THE DETERMINATION OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS IN WATER AND WASTEWATER BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

by

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DECLARATION

This dissertation is submitted in fulfilment of Master of Science degree in Chemistry to the University of South Africa. This work was carried out at the University of South Africa Laboratory, Department of chemistry South Africa. I declare that this work is my own and was not submitted elsewhere before.

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SIGNATURE DATE

Abstract

Emerging contaminants (EC) are unregulated substances that have entered the environment for as long as they have been produced through human activities. Within the ECs family, pharmaceuticals and personal-care products (PPCPs) is a class of the most common pollutants employed in everyday urban activities. Current regulatory approaches are inadequate to address these contaminants and the presence of such chemicals in the aquatic environment and their potential deleterious effects has received an increasing attention from the public and scientific community. Influent and effluent wastewater from Daspoort Waste Water Treatment Works (WWTW), which treats wastewater from the central Pretoria area, were sampled and analysed from January to December 2015 for pharmaceuticals and personal care products by gas chromatography time of flight mass spectrometry (GC/TOF-MS). Thirteen pharmaceuticals were selected for focused study in wastewater which include; (acetaminophen, bisphenol A, carbamazepine, diclofenac, 17α-estradiol, 17β-estradiol, estriol, famciclovir, fenoprofen, ibuprofen, primidone, progesterone and testosterone) based on the criteria of their prescription volumes in both private and public health sector in South Africa.

The development of a sensitive and reliable analytical method for the simultaneous determination of PPCPs in aquatic samples was carried out; using a combined solid-phase extraction (SPE) isolation and clean-up, followed by derivatization prior to GC/TOFMS determination. A seven points concentration levels linear calibration curve with correlation coefficient (R^2) ranged from 0.9988 to 0.9999 was obtained. The limits of detection (LOD) and the limit of quantification (LOQ) ranged from 0.01-0.27 µg L^{-1} and 0.03-0.91 µg L^{-1} respectively for target PPCPs. Repeatability studies gave % RSD within 3.41 – 11.72 % for peak area. The % RSD values for reproducibility studies were 2.88 – 9.91% for peak area over the three concentrations (ibuprofen: 0.4, 2 and 8 µg L^{-1}) evaluated during 5 days. These results indicated that the proposed method has excellent precision as evidenced by very stable peak area for the analytes. The recovery testing carried out, exhibited recoveries ranging from at least 82-115% and 81-115 % in tap water and Milli-Q water respectively, with % RSD less than 12%, showing that the overall PPCPs determination method including the extraction procedure was a repeatable method.. The method

was applied to target PPCPs from Daspoort influent and effluent wastewater in Pretoria (South Africa). Natural hormones and antiviral drug were not detected in all the samples analysed by this method. Bisphenol A, acetaminophen, carbamazepine, ibuprofen and diclofenac were detected at low concentrations, ranging from 0.052-135.42 μg L^{-1} in wastewater. The level of bisphenol A, primidone, carbamazepine, ibuprofen and diclofenac in effluent wastewater were found to be lower in comparison to the influent. Several other non-target compounds, such as benzophenone, caffeine, methocarbamol, efavirenz, atrazine, dioxaphetyl butyrate, nevirapine, androsta-1,4-diene-3,17-dione, cis-tramadol, batyl alcohol, paredrine, 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin, propylparaben, eugenol, cholesterol, stigmasterol, guaifenesin, benzyl benzoate, 4-tert-octylphenol, diethyltoluamide, dicyclomine, terbuthylazine, spiroxamine, bis(2-ethylhexyl) phthalate, bumetrizole , were also detected in the wastewater sample using the developed method.

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CHAPTER 1 INTRODUCTION

1.1 General introduction

The last few decades have witnessed a growing awareness of the environmental concerns caused by chemicals from various source. For this reason, the presence of such chemicals in the aquatic environment and their potential deleterious effects has received an increasing attention from the scientific community [1, 2, 3]. These chemicals, which are commonly referred to as emerging contaminants (ECs), have been introduced into the environment for as long as they have been produced but not much attention has been paid on them until about thirty years ago [4, 5, 6]. Within the ECs family, pharmaceuticals and personal care products (PPCPs) is a class of the most common pollutants employed in everyday urban activities [7, 8].

PPCPs consist of a large group of biologically active synthetic or natural chemicals with different physico-chemical properties. These chemicals have a wide range of uses and applications in our modern society. PPCPs are used for disease treatment, relieving pain and/or for the general improvement of the quality of life [9, 10]. These compounds include, but are not limited to, those found in human and veterinary drugs (antibiotics, tranquillizers, anti-epileptics, etc.), hormones (natural and synthetic), diagnostic agents such as X-ray contrast media, bioactive food supplements, soaps, detergents, shampoo, cosmetics, sun-screen products, fragrances, insect repellents, preservatives, etc. [11, 12, 13]. There are large quantities of these compounds used by both humans and animals, for example 3.6 tons of unused pharmaceutical products are reported disposed of annually through toilets and rubbish bins by Taiwanese consumers [14].

Conventional wastewater treatment plants remove these chemicals through dilution, oxidation by disinfectants, biological degradation, or sorption to solid materials settled out of the waste stream [15]. However, wastewater treatment plants are not designed or suited to effectively remove these compounds [16] and it has been reported that the removal efficiency of PPCPs from water/wastewater varies greatly and that their elimination is often incomplete [17, 18]. These substances and their transformation products are therefore continuously introduced in the aquatic environment through various effluents (at the range of ng L^{-1} to low µg L^{-1}) [19, 20], with potential to negatively affect aquatic and terrestrial organisms as they are designed to produce biological effects even at very low concentrations [8, 3]. To the best of our knowledge evidence of deleterious effects on human has not been reported. Defects due to exposure to PPCPs have definitely been detected in aquatic life and for other animals [21, 22, 23, 24]. Bacteria continually exposed to antibiotics, may developed antimicrobial-resistant strains which could affect directly lower life form and or indirectly influence humans. Feminization in fish and alligators exposed to PPCPs has been reported [25, 26]. In addition, PPCPs has been reported to affect the behaviour and migratory patterns of salmon [27]. The UV filter 3-benzylidene camphor (3BC) has been shown in vitro and in vivo to affect the reproduction of reproductively mature fathead minnows, in addition 3BC was shown to accumulate in fish [16]. Diclofenac has been directly linked with the near extinction of the vulture population in India [28]. The consequences of PPCPs even at trace levels in the environment have been previously demonstrated on certain wildlife [29, 30]. However, there still no reported evidence of any adverse effects in humans, in addition, some PPCPs are bio accumulative [31, 32].

The extensive human and veterinary use of PPCPs results in their subsequent discharge in the aquatic environment, mainly through household drainage systems and run off, and has caused widespread concern. Humans typically excrete 50 % to 90 % of the active ingredients of some pharmaceuticals, either as the native compounds or as their metabolites [28]. Furthermore, their introduction to the environment has no geographic boundaries; they are released into the environment wherever people live or visit, regardless of the time of year. After their intended usages they enter into the environment through wastewater effluents discharged to streams, lakes, estuaries, and groundwater, as well as surface-water runoffs and soil leaching after agricultural applications of manure or treated sludge [33]. These contaminants do not need to be persistent in the environment to cause negative effects; their continued low-level recurrent introduction to receiving waters is enough to induce chronic responses [13].

As the global population increases and drought conditions worsen in many areas, the demand for water continues to grow. Water supplies in many areas, particularly in those with limited water resources, must be reused to satisfy a multitude of needs. Increasingly, treated municipal wastewaters now, functions as a source of water for the maintenance of ecosystems and ultimately as the source of drinking water. Consequently, the determination of the presence of bioavailable mixtures of anthropogenic contaminants and their ultimate effects on ecosystems in environmental waters is critical [34]. Although the concentrations of the PPCPs found in the environment are at sub-therapeutic doses, effects of repeated low-level exposure to aquatic organisms are only beginning to be researched due to analytical methods having become sufficiently sensitive and selective [16, 35].

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Therefore, there is a need to develop appropriate procedures and tools for the determination and quantitation of these compounds in the environment thus enabling accurate, environmental risk assessment of PPCPs, furthermore the outputs will be of use to legislators, regulators, industry and the scientific community [36]. Several analytical approaches have been reported in the literature for the investigation of PPCPs in environmental water. They usually include sample preparation steps, chromatographic separation and mass spectrometry detection. It should be noted that not all sample preparation steps are always necessary [37].

The determination of PPCPs in environmental aquatic samples begins with the sample collection. Water sample are usually collected at a specific time by discrete grab of a sample. However for more accurate risk assessment purpose collection of sample could be via passive sampling approaches that provides a time-weighted average, rather than instantaneous determination of PPCPs concentrations [38, 39]. After sampling aquatic samples may be preserved, filtered or undergo pH adjustment prior analytes extraction and final determination. For example, the addition of 1 % formaldehyde solution to inhibits micro-bacterial growth and storage of the samples at 4 ºC in amber bottles led to no significant losses of the oestrogens after 24 days [40]. Several extraction techniques are available, to enrich the analytes present at very low concentration levels in aquatic samples. Solid phase extraction (SPE) is the most commonly used method and alternatives to SPE are greener and miniaturised techniques such as microextraction [37, 41].

Previously, PPCPs were determined by gas chromatography separation coupled to different detectors such as electron capture detector (ECD), flame ionisation detector (FID), nitrogen

phosphorus detector (NPD) and mass spectrometer (MS). In addition liquid chromatography separation has also been used in combination with detectors such as photodiode array detector (DAD), fluorescence detector (FLD) and mass spectrometry. GC-MS as a method was selected due to its superior separation and identification capabilities [37]. However, GC-MS requires that compounds to be separated are volatile and thermally stable which leads to limitation of the approach since only about 5 % of organic compound fulfil this requirement. Therefore highly-polar, thermally-fragile compounds are chemically modified during a process called derivatization in order to overcome this limitation [37].

A number of methods have been successfully applied on the analysis of single class of compound having similar physico-chemical properties (e.g. Molecular weight, pK_a, polarity, water solubility, etc.). A single method for the simultaneous analysis of different classes of PPCPs present many advantages, such as reduce overall analysis time, field sampling and costs [41, 42, 43]. Nonetheless, the determination of these compounds in wastewater and water samples is a challenging task because they vary widely in their physico-chemical properties (e.g. Molecular weight, pK_a, polarity, water solubility, etc.). In addition, wastewater and water is an extremely complex sample containing countless micropollutants [44].

1.2 Occurrence of pharmaceutical and personal care products in the aquatic environment

Generally, wastewater treatment plants are the barriers before the PPCPs are released into the aquatic environment. However, most wastewater treatment technologies were not designed to completely remove PPCPs in sewage and wastewater effluent [45, 46]. Hence, various

pharmaceutically active compounds have been released by WWTPs into the environment for many years [47, 48].

1.2.1 Pharmaceutical and personal care products in surface and ground water

PPCPs which apply for both human and veterinary use have a final sink into the natural ecosystem through various routes, as depicted by Figure 1.1. PPCPs are released into sewage water and from individual domestic households through various routes. These include metabolised and non-metabolised form excreted via urine and faeces, improper disposal of these compounds down the drain and those washed off from skin by bathing. Wastewater is directed to the municipal sewer systems through wastewater treatment plant for processing. The treated effluent wastewater is released into surface waters that is subsequently reused via several avenues such as ground water recharge, and ultimately used as a source of agricultural and drinking water. In addition, compounds from veterinary medicine and growth promoters for animals use are also released in surface water (e.g. aquaculture, runoff from soil onto which manure has been applied) through the application of manure to soil [49]. The disposal of drugs through landfill, treated sewage sludge released from wastewater treatment may all contribute to some extend or to the overall presence of pharmaceuticals in the environment. PPCPs in effluent from manufacturing plants are regarded as marginal under the normal operating condition of the manufacturing facilities, due to the rigorous regulations surrounding their production and the cost of chemicals and solvent used. However, high emissions are possible in some rare cases of accidents.

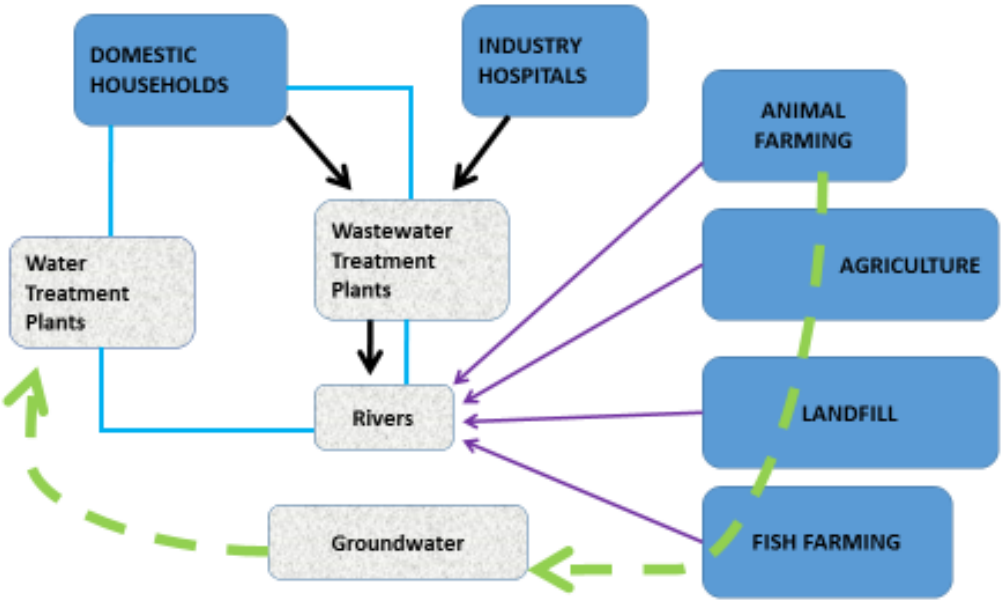


Figure 1-15 Sources, distribution and sinks of pharmaceuticals in the environment (The black arrows represent the main route of transport of PPCPs, purple arrows contribution are secondary)

The first reported case of the presence of PPCP was the accidental detection of clofibric acid in 1991 during a study involving the determination of the occurrence of herbicides residue in ground water around Berlin wall [50]. This discovery opened studies which led to subsequent discovery of the presence of other pharmaceutical in water system. Since then, the discovery sparked an explosion of interest in research concerning this new group of emerging contaminants in almost all the aquatic compartment (i.e. influent and effluent of wastewater, ground and drinking water) [51, 52]. The interest on pharmaceutical residues in the environment has extended to the risk associated with such occurrence [53]. Table 1.1 below highlights a few of the numerous findings

that have been reported across the globe on the occurrence of different pharmaceutical compounds in wastewater influents and effluents as well as in surface and ground water samples.

Table 1.5 Studies of pharmaceuticals and other emerging contaminants in wastewater influents, effluents, surface water, and ground water reported in the literature since 2004

Compound	WWTP Influent Concentration µg L^{-1}	WWTP Effluent Concentration µg L^{-1}	surface-Water Concentration µg L^{-1}	Ground Water Concentration µg L^{-1}	Reference /Country
Ibuprofen **Fenoprofen** **Mefenamic acid** **Ketoprofen** **Naproxen**	69 - 1090 ng L^{-1} nd - 20 ng L^{-1} 143-1580 ngL^{-} [1]160-1060 ng L^{-1} 179 -305 ng L^{-1}	nd - 40 ng/L nd 265 - 72 ng/L 107 - 64 ng/L 166 -74 ng/L			[3]/ Japan
Ibuprofen **Naproxen** **Diclofenac** **Galaxolide** **Tonalide**		0.2 µg L^{-1} 0.2 µg L^{-1} 1.1 µg L^{-1} 1.07 µg L^{-1} 0.37 µg L^{-1}			[8]/ UK
Acetaminophen **Erythromycin-H_2O**		75.5 µg L^{-1} 7.84 µg L^{-1}	15.7 µg L^{-1} 417.5 µg L^{-1}		[54]/Taiwan
Estrone **17β-estradiol** **17α-estrodial** **Estriol** **17αethynylestradiol** **Diethylstilbestrol** **Mestranol** **Nolyphenol** **Bisphenol A** **Triclosan** **Methylparaben** **Propylparaben** **Butylparaben** **2-phenylphenol**			65 ng L^{-1} ≤ 2 ng L^{-1} ≤ 2 ng L^{-1} 1 ng L^{-1} 1 ng L^{-1} 1 ng L^{-1} nd 36-33231ngL^{-1} 6 -881 ng L^{-1} 35-1023 ngL^{-1} 2 -1062 ng L^{-1} 5 -3142 ng L^{-1} Nd 8 -2506 ng L^{-1}		[55]/ China

Compound	WWTP Influent Concentration µg L^{-1}	WWTP Effluent Concentration µg L^{-1}	surface-Water Concentration µg L^{-1}	Ground Water Concentration µg L^{-1}	Reference /Country
Salicylic acid			9 -2098 ng L^{-1}		
Clofibric acid			22 -248 ng L^{-1}		
Ibuprofen			4 -1417 ng L^{-1}		
Naproxen			5-328 ng L^{-1}		
Gemfibrozil			nd		
5β-coprostanol			10-2717 ngL^{-1}		
Caffeine	2.4488-4.8658	0.0039-0.0231			[56]/USA
Carbamazepine	0.0248-0.0509	0.0337-0.1112			
Ciprofloxacin	0.0114-0.3772	0.0088-0.1099			
Clarithromycin	0.1057-0.7242	0.0702-0.6106			
Clindamycin	0.0068-0.0133	0.0149-0.0325			
Diclofenac	0.0095-0.0139	0.0083-0.1771			
Diltiazem	0.0405-0.0691	0.0939-0.1073			
Gemfibrozil	0.1818-0.4513	0.0421-0.0835			
Salicylic acid	0.4339-8.0361	0.0252-0.0472			
Sulfamethoxazole	0.0135-0.2610	0.0794-0.4724			
Tetracycline	0.0293-0.0389	0.0310-0.0344			
Cimetidine,	< MLQ	< MLQ			
Clofibric acid	< MLQ	< MLQ			
Sulfamethizole	< MLQ	< MLQ			
Sulfathiazole	< MLQ	< MLQ			
Cotinine	0.0412-1.5811				
Sulfadimethoxine	0.0026	0.0019			
Sulfamethazine	0.0269				
Sulfisoxazole	0.0221	0.0119			
Valproic acid	150 ng L^{-1}				[57]/USA
5-chloro-m-cresol	100 ng L^{-1}				
Biosol	250 ng L^{-1}	40 ng L^{-1}			
p-chloro-m-xylenol	350 ng L^{-1}	100 ng L^{-1}			
Biphenylol	820 ng L^{-1}	150 ng L^{-1}			
Ibuprofen	3700 ng L^{-1}				
Gabapentin	130 ng L^{-1}				

Compound	WWTP Influent Concentration µg L^{-1}	WWTP Effluent Concentration µg L^{-1}	surface-Water Concentration µg L^{-1}	Ground Water Concentration µg L^{-1}	Reference /Country
Acetaminophen	960 ng L^{-1}	130 ng L^{-1}			
Gemfibrozil	510 ng L^{-1}				
5-fluorouracil	nd	140 ng L^{-1}			
Chlorophene	610 ng L^{-1}	270 ng L^{-1}			
Naproxen	3800 ng L^{-1}				
Secobarbital	nd	170 ng L^{-1}			
Triclosan	850 ng L^{-1}	150 ng L^{-1}			
Ketoprofen	1200 ng L^{-1}	240 ng L^{-1}			
Diclofenac	230 ng L^{-1}				
Phenobarbital	110 ng L^{-1}	120 ng L^{-1}			
Phenytoin	410 ng L^{-1}				
Paracetamol			78%,156 ngL^{-1}		[19]/Greece
Phenazone			13%,95 ng L^{-1}		
Salicylic acid			100%,3001ngL^{-1}		
Diclofenac			75%, 457ngL^{-1}		
Ibuprofen			81%,1351ngL^{-1}		
Ketoprofen			34%,91 ng L^{-1}		
Indomethacin			0%, nd		
Mefenemic acid			19%, < MLQ		
Caffeine			100%,3506ngL^{-1}		
Bezafibrate			34%, < MLQ		
Fenofibrate			28%,91 ng L^{-1}		
Gemfibrozil			50%,602 ng L^{-1}		
Sulfamethazine			0%, nd		
Sulfamethoxazole			31%,190 ng L^{-1}		
Ciprofloxacin			13%,115 ng L^{-1}		
Erythromycin			13%,137 ng L^{-1}		
Carbamazepine			34%,406 ng L^{-1}		
Risperidone			22%, < MLQ		
Budesonide			53%, < MLQ		
Triclosan			6%,150 ng L^{-1}		
Atenolol			16%, < MLQ		

Compound	WWTP Influent Concentration µg L^{-1}	WWTP Effluent Concentration µg L^{-1}	surface-Water Concentration µg L^{-1}	Ground Water Concentration µg L^{-1}	Reference /Country
Cimetidine			16%,20 ng/L		
Oestriol			13% , < MLQ		
Bezafibrate			0.015		[58]/Canada
Indomethacin			0.013		
Diclofenac			0.139		
Ketoprofen			0.055		
Gemfibrozil			0.028		
Fenoprofen			0.014		
Naproxen			0.176		
Clofibric acid			0.095		
Ibuprofen			0.102		
Cotinine			0.063		
Caffeine			0.278		
Trimethoprim			0.053		
Pentoxyfilline			0.012		
Cyclophosphamide			< MLQ		
Carbamazepine			0.067		
Acetaminophen				0.32%, 1.89	[20]/USA
Caffeine				0.24%, 0.29	
Carbamazepine				1.46%, 0.42	
Codeine				0.16%, 0.214	
p-xanthine				0.08%, 0.12	
Sulfamethoxazole				0.41%, 0.17	
Trimethoprim				0.08%, 0.018	
Cotinine				nd	
Dehydronifedipine				nd	
Diltiazem				nd	
Diphenhydramine				nd	
Thiabendazole				nd	
Warfarin				nd	
Albuterol				nd	

Notes: nd, not detected; < MLQ, below the method limit of quantitation; x%, detection frequency.

1.2.2 Pharmaceutical and personal care products in Drinking Water

The population increase and finite water supplies will inevitably lead to water shortages. In many countries the possibility of recycling wastewater is now being seriously considered as an attractive solution for solving water shortages [59]. The reported concentrations of PPCPs are typically at trace or ultra-trace levels (low ng L^{-1} to low μg L^{-1}in surface water and groundwater sources that are at the receiving end of wastewater discharges. Some of this water feed into water reservoir that also feed into drinking-water reservoirs.

Information on the occurrence of PPCPs in the water cycle is well documented, although it is seldom that pharmaceuticals or their metabolites in drinking water are systematically detected. Designing programs for monitoring and treatment of these compounds in drinking water present challenges due to the factors affecting their removal [53, 60]. Maria Huerta-Fontela et al (2011) reported detection of fifty-five pharmaceuticals, hormones and their metabolites in raw water [61] (Llobregat river) used for drinking water production in Spain. Thirty-five of the targeted compounds were detected at the intake of the drinking water treatment plant, while five compounds were found in the finished waters at mean concentration levels of; phenytoin (9 ng L^{-1}), atenolol (12 ng L^{-1}), hydrochlorothiazide (7 ng L^{-1}), carbamazepine epoxide (1 to 2 ng L^{-1}) and sotalol (1 to 3 ng L^{-1}). The removal efficiency of these PPCPs from the river for drinking water purpose varied from 96 to 98 %. Drinking water supplies sampled from several countries that include, Canada, France, Greece, Germany, Italy, Spain, the United States, and the United Kingdom revealed the presence of pharmaceuticals compounds, including lipid regulators, antiepilepsy drugs, analgesic and anti-inflammatory drugs, psychiatric drugs, and antibiotics, at concentrations generally less than 50 ng L^{-1}[53].

Table 1.2 lists a non-exhaustive recommended guideline values for PPCPs in drinking water developed by the Australian Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council [62]. This list includes maximum concentrations of PPCPs compiled from a range of Australian and international data sets that have been detected in secondary treated sewage. Some of the pharmaceuticals selected for this study are highlighted in Table 1.2.

Table 1-6 Maximum concentrations of PPCPs detected in secondary treated sewage, and recommended drinking water guideline values [62]

Chemicals/Name	**Maximum concentration**	**Recommended drinking water guideline**
Fragrances		
4-Acetyl-6-t-butyl-1,1-dimethylindan	8 ng L^{-1}	7 µg L^{-1}
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	88 ng L^{-1}	4 µg L^{-1}
Musk ketone	410 ng L^{-1}	350 µg L^{-1}
Musk tibetene	0.04 ng L^{-1}	0.35 µg L^{-1}
Pharmaceuticals and their metabolites		
Antibiotics		
Amoxycillin	0.02 µg L^{-1}	1.5 µg L^{-1}
Chloramphenicol	0.56 µg L^{-1}	175 µg L^{-1}
Ciprofloxacin	0.4 µg L^{-1}	250 µg L^{-1}
Sulfamethoxazole	1.9 µg L^{-1}	35 µg L^{-1}
Trimethoprim	0.35 µg L^{-1}	70 µg L^{-1}
Non-steroidal anti-inflammatories		
Aspirin (Acetylsalicylic acid)	2.1 µg L^{-1}	29 µg L^{-1}
Diclofenac	0.81 µg L^{-1}	1.8 µg L^{-1}
Dipyrone (vet)	7.5 µg L^{-1}	525 µg L^{-1}
Fenoprofen	0.76 µg L^{-1}	450 µg L^{-1}
Ibuprofen	28 µg L^{-1}	400 µg L^{-1}
ß-adrenergic blockers		
Betaxolol	0.19 µg L^{-1}	10 µg L^{-1}
Bisoprolol	0.37 µg L^{-1}	0.63 µg L^{-1}
Carazolol	0.12 µg L^{-1}	0.35 µg L^{-1}
Metoprolol	2.2 µg L^{-1}	25 µg L^{-1}
Propranolol	0.29 µg L^{-1}	40 µg L^{-1}
Estrogenic hormones		
17α-estradiol	74 ng L^{-1}	175 ng L^{-1}
17α-ethinyl estradiol	270 ng L^{-1}	1.5 ng L^{-1}
17β-estradiol	93 ng L^{-1}	175 ng L^{-1}
Estriol	51 ng L^{-1}	50 ng L^{-1}
Progesterone	200 ng L^{-1}	105 µg L^{-1}

Chemicals/Name	Maximum concentration	Recommended drinking water guideline
Androgens		
Androsterone	0.21 µg L^{-1}	14 µg L^{-1}
Testosterone	0.21 µg L^{-1}	7 µg L^{-1}
General pharmaceuticals		
Alprazolam	0.62 µg L^{-1}	0.25 µg L^{-1}
Antipyrine (phenazone)	0.41 µg L^{-1}	1,000 µg L^{-1}
Atorvastatin	0.04 µg L^{-1}	5 µg L^{-1}
Bezafibrate	4.6 µg L^{-1}	300 µg L^{-1}
Caffeine	44 µg L^{-1}	0.35 µg L^{-1}
Carbamazepine	27 µg L^{-1}	100 µg L^{-1}
Cimetidine	0.58 µg L^{-1}	200 µg L^{-1}
Clenbuterol	0.05 µg L^{-1}	15 µg L^{-1}
Clofibric acid	1.6 µg L^{-1}	750 µg L^{-1}
Codeine	9.1 µg L^{-1}	50 µg L^{-1}
Enalaprilat	0.046 µg L^{-1}	1.3 µg L^{-1}
Fluoxetine (Prozac)	0.142 µg L^{-1}	10 µg L^{-1}
Gemfibrozil	1.5 µg L^{-1}	600 µg L^{-1}
Isophosphamide	2.9 µg L^{-1}	3.5 µg L^{-1}
Metformin (1,1-Dimethylbiguanide)	0.15 µg L^{-1}	250 µg L^{-1}
Paracetamol (acetaminophen)	4.3 µg L^{-1}	175 µg L^{-1}
Salbutamol	0.035 µg L^{-1}	3 µg L^{-1}
Salicylic acid	60 µg L^{-1}	105 µg L^{-1}
Valium (Diazepam)	2.92 µg L^{-1}	2.5 µg L^{-1}
Miscellaneous organic chemicals		
Bisphenol A	12 µg L^{-1}	200 µg L^{-1}
Cholesterol	10 µg L^{-1}	7 µg L^{-1}
Personal care product		
Triclosan	0.4 µg L^{-1}	0.35 µg L^{-1}

1.3 The removal of pharmaceutical and personal care products from wastewater and drinking water treatment

PPCPs will be excreted (and for those applied externally washed off from the body), as a mixture of metabolites or as non-metabolized substances. When they enter a wastewater treatment plant, depending on their physicochemical properties mainly their hydrophobicity (or hydrophilicity), PPCPs may partially settle out of the aqueous phase in the sludge. Alternatively, they are converted to a more hydrophilic form which results into their discharge in the receiving waters

through wastewater effluent [63]. This section therefore focuses on the different existing processes for water treatment and on how efficient these are in removing organic pollutants from wastewater and water.

