

OZONE TREATMENT OF EXCESS BIOLOGICAL SLUDGE AND
XENOBIOTICS REMOVAL

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

MELİS MUZ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

MAY 2012

Approval of the thesis:

**OZONE TREATMENT OF EXCESS BIOLOGICAL SLUDGE AND
XENOBIOTICS REMOVAL**

submitted by **MELİS MUZ** in partial fulfillment of the requirements for the degree
of **Master of Science in Environmental Engineering Department, Middle East
Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Göksel N. Demirer _____
Head of Department, **Environmental Engineering**

Prof. Dr. Celal F. Gökçay _____
Supervisor, **Environmental Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Filiz B. Dilek _____
Environmental Engineering Dept., METU

Prof. Dr. Celal F. Gökçay _____
Environmental Engineering Dept., METU

Prof. Dr. F. Dilek Sanin _____
Environmental Engineering Dept., METU

Assist. Prof. Dr. Tuba H. Ergüder _____
Environmental Engineering Dept., METU

Prof. Dr. Ayşenur Uğurlu _____
Environmental Engineering Dept., Hacettepe University

Date: 18 May 2012

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name :

Signature :

ABSTRACT

OZONE TREATMENT OF EXCESS BIOLOGICAL SLUDGE AND XENOBIOTICS REMOVAL

Muz, Melis

M.Sc., Department of Environmental Engineering

Supervisor: Prof. Dr. Celal F. GÖKÇAY

May 2012, 107 Pages

A novel ozone-assisted aerobic sludge digestion process to stabilize and decrease the amount of excess sludge produced during biological treatment is presented in this study.

Excess sludge production is a well known burden for the treatment plants both legally and financially. Moreover, with the arise in the knowledge in recalcitrant compounds it is understood that it can act as a significant secondary pollutant.

With the developed pulse ozonation method, waste activated sludge samples from Ankara Tatlar and other Wastewater Treatment Plants (WWTP) were ozonated for different periods in Erlenmeyer flasks once a day on each of four consecutive days. Flasks were continuously aerated between ozone applications on an orbital shaker. The MLVSS, MLSS, COD and OUR parameters were measured routinely during the course of four days of digestion in order to optimize the process. Also pH, CST(capillary suction time) and SVI (sludge volume index) were followed. As a result MLVSS reductions of up to 95% were achieved with an ozone dose of only 0.0056 kg O₃/kg-initial MLSS, at the end of the fourth day.

In another experimental set, ozone dose was increased on the last day in order to destroy the selected endocrine disrupting compounds, namely diltiazem, carbamazepine, butyl benzyl phthalate and acetaminophen and two natural hormones estrone and progesterone, which accumulated onto the sludge. Over 99%

removal of these contaminants were achieved on the fourth day. The analyses were conducted by using LC(ESI) MS/MS after solid phase extraction (SPE).

By this process it became possible to save on contact time, as well as achieving a bio-solids digestion far exceeding the standard aerobic process at the expense of a minimum of ozone dose with the additional micropollutants removal. The developed process is deemed superior over side-stream ozonation of activated sludge in that it does not cause any reduction in active biomass amount that should be maintained in the aeration tank.

Keywords: ozone, biological sludge reduction, endocrine disrupting chemicals (EDC), LC-MS/MS, SPE

ÖZ

ATIK BİYOLOJİK ÇAMURLARIN OZONLA MUAMELESİ VE SENTETİK ORGANİK KİRLETİCİLERİN ARITILMASI

Muz, Melis

Yüksek Lisans, Çevre Mühendisliği Bölümü

Tez Yöneticisi: Prof. Dr. Celal. F. GÖKÇAY

Mayıs 2012, 107 Sayfa

Bu çalışmada, biyolojik arıtım sırasında ortaya çıkan atık çamurun minimizasyonu ve stabilizasyonunu sağlayan yeni bir ozon destekli havasal çamur çürütme yöntemi sunulmuştur.

Atık çamur üretimi arıtım tesisleri için hem yasal hem de finansal olarak yük teşkil etmektedir. Buna ek olarak, kalıcı bileşiklerle ilgili bilinenlerin artmasıyla birlikte, atık çamurun önemli bir ikincil kirletici olarak davranabileceği da anlaşılmıştır.

Geliştirilen kesikli ozonlama metoduyla, Ankara Tatlar Arıtma Tesisi'nden ve diğer arıtma tesislerinden alınan aktif çamur örnekleri Erlenmeyer şişelerinde değişik sürelerle, peş peşe dört gün boyunca, her gün sadece bir doz olacak şekilde ozonlanmıştır. Ozon uygulamalarının dışındaki sürelerde şişeler orbital karıştırıcıda devamlı olarak havalandırılmıştır. Prosesi optimize etmek adına dört gün süresince UKM, AKM, KOİ ve OTH parametreleri rutin olarak takip edilmiştir. Ayrıca pH, KES (kapiler emme süresi) ve çamur hacim indeksi (SVI) da ölçülmüştür. Sonuç olarak, dördüncü günün sonunda toplam 0.0056 kg O₃/kg-başlangıç AKM ozon dozu uygulanarak, %95'e varan bir UKM azalımı elde edilmiştir.

Diğer deney setinde çamurda biriken, seçilen endokrin bozucu maddeleri- diltiazem, carbamazepine, butyl benzyl phthalate ve acetaminophen- ve iki doğal hormonu -estrone ve progesterone- yok etmek adına son günkü ozon dozu

arttırılmıştır. Dördüncü gün sonunda %99'un üzerinde arıtım sağlanmıştır. Analizler katı faz ekstraksiyonu (SPE) ardından LC (ESI) MS/MS cihazı ile yapılmıştır.

Bu proses sayesinde hem kontak süresinin azaltılması olanaklı hale gelmiş, hem de minimum ozon dozu kullanılarak standard havasal prosesten çok daha fazla miktarda çamur çürütmesi mikrokirletici arıtımıyla birlikte sağlanmıştır. Geliştirilen bu yöntem çamurun yan akımda ozonlanmasından, havalandırma tankında muhafaza edilmesi gereken aktif biokütle miktarını düşürmediği için de daha üstündür.

Anahtar kelimeler: ozon, biyolojik çamur azaltımı, endokrin bozucu maddeler (EBM), LC-MS/MS, SPE

To my grandmother...

ACKNOWLEDGEMENTS

I would like to express my profound thanks to my supervisor Prof. Dr. Celal F. Gökçay, for supporting me in every step of my research process. His prescient vision not only help me to gain an incredible knowledge but also lead me to a bright path for my career.

I would also give my sincere thanks to Okan T. Komesli who helped me every time I lost my way and Kemal Demirtaş who taught us to embrace our laboratory.

I owe my deepest gratitude to my family, my parents, aunts and uncle for their ongoing support during my studies, not only in this research but throughout my whole life.

Many thanks to İbrahim Ayhan Aysal for his endless support. I will always remember him as the superhero of our project.

I would like to show my gratitude to my beloved friends and my cousins Övünç Ongur, Mert Kozan and Burcu Muz. Without them I will not get over this process conveniently. You all make my life blissful. I also like to thank my dear Onur Erkan who literally feeded me while I was trying to write my thesis.

Finally, I would like to thank TÜBİTAK ÇAYDAG (Project Code: 10Y272) for financial support.

TABLE OF CONTENTS

ABSTRACT	iv
ÖZ	vi
ACKNOWLEDGMENTS	ix
TABLE OF CONTENTS	x
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xix

CHAPTERS

1. INTRODUCTION.....	1
1.1 Excess Biological Sludge Problem.....	1
1.2 Ozonation for Sludge Minimization and Stabilization.....	1
1.3 Endocrine Disrupting Chemicals.....	2
1.4 EDC treatment with ozonation.....	2
1.5 Rationale for research.....	3
2. LITERATURE REVIEW.....	4
2.1 Excess Sludge Production.....	4
2.2 Sludge Minimization Techniques.....	5
2.2.1 Lysis-cryptic growth.....	5
2.3 Ozone.....	7
2.3.1 Chemical Structure of Ozone.....	7
2.3.2 Ozonation in Sludge Treatment.....	8
2.4 Endocrine Disrupting Compounds.....	10
2.4.1. Analytical Methods.....	12
2.4.2 Compounds of Interest.....	13
2.4.3 Removal of EDC's From Wastewater and Sludge.....	20
2.5 Aim of the Study.....	21
3. MATERIALS AND METHODS.....	23
3.1 Reagents and Chemicals.....	23

3.2 Instrumental Analysis with LC/ESI/MS/MS.....	24
3.2.1 Reference Standard Preparation and Calibration.....	24
3.3 Extraction Procedure.....	24
3.3.1 Ultrasound Aided Sequential Extraction.....	24
3.3.2 Solid Phase Extraction.....	26
3.4 Selected Sewage Treatment Works.....	26
3.5 Pulsed Ozone Treatment Process.....	28
3.5.1 Ozone Generator.....	28
3.5.2 Ozone Dose Optimization.....	28
3.5.3 Ozone Experiment Procedure.....	29
3.5.4 Analysis.....	29
4. RESULTS & DISCUSSIONS	31
4.1 EDC Analysis.....	31
4.1.1 Optimization of MS/MS Parameters.....	31
4.1.2 Calibration Curves.....	33
4.1.3 Wastewater Extraction Optimization.....	34
4.1.4 Optimization of Sludge Extraction Procedure.....	37
4.2 Pulse Ozonation Experiments.....	41
4.2.1 First Set of Experiments.....	41
4.2.2 Second Set of Experiments.....	47
4.2.3 Effect of Ozonation on Phosphorus Content of Sludge.....	50
4.3 Removal of EDCs with Ozone Application.....	55
5. CONCLUSION.....	66
REFERENCES.....	68
APPENDICES	
A.FIGURES OF EDC OPTIMIZATION AND REMOVAL.....	78

LIST OF TABLES

TABLES

Table 2.1 Sludge reduction percentages with corresponding ozone doses given in literature	10
Table 2.2 Detection frequency of Dtz, Cbz and Atp in selected sampling locations of 10 different WWTPs	13
Table 2.3 Average concentrations of Dtz, Cbz and Atp in selected sampling locations of 10 different WWTPs ($\mu\text{g/L}$)	14
Table 2.4 LD 50 concentrations of Atp, Cbz and Dtz in mice	14
Table 2.5 Physicochemical properties of Atp, Cbz and Dtz	15
Table 2.6 Physicochemical properties of Butyl Benzyl Phthalate	17
Table 3.1 Operational parameters for the instrument	25
Table 3.2 Characteristics of WWTPs within the scope of this study	27
Table 4.1 Mobile phase sets prepared for optimization	31
Table 4.2 Initial Parameters used prior to optimization of the compounds	32
Table 4.3 Optimization Parameters for Target Compounds	33
Table 4.4 Percent (%) recoveries obtained during pH optimization	35
Table 4.5 Analytical Figures of Merit	37
Table 4.6 Extraction efficiencies of the analytes of interest from dried sludge	38
Table 4.7 Analytical Figures of Merit	41
Table 4.8 The OUR readings obtained for 4 and 6 minutes ozonated samples and control groups	44
Table 4.9 Results of sludge samples before and after ozonation on the 1 st and 4 th day.....	45
Table 4.10 The pH comparison of ozonated sample before and after ozonation versus control group on the 1 st and 4 th days	45
Table 4.11 MLSS and MLVSS removal percentages of sludge samples	50
Table 4.12 Ozone doses applied to sludge samples	50
Table 4.13 Results of the ozonation experiment for WWTP1	51

Table 4.14 Results of the ozonation experiment for WWTP4	52
Table 4.15 Amount of phosphorus accumulated in sludge	52
Table 4.16 Summary of Experimental Plan for the EDC Removal Experiments ...	56
Table 4.17 Final EDC concentrations of experiment conducted on 28.04 (50'-150')	
.....	57
Table 4.18 Final EDC concentrations of experiment conducted on 03.05 (50'-150')	
.....	58
Table 4.19 Final EDC concentrations of experiment conducted on 14.08 (6'-40')	
.....	59
Table 4.20 Final EDC concentrations of experiment conducted on 19.12 (6'-40')	
.....	60
Table 4.21 Removal percentages of EDC concentrations of experiment conducted	
on 28.04 (50'-150')	60
Table 4.22 Removal percentages of EDC concentrations of experiment conducted	
on 03.05 (50'-150')	61
Table 4.23 Removal percentages of EDC concentrations of experiment conducted	
on 14.08 (6'-40')	61
Table 4.24 Removal percentages of EDC concentrations of experiment conducted	
on 19.12 (6'-40')	62
Table 4.25 Ozonation periods corresponding to applied ozone doses	65

LIST OF FIGURES

FIGURES

Figure 2.1 Effect of an external source on lysis-cryptic growth	6
Figure 2.2 Chemical structure of an ozone molecule	7
Figure 2.3 Mechanism of the indirect and direct ozonation	8
Figure 2.4 Components of a (partially) closed water cycle with indirect potable reuse	11
Figure 2.5 Chemical structure of Diltiazem	16
Figure 2.6 Chemical structure of Carbamazepine	16
Figure 2.7 Chemical structure of Acetaminophen	16
Figure 2.8 Chemical structure of Butyl Benzyl Phthalate	18
Figure 2.9 Chemical structure of Estrone	19
Figure 2.10 Chemical structure of Progesteron	20
Figure 3.1 Ozonation periods versus ozone imparted into water	28
Figure 4.1 Description of LOD and LOQ via signal to noise ratio	36
Figure 4.2 TIC comparison of EDCs in aliquots after centrifugation	39
Figure 4.3 TIC comparison of 3 different sets of sludge extraction method	40
Figure 4.4 The soluble COD values for 2, 3, 4 and 6 minutes ozonation versus control group	42
Figure 4.5 The MLSS values for 2, 3, 4 and 6 minutes ozonation versus control group	43
Figure 4.6 pH variations in the aliquotes for ozonated samples versus control group	46
Figure 4.7 pH variations in the sludge for ozonated samples versus control group	47
Figure 4.8 MLSS results of 4 and 6 minutes ozonation versus control group	48
Figure 4.9 MLVSS results of 4 and 6 minutes ozonation versus control group	48
Figure 4.10 Soluble COD results of 4 and 6 minutes ozonation versus control group	49
Figure 4.11 MLSS and MLVSS results for WWTP1 during ozonation	53

Figure 4.12 TP&OP results for WWTP1 during ozonation	54
Figure 4.13 MLSS and MLVSS results for WWTP4 during ozonation	54
Figure 4.14 TP&OP results for WWTP4 during ozonation	55
Figure 4.15 Concentrations of Diltiazem after last ozone application for all sets of experiments	62
Figure 4.16 Concentrations of Progesterone after last ozone application for all sets of experiments.....	63
Figure 4.17 Concentrations of BBP after last ozone application for all sets of experiments.....	63
Figure 4.18 Concentrations of Estrone after last ozone application for all sets of experiments.....	64
Figure 4.19 Concentrations of Carbamazepine after last ozone application for all sets of experiments.....	64
Figure 4.20 Concentrations of Acetaminophen after last ozone application for all sets of experiments.....	65
Figure A-1 MS2 Scan Peak of Carbamazepine for Mobile phase set with %0.1 F.A.	78
Figure A-2 MS2 Scan Peak of Carbamazepine for Mobile phase set with 26 mM A.F.	78
Figure A-3 MS2 Scan Peak of Carbamazepine for Mobile phase set with %0,1 F.A. + 5 mM A.F.	79
Figure A-4 MS2 Scan Peak of Progesterone for Mobile phase set with %0.1 F.A.	79
Figure A-5 MS2 Scan Peak of Progesterone for Mobile phase set with 26 mM A.F.	80
Figure A-6 MS2 Scan Peak of Progesterone for Mobile phase set with %0,1 F.A. + 5 mM A.F.	80
Figure A-7 Chromatogram of Diltiazem in negative mode	81
Figure A-8 Chromatogram of Diltiazem in positive mode	81
Figure A-9 Chromatogram of Acetaminophen in negative mode	82
Figure A-10 Chromatogram of Acetaminophen in positive mode	82

Figure A-11 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with %0.1 F.A.	83
Figure A-12 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with 26 mM A.F.	83
Figure A-13 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with %0,1 F.A. + 5 mM A.F.	84
Figure A-14 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with %0.1 F.A.	84
Figure A-15 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with 26 mM A.F.	85
Figure A-16 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with %0,1 F.A. + 5 mM A.F.	85
Figure A-17 Product Ion Analysis for Progesterone	86
Figure A-18 Product Ion Analysis for Carbamazepine	86
Figure A-19 Product Ion Analysis for Estrone.....	87
Figure A-20 Product Ion Analysis for BBP	87
Figure A-21 Product Ion Analysis for Acetaminophen	88
Figure A-22 Product Ion Analysis for Diltiazem	88
Figure A-23 Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with %0.1 F.A.	89
Figure A-24 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with %0.1 F.A.	89
Figure A-25 26 mM Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with 26 mM A.F.	90
Figure A-26 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with 26 mM A.F.	90
Figure A-27 Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with %0,1 F.A. + 5 mM A.F.	91
Figure A-28 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with %0,1 F.A. + 5 mM A.F.	91
Figure A-29 Peak signals of all analytes with the mobile phase set with%0,1 F.A. + 5 mM A.F.	92

Figure A-30 Calibration curve for Estrone	92
Figure A-31 Calibration curve for Progesterone	93
Figure A-32 Calibration curve for Carbamazepine	93
Figure A-33 Calibration curve for BBP.....	94
Figure A-34 Calibration curve for Diltiazem	94
Figure A-35 Calibration curve for Acetaminophen	95
Figure A-36 Peak chromatograms of Diltiazem before and after extraction at pH 7	95
Figure A-37 Peak chromatograms of Progesterone before and after extraction at pH 7	96
Figure A-38 Peak chromatograms of BBP before and after extraction at pH 7	96
Figure A-39 Peak chromatograms of Estrone before and after extraction at pH 7..	97
Figure A-40 Peak chromatograms of Cbz before and after extraction at pH 7	97
Figure A-41 Peak chromatograms of Atp before and after extraction at pH 7	98
Figure A-42 Chromatograms of the analytes with a flow rate of 0.1 ml/min	98
Figure A-43 Chromatograms of the analytes with a flow rate of 0.2 ml/min	99
Figure A-44 Chromatograms of the analytes with a flow rate of 0.3 ml/min	99
Figure A-45 Chromatograms of the analytes with a flow rate of 0.4 ml/min	100
Figure A-46 Chromatograms of the analytes with a flow rate of 0.5 ml/min	100
Figure A-47 Diltiazem concentrations of 20 ppb mix solution and spiked sample after sludge extraction	101
Figure A-48 Progesterone concentrations of 20 ppb mix solution and spiked sample after sludge extraction	101
Figure A-49 BBP concentrations of 20 ppb mix solution and spiked sample after sludge extraction	102
Figure A-50 Cbz concentrations of 20 ppb mix solution and spiked sample after sludge extraction	102
Figure A-51 Acetaminophen concentrations of 20 ppb mix solution and spiked sample after sludge extraction	103
Figure A-52 Lyophilized vs dried sludge	103
Figure A-53 Total Ion Chromatogram Comparison of Methanol versus Dichloromethane	104

Figure A-54 6' and 30' ozonated flasks before passing through ordinary filter paper	105
Figure A-55 75' ozonated flask before passing through ordinary filter paper	106
Figure A-56 150' ozonated flask after passing through ordinary filter paper	107

LIST OF ABBREVIATIONS

ATP: Acetaminophen
BBP: Butyl Benzyl Phthalate
BNR: Biological Nutrient Removal
CBZ: Carbamazepine
CE: Collision Energy
CST: Capillary Suction Time
DTZ: Diltiazem
EDC: Endocrine Disrupting Compounds
ESI: Electrone Spray Ionization
FV: Fragmentor Voltage
GC: Gas Chromatography
GC/MS/MS: Gas Chromatography Tandem Mass Spectrometry
HPLC: High Performance Liquid Chromatography
LC/MS/MS: Liquid Chromatography Tandem Mass Spectrometry
LOD: Limit of Detection
LOQ: Limit of Quantification
MeOH: Methanol
MLSS: Mixed Liquour Suspended Solids
MLVSS: Mixed Liquour Volatile Suspended Solids
MRM: Multiple Reaction Monitoring
O₃: Ozone
OP: Ortho-Phosphate
OUR: Oxygen Uptake Rate
PPB: Parts Per Billion
PPM: Parts Per Million
PPT:Parts Per Trillion
RAS: Return Activated Sludge
sCOD: Soluble Chemical Oxygen Demand
SIM: Selected Ion Monitoring
SPE: Solid Phase Extraction

SRT: Solids Retention Time

SVI: Sludge Volume Index

TIC: Total Ion Chromatogram

TOC: Total Organic Carbon

TP: Total Phosphorus

TSS: Total Suspended Solids

WAS: Waste Activated Sludge

WWTP: Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

1.1 Excess Biological Sludge Problem

Activated sludge is the most widely used biological treatment process that relies on a dense microbial population being mixed with wastewater under aerobic conditions (Gray, 1989). However, excess biological sludge produced during the process is a rapidly emerging problem for the wastewater treatment facilities and sludge management is becoming an important environmental and legal issue worldwide. EU and Turkey are implementing stricter regulations on discharge, stabilization, minimization and disposal of sludge; which compel municipalities for more action to be taken and for more installations to be installed or upgrading of existing facilities to comply with the regulations (Spinosa, 2007). As Liu (2003) indicated, besides from the initial and operational costs of a treatment plant, treatment and disposal of sewage sludge accounts for upto 65% of the total operation cost (Zhao & Kugel, 1997). Therefore, the need for solution motivated more research to be channeled to find alternative sludge minimization technologies.

1.2 Ozonation for Sludge Minimization and Stabilization

Numerous full scale and lab scale processes have been developed to minimize excess sludge produced from biological wastewater treatment plant. These include thermal treatment, chemical treatment using acids or alkali, mechanical treatment by ultrasound, biological hydrolysis by enzyme addition, advanced oxidation and combination of these (Wei et al., 2003). All these applications aim to induce lysis and cryptic growth towards this outcome. Ozone, which is a strong oxidant, destroys cell wall of microbes and causes intracellular material to release into the medium (Chu et al., 2009).

Its success at full scale applications and the high efficiency makes ozonation one of the most widely used technique among other pretreatment mechanisms (Camacho et al., 2002; Yasui et al., 1996). Unlike the previously reported studies in the literature where stabilization of excess sludge is achieved by applying continuous or intermittent ozonation to the recycle stream (Zhang et al., 2008; Park et al., 2008; Kamiya & Hirotsuji, 1998); pulse ozonation of excess sludge in a segregated digester, in order to enhance aerobic digestion, is studied in this thesis.

1.3 Endocrine Disrupting Chemicals

Emerging contaminants which include Endocrine Disrupting Chemicals (EDCs) and Personal Care Products (PPCPs) have grabbed the attention of the scientific community in the recent years. The PPCPs have numerous areas of usage in daily life, industry and agriculture. Studies confirmed that they can cause growth and reproduction abnormalities, sexual orientation alterations in both wildlife and humans by interfering with the endocrine system (Lister & Kraak, 2001). Environment Agency of England and Wales and NERC both studied reproductive shifts in five different rivers in the UK and came up with a striking result that feminisation of male fish has been linked to the discharges from treatment plants (WWF, 1998). Thus, diverting concerns to the treatability of these compounds in water/wastewater treatment processes and to their removal mechanisms.

1.4 EDC treatment with ozonation

Variations in properties of EDCs result in differences in degree of their treatment in sewage treatment works. Although some are biodegradable and removed in the usual biological processes, hydrophobics tend to accumulate in sludge. Therefore, it is necessary to remove these contaminants from sludge, especially when land application of sludge is considered. Land application of sludge as fertilizer/soil conditioner is a viable option of disposal, but creates a threat for the environment as uptake by food crops may introduce these to the food chain (McClellan & Halden, 2010). Some of the available treatment techniques, at least some extent, which may be applicable to EDCs include nanofiltration, membrane bioreactors, physicochemical treatments such as coagulation/flocculation, and advanced treatment methods such as chlorination, photolysis, ozonation and other

miscellaneous advanced oxidation methods (Bolong et al., 2009). Ozonation is the dark oxidation process that can affect removal of more than 90% of several emerging contaminants, such as pesticides, anti-inflammatories, antiepileptics, antibiotics and natural and synthetic estrogens in waters and wastewaters (Esplugas et al., 2007).

1.5 Rationale for research

Ozonation is a very effective technique in sludge minimization, among others. However, continuous ozonation is costly. Moreover, ozone application to sludge recycle line (RAS) cause decrease in the viable biomass concentration that should be maintained in the aeration tank, thus requiring a larger tank volume and larger footprint for a given treatment target. The aim of this thesis was therefore set to find an optimum ozone pulsing strategy which will affect both maximum removal of sludge and endocrine disrupting compounds bound to sludge, simultaneously, without affecting the biomass held in the aeration tank.

CHAPTER 2

LITERATURE REVIEW

2.1 Excess Sludge Production

Product of biological treatment processes is an undesirable byproduct, excess sludge. Characteristics of excess sludge vary according to operating conditions and type of processes that the wastewater undergoes. Originally sludge is a suspension of inorganic and organic solids which constitute 1-5% of the mixture. It includes live bacteria, nutrients, pathogens and sometimes heavy metals and other constituents (Vesilind & Spinosa, 2001; Tchobanoglous et al., 2004). Typical amount of sludge produced is taken as 90 grams dry weight per day per capita for almost all EU countries. Following the implementation of Urban Wastewater Treatment Directive (91/271/EEC), sludge production in EU was forecasted to increase to 10.1 million tonnes of dry weight / year (Coulomb et al., 1997). In China, sludge that has to be disposed of was expected to be five times more in 2010 than 2007 (Zhao et al., 2007).

In Turkey, the first wastewater treatment plant was built in 1982. At the end of 1994 there were 45 treatment plants; of which 41 were biological treatment (Filibeli & Ayol, 2007). This number increased to 236 by the year 2008 (DIE, 2008).

The current national legislations stimulate municipalities to build sewerage systems, construct treatment plants and meet the tightened sludge disposal criteria. In other words, sludge production increases day by day but it becomes that much harder to dispose. The most important regulations in Turkey regarding disposal, transportation, incineration and agricultural use of sludge are Soil Pollution Control Regulation (revised 2010), Solid Waste Control Regulation (revised 2011), Urban Wastewater Treatment Regulation (revised 2009), Water Pollution Control Regulation (revised 2011), Hazardous Waste Regulation(revised 2011) and a new draft named: Use of Domestic and Urban Sewage Sludge on Soil (2010).

Methods for sludge disposal involve landfilling, incineration, land application and sea disposal; all which have negative impacts on the environment and create new problems, such as handling incineration ashes which are considered hazardous waste and consequently increasing disposal costs. Sea disposal has been banned and land application became undesirable due to heavy metal and persistent contaminant contents of sludge. Most parties tend to reuse sludge in different alternatives; such as an energy source or construction material, which clearly indicates that hereafter deposition of sludge on land will no longer be an acceptable solution (Ramakrishna & Viraraghavan, 2005).

In the light of these facts, sludge minimization techniques have come into focus as a means of sludge post treatment.

2.2 Sludge Minimization Techniques

There has been many biological minimization techniques explored to this effect. These can be categorized as: lysis-cryptic growth, uncoupling metabolism, maintenance metabolism and predation on bacteria (Wei et al., 2003).

2.2.1 Lysis-cryptic growth

Cell lysis technique is to mean the release of intracellular compounds of bio-solids leading to re-utilisation of the released material as substrate for the microbial metabolism; thereby causing an overall reduction in biomass amount. In 1971, Gaudy first demonstrated the lysis-cryptic growth process following sonication of sludge and it has since been established as a sludge reduction technique (Gaudy et al., 1971). Figure 2.1 is a schematic representation the effect of an external treatment on cryptic growth in sludge production.

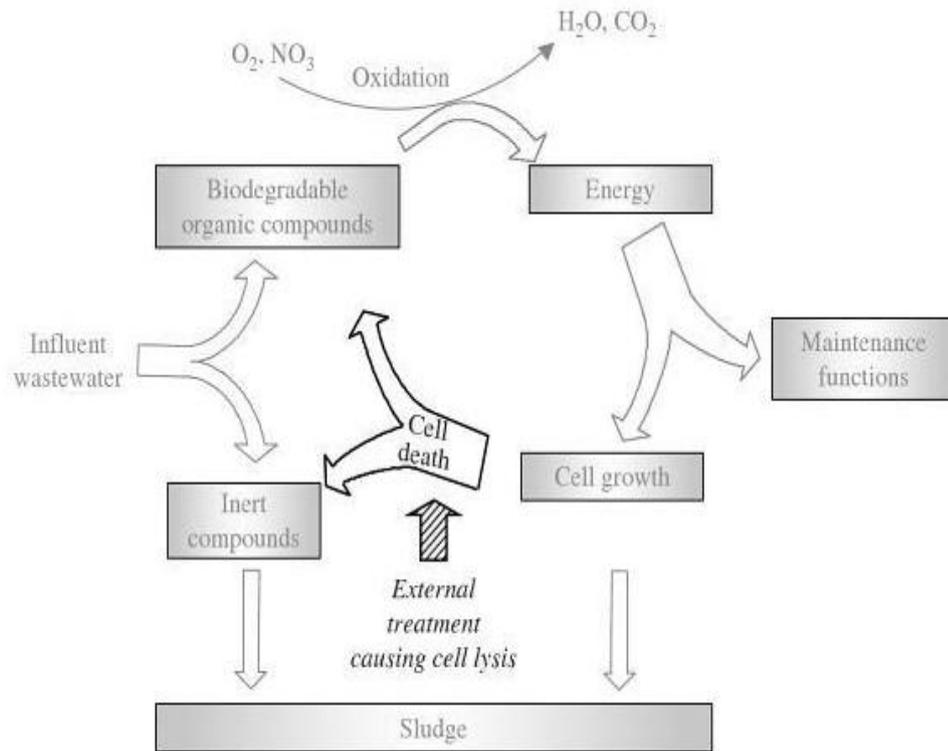


Figure 2.1 Effect of an external source on lysis-cryptic growth (Foladori et al., 2010)

Lysis-cryptic growth can be obtained by alternative methods such as :

Thermal/thermo-chemical treatment: High temperatures and combining high temperatures with acidic or alkali treatment potentiate cell lysates. NaOH was found to be an effective chemical in thermal alkali conditioning (Rocher et al., 1999).

Chlorination: Chlorine can be defined as a cheaper substitute for ozone to induce cell lysis. 65% of excess sludge reduction was achieved with an applied dose of 0.066 g Cl₂/g MLSS. The main issue with this method is the formation of THMs, which are known carcinogens, and its adverse effect on sludge settlability (Saby et al., 2002).

Ozonation: ozone is a powerful chemical to oxidize biomass and inducing cell lysis. Successful full scale applications, improvements in sludge settlability and zero excess sludge production - using proper dose- makes ozone an appropriate chemical for sludge minimization.

Other cell lysis strategies: high purity oxygen, enzymatic reactions, ultrasonic treatment (Pe´rez-Elvira et al., 2006).

2.3 Ozone

2.3.1 Chemical Structure of Ozone

Ozone, which is an allotrope of oxygen, is a very strong oxidizing agent, and is thirteen times more soluble than oxygen at standard temperature and pressure. It is an unstable structure in a watery solution and has a half-life about twenty minutes. Its reactivity is associated with the electron configuration. Figure 2.2 demonstrates the chemical structure of an ozone molecule.

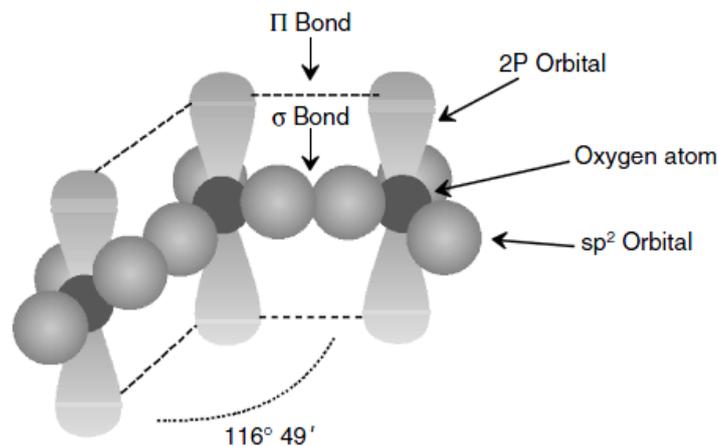
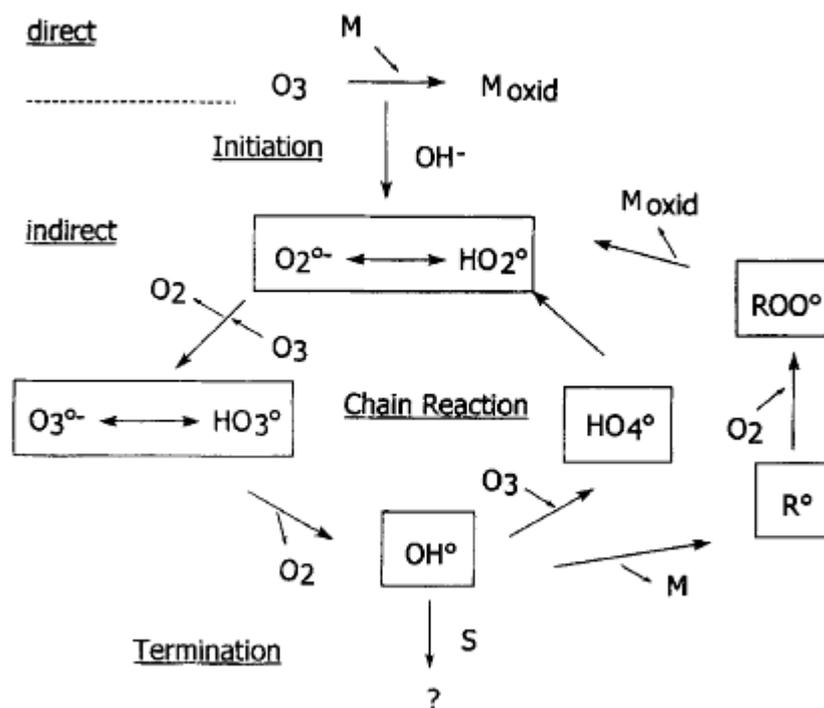


Figure 2.2 Chemical structure of an ozone molecule (Beltran, 2005)

Ozonation reactions proceed in two ways by direct and indirect manner which is shown in Figure 2.3; both leading to different oxidation products with different kinetics.



M: micropollutants; S: scavengers ; R: Reaction Products

Figure 2.3 Mechanism of the indirect and direct ozonation (Gottschalk et al., 2000)

2.3.2 Ozonation in Sludge Treatment

Ozonation has been highly used in both drinking water and wastewater treatment also in purification of ground and surface waters (Gottschalk et al., 2000). Scheminski (Scheminski et al., 2000) claimed that ozone destroys the cell walls of microorganisms which result in free cellular components in the sludge liquor. This destruction occurs in two steps, which are solubilization of cellular material and mineralization of soluble organic matter due to oxidation (Ahn et al., 2002). Among several disintegration methods, like thermal and mechanical treatment, ozonation provides the highest solubilization of organic matter (Müller, 2000).

Among many studies that use ozone as a disintegration method, ozone has been applied to different steps of the wastewater treatment process. These can be categorized as: application to the return activated sludge line (Fabiyyi et al., 2007; Yasui et al., 1996; Kamiya & Hirotsuji, 1998; Egemen, et al., 2001; Dytczak et al.,

2007), in a separate tank (Ahn et al., 2002), pretreating sludge to enhance anaerobic digestion (Scheminski et al., 2000; Weemaes et al., 2000), waste activated sludge (Park et al., 2003; Mines et al., 2008), biological reactor (Paul & Debellefontaine, 2007). Parameters that have been followed during application of ozone on sludge are those expressing sludge characteristics and its soluble products. These being sCOD, TOC, settlability, dewaterability, filterability, oxygen uptake rate (OUR), phosphorus release, pH, TSS, MLVSS, MLSS, effect on nitrification/denitrification processes and gas production in anaerobic processes. In the light of these studies, it is confirmed that ozone increases settlability of sludge and prevents bulking owing to destruction of filamentous organisms; enhances both solubilization and biodegradability, improves nitrogen removal and creates a precious carbon source for denitrification. Ozone stabilizes the sludge, augments dewaterability and reduces excess sludge. Moreover, it has a drastic effect on methane production in anaerobic digestion. However, filterability may deteriorate and a slight increase in effluent COD is observed. Another interesting point to note in the use of ozone in sludge treatment is that it enables phosphorus recovery which is a valuable product since phosphorus resources is being exhausted (Saktaywin et al., 2006).

The two common points of all these studies are high ozone amount usage and continuous ozone application. Table 2.1 summarizes the sludge reduction percentages with the corresponding ozone doses.

Table 2.1 Sludge reduction percentages with corresponding ozone doses given in literature

Article	Ozone dose (g O ₃ /g biomass)	Excess Sludge Reduction %	Application point
Yasui et al., 1996	0.05	100%	RAS
Kamiya&Hirotsuji, 1998	0.03	50%	RAS
Egemen et al., 2001	0.2 (g O ₃ / g SS per hour)	40-60%	RAS
Saktaywin et al., 2006	0.03-0.04	60%	RAS
Sievers et al., 2004	0.06	20-35%	RAS
Park et al.,2003	0.5	70%	WAS
Paul& Debellefontaine, 2007	0.07 (g O ₃ / g COD _{removed})	100%	Aeration basin

2.4 Endocrine Disrupting Compounds

In the last two decades, mainly after the release of the book "Our Stolen Future" in 1996 (Colborn et al., 1996), synthetic chemicals/natural hormones named, endocrine disrupting compounds, have grabbed the attention of the scientific and public parties (Lintelmann et al., 2003). It has gained even more importance in time, as their adverse effects on wildlife and humans have been proven by the increasing endocrine related illnesses in humans, alterations in the wildlife, fish and ecosystems and laboratory experiments that have been carried on animals (Damstra et al., 2002). Some of these alterations can be summarized as; sex changes, (Purdom et al., 1994) reduction in pheromone production which cause abnormalities in breeding of fish, sex reversal and deviance in egg shell formation in birds (Waring & Harris, 2005), genital track abnormalities, fertility defects (Degen & Bolt, 2000), susceptibility to cancer, tumor formation with leukemia

(Birnbaum & Fenton, 2003) and reduced immune function (Weisglas-Kuperus et al., 2004) in humans. These are generally attributed to xenoestrogens-chemicals with estrogenic activity.

