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EGE UNIVERSITY
Graduate School of Applied and Natural Science



ENRICHMENT OF RESIDUAL UV FILTERS IN ENVIRONMENTAL SAMPLES BY MICROEXTRACTION TECHNIQUES PRIOR TO CHROMATOGRAPHIC ANALYSIS

PhD Thesis

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Supervisor: Prof. F. Nil ERTAŞ

Chemistry Department

İzmir

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EÜ Lisansüstü Eğitim ve Öğretim Yönetmeliğinin ilgili hükümleri uyarınca Doktora Tezi olarak sunduğum “***Enrichment of residual UV filters in environmental samples by microextraction techniques prior to chromatographic analysis (Çevresel örneklerde Kalıntı UV Filtrelerinin Kromatografik Analiz öncesi Mikroekstraksiyon Teknikleriyle Zenginleştirilmesi)***” 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.

18 / 11 / 2022

İmzası

Cemile YÜCEL

ÖZET**Çevresel örneklerde Kalıntı UV Filtrelerinin Kromatografik Analiz Öncesi Mikroekstraksiyon Teknikleriyle Zenginleştirilmesi**

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UV filtreler kişisel bakım ürünlerinde ve endüstriyel uygulamalarda kullanılmaktadır ve son zamanlardaki yaygın kullanımları çamur, toprak, su gibi çevresel örneklerde derişimlerinin artmasına neden olmuştur. Bu bileşiklerin endokrin bozucu özellikleri sucul yaşamı ve insan sağlığını olumsuz etkilemektedir.

Bu tez kapsamında, UV filtrelerin atıksu ve yüzey sularından mikroekstraksiyon teknikleri ile zenginleştirilmesinden sonra GC MS/MS yönteminin uygulanabilirliği amaçlanmaktadır. Bu teknikler, daha az çözücü tüketilmesi ve daha az zamanın yeterli olması bakımından geleneksel yöntemlerden üstündür. Tezin ilk bölümünde, dispersif sıvı-sıvı mikroekstraksiyonu ile birleştirilmiş katılaşmış yüzen organic damlacık (DLLME-SFOD) metodu uygulanarak UV filtrelerin ekstraksiyonu gerçekleştirildi. Bunu takiben in-port türevlendirme ve gaz kromatografi-tandem kütle spektrometrisini (GC-MS/MS) birleştiren yeni, verimli, hızlı ve alternatif bir yöntem geliştirilmiştir. Optimum koşullar, yanıt yüzey yöntemi ile belirlenmiştir. Tezin ikinci bölümünde, UV filtrelerinin atık sudan SPME yöntemi ile ticari PA fiber kullanılarak ekstraksiyonu ve GC-MS/MS ile saptanabilirliği araştırılmıştır. Bu amaçla optimum koşullar belirlenmiş ve yöntem bu koşullar altında valide edilmiştir. Poliakrilonitril (PAN) lifinin elektroegirme yöntemi ile kaplanması ve bu lifin atık su numunelerinde UV filtrelerinin seçici ekstraksiyonu ve analizi için kullanılması tezin üçüncü bölümünü oluşturmaktadır. SPME yönteminin validasyonu, PA fiberinin optimize edilmiş koşulları altında PAN fiber kullanılarak gerçekleştirilmiştir.

Anahtar Kelimeler: UV filtre, DLLME-SFOD, Electro-çekim, SPME



ABSTRACT**ENRICHMENT OF RESIDUAL UV FILTERS IN ENVIRONMENTAL
SAMPLES BY MICROEXTRACTION TECHNIQUES PRIOR TO
CHROMATOGRAPHIC ANALYSIS**

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PhD in Chemistry Department (Analytical Chemistry)

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UV filters are used in personal care products and in industrial applications and their wide use has led to an increase in their concentrations in environmental samples such as sludge, soil, and water. The endocrine disrupting properties of these compounds adversely affect aquatic life and human health.

Within the scope of this thesis, the applicability of GC MS/MS method is aimed after enrichment of UV filters from wastewater and surface waters by microextraction techniques. These techniques are superior to conventional methods in that less solvent is consumed and less time is sufficient. In the first part of the thesis, the extraction of UV filters was carried out by applying the solidified floating organic droplet (DLLME-SFOD) method combined with dispersive liquid-liquid microextraction. Following this, a new, efficient, rapid and alternative method was developed combining in-port derivatization and gas chromatography-tandem mass spectrometry (GC-MS/MS). Optimal conditions were determined by the response surface method. In the second part of the thesis, the extraction of UV filters from wastewater by SPME method using commercial PA fiber and their detectability by GC-MS/MS were investigated. For this purpose, optimum conditions were determined and the method was validated under these conditions. Coating of polyacrylonitrile (PAN) fiber by electrospinning method and using this fiber for selective extraction and analysis of UV filters in wastewater samples constitute the third part of the thesis. Validation of SPME method was performed using PAN fiber under optimized conditions of PA fiber.

Keywords: DLLME-SFOD, Endocrine Disrupter, SPME, Electrospinning

ÖNSÖZ

Güneş kremleri günlük hayatımızda çok fazla kullandığımız ürünlerden olup, bu ürünlerin içerisindeki cildi güneş ışınlarına karşı koruyan UV filtreler sucul yaşam ve insan sağlığı açısından risk oluşturmaktadır. Endokrin bozucu etkileri son on yılda yapılan toksisite çalışmalarıyla daha iyi anlaşılmaktadır. Çok az düzeyde bile zararlı etkileri olan bu bileşiklerin duyar, seçici ve doğru bir şekilde analiz edilebilmeleri ileride yapılacak izleme çalışmalarını da etkileyecek niteliktedir.

Bu tez kapsamında yürüttüğüm ilk çalışma, düşük derişimdeki UV filtrelerin belirlenmesini sağlayacak çevre dostu bir yöntem geliştirilmesini amaçlamaktadır. Tezimin ikinci kısmında bu yöntemlerde kullanılan ticari fiberlere alternative olarak ucuz ve atık kumaşlardan üretilen bir fiber geliştirilmesi üzerine yaptığım çalışmaları içermektedir. Her iki çalışmanın da ileride yapılacak araştırmalara yol gösterici olmasını ve ülkemizdeki çevresel numunelerdeki UV filtre derişim seviyelerinin belirlenmesine olanak sağlamasını diliyorum.

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ABBREVIATIONS AND SYMBBOLS

BA μ E: Bar adsorptive micro-extraction

AuNPs: Gold nanoparticles

AC: Acetone

AcN: Acetonitrile

CHL: Kloroform

CHLB: Klorobenzene

DCM: Dichloromethane,

DMF: N,N-dimethylformamide DLLME: Dispersive liquid liquid microextraction

EDC: 1-ethyl-3-[3- dimethyl amino propyl]-carbodiimide hydrochloride

HFLPME: hollow fiber liquid phase microextraction

iSAME: In situ suspended aggregate microextraction

KWLPME: knitting wool supported liquid-phase microextraction

MEPS: microextraction by packed sorbent

MOFs: Metal organic frameworks

NOEC: Not observed effect concentration

PANI: Polyaniline

SA-DLLME: surfactant-assisted DLLME

SBSE: Stir bar sorptive extraction

SBSDME: stir bar-sorptive dispersive microextraction

SFOD: Solidified floating organic droplet

SM-DLLME: supramolecular-based DLLME

SPME: Solid phase microextraction

ST-DLLME: Solvent terminated DLLME

TCE: trichloroethylene

UVFs: UV filters



1. INTRODUCTION

In water quality studies, priority pollutants including pesticides, industrial chemicals, petroleum hydrocarbons, heavy metals, nutrients, and bacteria are considered. However, recent studies have revealed that wastewater is contaminated with many different organic chemicals and urban surface waters are affected by these pollutants. (Pal et al., 2014). These new pollutants are called as ***Emerging Organic Pollutants*** (EOCs) and a variety of pollutants such as hormones, antibiotics, surfactants, endocrine disruptors, human and veterinary drugs, X-ray contrast agents, pesticides and their metabolites, disinfection by-products, algal toxins, taste, and odor compounds.

Although the endocrine disrupting effects of some of these compounds are known, wastewater discharge limits have not been published yet. Among these pollutants, the European Union (EU) can make continuous evaluations for EDC, which has the potential to threaten human health. As a result of the evaluations, an ***Emerging pollutant*** (EP) list is published, and these chemicals are included in the ***Watch List*** (Watch list, 2015). These chemicals also include ***UV filters*** (UVFs) known as sunscreen agents, and action plans are expected to be created for determining /monitoring their concentrations in surface and wastewaters.

In daily life, we are exposed to sun rays and ultraviolet (UV) irradiation that can reach the Earth's surface and damage our skin. Acute damages of solar radiation are reversible for a short time, such as sunburn and tanning but, chronic damage can even be life-threatening and includes eye damage, suppression of the immune system, and skin cancer (Zgoła-Grześkowiak and Grześkowiak, 2011; Zhang and Lee, 2013). Therefore, organic chemicals, also called UV filters, are utilized in sunscreen products to protect human skin against direct exposure to deleterious UVA (400–315 nm) or UVB (315–280 nm) radiation (Marcinkowska et al., 2019).

Besides personal care products (PCPs) consisting of sunscreens, lotions, lip care products, shampoos, body washes, hair sprays and make-up products, these compounds are also used to prevent pigment photodegradation in other industrial products such as food packaging, plastics, paints, and textiles. (Diaz-Cruz et al., 2008). Then, these compounds access the rivers, lakes, and ocean by wastewater treatment plants (WWTP) effluents (indirect input) or from skin washing during swimming, bathing, or recreational activities (direct inputs) (Wang et al., 2017).

Subsequently, their entry into the environment has also increased and the presence of UVFs has been reported in aqueous media (MacManus-Spencer et al., 2011) as well as in sediments (Li et al. 2014a), soil (Li et al. 2014b), and sludges (Jeon et al., 2006). Therefore, it is of great importance to develop and implement environmentally friendly analytical methods for reducing the matrix effect to negligible levels and provide a fast and sensitive method with high sensitivity and good recovery values.

Overviews of analytical methods for determining UV-filters in the environment (Samos et al., 2015, Chisvert et. al., 2018) have been published and it was pointed out that the success of these methods is based on the possibility of selective and sensitive analysis for samples containing various organic pollutants and minimizing the interferences during the measurement phase. Therefore, sample preparation is the most critical step in the analysis of UVFs, with a concentration of sub- $\mu\text{g L}^{-1}$ in environmental water. A well-designed sample preparation step, which allows the separation and pre-concentration of trace pollutants from complex matrices prior to the measurement has a great effect on sensitivity and accuracy.

Present thesis aims to develop a chromatographic determination method for some selected UV filters (3-BC, BP-1, BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC) in water samples. Recently developed microextraction techniques such as dispersive liquid liquid microextraction (DLLME) based on solidification of floating organic droplet (SFOD) and solid phase microextraction (SPME) will be utilized for this purpose.

In addition, Injection Port Silylation technique will be used in derivatization stage. Experimental parameters will be optimized by Central Composite Design (CCD). After the validation studies, the performance of the different microextraction techniques will be compared.

2. GENERAL INFORMATION

2.1 Chemical Structures of UV Filters

UV-filters were grouped in 11 chemical families as given below.

Para-amino benzoate (PABA) is one of the first UV filters to appear on the US market and has an λ_{max} of approximately 290 nm. PABA is a molecule in which the electron donating COOR functional group is positioned at the para position to NR₂ which is electron accepting group. This PABA structure is the basis for UV filters such as ethylhexyl dimethyl PABA (Padimate-O, EDP), ethyl hexyl triazone and diethyl hexyl butamido triazone (Shaath et al., 2010, Bester et al., 2007, Crista et al., 2014).

Salicylates, containing a monohydroxy benzoic acid group, are a group of molecules that can form internal hydrogen bonding, thereby reducing their energy requirements and producing a UV absorbance at about 300 nm (Shaath et al. 2010).

Cinnamates are molecules with an aromatic structure that is substituted with an electron-donating ester group conjugated with a double bond, and an electron-accepting -OCH₃ group. This structure allows for extended displacement of electrons, providing absorption in the 310 nm region (Shaath et al., 2010).

Benzophenones are compounds containing aromatic ketones unlike other UV filters. The presence of an electron-releasing group in the ortho and/or para position in benzophenones promotes resonance delocalization, resulting in absorption at two wavelengths at 286 nm (UVB) and 324 nm (UVA) (Shaath et al., 2010).

Camphor derivatives belongs to a group of organic compounds classified as terpenoid ketones. These compounds generally have high molar absorption coefficients and high photostability due to reversible photo-isomerization (Shaath et al., 2010).

Octocrylene (OC) is an organic type of sunscreen that absorbs UVA and UVB. It is often used to increase the sun protection of a sunscreen and to stabilize other UV filters such as avobenzone. However, OC can form its own stable substances by releasing oxygen radicals when exposed to UV radiation (Berardesca et al., 2009; Meng et al., 2021)

2.2 Hazardous Effects of UV filters

UVFs have been widely used in different applications ranging from personal care products to different industrial production lines. These compounds are found in food packaging, plastics, paints, and textiles, detergents, and disinfection products. Their main function is to stabilize the quality of the industrial product like polymers and paints and prevent them from photodegradation (Ramos et al., 2019).

UVFs are being extensively consumed for self-protection of humans against harmful sun rays, in the form of sunscreens and personal care products (Li et al., 2014a), although their melanoma preventive ability is still under discussion. Extensive consumption of these chemicals resulted at high concentration in different environmental components as well as the human body. These compounds have been detected in fish (Díaz-Cruz and Barceló, 2009; Balmer et al., 2005), in lake water, (Kunz and Fent, 2006) soils, (Li et al., 2014b) sludges (Jeon et al., 2006), human milk (Poiger et al., 2004), and plasma and urine (Jiang et al., 1999).

The starting point of this UVFs contamination cycle is the uncontrolled disposal of UVF containing personal care products. The later phase is the UVFs in the human body through either dermal exposure or the food chain (Hayden et al., 1997). The wash-off remained UVF on the skin, swimming, washing UVFs spoiled clothes and discharge of absorbed UVFs through body urine are the components of human originated UVFs contamination cycle (Janjua et al., 2004). The other contributors to UVFs contamination are untreated industrial and domestic wastewater (Plagellat et al., 2006)

Recent studies revealed that UVFs are endocrine disrupter chemicals that cause problems in the hormonal system, tumors formation, birth defects, and other developmental disorders (Lorigo et al., 2018; Janjua et al., 2004; Schlumpf et al., 2004; Krause et al., 2012; Kim and Choi, 2014). In vitro and in vivo studies about their environmental estrogenic impacts increased the concern about UVFs (Schlumpf et al., 2001; Schreurs et al., 2002). These adverse effects are more significant for UVFs with log K_{ow} higher than 3 which enhances the bioaccumulation. The other indicator of contaminant's adverse effect on organisms is **NOEC** value short for **No Observed Effect Concentration** which is defined as the highest concentration tested at which the measured parameter shows no significant inhibition and typically obtained from chronic aquatic and terrestrial toxicity studies.

The NOEC values of UVFs which are lower than 0.01 mg/L are in great concern due to its high priority for further work (Brooke et al., 2008). Ramos et al. reviewed the toxicological and endocrine disrupting effects of UV filters and specified the UV filters of priority importance (2019). Since these UVFs receive more attention in analytical method development as well as removal from the contaminated sites, 10 of these UV filters (3-BC, BP-1, BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC) were selected in this study.

2.3 Physicochemical properties of UV filters.

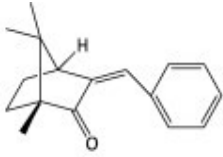
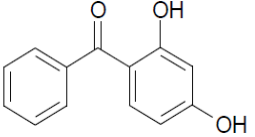
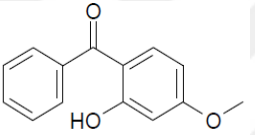
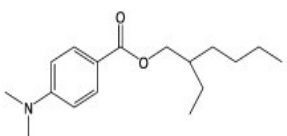
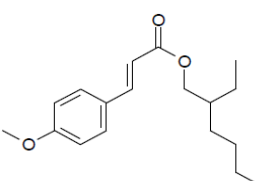
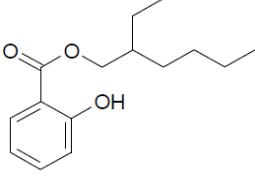
Physicochemical properties of a pollutant regarding its solubility in water, boiling points (BP) and octanol–water partition coefficients (K_{ow}) are important both for the prediction of their behavior in the environmental media and for the selection of the sample preparation and the instrumentation for their determination in environmental waters.

The boiling points and vapor pressures (VP) of targeted UV filters are in the range of 300-400°C and 3.2×10^{-9} - 1.88×10^{-4} mm Hg, respectively. Therefore, evaporation of UVFs from water is negligible and they are not considered as volatile compounds (Mao et al., 2019). The UVFs examined in this thesis belong to different derivative groups and contain different physicochemical properties as shown in **Table 2.1**. Among different derivative groups, targeted analytes were selected based on their **NOEC** values.

Water solubility provides information about the environment and human exposure to the UVFs passing from soil/sludge/sediment areas to water. UV filters are slightly soluble. (Ramos et al., 2015).

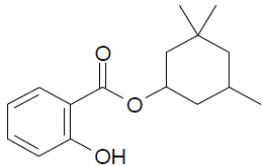
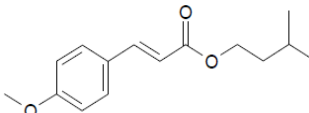
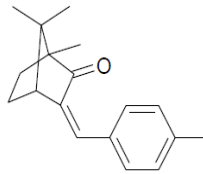
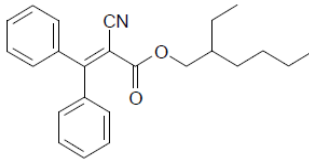
The K_{ow} is a measure of the distribution of chemicals between its solubility in oil and water. Compounds with a log K_{ow} value of less than 2.5 have low sorption on the particulate phase. Compounds with a log K_{ow} value of more than 4 have high sorption on the particulate phase while the compounds with a log K_{ow} value between 2.5 and 4 have a medium sorption potential (Tan et al, 2018; Luo et al., 2014). Since K_{ow} values are greater than 4, targeted UV filters (except BP-1 and BP-3) are hydrophobic. In the light of these information, various analytical methodologies have been developed for their determination in surface waters and wastewaters.

Table 2.1 The physicochemical properties of UV filters

UV filter, IUPAC name, NOEC Value	Chemical structure	Physicochemical Properties
3-BC: 3- Benzyline camphor (3-Benzylidene-1,7,7-trimethyl bicyclo [2.2.1] heptan-2-one) CAS 15087-24-8 NOEC> 0.022 mg L ⁻¹		MW: 240.35 g mol ⁻¹ MP: 110°C BP: 338°C VP: 3.8x10 ⁻⁵ mm Hg S: 0.69 mg L ⁻¹ logK _{ow} : 5.37 pK _a : 8.00
BP-1: Benzophenone-1 (2,4- dihydroxy benzophenone) CAS 131-56-5 NOEC> 0.01 mg L ⁻¹		MW: 214.22 g mol ⁻¹ MP: 144°C BP: 305.4°C VP: 1.4x10 ⁻⁷ mmHg S: insoluble log K _{ow} : 2.96 pK _a : 7.72
BP-3: Benzophenone-3 (2-Hydroxy-4-methoxy phenyl)- phenyl methanone CAS 131-57-7 NOEC> 0.01 mg L ⁻¹		MW: 228.25 g mol ⁻¹ MP: 65°C BP: 363°C VP: 1.42x10 ⁻⁶ mmHg S: 68.6 mg L ⁻¹ logK _{ow} : 3.79 pK _a : 7.56
EDP: 2-ethyl hexyl-4-(dimethyl amino) benzoate CAS 21245-02-3 NOEC= 0.012 mg mL ⁻¹		MW: 277.41 g mol ⁻¹ MP: 97°C BP: 345°C VP: 3.54x10 ⁻⁵ mmHg S: 0.20 mg L ⁻¹ logK _{ow} : 5.77 pK _a : 8.00
EHMC: 2-ethyl-hexyl-4-trimethoxy cinnamate CAS 5466-77-3 NOEC= 0.003 mg L ⁻¹		MW: 290.41 g mol ⁻¹ MP: 100°C BP: 361°C VP: 1.38x10 ⁻⁵ mmHg S: 0.2 mg L ⁻¹ logK _{ow} : 6.1 pK _a : 8
EHS: 2-ethylhexyl 2-hydroxy benzoate (Ethylhexylsalicylate) CAS 118-60-5 NOEC= 0.008 mg L ⁻¹		MW: 250.34 g mol ⁻¹ MP: 139°C BP: 345°C VP: 3.17x10 ⁻⁶ mmHg S: 0.5 mg L ⁻¹ logK _{ow} : 5.97 pK _a : 8.13

MP: Melting point, BP: Boiling point, VP: Vapor pressure, S: Solubility

Table 2.1 Continue

HMS: 3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate (Homosalate) CAS 118-56-9 NOEC= 0.005 mg L ⁻¹		MW: 262.36 g mol ⁻¹ MP: 121°C BP: 356°C VP: 2.76x10 ⁻⁶ mmHg S: 0.42 mg L ⁻¹ logK _{ow} : 6.16 pKa: 8.09
IAMC: Isoamyl p-methoxy cinnamate (3-Methylbutyl (2E)-3-(4-methoxyphenyl) acrylate) CAS 71617-10-2 NOEC= 0.013 mg L ⁻¹		MW: 248.32 g mol ⁻¹ MP: 72°C BP: 324°C VP: 1.88x10 ⁻⁴ mmHg S: 4.9 mg L ⁻¹ log K _{ow} : 4.33
4-MBC: 4-Methylbenzylidene camphor CAS 36861-47-9 NOEC=0.008 mg L ⁻¹		MW: 254.37 g mol ⁻¹ MP: 121°C BP: 349°C VP: 1.52x10 ⁻⁵ mmHg S: 0.20 mg L ⁻¹ logK _{ow} : 5.92 pKa: 8.00
OC: Octocrylene CAS 6197-30-4 NOEC= 0.00084 mg L ⁻¹		MW: 361.50 g mol ⁻¹ MP: 181°C BP: 473°C VP: 3.2x10 ⁻⁹ mmHg S: 1.3 mg L ⁻¹ logK _{ow} : 6.88 pKa: 8.00

2.4 Legal Aspects

The well-known control tools to protect human and environmental health and to ensure free mobility of substances and mixtures are to publish regulation. Classification and Labelling and Packaging (CLP) is one of these regulations but, UVFs are not included although their extensive utilization in the production of packaging materials. The **Watch List of European Union Water Framework Directive** (Directive 2008/105/EC) on the other hand, includes only one UV filter; EHMC (Sousa et al., 2018) as a potential risk for human.

In addition to the hazardous effects of the UVFs, their metabolites should also be considered both in the analysis and in their removal from contaminated areas. It has been reported that they have the risk of triggering breast cancer, transferring from breast milk to newborn infants (Diaz-Cruz et al., 2009) and they can interfere with endocrine systems (Schmutzler et al., 2004; Seidlová-Wuttke et al., 2006).

Benzophenone-1 (BP-1) and Benzophenone-8 (BP-8) are the metabolites of BP-3 and the estrogenic activity of BP-1 which causes estrogen-dependent disease endometriosis, is higher than BP-3 (Kunisue et al., 2012; Kawamura et al., 2003, Suzuki, et al., 2005; Nashev, et al., 2010; Kunz and Fent, 2006).

Ethyl hexyl methoxycinnamate (EHMC) can be absorbed from the gastrointestinal tract (Kadry et al., 1995) and then, it can be transferred to human milk which could threaten infant life (Schlumpf et al., 2001; Kunisue et al., 2012; Schlumpf et al., 2008). EHMC showed estrogenic, anti-progestogenic, and anti-thyroid activity in rats and humans (Lorigo et al., 2018). 3- Benzyldene camphor (3-BC) (Kunz et al., 2006) and Benzophenone-2 (BP-2) (Weisbrod et al., 2007) caused adverse effects on fecundity and reproduction.

Homosalate (HMS) acts as an endocrine-disrupting chemical (Schreurs et al, 2004). Reproduction and development of aquatic invertebrates are affected by 3-Benzylidenecamphor and 3-(40-methyl benzylidene)-camphor (Langford and Thomas, 2008). Finally, UV-filters have been linked to coral reef bleaching (Danovaro et al., 2008). These are some of the results of the scientific studies which clearly states that UVFs and their metabolites in the environment should be controlled for the sake of aquatic life, human health, and especially for breastfeeding newborns.

2.5 Occurrence of UVFs in Environmental Waters

UVFs reach the aquatic environment mostly through untreated municipal wastewater discharge. Therefore, in river water, UV filters belonging to different groups are found at different concentrations. The concentrations of some UV filters in river waters in different countries are given in **Table 2.2**.

These compounds enter sewage treatment plants (STPs) by being discharged from point sources because of people bathing and washing their clothes. Unfortunately, these compounds, which are not sufficiently treated in wastewater treatment plants, pose a health threat to aquatic organisms as they are found in high concentrations in the effluent (Pedrouzo et al., 2010). **Table 2.3** shows the concentrations of UVFs in the studies carried out to date.

Table 2.2 The concentrations of some UV filters in river waters in last fifteen years

UVF	Conc.	Reference	UVF	Conc.	Reference
BP-3	<7.1 ng L ⁻¹	da Silva, 2015	EHMC	<23.5 ng L ⁻¹	da Silva, 2015
	86-116 ng L ⁻¹	Tsiu, 2014		88-95 ng L ⁻¹	Tsui, 2014
	nd-37.8 ng L ⁻¹	Gago-Ferrero, 2013		nd-1040 ng L ⁻¹	Kameda, 2011
	12-67 ng L ⁻¹	Grabicova, 2013		240-770 ng L ⁻¹	Román, 2011
	12.3-15.4 ng L ⁻¹	Wu et al, 2013	EHS	nd-41 ng L ⁻¹	Fent, 2010
	3.0 ng L ⁻¹	Ho&Ding, 2012		12.1 ng L ⁻¹	da Silva, 2015
	nd-41 ng L ⁻¹	Kameda, 2011		28-56 ng L ⁻¹	Tsui, 2014
	428-993 ng L ⁻¹	Román, 2011		nd-266 ng L ⁻¹	Kameda, 2011
	6-28 ng L ⁻¹	Pedrouzo, 2010		14-586 ng L ⁻¹	Román, 2011
	56-68 ng L ⁻¹	Fent, 2010		8-56.20 ng L ⁻¹	Liu, 2010
	nd-5390 ng L ⁻¹	Liu et al., 2010	HMS	<5 ng L ⁻¹	Negreira, 2009
	<LOQ-44 µg L ⁻¹	Kasprzyk, 2009		<4 ng L ⁻¹	Rodil, 2008a
	52 ng L ⁻¹	Negreira, 2009		29-59 ng L ⁻¹	Tsui, 2014
	28-37 ng L ⁻¹	Kasprzyk, 2008a		nd-29 ng L ⁻¹	Kameda, 2011
BP-1	<30-220 ng L ⁻¹	Kasprzyk, 2008b	IAMC	342-712 ng L ⁻¹	Román, 2011
	14 ng L ⁻¹	Kawaguchi, 2008		<1-5 ng L ⁻¹	Rodil, 2008a
	<11-30 ng L ⁻¹	Rodil, 2008	4-MBC	29-59 ng L ⁻¹	Román, 2011
				<2 ng L ⁻¹	Rodil, 2008a
	127-166 ng L ⁻¹	Tsui, 2014	OC	3.5-12.6 ng L ⁻¹	Gago-Ferrero, 2013b
	nd-7.54 ng L ⁻¹	Gago-Ferrero, 2013b		264-794 ng L ⁻¹	Román, 2011
	nd-6.1 ng L ⁻¹	Wu et al., 2013		nd-106 ng L ⁻¹	Fent, 2010
	1.8 ng L ⁻¹	Ho&Ding, 2012		10-5790 ng L ⁻¹	Liu, 2010
	0.9-29 ng L ⁻¹	Wick, 2010		5-15 ng L ⁻¹	Rodil, 2008a
	0.3-17 µg L ⁻¹	Kasprzyk, 2009	EDP	<19.3 ng L ⁻¹	da Silva, 2015
	37 ng L ⁻¹	Negreira, 2009		153-205 ng L ⁻¹	Tsui, 2014
	6-9 ng L ⁻¹	Kasprzyk, 2008a		nd-14 ng L ⁻¹	Kameda, 2011
	<0.3 ng L ⁻¹	Kasprzyk, 2008b		<1.8-440 ng L ⁻¹	Román, 2011
				<2.5 ng L ⁻¹	Pedrouzo, 2010
				29-5180 ng L ⁻¹	Liu, 2010
				<7-16 ng L ⁻¹	Rodil, 2008a
EDP	56-531 ng L ⁻¹	Román, 2011			
	<0.2-3 ng L ⁻¹	Rodil, 2008a			

Table 2.3 Concentration of organic UV filter residues detected in wastewater treatment samples in previous studies

UVFs	Concentration		Reference
	Influent	Effluent	
BP-3	216-462	nd-44 ng L ⁻¹	Negreira et al. 2009
	Nd	-	Li and Yang, 2014
	2.62 ng L ⁻¹	1.38 ng L ⁻¹	Wang et al. 2015
	-	1.12 ng L ⁻¹	Wang et al, 2016
	-	3.07 ng L ⁻¹	Ma et al, 2017
	-	181 ng L ⁻¹	Gomez et al, 2011
	32-551 ng L ⁻¹	5-21 ng L ⁻¹	Magi et al, 2012
	0.1 µg L ⁻¹	0.1 µg L ⁻¹	Pintado-Herrera et al, 2013
	<LOQ-328 ng L ⁻¹	<LOQ-155 ng L ⁻¹	Gilart et al, 2013
	-	82 n ng L ⁻¹	Pintado-Herrera et al, 2014
	-	10.9 ng/L, 13.5 ng L ⁻¹	Chung et al, 2015
	-	431 ng L ⁻¹	Moeder et al, 2010
	<40-236 ng L ⁻¹	<40 ng L ⁻¹	Negreira et al, 2011b
	17-227 ng L ⁻¹	nd, 10 ng/L, 58 ng L ⁻¹	Negreira et al, 2010
	6.6-273.3 ng L ⁻¹	<LOQ-68.2 ng L ⁻¹	Cunha et al, 2015
BP-1	-	12.5-21.4 ng L ⁻¹	Wu et al, 2013
	-	754.6-1154.3 ng L ⁻¹	Li, et al 2017
	-	1.67-1.83 µg L ⁻¹	Xue et al, 2013
	131-245 ng L ⁻¹	nd-41 ng L ⁻¹	Negreira et al, 2009
	-	7.3 ng/L, 11.6 ng L ⁻¹	Chung et al, 2015
OD-PABA (EDP)	88.5-408.5 ng L ⁻¹	Nd	Cunha et al, 2015
	-	7.7-16.8 ng L ⁻¹	Wu et al, 2013
	-	216-490.3 ng L ⁻¹	Li et al, 2017
	3.43 ng/L, 6.97 ng L ⁻¹	-	Li and Yang, 2014
	3.16 ng L ⁻¹	1.93 ng L ⁻¹	Wang et al, 2015
EHMC	-	1.58 ng L ⁻¹	Wang et al, 2016
	-	4.22 ng L ⁻¹	Ma et al, 2017
	-	2 ng/L, 7 ng L ⁻¹	Rodil et al, 2008
	-	0.62-0.91 ng L ⁻¹	Xue et al, 2013
	2.28-4.76 ng L ⁻¹	-	Li and Yang, 2014
	2.28 ng L ⁻¹	1.64 ng L ⁻¹	Wang et al, 2015
	-	1.25 ng L ⁻¹	Wang et al, 2016
	-	2.39 ng L ⁻¹	Ma et al, 2017
	-	42 ng L ⁻¹ , 54 ng L ⁻¹	Rodil et al, 2008
	-	43 ng L ⁻¹	Gomez et al, 2011
	<2.8-23 ng L ⁻¹	<2.8 ng L ⁻¹	Magi et al, 2012
	-	31 ng L ⁻¹	Pintado-Herrera et al, 2014
EHS	-	332 ng L ⁻¹	Moeder et al, 2010
	27,129,757 ng L ⁻¹	<15,31 ng L ⁻¹	Negreira et al, 2011b
	37.9-689.5 ng L ⁻¹	35.1-483.4 ng L ⁻¹	Cunha et al, 2015
	-	0.71-0.91 µg L ⁻¹	Xue et al, 2013
	Nd	-	Li and Yang et al, 2014
	-	1.29 µg L ⁻¹	Wang et al, 2016
	-	nd	Ma, et al 2017
	-	<4	Rodil et al, 2008
HMS	-	3 ng L ⁻¹	Pintado-Herrera et al, 2014
	22,36,105 ng L ⁻¹	4.5,4.8,5.5 ng L ⁻¹	Negreira et al, 2011b
	6,18,32 ng L ⁻¹	nd	Negreira et al, 2010
	-	nd, 6.1	Wu et al, 2013
	-	nd	Xue et al, 2013
	n.d-28 ng L ⁻¹	nd-7.5 ng L ⁻¹	Negreira et al., 2009
	-	8,9 ng L ⁻¹	Rodil, 2008
	-	22 ng L ⁻¹	Pintado-Herrera, 2014
HMS	14,25,40 ng L ⁻¹	<8-14 ng L ⁻¹	Negreira, 2011b
	nd, 11, 20 ng L ⁻¹	Nd	Negreira, 2010
	-	nd	Wu, 2013
	-	-	-

Table 2.3 Continue

UVFs	Concentration		Reference
	Influent	Effluent	
IAMC	- nd	<2-3 ng L ⁻¹ nd	Rodil, 2008 Negreira, 2010
4-MBC	- - - - 17,61,131 ng L ⁻¹ 7,103,153 ng L ⁻¹ 45.8-154.9 ng L ⁻¹	38 ng L ⁻¹ 142 ng L ⁻¹ 49 ng L ⁻¹ 102 ng L ⁻¹ <10, 140, 223 ng L ⁻¹ nd, 66, 94 ng L ⁻¹ <6-84.6 ng L ⁻¹	Rodil, 2008 Gomez, 2011 Pintado-Herrera, 2014 Moeder, 2010 Negreira, 2011b Negreira, 2010 Cunha, 2015
OC	- 28-463 ng L ⁻¹ 1.3 µg L ⁻¹ - - 209-831 ng L ⁻¹ 66-440 ng L ⁻¹ <LOQ-659.6 ng L ⁻¹	10,18 <3.3-13 ng L ⁻¹ 0.2 µg L ⁻¹ 56 ng L ⁻¹ 461 ng L ⁻¹ 65-111 ng L ⁻¹ 15-59 ng L ⁻¹ 0.3-357 ng L ⁻¹	Rodil, 2008 Magi, 2012 Moeder, 2010 Pintado-Herrera, 2013 Moeder, 2010 Negreira, 2011b Negreira, 2010 Cunha, 2015

As can be followed from the tables above, UVFs can be encountered in surface and wastewater samples in ng L⁻¹ levels and therefore, selective, and sensitive analytical methods are required for their reliable analysis. Next heading explains the details of the methods used for their analysis.

2.6 Analytical Methodologies

The analysis of UVFs is accomplished with gas chromatographic methods coupled with mass detector (GC-MS) (Negreira et al., 2011; Tarazona et al., 2010, Liu et al., 2011; Clavijo et al., 2016; Zhang and Lee, 2012a; Benedé et al., 2014; Trujillo-Rodriguez et al., 2020) and tandem mass (MS/MS) (Vila et al., 2016). Liquid chromatographic (LC) methods coupled with ultraviolet (UV) (Li et al., 2013, 2014c; Ma et al., 2017; Yang et al, 2014; Vidal, 2010; Oenning et al., 2017; Li et al., 2015; Kirschner et al., 2017; Mei and Huang, 2017), photodiodearray PDA (Zhang et al, 2011), diode array (DAD) (Ge et al., 2018), time of flight tandem mass (TOF-MS) (An and Anderson, 2018) and tandem mass (MS/MS) (Li et al., 2017; Piovesana et al., 2017; Aparicio et al., 2017; Ahmadi et al., 2017) have been also developed for attaining selective and sensitive determination.

Since their high boiling points and high molecular weights as mentioned in **Table 2.1** above, UVFs are not classified as volatile compounds. Their direct analysis can be accomplished with LC systems and GC analysis can be performed with the aid of derivatization.

Multiple reaction monitoring (MRM) techniques used in GC-MS/MS provide high sensitivity and selectivity reducing matrix effects and interference in the identification of trace UV filters (Hayden et al., 1997; Janjua et al., 2004; Calafat et al., 2008; Liu et al., 2011).

Considering the whole processes, each step in the analytical procedure has a critical role to perform sensitive and selective analysis of UV filters. For this reason, each step starting from sampling and storage to the evaluation of the data with the chemometric approach was given below in detail.

2.7 Sample Preparation Methods

Sample preparation requires many different pre-purification techniques to ensure that the analytes are present at an appropriate concentration level. Since it is very difficult to determine low concentration UV filters in complex environmental water samples (Padron et al., 2014).

Determination of UVFs in environmental samples including river water (Li, 2020; Da Silva, 2013), lake water (Rodil and M. Moeder, 2008; Trujillo-Rodríguez et al., 2020), tap water (Capriotti et al., 2014; Basaglia and Pietrogrande, 2012), swimming pool water (Mokhtari et al., 2020; Trujillo-Rodríguez, 2018), sea water (Diaz-Cruz et al., 2012; Cunha et al., 2015; Rodil, 2009; Benedé, 2014a, 2014b; Vila, 2016), wastewater (Mei and Huang, 2017; Cunha et al. 2015), groundwater (Liu et al., 2011), sediments (Langford et al., 2015; Votani et al., 2020), soils (Votani et al., 2020; Jeon et al., 2006; Abril et al., 2018), and sewage sludges (Plagellat et al., 2006; Negreira et al., 2011a) require an efficient sample pretreatment method.

Today, several techniques have been applied for the extraction of UVFs from complex matrices as shown in **Figure 2.1**. We can examine the sample preparation techniques published to date in the analysis of UV filters under two main headings: non-comprehensive methods which are greener, simple, saving material and traditional methods that have shortcomings as laborious, time-consuming, and exhausting large volumes of toxic solvents.

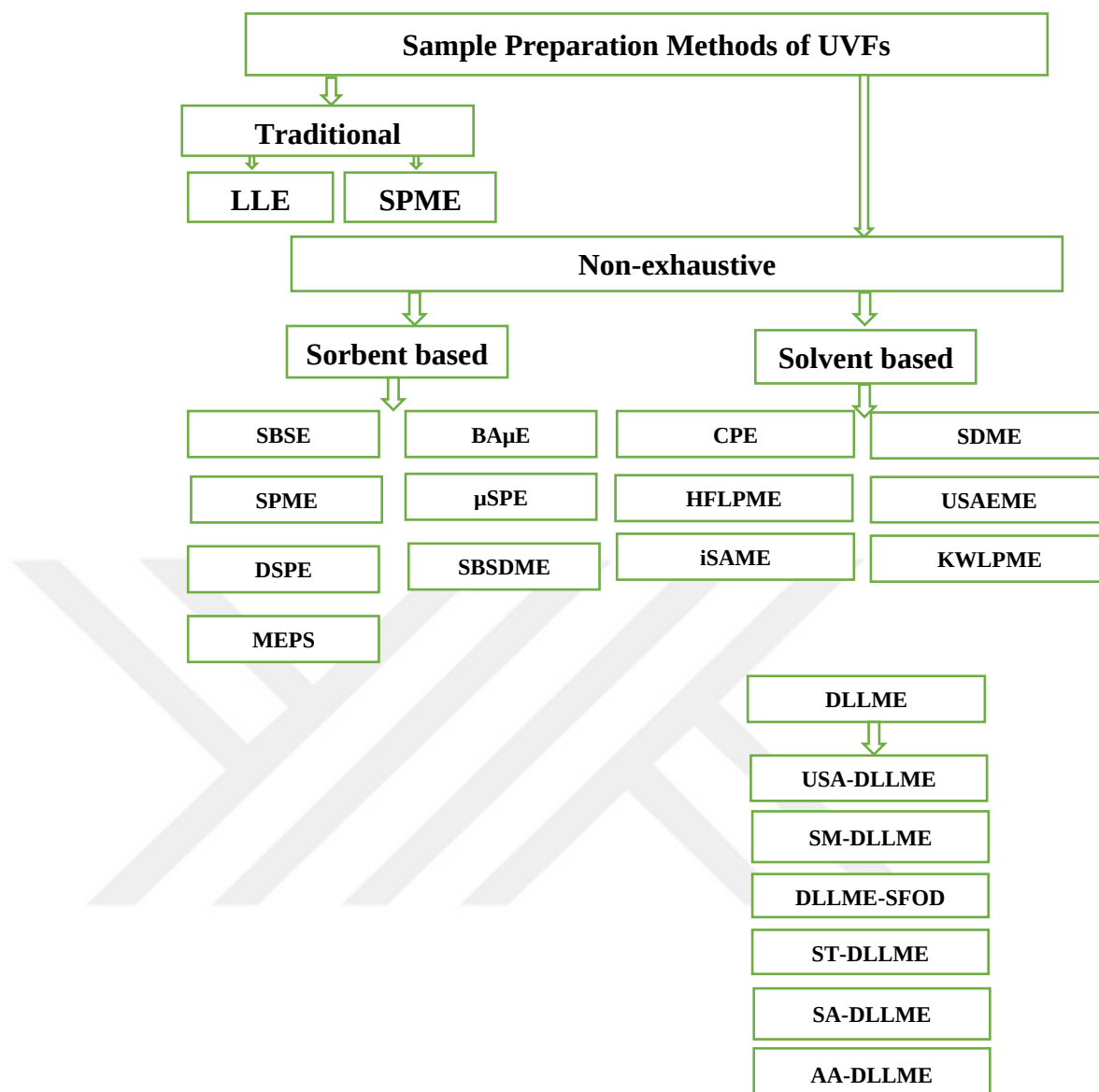


Figure 2.1 Sample preparation methods for the analysis of UVFs.

2.7.1 Liquid Liquid extraction (LLE)

Traditional liquid-liquid extraction (LLE) technique is based on the solubility of two phases which are immiscible in each other. Analytes in the aqueous phase transport the other phase according to their distribution between two phases. LLE techniques are used to extract the UVFs from the matrix (Jeon et al., 2006). Although this technique is simple and fast, it displays disadvantages such as consuming too much solvent and low selectivity.

2.7.2 Solid phase extraction (SPE)

In this technique, isolation and concentration of the desired components and removal of the interferences from the analyte can be maintained by using a mini-extraction column or discs filled with various sorbents (Rodil et al., 2009a, Tsui et al., 2014; Negreira et al., 2011b). The advantages of the SPE lie in its simplicity, flexibility, and selectivity. High sample volumes are used to attain high concentration factors but, the procedure is time-consuming and requires large volume of solvents. Therefore, special attention was paid for the technique and details of the methods coupled with chromatographic systems.

The critical step in the SPE technique is the choice of the adsorbent type. Today, a wide variety of sorbents for analytes with different physicochemical properties are available. There are three classes of sorbents mainly used which are high polymer-based sorbent (HLB) (Rodil et al., 2009a, Liu et al., 2012; Li et al., 2010), silica-based sorbents (C18) (Tsui et al., 2014; Kim et al., 2017) and more recently, sorbents packed with inorganic materials such as, florisil (Emnet et al., 2015). HLB is the most effective one for the extraction of UVFs due to its selectivity for a wide range of polarity.

