DETECTION AND QUANTIFICATION OF EMERGING MICRO-POLLUTANTS USING CAPILLARY ELECTROPHORESIS

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

Detection and quantification of pollutants in water are essential in giving a clearer picture on how safe our water is. Over the decades, many classes of pollutants such as phenolic compounds, pharmaceuticals and personal care products (PPCP), endocrine disrupting compounds (EDCs), phthalates, flame retardants, perfluorinated amongst others, have been reported to be present in water. These classes of contaminants are also known as emerging contaminants. Many of these contaminants are detected using methods such high-performance liquid chromatography (HPLC), gas chromatography and liquid chromatography coupled with mass spectrometry (GC-MS, LC-MS), electrochemistry and spectrophotometry. These techniques, however, require complex sample preparation processes because it is difficult to identify or quantify pollutants at low concentrations, thus requiring pre-concentration and sample clean-up. These limitations have led to the exploration of capillary electrophoresis (CE) as a technique that could eliminate some of the complexity and costs of analysis.

Electrophoresis is the migration of charged particles or molecules in a medium under the influence of an applied electric field. Literature shows that some advantages of CE over traditional analytical techniques include: analysis of solutes with limited UV chromophores, a reduced method development time, reduced operating costs, low solvent consumption and higher separation efficiencies. Other specific advantages are (i) the limit of detection of CE is one-thousandth that of GC or HPLC, (ii) the capillaries can also be conditioned easily with buffer before the analysis starts, and (iii) with CE, nearly one-thousandth of the solvent is used compared with HPLC. However, the method should be further developed for the identification of all types of emerging contaminants. This study covered characterisation of water samples using LC-MS, and capillary electrophoresis, including optimisation and method validation. During the extraction and recovery process the SPE cartridge eluant was also checked and it was shown that the eluant used (water to methanol ratio) could be the cause of the loss of more polar compounds such as acetaminophen during sample extraction protocols, giving false negative results.

Capillary electrophoresis methods were developed to identify and quantify specific pharmaceutical compounds (including acetaminophen, diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole) present in the influent and effluent of sewage water treatment plants, environmental water samples, including drinking water supplied to homes. CE was also used to identify and quantify the steroid hormone compounds (including androstenedione, testosterone, 17-ß-estradiol and progesterone) present in drinking water, influent and effluent of sewage water treatment plants, using the partial-filling micellar electrokinetic capillary chromatography methods. In terms of the optimisation of the capillary electrophoresis (CE) instrument, this study has been able to highlight the different parameters that affect the performance of the equipment. These parameters include: voltage which affects the field strength, the pH of the electrolyte solution that affects the separation and dissociation of the analytes according to their pKa values, and the injection type used which affects the detection of the analytes. Furthermore, the capillary zone electrophoresis (CZE) with UV detection could be used effectively in the identification and quantification of the selected pharmaceuticals at low concentration levels.

For sewage water treatment plant influent A, aspirin concentration was quantified to be 13.52 ng/L, diclofenac as 14.15 ng/L, salicylic acid as 6.514 ng/L and sulphamethoxazole as 11.79 ng/L respectively. The influent B water sample contained 4.23 ng/L of aspirin, 8.235 ng/L of diclofenac, 1.199 ng/L of salicylic acid, 1.095 ng/L of ibuprofen and 13.170 ng/L of sulphamethoxazole respectively. And in the effluent water samples of sewage water treatment plants the measurable pharmaceuticals quantities included 0.836 ng/L of aspirin, 0.802 ng/L of diclofenac, 1.343 ng/L of salicylic acid, 0.842 ng/L of ibuprofen and 10.241 ng/L of sulphamethoxazole. Furthermore, the partial-filling micellar electrokinetic capillary chromatography (PF-MEKC) method was adopted for the identification and quantification of steroid hormones. In the sewage water treatment plant's influent water sample A, androstenedione was quantified to be 2.224 ng/L, testosterone was quantified to be 3.474 ng/L, 17-ß-estradiol was quantified to be 0.96 ng/L and progesterone was 1.503 ng/L. The influent B water sample contained 2.224 ng/L of androstenedione, 3.142 ng/L of testosterone, 0.954 ng/L of 17-ß-estradiol and 0.691 ng/L of progesterone respectively. While in the treated effluent water samples of sewage water treatment plant, the measurable steroid hormones quantities include 1.205 ng/L of androstenedione, 3.037 ng/L of testosterone, 0.550 ng/L of 17-ß-estradiol and 0.440 ng/L of progesterone respectively. The steroid compounds content of the tap water (hot and cold) was also measured. For androstenedione, concentrations of 0.031 ng/L and 0.025 ng/L were quantified for hot tap water and cold tap water respectively; testosterone levels were 0.016 ng/L and 0.013 ng/L for hot tap water and cold tap water respectively; 17-ß-estradiol was 0.11 ng/L and 0.09 ng/L in hot tap water and cold tap water respectively; while progesterone concentrations were 0.049 ng/L and 0.031 ng/L in hot tap water and cold tap water respectively.

In Table A it can be seen that the limit of detection (LOD) and the limit of quantification (LOQ) for the respective compounds in capillary electrophoresis are generally lower compared to the LC method.

Table A: LOD and LOQ comparison for CE and LC

ANALYTES	CE		LC	
	LOD	**LOQ**	**LOD**	**LOQ**
Acetaminophen	0.230	0.690	0.286	0.857
Aspirin	0.137	0.411	0.195	0.584
Sulphamethoxazole	0.186	0.557	0.224	0.673
Diclofenac	0.073	0.219	0.412	1.236
Ibuprofen	0.085	0.255	0.291	0.873
17-beta estradiol	0.096	0.195	0.331	0.993

The advantages of the CE method are the high efficiency of separation, rapidness, simplicity, small sample volume (several nanolitres), and a lower consumption of reagents. The novelty of this research study is the demonstration of the CZE-UV method for the determination of pharmaceuticals as well as inorganic ions, and the use of the PF-MEKC-UV method for the determination of steroids in influent and effluent wastewaters, as well as in cold and hot tap water. From the performance, CE can be considered highly sensitive and suitable for rapid determination and quantification of contaminants in environmental samples that require low detection limits.

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ACRONYMS & ABBREVIATIONS

ACT	Acetaminophen
AVG	Average
BGE	Buffer Electrolyte Solution
BLQ	Below the limit of Quantification
BOD	Biochemical Oxygen Demand
BPA	Bisphenol A
CAF	Caffeine
CAR	Carbamazepine
CE	Capillary Electrophoresis
CEC	Chemicals of Emerging Concern
COD	Chemical Oxygen Demand
CZE	Capillary Zone Electrophoresis
CZE-UV	Capillary Zone Electrophoresis with UV detection
DCF	Diclofenac
DWA	Department of Water Affairs
EC	Emerging Contaminants
EDC	Endocrine Disrupting Compounds
EOF	Electroosmotic Flow
EPA	Environmental Protection Agency
ESI	Electrospray Ionisation
GC-MS	Gas Chromatography-Mass Spectrometry
HAA	Haloacetic Acids
HPCE	High-performance Capillary Electrophoresis
HPLC	High Pressure Liquid Chromatograph
ICH	International Conference of Harmonization
LA	Lamivudine
LC-MS	Liquid Chromatography-Mass Spectroscopy
LOD	Limit of Detection
LOQ	Limit of Quantification
MBR	Membrane Bioreactor
MS	Mass Spectrometer
NDMA	Nitrosodimethylamine
PCP	Personal Care Products
PFC	Perfluorinated Organic Compounds
PFDA	Perfluorodecanoic acid
PFHpA	Perfluoroheptanoic acid
PF-MEKC	Partial-filling Micellar Electrokinetic Capillary Chromatography
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid

PFOS	Perfluorooctane sulfonate
PFUnDA	Perfluoroundecanoic acid
PHE	Phenytoin
POP	Persistent Organic Pollutants
PPCP	Pharmaceuticals and Personal Care Products
PTFE	Polytetrafluoroethylene
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
SANS	South African National Standard
SDS	Sodium Dodecyl Sulphate
SERS	Surface Enhanced Raman Spectroscopy
SMX	Sulfamethoxazole
SPE	Solid-Phase Extraction
STD DEV	Standard Deviation
TOC	Total Organic Carbon
TS	Triclosan
UNEP	United Nations Environment Programme
UPLC	Ultra Performance Liquid-Chromatography
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WWTP	wastewater treatment plant
2-N	2 nitrophenol

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The contamination of water is a major global issue contingent upon the rapid increase in human development, increased urbanisation and industrialisation (Ojemaye and Petrik, 2019). This has resulted in unsustainable use of environmental resources and the indiscriminate disposal of contaminated wastewater and solid waste into different environmental matrices. Among the waste contaminants are many emerging contaminants, nutrients, toxic metals, and many other synthetic organic chemical compounds. These emerging contaminants (ECs) include organic phenolic compounds, pharmaceuticals and personal care products (PPCP), veterinary medicines, endocrine disrupting compounds (EDCs), phthalates, flame retardants, perfluorinated and brominated substances, pesticides and herbicides, nano-materials amongst others (Houtman, 2010; Fawell & Ong, 2012).

The non-availability of clean potable water is now a concern globally, attracting scientists' attention all over the world. The unavailability of sufficient and sustainable sources of freshwater for drinking water production is also linked to factors such as climate change, rapid industrialisation, overpopulation, agricultural practices and lack of functional water treatment plants. It is important to know that in spite of the efforts put in place by health organisations globally, United Nations Environment Programme (UNEP) and World Health Organization (WHO) reported in 2017 that approximately 2.2 billion people do not have access to reliable drinking water. It is expected that by 2025, more than two-thirds of the world population will be without access to quality potable water (Belgiorno et al., 2007; Fawell & Ong, 2012). According to South African Council for Scientific and Industrial Research, 2.11 million South Africans lack access to safe water infrastructure (Edokpayi et al., 2015). Amidst the serious concerns of persistent water shortages, the limited available potable water is not usually completely suitable due to the presence of low quantities of persistent and emerging contaminants, which are potentially harmful to humans and other living organisms (Boithias et al., 2014; Petrie et al., 2015; Steffen et al., 2011). Despite the growing awareness and stringent environmental regulations, environmental pollution still remains ubiquitous. Therefore, it is imperative to seek urgent interventions as the direct discharge of domestic and untreated industrial wastewater containing high pH, high chemical oxygen demand (COD), biochemical oxygen demand (BOD), unpleasant odour, strong colour, high total organic carbon (TOC) and certain toxic soluble substances into water bodies continues to degrade water quality (Hussaini et al., 2013). Many chemicals have infiltrated into water bodies as a result of anthropogenic activities. Even as some harmful chemicals are being phased out, new replacement chemicals are being produced and introduced into products to meet human needs. Many of these groups of chemicals are of serious public health concern and are referred to collectively as emerging contaminants (ECs) or chemicals of emerging concern (CEC) (Richardson and Ternes, 2011; Kolle et al.,

2013). A report by the US Geological survey, (2014), stated that CEC are new chemicals having no regulatory status, which modulate hormones in the endocrine system and disrupt the physiological activities of the endogenous hormones. Subsequently, the exposure to these chemicals through drinking water, or perhaps, the consumption of food crops irrigated with reclaimed water having these substances, have been reported to disrupt hormonal body functions and lead to birth defects, cancerous tumours, early puberty, heart disease, obesity and other abnormalities, especially in aquatic species (Kolle et al., 2013; Tijani et al., 2013).

The advancement of analytical instrumentation and detection techniques has led to the identification and quantification by different scientists of a sizeable number of CEC at low concentrations in different aqueous matrices as well as drinking water (Magureanu et al., 2010; Trapido et al., 2014). This research will look into the suitability of capillary electrophoresis (CE) as analytical equipment for detection and quantification of emerging pollutants. The CE analytical technique was investigated to establish its potential advantages and sensitivity compared to other, more conventional methods such as HPLC.

1.2 PROJECT AIMS

The aim of the project was to investigate emerging pollutants in water by developing the capillary electrophoresis (CE) technique. This technique was investigated for the detection and quantification of difference classes of emerging and persistent contaminants. In order to achieve the aim, the following objectives were considered;

(i) Development of clear, concise and suitable effluent sampling procedures
(ii) Development of suitable extraction procedures for the detection of a variety of emerging micro-pollutants
(iii) Analysed water samples for emerging pollutants using LC-MS technique
(iv) Develop and validate method for detection and quantification of emerging pollutants in water using capillary electrophoresis

The overall objective was the comparison of the CE technique with existing analytical procedures such as LC-MS in order to determine the advantage of this technique over the existing ones.

1.3 SCOPE AND LIMITATIONS

This study only considered detection and quantification of certain selected pharmaceuticals and hormones, because standards for these compounds were commercially available for their quantification. Selected environmental, effluent and potable water samples were chosen as being representative of different water matrices, in order to show the technique's sensitivity, not as an exhaustive monitoring exercise.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Emerging contaminants (EC) are chemicals that are legally synthesised and their presence in the environment should be monitored to conform to local and international standards for environmental protection (WRC Report No. TT 742/2/17). The formulation of chemical products is generally intended for improving the condition of humans, animals and plants. Meanwhile, once these chemicals are released into the environment, they undergo bioaccumulation, bioconcentration and persistence in the aquatic environment (Clarke & Cummins, 2015). Emerging contaminants or chemicals of emerging concern cannot be specifically defined and there is no comprehensive list yet. These terms have been used interchangeably among researchers due to some misconceptions. In the opinion of Houtman, (2010), emerging contaminants do not only mean compounds that are newly developed or detected in the environment; he rather classified them into three categories. The first category is made up of compounds released into the environment recently. The second category consists of compounds already existing in the environment for a longer period of time but that have recently detected as a result of development in analytical techniques. The third category points to compounds of which their negative health effects are just manifesting.

2.2 CHARACTERISTICS AND CLASSIFICATION OF EMERGING CONTAMINANTS

When Emerging Contaminants (ECs) are present in the environment, they are more acidic, alkaline and polar than natural chemicals, and their biological activity makes them potentially toxic even at low concentrations. Several types of persistent organic pollutants or emerging contaminants, grouped generally by their initial use, and at times, by structure or mechanism of action include the following:

2.2.1 PHARMACEUTICALS

Pharmaceuticals are a broad range of chemicals, which include diagnostic agents, prescribed drugs, veterinary drugs and vitamins which could either be synthetic or natural medicines. They are administered or used for the alteration of physiological and biochemical processes in animals and humans, for the purpose of diagnosis, treatment and prevention of diseases. The very widespread occurrence of these pharmaceuticals in the aquatic environment, freshwater, groundwater, seawater and wastewaters is a clear indication of poor bioavailability in medicinal formulations, low metabolism of medications, over-prescription, or contamination through direct disposal of expired and unwanted medicines in household wastes, landfills and toilets. Many such compounds induce antibiotic resistance to disease-causing organisms and increase the rate of cancer and organ damage (Bound & Voulvoulis, 2005). Their

occurrence in water needs to be considered on a country-by-country basis. This is because there is a wide variation in what is being prescribed or used in different countries, and also because the means by which they enter water sources differ (WHO, 2011). In some developing countries in Asia, the discharge of pharmaceutical waste into lakes and rivers is reported to result in levels as high as over 30 mg/L in places close to factory outlets. The concern is the fact that the water may be reused in households and also for agriculture (Lubick, 2009). South Africa is a water-stressed country with continually increasing demand on its natural resources. Regulations have been put in place for industries to recycle and reuse water to minimise the intake of fresh water from rivers by the water utility companies and eliminate the continuous decantation of polluted water into the environment and local river systems (Bell et al., 2001).

Efforts have been made to detect some of the emerging micropollutants in waste water treatment systems in South Africa, but only a selected range of emerging micro pollutants have been identified and monitored (Swartz et al, 2017). Wastewater reuse is a possible exposure pathway to a significant number of emerging contaminants and their metabolites. It is therefore important to identify and monitor emerging pollutants in the recovered water so as to determine its fitness for use. The extensive use of organic compounds in diverse applications, amongst them consumer products has increased the presence of these chemicals in the environment. In South Africa, several chemicals of emerging concern have been identified in water and wastewater sources due to industrial processes and disposal of untreated wastewater. Patterton (2013), conducted a scoping study on emerging contaminants, and discovered over 32 compounds, comprising predominantly pharmaceuticals and pesticides in drinking water in several cities in South Africa.

2.2.2 ENDOCRINE DISRUPTING COMPOUNDS (EDCs)

The WHO defines an endocrine disruptor as “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny” (WHO, 2002). Endocrine disrupting compounds (EDCs) are a diverse group of substances, which include human hormones and a range of industrial chemicals. These chemicals impact and affect the hormonal control in humans and other living organisms, as well as sexual and reproductive behaviour (Bradley & Journey, 2014). EDCs were the first of the emerging contaminants that drew media attention, owing to the observed feminisation of male fish just downstream of wastewater discharges. These compounds all have in common the ability to act as hormones or mimic hormones or even to interfere with the endocrine system at different concentrations (Watts et al., 2002). Apart from oestrogenicity, much less is known about the effects of the EDCs in the environment. Human oestrogens naturally excreted in the urine as well as the artificial oestrogens used in oral contraceptives are the most potent EDCs. Also, there are many other compounds and industrial substances such as organotins, detergent building blocks such as the alkylphenols, and bisphenol A that cause oestrogenicity (Lam et al., 2011).

2.2.3 PERSONAL CARE PRODUCTS (PCPs)

Personal care products are often grouped with the pharmaceuticals in “pharmaceuticals and personal care products” (PPCPs). However, they are comprised of a wide range of substances used in domestic cleaning, air-freshening products, make-up, toiletries and for other purposes, such as in insect repellents, and various UV/Sunscreen filters in personal care products. Examples of PCPs include benzophenone-3, octyldimethyl-p-aminobenzoic acid, siloxanes and disinfection products like triclosan. These are used in body sprays, cosmetics, lipsticks and a wide range of home products. The compounds used for the purpose of disinfection, such as chlorophene and triclosan are often used on a larger scale compared to pharmaceuticals. For example, triclosan has been in use for several years in many consumer products, ranging from body creams, deodorants, perfumes, toothpaste, hand soaps, to toys. These compounds enter the aquatic environment through bathing, washing, domestic grey water, swimming. When some organisms are exposed, there may be an accumulation of these compounds in them, thereby leading to endocrine disruption, carcinogenicity, cytotoxicity and genotoxicity (Fawell & Ong, 2012). For instance, an example of triclosan and chlorophene found in bile from bream in the Dutch River Dommel has been reported (Houtman et al., 2004). Table 1 reflects a selection of other reported incidences.

Table 1: Concentrations of pharmaceuticals and endocrine disrupting hormones found in the environment (Miraji et al., 2016)

Class	Drug/hormones	Amount reported (µg/L)/ Country
Antimalaria	Artemether Lumefantrine	No immediate report yet.
Analgesics for pain relief	Acetaminophen	0.211 (USA), 10.19 (Spain), 10 (USA),
	Ibuprofen	516 (USA), 0.174 (China), 2.5 (Poland), 6.0 (Spain), 70.35 (USA)
Antipyretics for fever reduction	Aspirin	0.22 (Germany), 13 (Greek, Spain)
	Naproxen	<0.1 (USA), 0.958 (EU), 0.108 (Spain), 0.7 (Poland)
Oestrogen, Endocrine disrupting hormones	Estradiol	0.017 (USA), 0.0014-0.002 (Netherlands), 0-0.670 0-0.670 (Equador), 0.0002 (USA)
	Estriol	0.0004 (USA), 0.0049-0.0121 (France), 0.005 (USA)
Hormone replacement	Estrone	0.0004 (USA), 0.0001-0.00157 (France), 0-0.67 (Ecuador), 0.0001-0.017 (USA)
	Progesterone	1.0 (USA), 3.1 (USA), 0.005 (USA)

Class	Drug/hormones	Amount reported (µg/L)/ Country
β-blocker for abnormal heart rhythm	Atenolol	0.4 (Spain), 0.036 (USA), 0.86 (USA), 1.872 (Spain), 0.026 (USA)
Antibiotic for bacterial infection	Metronidazole	0.176 (Spain), 0.9 (Switzerland)
	Tetracycline	0.10 (USA), 0.4 (Serbia), 0.69 (Spain), 0.023 (Spain) 0.12 (Spain), 2.69-31.71 (Tanzania)
	Amoxicillin	

Table 3 shows the details of some pharmaceuticals and endocrine disrupting hormones with their respective quantities reported in different countries. Ojemaye and Petrik (2018) provide an extensive review that shows that these compounds find their way into the environment including into marine organisms and sediments. These authors also report many persistent organic pollutants from sewage being present in the near shore environment and found bioaccumulated in benthic organisms as well as in fish caught in the environs of the Cape Peninsula, South Africa (Ojemaye et al, 2020; Ojemaye et al, 2019; Petrik et al, 2017).

2.2.4 DISINFECTION BY-PRODUCTS

Disinfection agents are oxidising agents used in the course of water treatment, for example, chlorine and chloramine. Their mode of operation involves the destruction of pathogenic microorganisms and oxidation of taste and odour-forming compounds (Miraji et al., 2016). This degradation process forms disinfectant residues, which prevent the further growth or contamination by microbes along the line. However, the reaction between the disinfecting chemicals with natural fluvic acid, amino acids, humic acid, iodide and bromide ions gives rise to chemicals such as haloacetic acids, HAAs (chloroacetic acid, bromoacetic acid, dibromoacetic acid, dichloroacetic acid and trichloroacetic acid), trihalomethanes, THMs (bromoform, bromodichloromethane, chloroform and dibromochloromethane), bromates and chlorates (USEPA, 2013). Other chemicals include bromonitromethanes and nitrosodimethylamine. However, brominated disinfectants are more harmful than the chlorinated ones, therefore, the use of chlorinated disinfectants is favoured, particularly chloroamines (Battaglin et al., 2007). Dermal absorption, direct ingestion and showering are the means through which DBPs enter into humans. A continuous exposure for a long time has been associated with genotoxicity and carcinogenic effects (Miraji et al., 2016).

2.2.5 PERFLUORINATED SURFACTANTS

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are perfluorinated substances that have been used widely as building blocks in non-stick coatings such as grease-repellent coatings, sprays for leather and textiles and dirt-repellent coatings, while polytetrafluoroethylene (PTFE) is used in fire-fighting foams and non-stick cookware (Fawell & Ong, 2012). These substances are persistent in the

environment and often found in groundwater. They are common owing to the fact that they are water-soluble (Fawell & Ong, 2012). There is a growing concern about perfluorinated compounds owing to their persistence, potential for accumulation in organisms and their toxic properties, which include developmental toxicity and possibly carcinogenicity (Skutlarek et al., 2006; McLachlan et al., 2007). Detection of perfluorinated compounds have been reported in surface waters across Europe (Ahrens et al., 2009; Loos et al., 2010; Kwadijk et al., 2010) and are widespread in the near shore marine environment of Cape Town (Petrik et al., 2017). They pose a serious problem for groundwater and seawater, where they are capable of remaining for a very long time due to their persistence, even when the source of contamination is removed.

2.2.6 PERCHLORATE

Perchlorates occur environmentally both naturally and synthetically. They find their applications in energetic boosters such as explosives, fertilisers, fireworks rocket fuel, and missiles. The available reports about perchlorates show their abundance as a contaminant in aquifers, sewages and natural waters. Perchlorates in the environment are not volatile but very soluble in water, and very stable (Miraji et al., 2016). In a short time of exposure to perchlorates, eye as well as respiratory tract and skin irritation, coughing, diarrhoea, nausea and vomiting ensues (EPA, 2013). Perchlorate contamination also disrupts the thyroid's ability to produce hormones. Urbansky and Schock, (1999), reported that EPA has set a perchlorate risk level of 5 ng/L, and this was considered to be below the ion chromatography detection limit. Therefore, owing to the EPA standards, just less than 2.5 ng/L of perchlorate is allowed in the water supply. An average of perchlorate concentration of about 2 μg/L was reported in New York (Parker, 2009).

2.2.7 NANOMATERIALS (NMs)

Nanomaterials have been classified by the US Environmental Protection Agency (EPA) as emerging contaminants. As a result of their unique properties, they are used in a wide range of industrial, scientific and medical applications. Their occurrence as nanoparticles in the environment has not been monitored yet, but there is a major concern about the lack of environmental health and safety data for nanomaterials. Currently, there is not sufficient scientific data to determine if nanomaterials may have adverse health effects on humans under pragmatic exposure conditions, because there are currently no specific standards or guidelines that regulate nanomaterials. Some major nanomaterials sources having potential impacts upon both surface and groundwater result from industrial production, which include nanomaterials such as carbon black and fullerenes, silica, and titanium and zinc oxide (Borm et al., 2006). Certain nanomaterials may also generate reactive oxygen species (ROS) that can lead to cell or membrane damage (Li et al., 2010).

2.3 POLLUTANT CONCENTRATION IN WATER

Due to the higher population density and as a result of the ever increasing use of pharmaceuticals and other emerging contaminants all over the world, their presence in treated wastewater is ubiquitous. In Europe alone, there are more than 4,000 estimated pharmaceuticals registered for use as human and veterinary medicines (Hayward, 2011), but it is uncertain how many of these compounds are likely to reach the aquatic environment. These are also not the only classes of persistent contaminants being discarded into receiving waters. The expansion of cities and megacities can be a vital yardstick in the assessment of whether these contaminants are present in surface waters. Also, the strain or pressure on water resources from the increasing population and expansion of cities has resulted in a significant increase in the reliance on surface water for drinking water. The concentrations of persistent organic pollutants in drinking water have not been given sufficient attention compared to the attention received in the case of effluents and surface waters. Some data from Europe and the US indicate that the levels of persistent organic pollutants detected may still be below the concentrations that could be of serious health concern (Bull et al., 2011; Watts & Crane Associates, 2007; WHO, 2011) but that does not take into account synergistic effects of interactions of many different compounds present in water, nor the degradation by-product toxicity. Patterton, (2013), conducted a scoping study on emerging contaminants, and quantified over 32 compounds, comprising predominantly pharmaceuticals and pesticides in drinking water in several cities in South Africa.

There are an increasing number of reports concerning the presence of pharmaceuticals and other emerging contaminants in drinking water and source water. These reports usually are from ad hoc surveys or some targeted research projects. But to date, no systematic investigations have provided a thorough overview of the occurrence and concentrations of different contaminants in different part of the water cycle over time. Also, routine monitoring studies to measure pharmaceuticals and other emerging contaminants in drinking water is difficult and expensive, and most regulatory agencies do not require them. This makes it difficult to give an account concerning the presence of certain specific substances and their concentrations. The concentrations of persistent organic pollutants are very low and vary depending on the dilution in the receiving waters and the extent of use, except in some specific situations. They are also difficult to quantify and identify unless the right instrumentation with sufficient sensitivity is available. The WHO group of working experts on pharmaceuticals in drinking water gave the conclusion that “available studies have reported that concentrations of pharmaceuticals in surface waters, groundwater and partially treated water are typically less than 0.1 µg/L and concentrations in treated drinking water are generally below 0.05 µg/L” (WHO, 2011). In the surveys where pharmaceuticals have been studied, there are just a few individual substances which have been found in drinking water. Some other substances which `have been identified in Europe and the US are at levels below the lowest clinical dose (Loos et al., 2010; Reddersen, 2002), but this does not take into account chronic risks. Patterton, (2013) presented a scoping study and research

strategy development on currently known and emerging contaminants influencing drinking water quality showing many different pharmaceuticals being simultaneously present in drinking water sampled in South Africa. Our own recent studies have shown significant contamination of seawater, fish and marine organisms with pharmaceuticals (Ojemaye & Petrik, 2021; Oyemaje & Petrik, 2019 a&b; Petrik et al, 2017), not all drinking water and source waters may contain emerging contaminants. In cases where contaminants are present, they vary significantly in concentration, type and number relative to location and circumstances (Bull et al., 2011; Focazio et al., 2008; Ternes, 2001; Mons et al., 2003).

Natural and synthetic hormones are other emerging contaminants found at concentrations in the nanogram-per-litre range. The majority of these substances are hydrophobic and may have the tendency to adsorb onto particulate matter and sediment. As a result, there may be a reduction in the bioavailability to pelagic organisms, yet they could be bioavailable to sediment-dwelling organisms (Fawell & Ong, 2012). The adsorption to particulates is noted due to a reduction in concentration going downstream and these compounds may not be found in groundwater except if it is shallow, but could be present in drinking water when it is not properly treated or not treated at all (Fawell & Ong, 2012). Israel et al (2018) showed that pollution plumes from municipal landfills may cause aquifer contamination in Atlantis, Western Cape.

Extensive studies were done on perfluorinated organic compounds (PFCs) in some Asian countries over the last decade, in contrast to pharmaceuticals. Perfluorooctanic acid (PFOA) happened to be the most dominant compound among the various PFCs found in surface water and aquatic animals which can be detected (Kunacheva et al., 2011; Nguyen et al., 2011). In the majority of the findings, it could be suggested that human and industrial activities revealed proximal correlations to the concentrations found in waters. The detected concentrations were often in the range of 1-100 ng/L in lake, reservoir and river water (Nguyen et al., 2011; Zhang et al., 2011). These concentrations were generally higher in dry weather, compared to seasons of storm water flow (Nguyen et al., 2011). According to Ericson et al., (2009), perfluorooctane sulphonate (PFOS) was also detected in Asian water bodies and surface water in Europe. PFOA and PFOS estimated concentrations in drinking water in various cities in China ranged from 0.12 to 0.92 ng/L, and the estimated daily intake of PFOA and PFOS through drinking water ranged from 0.006 to 0.15 ng per kg body weight per day (Sun et al., 2011). In the authors' conclusion, it was found that drinking water was a minor source of PFC exposure among the adults in the cities studied. Also, the result of 62 samples of potable water sampled from 34 locations throughout Australia proved a similar conclusion; that the combined PFCs in drinking water was generally low, being below 2-5 ng per kg body weight (Thompson et al., 2011).

Currently, there is an absence of guidance for determining compliance in water quality guidelines from the Department of Environmental Affairs as almost no organic chemical pollutants are specified in the SANS Drinking water guidelines. Our drinking water standards are not adequate as they do not contain maximum regulated levels for diverse classes of persistent chemical compounds. Regulators and municipalities are

thus left with considerable leeway to state that the waste effluent, or drinking water complies with standards, but the water will only be in compliance for the very limited specified parameters in the SANS Drinking water guidelines. South African National Drinking Water Standard (SANS) 241: 2005 or 2015, requires a 95% compliance to class I and 99% compliance to class II as a delivery specification, but does not specify safe levels for the myriad of chemicals of emerging concern now being detected in our drinking and environmental waters (Petrik et al, 2017).

As a result of these deficits, there is a growing concern that long-term, chronic exposure to antibiotics or pharmaceuticals used in human and veterinary medicine may be promoting the selection of resistant bacteria in our environment, or may have significant implications on human health (Boxall et al., 2003). Exposure to many different chemicals is possible through drinking water, or perhaps via consumption of crops irrigated with recycled water containing chemicals of emerging concern, or by eating contaminated fish (Ojemaye et al, 2019). Therefore, there is a need for proper identification and quantification of these compounds, monitoring their presence in our waters to fully understand their chemical properties, and their effect on human health and ecosystems.

It is however a difficult task to monitor organic pollutants in water, as a result of the complexity of most of the organic pollutants. For instance, out of thousands of organic pollutants that may have endocrine disrupting effects, only a few compounds have robust analytical methods in place to extract, identify and quantify them, thus many compounds remain undetected. With the advancement in detection techniques and analytical instrumentation, a significant number of chemicals of emerging concern (CEC) have been identified successfully and quantified at low concentrations in different water sources including drinking water by scientists (Magureanu et al., 2008; Trapido et al., 2014).

One of the challenges in identifying some of these micro pollutants is the suppression of low concentrations of one kind by the higher concentration of other compounds during the analysis due to the presence of several thousands of different compounds in water. As result of this, there is a great need to develop a rapid separation technique whereby the micro pollutants will be separated for easy identification and quantification. It is necessary therefore, to develop detection and monitoring methods that can be commonly applied to identify and quantify several classes of emerging micropollutants. This will enhance a good understanding of the water composition before and after treatment, as this is beneficial to the selection of adequate water treatment methods. Moreover, correct identification and quantification of the range of various persistent organic pollutants before treatment or remaining after treatment is significant for improving the treatment process.

2.4 OVERVIEW OF CAPPILLARY ELECTROPHORESIS

A new analytical method using capillary electrophoresis coupled with mass spectrometry to identify and quantify various classes of persistent and organic contaminants will be investigated in this study. Capillary electrophoresis (CE) has been identified as a separation technique that can be used to rapidly separate organic compound. CE alongside the most widely used conventional analytical techniques such as HPLC, GCMS, and surface enhanced Raman spectroscopy (SERS) could also be employed to identify persistent organic pollutants. Capillary electrophoresis coupled to mass spectrometry or UV/VIS Photodiode array will be employed in this study for separation, identification and quantification of emerging micropollutants. This method will be compared to existing analytical methods to determine the advantage of the new method over the existing ones.

Electrophoresis is a technique used to separate macromolecules based on size. Electrophoresis is the migration of charged particles or molecules in a medium under the influence of an applied electric field. In electrophoresis, the mobility or rate of migration of a molecule increases with increased applied voltage and increased net charge on the molecule. Conversely, the mobility of a molecule decreases with increased molecular friction, or resistance to flow through the viscous medium, caused by molecular size and shape. The total actual movement of the molecules increases with increased time, since mobility is defined as the rate of migration. The above is the general principle that governs all types of electrophoresis. Electrophoresis is a simple, rapid and highly sensitive analytical tool used to study the properties of a single charged species, and as a separation technique. This technique provides the basis for a number of analytical techniques used for separating molecules by size, charge, or binding affinity.

Electrophoresis is often classified according to the presence or absence of a solid supporting medium or matrix through which the charged molecules move in the electrophoretic system. The different types of electrophoresis include; routine electrophoresis, which is the traditional clinical laboratory electrophoresis performed on a rectangle-shaped slab gel, used for the separation of proteins and nucleic acids. High resolution electrophoresis is another type of electrophoresis which is often used where greater resolution of molecules is needed. For example, it is used in the separation of proteins for the diagnosis of multiple sclerosis and light chains in urine for early detection of lymph proliferative disorders such as multiple myeloma. Another type of electrophoresis is polyacrylamide gel electrophoresis, which is used to study individual proteins in serum, especially genetic variants and isoenzymes (polyacrylamide gel may yield 20 or more fractions). Isoelectric focusing is a type of electrophoresis used for the separation of isoenzymes and variant haemoglobins in prenatal screening. In isoelectric focusing, the gel is infused with chemicals that make a pH gradient across the surface of the gel (ampholytes). Using very high voltage, proteins will then migrate to the point on the gel where they have no net charge, i.e. their isoelectric point. Pulsed field electrophoresis is a type of electrophoresis where fragment separation is achieved by alternately applying

the power to different pairs of electrodes. The most common method alternates the positive and negative electrodes in cycles during electrophoresis. This type of electrophoresis separates larger fragments of DNA (> 50 kilobases) that cannot be separated in other electrophoresis systems. Another type of electrophoresis is capillary electrophoresis, which combines electrophoresis and high performance liquid chromatography.

2.5 FUNDAMENTALS AND BASIC PRINCIPLES OF CAPILLARY ELECTROPHORESIS

Capillary Electrophoresis (CE) (or high-performance CE, HPCE), the modern approach to instrumental electrophoresis, is arguably the most rapidly expanding analytical technique in recent years. CE has shown great potential, since its introduction, not only in biopolymer analysis, in which electrophoresis has long been applied, but also in areas (e.g. inorganic ion and drug analyses) where electrophoretic techniques have never been used before, for example, inorganic ion and drug analyses (Tagliaro et al, 1997). It is a concept that uses an electrical field to separate the components of a mixture. Electrophoresis in a capillary differs from other forms of electrophoresis because it is carried out within a narrow tube without supporting material for analytes to help their separation.

