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Development, optimisation and validation of a liquid chromatography-mass spectrometry method for the detection of drugs of abuse and pharmaceuticals in drinking water

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A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

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DECLARATION

I DECLARE THAT THIS RESEARCH IS MY ORIGINAL WORK EXCEPT WHERE REFERENCES AND ACKNOWLEDGEMENTS ARE MADE

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ABSTRACT
FACULTY OF SCIENCE AND TECHNOLOGY
DOCTOR OF PHILOSOPHY
DEVELOPMENT, OPTIMISATION AND VALIDATION OF A LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR THE DETECTION OF DRUGS OF ABUSE AND PHARMACEUTICALS IN DRINKING WATER
YAN PENG
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The quality of drinking water is fundamental to human health and welfare and therefore it is important to remove contaminants. Recent research has focused on the presence of drugs of abuse and pharmaceuticals in water which could have an adverse effect on human health via bio-accumulation. Therefore, the focus of this research is to develop a method to simultaneously analyse 20 traditional illicit drugs, novel psychoactive substances (NPS) and antidepressants in drinking water from the East Anglian, UK, which has never been investigated before. Furthermore, removal efficiencies were also determined to assess the drinking water treatment plants effectiveness in treating and eliminating such compounds.

The analysis was based on solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS) using a C_{18} column for identification and quantification, followed by a biphenyl column for confirmation. 65 - 107 % SPE recoveries were achieved for 17 analytes. For the C_{18} column, precision was below 7.57 % and 15.04 % relative standard deviations for higher and lower concentrations and method accuracy was below ± 8.66 % bias at low, medium and high concentrations. Method detection and quantification limits (0.0056 - 1.0918 ng/L and 0.0187 - 3.6394 ng/L) were at sub ng/Ls. For the biphenyl column, the method was selective and instrumental detection limits ranged from 0.0115 to 0.4795 ng/mL. This is the first reported method for the analysis of 20 drugs of abuse and pharmaceuticals in drinking water using LC-MS.

Cocaine, methamphetamine, citalopram, fluoxetine, ketamine, mephedrone and methylone were detected in drinking water between 0.139 and 2.814 ng/L. The latter two NPS have been found in drinking water for the first time. In addition, the removal efficiencies of drinking water treatment plants were determined for methamphetamine, fluoxetine, ketamine and mephedrone from -25.27 % to 98.76 %.

The findings could help to identify and recognise the ever-changing composition of contaminants in drinking water, which can aid in the development of water treatments for their removal. Moreover, this research could inform drinking water regulatory bodies of the presence of drugs of abuse and pharmaceuticals, as they are currently not included within the regulatory framework.

Keywords: drugs of abuse, pharmaceuticals, solid phase extraction, liquid chromatography-mass spectrometry, drinking water

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

3-CPP	1-(3-chlorophenyl)piperazine
3-TFMPP	1-(3-trifluoromethylphenyl)piperazine
4-FPP	1-(4-fluorophenyl)piperazine
4-MeOPP	1-(4-methoxyphenyl)piperazine
4-TFMPP	1-(4-trifluoromethylphenyl)piperazine
AC	Alternating current
ACMD	Advisory Council on the Misuse of Drugs
ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionisation
API	Atmospheric pressure ionisation
BZP	1-benzylpiperazine
CSEW	Crime Survey for England and Wales
DAD	Diode array detector
DC	Direct current
DDT	Dichlorodiphenyltrichloroethane
DL	Desolvation line
DWTP	Drinking water treatment plant
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESI	Electrospray ionisation
EtOAC	Ethyl acetate
GAC	Granular activated carbon
GC-MS	Gas chromatography-mass spectrometry
HDPE	High-density polyethylene
HPLC-DAD	High performance liquid chromatography-diode array detection
HSCIC	Health and Social Care Information Centre
ICH	International Conference on Harmonisation
IDL	Instrumental detection limit

IPA	Isopropanol
IQL	Instrumental quantification limit
IUPAC	International Union of Pure and Applied Chemistry
JWH-073	(1-butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone
JWH-398	(4-chloronaphthalen-1-yl)-(1-pentylindol-3-yl)methanone
kg	Kilograms
k_{ow}	Octanol-water partition coefficient
L	Litre
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MBZP	1-methyl-4-benzylpiperazine
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MDL	Method detection limit
MDMA	3,4-methylenedioxymethamphetamine
MDPV	Methylenedioxypyrovalerone
MeOH	Methanol
mg	Milligram
mg/L	Milligram per litre
mL	Millilitre
MQL	Method quantification limit
MS	Mass spectrometry
m/z	Mass-to-charge ratio
ng/L	Nanogram per litre
ng/mL	Nanogram per millilitre
NPS	Novel psychoactive substances
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organisation for Economic Co-operation and Development

PAR	Peak area ratio
pK_a	Logarithmic acid dissociation constant
Q	Quadrupole
QC	Quality control
QqQ	Triple quadrupole
R^2	Coefficient of determination
RI	Retention index
RMSE	Root mean square error
RSD	Relative standard deviation
RT	Retention time
SIC	Selected ion chromatogram
SIM	Selected ion monitoring
S/N	Signal-to-noise ratio
SPE	Solid phase extraction
SSRIs	Selective serotonin reuptake inhibitors
TCAs	Tricyclic antidepressants
THC	Δ^9-tetrahydrocannabinol
µg/L	Microgram per litre
UK	United Kingdom
µL	Microlitre
UNODC	United Nations Office on Drugs and Crime
USA	United States of America
US EPA	US Environmental Protection Agency
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
WWTP	Waste water treatment plant

LIST OF APPENDICES

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DEVELOPMENT, OPTIMISATION AND VALIDATION OF A LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR THE DETECTION OF DRUGS OF ABUSE AND PHARMACEUTICALS IN DRINKING WATER

YAN PENG

CHAPTER 1 INTRODUCTION

This research has developed and validated a method based on solid phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS). These methods were then used to determine drugs of abuse and pharmaceuticals in drinking water. This chapter introduces the theoretical background and publications underpinning this research. It discusses drugs of abuse and pharmaceuticals as emerging water contaminants and their impact, occurrence in the environment and distribution in drinking water. Therefore, a review of the drinking water treatment methods currently used is also included. The chapter discusses published findings regarding the presence and concerns of drugs of abuse and pharmaceuticals in drinking water, as well as the possible adverse effects on human health through possible bio-accumulation, which highlights the importance of this research. The chapter concludes with the drugs of abuse and pharmaceuticals chosen in this investigation, including the reasoning behind their selection, as informed by the reviewed literature. Additionally, the type of sample preparation and analytical technique used are reviewed and the parameters of method validation are also included. This chapter finishes with the aims of this research.

1.1 Sustainable drinking water

Daily water intake is essential to humans for keeping the body hydrated and maintaining normal body functions. However, the majority (97 %) of Earth's available water is saline and non-potable, while 2 % is locked away in glaciers and stagnant ice. Only 1 % of water meets with the needs of humanity (Royal Society of Chemistry, 2007). This means that there is a shortage of potable water resources on Earth (*ibid*). Moreover, in the face of an increasing global population, potable water has inevitably been polluted by various human activities (Harrison, 2014). Providing sufficient drinking water to satisfy the demand for human needs and an increasing population has become one of the key challenges being facing mankind in the 21st century (Royal Society of Chemistry, 2007). The sustainable management of this precious water resource plays an important role in helping to deal with this challenge by retaining water at an appropriate standard for human consumption on a long-term basis (*ibid*). In order to achieve this goal, it is important to identify and recognise

the ever-changing composition of contaminants and pollutants in drinking water, which can aid the development of water treatments for their removal and inform policy and regulations (Kümmerer, 2009; Escher, et al., 2011).

It is important to distinguish what a water contaminant and a water pollutant is. A water contaminant is a substance that is present in the aquatic environment but should not normally occur at concentrations over natural background (Chapman, 2007). When a water contaminant poses as an adverse biological effect to resident communities, it crosses over to pollutant status and is defined as a water pollutant (Chapman, 2007; Harrison, 2014). As an example with regards to drinking water, pesticides are known as water pollutants, due to the existing evidence and publication on their harm to human health. However, drugs of abuse and pharmaceuticals are still defined as water contaminants, since no human health consequences associated with exposure of these trace substances via drinking water have been reported so far.

1.2 Pollutants and contaminants in drinking water

The presence of pollutants and contaminants in drinking water has always received a considerable amount of public attention and scientific interest, particularly with respect to their effects on human health (Peng, Hall and Gautam, 2016). Historically, as an example, a substantial number of papers have highlighted the detection of endocrine disrupting compounds in drinking water (Falconer, et al., 2006; Benotti, et al., 2009). These have included compounds such as herbicides (atrazine and simazine) and insecticides (monocrotophos and triazofos) (Rodriguez-Mozaz, López de Alda and Barceló, 2004; Sinha, et al., 2011). These pollutants are present in run-offs from crops due to their solubility in water, which is their primary route into surface water and ground water (Aydinalp and Porca, 2004; Konstantinou, Hela and Albanis, 2006). This, then, eventually contaminates drinking water, as surface water and ground water are normally used as sources of drinking water. This is discussed further in Section 1.4. As an example, in the central and eastern regions of the United Kingdom (UK), 58 % of drinking water comes from treated surface water, 32.5 % from ground water and 9.5 % from either surface or

ground water (Drinking Water Inspectorate, 2014). Concerning the direct consumption of pesticide-contaminated water, there is increasing anxiety about the impact on the quality of drinking water and the results of serious consequences, especially regarding human health. For example, there is evidence for the possibility of increased rates of cancer and adverse effects on reproduction as some pesticides are suspected of being carcinogens or are able to disrupt endocrine activities (Ejaz, et al., 2004; Harrison, 2014). Thus, many developed countries such as the United States of America (USA) and European countries have introduced a variety of actions to regulate the use of pesticides and have even banned key pesticides that are associated with serious health effects, such as the organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and aldrin, in order to protect the waters (Ongley, 1996; World Health Organization, 2003; Rodriguez-Mozaz, López de Alda and Barceló, 2004; World Health Organization, 2004; Pesticide Action Network Europe, 2008). As a result, the amount of pesticides used showed a significant reduction worldwide due to proper management through regulation (Pimentel, et al., 1991).

In the last few years, the focus has shifted towards other emerging contaminants in the field of drinking water quality and analysis (Wille, et al., 2012). Drugs of abuse and pharmaceuticals are included in this group. Although they have been used for a long time, they have only recently been detected in drinking water due to advances in detection methods (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013; Peng, Hall and Gautam, 2016). For example, cocaine has been widely used as an illicit drug since the 20th century (Isralowitz and Myers, 2011), but it was only reported to have been detected in drinking water in 2008 using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Boleda, et al., 2011), which is a highly selective and sensitive technique popularly used for water analysis in recent years (discussed later in Section 1.9.2).

Alongside review papers on water analysis, the majority of publications and research focuses on the presence of drugs of abuse and pharmaceuticals in waste water and surface water (Mompelat, Le Bot and Thomas, 2009; Peng, Hall and Gautam, 2016). These findings are used both in a forensic perspective to estimate community drug usage

patterns and in an environmental perspective to evaluate the possible adverse effects on aquatic organisms' health (Baker and Kasprzyk-Hordern, 2011a). For example, cocaine has been detected in surface water in the range of 0.4 to 44 ng/L (Pal, et al., 2012). These concentrations of cocaine found in the aquatic environment have been shown to induce cyto-genotoxic effects in the mollusc Zebra mussel, such as primary DNA damage, a marked increase in micronucleated cells and a clear rise in apoptosis (Binelli, et al., 2012). Recently, the concern of the public and scientists is more focused on the detection of drugs of abuse and pharmaceuticals in drinking water (Peng, Hall and Gautam, 2016). These are present at very low concentrations in drinking water (Section 1.6), but are thought to be a potential threat to human health because these compounds are biologically active and may induce adverse effects. This is discussed further in Section 1.7.

1.3 United Kingdom (UK) policies governing drinking water quality

In order to provide wholesome and clean water intended for human consumption, the quality of drinking water in Europe is governed by the Drinking Water Directive (Council Directive 98/83/EC) through regularly monitoring and testing 48 microbiological, chemical and indicator parameters (European Commission, 2016b). With regards to the UK, the Drinking Water Directive has been translated into the national legislation, Water Supply (Water Quality) Regulations 2016, which relates to the water quality of the UK (Department for Environment, Food & Rural Affairs, 2016; European Commission, 2016b). This UK legislation is imposed in England and Wales and its legal standards are those which are laid down in the Drinking Water Directive of Europe, together with added national requirements for some parameters (European Commission, 2016b; The Water Supply (Water Quality) Regulations 2016). For example, pesticides in drinking water are monitored by the Water Supply (Water Quality) Regulations 2016 in order to maintain high-quality drinking water and the maximum concentration of the total pesticides should not be above 0.5 µg/L (microgram per litre). However, emerging contaminants, such as drugs of abuse and pharmaceuticals, are currently not included in the above-mentioned European and UK legislation for drinking water (Council Directive 98/83/EC; The Water Supply (Water Quality) Regulations 2016).

In 2013, the European Water Framework Directive (Directive 2013/39/EU) was introduced for the monitoring of priority substances in surface water on a European basis. As a result, diclofenac, a pharmaceutical compound belonging to a class of non-steroidal anti-inflammatory drugs (NSAIDs), has been added to the first watch list for collecting information regarding the risk is poses to the aquatic environment (Directive 2013/39/EU; Thermo Scientific, 2015). Although only one pharmaceutical compound is being monitored in surface water, this is a positive move in understanding the emerging contaminants in aquatic environments. As the drinking water standards are set based on the latest scientific evidence regarding the occurrence and level of pollutants and contaminants in drinking water (European Commission, 2016a), this highlights the need for studies for the evaluation of the presence of drugs of abuse and pharmaceuticals in drinking water, which this research aims to provide.

1.4 Occurrence of drugs of abuse and pharmaceuticals in drinking water

A number of research papers have been published on the transport of drugs of abuse and pharmaceuticals into the aquatic environment (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013). They are known to be present in waste water from human waste (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013; Peng, Hall and Gautam, 2016). When these compounds are consumed, they are distributed by the body and are either excreted unchanged as the parent compounds or as metabolites (Mompelat, et al., 2010). These are then released through faeces and urine into waste water (Repice, et al., 2013). In addition, improper disposal of unused or expired pharmaceuticals into toilets is also known to be a minor route into waste water (Gros, Petrović and Barceló, 2007). The waste water containing drugs of abuse and pharmaceuticals does pass through waste water treatment plants (WWTPs), but the processes of WWTPs are not designed to specifically eliminate these types of contaminants and therefore they are not always completely removed and are still present in the effluent waste water (Repice, et al., 2013). Subsequently, these contaminants are then released into surface water, such as rivers and lakes (Gros, Petrović and Barceló, 2007; Pal, et al., 2013). These drug residues are now being reported to have reached ground water, which is thought to be caused from either water leakage

from waste water systems or seepage from surface waters (Jurado, et al., 2012). With regards to drinking water, surface and ground waters that may contain drugs of abuse and pharmaceuticals are also used as a source of raw water for drinking water production (Mompelat, Le Bot and Thomas, 2009; Peng, Hall and Gautam, 2016). The raw water is treated by drinking water treatment plants (DWTPs) for human consumption by removing contaminants, pH adjustment and then additionally treated to improve taste, odour and colour (Drinking Water Inspectorate, 2010a). However, the treatment processes of DWTPs are generally designed to address the reduction in micro-organisms, metals and chemicals such as nitrates and pesticides, but not necessarily for drugs of abuse and pharmaceuticals (Drinking Water Inspectorate, 2010b). Therefore, the incomplete removal of drugs of abuse and pharmaceuticals is considered to be the main reason why these contaminants still exist and eventually end up in drinking water (Section 1.6).

The presence of drugs of abuse and pharmaceuticals in drinking water supplies raises concern over the removal efficiencies of these contaminants during current treatment processes of DWTPs. To date, a few studies have reported drugs of abuse and pharmaceuticals in both raw water and finished drinking water and have evaluated the removal rates of these compounds, as studied in a review paper (Peng, Hall and Gautam, 2016). In general, most of the studied drugs of abuse and pharmaceuticals are eliminated during DWTPs; however, some are still being detected in finished drinking water, which illustrates that they survive current water treatment processes. For example, the partial elimination of methadone (91 %) and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) (88 %) were reported when methods used by a DWTP consisted of pre-chlorination, clarification, post-ozonation, granular activated carbon (GAC) filtration and post-chlorination (Boleda, Galceran and Ventura, 2009). Their partial removal efficiencies may be due to several factors, such as their physico-chemical characteristics and the treatment processes applied (Huerta-Fontela, Galceran and Ventura, 2008). These are discussed further in Section 1.5. The various treatment processes of drinking water and specific examples of how drugs of abuse are removed, or partially removed, are discussed further in Section 1.5. The review

of drugs of abuse and pharmaceuticals detected in drinking water is also included in Section 1.6.

1.5 Raw water treatment methods for the production of drinking water

Water used for drinking water supplies is abstracted from surface water sources such as rivers and lakes and ground water sources (aquifers) (Drinking Water Inspectorate, 2014). This water, that has not undergone drinking water treatments, is referred to as raw water (Peng, Hall and Gautam, 2016). Drinking water treatment processes are used to remove or deactivate particles, pathogens, organic and inorganic pollutants from raw water and give a residual disinfectant (Royal Society of Chemistry, 2007). Various water treatment methods are applied, depending on the conditions of the raw water; generally, however, drinking water goes through six main stages of treatment which involve physical and chemical processes. These processes are known as pre-treatment, pre-oxidation, clarification, primary disinfection, filtration and secondary disinfection (LeChevallier and Au, 2004; Huerta-Fontela, Galceran and Ventura, 2008) and are described in the following sub-sections.

1.5.1 Pre-treatment

This stage is mainly used for treating the raw water abstracted from surface water sources, which is then stored in reservoirs for a long storage time (Thames Water, 2014). This process, known as self-purification, allows for water quality improvement, by the settling of suspended solids and adsorbed substances such as turbidity and heavy metals, biodegradation of organic substances by sunlight and die-off of faecal bacteria and viruses (LeChevallier and Au, 2004). In addition, filtering screens are also employed in pre-treatment to remove floating objects such as leaves from the water as these can cause blockage and damage in treatment processes (Thames Water, 2014).

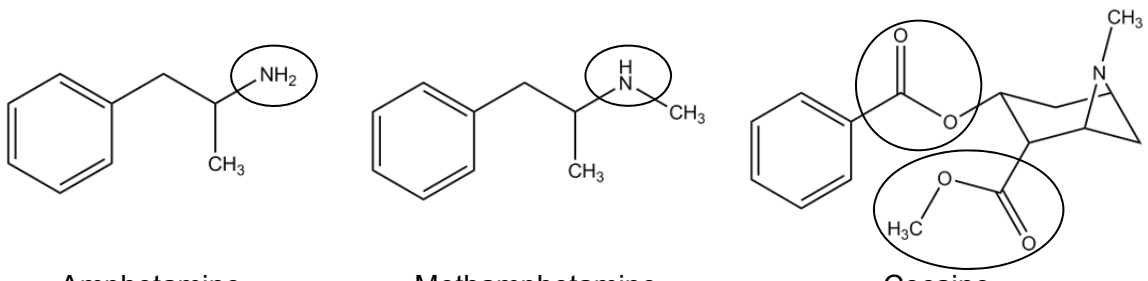
1.5.2 Pre-oxidation

This initial treatment is normally applied to raw water from surface sources (LeChevallier and Au, 2004; Huerta-Fontela, Galceran and Ventura, 2008). Usually, the oxidants are

added in this early stage treatment process in order to provide a long contact time for continual oxidation (LeChevallier and Au, 2004). Chlorine is often used in DWTPs, particularly in Europe, and are hence called pre-chlorination (Huerta-Fontela, Galceran and Ventura, 2008; Environmental Protection Agency, 2011). Thus, the oxidation reactions of chlorine and the effectiveness of removing drugs of abuse and pharmaceuticals by chlorination are discussed in this section. In addition, ozone, another powerful oxidant, is also used by some DWTPs instead of chlorine for pre-oxidation, which is referred to as pre-ozonation (Environmental Protection Agency, 2011). This is probably because ozone oxidises more compounds and produces fewer undesirable by-products compared to chlorine (Zwiener, 2007). However, ozonation is widely used in the primary disinfection stage and hence is further discussed in Section 1.5.4.

During the pre-chlorination stage, chlorine gas is added to water until it has undergone oxidation reactions and is then available as free chlorine residual, which has a high disinfecting ability (Huerta-Fontela, Galceran and Ventura, 2008; Environmental Protection Agency, 2011). Thus, the application of chlorine is for the purpose of disinfection, e.g. to kill microorganisms (LeChevallier and Au, 2004). Moreover, chlorine is also capable of oxidising unwanted inorganic compounds (e.g. iron or manganese) and organic compounds (e.g. drugs of abuse and pharmaceuticals) from water (*ibid*). The oxidation of these organic compounds by chlorine is related to electrophilic substitution. Their reactivity is influenced by the chemical properties of the compounds, such as whether they have amines and phenols as the electron-donating functional groups, which are the sites that are reactive to chlorine (Pinkston and Sedlak, 2004; Westerhoff, et al., 2005; Huerta-Fontela, Galceran and Ventura, 2008). Amino and phenolic groups have high electron densities, possessing a lone pair of electrons, which enable them to donate electrons to a neighbouring atom and thus are more likely to play a part in electrophilic substitution reactions (Miller and Solomon, 2000). Chlorine acts as the electrophile to selectively attack amino and phenolic groups in order to yield chlorinated amines and ortho- or para-substituted chlorophenols (Pinkston and Sedlak, 2004). Thus, the amine and/or phenol-containing compounds undergo a rapid reaction with chlorine, leading to

high removal during the pre-chlorination treatment (Westerhoff, et al., 2005; Huerta-Fontela, Galceran and Ventura, 2008). In contrast, if compounds contain electron-withdrawing functional groups (e.g. carboxyl and ester), they are less likely to undergo electrophilic substitution, as these groups are more likely to draw electron density from neighbouring atoms towards themselves (Miller and Solomon, 2000). This results in the slow reaction between carboxyl and/or ester-containing compounds and chlorine, leading to low removal during the pre-chlorination treatment (Westerhoff, et al., 2005). This correlates with a report by Huerta-Fontela, Galceran and Ventura (2008), wherein amphetamine that contains a primary amine and methamphetamine that contains a secondary amine, were removed at high percentages (> 99 %) by pre-chlorination, whereas cocaine with ester groups exhibited poor removal percentage (13 %). The structures of three drugs of abuse, as described above, can be found in Figure 1.1.



Amphetamine Methamphetamine Cocaine

Figure 1.1: Structures of amphetamine, methamphetamine and cocaine (drawn using ChemDraw Pro 13.0) and their functional groups relevant for pre-chlorination (circled)

Many drugs of abuse and pharmaceuticals exhibit amino or phenolic groups in their structure (shown in Table 1.3), thus pre-oxidation with chlorine is an effective treatment to remove these compounds. However, further treatments are essential for removing some drugs of abuse and pharmaceuticals that contain groups such as carboxyl and ester, or exhibit different chemical properties that prevent their total removal via pre-oxidation.

1.5.3 Clarification

Clarification is the second stage of drinking water treatment for the further removal of suspended sediments and dissolved organic carbon, which is normally applied to raw

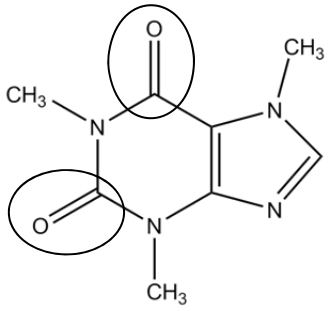
water from surface sources (Westerhoff, et al., 2005; Stackelberg, et al., 2007; Huerta-Fontela, Galceran and Ventura, 2008). In this stage, several steps are used, including coagulation, flocculation, sedimentation and sand filtration, (Huerta-Fontela, Galceran and Ventura, 2008). Firstly, coagulants such as ferric chloride are added to cause coagulation (Stackelberg, et al., 2007). The purpose of adding coagulants is to destabilise particles by reducing their surface electrical charge and to form the hydrolysis products that allow inter-particle attraction to form large particles (LeChevallier and Au, 2004). Flocculation is the next step, which is a physical process to promote particle aggregation by gentle mixing (*ibid*). Large particles that have sufficient settling velocities precipitate into the sedimentation basin, which can be separated from the water (Thames Water, 2014). Finally, the water undergoes gravity filtration through a sand filter to further remove suspended particles (LeChevallier and Au, 2004).

Clarification has been shown to be an efficient process to remove high molecular organic material in water, such as humic acids, whose molecular weights are from 700 to 200,000 (Ishiwatari, 1971; Vieno, Tuhkanen and Kronberg, 2006). However, this treatment is not an effective method to remove drugs of abuse and pharmaceuticals from the drinking water. According to Vieno, Tuhkanen and Kronberg (2006), five selected pharmaceuticals (diclofenac, ibuprofen, bezafibrate, carbamazepine and sulfamethoxazole) were poorly removed (all below 10 % removal, apart from diclofenac with 30 % removal) by the clarification process, which is probably due to their relatively lower molecular weights (< 361.83).

1.5.4 Primary disinfection

Primary disinfection is the process of chemical oxidation and ozone is typically used as the oxidant in this stage (LeChevallier and Au, 2004). This treatment is referred to as post-ozonation and seems to be considerably more efficient at removing organic compounds compared to other treatments. For example, post-ozonation followed by GAC filtration has been applied at numerous DWTPs globally for pesticides removal (Royal Society of Chemistry, 2007). The role of post-ozonation is to break down pesticides into compounds, which are readily absorbed by subsequent GAC filtration (*ibid*).

Like chlorine, the addition of ozone is for the purpose of disinfection and oxidation (von Gunten, 2003a). Ozone is a better disinfectant as it is able to inactivate even protozoa where other conventional disinfectants fail (von Gunten, 2003b). In addition, it is a more selective oxidant when compared to chlorine, because it oxidises not only amines and phenols (same reactive sites as chlorine), but also compounds with double bonds (Westerhoff, et al., 2005). Double bonds comprise a region of high electron density that is attractive to ozone electrophiles (von Gunten, 2003a). The mode of reactivity is related to electrophilic substitution, which is the same reaction as with chlorine (Section 1.5.2). Therefore, drugs of abuse and pharmaceuticals that contain amines, phenols or double bonds may show high reactivity with ozone and result in the high removal percentages during the post-ozonation treatment (Westerhoff, et al., 2005). For example, caffeine, which has double bonds in the structure (shown in Figure 1.2), was eliminated by up to 76 % in the post-ozonation treatment, whereas a lower removal percentage (38 %) was obtained using the pre-chlorination treatment, as chlorine only reacts with amines and phenols and caffeine lacks these two reactive sites (Huerta-Fontela, Galceran and Ventura, 2008).



Caffeine

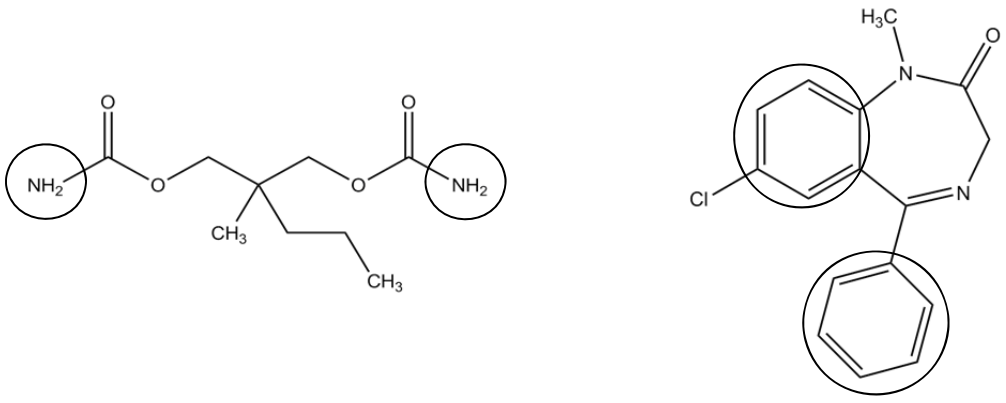
Figure 1.2: Structure of caffeine (drawn using ChemDraw Pro 13.0) and its functional groups relevant for post-ozonation (circled)

1.5.5 Filtration

Various filtrations are used in this stage, such as slow sand filtration and membrane filtration, but activated carbon filtration is the most commonly used process for drinking water treatment (LeChevallier and Au, 2004). This is because the adsorption process with activated carbon is considered as an efficient way to remove most of the organic compounds, such as pesticides (Royal Society of Chemistry, 2007). Adsorption is related to the properties of the compounds (Huerta-Fontela, Galceran and Ventura, 2008). The octanol-water partition coefficient (k_{ow}) of compounds, which represents the ratio of the solubility of a compound in octanol to its solubility in water, therefore influences the adsorption rates with the log k_{ow} used as a relative indicator (Miller, et al., 1985; Yu, Peldszus and Huck, 2008). If log k_{ow} value is higher, the compound is more hydrophobic (less soluble in water, but more soluble in octanol). In contrast, if log k_{ow} value is lower, the compound is less hydrophobic but more hydrophilic (more soluble in water) (Miller, et al., 1985). As adsorption is controlled by hydrophobic interactions between the activated carbon sorbent and the compound, compounds with a higher log k_{ow} value should have a higher adsorption affinity on activated carbon, whereas compounds with a lower log k_{ow} value have a relatively lower adsorption affinity (Huerta-Fontela, Galceran and Ventura, 2008; Yu, Peldszus and Huck, 2008).

This can be explained by the comparison of the removal efficiencies of two pharmaceuticals, meprobamate and diazepam. As meprobamate contains two primary

amines (Figure 1.3), which are hydrophilic functional groups, this compound has a lower log k_{ow} (0.70) and lower adsorption affinity on activated carbon (Westerhoff, et al., 2005; Harrold and Zavod, 2013). Thus, in the paper published by Westerhoff, et al. (2005), meprobamate was found to be difficult to remove (33 %) when treated with activated carbon. Whereas, diazepam exhibited a higher (67 %) removal as this compound has a higher log k_{ow} (2.82) due to its hydrophobic functional groups (Figure 1.3), such as aromatic rings (Westerhoff, et al., 2005; Harrold and Zavod, 2013).



Meprobamate Diazepam

Figure 1.3: Structures of meprobamate and diazepam (drawn using ChemDraw Pro 13.0) and their functional groups relevant for activated carbon filtration (circled)

1.5.6 Secondary disinfection

Finally, secondary disinfection is used to maintain the quality of finished drinking water in distribution systems (LeChevallier and Au, 2004). Chlorine is added to the water to retain the free chlorine residual throughout the drinking water supply system, which is known as the post-chlorination treatment (Boleda, Galceran and Ventura, 2009). Unlike pre-oxidation with chlorine, the main aim of this treatment is to kill any remaining pathogens and control bacterial growth in order to keep the water safe as it travels through the distribution system, eventually reaching the domestic supply tap (Thames Water, 2014). Post-chlorination treatment is necessary as any microbial contamination can cause a public health risk (LeChevallier and Au, 2004).

1.6 Review of drugs of abuse and pharmaceuticals in drinking water

As drugs of abuse and pharmaceuticals cannot be totally removed by current drinking water treatments as highlighted in Section 1.5, to date, some drugs of abuse and pharmaceuticals have been quantified in drinking water at nanogram per litre (ng/L) levels (Peng, Hall and Gautam, 2016). There is limited literature in this area, probably because of the analytical problems in quantifying these compounds at ultra-trace levels and this is further discussed in Section 1.9. A review summarised that 24 pharmaceuticals and metabolites have been detected in drinking water and therapeutic classes of pharmaceuticals such as NSAIDs and anticonvulsants are generally analysed (Mompelat, Le Bot and Thomas, 2009). However, only a few reports are available on the presence of other pharmaceutical classes in drinking water, such as antidepressants. This is the reason for the selection of antidepressants as the representative of pharmaceuticals in this research, which is further discussed in Section 1.8.3. Table 1.1 lists some pharmaceuticals and their concentrations as reported in drinking water.

Table 1.1: Concentrations of pharmaceuticals reported in drinking water

COMPOUND	COUNTRY	CONC. / ng/L	REFERENCE
Anticonvulsants			
Carbamazepine	France	43.2 [a]	Togola and Budzinski, 2008
	Germany	60 [b]	Heberer, et al., 2004
	USA	258 [a]	Stackelberg, et al., 2004
Dilantin	USA	1.3 [b]	Vanderford and Snyder, 2006
Primidone	Germany	40 [b]	Heberer, et al., 2004
Antidepressants			
Amitriptyline	China	0.1 - 0.5 [c]	Wu, et al., 2015
Citalopram	Poland	1.5 [a]	Giebułtowicz and Nałęcz-Jawecki, 2014
Fluoxetine	China	0.1 - 0.2 [c]	Wu, et al., 2015
	Spain	2.74 [b]	López-Serna, et al., 2010
	USA	0.59 - 0.82 [c]	Benotti, et al., 2009
	USA	19.2 [a]	Padhye, et al., 2014
	USA	< 0.5 [d]	Vanderford and Snyder, 2006
Mianserin	China	0.1	Wu, et al., 2015
Antineoplastics			
Bleomycin	UK	5 - 13 [c]	Aherne, Hardcastle and Nield, 1990
Psycho-stimulants			
Caffeine	France	22.9 [a]	Togola and Budzinski, 2008
	USA	119 [a]	Stackelberg, et al., 2004

United States of America (USA); United Kingdom (UK)

[a] Maximum concentration; [b] Mean concentration; [c] Concentration range; [d] Mean concentration below quantification limit but above detection limit

Very few studies have reported drugs of abuse in drinking water (Stackelberg, et al., 2007; Huerta-Fontela, Galceran and Ventura, 2008; Boleda, Galceran and Ventura, 2009; Boleda, et al., 2011; Boleda, Galceran and Ventura, 2011; Valcárcel, et al., 2012; Carmona, Andreu and Picó, 2014; Mendoza, et al., 2014; Mendoza, et al., 2016; Rodayan, et al., 2016). These were mainly focused on the analysis of traditional illicit drugs, including amphetamines, cannabinoids, cocainics, dissociative anaesthetics and opioid analgesics. Amphetamines have been quantified in a few samples from Canada, Spain and Latin American countries at trace levels (0.2 - 3.13 ng/L) (Boleda, et al., 2011; Valcárcel, et al., 2012; Mendoza, et al., 2016; Rodayan, et al., 2016). These low concentrations could be due to the high removal efficiency at DWTPs (96 - 100 %) (Huerta-Fontela, Galceran and Ventura, 2008; Boleda, Galceran and Ventura, 2011).

Cannabinoids have also reported in three drinking water samples from Spain (Valcárcel, et al., 2012; Carmona, Andreu and Picó, 2014). However, the low concentrations (0.49 - 5.53 ng/L) and low detection frequencies (12.5 - 33.3 %) determined do not correlate with the high consumption levels of cannabinoids, but probably due to their lower solubility in water (log k_{ow} > 5) (Mendoza, et al., 2014; United Nations Office on Drugs and Crime, UNODC, 2015).

Cocaine and its metabolites have been detected in drinking water from Canada, Japan, European and Latin American countries with concentrations (0.1 - 130 ng/L) varying between countries (Huerta-Fontela, Galceran and Ventura, 2008; Boleda, et al., 2011; Boleda, Galceran and Ventura, 2011; Mendoza, et al., 2014; Mendoza, et al., 2016; Rodayan, et al., 2016). These differences correlate with their regional consumption rates and removal efficiencies of DWTPs, which is further discussed in Section 5.3.1. For opioid analgesics, nine parent compounds and two metabolites were present in drinking water from Canada, Japan, the USA, European and Latin American countries between 0.1 and 44 ng/L (Stackelberg, et al., 2007; Boleda, Galceran and Ventura, 2009; Boleda, et al., 2011; Valcárcel, et al., 2012; Mendoza, et al., 2016; Rodayan, et al., 2016). Ketamine (15 ng/L) is the only NPS that has currently been detected in drinking water (Rodayan, et al., 2016). This is further discussed and compared with the results of this research in Section 5.3.3.

Table 1.2: Concentrations of drugs of abuse reported in drinking water

COMPOUND	COUNTRY	CONC. / ng/L	REFERENCE
Amphetamines			
Amphetamine	Spain	< 1.0 [c]; 1.7 [d]	Boleda, et al., 2011
MDMA	Latin America [a]	< 0.2 [c]; 0.4 [d]	Boleda, et al., 2011
	Spain	1.51	Valcárcel, et al., 2012
	Spain	1.47	Mendoza, et al., 2016
Methamphetamine	Latin America [a]	< 0.5 [c]; 0.6 [d]	Boleda, et al., 2011
	Spain	< 0.5 [c]; 1.4 [d]	Boleda, et al., 2011
	Spain	3.13	Mendoza, et al., 2016
Cannabinoids			
THC	Spain	5.53	Valcárcel, et al., 2012
Cocainics			
Cocaine	Canada	4.3 [e]	Rodayan, et al., 2016
	Europe [b]	0.1 [e]	Boleda, et al., 2011
	Japan	< 0.1 [c]	Boleda, et al., 2011
	Latin America [a]	0.6 [e]	Boleda, et al., 2011
	Spain	0.4 [e]; 2.3 [d]	Boleda, et al., 2011
	Spain	1.61	Mendoza, et al., 2014
	Spain	0.11 - 85.67 [f]	Mendoza, et al., 2016
Dissociative Anaesthetics			
Ketamine	Canada	15.0 [e]	Rodayan, et al., 2016
Opioid Analgesics			
Codeine	Canada	44.0 [e]	Rodayan, et al., 2016
	USA	30 [d]	Stackelberg, et al., 2007
Fentanyl	Canada	12.0 [e]	Rodayan, et al., 2016
	Spain	< 1.0 [c]; 1.4 [d]	Boleda, et al., 2011
Methadone	Europe [b]	0.1 [e]	Boleda, et al., 2011
	Latin America [a]	0.2 [e]	Boleda, et al., 2011
	Spain	0.2 [e]; 2.7 [d]	Boleda, et al., 2011
	Spain	0.99	Valcárcel, et al., 2012
	Spain	0.11 - 0.31 [f]	Mendoza, et al., 2016
Morphine	Canada	6.4 [e]	Rodayan, et al., 2016
Oxycodone	Canada	5.1 [e]	Rodayan, et al., 2016
Tramadol	Canada	5.4 [e]	Rodayan, et al., 2016

3,4-methylenedioxymethamphetamine (MDMA); Δ^9-tetrahydrocannabinol (THC)

[a] Includes Argentina, Brazil, Chile, Colombia, Panama, Peru and Uruguay; [b] Includes Austria, France, Germany, Iceland, Slovakia, Switzerland and the UK; [c] Mean concentration below quantification limit but above detection limit; [d] Maximum concentration; [e] Mean concentration; [f] Concentration range

The published review has revealed a lack of research regarding other drugs of abuse in drinking water, such as novel psychoactive substances (NPS) (Peng, Hall and Gautam, 2016). This is discussed further in Section 1.8.2. Available data regarding the concentrations of some drugs of abuse in drinking water is summarised in Table 1.2. Many drugs of abuse have been only determined in one study, such as amphetamine, Δ^9-tetrahydrocannabinol (THC), ketamine, morphine, oxycodone and tramadol (Boleda, et al., 2011; Valcárcel, et al., 2012; Rodayan, et al., 2016). Thus, there is a real need to undertake more studies to enable a better comparison of data and the prevalence of drugs of abuse in drinking water.

1.7 Human health impacts

A major concern about the presence of drugs of abuse and pharmaceuticals in drinking water is the possible adverse effect on human health (Mompelat, Le Bot and Thomas, 2009; Peng, Hall and Gautam, 2016). Some of the drugs of abuse and pharmaceuticals as described above have been detected in drinking water with concentrations generally reported in the ng/L range (Section 1.6). Although these concentrations are described as lower than those known to cause pharmaceutical and toxicological effects, there are still concerns with the continuous trace level exposure to drugs of abuse and pharmaceuticals causing a chronic human health risk, as they can bio-accumulate in human body (Peng, Hall and Gautam, 2016). For instance, carbamazepine, an anticonvulsant, has been detected in drinking water at the concentration of 0.258×10^{-3} mg/L (milligram per litre) (Stackelberg, et al., 2004). Assuming that an individual drinking two litres (L) of water per day would ingest an amount of carbamazepine equivalent to 0.516×10^{-3} milligram (mg), being 0.188 mg over a year, whereas a single therapeutic dose of carbamazepine is 100 mg (*ibid*). Therefore, the likelihood of acute human health risk is extremely low.

However, some drugs of abuse and pharmaceuticals, such as cocaine and diazepam, are toxic, persistent and lipophilic, leading to accumulation in bone, fat or other body compartments (Nayak, Misra and Mulé, 1976; Friedman, et al., 1985; Cone and Weddington, 1989). These drugs could then be retained in the body for a long time, as

known with chronic use, although this could also be the case through chronic exposure by contaminated drinking water.

Moreover, it is also necessary to be aware of the problems of possible drug-drug reactions, which might cause the synergistic effects. For example, mixing cocaine with heroin can amplify the effects of both drugs and increase the risk of death (Duvauchelle, Sapoznik and Kornetsky, 1998). As it is essential to drink water every day to maintain hydration, with drugs of abuse and pharmaceuticals now being reported to be present in drinking water, even at trace levels, their bio-accumulation in the human body and drug interaction are a matter of concern.

Therefore, the potential for chronic adverse effects of drugs of abuse and pharmaceuticals in drinking water should not be overlooked, especially for vulnerable people. For instance, young and elderly people are more sensitive as they have a reduced capability to remove toxic compounds from their bodies compared to healthy adults (Jones, Lester and Voulvoulis, 2005). Another concern is the exposure of toxic compounds to foetuses. As an example, antineoplastics (anticancer pharmaceuticals) have received increasingly reported concerns about their adverse effects on foetus health. As these pharmaceuticals, as many others do, cross the placenta, the foetus could be exposed to antineoplastics from their mother taking such prescribed medications (Bawle, Conard and Weiss, 1998; Paskulin, et al., 2005). Abnormal foetus development, such as growth retardation, craniofacial and digital anomalies, has been reported (Johnson, et al., 2008; Cancer Research UK, 2016). Some antineoplatstics have also been detected in drinking water, for example bleomycin was present at the concentration of 13 ng/L (Aherne, Hardcastle and Nield, 1990). Therefore, drinking water is another exposure route for such compounds. The quantity of antineoplastics found in drinking water may not be a problem for adults, but there may be sufficient levels to pose a health risk for the foetus (Derbyshire, 2008).

To date, no substantial human health consequence associated with the exposure of drugs of abuse and pharmaceuticals via drinking water has been reported. However, as this is a

relatively new area of research, only their detection in drinking water has been proved so far. However, drugs of abuse and pharmaceuticals in the environment can still be recognised as potential threats to human health due to their residues in drinking water and their potential toxic effects through accumulation (Hernando, et al., 2006; Peng, Hall and Gautam, 2016). Therefore, it is still necessary to assess the human health risk of drugs of abuse and pharmaceuticals in drinking water. As the risk can be evaluated by analysing the concentration of such contaminants in drinking water and comparing this to the level that causes adverse effects on human health (Johnson, et al., 2008), studies regarding the analysis of drugs of abuse and pharmaceuticals in drinking water are prerequisite for proving their environmental concentrations, which is the aim of this research.

1.8 Selection of drugs of abuse, pharmaceuticals and internal standards for this research

As mentioned in Section 1.7, there is a need for further research exploring the presence of drugs of abuse and pharmaceuticals in drinking water. However, it is impossible to analyse all drugs of abuse and pharmaceuticals that may be present in the drinking water in one study. Therefore, the rationale of defining major compounds of interest within this research is of importance, as detailed in the following sub-sections.

Studies regarding drinking water analysis mainly focus on traditional illicit drugs. However, as mentioned in Section 1.6, there is a limited amount of research in this area and literature reviews have revealed a lack of research in the UK (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013; Peng, Hall and Gautam, 2016). Thus, traditional illicit drugs were analysed in drinking water collected from the East Anglia region of the UK in this research, which can be compared with other published studies. Therefore, the most commonly traditional illicit drugs in the UK and Europe were chosen as target analytes (Mixmag, 2012; European Monitoring Centre for Drugs and Drug Addiction, EMCDDA, 2014a). While the majority of studies regarding the presence of pharmaceutical residues in aquatic environments mainly focused on four therapeutic classes (NSAIDs, anticonvulsants, antibiotics and lipid regulators), only 15 % of studies include antidepressants (Mompelat,

Le Bot and Thomas, 2009). Due to the shortfall in studies regarding antidepressants, this therapeutic class was chosen in this research as a representative of pharmaceuticals and the most frequently prescribed antidepressants in England according to the Health & Social Care Information Centre (HSCIC) report (2014) were selected in this research. Traditional illicit drugs and antidepressants are further discussed in Section 1.8.1 and Section 1.8.3.

The emphasis of this research is also on NPS, such as ketamine, cathinones, piperazines and synthetic cannabinoids, since they are progressively being abused (Home Office, 2012) and no data regarding drinking water analysis is available, except for ketamine (Rodayan, et al., 2016). The choice of studied NPS was based on findings from the Advisory Council on the Misuse of Drugs (ACMD) report (2010), Mixmag survey (2012), EMCDDA reports (2013; 2014a; 2015a; 2015e), UNODC report (2014) and published studies (Davies, et al., 2010; Ramsey, et al., 2010; Baker and Kasprzyk-Hordern, 2011b; Dargan, et al., 2011; Mwenesongole, et al., 2013). Further background and discussion to the NPS investigated in this research is included in Section 1.8.2.

In summary, this research aims to analyse not only traditional illicit drugs and pharmaceuticals, but also NPS in drinking water.

1.8.1 Traditional illicit drugs

Traditional illicit drugs have always received considerable attention by law enforcement agencies. The use of these drugs is a global problem and is normally related to physical and psychological harm in addition to drug dependence, such as the user continuing to take drugs despite associated problems (Degenhardt, et al., 2004; Pal, et al., 2013). Globally, between 3.5 % and 7.0 % of the world population aged 15 - 64 have reported using a traditional illicit drug in 2012, mainly a substance belonging to the cannabis, opioids, cocaine or amphetamines group (UNODC, 2014). Thus, the production, trafficking and consumption of traditional illicit drugs are prohibited by national or international laws (Degenhardt, et al., 2004). Traditional illicit drugs are also popular in the field of drinking

water analysis. Studies have generally focused on the detection and quantification of a mix of commonly used traditional illicit drugs. The most studied traditional illicit drugs in drinking water are amphetamine, methamphetamine and cocaine because of their high consumption (Pal, et al., 2013; Peng, Hall and Gautam, 2016). Amphetamines such as amphetamine and methamphetamine are a class of synthetic stimulant drugs, containing phenethylamine, and have sympathomimetic activity, which is designed to stimulate the central nervous system (Moore, 2003). Cocaine is a psychotropic drug, but it is also considered as a powerful, addictive stimulant (Isenschmid, 2003).

The number of seizures of illicit drugs in the European Union from drug users, traffickers and producers were reported to be around one million in 2012 (EMCDDA, 2014a). The report indicated that 9 % of seizures were cocaine, 3 % amphetamine and 1 % methamphetamine. Among all seizures, two-thirds were reported by Spain and the UK. This trend reflects the results of the Mixmag survey based in the UK, where 41.8 % of respondents have taken cocaine, 4.8 % amphetamine and 0.8 % methamphetamine in 2011 (Mixmag, 2012). Due to their high prevalence, cocaine, amphetamine and methamphetamine have recently been analysed in drinking water in Europe (Boleda, et al., 2011; Mendoza, et al., 2014; Mendoza, et al., 2016). However, in these studies, only two samples were collected from large cities in the UK. Hence, cocaine, amphetamine and methamphetamine were included in this research for comparative purposes, as mentioned earlier.

1.8.2 Novel psychoactive substances

NPS have gained popularity among drug users as they are available over the internet and can be considered as alternatives to controlled drugs. For example, NPS are designed to mimic the action and effects of known specific controlled drugs, such as amphetamines and cannabis (Home Office, 2012). Hence, NPS (such as ketamine, cathinones, piperazines and synthetic cannabinoids) have received a considerable amount of attention from the Home Office as their consumption has continuously grown in the UK (*ibid*). In April 2016, the Psychoactive Substances Bill, a new legislation in relation to the NPS, was

announced by the Government, which intends to introduce a ban on their production, distribution, sale and supply in order to reduce the easy availability of these substances (Sumnall and Atkinson, 2015; Psychoactive Substances Act, 2016).

However, NPS have received minimal attention in water analysis. In the UK, NPS have been studied in waste and surface waters (Baker and Kasprzyk-Hordern, 2011b; Mwenesongole, et al., 2013) and their presence has been reported in surface water, including ketamine, 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (3-TFMPP) (Baker and Kasprzyk-Hordern, 2011b). It is not surprising that NPS could make their way into drinking water, similar to reports of traditional illicit drugs. However, so far only ketamine has been reported in drinking water in Canada (Rodayan, et al., 2016) and no data regarding other NPS is available. Therefore, this research aims to address this gap in knowledge by analysing NPS in drinking water.

According to the UNODC report (2014), ketamine, cathinones, piperazines and synthetic cannabinoids are the most widely used NPS. Ketamine was used to treat asthmaticus due to its anaesthetic effect, but it also has a hallucinogenic effect, which makes it a popular drug of abuse and an agent of sexual assault (Jenkins, 2003). According to the Mixmag survey, 24.5 % of UK respondents have taken ketamine in 2011 (Mixmag, 2012). In addition, a study revealed that ketamine was detected in UK surface water at a concentration of 21.3 ng/L (Baker and Kasprzyk-Hordern, 2011b). Therefore, due to its prevalence and its presence in surface water, ketamine was included in this research.

Cathinone, an alkaloid of the khat plant, and its derivatives are structurally close to the phenethylamine family, thus their pharmacological effects are similar to amphetamines (EMCDDA, 2015e). For example, methcathinone, the first derivative, is the cathinone analogue of methylamphetamine, while methylone is the cathinone analogue of MDMA (ACMD, 2010). Thus, cathinones are considered as substitutes of amphetamines and have gained popularity among drug users (Ammann, et al., 2012b). EMCDDA summarised that butylone, mephedrone, methylone, methcathinone and methylenedioxypyrovalerone

(MDPV) are the most commonly sold cathinones on the recreational market in Europe (ACMD, 2010; EMCDDA, 2015e). Moreover, more than 200 kilograms (kg) of MDPV have been seized in Europe between 2008 and 2013 (EMCDDA, 2014a) and 1.1 % of adults (aged 16 - 59) reported using mephedrone in England and Wales in 2012 (EMCDDA, 2013). Another relevant study carried out in the UK analysed drug products purchased from various internet suppliers. After analysis, nine of the 18 products contained butylone and MDPV, followed by mephedrone (one) and methylone (one) (Ramsey, et al., 2010). Thus, butylone, mephedrone, methcathinone, MDPV and methylone are the most frequently found cathinones on the recreational market and they were selected in this research.

Piperazines are a group of synthetic drugs, which contain the six member ring and two opposing nitrogen groups (EMCDDA, 2015a). They are central nervous system stimulants with hallucinogenic effects similar to those produced by amphetamines (Vorce, et al., 2008). This makes piperazines good alternatives to amphetamines. According to EMCDDA (2015a), 1-(3-chlorophenyl)piperazine (3-CPP), 3-TFMPP and BZP are the commonly found substituted piperazines in Europe. In a recent study, Davies, et al. (2010) purchased 26 drug products from five internet suppliers and the results showed that nine drug products contained 3-TFMPP, followed by 1-methyl-4-benzylpiperazine (MBZP) (five), BZP (four), 3-CPP (three) and 1-(4-fluorophenyl)piperazine (4-FPP) (two). Thus, these five piperazines were selected as the target analytes in this research. Moreover, 1-(4-methoxyphenyl)piperazine (4-MeOPP) and 1-(4-trifluoromethylphenyl)piperazine (4-TFMPP) have been studied in waste water collected from Cambridgeshire (Mwenesongole, et al., 2013). These two piperazines were also chosen, as this will provide knowledge of the transport from waste water to drinking water in Cambridgeshire.

Synthetic cannabinoids are one of the most commonly reported NPS on the global market. For example, the number of newly identified NPS at the global level rose from 251 in 2012 to 348 in 2013 and their overall increase was mostly due to synthetic cannabinoids (UNODC, 2014). Synthetic cannabinoids are similar to THC, which is the active component

of cannabis, as they create cannabis-like effects, such as hallucinogenic, sedative and depressant effects (EMCDDA, 2015d). Therefore, synthetic cannabinoids are considered as alternatives to cannabis and are becoming popular. In the UK, a survey revealed that 3.3 % of respondents have tried synthetic cannabinoids in 2011 (Mixmag, 2012). The usage of synthetic cannabinoids was even higher than methamphetamine (0.8 %), one of the traditional illicit drugs (*ibid*). There is, therefore, a possibility that synthetic cannabinoids could also be present in the UK's drinking water, as with traditional illicit drugs and thus they were analysed in this research. Dargan, et al. (2011) analysed 20 products from a number of legal high websites and found that 70 % of products contained (1-butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone (JWH-073) and 40 % contained (4-chloronaphthalen-1-yl)-(1-pentylindol-3-yl)methanone (JWH-398). Therefore, as JWH-073 and JWH-398 are the most frequently found synthetic cannabinoids on the recreational market, they were selected as representatives of synthetic cannabinoids in this research.

1.8.3 Antidepressants

As mentioned earlier, there are a limited number of studies regarding the analysis of antidepressants in drinking water, thus antidepressants were chosen as the representative of pharmaceuticals and were analysed in this research. The selection of antidepressants was owing to the ever-increasing number of these pharmaceuticals. Antidepressants are the most effective medicines to treat depression, various anxiety disorder, obsessive-compulsive disorder and panic attacks (HSCIC, 2014). In 2013, the number of antidepressants dispensed in England was increased by 6.3 %, which was the largest increase in the volume of prescription in terms of therapeutic area (*ibid*). This is probably because psychiatric diseases are becoming increasingly prevalent, which is a growing concern for mental health. For example, in the UK, 20 % of adults aged 16 and older showed signs of depression or anxiety in 2013 (Mental Health Foundation, 2015).

To date, selective serotonin reuptake inhibitors (SSRIs), such as citalopram and fluoxetine, are the most widely used antidepressants over their predecessors, tricyclic antidepressants (TCAs), such as amitriptyline and dosulepin, because these newer

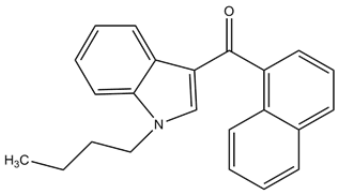
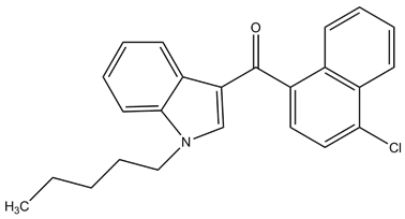
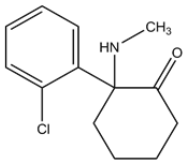
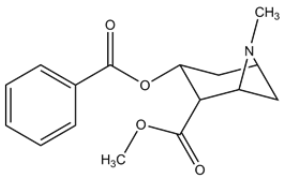
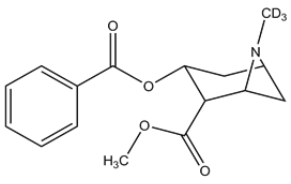
antidepressants have less toxicity and troublesome side effects (adrenergic, antihistaminic and anticholinergic) compared to TCAs (Anderson, 2003; HSCIC, 2014). A report published by the British Journal of Psychiatry described that prescriptions of seven TCAs were 74 million for the period 2000 - 2006, lower than the total prescriptions of five SSRIs (119 million) (Hawton, et al., 2010). Among the group of SSIRs, citalopram and fluoxetine were the first and second most frequently prescribed antidepressants in England for 2003 - 2013 (HSCIC, 2014). Because of their high consumption, it is reasonable to assume that citalopram and fluoxetine could be present in drinking water, so these two antidepressants were covered in this research.

1.8.4 Studied drugs of abuse, pharmaceuticals and internal standards for this research

In this research, deuterated internal standards were added to the mixed standards and samples prior to the SPE and LC-MS analysis. Their signals were used to calculate peak area ratio of the analyte to the internal standard (PAR), which were used for data analysis. Deuterated internal standards are the analogues of the analyte in which several atoms are labelled with deuterium and thus will theoretically co-elute with the analyte (Chambers and Diehl, 2007). This helps to compensate for matrix effects (further discussed in Section 1.9.2.2.1), injection variation and fluctuations in instrumental response, as these factors will affect the signal of the internal standard to the same degree as the analyte signal (Cody, 2003). As many studied NPS lack available deuterated analogues and the cost of using a deuterated internal standard for each analyte is high, three representative internal standards (amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6) were chosen for 20 analytes under investigation (Couchman and Morgan, 2011; Pedrouzo, et al., 2011). These internal standards were stable and didn't interfere with target analytes (Section 3.1.2.1 and Section 4.2.1), thus allowing for reliable quantification. Based on their retention times (Table 3.3), amphetamine-d_6 was chosen as an internal standard for analytes that elute near the beginning of the analysis. Cocaine-d_3 and fluoxetine-d_6 were used for analytes that elute in the middle and at the end of the analysis, respectively.

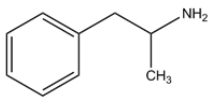
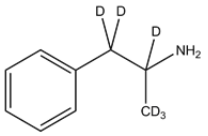
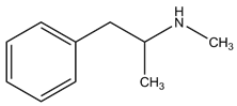
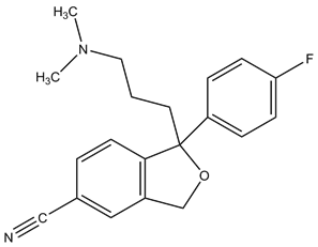
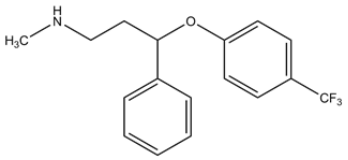
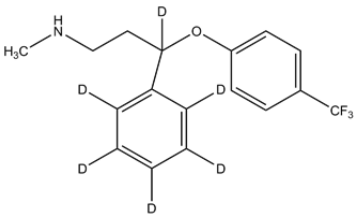
Table 1.3 lists the drugs of abuse and pharmaceuticals investigated in this research and internal standards used, including structures (drawn using ChemDraw Pro 13.0), empirical formulas, molar masses and logarithmic acid dissociation constant (pK_a) values. The compounds belong to a large range of chemical classes, namely, amphetamines (amphetamine), antidepressants (fluoxetine), cathinones (mephedrone), cocainics (cocaine), dissociative anaesthetics (ketamine), piperazines (BZP) and synthetic cannabinoids (JWH-073). This classification of drugs of abuse and pharmaceuticals was based on the UNODC (2014) and HSCIC (2014) reports. This research is considered as an initial research into drinking water from the East Anglia region of the UK, the purpose being to develop and validate a method for drugs of abuse and pharmaceuticals from various chemical classes for the detection and quantification. As 20 drugs of abuse and pharmaceuticals from seven chemical classes were to be simultaneously analysed in this research, the metabolites were not included owing to limits and scope of this analysis and its resulting validation.

Table 1.3: Drugs of abuse, pharmaceuticals and internal standards included in this research

COMPOUND	STRUCTURE	EMPIRICAL FORMULA	MOLAR MASS (g/mol)	pK_a
Synthetic Cannabinoids				
(1-butyl-1*H*-indol-3-yl)(naphthalen-1-yl) methanone (JWH-073)		$C_{23}H_{21}NO$	327.4	–
(4-chloronaphthalen-1-yl)-(1-pentylindol-3-yl)methanone (JWH-398)		$C_{24}H_{22}ClNO$	375.9	–
Dissociative Anaesthetics				
Ketamine		$C_{13}H_{16}ClNO$	237.7	7.5 [a]
Cocainics				
Cocaine		$C_{17}H_{21}NO_4$	303.4	8.7 [a]
Cocaine-d_3		$C_{17}H_{18}D_3NO_4$	306.4	–

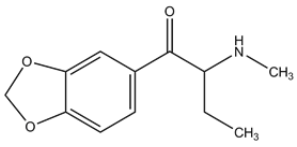
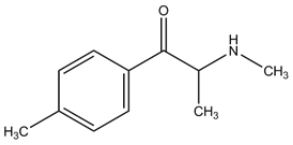
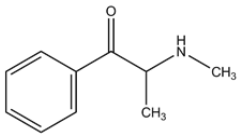
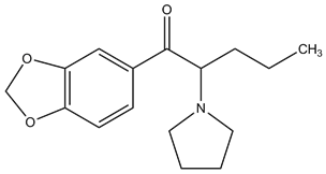
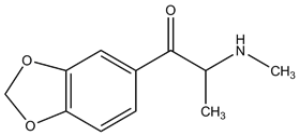
[a] Moffat, et al., 2011

Table 1.3 *cont'd*: Drugs of abuse, pharmaceuticals and internal standards included in this research

COMPOUND	STRUCTURE	EMPIRICAL FORMULA	MOLAR MASS (g/mol)	pK_a
Amphetamines				
Amphetamine		$C_9H_{13}N$	135.2	9.9 [a]
Amphetamine-d_6		$C_9H_7D_6N$	141.2	–
Methamphetamine		$C_{10}H_{15}N$	149.2	9.9 [a]
Antidepressants				
Citalopram		$C_{20}H_{21}FN_2O$	324.4	9.6 [b]
Fluoxetine		$C_{17}H_{18}F_3NO$	309.3	10.1 [b]
Fluoxetine-d_6		$C_{17}H_{12}D_6F_3NO$	315.3	–

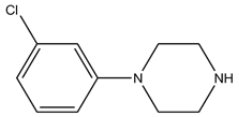
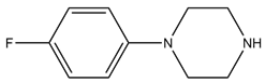
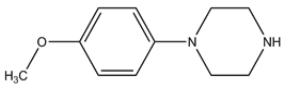
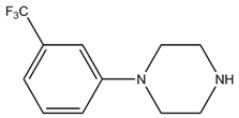
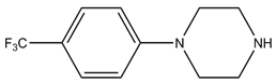
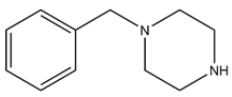
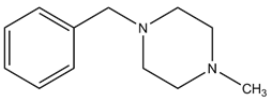
[a] Moffat, et al., 2011; [b] Kwon and Armbrust, 2008

Table 1.3 *cont'd*: Drugs of abuse, pharmaceuticals and internal standards included in this research

COMPOUND	STRUCTURE	EMPIRICAL FORMULA	MOLAR MASS (g/mol)	pK_a
Cathinones				
Butylone		$C_{12}H_{15}NO_3$	221.3	–
Mephedrone		$C_{11}H_{15}NO$	177.2	8.7 [c]
Methcathinone		$C_{10}H_{13}NO$	163.2	7.1 [d]
Methylenedioxypyrovalerone (MDPV)		$C_{16}H_{21}NO_3$	275.3	–
Methylone		$C_{11}H_{13}NO_3$	207.2	–

[c] Santali, et al., 2011; [d] Baker and Kasprzyk-Hordern, 2013

Table 1.3 *cont'd*: Drugs of abuse, pharmaceuticals and internal standards included in this research

COMPOUND	STRUCTURE	EMPIRICAL FORMULA	MOLAR MASS (g/mol)	pK_a
Piperazines				
1-(3-chlorophenyl) piperazine (3-CPP)		$C_{10}H_{13}ClN_2$	196.7	8.6 [a]
1-(4-fluorophenyl) piperazine (4-FPP)		$C_{10}H_{13}FN_2$	180.2	–
1-(4-methoxyphenyl) piperazine (4-MeOPP)		$C_{11}H_{16}N_2O$	192.3	9.0 [a]
1-(3-trifluoromethylphenyl) piperazine (3-TFMPP)		$C_{11}H_{13}F_3N_2$	230.2	8.7 [a]
1-(4-trifluoromethylphenyl) piperazine (4-TFMPP)		$C_{11}H_{13}F_3N_2$	230.2	–
1-benzylpiperazine (BZP)		$C_{11}H_{16}N_2$	176.3	9.6 [a]
1-methyl-4-benzylpiperazine (MBZP)		$C_{12}H_{18}N_2$	190.3	–

[a] Moffat, et al., 2011

1.9 Introduction to analytical methods for the detection of drugs of abuse and pharmaceuticals in drinking water

The lack of knowledge regarding the presence of drugs of abuse and pharmaceuticals in drinking water, as described in Section 1.6, can also be explained by the analytical difficulties involved in carrying out the quantification of drugs of abuse and pharmaceuticals at ultra-trace levels. Generally, the concentrations of drugs of abuse and pharmaceuticals in drinking water are found in the ng/L range (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013; Peng, Hall and Gautam, 2016), which are close to or even lower than the limits of quantification from published methods (Stackelberg, et al., 2007; Boleda, et al., 2011; Wu, et al., 2015; Rodayan, et al., 2016). Limit of quantification is the lowest concentration that can be measured reliably (Armbruster and Pry, 2008), which is further discussed in Section 1.10.5. Thus, if the concentration of an analyte in a sample is lower than its quantification limit, this analytical method is not sensitive enough for quantification. For example, Boleda, et al. (2011) used SPE followed by LC-MS/MS to examine 50 drinking water samples collected from Spain. Cocaine was detected and quantified in 33 samples, while amphetamine was detected in just one sample. This might be because the number of seizures reported for cocaine in Europe was twice the number for amphetamines in 2012 (EMCDDA, 2014a), although it could also be related to their limits of quantification. In the aforementioned research, the limit of quantification for amphetamine was 1 ng/L, a tenfold higher than cocaine, 0.1 ng/L (Boleda, et al., 2011). This means that, by using this method, one could quantify cocaine but not amphetamine if the concentrations of amphetamine and cocaine are both at 0.5 ng/L. Hence, more concerns should focus on overcoming analytical difficulties in drinking water analysis, such as achieving lower limits of quantification, thus resulting in higher method sensitivity. In addition, in this research, 23 analytes (20 target drugs of abuse and pharmaceuticals plus three internal standards) were simultaneously analysed in one analytical run (Table 1.3). Therefore, it is crucial to develop a method that uses a suitable sample preparation and analytical technique, which could offer both selectivity and sensitivity.

1.9.1 Sample preparation - solid phase extraction (SPE)

Although the matrix of a drinking water sample is comparatively clean, sample preparation prior to analysis cannot be overlooked. SPE, which uses a solid phase to separate the different components of a liquid sample, is the most reported sample preparation method used to extract the drugs of abuse and pharmaceuticals from drinking water (Postigo, Lopez de Alda and Barceló, 2008; Waters, 2016). The use of SPE can remove undesired interferences from water samples, retain analytes as much as possible and, more importantly, significantly concentrate the water samples, which is essential for a drinking water analysis, as target analytes are likely to be present in water samples at extremely low concentrations (Peng, Hall and Gautam, 2016; Waters, 2016). Thus, SPE was chosen as the sample preparation method in this research. Further background information regarding the SPE sorbents and discussion to the extraction protocol used in this research is provided in Section 1.9.1.1 and Section 1.9.1.2, respectively, and the benefits are discussed in Section 1.9.1.3.

1.9.1.1 SPE sorbents

SPE sorbents are available with many different packing materials, which result in different chemical behaviours. Based on their behaviours, SPE sorbents can be divided into five categories, namely reversed phase, normal phase, cation-exchange, anion-exchange and mixed-mode, which exhibits reversed phase behaviour in combination with either the cation-exchange or anion-exchange (Thurman and Mills, 1998). The selection of the most suitable SPE sorbent in this research was based on the sample matrix (drinking water) and the chemical properties of the investigated target analytes (drugs of abuse and pharmaceuticals). In this research, the studied drugs of abuse, pharmaceuticals and internal standards contain amines and aromatic rings (Table 1.3), which make them capable of interacting with both acidic and non-polar groups in the SPE sorbents. Therefore, mixed-mode cation-exchange sorbent exhibiting acidic and non-polar surface groups (Tzanavaras and Zacharis, 2010) were chosen for their extraction in drinking water (Section 3.2).

1.9.1.2 SPE Process

Most extraction protocols of the mixed-mode cation-exchange SPE involve six separate steps, including sample pre-treatment, column conditioning, column equilibration, sample loading, column washing and analyte elution. These protocol steps are described in the following sub-sections.

1.9.1.2.1 Sample pre-treatment

Before applying the sample to the SPE for extraction, it is necessary to pre-treat the sample for the purpose of promoting analyte retention during the sample loading step (Simpson, 2000). In order to retain a basic analyte on the surface of mixed-mode cation-exchange sorbent, there are two elements involved: (1) positive charged basic groups on the analytes, and (2) negative charged acidic groups on the sorbent, as the retention is facilitated based on ionic interactions (Sigma-Aldrich, 1998). Basic groups of the analytes can be charged in this step by pH control and charging the acidic groups on the SPE sorbent is related to the column equilibration step, which is discussed in Section 1.9.1.2.3. Dropping the sample pH by the addition of an acid will increase the concentration of the charged form of the basic analyte. For all basic groups to exist in the charged state, the sample pH is required to be at least two pH units below the pK_a of relevant analyte (Harris, 2010). The pK_a values of drugs of abuse and pharmaceuticals investigated in this research range from 7.1 (methcathinone) to 10.1 (fluoxetine) (Table 1.3). The pK_a values of some cathinones and piperazines have not yet been reported; however, their pK_a values can be predicted to be in the range of 7 - 10 based on the known pK_a of other cathinones and piperazines. For synthetic cannabinoids, no such information is available. As synthetic cannabinoids are the alternatives to cannabis, the pK_a values of JWH-073 and JWH-398 can be considered as similar as the known pK_a of THC (10.6), which is the principal active constituent of cannabis (Huestis, 2003; Boleda, et al., 2011). Thus, it was necessary to lower the pH of the water sample to below five in this research. 2 % v/v formic acid and 0.1 M hydrochloric acid were used for Oasis MCX and Strata-X-Drug B, respectively, based on their generic protocols (Section 2.3.3.1).

1.9.1.2.2 Column conditioning

Column conditioning is the first step used to activate the sorbent bed to allow for a proper phase interface with the applied sample (Simpson, 2000). As mixed-mode cation-exchange sorbents are hydrophobic, they are unable to be wet with polar solvent, such as water. When an aqueous sample is applied for extraction, it is necessary to first treat the sorbent bed with a water-miscible organic solvent, such as methanol, before sample loading.

1.9.1.2.3 Column equilibration

The aim of column equilibration is to adjust the pH of the sorbent in order to convert all acidic groups to their charged state, which can easily interact with the analytes (Thurman and Mills, 1998). The retention mechanism is discussed in Section 1.9.1.2.1. The pH of the sorbent is recommended to be a minimum of two pH units above the pK_a of the sorbent (Simpson, 2000). For a mixed-mode cation-exchange sorbent, this can be accomplished by passing acidified water through the SPE column (Baker and Kasprzyk-Hordern, 2011a; Peng, Hall and Gautam, 2016). Hence, 2 % v/v formic acid (for Oasis MCX cartridge) and 0.1 M hydrochloric acid (for Strata-X-Drug B cartridge) were added into water in this research (Section 2.3.3.1).

1.9.1.2.4 Sample loading

During sample loading, it is necessary to optimise the sample loading volume for the final protocol (Section 2.3.3.3 and Section 3.2.3). The main aim is to load as large a volume of sample as possible, because the larger the volume of sample applied, the higher the enrichment factor obtained, thereby leading to increased method sensitivity (discussed later in Section 1.9.1.3). This is even more important for a drinking water analysis, as target analytes are present in trace amounts (ng/L) (Peng, Hall and Gautam, 2016). It is important to control the flow rate of the sample when it passes through the column. Sample application with an adequately slow flow rate is recommended, as the residence time of analytes in the column must be sufficient for the retention to occur (Simpson, 2000).

1.9.1.2.5 Column washing

Column washing is when a solvent is used to selectivity remove as many unwanted interferences as possible, while leaving the analytes retained on the sorbent (Simpson, 2000). If the wash solvent is too weak, interferences may still be retained on the column and, subsequently, eluted along with the analytes in the elution step. On the other hand, if the wash solvent is too strong, the desired analytes may be eluted in this step, leading to loss of analyte. Therefore, the selection of wash solvent is important in order to ensure good-quality extracts. In this research, the wash solvent was selected based on the generic protocol of used SPE cartridges, Oasis MCX and Strata-X-Drug B (Section 2.3.3.1).

1.9.1.2.6 Analyte elution

The aim of this step is to elute the desired analytes from the sorbent by disrupting the interactions between the analytes and the sorbent (Waters, 2001). As mentioned in Section 1.9.1.2.1, the retention mechanism of mixed-mode cation-exchange is the interaction of positively charged basic groups on the analytes and negatively charged acidic groups on the sorbent. This interaction can be disrupted by neutralising either the analyte or the sorbent. Normally, it is better to neutralise the analyte rather than the sorbent as the neutralisation of sorbent will release species retained on the sorbent, including the undesired interferences. To neutralise the analyte, the pH of elution solvent should be adjusted to at least two pH units above the analyte pK_a (Sigma-Aldrich, 1998). According to the pK_a values of drugs of abuse and pharmaceuticals investigated in this research (Table 1.3), the pH of the elution solvent needs to be adjusted above 12. Based on the generic protocol of used SPE cartridges, Oasis MCX and Strata-X-Drug B, 5 % and 10 % of ammonium hydroxide was added for pH control, respectively (Section 2.3.3.1). Moreover, the strength of the elution solvent is also of importance. If the elution solvent is too weak, analytes cannot be completely removed from the column, resulting in only partial elution. On the other hand, if the elute solvent is too strong, the undesired interferences may be eluted in this step, leading to the poor-quality extracts. Thus, elute solvent needs to be optimised in order to achieve the complete recovery of analytes from the sorbents (Section 2.3.3.2 and Section 3.2.2).

1.9.1.3 Benefits of SPE for the detection of drugs of abuse and pharmaceuticals in drinking water

One benefit of using SPE is to remove potential interferences and purify compounds from the sample matrix (Waters, 2016). The application of an effective sample clean-up could reduce matrix effects, as components in the sample matrix are the common contributor to signal suppression during the ionisation process in LC-MS. Matrix effects are further discussed in Section 1.9.2.2.1.

The use of SPE can also concentrate the water samples (Waters, 2016), which is more important for this research as mentioned earlier. Reported concentration levels of drugs of abuse and pharmaceuticals in water samples are in the sub ng/L range (Mompelat, Le Bot and Thomas, 2009; Pal, et al., 2013; Peng, Hall and Gautam, 2016), far lower than the sensitivity capability of the commonly used analytical instruments. For example, the published instrumental detection limits of LC-MS/MS regarding the analysis of drugs of abuse and pharmaceuticals were at nanogram per millilitre (ng/mL) (Kasprzyk-Hordern, Dinsdale and Guwy, 2007; Baker and Kasprzyk-Hordern, 2011b). Hence, the use of analytical techniques alone is insufficient to determine most drugs of abuse and pharmaceuticals in drinking water. This sensitivity gap could be bridged by using SPE to reduce the volume of the water sample for the purpose of analyte enrichment. This is normally performed by isolating analytes of interest from large volumes of water sample, such as a few 100 millilitres (mL), and re-dissolving them in small volumes of injection solvent for analysis (single mLs) (Baker and Kasprzyk-Hordern, 2011a; Peng, Hall and Gautam, 2016). Published research, which analysed drugs of abuse in waste and surface water, has reported the use of SPE to enrich river water samples, with 500 mL of river water sample reduced to 0.5 mL of extracted LC-MS/MS sample (Baker and Kasprzyk-Hordern, 2011b). The enrichment factor of this method was 1000, which was calculated as the ratio of the loaded sample volume (500 mL) to the extracted sample volume (0.5 mL) (*ibid*). Therefore, the application of SPE has significantly lowered the quantification limits of analytical methods for the studied drugs of abuse. For example, in the aforementioned research (Baker and Kasprzyk-Hordern, 2011b), the limit of

quantification for cocaine was 0.1 μg/L when using LC-MS/MS only, which is far higher than the reported environmental concentration (ng/L) (Pal, et al., 2013). However, when using SPE for the sample enrichment, its limit of quantification was reduced to 0.1 ng/L, which is capable of detecting cocaine in water samples. Therefore, SPE is considered an effective pre-concentration method, which enables analytes to be detected and improves the quantification limit of the analytical method (Baker and Kasprzyk-Hordern, 2011a).

1.9.2 Instrumental techniques - liquid chromatography-mass spectrometry (LC-MS) and high performance liquid chromatography-diode array detection (HPLC-DAD)

Since the 1970s, different analytical techniques, mainly varying between gas chromatography-mass spectrometry (GC-MS) and LC-MS/MS, have been utilised for the analysis of drugs of abuse and pharmaceuticals in a wide range of aqueous matrices, from waste water to drinking water (Castiglioni, et al., 2008; Noguera-Oviedo and Aga, 2016; Peng, Hall and Gautam, 2016). GC-MS has been used to detect drugs of abuse in waste water (Mwenesongole, 2015), where this technique generates much less solvent waste compared to LC-MS/MS and mass spectra are able to be compared between instruments and to a reference database for their identification (Peng, Hall and Gautam, 2016). However, its disadvantage is the need for lengthy derivatisation procedures when detecting low volatility and high polarity compounds (Kasprzyk-Hordern, Dinsdale and Guwy, 2007). Recently, LC-MS/MS has become the most commonly applied analytical technique when detecting drugs of abuse and pharmaceuticals in water samples, since most of these analytes are non-volatile and with a low-to-medium polarity, which are not amenable to GC-MS analysis (Postigo, Lopez de Alda and Barceló, 2008). In comparison to GC-MS, the analysis time of LC-MS/MS could be significantly reduced due to non-essential pre-column derivatisation (Mwenesongole, et al., 2012). This trend of LC-MS/MS over GC-MS is consistent with the recently published review (Vazquez-Roig, Blasco and Picó, 2013). In previous studies (n = 19) regarding the analysis of illegal drugs and pharmaceuticals in water samples, 15 used LC-MS/MS for detection, while only two used GC-MS. Thus, using LC-MS/MS for the analysis of drugs of abuse and pharmaceuticals in waters is increasingly popular.

In forensic toxicology, LC-MS has been used to analyse drugs of abuse and pharmaceuticals in biological samples such as blood, plasma and urine (Maurer, 1998). However, to the author's knowledge, it is less commonly used in the field of water analysis. A recent review showed that only ten out of 29 studies for determining pharmaceuticals in water used LC-MS and all studies that used LC-MS analysed pharmaceuticals from only one chemical class (Kosjek and Heath, 2008). In addition, LC-MS has never been used to analyse drugs of abuse in water samples (Petrovic, et al., 2010; Peng, Hall and Gautam, 2016). Even though LC-MS/MS is increasing in popularity for the analysis of drugs of abuse and pharmaceuticals in water, LC-MS also has several advantages. It is less expensive compared with LC-MS/MS, which means it is still the instrument of choice for many laboratories (Holčapek, Jirásko and Lísa, 2012). Díaz-Cruz, et al. (2003) reported that the instrumental sensitivities achieved by LC-MS and LC-MS/MS were comparable. As there is little information published on the investigation of drugs of abuse and pharmaceuticals in water using LC-MS and as this was the instrument available for this research, it was decided to investigate LC-MS as an alternative to the popularly used LC-MS/MS in the analysis of drugs of abuse and pharmaceuticals in drinking water. This research is novel, as there have been no other studies to simultaneously analyse drugs of abuse and pharmaceuticals in drinking water using LC-MS.

LC-MS is a powerful hyphenated technique that provides both high selectivity and sensitivity, as it combines the separation power of liquid chromatography (LC) with the detection and identification power of mass spectrometry (MS). LC separates compounds of a mixture by their chemical properties (Sargent, 2013), while MS is a detector with great sensitivity and the ability to identify compounds by their mass-to-charge ratio (m/z) values (Harris, 2010). In Section 1.9.2.1 and Section 1.9.2.2, LC and MS are further discussed. High performance liquid chromatography-diode array detection (HPLC-DAD) for the development of the LC-MS method in this research was also used (Section 2.3.1 and Section 3.1.1), as solvents and water grades are cheaper than those needed at LC-MS grade, leading to a cost reduction in the method development stage. The diode array detector (DAD) is described in Section 1.9.2.3.

1.9.2.1 Liquid chromatography (LC)

In LC, an autosampler injects a small amount of a liquid sample into a flow of polar mobile phase, then the mobile phase carries the sample through the non-polar stationary phase where separation occurs (Fallon, Booth and Bell, 1987). The components of the sample not only move and interact with the mobile phase, but also partition into this stationary phase. Due to their different chemical properties, the components in the sample interact slightly differently with the mobile phase and stationary phase (Braithwaite and Smith, 1985).

The elution times of components can be manipulated through polarity changes in the stationary phase or mobile phase characteristics in order to achieve better separation. Typically, the mobile phase is a mixture of water with a relatively non-polar organic solvent that is miscible with water, such as methanol or acetonitrile (Sargent, 2013). The organic solvent is referred to as an organic modifier, whereby increasing the percentage of organic modifier can make the mobile phase more non-polar. Thus, with control of the mobile phase composition (mainly the choice of the organic modifier and its percentage), the elution times of components can be changed, resulting in better separation. These variables were studied under the method development and optimisation (Section 2.3.1 and Section 3.1.1).

Moreover, the flow rate of the mobile phase is normally applied as 1 mL/min for DAD. However, when using MS as the detector, a lower flow rate should be applied in order to produce smaller droplets, as droplet size is related to the effectiveness of nebulisation and desolvation processes in the electrospray ionisation (ESI) interface and the generation of gas phase analyte ions, which are further discussed in Section 1.9.2.2.1. The typical flow rate of the mobile phase can be from 0.2 to 0.5 mL/min for LC-MS (Naegele, 2011).

1.9.2.2 Mass spectrometry (MS)

MS is a powerful technique that is used to detect and identify the separated analytes from LC. The analytes introduced from the LC are ionised at atmospheric pressure by an

atmospheric pressure ionisation (API) probe, such as ESI or atmospheric pressure chemical ionisation (APCI) (Sargent, 2013). The principle of ionisation is described in Section 1.9.2.2.1. Once ions are generated, they are introduced by the desolvation line (DL) into the vacuum and then confined and converged by the lens system including qarray, skimmer, octapole and entrance lens (Shimadzu, 2011). By optimising the voltages of DL, qarray DC and qarray RF for selected ions, enhanced peak intensity can be achieved because these voltages act as a force to introduce the selected ions to the mass analyser (Shimadzu, 2008). The more the ions are converged as they enter the mass analyser, the higher the peak intensity obtained. It is necessary to investigate the optimum voltage values for the DL and qarray based on the selected ions. This parameter was studied under method optimisation (Section 2.3.2 and Section 3.1.2.3). Subsequently, the ions are separated in relation to their m/z values by the mass analyser (discussed later in Section 1.9.2.2.2). Then ion energy is converted into the electrical signal and measured by the detector (Shimadzu, 2011).

1.9.2.2.1 Atmospheric pressure ionisation - electrospray ionisation

The sample and the liquid mobile phase after LC separation enter the API unit, where ionisation occurs at atmospheric pressure (Sargent, 2013). The ionisation mechanisms for the ESI and APCI, which are the most commonly used API techniques, are different. However, only the ionisation principle of ESI is described in this section as APCI was not used in this research.

ESI is a method of ionisation to transfer ions present in the sample from the liquid phase into the gas phase (Watson and Sparkman, 2007). The sample and mobile phase from the LC eluent is drawn into a metal capillary tube contained within the ESI probe and charged by the application of a potential difference between the capillary tip and sampling cone (Shimadzu, 2011). If a positive potential is applied to the capillary tip, it causes positive ions to predominantly populate the fine droplets. It is referred to as positive ion mode, which is normally used for the analysis of basic compounds (Molin and Traldi, 2007). On the other hand, negative ion mode is used to form negative ions by applying the negative potential to

the capillary tip. This mode is suitable for the analysis of acidic compounds (*ibid*).

Nitrogen is then blown out around the outside of the capillary tip as nebuliser gas in order to spray the solution. This process is known as nebulisation. The purpose of nebulisation is to generate small charged droplets that contain positive or negative ions, because the droplet size can affect the effectiveness of the following step (Watson and Sparkman, 2007). The effectiveness of the nebulisation process is related to the mobile phase composition. Small charged droplets may form when the mobile phase has a high percentage of organic solvent due to the low surface tension of mobile phase (Sargent, 2013). If the mobile phase has a high percentage of water content, its surface tension is high and large liquid droplets may form. In this case, the higher potential difference is required to induce the small size droplets (*ibid*). Therefore, it is necessary to investigate the optimum values for the potential difference applied based on the mobile phase composition. This parameter was fixed based on the tuning file of the LC-MS (Section 2.3.2.2).

Once the finely charged droplets are formed, they are attracted by an electrostatic field from the capillary tip to the sampling cone (counter electrode) and then enter into the DL. During the course of the movement, the solvent of the droplets is vaporised by using a heated drying gas (Shimadzu, 2011). This process is referred to as desolvation and is used to reduce the droplet size and increase the charge density on its surface (Sargent, 2013). When the surface charge increases to a point where the repulsion forces between ions with like charges exceed the tension forces of the surface, the Rayleigh limit is reached and the droplets disintegrate into even smaller droplets (Molin and Traldi, 2007). The repetition of vaporisation and disintegration leads to very fine droplets and, ultimately, it is thought that sample ions are desorbed into the gas phase (Sargent, 2013). Once in their gas phase, these ions are introduced into a lens system and mass analyser for separation and detection (Shimadzu, 2011).

In the ESI process, a commonly encountered problem is that the ionisation efficiency of analytes is susceptible to the components present in the sample matrix, which refers to

matrix effects (Hajšlová and Zrostlíková, 2003; Chambers, et al., 2007). Ions present in the charged droplets have different abilities to move to the droplet surface, which are affected by the number of charges and the size of their molecules in solution (Tang and Kebarle, 1993; Taylor, 2016). Those ions at the surface of droplets have a better chance of escaping into the gas phase and being detected by the mass analyser (Taylor, 2016). If the ions of matrix components move to the surface preferentially to the ions of analytes, more matrix ions will be at the surface spaces. During ESI ionisation, matrix ions will compete and interfere with analyte ions to evaporate into the gas phase, resulting in a decreased number of analyte ions entering the mass analyser for detection (Chambers, et al., 2007). As a result, the analyte signals will be suppressed and unrepeatable, which will lead to decreased method sensitivity and impact on accuracy, precision and reproducibility of analytical method (Chambers, et al., 2007; Kasprzyk-Hordern, Dinsdale and Guwy, 2007). Therefore, it is of importance to reduce the matrix effects. In this research, the SPE was used to remove matrix components and standard addition method was applied in order to compensate for the matrix effects.

1.9.2.2.2 Analysis unit

To date, several mass analysers have been developed and improved in order to achieve better performance, such as high-resolving power in order to resolve peaks in the mass spectra, higher mass accuracy, wider mass range, faster acquisition time, wider linear dynamic range and reduced cost of instrumentation (Holčapek, Jirásko and Lísa, 2012). These include the quadrupole (single and triple), ion trap, time of flight, orbitrap and ion cyclotron resonance. With respect to the analysis of drugs of abuse and pharmaceuticals in environmental aqueous samples, the majority of researchers have used the triple quadrupole (QqQ) mass analyser (Kosjek and Heath, 2008; Postigo, Lopez de Alda and Barceló, 2008; Petrovic, et al., 2010; Peng, Hall and Gautam, 2016). However, there is little information published regarding the use of the single quadrupole (Q), this being the mass analyser available. Thus, the Q mass analyser was chosen for this research.

The Q mass analyser is composed of four rods, which are arranged at an equal distance from the central axis (Shimadzu, 2011). Depending on the electrical potential, these four rods are divided into two sets; one is at a positive electrical potential, the other is at a negative electrical potential (Cody, 2003). Two voltages, direct current (DC) and alternating current (AC), are involved and a combination of DC and AC voltages is applied on each set of rods. Thus, an electric field is generated within the quadrupole analyser (Shimadzu, 2011). The quadrupole analyser acts as a mass filter based on the amplitude of DC and AC voltages. Only the ions of given m/z values have a stable trajectory to pass through the four rods and be detected (*ibid*).

The Q mass analyser can be used in two acquisition modes, being scan mode or selected ion monitoring (SIM) mode. In scan mode, the mass analyser is set to observe a range of m/z values, whereas in SIM mode only a few specific m/z values are monitored (Harris, 2010). Scan mode can provide ion information over a specific m/z range. Therefore, in this research, scan mode was initially used to analyse the drugs of abuse and pharmaceutical standards in order to obtain their mass spectra, which help to select the diagnostic ions of each studied compound to be monitored in SIM mode (Section 2.3.2.2). SIM mode is significantly more sensitive than scan mode as more time can be spent on each m/z. In this research, SIM mode was used to monitor the diagnostic ions obtained from the scan mode for method development, method validation and water sample analysis.

1.9.2.3 Diode array detector (DAD)

The DAD detects the absorbance of compounds in the ultraviolet-visible (UV-Vis) spectral region and thus can identify analytes being eluted from the LC column (Cole and Levine, 2003). The resulting spectrum is the absorbance of an analyte over a range of wavelength. Usually the highest wavelength from the spectrum is chosen for quantification. Thus, it is important to choose the optimal wavelength in order to achieve the appreciable absorbance for all analytes of interest (discussed in Section 3.1.1).

1.10 Method validation parameters for this research

As mentioned in Section 1.8.2 and Section 1.9.2, this research is novel as the presence of 14 NPS (cathinones, piperazines and synthetic cannabinoids) in drinking water has never been investigated before. Also, LC-MS is much less used in the field of drinking water analysis, especially for multi-residue analysis. Thus, the new analytical method used in this research needs to be developed, optimised and then validated before applying to drinking water samples.