All sorbents type and extraction conditions coupled with subsequent GC or LC detection in drinking water (DW), mineral water (MW), ground water (GW), tap water (TW), river water (RW), lake water (LW), pool water (PW), sea water (SW) and wastewater (WW) have been summarized in **Table 2.4 and Table 2.5**, respectively.

Since the most of the UVFs are relatively polar, ethyl acetate (EtAC) and dichloromethane (DCM) are used as the elution solvents. However, in the analysis of more polar UVFs such as BP-4, BP-1, PBSA, mixtures with more polar solvents can be chosen but, co-elution of other organic compounds should be considered (Rodil et al., 2012).

Table 2.4 Extraction conditions and analytical performance of SPE in water samples by using gas chromatography

	Reference	Langford and Thomas, 2008	Zenker et al., 2008	Liu et al, 2011	Ho and Ding, 2012	Da Siva et al., 2013	Tashiro and Kameda, 2013	Emnet et al., 2015	Kim et al., 2017	Nurerk et al., 2020
Sample	Water type	SW	SW	GW WW	WW, RW	RW, TW	SW	SW, WW	SW, WW, RW	MW, RW, WW, PW, SW
	UV Filter	BP-3, 4-MBC, OC, EHMC	EHMC, 4-MBC, BP-3, 3-BC	BP-3, 4-MBC, EHMC, UV-326, UV-329, OC	BP1, BP3, BP8	EHS, BP-3, EHMC OC	BP-3, 4-MBC OC, EHMC, OD-PABA EHS, HMS	4-MBC, BP-3, BP-1	BP-3, 4-MBC, EHMC, OD-PABA, EHS, BP	EHS, HMS, IAMC BP3, OC 4-MBC, MA Eto, EHPABA EHMC
Sample Treat.	Filtration		-	GF	0.45µm membrane filter	GF, 0.6 µm	GF	GF		-
	pH		-	2	3	3		2	3	-
SPE Step	Cartridge type	STRATA-X	POCIS 200 mg (80:20) (w/w) Isolute ENV+: Ambersorb 572	HLB	HLB		Discovery DSC-18LT DSC-P	HLB, Florosil	C18 Extraction disk	MIL-101
	Conditioning	6 mL EtAc, 6 mL MeOH, 6 mL UPW	AC, 2 mLUPW	6 mL DCM, 6 mL MeOH, 6 mL UPW	2 mL EtAc, 3 mL MeOH, 3 mL UPW	5 mL EtAc, 5 mL MeOH, 5mL UPW	-	4 x 10 mL (95:5) DCM/MeOH 2x10 mL MeOH, 2 x 10 mL UPW FLO cartridges 2 x 5 mL AC,	5 mL 1:1 MC:EtAc 5 mL MeOH, 5 mL UPW 3mL/min	1.0 mL EtAc, 1.0 mL UPW
SPE Step	Sample volume Loading rate	1L sample	POCIS (200 mg)	1 L sample 5–10 mLmin ⁻¹ . 2x50 mL UPW (5% MeOH)	100-mL 4-6 mL/min.	500 mL , 10 mL/min	1000 mL sample, 10 mL/min	1 L sewage, 4 L ocean water samples, 10 mL min ⁻¹	400 mL	40 mL sample
	Washing	UPW	20 mL UPW		2 mL UPW (5% MeOH)	50 mL UPW (5% MeOH)	-	-	UPW, 1 min	-
	Drying	Drying	45 min under N ₂	1 h under vacuum	15 min under N ₂	Under vacuum 500 µL MeOH	Centrifugion	Drying: ~3– 4 h.	8 min.	-
	Elution	6 mL EtAc, 3 mL DCM	2 mL MeOH 7 mL (1:1:8) MeOH, Tol, DCM	3x2 mL (1:1) MeOH/DCM	3 mL EtAc	3x2 mL EtAc	10 mL DCM	6 x 5 mL (95:5) DCM/MeOH	5 mL MC, EtAc, (1:1) MC:EtAc	2.0 mL EtAc

Table 2.4 Continue

	Reference	Langford and Thomas, 2008	Zenker et al., 2008	Liu et al., 2011	Ho and Ding, 2012	Tashiro and Kameda, 2013	Da Siva et al., 2013	Emnet et al., 2015	Kim et al., 2017	Nurerk et al., 2020
Post SPE	Evaporation	Evop. under N ₂ till 0,2 mL redissolved in 0,98 mL CHX	till dryness under N ₂ redissolved in 50 µL EtOH	till dryness under N ₂ redissolved in 0,95 mL AC	till dryness under N ₂ redissolved in DCM	Evop. under N ₂ till 0,2 mL	Evop. under N ₂ till 1 mL	till dryness under N ₂	Evop. under N ₂ till 0,5 mL	till dryness under N ₂ redissolved in EtAc
	Derivatization	-	-	-	1 µL MSTFA, PTV IPS (260°C)	-	-	200 µL (98:2) BSTFA/TMS 80°C	-	-
Measurement Step	Instrument	GC-ToF-MS	GC-MS, LC-MS	GC-MS/MS	GC-MS/MS	GC-MS	GC-MS/MS	GC-MS	GC-MS/MS	GC-MS/MS
	Linear Range	-	GC: 10-320 ng L ⁻¹ LC: 40-1280 ng L ⁻¹	-	5-500 ngmL ⁻¹ (In sample)	-	20-4000 ng L ⁻¹ (ES, BP-3) 50-4000 ng L ⁻¹ (EHMC, OC) (In a vial)	0-250 µg L ⁻¹	0.3-2.0 ng L ⁻¹	0.5–100 µg L ⁻¹ (In sample)
	LOD (ng L ⁻¹)	1-5	20-77	0.5-14.1	0,3-1	0.1-3.0	7.2-24.1	0,59-1,00	-	1.0-11.7
	Recovery	-	76.1 -98.0%	82–127%	80-106%	80-113 %	R: 62-107%	94-124%	79-118 %	82- 105%
	RSD	-	0.3-4.1%	<14%	<11%	-	RSD: <14 %	-		1.3-6.6%

Table 2.5 Extraction Conditions and Analytical Performance of SPE in water samples by using Liquid Chromatography (LC)

	Reference	Rodil et al., 2009a	Capriotti et al., 2014	Tsui et al., 2014	Langford et al., 2015	Mokhtari et al., 2020	Li et al., 2020
Sample	Water type	SW, WW	TW, LW	SW	WW	WW, PW	WW, RW
	UV Filter	BP-3, BP-4, IAMC 4MBC, OC, OD-PABA, PBSA	BP1, BP2, 4-HB, BP3, BP4, OC 4-MBC, BP-8, PBSA, BMDM, OD-PABA, EHMC, DHHB, Et-PABA, PABA, 4-DHB	BP- 3, BP-4, IAMC, 4- MBC, OC, EHMC, OD-PABA, EHS, HMS, BMDM	BP3, EDP, EHMC, OC, UV-324, UV-327 UV-328, UV-329	4-HB, BP-1 4,4'-DHB	BP-3, OC, BMDM, EHMC
Sample Treatment	Filtration	-	GF for LW		-	20–25 µm cellulose membranes	-
	pH	7.0		-	-	6.5	2.5
	Added reagent	-	Na ₂ S ₂ O ₃ ·5H ₂ O for DW	5% Na ₂ EDTA	-		Diluted to 0.5 L
SPE Step	Cartridge type	HLB	GCB (Graphite-4)	Bond Elut C18	HLB	MCM-41*-SO ₃ H	HLB
	Conditioning	5 mL MeOH, 5 mL UPW	5 mL (80:20) DCM/ MeOH + 10 mM AF, 3 mL MeOH, 10 mL 10 mM HCl, 5 mL UPW.	15 mL (1:1) MeOH/EtAc, 15 mL UPW	6 mL DCM, 6 mL MeOH, 12 mL UPW	5 mL UPW	6mL DCM, 6mL MeOH, 6mL UPW (~1 mL min ⁻¹)
	Sample volume Loading rate	200 mL sample	200 mL sample 15–20 mL min ⁻¹	250 mL samples 2-3 mL min ⁻¹	1 L Sample	50 mL sample 10% NaCl 5 mL min ⁻¹	1 L RW, 20 mL influent, 500 mL effluent, 10 mL min ⁻¹
	Washing	3 mL UPW	10 mL UPW	10 mL UPW	-	5 mL UPW	-
	Drying	under N ₂ , 30 min	with 200 µL MeOH, 1 min under N ₂	under vacuum, 10 min centrifugation, 3000 rpm, 2 min	-	5 min under N ₂	-
	Elution	3×10 MeOH	in backflushing mode, 1 mL MeOH,	3×4 mL (1:1) MeOH: EtAc	20 ml (50/50) EtAc//DCM	2 mL EtAc	7 mL DCM, 7 mL MeOH

Table 2.5 continue

	Ref	Rodil et al., 2009a	Capriotti et al., 2014	Tsui et al., 2014	Langford et al., 2015	Mokhtari et al., 2020	Li et al., 2020
Post SPE	Evaporation	At 50 °C till 0.2 mL adjusted to 1mL with (1:1) MeOH:UPW	till dryness under N ₂ redissolved in 500 µL (70:30) (0.1% FA) UPW/MeOH	till < 0.5 mL under N ₂ adjusted to 0.5 mL with MeOH.	At 35 °C till 2 mL	till dryness under N ₂ redissolved in 0.5 mL (20:80) MeOH:UPW	to 1.0 mL, 28 °C
Measurement Step	Instrument	LC-MS/MS	UHPLC-MS/MS	HPLC-MS/MS	LC-HRMS GC-HRMS	UPLC-QqQ-MS/MS	LC-MS/MS
Analytical merits	Linear Range	5-1500 µg L ⁻¹ (in vial)	LOQ-50 ng L ⁻¹ (in sample)	0.1-800 ng mL ⁻¹ (in vial)	-	1-100 µg L ⁻¹ (in vial)	1-500 ng mL ⁻¹
	LOD	0.8-30 ng L ⁻¹	0.7–3.5 ng L ⁻¹	0.03-1.04 ng L ⁻¹	1,5-3 ng L ⁻¹	0.15-1.5 ng L ⁻¹	1-67 ng L ⁻¹
	Recovery	76-128%	51-120%	63-104%	14-78%	74.7–99.6 %	69.8-94.2%
	RSD	<15%	1-15%	<10%	4-38%	2.8-6.8%	<12

MCM-41: A silica based mesoporous material.

2.7.3 Dispersive Liquid Liquid Microextraction

Due to the drawbacks of LLE, researchers focused on developing more selective, greener, and user-friendly techniques. Dispersive liquid-liquid microextraction (DLLME) is a simple, fast, low-cost, micro-extraction method with low sample and solvent consumption, based on the transfer of the analyte from the aqueous phase to the extraction solvent.

DLLME includes two solvent phases first one is the dispersive solvent that is miscible in both extraction and liquid phase and the second one is the extraction solvent which should be immiscible with water and miscible with the dispersive solvent. And the previously mixed these two solvents are rapidly injected into the liquid phase to form a cloudy phase by dispersing small organic droplets. By this means, organic analytes are transferred from aqueous matrix to the small droplets of organic solvents with high efficiency due to the effective interactions. After centrifugation, the aqueous and organic phases are separated, and the organic phase is injected to the GC or LC systems. The DLLME technique was developed by varying the density of extraction solvents and using different processes to expand the surface area where the analyte and extraction solvent interact.

In the analysis of UVFs by classical DLLME, organic solvents which have a density higher than water such as chloroform, chlorobenzene, trichloroethylene, perchloroethylene and octanol have been used (Negreira et al., 2010; Tarazona, 2010; Benedé et al., 2014; Cunha et al., 2015; Wu et al., 2013; Zhang and Lee, 2012c). However, these solvents are toxic and hazardous to the environment. As a result of concern about the use of toxic properties and high vapor pressure of solvents used in the classical DLLME method, the studies focused on the use of ionic liquids (ILs) due to their low toxicity, thermal stability, and their negligible vapor pressure (Zhang and Lee, 2013; Zhang and Lee, 2012b, Ku et al., 2013; Xue et al., 2013; Suárez et al., 2016).

The application of DLLME method to extraction of UV filters followed by GC and LC were shown in **Table 2.6**. The type of sample, the applied extraction procedure and optimization parameters along with the chemometric approaches used in optimization were also covered. Mostly One Factor At a Time (OFAT), Fractional Factorial Design (FFD) and Central Composite Design (CCD) and Two Level Full Factorial design (2^k FFD) have been used for the practical purposes.

Chromatographic conditions such as temperature programming for the GC-MS systems and gradient program for the LC systems have also been included to give a comprehensive information about the methods developed so far. Along with the instrumental and the detector parameters, analytical merits of the methods have been summarized for comparison.

Temperature-controlled IL dispersive liquid-phase microextraction (TC-IL-DLPME) technique was applied by Zhang et al. (2013) for the extraction of UV filters. In this method, high-temperature application ensures the dispersion of the extraction solvent in the aqueous phase. Thus, there is no need for toxic and expensive dispersive solvents. However, ILs are not directly compatible with GC systems due to their non-volatility. Injection of ILs into the GC results in the IL remaining in the injection port of the GC and system contaminations that require frequent maintenance, such as daily replacement of quartz wool and cleaning of the liner for the GC (Ma et al., 2011, from Marcinkowska et al., 2019).

The applicability of different methods based on DLLME modes has been investigated in UVF analysis such as ultrasound assisted (USA) procedure with in-situ derivatization (Wu et al., 2013). Here ultrasound radiation facilitated to increase the mass transfer of analyte aqueous phase to the organic phase. The performance of the method, abbreviated as USA-DLLME-GC-MS, has been compared with the other USA-DLLME combined with LC without derivatization (Wang et al., 2017; Zhang and Lee, 2012b; Xue et al., 2013). It was noted that LOD values were much lower than LC counterpart.

In another study, the transfer of analyte to organic solvent was achieved by vortex mixing without using a dispersive solvent (Zhang and Lee, 2012c). Since the phase separation occurs immediately after extraction, there is no need for centrifugation in VA-DLLME. More recently, the dispersion of the extractant phase into the aqueous solution was achieved by the combination of microwave assistance (MWA) with dispersive liquid-liquid microextraction. When MWA-DLLME is compared with stir bar sorptive extraction (SBSE) (Kawaguchi et al., 2008; Rodil and Moeder, 2008; Gilart et al., 2013; Aparicio et al., 2017) and solid-phase extraction (SPE) (Ho and Ding, 2012; Liu et al., 2011; Da Silva et al., 2013; Nurerk et al., 2020), lower detection limits have been achieved in addition to the ease and rapidness of this technique (Li et al., 2017).

Table 2.6 Details of DLLME-GC methods developed for the determination of UV filters in water samples

UV filters	Sample type Extraction procedure	Ext optimization	Chromatographic Condition / Temperature Optimization	Instruments/ MS parameters	Analytical performance	Reference															
BP-1, BP-3, BP-8	SW DLLME :5mL (pH 4), 10% NaCl)+1mL AC, 60 μ L CHL, Centrifuged, 3500 rpm, 3 min, Evaporated to dryness, Derivatized with 0.06 mL BSTFA at 75 $^{\circ}$ C for 30 min	OFAT CHL, DCM, CCl ₄ (40-80 μ L) AC, AcN, EtOH (250-1000 μ L) pH: 2-8 NaCl: 0-15% Derivatization: Deriv. Agent: MSTFA, BSTFA Deriv. Temp: 25-75 $^{\circ}$ C, Deriv. Time: 0-50 min	Inj: 1 μ L splitless, 280 $^{\circ}$ C, Purge: 1.5 min, Flow rate: 1 mL min ⁻¹ , <table><tr><th>Ramp ($^{\circ}$C min⁻¹)</th><th>Temp. ($^{\circ}$C)</th><th>Hold time min</th></tr><tr><td></td><td>130</td><td>1</td></tr><tr><td>20</td><td>200</td><td>0</td></tr><tr><td>3</td><td>250</td><td>0</td></tr><tr><td>20</td><td>280</td><td>3</td></tr></table>	Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min		130	1	20	200	0	3	250	0	20	280	3	GC-MS (EI) Ion source T:225 $^{\circ}$ C Interface T: 280 $^{\circ}$ C	LOD: 32-33 ng L ⁻¹ Rec: 65-169 % RSD: 5-6 %	Tarazona et al., 2010
Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min																			
	130	1																			
20	200	0																			
3	250	0																			
20	280	3																			
BP-3, EDP, EHMC, ES, HMS, IAMC, MBC, OC	RW, PW, WW DLLME: 10mL+1mL AC, 60 μ L CHLB, 3200 rpm, 3 min, 45 μ L segmented phase injection	2⁴⁻¹ FFD CH ₃ CCl ₃ , CHLB (0.5- 1.5 mL) pH: 2-9, NaCl:0-5 % tE: 1-10 min. OFAT AC, MeOH, AcN Multilayer Fact. Exp Design AC (5-2 mL) CHLB: 0.06- 0.1 mL	Inj: 2 μ L, splitless, 280 $^{\circ}$ C, Purge: 1.0 min, Flow rate: 1.2 mL min ⁻¹ , <table><tr><th>Ramp ($^{\circ}$C min⁻¹)</th><th>Temp. ($^{\circ}$C)</th><th>Hold time min</th></tr><tr><td></td><td>125</td><td>1</td></tr><tr><td>9</td><td>280</td><td>10</td></tr></table>	Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min		125	1	9	280	10	GC-MS (EI ⁺) Ion source T: 230 $^{\circ}$ C Interface T: 280 $^{\circ}$ C Quadrupole: 150 $^{\circ}$ C	LOD: 0.6-4.2 ng L ⁻¹ Rec: 80-117% RSD: 2-14%	Negreira et al., 2010						
Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min																			
	125	1																			
9	280	10																			
BP, BP-1, BP-3, ES, HMS	RW VA-DLLME: 10 mL (pH 4), 3 min, vortexed at 3500 rpm, 20 μ L segmented phase evaporated Derivatized with 0.03 mL BSTFA at 75 $^{\circ}$ C for 30 min	CHL, CHB, PERC (40-70 μ L) pH (2-6) NaCl (0-200 g/L) tE: (0.5-5 min)	Inj: 1 μ L, splitless, 280 $^{\circ}$ C, Purge: 1.0 min, Flow rate: 1 mL min ⁻¹ , <table><tr><th>Ramp ($^{\circ}$C min⁻¹)</th><th>Temp. ($^{\circ}$C)</th><th>Hold time min</th></tr><tr><td></td><td>110</td><td>2</td></tr><tr><td>20</td><td>170</td><td>0</td></tr><tr><td>6</td><td>210</td><td>0</td></tr><tr><td>20</td><td>290</td><td>4</td></tr></table>	Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min		110	2	20	170	0	6	210	0	20	290	4	GC-MS (EI+) Interface T: 280 $^{\circ}$ C	LOD: 8-30 ng L ⁻¹ Rec: 76-120% RSD: 6-13%	Zhang and Lee, 2012c
Ramp ($^{\circ}$ C min ⁻¹)	Temp. ($^{\circ}$ C)	Hold time min																			
	110	2																			
20	170	0																			
6	210	0																			
20	290	4																			

Table 2.6 Continue

UV filters	Sample type Extraction procedure	Ext optimization	Chromatographic Condition / Temperature Optimization	Instruments/ MS parameters	Analytical performance	Reference															
BP-1, BP-3, BP-8, EHS HMS	RW, WW USA-DLLME: 10 mL (pH 7+ 5 % NaCl), 15 µL PERC+0.75 mL MeOH, + in-situ deriv. 0.02 mL BSTFA, sonicated 2 min, centrifuged at 5000 rpm for 10 min 5 µL segmented phase is injected	OFAT EtOH, IPA, AC CCl ₄ , PERC, CHLB BSTFA, MSTFA tU: 0,5-10 min, NaCl: 0-20 %, pH: 3-8 CCD Dispersive solvent: 500-750 µL, Extractant solvent: 15-25 µL BSTFA: 20-30 µL	Inj: 5 µL, PTV, liner: 3.4 mm i.d (70°C for 2.5 → 260°C) Flow rate: 1 mL min ⁻¹ , <table><tr><th>Ramp (°C min⁻¹)</th><th>Temp. (°C)</th><th>Hold time min</th></tr><tr><td></td><td>100</td><td>4</td></tr><tr><td>25</td><td>300</td><td>3</td></tr></table>	Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min		100	4	25	300	3	GC-MS (EI+) Ion trap T: 200°C Ion source T: 230 °C Quadrupole : 150 °C Interface T: 280°C	LOD: 1-2 ng L ⁻¹ Rec: 70-93% RSD: 2-9%	Wu et al., 2013						
Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min																			
	100	4																			
25	300	3																			
BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC	SW DLLME 5 mL (pH 2.5) +0.25 mL AC, 0.05 µL CHL centrifuged at 3500 rpm for 5 min	OFAT tU: 5-20 min (before DLLME procedure) CHL, DCM (40-80 µL) EtOH AC, AcN (250 - 1000 µL) pH: 2,5-8 Sample: 2.5- 7.5 mL NaCl: 0-20 %	Inj: 2 µL, splitless, 280°C, Purge: 1.5 min, Flow rate: 1 mL min ⁻¹ <table><tr><th>Ramp (°C min⁻¹)</th><th>Temp. (°C)</th><th>Hold time min</th></tr><tr><td></td><td>70</td><td>1</td></tr><tr><td>10</td><td>170</td><td>0</td></tr><tr><td>2</td><td>200</td><td>0</td></tr><tr><td>10</td><td>280</td><td>6</td></tr></table>	Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min		70	1	10	170	0	2	200	0	10	280	6	GC-MS (EI+) Ion source T: 250 Interface T: 280°C Quadrupole: 150 °C	LOD: 10-30 ng L ⁻¹ Rec: 82-117% RSD: 2-14%	Benedé et al., 2014
Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min																			
	70	1																			
10	170	0																			
2	200	0																			
10	280	6																			
BMDM, BP-1, BP-3, BP-8, DHHB HMS EDP, EHS, EHMC, IAMC, MBC, OC	WW DLLME: 10mL (pH 3), 0.1 mL AC, 0.05 mL C ₂ H ₃ Cl ₃ , 3500 rpm, 1 min. 38 µL segmented phase evaporated and derivatized with 0.04 mL BSTFA, 5 min, 600 W with Microwave derivatization system	OFAT C ₂ H ₃ Cl ₃ , C ₂ HCl ₃ , C ₆ H ₅ Cl, C ₂ Cl ₄ , C ₂ Cl ₄ , C ₈ H ₁₈ O (3:1) (50-150 µL) AC, AcN, MeOH 500 -1500 µL pH: 2-7 NaCl: 5-10%	Inj: 1 µL, splitless, 250°C, Purge: 1 min, Flow rate: 1 mL min ⁻¹ <table><tr><th>Ramp (°C min⁻¹)</th><th>Temp. (°C)</th><th>Hold time min</th></tr><tr><td></td><td>95</td><td>1</td></tr><tr><td>40</td><td>180</td><td>0</td></tr><tr><td>5</td><td>230</td><td>0</td></tr><tr><td>25</td><td>290</td><td>6.47</td></tr></table>	Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min		95	1	40	180	0	5	230	0	25	290	6.47	GC-MS (EI+) Ion source: 230°C Interface T: 280°C Quadrupole: 150 °C	LOD: 2-26 ng L ⁻¹ R: n.a. RSD: 2-16%	Cunha et al., 2015
Ramp (°C min ⁻¹)	Temp. (°C)	Hold time min																			
	95	1																			
40	180	0																			
5	230	0																			
25	290	6.47																			

Table 2.6 Continue

UV filters	Sample type Extraction procedure	Ext optimization	Chromatographic Condition / Temp. Prog/ LC Prog	Instruments	Analytical performance	Reference		
BP-3, EHMC, EHS, HMS, 4-MBC, OC	PW, SW In syringe MSA-DLLME : 3,7 mL (pH 7),0,35 mL TCE, 0.6 mL AC, 160 sn, 0,7 mol/L NaCl,0.35 mL BSTFA, 2.6 min, Direct injection (on-line)	OFAT CHL, CCl ₄ , DCM, TCE AC, AcN, MeOH Derivatization : MSTFA, BSTFA 2^k FFD AC (500-1000 μL), TCE: BSTFA (200-400 μL) NaCl (0-1 mol/L), pH (5-7), Stir time (5-120 sn) CCD BSTFA (250-350 μL), AC (400-600μL) Stiring time:75-165 s	Inj :1 μL, pulsed splitless (0.1 psi, 0.5 min) 280°C, Flow rate : 1 mL min ⁻¹	GC-MS (EI+) Ion source T: 230°C Interface T: 280°C Quadrupole : 150°C	LOD: 19-160 ng L ⁻¹ R: 82-122% RSD: 4-17%*	Clavijo et al., 2016		
			Ramp (°C min ⁻¹)				Temp. (°C)	Hold time min
							130	1
			20				170	0
			2				200	0
10	280	4						
BP-1, BP-3	LW MSA-DLLME : 20 mL sample (pH 7), 40 μL octanol, 20 min, 1300 rpm, Diluted to 0.08 mL with MeOH	OFAT 1-octanol: 20- 60 μL, pH: 3-7, NaCl: 5–100 mM tE: 2-50 min Stirring rate: 260-1300 rpm	Inj :10 μL, Flow rate : 0.75 mL min ⁻¹ Isocratic (60:40). MeOH: 1% AcAc	LC-UV λ: 254 nm	LOD: 200-800 ngL ⁻¹ Rec: 91-97% RSD: 1-5%	Zhang et al., 2011		
BP BP-3 EHS HMS	RW, PW, TW IL-USA-DLLME : 10 mL (pH 4) 20 μL [C ₆ MIM][FAP], 0.1 mL MeOH, 25 C, 3 min. Cent, 4000 rpm, 5 min.	OFAT [HMIM][PF ₆], [BTMA][NTf ₂]: 20- 60 μL [BMPL][FAP], [HMIM][FAP]: 20- 60 μL MeOH, EtOH, AC, AcN: 0.1- 1.0 mL pH: 2–6, NaCl: 0-200 mg mL ⁻¹	Inj :10 μL, Temp : 40°C Flow rate : 0.4 mL min ⁻¹	LC-UV λ:305 nm (BP-3, EHS, HMS) λ: 254 nm (BP)	LOD: 200-5000 ng L ⁻¹ Rec: 71-118% RSD: 4-6%	Zhang and Lee, 2012b		

Table 2.6 Continue

UV filters	Sample type Extraction procedure	Ext optimization	Chromatographic Condition / Gradient Prog	Instruments	Analytical performance	Reference															
BPi BP-3, BP-8	PW, WW, LW IL-DLLME: 40 μL [C ₆ MIM][PF ₆], 5 mL+ pH:7, 0.2 mL MeOH, shaked ,3 min, 360 rpm. Centrifuged, 5 min, 5000 rpm, 20 μL sedimented phase diluted with 0.02 mL MeOH	OFAT C ₄ MIM][PF ₆], [C ₆ MIM][PF ₆]: 30-70 μL [C ₈ MIM][PF ₆]. [C ₄ MIM][PF ₆]: 30-70 μL MeOH, AcN, AC: 100-400 μL NaCl: 0-30 %	Inj: 10 μL, Temp: 40°C Flow rate: 0.4 mL min ⁻¹ <table><tr><th>0.05% TFA in UPW</th><th>0.05% TFA in ACN</th><th>Time (min)</th></tr><tr><td>95</td><td>5</td><td>0</td></tr><tr><td>0</td><td>100</td><td>8</td></tr><tr><td>0</td><td>100</td><td>10</td></tr></table>	0.05% TFA in UPW	0.05% TFA in ACN	Time (min)	95	5	0	0	100	8	0	100	10	λ: 254 (BP) λ: 330 nm (BP-8 and BP-3).	LOD: 200-1300 ng L ⁻¹ Rec: 92-120% RSD: 1-3%	Ku et al., 2013			
0.05% TFA in UPW	0.05% TFA in ACN	Time (min)																			
95	5	0																			
0	100	8																			
0	100	10																			
BP-3, EDP, EHMC, EHS	RW, WW IL-USA-DLLME: 10 mL (pH 7) 30 μL [C ₈ MIM][PF ₆], +0.1 mL MeOH, sonicate, 25°C, 5 min centr. 4500 rpm, 5 min, diluted with 0.03 mL MeOH	OFAT [BMIM][PF ₆], [HMIM][PF ₆]: 30 μL [OMIM][PF ₆], [BMIM][TF ₂ N]: 30 μL MeOH, EtOH, AC, AcN: 100-900 μL pH: 2-8, NaCl: 0-20% tUS: 1-6 min, Centrifuge time: 1–10 min	Inj: 20 μL, Isocratic (90:10) MeOH: UPW	LC-UV λ: 310 nm	LOD: 60-160 ng L ⁻¹ Rec: 93-114% RSD: 3-8%	Xue et al., 2013															
BP, BP-3, EHS, HMS	PW, TW TC-IL-DLPME: 10 m +20 μL [C ₆ MIM][FAP], 50°C, cooled down to 0°C, centr. 4000 rpm, 20 min	OFAT [BTMA][NTf ₂], [HMIM]: 20–60 μL [PF ₆] [HMIM][FAP]: 20–60 μL NaCl: 0 -200 mg mL ⁻¹ TE: 50- 90°C, tE: 0-30 min	Inj: 10 μL, Flow rate: 1 mL min ⁻¹ <table><tr><th>A % UPW</th><th>B% ACN</th><th>Time (min)</th></tr><tr><td>30</td><td>70</td><td>9</td></tr><tr><td>0</td><td>100</td><td>15</td></tr><tr><td>0</td><td>100</td><td>20</td></tr><tr><td>30</td><td>70</td><td>25</td></tr></table>	A % UPW	B% ACN	Time (min)	30	70	9	0	100	15	0	100	20	30	70	25	LC-UV λ: 305 nm (BP-3, EHS, HMS) λ: 254 nm (BP)	LOD: 300-5000 ng L ⁻¹ R: 88-116% RSD: 1-8%	Zhang and Lee, 2013a
A % UPW	B% ACN	Time (min)																			
30	70	9																			
0	100	15																			
0	100	20																			
30	70	25																			

Table 2.6 Continue

UV filters	Sample type Extraction procedure	Ext optimization	Chromatographic Condition / Gradient Prog	Instruments	Analytical performance	Reference								
(on-line) BP-3, EHS, HMS, 4-MBC, OC	PW, SW In syringe IL-MSA- DLLME: 190 µL [C6MIM][PF6], 3.5 mL, + 0.2 mL ACN, 2.5 min. diluted with ACN	2⁵ FFD [C4MIM][PF6],[C6MIM][PF6]: 80-240 µg [C8MIM][PF6] ACN, MeOH, AC: 200-600 µL pH: 3–8, NaCl: 0–59 g L ⁻¹ tE: 60–300 s CCD NaCl: 0–59 g L ⁻¹ [C6MIM][PF6]: 160–320 mg, ACN: 200–600 µL	Inj: 20 µL, Flow rate: 0.8 mL min ⁻¹ Isocratic A: 85%, ACN (0.5% AcAc) B: 15%, UPW (0.5% AcAc)	LC-UV λ: 307 nm	LOD: 0.08-12 µg L ⁻¹ Rec: 86-114% RSD: 4-8%	Suárez et al., 2016								
BP-1, BP-3, BP-8	RW, TW, WW MWA-DLLME: 1 mL sample, (100 µL CHL+ 200 µL D0-/D3- MIAA+100 µL DMAP+60µL EDC), 0.5 mL AC, 5 min MW 300 s, 240 W, Evaporated and redissolved in 0.5 mL ACN	OFAT CHL, MeOH, DCM, DMF D0-/D3-MIAA: analytes.: 2/1; 2: 4/1; 3: 6:1; 4: 8:1; 5: 10;1; 6: 12:1). Catalyst (pyridine, 2-methylpyridine, trimethylamine, DMAP Cond agent (DCC, CDI, DIC, EDC)	Inj: 0.5 µL, Flow rate: 0.2 mL min ⁻¹ Temp: 30°C <table><tr><td>A %: AcN/ UPW: 5/95, (0.1% FA)</td><td>B %: AcN (0.1% FA)</td></tr><tr><td>30</td><td>0</td></tr><tr><td>100</td><td>2</td></tr><tr><td>100</td><td>6</td></tr></table>	A %: AcN/ UPW: 5/95, (0.1% FA)	B %: AcN (0.1% FA)	30	0	100	2	100	6	UPLC-MS/MS (ESI) drying gas T:300 °C; drying gas flow rate: 9 L min ⁻¹ ; nebulizer gas: 40 psi; sheath gas T: 250 °C; sheat gas flow: 8 L min ⁻¹ capillary V:3.5 kV	LOD: 0.96-1.2 ng L ⁻¹ Rec: 93-104% RSD: 1-3%,	Li et al., 2017
A %: AcN/ UPW: 5/95, (0.1% FA)	B %: AcN (0.1% FA)													
30	0													
100	2													
100	6													

*tE: Extraction time, TE: Extraction Temperature, MSTFA: N-Trimethylsilyl-N-methyl trifluoroacetamide, BSTFA: **N, O-Bis(trimethylsilyl)trifluoroacetamide**, ES: Extraction Solvent, DS: Dispersive Solvent, TCE: Trichloroethylene, DES: Deep eutectic solvent, TAC: Trioctylmethylammonium chloride, DecA: Decanoic acid. AcAc: Acetic Acid, CCCD: Circumscribed Central Composite Design, Nebulizer gas (GS1), heater gas (GS2), IS: Ion spray voltage, dicyclohexylcarbodiimide (DCC), N, N'-carbonyldiimidazole (CDI), N,N-diisopropyl carbodiimide (DIC) and N-Ethyl-N-(3-dimethyl aminopropyl) carbodiimide (EDC), 4-dimethyl aminopyridine (DMAP), d0-MIAA (light labeled) and d3-MIAA (heavy labeled). DecA: Decanoic acid

Magnetic stirring assisted (MSA) extraction is known to enhance the interaction and improves the recovery of analytes. UVFs were determined using both LC-UV (Zhang et al., 2011) and GC-MS (Clavijo et al., 2016) combined with the MSA-DLLME method without using dispersive solvent and centrifugation. The homemade device facilitates separating the low-density extractant from the aqueous phase. This method has shown successful results in determining low concentration (ng L^{-1}) of UVFs using large sample volumes (Zhang et al., 2011).

An online in-syringe MSA-DLLME method and simultaneous derivatization have been developed for analyzing of UVFs by using GC-MS system (Clavijo et al., 2016). When this method is compared with other studies (Zhang and Lee, 2012), as the derivatization and extraction process is performed simultaneously, the analysis time has decreased considerably, but higher LOD was obtained due to using low sample volumes.

2.7.4 DLLME-SFOD

Since the solvents used in DLLME are heavier than water, the organic droplet is collected at the bottom of the centrifuge tube. These organic solvents are both highly toxic and limited in number. Because of these disadvantages, Zanjani et al., developed a new LLME technique based on stirring the sample solution after adding floating solidified organic solvent (2007). The organic phase in the sample solution placed in the ice bath was allowed to solidify. The solidified floating organic droplet (SFOD) was then transferred to a conical cup using a spatula or forceps and allowed to melt at room temperature as illustrated in **Figure 2.2**.

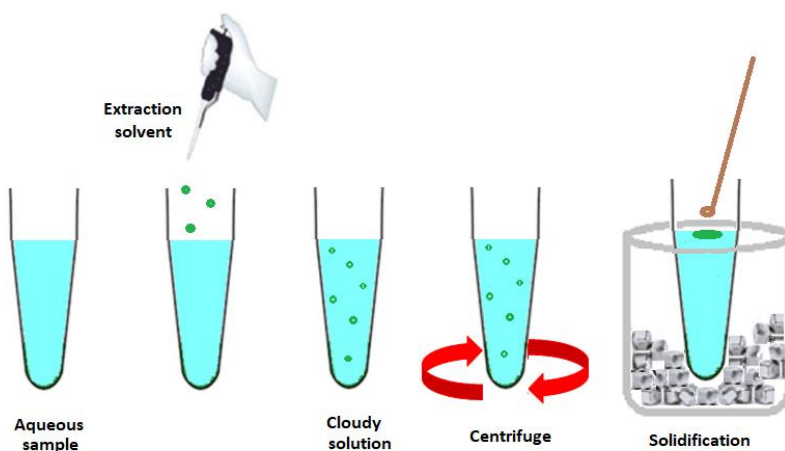


Figure 2.2 Extraction steps in Solidified Floating Organic Drop Technique

The use of simpler and more environmentally friendly organic solvents listed in **Table 2.7** is an advantage of the SFOD method over DLLME. However, the spherical geometry of the solidified organic droplet in this method resulted in a decrease in the interface area, thus reducing the extraction efficiency. To overcome this problem, a dispersive solvent was used which allows the organic droplet to diffuse into the sample (Leong and Huang, 2009; Xu et al., 2009; Mansour and Danielson, 2017).

This method, which combines the advantages of both methods, is called the DLLME-SFOD method. The use of less toxic non-chlorinated solvents, the floating characteristic of the solidified solvents facilitates the collection of the extract.

Table 2.7 Organic solvents commonly used in DLLME-SFOD

	Solvent	MP (°C)	BP (°C)	d (g mL ⁻¹)
Extraction solvents	1-Bromohexadecane	17-18	190	0.99
	1-decanol	6.4	233	0.83
	1-dodecanol	22-24	259	0.83
	1-undecanoic acid	28.6	284	0.89
	1-undecanol	13-15	243	0.83
	2-undecanol	17-18	249	0.80
	Cyclohexanol	25,9	162	0.96
	n-hexadecane	18	287	0.77
	2-dodecanone	21	245	0.83
	2-dodecanol	19	129	0.83
Dispersion solvents	AC	-94.7	56.05	0.7845
	MeOH	-98	64.6	0.791
	AcN	-43.8	81.65	0.7857
	EtOH	-114.1	78.5	0.789
	1-propanol	-126	97	0.803

The extraction technique has the same variables as those of LLE since the concentration of the analyte in the organic solvent remains constant after a certain period and the extraction reaches equilibrium.

The amount of substance (q) remaining in the aqueous sample phase after n numbered extractions is calculated by **Equation 2.1** given below where V_{aq} is the sample volume, V_o is the volume of organic extracting solvent and K is the partition coefficient of the reaction.

$$q = [V_{aq} / (V_{aq} + K V_o)]^n \quad \text{Eq. 2.1}$$

2.7.5 Solid phase microextraction (SPME)

This technique utilizes a 1-2 cm long thin polymer film-coated fiber for extraction by dipping it into the sample solution or exposing its headspace for collection of volatile components. It is a simple, efficient, and solvent-free technique based on mass transfer of analytes from the sample to the sorbent surface as gas-liquid or liquid-liquid partitioning. SPME apparatus is shown in **Figure 2.3**.

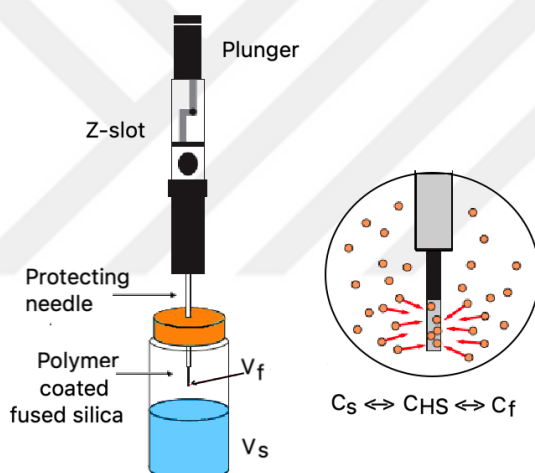


Figure 2.3 SPME apparatus operated in Head Space mode

There are two types of SPME coatings such as liquid polyacrylate (PA) and polydimethylsiloxane (PDMS) and solid polymer coatings such as divinylbenzene (DVB) blended with Carbowax and Carboxen (CAR). The liquid coatings are based on an absorption mechanism where the analyte is partitioned between two liquid phases such as organic solvent and water (Zewe et al., 2010).

In this mechanism, the target analyte is transferred between these two phases until equilibrium is reached. The calculation of equilibrium constant is shown in **Equation 2.2** where the n is the amount of analyte in mole extracted at equilibrium, V_s is the sample volume, V_f is the volume of the SPME fiber coating, and C_o is the initial concentration of analyte in the sample (Zewe et al., 2010).

$$K = nV_s / (V_f (C_o V_s - n)) \quad \text{Eq. 2.2}$$

In the SPME method, the fiber is exposed to the aqueous phase or the gas phase under temperature-controlled conditions. When the equilibrium is reached in these phases, target analytes transfer into the fiber (adsorption). In the next step, the fiber is placed directly into the injection port of the GC, thermal desorption occurs at certain temperature and the analytes are separated in the column.

Many commercial fibers have been used in the analysis of UV filters in previous studies as shown in **Table 2.8**. The traditionally available single fiber coatings including PDMS (Lambropoulou et al., 2002; Liu et al., 2010), PA (Basaglia and Pietrogrande, 2012; Vila et al., 2016), PDMS-DVB (Negreira et al., 2009) and DMS/DVB/CAR (Vila et al., 2017) were used to extract the UVFs from the environmental waters.

UVFs (BMDM, BP-3, DHHB, DRT, EDP, EHMC, ES, HMS, IAMC, MA, MBC, OC), in wide range polarities, were determined with PA fiber followed by GC-MS without derivatization in bathing water (Basaglia and Pietrogrande, 2012). The results obtained in the recovery studies on sea water, swimming pool water, river water, and SPA water are within the acceptable range (0.16–12 ng L⁻¹). In another study of the same authors, UV filters, which were derivatized by treating with 0.2 mL of acetic anhydride at 100°C for 30 min, were detected by GC-MS/MS following SPME using DVB/CAR/PDMS fiber (Vila et al., 2017). The LOD and recovery values are in the range of 0.045–8.2 ng L⁻¹ and 80%–106%, respectively.

The extraction efficiency of BP-3, EDP was investigated with different SPME fiber such as 100 µm PDMS, 65 µm CW-DVB and 65 µm PDMS-DVB (Lambropoulou et al., 2010). PDMS and PA fiber coatings were selected due to high extraction efficiencies of UV filters. 4-MBC, OC were extracted from environmental water with PDMS fiber followed by GC (Liu et al., 2010).

In another study using PDMS fiber, film thicknesses were investigated in the extraction of 4-MBC, OC and polycyclic musks (Liu et al., 2010). In the analysis of these compounds, 30 µm PDMS fiber was chosen since 100 µm fiber requires long time for extraction and desorption of the target analytes.