In Capillary Electrophoresis, a conductive fluid at a certain pH is fed into a capillary. This fluid represents the buffer solution in which the sample will be separated. A sample is introduced into the capillary either by electrokinetic injection or by pressure injection, and a high voltage is then generated over the capillary. Due to this electric field, the sample components migrate through the capillary at different speeds based on their masses and charges. Molecules can either be positively or negatively charged. When the numbers of positively or negative charges are the same, the charges nullify, thereby creating a neutral molecule. When the molecules are given freedom to move, the charged particles seek regions, such an electrode with an opposite charge that attracts the charged particles. For example, Figure 1, explains an electrolysis environment when both electrodes are in the same solvent as a mixture of ionic substances which is dissolved in a suitable solvent such as water. The motion of these ions is essentially random in the absence of electro-osmosis in an electric field. But under influence of electro-osmosis and uniform electric field, the charged species begin to move. As a result, a crude separation occurs, this result in a less random distribution of charged particles. Cations move toward the cathode and anions move toward the anode. The significance of the mass charge ratio (m/z) which is another aspect of electrophoresis in solution, is also illustrated in Figure 1. In this figure, four types of charged particles can be identified: large and small positively charged, and large and small negatively charged. When the capillary is viewed at a certain place with a detector, the fast components pass first, and later on the slower components.

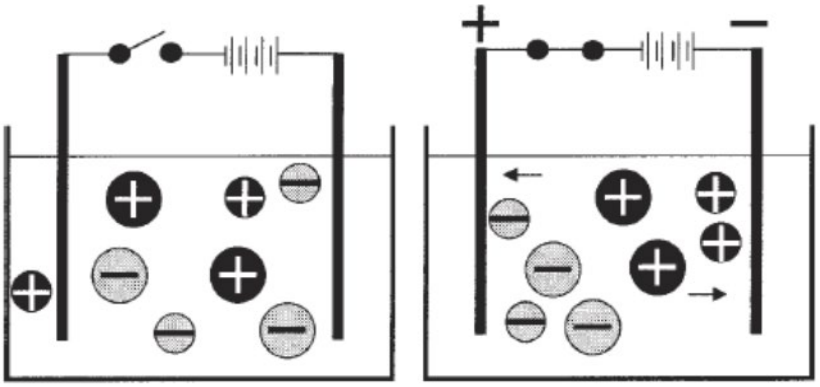


Figure 1: Simple electrolysis separates anions from cations (movement in different directions), in electrophoresis the ions are also separated, but electro-osmosis moves them all in the same direction

When the capillary is placed between the inlet solvent reservoir and the outlet, with a detector fixed in the outlet part of the capillary, the fastest moving component ions pass first, and slowest moving ions enter the detector the last. When each compound possesses a single charge, the absolute value of the force on each particle will be same. The acceleration created by this force is calculated by the relationship: Force = mass x acceleration (F = ma) (Whatley, 2001). The separation medium's viscosity opposes the acceleration resulting in a steady velocity being achieved under constant conditions. This shows that the system cannot only separate compounds having opposite charges, but can also separate compounds of the same charge provided there are other differences between them. Electrophoresis science is based on creating systems that exploit the differences between molecules. However, the analyst may wish to develop a system which creates alternative differences between molecules. An example is by varying the pH of the separation method (Whatley, 2001). At the pH of 10.0, glycine and acetic acid will have the same charge (-1). At pH = 7.0, glycine will have a very small net charge whereas acetic acid will still have a charge of -1. Separating these two molecules would thus be different at pH = 7.0 than at pH = 10.0.

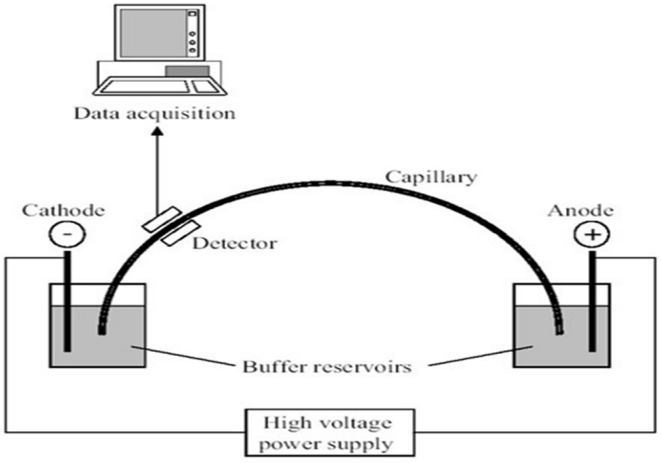


Figure 2: Principle of Capillary Electrophoresis

Figure 2 illustrates the principle of Capillary Electrophoresis. The mobility of any component is dependent on the size and charge of a specific species. This size is a combination of the sample component and the shield of water bound to such a component under the applied electric field. Generally, the bigger the component, the slower it migrates through the buffer. Another important factor is the charge of ions, which strongly depends on the pH value. Therefore, a buffer at a certain pH value is used to carry out separations. By altering the pH of a buffer system, the mobilities of the different components can be altered to achieve the best separation. Meanwhile, the best pH for any separation is between the dissociation values (pK values) of the components.

Capillary electrophoresis (CE) takes place in a very thin, fused silica capillary tube, (the inside being uncoated or coated) but coated with polyacrylamide or agarose gel on its outside. When the capillary is filled with agarose gel the electrophoresis system is commonly used for separation of biological compounds, this type of electrophoresis is commonly used for separation of biological compounds in the clinical laboratory. CE is very rapid, efficient, easily automated, computerised, and requires only a micro volume of sample. These features have made electrophoresis a technique that has attracted the interest of researchers. Capillary electrophoresis is rapidly finding increasing application in biochemical research. Capillary electrophoresis is a new method of analytical electrophoresis that is rapidly finding increasing application in biochemical research. This technique involves samples in liquids to be analysed and the buffer electrolyte for electrophoresis medium (a conducting liquid, usually aqueous electrolytes), which are placed in a long, fine-bore capillary tube, typically 50 to 100 cm long and 25 to 100 μm inside diameter. A very small sample (in the nano-litre range) is introduced to the inlet end of the capillary and subjected to electrophoresis under fields up to ± 20 to 30 kV. The analytes are separated by the principles described for general electrophoresis (with electrolysis and electro-osmosis) and detected as they emerge from the other end of the capillary by UV, LIF, or MS methods.

Capillary electrophoresis offers the advantages of extremely high resolution, speed, and high sensitivity for the analysis of extremely small samples, but also for macromolecules. It has proven especially useful in the separation of DNA molecules that differ in size by as little as only a single nucleotide. Because of its high resolution, capillary electrophoresis is the basis of separation of polynucleotides in some of the newer designs of DNA sequencers. Capillary electrophoresis can also be adapted to the separation of uncharged molecules by modifying buffering electrolytes with detergents to form charged micelles (such as sodium dodecyl sulphate (SDS)) in the aqueous electrophoresis medium. If a mixture of solute molecules that partition between the aqueous medium and the hydrophobic interior of the micelles is introduced into such a system, they can be separated by electrophoresis. Capillary electrophoresis is a highly adaptable method, and the range of its applications and optimal methodology are still being explored (Whatley, 2001). There has been a lot of progress in CE over the recent past, i.e. microchip systems, etc.

2.6 CAPILLARY ELECTROPHORESIS APPLICATIONS

CE application started in the fields of traditional electrophoresis, and has been applied extensively in the analysis and separation of components such as peptides, proteins, nucleotides and DNA. In recent times, its applications have now spread over many other areas, invading those typical of chromatography, which include the analysis of drugs and pharmaceuticals (Altria, 1999), and clinical and forensic pharmacology and toxicology (Thorman, et al., 1994). In reality, CE has been successfully applied in the separation of ionic, hydrophilic and lipophilic compounds, including organic and inorganic ions, amino acids, biological amines, biopolymers, drugs and nucleic acids (Tagliaro et al., 1995).

Numerous other factors besides pH affect electrophoretic separations, and these include hydrodynamic radius of the molecules, the temperature and the viscosity of the separation medium. Meanwhile, there are other forces in real systems, in addition to the electrical field, acting on the charged molecules, for example, the fluid mass may entirely be moving relative to the vessel in which it is contained. The solvent does not move from the vials used for separation. Only ions move. When the solvent needs to move it is flushed with high pressure using the solvent for 1 min (60 cm capillary used). But naturally, if there is no electrolyte vial for conditioning the capillary the level of the inlet vial solution decreases when it is used. Electrophoresis can be affected by some of these factors in a complex manner; for instance, the temperature of a liquid can be raised by the passage of current through it. Electrophoresis can be affected by the temperature of a liquid, if the separation is not done under controlled and optimized conditions, or the ionic strength of the buffer is very high. The electrical resistance (and hence the current), the velocity, and the viscosity of the molecules moving in the field and of the electrolyte buffer can be influenced by this change in temperature. Hence, each separation requires optimization by consideration of these factors.

2.7 THE BENEFITS OF CE OVER CONVENTIONAL TECHNIQUES

CE in terms of development can be broadly defined to be in a refining stage; with routine implementation in certain areas when compared to other conventional methods of analysis. These areas include chiral analysis DNA analysis, metal ion/inorganic anion analysis and clinical applications, CE can always be proved to be an obvious improvement over rival techniques, in terms of cost, efficiency, and reliability, and routine methods have also been established (Altria, 1999). Among the benefits CE possesses over other traditional analytical methods are: analysis of solutes with limited UV chromophores, reduced operating costs, a reduced method development time, solvent consumption and higher separation efficiencies (Altria, 1999). Capillary electrophoresis is also superior to chromatographic separation techniques in many other aspects such as:

- CE is more effective comparatively with common liquid and gas techniques as a result of maximum theoretical plate number.

- The limit of detection of CE is one-thousandth that of GC or HPLC.
- The capillaries can also be conditioned easily with buffer before the analysis starts.
- With CE, nearly one-thousandth of the solvent is used compared with HPLC.
- In CE, detection limit as low as 10 yoctomolar (10×10^{-24} *M*) level (six molecules) may be attained using laser-induced fluorescence (LIF) detection devices.
- With the CE, a wide range of analytes with small to macro molecular structures can easily be analysed.

The major drawback of capillary electrophoresis is that it is affected by the mode of its detection system. This is because the CE laser-induced fluorescence and photothermal systems produce sensitive detection at the trace levels, but other detection systems , like universally used UV detection are incapable of adequate detection limits (Malik & Faubel, 2001). Table 2 presents a comparison of LC-MS, GC-MS, CE and SERS.

Table 2: Summary of the comparison of the LC, GC, CE instruments and SERS. Sources (Megson et al., 2016, Zachhuber et al., 2012, Holcapek et al., 2012)

INSTRUMENT	**LC**	**GC**	**CE**	**SERS**
Detectable analytes	Inorganic, organic molecules, biomolecules, ions, neutral compounds	Inorganic and organic molecules: need to be volatile, derivatization needed in many cases	Inorganic and organic molecules, ions, lipophilic and hydrophilic compounds, biomolecules, neutral compounds	Inorganic and organic compounds, ions
Instrument size	Takes up space	Takes up space	Small size/portable	Portable
Analysis cost	Very high	High	Low	Low
Operational cost	High	High	Low	Low
Sample phase	Liquid	Gas (semi-volatile and volatile)	Liquid, sample preparation when other sample phases are used	Solid, liquid, slurries
Sample volume	1 mL	0.5 uL	Nanolitre	Droplets in microlitre range
Temperature Pressure	No temperature (adjustable gradients for temperature) Pressure based on the mobile phase (Pressure-driven)	Oven temperature (adjustable gradients for temperature) Pressure based on the flow of the carrier gas (Pressure-driven)	Stabile temperature by cooling with air or coolant because of the high voltage No pressure driven system, only electro-osmotic flow (electrical flow)	Not required Not required
Sample mobility method	High pressure pump with liquid	Carrier gas (N_2, Ar, He)	Electro-osmosis and electrolysis	Not required
Machine cost	Expensive	Relatively cheap	Very cheap	Cheap
Flow	Laminar/parabolic	Laminar/parabolic	Plug-like	None
Column efficiency	Poor	Moderate	High performance	Not required
Maintenance	High	High	Little maintenance	Low
Detectors	UV/VIS (photodiode array, UV spectrophotometer), LIF (argon laser, He-Cd laser), EC, and MS (MS/MS) detectors can be on-line coupled.	Thermal conductivity detector(TCD), Flame Ionisation (FID), Electron Capture (ECD), MS (MS/MS)	SERS components(NIR detectors, UV, Intensified CCD detectors)), fluorescence detectors (FL, LIF), MS (MS/MS)	NIR detectors, Intensified CCD detectors
Method of separation	Via liquid chromatography	Via gas chromatography	Via capillary electrophoresis	To avoid matrix effects it should be coupled to a separation technique

INSTRUMENT	**LC**	**GC**	**CE**	**SERS**
Analysis time	Quick analysis	Slow	Real time response/very short	Real time response/very short
Sample preparation Solvents	Simple Large volumes of solvents required	Extensive Large volumes of solvents required	Little or not required Very little required	Little or not required Small volumes or none are used
Column Cartridges	Short and wide Packed column Require cartridges	Long and narrow Packed/Capillary column, Requires column holders	Narrow bore, short Fused Silica capillary	Not required
Destructive	Yes	Yes	No	No
Application	Separation, identification and quantification	Separation, identification and quantification	Separation, Identification and quantification	Identification, quantification possible but not easy

CHAPTER 3: SAMPLING AND CHARACTERISATION OF WATER USING LC-MS

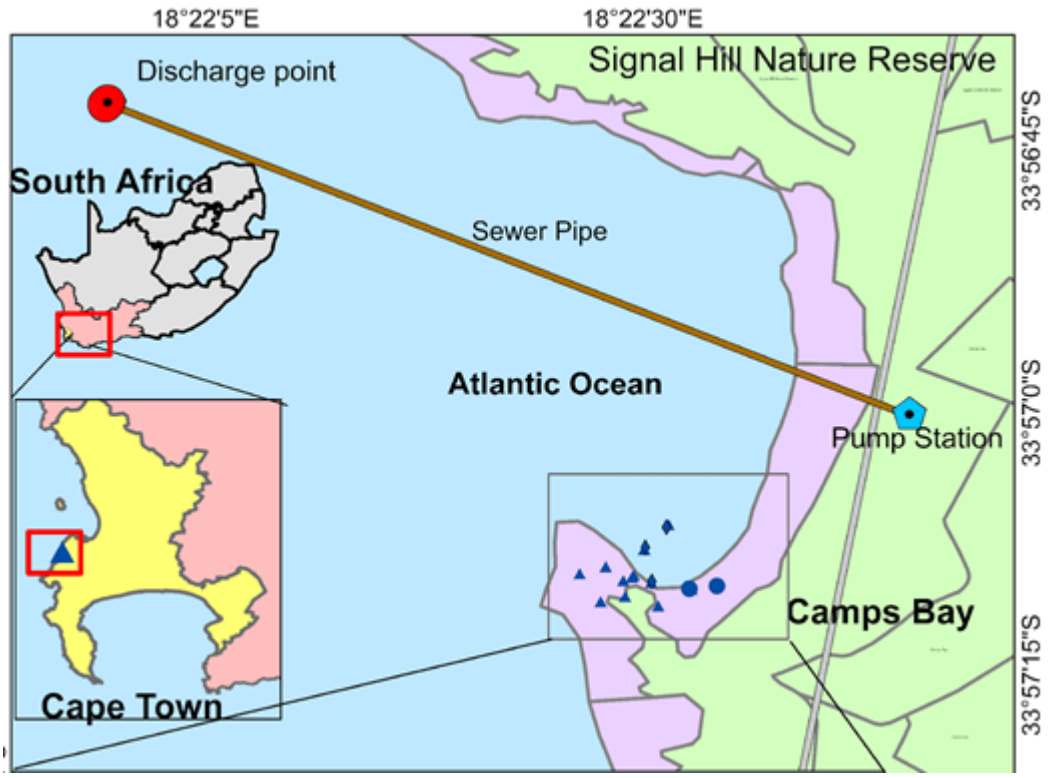
3.1 INTRODUCTION

Different classes of emerging contaminants exist depending on their chemical compositions, sources, effects in the ecosystem, mode of action and interaction. Although these contaminants are initially useful compounds, their original and metabolite forms cause deleterious effects to the environment and humans in the wrong environmental context, thereby raising concerns about their fate and effects in the environment. Globally, there is a huge number of emerging contaminants or POPs released to the environment. POPs are persistent organic pollutants, such as pharmaceuticals, personal care products and industrial chemicals and include a large number of organic chemical compounds, which are extensively used in the society. For this report, water samples were collected from marine sites, waste water treatment plants and tap water around Western Cape, RSA, and Finland, and were characterised to determine the presence of pharmaceuticals, steroids and endocrine-disrupting chemicals in them.

3.2 SEA WATER ANALYSES

3.2.1 SAMPLING

The sea water sampling was done in Camps Bay. Camps Bay is a small, shallow bay, approximately 850 m wide, located at 33°57′00″S 18°23′00″E of Cape Town. Figure 3 is a map showing various sampling points and the marine outfall discharge point as well as the position of the sewage pump station. Three samples of seawater were collected from the sampling site, one was collected from a dug site very close to the beach (marked 3 on the map), the second sample (marked 4) was collected where most activities take place on the beach while the third sample (marked 5) was collected further into the ocean. Moreover, the marine outfall discharges the sewage generated by just the households and hotels of the suburb itself, which is enclosed on three sides by mountains, cutting it off from the rest of Cape Town. There are no industries, apart from hotels and restaurants, releasing effluent into this bay. Before seawater collection, clean and new bottles were rinsed three times with ambient water and samples were taken from under the surface of the water to avoid floating debris. Triplicate seawater samples were collected for analyses of organic compounds. Samples were collected in pre-washed amber glass bottles with screw caps. Field blanks were also prepared by filling pre-washed bottles with ultrapure water that were transported to the sampling site, and subjected to all the field conditions. All samples were kept on ice and transported back to the laboratory. Samples and field blanks were kept in the refrigerator at 4°C.



Location	Coordinates	
SW3	33°57'09.7" S	18°22'30.5" E
SW4	33°57'07.8" S	18°22'30.1" E
SW5	33°57'06.4" S	18°22'31.5" E
Camps Bay marine outfall discharge point	33° 56' 42.214" S	18° 21'59.257" E

Figure 3: Sampling sites/points in Camps Bay, Cape Town

3.2.2 SAMPLE PREPARATION (EXTRACTION AND CLEAN-UP)

For sea water extraction, the method was based on that of Valdés et al., (2014) method with certain adjustment and modifications. Samples were extracted in triplicate. Solid-phase extraction (SPE) was applied by using a vacuum manifold. Up to twenty-four SPE (200 mg, 6 cc HLB) cartridges were connected to the manifold and the manifold was directly connected to a vacuum supply with tubes. 500 mL of each seawater sample was filtered through filter paper (Particle retention 1.2 µm, GF/C diameter 47 mm) to avoid sorbent clogging. In order to avoid the analytes of interest being lost through filtration, the filter papers were then washed with 2 mL methanol. Prior to extraction, the methanol extract was collected and added to the

filtered sample. The pH of the filtered solution was adjusted to 6 with 0.1 M of HCl and NaOH. SPE was carried out with each HLB cartridge preconditioned with 7 mL of methanol followed by 7 mL Milli-Q water, and care was taken not to dry the cartridges during the loading process. 500 mL of each filtered seawater sample was separately loaded in a preconditioned cartridge, with a flow rate of 5 mL/min. The cartridges were left to dry under a gentle stream of nitrogen. Analytes were eluted with 7 mL of methanol at a flow rate of 1 mL/min. The eluate was subsequently concentrated to 2 mL under a gentle stream of nitrogen and was further centrifuged for 25 mins prior to analysis.

3.2.3 CHROMATOGRAPHIC CONDITIONS

Analyses were carried out using Waters ACQUITY UPLC™ system consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. Separation of the compounds of interest was achieved using an ACQUITY UPLC BEH C18 column (1.7 µm; 2.1 mm × 100 mm) with an ACQUITY BEH C18 VanGuard™ precolumn (1.7 µm; 2.1 mm×5 mm) (Waters, Mildford, MA, USA). The column was kept at 50°C. The mobile phase was a mixture of 0.02 M formic acid (solvent A) in water, and acetonitrile (solvent B). Linear gradient elution of 0.35 mL/min with a mixture of 80 % solvent A and 20 % solvent B was used for 9 min and at 10 min, the acetonitrile percentage was increased linearly from 90 to 100 % and was maintained at 80 % of solvent A and 20 % of solvent B. 5 µL of each sample was injected into the LC/MS system. Standards and the test samples were each subjected to 12 min chromatographic run.

3.2.4 MASS SPECTROMETRY (MS)

The UPLC was coupled to a triple quadrupole mass spectrometer (Xevo TQ-MS), with an electrospray ionisation (ESI) source. A multiple reaction monitoring (MRM) scan mode was generated for all analytes during optimisation. Source cone voltage, temperature, cone gas flows, capillary voltage and desolvation temperatures were used to obtain the maximum sensitivity. It was achieved by direct injection of 10 µg/mL concentration of the stock solutions. The capillary voltage of 3.5 kV, desolvation gas (N_2) flow of 800 L/h, source temperature of 140˚C and desolvation temperature of 400°C were applied. Masslynx software was used to collect and analyse the obtained data. The gradient elution method and the summary of the instrument and the analytical conditions are presented in Table 3.

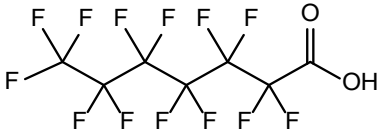
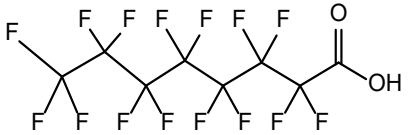
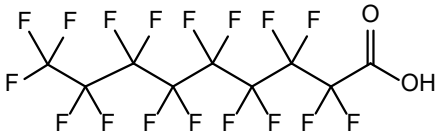
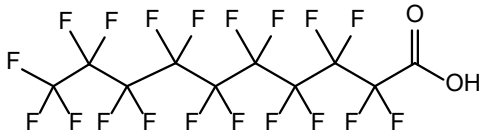
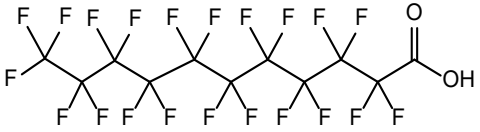
Table 3: Summary of instrumentation and analytical conditions

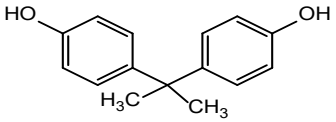
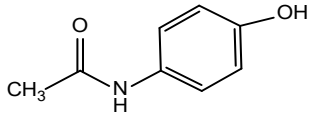
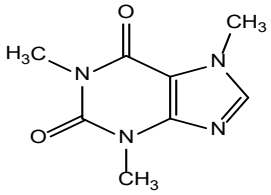
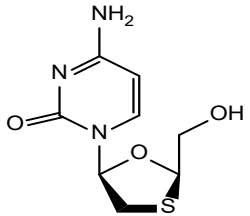
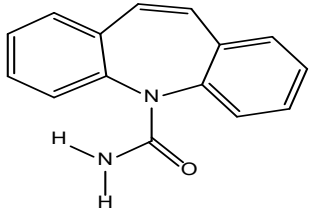
UPLC conditions	
LC System	Acquity Ultra Performance LC (Waters)
LC Column	Acquity UPLC BEH C18, 2.1x100 mm, 1.7µm (Waters)
Column temperature	50°C
Eluent	
(A) 2 mM ammonium acetate in milli-Q water	
(B) 2 mM ammonium acetate in methanol	
Run time	12.00 min
MS condition	
MS System	Xevo TQ-MS
Ion Mode	ESI+
Desolvation Temperature	400°C
Desolution gas (L/h)	800
R F lense (v)	1.0
Capillary Voltage (KV)	3.5

3.2.5 PPCPs, PFCs and EDC OCCURRENCE IN CAMPS BAY SEAWATER

In this section, three classes of contaminants were evaluated namely: pharmaceuticals and personal care product, perfluorinated compounds and industrial chemicals in seawater. Table 6 provides the LC-MS retention time, transition and collision energy, limit of detection (LOD), and limit of quantification (LOQ) while Table 4 provides a summary of the PPCPs, PFCs and industrial chemical compounds detected in seawater samples collected at Camps Bay. The analysis of variance conducted on all data obtained showed that the level of all contaminants varies significantly above the background ($p<0.05$). Furthermore, statistical analysis of all data obtained in this study (Table 5) showed that the results obtained for the quantification of PPCPs, PFCs and EDCs in the seawater samples reported in this study are replicated, going by the values obtained for standard deviation.

Table 4: LC-MS retention time, transition and collision energy, limit of detection (LOD), and limit of quantification (LOQ)

Compound Name	Mol. weight (g/mol)	Molecular structure	Retention Time (min)	Ion transition (m/z)	Collision energy (eV)	LOD	LOQ	% Recovery
						Seawater (ng/L)	Seawater (ng/L)	Seawater
PFHpA (Perfluoroheptanoic acid)	364.06		6.82	363 to 319	15	0.03	0.08	96.5
PFOA (Perfluorooctanoic acid)	414.07		7.39	413 to 369	15	0.003	0.01	97.3
PFNA (Perfluorononanoic acid)	464.08		7.88	463 to 419	15	0.01	0.02	98.0
PFDA (Perfluorodecanoic acid)	514.09		8.24	513 to 469	15	0.02	0.06	99.6
PFUnDA (Perfluoroundecanoic acid)	564.09		8.57	563 to 523	15	0.04	0.11	97.0

Bisphenol A (BPA)	228.29		5.87	227 to 212	28	0.01	0.05	96.2
Acetaminophen (ACT)	151.16		2.01	152 to 110	15	0.02	0.07	98.1
Caffeine (CAF)	194.19		3.41	195 to 138	20	0.03	0.08	97.8
Lamivudine (LA)	229.26		1.74	230 to 112	15	0.03	0.09	96.0
Carbamazepine (CAR)	236.27		6.43	237 to 194	20	0.01	0.03	99.3

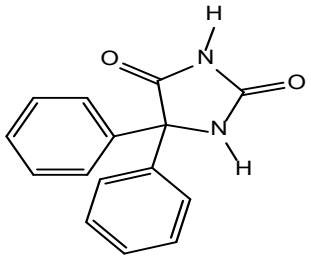
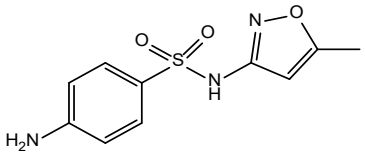
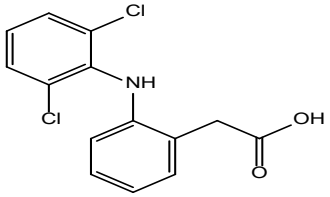
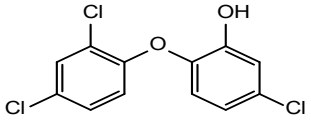
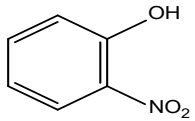
Phenytoin (PHE)	252.27		6.18	253 to 182	15	0.27	0.81	99.0
Sulfamethoxazole (SMX)	253.28		3.23	254 to 188	25	0.02	0.06	96.0
Diclofenac (DCF)	296.15		6.72	296 to 250	15	0.03	0.09	98.6
Triclosan (TS)	289.54		9.09	288 to 36.80	10	0.02	0.08	95.9
2 nitrophenol (2-N)	139.11		-	139 to 121	15	-	-	-

Table 5: Concentration of PFCs, EDCs and PPCPs (ng/L) in sea water.

	Seawater 1	Seawater 2	Seawater 3
Perfluorinated compounds			
PFHpA	0.21±0.01	0.46±0.02	0.27±0.00
PFOA	<LOQ	0.76±0.01	<LOQ
PFNA	0.02±0.00	0.32±0.00	<LOQ
PFDA	2.44±0.05	0.28±0.00	<LOQ
PFUnDA	<LOQ	<LOQ	<LOQ
Industrial Chemicals			
2-N	<LOD	<LOD	<LOD
BPA	<LOQ	<LOQ	<LOQ
Pharmaceuticals and personal care product			
TS	<LOQ	<LOQ	<LOQ
DCF	0.73±0.05	2.86±0.03	<LOQ
SMX	<LOQ	<LOQ	<LOQ
PHE	<LOQ	<LOQ	0.94±0.01
CAR	0.05±0.01	0.14±0.00	0.04±0.00
LA	<LOQ	<LOQ	<LOQ
CAF	<LOQ	<LOQ	<LOQ
ACT	0.09±0.00	0.10±0.00	<LOQ

Concentration expressed in ng/L for water, Mean±standard deviation (n=4, replicate samples taken at the same time), < LOD= below limit of detection: <LOQ= below limit of quantification.

Of the fifteen target compounds, eight were detected in at least one of the sea water samples. The compounds detected in the highest concentrations were among those most frequently detected. The detected compounds represent a variety of chemical types and therapeutic uses, and most have been frequently observed in other marine/sea water studies (Birch et al., 2015; Petrik et al, 2017; Kim et al., 2017). PFDA (Perfluorodecanoic acid) and diclofenac were the two compounds detected in the highest concentration in seawater samples (2.44 and 2.86 ng/L respectively), and both carbamazepine and PFHpA (Perfluoroheptanoic acid) were detected in all water samples in this study. Only a few studies have previously investigated their occurrence in the environment (Munaron et al., 2012; Petrik et al., 2017). Several other compounds that have been suggested as tracers of wastewater contamination due to incomplete metabolism in humans, low removal efficiency during wastewater treatment, and persistence in the environment (Boxall et al., 2012; Lambropoulou and Nollet, 2014) were also detected in the Camps Bay seawater samples, these include acetaminophen and diclofenac.

The concentration of the various compounds detected in all the sea water samples was less than 5 ng/L as presented in Table 5. These concentrations are lower than those typically reported for sites in freshwater

systems, which are often located near wastewater outfalls (Xie et al., 2015; Zhou and Broodbank, 2014) and are similar to concentrations reported for other marine and estuarine environments, where wastewater discharges are also common but dilution occurs to a greater extent (Birch et al., 2015).

Recently, the CSIR report (CSIR, 2017) published in 2017 detected the following pollutants carbamazepine, diclofenac and paracetamol (acetaminophen) with concentrations shown in Table 6 in Camps Bay sewage effluent discharge sampled at the pump station. Hence, the dilution factor in the ocean can be assumed to range between 16 to above 1000 times for carbamazepine, diclofenac and paracetamol as shown in Table 6, considering the concentration difference between the concentrated sewage at the Camps Bay pump station and the diluted sewage in the ocean surrounding the discharge.

Table 6: Dilution factor of diverse compounds discharged into the marine environment in Camps Bay

Compounds	**Sewage at pump station (ng/L) (CSIR, 2017)**	**Seawater (ng/L) (This study)**	**Dilution factor**
Carbamazepine	280-580	0.05-0.14	4142×
Diclofenac	630-1500	0.73-2.86	524×
Paracetamol	250000-950000	0.09-0.10	9700000×

Despite the purported adequate dilution of the chemicals discharged in the sewage by the ocean according to the City and the outfall design criteria (CSIR, 2017), these compounds were shown to be present in measurable amounts in the ocean water, albeit not equally dispersed, which points to the uneven and slow dilution and dispersion of contaminants in a concentrated sewage plume constantly being released into a marine environment. It also shows that the dispersion rate of chemical compounds is related to the type of chemical, since these compounds are not all equally hydrophilic. In addition, changes in pH and salt concentration greatly influence the electrostatic properties of these chemicals, which may have multiple ionisable functional groups with substantially different acid dissociation constant (pK_a) values thus influencing their environmental partition in seawater (Fabbri and Franzellitti, 2016).

Although the study showed that conventional UPLC that was coupled to a triple quadrupole mass spectrometer (Xevo TQ-MS), with an electrospray ionisation (ESI) source could accurately determine these compounds in a complex matrix such as seawater, some of the compounds were below the limit of detection and some below the limit of quantification of the instrument, indicating greater instrumental sensitivity is needed for accurately determining the presence of low levels of such compounds in the marine environment.

3.3 WASTE WATER ANALYSES

This section investigates certain persistent organic pollutants in the effluent wastewater sample from a wastewater treatment plant (WWTP) using the Liquid Chromatography-Mass Spectroscopy (LC-MS) method. The analysis of pharmaceutical compounds will include acetaminophen, aspirin (ASA), diclofenac, ibuprofen,

and sulphamethoxazole. LC-MS has been the most adequate and reliable tool for polar and thermo-labile pharmaceutical compound analysis for quantification and identification because of its sensitivities and ease of analysis. A lot of scientists have performed extensive work on these POPs and hence have validated LC-MS as the analytical tool for pharmaceutical analysis. The LC-MS is well known for its sensitivity, specificity and selectivity in the analysis of trace compounds (Fatta et al., 2007; Dams et al., 2003). LC-MS is suitable for analysis of large molecular weights and thermally liable polar compounds not suitable for GC-MS.

3.3.1 CHEMICALS

17-beta-Estradiol ($C_{18}H_{24}O_2$, 98.4%) was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany); Sulphamethoxazole ($C_{10}H_{11}N_3O_3S$, 98%) and Acetaminophen (98%) were purchased from Alfa Aesar, Germany; Ibuprofen ($C_{13}H_{18}O_2$, 98%), Diclofenac ($C_{14}H_{11}Cl_2NNaO_2$, 98.5%), and Aspirin (99.0%) were purchased from Sigma Aldrich, Germany. Other chemicals were Methanol (HPLC grade) from Fisher Scientific (UK). Hydrochloric acid (1.0 M, analysis result 0.9995 mol/L, ±0.0021 mol/L) and sodium hydroxide (1M, analysis result 1.0003 mol/L, ±0.0021 mol/L) were purchased from Oy FF Chemicals Ab (Finland). Methanol was used as the solvent in standards. Ultrapure water used in the analyses was purified with a Direct-Q UV Millipore water purification system (Millipore S.A., Molsheim, France). The steroid (17-beta-Estradiol ($C_{18}H_{24}O_2$, 98.4%) was used as received and stored in a dark and cold room (+4°C).

3.3.2 INSTRUMENTS AND METHODS

A Shimadzu LC 20AD HPLC system coupled to a Xevo TQ-MS mass spectrometer (MS/MS) (Waters, Milford, MA, USA) was used for high-resolution LC-MS/MS analysis. Separation of the analytes of interest was achieved using a Poroshell 20 EC C_{18} column (2.1 x 100 mm; 3 µm particle size) at the temperature of 40˚C and a flow rate of 0.4 mL/min. The mobile phase was a mixture of 0.02 M formic acid (solvent A) in water, and acetonitrile (solvent B). Linear gradient elution of 0.4 mL/min with a mixture of 70 % solvent A and 30 % solvent B was used for 4 minutes, after which the acetonitrile percentage was increased linearly from 30 % to 90 % until 6.5 minutes. And at 7.5 minute, 70 % of solvent A and 30 % of solvent B was achieved and maintained until the run was 10 minutes. 5 µL of each sample was injected into the LC/MS system.

3.3.3 MASS SPECTROMETRY (MS)

The HPLC system was coupled to a triple quadruple mass spectrometer (Shimadzu LCMS-8040), with an electrospray ionisation (ESI) source. Source cone voltage, temperature, cone gas flows, capillary voltage and desolvation temperatures were used to obtain the maximum sensitivity. It was achieved by direct injection of 10 µg/mL concentration of the stock solutions. The capillary voltage of 3.5 kV, drying gas flow of 15 L/min, nebulizing gas 3 L/min, source heat block temperature of 400˚C and desolvation temperature of 250˚C were applied. Table 7 and Table 8 present the gradient elution method and summary of the instrumentation and conditions of the analytical method, respectively.

Table 7: Gradient elution method

Time (min)	Flow (mL/min)	%A	%B
0.5	0.400	70	30
4.0	0.400	10	90
6.5	0.400	10	90
7.5	0.400	70	30
10.0	0.400	70	30

Table 8: Summary of instrumentation and analytical conditions

HPLC conditions	
LC System	Shimadzu LC 20AD HPLC
LC Column	Poroshell 20 EC C18, 3 x 100, 2.7 µm
Column temperature	40°C
Eluent	(A) 0.02 FA v/v; 2 mM Ammonium formate in Milli-Q water
	(B) 0.02 FA v/v; 2 Mm Ammonium formate in Acetonitrile
Run time	10.00 min
MS conditions	
MS System	Shimadzu LCMS-8040
Ion Mode	ESI+
Desolvation Temperature	250°C
Nebulizing gas (L/min)	3
Drying gas (L/min)	15
Capillary Voltage (KV)	3.5

3.3.4 DETERMINATION OF ACCURACY, LINEARITY, PRECISION AND SELECTIVITY

Accuracy was expressed as a function of recovery percentage, and determined by comparing the concentrations found in the spiked samples with the added concentration. Linearity was assessed by using calibration curves at the concentrations range and peak area was plotted against the concentration of each analyte. Determination of precision was achieved by the replicate standards injection. Selectivity evaluation was carried out by the qualitative comparison of the retention time of the peaks obtained with those of a standard solution, and the identification of the analytes was simultaneously confirmed by comparing the spectra of the peaks in the chromatograms of the sample and standard solutions.