1.3.1 Conventional activated sludge and membrane bioreactor processes

1.3.1.1 Conventional activated sludge

Conventional activated sludge (CAS) is the most commonly used wastewater treatment process, during which bacteria and other micro-organisms (i.e. activated sludge) are grown in aeration tanks containing wastewater in order to sanitize water. In the presence of dissolved oxygen, the development of this biomass usually results in the removal or decrease of the concentrations of dissolved organic matter, inorganic pollutants and pathogens in sewage water [64]. The removal of as much as 90 % of the suspended and dissolved organic material in influent wastewater has been reported for a well-operated treatment plant.

1.3.1.2 Membrane bioreactor (MBR) processes

The secondary clarifier in the CAS process can be replaced by membrane filtration, in order to obtain a treatment process called Membrane Bioreactors (MBR). In MBR, water from biological treatment is allowed to pass through thin synthetic plastics membrane with pore size below 0.1 μm, resulting in production of a higher quality effluent with a concentration of suspended solids virtually being zero. These membranes can be design to be either inside or outside the aeration basin. The removal of PPCPs in MBR occurred mainly through biodegradation or biotransformation. Other mechanisms of removal may include size exclusion, charge repulsion and adsorption which is controlled by the membranes charge, hydrophobicity and roughness, on

the one hand; but also by the micro pollutant size, charge and hydrophobicity, on the other hand; and also by the water properties such as temperature, ionic strength, etc. [64].

1.3.1.3. Removal efficiency of CAS vs. MBR treatment

Jelena Radjenovic et al (2009) assessed the performance of a full scale CAS treatment and two pilot-scale MBRs in eliminating pharmaceuticals belonging to different therapeutic groups. One of the MBRs was equipped with hollow-fibre (HF) ultra-filtration membranes (pore size 0.05 μm) while the other was operated with micro-filtration flat-sheet (FS) membrane with nominal porosity of 0.4 μm. Table 1.3 shows the removal efficiency of pharmaceuticals in the aqueous phase during CAS and both FS and HF MBR treatments which was calculated as the mean values with their relative standard deviation [65].

Table 1-7 Mean removals (n=9) of selected pharmaceuticals from the aqueous phase in wastewater treatment plant Terrassa [65]

Compound	**Elimination from the aqueous phase (%)**		
	CAS	**FS MBR**	**HF MBR**
Analgesics and anti-inflammatory drugs			
Ibuprofen	99.1 ± 1.8	99.2 ± 1.8	99.5 ± 1.6
Naproxen	71.8 ± 14.3	90.2 ± 3.2	91.6 ± 8.1
Ketoprofen	54.6 ± 19.7	43.9 ± 27.7	44.0 ± 20.6
Diclofenac	21.8 ± 28.5	65.8 ± 13.1	62.6 ± 18.3
Mefenamic acid	n.e.	40.5 ± 23.7	35.5 ± 28.3
Propyphenazone	37.6 ± 10.8	64.5 ± 16.0	60.7 ± 18.7
Acetaminophen	99.9 ± 0.1	99.8 ± 0.2	99.9 ± 0.1
Indomethacin	n.e.	41.4 ± 20.6	39.7 ± 26.2
Anti-histamines			
Ranitidine	24.7 ± 44.9	44.2 ± 29.6	29.5 ± 47.9
Loratidine	15.0 ± 43.9	n.e.	33.5 ± 52.2
Famotidine	60.1 ± 22.3	64.6 ± 24.5	47.4 ± 63.0
Anti-epileptic drug			
Carbamazepine	n.e.	n.e.	n.e.
Psychiatric drugs			
Fluoxetine	33.1 ± 28.9	98.0 ± 1.9	98.0 ± 1.6
Antibiotics			
Erythromycin	35.4 ± 50.5	43.0 ± 51.5	25.2 ± 108.9
Sulfamethoxazole	73.8 ± 12.7	80.8 ± 12.2	78.3 ± 13.9
Ofloxacin	75.8 ± 13.8	95.2 ± 2.8	91.3 ± 10.8
Trimethoprim	40.4 ± 25.4	66.7 ± 20.6	47.5 ± 22.5
β-blockers			
Atenolol	61.2 ± 18.6	76.7 ± 12.6	69.5 ± 12.5
Sotalol	21.4 ± 31.5	53.1 ± 24.1	30.4 ± 25.3
Metroprolol	24.7 ± 44.9	44.2 ± 29.6	29.5 ± 47.9
Propanolol	58.8 ± 24.5	77.6 ± 12.2	65.5 ± 22.4
Hypoglycaemic agents			
Glibenclamide	46.1 ± 40.8	95.6 ± 4.4	82.2 ± 28.6
Lipid regulator and cholesterol lowering statin drugs			
Gemfibrozil	n.e.	42.2 ± 36.7	32.5 ± 49.3
Bezafibrate	80.8 ± 20.9	90.3 ± 10.1	88.2 ± 15.3
Pravastatin	59.4 ± 16.2	86.1 ± 9.1	83.1 ± 12.5
Diuretics			
Hydrochlorothiazide	n.e.	n.e.	n.e.

n.e. : no elimination, defined for the mean elimination efficiency less than 10 %
where; **CAS =** Conventional activated sludge
FS MBR = flat-sheet membrane Bioreactor
HF MBR = hollow-fibre membrane Bioreactor

Radjenovic and co-worker found that the MBR generally perform better than CAS treatment in removing the targeted analytes from the wastewater even for the most recalcitrant. Subsequently,

Jan Sipma et al (2010) compared the removal efficiency for 30 pharmaceuticals in the MBRs and in CAS treatment systems. They observed that easily removed pharmaceuticals such as acetaminophen, ibuprofen and paroxetine were efficiently removed in both systems. In addition, moderately removed pharmaceuticals in CAS generally showed improved removal efficiency in MBR, although the removal was incomplete in most cases. However, the removal efficiency for pharmaceuticals such as sotalol and hydrochlorothiazide were worse in MBR compared to the values obtained with CAS. Other pharmaceuticals like Carbamazepine and some macrolides showed negative removal efficiency which was explained by the release of the parent compounds during the treatment processes. This study concluded that the efficiency for the removal of PPCPs with MBR technology was not that pronounced compared to CAS treatment. Yet, even though MBR did not show 100 percent removal efficiency for all micro constituents in wastewater the resulting effluent had higher quality compared to those obtained from CAS treatment. Hence, MBR treatment once again provided better water quality for subsequent water treatment processes [66].

1.3.2 Advance treatment processes

1.3.2.1 Reverse osmosis

Reverse osmosis (RO) is a membrane treatment process in which contaminants are removed from water by forcing it, under a pressure greater than the osmotic pressure, through a membrane [64].

1.3.2.2 Activated Carbon Adsorption

Highly porous charcoal (activated carbon) having a large surface area is used to remove PPCPs compounds from water through adsorption. The dissolved compounds are transported via diffusion into the porous surface of the adsorbent. Compounds are attached to the solid surface through either chemisorption (irreversible specific chemical bond formation process) or physical attraction (reversible nonspecific binding mechanisms). The compounds are not degraded in this mechanism, but simply transferred to the activated carbon surface [64].

1.3.2.3 Oxidation and advanced oxidation processes

Organic micro pollutants in water and wastewater can be chemically oxidize into carbon dioxide, water and minerals acids, through various oxidation processes instead of only being separated from it. Generally, oxidants such as chlorine gas, hypochlorous acid, hypochlorite, chlorine dioxide, ozone, hydrogen peroxide and permanganate are used in conventional chemical oxidation. The selection of which oxidant to use is usually dependent of the type of compounds targeted for degradation. A combination of UV radiation and a chemical oxidant, or some combinations of oxidants may result in the increase of the oxidation rate, resulting in some processes classified as advanced oxidation processes. The most commonly use advanced oxidation process include, UV/ozone, UV/hydrogen peroxide, UV/titanium oxide, and hydrogen peroxide/iron salt. Oxidation may occur, either, through direct chemical oxidation of bonds, or through generation of reactive free radicals in the targeted free molecules [64].

Xing Yang and coworkers investigated the occurrence and removal of pharmaceutically active compounds and personal care products at various stages of treatment using an advanced wastewater treatment plant in the USA [67]. They showed that caffeine, acetaminophen, ibuprofen, diethyltoluamide, tetracycline and 17α-ethynylestradiol had removal efficiency at a

level over 90 % under activated sludge treatment and membrane filtration. Erythromycin and carbamazepine which were recalcitrant to biological treatment, were eliminated on average by 74 to 88 % via granular activated carbon, while primidone, diethyltoluamide and caffeine remaining unaffected by the process. The remaining compounds, except for primidone and diethyltoluamide, were oxidized by ozonation with a removal efficiency of over 60 % [67].

1.4 Objectives

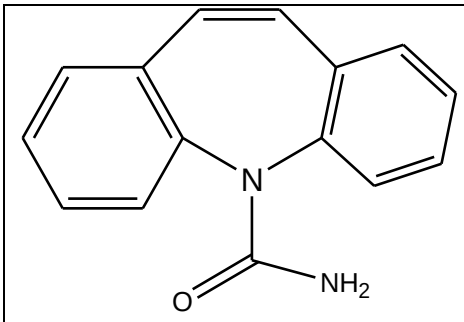
For the purpose of this study, thirteen pharmaceuticals were selected primarily on the basis of their prescription volumes in South Africa and their known impact on aquatic organisms. The selected pharmaceuticals, as shown in Table 1.4 below were divided into 6 classes [68]:

1. **Anticonvulsant and antiepileptic drugs (AEDs)**: the drugs carbamazepine and primidone are in this group and are used for sedation and for treatment of people suffering from epileptic seizure. AEDs act by reducing the central nervous system excessive activity during seizures, and also prevent seizure within the brain to spread.
2. **Antiviral**: Famciclovir is one of the most widely use antiviral drug for prophylaxis and treatment of herpes virus infections, including varicella infections.
3. **Natural androgen**: Natural androgens such as testosterone are used for treatment of hypogonadism, delayed puberty, and breast cancer in females. It promotes the growth and development of the male sex organs as well as maintaining secondary sex characteristics.
4. **Natural oestrogen**: Oestrogens are naturally occurring hormones secreted primarily by the ovarian follicles and also by the adrenals, corpus luteum, placenta, and testes. They are used pharmaceutically for female birth control, to treat prostate cancer, and to treat symptoms of menopause.

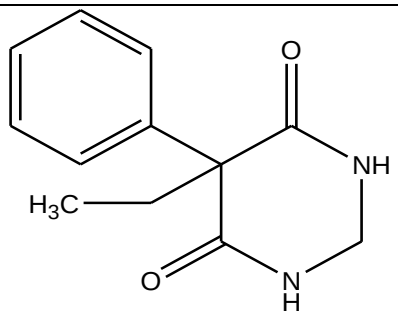
5. **Nonsteroidal anti-inflammatory drugs (NSAID)**: Acetaminophen, diclofenac, fenoprofen, ibuprofen are in this class of nonsteroidal anti-inflammatory drugs (NSAIDs). Their effects include pain-killing (analgesic), fever-reducing (antipyretic) and anti-inflammation.

~~6.~~ **Xenoestrogen**: Bisphenol A is a monomer used in the manufacture of polycarbonates plastic and epoxy resins, which is mainly used as a lining in food and beverage cans, as a dental sealant, baby-milk bottles, powder paints, optical lenses and as an additive in several consumer and personal-care products. It has been demonstrated that bisphenol A have both estrogenic and anti-androgenic activity [69, 70].

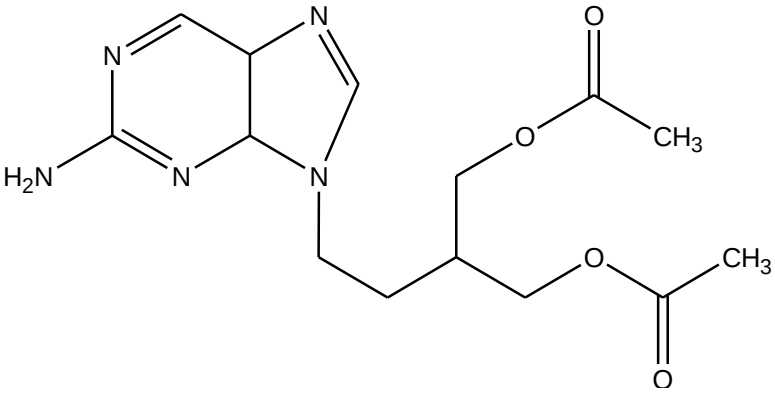
Table 1-4 Names and structures of the selected pharmaceuticals



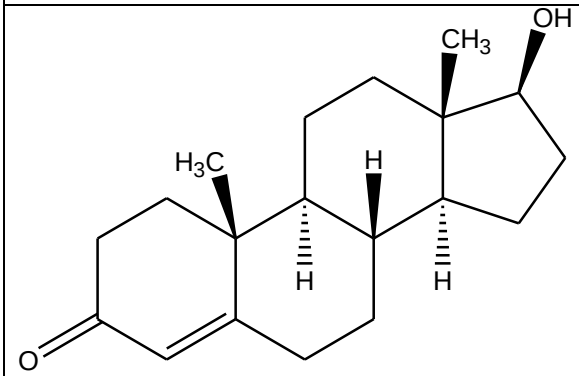
Carbamazepine (Anti-seizure)



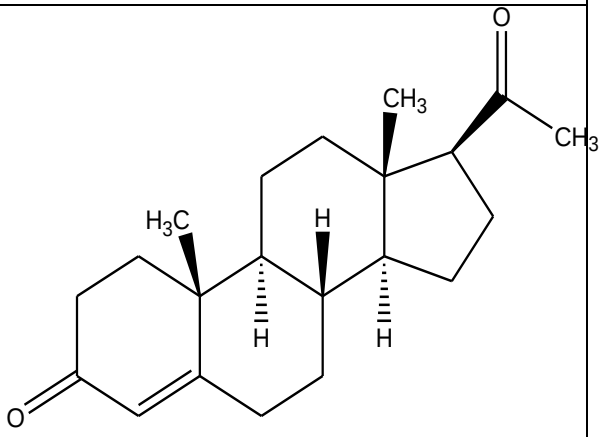
Primidone (AEDs)



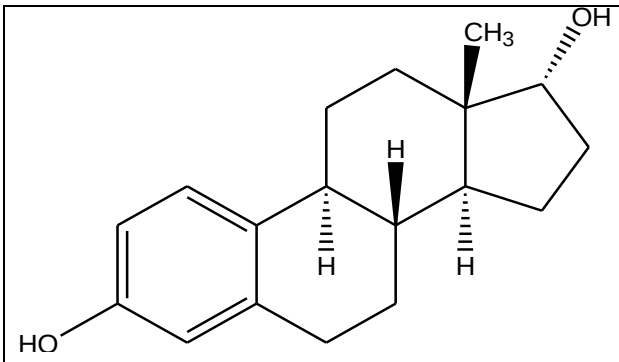
Famciclovir (Antiviral)



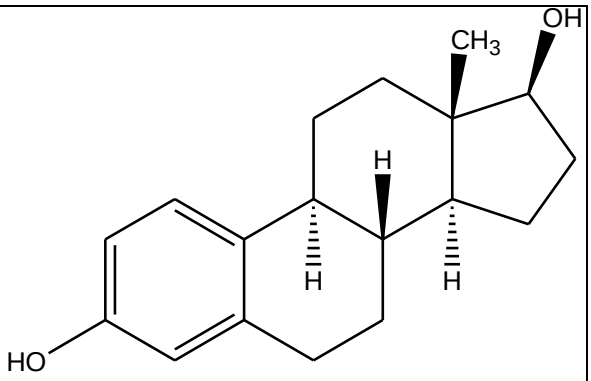
Testosterone (Natural androgen)



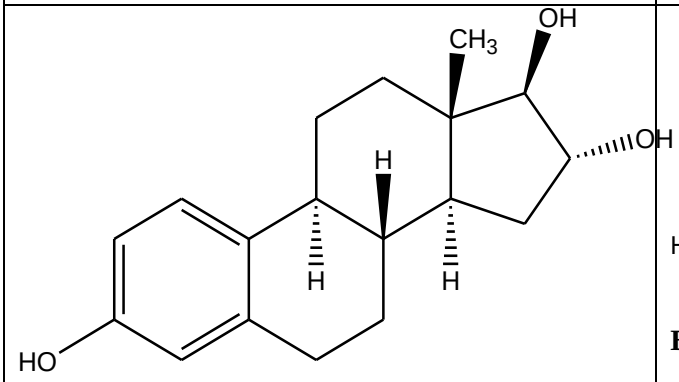
Progesterone (Natural oestrogen)



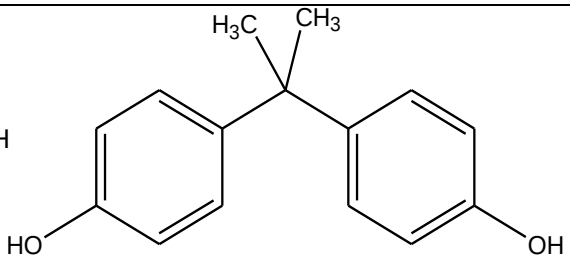
17α-Estradiol (Natural oestrogen)



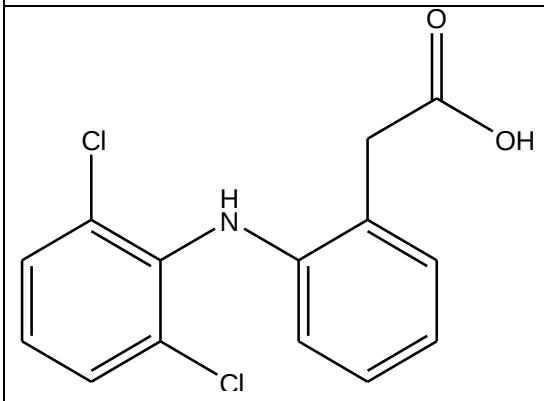
17β-Estradiol (Natural oestrogen)



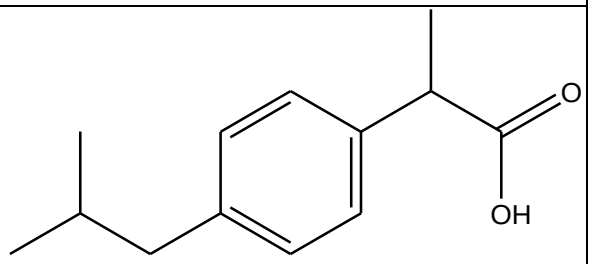
Estriol (Natural oestrogen)



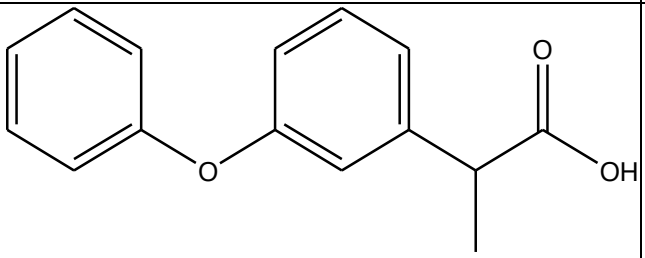
Bisphenol A (Xenoestrogen)



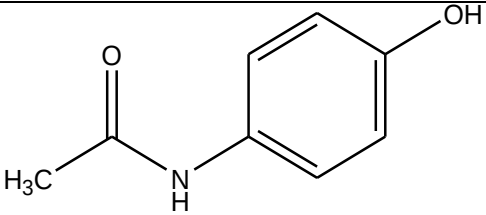
Diclofenac (Anti-arthritic)



Ibuprofen (Analgesic)



Fenoprofen (NSAID)



Acetaminophen (Analgesic)

In order to determine the occurrence of the target compounds in wastewater, a trace analytical method with high precision and proficiency was required for simultaneously determining the selected PPCPs. Although several papers describe multi-residue analytical methods for determining micro-pollutants in water with GC/MS none of them were suitable for the selected compounds. The published methods were either focusing on a group of specific compounds with similar properties or they utilized complex sample preparation schemes often including sequential elution and separate derivatization of different groups of compounds. Therefore, the aim of this study was to develop and validate a fairly simple multi-residue GC-MS method for the quantification of targeted PPCPs in wastewater samples from a selected wastewater treatment plants in Gauteng.

Specific objectives

- Optimize pH for extraction of targeted PPCPs using off-line solid phase extraction (SPE)
- Optimize derivatization conditions for the targeted PPCPs
- Validation of the developed gas-chromatography mass spectrometry method
- Quantification of the PPCPs using the developed GC-MS method

A more detailed discussion of the objectives will be presented in Chapter 2, following a comprehensive literature review on relevant information regarding PPCPs in the environment.

CHAPTER 2 LITERATURE REVIEW

2.1 Sample preparation methods for analysis of pharmaceutical and personal care products in waters

Extensive extraction and clean-up procedures, coupled with specific and sensitive analytical procedures are required for PPCPs analysis. PPCPs typically occur at very low concentrations, and the complexity of the matrix involved which contains a variety of micro-pollutants and suspended solids makes the task challenging. A typical scheme for the analytical process when determining PPCPs in water is presented in Figure 2.1. Water samples are filtered to remove the particulate and/or the pH of the sample is adjusted, in order to maximise analyte retention on the SPE sorbent, prior to the extraction and enrichment of the compounds. Following the enrichment process, if necessary a clean-up step may be done, and depending on the instrumental technique, the extract may undergo certain treatment prior to analysis.

Analyte recovery/enrichment is calculated according to the following equation:

% Recovery = (Area **A** / Area **B**) × 100 %

Where **A** is the peak area of an extracted sample and **B** is the peak area of an extracted matrix in which the compounds are added post extraction.

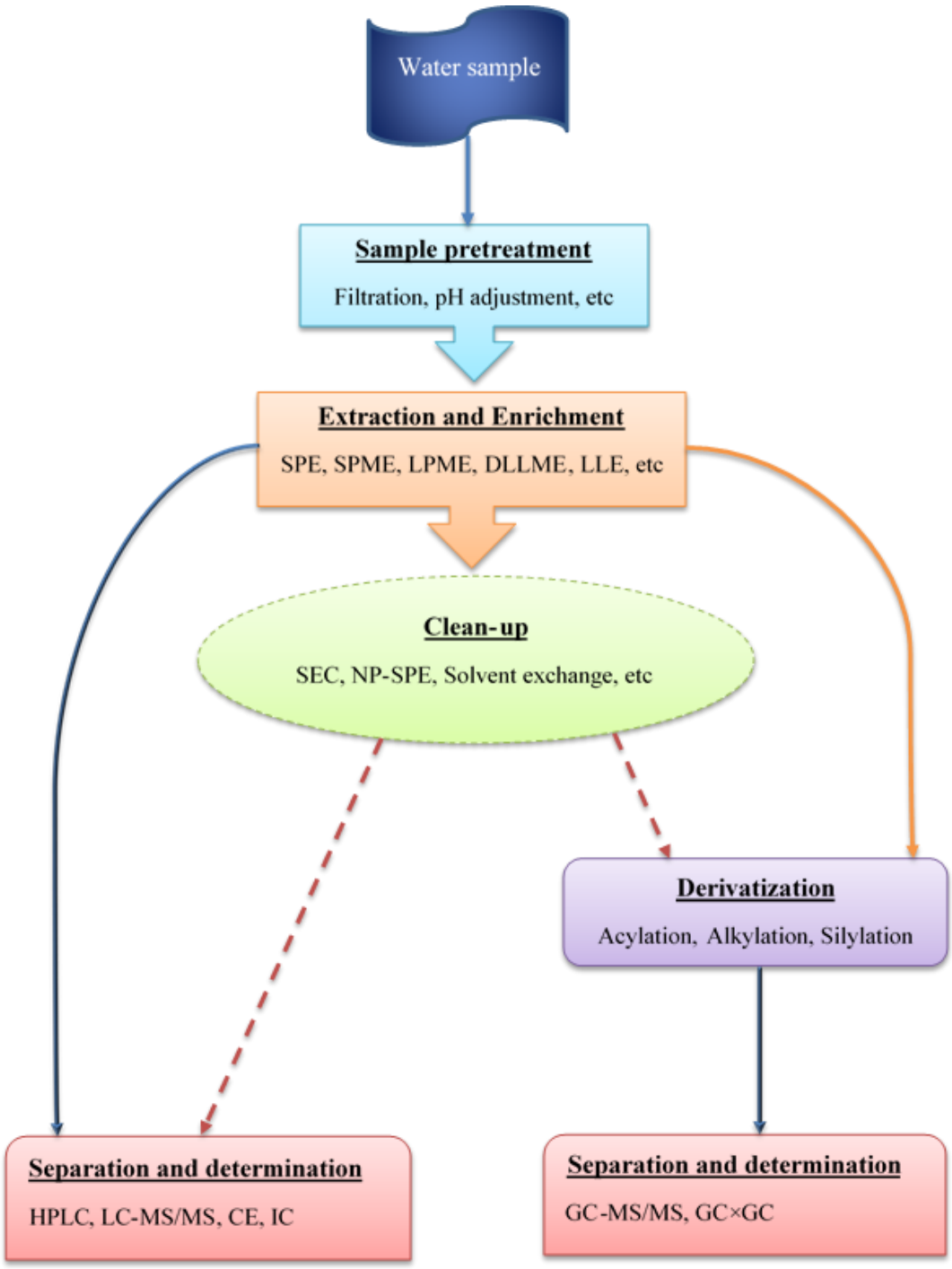


Figure 0-1 Scheme of the general analytical procedure for analysis of PPCPs in aqueous samples [42].

2.1.1 Sampling

Sampling is the most critical step of any environmental analysis as incorrect sampling procedures would nullify all the subsequent data generated. The selection of an appropriate sampling procedure is important to obtain representative results about the occurrence of the compounds of interest in the environment.

