EGE UNIVERSITY
GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCE

INVESTIGATION OF SAMPLE PREPARATION TECHNIQUES FOR CHROMATOGRAPHIC ANALYSIS OF FATTY MATRICES

Betül ŞAKRAK

Supervisor: Assoc. Prof. Dr. Hasan ERTAŞ

Department of Chemistry

Presentation Date: 10.03.2017

Bornova-İZMİR
2017

Betül ŞAKRAK tarafından Yüksek Lisans tezi olarak sunulan "***Investigation of Sample Preparation Techniques for Chromatographic Analysis of Fatty Matrices***" başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesinin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **10.03.2017** tarihinde yapılan tez savunma sınavında aday oybirliği/~~oyçokluğu~~ ile başarılı bulunmuştur.

Jüri Üyeleri:		**İmza**
Jüri Başkanı	**: Doç.Dr. Hasan Ertaş**	
Raportör Üye	**: Doç.Dr.Füsun Okçu Pelit**	
Üye	**: Doç.Dr.Süleyman Koçak**	

EGE ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
ETİK KURALLARA UYGUNLUK BEYANI

E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliğinin ilgili hükümleri uyarınca Yüksek Lisans Tezi olarak sunduğum "***Investigation of Sample Preparation Techniques for Chromatographic Analysis of Fatty Matrices***" başlıklı bu tezin kendi çalışmam olduğunu, sunduğum tüm sonuç, doküman, bilgi ve belgeleri bizzat ve bu tez çalışması kapsamında elde ettiğimi, bu tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara atıf yaptığımı ve bunları kaynaklar listesinde usulüne uygun olarak verdiğimi, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını, bu tezin herhangi bir bölümünü bu üniversite veya diğer bir üniversitede başka bir tez çalışması içinde sunmadığımı, bu tezin planlanmasından yazımına kadar bütün safhalarda bilimsel etik kurallarına uygun olarak davrandığımı ve aksinin ortaya çıkması durumunda her türlü yasal sonucu kabul edeceğimi beyan ederim.

10/05/2017

İmzası

Adı-Soyadı
Betül ŞAKRAK

ÖZET

YAĞLI ÖRNEKLERDE KROMATOGRAFIK ANALIZ IÇIN ÖRNEK HAZIRLAMA TEKNIKLERININ İNCELENMESI

ŞAKRAK, Betül

Yüksek Lisans Tezi, Kimya Anabilim Dalı
Tez Danışmanı: Doç. Dr. Hasan ERTAŞ
Mart, 2017, 47 sayfa

Pestisitler, tarımda zararlı ot ve hayvanların kontrolünde kullanılan etkili kimyasallar olmakla birlikte bu pestisit kalıntılarının izlenmesi gıda güvenliği açısından büyük önem taşımaktadır. Analizde kullanılan geleneksel yöntemler zaman alıcı ve emek yoğun işlemler içermekte özellikle yağlı gıda ürünlerinin analizinde büyük sorunlarla karşılaşılmaktadır.

Bu tez çalışmasında siyah zeytin örneklerinde pestisit kalıntılarının kütle spektroskopisiyle eşleşmiş sıvı kromatografi (LC-MS/MS) yöntemiyle analizi incelenmiştir. Siyah zeytin örneklerinin yağlı yapısı dikkate alınarak, hızlı, kolay, ucuz, verimli, sağlam ve güvenli sözcüklerinden oluşan QuEChERS yöntemi seçilmiştir. Tez çalışmasının birinci kısmında siyah zeytin örneklerine orijinal yöntem uygulanarak, 46 etken maddenin kromatografik pik alanlarından gidilerek, nicel tayin gerçekleştirilmiş ve ppb düzeyinde kalibrasyon grafikleri çizilmiştir.

Tezin ikinci kısmında ise bu orijinal yöntemin dispersive SPE örnek temizleme aşamasında kimi modifikasyonlar planlanmış ve $MgSO_4$ yerine ilk kez kalsiyum karbonat kullanılmış ve PSA yerine de tersiyerbutilamin (TBA) denenmiştir. Bu sorbentlerin yanında kullanılan C18 ile birlikte miktarlarının gerikazanım değerleri üzerine etkisini incelemek üzere iki düzeyde tam faktöriyel deneysel tasarım uygulanmıştır. Gerikazanım değerleri iki ayrı derişim için belirlenmiş ve sonuçlar orjinal yöntemin sonuçları ile kıyaslanmıştır. Elde edilen tüm veriler birlikte değerlendirildiğinde geniş bir aralıkta dağıldığı ve bir kısmının Kabul edilen 70-120% aralığının dışında kaldığı görülmüştür. Bu durumun sebeplerinden biri çalışılan pestisitlerin polarite ve hidrofobiklik değerlerinin geniş bir aralıkta dağılmasıdır. Bir diğer neden ise kalsiyum karbonatın magnezyum sülfat kadar su çekici olmayışıdır.

Anahtar sözcükler: QuEChERS, pestisit, yağlı gıda, zeytin, LC–MS/MS

ABSTRACT

INVESTIGATION OF SAMPLE PREPARATION TECHNIQUES FOR CHROMATOGRAPHIC ANALYSIS OF FATTY MATRICES

ŞAKRAK, Betül

MSc in Chemistry
Supervisor: Assoc. Prof. Dr. Hasan ERTAŞ
March, 2017, 47 pages

Pesticides are efficient in controlling weeds and pets during planting however; their residue in food should be monitored for safety of the food. Traditional analysis methods are complicated and labor-intensive and fatty foods are particularly challenging.

In this thesis, determination of pesticide residues in black olive samples by Liquid chromatography tandem mass spectrometry (LC-MS/MS) method was investigated. Considering the fatty matrix of the black olives, QuEChERS method which stands for the quick, easy, cheap, efficient, rugged and safe method was chosen. In the first part of the study, original method was applied to the black olive samples and pesticide residues for 46 active ingredients were quantified according to their chromatographic peak areas. The calibration graphs were drawn in ppb ranges.

In the second part of the thesis, some modifications were made to the original method to improve the d-SPE clean up step and $MgSO_4$ was replaced with $CaCO_3$ for the first time where tert-butyl ammine (TBA) was used instead of PSA. In order to reveal the effect of the amount of these sorbents on the recovery values along with the C18 used in dSPE step, full factorial experimental design was applied at two levels. Recovery studies have been performed at two concentration levels and the results have been compared with those of original QuEChERS method. Overall results have demonstrated that recovery values were distributed in a wide range and were found out of the 70-120% range for the 8 experiments in the design. One of the reasons to this outcome is the pesticides studied display a wide range of polarity and hydrophobicity due to the structural differences. Another reason is that $CaCO_3$ is not hygroscopic as $MgSO_4$ used in QuEChERS method.

Keywords: QuEChERS, pesticide, fatty food, olive, LC-MS/MS

ACKNOWLEDGEMENT

I would like to express my appreciation to my supervisor Assoc. Prof. Dr. Hasan ERTAŞ for his support and guidance. I would like to thank to Prof. Dr. F. Nil ERTAŞ, for her kind supervision, valuable suggestion and helpful discussions.

I would like to thank to my husband Selim ŞAKRAK for helpful understanding ,and also thank to my colleague Gürkan KEÇELİ for his help and understanding.

I would like to acknowledge to all my dear friends who providing full support to me and for their real friendship.

I would like to thank my family for their encouragement, understanding attitude throughout my study.

CONTENTS

Page

CONTENTS (Continue)

FIGURE LIST

TABLE LIST

Table Page

ABBREVIATIONS AND SYMBOLS

Abbreviations

ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
APPI	Atmospheric pressure photoionization
CID	Collision induced dissociation
d-SPE	Dipersive Solid phase extraction
ESI	Electrospray ionization
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
LC-MS/MS	Liquid chromatography-mass spectrometry (tandem)
LLE	Liquid-liquid extraction
LOD	Limit of Detection
LOQ	Limit of Quantitation
MeOH	Methanol
SPE	Solid phase extraction
SIM	Selected ion monitoring mode
SPME	Solid phase micro extraction

1. Introduction

1.1. Pesticides

Pesticides are all chemicals and biological products that can be used to remove or destroy microorganisms and harmful microorganisms that damage food during the production, consumption and storage of foodstuffs and also to regulate the growth of plants (www.epa.gov).

The first chemicals used as pesticides are arsenic and sulfur species. Then, until the 2nd world war, a few chemical substances have been used. These are copper and mercury salts and sulfur that it used for fungicide, for insects used arsenic and cyanide. Pesticides widely started to use in 1940s with DDT use and the first pesticide law was published at ABD in 1947. Thereafter, in 1970, Environmental Protection Agency (EPA) was constructed. Pesticides are usually classified by target organisms (Insecticides, Rodenticides, Fungicides, Herbicides, Acaricides), or by chemical structure.

Organochlorine pesticides: These insecticides were used to apply widely in the past, but their health and environmental effects have leaded their removal from the market due to and their persistence. Dieldrin, aldrin, DDT, heptachlor, lindane and chlordane are the examples.

Organophosphorus pesticides: These chemicals are usually used as insecticides and affect the nervous system. Organophosphorus pesticides disrupt the enzyme which regulates acetylcholine. Some of these pesticides have been used as nerve agents in World War II. Fortunately, they were not persistent in the environment. Among them, chlorpyrifos, dichlorvos, malathion and trichlorfon can be counted.

Carbamates: This group of chemicals has several subgroups and again affects the nervous system. Examples include aldicarb, carbaryl, carbofuran and methomyl.

Pyrethroid Pesticides: Pyrethrin is a naturally occurring pesticide and to increase their stability, synthetic versions have been produced. Some of them are toxic to the nervous system. Cypermethrin, cyfluthrin and bifenthrin are encountered.

Biopesticides: These types of pesticides are derived from animals, bacteria, plants and certain minerals. For instance, canola oil and baking soda can be used for this purpose.

Recently population has grown so rapidly, the use of pesticides has also increased. In case of improper use, pesticides can lead harmful effects on the public health as well as the environment in addition to the target pest. Exposure to the pesticides can be via inhalation, dermal or oral exposure.

In terms of food quality and safety, the most important task of the food industry is the control of contaminants as well as the harmful residues of pesticides. Therefore, maximum residue limits (MRL) have been determined for controlling the pesticide use (EC Regulations, 2005). Consequently, determination of trace amount of pesticide residues in agricultural products is essential for controlling the pesticide use and also for public health.

1.2. Determination Methods for Pesticide Residues

Trace analysis of pesticide residues in food are usually achieved by using chromatographic methods. To comply with the EU Maximum residue limits, these methods should be validated to allow determination of the pesticide residues in foodstuff. For screening purposes, generally multiresidue methods are preferred since they cover a number of compounds (Villaverde et al., 2016).

These methods are chosen to meet the high demand for residue analysis and to reduce the cost of analyses as well. Generally, liquid chromatography (LC) with mass detector (MS) is used. More recently, ultra-HPLC coupled with tandem mass spectrometry (UHPLC–MS/MS) are preferred since these systems gives more sensitive results in shorter analysis time along with increased sample throughput (Romero-González, 2011).

Sample preparation step prior to the chromatographic analysis is equally important with the analytical instrument since the interferences from complex matrices of food can be eliminated by applying a separation and preconcentration step. Classical sample preparation methods include liquid-liquid extraction techniques (Mitra, 2003). However, this technique is time consuming and requires a deal of efforts in addition to the large volume of toxic solvents wasted.

In the last three decades, solid phase extraction is replaced this technique in many standard methods but, still producing waste solvents. A more recent development in this area is the use of a polymeric fiber as a solid phase micro extraction (SPME) tool. This technique is solvent free and provides a simple, fast and inexpensive alternative for sensitive determination of trace organic compounds. However, the capacity of fiber is limited and it may not respond well in fatty food matrices (Mitra, 2003).

A multi-residue method DFG S19 has been applied in control laboratories and in food industry (DFG, 1999) in the last two decades, This method consist of different modules including extraction steps, clean-up with GPC and fractionation on silica gel (SiO_2) column and finally GC with various selective detection methods (NPD, ECD, FPD) (Lee, 2003). However, the method procedures are labor intensive and time-consuming and alternatives have been searched.

Nowadays, in addition to minimizing the solvent consumption, more selective and sensitive methods have been searched by using less volume of sample (Pizzutti et al., 2009). Vast number of papers dealing with multi-residue methods for pesticides in fruits and vegetables have lead the use of quick, easy, cheap, effective, rugged and safe methods summarized as ***QuEChERS*** in sample preparation, followed by a chromatographic detection system. This method provides a practical alternative to traditional LLE and SPE techniques since minimum volume of solvent is consumed (Gonzales-Curbelo, 2015).

QuEChERS method was first published in 2003 by Anastassiades and Lehotay as a novel multi-residue method employing acetonitrile for extraction and "***dispersive solid-phase extraction***" (dSPE) for the determination of pesticide residues in food samples. The method was modified in years as given in Figure 1.

Several types of sorbents have already been applied for sample clean-up and sample preparation purposes as well as in SPE cartridges. Here, successive extraction and clean-up steps makes the method quick and easy as well as cheap and effective as the name implies. In the original procedure, after homogenization of the carefully weighed samples (usually 10-15 g), solvent extraction is applied. Then the extract is cleaned up by d-SPE for avoiding the interfering compounds from the food matrix (Romero-Gonzalez, 2011).

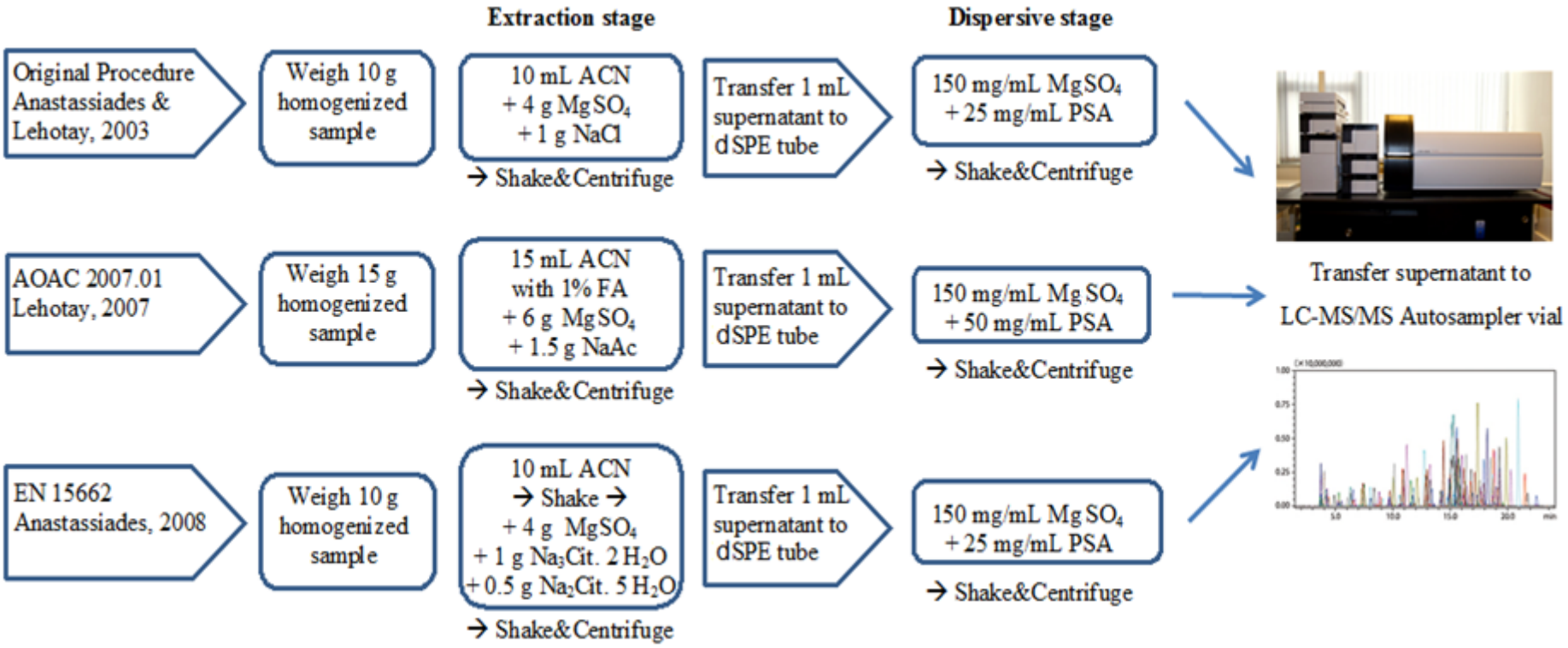


Figure 1. The stages of QuEChERs method and developments over years.