As these emerging chemicals become more widely used in the community, their encounter has been more frequent in the environment. Although there are different views about how they smear with the environment, the common belief is that the main source of many EDCs is treatment plant discharges (Spring, 2004). It is not difficult to imagine how they contribute to municipal wastewater, having such a broad area of utilization. For example bathing, cleaning, laundry and disposal of unused pharmaceuticals and human wastes (EPA , 2009) end up in the water cycle. None of the current treatment processes are capable of removing these emerging contaminants and their metabolites from effluents (Petrovic et al., 2003). Most certainly there are many other contributors and contribution routines for these contaminants in the environment. Figure 2.4 indicates the extensive routes of contamination.

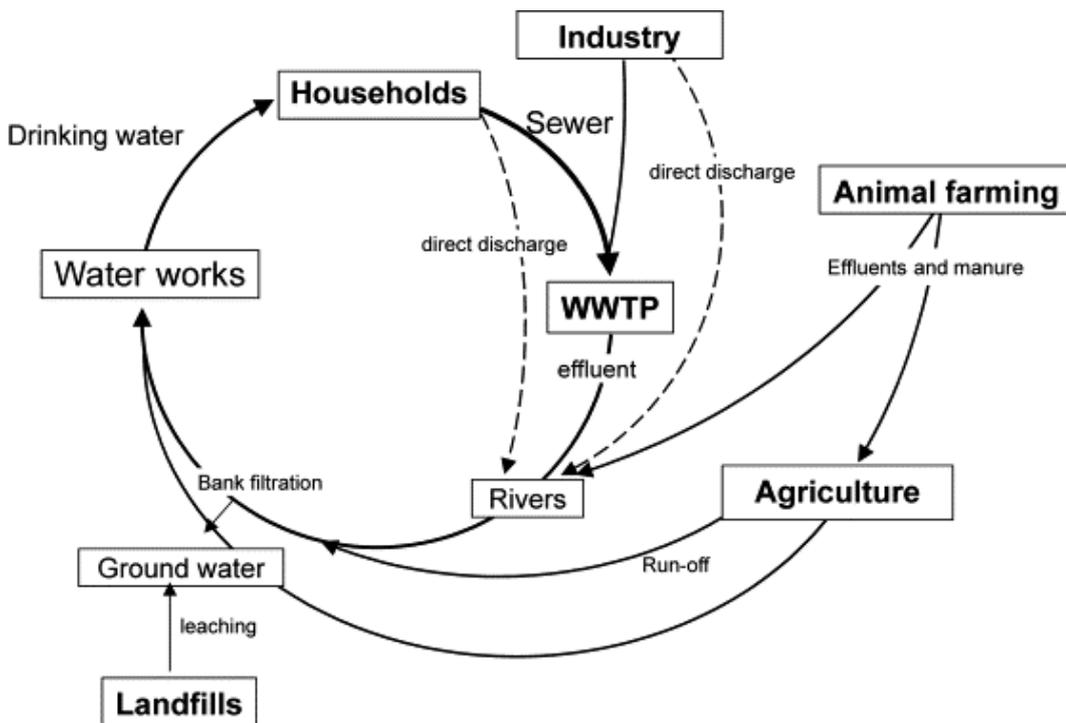


Figure 2.4 Components of a (partially) closed water cycle with indirect potable reuse (Kelvin, 2008).

2.4.1. Analytical Methods

Most widely used analysis techniques for EDCs include gas chromatography and liquid chromatography apart from biological assays. Up to now successful methods have been applied to measure these compounds by using GC/MS, GC/MS/MS, HPLC, LC/MS and LC/MS/MS in different aqueous matrices (Ternes T. A., 2001). In order to detect these contaminants in environmental samples whose concentrations are as low as ng/L levels, an appropriate instrument should be selected is suitable for the chemical and physical structure of the compound. Although there are a lot of successful applications of gas chromatographic analysis (Soliman et al., 2004) (Fromme et al., 2002) (Jiang et al., 2005), the time consuming derivatization step, which is used to reduce the polarity of the chemicals to achieve distinct chromatographic peaks (Liu et al., 2004), makes these methods difficult to apply.

It is known that the greater part of the EDCs is more polar than traditional contaminants and they are present in trace amounts in the environment. At this point, liquid chromatography, especially liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), serves the researchers working in this field. Most common ionization options in LC/MS are electrospray ionization (ESI), atmospheric chemical ionization (APCI) and atmospheric pressure photoionization (APPI) (Snyder et al., 2003).

Unlike GC/MS, in LC/MS systems, analytes reach to the ion source in liquid phase. The ions formed are carried through under vacuum and are analyzed in the mass spectrometer. The idea behind the selection of the ions is that, when a certain voltage is applied, only ions that have the specific m/z value pass through the quadrupole and reach the detector. In single quadrupole systems, origin of product ions cannot be differentiated; since all the ions formed from the source are transferred to the quadrupole whether fragmented or not. On the other hand, in triple quadrupole systems *selected reaction monitoring* mode (SRM) enables the user to monitor a specific precursor ion and a specific product ion simultaneously, and it is possible to run multiple SRMs together, which is called *MRM-multiple reaction monitoring* (Agilent, 2009). The availability of selecting two transitions (qualification and quantification) enables high degree of selectivity and effective

monitoring of trace level concentrations of EDCs in aqueous medium (Henriques et al., 2010).

Nevertheless, these low concentrations and the complexity of the matrices of the environmental samples require a pre-cleaning/concentration step (Picó et al., 2007). The methods that have been generally used can be classified as; liquid-liquid extraction, soxhlet extraction, solid-phase extraction (SPE) and solid-phase micro extraction (SPME) (Jeannot et al., 2002). Of these SPE system is the most extensively used technique. The wide diversity of available solid phases in packed cartridges, their selectivity, speed of use and high recovery percentages make SPE a preferable option (Alda & Barcelo, 2001) (Alda et al., 2003). Hernando et al., (2006) is one of many who proved the effectiveness of SPE prior to LC/MS/MS analysis with high recoveries and its suitability in monitoring trace amounts of emerging contaminants.

2.4.2 Compounds of Interest

a) Diltiazem, Carbamazepine and Acetaminophen

Diltiazem (Dtz), which is an antihypertensive drug, Carbamazepine (Cbz), an anticonvulsant and Acetaminophen (Atp), an analgesic and antipyretic, have all been detected widely in the aquatic environments. The results of a study where samples were collected from upstream, two points downstream and effluent of 10 different wastewater treatment plants can be seen from Table 2.2 and 2.3.

Table 2.2 Detection frequency of Dtz, Cbz and Atp in selected sampling locations of 10 different WWTPs.

Sampling points	Upstream	WWTP effluent	Downstream 1	Downstream 2
Compounds				
Diltiazem	22%	91%	80%	70%
Carbamazepine	33%	91%	100%	100%
Acetaminophen	44%	73%	40%	40%

Table 2.3 Average concentrations of Dtz, Cbz and Atp in selected sampling locations of 10 different WWTPs ($\mu\text{g/L}$) (Glassmeyer et al., 2005).

Sampling points	Upstream	WWTP effluent	Downstream 1	Downstream 2
Compounds				
Diltiazem	<RL	0.049	0.016	0.010
Carbamazepine	<RL	0.080	0.079	0.075
Acetaminophen	<RL	0.006	<RL	<RL

RL= reporting level

In another study, grab samples of influent, effluent and biosolids from three wastewater treatment plants were analyzed and maximum $12.8216 \mu\text{g/kg}$ dry mass diltiazem was observed in biosolids. This value was $12.8581 \mu\text{g/kg}$ dry mass for carbamazepine (Spongberg & Witter, 2008). Ding et al., (2011) observed $88.6\text{--}370.4 \mu\text{g/kg}$ acetaminophen in biosolids of three WWTP. Moreover, the LD50 concentrations of these compounds can be seen from table 2.4.

Table 2.4 LD 50 concentrations of Atp, Cbz and Dtz in mice

	ATP	CBZ	DTZ
LD50	$340 \text{ mg/kg}^{\text{b}}$	$212.02 \text{ mg/kg}^{\text{a}}$	$508 \text{ mg/kg}^{\text{c}}$

^a(Samini et al., 1997), ^b (Nelson et al., 1980) ^cMSDS of diltiazem

Scheytt et al., (2005) showed that carbamazepine is a hydrophobic compound with and its main sorption mechanism is hydrophobic sorption. The log

Kow values show that Cbz and Dtz have relatively higher hydrophobic natures than Atp which means that they tend to sorbe onto the sewage sludge in wastewater treatment plants. Table 2.5 indicates the physicochemical properties of these compounds.

Table 2.5 Physicochemical properties of Atp, Cbz and Dtz (Kim et al., 2007)

	ATP	CBZ	DTZ
CAS No.	103-90-2	298-46-4	42399-41-7
Molecular weight(g/mol)	151.2	236.3	414.5
Formula	C ₈ H ₉ NO ₂	C ₁₅ H ₁₂ N ₂ C	C ₂₂ H ₂₆ N ₂ O ₄ S
pKa	9.38	14.00*	8.90
Log K _{ow}	0.46	2.45	2.79

*pKa value of Carbamazepine is taken from (Scheytt et al., 2005)

Ding et al., (2011) successfully revealed that hydrophobic chemicals cannot be removed completely in treatment processes and tend to accumulate in sludge. Consequently, land application of this contaminated sludge lead to a toxicity in soil and is transferred by ecological chain.

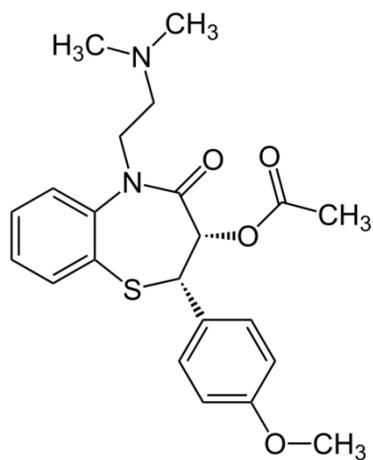


Figure 2.5 Chemical structure of Diltiazem

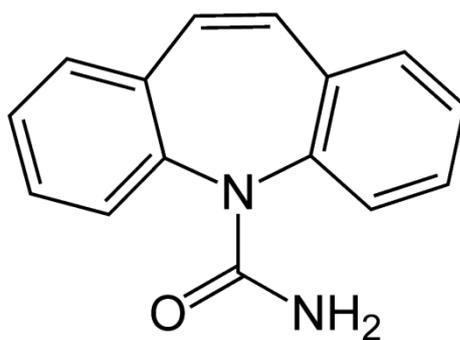


Figure 2.6 Chemical structure of Carbamazepine

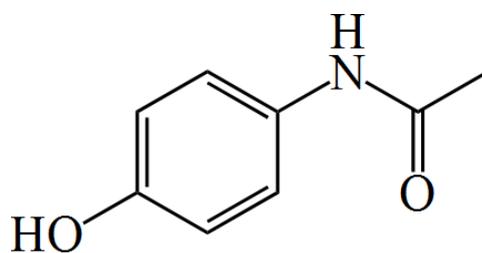


Figure 2.7 Chemical structure of Acetaminophen

b)Butyl Benzyl Phthalate(BBP)

BBP is a widely used chemical and is mainly used as plasticizer in polyvinyl chloride flooring, in paints, coatings (Long & Meek, 2001) and also as solvent and fixative in perfumes. According to National Library of Medicine, USA, 170 000 kg was released into the air, 620 kg discharged into water and 1200 kg was disposed to land in 1993 (IARC, 1999). Its estrogenic activity classified it as an endocrine disrupter in the last decades. In a study where rats in utero were exposed to 10-1000 µg/L BBP by adding the chemical into the drinking water of the pregnant rats, culminated in a distinct decrease in testis size and sperm production in the male offsprings (Tyler et al., 1998).

Table 2.6 Physicochemical properties of Butyl Benzyl Phthalate (Gledhill et al., 1980)

Vapor pressure,20°C	8.6 x 10 ⁻⁶ mmHg
Vapor pressure,200°C	1.9 mmHg
Aqueous solubility, deionized water	2.9 ±1.2 mg/L
Octanol/water partition coefficient	5.9± 4.3 x 10 ⁴
Calculated bioconcentration factor	510
Soil adsorption coefficient (measured,20°C)	68-350

Solubility of BBP in water is relatively low compared to its adsorption capacity onto solids, as can be seen from octanol water partition coefficient in Table 2.6. In other words, its tendency is to adsorb onto sediments, sludge and biosolids present in the environment. In a study conducted by Roslev et al., (2007) samples taken from Aalborg East WWTP showed mean BBP concentrations of 37.87 µg/L in influent, 3.13 µg/L in effluent and 3.41 mg/kg dw in dewatered sludge , which corresponds to 90.2 kg/day % degradation. Also in the study by Gledhill et al., (1980) concentrations of 8.0, 1.3 and 1.0 µg/L BBP in influent, effluent and

aeration tank, respectively, were observed in a local domestic activated sludge treatment plant.

Main removal mechanism of BBP is reported as biodegradation in activated sludge (93-99%) (Gledhill et al., 1980) and the pathway can be summarized as the addition of hydrolysis products which are benzyl alcohol and butanol leading to the TCA cycle (Chatterjee & Karlovsky, 2010).

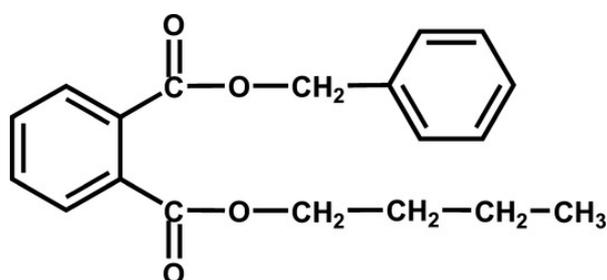


Figure 2.8 Chemical structure of Butyl Benzyl Phthalate

c) Estrone, Progesterone

Estrogens that are found in wastewaters also pose a threat due to their endocrine disrupting property. Estrone and progesterone are two common natural hormones that are present in wastewaters. Their removal percentages differ from type and operational conditions of wastewater treatment plants. However it is a known fact that they cannot be completely removed in conventional treatment systems (Pholchan et al., 2008).

Moreover, manure and sewage sludge are serious sources of estrone and progesterone on the agricultural land. By this way, they can contribute to groundwater by leaching or to surface waters by run-offs. In addition to these sources another increasing trend for estrogen and progestogen contamination is fish farming since hormone containing feed additives are discharged straight into the water (Kuster et al., 2004).

Estrone is excreted on an average of 10.5 $\mu\text{g/day}$ per capita which is the main source of estrone in sewage treatment works. The $\log K_{ow}$ for Estrone is 3.4, a relatively high value. Thus, sorption to solid particles is an important aspect of estrone's behavior in treatment plants (Braga et al., 2005). The case is the same for progesterone as well. The K_d value for progesterone was found as 204 and $\log K_{ow}$ was found 3.87 which indicates its tendency to accumulate on soil phases (Kuster et al., 2005).

Occurrence of estrone and progesterone in wastewater treatment plants is very frequent. In a study conducted by Liu et al., (2011) samples taken from two different BNR plants showed that the concentrations in the influents were 6.1 and 5.4 ng/L for progesterone and 40.6 and 21.7 ng/L estrone. Progesterone could not be detected in the effluents of both plants; whereas estrone concentrations were 8.5 and 3.1 ng/L in the effluents. The data shows that these compounds tend to sorb onto sludge, since progesterone concentration for the dewatered sludges of the two plants' were 24.6 and 6.0 ng/g and estrone were 4.8 and 5.4 ng/g.

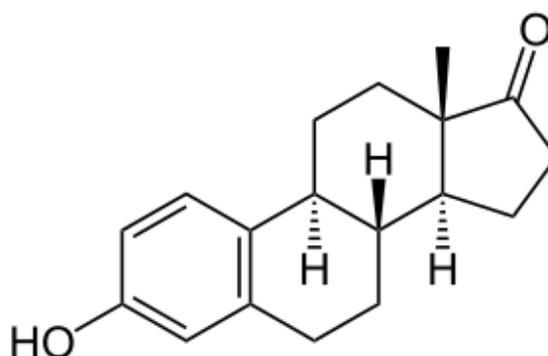


Figure 2.9 Chemical structure of Estrone

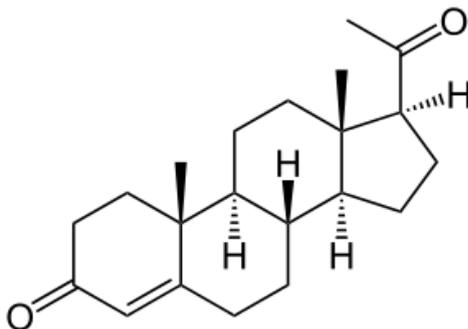


Figure 2.10 Chemical structure of Progesteron

2.4.3 Removal of EDC's From Wastewater and Sludge

Aforementioned studies have shown that conventional treatment systems do not achieve complete removal of most of the EDCs. Although a considerable amount stays in the aqueous phase, most are adsorbed onto sludge. EDC removal techniques studied can be summarized as;

-Physical Treatment: Sedimentation and mechanical separation techniques have been studied to remove EDCs from aqueous phase since these compounds tend to accumulate on sewage sludge (Auriol et al., 2006). Membrane bioreactor systems (Hu et al., 2007), nanofiltration and ultrafiltration membranes (Yoon et al., 2007) have proved successful in separating these trace contaminants into the concentrate by size exclusion or adsorption mechanisms. However, a further treatment is needed to eliminate these substances from the concentrate.

-Biological Treatment: Aerobic and anaerobic degradation processes are noted for the removal of EDCs biologically. Ternes et al., (1999) observed that activated sludge treatment is more efficient than trickling filters in removing these compounds from the waste stream since 64% removal for 17 α -ethinylestradiol was achieved in the effluent of a trickling filter whereas removal was 99.9% in the effluent of an activated sludge. In another study by Andersen et al., (2003) it has been shown that natural estrogens were largely degraded in the denitrifying and aerated nitrifying tanks. High removal efficiencies were achieved for natural estrogens in anaerobic digestion but no elimination was observed for Carbamazepine (Carballa et al., 2007).

-Advanced Treatment: Advanced treatment methods can be classified as chlorination, manganese oxide treatment, photolysis reactions and advanced oxidation methods which involve ozonation (Auriol et al., 2006). Between these methods, ozonation and its combination with peroxides gave the best results. Snyder et.al succeeded in removing 22 compounds; including carbamazepine, estrone and acetaminophen, to below detection level in wastewater using 2.5 mg/L O₃. 95% progesterone removal was observed with a higher ozone dose. The H₂O₂ addition increased removal percentages by 5-10% (Snyder et al., 2006). In another multifaceted study, an ozonation unit was installed to a BNR plant in order to achieve excess sludge reduction, phosphorus recovery and edc treatment at the same time. With an ozone dose of 40–50 mgO₃/g SS 90% sludge reduction was accomplished and E2 (estradiol) concentration was lowered to below detection limit (Tsuno et al., 2008).

2.5 Aim of the Study

Until this day studies were carried out in situ side-stream continuous ozonation; which, in our opinion has important drawbacks. Firstly, ozone, which is an expensive chemical to generate, should not be over used for the sake of economy. Secondly, its use in the aeration tank and its peripheries leads to reduced active biomass amount in the aeration tank. This in turn will require larger tank volumes to achieve the same degree of treatment. Thirdly, it will cause longer SRT which may impair flocculation of sludge. Long SRT will cause a higher proportion of non-active biomass in the tank and lower reaction rate. Hence even larger tank volumes will be required. Sievers et al., (2004) frankly disprove the idealization of zero excess sludge production conducted by Yasui et al., (1996) and their subsequent studies. In order to achieve zero excess sludge, the volume of the basin had to be increased twice the size to compensate SRT; which is not comparable with the SRT of a conventional process. Therefore, it is deemed necessary that ozone application on sludge be optimized by partial or pulse ozonation over that which is continuous. Moreover ozone should be administered on sludge in a separate compartment, such as a digester, for optimum effect. This thesis aims at investigating feasibility of the latter application.

As can be seen from the excessive number of studies already reviewed, EDCs tend to accumulate in sludge due to their hydrophobicity. However these studies have generally concentrated on removal of EDCs from wastewater or drinking water but not sludge. Sewage sludge that contains a concentrated amount of micropollutants is also a threat for the environment. Unless sludge is freed from these pollutants, treatment cycle is not complete. The present thesis also aims at reducing/removal of these contaminants in sludge.

In conclusion, the aim of this study was set to achieve excess sludge decrement in biological treatment at a reasonable ozone dose and to remove EDCs at the same time. The side goals of the thesis were to develop appropriate analysis methods for the detection of the EDCs of interest, by using LC(ESI)/MS/MS, at trace quantities.

CHAPTER 3

MATERIALS AND METHODS

3.1 Reagents and Chemicals

The chemicals used in this study were of analytical grade. The compounds selected, estrone (>99%), diltiazem (>99%), progesterone (>99%) were purchased from Sigma, Benzyl Butyl Phthalate (BBP) (>98%) was obtained from Aldrich and carbamazepine (>99%) and acetaminophen (>99%) were from Sigma-Aldrich. LC-MS grade methanol, toluene and acetone were obtained from Merck (Darmstadt, Germany). Formic acid and ammonia used in mobile phase preparation were purchased from Merck. Oasis HLB extraction cartridges used for pre-cleaning and pre-concentration were purchased from Waters (Milford, MA, USA). Glass-fiber prefilters (0.7 μm pore size, 47 mm diameter) were obtained from PAL Life Sciences (Mexico). Sulfuric acid used for the pH adjustment was obtained from Merck.

Ultrapure de-ionized water was obtained from Milli-Q water purification system (Millipore, USA). Ultrapure de-ionized water was used in all dilutions and sample preparations.

In order to minimize adsorption of EDCs on glass wall, all glassware was coated with silane due to the high hydrophobicity of EDCs. The coating procedure of glassware was performed according the study by Yu et al., (2007). In this procedure, all glassware were rinsed with dichloromethylsilane (DCMS) prepared in toluene 10% (v/v), and then rinsing three times with toluene followed by three times with acetone. Glassware was then heated to 150 °C for 12 h to fix the silylation reagent onto the glass wall.

3.2 Instrumental Analysis with LC/ESI/MS/MS

In trace organics analysis an Agilent 6410A type LC-ES-MS/MS instrument equipped with Electrospray Ionization (ESI), quadropole MS detector, autosampler, degasser and binary pump was used. In order to obtain high sensitivity, all of the ES-MS/MS parameters were optimized using the instrument control software program. In the separation of EDCs from each other, Agilent 1200 brand HPLC system was used. Nitrogen gas was used as the collision gas. Agilent, Zorbax, SB-C8 (100 x 2.1 mm x 3.5 μ m) was used as reverse phase column. Mass spectrometer was operated in positive mode by multiple reaction monitoring (MRM). Gradient elution was used to get sufficient separation of analytes. Table 3.1 shows the operational parameters of LC/MS/MS.

Other parameters used in the ESI-MS/MS measurements were: nebulizer pressure 50 psi; emv 400 V; drying gas (N_2) temperature and volume 350 $^{\circ}C$, 11.0 L/min respectively; injection volume 20 μ L; flow rate 0.5 mL/min and draw speed 200 μ L/min.

3.2.1 Reference Standard Preparation and Calibration

All working standards were daily prepared by using 1000 mg/L stock solutions which were prepared in methanol and stored at 4 $^{\circ}C$. Standard solutions were prepared in 25% methanol/water v/v in 10 ml volumetric flasks and used to calibrate the response of LC/MS/MS with respect to the analyte concentration. Calibration curves were drawn by using at least 4 points and always a new calibration set was prepared together with every set of sample analyzed.

3.3 Extraction Procedure

3.3.1 Ultrasound Aided Sequential Extraction

A new extraction procedure was developed for the analysis of sludge samples. A 0.5 g 105 $^{\circ}C$ -dried and homogenized sludge sample was placed into 100 mL Erlenmeyer flask and 100 mL of methanol (MERCK- LC Grade) was added on top. Flasks were then placed into a FALC Ultrasonic Bath (50 KHz, 160 W) for 30 mins.

Table 3.1 Operational parameters for the instrument

Parameter	HPLC
Mobile Phase Program	i) 0-0.3 min 90% of 0.1% Formic Acid + 5.0 mM Ammonium Format in ultra pure H ₂ O (Mobile Phase A) 10% of 0.1% Formic Acid + 5.0 mM Ammonium Format in CH ₃ OH (Mobile Phase B)
	ii) 0.3-1.0 min 90-5.0% of Mobile Phase A 10-95% of Mobile Phase B
	iii) 1-5 min 5% of Mobile Phase A 95% of Mobile Phase B
	iv) 5-5.1 min 5-90% of Mobile Phase A 95-10% of Mobile Phase B
	v) 5.1-10 min 90% of Mobile Phase A 10% of Mobile Phase B
Flow Rate, mL/min	0.5
Loop Volume, μ L	20.0

At the end of 30 mins, samples were centrifuged at 3400 rpm for 10 min in order to collect the aliquots. This procedure is repeated three times and 300 mL of extraction solution was obtained at the end. The 300 mL aliquot obtained was evaporated to dryness by heating, thus leaving the EDCs on the glassware. Then glassware was washed with 3.0 mL 25% methanol-ultra de-ionized water (v/v) mixture.

3.3.2 Solid Phase Extraction

Following ozonation nearly all the solid particles were destroyed so solid phase extraction was carried out on the remaining aqueous part. Samples were filtered through ordinary filter paper and then through glass fiber filter with pore size of 0.7 μm . Filtered samples were then applied onto the SPE cartridges. The Oasis HLB SPE cartridges were pre-conditioned by passing 10 mL methanol and 10 mL ultra-distilled water through. Filtered samples were then passed through the SPE column cartridges at a flow rate of 10 mL/min under vacuum. This was followed by a drying process under vacuum for 15 min by air. As a result, EDCs were sorbed by the cartridges. Then, sorbed EDCs were eluted using 25 mL methanol. Eluates were then dried under a gentle stream of nitrogen gas until complete evaporation was achieved. In order to match the matrices of both the samples and the calibration standards, compounds were taken into 1.0 mL of methanol/ultra-distilled water mixture (25% methanol, v/v).

3.4 Selected Sewage Treatment Works

a) METU VRM Wastewater Treatment Plant

METU owns a membrane bioreactor plant to treat wastewaters from part of the campus and it is operated by the METU Environmental Engineering Department. The daily capacity is about 150 m^3 . Effluent from the Vacuum Rotating Membrane system is used for irrigation by the METU Technopolis administration

b)Tatlar (Ankara) Wastewater Treatment Plant

This is a conventional activated sludge plant capable of treating around 971 000 m³ wastewater daily. The plant does not contain nutrient removal facilities and operates with a very short sludge age. Sludge samples were taken from the return activated sludge (RAS) line

c) Kayseri Wastewater Treatment Plant

Kayseri WWTP is a Biological Nutrient Removing (BNR) plant with a capacity of 110.000 m³/day. Samples were taken from the aeration tank of the plant.

d) Konacık (Bodrum) Wastewater Treatment Plant

Konacık plant is a static, flat sheet membrane treatment plant. The daily capacity of the plant is 1200 m³; operating at a high SRT. Samples from this plant were taken from the RAS line. Other properties of the plants were given in Table 3.2

Table 3.2 Characteristics of WWTPs within the scope of this study

Characteristics	Tatlar WWTP	Kayseri WWTP	Konacık WWTP	METU WWTP	VRM
Type	Conventional activated sludge plant	Biological Nutrient Removal (BNR)	Static membrane plant	Vacuum membrane	rotating plant
SRT (days)	2-4	20-25	40-50	10	
Sustainable flow handled m³/day	971.000	110.000	1200	200	

3.5 Pulsed Ozone Treatment Process

3.5.1 Ozone Generator

Ozone for the experiments was supplied from an OSC-Modular 4HC, WEDECO ITT INDUSTRIES (2007) ozone generator, by sparging through the aqueous liquid. Operating pressure was 5 bars and gas flow rate was adjustable between 10-140 L/h with a rated capacity of 4 g/h. The ozone generator generates up to 300 L/h oxygen with a purity of 90-95%.

3.5.2 Ozone Dose Optimization

The amount of ozone imparted into the liquid was determined by measuring ozone concentration in the liquid spectrophotometrically according to the Standard Method 8021 (DPD chlorine reagent) (APHA, 1998) and consulting a calibration curve. The amount of ozone imparted into the ultra pure water by using the ozone generator was linearly proportional with the duration of ozonation, as shown in Figure 3.1. The medium temperature was 27.2°C and the ultra pure water temperature was 23.3 °C. Flow rate is 30L/h(0.5 bar, 20 °C). As can be seen from this figure, 0.122 mg O₃/ L-min was imparted. After 15 minutes of ozone application, the ozone- in-water curve levels off indicating that ozone saturation in the water is reached.

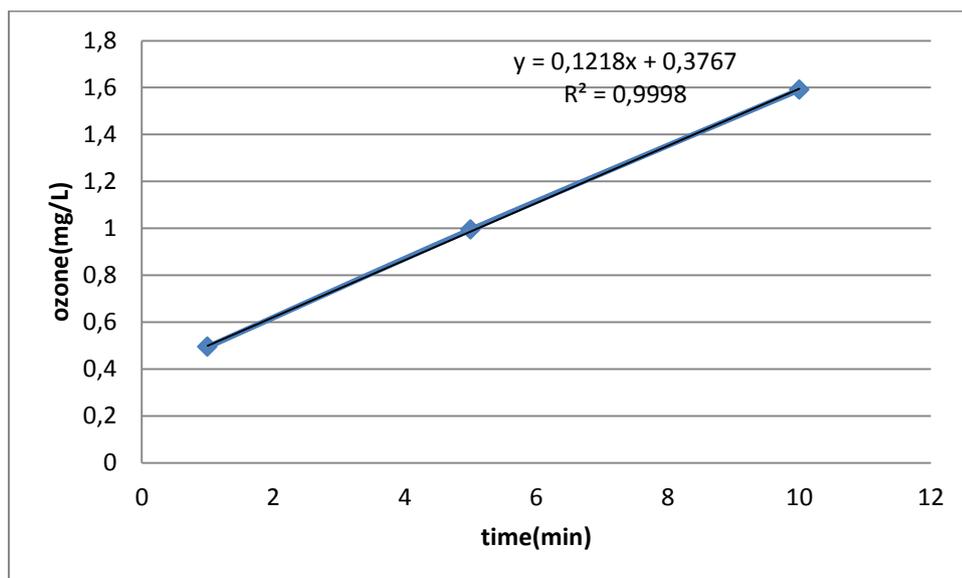


Figure 3.1 Ozonation periods versus ozone imparted into water

3.5.3 Ozone Experiment Procedure

Two sets of experiments were conducted to optimize the ozone dosage for sludge minimization. In the first set of experiments, in order to prevent COD interference from the medium, sludge was washed twice with a buffer solution at pH=7 (0.013M KH₂PO₄/K₂HPO₄); aliquots were discarded and pellets remaining in the centrifuge bottles were collected and brought up to 300 mL with buffer and again supernatants were discarded. This procedure was applied to both control and parallel groups. Therefore, any soluble COD measured in the flask supernatants should be originating from the biomass in the medium. In the second set experiments, washing of sludge samples with phosphate buffer was discontinued.

During 4 days of experimentation, samples were ozonated for set periods on each day at the same hour of the day and chemical analysis (COD, MLSS,MLVSS) were carried out routinely before and after ozonation. After each ozonation, flasks were incubated for 24 hours at 25°C in an orbital shaker at 75 rev/min.

3.5.4 Analysis

a) Chemical Oxygen Demand

The soluble COD release into the supernatants during the experiments was measured by using high range (150-1500 mg/L COD) and low range (15-150 mg/L COD) Hach Lange kits according to HACH 8000 (U.S. EPA approved) method before and after ozonation every day.

b) Mixed Liquor Suspended Solids-Mixed Liquor Volatile Suspended Solids

The MLSS measurements were carried out according to the Method 2540B. MLVSS was measured according to Method 2540, solids method. Measurements were conducted before and after ozonation.

c) Total Coliform

Total coliform count before and after the ozonation period were carried out according to the Method 9132-Membrane Filter Method. Sample is filtered under vacuum and filter (0.45 µm pore size) which retains the bacteria found in the sample was placed to an M-Endo agar.

d) Total Phosphorus and Ortho-Phosphate

Total-P (TP) was analyzed by using Method 365.4; ortho-phosphate (OP) by Method 365.3 (EPA). The LCK350 Hach kits were also used for TP and OP analysis (unit: PO₄-P). Ortho-phosphate experiments were performed by using the substrate and total phosphorus by using the whole well-mixed sample.

e) Capillary Suction Time

De-waterability of sludge samples before and after ozonation was measured by using Geneq Model 304M CST unit. CST values were calculated according to the period of the sample to reach from one electrode to the other one in the instrument.

f) Sludge Volume Index-SVI

The SVI of sludge was measured to have an idea about the settlability of sludge. The method was applied before ozonation at the first day and after ozonation at the last day. Sludge sample was put into a 1 liter measuring cylindrical and volume of settled sludge was measured after 30 mins where SVI unit is ml/g and V₃₀ unit is ml.

$$SVI = \frac{V_{30} * 1000}{MLSS}$$

g) Oxygen Uptake Rate-OUR

OUR experiments were carried out by using YSI model 51B dissolved oxygen meter and 5700 series oxygen probe (Ohio, USA). Display of the D.O meter unit was in mg/L oxygen. Readings were commenced after calibrating the instrument.

f) pH

The pH was measured using a HQ40d Portable pH meter (Hach, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 EDC Analysis

4.1.1 Optimization of MS/MS Parameters

a) Mobile Phase Optimization

Mass spectrometry is based on the analysis of ions moving through a vacuum. In order to obtain distinct results, an optimization step is obligatory. The parameters to optimize can be listed as; mobile phase, flow rate and target compound parameters aside from column optimization which is not in the content of this optimization process.

Prior to conducting instrument optimization for selected compounds, a mobile phase optimization is necessary. Table 4.1 shows the three different sets of mobile phases prepared and used for the analysis of compounds of interest. A gradient separation was achieved by mixing two different mobile phases; one prepared with ultra-pure H₂O and the other with methanol.

Table 4.1 Mobile phase sets prepared for optimization

Set	Mobile Phase-A	Mobile Phase-B
1	Ultra pure H ₂ O+ %0,1 F.A.	Methanol+ %0,1 F.A.
2	Ultra pure H ₂ O + 26 mM A.F.	Methanol+ 26 mM A.F.
3	Ultra pure H ₂ O + %0,1 F.A. + 5 mM A.F.	Methanol+ %0,1 F.A. + 5 mM A.F.

F.A.= Formic Acid, A.F.= Ammonium Formate

The 10 ppm standard solutions were prepared for each compound from stock solutions. The standard solutions were analyzed with each set of mobile phase. Initial parameters used for the series of analysis are given in Table 4.2.

Table 4.2 Initial Parameters used prior to optimization of the compounds

Parameter	Value
Flow rate	0.4 ml/min
Mobile Phase A	%95
Mobile Phase B	%5
Nebulizer Pressure	50 psi
emv	400 V

The MS2 Scan Mode chromatograms of carbamazepine and progesterone with each set of mobile phases are given as an example in Appendix A (A-1 to A-6).

As can be seen from the chromatograms, the TIC (Total Ion Chromatogram) results for different mobile sets do not show a significant variation. Hence in order to obtain the best result, optimization of the compounds was carried out with each set of mobile phases one at a time.

b) Optimization of Parameters for the Target Compounds

Compounds of interest were scanned both in positive and negative modes and it was found that all gave better peak shapes (sharp peaks without tailing) and higher peak area values at the positive mode. Sample chromatograms for diltiazem and acetaminophen are appended in Appendix A (A-7 to A-10). Following the decision on polarity and identifying precursor ions for every compound, fragmentor voltages were varied between 70-150 Volts in MS2 SIM mode using different mobile phase sets. Results of fragmentor voltage optimization for carbamazepine

and progesterone are shown in Appendix A (A-11 to A-16). After deciding on optimized fragmentor voltage, product ions were observed with scan type “Product Ion” (A-17 to A-22). Finally collision energy optimizations were completed in MRM mode for both of the product ions and thus quantifier and qualifier ions were set. Progesterone is given as an example in the appendix (A-23 to A-28). In the light of these results, it was decided to continue with Set 3 (*0,1 F.A. + 5 mM A.F.*). Figure A-29 shows chromatograms of the selected compounds with the selected mobile phase. The optimization summary for each compound is given in Table 4.3.

Table 4.3 Optimization Parameters for Target Compounds

Compound	MW	Q1 (precursor)	Q2 (Quantifier)	Q3 (Qualifier)	FV	CE	Polarity
Diltiazem	414.5	415	177.9	309.9	130	24-30	Positive
Progesterone	314.5	309	109	97	120	30-23	Positive
BBP	312.4	313	91	148.9	70	20-9	Positive
Estrone	270.4	271	253	159	110	9-20	Positive
Cbz	236.3	237	194	192	120	18-22	Positive
Atp	151.2	152	110	93.1	90	14-22	Positive

4.1.2 Calibration Curves

After completing optimization, standard solutions were prepared from the stock solutions. The concentrations of the standards were prepared as 100, 50, 25, 10, 5, 1, 0.5, 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 ppb (in 25% MeOH/H₂O v/v) prepared with ultra-pure water and the calibration curves were drawn using the instrument software. Sample calibration curves for the analytes are given in the appendix A, Figure A-30 to A-35.

4.1.3 Wastewater Extraction Optimization

a) pH optimization

It is known that Oasis HLB cartridges can perform equally well at a pH range of 1-14; an optimization study was conducted to optimize the pH of the samples and to calculate the recoveries. Aforementioned extraction procedure was applied to the samples with pHs adjusted to 2, 2.5, 3, 3.5, 4, 4.5, 5, 7, 8 and 9 spiked with a mixture of standards containing 50 ng mL⁻¹ of the analytes. The peak areas of the analytes before and after passing through the cartridges were compared and were used to calculate the recoveries. As can be seen from the recoveries in Table 4.4, pH 7 stood out as the optimum for all the analytes. Chromatograms at pH 7 are provided in Appendix A (A-36 to A-41).