Sampling protocols should take into consideration sampling location and time of sample collection. Great care must be taken to ensure that the sampling procedure and transport of the sample do not affect the matrix. For instance, wastewater sample must not be exposed to light, oxygen, and high temperatures, in order to prevent possible transformation of the analytes or other organic components present in the samples. Usually, wastewater samples are collected in amber glass containers that have been properly cleaned. The collected samples must be promptly refrigerated at a temperature below 4°C. Preservative may be added to the samples to prevent bacterial growth during storage. Alternatively, the samples may be extracted and then stored until analysis [71, 72]. The U.S. Environmental Protection Agency Office of Water, 2010, recommended the following holding times and preservation conditions for sewage influent and effluent water [73]:

- The use of amber high density polyethylene or glass containers bottles, ascorbic acid as a dechlorinating reagent.
- That shipping and storage should be carried in the dark at temperature below 6 °C (optional freezing for biosolids).
- Samples extraction must be done within seven days and the analysis of extracts as soon as possible and should not exceed 30 days (10 days in the case of tetracyclines).

The traditional sampling procedure involves periodically grabbing a water sample. Unfortunately, the resulting samples when employing this approach only represents the concentration of chemicals at the instant of sampling, or over the sampling period. Such traditional approach of sampling is not convenient for continuous PPCP monitoring over extended periods of time [39]. An alternative approach entails the use of passive sampling devices such as semi permeable membrane device (SPMD), and polar organic chemical integrative sampler (POCIS) which can be deployed over extended time periods (days or weeks).

2.1.2 Sample Pre-treatment

The analysis of trace pollutants in environmental matrices require that the sample undergo pretreatment procedures which provide a clean-up and/or pre-concentration of sample with all the target analytes [74]. Filtration is the first step of wastewater sample treatment. It serves to remove the suspended solid substances present in the sample. Adjustment of the samples pH may be made to improve the extractability of the analyte of interest. The extraction is performed to enrich the analytes present at very low levels. The extraction methods are chosen based on their selectivity dependent on the compound/s of interest. Numerous extraction techniques for aqueous samples are available in the practice of modern analytical chemistry such as solid phase extraction (SPE) which is the most commonly used method and liquid phase micro extraction (LPME) [37].

2.1.3 Solid Phase Extraction (SPE)

SPE is one of the most widely used sample preparation techniques for the isolation of contaminants at low concentrations in aqueous samples. It overcomes many of the disadvantages associated with classical liquid-liquid extraction, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents [75]. Both the varied choice of sorbent materials and the possibility of automation of SPE enhance its activeness in a broad range of applications such as environmental analyses, pharmaceutical and biochemical analyses, organic chemistry and food analyses [75, 76]. Table 2.6 summarizes the most recent applications in PPCPs analysis.

The nature of the functional groups on SPE sorbents material enable their classification into four different classes; adsorption, partition, ion exchange and gel permeation/size exclusion. Adsorption takes advantage of polar functional groups; such as cyano, amino and diol, which are usually used to retain polar compounds from non-polar matrices. In addition, modified phases, for non-polar functional groups (reverse phase), such as octadecyl, octyl and methyl; and generally non-polar to moderately polar compounds in a polar matrix are likely to be retained. Finally, **ion-exchange** sorbents, carry either negatively or positively charged functional groups and when in the ionized form attract compounds of the opposite charge.

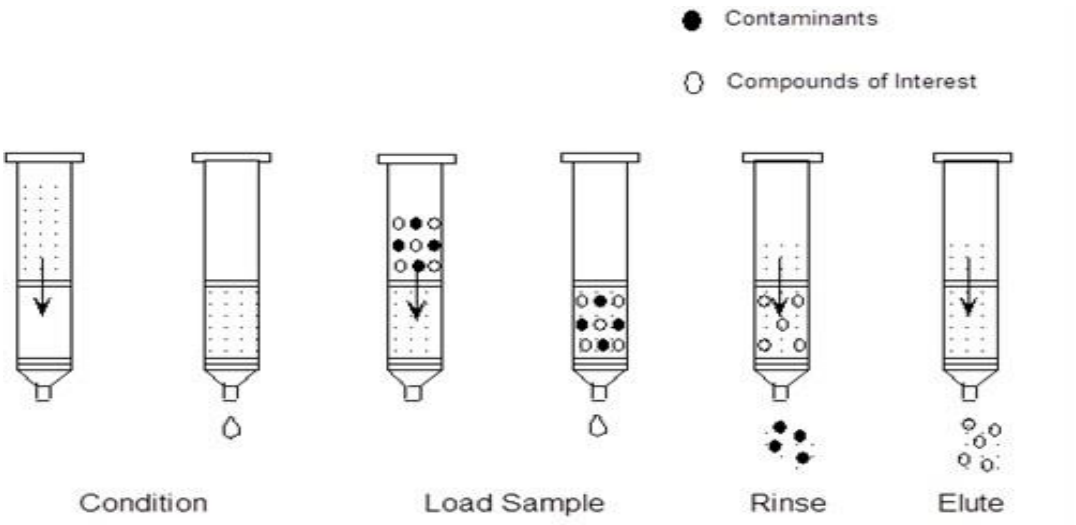


Figure 0-2 Schematic of solid phase extraction procedure [77].

Figure 2.2 shows a typical SPE procedure. The first step involves conditioning and or equilibrating the cartridges with the appropriate solvents then loading the water sample. Initial conditions are carefully selected to allow for the complete adsorption of the analytes. Interfering material can be washed off from the sorbent by passing an appropriate solvent. Lastly a suitable solvent the pre-absorbed and desired analytes can then be eluted from the sorbent material. This eluate is then collected for analysis, and if needed it can be further clean-up and/or pre-concentrated. The eluent is sometime evaporated to further concentrate the analytes, or to allow the reconstitution of the analytes in a solvent that is more compatible with the subsequent chromatographic technique [72, 74, 78].

In a 2006 study, Verenitch and co-workers emphasized the importance of both sample preparation and instrumental techniques for analysis of various acidic drugs and caffeine in surface water and municipal wastewater, including acetylsalicylic acid, ibuprofen, gemfibrozil, fenoprofen,

naproxen, ketoprofen, diclofenac, and caffeine [79]. Water samples were filtered and the target analytes were extracted by solid-phase extraction (SPE). Two types SPE cartridges mainly Supelco LC-18 and Waters Oasis HLB were used to pre-concentrate samples for acidic drugs and caffeine, respectively. A methylation process was applied to acidic drugs prior to analysis while caffeine was analyzed directly. Gas chromatography–ion trap tandem mass spectrometry for the analysis of the target acidic pharmaceuticals and caffeine were developed. Parameters such as collision-induced dissociation (CID) voltage, isolation time, excitation time, excitation storage level, and electron energy were adjusted in order to optimize the instrument analytical performance. After optimization, an instrument detection limit of 0.5–20 pg L^{-1} with signal-to-noise (S/N) not less than 5 was achieved for all target analytes. The method showed a good linearity within the range of 10–2000 pg L^{-1}. The application of the optimized GC–MS/MS parameters in conjunction with sample preparation procedure resulted in method detection limits (MDLs) of 0.1–1.0 and 20 ng L^{-1} for the determination of acidic drugs and caffeine, respectively, in such samples as surface water, effluent from municipal wastewater plants, as well as receiving waters.

Table 0-1 Analytical methods of PPCPs in water using solid-phase extraction (SPE)

Matrix	Analytes	Extraction and preparation	% recovery	Analytical Method	MDL (ng L^{-1})	Reference
WWTP influent and effluent	18 neutral PhACs 11 Acidic PhACs 2 Antibiotics 3 Estrogens	for the acidic PhACs. • Acidified to pH 2-3 • SPE (OASIS HLB); eluent MeOH for the neutral PhACs. • pH adjusted to 7–7.5 SPE (RP-C_{18ec}); eluent MeOH		LC-MS		[80]
sewage influent and effluent, surface water and ground water	4 beta blockers 1 antiepileptic drug 3 fluoroquinolone	• pH adjusted to 10 • SPE (OASIS HLB); eluent MeOH		LC-$ESIMS^2$		[81]
wastewater influents and effluents	Ibuprofen Ketoprofen Naproxen Diclofenac Indomethacin Acetaminophen Mefenamic acid Propyphenazone Clofibric acid Gemfibrozil Bezafibrate Pravastatin Mevastatin Carbamazepine Fluoxetine Paroxetine Lansoprazole Famotidine Ranitidine Loratidine Erythromycin	• pH adjusted to 7 • SPE (OASIS HLB); eluent MeOH	68.8-131 59.1-71.3 49.2-59.4 83.3-95 110-120 56-123 91.5-93.3 60-71 74.5-104 87.5-108 89.4-106 78-96 103-134 84-89.5 46.7-93.7 62.2-109 70-87 55.4-66.6 41.5-125 64.5-78 50-67.7	LC-$ESIMS^2$	20-98 74-190 20-79 40-160 31-150 5.35-20.9 1.85-5.70 1.45-4.80 3.75-16.3 2.20-8.70 4.35-18.5 30.9-120 1.30-9.30	[82]

Matrix	Analytes	Extraction and preparation	% recovery	Analytical Method	MDL (ng L^{-1})	Reference
	Azithromycin Sulfamethoxazole Trimethoprim Ofloxacin Atenolol Sotalol Metoprolol Propranolol Hydrochlorothiazide Glibenclamide		30-73 33.7-95.5 58.8-128 135-142 60.8-131 31.9-52 36.7-120 60.2-90.8 39.8-73.4 98.5-107		0.60-2.20 1.70-19.8 0.65-3.50 4.20-10.9 0.4-3.10 0.3-1.40 2.75-8 2-12.4 0.3-1 3.1-16.1 0.35-1.3 7.85-29.3 0.75-1.70 0.7-4.80 1.6-6.30 0.3-2.60 0.9-4.50 2.3-19.2	
hospital effluent wastewaters	1 anti-epileptic 7 analgesic/anti-inflammatory drugs 1 analgesic opiate 2 antidepressants 2 β-blockers 3 antibiotic 1 anti-ulcer	• pH adjusted to 7 • SPE (OASIS HLB); eluent MeOH		LC-$ESIMS^2$		[83]
Drinking water	2 β-blockers 3 Analgesic/Anti-inflammatory 1 Antibiotics 1 Lipid regulator 1 Psychiatric drug	• SPE (OASIS HLB); eluent MeOH		LC-$ESIMS^2$		[43]
WWTP effluents, surface water	Triclosan Chlorophene Dichlorophene Oxybenzone 1-Naphthol 2-Naphthol Atrazine Carbamazepine	• Acidified to pH 2 • SPE (Bond Elut Plexa);	79-127	LC-MS^2	1-46 3-25 9-41 20-127 15-86 12-50 10-125 3-66	[84]

Matrix	Analytes	Extraction and preparation	% recovery	Analytical Method	MDL (ng L^{-1})	Reference
	Ibuprofen Naproxen Venlafaxine	eluent MeOH			2-74 3-85 1-302	
surface water	8 analgesics/anti-inflammatories 3 lipid regulators 4 antibiotics 1 antiepileptics 1 antipsychotics 1 psychomotor stimulants 1 glucocorticoid steroids 1 disinfectants 1 beta-blockers 1 H2 receptor antagonists 1 oestrogens	• SPE (SDB-RPS disk); eluent MeOH		LC-UV/VIS-ESI-MS		[19]

Where PhACs = pharmaceutical active compounds;

2.1.4 Solid phase micro extraction (SPME)

Alternatives to SPE in the extraction of environmental contaminants in water samples include micro extraction techniques, such as solid phase micro extraction (SPME) for environmental water analysis. The main advantage of this technique over SPE is that it minimizes the amount of extraction solvent and sample used [37]. It is a solvent free, one step extraction technique that can be applied for isolating organic molecules from gaseous, liquid and solid samples. In SPME, the analytes are first concentrated onto a sorbent, coated on a short piece of a fused silica fiber that is either exposed directly to the sample or to its headspace until equilibrium is reached between the coating and the sample. The analytes can be desorbed either thermally by immersing the fiber into a GC injector or by an organic solvent if they are to be analysed by LC or CE [37, 85].

Organic contaminant such as fragrances, UV-filters, antiseptics, oestrogens, anti-inflammatory drugs, and antioxidant and plastics component can be extracted from effluents and influents sewage water from industrial and domestic origin using on fiber SPME derivatization. The extraction recovery parameters can be optimized by systematically investigating the influence of factors such as extraction temperature and time, derivatization time, desorption temperature and time. Limits of quantification (LOQs) ranging from 1.7 to 29.7 ng L^{-1} can be achieved with acceptable precision [86]. Table 2.2 summarized various analytical methods for the determination of PPCPs in water sample using SPME as the extraction technique.

Table 0-2 Analytical methods of PPCPs in water using solid-phase micro-extraction (SPME)

Matrix	**Analytes**	**Extraction and preparation**	**% recovery**	**Analytical method**	**LOD** (ng L^{-1})	**Reference**
WWTP influent and effluent	7 Musks	SPME (PDMS/DVB)		GC-MS		[80]
WWTP Influent and final effluent, non-treated domestic and industrial sewage, river water	Ibuprofen Fenoprofen Naproxen Ketoprofen Flurbiprofen Fluoxetine Estrone 17-β Estradiol Simvastatine	SPME (PDMS/DVB PDMS, PDMS/CAR, PA)		GC-MS	1050 1540 1340 1600 1570 1630 1600 952 1190	[87]
effluent of an urban treatment plant, industrial and domestic influents	Fragrances UV-filters Antiseptics Estrogens Anti-inflammatory drugs Antioxidant Plastics component	HS-SPME (PA)		GC/MS		[86]
		Where: PDMS/DVB, = polydimethylsiloxane/divinylbenzene; **PDMS =** polydimethylsiloxane; **PDMS/CAR =** polydimethylsiloxane/carboxene; **PA =** polyacrylate;				

2.1.5 Liquid phase micro extraction (LPME)

LPME is carried out using a membrane as an interface between the sample (donor) and the organic solvent (acceptor). Analytes are extracted by partition between the acceptor and the donor phase. The phases is either in direct contact, or a porous membrane may use as an interface. The main advantages of LPME are:

1. very low consumption of organic solvent;
2. low cost;
3. high selectivity; and
4. clean extracts;

With this technique extraction may be carried out as a two-phase or three-phase system. In a two-phase system, the organic solvent immiscible in water fills the hollow fiber and the pores of the membrane, acting both as interface and acceptor phases. In the three-phase system, on the other hand, the membrane pores filled with the organic solvent act as the interface, while the acceptor solution is aqueous like the sample. In addition, the pH of the donor and acceptor phase should be adjusted in such a way that the analyte solubility is reduced in the donor phase and enhanced in the acceptor phase [37]. As described in Table 2.3, several classes of different PPCPs can be extracted by LPME. Sergiane Souza Caldas et al. evaluated the applicability of solvent-based de-emulsification dispersive liquid-liquid micro-extraction (SD-LLME) for the extraction of 58 pharmaceuticals and personal care products and pesticides (e.g., triclosan, ibuprofen, bisphenol A, carbamazepine, and diltiazem hydrochloride) from water samples prior to the determination by LC-MS/MS. The recoveries range from 60 % to 120 % for 84 % of the compounds [88].

Table 0-3 Analytical methods of PPCPs in water using liquid-phase micro-extraction (LPME)

Matrix	**Analytes**	**Extraction and preparation**	**% recovery**	**Analytical method**	**LOD (ng L^{-1})**	**Reference**
Treated wastewater, raw wastewater, river water	Triclosan methyltriclosan	DLLME ternary mixture: methanol 1,1,1trichloroethane, MTBSTFA	93.4-103.3 81.7-105.4	GC–MS/MS	2 5	[89]
WWTP influent and effluent, river water	Pharmaceuticals Antimicrobials Preservative UV filters insect repellent	SPE(Oasis HLB)–DLLME (ternary mixture acetonitrile/methanol/dichloromethane)		UHPLC–MS/MS		[90]
surface water	Salicylic acid Albendazole Amitriptyline Avobenzone Bisphenol A Carbamazepine Clarithromycin Diltiazem hydrochloride, Flurazepam hydrochloride, Chlorpropamide, Sodium diclofenac, Eusolex 6300, Furosemide, Gemfibrozil, Glibenclamide, Haloperidol, Ibuprofen, Mebendazole, Methylparaben, Nimesulide, Miconazole nitrate, Propranolol, Propylparaben, Sulfamethoxazole Triclocarban, Triclosan	SD-DLLME ternary mixture: water, octanol and acetone		LC–MS/ MS		[88]

2.2 Instrumental methods for analysis of of pharmaceutical and personal care products in waters

The use of multi-residue methods has become widespread in response to the need to monitor a wide range of pharmaceuticals belonging to diverse drug classes in wastewater, surface water, and groundwater. This approach has the advantages to provide a broad picture of the occurrence and fate of contaminants in the environment. The simultaneous determination of a large number of analytes by a single method also represents a more economical and less time-consuming approach. Various multi-residue methods can be found in the literature which can target analytes usually on the basis of the consumption patterns ascribed to the country where the study is being conducted, the rate of metabolism of drugs, the environmental occurrence, and persistence in the environment [7]. Chromatographic techniques have the ability to separate a mixture of compounds and determine their respective identities in complex samples. The separation and detection of most PPCPs are usually achieved by GC or LC coupled with several types of detectors, such as electron capture detector (ECD), MS detector and photodiode array detector, etc [91]. The coupling of chromatographic separation with mass spectrometry results in detection of PPCPs at very low levels (ng L^{-1}-µg L^{-1}). Table 2.4 and 2.5 show typical examples of the levels of PPCPs detected.

2.2.1 Gas Chromatography-Mass spectrometry (GC-MS)

In gas chromatography, the sample (usually liquid) to be analyzed is injected into an injection port where it is flash vaporized then swept into a chromatographic column. The column is held in a temperature-controlled oven and the solute being separated are transported by an inert gas stream flow, the carrier gas which usually is either nitrogen, helium, or hydrogen. Separation occurs because sample components have differing affinities for the stationary and mobile phases, boiling

points and therefore move at different rates along the column. The baseline resolved volatile sample components, obtained within a reasonable run time, are detected as they come off the column end, and into the interface with the mass spectrometer. In the mass spectrometer sample molecules are usually ionized by a number of sources, with electron ionisation (EI) and chemical ionization (CI) being the two commonly used ionization methods for GC-MS. In EI, a stream of electrons of 70 eV interact with sample molecules which loses an electron resulting in the formation of positively charged molecular ion and characteristic smaller fragmented ion. These ions are subsequently propelled and focused through a magnetic mass analyzer, and strike the surface of the detector, which convert this to a signal for the data system to process [92]. GC-MS is a combination of two different analytical techniques, namely Gas Chromatography and Mass Spectrometry, which provide a powerful tool for the separation, identification and quantification of compounds in complex mixtures.

2.2.1.1 Derivertization

Gas chromatography is used to separate volatile organic compounds. However, some of the PPCPs are highly polar, not thermally stable compounds, and may need to be chemically modified to improve their separation and detection by GC. By modifying the functionality of a molecule in order to increase – or sometimes decrease – volatility, compounds that otherwise are not readily monitored by GC can be analyzed. Hence, derivatization entails the process by which a compound is chemically changed in order to produce a new compound with properties that are more amenable to a particular analytical method. Compounds that have poor volatility and poor thermal stability, or those that can be adsorbed in the injector will exhibit non-reproducible peak areas, heights and shapes. In addition to improving suitability and response, derivatization can improve resolution between co-eluting compounds and overlapping peaks [93].

The choice of the methods and that of the derivatives to be used for the purpose of analysis is governed by nature of the compounds to be analyzed and their chemical properties, more than any other factors. Overall, derivatization serves to accentuate the differences in the sample compounds in order to facilitate chromatographic separation. According to Sellers (2010), there are three basic types of derivatization reactions that may be employed including: **silylation**, **acylation**, and **alkylation** [93]. Silylation is the most versatile approach currently used. It enhances GC performance by blocking protic sites, thereby reducing dipole-dipole interactions and increasing volatility. Silyl derivatives are usually formed by the replacement of active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A wide variety of reagents are available for such introduction of the trimethylsilyl group. These reagents differ in their reactivity, selectivity, side reactions and the character of the reaction byproducts from the silylation reagent.

The ease of the derivatization of various functional groups for a given silylating agent follows the order that is described below:

Alcohol > Phenol > Carboxylic acid > Amine > Amide

Within this sequence reactivity towards a particular silylating reagent will also be influenced by steric hindrance.

Acylating reagents react with highly polar functional groups such as amino acids or carbohydrates. In fact, acylation involves the introduction of an acyl group into molecule with a replaceable hydrogen atom. The acylating agent R-C(:O)-X can lose the group –X by electrophilic, nucleophilic or free radical mechanisms, with the most common mode of acylation being

electrophilic acylation. Acyl derivatives are usually made with three main types of acylating reagent, including acyl anhydrides, acyl halides, and activated acyl amide reagents. However, the anhydrides and acyl halides normally form acid by-products which must be removed prior GC-MS analysis. Alkylating reagents target active hydrogens on amines and acidic hydroxyl groups. As with other derivatization reagents, alkylation reagents reduce molecular polarity by replacing active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides—or they can be used in conjunction with acylation or silylation reagents. Multiple derivatizing reagents may be necessary for compounds containing several different functional groups [93].

Table 0-4 Gas chromatography mass-spectrometry methods for the determination of PPCPs in water

Class/compounds	Sample matrix	Derivertization agents	MS system	Environmental concentrations (ng L^{-1})	Reference
7 Musks	WWTP influent and effluent		QMD 1000 Carlo Erba MSD, full scan	1-11,463	[80]
Triclosan methyltriclosan	Treated wastewater, raw wastewater, river water	MTBSTFA	Varian Saturn 2100 ion-trap mass spectrometry	n.d-728	[89]
anti-inflammatory antidepressant hormones lipid regulator	WWTP Influent and final effluent, non-treated domestic and industrial sewage, river water	ECF	Shimadzu GC-MS QP-5000	< LOQ-1050	[87]
Fragrances UV-filters Antiseptics Estrogens Anti-inflammatory drugs Antioxidant Plastics component	effluent of an urban treatment plant, industrial and domestic influents	BSTFA (1% TMCS)	Thermo Fisher Scientific, PolarisQ GCMS Benchtop IT mass spectrometer	< LOQ-16	[86]
4-tert-octylphenol 4-nonylphenol bisphenol-A estrone estradiol triclosan Diethylstilbestrol Clofibric acid Ibuprofen Gemfibrozil Bentazone	River water	PFBOCl PFBBr	Agilent 5975B MSD mass spectrometer with a chemical ionization (CI) source	n.d-8890	[94]

Class/compounds	Sample matrix	Derivertization agents	MS system	Environmental concentrations (ng L^{-1})	Reference
Fenoprofen Naproxen Ketoprofen Mefenamic acid Tolfenamic acid Diclofenac Meclofenamic acid Indomethacin					

Moder and coworkers developed an analytical method for the determination of acidic pharmaceutical and endocrine disrupting compounds at low ng L^{-1} levels in surface and wastewater [95]. The water samples under study were prepared using solid phase extraction (SPE) for analyte enrichment and/or clean up. Prior to GC-NCI-MS, the polar analytes were derivatized using pentafluorobenzyl bromide. The performance of this analytical method has for example been revealed in fortification experiments with limits of detection ranging from 0.01 to 0.2 ng L^{-1} for ibuprofen and 17α-ethinyl estradiol, respectively. In this analytical procedure, the SPE was the most rate determining step. The evaluation of the method demonstrated that even complex sample matrices, such as model wastewater or even synthetic humic acids, did not interfere with the quantification and identification of target analytes. Further, the performance of the analytical method developed for water monitoring was emphasized by the investigation of surface water of the river Saale and effluents of the wastewater treatment plant (WWTP) at Halle, Saxony-Anhalt, in Germany. The selected samples presented acidic pharmaceuticals and corresponding metabolites at 0.1 ng L^{-1} for clofibric acid up to 498 ng L^{-1} for bezafibrate. Technical nonylphenol

and bisphenol A were also found in every water sample; whereas, 17α-ethinylestradiol was determined only in one WWTP effluent sample at 1 ng L^{-1}.

2.2.2 Liquid Chromatography-Mass spectrometry (LC-MS)

A large majority of PPCPs are ionic and or thermally labile, and this makes them ideal candidates for determination by LC-MS in comparison to GC-MS which require derivatization [37]. In the LC-MS technique, the mobile phase is nebulized via electrospray ionization (ESI) into a MS source, resulting in the creation of a single ion in the source, typically the molecular ion plus or minus hydrogen. The single ion produced may be either a positive $(M+H)^+$, or a negative $(M-H)^-$, if in positive ionization mode or negative ionization mode respectively. However, the formation of a single ion is also a limitation of LC-MS, since this is making the identification of analytes in complex environmental matrices quite unreliable. The use of LC-MS/MS techniques overcomes this setback. In the tandem MS techniques the precursor ion (the $(M+H)^+$ or $(M-H)^-$ ion) formed in the MS source is energized and collided, either in a triple quadrupole ion trap or a magnetic sector mass spectrometer, thereby creating ion products which correspond to the loss of various functional groups from the analytes such as $(M+H-OH)^+$ or $(M+H-CH_3)^+$ [96]. A summary of the various LC-MS method for the determination of PPCPs in water samples from different sources is provided in Table 2.5.

Table 0-5 Liquid chromatography mass-spectrometry methods for the determination of PPCPs in water

Class/compounds	Sample matrix	Additive (s) in LC mobile phase	MS system	Environmental concentrations (ng/L)	Reference
18 neutral PhACs 11 Acidic PhACs 2 Antibiotics 3 Estrogens	WWTP influent and effluent	formic acid	quadrupole VG Platform spectrometer, ESI (+), full scan	20-106,490	[67]
4 beta blockers 1 antiepileptic drug 3 fluoroquinolone	sewage influent and effluent, surface water, ground water	acetic acid	Micromass Quattro Micro triple-quadrupole, ESI (+), MRM	2-1350	[71]
8 analgesics and anti-inflammatory drugs 5 lipid regulators and cholesterol-lowering statin drugs 5 antibiotics 2 psychiatric drugs 1 antiepileptic drug 4 β-blockers 2 anti-histaminics 2 anti-ulcer agents 1 anti-diabetic 1 diuretic	wastewater influents and effluents	Ammonium acetate, acetic acid	Micromass Quattro triple quadrupole, ESI (+), ESI (-), MRM		[72]
1 anti-epileptic 7 analgesic/anti-inflammatory drugs 1 analgesic opiate 2 antidepressants 2 β-blockers 3 antibiotic 1 anti-ulcer	hospital effluent wastewaters	formic acid	Micromass Quattro triple quadrupole, ESI (+), ESI (-), MRM	10-151,000	[74]
2 β-blockers 3 Analgesic/Anti-inflammatory 1 Antibiotics 1 Lipid regulator 1 Psychiatric drug	drinking water samples	formic acid	Micromass Quattro API triple quadrupole, ESI (+), ESI (-), MRM	< QL	[42]
8 analgesics/anti-inflammatories 3 lipid regulators 4 antibiotics 1 antiepileptics 1 antipsychotics 1 psychomotor stimulants 1 glucocorticoid steroids	Surface water	formic acid	MS 2010 EV MSD, ESI (+), ESI (-)	< MQL-3506	[7]

Class/compounds	Sample matrix	Additive (s) in LC mobile phase	MS system	Environmental concentrations (ng/L)	Reference
1 disinfectants 1 beta-blockers 1 H2 receptor antagonists 1 oestrogens					
Analgesics and anti-inflammatory Cholesterol lowering statin drugs lipid regulators Antidepressants Anti-ulcer agents Psychiatric drugs Ansiolitics Cardiovasculars Macrolide antibiotics Quinolone antibiotics Sulfonamide antibiotics Lincosamide antibiotics Other antibiotics	influent and effluent wastewater		Waters Corp triple quadrupole MS, ESI (+), ESI (-), SRM	n.d-236,000	[87]

Sara Castiglioni and co-workers developed and validated an analytical method with two extraction steps for the simultaneous determination of 30 pharmaceuticals belonging to various therapeutic categories in urban wastewater [97]. This approach was aimed at boosting the little available information on drugs fates in sewage treatment plants (STPs) and in receiving surface water. Aqueous samples were divided into two aliquots, each extracted by a different solid-phase extraction (SPE) method and analyzed by reversed-phase liquid chromatography tandem mass spectrometry (HPLC–MS/MS). The recovery of the pharmaceuticals was mostly greater than 70 % and the overall variability of the method was below 8 %. While the instrumental quantification limit (IQL) varied between 30 and 400 pg injected, the limits of quantification (LOQ) were in the low ng L^{-1} range. Nineteen pharmaceuticals were detected in concentrations between 0.5 and 2000 ng L^{-1} in effluents collected from several STPs in Italy. Atenolol, ciprofloxacin, furosemide,

hydrochlorothiazide, ofloxacin, ranitidine and sulphamethoxazole were the most abundant compounds.