The modifications of original QuEChERS method are usually made in type of extraction solvent and the salt added along with the sorbents used in dSPE stage according to the sample type. In the extraction step, usually the sample composition determines the solvent type and volume, and extraction time and temperature. The most widely used solvents for the multi-residue analysis of pesticides are acetone (Ac), acetonitrile (ACN) and ethyl acetate (EtAc). Among them, ACN is more suitable to extract pesticides having a wide range of polarities with higher capacity and selectivity than Ac and EtAc. In addition, the extraction of lipophilic material is greatly reduced in comparison to the other solvents (Gonzales-Curbelo, 2015).

Another advantage of the ACN is that it forms separate partitioning phases with non-polar solvents which are important in the subsequent liquid-liquid clean-up step. Although the d-SPE procedure was preferred in this step, nonpolar co-solvents and/or salts are used are some cases. Drying salts are usually Na_2SO_4 or $MgSO_4$ where the latter provides more complete phase separation. In some cases, NaCl is used along with $MgSO_4$ to obtain more satisfactory recoveries.

It is well known that high temperatures accelerate the extraction of non-polar species and usually a temperature range of 40–45°C is favored. The sample pH, on the other hand, should also be taken into account since some of the pesticides are less stable at basic media while some can be lost in acidic solutions. In AOAC Official Method 2007.01, developed by Lehotay includes pH 5 acetate buffer for controlling the recovery values. Anastassiades and co-workers have also modified the original unbuffered procedure by inserting citrate buffer as given in EN Standard Method EN 15662 (2008).

In both modifications, the sample pH is adjusted to 5 to ensure an acceptable extraction of the pesticides sensitive under acidic or basic conditions. However, such use of buffers may lead an increase in co-extractives and therefore, it is not recommended for samples with high lipid content (Gonzales-Curbelo, 2015). It was also reported that fatty acid recovery has increased at low pHs and this co-extraction can be avoided by adjusting the salt amount added to the sample.

In addition to the sample pH, the solvent pH can also affect the stability of the pesticides. For example primer seconder ammines (PSA) are reported to increase the pH of the extracts.

In the dSPE step where the sample clean-up and drying of the ACN phase are accomplished; the choice of the sorbent is important task. Polar interferences from the sample matrix including fatty acids, sugar and pigments can be eliminated by using several sorbents in a wide range of polarity. For this purpose; a strong-anion exchanger, co-polymeric sorbent, PSA, cyanopropyl, amino-propyl and octadecylsilane (C18) have been used.

These sorbents each have advantages and drawbacks such as polar interferents including fatty acids, sugars and pigments are removed by PSA, but pesticides are not whereas pigments are efficiently eliminated by graphitized carbon Black (GCB) but, display a low recovery to planar pesticides. For fatty matrices, PSA and C18 (Georgakopoulos et al., 2011; Herrmann and Poulsen, 2015) are preferred to obtain cleaner extracts.

Recently, alternative sorbents are searched for complex samples and promising results have been obtained with zirconium dioxide based sorbents for purification of fatty samples (Dias et al., 2016; Vázquez et al., 2016; Rejczak and Tuzimski, 2017). By this means enhanced extraction efficiency, better analyte recovery and reproducibility than traditional PSA and C18 sorbents have been reported. Other modifications include the addition of carbon based nanoparticles as MWCNTs (Hou et al., 2004), alumina (Koesukwiwat et al., 2008), Florisil (Nguyen et al., 2010) and magnetic nanoparticles (Li et al., 2014).

An additional freezing step was introduced recently to the procedure for the precipitation of lipids at low temperature (Gonzales-Curbelo, 2015). This step takes 1 or 2 hours but, it was revealed that the extraction of 22 organochlorine pesticides and seven polychloro biphenyls can be extracted effectively from fish (Norli et al., 2010).

Final modification in the procedure includes the use of SPE instead of d-SPE procedure. For this purpose amminopropyl and graphitized carbon black columns and PSA were used (Schenk et al., 2008 and Hunter et al., 2010). Even though the SPE technique provides better clean-up for the final extract, it was reported that d-SPE cannot be replaced due to its speed and convenience for a wide variety of samples. In conclusion, QuEChERS provides very satisfactory results for a large number of pesticides and the procedure can be adapted to different types of matrixes by modifying the stages by inserting different solvents or new materials.

1.3. Fatty Food Samples

According to the U.S. Food and Drug Administration, fatty food is the food material which has more than 2% fat composition (Lehotay et al., 2005). Considering the huge difference in the analysis of milk and very fatty lard samples, the terminology has been divided into non-fatty (<2% fat), low fatty (2–20%), and high fatty (>20%) foods on a wet weight basis.

Nowadays, analysis of pesticides in fatty food samples is a challenging issue since the sample preparation is a critical step (Gilbert-López et al., 2009). This analysis is usually accomplished by using acetone, hexane, EtAc, and dichloromethane as the extraction solvents to dissolve the lipids but, in sample clean-up step gel-permeation chromatography (GPC) is required to remove the co-extracted fat from the extracts prior to the measurement (Lehotay et al., 2005).

In a comparative study carried out with most vegetable oils and animal fats, the performance of the GPC clean-up on the five column types was examined and it was found satisfactory results were obtained for most of the pesticides (David et al., 2017). However, low recoveries were obtained for the low molecular weight triglyceride containing fatty foods such as butter. Therefore, it was concluded that for high fatty matrixes containing >20% lipids, nonpolar solvents should be used.

In another study carried out for a large number of pesticide analysis in edible oil samples, the samples were freezed-out with dry ice and in d-SPE stage a new material called Enhanced Matrix Removal-Lipid was used (Vázquez et al., 2016).

1.4. The Aim of the Thesis

In this thesis, it was planned to develop a method for pesticide residue analysis in olive samples. For this purpose, some modifications in QuEChERS method have been planned and the use of calcium carbonate instead of $MgSO_4$ and tertbutyl ammine (TBA) instead of PSA was tried. Table 1 lists the pesticides studied in this thesis.

Table 1. Chemical structure, type and MRL values of the pesticides studied.

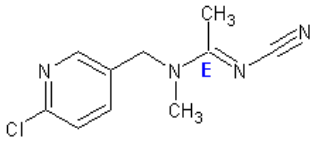
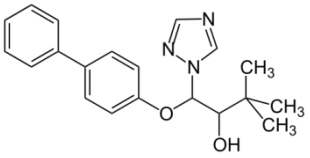
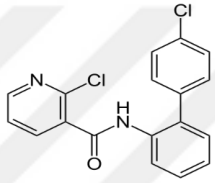
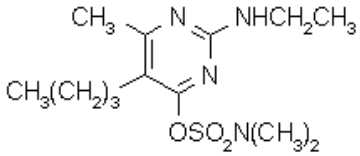
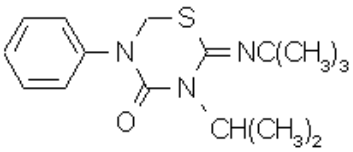
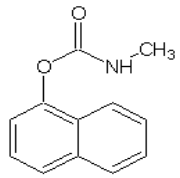
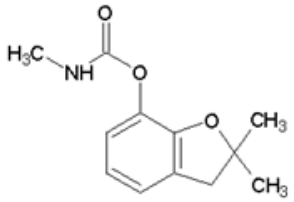
Name/Type/MRLvalue	Chemical Structure / IUPAC Name
Acetamiprid **Type:** Insecticide **MRL:** 0,9 mg/kg **MW:** 222.7 g/mol	 N-[(6-chloro-3-pyridyl)methyl]-N'-cyano-N-methyl-acetamidine
Bitertanol **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 337,4 g/mol	 (1RS,2RS;1RS,2SR)-1-(Biphenyl-4-yloxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butane-2-ol
Boscalid **Type**: Fungicide **MRL:** 0,01 mg/kg **MW:** 343.2 g/mol	 2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Bupirimate **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 316,4 g/mol	 5-Butyl-2-ethylamino-6-methylpyrimidin-4-yldimethylsulphamate
Buprofezin **Type:** Acaricide **MRL:** 5 mg/kg **MW:** 305,4 g/mol	 (2Z)-3-Isopropyl-2-[(2-methyl-2-propanyl)imino]-5-phenyl-1,3,5-thiadiazinan-4-one
Carbaryl **Type:** Insecticide **MRL:** 0,01 mg/kg **MW:** 201,23 g/mol	 1-naphthyl methylcarbamate
Carbofuran **Type:** Insecticide **MRL:** 0,003 mg/kg **MW:** 221,3 g/mol	 2 2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate

Table 1. Continue

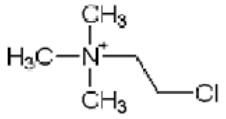
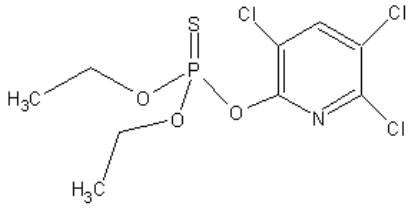
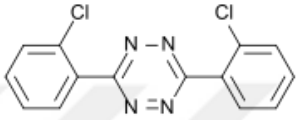
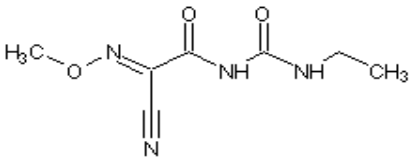
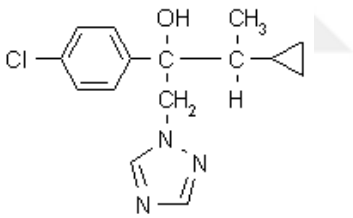
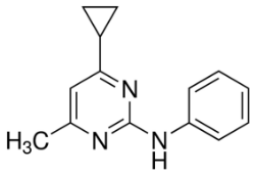
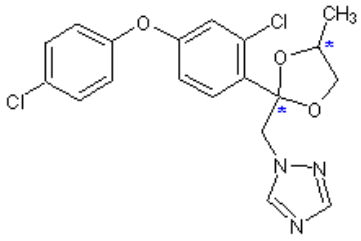
Chlormequat Chloride **Type:** Biopesticide **MRL:** 0,1 mg/kg **MW:** 202,6 g/mol	 Metil 2-Chloro-N,N,N-trimethylethanaminium
Chlorpyrifos **Type:** Insecticide **MRL**: 0,05 mg/kg **MW:** 350.6 g/mol	 O,O-Dietil O-3,5,6-trikloropiridin-2-il fosforothiyoat
Clofentezine **Type:** Acaricide **MRL:** 0,02 mg/kg **MW:** 303,1 g/mol	 3,6-bis(2-chlorophényl)-1,2,4,5-tétrazine
Cymoxanil **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 198,18 g/mol	 2-Cyan-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamid
Cyproconazole **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 291,8 g/mol	 2-(4-Chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol
Cyprodinil **Type:** Fungicide **MRL:** 0,02 mg/kg **MW:** 225.3 g/mol	 (4-cyclopropyl-6-methyl-pyrimidin-2-il)-phenyl-amin
Difenoconazole **Type:** Fungicide **MRL:** 2 mg/kg **MW:** 406,3 g/mol	 1-[(2-[2-Chlor-4-(4-chlor-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-yl)methyl]-1H-1,2,4-triazol

Table 1. Continue

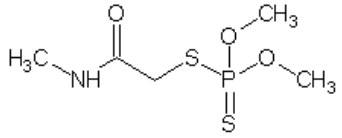
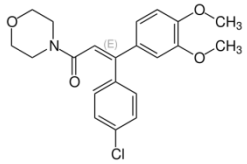
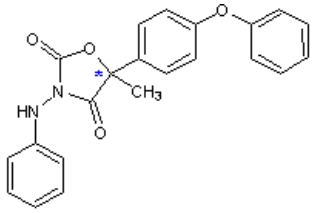
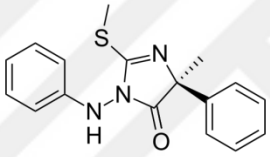
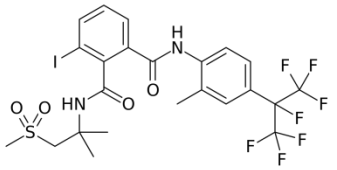
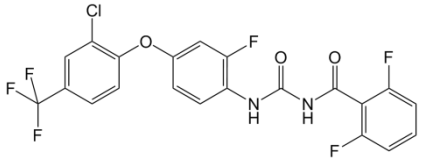
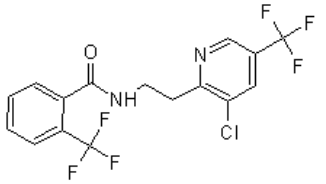
Dimethoate **Type:** Insecticide **MRL:** 2 mg/kg **MW:** 229.3 g/mol	 O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate
Dimethomorph **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 387,9 g/mol	 (E,Z)-4-[3-(4-Chlorphenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholin
Famoxadone **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 374,4 g/mol	 (RS)-5-Methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-1,3-oxazolidine-2,4-dione
Fenamidone **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 311,4 g/mol	 (5S)-5-Methyl-2-(methylsulfanyl)-5-phenyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one
Flubendiamide **Type:** Insecticide **MRL:** 0,01 mg/kg **MW:** 682,4 g/mol	 1-N-[4-(1,1,1,2,3,3,3-heptafluoropropan-2-yl)-2-methylphenyl]-3-iodo-2-N-(2-methyl-1-methylsulfonylpropan-2-yl)benzene-1,2-dicarboxamide
Flufenoxuron **Type:** Acaricide **MRL:** 0,05 mg/kg **MW:** 488,8 g/mol	 1-[4-(2-chloor-α,α,α-trifluor-p-tolyloxy)-2-fluorfenyl]-3-(2,6-difluorbenzoyl)ureum
Fluopyram **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 396,7 g/mol	 N-{2-[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl}-2-(trifluoromethyl)benzamide

Table 1. Continue

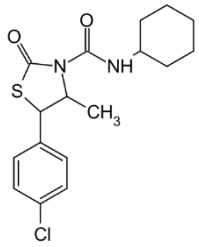
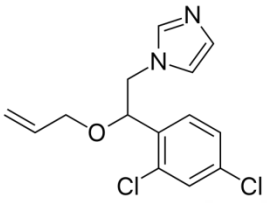
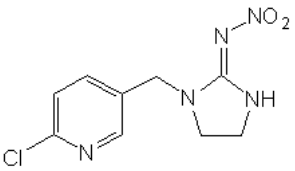
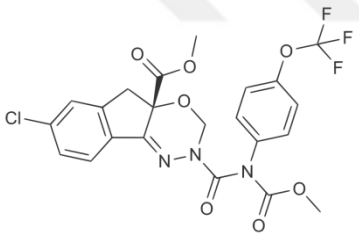
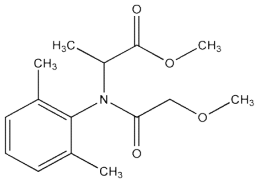
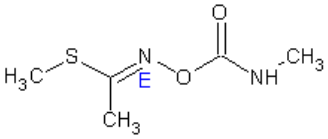
Hexythiazox **Type:** Insecticide **MRL:** 0,5 mg/kg **MW:** 352,9 g/mol	 (4RS,5RS)-5-(4-Chlorphenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidin-3-carboxamid
Imazalil **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 297,2 g/mol	 1-{2-(2,4-Dichlorophenyl)-2-[(prop-2-en-1-yl)oxy]ethyl}-1H-imidazole
Imidacloprid **Type:**Insecticide **MRL:** 0,5 mg/kg **MW:** 255.6 g/mol	 N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide
Indoxacarb **Type:** Insecticide **MRL:** 0,02 mg/kg **MW:** 527.8 g/mol	 Methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate
Metalaxyl **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 279.33 g/mol	 2-[(2,6-dimetilfenil)- (2-metoksi-1-oksoetil) amino] propanoik asid metil ester
Methomyl **Type:** Insecticide **MRL:** 0,02 mg/kg **MW:** 162.2 g/mol	 (E,Z)-methyl N-{[(methylamino) carbonyl]oxy} ethanimidothioate

