

Article

Investigating the Utility of Fabric Phase Sorptive Extraction and HPLC-UV-Vis/DAD to Determine Antidepressant Drugs in Environmental Aqueous Samples

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Abstract: Depression is considered to be one of the most prevalent mental disorders in humans. Antidepressant drugs are released in large concentrations and cause adverse effects on the environment and/or human health. Fabric Phase Sorptive Extraction (FPSE), a contemporary solid sorbent-handling technique, is a quick, sensitive, and simple analytical process. This paper describes a micro-extraction FPSE procedure coupled with High-Performance Liquid-Chromatography–Photodiode Array Detection (FPSE-HPLC–DAD) for the simultaneous extraction and analysis of five antidepressants, namely citalopram, clozapine, mirtazapine, bupropion and sertraline. Three fabric media (Whatman Cellulose filter, Whatman Microfiber Glass filter and Polylactic acid disks) and two different sol–gel sorbents (polyethylene glycol (PEG 300), alongside poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEG-PPG-PEG 5.800)) were tested. The best FPSE device was observed to be the microfiber glass filter coated with PEG 300 sol–gel sorbent. In addition, the parameters that affect the efficiency of the process (FPSE media and sorbents, sample pH, extraction time, elution time, etc.) were optimized. The proposed methodology displays a linear range with absolute recovery values higher than 60%, RSD% of less than 13% and LOQs in the range between 1.9–10.7 $\mu\text{g}\cdot\text{L}^{-1}$. Finally, the method was applied in hospital and urban effluents and lake water samples, but none of the analytes were detected.

Keywords: antidepressants; determination; experimental design; emerging contaminants

1. Introduction

Depression (major depressive disorder) is one of the most ordinary and widespread mental diseases caused by various social, psychological, and biological factors interacting and correlating among each other. It negatively affects how someone feels, their way of thinking and how the person acts. Depending on the nature and period of the symptoms, it may cause a severe health disorder that causes restricted psychosocial function, a decreased quality of life and satisfaction, feelings of culpability or low confidence, reduced sleep or hunger, poor performance, and may even result in suicide.

The pharmacological treatment of depression began in the 1950s, with the introduction of the first-generation antidepressant drugs (ADs), such as tricyclic and tetracyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs). Two decades later, the second-generation agents were

introduced, namely selective serotonin reuptake inhibitors (SSRIs). The newest and most used antidepressants drugs appeared after the 1990s, (serotonin and norepinephrine reuptake inhibitors) [1].

A concerning overconsumption of ADs has been observed in recent years. A big variety of ADs have already been detected in the range of a few $\text{ng}\cdot\text{L}^{-1}$ to several tens of $\mu\text{g}\cdot\text{L}^{-1}$ in surface waters and wastewater [2–5]. Therefore, the identification and quantification of such drugs is now of great importance in natural water, since concentration levels up to $78.3\ \mu\text{g}\cdot\text{L}^{-1}$ have been reported [5].

The literature has documented numerous analytical techniques for the determination of ADs. The most commonly used are high performance liquid chromatography (HPLC) coupled to UV–Vis or a mass spectrometric detector (MS) [6–13]. Gas chromatography (GC) coupled to MS [14–17] or tandem MS [18], and the capillary electrophoretic technique (CE) have also been reported [19]. A significant drawback of GC methods is the restriction of the target compound's volatility [20]. In comparison, HPLC can evaluate both volatile and non-volatile compounds, contributing to an adequately user-friendly separation technique [21].

Pretreatment of the sample is the most important step in the workflow of chromatographic analysis, since most samples are not able to be immediately injected into the chromatographic instrument. Various sample preparation approaches coupled with HPLC for the determination and preconcentration of antidepressant drugs in biological fluids have been reported. The most frequently employed techniques are liquid–liquid extraction (LLE) [22] and solid-phase extraction (SPE) techniques [23]. Fabric-Phase Sorptive Extraction (FPSE) has recently attracted the interest of the scientific community, particularly during its application for the determination of pharmaceutical compounds in biological samples [24,25].