In order to assess recovery of the extraction method conclusively, an experiment was carried out. A 20 ppb of mix standard was spiked to 1 L of wastewater that was pre-filtered from glass fiber filters and arranged to pH 7. The filtered sample was split into two. One part was directly analyzed by LC/MS/MS. The other part was passed through the SPE cartridge. A 1 ml sample was taken into a vial from the aliquote left under the cartridge and analyzed in LC/MS/MS. The extraction method was continued. At the end of the procedure, the analytes were concentrated in a final volume of 1 ml which then added to the initial 499 ml. A total volume of 500 ml was obtained and analyzed directly with LC/MS/MS. The peak areas of the chromatograms were compared and no significant difference was observed with the pH optimization recoveries.

Table 4.4 Percent (%) recoveries obtained during pH optimization (n = 3)

pH	Diltiazem	Progesterone	BBP	Estrone	Cbz	Atp
2	68,46±0,06	99,82±0,04	90,35±1,78	50,32±0,04	87,85±0,12	-5,52± 20,1
2.5	71,66±0,06	99,02±0,02	86,92±1,71	51,19±0,03	83,59±0,11	-14,91±17,55
3	84,02±0,08	99,83±0,04	87,70±1,73	74,61±0,04	52,73±0,07	-0,44±1,67
3.5	36,32±0,03	99,84±0,04	87,65±1,73	72,51±0,02	96,10±0,13	2,67±1,4
4	71,69±0,01	99,80±0,07	85,30±1,68	85,06±0,02	93,88±0,13	3,68±1,25
4.5	99,77±0,09	99,85±0,01	88,30±1,74	81,82±0,02	97,81±0,13	1,94±1,27
5	99,68±0,09	99,89±0,02	90,38±1,78	100,00±0,02	57,37±0,08	8,02±0,78
7	99,56±0,09	99,83±0,02	92,29±1,82	100,33±0,02	99,62±0,13	30,11±3,87
8	99,25±0,09	98,71±0,19	86,94±1,71	87,90±0,82	99,21±0,13	-0,94±3,43
9	99,2±00,09	99,54±0,02	81,15±1,60	99,57±0,02	99,36±0,13	45,76±5,26

b) Flow rate optimization

Different flow rates through the LC column (0.1, 0.2, 0.3, 0.4, 0.5 ml/min) were tested using Zorbax C-8 column and higher and sharper peaks were observed with a flow rate of 0.5 ml/min. Related chromatograms are given in Appendix A (Figures A-42 to A-46)

c) Analytical figures of merit

Limit of detection LOD, is the lowest amount of analyte in a sample that can be detected but cannot be quantified safely. On the other hand, limit of

quantification LOQ, is the lowest analyte concentrate that can be quantified accurately. A typical LOD and LOQ calculation for LC/MS/MS instrument is given below in Figure 4.1.

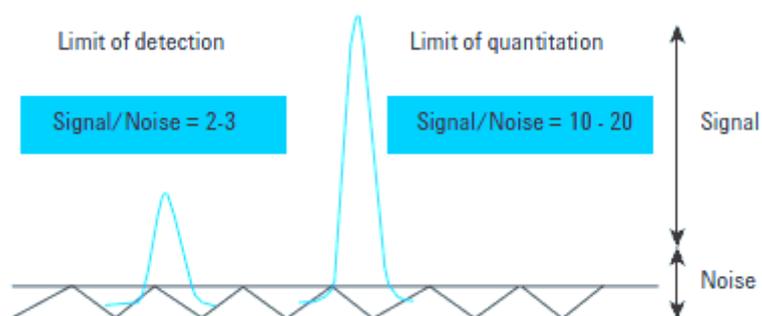


Figure 4.1 Description of LOD and LOQ via signal to noise ratio (Huber, 2010)

A 0.25 $\mu\text{g/L}$ mixed standard was prepared and analyzed ten times. The following formulae were used to calculate LOD and LOQ values using the peak areas.

LOD= $3 \times$ Standard deviation of the 0.25 $\mu\text{g/L}$ mixed standard solution/slope of calibration plot

LOQ= $10 \times$ Standard deviation of 0.25 $\mu\text{g/L}$ mixed standard solution/slope of calibration plot

Table 4.5 summarizes the analytical figures of merit for the selected compounds.

Table 4.5 Analytical Figures of Merit

Analytes	Linear Range µg/L	R²	LOD, µg/L	LOQ, µg/L
Diltiazem	0.25 – 50.0	0.9999	0.13	0.43
Progesterone	0.25 - 20.0	0.9997	0.12	0.40
BBP	0.10 – 20.0	0.9982	0.04	0.13
Estrone	0.25 – 100.0	0.9998	0.13	0.43
Cbz	0.25 - 20.0	0.9998	0.12	0.40
Atp	0.10 – 50.0	0.9997	0.05	0.17

4.1.4 Optimization of Sludge Extraction Procedure

a) Recovery Studies

A 0.5 g dried sludge sample with known EDC content was spiked by adding 1 ml of a standard solution having 20 ng/ml of each analyte. The sample was dried at 105 °C, thus leaving EDCs on the sludge sample. Then, sludge extraction method was applied and the peak areas of the spike solution and the sample were compared. The peak areas were used to calculate the recovery of each analyte. Chromatograms that were used to calculate the recoveries are provided in Appendix A (A-47 to A-51). Table 4.6 shows the recovery percentages of the selected compounds of sludge extraction method. These results were obtained from one set of experiment. Two other sets were conducted with two different sludge samples and the results were found similar.

Table 4.6 Extraction efficiencies of the analytes of interest from dried sludge.

Compounds	Recovery (%)
Diltiazem	96.2 ± 0.3
Progesteron	97.5 ± 1.1
BBP	93.0 ± 0.3
Estrone	97.2 ± 0.6
Carbamezapine	95.3 ± 0.4
Acetaminophen	95.8 ± 0.3

The sequential sludge extraction method consists of three steps; in other words the same procedure was repeated three times and at each time a 100 ml of aliquot was collected to total up to 300 ml final volume. In order to judge effectiveness of the extraction procedure, a 1 ml sample was taken after each step and analyzed. It became clear from Figure 4.2, that most of the analytes were extracted in the first step while much less were obtained in the latter two steps. In the light of this information it was decided to continue with the three step procedure to collect all the analytes present in the sample.

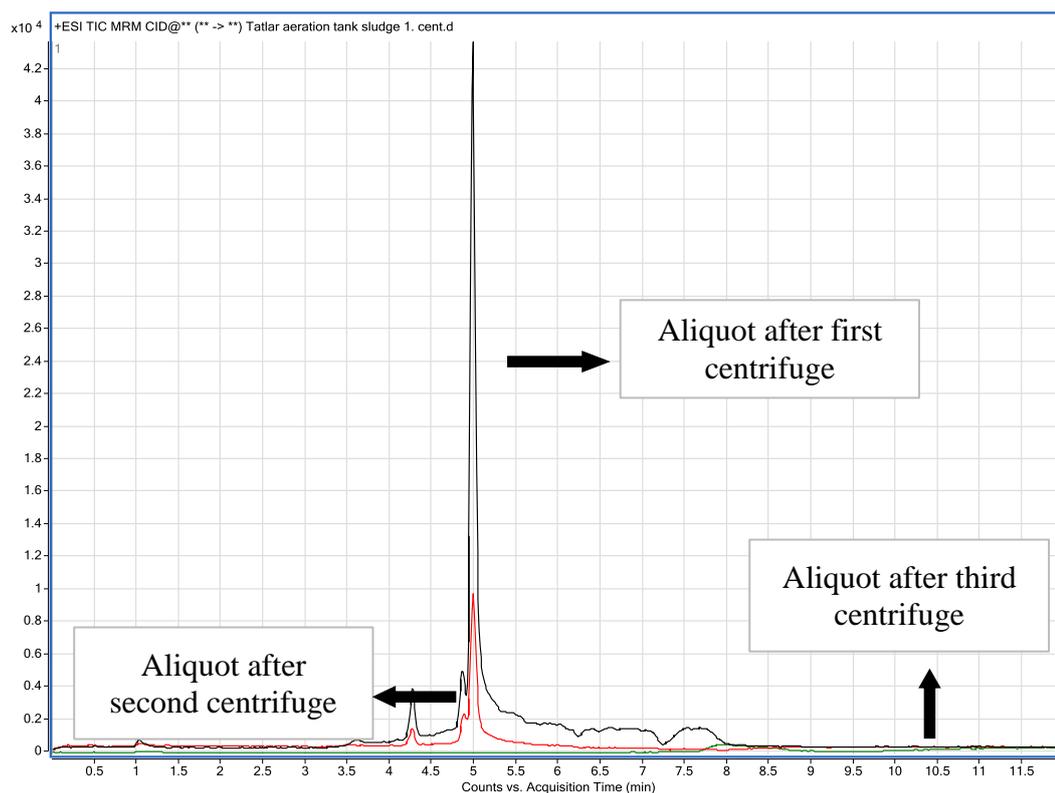


Figure 4.2 TIC comparison of EDCs in aliquots after centrifugation

b) Dried sludge vs Lyophilized sludge

Sludge samples taken from METU VRM plant was divided into two parts. One part was lyophilized under vacuum and on liquid ice and the other part was simply dried at 105°C. Then the sludge extraction method was applied to both samples. Since there were no significant differences observed between the results, it was decided to continue with drying of the sludge samples by heat. Chromatograms of lyophilized and dried sludge are given in appendix A (A-52).

c) Solvent Optimization

Two different solvents were tested for effective sludge extraction: dichloromethane (DCM) and methanol (MEOH). Total ion chromatogram TIC, results of methanol versus dichloromethane reveals that methanol is a superior

solvent over DCM for the ultrasound aided sequential extraction method. Total ion chromatogram comparison is given in figure A-53.

d) Method Optimization

The 3 sets of experiments were performed to optimize the sludge extraction method. In the first set, the aforementioned method (normal procedure) was applied, which consists of 3 sequential sonication for 30 minutes and collecting 100 ml solvent in each step. In the second set the sonication time was increased to 45 minutes instead of 30 minutes. Finally in the third set, rather than collecting a 300 ml aliquot in three steps, six steps were applied by collecting 50 ml aliquot instead of 100 ml in each step totaling a final volume of 300ml. Figure 4.3 shows that the first set, which is the normal procedure, gives much higher peaks than the other alternatives.

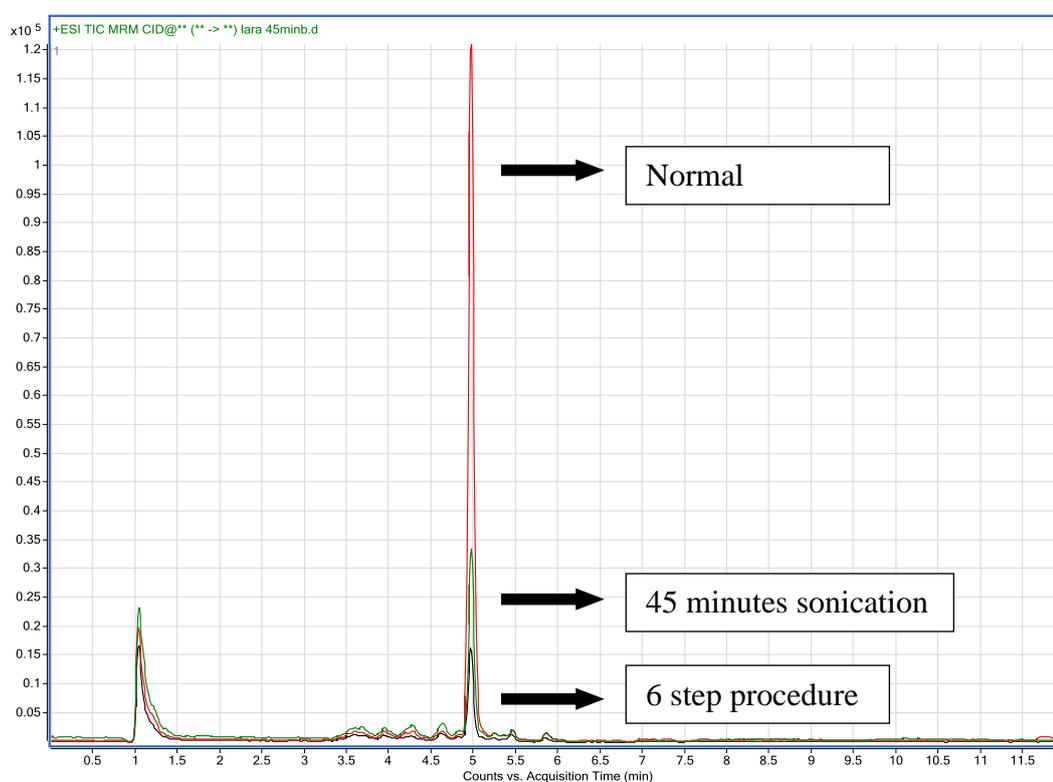


Figure 4.3 TIC comparison of 3 different sets of sludge extraction method

e) Analytical Figures of Merit

A 0.25 µg/L standard mix solution was analyzed ten times. Following formula was used to calculate LOD values.

LOD= 3xStandard deviation of the 0.25 µg/L mixed standard solution/slope of calibration plot

Table 4.7 presents the analytical figures of merit for each compound.

Table 4.7 Analytical Figures of Merit

Analytes	Equation (y=mx+n)	Linear Range (µg/L)	R²	LOD for sample, µg/kg
Diltiazem	$y = 14265x + 2526.8$	0.50-20	0.997	0.78
Progesterone	$y = 7433.2x + 2662.2$	0.10-100	0.999	0.72
BBP	$y = 52969x + 17875$	0.10-20	0.998	0.24
Estrone	$y = 749.71x - 166.28$	0.50-100	0.999	0.75
Carbamazepine	$y = 49852x + 1519.5$	0.5-50	0.999	0.72
Acetaminophen	$y = 5885x - 1785.3$	0.2-50	0.999	0.71

4.2 Pulse Ozonation Experiments

4.2.1 First Set of Experiments

In the first set 2, 3, 4 and 6 minutes pulse ozonation were applied to the flasks on each of four consecutive days. The control flask, which did not receive ozone treatment, was simply incubated alongside the test flasks. Soluble COD results in the flask supernatants are given in Figure 4.4, immediately before and after every ozone treatment. Following every ozone application COD in the

supernatants rapidly increased in all the flasks except for the control group. This supports the hypothesis that ozone disrupts cell walls releasing intracellular materials into the medium. Moreover, declining trend of COD following each ozonation was taken as indication of cryptic growth of the biomass on the released organic matter. The COD release upon ozonation and subsequent uptake by the remaining biomass is shown in Figure 4.4. The MLSS data given in Figure 4.5 also supports this view.

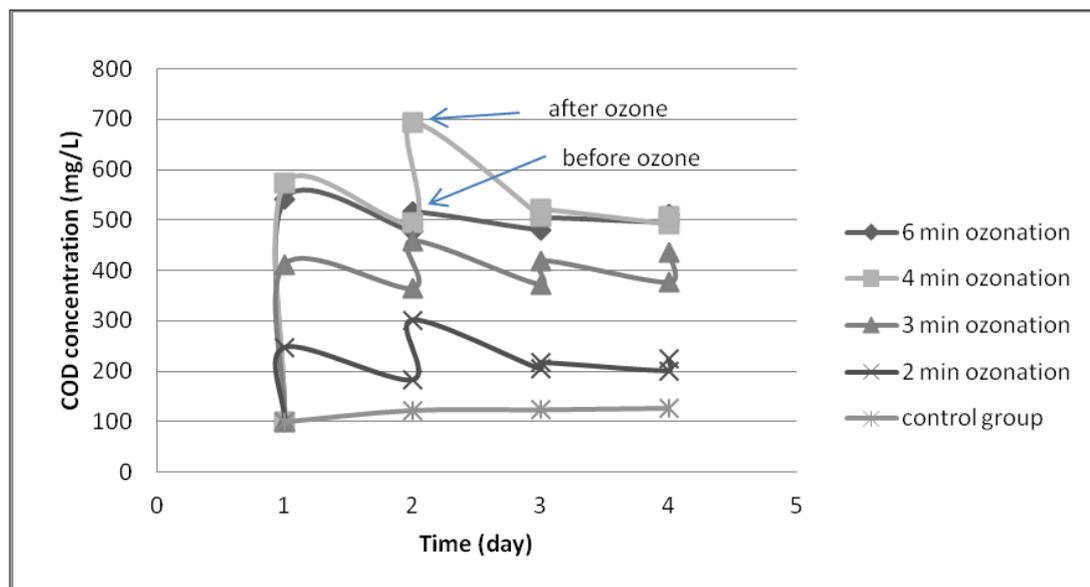


Figure 4.4 The soluble COD values for 2, 3, 4 and 6 minutes ozonation versus control group

It was concluded from Figure 4.4 and Figure 4.5 that 2 and 3 minutes of ozonation were ineffective due to unappreciable COD release and lower MLSS removals. It is also clear that COD release for 4 and 6 minutes of ozonation were nearly the same. This may be due to the fact that 6 minutes ozonation destroyed all the active bacteria after 2nd ozone application. Consequently, in the absence of an active biomass no further uptake of the released COD material could be observed in this flask from the second day on. This phenomenon also suggested the possibility that the first ozonation destroyed all the biomass and released COD to the filtrate and further ozonation had no further effect on the COD release. This may actually

be the case since from Figure 4.5 it can be deduced that repeated ozonation affected MLSS destruction to the highest extent but evidently did not cause appreciable soluble COD release and subsequent removal after day 2, in 6 minutes flask. In order to analyze this observation OUR experiments were performed for 4 and 6 minutes ozonation.

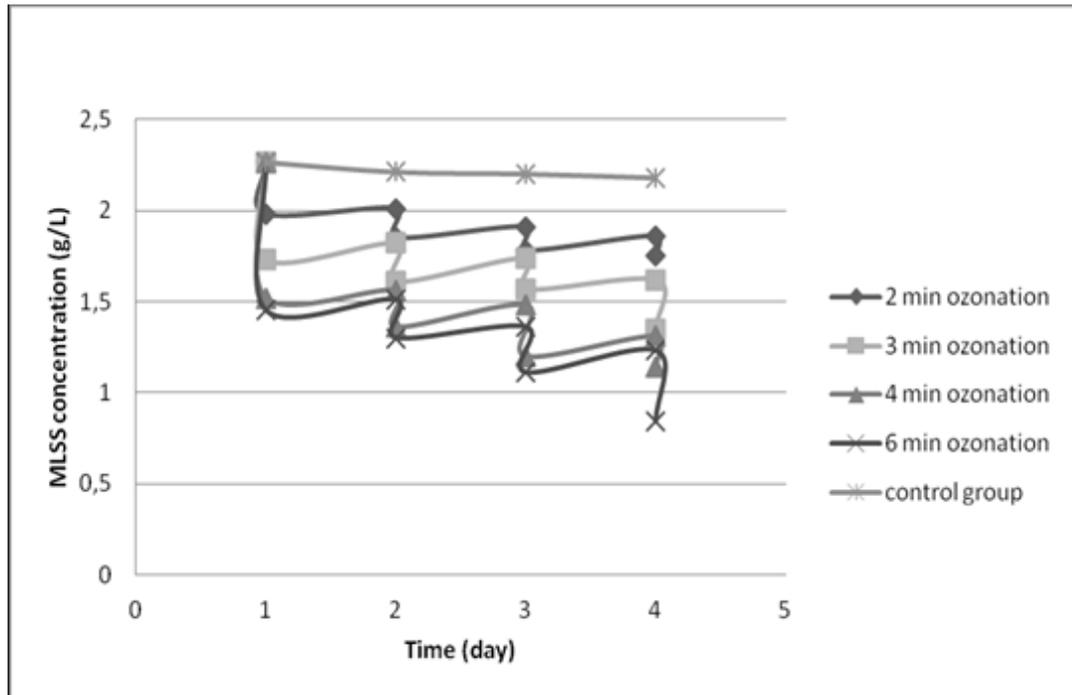


Figure 4.5 The MLSS values for 2, 3, 4 and 6 minutes ozonation versus control group

a) Oxygen Uptake Rate Experiments

The OUR results are given in Table 4.8. It is clear that the oxygen uptake rate decreased in all the flasks during the experiments. Indeed in 6' ozone treatment OUR reading was almost zero on the third day confirming the view that all the biomass was killed after second day application and no further soluble COD removal could be detected from then on.

In order to understand whether the remaining released COD was biodegradable or not, a seed sample with known OUR was added to the 6' sample at

the end of 4th day following the last ozonation and OUR of this seeded sample was checked. Since the endogeneous OUR value obtained for the seed was -0.0004 mg/L-sec and that of the seeded sample was -0.0006 mg/L-sec it was concluded that COD released from the 6'-ozonated sample was still biodegradable but in the absence of a viable seed soluble COD was not removed. In other words, the COD in the medium was biodegradable but high ozone amount killed all the active biomass.

Table 4.8 The OUR readings obtained for 4 and 6 minutes ozonated samples and control groups

OUR (dO/dt) (mg/L*sec)	Control group	4 minutes ozonation	6 minutes ozonation
1 st day	-0.0008	-0.0008	-0.0008
2 nd day	-0.0006	-0.0008	-0.0003
3 rd day	-0.0005	-0.0003	-2*10 ⁻⁵
4 th day	-0.0004	-0.0002	-5*10 ⁻⁵

b) The Capillary Suction Time, CST, SVI, pH and Disinfection Experiments

Experiments were performed with sludge samples taken from METU-VRM plant aeration tank. Samples were ozonated for 4 minutes on every day and in addition to the routine COD (in aliquote), MLSS and MLVSS analysis, CST, SVI and pH variations were also analyzed. The experiments were conducted in replicates. The pH analysis were carried out both in completely mixed sample (sludge) and in aliquotes obtained after settling of the sludge. pH experiments were run in parallel. Control groups were not ozonated.

Table 4.9 Results of sludge samples before and after ozonation on the 1st and 4th day

	MLSS (g/L)	MLVSS(g/L)	Soluble COD (mg/L)	SVI (ml/g)	CST(sec)
1 st day before ozonation	2,3	1,66	43	36,1	10,8
4 th day after ozonation	1,33	0,68	877,5	82,7	10,2

Table 4.10 The pH comparison of ozonated sample before and after ozonation versus control group on the 1st and 4th days

pH	4 minutes ozonated (average)		Control group(average)	
	Mixed sludge	aliquote	Mixed sludge	aliquot
1 st day before ozonation	7,1	7,08	(1 st day) 7,1	7,08
4 th day after ozonation	7,04	6,42	(4 th day) 7,06	7,06

As seen in Table 4.9 the sludge de-waterability was not affected after ozonation, as understood from the CST values. Settability of sludge, which is indicated by the SVI value, somewhat deteriorated. Although some pin-floc formation was expected with the initial SVI value of the sludge, it was well settleable with a clear supernatant. No pin floc formation was observed. After last ozone application on the 4th day, the supernatant of the sample showed a turbid nature. However, the SVI value obtained after ozonation still lies in the well-settleable

sludge range since in activated sludge plants, a sludge with an SVI less than or equal to 100 is considered a well-settling sludge (Vesilind, 2003). Table 4.10 shows the average pH trends of both ozonated and control groups in both aliquotes after settling and sludge mixtures. There wasn't significant change in the pH of sludge mixture for the ozonated sample but only a slight decrease in the pH of the aliquote could be observed. The pH variations in aliquots and sludge mixtures are presented in Figure 4.6 and Figure 4.7.

In order to obtain an idea on the disinfection quality of ozone total coliform count was performed. Total coliform count was recorded as 800 colonies /100 mL on the first day, prior to ozonation. Whereas no total coliform colony could be observed on the plates on the last day of ozonation.

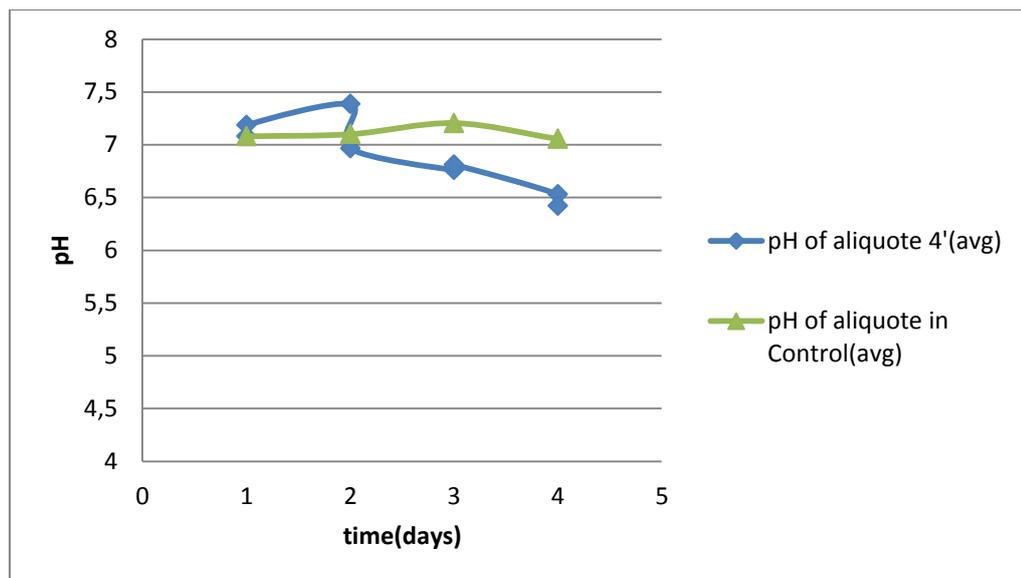


Figure 4.6 pH variations in the aliquotes for ozonated samples versus control group

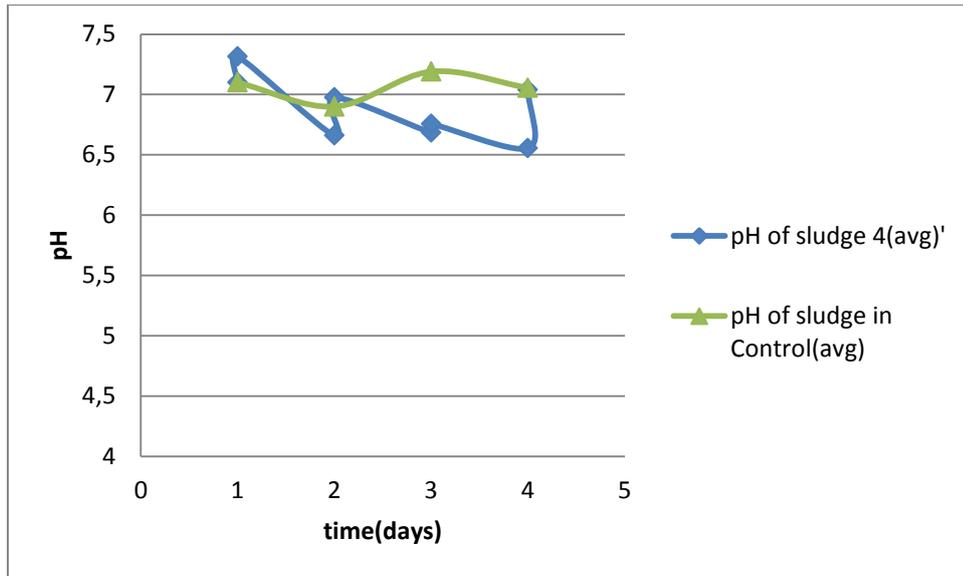


Figure 4.7 pH variations in the sludge for ozonated samples versus control group

The ozone amounts imparted according to the calibration curves are 0.6203 mg O₃/L for 2 minutes, 0.7421 mg O₃/L for 3 minutes, 0,8639 mg O₃/L for 4 minutes and 1.1 mg O₃/L for 6 minutes.

4.2.2 Second Set of Experiments

The second set of experiments was conducted with sludge samples not washed with phosphate buffer. Two parallel sets were prepared, where one was ozonated for 4 minutes and the other for 6 minutes on each day for 4 days. The MLSS and MLVSS reductions were 73% and 75% for 4 minutes and 78% and 84% for 6 minutes ozonated samples. Figures 4.8 and 4.9 show the MLSS and MLVSS trends in the samples versus the control group. Experiments were conducted in parallels. MLSS and MLVSS values were obtained from one flask. COD analysis were conducted from the aliquots of both flasks of each group (4', 6' and control) and the values were averaged.

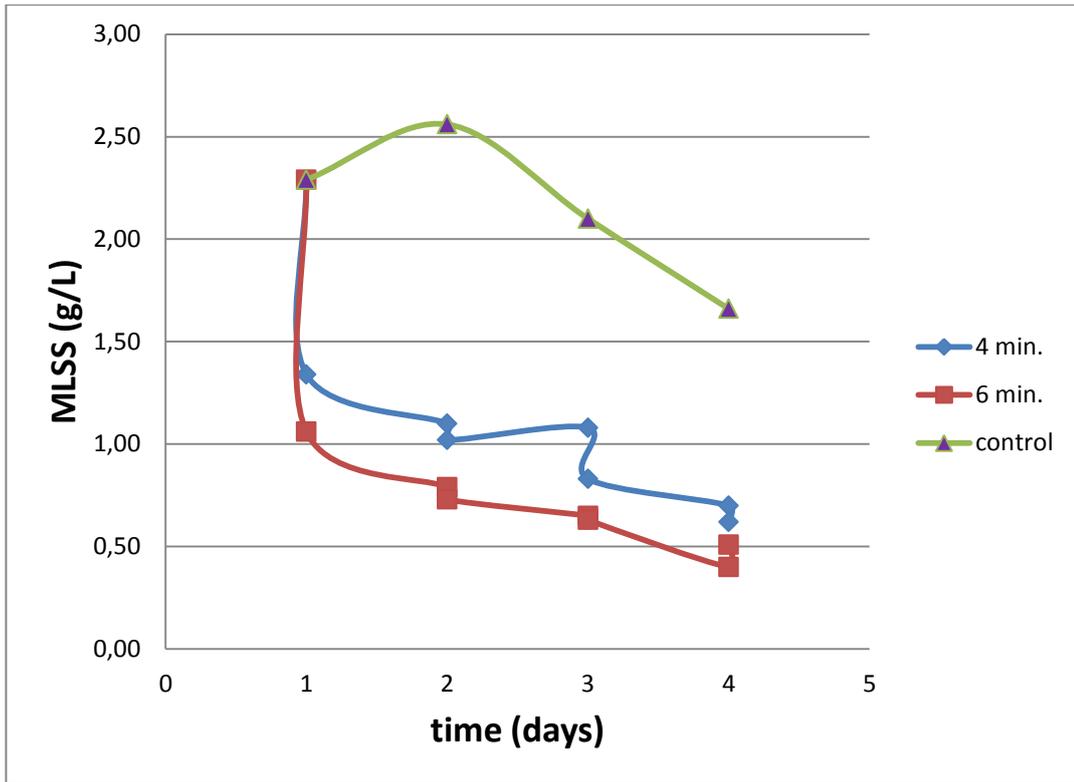


Figure 4.8 MLSS results of 4 and 6 minutes ozonation versus control group

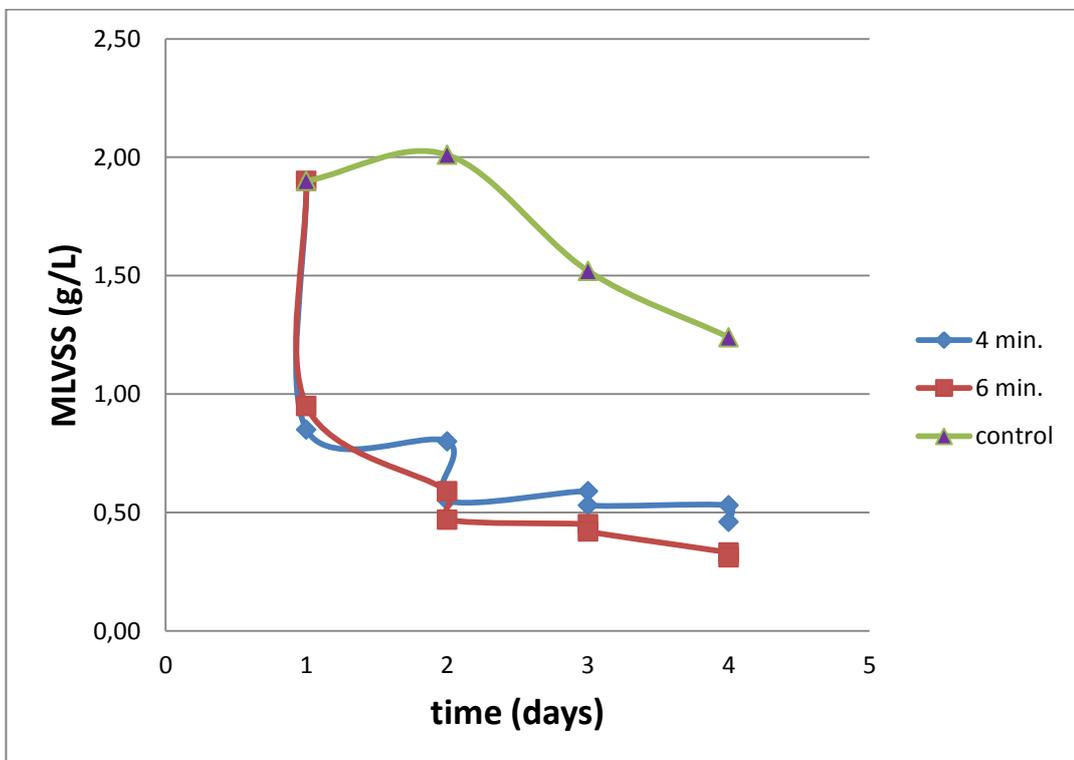


Figure 4.9 MLVSS results of 4 and 6 minutes ozonation versus control group

The observed soluble COD values in the aliquotes were very close to each other in the last day as shown in Figure 4.10

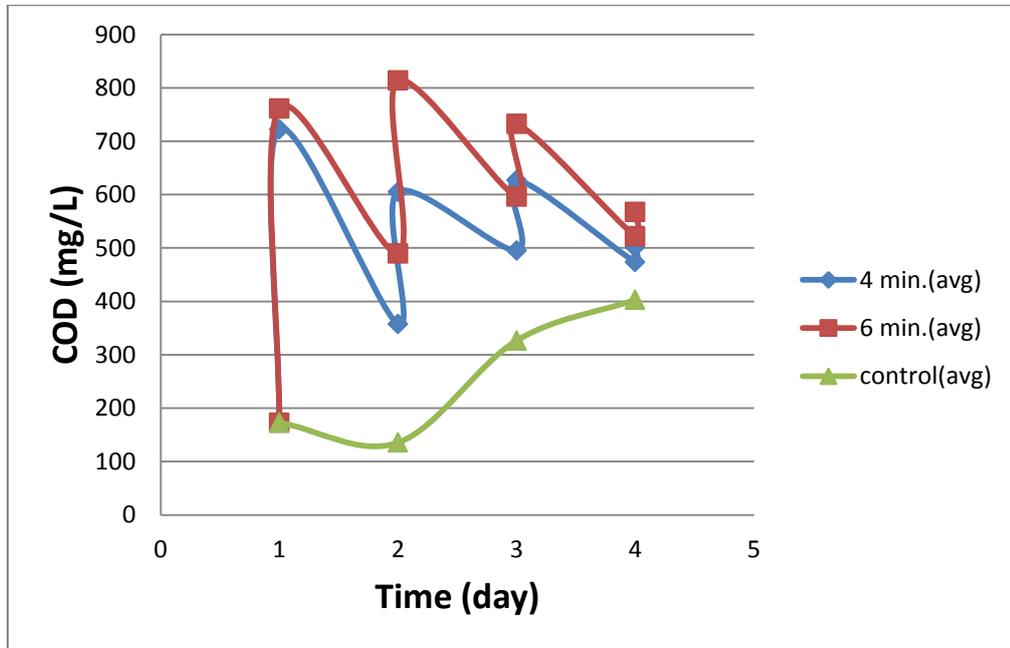


Figure 4.10 Soluble COD results of 4 and 6 minutes ozonation versus control group

Although there was no significant difference in COD release between 4 and 6 minutes ozonation, 6 minutes ozonation caused higher MLSS removal and a more stabilized sludge, as can be understood from the OUR results. Hence, it was concluded to proceed with an ozonation strategy of 4 minutes in the first three days and 6 minutes on the last day (4'+4'+4'+6') which means a total ozonation period of 18 minutes corresponding to 2.57 mg O₃/L.

This procedure was then applied to four different sludge samples obtained from different wastewater treatment plants, namely: Ankara Tatlar (WWTP1), Bodrum Konacık (WWTP2), Kayseri (WWTP3) and METU-VRM (WWTP4). The MLSS concentrations for WWTP1, WWTP2, WWTP3 and WWTP4 were 3.3 g/L, 3.08 g/L, 4.63 g/L and 3.1 g/L, respectively. The percentage of MLSS and MLVSS removals at the end of the experiment are given in Table 4.11. Corresponding

normalized ozone doses on the bases of removed and initial MLSS concentrations are given in Table 4.12.

Table 4.11 MLSS and MLVSS removal percentages of sludge samples

%	WWTP1	WWTP2	WWTP3	WWTP4
MLSS	85,9%	82%	77%	72,6%
removal (initial-final)	(3.33g/l- 0.47g/l)	(3.08g/l-0.53 g/l)	(4.63g/l- 1.08g/l)	(3.1g/l -0.85 g/l)
MLVSS	87,5%	95%	77%	90,7%
removal (initial-final)	(2.73g/l- 0.34g/l)	(2.12g/l-0.1 g/l)	(3.07g/l -0.68 g/l)	(2.25 g/l-0.21 g/l)
Sample Location	RAS	RAS	RAS	Aeration Tank

Table 4.12 Ozone doses applied to sludge samples

WWTP	Ozone dose applied, kg O ₃ /kg MLSS removed	Ozone dose applied, kg O ₃ /kg initial MLSS
WWTP 1	0.00130	0.00117
WWTP 2	0.00146	0.00121
WWTP 3	0.00105	0.000803
WWTP 4	0,00165	0.00120

Total ozone dose applied: $(0.87 \text{ mg O}_3/\text{L} * 3) + 1.11 \text{ mg O}_3/\text{L} = 3.72 \text{ mg O}_3/\text{L}$
 $3.72 \text{ mg O}_3/\text{L} * 0.3 \text{ L} = 1.116 \text{ mg O}_3$ (Volume of all the samples were 300 ml)

For WWTP2:

$$1.116 \text{ mg O}_3 / (3080 \text{ mg initial MLSS /L} * 0.3\text{L}) = 0.00121 \text{ kg O}_3/\text{kg initial MLSS}$$

$$1.116 \text{ mg O}_3 / (3080-530 \text{ mg MLSS /L} * 0.3\text{L}) = 0.0146 \text{ kg O}_3/\text{kg MLSS removed}$$

The values for the other WWTPs were calculated accordingly.