Method validation can be defined as the procedure of providing evidence and statistical interpretation to show that the method being developed is reliable and reproducible for its intended purpose (Harris, 2010). At this point, the use of a validated method provides a reasonable degree of confidence, which can generate reproducible data of sufficiently reliable quality with statistical grounds (Peters, Drummer and Musshoff, 2007). Before validating a method, it is suggested that one should select the validation parameters and define the evaluation criteria by considering the scopes and requirements of the method (Singh, 2013). In this research, the method uses SPE followed by LC-MS to identify and quantify selected drugs of abuse and pharmaceuticals (Table 1.3) in drinking water. A simple interpretation of its validation is to ensure that the methodology can measure the correct analytes (drugs of abuse and pharmaceuticals) in the correct amount over the specified range in the sample matrix (drinking water). For quantitative procedures, according to the recommendations of some major regulatory authorities, such as the International Union of Pure and Applied Chemistry (IUPAC) and the International Conference on Harmonisation (ICH), the following parameters should be validated: selectivity, stability, calibration model (linearity), precision, accuracy, limit of detection and limit of quantification (Peters, Drummer and Musshoff, 2007). Thus, these validation parameters are deemed relevant for this research and are described in detail in the following sub-sections.

It is suggested to begin the validation with the selectivity study, as major modifications of the method might be needed if this parameter is not fulfilled (Swartz and Krull, 1997; Wille,

et al., 2011). If the method is found to be selective, one can proceed to the autosampler storage stability study in order to ensure that samples and standards are stable during analytical run (Peters, Drummer and Musshoff, 2007). The next step is to undertake the linearity experiment in order to determine the model of calibration for the analytical method (Huber, 2007). After ascertaining the calibration model, precision and accuracy are evaluated. The results can be used to further ensure that the data obtained from the analytical method is accurate and reproducible (Swartz and Krull, 1997). In addition, limits of detection and quantification are also determined as the indicators of instrument and method sensitivity (Singh, 2013).

1.10.1 Selectivity

Selectivity is defined as the ability of a specified analytical method to measure explicitly the analyte of interest when other compounds are present (Swartz and Krull, 1997). To demonstrate the selectivity of the method, it is important to ensure that the peak of the target analyte is due only to a single component (Peters, Drummer and Musshoff, 2007). Therefore, it is suggested to prove that the developed method is selective enough to separate all studied drugs of abuse, pharmaceuticals and internal standards from each other (Huber, 2007). Moreover, it is also important to ensure that there is no interference from the matrix blank (Peters, Drummer and Musshoff, 2007). Selectivity was assessed by monitoring a matrix blank following the procedures described in Section 2.4.1 and then comparing this to a mixed standard containing 20 target analytes and three internal standards to check interferences (Section 4.1).

1.10.2 Autosampler storage stability

The stability of target analytes and internal standards during the analytical run should be assessed prior to other validation experiments, as it is a prerequisite for reliable and reproducible quantification (Wille, et al., 2011). In this research, mixed standards were prepared every five working days and then put in the autosampler until the LC-MS analysis. Thus, autosampler storage stability was assessed for the selected drugs of abuse, pharmaceuticals and internal standards in the LC-MS injection solvent (0.5 % formic

acid/4.975 % acetonitrile/94.525 % water) during the maximum length of time standards stored at 10 °C on the temperature controlled autosampler (Peters, Drummer and Musshoff, 2007). The typical analysis time for water sample analysis ranged from 24 to 36 hours and the maximum anticipated run time for method validation experiments was four days. Based on the experiment schedule, a period of five days was enough to cover the typical working time. Therefore, this was chosen as the time interval to be investigated. To test the stability of the studied drugs of abuse, pharmaceuticals and internal standards, repeated injections of mixed standards were analysed at a five-day interval (Section 2.4.2). An analysis of instrumental response plotted against the injection time was employed for each analyte, while the internal standard and *p*-value obtained from the bar graph was used to evaluate the stability (Section 4.2). The acceptance criterion of stability is discussed in Section 4.2.

1.10.3 Calibration model - linearity

The purpose of calibration is to determine the concentration of a substance in an unknown sample (Huber, 2007). Firstly, it is essential to ascertain the model of calibration for the analytical method. There are different models for calibration, such as linear, curvilinear and non-linear (Van Loco, et al., 2002). The linear model is widely used for the calibration of an analytical method. This is because, when using this mode, it is easier to estimate the equation of the calibration curve and compute the coefficients and standard deviations when compared to other models (Wille, et al., 2011). Thus, undertaking the linearity experiment is a prerequisite in order to verify whether the method shows that instrumental response is proportional to the analyte concentration (Hartmann, et al., 1998). The linearity was assessed over a wide range of concentrations (Section 2.4.3) and the results were presented as a linear regression plot of instrumental response against concentration (Section 4.3.1). The linearity was evaluated by calculating the linear regression trend line by the ordinary least squares method. The method of ordinary least squares is used to find the best fit line (not forced through the origin) to a number of data points by minimising the sum of the squares of the distances from the actual data points to the determined line (Linoff and Berry, 2011). The coefficient of determination, which was obtained from the

linear regression plot, was used to determine the linear range. Then, the linearity was additionally assessed by the plot of relative response against the log concentration (Section 4.3.2). The acceptance criteria of linearity are described in Section 4.3. The linearity result can also aid to select the appropriate working range to be considered during sample analysis (UNODC, 2009).

1.10.4 Precision and accuracy

The precision of an analytical method describes how well replicate measurements agree with one another and is normally expressed as the percent relative standard deviation (RSD) of replicates (Harris, 2010). Precision in this research was assessed using intra-assay precision and intermediate precision (Swartz and Krull, 1997). Intra-assay precision is a measure of precision for analysis operating over a short time interval under the same conditions, such as the same operator, same equipment and same laboratory (Peters, Drummer and Musshoff, 2007). The experimental procedure is described in Section 2.4.4.1 and Section 2.4.4.3. Intermediate precision is the result of the method operating under different conditions (Swartz and Krull, 1997). In this research, the difference in experimental period (over days) was examined (Section 2.4.4.2). The acceptance criteria of precision are described in Section 4.4.

The accuracy of an analytical method is the closeness of agreement between the calculated values obtained by calibration curve and the standard concentrations (Wille, et al., 2011). Accuracy can be expressed as a percent deviation from the true value (Peters, Drummer and Musshoff, 2007). The experimental procedure and acceptance criteria of accuracy are described in Section 2.4.4.3 and Section 4.4.

1.10.5 Detection and quantification limits

The study of detection limit and quantification limit is to specify the capabilities of an analytical method for detection and quantification (Singh, 2013). In this research, the method was based on using LC-MS for analysis and SPE for sample preparation. Two sets of detection limit and quantification limit need to be determined (Corley, 2003). One set

includes instrumental detection limit (IDL) and instrumental quantification limit (IQL), while the other set includes method detection limit (MDL) and method quantification limit (MQL).

1.10.5.1 Instrumental detection and quantification limits

IDL and IQL are used to define only the limitations of the LC-MS instrument (Bernal, 2014). In this research, IDL and IQL are determined using the mixed drug and pharmaceutical standards in order to prove that the LC-MS is sensitive enough to perform the detection and quantification of the studied drugs of abuse and pharmaceuticals (Section 2.4.5). IDL is the lowest concentration of an analyte that can be reliably distinguished from the background on an instrument, while IQL is the lowest concentration of an analyte that can be quantitatively determined by the instrument (*ibid*).

The signal-to-noise ratio (S/N) method was used to estimate the IDLs and IQLs for the studied drugs of abuse and pharmaceuticals and comparisons are made to the published methods using LC-MS/MS (Section 4.5.1). The S/N is defined as the ratio of analyte peak signal to baseline noise in a certain area around the analyte peak (Huber, 2007). The IDL is taken as the concentration of analyte that gives the S/N of 3:1 (Harris, 2010). At this concentration, the presence of an analyte can be deduced and reported with a confidence level of 99.86 % for a normal distribution (Corley, 2003). For IQL, the concentration of analyte that gives the S/N of 10:1 is chosen (Singh, 2013).

In addition to this, the Root Mean Square Error (RMSE) method, which is recommended by the US Environmental Protection Agency (US EPA), was also chosen as another means of calculating IDLs and IQLs. The RMSE approach is more accurate and reliable compared to the S/N method. Using the S/N method, interpretation is very subjective because the S/N is dependent on the chosen region of the baseline where the noise is calculated (Wells, Prest and Russ, 2011). If the selected time window is very narrow and away from the analyte peak where only small noise peaks occur, the instrumental noise could be relatively low, leading to higher S/N for IDL and IQL determinations, therefore resulting in overestimation. For the RMSE approach, the variability between laboratories and analysts is low as it uses

the root mean square error (the difference between the predicted response and measured response) instead of calculating the noise (Corley, 2003; Bernal, 2014). To undertake the RMSE method, it is first required to generate a calibration curve that is near the estimated IDL and IQL range and then calculate the error based on the obtained linear regression equation (Corley, 2003). The concentrations of mixed standards for calculating the IDLs and IQLs of two LC-MS methods (C_{18} column and biphenyl column) using the RMSE approach is shown in Section 2.4.5 and the calculation process is further discussed in Section 4.5.1.

1.10.5.2 Method detection and quantification limits

Determination of MDL and MQL are also required when the method involves the sample preparation step. They are applied to the whole analytical method, including sample preparation and instrument analysis, for the test of an analyte within the sample matrix (Bernal, 2014). MDL and MQL are dependent on various factors, such as sample matrix interference, recovery in the sample extraction and the concentration factor (enrichment or dilution factor) of sample preparation (Corley, 2003). MDL and MQL can be calculated based on the values of IDL and IQL, the recovery of analyte from the matrix and concentration factor (Baker and Kasprzyk-Hordern, 2011b). The calculation processes are discussed in detail in Section of 4.5.2.

1.11 Research aims

In light of the above discussion, this research aims to address the aforementioned gaps in knowledge. Firstly, as very little published information regarding the occurrence of drugs of abuse and pharmaceuticals in drinking water is available (Section 1.6), traditional illicit drugs (Section 1.8.1) and antidepressants (Section 1.8.3) were chosen to be analysed in drinking water. In addition, newer drugs of abuse, NPS, were also investigated in this research as they are increasingly being abused but have never been studied in drinking water before, except for ketamine (Section 1.8.2). Secondly, this research was carried out in order to develop and validate a methodology based on using SPE for sample preparation, followed by LC-MS as the detection and quantification technique for the

simultaneous analysis of 20 selected drugs of abuse and pharmaceuticals in drinking water. This method is novel, as there have been no other published studies which use LC-MS for the purpose of multi-residue analysis in drinking water (Section 1.9.2). HPLC-DAD was also used as an analytical instrument during a preliminary study for chromatographic separation development (Section 2.3.1 and Section 3.1.1). Finally, water samples, both raw water and drinking water, were collected from several DWTPs and taps in the East Anglia region of the UK (Section 2.2), which have never been investigated before.

This research focuses on the analysis of the presence of selected drugs of abuse and pharmaceuticals in water samples and the evaluation of their removal efficiencies in DWTPs. It is hoped that the findings of this research could inform drinking water regulatory bodies of the presence of these contaminants, as they are currently not included within the legislation and regulatory framework for drinking water (Section 1.3). It is also hoped they will highlight the need for investing new and effective treatment processes, which are designed to remove drugs of abuse and pharmaceuticals from DWTPs. Moreover, this research can be considered as a preliminary test, as it is impossible to monitor every substance that may be present in drinking water. If the result of this research does reveal that trace amounts of drugs of abuse and pharmaceuticals are present in drinking water, it indicates the need to study a complete suite of parent compounds and their metabolites in order to fully characterise their transport through DWTPs and the potential for exposure through drinking water.

CHAPTER 2 EXPERIMENTAL PROCEDURES

This chapter first describes the standards and solvents used during the development and validation of the method for the determination of drugs of abuse and pharmaceuticals in drinking water. This is followed by the methods of sample collection and storage protocols used during drinking water sample analysis. The chapter is then divided into sub-sections which describe the experimental methods used, including method development and optimisation, method validation and drinking water analysis.

2.1 Drug and pharmaceutical standards and solvents

All standards were of an analytical grade, at a purity > 97 %. Amphetamine hydrochloride, cocaine hydrochloride, methamphetamine hydrochloride, ketamine hydrochloride, butylone hydrochloride, mephedrone hydrochloride, methylone hydrochloride, 1-(3-chlorophenyl)piperazine hydrochloride, 1-(4-fluorophenyl)piperazine, 1-(4-methoxyphenyl)piperazine, 1-(4-trifluoromethylphenyl)piperazine, 1-benzylpiperazine, 1-methyl-4-benzylpiperazine, citalopram hydrobromide and fluoxetine hydrochloride were purchased as powders from Sigma-Aldrich (UK). Methylenedioxypyrovalerone hydrochloride, methcathinone hydrochloride, 1-(3-trifluoromethylphenyl)piperazine hydrochloride, (1-butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone and (4-chloronaphthalen-1-yl)-(1-pentylindol-3-yl) methanone were purchased as powders from LGC Standards (UK). Amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 were used as internal standards and were purchased as 0.1 mg/mL solutions in methanol or acetonitrile (Sigma-Aldrich, UK). Stock solutions of all drug and pharmaceutical standards were prepared from their solids in methanol at a concentration of 1 mg/mL and were stored at -20 °C. Ultra-pure water was obtained from an Elga Purelab Ultra (Veolia, UK) and deionised water was obtained from an Elga Purelab Prima (Veolia, UK). Other solvents used and purchased are listed in Table 2.1. Silanised vials, LC-MS autosampler vials and inserts were purchased from Fisher Scientific (UK) and Hichrom (UK).

Table 2.1: Solvents used and their suppliers in this research

SOLVENTS	GRADE	SUPPLIERS
2-propanol	HPLC	Fisher Scientific (UK)
Acetonitrile	HPLC	Fisher Scientific (UK)
	LC-MS	Sigma-Aldrich (UK)
Ammonium hydroxide	HPLC	Sigma-Aldrich (UK)
Ethyl acetate	HPLC	Fisher Scientific (UK)
Formic acid	LC-MS	Sigma-Aldrich (UK)
Hydrochloric acid	HPLC	Sigma-Aldrich (UK)
Methanol	HPLC	Fisher Scientific (UK)
	LC-MS	Sigma-Aldrich (UK)
Water	LC-MS	Sigma-Aldrich (UK)

2.2 Sample collection and storage

Water samples used for method development and validation (Section 2.3 and Section 2.4) were ultra-pure water and raw water, while different water samples including raw water, finished drinking water and tap water were used during the course of drinking water analysis (Section 2.5). Raw water (water samples collected before drinking water treatments) and finished drinking water (water samples collected after drinking water treatments) were grab samples collected from three DWTPs of Anglian Water and Essex and Suffolk Water. Sampling dates were all on Mondays in February 2016. A number of 2.00 L high-density polyethylene (HDPE) containers (Fisher Scientific, UK) were used for sample collection and storage. After collection, the filled containers were immediately transported to the laboratory. Raw water and finished drinking water samples were stored at 10 °C and extracted within 12 hours of collection, as drugs of abuse and pharmaceuticals may start degrading after 24 hours of collection (Togola and Budzinski, 2008; Boleda, et al., 2011; Valcárcel, et al., 2011; Valcárcel, et al., 2012). In addition, two tap water samples were collected from Anglia Ruskin University and a privacy residence in Cambridge, UK, in 2.00 L HDPE containers and were extracted on the day of sample collection. As no human or animal participants were involved in this research, formal ethics approval was not required.

2.3 Method development and optimisation experiments

This section provides experimental procedures and is divided into three parts, namely the development and optimisation of HPLC-DAD, LC-MS and SPE.

2.3.1 Development of LC method by HPLC-DAD

2.3.1.1 Drug and pharmaceutical standards for LC method development

Mixed standards were prepared from 20 individual standard stock solutions (1 mg/mL). They were then evaporated using the miVac DNA concentrator (Genevac, UK) and reconstituted in HPLC injection solvent (0.5 % formic acid/4.975 % acetonitrile or methanol/94.525 % water, v/v), resulting in the concentration of 1 mg/mL.

2.3.1.2 Instrumental parameters and gradient elution profiles for LC method development using a C_{18} column

Chromatography was performed on a Shimadzu VP HPLC system (Shimadzu, Japan) using an ACE UltraCore SuperC_{18} UHPLC/HPLC column (75 x 4.6 mm i.d., 2.5 µm particle size) (Hichrom, UK). Separation was performed with mobile phase A (acetonitrile or methanol with 0.5 % v/v formic acid, pH 2.1) and mobile phase B (water with 0.5 % v/v formic acid, pH 2.1) at a flow rate of 1 mL/min. An injection volume of 1 microlitre (µL) was used and the column was maintained at 30 °C. The DAD was set from 190 nm to 800 nm. Data was collected, analysed and processed using LABsolutions software.

The time programme of gradient elution is shown in Table 2.2 when using acetonitrile as the organic modifier. Mobile phase A was acetonitrile with 0.5 % v/v formic acid. Mobile phase B was water with 0.5 % v/v formic acid. The obtained results are discussed in Section 3.1.1.

Table 2.2: Time programme of gradient elution for HPLC-DAD analysis with acetonitrile and a C_{18} column

TIME / min	MOBILE PHASE A / % v/v (0.5 % formic acid/99.5 % acetonitrile)	MOBILE PHASE B / % v/v (0.5 % formic acid/99.5 % water)
0	10	90
1	10	90
21	60	40
22	100	0
29	100	0
30	10	90
35	10	90

The time programme of gradient elution is shown in Table 2.3 when using methanol as the organic modifier. Mobile phase A was methanol with 0.5 % v/v formic acid. Mobile phase B was water with 0.5 % v/v formic acid. Obtained results are discussed in Section 3.1.1.

Table 2.3: Time programme of gradient elution for HPLC-DAD analysis with methanol and a C_{18} column

TIME / min	MOBILE PHASE A / % v/v (0.5 % formic acid/99.5 % methanol)	MOBILE PHASE B / % v/v (0.5 % formic acid/99.5 % water)
0	10	90
12	10	90
24	60	40
25	100	0
32	100	0
33	10	90
41	10	90

2.3.1.3 Instrumental parameters and gradient elution profile for LC method development using a biphenyl column

Chromatography was performed on a Shimadzu VP HPLC system (Shimadzu, Japan) using a Kinetex biphenyl 100 Å LC column (100 x 4.6 mm i.d., 2.6 µm particle size) and a matching SecurityGuard ULTRA cartridge UHPLC biphenyl (4.6 mm i.d.) (Phenomenex, UK). Separation was performed with mobile phase A (0.5 % formic acid/59.7 % methanol/39.8 % acetonitrile, v/v, pH 2.1) and mobile phase B (0.5 % formic acid/99.5 % water, v/v, pH 2.1) at a flow rate of 1 mL/min. Injection volume, column temperature and DAD setting were the same as the HPLC-DAD method using the C_{18} column, which are

mentioned in Section 2.3.1.2. Data was collected, analysed and processed using LABsolutions software. The time programme of gradient elution is shown in Table 2.4 and obtained results are discussed in Section 3.1.1.

Table 2.4: Time programme of gradient elution for HPLC-DAD analysis with a biphenyl column

TIME / min	MOBILE PHASE A / % v/v (0.5 % formic acid/59.7 % methanol/39.8 % acetonitrile)	MOBILE PHASE B / % v/v (0.5 % formic acid/99.5 % water)
0	10	90
1	10	90
26	60	40
27	100	0
34	100	0
35	10	90
40	10	90

2.3.2 Development and optimisation of MS method for LC-MS

2.3.2.1 Drug and pharmaceutical standards for MS method development and optimisation

Mixed standards were prepared from 20 individual standard stock solutions (1 mg/mL) and three internal standards (0.1 mg/mL). Mixed standards were evaporated using the miVac DNA concentrator (Genevac, UK) and reconstituted in LC-MS injection solvent (0.5 % formic acid/4.975 % acetonitrile/94.525 % water, v/v). The concentration of mixed standards was 0.01 mg/mL for the scan mode (Section 3.1.2.1) as well as the investigation of DL, qarray DC and RF settings (Section 3.1.2.3) and 100 ng/mL for the SIM mode (Section 3.1.2.1), as well as time segmentation (Section 3.1.2.2).

2.3.2.2 Instrumental parameters for MS method development and optimisation

For LC conditions, the flow rate was fixed at 0.2 mL/min and an injection volume of 10 µL was used. The column was maintained at 30 °C and the temperature of the autosampler was set at 10 °C. Two analytical columns were used. A C_{18} column was used for the identification and quantification of the studied drugs of abuse and pharmaceuticals, while a biphenyl column was used for confirmation.

For interface conditions, an LCMS-2020 single quadrupole mass spectrometer (Shimadzu, Japan) with an ESI source was used in positive ionisation mode. Interface conditions were fixed based on the tuning file, as follows: interface temperature, 350 °C; DL temperature, 250 °C; heat block temperature, 200 °C; nebulising gas flow, 1.5 L/min; drying gas flow, 15 L/min. Nitrogen was used as the nebulising and drying gas and was supplied by a nitrogen generator (Parker, UK).

For MS conditions, data acquisition was carried out in both scan mode and SIM mode. Scan mode was used to obtain the mass spectra of the studied drugs of abuse, pharmaceuticals and internal standards from individual standards. The m/z range was set to scan from 40 to 800 m/z and the event time was 1 sec. For SIM mode, the diagnostic ions of target analytes and internal standards were obtained from their mass spectra (Appendix I) by selecting the most abundant ion and are listed in Table 2.6 (C_{18} column) and Table 2.8 (biphenyl column). Event time was 0.03 min. The interface voltage was 4.5 kV and the detector voltage was -1.4 kV based on the tuning file. Data was collected, analysed and processed using LABSolutions software.

2.3.2.3 LC gradient programme, MS segmentation, Desolvation line (DL) voltages and lens system voltages for MS method development and optimisation using a C_{18} column

Chromatographic separation was carried out using Shimadzu Nexera UHPLC system (Shimadzu, Japan) equipped with an Acquity UPLC BEH C_{18} column (150 x 2.1 mm i.d., 1.7 µm particle size) and a matching VanGuard pre-column (5 x 2.1 mm i.d., 1.7 µm particle size) (Waters, UK). Separation was performed with mobile phase A (acetonitrile with 0.5 % v/v formic acid, pH 2.1) and mobile phase B (water with 0.5 % v/v formic acid, pH 2.1). The gradient programme is shown in Table 2.5.

Table 2.5: Time programme of gradient elution for LC-MS analysis with a C_{18} column

TIME / min	MOBILE PHASE A / % v/v (0.5 % formic acid/99.5 % acetonitrile)	MOBILE PHASE B / % v/v (0.5 % formic acid/99.5 % water)
0	10	90
1.5	10	90
14	60	40
15.5	100	0
22.5	100	0
24	10	90
44	10	90

MS analysis time was divided into ten segments and their time intervals are shown in Table 2.6. Quantifier ions of the studied analytes and internal standards were selected based on their mass spectra (Appendix I). DL voltage and lens system voltages (qarray DC and qarray RF) were optimised for each studied analyte. Their voltage values, as shown in Table 2.6, were determined from LC-MS optimisation experiments by systematically changing these MS parameters and selecting the voltages that gave the best instrumental response as optimal. Result graphs were produced using Microsoft Office Excel 2007.

Table 2.6: MS parameters of SIM mode for LC-MS analysis with a C_{18} column

TIME / min	COMPOUND	QUANTIFIER ION / m/z	DL VOLTAGE / kV	QARRAY DC VOLTAEG/ kV	QARRAY RF VOLTAEG / kV
0.00 - 4.25	BZP	177	9.6	6.4	35.2
	MBZP	191	12.8	9.6	38.4
4.25 - 7.05	Methcathinone	164	12.8	9.6	32.0
	Methylone	208	16.0	6.4	35.2
7.05 - 8.30	4-MeOPP	193	6.4	6.4	32.0
	Amphetamine-d_6	142	6.4	9.6	32.0
	Amphetamine	136	25.6	16.0	28.8
8.30 - 10.95	Methamphetamine	150	38.4	9.6	32.0
	4-FPP	181	6.4	6.4	38.4
	Butylone	222	16.0	6.4	41.6
10.95 - 12.25	Mephedrone	178	16.0	9.6	32.0
	Ketamine	238	12.8	6.4	35.2
12.25 - 14.60	3-CPP	197	9.6	6.4	32.0
	MDPV	276	96.0	6.4	41.6
	Cocaine-d_3	307	12.8	0.0	51.2
	Cocaine	304	32.0	0.0	48.0
14.60 - 15.70	3-TFMPP	231	32.0	16.0	38.4
	4-TFMPP	231	32.0	16.0	38.4
15.70 - 17.40	Citalopram	325	32.0	0.0	44.8
17.40 - 19.20	Fluoxetine-d_6	316	22.4	16.0	48.0
	Fluoxetine	310	16.0	16.0	41.6
19.20 - 35.00	JWH-073	328	32.0	19.2	48.0
	JWH-398	376	0.0	19.2	57.6

2.3.2.4 LC gradient programme, MS segmentation, DL voltages and lens system voltages for MS method development and optimisation using a biphenyl column

Chromatographic separation was carried out using Shimadzu Nexera UHPLC system (Shimadzu, Japan) equipped with an Kinetex biphenyl 100 Å LC column (100 x 4.6 mm i.d., 2.6 µm particle size) and a matching SecurityGuard ULTRA cartridge UHPLC biphenyl (4.6 mm i.d.) (Phenomenex, UK). Separation was performed with mobile phase A (0.5 % formic acid/59.7 % methanol/39.8 % acetonitrile, v/v, pH 2.1) and mobile phase B (0.5 % formic acid/99.5 % water, v/v, pH 2.1). The gradient programme is shown in Table 2.7.

Table 2.7: Time programme of gradient elution for LC-MS analysis with a biphenyl column

TIME / min	MOBILE PHASE A / % v/v (0.5 % formic acid/59.7 % methanol/39.8 % acetonitrile)	MOBILE PHASE B / % v/v (0.5 % formic acid/99.5 % water)
0	30	70
4	30	70
19	60	40
20	100	0
29	100	0
30	30	70
50	30	70

MS analysis time was divided into ten segments and their time intervals are shown in Table 2.8. Confirmation ions were selected for studied analytes and internal standards. The procedures are described in Section 2.3.2.3 and their values are shown in Table 2.8. DL voltages and lens system voltages (qarray DC and qarray RF) were the same as the LC-MS method using the C_{18} column and are also included in Table 2.8.

Table 2.8: MS parameters of SIM mode for LC-MS analysis with a biphenyl column

TIME / min	COMPOUND	CONFIRMATION ION / m/z	DL VOLTAGE / kV	QARRAY DC VOLTAEG / kV	QARRAY RF VOLTAEG / kV
0.00 - 6.80	BZP	177	9.6	6.4	35.2
	MBZP	191	12.8	9.6	38.4
6.80 - 8.55	Methcathinone	164	12.8	9.6	32.0
	Amphetamine-d_6	142	6.4	9.6	32.0
	Amphetamine	136	25.6	16.0	28.8
8.55 - 10.40	Methylone	208	16.0	6.4	35.2
	Methamphetamine	150	38.4	9.6	32.0
	4-MeOPP	193	6.4	6.4	32.0
10.40 - 13.50	4-FPP	181	6.4	6.4	38.4
	Mephedrone	178	16.0	9.6	32.0
	Butylone	222	16.0	6.4	41.6
13.50 - 16.60	Ketamine	238	12.8	6.4	35.2
16.60 - 18.40	3-CPP	197	9.6	6.4	32.0
18.40 - 23.00	3-TFMPP	231	32.0	16.0	38.4
	4-TFMPP	231	32.0	16.0	38.4
	Cocaine-d_3	307	12.8	0.0	51.2
	Cocaine	304	32.0	0.0	48.0
	MDPV	276	96.0	6.4	41.6
23.00 - 27.60	Citalopram	325	32.0	0.0	44.8
27.60 - 31.00	Fluoxetine-d_6	316	22.4	16.0	48.0
	Fluoxetine	310	16.0	16.0	41.6
31.00 - 38.00	JWH-073	328	32.0	19.2	48.0
	JWH-398	376	0.0	19.2	57.6

2.3.3 Development and optimisation of SPE method

A PRESSURE+ 48 positive pressure manifold with 48 wells (Biotage, UK) was used for all SPE extractions and a miVac DNA concentrator (Genevac, UK) was used for sample evaporation. The LC-MS protocol (C_{18} column) used was optimised during method development and optimization, as discussed in Section 2.3.2. SIM mode was used for the quantification of all water samples. Samples were extracted three times by SPE and then analysed in triplicate by LC-MS; therefore, nine measurements were obtained for each sample. A solvent blank was injected between each standard run.

2.3.3.1 Comparison of SPE cartridges, Oasis MCX (3 mL) and Strata-X-Drug B (3 mL)

Oasis MCX and Strata-X-Drug B cartridges were investigated using generic protocols (Waters, 2003; Phenomenex, 2011), as shown in Table 2.9. Three cartridges (60 mg, 3 mL) were analysed simultaneously for each protocol. 2.00 mL of raw water was spiked with a mixed standard of 20 target analytes and three internal standards (10 ng for each, with a final concentration of 5 ng/mL) and was extracted for each cartridge. Acids were added into raw water for pH adjustment: 2 % v/v formic acid for Oasis MCX cartridges and 0.1 M hydrochloric acid for Strata-X-Drug B cartridges. Eluants were then evaporated and reconstituted in 100 µL LC-MS injection solvent (0.5 % formic acid/4.975 % acetonitrile/94.525 % water, v/v). Three lots of 2.00 mL non-spiked raw water were analysed simultaneously using the same protocol as shown in Table 2.9. After evaporation, extracted non-spiked raw waters were also reconstituted in 100 µL LC-MS injection solvent and spiked with the same quantity of target analytes and internal standards (10 ng for each, with a final concentration of 100 ng/mL) for recovery calculation (Section 3.2.1).

Table 2.9: Generic protocols for SPE with Oasis MCX and Strata-X-Drug B cartridges

PROTOCOL	SPE CARTRIDGES	
	Oasis MCX (60 mg, 3 mL)	**Strata-X-Drug B (60 mg, 3 mL)**
Condition	2.00 mL methanol	2.00 mL methanol
Equilibrate	2.00 mL deionised water (2 % v/v formic acid)	2.00 mL deionised water (0.1 M hydrochloric acid)
Load	2.00 mL water sample (2 % v/v formic acid)	2.00 mL water sample (0.1 M hydrochloric acid)
Wash	2.00 mL deionised water (2 % v/v formic acid)	2.00 mL deionised water (0.1 M hydrochloric acid)
Eluent 1	2.00 mL methanol	2.00 mL methanol
Eluent 2	2 x 2.00 mL methanol (5 % v/v ammonium hydroxide)	2 x 2.00 mL ethyl acetate/isopropanol/ammonium hydroxide (70:20:10, v/v)
Evaporate	miVac DNA concentrator	miVac DNA concentrator
Reconstitute	100 µL LC-MS injection solvent	100 µL LC-MS injection solvent

2.3.3.2 Optimisation of SPE elution solvent for Strata-X-Drug B (3 mL)

Using the Strata-X-Drug B protocol in Table 2.9, three elution solvents, namely methanol, acetonitrile and ethyl acetate/isopropanol (85:15, v/v) were investigated separately for the step of eluent 1. Three cartridges (60 mg, 3 mL) were used simultaneously for each elution solvent. A mixed standard containing 20 target analytes and three internal standards (10 ng for each) was added into 2.00 mL of acidic raw water (0.1 M hydrochloric acid) resulting in the concentration of 5 ng/mL and was extracted for each cartridge. Eluants were then evaporated and reconstituted in 100 µL LC-MS injection solvent (0.5 % formic acid/4.975 % acetonitrile/94.525 % water, v/v). Three lots of 2.00 mL non-spiked raw water were analysed simultaneously and then spiked with the same quantity of target analytes. Internal standards (10 ng for each) were dissolved in 100 µL LC-MS injection solvent, with a final concentration of 100 ng/mL, after evaporation for recovery calculation (Section 3.2.2).

2.3.3.3 Optimisation of SPE sample loading volume for Strata-X-Drug B (6 mL)

The sample loading volume was investigated using optimised protocol as shown in Table 2.10. Higher sample loading volume (200 mL) and larger capacity of Strata-X-Drug B (60 mg, 6 mL) cartridge were incorporated into this SPE method. Three cartridges (60 mg, 6

mL) were analysed simultaneously. A mixed standard containing 20 target analytes and three internal standards (20 ng for each) was added into 200 mL acidic raw water (0.1 M hydrochloric acid) resulting in the concentration of 0.1 ng/mL and extracted for each cartridge. Eluants were then evaporated and reconstituted in 100 µL LC-MS injection solvent (0.5 % formic acid/4.975 % acetonitrile/94.525 % water, v/v) for LC-MS analysis. Three lots of 200 mL non-spiked raw water were analysed simultaneously and then spiked with the same quantity of target analytes and internal standards (20 ng for each) dissolved in 100 µL LC-MS injection solvent, resulting in a final concentration of 200 ng/mL, after evaporation for recovery calculation (Section 3.2.3).

Table 2.10: Optimised protocol for SPE with Strata-X-Drug B cartridges

PROTOCOL	SPE CARTRIDGES
	STRATA-X-DRUG B (60 mg, 6 mL)
Condition	2.00 mL methanol
Equilibrate	2.00 mL deionised water (0.1 M hydrochloric acid)
Load	200 mL water sample (0.1 M hydrochloric acid)
Wash	2.00 mL deionised water (0.1 M hydrochloric acid)
Eluent 1	2.00 mL ethyl acetate/isopropanol (85:15, v/v)
Eluent 2	2 x 2.00 mL ethyl acetate/isopropanol/ammonium hydroxide (70:20:10, v/v)
Evaporate	miVac DNA concentrator
Reconstitute	100 µL LC-MS injection solvent

2.4 Method validation experiments

In this section, method validation parameters (Section 1.10) were assessed by the analysis of mixed standards reconstituted in LC-MS injection solvent and waters spiked with known concentrations of target analytes. For internal standards, 5 ng/mL of amphetamine-d_6, 0.1 ng/mL of cocaine-d_3 and 0.75 ng/mL of fluoxetine-d_6 were added to all mixed standards for autosampler storage stability, instrumental linearity, instrumental precision, IDL and IQL, whereas 50 ng/L of amphetamine-d_6, 5 ng/L of cocaine-d_3 and 25 ng/L of fluoxetine-d_6 were added to spiked waters for analytical method precision and accuracy. The LC-MS protocols using a C_{18} column and biphenyl column optimised during the method development and optimisation (Section 2.3.2) were used. SIM mode was used for all method validation

studies. The optimised SPE protocol (Table 2.10) was used for the extraction of spiked waters and the loading volume was 200 mL. Microsoft Office Excel 2007 was used to set out the analysis of the results.

2.4.1 Selectivity

The selectivity of the method (Section 4.1) was studied using a 100 ng/mL mixed standard dissolved in LC-MS injection solvent containing 20 target analytes and three internal standards. Ultra-pure water was also analysed as the matrix blank.

2.4.2 Autosampler storage stability

Mixed standards were prepared in LC-MS injection solvent at two concentrations (10 and 500 ng/mL). Standards remained on the autosampler at 10 °C for five days. Replicate injections (n = 8) at each concentration were analysed per day and the solvent blank was injected between each standard run. The stabilities of studied target analytes and internal standards are discussed in Section 4.2.1 and Section 4.2.2.

2.4.3 Instrumental linearity

The calibration model (Section 4.3) was studied using 19 mixed standards, which were prepared in LC-MS injection solvent at the concentration of 0.001, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 100, 500, 1000, 5000 and 10000 ng/mL. Replicate injections (n = 3) were analysed for each concentration and the solvent blank was injected between each standard run.

2.4.4 Precision and accuracy

2.4.4.1 Instrumental intra-assay precision

Mixed standards were prepared in LC-MS injection solvent at low (5 ng/mL), medium (50 ng/mL) and high (500 ng/mL) concentrations. Replicate injections (n = 6) were analysed at each concentration for instrumental intra-assay precision (Section 4.4.1) and the solvent blank was injected between each standard run.

2.4.4.2 Instrumental intermediate precision

Instrumental intermediate precision was verified at three concentrations (low, medium and high) on three separate days for instrumental intermediate precision (Section 4.4.2). These three concentrations were the same as described above for intra-assay precision. Replicate injections (n = 3) at each concentration were analysed on each day and the solvent blank was injected between each standard run.

2.4.4.3 Method precision and accuracy

Five calibrators were prepared in ultra-pure water at the concentration of 5, 30, 50, 70 and 100 ng/L. In addition, three quality controls (QCs), which were used to estimate the method accuracy (Section 4.4.3), were independently prepared by spiking ultra-pure water with mixed standards, resulting in the concentrations of 10, 40 and 80 ng/L. 0.1 M hydrochloric acid was added to all calibrators and QCs for pH control. Calibrators and QCs were extracted and then were analysed with replicate injection (n = 3) for method precision (Section 4.4.3).

2.4.5 Detection and quantification limits

Five mixed standards dissolved in LC-MS injection solvent were studied for each analyte in order to calculate the IDLs and IQLs for a C_{18} column (Section 4.5.1.1) and biphenyl column (Section 4.5.1.2). The concentrations of mixed standards are listed in Table 2.11. Replicate injections (n = 3) at each concentration were analysed and the solvent blank was injected between each standard. MDLs and MQLs for the studied drugs of abuse and pharmaceuticals were calculated based on the IDLs and IQLs, SPE recovery results and enrichment factor (2000 in this research). The calculation equations and results are shown in Section 4.5.2.

Table 2.11: Individual concentrations in the mixed standards used for the calculation of IDLs and IQLs for LC-MS analysis with a C_{18} column and biphenyl column

COMPOUND	MIXED STANDARD CONCENTRATIONS / ng/mL									
	C_{18} Column					Biphenyl Column				
	1	2	3	4	5	1	2	3	4	5
BZP	0.100	0.250	0.500	0.750	1.000	0.025	0.050	0.075	0.100	0.250
MBZP	0.050	0.075	0.100	0.250	0.500	0.025	0.050	0.075	0.100	0.250
Methcathinone	0.050	0.075	0.100	0.250	0.500	0.100	0.250	0.500	0.750	1.000
Methylone	0.100	0.250	0.500	0.750	1.000	0.010	0.025	0.050	0.075	0.100
4-MeOPP	0.750	1.000	2.500	5.000	7.500	0.075	0.100	0.250	0.500	0.750
Amphetamine	0.500	0.750	1.000	2.500	5.000	0.500	0.750	1.000	2.500	5.000
Methamphetamine	0.250	0.500	0.750	1.000	2.500	0.250	0.500	0.750	1.000	2.500
4-FPP	0.075	0.100	0.250	0.500	0.750	0.050	0.075	0.100	0.250	0.500
Butylone	0.010	0.025	0.050	0.075	0.100	0.025	0.050	0.075	0.100	0.250
Mephedrone	0.025	0.050	0.075	0.100	0.250	0.100	0.250	0.500	0.750	1.000
Ketamine	0.010	0.025	0.050	0.075	0.100	0.025	0.050	0.075	0.100	0.250
3-CPP	0.075	0.100	0.250	0.500	0.750	0.250	0.500	0.750	1.000	2.500
MDPV	0.025	0.050	0.075	0.100	0.250	0.025	0.050	0.075	0.100	0.250
Cocaine	0.010	0.025	0.050	0.075	0.100	0.025	0.050	0.075	0.100	0.250
3-TFMPP	0.025	0.050	0.075	0.100	0.250	0.075	0.100	0.250	0.500	0.750
4-TFMPP	0.025	0.050	0.075	0.100	0.250	0.075	0.100	0.250	0.500	0.750
Citalopram	0.010	0.025	0.050	0.075	0.100	0.025	0.050	0.075	0.100	0.250
Fluoxetine	0.100	0.250	0.500	0.750	1.000	0.050	0.075	0.100	0.250	0.500
JWH-073	0.750	1.000	2.500	5.000	7.500	0.250	0.500	0.750	1.000	2.500
JWH-398	0.750	1.000	2.500	5.000	7.500	0.500	0.750	1.000	2.500	5.000

2.5 Drinking water analysis

Five drinking water samples and three raw water samples collected from the East Anglia region of the UK were prepared and analysed in triplicate using a C_{18} column. In addition, all were confirmed using a biphenyl column. For each sample, six lots of 200 mL water were measured and 0.1 M hydrochloric acid was added for pH control. Three lots of 200 mL water were used as non-spiked samples and the other three were spiked with mixed standards, resulting in the added concentrations of 5, 50 and 100 ng/L. Amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 were added to all samples (three non-spiked samples and three spiked samples) at the concentration of 50, 5 and 25 ng/L, respectively. Each non-spiked sample was extracted three times using the SPE protocol in Table 2.10 and then analysed with replicate injection (n = 3) using the LC-MS method (C_{18} column) in Section 2.3.2.3. Three spiked samples were also extracted and analysed in the same analytical batch. Then, all samples were analysed using the LC-MS method (biphenyl column), as described in Section 2.3.2.4 for confirmation. Moreover, ultra-pure water as method blank and mixed standards (50 ng/L) as positive control for the confirmation of the retention time were also analysed. The results of water samples (raw and drinking waters) were analysed using Microsoft Office Excel 2007 and are discussed in Chapter 5.

CHAPTER 3 RESULTS AND DISCUSSION: METHOD DEVELOPMENT AND OPTIMISATION

This chapter includes the development and optimisation of the simultaneous method for the identification and quantification of 20 drugs of abuse and pharmaceuticals in drinking water. LC-MS methods using two analytical columns were developed and validated and are discussed in Section 3.1. A C_{18} column was used as the main method for identification and quantification and a biphenyl column was used for the purpose of confirmation. The reason for using two different analytical columns is discussed in Section 3.1.2.1. Finally, Section 3.2 includes the development and optimisation process of the sample preparation and extraction method based on SPE.

3.1 Development and optimisation of LC-MS method

During the course of the LC-MS method development and optimisation, preliminary experiments of the method development regarding LC conditions (Section 3.1.1) were initially undertaken using a HPLC-DAD. Then the developed LC method was transferred onto a LC-MS and MS conditions were developed and optimised further. The results are described and discussed in Section 3.1.2.

3.1.1 Development of LC method by HPLC-DAD

When developing a new LC method for the analysis of ionisable compounds, the optimal pH of the mobile phase is one of the most crucial decisions to make. As the pH of the mobile phase is close to the pKa of the analyte, compounds will exist in both the non-ionised and ionised forms, resulting in different retention times (Kazakevich and LoBrutto, 2007). In this research, all analytes investigated are basic compounds and their pK_a values are between 7.1 (methcathinone) and 10.1 (fluoxetine) as detailed in Table 1.3. Hence, the pH of the mobile phase should be adjusted either below 5.1 or above 12.1. If below pH 5.1, all basic compounds will exist in the ionised form, as the mobile phase pH is more than two pH units below the pK_a values of relevant analytes (discussed in Section 1.9.1.2.1). In contrast, when the mobile phase pH is above 12.1, all compounds will exist in the non-ionised form due to the pH being more than two pH units above their pK_a values. In

addition, the low pH can also improve the peak shape and remove the peak tailing when analysing basic compounds. Peak tailing is often the characteristic of a protonated basic compound interacting with deprotonated silanol sites on the surface of the silica particle (Snyder, Kirkland and Glajch, 1997). In order to overcome this ionic interaction between the basic compounds and the column, the mobile phase pH should be controlled at around 2 to protonate the silica, as the pK_a value of silica ranges from 4 to 5 (Dolan, 2011). Thus, 0.5 % v/v formic acid was added to the mobile phase to adjust the pH to 2.

The gradient elution profiles for a C_{18} column were then developed and the influence of two organic modifiers in mobile phase, acetonitrile and methanol, on the chromatographic separation was studied and compared in order to obtain the best resolution, together with shorter analysis time. Following the gradient elution as detailed in Table 2.2 and Table 2.3, the chromatograms of a mixed standard containing 20 analytes were obtained using acetonitrile (Figure 3.1) and methanol (Figure 3.2). The wavelength 265 nm was chosen, as all target analytes showed appreciable absorbance at this wavelength. The retention times of the studied drugs of abuse and pharmaceuticals are listed in Table 3.1.

Table 3.1: Retention times for 20 analytes obtained from a HPLC-DAD analysis with a C_{18} column and biphenyl column

COMPOUND	RETENTION TIME / min		
	C_{18} Column		Biphenyl Column
	Acetonitrile	Methanol	Methanol/Acetonitrile
BZP	1.54	1.30	2.59
MBZP	3.11	2.32	5.55
Methcathinone	3.48	2.54	7.34
4-MeOPP	4.38	2.93	9.76
Methylone	4.57	3.07	9.47
Amphetamine	4.86	3.94	7.64
Methamphetamine	5.66	4.13	9.30
4-FPP	5.91	3.89	10.86
Butylone	6.36	5.51	11.73
Mephedrone	6.60	6.14	11.37
Ketamine	6.99	7.62	13.62
3-CPP	8.63	12.08	14.82
MDPV	9.18	16.90	16.49
Cocaine	9.21	12.79	16.53
3-TFMPP	10.27	19.15	15.96
4-TFMPP	10.57	19.36	16.68
Citalopram	12.58	21.40	21.68
Fluoxetine	15.03	24.33	21.72
JWH-073	24.80	27.68	23.74
JWH-398	26.01	28.19	23.77

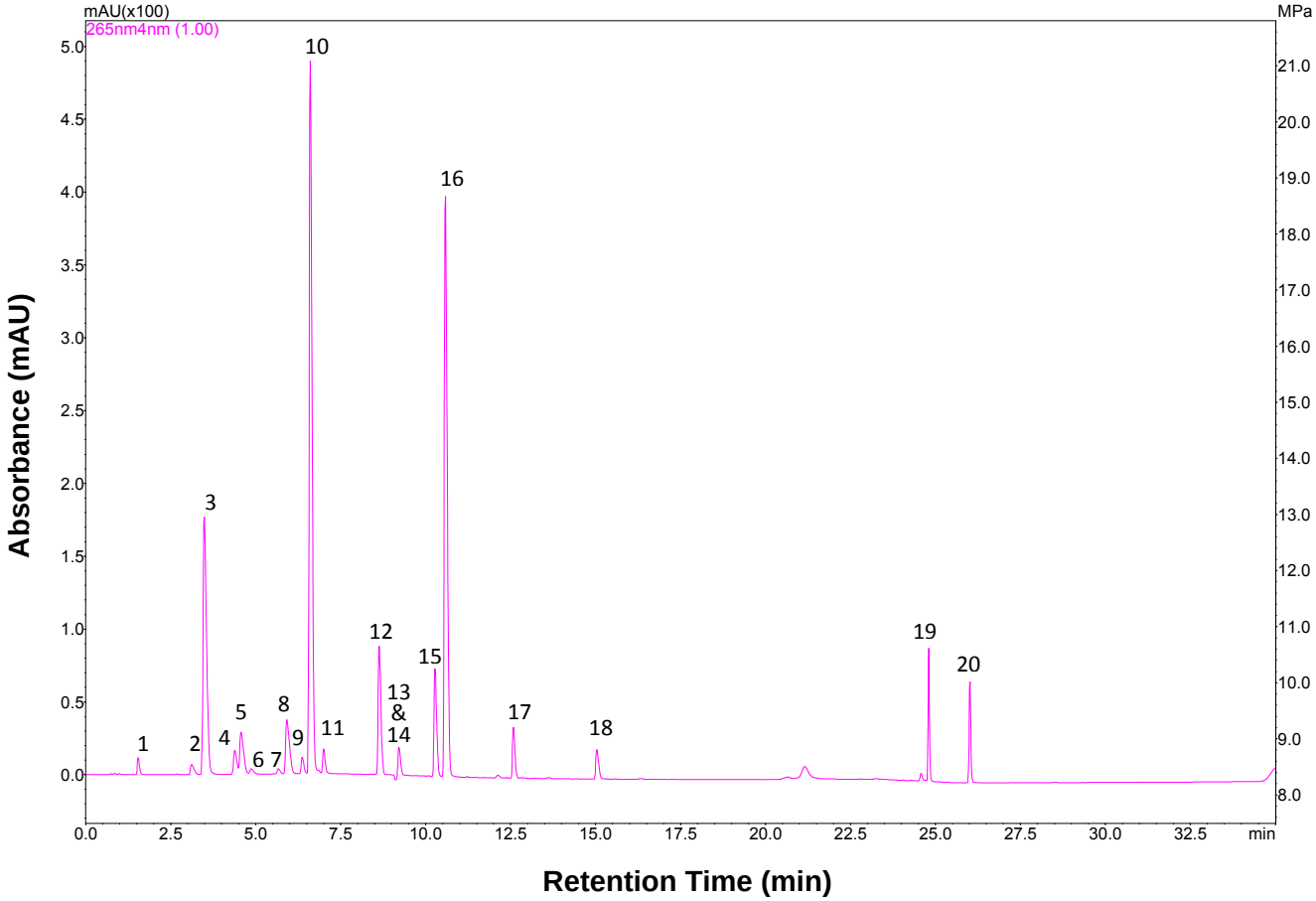


Figure 3.1: Chromatogram of a mixed standard containing 20 analytes at 1 mg/mL from a HPLC-DAD analysis obtained with acetonitrile and a C_{18} column

(1) BZP, (2) MBZP, (3) methcathinone, (4) 4-MeOPP, (5) methylone, (6) amphetamine, (7) methamphetamine, (8) 4-FPP, (9) butylone, (10) mephedrone, (11) ketamine, (12) 3-CPP, (13) MDPV, (14) cocaine, (15) 3-TFMPP, (16) 4-TFMPP, (17) citalopram, (18) fluoxetine, (19) JWH-073, (20) JWH-398

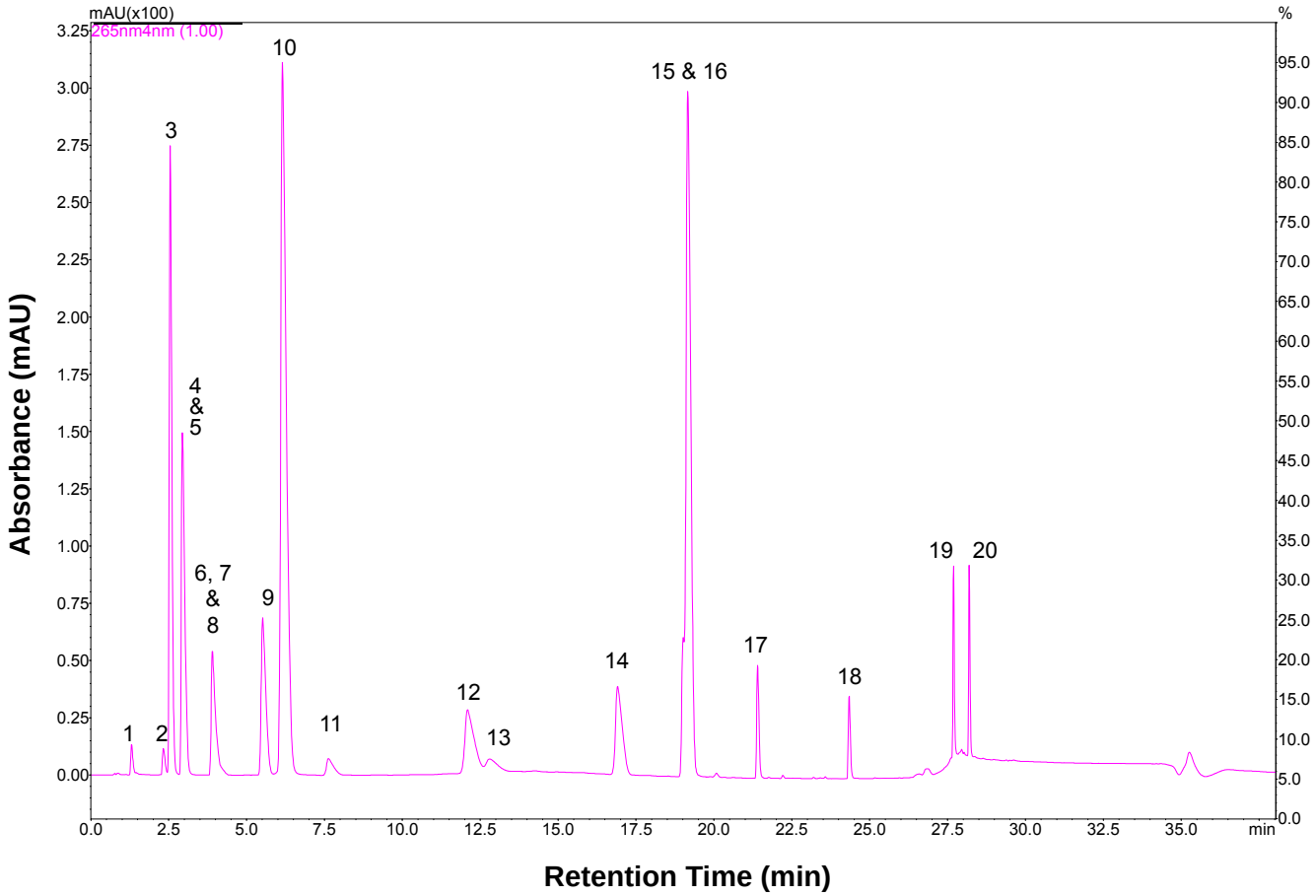


Figure 3.2: Chromatogram of a mixed standard containing 20 analytes at 1mg/mL from a HPLC-DAD analysis obtained with methanol and a C_{18} column

(1) BZP, (2) MBZP, (3) methcathinone, (4) 4-MeOPP, (5) methylone, (6) 4-FPP, (7) amphetamine, (8) methamphetamine, (9) butylone, (10) mephedrone, (11) ketamine, (12) 3-CPP, (13) cocaine, (14) MDPV, (15) 3-TFMPP, (16) 4-TFMPP, (17) citalopram, (18) fluoxetine, (19) JWH-073, (20) JWH-398

In terms of acetonitrile, the application of gradient elution, as described in Table 2.2, gave a good chromatographic separation of the 20 drugs of abuse and pharmaceuticals. As shown in Figure 3.1 and Table 3.1, the majority of target analytes (16) are well separated in 27 min using acetonitrile based on their retention times, including the positional isomers 3-TFMPP and 4-TFMPP. However, a co-elution between MDPV and cocaine was obtained and two analytes (4-MeOPP and methylone) were eluted at similar retention times; thus, it is impossible to identify these analytes by simply using their retention times.

On the other hand, an acceptable separation of most target analytes (13) can be achieved in 29 min when methanol was used as the organic modifier. As can be seen from Figure 3.2 and Table 3.1, one set of positional isomers 3-TFMPP and 4-TFMPP were co-eluted and cannot be separated based on their retention times. In addition, two groups of analytes showed overlapping peaks: (1) 4-MeOPP and methylone and (2) 4-FPP, amphetamine and methamphetamine.

Of the two organic modifiers, it is found that acetonitrile was more selective and suitable for the analysis of the studied drugs of abuse and pharmaceuticals compared to methanol when using the C_{18} column. This is because more analytes can be separated based on the retention times. Although some analyte peaks such as MDPV and cocaine overlapped using acetonitrile (Table 3.1), the co-elution does not represent a drawback, since this HPLC-DAD method was later transferred onto LC-MS, where these analytes can be identified by their quantifier ions (which is shown later in Table 3.3). Moreover, the most important reason for choosing acetonitrile is that the one set of positional isomers 3-TFMPP and 4-TFMPP can only be separated when acetonitrile was used as the organic modifier (Figure 3.1 and Figure 3.2). As 3-TFMPP and 4-TFMPP have the same molecular weight (Table 1.3) and share the same quantifier ion (Table 3.3), it is necessary to achieve the chromatographic separation between them, otherwise they cannot be identified even when using a MS detector (discussed in Section 3.1.2.1). Therefore, acetonitrile was chosen as the organic modifier and gradient elution, as shown in Table 2.2, was the final developed HPLC-DAD method to be transferred to LC-MS.

Methanol and acetonitrile were also run for a biphenyl column and the mix of methanol and acetonitrile (6:4, v/v) was chosen as an organic modifier. Several gradient runs were undertaken in order to achieve better separation. The final time programme of the gradient elution is detailed in Table 2.4. The chromatogram of a mixed standard containing 20 analytes obtained by this gradient elution is shown in Figure 3.3 and their retention times are listed in Table 3.1.

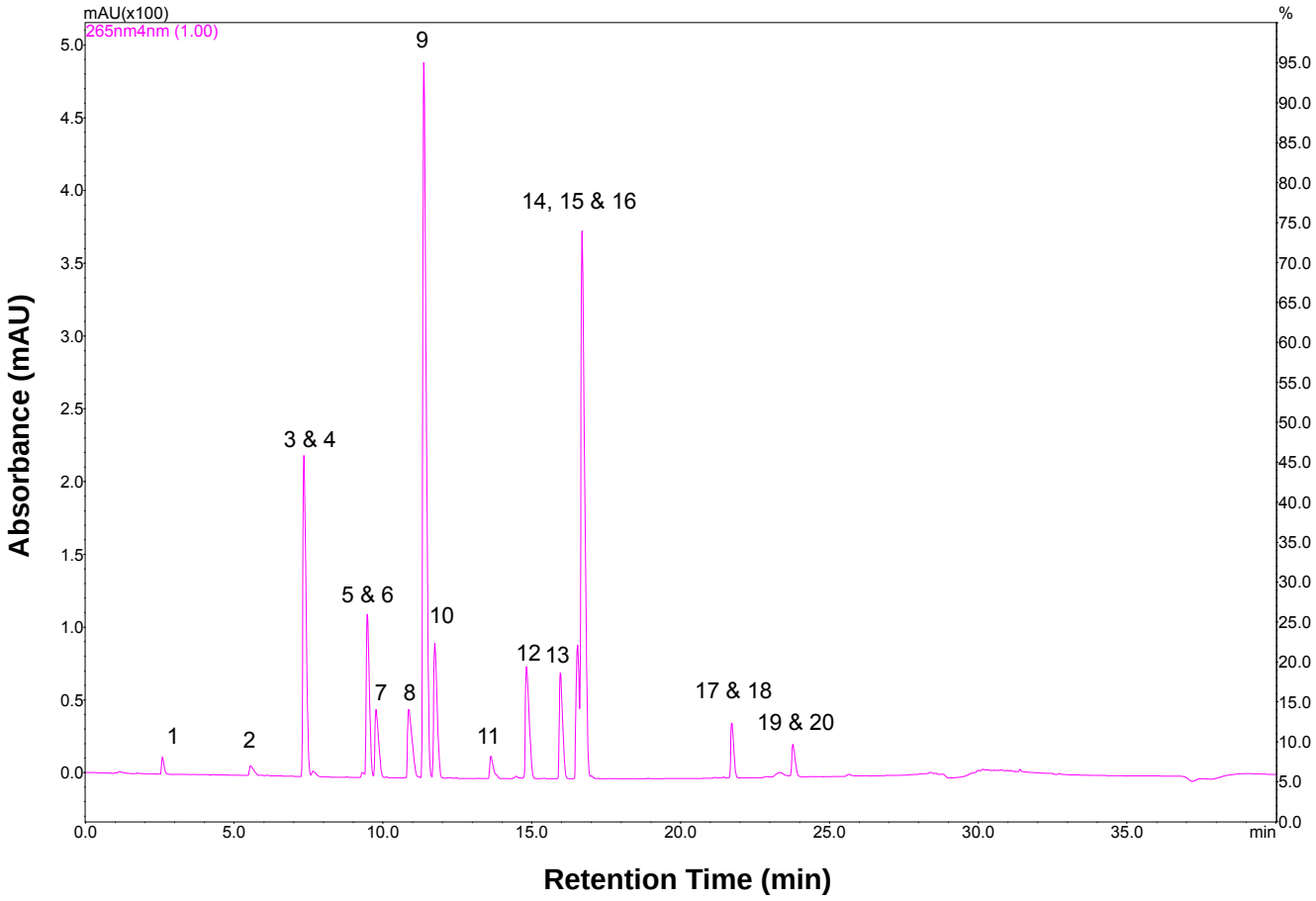


Figure 3.3: Chromatogram of a mixed standard containing 20 analytes at 1 mg/mL from a HPLC-DAD analysis obtained with a biphenyl column

(1) BZP, (2) MBZP, (3) methcathinone, (4) amphetamine, (5) methamphetamine, (6) methylone, (7) 4-MeOPP, (8) 4-FPP, (9) mephedrone, (10) butylone, (11) ketamine, (12) 3-CPP, (13) 3-TFMPP, (14) MDPV, (15) cocaine, (16) 4-TFMPP, (17) citalopram, (18) fluoxetine, (19) JWH-073, (20) JWH-398

In Figure 3.3, the developed HPLC-DAD method using the biphenyl column gave an acceptable chromatographic separation in 24 min and many target analytes can be separated based on their retention times, including 3-TFMPP (15.96 min) and 4-TFMPP (16.68 min). The separation of 3-TFMPP and 4-TFMPP was important, as this method was then transferred to LC-MS and these two positional isomers cannot be separated based on diagnostic ions, as mentioned earlier. Thus, this method was suitable for the separation of the studied drugs of abuse and pharmaceuticals in this research.

3.1.2 Development and optimisation of MS method by LC-MS

During the course of the MS method development and optimisation, two HPLC-DAD methods using the C_{18} column and biphenyl column were first transferred onto LC-MS. The flow rate of the mobile phase was reduced to 0.2 mL/min in order to make the method compatible with MS, as described in Section 1.9.2.1. In addition, the gradient elution profiles of the HPLC-DAD methods were changed based on the altered flow rate and LC-MS column dimension (150 x 2.1 mm, i.d.) and are detailed in Table 2.5 (C_{18} column) and Table 2.7 (biphenyl column). The method development experiments regarding diagnostic ions and peak separation were then undertaken to further improve method selectivity (Section 3.1.2.1). In addition, a variety of factors with regards to MS conditions needed to be optimised in order to increase method sensitivity, including time segmentation (Section 3.1.2.2) and the optimisation of DL, qarray DC and RF voltages (Section 3.1.2.3).

3.1.2.1 Diagnostic ions and peak separation

After changing the gradient elution profile and flow rate, the diagnostic ions of the studied drugs of abuse, pharmaceuticals and internal standards were determined and then monitored in SIM mode in order to increase the selectivity and sensitivity, as mentioned in Section 1.9.2.2.2. The diagnostic ions normally include ions used for quantification and confirmation. The quantifier ions of target analytes monitored in the LC-MS method using a C_{18} column were obtained from their mass spectra by selecting the most abundant ion. The corresponding mass spectra of all studied drugs of abuse, pharmaceuticals and internal

standards can be found in Appendix I. Based on their mass spectra, the ion with the highest relative intensity was the protonated molecular ion $[M+H]^+$ for each analyte, except for amphetamine-d_6. For example, the molar mass of 4-MeOPP is 192.3 g/mol (Table 1.3) and its most abundant ion is m/z 193 (as shown in Figure 3.4 below). The molar masses of the studied drugs of abuse and pharmaceuticals are shown in Table 1.3. Thus, the protonated molecular ion was monitored for each analyte as the quantifier ion for the purpose of quantification, as shown in Table 3.2. For amphetamine-d_6, m/z 142 was chosen as the quantifier ion, as it is the protonated molecular ion of this compound and also has a high intensity in the mass spectrum (which is shown in Figure 3.5 A). The selected quantifier ions are in agreement with published literature (de Castro, et al., 2008; Elliott and Smith, 2008; Baker and Kasprzyk-Hordern, 2011b; Sørensen, 2011; Ammann, et al., 2012a; Ammann, et al., 2012b).

Table 3.2: Quantifier ion, confirmation ions and ion ratios for 20 analytes and three internal standards obtained from a LC-MS analysis with a C_{18} column

COMPOUND	Quantifier Ion (Q)		Confirmation Ion 1 (C1)		Confirmation Ion 2 (C2)		Ion Ratios	
	Mass to charge ratio / m/z	Relative Intensity / %	Mass to charge ratio / m/z	Relative Intensity / %	Mass to charge ratio / m/z	Relative Intensity / %	Q/C1	Q/C2
BZP	177	100	208	19	214	17	5.26	5.88
MBZP	191	100	417	11	213	7	9.09	14.29
Methcathinone	164	100	205	49	377	35	2.04	2.86
Methylone	208	100	249	9	517	6	11.11	16.67
4-MeOPP	193	100	42	21	385	19	4.76	5.26
Amphetamine-d_6 [a]	142	100	142	69	214	21	1.45	4.76
Amphetamine	136	100	177	66	115	36	1.52	2.78
Methamphetamine	150	100	191	26	413	16	3.85	6.25
4-FPP	181	100	222	15	373	11	6.67	9.09
Butylone	222	100	263	6	–	–	16.67	–
Mephedrone	178	100	219	21	458	20	4.76	5.00
Ketamine	238	100	–	–	–	–	–	–
3-CPP	197	100	238	42	390	8	2.38	12.50
MDPV	276	100	–	–	–	–	–	–
Cocaine-d_3 [a]	307	100	–	–	–	–	–	–
Cocaine	304	100	–	–	–	–	–	–
3-TFMPP	231	100	272	82	115	7	1.22	14.29
4-TFMPP	231	100	272	85	115	11	1.18	9.09
Citalopram	325	100	341	15	–	–	6.67	–
Fluoxetine-d_6 [a]	316	100	357	46	134	10	2.17	10.00
Fluoxetine	310	100	351	35	350	17	2.86	5.88
JWH-073	328	100	391	35	677	12	2.86	8.33
JWH-398	376	100	341	39	378	37	2.56	2.70

[a] Amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 to be used as internal standards

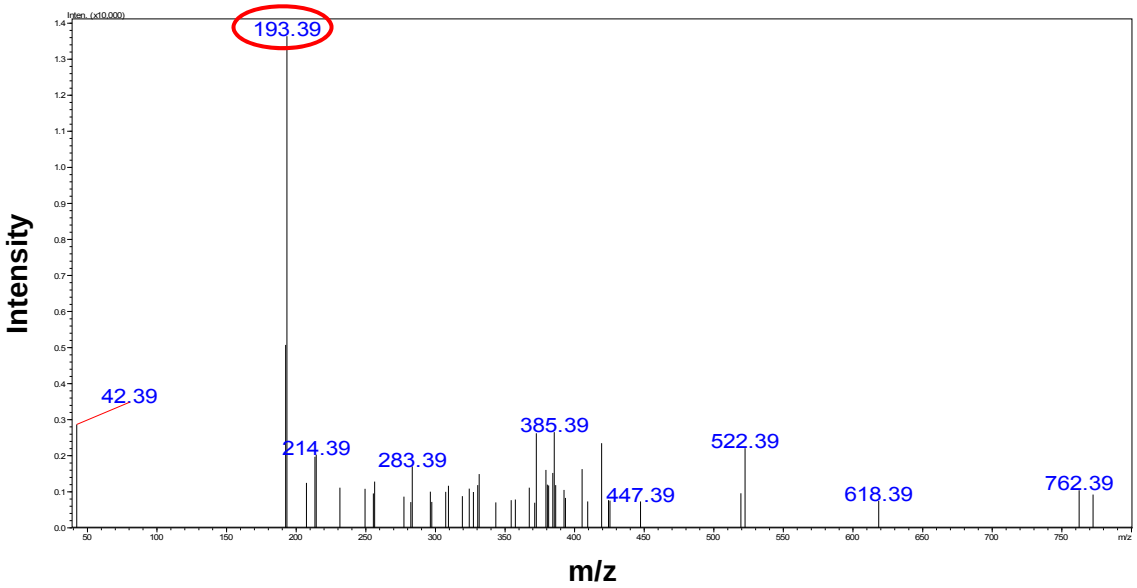


Figure 3.4: Mass spectrum of 4-MeOPP at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing its quantifier ion (circled)

In this research, amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 were used as internal standards for the quantitative analysis. If they contain fragment ions with the same m/z as the quantifier ions of their undeuterated analogues, the analyte signals would be overestimated, leading to the incorrectness of analyte concentrations in the standards and samples (Bogusz, 1997). Figure 3.5 shows the ion m/z 136, which is the quantifier ion of amphetamine, which was not present in the mass spectrum of amphetamine-d_6. It demonstrates that no cross-contribution (Peters, Drummer and Musshoff, 2007) between amphetamine-d_6 (internal standard) and amphetamine (its undeuterated analogue) occurred and thus amphetamine-d_6 can be used as the internal standard to provide reliable quantification. The same applied to the other two internal standards, cocaine-d_3 and fluoxetine-d_6 (Appendix I).

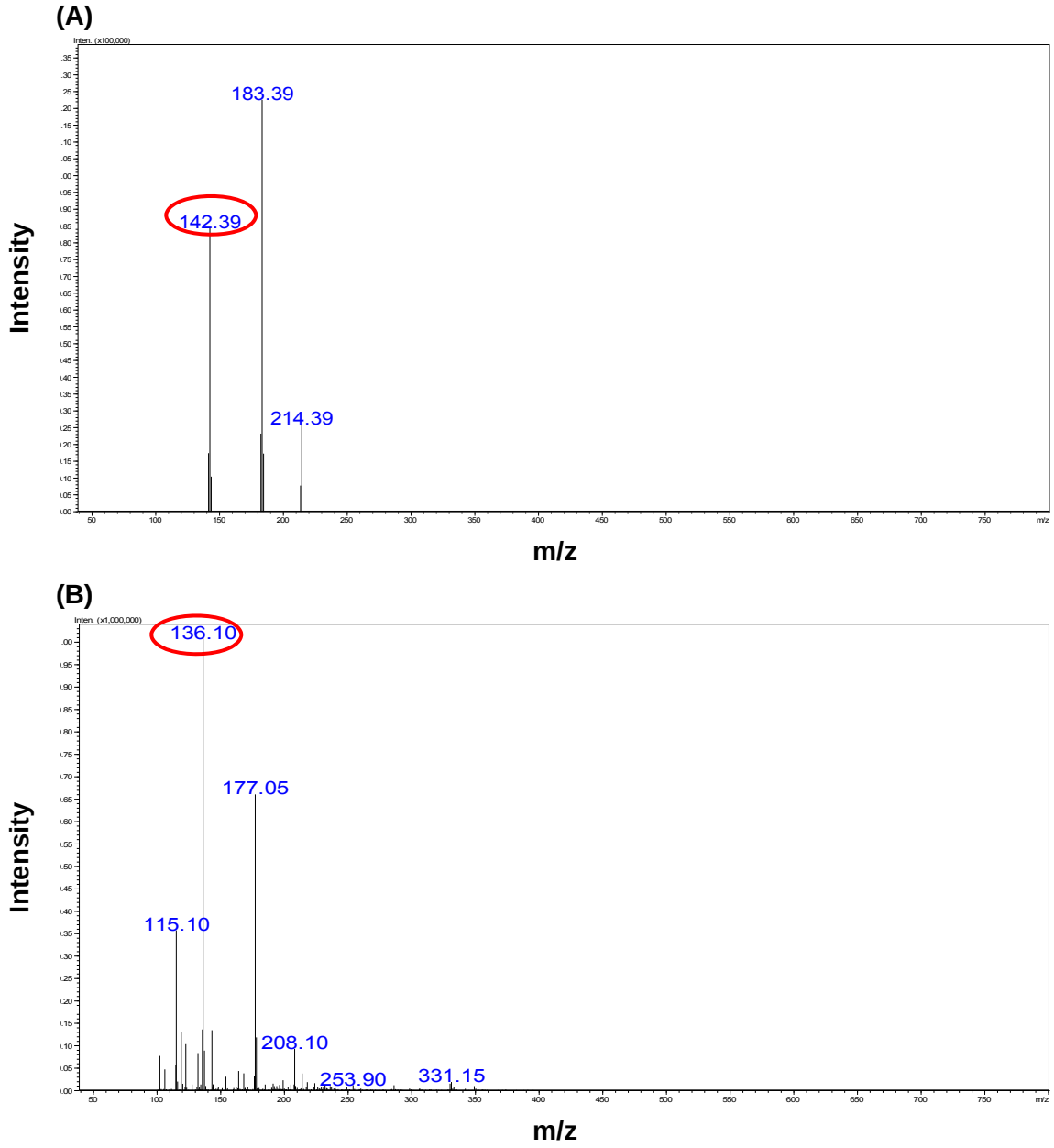


Figure 3.5: Mass spectra of internal standard (A) amphetamine-d_6 at 0.01 mg/mL and undeuterated analogue (B) amphetamine at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their quantifier ions (circled)

Moreover, it is recommended that two ions should be monitored for each analyte as confirmation ions and the ion ratio of quantifier ion to confirmation ion should be calculated in order to improve the reliability of confirmation (Commission Decision 2002/657/EC; Rivier, 2003; Boleda, et al., 2011). For example, m/z 328 was used as the quantifier ion of JWH-073 because it was the base peak and m/z 391 and m/z 677, the other two predominant ions, should be used as confirmation ions (Figure 3.6 A). These ions and their ratios are shown in Table 3.2. However, using LC-MS, some of the analytes had only one or two predominant ions in their mass spectra, including butylone, ketamine, MDPV, cocaine and citalopram (Table 3.2). The mass spectrum of cocaine, as an example, is depicted in Figure 3.6 B. Only its protonated molecular ion, m/z 304, was present. This is because the ESI technique was used for the LC-MS method, which provoked light fragmentation and yielded less characteristic ions (López de Alda and Barceló, 2000). Hence, it is not always possible to monitor three diagnostic ions for all studied analytes in this research with just the use of LC-MS and ESI. In this research, another approach for the confirmation assessment was used, which is the use of two analytical columns with different selectivity. This approach is further explained in this Section.

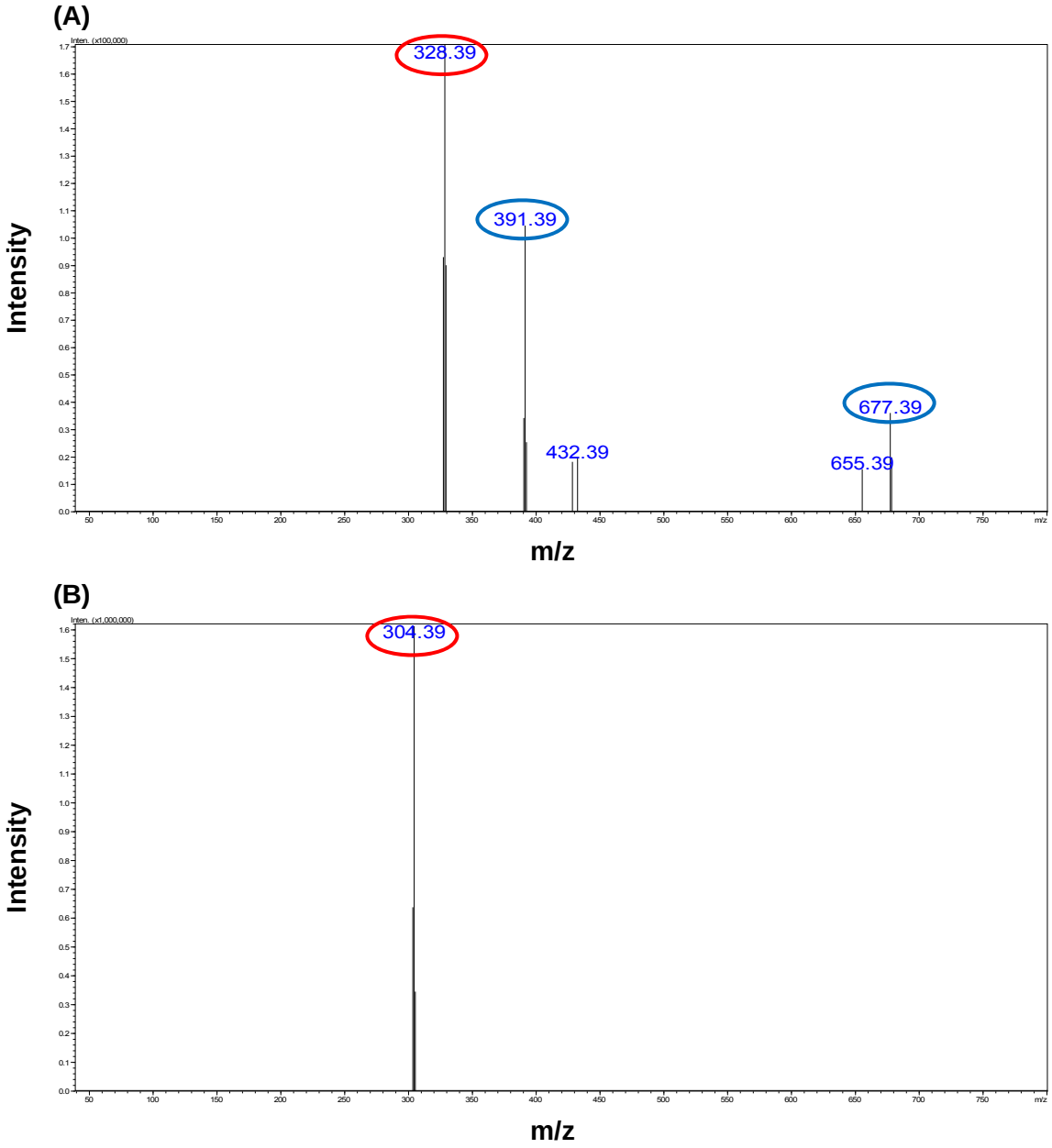


Figure 3.6: Mass spectra of (A) JWH-073 at 0.01 mg/mL and (B) cocaine at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their quantifier ions (red circled) and confirmation ions (blue circled)

This limitation is also observed by López de Alda and Barceló (2000) when using LC-MS with ESI to analyse steroid sex hormones and associated synthetic compounds in water and the authors have recommended using another approach for the confirmation assessment. In this publication, two analytical columns with different selectivity were used to run the samples. One column was used for identification and quantification, while the other column was used for further confirmation (López de Alda and Barceló, 2000). This approach was used in this research. A C_{18} column was used for identification and quantification and a biphenyl column was used for confirmation by comparison to standards. For the biphenyl column, the protonated molecular ion $[M+H]^+$ of each analyte was used as the confirmation ion based on the mass spectra of studied analytes (Appendix I) and their m/z values are included in Table 3.3. The quantifier ions of target analytes monitored in the LC-MS method using a C_{18} column are also shown in Table 3.3. The criteria and procedures for the identification and confirmation of the studied drugs of abuse and pharmaceuticals in drinking water samples are discussed in Section 5.1.1 and Section 5.1.2.

Table 3.3: Retention times (RT), retention indexes (RI) and diagnostic ions for 20 analytes and three internal standards obtained from a LC-MS analysis with a C_{18} column and biphenyl column

COMPOUND	C_{18} COLUMN			BIPHENYL COLUMN		
	RT / min	RI	Quantifier Ion / m/z	RT / min	RI	Confirmation Ion / m/z
BZP	2.14	0.29 [a]	177	4.98	0.62 [a]	177
MBZP	2.95	0.40 [a]	191	5.39	0.67 [a]	191
Methcathinone	5.42	0.74 [a]	164	7.52	0.93 [a]	164
Methylone	6.43	0.87 [a]	208	8.91	1.11 [a]	208
4-MeOPP	7.26	0.99 [a]	193	9.46	1.18 [a]	193
Amphetamine-d_6	7.35	–	142	8.05	–	142
Amphetamine	7.42	1.01 [a]	136	8.12	1.01 [a]	136
Methamphetamine	9.20	1.25 [a]	150	9.18	1.14 [a]	150
4-FPP	9.65	1.31 [a]	181	10.89	1.35 [a]	181
Butylone	10.63	1.45 [a]	222	11.60	1.44 [a]	222
Mephedrone	11.16	0.80 [b]	178	11.36	1.41 [a]	178
Ketamine	11.78	0.85 [b]	238	14.99	0.75 [b]	238
3-CPP	13.33	0.96 [b]	197	17.49	0.88 [b]	197
MDPV	13.78	0.99 [b]	276	20.25	1.01 [b]	276
Cocaine-d_3	13.91	–	307	19.96	–	307
Cocaine	13.91	1.00 [b]	304	19.99	1.00 [b]	304
3-TFMPP	14.66	1.05 [b]	231	19.14	0.96 [b]	231
4-TFMPP	15.00	1.08 [b]	231	20.12	1.01 [b]	231
Citalopram	16.31	0.90 [c]	325	26.55	0.93 [c]	325
Fluoxetine-d_6	18.11	–	316	28.67	–	316
Fluoxetine	18.15	1.00 [c]	310	28.74	1.00 [c]	310
JWH-073	24.01	1.33 [c]	328	33.00	1.15 [c]	328
JWH-398	25.57	1.41 [c]	376	34.76	1.21 [c]	376

[a] Amphetamine-d_6 to be used as internal standard; [b] Cocaine-d_3 to be used as internal standard; [c] Fluoxetine-d_6 to be used as internal standard

For the C_{18} column, 23 selected quantifier ions were monitored in SIM mode using the method stated in Section 2.3.2.3 in order to achieve the simultaneous identification of the studied drugs of abuse, pharmaceuticals and internal standards. Figure 3.7 shows the selected ion chromatogram of a mixed standard containing 20 analytes and three internal standards obtained by the SIM mode using a C_{18} column. The retention times (RTs) were obtained by comparing them to individual standards. The results are shown in Table 3.3, including the retention times of the drugs of abuse, pharmaceuticals and internal standards and the analytes identified to be used relative to the internal standards for later

identification, quantification and method validation. Table 3.3 also includes diagnostic ions and retention index (RI), which is the ratio of the retention time of analyte to the retention time of corresponding internal standard. The results of retention index were used in Chapter 5 for the identification and confirmation of the studied drugs of abuse and pharmaceuticals in collected raw and drinking waters, as the use of retention index could help to reduce the effects of variations that affect the retention time (Cody, 2003).

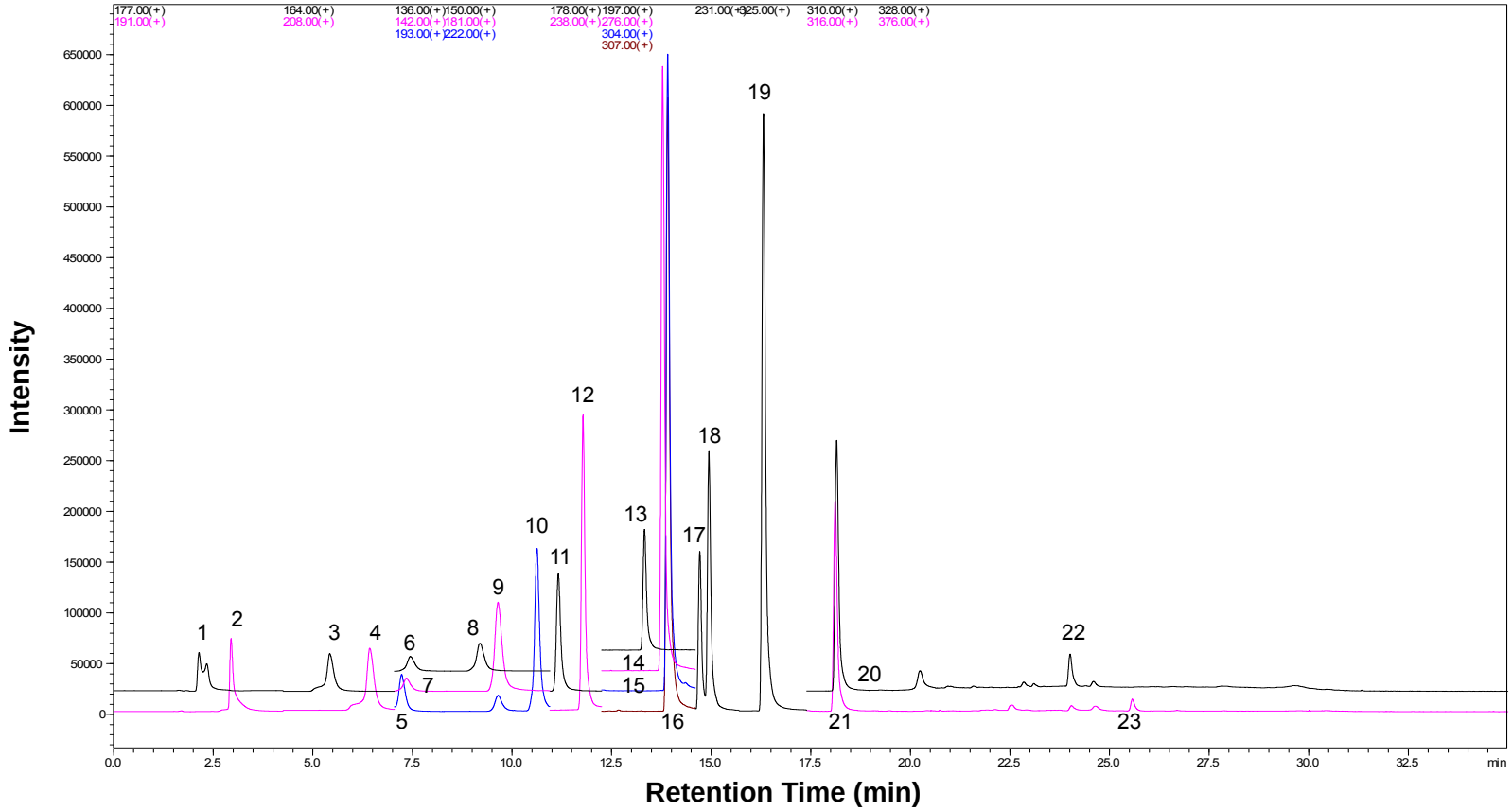


Figure 3.7: Selected ion chromatogram of a mixed standard containing 20 analytes and three internal standards at 100 ng/mL from a LC-MS analysis obtained with SIM mode and a C_{18} column

(1) m/z 177 BZP, (2) m/z 191 MBZP, (3) m/z 164 methcathinone, (4) m/z 208 methylone, (5) m/z 193 4-MeOPP, (6) m/z 136 amphetamine, (7) m/z 142 amphetamine-d_6, (8) m/z 150 methamphetamine, (9) m/z 181 4-FPP, (10) m/z 222 butylone, (11) m/z 178 mephedrone, (12) m/z 238 ketamine, (13) m/z 197 3-CPP, (14) m/z 276 MDPV, (15) m/z 304 cocaine, (16) m/z 307 cocaine-d_3, (17) m/z 231 3-TFMPP, (18) m/z 231 4-TFMPP, (19) m/z 325 citalopram, (20) m/z 310 fluoxetine, (21) m/z 316 fluoxetine-d_6, (22) m/z 328 JWH-073, (23) m/z 376 JWH-398

As shown in Figure 3.7 and Table 3.3, the majority of the studied drugs of abuse and pharmaceuticals, including 3-TFMPP and 4-TFMPP, can be separated based on their retention times using SIM mode. These isomers, 3-TFMPP and 4-TFMPP, shared the same quantifier ion (m/z 231) but occurred at different retention times (14.66 and 15.00 min) (Table 3.3). Hence, they can be separated and independently quantified, as shown in the selected ion chromatogram from SIM mode (Figure 3.7). These results prove that the proposed LC-MS method using the C_{18} column was selective enough to separate the isomers 3-TFMPP and 4-TFMPP compared to other studies using MS, as the detector that had found limitations with overlapping retention times (Elliott and Smith, 2008).

On the other hand, the use of different quantifier ions in SIM mode can also enable separation if co-eluting compounds do have similar retention times. According to Figure 3.7 and Table 3.3, there was co-elution of 4-MeOPP (7.26 min) and amphetamine (7.42 min), but they can be differentiated by their different quantifier ions, m/z 193 for 4-MeOPP and m/z 136 for amphetamine. The same concept was applied to the co-elution of MDPV (m/z 276) and cocaine (m/z 304) (Table 3.3). Although the internal standards (amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6) and their respective undeuterated analogues had similar retention times, the quantifier ions for these co-eluting compounds were different, as shown in Table 3.3. Thus, they can also be distinguished from each other.

Overall, by their retention times and quantifier ions, the SIM mode method using the C_{18} column can distinguish all studied drugs of abuse, pharmaceuticals and internal standards from each other, as evidenced by the selected ion chromatogram of a mixed standard (Figure 3.7). This separation also includes three groups of co-eluting compounds, which cannot be separated when using the scan mode, as their quantifier ions are different (Table 3.3). It is concluded that SIM mode was far more selective than scan mode (Harris, 2010) and hence enabled reliable identification of target drugs of abuse, pharmaceuticals and internal standards.

With using the biphenyl column, the selected ion chromatogram of a mixed standard containing 20 target analytes and three internal standards obtained by the SIM mode is shown in Figure 3.8 and their retention times are listed in Table 3.3. Based on their retention times and confirmation ions, all drugs of abuse, pharmaceuticals and internal standards can be distinguished from each other by using this SIM mode method. Thus, the LC-MS method using the biphenyl column, as described in Section 2.3.2.2 and Section 2.3.2.4, was suitable for the purpose of confirmation.