Table 2.8 Extraction procedure and analytical performance of commercial SPME Fibers used for UVF determination

Extraction Mode/Fiber type/Extr conditions	UVFs	Sample type	Analytical performance			Reference
			LOD ng L ⁻¹	Rec%	RSD%	
(DI)SPME, (HS)SPME PDMS, 25 °C, 45 min 5 mL pH 2, +1% NaCl, TD: 200 °C, 8 min	BP-3	PW	515	98	6.7	Lambropoulou et al., 2002
		SW		94		
	EDP	PW	264	94	4.5	
		SW		82		
(DI)SPME PDMS-DVB, 20 °C, 30 min 10 mL, pH 3 Deriv: 0.02 mL MSTFA, 45 °C, 10 min TD: 270 °C, 3 min	EHS	RW	5	110	2-7	Negreira et al., 2009
		Influent		95		
		Effluent		53		
	HMS	RW	5	109	1-7	
		Influent		89		
		Effluent		48		
	BP-3	RW	0.5	108	6-8	
		Influent		115		
		Effluent		93		
	BP-1	RW	10	99	10-13	
		Influent		97		
		Effluent		92		
	BP-8	RW	2	97	8-12	
		Influent		108		
		Effluent		80		
(DI)SPME PDMS, 3 mL, 24 °C, 90 min TD: 280 °C, 7 min	4-MBC	RW	21	72.5-115	<10	Liu et al., 2010
	OC		42.9	100-114		
(HS)SPME PA, 40 mL, 40 °C, 125 min, Deriv: 0.04 mL BSTFA, 35 °C, 30.5 min TD: 300 °C, 2 min	BP-3	TW	9	na	<20	Basaglia and Pietrogrande, 2012
	BP		2		<10	
HS SPME PA, 10 mL (35% NaCl, Na2S2O3), 100 °C, 20 min TD: 290 °C, 20 min	BMDM	SW	12	-	-	Vila et al., 2016
		SPA		118	1.8	
		PW		96-115	22	
		RW		72	5.8	
	EHS	SW	0.69	92-112	16	
		SPA		86-114	13	
		PW		67-85	6.2	
		RW		78-116	6.5	
	BS	SW,	0.12	92-123	10	
		SPA		97-102	6.3	
		PW		83-110	10	
		RW		88-94	3.5	

Table 2.8 Continue

Extraction Mode/Fiber type/Extr conditions	UVFs	Sample type	Analytical performance			Reference
			LOD ng L ⁻¹	Rec%	RSD%	
HS SPME PA, 10 mL (35% NaCl, Na ₂ S ₂ O ₃), 100 °C, 20 min TD: 290 °C, 20 min	HMS	SW	0.34	89-117	15	Vila et al., 2016 Continue
		SPA		106-126	12	
		PW		70-104	9.1	
		RW		84-97	6.2	
	IAMC	SW	0.068	94-108	15	
		SPA		87-98	13	
		PW		79-98	5.2	
		RW		88-94	10	
	BP-3	SW	1.5	73-115	17	
		SPA		82-102	9.2	
		PW		84-101	16	
		RW		73-98	13	
	4MBC	RW	1.5	73-108	12	
		SW		90-105	5.6	
		SPA		92-127	9.8	
		PW		68-101	19	
	EDP	RW	0.25	82-106	4.9	
		SW		80-108	12	
		SPA		64-93	13	
		PW		89-95	21	
	EHMC	RW	0.22	75-117	10	
		SW		74-106	20	
		SPA		74-97	8.2	
		PW		82-92	9.5	
	OC	RW	0.16	104-128	12	
		SW		105	21	
		SPA		111	6.2	
		PW		-	-	
(DI)SPME DVB/CAR/PDMS 10 mL +0.1 g K ₂ CO ₃ + 0.2 mL acetic anhydride, 100 C, 30min TD: 260 °C, 5 min	EHS	RW, PW, SPA, SW	0.066	95.9	6.3-9.7	Vila et al., 2017
	IAMC		0.069	96.5	3-5.6	
	HMS		0.15	90.8	76.7.3	
	BS		0.084	91.4	6.1-10	
	4-MBC		0.84	90.6	6.5-18	
	BP3		0.30	86.9	9.6-11	
	BP1		6.1	103	8.4-8.7	
	EDP		0.096	95.9	7.3-8.3	
	EHMC		0.060	86.8	9.0-9.2	
	BP8		8.2	102	9.0-9.4	
	MA		0.045	93.9	6.9-14	
	OC		0.18	102	4.2-5.5	

In a study conducted with PDMS/DVB fiber was selected for extraction of derivatized salicylate and benzophenone type UV filters, the author compared the performances of PA, PDMS/DVB, PDMS, CAR/PDMS fibers (Negreira et al., 2009). Due to the large molecular size of the target analytes, the analytes could not diffuse into the porous structure of the carboxene sorbent. PA fiber coating gave high response only for BP-1 analyte. Therefore, PDMS coated fiber was chosen, which provides the highest extraction efficiency in the extraction of target analytes.

Laboratory-made SPME can also be prepared in a wide range of physical properties and can be used in more selective extraction of the analytes. There are different class of sorbents such as metal or metal oxide nanoparticles (Li et al., 2014a, 2014b; Son et al., 2015; Ma et al., 2017), organic/inorganic coated fibers (Yang et al., 2014; Li et al., 2013), polymeric ionic liquids (PIL) (Trujillo-Rodríguez, 2020; An and Anderson, 2018) developed for UV filter analysis as summarized in **Table 2.9**.

Plunger-in-needle headspace solid-phase microextraction method with graphitized sol gel-based fiber is applied to extract the UVFs following by derivatization of analytes with MSTFA at 45°C for 15 min (Zhang and Lee, 2012a). Recovery (99–114%) and LOD (0.5–6.8 ng L⁻¹) values are satisfactory. In another study, it is used natural products as fiber for the extraction of UVFs (Silva et al., 2017). When the surfaces of wine corks containing lignin and suberin are exposed to heat, they become suitable for interaction with analytes. Good recovery (67–117%) and repeatable results (4-30 ng L⁻¹) have been achieved with the SPME technique where this natural and renewable material is used as fiber.

PILs coated fibers are favorable for extraction of UVFs from the water followed by a GC or LC detection. Polyionic liquids formed because of the polymerization of ionic liquids show the same properties of ionic liquids being chemically and electrochemically stable and their vapor pressures are very low. They are thermally stable due to their viscosity (Yuan et al., 2013; Clark et al., 2017).

Table 2.9 Extraction procedure and analytical performance of UV filters with lab-made SPME Fiber

UVFs/Water type	Extraction	SPME fiber	Instruments	Analytical merits	Ref
BP, BP3, EHS, HMS, 4-MBC RW	(DI)SPME 7 mL (pH 7), 25 °C, 40 min, 1000 rpm; Deriv. with 0.04 mL MSTFA, 45°C, 15 min TD: 280 °C, 1 min	Graphene-coated fiber	GC-MS	LOD: 0.5–6.8 ng L ⁻¹ Rec: 99–114% RSD: 1–6%	Zhang and Lee, 2012a
EDP, 4-MBC RW	(DI)SPME 25 mL (pH 4, 9% NaCl), 80 °C, 70 min TD: 260 °C, 15 min	Cork-coated fiber	GC-MS	LOD: 4–30 ng L ⁻¹ Rec: 67–117% RSD: 3–18%	Silva et al., 2017
EHS, HMS, BS, BP3, MA EH-PABA, Eto, 2EHMC, OC, TW, PW, LW	DI SPME 10 mL (2.5% NaCl), 100°C, 30 min. TD: 260 °C, 2 min	PIL Poly ([VBC1 ₆ IM] [NTf ₂]-co-[(VBIM) ₂ C ₁₂ ²⁺]) 2[NTf ₂ -]	GC-MS	LOD: 2.8-26 ng L ⁻¹ Rec: 63.5-119% RSD: 20.5-15%	Trujillo-Rodriguez et al., 2018
BP3, EHMC, OD-PABA, EHS RW, WW	DI-SPME 15 mL, pH7, 5% NaCl, 45°C, 40 min, 300 rpm SD: 1mL min ⁻¹ , MeOH:UPW (88:12)	Zn-ZnO nanosheets	HPLC-UV	LOD: 0.02-0.05 µg L ⁻¹ Rec: 85.8- 105% RSD: 5.18- 7.56%	Song et al., 2015
BP3, EHMC, OD-PABA, EHS RW WW	DI-SPME 15 mL. (pH 7.5% NaCl), 55°C, 60 min SD: MeOH/water, 8 min, 1mLmin ⁻¹	N-/TiO ₂ NTs/Ti,	HPLC-UV	LOD: 0.03- 0.05 µg L ⁻¹ Rec: 86.2- 113% RSD: 5.7 -7.7%.	Ma et al., 2017
BP3, 4-MBC, EHMC, OD-PABA, EHS LW, RW, WW	IT/SPME 1.5 mL, pH 9, no salt	PIL-MCC/MNPs	HPLC/UV	LOD: 0.04–0.26 µg L ⁻¹ Rec: 44.0–100% RSD: 0.8- 9.9%	Mei and X. Huang, 2017

IT/SPME: in-tube solid phase microextraction, SD: solvent desorption, MCC: monolithic capillary column, MNPs: nanoparticles, N-CNP: nitrogen-containing carbon nanoparticle

Table 2.9 continue

UVFs/Water type	Extraction	SPME fiber	Instruments	Analytical merits	Ref
OD-PABA, EPMC, EHS RW, WW	SPME 15 mL, (pH 7.0, 10% NaCl), 30 min, 35°C, 4 min	TiO ₂ -nanosheets	HPLC-UV	LOD: 0.026-0.089 µg L ⁻¹ Rec: 88.8- 107% RSD: 4.50 -8.76%	Li et al., 2014b
BP-3, OD-PABA, EPMC and EHS RW, Rainwater	(DI) SPME: 8 mL sample SD: MeOH:UPW (88:12)	Ph-TiO ₂ -Ti	HPLC-UV	LOD: 0.1 -50 ng L ⁻¹ Rec: 86.2- 105.5% RSD:5.5 to 9.1%.	Li et al., 2015
BP-3, OD-PABA, EPMC, EHS RW, WW	(DI)SPME 15 mL, pH 7.0, 30 min, 35°C	ZrO ₂ NPs-coated Ti-TiO ₂ fiber,	HPLC-UV	LOD: 32-82 ng L ⁻¹ Rec: 81-116% RSD: 5-12%	Li et al. 2014a
BP-1, BP-2, BP-3, PBSA, OC, EPMC, BMDM, WW	SPME PASSIVE Sampler, 90 days SD: MeOH:AcN:IP A(50:25:25), 30 min	TWA-TFME HL/B coating	LC-MS/MS	LOD: - RSD:7-19%	Ahmadi et al., 2017
BP-,MBC, EPMC RW	96-well plate 1.5 mL, pH6, 15% NaCl, 240 min, MeOH:AcN (1:1), 30 min	TF Recycled diatomaceous earth	LC-UV	LOD: 1-8 µg L ⁻¹ Rec:70-117 % RSD:3-23%	Kirschner et al., 2017
ES, HS, BS, BP-3, Ma, EHPABA, OC, 2-EPMC, TW, LW	VASE 10 mL sample, pH 3.5, 70°C, 14 h, 290°C, 2 min	Silonite coated cylinder packed with 70 mg of Tenax	GC-MS	LOD: 0.5-80 ng L ⁻¹ Rec: 70.0 and 120%. RSD:3.5-11%	Trujillo-Rodríguez et al., 2020
BP-3, BS, EMC, EDP, ES, OC, HS, BMDM TW, PW, LW	(DI)SPME 10 ml sample (25% NaCl), 60-75 min, room temp	PIL	LC-TOF-MS	LOD: 0.1-5 µg L ⁻¹ Rec: 66.6-117.9 % RSD: 1.8-11.6 %	An and Anderson, 2018
BZ, BZ3 RW	(DI)SPME 10 mL (20% NaCl), 60 min LD: 0.2 mL MeOH, 10 min	C12-coated silver wire	HPLC-UV	LOD 580-1860 ng L ⁻¹ R: 70-102% RSD: 3-15%	Li et al., 2013
BP-3, EPMC, OD-PABA, EHS	(DI) SPME: 15 mL, LD:500 mL.	AuNPs/SS	HPLC-UV	ND	Zhang et al., 2015
BZ, BZ3, EDP, EPMC, ES RW, WW	(DI) SPME: 15 mL (pH 7), 45 C, 50 min SD: MeOH: UPW (86:14), 6 min	N-CNPP/PANI coated SS	LC-UV	LOD: 6-200 ng L ⁻¹ Rec: 84-119% RSD: 4-9%	Wang et al., 2015

An and Anderson (2018) polymerized IL monomers of 1-vinyl-3-decyl-imidazolium styrenesulfonate ($[\text{VImC}_{10}][\text{SS}]$) and 1,12-di (3-vinyl-benzyl imidazolium) dodecane distyrenesulfonate ($[(\text{VBIm})_2\text{C}_{12}][2[\text{SS}]]$) to generate PIL sorbent coating and used it in UVF analysis coupled to HPLC. In another study, different crosslinked PIL-based sorbent coatings have been produced and used in HS mode in SPME. $[\text{VBC}_{16}\text{IM}^+][\text{NTf}_2^-]$, $[\text{VC}_{16}\text{IM}^+][\text{NTf}_2^-]$ and $[\text{VC}_{10}\text{IM}^+][\text{SS}^-]$ are used as monomers $[(\text{VBIM})_2\text{C}_{12}^{2+}][2[\text{NTf}_2^-]]$ $[(\text{VIM})_2\text{C}_{12}^{2+}][2[\text{NTf}_2^-]]$ and $[(\text{VBIM})_2\text{C}_{12}^{2+}][2[\text{SS}^-]]$ are used as crosslinker (Trujillo-Rodriguez et al., 2018). Analytical performances were comparable with commercial PA fibers.

Metal or metal oxide nanoparticles-based fibers have been used in the extraction of UVFs (Li et al., 2014a, 2014b; Song et al., 2015; Ma et al., 2017; Li et al., 2015). Li et al. have fabricated fibers that are formed by the anodization of TiO_2 nanosheets on Ti wire provide a wider surface area and better selectivity compared to commercial fibers (2014a and 2014b). The same group has prepared phenyl functionalized TiO_2 -NPs SPME fiber by anodization of Ti wire and then, trichlorophenylsilane was self-assembled on the surface (Li et al., 2015).

In study conducted by Song et al. (2015), coating process of Zn on stainless steel substrates was applied with potentiostatic technique instead of hydrothermal technique and Li and Yang prepared Ti- TiO_2 - ZrO_2 fiber formed by coating the combination of TiO_2 -based nanostructure and ZrO_2 electrodeposition to the Ti wire with the cyclic voltammetry technique and reported a good limit of detection concentration and reproducible results (2014). In the study of Ma et al. (2017) after TiO_2 NTs arrays were formed by anodization on Ti wire, Ti wire was immersed in a mixture of acid and aniline and coated with polyaniline (PANI) by direct electropolymerization method.

A method combining the advantages of the SBSE and vacuum HS-SPME method was applied for the extraction and desorption of UVFs (Trujillo-Rodríguez et al., 2020). Commercially available Silonite coated Tenax filled sorbent pens (SPs) were attached on vials filled with a 1-40 mL water sample. The evacuation of air in the interior of the SPs and vials was carried out by the vacuum pump. Vials were placed in the Sorbent Pen Extraction System (SPES) and vacuum assisted sorbent extraction (VASE) was performed at certain temperatures and mixing speeds. Water vapor inside the SP attached vials has been reduced by keeping vials at -17°C . At the final stage, the thermal desorption of analytes on the surface of the SPs was performed in the SP desorption unit (SPDU) in the GC injection port.

Organic/inorganic composite coated fibers fabricated by. Zhang and Song (2015). AuNP coating was performed by self-bonding mercapto-undecanol on the EES wire by utilizing the direct chemical accumulation method and thus a wider surface area and active points were obtained. But the extraction efficiencies of UVFs are not in the acceptable range. In another study, 1,8-octanedithiol has been self assembled into ordered AuNPs which can compete with PDMS and PA in terms of extraction efficiency and selectivity (Yang et al., 2014).

A passive sampler based on a thin-film solid-phase microextraction (TF-SPME) technique was prepared by Ahmadi, 2017. In this technique retracted HLB thin film is used to enhance the surface, capacity, and sensitivity of the method. In another study, an environmentally friendly chemical, diatomaceous earth, was used as a sorbent, and by using 96 well plates, extraction time was decreased to 2.8 min per sample (Kirschner et al., 2017).

In-tube SPME is a mode of SPME that typically uses a GC capillary column with a suitable coating on its internal surface to extract the analytes. Polymeric ionic liquid (PIL)-based ME/MB(IT-SPME) was prepared by the synthesis of modified magnetic nanoparticles ($\text{Fe}_3\text{O}_4@\text{SiO}_2@\gamma\text{-MAPS}$) and mixing it with 1-allyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide (AM), Ethylene dimethacrylate (ED), 1-propanol, 1,4-butanediol and Azobis isobutyro-nitrile (AIBN) for the in-situ polymerization (Mei and Huang, 2017). Then, the fused silica capillary column filled with this solution was assembled to HPLC-DAD system. As a result, the extraction, concentration, injection, separation, and detection were performed in a single step. This method requires less than 20 minutes for analysis of UVFs while some other methods are applied in more than 30 minutes. Since ME/MB (IT-SPME) method does not need SPME-HPLC interface systems used in the static desorption method, it is more economic than other studied methods.

2.7.6 Preparation Techniques for Lab-made SPME Fiber

There have been several different methods of SPME fiber preparation described in the literature such as dipping and physical agglutinating, sol-gel technology, chemical grafting, electrochemical methods, electrospinning, liquid phase deposition and hydrothermal method (Aziz-Sanjani and Mehdinia, 2014). Among these methods, electrospinning is a suitable method to produce nanofibers with controlled diameter, size, morphology and composition polarities and pores.

All soluble or fusible polymers, such as natural polymers, synthetic polymers, or a mixture of both, can be used in fiber production by electrospinning (Greiner and Wendorff, 2007; Islam et al., 2019). Electrospun nanofibers, apart from SPME (Zewe et al., 2010; Bagheri and Aghakhani, 2011) can be used in solid phase extraction (SPE) (Kang et al., 2007; Xu et al.; Bagheri and Aghakhani, 2011) semi-microsolid phase extraction, (Chigome et al., 2010; Bagheri and Aghakhani, 2011) and affinity membranes (Yoshimatsu, Ye and Lindberg, 2008).

In electrospinning method, a high-viscosity, high-molecular-weight polymer solution is produced from a small needle by applying high voltage with an electrospinning apparatus consisting of a spinneret, high-voltage source, low-voltage collector, and small-diameter needle capillary tube (Hussain et al., 2020). When the electric field reaches a critical value to overcome the surface tension of the drop, a Taylor cone is formed, and the polymeric nanofibers are ejected towards the conductive collector. As the solvent evaporates, the polymer solidifies and nanofiber mat is collected in nano- to micro-scale diameters (Teo and Ramakrishna, 2006; Bagheri et al., 2011). **Figure 2.4** shows the electrospinning main parameters effecting on the process (Rivero, et al., 2020).

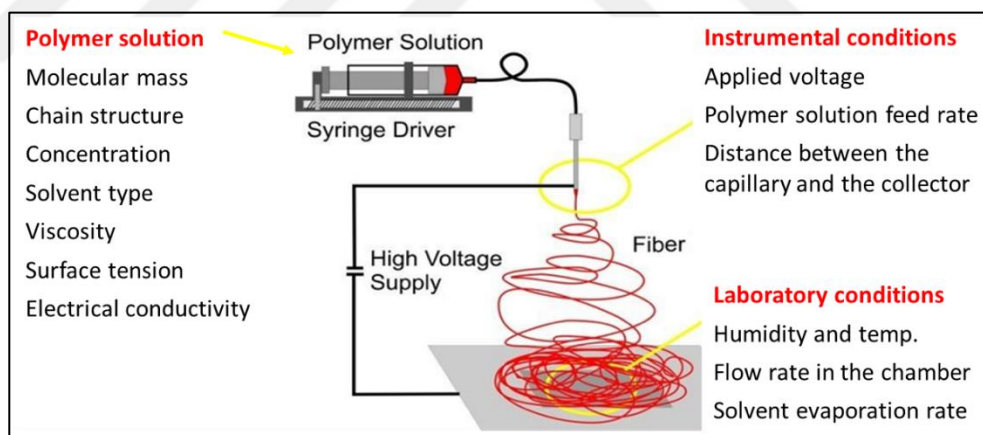


Figure 2.4 Electrospinning process and the main factors on the fiber production

2.8 Derivatization of UV filters

Some compounds contain polar and low volatility functional groups (OH, COOH, NH and SH), and some polar compounds have high affinity for active sites. Derivatization should be applied to increase the volatility and stability of such analytes (Wang et al., 2013).

Derivatization reactions can be carried out in both off-line and on-line mode. Off-line procedures are cumbersome, time-consuming, and harmful to humans and the environment. In addition, if the derivatization reagent is exposed to moisture, side reactions may occur (Wu et al., 2009). Since the DLLME technique is a miniaturized technique, a low derivatization reagent is sufficient. Derivatization techniques are examined under 4 main headings:

Derivatization before extraction: In this technique, derivatization is done in the sample solution before extraction. If the extraction rate is higher than the derivatization rate, the pre-derivatization technique is preferred instead of the simultaneous derivatization technique (Hu et al., 2014; Sajid et al., 2018).

Post-extraction derivatization: This technique is used when derivatization will adversely affect extraction and the derivatization reagent is more reactive to the components in the matrix than the analytes. This method has been used in many previous publications (Gonzalez et al., 2015; Tarazona et al., 2010; Zhang et al., 2012a; Cunha et al., 2015; Wu et al., 2015).

In-situ derivatization: It is a technique applied when extraction and derivatization are simultaneous, the sample matrix is clean and there are no interferences, and it has been applied in many previous studies (Fattahi et al., 2007; Xu et al., 2011; Chiang and Huang, 2008; Farajzadeh et al., 2012; Montes et al., 2009; Luo et al., 2010).

Most derivatization procedures involve offline coupling with microextractions. Such protocols are performed prior to analysis. However, in some cases, off-line derivatizations lead to experimental errors due to analyte loss through the evaporation, transfer, and resuspension steps. Besides, some of the derivatization reagents and resulting derivatives are very sensitive to water, so mixing moisture in the reaction system can cause some major problems.

In-port derivatization: This technique increases the thermostability and detectability of analytes and improves sensitivity, accuracy, and resolution. It is a fast, one-step on-line derivatization technique with low reagent consumption. Sample extract and derivatization reagent injected into the GC simultaneously, often called as sandwiches technique, or individually are derivatized in the injection block at high temperatures as reported in previous studies (Mudiam et al., 2014a and b; Marsol-Vall et al., 2017; Chung et al., 2015; Tsai et al., 2014).

2.9 The Aim of the Thesis

Nowadays, organic UV filters are becoming important due to their toxicity and endocrine disrupting potency in waters. The UVFs with NOEC $<0,01$ mg/L are in great concern and therefore, the main objective of the thesis is to develop a chromatographic determination method for some selected UV filters from each group (HMS, OC, EHMC, IAMC, 4-MBC, 3-BC, EHS, EDP, BP-1, BP-3) in water samples. No study was encountered published in Turkey and the outputs of the thesis we believe, will constitute the starting point for future studies in our country.

Recently, there has been a great interest in the development and application of environmentally friendly methods that provide a fast and sensitive method with high sensitivity and good recovery values, reducing the matrix effect to negligible levels. Gas chromatography is the method of choice but, the sample preparation step is also crucial for a selective and sensitive analysis since UV filters are usually at sub ppb concentration in environmental waters. Particularly in complex structures, a well-designed sample preparation step that allows traces of contaminants to be separated and pre-concentrated prior to measurement has a major impact on sensitivity and accuracy.

In this thesis, vortex assisted DLLME-SFOD method for the determination of UV filters has been developed for the first time in the literature. This environmentally friendly method consumes less solvent than conventional SPE and LLE methods. In addition, since non-toxic, low density and solidifying organic solvents were used in this method, it was much easier to separate the analytes from the water phase.

In addition to this green and effective microextraction method, chemometric approach was adopted in optimization step which provides more data with fewer trials to reduce the solvent consumption and waste amount. Chemometric data also gives information about the interactions between factors such as dispersive solvent type and volume, pH of sample, NaCl amount.

Considering polar nature of some of the target analytes (BP-1, BP-3, EHS and HMS), a derivatization step was also included in the procedure. Injection Port Silylation was chosen for a rapid and greener technique that allows low reagent consumption. Experimental parameters were optimized by Central Composite Design (CCD).

In the second part of the thesis, another green microextraction technique, solid phase microextraction (SPME) was studied. The performance of the commercial fiber (PA) was investigated and optimization of the SPME parameters namely sample volume and pH, ionic strength, extraction time and temperature, stirring rate and finally desorption time and temperature was optimized by using Experimental Design Methodology to evaluate the simultaneous effects of the experimental parameters. In validation studies, method parameters have been studied in wastewater samples.

In context of the thesis, the use of lab-made fibers for the SPME analysis was also attempted. Electrospinning technique was chosen since its capability to produce nanofibers with controlled diameter and composition desired for selectivity and sensitivity. Among the soluble polymers, polyacrylonitrile (PAN) which is a copolymer of acrylonitrile and vinyl acetate was chosen for the SPME fiber fabrication for UVF extraction. This co-polymer has high melting point and great carbon yield makes it hard and relatively insoluble in a wide range of solvents. Besides, it was obtained from waste textile scraps and this is another green aspect of the study. The performance of the fibers was investigated under headspace conditions and the results have been compared with those obtained with commercial PA fiber.

Consequently, development of a sensitive, accurate and reliable method for the analysis of UVFs in surface and wastewater samples will provide important information for future work.

3. MATERIALS AND METHODS

3.1 Materials and Reagents

All the reagents were of analytical grade. UV filters namely, 2-Ethylhexyl salicylate (EHS), 2-hydroxy-4-methoxybenzophenone (benzophenone-3, BP-3) > 98%, 3,3,5-trimethylcyclohexylsalicylate (Homosalate, HMS) >98%, 2-Ethylhexyl 2-cyano-3,3-diphenyl acrylate (octocrylene, OC) >98%, 4-Methyl benzylidene camphor (4-MBC) 99%, 2-ethyl-hexyl-4-trimethoxy cinnamate (EHMC) 99%, were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 3- Benzylidene camphor (3-BC) 99%, Isoamyl p-methoxycinnamate (IAMC) and 2,4- dihydroxy benzophenone (benzophenone-1, BP-1) 98% were acquired from Sigma Aldrich (St. Louis, USA), Toronto Research Chemicals (Toronto, Canada) and Chemservice (West Chester, USA) respectively.

LC-MS-grade methanol and acetonitrile, GC-MS grade ethyl acetate and acetone, sodium chloride, sodium sulfate, and orthophosphoric acid were purchased from Merck (Darmstadt, Germany). Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) from Macharey-Nagel to be 99:1 (v/v) was used as derivatization reagent. Extraction solvents; 2-dodecanol, 2-dodecanone, and 1-undecanol were obtained from Sigma.

Surface water and wastewater samples from local rivers were collected in amber glass bottles that were pre-rinsed with methanol, ultrapure water (UPW), acetone, hexane and kept in an oven at 250°C for at least 4 hours. Samples were transported immediately to the laboratory and were stored in the dark, at 4°C. The use of hand cream by the laboratory staff was avoided and gloves were used during analysis to prevent any UV filter contamination.

Commercially available 85 µm polyacrylate (PA) fiber for PAL autosampler was obtained from Agilent (Bellefonte, USA). The fiber was conditioned at 280°C for 25 min by inserting in GC-MS/MS injection port with carrier gas flow. Lab-made SPME fibers were coated with PAN and this material was obtained from waste textile products with the help of Assoc. Prof. Dr. Ahmet Çay from Ege University Textile Engineering Department.

3.2 Preparation of Standard Solutions

The individual stock solutions of UV Filters were dissolved in methanol (MeOH) at $1000 \mu\text{g mL}^{-1}$ and stored at -20°C . Standard solutions were prepared by using different solvents according to the nature of the method. Intermediate stock solutions of UVFs at $50 \mu\text{g mL}^{-1}$ and $1 \mu\text{g mL}^{-1}$ concentrations were prepared in methanol for the extractions studies namely DLLME-SFOD and SPME, and in ethylacetate for optimization of injection port derivatization (IPD) studies. Daily prepared mixed standard solutions of $100\text{--}500 \text{ ng L}^{-1}$ prepared in either methanol or ethylacetate were used in experimental studies. The calibration standards ($10\text{--}500 \text{ ng L}^{-1}$) and working solutions were prepared from mix standards at different concentrations by using environmental waters.

For the SPME studies, the calibration curves have been constructed diluting the concentrated MeOH solution with wastewater in the range of $20\text{--}500 \text{ ng L}^{-1}$ and $50\text{--}500 \text{ ng L}^{-1}$ for polyacrilate and paolyacrilonitrile fiber, respectively. All calibration solutions were obtained by appropriately diluting the concentrate solutions in 10 mL of wastewater samples. Since the methanol content changes the extraction efficiency, wastewater samples were spiked with methanol to obtain a constant methanol content of 0.5% (v/v).

3.3 Instrumentation

GC–MS/MS measurements have been performed by using an Agilent 7890B gas chromatograph coupled to a triple quadrupole mass spectrometer (MS 7000C) and a PAL autosampler (GC Sampler 80) located in Dokuz Eylül University Environmental Engineering Department. Separation was accomplished by using a 5% phenyl-arylene/ 95%-dimethylpolysiloxane HP-5MS ($30 \text{ m} \times 0.25 \text{ mm i.d.}$, 0.25 m film thickness) coloumn supplied by Phenomenex (Torrance, CA, USA). A design expert software (Trial Version 13.0 Stat-Ease Inc., Minneapolis, MN, USA) was used for statistical evaluation of the data. The operation parameters of the GC–MS/MS system in VA-DLLME-SFOD and SPME methods are given in **Table 3.1** and **Table 3.2** respectively.

Electrospinning studies were carried out in Ege University, Textile Engineering Faculty under supervision of Assoc. Prof. Dr. Ahmet Çay. The oven used in stabilization of lab-made electrospun fibers was Protherm PLO 450/50 PC442T and the instrument for coating fiber was NanoSpinner, NS Plus.

Table 3.1. Operational parameters of GC MS/MS used in determination of UV filters by VA-DLLME-SFOD technique.

Oven Temperature program			
Temperature (°C)		Ramp (°C min ⁻¹)	Hold time (min)
70			4
180		25	0
230		5	0
300		25	10
GC MS/MS parameters			
Ion Source Temperature	280°C	Column	Guard column, DB-5MS (30 m×0.25 mm I.D.×0.25 µm)
Interface Temperature	300°C	Injection Mode	Programmable Temperature Vaporizer (PTV)
Carrier gas flow rate	1.0 mL min ⁻¹	PTV	70°C (3 min) 260°C (250°C min ⁻¹)
Quadrupole Temperature	150°C	Purge off time	4.5 min
Derivatization	Sandwiches mode	Sample:BSTFA	2:1 (v/v)

Table 3.2. Operational parameters of GC MS/MS used in determination of UV filters by SPME technique

Oven Temperature programme			
Temperature (°C)		Ramp (°C min ⁻¹)	Hold time (min)
60		-	3
300		20	3
SPME Conditions			
Conditioning time and temperature		10 min, 87.6°C	
Extraction time and temperature		30 min, 87.6°C	
Extraction stirring rate		250 rpm	
Desorption time and temperature		4.7 min, 285°C	
GC MS/MS parameters			
Ion Source Temperature	280°C	Column type	Guard column, DB-5MS (30 m×0.25 mm I.D.×0.25 µm)
Interface Temperature	300°C	Injection Mode	Splitless
Carrier gas flow rate	1.0 mL min ⁻¹	Injection Temperature	285°C
Quadrupole Temperature	150°C	Purge off time	4.7 min

The individual solutions of UV filters in ethyl acetate were studied to obtain the parent ions of the UV filter with in-port derivatization in VA-DLLME-SFOD method and without derivatization in SPME method. The Total Ion Chromatograms (TICs) of the UV filters were obtained after scanning between 50 and 500 amu mass range in Full Scan mode at a concentration of 1 $\mu\text{g mL}^{-1}$. The parent and product ions, retention times (R_t) and collision energy (CE) values for the analysis of UV filters by DLLME-SFOD and SPME techniques are shown in **Table 3.3** and **Table 3.4**, respectively. In the spectra, the ions with high mass and high relative abundance were selected for quantification and indicated in bold figures. The MS system was routinely set in multiple reaction monitoring (MRM) mode and each analyte was quantified based on peak area using one target and qualifier ion(s).

Table 3.3. Parent and product ions of UVFs for the VA-DLLME-SFOD Method.

UV Filters	R_t (min)	Parent ions (m/z)	Product ions (m/z)	CE (mV)
3-BC	14.889	240	149.2 , 225.1, 92.1	5, 9, 12
EHS	14.821	195	177 , 159,75	15, 25, 30
HMS	15.848	195	177 ,159, 75	15, 25, 27
IAMC	15.948	178.1 , 161	161.1 , 133	15,10
4-MBC	16.358	254	239 , 105	15, 25
BP3	16.689	285	242	25
BP1	17.570	343	271	25
EDP	18.997	277 , 148, 165	164.9 , 104.2, 148.6	10, 30, 32
EHMC	19.451	161 , 178, 290	133.1 , 133.1, 178.1	8, 22, 6
OC	21.719	360 , 249	276 , 204	30, 22

Table 3.4. Parent and product ions of UVFs for the HS-SPME Method

UV Filters	R_t (min)	Parent ions (m/z)	Product ions (m/z)	CE (mV)
EHS	11.821	138	120 , 159,75	15, 25, 30
3-BC	12.582	240	149.2 , 225.1, 92.1	5, 9, 12
HMS	12.295	138	120 ,159, 75	15, 25, 27
IAMC	12.955	178.1 , 161	161.1 , 133	15,10
4-MBC	13.156	254	105 , 239	15, 25
BP3	13.001	227	184	25
EDP	14.002	165.2	164	10, 30, 32
EHMC	14.201	178.1	161.1	8, 22, 6
OC	15.719	248	165	30, 22

3.4 Preparation of Laboratory-made PAN Fibers by Electrospinning

A laboratory-made SPME fiber was fabricated by electrospinning the polymer onto a stainless-steel (SS) wire. Since the diameter of the wire is large for the GC injection block, it was treated with acid solution to thin out the wire chemically. Therefore, a stainless-steel wire was immersed about 1 cm in concentrated HCl solution at 50-60°C for 25 minutes. Then, the whole wire was immersed into solution for another 15 min. The wires were then rinsed with UPW and placed in a sonicator filled with saturated oxalic acid solutions for 10 min and then, cleaned with acetone for another 10 min.

The co-polymer, PAN was obtained from waste textile materials and dissolved in DMF to be 18% (w/v) by stirring for homogenization. Sufficient volume of the solution was withdrawn into a 5 mL syringe and located in a syringe pump. The wire was attached to a small electrical motor and electrospinning was performed. The electrospun fiber-coated SS wire was mounted inside the protective body of commercial fibers and attached to the screw holder.

The apparatus used for electrospinning was given in **Figure 3.1**. In each experiment, a single SS wire was connected to the protective body and a voltage of 9 kV was applied. A flow rate of 0.1 mL h⁻¹ was chosen for the prepared PAN solutions. The distance between the syringe tip and the collector wire was set to 10 cm, and the fibers were electrospun for 60 s to coat the fiber onto the wire. Close inspection of the surface was made by using Scanning Electron Microscopy (SEM) Zeiss Gemini Sigma 300VP.

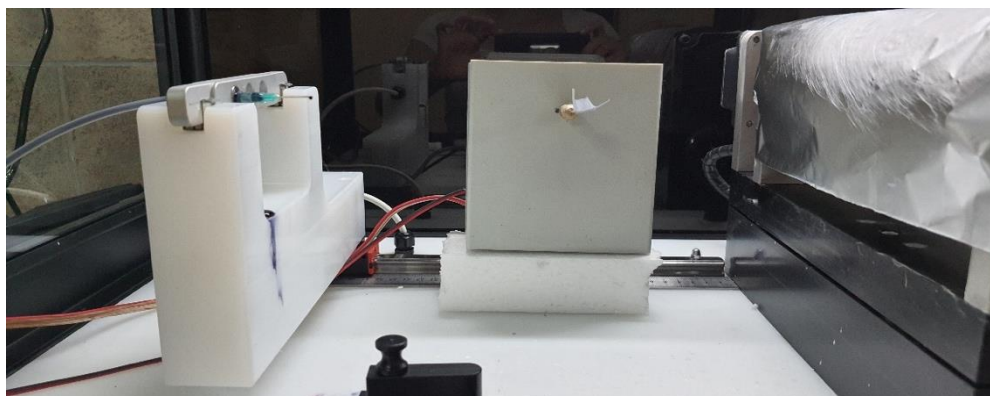


Figure 3.1 Apparatus used for fabrication of electrospun nanofiber-coated solid phase microextraction fibers.

The wires were then thermally treated for stabilization and the expected structure being formed was shown in **Figure 3.2**. The nitrile group is cleaved in the PAN nanofiber membrane and a C=C double bond is formed at high temperatures, with minimum volatilization of carbonaceous material. Thus, a heterocyclic structure is formed, and stability is increased in a range of 180-300°C (Clingerman et al., 2001; Paiva et al., 2003, from Rahaman et al.; 2007). Therefore, the fibers obtained were kept in oven set at 250°C for 5 hours for stabilization.

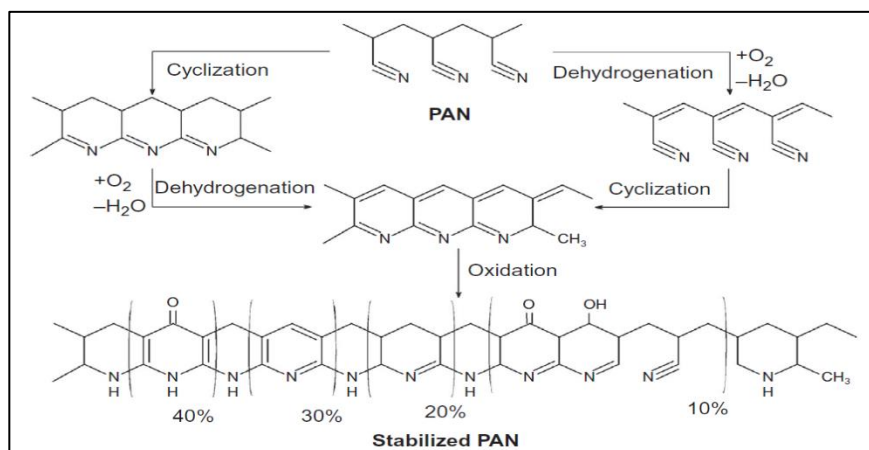


Figure 3.2 Plausible mechanism in the stabilization step of the PAN fiber (Rahaman et al., 2007)

3.5 Procedure of VA-DLLME-SFOD and SPME Method

In VA-DLLME-SFOD method, 10 mL of surface water or wastewater sample (pH 2.5) was placed in a 15 ml centrifuge tube and 1.0 g of sodium chloride was added as shown in **Figure 3.3**. A mixed solution of 2-dodecanone (30 μ L) and acetone (460 μ L) were pipetted into the sample by pressing firmly to form a cloudy solution. The solution was vortexed for 3 minutes and then, it was centrifuged at 4000 rpm for 5 minutes.

After cooling at -20°C for 7 min, aqueous phase was decanted with a glass pasteur pipette, and the remaining frozen organic solvent was allowed to melt at room temperature. About 25 μ L of the organic phase was taken into a 150 μ L insert and small amount of sodium sulfate was added to remove the water content. After the insert was centrifuged at 4000 rpm for 5 minutes, organic phase was transferred to another 150 μ L conical vial with a micro syringe. 2.0 μ L of this solution were injected into the GC-MS/MS injection port along with 1.0 μ L BSTFA: TCMS (99:1) in sandwich mode to carry out derivatization.

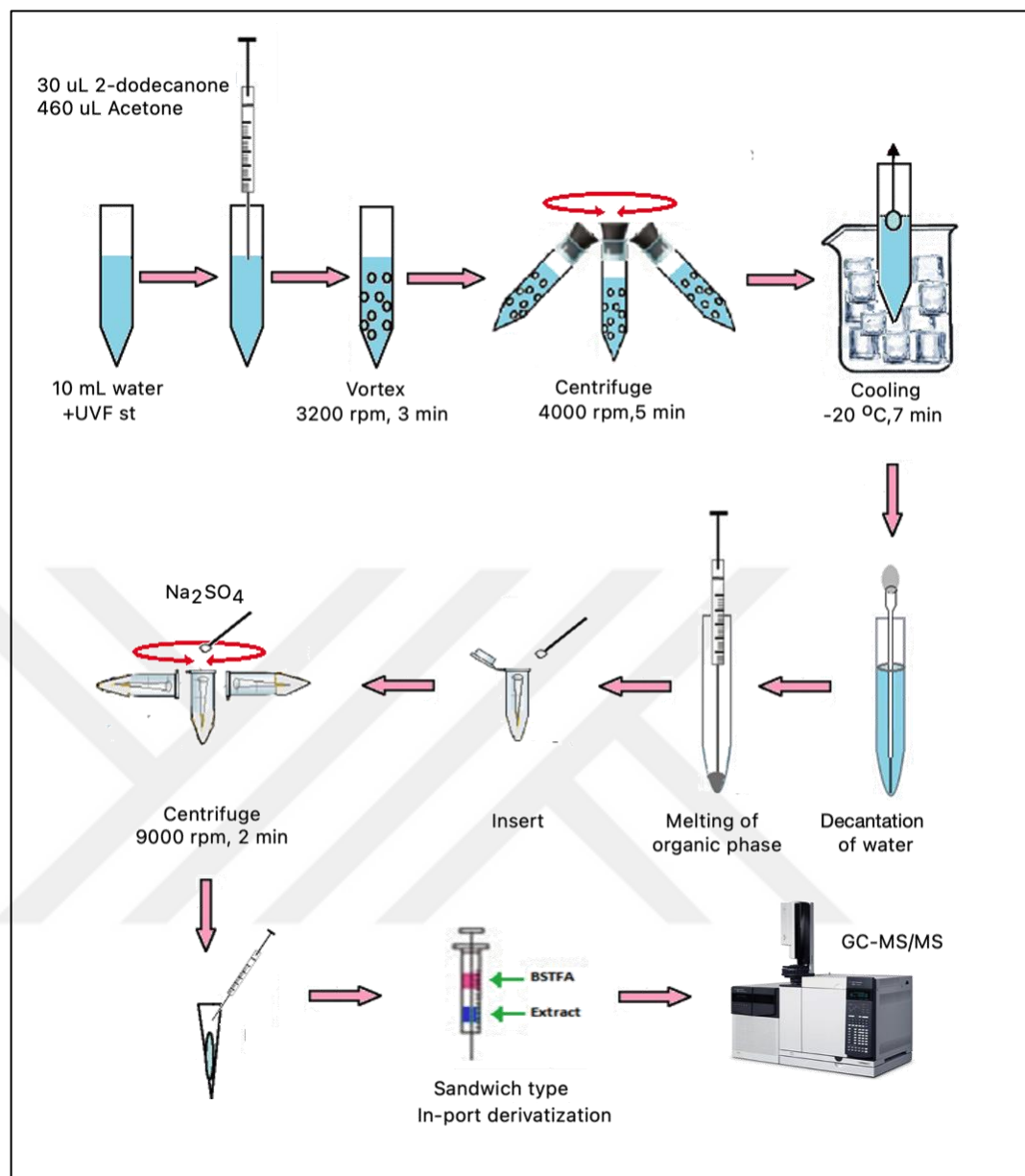


Figure 3.3 VA-DLLME-SFOD method for extraction of UV filters

Solid phase microextraction of the UV filters with PA fiber was carried out in the PAL automated system. Lab-made PAN fiber was used manually at conditions optimized for PA. Fibers were pre-conditioned in the injection block, introduced into the vial containing 10 mL of sample placed in a 20 mL magnetic cap glass bottle. The fiber was exposed to the headspace of sample solutions at 87.6°C for 30 min. The solution was previously equilibrated at the same temperature for 10 min. Desorption of the analytes was carried out in the injection-port of GC-MS/MS at 285°C for 4.7 min as illustrated in **Figure 3.4**. To avoid carry out effect, the fiber was immersed into empty vial between each extraction.