Ultimately, the aim of the study conducted in 2010 by Najat Ahmed Al-Odaini and co-workers was to develop a sensitive and selective multi-residue method for simultaneous determination and quantification of 23 pharmaceuticals and synthetic hormones from different therapeutic classes in water samples [98]. Target pharmaceuticals included anti-diabetic, antihypertensive, hypolipidemic agents, 2 adrenergic receptor agonist, antihistamine, analgesic and sex hormones. The method involved solid phase extraction (SPE) followed by instrumental analysis using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) with 30 min total run time. River water samples (150 mL) and STP effluents (100 mL) adjusted to pH 2, were loaded into Waters MCX (3 cm^3, 60 mg) cartridge and eluted with four different reagents for maximum recovery. Then, quantification was achieved by using eight isotopically labeled internal standards (I.S.) that effectively correct for losses during sample preparation and matrix effects during LC–ESI-MS/MS analysis. Good recoveries higher than 70 % were obtained for most of target analytes in all matrices, and method detection limit (MDL) ranged from 0.2 to 281 ng L^{-1}. The developed method was applied to determine the levels of target analytes in various samples, including river water and STP effluents. Among the emerging pollutants that were tested, chlorothiazide was found at the highest level, with concentrations reaching up to 865 ng L^{-1} in STP effluent, and 182 ng L^{-1} in river water.

2.3 Definition, classification and selection of pharmaceutical and personal care products

PPCPs have a wide span of physico-chemical properties which can be attributed to their large and complex structure and the functional groups that they contain such as hydroxyl, carboxyl, ketone, and amine. It is important to have information on these properties because this determines their fate in the environment and removal during wastewater treatment. In addition, this will guide the choice of analytical procedures. Most PPCPs are either weakly acidic and or weakly basic or neutral in nature because of the functional group they possess. Their ionization rate depends on the acidic dissociation constants (pK_a values) and is controlled by solution pH. These compounds dissociate partially in aqueous media and an equilibrium exists between the various ionized and non-ionized species [85, 99, 100]. The key physico-chemical properties of organic compounds most applied in the field of environmental chemistry include:

1. The solubility in water;
2. The partition coefficient between air;
3. Water and octanol: the octanol-water partition coefficient K_{ow} is an indicator of the partitioning tendency from water to organic media (hydrophobicity) of a non-charged compound. Since values of K_{ow} range from about 0.001 to over 10,000,000, log K_{ow} values are used instead, and range from −3 to 7. A log K_{ow} values ranging from 3-4 to 6.5 is characteristic for hydrophobic compounds, whereas a low log K_{ow} (log $K_{ow} < 2.5$) signifies a compound soluble in water, compounds with intermediate hydrophobicity (or solubility in water) have log K_{ow} value between 2.5-4.0.
4. And, when relevant the dissociation constant in water (K_a) which is a measure of (weak) acid strength.

Pharmaceuticals substances are formulated in such manner that they are biologically active and hydrophilic so that the body can absorb them easily. These substances can be categorized according to their therapeutic use or their chemical class (the chemical type of the active ingredient). Personal care products such as fragrances are generally semi-volatile organic compounds which are fairly hydrophobic and having a multitude of applications (e.g., perfumes, deodorants, washing and cleaning agents, and cosmetics). Large amounts of PCPs can be directly introduced in the environment, by direct release into recreational waters or volatilized into the air (e.g., musks) [59].

Numerous drugs are commonly prescribed in both private and public health sector in South Africa but often, much higher quantities are sold without prescription as over the counter drugs. Table 2.6 shows the top 50 most prescribed drugs in South Africa private health sector in 2012. In term of prescription volume this indicate that the analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) will most likely be found in relatively large quantities in the sewage system compared to other class of pharmaceuticals such as anti-acids or contraceptive [101].

Table 0-6 Top 50 most prescribed drugs in South Africa private health sector in 2012

	Product	Drug	Class	Prescriptions
1	Adco-dol	Paracetamol (450mg), Codeine, Caffeine	Analgesic	6670821
2	Alcophyllex	Diphenhydramine, Theophyline, Etofyline	Expectorants	6257534
3	Allergex	Chlorpheniramine maleate	Antihistamines	6014431
4	Dph	Diphenhydramine	Antihistamines	3944118
5	Panamor	Diclofenac sodium (25-100 mg)	Anti-inflammatory	3724619
6	Panado	Paracetamol (500 mg), Codeine (8 mg)	Analgesic	2880919
7	Broncleer with COD	Diphenhydramine (125 mg), Codeine (10 mg)	Expectorants	2859364
8	Corenza c	Aspirin, Chlorprophenpyridamine	Cold and Flu	2334721
9	Sinucon	Phenylpropanolamine	Antihistamines	2158663
10	Sinuend	Chlorpheniramine, Phenyltoloxamin	Antihistamines	2098621
11	Asthavent	Salbutamol (100 µg)	Asthmatic	2068270
12	Mayogel	Aluminium oxide, Magnesium oxide	Anti-acids	1825719
13	Gen-payne	Ibuprofen, Paracetamol, Codeine	Analgesic	1697405
14	Theophen compound	Theophyline, β-Hydroxyethyl Theophyline	Expectorants	1634112
15	Mybulen	Ibuprofen, Paracetamol, Codeine	Analgesic	1625431
16	Benylin four flu	Paracetamol, Diphenhydramine HCl	Analgesic	1620013
17	Persivate	Etamethasone (5 mg)	Corticosteroid	1570532
18	Stilpane	Paracetamol, Codeine, Caffeine	Analgesic	1515511
19	Adco-simvastatin	Simvastatin, Butylhydroxyanisol	Anti-cholesterol	1513178
20	Napamol	Paracetamol (500 mg)	Analgesic	1451078
21	Acc 200	N-Acetylcysteine (200 mg)	Mucolytic agent	1448168
22	Flutex	Paracetamol, Caffeine, SCP	Cold and Influenza	1409604
23	Disprin	Aspirin	NSAIDs	1408014
24	Myprodol	Ibuprofen, Paracetamol, Codeine	Analgesic	1392375
25	Purbac	Trimethoprim, Sulphamethoxazole	Antibiotics	1376298
26	Adco-linctopent	Bromhexine HCl, Orciprenaline	Expectorants	1360582
27	Venteze	Salbutamol	Asthmatic	1337780
28	Bactroban	Mupirocin	Antibiotics	1303609
29	Glucophage	Metformin HCl (500 mg)	Diabetic	1297591
30	Mylan diclofenac	Diclofenac sodium	NSAIDs	1293478

	Product	Drug	Class	Prescriptions
31	Gaviscon	Alginic acid, Bicarbonate	Anti-acids	1227850
32	Sinutab	Paracetamol, Pseudoephedrine HCl	AND	1227631
33	Centrum	Multivitamin supplement	Vitamins	1197453
34	Iliadin	Oximetazoline HCl, Benzalkonium chloride	Decongestants	1173866
35	Lenazine	Codeine, Ephedrine HCl, Promethazine HCl	Expectorants	1165048
36	Betapyn	Paracetamol, Codeine, Caffeine	Analgesic	1163355
37	Andolex-c	Benzydamine HCl, Chlorhexidine	NSAIDs	1144247
38	Cataflam	Diclofenac $KCaPO_3$	NSAIDs	1138718
39	Adco-sinal co	Paracetamol, Codeine Phenylpropanolamine	NSAIDs	1126793
40	Hyospamol	Hyoscine butylbromide	Antispasmodics	1115273
41	Flusin	Paracetamol, Pseudoephedrine	AAA	1113084
42	Augmentin gsk	Amoxicillin	Antibiotics	1105783
43	Vermox	Mebendazole	Anthelmintics	1090839
44	Crestor	Rosuvastatin calcium	Anticholesterol	1076578
45	Syndol	Paracetamol, Codeine, Caffeine	Antihistamine	1050326
46	Strepsils	Amylmetacresol	Antiseptics	1034799
47	Chloramex	Chloramphenicol (10 mg)	Antibiotics	1029357
48	Aspavor	Atorvastatin	Anticholesterol	1021177
49	Yasmin	Drospirenone, Ethinyl estradiol	Contraceptive	1002940
50	Norflex co	Orphenadrine, Aspirin, Caffeine	Arthritis	978804

CHAPTER 3 EXPERIMENTAL

All experimental work is presented in this section. The first section provides the list of different chemicals and reagents used. The subsequent sections provide comprehensive details on the sample collection, analytical methods and experimental details on occurrence of the PPCPs in wastewater. Quality control and data analysis are covered in the last sections.

3.1 Chemicals and reagents

Thirteen compounds were selected to represent different chemical classes. The choice was based on the widest occurrence in the aquatic environment, their prescription volumes in South Africa and their known impact on aquatic organisms. These PPCPs included a broad class of NSAIDs, anticonvulsant, antiepileptic, antiviral, industrial chemical, natural androgen and oestrogen. The detailed list of target compounds and classification is provided in Table 3.1. Acetaminophen, bisphenol A, carbamazepine, diclofenac sodium salt, α-estradiol, β-estradiol, estriol, famciclovir, fenoprofen calcium salt hydrate, ibuprofen, primidone, progesterone testosterone, 1, 2-benzanthracene and anthracene were all purchased from Sigma-Aldrich (Steinheim, Germany).

HPLC and LC-MS grade methanol, HPLC grade ethyl acetate, HPLC grade pyridine were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid 32 %, nitric acid 55 % and ammonia solution 25 % were purchased from Merck KGaA (Darmstadt, Germany). Silicone oil was obtained from Promark Chemicals. The derivatization reagent BSTFA + 1% trimethylchlorosilane (TMCS) were supplied by Sigma-Aldrich (Steinheim, Germany). Silane treated glass wool, silanized 2 mL amber vials were purchased from Sigma-Aldrich (Steinheim, Germany). Waters Oasis HLB solid phase extraction cartridges (12 cc, 500 mg) were purchased

from Waters (Milford, MA, USA). Ultrahigh purity (UHP) water was processed using Milli-Q water system (18.3 M Ω. cm) (Millipore, Billerica, MA).

Table 3-8 List of the 13 studied target compounds belonging to different PPCPs classes with their, CAS number, physico-chemical properties: molecular weight (MW), the pK_a, water solubility, octanol–water partition K_{ow}

Name of Compounds	Main category	CAS	Mol wt. (g/mol)	pK_a	Water solubility (mg/L) at 25 C^0	$logK_{ow}$
Estriol	Natural estrogen	50-27-1	288.38	10.54	27.34	2.45
17 α-Estradiol	Natural estrogen	57-91-0	272.4	9.85	3.9	4.01
17 β-Estradiol	Natural estrogen	50-28-2	272.4	10.71	3.6	4.01
Testosterone	Natural androgen	58-22-0	288.42	17.4	23.4	3.32
Progesterone	Natural androgen	57-83-0	314.46	N.A	8.81	3.87
Bisphenol A	Industrial chemical	80-05-7	228.287	9.6	300	3.32
Acetaminophen	NSAID	103-90-2	151.163	9.38	14,000	0.46
Fenoprofen Ca salt hydrate	NSAID	53746-45-5	522.60	N.A	N.A	N.A
Ibuprofen	NSAID	15687-27-1	206.28	4.91-5.2	21	3.97
Diclofenac sodium salt	NSAID	15307-79-6	318.13	4.15	50,000	4.51
Carbamazepine	Anticonvulsant	298-46-4	236.3	13.9	18	2.45
Primidone	Antiepileptic	125-33-7	218.26	11.1-12.2	500	0.91
Famciclovir	Antiviral	104227-87-4	321.33	N.A	N.A	N.A

The following information was generated from, the Hazardous Substances Data Bank (HSDB), a database of the National Library of Medicine's TOXNET system [Ref](http://toxnet.nlm.nih.gov) on May 30, 2016;[104]; [105]; [106]; [87]; N.A: not available

3.2 Instrumentation

A LECO Pegasus HT time of flight mass spectrometry (TOFMS) (LECO Corporation, St Joseph, Michigan, USA) coupled to an Agilent 7890A GC (Agilent Technology, Santa Clara, California, USA) was used. The samples were loaded onto a Gerstel MPS2 Liquid/HS/SPME auto-sampler for analysis. Separation was through a J & W capillary column HP-5 30 m × 0.25 mm I.D. with 0.25 μm film thickness. Separation occurred under the following chromatographic conditions: Injection of a 1 μL sample was performed with a splitless injector at a temperature of 250 °C, the GC oven was programmed as follows: 2 min at 70 °C, then ramp 15 °C min^{-1} to 280 °C held for 10 minute. The total analysis time was approximately 19 min. High purity helium (99.999 %) (Afrox, Johannesburg, RSA) was used as the carrier gas (constant flow at 1.5 mL min^{-1}). The GC/TOFMS interface temperature was set at 280 °C and mass spectra were obtained at 70 eV in full scan mode (scanning *m/z* ranging from 50 to 550). Data acquisition was achieved using ChromaTOF software. All weighing and pH measurement were done using Mettler Toledo microbalance and pH-meter respectively (Greifensee, Switzerland).

3.3 Preparation of standard solution

Stock solution of individual compounds as well as internal standards were prepared in LC-MS grade methanol at the concentrations of 1000 mg L^{-1} in amber volumetric flasks, and stored - 5 ^{0}C for later use. Working standards mixture of the target compounds were diluted with methanol. 5, 10, 20, 50 % (V/V) of pyridine was prepared by diluting with ethyl acetate.

3.4 Sampling

All glassware were soaked in detergent for 24 hours, rinsed thoroughly with distilled water, then soaked further in 10 % nitric acid (HNO_3) or aqua regia solution for another 24 hours followed by rinsing with UHP water (18.3 M Ω. cm) in order to minimize the possible contaminants on the glass walls. All glassware, except for volumetric flasks were then heated to 150 ^{0}C for at least 8 hours . Sampling was conducted from January to December 2015 at the Daspoort Waste Water Treatment Works (WWTW). Samples of influent and effluent were collected once a week over a 12 months period. The WWTW is located on the corner of DF Malan Drive (M1) and Bazaar Street in Pretoria (GPS: 25° 43' 16.72" S, 28° 03' 14.07" E), where the Apies River and the Skinnerspruit meet.

Figure 3-16 Daspoort wastewater treatment plant map

The Daspoort WWTW draws raw wastewater from the main wastepipe sewer that collects wastewater from the Central Pretoria area at two points. The first inlet of wastewater goes through its Eastern Works and the second inlet through the Western Works. The Eastern Works is a trickling filter (TF) plant while the Western Works is a conventional biological nutrient removal

activated sludge system. The main sewage drainage runs alongside the Apies River past the DWWTW to the Rooiwal Waste Water Treatment Works.

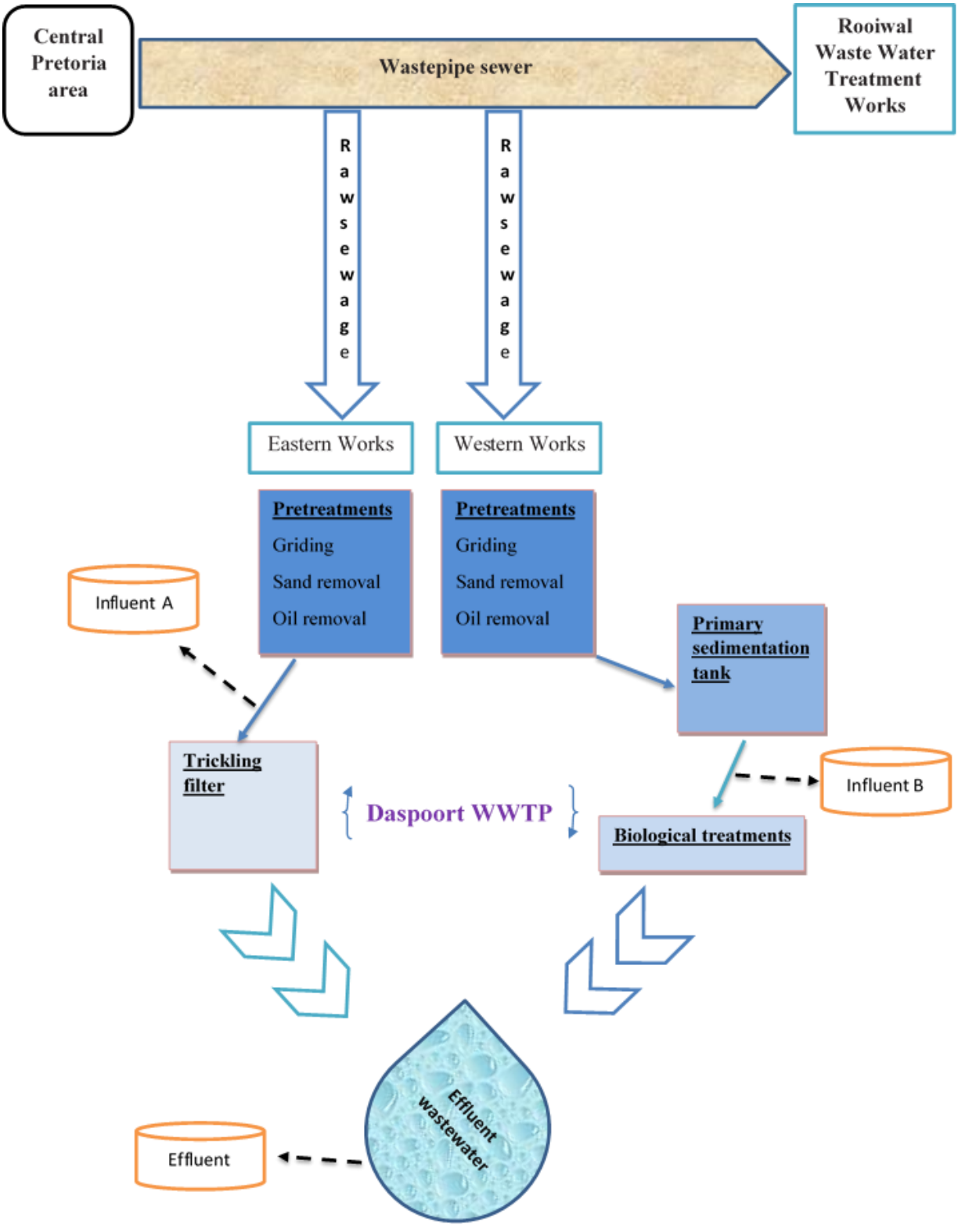


Figure 3-17 Schematic of the wastewater sampling point

Influent A

Effluent

Influent B (collection point on the left)

Figure 3-18 Wastewater sampling point

Influent wastewater samples from the Eastern work (Influent A) were collected after the wastewater went through mechanical screening and grit removal. Influent wastewater samples from the Western works (Influent B) were sampled after the wastewater has gone through mechanical screening, grit removal and primary settling in Dortmund-type vertical flow settling tanks. Effluent wastewaters were also collected at the Daspoort WWTP, where the treated effluent water is discharged to the downstream Apies River (see Figure 3.2 and 3.3). All grab samples were transferred on site in amber glass bottles which were pre-cleaned as mention above. Samples were transported to the laboratory and stored at -5 ^{0}C in a refrigerator for a predetermined time intervals after which they were processed and analysed in triplicate.

3.5 Optimization of silylation with BSTFA:TMCS (99:1)

Generally the factors taken into account during the optimization of the silylation method include: solvent, silylation reagent, catalyst volume, temperature and the reaction time. In this study the effects of reaction time, temperature, and catalyst volume on the final derivatization product were investigated systematically by employing a number of experiments.

3.5.1 Effect of pyridine catalyst on silylation

The effect of 5, 10, 20, 50 % pyridine in ethyl acetate, as a catalyst for the derivatization was investigated as follow: 50 µL of working standard mixture in methanol, containing all the target compounds at a concentration of 2 mg L-^{1} together with the internal standard 1, 2-benzanthracene at a concentration of 1 mg L^{-1}, were transferred to three 2 mL reaction vials and evaporated to dryness under a gentle stream of nitrogen. Each of the vials was then treated individually. To the dry residues, 0 µL, 25 µL and 50 µL of 5, 10, 20, 50 % pyridine in ethyl acetate were added

respectively. Fifty microliter of BSTFA:TMCS (99:1) were then added in each of the vials and sealed with a Teflon cap, and subsequently heated in a silicone oil bath at 60 °C for 30 min. After derivatization, the reaction vials were cooled to room temperature, then dried under a gentle stream of nitrogen followed by reconstitution in 50 µL of ethyl acetate and finally analysed by GC-TOFMS. Each derivatization was performed in five replicates

3.5.2 Effect of reaction time on the silylation

The effect of incubation time on the silylation was studied: Fifty microliters of working standard mixture in methanol, containing all the target compounds at a concentration of 2 mg L^{-1} together with the internal standard 1, 2-benzanthracene at a concentration of 1 mg L^{-1}, were transferred to three 2 mL reaction vials and evaporated to dryness under a gentle stream of nitrogen. Each of the vials was then treated individually; to the dry residues 25 µL 10 % pyridine in ethyl acetate were added. 50 µL of BSTFA:TMCS (99:1) was then added in each of the vials and sealed with a Teflon cap, and subsequently heated in a silicone oil bath at 60 °C for 30 min, 45 min and 60 min. After derivatization, the reaction vials were cooled to room temperature, then dried under a gentle stream of nitrogen followed by reconstitution in 50 µL of ethyl acetate and finally analysed by GC-TOFMS. Each derivatization was performed in five replicates.

3.5.3 Effect of reaction temperature on the silylation

Finally the effect of incubation temperature on the silylation was examined: Five triplicate working standards were treated as already described above using optimum volume of catalyst and reaction time. The derivatization procedure was monitored at 60 °C, 90 °C and 120 °C for 30 min. After derivatization, the reaction vials were cool to room temperature, then dried under a gentle stream of nitrogen followed by reconstitution in 50 µL of ethyl acetate and finally analysed by GC-TOFMS. Each derivatization was performed in five replicates.

3.6 Optimization of solid phase extraction

The pH of 1 L of UHP water was adjusted to pH 5, 7 and 9, respectively, using hydrochloric acid 32 % and ammonia solution 25 %. Subsequently, 200 mL of the pH adjusted UHP water was spiked with a standard mixture of the pharmaceuticals containing each target compound, so that the concentration of each analyte in the spiked water was 50 µg L^{-1}. The water sample was extracted using Waters Oasis HLB cartridges (12 cc, 500 mg). Before extraction, each Waters HLB cartridge was pre-conditioned with 3 mL of methanol, and then rinsed by 3 mL of deionized water on an SPE manifold. A 200 mL of the water sample was then passed through the HLB cartridge. After extraction the cartridge was washed with 1 mL of 5 % methanol in water, and subsequently air-dried under vacuum for at least 20 min. The drug residues were then eluted from the cartridge with 2 portions of 5 mL of methanol (HPLC grade). This extraction was done in five replicates. All the extracts were completely evaporated to dryness by a gentle stream of nitrogen and derivatized as described above under the optimal conditions. After derivatization, the reaction vials were cooled to room temperature, then dried under a gentle stream of nitrogen followed by reconstitution in 100 µL of ethyl acetate and finally analysed by GC-TOFMS.

3.7 Identification and quantification

Calibration standard solutions were prepared by using spiked UHP water at seven different concentration levels ranging from 0.025 to 12 µg L^{-1}, depending on target compound. Each concentration level was prepared in five replicates and injected twice. The calibration curves were then constructed using the average peak area versus the concentration.

3.7.1 Recoveries, detection limits and method precision

Relative recoveries were determined by processing ultrapure water spiked at 0.5, 1 and 1.5 time the maximum residue limit in the recommended guideline values for PPCPs in drinking water developed by the Australian Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council (see Table 1.2). For example, 17α-estradiol at concentrations of 87.5 ng L^{-1}, **175 ng L^{-1}** and 262.5 ng L^{-1}, with 5 replicates each. The limit of detection (LOD) of individual compounds was determined using the following equation

Detection limit = $3 \times \frac{S}{m}$

where *s* is the standard deviation of a low-concentration sample and *m* is the slope of the calibration curve. A signal that is 10 times as great as the noise is defined as the lower limit of quantitation**,** it is the smallest quantity that can be measured accurately, and can be calculated using the following equation [107] [108].

$$\text{Quantitation limit} = 10 \times \frac{S}{m}$$

The method precision was determined by evaluating intra-day and inter-day variability. For intra-day, three concentration levels, representing the lower, mid and upper range of the calibration were used. Five replicates of each the selected concentration levels were injected twice in the GC-TOFMS, and this was done three times a day at eight hours interval. For the inter-day variability, the entire process was repeated for five days with two days interval in between.

3.7.2 Application of the analytical method to real samples

The samples stored in the refrigerator were removed and allowed to equilibrate to room temperature. Thereafter samples were filtered using a glass column packed with silane treated

glass wool, followed by filtration with 1.2 mm glass fiber filters (GF/C, Whatman, UK). Subsequently the wastewater sample was pH adjusted prior SPE extraction. Waters Oasis HLB cartridge (12 cc, 500 mg) were pre-conditioned with 5 mL of HPLC grade methanol, and then rinsed by 5 mL of deionized water on an SPE manifold. Wastewater sample 1 L was passed through the Waters HLB cartridge. After completion of the extraction the cartridge was washed with 3 mL of 5% methanol in water, and subsequently air-dried under vacuum for at least 20 min. The drug residues were then eluted from the cartridge with 5 mL of methanol (HPLC grade). All the extracts were completely evaporated to dryness by a gentle stream of nitrogen. The residues were then redissolved in 500 μL of ethyl acetate, 20 μL of the residue were derivatized under optimal conditions. The derivatized extract were dried with nitrogen and reconstituted in 40 μL for the effluent and 100 μL for the influent, in ethyl acetate solvent, followed by GC-TOFMS analysis. Then entire process was replicated 3 times.