Table 1. Continue

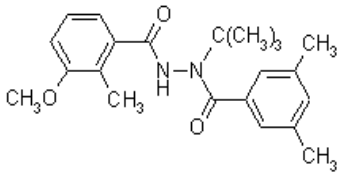
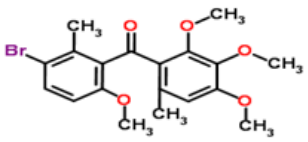
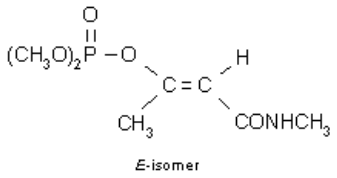
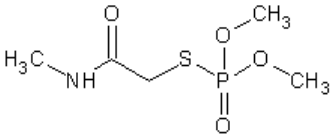
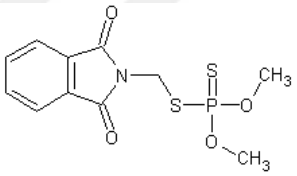
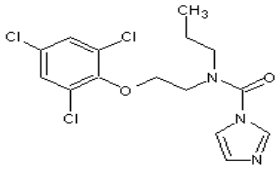
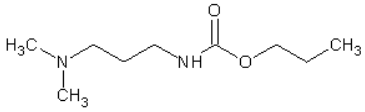
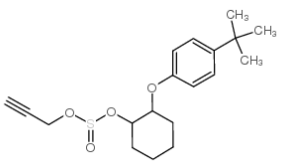
Methoxyfenozide **Type:** Insecticide **MRL:** 0,01 mg/kg **MW:** 368,5 g/mol	 N-tert-Butyl-N'-(3-methoxy-o-toluol)-3,5-xylohydrazid
Metrafenone **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 409.3 g/mol	 3'-bromo-2,3,4,6'-tetrametoksi-2',6-dimetilbenzofenon
Monocrotophos **Type:** Insecticide **MRL:** 0,01 mg/kg **MW:** 223,2 g/mol	 Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate
Omethoate **Type**: Insectisit **MRL**: 2 mg/kg **MW:** 213,2 g/mol	 2-[(Dimethoxyphosphoryl)sulfanyl]-N-methyl-acetamide
Phosmet **Type:** Insecticide **MRL:** 3 mg/kg **MW:** 317,32 g/mol	 (2-(Dimethoxyphosphinothioylthiomethyl)isoindoline-1,3-dione
Prochloraz **Type:** Fungicide **MRL:** 0,05 mg/kg **MW:** 376,7 g/mol	 N-Propyl-N-(2-(2,4,6-trichlorphenoxy)ethyl)-1H-imidazol-1-carboxamid
Propamocarb **Type:** Fungicide **MRL:** 0,01 mg/kg **MW:** 188.3 g/mol	 Propyl [3-(dimethylamino)propyl]carbamate
Propargite **Type:** Akarisit **MRL:** 0,01 mg/kg **MW:** 350.5 g/mol	 2-(4-tert-butilfenoksi)siklohekzil prop-2-in-1-sulfonat

Table 1. Continue

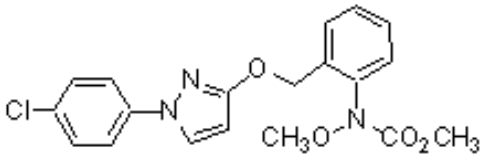
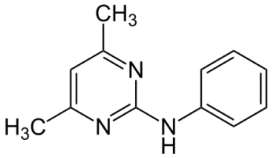
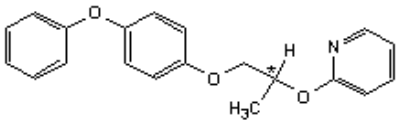
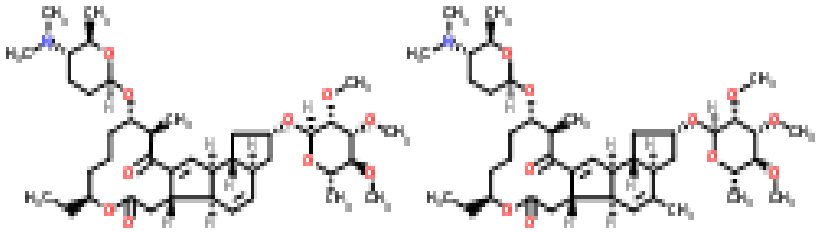
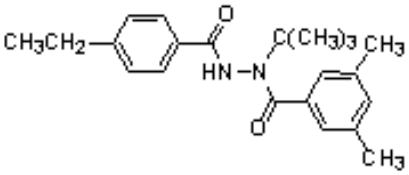
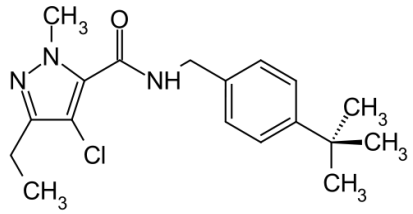
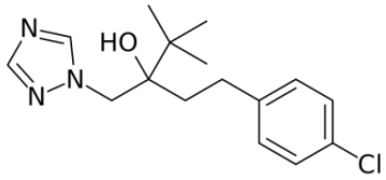
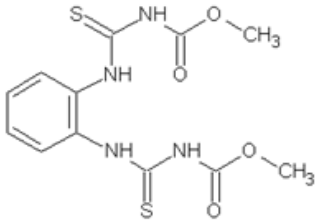
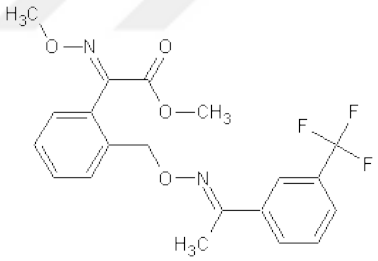
Pyraclostrobine **Type:** Fungicide **MRL:** 0,02 mg/kg **MW:** 387,8 g/mol	 (RS)-5-Methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-1,3-oxazolidine-2,4-dione
Pyrimethanil **Type:** Fungisit **MRL:** 0,02 mg/kg **MW:** 199.3 g/mol	 4,6-Dimetil-N-fenilpirimidin-2-amin
Pyriproxyfen **Type:** Insecticide **MRL:** 0,05 mg/kg **MW:** 321,4 g/mol	 4-Phenoxyphenyl (R/S)-2-(2-pyridyloxy)propyl ether 2-[1-(4-Phenoxyphenoxy)propan-2-yloxy]pyridine
Spinosyn A - D **Type:** Insecticide **MRL:** 0,02 mg/kg **MW:** 731,98 g/mol	 (2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR)-2-(6-deoxy-2,3,4-tri-O-methyl-α-Lmannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-Derythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16bhexadecahydro-14-methyl-1H-8-oxacyclododeca[b]as-indacene-7,15-dione
Tebufenozide **Type:** Insecticide **MRL:** 0,05 mg/kg **MW:** 352,5 g/mol	 N'-(4-Ethylbenzoyl)-3,5-dimethyl-N-(2-methyl-2-propanyl)benzohydrazide

Table 1. Continue

Tebufenpyrate **Type:** Insecticide **MRL:** 0,05 mg/kg **MW:** 333.9 g/mol	 N-[(4-tert-butilfenil)metil]-4-klor-5-etil-2-metilpirazol-3-karboksamid
Tebuconazole **Type:** Fungisit **MRL:** 0,05 mg/kg **MW:** 307.8 g/mol	 (RS)- 1-(4-Klorofenil)- 4,4-dimetil-3-(1H, 1,2,4-triazol-1-ilmetil) pentan- 3-ol
Thiophanate Methyl **Type:** Fungicide **MRL:** 0,1 mg/kg **MW:** 342,4 g/mol	 dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate)
Trifloxystrobine **Type:** Fungicide **MRL:** 0,3 mg/kg **MW:** 408.4 g/mol	 methyl (E)-metoxymino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylidenaminooxy]-o-tolyl}acetate

1.5. Theoretical Aspects of the Methods Used

Trace analysis of pesticide residues in food and in environmental matrices are often accomplished with chromatographic methods. Interferences from complex matrices can be eliminated by applying a separation and preconcentration step prior to analysis.

1.5.1. Sample Preparation Methods

Fatty food analyses are challenging since it constitutes a rather complex matrix. Therefore, well designed sample preparation techniques are required. The techniques used in sample preparation include liquid-liquid extraction (LLE) and solid phase extraction (SPE). The latter has replaced the former in many standard methods since its low solvent consumption, time saving aspects and its potential for automation.

Another green technique developed in the last two decades is solid phase micro extraction (SPME) which is very popular since it provides a simple, solvent free, fast and inexpensive alternative. The extracting phase is a thin polymer film coated fiber where the analyte preconcentrated on it. Then, the fiber is rapidly delivered to a gas chromatographic column. The method can also be adopted to the volatile organic compounds by using headspace (HS) technique. However, in fatty food samples, this technique was not satisfactory. Therefore SPE cartridges and dispersive SPE methods have been utilized in this thesis. Here, the fundamentals of these techniques are given.

Solid Phase Extraction is a rapid sample preparation technique performed with a cartridge packed with a solid stationary phase. The sorbent is hold in the barrel by the frits which also act as a particulate filter. Here, the separation mechanism is quite similar to the liquid chromatography (LC).

The methodology consists of four steps (Figure 2). First of all, the column is conditioned by using appropriate solvents to prepare the sorbent for subsequent sample loading step. The analytes interact with the sorbent via several mechanism including adsorption, ion exchange, dipol-dipol interactions or intermolecular interactions. Then, the column is washed with a mild solvent to remove impurities and then with a strong solvent to elute the analytes. The interaction parameters are optimized to effect retention or elution of the analytes.

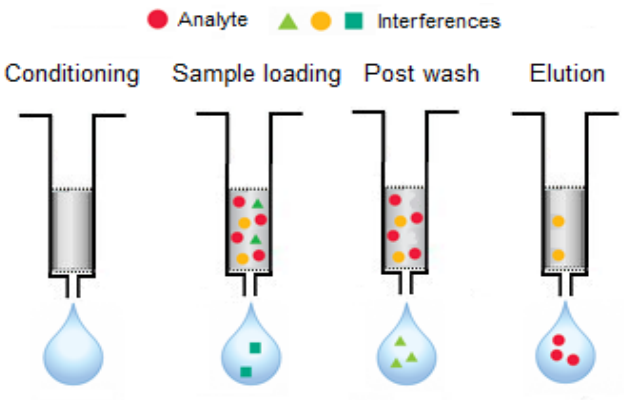


Figure 2. The stages of solid phase extraction technique

A variety of sorbents are available including silica and chemically modified reverse phase silica sorbents such as C8, C18, amminopropyl, cyanopropyl silica sorbents similar to the bonded phases used in HPLC but, larger in particle size, typically 40–60 µm diameters (Chomatography, 1998). Other sorbents include weak and strong ion exchangers, polymeric sorbents and graphitized carbon black. The sorbent selection depends on the interactions of the analyte with the solid phase including dipol-dipol interactions, hydrophobic and/or ionic interactions.

Dispersive solid phase extraction (d-SPE) analysis is a suitable and simple technique for the preparation of a variety of food to analysis. It was first introduced in 2003 as an important step of QuEChERS. It simply includes the interaction of the sample with the carefully weighed amount of solid sorbent without conditioning and an appropriate type of salt is also added to enhance ionic strength. After shaking the sample mixture manually or by using a vortex, the mixture is centrifuged and then, the supernatant is transferred to the autosampler vials of the chromatographic system. This technique is mainly used for clean-up purposes for matrix removal. Among the sorbents used, non-polar interferences including fats are removed by C18 removes very non-polar, while sugars and organic acids are removed by PSA. Pigments in the matrices can be easily eliminated by using graphitized carbon black (GCB).

1.5.2. Liquid Chromatography with Tandem Mass Spectroscopy

High performance liquid chromatography (HPLC) mainly consists of a separation and a detection unit. Separation is provided with a column packed with an appropriate stationary phase to separate the analytes and selective detection of each analyte is performed in the latter unit (http://www.waters.com).

The sample is injected into the mobile phase stream which is pumped through the column via a high-pressure pump at a certain flow rate. There are two types of liquid chromatography depending on the polarity of these two phases. In normal or classical chromatography, such as Tswett's study, the packing material is polar and the mobile phase consists of nonpolar solvents. In contrast, the solvent is polar in reverse phase chromatography with respect to the stationary phase. In this mode, nonpolar analytes retained on the nonpolar stationary phase such as C18 and C8 and polar aqueous organic mixtures such as methanol-water elutes the analytes in order.

Various detectors can be coupled with chromatographic system but, for identification and quantification of pesticide residues in food, mass spectrometers (MS) are most popular. MS detectors function by sorting the ionized molecules and identifying them according to their mass-to-charge (m/z) ratios.

The ion source is one of the most important parts of the system. Several different types of ion sources are available to meet the requirements. In traditional electron ionization system, the analyte molecules are ionized under vacuum. In atmospheric pressure ionization (API) system, as the name implies, the analyte molecules are ionized at atmospheric pressure. The product ions are separated from neutral molecules electrostatically.

The most common API techniques are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). In the former system, the electrostatic field causes additional dissociation of the analytes. The heated drying gas is used to evaporate the solvent droplets and the charge concentration in the droplet increases as the droplets shrink. Finally, the ions are freed into the gas phase and then, pass into the mass analyzer.

In APCI system, on the other hand, the LC eluent is sprayed through a heated vaporizer and the gas-phase solvent molecules are ionized by electrons discharged from a corona needle. The charge is transferred by solvent ions to the analytes through chemical ionization and the analytes enters the mass analyzer. Another system used is atmospheric pressure photoionization (APPI) which utilize a discharge lamp to generate photons in a narrow range of ionization energies.

Different sorts of mass Analyzers are available. ***Quadrupole*** is the well-known system consisting of four parallel rods organized as a square. A voltage is applied to the rods to generate electromagnetic fields and the analyte ions directed to the center are affected by this field. Only certain mass-to-charge ratio (m/z) of ions will pass through the filter at a certain time interval. Quadrupole can operate in scan mode where the mass analyzer monitors a range of mass-to-charge ratios and selected ion monitoring (SIM) mode where only a few m/z ratios are monitored. Scan mode is commonly used for qualitative analyses while quantitation of target compounds are accomplished by using the SIM mode.

Time-of-flight (TOF) analyzers are very accurate in mass measurements. In TOF system, a uniform electromagnetic force is applied to the ions to accelerate them down a flight tube. Lighter ions move faster and thus, arrive first to the detector. Therefore, m/z ratios of the ions are determined by their arrival times.

Ion trap is a system where the ions entered a chamber and trapped there by electromagnetic fields. The ions are ejected from the trap selectively by applying another field. Other systems include Fourier transform-ion cyclotron resonance (FT-ICR or FT-MS) and each has advantages and disadvantages depending on the specific analysis.