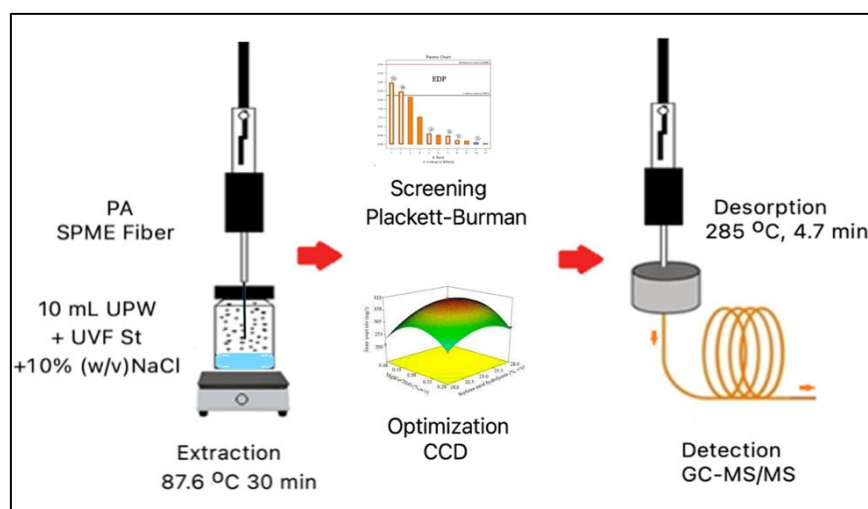


Figure 3.4 Schematic illustration of HS-SPME-GC-MS/MS method applied for UV filters

3.6 Optimization of VA-DLLME-SFOD and SPME Method

The design of experiment (DoE) is usually carried out in three stages:

- screening (identification of influential factors),
- optimization (definition of optimal conditions),
- validation of the model (validation of predictions). (Ferreira et al., 2007).

It is widely used for determining the factors that affect the results obtained in a test method, the interactions between these factors, and the intensity of impact on the response (Leardi et al., 2009; Mousavi et al., 2018). As a result of well-designed experimental conditions, time and cost are saved. Many articles have been published on the application of this technique in determination of UV filters in waters as shown in **Table 2.6**.

The Plackett–Burman design (PBD) type is a two-level fractional factorial scanning design in which the effects of the main factors are examined and the interactions between the factors are not considered.

The most attractive aspect of this design is that it is cost-effective when applied in a method such as SPME, where the efficiency of the fiber depends on the number of samples analysed. In this study PBD was used to investigate the significant factors for determination of UV filters with SPME method.

The central composite design (CCD) is one of the most recognised response surface designs for the analysis of UV filters (Chung et al., 2015; G. Basaglia and Pietrogrande, 2012; Silva et al., 2017; Wu et al., 2013). Star points are located $\pm k$ from the center of the experimental field at 0. Considering that studying the axial points at different intervals would cause experimental complexity, the distance of the axial points was chosen as ± 1 in Central Composite Face Centered Design (CCFCD) (Najafi et al., 2012, Maran et al., 2017).

In the present thesis, one factor at a time (OFAT) and central composite design methods have been used. For VA-DLLME-SFOD methods CCFCD has been used and the effects of four factors including volume of extraction and dispersive solvents, the amount of sodium chloride and vortex time by CCFCD.

The range of factors were selected as 30-110 mL, 200-600 mL, 0-10% and 1-6 minutes, respectively. A total of 46 experiments including 16 factorial points ($n=2$), eight-star points ($n=1$) and 6 center points were conducted according to following equation. In **Equation 3.1**, the total number of experiments the number of factors and the number of center points are represented as terms of N , n and c , respectively.

$$N = 2^n + 2n + n_c \quad \text{Eq. 3.1}$$

A quadratic polynomial equation including four factors, their squares, and two-factor interactions was used to develop an empirical model that correlates responses to independent variables. The dependence of each experimental response, y , on factors was calculated by the following **Equation 3.2**. In this equation β_0 is the constant term, β_i , β_{ii} and β_{ij} are the coefficients, ε is the error, x_i and x_j are the variables and n is the number of variables (Mangili 2015; Maran, 2017).

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum \sum_{< j=2}^k \beta_{ij} X_i X_j + e_i \quad \text{Eq. 3.2}$$

3.7 Validation Studies

In validation, method parameters are examined to determine that the method complies with the necessary principles. Non-standard methods, methods developed in the laboratory, standard methods used outside of their intended purpose, or modified standard methods must be validated in accordance with section 5.4.5.2 of ISO/IEC 17025.

In the present thesis, the linear range, LOD (Limit of Detection), LOQ (Limit of Detection), recovery percentages and precision as intra-day, inter-day repeatability along with the selectivity of the method developed were investigated.

Linear range is the range of concentrations where the signals are directly proportional to the concentration of the analyte in the sample. It is often confused with dynamic range which is the range of standard solution concentrations used in calibration studies. Linear range is started with the **LOQ** which is the concentration level at which the analyte can be quantified with acceptable precision and accuracy.

LOD is the lowest concentration at which the analyte can be observed without being within quantitative limits. Various approaches are possible to determine the LOD of the applied method. Generally, the following approaches are used based on

- Signal-to-Noise Ratio
- Standard Deviation of the Response and the Slope
- Standard deviation of sample spiked with minimum concentration of analyte

Signal-to-Noise Ratio: This approach is applicable only to analytical procedures that exhibit baseline noise and is determined by comparing the measured signals obtained for low concentrations of the analyte to those in blank samples. A signal-to-noise ratio (S/N) of 3:1 is considered the LOD, while S/N of 10:1 considered the LOQ values.

Standard Deviation of the Response and the Slope: LOD and LOQ values were calculated as $3.3 \times (\sigma/s)$ and $10 \times (\sigma/s)$, respectively. In this equation σ is the standard deviation of y-intercept, s is the slope of the calibration curve. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Standard deviation of sample spiked with minimum concentration of analyte: LOD and LOQ values were calculated as $3 \times SD$ and $10 \times SD$, respectively. In this equation SD is the standard deviations of UV filters.

Accuracy is one of the most critical parameters in method validation. This term confirms the suitability of method to the accurate quantification of analyte in presence of sample matrix. Accuracy, according to the ISO 17025 comprises two components: trueness and precision.

Trueness is assessed against a reference value of the certified value of a CRM, or against a reference method or in a more practical way, it can be assessed in large concentration ranges with recovery studies.

Recovery is defined as the proportion of the amount of analyte, present or added to the analytical portion of test material, which is extracted and presented for measurement. In matrix match studies, mixed standard was added to the real sample at a concentration of low, medium, and high points of calibration curve and the method is applied for subsequent days.

In the SPME studies, Recovery values of UV filters were calculated as **Eq. 3.3** where $C_{preSPME}$ is the concentration of wastewater in which analytes was added before the extraction, C_{std} is the concentration of analytes which was added and C_{sample} is the concentration of UV filters in wastewater without the addition of analyte.

$$Recovery \% = \frac{C_{preSPME} - C_{sample}}{C_{std}} \times 100 \quad \text{Eq. 3.3}$$

Precision is usually defined as the closeness of a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Repeatability and Reproducibility are the two components of precision in a measurement system. Repeatability (intra-day) and intermediate precision (inter-day) values were calculated by ANOVA. Intra-day and inter-day repeatability studies are usually performed at low, medium, and high concentrations of the calibration curve with 3 replicates.

The standard deviation of repeatability (S_r), also called as within-group standard deviation, is calculated by taking the square root of the within-group mean square term (MS_w) as shown in **Eq. 3.4** and the contribution of the grouping factor to the total variation can be calculated by using the square root of the between-group mean square term (MS_b) **Eq. 3.5**. Then intermediate precision (S_I) can be determined by combining the within-group and between-group variance ($S_{between}$) components as shown in **Eq. 3.6**.

$$S_r = \sqrt{MS_w} \quad \text{Eq. 3.4}$$

$$S_{between} = \sqrt{\frac{MS_b - MS_w}{n}} \quad \text{Eq. 3.5}$$

$$S_I = \sqrt{S_r^2 + S_{between}^2} \quad \text{Eq.3.6}$$

The % RSD values are calculated as given in **Eq. 3.7**, where σ is the standard deviation and, $\bar{x}_{\text{mean}}$ is the mean of the response.

$$\text{RSD}\% = \sigma / \bar{x}_{\text{mean}} \times 100 \quad \text{Eq. 3.7}$$



4. RESULTS AND DISCUSSION

Initial studies comprise the optimization of collision energy and selecting the parent and product ions for qualification and quantification of selected UV Filters.

In the second part of the study, the use of VA-DLLME-SFOD technique prior to the GC-MS/MS detection was investigated. Derivatization conditions were also optimized.

The last part of the thesis includes the use of commercial and lab-made SPME fibers in headspace analysis of the UVFs. Optimization and validation studies have been carried out in wastewater samples.

4.1 Optimization of GC-MS/MS Parameters

In the analysis of UV filters in environmental waters, GC-MS/MS system was used in MRM mode. For more precise identification of UV filters, the most selective and abundant ion of each compound should be selected in full scan mode in the MS/MS system. After the main ions were selected, the product ions formed by the fragmentation of the main ions in the production ion mode were determined. The collision energy (CE) in the chemical ionization system is a critical parameter that is often optimized to increase the fragment ion density in GC-MS/MS system. Although high CE values result in greater fragmentation, extremely high or low levels of this energy can greatly reduce the sensitivity of the MRM method. For this reason, suitable product ions were determined by applying a collision energy of 5-40 eV to the target UV filters in the GC-MS/MS system as given in **Table 4.1**.

Another important point is that four of the UV filters, BP-1, BP-3, EHS and HMS, are polar in nature due to their -OH groups and may interact with aqueous solution through strong hydrogen bonds. Their low volatility results in poor chromatographic peaks. Besides, these -OH groups may interact with silanol groups in the chromatographic system and/or with sample residues in the injector or column resulting in peak loss or tailed peak. Therefore, a derivatization procedure is applied for these analytes and their parent and product ions are changed accordingly. After derivatization, base peak forms only $[B]^+$ ion and molecular ion $[M]^+$ is formed as well. Silylated $[M]^+$ ion and TMS^+ ion (m/z : 73) were obtained from all silylated compounds.

Peak base ions of $[M-CH_3]^+$ (BP-3, m/z: 285; BP-1, m/z :343) of the UV filters were obtained. The quantification ion (base peak) at m/z: 195 for both EHS and HMS was attributed to the loss of OC_8H_{17} and OC_9H_{17} from the TMS-derivatives, respectively.

Table 4.1 The peak areas of UV filters with 3 different CE values

UVFs	Parent ion	Product ion (m/z)	Peak Area	CE (eV)	UVFs	Parent ion (m/z)	Product ion	Peak Area	CE (eV)
BP-3	343	271	87862	30	4-MBC	254	105	81748	27
	343	271	88635	28		254	105	84658	25
	343	271	85893	26		254	105	72589	20
BP1	284.9	242	379610	30		254	239	71258	20
	284.9	242	418735	25		254	239	73256	15
	284.9	242	369926	20		254	239	69970	10
HMS	194.9	177	98109	17	EHMC	178	133.1	209962	20
	194.9	177	106072	15		178	133.1	232695	22
	194.9	177	104232	13		178	133.1	216474	25
	194.9	159	70270	22		161	133.1	193870	5
	194.9	159	75034	25		161	133.1	283966	8
	194.9	159	63295	27		161	133.1	236024	10
	194.9	74.9	88756	25		290.1	178.1	62131	5
	194.9	74.9	94485	27		290.1	178.1	65815	6
	194.9	74.9	76029	30		290.1	178.1	64018	10
EHS	194.9	177	98109	17	3-BC	240	225	70886	12
	194.9	177	106072	15		240	225	73780	9
	194.9	177	104232	13		240	225	71086	7
	194.9	159	74105	27		240	92.1	119645	15
	194.9	159	75034	25		240	92.1	120300	12
	194.9	159	70270	22		240	92.1	112104	10
	194.9	74.9	94485	27	EDP	161	133.1	96619	15
	194.9	74.9	88756	30		161	133.1	130237	10
	194.9	74.9	76029	22		161	133.1	121007	7
OC	249	204	46741	34		277	164.9	504658	17
	249	204	48428	30		277	164.9	534961	15
	249	204	48227	28		277	164.9	544291	10
	165	148.6	105183	25		148	104.2	105700	27
	165	148.6	119851	22		148	104.2	111234	30
	165	148.6	116226	23		148	104.2	110872	32
IAMC	178	161	98460	17					
	178	161	106843	15					
	178	161	97361	12					

The $[M]^+$ ion, which is fragmented by applying a certain collision energy, loses 58 for BP-3 and forms 242 ions, for BP-1 it loses 87 ions to form 227 ions. The parent ions were shown in **Supplementary Section (Figure A1-A9)**

As shown in **Table 4.1**, the peak areas with 3 different CE values were given. Among the product ions used for the UV filters, the ions with the highest peak area were used as the quantifier ion, and the other ions with a relatively lower peak area were used as the qualifying ion. A product ion with sufficient intensity value for BP-1 and BP-3 analytes could not be detected.

4.2 Development and Applications of VA-DLLME-SFOD Method

4.2.1 Optimization Studies

There are several parameters affecting the efficiency of the VA-DLLME-SFOD method for the determination of UV filters in surface and wastewaters. Initially, the type of dispersive solvent, the pH of the sample and the mixing type were determined by General Factorial Design. Then, the volume of dispersive solvent, volume of extraction solvent, ionic strength of the sample and vortex time applied to the sample were optimized using CCFC.

Considering the polar nature of some of the UV filters, their strong interaction with aqueous solution will prevent their volatilization and therefore, their derivatization by using an appropriate reagent is necessary. In the present study, silylation of these UV filters was accomplished by using 2 μ L BSTFA: TMCS mixture (99:1). In-port derivatization is the method of choice since it is a fast and one-step technique and requires low volume of reagent. Therefore, derivatization reaction was placed in the injector port of GC-MS/MS by using the PTV mode.

The choice of extraction and dispersive solvent type: Extraction solvent type is a crucial parameter in optimization study of VA-DLLME-SFOD method. The solvent was chosen among solvents with a low melting point (10-30°C) and a lower density than water.

Organic solvents having similar K_{ow} values with the UV filters were considered and the performance of 1-undecanol, 2-dodecanol and 2-dodecanone were tested by using General Factorial Design.

Here, another solvent, i.e., dispersive solvent must be miscible with both the extraction solvent and the aqueous solution. The role of this solvent is to disperse the extraction solvent in the aqueous sample as small droplets to form a cloudy solution and increase the surface contact of the analytes with the extraction solvent. Increasing the surface contact is known to enhance the extraction efficiency.

In this study, the effects of methanol (MeOH), acetonitrile (AcN), and acetone (AC), which were the most widely used as dispersive solvents, on the extraction efficiency were investigated. For this purpose, 10 mL ultrapure water (UPW) having $\text{pH } 6.3 \pm 0.2$ was spiked with 50 μL of $100 \mu\text{g L}^{-1}$ standard solution to obtain a solution of 500 ng L^{-1} UV filter. Extraction solvent (100 μL) and dispersive solvent (400 μL) mixture was added to solution with 1 mL microsyringe. Their volumes were signified as V_{ext} and V_{disp} , respectively. After centrifuging at 4000 rpm for 3 min, the mixture was cooled at -20°C for 7 min and then, allowed to melt at room temperature. 2.0 μL of the extract was collected and subjected to in-port derivatization using 2.0 μL BSTFA. In PTV injection mode, inlet initial temperature and purge off time were 240°C and 4 minutes, respectively.

General factorial design with 3 replicates was used to select dispersive and extraction solvent which significantly affect the peak area. **Table 4.2** depicts the variation of UV filter peak areas in GC-MS/MS with corresponding solvents. The results indicated that the highest peak areas for all the types of UV filters can be obtained when 2-dodecanone-acetone solvents were used probably due to the lower polarity of 2-dodecanone compared to other ones.

Analysis of variance (ANOVA) supports these data with the p -values ($p < 0.05$) about the significance of extraction solvent (except for IAMC) and dispersive solvent (except for EHS, HMS, IAMC, BP-1) as given in **Table 4.3**.

Overall results were illustrated in **Figure 4.1**. The K_{ow} values of the extraction solvents are 4.72, 4.76 and 4.99 for 1-undecanol, 2-dodecanol, 2-dodecanone, respectively. The polarity of 2-dodecanone is lower compared to other solvents. This may explain the highest peak area values were obtained for all UV filters when this solvent was used. Further studies was conducted with 2-dodecanone.

Table 4.2 General factorial design model and the peak area values of the UV filters

Extraction solvent	Dispersive solvent	3-BC	EHS	HMS	IAMC	4-MBC	BP-3	BP-1	EDP	EHMC	OC
1-undecanol	MeOH	1558	3877	5930	7952	1149	475	126	5279	6117	1069
2-Dodecanone	AC	4327	59140	38161	19833	3462	31241	10159	32468	28956	6570
2-dodecanol	AC	1680	1797	922	13153	1256	1158	477	10424	6436	1482
2-Dodecanone	AC	4236	50991	33590	19831	3329	33304	11381	25049	24969	4679
2-dodecanol	MeOH	1678	7084	1681	11806	1111	2392	1103	9017	6031	1434
1-undecanol	AcN	1323	5302	723	7417	932	640	199	6604	7023	1701
2-Dodecanone	MeOH	2632	46935	29438	10700	2180	25497	9483	16546	14349	3294
2-Dodecanone	AC	3381	42950	27299	15554	2681	27265	9607	22731	24255	3786
1-undecanol	AC	3100	4205	831	18632	2600	12492	485	7700	16966	4728
2-Dodecanone	MeOH	2887	33850	19268	15106	2132	19662	6617	17895	9974	3408
1-undecanol	MeOH	1461	6175	774	8118	1169	909	327	6302	6774	1567
1-undecanol	AC	1577	5164	562	7930	1250	855	344	7655	8801	2419
2-dodecanol	AC	1394	1863	912	10185	1030	1100	560	8256	5849	1536
2-dodecanol	MeOH	2018	2067	1123	14695	1464	1680	745	11030	6594	1350
2-Dodecanone	AcN	3003	55149	35223	11278	2463	3160	11625	19692	11290	3235
2-dodecanol	AcN	1149	5907	1372	8055	903	1237	498	7709	5210	1211
1-undecanol	AcN	2191	1395	571	25074	1336	1143	244	10532	22155	5153
1-undecanol	AC	2520	4300	591	14181	1970	1081	283	10740	14341	3423

Table 4.2 Continue

Extraction solvent	Dispersive Solvent	3-BC	EHS	HMS	IAMC	4-MBC	BP-3	BP-1	EDP	EHMC	OC
2-dodecanol	AC	886	4317	925	6415	588	607	185	7307	4942	1365
1-undecanol	AcN	1548	1354	506	9322	1163	1126	513	6060	7499	1359
2-Dodecanone	AcN	1361	23779	12662	8233	1111	7040	2568	11702	6902	2589
2-dodecanol	AcN	1849	7329	1834	13398	1472	1965	906	11694	8348	1999
2-dodecanol	AcN	2022	7563	1904	14998	1523	2651	1111	15957	11045	2670
2-Dodecanone	MeOH	1860	10329	5665	10870	1415	6865	2294	10946	6265	2295
2-Dodecanone	AcN	1826	23365	12708	12708	1122	1361	10154	4169	13738	3094
1-undecanol	MeOH	1877	5176	682	9387	1318	845	330	5180	7591	1597
2-dodecanol	MeOH	1593	4663	880	10956	1097	1048	471	10339	7284	1567

Table 4.3 ANOVA data for the VA-DLLME-SFOD-GC-MS/MS Method

Factors	3BC					EHS				
	SS	df	MS	F	p	SS	Df	MS	F	p
A*	7.83E+006	2	3.91E+006	14.16	0.0002	6.78E+12	2	3.39E+12	38.07	< 0.0001
B	3.25E+006	2	1.62E+006	5.88	0.0114	1.80E+11	2	8.98E+10	1.10	0.3854
AB	4.61E+006	4	1.15E+006	4.17	0.0156	5.79E+11	4	1.45E+11	1.63	0.2137
	HMS					IAMC				
	SS	df	MS	F	p	SS	df	MS	F	p
A	2.99E+12	2	1.49E+12	36.22	< 0.0001	5.65E+10	2	2.82E+10	3.14	0.0692
B	1.03E+11	2	5.17E+10	1.25	0.3108	6.09E+10	2	3.05E+10	3.39	0.0578
AB	2.94E+11	4	7.36E+10	1.78	0.1788	9.95E+10	4	2.49E+10	2.77	0.0613
	4-MBC					BP-3				
	SS	df	MS	F	p	SS	df	MS	F	p
A	5.38E+09	2	2.69E+09	13.32	0.0003	1.44E+12	2	7.21E+11	39.77	< 0.0001
B	2.43E+09	2	1.22E+09	6.02	0.0105	4.56E+11	2	2.28E+11	12.57	0.0004
AB	3.18E+09	4	7.96E+08	3.94	0.0193	6.50E+11	4	1.62E+11	8.96	0.0004
	BP-1					EDP				
	SS	df	MS	F	p	SS	df	MS	F	p
A	3.49E+11	2	1.74E+11	39.16	< 0.0001	5.64E+11	2	2.82E+11	19.34	< 0.0001
B	7.99E+09	2	4.00E+09	0.90	0.4258	1.27E+11	2	6.34E+10	4.35	0.0299
AB	1.95E+10	4	4.87E+09	1.10	0.3905	2.71E+11	4	6.78E+10	4.65	0.0102
	EHMC					OC				
	SS	df	MS	F	p	SS	df	MS	F	p
A	3.70E+11	2	1.85E+11	25.28	< 0.0001	1.98E+10	2	9.89E+09	12.88	0.0002
B	2.73E+11	2	1.37E+11	18.66	< 0.0001	9.65E+09	2	4.83E+09	6.29	0.0072
AB	3.02E+11	4	7.54E+10	10.30	0.0002					

*A: Extraction solvent type, B: Dispersive solvent type, AB: Interaction of extraction and dispersive solvents.

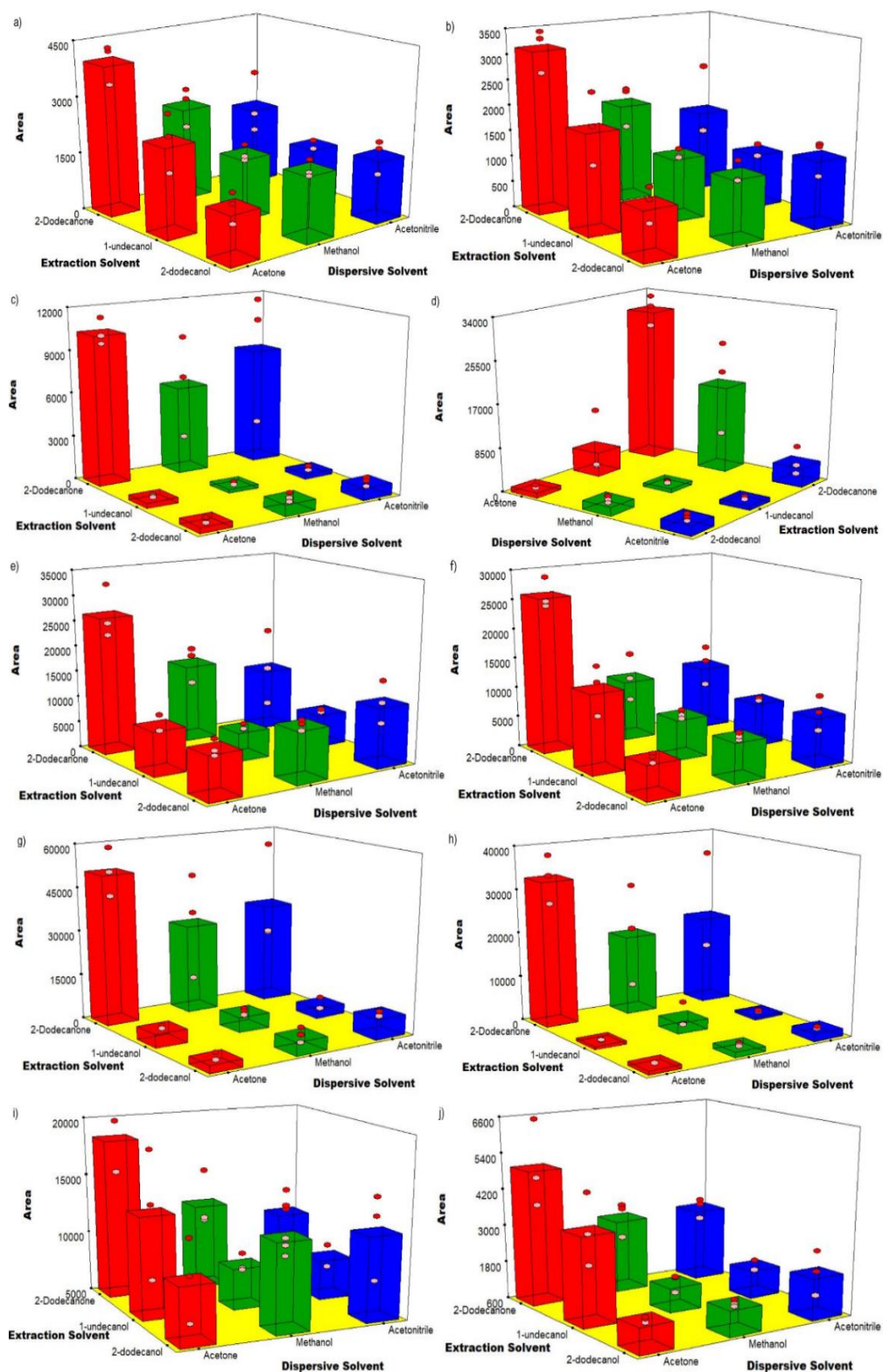


Figure 4.1 General factorial design of extraction and dispersive solvent type.

(a) 3-BC, (b) 4-MBC (c) BP-1 (d) BP-3 (e) EDP (f) EHMC (g) EHS (h) HMS (i) IAMC (j) OC.

UPW volume: 10 mL UPW (pH: 6.63), UVFs: 500 ng L⁻¹; V_{ext}: 100 µL, V_{disp}: 400 µL.

Dispersing solvent has also critical role by maintaining a cloudy solution with high contact area for efficient transfer of the analyte from aqueous solution to the organic solvent drop and providing an appropriate medium for further reactions. When MeOH is used as the dispersive solvent, poorly resolved chromatographic peaks were observed for polar analytes probably due to their high interaction with water. Another disadvantage of methanol lies in the further reaction. The role of the derivatization reagent is to bind to the -OH bonds, reducing the polarity of the analyte and thereby increasing its volatility. However, this reagent also interacts with MeOH and thus, derivatization reaction is hindered.

Acetonitrile, on the other hand, not properly dispersed the extracting solvents in aqueous solution probably due to the low solubility of 2-dodecanol and 1-undecanol in this solvent. Another solvent, 1-undecanol has a low freezing point and therefore, melts quickly leading the loss of the analytes. Among the solvents tested, acetone has given the best results by providing a good phase separation and higher droplet density compared to other solvents.

The effect of pH: It is well known that in the extraction of weak organic acids, solution pH might play a role on the resulting peak areas. In previous studies, the highest recoveries of UV filters were reported at different pHs such as 2.5 (Tsiu et al., 2014), 3.0 (Cunha et al., 2015), 4.0 (Zhang and Lee, 2012b, 2012c) and 7.0 (Xue et al., 2013; Ku et al., 2013).

Considering that acidity constants of UV filters studied varies in a range of 7.56 - 8.13, the effect of pH on the extraction efficiency was examined by using pH 2.5, 4.0 and 7.0 media by adding 0.1 M phosphoric acid into 10 mL of UPW. The solution was spiked with 500 ng L⁻¹ of UV filter standard solution and pre-mixed solvents i.e., 100 µL of 2-dodecanone and 400 µL of acetone were added to this solution. Cloudy solution was formed and after centrifuging, it was cooled down and melted again at room temperature as described above. In derivatization step, 2 µL of the final solution along with 2 µL of BSTFA were injected into GC-MS/MS injection port at 240°C (inlet temperature) for 4 min (purge-off). Overall results obtained were given in **Table 4.4** for three pHs.

The best peak formation was obtained in pH 2.5 as shown in **Figure 4.2**. Considering the pK_a values, the analytes are in their neutral form in highly acidic media, which increases the transfer of analytes to the organic phase and this pH was used for further studies.

Table 4.4 The peak areas obtained for 500 ng L⁻¹ UV filters at three different pHs

pH	3BC	EHS	HMS	IAMC	4-MBC	BP3	BP1	EDP	EHMC	OC
2.5	6034	126767	80098	41232	5567	66401	28669	73607	39504	14778
	6806	129563	80580	44834	6329	66091	27040	72263	37848	13947
	6527	121593	74573	46282	6289	61179	25553	74262	40846	15057
	7120	162426	104431	49045	6854	70144	29194	84615	47283	29701
	6677	156832	104848	43519	6226	78474	34545	76924	46282	22893
	6491	159235	106538	42761	5749	96527	43604	69871	36848	13199
4	5626	112565	69309	34325	5236	72248	30964	62351	31956	11406
	5242	106322	67176	35094	4845	47987	30964	57522	31896	10351
	5457	93403	58585	39284	5034	49204	19525	58313	34366	10869
	5620	108081	65072	39104	5181	50307	22091	61932	36126	10343
	4699	105023	67222	38654	4625	59628	25567	58671	33107	9659
	5581	93531	56801	39716	5073	45908	19885	56993	34425	10837
7	3829	52252	30729	19678	3460	33585	15319	30333	13207	6866
	4647	62528	37917	27567	3947	43229	20620	36006	16964	5801
	4458	74492	47063	28976	4011	47802	23279	35214	16984	7984
	5822	86886	57377	37759	4947	71481	30951	41836	20708	7367
	5218	68140	40742	32577	4507	47848	21675	39954	18135	6592
	5239	86861	56188	32681	4356	60429	27880	36491	18044	6069

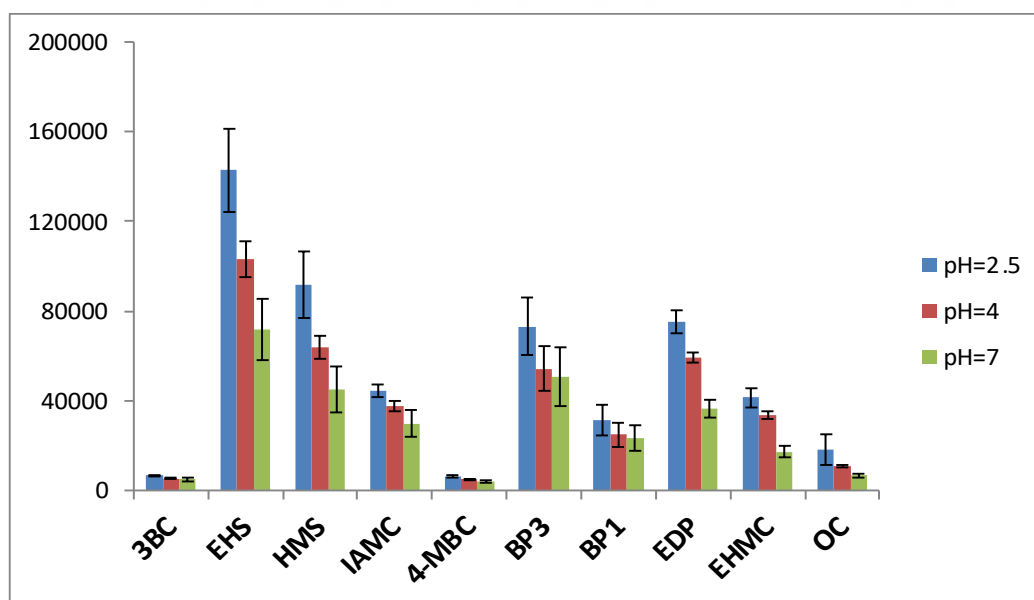


Fig. 4.2 The peak area values obtained for each UV filter at three different pHs (2.5, 4.0 and 7.0).
UPW volume: 10 mL, analyte concentration: 500 ng L⁻¹; 2-dodecanone: 100 μ L, acetone: 400 μ L.

The effect of mixing type on the extraction efficiency: In extractions proceed in a liquid phase, high extraction efficiency can be achieved by mixing the solutions well enough to ensure the interaction of analyte with organic solvent. Different modes of interactions such as Ultrasound-Assisted (USA-DLLME) (Zhang and Lee, 2012b; Wu et al., 2013; Xue et al., 2013), Vortex Assisted (VA-DLLME) (Zhang and Lee, 2012c), Microwave assisted (MWA-DLLME) (Li et al., 2017), Magnetic Stirring Assisted (MSA-DLLME) (Zhang et al., 2011), Air assisted (AA) LPME (Ge et al., 2018) have been developed so far.

The effects of sonication and vortex modes on the extraction efficiency were investigated to increase the transition of UV filters from the aqueous phase to the organic phase in the DLLME method. 10.0 mL of UPW spiked with UVF standards to be 500 ng L⁻¹ was mixed with 400 µL acetone and 100 µL 2-dodecanone mixture by using either a vortex set at 3200 rpm for 5 minutes or an ultrasonic bath set at 35 kHz for 5 minutes. The same procedure was repeated without any mixing step to reveal the difference. After centrifuging and cooling steps, 2 µL of the final solution was injected into GC-MS/MS injection port along with 2 µL of BSTFA. Injection temperature is 240°C and purge off time is 4 min. **Table 4.5** depicts the variation of UV filter peak areas in GC-MS/MS with mixing types. The highest efficiency was obtained with the VA-DLLME-SFOD procedure compared to the UA-DLLME-SFOD and DLLME-SFOD methods as shown in **Figure 4.3**.

Table 4.5 The peak areas of UVFs obtained with DLLME-SFOD-GC-MS/MS method by mixing with VA, UA and without mixing mode.

Mixing type	Peak Area									
	3BC	EHS	HMS	IAMC	4-MBC	BP3	BP1	EDP	EHMC	OC
VA	6046	120525	77405	55187	7387	77695	27758	95353	62382	21340
	7478	157435	118389	50208	7468	101881	37447	100808	61576	20141
	6064	117277	73652	62391	8065	136582	39425	109978	64576	21539
UA	7199	166728	107928	45544	6871	111308	36996	90041	50327	17001
	6305	110293	68578	42697	5885	72476	24172	77332	45290	14599
	6291	85071	50573	50742	5857	46621	18561	77237	50347	15331
None	5579	142626	92014	38674	5380	87439	27607	71256	41114	14084
	5279	137833	86413	36125	5167	84950	26563	66402	37839	13622
	4410	108608	54566	35155	3999	63013	24214	52052	33076	9682

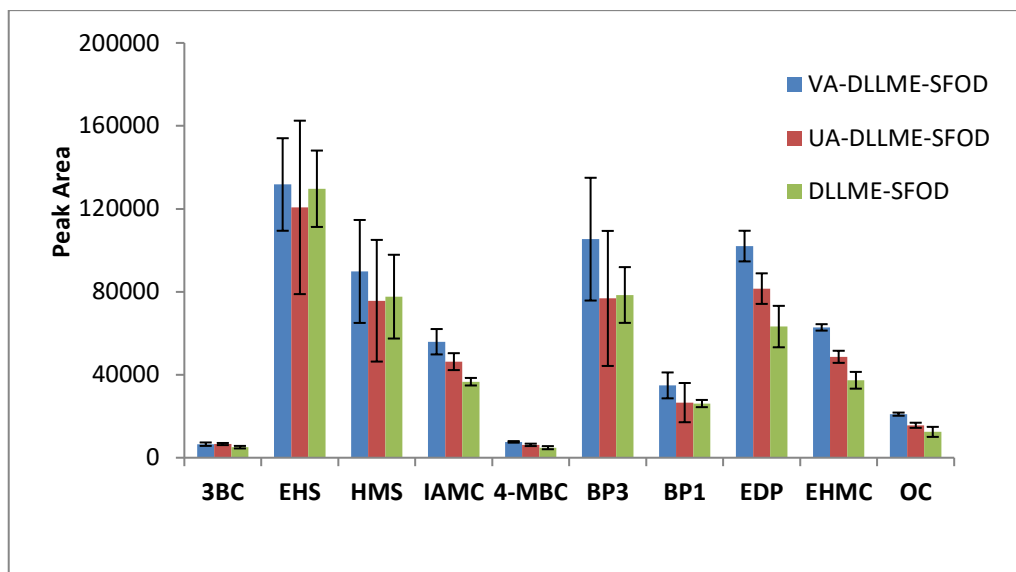


Figure 4.3 The effect of mixing type on the extraction efficiency. UPW volume: 10 mL, analyte concentration: 500 ng L⁻¹; 2-dodecanone volume: 100 µL, acetone volume: 400 µL, pH:2.5

Further studies were conducted to investigate other four parameters namely, (A) the volumes of extraction solvent (V_{2-DD}), (B) volume of dispersion solvents (V_{AC}) (C) amount of NaCl to reveal any salting-out effect (NaCl % w/v) and (D) the vortex time (t_{vtx}) at 3200 rpm. A series of experiments were carried out by using Central Composite Face Centered (CCFC) Design to find the optimal conditions of VA-DLLME-SFOD procedures for UV filters. The factors were optimized in UPW samples spiked with 500 ng L⁻¹ of UVFs at pH 2.5. The peak area of two replicates for each parameter and a total of 46 experiments were performed with 6 central points was shown in **Table 4.6**.

After optimization of the VA-DLLME-SFOD method, obtained results were evaluated by using ANOVA. As shown in **Table 4.7**, the factors with a $p < 0.05$ are considered significantly effective. The **Predicted R^2** values are in reasonable agreement with the **Adjusted R^2** values as shown in **Table 4.8**. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. This model can be used to navigate the design space. As a result of ANOVA the "Effects" values (**Table 4.9**) show the direction and extent of the effects. **Figure 4.4**-**Figure 4.13** shows the surface response for each UV filter for each parameter.

Table 4.6 The data obtained by using CCFCd for VA-DLLME-SFOD-GC-MS/MS method

A V _{2-DD} (μL)	B V _{Ac} (μL)	C NaCl % (w/v)	D t _{vx} (min)	3BC	EHS	HMS	IAMC	4MBC	BP-3	BP-1	EDP	EHMC	OC
30	600	0.0	6.0	13923	362589	254672	81114	13788	169804	73625	98128	86271	21448
30	600	10.0	6.0	22590	728054	497000	138157	12201	355575	191614	165728	154084	37063
30	200	10.0	1.0	21718	494523	333206	82839	16704	381807	163858	128872	107271	37154
30	200	10.0	1.0	21718	592297	311753	91876	19000	405273	142758	152696	124420	41797
30	600	10.0	1.0	19718	617019	427613	117554	17254	305551	158965	144232	140509	27936
110	600	0.0	1.0	5707	73735	50201	17955	5487	33296	14893	18569	17942	4402
70	400	5.0	3.5	9757	224530	155017	52309	2502	115069	53397	56780	59295	16778
110	400	5.0	3.5	9541	305771	186536	64348	13452	51444	17606	86692	79734	21917
30	200	0.0	6.0	32052	448545	263603	161511	29865	258970	103507	223914	138475	46478
30	600	0.0	1.0	21283	504395	364004	98802	21283	308634	153750	127138	111868	39651
110	200	0.0	1.0	5788	102176	73996	35836	2816	66038	31458	40014	40579	12083
110	200	0.0	6.0	3785	110748	74543	20708	3817	60650	21086	28766	27585	9389
30	600	0.0	6.0	17642	426902	307069	118686	12987	252313	125988	111429	103179	27626
110	200	10.0	6.0	3465	94817	63616	22694	3149	61644	23080	23251	24114	7351
110	600	10.0	6.00	7326	131329	75668	37554	7142	61719	24828	59144	41198	15664
110	200	0.0	6.0	3842	94734	66594	26083	3734	57183	20205	28222	32701	8438
70	400	0.0	3.5	10823	250022	200265	69746	11495	143132	68377	78170	70766	18335
30	200	0.0	6.0	32052	448545	263603	161511	29865	258970	103507	223914	138475	46478
70	600	5.0	3.5	10586	163919	104326	62507	10064	97945	37929	81535	58574	18891
700	400	5.0	3.5	9541	149550	94554	50498	8279	107795	39862	69356	48397	18007
70	400	5.0	6.0	10862	29843	221891	63073	7651	197433	98705	71447	64501	19485
30	600	0.0	1.0	23889	59535	416512	123038	21143	408443	184308	169451	163871	42298
110	600	0.0	6.0	6272	117775	70782	31418	5440	68464	23097	47356	39287	17233

Table 4.6 Continue

A	B	C	D	3BC	EHS	HMS	IAMC	4MBC	BP-3	BP-1	EDP	EHMC	OC
V _{2-DP} (μL)	V _{AC} (μL)	NaCl % (w/v)	t _{VR} (min)										
110	600	10.0	1.0	7602	141607	83038	33093	6883	69931	28827	56177	32583	14404
110	600	0.0	6.0	5585	100943	64928	33398	4847	60840	17480	39317	50850	16736
70	400	10.0	3.5	11725	378836	272169	75747	12790	154331	66323	87092	84029	19439
70	200	5.0	3.5	15058	748713	540869	118486	18386	201616	89078	159502	152977	33052
70	400	5.0	3.5	9844	158341	100270	56664	9047	115507	43819	71816	58345	17623
110	200	0.0	1.0	5611	108579	74456	42874	4402	61767	26464	58389	58558	19436
110	200	10.0	1.0	3944	54117	31832	21600	3500	29555	10920	28727	20985	8603
70	400	5.0	3.5	9959	203766	145513	46514	7792	95918	45561	57593	57135	15613
110	600	0.0	1.0	5420	55946	33413	34354	4812	42258	15856	36245	32313	9815
30	200	10.0	6.0	24616	560922	382308	75557	16529	236484	128917	116865	126109	31242
30	200	10.0	6.0	24616	367808	236598	126568	21506	258949	139697	179024	133912	38696
30	400	5.0	3.5	27815	439962	288886	142212	22123	301311	127488	179660	168245	47355
70	400	5.0	3.5	9588	166601	108795	58125	8958	80361	26585	64387	75507	35314
110	200	10.0	6.0	4347	103261	70811	27712	3923	70703	26099	28965	28041	10456
70	400	5.0	1.0	9590	224702	140813	45322	8716	157398	55404	71554	67053	36653
30	200	0.0	1.0	21536	336260	237280	120044	19667	283157	138388	100578	81087	24778
30	600	10.0	1.0	23559	793773	566852	134617	16987	409483	239622	172192	164187	38752.4
110	200	10.0	1.0	3944	40311	25210	30628	4409	26224	8012	30493	33585	20120
110	600	10.0	6.0	6905	224189	160010	39653	6868	104603	50403	50231	49095	15623
30	600	10.0	6.0	23779	766373	523157	145428	16251	374290	201699	174450	162194	39014
110	600	10.0	1.0	6388	153498	114890	34781	6214	84445	39192	43458	39695	11124
70	400	5.0	3.5	9757	256574	180430	72960	8915	164927	92915	78522	75592	19634
30	200	0.0	1.0	21536	428772	312610	84483	18322	290753	146431	107889	107807	27665

Table 4.7 *p*-values obtained with CCFCD for the VA-DDLME-SFOD method

Factors	V _{2-DD} (μL)	V _{AC} (μL)	NaCl % (w/v)	t _{vtx} (min)
3-BC	< 0.0001	0.2685	0.9304	0.2948
EHS	< 0.0001	0.0015	0.0004	0.6323
HMS	< 0.0001	< 0.0001	0.0006	0.9976
IAMC	< 0.0001	0.3071	0.7974	0.1122
4-MBC	< 0.0001	0.2801	0.1977	0.9091
BP-3	< 0.0001	0.1484	0.0160	0.0488
BP-1	< 0.0001	0.0092	0.0028	0.1195
EDP	< 0.0001	0.8298	0.4889	0.2265
EHMC	< 0.0001	0.0721	0.1068	0.5770
OC	< 0.0001	0.6908	0.5959	0.8432

Table 4.8 The Regression analysis of CCFCD for VA-DDLME-SFOD method

3-BC				EHS			
SD	2365.47	R-Squared	0.9466	SD	72328.50	R-Squared	0.9208
Mean	13367.69	Adj R-Squared	0.9217	Mean	2,92E+08	Adj R-Squared	0.8838
C.V. %	17.70	Pred R-Squared	0.8603	C.V. %	24.78	Pred R-Squared	0.7892
PRESS	4,39E+11	Adeq Precision	18.862	PRESS	4,18E+14	Adeq Precision	16.234
HMS				IAMC			
SD	47101.41	R-Squared	0.9259	SD	16887.81	R-Squared	0.8921
Mean	2,01E+08	Adj R-Squared	0.8913	Mean	71381.13	Adj R-Squared	0.8417
C.V. %	23.47	Pred R-Squared	0.7992	C.V. %	23.66	Pred R-Squared	0.7324
PRESS	1,80E+14	Adeq Precision	17.641	PRESS	2,12E+13	Adeq Precision	12.541
4-MBC				BP-3			
SD	2924.19	R-Squared	0.8877	SD	38027.70	R-Squared	0.9335
Mean	11390.64	Adj R-Squared	0.8353	Mean	1,71E+08	Adj R-Squared	0.9024
C.V. %	25.67	Pred R-Squared	0.6710	C.V. %	22.22	Pred R-Squared	0.8441
PRESS	7,51E+11	Adeq Precision	13.744	PRESS	1,02E+14	Adeq Precision	16.331
BP-1				EDP			
SD	19858.98	R-Squared	0.9314	SD	25480.98	R-Squared	0.8594
Mean	78944.56	Adj R-Squared	0.8993	Mean	90409.52	Adj R-Squared	0.7938
C.V. %	25.16	Pred R-Squared	0.8450	C.V. %	28.18	Pred R-Squared	0.6443
PRESS	2,67E+13	Adeq Precision	17.425	PRESS	4,93E+13	Adeq Precision	10.872
EHMC				OC			
SD	16958.43	R-Squared	0.9058	SD	16958.43	R-Squared	0.9058
Mean	79555.61	Adj R-Squared	0.8619	Mean	79555.61	Adj R-Squared	0.8619
C.V. %	21.32	Pred R-Squared	0.7658	C.V. %	21.32	Pred R-Squared	0.7658
PRESS	2,15E+13	Adeq Precision	14.686	PRESS	2,15E+13	Adeq Precision	14.686

Table 4.9 The coded effects of factors to response in optimisation of UV filters by CCFCF.