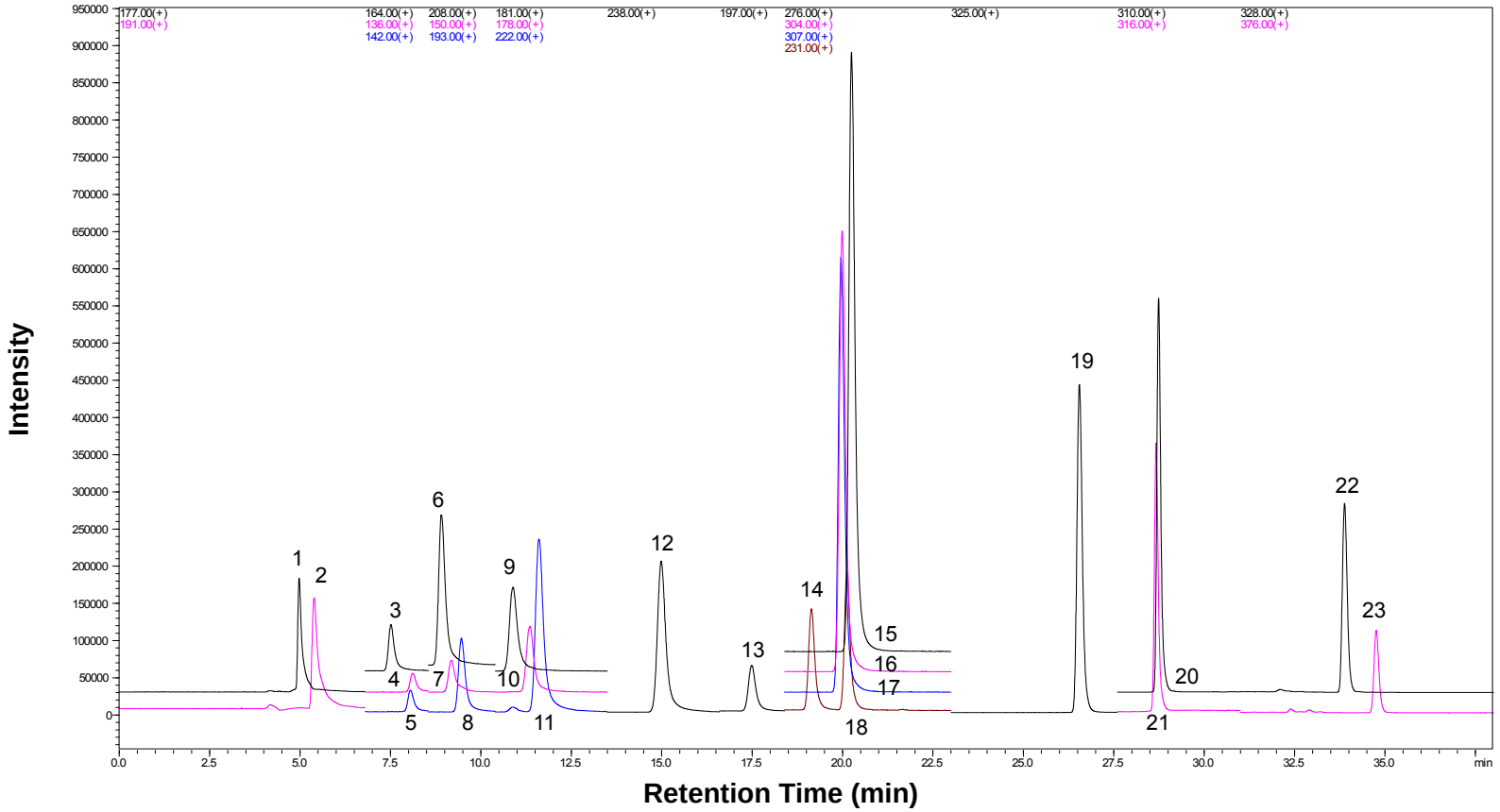


Figure 3.8: Selected ion chromatogram of a mixed standard containing 20 analytes and three internal standards at 100 ng/m L from a LC-MS analysis obtained with SIM mode and a biphenyl column

(1) m/z 177 BZP, (2) m/z 191 MBZP, (3) m/z 164 methcathinone, (4) m/z 136 amphetamine, (5) m/z 142 amphetamine-d_6, (6) m/z 208 methylone, (7) m/z 150 methamphetamine, (8) m/z 193 4-MeOPP, (9) m/z 181 4-FPP, (10) m/z 178 mephedrone, (11) m/z 222 butylone, (12) m/z 238 ketamine, (13) m/z 197 3-CPP, (14) m/z 231 3-TFMPP, (15) m/z 276 MDPV, (16) m/z 304 cocaine, (17) m/z 307 cocaine-d_3, (18) m/z 231 4-TFMPP, (19) m/z 325 citalopram, (20) m/z 310 fluoxetine, (21) m/z 316 fluoxetine-d_6, (22) m/z 328 JWH-073, (23) m/z 376 JWH-398

3.1.2.2 Time segmentation

The SIM analysis using time segmentation was carried out in order to increase the sensitivity, which is of importance for this research, as the method is required to detect and quantify trace levels of drugs of abuse and pharmaceuticals in drinking water. The application of time segmentation is to divide the entire analysis time into multiple predefined time segments and, during each time segment, the MS is programmed to only monitor a subset of the diagnostic ions of target analytes that elute in this given segment (Stone, et al., 2009). The benefit of time segmentation is that there are fewer ions to be analysed during that time segment compared to the SIM analysis, which monitors for all target analytes during the entire analysis time. It results in the increase of method sensitivity because the number of diagnostic ions decreases and more acquisition time can be spent on each ion (Agilent Technologies, 2011). In this research, various time segments were investigated and the final parameters are shown in Table 2.6 (C_{18} column) and Table 2.8 (biphenyl column). For 20 drugs of abuse and pharmaceuticals plus three internal standards under investigation, ten time segments containing one to four ions were used. Through the use of this ten time segments method (S10), the peak areas of 20 analytes of interest and three internal standards have all increased compared to the method without segmentation (S1) for these two analytical columns. Figure 3.9 shows the peak areas comparison of a mixed standard using the S1 and S10 methods for a C_{18} column as an example and all target analytes and internal standards gave at least 10 % increase in peak area.

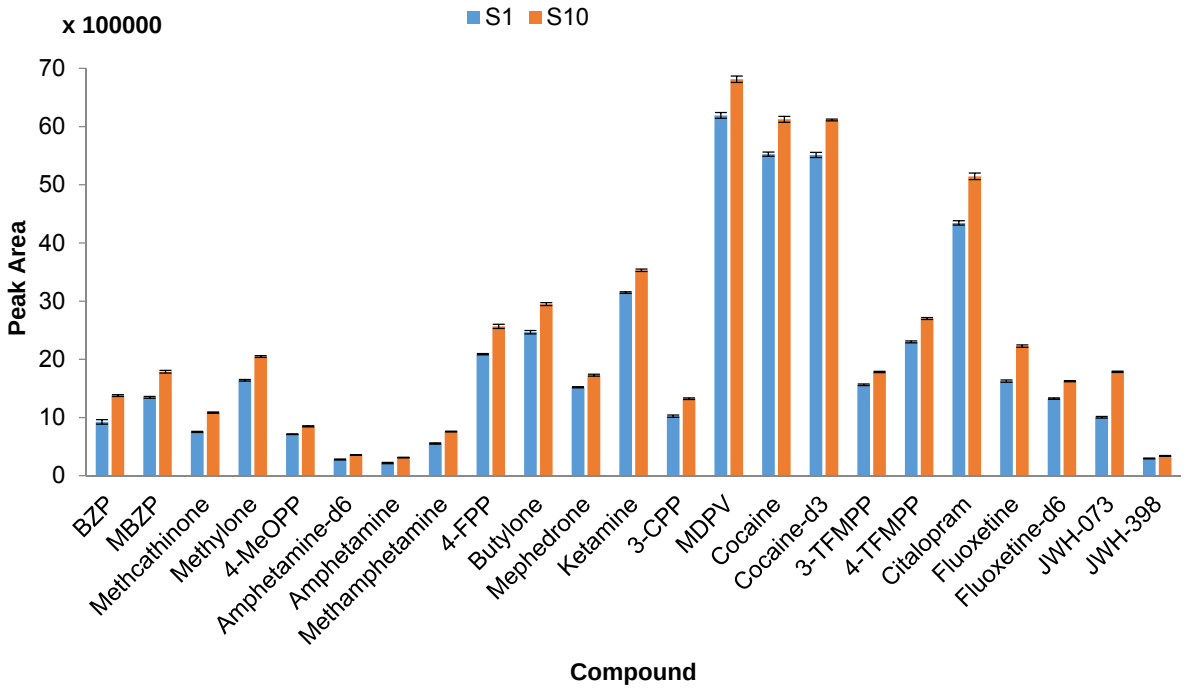


Figure 3.9: Comparison of peak areas of 20 analytes and three internal standards at 100 ng/mL obtained from a LC-MS analysis and a C_{18} column using SIM mode without segmentation (S1) and SIM mode with ten time segments (S10), n=3

3.1.2.3 Optimisation of DL, qarray DC and RF voltages

The voltages of DL and lens system (qarray DC and RF) on the MS (which are described in Section 1.9.2.2) were then optimised. As mentioned in Section 1.9.2.2, these voltages are used to introduce the selected ions to the mass analyser for detection; thus, they also have a significant impact on the peak intensity. The DL, qarray DC and RF voltages were adjusted to give the best peak intensity and hence selected as optimal. These voltages were the same for both the C_{18} column and biphenyl column and are shown in Table 2.6 and Table 2.8, respectively. In order to study the effect of optimised DL, qarray DC and RF voltages, a mixed standard with internal standards were analysed using (1) the optimised values and (2) the default values (0 kV for DL, qarray DC and RF voltages). The results of the comparison of peak areas for 20 target analytes and three internal standards using the optimised values and the default values for a C_{18} column are shown in Figure 3.10. The peak areas of all analysed compounds have improved by at least 25 % after the optimisation of DL, qarray DC and RF voltages.

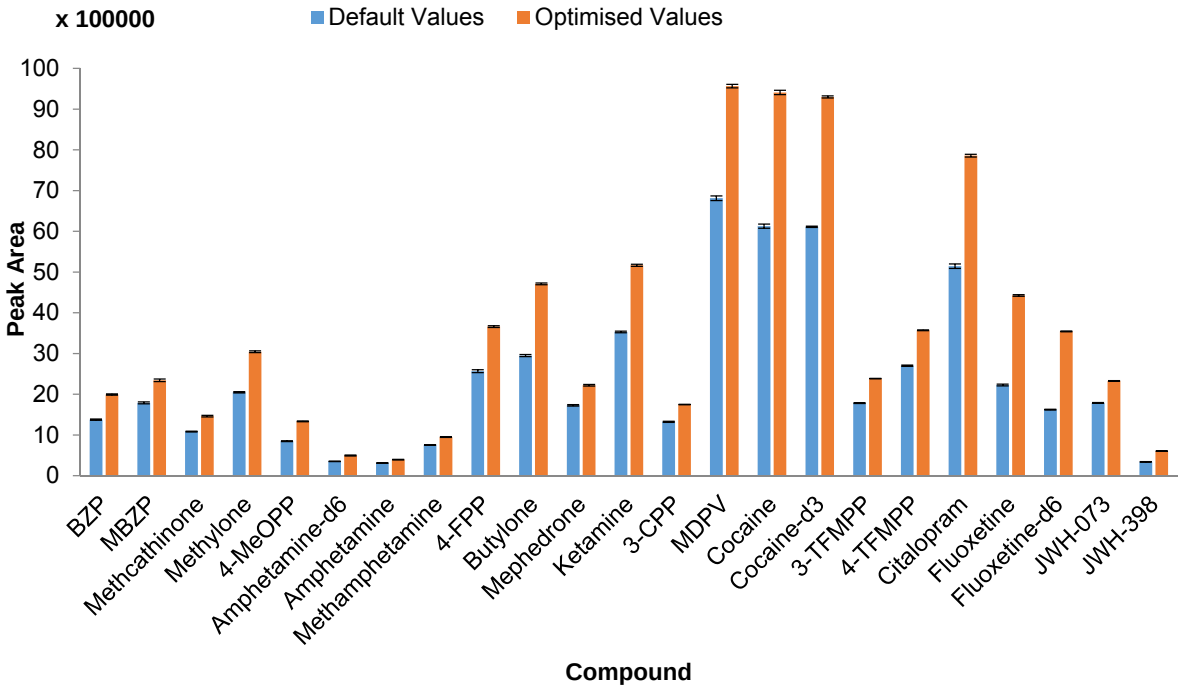


Figure 3.10: Comparison of peak areas of 20 analytes and three internal standards at 0.01 mg/mL obtained from a LC-MS analysis and a C_{18} column using default values and optimised values of DL, qarray DC and RF voltages, n=3

In summary, the developed and optimised LC-MS methods using the C_{18} column and biphenyl column, as detailed in Section 2.3.2, were selected due to their good selectivity, reliable compound identification and high sensitivity. These two methods can separate 20 drugs of abuse and pharmaceuticals plus three internal standards based on their retention times and diagnostic ions. Thus, the LC-MS methods using the C_{18} column and biphenyl column were used for method validation studies, as discussed in Chapter 4, and were applied to drinking water samples, as discussed in Chapter 5.

3.2 Comparison, development and optimisation of SPE method

As discussed in Section 1.9.1.2.1, the separation mechanism is based on the electrostatic interaction between the functional groups of the analyte and the functional groups on the SPE sorbent. Depending on the nature of the target analytes, it is necessary to screen various types of SPE cartridges in order to select the most suitable sorbent, which can provide maximum analyte retention and highest recovery (Section 3.2.1). The SPE extraction process also relies on the solvents selected and volumes used, which were also optimised (Section 3.2.2). Finally, a large loading volume of sample was examined to ascertain whether this increased the chances of detecting and quantifying the studied drugs of abuse and pharmaceuticals in water samples (Section 3.2.3).

Two sets of samples were prepared and analysed during the course of SPE development and optimisation. These comprised water samples that were spiked with target analytes and internal standards and then extracted via SPE (set 1) and water samples (from the same source) that were extracted via SPE but spiked after extraction (set 2). Raw water was used to prepare the set 1 samples for all SPE experiments, as the SPE method developed in this research was required to extract both raw water and drinking water. There are more matrix components present in raw water that need to be removed during extraction compared to drinking water. Moreover, the raw water collected from the same source was also used for the set 2 samples to ensure that target analytes and matrix components that were already present in the set 1 and 2 samples were the same, resulting in an accurate comparison. The ratio of analyte peak area to internal standard peak area (PAR) in the set 1 and 2 samples were used to calculate the recovery, which assess the effectiveness of the SPE method and also enable comparisons between the different SPE cartridges and different extraction methods (Peters, Drummer and Musshoff, 2007). Recovery was calculated using Equation 3.1, as the percentage of the PAR of an analyte in set 1 sample in relation to those in set 2 sample (Chambers, et al., 2007).

$$\% \text{ Recovery} = (\text{PAR}_{\text{sample spiked before extraction}}/\text{PAR}_{\text{sample spiked after extraction}}) \times 100$$

(Equation 3.1)

Where, PAR of the sample spiked before extraction is the mean PAR of the analyte in the set 1 sample and PAR of the sample spiked after extraction is the mean PAR for the same quantity of analyte in set 2 sample.

In addition, the precision of the SPE method was also assessed and 15 % RSD is accepted, indicating good replicate measurements (Peters, Drummer and Musshoff, 2007; Tarcomnicu, et al., 2011).

3.2.1 Comparison of SPE cartridges, Oasis MCX (3 mL) and Strata-X-Drug B (3 mL)

The first step of SPE development was to choose the most appropriate sorbent and SPE cartridge. As mentioned in Section 1.9.1.1, mixed-mode cation-exchange has been the most widely used sorbent in water analysis, as this sorbent can be used to extract a wide range of different analytes, including basic compounds as well as neutral compounds, from aqueous samples due to both functional groups being present (acidic and non-polar groups). Thus, mixed-mode cation-exchange sorbent was chosen to extract drugs of abuse and pharmaceuticals from raw water and drinking water in this research. Two different mixed-mode cation-exchange cartridges from different manufacturers were selected for the evaluation of the recoveries of target analytes from water: the Oasis MCX from Waters and the Strata-X-Drug B from Phenomenex. The extractions using Oasis MCX and Strata-X-Drug B were conducted according to their generic protocols (Table 2.9), adapted from Waters (2003) and Phenomenex (2011), and other parameters including sample volume, analyte concentration and instrumental method were the same (Section 2.3.3 and Section 2.3.3.1).

Recovery results of 20 studied drugs of abuse and pharmaceuticals obtained by using Oasis MCX and Strata-X-Drug B cartridges were calculated using Equation 3.1 and are listed in Table 3.4 for comparison. Moreover, the precision results (RSD) were obtained

using three replicates. Results highlighted in green indicate the higher recovery obtained for that particular analyte, while the results highlighted in red show that the RSD is below 15 %, which means good repeatability.

Table 3.4: Comparison of recoveries and relative standard deviations (RSD) of 20 analytes obtained from a SPE analysis with 3 mL Oasis MCX and 3 mL Strata-X-Drug B cartridges, n=3

COMPOUND / 10 ng	3 mL Oasis MCX Cartridge		3 mL Strata-X-Drug B Cartridge	
	Recovery / %	RSD / %	Recovery / %	RSD / %
BZP	137	26.2	63	6.2
MBZP	161	20.9	70	4.5
Methcathinone	52	26.1	102	14.3
Methylone	108	4.7	87	9.1
4-MeOPP	171	39.4	84	3.9
Amphetamine	102	1.2	99	1.1
Methamphetamine	78	7.6	87	13.7
4-FPP	150	13.4	88	2.7
Butylone	118	16.0	87	9.8
Mephedrone	72	17.1	84	10.5
Ketamine	95	17.6	85	7.3
3-CPP	110	36.7	84	7.6
MDPV	93	29.7	100	1.4
Cocaine	101	0.2	97	0.3
3-TFMPP	100	35.1	106	8.1
4-TFMPP	102	31.3	97	8.0
Citalopram	79	13.5	96	0.8
Fluoxetine	87	2.1	98	1.4
JWH-073	55	38.6	66	14.4
JWH-398	33	94.6	43	13.3

For the Oasis MCX cartridge, as shown in Table 3.4, the majority of selected analytes (17 out of 20) had moderate to high recoveries between 72 - 171 %. Low recoveries were only observed for JWH-398 (33 %), methcathinone (52 %) and JWH-073 (55 %). This indicates that the Oasis MCX generic protocol (Table 2.9) was able to retain many target analytes and then elute most of them from the SPE cartridge. However, for methcathinone, JWH-073 and JWH-398, this generic protocol was not ideal for their extractions, possibly due to inappropriate pH and a weaker elution solvent applied (Section 1.9.1.2.1 and

Section 1.9.1.2.6). Therefore, analytes were lost during the sample loading and/or the analyte elution step. As a result, the PARs of sample spiked before extraction (set 1) were significantly lower than those of sample spiked after extraction (set 2), which resulted in low recoveries (33 - 55 %). Although the moderate to high recoveries were achieved for nearly all target analytes, the RSD results for most analytes were over 15 % and only seven analytes had acceptable RSD (< 15 %) (Peters, Drummer and Musshoff, 2007), which are highlighted in red as shown in Table 3.4. This phenomenon could be because the applied generic protocol was not able to remove the matrix components and thus caused matrix effects. As a result, the number of analyte ions escaping into the gas phase and being detected varied from run to run, resulting in poor repeatability for most analytes (as stated in Section 1.9.2.2.1). Thus, Oasis MCX might be not suitable for extraction in this research, as poor repeatability can lead to erroneous results (*ibid*).

According to Table 3.4, the Strata-X-Drug B cartridge also gave moderate to high recovery values for all analytes of interest, ranging from 63 - 106 %, except for low recovery for JWH-398 (43 %). Moreover, the RSD results of the studied analytes for Strata-X-Drug B cartridge were all below 15 % criteria (highlighted in red), indicating good repeatability for all studied drugs of abuse and pharmaceuticals when using this type of SPE cartridge.

When comparing results from Table 3.4, higher recovery results (highlighted in green) were obtained for 11 target analytes when using Oasis MCX cartridge and nine analytes of interest show better recoveries when using Strata-X-Drug B cartridge, but only Strata-X-Drug B can provide acceptable RSDs (< 15 %) for all studied drugs of abuse and pharmaceuticals. It is worth noting that the SPE method in this research was developed to simultaneously extract 20 drugs of abuse and pharmaceuticals from different classes (Table 1.3). Therefore, it is difficult to achieve high recoveries for all target analytes as non-ideal pH and elution solvent might be applied to some analytes, as discussed above. However, low RSDs should be required when selecting the most suitable SPE cartridge in order to ensure repeatable and precise recovery is obtained (Food and Drug Administration, 2001; Tarcomnicu, et al., 2011; Mwenesongole, 2015). In this regard,

Strata-X-Drug B was chosen in this research for the extraction of the studied drugs of abuse and pharmaceuticals in raw and drinking waters due to its good repeatability.

3.2.2 Optimisation of elution solvent for Strata-X-Drug B (3 mL)

After the selection of the Strata-X-Drug B cartridge, it was necessary to further develop and optimise the method in order to increase the recovery. As the Strata-X-Drug B cartridge contains a mixed-mode cation-exchange sorbent and has two different functional groups (non-polar and acidic groups) on its sorbent surface (Section 1.9.1.1), two elution solvents, as shown in Table 2.9, are used in tandem for this cartridge. Methanol is first applied to disrupt the interactions between the neutral analytes and non-polar groups. Among 20 studied drugs of abuse and pharmaceuticals in this research, only two synthetic cannabinoids (JWH-073 and JWH-398) were eluted during this step, as they are more hydrophobic and mainly interact with non-polar surface groups. Then, ethyl acetate/isopropanol/ammonium hydroxide (70:20:10, v/v) is used as the second elution solvent in order to break the interactions between basic analytes and acidic groups. The remaining analytes of interest are basic compounds, including amphetamines, cocaine, ketamine, cathinones, piperazines and antidepressants (Table 1.3), and thus were eluted by this basified elution solvent.

Based on the results in Table 3.4, good and repeatable recoveries were obtained for the majority of the studied drugs of abuse and pharmaceuticals when using the generic protocol of Strata-X-Drug B. Only JWH-398 that eluted in the first elution step showed low recovery (43 %) and thus the first elution solvent needed to be optimised. Methanol and acetonitrile are the most commonly used organic solvents for the elution of neutral analytes in the literature (Baker and Kasprzyk-Hordern, 2011a; Peng, Hall and Gautam, 2016). Ethyl acetate/isopropanol (85:15, v/v) is the recommended elution solvent for the extraction of marijuana metabolites such as THC, according to the technical document for the Strata-X-Drug B cartridge (Phenomenex, 2011). Thus, these three elution solvents, namely methanol (MeOH), acetonitrile (ACN) and ethyl acetate/isopropanol (EtOAC/IPA), were used following the procedures described in Section 2.3.3.2. The recovery results obtained

by using different first elution solvents were calculated based on Equation 3.1 and their precisions were obtained using three replicates (Table 3.5). Green recoveries indicate the highest value obtained for that particular analyte, while RSDs highlighted in red are below 15 %, which mean good repeatability.

Table 3.5: Comparison of recoveries and relative standard deviations (RSD) of 20 analytes obtained from a SPE analysis with different first elution solvents (MeOH, ACN and EtOAC/IPA) for 3 mL Strata-X-Drug B cartridges, n=3

COMPOUND / 10 ng	MeOH		ACN		EtOAC/IPA	
	Recovery / %	RSD / %	Recovery / %	RSD / %	Recovery / %	RSD / %
BZP	63	6.2	70	11.0	103	10.1
MBZP	70	4.5	70	8.9	98	8.5
Methcathinone	102	14.3	105	8.0	107	11.1
Methylone	87	9.1	86	1.6	102	5.8
4-MeOPP	84	3.9	111	14.6	93	14.9
Amphetamine	99	1.1	98	0.9	97	1.2
Methamphetamine	87	13.7	79	3.2	83	4.4
4-FPP	88	2.7	88	5.0	95	6.1
Butylone	87	9.8	86	1.4	101	5.2
Mephedrone	84	10.5	91	5.8	83	7.2
Ketamine	85	7.3	83	7.1	72	10.3
3-CPP	84	7.6	92	0.8	84	1.8
MDPV	100	1.4	83	1.3	97	1.0
Cocaine	97	0.3	92	0.4	98	0.6
3-TFMPP	106	8.1	104	0.8	81	0.8
4-TFMPP	97	8.0	99	0.8	80	1.8
Citalopram	96	0.8	108	2.3	94	3.9
Fluoxetine	98	1.4	102	0.3	98	0.9
JWH-073	66	14.4	55	12.1	78	14.3
JWH-398	43	13.3	59	13.6	87	14.8

In Table 3.5, the comparison of the results from methods 1, 2 and 3 indicated that, for JWH-398, elution with ethyl acetate/isopropanol (85:15, v/v) in method 3 resulted in high recovery (87 %) with good repeatability (RSD = 14.8 %) and is better than elution with the other two organic solvents used in methods 2 and 3, namely methanol (43 %) and acetonitrile (59 %). For JWH-073, another analyte that eluted in the first elution step, using ethyl acetate/isopropanol (85:15, v/v) provided good repeatability (RSD = 14.3 %) and also

increased its recovery to 78 %. In addition, the remaining 18 analytes that eluted in the second elution step were not affected by changing the organic solvent for the first elution step and hence high recoveries (72 - 107 %) as well as low RSDs (< 15 %) were still obtained. Therefore, ethyl acetate/isopropanol (85:15, v/v) was the most suitable elution solvent for the first elution step owing to better recovery and good repeatability and was used for the final SPE method (Table 2.10).

3.2.3 Optimisation of sample loading volume for Strata-X-Drug B (6 mL)

As mentioned in Section 1.9.1.3, the larger the sample loading volume applied, the more the target analytes can be retained by SPE sorbent, thereby leading to increased chances of detection and quantification. It is of importance for this research as the studied drugs of abuse and pharmaceuticals are likely to be present in drinking water at sub ng/L levels. Thus, sample loading volume needs to be optimised in order to improve the method sensitivity. Following the procedures as detailed in Section 2.3.3.3, a higher volume of raw water (200 mL) and a larger capacity of Strata-X-Drug B cartridge (6 mL) were incorporated into the optimised SPE method (Table 2.10). Table 3.6 shows the results, including recovery, which were calculated based on Equation 3.1, and RSD, which was obtained by three replicates.

Table 3.6: Recoveries and relative standard deviations (RSD) for 20 analytes obtained from a SPE analysis with 200 mL sample loading volume for 6 mL Strata-X-Drug B cartridges, n=3

COMPOUND	6 mL Strata-X-Drug B Cartridge	
/ 20 ng	Recovery / %	RSD / %
BZP	72	11.2
MBZP	65	14.5
Methcathinone	30	13.3
Methylone	70	12.0
4-MeOPP	39	14.9
Amphetamine	97	0.9
Methamphetamine	97	7.4
4-FPP	81	2.8
Butylone	67	12.9
Mephedrone	47	14.6
Ketamine	90	13.7
3-CPP	79	8.6
MDPV	96	7.1
Cocaine	100	0.3
3-TFMPP	86	7.9
4-TFMPP	65	14.1
Citalopram	98	13.8
Fluoxetine	103	2.6
JWH-073	107	14.5
JWH-398	99	14.9

In Table 3.6, most of the studied drugs of abuse and pharmaceuticals (17 out of 20) had moderate and high recoveries falling between 65 and 107 % and showed good precisions (RSD < 15 %) when loading a large volume of raw water (200 mL). Only three target analytes, namely methcathinone, 4-MeOPP and mephedrone, exhibited low recoveries (30, 39 and 47 %). This is possibly due to the loss of analytes during the sample loading step, as higher recoveries (107, 93 and 83 %) have previously been observed when the sample loading volume was 2 mL (column F, Table 3.5).

As this research aims to develop a simultaneous method and therefore is impossible to suit all studied drugs of abuse and pharmaceuticals from different classes, the acceptance criterion of recovery is to make sure that the value is repeatable and precise, regardless of the percent value obtained (Section 3.2.1). In this regard, low recoveries for methcathinone, 4-MeOPP and mephedrone were acceptable as their RSD values were still below 15 %,

indicating good repeatability. Therefore, 200 mL was selected as the sample loading volume and was used for the final SPE method (Table 2.10).

As shown in Table 3.7, the recovery results of seven drugs of abuse as well as two antidepressants from this research are compared with previously published research. These include amphetamine, methamphetamine, cocaine, ketamine, methcathinone, BZP, 3-TFMPP, citalopram and fluoxetine. These eight references are chosen for comparison, as surface and drinking waters were used, which are similar to the sample matrix in this research (raw water from surface water source). Results regarding SPE recovery for other studied drugs of abuse have not been found in the literature.

Table 3.7: Comparison of SPE recovery results in this research with published literature

COMPOUND	RECOVERY / %								
	This Research	Published Literature							
		1 [a]	2 [b]	3 [c]	4 [d]	5 [e]	6 [f]	7 [g]	8 [h]
	RW	DW	SW	SW	DW	SW	DW	RW	DW
Amphetamine	97	–	121	101	–	99	92	–	92
Methamphetamine	97	–	–	108	–	92	75	–	98
Cocaine	100	–	99	105	–	102	91	–	86
Ketamine	90	–	–	–	–	100	93	–	–
Methcathinone	30	–	–	–	–	71	–	–	–
BZP	72	–	–	–	–	99	–	–	–
3-TFMPP	86	–	–	–	–	101	–	–	–
Citalopram	98	–	–	–		–	–	97	–
Fluoxetine	103	102	–	–	102	101	–	–	–

RW, raw water from surface water source; DW, drinking water; SW, surface water

[a] Vanderford and Snyder, 2006; [b] Kasprzyk-Hordern, Dinsdale and Guwy, 2007; [c] Zuccato, et al., 2008; [d] López-Serna, et al., 2010; [e] Baker and Kasprzyk-Hordern, 2011b; [f] Boleda, et al., 2011; [g] Gros, Rodríguez-Mozaz and Barceló, 2012;

[h] Valcárcel, et al., 2012

The comparison above shows that the recovery results of six target analytes in this research, including amphetamine, methamphetamine, cocaine, ketamine, citalopram and fluoxetine, are very close to those values from published literature (Table 3.7). However, the recoveries of methcathinone (30 %), BZP (72 %) and 3-TFMPP (86 %) are lower than

the recoveries of 71, 99 and 101 %, respectively, as reported by Baker and Kasprzyk-Hordern (2011b). This could be attributed to the different SPE cartridges and extraction processes used in these two methods, namely Strata-X-Drug B in this research and Oasis MCX in the previously published research, although both of them are mixed-mode cation-exchange sorbent. The differences have also been observed for some target analytes in Table 3.4 when determining the suitable SPE cartridge during this research.

Overall, recoveries displayed in Table 3.6 show that this optimised SPE method (Table 2.10) was suitable for the extraction of the studied drugs of abuse and pharmaceuticals in drinking water in this research and the obtained results were used for the calculation of MDLs and MQLs in Section 4.5.2.

3.3 Overall discussion and conclusion of method development

The method applied in this research was based on using SPE followed by LC-MS. Two LC-MS analytical columns were used for the analysis of drugs of abuse and pharmaceuticals in drinking water, namely a C_{18} column for identification and quantification and a biphenyl column for confirmation. The first step was to develop the LC-MS method in order to simultaneously detect 20 target analytes and three internal standards. LC conditions were initially investigated by means of HPLC-DAD to achieve good chromatographic separation for the studied analytes, especially for the two isomers, 3-TFMPP and 4-TFMPP. As a result, acetonitrile was chosen as the organic modifier of mobile phases for the C_{18} column and a mixture of methanol and acetonitrile (6:4, v/v) was selected for the biphenyl column (Section 3.1.1).

The final gradient elution profiles for these two columns, as shown in Table 2.2 and Table 2.4, were then transferred onto LC-MS and their peak separations were further investigated (Section 3.1.2.1). Scan mode was used to obtain the mass spectra of 20 studied analytes and three internal standards (Appendix I) for selecting the diagnostic ions to be monitored in SIM mode. Based on their mass spectra, three predominant ions cannot

be found for all analytes and thus two columns were used to test the water samples, namely the C_{18} column for quantification and the biphenyl column for confirmation. The protonated molecular ion $[M+H]^+$ of each analyte, which is the most abundant ion, was selected as the diagnostic ion (Table 3.8) and monitored in SIM mode in order to improve the method selectivity. As shown in Table 3.8, the developed LC-MS method using the C_{18} column and biphenyl column can distinguish all studied drugs of abuse, pharmaceuticals and internal standards from each other by their retention times and diagnostic ions and the following chapter discusses the validation of selectivity (Section 4.1). In addition, the MS analysis time was divided into multiple time segments and DL, qarray DC and RF voltages were optimised for each analyte of interest and internal standard in order to increase the method sensitivity (Section 3.1.2.2). The MS segmentation programmes are detailed in Table 2.6 (C_{18} column) and Table 2.8 (biphenyl column) and these voltages are displayed in Table 3.8. The sensitivity of these two developed LC-MS methods is further investigated and discussed in the following chapter (Section 4.5).

Finally, a sample preparation method based on SPE was developed and optimised. Strata-X-Drug B cartridges from Phenomenex were selected as good and repeatable recoveries were obtained for all target analytes (Section 3.2.1). The generic protocol of this cartridge was further optimised in order to increase the recovery. As a result, ethyl acetate/isopropanol (85:15, v/v) was chosen as the first elution solvent and 200 mL was used as the sample loading volume (Section 3.2.2 and Section 3.2.3). The recovery results obtained are shown in Table 3.8 by using the final SPE method, as described in Table 2.10.

Table 3.8: Summary of method development and optimisation results (C_{18} column and biphenyl column)

COMPOUND	METHOD DEVELOPMENT AND OPTIMISATION RESULTS								
	LC-MS Method								SPE Method
	Diagnostic ion / m/z	Retention Time / min		Retention Index		DL Voltage / kV	Qarray DC Voltage / kV	Qarray RF Voltage / kV	Recovery / %
	C_{18} and Biphenyl	C_{18}	Biphenyl	C_{18}	Biphenyl	C_{18} and Biphenyl	C_{18} and Biphenyl	C_{18} and Biphenyl	Raw Water
BZP	177	2.14	4.98	0.29 [a]	0.62 [a]	9.6	6.4	35.2	72
MBZP	191	2.95	5.39	0.40 [a]	0.67 [a]	12.8	9.6	38.4	65
Methcathinone	164	5.42	7.52	0.74 [a]	0.93 [a]	12.8	9.6	32.0	30
Methylone	208	6.43	8.91	0.87 [a]	1.11 [a]	16.0	6.4	35.2	70
4-MeOPP	193	7.26	9.46	0.99 [a]	1.18 [a]	6.4	6.4	32.0	39
Amphetamine-d_6	142	7.35	8.05	–	–	6.4	9.6	32.0	–
Amphetamine	136	7.42	8.12	1.01 [a]	1.01 [a]	25.6	16.0	28.8	97
Methamphetamine	150	9.20	9.18	1.25 [a]	1.14 [a]	38.4	9.6	32.0	97
4-FPP	181	9.65	10.89	1.31 [a]	1.35 [a]	6.4	6.4	38.4	81
Butylone	222	10.63	11.60	1.45 [a]	1.44 [a]	16.0	6.4	41.6	67
Mephedrone	178	11.16	11.36	0.80 [b]	1.41 [a]	16.0	9.6	32.0	47
Ketamine	238	11.78	14.99	0.85 [b]	0.75 [b]	12.8	6.4	35.2	90
3-CPP	197	13.33	17.49	0.96 [b]	0.88 [b]	9.6	6.4	32.0	79
MDPV	276	13.78	20.25	0.99 [b]	1.01 [b]	96.0	6.4	41.6	96
Cocaine-d_3	307	13.91	19.96	–	–	12.8	0.0	51.2	–
Cocaine	304	13.91	19.99	1.00 [b]	1.00 [b]	32.0	0.0	48.0	100
3-TFMPP	231	14.66	19.14	1.05 [b]	0.96 [b]	32.0	16.0	38.4	86
4-TFMPP	231	15.00	20.12	1.08 [b]	1.01 [b]	32.0	16.0	38.4	65
Citalopram	325	16.31	26.55	0.90 [c]	0.93 [c]	32.0	0.0	44.8	98
Fluoxetine-d_6	316	18.11	28.67	–	–	22.4	16.0	48.0	–
Fluoxetine	310	18.15	28.74	1.00 [c]	1.00 [c]	16.0	16.0	41.6	103
JWH-073	328	24.01	33.00	1.33 [c]	1.15 [c]	32.0	19.2	48.0	107
JWH-398	376	25.57	34.76	1.41 [c]	1.21 [c]	0.0	19.2	57.6	99

[a] Amphetamine-d_6 to be used as internal standard; [b] Cocaine-d_3 to be used as internal standard; [c] Fluoxetine-d_6 to be used as internal standard

CHAPTER 4 RESULTS AND DISCUSSION: VALIDATION OF THE OPTIMISED METHOD

This chapter includes the validation results of the instrument and method using the final optimised SPE and LC-MS methods, subsequent to their development and optimization, as discussed in Chapter 3. These results prove that the instrument and method were selective, sensitive and capable for identification, quantification and confirmation. This chapter interprets and discusses the results of several validation experiments, including selectivity, autosampler storage stability, instrumental linearity, precision, accuracy, detection and quantification limits for the instrument and method. All parameters were validated for the LC-MS method using a C_{18} column (Section 2.3.2.3) as this method was used for quantification. In addition, selectivity and the instrumental detection limit were studied using a biphenyl column (Section 2.3.2.4), as this method was only used for the purpose of confirmation.

4.1 Selectivity

As the methods used in this research were to simultaneously analyse 20 drugs of abuse and pharmaceuticals, as well as three internal standards, it is important to demonstrate the ability of the method for identification with selectivity. For the C_{18} column and biphenyl column, all studied drugs of abuse, pharmaceuticals and internal standards can be distinguished from each other either by their retention times or by diagnostic ions using the SIM mode, as shown and discussed in Section 3.1.2.1. Therefore, these two SIM mode methods were selective enough to enable the reliable identification of the target analytes, as evidenced by the selected ion chromatograms of a mixed standard with internal standards obtained by the use of the C_{18} column (Figure 3.7) and biphenyl column (Figure 3.8). In addition, ultra-pure water was used as a matrix blank and analysed using a C_{18} column and biphenyl column (Section 2.3.2.3 and Section 2.3.2.4) in order to check the interferences from the matrix. Figure 4.1 A and Figure 4.1 B show the selected ion chromatograms of a matrix blank obtained in SIM mode using the C_{18} column and biphenyl column, respectively.

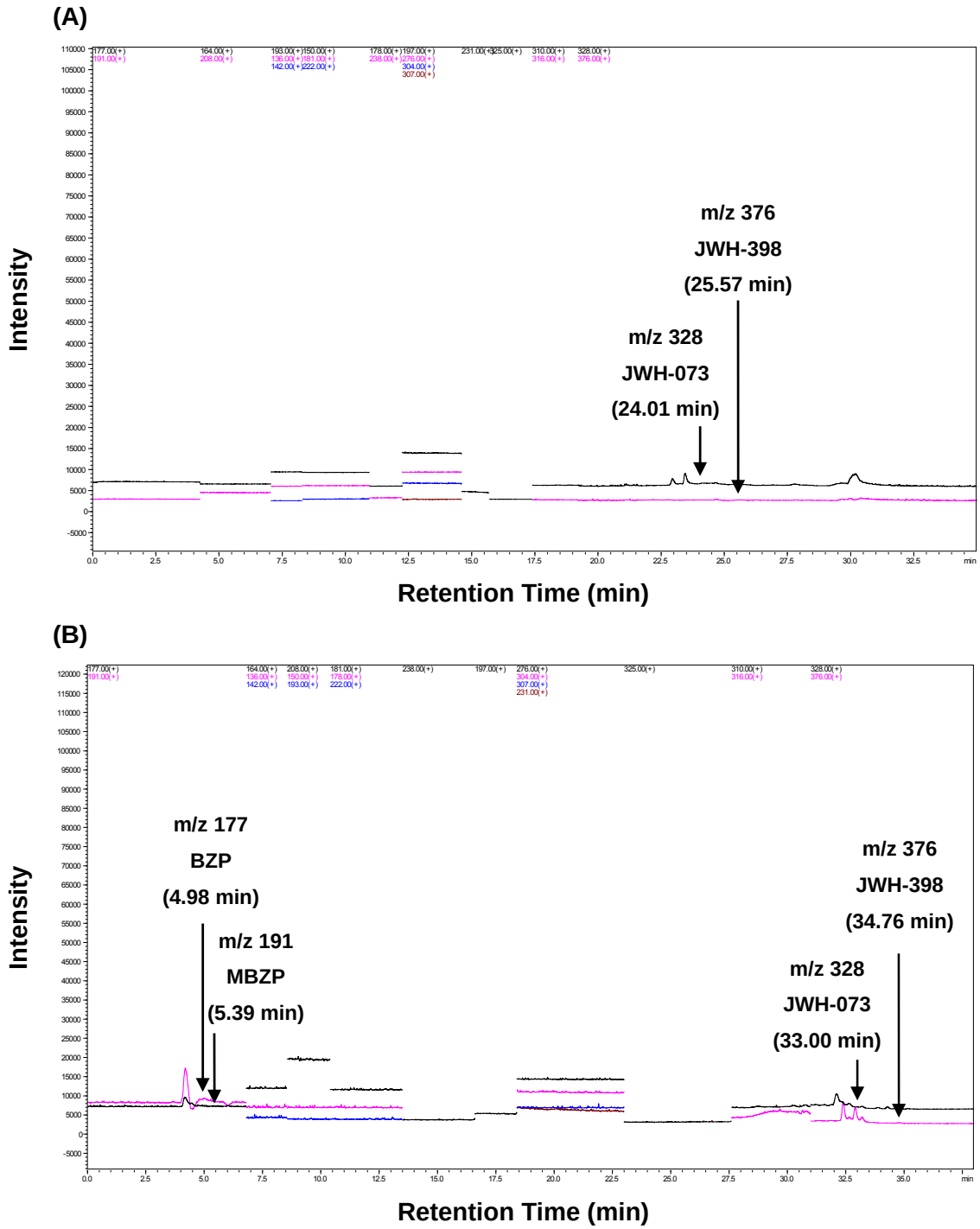


Figure 4.1: Selected ion chromatograms of a matrix blank from a SPE-LC-MS analysis obtained with SIM mode and (A) a C_{18} column and (B) a biphenyl column

In Figure 4.1 A, there were some peaks with the m/z 328 ion and m/z 376 ion present, which were the quantifier ions for JWH-073 and JWH-398. The signals may be from some components in the sample matrix. However, by comparing the selected ion chromatogram of a matrix blank (Figure 4.1 A) to the selected ion chromatogram of a mixed standard

containing the internal standards (Figure 3.7), these peaks were not present at the retention times of JWH-073 (24.01 min) and JWH-398 (25.57 min), which are labelled in Figure 4.1 A using the C_{18} column, so do not interfere. Moreover, there were no interference peaks observed in the matrix blank for other target analytes and internal standards. This proves that the peaks of all target analytes and internal standards were only as a result of the compound and not the matrix blank. Therefore, the LC-MS method in SIM mode using the C_{18} column was selective and can be used during method validation studies and water sample analysis in order to provide reliable identification.

For the biphenyl column, by comparing the selected ion chromatogram of a matrix blank (Figure 4.1 B) to the selected ion chromatogram of a mixed standard containing the internal standards (Figure 3.8), some ions were detected for BZP, MBZP, JWH-073 and JWH-398 in the matrix blank. However, they were not present at the retention times of these analytes, which are labelled in Figure 4.1 B. Hence, there were no interferences observed in the matrix blank, indicating that this LC-MS method in SIM mode using the biphenyl column was selective enough for the identification of 20 studied drugs of abuse and pharmaceuticals as well as three internal standards.

4.2 Autosampler storage stability

Firstly, three internal standards were added to mixed standards (10 ng/mL) at the concentrations of 5 ng/mL (amphetamine-d_6), 0.1 ng/mL (cocaine-d_3) and 0.75 ng/mL (fluoxetine-d_6). Their autosampler storage stability in the LC-MS injection solvent (0.5 % formic acid/4.975 % acetonitrile/94.525 % water) was assessed over five days at 10 °C, which was enough to cover the typical working time for validation experiments and water sample analysis (Section 4.2.1). The autosampler storage stability study was then conducted on two mixed standards in LC-MS injection solvent, one at low concentration (10 ng/mL) and the other at high concentration (500 ng/mL), in order to determine the stability of the studied drugs of abuse and pharmaceuticals (Section 4.2.2). The LC-MS method using a C_{18} column (Section 2.3.2.3) was used for the autosampler storage stability study.

4.2.1 Stability and instrumental response of internal standards

In this research, PAR was used for data analysis, such as the analysis of autosampler storage stability, the establishment of the linear range, the assessment of precision and accuracy, the calculation of IDL and IQL as well as the quantification of target analytes in water samples. Hence, the investigation of the stability of amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 is of importance before evaluating other validation parameters.

The concentrations of the three internal standards that are added to the mixed standards need to be selected. The concentration of internal standard should yield a similar response to that of the analyte of interest. If the response of the internal standard is too high, it will result in low PAR, leading to increased error when using it for the purpose of quantification. Moreover, the peak of internal standard should always be visible on the chromatogram and should be accurate and reproducible. Thus, the concentrations of internal standards were selected based on their respective IQLs, ensuring the peak areas won't be too large and can be measured with suitable precision, typically within 20 % RSD (Peters, Drummer and Musshoff, 2007; UNODC, 2009). The IQL was estimated based on the S/N, which is typically required to be equal to or greater than 10 (Huber, 2007). The measurement of signal was the maximum height of analyte peak above the baseline and the noise was measured based on the amplitude between the highest and lowest point of baseline (Wells, Prest and Russ, 2011). Based on the results in Table 4.1, the concentration of amphetamine-d_6 was chosen at 5 ng/mL. This is because, at this concentration, its S/N was 17.23 and acceptable precision (RSD = 7.46 %) was attained. Cocaine-d_3 and fluoxetine-d_6 were selected at the concentrations of 0.1 ng/mL and 0.75 ng/mL, respectively, based on the criteria shown in Table 4.1.

Table 4.1: Signal-to-noise ratios (S/N) and relative standard deviations (RSD) for three internal standards at their respective concentrations obtained from a LC-MS analysis with a C_{18} column, n=3

INTERNAL STANDARD	CONCENTRATION / ng/mL	S/N	RSD / %
Amphetamine-d_6	5.00	17.23	7.46
Cocaine-d_3	0.10	15.02	7.64
Fluoxetine-d_6	0.75	17.58	4.13

The stability of internal standards in LC-MS injection solvent was then evaluated by plotting the peak area of each internal standard at each concentration against the injection time (every three hours). Bar graphs of three internal standards can be found in Appendix II-a, while an example of amphetamine-d_6 is provided in Figure 4.2.

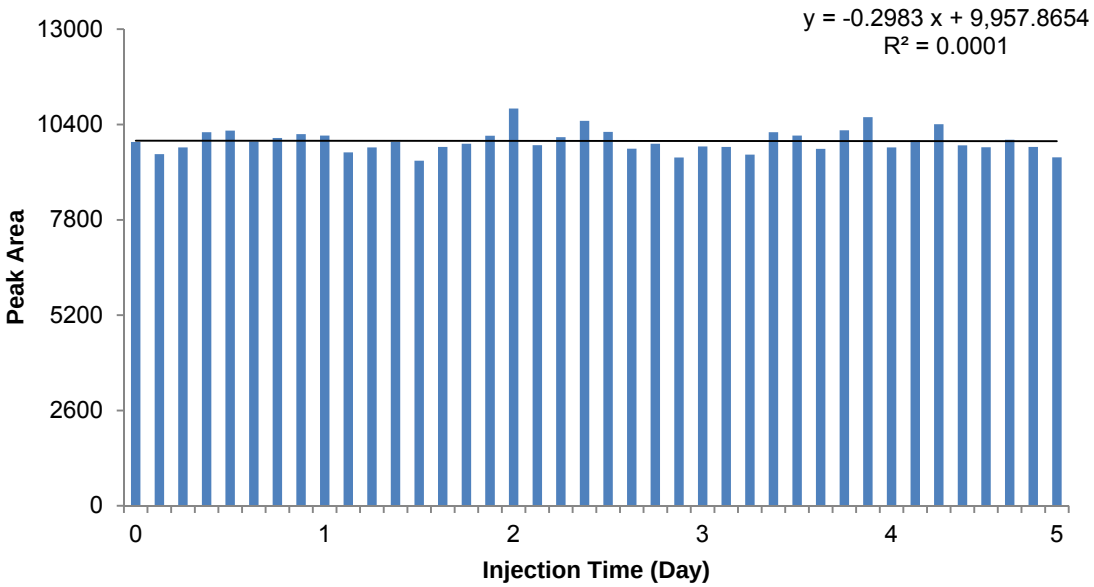


Figure 4.2: Bar graph of peak area against injection time for amphetamine-d_6 at 5 ng/mL for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

The instability is indicated by a slope, which is significantly different from zero ($p \leq 0.05$) (Saar, et al., 2010). Slope and *p*-value were calculated using linear regression analysis with Microsoft Excel 2007. The *p*-value was used for testing the null hypothesis that the slope of the linear regression line is equal to zero. If *p*-value is lower or equal than 0.05, it indicates that the null hypothesis is rejected and the slope is significantly different from zero, which shows the instability of the tested internal standard. If the *p*-value is above 0.05,

it indicates that the null hypothesis is retained and the slope is not significantly different from zero, which shows the difference is random and the tested internal standard is stable (*ibid*). Results regarding the *p*-values of three internal standards for autosampler storage stability are shown in Table 4.2.

Table 4.2: Results of autosampler storage stability for three internal standards obtained from a LC-MS analysis with a C_{18} column

COMPOUND	AUTOSAMPLER STORAGE STABILITY		
	Concentration / ng/mL	*p*-value [a]	Is the Null Hypothesis Retained?
Amphetamine-d_6	5.00	0.94	Yes
Cocaine-d_3	0.10	0.71	Yes
Fluoxetine-d_6	0.75	0.87	Yes

[a] *p*-value > 0.05 and therefore the null hypothesis was retained, indicating that the internal standard was stable

The results in Table 4.2 show that amphetamine-d_6 was stable in LC-MS injection solvent at the concentration of 5 ng/mL over the five-day period at 10 °C as its *p*-value was 0.94 ($p > 0.05$), while the same was applied to cocaine-d_3 and fluoxetine-d_6. They were stable in LC-MS injection solvent at the concentrations of 0.1 and 0.75 ng/mL, respectively, over the five-day period at 10 °C.

4.2.2 Stability of studied drugs of abuse and pharmaceuticals

The stability of the studied drugs of abuse and pharmaceuticals in LC-MS injection solvent was also evaluated by linear regression analysis using a plot of the PAR of each analyte at each concentration against injection time (every three hours). Bar graphs of the studied drugs of abuse and pharmaceuticals at two concentrations (10 and 500 ng/mL) can be found in Appendix II-b. An example of citalopram is provided in Figure 4.3.

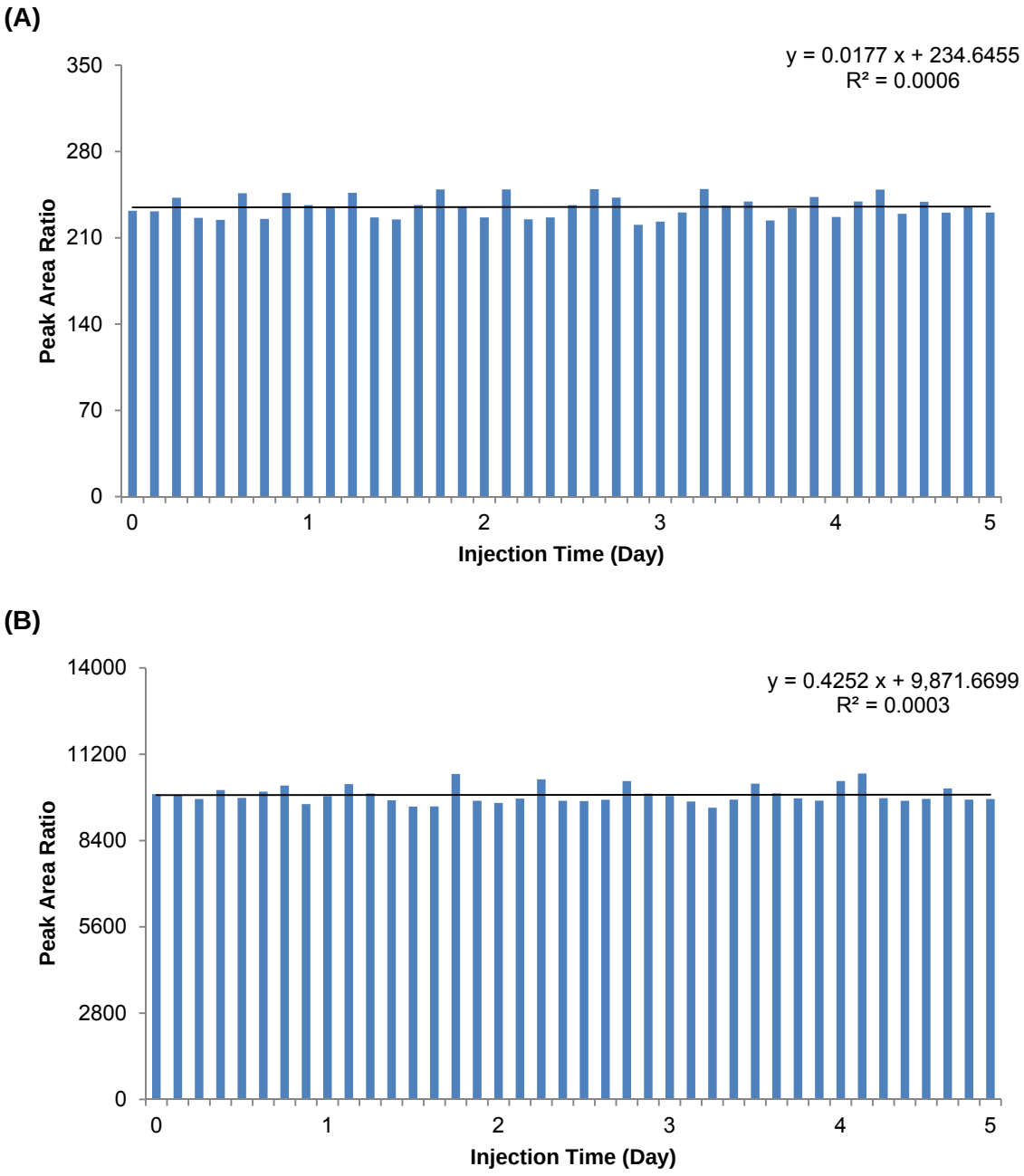


Figure 4.3: Bar graphs of peak area ratio against injection time for citalopram at (A) 10 ng/mL and (B) 500 ng/mL for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

PAR was calculated as the ratio of analyte peak area to internal standard peak area. As shown in Section 4.2.1, amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 have already been proved to be stable at their added concentrations (5 ng/mL, 0.1 ng/mL and 0.75 ng/mL, respectively). Thus, if the acceptance criterion of stability is fulfilled, it indicates that the studied drugs of abuse and pharmaceuticals were stable in LC-MS injection solvent during

five days at 10 °C. The stability criterion is as stated in Section 4.2.1 with the *p*-value greater than 0.05. The results of the autosampler storage stability for the studied drugs of abuse and pharmaceuticals are shown in Table 4.3, including *p*-value, which was calculated as detailed in Section 4.2.1.

Table 4.3: Results of autosampler storage stability for 20 analytes obtained from a LC-MS analysis with a C_{18} column

COMPOUND	AUTOSAMPLER STORAGE STABILITY			
	Low Concentration / 10 ng/mL		High Concentration / 500 ng/mL	
	p-value [a]	Is the Null Hypothesis Retained?	*p*-value [a]	Is the Null Hypothesis Retained?
BZP	0.16	Yes	0.48	Yes
MBZP	0.86	Yes	0.84	Yes
Methcathinone	0.48	Yes	0.69	Yes
Methylone	0.41	Yes	0.73	Yes
4-MeOPP	0.74	Yes	0.25	Yes
Amphetamine	0.71	Yes	0.45	Yes
Methamphetamine	0.71	Yes	0.45	Yes
4-FPP	0.68	Yes	0.24	Yes
Butylone	0.50	Yes	0.78	Yes
Mephedrone	0.18	Yes	0.51	Yes
Ketamine	0.71	Yes	0.77	Yes
3-CPP	0.85	Yes	0.83	Yes
MDPV	0.91	Yes	0.71	Yes
Cocaine	0.21	Yes	0.96	Yes
3-TFMPP	0.89	Yes	0.91	Yes
4-TFMPP	0.95	Yes	0.73	Yes
Citalopram	0.89	Yes	0.91	Yes
Fluoxetine	0.72	Yes	0.85	Yes
JWH-073	0.88	Yes	0.25	Yes
JWH-398	0.76	Yes	0.61	Yes

[a] *p*-value > 0.05 and therefore the null hypothesis was retained, indicating that the studied analyte was stable

Based on the results in Table 4.3, the *p*-values for all 20 studied drugs of abuse and pharmaceuticals were greater than 0.05. Thus, the slopes were not significantly different from zero, indicating stability over autosampler storage at both low and high concentrations.

The stability study has confirmed that the investigated drugs of abuse, pharmaceuticals and internal standards were stable in LC-MS injection solvent during LC-MS analysis on the autosampler for the duration of five days. Therefore, mixed standards and water samples dissolved in LC-MS injection solvent can be stable and stored for up to five days at 10 °C. This shows that long sequences for five days can be prepared. In practice, mixed standards in LC-MS injection solvent were made every five working days and kept in a fridge or on the autosampler at 10 °C in order to prevent any significant degradation.

4.3 Instrumental linearity

The LC-MS method using a C_{18} column (Section 2.3.2.3) was tested for linearity in this research. A series of data points were obtained by analysing 19 mixed standards between 0.001 to 10000 ng/mL (0.001, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 100, 500, 1000, 5000 and 10000 ng/mL). To assess linearity, a linear regression plot (Section 4.3.1) was first used to determine the initial linear range of the LC-MS instrument, especially for the elimination of higher concentration points. Then, the initial linear range was further tested by the plot of relative response against log concentration (Section 4.3.2) in order to remove lower concentration points and obtain the final instrumental linear range. This is because the deviations from linearity at the low concentrations are too small to detect and can often go unnoticed in linear regression plot (Singh, 2013).

4.3.1 Linear regression plot for initial linearity assessment

The linear regression plots for the studied drugs of abuse and pharmaceuticals were obtained by plotting the mean PARs of standards against corresponding standard concentrations. An example of a linear regression plot can be seen in Figure 4.4 for ketamine over the 0.001 to 10000 ng/mL concentration range.

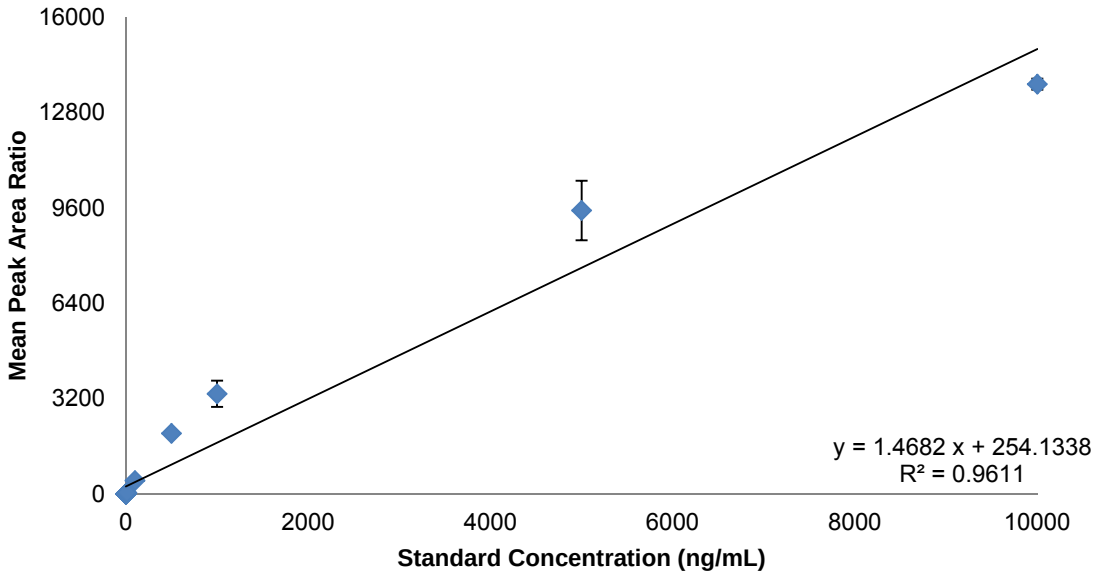


Figure 4.4: Linear regression plot of mean peak area ratio against standard concentration for ketamine over 0.001 to 10000 ng/mL for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

It is apparent by visual examination of this linear regression plot for ketamine that the mean PARs at the highest three concentrations (1000, 5000 and 10000 ng/mL) were beginning to plateau and were not directly proportional to standard concentration. This indicates that these three concentrations were beyond the instrumental linear range and the response of ketamine was non-linear over the whole concentration range, being 0.001 to 10000 ng/mL.

Besides visual examination, linearity can also be evaluated by the coefficient of determination (R^2), which was obtained from the linear regression plot. The R^2 value can be considered as an indicator to measure the degree of linear association between x and y variables (standard concentration and mean PAR). Thus, the closer the R^2 value is to 1, the closer the correlation. An R^2 value of 0.9900 or better is deemed as an acceptable measure of linearity (UNODC, 2009). In order to determine the initial instrumental linear ranges for the studied drugs of abuse and pharmaceuticals, the mean PAR results that showed plateau responses at the higher concentrations were removed until the R^2 value was equal to or greater than 0.9900. For example, in the linear regression plot for ketamine (Figure 4.4), the R^2 was calculated at 0.9611, which was outside the accepted 0.9900. After the removal of the highest three concentrations, the accepted R^2 (0.9996) was

obtained (Figure 4.5). Thus, the initial instrumental linear range of ketamine was 0.001 to 500 ng/mL.

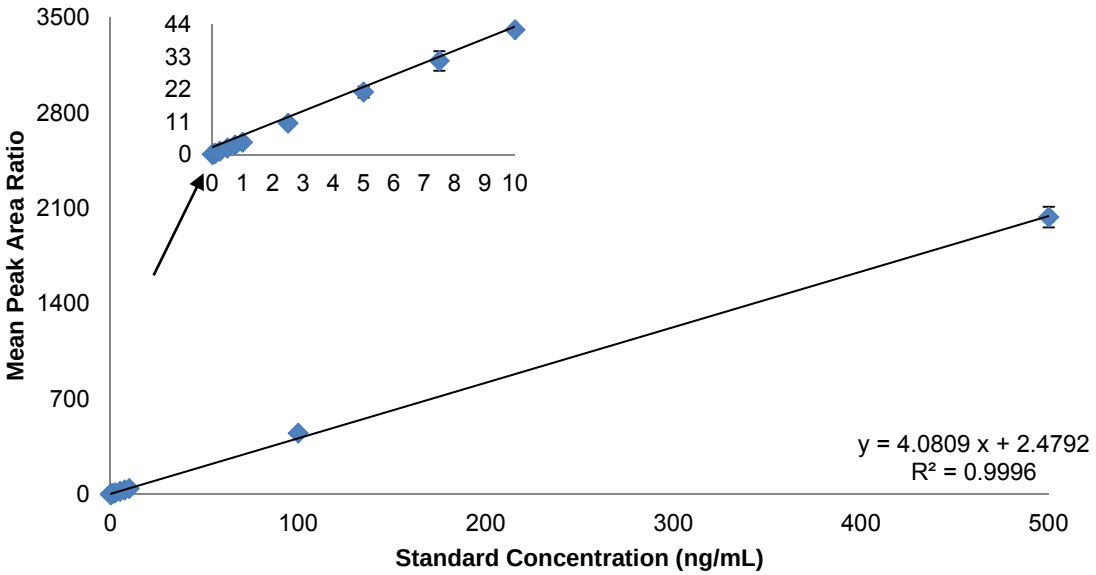


Figure 4.5: Linear regression plot of mean peak area ratio against standard concentration for ketamine over 0.001 to 500 ng/mL for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

The linear regression plots for drugs of abuse and pharmaceuticals investigated in this research are displayed in Appendix III and their coefficient of determination values are listed in Table 4.4 (column B). As the R^2 values were all greater than 0.9900, this indicates that good linearity was obtained for all assessed drugs of abuse and pharmaceuticals over the concentration range shown in Table 4.4 (column C), which is known as the initial instrumental linear range in this research. The linearity was examined further in the following Section 4.3.2 using the plot of relative response against log concentration.

4.3.2 Plot of relative response against log concentration for further examination of linearity

The instrumental linear ranges obtained from the linear regression plots were then further tested by the plots of relative response against log concentration. Relative responses, which were calculated by dividing the responses of data point (mean PAR) by their corresponding standard concentrations, were plotted in the y-axis against corresponding standard concentrations on a log scale in the x-axis due to the wide linear range. This helps to normalise the response to the concentration of the analyte (Huber, 2007). Moreover, this plot could also be used to check the behaviour of variance. If the method is linear, the relative responses should be statistically the same, which would indicate that the relative responses do not change with concentrations, because the ordinary least squares model is based on the assumption that the variance is constant (Hartmann, et al., 1998). If an ideal linearity is achieved, the obtained line should be horizontal and show the data points within ± 5 % of the mean relative response (Huber, 2007).

The plot of relative response against log concentration for ketamine is shown in Figure 4.6, where Rc is the line of mean relative response while 0.95 Rc and 1.05 Rc indicate 95 % and 105 % of the horizontal line, respectively (Hartmann, et al., 1998; Huber, 2007). This shows the plot for ketamine where data points beyond the line of 1.05 Rc were identified at the lowest three concentrations, 0.001, 0.01 and 0.025 ng/mL. Thus, these three data points were deleted from the linear range and the final instrumental linear range was changed to 0.05 to 500 ng/mL.

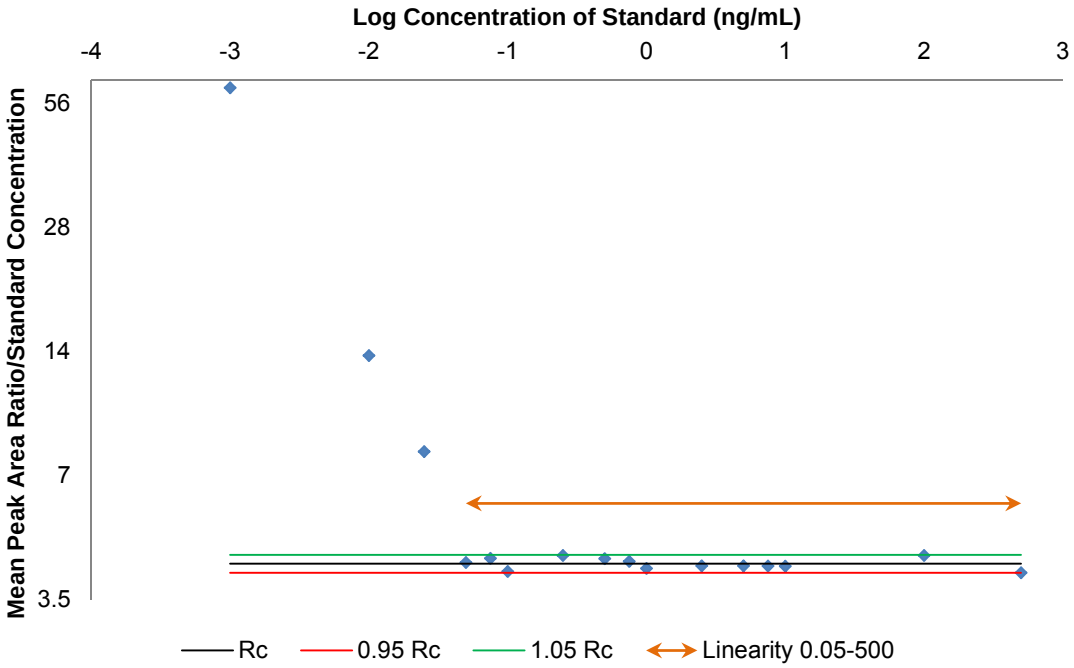


Figure 4.6: Relative response/log concentration plot of the ratio of mean peak area ratio to standard concentration against log concentration of standard for ketamine over 0.001 to 500 ng/mL for instrumental linearity obtained from a LC-MS analysis with a C_{18} column

Other plots of relative response against log concentration are displayed in Appendix IV. The same approach for removing concentration points (column D, Table 4.4) that were beyond the 95 % and 105 % limits was used.

Table 4.4: Results of instrumental linearity for 20 analytes obtained from a LC-MS analysis with a C_{18} column

COMPOUND	INSTRUMENTAL LINEARITY			
	Linear Regression Plot		Relative Response/ Log Concentration Plot	
	Coefficient of Determination / R^2	Initial Linear Range / ng/mL	Deleted Concentration Points / ng/mL	Final Linear Range / ng/mL
BZP	0.9998	0.001 - 1000	7 Points (0.001 - 0.25)	0.5 - 1000
MBZP	0.9997	0.001 - 1000	5 Points (0.001 - 0.075)	0.1 - 1000
Methcathinone	0.9995	0.001 - 1000	6 Points (0.001 - 0.1)	0.25 - 1000
Methylone	0.9997	0.001 - 1000	7 Points (0.001 - 0.25)	0.5 - 1000
4-MeOPP	0.9992	0.001 - 1000	11 Points (0.001 - 2.5)	5 - 1000
Amphetamine	0.9993	0.001 - 1000	10 Points (0.001 - 1)	2.5 - 1000
Methamphetamine	0.9994	0.001 - 1000	8 Points (0.001 - 0.5)	0.75 - 1000
4-FPP	0.9996	0.001 - 1000	6 Points (0.001 - 0.1)	0.25 - 1000
Butylone	0.9997	0.001 - 500	3 Points (0.001 - 0.025)	0.05 - 500
Mephedrone	0.9998	0.001 - 1000	3 Points (0.001 - 0.025)	0.05 - 1000
Ketamine	0.9996	0.001 - 500	3 Points (0.001 - 0.025)	0.05 - 500
3-CPP	0.9997	0.001 - 1000	6 Points (0.001 - 0.1)	0.25 - 1000
MDPV	0.9994	0.001 - 1000	5 Points (0.001 - 0.075)	0.1 - 1000
Cocaine	0.9999	0.001 - 500	3 Points (0.001 - 0.025)	0.05 - 500
3-TFMPP	0.9997	0.001 - 1000	3 Points (0.001 - 0.025)	0.05 - 1000
4-TFMPP	0.9998	0.001 - 1000	3 Points (0.001 - 0.025)	0.05 - 1000
Citalopram	0.9996	0.001 - 500	2 Points (0.001 - 0.01)	0.025 - 500
Fluoxetine	0.9998	0.001 - 1000	7 Points (0.001 - 0.25)	0.5 - 1000
JWH-073	0.9998	0.001 - 1000	11 Points (0.001 - 2.5)	5 - 1000
JWH-398	0.9997	0.001 - 1000	11 Points (0.001 - 2.5)	5 - 1000

4.3.3 Instrumental linear ranges

The final instrumental linear ranges for the studied drugs of abuse and pharmaceuticals are shown in Table 4.4 (column E). It is apparent that the instrumental linear ranges are different for various analytes. For example, the instrumental linear range for ketamine was 0.05 to 500 ng/mL, whereas the instrumental linear range for BZP was 0.5 to 1000 ng/mL. This is probably because, whilst the concentration is the same, not all analytes yield the same instrumental response. According to the selected ion chromatogram of a mixed standard at the concentration of 100 ng/mL (Figure 3.7), the instrumental intensity (peak height) of ketamine (286,371) was significantly higher than BZP (37,457). The highest concentration in the instrumental linear range of ketamine was 500 ng/mL and, beyond this concentration, the peak area was not directly proportional to the concentration due to a too strong signal causing detector saturation. Figure 4.7 A shows the selected ion chromatogram of ketamine at the concentration of 1000 ng/mL, resulting in a strong signal and causing flatting at the top of the peak. For BZP, its instrumental response was lower than ketamine and hence good peak shape was observed at the concentration of 1000 ng/mL without detector saturation, as this concentration was within its instrumental linear range (Figure 4.7 B). This explains the different highest concentrations of instrumental linear range that were obtained for different analytes, such as 500 ng/mL for ketamine and 1000 ng/mL for BZP.

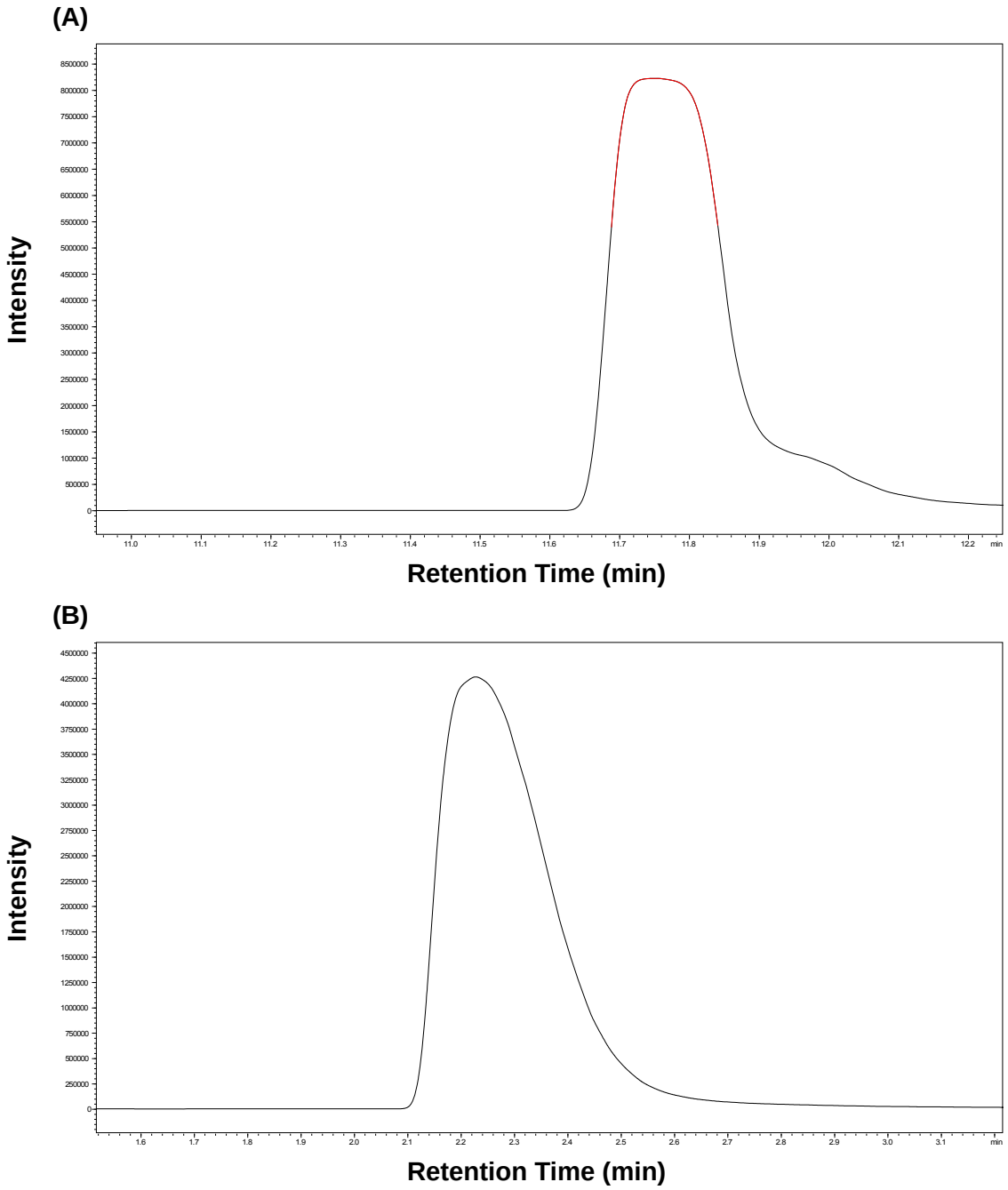


Figure 4.7: Selected ion chromatograms of (A) m/z 238 ketamine at 1000 ng/mL and (B) m/z 177 BZP at 1000 ng/mL from a LC-MS analysis obtained with SIM mode and a C_{18} column

In addition, the instrumental responses of an analyte can be too weak at lower concentrations and therefore be unusable for the purpose of quantification. This is because the concentration at the lower end can be beyond the IQL for this analyte, which is the lowest concentration that can be quantitatively determined by the instrument (Section 1.10.5.1). Since the instrumental response of ketamine was higher than BZP (Figure 3.7), the lowest concentration end of ketamine (0.05 ng/mL) was significantly lower than BZP (0.5 ng/mL), as shown in Table 4.4 (column E). Figure 4.8 shows the selected ion chromatograms of ketamine and BZP at the same concentration of 0.05 ng/mL. Good instrumental response (clear peak) was observed for ketamine (Figure 4.8 A), as this concentration (0.05 ng/mL) is higher than the IQL of ketamine (0.0412 ng/mL, Table 4.12), whilst no peak of BZP was detected (Figure 4.8 B) as 0.05 ng/mL is significantly lower than the IQL of BZP (0.4087 ng/mL, Table 4.12).

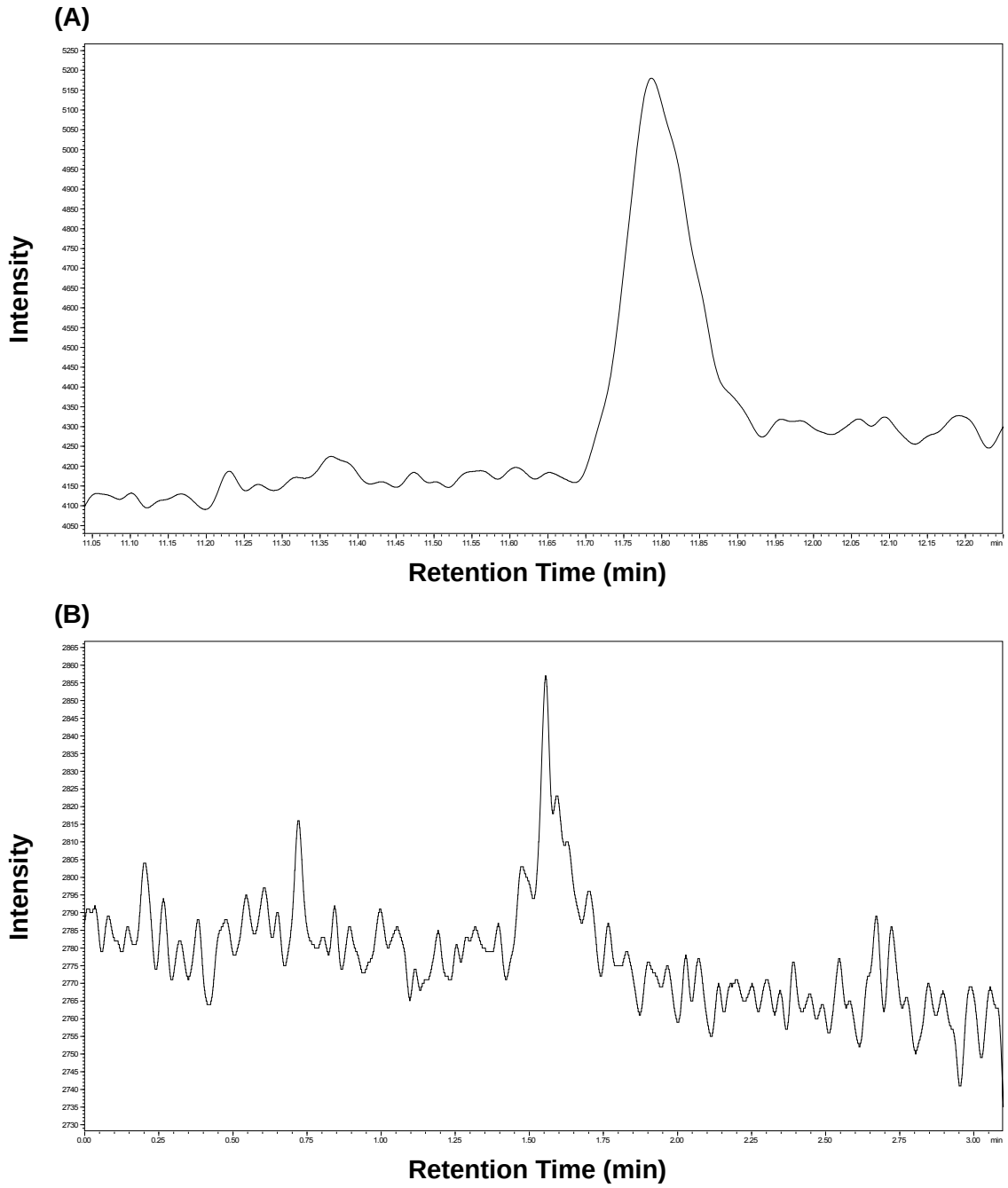


Figure 4.8: Selected ion chromatograms of (A) m/z 238 ketamine at 0.05 ng/mL and (B) m/z 177 BZP at 0.05 ng/mL from a LC-MS analysis obtained with SIM mode and a C_{18} column

Instrumental linear ranges for the studied drugs of abuse and pharmaceuticals were investigated in this research between 0.001 and 10000 ng/mL, over seven orders of magnitude. Good linearity was obtained for all target analytes, over four to five orders of magnitude (column E, Table 4.4). This is consistent with a published review (Holčapek, Jirásko and Lísa, 2012). This indicates that the LC-MS could provide a good linear

dynamic range, which is common of five to six orders of magnitude. In addition, instrumental linear ranges of 16 target analytes included in this research are compared with those from published literature (Table 4.5), which were based on using LC-MS/MS (Baker and Kasprzyk-Hordern, 2011b; Reid, Derry and Thomas, 2014; Baz-Lomba, Reid and Thomas, 2016; González-Mariño, et al., 2016a; González-Mariño, et al., 2016b; Petrie, et al., 2016; Gao, et al., 2017). As described in Section 1.6, previously published work is limited with respect to such analytes in drinking water, while the instrumental linear ranges for MBZP, 4-MeOPP, 4-FPP and 4-TFMPP have not been reported yet.

Table 4.5: Comparison of instrumental linear ranges in this research with published literature

COMPOUND	INSTRUMENTAL LINEAR RANGE / ng/mL							
	This Research	Published Literature						
		1 [a]	2 [b]	3 [c]	4 [d]	5 [e]	6 [f]	7 [g]
	LC-MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
BZP	0.5 - 1000	0.5 - 1000	–	–	–	–	–	0.5 - 200
Methcathinone	0.25 - 1000	0.075 - 1000	–	1 - 400	–	–	–	–
Methylone	0.5 - 1000	–	–	0.25 - 400	–	–	–	–
Amphetamine	2.5 - 1000	0.1 - 1000	–	5 - 400	–	–	0.1 - 500	–
Methamphetamine	0.75 - 1000	0.025 - 1000	–	2 - 200	–	–	0.1 - 500	–
Butylone	0.05 - 500	–	–	–	–	0.07 - 100	–	–
Mephedrone	0.05 - 1000	–	–	2 - 400	–	–	0.05 - 500	0.5 - 200
Ketamine	0.05 - 500	0.025 - 1000	–	0.5 - 400	–	–	0.05 - 500	–
3-CPP	0.25 - 1000	–	–	–	–	–	–	0.5 - 200
MDPV	0.1 - 1000	–	–	–	–	–	0.05 - 500	0.1 - 200
Cocaine	0.05 - 500	0.025 - 1000	–	0.25 - 200	–	–	0.05 - 500	–
3-TFMPP	0.05 - 1000	0.025 - 1000	–	–	–	–	–	0.5 - 200
Citalopram	0.025 - 500	–	–	0.25 - 200	–	–	0.5 - 1000	–
Fluoxetine	0.5 - 1000	0.075 - 1000	–	–	–	–	0.05 - 1000	–
JWH-073	5 - 1000	–	15 - 500	–	–	–	–	–
JWH-398	5 - 1000	–	–	–	1 - 100	–	–	–

[a] Baker and Kasprzyk-Hordern, 2011b; [b] Reid, Derry and Thomas, 2014; [c] Baz-Lomba, Reid and Thomas, 2016; [d] González-Mariño, et al., 2016a; [e] González-Mariño, et al., 2016b; [f] Petrie, et al., 2016; [g] Gao, et al., 2017

According to Table 4.5, instrumental linear ranges from this research and published literature are comparable. Results indicate that LC-MS was able to provide as good linearity as LC-MS/MS and their lowest concentration ends of instrumental linear range are at the same levels. This proves the potential of LC-MS for the analysis of drugs of abuse and pharmaceuticals in drinking water at ultra-trace level, as these LC-MS/MS methods have been applied to waste water and surface water.

4.4 Precision and accuracy

Instrumental precision was studied in order to assess the (1) instrumental intra-assay precision (Section 4.4.1), the repeatability of instrument under repeatable condition (operating the same conditions over a short time interval), and (2) instrumental intermediate precision (Section 4.4.2), the repeatability of instrument under reproducible condition (operating under the same conditions over different days). In addition, the precision and accuracy of the analytical method were also conducted in order to test the ability of the method for the purpose of quantification, the results of which are shown and discussed in Section 4.4.3. The LC-MS method, using a C_{18} column (Section 2.3.2.3), was used for all experiments in this section and the SPE protocol in Table 2.10 was the sample preparation method for spiked waters.

4.4.1 Instrumental intra-assay precision

The instrumental intra-assay precision study was assessed over a short time interval (26.4 hours) under the same instrumental conditions and its experimental procedure is described in Section 2.4.4.1. The precision was measured by six replicates at low (5 ng/mL), medium (50 ng/mL) and high (500 ng/mL) concentration levels and RSD results should not exceed 15 %, except for the lower concentration level, where 20 % RSD is acceptable (Peters, Drummer and Musshoff, 2007). The RSD (%) of instrumental intra-assay precision for the studied drugs of abuse and pharmaceuticals are listed in Table 4.6.

Table 4.6: Results of instrumental intra-assay precision for 20 analytes obtained from a LC-MS analysis with a C_{18} column, n = 6

COMPOUND	INSTRUMENTAL INTRA-ASSAY PRECISION		
	Low Concentration / 5 ng/mL	Medium Concentration / 50 ng/mL	High Concentration / 500 ng/mL
	RSD / %	RSD / %	RSD / %
BZP	15.04	1.52	1.87
MBZP	7.67	1.68	1.23
Methcathinone	7.98	4.04	1.28
Methylone	6.70	2.33	0.71
4-MeOPP	7.21	2.08	3.14
Amphetamine	5.03	2.59	1.02
Methamphetamine	4.51	3.35	1.44
4-FPP	7.79	1.34	0.74
Butylone	2.98	2.24	1.66
Mephedrone	6.05	2.12	0.81
Ketamine	3.05	3.30	0.67
3-CPP	5.46	4.06	0.81
MDPV	5.21	2.08	0.52
Cocaine	3.98	1.99	0.33
3-TFMPP	4.87	4.55	2.27
4-TFMPP	6.04	2.39	0.90
Citalopram	2.51	4.24	0.56
Fluoxetine	6.50	6.61	2.54
JWH-073	11.97	5.01	1.17
JWH-398	9.28	3.46	1.27

The RSDs for the studied drugs of abuse and pharmaceuticals at low concentration (5 ng/mL) were all below the 20 % acceptance criterion of precision normally set for the lower concentration, ranging from 2.51 % (citalopram) to 15.04 % (BZP) (as shown in column B, Table 4.6). It is apparent that the RSDs for low concentration (5 ng/mL) are higher compared to those measured at the higher concentration, medium (50 ng/mL) and high (500 ng/mL) levels. This is probably due to higher fluctuations in instrumental response occurring at low concentrations. Moreover, the RSDs as shown in Table 4.6 ranged from 1.34 % (4-FPP) to 6.61 % (fluoxetine) for medium concentration (column C) and 0.33 % (cocaine) to 3.14 % (4-MeOPP) for high concentration (column D). Lower RSDs were obtained at the medium (50 ng/mL) and high (500 ng/mL) concentrations (< 6.61 % and < 3.14 %, respectively) and were below the 15 % acceptance criterion of precision normally set for higher concentration. The results of instrumental intra-assay precision indicate good repeatability of the LC-MS method under repeatable conditions.

4.4.2 Instrumental intermediate precision

Instrumental intermediate precision was also verified at low (5 ng/mL), medium (50 ng/mL) and high (500 ng/mL) concentrations on three separate days under the same instrumental conditions (Section 2.4.4.2). Mixed standards were stored at 10 °C during these three days. The acceptance criteria of precision are the same as described above for instrumental intra-assay precision (Section 4.4.1), as RSD should be lower than 15 % at medium and high levels and below 20 % for low level. The RSDs (%) of instrumental intermediate precision for all studied drugs of abuse and pharmaceuticals were obtained by three replicates and are shown in Table 4.7.

Table 4.7: Results of instrumental intermediate precision for 20 analytes obtained from a LC-MS analysis with a C_{18} column, n = 3

COMPOUND	INSTRUMENTAL INTERMEDIATE PRECISION		
	Low Concentration / 5 ng/mL	Medium Concentration / 50 ng/mL	High Concentration / 500 ng/mL
	RSD / %	RSD / %	RSD / %
BZP	6.30	1.49	1.94
MBZP	2.62	1.63	1.28
Methcathinone	3.05	1.03	1.24
Methylone	2.82	2.42	0.70
4-MeOPP	6.34	2.12	3.27
Amphetamine	5.23	2.54	1.06
Methamphetamine	2.60	3.42	1.40
4-FPP	7.62	1.41	0.77
Butylone	6.17	2.18	1.63
Mephedrone	6.23	2.20	0.79
Ketamine	6.04	3.23	0.66
3-CPP	5.35	1.10	0.80
MDPV	5.11	2.16	1.54
Cocaine	6.84	2.03	0.75
3-TFMPP	5.06	4.46	2.23
4-TFMPP	5.92	2.48	0.88
Citalopram	6.12	3.12	0.54
Fluoxetine	4.32	6.87	2.49
JWH-073	8.70	2.21	1.14
JWH-398	6.52	6.33	1.32

For the lower concentration (5 ng/mL), RSDs (column B, Table 4.7) ranged from 2.60 % (methamphetamine) to 8.70 % (JWH-073), all below the 20 % acceptance criterion. For the higher concentration (50 and 500 ng/mL), RSDs ranged from 1.03 % (methcathinone) to 6.87 % (fluoxetine) at medium concentration (column C) and from 0.54 % (citalopram) to 3.27 % (4-MeOPP) at high concentration (column D), which are all below the 15 % acceptance criterion. As observed with the instrumental intra-assay precision study, higher RSDs (< 8.70 %) were observed for low concentration level, while relatively lower RSDs (< 6.87 %) were for medium and high concentration levels. The results of instrumental intermediate precision indicate good repeatability of the LC-MS method under reproducible conditions.

4.4.3 Method precision and accuracy

In order to test the precision and accuracy of the method, five calibrators (5, 30, 50, 70 and 100 ng/L) and three QCs (10, 40 and 80 ng/L) were extracted by SPE and analysed by LC-MS, following the procedures described in Section 2.4.4.3. A linear regression plot was generated for each analyte of interest from five calibrators, by plotting the mean PARs of calibrators against corresponding calibrator concentrations. The multi-level calibration curve obtained from this plot was used later for the calculation of method precision and accuracy (Appendix V). An example of a linear regression plot for methamphetamine is shown in Figure 4.9. An acceptable R^2 (0.9983) was obtained, which indicates good linearity in this concentration range (5 - 100 ng/L) and therefore was suitable for reliable quantification. The linear regression plots of the studied drugs of abuse and pharmaceuticals are shown in Appendix V and all calibration curves were linear with $R^2 \geq 0.9900$ (UNODC, 2009).