API techniques given above are relatively soft techniques and generated ions are molecular ions (M^+ or M^-), protonated molecules (MH^+) or simple adduct ions. In addition to the molecular weight, complementary structural information is needed and therefore, analyte ions are forced to collide with neutral molecules by applying voltage to create more fragmentation. This process is known as collision induced dissociation (CID).

In tandem MS systems (MS/MS) more structural information can be obtained. It is also called triple-quadrupole instruments where the first quadrupole is used to select the precursor ion (Figure 3). CID, so-called collision cell, is in the second stage. In the third stage (quadrupole or TOF) a spectrum of the resulting product ions is obtained. The ions apart from the analyte are discarded by this means. This system can also function in SIM mode.

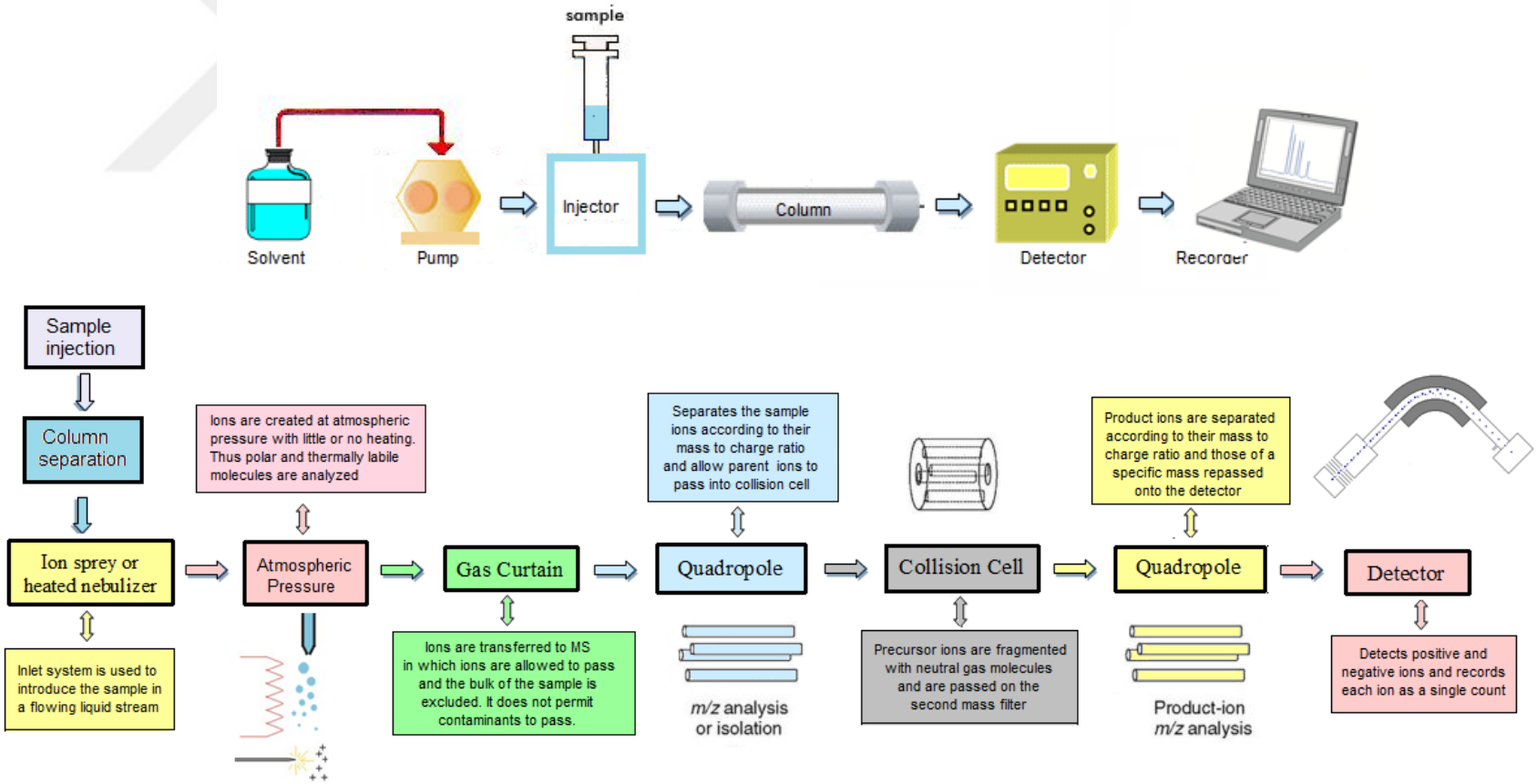


Figure 3. Schematic representation of the LC-MS/MS system with a triple-quadrupole mass spectrometer (www.agilent.com/chem)

1.5.3. Chemometric Evaluation of the Results

The results obtained by these systems are usually treated with chemometric approach (Brereton, 2007). Experimental designs are used for screening, optimizing and quantitative modeling of the system. These designs usually consist of a series of experiments achieved under different conditions. The data obtained reflect the mathematical relationship between the factors or independent variables. For example, assuming that three factors are present in an experiment, estimated response ($\hat{y}$) can be written as below;

$$\hat{y} = \underbrace{b_0}_{\text{intercept}} + \underbrace{b_1x_1 + b_2x_2 + b_3x_3}_{\text{linear terms}} + \underbrace{b_{11}x_{12} + b_{22}x_{22} + b_{33}x_{32}}_{\text{quadratic terms}} + \underbrace{b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3}_{\text{interaction terms}}$$

The intercept is an average in some cases and the linear terms reflects the direct relationship between the response and a given factor. There might be only linear terms for some data, but usually the factors are not independent and thus, interaction terms arise. The design matrix consists of individual parameters in the mathematical model and the response to corresponding values. The number and arrangement of the experiments including replication must be considered and the mathematical model to be tested. For convenience, experimental data is coded and the highest value is coded as +1 and the lowest is coded as −1.

When there are a large number of factors, ***Factorial Design is*** used for screening. Full factorial design is arranged at two levels and in first step, high and low levels are chosen for each factor. Then, the standard design is used where the value of each factor is usually coded and after performing experiments, the data are evaluated by setting up a design matrix based on the below model.

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1x_2$$

Each of the columns in the matrix is different distinguishing each term from one another. Next step is to calculate and then, interpret the coefficients. Major disadvantage of full factorial design is that large number of experiments must be achieved and this number can be reduced by ½, ¼ and so on with two level fractional factorial designs (Brereton, 2007).

1.5.4. Method Validation

For revealing if the analytical method meet the requirements for its intended purpose, validation of the method are searched (Green, 1999). The parameters used in validation of chromatographic methods are given below.

Selectivity is one of the most important parameters. It can be described as the ability of a method to quantify an analyte in the presence of other components in a sample. It is desired to distinguish the analyte signal from that of other components. The results directly proportional to the concentration of the analytes within a range of concentrations are called ***Linearity***. In calibration studies, different aliquots taken from a homogenous sample are repeatedly analyzed to reveal closeness of the results. ***Precision*** is a measure of random error and evaluated by the means of the Relative Standard Deviation (RSD).

%RSD = (standard deviation/mean) x 100

Precision is stated in three levels; if the experiments are repeated under the same operating conditions over a short interval called ***repeatability***. However, if the experiments are repeated at different days with different analytes and/or, different equipment, it is called ***intermediate precision***. Finally, ***reproducibility*** expresses the precision between laboratories.

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected as three fold of signal to noise ratio, (*S/N*x3). ***Limit of Quantitation (LOQ)*** is the minimal concentration that can be determined with acceptable precision (*S/N*x10).

The accuracy of the method is usually given as the ***Recovery*** which is the percent ratio of found concentration to the theoretical concentration. ***Stability*** reflects the effect of time, storage and the sample containers on the concentration.

2. EXPERIMENTAL

2.1. Reagents and Apparatus

In all experiments, analytical grade reagents were used. The pesticide standards were purchased from Dr. Ehrensttrofer and individual stock solutions at 1000 mg/L were prepared in acetonitrile and acetone. The working solutions were prepared by acetonitrile. HPLC grade acetonitrile was maintained from Merck (Darmstadt, Germany).

For pesticides analysis Q-sep QuEChERS extraction salts was purchased from Restek (USA), d-SPE clean up were $MgSO_4$ Merck (Darmstadt, Germany), C18 sorbent Merck (Darmstadt, Germany) and triethyl tert-butylammine.

Chromatographic measurements were performed by using Shimadzu LC-MS/MS 8040 with an electrospray ionization interface (ESI). For data processing, Lab solutions Main Browser software from Shimadzu was used.

The column used was an Inertsil ODS-4 C18 (2.1mm x 50mm, 2.1 μm). The oven temperature was set to 40^{o}C and injection volume was set to 20 μL in fool loop mode. The MS conditions are listed below in Table 2. The mobile phases were 5 mM ammonium formate containing methanol and ultrapure water maintained from Smart 2 Pure equipment.

Table 2. The conditions of MS/MS unit

Nebulizing gas flow	3 L/min
DL temperature	250°C
Heat block temperature	400°C
Drying gas flow	12 L/min
Interface	ESI
Interface voltage	4,5 kV,
Detector voltage	1,96 kV
IG vacuum	1,7 mPa
CID gas	230 kPa

The samples have been analyzed in positive ionization by using selected reaction monitoring (SRM) mode. Gradient elution program was applied with 0.4 mL/min flow rate. Precursor (Parent mass) and product ion masses as well as the individual are shown in **Table 3**.

Table 3. SRM mode conditions of the MS/MS detector

Pesticide	**R_T (min)**	**Parent/Product ion**	**Parent/Product ion**
Acetamiprid	2,857	223,00>126,00	223,00>56,00
Azoxystrobin	3,907	404,10>371,95	404,10>329,00
Bitertanol	4,279	338,10>70,00	338,10>99,00
Boscalid	3,921	343,00>307,00	343,00>139,90
Buprimate	3,812	317,10>108,00	317,10>166,10
Buprofezin	4,324	306,10>56,95	306,10>201,10
Carbaryl	3,549	202,15>145,00	202,15>126,95
Carbofuran	3,418	222,00>165,00	222,00>123,05
Chlofentezine	4,394	303,00>138,00	303,00>102,00
Chlormequat Chloride	0,466	122,00>58,10	122,00>62,90
Chlorpyrifos	4,604	349,90>96,90	349,90>197,85
Cymoxanil	2,962	199,00>127,95	199,00>111,00
Cyproconazole	4,024	292,10>70,00	292,10>124,95
Cyprodinil	3,933	226,20>92,95	226,20>108,00
Difenocazole	4,324	406,00>250,90	406,00>110,90
Dimethoate	2,83	229,90>198,95	229,90>124,95
Dimethomorph	3,942	388,10>300,95	388,10>165,00
Famaxadone	4,237	392,10>331,00	392,10>93,00
Fenamidone	3,92	312,00>92,10	312,00>236,10
Flubendiamide	4,108	683,10>408,00	683,10>273,00
Flufenoxuron	4,557	489,00>158,00	489,00>141,00
Fluopyram	4,013	397,20>208,00	397,20>173,00
Hexythiazox	4,576	353,10>227,90	353,10>168,00
Imazalil	3,515	296,90>159,00	296,90>254,90
Imidachloropid	2,67	256,00>208,95	256,00>175,05
Indoxacarb	4,308	527,95>202,90	527,95>217,95
Metalaxyl	3,711	280,00>220,15	280,00>160,10
Methomyl	2,378	163,10>88,10	163,10>106,00
Methoxyfenozide	4,008	369,10>149,10	369,10>313,20
Metrofenone	4,348	409,00>209,00	409,00>227,00
Monocrotophos	2,464	224,00>127,00	224,00>98,00

Table 3. Continue

Omethoate	1,833	214,10>125,00	214,10>182,90
Phosmet	3,909	317,95>159,95	317,95>132,95
Prochloraz	4,091	375,90>307,80	375,90>266,00
Propamocarb	1,891	189,00>102,00	189,20>143,90
Propargite	4,589	368,15>231,10	368,15>175,10
Pyraclostrobin	4,303	388,00>163,00	388,00>104,00
Pyrimethanil	3,549	199,90>107,00	199,80>82,00
Pyriproxypen	4,601	322,10>96,00	322,10>184,95
Spinosyn A	4,171	732,30>142,10	732,30>98,15
Spinosyn D	4,248	746,35>142,10	746,35>98,15
Tebuconazole	4,181	308,10>70,05	308,10>125,00
Tebufenozide	4,147	353,20>133,10	353,20>105,00
Tebufenpyrad	4,456	334,00>117,00	334,00>145,05
Thiaphionat Methyl	3,367	343,00>150,95	343,00>310,85
Trifloxystrobin	4,364	409,10>186,00	409,10>144,95

2.2. Sample Preparation Procedure

First of all, original QuEChERS method was applied to olive samples. For this purpose, 15 g of homogenized olive sample was transferred to a 50mL centrifuge tube where it was added to 15 mL of acetonitrile containing of %1 formic acid. The mixture was shaken for 1 min and then, extraction salts; 6 g of $MgSO_4$ and 1.5 g NaOAc were added. It was vortexed or shaken vigorously by hand for 1 min. The sample was then, centrifuged at 5000 rpm for 1 min and the 8 mL of supernatant was transferred to the dSPE clean-up tube including 1.5 g $MgSO_4$, 0.4 g of C18 and 0.4 g of PSA. After shaken of the sample by hand or by using a vortex for 1 min, the sample was centrifuged at 5000 rpm for 1 min. The supernatant was transferred to an autosampler vial as given in **Figure 4**.

The modifications of this procedure were made in clean-up stages. Several salt mixtures were tested for screening purposes and their performances were compared. The effect of these salts and their amount was evaluated by applying full factorial design at two levels for three factors. Therefore, $2^3 = 8$ experiment was design as given in **Table 4**. This design was repeated for the pesticide concentrations of 10 and 50 ng/mL.

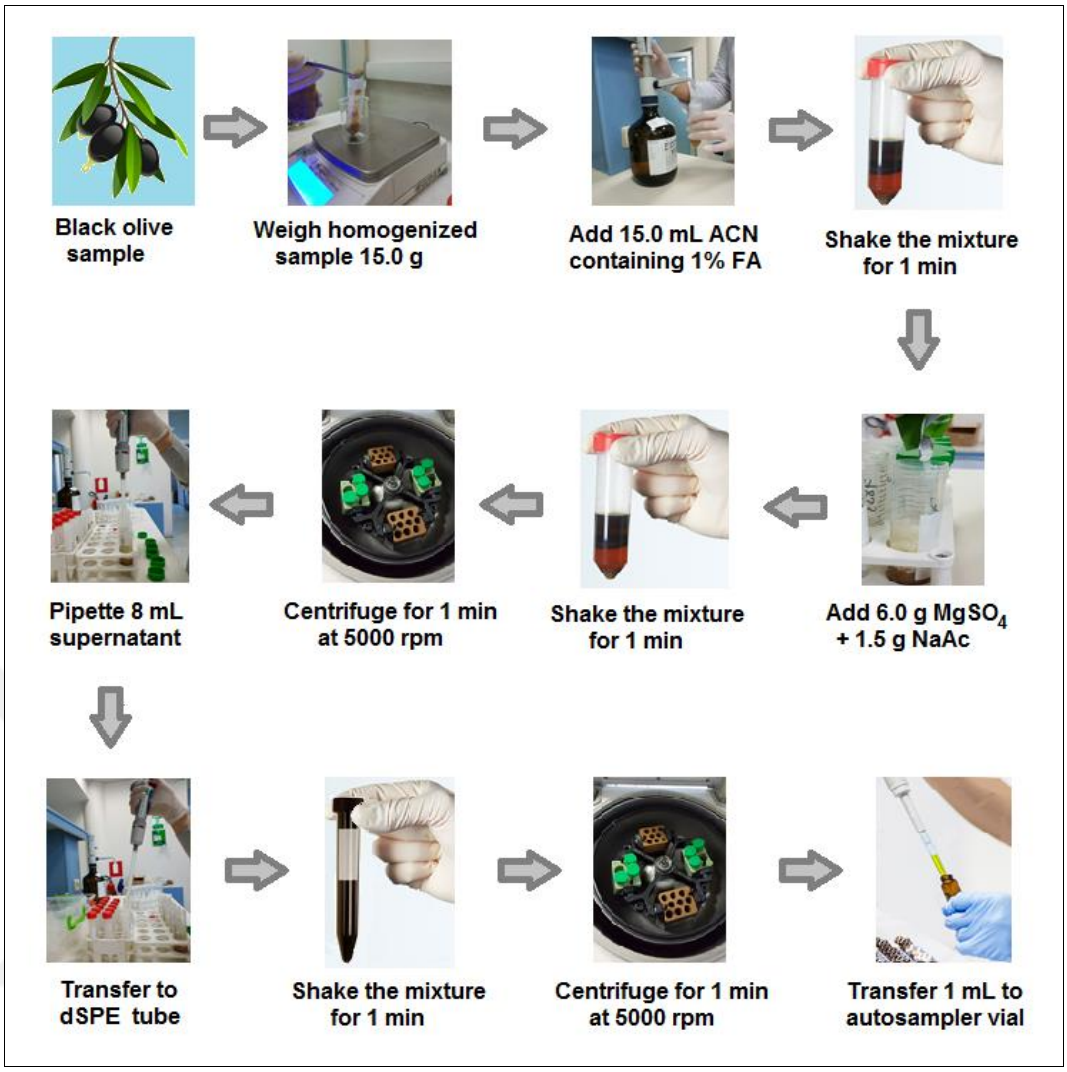


Figure 4. The stages of original QuEChERS method used for olive samples

Table 4. Full factorial design applied for the olive samples.