3.3.5 DETERMINATION OF LOD AND LOQ

The determination of the limits of detection (LOD) and quantification (LOQ) was based on the standard deviation of blank-sample responses and the slope of the calibration curve for each analyte. For each compound, the instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined. The LODs and LOQs were calculated using the following formulas: LOD = 3.3 α/S and LOQ = 10 α/S, where α is the standard deviation of the response and S is the average slope of the calibration curves, with the same curves. The method was validated against a set of quality control parameters which include laboratory and field blanks, matrix spikes and triplicate samples. Precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware, as blank contamination is the most common problem observed in the determination of emerging contaminants. Along with the samples and laboratory spikes to monitor potential laboratory contamination of the studied compounds, blank samples of Milli-Q water were extracted and analysed. Methanol blanks were also run between samples in order to monitor instrumental contamination and carryover. Signal noise, chromatographic peak area and height were used to characterize the analytes of interest; peak area was used to measure the optimal signal intensities for quantification.

3.3.6 PREPARATION OF STANDARD SOLUTIONS

The stock solutions of the standards at 1000 µg/mL were prepared in methanol for individual analytes, and stored at 4˚C in glass vessels until used. The preparation of the working solutions from the stocks was achieved by diluting specific concentration into Milli-Q water. The stock solutions were allowed to warm up to room temperature and mixed with a mixer before use. The working solutions made of the analyte standards were 500, 100, 20, 4, 0.8, 0.16 and 0.032 ng/mL. Working standard solutions were used for preparation of the calibration curves and for spiking samples in the validation study.

3.3.7 SAMPLING

Effluent water sample was taken from the wastewater treatment plant located in Bellville, Cape Town, South Africa. The sampling was carried out after the membrane bioreactor (MBR) stage treatment. 2 L of the effluent wastewater was taken into 2.5 L amber coloured glass bottle. The water sample collected was kept in an ice chest during transportation to the laboratory for analysis (Nikolaou et al., 2007). The bottle was tightly capped and taken to the laboratory where it was kept in a dark place at 4˚C until the water was used, and the extraction procedure was performed before 3 days after sampling.

3.3.8 SAMPLE PREPARATION WITH SOLID PHASE EXTRACTION (SPE)

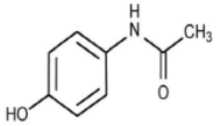
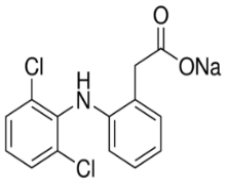
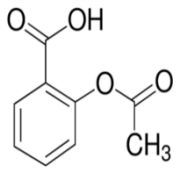
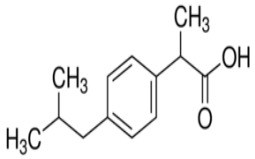
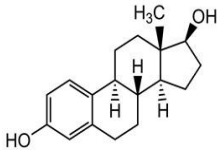
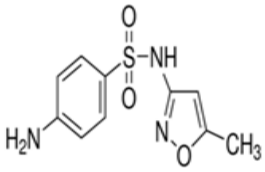
The SPE device VacMaster (Biotage® VacMaster™ 20 Sample Processing Station) was used for sample concentration (solid phase extraction of the water samples). The water samples were pre-concentrated with Strata-X 33 µm polymeric C_{18} reverse phase columns (500 mg/6 mL) which were obtained from Phenomenex (Copenhagen, Denmark). The Reacti-Vap Evaporation unit (Thermo Scientific) was used for evaporation of the extracts under N_2 gas. All waters used were purified with a Direct-Q UV Millipore water purification system (Millipore S.A., Molsheim, France).

2 L each of effluent wastewater sample, distilled water, cold tap water and hot tap water samples were preconcentrated by running through separate SPE cartridge (for each 2 L of samples). Prior to the extraction process, the SPE cartridges (C_{18} columns) were preconditioned with 6 mL absolute methanol (HPLC grade) and 6 mL Milli-Q water was also used to flush the cartridges after. The respective water samples were run through the cartridges at a slow rate, thereafter, the sorbent materials (SPE cartridges) were left to dry for 12 hours. Extraction from the sorbent materials was then carried out by running 6 mL methanol slowly through each one. The eluates were collected in test tubes and separately evaporated under nitrogen with mild heating (40˚C) to dryness, followed by dissolution with 2 mL methanol with agitation. The sample volumes from the C_{18} columns were 2 mL in each case. The final sample volume of 250 µL was separated from the pre-concentrated analytical sample for the analysis and the study was performed with eight replicates.

3.3.9 OPTIMISATION OF HPLC METHOD

A method for the quantitation of acetaminophen, diclofenac, aspirin, ibuprofen, sulphamethoxazole, and 17-beta-estradiol was developed based on a High Pressure Liquid Chromatograph (HPLC) coupled to a triple quadruple mass spectrometer (Shimadzu LCMS-8040), with an electrospray ionisation (ESI) source, and validated following the international conference of harmonization (ICH) procedures/principles. These include triplicate runs of 5-set calibration standards for each analyte, plots of calibration curves, determining the equations for the linear regression lines from the calibration curves, determination of the limit of detection (LOD) and limit of quantification (LOQ) for each analyte, evaluation of the reproducibility and repeatability of method selectivity and sensitivity for each analyte. Table 9 shows the structures of acetaminophen, diclofenac, aspirin, salicylic acid, ibuprofen, sulphamethoxazole and 17-beta-estradiol; with their theoretical and experimentally measured molar masses with retention times.

Table 9: Structures of acetaminophen, diclofenac, aspirin, salicylic acid, ibuprofen, sulphamethoxazole and 17-beta-estradiol. Theoretical and experimentally measured exact molar masses with retention times

Compound	Structure	Molar mass [g/mol]	Experimental molar mass [M + H]	Retention time [min]
Acetaminophen $C_8H_9O_2$		151.17	152.071	2.370
Diclofenac $C_{14}H_{10}Cl_2NNaO_2$		318.10	319.024	6.640
Aspirin $C_9H_8O_4$		180.16	181.05	10.110
Ibuprofen $C_{13}H_{18}O_2$		206.29	207.138	6.406
17-ß-estradiol		272.38	273.185	6.006
Sulphamethoxazole $C_{10}H_{11}N_3O_3S$		253.28	254.059	4.810

3.3.10 CHROMATOGRAMS OF STANDARD ANALYTE SAMPLES

The optimised method for the identification and quantification of the analytes was adopted for subsequent analyses. The respective retention times can be seen in the chromatograms as given in the subsequent Figures 4 to 9.

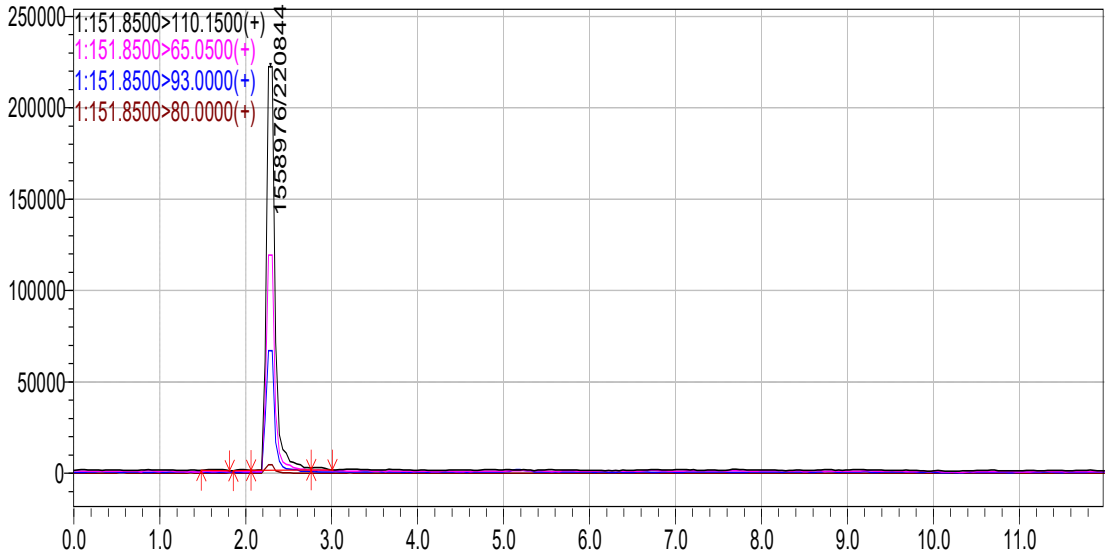


Figure 4: Chromatogram for Acetaminophen

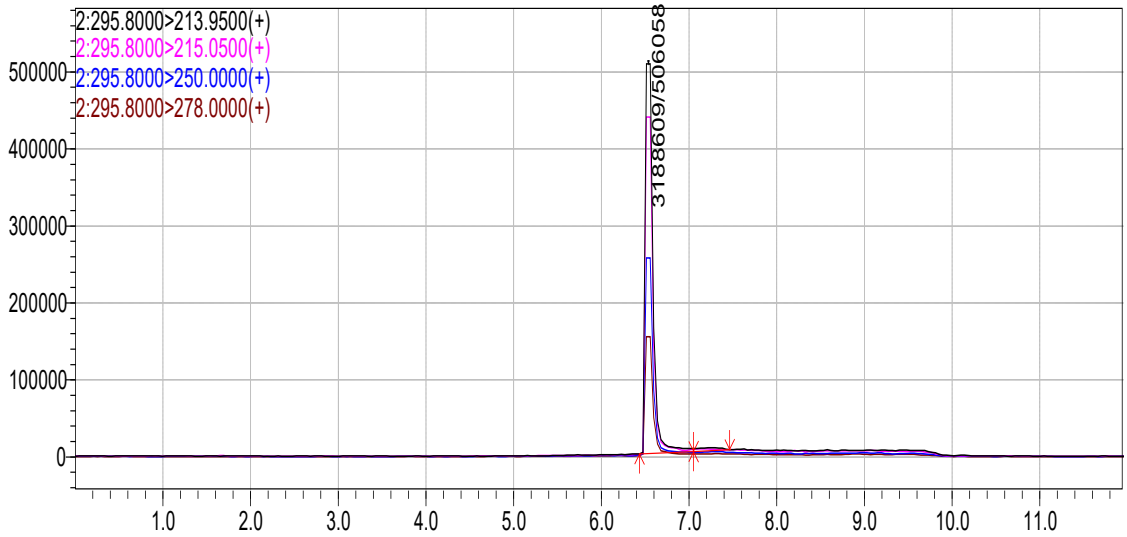


Figure 5: Chromatogram for Diclofenac

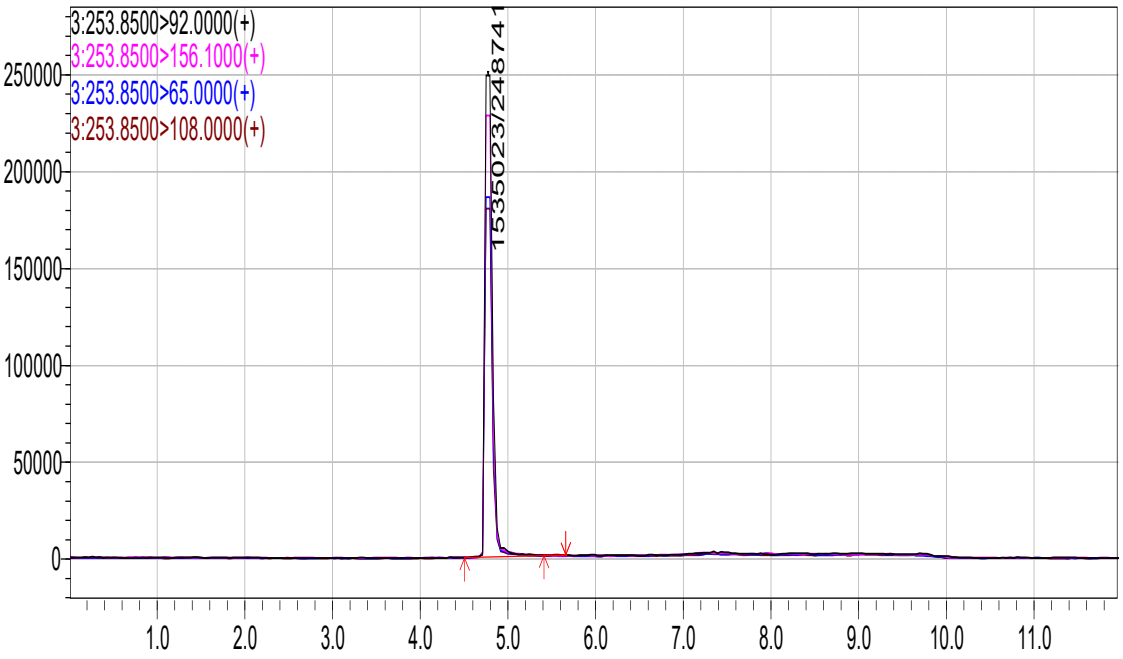


Figure 6: Chromatogram for Sulphamethoxazole

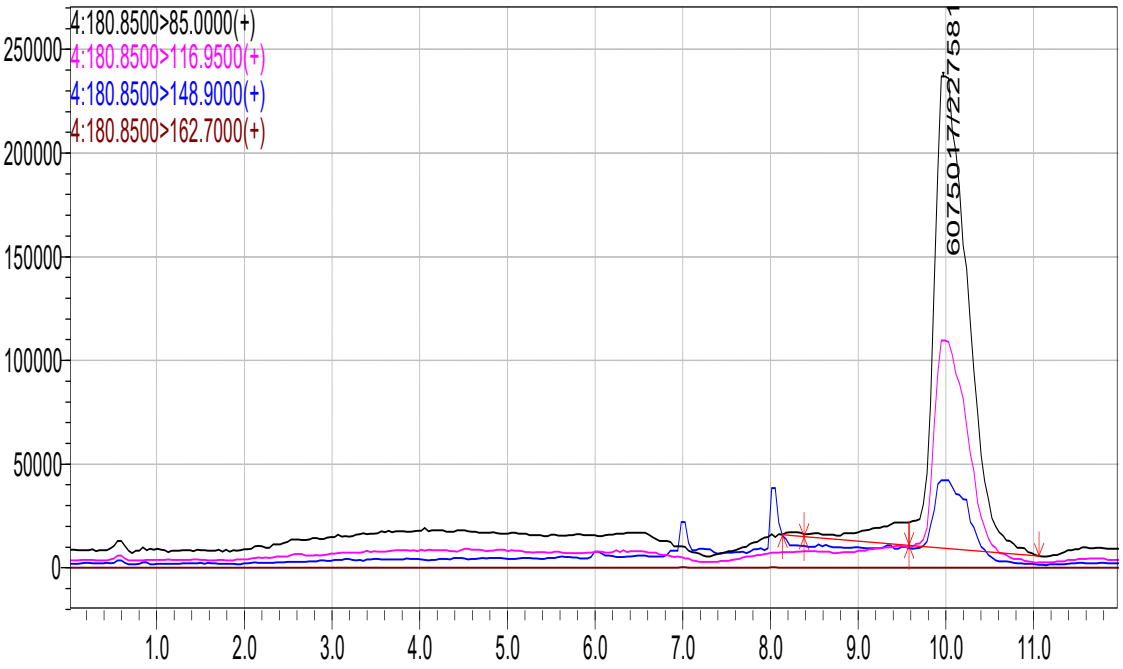


Figure 7: Chromatogram for Aspirin

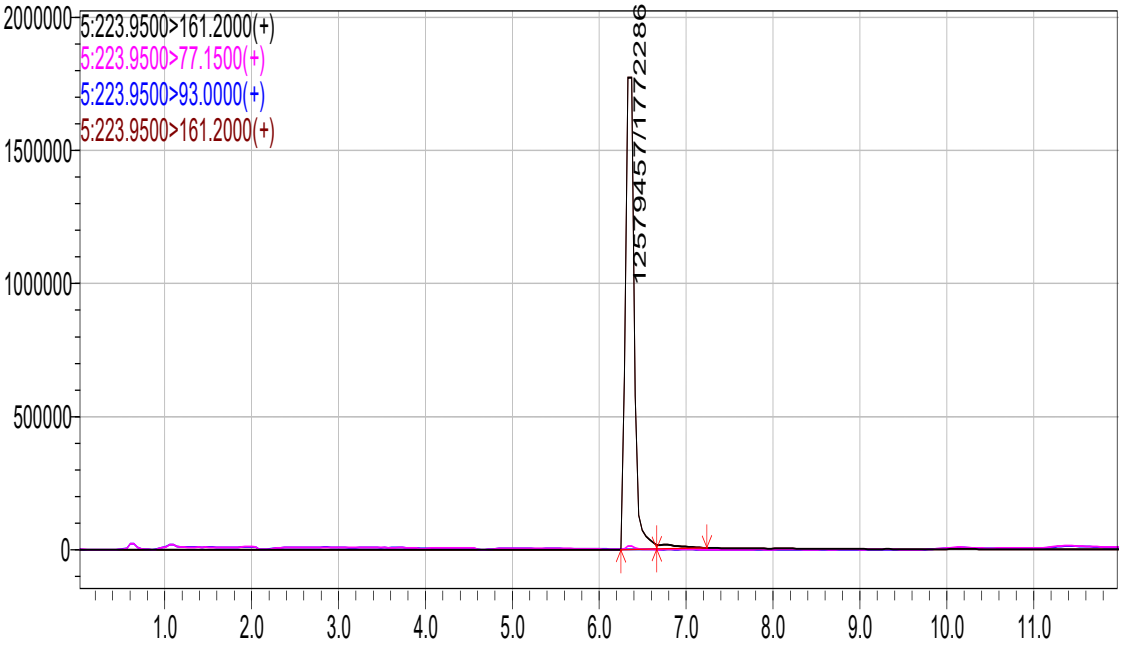


Figure 8: Chromatogram for Ibuprofen

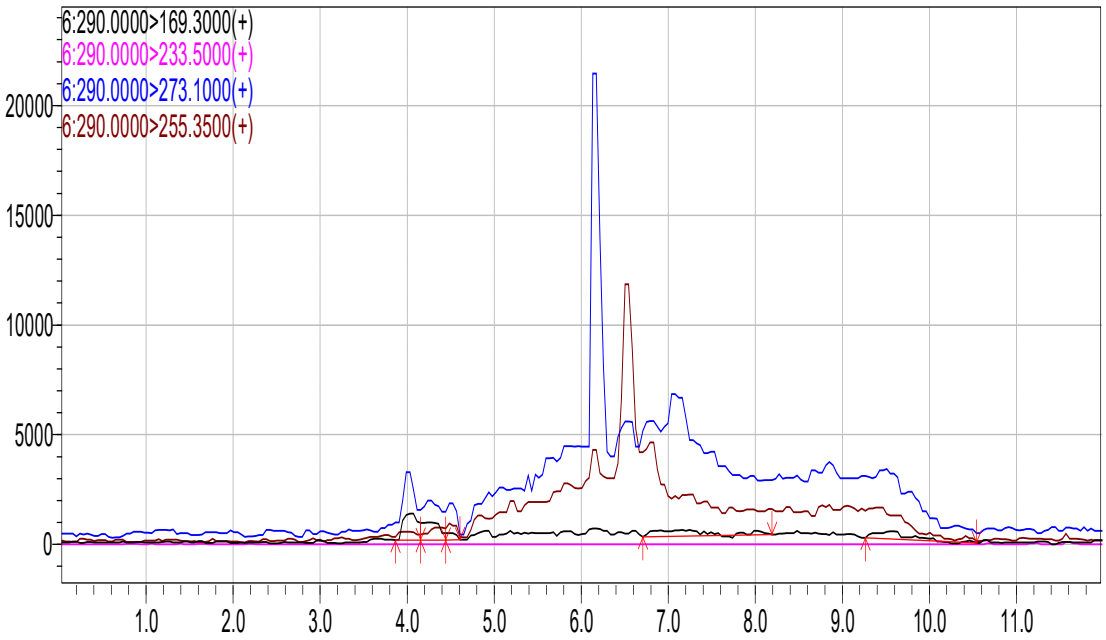


Figure 9: Chromatogram for 17-beta-estradiol

The respective chromatograms show the retention times for the different analytes. The instrument response to the detection of each of the analytes showed linear sensitivity to increasing concentrations with the R^2 values of all the analytes. In Table 10, the method's LOD and LOQ values for each analyte were statistically calculated.

Table 10: Calibration data for standard analytes

Standards	Calibration Range[ng/mL]	Linear equation	R^2	λ_{max} nm	LOD ng/mL	LOQ ng/mL
Acetaminophen	0.032-500	*y = 5.28x + 438*	0.9902	210	0.286	0.857
Aspirin	0.032-500	*y = 3.96x + 124*	0.9920	210	0.195	0.584
Sulphamethoxazole	0.032-500	*y = 2.86x + 584*	0.9949	210	0.224	0.673
Diclofenac	0.032-500	*y = 1.28x + 248*	0.9981	210	0.412	1.236
Ibuprofen	0.032-500	*y = 582x + 884*	0.9707	210	0.291	0.873
17-beta estradiol	0.032-500	*y = 1.32x + 221*	0.9389	254	0.331	0.993

3.3.11 RECOVERY STUDIES FOR STANDARD ANALYTES USING SPE

Sample extraction and clean-up of environmental matrices are often required before LC-MS analysis, so as not to compromise the integrity of expensive instrumentation. As a result, analytical recovery data regarding environmental sample clean-up is becoming increasingly important in the development of an effective sample clean-up strategy. This section reports on an analytical experiment focused at determining the percent recovery of analytes after extraction and theoretical SPE clean-up phase of three pharmaceutical analytes of varying polarity, which was aimed at roughly covering the polarity spectrum of organic compounds of emerging concern being found in the environment today. Currently, environmental monitoring of persistent organic pollutants POPs in environmental matrices often requires multiple extraction and clean-up steps before the sample can be suitably analyzed by Mass Spectrometry (MS). Because there is no universal extraction method for all pharmaceutical analytes, typically it is necessary to develop standard, routine clean-up and extraction procedures for each specific targeted analyte based on a compound's class and relative molecular polarity. Such procedures can be very time consuming and slow, especially since our prior studies have shown that a cocktail of persistent organic compounds such as acetaminophen, sulfamethoxazole, and carbamazepine as well as many other contaminant compounds are present in each environmental sample, e.g. waste water or seawater. Therefore, there is merit in developing a standardized, single protocol for extracting multiple analytes from a single sample. Using the same procedure for analyte extraction of multiple POPs present in a sample during the SPE clean-up phase prior to analysis can greatly reduce the cost and time of environmental monitoring. The major downside to developing a single extraction procedure for extracting multiple analytes simultaneously from a sample during the SPE clean-up phase is not obtaining effective and complete recovery of all analytes present in the sample. It is therefore probable that inadequate extraction methods have resulted in environmental analyte concentrations being under reported in the past.

3.3.12 SPE RECOVERY PERCENTAGES

The efficiencies of the quantitative recovery of the standard analytes (acetaminophen, aspirin, sulphamethoxazole, diclofenac, ibuprofen and 17-beta estradiol) using the SPE method were evaluated using

the data from recovery experiments. The analytes were recovered from a solution of 5 mg/L cocktail analyte standards in Milli-Q water. The relative percentage recovery of the analytes is given in Table 11.

Table 11: Mean recovery percentages for standard analytes by SPE method

Standards Analytes	Expected concentration (mg/L)	Measured concentration (mg/L)	Average recovery (%)
Acetaminophen	5	4.81	96.2
Aspirin	5	4.96	99.2
Sulphamethoxazole	5	4.88	97.6
Diclofenac	5	4.76	95.2
Ibuprofen	5	4.67	93.4
17-beta estradiol	5	4.69	93.8

3.3.13 PHARMACEUTICAL ANALYTES PRESENT IN WATER SAMPLES

Four water samples studied in this work include an effluent sample from the wastewater treatment plant, tap water (cold and hot), and Milli-Q water. The result from the analysis is presented in Table 12 and Figure 10. In the effluent sample, 3.306 ng/mL of diclofenac and 1.18 ng/mL of sulphamethoxazole were quantified to be present. Other pharmaceuticals under study were below the limit of quantification (BLQ). Nevertheless, in the other water samples, none of the analytes under investigation was detected.

Table 12: Identification of pharmaceuticals in the Effluent water sample. Determination made with HPLC. Effluent water sample purified with C18 (Strata-X) nonpolar sorbent.

Analytes	Effluent [ng/mL]	Hot tap water [ng/mL]	Cold tap water [ng/mL]	Milli-Q water [ng/mL]
Acetaminophen	BLQ	BLQ	BLQ	BLQ
Aspirin	BLQ	BLQ	BLQ	BLQ
Sulphamethoxazole	1.18	BLQ	BLQ	BLQ
Diclofenac	3.306	BLQ	BLQ	BLQ
Ibuprofen	BLQ	BLQ	BLQ	BLQ
17-beta estradiol	BLQ	BLQ	BLQ	BLQ

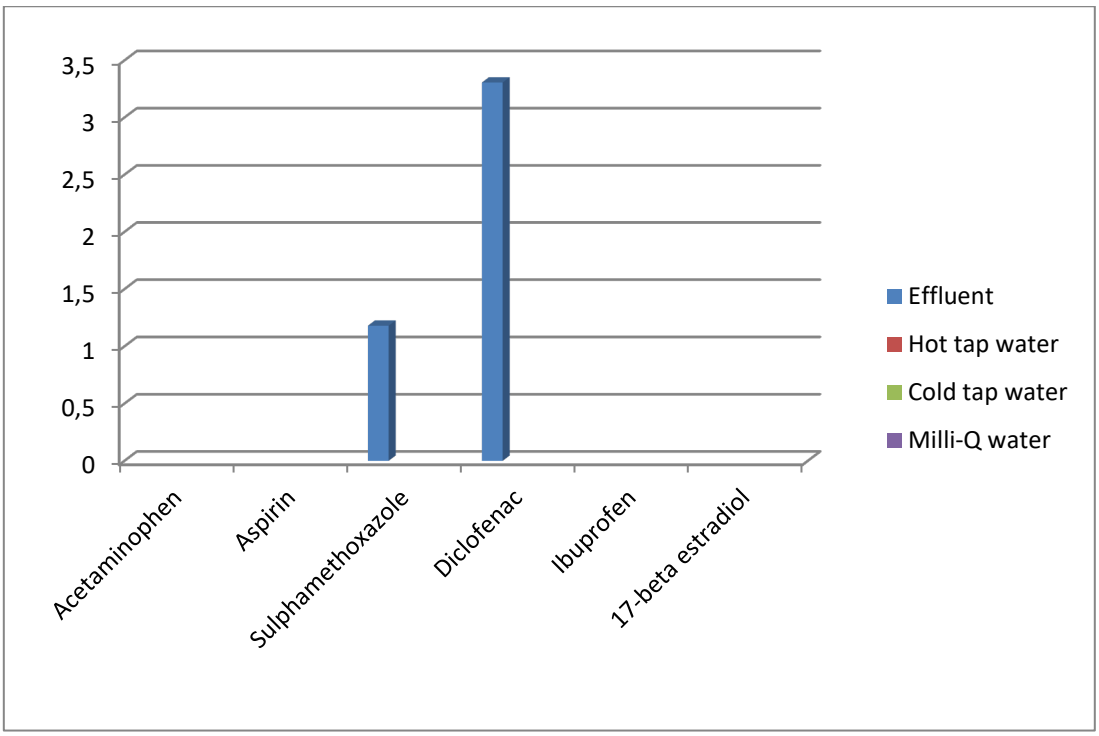


Figure 10: Pharmaceutical analyte concentration amounts in the water samples.

3.3.14 REPEAT STUDIES ON ANALYTE RECOVERY

This section reports the results of the extraction of three standard pharmaceutical analytes, namely, acetaminophen, sulfamethoxazole, and carbamazepine, of varying polarity, which roughly cover the polarity spectrum of organic compounds of emerging concern in the environment, using varying ratios of two different solvents followed by the recovery from the SPE. These standard compounds were selected based on their relative polarities, to determine their probable respective analyte recovery percentages in the SPE step of common environmental samples. In many cases instrumental operators or technicians insist on sample clean prior to injecting the sample onto HPLC columns. There may be a loss of analyte, which is what this section aims to illustrate.

3.3.15 METHODOLOGY

A 1 ug/mL analyte mixture containing each analyte (namely, acetaminophen, sulfamethoxazole, and carbamazepine) was prepared in a 2 mL Eppendorf vial using previously prepared 1 mg/mL standard stock solutions of acetaminophen, sulfamethoxazole, and carbamazepine prepared in HPLC grade methanol. The 1 μg/mL analyte mixture was vortexed for 5 minutes, and then diluted 10-fold using Milli-Q water to obtain a 100 ng/mL analyte mixture in 10 % methanol solution. The resulting analyte mixture was vortexed for 5 minutes to ensure complete dissolution of analytes. Six SPE wash step solutions were made up using HPLC grade methanol and Milli-Q water with concentrations ranging from pure Milli-Q water up to 50 % methanol in five 10 % increments. Six HLB PRiME 3cc 150 mg SPE cartridges were then loaded into a CEREX 48 SPE WARE manifold with nitrogen as the carrier gas. The cartridges were then pre-conditioned using 3 mL Mill-Q water at a flow rate of 1 mL/min. To each SPE cartridge, 1 mL of the 100 ng/mL analyte mixture solutions were loaded.

The 2 mL Eppendorf vial was then rinsed using an additional 1 mL of a 10 % methanol solution to ensure that the full 100 ng was transferred to the SPE cartridge. The solutions were allowed to pass through the SPE cartridge at a flow rate of 1 mL/min. The 2 mL run-off solutions were collected to determine if any analytes were not being retained on the stationary phase of the SPE cartridge. Cartridges 1 through 6 were washed twice using 2,5 mL of Milli-Q water, 10, 20, 30, 40 and 50 % methanol solutions respectively. In each case, the total wash volume collected per cartridge for further analysis was 5 mL. The remaining analytes were then eluted using 5 mL of HPLC grade methanol and collected for analysis. Each collection was allowed to dry by vacuum under reduced pressure. The collected samples were then reconstituted with 200 μL of methanol before analysis.

A Shimadzu 8040 LC-MS equipped with an InfinityLab Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7 μm) column at 30˚C was used to analyze each experimental sample in both the positive and negative electrospray ionization (ESI) modes. All analytes were setup for detection within the calibration range of 0.32-1000 ng/mL. A blank sample was injected after the highest standard to establish carry-over in relation to the lower limits of quantification. ESI-method: Mobile phase A – 10 mM ammonium acetate in water; Mobile phase B – Acetonitrile. The flowrate was set to a constant 0.5 mL/min with a gradient elution of 60 to 95 % B over 3 minutes; 93 % up to 3.5 minutes; 95 to 60 % up to 4 minutes; equilibrate at 60 % until 7 minutes. ESI+ method: Mobile Phase A – 2 mM Ammonium formate with 0.1 % formic acid in water:acetonitrile 95:5 by volume; Mobile Phase B – 2 mM Ammonium formate with 0.1 % formic acid in acetonitrile:water 95:5 by volume. The flowrate was set to a constant 0.5 mL/min with a gradient elution of 10 to 95 % B over 5 minutes; 95 % up to 5.5 minutes; 95 to 10 % up to 6 minutes; equilibrate at 10 % until 9 minutes.

3.3.16 RESULTS AND DISCUSSION

Table 13 summarizes the LC-MS results based on peak areas for each of the loading, wash, and elution steps. Calculated is also the percent recovery of each analyte at varying concentrations of methanol used during the wash steps. Percent recovery is calculated by dividing the elution peak area by the sum of all step peak areas, and multiplying by 100. It is assumed that all analytes are eluted using 5 mL of methanol.

Table 13: Peak areas for various cartridges at different SPE steps and total percent recovery.

Chemical analyte:	Cartridge number:	% Methanol used in wash step:	Loading step peak area:	Wash step peak area:	Elution step peak area:	Percent recovery:
Acetaminophen	1	0 %	564378	111062	1981071	74,57
	2	10 %	506480	1301270	2108579	53,84
	3	20 %	757052	1228751	831 181	29,51
	4	30 %	543414	2116466	1553654	36,87
	5	40 %	592831	3900772	173901	3,73
	6	50 %	592831	5440066	19510	0,32
Sulfamethoxazole	1	0 %	1765	2695	487486	99,09
	2	10 %	2 131	0	520645	99,59
	3	20 %	899	5029	664249	99,12
	4	30 %	0	5905	496696	98,83
	5	40 %	1198	217437	557301	71,82
	6	50 %	1198	606476	19489	3,11
Carbamazepine	1	0 %	16348	18099	62622155	99,95
	2	10 %	25 639	20893	49019835	99,91
	3	20 %	30 685	31899	56949374	99,89
	4	30 %	6 398	8623	58648086	99,97
	5	40 %	19768	49003	73191261	99,91
	6	50 %	19768	15804745	53445180	77,16

Any attempts to use various solutions in a procedure to clean up environmental samples are shown to have a significant effect on analyte recoveries in the SPE step of the experimental procedure. The compound which is chemically more polar, such as acetaminophen in this case, was found to have a maximum achievable recovery of about 75 percent when using 10 % methanol as the sample clean-up solution. This is due to the analyte having an affinity to the 10 % methanol solution in which it is initially dissolved, as well as being the most water-soluble analyte of the three compounds.

It should be noted that using Milli-Q water as the clean-up step solvent will not have any significant effect on removing organic sample matrix interferences, and therefore a sample pretreatment or clean-up procedure such as this will not work effectively for acetaminophen. For more lipophilic analytes such as sulfamethoxazole and carbamazepine, washing with 5 mL of methanol solution up to 30 % may be acceptable, where recoveries of 98,83 and 99,97 % for sulfamethoxazole and carbamazepine respectively were attained. What is certain is that clean-up or washing prior to elution definitely takes down the analyte of interest. These results suggest that additional research is required to optimize any implemented clean-up methodologies which are designed for simultaneous analyte quantification in complex matrices.

CHAPTER 4: DETECTION AND QUANTIFICATION OR EMERGING POLLUTANTS IN WATER USING CAPILLARY ELECTROPHORESIS

4.1 INTRODUCTION

The aim of the optimisation process is to investigate the chemical and instrumental parameters affecting the performance in capillary electrophoresis (CE) for method validation. Especially, the parameters studied are voltage (affecting the field strength), pH of the electrolyte solution (affecting the separation of the analytes and dissociation of the analytes), and injection type (sample introduction to analysis and detectability).

4.2 INSTRUMENTATION

A commercial CE instrument (Agilent Technologies, Waldbronn, Germany) with diode array detector and air cooler system was used for the determination. The CE instrument was applied with ChemStation programmes (Agilent) for instrument running and data handling. PeakMaster software was used to predict the migration order of the analytes. Bare fused silica capillaries (inner diameter 50 µm, outer diameter 375 µm, total length 48.5 cm, length to detector 40.0 cm).