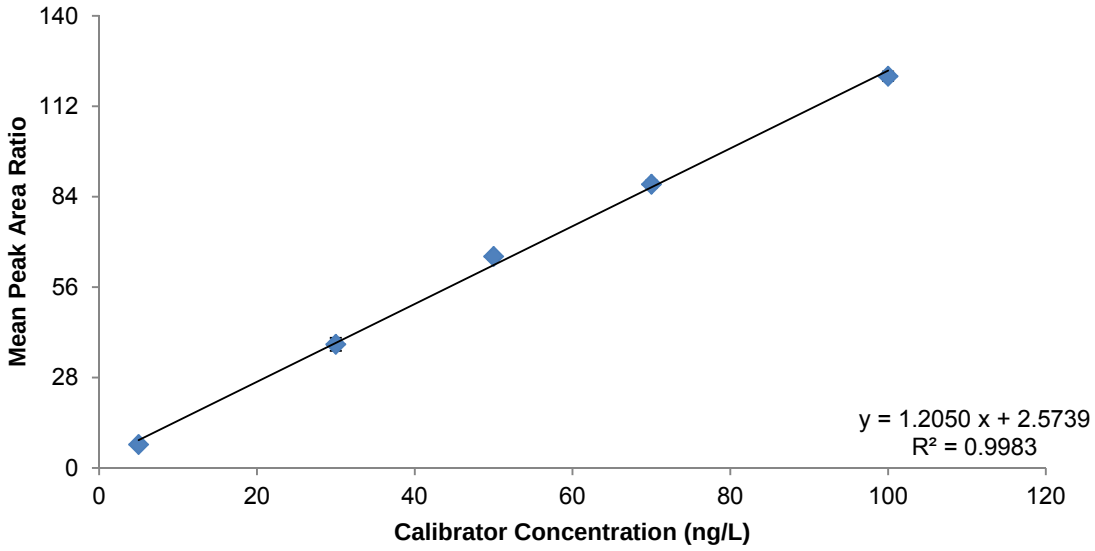


Figure 4.9: Linear regression plot of mean peak area ratio against calibrator concentration for methamphetamine over 5 to 100 ng/L for method precision and accuracy obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

The precision and accuracy of the method were calculated based on the results of three QCs. Precision was assessed by calculating the RSD (%) of three PAR values for each of the three QCs, while accuracy was the deviation (%) of mean calculated concentration from true concentration (10, 40 and 80 ng/L). The calculated concentration was obtained by using the generated calibration curve, e.g. the accuracy of the method for methamphetamine was calculated based on the linear regression equation (y = 1.2050 x + 2.5739) from the plot in Figure 4.9. Table 4.8 shows the calculation process of method precision and accuracy for methamphetamine.

Table 4.8: Calculation of relative standard deviations (RSD) and biases of methamphetamine for method precision and accuracy from a SPE-LC-MS analysis with a C_{18} column, n=3

QC	MEAN PAR	Std Dev	RSD / %	CALCULATED CONC. [a] / ng/L	TRUE CONC. / ng/L	BIAS / %
Low	14.66	1.22	8.32	10.03	10	0.30
Medium	52.71	0.77	1.46	41.61	40	4.03
High	101.23	0.64	0.63	81.87	80	2.34

[a] Calculation based on linear regression equation y = 1.2050 x + 2.5739

Following the calculation process as described above, the results of the method precision and accuracy for all studied drugs of abuse and pharmaceuticals are presented in Table 4.9 and Table 4.10, respectively. The acceptance criteria are within ± 15 % bias of the true value for accuracy and within 15 % RSD for the precision at each concentration level, except 20 % for the lower concentration (Peters, Drummer and Musshoff, 2007; Wille, et al., 2011).

Table 4.9: Results of method precision for 20 analytes obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

COMPOUND	METHOD PRECISION		
	Low Concentration / 10 ng/L	Medium Concentration / 40 ng/L	High Concentration / 80 ng/L
	RSD / %	RSD / %	RSD / %
BZP	7.87	6.44	1.56
MBZP	4.48	1.97	1.02
Methcathinone	5.34	3.99	3.24
Methylone	7.01	0.67	1.77
4-MeOPP	8.06	5.37	2.30
Amphetamine	3.25	2.42	0.92
Methamphetamine	8.32	1.46	0.63
4-FPP	6.06	1.01	2.41
Butylone	3.88	4.65	1.62
Mephedrone	5.73	6.17	6.07
Ketamine	7.52	3.10	1.46
3-CPP	3.18	5.11	6.21
MDPV	4.91	6.59	2.69
Cocaine	1.92	7.57	2.25
3-TFMPP	5.76	1.30	6.77
4-TFMPP	3.55	7.24	7.15
Citalopram	1.79	6.13	4.50
Fluoxetine	5.29	4.78	5.04
JWH-073	4.94	4.64	6.11
JWH-398	3.55	4.27	4.04

The results in Table 4.9 show that the RSDs of method precision for all studied drugs of abuse and pharmaceuticals were in the range of 1.79 % (citalopram) and 8.32 % (methamphetamine) for the low concentration (10 ng/L), 0.67 % (methylone) and 7.57 % (cocaine) for the medium concentration (40 ng/L) and 0.63 % (methamphetamine) and 7.15 % (4-TFMPP) for the high concentration (80 ng/L). These RSD values were all below the acceptance criterion of precision (20 % for low concentration and 15 % for medium and high concentrations). The results of the method precision indicate good repeatability of this analytical method under repeatable condition.

Table 4.10: Results of method accuracy for 20 analytes obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

COMPOUND	METHOD ACCURACY / %		
	Low Concentration / 10 ng/L	Medium Concentration / 40 ng/L	High Concentration / 80 ng/L
	Bias / %	Bias / %	Bias / %
BZP	7.24	- 1.88	7.69
MBZP	- 8.66	3.84	0.63
Methcathinone	- 7.75	- 3.60	0.64
Methylone	- 5.00	0.80	0.52
4-MeOPP	8.34	7.98	0.71
Amphetamine	2.17	0.72	0.65
Methamphetamine	0.30	4.03	2.34
4-FPP	- 8.05	- 1.41	- 2.79
Butylone	- 0.59	2.56	1.00
Mephedrone	2.33	- 5.54	0.68
Ketamine	7.83	0.17	0.26
3-CPP	- 3.46	2.23	- 4.72
MDPV	- 0.28	1.21	0.91
Cocaine	- 6.79	- 2.42	6.04
3-TFMPP	- 5.12	1.34	0.17
4-TFMPP	3.81	- 4.55	- 0.16
Citalopram	- 5.40	- 3.96	2.34
Fluoxetine	- 6.77	0.47	1.49
JWH-073	7.63	- 4.68	- 3.55
JWH-398	4.86	- 3.48	- 6.50

In addition, the biases of method accuracy (Table 4.10) ranged from -0.28 % (MDPV) to -8.66 % (MBZP) for the low concentration (10 ng/L), 0.17 % (ketamine) to 7.98 % (4-MeOPP) for the medium concentration (40 ng/L) and -0.16 % (4-TFMPP) to 7.69 % (BZP) for the high concentration (80 ng/L). It is obvious from the results that good method accuracy was obtained for all studied drugs of abuse and pharmaceuticals, as the criterion of acceptable accuracy is ± 20 % for low concentration and ± 15 % for medium and high concentrations.

Therefore, as observed with the studies of method precision and accuracy, this analytical method was suitable for the quantification of 20 drugs of abuse and pharmaceuticals in drinking water at low, medium and high concentrations.

4.5 Detection and quantification limits

As mentioned in Section 1.10.5, two sets of detection limit and quantification limit were determined in this research, including IDL and IQL, for defining the limitations of the LC-MS instrument (Section 4.5.1) and MDL and MQL for specifying the capabilities of the analytical method based on SPE followed by LC-MS (Section 4.5.2).

4.5.1 Instrumental detection and quantification limits (IDL and IQL)

This sub-section is divided into two parts, one for the LC-MS method using a C_{18} column (Section 4.5.1.1) and the other for the LC-MS method using a biphenyl column (Section 4.5.1.2). IDL and IQL were determined using the RMSE approach, as mentioned in Section 1.10.5.1.

4.5.1.1 IDLs and IQLs of LC-MS method using a C_{18} column

Following the procedures as detailed in Section 2.4.5, five mixed standards were analysed for calculating the IDLs and IQLs of the LC-MS method using a C_{18} column (Section 2.3.2.3). Concentrations for each analyte were selected near its estimated IDL and IQL range (Table 2.11).

The root mean square error approach was used to calculate the IDL and IQL for each analyte, as it is more accurate and reliable compared to other methods for determining the detection and quantification limits, as discussed in Section 1.10.5.1. Firstly, a linear regression plot of measured mean PARs against the concentrations of five mixed standards was generated for each target analyte. The linear regression equation was then obtained, which was used later for calculating the IDLs and IQLs. Figure 4.10 is an example of a linear regression plot of mean PAR against a concentration for methamphetamine with a R^2 of 0.9925. The linear regression plots of other studied drugs

of abuse and pharmaceuticals used for the RMSE approach to calculate their IDLs and IQLs are shown in Appendix VI-a.

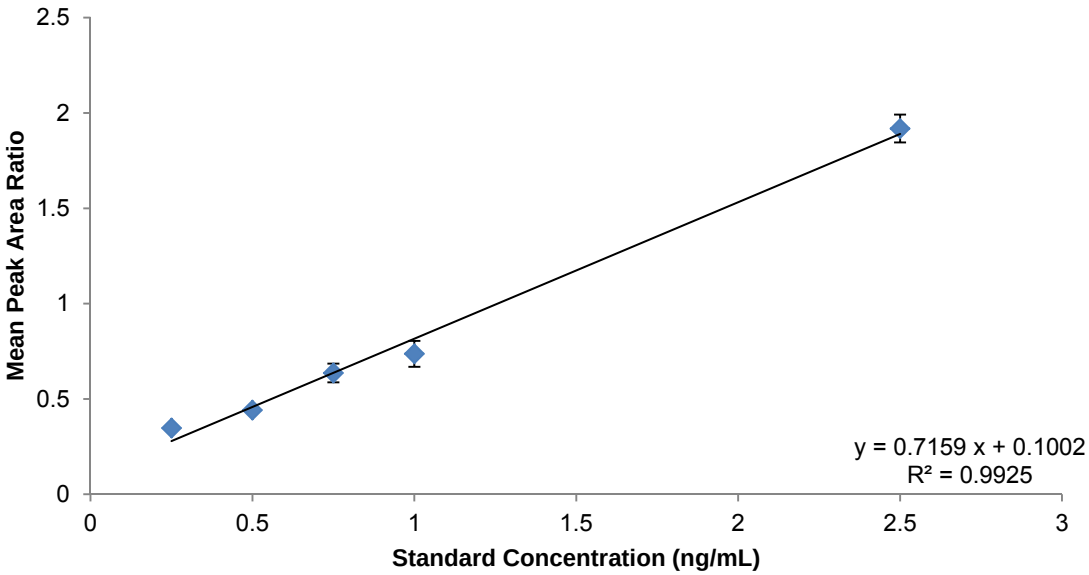


Figure 4.10: Linear regression plot of mean peak area ratio against standard concentration for methamphetamine over 0.25 to 2.5 ng/mL for the calculation of instrumental detection and quantification limits using root mean square error approach obtained from a LC-MS analysis with a C_{18} column, n = 3

The concentrations of five mixed standards (as shown in Table 2.11) were then used in the linear regression equation (y = 0.7159 x + 0.1002) and PARs were calculated for all concentration levels. The square error for each concentration was determined by the square of the difference between calculated PAR and measured PAR. RMSE was then calculated as follows (Corley, 2003):

$$RMSE = [(E^2 / (n-2)]^{1/2}$$

(Equation 4.1)

Where, E^2 is defined as the sum of square errors for all concentration levels and n represents the number of concentrations, which was 5 in this research.

Finally, the IDL and IQL can be calculated as follows (*ibid*):

$$IDL = 3 \times (RMSE/m)$$

(Equation 4.2)

$$IQL = 10 \times (RMSE/m)$$

(Equation 4.3)

Where, m represents the slope obtained by the linear regression equation.

Table 4.11 shows the calculation process of IDL and IQL for methamphetamine using the RMSE approach based on Equation 4.1 to Equation 4.3.

Table 4.11: Calculation of instrumental detection and quantification limits (IDL and IQL) of methamphetamine using root mean square error (RMSE) approach from a LC-MS analysis with a C_{18} column, n=3

CONC. / ng/mL	MEASURED MEAN PAR	CALCULATED MEAN PAR [a]	SQUARE ERROR	SUM OF SQUARE ERROR	RMSE	IDL / ng/mL	IQL / ng/mL
0.25	0.3477	0.2792	0.004692	0.0121	0.0635	0.2661	0.8870
0.50	0.4415	0.4582	0.000279				
0.75	0.6362	0.6371	0.000001				
1.00	0.7365	0.8161	0.006336				
2.50	1.9185	1.8900	0.000812				

[a] Calculation based on linear regression equation y = 0.7159 x + 0.1002

Following the calculation process using the RMSE method, as described above, the IDLs and IQLs for all studied drugs of abuse and pharmaceuticals are presented in Table 4.12. Furthermore, the S/N method was also used and the obtained results are included in Table 4.12 to check these IDLs and IQLs values. The IDL is taken as the concentration of analyte that gives the S/N of 3:1 and IQL is taken as 10:1.

Table 4.12: Results of instrumental detection and quantification limits (IDL and IQL) for 20 analytes obtained from a LC-MS analysis with a C_{18} column

COMPOUND	INSTRUMENTAL DETECTION AND QUANTIFICATION LIMITS			
	C_{18} Column			
	IDL / ng/mL		IQL / ng/mL	
	RMSE Method [a]	S/N Method [b]	RMSE Method [c]	S/N Method [d]
BZP	0.1226	0.2500	0.4087	0.7500
MBZP	0.0503	0.0500	0.1675	0.5000
Methcathinone	0.0651	0.0750	0.2168	0.5000
Methylone	0.1270	0.2500	0.4232	0.7500
4-MeOPP	0.8516	0.7500	2.8387	2.5000
Amphetamine	0.5342	0.7500	1.7807	2.5000
Methamphetamine	0.2661	0.2500	0.8870	1.0000
4-FPP	0.0852	0.1000	0.2842	0.5000
Butylone	0.0125	0.0250	0.0415	0.0750
Mephedrone	0.0251	0.0100	0.0837	0.0500
Ketamine	0.0123	0.0100	0.0412	0.0500
3-CPP	0.0823	0.0750	0.2745	0.5000
MDPV	0.0269	0.0500	0.0896	0.1000
Cocaine	0.0113	0.0250	0.0378	0.0750
3-TFMPP	0.0257	0.0500	0.0858	0.1000
4-TFMPP	0.0267	0.0500	0.0891	0.1000
Citalopram	0.0110	0.0100	0.0366	0.0500
Fluoxetine	0.1267	0.2500	0.4222	0.7500
JWH-073	0.8814	1.0000	2.9379	5.0000
JWH-398	0.9253	1.0000	3.0844	5.0000

[a] IDL = 3 x (RMSE/m); [b] IDL = 3 x (S/N); [c] IQL = 10 x (RMSE/m); [d] IQL = 10 x (S/N)

IDLs across the studied drugs of abuse and pharmaceuticals are between 0.0110 and 0.9253 ng/mL using the RMSE method and between 0.0100 and 1.0000 ng/mL using the S/N method, whilst the IQLs range from 0.0366 to 3.0844 ng/mL using the RMSE method and from 0.0500 to 5.0000 ng/mL using the S/N method.

The results of IDLs and IQLs for seven drugs of abuse and one pharmaceutical from this research and available literature (Kasprzyk-Hordern, Dinsdale and Guwy, 2007; Baker and Kasprzyk-Hordern, 2011b) are compared and shown in Table 4.13. LC-MS was the analytical instrument in this research and LC-MS/MS was used in the previously published studies. The S/N method was used for calculating the IDLs and IQLs. The IDL and IQL

values for other studied drugs of abuse and pharmaceuticals have not been reported in the literature.

Table 4.13: Comparison of instrumental detection and quantification limits (IDL and IQL) in this research with published literature

COMPOUND	INSTRUMENTAL DETECTION AND QUANTIFICATION LIMITS					
	This Research		Published Literature			
			1 [a]		2 [b]	
	LC-MS		LC-MS/MS		LC-MS/MS	
	IDL [c] / ng/mL	IQL [d] / ng/mL	IDL [c] / ng/mL	IQL [d] / ng/mL	IDL [c] / ng/mL	IQL [d] / ng/mL
BZP	0.250	0.750	–	–	0.500	1.000
Methcathinone	0.075	0.500	–	–	0.075	0.500
Amphetamine	0.750	2.500	0.300	1.000	0.100	0.500
Methamphetamine	0.250	1.000	–	–	0.025	0.100
Ketamine	0.010	0.050	–	–	0.025	0.100
Cocaine	0.025	0.075	0.050	0.200	0.025	0.100
3-TFMPP	0.050	0.100	–	–	0.025	0.100
Fluoxetine	0.250	0.750	–	–	0.075	0.500

[a] Kasprzyk-Hordern, Dinsdale and Guwy, 2007; [b] Baker and Kasprzyk-Hordern, 2011b; [c] IDL = 3 x (S/N); [d] IQL = 10 x (S/N)

The IDLs and IQLs from this research are lower or similar when compared to the two cited studies (Table 4.13) in the case of BZP, methcathinone, ketamine, cocaine and 3-TFMPP. The IDL and IQL values for amphetamine, methamphetamine and fluoxetine are higher but still comparable with the reported results. This indicates that the IDL and IQL values obtained by the LC-MS method using a C_{18} column in this research are very close to those reported values based on LC-MS/MS, proving the potential of LC-MS for the detection and quantification of these chosen drugs of abuse and pharmaceuticals at ultra-trace levels.

4.5.1.2 IDLs of LC-MS method using a biphenyl column

Five mixed standards were analysed for calculating the IDLs of the LC-MS method using a biphenyl column (Section 2.3.2.4) and their concentrations are shown in Table 2.11. The RMSE and S/N methods were used to calculate the IDLs for all studied drugs of abuse and pharmaceuticals following the procedures as detailed in Section 4.5.1.1. The linear

regression plots of the studied drugs of abuse and pharmaceuticals used for the RMSE approach are shown in Appendix VI-b, while the values of IDL for all target analytes are shown in Table 4.14 (column B and C). The results of IDLs for the studied drugs of abuse and pharmaceuticals from the LC-MS method using the biphenyl column are also compared with those IDL and IQL values from LC-MS method using the C_{18} column. These results are listed in Table 4.14 (column D to G).

Table 4.14: Comparison of instrumental detection and quantification limits (IDL and IQL) of 20 analytes obtained from a LC-MS analysis with a biphenyl column and C_{18} column

COMPOUND	INSTRUMENTAL DETECTION AND QUANTIFICATION LIMITS					
	Biphenyl Column		C_{18} Column			
	IDL / ng/mL		IDL / ng/mL		IQL / ng/mL	
	RMSE Method [a]	S/N Method [b]	RMSE Method [a]	S/N Method [b]	RMSE Method [c]	S/N Method [d]
BZP	0.0286	0.0500	0.1226	0.2500	0.4087	0.7500
MBZP	0.0266	0.0500	0.0503	0.0500	0.1675	0.5000
Methcathinone	0.0994	0.2500	0.0651	0.0750	0.2168	0.5000
Amphetamine	0.4795	0.5000	0.5342	0.2500	1.7807	0.7500
Methylone	0.0115	0.0250	0.1270	0.7500	0.4232	2.5000
Methamphetamine	0.2260	0.2500	0.2661	0.7500	0.8870	2.5000
4-MeOPP	0.0831	0.2500	0.8516	0.2500	2.8387	1.0000
4-FPP	0.0635	0.0250	0.0852	0.1000	0.2842	0.5000
Mephedrone	0.1095	0.0500	0.0251	0.0250	0.0837	0.0750
Butylone	0.0198	0.0500	0.0125	0.0100	0.0415	0.0500
Ketamine	0.0288	0.0500	0.0123	0.0100	0.0412	0.0500
3-CPP	0.2973	0.2500	0.0823	0.0750	0.2745	0.5000
3-TFMPP	0.0751	0.1000	0.0257	0.0500	0.0858	0.1000
4-TFMPP	0.0782	0.1000	0.0267	0.0250	0.0891	0.0750
Cocaine	0.0210	0.0075	0.0113	0.0500	0.0378	0.1000
MDPV	0.0217	0.0250	0.0269	0.0500	0.0896	0.1000
Citalopram	0.0292	0.0100	0.0110	0.0100	0.0366	0.0500
Fluoxetine	0.0314	0.0750	0.1267	0.2500	0.4222	0.7500
JWH-073	0.2764	0.1000	0.8814	1.0000	2.9379	5.0000
JWH-398	0.4233	0.1000	0.9253	1.0000	3.0844	5.0000

[a] IDL = 3 x (RMSE/m); [b] IDL = 3 x (S/N); [c] IQL = 10 x (RMSE/m); [d] IQL = 10 x (S/N)

In Table 4.14, the IDL values for 13 target analytes (BZP, MBZP, amphetamine, methylone, methamphetamine, 4-MeOPP, 4-FPP, cocaine, MDPV, citalopram, fluoxetine, JWH-073

and JWH-398) obtained by using the biphenyl column are lower or similar when compared to the IDLs of the C_{18} column. For the rest of the target analytes, their IDLs when using the biphenyl column are higher than those IDL values obtained by using the C_{18} column, but are still lower than or comparable with the IQLs of the C_{18} column. Overall, the IDLs obtained by the LC-MS method using the biphenyl column are lower than or very close to those IQL values based on the LC-MS method using the C_{18} column. Therefore, this indicates that the LC-MS method using the biphenyl was suitable for the detection of the 20 studied drugs of abuse and pharmaceuticals when they were quantified in water samples by using the C_{18} column, proving the potential of this method for the purpose of confirmation.

4.5.2 Method detection and quantification limits (MDL and MQL)

In this research, MDL and MQL were used to specify the capabilities of SPE-LC-MS method for the detection and quantification of the studied drugs of abuse and pharmaceuticals in water samples (Section 1.10.5.2) and were calculated according to Section 2.4.5, respectively. The equations applied for determining MDL and MQL are based on previously published equations by Baker and Kasprzyk-Hordern (2011b). SPE recovery results present in Table 3.6 (column B) were used for the calculation and the results of IDL and IQL obtained by using the RMSE method are based on Table 4.12. The enrichment factor for this method was 2000, which is the ratio of loaded sample volume (200 mL) to extracted sample volume (0.1 mL).

$$MDL = [IDL / (SPE\ Recovery \times Enrichment\ Factor)] \times 100$$

(Equation 4.4)

$$MQL = [IQL / (SPE\ Recovery \times Enrichment\ Factor)] \times 100$$

(Equation 4.5)

Based on Equation 4.4 and Equation 4.5, the MDLs and MQLs of a C_{18} column for all studied drugs of abuse and pharmaceuticals are presented in Table 4.15. In this research, IDLs and IQLs of the LC-MS method using a C_{18} column are all in the ng/mL range, as

shown in Table 4.12, but the MDL and MQL of the same target analyte can drop down to the ng/L range after the application of SPE as the sample preparation method (Table 4.15). This is because SPE can highly concentrate the water samples (enrichment factor was 2000) and thus could enable the studied drugs of abuse and pharmaceuticals to be detected in drinking water, which are expected to be present at sub ng/L levels (Section 1.6).

Table 4.15: Results of method detection and quantification limits (MDL and MQL) for 20 analytes obtained from calculation for a C_{18} column

COMPOUND	METHOD DETECTION AND QUANTIFICATION LIMITS	
	C_{18} Column	
	MDL / ng/L	MQL / ng/L
BZP	0.0851	0.2838
MBZP	0.0387	0.1288
Methcathinone	0.1085	0.3613
Methylone	0.0907	0.3023
4-MeOPP	1.0918	3.6394
Amphetamine	0.2754	0.9179
Methamphetamine	0.1372	0.4572
4-FPP	0.0526	0.1754
Butylone	0.0093	0.0310
Mephedrone	0.0267	0.0890
Ketamine	0.0068	0.0229
3-CPP	0.0521	0.1737
MDPV	0.0140	0.0467
Cocaine	0.0057	0.0189
3-TFMPP	0.0149	0.0499
4-TFMPP	0.0205	0.0685
Citalopram	0.0056	0.0187
Fluoxetine	0.0615	0.2050
JWH-073	0.4119	1.3729
JWH-398	0.4673	1.5578

In Table 4.15, citalopram has the lowest MDL (0.0056 ng/L) and MQL (0.0187 ng/L) values across all studied drugs of abuse and pharmaceuticals. This result is consistent with the findings of IDL and IQL (Table 4.12), which indicate that the lowest IDL and IQL values are also for citalopram. On the other hand, the MDL (1.0918 ng/L) and MQL (3.6394 ng/L) values of 4-MeOPP are higher than JWH-398, which has the highest IDL and IQL (Table

4.12). This is probably due to relatively low SPE recovery (39 %) obtained for 4-MeOPP, compared to JWH-398 (99 %) (Table 3.6).

MDL and MQL results from this research using LC-MS and published references using LC-MS/MS (Cahill, et al., 2004; Gros, Petrović and Barceló, 2006; Kasprzyk-Hordern, Dinsdale and Guwy, 2007; Zuccato, et al., 2008; Bijlsma, et al., 2009; Alonso, et al., 2010; Baker and Kasprzyk-Hordern, 2011b; Boleda, et al., 2011; Valcárcel, et al., 2012) are compared and presented in Table 4.16 and Table 4.17, respectively. These are amphetamine, methamphetamine, cocaine, ketamine, methcathinone, BZP, 3-TFMPP, citalopram and fluoxetine. MDLs and MQLs for other studied drugs of abuse have not been reported in the literature. In these two tables, relatively clean water samples, including surface water, ground water, drinking water and Milli-Q water, were used for reference methods, which are similar to this research (raw water from surface water source).

Table 4.16: Comparison of method detection limits (MDL) in this research with published literature

COMPOUND	METHOD DETECTION LIMIT						
	This Research	Published Literature SPE-LC-MS/MS					
	SPE-LC-MS	1 [a]	2 [b]	3 [c]	4 [d]	5 [e]	6 [f]
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Amphetamine	0.2754	–	–	0.2	0.19	2	0.50
Methamphetamine	0.1372	–	–	–	0.12	0.6	0.05
Cocaine	0.0057	–	–	0.1	0.04	0.8	0.05
Ketamine	0.0068	–	–	–	–	–	0.08
Methcathinone	0.1085	–	–	–	–	–	0.10
BZP	0.0851	–	–	–	–	–	1.00
3-TFMPP	0.0149	–	–	–	–	–	0.05
Fluoxetine	0.0615	18	20	–	–	–	1.00

[a] Cahill, et al., 2004; [b] Gros, Petrović and Barceló, 2006; [c] Kasprzyk-Hordern, Dinsdale and Guwy, 2007; [d] Zuccato, et al., 2008; [e] Bijlsma, et al., 2009; [f] Baker and Kasprzyk-Hordern, 2011b

In this research, MDL values for cocaine, ketamine, BZP and fluoxetine are significantly lower when compared to all published references included in Table 4.16. The MDL of 3-TFMPP is slightly lower than the MDL reported by Baker and Kasprzyk-Hordern (2011b).

For amphetamine, methamphetamine and methcathinone, MDL values are comparable to the available literature (Table 4.16).

Table 4.17: Comparison of method quantification limits (MQL) in this research with published literature

COMPOUND	METHOD QUANTIFICATION LIMIT							
	This Research	Published Literature SPE-LC-MS/MS						
	SPE-LC-MS	1 [a]	2 [b]	3 [c]	4 [d]	5 [e]	6 [f]	7 [g]
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Amphetamine	0.9179	–	1	0.65	–	1.00	1.0	4.28
Methamphetamine	0.4572	–	–	0.41	–	0.10	0.5	1.28
Cocaine	0.0189	–	0.3	0.13	–	0.10	0.1	0.13
Ketamine	0.0229	–	–	–	–	0.50	1.5	–
Methcathinone	0.3613	–	–	–	–	1.00	–	–
BZP	0.2838	–	–	–	–	5.00	–	–
3-TFMPP	0.0499	–	–	–	–	0.10	–	–
Citalopram	0.0187	–	–	–	10	–	–	–
Fluoxetine	0.2050	66	–	–	10	5.00	–	–

[a] Gros, Petrović and Barceló, 2006; [b] Kasprzyk-Hordern, Dinsdale and Guwy, 2007; [c] Zuccato, et al., 2008; [d] Alonso, et al., 2010; [e] Baker and Kasprzyk-Hordern, 2011b; [f] Boleda, et al., 2011; [g] Valcárcel, et al., 2012

For MQLs, the comparison results are similar to MDLs. As shown in Table 4.17, the MQL values of four analytes (cocaine, ketamine, BZP and fluoxetine) in this research are still significantly lower than the published results, as well as citalopram. The MQLs of methcathinone and 3-TFMPP are slightly lower than the MQLs reported by Baker and Kasprzyk-Hordern (2011b). For the other two analytes (amphetamine and methamphetamine), their MQL values in this research are comparable with the published results (Table 4.17).

Thus, the low MDLs and MQLs obtained in this research prove the capability of this novel SPE-LC-MS method for the detection and quantification of the studied drugs of abuse and pharmaceuticals in drinking water. This is because their values are lower than or similar to those published methods using SPE followed by LC-MS/MS, which have already been applied in order to analyse surface water, ground water and tap water.

4.6 Overall discussion and conclusion of method validation

The LC-MS methods using a C_{18} column (as detailed in Section 2.3.2.3) and biphenyl column (as detailed in Section 2.3.2.4) were developed and optimised in Chapter 3 to simultaneously detect 20 drugs of abuse and pharmaceuticals. In order to improve the selectivity and sensitivity of the methods, the analysis was undertaken in SIM mode, the diagnostic ions were monitored in ten time segments and the voltages of DL, qarray DC and RF that gave the best peak intensity were used. These two methods were then validated in this chapter. The LC-MS method using a C_{18} column was validated in terms of selectivity, autosampler storage stability, instrumental linearity, precision, accuracy, instrumental and method limits of detection and quantification, as this method was used for quantitative purpose. Validation studies, including selectivity and instrumental detection limit, were only undertaken for the LC-MS method using a biphenyl column as it was used for confirmation only.

The first step in the validation was to demonstrate the selectivity of the method. Through the monitoring of matrix blank, no interference peaks were identified at the retention times of the drugs of abuse, pharmaceuticals and internal standards studied for both columns. Thus, these LC-MS methods (C_{18} column and biphenyl column) have been proven to be selective, as the peak of target analyte detected was due to the target compound itself.

A five day autosampler storage stability was carried out after investigating the selectivity in order to determine the stability of 20 drugs of abuse and pharmaceuticals as well as three internal standards during LC-MS analysis. The mixed standards in the LC-MS injection solvent were prepared at both low (10 ng/mL) and high concentrations (500 ng/mL) and their stability was assessed by plotting instrumental response (PAR) against injection time (every three hours). All drugs of abuse and pharmaceuticals investigated were found to be stable at both low and high concentrations during the five-day analysis period, as the slopes of their bar graphs were not significantly different from zero ($p > 0.05$) (Table 4.3). Moreover, three internal standards, namely amphetamine-d_6 (5 ng/mL), cocaine-d_3 (0.1 ng/mL) and fluoxetine-d_6 (0.75 ng/mL), were stable for up to five days in the autosampler

(p > 0.05) (Table 4.2). Therefore, based on these stability results, the mixed standards dissolved in the LC-MS injection solvent can be stored on the autosampler at 10 °C for the duration of five days, which was sufficient time to cover the typical working time for method validation and water sample analysis.

After this, the instrumental linearity for the studied drugs of abuse and pharmaceuticals was assessed using mixed standards at 19 different concentrations between the range of 0.001 ng/mL and 10000 ng/mL. The instrumental linear range was determined by the linear regression plot for the elimination of higher concentration points and the plot of relative response against log concentration for the elimination of lower concentration points. The R^2 of all linear regression plots were higher than 0.9900 (Table 4.4) and the relative responses of all data points fell within ± 5 % of the mean relative response after their removal. Hence, the results fulfilled the acceptance criteria of linearity (Huber, 2007; UNODC, 2009). Good instrumental linear ranges were obtained for all studied drugs of abuse and pharmaceuticals over four to five orders of magnitude. These linear ranges were wide enough to cover the expected working range to be considered during the sample analysis, as evidenced by previous publications (Kasprzyk-Hordern, Dinsdale and Guwy, 2007; Baker and Kasprzyk-Hordern, 2011b).

Through instrumental intra- and inter-day analysis, the precision of the LC-MS method was determined and assessed by the RSDs of PAR results obtained from the mixed standards, which were prepared at low (5 ng/mL), medium (50 ng/mL) and high (500 ng/mL) concentrations within the linear range. The instrumental intra-assay precision was determined over a 26.4-hour period under the same instrumental conditions. The RSDs of all studied drugs of abuse and pharmaceuticals were less than 6.61 % across medium and high concentrations and were less than 15.04 % at low concentration (Table 4.6). The RSD results were within the acceptance criteria of precision, 15 % for medium and high concentrations and 20 % for low concentration, indicating good repeatability of the LC-MS method (Peters, Drummer and Musshoff, 2007). In addition, the instrumental intermediate precision was determined by analysing the mixed standards at the same three

concentrations (5, 50 and 500 ng/mL) on three separate days. At the low concentration, all drugs of abuse and pharmaceuticals studied were, in general, less than 8.70 % RSD, which is within the accepted 20 % and, as the concentration increased, the corresponding RSDs reduced to 6.87 % and fell within the required 15 % (Table 4.7). This, therefore, indicates good repeatability of the LC-MS method over three days of analysis.

Method precision and accuracy were determined by analysing five calibrators (5, 30, 50, 70 and 100 ng/L) as well as three QCs (10, 40 and 80 ng/L), which were all prepared by spiking 20 analytes of interest and three internal standards in ultra-pure water. The results of RSD and bias for low concentration were all below 8.32 % and ± 8.66 %, respectively, while 7.57 % and ± 7.98 % for medium concentration and 7.15 % and ± 7.69 % for high concentration were achieved (Table 4.9 and Table 4.10). This, therefore, proves the potential of this analytical method for the quantification of these chosen drugs of abuse and pharmaceuticals in drinking water.

For the LC-MS method using a C_{18} column, the IDLs and IQLs for all studied drugs of abuse and pharmaceuticals are presented in Table 4.18. The IDL and IQL were calculated by using the RMSE and S/N methods (Section 1.10.5.1). IDLs across the studied drugs of abuse and pharmaceuticals are between 0.0110 and 0.9253 ng/mL using the RMSE method and between 0.0100 and 1.0000 ng/mL using the S/N method, whilst the IQLs range from 0.0366 to 3.0844 ng/mL using the RMSE method and from 0.0500 to 5.0000 ng/mL using the S/N method. In addition, the MDL and MQL were calculated according to Equation 4.4 and Equation 4.5 and presented in Table 4.18. MDLs across the studied drugs of abuse and pharmaceuticals ranged from 0.0056 ng/L for citalopram to 1.0918 ng/L for 4-MeOPP, whilst the MQLs ranged from 0.0187 ng/L for citalopram to 3.6394 ng/L for 4-MeOPP. In comparison with published literature, the IDLs and IQLs obtained in this research are similar or in some cases lower than those reported by published references (Table 4.13, Table 4.16 and Table 4.17).

For the LC-MS method using a biphenyl column, the IDLs were determined for all studied drugs of abuse and pharmaceuticals and are presented in Table 4.18. The IDLs obtained in this research are comparable with the IDLs and IQLs results from the LC-MS method using a C_{18} column, which indicates the potential of this method when using a biphenyl column for the purpose of confirmation when analysing water samples suspected of containing target drugs of abuse and pharmaceuticals.

Based on the above discussion, the LC-MS method using the C_{18} column has been validated to allow for the identification of target analytes in SIM mode with high selectivity and also be capable for the purpose of quantification with high sensitivity, accuracy and precision. Therefore, this LC-MS method has been proved to be successful as a simultaneous identification and quantification method for 20 drugs of abuse and pharmaceuticals. Moreover, the LC-MS method using the biphenyl column has been proved to be selective and sensitive enough and thus has the ability in the confirmation of target drugs of abuse and pharmaceuticals in drinking water. The application of these two newly validated methods to real sample analysis is included in Chapter 5.

Table 4.18: Summary of method validation results (C_{18} column and biphenyl column)

COMPOUND	METHOD VALIDATION RESULTS								
	C_{18} Column							Biphenyl Column [a]	
	Retention Time	Quantifier ion	Linearity	IDL	IQL	MDL	MQL	Retention Time	IDL
	/ min	/ m/z	/ ng/mL	/ ng/mL	/ ng/mL	/ ng/L	/ ng/L	/ min	/ ng/mL
BZP	2.14	177	0.5 - 1000	0.1226	0.4087	0.0851	0.2838	4.98	0.0286
MBZP	2.95	191	0.1 - 1000	0.0503	0.1675	0.0387	0.1288	5.39	0.0266
Methcathinone	5.42	164	0.25 - 1000	0.0651	0.2168	0.1085	0.3613	7.52	0.0994
Methylone	6.43	208	0.5 - 1000	0.1270	0.4232	0.0907	0.3023	8.91	0.0115
4-MeOPP	7.26	193	5 - 1000	0.8516	2.8387	1.0918	3.6394	9.46	0.0831
Amphetamine-d_6	7.35	142	–	–	–	–	–	8.05	–
Amphetamine	7.42	136	2.5 - 1000	0.5342	1.7807	0.2754	0.9179	8.12	0.4795
Methamphetamine	9.20	150	0.75 - 1000	0.2661	0.8870	0.1372	0.4572	9.18	0.2260
4-FPP	9.65	181	0.25 - 1000	0.0852	0.2842	0.0526	0.1754	10.89	0.0635
Butylone	10.63	222	0.05 - 500	0.0125	0.0415	0.0093	0.0310	11.60	0.0198
Mephedrone	11.16	178	0.05 - 1000	0.0251	0.0837	0.0267	0.0890	11.36	0.1095
Ketamine	11.78	238	0.05 - 500	0.0123	0.0412	0.0068	0.0229	14.99	0.0288
3-CPP	13.33	197	0.25 - 1000	0.0823	0.2745	0.0521	0.1737	17.49	0.2973
MDPV	13.78	276	0.1 - 1000	0.0269	0.0896	0.0140	0.0467	20.25	0.0217
Cocaine	13.91	304	0.05 - 500	0.0113	0.0378	0.0057	0.0189	19.99	0.0210
Cocaine-d_3	13.91	307	–	–	–	–	–	19.96	–
3-TFMPP	14.66	231	0.05 - 1000	0.0257	0.0858	0.0149	0.0499	19.14	0.0751
4-TFMPP	15.00	231	0.05 - 1000	0.0267	0.0891	0.0205	0.0685	20.12	0.0782
Citalopram	16.31	325	0.025 - 500	0.0110	0.0366	0.0056	0.0187	26.55	0.0292
Fluoxetine-d_6	18.11	316	–	–	–	–	–	28.67	–
Fluoxetine	18.15	310	0.5 - 1000	0.1267	0.4222	0.0615	0.2050	28.74	0.0314
JWH-073	24.01	328	5 - 1000	0.8814	2.9379	0.4119	1.3729	33.00	0.2764
JWH-398	25.57	376	5 - 1000	0.9253	3.0844	0.4673	1.5578	34.76	0.4233

[a] The confirmation ions of LC-MS method using a biphenyl column were same with the quantifier ions of LC-MS method using a C_{18} column (column C)

CHAPTER 5 RESULTS AND DISCUSSION: DRINKING WATER ANALYSIS

This chapter first includes the procedures of analysing the raw and drinking water samples that were collected from the East Anglia region of the UK (Section 5.1) and then discusses their results (Section 5.2). Drugs of abuse and pharmaceuticals detected in drinking water in this research are also compared with those detected in other countries (Section 5.3). Finally, the removal efficiencies of detected drugs of abuse and pharmaceuticals during DWTPs were evaluated by comparing their concentration in raw and drinking waters and then comparing the levels present with previously published data (Section 5.4).

5.1 Analysis of drugs of abuse and pharmaceuticals in raw and drinking water samples

Following the procedures of sample collection and storage as detailed in Section 2.2, five types of drinking water samples were collected from the East Anglia region, UK (two as tap water and three from three DWTPs). In addition to this, three raw water samples were collected at the same time from the same DWTPs in order to evaluate the removal efficiencies of the studied drugs of abuse and pharmaceuticals. Three non-spiked water samples as well as three spiked water samples were prepared (Section 2.5) and then extracted using the optimised SPE method (Table 2.10). Regarding the LC-MS analysis, a C_{18} column (Section 2.3.2.3) was first used for the identification and quantification of the studied drugs of abuse and pharmaceuticals. The processes of identification and quantification are further discussed in Section 5.1.1 and Section 5.1.3, respectively. When samples were suspected of containing target analytes, a biphenyl column (Section 2.3.2.4) was used for confirmation, which is discussed in Section 5.1.2.

5.1.1 Identification of detected drugs of abuse and pharmaceuticals

Raw and drinking water samples collected from the East Anglia region of the UK, including non-spiked and spiked water samples, were first analysed using a C_{18} column. Drugs of abuse and pharmaceuticals detected from non-spiked water samples were identified based on two parameters, including the quantifier ion monitored in SIM mode and the difference of retention index (retention time of target analyte/retention time of internal

standard) between the non-spiked water sample and positive control (a mixed standard). The reason for using the retention index instead of retention time is discussed in Section 3.1.2.1. As an illustration, Figure 5.1 depicts the overlapping selected ion chromatograms of a non-spiked drinking water (collected from the DWTP A of Anglian Water) and a mixed standard (50 ng/L), which were used to identify the methamphetamine in the drinking water sample.

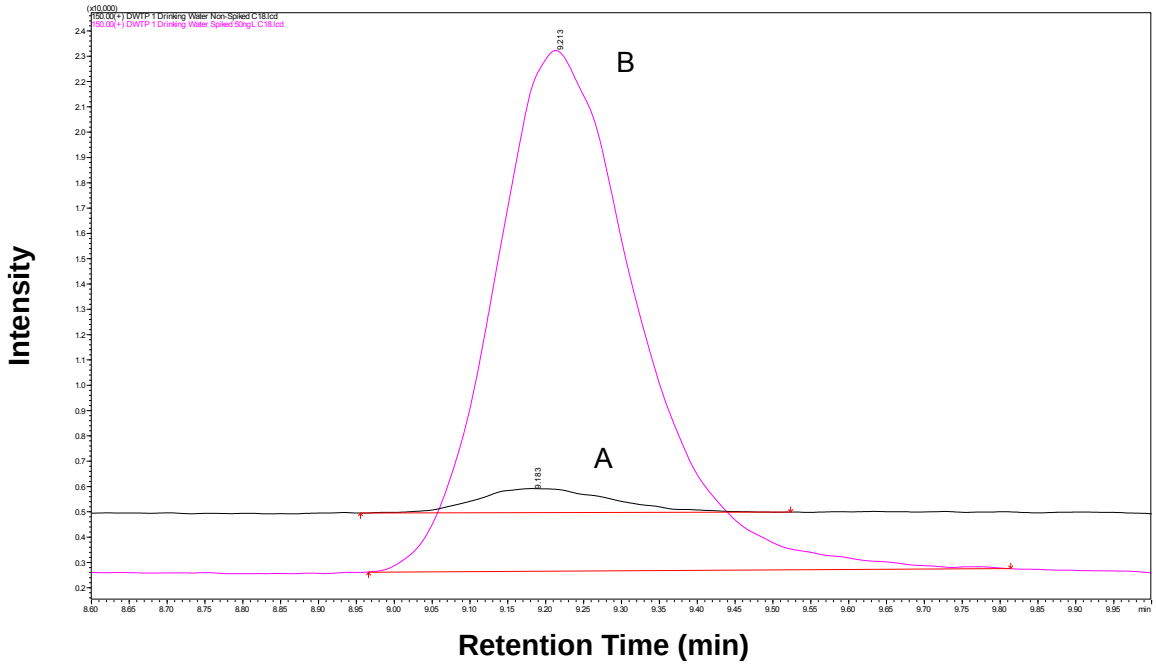


Figure 5.1: Identification of methamphetamine in the drinking water from the DWTP A of Anglian Water, showing overlapping selected ion chromatograms (m/z 150) of (A) a non-spiked water sample and (B) a mixed standard at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

In the selected ion chromatogram of the non-spiked water sample (Figure 5.1 A), there was a peak present with the m/z 150, which was the quantifier ion of methamphetamine, and its retention index was 1.239. This corresponds with the retention index of methamphetamine in the mixed standard (positive control) analysed in the same batch, being 1.242 (Figure 5.1 B). The difference between these two retention indexes was -0.24 %, thereby meeting the requirement that the retention index of the target analyte in a sample shall correspond to that of the same substance in positive control at a tolerance of ± 1.00 % (World Anti-doping Agency, 2010). Therefore, this compound found in the water sample was

identified as methamphetamine. In addition, a solvent blank was analysed directly before the non-spiked water sample and demonstrates that the presence of methamphetamine in the drinking water sample was not due to carryover from the mixed standard or spiked water samples.

For the rest of the detected drugs of abuse and pharmaceuticals, the overlapping selected ion chromatograms of non-spiked water samples collected from the East Anglia region (UK) and mixed standards (50 ng/L) used for identification are included in Appendix VII (raw water samples) and Appendix X (drinking water samples). The retention index differences between the non-spiked water samples and mixed standards (positive control) for all detected analytes were within ± 1.00 % (as shown in Table 5.1) and no peaks were present in the solvent blanks at the corresponding retention times.

Table 5.1: Retention indexes (RI) and diagnostic ions for drugs of abuse and pharmaceuticals detected in raw and drinking water samples obtained from a SPE-LC-MS analysis with a C_{18} column and biphenyl column

	COMPOUND	C_{18} COLUMN				BIPHENYL COLUMN			
		Sample RI	Standard RI	RI Difference / %	Quantifier Ion / m/z	Sample RI	Standard RI	RI Difference / %	Confirmation Ion / m/z
Raw Water									
1 [a]	Methamphetamine	1.236	1.240	- 0.32	150	1.138	1.142	- 0.35	150
	Mephedrone	0.805	0.807	- 0.25	178	1.407	1.405	0.14	178
	Ketamine	0.846	0.847	- 0.12	238	0.750	0.752	- 0.27	238
2 [b]	Fluoxetine	0.996	1.002	- 0.60	310	0.998	1.003	- 0.50	310
3 [c]	Mephedrone	0.812	0.808	0.49	178	1.415	1.411	0.28	178
	Ketamine	0.855	0.849	0.70	238	0.749	0.753	- 0.53	238
Drinking Water									
1 [a]	Methamphetamine	1.239	1.242	- 0.24	150	1.140	1.142	- 0.18	150
	Mephedrone	0.806	0.809	- 0.37	178	1.410	1.404	0.43	178
	Ketamine	0.853	0.854	- 0.12	238	0.750	0.748	0.27	238
	Cocaine	1.010	1.011	- 0.10	304	1.003	1.001	0.20	304
2 [b]	Fluoxetine	0.998	1.002	- 0.40	310	0.999	1.002	- 0.30	310
3 [c]	Mephedrone	0.810	0.808	0.25	178	1.413	1.411	0.14	178
	Ketamine	0.857	0.852	0.58	238	0.751	0.748	0.40	238
4 [d]	Methylone	0.893	0.897	- 0.45	208	1.106	1.110	- 0.36	208
	Mephedrone	0.806	0.808	- 0.25	178	1.406	1.397	0.64	178
	Ketamine	0.850	0.851	- 0.12	238	0.749	0.751	- 0.27	238
	Cocaine	1.009	1.011	- 0.20	304	1.002	1.004	- 0.20	304
	Citalopram	0.898	0.897	0.11	325	0.932	0.930	0.21	325
5 [d]	Mephedrone	0.808	0.807	0.12	178	1.412	1.410	0.14	178
	Ketamine	0.850	0.853	- 0.35	238	0.749	0.753	- 0.53	238
	Citalopram	0.899	0.897	0.22	325	0.935	0.932	0.32	325

[a] Sample from DWTP A of Anglian Water; [b] Sample from DWTP B of Anglian Water; [c] Sample from DWTP C of Essex and Suffolk Water; [d] Sample from taps, City of Cambridge

5.1.2 Confirmation of detected drugs of abuse and pharmaceuticals

Initially, raw and drinking water samples were analysed using a C_{18} column and the target analytes were then further confirmed using a biphenyl column (Section 2.3.2.4), which were evaluated by comparing their retention indexes with the mixed standards. Figure 5.2 depicts the overlapping selected ion chromatograms of a non-spiked water sample and a mixed standard (50 ng/L) was used to confirm the identity of methamphetamine in the drinking water sample as an example, which was collected from the DWTP A of Anglian Water.

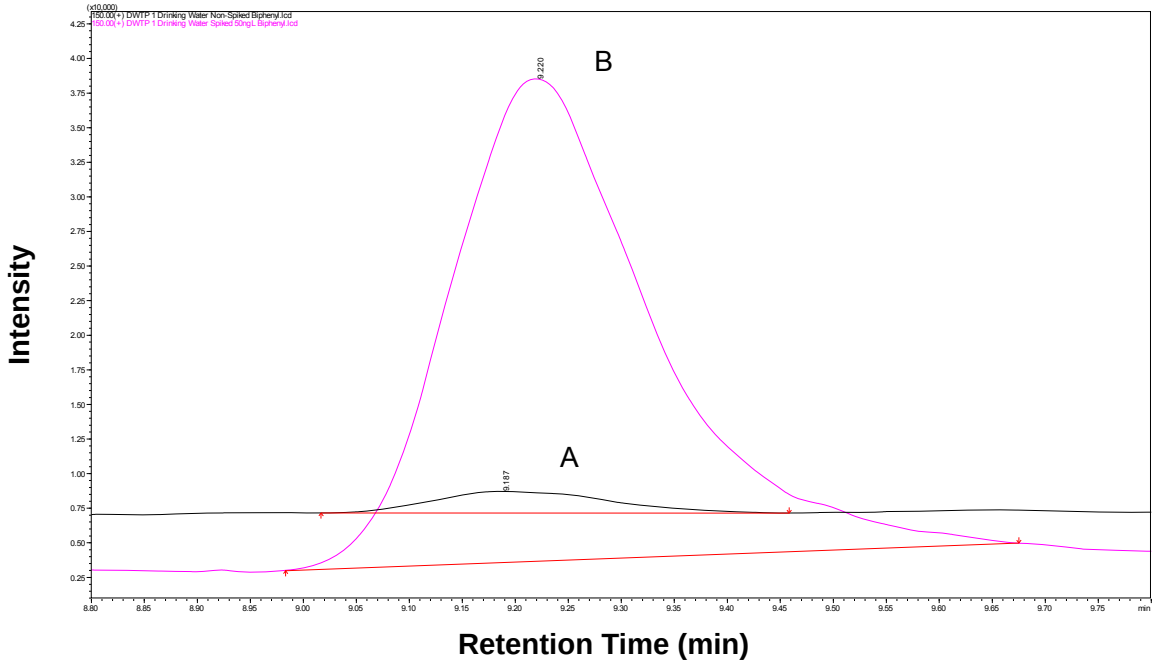


Figure 5.2: Confirmation of methamphetamine in the drinking water from the DWTP A of Anglian Water, showing overlapping selected ion chromatograms (m/z 150) of (A) a non-spiked water sample and (B) a mixed standard at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

The retention index of the methamphetamine peak in the non-spiked water sample (1.140) corresponds with its retention index in the mixed standard (1.142). The difference in their retention indexes was -0.18 % and thus fulfills the acceptance criterion of retention index difference as described in Section 5.1.1, which confirms the presence of methamphetamine in this water sample. The retention index differences for the rest of the detected drugs of abuse and pharmaceuticals in raw and drinking water samples were all

within ± 1.00 % (Table 5.1) and their confirmation of selected ion chromatograms are shown in Appendix VIII and XI, respectively. Moreover, no peaks were present at the retention times of the studied drugs of abuse and pharmaceuticals in the solvent blanks, which were analysed before non-spiked water samples, indicating that no carryover occurred.

To the author's knowledge, there are no specific guidelines in relation to the criteria for the identification and confirmation of drugs of abuse and pharmaceuticals in raw and drinking water samples. However, the Commission Decision 2002/657/EC published by the European Union has been used (Boleda, Galceran and Ventura, 2009; Baker and Kasprzyk-Hordern, 2011b; Boleda, et al., 2011). This is a guideline in respect of the detection of drugs residues at trace levels in live animals and animal products, which states that a minimum of three identification points are recommended for confirmation (Commission Decision 2002/657/EC). Therefore, the identification and confirmation of target drugs of abuse and pharmaceuticals detected in raw and drinking water samples were carried out in this research using three identification points: (1) one retention index obtained from a C_{18} column, (2) one retention index obtained from a biphenyl column and (3) one ion monitored in SIM mode for both the C_{18} column and biphenyl column. Retention indexes and diagnostic ions for drugs of abuse and pharmaceuticals detected in raw and drinking water samples are shown in Table 5.1.

5.1.3 Quantification of detected drugs of abuse and pharmaceuticals

The quantification of detected drugs of abuse and pharmaceuticals in raw and drinking waters was conducted using the standard addition method. This method can compensate for matrix effects when analysing drugs of abuse and pharmaceuticals in water samples (Petrović, et al., 2005; Chiaia, Banta-Green and Field, 2008; Peng, Hall and Gautam, 2016). This method is of importance as matrix effects are a common problem associated with the ionisation process in LC-MS (Furey, et al., 2013), which was the analytical instrument used in this research. Matrix effects can impact on the accuracy, precision and reproducibility of an analytical method (Chambers, et al., 2007), as the signals of analytes

and internal standards may be suppressed or enhanced due to interferences from components in the sample matrix (stated in Section 1.9.2.2.1). Thus, calibrators of the standard addition method are prepared within the same matrix as the sample, resulting in more accurate quantification, as any interferences in the sample matrix that affect the signals of analytes and internal standards in a sample will also affect their signals in calibrators to the same degree (Quintana and Reemtsma, 2004). In this regard, raw and drinking water samples collected from the East Anglia region, UK, were spiked with mixed standards containing 20 studied drugs of abuse and pharmaceuticals as well as three internal standards and were used as calibrators for quantification.

For each water sample, three non-spiked samples (non-spiked sample 1, 2 and 3) and three spiked samples (added concentrations of 5, 50 and 100 ng/L) were extracted by SPE and each eluent was then analysed with triplicate injections by LC-MS using a C_{18} column (Section 2.5). Results were grouped into three sections, namely (1) non-spiked sample 1 and three spiked samples, (2) non-spiked sample 2 and three spiked samples, and (3) non-spiked sample 3 and three spiked samples. Three linear regression trend lines were gained for each analyte by plotting the mean PARs of the four samples in each section against the corresponding added standard concentrations (0, 5, 50 and 100 ng/L), as shown in Figure 5.3. This resulted in the generation of three linear regression equations and thus three concentrations of each analyte in the same sample were calculated and the standard deviation (Std Dev) and RSD (%) were also calculated. Figure 5.3 gives an example of the standard addition plot for methamphetamine in the drinking water sample that was collected from the DWTP A of Anglian Water.

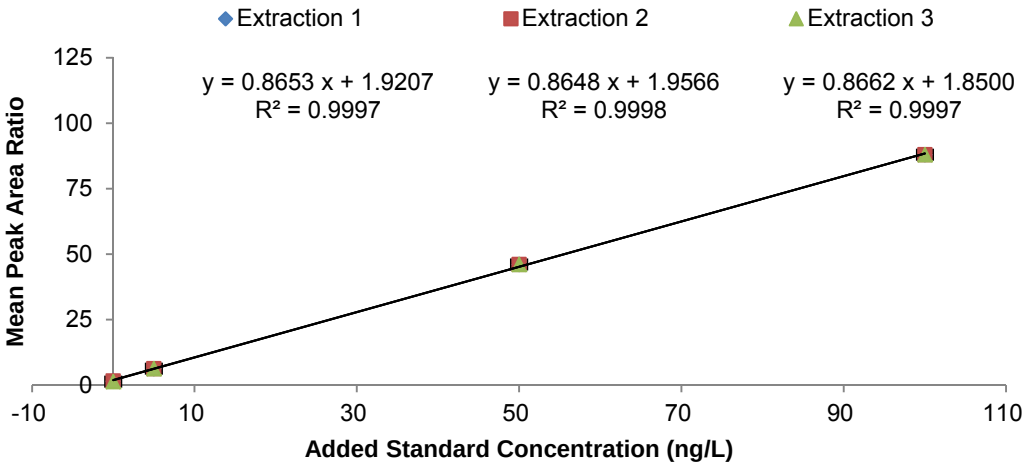


Figure 5.3: Standard addition plot of mean peak area ratio against added standard concentration for methamphetamine over 0 to 100 ng/L for the quantification of its concentration in the drinking water from the DWTP A of Anglian Water obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

The coefficient of determination (R^2) values of three linear regression trend lines were 0.9997, 0.9998 and 0.9997, which all fulfill the acceptance criterion of linearity (> 0.9900), as described in Section 4.3.1, meaning that they were suitable for quantification. In addition, Table 5.2 depicts the process of calculating the concentration of methamphetamine in this drinking water sample using the standard addition method.

Table 5.2: Calculation of methamphetamine concentration in the drinking water from the DWTP A of Anglian Water using standard addition, n=3

	ADDED CONC. / ng/L	MEAN PAR	GRADIENT	INTERCEPT	SAMPLE CONC. / ng/L	
Extraction 1	0	1.469	0.8653	1.9207	2.220	
	5	6.244				
	50	46.094				
	100	87.995				
Extraction 2	0	1.544	0.8648	1.9566	2.262	
	5	6.244				
	50	46.094				
	100	87.995				
Extraction 3	0	1.322	0.8662	1.8500	2.136	
	5	6.244				
	50	46.094				
	100	87.995				
					2.206	Mean Conc.
					0.064	Std Dev
					2.901	RSD / %

As a result, the mean concentration of methamphetamine in the drinking water sample collected from the DWTP A of Anglian Water was 2.206 ± 0.064 ng/L. Also, good repeatability was obtained for the analysis of three non-spiked samples as its RSD was 2.922 %, below the 20 % acceptance criterion of precision at low concentration (Section 4.4).

Standard addition plots for other studied drugs of abuse and pharmaceuticals detected in raw and drinking water samples are included in Appendix IX and XII. All R^2 values were higher than 0.9900 and RSDs did not exceed 20 %, indicating good linearity and repeatability. Based on the calculation as shown in Table 5.2, the concentrations of drugs of abuse and pharmaceuticals detected in the raw and drinking water samples were calculated and are shown in Table 5.3.

Table 5.3: Concentrations and detection frequencies of drugs of abuse and pharmaceuticals detected in three raw water samples and five drinking water samples collected from the East Anglia region, UK, n = 3

	COMPOUND CONCENTRATION / ng/L						
	Methylone	**Methamphetamine**	**Mephedrone**	**Ketamine**	**Cocaine**	**Citalopram**	**Fluoxetine**
Raw Water Sample							
1 [a]	n.d.	1.761 ± 0.015	6.471 ± 0.417	11.199 ± 0.318	n.d.	n.d.	n.d.
2 [b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.675 ± 0.153
3 [c]	n.d.	n.d.	5.742 ± 0.195	6.217 ± 0.108	n.d.	n.d.	n.d.
Conc. Range	n.a.	n.a.	5.742 - 6.471	6.217 - 11.199	n.a.	n.a.	n.a.
Detection Freq. (%)	0	33	67	67	0	0	33
Drinking Water Sample							
1 [a]	n.d.	2.206 ± 0.064	1.871 ± 0.099	0.139 ± 0.010	0.185 ± 0.018	n.d.	n.d.
2 [b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.270 ± 0.023
3 [c]	n.d.	n.d.	2.814 ± 0.023	0.977 ± 0.034	n.d.	n.d.	n.d.
4 [d]	1.368 ± 0.055	n.d.	0.767 ± 0.058	1.124 ± 0.060	0.836 ± 0.080	2.800 ± 0.022	n.d.
5 [d]	n.d.	n.d.	2.519 ± 0.074	0.736 ± 0.028	n.d.	2.257 ± 0.144	n.d.
Conc. Range	n.a.	n.a.	0.767 - 2.814	0.139 - 1.124	0.185 - 0.836	2.257 - 2.800	n.a.
Detection Freq. (%)	20	20	80	80	40	40	20

n.d., not detected; n.a., not available

[a] Sample from DWTP A of Anglian Water; [b] Sample from DWTP B of Anglian Water; [c] Sample from DWTP C of Essex and Suffolk Water; [d] Sample from taps, City of Cambridge

5.2 Results and discussion of drugs of abuse and pharmaceuticals in raw and drinking waters from the East Anglia region of the UK

Following the analysis procedures as described in Section 5.1, seven studied drugs of abuse and pharmaceuticals were quantified in raw and drinking waters from the East Anglia region in the UK. Table 5.3 shows the results of this research, including detected compounds and their detection frequencies and concentrations in drinking water and raw water. These results are discussed in Section 5.2.1 and Section 5.2.2, respectively. Moreover, this research is discussed further in Section 5.4 in relation to removal efficiency during DWTPs.

5.2.1 Drugs of abuse and pharmaceuticals detected in drinking water from the East Anglia region of the UK

As shown in Table 5.3, out of the 20 target analytes, five drugs of abuse and two pharmaceuticals were determined in drinking water samples with concentrations all above their MQL values (Table 4.15). These include traditional illicit drugs (cocaine and methamphetamine), antidepressants (citalopram and fluoxetine) as well as NPS (ketamine, mephedrone and methylone). The concentrations of these detected drugs of abuse and pharmaceuticals were all in ng/L, from 0.139 ng/L for ketamine (sample 1) to 2.814 ng/L for mephedrone (sample 3). Different detection frequencies (number of positive samples/number of total samples) were observed for different analytes of interest in this research. For example, methylone was only detected in sample 4 collected from a tap in Cambridge, whereas ketamine was widely quantified in drinking water from the East Anglia region of the UK, except for sample 2, which was collected from the DWTP B. This can be due to many factors, such as different consumption rates and patterns in the UK and different removal efficiencies of DWTPs. These differences are further discussed in the following sub-sections.

5.2.1.1 Traditional illicit drugs

In Table 5.3, cocaine was quantified in two drinking water samples (1 and 4) in the concentration range of 0.185 - 0.836 ng/L, whereas methamphetamine was only detected in sample 1 at 2.206 ng/L. The detection frequency of cocaine was 40 %, which is higher than methamphetamine (20 %). This could be explained by the different MDLs and MQLs of these two traditional illicit drugs obtained in this research. Since cocaine had relatively lower MDL and MQL (0.0057 and 0.0189 ng/L), as shown in Table 4.15, this compound could have an increased chance of detection compared to methamphetamine, the MDL and MQL of which were 0.1372 and 0.4572 ng/L. Moreover, the concentration of methamphetamine present in the drinking water of the East Anglia region (2.206 ng/L) was slightly higher than the concentration range of cocaine (0.185 - 0.836 ng/L). This result correlates with findings from Mwenesongole (2015) in that the concentration of methamphetamine is higher than cocaine in waste water samples from Cambridge, UK. The detection of these two traditional illicit drugs has been reported by other publications (Boleda, et al., 2011; Mendoza, et al., 2014; Mendoza, et al., 2016; Rodayan, et al., 2016) and the results are described and compared with this research in Section 5.3.1.

Amphetamine was also analysed in this research but was not detected in the drinking water samples from the East Anglia region of the UK. This finding is interesting, since amphetamine continues to dominate the market for amphetamine-type stimulants in Europe (UNODC, 2015). In 2013, 34,000 seizures of amphetamine (6.7 tonnes) reported in Europe were far higher than methamphetamine with 7,000 seizures (0.5 tonnes) and more than 50 % were accounted for by Germany, the Netherlands and the UK (EMCDDA, 2015b). However, methamphetamine was detected and quantified, but amphetamine was not in this research. This is probably because the presence of amphetamine in drinking water samples from the East Anglia region was below its MDL level 0.2754 ng/L (Table 4.15) and hence cannot be detected. Thus, the method sensitivity for amphetamine needs to be further improved.

5.2.1.2 Antidepressants

Two antidepressants (citalopram and fluoxetine) have been detected in drinking water from the East Anglia region of the UK. The concentrations of citalopram found in water samples were 2.257 and 2.800 ng/L, which are all significantly higher than the concentration of fluoxetine 0.270 ng/L (Table 5.3). This is probably because 14.4 million items of citalopram distributed in England are far higher than fluoxetine, with 6.4 million items in 2015 (HSCIC, 2016). To date, a few publications have reported the detection of these two antidepressants in drinking water from China, Poland, Spain and the USA (Vanderford and Snyder, 2006; Benotti, et al., 2009; López-Serna, et al., 2010; Giebułtowicz and Nałęcz-Jawecki, 2014; Padhye, et al., 2014; Wu, et al., 2015). The results from these publications are summarised and then compared with this research in Section 5.3.2.

5.2.1.3 Novel psychoactive substances

In recent years, growing numbers of NPS have been seized from all over the world (UNODC, 2015). In 2013, 46,730 seizures of NPS weighing more than 3.1 tonnes were reported across Europe, which indicates a seven-fold increase between 2008 and 2013 (EMCDDA, 2015c). Strong growth in the drug market also results in the increase of non-fatal intoxications and deaths as well as broader social harms (*ibid*). As a result, NPS have been receiving considerable attention from law enforcement agencies. For instance, in the UK, some NPS such as mephedrone and ketamine have been added to the Crime Survey for England and Wales (CSEW) since 2010/11 to collect information regarding their use in the general population and they are also controlled under the Misuse of Drugs Act 1971 as Class B substances (The Misuse of Drugs Act 1971 (Ketamine etc.) (Amendment) Order 2014; Home Office, 2015). Recently, NPS have been prohibited by the Psychoactive Substances Act 2016 in order to control these substances in the UK (Psychoactive Substances Act, 2016). In Europe, the EU Early Warning System operated by EMCDDA gathers and analyses information regarding the use of NPS from the 28 European Union Member States, Turkey and Norway and provides a report in order to ensure a rapid response to emerging threats (EMCDDA, 2015c).

In this research, three NPS were detected in the drinking water of the East Anglia region including ketamine (Section 5.2.1.3.1), methylone and mephedrone (Section 5.2.1.3.2). In this research, the latter two NPS have been reported in drinking water for the first time. This proves that these newer emerging drugs of abuse are already present in the drinking water of the UK, which could be due to their increased illegal consumption in the UK as well as the legitimate use of ketamine. These results are discussed in more detail in the following sub-sections.

5.2.1.3.1 Ketamine

Worldwide, ketamine has been the most commonly reported NPS on the market over several years. According to the World Drug report by UNODC (2015), ketamine has been identified by 58 countries. This is probably because this NPS is also used as a prescribed anaesthetic for human and veterinary treatments (*ibid*). In addition, ketamine is one of the most highly abused drugs in the East Anglia region, as evidenced by the findings from Cambridgeshire Constabulary, where it was the third highest in terms of seizures in 2011 (Mwenesongole, 2015). Thus, due to its high level of consumption (illegal and medical use), the presence of ketamine has been reported in the aquatic environment in the UK. For example, in a study conducted in Cambridge, ketamine was detected in waste water at the concentration of 97 x 10^3 ng/L (Mwenesongole, 2013) and its presence (21.3 ng/L) was also found in surface water in a study conducted in Marsden (Baker and Kasprzyk-Hordern, 2011b). This research has further demonstrated the presence of ketamine in drinking water at the concentration range of 0.139 - 1.124 ng/L in the East Anglia region (Table 5.3). In addition, four in five collected samples tested positive for the presence of ketamine and hence a relatively higher detection frequency (80 %) was observed for ketamine (Table 5.3), which corroborates further that it is a commonly consumed drug in the East Anglia region. Based on the CSEW data supplied by Home Office (2015), the use of ketamine in the UK was highest among 20 to 24-year-olds between 2014 and 2015. This correlates well with the results of this research where positive identification of ketamine was observed for all samples collected from Cambridge, which, as a university city, has a high student population in the age range 20 - 24 (i.e. 14.6 % of all residents in 2011) (Office for National

Statistics, 2013). The detection of ketamine in drinking water has also been reported in the southern Ontario of Canada (Rodayan, et al., 2016), which is described and compared with this research in Section 5.3.3.

5.2.1.3.2 Cathinones

Two out of five cathinones were determined in drinking water, including mephedrone and methylone. This is not surprising considering that cathinones are one of the most highly abused NPS in Europe, comprising 23 % of the total seizures in 2013, and there was a 60-fold increase in the number of seizures of cathinones from 2008 to 2013 (EMCDDA, 2015c).

The highest concentration of a cathinone in drinking water was observed for mephedrone at the concentration of 2.814 ng/L (Table 5.3). This could be due to the high level of mephedrone use in the UK, as it is considered a substitute for ecstasy (EMCDDA, 2015c; UNODC, 2015). For example, according to the seizure data reported by Cambridgeshire Constabulary, mephedrone was the fourth most seized drug in 2011 (Mwenesongole, 2015). In addition, mephedrone was frequently detected in most drinking water samples except for sample 2 (Table 5.3). This indicates a wider usage of this drug in the East Anglia region of the UK, compared to methylone, which was only detected in one sample (sample 4). This is the first time to the author's knowledge that mephedrone has been detected in drinking water. It is interesting that the occurrence of mephedrone in drinking water has only been observed in the UK, as its presence on the drug market has been reported by 46 countries in recent years (UNODC, 2015). Therefore, it is necessary to carry out further studies regarding the analysis of mephedrone in drinking water in order to gather more information from other regions of the UK and from other countries.

As shown in Table 5.3, methylone, one cathinone analogue of MDMA (ACMD, 2010), was also detected in this research at the concentration of 1.368 ng/L, which is slightly lower than the concentrations of mephedrone in samples 1, 3 and 5. This result correlates with findings in the Mixmag drug survey (2012), where methylone was reported as one of the most abused cathinones in the UK, except for mephedrone.

Butylone, methcathinone and MDPV were also incorporated in this analytical method (Table 1.3) but were not detected. This could be due to their relatively lower consumption levels in the UK. However, butylone and methcathinone have previously been detected in waste water from Cambridge, UK, at the concentrations of 4 and 253 x 10^3 ng/L, respectively (Mwenesongole, 2015). Thus, it is worth collecting more water samples from Cambridge and further investigating whether or not these cathinones are present in drinking water.

5.2.1.3.3 Piperazines

According to the information supplied by Cambridgeshire Constabulary, piperazines were also one of the highest seized drugs in Cambridge in 2010 (Mwenesongole, 2015). However, this research has failed to detect all seven piperazines in the drinking water from the East Anglia region of the UK. In two other published studies, BZP and 3-TFMPP were detected in both waste and surface waters in the UK (Baker and Kasprzyk-Hordern, 2011b; Mwenesongole, 2015). Therefore, piperazines are known to be present in the UK's waste water and have even contaminated surface water. Therefore, it is surprising that this research did not detect piperazines in drinking water, even though their MDLs were low when using this method described in this research, such as 0.0851 ng/L for BZP and 0.0149 ng/L for 3-TFMPP (Table 4.15). Hence, more drinking water samples are needed for further analysis, either from this area or other regions of the UK.

5.2.1.3.4 Synthetic cannabinoids

Synthetic cannabinoids are preferred by some drug users as they can mimic the action of cannabis and may also not be picked up in a positive drug test result (UNODC, 2015).

Based on the seizure data reported by EMCDDA (2015c), over 21,000 seizures of synthetic cannabinoids amounting to 1.6 tonnes were found across Europe in 2013, which accounted for almost 40 % of the total number of seizures for NPS. However, synthetic cannabinoids have not been reported to be present in waste and surface waters. In this research, JWH-073 and JWH-398 were included in the analytical method (Table 1.3), but were not detected in drinking water samples from the East Anglia region of the UK. This is probably because these two synthetic cannabinoids had relatively higher MDLs (0.4119 ng/L for JWH-073 and 0.4673 ng/L for JWH-398, as shown in Table 4.15). Thus, it is worth carrying out a further study to enhance the sensitivity of the method for synthetic cannabinoids.

5.2.2 Drugs of abuse and pharmaceuticals detected in raw water from the East Anglia region of the UK

Four studied drugs of abuse and pharmaceuticals detected in drinking water samples were also found in raw water samples, including methamphetamine, mephedrone, ketamine and fluoxetine, and their concentrations ranged from 1.761 ng/L for methamphetamine to 11.199 ng/L for ketamine (Table 5.3). The results of raw water samples were then used for calculating the removal efficiencies of these analytes during DWTPs and are further discussed in Section 5.4. It is worth noting that methylone, cocaine and citalopram were only detected in drinking water samples collected from taps in Cambridge and therefore the removal efficiencies were not calculated. In one collection from DWTP 1, cocaine was detected in the drinking water, but not in the raw water sample. This may be because the samples were collected at the same time and residence time was not considered during the sampling. This is discussed further in Section 5.4.3.

5.3 Comparison of drugs of abuse and pharmaceuticals in drinking water with those detected in other countries

In this section, drugs of abuse and pharmaceuticals detected in drinking water from this research are compared to those from published literature, which could reflect their presence on a global scale.

Two traditional illicit drugs (cocaine, methamphetamine), two antidepressants (citalopram and fluoxetine) and one NPS (ketamine) were determined in drinking water from the East Anglia region of the UK in this research (Table 5.3) and they have also been quantified in drinking water from other countries. Available data including sample collection site, study period, detected compounds and their concentrations from this research and published references are presented in Table 5.4.

Table 5.4: Concentrations of drugs of abuse and pharmaceuticals detected in drinking water from different countries

COMPOUND	COLLECTION SITE	STUDY PERIOD	CONC. / ng/L	REFERENCES
Cocaine	Canada	2012	4.3 [c]	Rodayan, et al., 2016
	Europe [a]	2008 - 2009	0.1 [c]	Boleda, et al., 2011
	Japan	2008 - 2009	< 0.1 [d]	Boleda, et al., 2011
	Latin America [b]	2008 - 2009	0.6 [c]	Boleda, et al., 2011
	Spain	2008 - 2009	0.4 [c]	Boleda, et al., 2011
	Spain	2012	1.61	Mendoza, et al., 2014
	Spain	2013	0.11 - 85.67 [e]	Mendoza, et al., 2016
	UK	**2016**	**0.185 - 0.836 [e]**	**This Research**
Methamphetamine	Latin America [b]	2008 - 2009	< 0.5 [d]	Boleda, et al., 2011
	Spain	2008 - 2009	< 0.5 [d]	Boleda, et al., 2011
	Spain	2013	3.13	Mendoza, et al., 2016
	UK	**2016**	**2.206**	**This Research**
Ketamine	Canada	2012	15.0 [c]	Rodayan, et al., 2016
	UK	**2016**	**0.139 - 1.124 [e]**	**This Research**
Citalopram	Poland	2013	1.5 [f]	Giebułtowicz and Nałęcz-Jawecki, 2014
	UK	**2016**	**2.257 - 2.800 [e]**	**This Research**
Fluoxetine	China	2014	0.1 - 0.2 [e]	Wu, et al., 2015
	Spain	2009	2.74 [c]	López-Serna, et al., 2010
	USA	2006 - 2007	0.59 - 0.82 [e]	Benotti, et al., 2009
	USA	2009 - 2010	19.2 [f]	Padhye, et al., 2014
	USA	n.r.	< 0.5 [d]	Vanderford and Snyder, 2006
	UK	**2016**	**0.270**	**This Research**

n.r., not reported

[a] Includes Austria, France, Germany, Iceland, Slovakia, Switzerland and the UK; [b] Includes Argentina, Brazil, Chile, Colombia, Panama, Peru and Uruguay; [c] Mean concentration; [d] Mean concentration below quantification limit but above detection limit; [e] Concentration range; [f] Maximum concentration

As mentioned in Section 1.6, the occurrence data of drugs of abuse and pharmaceuticals in drinking water is limited. Hence, only ten published references are included in Table 5.4 for the comparison of their presence on a global scale. These are discussed in more detail in the following sub-sections. Only one research group in this table has analysed two drinking water samples collected from the UK (Boleda, et al., 2011). However, concentration results obtained from Boleda, et al. (2011) were the mean concentration of 15 samples collected from seven European countries. No information is available on their concentrations in drinking water from the UK only. Therefore, no conclusions regarding the occurrence of drugs of abuse and pharmaceuticals can be drawn on the UK.

Two NPS (methylone and mephedrone) were also detected in drinking water from this research (Table 5.3). However, there have been no other studies conducted on the analysis of methylone and mephedrone in drinking water before. Therefore, no data is available that allows for a comparison of their presence within the UK or globally.

5.3.1 Traditional illicit drugs

In this research, two traditional illicit drugs (methamphetamine and cocaine) were detected in drinking water from the East Anglia region of the UK. As shown in Table 5.4, methamphetamine was detected at the concentration of 2.206 ng/L from the UK in 2016, which is significantly higher than that reported from Spain (< 0.5 ng/L) and Latin American countries (< 0.5 ng/L) between 2008 and 2009. This could be due to the eight-year gap between this research and the publication by Boleda, et al. (2011). According to the EMCDDA (2014b) report, various European countries have seen an increase in the use of methamphetamine since 2012. Therefore, this might be the reason why higher concentrations of methamphetamine are being detected in the UK (2.206 ng/L). In addition, this correlates well with the results from Mendoza, et al. (2016) where 3.13 ng/L of this traditional illicit drug were reported in Spain in 2013.

For cocaine, the result for the UK (0.185 - 0.836 ng/L) is comparable to Japan (< 0.1 ng/L), Latin American countries (0.6 ng/L), Spain (0.4 ng/L) and other European countries (0.1

ng/L). However, a concentration of 1.61 ng/L was measured for this traditional illicit drug in Spain by another research group (Mendoza, et al., 2014), which showed a higher concentration than other published values as well as the result of this research. This is interesting, since the concentrations of cocaine in Spain are quite dissimilar in two publications (Boleda, et al., 2011; Mendoza, et al., 2014). This could be explained by differences in cocaine use in certain parts of Spain, as drinking waters were only collected from central Spain by Mendoza, et al. (2014), although Boleda, et al. (2011) sampled drinking water from all over the country. Rodayan, et al. (2016) have also reported a higher concentration of cocaine (4.3 ng/L) in the drinking water of Canada, which could be associated with less efficient water treatments (clarification and post-chlorination) being applied in the studied DWTP. In addition, a significantly higher concentration of cocaine (85.67 ng/L) has been reported in Spain by Mendoza, et al. (2016), which could be due to the dumping of large amounts of cocaine at or near to the sampling sites. As cocaine is largely metabolised by a carboxylesterase reaction to benzoylecgonine (20 - 60 %) and only 1 - 15 % is excreted unchanged as the parent compound in urine, the ratio of cocaine to benzoylecgonine should be below 0.75 when measured concentrations result from human consumption (Castiglioni, et al., 2008; Van Nuijs, et al., 2009). However, the concentration ratio of cocaine to benzoylecgonine was 1.62 in this publication (Mendoza, et al., 2016), which is considered as an abnormal ratio (> 0.75), suggesting that the measured value may not only result from human consumption (Van Nuijs, et al., 2009).

5.3.2 Antidepressants

To date, a handful of publications have included these two antidepressants in drinking water analysis. Five of them reported the detection of fluoxetine in China, Spain and the USA and only one publication reported the detection of citalopram in Poland (Vanderford and Snyder, 2006; Benotti, et al., 2009; López-Serna, et al., 2010; Giebułtowicz and Nałęcz-Jawecki, 2014; Padhye, et al., 2014; Wu, et al., 2015). From the results presented in Table 5.4, the concentration range of citalopram in drinking water for the UK (2.257 - 2.800 ng/L) is higher than that for Poland (1.5 ng/L). This difference is not surprising, considering that the prescribing patterns of antidepressants vary throughout the world, with

the UK having the sixth highest level of consumption in 2013 (Organisation for Economic Co-operation and Development, OECD, 2015).

A study conducted in the southeastern region of the USA reported the highest concentration of fluoxetine in drinking water (19.2 ng/L), followed by Spain (2.74 ng/L) and the Nevada state of the USA (< 0.50 ng/L). In addition, the concentration range of this antidepressant in the USA (0.59 - 0.82 ng/L) could be found in the publication by Benotti, et al. (2009), as drinking water samples were collected from 19 DWTPs across the USA. Based on these results, a conclusion might be drawn that the concentration of fluoxetine in drinking water from Spain (2.74 ng/L) is higher than its concentration range in the USA (0.59 - 0.82 ng/L), but is remarkably lower than that from some regions of the USA, such as the southeastern region (19.2 ng/L). This also correlates with the regional trends in mental health medication use in the USA (World Health Organization, 2011). In this report, the regions with the highest users of mental health medications were in the east south central section of the USA. In this research, fluoxetine was also detected in drinking water from the East Anglia region of the UK and its concentration (0.270 ng/L) is similar to that found in China (0.1 - 0.2 ng/L). It is interesting that this antidepressant is present in drinking water at such low concentration, because the UK reported a high consumption level of antidepressants in 2013 (OECD, 2015). However, with high removal efficiency (89.91 %), as discussed in Section 5.4.2, this may further explain the low concentration detected in this research.

5.3.3 Ketamine

Ketamine was also determined in drinking water in this research, the concentration range being from 0.139 ng/L to 1.124 ng/L. The detection of ketamine in drinking water has only been reported in Canada by Rodayan, et al. (2016) at a higher concentration of 15.0 ng/L. This could be due to low removal efficiency of ketamine during the studied DWTP (49 %), as only clarification and post-chlorination were applied (as mentioned in Section 5.3.1).

5.4 Removal efficiencies of drugs of abuse and pharmaceuticals during drinking water treatment plants from the East Anglia region of the UK

This section summarises and discusses the removal efficiency results of drugs of abuse and pharmaceuticals during DWTPs. In this research, raw water and drinking water were collected at the same time from three different DWTPs in the East Anglia region of the UK and four studied drugs of abuse and pharmaceutical were detected in both raw and drinking water samples. This includes methamphetamine, mephedrone, ketamine and fluoxetine. Their concentrations were used for the calculation of the removal efficiency of DWTPs. Table 5.5 lists the removal efficiencies of these four studied drugs of abuse and pharmaceuticals, which was calculated using the following Equation 5.1.

Removal % = [(Concentration of analyte in raw water - Concentration of analyte in drinking water)/Concentration of analyte in raw water] x 100 %

(Equation 5.1)

Table 5.5: Concentrations of drugs of abuse and pharmaceuticals detected in raw and drinking waters and their removal efficiencies of three DWTPs from the East Anglia region, UK

	COMPOUND			
	Methamphetamine	**Mephedrone**	**Ketamine**	**Fluoxetine**
DWTP 1				
Raw Water Conc. / ng/L	1.761	6.471	11.199	n.d.
Drinking Water Conc. / ng/L	2.206	1.871	0.139	n.d.
Removal / %	-25.27	71.09	98.76	n.a.
DWTP 2				
Raw Water Conc. / ng/L	n.d.	n.d.	n.d.	2.675
Drinking Water Conc. / ng/L	n.d.	n.d.	n.d.	0.270
Removal / %	n.a.	n.a.	n.a.	89.91
DWTP 3				
Raw Water Conc. / ng/L	n.d.	5.742	6.217	n.d.
Drinking Water Conc. / ng/L	n.d.	2.814	0.977	n.d.
Removal / %	n.a.	50.99	84.29	n.a.

n.d., not detected; n.a., not available

In Table 5.5, the higher removal efficiencies were obtained for ketamine (84.29 and 98.76 %), followed by fluoxetine (89.91 %), which indicate that the applied drinking water treatment methods were more efficient in removing them from water. This corresponds with low concentrations of ketamine and fluoxetine detected in drinking water samples (0.139 - 0.977 ng/L and 0.270 ng/L, respectively, as shown in Table 5.5). Removals were reported as 50.99 and 71.09 % for mephedrone, thus relatively higher concentrations were detected in drinking water at 1.871 and 2.814 ng/L (Table 5.5). In addition, the concentration of methamphetamine in drinking water (2.206 ng/L) was higher than its concentration in raw water (1.761 ng/L), resulting in a negative removal from DWTP (-25.27 %). This is discussed further in Section 5.4.3.

5.4.1 Ketamine

In this research, the removal efficiencies for ketamine were obtained from DWTP 1 and DWTP 3 (Table 5.5). Surface water was used as raw water for these two DWTPs as they employ a similar water treatment process, which consists of pre-treatment, pre-ozonation, clarification, post-ozonation, GAC filtration and post-chlorination. These water treatment methods are described in detail in Section 1.5. For DWTP 1, ammonia and phosphate were also dosed during the secondary disinfection stage to maintain a certain pH range and provide a protective film for lead pipes in order to minimise the likelihood of lead being present in drinking water (Maine Water Utilities Association, 2010; Drinking Water Inspectorate, 2014).

High removal efficiencies (84.29 - 98.76 %) were observed for ketamine in this research (Table 5.5), as this compound contains a secondary amine and a nonaromatic double bond (Table 1.3), which are the sites reactive to ozone and chlorine (Section 1.5.2 and Section 1.5.4). This is consistent with findings from the study by Boleda, Galceran and Ventura (2011). This research group has evaluated the removal efficiency of ketamine in a Spanish DWTP (surface water treatment works). These treatment methods consist of pre-chlorination, clarification, post-ozonation, GAC filtration and post-chlorination. Similar removal efficiency (92 %) was reported for ketamine, even though chlorine was applied in

the pre-oxidation stage instead of ozone. In addition, another surface water treatment works was also used for the evaluation of removal efficiency in this publication. Ketamine was almost completely removed (98 % removal) by a series of water treatments, including pre-chlorination, clarification, ultrafiltration, ultraviolet (UV) disinfection, reverse osmosis, remineralisation and post-chlorination. UV disinfection, membrane filtration (ultrafiltration and reverse osmosis) and remineralisation were applied in this DWTP instead of post-ozonation and GAC filtration. The results show that these water treatments are slightly more efficient at eliminating ketamine from water.

5.4.2 Fluoxetine

The removal efficiency of fluoxetine was only calculated based on the data from DWTP 2 (Table 5.5). Applied water treatments include aeration, filtration, chlorine disinfection and phosphate dosing. Fluoride was also added at the final step as a protection against tooth decay (Drinking Water Inspectorate, 2014). As this is a ground water treatment works, treatments are designed to remove dissolved gases, iron as well as manganese, while some oxidation processes are normally not required, such as ozonation and chlorination (Reddersen, Heberer and Dünnbier, 2002; Zwiener, 2007).

DWTP 2 in this research has largely eliminated the fluoxetine from water with the removal efficiency of 89.91 % (Table 5.5). This is because chlorine can react rapidly with the secondary amine of this pharmaceutical (Table 1.3), which indicates that chlorination was effective for the removal of fluoxetine. However, a lower removal was reported by Padhye, et al. (2014). An American DWTP (surface water treatment works), which consists of pre-ozonation, clarification, post-ozonation, media filtration and post-chlorination, provided a 66.7 % removal for fluoxetine in this publication. It is surprising that ozonation and chlorination treatments only removed two thirds of this compound from water, as its secondary amine functional group is the site reactive to ozone and chlorine (Section 1.5.2 and Section 1.5.4). This could be due to different raw water sources. Ground water was used as raw water for DWTP 2 in this research and surface water was used for the American DWTP (Padhye, et al., 2014).

5.4.3 Methamphetamine

In a study conducted in Spain, the removal efficiency of methamphetamine in a surface water treatment works was evaluated by Huerta-Fontela, Galceran and Ventura (2008). Methamphetamine was totally eliminated (100 % removal), as this compound contains a secondary amine (Table 1.3), which is the site that is reactive to both ozone and chlorine (Section 1.5.2 and Section 1.5.4) and therefore explains why it was not detected in drinking water. Water treatment methods for this DWTP include pre-treatment, pre-chlorination, clarification, post-ozonation, GAC filtration and post-chlorination. This research also monitored the removal of drugs of abuse and pharmaceuticals in DWTP 1, which used water treatments similar to the above-mentioned Spanish DWTP (as mentioned in Section 5.4.1). However, negative removal efficiency (-25.27 %) was observed for methamphetamine (Table 5.5). It is unusual to obtain a higher concentration in drinking water compared to that of raw water. An explanation for the negative removal of methamphetamine could be associated with residence time (Andrés-Costa, et al., 2014; Du, et al., 2015). In this research, raw and drinking waters were collected at the same time from the DWTP 1. As a result, there was a mismatch in timing between these two water samples, as it doesn't compensate for the time delay during treatments, i.e. residence time. If a pulse of low concentration for methamphetamine occurs in raw water during this mismatch and is collected for removal calculation, drinking water concentration might be higher and negative removal could be observed. The negative removal efficiency of methamphetamine has also been reported in a Chinese WWTP due to the same reason (Du, et al., 2015). Thus, raw and drinking waters should be re-collected from DWTP 1 and analysed in order to verify these results. In addition, it is better to collect samples from the same location over a specific time period in order to get a representative sample with average water conditions, which may result in more accurate quantification in the future.

5.4.4 Mephedrone

In this research, two removal efficiencies of mephedrone (71.09 and 50.99 %) were obtained from DWTP 1 and DWTP 3, respectively (Table 5.5), as this drug was detected in both raw and drinking water samples. Water treatments applied in these two DWTPs are discussed in Section 5.4.1. Mephedrone has the secondary amine and nonaromatic double bond as electron-donating functional groups (Table 1.3), thus this compound can undergo a rapid reaction with ozone and chlorine (Section 1.5.2 and Section 1.5.4), which removed more than half of the total amount of mephedrone from water. However, as far as the author is aware, the removal efficiency of DWTP has been reported for mephedrone for the first time and hence no comparative results are available in the literature.

5.5 Overall discussion and conclusion of drinking water analysis

Raw water (three samples) and drinking water (five samples) were collected from the East Anglia region of the UK (Section 2.5), as this sampling site has never been investigated before. All water samples were extracted three times by SPE and analysed in triplicate by LC-MS using a C_{18} column for identifying the studied drugs of abuse and pharmaceuticals. All were then confirmed by LC-MS using an additional biphenyl column. The identification and confirmation of detected target analytes were based on the quantifier and confirmation ions and the acceptable retention index difference between the water sample and positive control (± 1.00 %) (Section 5.1.1 and Section 5.1.2). After that, quantification was conducted using a standard addition method in order to compensate for matrix effects for LC-MS analysis. Thus, water samples were spiked with mixed standards containing the studied drugs of abuse, pharmaceuticals and internal standards, which were used as calibrators. Linear regression equations were generated for each analyte by plotting the mean PARs of one non-spiked sample and three spiked samples and the concentrations of detected target analytes were calculated based on the obtained equations (Section 5.1.3).