CHAPTER 4 RESULTS AND DISCUSSION

A simple analytical method for the simultaneous determination of 13 PPCPs by a GC-MS has been developed. Many of the target analytes are not volatile due to the presence of polar functional groups such as OH, COOH, NH, NH_2, SH in their chemical structures. Polar compounds may have poor volatility, thermal stability and non-reproducible detector response which result in unreliable qualitative and quantitative results. Derivatization is therefore a prerequisite for the determination of these non-volatile PPCPs by GC-MS.

There are three methods that could be used for derivatization of polar compounds, i.e. silylation, acylation and alkylation. Silylation is the most commonly method of derivatization used for PPCPs. The silylating reagent, *N*,*O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) at ratios of 99:1 (BSTFA:TMCS), was selected for derivatizing the compounds under this study because it was readily available. In addition, TMCS increases the reactivity of BSTFA mixture by ensuring that hydroxyls and other functional groups that had challenges of reacting with BSTFA on its own, could be derivatised. A trimethylsilyl group (–$Si(CH_3)_3$) from the silylation reagent, with a molecular mass of 73 Da, will substitute the protic hydrogen of the polar group of the PPCPs.

Figure 4.1 summarizes a typical silylation reaction using BSTFA: TMCS (99:1) with bisphenol A compound as an example. The protic hydrogen on the hydroxyl group is substituted by a trimethylsilyl group resulting in the formation of bisphenol A (di-TMS) derivative. Derivatization process is often time consuming. In the present study, several strategies were employed to enhance

this process, such as incubation temperature and time, as well as different ratio of pyridine in ethyl acetate.

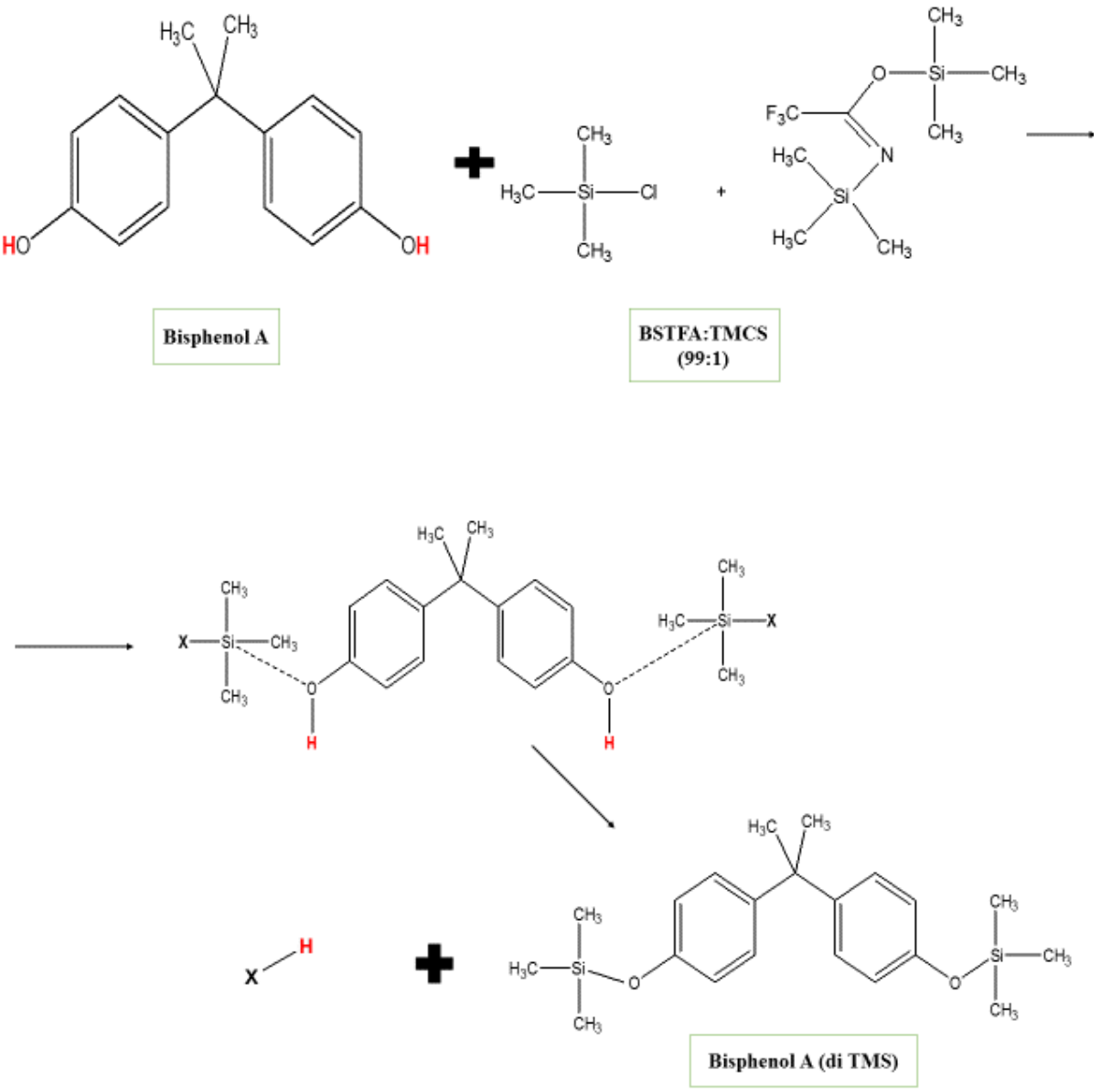


Figure 4-1 General silylation reaction with BSTFA: TMCS (99:1), here bisphenol A was used as the analyte, for BSTFA X = $CF_3CON\text{-}Si(CH_3)_3$, for TMCS X = Cl

4.1 Optimization of silylation derivatization of pharmaceutical and personal care products

4.1.1 Factor affecting the derivatization of pharmaceutical and personal care products

Peak areas from the derivatized analytes were compared with peak areas from the internal standard (anthracene), which is virtually inert to the derivatization reaction conditions used. The effectiveness (yield) of the derivatization when studying each condition was determined by computing the relative response of the detector to the species, derived according to the following equation [102]:

$$Response\ factor(F): \frac{\text{Area of analyte signal}}{\text{Concentration of analyte}} = F(\frac{\text{area of internal standard signal}}{\text{concentration of internal standard}})$$

After rearrangement

→ Response factor (F): $F = (A_S \times C_{IS}) / (A_{IS} \times C_S)$

where A_S and C_S is the target compound derivative peak area and concentration (mg L^{-1}) respectively, A_{IS} is the internal standard peak area (anthracene), C_{IS} is the internal standard concentration (mg L^{-1}). Increase in the response factor F was viewed as an indication of an improvement of the conversion of the target analytes to silyl derivative. The mean of the response factor F was calculated for the tested PPCPs, except for progesterone, famciclovir, primidone, carbamazepine and diclofenac which were not derivatized.

4.1.1.1 Effect of pyridine on the derivatization

The reaction medium for the target compounds consisted of ethyl acetate which is a neutral aprotic organic solvent that could be used to dissolve analyte during derivatization, and various ratio of the Lewis base pyridine. Lewis base catalysis of silylation with pyridine proceeded through the formation of silylpyridinium ion as intermediate as a result of the reaction of silyl chlorides with pyridine. Subsequent reaction of that intermediate with the polar group on the analyte yields the silyl derivative product together with the protonated pyridine base.

Table 4-1 Effect of volume of pyridine in ethylacetate on derivatization

Concentration ($mg.L^{-1}$)	**Compound**	**0 µL pyridine**		**25 µL pyridine**		**50 µL pyridine**	
		Peak area	**Response factor**	**Peak area**	**Response factor**	**Peak area**	**Response factor**
2	Ibuprofen*	15500	0.077	28674.733	0.142	22377.5	0.111
2	Paracetamol*	447121	2.220	503160.333	2.499	488152.7	2.424
2	Fenoprofen*	3164.1	0.016	4963.067	0.025	4661.55	0.023
2	Bisphenol A*	136142.5	0.676	151368.666	0.752	149956.5	0.745
2	α-Estradiol*	62005.5	0.308	67713.667	0.336	64741.5	0.322
2	β-Estradiol*	46844.5	0.233	55791	0.277	51430.5	0.255
2	Testosterone*	49388	0.245	55208.667	0.274	52404	0.260
2	Estriol*	19627.5	0.097	29573	0.147	28158	0.139

1	Anthracene (IS)	100680.5	1	100680.5	1	100680.5	1
* Trimethylsylil derivative, IS= internal standard							

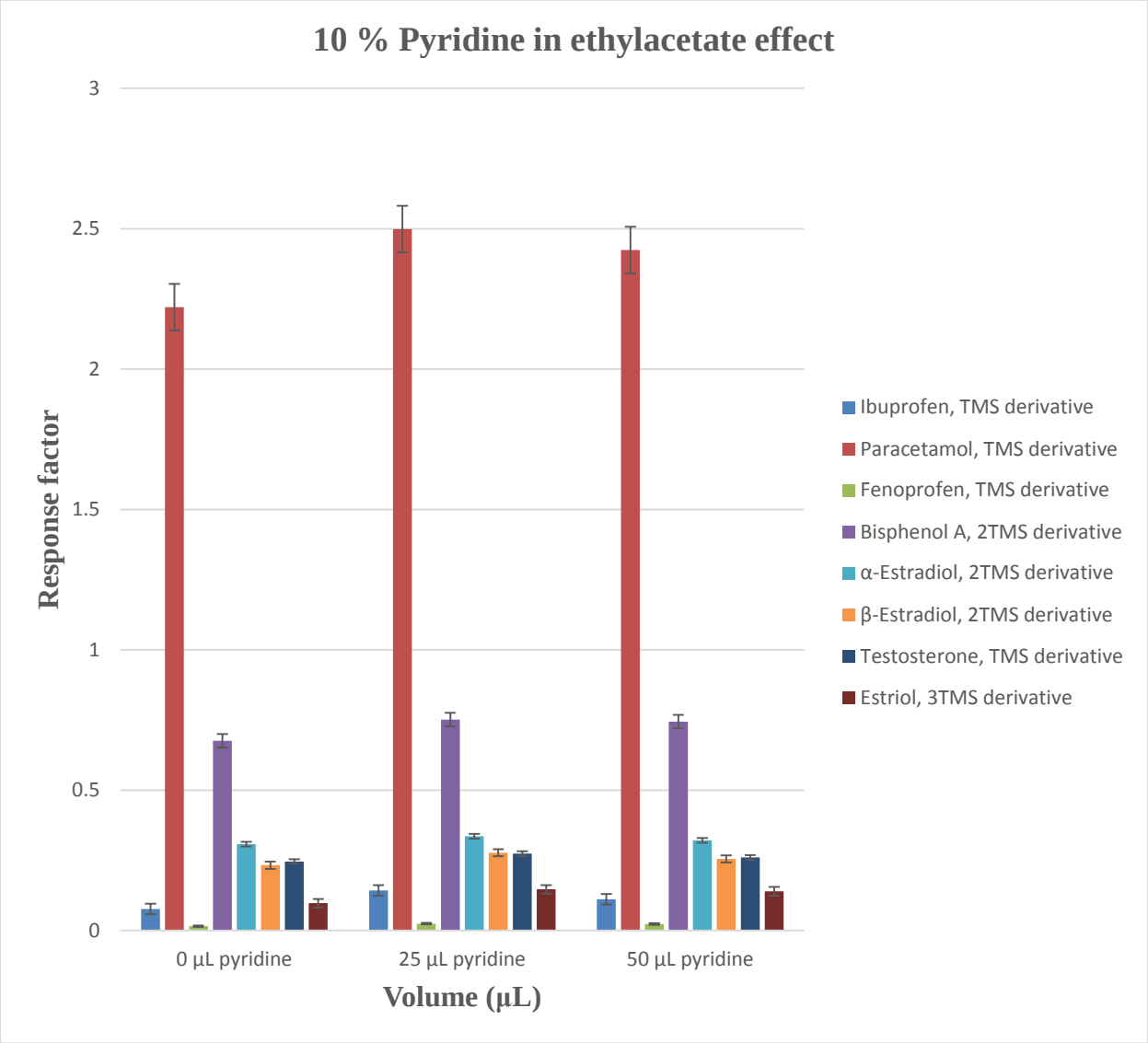


Figure 4-2 Effect of volume of pyridine in ethylacetate on derivatization

The effect of the presence of 10 % pyridine in ethyl acetate as a catalyst is shown in Figure 4-2. All the target compounds showed a significant improvement of the conversion to their respective

silyl derivative when 10 % pyridine in ethylacetate was added in the reaction medium. The response factor (RF) increased from 0.676 to 0.752 for bisphenol A 2TMS derivative, 0.016 to 0.025 for fenoprofen TMS derivative and 2.22 to 2.499 for paracetamol TMS derivative, without and with the addition of pyridine catalyst respectively. The pyridine catalyst increased the polarisation of the Si–X bonds. In addition, the silicon atom becomes a strong electrophile based on the positive charge in the activated intermediate. This confirmed that the addition of 10 % pyridine in ethyl acetate improved the efficiency of the derivatization reactions. A slight decrease of the efficiency of the silylation reaction for all the compounds was observed when the catalyst load was increased from 25 µL to 50 µL as demonstrated by lower RFs obtained, that is from 0.752 to 0.745 for bisphenol A 2TMS derivative, 0.025 to 0.023 for fenoprofen TMS derivative and 2.499 to 2.424 for paracetamol TMS derivative. The use of 25 µL of 10 % pyridine gave the optimal results as illustrated by higher response factor for all compounds under this condition (see Table 4-1). These results clearly demonstrate that the volume of 10 % pyridine in ethyl acetate plays a significant role in the derivatization procedure.

4.1.1.2 Effect of reaction time on the derivatization

The duration of derivatization reaction was investigated. The overall results suggested that there was a correlation between reaction duration and the degree of silylation of the target compound (Figure 4.3). Most of the analytes were derivatized after 30 min and no improvement was observed for long times, i.e. 60 min paracetamol TMS derivative (RF values 1.294 to 1.199), with the exception of ibuprofen TMS derivative (RF values 0.280 to 0.288), fenoprofen TMS derivative (RF values 0.004 to 0.009) and bisphenol A 2 TMS derivative (RF values 0.314 to 0.390). However, there was no significant difference for the majority of the analytes and therefore a time of 30 min was selected as reaction time for the derivatization in this work.

Table 4-2 Effect of incubation time on derivatization

Concentration ($mg.L^{-1}$)	**Compound**	**30 minute**		**60 minute**	
		Peak area	**Response factor**	**Peak area**	**Response factor**
2	Ibuprofen*	130779.7	0.280	134283.5	0.288
2	Paracetamol*	603607	1.294	559462	1.199
2	Fenoprofen*	2022.84	0.004	4464.583	0.009
2	Bisphenol A*	146277.3	0.314	182021.8	0.390
2	α-Estradiol*	81560.5	0.175	76932	0.165
2	β-Estradiol*	65363.67	0.140	63188	0.136
2	Testosterone*	68326.5	0.147	64999.67	0.139
2	Estriol*	38714.5	0.083	35291	0.076
1	Anthracene (IS)	233111.7	1	233111.7	1

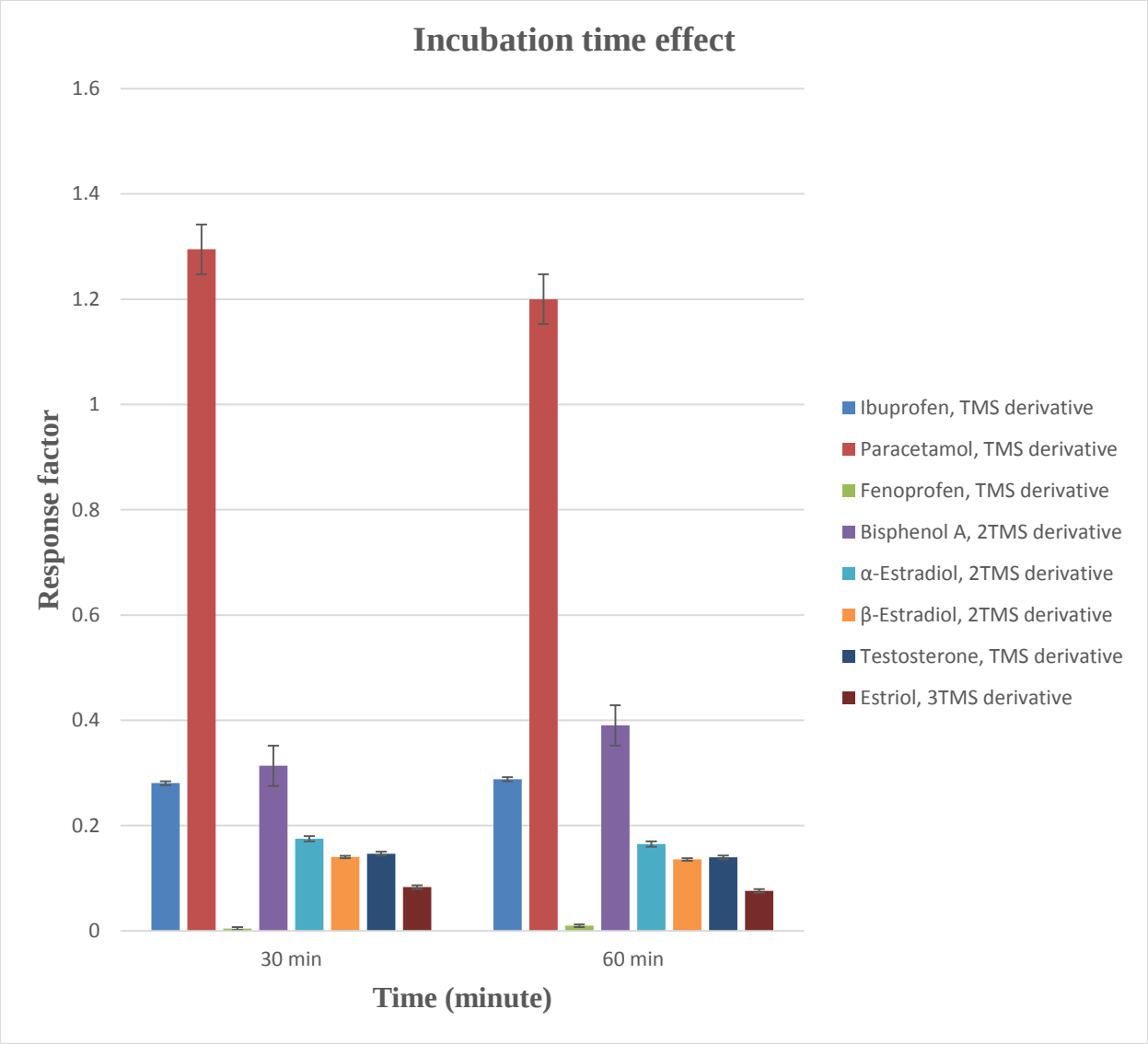


Figure 4-3 Effect of reaction time on derivatization

4.1.1.3 Effect of temperature on the derivatization

The reaction temperature slightly affected the derivatization yield for the eight target compounds as demonstrated by the RFs value recorded at 60 ºC, 90 ºC and 120 ºC. The RF values presented in increasing order of the temperatures are: bisphenol A 2 TMS derivative (0.386, 0.421 and 0.46), paracetamol TMS (1.24, 1.372 and 1.415), fenoprofen TMS derivative (0.006, 0.008 and 0.012).

Table 4-3 Effect of reaction temperature on derivatization

Concentration ($mg.L^{-1}$)	**Compound**	**60 °C**		**90 °C**		**120 °C**	
		Peak area	**Response factor**	**Peak area**	**Response factor**	**Peak area**	**Response factor**
2	Ibuprofen*	107813.6	0.332	109044.8	0.336	110967.3	0.342
2	Paracetamol*	402337.8	1.240	444952	1.372	459121.3	1.415
2	Fenoprofen*	1930.5	0.006	2536.6	0.008	3773.517	0.012
2	Bisphenol A*	125255.6	0.386	136604.5	0.421	149359.2	0.460
2	α-Estradiol*	61758.6	0.190	66122.83	0.204	69709.5	0.215
2	β-Estradiol*	50165.8	0.155	52254	0.161	54131.33	0.167
2	Testosterone*	50644.2	0.156	53891.33	0.166	53886.67	0.166
2	Estriol*	26199.17	0.081	26291	0.081	26313.5	0.081
1	Anthracene (IS)	162201.8	1	162201.8	1	162201.8	1
* Trimethylsylil derivative, IS= internal standard							

This rather moderate increase of RFs implies that the elevated temperatures are mainly beneficial for the dissolution of analytes and /or derivatives during derivatization. Figure 4.4 shows a histogram of results of the reaction temperature experiments. The optimal reaction temperature for the BSTFA:TMCS (99:1) derivatization were found to be around 90 ºC which gave RFs that were comparable to those observed at higher reaction temperature.

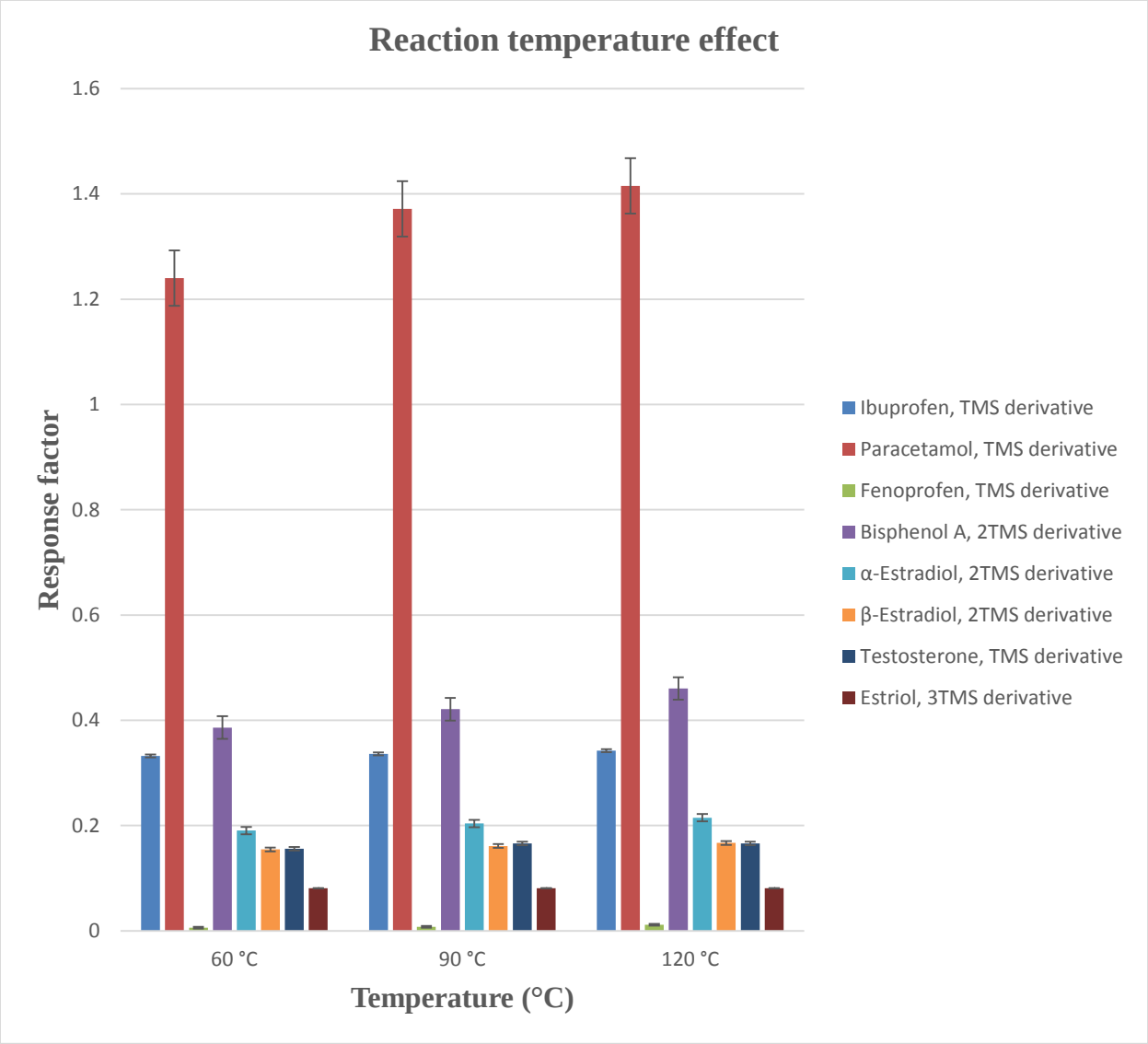


Figure 4-4 Effect of temperature on derivatization

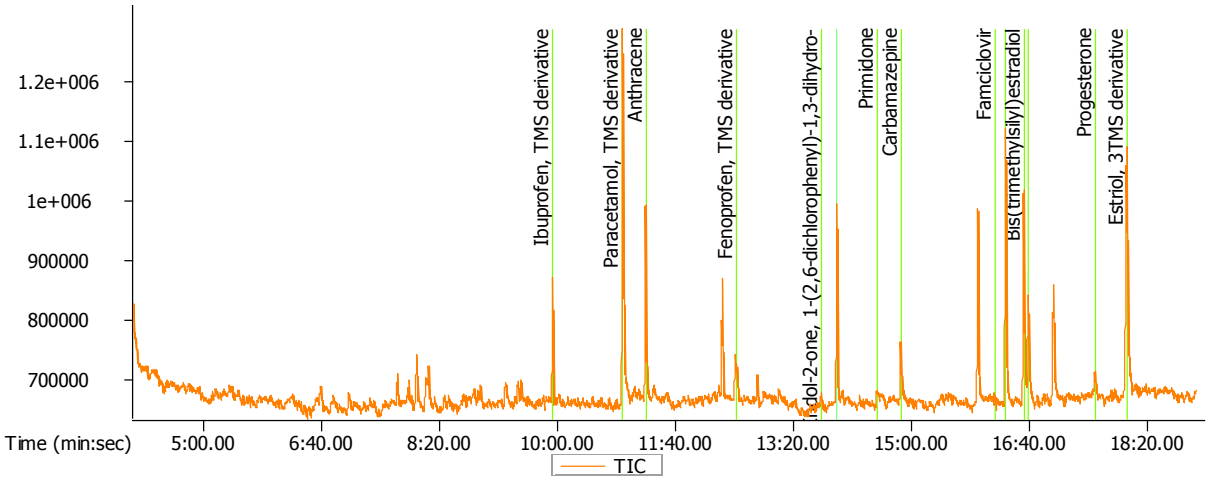
With respect to silylation reactions, the main effects of incubation time and temperature and the volume of 10 % pyridine in ethyl acetate were significant. By observing the histogram plots as shown in Figures 4.2 - 4.4 and Table 4.1- 4.3, the optimal conditions for the derivatization with 50 µL of BSTFA:TMCS (99:1) were found to be around in 25 µL of 10 % pyridine in ethylacetate at 90 ºC for 30 min. It should be noted that the optimal derivatization of a sample of unknown composition (e.g. wastewater samples) may depend on many factors including concentration of

the analytes of interest and that of other polar organic pollutants that may compete for the silylating reagent.