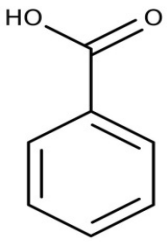
4.2.1 SAMPLE SOLUTIONS

4.2.1.1 STANDARD SOLUTION

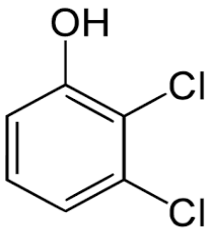
Solutions (standard solution, abbreviated as the MIX) of the six analytes; benzoic acid, 2,3-dichlorophenol, acetylic salicylic acid benzylamine, and imidazole (Figure 11) were prepared as a mixture for the qualitative studies. Their pKa-values, which inform their ionization in the electrolytes, are listed below:

- Benzoic acid (pKa = 4.2)
- 2,3-dichlorophenol (pKa = 7.5)
- 2,4-dinitrophenol (pKa = 4.0)
- Acetyl salicylic acid (Aspirin) (pKa = 3.5)
- Benzylamine (pKa = 9.4)
- Imidazole (pKa = 7.0)
- Salicylic acid (pKa = 2.98)

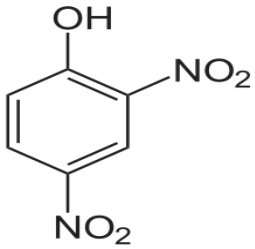
The working solution was made from 1000 µg/mL solutions (individual solutions for each standard in methanol; solutions are ready to use) by first preparing a mixture of 100 µg/mL from the stock solutions in methanol. Then, the final working solution at 20 µg/mL was made from the 100 µg/mL solution in purified water (MilliQ-water) in a 10 mL volumetric flask. A fresh stock was made each time for acetylsalicylic acid, since it is degraded easily to salicylic acid.



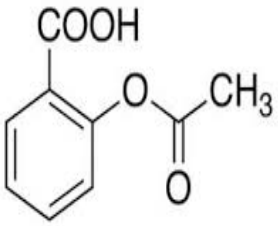
Benzoic acid



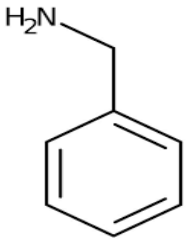
2,3-dichlorophenol



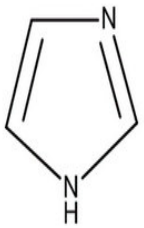
2,4-dinitrophenol



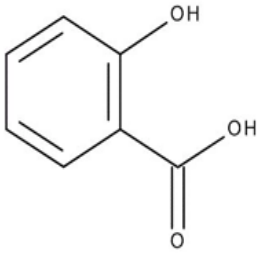
Acetyl salicylic acid (Aspirin)



Benzylamine



Imidazole



Salicylic acid

Figure 11: Structure of the standards.

4.2.2 METHANOL MILLIQ WATER SAMPLE

A methanol-MilliQ water (50:50, v/v) sample was needed for detecting the mobility of electro-osmosis.

4.3 CALIBRATION STANDARD FOR ASPIRIN QUANTIFICATION

Acetysalicylic acid (Aspirin) reference samples for quantification were prepared to determine the concentration of Aspirin in Disprin™ tablets. To measure the amount of acetylsalicylic acid (Aspirin) and the degradation product, salicylic acid, a concentration calibration curve with 2, 5, 10, 15, 20, and 25 µg/mL standard mixtures were prepared (only the two compounds in the mixture; preparation from 100 µg/mL solutions).

4.3.1 DISPIRIN SAMPLE

A sample was prepared from the pharmaceutical Disprin™ 500 mg. The pill contains acetylsalicylic acid 63% (w/w). The whole Disprin tablet was crushed in a mortar and 10 mg was taken for the analysis. It was dissolved in MilliQ-water with the use of sonication for 5 minute. A 1000 µg/mL concentration of Aspirin was prepared, and the sample was syringe-filtered (0.45 µm) and then diluted to a concentration of three subsamples of 25 µg/mL that were determined with CE.

4.3.2 ELECTROLYTE SOLUTION

Tricine (Figure 12) buffer was prepared from the 400 mM stock solution. Two electrolyte solutions were prepared from the stock solution. Their pHs were adjusted with 1.0 M NaOH and 0.1 M NaOH. The exact volume of each buffer was recorded. The freshly prepared buffered electrolyte solutions had nearly the same ionic strengths, but different concentrations and pH-values: **I**=10 Mm; **BGE1** (14 mM, **pH 8.5**) and **BGE2 (**50 mM, **pH 7.5**). The final volumes of the buffers were 50 mL made up with Milli-Q water. The final dilution to the mark of the volumetric flask was made after the pH adjustment. Both buffers were transferred to CE vials (800 µL) used in the CE instrument carousels.

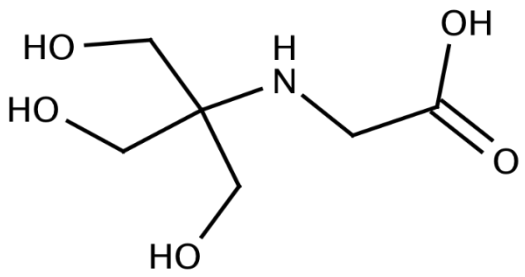


Figure 12: The structure of Tricine (pKa-value 8.15)

4.3.3 CONDITIONING OF THE CAPILLARY

Before any analysis, the capillary was conditioned by flushing it with 0.1 M NaOH for 5 minutes, followed by Milli-Q water for 10 minutes, then finally with the BGE1 electrolyte for 10 minutes respectively. Subsequently, when the BGE2 electrolyte was used, the capillary was rinsed with BGE2 electrolyte for 5 minutes before any analysis. In total, 20 vials were used altogether (NaOH, MilliQ water, 4BGE1 (for 6 standard MIX), 3BGE1 (for Disprin), 4BGE2 (for 6 standard MIX), standard MIX (containing all the 6 analytes), the pre-treated Disprin sample, 6 standard MIX solutions (concentrations from 5 to 25 µg/mL) for quantification of acetylsalicylic acid, and a waste vial).

4.4 METHOD DEVELOPMENT

The methods were prepared offline while conditioning the capillary; the vials were placed into the sample carousel, the needed methods were programmed and combined into a sequence. The temperature of the capillary cassette was 25°C, and the detection wavelength was 214 nm in all methods. (However, to measure the differences in intensities, 4 other wavelengths were chosen). Current was detected in each analysis; and between the runs, the capillary was flushed for 2 minutes with the BGE used (BGE1 or BGE2; depending on the analysis).

4.4.1 ANALYSES WITH BGE1 ELECTROLYTE (pH 8.5; I = 10 mM)

The constant pressure injection was 35 mbar, and the injection time was 10 seconds. The following analyses were carried out with the BGE1:

a. **Control analysis for electro-osmosis**
   A mixture of methanol and MilliQ-water (50:50, v/v) was injected as a control sample (blank) for the measurement of the EOF mobility. Analysis voltage was 25 kV and analysis time ranged from 5 to 13 minutes.
b. **Voltage series**
   Analyses of the standard sample with different voltages (10, 15, 20 and 25 kV). One run per voltage was made, and the analysis time ranged about 5-13 minutes.
c. **Repeatability and identification**
   Six repetitions of the standard solution (mixture of all the analytes) were carried out, with the voltage set at 25 kV. Analysis time was 5 minutes.
   The peakmaster 5.1-software was used to predict the migration order of the analytes.

4.4.2 ANALYSES WITH BGE2 ELECTROLYTE (PH 7.5; I = 10 MM)

Six analyses were carried out. The constant pressure injection was 35 mbar, and the injection voltage was 25 kV.

a. **Control analysis for electro-osmosis**
   Analysis of the MilliQ-water with 25 kV (a control sample, blank) for measuring the mobility of the EOF.
b. **Constant pressure injection**
   The standard sample (analyte mixture) was injected with constant pressure of 35 mbar with different injection times (5, 10, 15, 20, and 30 seconds). Analysis voltage was 25kV.

4.5 CE OPTIMISATION RESULT

The Peakmaster 5.1-software was employed to predict the migration order of the individual analytes, and the parameters that ensure correct prediction are shown in the Peakmaster table shown in Figure 13 below.

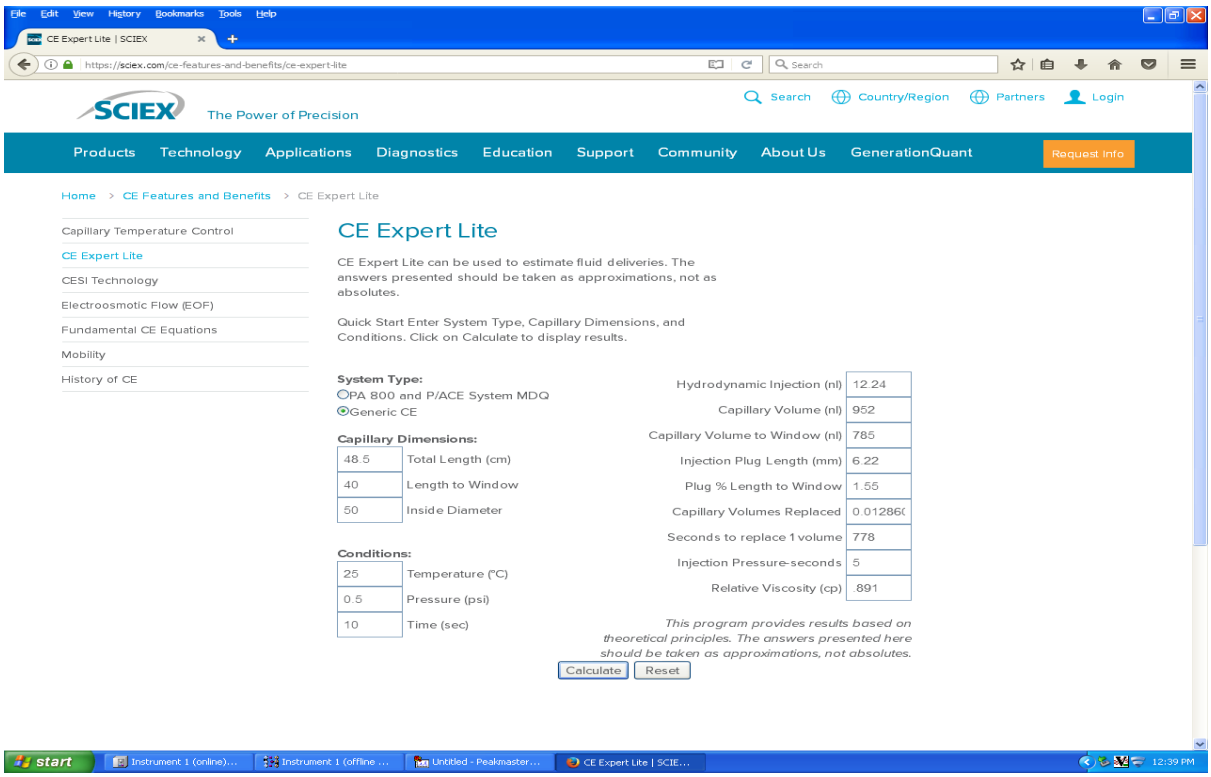


Figure 13: Peakmaster software

After the necessary information about the capillary length and other vital experimental values were entered into the menu boxes, then the migration order was calculated and predicted as in Figure 14.

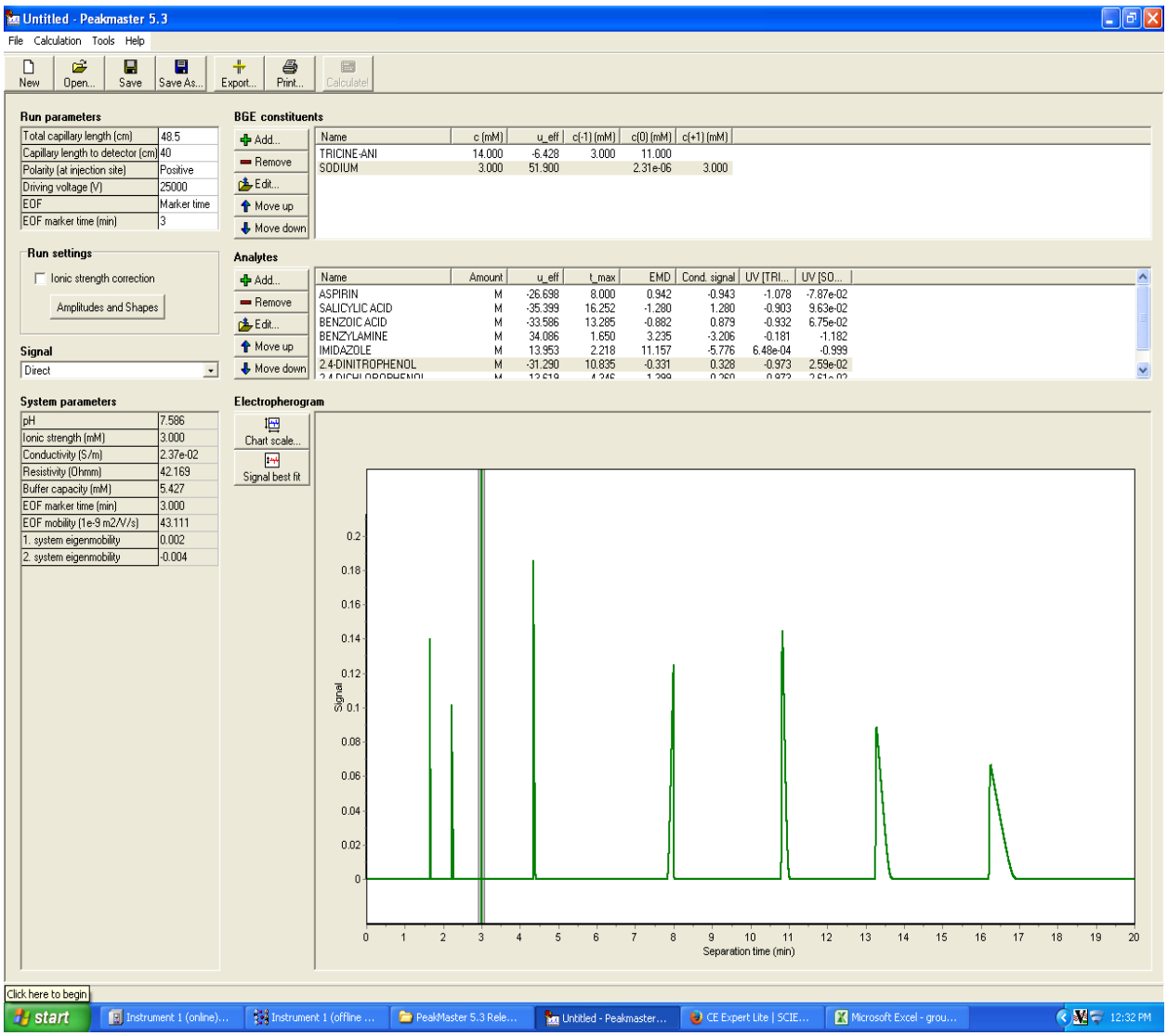


Figure 14: Individual analytes correctly predicted with Peakmaster software.

Thereafter, once the prediction and the experiments were carried out using both buffered electrolytes BGE1 and BGE2, the electropherogram of the result showing the individual analytes in their correctly predicted order was obtained. Figure 15 shows the electropherogram showing the analytes, with two analytes peaking before the electro-osmosis and five analytes peaking after the electro-osmosis in their order of prediction.

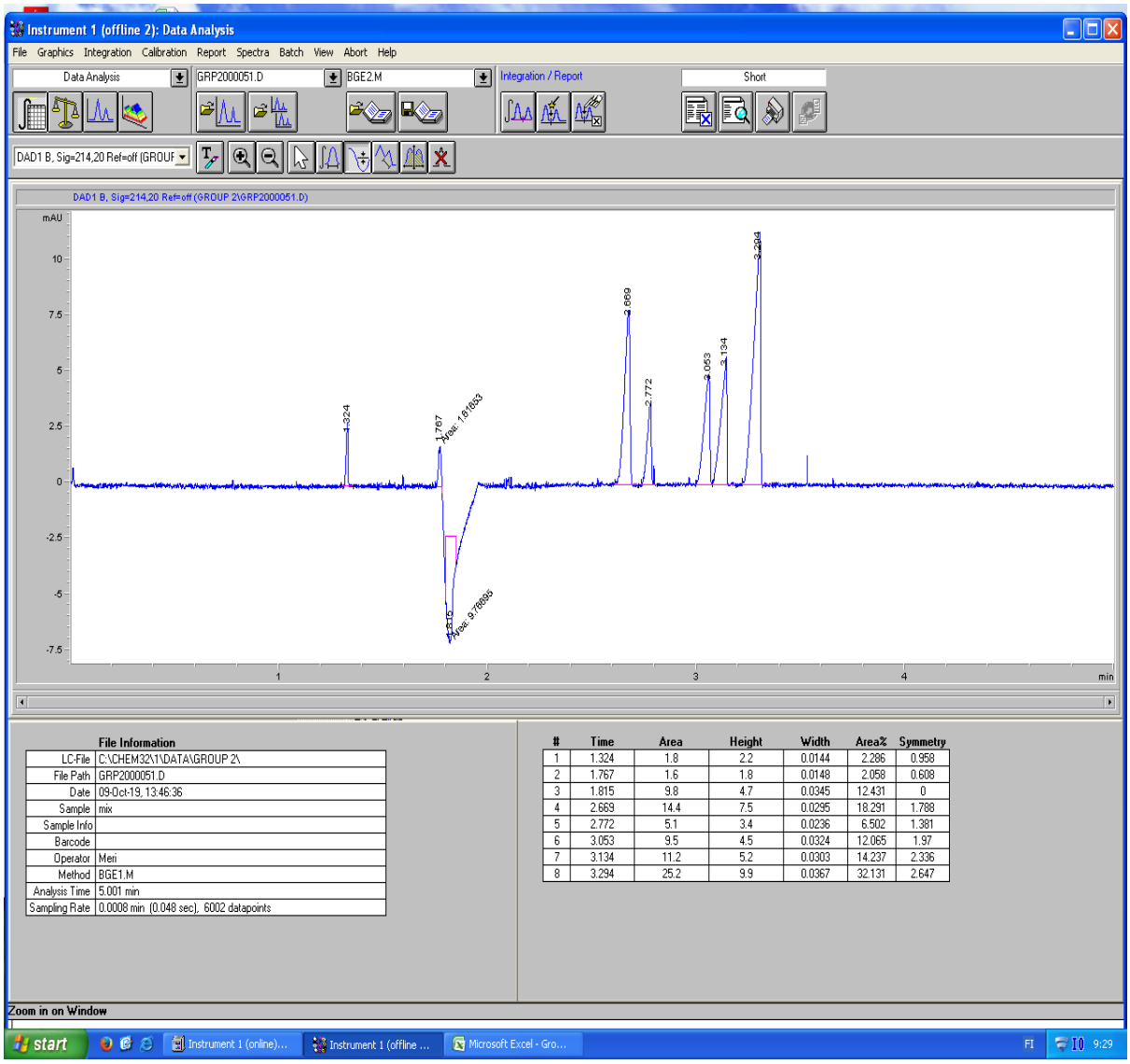


Figure 15: Electropherogram showing the peaks of individual analytes in BGE1 electrolyte.

In the electropherogram above, the analytes appeared in the correct order of prediction. The order is Benzylamine, Imidazole, Aspirin, 2,3-dichlorophenol, 2,4-dinitrophenol, Benzoic acid and Salicylic acid. The analyte peak appearing before the electro-osmosis have their electrophoretic mobilities μ_{ep} added to electro-osmotic mobility μ_{eo}, this is expressed in equation 1, the equation is for cations, but electro-osmosis is subtracted from the electrophoretic mobility when the compound migrates after electro-osmosis, which means that the anions have negative electro-osmosis values.

$$\mu_{tot} = \mu_{ep} + \mu_{eo} \quad \textbf{(1)}$$

In the equation 1, μ_{tot} is the total mobility of a compound. μ_{eo} is the mobility of the electro-osmosis (EOF).

4.6 ELECTROPHORETIC MOBILITY (μ_{EP})

This is the mobility due to the individual analyte in the buffer solution under the influence of the spatial uniform electric field. Electrophoretic mobility is calculated as illustrated below:

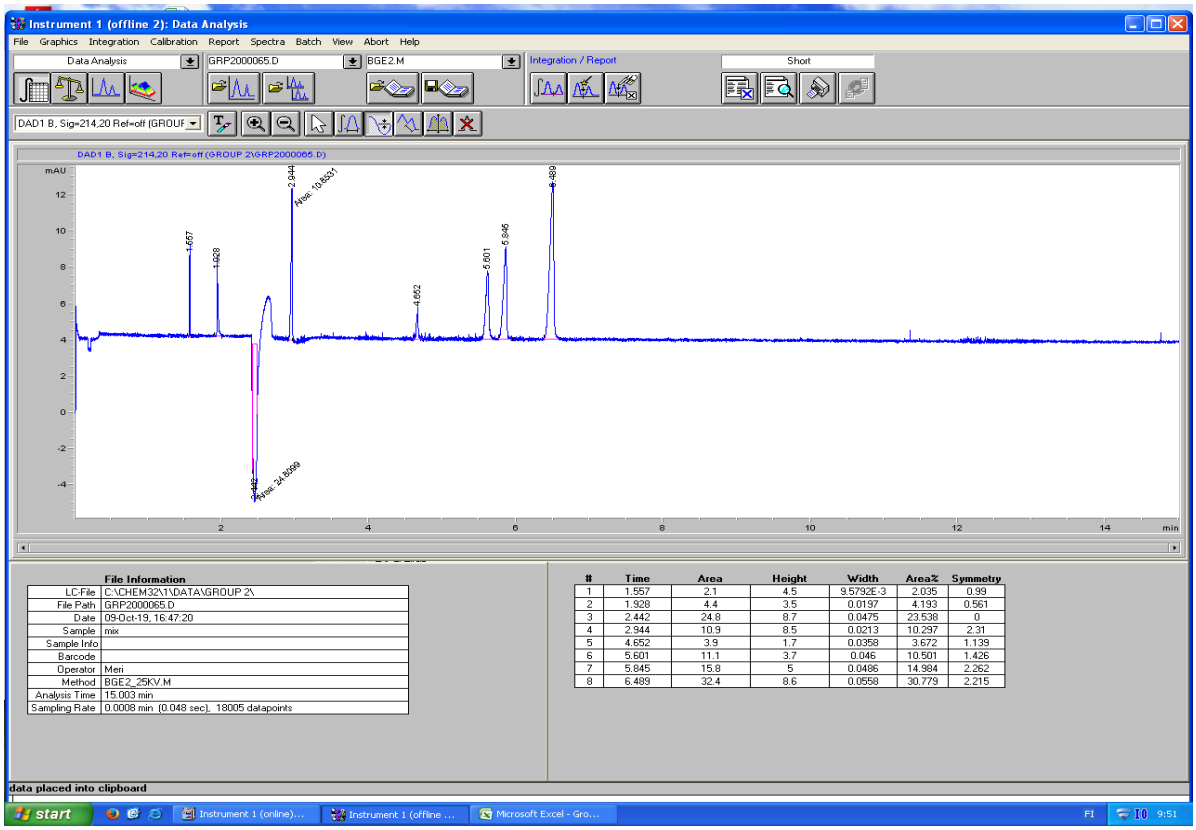
$$\mu_{ep} = \frac{L_{cap} \times L_{det}\,(m^2)}{Voltage\,(V) \times Time\,(s)}\,[m^2/Vs] \qquad (2)$$

Where Lcap = Length of the capillary (m);

Ldet = Length to the detector (m);

V = Voltage applied (V);

T = Migration time (s).



#	Time	Area	Height	Width	Area%	Symmetry
1	1.557	2.1	4.5	9.5792E-3	2.035	0.99
2	1.928	4.4	3.5	0.0197	4.193	0.561
3	2.442	24.8	8.7	0.0475	23.538	0
4	2.944	10.9	8.5	0.0213	10.297	2.31
5	4.652	3.9	1.7	0.0358	3.672	1.139
6	5.601	11.1	3.7	0.046	10.501	1.426
7	5.845	15.8	5	0.0486	14.984	2.262
8	6.489	32.4	8.6	0.0558	30.779	2.215

Figure 16: Electropherogram showing the peaks of individual analytes in BGE2 electrolyte.

It can be observed that there are visible changes in the electropherograms of BGE1 and BGE2 respectively. These differences are ascribed to the slight differences in instrumental and chemical parameters. In Figure 16, the BGE1 buffer (pH 8.5; I = 10 mM) electropherogram shares the same ionic strength with the BGE2 buffer, but the slight difference in separation efficiency and peak intensities of individual analytes can be attributed to the differences in the pH, degree of dissociation

4.7 REPEATABILITY STUDY

The results of the repeatability study carried out with the six analytes in order to validate the instrument's performance and reliability are presented in Table 14. In the repeatability study for Benzylamine, it can be observed that the migration times are very close to one another; this is evident in the closeness in values of

the respective electrophoretic mobility readings. The RSD value of 0.463 for the electrophoretic mobility shows the repeatability of the analysis (Table 14). In the same vein, the rest of the analytes gave similar good repeatability, with their standard deviation and relative standard deviation values; and the results are compiled in Tables 15-20, respectively.

Table 14: Results of the repeatability calculations for Benzylamine (pKa = 9.4) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\,[\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	79.44	1.796	7.163×10^{-8}	1.698×10^{-7}
2	79.26	1.806	7.169×10^{-8}	1.701×10^{-7}
3	79.14	2.189	7.178×10^{-8}	1.704×10^{-7}
4	78.72	1.501	7.190×10^{-8}	1.709×10^{-7}
5	79.74	1.681	7.120×10^{-8}	1.690×10^{-7}
6	79.68	1.708	7.099×10^{-8}	1.689×10^{-7}
AVG	79.33	1.780	7.153×10^{-8}	1.699×10^{-7}
STD DEV	0.378	0.229	0.036	0.008
RSD	0.477	12.835	0.498	0.463

Table 15: Results of the repeatability calculations for Imidazole (pKa = 7.0) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\,[\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	106.00	1.617	7.163×10^{-8}	1.451×10^{-7}
2	105.90	1.384	7.169×10^{-8}	1.454×10^{-7}
3	105.58	1.513	7.178×10^{-8}	1.457×10^{-7}
4	105.59	1.508	7.190×10^{-8}	1.458×10^{-7}
5	106.60	1.614	7.120×10^{-8}	1.440×10^{-7}
6	106.75	1.788	7.099×10^{-8}	1.441×10^{-7}
AVG	106.07	1.571	7.153×10^{-8}	1.450×10^{-7}
STD DEV	0.500	0.137	0.036	0.008
RSD	0.471	8.699	0.498	0.544

Table 16: Results of the repeatability calculations for Aspirin (Acetyl salicylic acid) (pKa = 3.5) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\,[\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	160.14	14.370	7.163×10^{-8}	-2.290×10^{-8}
2	159.56	13.145	7.169×10^{-8}	-2.281×10^{-8}
3	159.24	12.836	7.178×10^{-8}	-2.279×10^{-8}
4	159.78	12.639	7.190×10^{-8}	-2.308×10^{-8}
5	160.56	15.571	7.120×10^{-8}	-2.260×10^{-8}
6	160.74	14.229	7.099×10^{-8}	-2.250×10^{-8}
AVG	160.00	13.798	7.153×10^{-8}	-2.278×10^{-8}
STD DEV	0.583	1.127	0.036	0.021
RSD	0.365	8.168	0.498	0.913

Table 17: Results of the repeatability calculations for 2,3-dichlorophenol (pKa = 7.5) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\,[\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	166.32	5.109	7.163×10^{-8}	-2.470×10^{-8}
2	166.02	4.122	7.169×10^{-8}	-2.472×10^{-8}
3	165.78	4.439	7.178×10^{-8}	-2.473×10^{-8}
4	166.08	3.476	7.190×10^{-8}	-2.493×10^{-8}
5	167.10	3.979	7.120×10^{-8}	-2.450×10^{-8}
6	167.88	4.050	7.099×10^{-8}	-2.453×10^{-8}
AVG	166.53	4.196	7.153×10^{-8}	-2.469×10^{-8}
STD DEV	0.802	0.545	0.036	0.0156
RSD	0.482	12.988	0.498	0.632

Table 18: Results of the repeatability calculations for 2,4-dinitrophenol (pKa = 4.0) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\ [\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	183.18	9.479	7.163×10^{-8}	-2.905×10^{-8}
2	182.58	9.263	7.169×10^{-8}	-2.898×10^{-8}
3	182.58	8.967	7.178×10^{-8}	-2.906×10^{-8}
4	182.70	8.017	7.190×10^{-8}	-2.920×10^{-8}
5	183.90	9.406	7.120×10^{-8}	-2.879×10^{-8}
6	185.04	9.056	7.099×10^{-8}	-2.884×10^{-8}
AVG	183.33	9.031	7.153×10^{-8}	-2.899×10^{-8}
STD DEV	0.979	0.534	0.036	0.0152
RSD	0.534	5.918	0.498	0.524

Table 19: Results of the repeatability calculations for Benzoic acid (pKa = 4.2) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\ [\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	188.04	11.185	7.163×10^{-8}	-3.015×10^{-8}
2	187.32	10.748	7.169×10^{-8}	-3.010×10^{-8}
3	187.44	10.572	7.178×10^{-8}	-3.017×10^{-8}
4	187.56	9.408	7.190×10^{-8}	-3.031×10^{-8}
5	188.76	10.934	7.120×10^{-8}	-2.990×10^{-8}
6	189.96	10.718	7.099×10^{-8}	-2.993×10^{-8}
AVG	188.18	10.594	7.153×10^{-8}	-3.009×10^{-8}
STD DEV	1.019	0.618	0.036	0.016
RSD	0.542	5.836	0.498	0.515

Table 20: Results of the repeatability calculations for Salicylic acid (pKa = 2.97) using BGE 1

Repetition Number	Migration [s]	Area	$\mu\ [\frac{m^2}{Vs}]$	$\mu_{ep}[\frac{m^2}{Vs}]$
1	197.64	25.244	7.163×10^{-8}	-3.216×10^{-8}
2	196.88	25.261	7.169×10^{-8}	-3.208×10^{-8}
3	196.98	25.071	7.178×10^{-8}	-3.218×10^{-8}
4	197.09	21.502	7.190×10^{-8}	-3.230×10^{-8}
5	198.42	25.741	7.120×10^{-8}	-3.189×10^{-8}
6	199.78	26.040	7.099×10^{-8}	-3.195×10^{-8}
AVG	197.80	24.810	7.153×10^{-8}	-3.209×10^{-8}
STD DEV	1.127	1.660	0.036	0.015
RSD	0.570	6.692	0.498	0.476

In the voltage series analysis, the results are also compiled in tabular format to portray the changes that take place when the voltage is varied during capillary electrophoresis analysis. The voltage series analysis was also conducted for each of the analytes using the BGE1 buffer. The results are presented in Tables 21-27, respectively.

Table 21: Comparison of injection methods with varying voltage for Benzylamine (pKa = 9.4) using *BGE 1*

	10 kV	15kV	20kV	25kV
Migration time [min]	3.48	2.29	1.68	1.09
Area	5.0794	3.187	2.504	1.869
Velocity [m/s]	2.323 x 10^{-3}	3.5 x 10^{-3}	4.81 x 10^{-3}	7.42 x 10^{-3}
μ $[\frac{m^2}{Vs}]$	2.746 x 10^{-8}	4.132 x 10^{-8}	5.611 x 10^{-8}	6.241 x 10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	6.468 x 10^{-8}	9.772 x 10^{-8}	1.33 x 10^{-7}	1.619 x 10^{-7}

Table 22: Comparison of injection methods with varying voltage for Imidazole (pKa = 7.0) using BGE *1*

	10 kV	15kV	20kV	25kV
Migration time [min]	4.57	3.05	2.30	1.22
Area	4.535	3.137	2.313	1.241
Velocity [m/s]	1.77 x 10^{-3}	2.65 x 10^{-3}	3.52 x 10^{-3}	6.63 x 10^{-3}
μ $[\frac{m^2}{Vs}]$	2.746 x 10^{-8}	4.132 x 10^{-8}	5.611 x 10^{-8}	6.241 x 10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	5.618 x 10^{-8}	8.494 x 10^{-8}	9.899 x 10^{-8}	1.703 x 10^{-7}

Table 23: Comparison of injection methods with varying voltage for Aspirin (Acetyl salicylic acid) (pKa = 3.5) using BGE 1

	10 kV	15kV	20kV	25kV
Migration time [min]	6.70	4.50	3.35	2.15
Area	35.049	24.262	16.678	7.856
Velocity [m/s]	1.21×10^{-3}	1.80×10^{-3}	2.41×10^{-3}	3.76×10^{-3}
$\mu [\frac{m^2}{Vs}]$	2.746×10^{-8}	4.132×10^{-8}	5.611×10^{-8}	6.241×10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	-8.157×10^{-9}	-1.258×10^{-8}	-1.750×10^{-8}	-2.255×10^{-9}

Table 24: Comparison of injection methods with varying voltage for 2,3-dichlorophenol (pKa = 7.5) using BGE 1

	10 kV	15kV	20kV	25kV
Migration time [min]	7.29	4.71	3.49	2.32
Area	10.605	5.602	3.311	1.598
Velocity [m/s]	1.109×10^{-3}	1.716×10^{-3}	2.316×10^{-3}	3.484×10^{-3}
$\mu [\frac{m^2}{Vs}]$	2.746×10^{-8}	4.132×10^{-8}	5.611×10^{-8}	6.241×10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	-9.719×10^{-9}	-1.386×10^{-8}	-1.905×10^{-8}	-6.663×10^{-9}

Table 25: Comparison of injection methods with varying voltage for 2,4-dinitrophenol (pKa = 4.0) using BGE 1

	10 kV	15kV	20kV	25kV
Migration time [min]	7.64	5.19	3.86	2.13
Area	23.625	15.655	11.254	7.743
Velocity [m/s]	1.058×10^{-3}	1.558×10^{-3}	2.094×10^{-3}	3.795×10^{-3}
$\mu [\frac{m^2}{Vs}]$	2.746×10^{-8}	4.132×10^{-8}	5.611×10^{-8}	6.241×10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	-1.053×10^{-8}	-1.640×10^{-8}	-2.260×10^{-8}	-1.690×10^{-9}

Table 26: Comparison of injection methods with varying voltage for Benzoic acid (pKa = 4.2) using BGE 1

	10 kV	15kV	20kV	25kV
Migration time [min]	7.84	5.32	3.96	2.13
Area	28.308	18.885	13.378	9.431
Velocity [m/s]	1.031 10^{-3}	1.519 x 10^{-3}	2.041 x 10^{-3}	3.795 x 10^{-3}
$\mu\ [\frac{m^2}{Vs}]$	2.746 x 10^{-8}	4.132 x 10^{-8}	5.611 x 10^{-8}	6.241 x 10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	-1.096 x 10^{-8}	-1.701 x 10^{-8}	-2.345 x 10-8	-1.690 x 10^{-9}

Table 27: Comparison of injection methods with varying voltage for Salicylic acid (pKa = 2.97) using BGE 1

	10 kV	15kV	20kV	25kV
Migration time [min]	8.21	5.60	4.13	2.724
Area	68.58831787	47.27023125	34.7743454	26.756
Velocity [m/s]	9.846 x 10^{-4}	1.444 x 10^{-3}	1.957 x 10^{-3}	2.968 x 10^{-3}
$\mu\ [\frac{m^2}{Vs}]$	2.746 x 10^{-8}	4.132 x 10^{-8}	5.611 x 10^{-8}	6.241 x 10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	-1.171 x 10^{-8}	-1.823 x 10-8	-2.479 x 10^{-8}	-1.493 x 10^{-8}

In the comparison of the injection method with varying voltage, it can be observed that the migration time decreases as the voltage increases; and so it applies to other parameters including the electrophoretic mobility, which also decreases with increasing voltage. The plot of migration time against the voltage for Benzylamine shows an inverse proportionality between the two parameters (Figure 17). Figure 18 shows the plot of migration time against voltage.