	TBA	**$CaCO_3$**	**C18**
	0,3 g (-1)	0,8 g (-1)	0,3 g (-1)
Analysis No	0,5 g (+1)	1,5 g (+1)	0,5 g (+1)
1	1	1	1
2	1	1	-1
3	1	-1	1
4	1	-1	-1
5	-1	1	1
6	-1	1	-1
7	-1	-1	1
8	-1	-1	-1

3. Results and Discussion

Initial studies were devoted to the use of original procedure to reveal the analytical characteristics of the method. Therefore, original QuEChERS method was applied for the black olive samples as described in Experimental Section.

3.1. Method Development with Original QuEChERS Method

Following the sample preparation step, measurements were made according to the instrumental conditions given above. Standard addition method was applied for the samples and the chromatogram obtained was given in **Figure 5**.

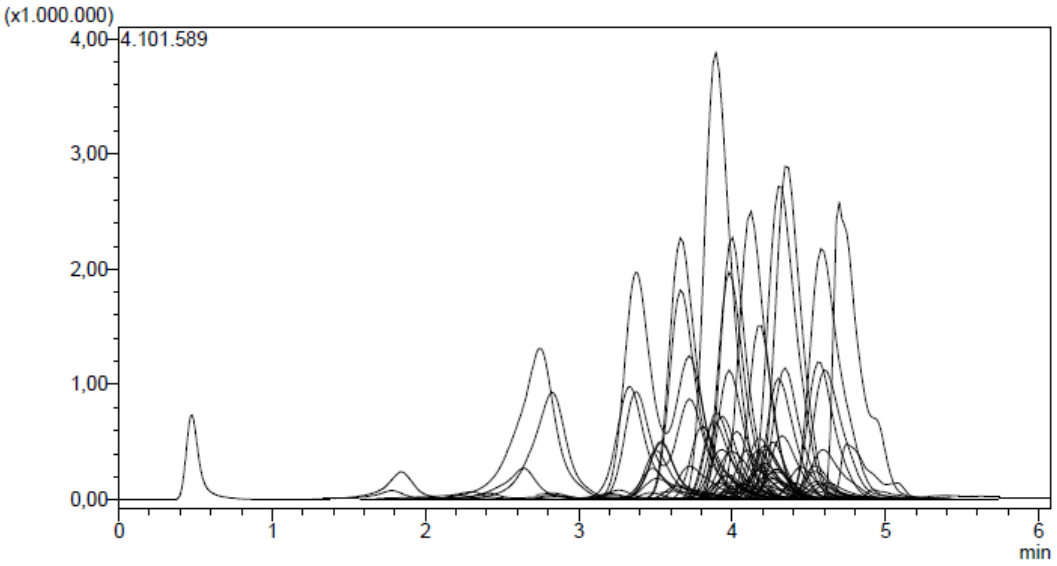


Figure 5. Standard addition LC-MS/MS chromatograms of the 46 pesticides after QuEChERS method was applied to black olive samples.

From the peak area values calculated from the chromatograms, the calibration curves obtained for all the pesticides were evaluated. The calibration graphs were found linear for the concentration range of 2.5-100 ng/mL and given in **Figure 6** where the m/z ratios were indicated as well.

The equation, correlation coefficients and limit of detection (LOD) along with limit of quantitation (LOQ) were given in **Table 5.**

Figure 6. The calibration curves obtained for the pesticides studied by LC-MS/MS technique.