Factors	3-BC	EHS	HMS	IAMC	4-MBC	BP-3	BP-1	EDP	EHMC	OC
A	-8781	-2.030 x10 ⁵	-1.372x10 ⁵	-42626	-6899	-1.250 x10 ⁵	-62488	-55063	-45974	-11548
B	-469	+44381	+38016	+3090	-566	+9932	+9736	+973	+5567	-495
C	+35	+49192	+30799	-750	-6609	+16643	+11070	+3062	+4835	+651
D	+432	-5996	-24.29	+4739	+57	-13394	-5457	+5396	+1639	-242
A*B	+1560	-25959	-26376	-591	+1797	-3685	-6726	+4833	-2868	-
A*C	+109	-37085	-23217	+1302	+1163	-13688	-9275	-1501	-6630	-
A*D	-599	+15770	+12554	-5224	-69	+23751	+9068	-6163	-782	-
B*C	+1124	+33885	+24937	+9864	+743	+9012	+8558	+10649	+6399	-
B*D	-1016	-4803	-3892	-2529	-1378	+2080	-765	-7091	-2872	-
C*D	+145	+5882	+9732	-334	-3063	+3714	+6788	-3186	+1647	-
A^2	+6982	+1.707 x10 ⁵	+78220	+35293	+6708	+46787	+20393	+48296	+48864	-
B^2	-1617	-87149	-1.075 x10 ⁵	-14473	-2197	-49740	-24859	-13553	-28491	-
C^2	-421	+1.122 x10 ⁵	+76726	+4759	+1063	+19141	+15196	-2248	+2272	-
D^2	-1469	-74927	+21861	-13789	-2895	+47825	+24900	-13379	-9347	-

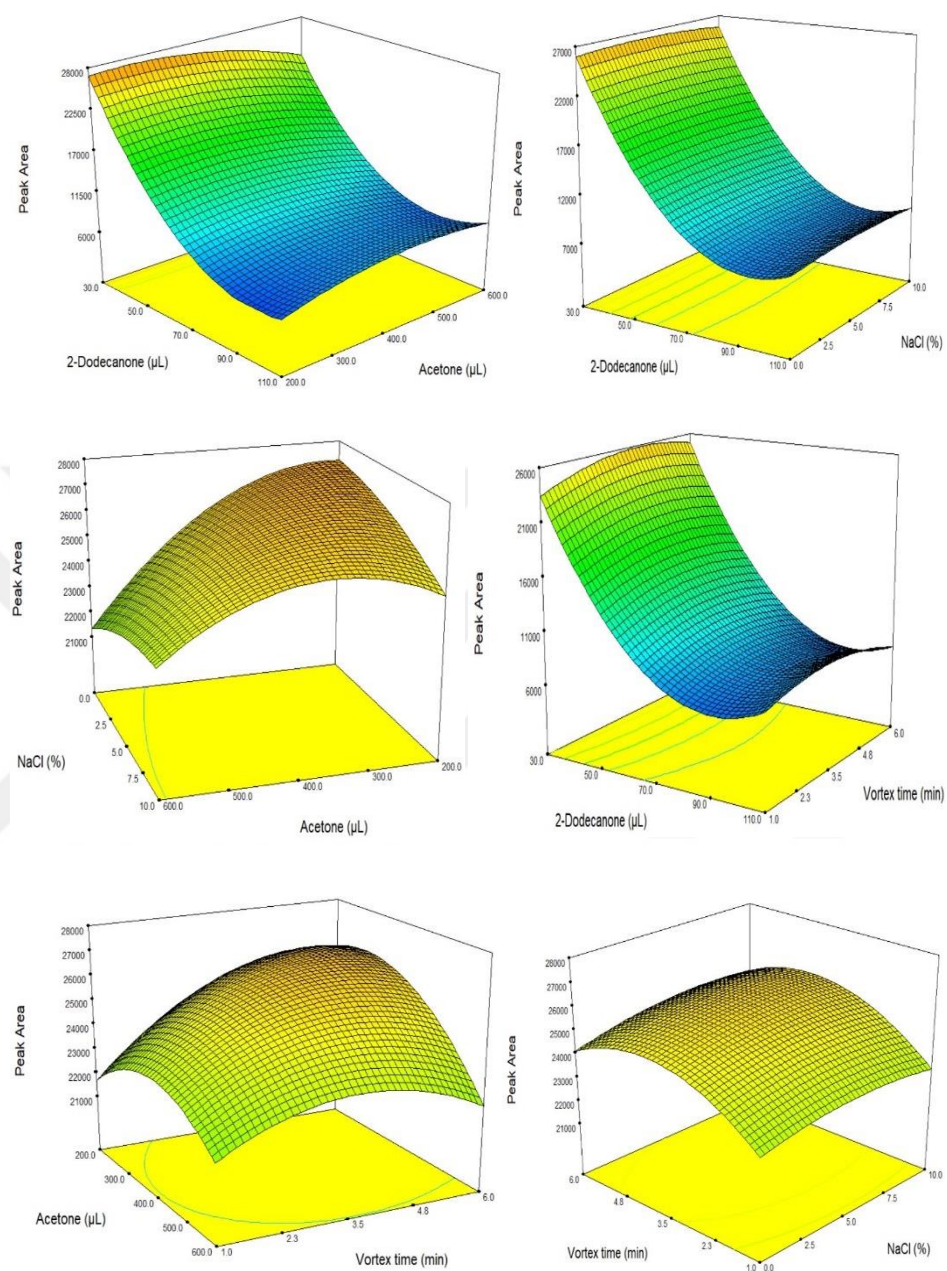


Figure 4.4 Estimated response surface for UV filters using CCFD for 3-BC

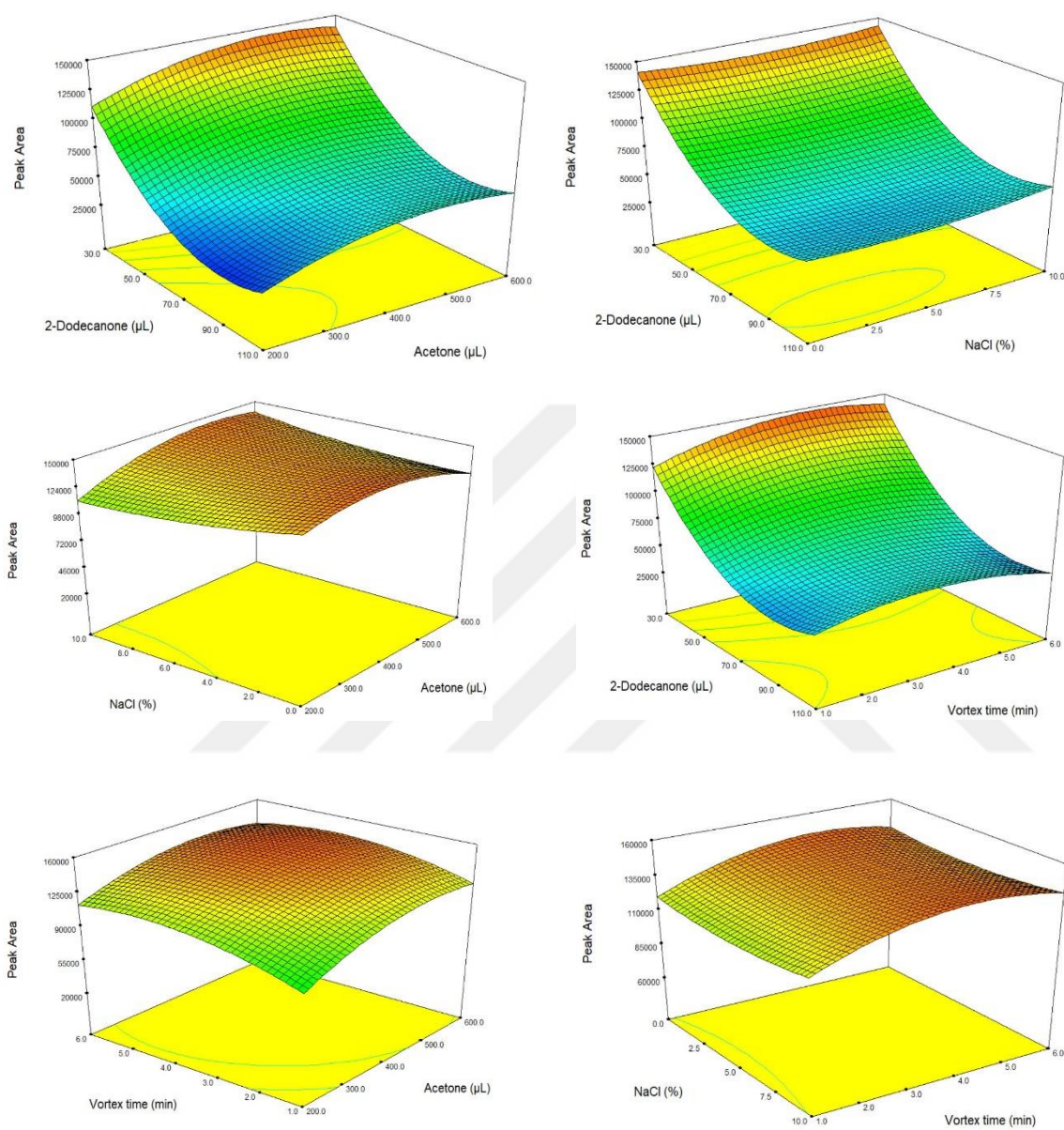


Figure 4.5 Estimated response surface for UV filters using CCFD for IAMC

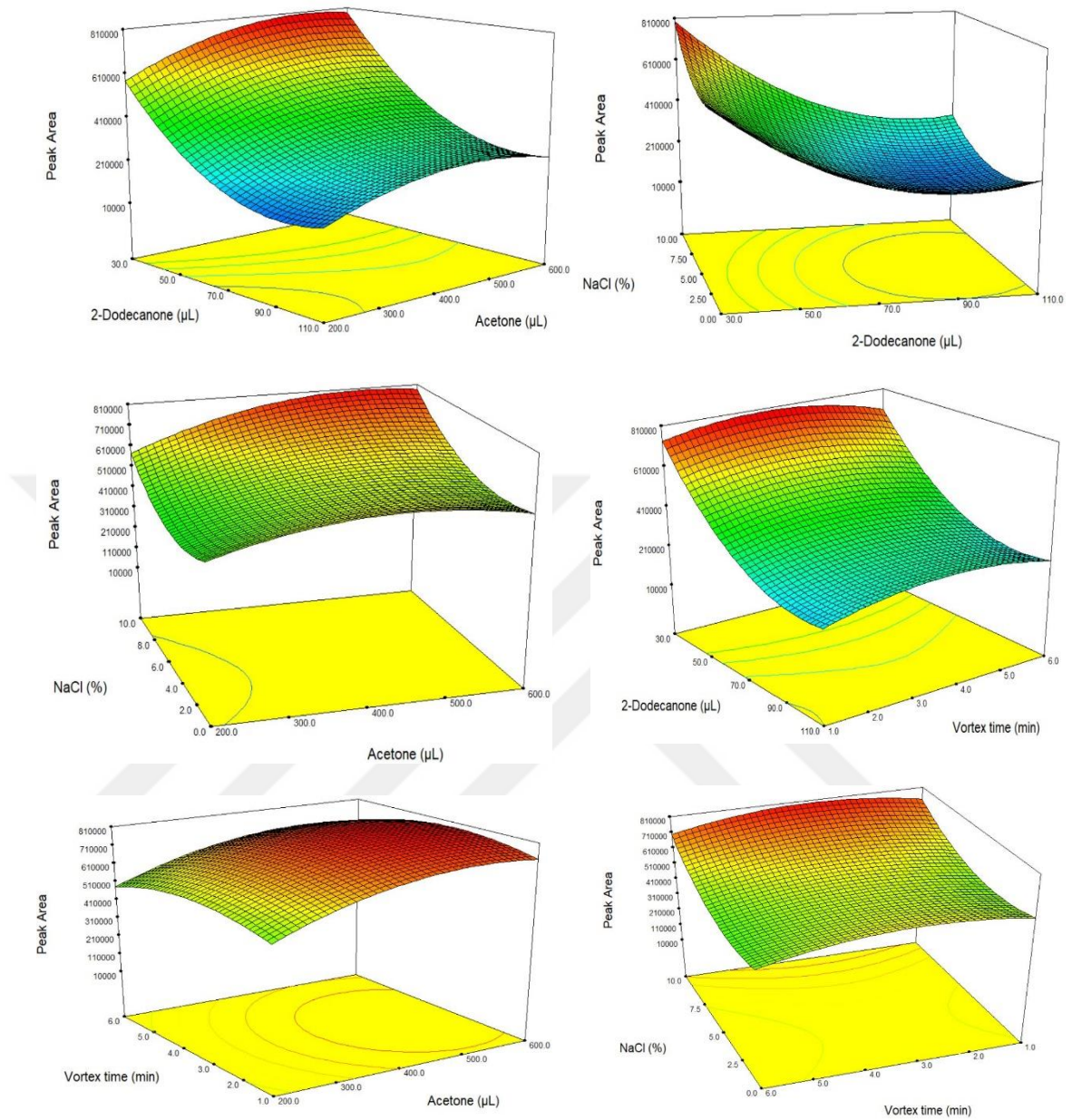


Figure 4.6 Estimated response surface for UV filters using CCFD for EHS.

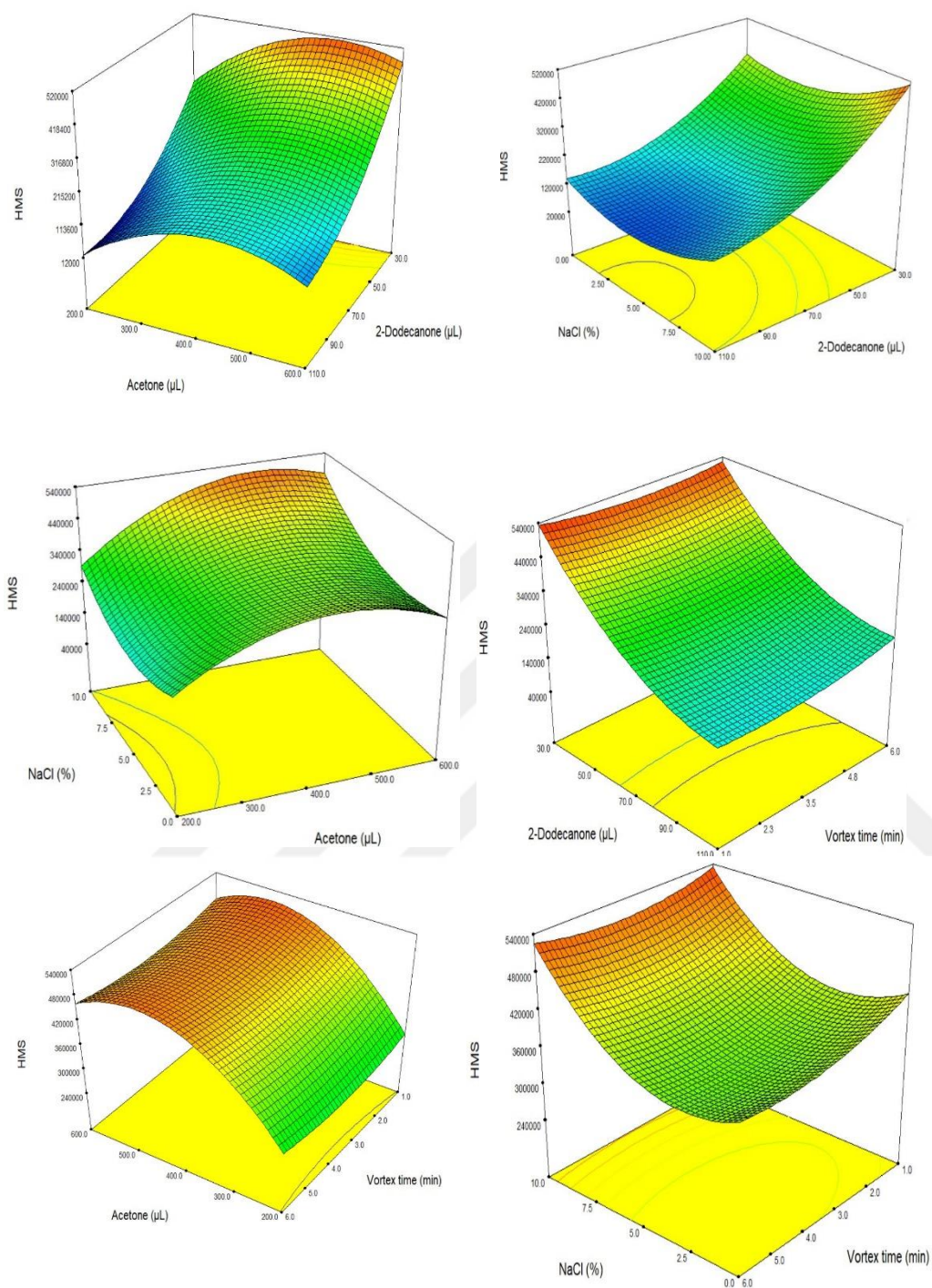


Figure 4.7 Estimated response surface for UV filters using CCFD for HMS.

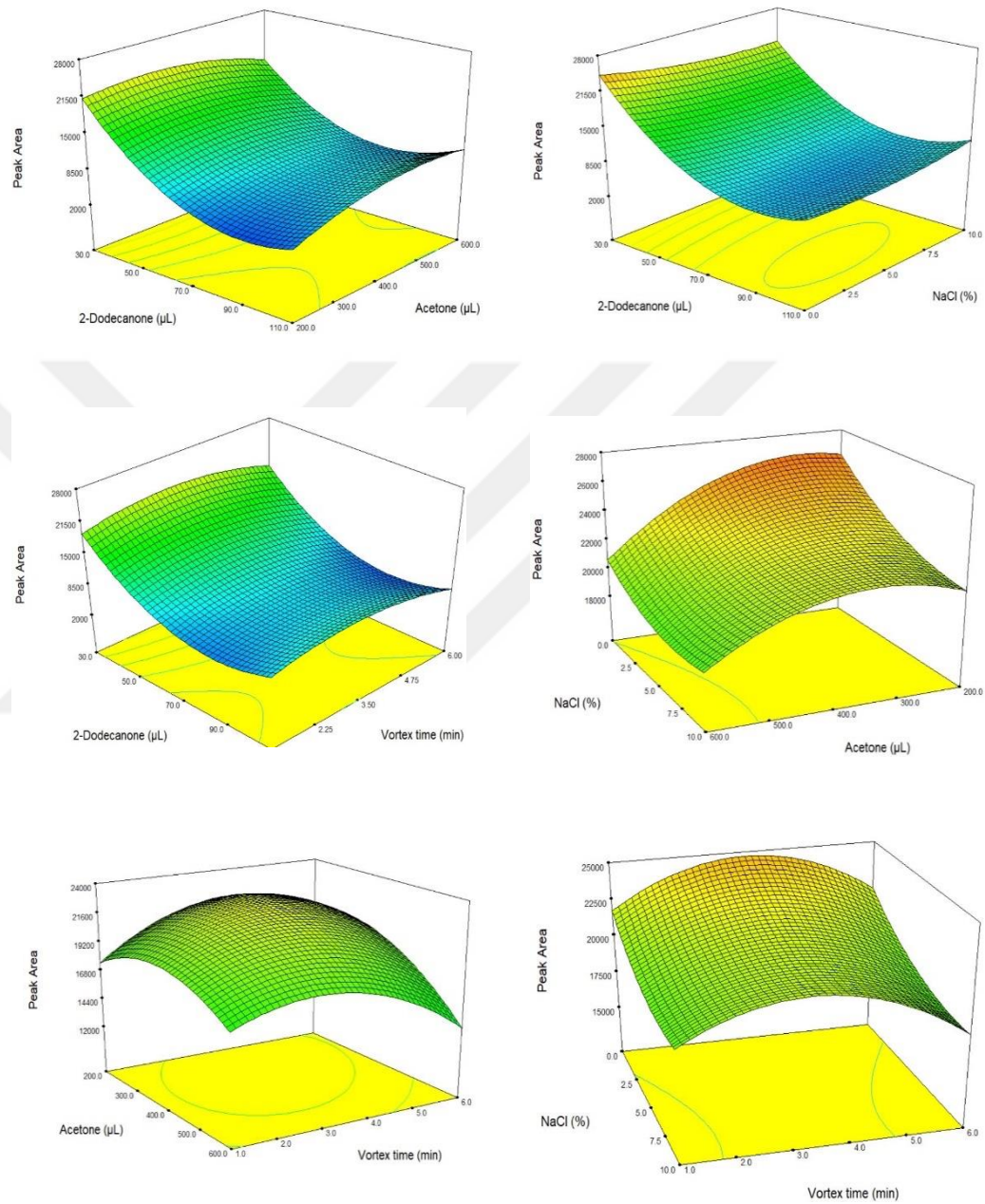


Figure 4.8 Estimated response surface for UV filters using CCFD for 4-MBC

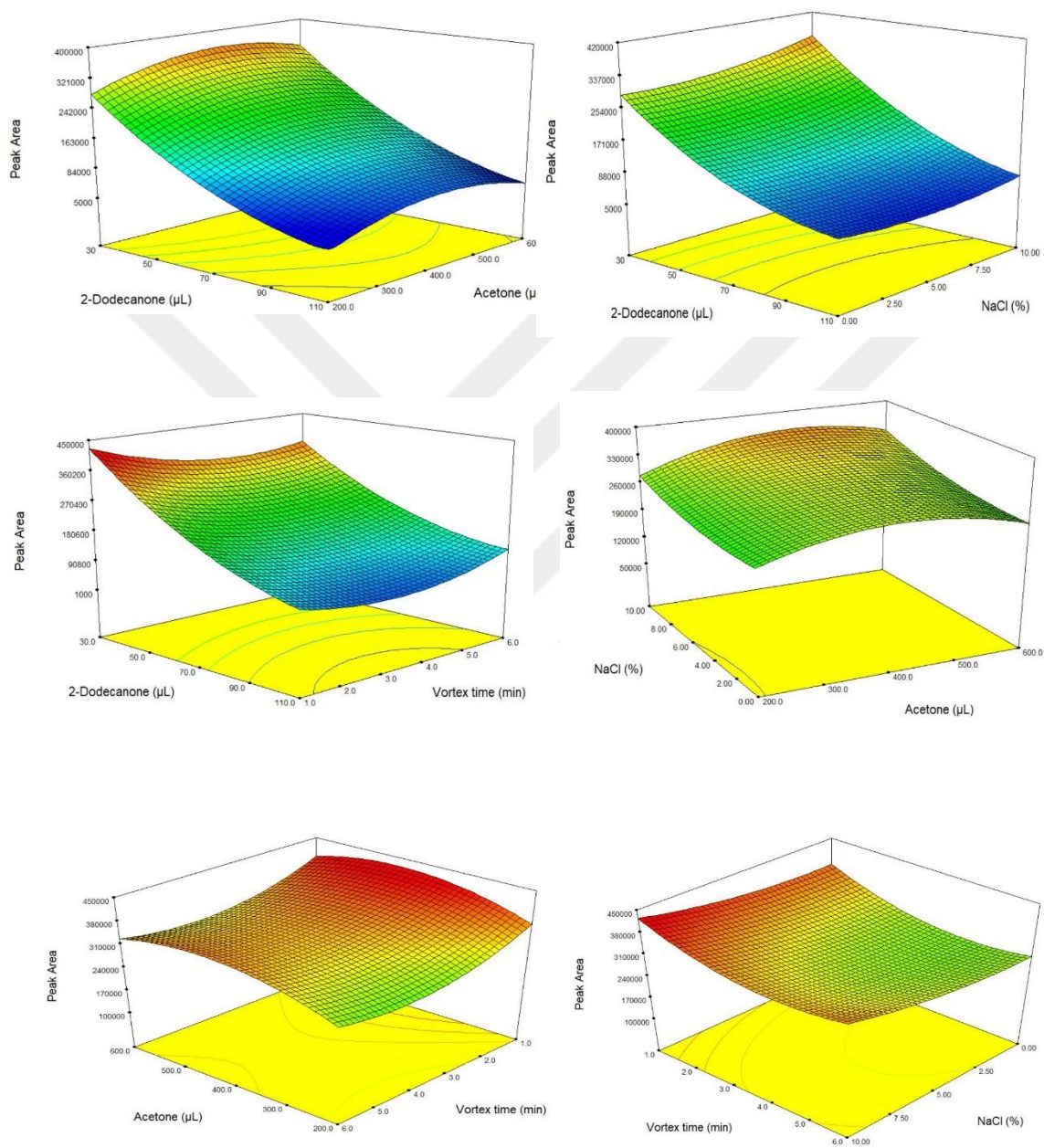


Figure 4.9 Estimated response surface for UV filters using CCFD for BP-3

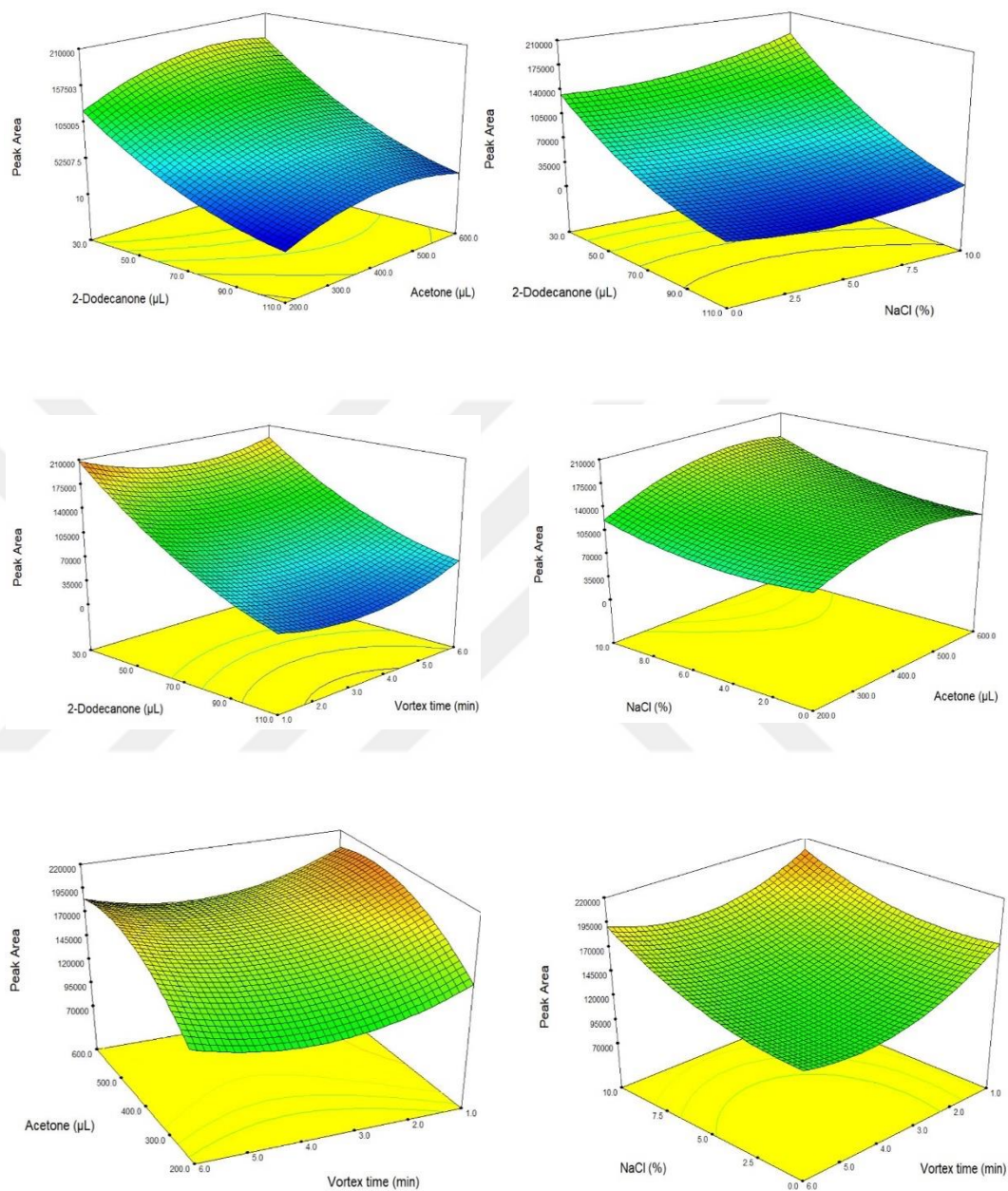


Figure 4.10 Estimated response surface for UV filters using CCFD for BP-1

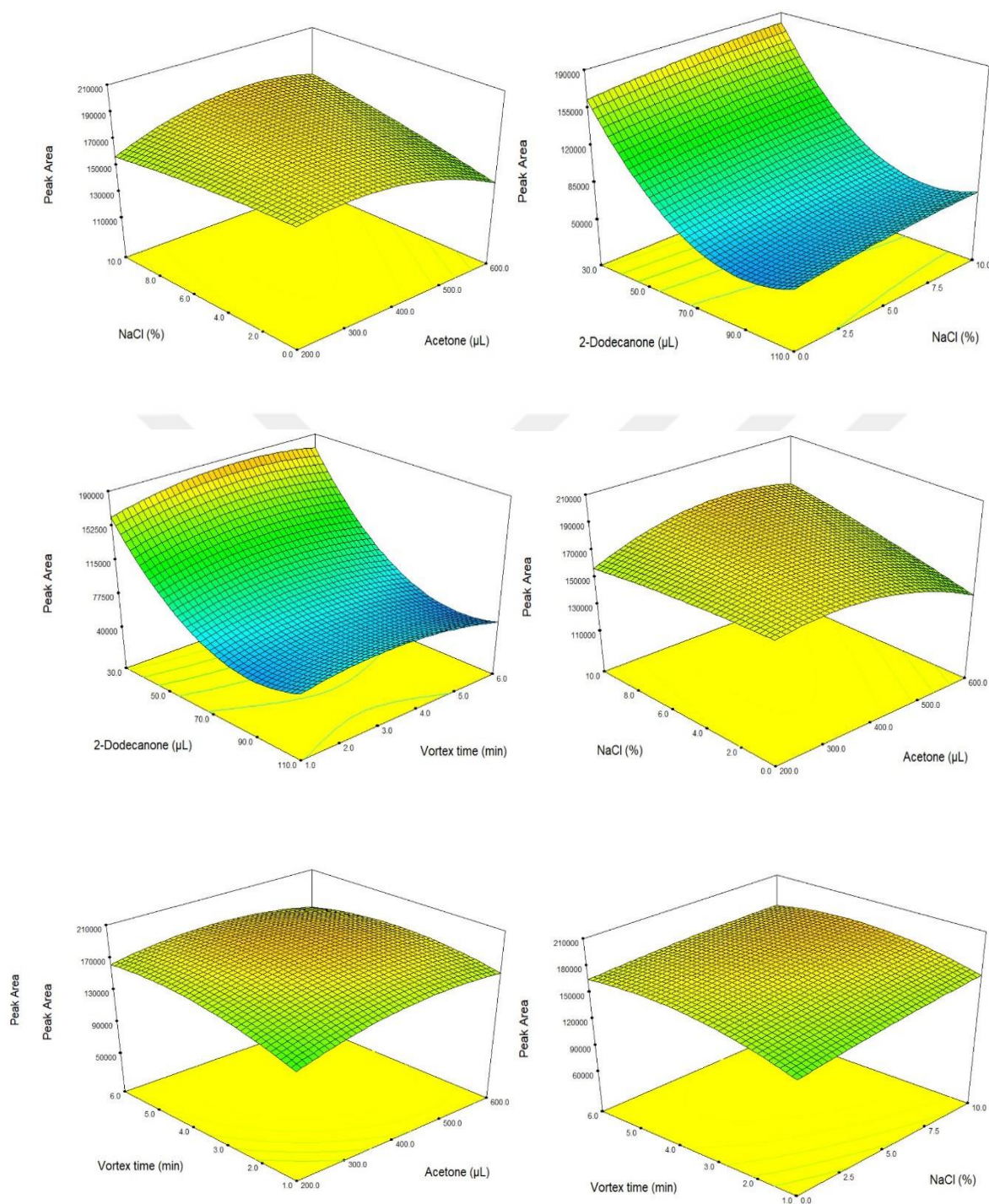


Figure 4.11 Estimated response surface for UV filters using CCFD for EDP.

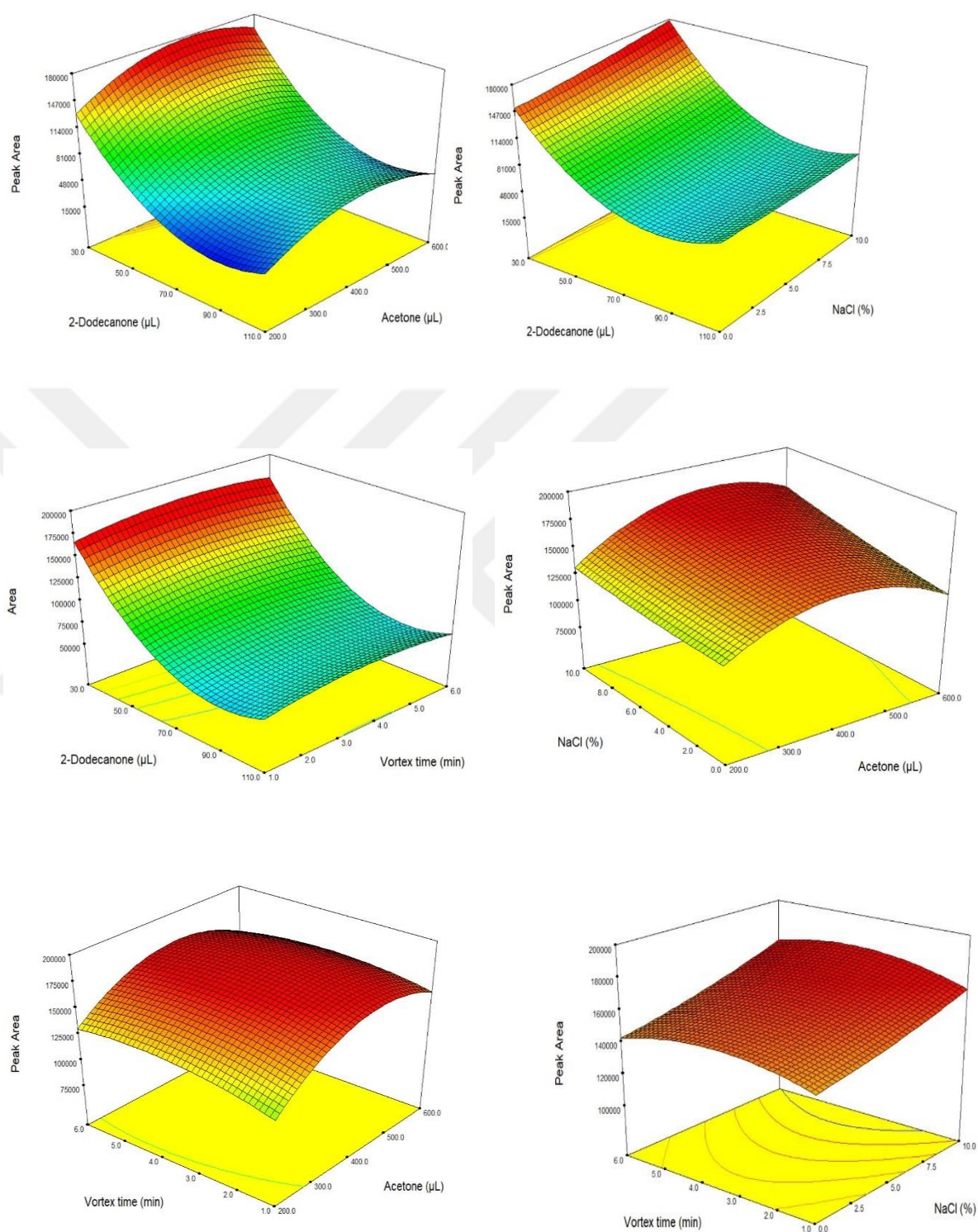


Figure 4.12 Estimated response surface for UV filters using CCFD for EHMC.

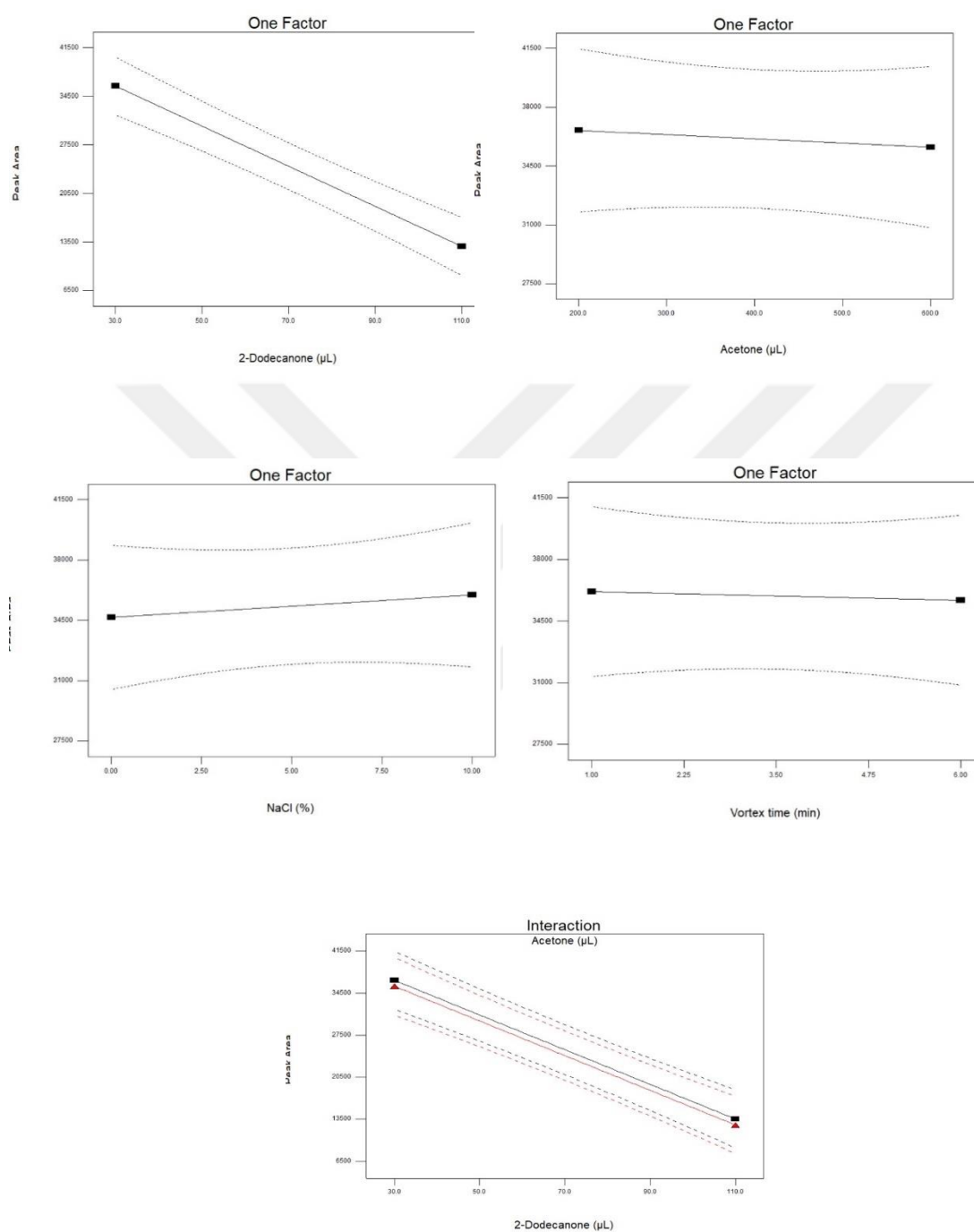


Figure 4.13 Estimated response surface for UV filters using CCFD for OC.

Considering all the data given above, each parameter was evaluated below.

The effect of extraction solvent volume: High volume solvent use results in dilution effect which limits the sensitivity. Therefore, this parameter should be optimized. The volume of 2-dodecanone was changed between 30 and 110 μL and The best extraction efficiency was obtained in 30 μL of extraction solvent. Lower volumes of extraction solvent was not favored for practical reasons.