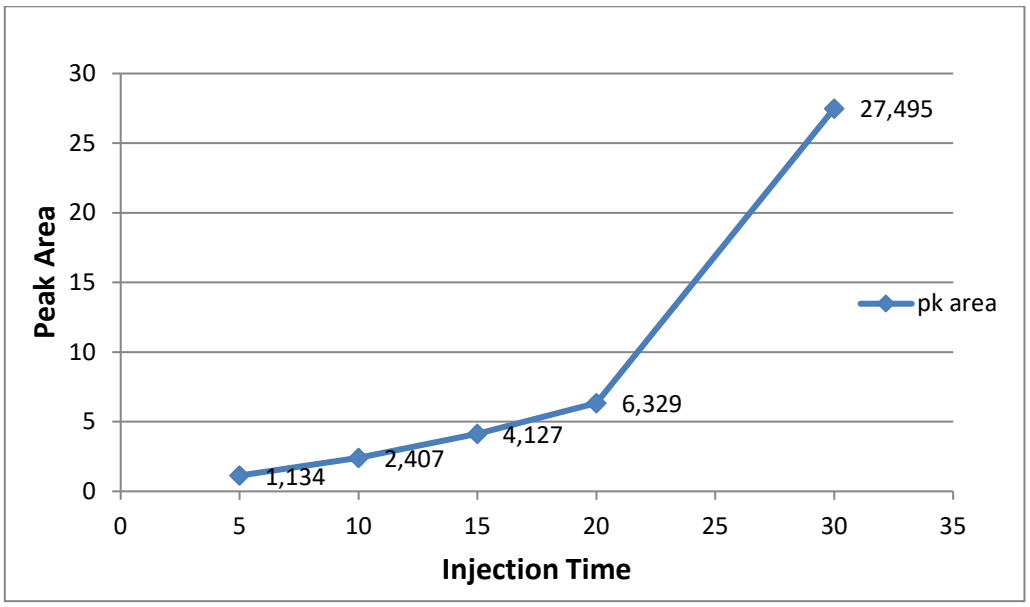


Figure 17: Injection time vs the peak area for Benzylamine

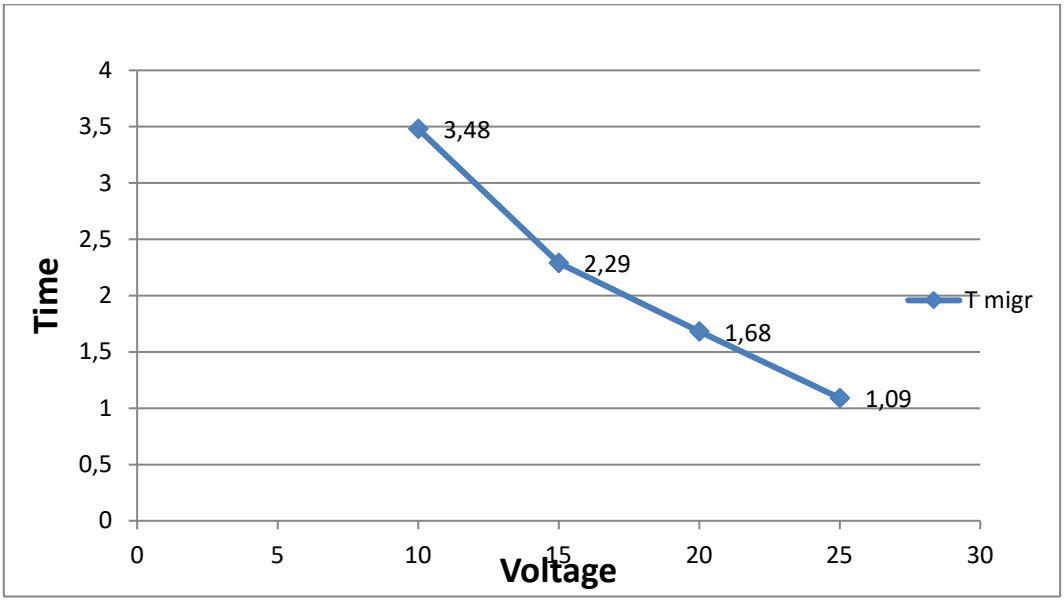


Figure 18: The plot of migration time against voltage.

It is however important to compare the electrophoretic mobilities calculated from the 10 seconds injection in BGE2 to the mobilities in BGE1. Benzylamine and Imidazole electrophoretic mobilities were compared to see the differences. In both cases, BGE1 and BGE2 share the same ionic strength but different pHs and concentrations. Both the injection time and voltage are the same for both buffers, the only difference being the pH. In comparing the constant pressure injection with varying injection time (Tables 28 and 29), it can be seen that the migration time increases with an increase in the injection time, and the peak areas. The electrophoretic mobility however decreases as the injection time increases.

Table 28: Comparison of constant pressure injection with varying injection times for Benzylamine (pKa = 9.4) using BGE 2

	5s	10s	15s	20s	30s
Migration time [min]	1.53	1.57	1.65	1.90	2.39
Area	1.134	2.407	4.127	6.329	27.495
Velocity [m/s]	5.283×10^{-3}	5.149×10^{-3}	4.899×10^{-3}	4.254 x 10-3	3.382×10^{-3}
$\mu\ [\frac{m^2}{Vs}]$	5.072×10^{-8}	5.194×10^{-8}	4.994×10^{-8}	4.293×10^{-8}	2.980×10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	1.353×10^{-7}	1.343×10^{-7}	1.283×10^{-7}	1.110×10^{-7}	8.391×10^{-8}

Table 29: Comparison of constant pressure injection with varying injection times for Imidazole (pKa = 7.0) using BGE 2

	5s	10s	15s	20s	30s
Migration time [min]	1.94	1.95	2.02	2.31	3.07
Area	2.854	4.456	7.516	12.009	12.478
Velocity [m/s]	4.167×10^{-3}	4.145×10^{-3}	4.002×10^{-3}	3.499×10^{-3}	2.633×10^{-3}
$\mu\ [\frac{m^2}{Vs}]$	5.072×10^{-8}	5.194×10^{-8}	4.994×10^{-8}	4.293×10^{-8}	2.980×10^{-8}
$\mu_{ep}[\frac{m^2}{Vs}]$	1.174×10^{-7}	1.183×10^{-7}	1.140×10^{-7}	9.892×10^{-8}	7.193×10^{-8}

Table 30 shows the electrophoretic mobilities calculated from the 10 seconds injection in BGE1 and BGE2. Both buffers have the same injection time and 25 kV analysis voltage. It can be observed in Table 30 that the electrophoretic mobilities in BGE1 are greater than the respective electrophoretic mobilities in BGE2. This confirms that the effect of pHs is significant in capillary electrophoresis.

Table 30: Electrophoretic mobilities calculated from the 10 seconds injection in BGE1 & BGE2

	Electrophoretic Mobility	
	Benzylamine	Imidazole
BGE1	1.699 x 10-7	1.450 x 10-7
BGE2	1.343×10^{-7}	1.183×10^{-7}

Figure 19 shows a display of the method sequence in capillary electrophoresis. Different analysis with different methods can be carried out simultaneously. Therefore, in this optimisation/validation analysis, all the analyses described were performed at once, and to ensure also that the commands are correct (injection times, method names, sample names, and vial number).

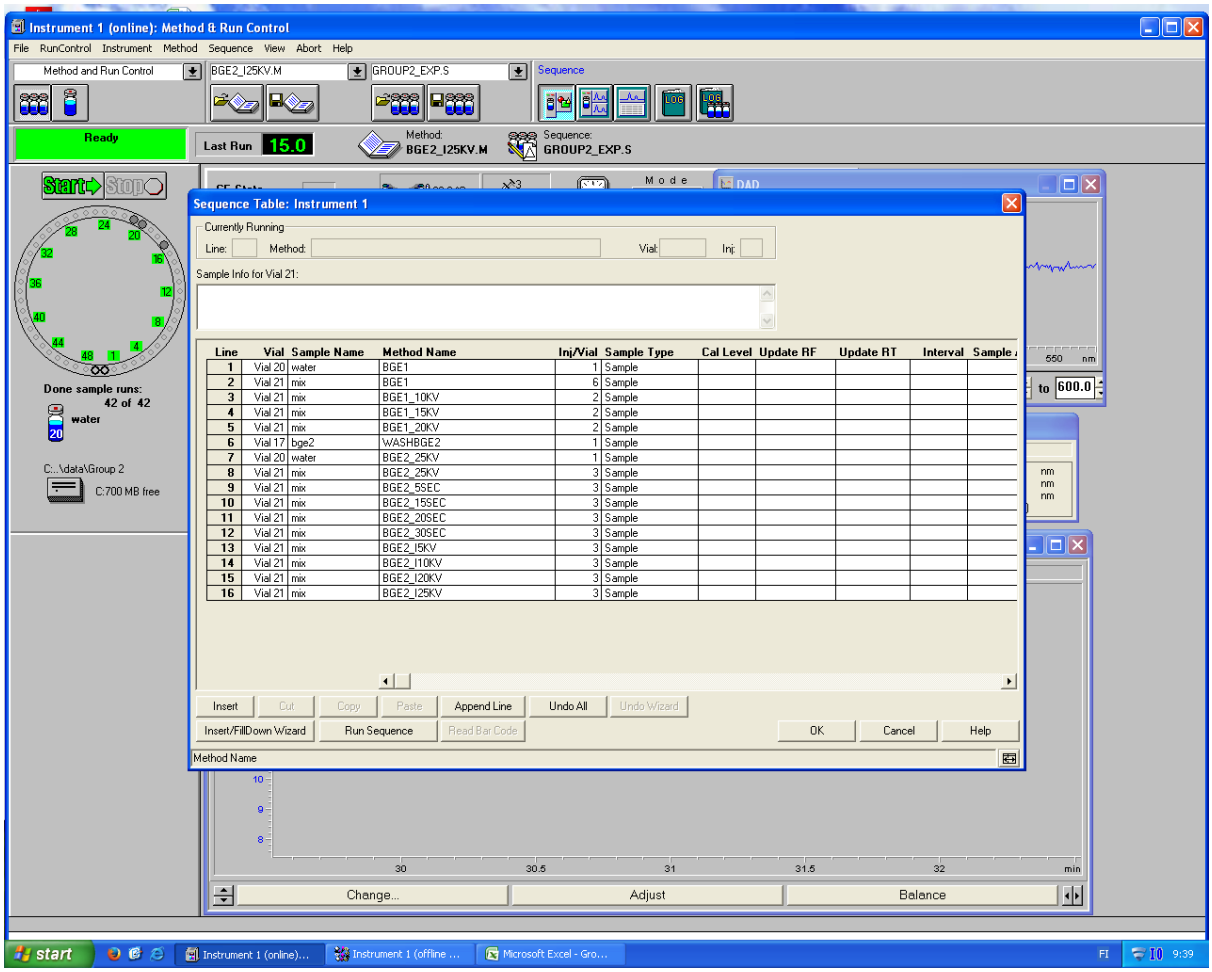


Figure 19: Method sequence in Capillary Electrophoresis.

A wavelength of 214 nm was chosen for the Capillary Zone Electrophoresis analysis, because this is the wavelength where the sensitivity is highest for all the analytes. Other wavelengths were also employed in the analysis to optimise/validate the best wavelength for the best sensitivity. These other wavelengths include: 200 nm, 220 nm, 254 nm, 320 nm.

Figure 20 shows the overlay of the different wavelength values to reveal the best wavelength value. This is shown by the sensitivity and peak intensity of each analyte in the electropherogram. The control analysis for electro-osmosis for the measurement of the mobility of the EOF for both BGE1 and BGE2 are represented in the electropherograms in Figures 21 and 22 respectively. In Figure 23, the overlay of the different wavelengths shows clearly that the peak height belonging to 214 nm gives the best signal intensity for all the analytes, thereby justifying the 214 nm wavelength used for the CE optimisation.

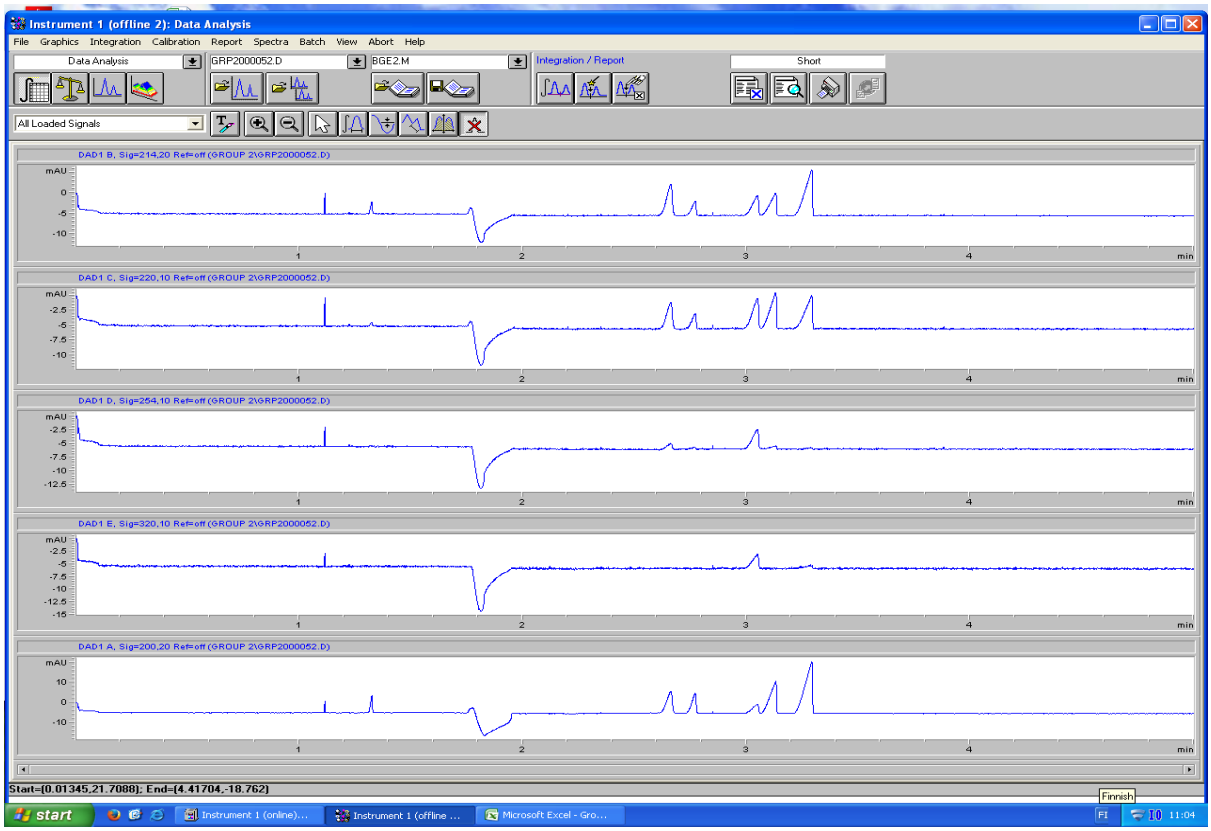


Figure 20: Wavelength overlay showing 214 nm as the best for all the analytes

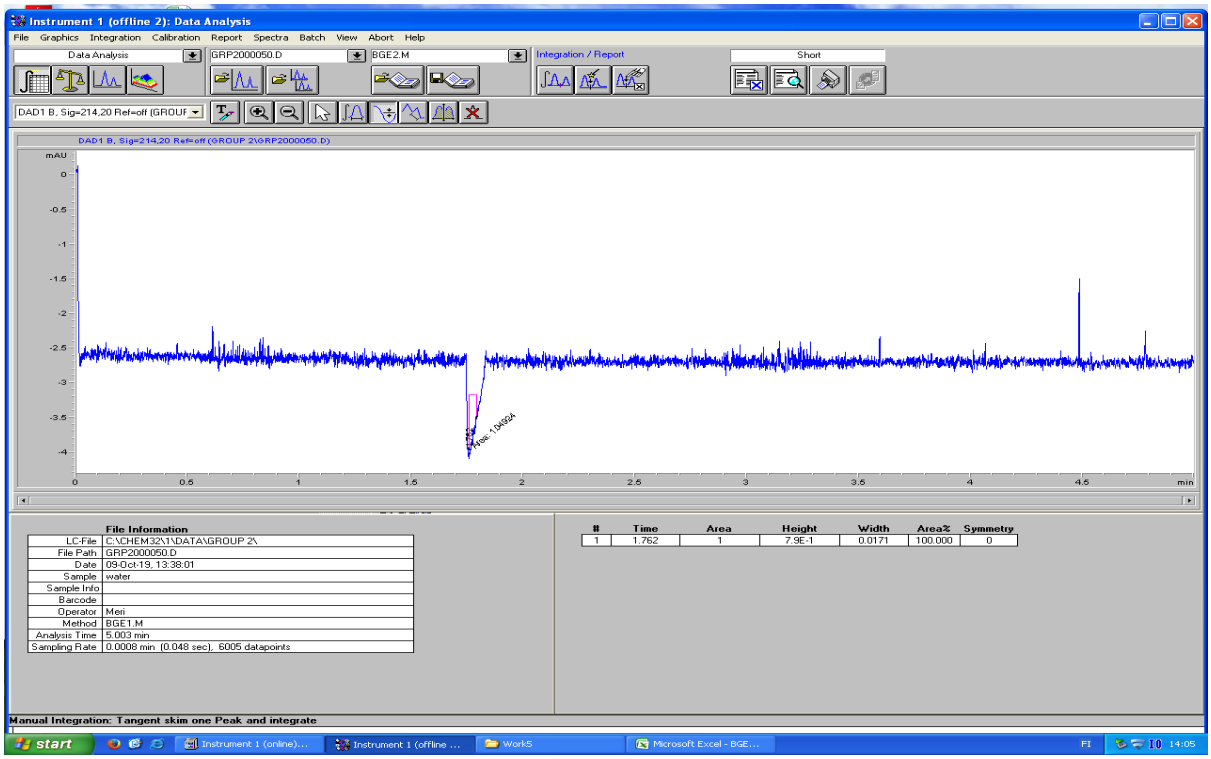


Figure 21: BGE1 control (blank) analysis for electro-osmosis for the measurement of the mobility of the EOF.

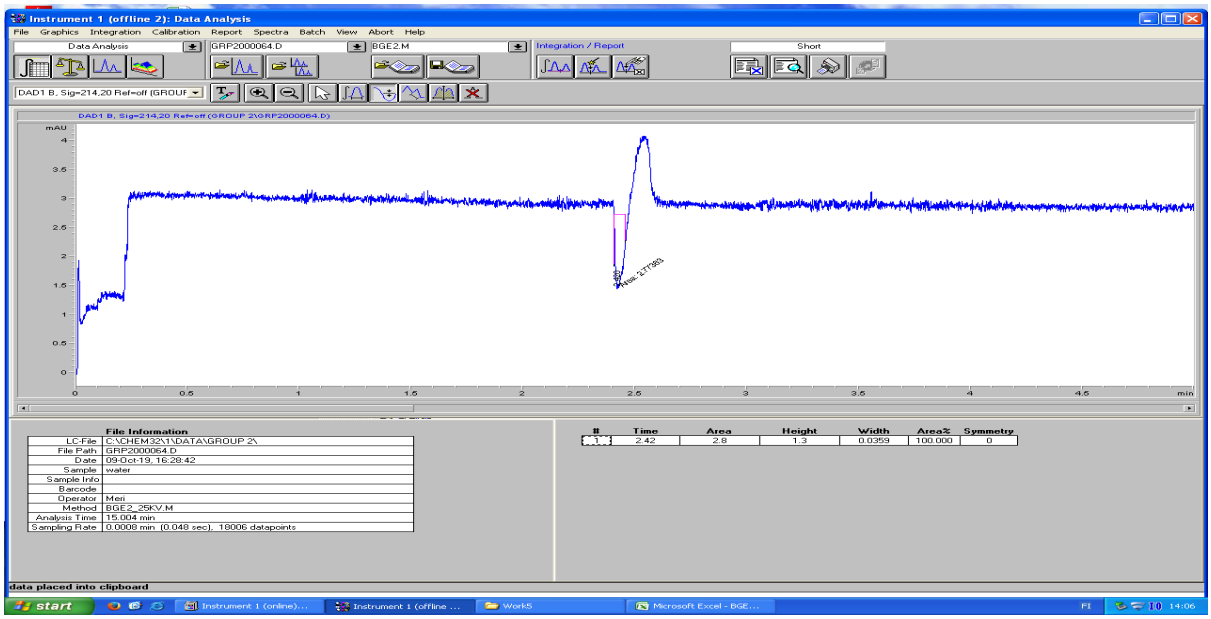


Figure 22: BGE2 control (blank) analysis for electro-osmosis for the measurement of the mobility of the EOF.

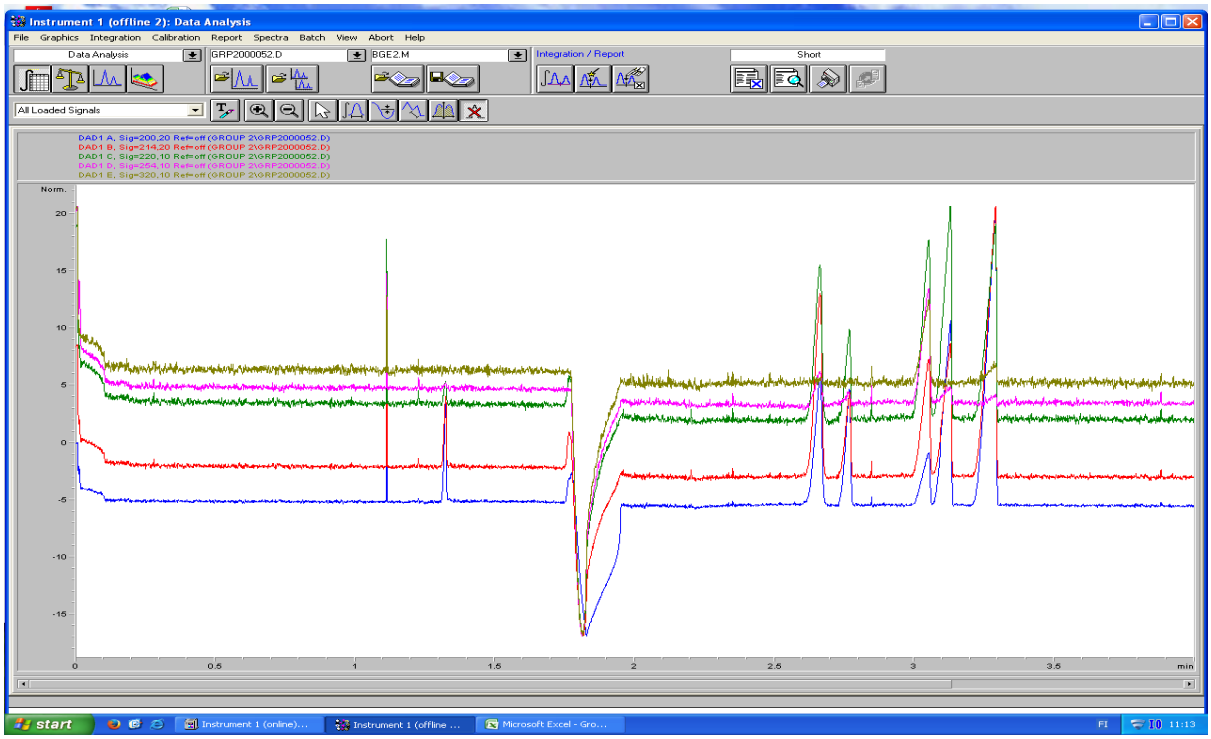


Figure 23: An overlay of the peak heights obtained from different wavelengths.

4.8 DISPIRIN ANALYSIS

Table 31 shows the electrophoretic mobility of aspirin and salicylic acid contained in a Disprin tablet. Acetylsalicylic acid (Aspirin) has a greater electrophoretic mobility value than the degradation product (salicylic acid), resulting in the faster migration time for acetylsalicylic acid as predicted in the peakmaster software in Figure 24.

Table 31: Electrophoretic mobility of Aspirin and Salicylic acid in Disprin Sample Electrophoretic Mobility (μep) [m2/Vs]

Method	Aspirin	Salicylic acid
BGE1	-2.485×10^{-8}	-3.188×10^{-8}

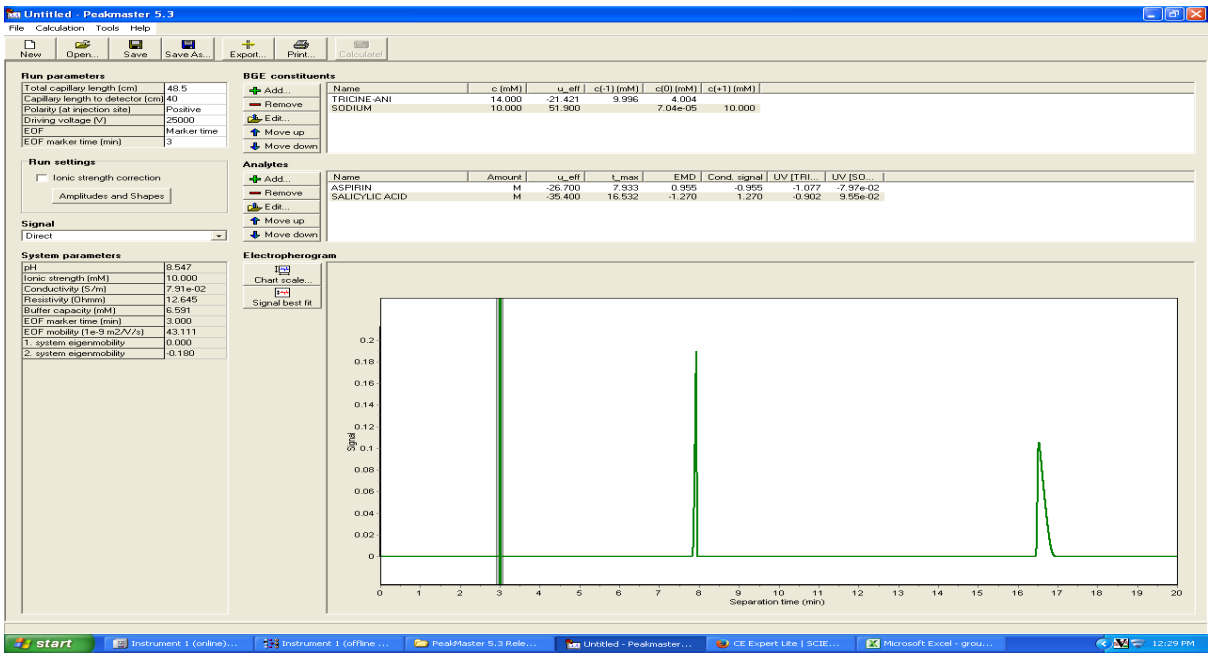


Figure 24: The peakmaster software migration order prediction for acetylsalicylic acid and salicylic acid in Disprin tablet.

Figure 25 shows the electropherogram of Disprin, with the electro-osmosis coming as the negative peak, followed by acetylsalicylic acid (aspirin) and salicylic acid (the degradation product). Table 32 shows the instrument's response to the detection of each of the analytes, and linear sensitivity to increasing concentrations with R^2 values > 0.99 for both acetylsalicylic acid and salicylic acid (Figures 26 and 27). In Table 34, the method's LOD and LOQ values for each analyte were statistically calculated.

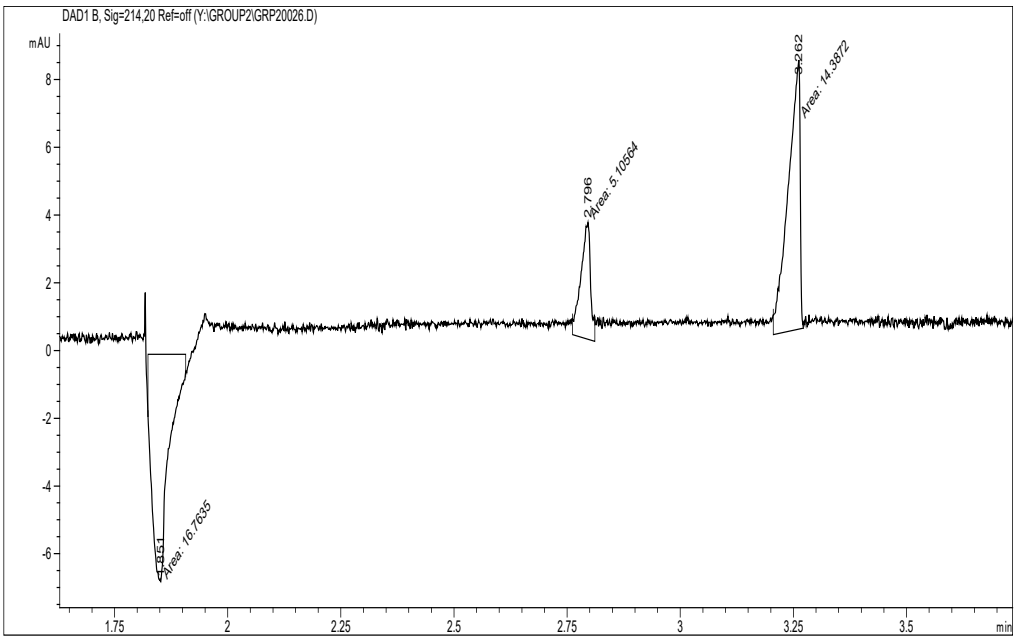


Figure 25: Electropherogram for acetylsalicylic acid and salicylic acid in Disprin tablet at 10 ppm calibration concentration.

Table 32: Calibration data for Asprin and Salicylic acid in Disprin

	Calibration					
Standards	**range [µg/mL]**	**Linear equation**	**R^2**	**λ_{max}**	**LOD**	**LOQ**
Aspirin	2-25	*y = 0.5136× - 0.4449*	0.9979	214 nm	0.004	0.012
Salicylic acid	2-25	*y = 1.6549× - 0.3462*	0.9976	214 nm	0.003	0.009

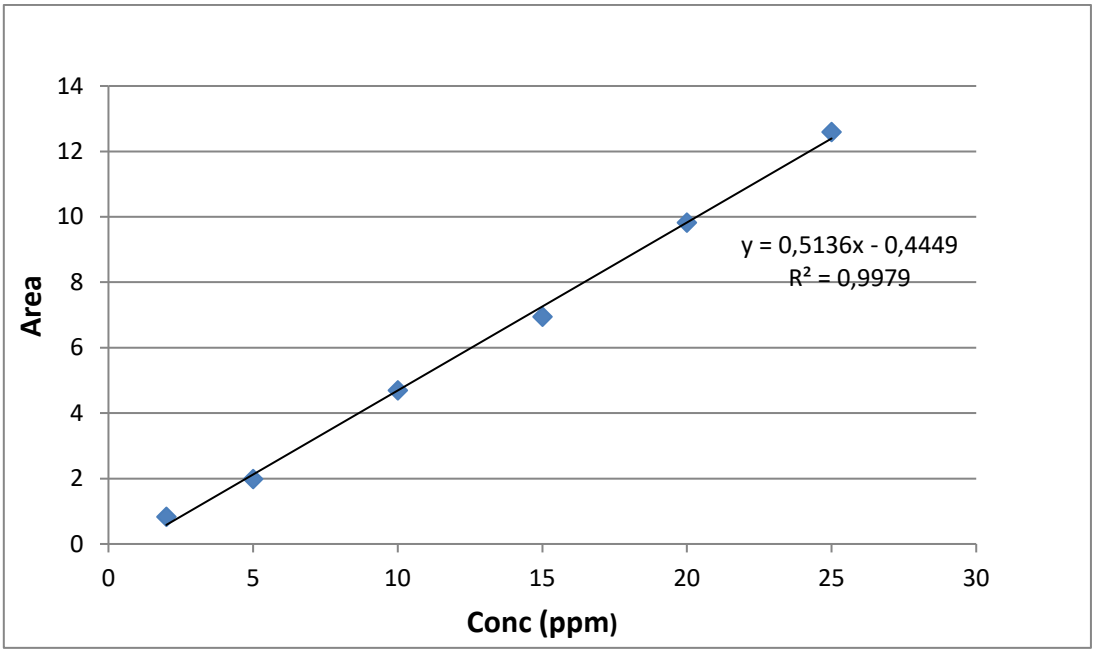


Figure 26: Calibration curve for Acetylsalicylic acid in Disprin

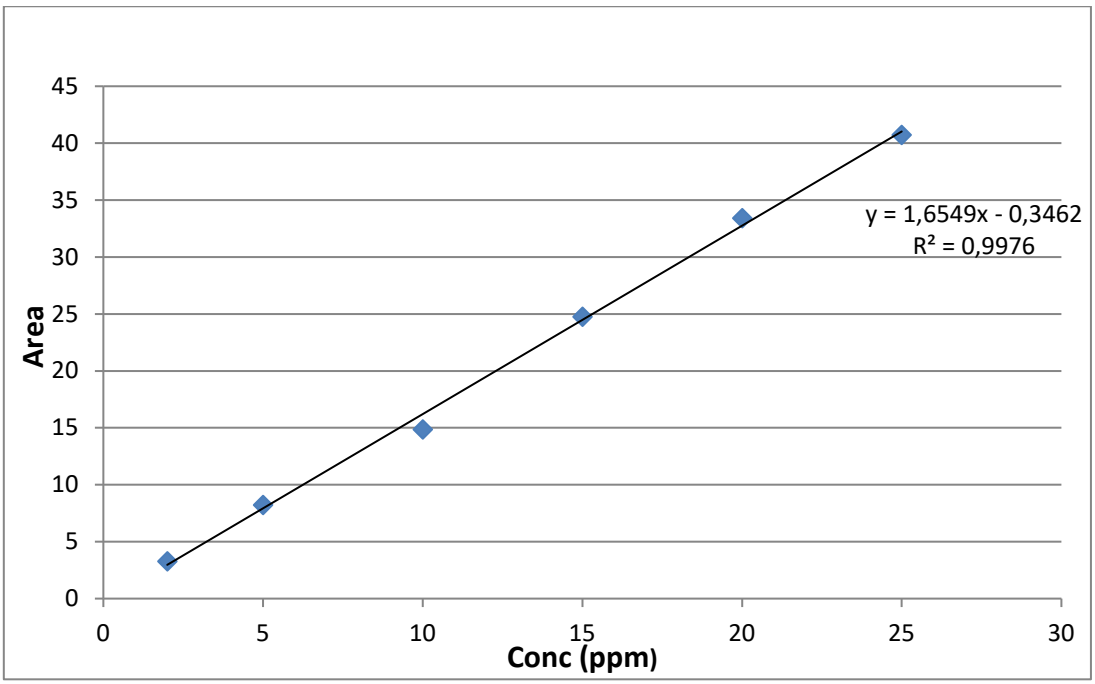


Figure 27: Calibration curve for Salicylic acid in Disprin

4.9 TAP WATER AND WASTE WATER SAMPLES FOR CE ANALYSES

4.9.1 SAMPLING

Cold tap water (1 × 2 L) and hot water (1 × 2 L) samples were taken within the University of Helsinki campus in Finland. The sampling was done into clean plastic bottles (2 L). These bottles were prewashed with ultra-pure water; for the cold water sampling, the tap water was let to flow (3 x 2 L volume) before the final sampling and the bottle was filled completely. For hot water sampling, the water was allowed to run for at least 10 minutes at its maximum flow to ensure the water temperature stability before the sample was taken. The bottles were tightly capped and taken to the laboratory where they were kept in a dark place at 4˚C until the water was used, and the extraction procedure was performed before 3 days after sampling. Subsequently, sampling was also done at the wastewater treatment plant (WWTP) of the Helsinki Metropolitan in Viikki suburb. Two different influent water samples were taken on different dates (Influents A & B), and an effluent sample was also taken, with the bottles tightly capped and taken to the laboratory where they were kept in a dark place at 4˚C until preconcentration was carried out.

4.9.2 INSTRUMENTS AND METHODS

A commercial CE instrument (Agilent Technologies, Waldbronn, Germany) with diode array detector and air cooler system was used for the determination. The CE instrument was applied with ChemStation programmes (Agilent) for instrument running and data handling. PeakMaster software was used to predict the migration order of the analytes. Bare fused silica capillaries (inner diameter 50 µm, outer diameter 375 µm, total length 70 cm, length to detector 61.5 cm). Prior to use, the capillaries were conditioned by sequentially flushing with

0.1 M NaOH, Milli-Q water, and the electrolyte solution, for 20 min each at 13.634 psi (940 mbar). Before each analysis, the capillary was flushed with 0.1 M NaOH and the electrolyte solution for 2-5 min depending on the method.

4.9.3 METHOD DEVELOPMENT

The methods were prepared offline while conditioning the capillary; the vials were placed into the sample carousel, the needed methods were programmed and combined into a sequence. The temperature of the capillary cassette was 25˚C, and the detection wavelength was 214 nm in all methods. (However, to measure the differences in intensities, 4 other wavelengths were chosen). Current was detected in each analysis; and between the runs, the capillary was flushed for 2 minutes with the buffer electrolyte solution (BGE) used. It can be BGE1 or BGE2 (electrolyte solution with different pH and ionic strength) depending on the analysis.