The detected drugs of abuse and pharmaceuticals present in raw and drinking water samples that were collected from the East Anglia region of the UK are shown in Table 5.6. These include two traditional illicit drugs (methamphetamine and cocaine), two

antidepressants (citalopram and fluoxetine) and three NPS (methylone, mephedrone, ketamine). In this research, the concentrations of seven studied drugs of abuse and pharmaceuticals found in the drinking water of the UK were all at trace levels (from 0.139 ng/L for ketamine to 2.814 ng/L for mephedrone). They were then compared with published references from other countries in order to understand their presence on a global scale. The concentrations of methamphetamine, cocaine, citalopram, fluoxetine and ketamine in drinking water vary between countries. These differences correlate with their global and regional consumption rates and patterns as well as the removal efficiencies of DWTPs. Moreover, it is worth noting that methylone and mephedrone have been determined in drinking water for the first time, which indicates NPS are already present in drinking water due to their high consumption levels in the UK.

Removal efficiencies of detected drugs of abuse and pharmaceuticals were evaluated for three DWTPs from Anglian Water and Essex and Suffolk Water, which are shown in Table 5.6. High removals were observed for fluoxetine (89.91 %) and ketamine (84.29 and 98.76 %), which are consistent with the reported removals in published literature. In contrast, mephedrone cannot be largely removed during DWTPs (50.99 and 71.09 %) and thus relatively higher concentrations (1.871 and 2.814 ng/L) were detected in drinking water. To the author's knowledge, the removal of DWTP has been reported for mephedrone for the first time. In addition, negative removal (-25.27 %) was obtained for methamphetamine as its concentration in drinking water is higher than in raw water. This is unusual and needs more investigations, as 100 % of removal for this traditional illicit drug has been reported in a Spanish DWTP, which used water treatments similar to the DWTP studied in this research (Huerta-Fontela, Galceran and Ventura, 2008). The findings in this research highlight the need for investing the more effective water treatments to remove drugs of abuse and pharmaceuticals from drinking water.

Table 5.6: Summary of drinking water analysis results

COMPOUND	CONC. IN RAW WATER / ng/L	CONC. IN DRINKING WATER / ng/L	REMOVAL EFFICIENCY OF DWTPs / %
Methylone	n.d.	1.368	n.a.
Methamphetamine	1.761	2.206	-25.27
Mephedrone	5.742 - 6.471	0.767 - 2.814	50.99 - 71.09
Ketamine	6.217 - 11.199	0.139 - 1.124	84.29 - 98.76
Cocaine	n.d.	0.185 - 0.836	n.a.
Citalopram	n.d.	2.257 - 2.800	n.a.
Fluoxetine	2.675	0.270	89.91

n.d., not detected; n.a., not available

CHAPTER 6 CONCLUSION AND FURTHER WORK

This chapter summarises the conclusions arising from a variety of studies undertaken in Chapter 3 (method development and optimisation), Chapter 4 (method validation) and Chapter 5 (drinking water analysis).

6.1 Conclusion

As the chemical industry, including the production of agrochemical, industrial and consumer chemicals, has expanded around the globe, the contamination of water sources has inevitably spread as a result of various human activities (Harrison, 2014). The presence of pollutants and contaminants in drinking water has led to increasing public attention and scientific interest regarding their effects on human health, because drinking water provides a direct route into the human body for any drug compounds that might be present. In recent years, scientific interest has focused on the study of drugs of abuse and pharmaceuticals in drinking water, as these water contaminants are biologically active and may induce adverse effects on human health. To date, there are only a few studies that focus on the occurrence and concentrations of traditional illicit drugs and pharmaceuticals in drinking water, most probably because they are present at sub ng/L levels or less and thus cannot be detected by most analytical methods. In addition, NPS such as cathinones and piperazine have also been found in surface water. As surface water is used as raw water for drinking water production, it is not surprising that NPS could be present in drinking water due to incomplete removal during DWTPs. As there have not been any analytical methods developed to detect and quantify this suite of drugs of abuse in drinking water, selective and sensitive analytical methods are needed for the determination of drugs of abuse, especially for NPS, and pharmaceuticals in drinking water.

This research aimed to develop and validate analytical methods for the simultaneous determination of 20 drugs of abuse and pharmaceuticals based on using SPE for sample preparation, followed by LC-MS as the detection and quantification technique. The selected compounds belong to a large spectrum of chemical classes, namely cocainics, amphetamines, dissociative anaesthetics, cathinones, piperazines, synthetic cannabinoids and antidepressants.

LC-MS methods were developed and optimised using a C_{18} column and a biphenyl column. Mobile phase pH (2.1), organic modifier (acetonitrile for the C_{18} column and a mixture of methanol and acetonitrile for the biphenyl column) and time segmentation (ten segments) were chosen, while diagnostic ions, DL and lens system voltages were also investigated in order to achieve good chromatographic separations and enable reliable mass identification. The C_{18} column was used for identification and quantification, while the biphenyl column was used for confirmation when samples were suspected of containing the studied drugs of abuse and pharmaceuticals. Moreover, in order to determine which SPE cartridge would be most appropriate for the extraction of the studied drugs of abuse and pharmaceuticals from drinking water, Oasis MCX and Strata-X-Drug B were compared. Strata-X-Drug B was selected based on high and reliable recoveries for most analytes of interest. Elution solvents were further investigated to improve the recovery. The optimised elution solvents finally resulted in the use of ethyl acetate/isopropanol (85:15, v/v), followed by ethyl acetate/isopropanol/ammonium hydroxide (70:20:10, v/v). After determining the applied SPE protocol, sample loading volume was optimised and 200 mL was chosen in order to increase the chances of detecting and quantifying the studied drugs of abuse and pharmaceuticals in water samples. Moderate to high recoveries (65 - 107 %) were achieved for the majority of the studied drugs of abuse and pharmaceuticals and good precisions (RSD < 15 %) were obtained for all analytes using a Strata-X-Drug B cartridge.

Following on from the studies of method development and optimisation, method validation was conducted in order to prove that these analytical methods were selective, sensitive and capable for the purpose of identification, quantification and confirmation. Key

performance tests were undertaken for the LC-MS method using a C_{18} column, which included selectivity, autosampler storage stability, instrumental linearity, precision, accuracy, instrumental and method detection and quantification limits. This method was selective enough to simultaneously identify 20 studied drugs of abuse and pharmaceuticals based on their retention times and diagnostic ions. This method showed good instrumental linearity for all target analytes of interest over four to five orders of magnitude. Method accuracy was reported below ± 8.66 % bias of true value at low, medium and high concentrations. The RSDs of instrumental and method precision were below 7.57 % at medium and high concentrations and below 15.04 % at low concentration. These results prove that this method was good for the purpose of quantification. Low detection and quantification limits were obtained for all studied drugs of abuse and pharmaceuticals using LC-MS (0.0110 - 0.9253 ng/mL for IDL, 0.0366 - 3.0844 ng/mL for IQL, 0.0056 - 1.0918 ng/L for MDL and 0.0187 - 3.6394 ng/L for MQL) and are consistent with or in some cases lower than previously published LC-MS/MS methods. This indicates that the potential of this newly developed LC-MS method using the C_{18} column to determine the studied drugs of abuse and pharmaceuticals is down to trace levels. As a result of autosampler storage stability, it is suggested to store mixed standards as well as water samples in the LC-MS injection solvent for up to five days. In addition, selectivity and instrumental detection limit were also validated for the LC-MS method using a biphenyl column. Results show that this developed method allows for the identification of the studied drugs of abuse and pharmaceuticals and can separate all analytes of interest based on their retention times and diagnostic ions. Furthermore, the method was sensitive enough for confirmation, as IDLs for all target analytes were 0.0115 to 0.4795 ng/mL, which are comparable to their IDLs and IQLs using a C_{18} column.

The analytical methods reported in this research are novel, as this is the first time a LC-MS method has been developed and validated for the identification and quantification of 14 NPS in drinking water, including five cathinones, seven piperazines and two synthetic cannabinoids. In addition, this research has proved the capability of LC-MS as a cheaper and useful alternative to LC-MS/MS in the multi-residue analysis of drugs of abuse and

pharmaceuticals in drinking water at trace levels (sub ng/Ls). This demonstrates that those laboratories that only have LC-MS can also conduct drinking water research, which is normally undertaken by LC-MS/MS.

The analytical method using a C_{18} column was successfully applied to raw water (three samples) and drinking water (five samples), which were collected from three DWTPs and two taps in the East Anglia region of the UK. To the author's knowledge, this sampling site has never been studied for the determination of drugs of abuse and pharmaceuticals in raw and drinking waters. When samples were suspected of containing the studied drugs of abuse and pharmaceuticals, the analytical method using a biphenyl column was used for confirmation. The identification and confirmation criteria were fulfilled, as three identification points were obtained, namely (1) one retention index obtained from a C_{18} column, (2) one retention index obtained from a biphenyl column and (3) one ion monitored in SIM mode for both the C_{18} column and biphenyl column. The standard addition method was used for calibration in order to compensate for any matrix interference and hence calibrators were prepared within the same matrix as the samples.

Seven studied drugs of abuse and pharmaceuticals were detected in drinking water samples above their MQLs, including methylone (1.368 ng/L), methamphetamine (2.206 ng/L), mephedrone (0.767 - 2.814 ng/L), ketamine (0.139 - 1.124 ng/L), cocaine (0.185 - 0.836 ng/L), citalopram (2.257 - 2.800 ng/L) and fluoxetine (0.270 ng/L). The findings from this research make a positive contribution to identify and recognise the ever-changing composition of water contaminants in drinking water. This is the first time, to the author's knowledge, that methylone and mephedrone have been determined in drinking water samples, which indicates these two NPS have already been present in drinking water due to their high consumptions in the UK. This research could inform drinking water regulatory bodies of the presence of drugs of abuse and pharmaceuticals, as they are currently not included within the regulatory framework. Moreover, the presence of methamphetamine, cocaine, citalopram, fluoxetine and ketamine in the UK's drinking water (this research) is compared to published references from other countries, which can reflect their presence

on a global scale. The concentrations of these traditional illicit drugs, antidepressants and NPS in drinking water vary between countries and these differences can be due to their different consumption patterns as well as different removal efficiencies of DWTPs.

In addition, the removal efficiencies of DWTPs were calculated for four studied drugs of abuse and pharmaceuticals, as they have been quantified in both raw water and drinking water. These are methamphetamine (-25.27 %), mephedrone (50.99 - 71.09 %), ketamine (84.29 - 98.76 %) and fluoxetine (89.91 %). This is in response to concerns that current treatment methods do not remove some of these contaminants and thus highlights the need for investing more effective water treatments to remove drugs of abuse and pharmaceuticals from drinking water.

6.2 Suggestions for further work

Although the LC-MS methods used in this research were able to identify and quantify the studied drugs of abuse and pharmaceuticals at trace levels, further work will be required to reduce the total analysis time in order to achieve high sample throughput, which is of importance for routine analysis. The total analysis times for the C_{18} column and biphenyl column were 44 min and 50 min, respectively, with an equilibration time of 20 min. Equilibration time is used to equilibrate the column to the initial column pressure and mobile phase composition and thus allow the column to be ready for subsequent runs. Enough equilibration is critical for running the gradient elution in order to ensure the reproducible retention times of analytes from run to run (Kazakevich and LoBrutto, 2007). Normally, it is suggested that ten column volumes of mobile phase are required for sufficient equilibration. Therefore, a small column internal diameter or a shorter length column can reduce the column volume, resulting in a shorter equilibration time (Separation Science, 2015). Hence, the LC-MS methods developed in this research can be further optimised using a column with a smaller column volume to decrease the waiting time.

Furthermore, the confirmation of the identity of the analytes present in water samples needs to be improved. As drinking water is variable in composition, matrix components that

share the same diagnostic ions with the analytes may be present and could lead to the false positive results. Although two retention indexes and one diagnostic ion were used for identification and confirmation, it is still recommended to monitor more ions and calculate the ion ratios in order to provide further confirmation of the identity of the analytes present. The application of using the ion ratios could help with analyte identification and confirmation, as it is unlikely that a matrix component present in drinking water would co-elute with an analyte and also share the same ion ratio with the analyte.

The LC-MS method using a biphenyl column was only validated for selectivity and instrumental detection limit as it was used for the purpose of confirmation. Other validation studies would be worth investigating, as this method has the potential for the quantification of trace drugs of abuse and pharmaceuticals in drinking water. Moreover, the chemistry of the biphenyl column allows for the combination of hydrophobic, polar and aromatic selectivity, which cannot be offered by the commonly used C_{18} column (Phenomenex, 2016). For example, in this research, the separation of one set of positional isomers (3-TFMPP and 4-TFMPP) using the biphenyl column is better than the C_{18} column. Thus, the biphenyl column is also a good choice for the analysis of drugs of abuse and pharmaceuticals as many of them exhibit aromatic rings, although it has been much less used compared to the C_{18} column. In addition, these two novel LC-MS methods need to be further validated, such as the robustness study, which will enhance the reliability of operating the methods by different analysts and different LC-MS instruments and prove their transferrable between laboratories.

In this research, water samples were extracted by SPE within 12 hours of collection. This is because drugs of abuse and pharmaceuticals may start degrading after 24 hours of collection, as informed by the published literature (Togola and Budzinski, 2008; Boleda, et al., 2011; Valcárcel, et al., 2011; Valcárcel, et al., 2012). Sample extracts were then dissolved in LC-MS injection solvent and stored in an autosampler until analysis. Thus, matrix-based stability was not considered and only the stability of analytes in the LC-MS injection solvent was studied. However, if water samples are collected from other areas

within the UK and other countries and arrive at the laboratory after 24 hours, the study of long-term matrix-based stability is required in order to continue refining the sample storage conditions during transport.

During SPE studies, the larger loading volume of water samples, such as 500 mL or 1000 mL, will be worth investigating if automatic SPE manifold is available. The larger the loading volume extracted, the higher the enrichment factor obtained, thereby resulting in increased method sensitivity and enhanced chances of determining the studied trace drugs of abuse and pharmaceuticals in drinking water.

In this research, drinking water samples were collected from only five sampling points in the East Anglia region of the UK, thus more samplings in this area as well as in other cities within the UK are needed in order to investigate the spatial and temporal occurrence of the studied drugs of abuse and pharmaceuticals in drinking water. Residence time should be considered when collecting raw and drinking water samples from DWTPs, which may result in a more accurate calculation of removal efficiency. In addition, it would still be worth evaluating the removal efficiency of the different steps of drinking water treatments by collecting and analysing samples before and after each treatment process. It is hoped that the results could provide valuable information about the behaviour of drugs of abuse and pharmaceuticals through drinking water treatments and help the drinking water companies and scientists to invest in effective treatment processes.

The result of this research reveals that some studied drugs of abuse and pharmaceuticals (cocaine, methamphetamine, citalopram, fluoxetine, ketamine, mephedrone and methylone) are present in drinking water. Therefore, it is important to study their metabolites in order to fully understand the transportation of drugs of abuse and pharmaceuticals through DWTPs and the potential of human exposure. The health impacts of these compounds in drinking water have been discussed in Section 1.7. The presence of their metabolites could pose an additional threat to humans, as some will still be pharmacologically active or even more potent than the parent compounds. For example,

morphine-6-glucuronide (an active metabolite of morphine) is more potent than its parent compound as an analgesic (Spiehler and Levine, 2003). In addition, the study of emerging drugs of abuse and pharmaceuticals (such as NPS) in drinking water could also further enhance this research area by helping to identify and recognise the ever-changing composition of such contaminants in drinking water.

If trace amounts of drugs of abuse, pharmaceuticals and their metabolites are being detected in drinking water, there is a need for further research exploring their bioaccumulation and possible drug-drug reactions. If these compounds do have an impact on health, even if in trace amounts, the future screening of such compounds need to be carried out to inform drinking water regulatory bodies. The findings could also aid in the development of water treatments for their removal in order to deliver a sustainable and safe drinking water. It is well documented that the analytical science has an important and challenging role in the management of good quality drinking water, where this research contributes by developing a LC-MS based method of testing for drugs of abuse and pharmaceuticals in drinking water. Other future challenges include the development of treatment methods to address these newer contaminants, as well as creating fast and more economical analytical methods (LC-MS instead of tandem MS) which allow for the simultaneous detection of emerging drugs of abuse and pharmaceutical compounds.

REFERENCES

Advisory Council on the Misuse of Drugs, 2010. *Consideration of the cathinones*. [pdf] London: Advisory Council on the Misuse of Drugs. Available at: <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/119173/acmd-cathinodes-report-2010.pdf> [Accessed 5 November 2016].

Agilent Technologies, 2011. *Agilent Triple Quadrupole LC/MS Quantitation of Pesticides*. [pdf] Santa Clara: Agilent Technologies. Available at: <http://www.ingenieria-analitica.com/downloads/dl/file/id/2990/product/110/triple_quadrupole_lc_ms_quantitation_of_pesticides_workflow_guide.pdf> [Accessed 5 November 2016].

Aherne, G. W., Hardcastle, A. and Nield, A. H., 1990. Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. *Journal of Pharmacy and Pharmacology*, 42 (10), pp. 741-742.

Alonso, S. G., Catalá, M., Maroto, R. R., Gil, J. L. R., de Miguel, Á. G. and Valcárcel, Y., 2010. Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain). *Environment International*, 36 (2), pp. 195-201.

Ammann, J., McLaren, J. M., Gerostamoulos, D. and Beyer, J., 2012a. Detection and Quantification of New Designer Drugs in Human Blood: Part 1 – Synthetic Cannabinoids. *Journal of Analytical Toxicology*, 36 (6), pp. 372-380.

Ammann, D., McLaren, J. M., Gerostamoulos, D. and Beyer, J., 2012b. Detection and Quantification of New Designer Drugs in Human Blood: Part 2 – Designer Cathinones. *Journal of Analytical Toxicology*, 36 (6), pp. 381-389.

Anderson, W. H., 2003. Therapeutic Drugs II: Antidepressant Drugs. In: B. Levine, ed. 2003. *Principles of Forensic Toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.18.

Andrés-Costa, M. J., Rubio-López, N., Suárez-Varela, M. M. and Pico, Y., 2014. Occurrence and removal of drugs of abuse in Wastewater Treatment Plants of Valencia (Spain). *Environmental Pollution*, 194, pp. 152-162.

Armbruster, D. A. and Pry, T., 2008. Limit of Blank, Limit of Detection and Limit of Quantitation. *The Clinical Biochemist Reviews*, *29* (i), pp. 49-52.

Aydinalp, C. and Porca, M. M., 2004. The effects of pesticides in water resources. *Journal of Central European Agriculture*, 5 (1), pp. 5-12.

Baker, D. R. and Kasprzyk-Hordern, B., 2011a. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1218 (44),

pp. 8036-8059.

Baker, D. R. and Kasprzyk-Hordern, B., 2011b. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A*, 1218 (12), pp. 1620-1631.

Baker, D. R. and Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. *Science of the Total Environment*, 454-455, pp. 442-456.

Bawle, E. V., Conard, J. V. and Weiss, L., 1998. Adult and Two Children With Fetal Methotrexate Syndrome. *Teratology*, 57, pp. 51-55.

Baz-Lomba, J. A., Reid, M. J. and Thomas, K. V., 2016. Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF. *Analytica Chimica Acta*, 914, pp. 81-90.

Benotti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D. and Snyder, S. A., 2009. Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. *Environmental Science & Technology*, 43 (3), pp. 597-603.

Bernal, E., 2014. Limit of Detection and Limit of Quantification Determination in Gas Chromatography. In: X. Guo, ed. 2014. *Advances in Gas Chromatography*. Rijeka: InTech. Ch.3.

Bijlsma, L., Sancho, J. V., Pitarch, E., Ibáñez, M. and Hernández, F., 2009. Simultaneous ultra-high-pressure liquid chromatography–tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. *Journal of Chromatography A*, 1216 (15), pp. 3078-3089.

Binelli, A., Pedriali, A., Riva, C. and Parolini, M., 2012. Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model *Dreissena polymorpha*. *Chemosphere*, 86 (9), pp. 906-911.

Bogusz, M. J., 1997. Large Amounts of Drugs May Considerably Influence the Peak Areas of Their Coinjected Deuterated Analogues Measured with APCI-LC–MS. *Journal of Analytical Toxicology*, 21, pp. 246-247.

Boleda, M. R., Galceran, M. T. and Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. *Water Research*, 43 (4), pp. 1126-1136.

Boleda, M. R., Galceran, M. T. and Ventura, F., 2011. Behavior of pharmaceuticals and drugs of abuse in a drinking water treatment plant (DWTP) using combined conventional and ultrafiltration and reverse osmosis (UF/RO) treatments. *Environmental Pollution*, 159 (6), pp. 1584-1591.

Boleda, M. R., Huerta-Fontela, M., Ventura, F. and Galceran, M. T., 2011. Evaluation of the presence of drugs of abuse in tap waters. *Chemosphere*, 84 (11), pp. 1601-1607.

Braithwaite, A. and Smith, F. J., 1985. *Chromatographic Methods*. 4th ed. New York: Chapman and Hall. Ch.6.

Cahill, J. D., Furlong, E. T., Burkhardt, M. R., Kolpin, D. and Anderson, L. G., 2004. Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A*, 1041 (1-2), pp. 171-180.

Cancer Research UK, 2016. *Fluorouracil*. [online] Available at: <http://www.cancerresearchuk.org/about-cancer/cancers-in-general/treatment/cancer-drugs/fluorouracil> [Accessed 5 November 2016].

Carmona, E., Andreu, V. and Picó, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water. *Science of the Total Environment*, 484, pp. 53-63.

Castiglioni, S., Zuccato, E., Chiabrando, C., Fanelli, R. and Bagnati, R., 2008. Mass spectrometric analysis of illicit drugs in wastewater and surface water. *Mass Spectrometry Reviews*, 27 (4), pp. 378-394.

Chambers, E. E. and Diehl, D. M., 2007. *The use of stable-isotope-labeled (SIL) internal standards to compensate for matrix effects: key considerations*. [pdf] Milford: Waters. Available at: <http://www.waters.com/webassets/cms/library/docs/720002438en.pdf> [Accessed 21 May 2017].

Chambers, E., Wagrowski-Diehl, D. M., Lu, Z. and Mazzeo, J. R., 2007. Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. *Journal of Chromatography B*, 852 (1-2), pp. 22-34.

Chapman, P. M., 2007. Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environment International,* 33 (4), pp. 492-501.

Chiaia, A. C., Banta-Green, C. and Field, J., 2008. Eliminating Solid Phase Extraction with Large-Volume Injection LC/MS/MS: Analysis of Illicit and Legal Drugs and Human Urine Indicators in US Wastewaters. *Environmental Science & Technology*, 42 (23), pp. 8841-8848.

Cody, J., 2003. Mass Spectrometry. In: B. Levine, ed. 2003. *Principles of Forensic Toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.9.

Cole, K. and Levine, B., 2003. Spectrophotometry. In: B. Levine, ed. 2003. *Principles of Forensic Toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.6.

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

Cone, E. J. and Weddington, W. W., 1989. Prolonged Occurrence of Cocaine In Human Saliva and Urine after Chronic Use. *Journal of Analytical Toxicology*, 13 (2), pp. 65-68.

Corley, J., 2003. Best practices in establishing detection and quantification limits for pesticide residues in foods. In: J. J. Murphy, ed. 2003. *Handbook of Residue Analytical Methods for Agrochemicals*. Chichester: John Wiley & Sons. Vol. 2.

Couchman, L. and Morgan, P. E., 2011. LC-MS in analytical toxicology: some practical considerations. *Biomedical Chromatography*, 25 (1-2), pp. 100-123.

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

Dargan, P. I., Hudson, S., Ramsey, J. and Wood, D. M., 2011. The impact of changes in UK classification of the synthetic cannabinoid receptor agonists in ‘Spice’. *International Journal of Drug Policy*, 22 (4), pp. 274-277.

Davies, S., Wood, D. M., Smith, G., Button, J., Ramsey, J., Archer, R., Holt, D. W. and Dargan, P. I., 2010. Purchasing 'legal highs' on the Internet—is there consistency in what you get?. *QJM: An International Journal of Medicine*, 103 (7), pp. 489-493.

de Castro, A., Concheiro, M., Quintela, O., Cruz, A. and López-Rivadulla, M., 2008. LC–MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma: Study of correlation between venlafaxine concentrations in both matrices. *Journal of Pharmaceutical and Biomedical Analysis*, 48 (1), pp. 183-193.

Degenhardt, L., Hall, W., Warner-Smith, M. and Lynskey, M., 2004. Illicit drug use. In: M. Ezzati, A. D. Lopez, A. Rodgers and C. J. L. Murray, eds. 2004. *Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors, Volume 1*. Geneva: World Health Organization. Ch.13.

Department for Environment, Food & Rural Affairs, 2016. *Consultation on the drinking water regulations 2016: Summary of responses and government response*. [pdf] London: Department for Environment, Food & Rural Affairs. Available at: <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/526137/drinking-water-consult-sum-resp.pdf> [Accessed 5 November, 2016].

Derbyshire, D., 2008. *Is our water being poisoned with a cocktail of drugs?*. [online] Available at: <http://www.dailymail.co.uk/health/article-1064203/Is-water-poisoned-cocktail-drugs.html> [Accessed 5 November 2016].

Díaz-Cruz, M. S., López de Alda, M. J., López, R. and Barceló, D., 2003. Determination of estrogens and progestogens by mass spectrometric techniques (GC/MS, LC/MS and LC/MS/MS). *Journal of Mass Spectrometry*, 38 (9), pp. 917-923.

Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

Dolan, J., 2011. *A Guide to HPLC and LC-MS Buffer Selection*. [pdf] ACE HPLC Columns. Available at: <http://www.hplc.eu/Downloads/ACE_Guide_BufferSelection.pdf> [Accessed 5 November 2016].

Drinking Water Inspectorate, 2010a. *The Water Supply (Water Quality) Regulations 2010.* [pdf] Welsh Statutory Instruments. Available at: <http://www.dwi.gov.uk/stakeholders/legislation/wsr2010wales.pdf> [Accessed 5 November 2016].

Drinking Water Inspectorate, 2010b. *What are the drinking water standards?*. [pdf] London: Drinking Water Inspectorate. Available at: <http://dwi.defra.gov.uk/consumers/advice-leaflets/standards.pdf> [Accessed 5 November 2016].

Drinking Water Inspectorate, 2014. *Drinking water 2013: Public water supplies in the Central and Eastern region of England*. [pdf] London: Drinking Water Inspectorate. Available at: <http://dwi.defra.gov.uk/about/annual-report/2013/central-eastern.pdf> [Accessed 5 November 2016].

Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., Zhang, H. and Li, X., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. *Water Research*, 84, pp. 76-84.

Duvauchelle, C. L., Sapoznik, T. and Kornetsky, C., 1998. The Synergistic Effects of Combining Cocaine and Heroin (“Speedball”) Using a Progressive-Ratio Schedule of Drug Reinforcement. *Pharmacology Biochemistry and Behavior*, 61, pp. 297-302.

Ejaz, S., Akram, W., Lim, C. W., Lee, J. J. and Hussain, I., 2004. Endocrine disrupting pesticides: a leading cause of cancer among rural people in Pakistan. *Experimental Oncology*, 26 (2), pp. 98-105.

Elliott, S. and Smith, C., 2008. Investigation of the First Deaths in the United Kingdom Involving the Detection and Quantitation of the Piperazines BZP and 3-TFMPP. *Journal of Analytical Toxicology*, 32 (2), pp. 172-177.

Environmental Protection Agency, 2011. *Water Treatment Manual: Disinfection*. [pdf] Wexford: Environmental Protection Agency. Available at: <https://www.epa.ie/pubs/advice/drinkingwater/Disinfection2_web.pdf> [Accessed 5 November 2016].

Escher, B. I., Baumgartner, R., Koller, M., Treyer, K., Lienert, J. and McArdell, C. S., 2011. Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater. *Water Research*, 45 (1), pp. 75-92.

European Commission, 2016a. *Drinking Water*. [online] Available at: <http://ec.europa.eu/environment/water/water-drink/index_en.html> [Accessed 5 November 2016].

European Commission, 2016b. *Legislation: The Directive overview*. [online] Available at: <http://ec.europa.eu/environment/water/water-drink/legislation_en.html> [Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2013. *European Drug Report 2013: Trends and developments*. [online] Available at: <http://www.emcdda.europa.eu/publications/edr/trends-developments/2013> [Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2014a. *European Drug Report 2014: Trends and developments*. [online] Available at: <http://www.emcdda.europa.eu/publications/edr/trends-developments/2014> [Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2014b. *Exploring methamphetamine trends in Europe*. [pdf] Luxembourg: Publications Office of the European Union. Available at: <http://www.emcdda.europa.eu/system/files/publications/787/TDAU14001ENN_460800.pdf> [Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2015a. *BZP and other piperazines drug profile*. [online] Available at:
<http://www.emcdda.europa.eu/publications/drug-profiles/bzp>
[Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2015b. *European Drug Report 2015: Trends and Developments*. [pdf] Luxembourg: Publications Office of the European Union. Available at:
<http://www.emcdda.europa.eu/attachements.cfm/att_239505_EN_TDAT15001ENN.pdf>
[Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2015c. *New psychoactive substances in Europe: An update from the EU Early Warning System*. [pdf] Luxembourg: Publications Office of the European Union. Available at:
<http://www.emcdda.europa.eu/system/files/publications/65/TD0415135ENN.pdf>
[Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2015d. *Synthetic cannabinoids and 'Spice' drug profile*. [online] Available at:
<http://www.emcdda.europa.eu/publications/drug-profiles/synthetic-cannabinoids>
[Accessed 5 November 2016].

European Monitoring Centre for Drugs and Drug Addiction, 2015e. *Synthetic cathinones drug profile*. [online] Available at:
<http://www.emcdda.europa.eu/publications/drug-profiles/synthetic-cathinones>
[Accessed 5 November 2016].

Falconer, I. R., Chapman, H. F., Moore, M. R. and Ranmuthugala, G., 2006. Endocrine-disrupting compounds: A review of their challenge to sustainable and safe water supply and water reuse. *Environmental Toxicology*, 21 (2), pp. 181-191.

Fallon, A., Booth, R. F. G. and Bell, L. D., 1987. Applications of HPLC in Biochemistry. In: R. H. Burdon and P. H. van Knippenberg, eds. 1987. *Laboratory Techniques in Biochemistry and Molecular Biology, Volume 17*. Amsterdam: Elsevier Science Publishers B. V.

Food and Drug Administration, 2001. *Guidance for Industry: Bioanalytical Method Validation*. [pdf] Food and Drug Administration. Available at:
<http://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf> [Accessed 5 November 2016].

Friedman, H., Ochs, H. R., Greenblatt, D. J. and Shader, R. I., 1985. Tissue Distribution of Diazepam and Its Metabolite Desmethyldiazepam: A Human Autopsy Study. *The Journal of Clinical Pharmacology*, 25 (8), pp. 613-615.

Furey, A., Moriarty, M., Bane, V., Kinsella, B. and Lehane, M., 2013. Ion suppression; A critical review on causes, evaluation, prevention and applications. *Talanta*, 115, pp. 104-122.

Gao, T., Du, P., Xu, Z. and Li, X., 2017. Occurrence of new psychoactive substances in wastewater of major Chinese cities. *Science of The Total Environment*, 575, pp. 963-969.

Giebułtowicz, J. and Nałęcz-Jawecki, G., 2014. Occurrence of antidepressant residues in the sewage-impacted Vistula and Utrata rivers and in tap water in Warsaw (Poland). *Ecotoxicology and Environmental Safety*, 104, pp. 103-109.

González-Mariño, I., Gracia-Lor, E., Bagnati, R., Martins, C. P., Zuccato, E. and Castiglioni, S., 2016a. Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, 408 (16), pp. 4297-4309.

González-Mariño, I., Gracia-Lor, E., Rousis, N. I., Castrignanò, E., Thomas, K. V., Quintana, J. B., Kasprzyk-Hordern, B., Zuccato, E. and Castiglioni, S., 2016b. Wastewater-based epidemiology to monitor synthetic cathinones use in different European countries. *Environmental Science and Technology*, 50 (18), pp. 10089-10096.

Gros, M., Petrović, M. and Barceló, D., 2006. Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta*, 70 (4), pp. 678-690.

Gros, M., Petrović, M. and Barceló, D., 2007. Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (Northeast Spain). *Environmental Toxicology and Chemistry*, 26 (8), pp. 1553-1562.

Gros, M., Rodríguez-Mozaz, S. and Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal of Chromatography A*, 1248, pp. 104-121.

Hajšlová, J. and Zrostlíková, J., 2003. Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices. *Journal of Chromatography A*, 1000, pp. 181-197.

Harris, D. C., 2010. *Quantitative Chemical Analysis*. 8th ed. New York: W. H. Freeman and Company. Ch.21.

Harrison, R. M., 2014. *Pollution: Causes, Effects and Control*. 5th ed. Cambridge: The Royal Society of Chemistry.

Harrold, M. W. and Zavod, R. M., 2013. *Basic Concepts in Medicinal Chemistry*. Bethesda: American Society of Health-System Pharmacists. Ch.2.

Hartmann, C., Smeyers-Verbeke, J., Massart, D. L. and McDowall, R. D., 1998. Validation of bioanalytical chromatographic methods. *Journal of Pharmaceutical and Biomedical Analysis*, 17 (2), pp. 193-218.

Hawton, K., Bergen, H., Simkin, S., Cooper, J., Waters, K., Gunnell, D. and Kapur, N., 2010. Toxicity of antidepressants: rates of suicide relative to prescribing and non-fatal overdose. *The British Journal of Psychiatry*, 196 (5), pp. 354-358.

Health & Social Care Information Centre, 2014. *Prescriptions Dispensed in the Community: England 2003-13*. [pdf] Health & Social Care Information Centre. Available at: <http://www.hscic.gov.uk/catalogue/PUB14414/pres-disp-com-eng-2003-13-rep.pdf> [Accessed 5 November 2016].

Health & Social Care Information Centre, 2016. *Prescriptions Dispensed in the Community: England 2005-2015*. [pdf] Health & Social Care Information Centre. Available at: <http://www.hscic.gov.uk/catalogue/PUB20664/pres-disp-com-eng-2005-15-rep.pdf> [Accessed 5 November 2016].

Heberer, T., Mechlinski, A., Fanck, B., Knappe, A., Massmann, G., Pekdeger, A. and Fritz, B., 2004. Field Studies on the Fate and Transport of Pharmaceutical Residues in Bank Filtration. *Groundwater Monitoring & Remediation*, 24 (2), pp, 70-77.

Hernando, M. D., Mezcua, M., Fernández-Alba, A. R. and Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, 69 (2), pp. 334-342.

Holčapek, M., Jirásko, R. and Lísa, M., 2012. Recent developments in liquid chromatography–mass spectrometry and related techniques. *Journal of Chromatography A*, 1259, pp. 3-15.

Home Office, 2012. *Annual Report on the Home Office Forensic Early Warning System (FEWS): A System to Identify New Psychoactive Substances in the UK*. [pdf] Home Office. Available at: <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/225840/fews.pdf> [Accessed 5 November 2016].

Home Office, 2015. *Drug Misuse: Findings from the 2014/15 Crime Survey for England and Wales*. [pdf] Home Office. Available at: <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/462885/drug-misuse-1415.pdf> [Accessed 5 November 2016].

Huber, L., 2007. *Validation and Qualification in Analytical Laboratories*. 2nd ed. New York: CRC Press.

Huerta-Fontela, M., Galceran, M. T. and Ventura, F., 2008. Stimulatory Drugs of Abuse in Surface Waters and Their Removal in a Conventional Drinking Water Treatment Plant. *Environmental Science & Technology*, 42 (18), pp. 6809-6816.

Huestis, M. A., 2003. Marijuana. In: B. Levine, ed. 2003. *Principles of forensic toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.14.

Isenschmid, D. S., 2003. Cocaine. In: B. Levine, ed. 2003. *Principles of forensic toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.13.

Ishiwatari, R., 1971. Molecular weight distribution of humic acids from lake and marine sediments. *Geochemical Journal*, 5 (3), pp. 121-132.

Isralowitz, R. E. and Myers, P. L., 2011. *Illicit Drugs*. Santa Barbara: ABC-CLIO. Ch.2.

Jenkins, A. J., 2003. Hallucinogens. In: B. Levine, ed. 2003. *Principles of forensic toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.16.

Johnson, A. C., Jürgens, M. D., Williams, R. J., Kümmerer, K., Kortenkamp, A. and Sumpter, J. P., 2008. Do cytotoxic chemotherapy drugs discharged into rivers pose a risk to the environment and human health? An overview and UK case study. *Journal of Hydrology*, 348 (1-2), pp. 167-175.

Jones, O. A., Lester, J. N. and Voulvoulis, N., 2005. Pharmaceuticals: a threat to drinking water?. *Trends in Biotechnology*, 23 (4), pp. 163-167.

Jurado, A., Vàzquez-Suñé, E., Carrera, J., López de Alda, M., Pujades, E. and Barceló, D., 2012. Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context. *Science of the Total Environment*, 440, pp. 82-94.

Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J., 2007. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A*, 1161 (1-2), pp. 132-145.

Kazakevich, Y. and LoBrutto, R., 2007. *HPLC for Pharmaceutical Scientists*. Hoboken: John Wiley & Sons.

Konstantinou, I. K., Hela, D. G. and Albanis, T. A., 2006. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environmental Pollution*, 141 (3), pp. 555-570.

Kosjek, T. and Heath, E., 2008. Applications of mass spectrometry to identifying pharmaceutical transformation products in water treatment. *Trends in Analytical Chemistry*, 27 (10), pp. 807-820.

Kümmerer, K., 2009. The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. *Journal of Environmental Management*, 90 (8), pp. 2354-2366.

Kwon, J. and Armbrust, K. L., 2008. Aqueous Solubility, *n*-Octanol–Water Partition Coefficient, and Sorption of Five Selective Serotonin Reuptake Inhibitors to Sediments and Soils. *Bulletin of Environmental Contamination and Toxicology*, 81 (2), pp. 128-135.

LeChevallier, M. W. and Au, K., 2004. *Water treatment and pathogen control: Process efficiency in achieving safe drinking water*. London: IWA Publishing.

Linoff, G. S. and Berry, M. J. A., 2011. *Data Mining Techniques: For Marketing, Sales, and Customer Relationship Management*. 3rd ed. Indianapolis: Wiley Publishing. Ch.6.

López de Alda, M. J. and Barceló, D., 2000. Determination of steroid sex hormones and related synthetic compounds considered as endocrine disrupters in water by liquid chromatography–diode array detection–mass spectrometry. *Journal of Chromatography A*, 892 (1-2), pp. 391-406.

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M. and Barceló, D., 2010. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry. *Talanta*, 83 (2), pp. 410-424.

Maine Water Utilities Association, 2010. *Science of Chloramination*. [pdf] Maine Water Utilities Association. Available at: <http://www.mwua.org/wp-content/uploads/2010/09/Science_of_Chloramination_-_Dina.pdf> [Accessed 5 November 2016].

Maurer, H. H., 1998. Liquid chromatography–mass spectrometry in forensic and clinical toxicology. *Journal of Chromatography B: Biomedical Sciences and Applications*, 713 (1), pp. 3-25.

Mendoza, A., Rodríguez-Gil, J. L., González-Alonso, S., Mastroianni, N., López de Alda, M., Barceló, D. and Valcárcel, Y., 2014. Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): Seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. *Environment International*, 70, pp. 76-87.

Mendoza, A., Zonja, B., Mastroianni, N., Negreira, N., López de Alda, M., Pérez, S., Barceló, D., Gil, A. and Valcárcel, Y., 2016. Drugs of abuse, cytostatic drugs and iodinated contrast media in tap water from the Madrid region (central Spain): A case study to analyse their occurrence and human health risk characterization. *Environment international*, 86, pp. 107-118.

Mental Health Foundation, 2015. *Fundamental Facts About Mental Health 2015*. [pdf] Mental Health Foundation. Available at: <https://www.mentalhealth.org.uk/sites/default/files/fundamental-facts-15.pdf> [Accessed 5 November 2016].

Miller, A. and Solomon, P. H., 2000. *Writing Reaction Mechanisms in Organic Chemistry*. 2nd ed. San Diego: Academic Press. Ch.4.

Miller, M. M., Wasik, S. P., Huang, G. L., Shiu, W. Y. and Mackay, D., 1985. Relationships between octanol-water partition coefficient and aqueous solubility. *Environmental Science & Technology*, 19 (6), pp. 522-529.

Mixmag, 2012. *Mixmag's drug survey: The results*. [online] Available at: <https://issuu.com/mixmagfashion/docs/drugs_survey_2012_2> [Accessed 5 November 2016].

Moffat, A. C., Osselton, M. D., Widdop, B. and Watts, J., eds., 2011. *Clarke's Analysis of Drugs and Poisons*. 4th ed. London: Pharmaceutical Press. Vol 2.

Molin, L. and Traldi, P., 2007. Basic aspects of electrospray ionization. In: A. Cappiello, ed. 2007. *Advances in LC-MS instrumentation*. Amsterdam: Elsevier Science B. V.. Ch.1.

Mompelat, S., Le Bot, B. and Thomas, O., 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. *Environment International*, 35 (5), pp. 803-814.

Mompelat, S., Le Bot, B., Thomas, O. and Roig, B., 2010. Exposure based on life cycle. In: B. Roig, ed. 2010. *Pharmaceuticals in the Environment: Current knowledge and need assessment to reduce presence and impact*. London: IWA Publishing. Ch.3.

Moore, K. A., 2003. Amphetamines/Sympathomimetic Amines. In: B. Levine, ed. 2003. *Principles of forensic toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.15.

Mwenesongole, E., Gautam, L., Hall, S. and Emmett, T., 2012. Estimating community drug usage patterns by the analysis of waste water. In: University of Salford, *Proceedings of the Salford Postgraduate Annual Research Conference*. Salford, UK, 2011. Salford: University of Salford.

Mwenesongole, E. M., 2015. *Simultaneous Detection of Drugs of Abuse in Waste Water Using Gas Chromatography-Mass Spectrometry*. Ph D. Anglia Ruskin University.

Mwenesongole, E. M., Gautam, L., Hall, S. W., Waterhouse, J. W. and Cole, M. D., 2013. Simultaneous detection of controlled substances in waste water. *Analytical Methods*, 5, pp. 3248-3254.

Naegele, E., 2011. *Making your LC Method Compatible with Mass Spectrometry*. [pdf] Agilent Technologies. Available at: <https://www.agilent.com/cs/library/technicaloverviews/public/5990--7413EN.pdf> [Accessed 5 November 2016].

Nayak, P. K., Misra, A. L. and Mulé, S. J., 1976. Physiological disposition and biotransformation of (3H) cocaine in acutely and chronically treated rats. The *Journal of Pharmacology and Experimental Therapeutics*, 196 (3), pp. 556-569.

Noguera-Oviedo, K. and Aga, D. S., 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials*, 316, pp. 242-251.

Office for National Statistics, 2013. *Age Structure, 2011 (KS102EW)*. [online] Available at: <http://www.neighbourhood.statistics.gov.uk/dissemination/LeadTableView.do?a=7&b=6275044&c=cambridge&d=13&e=62&g=6405296&i=1001x1003x1032x1004&m=0&r=1&s=1464012016906&enc=1&dsFamilyId=2474> [Accessed 5 November 2016].

Ongley, E. D., 1996. *Control of water pollution from agriculture*. Rome: Food and Agriculture Organization of the United Nations. Ch.4.

Organisation for Economic Co-operation and Development, 2015. *Health at a Glance 2015*. [online] Available at: <http://www.oecd-ilibrary.org/sites/health_glance-2015-en/10/04/index.html?contentType=&itemId=%2fcontent%2fchapter%2fhealth_glance-2015-68-en&mimeType=text%2fhtml&containerItemId=%2fcontent%2fserial%2f19991312&accessItemIds=> [Accessed 5 November 2016].

Padhye, L. P., Yao, H., Kung'u, F. T. and Huang, C., 2014. Year-long evaluation on the occurrence and fate of pharmaceuticals, personal care products, and endocrine disrupting chemicals in an urban drinking water treatment plant. *Water Research*, 51, pp. 266-276.

Pal, R., Megharaj, M., Kirkbride, K. P. and Naidu, R., 2013. Illicit drugs and the environment – A review. *Science of the Total Environment*, 463-464, pp. 1079-1092.

Paskulin, G. A., Gazzola Zen, P. R., de Camargo Pinto, L. L., Rosa, R. and Graziadio, C., 2005. Combined chemotherapy and teratogenicity. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 73 (9), pp. 634-637.

Pedrouzo, M., Borrull, F., Pocurull, E. and Marcé, R. M., 2011. Drugs of abuse and their metabolites in waste and surface waters by liquid chromatography-tandem mass spectrometry. *Journal of Separation Science*, 34 (10), pp. 1091-1101.

Peng, Y., Hall, S. and Gautam, L., 2016. Drugs of abuse in drinking water – a review of current detection methods, occurrence, elimination and health risks. *Trends in Analytical Chemistry*, [e-journal] 85 (C), pp. 232-240. http://dx.doi.org/10.1016/j.trac.2016.09.011.

Pesticide Action Network Europe, 2008. *Which Pesticides are Banned in Europe?*. [pdf] Pesticide Action Network Europe. Available at: <http://www.pan-europe.info/old/Resources/Links/Banned_in_the_EU.pdf> [Accessed 5 November 2016].

Peters, F. T., Drummer, O. H. and Musshoff, F., 2007. Validation of new methods. *Forensic Science International*, 165 (2-3), pp. 216-224.

Petrie, B., Youdan, J., Barden, R. and Kasprzyk-Hordern, B., 2016. Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, 1431, pp. 64-78.

Petrovic, M., Farré, M., Lopez de Alda, M., Perez, S., Postigo, C., Köck, M., Radjenovic, J., Gros, M. and Barcelo, D., 2010. Recent trends in the liquid chromatography–mass spectrometry analysis of organic contaminants in environmental samples. *Journal of Chromatography A*, 1217 (25), pp. 4004-4017.

Petrović, M., Hernando, M. D., Díaz-Cruz, M. S. and Barceló, D., 2005. Liquid chromatography–tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. *Journal of Chromatography A*, 1067 (1-2), pp. 1-14.

Phenomenex, 2011. *Strata-X-Drug B for Drugs of Abuse*. [pdf] Phenomenex. Available at: <http://az621941.vo.msecnd.net/documents/4c24faef-af6e-4b27-8394-84849d99b058.pdf> [Accessed 5 November 2016].

Phenomenex, 2016. *Kinetex biphenyl*. [Online] Available at: <http://www.phenomenex.com/Info/Page/2014knxbiphenyl> [Accessed 5 November 2016].

Pike, E., 2015. *Achieving excellent drugs-of-abuse analysis starts with the proper sample preparation technique*. [online] Available at: <http://www.mlo-online.com/articles/201501/achieving-excellent-drugs-of-abuse-analysis-starts-with-the-proper-sample-preparation-technique.php> [Accessed 5 November 2016].

Pimentel, D., McLaughlin, L., Zepp, A., Lakitan, B., Kraus, T., Kleinman, P., Vancini, F., Roach, W. J., Graap, E., Keeton, W. S. and Selig, G., 1991. Environmental and Economic Effects of Reducing Pesticide Use. *BioScience*, 41 (6), pp. 402-409.

Pinkston, K. E. and Sedlak, D. L., 2004. Transformation of Aromatic Ether- and Amine-Containing Pharmaceuticals during Chlorine Disinfection. *Environmental Science & Technology*, 38 (14), pp. 4019-4025.

Postigo, C., Lopez de Alda, M. J. and Barceló, D., 2008. Analysis of drugs of abuse and their human metabolites in water by LC-MS^2: A non-intrusive tool for drug abuse estimation at the community level. *Trends in Analytical Chemistry*, 27 (11), pp. 1053-1069.

Psychoactive Substances Act 2016. (c.2). London: The Stationery Office.

Quintana, J. B. and Reemtsma, T., 2004. Sensitive determination of acidic drugs and triclosan in surface and wastewater by ion-pair reverse-phase liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 18 (7), pp. 765-774.

Ramsey, J., Dargan, P. I., Smyllie, M., Davies, S., Button, J., Holt, D. W. and Wood, D. M., 2010. Buying 'legal' recreational drugs does not mean that you are not breaking the law. *QJM: An International Journal of Medicine*, 103 (10), pp. 777-783.

Reddersen, K., Heberer, T. and Dünnbier, U., 2002. Identification and significance of phenazone drugs and their metabolites in ground- and drinking water. *Chemosphere*, 49 (6), pp. 539-544.

Reid, M. J., Derry, L. and Thomas, K. V., 2014. Analysis of new classes of recreational drugs in sewage: Synthetic cannabinoids and amphetamine-like substances. *Drug Testing and Analysis*, 6 (1-2), pp. 72-79.

Repice, C., Dal Grande, M., Maggi, R. and Pedrazzani, R., 2013. Licit and illicit drugs in a wastewater treatment plant in Verona, Italy. *Science of the Total Environment*, 463-464, pp. 27-34.

Rivier, L., 2003. Criteria for the identification of compounds by liquid chromatography–mass spectrometry and liquid chromatography–multiple mass spectrometry in forensic toxicology and doping analysis. *Analytica Chimica Acta*, 492 (1-2), pp. 69-82.

Rodayan, A., Afana, S., Segura, P. A., Sultana, T., Metcalfe, C. D. and Yargeau, V., 2016. Linking drugs of abuse in wastewater to contamination of surface and drinking water. *Environmental Toxicology and Chemistry*, 35 (4), pp. 843-849.

Rodriguez-Mozaz, S., López de Alda, M. J. and Barceló, D., 2004. Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction–liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1045 (1-2), pp. 85-92.

Royal Society of Chemistry, 2007. *Sustainable Water: Chemical Science Priorities Summary Report*. [pdf] Royal Society of Chemistry. Available at: <https://www.yumpu.com/en/document/view/25882784/sustainable-water-chemical-science-priorities-royal-society-of-> [Accessed 5 November 2016].

Saar, E., Gerostamoulos, D., Drummer, O. H. and Beyer, J., 2010. Identification and quantification of 30 antipsychotics in blood using LC-MS/MS. *Journal of Mass Spectrometry*, 45 (8), pp. 915-925.

Santali, E. Y., Cadogan, A., Daeid, N. N., Savage, K. A. and Sutcliffe, O. B., 2011. Synthesis, full chemical characterisation and development of validated methods for the quantification of (±)-4'-methylmethcathinone (mephedrone): A new "legal high". *Journal of Pharmaceutical and Biomedical Analysis*, 56 (2), pp. 246-255.

Sargent, M., 2013. *Guide to achieving reliable quantitative LC-MS measurements*. [pdf] RSC Analytical Methods Committee. Available at: <http://www.rsc.org/images/AMC%20LCMS%20Guide_tcm18-240030.pdf> [Accessed 5 November 2016].

Separation Science, 2015. *HPLC Solutions #93: Poor Equilibration*. [online] Available at: <http://www.sepscience.com/Techniques/LC/Articles/171-/HPLC-Solutions-93-Poor-Equilibration> [Accessed 5 November 2016].

Shimadzu, 2008. *LabSolutions LCMS solution: Operators Guide*. Kyoto: Shimadzu.

Shimadzu, 2011. *High Performance Liquid Chromatograph Mass Spectrometer LCMS-2000: Instruction Manual*. Kyoto: Shimadzu.

Sigma-Aldrich, 1998. *Guide to Solid Phase Extraction*. [pdf] Sigma-Aldrich. Available at: <http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf> [Accessed 5 November 2016].

Simpson, N. J. K., 2000. *Solid-Phase Extraction: Principles, Techniques and Applications*. New York: Marcel Dekker.

Singh, R., 2013. HPLC method development and validation- an overview. *Journal of Pharmaceutical Education and Research*, 4 (1), pp. 26-33.

Sinha, S. N., Vasudev, K., Rao, M. V. V. and Odetokun, M., 2011. Quantification of organophosphate insecticides in drinking water in urban areas using lyophilization and high-performance liquid chromatography–electrospray ionization-mass spectrometry techniques. *International Journal of Mass Spectrometry*, 300 (1), pp. 12-20.

Snyder, L. R., Kirkland, J. J. and Glajch, J. L., 1997. *Practical HPLC Method Development*. 2nd ed. New Jersey: John Wiley & Sons.

Sørensen, L. K., 2011. Determination of cathinones and related ephedrines in forensic whole-blood samples by liquid-chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography B*, 879 (11-12), pp. 727-736.

Spiehler, V. and Levine, B., 2003. Pharmacokinetics and Pharmacodynamics. In: B. Levine, ed. 2003. *Principles of forensic toxicology*. 2nd ed. Washington, D.C.: AACC Press. Ch.4.

Stackelberg, P. E., Furlong, E. T., Meyer, M. T., Zaugg, S. D., Henderson, A. K. and Reissman, D. B., 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment*, 329 (1-3), pp. 99-113.

Stackelberg, P. E., Gibs, J., Furlong, E. T., Meyer, M. T., Zaugg, S. D. and Lippincott, R. L., 2007. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. *Science of the Total Environment*, 377 (2-3), pp. 255-272.

Stone, P., Glauner, T., Kuhlmann, F., Schlabach, T. and Miller, K., 2009. *New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses*. [pdf] Agilent Technologies. Available at: <https://www.agilent.com/cs/library/technicaloverviews/Public/5990-3595en_lo%20CMS.pdf> [Accessed 5 November 2016].

Sumnall, H. and Atkinson, A., 2015. *The new Psychoactive Substances Bill - a quick introduction*. [online] Available at: <http://www.cph.org.uk/blog/the-new-psychoactive-substances-bill-a-quick-introduction/> [Accessed 5 November 2016].

Swartz, M. E. and Krull, I. S., 1997. *Analytical Method Development and Validation*. New York: Marcel Dekker.

Tang, L. and Kebarle, P., 1993. *Dependence of ion intensity in electrospray mass spectrometry on the concentration of the analytes in the electrosprayed solution*. Analytical Chemistry, 65 (24), pp. 3654-3668.

Tarcomnicu, I., van Nuijs, A. L. N., Simons, W., Bervoets, L., Blust, R., Jorens, P. G., Neels, H. and Covaci, A., 2011. Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. *Talanta*, 83 (3), pp. 795-803.

Taylor, T., 2016. *Hidden problems in your LC-MS data?*. [online] Available at: <http://www.chromatographyonline.com/hidden-problems-your-lcms-data> [Accessed 22 May 2017].

Thames Water, 2014. *Drinking water treatment*. [online] Available at: <http://www.thameswater.co.uk/your-account/2915.htm> [Accessed 5 November 2016].

The Misuse of Drugs Act 1971 (Ketamine etc.) (Amendment) Order 2014. 2014 SI 2014/1106. London: The Stationery Office.

Thermo Scientific, 2015. *Compliance and Workflows: European Water Framework Directive 2013/39/EU*. [pdf] Thermo Scientific. Available at: <https://tools.thermofisher.com/content/sfs/brochures/BR-71154-Water-Analysis-EU-Water-Framework-BR71154-EN.pdf> [Accessed 5 November 2016].

The Water Supply (Water Quality) Regulations 2016. 2016 SI 2016/614. London: The Stationery Office.

Thurman, E. M. and Mills, M. S., 1998. *Solid-Phase Extraction: Principles and Practice*. New York: John Wiley & Sons.

Togola, A. and Budzinski, H., 2008. Multi-residue analysis of pharmaceutical compounds in aqueous samples. *Journal of Chromatography A*, 1177 (1), pp. 150-158.

Tzanavaras, P. D. and Zacharis, C. K., 2010. *Reviews in Pharmaceutical & Biomedical Analysis*. Sharjah: Bentham Science Publishers.

United Nations Office on Drugs and Crime, 2009. *Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens*. [pdf] Vienna: United Nations Publication. Available at: <http://www.unodc.org/documents/scientific/validation_E.pdf>
[Accessed 5 November 2016].

United Nations Office on Drugs and Crime, 2014. *World Drug Report 2014*. [pdf] United Nations Publication. Available at: <http://www.unodc.org/documents/wdr2014/World_Drug_Report_2014_web.pdf> [Accessed 5 November 2016].

United Nations Office on Drugs and Crime, 2015. *World Drug Report 2015*. [pdf] United Nations Publication. Available at: <https://www.unodc.org/documents/wdr2015/World_Drug_Report_2015.pdf> [Accessed 5 November 2016].

Valcárcel, Y., Alonso, S. G., Rodríguez-Gil, J. L., Gil, A. and Catalá, M., 2011. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere*, 84, pp. 1336-1348.

Valcárcel, Y., Martínez, F., González-Alonso, S., Segura, Y., Catalá, M., Molina, R., Montero-Rubio, J. C., Mastroianni, N., López de Alda, M., Postigo, C. and Barceló, D., 2012. Drugs of abuse in surface and tap waters of the Tagus River basin: Heterogeneous photo-Fenton process is effective in their degradation. *Environment International*, 41, pp. 35-43.

Vanderford, B. J. and Snyder, S. A., 2006. Analysis of Pharmaceuticals in Water by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry. *Environmental Science & Technology*, 40 (23), pp. 7312-7320.

Van Loco, J., Elskens, M., Croux, C. and Beernaert, H., 2002. Linearity of calibration curves: use and misuse of the correlation coefficient. *Accreditation and Quality Assurance*, 7 (7), pp. 281-285.

Van Nuijs, A. L. N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P. G., Bervoets, L., Blust, R., Meulemans, H., Neels, H. and Covaci, A., 2009. Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in Belgium. *Addiction*, 104 (5), pp. 734-741.

Vazquez-Roig, P., Blasco, C. and Picó, Y., 2013. Advances in the analysis of legal and illegal drugs in the aquatic environment. *Trends in Analytical Chemistry*, 50, pp. 65-77.

Vieno, N., Tuhkanen, T., and Kronberg, L., 2006. Removal of Pharmaceuticals in Drinking Water Treatment: Effect of Chemical Coagulation. *Environmental Technology*, 27 (2), pp. 183-192.

von Gunten, U., 2003a. Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Research*, 37 (7), pp. 1443-1467.

von Gunten, U., 2003b. Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. *Water Research*, 37 (7), pp. 1469-1487.

Vorce, S. P., Holler, J. M., Levine, B. and Past, M. R., 2008. Detection of 1-Benzylpiperazine and 1-(3-Trifluoromethylphenyl)-piperazine in Urine Analysis Specimens Using GC-MS and LC-ESI-MS. *Journal of Analytical Toxicology*, 32 (6), pp. 444-450.

Waters, 2001. *A Sample Preparation Primer and Guide to Solid Phase Extraction Methods Development*. [pdf] Waters. Available at:
<http://www.waters.com/webassets/cms/library/docs/wa20300.pdf>
[Accessed 5 November 2016].

Waters, 2003. *Methodology– Section 1: Oasis Generic Methodology*. [pdf] Waters. Available at:
<http://www.waters.com/webassets/cms/library/docs/oasis15.pdf> [Accessed 5 November 2016].

Waters, 2016. *Beginner's Guide to SPE [Solid-Phase Extraction]*. [online] Available at:
<http://www.waters.com/waters/en_GB/Beginner%27s-Guide-to-SPE-%5BSolid-Phase-Extraction%5D/nav.htm?cid=134721476&lset=1&locale=en_GB&changedCountry=Y>
[Accessed 5 November 2016].

Watson, J. T. and Sparkman, O. D., 2007. *Introduction to Mass Spectrometry: Instrumentation, Applications and Strategies for Data Interpretation*. 4th ed. Chichester: John Wiley and Sons. Ch.8.

Wells, G., Prest, H. and Russ, C. W., 2011. *Why use Signal-To-Noise as a Measure of MS Performance When it is Often Meaningless?*. [pdf] Agilent Technologies. Available at:
<http://www.agilent.com/cs/library/technicaloverviews/public/5990-8341EN.pdf>
[Accessed 5 November 2016].

Westerhoff, P., Yoon, Y., Snyder, S. and Wert, E., 2005. Fate of Endocrine-Disruptor, Pharmaceutical, and Personal Care Product Chemicals during Simulated Drinking Water Treatment Processes. *Environmental Science & Technology*, *39* (17), pp. 6649-6663.

Wille, K., De Brabander, H. F., Vanhaecke, L., De Wulf, E., Van Caeter, P. and Janssen, C. R., 2012. Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. *Trends in Analytical Chemistry*, 35, pp. 87-108.

Wille, S. M. R., Peters, F. T., Di Fazio, V. and Samyn, N., 2011. Practical aspects concerning validation and quality control for forensic and clinical bioanalytical quantitative methods. *Accreditation and Quality Assurance*, 16 (6), pp. 279-292.

World Anti-doping Agency, 2010. *Identification criteria for qualitative assays incorporating column chromatography and mass spectrometry*. [pdf] Montreal: World Anti-doping Agency. Available at: <https://wada-main-prod.s3.amazonaws.com/resources/files/WADA_TD2010IDCRv1.0_Identification%20Criteria%20for%20Qualitative%20Assays_May%2008%202010_EN.doc.pdf> [Accessed 5 November 2016].

World Health Organization, 2003. *Aldrin and Dieldrin in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality*. [pdf] Geneva: World Health Organization. Available at: <http://www.who.int/water_sanitation_health/dwq/chemicals/adrindieldrin.pdf> [Accessed 5 November 2016].

World Health Organization, 2004. *DDT and its Derivatives in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality*. [pdf] World Health Organization. Available at: <http://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/ddt.pdf?ua=1> [Accessed 5 November 2016].

World Health Organization, 2011. *America's State of Mind Report*. [pdf] World Health Organization. Available at: <http://apps.who.int/medicinedocs/documents/s19032en/s19032en.pdf> [Accessed 5 November 2016].

Wu, M., Xiang, J., Que, C., Chen, F. and Xu, G., 2015. Occurrence and fate of psychiatric pharmaceuticals in the urban water system of Shanghai, China. *Chemosphere*, 138, pp. 486-493.

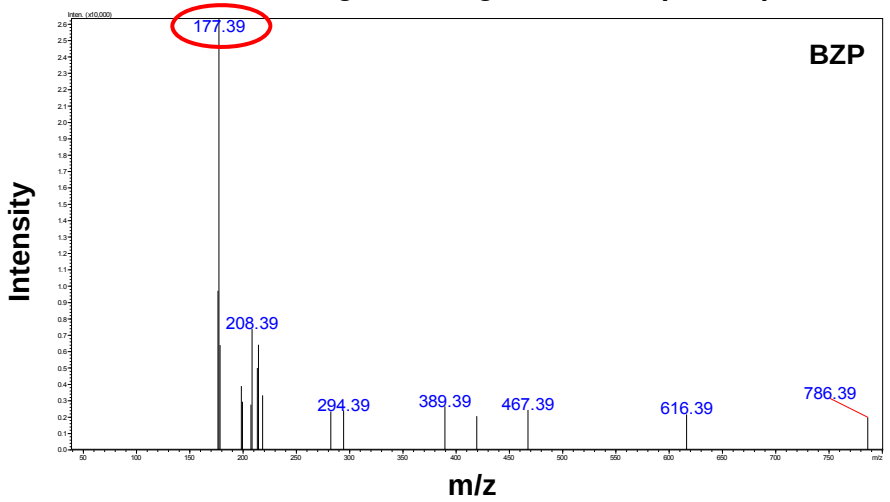
Yu, Z., Peldszus, S. and Huck, P. M., 2008. Adsorption characteristics of selected pharmaceuticals and an endocrine disrupting compound—Naproxen, carbamazepine and nonylphenol—on activated carbon. *Water Research*, 42 (12), pp. 2873-2882.

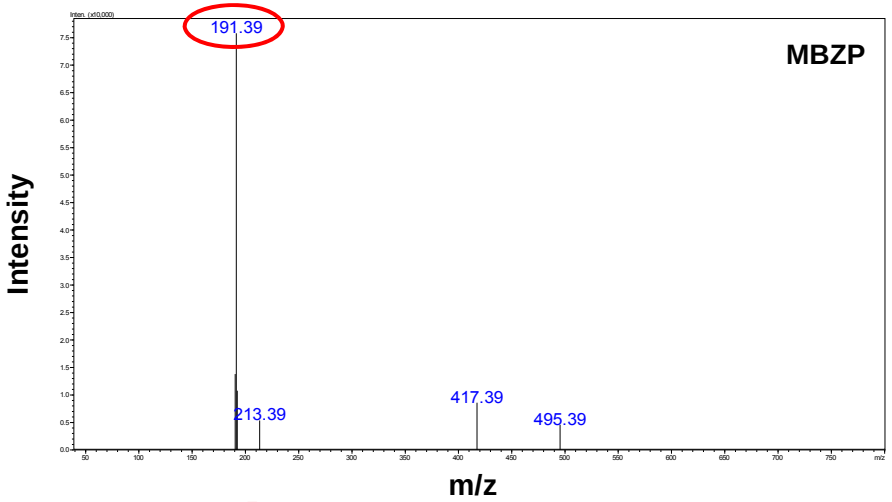
Zuccato, E., Castiglioni, S., Bagnati, R., Chiabrando, C., Grassi, P. and Fanelli, R., 2008. Illicit drugs, a novel group of environmental contaminants. *Water Research*, 42 (4-5), pp. 961-968.

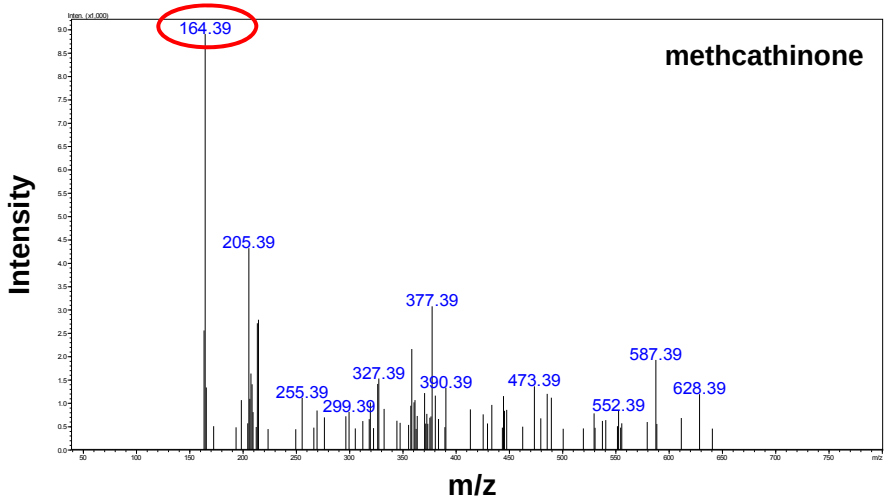
Zwiener, C., 2007. Occurrence and analysis of pharmaceuticals and their transformation products in drinking water treatment. *Analytical and Bioanalytical Chemistry*, 387 (4), pp. 1159-1162.

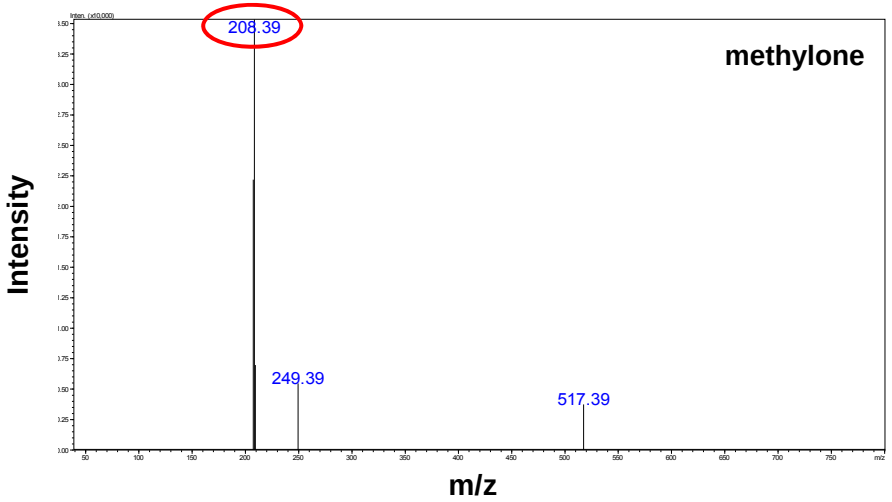
APPENDICES

Appendix I: Mass spectra for drugs of abuse, pharmaceuticals and internal standards at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their diagnostic ions (circled)









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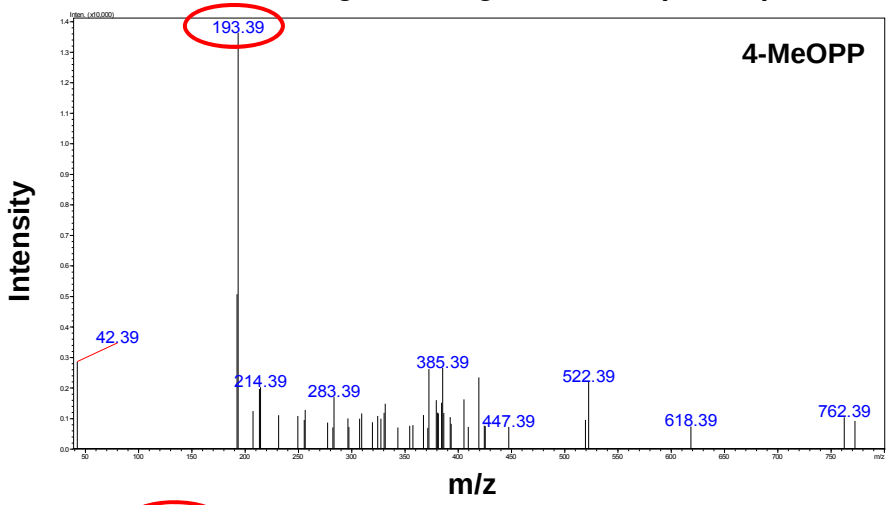
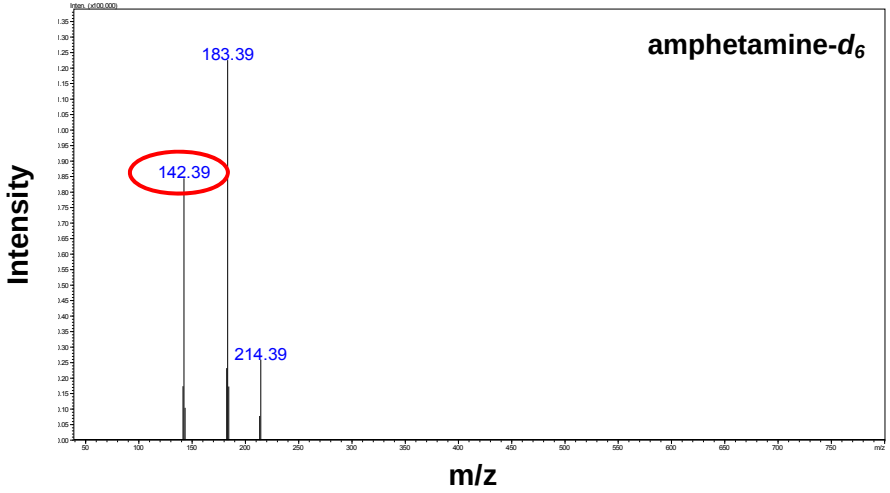
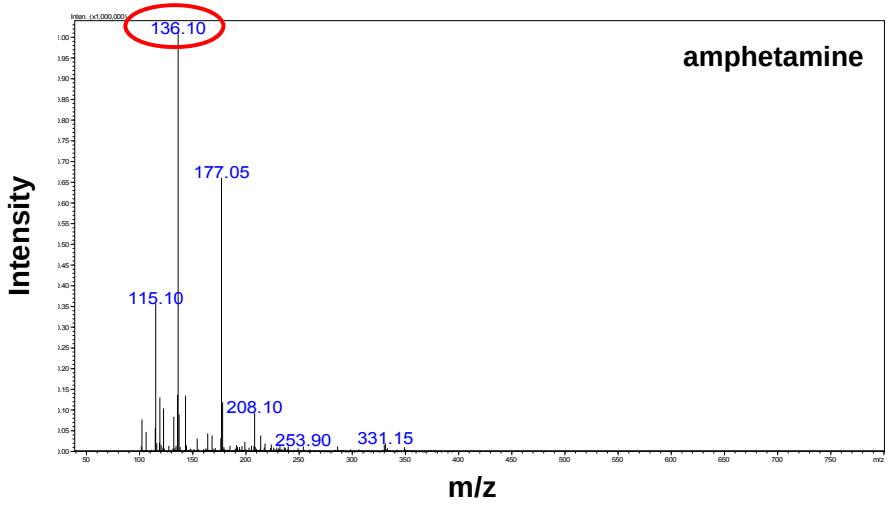
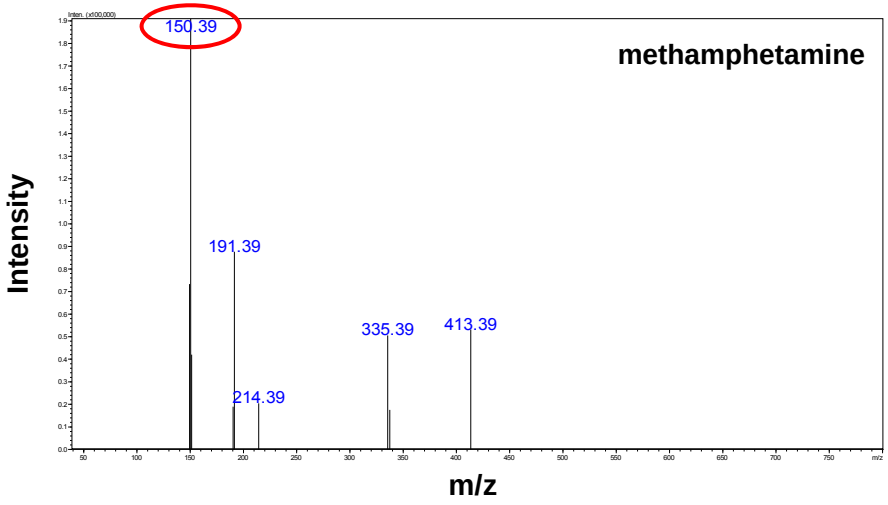
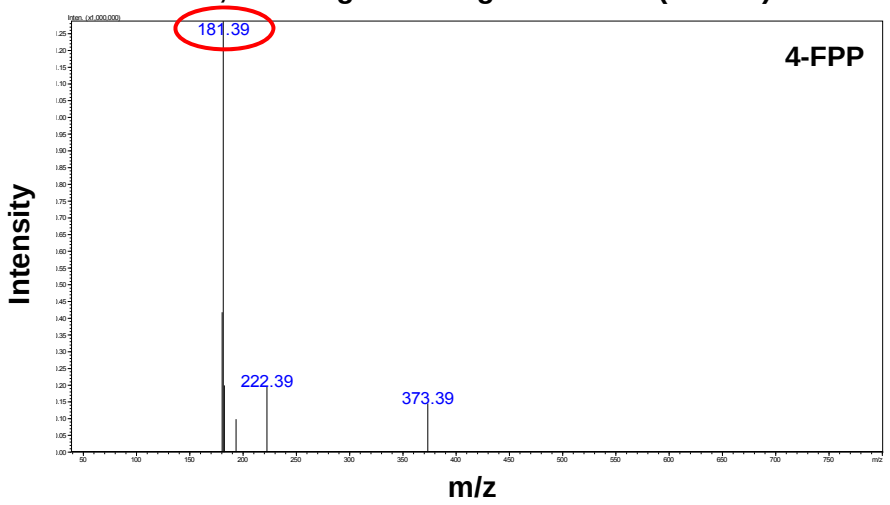


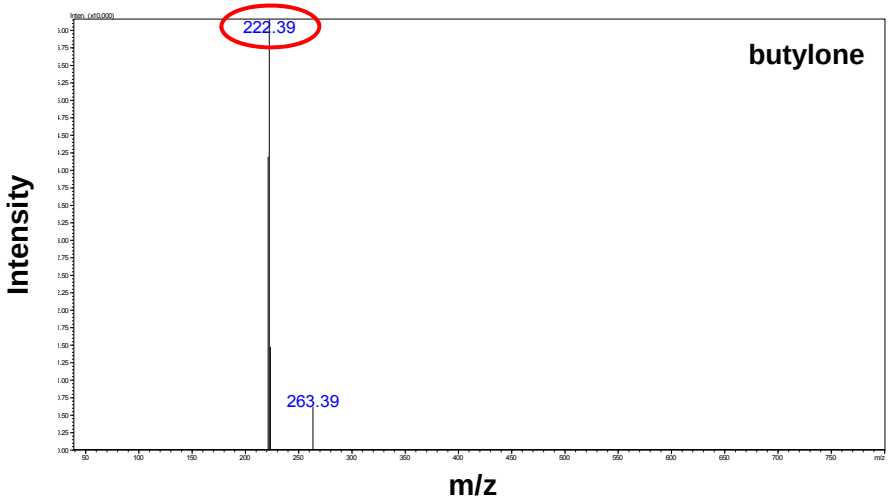


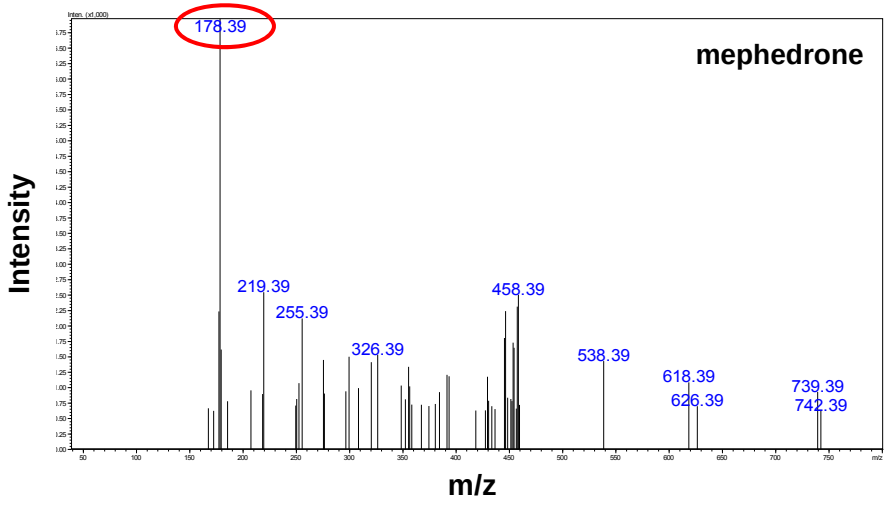


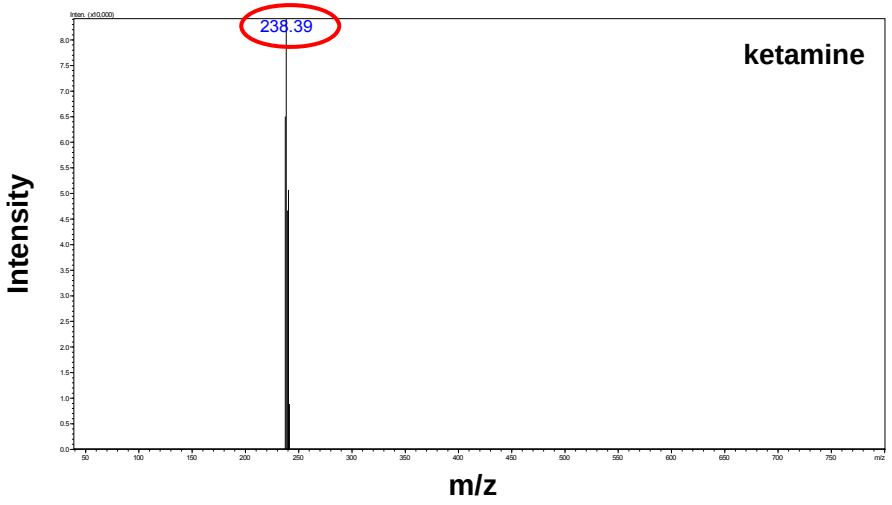


Appendix I: Mass spectra for drugs of abuse, pharmaceuticals and internal standards at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their diagnostic ions (circled)









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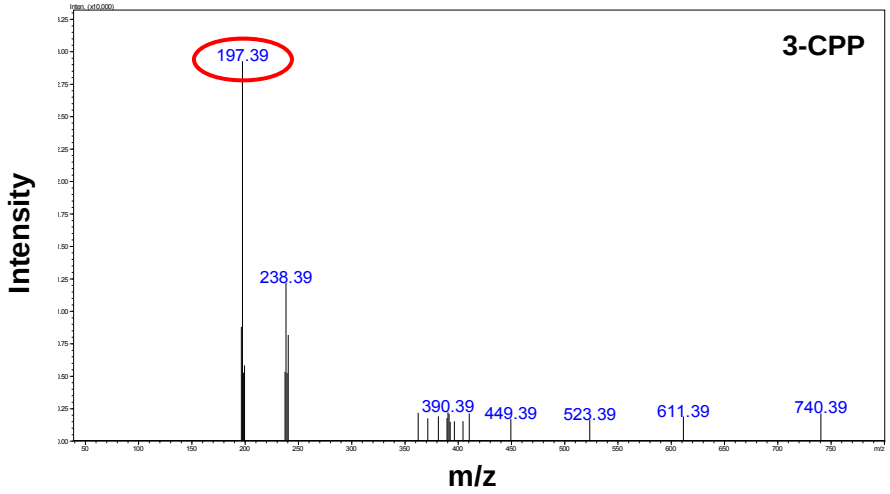
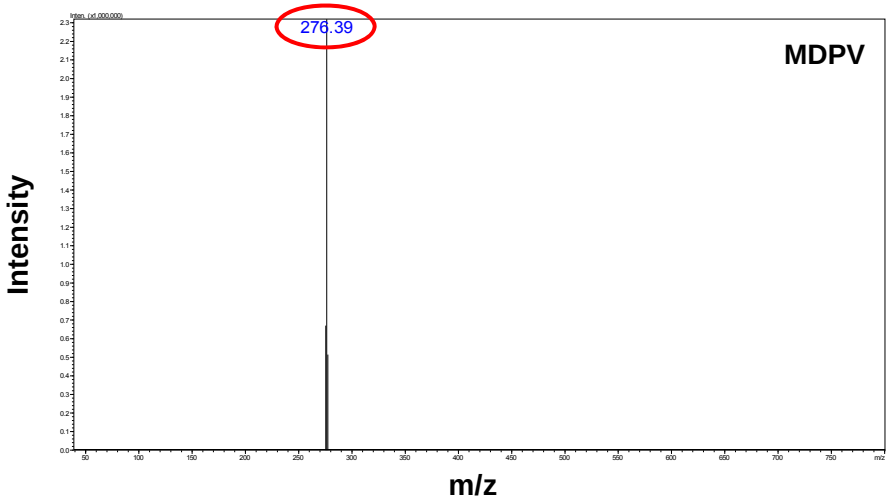
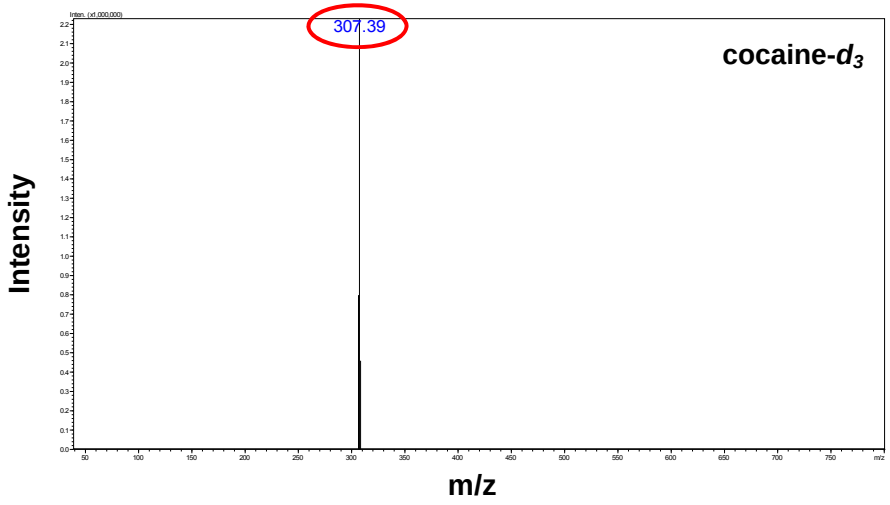
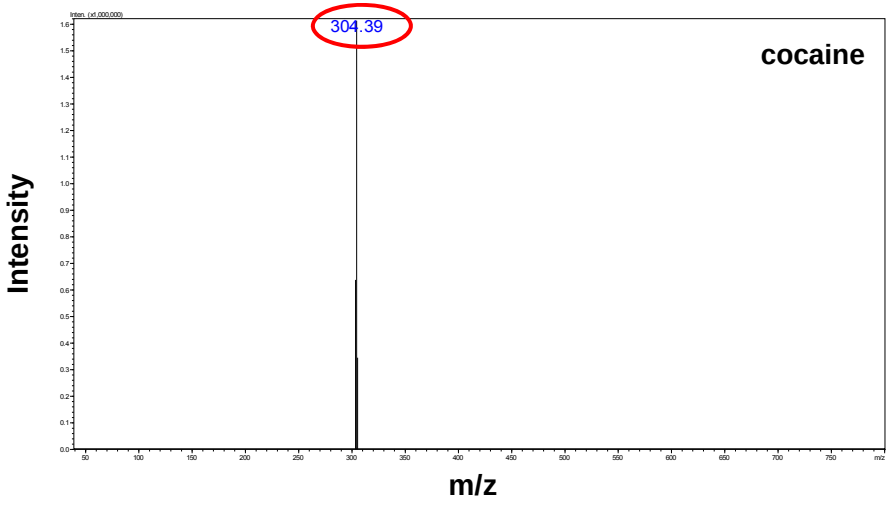
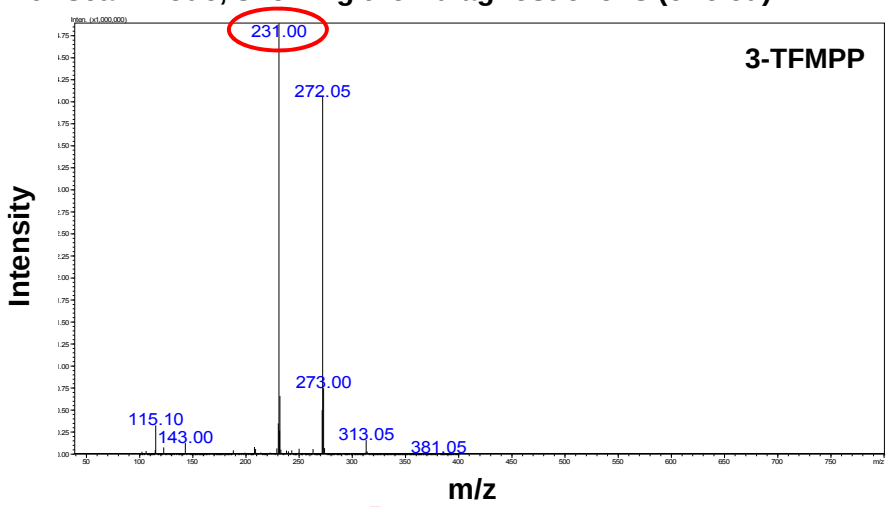


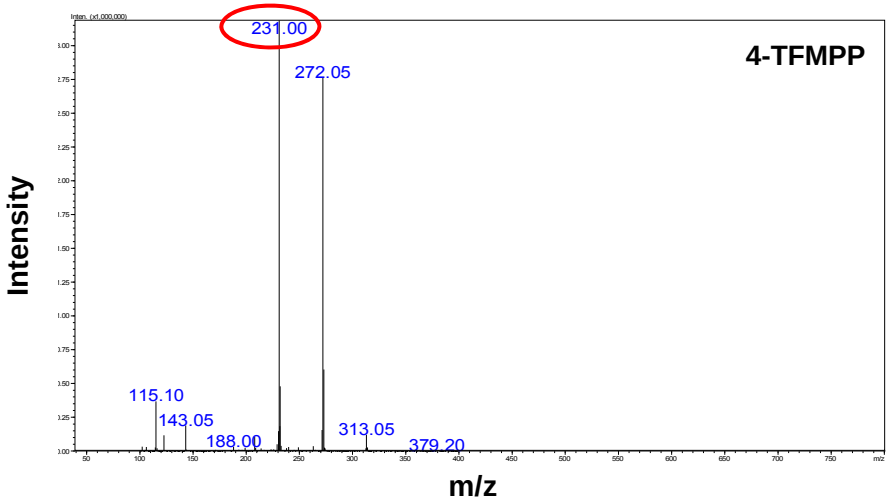


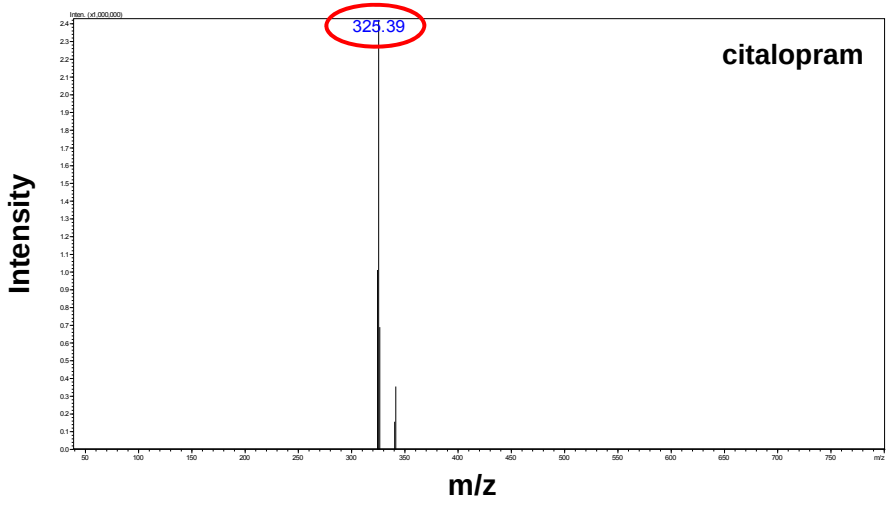


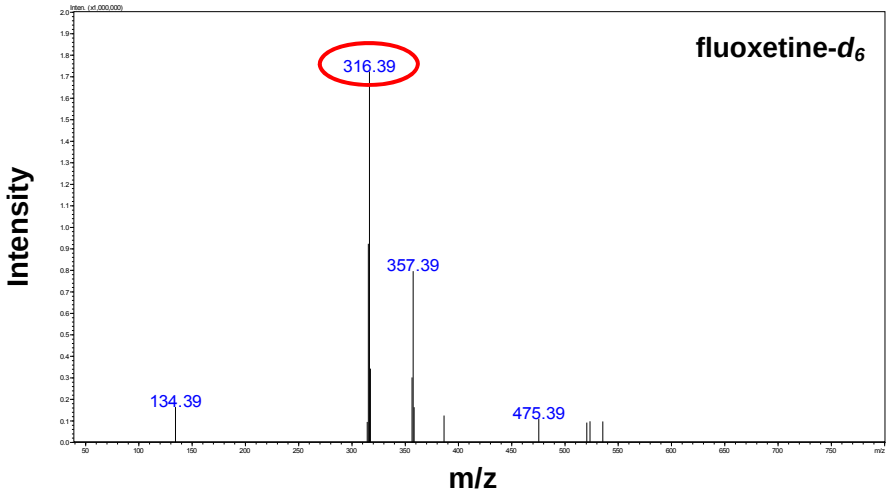


Appendix I: Mass spectra for drugs of abuse, pharmaceuticals and internal standards at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their diagnostic ions (circled)