4.2.3 Effect of Ozonation on Phosphorus Content of Sludge

Total phosphorus and ortho-phosphate measurements were conducted with WWTP1 and WWTP4 sludges to observe effect of ozonation on phosphorus release into the medium. Experiments were conducted in replicates. Table 4.13 shows P-release results in WWTP1 and Table 4.14 shows P-release in WWTP4 set. The amount of phosphorus accumulated in sludge is given in Table 4.15 and calculated by:

$$\text{Phosphorus accumulated in sludge (mg PO}_4\text{/g biomass)} = (TP_i - OP_i) / MLVSS_i$$

Where TP_i is initial total phosphorus and OP_i is initial ortho-phosphate.

Table 4.13 Results of the ozonation experiment for WWTP1

		MLSS (g/L)	MLVSS (g/L)	MLSS Reduction %	MLVSS Reduction %	Total Phosphorus (mg/l PO4-P)	Ortho- Phosphate (mg/l PO4-P)
1 st	day	3,78	2,99	-	-	21,95	6,85
before ozonation							
4 th	day	0,98	0,75	74,10	74,92	22,3	7,625
after ozonation							

Table 4.14 Results of the ozonation experiment for WWTP4

	MLSS (g/L)	MLVSS (g/L)	MLSS Reduction %	MLVSS Reduction %	Total Phosphorus (mg/l PO4-P)	Ortho- Phosphate (mg/l PO4-P)
1 st day before ozonation	3,1	2,25	-	-	19,80	4,61
4 th day after ozonation	0,85	0,21	72,58	90,67	19,00	6,73

Table 4.15 Amount of phosphorus accumulated in sludge

	WWTP1	WWTP4
1 st day before ozonation	4,52 mg PO4/g biomass	6,72mg PO4/g biomass
4 th day after ozonation	19,55 mg PO4/g biomass	62,52 mg PO4/g biomass

As can be seen from Table 4.13 and 4.14 no appreciable P release by the biomass could be observed at the end of ozone applications. From Table 4.15, it is readily understood that P in the sludge was concentrated 4.3 times after ozonation in the case of WWTP1 sludge; and almost 9.3 times in the case of WWTP4 sludge. The MLSS, MLVSS, TP and Ortho-P variations during the experiments are tabulated in Figure 4.11 to Figure 4.14. As can be seen from Figure 4.14 the release of phosphorus has stopped after 3rd day in the case of WWTP4. From Figure 4.12 it

can be deduced that a slight phosphate release into the medium occurred on the second day of treatment, but it seemed to be re-absorbed onto the sludge on the 3rd and 4th days of application in the case of WWTP1. This decrease in ortho-phosphate may also be due to precipitation of phosphorus ions instead of a reabsorbance mechanism. Since WWTP1 have some industrial wastewater sources, phosphate ions may be precipitated with the iron present in the wastewater. However, no further experiments were conducted for the heavy metal concentrations of the samples.

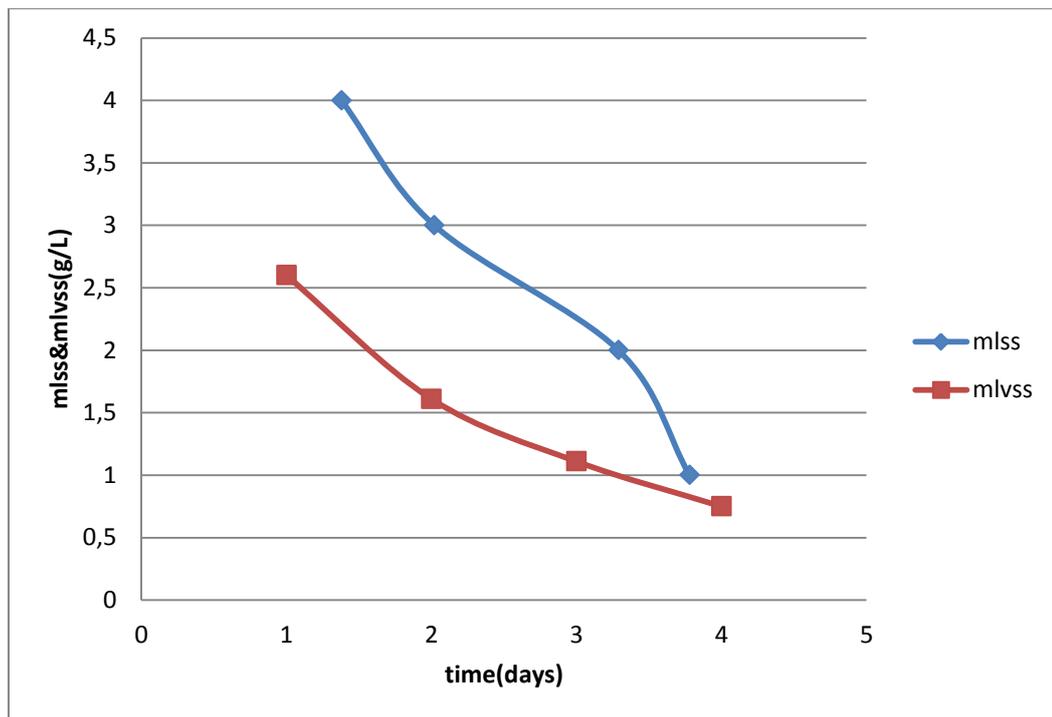


Figure 4.11 MLSS and MLVSS results for WWTP1 during ozonation

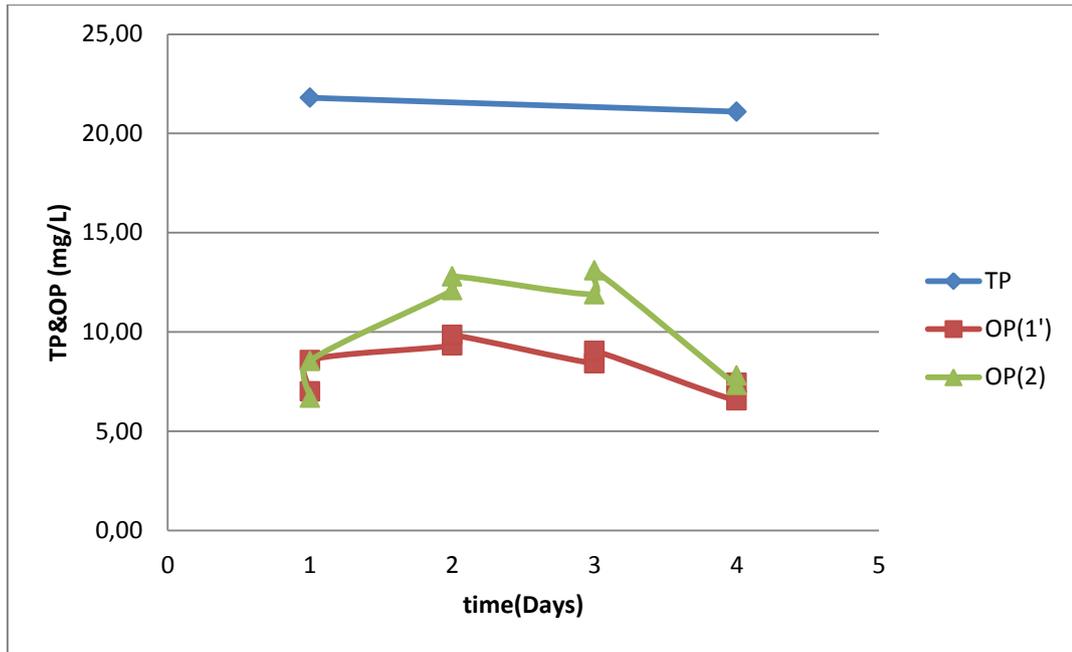


Figure 4.12 TP&OP results for WWTP1 during ozonation

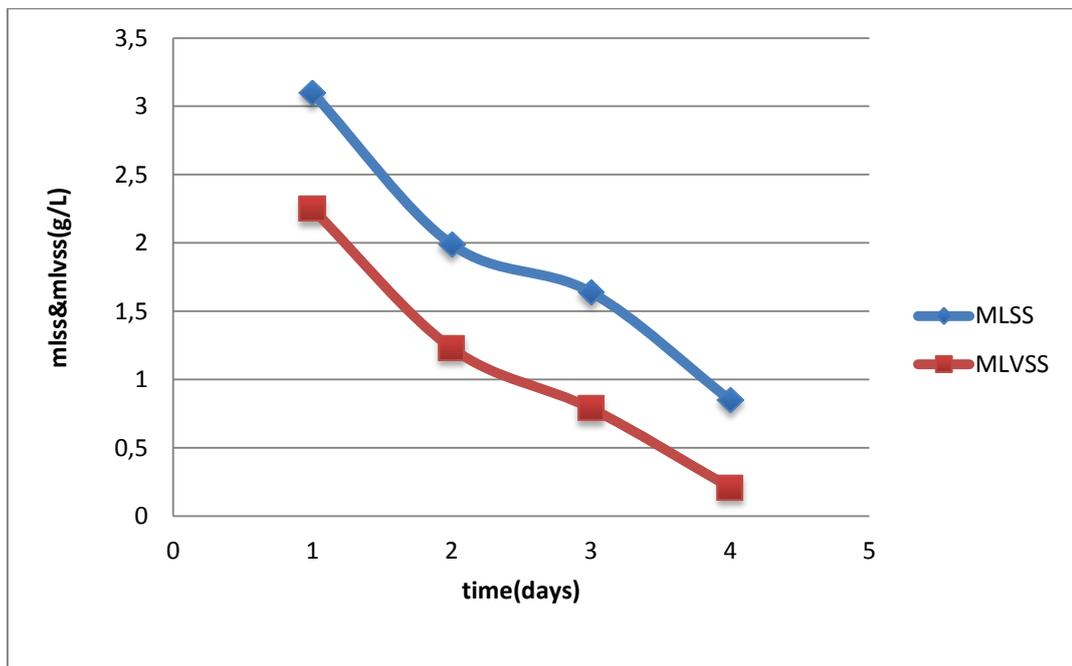


Figure 4.13 MLSS and MLVSS results for WWTP4 during ozonation

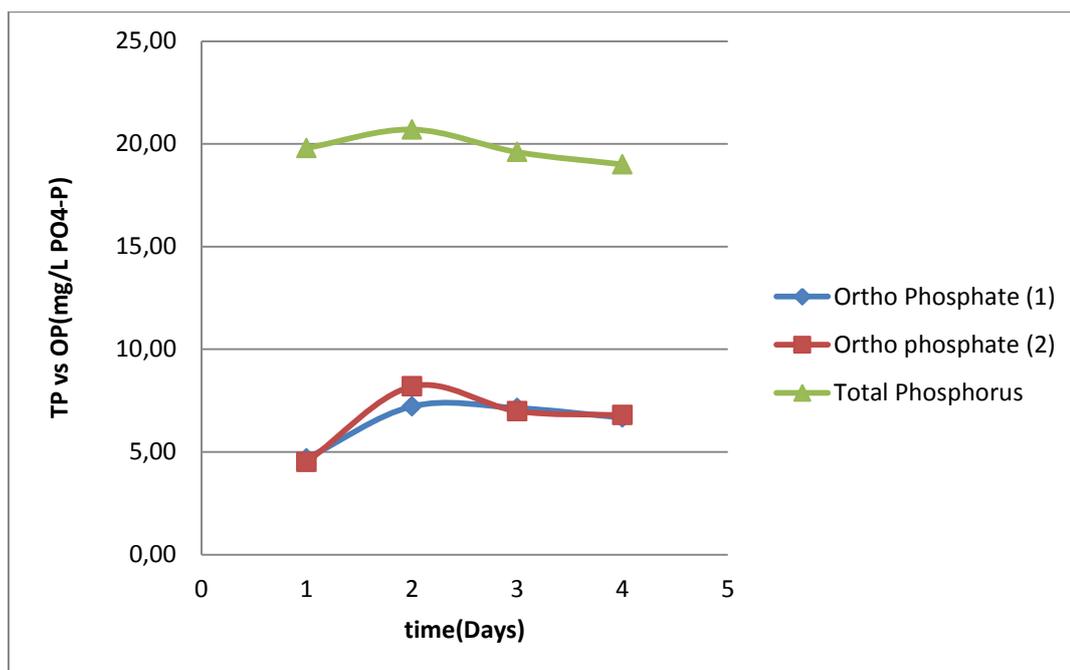


Figure 4.14 TP&OP results for WWTP4 during ozonation

At the end of the experiments the amount of remaining biomass was very small compared to the initial value due to the effect of ozonation; still very little amount of phosphorus was found released into the medium. Hence, it can be concluded that sludge was enriched with P following the ozone assisted digestion process.

4.3 Removal of EDCs with Ozone Application

A combination ozone strategy was tried upon observing the effects of different ozone doses on sludge. The aim was to further optimize the process. Accordingly sludge samples were spiked with 200 ppb standard mix containing all the analytes at this concentration. Samples were ozonated for 4 minutes for the first

three days and 6 minutes on the last day. The remaining MLSS and MLVSS concentrations were calculated after the last ozone administration on the fourth day.

Moreover final ozone dose on the 4th day was extended in experiments with four different sludge samples. The aim was to destroy the sorbed EDCs onto the sludge samples. The experimental plan is summarized in Table 4.16.

Table 4.16 Summary of Experimental Plan for the EDC Removal Experiments

date	28.04	03.05	14.08	19.12
Sample	METU-VRM	Tatlar Ankara	METU-VRM	METU-VRM
Location	aeration tank	aeration tank	aeration tank	aeration tank
Initial MLSS(g/L)	3,1	3,78	2,92	4,21
Initial MLVSS(g/L)	2,25	2,99	2,87	3,9
MLSS	72,58	74,10	68,15	73,12
removal (%)				
MLVSS	90,67	74,92	77,70	78,94
Removal(%)				
Last day				
ozone period (minutes)	50,75,100,150	50,75,100,150	6,10,20,30,40	6,10,20,30,40
Total ozone period (minutes)	(4'+4'+4'+50'+75'+100'+150')	(4'+4'+4'+50'+75'+100'+150')	(4'+4'+4'+6'+10'+20'+30'+40')	(4'+4'+4'+6'+10'+20'+30'+40')

The background EDC content of sludge samples were analyzed by using sludge extraction method described in Materials and Methods section prior to spiking. After receiving high ozone doses on the last day, all the solid particles were destroyed in the flasks which consequently resulted in a slightly turbid aqueous medium. Hence wastewater extraction method, rather than solid extraction method, was applied to the samples to calculate EDC concentrations remaining in the flasks. Photographs showing the samples after ozonation on the last day can be found in Appendix A (A-54 to A-56). Pictures clearly show that there was not much difference between filtered and non-filtered samples through ordinary filter paper. Results of these experiments are given in Table 4.17 - 4.20.

In the first two sets of experiments, the sludge samples were taken from WWTP4 (28.04) and WWTP1 (03.05). 4 flasks were prepared and each was ozonated for different periods of time on the last day's ozone application which were 50, 75, 100 and 150 minutes. Same procedure was applied to WWTP1.

Table 4.17 Final EDC concentrations of experiment conducted on 28.04 (50'-150') (n=3)

	diltiazem	progesterone	bbp	estrone	carbamazepine	acetaminophen
Initial EDC conc of sludge	n.d	n.d	1,302 ±0,064	n.d	0,167±0,102	3,325±0,642
ppb						
Initial EDC conc of sludge +200 ppb	200	200	201,302 ±0,064	200	200,167±0,102	203,325±0,642
ppb						
50' Ppt	3,92± 0,01	14,08± 0,11	26,22± 2,15	n.d	n.d (<LOD)	n.d
75' Ppt	3,61± 0,01	13,28± 0,12	19,49± 2,45	n.d	n.d	n.d
100' Ppt	3,54± 0,01	13,31± 0,24	25,90± 1,35	n.d	n.d	n.d
150' ppt	3,49± 0,005	13,09 ±0,27	53,20 ± 2,49	n.d	n.d	n.d

Table 4.18 Final EDC concentrations of experiment conducted on 03.05 (50'-150') (n=3)

	diltiazem	progesterone	bbp	estrone	carbamazepine	Acetaminophen
Initial conc of sludge ppb	n.d	n.d	n.d	n.d	n.d	24,641±4,304
Initial conc of sludge +200 ppb ppb	200	200	200	200	200	224,641±4,304
50' Ppt	1,75 ± 0,02	2,13 ± 0,32	3,36 ±0,48	335,91 ± 15,49	n.d	11,81 ± 1,89
75' Ppt	6,78 ±0,20	5,60 ± 0,31	n.d	370,73 ± 33,06	n.d	3,59 ± 1,18
100' Ppt	1,87 ±0,04	1,07 ± 0,15	n.d	351,99 ± 9,98	0,72 ± 0,02	18,25 ± 4,46
150' ppt	8,38 ±0,18	59,30 ±2,31	6,43 ±0,45	197,25 ±136,84	n.d	24,31 ± 2,91

From the results of these two sets of experiments with ozonation periods of 50, 75, 100 and 150 minutes on the last day, EDC concentrations higher than 200 ppb of each analyte were removed to ppt levels which corresponds to a removal higher than 99%. This means that most probably even 50' ozonation was more than enough to remove these selected compounds. Hence, it was decided to optimize the ozone dose between 6' and 40' in the other two sets of experiments conducted with WWTP4 sludge samples. Two sets of experiments were conducted on 14.08 and 19.12. 5 flasks were prepared and each was ozonated for different periods of time on the last day's ozone application which were 6, 10, 20, 30 and 40 minutes.

Table 4.19 Final EDC concentrations of experiment conducted on 14.08 (6'-40') (n=3)

	diltiazem	progesterone	bbp	estrone	carbamazepine	Acetaminophen
Initial conc of sludge ppb	n.d	2,149±0,168	n.d	13,982±1,620	n.d	9,398±0,078
Initial conc of sludge +200 ppb ppb	200	202,149±0,168	200	213,982±1,620	200	209,398±0,078
6' Ppt	n.d	1,86±0,27	3,33±0,20	1296,17±26,86	n.d	n.d
10' Ppt	n.d	1,19±0,08	4,58±0,18	1883,84±15,23	n.d	n.d
20' Ppt	n.d	1,42±0,11	5,20±0,20	2939,53±54,84	n.d	n.d
30' ppt	n.d	2,36±0,19	n.d	2408,05±105,15	n.d	n.d
40' ppt	n.d	2,39±0,14	1,27±0,57	4170,38±189,33	n.d	n.d

Table 4.20 Final EDC concentrations of experiment conducted on 19.12 (6'-40') (n=1)

	diltiazem	progesterone	bbp	estrone	carbamazepine	Acetaminophen
Initial conc of sludge ppb	0,230	n.d	n.d	63,63	n.d	n.d
Initial conc of sludge +200 ppb	200,230	200	200	263,63	200	200
6' Ppt	n.d	0,23	n.d	199,45	n.d	n.d
10' Ppt	0,77	0,25	n.d	164,84	n.d	n.d
20' Ppt	n.d (<LOD)	1,41	n.d	332,93	n.d	n.d
30' ppt	0,29	2,32	n.d	599,82	n.d	n.d
40' ppt	0,14	0,22	n.d	298,20	n.d	n.d

Results of these experiments showed that 6' ozonation was sufficient to decrease the concentrations of these compounds to non-detectable levels. Removal percentages of EDCs in the experiments are provided in Table 4.21 to 4.24.

Table 4.21 Removal percentages of EDC concentrations of experiment conducted on 28.04 (50'-150')

	diltiazem	progesterone	bbp	estrone	carbamazepine	acetaminophen
50'	99.998	99.992	99.987	>99.99	>99.99	>99.99
75'	99.998	99.993	99.990	>99.99	>99.99	>99.99
100'	99.998	99.993	99.987	>99.99	>99.99	>99.99
150'	99.998	99.993	99.974	>99.99	>99.99	>99.99

Table 4.22 Removal percentages of EDC concentrations of experiment conducted on 03.05 (50'-150')

	diltiazem	progesterone	bbp	estrone	carbamazepine	acetaminophen
50'	99.999	99.999	99.998	99.832	>99.99	99.999
75'	99.997	99.997	>99.99	99.815	>99.99	99.999
100'	99.999	99.999	>99.99	99.824	99.999	99.999
150'	99.996	99.970	99.997	99.901	>99.99	99.999

Table 4.23 Removal percentages of EDC concentrations of experiment conducted on 14.08 (6'-40')

	diltiazem	progesterone	bbp	estrone	carbamazepine	acetaminophen
6'	>99.99	99.999	99.998	99.394	>99.99	>99.99
10'	>99.99	99.999	99.997	99.120	>99.99	>99.99
20'	>99.99	99.999	99.996	98.626	>99.99	>99.99
30'	>99.99	99.998	>99.99	98.875	>99.99	>99.99
40'	>99.99	99.998	99.998	98.051	>99.99	>99.99

Table 4.24 Removal percentages of EDC concentrations of experiment conducted on 19.12 (6'-40')

	diltiazem	progesterone	bbp	estrone	carbamazepine	acetaminophen
6'	>99.99	99.999	>99.99	99.924	>99.99	>99.99
10'	99.999	99.999	>99.99	99.937	>99.99	>99.99
20'	>99.99	99.999	>99.99	99.874	>99.99	>99.99
30'	99.999	99.999	>99.99	99.772	>99.99	>99.99
40'	99.999	99.999	>99.99	99.887	>99.99	>99.99

Also, concentrations of analytes after last ozonation are given as graphs according to the dates of experiments and can be seen through Figure 4.15 to Figure 4.20. First two sets (28.04 and 03.05) were ozonated between 50-150 minutes. The other sets (19.12 and 14.08) were ozonated between 6-40 minutes.

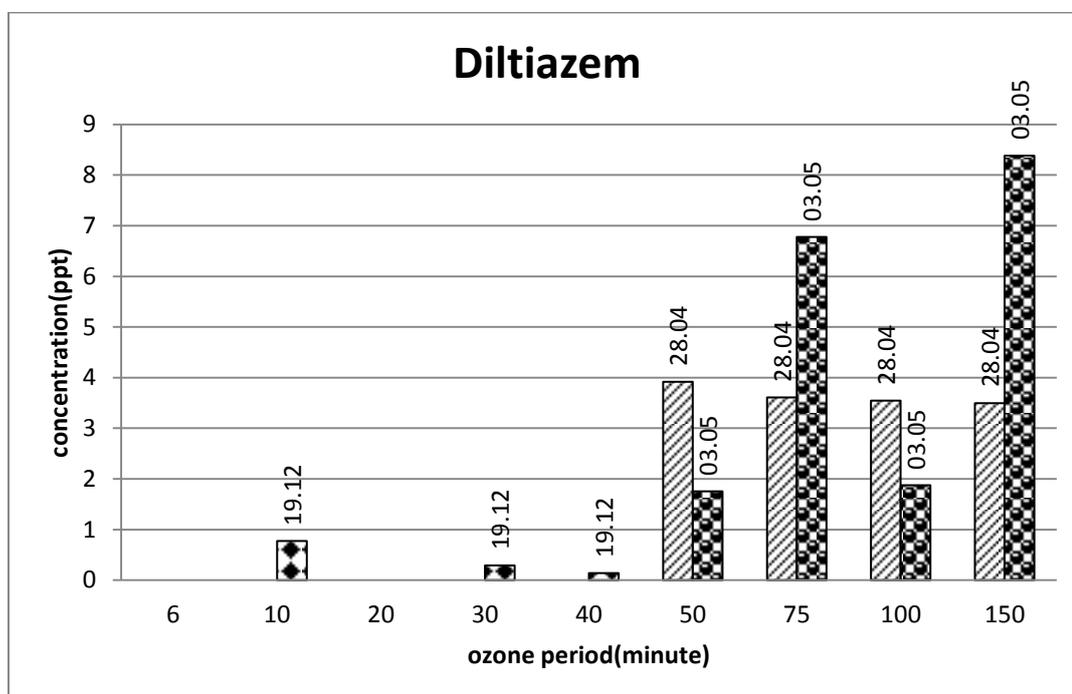


Figure 4.15 Concentrations of Diltiazem after last ozone application for all sets of experiments.

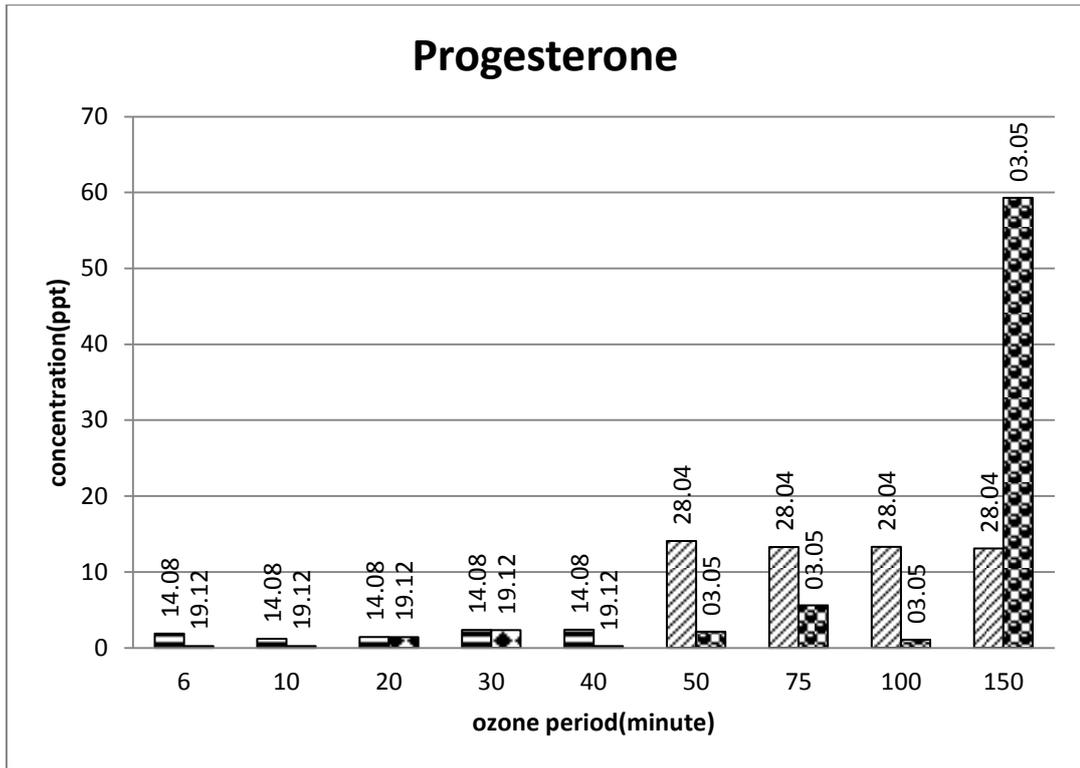


Figure 4.16 Concentrations of Progesterone after last ozone application for all sets of experiments.

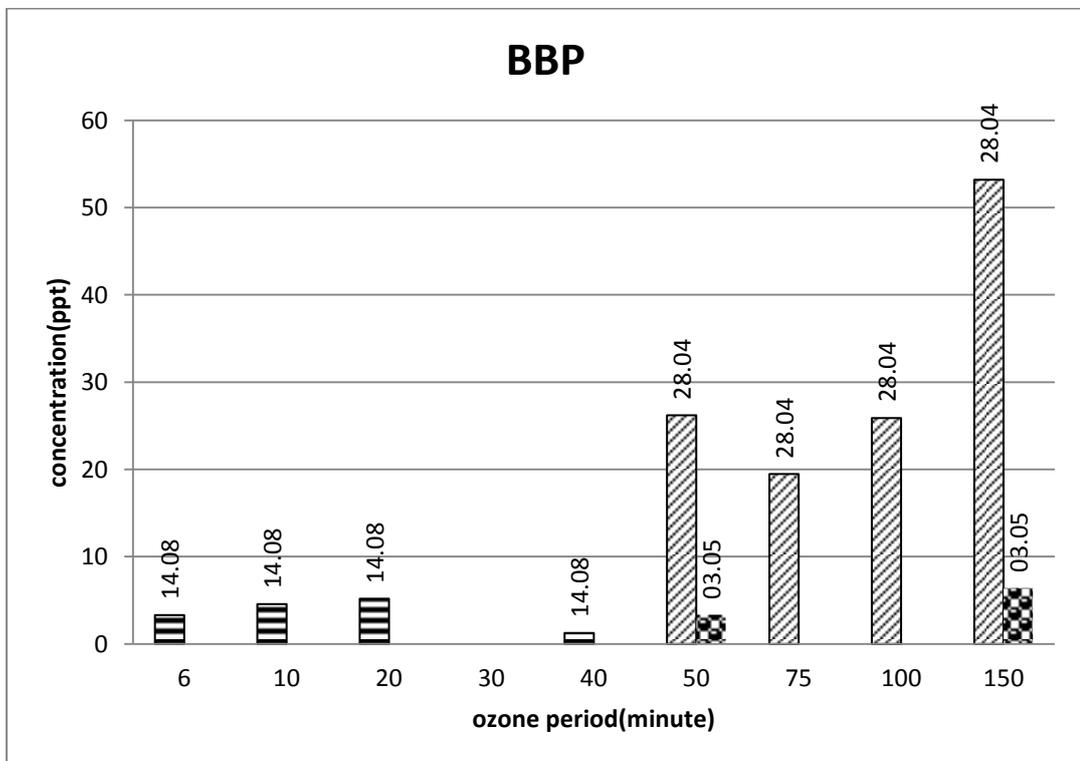


Figure 4.17 Concentrations of BBP after last ozone application for all sets of experiments.

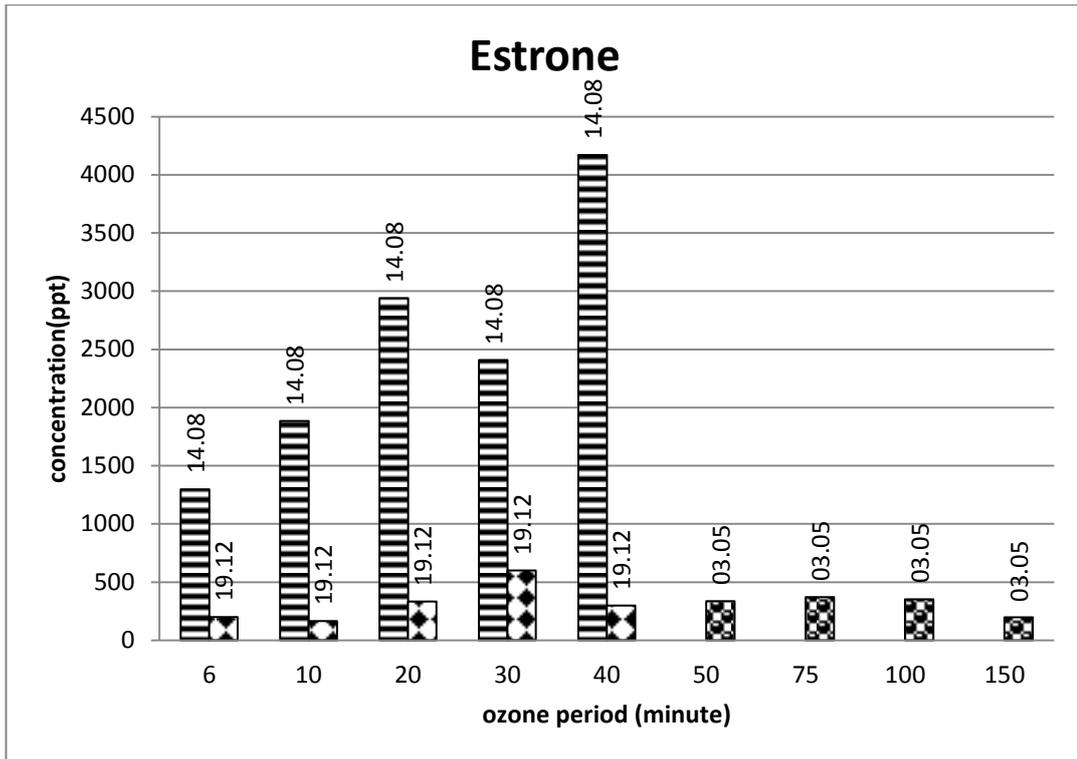


Figure 4.18 Concentrations of Estrone after last ozone application for all sets of experiments.

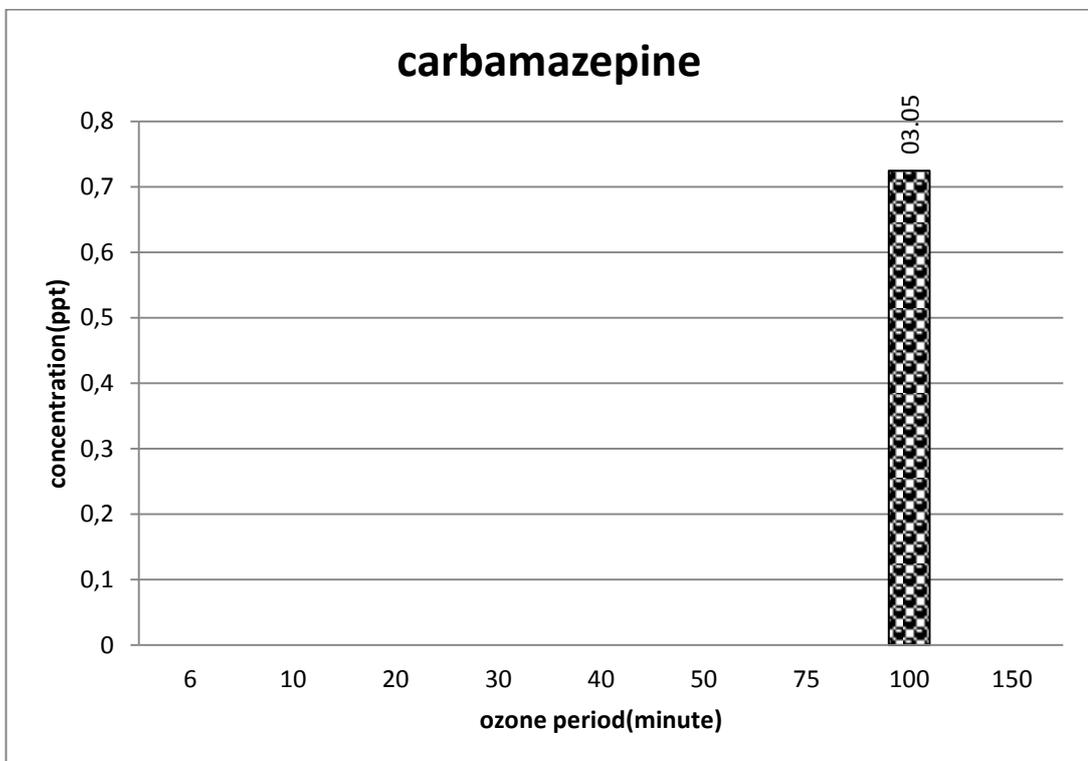


Figure 4.19 Concentrations of Carbamazepine after last ozone application for all sets of experiments.

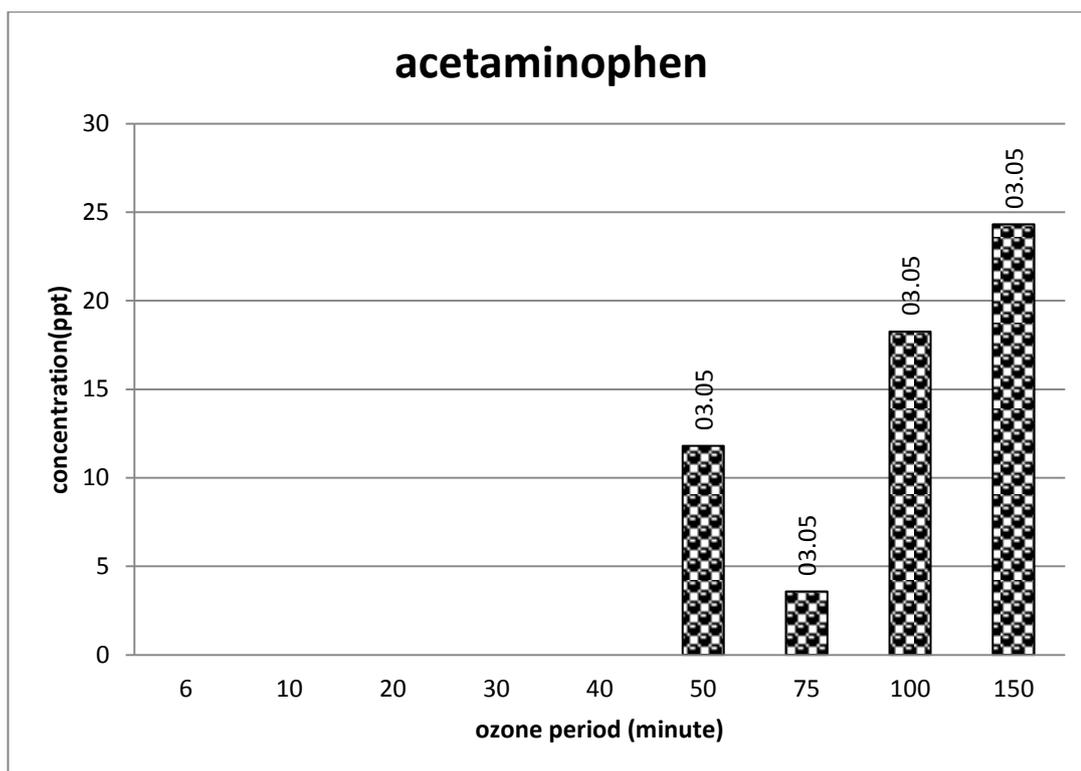


Figure 4.20 Concentrations of Acetaminophen after last ozone application for all sets of experiments.