Kabir and Furton first proposed FPSE as a two-step, novel and green sample preparation technique [26]. FPSE utilizes, as an extraction medium, a natural or synthetic fabric substrate, which is chemically coated in the form of an ultra-thin coating with a sol–gel organic–inorganic hybrid sorbent [27]. This microextraction technique is capable of extracting analytes within a wider polarity range, depending on the sol–gel's physicochemical properties. The porous network of sol–gel sorbent coating and the permeability of the fabric substratum contribute to the current method, which imitates the extraction of the solid phase. The desorption of analytes from the FPSE media into organic solvents is much more rapid without a significant chance of carryover. This technique overcomes the major drawbacks of other sorptive extraction procedures that exhibit limitations such as sample size and preparation time, system or coating damage, resistance to harsh chemical environments and organic solvents. FPSE has been widely used for the extraction and determination of different analytes from different sample matrices since its invention in 2014 [28–30].

In the present study, an easy, green and fast analytical methodology is proposed for the extraction of five psychoactive drugs that are commonly used in the treatment of mental diseases (citalopram, clozapine, mirtazapine, bupropion and sertraline) by employing FPSE coupled to High Performance Liquid Chromatography–Photodiode Array Detection (HPLC–DAD). The primary parameters affecting extraction efficiency were investigated and optimized using both univariate and multivariate experimental configurations of fractional factorial design followed by Response Surface Methodology. Finally, the applicability of the method was examined by obtaining natural water samples from wastewater treatment plants. To the best of our knowledge, FPSE has been extensively applied to environmental samples [31–34], but the extraction of antidepressants from real water samples has not been explored yet.

2. Materials and Methods

2.1. Reagents

The starting materials to be coated and used as FPSE media were Whatman microfiber glass filters of 110 mm and Whatman cellulose filter papers of 125 mm (Boston, MA, USA). The organic polymers polyethylene glycol (PEG 300) and poly(ethylene glycol)-block-poly(propylene

glycol)-block-poly(ethylene glycol) (PEG-PPG-PEG 5.800) were purchased from Sigma-Aldrich (Athens, Greece). Solvents (dichloromethane, acetonitrile (ACN), acetone, methanol (MeOH) and phosphoric acid) were purchased from Merck (Darmstadt, Germany). Trimethoxymethylsilane (MTMS), trifluoroacetic acid (TFA), sodium hydroxide, and hydrochloric acid were also supplied by Merck (Darmstadt, Germany).

Poly(lactic acid) (PLA) 3D-printed disks of $0.02 \times 0.02 \times 0.0015$ m were prepared using a Prusa i3 3D printer using the fused deposition modeling (FDM) technique [35]. The printing and specimen volume filling parameters for the 3D printing process were modified using slicer software. All the parts were printed (100% filling density) using rectilinear pattern filling, on a heated bed (60 °C) and the nozzle (diameter 4 µm) was maintained at 200 °C during the process [36].

Antidepressants citalopram (CIT), clozapine (CLO), mirtazapine (MIRT), bupropion (BUP) and sertraline (SERT) were purchased from TCI Tokyo Chemical Industry (Tokyo, Japan). All analytes have a purity higher than 98%. By dissolving the compound in methanol, stock solutions containing 1000 mg·L⁻¹ of each analyte were prepared, and the solutions were stored in glass-stopped bottles at -20 °C in the dark. Standard working solutions were prepared daily. The ultrapure water used was produced by a Milli-Q system (Evoqua, Pittsburg, PA, USA).

STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA) was used to produce the experimental matrix and to evaluate the results.