The effect of dispersive solvent volume: Usually dispersive solvent volume is higher than the extraction solvent to provide cloudy medium for the analyte and therefore, the experiments were performed by changing volume of dispersive solvent i.e., acetone, between 200 and 600 μL .

According to the data given above, the peak areas of the UV filters increased when the acetone volume was increased in the range of 200 - 460 μL . This may be explained as the low volume of dispersion solvent is not sufficient to form a cloudy solution necessary for the extraction in agreement with a former study (Tarazona, 2010). However, the peak areas obtained were decreased for higher volumes due to the solubility of the extraction solvent in aqueous solution. Indeed, the interaction between the dispersing solvent and the extraction solvent is significant for 3 analytes (BP-3, EHS, and HMS).

The effect of ionic strength: High ionic strength reduces the solubility of organic analytes in aqueous solution and easily transfer to the organic solvent with a process known as the salting out effect. Here, the upper limit is also important phase separation since in saline samples can not be observed due to partially dissolving of the solvent in water. Therefore, ionic strength is a critical parameter for the extraction efficiency.

Most of the authors investigate the effect of NaCl on extraction efficiency for determination of UVFs in DLLME method. In most of studies, OFAT technique was used to study the effect of ionic strength on extraction efficiency. And it was reported that the effect of the NaCl concentration in the sample is negligible (Negreira et al., 2010; Cunha et al., 2015; Zhang and Lee, 2012). In other studies, it was claimed that larger peak areas were obtained by increasing the salt concentration (Wu et al., 2013; Tarazona et al, 2010).

Most of studies on DLLME-SFOD method showed that NaCl concentration has significant effect on extraction efficiency for various analyte type (Leong et al., 2009; Xiong et al., 2014; Bolzan et al., 2016; Wu et al. 2010; Huang et al., 2020; Lili et al., 2010; Jing et al., 2018; Hou et al., 2013; Peng et al., 2015). Higher NaCl contents cause the extraction solvent to accumulate in an extremely thin layer on the aqueous phase, making it difficult to collect the extraction solvent. Moreover, it has been claimed that no phase separation takes place due to the increasing of viscosity and density of the aqueous phase when the sodium chloride concentration is very high (20%, w/v).

In this study, the effect of NaCl concentration was investigated in the range of 0-10%. The effect of NaCl concentration on the peak areas of UV filters is negligible for 6 analytes but, it was found significant for BP-1, BP-3, EHS and HMS. These compounds are more polar than the other analytes and their solubility in water decreases with increasing ionic strength. Therefore 10% NaCl concentration was chosen for further studies.

The effect of vortex time: In DLLME studies, well mixing of the solution is important to disperse the organic solvent in small droplets in the aqueous sample. As a result of the acceleration of the transfer of the analytes to the organic phase with the vortex effect, the highest peak areas of the UV filters were obtained within 3 min at 3200 rpm. Vortex times longer than 3 min had no significant effect on the peak areas in agreement with a previous study (Zhang and Lee, 2012c). Therefore, 3 minutes was chosen for the vortex time.

In conclusion, optimal parameters were determined as 30 μ L dodecanone as the extraction solvent, 460 μ L acetone as dispersing solvent, 10% (w/v) NaCl concentration and 3 min vortex time at 3200 rpm for further studies. Further studies include the optimization of in-port derivatization technique.

Literature survey has revealed derivatization process is applied in two modes, mainly off-line or in-port derivatization. Later technique was chosen as is faster and greener than the off-line mode since only a small amount of reagent is consumed. In most studies based on in-port derivatization technique, injection modes are usually programmable temperature evaporation (PTV); (Chung et al, 2015; Mudiam et al, 2014; Jain et al, 2013) and direct splitless injection (Vinas et al, 2011; Jaber et al, 2007; Mandrah et al, 2017).

In the present thesis, the PTV mode was selected, and the injector is heated from the initial temperature (T1) to a high temperature (T2). T1 is selected for both solvent evaporation and derivatization of analytes and T2 allows the derivatives to evaporate and enter the analytical column (Wang et al., 2013).

In the studies reported for optimization, injection temperature, purge off time and injected volume of the reagent and the extract are the most important factors (Chung et al., 2015; Mudiam et al., 2014; Jain et al., 2013; Vinas et al., 2011; Jaber et al., 2007; Mandrah et al., 2017). In this thesis, the influence of these parameters on the extraction efficiency of UVFs were investigated by using OFAT.

The effect of injection temperature: To examine the effect of injection temperature on the peak areas of the UV filters, the injection block was set at 240°C, 260°C and 280 °C. Injection was carried out in splitless mode with programmable temperature vaporization technique. Samples containing 500 ng L⁻¹ of UV filters were subjected to VA-DLLME-SFOD procedure. The injection was carried out at a temperature of 70°C (held for 1 min) and rapidly increased to 240, 260 and 280°C at a rate of 250°Cmin⁻¹, and after 4.5 minutes the split was used at a ratio of 75:1.

As shown in **Figure 4.14**, the highest peak area was obtained at 260°C for most of the UV filters. The decrease in the peak areas at 260°C may be resulted from insufficient evaporation of the high boiling point of UV filters at low temperature, while the decrease in the peak areas at 280°C could be due to the degradation of the trimethylsilyl (TMS) derivatized of UV filters.

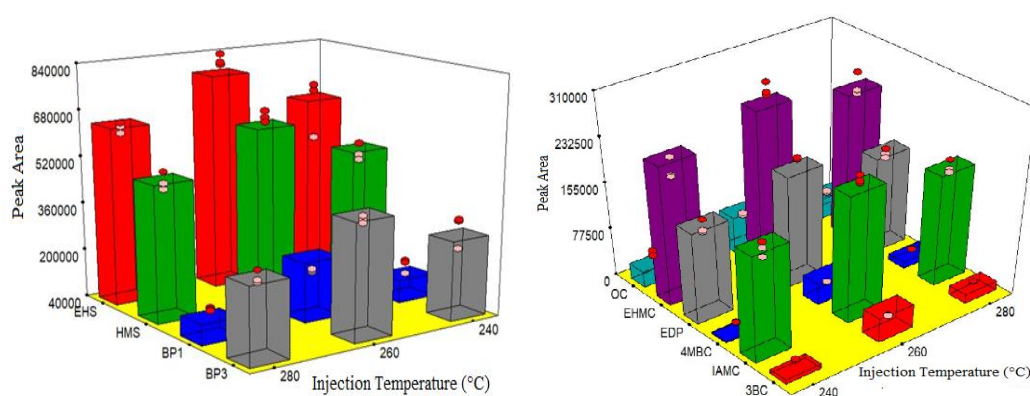


Figure 4.14 The effect of injection temperature on the signal of UV filters. Conditions: sample volume 10 mL, concentration level: 500 ng L⁻¹, the volume of acetone: 460 µL, volume of 2-dodecanone: 30 µL, pH: 2.5, vortex time: 3 min, amount of NaCl: 10% (w/v), sample/BSTFA volume: 2:2 µL, purge off time: 4.5 min.

The effect of purge off time: It was observed that the peak areas of the UV filters increased when the purge off time was increased from 2.5 to 3.5 min as shown in **Figure 4.15**. Although there was an increase in the peak area when the purge off time of 5.5 min was used, peak broadening was detected. Therefore 4.5 min was selected for the purge off time in PTV mode.

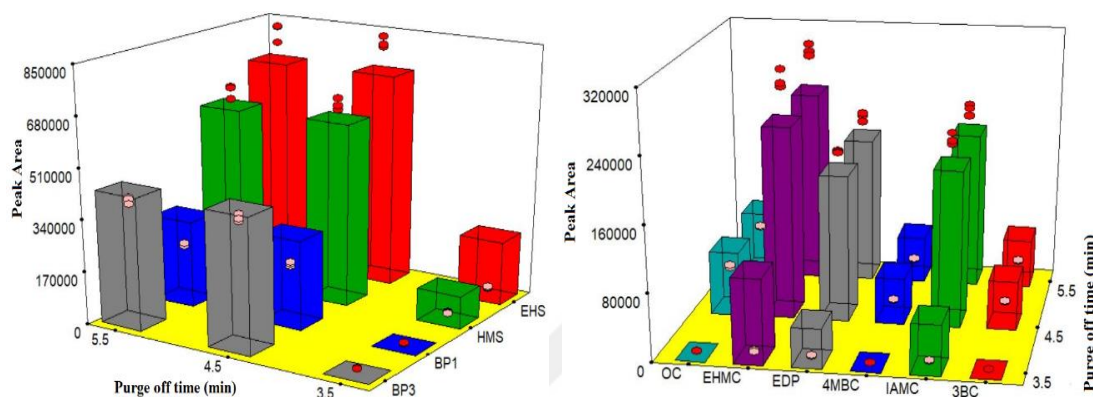


Figure 4.15 The effect of purge off time on the signal of UV filters. Conditions: sample volume: 10 mL, concentration: 500 ng L⁻¹, volume of acetone: 460 µL, volume of 2-dodecanone: 30 µL, pH: 2.5, vortex time: 3 min, amount of NaCl: 10% (w/v), sample/BSTFA volume: 2:2 µL, injection temperature: 260°C.

The effect of the volume of BSTFA and sample extract: The volume of derivatization reagents is another critical factor for derivatization efficiency. If the amount of this reagent is not sufficient, partial derivatization occurs and the peaks of the analytes can be impaired in the chromatogram (Toledano et al., 2012). Excess amount of the reagent, on the other hand, may disrupt the separation of the peaks and damage the column (Wang et al., 2013) and reduces the derivatization efficiency of analytes (Basheer et al., 2005).

In previous studies, the amount of derivatization reagent was optimized in terms of the absolute volume of reagent (Tzing et al., 2010; Wu et al., 2009; Quintana et al., 2007; Ho et al., 2012; Basheer et al., 2004; Cheng et al., 2011; Hyötyläinen et al., 1997) or the ratio of reagent to sample (Dobson et al., 1997; Vinas, 2011).

In this study, derivatization efficiency was investigated by changing the injection volume of final extract and BSTFA ($V_{\text{ext}}/V_{\text{BSTFA}}$) as 2 μL / 2 μL , 1 μL / 1 μL and 2 μL / 1 μL . As shown in **Figure 4.16**, the best extraction efficiency was obtained simultaneously injecting 2 μL of the final extract and 1 μL of BSTFA into the GC-MS/MS system in sandwich mode.

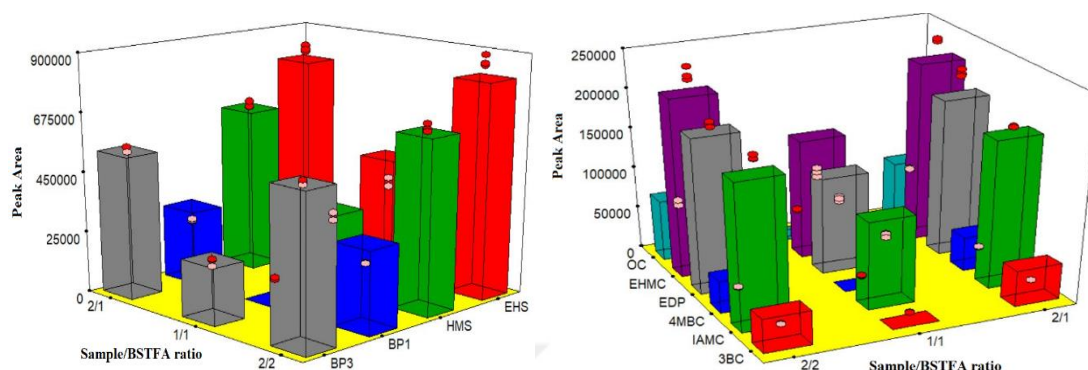


Figure 4.16 The effect of the volume of sample extract/ BSTFA on the signal of UV filters.

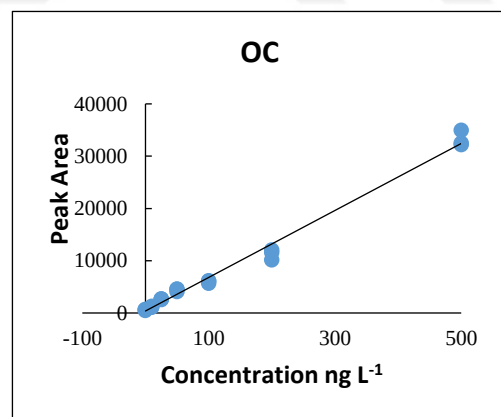
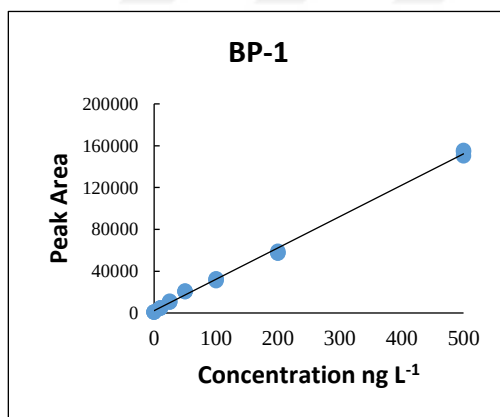
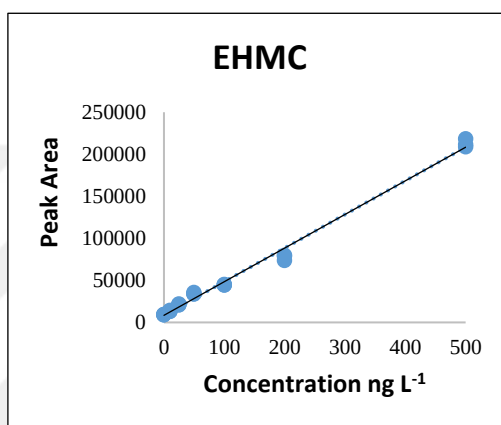
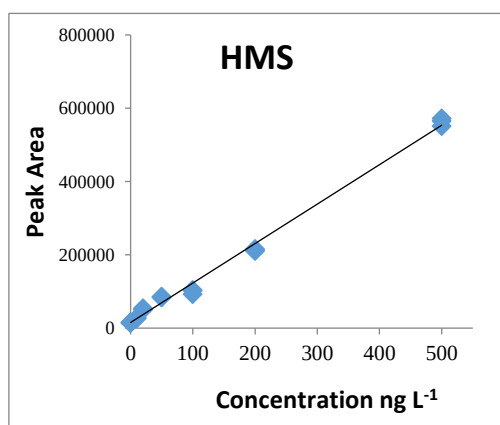
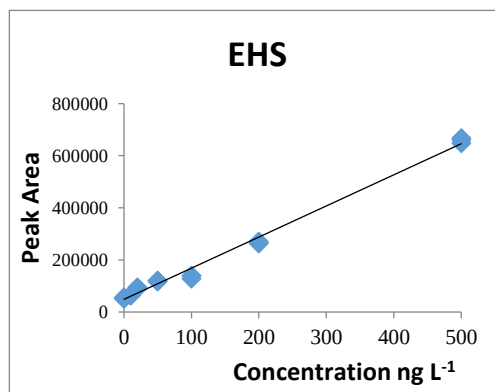
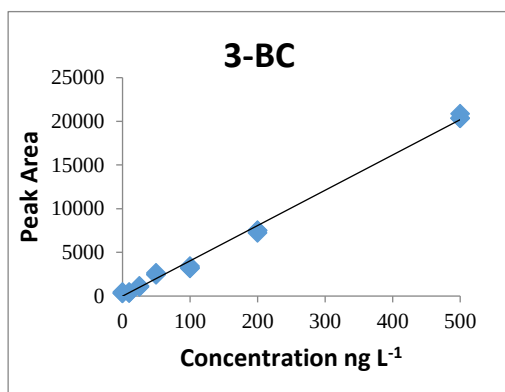
Conditions: sample volume: 10 mL, concentration level: 500 ng L⁻¹, volume of acetone: 460 μL , volume of 2-dodecanone: 30 μL , pH: 2.5, vortex time: 3 min, amount of NaCl: 10% (w/v), sample/BSTFA volume: 2:2 μL , injection temperature: 260°C. purge of time: 4.5 min

So far, all the parameters of the VA-DLLME-SFOD method along with the in-port derivatization conditions have been optimized. Further studies include validation of the method by using surface water and wastewater samples.

4.2.2 Method Validation of VA-DLLME-SFOD Method

Under optimized conditions, chromatographic analysis of the target analytes was performed to construct calibration curves by using matrix-match method. Surface water samples were spiked with mix-standard solutions of target analytes to be 0, 10, 25, 50, 100, 200 and 500 ng L⁻¹ in the sample.

The calibration graphs were constructed by subtracting the peak area values of the real water sample from the spiked sample and plotting against the concentration of UV filter added into the real water sample. Each concentration was studied in 3 replicates and linear equations can be seen in **Figure 4.17** respectively. and a correlation coefficient ranging from 0.9892 to 0.9988 was obtained for all target analytes as listed in **Table 4.10**.



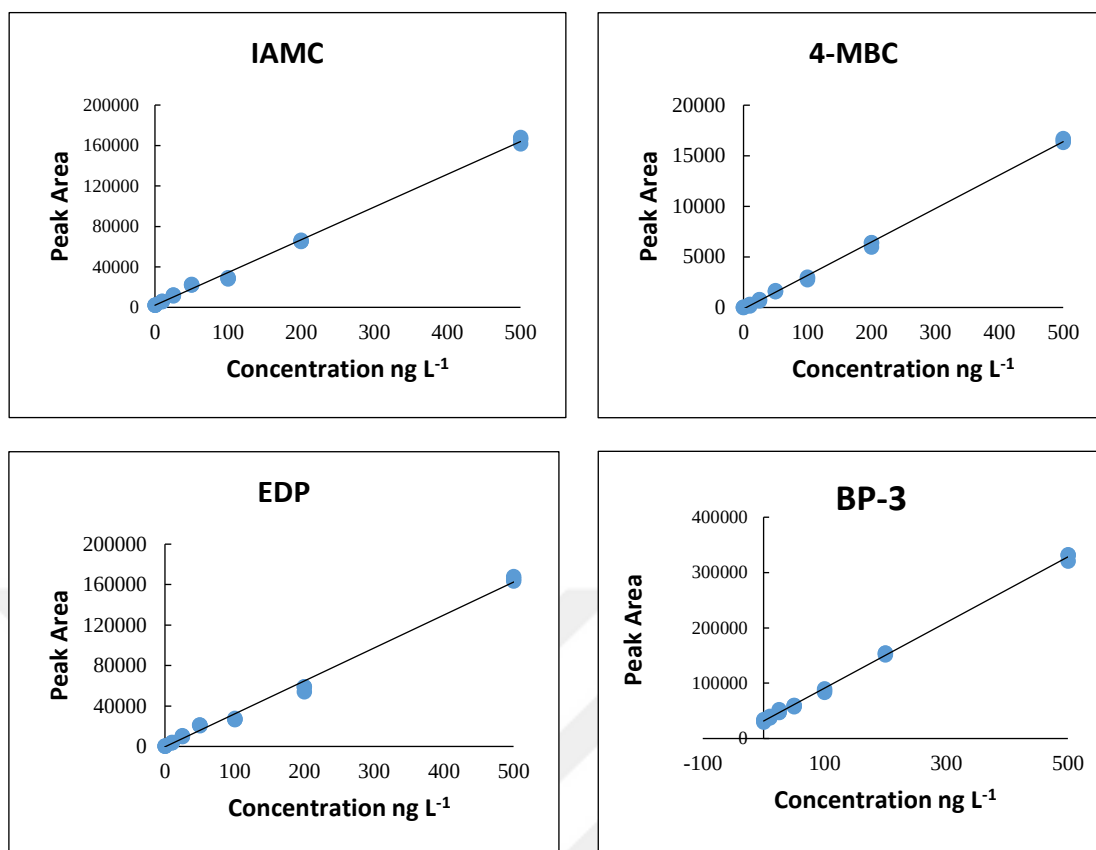


Figure 4.17 Calibration curves for UV filters injected onto GC column after derivatization

The LOD and LOQ values of optimized methods were calculated as $3.3 \times (\sigma/s)$ and $10 \times (\sigma/s)$, respectively, where σ is the standard deviation of y-intercept, s is the slope of the calibration curve (**Table 4.10**).

Table 4.10 also lists the extraction factor of the method developed. The term **Extraction Factor** (EF) is often employed for the overall concentration factor in an extraction process. For the DLLME-SFOD technique it can be described as the ratio of the target analyte concentration in the floated phase (C_{fl}) and the initial concentration of the analyte (C_o) in the aqueous phase. ($EF = C_{fl}/C_o$).

Table 4.10 Analytical performances and Extraction Factors of the VA-DLLME-SFOD method for the targeted UVFs.

UV Filter	Linear Equation	R^2	Linear range (ng L ⁻¹)	LOD (ng L ⁻¹)	EF
3-BC	$40.40x - 9.04$	0.9948	13-500	4.3	258
EHS	$1198.4x + 47\ 537$	0.9923	17-500	5.5	247
HMS	$1079.6x + 14\ 323$	0.9938	15-500	4.9	231
IAMC	$323.66x + 2092$	0.9968	10-500	3.4	232
4-MBC	$33.203x - 187.9$	0.9988	6.4-500	2.1	291
BP-3	$593.16x + 31\ 686$	0.9987	6.5-500	2.2	250
BP-1	$299.92x + 2235.6$	0.9975	8.8-500	2.9	254
EDP	$325.75x + 376.8$	0.9935	15-500	4.8	248
EHMC	$399.83x + 8456.8$	0.9927	5.8-500	1.9	263
OC	$64.09x + 383.39$	0.9892	3.1-500	1.1	350

Precision is usually expressed by statistical parameters which describe the variations in the results, typically the standard deviation or relative standard deviation, calculated from results obtained by carrying out replicate measurements on a suitable material under specified conditions. Deciding on the *specified conditions* is an important aspect of evaluating measurement precision.

For precision studies, mixed standard was added to the real surface water sample at a concentration of 50, 200, and 500 ng L⁻¹ and the VA-DLLME-SFOD method was applied to the surface water and wastewater and the analytes were derivatized in the injection port. Background correction was also made for surface and wastewater samples. The corrected peak areas of UV filters for surface water and wastewater were shown in **Table 4.11** and **Table 4.12**, respectively.

Table 4.11 The peak area of UV filters for the whole VA-DLLME-SFOD procedures applied to three parallel surface water samples on day-1.

Spiked conc. (ng L ⁻¹)	Inj No	Peak Areas of UV filters (n=3)									
		EHS	HMS	3BC	IAMC	4MBC	BP3	BP1	EDP	EHMC	OC
50	1	102089	68302	2168	16549	1259	57553	16186	13888	26834	3244
	2	101833	68875	1970	16376	1275	57726	16901	13552	26306	3564
	3	104268	70638	1854	16517	1230	59893	17658	14701	29541	3938
200	1	275988	229757	7904	65471	5501	148454	63217	69962	81751	15120
	2	268167	220681	7867	65197	5623	139812	60952	71071	80498	15161
	3	263595	218946	7882	62786	5703	134539	58544	72175	79413	15287
500	1	605105	536553	21164	162307	15039	336446	157323	163975	217459	36239
	2	626576	555843	21699	156642	16000	322334	149756	155267	202765	32590
	3	613694	549109	20950	161788	15643	330357	154336	165220	218634	36427

Table 4.12 The peak area of UV filters for the whole VA-DLLME-SFOD procedures applied to three parallel surface water samples on day-2.

Spiked conc. (ng L ⁻¹)	Inj No	Peak Areas of UV filters (n=3)									
		EHS	HMS	3BC	IAMC	4MBC	BP3	BP1	EDP	EHMC	OC
50	1	94140	61691	2138.1	16172	1158,7	49215	14802,1	15658	28399	3070
	2	95832	61957	1955	16180	1157	50443	15035	15530	28010	30874
	3	97482	64828	20092	16298	1273	52484,5	14995	16599	28269	3160
200	1	275988	226584	8880	75252	6321	155421	68555	60048	100381	11919
	2	268167	217508	9264	79023	6266	165615	72569	59225	99674	12202
	3	263594	215773	9404	79704	6360	165587	72341	58904	99440	12709
500	1	605104	536553	19679	158068	15429	370596	166812	141769	182589	25165
	2	626575	555843	19521	165147	15325	380749	175469	156731	205635	30924
	3	613694	549109	20606	162407	15527	367720	170782	154411	202261	30709

Table 4.13 The peak area of UV filters for the whole VA-DLLME-SFOD procedures applied to three parallel wastewater samples on day-1.

Spiked conc. (ng L ⁻¹)	Inj No	Mean Peak Area of UV filters								
		EHS	HMS	3BC	IAMC	4MBC	BP3	EDP	EHMC	OC
50	1	104952	74293	2137	18767	1351	54794	16878	28598	3342
	2	104210	72972	2418	19174	1325	57717	16480	27128	3350
	3	106856	73412	2387	18631	1355	58076	16723	27164	3135
200	1	313015	263685	8954	72657	6416	173266	65534	94765	11990
	2	304941	259261	8862	70550	6615	170305	66553	96679	12869
	3	314044	267739	9163	71613	6696	174606	69980	102336	14389

As a result of the validation studies, the intra-day and inter-day precision indicated as relative standard deviation of VA-DLLME-SFOD method applied to surface water were calculated and were shown in **Table 4.15**. VA-DLLME-SFOD method was applied to wastewater samples and inter-day repeatability and recovery values were not calculated.

Table 4.14. Recovery and RSD values of UV filters obtained by applying VA-DLLME-SFOD method to surface water sample spiked with different concentrations

UV Filter	Recovery (%)						RSD (%)					
	Intra-day (<i>n</i> = 3)			Inter-day (<i>n</i> = 2)			Intra-day (<i>n</i> = 3)			Inter-day (<i>n</i> = 2)		
	Concentration (ng L ⁻¹)											
	50	200	500	50	200	500	50	200	500	50	200	500
3-BC	99.3	96.1	105.3	94.4	96.1	98.1	6.82	2.66	4.06	7.76	3.07	7.04
EHS	92.1	92.5	94.7	86.3	98.1	98.6	2.93	3.37	1.79	9.91	8.56	6.93
HMS	101.8	96.7	98.7	95.8	98.2	98.3	3.09	2.66	2.02	9.72	4.89	3.02
IAMC	88.9	96.4	98.7	88.1	99.9	95.4	0.58	3.08	2.17	1.39	5.62	5.14
4-MBC	96.5	92.7	102.9	94.5	91.8	99.7	4.08	1.43	2.32	4.74	1.84	4.84
BP-3	90.0	92.1	100.1	77.1	99.7	107.4	4.22	5.47	1.88	7.33	11.7	2.84
BP-1	97.8	97.8	97.7	91.3	100.1	104.3	3.85	1.27	2.67	10.6	1.47	8.26
EDP	88.6	90.5	97.2	94.3	92.2	92.7	4.03	1.47	4.54	9.70	2.80	5.54
EHMC	95.5	87.4	100.2	97.2	93.0	94.6	6.72	1.20	5.70	12.2	8.55	6.22
OC	97.7	96.6	100.5	79.9	93.0	94.0	8.33	3.33	10.7	15.1	3.37	13.1

Table 4.15 Recovery and RSD values of UV filters obtained by applying VA-DLLME-SFOD method to wastewater sample spiked with different concentrations.

UV Filter	Recovery% (n=3)		RSD% (n=3)	
	50 ng L ⁻¹	200 ng L ⁻¹	50 ng L ⁻¹	200 ng L ⁻¹
EHS	96.5	109.8	2.36	1.89
HMS	109.7	115.4	1.14	1.70
3-BC	115.0	111.4	6.63	1.71
IAMC	103.6	107.4	1.69	1.52
4-MBC	96.1	109.9	3.35	2.17
BP-3	84.9	118.9	7.15	1.56
BP-1	107.8	120.1	1.69	3.02
EDP	100.2	102.8	1.23	3.48
EHMC	95.9	111.8	4.37	4.40
OC	90.2	99.1	4.21	9.56

Overall results indicated that method developed provide an accurate and reliable method for the UV Filters having a sensitivity in sub-ppb levels. The applicability of the developed method was confirmed by validating the method in surface water and wastewater samples

The VA-DLLME-SFOD method has been applied to river and wastewater. The extracts were then derivatized in the injection port and determined by GC-MS/MS method. The chromatograms obtained were given in **Figure 4.18**.

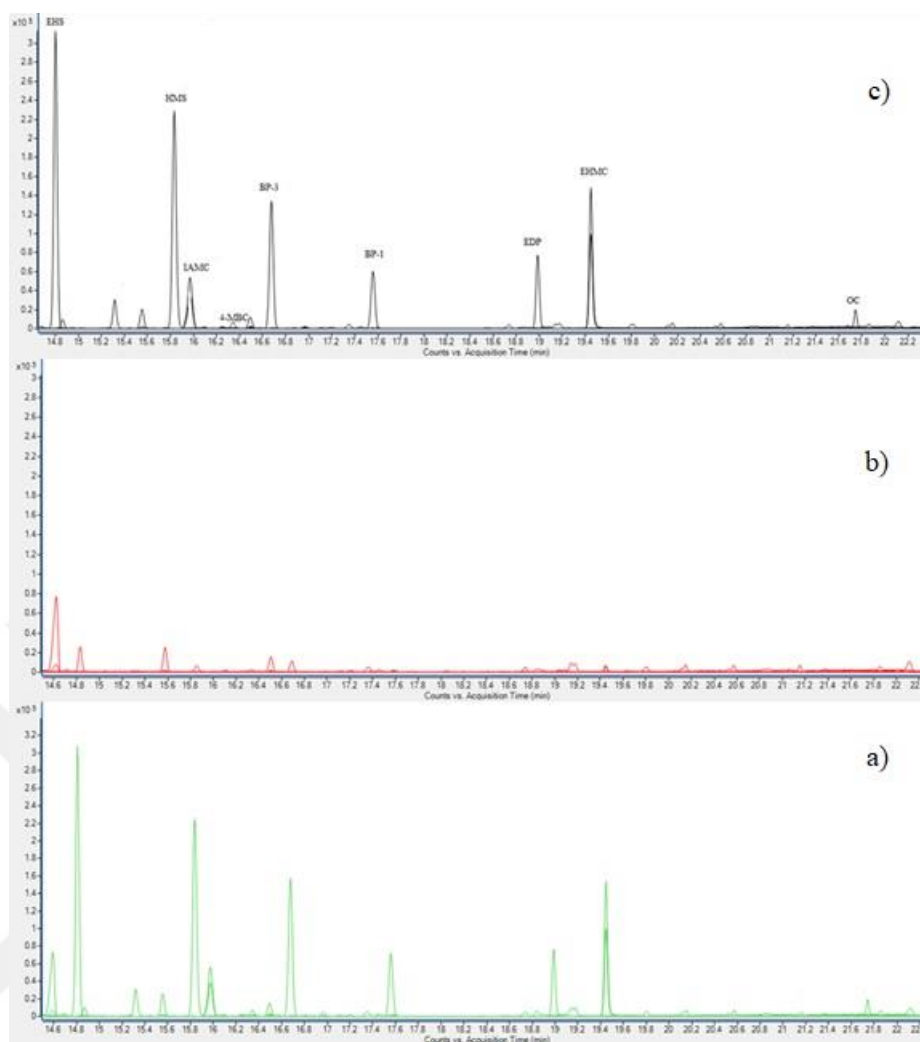


Figure 4.18 Chromatograms of the UV-filters in (a) ultra-pure water spiked with 500 ng L⁻¹ UV filters, (b) river water alone and (c) river water spiked with UV filters at 500 ng L⁻¹

The comparison of the analytical performance of the method with previously reported data are shown in **Table 4.16**. As can be deduced from the table, the method developed is quite sensitive and selective for the UV filter analysis with good precision and recovery values.

Table 4.16 Comparison of the VA-DLLME-SFOD method characteristics with previously reported other methods

UV Filter	Water matrix	Extraction/ Derivatization	Instrument	Analytical performance	Reference
BP-3, EDP, EMC, EHS, HMS, IAMC, 4-MBC, OC	RW SPW WW	DLLME None	GC-MS	LOD: 0.6-4.2 ng L ⁻¹ Rec: 80-117% RSD: 2-14%	Negreira et al., 2010
BP-1, BP-3	SW	DLLME Off-line post der.	GC-MS	LOD: 32-33 ng L ⁻¹ Rec: 65-169 % RSD: 5-6 %	Tarazona et al., 2010
BP-1, BP-3, EHS, HMS	SW	VA-DLLME Off-line post der.	GC-MS	LOD: 8-30 ng L ⁻¹ Rec: 76-120% RSD: 6-13%	Zhang and Lee, 2012
BP-3, BP-1	WW RW	SPE IPD	GC-MS/MS	LOD: 0.3-1 ng L ⁻¹ Rec: 80-105% RSD: 9-11%	Ho and Ding, 2012
BP-1, BP-3, ES, HS	RW WW	USA-DLLME Simultaneous der.	GC-MS	LOD: 1-2 ng L ⁻¹ Rec: 70-93% RSD: 2-9%	Wu et al., 2013
BP-3, EDP, EMC, EHS, HMS, IAMC, 4-MBC, OC	SW	DLLME None	GC-MS	LOD: 10-30 ng L ⁻¹ Rec: 82-117% RSD: 2-14%	Benede et al., 2014
BMDM, BP-1, BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC	WW	DLLME Off-line MW der.	GC-MS	LOD: 2-26 ng L ⁻¹ Rec: 55-105%. RSD: 2-22%	Cunha et al., 2015
EDP, EMC, EHS, HMS, IAMC, 4-MBC, OC	RW, SW, SPW	USAEME None	GC-MS/MS	LOD: 0.08-1.5 ng L ⁻¹ Rec: 60-108% RSD: 3-11%	Vila et al., 2016
BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC	RW, SW, SPW	SBDLME None	GC-MS	LOD: 9.9-26.7 ng L ⁻¹ R: 87-117% RSD: 1-15%	Chisvert et al., 2017
3-BC, EHS, HMS, IAMC, 4-MBC, BP-3, BP-1, EDP, EHMC, OC	RW	VA-DLLME-SFOD IPD	GC-MS/MS	LOD: 1.1-5.5 ng L ⁻¹ Rec: 87.4-105.3% RSD: 0.64-12.4%	Present work

4.3 Studies with the SPME Technique

In this part of the thesis, another microextraction technique, solid phase microextraction was utilized for the determination of UV filters in wastewaters. SPME is drawing great attention in the last three decades for the extraction of a wide range of pollutants from environmental samples. In this technique, volatile compounds can be collected from the dirty matrices without any solvent consumption and head space analysis provide a further selectivity for the analysis.

Here, a method development for UV filter analysis with a commercial fiber for method was intended and a polyacrylate (PA) fiber was chosen for this purpose since its structure displays medium polarity required for some of the targeted analytes. Considering the volatility of the target analytes, headspace (HS) mode was selected, and derivatization step was omitted due to the harmful effects of silylation reagent on the fiber. In the absence of this step, the most polar one of the targeted analytes i.e., BP-1 was omitted.

One major drawback of SPME fibers is their deterioration after a certain number of samples extractions which reflects in decreased efficiency of the fibers. Therefore, chemometric methods were used to optimize the conditions by performing a reduced number of experiments. The method, abbreviated as HS-SPME-GC-MS/MS was then, validated by using wastewater samples. In addition to the commercial fiber, a lab-made electrospun PAN coated SS fiber was also fabricated and their performances were compared under optimized conditions of PA fiber.

4.3.1 Optimization of HS-SPME-GC-MS/MS Method with PA Fiber

In this part of the thesis, optimization studies were started with screening the experimental parameters with Plackett-Burman (P-B) design to select the parameters with the greatest influence on the response with fewer experiments.

Only the main effects on the peak area values were considered and interactions between factors were neglected. The effects of 6 factors such as sample volume, extraction time and temperature, desorption time and temperature, and the ionic strength of the sample solutions were examined, and 5 dummy factors were also included to estimate the experimental error using in the statistical interpretation.

The levels of the 6 factors with low, central, and high values were shown in **Table 4.17** and **Table 4.18**. Total 17 studies including five center points were used. These studies were carried out with 10 mL of UPW spiked with UV filter standard solution to be 100 ng mL⁻¹ and placed in a vial set at 50-100°C for 10-30 min. Purge time was 4.7 min and flow rate was 1 mL min⁻¹.

Table 4.17 Factors and min, max values used in Plakett-Burman design

Factors		-1	+1
A	Extraction time (min)	10	30
B	Extraction temperature (°C)	50	100
C	Desorption time (min)	2.0	5.0
D	Desorption temperature (°C)	200	290
E	Sample volume (mL)	5.0	10
F	Salt amount (% w/v)	0	20

Table 4.18 Plackett-Burman 6 Factor Experimental Design for HS-SPME-GC-MS/MS

Run	Extraction time (min)	Extraction temp. (°C)	Desorption time (min)	Desorption temp. (°C)	Sample volume (mL)	Salt amount (% w/v)
1	10	100	5	200	10	20
2	20	75	3.5	245	7.5	10
3	10	100	2	290	10	0
4	10	50	5	200	10	20
5	20	75	3.5	245	7.5	10
6	30	50	5	290	5	20
7	10	100	5	290	5	0
8	10	50	2	200	5	0
9	20	75	3.5	245	7.5	10
10	30	100	5	200	5	0
11	30	50	5	290	10	0
12	30	50	2	200	10	0
13	10	50	2	290	5	20
14	20	75	3.5	245	7.5	10
15	30	100	2	200	5	20
16	30	100	2	290	10	20
17	20	75	3.5	245	7.5	10

The peak area values obtained for the UV filters after 17 runs were shown in **Table 4.19**. Regression analysis was performed according to these data.

Table 4.19 Peak area of UV filters obtained after Plackett-Burman experiments

Run	EHS	HMS	3-BC	IAMC	4-MBC	BP-3	EDP	EHMC	OC
1	75641	106434	3108	13030	3562	0	30682	7383	119
2	326238	331459	5703	30432	7769	4772	78829	30823	1444
3	174641	202457	1919	17089	3546	4777	127350	53829	2858
4	31444	29560	208	871	210	0	2810	1083	79
5	322604	330511	5154	26299	7455	4712	70354	27369	999
6	415678	367634	3888	19172	3882	18322	113217	62613	9768
7	228926	294426	4905	51742	9266	17237	337451	164660	10276
8	30089	32233	32	0	0	0	0	0	0
9	381275	395926	5768	29255	8467	4732	87441	33833	907
10	44058	71914	2062	1184	3069	0	46627	13542	322
11	281397	222076	436	4159	615	5580	45700	28894	5174
12	37394	50401	51	0	0	0	0	0	0
13	107435	84949	269	639	186	646	6787	4129	954
14	278972	307742	3703	18942	5438	3840	54775	19259	736
15	17271	31538	2272	10059	2395	0	18632	5212	164
16	199906	250701	16127	188180	24926	64530	450205	243689	13864
17	363403	388203	5718	27825	8581	3217	83688	25715	641

The most significant parameters were evaluated with the Design Expert 13 program using Pareto charts (**Fig. 4.20**). According to the ANOVA results as shown in **Table 4.20**, values of p values less than 0.05 for UV filters indicate that parameter is significant. Bold values shows the significant

Table 4.20 *p*-values of UV Filters according to ANOVA

	Intercept	$t_{\text{Ext.}}$ (min)	$T_{\text{Ext.}}$ (°C)	$t_{\text{Des.}}$ (min)	T_{Desor} (°C)	V_{Sam} (mL)	NaCl (%)
EHS	136990	28961	-13582.8	42534	97674	-3586	4239
<i>p</i> -values		0.1158	0.4355	0.0309	0.0002	0.8342	0.8047
HMS	145360	20350	14218.1	36647	91680	-1755.42	-224
<i>p</i> -values		0.2693	0.4320	0.0630	0.0005	0.9213	0.9899
3-BC	2939.75	1200	2126	-505	1651	702	1372
<i>p</i> -values		0.2116	0.0410	0.5850	0.0972	0.4517	0.1583
IAMC	25510.4	11615	21370	-10484	21320	11711	13148
<i>p</i> -values		0.3296	0.0905	0.3765	0.0911	0.3259	0.2734
4-MBC	4304.75	1510	3489	-871	2765	1172	1555
<i>p</i> -values		0.3252	0.0396	0.5632	0.0890	0.4401	0.3116
BP-3	9257.67	5481	5166,33	-2401.17	9257.67	3224	4658
<i>p</i> -values		0.1958	0,2203	0,5555	0.0426	0.4324	0.2653
EDP	98288.4	14108.4	70202.7	-2207.25	81829.9	11169.4	5434
<i>p</i> -values		0.6406	0.0397	0.9414	0.0207	0.7111	0.8566
EHMC	48752.8	10238,8	32633	-2390.33	44216.2	7060.17	5265
<i>p</i> -values		0.5374	0.0713	0.8843	0.0218	0.6688	0.7491
OC	3631,5	1250,5	969	658,167	3517.5	50.8333	526.5
<i>p</i> -values		0.1906	0.3012	0.4753	0.0032	0.9554	0.5659

As can be seen from the **Table 4.19** and **Figure 4.20**, the significant factors affecting the peak areas of the UV filters differ according to the analyte. The most important parameters were determined as extraction temperature, desorption time and temperature. Then, the optimization of the adsorption temperature, desorption temperature and desorption time parameters was performed for UV filters using a Central Composite Design (CCD). Sample volume, salt amount, and absorption time parameters were kept constant as 10.0 mL, 2 g and 30 min, respectively.