The slight increase of concentration of analytes with the increasing ozonation periods may be due to the elimination of matrix effect by ozonation. However the concentrations of the analytes were still in ppt (ng/L) levels which indicates a removal of more than 99%. Applied ozone doses can be seen from Table 4. 25

Table 4.25 Ozonation periods corresponding to applied ozone doses

Ozonation period (min)	6	10	20	30	40	50	75	100	150
Ozone dose(mg O ₃ /L)	1,1075	1,5947	2,8127	4,0307	5,2487	6,4667	9,5117	12,5567	18,6467

CHAPTER 5

CONCLUSION

Method for the detection of endocrine disrupting compounds and natural hormones namely diltiazem, progesterone, butyl benzyl phthalate, estrone, carbamazepine and acetaminophen in wastewater and sludge by HPLC(ESI)/MS/MS was successfully optimized in this study.

Extraction of wastewater samples by solid phase extraction provided more than 90% recoveries for all the compounds analyzed, except for acetaminophen.

Extraction of the analytes from sludge samples was carried out by an ultrasound aided sequential extraction procedure developed in this thesis work. More than 90% recoveries were achieved for all the compounds of interest.

For pulse ozone-assisted sludge minimization studies two set of experiments were conducted. As a result, a strategy to ozonate sludge samples for 4 minutes on the first three days and 6 minutes on the last day has been adopted for optimum results.

The COD, MLSS and MLVSS results proved cryptic growth of the biomass taking effect on the solubilized material upon pulse ozonation. Also the OUR experiments revealed that a stabilized sludge was obtained at the end of fourth day of ozonation.

Effects of ozone on sludge characteristics were followed by CST, SVI and pH experiments. In the light of the results, it can be concluded that sludge de-waterability and pH was not affected by ozonation. However, settlability of the sludge has slightly deteriorated (from 36.1 to 82.7).

Total coliform counts showed that sludge was disinfected at the end of ozone application.

The second set of results indicated that pulse ozonation was an equally powerful technique on different sludge samples obtained from diverse treatment plant configurations. Up to 86% MLSS and 95% MLVSS removal was achieved in these trials. The differences in removal percentages may be due to the variations in wastewater sources of the WWTPs. The lowest MLSS and MLVSS removals were

observed in Kayseri sludge (WWTP3) which also has industrial influent coming to the plant aside from domestic wastewater. Ozone was also consumed to oxidize many complex compounds and heavy metals coming from these industrial sources which may lead to lower removal rates in MLSS and MLVSS. WWTP4 and WWTP2's are domestic treatment plants.

Another interesting finding was that pulse ozonation did not cause a high phosphorus release to the medium. Conversely, a phosphorus rich sludge was obtained since the release of phosphorus was very small whereas sludge reduced was significant. As a result phosphorus content of sludge increased by up to 10 fold (from 6.72 mg PO₄/g biomass to 63 mg PO₄/g biomass for WWTP4 and 4.5 mg PO₄/g biomass to 20 mg PO₄/g biomass for WWTP1) while the amount of sludge reduced by more than 80%.

Another important gain of pulse ozonation experiments was micropollutants removal. In order to optimize the ozone dose for complete removal of the trace organics fourth day ozonation was extended to 6 -150 minutes. The results showed that 6 minutes ozonation on the last day was sufficient to achieve a 99% removal of the selected endocrine disrupting compounds; and need for extended ozonation was unjustifiable.

As summary, it is demonstrated that by using an ozone dose, a thousand times lower than the values reported in the literature, a stabilized, disinfected, phosphorus-rich sludge could be achieved at a considerably low cost. Compared to aerobic digestion, which provides 40-50% solids reduction in 10-15 days, a far more effective method is thus proposed establishing more than 80% solids reduction only in 4 days. Furthermore, sludge characteristics were not altered by ozone application and removal of micropollutants and Total coliforms were achieved at the same time.

REFERENCES

- Agilent. (2009). Agilent 6400 Series Triple Quad LC/MS System. Germany: Agilent Technologies, Inc.
- Ahn, K., Park, K., Maeng, S., Hwang, J., Lee, J., Song, K.-G., et al. (2002). Ozonation of Wastewater Sludge for Reduction and Recycling. *Water Sci. Technol.*, 46(10), 71-77.
- Alda, M. J., & Barcelo, D. (2001). Use of solid-phase extraction in various of its modalities for sample preparation in the determination of estrogens and progestogens in sediment and water. *J. Chromatogr. A*, 938(1-2), 145–153.
- Alda, M. L., Díaz-Cruz, S., Petrovic, M., & Barcelo, D. (2003). Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. *J. Chromatogr. A*, 1000(1-2), 503–526.
- Andersen, H., Siegrist, H., Halling-Sørensen, B., & Ternes, T. A. (2003). Fate of estrogens in a municipal sewage treatment plant. *Environ. Sci. Technol.*, 37(18), 4021-4026.
- Auriol, M., Filali-Meknassi, Y., Tyagi, R. D., Adams, C. D., & Surampalli, R. Y. (2006). Endocrine disrupting compounds removal from wastewater, a new challenge. *Process Biochem.*, 41(3), 525–539.
- Beltran, F. J. (2005). *Ozone Reaction Kinetics for Water and Wastewater Systems*. Florida: Lewis Publishers.
- Birnbaum, L. S., & Fenton, S. E. (2003). Cancer and Developmental Exposure to Endocrine Disruptors. *Environ. Health Perspect.*, 111(4), 389-394.
- Bolong, N., Ismail, A., Salim, M., & Matsuura, T. (2009). A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination*, 239(1-3), 229–246.

Braga, O., Smythe, G. A., Schafer, A. I., & Feitz, A. J. (2005). Fate of Steroid Estrogens in Australian Inland and Coastal Wastewater Treatment Plants. *Environ. Sci. Technol.*, 39(18), 3351-3358.

Camacho, P., Deleris, S., Geaugey, V., Ginestet, P., & Paul, E. (2002). A comparative study between mechanical, thermal and oxidative disintegration techniques of waste activated sludge. *Water Sci. Technol.*, 46(10), 79-87.

Carballa, M., Omil, F., Ternes, T., & Lema, J. M. (2007). Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Res.*, 41(10), 2139 – 2150.

Chatterjee, S., & Karlovsky, P. (2010). Removal of the endocrine disrupter butyl benzyl phthalate from the environment. *Appl. Microbiol. Biotechnol.*, 87(1), 61–73.

Chu, L., Yan, S., Xing, X., Sun, X., & Jurcik, B. (2009). Progress and perspectives of sludge ozonation as a powerful pretreatment method for minimization of excess sludge production. *Water Res.*, 43(7), 1811-1822.

Colborn, T., Dumanoski, D., & Myers, J. P. (1996). *Our Stolen Future*. United States: Dutton .

Coulomb, I., Deak, B., Matter, B., Saabye, A., Spinosa, L., & Utvik, Å. Ø. (1997). *Sludge Treatment and Disposal Management Approaches and Experiences*. Copenhagen: European Environment Agency.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R., & Kraak, G. V. (2002). *Global Assessment of the State of the Science of Endocrine Disruptors*. WHO: IPCS-International Programme on Chemical Safety.

Degen, G., & Bolt, H. (2000). Endocrine Disruptors: update on xenoestrogens. *Int. Arch. Occup. Environ. Health*, 73(7), 433-441.

DIE. (2008). *Municipalities Wastewater Statistical Data*. TUIK.

Ding, Y., Zhang, W., Gu, C., Xagorarakis, I., & Li, H. (2011). Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. *J. Chromatogr. A*, 1218(1), 10–16.

Dytczak, M., Londry, K., Siegrist, H., & Oleszkiewicz, J. (2007). Ozonation Reduces Sludge Production and Improves Denitrification. *Water Res.*, 41(3), 543-550.

Egemen, E., Corpening, J., & Nirmalakhandan, N. (2001). Evaluation of an Ozonation System for Reduced Waste Sludge Generation. *Water Sci. Technol.*, 44(2-3), 445-452.

EPA . (2009). Occurrence of Contaminants of Emerging Concern in Wastewater From Nine Publicly Owned Treatment Works. Washington DC: U.S. Environmental Protection Agency.

Esplugas, S., Bila, D. M., Krause, L. G., & Dezotti, M. (2007). Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. *J. Hazard Mater.*, 149(3), 631–642.

Fabiyi, M., Novak, R., Ried, A., Wieland, A., Capra, A., & Sandon, A. (2007). Sludge Reduction using Ozone Induced Lysis. World Congress on Ozone and Ultraviolet Technologies. Los Angeles: Praxair.

Filibeli, A., & Ayol, A. (2007). Historical development of sludge management in Turkey. Facing Sludge Diversities: Challenges, Risks and Opportunities, 39-44. Antalya: IWA.

Foladori, P., Andreottola, G., & Ziglio, G. (2010). Cell Lysis and Cryptic Growth. Sludge Reduction Technologies in Wastewater Treatment Plants (pg. 32). London: IWA .

Fromme, H., Kuchler, T., Otto, T., Pilz, K., Müller, J., & Wenzel, A. (2002). Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.*, 36(6), 1429–1438.

Gaudy, A. F., Yang, P., & Obayashi, A. (1971). Studies on the total oxidation of activated sludge with and without hydrolytic pretreatment. *J. Water Pollut. Con. F.*, 43(1), 40-54.

Glassmeyer, S., Furlong, E., Kolpin, D., Cahill, J. D., Zaugg, S., Werner, S. , Meyer, M. T., Kryak, D. D. (2005). Transport of Chemical and Microbial

Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination. *Environ. Sci. Technol.*, 39(14), 5157-5169.

Gledhill, W. E., Kaley, R. G., Adams, W. J., Hicks, O., Michael, P. R., & Saeger, V. W. (1980). An Environmental Safety Assessment of Butyl Benzyl Phthalate. *Environ. Sci. Technol.*, 14(3), 301-305.

Gottschalk, C., Libra, J. A., & Saupe, A. (2000). Ozonation of water and wastewater. Weinheim: Wiley.

Gray, N. (1989). Biology of wastewater treatment. New York: Oxford University Press.

Henriques, M., Cardoso, V. V., Rodrigues, A. M., Ferreira, E., Benoliel, M. J., & Almeida, C. M. (2010). Simultaneous Determination of Ten Endocrine Hormone Disrupters in Water Using SPE/LC-(ESI)MS-MS. *J. Water Resource Prot.*, 2, 818-829.

Hernando, M. D., Heath, E., Petrovic, M., & Barceló, D. (2006). Trace-level determination of pharmaceutical residues by LC-MS/MS in natural and treated waters. A pilot-survey study. *Anal. Bioanal. Chem.*, 385(6), 985-991.

Hu, J. Y., Chen, X., Tao, G., & Kekred, K. (2007). Fate of Endocrine Disrupting Compounds in Membrane Bioreactor Systems. *Environ. Sci. Technol.*, 41(11), 4097-4102.

IARC. (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon: World Health Organization.

Jeannot, R., Sabik, H., Sauvard, E., Dagnac, T., & K.Dohrendorf. (2002). Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry. *J. Chromatogr. A*, 974(1-2), 143-159.

Jiang, J., Yin, Q., Zhou, J., & Pearce, P. (2005). Occurrence and treatment trials of endocrine disrupting chemicals (EDCs) in wastewaters. *Chemosphere*, 61(4), 544-550.

Kamiya, T., & Hirotsuji, J. (1998). New combined system of biological process and intermittent ozonation for advanced wastewater treatment. *Water Sci. Technol.*, 38(8-9), 145–153.

Kelvin, K. Y. (2008, January). An Evaluation of the Factors Controlling Biodegradation of Endocrine Disrupting Chemicals During Wastewater Treatment. London: PhD thesis Imperial College London, Faculty of Medicine.

Kim, Y., Choi, K., Jung, J., Park, S., Kim, P., & Park, J. (2007). Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environ. Int.*, 33(3), 370–375.

Kuster, M., Alda, M. J., & Barcelo, D. (2004). Analysis and distribution of estrogens and progestogens in sewage sludge, soils and sediments. *Trac-Trend. Anal. Chem.*, 23(10-11), 790-798.

Kuster, M., Alda, M. J., & Barceló, D. (2005). Estrogens and Progestogens in Wastewater, Sludge, Sediments, and Soil. *The Handbook of Environmental Chemistry*, 5, 1-24.

Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., & Wenzel, A. (2003). Endocrine Disruptors in the Environment (IUPAC Technical Report). *Pure Appl. Chem.*, 75(5), 631–681.

Lister, A. L., & Kraak, G. J. (2001). Endocrine Disruption: Why Is It So Complicated? *Water Qual. Res. J. Canada*, 36(2), 175-190.

Liu, R., Zhou, J., & Wilding, A. (2004). Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction–gas chromatography–mass spectrometry. *J. Chromatogr. A*, 1022(1-2), 179–189.

Liu, S., Ying, G., Zhao, J., Chen, F., Yang, B., Zhou, L., et al. (2011). Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry. *J. Chromatogr. A*, 1218(10), 1367–1378.

Liu, Y. (2003). Chemically reduced excess sludge production in the activated sludge process. *Chemosphere*, 50(1), 1-7.

Long, G., & Meek, M. E. (2001). Butylbenzylphthalate: Hazard Characterization and Exposure-Response Analysis. *Environ. Carcino. Ecotox. Revs*, 19(1), 105-123.

McClellan, K., & Halden, R. U. (2010). Pharmaceuticals and personal care products in archived U.S.biosolids from the 2001 EPA national sewage sludge survey. *Water Res.*, 44(2), 658-668.

Mines, R., Northenor, C. B., & Murchison, M. (2008). Oxidation and ozonation of waste activated sludge. *J Environ Sci Health A*, 43(6), 610-618.

Müller, J. (2000). Pretreatment Processes for the Recycling and Reuse of Sewage Sludge. *Water Sci. Technol.*, 42(9), 167-174.

Nelson, E. B., Montes, M. & Goldstein, M. (1980). Effectiveness of metyrapone in the treatment of acetaminophen toxicity in mice. *Toxicology*, 17(1), 73-81.

Park, K. Y., Maeng, S. K., Song, K. G., & Ahn, K. H. (2008). Ozone treatment of wastewater sludge for reduction and stabilization. *J. Environ. Sci. Health A.*, 43(13), 1546-1550.

Park, K., Ahn, K.-H., Maeng, S., Hwang, J., & Kwon, J. (2003). Feasibility of Sludge Ozonation for Stabilization and Conditioning. *Ozone-Sci. Eng.*, 25(1), 73-80.

Paul, E., & Debellefontaine, H. (2007). Reduction of excess sludge produced by biological treatment processes: effect of ozonation on biomass and on sludge. *Ozone-Sci. Eng.*, 29(6), 415-427.

Pe´rez-Elvira, S. I., Diez, P. N., & Fdz-Polanco, F. (2006). Sludge minimisation technologies. *Rev. Environ. Sci. Biotechnol.*, 5(4), 375–398.

Petrovic, M., Gonzalez, S., & Barcelo, D. (2003). Analysis and removal of emerging contaminants in wastewater and drinking water. *Trac-Trend. Anal. Chem.*, 22(10), 685-696.

Pholchan, P., Jones, M., Donnelly, T., & Sallis, P. J. (2008). Fate of Estrogens during the Biological Treatment of Synthetic Wastewater in a Nitrite-Accumulating Sequencing Batch Reactor. *Environ. Sci. Technol.*, 42(16), 6141–6147.

Picó, Y., Fernández, M., Ruiz, M. J., & Font, G. (2007). Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *J. Biochem. Biophys. Methods*, 70(2), 117–131.

Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., & Sumpter, J. (1994). Estrogenic Effects of Effluents From Sewage Treatment Works. *Chem. Ecol.*, 8(4), 275-285.

Ramakrishna, D. M., & Viraraghavan, T. (2005). Strategies for sludge minimization in activated sludge process- A review. *Fresen. Environ. Bull.*, 14(1), 2-12.

Rocher, M., Goma, G., Begue, A. P., Louvel, L., & Rols, J. L. (1999). Towards a reduction in excess sludge production in activated sludge processes: Biomass physicochemical treatment and biodegradation. *Appl. Microbiol. Biotechnol.*, 51(6), 883-890.

Rosley, P., Vorkamp, K., Aarup, J., Frederiksen, K., & Nielsen, P. H. (2007). Degradation of phthalate esters in an activated sludge wastewater treatment plant. *Water Res.*, 41(5), 969 – 976.

Saby, S., Djafer, M., & Chen, G. (2002). Feasibility of using a chlorination step to reduce excess sludge in activated sludge process. *Water Res.*, 36(3), 656–666.

Saktaywin, W., Tsuno, H., Nagare, H., & Soyama, T. (2006). Operation of a New Sewage Treatment Process with Technologies of Excess Sludge Reduction and Phosphorus Recovery. *Water Sci. Technol.*, 53(12), 217-227.

Samini, M., Dehpour, A. R. & Hajinabi, K. (1997). The Effect of Lithium on Acute Toxicity of Carbamazepine in Mice. *Acta Med. Iranica*, 35(3-4), 74-76.

Scheminski, A., Krull, R., & Hempel, D. (2000). Oxidative Treatment of Digested Sewage Sludge With Ozone. *Water Sci. Technol.*, 42(9), 151-158.

Scheytt, T., Mersmann, P., Lindstadt, R., & Heberer, T. (2005). Determination of sorption coefficients of pharmaceutically active substances carbamazepine, diclofenac, and ibuprofen, in sandy sediments. *Chemosphere*, 60(2), 245–253.

Sievers, M., Ried, A., & Koll, R. (2004). Sludge Treatment by Ozonation-Evaluation of Full-Scale Results. *Water Sci. Technol.*, 49(4), 247-253.

Snyder, S. A., Wert, E. C., Rexing, D. J., & Zegers, R. E. (2006). Ozone Oxidation of Endocrine Disruptors and Pharmaceuticals in Surface Water and Wastewater. *Ozone-Sci. Eng.*, 28(6), 445-460.

Snyder, S., Vanderford, B., Pearson, R., Quinones, O., & Yoon, Y. (2003). Analytical Methods Used to Measure Endocrine Disrupting Compounds in Water. *Pract. Period. Hazard. Toxic Radioact. Waste Manage.*, 7(4), 224-234.

Soliman, M. A., Pedersen, J. A., & Suffet, I. (2004). Rapid gas chromatography-mass spectrometry screening method for human pharmaceuticals, hormones, antioxidants and plasticizers in water. *J. Chromatogr. A*, 1029(1-2), 223-237.

Spinosa, L. (2007). Sludge management: current questions and future prospects. Facing Sludge Diversities; Challenges, Risks, and Opportunities , 9-14. Antalya: IWA.

Spongberg, S., & Witter, J. (2008). Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. *Sci. Total Environ.*, 397(1-3), 148 – 157.

Spring, A. J. (2004). Advanced Treatment Methods for the Removal of Endocrine Disrupting Compounds from Municipal Wastewater. University of Toronto: MSc Thesis, Graduate Department of Civil Engineering.

Tchobanoglous, G., Burton, F. L., & Stensel, H. D. (2004). Treatment, Reuse and Disposal of Solids and Biosolids. *Metcalf&Eddy Wastewater Engineering Treatment and Reuse* (pg. 1451-1452). New York: McGraw Hill.

Ternes, T. A. (2001). Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. *Trac-Trend. Anal. Chem.*, 20(8), 419-434.

Ternes, T., Stumpf, M., Mueller, J., Haberer, K., Wilken, R. D., & Servos, M. (1999). Behavior and occurrence of estrogens in municipal sewage treatment plants- I. Investigations in Germany, Canada and Brazil. *Sci. Total Environ.*, 225(1-2), 81-90.

Tsuno, H., Arakawa, K., Kato, Y., & Nagare, H. (2008). Advanced Sewage Treatment with Ozone Under Excess Sludge Reduction, Disinfection and Removal of EDCs. *Ozone-Sci. Eng.*, 30(3), 238–245.

Tyler, C. R., Jobling, S., & Sumpter, J. P. (1998). Endocrine Disruption in Wildlife: A Critical Review of the Evidence. *Crit. Rev. Toxicol.*, 28(4), 319–361.

Vesilind, P., & Spinosa, L. (2001). Production and Regulations. P. Vesilind, & L. Spinosa, Sludge into biosolids: processing, disposal, utilization (s. 3-4). London: IWA Publishing.

Vesilind, P. A. 2003. Wastewater Treatment Plant Design. Alexandria, USA : IWA, 2003.

Waring, R., & Harris, R. (2005). Endocrine disruptors: A human risk? *Mol. Cell Endocrinol.*, 244(1-2), 2-9.

Weemaes, M., Grootaerd, H., Simoens, F., & Verstraete, W. (2000). Anaerobic Digestion of Ozonized Biosolids. *Water Res.*, 34(8), 2330-2336.

Wei, Y., Van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003). Minimization of excess sludge production for biological wastewater treatment. *Water Res.*, 37(18), 4453–4467.

Weisglas-Kuperus, N., Vreugdenhil, H., & Mulder, P. (2004). Immunological effects of environmental exposure to PCBs and dioxins in Dutch children. *Toxicol Lett.*, 149(1-3), 281-285.

WWF. (1998). The Effect of Endocrine Disrupting Chemicals on Fish. Briefing for OSPAR
MMC:
<http://www.ngo.grida.no/wwfneap/Publication/briefings/Nonylphenol.pdf>

Yasui, H., Nakamura, K., Sakuma, S., Iwasaki, M., & Sakai, Y. (1996). A full-scale operation of a novel activated sludge process without excess sludge production. *Water Sci. Technol.*, 34(3-4), 395-404.

Yoon, Y., Westerhoff, P., Snyder, S. A., Wert, E. C., & Yoon, J. (2007). Removal of endocrine disrupting compounds and pharmaceuticals by nanofiltration and ultrafiltration membranes. *Desalination*, 270(1-2), 16–23.

Yu, Z., Peldszus, S., & Huck, P. M. (2007). Optimizing gas chromatographic–mass spectrometric analysis of selected pharmaceuticals and endocrine-disrupting substances in water using factorial experimental design. *J. Chromatogr. A*, 1148(1), 65-77.

Zhang, G., Yang, J., Liu, H., & Zhang, J. (2008). Sludge ozonation: Disintegration, supernatant changes and mechanisms. *Bioresource Technol.*, 100(3), 1505-1509.

Zhao, Q., & Kugel, G. (1997). Thermophilic/mesophilic digestion of sewage sludge and organic waste. *J. Environ. Sci. Health*, A31(9), 2211-2231.

Zhao, Y. X., Yin, J., Wang, J. H., Zhang, L., & Sun, Z. Y. (2007). Observations on Ozone Treatment of Excess Sludge. *Facing Sludge Diversities: Challenges, Risks and Opportunities*, 807-816. Antalya: IWA.

APPENDIX A

FIGURES OF EDC OPTIMIZATION AND REMOVAL

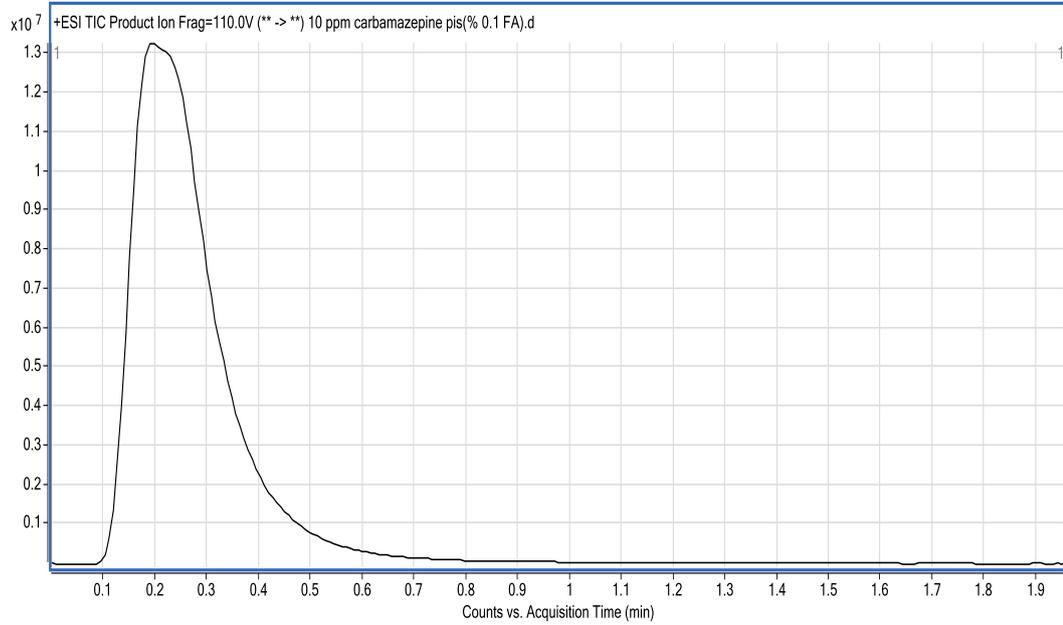


Figure A-1 MS2 Scan Peak of Carbamazepine for Mobile phase set with %0.1 F.A.

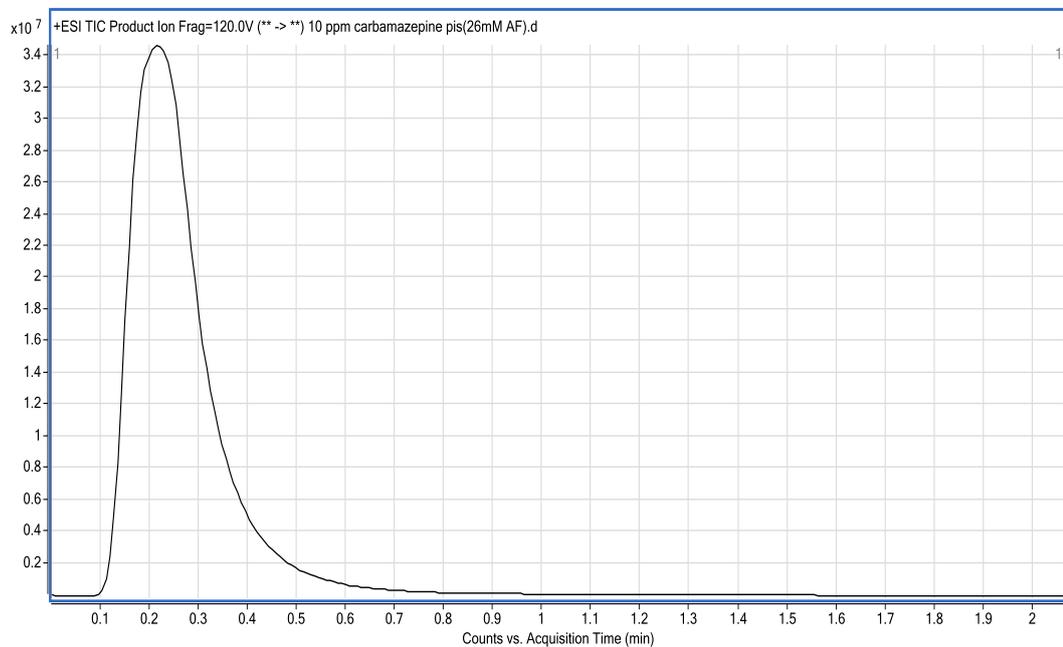


Figure A-2 MS2 Scan Peak of Carbamazepine for Mobile phase set with 26 mM

A.F.

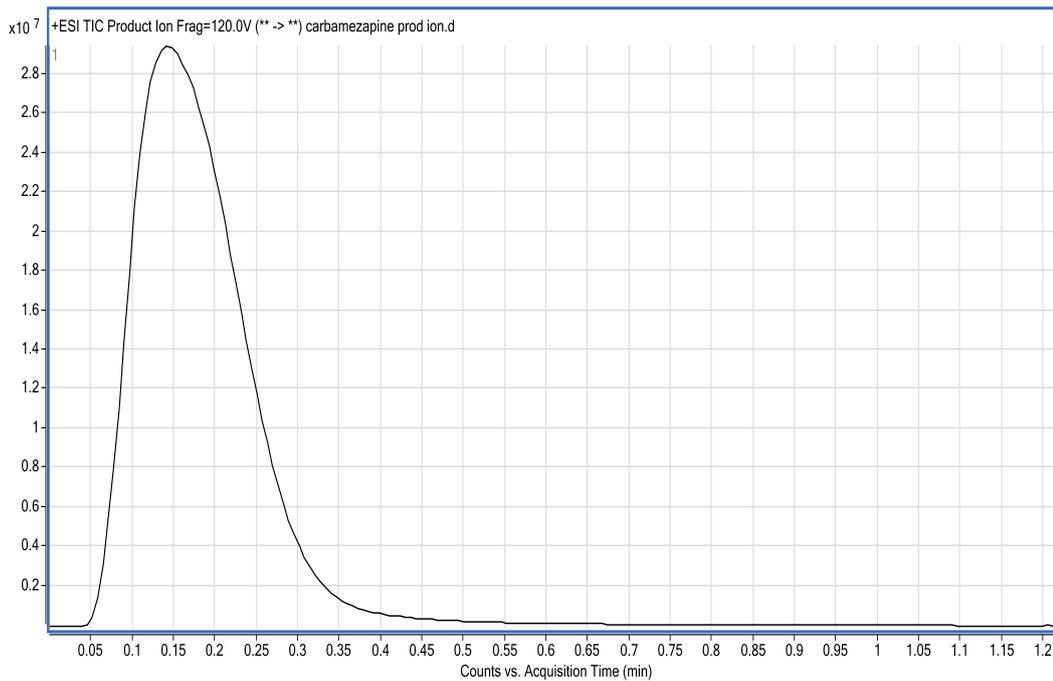


Figure A-3 MS2 Scan Peak of Carbamazepine for Mobile phase set with %0.1 F.A. + 5 mM A.F.

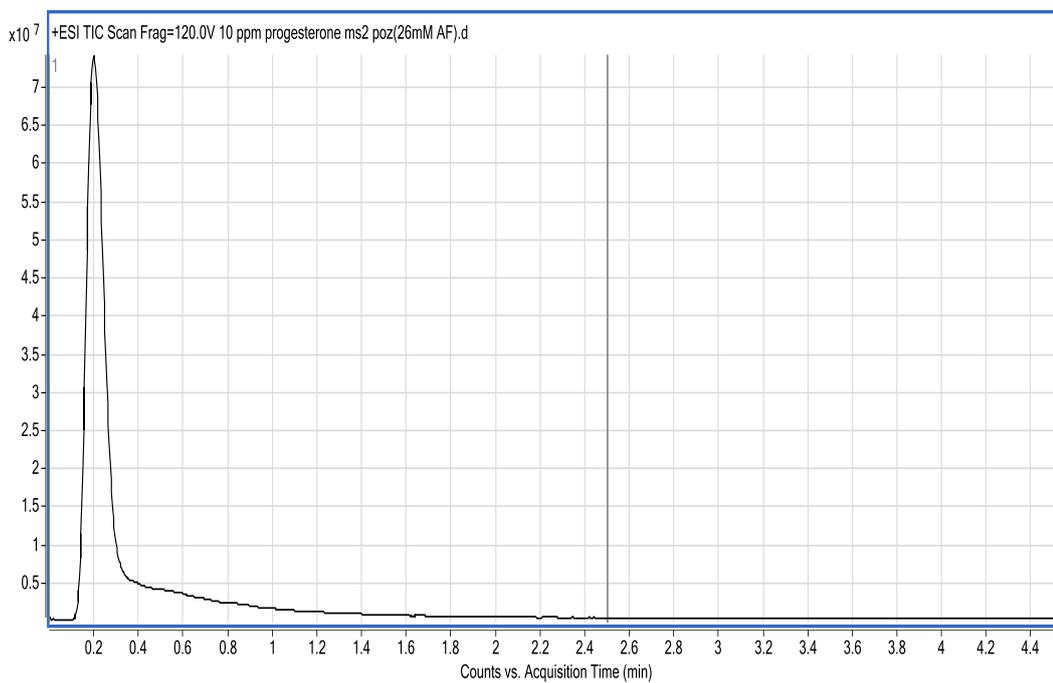


Figure A-4 MS2 Scan Peak of Progesterone for Mobile phase set with %0.1 F.A.

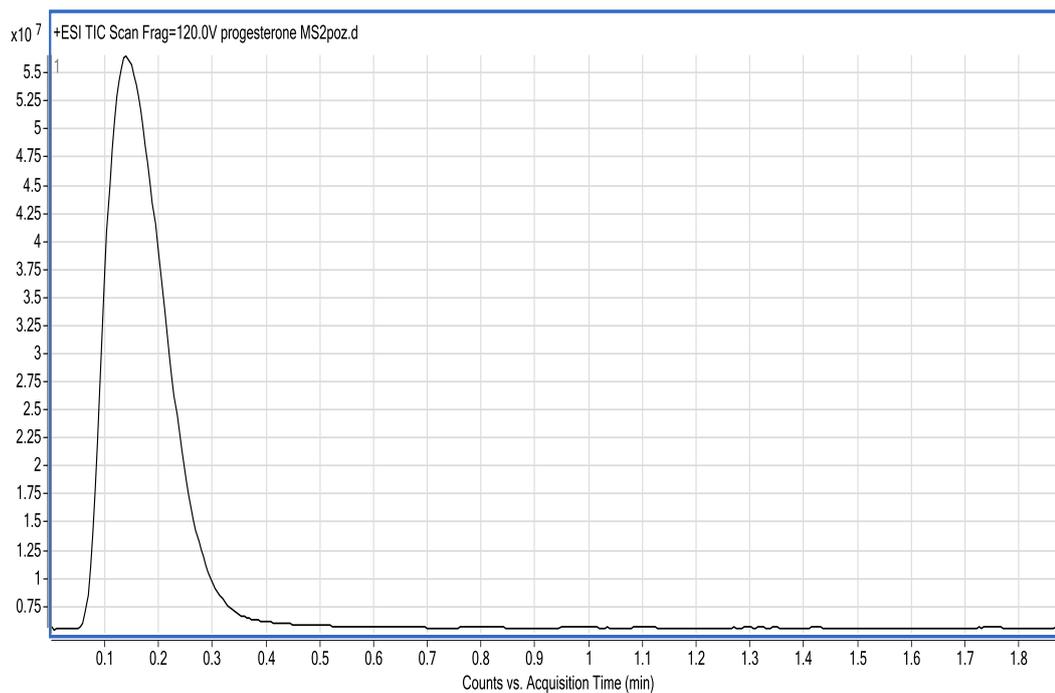


Figure A-5 MS2 Scan Peak of Progesterone for Mobile phase set with 26 mM A.F.

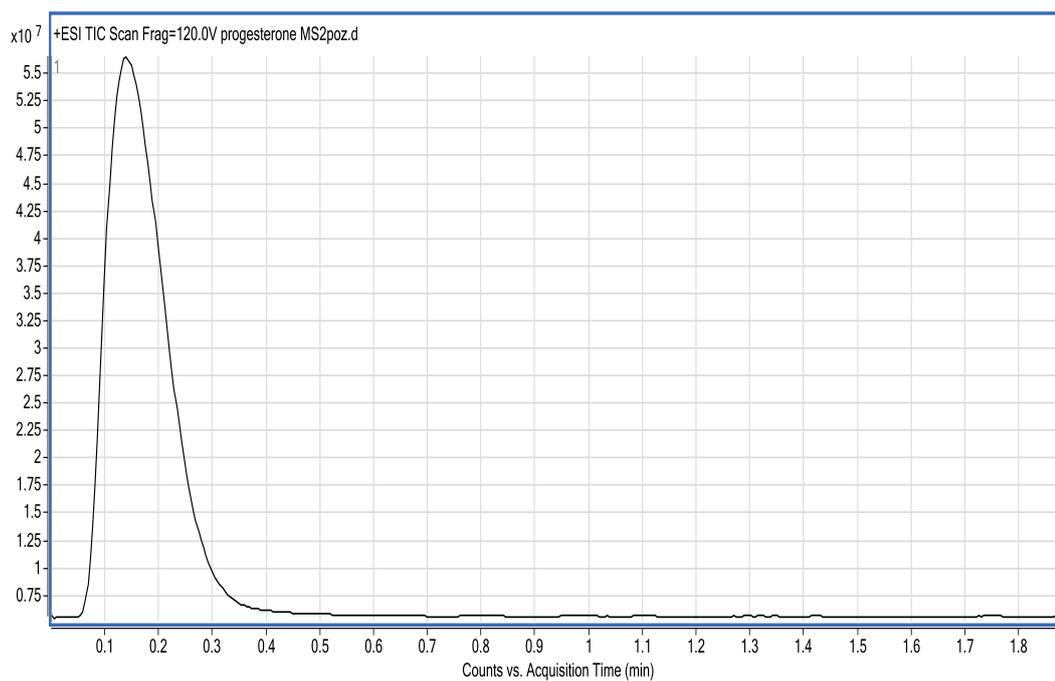


Figure A-6 MS2 Scan Peak of Progesterone for Mobile phase set with %0,1 F.A. + 5 mM A.F.