2.2. Sample Collection

Wastewater samples were collected from the outflow of municipal and university hospital wastewater treatment plants in Ioannina city, located in the Epirus region, NW Greece. In the municipal wastewater treatment plant (WWTP1) the wastewaters of the whole Ioannina city are deposited, with a population of more than 100,000 people. All household and industrial waste and rain waters are collected from the WWTP1, frequently surpassing the WWTP's capacity to handle this volume. In addition, wastewater from Ioannina hospital's WWTP effluent (WWTP2) was also sampled. Ioannina University Hospital is also the center of learning and applied medical science linked to the School of Medicine and School of Nursing at Ioannina University. About 45,000 patients are treated annually in the Care Clinics of the medical center. Furthermore, the hospital has a Department of Psychiatry, making it interesting to research the occurrence of psychotropic drugs in the hospital's WWTP effluent. The hospital's plant (WWTP2) sheds its treated wastewater into the urban network that eventually goes straight to the municipal WWTP; thus we have a significant interest in assessing its efficiency in order to determine the presence of ADs in the municipal WWTP.

Moreover, surface water was collected from Pamvotis Lake in Ioannina. Covering an area of 19.4 km², Pamvotis Lake is one of the largest lakes in Greece. The pollution of the lake is due to the illegal dumping of waste from tankers, livestock and industrial plants, pesticide residues, illegal landfills, and the fact that parts of the city have not been connected to the city's wastewater network. All the samples were collected in clean amber glass bottles. Finally, the samples were transported to the laboratory immediately and placed in the refrigerator at 5 °C before extraction and analysis.

2.3. HPLC Analysis

To determine the selected analytes, a Thermo Fisher Scientific UltiMate 3000 HPLC system equipped with a diode array detector and Chromeleon Thermo Fischer software were used. The equipment consists of a Binary Solvent Manager (BSM), a WPS-3000SL autosampler and a column manager, all from Waters Thermo Fisher Scientific (Waltham, MA, USA). The parameters for the detection of the targeted analytes were optimized by using a standard solution of a mixture of all the compounds.

The injection volume into the chromatographic equipment was 20 µL, and the analytical column was a Hypersil GOLD 150 × 4.6 mm, with a particle size of 5 µm (Thermo Scientific, Waltham, MA, USA), operating at 25 °C.

The mobile phase consists of methanol (A) and water with 0.3% phosphoric acid (B), at 1.0 mL/min flow in gradient mode. The gradient started at 15:85 (A:B) (*v/v*) and increased after 3 min to 50% methanol. The percentage of methanol increased further up to 80% after 7 min and the gradient returned, after 2 min, to its initial state 15:85 (equilibrium time 3 min). The whole chromatographic separation process finished in 15 min. The parameters of the diode array detector (DAD) were chosen for the quantitative analysis of each compound. The UV detector was set at 245 nm for sertraline, clozapine and citalopram, at 230 nm for mirtazapine and at 254 nm for bupropion, with full spectral scanning from 200 to 400 nm.

2.4. Preparation of Sol–Gel Sorbent-Coated FPSE Media

2.4.1. Pretreatment of Media for Sol–Gel Coatings

All fabrics chosen to produce sol–gel covered sorbents have remaining finishing additives or other contaminants that agglomerated over time on their surface, which required thorough cleaning. Additionally, the surface areas of these fabrics require activation to optimize the sol–gel sorbent loading during the polymer sorbent coating phase.

This was done after we implemented a systematic cleaning procedure. Briefly, a 95 cm² (11 cm diameter) circular piece of the Whatman Cellulose filter was soaked with deionized water under constant sonication for 15 min, followed by NaOH treatment (1.0 M, 1 h) and then HCl (0.1 M, 0.1 h), under sonication, respectively. After each one of these three cleaning steps the fabric media were washed with a profuse amount of deionized water. The media were then left to dry in ambient air at room temperature for 24 h, following the coating step. This procedure was followed to increase the availability of all the hydroxyl groups of the cellulose chemical structure that lead to more chemical reactions with the sol–gel inorganic–organic network.

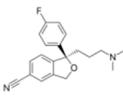
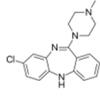
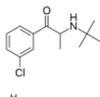
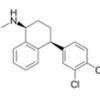
Almost the same procedure was adopted for the Whatman Microfiber Glass filter pretreatment to activate the surface silanol groups. The difference with the abovementioned procedure was that the pretreatment time with NaOH (1.0 M) and then HCl (0.1 M) was for 8 h, respectively.