4.2.1 Optimization of the GC-MS conditions

The identification and qualitative method of the target analytes was developed in the full scan mode. Detection was achieved after the derivatization with 50 μL BSTFA: 1 % TMCS and 25 μL of 10 % pyridine in ethyl acetate mixture. A synthetic mixture of the commercial standard was used for method development. Compounds were separated and identified in the synthetic mixture after derivatization process. The trimethylsilyl (TMS) derivative products were used for monitoring. A mass spectral NIST was used for the identification of compounds of interest. A good baseline separation was achieved for all the compounds of interest with the exception for β-estradiol and testosterone. For this purpose, different temperature programs and flow rates (0.9 to 2 mL min^{-1}) were investigated to improve their chromatographic elution. The best separations are usually observed for temperature programming, taking advantage of the differences in polarity and in boiling points of the compounds being separated. The flow rate of the carrier gas affect how the components of a mixture interact with the stationary phase while being pushed through the column.

The peak deconvolution was used to detect each compound and the internal standards (I.S.) The quantifier ions were mostly the ion peaks with the largest abundance (Table 4-4, Figure 4.5).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Primidone
Carbamazepine
Famciclovir
Bis(trimethylsilyl)estradiol
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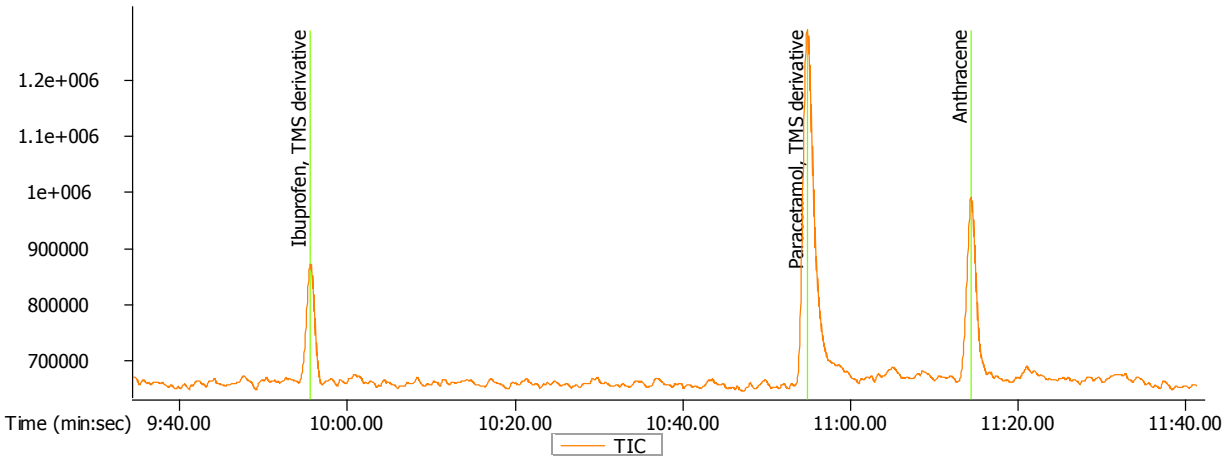
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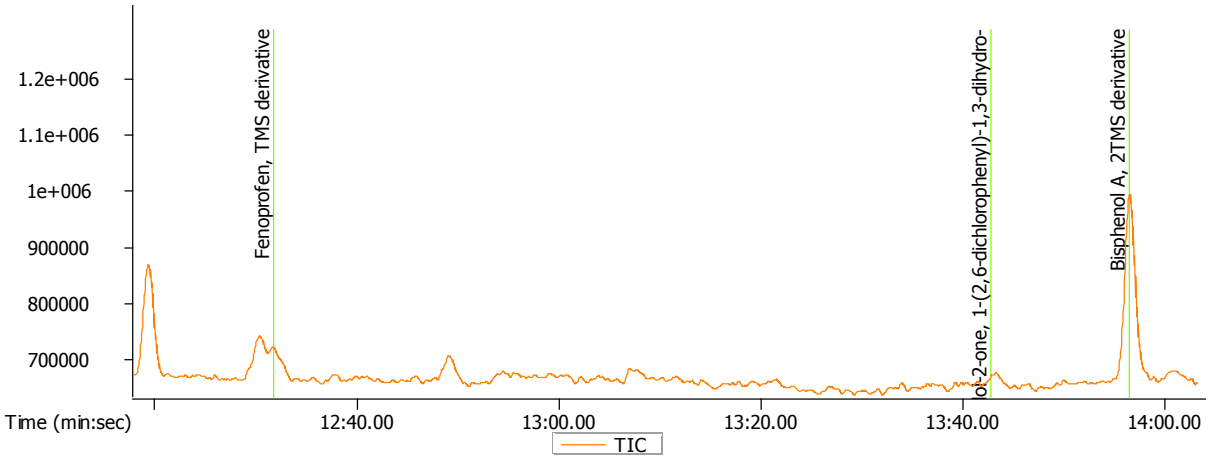
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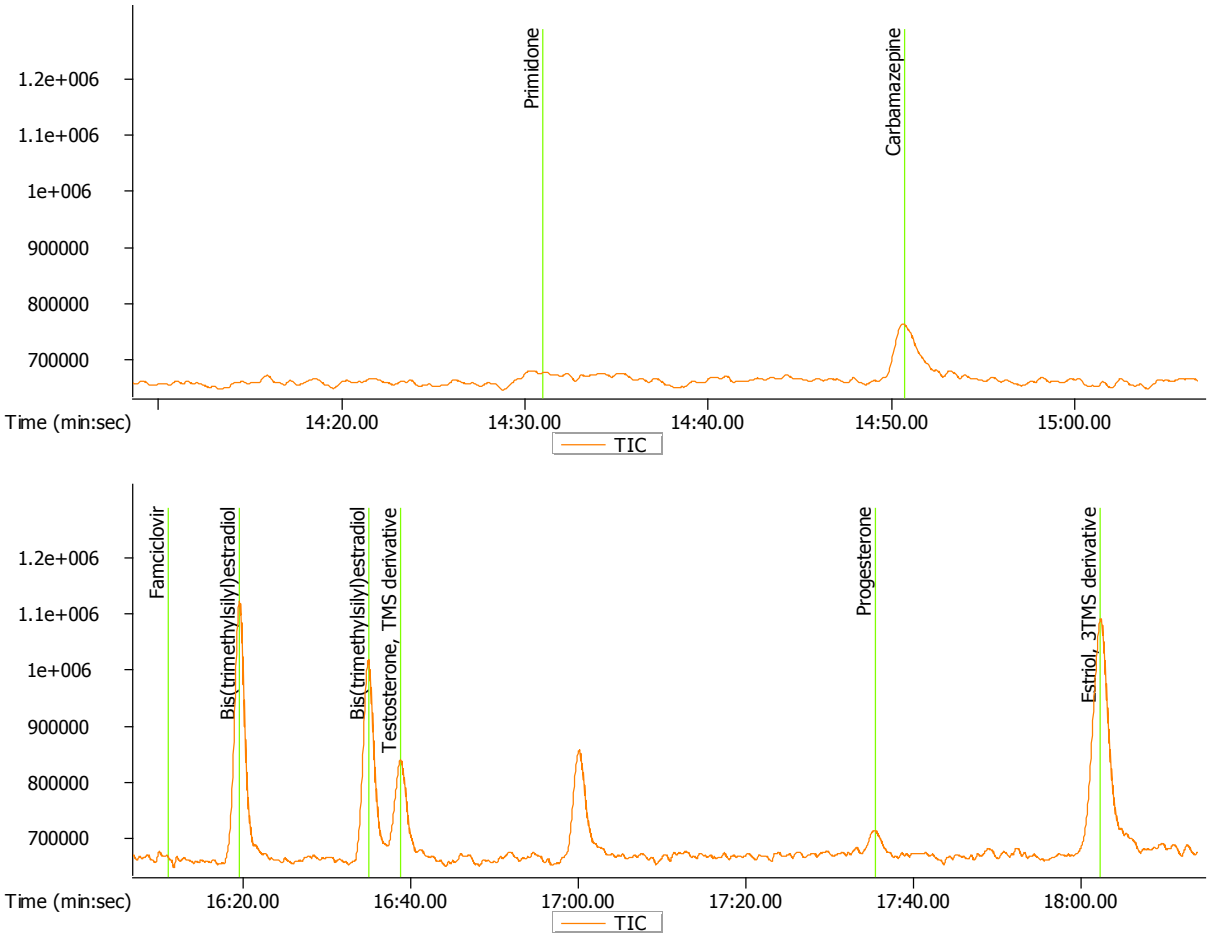
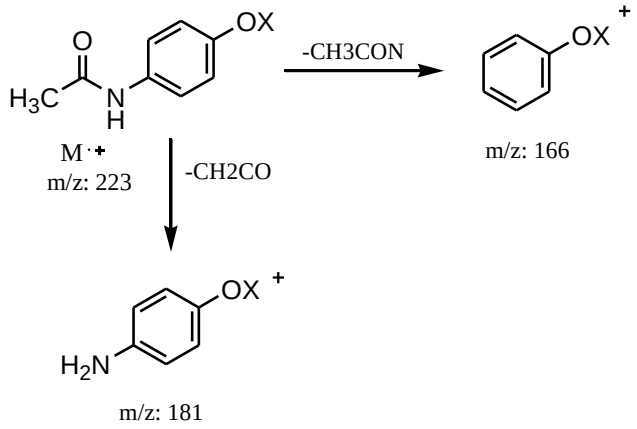


Figure 4-5 Total ion chromatogram separation of 4 mg L^{-1} of analytes using HP-5 J&W capillary column, constant flow rate of 1.5 mL min^{-1}, 2 min at 70 ^{0}C, then ramp 15 ^{0}C min^{-1} to 280 ^{0}C held for 10 min

The molecular mass of paracetamol for example is 151.163 Da, and the structure has one hydroxyl group. The Mass spectrum of paracetamol TMS derivative showed an abundance singly charged molecular ions $[(M-H)+73]^+$ at *m/z* of 223 providing evidence of paracetamol + TMS product. Also fragments of *m/z* 166 and 181 were detected. The base peak at mass to charge ratio *m/z* of 166 was formed by hydrogen transfer from the nitrogen group of the amide moiety to the ionized benzene ring followed by alpha cleavage. Also the formation of a peak at *m/z* at 181 can be explained by hydrogen transfer from the methyl group of the acetyl moiety to the ionized nitrogen

followed by alpha cleavage. As observed in Figure 4-6, the intensity of the molecular ion with *m/z* of 223 was relatively low in comparison with the other fragments due to fact that the EI source is known to result in fragmentation in comparison with other soft ionisation techniques such as chemical ionisation.



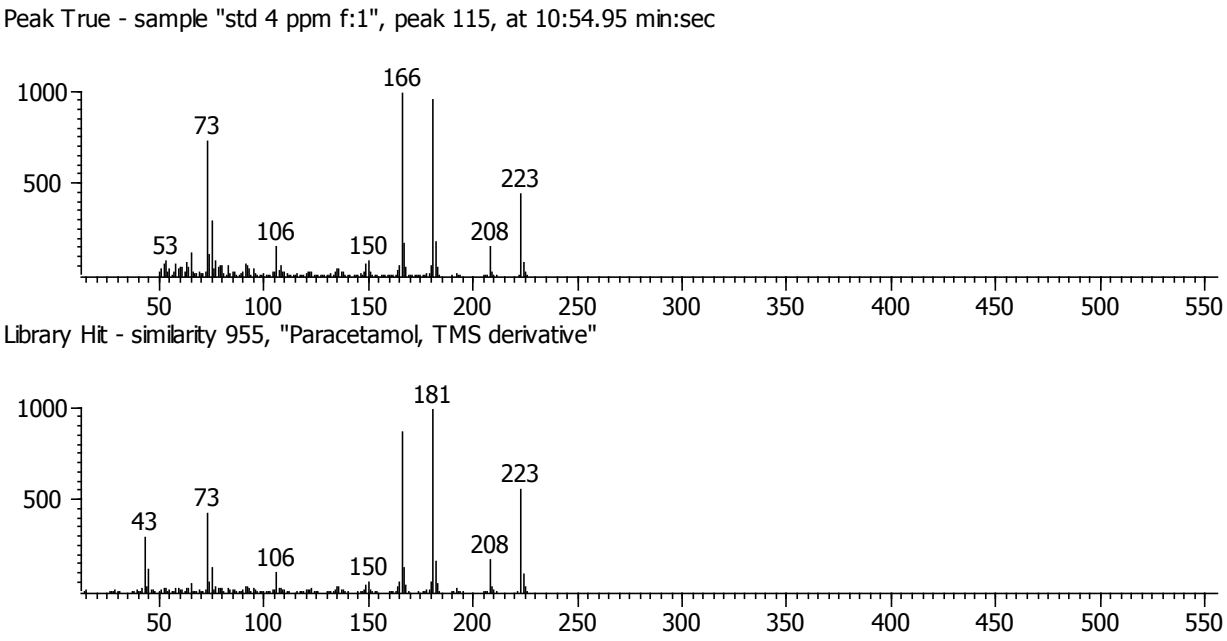
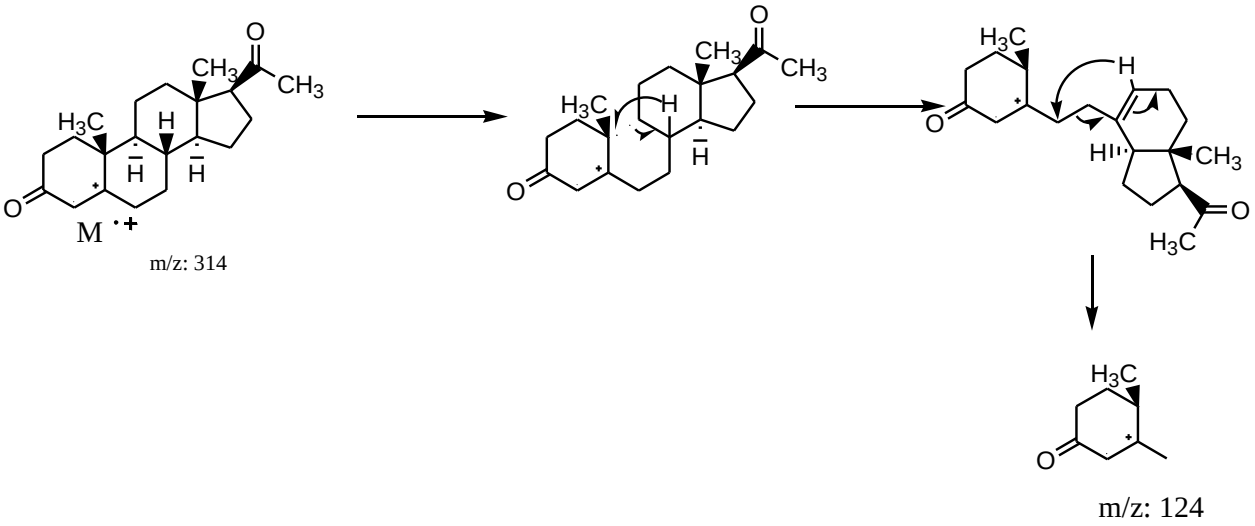


Figure 4-6 Proposed fragmentation mechanism, library hit and peak true of paracetamol-TMS derivative mass spectrum. The molecular ion *m/z* is 223

The molecular mass of progesterone is 314 Da and its structure has two ketone groups. The MS detection of progesterone showed a relatively low intensity of a charged molecular ion $[M]^{+\cdot}$ at *m/z* of 314(Figure 4.7). Homolytic σ-bond breakage followed by 2 consecutive H-radical rearrangement lead to a base peak ion at *m/z* 124 which is characteristic of α, β-unsaturated 3-keto steroids. Peaks at *m/z* 91 and *m/z* 105 were also observed.



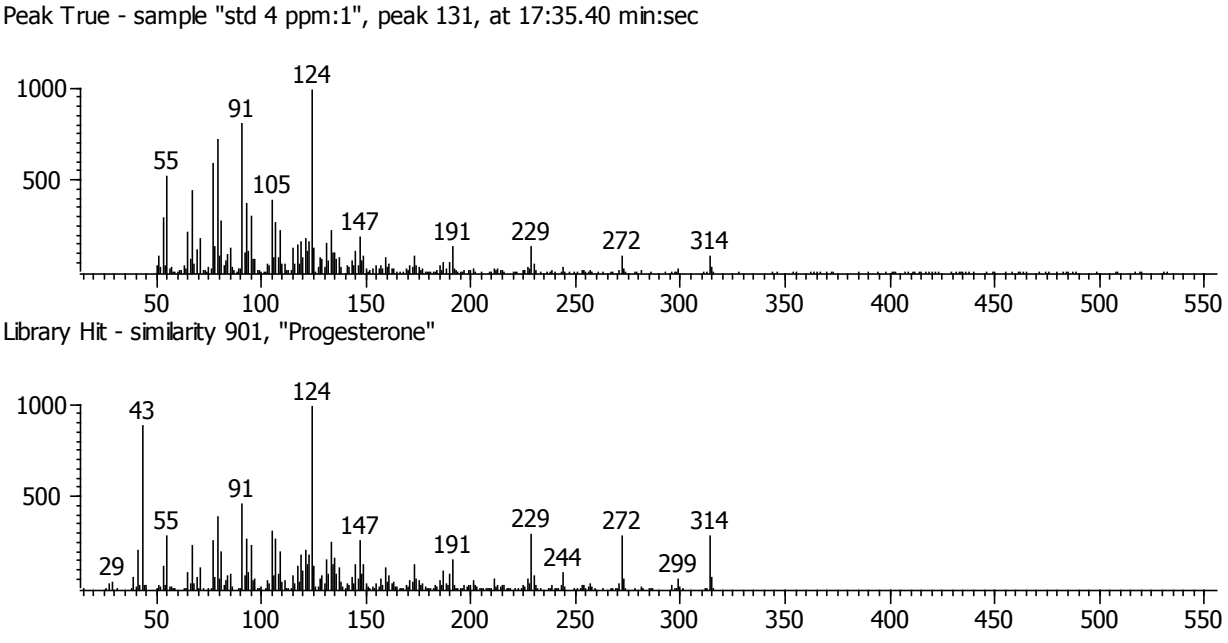
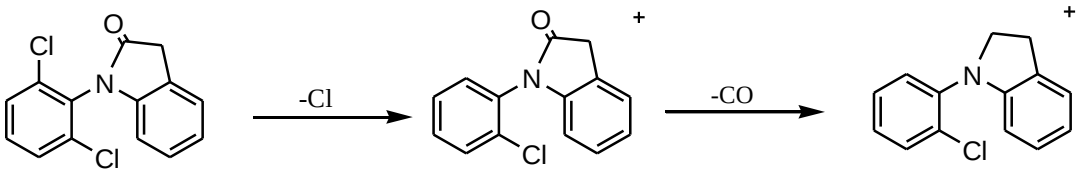


Figure 4-7: Proposed fragmentation mechanism, library hit and peak true of progesterone mass spectrum

According to European Pharmacopoeia 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one is derived from diclofenac either as an impurity during its synthesis, or may also be formed by the presence of the free OH radicals or as a resultant of thermal decomposition of diclofenac during GC analysis. It is formed by dehydration with simultaneous intramolecular lactame formation of diclofenac [103].

The molecular mass of 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one is 278.1334 Da. The structure of 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one has one ketone group and its MS detection showed one charged ion $[M-H]^+$ at *m/z* 277 (Figure 4.8). The MS spectrum also showed an ion at *m/z* 242 which originated from the loss of Cl from an ion at *m/z* 277 and additional neutral loss of CO (28 Da), produced a $[M-H-Cl-CO]^+$ ion at *m/z* 214 which is the most abundant product ion. Further cleavage of the second chlorine as a radical gave *m/z* 179, $[M-2Cl-CO]^+$.



M $^{\cdot +}$
m/z: 277

m/z: 242

m/z: 214

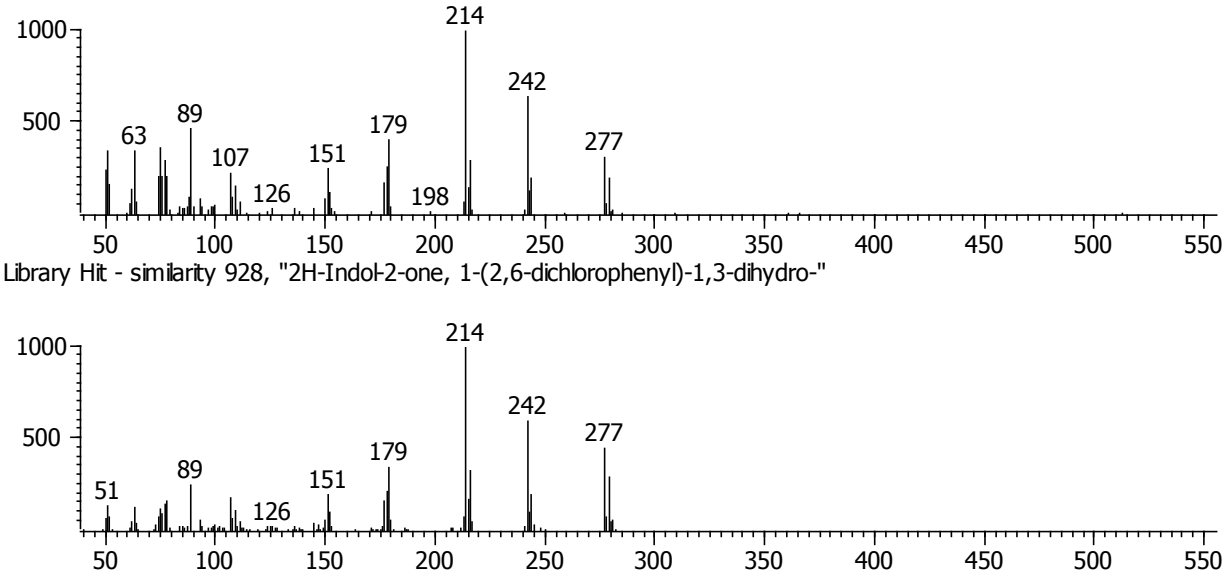


Figure 4-8 Proposed fragmentation mechanism, library hit and peak true of 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one mass spectrum

Table 4-4 Compounds of study with retention times, molecular mass of product ion and characteristic ions

Compounds	**Retention time (min)**	**Molecular weight after Derivatization (g/mol)**	**Quantitation and qualification ion (*m/z*)**
Ibuprofen *	9:58	278.47	117, **160**, 234
Paracetamol *	10:58	223.35	**166**, 181
Fenoprofen*	12:33	314.46	196, **270**, 314
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	13:45	278.13	179, **214**, 242
Bisphenol A *	13:59	372.67	**357**, 372
Primidone	14:36	218.25	**146**, 161, 190
Carbamazepine	14:55	236.27	165, **193**
Famciclovir	16:14	321.33	**136**, 262
α-Estradiol *	16:23	416.76	129, 232, **285**
β-Estradiol *	16:39	416.76	129, 232, **285**
Testosterone*	16:42	360.61	91, **129**
Progesterone	17:40	314.46	91, **124**, 314
Estriol *	18:08	504.95	129, 147, **311**
Anthracene	11:14	178.23	76, 152, **178**
* Trimethylsylil derivative			

The developed method was validated and used for quantitative determination of compounds of interest from the real samples.

4.3 Optimization of SPE procedure

Solid phase extraction was used to achieve effective sample clean-up and enrichment in this study. The physiochemical diversity of selected target compounds makes the extraction of these

compounds challenging. Therefore, there was a need to optimise the pH for overall extraction of the selected compounds. Waters Oasis HLB SPE was selected for this work. This SPE sorbent consists of, the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone groups which are stable from pH 1 to 14. The Oasis HLB mixed mode cartridges are excellent in the extraction of a variety of polar and non-polar compounds via their aromatic rings and hydrophilic groups [104].

The optimum pH for extraction of PPCPs from water was investigated at three pH levels (pH 5, 7 and 9, respectively) at a concentration level of 0.5 µg L^{-1}. Figure 4.9 shows the results of recoveries for the target PPCPs. All target PPCPs extracted well with recoveries of 77-106 %, 87 – 106 and 87 % – 112 % for pH 5, 7 and 9 respectively. At pH 5.0, basic PPCPs exist in their protonized form (pH< pK_a). The carbonyl group on N-vinylpyrrolidone react with the weakly nucleophilic water molecule and forms the positively polarized conjugate acid. The retention of the basic analytes is weakened by the charge-to-charge repulsion between the solutes and the sorbent, resulting in lower recoveries relative to pH 9.0 (see famciclovir). At pH 9.0, the acidic PPCPs are in their deprotonated form (pH> pK_a), and the sorbent forms the negatively charged alkoxide ion through the hydration. The retention of the acidic analytes is weakened by the charge-to-charge repulsion between the solutes and the sorbent, resulting in lower recoveries relative to pH 5.0 (see ibuprofen). The combination of different interactions, such as Van der Waals forces, hydrogen bonds and π interaction between the lipophilic divinylbenzene monomer of Waters Oasis HLB with analytes containing aromatic rings may explain the slightly improved recoveries observed at pH 9. From these results it was concluded that best extraction was under basic conditions, namely pH 9.

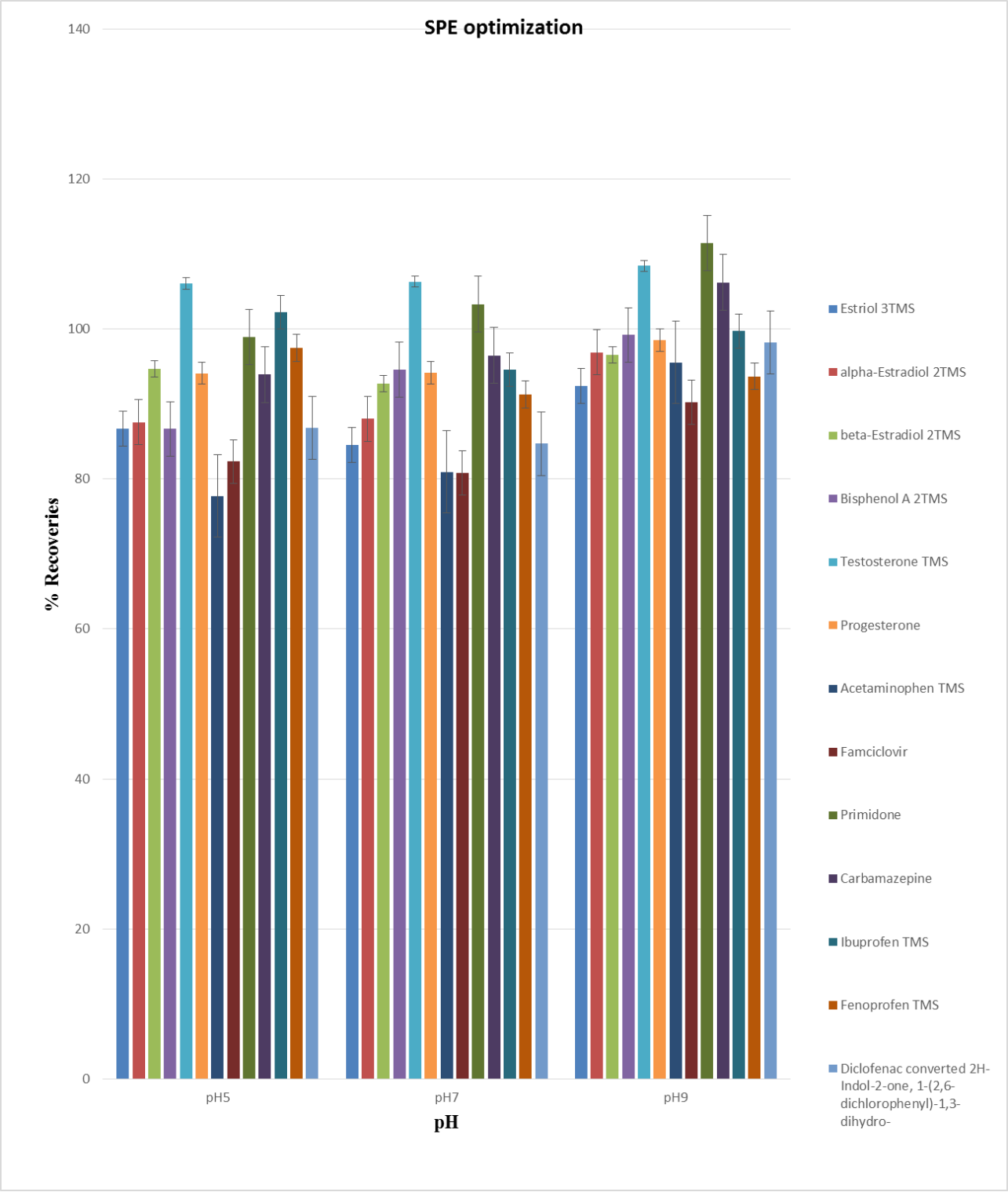


Figure 4-9 Influence of pH on the solid phase extraction of target pharmaceuticals

4.4 SPE method validation

4.4.1 Linearity, limit of detection, limit of quantitation, recovery and precision

The SPE method for the extraction of the target PPCPs was validated using seven concentration levels (n) calibration curves, and each point was replicated five times (m) and injected twice on the instrument. Table 4.5 shows validation data for the target PPCPs in this study. The correlation of determination (r^2) ranged from 0.9988 to 0.9999 and the calibration curves were linear in the concentration range of 0.2-10 µg L^{-1} for ibuprofen, 0.09-4 µg L^{-1} for paracetamol, 0.08-30 µg L^{-1} for fenoprofen, 0.45-14 µg L^{-1} for diclofenac (as 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one,), 0.1-6 µg L^{-1} for bisphenol A, 0.05-0.9 µg L^{-1} for primidone, 0.05-2 µg L^{-1} for carbamazepine, 0.5-10 µg L^{-1} for famciclovir, 0.09-1 µg L^{-1} for α-estradiol, β-estradiol, 3.5-12 µg L^{-1} for testosterone, 0.05-0.8 µg L^{-1} for progesterone and 0.03-0.35 µg L^{-1} for estriol. The limits of detection (LOD) and the limit of quantification (LOQ) ranged from 0.01-0.27 µg L^{-1} and 0.03 – 0.91 µg L^{-1} respectively for target PPCPs.