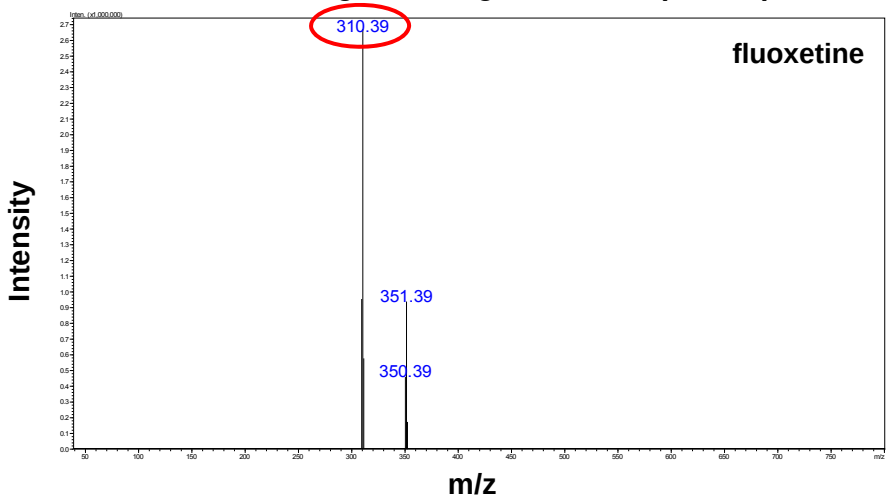


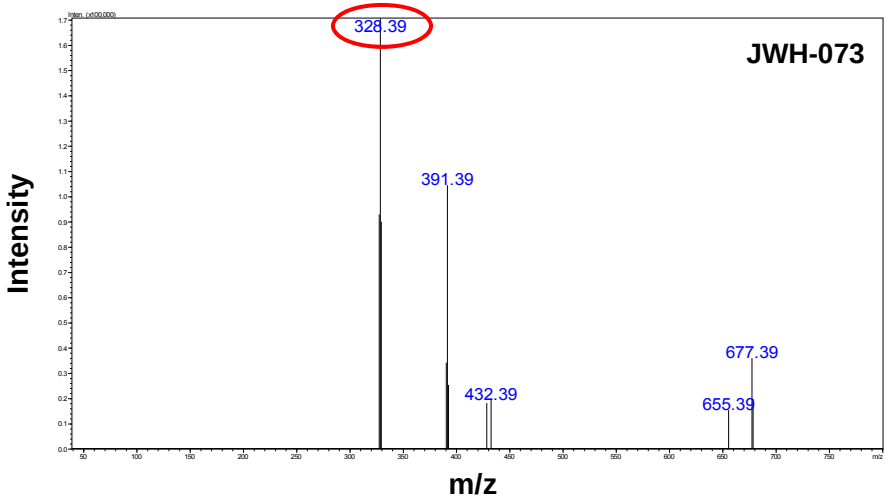


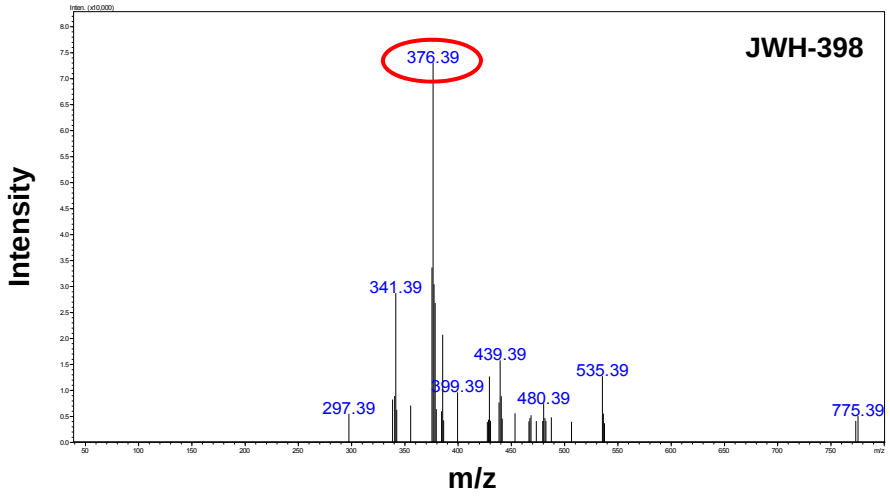




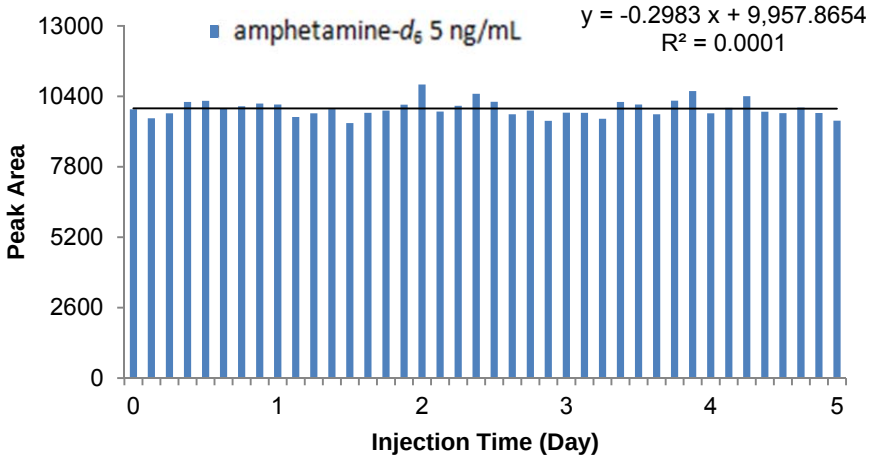
Appendix I: Mass spectra for drugs of abuse, pharmaceuticals and internal standards at 0.01 mg/mL from a LC-MS analysis obtained with scan mode, showing their diagnostic ions (circled)

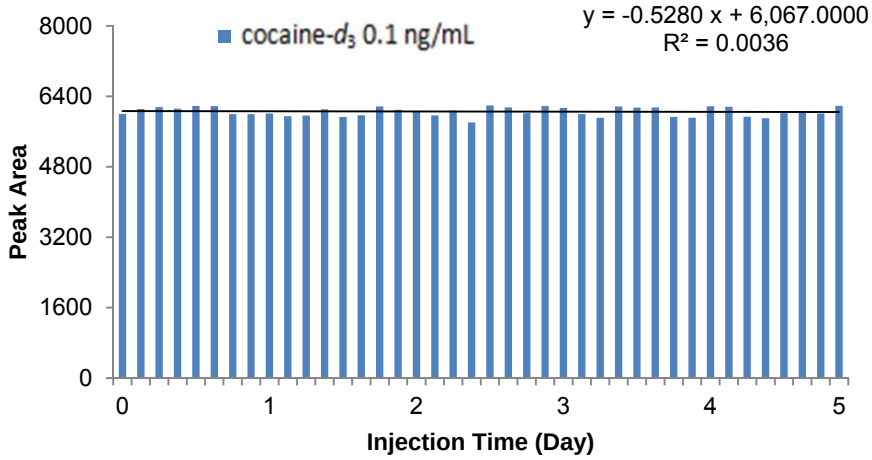


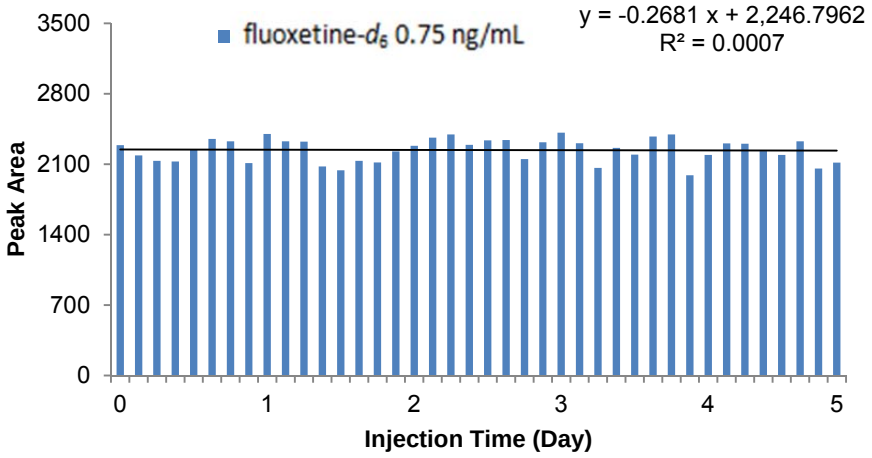




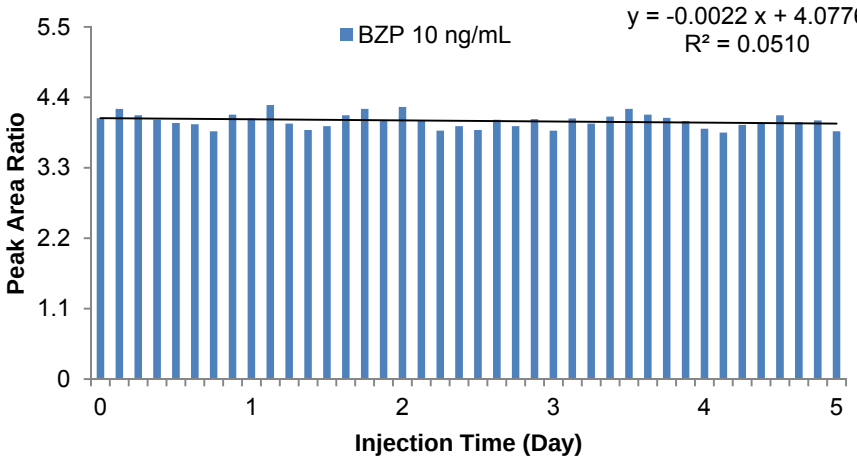
Appendix II-a: Bar graphs of peak area against injection time for internal standards for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

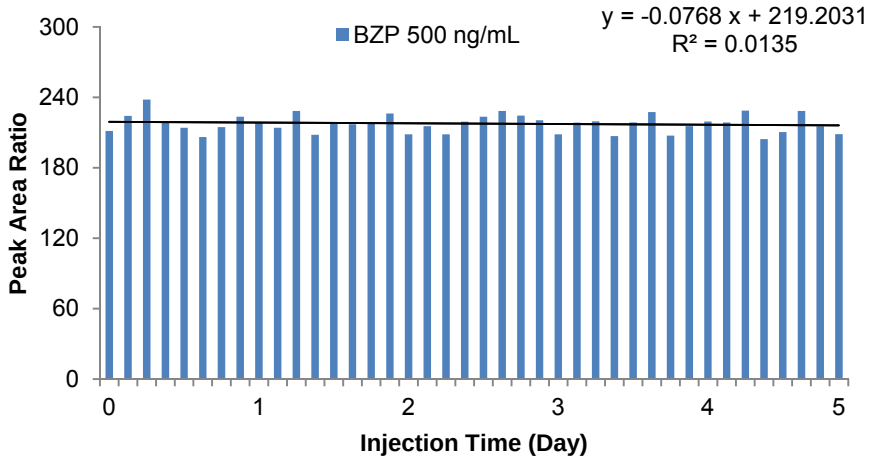


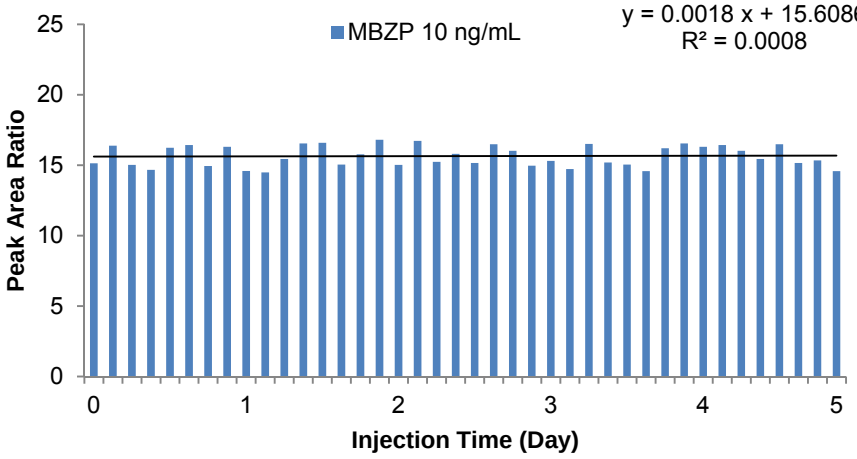


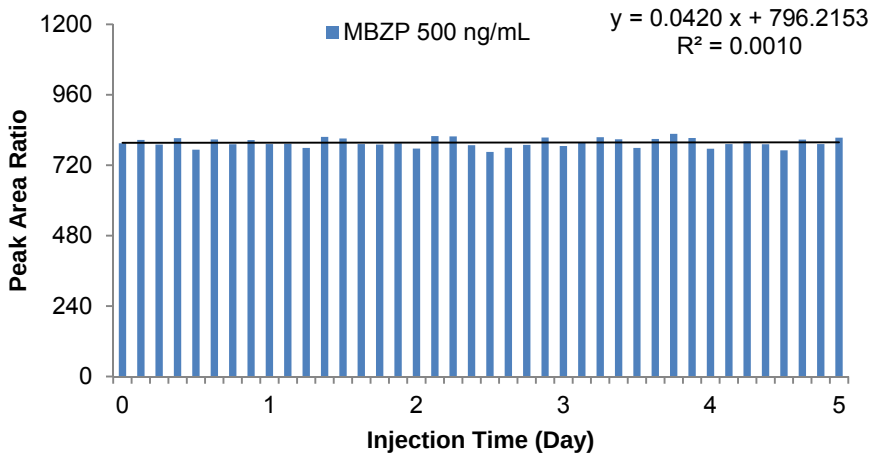


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

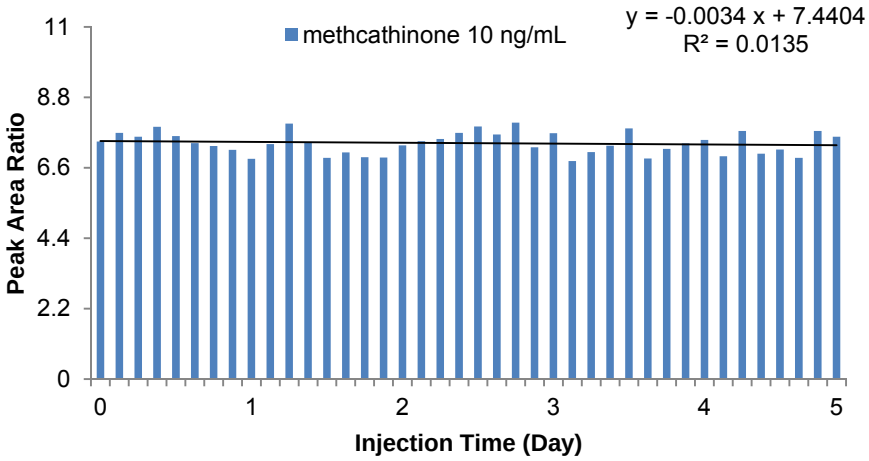


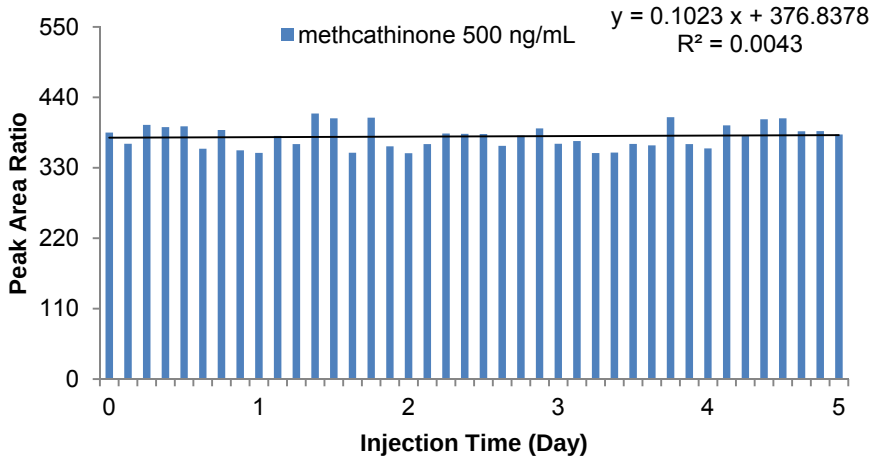


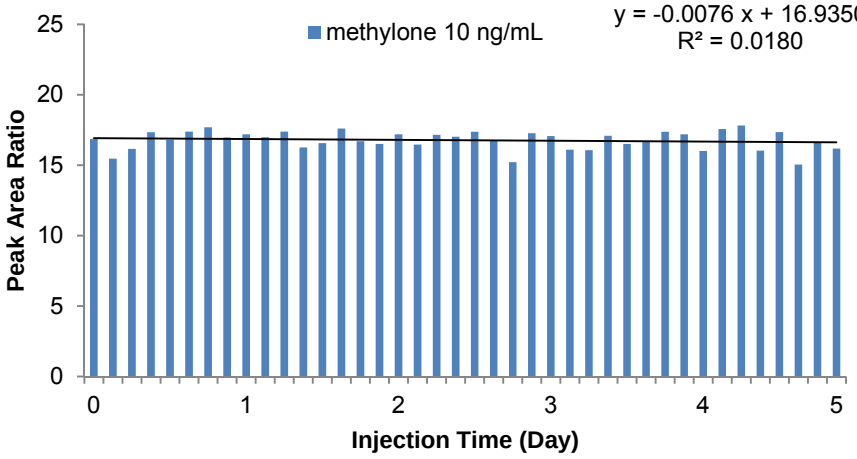


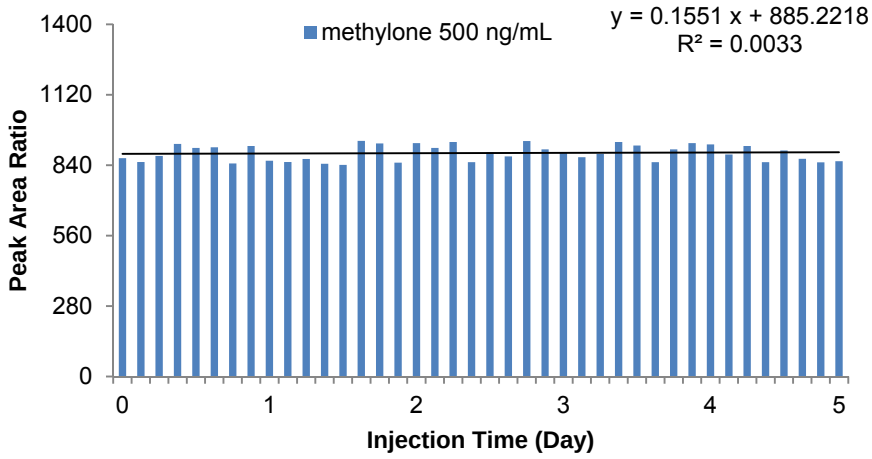


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

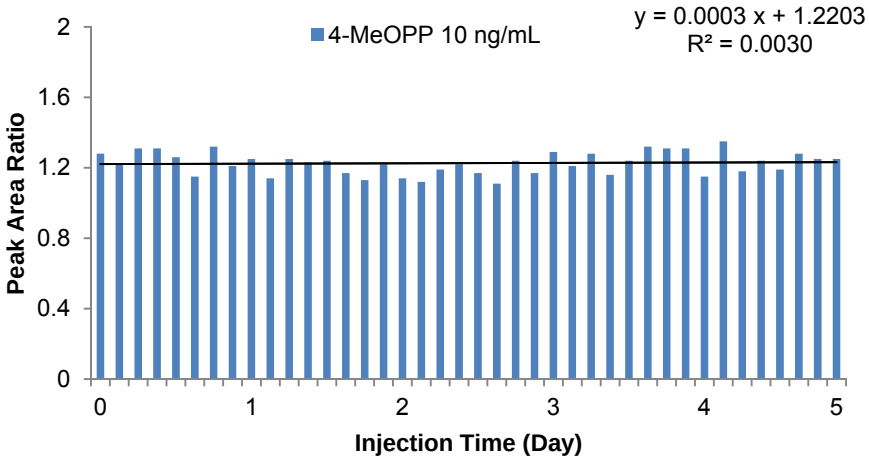


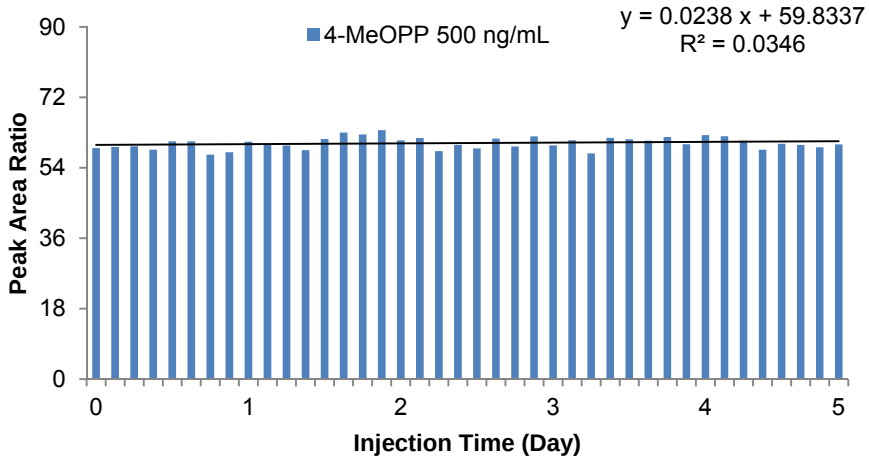


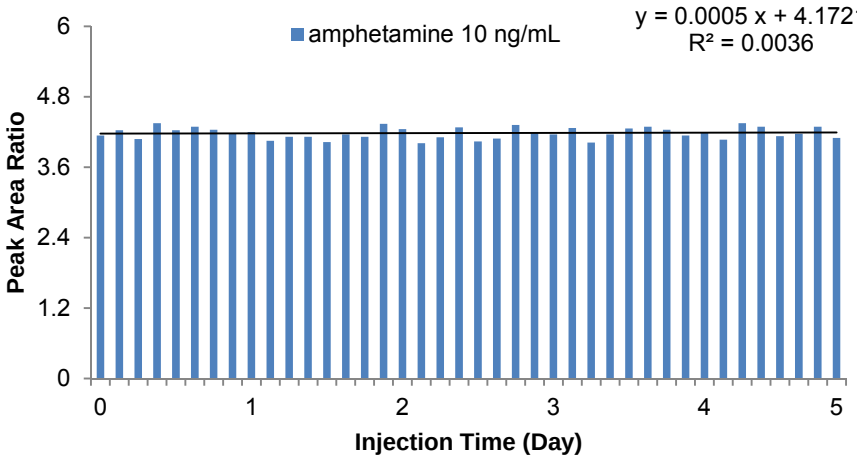


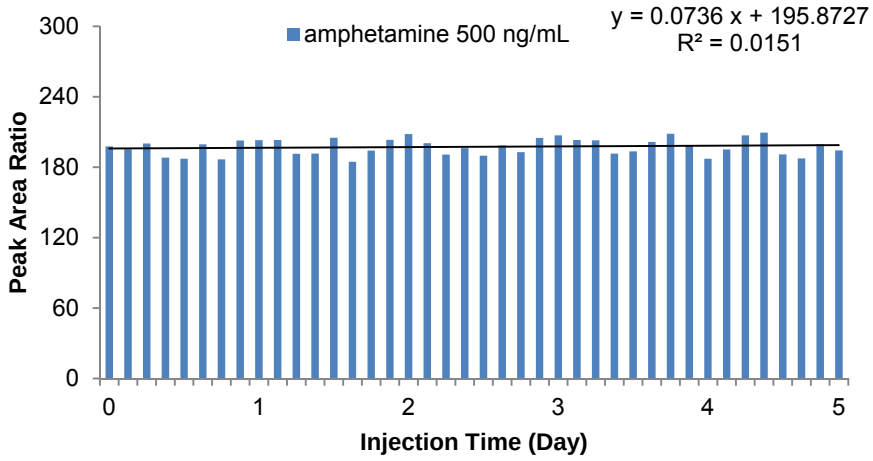


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

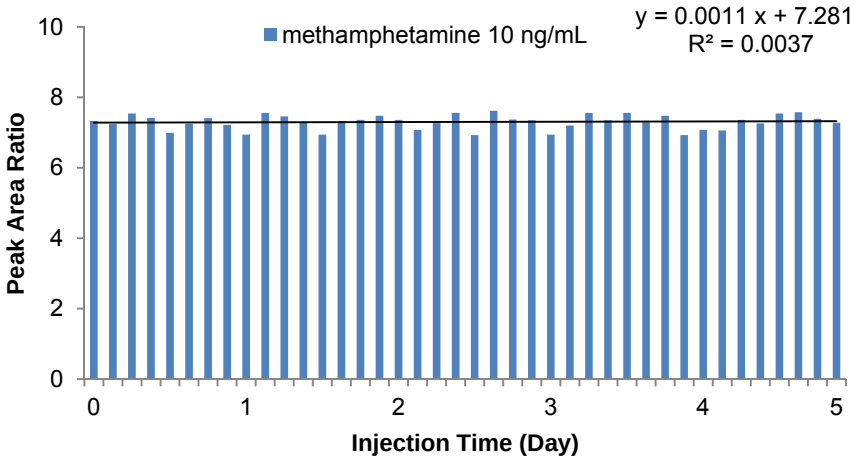


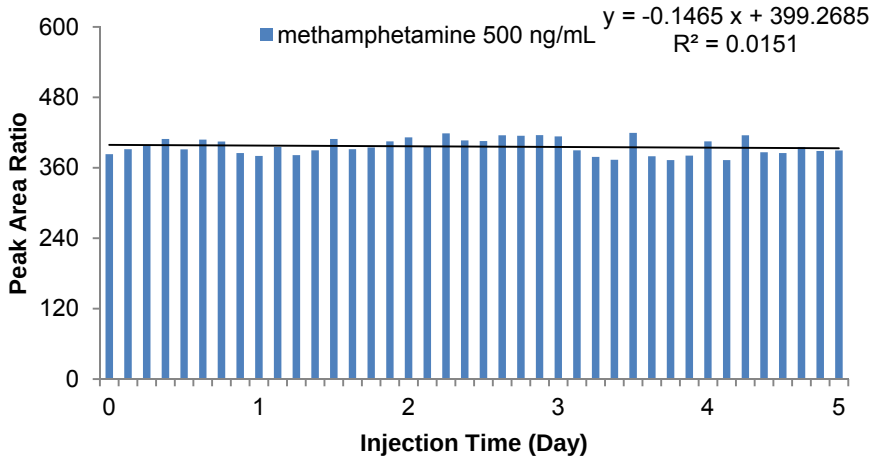


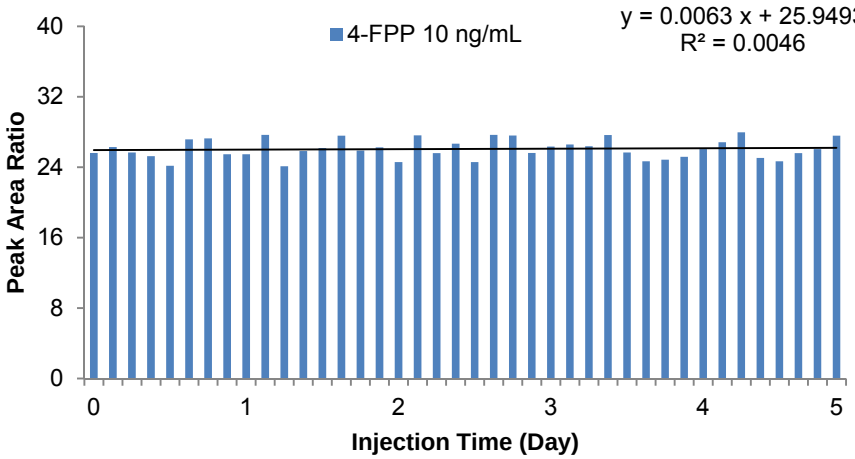


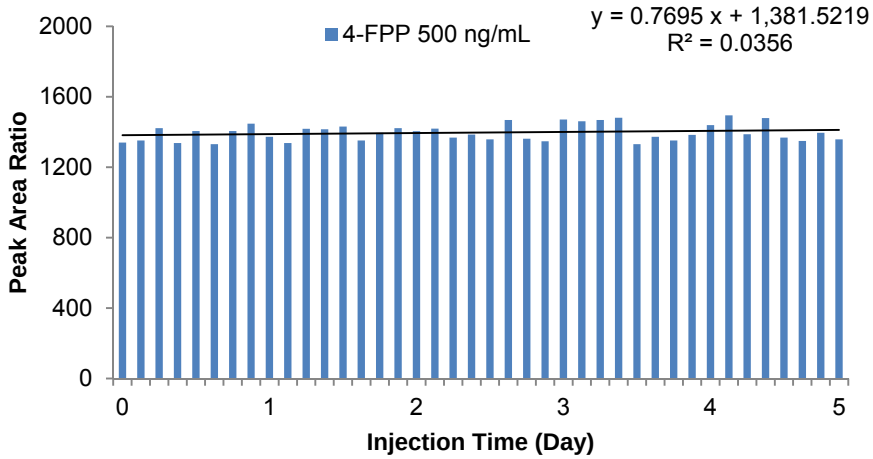


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

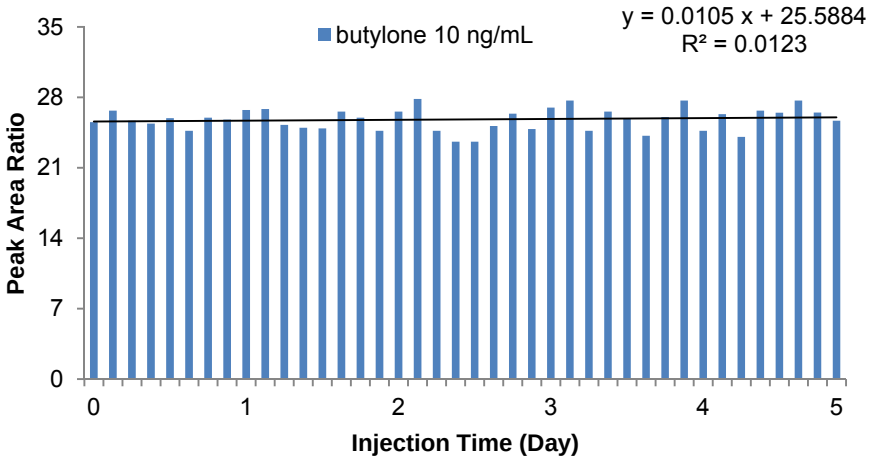


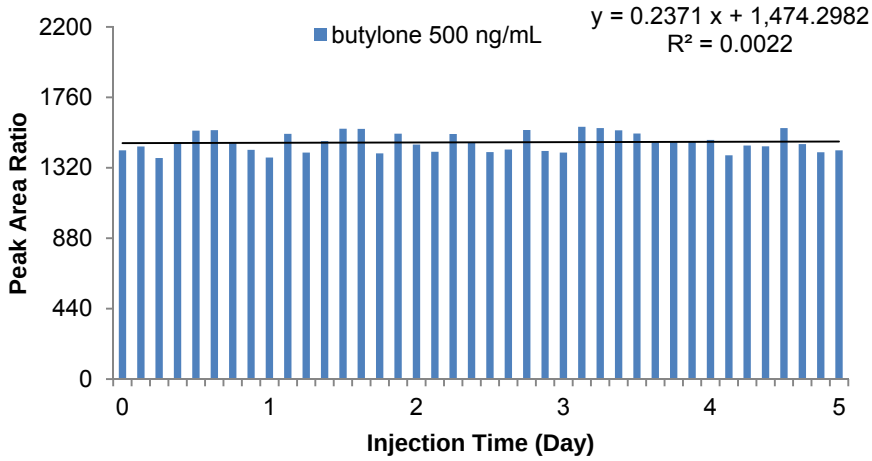


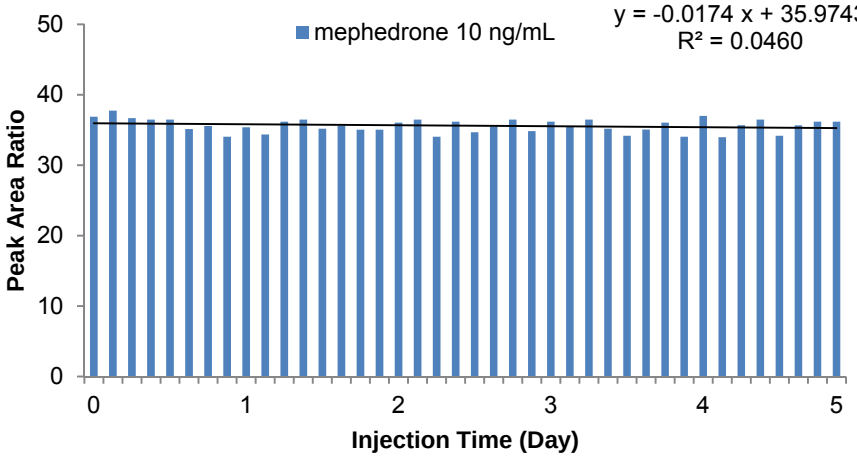


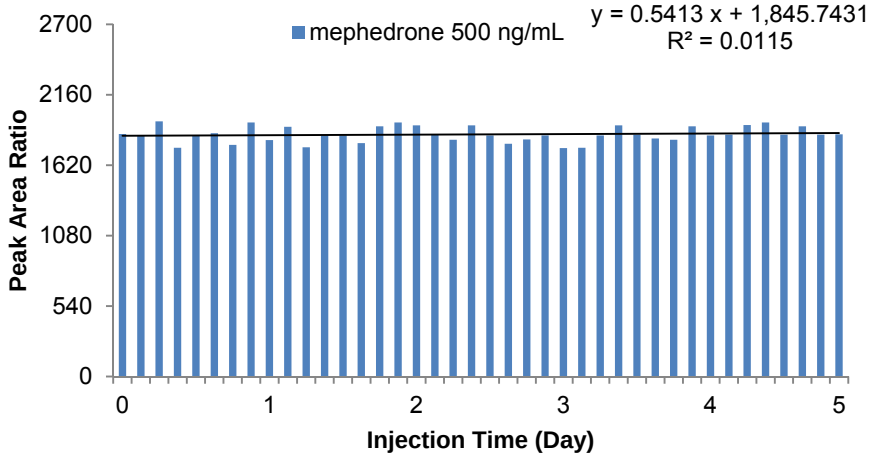


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

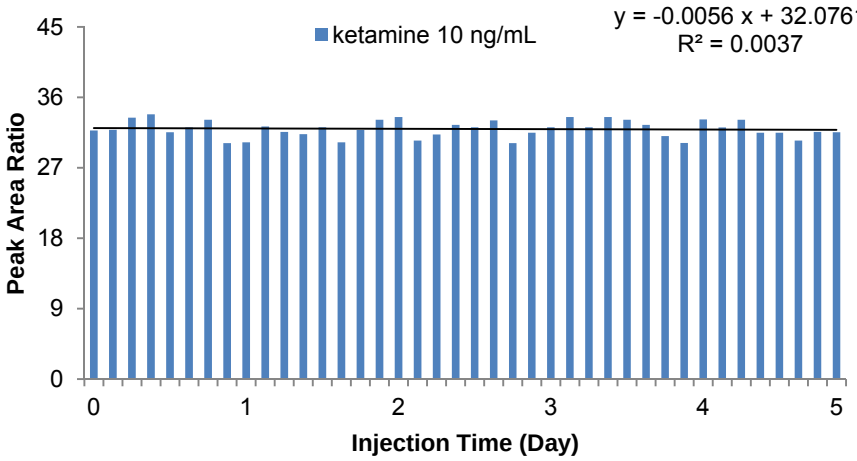


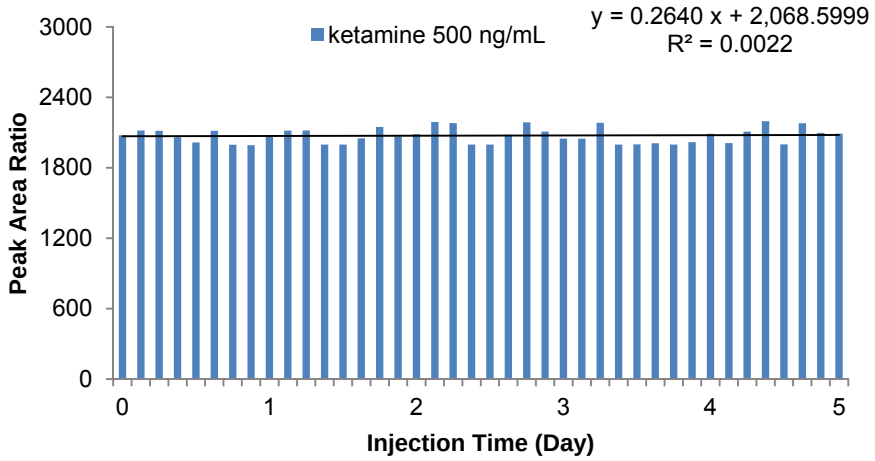


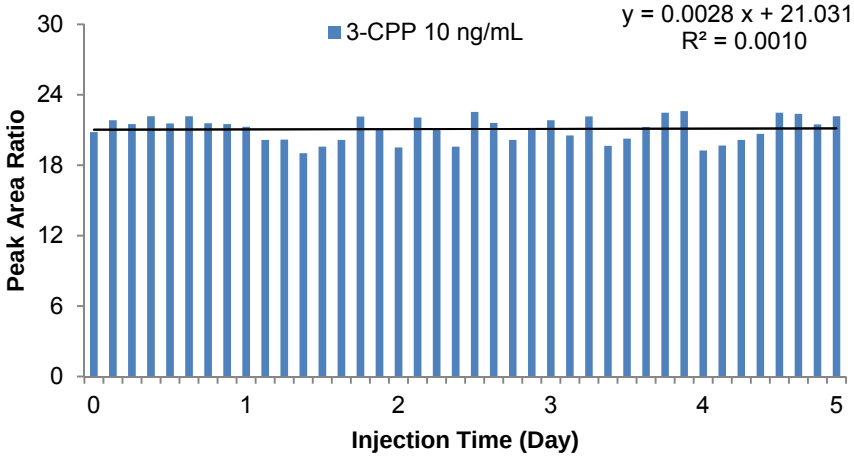


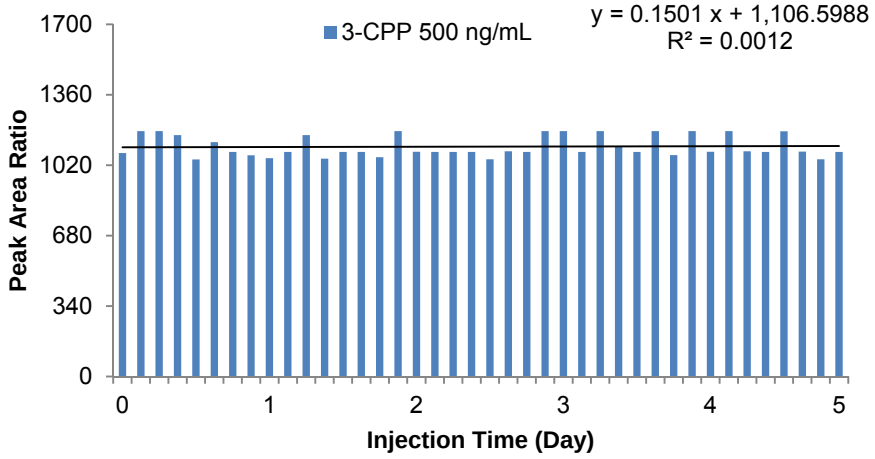


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

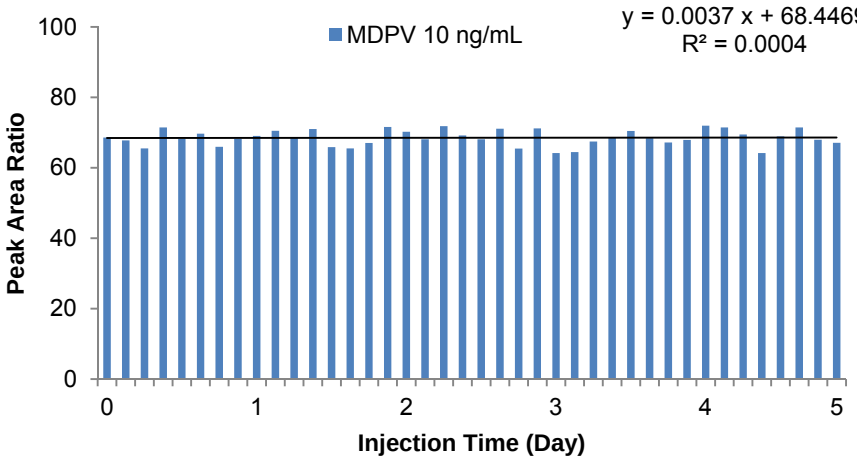


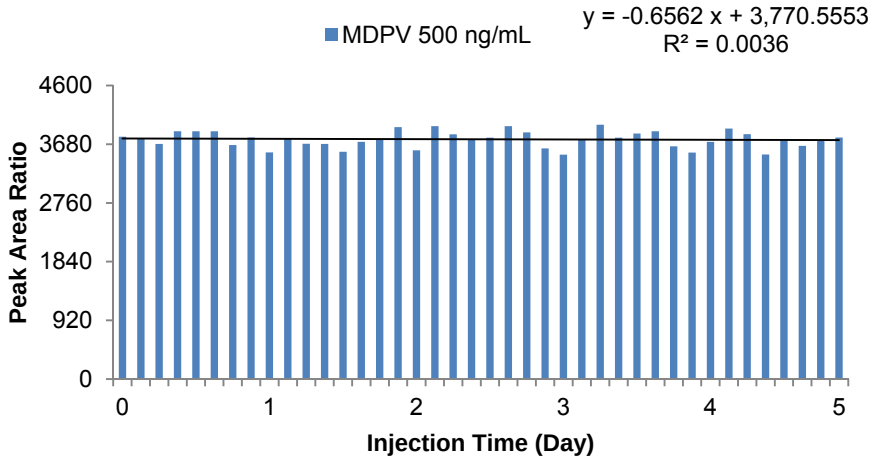


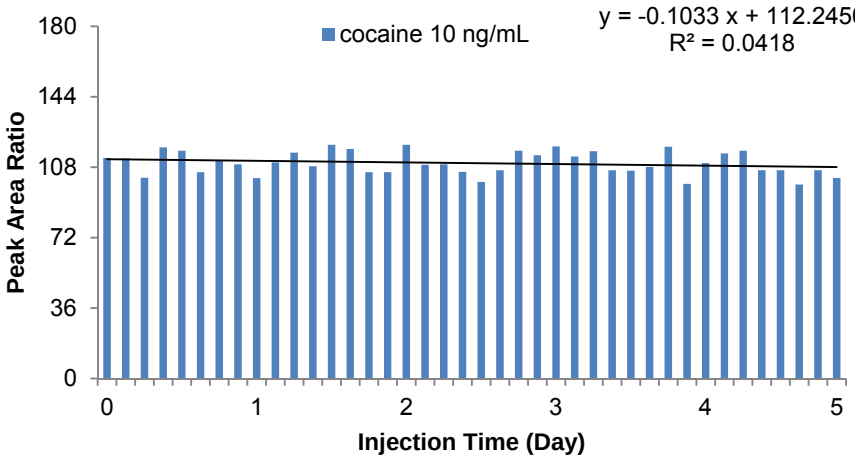


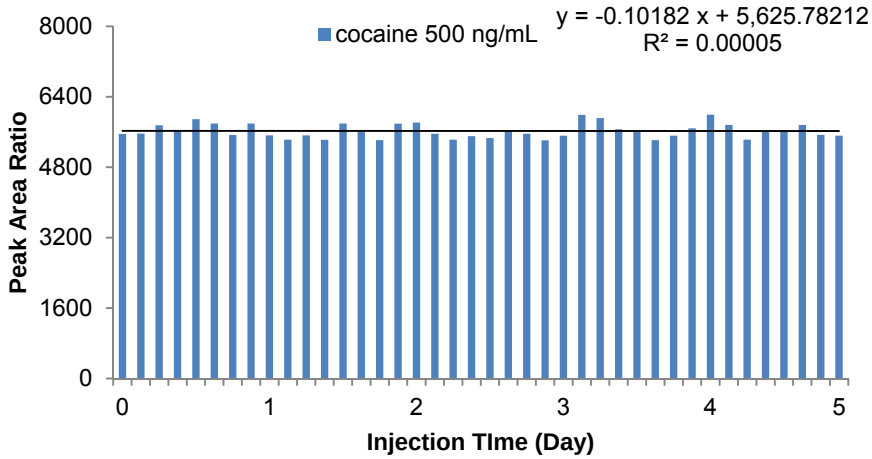


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

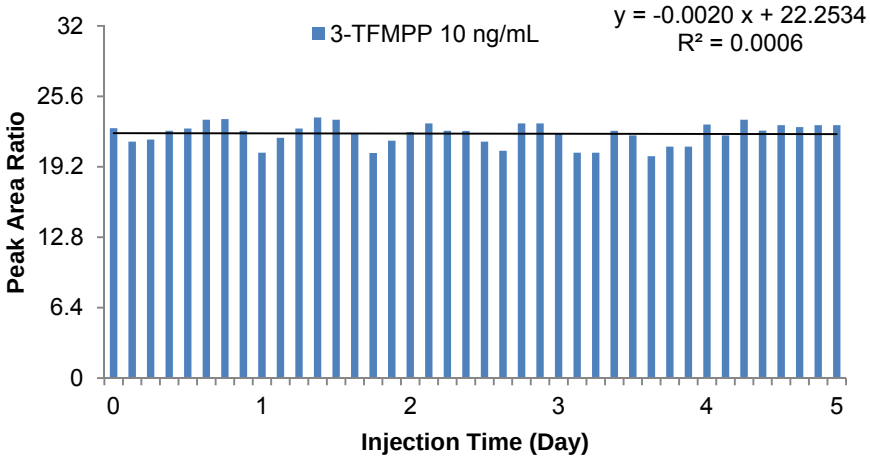


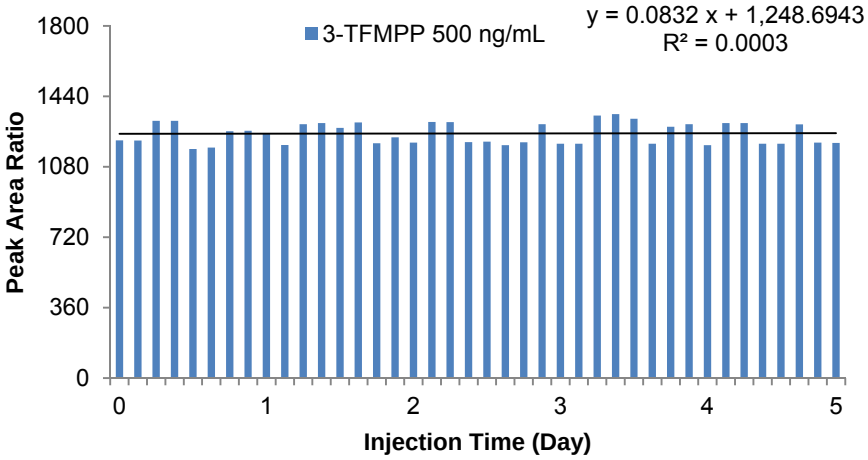


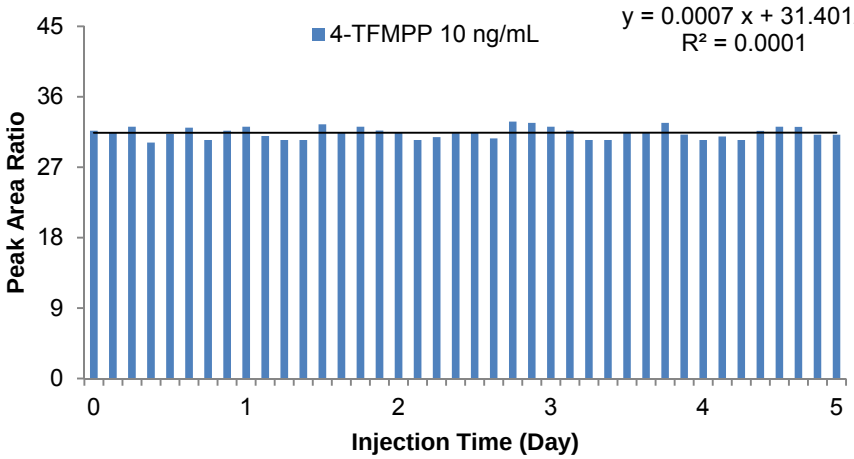


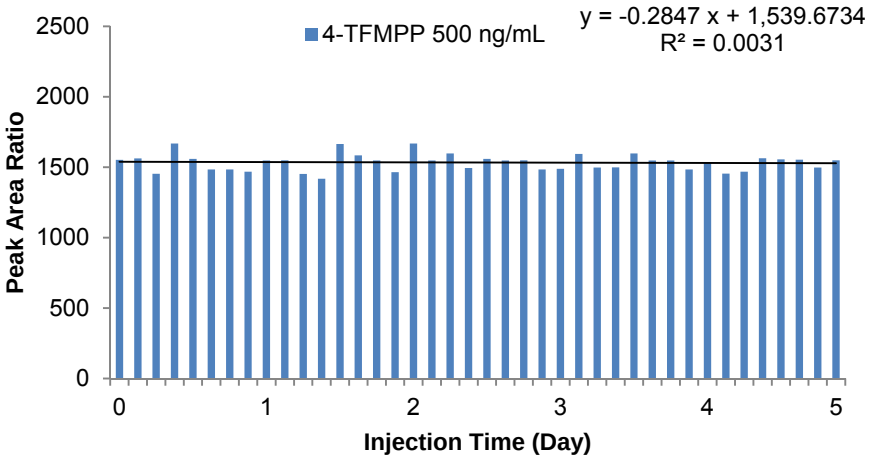


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

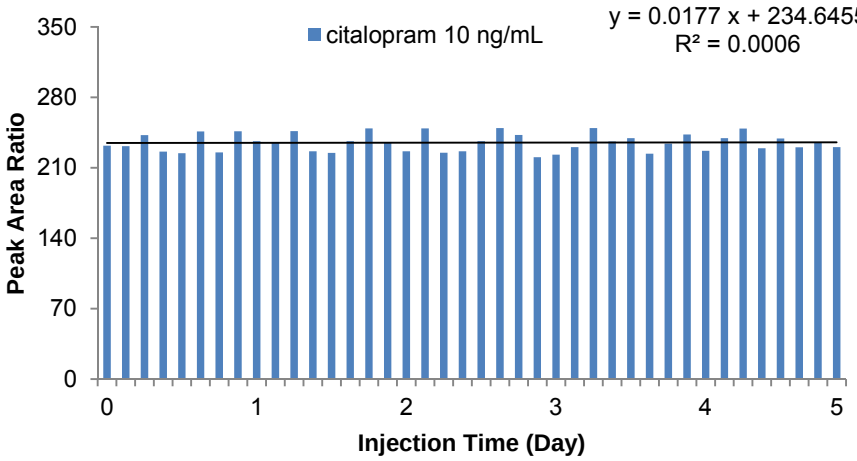


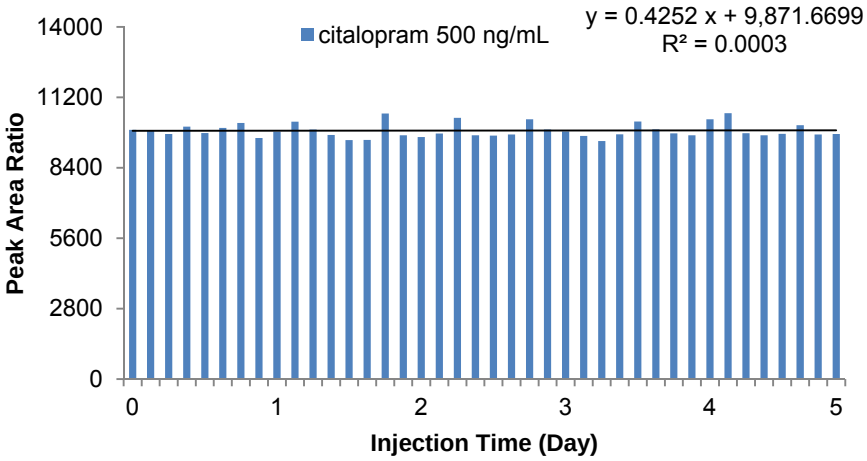


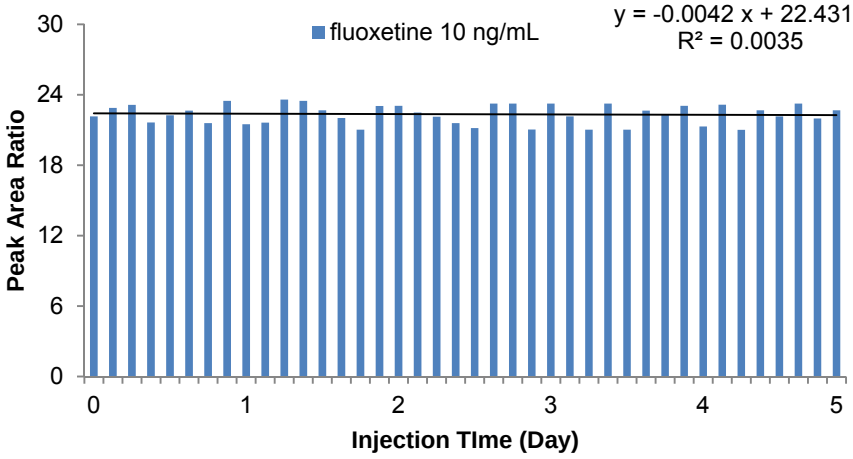


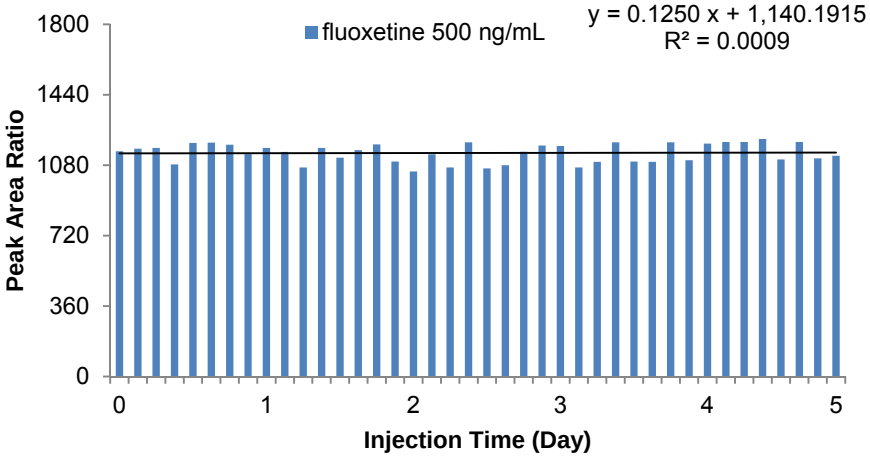


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

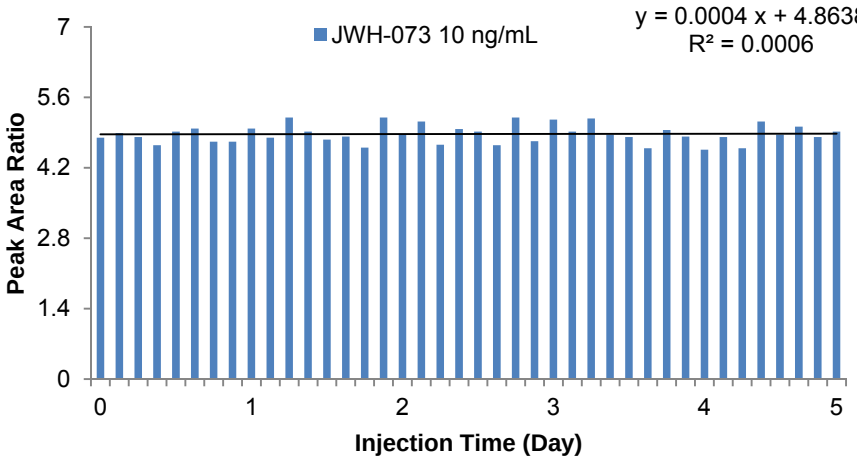


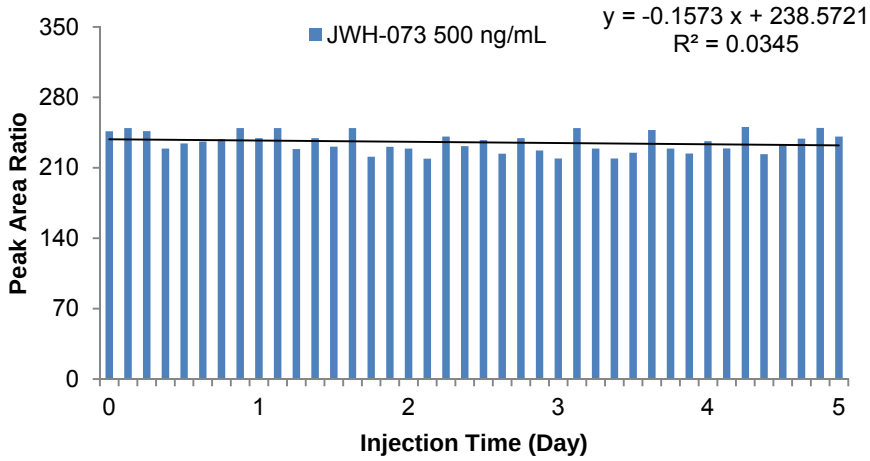


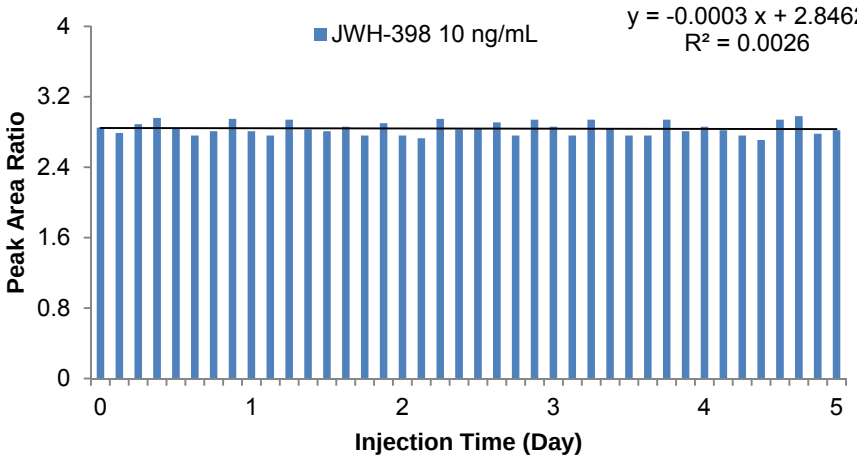


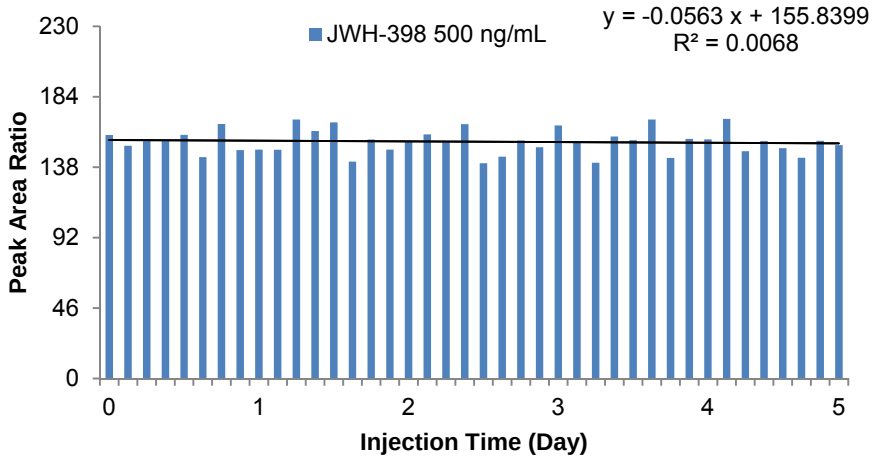


Appendix II-b: Bar graphs of peak area ratio against injection time for drugs of abuse and pharmaceuticals for autosampler storage stability obtained from a LC-MS analysis with a C_{18} column, n = 40

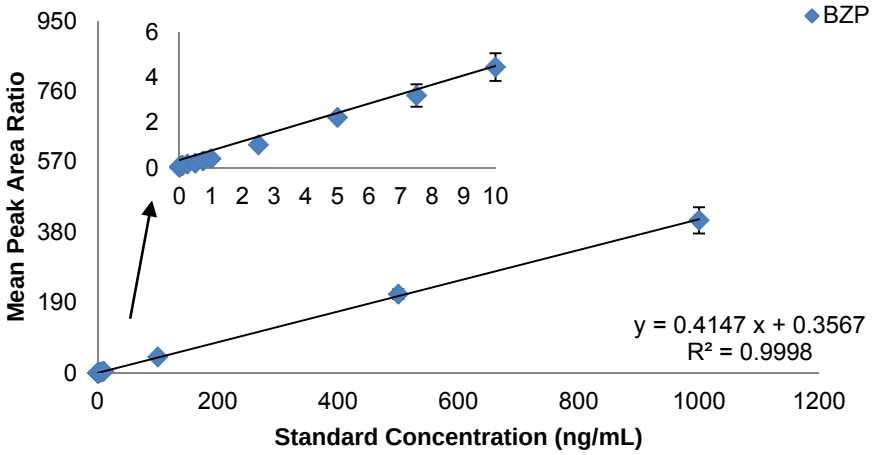


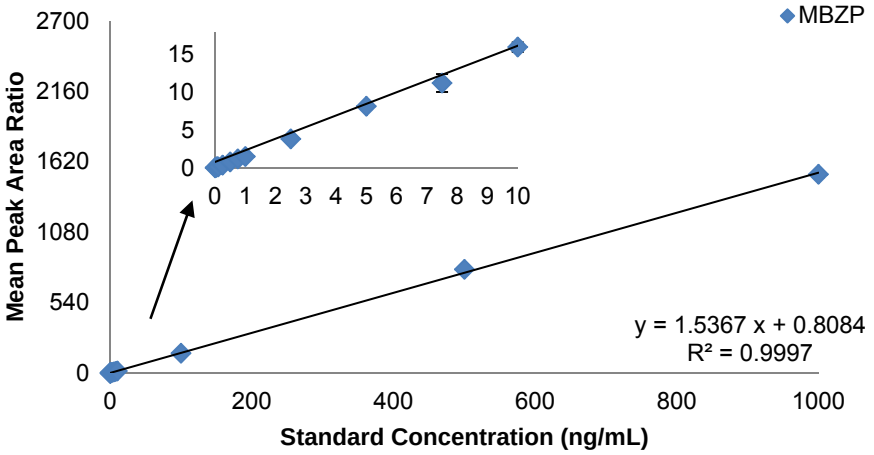


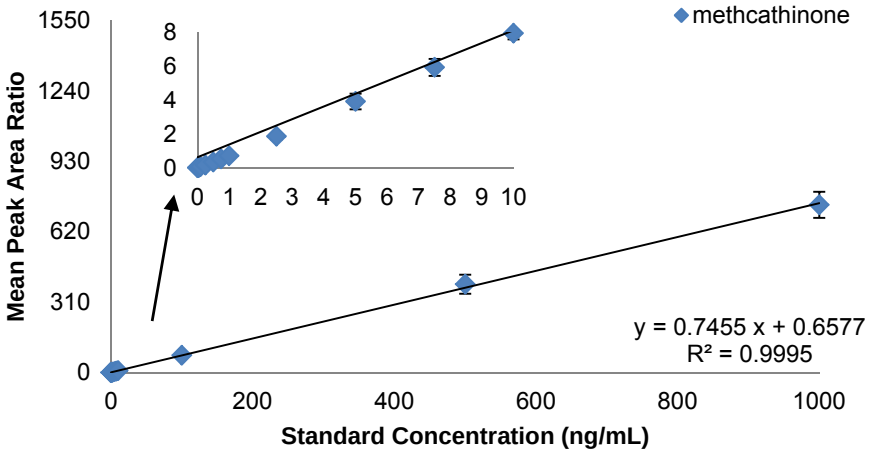


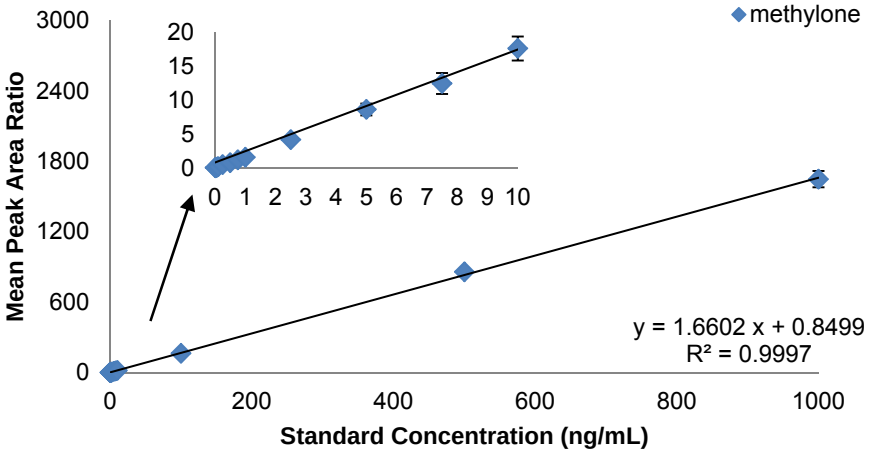


Appendix III: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

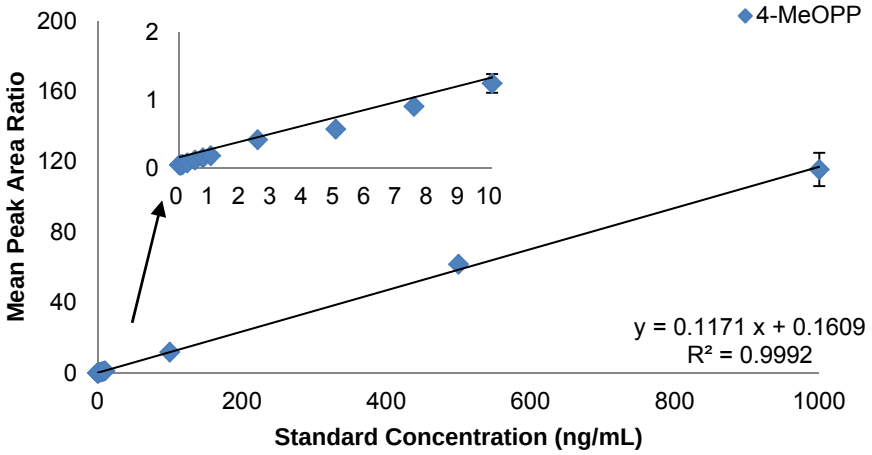


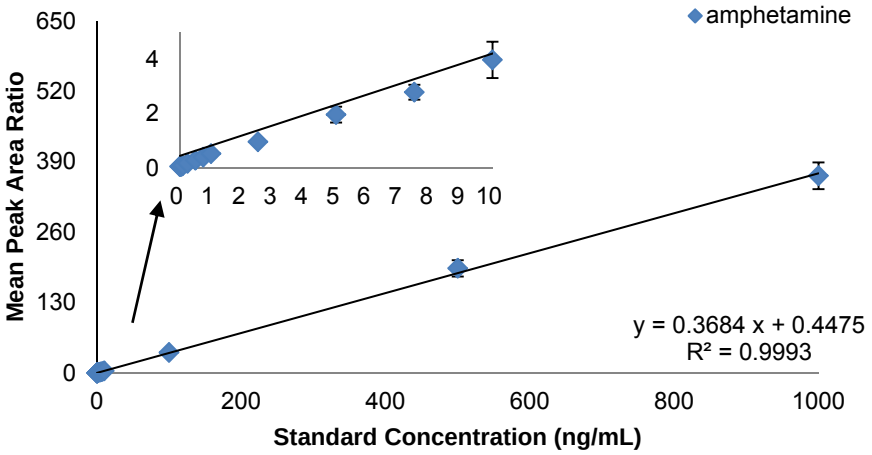


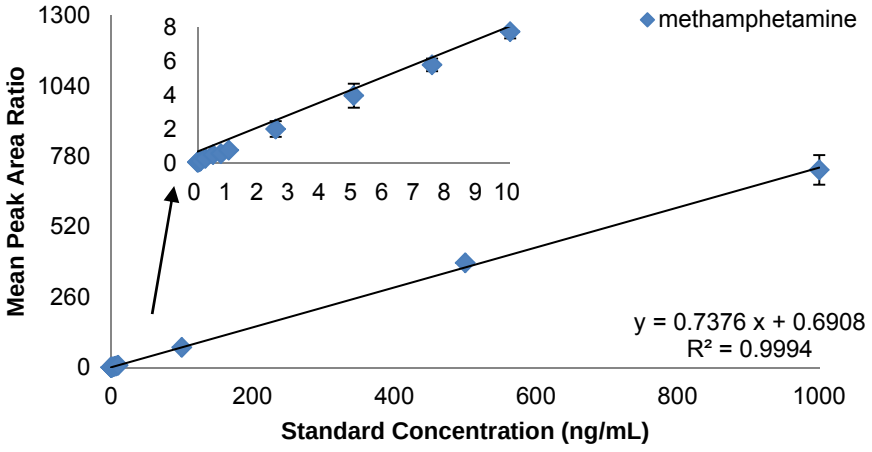


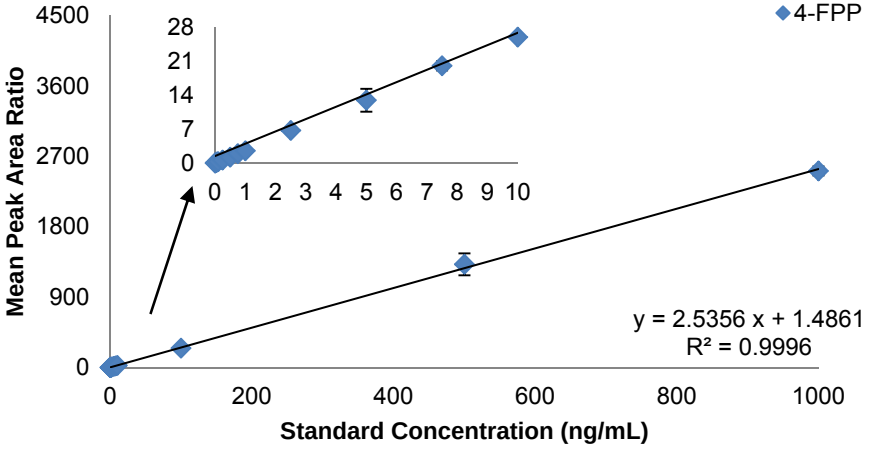


Appendix III: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

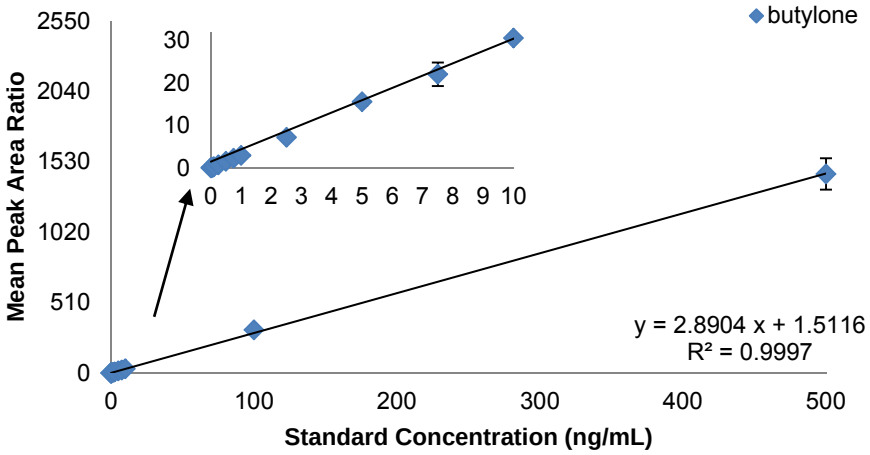


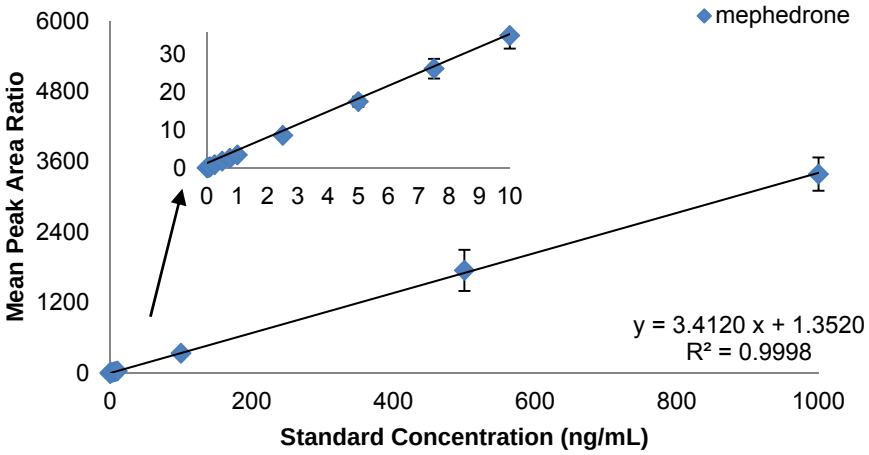


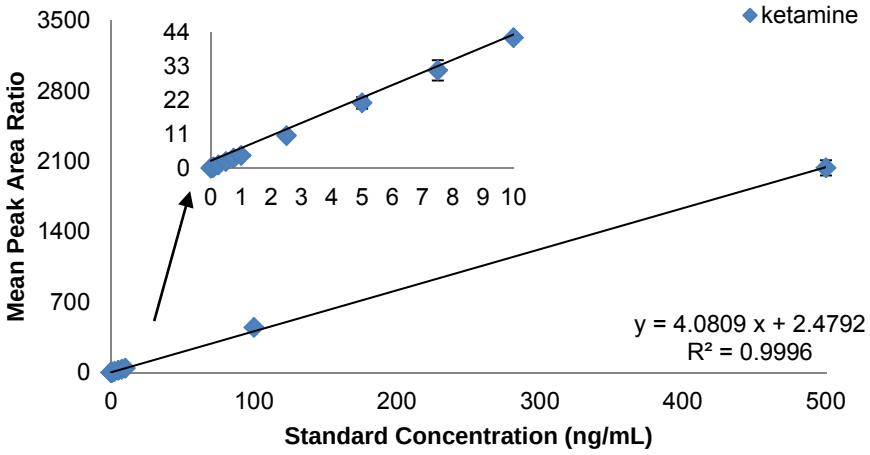


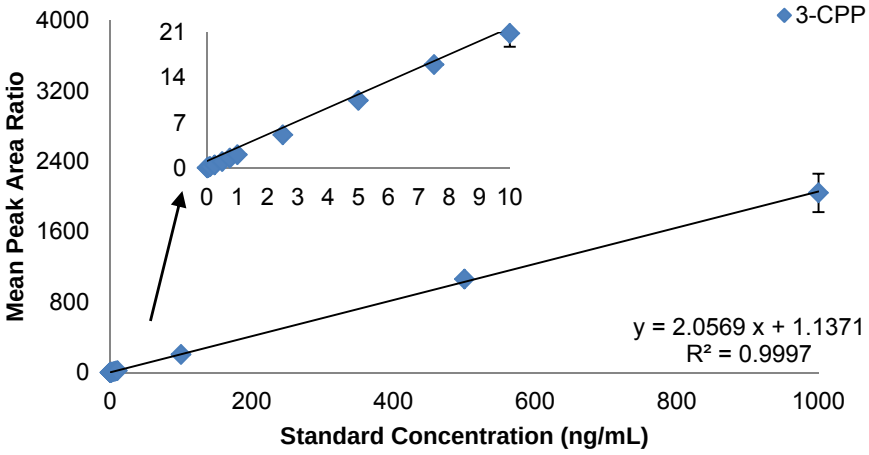


Appendix III: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

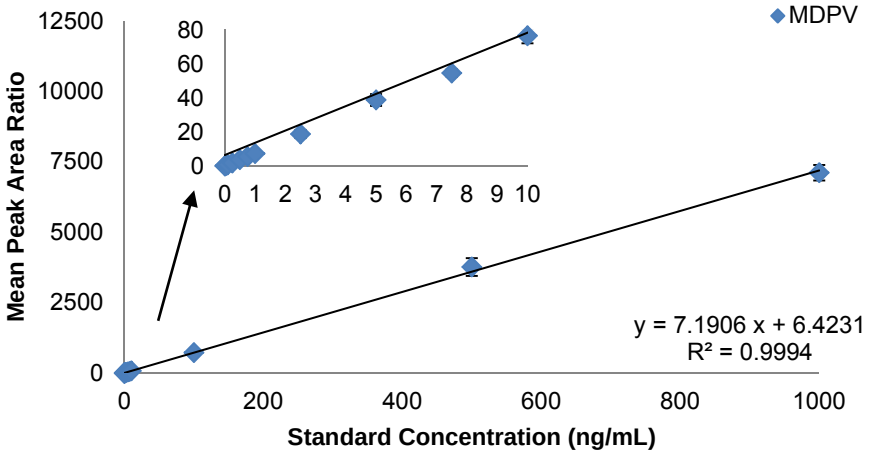


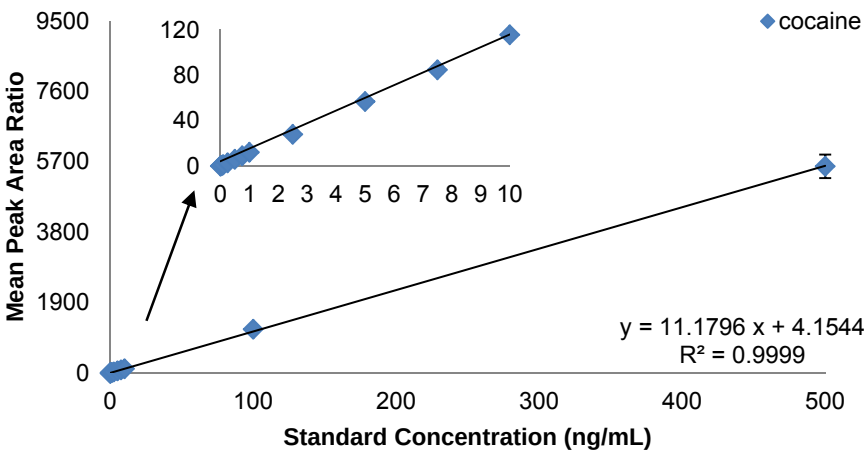


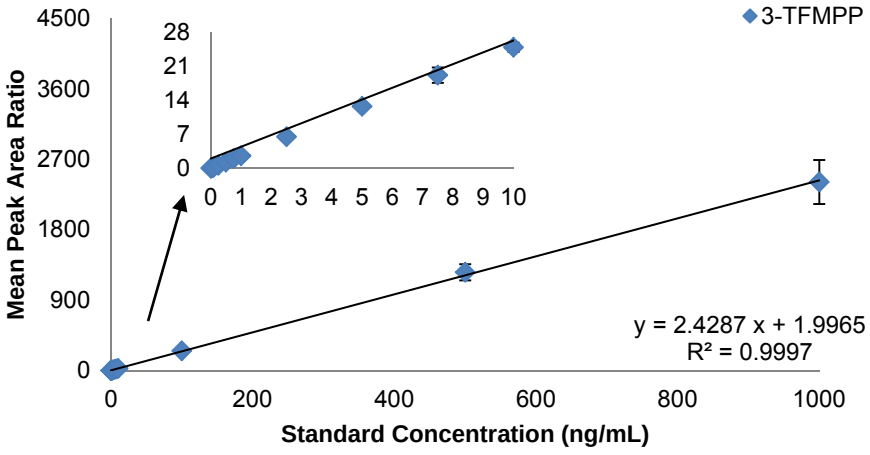


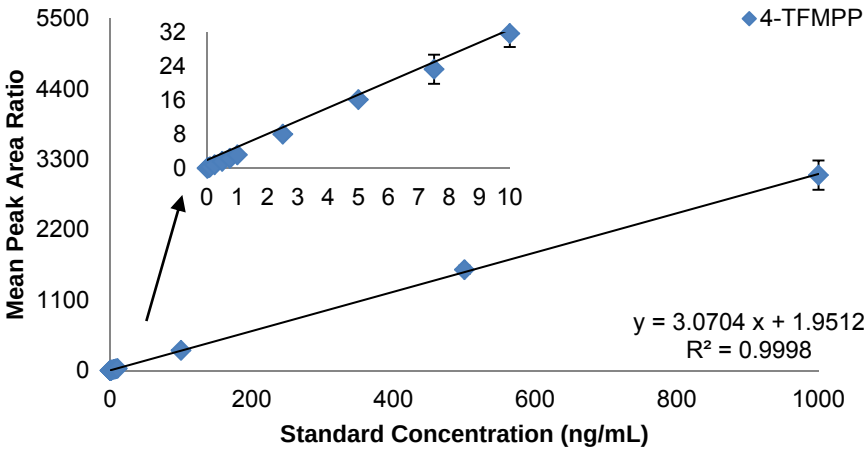


Appendix III: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

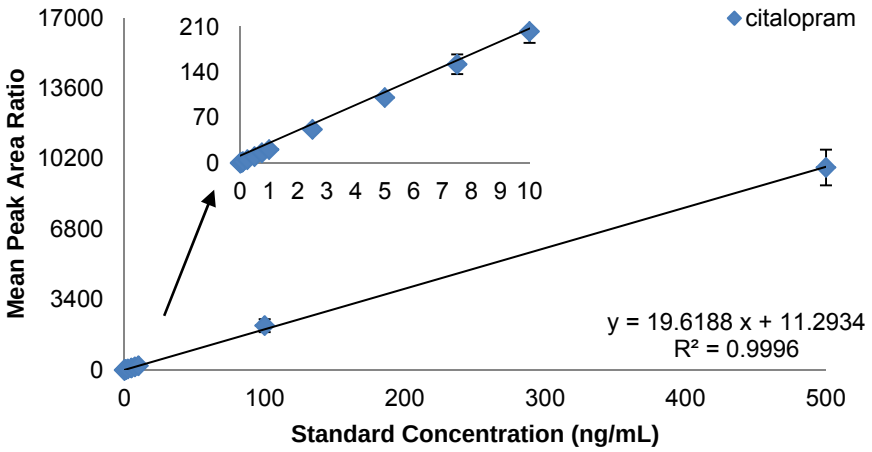


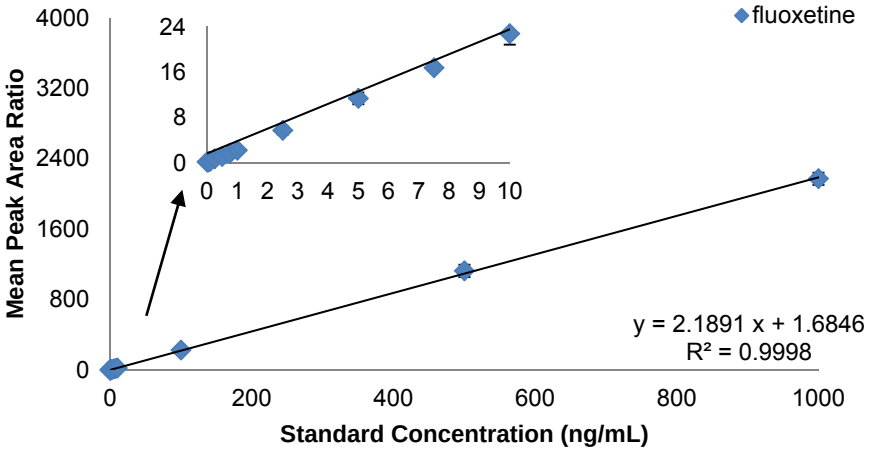


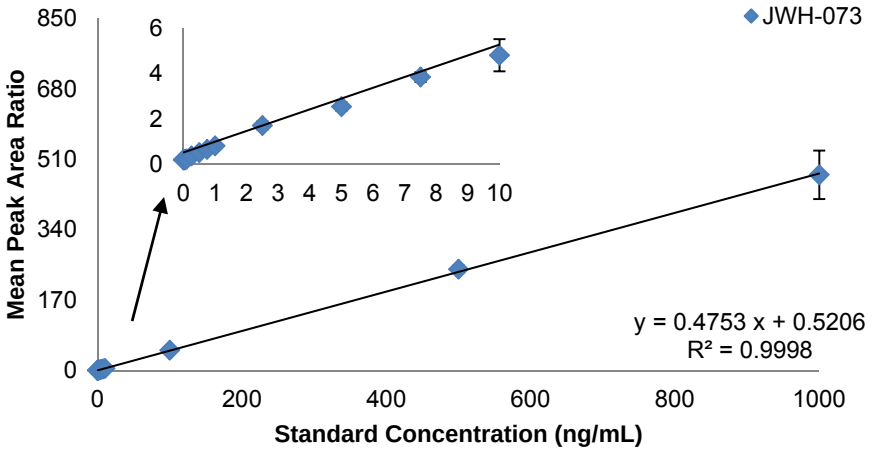


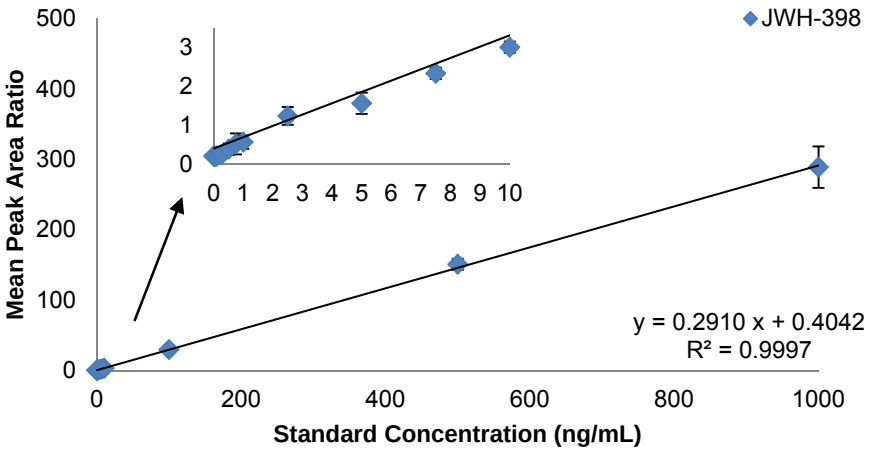


Appendix III: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column, n = 3

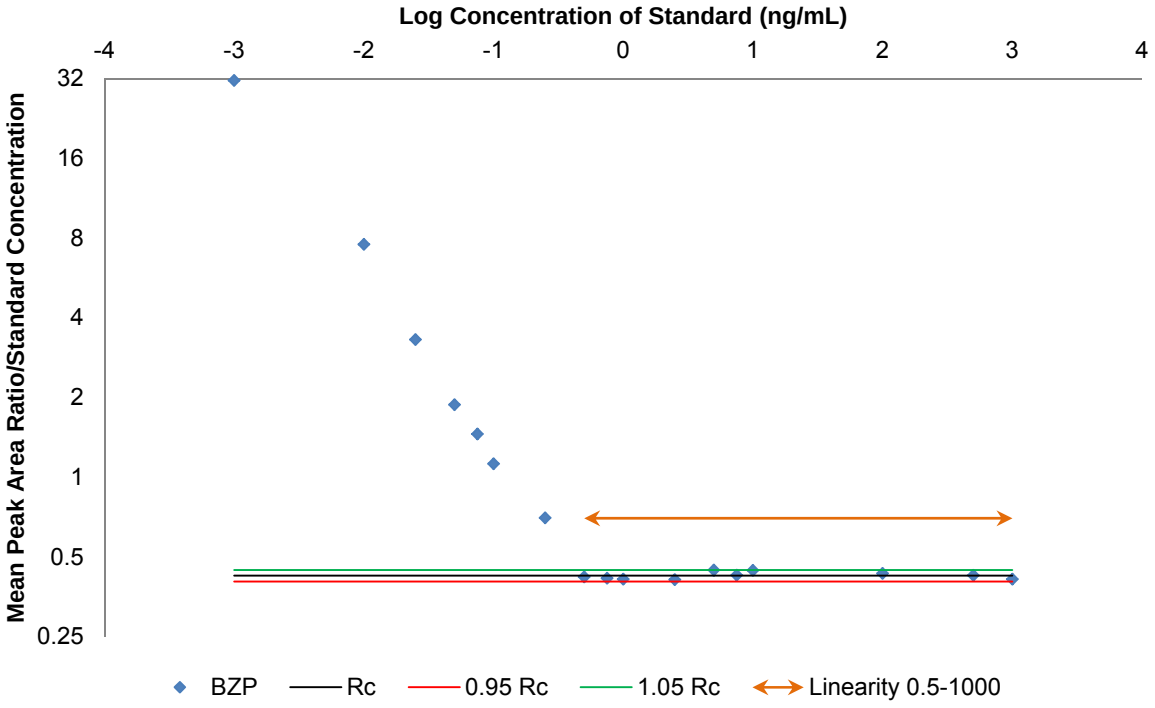


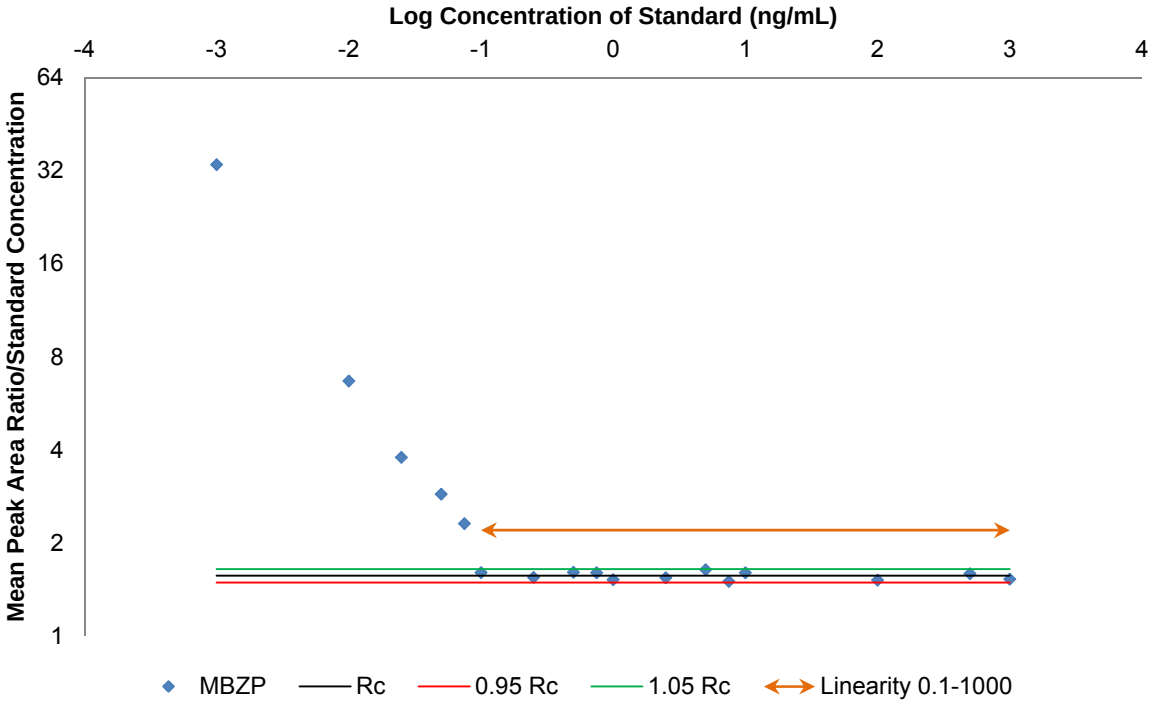




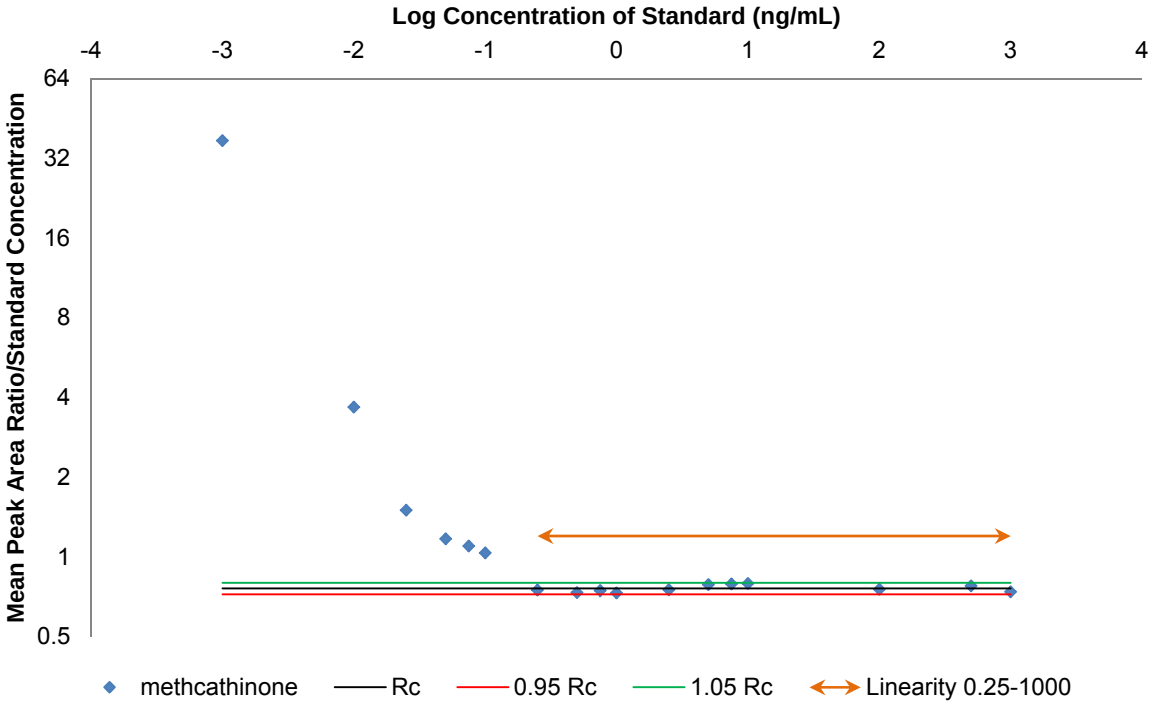


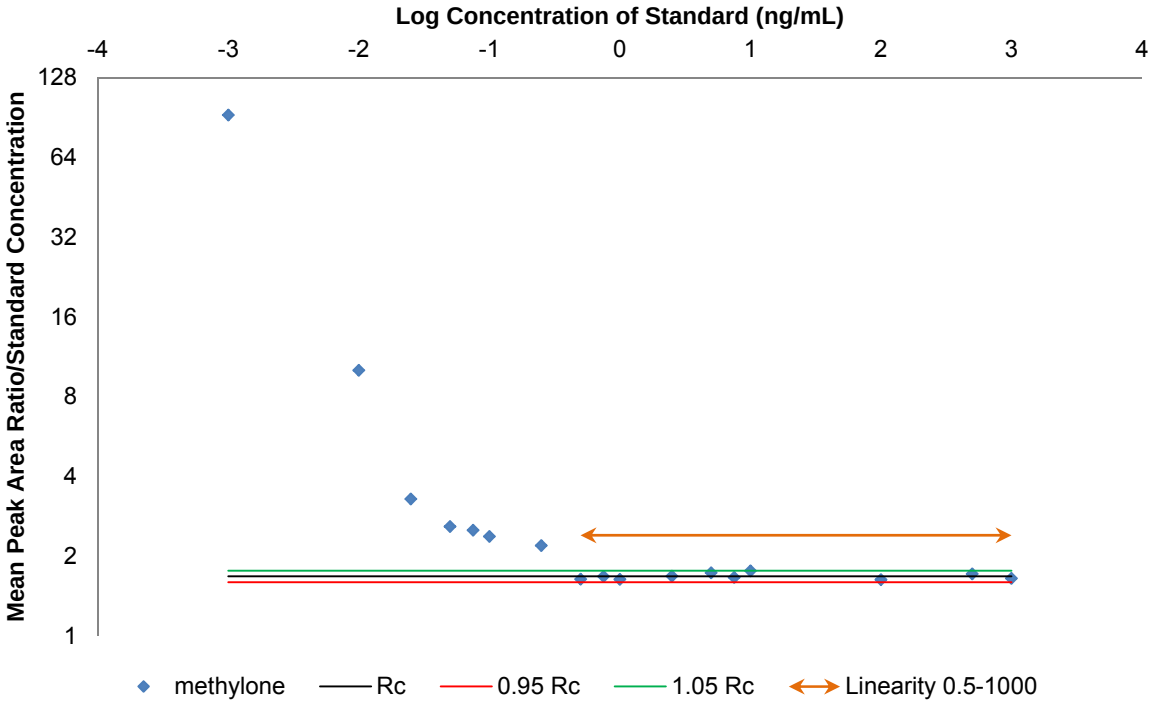
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