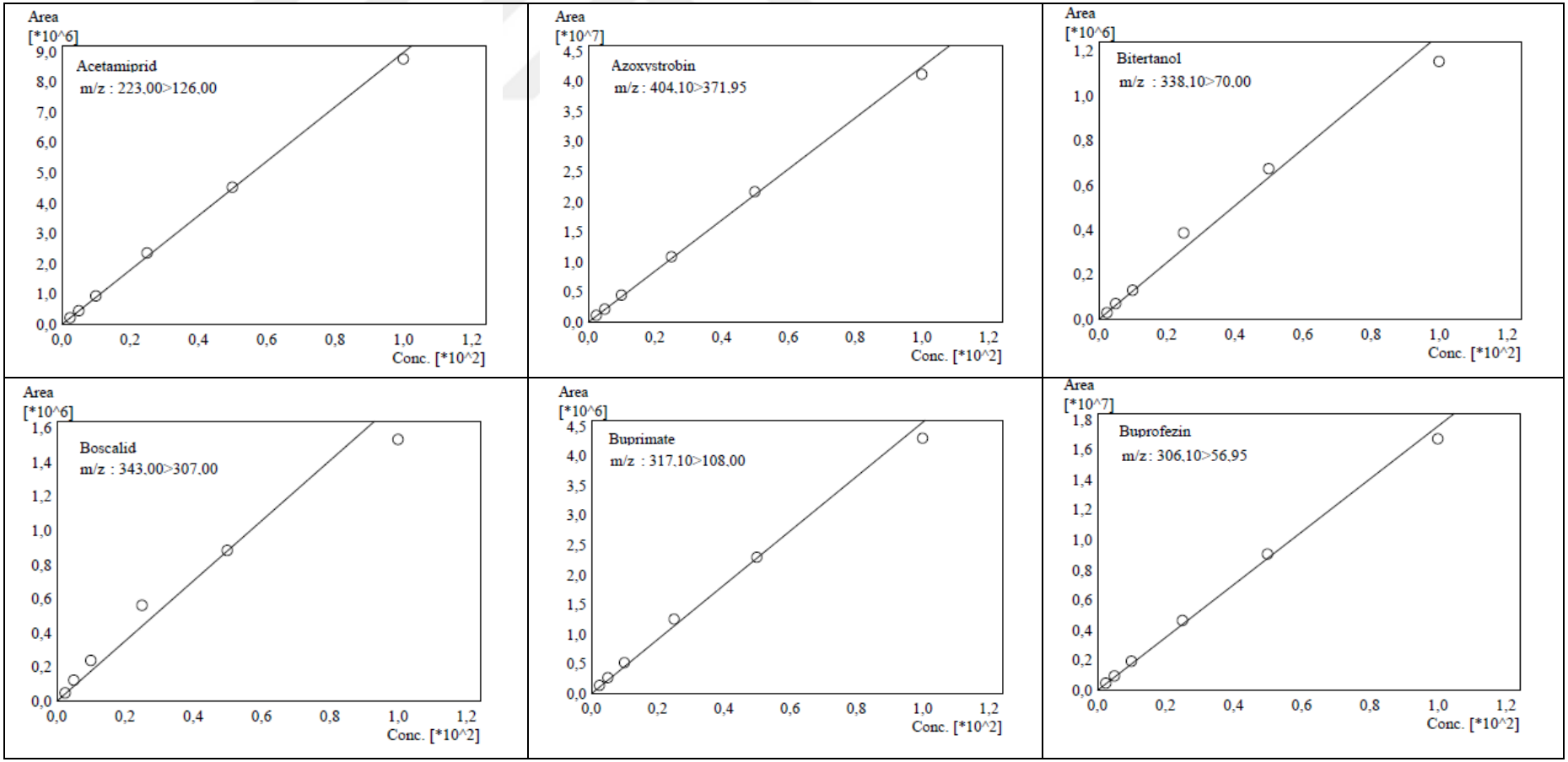


Figure 6. Continue

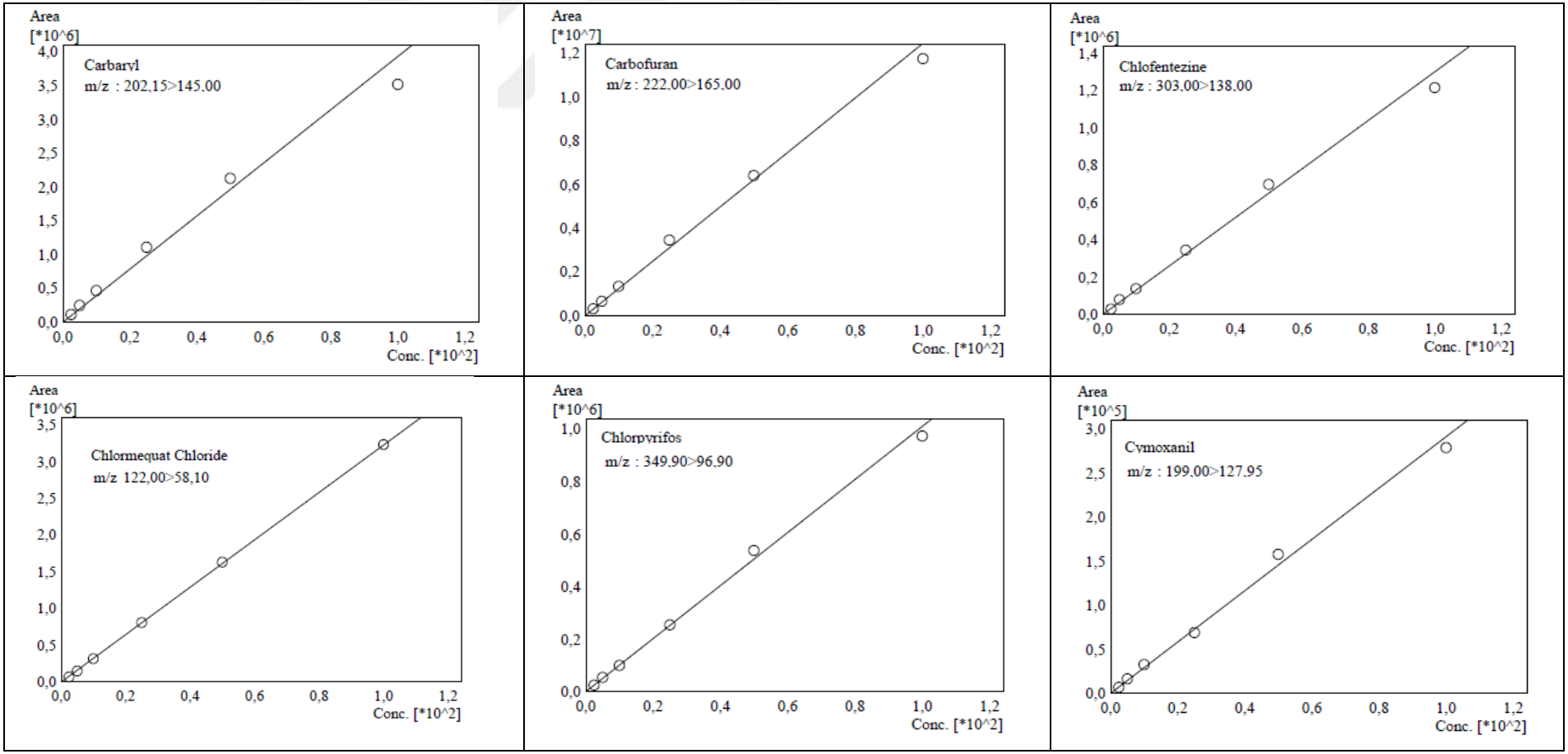


Figure 6. Continue

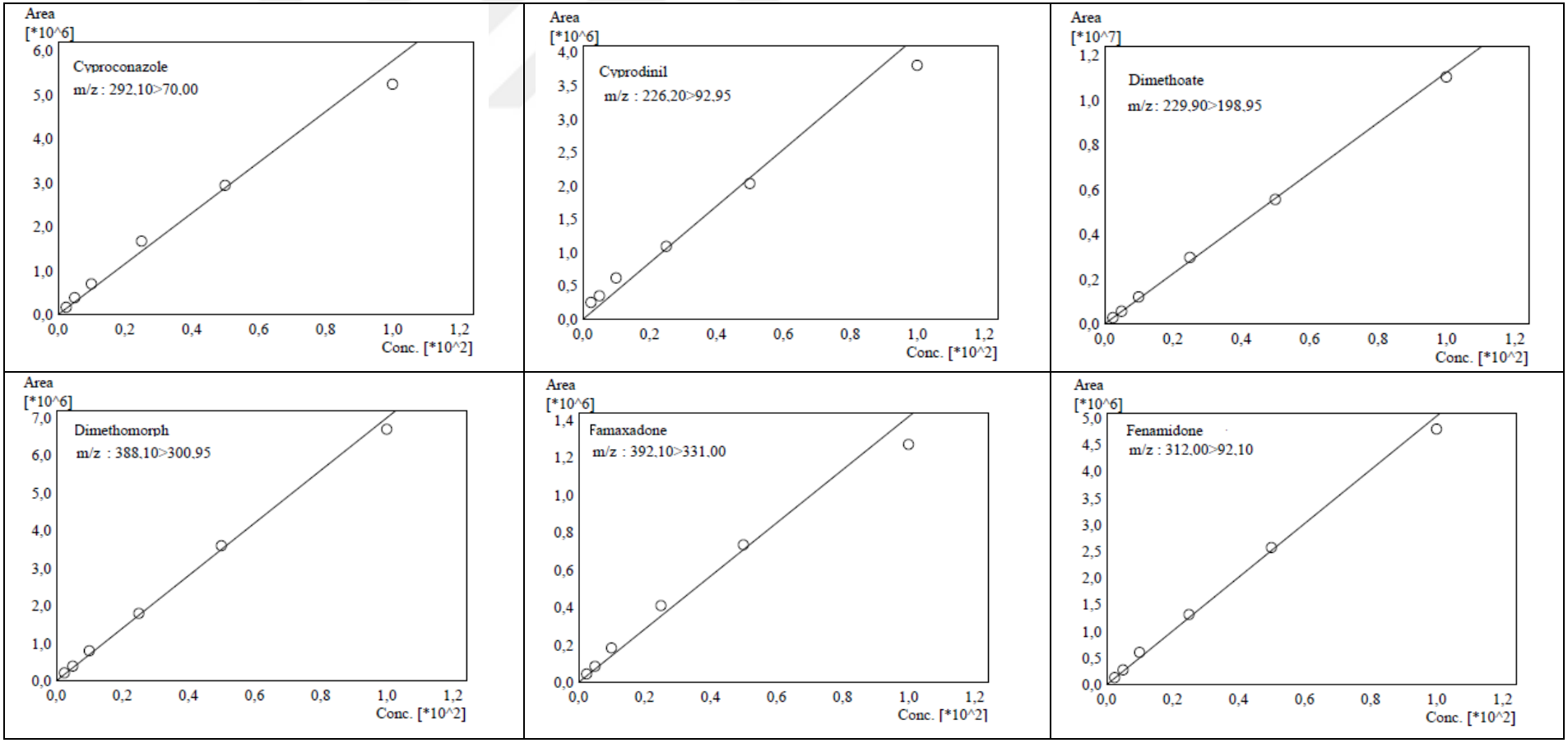


Figure 6. Continue

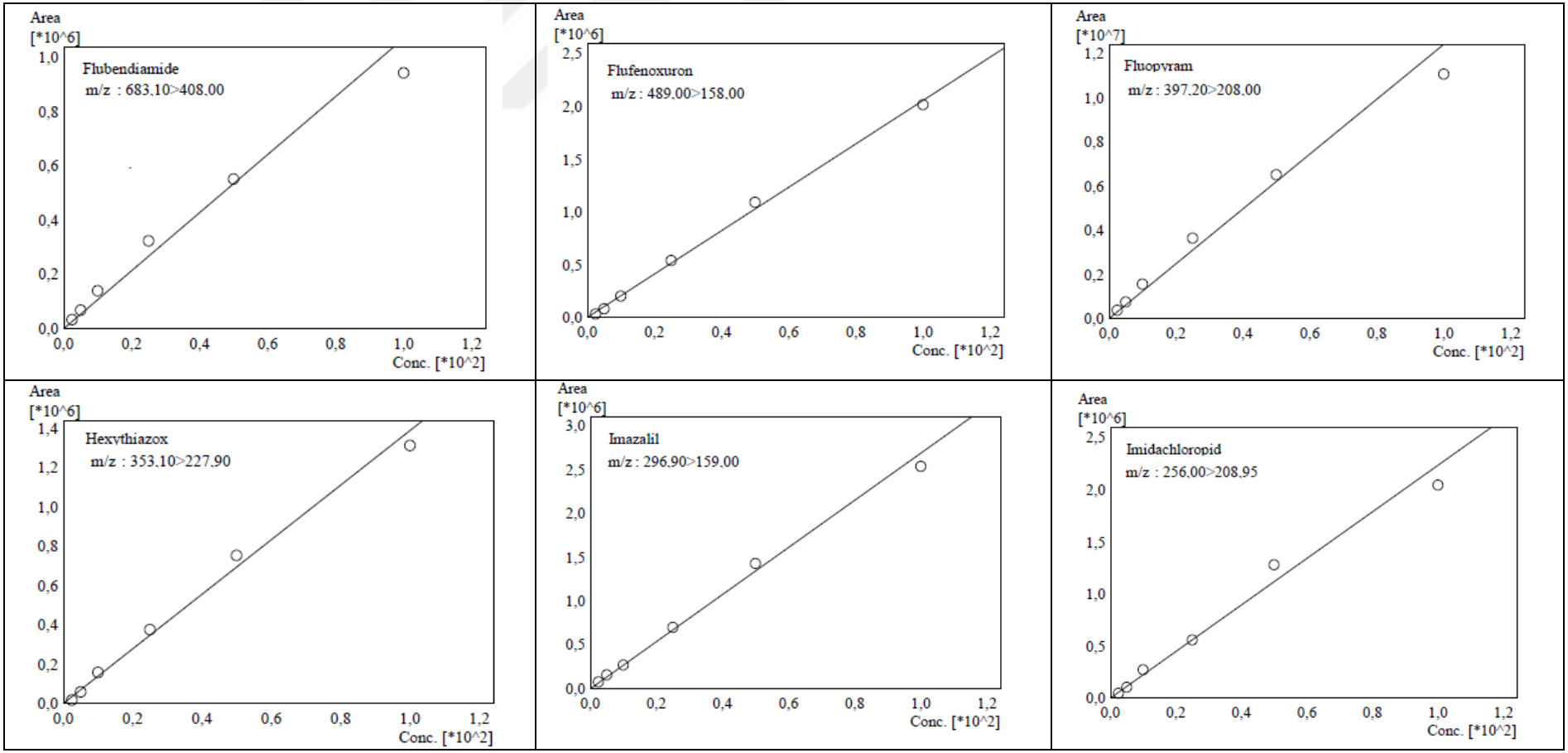


Figure 6. Continue

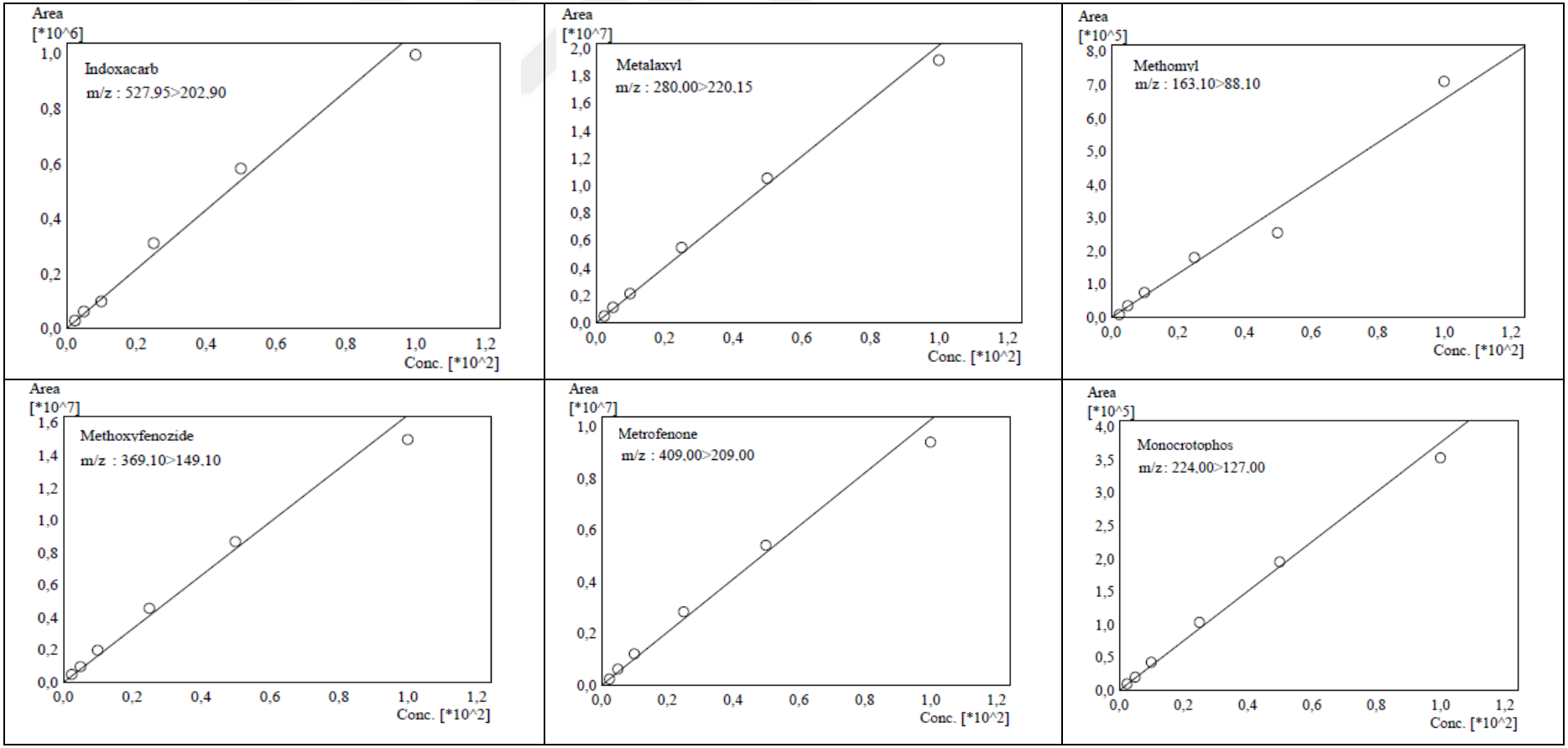


Figure 6. Continue

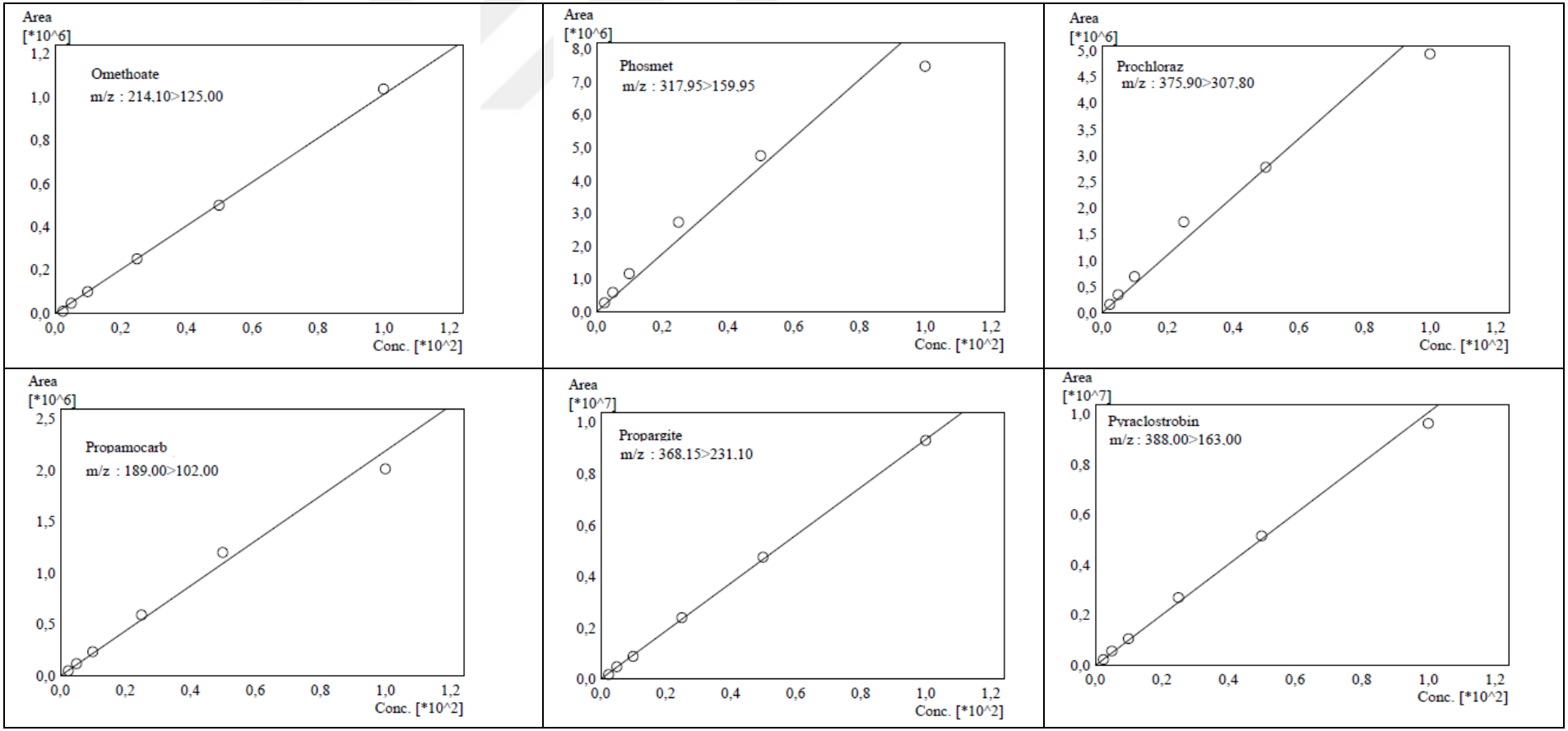


Figure 6. Continue

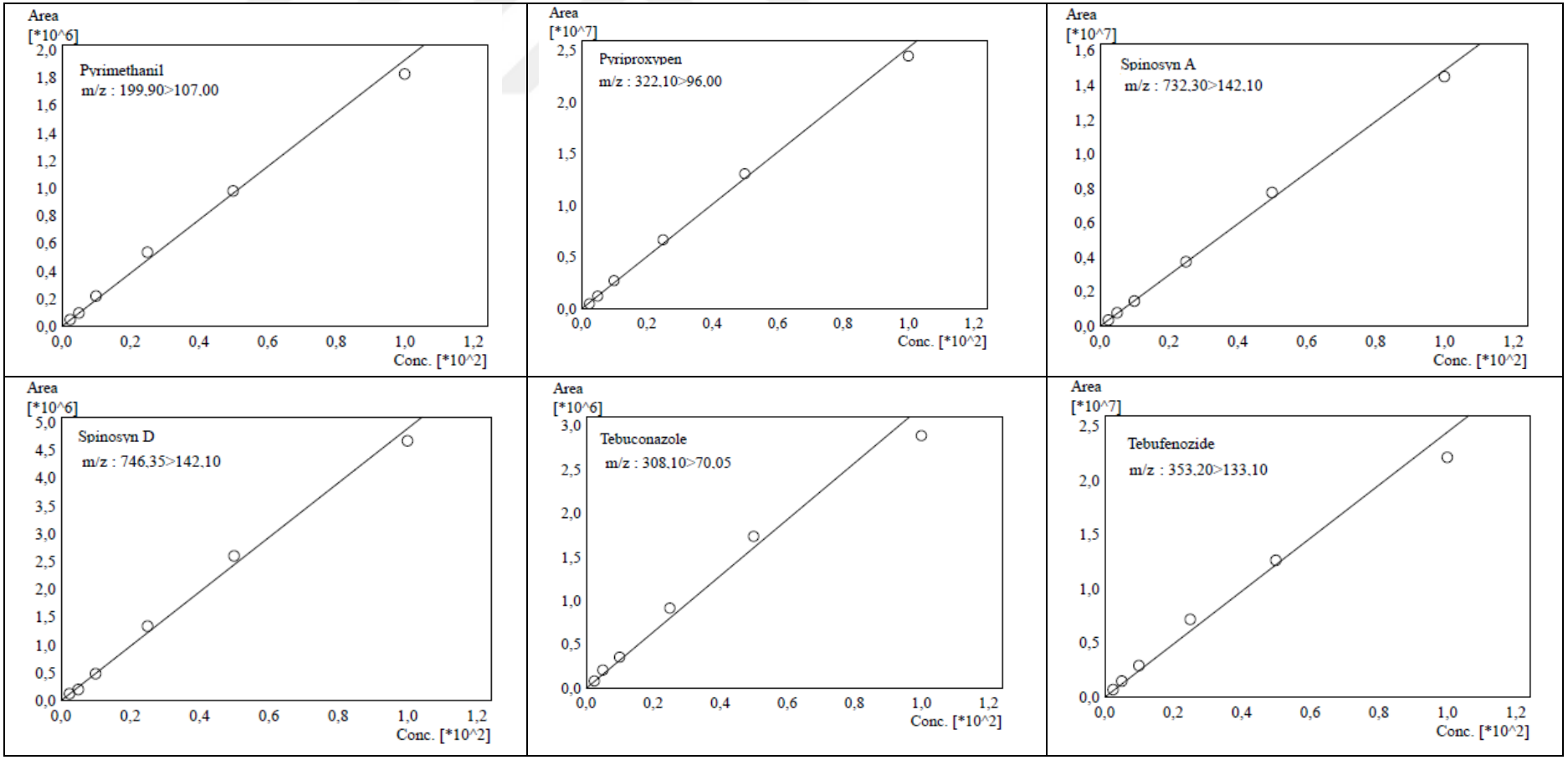


Figure 6. Continue

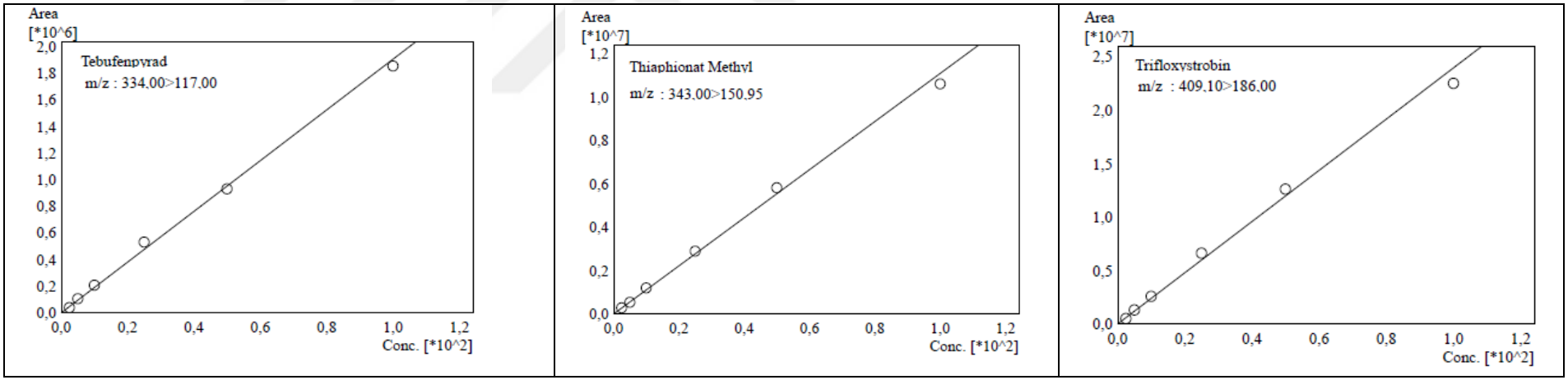


Table 5. Analytical characteristics of the QuEChERS method applied to the black olive samples.