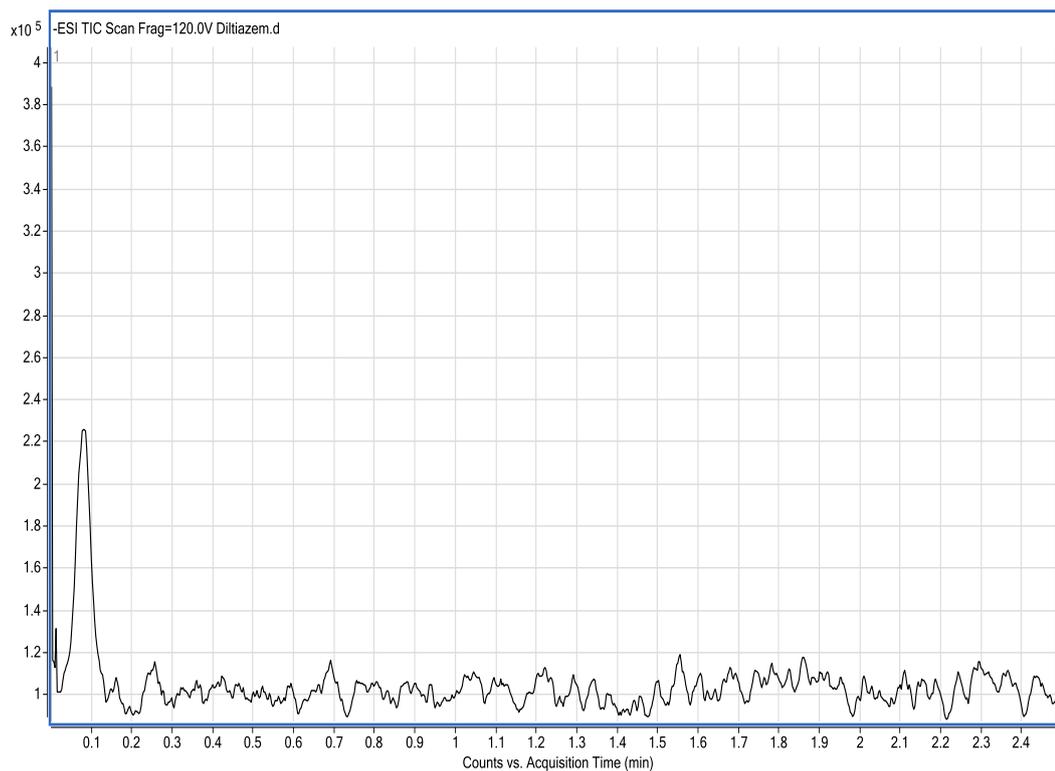


Figure A-7 Chromatogram of Diltiazem in negative mode

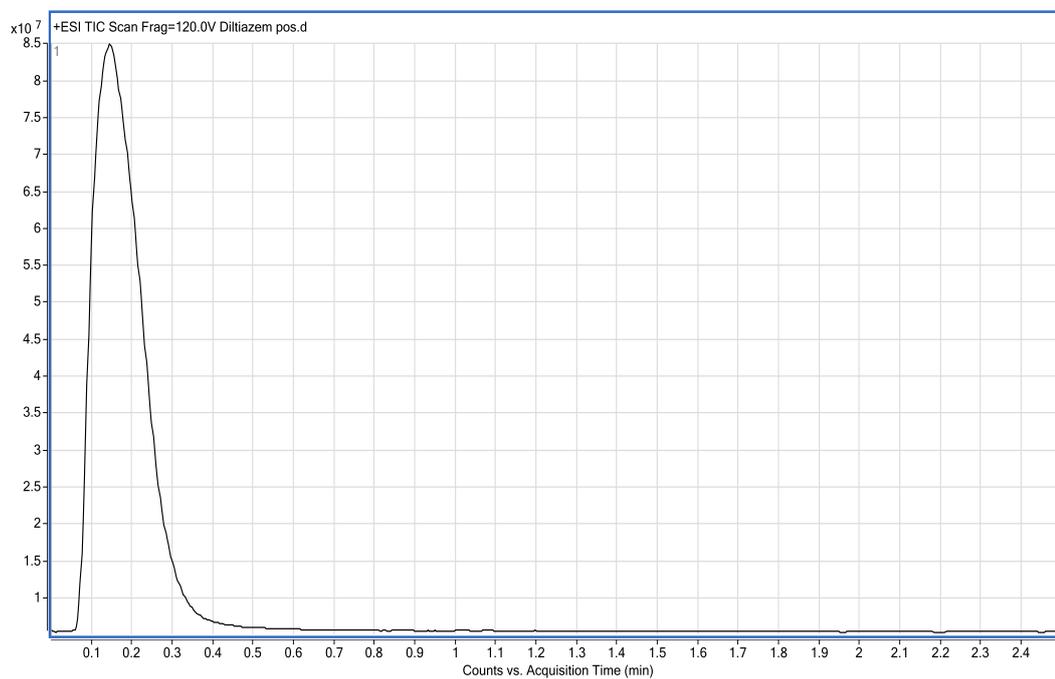


Figure A-8 Chromatogram of Diltiazem in positive mode

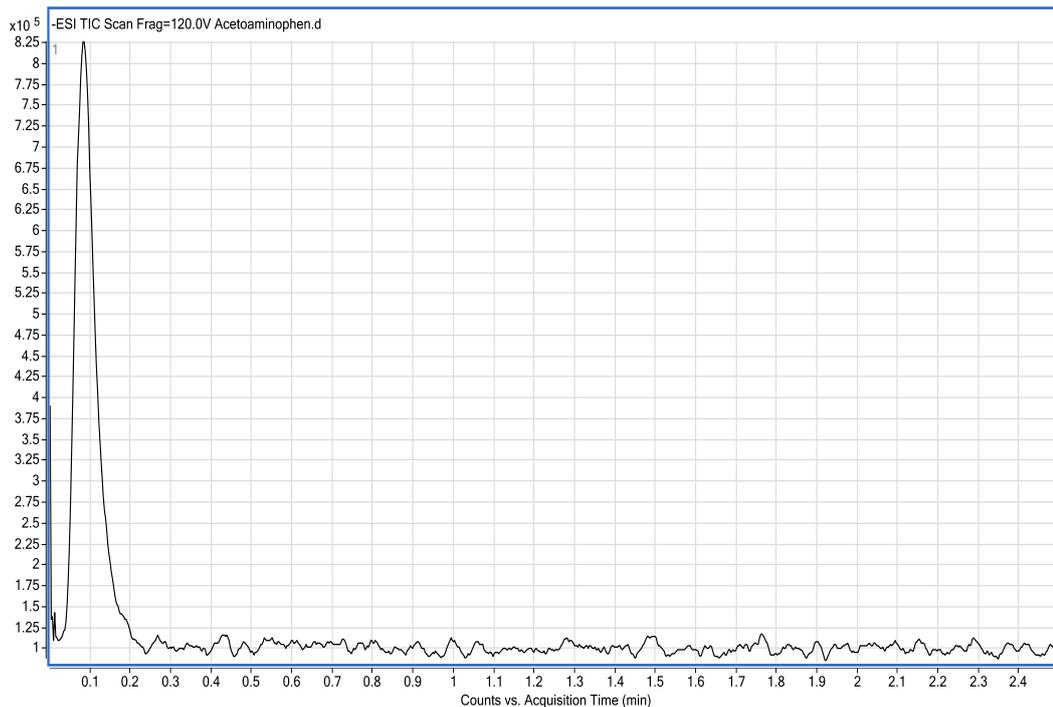


Figure A-9 Chromatogram of Acetaminophen in negative mode

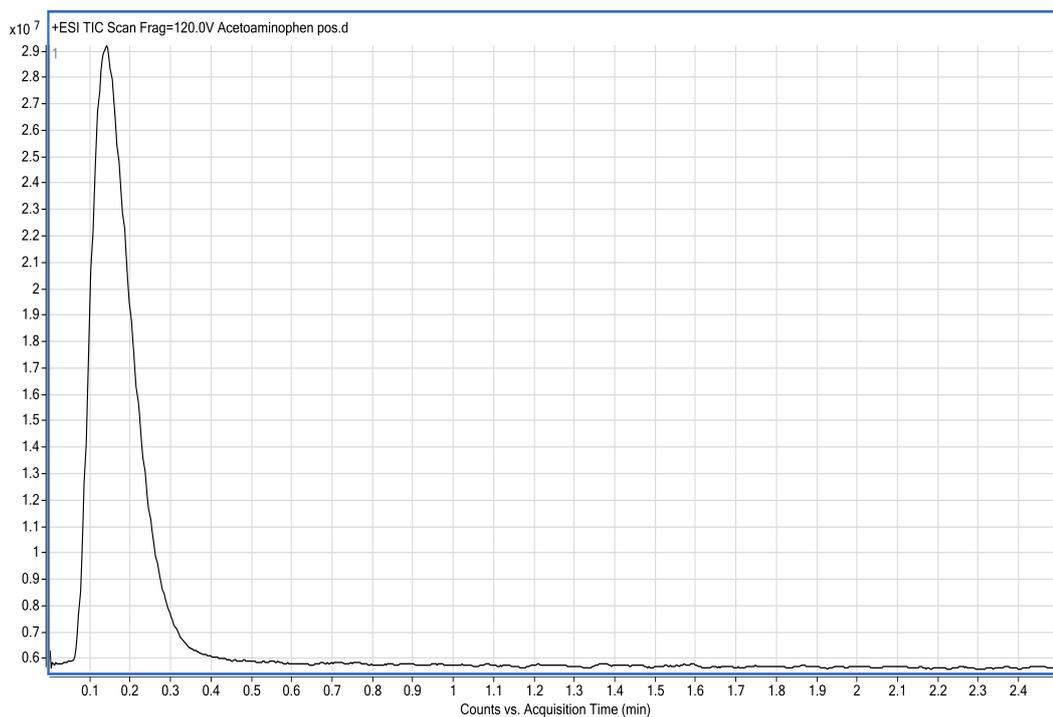


Figure A-10 Chromatogram of Acetaminophen in positive mode

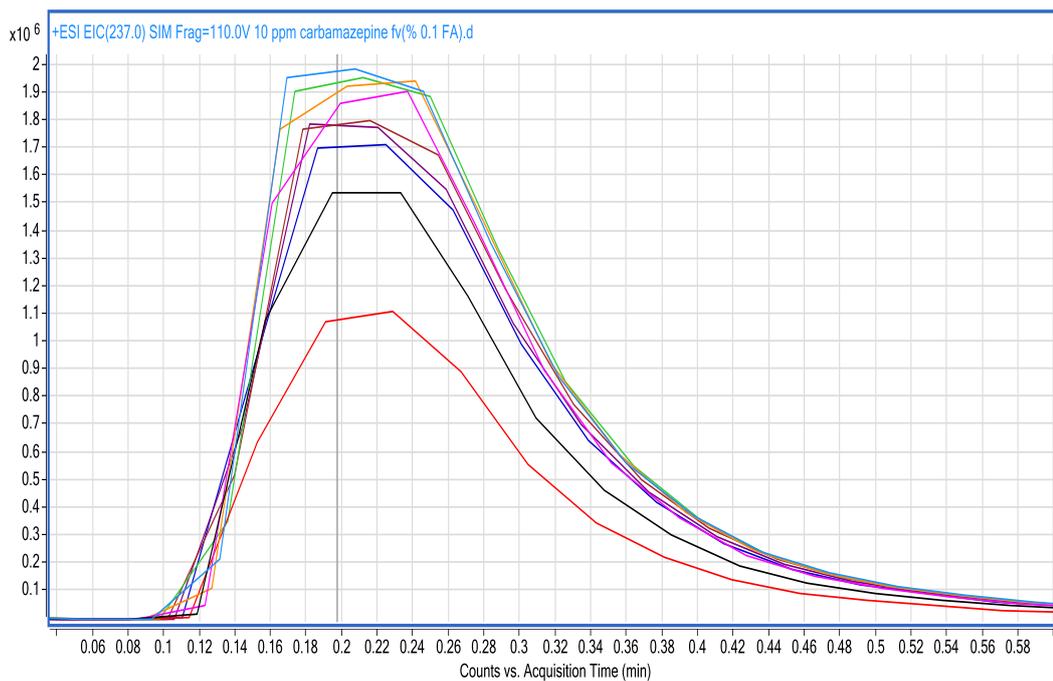


Figure A-11 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with %0.1 F.A.

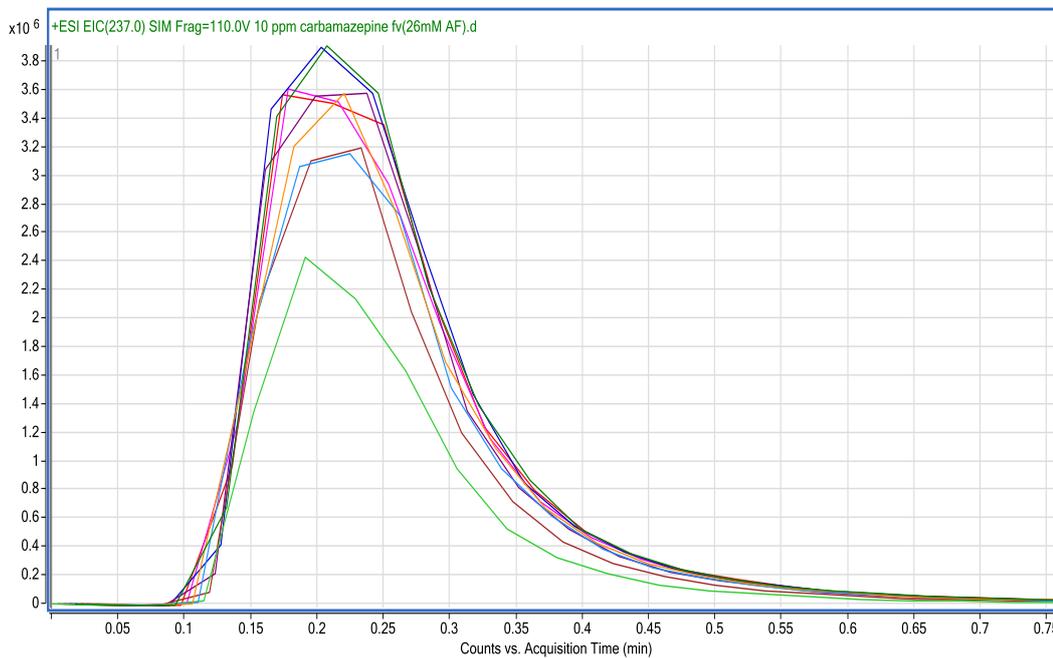


Figure A-12 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with 26 mM A.F.

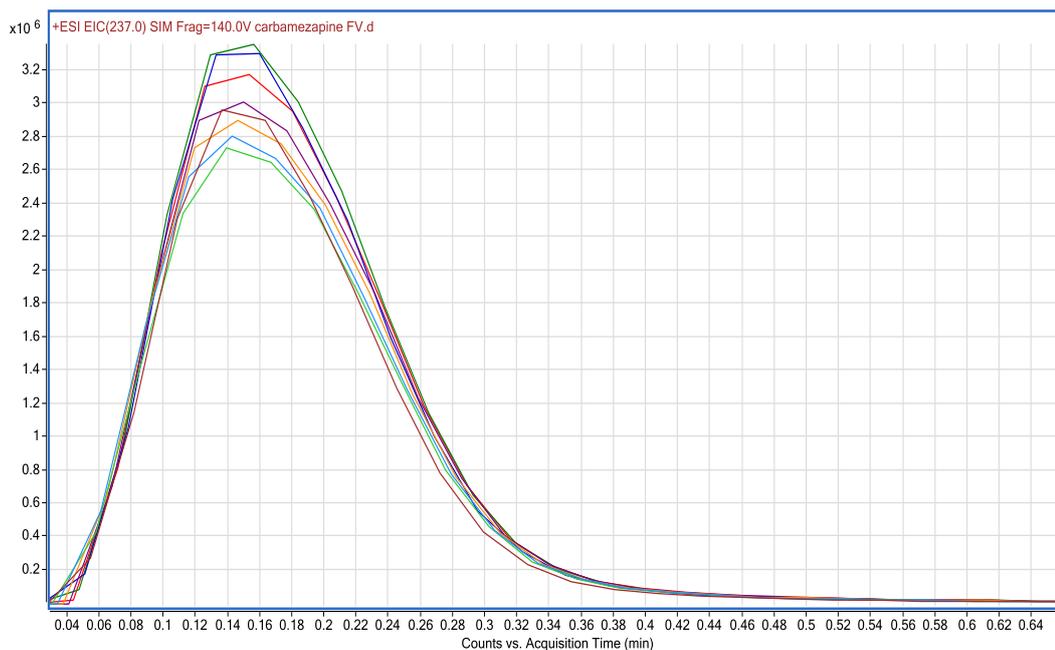


Figure A-13 Fragmentor Voltage Optimization of Carbamazepine for Mobile phase set with %0,1 F.A. + 5 mM A.F.

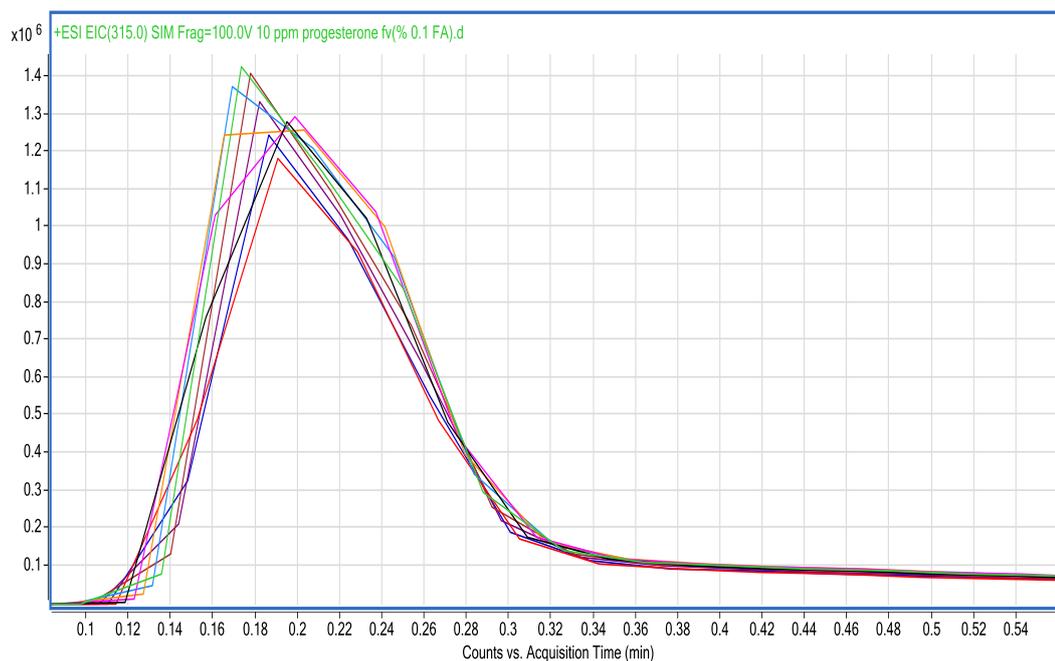


Figure A-14 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with %0.1 F.A.

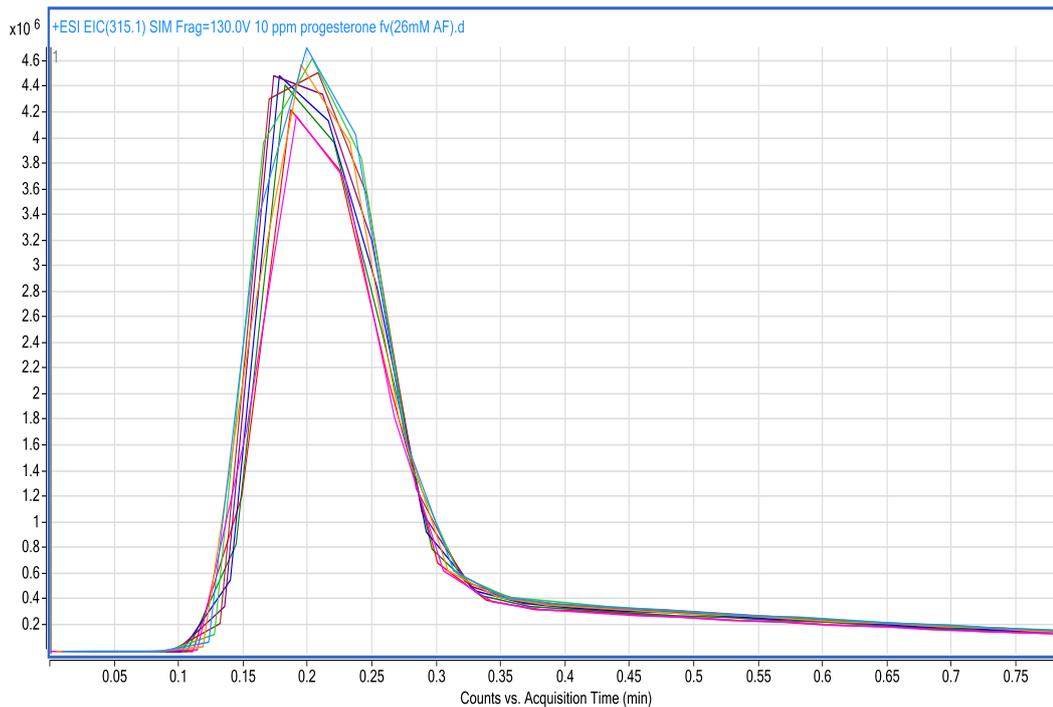


Figure A-15 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with 26 mM A.F.

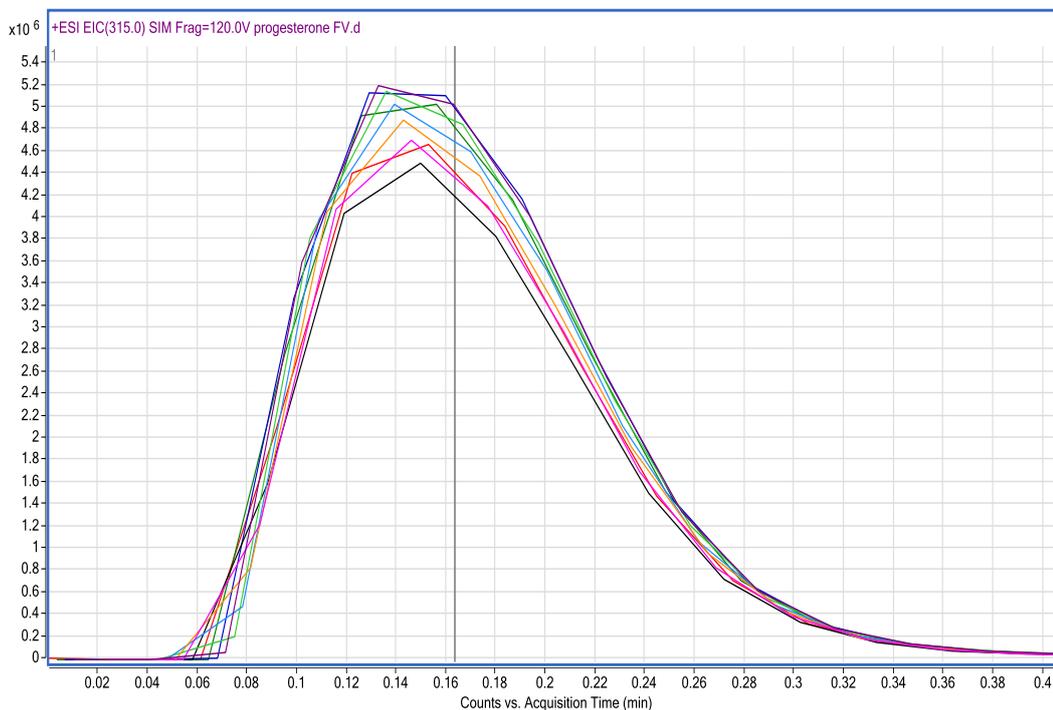


Figure A-16 Fragmentor Voltage Optimization of Progesterone for Mobile phase set with %0,1 F.A. + 5 mM A.F.

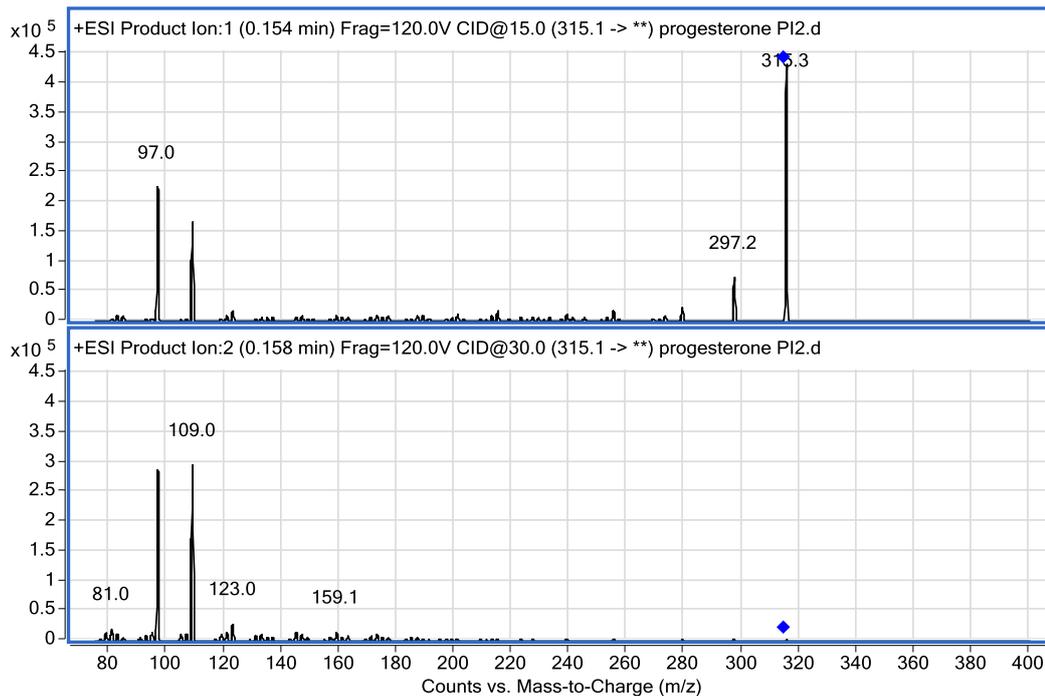


Figure A-17 Product Ion Analysis for Progesterone

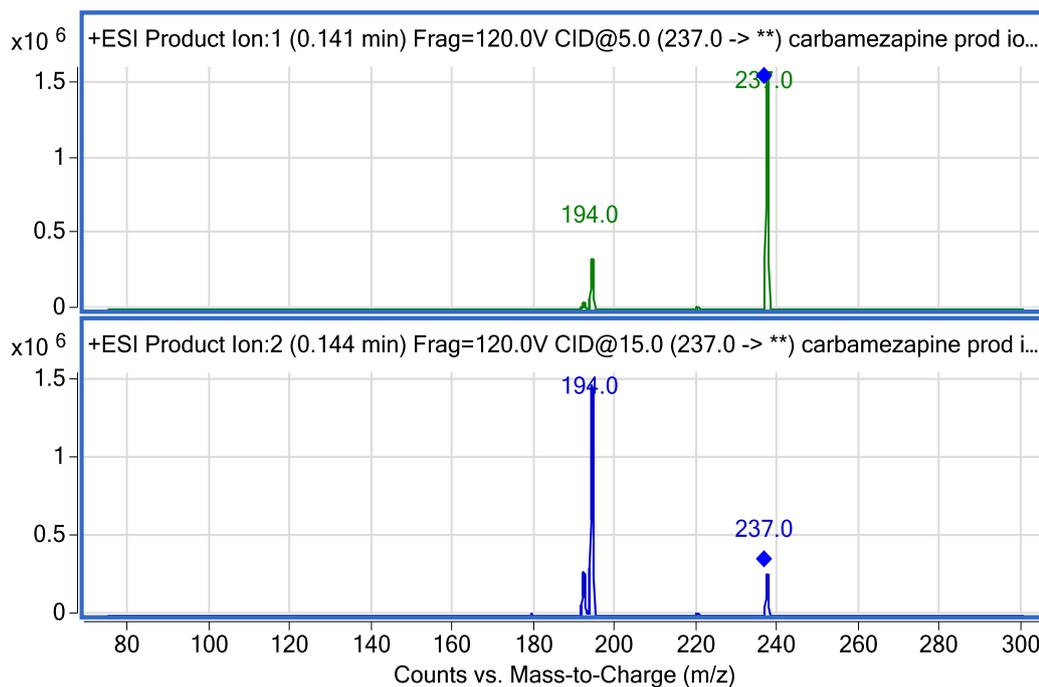


Figure A-18 Product Ion Analysis for Carbamazepine

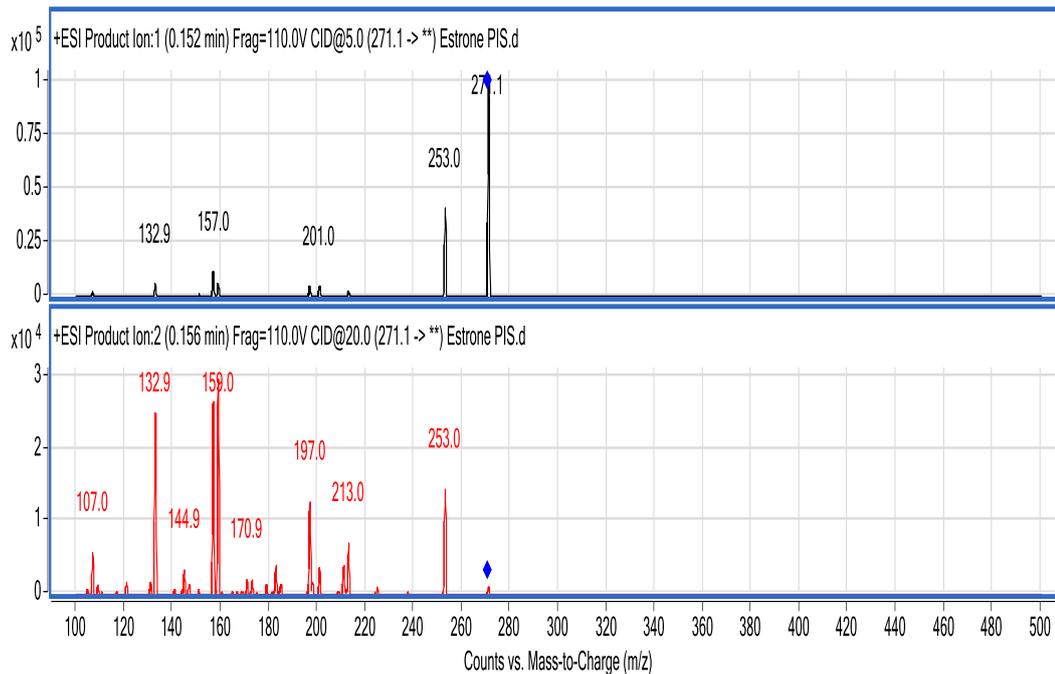


Figure A-19 Product Ion Analysis for Estrone

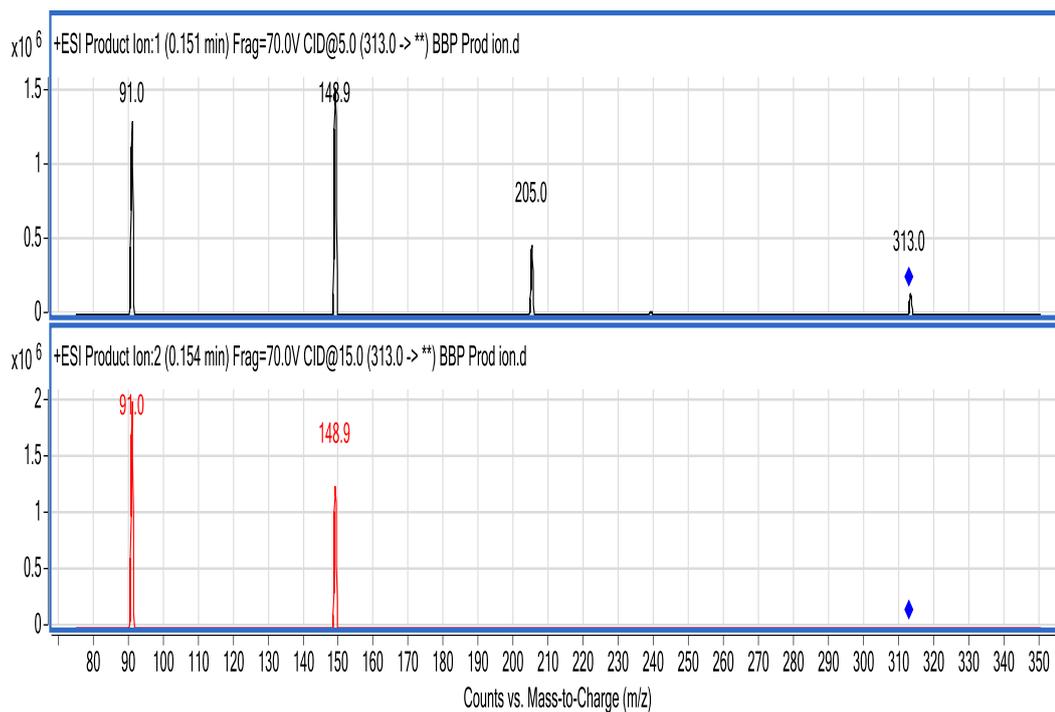


Figure A-20 Product Ion Analysis for BBP

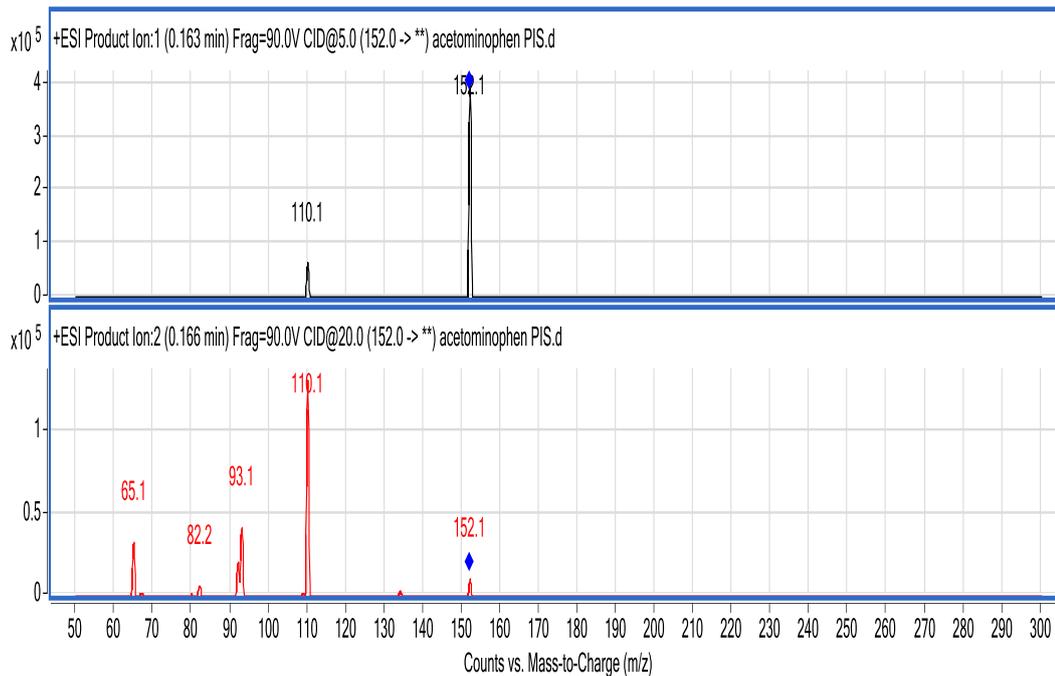


Figure A-21 Product Ion Analysis for Acetaminophen

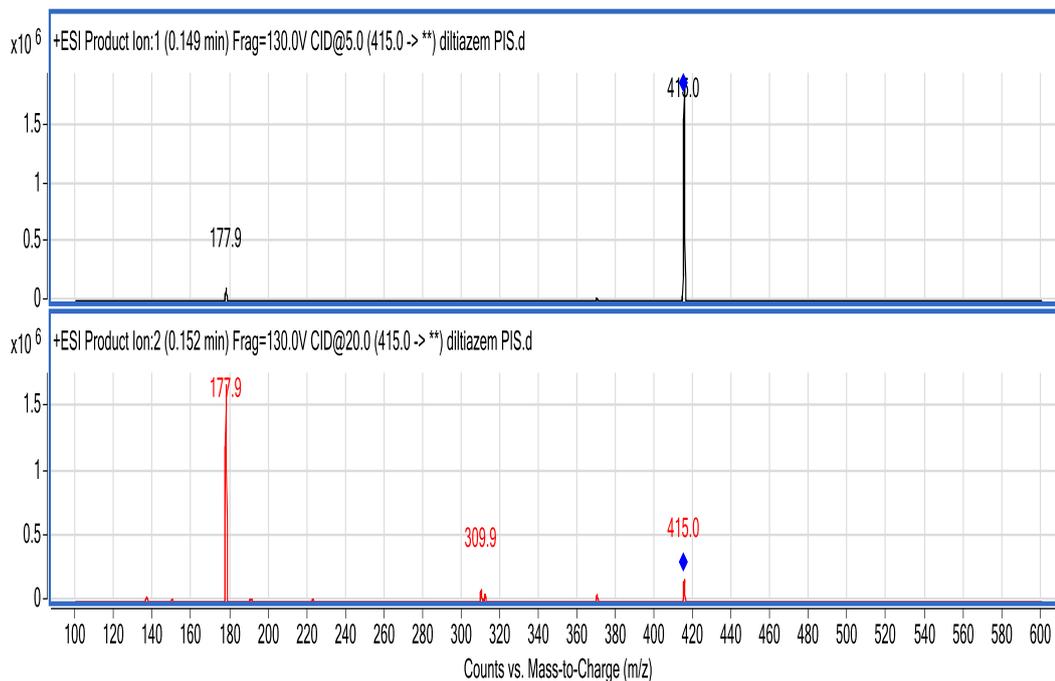


Figure A-22 Product Ion Analysis for Diltiazem

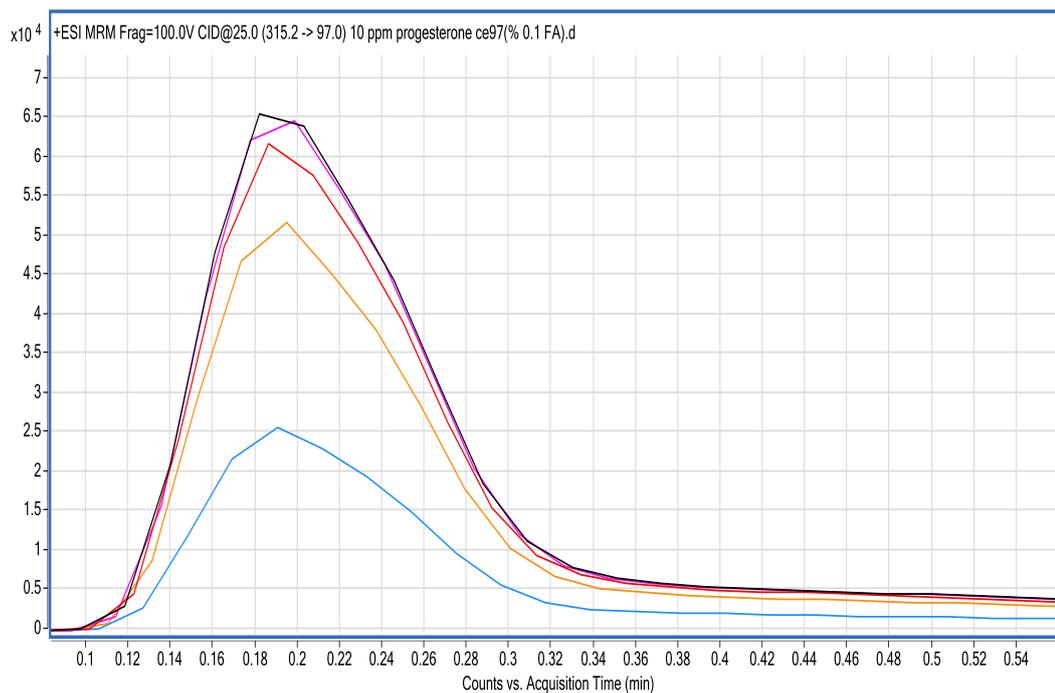


Figure A-23 Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with %0.1 F.A

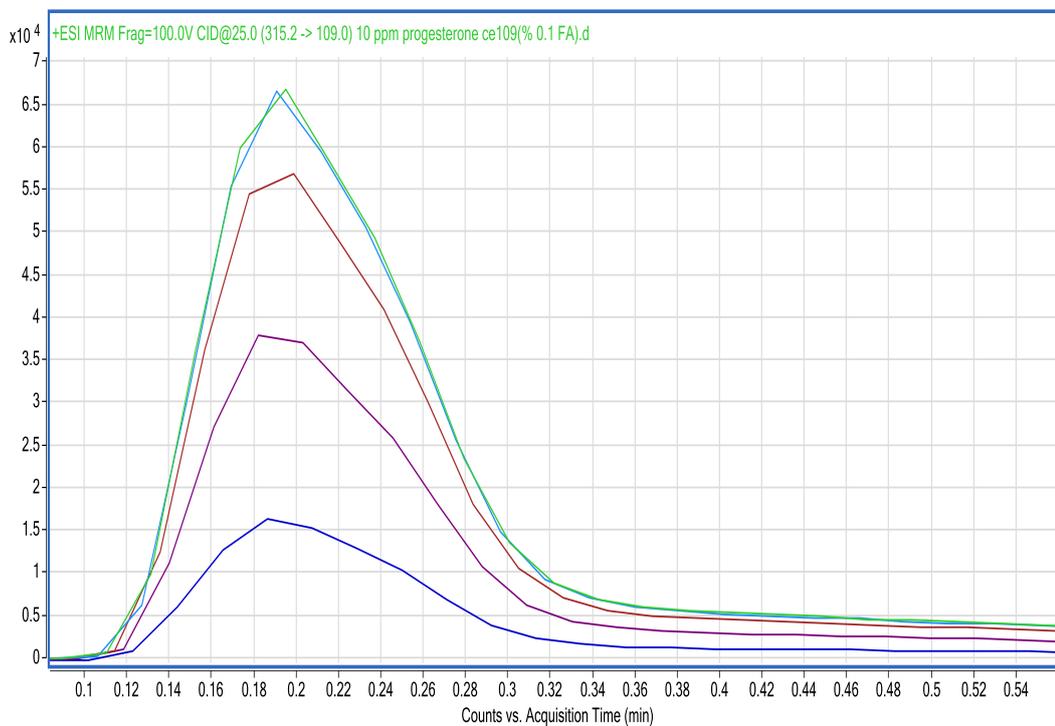


Figure A-24 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with %0.1 F.A

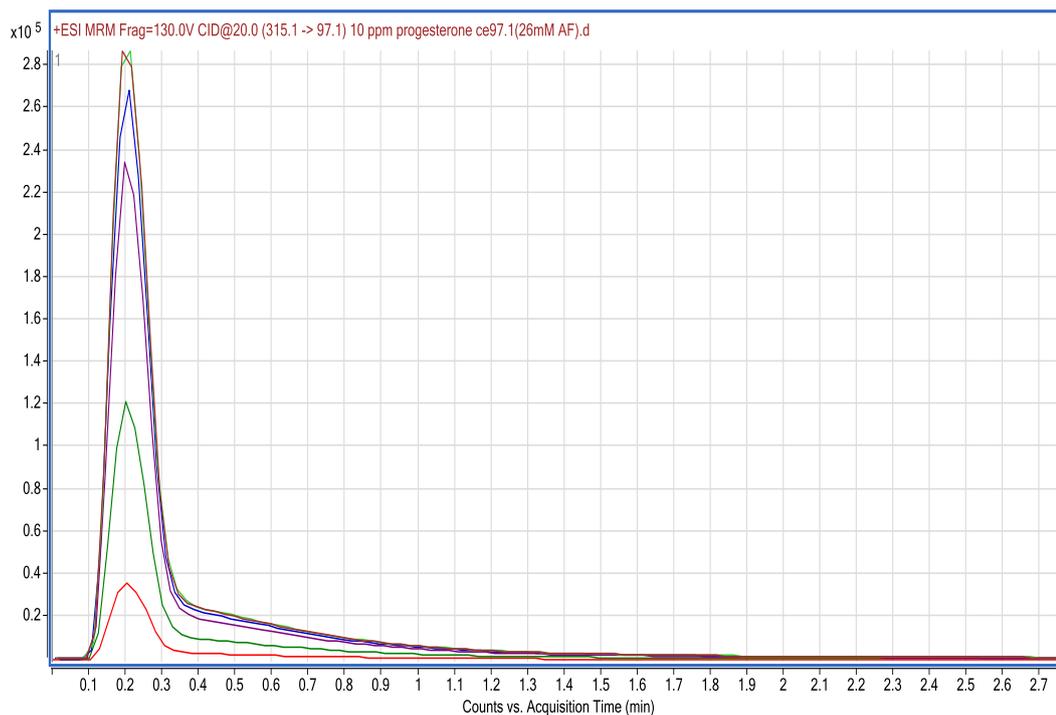


Figure A-25 26 mM Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with 26 mM A.F.