4.9.4 ANALYSES WITH BGE1 ELECTROLYTE (pH 8.5; I = 10 mM)

The constant pressure injection was 35 mbar, and the injection time was 10 seconds. The following analyses were carried out with the BGE1 electrolyte:

a. **Control analysis for electroosmosis**
   A mixture of methanol and MilliQ-water (50:50, v/v) was injected as a control sample (blank) for the measurement of the electroosmotic flow (EOF) mobility. Analysis voltage was 25 kV and analysis time ranged from 5 to 13 minutes.
b. **Voltage series**
   Analysis of the standard sample with different voltages (10, 15, 20 and 25 kV). One run per voltage was made, and the analysis time ranged about 5-13 minutes.
c. **Repeatability and identification**
   Six repetitions of the standard solution (mixture of all the analytes) were carried out, with the voltage set at 25 kV. Analysis time was 5 minutes.
   The peakmaster 5.1-software was used to predict the migration order of the analytes.

4.9.5 ANALYSES WITH BGE2 ELECTROLYTE (pH 7.5; I = 10 mM)

Six analyses were carried out. The constant pressure injection was 35 mbar, and the injection voltage was 25 kV.

a. **Control analysis for electro-osmosis**
   Analysis of the MilliQ-water with 25 kV (a control sample, blank) for measuring the mobility of the EOF.
b. **Constant pressure injection**
   The standard sample (analyte mixture) was injected with constant pressure of 35 mbar with different injection times (5, 10, 15, 20, and 30 seconds). Analysis voltage was 25 kV.

4.9.6 CE ANALYSIS METHODS FOR STEROIDS AND PHARMACEUTICALS

Four types of analysis methods were used: a partial filling micellar electrokinetic chromatography (PF-MEKC), capillary zone electrophoresis with direct UV detection (CZE-UV) and two capillary zone electrophoresis methods with indirect UV detection (CZE with indirect-UV). The temperature during the analyses was +25°C. Positive polarity and voltage of 25 kV was set for the steroids analysis, and 20 kV set as the constant value for pharmaceuticals and inorganic cation analyses, respectively. Negative polarity of voltage -20 kV was used for inorganic anion analyses. In the PF-MEKC, the electrolyte solution was prepared to give a current of 17 µA, while in the cation and anion analyses the current was between 30 and 40 µA. The analysis times were 20 minutes, 10 minutes, 10 minutes, and 10 minutes for steroids, pharmaceuticals, cations, and anions, respectively. To quantify steroids and pharmaceuticals the samples were injected with 0.50 psi (35 mbar) for 6 s, 0.7 psi (50 mbar) for 10 s and with 0.73 psi (50 mbar) pressure for 10 s and 5 s, respectively. In PF-MEKC, the steroids were detected at 214, 220, and 247 nm.

4.9.7 ELECTROLYTES FOR STEROIDS AND PHARMACEUTICALS ANALYSES

The electrolyte solutions in the analyses of steroids, pharmaceuticals, and inorganic ions are different, and based on the method and composition of the relevant samples. For steroid analysis, the electrolyte solution in the partially-filled micelle composition was 30 mM ammonium acetate, with pH adjusted to pH 9.68 with 25% ammonia. The eventual micelle composition was prepared by adding 1000 µL of 30 mM ammonium acetate (AA), 440 µL of 100 mM sodium dodecyl sulphate (SDS) to 30 mM AA solution followed by addition of 50 µL of 100 mM sodium taurocholate solution, made in MQ water (lab quality 1), in this specific order. The micelle and the electrolyte solutions were sequentially introduced into the capillary. The micelle plug was placed between the electrolyte solution and the sample solutions.

For the electrolyte solution in the CZE-UV method for the pharmaceuticals analysis, 30 mM ammonium acetate adjusted to the pH 9.68 was the main buffer electrolyte. Subsequently, in the inorganic ions analysis, the cations were separated in the buffer solution containing 9 mM pyridine-12 mM glycol acid-5 mM 18-crown-6 ether in milli-Q water (pH adjusted with 0.1 M HCl). Also, in the optimised CE method, the anions were separated in a buffer solution containing 2.25 mM pyromellitic acid, 6.50 mM NaOH, 0.75 mM hexamethonium hydroxide and 1.60 mM triethanolamine (pH 7.7 ± 0.2, Fluka). The pyromellitic acid electrolyte (BGE) pH 7.7 for HPCE separation of anions was from Fluka Chemie AG (Buchs, Switzerland). The pH of the electrolyte for anions was 7.7. The pH values were adjusted and checked using InoLab pH 7110 (WTW) instrument. The electrodes were calibrated with commercial buffer solutions at pH 4.00, 7.00 and 10.00 (Fisher Scientific, Loughborough, UK).

4.9.8 SAMPLE PREPARATION WITH SOLID PHASE EXTRACTION (SPE)

The SPE device VacMaster (Biotage® VacMaster™ 20 Sample Processing Station) was used for sample concentration (solid phase extraction of the water samples). The water samples were pre-concentrated with Strata-X 33 µm polymeric C_{18} reverse phase columns (500 mg/6 mL) which were obtained from Phenomenex (Copenhagen, Denmark). The Reacti-Vap Evaporation unit (Thermo Scientific, Vantaa Finland) was used for

evaporation of the extracts under N_2 gas. All waters used were purified with a Direct-Q UV Millipore water purification system (Millipore S.A., Molsheim, France).

2 L each of hot tap water, cold tap water, and Milli-Q were pre-concentrated by running through the SPE cartridge (2 cartridges for each 2 L of samples). Prior to the extraction process, the SPE cartridges (C_{18} columns) were preconditioned with 6 mL absolute methanol (HPLC grade) and 6 mL Milli-Q water was also used to flush the cartridges after. The respective water samples were run through the cartridges at a slow rate, thereafter, the sorbent materials (SPE cartridges) were left to dry for 12 hours. Extraction from the sorbent materials was then carried out by running 6 mL methanol slowly through each one. The eluates were collected in test tubes and separately evaporated under Nitrogen with mild heating (40 ° C) to dryness, followed by dissolution with 2 mL methanol with agitation. The sample volumes from the C_{18} columns were 2 mL in each case. The final sample volume of 250 µL was separated from the pre-concentrated analytical sample for the analysis and the study was performed with four replicates and with eight sequential analyses.

4.9.9 DETERMINATION OF PHARMACEUTICAL ANALYTES IN WATER

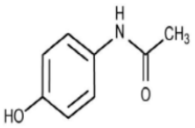
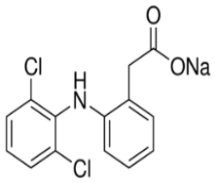
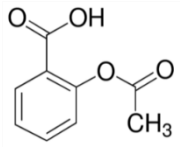
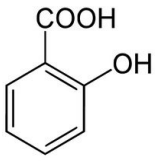
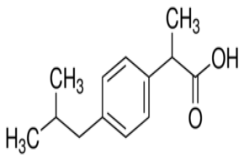
This section focuses on measuring the persistent organic pollutants in hot tap water, cold tap water, Milli-Q water and wastewater sampled from the University of Helsinki, Finland's WWTP. Pharmaceutical compounds such as acetaminophen, diclofenac, aspirin, ibuprofen, sulphamethoxazole, etc. are usually determined in environmental water. The water samples studied include: two different influent water samples and an effluent water sample from sewage water treatment plants, hot tap water, cold tap water, and Milli-Q water. In the two influent waste water samples influent A and influent B, the pharmaceuticals found in the most quantities include aspirin, diclofenac and sulphamethoxazole respectively. In the influent A water sample, aspirin was quantified to be 13.52 ng/L, diclofenac as 14.15 ng/L, salicylic acid as 6.514 ng/L and sulphamethoxazole as 11.79 ng/L respectively. Acetaminophen and ibuprofen were not detected in influent A water sample. In the same vein, influent B water sample contained 4.23 ng/L of aspirin, 8.235 ng/L of diclofenac, 1.199 ng/L of salicylic acid, 1.095 ng/L of ibuprofen and 13.170 ng/L of sulphamethoxazole respectively. In the purified effluent water samples of the sewage water treatment plants the measurable pharmaceuticals quantities include 0.836 ng/L of aspirin, 0.802 ng/L of diclofenac, 1.343 ng/L of salicylic acid, 0.842 ng/L of ibuprofen and 10.241 ng/L of sulphamethoxazole.

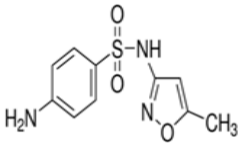
Acetaminophen was not found in either influent or effluent samples from the WWTP. While ibuprofen was also not found in the influent A water sample, only acetaminophen was not detected in the influent B water sample. It is not surprising that both the influent and effluent water samples for the sewage treatment plant contain some of these pharmaceuticals under study.

Since the concentrations are perceived to be low, the pharmaceuticals in the water sample need to be enriched and extracted from the main matrix. Therefore, in the present study the sampled water volume was 2 L in order to enrich the pharmaceutical concentrations for maximal UV detection in the CZE analyses and to validate the system capability for long-term pharmaceuticals monitoring. In the cleaning process and enrichment with SPE, the pharmaceuticals were eluted at 20 times more concentrated than the original plant sample.

The measured quantities of these pharmaceuticals determined from the electropherograms of the influent and effluent water samples are ascertained by comparing the respective electrophoretic mobilities of the individual analytes in the standards calibration to the electrophoretic mobilities of the individual analytes in the influent and effluent water samples. Naturally, the quantities in the effluents were lower than the quantities in the influents. When the analyte in a sample concentrate was clearly identified (based on specific wavelengths, absolute migration time, relative migration time and electrophoretic mobility) in the electropherogram, the sample was spiked with a 2 µg/mL standard and quantified using the standard addition method. As a result, it was possible to confirm the respective peaks belonging to the analytes under investigation. Table 33 shows the pharmaceutical analytes with their structures, theoretical and experimentally measured exact molar masses with migration times and electrophoretic mobilities.

Table 33: Structures of acetaminophen, diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole. Theoretical and experimentally measured exact molar masses with migration times in CE.

Compound	Structure	Molar mass [g/mol]	Experimental molar mass [M + H]	CZE-UV Migration time [min]	(μ_{ep}) [m^2/Vs]
Acetaminophen $C_8H_9O_2$		151.17	152.071	4.980	-9.70 x 10^{-9}
Diclofenac $C_{14}H_{10}Cl_2NNaO_2$		318.1	319.024	6.510	-2.66 x 10^{-8}
Aspirin $C_9H_8O_4$		180.16	181.05	6.598	-2.73 x 10^{-8}
Salicylic acid $C_7H_6O_3$		138.12	139.039	6.803	-2.89 x 10^{-8}
Ibuprofen $C_{13}H_{18}O_2$		206.29	207.138	7.406	-3.33 x 10^{-8}

Sulphamethoxazole **$C_{10}H_{11}N_3O_3S$**		253.28	254.059	9.320	-4.32 x 10^{-8}

Appendix I Figure 1 shows the electropherogram for influent A sample which reveals four of the analytes under investigation, including diclofenac, aspirin, salicylic acid and sulphamethoxazole; acetaminophen and ibuprofen were not detected in this influent sample. The respective analyte peaks were identified based on the calculation of their individual electrophoretic mobilities, in comparison or correlation to their respective mobilities in the standard analyte electrophoretic mobility calculations. These individual analyte peaks are ascertained by the nearness in value to their respective peaks in the standard analyte electrophoretic mobility calculations by ± 0.2. This is showcased in Table 34, where the electrophoretic mobilities calculations for the standard analytes, influent A, influent B and effluent water samples are displayed. Appendix I Figure 2 shows the electrophoregram for the influent B water sample and it contains five of the pharmaceutical analytes under investigation, including diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole respectively.

Table 34: Correlation between the electrophoretic mobilities of the standards analytes with influent and effluents water samples.

Compounds	**Standards (μ_{ep}) [m^2/Vs]**	**Influent A (μ_{ep}) [m^2/Vs]**	**Influent B (μ_{ep}) [m^2/Vs]**	**Effluent (μ_{ep}) [m^2/Vs]**
Acetaminophen	-9.70 x 10^{-9}	ND	ND	ND
Diclofenac	-2.66 x 10^{-8}	-2.78 x 10^{-8}	-2.55 x 10^{-8}	-2.59 x 10^{-8}
Aspirin (ASA)	-2.73 x 10^{-8}	-2.84 x 10^{-8}	-2.71 x 10^{-8}	-2.79 x 10^{-8}
Salicylic acid (SA)	-2.89 x 10^{-8}	-2.99 x 10^{-8}	-2.78 x 10^{-8}	-2.92 x 10^{-8}
Ibuprofen	-3.33 x 10^{-8}	ND	-2.93 x 10^{-8}	-3.51 x 10^{-8}
Sulphamethoxazole	-4.32 x 10^{-8}	-4.19 x 10^{-8}	-4.10 x 10^{-8}	-4.14 x 10^{-8}

Tables 35-37 give the detailed analysis account of the influent A, influent B and effluent water samples respectively, including the measured quantities of the analytes, the mean, standard deviation and relative standard deviation of the parameters such as the migration time, peak area and peak height respectively.

Table 35: Identification of pharmaceuticals in the Influent A sample. Determination made with CZE. Influent B water sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Acetaminophen				
Mean				
SD	**Not Found**	**Not Found**	**Not Found**	**Not Found**
RSD%				
Diclofenac				
Mean	7.042	32.052	11.250	
SD	0.275	16.852	5.496	14.150
RSD%	3.906	52.576	48.856	
Aspirin				
Mean	7.142	17.258	6.412	13.520
SD	0.271	6.648	2.463	
RSD%	3.790	38.522	38.418	
Salicylic acid				
Mean	7.346	3.889	1.123	
SD	0.300	1.621	0.351	6.514
RSD%	4.088	41.694	31.297	
Ibuprofen				
Mean				
SD	**Not Found**	**Not Found**	**Not Found**	**Not Found**
RSD%				
Sulphamethoxazole				
Mean	9.741	14.710	3.513	
SD	0.532	1.133	0.212	11.790
RSD%	5.463	7.703	6.022	

Table 36: Identification of pharmaceuticals in the Influent B sample. Determination made with CZE. Influent B water sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Acetaminophen				
Mean	**Not found**	**Not found**	**Not found**	**Not found**
SD				
RSD%				
Diclofenac				
Mean	7.238	17.635	6.964	
SD	0.036	1.084	0.153	8.235
RSD%	0.492	6.146	2.195	
Aspirin				
Mean	7.334	5.407	2.165	4.226
SD	0.035	0.557	0.066	
RSD%	0.476	10.293	3.051	
Salicylic acid				
Mean	7.544	0.854	0.271	
SD	0.098	0.104	0.028	1.199
RSD%	1.296	16.331	10.404	
Ibuprofen				
Mean	8.528	0.848	0.221	1.095
SD	0.058	0.139	0.013	
RSD%	0.675	16.331	5.979	
Sulphamethoxazole				
Mean	10.057	16.564	3.940	13.170
SD	0.055	0.904	0.037	
RSD%	0.545	5.459	0.932	

Table 37: Identification of pharmaceuticals in the Effluent water sample. Determination made with CZE. Effluent water sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Acetaminophen				
Mean				
SD	**Not found**	**Not found**	**Not found**	**Not found**
RSD%				
Diclofenac				
Mean	7.052	1.137	0.268	
SD	0.038	0.267	0.054	0.802
RSD%	0.538	23.511	20.063	
Aspirin				
Mean	7.607	1.084	0.304	
SD	0.048	0.059	0.014	0.836
RSD%	0.627	5.420	4.716	
Salicylic acid				
Mean	8.627	0.598	0.157	
SD	0.036	0.184	0.008	1.343
RSD%	0.411	30.767	4.947	
Ibuprofen				
Mean	8.627	0.599	0.157	
SD	0.036	0.184	0.008	0.842
RSD%	0.411	30.767	4.947	
Sulphamethoxazole				
Mean	10.251	12.813	3.061	
SD	0.089	1.998	0.064	10.241
RSD%	0.873	15.591	2.074	

Appendix I Figure 3 shows the electrophoregram for the effluent water sample and it contains five of the pharmaceutical analytes under investigation, which includes diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole respectively. There are other peaks in the electropherogram for the effluent water sample also, which are not among the analytes being investigated in this study. But the electropherogram profile shows

the water sample contains several other components, indicating the presence of other pharmaceutical compounds in the effluent water sample.

Appendix I Figure 4, the electropherogram shows the overlay of the standard mixture profile with the influent B water sample profile. It can be observed in the electropherogram that the influent B water sample profile correlates with the standard sample profile in terms of the observable peaks belonging to the studied analytes in the two profiles, except for the shift in the position of one profile relative to the other due to the effect of the matrix. In both profiles, the peaks belonging to diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole can be observed, in spite of the shift due to the matrix effect.

However, to ascertain the authenticity of the analytes peaks, 2 µg/mL of the standard of the specified analyte was spiked into the influent and the effluent water samples to double-check if the peaks actually belong to them or not. Appendix I Figure 5 describes the spiking of the effluent water sample with 2 µg/mL sulphamethoxazole in an overlay with the standard analytes mixture profile, and showing the peak really belongs to sulphamethoxazole. The spiked profile can be seen in colour red.

In the same vein, Appendix I Figure 6 shows the electropherogram showing the overlay profiles of the influent B water sample, both spiked and unspiked. The spiked profile is in colour blue and shows an intense peak than the other. While in Appendix I Figure 7, the influent A water sample was spiked with 2 µg/mL diclofenac standard and the electropherogram profile compared to that of the standard analyte mixture. The shift in the positioning of the peaks can be attributed to the matrix effect in the influent water sample, but the profile explicitly depicts how the analytes peaks are positioned in the profile.

Appendix I Figure 8 shows the electropherogram profiles of influent B profiles. The electropherogram in blue colour represents the spiked profile with 2 µg/mL of sulphamethoxazole, and the profile in red represents the unspiked.

4.10 SPE EXTRACTION OF HOT WATER, COLD WATER AND MILLI-Q WATER IN CZE ANALYSIS

The SPE extraction was also carried out for hot tap water, cold tap water and milli-Q water respectively. The electrophoretic mobilities of the observable peaks thereof are presented in the Table 38. Appendix I Figures 9, 10 and 11 show the electropherogram profiles of the three samples (hot tap water, cold tap water and milli-Q water) these peaks are synonymous to the three samples. However, one of the two peaks gives an electrophoretic mobility value and peak profile exactly the same as that of sulphamethoxazole; and the other peak does not share any similar profile with the analytes under investigation. This shows that even in the purified water for household consumption supplied in Helsinki, Finland, and milli-Q water for laboratory analysis, there exists some minute quantities of known and unknown persistent organic pollutants. It is obvious by virtue of electrophoretic correlation of the sulphamethozaxole standard to that of the peak identified in the three samples that the unknown peak identified, belongs to sulphamethoxazole. The values given in Table 38 confirm its authenticity.

Table 38: Electrophoretic mobilities of the peaks in hot, cold and milli-Q water samples.

Compounds	Standards (μ_{ep}) [m^2/Vs]	Cold water (μ_{ep}) [m^2/Vs]	Hot water (μ_{ep}) [m^2/Vs]	Milli-Q water (μ_{ep}) [m^2/Vs]
Unknown	-	-1.87 x 10-8	-1.74 x 10-8	-1.98 x 10-8
Sulphamethoxazole	-4.32×10^{-8}	-4.17×10^{-8}	-4.17×10^{-8}	-4.16×10^{-8}

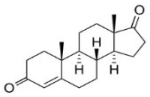
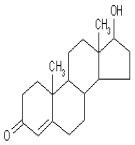
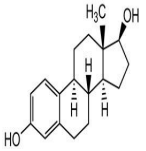
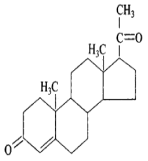
4.10.1 DETERMINATION OF STEROID HORMONES IN WATER

This section focuses on measuring the steroid hormones (human-based androgens, oestrogens, and synthetic progesterone) in environmental wastewater. Steroid hormones compounds such as androstenedione, testosterone, 17-ß-estradiol, and progesterone, etc. are usually determined in environmental water (Jobling et al., 2005: Liu et al., 2011; Nelson & Lee, 1996; Thorpe et al., 2003). The water samples studied for steroid hormones include: two different influent water samples and an effluent water sample from sewage water treatment plants, hot tap water, cold tap water, and Milli-Q water respectively. The steroid hormones in the water samples had to be enriched and extracted from the major environmental matrix owing to the very low concentration levels. As a result, in this analysis of steroids, the sampled water volume used was 2 L in order to enrich the steroid concentrations for maximal UV detection in the partial-filling micellar electrokinetic capillary chromatography (PF-MEKC) analyses, and to also embark on system validation to enhance its capability for long-term steroid monitoring. The measured quantities of steroid hormones were determined from the electropherograms of the influent and effluent water samples and were ascertained by comparing the respective electrophoretic mobilities of the individual steroid analytes in the standards calibration to the electrophoretic mobilities of the individual steroid analytes in the influent and effluent water samples. When the analyte in a sample concentrate was clearly identified (based on specific wavelengths, absolute migration time, relative migration time and electrophoretic mobility) in the electropherogram, the sample was spiked with a 2 µg/mL standard and quantified using the standard addition method. As a result, it was possible to ascertain the respective peaks belonging to the analytes under investigation.

For both waste water influent water samples influent, A and B, the steroid hormones found in the highest quantities include androstenedione and testosterone. In the influent A water sample, androstenedione was quantified to be 2.224 ng/L, testosterone was quantified to be 3.474 ng/L, 17-ß-estradiol was quantified to be 0.96 ng/L and progesterone was 1.503 ng/L. Subsequently, the influent B water sample contained 2.224 ng/L of androstenedione, 3.142 ng/L of testosterone, 0.954 ng/L of 17-ß-estradiol and 0.691 ng/L of progesterone respectively. In the effluent water samples from the waste water treatment plants however, the measurable steroid hormones quantities include 1.205 ng/L of androstenedione, 3.037 ng/L of testosterone, 0.550 ng/L of 17-ß-estradiol and 0.440 ng/L of progesterone respectively. These levels are not surprising, based on the concentration levels of both influent and effluent water samples, as almost 80% of effluent water samples from waste water treatment plants have been noticed to contain female hormones even after clean-up of the water.

Furthermore, the measured quantities in the effluents were lower than the quantities in the influent samples. Table 39 shows the steroid analytes with their structures, theoretical and experimentally-measured exact molar masses with migration times and electrophoretic mobilities.

Table 39: Structures of androstenedione, testosterone, 17-ß-estradiol and progesterone. Theoretical and experimentally-measured exact masses with migration times in CE.

Compound	Structure	Molar mass [g/mol]	Experimental molar mass [M + H]	PF-MEKC-UV Migration time [min]	(μ_{ep}) [m^2/Vs]
Androstenedione $C_{19}H_{26}O_2$		286.410	287.200	10.770	-2.630 x 10^{-8}
Testosterone $C_{19}H_{28}O_2$		288.420	289.216	12.550	-3.100 x 10^{-8}
17-ß-estradiol		272.380	273.185	12.798	-3.200 x 10^{-8}
Progesterone $C_{21}H_{30}O_2$		314.462	315.232	13.360	-3.280 x 10^{-8}

These individual analyte peaks are ascertained by the nearness (within ± 0.2) in value to their respective peaks in the standard analyte electrophoretic mobility calculations. This is showcased in Table 40, where the electrophoretic mobilities calculations for the standard analytes, influent A, influent B and effluent water samples are displayed.

Table 40: Correlation between the electrophoretic mobilities of the steroid standards analytes with influent and effluents water samples.

Compounds	Standards (μ_{ep}) [m^2/Vs]	Influent A (μ_{ep}) [m^2/Vs]	Influent B (μ_{ep}) [m^2/Vs]	Effluent (μ_{ep}) [m^2/Vs]
Androstendione	-2.630×10^{-8}	-2.200×10^{-8}	-2.640×10^{-8}	-2.200×10^{-8}
Testosterone	-3.100×10^{-8}	-2.980×10^{-8}	-2.874×10^{-8}	$-.2.750 \times 10^{-8}$
17-ß-estradiol	-3.200×10^{-8}	-3.310×10^{-8}	-2.988×10^{-8}	-2.970×10^{-8}
Progesterone	-3.280×10^{-8}	-3.410×10^{-8}	-3.510×10^{-8}	-3.450×10^{-8}

The electropherogram for influent A sample can be seen in Appendix I Figure 12, and shows the steroid analytes being investigated, which include androstenedione, testosterone, 17-ß-estradiol and progesterone. Appendix I Figure 13 shows the electrophoregram for the influent B water sample and it contains all the four steroid analytes under investigation, including androstenedione (1), testosterone (2), 17-ß-estradiol (3) and progesterone (4) respectively.

The respective analyte peaks were identified based on the calculation of their individual electrophoretic mobilities, in relation to their respective mobilities in the standard analytes electrophoretic mobility calculations. There were other peaks present within the electropherogram belonging to other metabolites of the steroids being studied. However, their electrophoretic mobilities did not correlate with the electrophoretic mobility calculations of the steroids standard analytes, hence they were not taken into consideration.

Tables 41-43 give the detailed analysis of the influent A, influent B and effluent water samples respectively, including the measured quantities of the steroid analytes, the mean, standard deviation and relative standard deviation of the parameters such as the migration time, peak area and peak height respectively. The content of steroids in all these samples were determined with PF-MEKC, after the influent water samples were purified with C18 (Strata-X) nonpolar sorbent. Table 41 shows the identification of steroid hormones in the Influent A sample, and Table 42 shows the identification of steroid hormones in the Influent B sample. Table 43 shows the identification of steroid hormones in the Effluent water sample.

Table 41: Identification of steroid hormones in the Influent A sample. Determination with PF-MEKC. Influent water sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Androstenedione				
Mean	9.161	2.455	0.938	2.224
SD	0.032	0.231	0.058	
RSD%	0.355	9.417	6.203	
Testosterone				
Mean	11.430	3.206	0.854	3.474
SD	0.056	0.310	0.066	
RSD%	0.487	9.676	7.721	
17-ß-estradiol				
Mean	12.783	0.842	0.842	0.96
SD	0.036	0.015	0.015	
RSD%	0.280	1.810	1.810	
Progesterone				
Mean	13.797	2.060	0.926	1.503
SD	0.221	0.417	0.061	
RSD%	1.599	20.235	6.569	

Table 42: Identification of steroid hormones in the Influent B sample. Determination with PF-MEKC. Influent sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Androstenedione				
Mean	10.396	1.170	0.263	1.349
SD	0.079	0.108	0.024	
RSD%	0.769	9.200	9.281	
Testosterone				
Mean	11.448	2.866	0.939	3.142
SD	0.187	0.581	0.030	
RSD%	1.630	20.277	3.137	
17-ß-estradiol				
Mean	11.515	0.830	0.429	0.954
SD	0.011	0.032	0.096	
RSD%	0.099	3.895	22.257	
Progesterone				
Mean	14.416	0.447	1.041	1.691
SD	0.124	0.024	0.070	
RSD%	0.859	5.394	6.743	

Table 43: Identification of steroid hormones in the Effluent water sample. Determination made with PF-MEKC. Effluent sample purified with C18 (Strata-X) nonpolar sorbent.

Compound	Migration time [min]	Peaks area [min × mAU]	Peak height [mAU]	Amount [ng/L]
Androstenedione				
Mean	11.01	0.959	0.264	1.205
SD	0.045	0.063	0.051	
RSD%	0.405	6.529	19.177	
Testosterone				
Mean	11.777	2.758	0.728	3.037
SD	0.063	0.243	0.049	
RSD%	0.538	8.820	6.674	
17-ß-estradiol				
Mean	14.005	1.206	0.407	0.550
SD	0.337	0.128	0.062	
RSD%	2.405	10.570	15.314	
Progesterone				
Mean	14.662	2.729	1.263	0.940
SD	0.357	0.099	0.063	
RSD%	2.436	3.641	5.025	

In Figure 28, the steroid hormone concentration in the influent A, influent B and effluent water samples are presented. Figure 29 gives the steroid hormones concentration in the hot tap water and cold tap water samples.

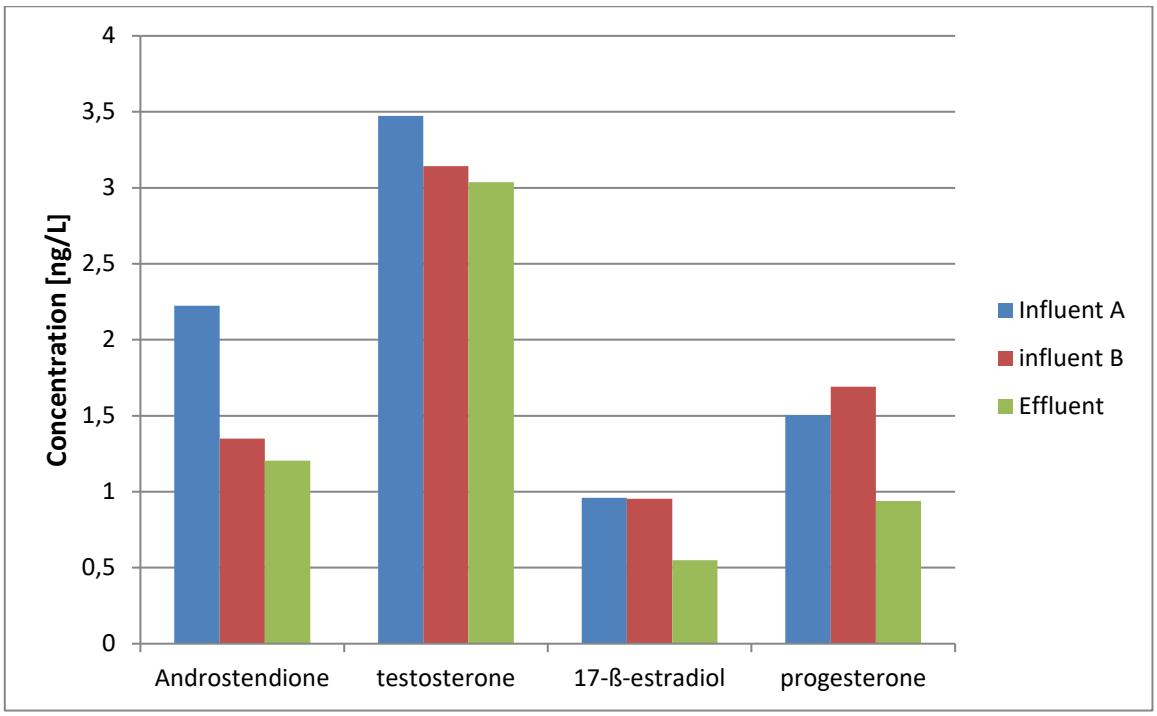


Figure 28: Steroid hormone concentration in the influent A, influent B and effluent water samples.

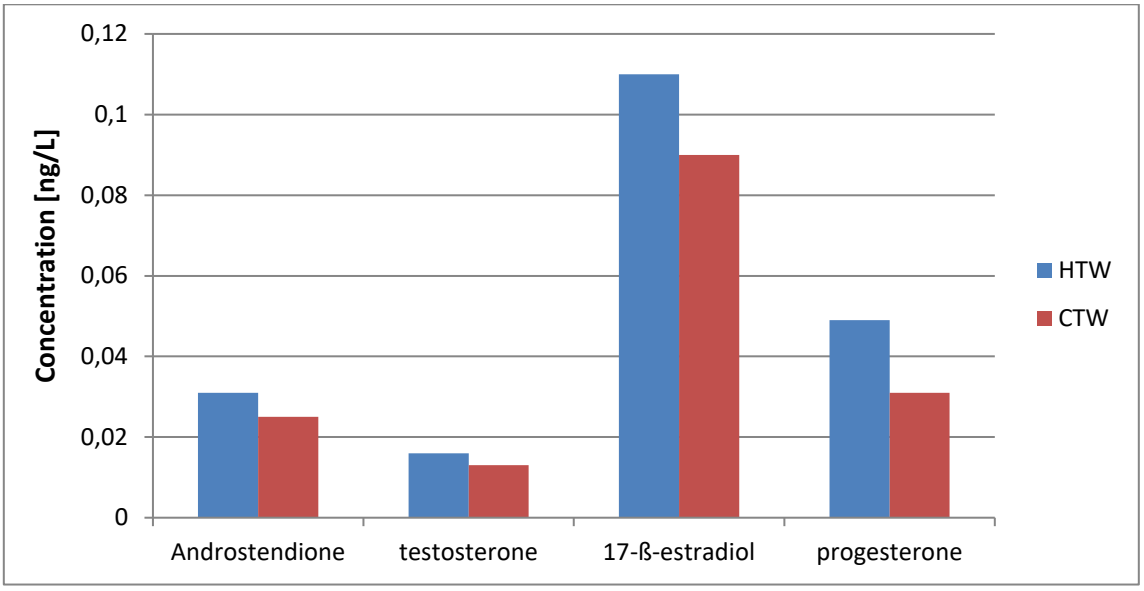


Figure 29: Steroid hormones concentration amounts in the hot tap water (HTW) and cold tap water (CTW) samples.

Appendix I Figure 14, shows the electrophoregram for the effluent water sample and it contains the four steroid analytes under investigation, which includes androstenedione (1), testosterone (2), 17-ß-estradiol (3) and progesterone (4) respectively. There were other peaks in the electropherogram for the effluent water sample also, which are not among the analytes being investigated in this study. But the electropherogram profile shows the water sample contains several other metabolites of steroid components, indicating the presence of other steroid hormonal compounds in the effluent water sample. In Appendix I Figures 15, 16 and 17, the electropherograms show the overlay of the steroid standard mixture profiles with the influent A, influent B and

effluent water sample profiles. It can be observed in the electropherograms that the influent and effluent water sample profiles contain other peaks aside from the analytes being investigated; at the same time, the matrix effect of the influent water samples also affected the non-alignment of the respective peaks. However, these individual peaks belonging to individual analytes were ascertained and confirmed from the calculations of their respective electrophoretic mobilities which correlate with those of the steroid analytes in the standard mixture. However, to ascertain the authenticity of the analytes peaks, 2 µg/mL of the steroid standard of the specified analyte was spiked into the influent and the effluent water samples to double-check the authenticity of the particular peaks. Appendix I Figure 18 describes the spiking of the effluent water sample with 2 µg/mL of androstenedione and testosterone in an overlay with both spiked and unspiked electropherograms.

4.11 COMPARISON BETWEEN THE LODS AND LOQS OF CE & LC

The comparison of the limit of detection (LOD) and limit of quantification (LOQ) between the methods employed in capillary electrophoresis (CE) and liquid chromatography (LC) is expressed in Table 44. It can be seen that the limit of detection (LOD) and the limit of quantification (LOQ) for the respective compounds in capillary electrophoresis are generally lower compared to the LC method, showing its greater sensitivity overall.

Table 44: LOD and LOQ comparison for CE and LC

ANALYTES	CE		LC	
	LOD	**LOQ**	**LOD**	**LOQ**
Acetaminophen	0.230	0.690	0.286	0.857
Aspirin	0.137	0.411	0.195	0.584
Sulphamethoxazole	0.186	0.557	0.224	0.673
Diclofenac	0.073	0.219	0.412	1.236
Ibuprofen	0.085	0.255	0.291	0.873
17-beta estradiol	0.096	0.195	0.331	0.993

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 INTRODUCTION

In this study, the major objectives were to develop sampling protocols for detecting specific compounds of emerging concern in different aqueous matrixes and optimise the operating parameters (both chemical and instrumental) affecting LC-MS and the capillary electrophoresis. The LC-MS retention time, transition and collision energy, limit of detection (LOD), and limit of quantification (LOQ) and summary of the (PPCPs), (PFCs) and EDC compounds detected in seawater samples collected at Camps Bay were presented. The results showed the limit of detection (LOD), and limit of quantification (LOQ) when various anthropogenic compounds (PPCPs, PFCs and EDC) derived from sewage discharged from the marine outfall were detected as being present in low ng/L concentrations in sea water samples collected at Camps Bay.

Of the fifteen target compounds, eight were detected in at least one of the sea water samples and two of these compounds were detected in all the samples. PFDA (Perfluorodecanoic acid) and diclofenac were the two compounds detected in the highest concentration in seawater samples (2.44 and 2.86 ng/L respectively), and both carbamazepine and PFHpA (Perfluoroheptanoic acid) were detected in all water samples in this study. The compounds detected in the highest concentrations were among those most frequently detected. The detected compounds represent a variety of chemical types and therapeutic uses, and some have been suggested as anthropogenic tracers of wastewater contamination due to their incomplete metabolism in humans, low removal efficiency during wastewater treatment, and persistence in the environment; these include acetaminophen and diclofenac detected in the Camps Bay seawater samples.