Pesticide Name	**Equation**	**R^2**	**LOD (ng/mL)**	**LOQ (ng/mL)**
Acetamiprid	f(x)=89721,2*x+0	0.99	1.77	5.39
Azoxystrobin	f(x)=424226*x+0	0.99	2.06	6.24
Bitertanol	f(x)=12699,3*x+0	0.98	8.35	28.31
Boscalid	f(x)=17616,5*x+0	0.97	9.16	27.77
Buprimate	f(x)=45642,4*x+0	0.99	3.24	9.81
Buprofezin	f(x)=175543*x+0	0.99	3.30	10.02
Carbaryl	f(x)=39359,6*x+0	0.98	8.22	24.92
Carbofuran	f(x)=124391*x+0	0.99	4.26	12.91
Chlofentezine	f(x)=13021,5*x+0	0.99	5.74	17.39
Chlormequat Chloride	f(x)=32274,3*x+0	0.99	0.54	1.65
Chlorpyrifos	f(x)=10109,8*x+0	0.99	4.06	12.32
Cymoxanil	f(x)=2912,84*x+0	0.99	5.29	16.05
Cyproconazole	f(x)=57720,5*x+0	0.98	5.74	17.42
Cyprodinil	f(x)=42438,1*x+0	0.99	2.08	6.30
Difenoconazole	f(x)=63086,1*x+0	0.99	4.32	13.09
Dimethoate	f(x)=112295*x+0	0.99	1.48	4.51
Dimethomorph	f(x)=70159,4*x+0	0.99	2.72	8.25
Famaxadone	f(x)=14191,5*x+0	0.98	6.79	20.60
Fenamidone	f(x)=50453,6*x+0	0.99	3.06	9.29
Flubendiamide	f(x)=10713,0*x+0	0.98	8.11	24.59
Flufenoxuron	f(x)=20594,0*x+0	0.99	3.74	11.36
Fluopyram	f(x)=124118*x+0	0.98	7.71	23.36
Hexythiazox	f(x)=13898,9*x+0	0.99	6.46	19.58
Imazalil	f(x)=26858,6*x+0	0.99	4.81	14.59
Imidachloropid	f(x)=22360,4*x+0	0.98	9.79	29.67
Indoxacarb	f(x)=10806,0*x+0	0.98	7.50	22.72
Metalaxyl	f(x)=202521*x+0	0.99	4.27	12.96
Methomyl	f(x)=6568,41*x+0	0.97	6.54	19.82
Methoxyfenozide	f(x)=164723*x+0	0.99	6.36	19.28
Metrofenone	f(x)=102770*x+0	0.98	6.24	18.93
Monocrotophos	f(x)=3764,89*x+0	0.99	4.59	13.91
Omethoate	f(x)=32274,3*x+0	0.99	1.44	4.36
Phosmet	f(x)=88326,7*x+0	0.97	11.65	35.32
Prochloraz	f(x)=55417,5*x+0	0.98	7.93	24.04
Propamocarb	f(x)=21910,8*x+0	0.99	7.58	22.98
Propargite	f(x)=93410,9*x+0	0.99	1,33	4,04
Pyraclostrobin	f(x)=100803*x+0	0.99	3.16	9.58
Pyrimethanil	f(x)=19299,9*x+0	0.99	3.92	11.88
Pyriproxypen	f(x)=253177*x+0	0.99	1.08	9.36
Spinosyn A	f(x)=148558*x+0	0.99	2.77	8.40
Spinosyn D	f(x)=48931,1*x+0	0.99	5.11	15.51
Tebuconazole	f(x)=32118,3*x+0	0.98	8.17	24.77
Tebufenozide	f(x)=244704*x+0	0.98	6.670	20.22
Tebufenpyrad	f(x)=19088,4*x+0	0.99	2.97	9.00
ThiaphionateMethyl	f(x)=110885*x+0	0.99	3.90	11.83
Trifloxystrobin	f(x)=239786*x+0	0.99	5.27	15.98

As shown in **Table 5**, the correlation coefficients were found close to unity and LOD level were all well below the MRL values of pesticides. Following section includes the studies on the modified QuEChERS method.

3.2. Studies with Modifications in QuEChERS Method

In this part of thesis, the modifications were made in clean-up steps. $CaCO_3$ is used as a salt, instead of $MgSO_4$ as mentioned above. In addition, PSA is replaced by TBA in the same step. The analytical procedure is applied as given in Experimental Section and full factorial design was chosen to evaluate the effects of salt amount on the recovery values. The recoveries were given for each pesticides at two concentration levels in **Table 6.**

As can be concluded from the Table, the type and amount of salt has a significant effect on the results obtained and recovery values varies in a wide range. The salts are chosen as representing a wide range of polarity being C18 non-polar, $CaCO_3$ as the polar constituent and TBA as the salt with medium polarity. As given in **Table 4**, the highest amount of each salt along with C18 constitutes experiment number 1 and others were also indicated in the table.

As a rule, the recovery values between the limits of 70-120% are generally accepted. Here, the results are distributed in a wider range and the recovery values greater than 70% were made bold in **Table 6.** According to these results, several combinations have resulted higher recoveries while the rest are below or higher than acceptable levels.

Table 6. The recovery values obtained for each pesticide from full factorial design and original QuEChERS method at two concentration levels

Pesticide	**Conc. (ng/mL)**	**Full Factorial experiment number (TBA, $CaCO_3$, C18)**								
		1 (+ + +)	**2 (+ + -)**	**3 (+ - +)**	**4 (+ - -)**	**5 (- + +)**	**6 (- + -)**	**7 (- - +)**	**8 (- - -)**	**Original**
Acetamiprid	10	41.9	53.2	32.7	**77.5**	43.5	57.7	53.2	32.7	81.4
	50	60.0	44.0	41.8	61.3	41.8	**84.5**	60.0	55.3	78.4
Azoxystrobin	10	**77.2**	59.8	58.3	**81.8**	55.2	59.0	59.8	58.3	79.4
	50	57.8	59.4	56.4	58.9	56.4	**76.8**	57.8	54.7	79.2
Bitertanol	10	**79.0**	**88.4**	47.9	61.6	59.7	66.6	53.4	42.1	80.9
	50	55.9	70.2	65.3	**70.5**	54.7	62.7	**86.3**	63.8	80.4
Boscalid	10	75.5	66.5	50.0	**88.0**	57.3	**75.5**	66.5	50.0	82.4
	50	68.3	50.2	51.7	66.3	51.7	**83.0**	68.3	58.1	81.4
Buprimate	10	**71.0**	41.4	29.4	**81.8**	58.3	61.8	41.4	29.4	78.5
	50	58.6	53.2	48.7	62.3	44.4	**72.3**	58.6	56.7	85.6
Buprofezin	10	**78.8**	28.3	23.5	**83.5**	59.6	53.9	28.3	23.5	82.4
	50	56.2	25.5	23.1	62.5	22.2	**79.8**	56.2	54.0	83.6
Carbaryl	10	60.6	46.9	34.3	66.0	48.7	61.3	46.9	34.3	84.6
	50	56.8	41.2	40.6	57.5	40.6	**72.9**	56.8	52.9	80.6
Carbofuran	10	52.0	44.1	32.7	**75.6**	41.5	59.3	44.1	32.7	84.3
	50	56.2	39.9	39.1	56.8	39.1	**76.4**	56.2	50.2	85.4
Chlofentezine	10	56.5	15.2	14.6	84.7	49.0	33.3	15.2	14.6	83.0
	50	36.7	11.4	12.9	50.8	16.9	68.0	36.7	47.5	86.4
Chlormequat Chloride	10	49.4	30.5	28.9	**78.0**	39.2	58.7	30.5	28.9	83.8
	50	65.6	30.0	31.7	67.3	28.1	66.4	65.6	61.8	84,7

Table 6. Continue

Pesticide	Conc. (ng/mL)	Full Factorial experiment number (TBA, $CaCO_3$, C18)								
		1 (+ + +)	**2 (+ + -)**	**3 (+ - +)**	**4 (+ - -)**	**5 (- + +)**	**6 (- + -)**	**7 (- - +)**	**8 (- - -)**	**Original**
Chlorpyrifos	10	57.8	10.0	35.0	59.7	33.3	23.2	25.0	25.0	77.4
	50	34.8	1.8	1.1	49.8	1.1	64.2	34.8	45.5	79.6
Cymoxanil	10	57.4	62.4	31.1	38.7	17.8	59.0	62.4	31.1	77.8
	50	**71.9**	52.2	43.8	65.8	43.8	**80.8**	**71.9**	56.8	79.2
Cyproconazole	10	**85.7**	**72.5**	69.1	**78.3**	65.4	**72.1**	**72.5**	69.1	77.2
	50	62.8	62.8	58.6	67.0	58.6	**87.8**	62.8	67.0	82.6
Cyprodinil	10	266.1	61.5	50.4	**73.0**	**83.6**	64.5	61.5	**101.6**	82.8
	50	60.4	35.5	33.5	61.7	30.4	**79.4**	60.4	51.9	85.2
Difenocozole	10	**87.5**	48.4	30.7	**80.9**	67.5	**77.5**	48.4	30.7	82.9
	50	**71.7**	36.8	28.7	65.9	35.4	**73.3**	**71.7**	60.0	81.6
Dimethoate	10	40.4	47.4	36.4	**73.1**	34.1	59.4	47.4	36.4	84.2
	50	63.9	48.7	49.0	65.4	49.0	**76.9**	63.9	62.3	85.2
Dimethomorph	10	**76.7**	**81.4**	67.8	**89.9**	69.4	**74.3**	**81.4**	67.8	84.1
	50	65.9	**72.0**	67.4	64.7	67.4	**73.9**	65.9	58.0	80.6
Famaxadone	10	**83.1**	49.1	43.9	**90.4**	**72.3**	60.2	49.1	43.9	81.5
	50	61.6	46.7	41.6	60.6	39.7	**84.8**	61.6	55.0	82.8
Fenamidone	10	**90.7**	**70.1**	52.4	**90.1**	64.6	**71.7**	**70.1**	60.2	77.9
	50	**70.1**	66.7	68.1	68.2	68.1	68.9	**70.1**	60.3	78.2
Flubendiamide	10	**75.1**	**76.3**	69.4	**70.2**	**71.6**	69.6	**76.3**	69.4	82.0
	50	**70.7**	64.6	60.9	68.9	53.1	**72.3**	64.9	65.1	85.6

Table 6. Continue

Pesticide	Conc. (ng/mL)	Full Factorial experiment number (TBA, $CaCO_3$, C18)								
		1 (+ + +)	**2 (+ + -)**	**3 (+ - +)**	**4 (+ - -)**	**5 (- + +)**	**6 (- + -)**	**7 (- - +)**	**8 (- - -)**	**Original**
Flufenoxuron	10	69.1	12.6	6.4	81.1	28.2	23.4	12.6	6.4	84.4
	50	30.7	6.3	6.7	50.7	6.2	65.9	30.7	48.6	84.2
Fluopyram	10	**89.2**	57.0	46.0	**89.1**	61.1	65.4	57.0	46.0	82.2
	50	60.2	45.7	40.9	61.7	36.9	**79.3**	60.2	55.3	86.2
Hexythiazox	10	64.4	3.5	2.8	**77.4**	34.1	25.9	3.5	2.8	85.6
	50	28.0	1.0	1.8	46.0	1.8	60.1	28.0	41.9	88.4
Imazalil	10	59.3	67.7	**72.3**	**85.3**	53.4	63.4	67.7	**72.3**	84.6
	50	58.9	64.9	66.7	65.8	66.7	**85.6**	58.9	58.6	83.8
Imidachloropid	10	**89.2**	58.8	60.4	**95.8**	**80.4**	**72.1**	58.8	60.4	81.3
	50	63.5	48.3	44.5	**74.3**	44.5	69.2	63.5	64.3	80.4
Indoxacarb	10	**87.1**	59.3	50.8	**81.9**	**75.8**	**76.6**	59.3	50.8	80.1
	50	**78.1**	46.1	48.5	**74.5**	44.7	**74.3**	**83.3**	**70.0**	82.8
Metalaxyl	10	**77.4**	**78.2**	**75.0**	**91.8**	55.2	67.2	**78.2**	**75.0**	80.1
	50	69.8	**72.0**	**76.7**	**71.1**	**76.7**	**80.0**	69.8	63.4	81.2
Methomyl	10	64.0	56.3	29.2	**80.1**	33.6	58.4	56.3	29.2	85.8
	50	61.1	57.1	68.0	60.9	68.0	**86.3**	61.1	57.3	86.4
Methoxyfenozide	10	**85.1**	69.4	64.4	**95.6**	65.7	64.8	69.4	64.4	88.6
	50	68.3	62.0	59.2	**70.0**	53.4	68.4	68.3	64.0	85.6
Metrofenone	10	**95.2**	36.5	31.5	63.0	47.9	48.1	36.5	31.5	80.2
	50	50.3	29.6	28.5	60.3	27.1	**71.3**	50.3	57.5	87.0

Table 6. Continue

Pesticide	Conc. (ng/mL)	Full Factorial experiment number (TBA, $CaCO_3$, C18)								
		1 (+ + +)	**2 (+ + -)**	**3 (+ - +)**	**4 (+ - -)**	**5 (- + +)**	**6 (- + -)**	**7 (- - +)**	**8 (- - -)**	**Original**
Monocrotophos	10	**76.7**	53.9	29.4	59.3	21.8	54.0	53.9	29.4	85.4
	50	60.8	68.9	52.1	65.3	52.1	69.1	60.8	59.7	83.6
Omethoate	10	**78.6**	35.4	12.0	57.9	54.1	52.9	190.3	33.5	88.3
	50	57.5	43.0	**83.1**	60.4	64.8	**82.8**	57.5	51.9	88.8
Phosmet	10	**77.0**	57.3	48.4	**85.1**	66.8	**70.1**	57.3	48.4	88.9
	50	66.3	49.7	45.2	64.7	45.2	**83.1**	66.3	59.7	86.4
Prochloraz	10	**90.5**	57.8	53.9	**83.7**	**74.5**	**77.1**	57.8	53.9	85.7
	50	64.7	54.3	52.1	**77.4**	46.8	**81.1**	**81.0**	69.7	83.4
Propamocarb	10	**74.7**	**79.9**	66.5	**89.9**	56.6	**70.1**	**92.8**	**86.8**	81.5
	50	**74.4**	**72.6**	56.1	**77.6**	69.4	**86.9**	**74.4**	63.3	80.2
Propargite	10	57.8	6.3	8.9	**81.8**	33.7	30.2	6.3	8.9	81.3
	50	37.4	7.9	9.9	48.6	11.1	61.6	37.4	45.2	81.8
Pyraclostrobin	10	**81.5**	43.7	47.5	**75.9**	69.7	66.6	43.7	47.5	82.8
	50	63.8	37.4	38.0	**70.1**	33.1	**74.9**	63.8	67.1	88.0
Pyrimethanil	10	42.8	40.7	21.9	**92.5**	38.2	50.1	40.7	21.9	84.5
	50	58.8	38.0	39.1	55.9	33.7	58.2	52.6	46.8	79.6
Pyriproxypen	10	**77.3**	12.3	13.5	55.5	45.8	38.3	12.3	13.5	83.0
	50	43.2	12.9	14.1	56.6	16.2	65.0	43.2	50.3	80.4
Spinosyn A	10	**75.7**	29.4	29.9	**86.8**	58.9	65.0	29.4	29.9	80.9
	50	**70.6**	32.3	34.4	69.8	31.9	**77.1**	**70.6**	64.6	82.6