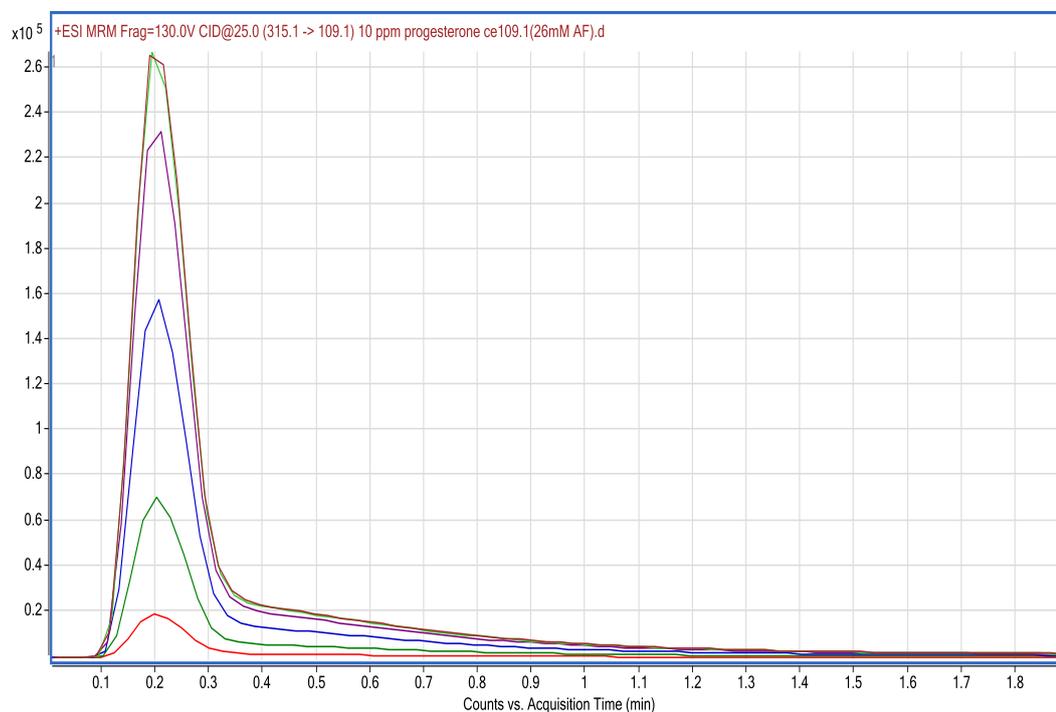


Figure A-26 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with 26 mM A.F.

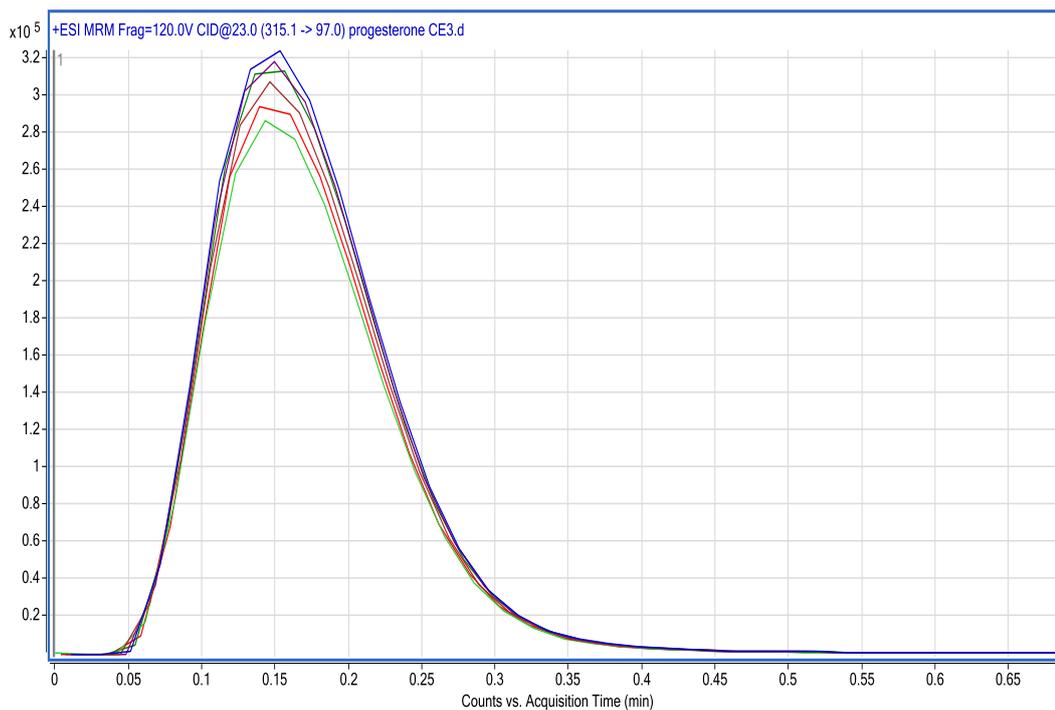


Figure A-27 Collision Energy Optimization of Progesterone 315>>97 for Mobile phase set with %0,1 F.A. + 5 mM A.F

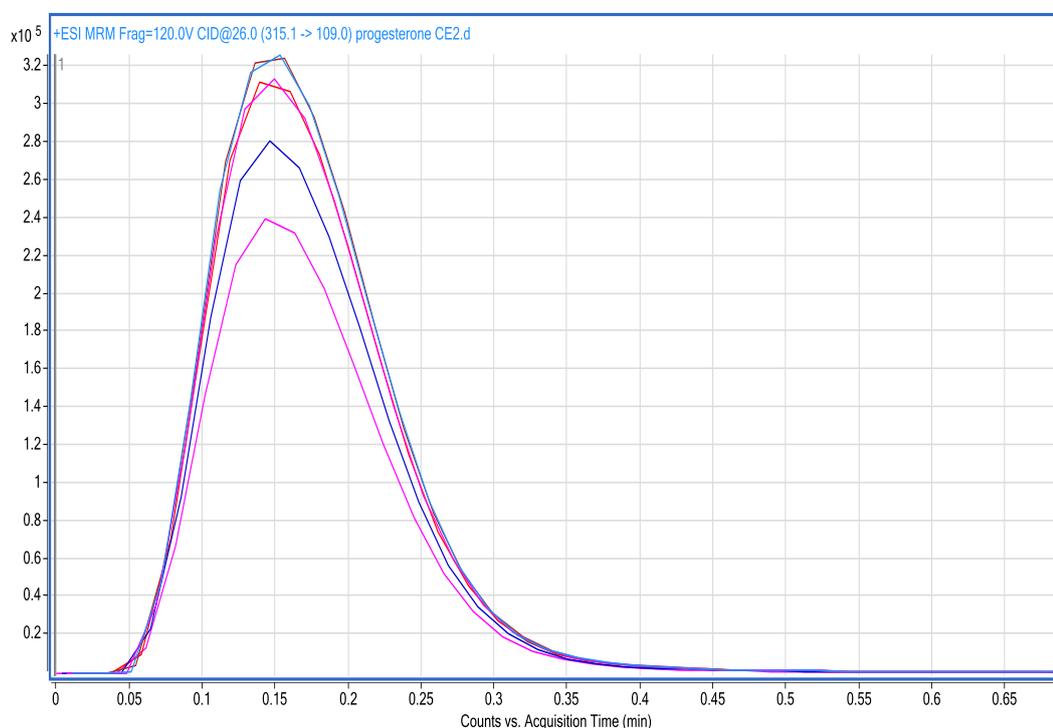


Figure A-28 Collision Energy Optimization of Progesterone 315>>109 for Mobile phase set with %0,1 F.A. + 5 mM A.F

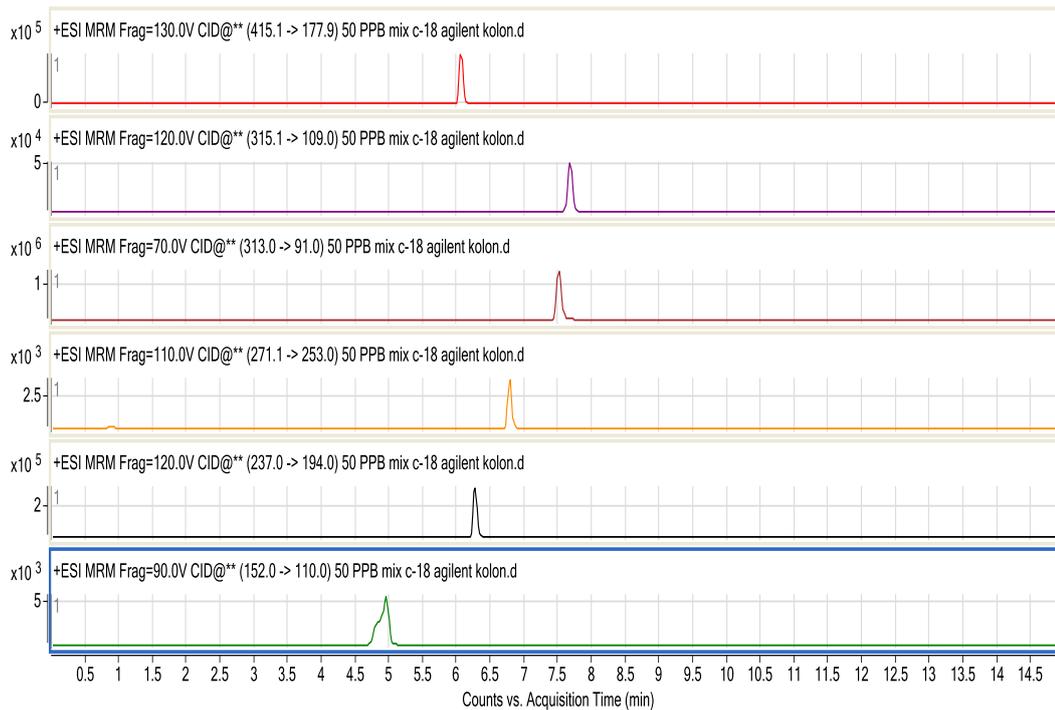


Figure A-29 Peak signals of all analytes with the mobile phase set with %0,1 F.A. + 5 mM A.F

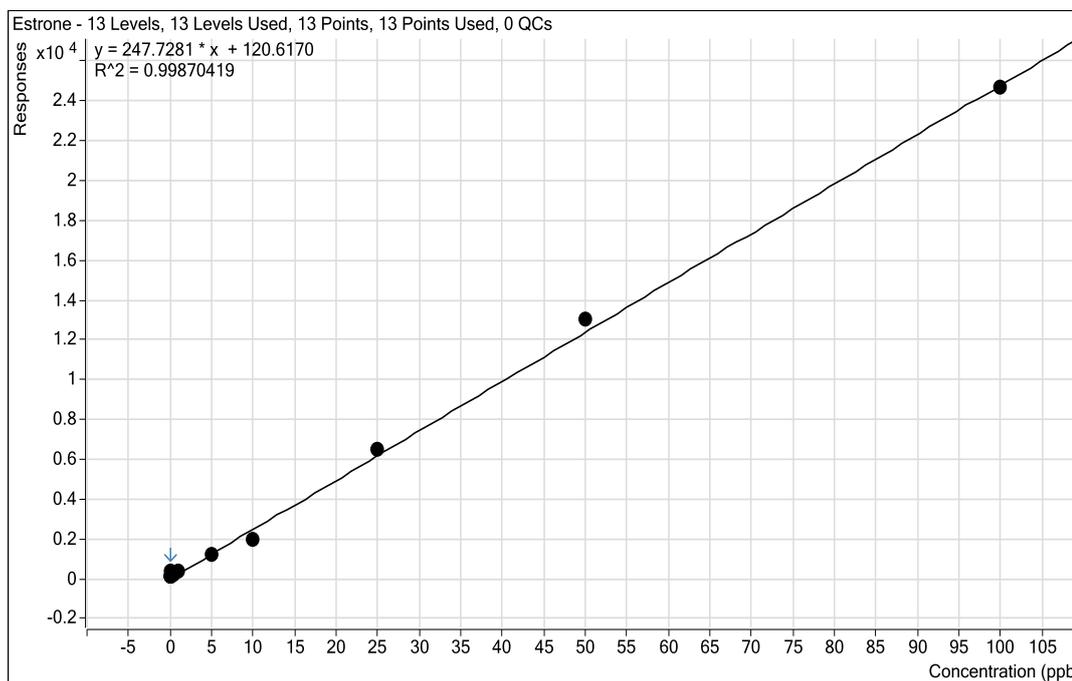


Figure A-30 Calibration curve for Estrone

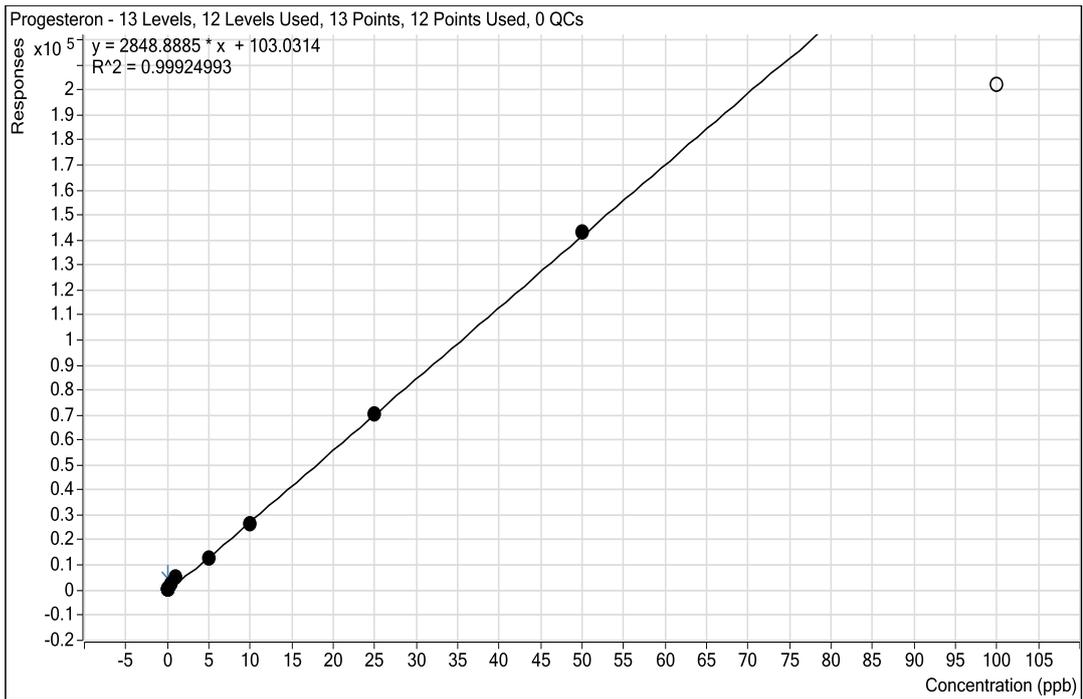


Figure A-31 Calibration curve for Progesterone

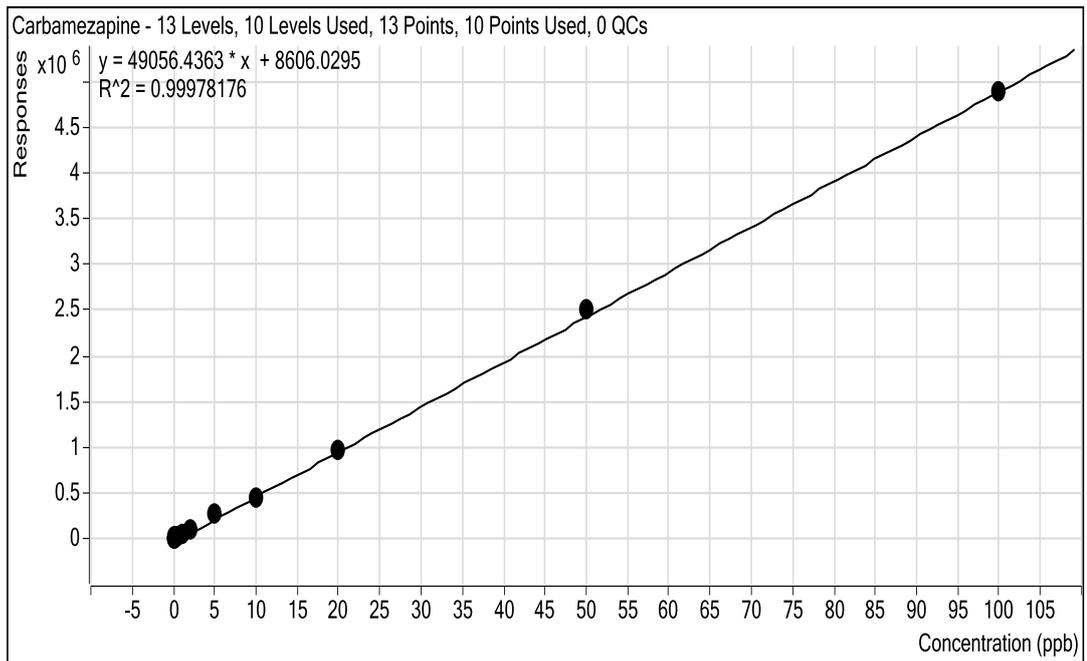


Figure A-32 Calibration curve for Carbamazepine

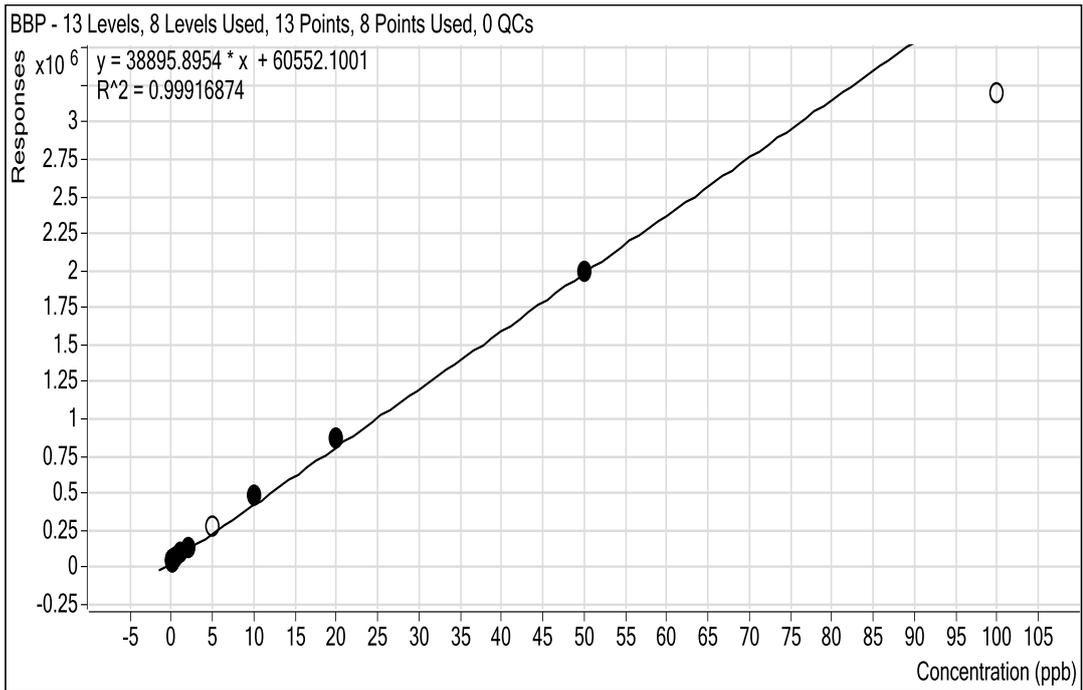


Figure A-33 Calibration curve for BBP

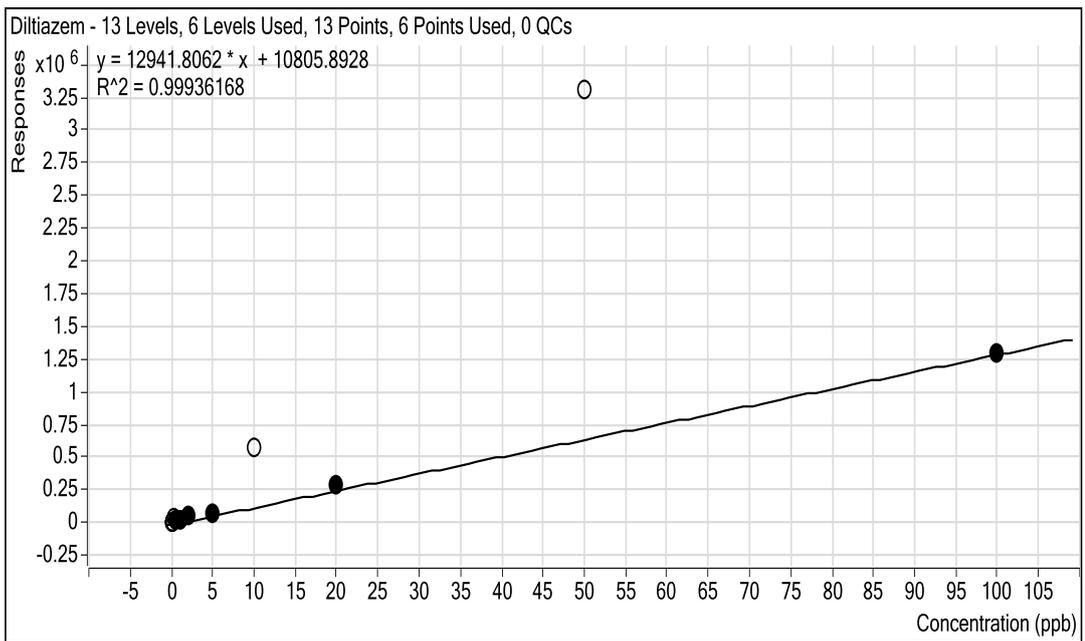


Figure A-34 Calibration curve for Diltiazem

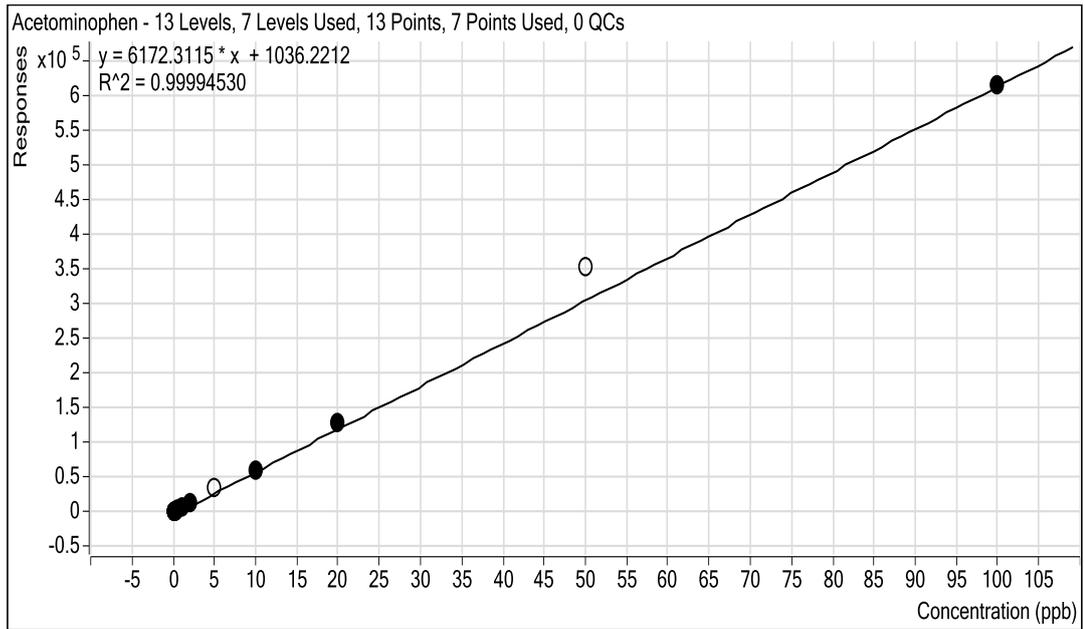


Figure A-35 Calibration curve for Acetaminophen

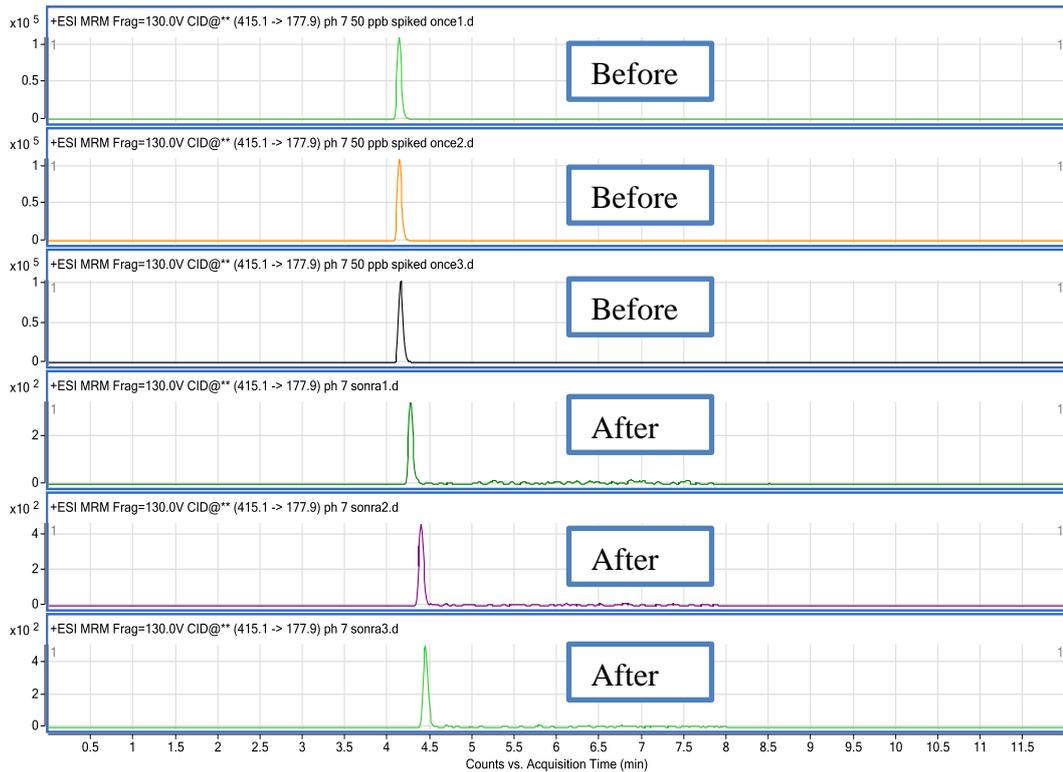


Figure A-36 Peak chromatograms of Diltiazem before and after extraction at pH 7 (n=3)

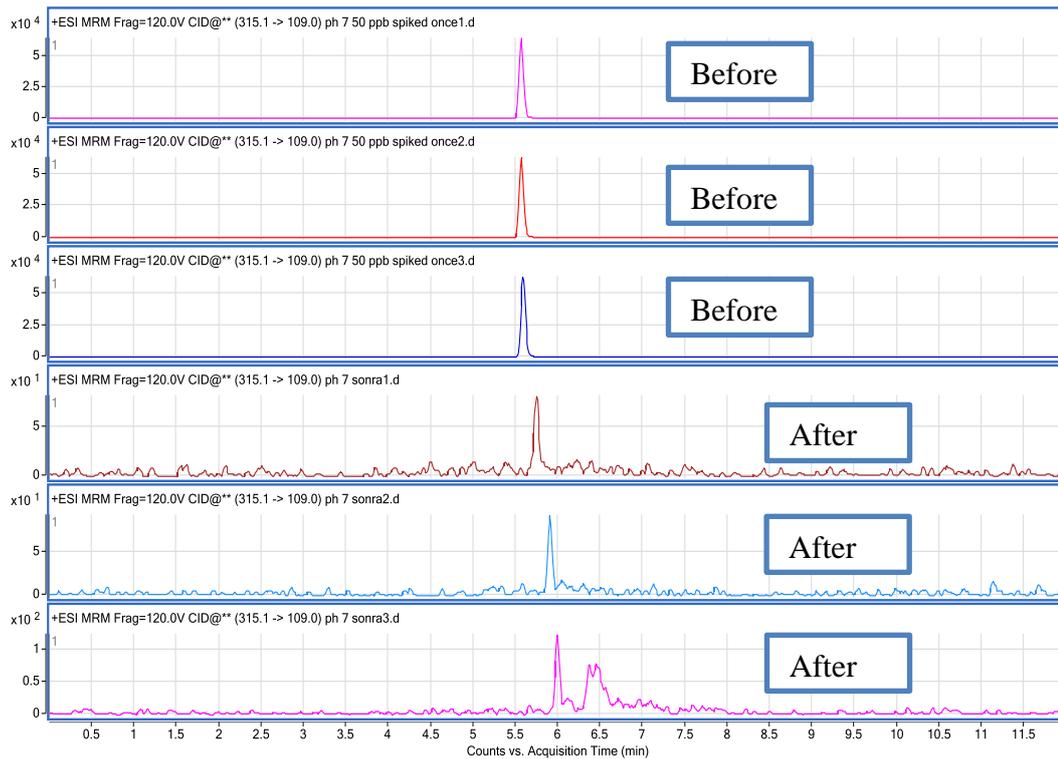


Figure A-37 Peak chromatograms of Progesterone before and after extraction at pH 7 (n=3)

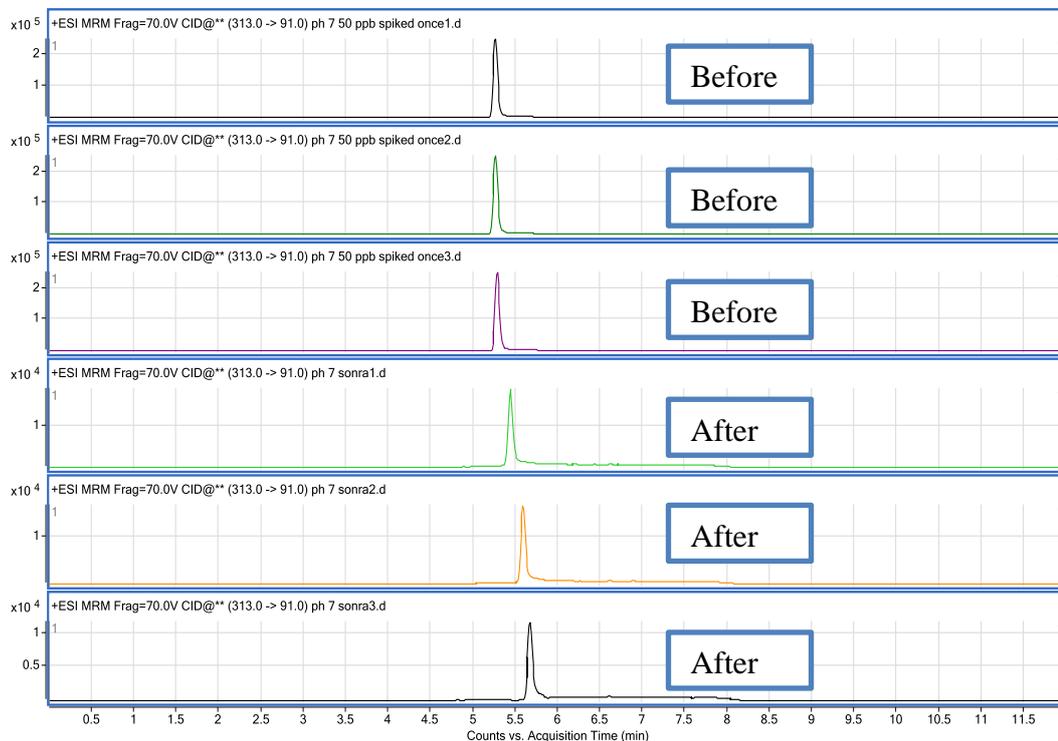


Figure A-38 Peak chromatograms of BBP before and after extraction at pH 7 (n=3)