Despite the purported adequate dilution of the chemicals and sewage by the ocean according to the City and the outfall design criteria, these compounds were shown to be present in measurable amounts in the ocean water, albeit not uniformly dispersed, which points to the uneven and slow dilution and dispersion of persistent contaminants in a concentrated, untreated sewage plume released into a marine environment. It also shows that the dispersion rate of chemical compounds is related to the type of chemical, since these compounds are not all equally hydrophilic. In addition changes in pH and salt concentration greatly influence the electrostatic properties of these chemicals, which may have multiple ionisable functional groups with substantially different acid dissociation constant (pKa) values thus influencing their environmental partition in seawater.

Overall, for the LC-MS technique, the analysis of variance conducted on all data obtained showed that the level of all contaminants varies significantly ($p<0.05$) across all environmental samples assessed in this study. Furthermore, statistical analysis of all data obtained in this study showed that the result obtained for the quantification of PPCPs, PCFs and EDCs in the environmental samples reported in this study are replicated, going by the values obtained for standard deviation. Moreover, statistical analysis of the data obtained showed that the results obtained for the quantification of PPCPs, PCFs and EDCs in the seawater samples are replicated, going by the values obtained for standard deviation, indicating that the sampling protocol developed and the LC-MS analytical technique was reliable for detecting very low concentrations of these compounds in

a complex aqueous matrix such as seawater. But some compounds were below limits of detection/quantification showing the lack of sensitivity of this instrumental technique.

Various other water samples including effluent wastewater from the wastewater treatment plant in Bellville, Cape Town, cold tap water, hot tap water and milli-Q water were sampled to understand the influence of the aqueous matrix in which these compounds were present. The study investigated some pharmaceuticals including acetaminophen, aspirin, diclofenac, ibuprofen, sulphamethoxazole, and 17-beta estradiol, a steroid hormone compound. Solid phase extraction (SPE) method was employed to concentrate the analyte and LC-MS was used for the analysis. However, of the selected compounds, only diclofenac (3.306 ng/mL) and sulphamethoxazole (1.18 ng/mL) were detected in the effluent samples; while none of the selected compounds were detectable in hot or cold tap water or in Milli-Q water samples by HPLC-MS. This could be due either to poor instrument sensitivity, or due to their absence. The SPE cartridge eluant used during any clean up step was also checked and it was shown that the eluant used could be the cause of the loss of more polar compounds such as acetaminophen during sample extraction protocols, giving false negative results. These results suggest that additional research is required to optimize the current environmental clean-up methodologies which are designed for simultaneous analyte quantification.

Thereafter, the potential of capillary electrophoresis as an alternative route to rapid and accurate quantification of a similar suite of persistent contaminants in several environmental waters was explored. In this CE optimisation/validation study, the chemical and instrumental parameters affecting the performance in capillary electrophoresis (CE) were investigated; this study shows that voltage affects the field strength, pH of the electrolyte solution affects the separation of the analytes and dissociation of the analytes, injection type affects the analysis and detectability of the instrument, the pKa values (dissociation constants) of both the buffered electrolytes and the individual analytes are also factors in the migration order of analytes to the detector.

The further objective was to optimise the operating parameters (both chemical and instrumental) affecting the capillary electrophoresis; to identify and quantify selected pharmaceutical compounds (including acetaminophen, diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole) present in water, including the influent and effluent environmental water samples, as well as drinking water supplied to homes, using the capillary zone electrophoresis (CZE) methods; to also identify the steroid hormone compounds (including androstenedione, testosterone, 17-ß-estradiol and progesterone) present in the drinking water, influent and effluent environmental samples, using the partial-filling micellar electrokinetic capillary chromatography methods.

In terms of the optimisation of the capillary electrophoresis (CE) instrument, the results showed the different parameters that affect the performance of the CE equipment. These parameters include: voltage which affects the field strength; the pH of the electrolyte solution that affects the separation and dissociation of the analytes according to their pKa values; and the injection type used which affects the detectability of the instrument. Furthermore, the capillary zone electrophoresis (CZE) with UV detection was employed in the identification and quantification of pharmaceuticals. For waste water influent A sample, aspirin was quantified to be 13.52 ng/L, diclofenac as 14.15 ng/L, salicylic acid as 6.514 [ng/L] and sulphamethoxazole as 11.79 ng/L respectively. The influent B water sample contained 4.23 ng/L of aspirin, 8.235 ng/L of diclofenac, 1.199 ng/L

of salicylic acid, 1.095 ng/L of ibuprofen and 13.170 ng/L of sulphamethoxazole respectively. And in the effluent water samples of sewage water treatment plants the measurable pharmaceuticals quantities included 0.836 ng/L of aspirin, 0.802 ng/L of diclofenac, 1.343 ng/L of salicylic acid, 0.842 ng/L of ibuprofen and 10.241 ng/L of sulphamethoxazole.

Thereafter, the partial-filling micellar electrokinetic capillary chromatography (PF-MEKC) method was adopted for the identification and quantification of the steroid hormones. In the waste water influent A water sample, androstenedione was quantified to be 2.224 ng/L, testosterone was quantified to be 3.474 ng/L, 17-ß-estradiol was quantified to be 0.96 ng/L and progesterone was 1.503 ng/L. The influent B water sample contained 2.224 ng/L of androstenedione, 3.142 ng/L of testosterone, 0.954 ng/L of 17-ß-estradiol and 0.691 ng/L of progesterone respectively. While in the purified effluent water samples of sewage water treatment plants, the measurable steroid hormones quantities include 1.205 ng/L of androstenedione, 3.037 ng/L of testosterone, 0.550 ng/L of 17-ß-estradiol and 0.440 ng/L of progesterone respectively, showing that these steroid compounds are not adequately removed by the waste water treatment process and escape into the environment.

The steroid compounds content of the tap water (hot and cold) was also measured. For androstenedione, 0.031 ng/L and 0.025 ng/L were quantified for hot tap water and cold tap water respectively; testosterone accounted for 0.016 ng/L and 0.013 ng/L of hot tap water and cold tap water respectively; 17-ß-estradiol accounted for 0.11 ng/L and 0.09 ng/L of hot tap water and cold tap water respectively; while progesterone gave 0.049 ng/L and 0.031 ng/L of hot tap water and cold tap water respectively. It is of concern that low quantities of these compounds are found in potable water supplies.

The advantages of the CE method are the high efficiency of separation, rapidness, simplicity, small sample volume (several nanolitres), and a lower consumption of reagents. The novelty of this research study is the demonstration of the CZE-UV method for the determination of pharmaceuticals as well as inorganic ions, and the use of the PF-MEKC-UV method for the determination of steroids in influent and effluent wastewaters, as well as in cold and hot tap water. From the performance, CE can be considered highly sensitive and suitable for rapid determination and quantification of contaminants in environmental samples that require low detection limits. Overall, the LOQ and LOD of the electro-capillary method showed greater sensitivity for detecting low nanogram concentrations of selected analytes in diverse matrices than HPLC-MS.

5.2 RECOMMENDATIONS

Apart from extending the CE method development to analyse further pharmaceuticals and other compounds such as personal care products, pesticides, etc. a reasonable suggestion for future studies would be to first attempt to remove the more polar analytes on the SPE cartridge, which can be achieved by washing the adsorbed sample with larger quantities of water or low concentrations of methanol. This could effectively be used to remove and recover polar analytes such as acetaminophen during the first wash step, by which it can then be isolated and analysed without any major organic interferences. The SPE cartridge could then be washed using increasing concentrations of organic solvents such as methanol to elute and further isolate more non-polar analytes, such as sulfamethoxazole at 50 % methanol concentration.

It is also suggested that future experimental procedures be investigated around absorbing matrix interferences to the stationary phase of the cartridge while analytes pass through the cartridge unretained and can then be recovered purified from the filtrate. Alternatively, if experimental SPE recovery determinations such as the present study are performed before extracting environmental samples, one could statistically extrapolate and correct for analytes with marginally poor recoveries, although one should keep in mind that the matrix of a sample can also affect an analyte's potential to be retained upon the stationary phase, especially if such an SPE cartridge becomes saturated by a sample's organic matrix.

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APPENDICES

APPENDIX I

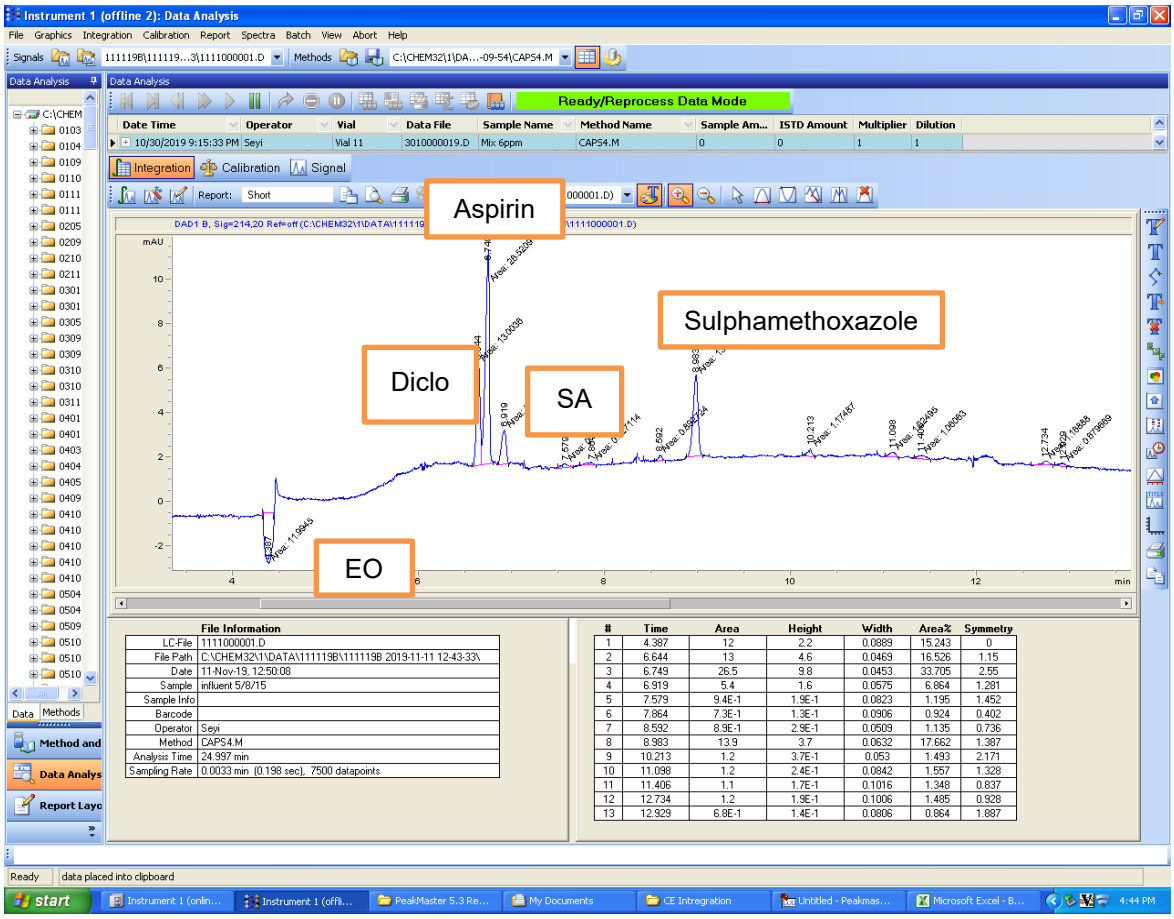

Figure 1: Electropherogram for influent A sample showing the peaks for the identified peaks including diclofenac, aspirin (ASA), salicylic acid (SA), and sulphamethoxazole.

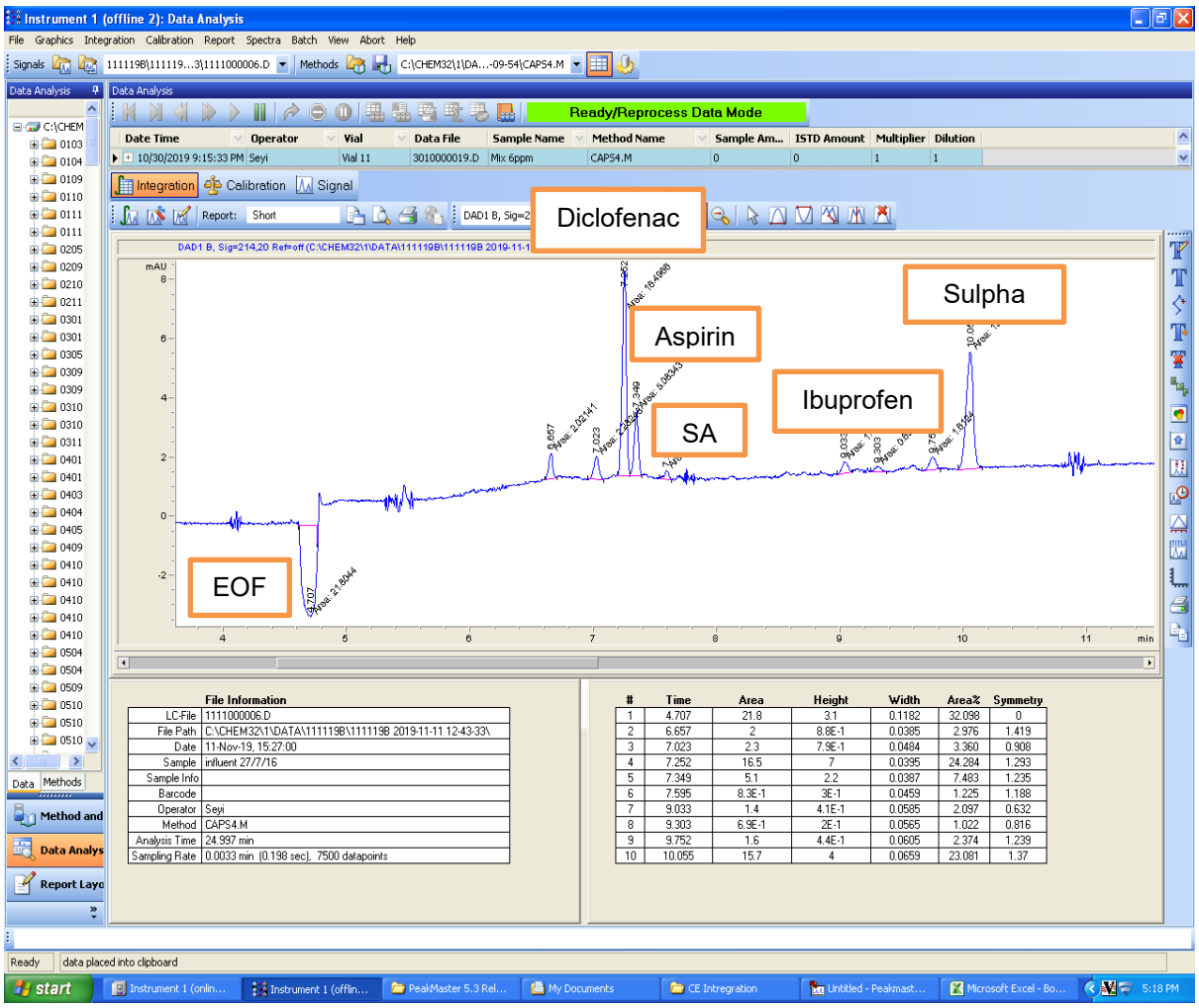

Figure 2: Electropherogram for influent B sample showing the peaks for the identified peaks: Diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole.

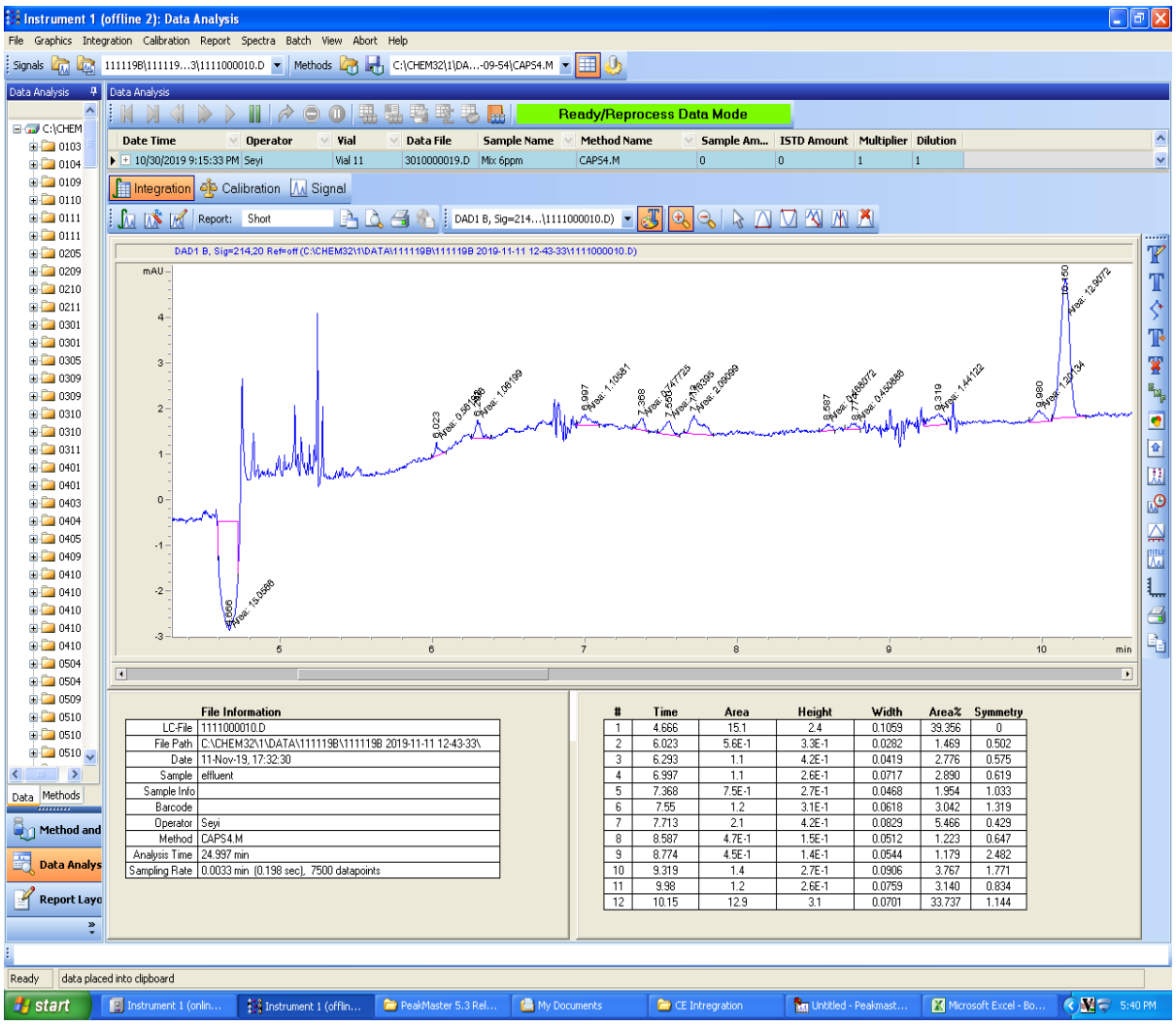

Figure 3: Electropherogram for effluent sample showing the peaks for the identified peaks including diclofenac, aspirin, salicylic acid, ibuprofen and sulphamethoxazole.

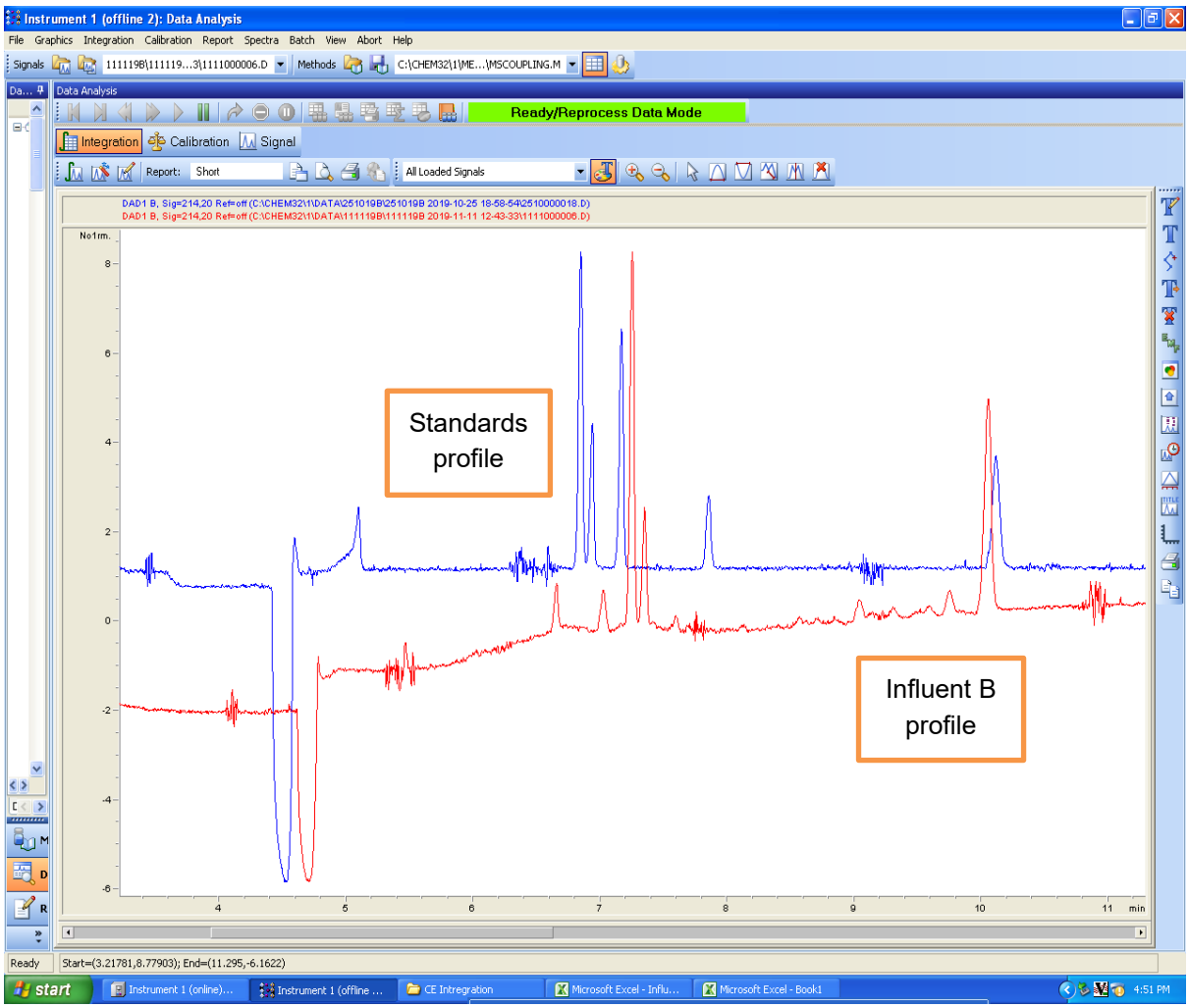

Figure 4: Electropherogram profile showing the standards mixture profile (blue) overlay with the influent B sample profile (red).

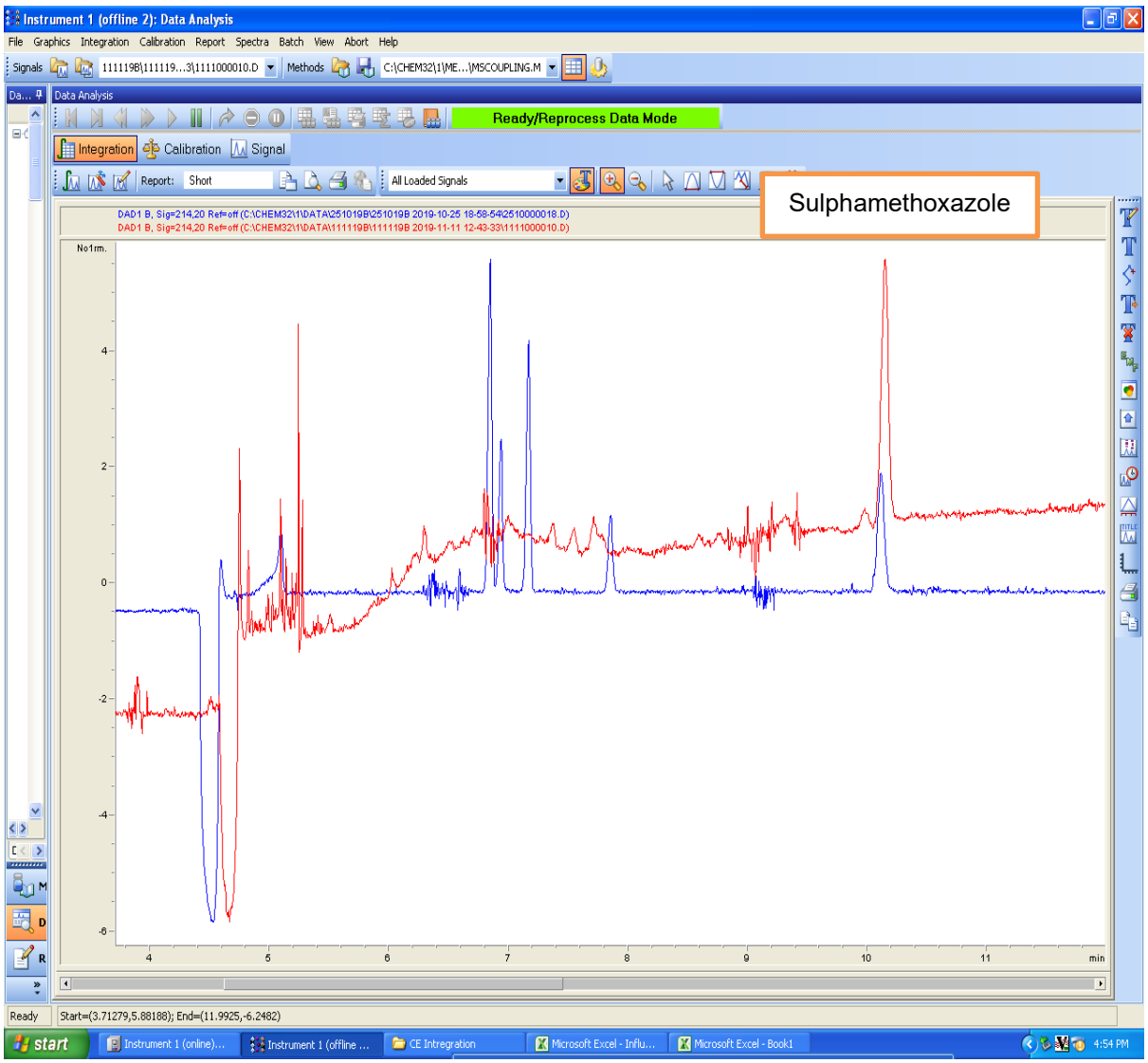

Figure 5: Electropherogram profile showing the standards mixture profile overlay with 2 µg/mL sulphamethoxazole-spiked effluent water sample profile.

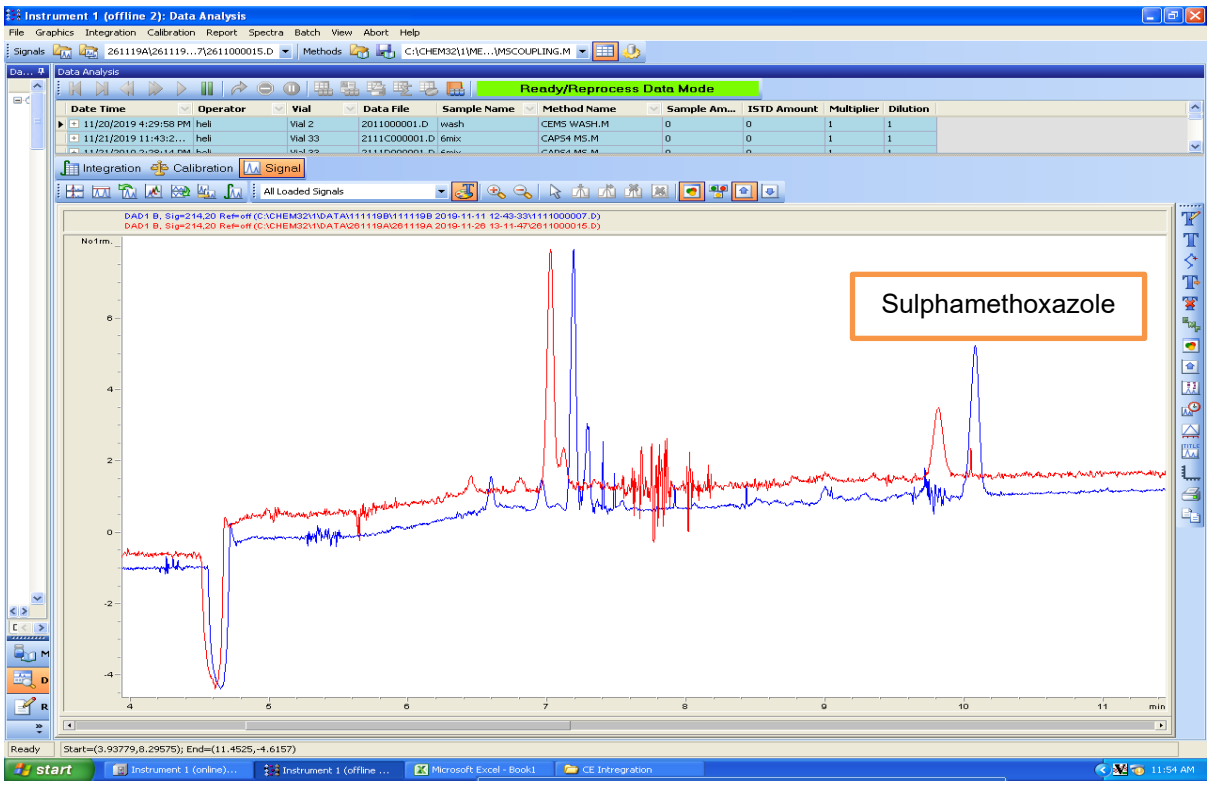

Figure 6: Electropherogram profile overlay of both spiked (blue) and unspiked effluent water sample profile.

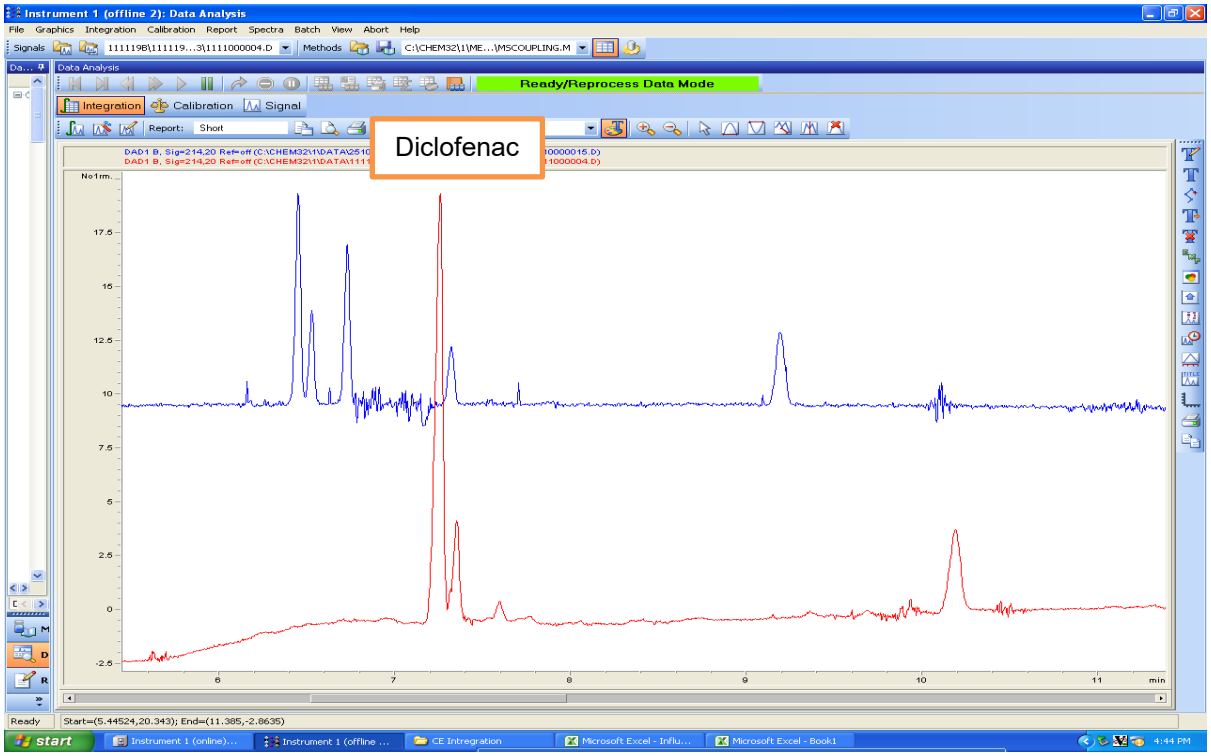

Figure 7: Electropherogram profile showing the standards mixture profile overlay with 2 µg/mL diclofenac-spiked influent A water sample profile.

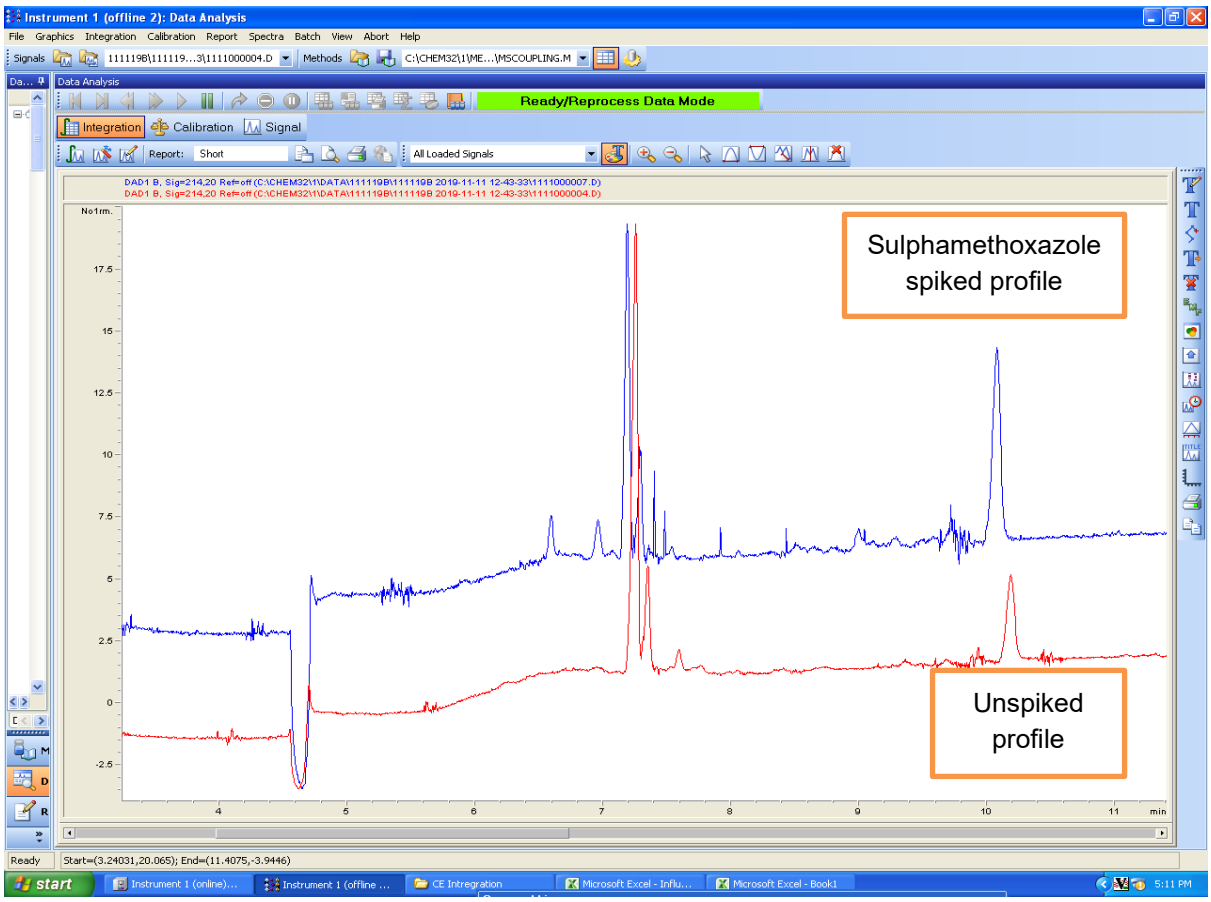

Figure 8: Electropherogram profile overlay of both spiked (blue) and unspiked (red) influent B water sample profiles.