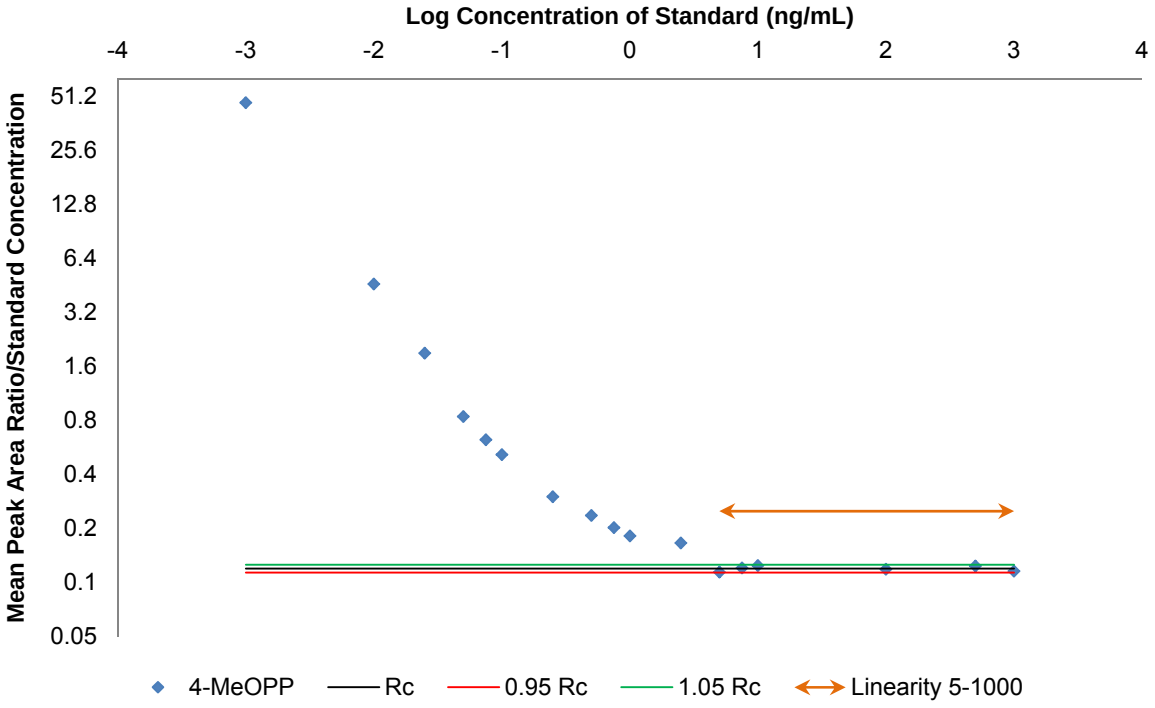


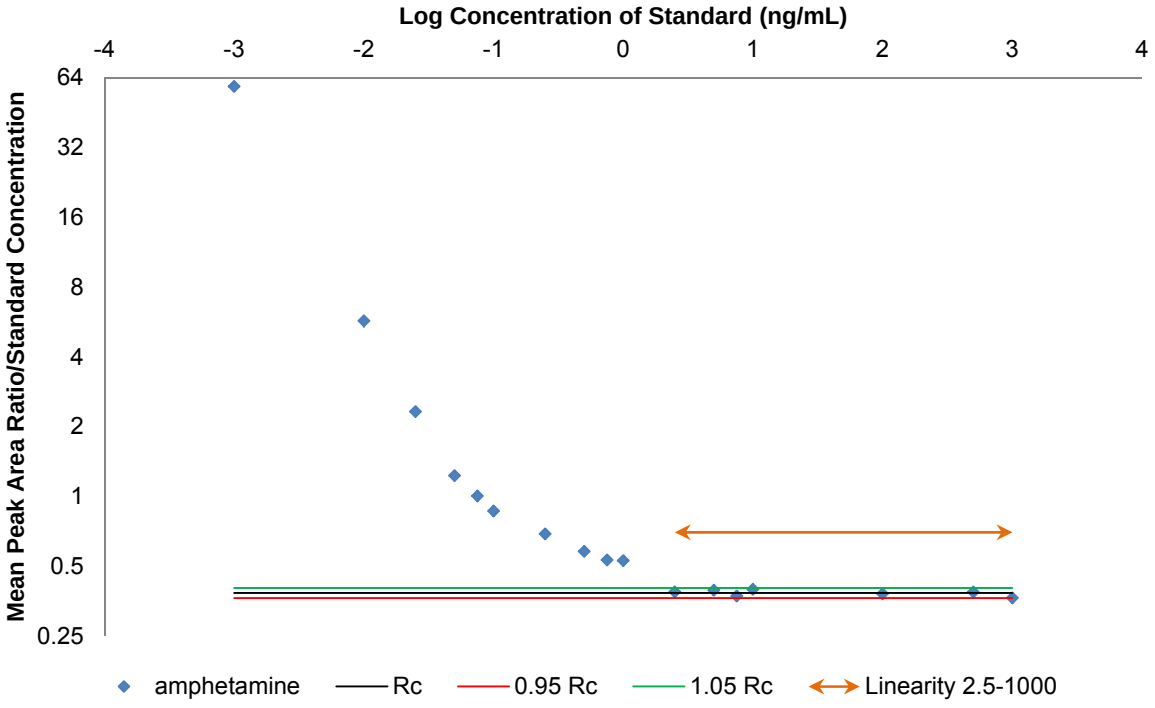
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



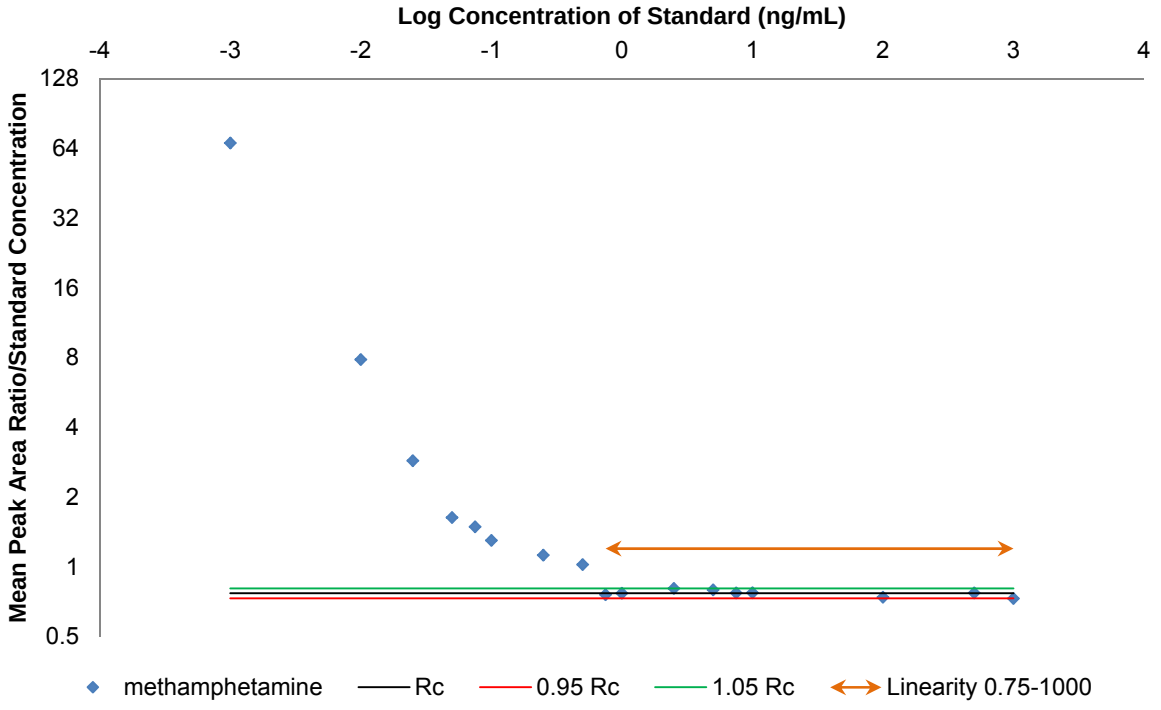


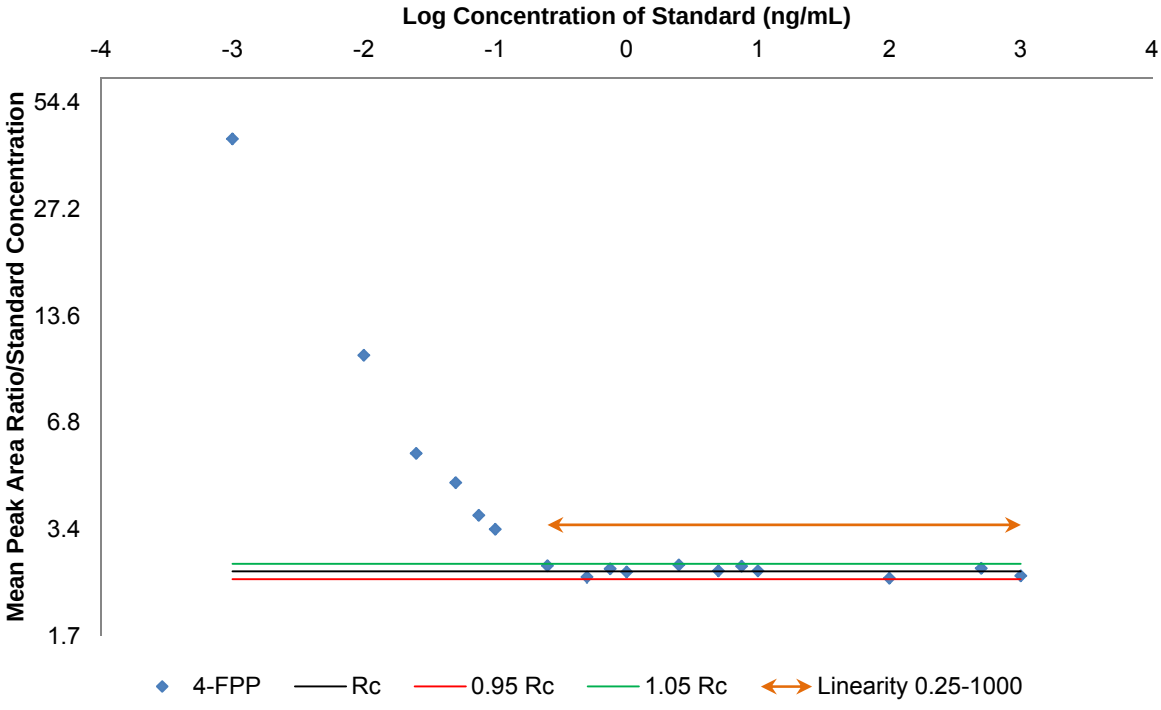
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



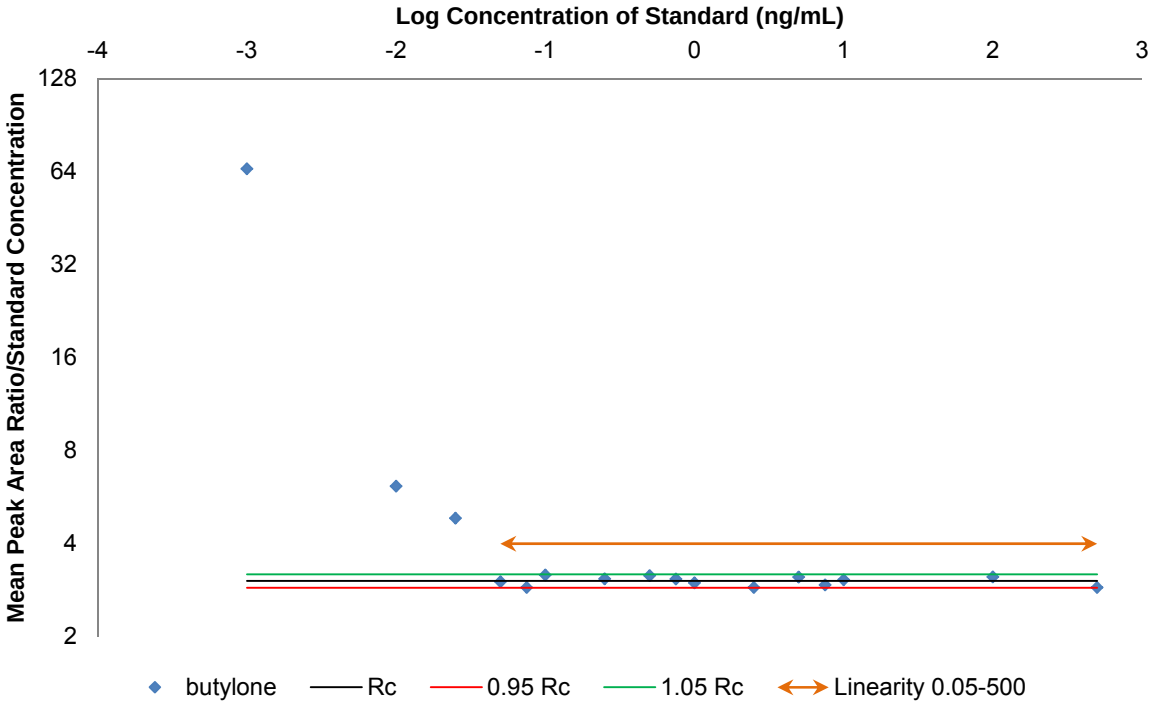


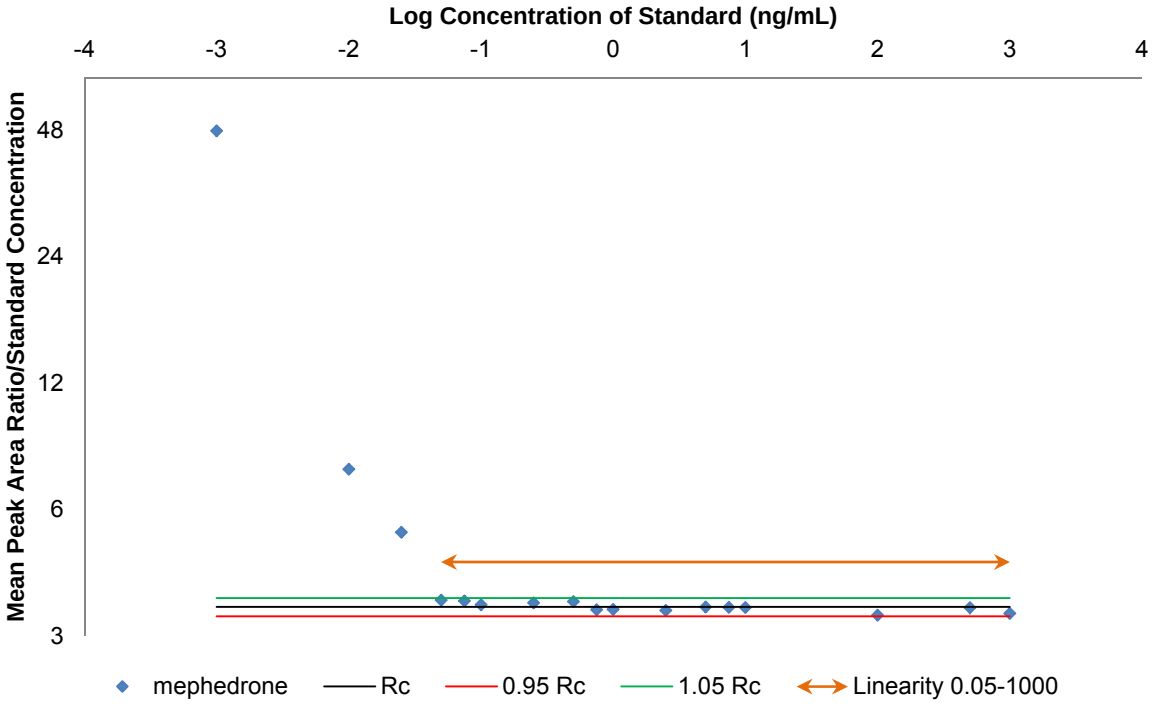
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



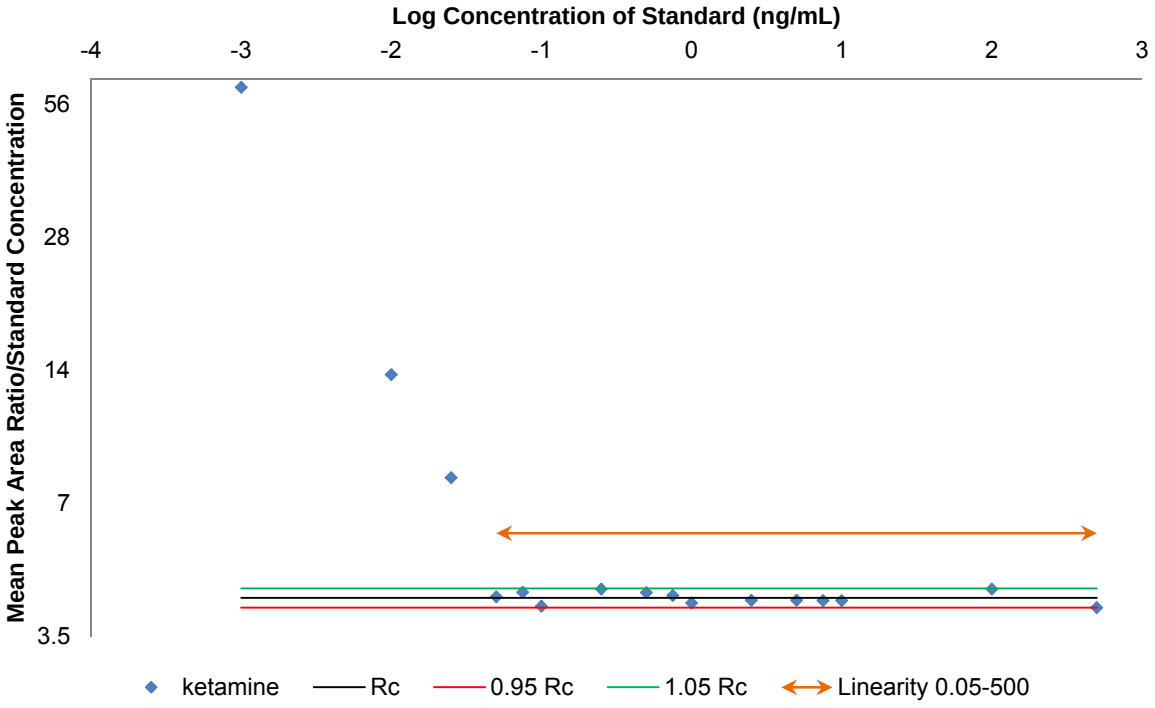


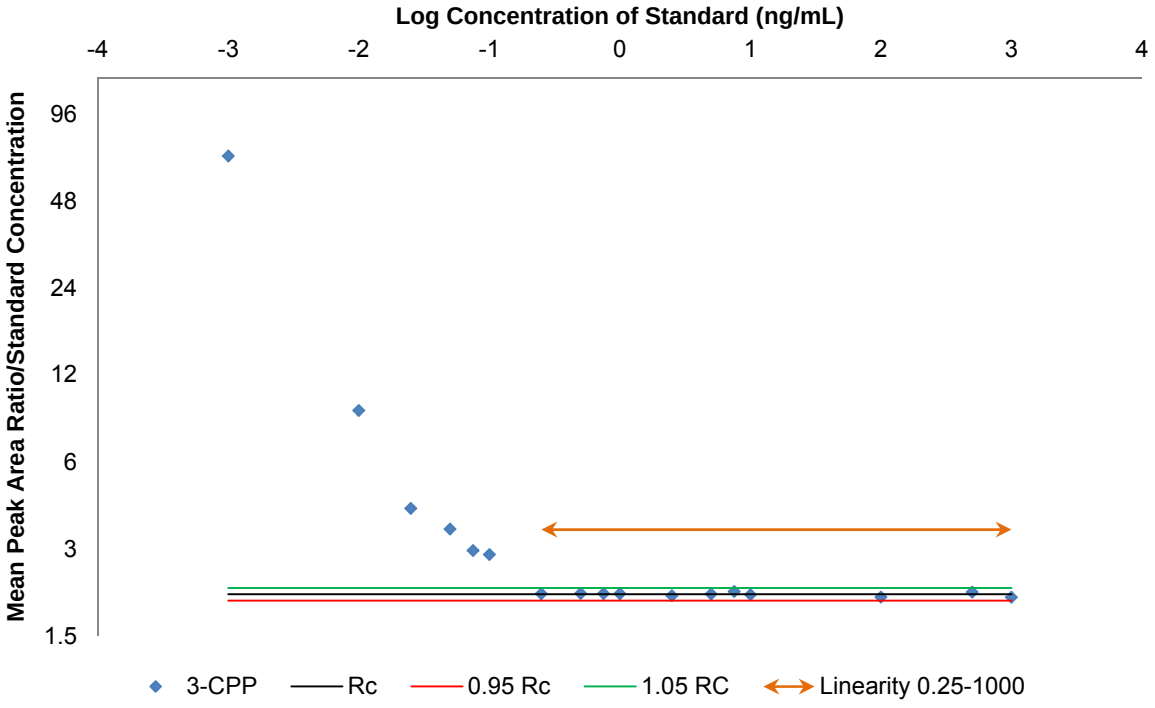
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



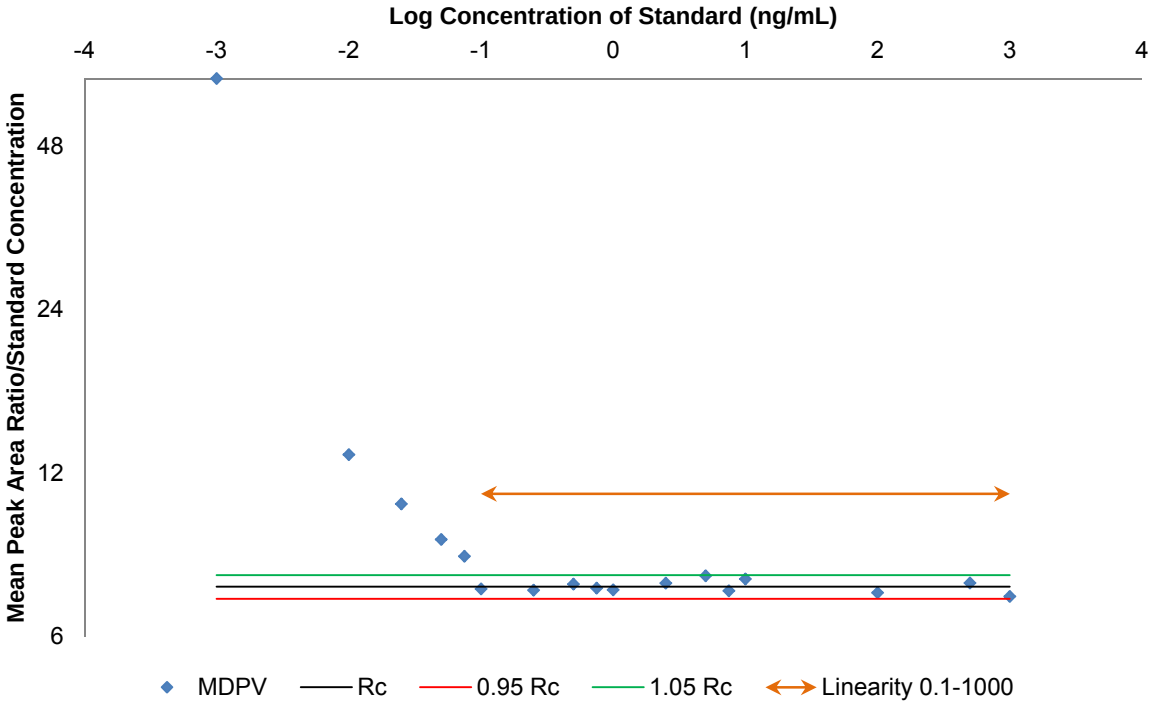


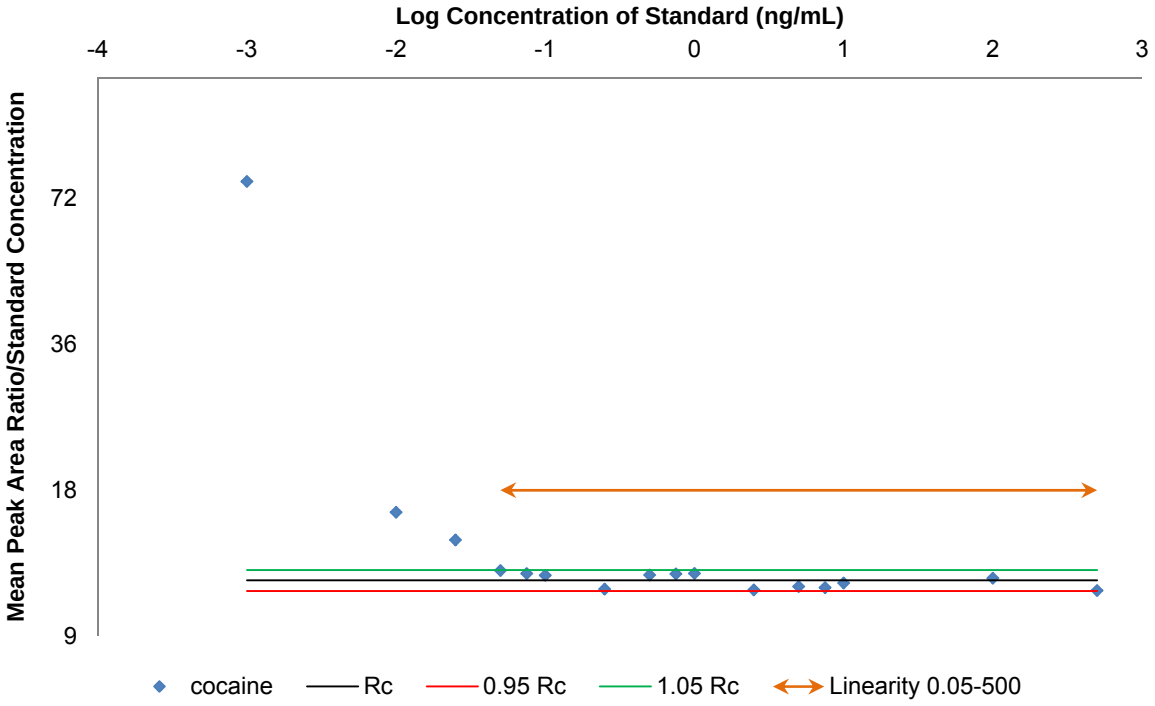
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



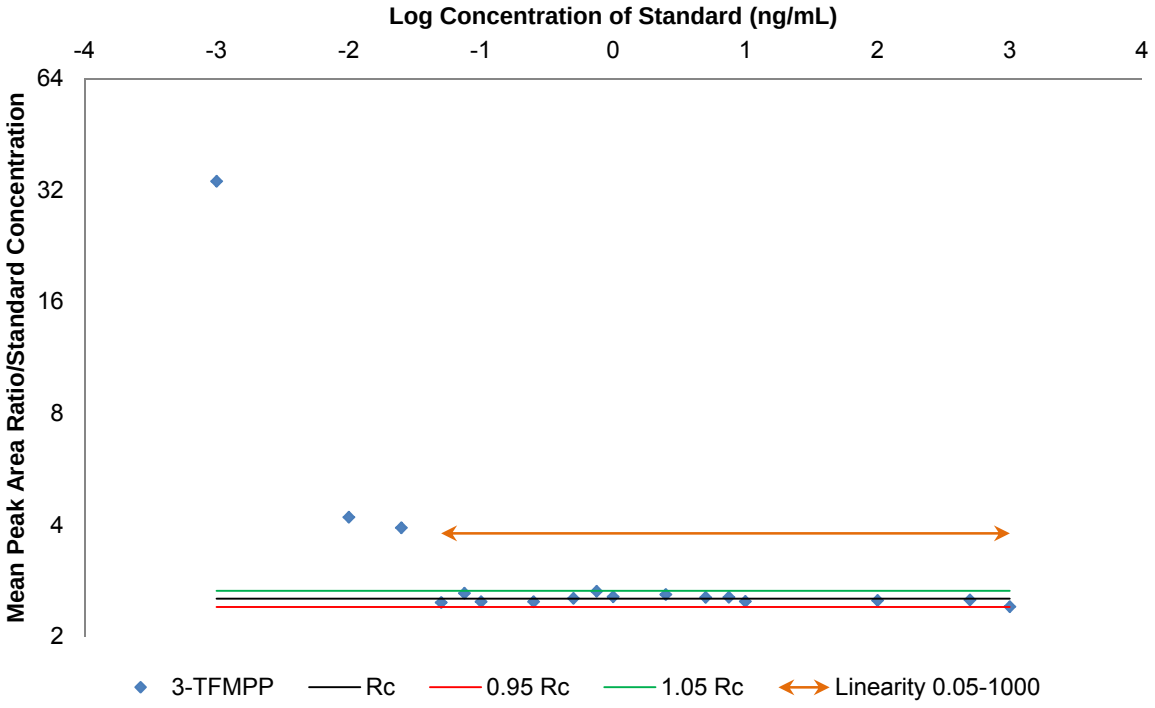


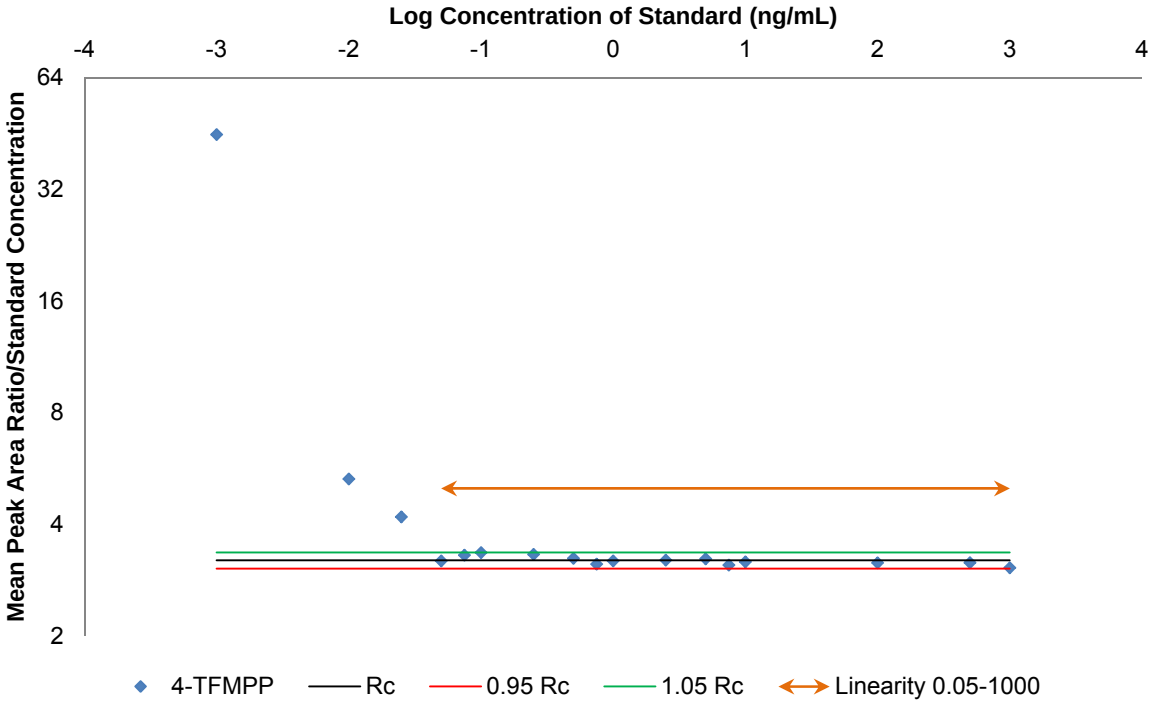
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



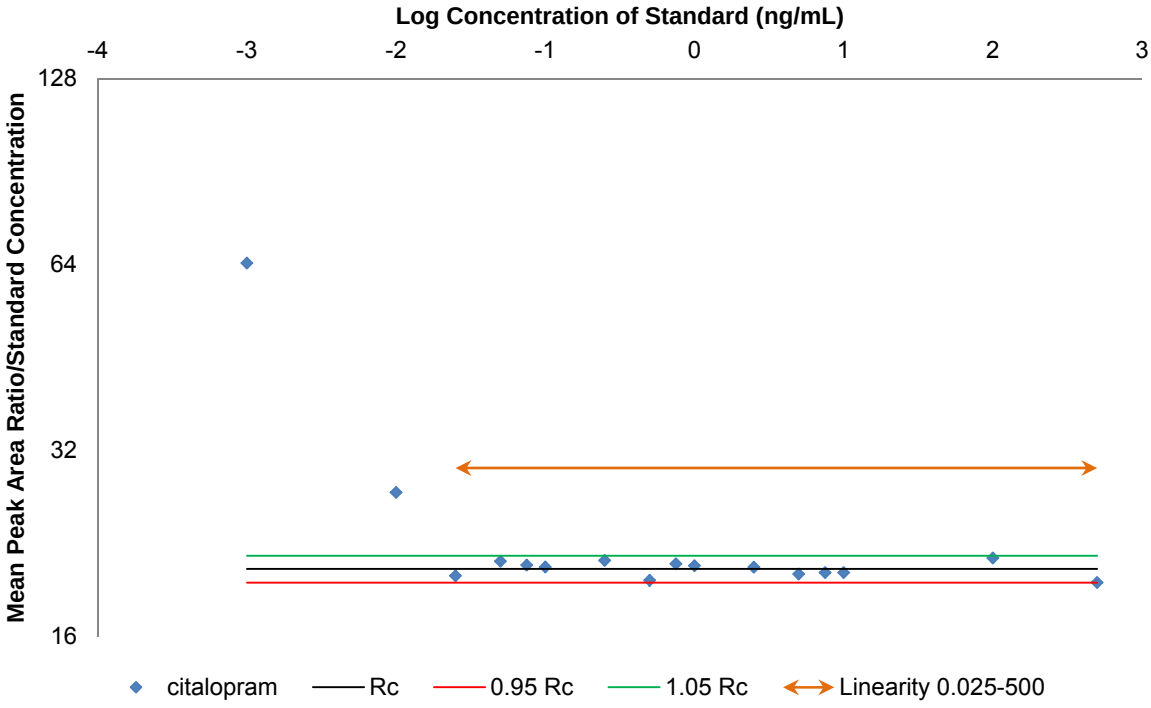


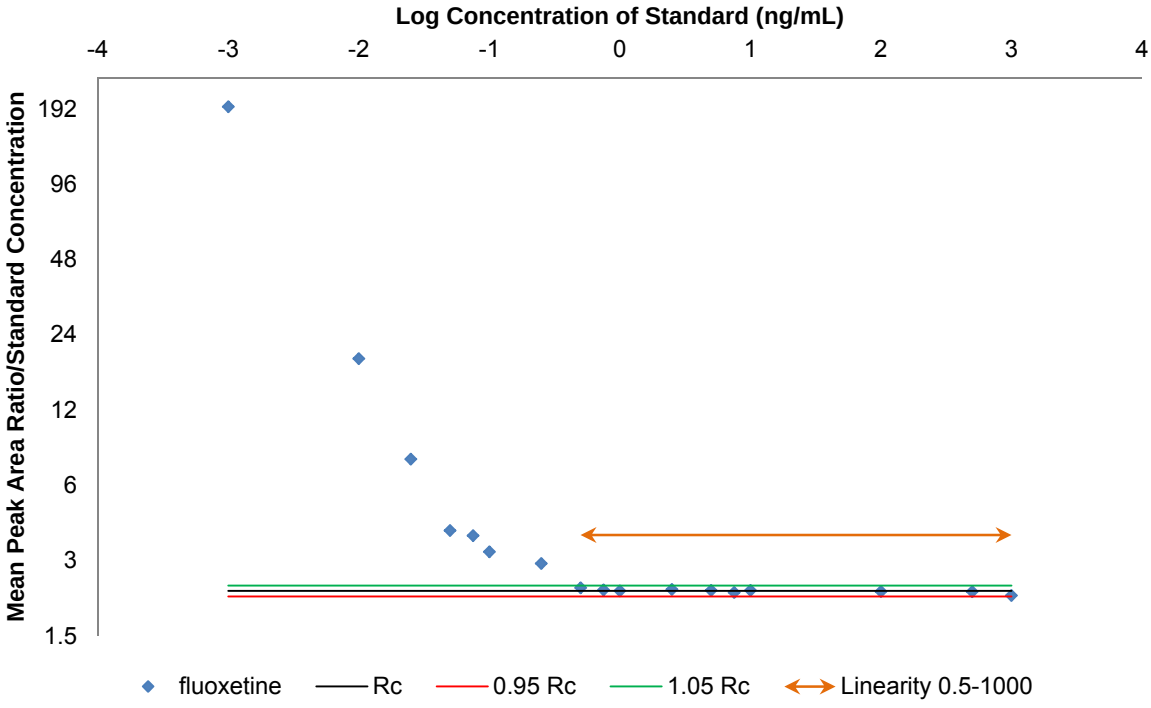
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



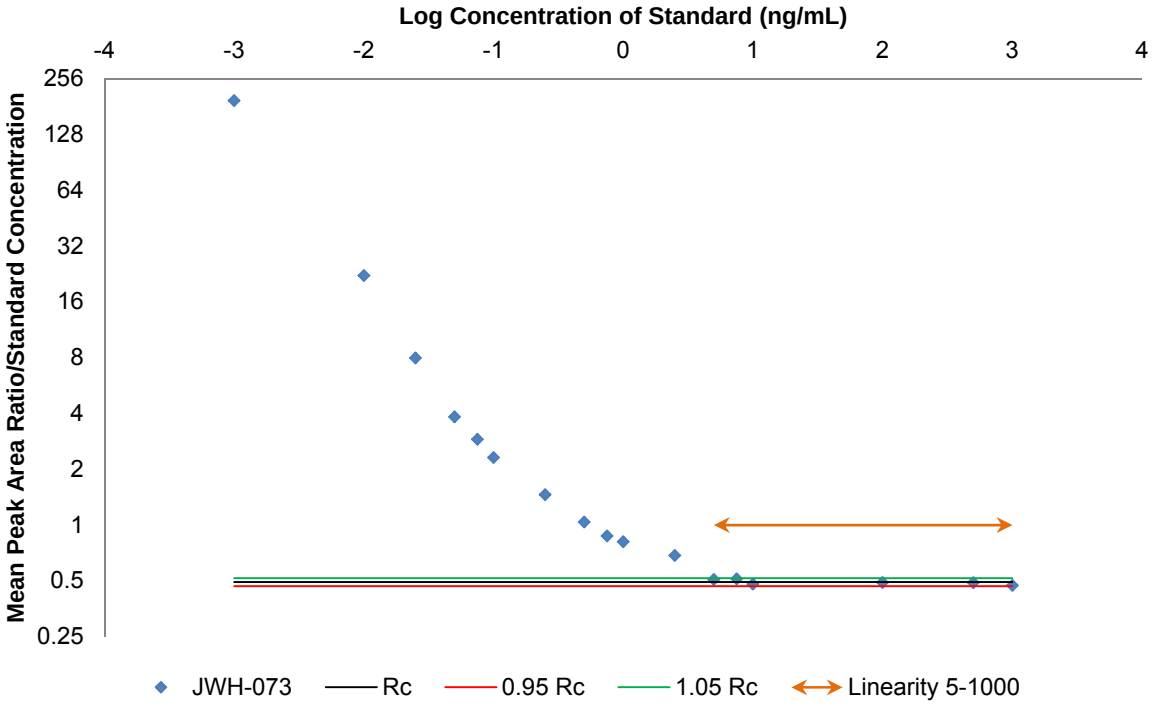


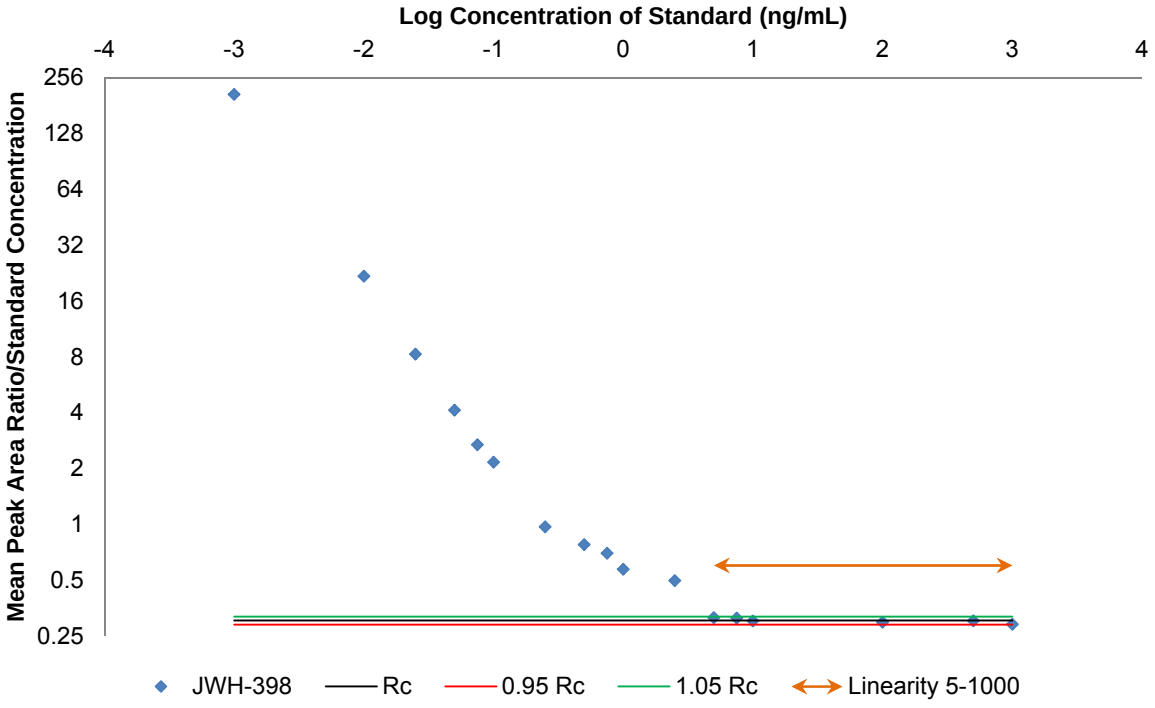
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)



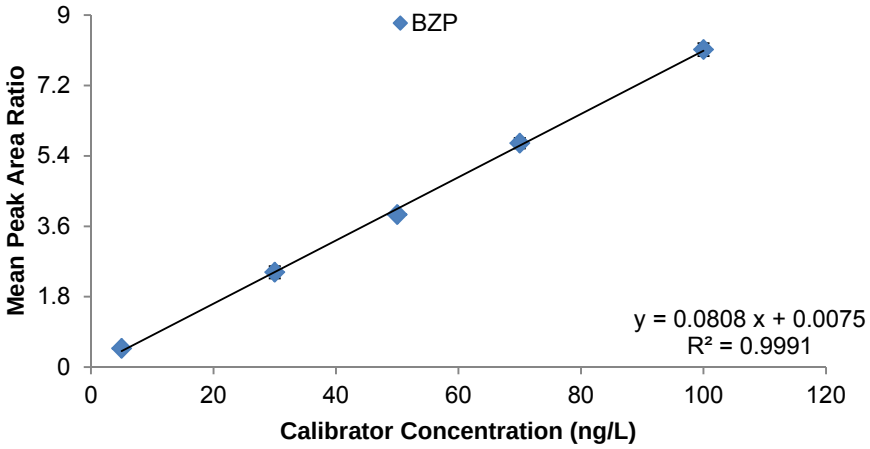


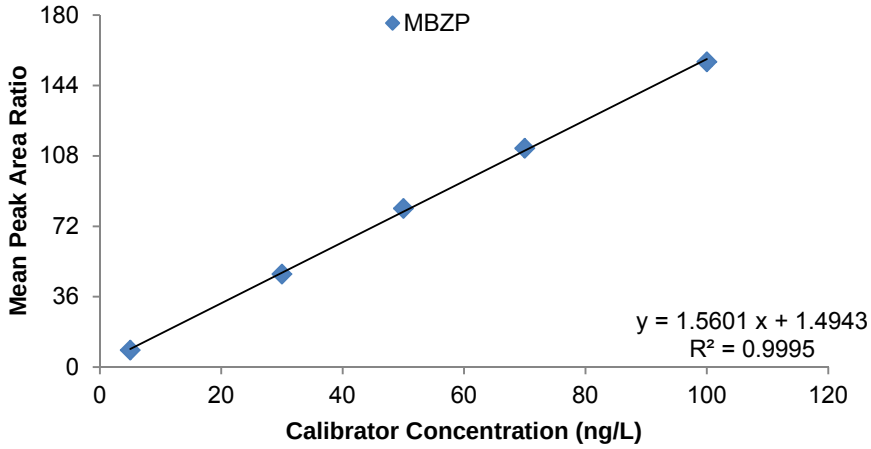
Appendix IV: Relative response/log concentration plots of the ratio of mean peak area ratio to standard concentration against log concentration of standard for drugs of abuse and pharmaceuticals over initial linear range for instrumental linearity obtained from a LC-MS analysis with a C_{18} column (Rc is the line of mean relative response)

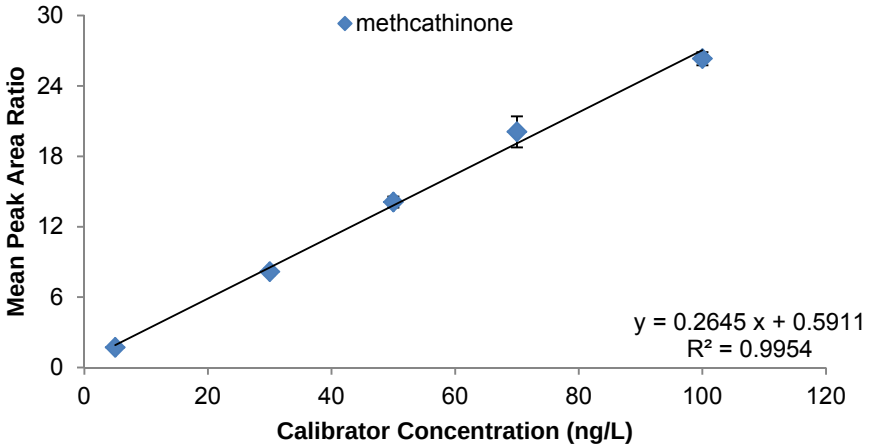


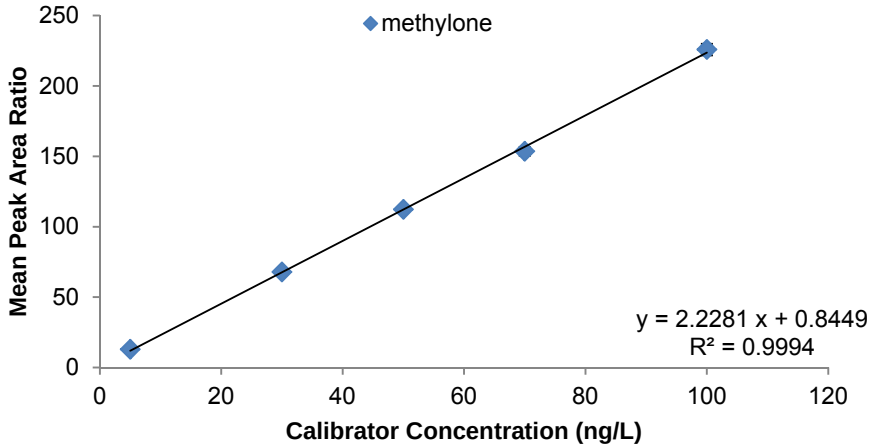


Appendix V: Linear regression plots of mean peak area ratio against calibrator concentration for drugs of abuse and pharmaceuticals over 5 to 100 ng/L for method precision and accuracy obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

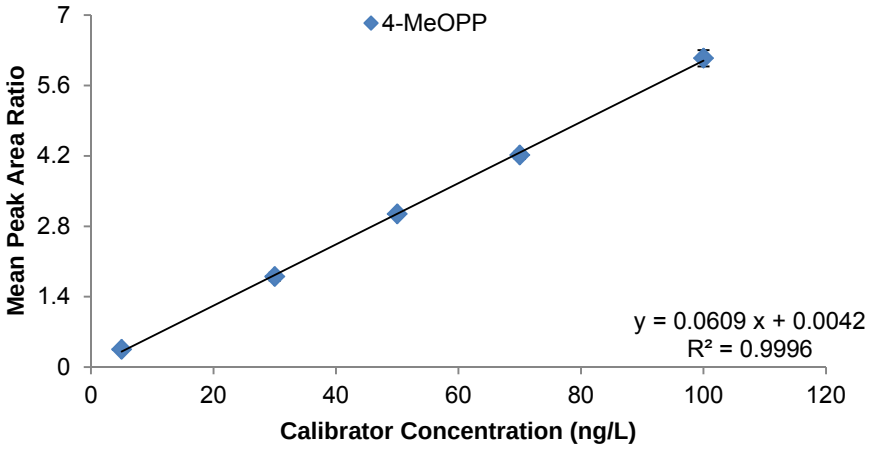


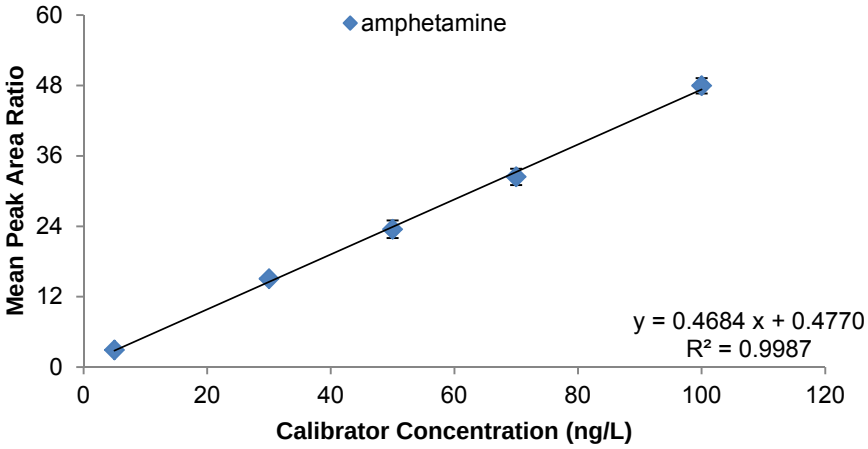


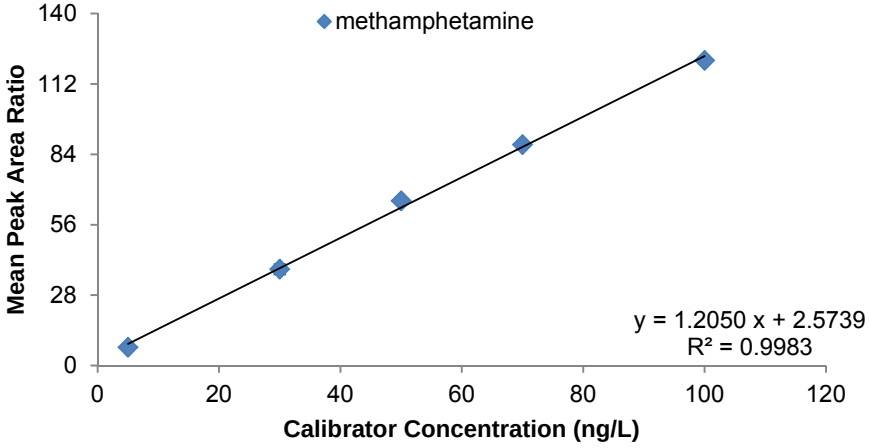


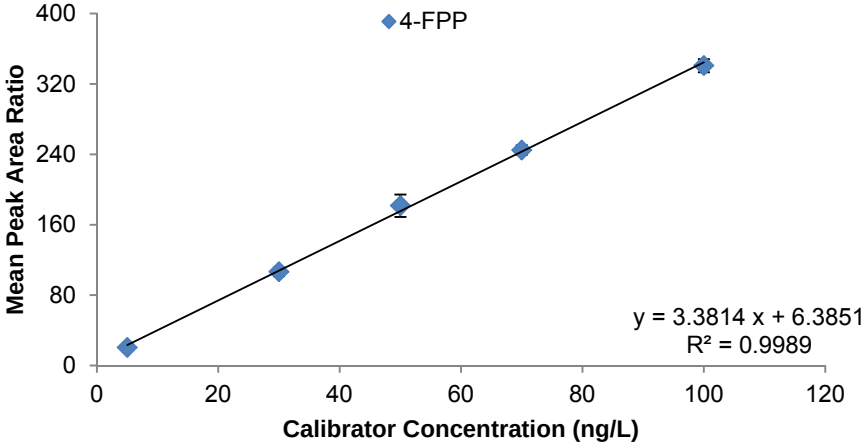


Appendix V: Linear regression plots of mean peak area ratio against calibrator concentration for drugs of abuse and pharmaceuticals over 5 to 100 ng/L for method precision and accuracy obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

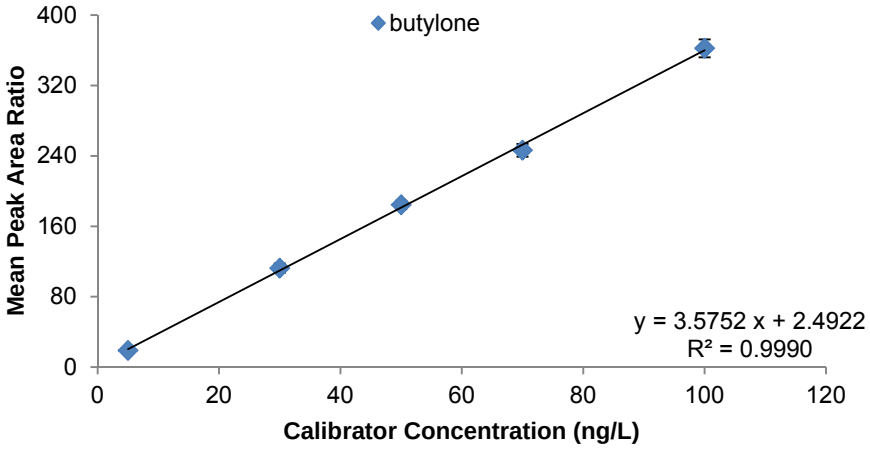


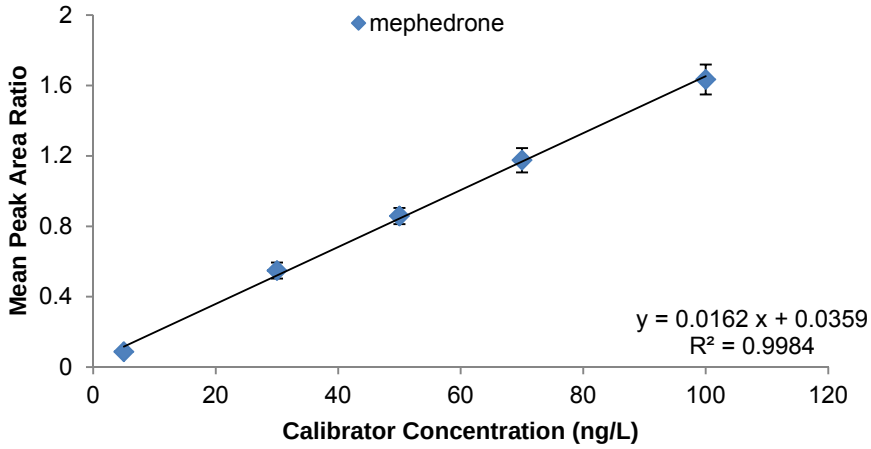


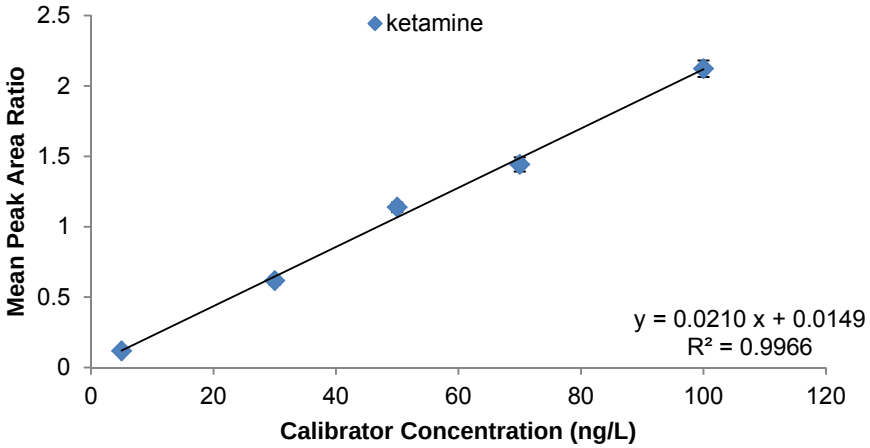


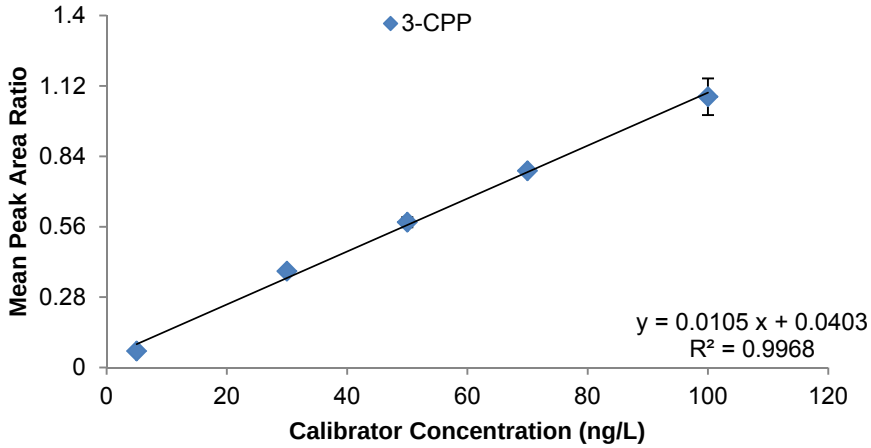


Appendix V: Linear regression plots of mean peak area ratio against calibrator concentration for drugs of abuse and pharmaceuticals over 5 to 100 ng/L for method precision and accuracy obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3









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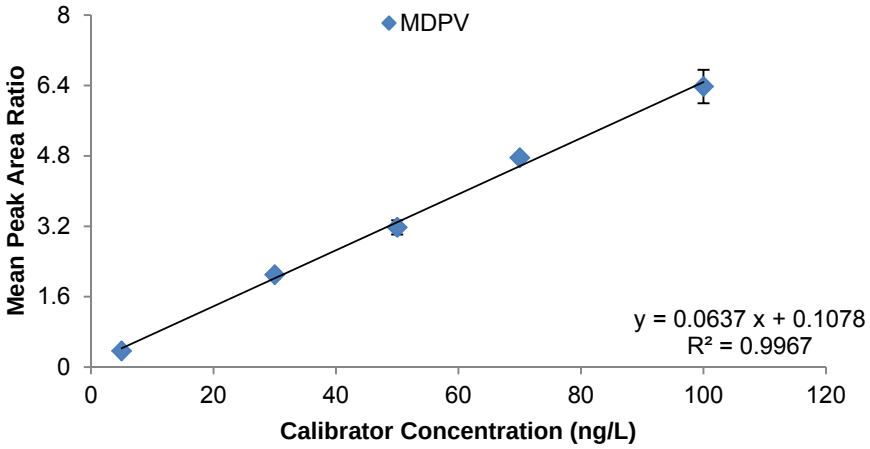

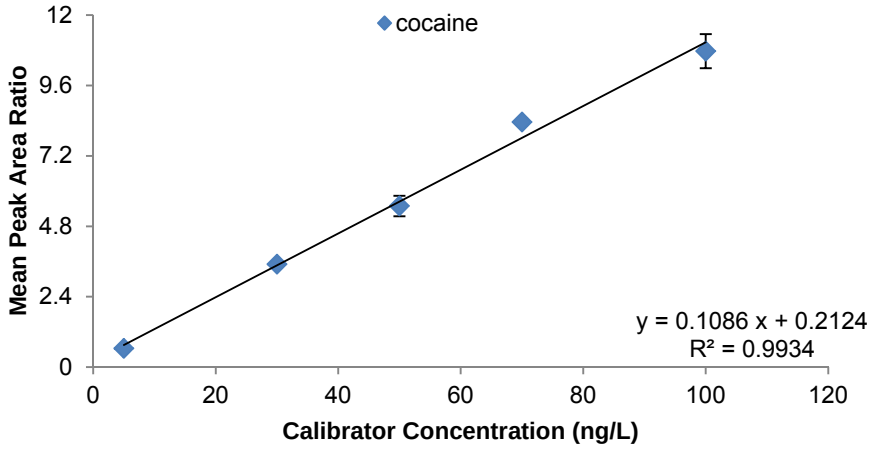

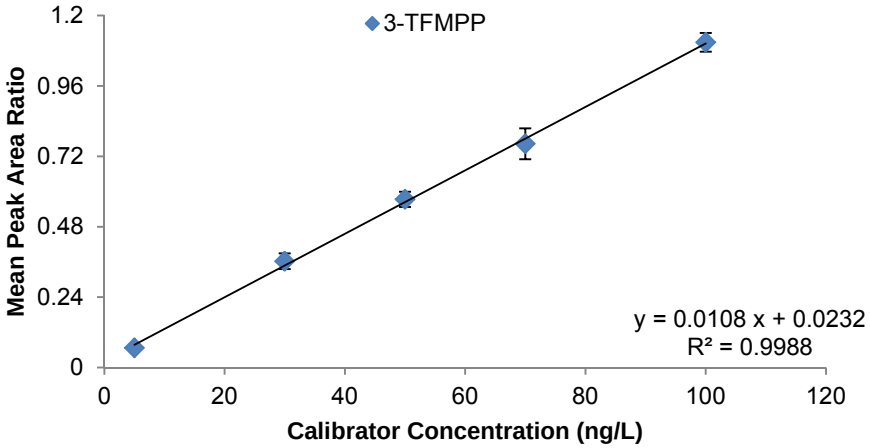

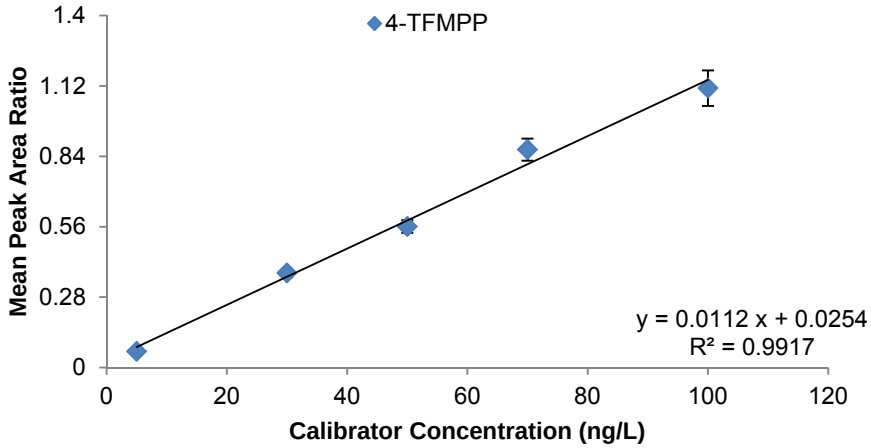

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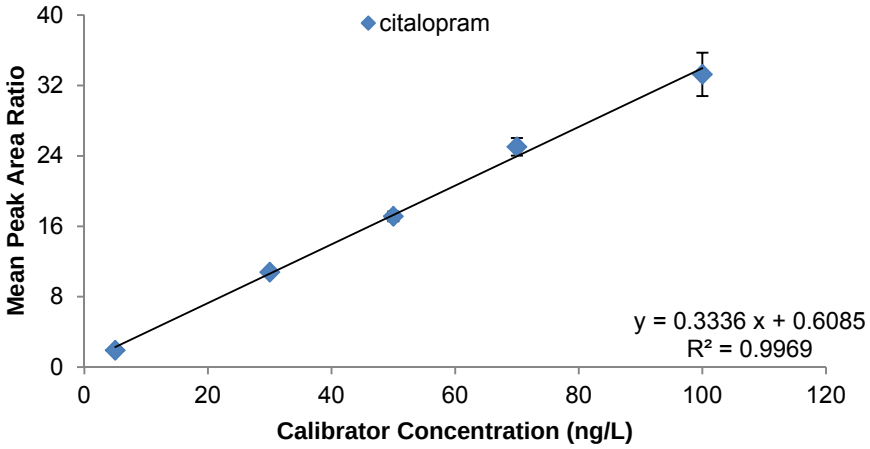
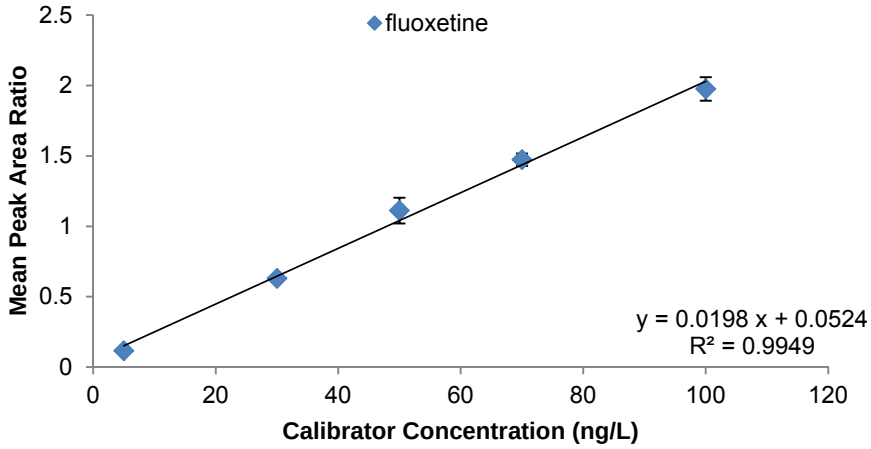
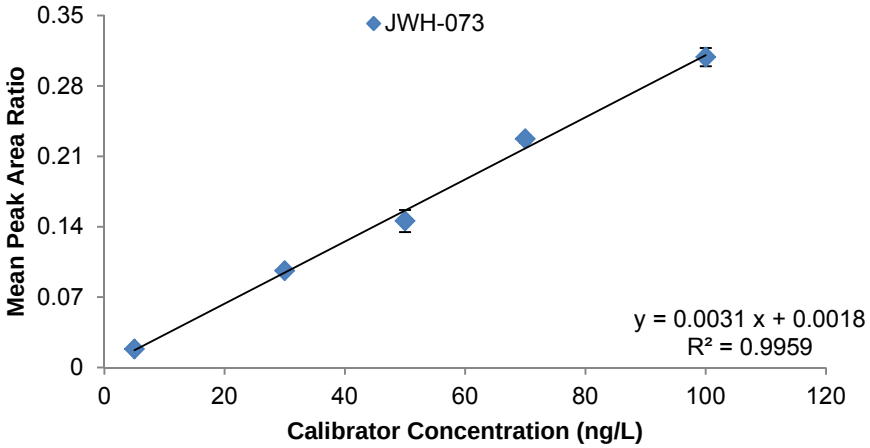
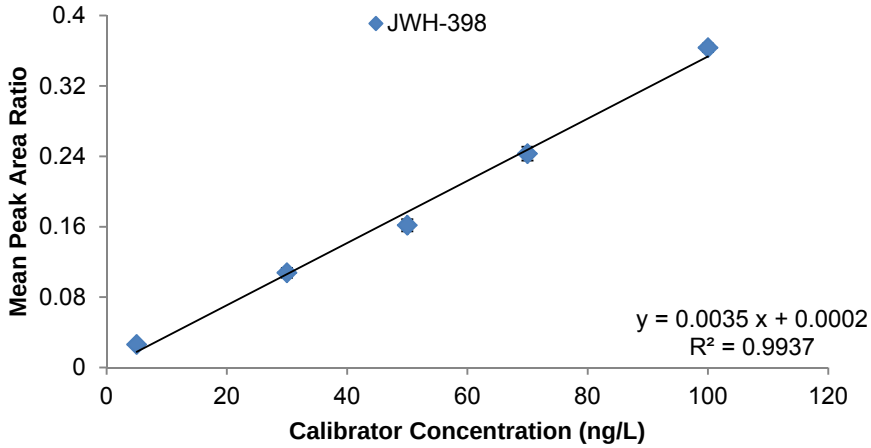
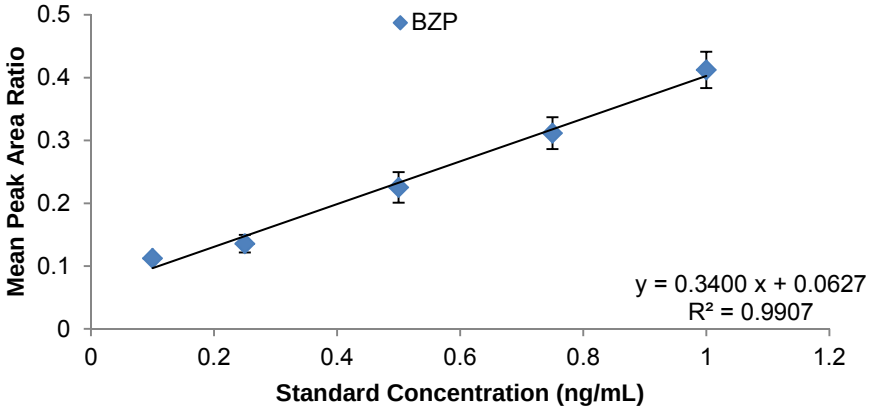


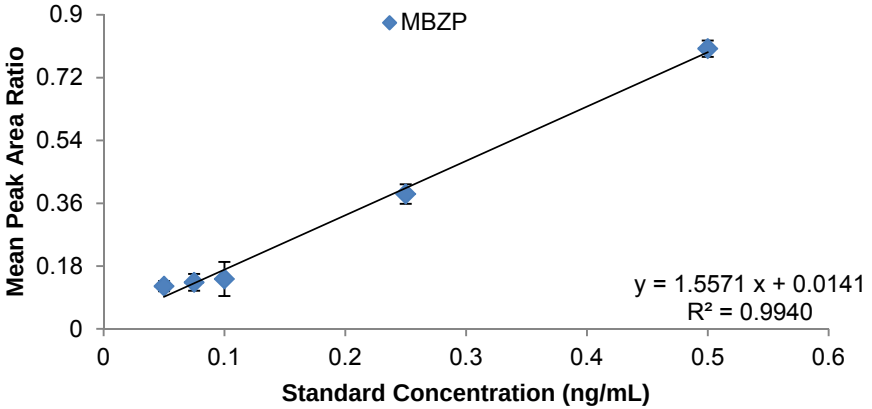


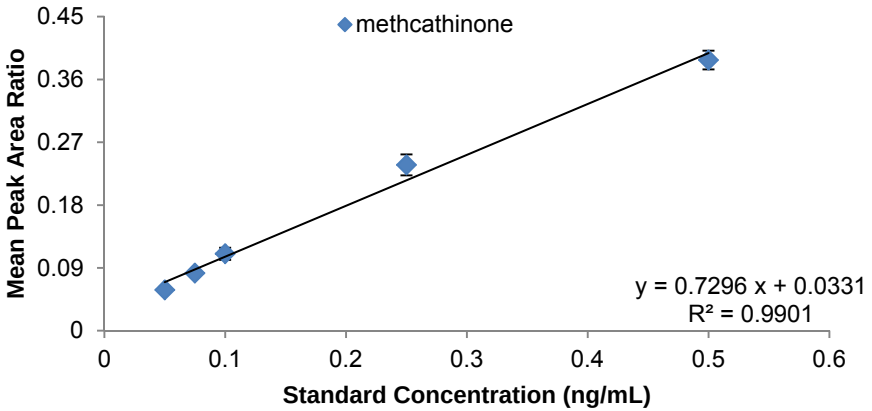


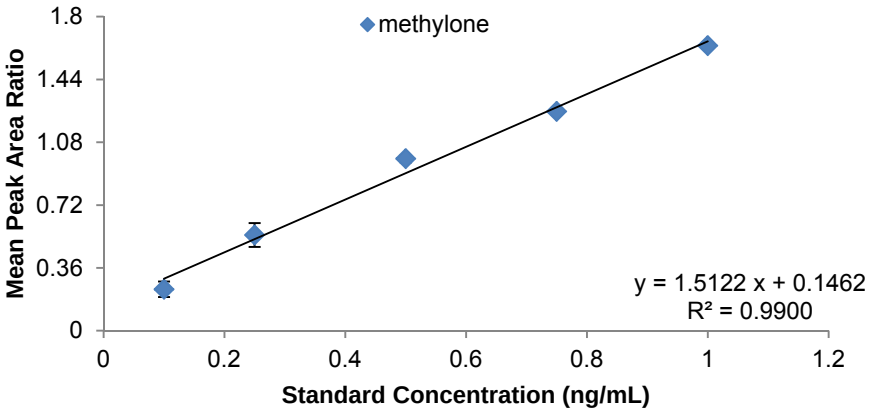


Appendix VI-a: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals for the calculation of instrumental detection and quantification limits using root mean square error approach obtained from a LC-MS analysis with a C_{18} column, n = 3









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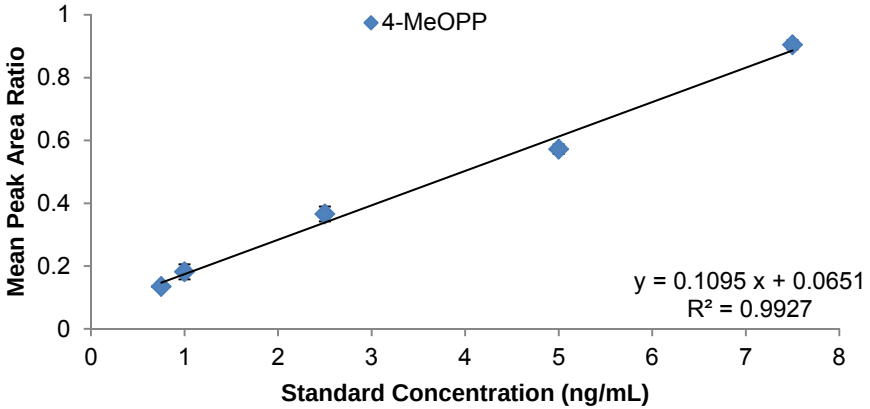

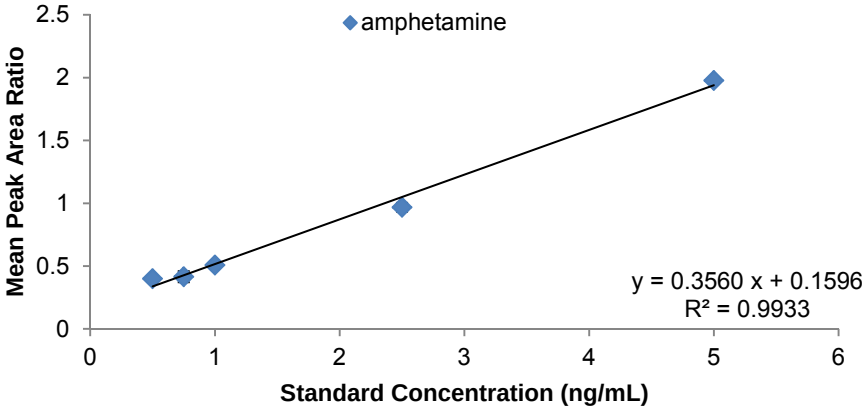

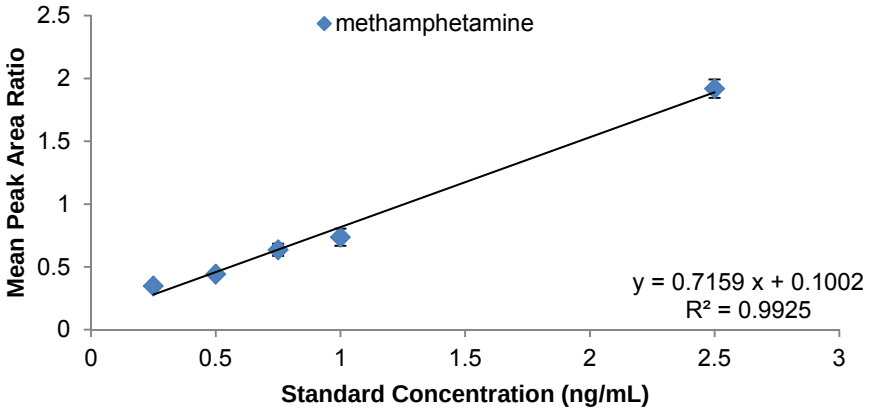

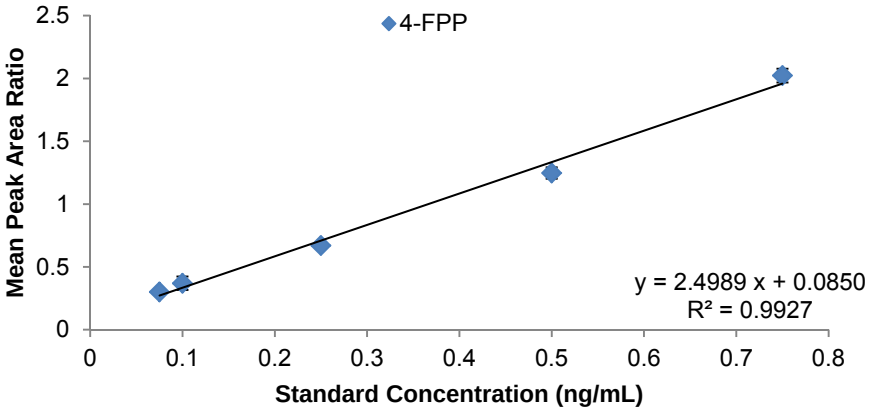

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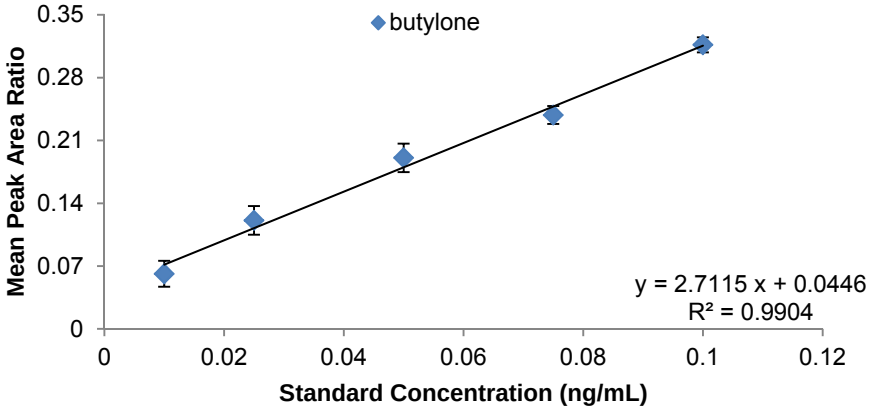

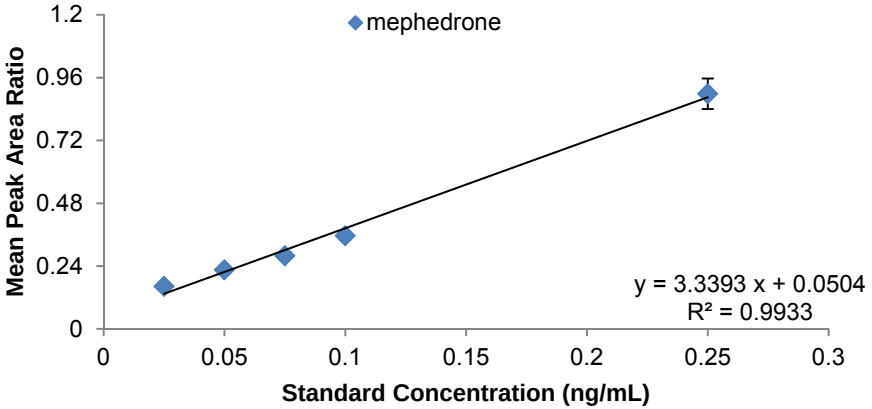

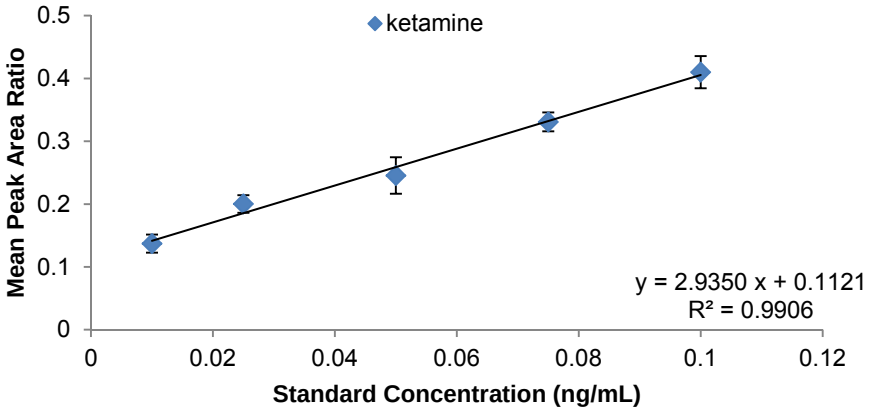

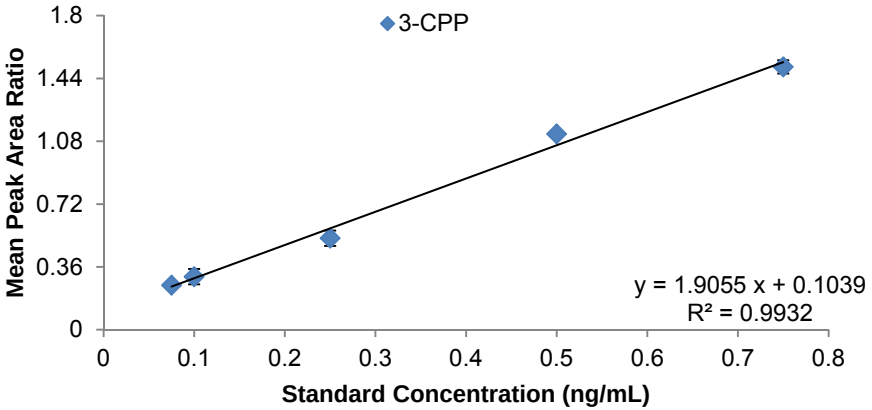

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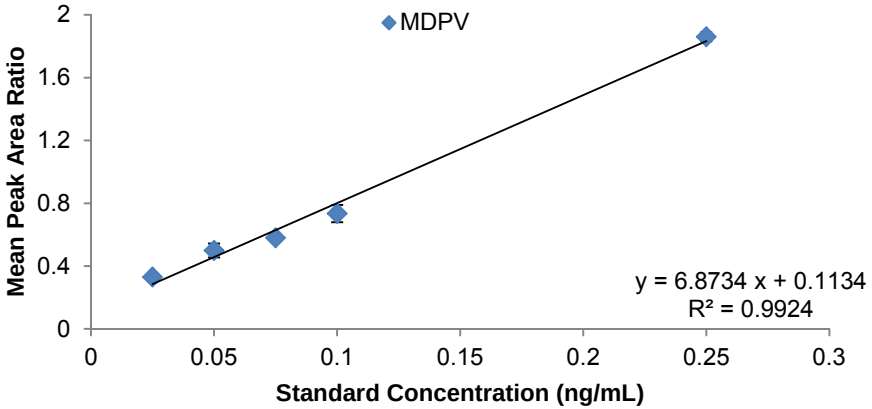