Table 6. Continue

Pesticide	**Conc. (ng/mL)**	**Full Factorial experiment number (TBA, $CaCO_3$, C18)**								
		1 (+ + +)	**2 (+ + -)**	**3 (+ - +)**	**4 (+ - -)**	**5 (- + +)**	**6 (- + -)**	**7 (- - +)**	**8 (- - -)**	**Original**
Spinosyn D	10	**88.9**	29.8	22.2	69.5	58.3	47.6	29.8	22.2	81.0
	50	56.8	29.8	24.6	60.3	23.5	74.2	56.8	55.9	81.8
Tebuconazole	10	**79.9**	**71.8**	61.4	65.2	**82.0**	66.6	**71.8**	61.4	82.2
	50	**76.7**	61.7	57.1	66.2	51.8	72.1	**76.7**	59.1	80.8
Tebufenozide	10	**86.2**	**72.3**	63.9	87.9	65.2	66.7	**72.3**	63.9	85.5
	50	61.2	58.1	53.3	64.5	47.2	**82.7**	61.2	58.8	86.8
Tebufenpyrad	10	60.2	14.3	29.4	**80.3**	38.0	41.0	14.3	29.4	81.5
	50	52.5	15.5	13.8	64.1	16.7	**71.7**	52.5	58.1	83.8
ThiaphionateMethyl	10	40.8	36.0	32.4	55.0	30.7	42.2	36.0	32.4	79.4
	50	50.2	40.0	36.0	52.4	36.0	66.8	50.2	46.9	86.0
Trifloxystrobin	10	**79.3**	24.9	24.5	**77.4**	49.9	52.0	24.9	24.5	86.3
	50	51.0	22.4	20.8	55.7	26.7	69.4	51.0	52.1	83.6

Figure 7 shows the distribution of pesticides with recovery values coincides within the range of 70-120% over the experiment number for two concentration levels being 10 and 50 ng/mL. As can be concluded from the bar graph, experiment numbers 1, 4 and 6 have displayed high efficiency for both concentrations. However, the rest of the data were found unsatisfactory to carry out the validation studies. Therefore, alternative adsorbents should be considered for further studies.

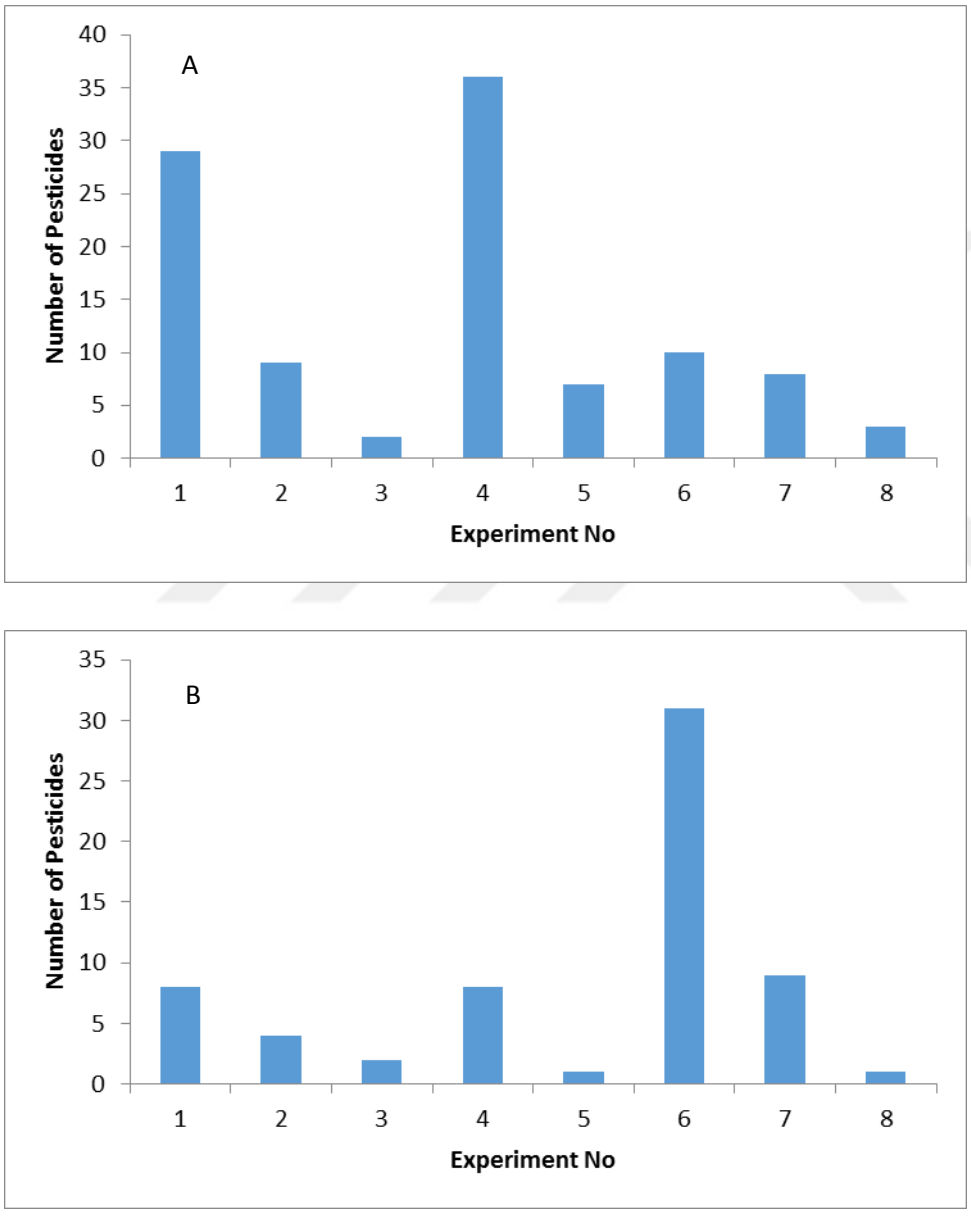


Figure 7. The distribution of pesticides with recovery values between 70-120% according to the experiment number for A) 10 ng/mL and B) 50 ng/mL standard addition.

4. CONCLUSION

The main objective of the present thesis is to develop a method for determination of pesticide residue in olive samples by LC-MS/MS system. Considering the fatty matrix of the black olives, the attention was paid to the sample preparation step where QuEChERS method is preferred. In the first part of the study, original method was applied to the black olive samples and pesticide residues for 46 active ingredients were quantified according to their chromatographic peak areas. The calibration graphs were drawn in ppb ranges and LOD levels were calculated.

In the second part of the thesis, some modifications were made to the original method to improve the d-SPE clean up step and possibly reduce the expenses. Therefore, $MgSO_4$ was replaced with $CaCO_3$ which can be easily available at a reduced cost. In addition, tert-butyl ammine (TBA) was used instead of PSA. In order to reveal the effect of the amount of these sorbents on the recovery values along with the C18 used in dSPE step, full factorial experimental design was applied at two levels. Recovery studies have been performed at two concentration levels being 10 and 50 ng/mL and the results have been compared with those of original QuEChERS method.

Overall results have demonstrated that recovery values were distributed in a wide range and were found out of the 70-120% range for the 8 experiments in the design. Therefore, the use of $CaCO_3$ was not satisfactory for the clean-up step. One of the reasons to this outcome is the pesticides studied display a wide range of polarity and hydrophobicity due to the structural differences. Another reason is that $CaCO_3$ is not hygroscopic as $MgSO_4$ used in QuEChERS method.

A closer look to the data has revealed that experiments 1, 4 and 6 have resulted better recoveries for all the pesticide types. However, this modification is not satisfactory for validation studies. Further studies will be dealing with alternative modifications in the method for higher recovery values.

REFERENCES

Anastassiades et al., 2003 "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce" Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., J AOAC Int. 86: 412-31.

AOAC Official Method 2007.01, "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry", First Action 2007

Brereton, R.G., 2007 "Applied Chemometrics for Scientists", John Wiley and Sons, England, 379p.

Chomatography, 1998. "The catalog of Macherey-Nagel"

Commission Regulation (EC) 2005 "Maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending", Council Directive 91/414/EEC.

David et al., 2017 "Determination of pesticides in fatty matrices using gel permeation clean-up followed by GC-MS/MS and LC-MS/MS analysis: A comparison of low- and high-pressure gel permeation columns" David, F., Dumont, C.D.E., Yang, Z. Sandra, P., Huertas-Pérez, J.F., Talanta 165: 201–210

Dias et al., 2016 "Determination of pesticides in edible oils by liquid chromatography-tandem mass spectrometry employing new generation materials for dispersive solid phase extraction clean-up" J.V. Dias, V. Cutillas, A. Lozano, I. R. Pizzutti, A.R. Fernández-Alba, Journal of Chromatography A, 1462: 8–18

EN Standard Method EN 15662 (2008). "Foods of plant origin. Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE. QuEChERS-method"

Georgakopoulos et al., 2011 "Optimisation of octadecyl (C18) sorbent amount in QuEChERS analytical method for the accurate organophosphorus pesticide residues determination in low-fatty baby foods with response surface methodology" P. Georgakopoulos, R. Zachari, M. Mataragas, P. Athanasopoulos, E. H. Drosinos, P.N. Skandamis, Food Chemistry 128: 536–542

Gilbert-López et al., 2009 "Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Talanta 79: 109–128

Green, J.M., 1999, "A Practical Guide to Analytical Method Validation", Analytical Chemistry News and Features, 1:305-309

Gonzalez-Curbelo et al., 2015 "Evolution and applications of the QuEChERS method" Gonzalez-Curbelo, M.A., Socas-Rodriguez, B., Herrera-Herrera, A.V., Gonzalez-Salamo, J., Hernandez-Borges, J., Rodriguez-Delgado, M.A., Trends in Analytical Chemistry 71: 169–185

REFERENCES(Continue)

Herrmann and Poulsen, 2015 "Clean-up of cereal extracts for gas chromatography–tandem quadrupole mass spectrometry pesticide residues analysis using primary secondary amine and C18", Herrmann, S.S., Poulsen, M.E. Journal of Chromatography A, 1423: 47–53

Hou et al., 2014 "A multi-residue method for the determination of pesticides in tea using multi-walled carbon nanotubes as a dispersive solid phase extraction absorbent" Hou, X. Lei, S.R. Qiu, S.T. Guo, L.A. Yi, S.G. Liu, W., Food Chem. 153: 121–129.

Hunter et al., 2010 "Method for the determination of organophosphorus and pyrethroid pesticides in food via gas chromatography with electron-capture detection", Hunter R.E., Riederer, A.M., Ryan, P.B. J. Agric. Food Chem. 58: 1396–1402.

Kaczyńskia et al., 2017 "Multiclass pesticide residue analysis in fish muscle and liver on one-step extraction-cleanup strategy coupled with liquid chromatography tandem mass spectrometry", P. Kaczyńskia, B. Łozowicka, M. Perkowski, J. Szabuńko, Ecotoxicology and Environmental Safety, 138: 179–189

Koesukwiwat et al., 2008 "Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography–tandem mass spectrometry", Koesukwiwat, U. Sanguankaew, K. Leepipatpiboon, N. Anal. Chim. Acta 626: 10–20.

Lee, P.W, 2003 "Handbook of Residue Analytical Methods for Agrochemicals", John Wiley & Sons

Lehotay et al., 2005 "Evaluation of Two Fast and Easy Methods for Pesticide Residue Analysis in Fatty Food Matrixes" Lehotay, S.J., Maòtovská, K., Yun, S.J., Journal of AOAC International, 88: 630-638

Li et al., 2014 "Determination of multiple pesticides in fruits and vegetables using a modified quick, easy, cheap, effective, rugged and safe method with magnetic nanoparticles and gas chromatography tandem mass spectrometry", Li, Y.-F., Qiao, L.-Q., Li, F.-W., Ding, Y., Yang, Z.-J., Wang, M.L., J. Chromatogr. A 1361: 77–87.

Mitra, S., 2003 "Sample Preparation Techniques in Analytical Chemistry", John Wiley &Sons

Nguyen et al., 2010 "Rapid determination of 95 pesticides in soybean oil using liquid–liquid extraction followed by centrifugation, freezing and dispersive solid phase extraction as clean-up steps and gas chromatography with mass spectrometric detection", Nguyen, T.D., Lee, M.H., Lee, G.H. Microchem. J. 95 113–119

Norli et al., 2011 "Application of QuEChERS method for extraction of selected persisten organic pollutants in fish tissue and analysis by gas chromatography

REFERENCES(Continue)

mass spectrometry", Norli, H.R. Christiansen, A. Deribe, E. J. Chromatogr. A 1218 (2011) 7234–7241.

Rejczak and Tuzimski, 2017 "QuEChERS-based extraction with dispersive solid phase extraction clean-up using PSA and ZrO2-based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD" T. Rejczak and T. Tuzimski, Food Chemistry 217: 225–233

Romero-González et al., 2011 "Simultaneous determination of pesticides, biopesticides and mycotoxins inorganic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry", Romero-González, R., Frenich, A.G., Vidal, J.L.M., Prestes, O.D., Grio, S.L., Journal of Chromatography A, 1218: 1477–1485

Schenk et al., 2008 "A rapid multiresidue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction/partitioning and solid-phase extraction column clean-up" Schenk, F.J., Brown, A.N., Podhorniak, L.V. J. AOAC Int. 91: 422–438.

Vázquez et al., 2016, "Large multiresidue analysis of pesticides in edible vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy, cheap, effective, rugged and safe methodology followed by gas chromatography–tandem mass spectrometry" P. P. Vázquez, E. Hakme, S. Uclés, V. Cutillas, M.M. Galera, A.R. Mughari, A.R. Fernández-Alba, Journal of Chromatography A, 1463: 20–31

Villaverde et al., 2016 "Trends in analysis of pesticide residues to fulfil the European Regulation (EC) No. 1107/2009" Villaverde, J.J., Sevilla-Morán, B., López-Goti, C., Alonso-Prados, J.L., Sandín-España, P., Trends in Analytical Chemistry 80: 568–580

(www.agilent.com/chem)

www.epa.gov.tr, Last assesment January 2017.

www.waters.com/waters/en_TR/QuEChERS

CURRICULUM VITAE

Name and Surname : Betül ŞAKRAK

Birth Place and Date : Manisa, 07/08/1987

Specialization : Chemist

Driving Licence : B

Email : **betulkocel@hotmail.com**

EDUCATION

2013 – 2014 MSc Student at Ege University Graduate School of Natural and Applied Science

2005 – 2009 Karadeniz Technical University Faculty of Science, Chemistry Department

PROFESSIONAL EXPERIENCE

2009- Laboratory Director in İntertek (Manisa Özel Gıda Kontrol Laboratuvarı)