PLA was treated with a mixture of 0.25 M NaOH/EtOH for 4 h to improve its hydrophilicity and cell affinity. To stop any major deterioration of the product, an alkali solution was used. Ethanol has been found to aid the hydroxide nucleophilic effect on ester bonds of PLA. Then, the conditioned PLA was soaked with deionized water and eventually dried overnight under N₂ gas flow in inert conditions.

2.4.2. Preparation of the Sol–Gel Solutions for Coating on the Substrate Surface

In the sol–gel process, polymer choice is important, depending on the target analytes' polarity/functionality. Taking into account the target analytes' physicochemical characteristics (Table 1), two sol–gel sorbents were prepared, including polyethylene glycol 300 (PEG 300) and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) 5.800 (PEG-PPG-PEG 5.800). Sol–gel solutions consist of a solvent system, a sol–gel precursor, an organic polymer, and an aqua solution of the catalyst. In our study, both polymer solutions were developed using methyltrimethoxysilane (MTMS) as the sol–gel precursor, a trifluoroacetic acid (TFA) catalyst containing 5% water, and a combination of acetone and dichloromethane (50/50 *v/v*) as the organic solvent. The molar ratio between polymer:precursor:organic solvent:catalyst was optimized and maintained at 0.5:0.2:1.0:0.5, respectively.

Table 1. Structures and respective physico-chemical characteristics of target analytes.

Analyte	Molecular Weight, g/mole	Chemical Structure	pKa	logK _{ow}
Citalopram (CIT)	324.39		9.78	3.76
Clozapine (CLO)	326.82		7.50	3.23
Mirtazapine (MIRT)	265.35		7.70	2.90
Bupropion (BUP)	239.74		8.22	3.85
Sertraline (SERT)	306.20		9.48	5.10

A modified version of a previously described procedure [37] was adopted for the preparation of the sol solution. Briefly, 5 g of PEG 300 was dissolved in 5 mL of methyltrimethoxysilane (MTMS, sol-gel precursor). The mixture was then vortexed (1 min). Following this, 10 mL of the mixture of acetone/dichloromethane in ratio 50/50 *v/v* (solvent) was added, and then was vortexed again for 1 min. After that, 2 mL of TFA catalyst was added. MTMS is hydrolyzed by the addition of the catalyst, and a network of the hydrolyzed MTMS molecules is created through the polycondensation. While the sol-gel network is growing, the chosen polymer incorporates randomly into it. The entire mixture was then vortexed (5 min) and centrifuged for 5 min at 5000 rpm, and the transparent sol solution supernatant was moved to another clean flask. The fabric media were immersed there for 4 h. The coated material was separated from the sol-gel solution and was held overnight in a desiccator for solvent evaporation. The sol-gel-coated FPSE device was then rinsed as a final move with a profuse amount of the organic solvent (acetone/dichloromethane: 50/50 *v/v*) under constant sonication for 30 min to remove unreacted and unbonded residual coating ingredients from the fabric surface. The sol-gel-coated fabric was then cut into 1-cm diameter circular pieces and placed in a sealed container so that unwanted analytes from the atmosphere did not accumulate. The successful preparation of the whole process was confirmed by FTIR analysis (Figure S1).

2.5. Fabric Phase Sorptive Extraction Process

First, the coated materials were soaked for 5 min in 2 mL of methanol:acetonitrile (1:1) solution, and next were treated with 2 mL deionized water for 5 min to remove the previous organic solvents. This step was conducted to clean and activate the coating of the fabric substrate. Afterwards, the FPSE media were immersed in the aquatic sample. Adsorption studies were conducted (spiking level 100 µg·L⁻¹, Milli-Q water, continuous stirring at 300 rpm provided by magnetic stir bar) with the appropriate fabrics to choose the one that could be most effective for the target compounds. After this, the target analytes were eluted from the media in a sufficient volume of solvent for an optimal period. The collected solvent was adjusted to 100 µL with a gentle N₂ stream, before HPLC analysis. The schematic procedure of FPSE is shown in Figure S2.