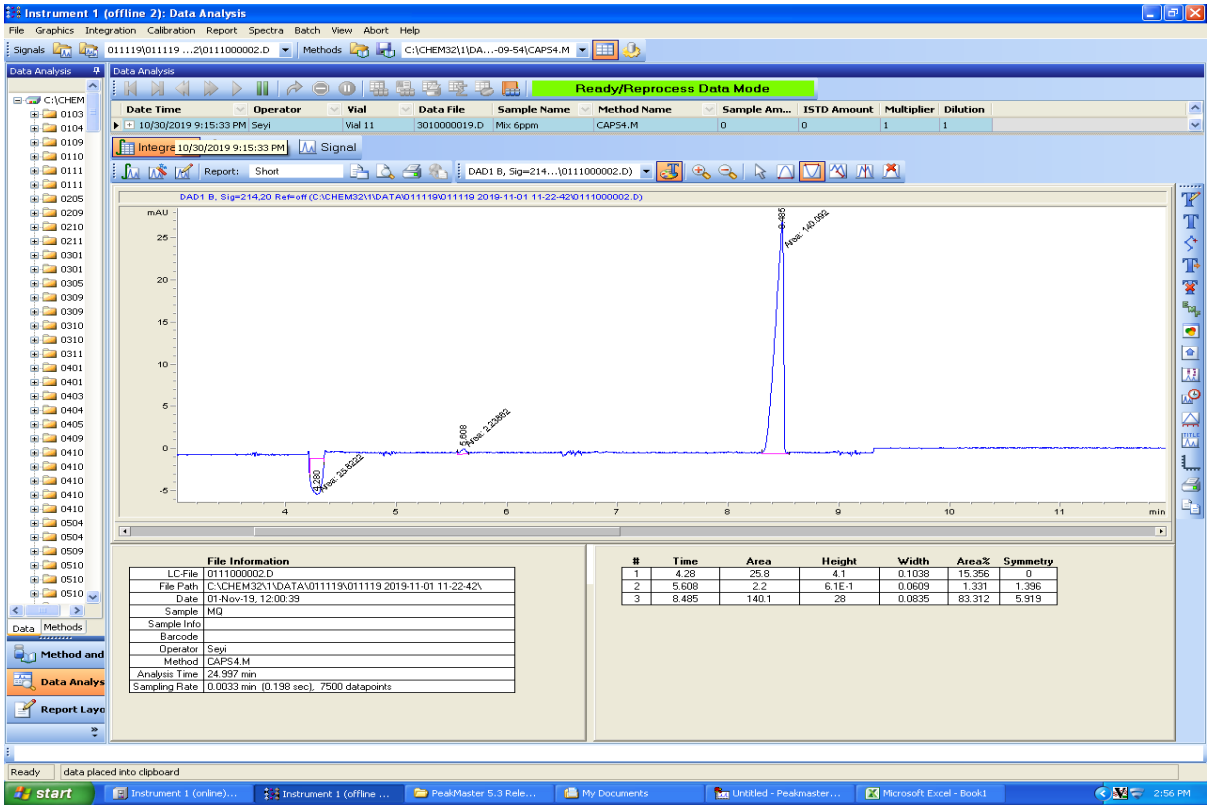

Figure 9: Electropherogram profile for Milli-Q water.

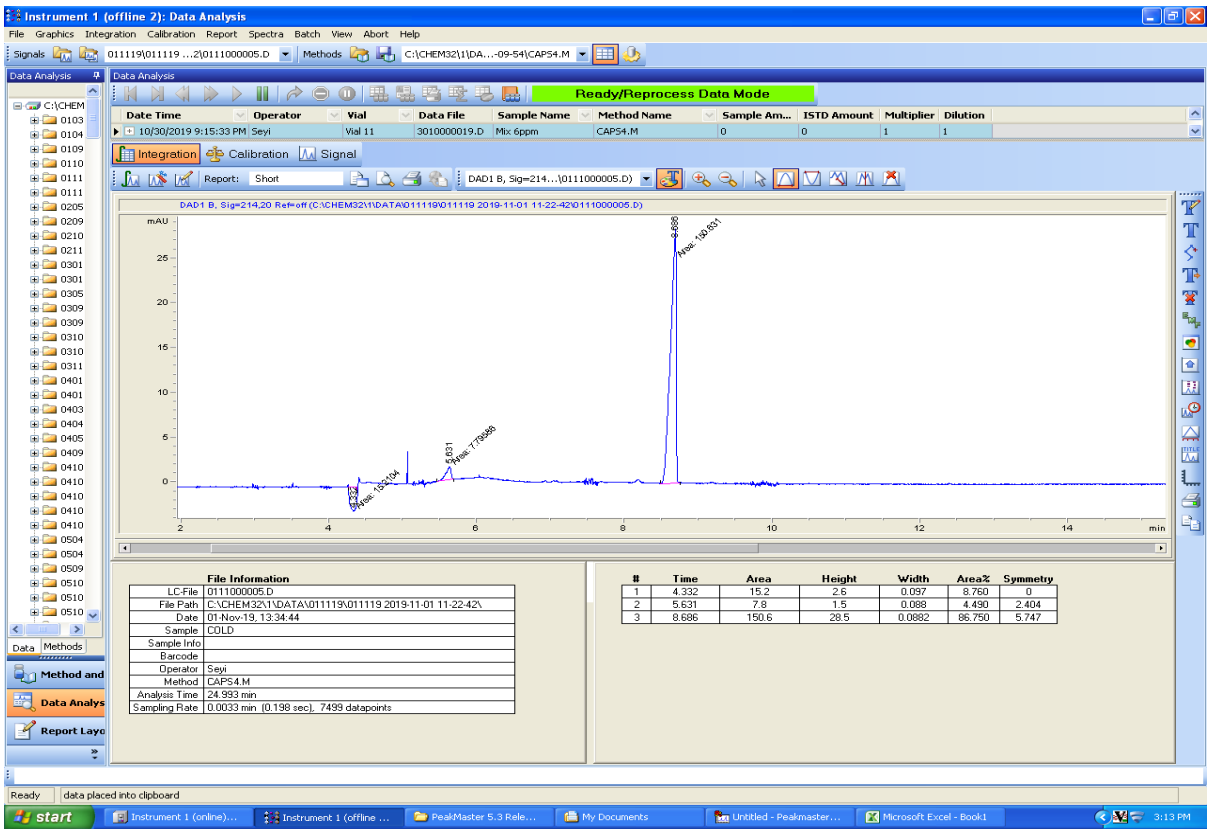

Figure 10: Electropherogram profile for cold water.

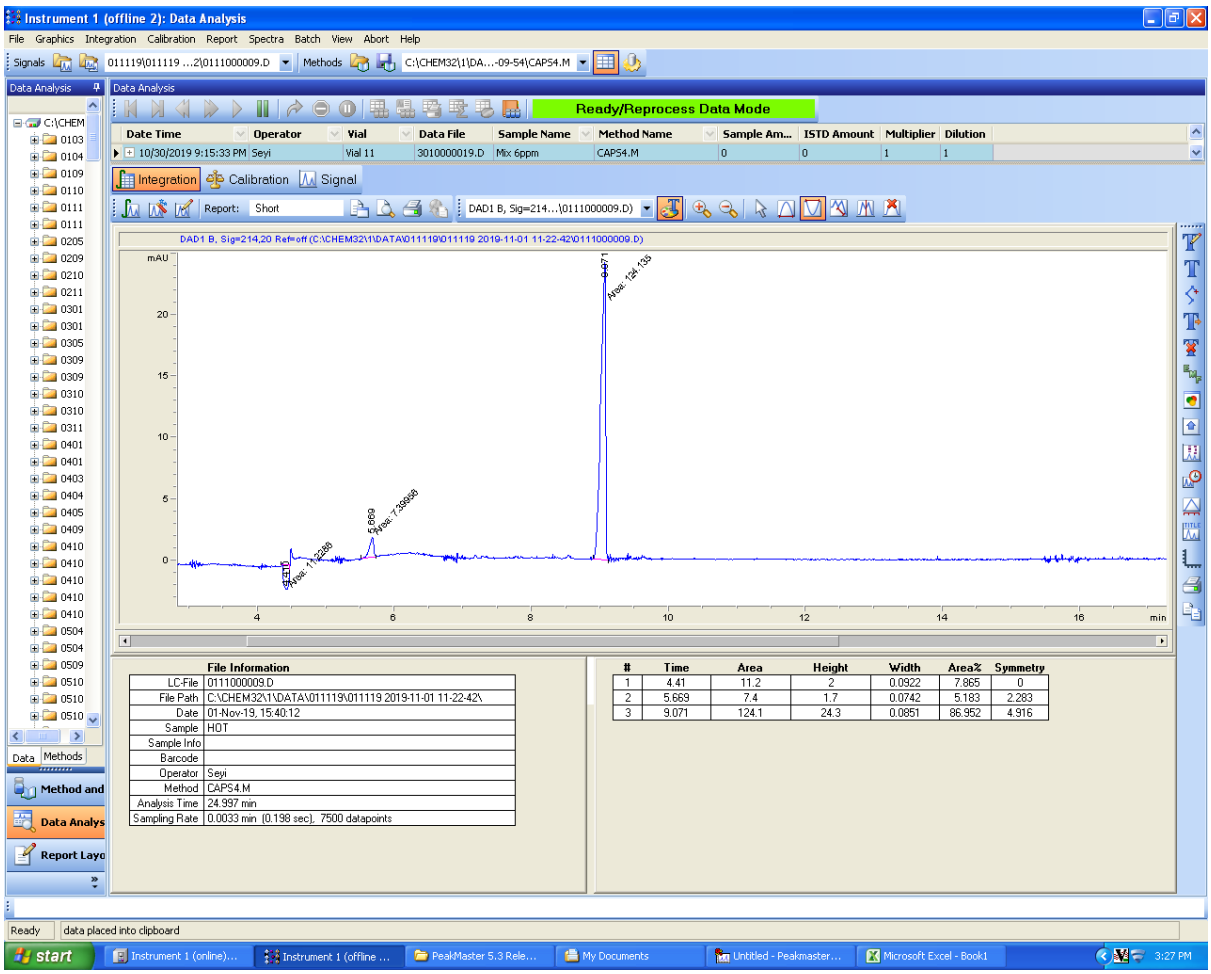

Figure 11: Electropherogram profile for cold water.

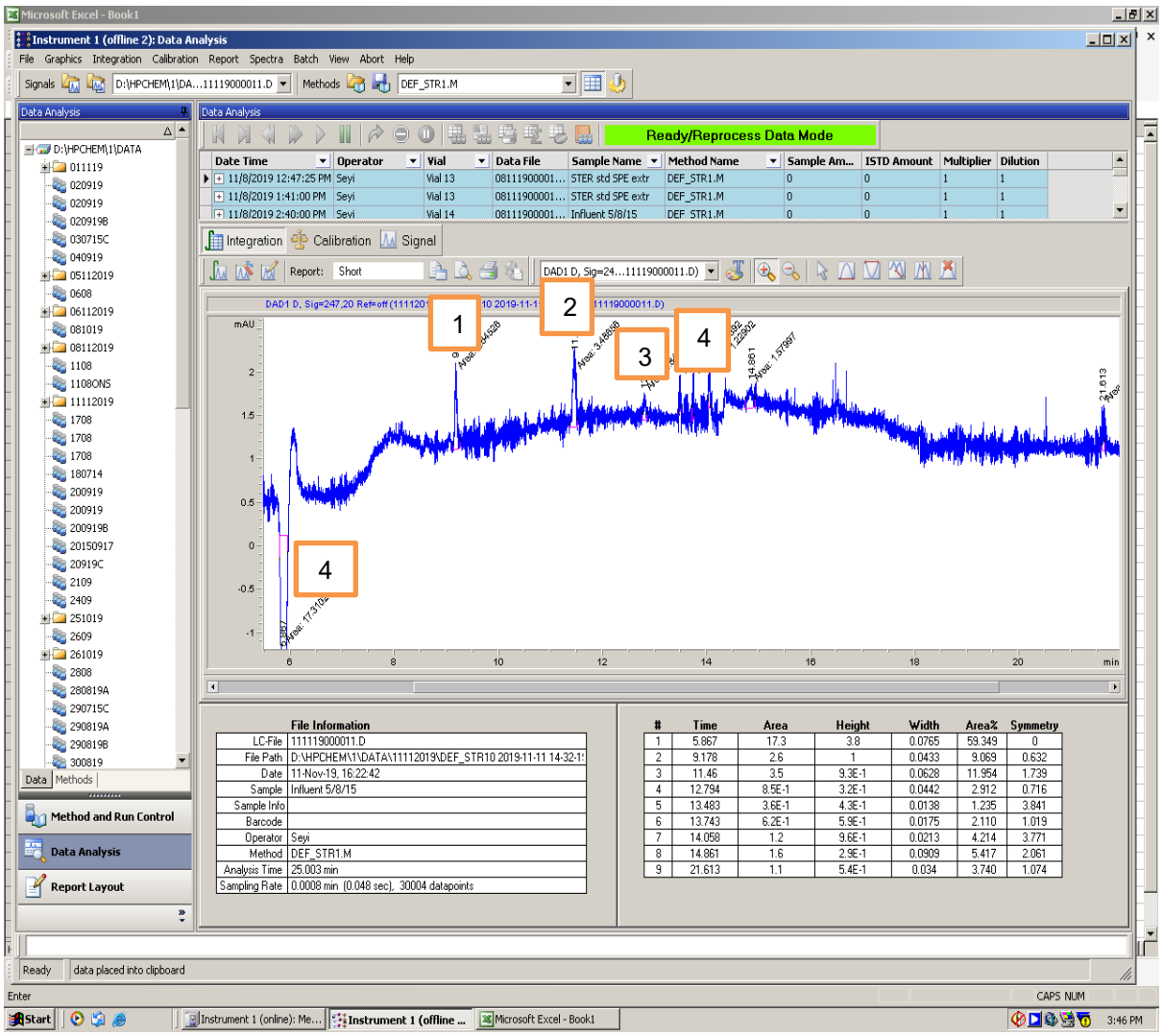

Figure 12: Electropherogram for influent A sample showing the peaks for the identified peaks including androstenedione (1), testosterone (2), 17-ß-estradiol (3) and progesterone (4).

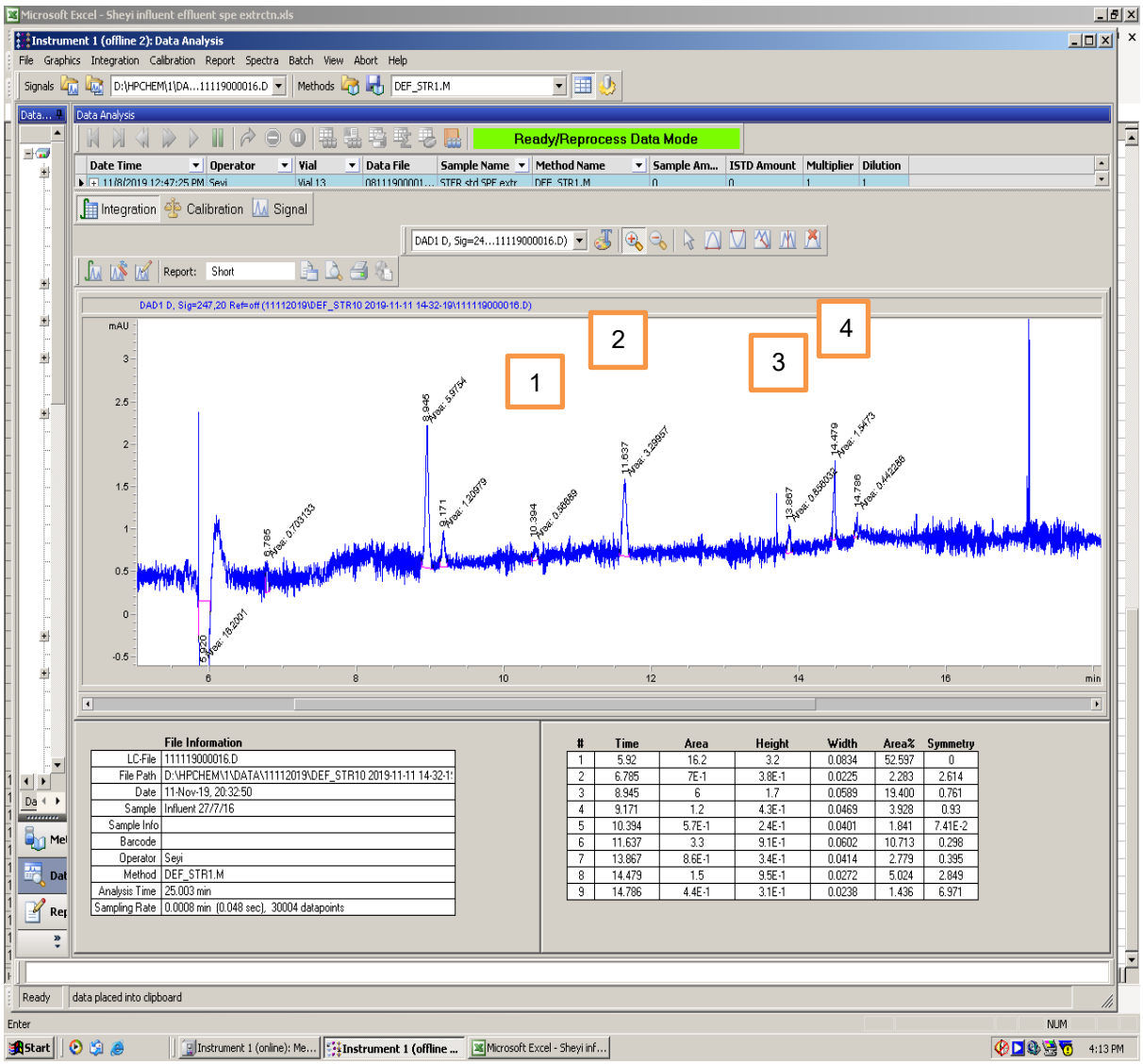

Figure 13: Electropherogram for influent B sample showing the peaks for the identified peaks including androstenedione (1), testosterone (2), 17-ß-estradiol (3) and progesterone (4).

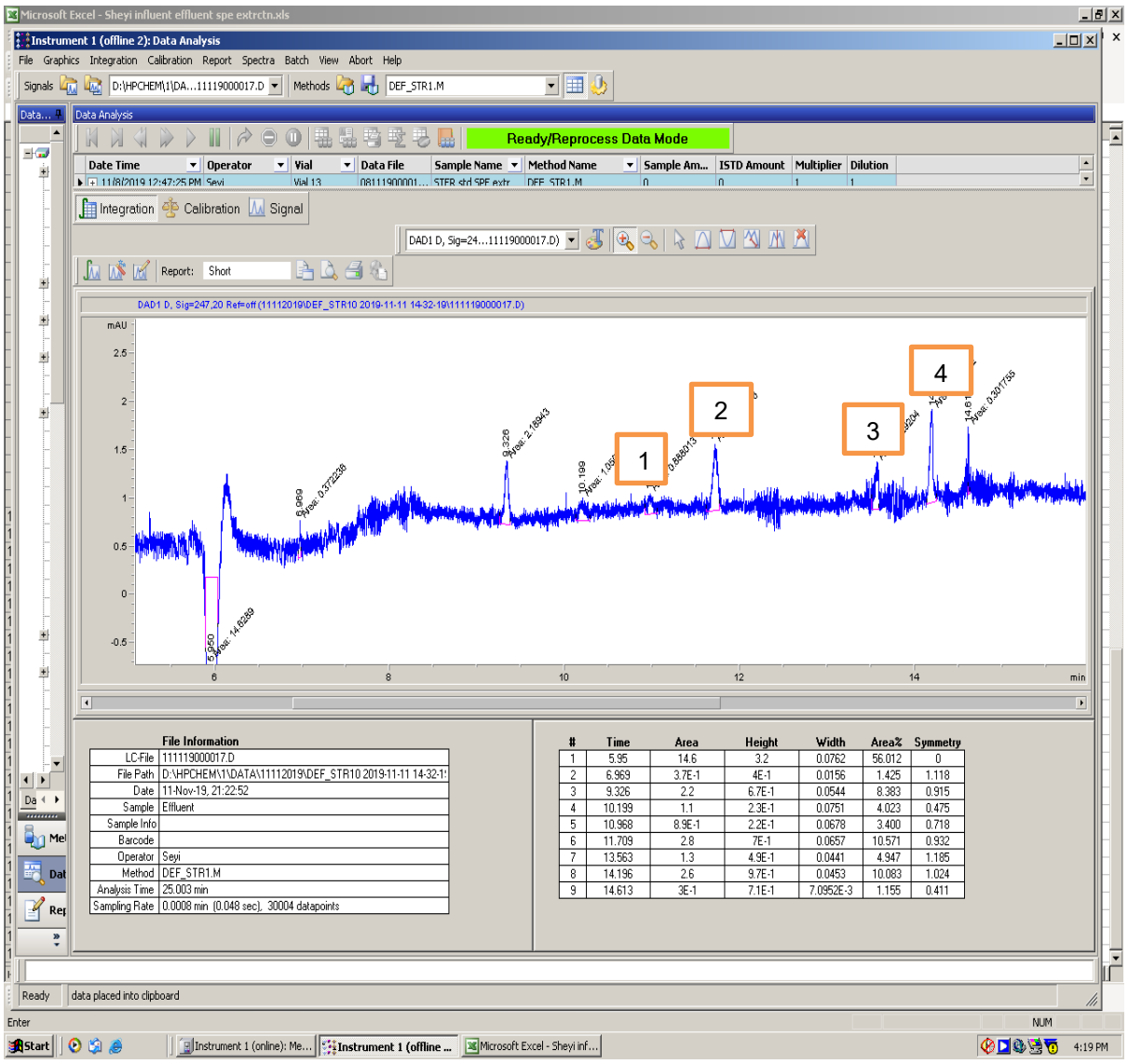

Figure 14: Electropherogram for effluent sample showing the peaks for the identified peaks including androstenedione (1), testosterone (2), 17-ß-estradiol (3) and progesterone (4).

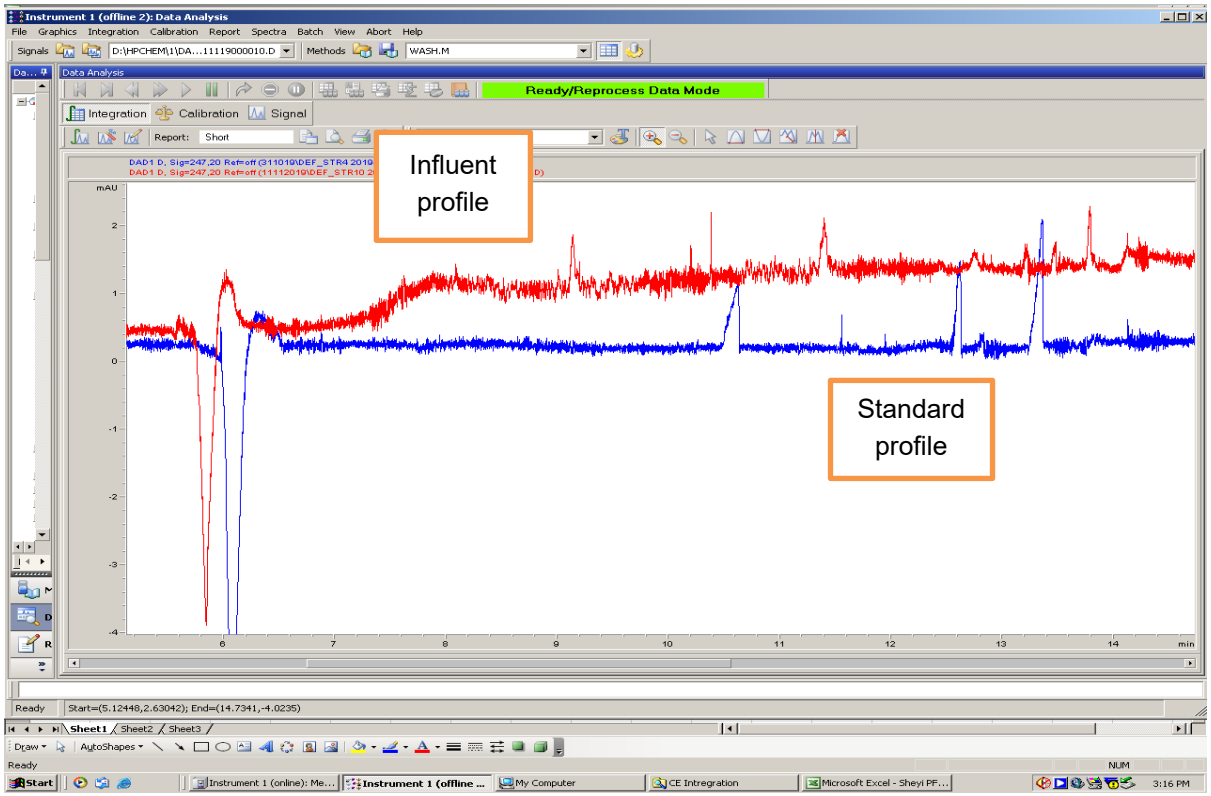

Figure 15: Electropherogram profile showing the steroids standards mixture profile overlay with the influent A sample profile.

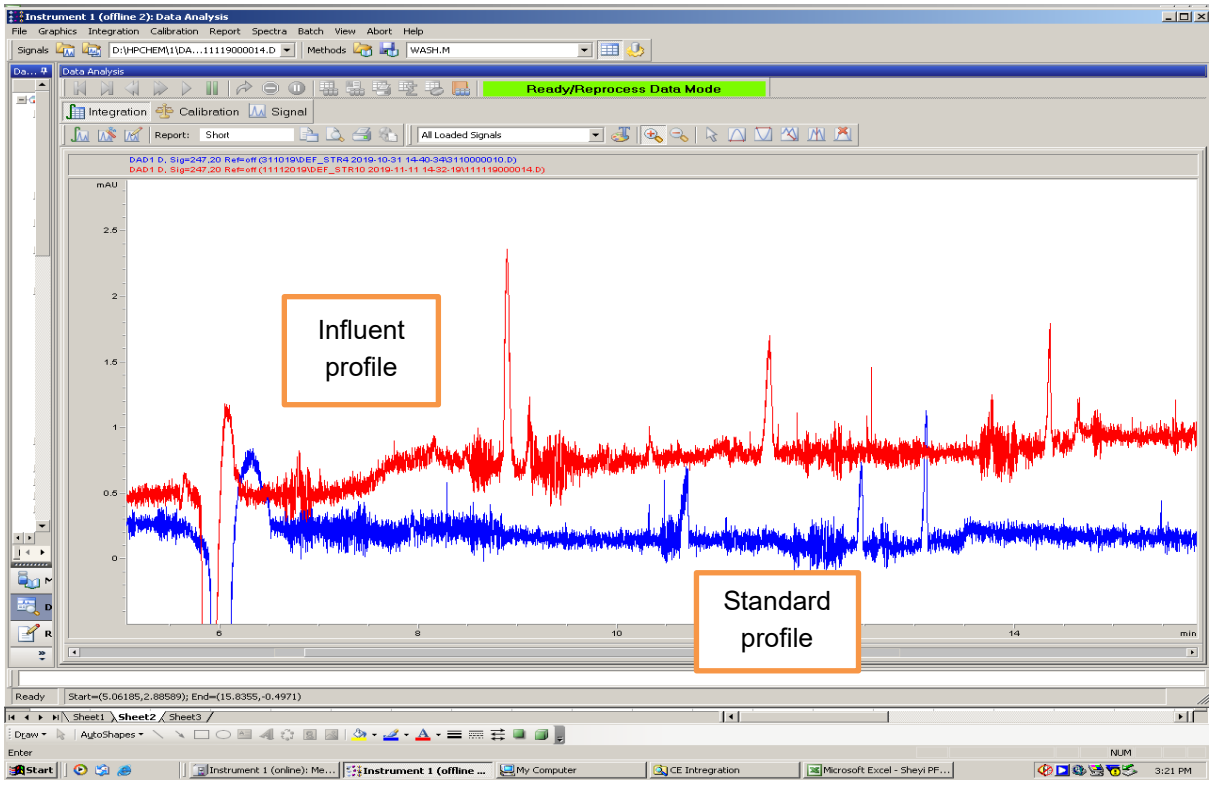

Figure 16: Electropherogram profile showing the steroids standards mixture profile overlay with the influent B sample profile.

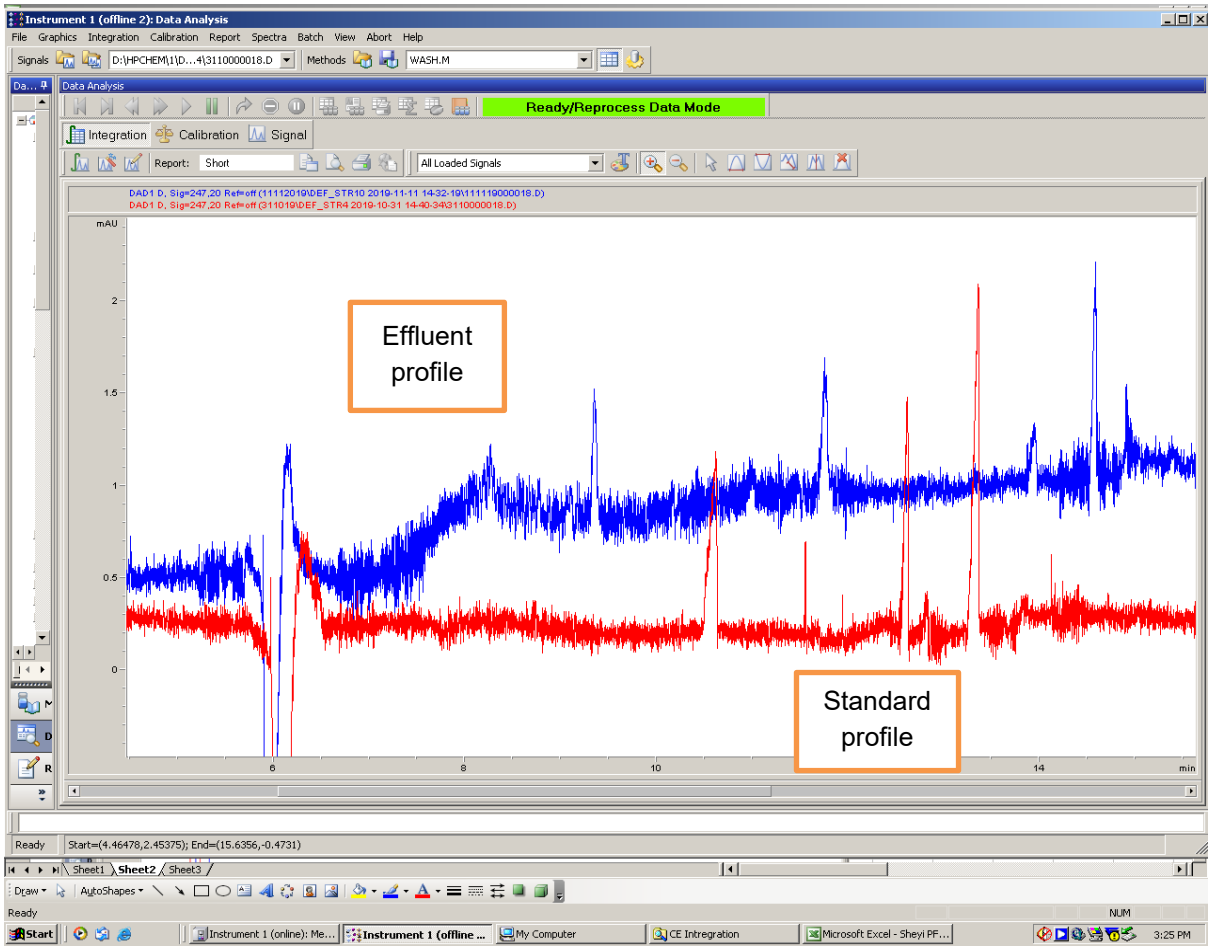

Figure 17: Electropherogram profile showing the steroids standards mixture profile overlay with the effluent sample profile.

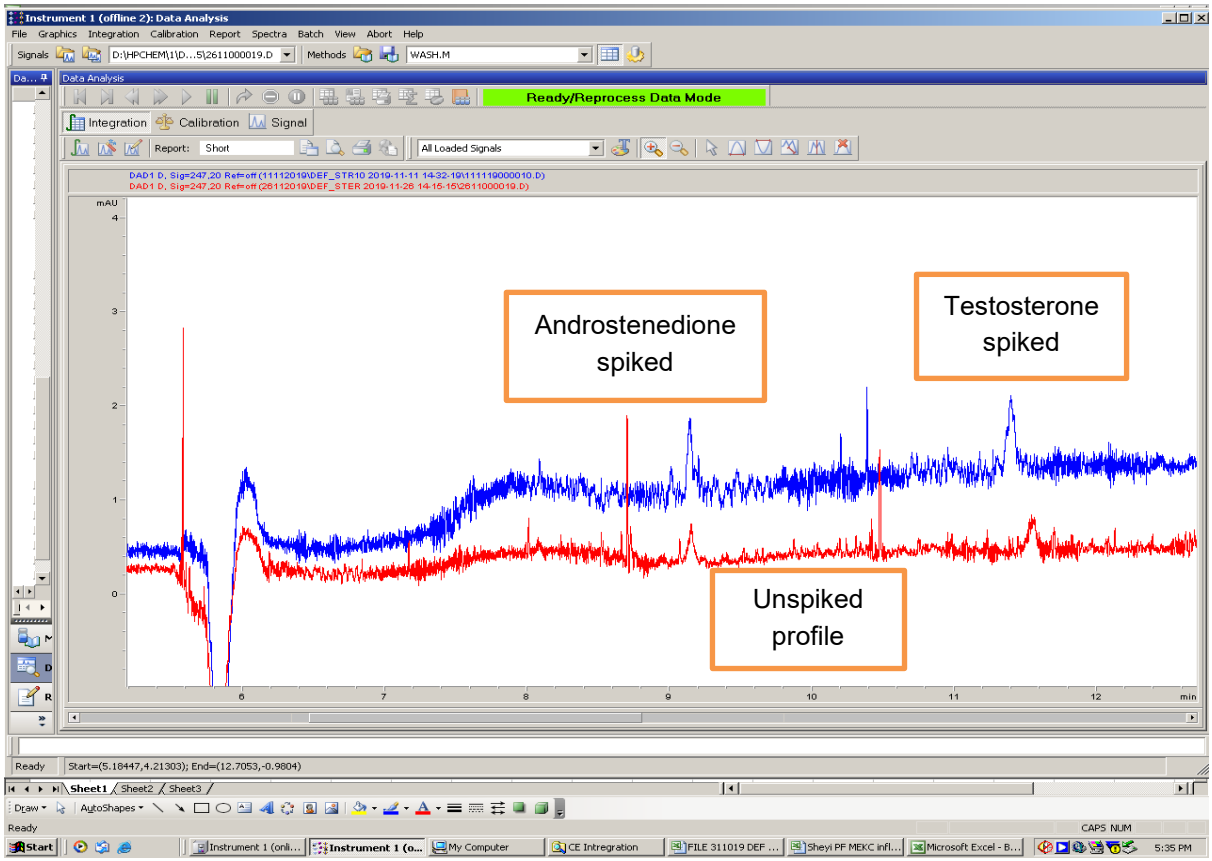

Figure 18: Electropherogram profile showing the androstenedione and testosterone-spiked and unspiked effluent profile.