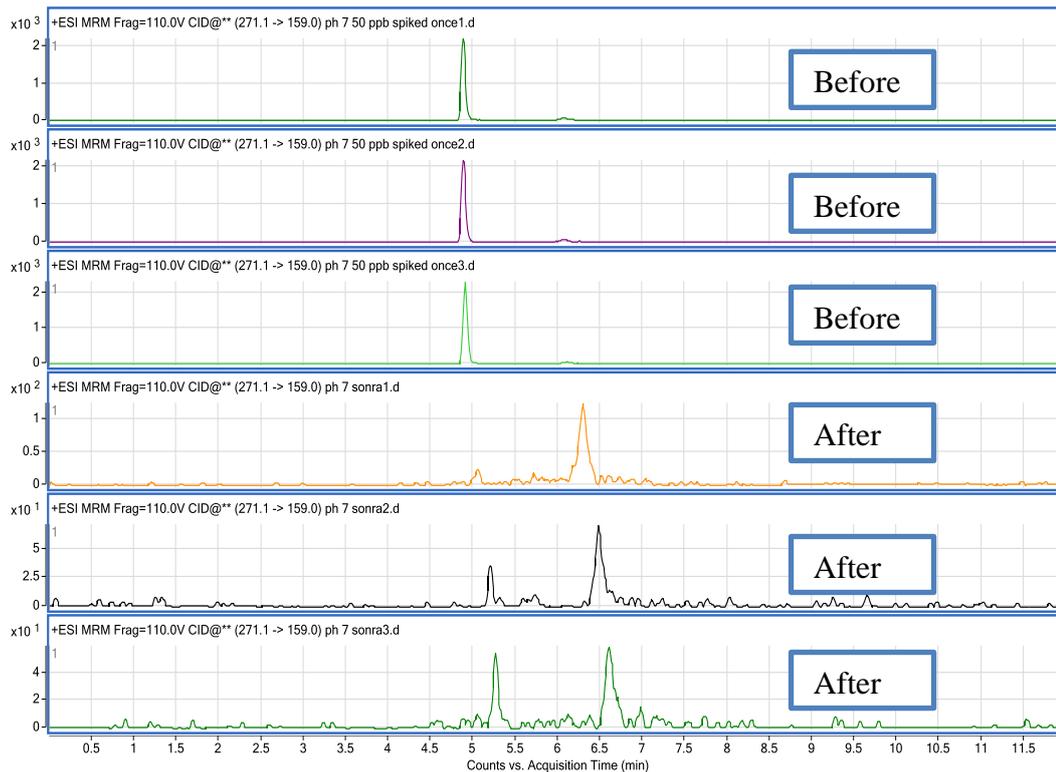


Figure A-39 Peak chromatograms of Estrone before and after extraction at pH 7 (n=3)

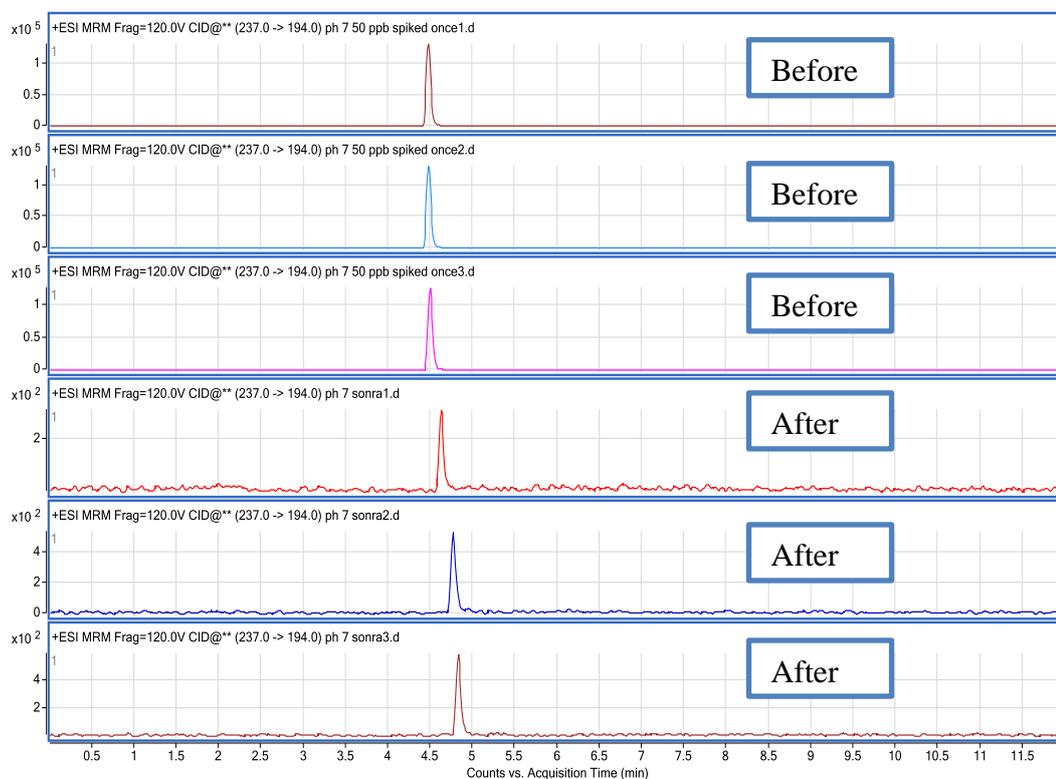


Figure A-40 Peak chromatograms of Cbz before and after extraction at pH 7 (n=3)

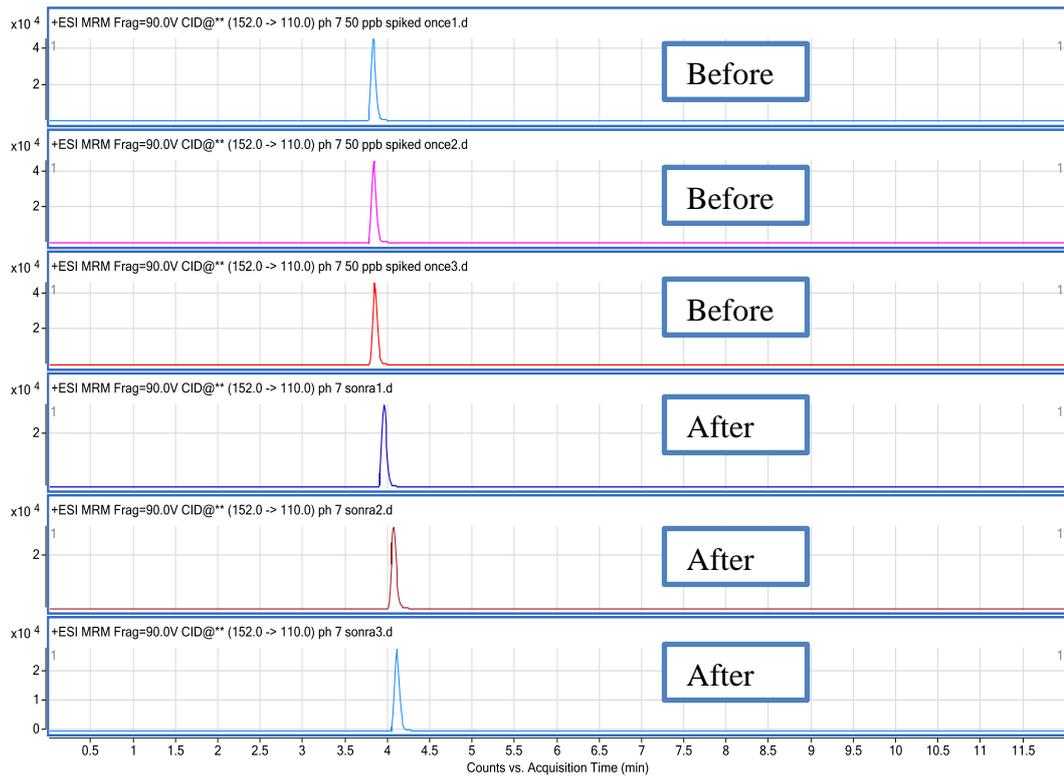


Figure A-41 Peak chromatograms of Atp before and after extraction at pH 7 (n=3)

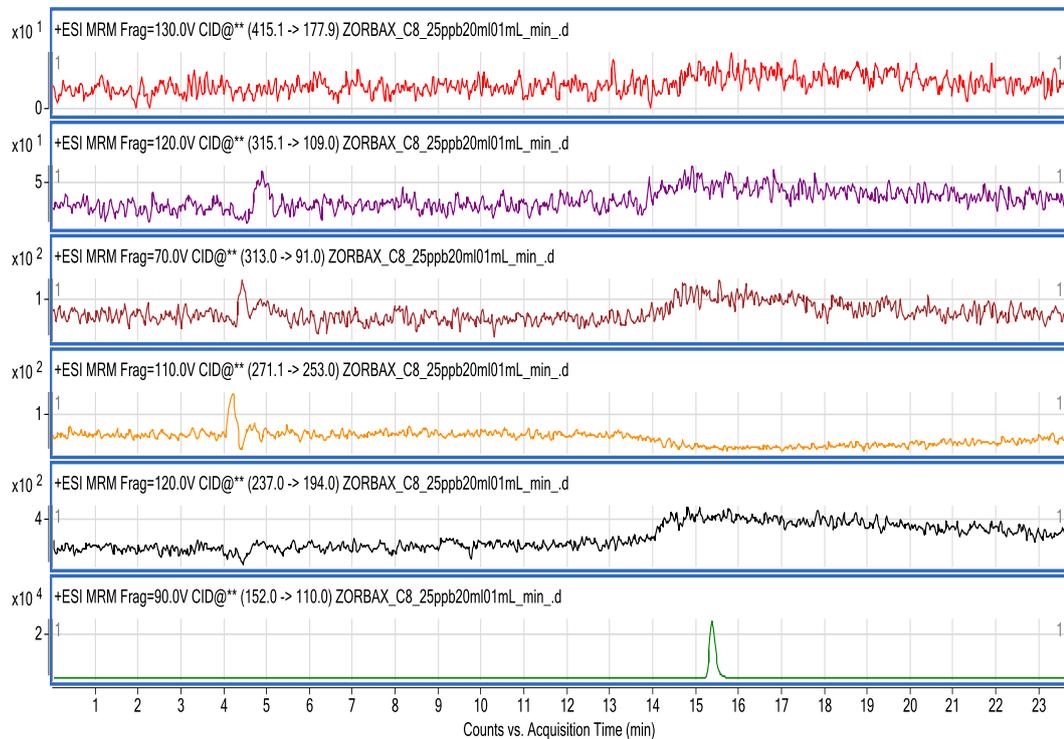


Figure A-42 Chromatograms of the analytes with a flow rate of 0.1 ml/min

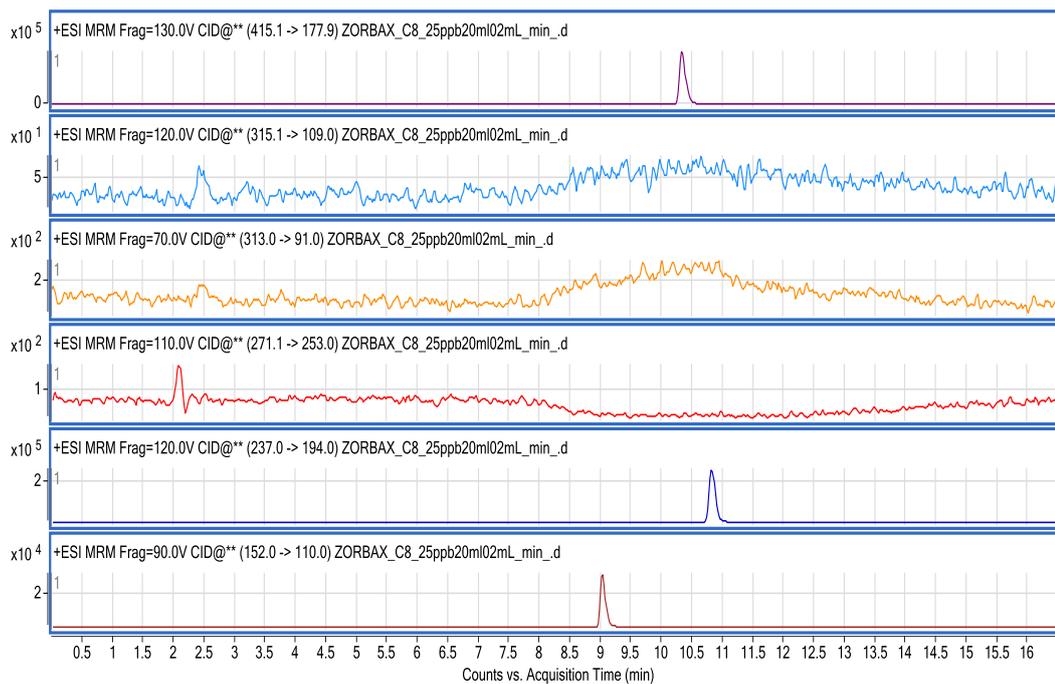


Figure A-43 Chromatograms of the analytes with a flow rate of 0.2 ml/min

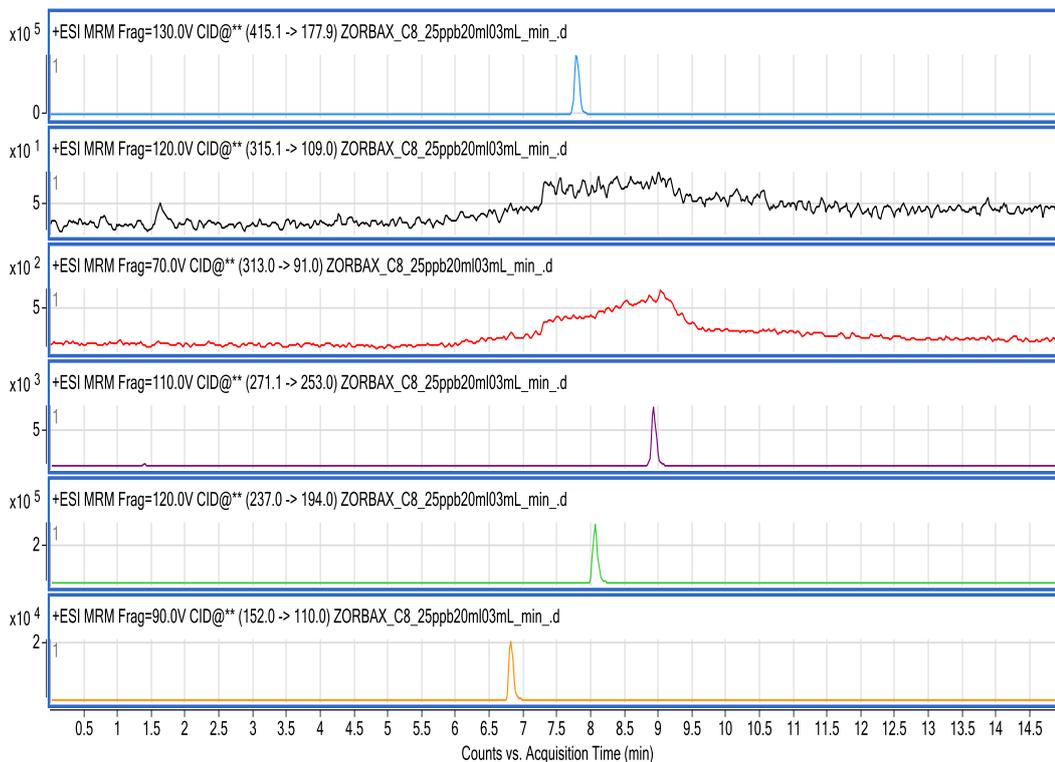


Figure A-44 Chromatograms of the analytes with a flow rate of 0.3 ml/min

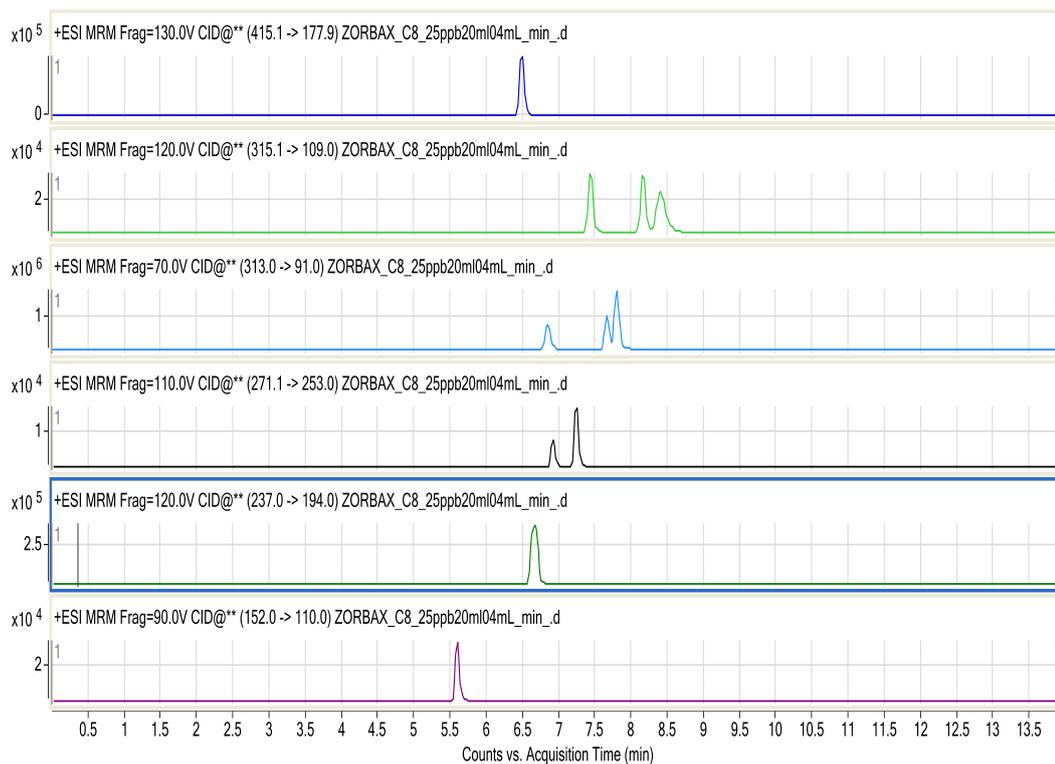


Figure A-45 Chromatograms of the analytes with a flow rate of 0.4 ml/min

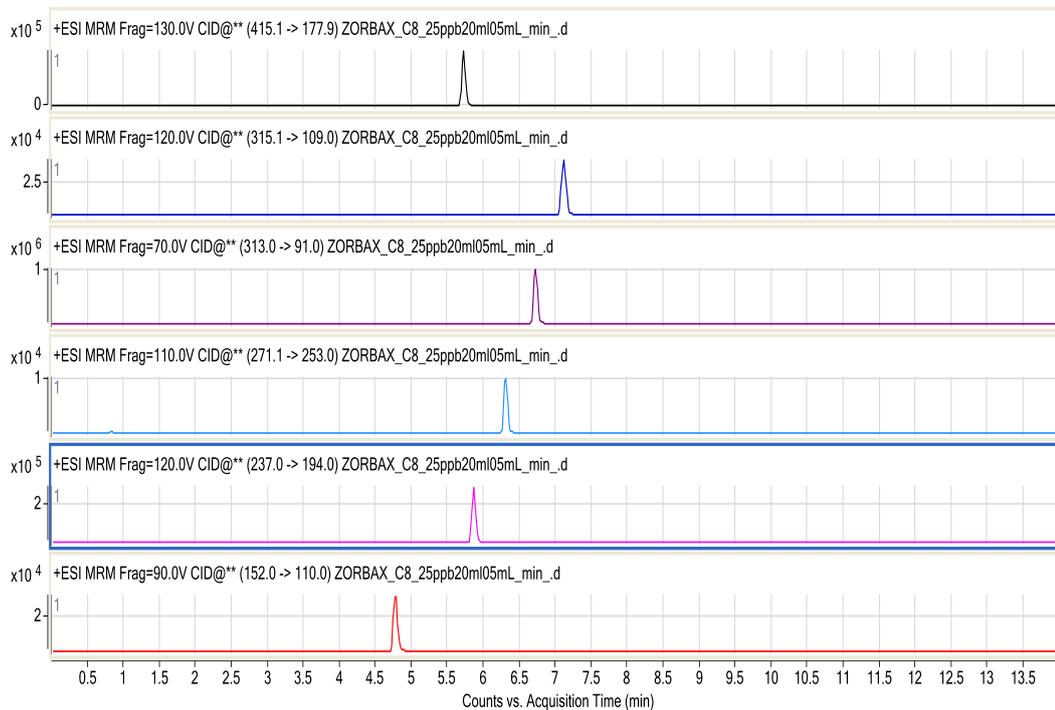


Figure A-46 Chromatograms of the analytes with a flow rate of 0.5 ml/min

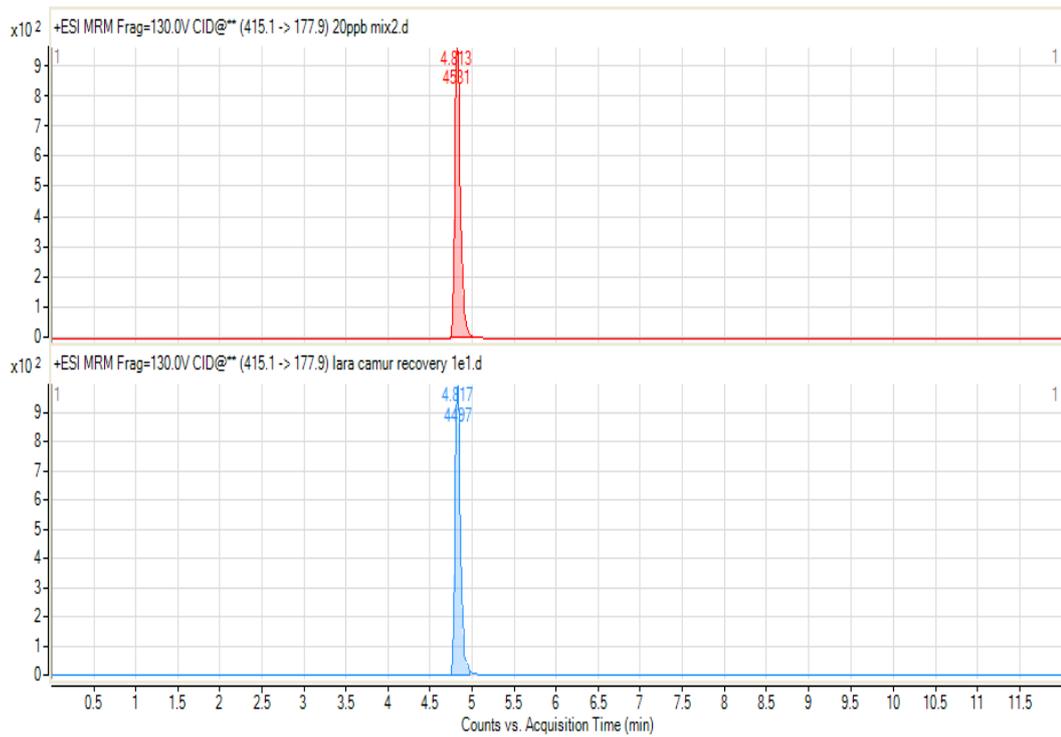


Figure A-47 Diltiazem concentrations of 20 ppb mix solution and spiked sample after sludge extraction

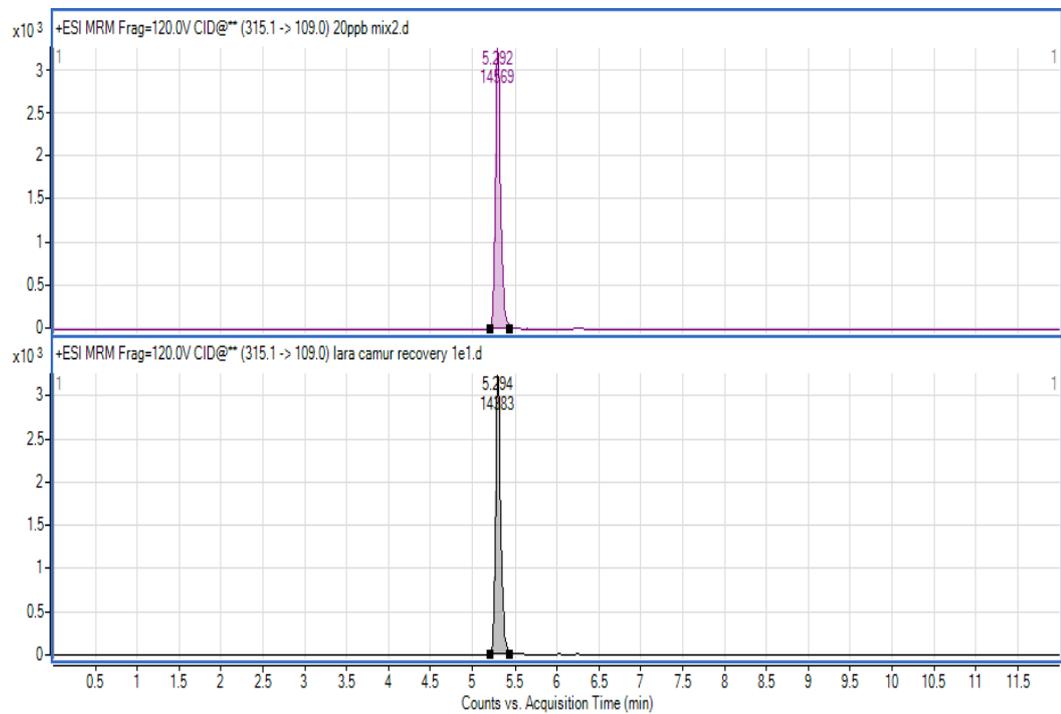


Figure A-48 Progesterone concentrations of 20 ppb mix solution and spiked sample after sludge extraction

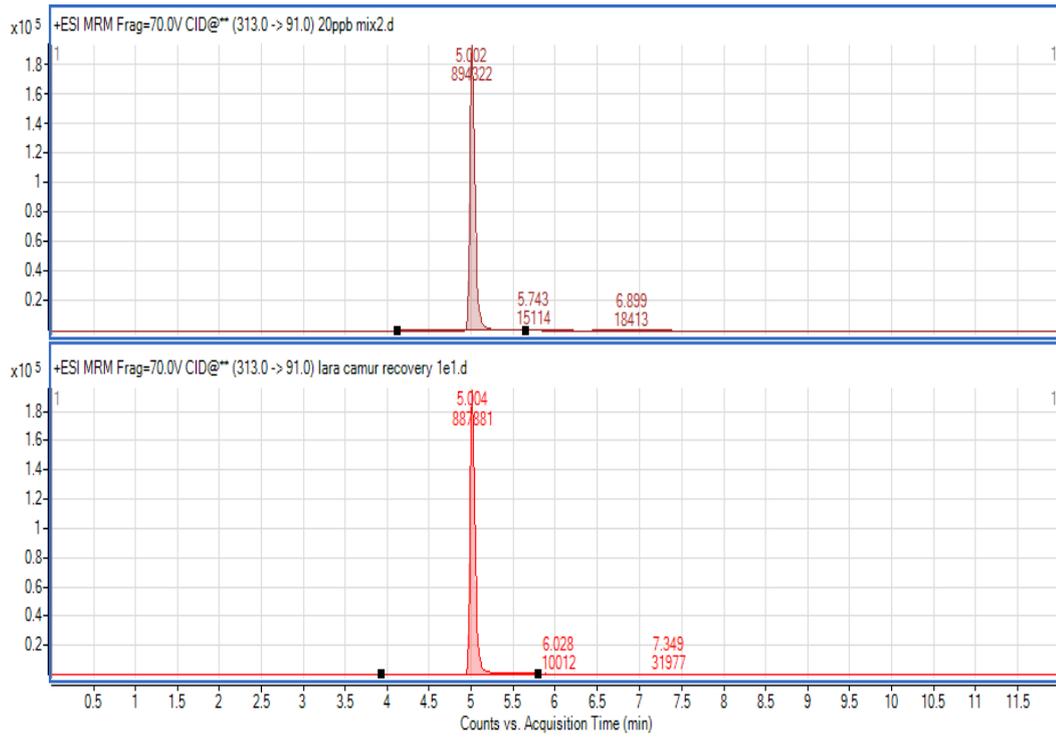


Figure A-49 BBP concentrations of 20 ppb mix solution and spiked sample after sludge extraction

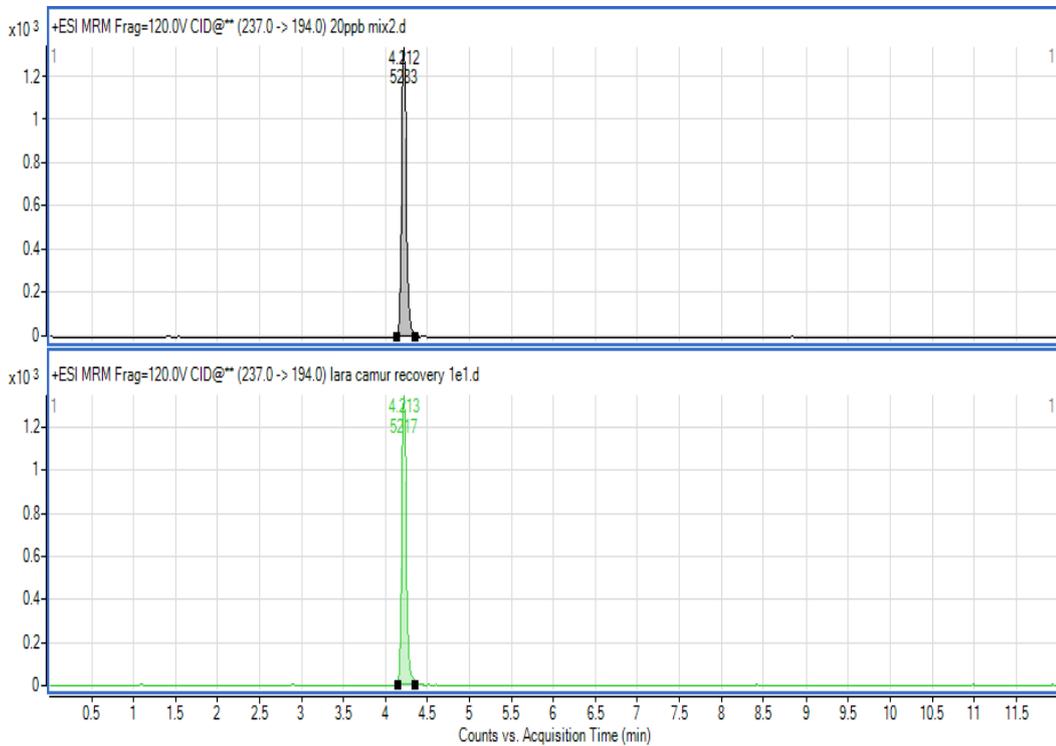


Figure A-50 Cbz concentrations of 20 ppb mix solution and spiked sample after sludge extraction

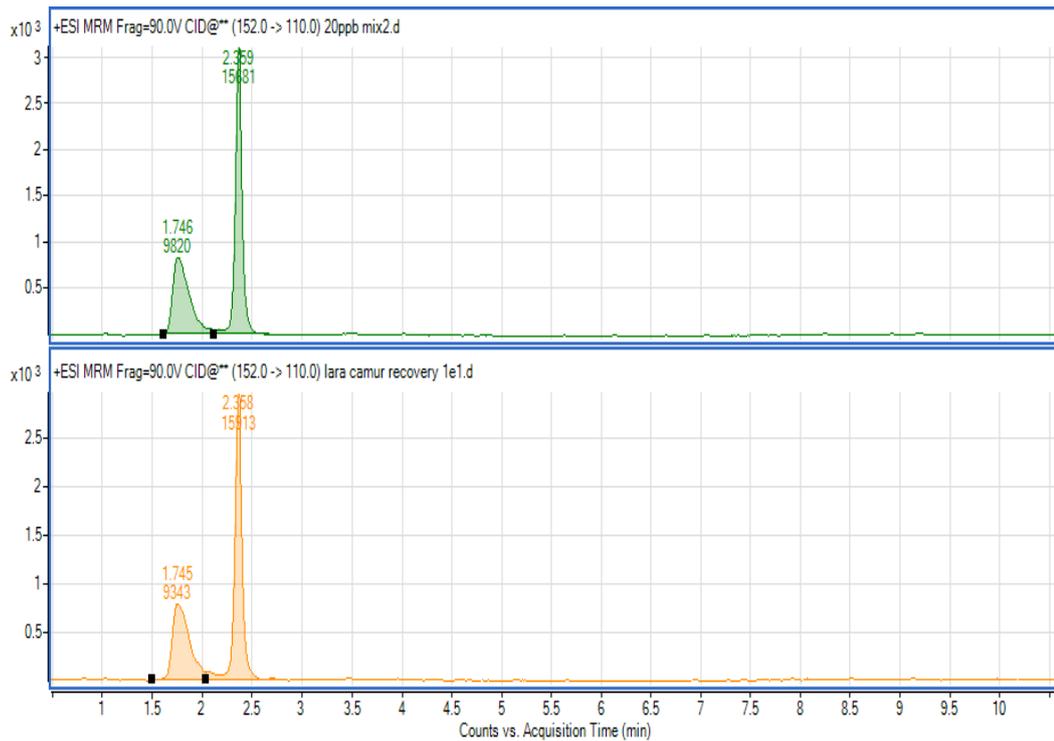


Figure A-51 Acetaminophen concentrations of 20 ppb mix solution and spiked sample after sludge extraction

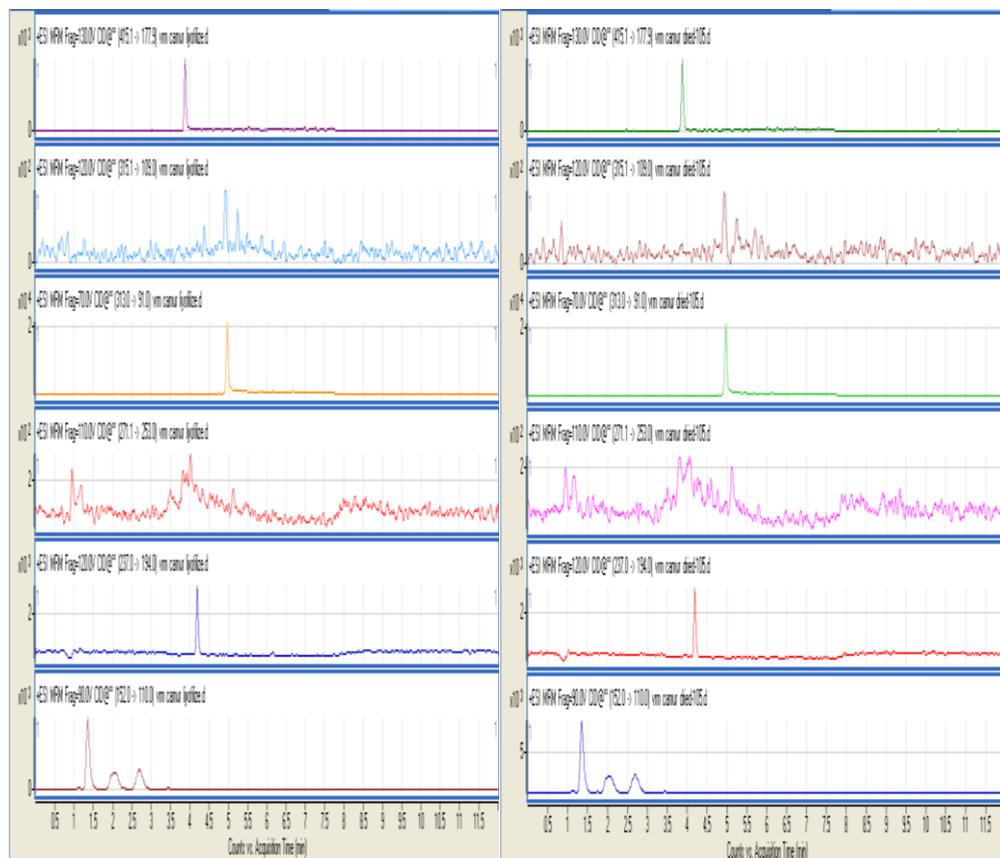


Figure A-52 Lyophilized vs dried sludge

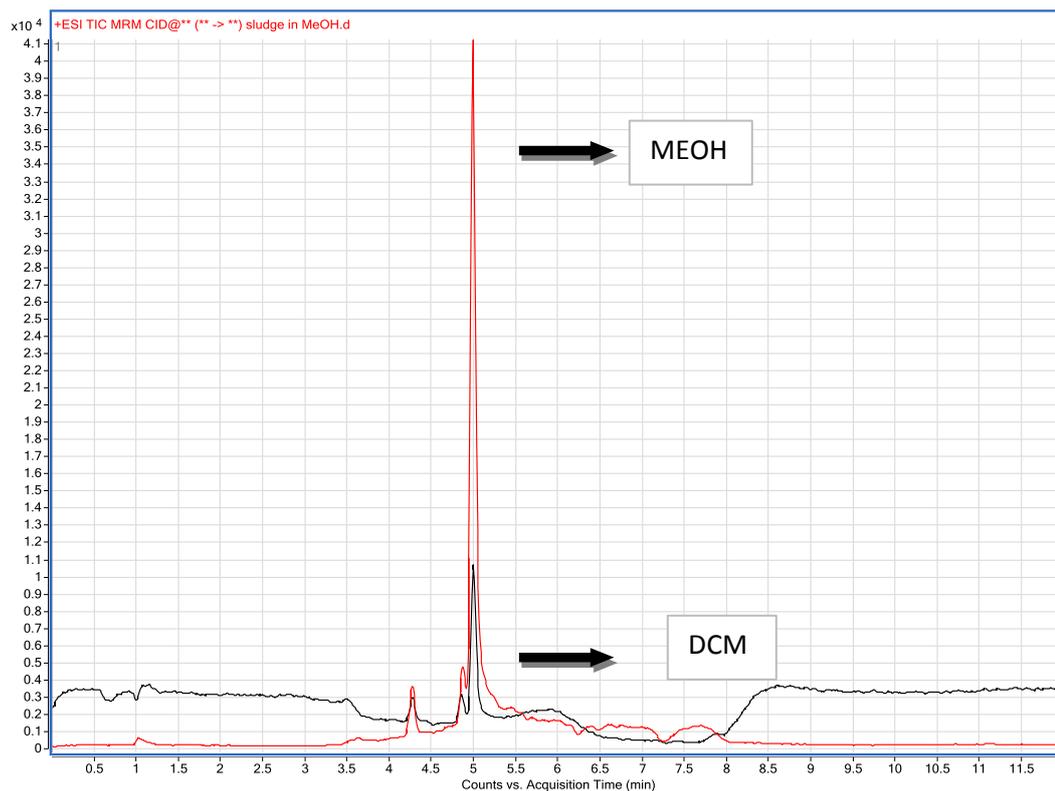


Figure A-53 Total Ion Chromatogram Comparison of Methanol versus Dichloromethane



Figure A-54 6' and 30' ozonated flasks before passing through ordinary filter paper.



Figure A-55 75' ozonated flask before passing through ordinary filter paper.



Figure A-56 150' ozonated flask after passing through ordinary filter paper.