Table 4-5 Linear range, linearity, regression equation, limit of detection (LODs) and limit of quantitation (LOQs) of the analytes in the GC-TOFMS system

Compounds	**Linear range (µg/L)**	**Linearity (R^2)**	**Equation Y= mx + b**	**LODs (µg L^{-1})**	**LOQs (µg L^{-1})**
Ibuprofen*	0.2 - 10	0.9998	Y= 46.582 **x** - 876.015	0.11	0.36
Paracetamol*	0.09 - 30	0.9997	Y= 226.893 **x** - 4.189	0.08	0.27
Fenoprofen,	0.08 - 2	0.9998	Y= 9.403 **x** + 268.491	0.03	0.09
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	0.45 - 14	0.9994	Y= 8751.572 **x** - 405.275	0.18	0.60
Bisphenol A*	0.1 - 6	0.9999	Y= 394.437 **x** - 316.192	0.04	0.12
Primidone	0.05 - 0.9	0.9990	Y= 112.679 **x** - 992.393	0.03	0.11
Carbamazepine	0.05 – 2	0.9986	Y= 95.599 **x** - 187.254	0.03	0.11
Famciclovir	0.5 - 10	0.9993	Y= 4716.338 **x** - 1007.19	0.27	0.91
α-Estradiol*	0.09 - 1	0.9991	Y= 48.533 **x** + 552.283	0.03	0.11
β-Estradiol*	0.09 - 1	0.9997	Y= 50.168 **x** – 1048.48	0.02	0.05

Compounds	Linear range (µg/L)	Linearity (R^2)	Equation Y= mx + b	LODs (µg L^{-1})	LOQs (µg L^{-1})
Testosterone*	3.5 - 12	0.9993	Y= 97357.38 **x** + 1759.441	0.23	0.76
Progesterone	0.05 - 0.8	0.9988	Y= 33.151 **x** - 68.562	0.03	0.10
Estriol*	0.03 - 0.35	0.9994	Y= 16.514 **x** - 73.773	0.01	0.03
* Trimethylsilyl derivative					

Accuracy and precision were determined at three concentrations levels for each analyte: for example for ibuprofen at 0.4, 2 and 8 µg L^{-1}. The repeatability of the method was estimated from 2 consecutive injections of five replicates of a standard mixture of the thirteen PPCPs at the three concentrations levels (retention times, peak areas were determined). The same mixture of PPCPs was injected three times a days, for five consecutive days to determine reproducibility (see Table 4.6). Repeatability studies gave % RSD between 3.41 – 11.72 % for peak area. The % RSD values for reproducibility studies were 2.88 – 9.91 % for peak area over the three concentrations (ibuprofen: 0.4, 2 and 8 µg L^{-1}) evaluated during 5 days. These results indicated that the proposed method has excellent precision as evidenced by very stable peak area for the analytes.

Table 4-6 Intraday and inter day percentage relative standard deviation

Compounds	Intraday % RSD	Inter day % RSD
Ibuprofen, TMS derivative	5.28	4.46
Paracetamol, TMS derivative	6.44	5.44
Fenoprofen, TMS derivative	9.15	7.73
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	3.41	2.88
Bisphenol A, 2TMS derivative	5.08	4.30
Primidone	5.21	4.40
Carbamazepine	7.33	6.20
Famciclovir	10.67	9.02
α-Estradiol, 2TMS derivative	8.17	6.90
β-Estradiol, 2TMS derivative	8.78	7.42
Testosterone, TMS derivative	11.72	9.91
Progesterone	8.41	7.11
Estriol, 3TMS derivative	6.93	5.86

To further validate the precision and accuracy of the method, recovery testing was carried out by spiking a known amount of the standard mixture into tap water (250 ng L^{-1}) and Milli-Q water samples (also shown in Table 4.7 and Table 4.8) in five replicates. All spiked concentrations exhibited recoveries ranging from at least 82-115 % and 81-115 % in tap water and Milli-Q water respectively, with % RSD less than 12 %, showing that the overall PPCPs determination method including the extraction procedure was a repeatable method.

Table 4-7 Mean recoveries of analytes from tap water using SPE technique and % RSD values

Compounds	Tap Water	
	% Recovery	% RSD
Estriol (tri TMS)	102.20	0.8
α-Estradiol (di TMS)	104.89	1.7
β-Estradiol (di TMS)	99.74	0.1
Bisphenol A (di TMS)	104.49	1.6
Testosterone (mono TMS)	101.92	0.7
Progesterone	115.54	5.5
Acetaminophen (mono TMS)	82.37	6.2
Famciclovir	93.99	2.1
Primidone	112.41	4.4
Carbamazepine	102.65	0.9
Fenoprofen (mono TMS)	114.69	5.2
Ibuprofen (mono TMS)	96.72	1.2
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	99.14	0.3

Table 4-8 Mean recoveries of analytes from Milli-Q water using SPE technique and % RSD values n=5

Milli-Q water		
Compounds	**Spiking levels**	**% Recoveries (%RSD)**
Ibuprofen, TMS derivative	200 ng L^{-1} 400 ng L^{-1} 800 ng L^{-1}	115.41 (5.3) 101.56 (0.3) 93.58 (1.4)
Paracetamol, TMS derivative	87,5 ng L^{-1} 175 ng L^{-1} 262,5 ng L^{-1}	81.23 (9.6) 111.96 (7.8) 98.94 (3.4)
Fenoprofen, TMS derivative	225 ng L^{-1} 450 ng L^{-1} 675 ng L^{-1}	96.18 (6.1) 100.68 (0.9) 100.29 (4.5)
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	0,9 ng L^{-1} 1,8 ng L^{-1} 2,7 ng L^{-1}	103.59 (8.1) 100.51(7.0) 100.29 (0.4)
Bisphenol A, 2TMS derivative	100 ng L^{-1} 200 ng L^{-1} 300 ng L^{-1}	87.05 (4.6) 108.22 (6.2) 96.12 (3.3)
Primidone	150 ng L^{-1} 200 ng L^{-1} 300 ng L^{-1}	100.54 (5.3) 99.36 (3.1) 94.01 (0.07)
Carbamazepine	150 ng L^{-1} 200 ng L^{-1} 300 ng L^{-1}	104.40 (10.9) 100.28 (5.7) 101.80 (3.3)
Famciclovir	1 µg L^{-1} 1,5 µg L^{-1} 3 µg L^{-1}	102.99 (1.8) 97.78 (1.6) 95.46 (2.2)
α-Estradiol, 2TMS derivative	175 ng L^{-1} 262,5 ng L^{-1} 350 ng L^{-1}	109.54 (7.4) 94.17 (6.9) 99.33 (8.1)
β-Estradiol, 2TMS derivative	87,5 ng L^{-1} 175 ng L^{-1} 262,5 ng L^{-1}	110.11 (9.7) 96.10 (7.6) 99.72 (3.2)
Testosterone, TMS derivative	3,5 µg L^{-1} 5 µg L^{-1} 7 µg L^{-1}	99.44 (4.0) 101.79 (8.9) 99.32 (1.4)
Progesterone	105 ng L^{-1} 157,5 ng L^{-1} 200 ng L^{-1}	98.21 (3.8) 94.20 (2.3) 99.52 (6.6)
Estriol, 3TMS derivative	50 ng L^{-1} 75 ng L^{-1} 100 ng L^{-1}	102.19 (5.5) 95.58 (1.8) 98.90 (3.8)

4.5 Application of the analytical method to the investigation of wastewater samples

The developed method was applied to the determination of the target compounds in influent and effluent wastewater from the Daspoort wastewater treatment plant in Pretoria (South Africa). Figure 4.10 shows an example of GC-TOFMS selected ion chromatogram of wastewater sample and the target compounds detected. The findings of the study are presented in Table 4.9, 4.10 and 4.11. Five of the thirteen investigated pharmaceuticals were found in influent wastewater samples from the eastern work (Influent A), while four were detected in influent wastewater from the western works (Influent B) and five were found in the treated effluent wastewater.

The highest concentration was found for paracetamol (135.42 μg L^{-1}) in untreated wastewater. The most commonly detected compounds in wastewater sample were bisphenol A, acetaminophen and ibuprofen. Interestingly, the absence of acetaminophen was noticeable in the effluent of WWTP. Unfortunately, the influent and effluent of the WWTPs cannot be linked together since it is not possible to analyse the same influent wastewater as effluent. However, it is worthy to note that there seems to be a reduction of acetaminophen by WWTP.

The photo transformation products of diclofenac, 1-(2, 6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one were detected mostly in effluent wastewater. Primidone and carbamazepine were also detected in influent and effluent wastewater. The oestrogenic hormones estriol, α-estradiol, β-estradiol, testosterone, progesterone, and the antiretroviral famciclovir were not detected in all the samples analysed.

Table 4-9 Concentration of pharmaceuticals in influent wastewater samples from Daspoort municipal WWTP in Pretoria (South Africa)

Influent A (concentration in µg L^{-1})									
samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Pharmaceuticals									
Estriol (tri TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
α-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
β-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Bisphenol A (di TMS)	0.36	2.92	5.89	3.89	0.78	2.48	2.31	8.07	7.42
Testosterone(mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Progesterone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Acetaminophen(mono TMS)	6.59	46.62	43.81	37.38	39.41	59.66		82.37	
Famciclovir	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Primidone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Carbamazepine	5.07	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fenoprofen (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ibuprofen (mono TMS)	2.49	5.12	n.d	n.d	14.71	45.27	n.d	n.d	n.d
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	17.54	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d, not detected (below LOD)

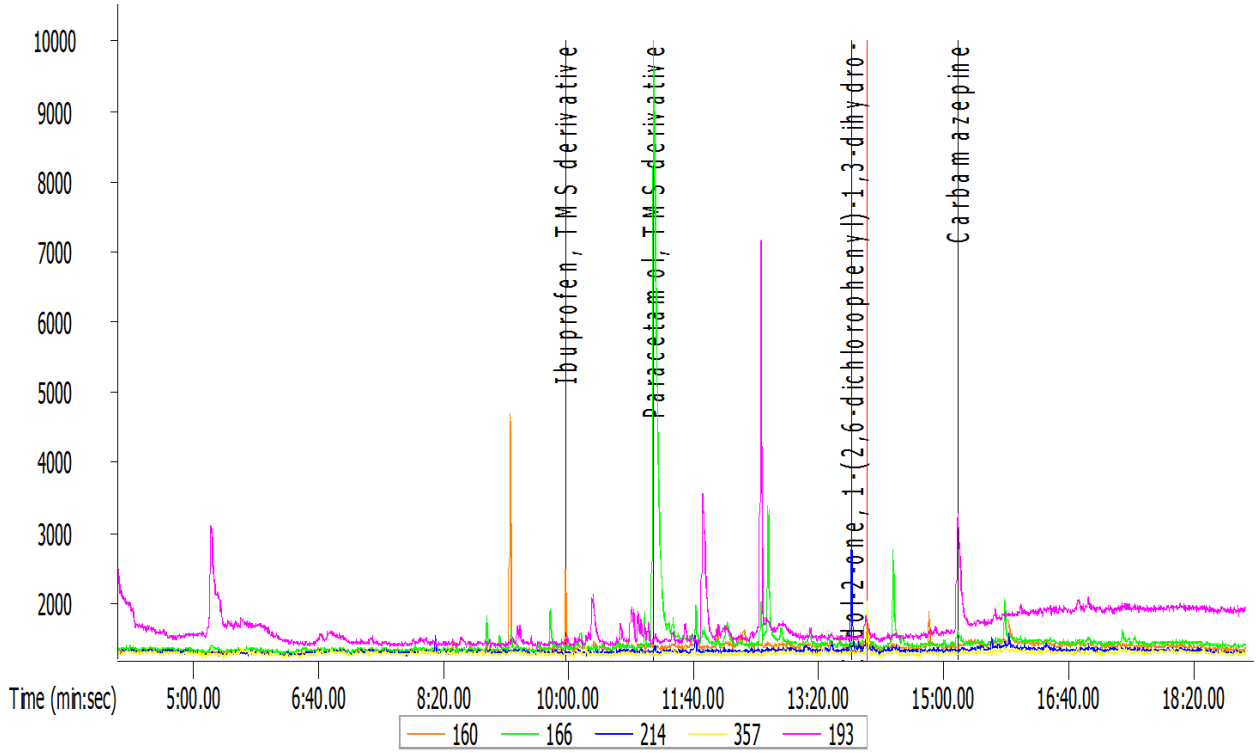


Figure 4-10 Selected ion chromatogram from the influent wastewater (influent A sample 1)

Table 4-10 Concentration of pharmaceuticals in influent wastewater samples from Daspoort municipal WWTP in Pretoria (South Africa)

Influent B (concentration in µg L^{-1})									
samples	**1**	**2**	**3**	**3**	**5**	**6**	**7**	**8**	**9**
Pharmaceuticals									
Estriol (tri TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
α-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
β-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Bisphenol A (di TMS)	0.28	1.04	5.20	6.29	0.94	2.08	2.81	4.72	4.18
Testosterone (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Progesterone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Acetaminophen (mono TMS)	21.06	54.68	77.38	67.18	47.01	135.42		103.97	
Famciclovir	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Primidone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Carbamazepine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fenoprofen (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ibuprofen (mono TMS)	1.77	7.70	n.d	n.d	12.04	7.99	n.d	n.d	n.d
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	11,72	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d, not detected (below LOD)

Table 4-11 Concentration of pharmaceuticals in effluent wastewater samples from Daspoort municipal WWTP in Pretoria (South Africa)

Effluent (concentration in µg L^{-1})									
samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Pharmaceuticals									
Estriol (tri TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
α-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
β-Estradiol (di TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Bisphenol A (di TMS)	0.052 < LOQ	0.066 < LOQ	0.435	0.290	0.089 < LOQ	1.601	0.816	1.592	0.548
Testosterone (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Progesterone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Acetaminophen (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Famciclovir	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Primidone	0.76	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Carbamazepine	1.85	n.d	n.d	n.d	1.24	n.d	n.d	3.71	n.d
Fenoprofen (mono TMS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ibuprofen (mono TMS)	0.78	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-Indol-2-one	27.30	3.12	n.d	n.d	6.13	n.d	n.d	n.d	10.51

n.d, not detected (below LOD)

4.5.1 Detection of non-targeted-compounds in wastewater

The aim of the project was to develop a method of determination of 13 targeted PPCPs in wastewater using GC-MS via derivatization. However, in the processing of analysis of wastewater several other non-targeted compounds were detected. Table 4-12 shows several compounds detected in both influent and effluent wastewater.

Table 4-12 Non targeted compounds detected in influent and effluent wastewater through the developed GC-MS method

#	Name	Use	Source	Ref.
1	Tyrosol	natural phenolic antioxidant	Effluent	[105]
2	Acetin	cosmetic agents	Effluent	[106]
3	Diethyltoluamide	insect repellent	Influent A, Effluent	[107]
4	Benzophenone	fragrance enhancer, ultraviolet curing agent	Effluent	[108]
5	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	fragrances, shampoos, toilet soaps	Effluent	[109]
6	Butylparaben	Flavouring agent; Preservative	Effluent	[110]
7	Methanone, (1-hydroxycyclohexyl)phenyl-	binding agents	Influent B	[111]
8	4-tert-Amylphenol	virucidal, fungicidal, bactericidal, pseudomonacidal, or staphylocidal	Influent A	[112]
9	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	Synthetic musk	Influent A	[113]
10	2-Propanol, 1-chloro-, phosphate (3:1)	flame retardant	Effluent	[114]
11	Caffeine	Central nervous system stimulant	Influent A, Influent B, Effluent	[115]
12	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	plasticizer	Influent A, Influent B, Effluent	[116]
13	Methocarbamol	muscle relaxant	Effluent	[117]
14	Bumetrizole	UV absorber	Effluent	[118]
15	Efavirenz	antiviral	Effluent	[119]
16	Bis(2-ethylhexyl) phthalate	plasticizer	Influent A, Influent B, Effluent	[120]
17	Spiroxamine	fungicide	Effluent	[121]

#	Name	Use	Source	Ref.
18	Terbuthylazine	herbicide	Effluent	[122]
19	Atrazine	herbicide	Effluent	[123]
20	Methaqualone (Quaaludes)	anxiolytic and a sedative-hypnotic drug	Effluent	[124]
21	Dioxaphetyl butyrate	opioid analgesic	Effluent	[125]
22	Nevirapine	antiviral	Effluent	[126]
23	Androsta-1,4-diene-3,17-dione	Exogenous anabolic androgenic steroid	Effluent	[127]
24	20á-Progerol	metabolite of progesterone	Influent A , Influent B, Effluent	[128]
25	Behenic alcohol	Binder; Emulsion Stabilizer; Viscosity Increasing Agent		[129]
26	cis-Tramadol	Opioid analgesic.	Influent B	[130]
27	Dicyclomine	antispasmodic and anticholinergic agent	Influent B	[131]
28	2(3H)-Furanone, 5-hexyldihydro-	Flavouring agent	Influent A , Influent B	[132]
29	Batyl alcohol	Skin-conditioning agent	Influent B	[133]
30	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	Sunscreen	Influent B	[134]
40	Monolaurin	food additive, cosmetics	Influent A	[135]
41	Paredrine	Sympathomimetic agent	Influent A , Influent B	[136]
42	4-tert-Octylphenol	adjuvants for pesticide use dilutions	Influent A , Influent B	[137]
43	Benzyl Benzoate	Scabicide	Influent A	[138]
44	Linolool oxide, (Z)	Flavouring ingredient	Influent A , Influent B	[139]
45	Propylparaben	antimicrobial preservative	Influent A, Influent B	[140]
46	Butoxytriglycol	Anti-Freeze and De-icing Products	Influent A	[141]

#	Name	Use	Source	Ref.
47	Farnesol, (E,E)-	perfume	Influent A	[142]
48	Eugenol	perfumeries, flavorings, essential oils ,local antiseptic and anaesthetic.	Influent A	[143]
49	Guaifenesin	expectorant	Influent B	[144]
50	Stigmasterol	food additive, cholesterol-lowering properties	Effluent	[145]
51	Nandrolone	Anabolic androgenic steroid	Effluent	[146]
52	5à-Androstan-3à-ol-17-one	metabolite of testosterone and dihy drotestosterone	Influent A , Influent B	[147]
53	Terbutol	Herbicide	Effluent	[148]
54	Methanone, (4 methylphenyl)phenyl-	stabilizing agents	Effluent	[149]
55	Benzo[h]quinoline	Carcinogenic aza-arenes in the environmental pollutants such as cigarette smoke and urban air	Effluent	[150]
56	Benzo[f]quinoline	Carcinogenic aza-arenes in the environmental pollutants such as cigarette smoke and urban air	Effluent	[150]
57	Dibutyl phthalate	plasticizer	Influent A , Influent B	[151]
58	Benzyl butyl phthalate	Adhesives and Sealants, plasticizer, Floor Coverings	Influent A	[152]
59	Triphenyl phosphate	flame retardant	Effluent	[153]
60	Ethanol, 2-butoxy-, phosphate (3:1)	Fungicide , Microbiocide	Influent A, Effluent	[154]
61	Cholesterol	precursor for the biosynthesis of steroid hormones	Influent A , Influent B	[155]
62	Androstane-3,17-diol,	major metabolite of testosterone with androgenic activity	Influent A , Influent B	[156]

#	Name	Use	Source	Ref.
63	Squalene	component of some adjuvants that are added to vaccines to enhance the immune response	Influent A , Influent B	[157]
64	Aminocaproic acid	antifibrinolytic agent	Effluent	[158]
65	Tributyl acetylcitrate	flavor and fragrance agents	Influent A	[159]
66	11-Ketoetiocholanolone	endogenous anabolic androgenic steroid	Influent A , Influent B	[160]
67	2(3H)-Furanone, 5-dodecyldihydro-	flavoring agents	Effluent	[161]
68	Benadryl	antihistamine	Effluent	[162]
69	Benzene ethanamine, N[(pentafluorophenyl)methylene]	potential biomarkers for kidney diseases for distinguishing between mesangial proliferative glomerulonephritis patients and IgA nephropathy patients	Influent A, Influent B,	[163]
70	Carbamazepine-10,11-dihydrodiol	Unconjugated metabolite of carbamazepine	Effluent	[164]
71	Benzedrex	Decongestants	Influent A	[165]
72	Ephedrine	Decongestant, bronchodilator	Influent A	[166]
73	Pseudoephedrine	Decongestant	Influent B	[167]
74	Phentermine	Appetite suppresssant	Influent A	[168]
76	Cetrimonium Bromide	Antistatic agent, cosmetic biocide	Influent A	[169]
77	4-tert-Amylphenol	M, Germicide	Influent A	[170]
78	Butanedioic acid, diethyl ether	Flavour ingredient	Influent A	[171]
79	Oxomemazine	Sedative agent, antiallergic	Influent A	[172]

CHAPTER 5 CONCLUSIONS

A multi-residue analytical method based on GC/MS –ToF was successfully developed and validated for the simultaneous determination of thirteen selected PPCPs in wastewater samples. Accuracy and precision were determined at three analyte concentrations levels and these results indicated that the proposed method has excellent precision as evidenced by very stable peak area for the analytes. The limits of detection (LOD) and the limit of quantification (LOQ) for the target analytes were as follow: ibuprofen 0.11 µg L^{-1}, paracetamol 0.08 µg L^{-1}, fenoprofen 0.03 µg L^{-1}, 2h-indol-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro 0.18 µg L^{-1}, bisphenol a 0.04 µg L^{-1}, primidone 0.03 µg L^{-1}, carbamazepine 0.03 µg L^{-1}, famciclovir 0.27 µg L^{-1}, α-estradiol 0.03 µg L^{-1}, β-estradiol 0.02 µg L^{-1}, testosterone 0.23 µg L^{-1}, progesterone 0.03 µg $L^{-1}$and estriol 0.01 µg L^{-1}. Fortification experiments were carried out on both tap water and UHP water and exhibited recoveries ranging from at least 82-115 % and 81-115 % in tap water and Milli-Q water respectively, with % RSD less than 12 %, showing that the overall PPCPs determination method including the extraction procedure was a repeatable method.

Solid phase extraction was used to achieve effective sample clean-up and enrichment in this study. The pH of 1 L of UHP water was adjusted to pH 5, 7 and 9, respectively, using hydrochloric acid 32 % and ammonia solution 25 %. Subsequently, 200 mL of the pH adjusted UHP water was spiked with a standard mixture of the pharmaceuticals containing each target compound, so that the concentration of each analytes in the spiked water was 0.5 µg L^{-1}. The water sample was extracted using Waters Oasis HLB cartridges (12 cc, 500 mg). All the target PPCPs extracted well with recoveries of 77-106 %, 87 – 106 and 87 % – 112 % for pH 5, 7 and 9 respectively

The optimal derivatization conditions were determined systematically by investigating the effects of reaction time, temperature, and catalyst volume on the final derivatization output. . First the effect of 5, 10, 20, 50 % pyridine in ethyl acetate, as a catalyst for the derivatization was inspected, then the effect of incubation time on the silylation was studied at 30 min, 45 min and 60 min. Finally the effect of incubation temperature on the silylation was examined at 60 °C, 90 °C and 120 °C. The optimal derivatization conditions were as follow: 10 % pyridine in ethyl acetate, 30 min and 90 °C as incubation time and temperature respectively.

The developed analytical method was successfully applied to wastewater influent and effluent from Daspoort Waste Water Treatment Works, where compounds such as bisphenol A, primidone, carbamazepine, acetaminophen, ibuprofen and diclofenac were identified as the most common contaminants, and thus should be of more concern

Fve out of the targeted thirteen PPCPs (bisphenol A, acetaminophen, carbamazepine, ibuprofen and diclofenac) were detected from eastern influent of the WWTP at concentrations ranging from 0.36 µg L^{-1} to 82.37 µg L^{-1}. Three of the target PPCPs out of thirteen (bisphenol A, acetaminophen, ibuprofen and diclofenac) were detected at concentrations ranging from 0.28 µg L^{-1} to 135.42 µg L^{-1} from the western works influent.

Five of the thirteen selected PPCPs (bisphenol A, primidone, carbamazepine, ibuprofen and diclofenac) were measured at concentrations ranging from < 0.052 µg L^{-1} to 27.3 µg L^{-1} from the effluent samples This demonstrated that the treatment process reduce significantly the quantity of

PPCPs in sewage water but does not necessary eliminate all the compounds. The anticonvulsant primidone was detected in one instance in effluent wastewater. Since acetaminophen was not detected in all effluent samples, we could infer that it might have been completely eliminated during the treatment processes.