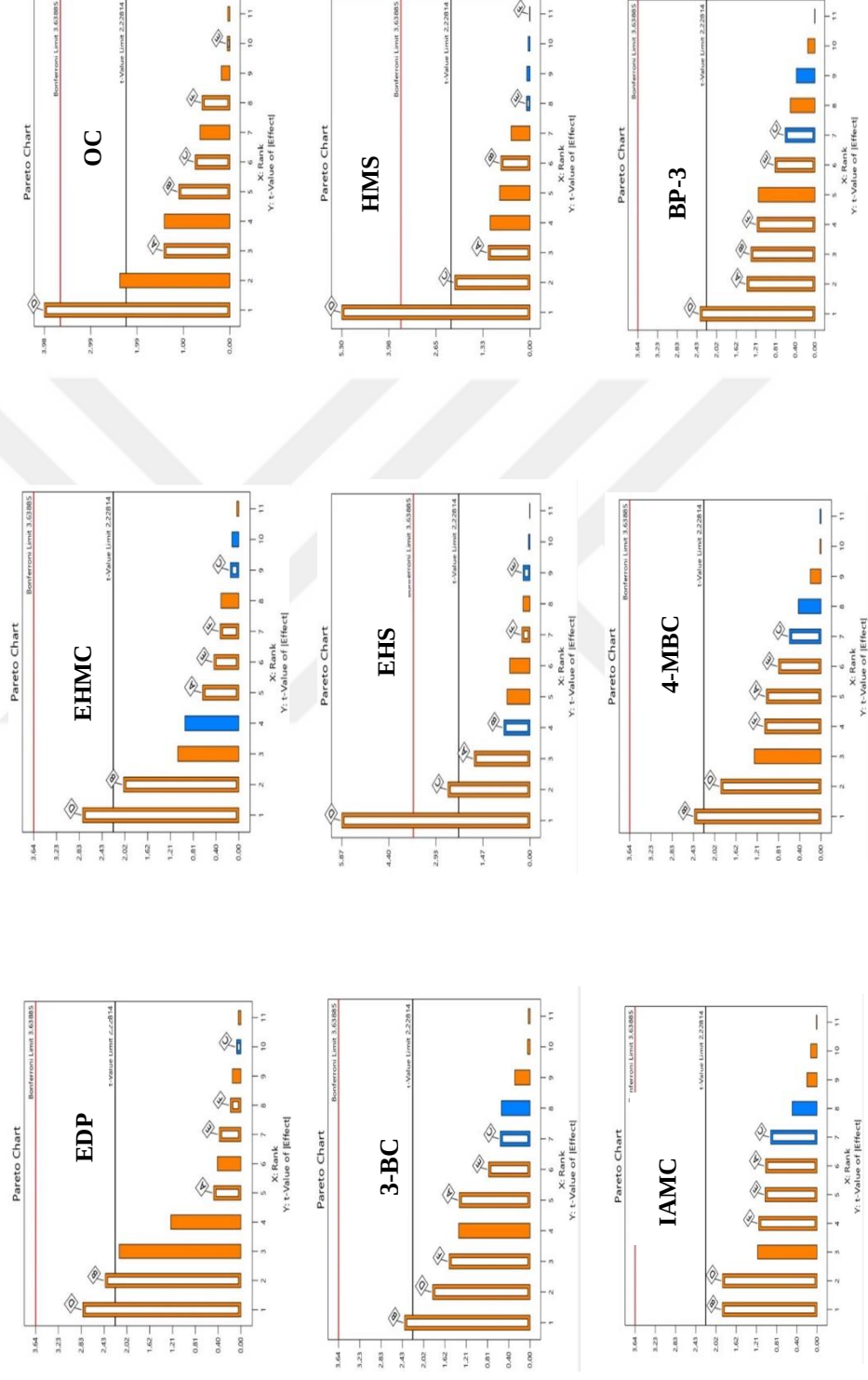


Figure 4.19 Pareto chart of UV filters for the Plackett–Burman design of screening experiment. A: Extraction time
B: Extraction temperature, C: Desorption time, D: Desorption temperature, E: Sample volume, F: Salt amount

CCD is the second-order multivariate design most widely used among response surface methodologies. This design consists of a two-level factorial design that includes all possible combinations between high- and low-level variables, a central point where all factors have central coding, and finally axial points where all factors are in the center except for the values at $+\alpha$ and $-\alpha$ levels. The number of experiments (33) was determined according to $2^k + 2k + C_p$. In this equation, C_p (5) is the number of the center points and k (3) is the number of factors. The factors affecting the peak abundance of UV filters and were determined as absorption time, desorption temperature and desorption time. The levels of the three factors with low, central, and high were shown in **Table 4.21**.

Table 4.21 The levels of the three factors with low, medium, and high in CCD

Factor	Name	Units	-1	+1	Coded Low	Coded High	Mean	SD
A	Extraction Temp.	°C	62.50	99.50	70	92	81.0	10.16
B	Desorption Temp.	°C	251.16	289.84	259	282	270.5	10.62
C	Desorption time	min	1.32	4.68	2.0	4.0	3.0	0.924

A total of 33 experiments involving two replicates for each parameter and 5 central points were performed, and the peak areas of UV filters were shown in **Table 4.22**.

Table 4.22 The results of the experiments by using CCD

A T _{Ext} (°C)	B T _{Desorp} (°C)	C t _{Desorp} (min)	Peak Area of UV filters								
			EHS	HMS	3-BC	IAMC	4-MBC	BP-3	EDP	EHMC	OC
92	259	4	241179	254710	13003	85050	17465	36475	217301	87137	4721
81	251.159	3	233465	227402	7631	27464	8427	3865	57534	23458	440
70	259	4	294145	291595	4207	11762	4109	3291	16275	7125	549
81	270.5	3	357998	326443	9003	41533	11452	14668	114375	43707	2675
70	282	4	312382	301396	6053	42563	9058	21652	145125	84470	6387
99.4997	270.5	3	196629	225457	16559	119199	23665	36909	352404	141115	8087
62.5003	270.5	3	307910	319122	5207	13228	4576	4878	10809	5116	659
81	289.841	3	363762	358738	14733	129171	22423	48014	368725	174311	12463
62.5003	270.5	3	288213	277789	3473	8974	3406	3788	10383	4968	577
92	282	2	209979	238928	9872	65539	12982	17565	173827	80323	2597
81	270.5	3	345598	326645	12764	44160	12001	14896	128184	46428	2465
81	270.5	4.68179	407562	398309	10969	64335	16038	29158	202301	81715	3495
81	270.5	3	371719	358993	12764	64515	17143	21129	196759	100254	2750
92	259	2	170351	192697	9119	48864	11189	15105	137645	58148	1940
92	282	2	232110	235292	8008	46953	10743	23053	183031	76508	3401
70	259	2	179585	188634	2789	6803	2667	1189	7178	2667	181
92	282	4	347033	349454	16837	121695	27715	44430	421606	187793	14449
92	259	4	168382	210711	12597	89815	17618	38149	254975	103234	5114
81	270.5	3	347949	335502	12083	62189	19719	19393	166556	60654	2090
81	270.5	3	387896	366811	10874	49141	13460	18135	139456	49758	2561
70	259	4	263680	266293	3746	11572	3838	2965	17542	7071	518
70	282	2	275176	263655	5476	19140	5598	6860	29869	13967	1071
70	282	2	213528	208711	4251	23094	5090	7516	60891	28065	1400
81	270.5	1.31821	230169	215298	7744	30567	9586	7083	93504	34347	981
92	282	4	273323	296368	18077	151222	26988	50934	483309	212972	13800
92	259	2	177784	197684	9273	55184	12425	17019	167778	55184	2197
70	282	4	305005	308338	9505	60596	13174	20907	148692	73122	4745
81	251.159	3	228672	241026	6125	22333	7149	4448	55472	21796	374
81	270.5	1.31821	281501	268809	9639	40869	12168	8672	122037	43380	928
81	289.841	3	383999	367350	14861	100155	21703	37062	297373	131357	10235
70	259	2	183191	209811	2882	7016	2776	1158	12245	4584	159
99.4997	270.5	3	185517	215049	17897	125720	24745	39205	385490	156779	10570
81	270.5	4.68179	431365	407257	11414	61095	15071	24366	165770	66709	2897

Table 4.23 The Fit Statistics of CCD Model

EHS				HMS			
SD	38512	R ²	0.8207	SD	32498.12	R ²	0.8151
Mean	2.787E+05	Adjusted R ²	0.7505	Mean	2.803E+05	Adjusted R ²	0.7427
C.V. %	13.82	Predicted R ²	0.6144	C.V. %	11.59	Predicted R ²	0.6069
		Adeq Precision	10.069			Adeq Precision	10.221
3-BC				IAMC			
SD	1760.46	R ²	0.8207	SD	14536.11	R ²	0.8915
Mean	9679.85	Adjusted R ²	0.7505	Mean	56106.55	Adjusted R ²	0.8664
C.V. %	18.19	Predicted R ²	0.6144	C.V. %	25.91	Predicted R ²	0.8252
		Adeq Precision	10.0691			Adeq Precision	20.6523
4-MBC				BP-3			
SD	2720.64	R ²	0.8996	SD	4795.64	R ²	0.9144
Mean	12914.15	Adjusted R ²	0.8603	Mean	19513.24	Adjusted R ²	0.8947
C.V. %	21.07	Predicted R ²	0.8012	C.V. %	24.58	Predicted R ²	0.8642
		Adeq Precision	16.784			Adeq Precision	22.8799
EDP				EHMC			
SD	39043.43	R ²	0.9250	SD	17984.14	R ²	0.9278
Mean	1,620E+05	Adjusted R ²	0.9077	Mean	68734.00	Adjusted R ²	0.8995
C.V. %	24.11	Predicted R ²	0.8810	C.V. %	26.16	Predicted R ²	0.8574
		Adeq Precision	25.957			Adeq Precision	20.735
OC							
	SD	1386.05	R ²	0.9184			
	Mean	3862.91	Adjusted R ²	0.8865			
	C.V. %	35.88	Predicted R ²	0.8247			
			Adeq Precision	19.699			

After optimization of the SPME method, the results were evaluated by using ANOVA. In Fit Statistics of design, The Predicted R^2 values are in reasonable agreement with the Adjusted R^2 values as shown in **Table 4.23**. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. This model can be used to navigate the design space. The factors with a $p < 0.05$ were also considered significantly effective and given in bold in **Table 4.24**. The *Effects* values show the direction and extent of the effects.

When the coefficients of the main factors are found positive, the high-value factors will have high peak areas. For negative coefficients, the peak areas are high at low values of the main factors. Positive coefficients indicate a synergistic effect of AxB, AxC and BxC interactions, while negative coefficients indicate a reverse effect.

The effect of 3 factors with p values < 0.05 was found to be significant with 95% probability. Desorption temperature and desorption time showed positive effects for all analytes, while extraction temperature showed positive effects for analytes except EHS and HMS. Also, it was revealed that the interactions between the extraction temperature (A) and the desorption time (C) and between the desorption temperature (B) and the desorption time (C) were significant at the 95% confidence interval. The effect of A*C and B*C interactions was significant except for EHS and HMS and has a positive effect on extraction.

In addition, quadratic effects such as A^2 , B^2 and C^2 were also studied. As a result, the effects of extraction temperature (A^2) and desorption temperature on extraction efficiency were negative for EHS and HMS, and positive for OC. The quadratic effect of the desorption time on the extraction efficiency is negative for EHS, HMS and 3-BC. In the quadratic term, the model is convex if the sign is positive, and the curve is concave if it is negative. A positive quadratic term may indicate that the relationship is exponential.

Table 4.24 *p*-values and coded effects of factors to response in optimisation of UV filters with HS-SPME-GC-MS/MS method by using CCD.

	Intercept	A	B	C	AB	AC	BC	A²	B²	C²
EHS	366126	-20737	35535	40778	7454	-5252	3155	-53007	-32534	-20097
p-values		0.0098	<0.0001	< 0.0001	0.4467	0.5906	0.7461	< 0.0001	0.0008	0.0262
HMS	345937	-11922	30143	39690	8654	-3135	4655	-38490	-24604	-16194
p-values		0.0677	<0.0001	< 0.0001	0.2978	0.7031	0.5722	< 0.0001	0.0022	0.0329
3-BC	11650.7	3706	1724	1492	-179	1008	836	-701	-682	-999
p-values		< 0.0001	<0.0001	0,0002	0.6885	0.0315	0.0701	0.0829	0.0910	0.0166
IAMC	56106.5	31352	18916	14369	-109	10050	8813			
p-values		< 0.0001	<0.0001	< 0.0001	0.9763	0,0103	0.0225			
4-MBC	14953.9	5814	3195	2644	12.5	1775	1784	-815	-522	-1127
p-values		< 0.0001	<0.0001	< 0.0001	0.9854	0.0157	0.0152	0.1856	0.3908	0.0719
BP-3	19513.2	10640	7566	7060	-1193	4072	2282			
p-values		< 0.0001	<0.0001	< 0.0001	0.3286	0.0022	0.0681			
EDP	161952	102769	63909	43527	9546	31091	35617			
p-values		< 0.0001	<0.0001	< 0.0001	0.3371	0.0037	0.0012			
EHMC	60695.8	41161	31853	20589	4732	12404	17219	4382	8178	-2850
p-values		< 0.0001	<0.0001	< 0.0001	0.3035	0.0112	0.0009	0.2785	0.0497	0.4777
OC	2544.23	2288	2536	1643	505	1160	1530	766	1085	-259
p-values		< 0.0001	<0.0001	< 0.0001	0.1586	0.0028	0.0002	0.0193	0.0016	0.4042

The extraction efficiency of the SPME method depends on the extraction temperature (A), desorption temperature (B) and desorption time. As shown in **Figure 4.20-4.22**, the peak areas of UV filters are at the highest value when the extraction temperature and desorption temperature are 75°C and 270°C for EHS and HMS, respectively. For other analytes, the peak area values decrease below 92°C and 290 °C, respectively. The peak areas of all analytes decrease below 4.7 min of desorption time.

The optimized values of factors in HS-SPME method were determined as 87.6 °C, 4.7 min and 290°C as the extraction temperature and desorption time and temperature, respectively.



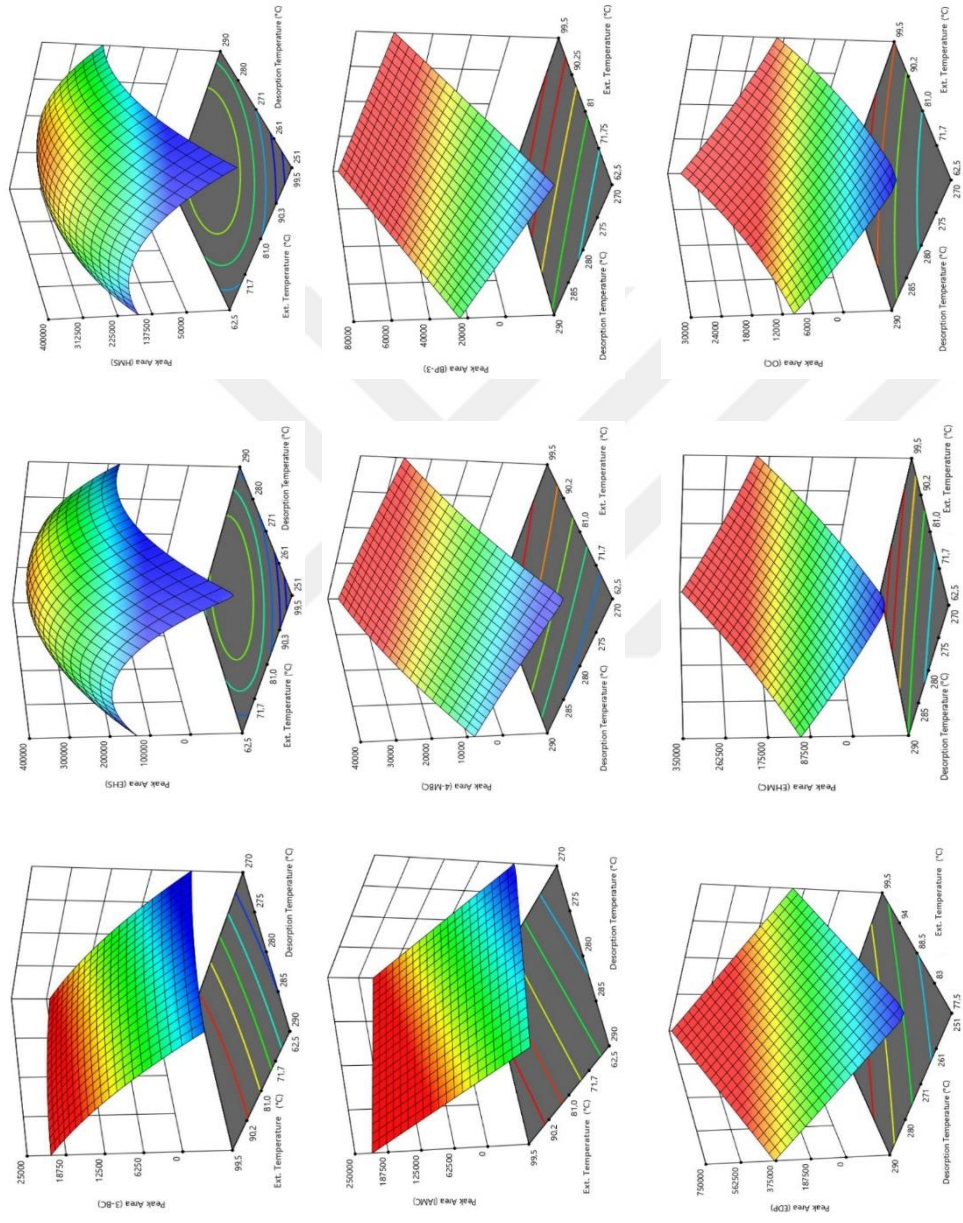


Figure 4.20 Estimated response surfaces with related contours by plotting peak area versus extraction temperature and desorption temperature. Desorption time is 4.7 min.

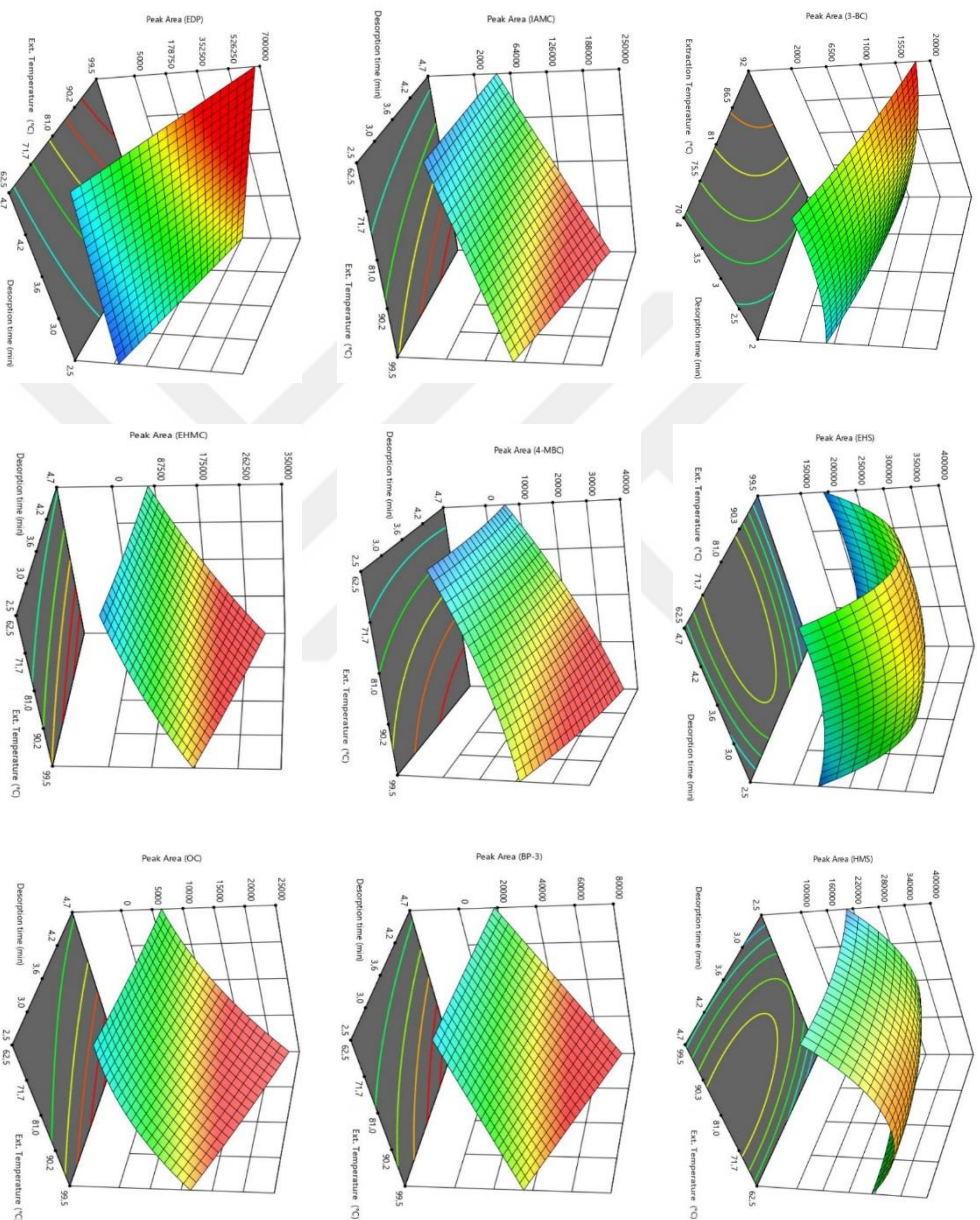


Figure 4.21 Estimated response surfaces with related contours by plotting peak area versus extraction temperature and desorption time. Desorption time is 4.7 °C

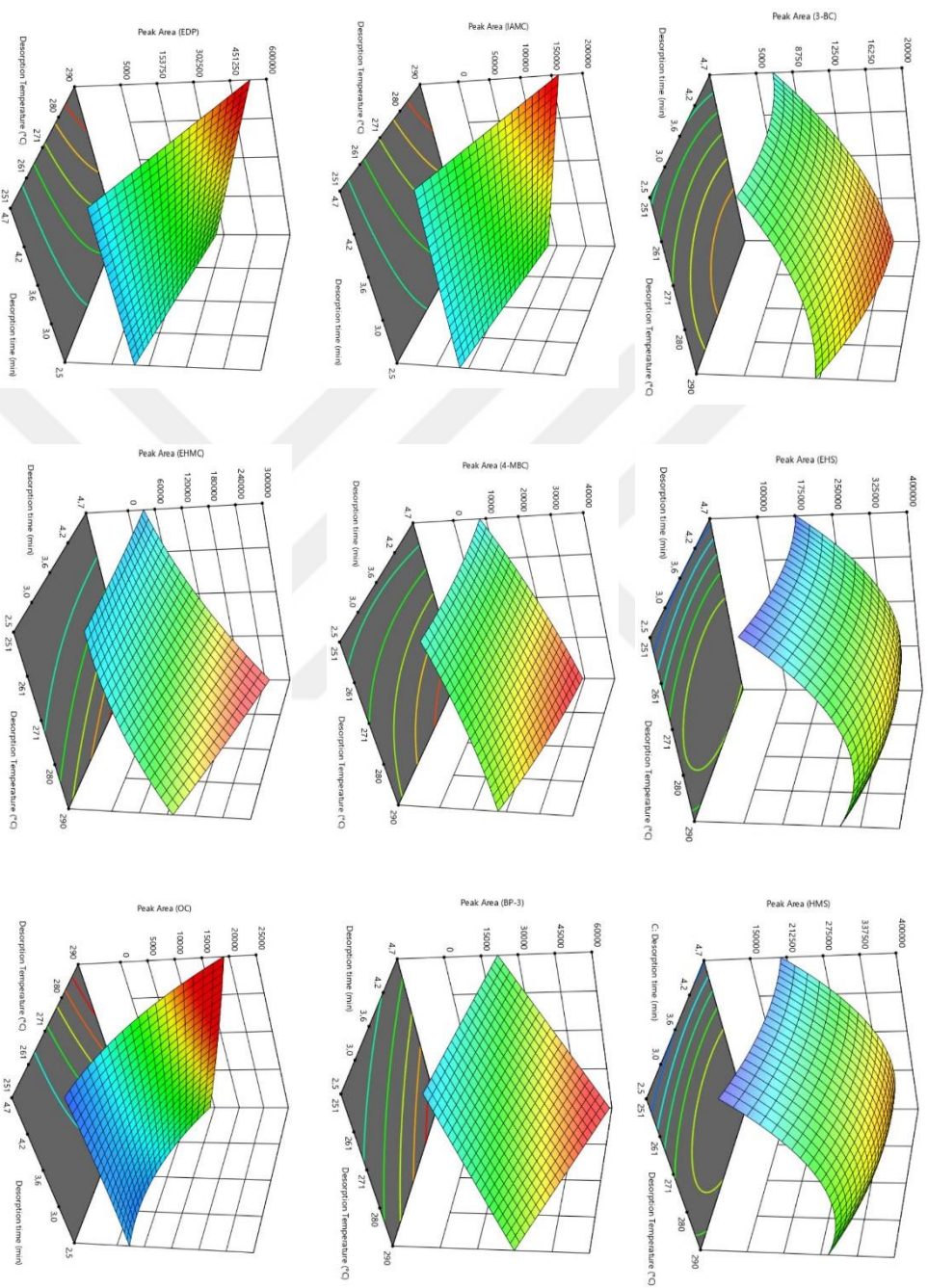


Figure 4.22 Estimated response surfaces with related contours by plotting peak area versus desorption temperature and desorption time. Extraction temperature is 86,7 °C

4.3.2 Validation of HS-SPME-GC-MS/MS Method

Chromatograms of UV filters obtained under optimized conditions were given below. **Figure 4.23** shows the chromatogram recorded for ultrapure water spiked with UV filters at 200 ng L⁻¹ concentration. The developed and validated HS-SPME method has been applied to wastewater sample and the chromatograms obtained for wastewater and the wastewater spiked with UV filters at 200 ng L⁻¹, were given in **Figure 4.24** and **Figure 4.25**, respectively.

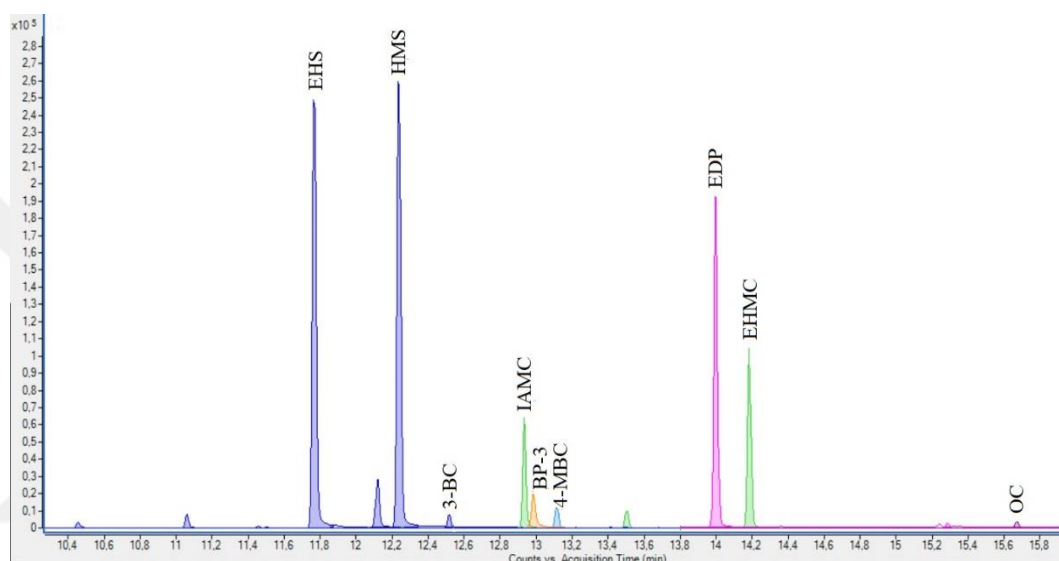


Figure 4.23 Chromatograms of UV filters in UPW obtained under optimized conditions.

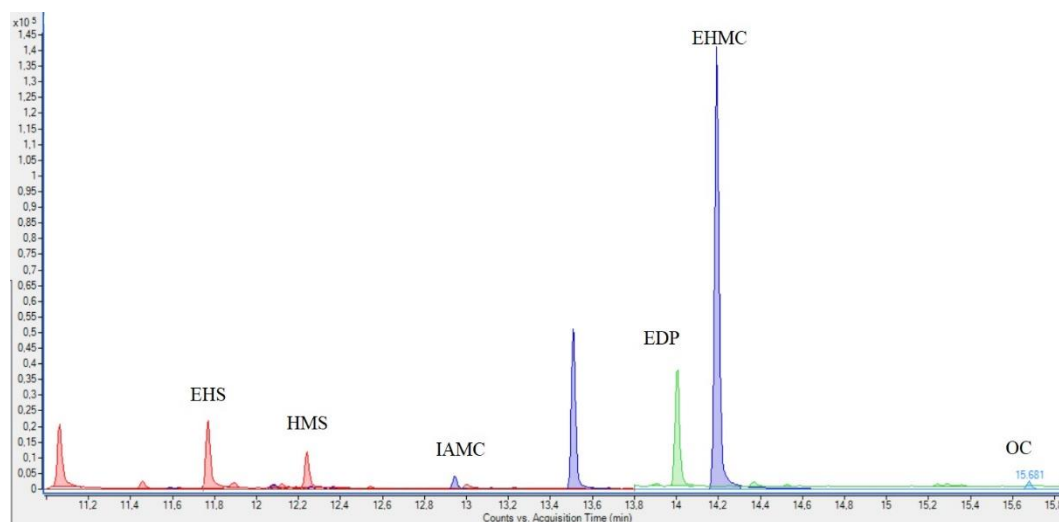


Figure 4.24 Chromatogram of UV Filters in wastewater sample.

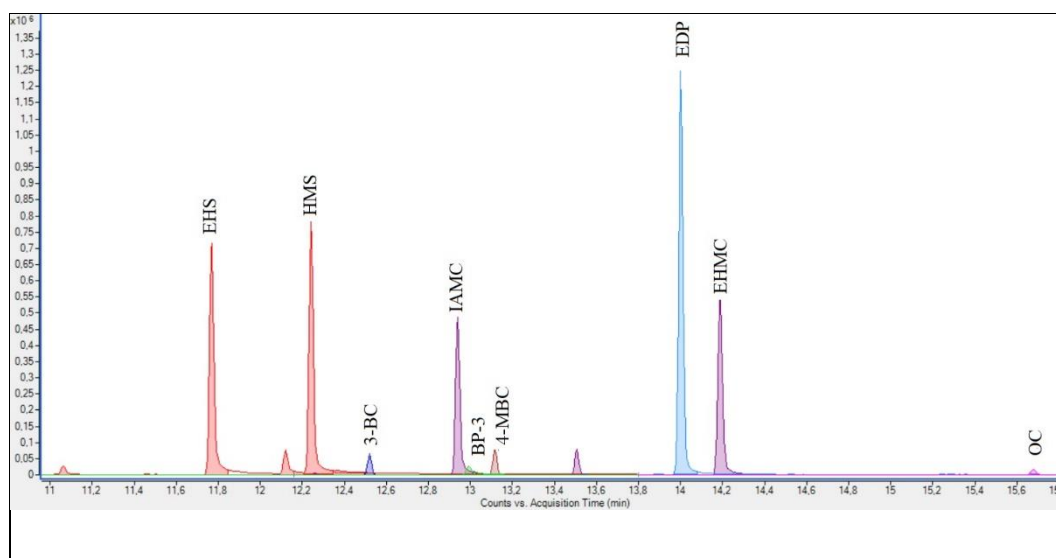


Figure 4.25 Chromatogram of the wastewater sample spiked with 200 ng L⁻¹ UV Filters.

As can be followed from the chromatograms, well separated and symmetrical peaks were observed for the targeted analytes. As it is clear from **Figure 4.24**, traces of EHS, EMS, IAMC, EDP, EHMC and OC was observed for the real sample and the peak heights have increased upon spiking with the standard solutions as can be seen from **Figure 4.25**.

In this part of the thesis, the method was validated for the detection of UV filters in wastewater. The peak area values obtained for the wastewater sample was extracted from the peak area obtained for the spiked sample.

The linear range was determined after the determination of optimum parameters, calibration curves were constructed with matrix-match standards after performing the SPME method with PA fiber using seven different concentrations such as 20, 50, 100, 200 and 400 ng L⁻¹. Each concentration was with a correlation coefficient ranging from 0.9912 to 0.9986 was obtained for all target analytes. The calibration graphs were shown in **Figure 4.26** and **Figure 4.27**.

The LOD and LOQ values were calculated as described in Experimental Section and **Table 4.25** shows these values based on Signal-to-Noise Ratio and on the standard deviation of the response and the slope. All regression tables and LOD and LOQ values of UV filters were shown in **S1**. LOD and LOQ values were also calculated according to the standard deviation (SD) of sample spiked with minimum concentration of analyte as 3×SD and 10×SD, respectively. As shown in **Table 4.26**, SPME is a method that offers acceptable sensitivity in the analysis of UV filters in wastewater samples.

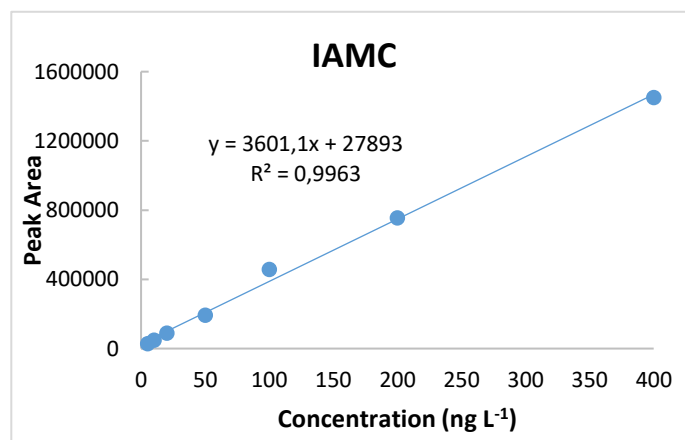
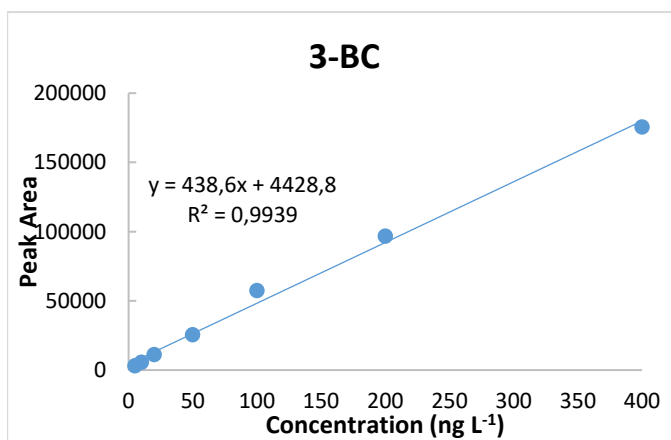
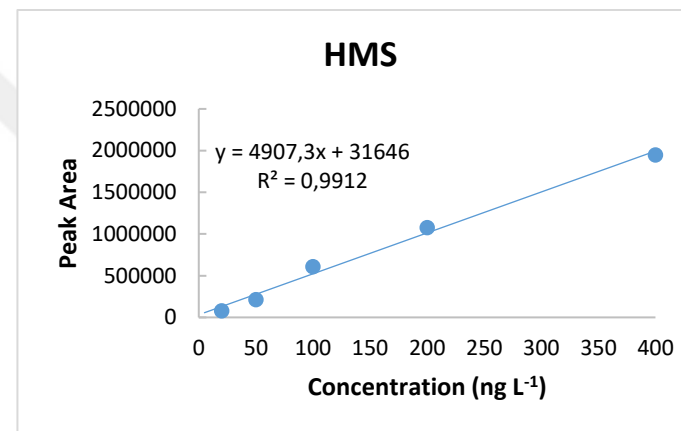
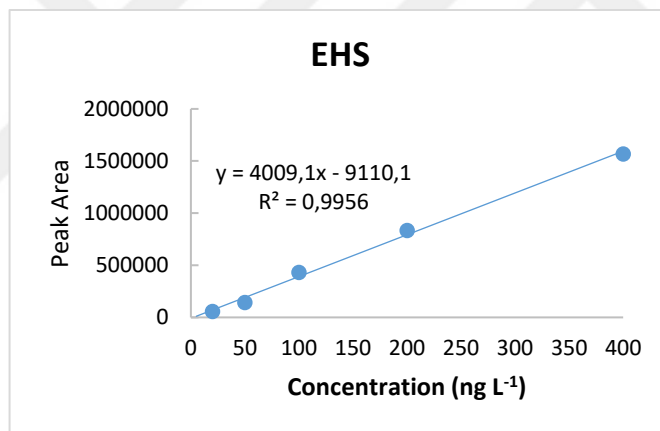


Figure 4.26 Calibration curves for EHS, HMS, 3-BC and IAMC obtained with HS-SMPE-GC-MS/MS method.

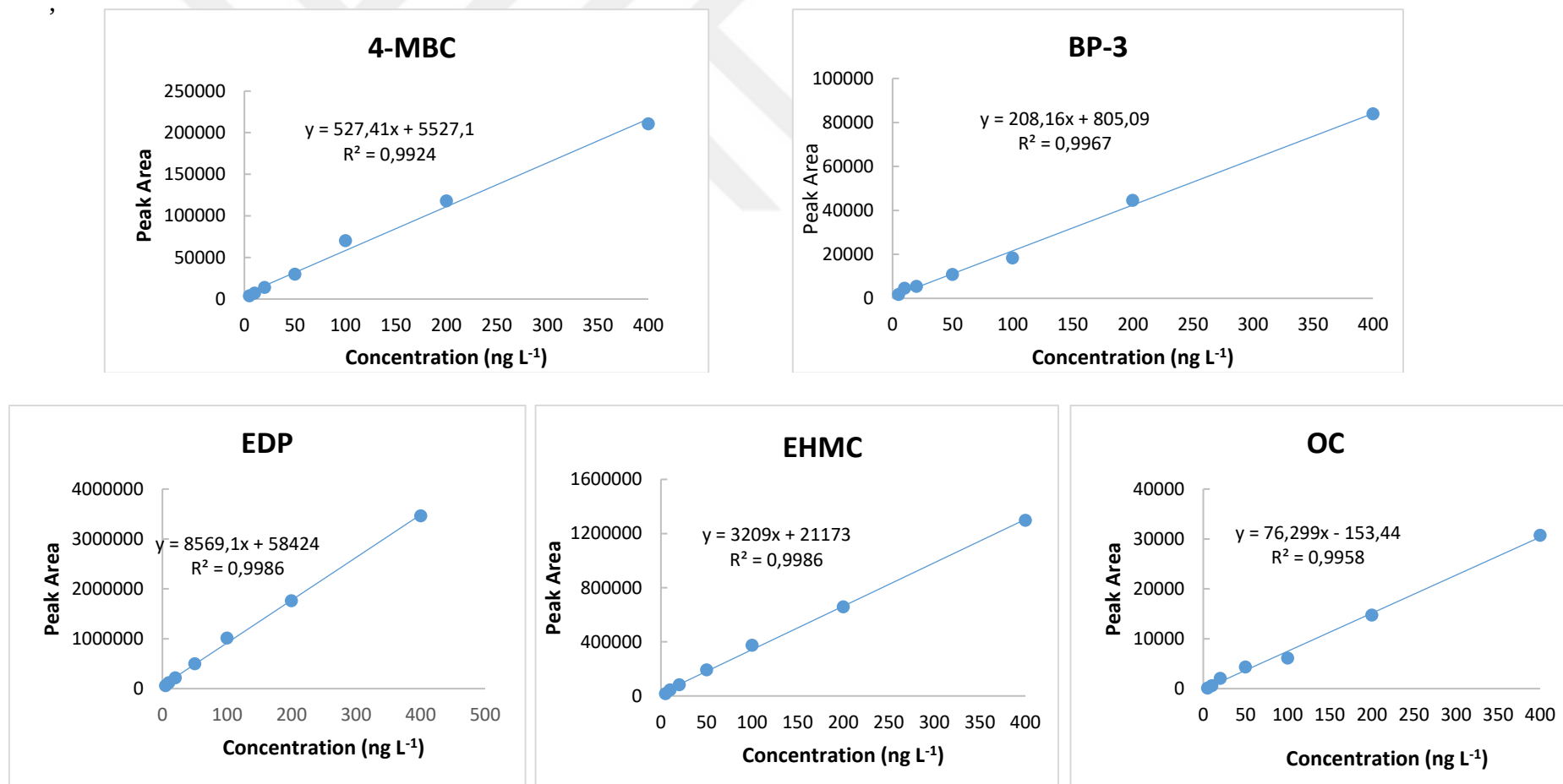


Figure 4.27 Calibration curves for 4-MCB, BP-3, EDP, EHMC and OC obtained with HS-SMPE-GC-MS/MS method.

Table 4.25 LOD and LOQ of UV filters calculated based on S/N Ratio and on the standard deviation of the response and the slope

Analyte	S/N Ratio		SD/slope		
	LOD	LOQ	σ/s	LOD	LOQ
EHS	12.0	40.0	7.92	26.2	79.2
HMS	9.92	33.0	11.2	37.1	112.4
3BC	10.07	39.6	6.12	20.2	61.2
IAMC	9.52	31.7	4.74	15.6	47.4
4MBC	8.00	26.7	6.82	22.5	68.2
BP-3	8.44	28.2	4.52	14.9	45.2
EDP	4.26	14.21	2.91	9.61	29.1
EHMC	4.21	13.9	2.89	9.52	28.9
OC	10.6	35.1	5.06	16.7	50.6

Table 4.26 LOD and LOQ levels calculated based on standard deviation of sample spiked with minimum concentration of analyte

	Day	Spiked conc. (ng L ⁻¹)			SD	LOD	LOQ
EHS	1	22.50	17.09	15.89	3.54	10.63	35.44
	2	22.50	29.56	26.92			
HMS	1	13.71	10.21	9.40	2.86	8.59	28.64
	2	11.68	17.75	17.14			
3BC	1	10.46	13.84	13.47	2.38	7.14	23.80
	2	13.84	18.62	18.78			
IAMC	1	10.72	14.60	15.19	3.26	9.79	32.66
	2	16.99	24.54	22.65			
4MBC	1	15.54	12.73	12.81	3.33	9.99	33.32
	2	10.6	18.21	19.01			
BP3	1	15.24	17.06	20.72	2.50	7.52	25.09
	2	18.61	20.92	22.99			
EDP	1	12.39	17.22	18.93	4.28	12.84	42.82
	2	17.19	25.80	25.95			
EHMC	1	19.30	17.81	20.70	4.13	12.39	41.32
	2	20.31	30.31	29.90			
OC	1	18.83	25.54	29.51	4.20	12.62	42.08
	2	25.46	23.05	28.05			

Accuracy was tested upon recovery studies. In matrix match studies, mixed standard was added to the real wastewater sample at a concentration of 20, 100, and 200 ng/L and SPME method was applied to the real wastewater sample for two days. The peak areas and mean concentration of UV filters on the first day (day-1) were shown **Table 4.27** and **Table 4.28**, respectively.

Table 4.27 The peak area of UV filters for the whole SPME procedures applied to three parallel wastewater samples by three injections on day-1.

Spiked conc.		Mean Peak Area of UV filters								
		EHS	HMS	3BC	IAMC	4MBC	BP3	EDP	EHMC	OC
Sample alone	mean	23278	25306	745	4986	1502	2313	25804	18018	479
20 ng L ⁻¹	1	104362	124240	9760	71480	15227	6290	190426	101121	1762
	2	82691	107045	11243	85438	13743	6670	231761	96330	2274
	3	77877	103104	11083	87582	13785	7431	246441	105627	2577
100 ng L ⁻¹	1	365010	486085	53384	406438	61867	22549	989063	398445	7451
	2	415609	542335	56644	414168	66875	25109	1026644	410169	9252
	3	456974	635796	58138	461809	71518	26624	1038904	392466	6607
200 ng L ⁻¹	1	859210	1104486	97436	760465	119473	46805	1788638	676850	15230
	2	854783	1134084	107441	861214	132359	49750	2161322	849419	15230
	3	775887	1062079	105593	860752	126680	52538	2081054	788482	15230

Table 4.28 Mean concentration of UV filters spiked to wastewater on day-1

	Mean concentration (ng L ⁻¹)		
UV filters	20	100	200
EHS	18.5	99.4	203.5
HMS	11.1	101.4	212.6
3BC	12.6	116.0	224.2
IAMC	13.5	109.6	220.7
4MBC	11.6	113.2	225.9
BP-3	17.7	104.0	223.8
EDP	16.2	109.0	224.8
EHMC	16.9	112.5	228.2
OC	24.6	97.6	212.7

The peak areas and mean concentration of UV filters on day-2 were shown in **Table 4.29** and **Table 4.30**, respectively.