3. Results and Discussion

3.1. Selection of FPSE Substrate and Sol-Gel Coating

The selectivity of the extraction depends on the hydrophilic/hydrophobic properties of the media and on the polarity of the polymer, which, together, define the extraction performance of the FPSE

device. A Milli-Q water solution spiked (at $100 \mu\text{g}\cdot\text{L}^{-1}$) with the five antidepressant drugs was prepared to determine the efficiency of the process ($N = 3$). The concentration of the analytes was measured by HPLC-UV-Vis/DAD before and after the extraction step, as well as after the elution step, in order to determine the adsorption and desorption efficiency, respectively. The performance of extraction was calculated as the ratio of the initial aqueous concentration in the Milli-Q water sample to the final concentration.

During preliminary studies, in which the type and shape of substrate as well as the polymer used in the creation of the sol-gel solution were tested, we decided to assess the following three different substrates: Whatman microfiber glass filter (FG), Whatman cellulose filter (WC), and polylactic acid disks (PLA). Two different sol-gel coatings were developed, high-polarity PEG 300 and medium-polarity PEG-PPG-PEG 5.800, which were finally evaluated. From the results obtained, it was found that the circle-shaped 100% glass microfiber filter (FG) with a diameter of 1 cm coated with PEG300 displayed the greatest adsorption and desorption yields, so this combination was chosen for further optimization.

3.2. Optimization of the Experimental Conditions

3.2.1. Preliminary Experiments

For the optimization of the FPSE process, both univariate and multivariate analysis approaches were carried out to encourage sound, rational thinking and responsibility instead of 'push-the-button' automated data. There are many variables that may influence FPSE extraction efficiency, among them are sample volume, ionic strength, pH, extraction time and the solvent, as well as the elution solvent, time and volume, etc. Four of them were initially investigated to check their suitability to efficiently recover the target analytes: sample volume (1 and 10 mL), extraction time (10, 20, 30 and 40 min), pH (3, 7, 10 and 12) and ionic strength of the sample (0% and 10% *w/v*).

The volume of the aqueous sample as well as the final volume of the extract are closely related to the technique's preconcentration ability. Two sample volumes (1 and 10 mL) with a fixed elution volume solvent (1 mL, MeOH) were examined initially under continuous stirring at 300 rpm by a magnetic stir bar. The rate of adsorption for 1 mL was higher than for 10 mL (Figure 1a) for a fixed adsorption time of 10 min, so 1 mL was selected to reach faster equilibrium conditions.

The extraction time is directly associated with the distribution parameters that determine the interaction between the FPSE material and the analytes. Four extraction times were investigated: 10, 20, 30 and 40 min. The results revealed that extraction times of 10 and 20 min were marginally worse than longer extraction times of 30 and 40 min (Figure 1b). It can be seen that, after 30 min, the equilibrium is established, so 30 min was chosen as the optimum extraction time.

For the 1 mL sample and 30 min extraction time, four separate pH values were measured: pH 3, 7, 10 and 12. At the alkaline pH region (pH 12) the highest extraction efficiency was obtained for all target analytes (Figure 1c). Considering the fact that the analytes act as weak bases (pK_a s are in the range between 7.5–9.8, Table 1), they are positively charged at environmentally relevant pH values, while, at pH values two units higher than their pK_a ($\text{pK}_a + 2$), they are in neutral form. This explains the higher adsorption yield that is observed at alkaline pH levels (pH 12).