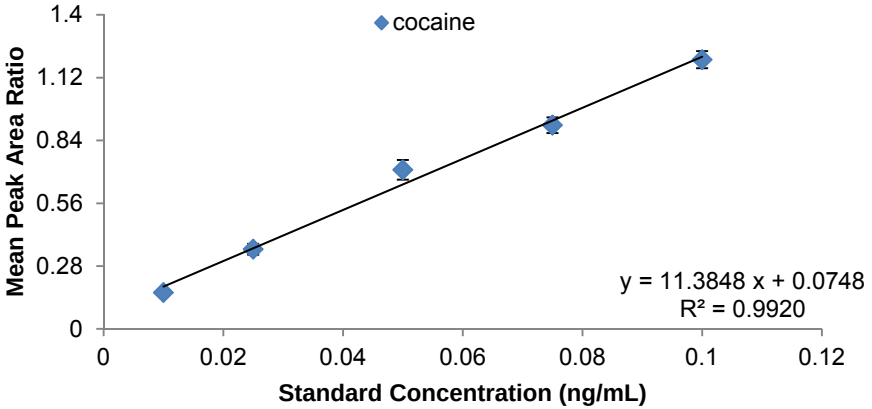

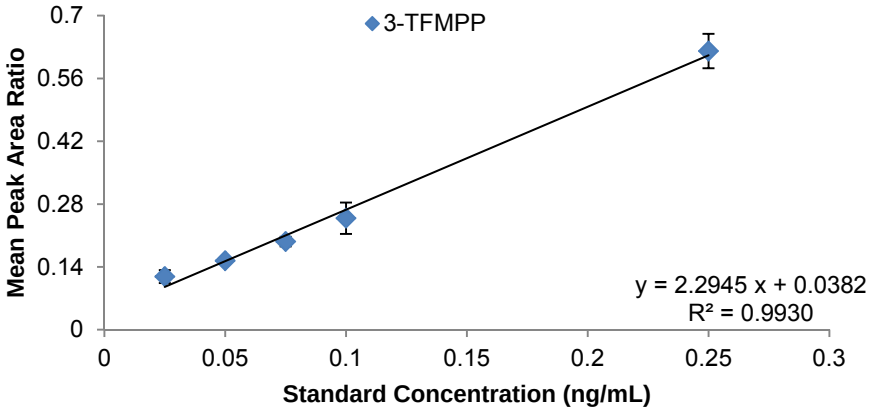

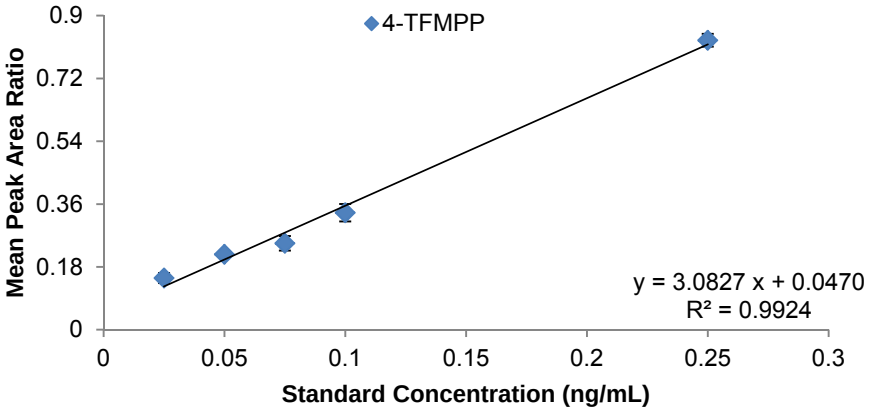

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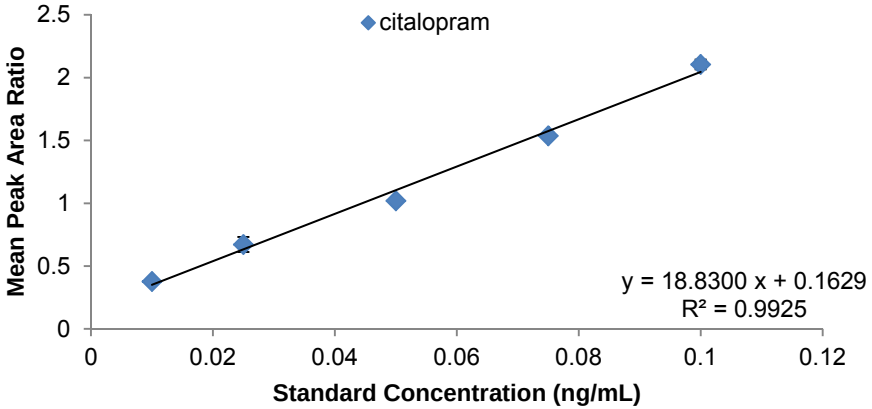
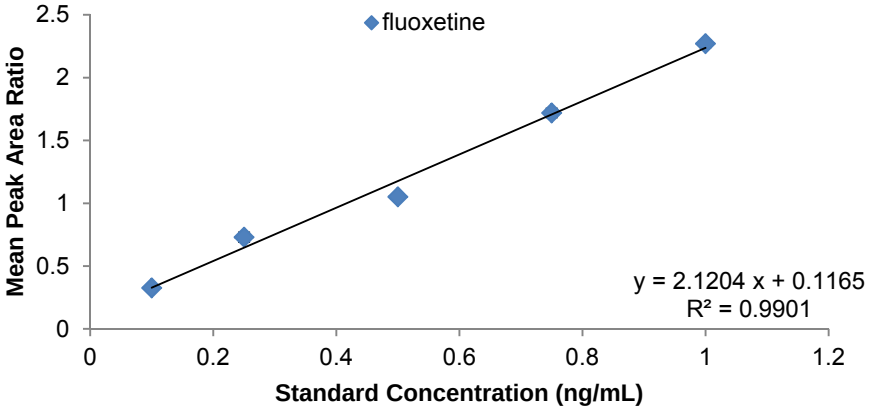
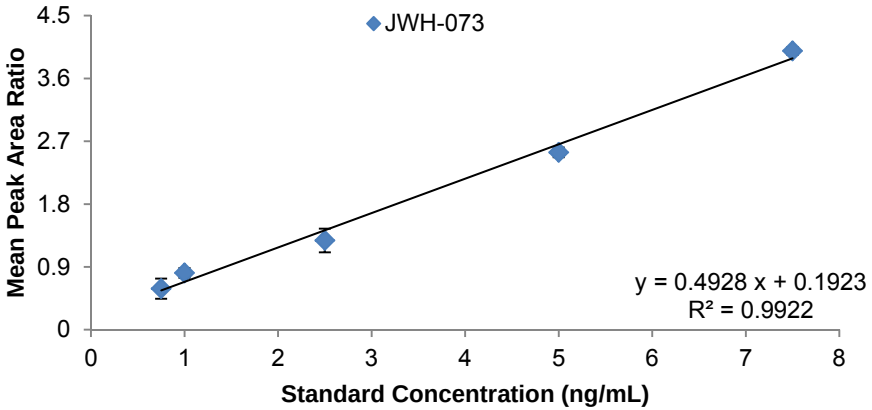
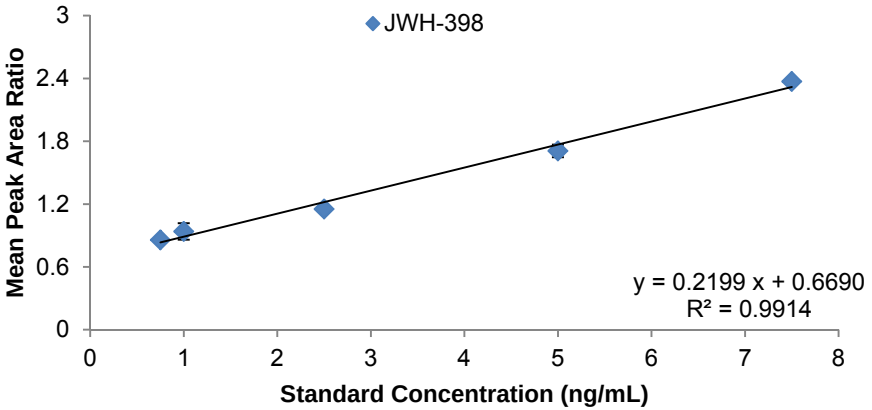
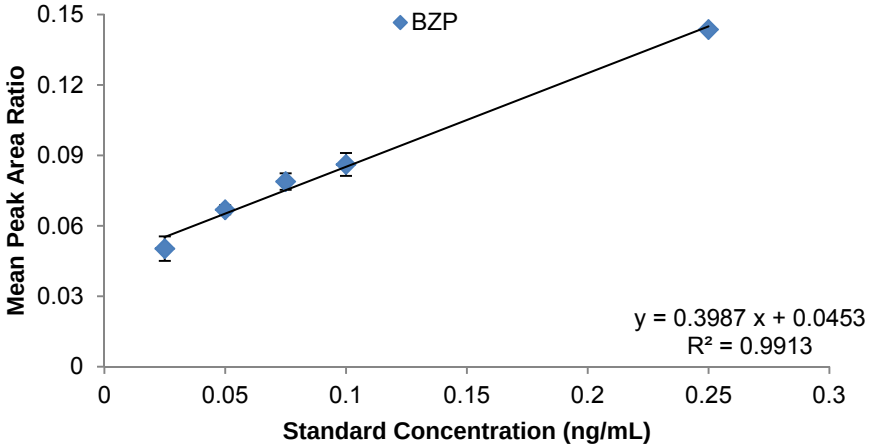


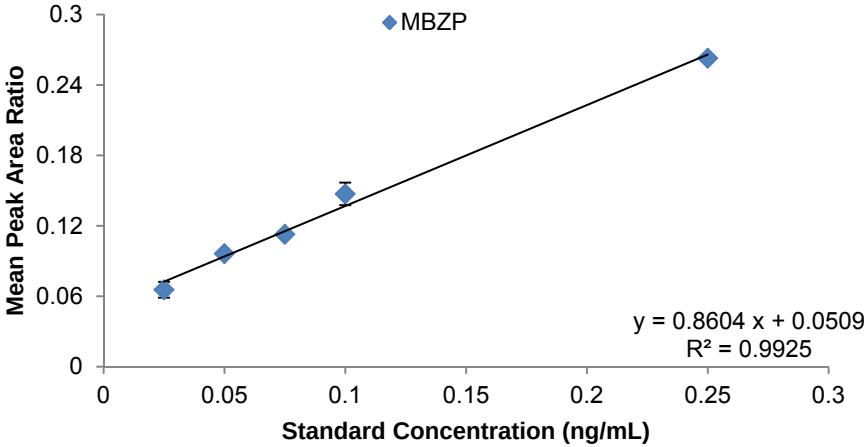


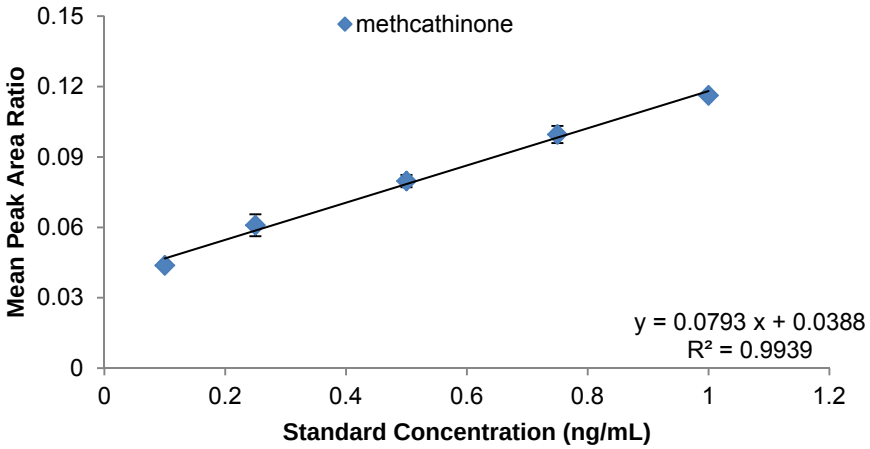


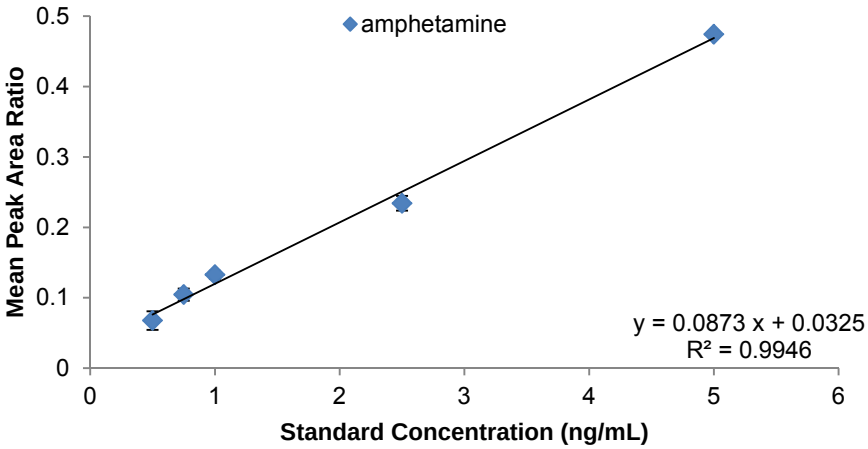


Appendix VI-b: Linear regression plots of mean peak area ratio against standard concentration for drugs of abuse and pharmaceuticals for the calculation of instrumental detection and quantification limits using root mean square error approach obtained from a LC-MS analysis with a biphenyl column, n = 3









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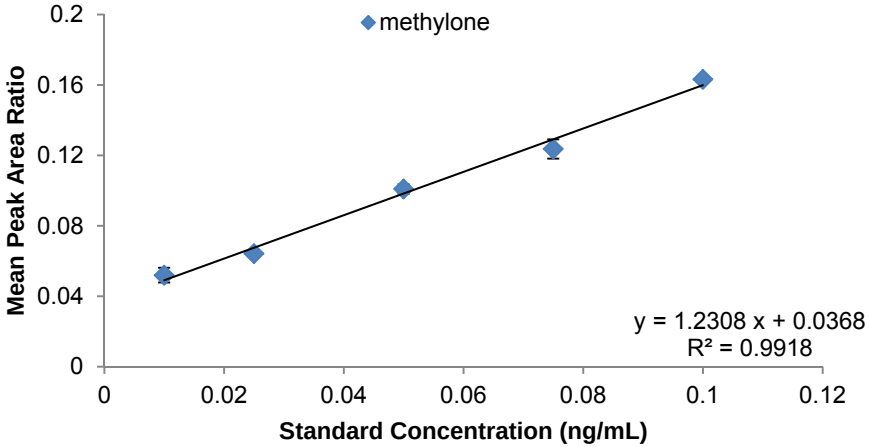

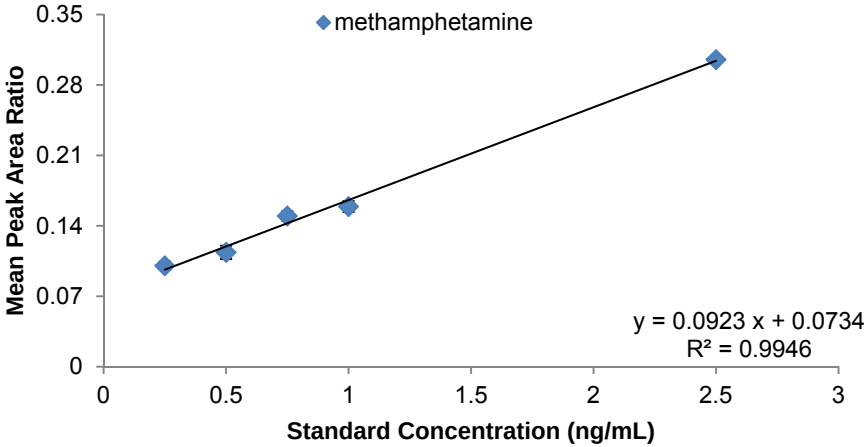

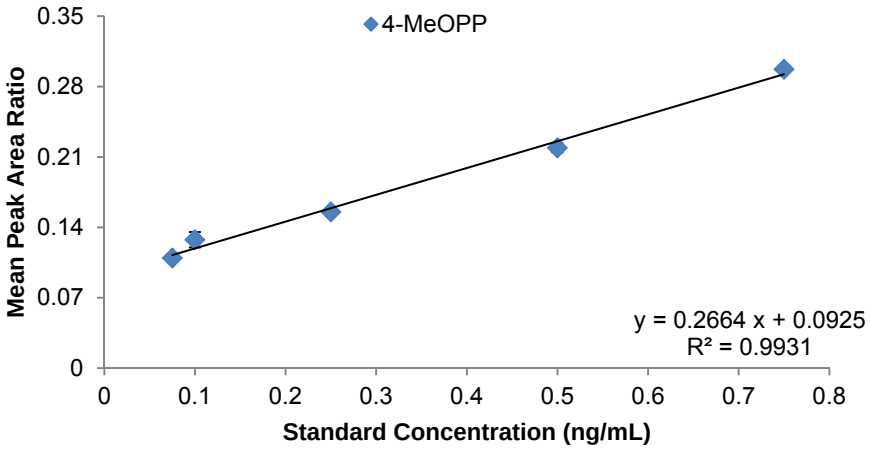

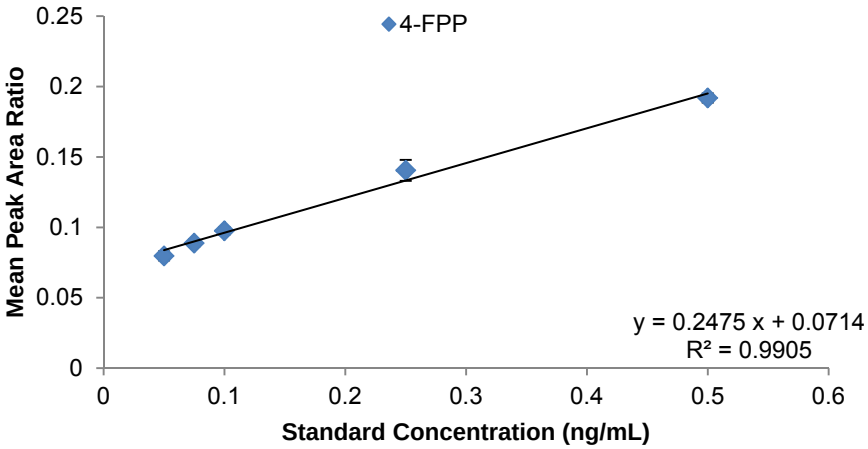

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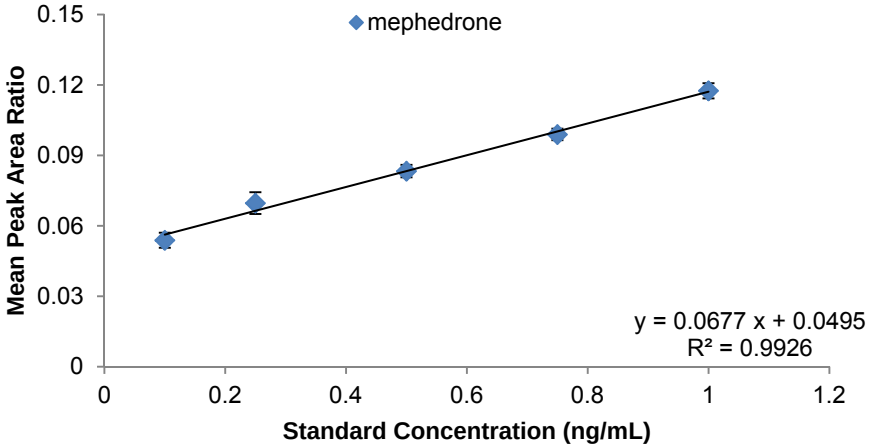

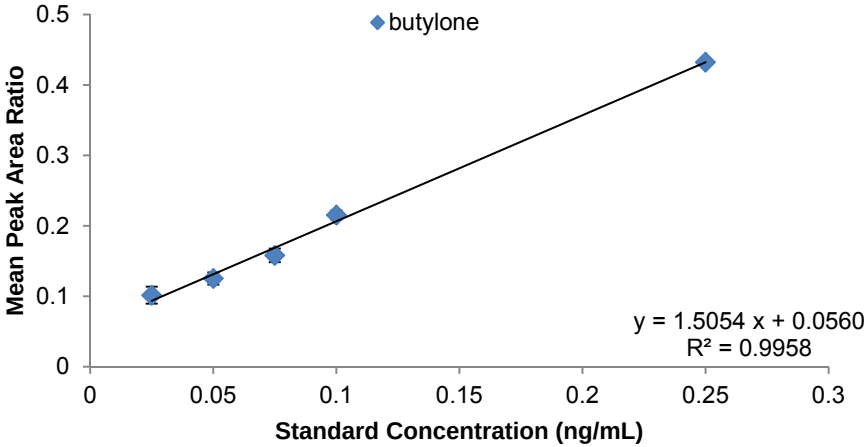

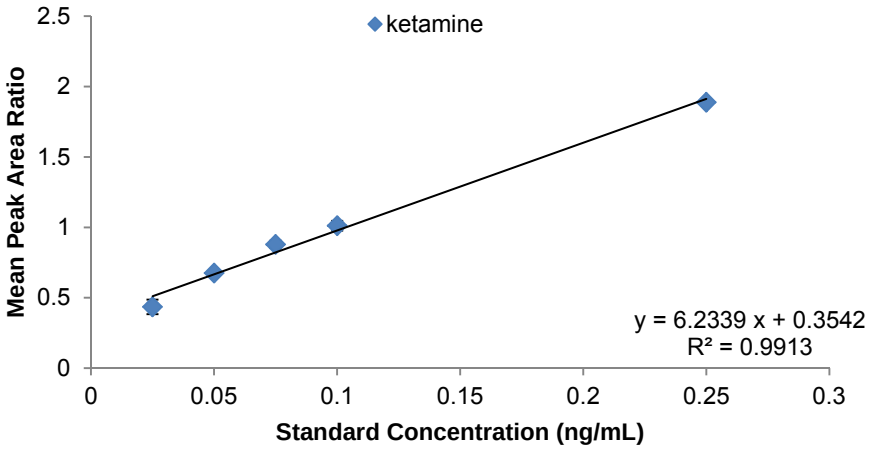

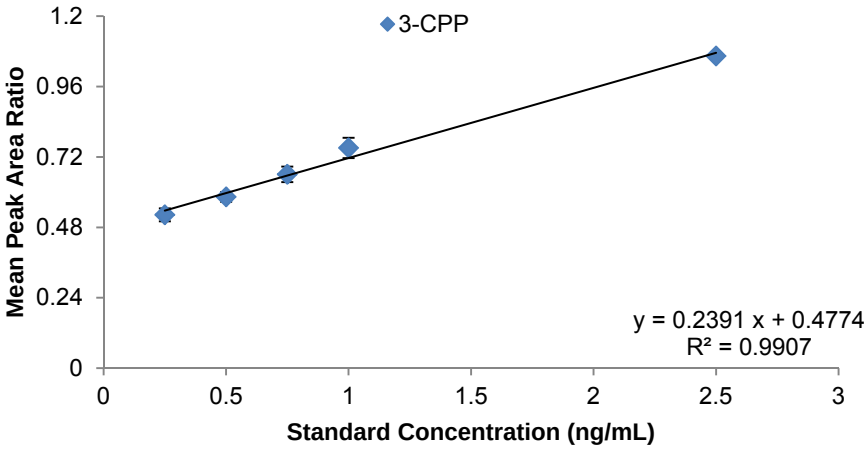

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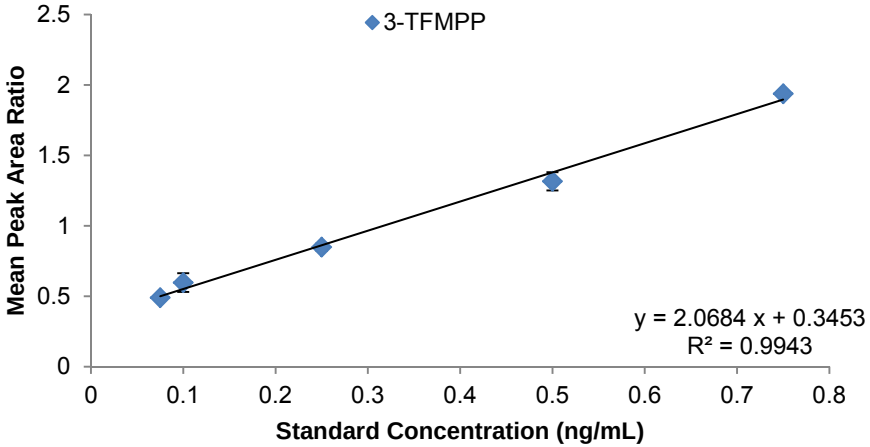

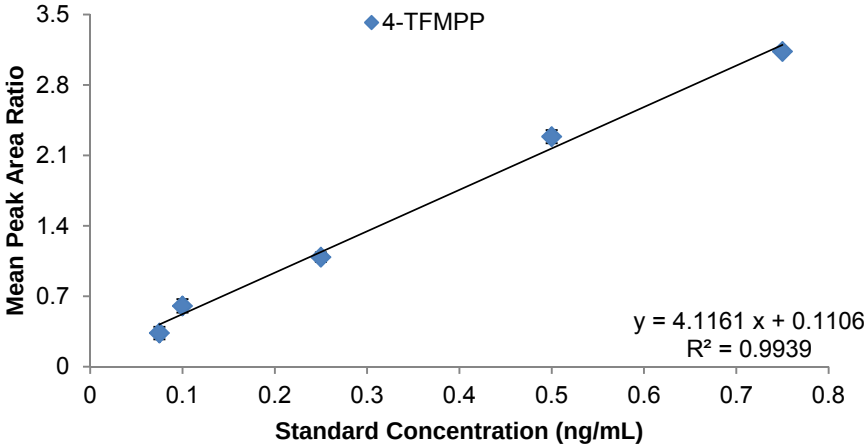

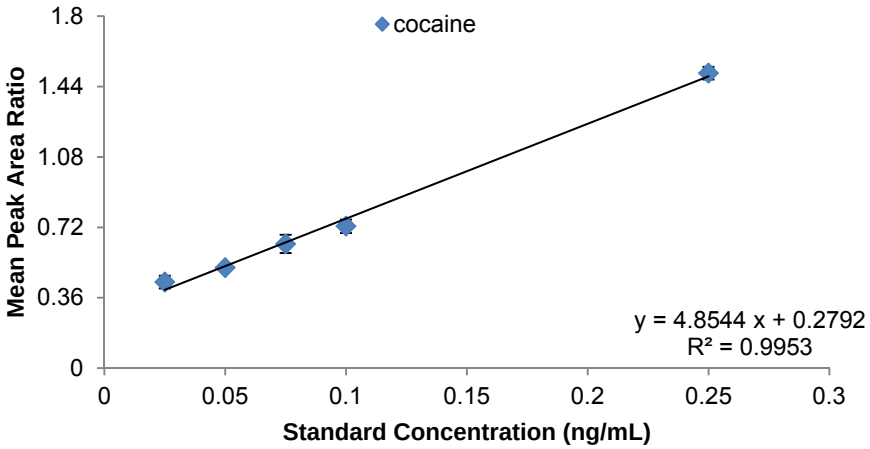

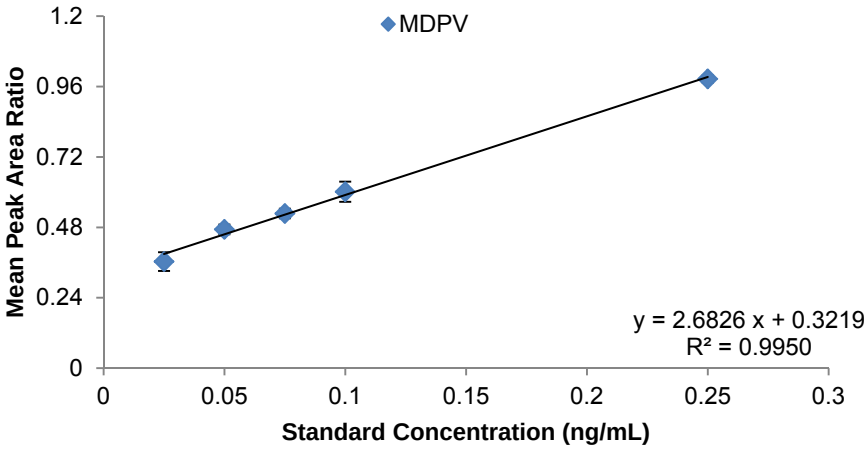

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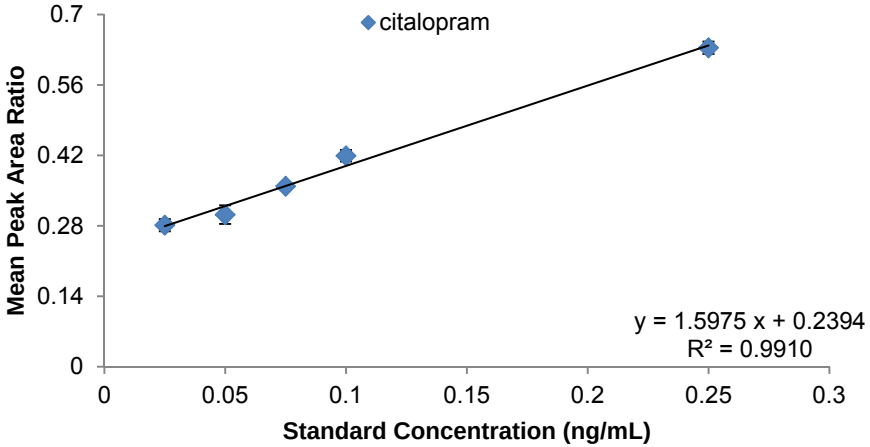
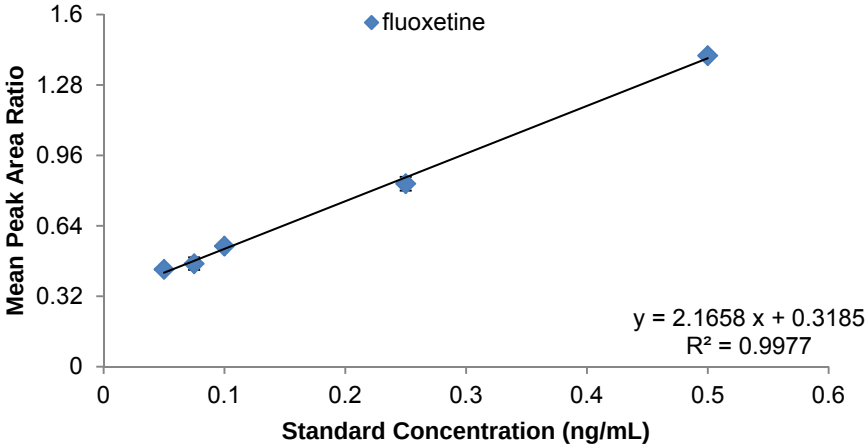
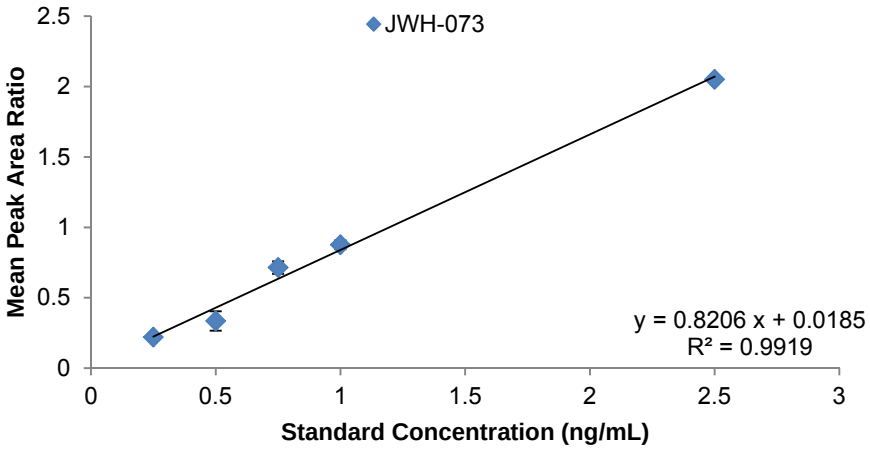
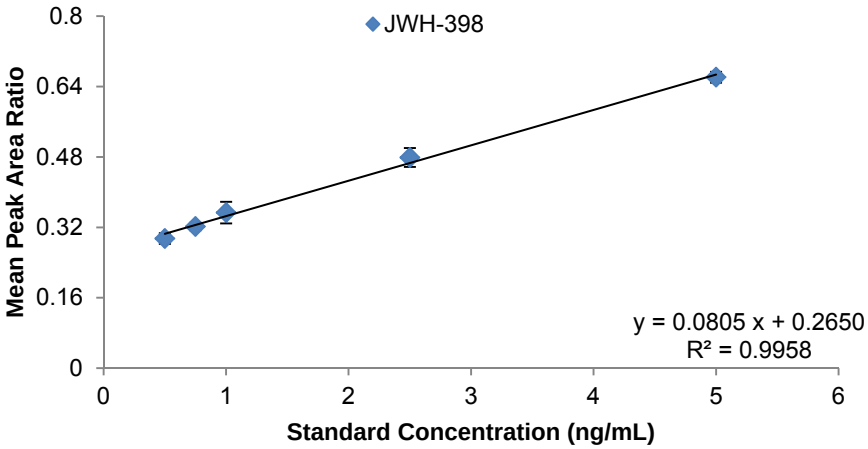




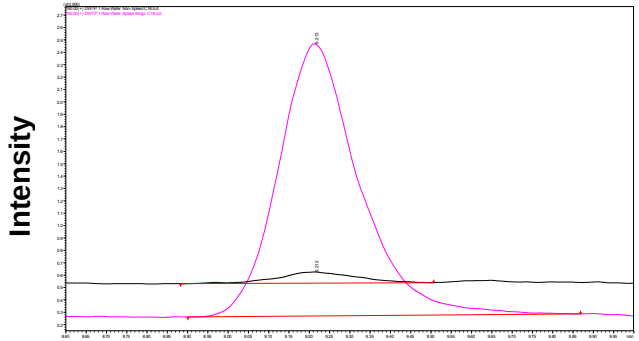




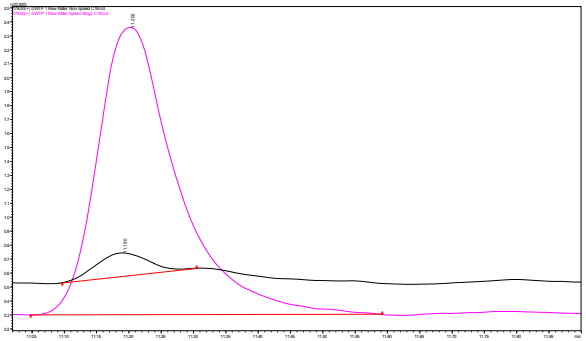
Appendix VII: Identification of drugs of abuse and pharmaceuticals in the raw water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

a) Samples from DWTP A

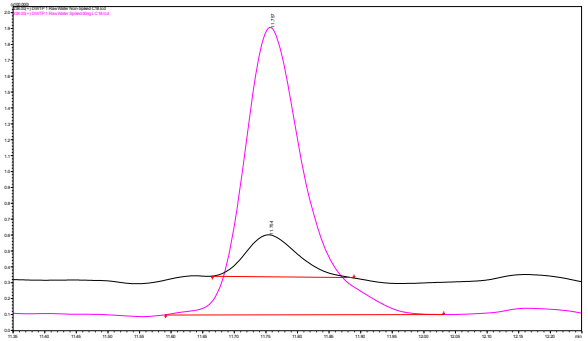
methamphetamine (m/z 150)



mephedrone (m/z 178)

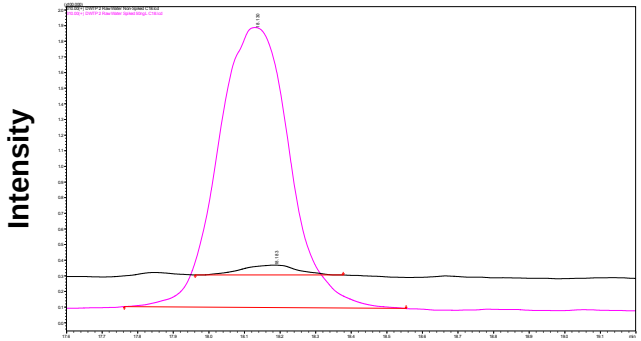


ketamine (m/z 238)



b) Samples from DWTP B

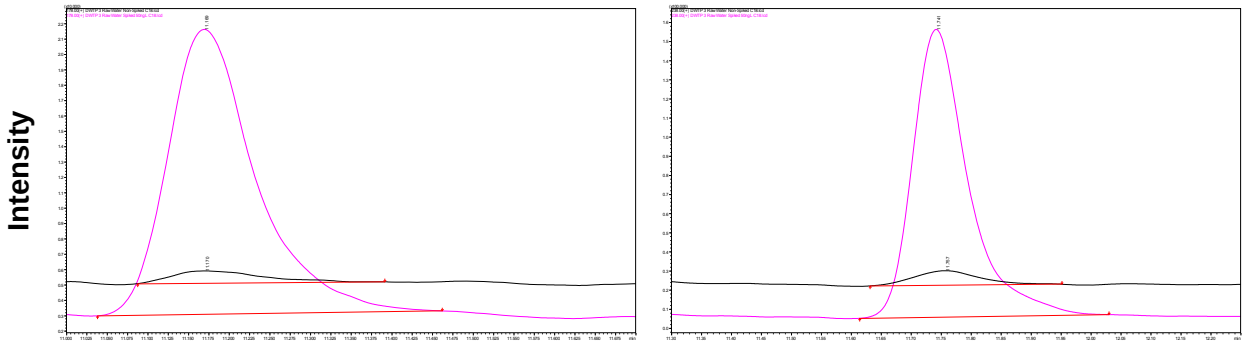
fluoxetine (m/z 310)



Appendix VII: Identification of drugs of abuse and pharmaceuticals in the raw water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

c) Samples from DWTP C

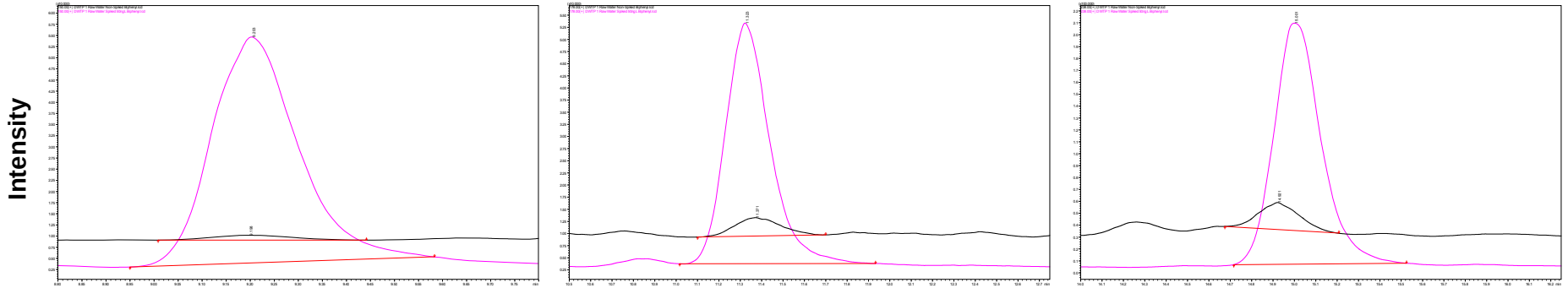
mephedrone (m/z 178) **ketamine (m/z 238)**



Appendix VIII: Confirmation of drugs of abuse and pharmaceuticals in the raw water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

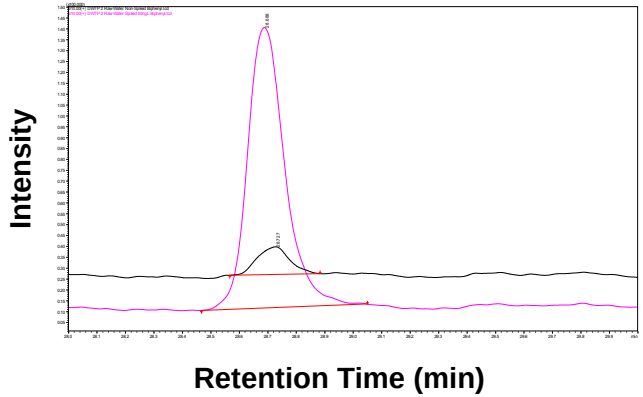
a) Samples from DWTP A

methamphetamine (m/z 150) **mephedrone (m/z 178)** **ketamine (m/z 238)**



b) Samples from DWTP B

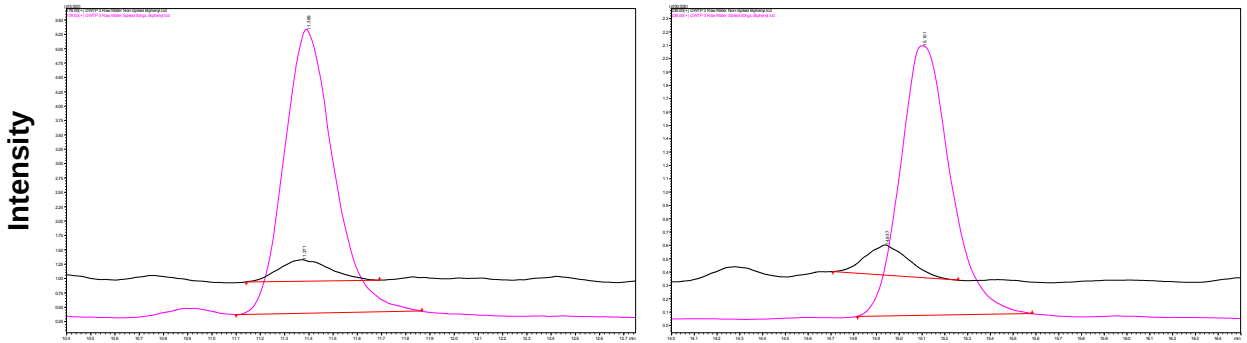
fluoxetine (m/z 310)



Appendix VIII: Confirmation of drugs of abuse and pharmaceuticals in the raw water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

c) Samples from DWTP C

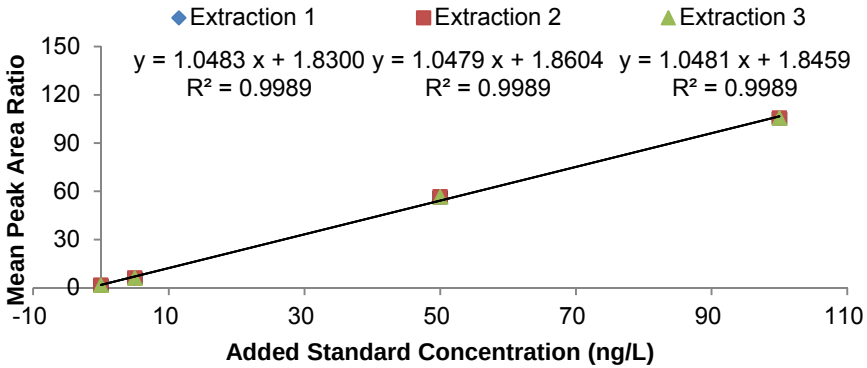
mephedrone (m/z 178) **ketamine (m/z 238)**



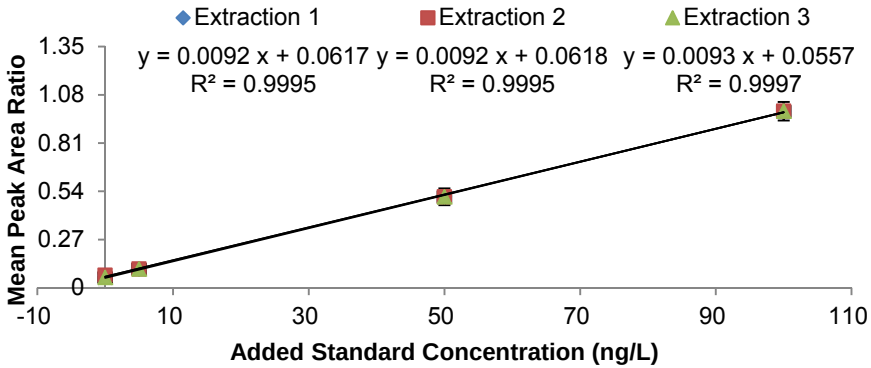
Appendix IX: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the raw water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

a) Samples from DWTP A

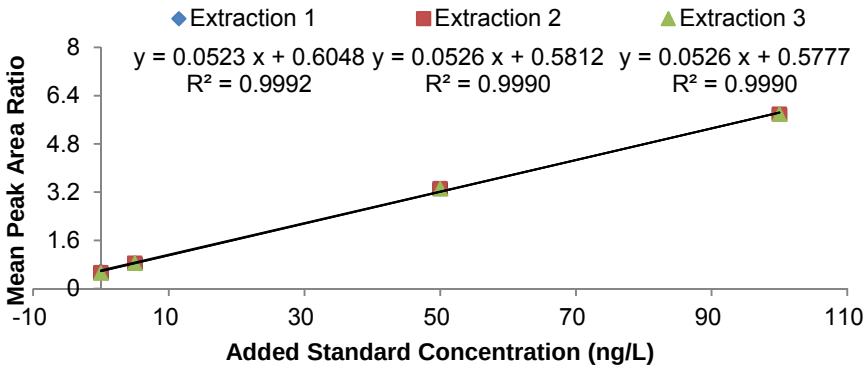
methamphetamine



mephedrone



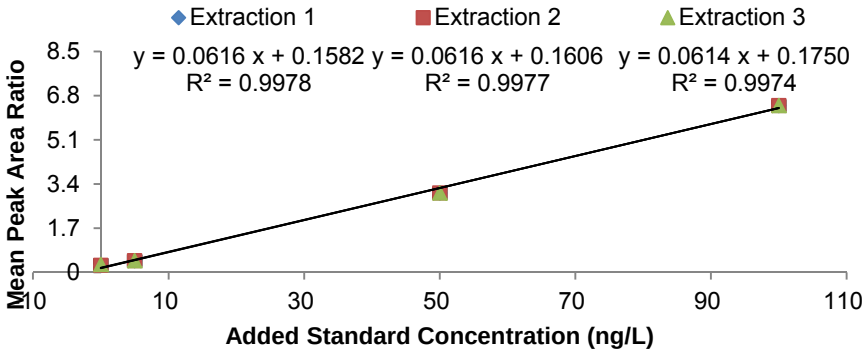
ketamine



Appendix IX: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the raw water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

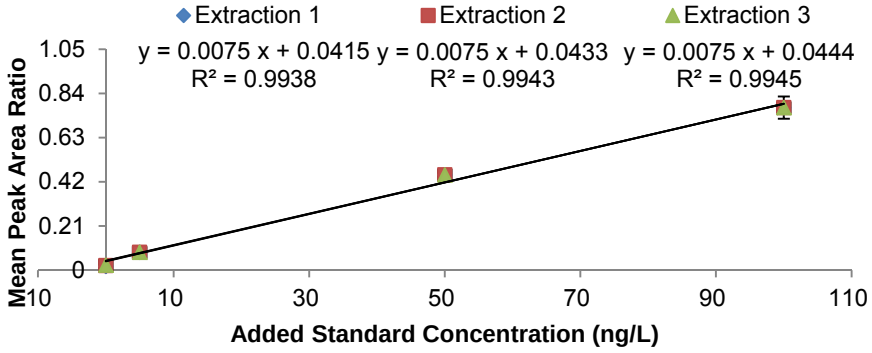
b) Samples from DWTP B

fluoxetine

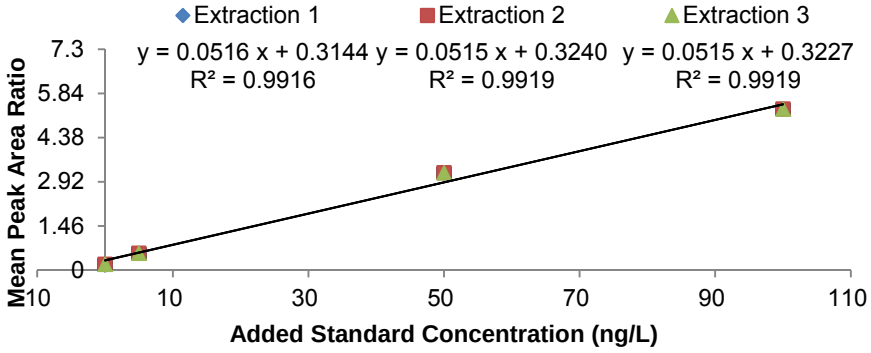


c) Samples from DWTP C

mephedrone



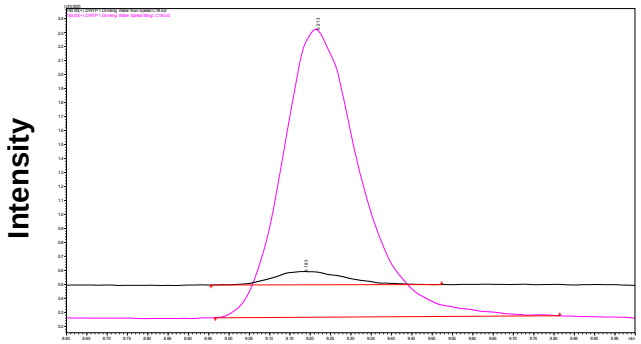
ketamine



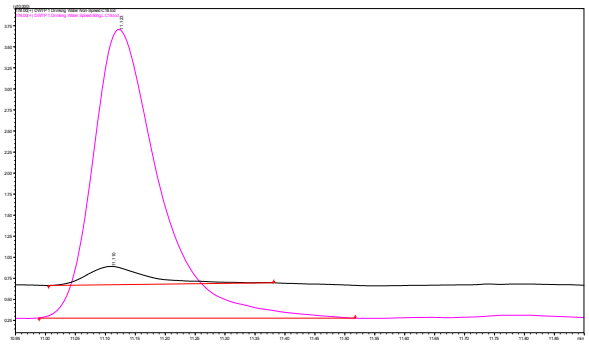
Appendix X: Identification of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

a) Samples from DWTP A

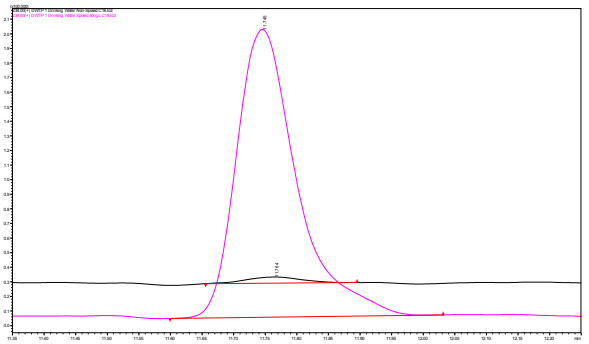
methamphetamine (m/z 150)



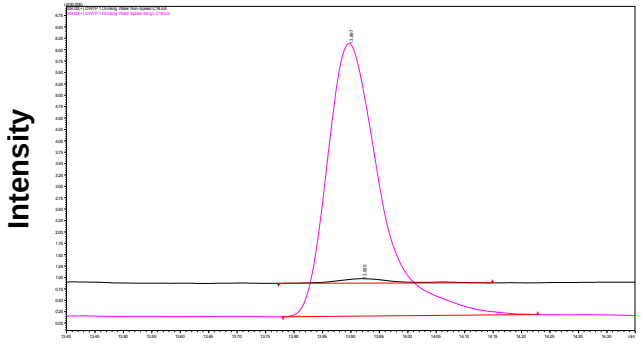
mephedrone (m/z 178)



ketamine (m/z 238)



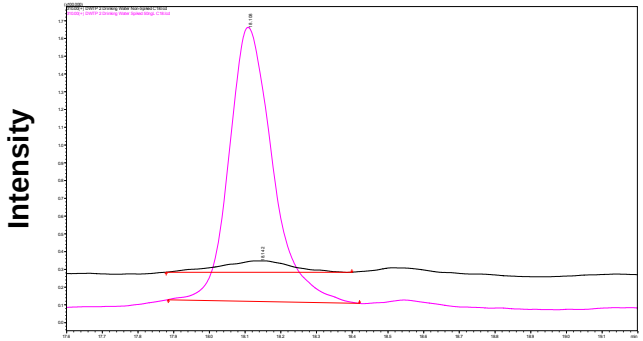
cocaine (m/z 304)



Appendix X: Identification of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

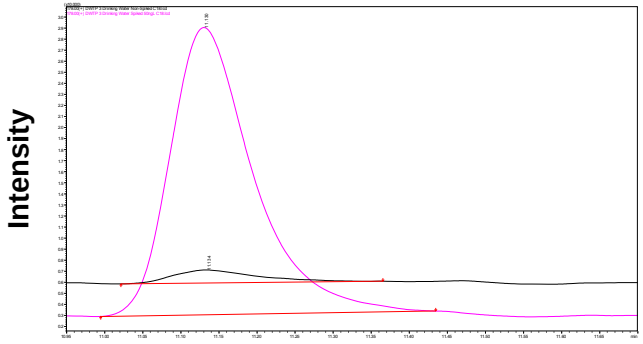
b) Samples from DWTP B

fluoxetine (m/z 310)

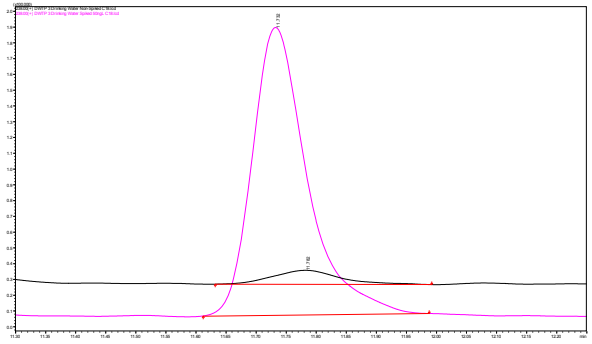


c) Samples from DWTP C

mephedrone (m/z 178)

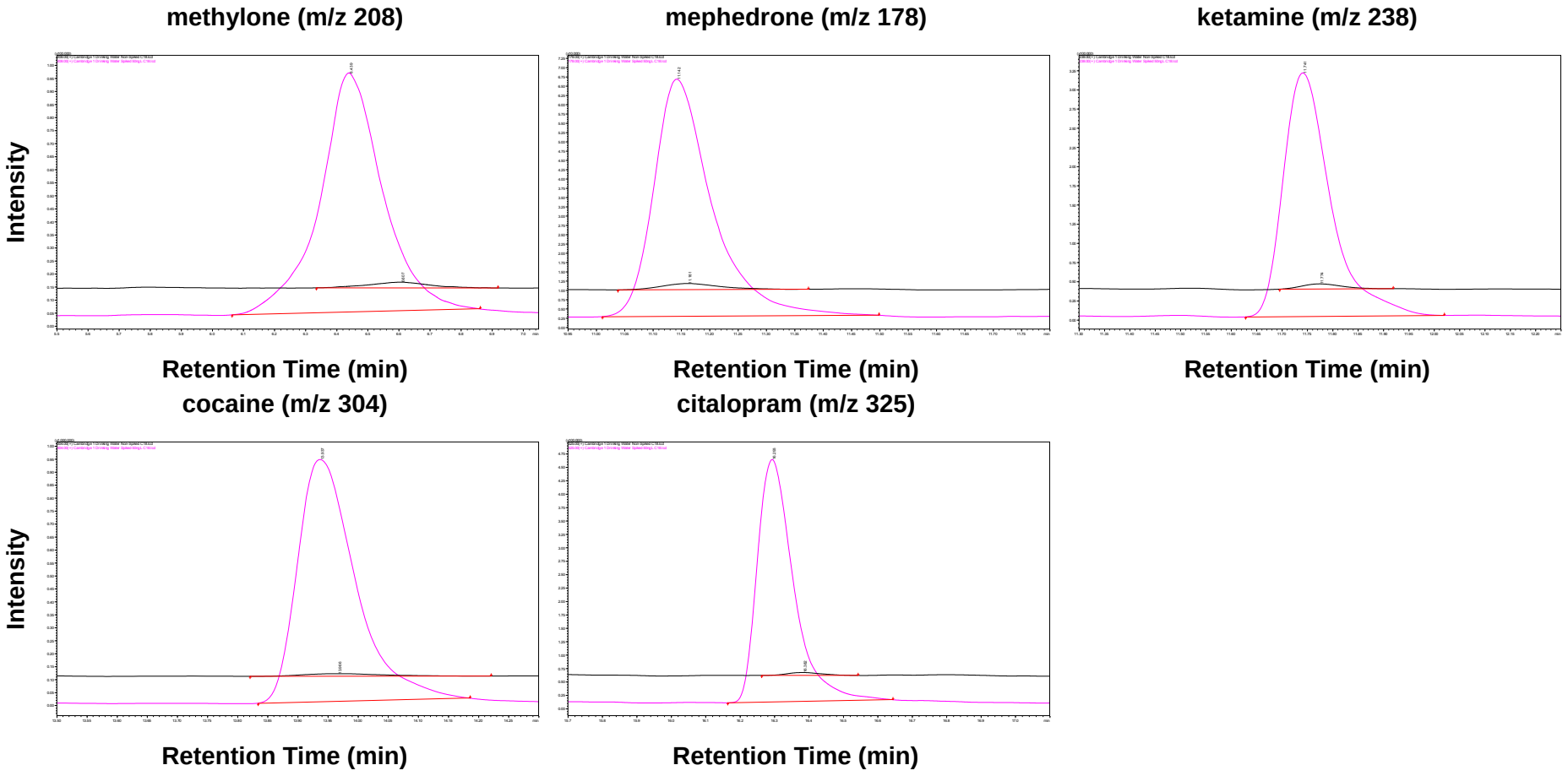


ketamine (m/z 238)



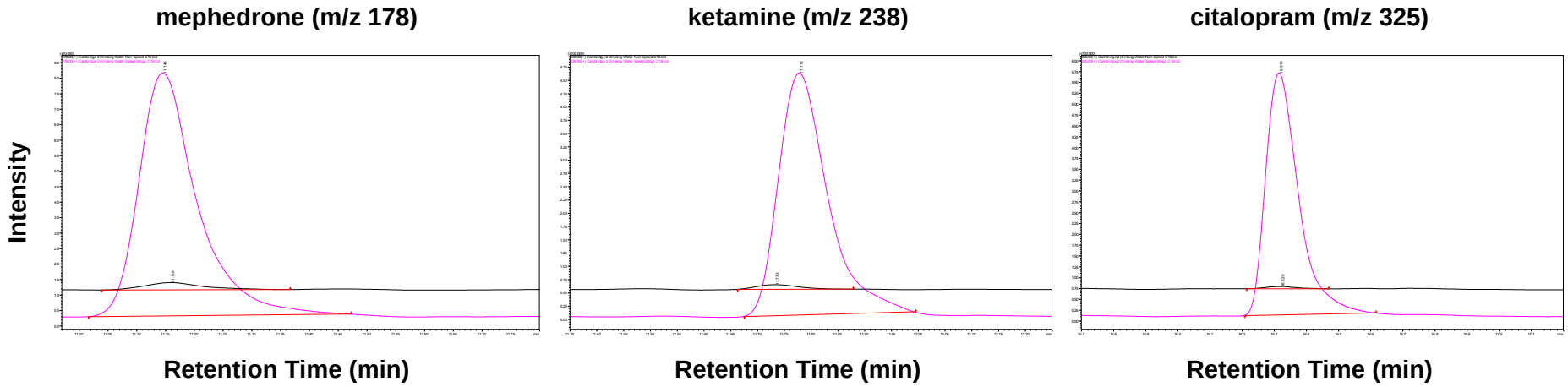
Appendix X: Identification of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

d) Samples from tap 1, City of Cambridge



Appendix X: Identification of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a C_{18} column

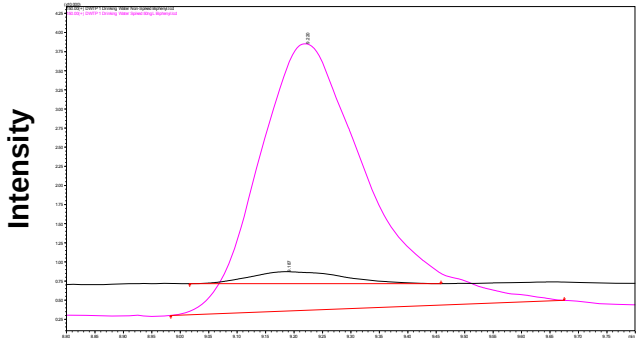
e) Samples from tap 2, City of Cambridge



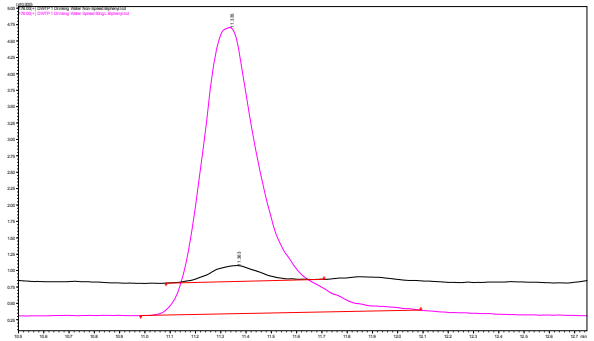
Appendix XI: Confirmation of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

a) Samples from DWTP A

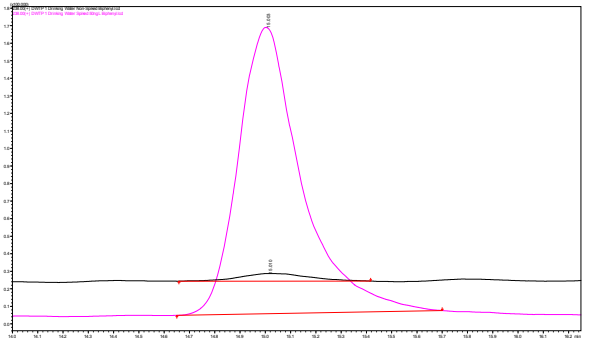
methamphetamine (m/z 150)



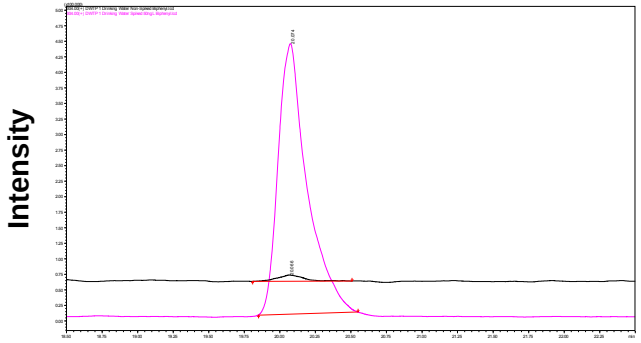
mephedrone (m/z 178)



ketamine (m/z 238)



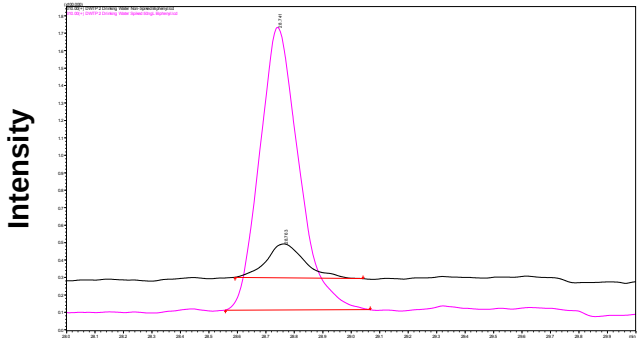
cocaine (m/z 304)



Appendix XI: Confirmation of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

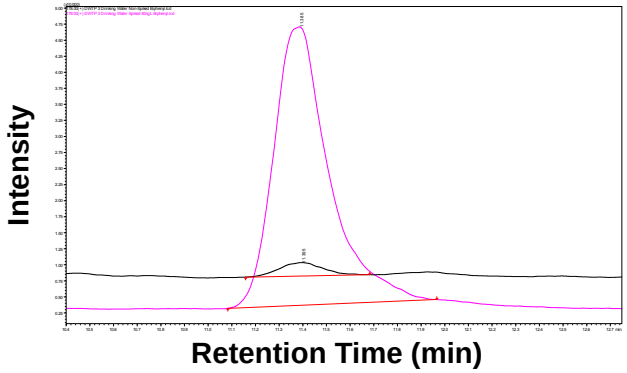
b) Samples from DWTP B

fluoxetine (m/z 310)

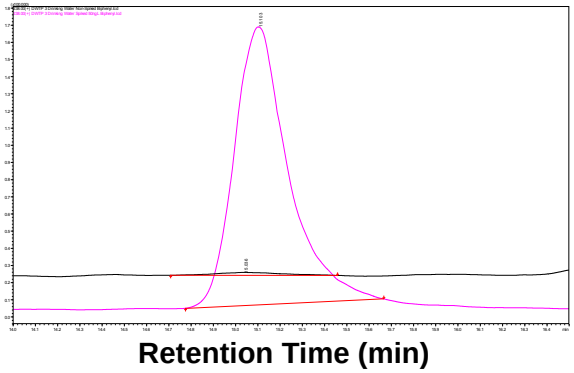


c) Samples from DWTP C

mephedrone (m/z 178)



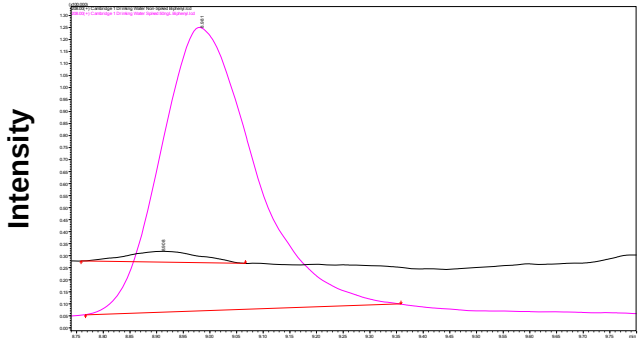
ketamine (m/z 238)



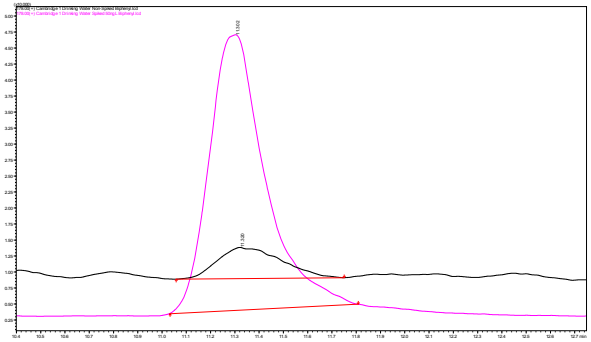
Appendix XI: Confirmation of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

d) Samples from tap 1, City of Cambridge

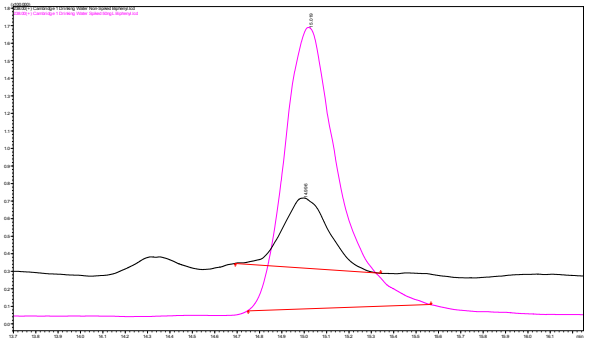
methylone (m/z 208)



mephedrone (m/z 178)



ketamine (m/z 238)



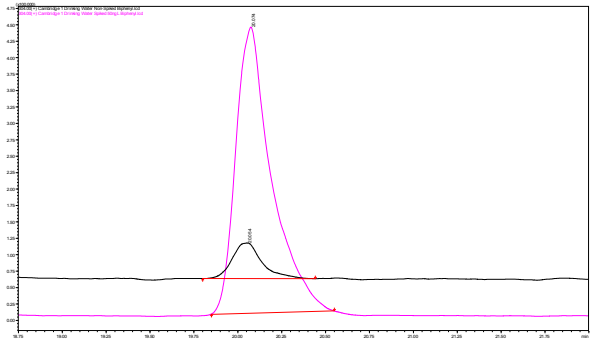
Intensity

Retention Time (min)

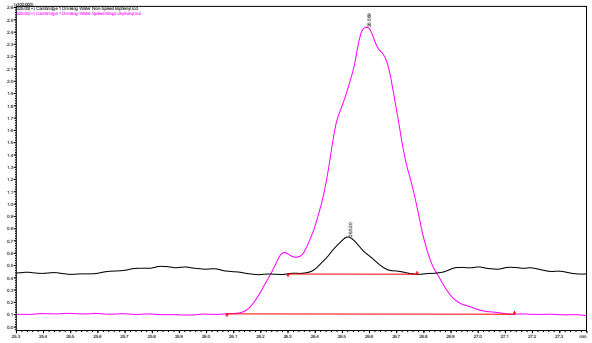
Retention Time (min)

Retention Time (min)

cocaine (m/z 304)



citalopram (m/z 325)



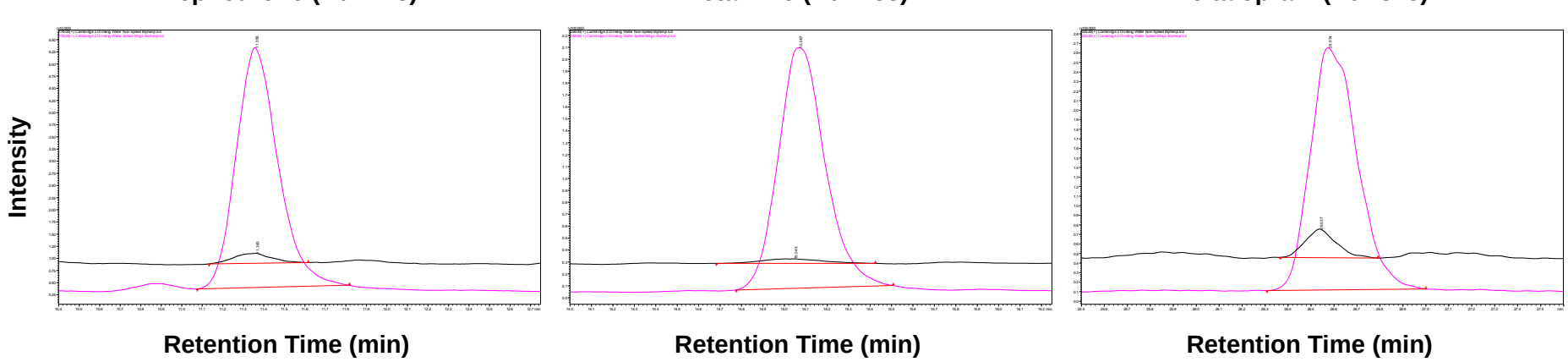
Intensity

Retention Time (min)

Retention Time (min)

Appendix XI: Confirmation of drugs of abuse and pharmaceuticals in the drinking water from The East Anglia region, UK, showing overlapping selected ion chromatograms of non-spiked water samples (black) and mixed standards (pink) at 50 ng/L from a SPE-LC-MS analysis obtained with SIM mode and a biphenyl column

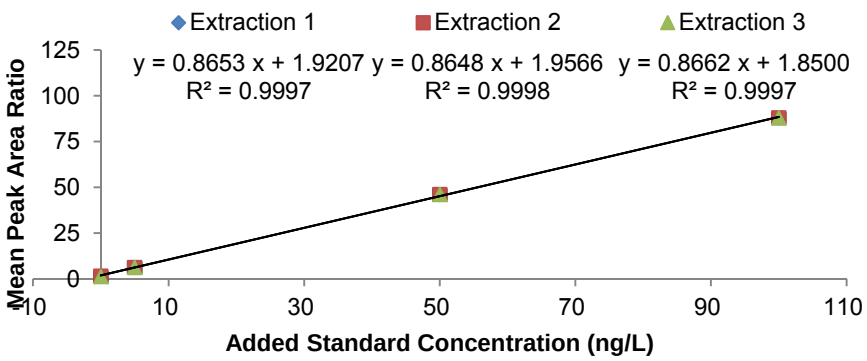
e) Samples from tap 2, City of Cambridge



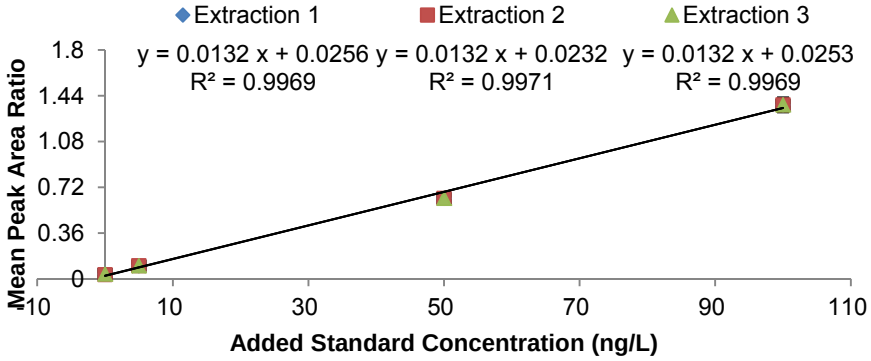
Appendix XII: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the drinking water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

a) Samples from DWTP A

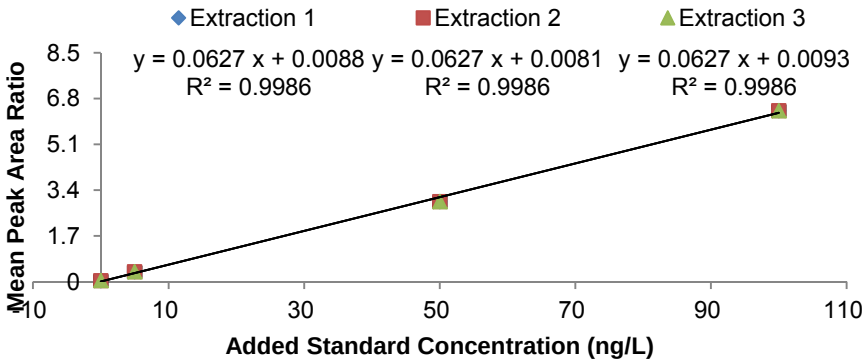
methamphetamine



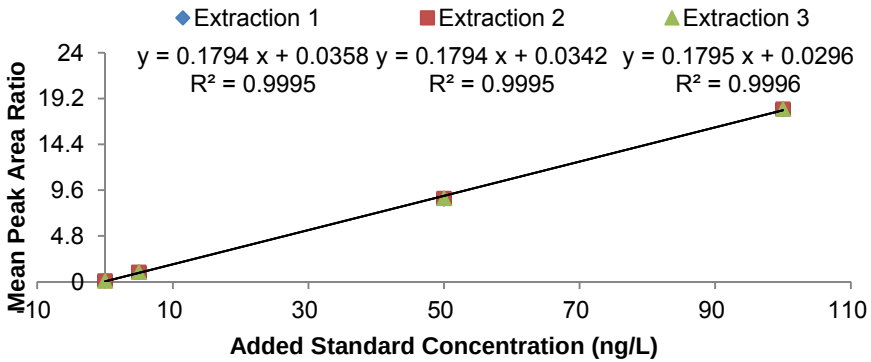
mephedrone



ketamine



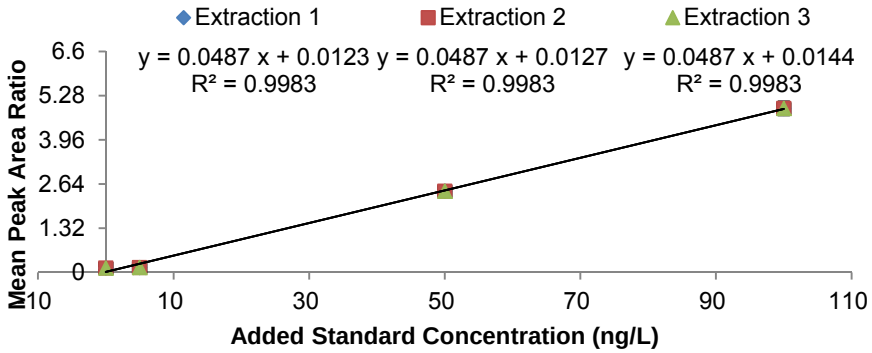
cocaine



Appendix XII: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the drinking water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

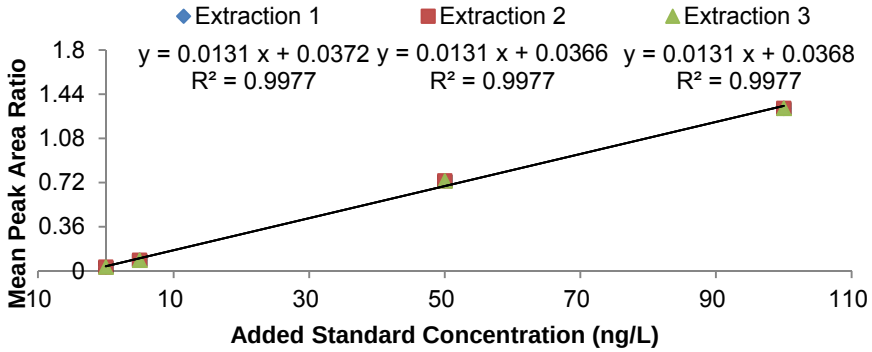
b) Samples from DWTP B

fluoxetine

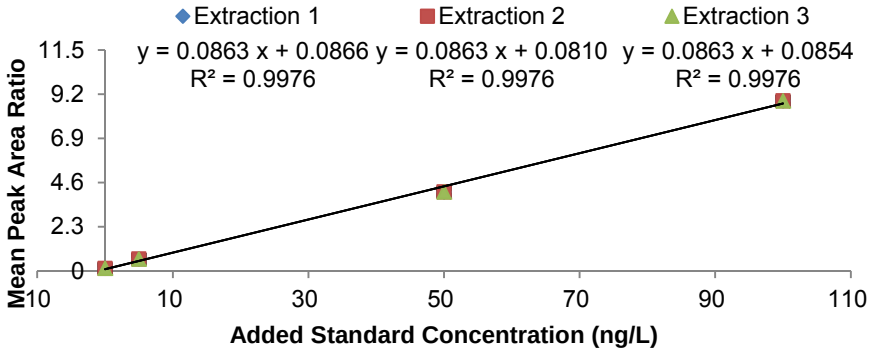


c) Samples from DWTP C

mephedrone



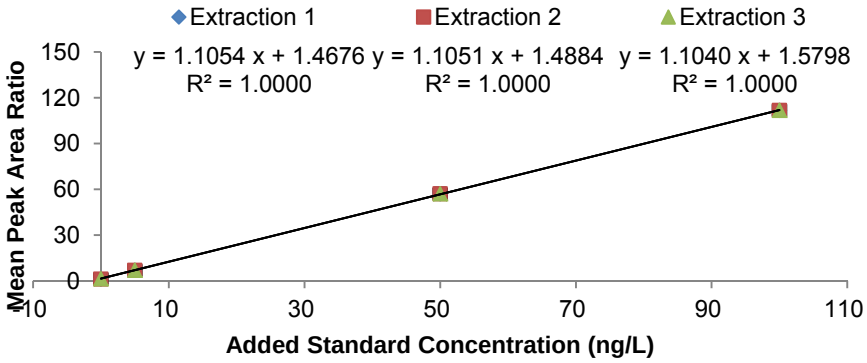
ketamine



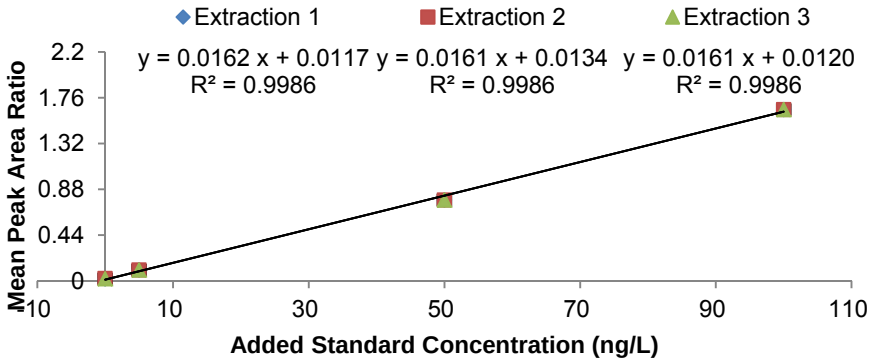
Appendix XII: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the drinking water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

d) Samples from tap 1, City of Cambridge

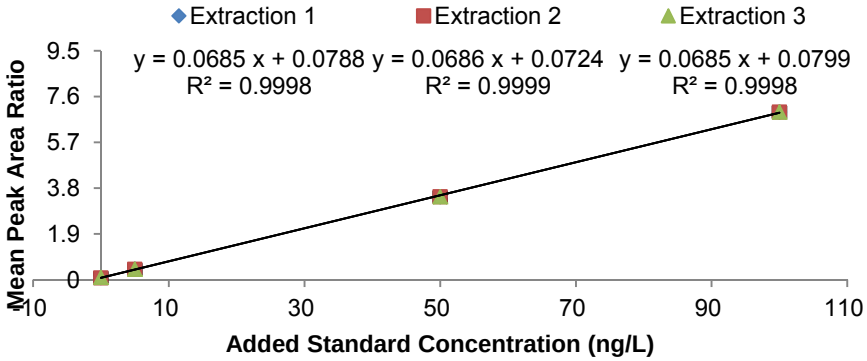
methylone



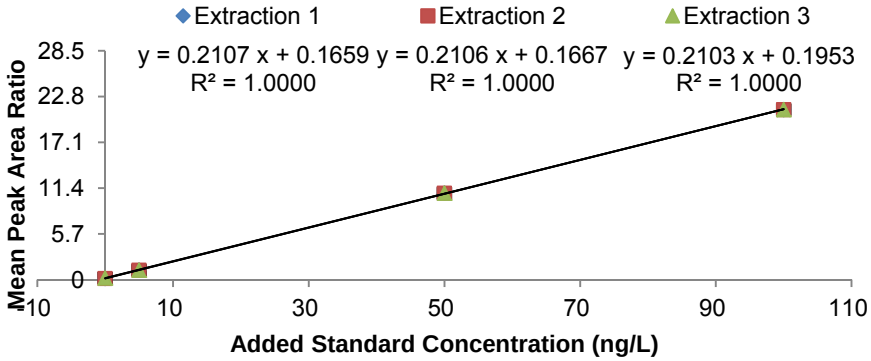
mephedrone



ketamine



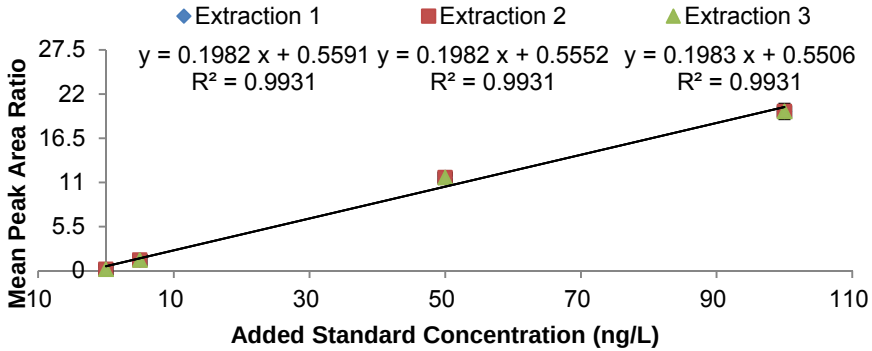
cocaine



Appendix XII: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the drinking water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

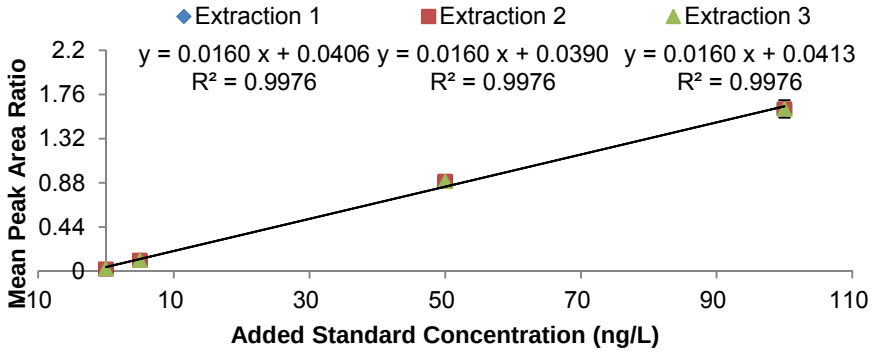
Samples from tap 1, City of Cambridge

citalopram

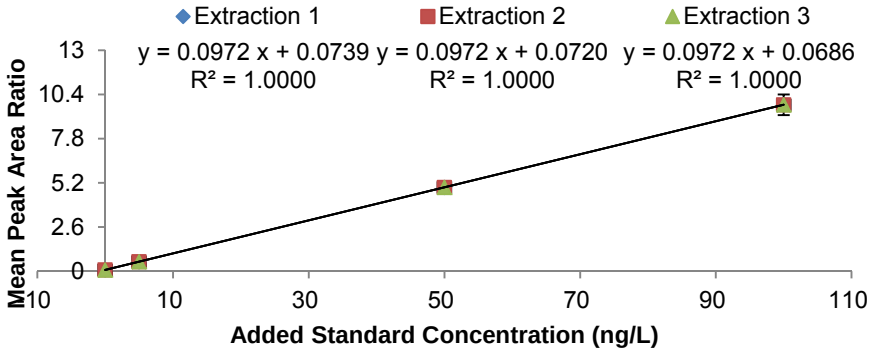


e) Samples from tap 2, City of Cambridge

mephedrone



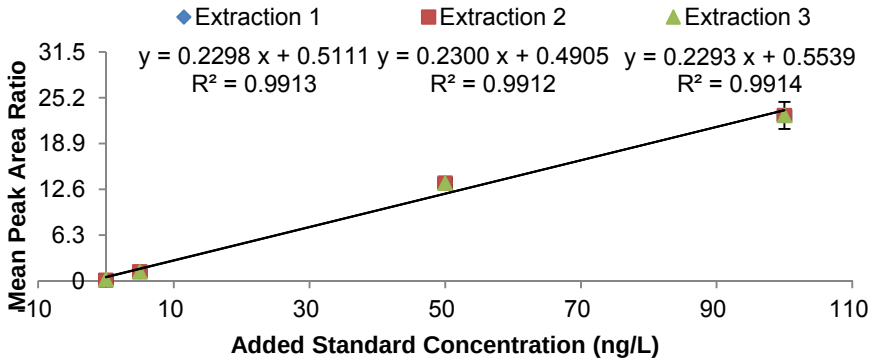
ketamine



Appendix XII: Standard addition plots of mean peak area ratio against added standard concentration for drugs of abuse and pharmaceuticals over 0 to 100 ng/L for the quantification of their concentrations in the drinking water from The East Anglia region, UK, obtained from a SPE-LC-MS analysis with a C_{18} column, n = 3

e) Samples from tap 2, City of Cambridge

citalopram



Appendix XIII: Publication and presentations

a) Publication

Peng, Y., Hall, S. and Gautam, L., 2016. Drugs of abuse in drinking water – a review of current detection methods, occurrence, elimination and health risks. *Trends in Analytical Chemistry*, [e-journal] 85 (C), pp. 232-240. http://dx.doi.org/10.1016/j.trac.2016.09.011.

b) Presentations

Peng, Y., Hall, S. and Gautam, L., 2016. Detection of drugs of abuse and pharmaceuticals in drinking water (poster presentation). *6th Annual Research and Scholarship Conference*, Chelmsford, UK, 6 July 2016.

Peng, Y., Hall, S. and Gautam, L., 2016. Drugs in our drink (oral presentation). *Anglia Ruskin University Biomedical & Forensic Sciences Departmental Research Seminars*, Cambridge, UK, 11 May 2016.

Peng, Y., Hall, S. and Gautam, L., 2016. Detection of pharmaceuticals and drugs of abuse in drinking water (oral presentation). *International Conference on Environmental Pollution and Public Health*, Suzhou, China, 13-15 April 2016.

Peng, Y., Hall, S. and Gautam, L., 2014. Simultaneous detection of drugs of abuse and pharmaceuticals in drinking water using liquid chromatography-mass spectrometry (oral presentation). *Anglia Ruskin University Forensic and Investigative Science Research Group Seminar*, Cambridge, UK, 26 November 2014.

Peng, Y., Hall, S. and Gautam, L., 2014. Detection of drugs of abuse in drinking water (poster presentation). *8th Annual Research Student Conference*, Chelmsford, UK, 13 June 2014.

Peng, Y., Hall, S. and Gautam, L., 2014. Detection of drugs of abuse and pharmaceuticals in drinking water (poster presentation). *4th Annual Research and Scholarship Conference*, Cambridge, UK, 14 May 2014.