The natural androgen testosterone and natural oestrogen, estriol, α-estradiol, β-estradiol, progesterone, and the antiretroviral famciclovir were not detected in all the sewage samples analysed. Several other compounds that were not preselected were found in wastewater influent and effluent such as benzophenone, caffeine, methocarbamol, efavirenz, atrazine, dioxaphetyl butyrate, nevirapine, androsta-1,4-diene-3,17-dione, cis-tramadol, batyl alcohol, paredrine, 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin, propylparaben, eugenol, cholesterol, stigmasterol, guaifenesin, benzyl benzoate, 4-tert-octylphenol, diethyltoluamide, dicyclomine, terbuthylazine, spiroxamine, bis(2-ethylhexyl) phthalate, bumetrizole with a $\geq$ 90 % probability library match.

5.1 Recommendation for further Studies

During the present study, some areas of significant interest were revealed for future research. This study provided details on several other class of contaminants that were recurrently detected in wastewater. These compounds belonged to various group such as antihistamine and antiviral drug, flavouring agent, flame retardants, UV absorber, fragrance agent, pesticide and synthetic musk. Future research should be directed towards studying a wider range of pollutants.

The use of passive sampling technology should be implemented for the continuous monitoring of emerging contaminants in order to have information on their occurrence especially since their concentrations in wastewater and receiving surface water fluctuate substantially.

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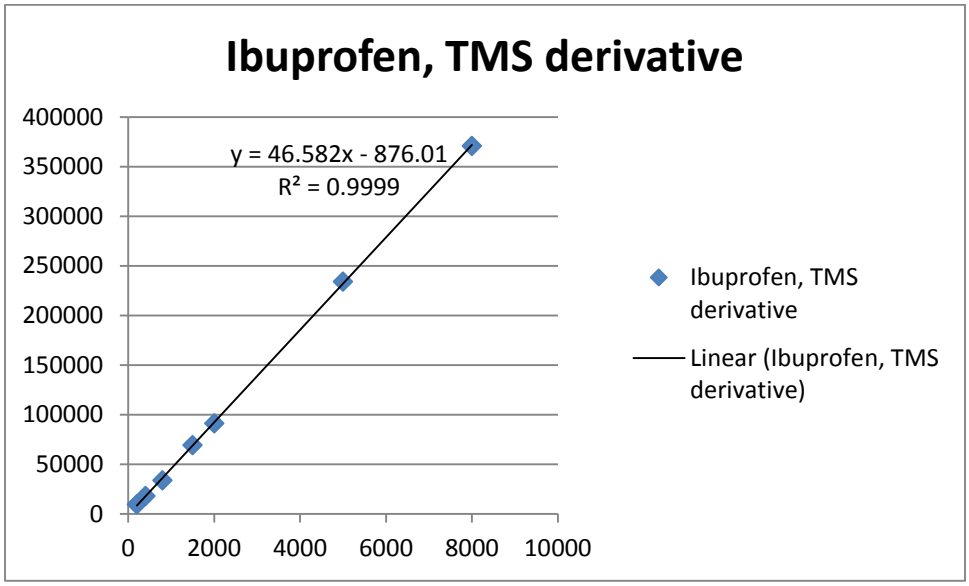
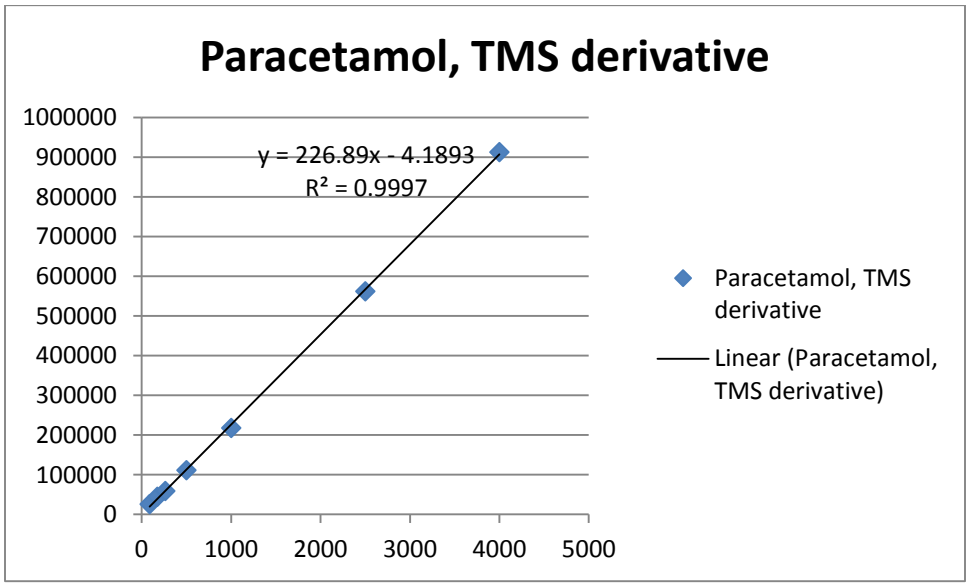
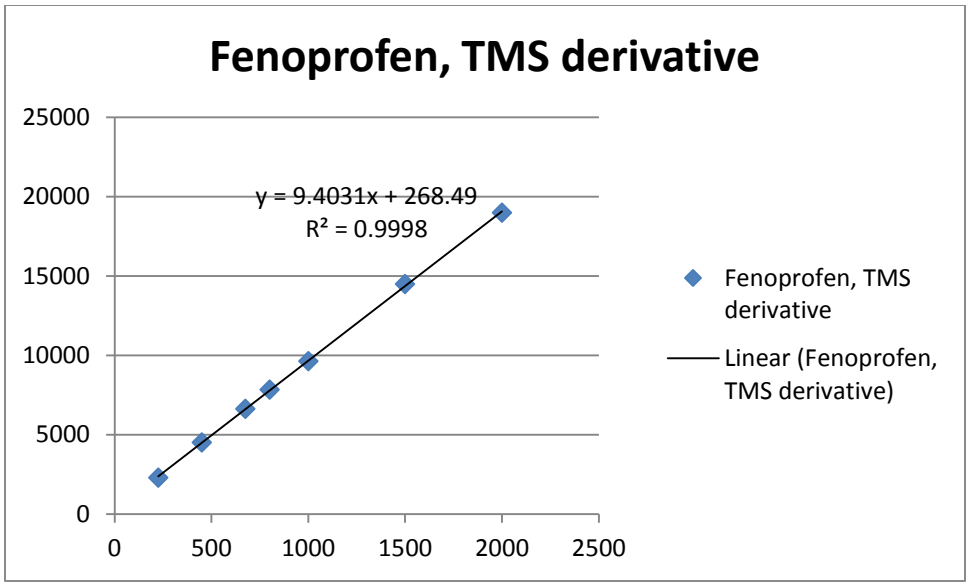
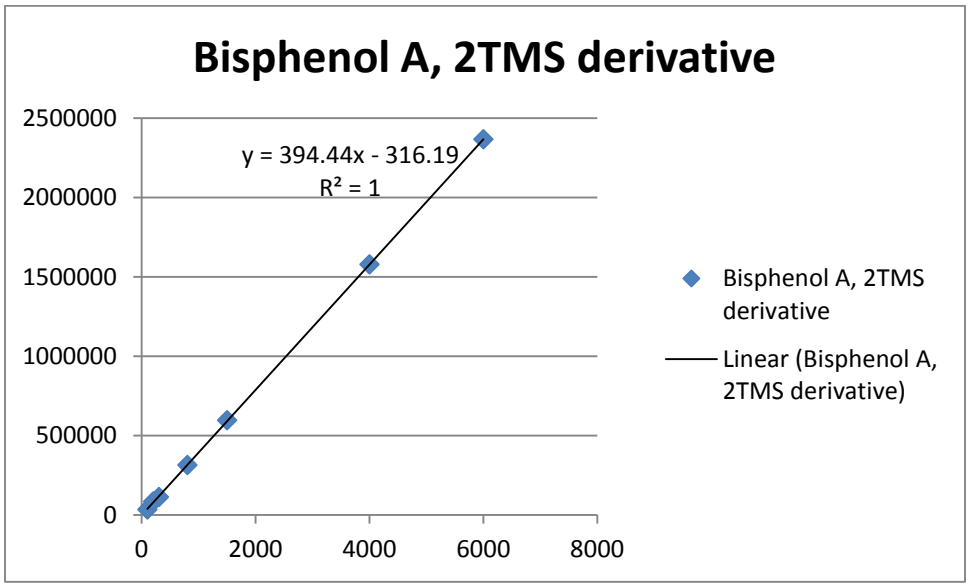
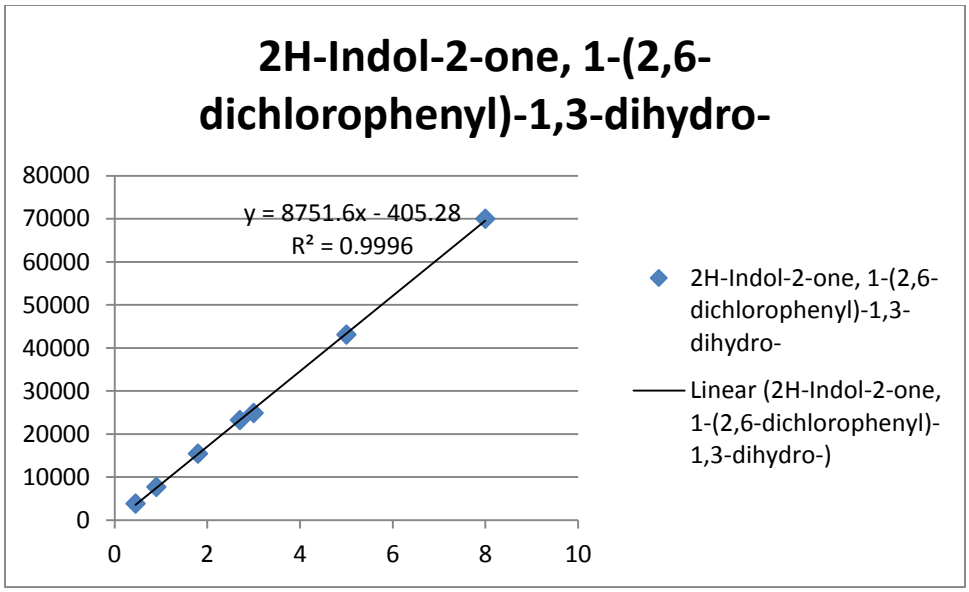
Appendix A. Calibration curves

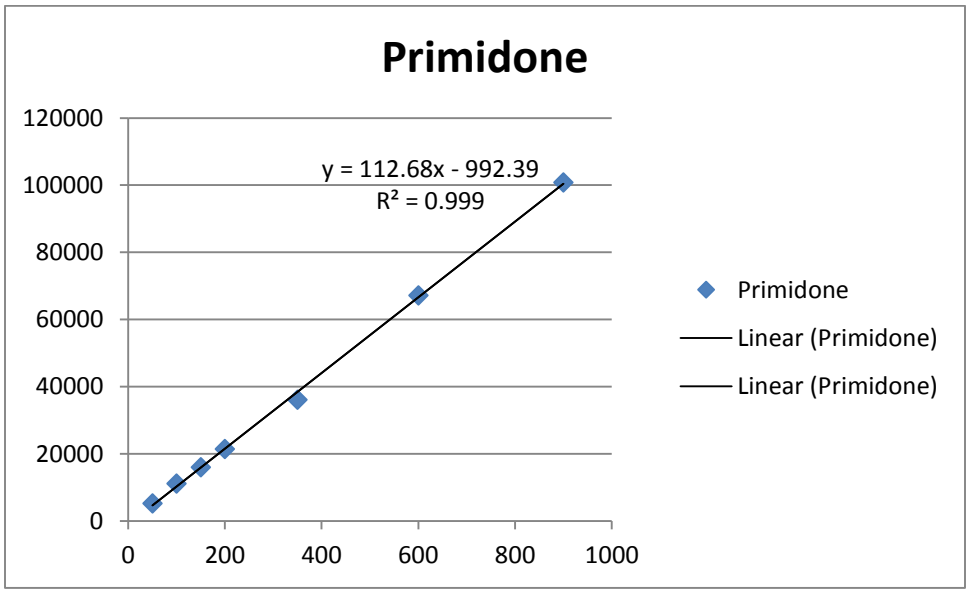


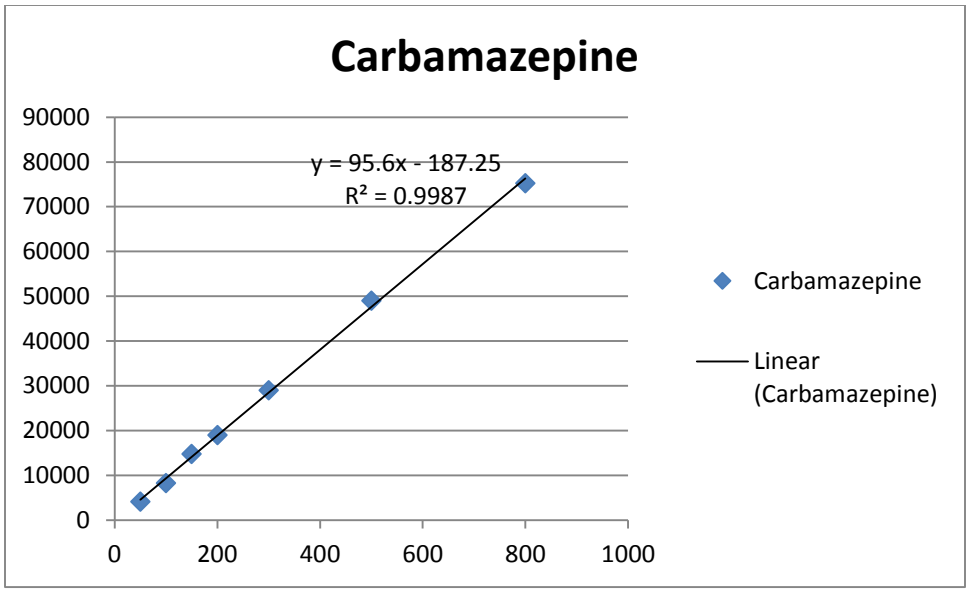


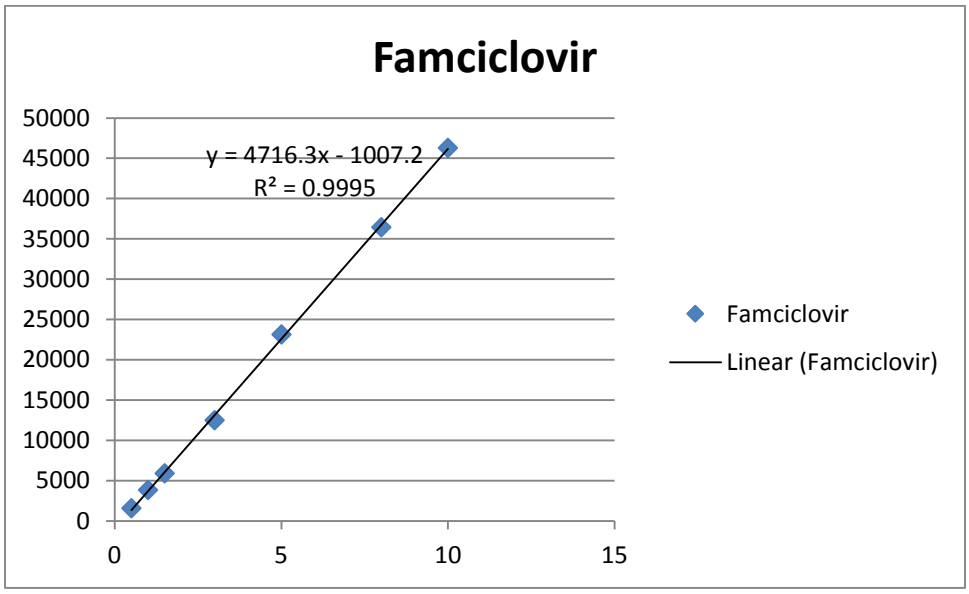

Fenoprofen, TMS derivative
25000
20000
15000
10000
5000
0
0
500
1000
1500
2000
2500
y = 9.4031x + 268.49
R² = 0.9998
Fenoprofen, TMS derivative
Linear (Fenoprofen, TMS derivative)

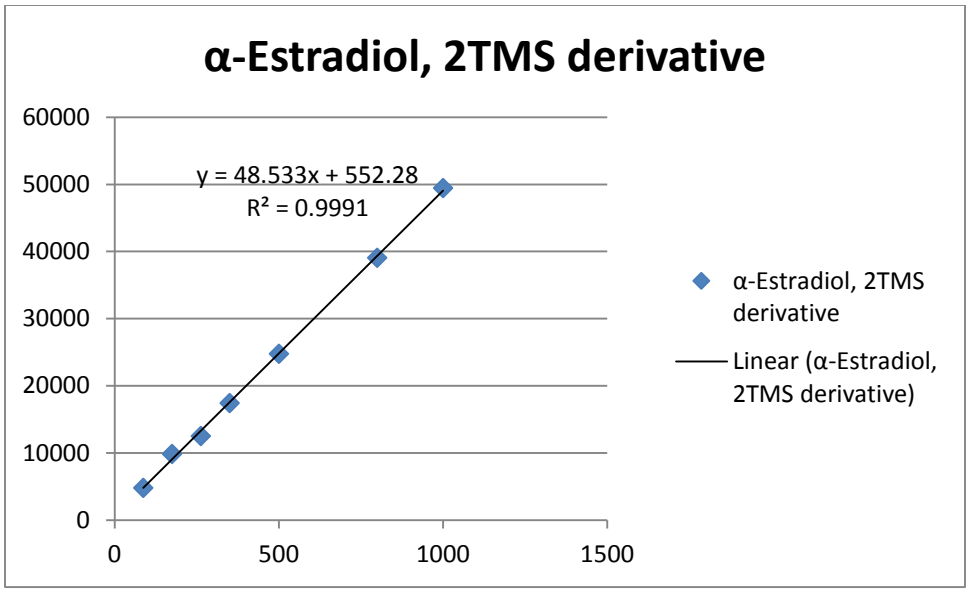

Bisphenol A, 2TMS derivative
2500000
2000000
1500000
1000000
500000
0
0
2000
4000
6000
8000
y = 394.44x - 316.19
R² = 1
Bisphenol A, 2TMS derivative
Linear (Bisphenol A, 2TMS derivative)

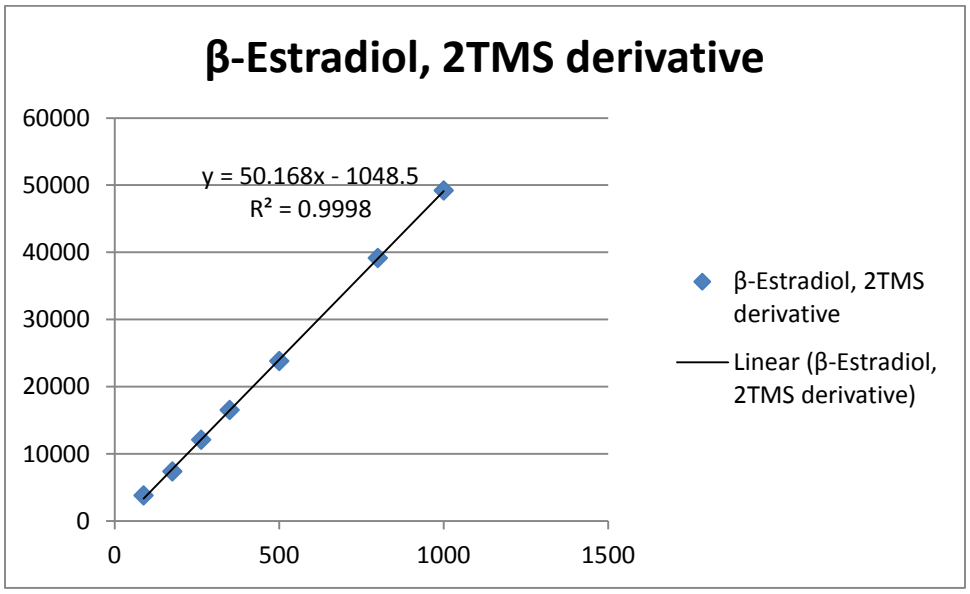

2H-Indol-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro-
y = 8751.6x - 405.28
R² = 0.9996
80000
70000
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
2H-Indol-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro-
Linear (2H-Indol-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro-)

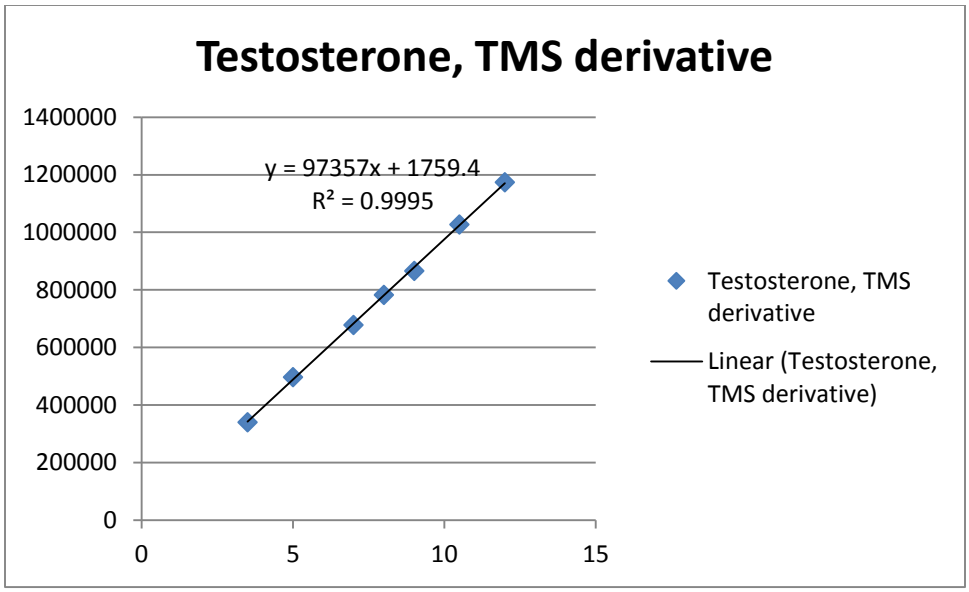

Primidone
y = 112.68x - 992.39
R² = 0.999
120000
100000
80000
60000
40000
20000
0
0
200
400
600
800
1000
Primidone
Linear (Primidone)
Linear (Primidone)

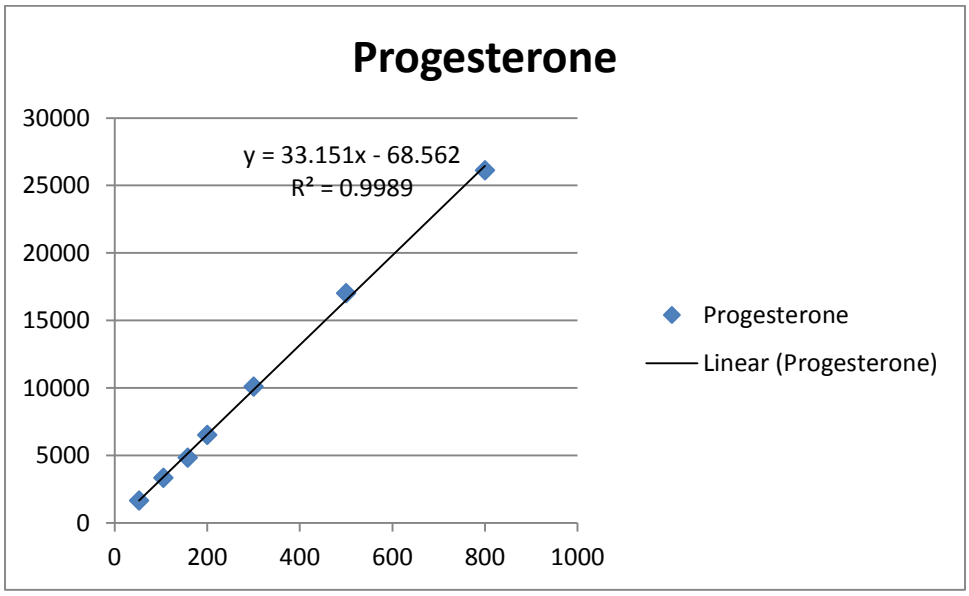

Carbamazepine
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
200
400
600
800
1000
y = 95.6x - 187.25
R² = 0.9987
Carbamazepine
Linear (Carbamazepine)

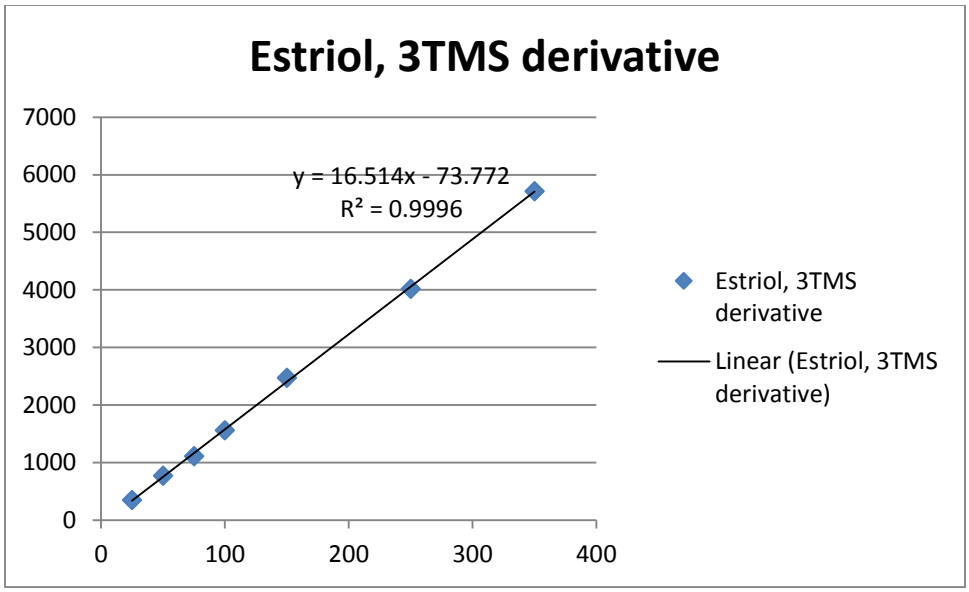

Famciclovir
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
5
10
15
y = 4716.3x - 1007.2
R² = 0.9995
Famciclovir
Linear (Famciclovir)


α-Estradiol, 2TMS derivative
60000
50000
40000
30000
20000
10000
0
0
500
1000
1500
y = 48.533x + 552.28
R² = 0.9991
α-Estradiol, 2TMS derivative
Linear (α-Estradiol, 2TMS derivative)


β-Estradiol, 2TMS derivative
60000
50000
40000
30000
20000
10000
0
0
500
1000
1500
y = 50.168x - 1048.5
R² = 0.9998
β-Estradiol, 2TMS derivative
Linear (β-Estradiol, 2TMS derivative)


Testosterone, TMS derivative
1400000
1200000
1000000
800000
600000
400000
200000
0
0
5
10
15
y = 97357x + 1759.4
R² = 0.9995
Testosterone, TMS derivative
Linear (Testosterone, TMS derivative)


Progesterone
30000
25000
20000
15000
10000
5000
0
0
200
400
600
800
1000
y = 33.151x - 68.562
R² = 0.9989
Progesterone
Linear (Progesterone)


Estriol, 3TMS derivative
7000
6000
5000
4000
3000
2000
1000
0
0
100
200
300
400
y = 16.514x - 73.772
R² = 0.9996
Estriol, 3TMS derivative
Linear (Estriol, 3TMS derivative)