Table 4.29 The peak area of UV filters for the whole SPME procedures applied to three parallel wastewater samples by three injections on the day-2

Spiked conc.	Run	Mean Peak Area of UV filters								
		EHS	HMS	3BC	IAMC	4MBC	BP3	EDP	EHMC	OC
Sample alone	mean	23278	25306	745	4986	1502	2313	25804	18018	479
20 ng L ⁻¹	1	104362	114293	11243	94057	1282	6991	231507	104352	2268
	2	132685	144073	13341	121252	16633	7473	305348	136465	2084
	3	122089	141073	13410	114445	17055	7903	306614	135129	2466
100 ng L ⁻¹	1	445121	588858	52207	390683	62266	21745	915184	361140	5237
	2	337579	514026	57342	442607	68195	24919	1102248	433498	7669
	3	341570	496576	55999	436955	68276	25452	1115625	440444	7489
200 ng L ⁻¹	1	640624	861222	93630	712485	110697	36966	1817189	714419	9242
	2	698914	933161	101505	774263	119837	42562	2004291	756451	12123
	3	104362	114293	11243	94057	12812	6991	231507	104352	2268

Table 4.30 Mean concentration of UV filters spiked to wastewater on day-2.

UV Filters	Mean concentration (ng L ⁻¹)		
	20	100	200
EHS	26.3	89.9	167.2
HMS	15.5	97.0	182.0
3BC	17.1	114.0	218.5
IAMC	21.4	108.5	206.0
4MBC	16.1	112.3	213.3
BP3	20.8	100.5	181.0
EDP	23.0	112.0	218.8
EHMC	26.8	116.1	219.8
OC	25.5	84.8	144.9

Recovery Recovery values of UV filters were calculated by using the concentration calculated as given in **Eq 3.3**. Calculated recovery percentages of UV filters were shown in **Table 4.31**.

Table 4.31 Recovery of UV filters determined by SPME method applied to spiked wastewaters.

UVF	Intra-day Recovery% (n=3)			Inter-day Recovery% (n=3)		
	20 ng L ⁻¹	100 ng L ⁻¹	200 ng L ⁻¹	20 ng L ⁻¹	100 ng L ⁻¹	200 ng L ⁻¹
EHS	92.5	99.4	101.7	112.0	94.6	92.7
HMS	55.5	101.4	106.3	66.6	99.2	98.7
3BC	62.9	116.0	112.1	74.2	115.0	110.7
IAMC	67.5	109.6	110.3	87.2	109.0	106.7
4MBC	58.1	113.2	112.9	74.4	112.8	109.8
BP3	88.4	104.0	111.9	96.3	102.2	101.2
EDP	80.9	109.0	112.4	97.9	110.5	110.9
EHMC	84.5	112.5	114.1	115.3	114.3	112.0
OC	123.1	97.6	106.4	125.4	91.2	89.4

Precision: Intra-day and inter-day repeatability studies were performed at low (20 ng L⁻¹), medium (100 ng L⁻¹), and high (200 ng L⁻¹) concentrations, with 3 replicates. Repeatability (intra-day) and intermediate precision (inter-day) values were calculated by ANOVA. The standard deviation of repeatability (S_r), standard deviation of between-group ($S_{between}$) and intermediate precision (S_I) were calculated as shown in **Eq. 3.4 and 3.5 and 3.6**, respectively. The intra-day (n = 3) and inter-day (n = 3, days) relative standard deviations (RSD%) of UV filters by HS-SPME were obtained with spiked solutions of the analytes at different concentration levels and shown in **Table 4.32**.

Table 4.32 Intra-day and inter-day repeatability of UV filters determined by SPME method applied to spiked wastewaters based on ANOVA.

UV Filters	Intra-day RSD% (n=3)			Inter-day RSD% (n=3)		
	20 ng L ⁻¹	100 ng L ⁻¹	200 ng L ⁻¹	20 ng L ⁻¹	100 ng L ⁻¹	200 ng L ⁻¹
EHS	15.8	14.2	5.80	32.2	15.2	17.4
HMS	21.5	13.1	7.68	29.3	15.5	14.5
3-BC	26.1	5.05	6.48	25.1	6.0	7.61
IAMC	18.7	7.45	7.84	35.4	9.08	8.13
4MBC	22.4	7.05	18.5	23.3	8.61	23.6
BP-3	13.0	9.55	7.37	15.7	11.3	19.0
EDP	21.3	8.59	8.66	30.3	10.2	10.3
EHMC	17.9	8.64	8.98	27.4	10.2	10.5
OC	18.8	18.2	14.7	21.9	26.6	34.4

Recovery values were above 66.6% and RSD values were in the range of 6.0 to 34.4%. The use of HS-SPME method following GC-MS/MS allowed us to reach LODs below 12 ng L⁻¹, which is sufficient for the analysis of these emerging pollutants in wastewater. In the proposed method, the absence of derivatization reduces fiber damage and stripping and allows the fiber to be used for further analysis. However, BP-1 analyzed in the method including the derivatization step after VA-DLLME-SFOD could not be determined by the HS-SPME method. Because derivatization is necessary for the determination of UV filters with high polarity such as BP-1.

4.3.3 Evaluation of the Method Performance

The method exhibited wide linear ranges for the three analytes with good R² values ranging from 0.9912 to 0.9986. As given above, the LOD and LOQ values were calculated in three different methodologies by using wastewater samples. According to the S/N values these limits were found in a range of 4.21-12.0 ng L⁻¹ and 13.9-40.0 ng L⁻¹, respectively. Quite similar limits were obtained based on standard deviation of sample spiked with minimum concentration of analyte and the LOD and LOQ levels were found in the range of 7.52-12.84 ng L⁻¹ and 25.09-42.82 ng L⁻¹ respectively.

Literature survey revealed that there is only one study focused on the determination of UV filters (EHS, HMS, BP-3) in a wastewater sample (Negreira et al., 2009). In this study, the calibration curve was constructed by adding analytes to the procedural blank and the LOQ (0.5-5 ng L⁻¹) levels belong to pure solutions, rather than real matrix. For the wastewater sample spiked with 200 ng L⁻¹ standard solution, the recovery and reproducibility values were calculated in the range of 95-115% and 4-11%, respectively. Considering complex matrices such as wastewater sample, the LOD and LOQ levels found in this thesis, can be evaluated quite good for the determination of UV filters in such samples. In addition, the repeatability values at 20 ng L⁻¹ were within acceptable limits. Comparison of the developed method with previous studies were given in **Table 4.33**.



Table 4.33 Comparison of HS-SPME method with previous studies.

UV filters	Matrix	Sample preparation method	Calculation method of LOD	Analytical performance	Reference
BP-3, EDP	SP, SW	(DI)SPME, (HS)SPME PDMS	S/N	LOD: 220–1340 ng L ⁻¹ Rec: 82–98% RSD: 5–8%	Lambropoulou et al. 2002
BP-1, BP-3, EHS, HMS	RW, WW	(DI)SPME PDMS-DVB Deriv. with MSTFA	SD/slope	LOD: 0.15–3 ng L ⁻¹ Rec: 48–115% RSD: 1–13%	Negreira et al., 2009
4-MBC, OC	RW	(DI)SPME PDMS	3 SD	LOD : 1.3–2.0 ng L ⁻¹ Rec: 64–117% RSD: n.a.	Liu et al., 2010
BP3	TW	(HS)SPME PA Deriv. with BSTFA	S/N	LOD: 2–9 ng L ⁻¹ R: n.a. RSD: < 20%	Basaglia and Pietrogrande, 2012
BP-3, EDP, EHMC, EHS, HMS, IAMC, OC, 4-MBC	RW, SP, SW	HS SPME PA	S/N	LOD: 0.16–12 ng L ⁻¹ Rec: 64–128% RSD: 3–19%*	Vila et al., 2016
BP-1, BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC	RW, SPW, SW	(DI)SPME DVB/CAR/PDMS (0.1 g K ₂ CO ₃), Pre-deriv. Acylation	S/N	LOD: 0.045–8.2 ng L ⁻¹ Rec: 80–106% RSD: 3–18%	Vila et al., 2017
BP-3, EDP, EHMC, EHS, HMS, IAMC, 3-BC, 4-MBC, OC	WW	HS SPME PA	S/N	LOD: 4.2–12.0 ng L ⁻¹ Rec: 55.5–123.1% RSD: 5.05–34.4%	This study

4.4 The Studies with Lab-made PAN Fiber

HS-SPME-GC-MS/MS method was also applied to wastewater samples by using laboratory made PAN coated SS fiber. **Figure 4.28** shows the chromatograms obtained with PAN-SPME fiber applied to wastewater spiked with 100 ng L⁻¹ of UV filters.

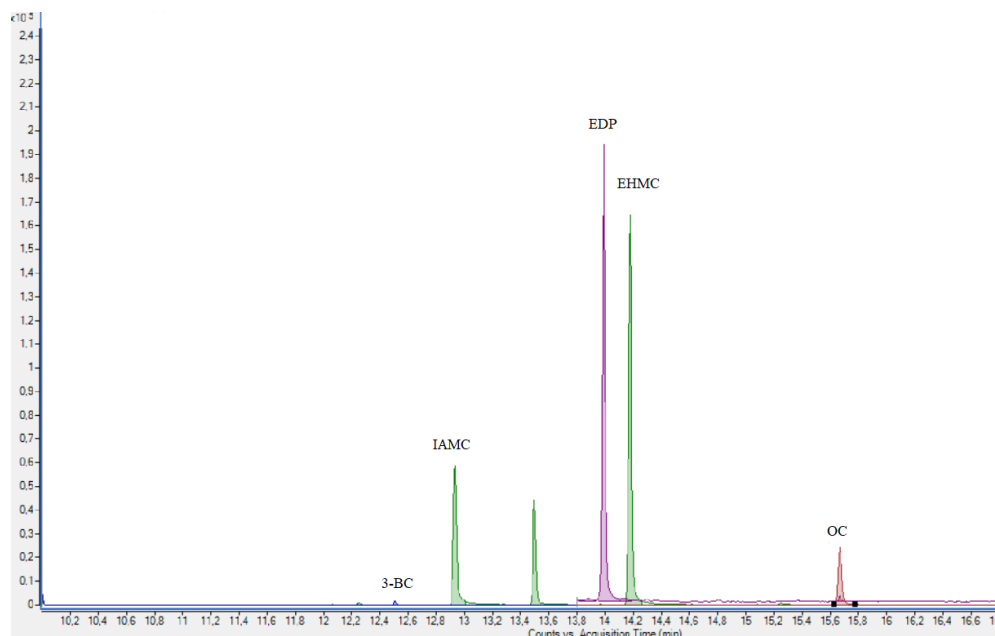


Figure 4.28 GC-MS/MS chromatogram obtained with PAN-SPME fiber applied to the wastewater sample spiked with 100 ng L⁻¹ UV filter standards.

As can be seen from the chromatogram, well-formed peaks of five UV filters can be observed namely, 3-BC, IAMC, BP-3; EDP, EHMC, OC. The procedure optimized with PA fiber was used for target UV filters and chromatographic analysis was performed by using matrix-match method. Wastewater samples were spiked with mix-standard solutions of target analytes to be, 20, 50, 100, 200 and 400 ng L⁻¹ in the final calibration solution. Two different fibers have been used to reveal fiber-to-fiber reproducibility and to observe the effect of conditions during electrospinning process.

The calibration graphs were constructed by subtracting the peak area values of the real wastewater sample from the spiked sample and plotting against the concentration of UV filter standards added into the real wastewater sample. Each concentration was with a correlation coefficient ranging from 0.9722 to 0.9988 was obtained for all target analytes except BP-3 for the first fiber.

For the second fiber it was between 0.9522-0.9956 except for 3-BC. Each concentration was studied in 3 replicates for two different fibers and linear equations can be seen in **Figure 4.29** and **4.30** respectively.

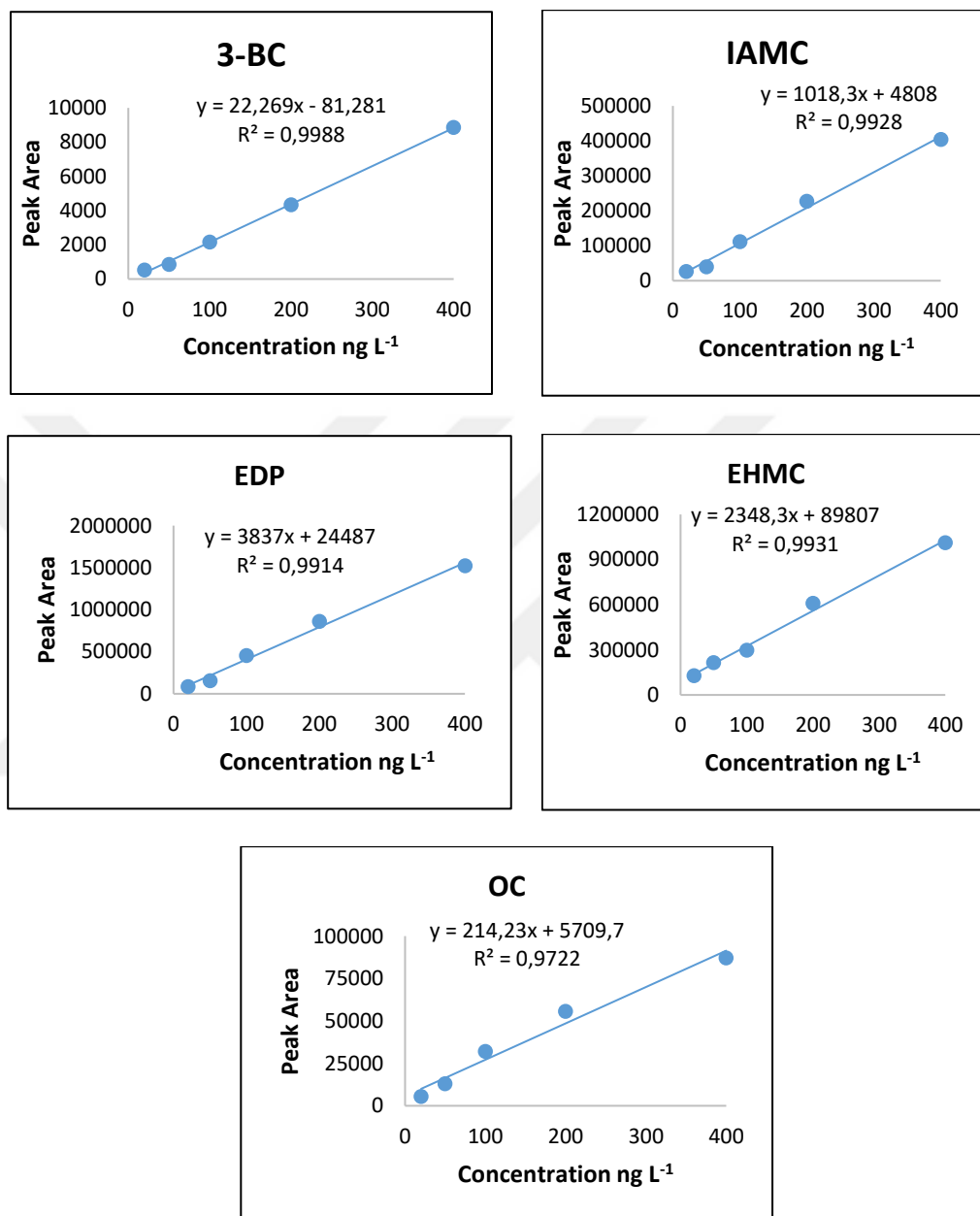


Figure 4.29 Calibration curves for UV filters injected onto GC column with the first fiber

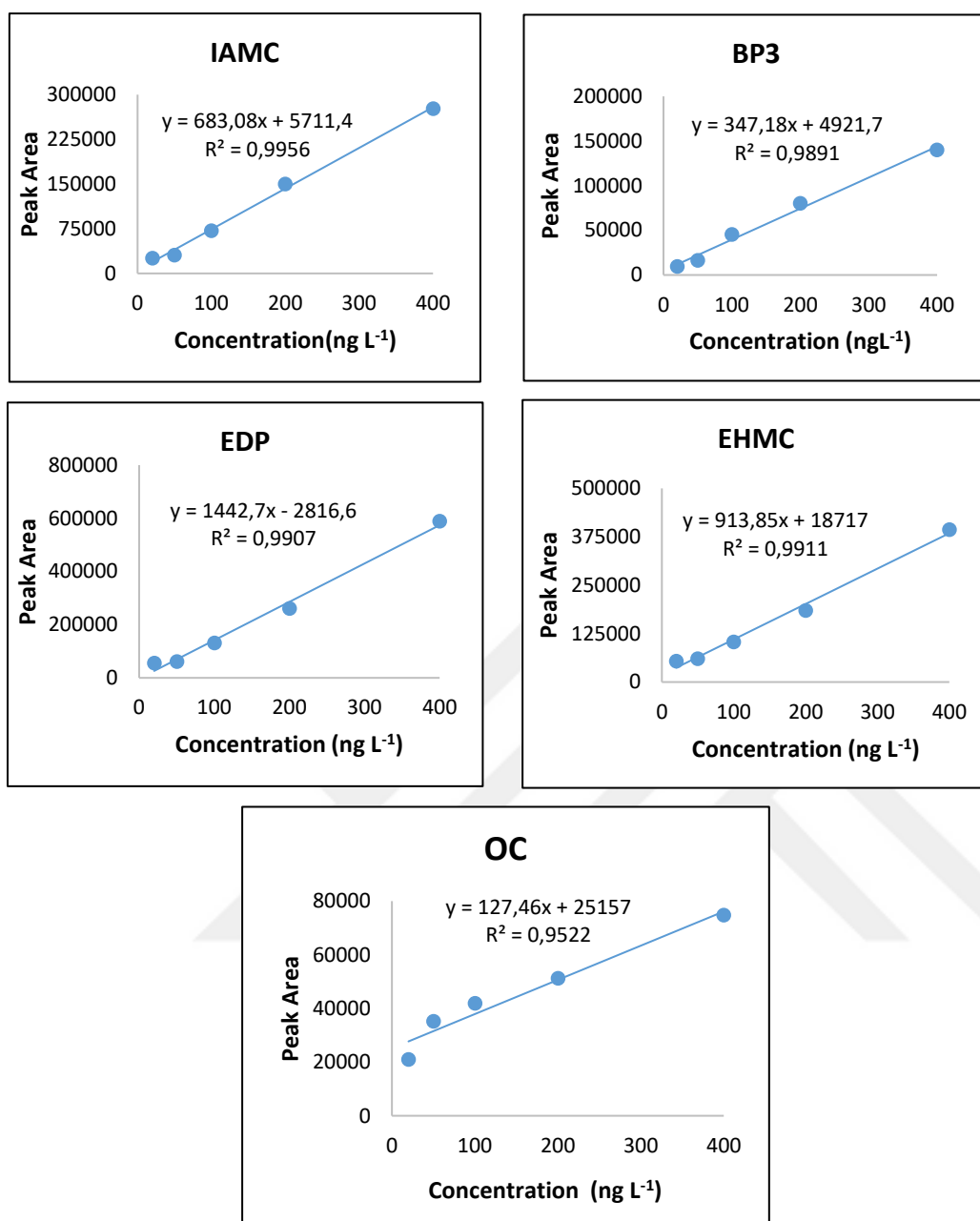


Figure 4.30 Calibration curves for UV filters injected onto GC column with the second fiber

LOD and LOQ values were calculated according to three different techniques with both fibers and the data obtained were given in **Table 4.34- Table 4.35**.

Table 4.34 LOD and LOQ of UV filters calculated based on S/N and on the standard deviation of the response and the slope with both fibers.

UV Filter	Fiber	S/N Ratio		SD/slope		
		LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)	σ/s	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)
3-BC	First fiber	30.6	101	4.19	12.6	41.9
	Second fiber	-	-	-	-	-
IAMC	First fiber	16.1	53.2	10.2	30.5	101.5
	Second fiber	6.43	21.2	9.97	29.9	99.8
EDP	First fiber	13.6	45.0	11.1	33.4	111.2
	Second fiber	2.62	8.65	8.63	25.9	86.3
EHMC	First fiber	15.5	51.0	9.91	29.7	99.1
	Second fiber	2.87	9.50	9.48	28.4	94.8
OC	First fiber	29.4	97.1	20.2	60.5	201.6
	Second fiber	-	-	-	-	-

Table 4.35 The LOD and LOQ values of each UV filters based on standard deviation of sample spiked with minimum concentration (50 ng L⁻¹) of analyte

UVF	First fiber			Second fiber		
	SD	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)	SD	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)
3BC	3.73	11.20	37.3	-	-	-
IAMC	2.68	8.04	26.8	0.48	1.44	4.82
EDP	1.56	4.69	15.6	6.85	20.6	68.5
EHMC	4.79	14.36	47.9	7.58	22.7	75.8
OC	37.33	111.99	373.3	-	-	-

As can be seen from the Table, the LOD and LOQ levels obtained for both fibers are quite different indicating fiber-to-fiber reproducibility largely depends on the electrospinning conditions. First fiber was observed to be more homogeneous and consequently, more analytes could be identified with this fiber.

Accuracy: In matrix match studies, wastewater sample was spiked with UVF standards to be 50, 200, and 400 ng L⁻¹ in the vial and then, the SPME method was applied as described before. The peak areas obtained, and mean concentrations calculated were given in **Table 4.36** and **Table 4.37**, respectively

Table 4.36 The peak area of UV filters for the whole SPME procedures applied to three parallel wastewater samples by three injections with first fiber.

Spiked conc.	Run	Mean Peak Area obtained with first fiber					Second fiber		
		3-BC	IAMC	EDP	EHMC	OC	IAMC	EHMC	EDP
50 ng L ⁻¹	1	665	46567	153512	52991	24287	18421	65365	69216
	2	828	42713	142671	70091	14070	17759	62093	87011
	3	718	41291	143651	42844	8521	17990	52060	70646
200 ng L ⁻¹	1	4473	204118	648168	149881	51288	32568	99966	103962
	2	4195	230981	679082	170398	68894	37166	106908	127735
	3	4076	209407	868705	213748	30843	36576	87595	100152
400 ng L ⁻¹	1	7347	337074	1368798	502051	70175	158968	231012	250665
	2	7688	357374	1516168	605126	73402	10552	448407	632012
	3	7044	353949	1531894	476565	72091	107985	438680	343008

Table 4.37 Mean concentration of UV filters spiked to wastewater for the whole SPME procedures with first fiber.

UVF	First Fiber						Second Fiber					
	Mean conc. (ng L ⁻¹)			Recovery% (n=3)			Mean conc. (ng L ⁻¹)			Recovery% (n=3)		
	50	200	400	50	200	400	50	100	300	50	100	300
3-BC	36.8	194.4	334.1	73.5	97.2	83.5	-	-	-	-	-	-
IAMC	38.0	206.3	338.5	76.0	103.1	84.6	23.8	48.7	189.9	47.6	48.7	63.3
EDP	31.8	184.4	377.3	63.7	92.2	94.3	44.9	86.9	308.2	89.9	86.9	102.7
EHMC	45.3	186.6	366.0	75.1	93.3	91.5	54.4	78.6	285.1	108.7	78.6	95.0
OC	46.3	208.3	308.9	92.6	104.2	77.2	-	-	-	-	-	-

Precision: Intra-day and inter-day repeatability studies were performed at low (50 ng L⁻¹), medium (200 ng L⁻¹), and high (400 ng L⁻¹) concentrations, with 3 replicates. Repeatability (intra-day) values expressed as RSD% were calculated as $(\sigma/x_{mean}) \times 100$, where σ is the standard deviation and, x_{mean} is mean of the response. and the results were shown in **Table 4.38**.

Table 4.38 Intra-day repeatability of UV filters determined by SPME method applied to spiked wastewaters with the first fiber (n=3).

UVF	First fiber RSD%			Second fiber RSD%		
	50 ng L ⁻¹	200 ng L ⁻¹	400 ng L ⁻¹	50 ng L ⁻¹	100 ng L ⁻¹	300 ng L ⁻¹
3-BC	10.02	4.70	4.33	-	-	-
IAMC	7.05	6.77	3.15	2.02	7.36	19.2
EDP	4.90	16.9	6.21	16.9	12.3	45.5
EHMC	36.9	15.5	3.23	12.6	13.2	48.3
OC	80.6	42.6	2.45	-	-	-

According to the tables, first fiber has provided better RSD values for most of the targeted analytes except the OC at 50 ng L⁻¹. Among the concentrations for which reproducibility studies were performed, the closest concentration to the calculated LOQ for OC was 200 ng L⁻¹, and the RSD % value was acceptable at sub-ppb concentrations. The LOQ values calculated for other UV filters and the RSD values obtained at this concentration were at an acceptable level.

Since the conditions used in electrospinning technique effect the analytical characteristics of the method, the fiber surface was examined closely to see the surface morphology. **Figure 4.32** shows the SEM images of PAN fiber at 25 000x magnification.

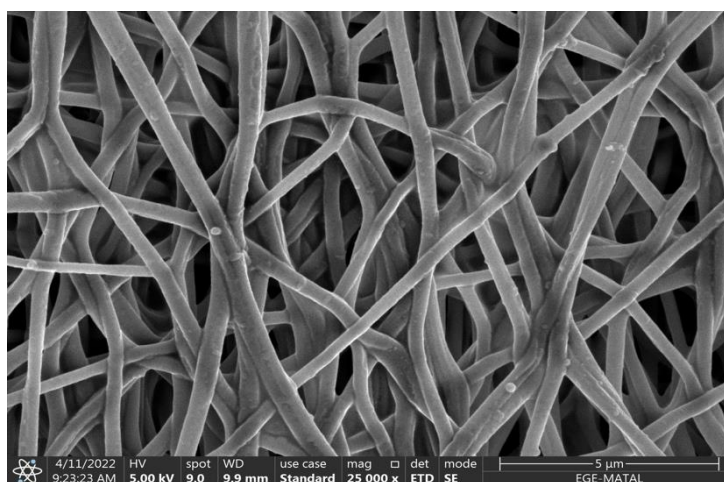


Figure 4.31 SEM images of PAN-SPME fiber obtained.

As can be clearly seen in figure, stainless steel surface was covered with nanofibers which enlarge the surface area suitable for the attachment of volatile species such as UV filters. Thus, high extraction efficiency can be obtained by this means. Overall data has revealed that lab-made PAN fiber has a great potential for UV filter analysis but, fiber-to-fiber reproducibility was not satisfactory.



5. CONCLUSION

Organic UV filters have been widely used in cosmetics and industry for UV protection and their growing use increases environmental contamination and exposure through the food chain. Therefore, accurate and sensitive methods are being searched for their trace level residues in environmental water samples.

In this thesis, the main objective was to develop novel green analytical methods for trace analysis of UV filters in surface water and wastewater samples with good recovery values. For this purpose, ten UV filters (3-BC, BP-1, BP-3, EDP, EHMC, EHS, HMS, IAMC, 4-MBC, OC) representing different groups were selected according to their NOEC values. and both solvent-based and sorbent-based microextraction techniques have been utilized for the subsequent GC-MS/MS detection of UV filters.

In the first part of the thesis, dispersive liquid liquid microextraction (DLLME) based on solidification of floating organic droplet (SFOD) was developed for targeted analytes. Following the extraction, in-port silylation was used for the polar analytes to obtain more volatile derivatives.

Central Composite Faced Design (CCFD) was used for the statistical optimization of experimental parameters. Solution parameters were established as 460 μL acetone and 30 μL of 2-dodecanone as the dispersion and extraction solvent. Since very small amount of less toxic nonchlorinated solvent is required, this technique provides an eco-friendly approach for the extraction of the analytes. Floating characteristic of the solidified solvents have facilitated the easy collection of the extract.

The extract was then injected into the system together with derivatization reagent, BSTFA, in sandwich mode. This constitutes another green aspect of the method since a very small volume of reagent is used and this process shortens the analysis time about 30 min. Whole procedure takes 25 min till injection.

Validation studies have been carried out in surface water samples and the LOD levels were calculated in the range of 1.1-5.5 ng L^{-1} where the extraction efficiency which shows the enrichment degree was in the range of 231-350. Method performance in determining the UV filters of different polarities was found comparable to the previous studies in terms of sensitivity and precision.

Second part of the thesis include studies based on solid phase microextraction (SPME) carried out with commercial PA fiber and a lab-made PAN coated SS wire. This technique is known as one of the green extraction techniques since it does not require any solvent. Method development for UV filter analysis was accomplished with a polyacrylate (PA) fiber which displays medium polarity required for some of the targeted analytes. Considering the volatility of the target analytes, headspace (HS) mode was selected, and derivatization step was omitted due to the harmful effects of silylation reagent on the fiber. In the absence of this step, the BP-1 which is the most polar analyte was omitted.

Chemometric approach was adopted to optimize the conditions by performing a reduced number of experiments to prevent deterioration of the fiber coating. Solution and instrumental parameters have been optimized and the most significant factors were established as extraction temperature along with desorption time and temperature. Then, the HS-SPME-GC-MS/MS method was validated in wastewater samples and the LOD levels have been determined by using three different approaches to see the differences. Relatively high LOD levels were obtained than the literature however, the calibration studies of previous reports were mostly conducted in pure standards while this study was carried out in wastewater samples, a rather complicated matrix. Headspace technique helps extend fiber life and provides a practical way for UV filter detection in trace levels.

In addition to the commercial fiber, a lab-made electrospun PAN coated SS fiber was also fabricated and their performances were compared under optimized conditions of PA fiber. The data obtained has revealed that lab-made PAN fiber has a great potential for UV filter analysis but, electrospinning conditions should be optimized to enhance the fiber-to-fiber reproducibility. Since this fiber is produced from waste textile products, it represents a novel and valuable material converted from waste. Future studies will include the optimization of electrospinning conditions as well as subsequent oxidation conditions to achieve better analytical performance.

In conclusion, UV filter residues can be determined sensitively in surface and wastewater samples by using both methods. Both methods will contribute to monitor and control the level of these emerging pollutants in surface waters as well as wastewater samples.

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APPENDICES

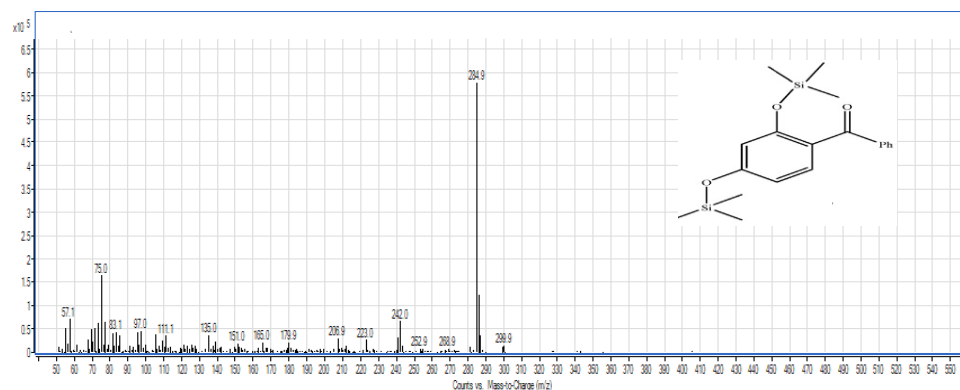


Figure A1. Mass spectra of BP-3 in full scan mode.

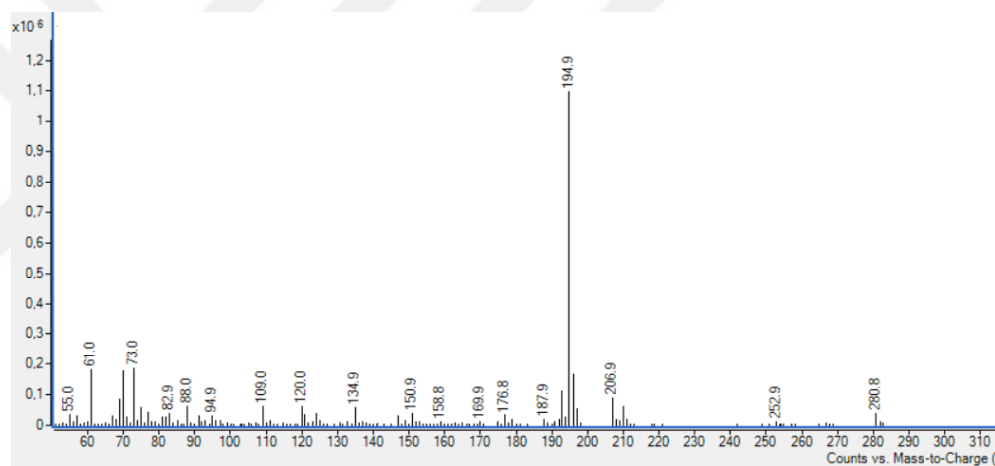


Figure A2. Mass spectra of EHS and HMS.

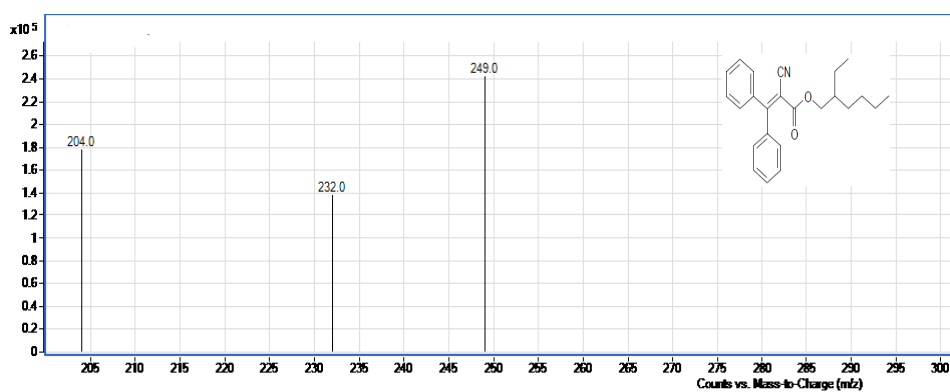


Figure A3. Mass spectra of OC in full scan mode.

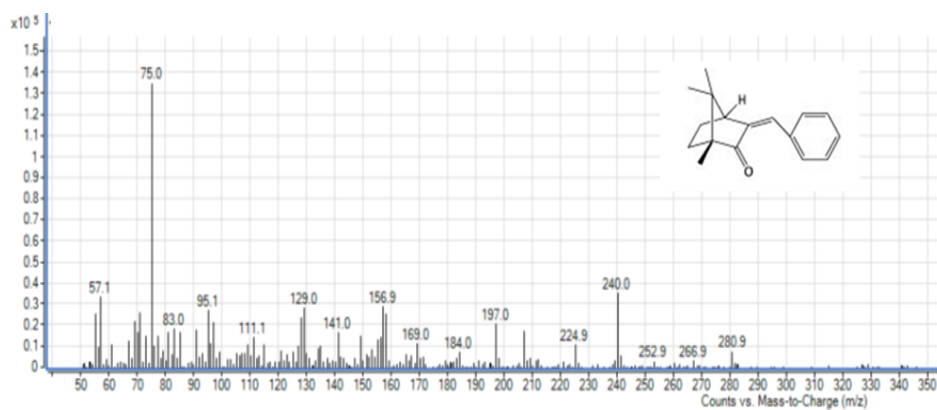


Figure A4. Mass spectra of 3-BC in full scan mode.

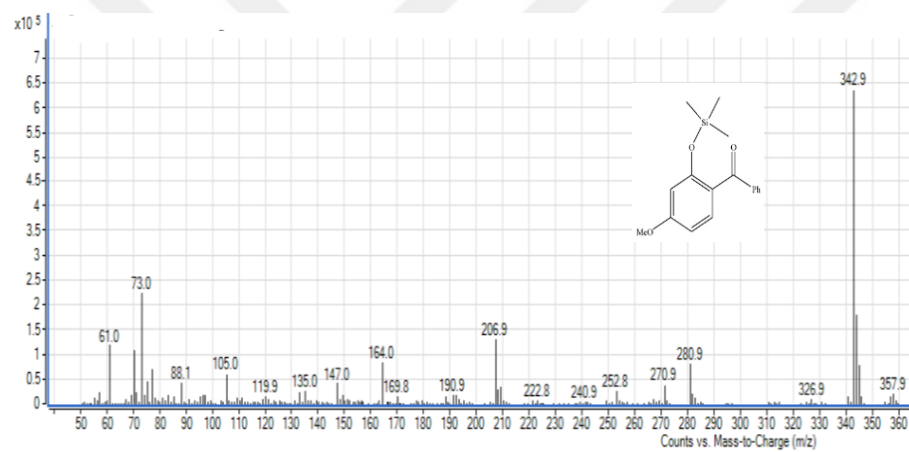


Figure A5. Mass spectra of BP-1 in full scan mode.

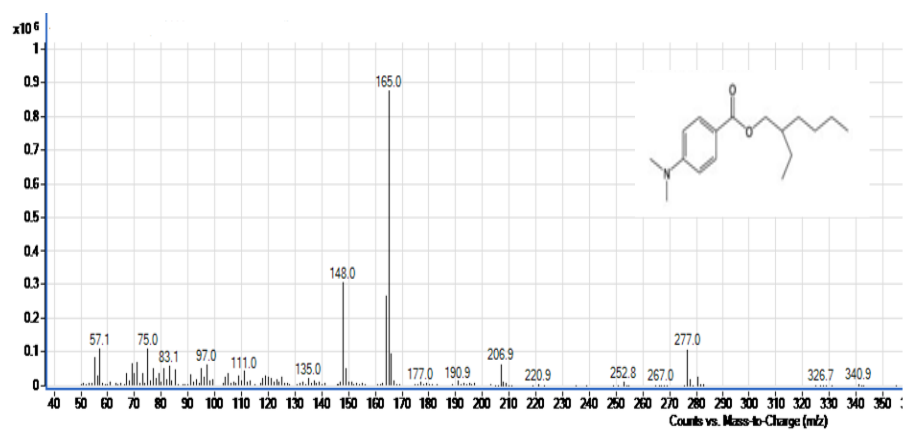


Figure A6. Mass spectra of EDP in full scan mode.

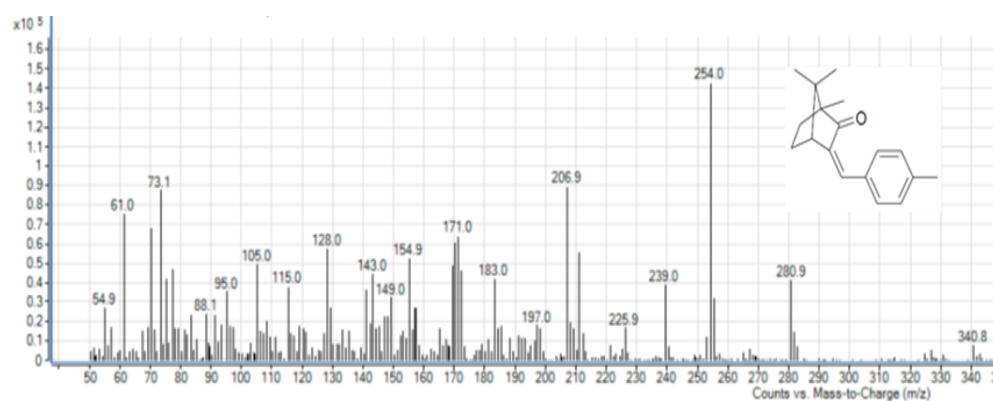


Figure A7. Mass spectra of 4-MBC in full scan mode.

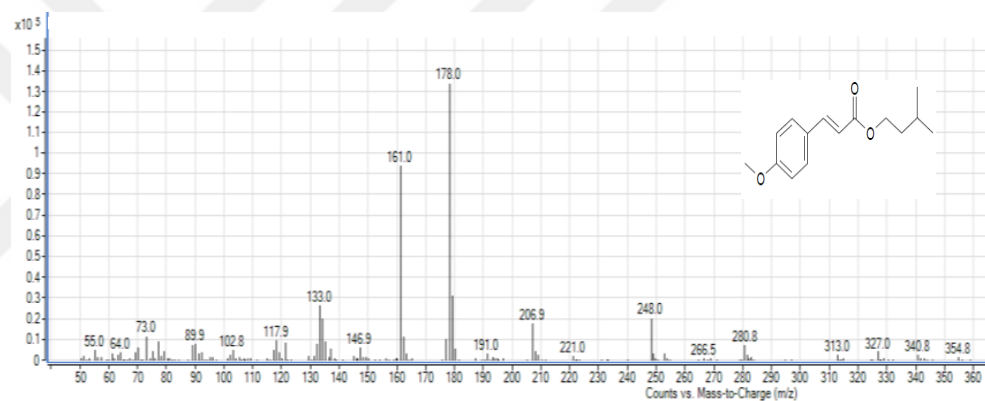


Figure A8. Mass spectra of IAMC in full scan mode.

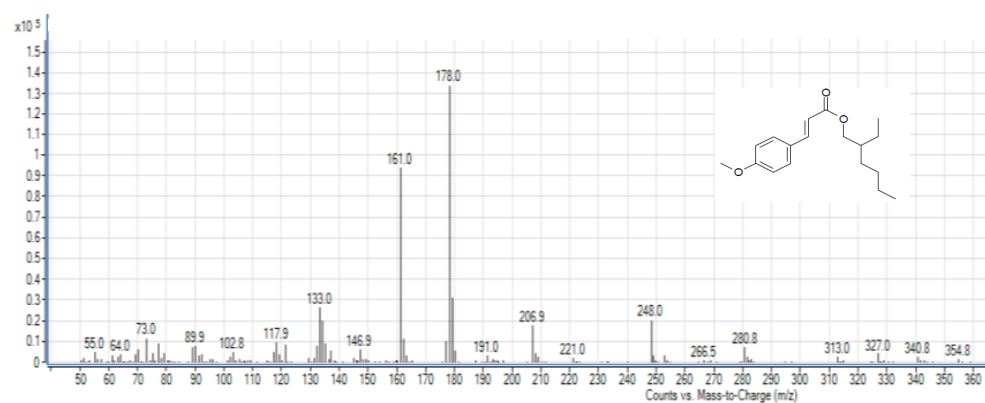


Figure A9. Mass spectra of EHMC in full scan mode.

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Authors	Publication Title	Journal Name	Volume /Page	Date
C. Yücel , H. Ertaş, F.N. Ertaş,	Gas Chromatographic Determination and Method Validation of Stigmasterol, B-Sitosterol, Campesterol and Brassicasterol Contents of Turkish Cottonseed Oil Samples	Turkish Journal of Agriculture - Food Science and Technology,	5/ 1274-1278	2017

C. Yücel, H. Ertaş, F.N Ertaş, İ. Karapınar	Determination of UV Filters in Surface Water by VA-DLLME-SFOD Technique Coupled with GC-MS/MS	<i>Clean – Soil, Air, Water</i>	50/ 2100246	2022
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International Congress

Authors	Paper Title	Conference Name	Date
Cemile Yücel, Serenay Ceren Tüzün, Hasan Ertaş, İlgi Karapınar, F. N. Ertaş	Development of DLLME- SFOD/GC-MS Method for UV Filter Determination	3rd International Congress on Analytical and Bioanalytical Chemistry, Online, Türkiye	22-26 Mart 2021
Cemile Yücel, Serenay Ceren Tüzün, Hasan Ertaş, F. N. Ertaş, İlgi Karapınar.	Validation of QuEChERS- GC MS/MS Method for determination of UV Filters in Sludges	4th International Congress on Analytical and Bioanalytical Chemistry 3rd Online-Turkey	23-26 Mart 2022

Certificates

Training Name	Institution	Date
ISO/IEC 17025	European Union Project	2007
Volumetric Calibration	TSE-SOJUZTEST	2003
GC-MS/MS	Agilent Technologies	2014
LC- MS/MS Method Thermo LC-MS/MS Cihaz Kullanıcı Eğitimi Redoks Lab. Cihazları	RedoxLab	2014
Validation and Measurement Uncertainty	European Union Project	2015