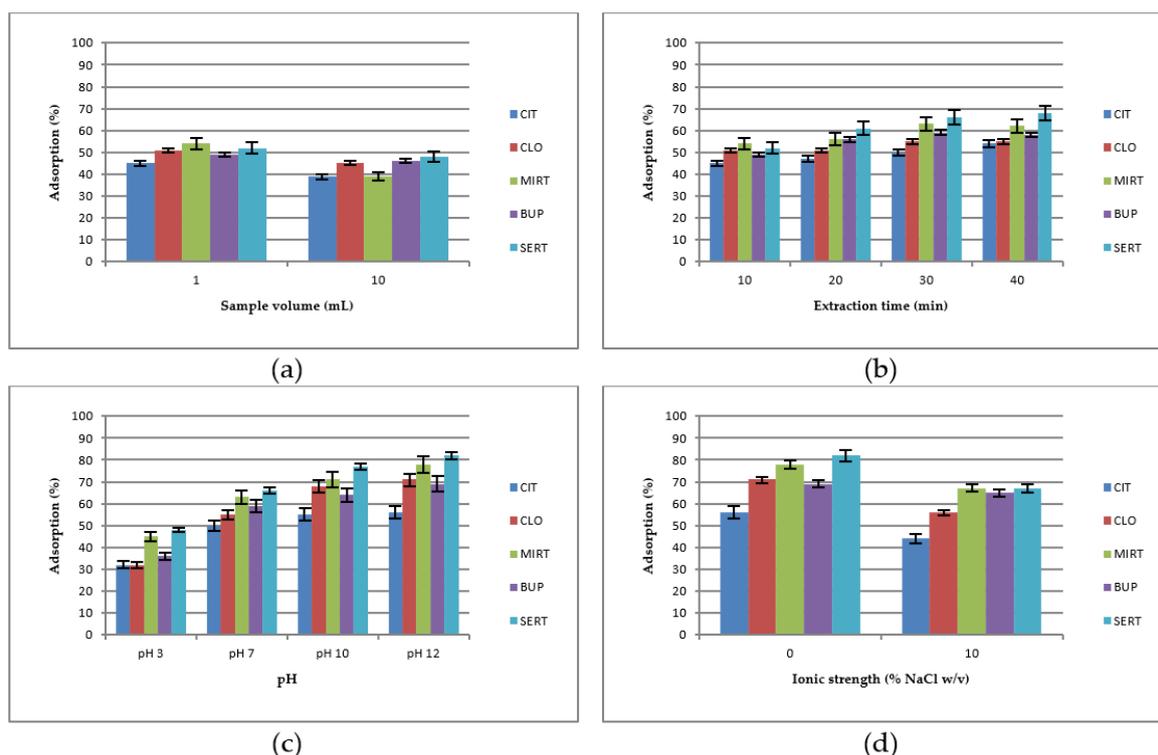


Figure 1. Efficiencies of the five antidepressants (ADs) during the adsorption step depending on (a) the volume of the sample, (b) extraction time for 10, 20 30 and 40 min under stirring, (c) different pH values, and (d) ionic strength; citalopram (CIT), clozapine (CLO), mirtazapine (MIRT), bupropion (BUP), sertraline (SERT).

An increased concentration of ions in a solution is advantageous for increasing the recovery of organic contaminants from aqueous solution. The effect of ionic strength on analyte adsorption was studied, while the remaining parameters were fixed (1 mL sample volume, 30 min of extraction and pH adjusted at 12). For this reason, 10% (*w/v*) of NaCl was added to the aqueous solution containing analytes (Figure 1d). The results have shown that the adsorption efficiency declines with increasing ionic strength. The decreased adsorption efficiency may be attributed to the increase in the aqueous matrix’s viscosity, resulting in a sluggish distribution of analytes to the FPSE media.

3.2.2. Experimental Design Optimization

In the next step, we decided to select the appropriate elution variables to build a model that best reflected the variation of the microextraction process. The elution procedure was optimized through chemometric tools such as Design of Experiments (DoE) and Response Surface Methodology (RSM) (STATISTICA Software v7). The experimental configuration was initially conducted with four variables (two levels for each) to define how these affect the extraction performance (elution solvent: MeOH/ACN, elution process: vortex/stirring, elution time: 3/6 min, elution volume 0.1/0.5 mL). Methanol was far more efficient than ACN, followed by stirring at 300 rpm against a vortex as the desorption process, while both a higher elution time and solvent volume had a positive effect on the efficiency of the FPSE process (Figure 2).

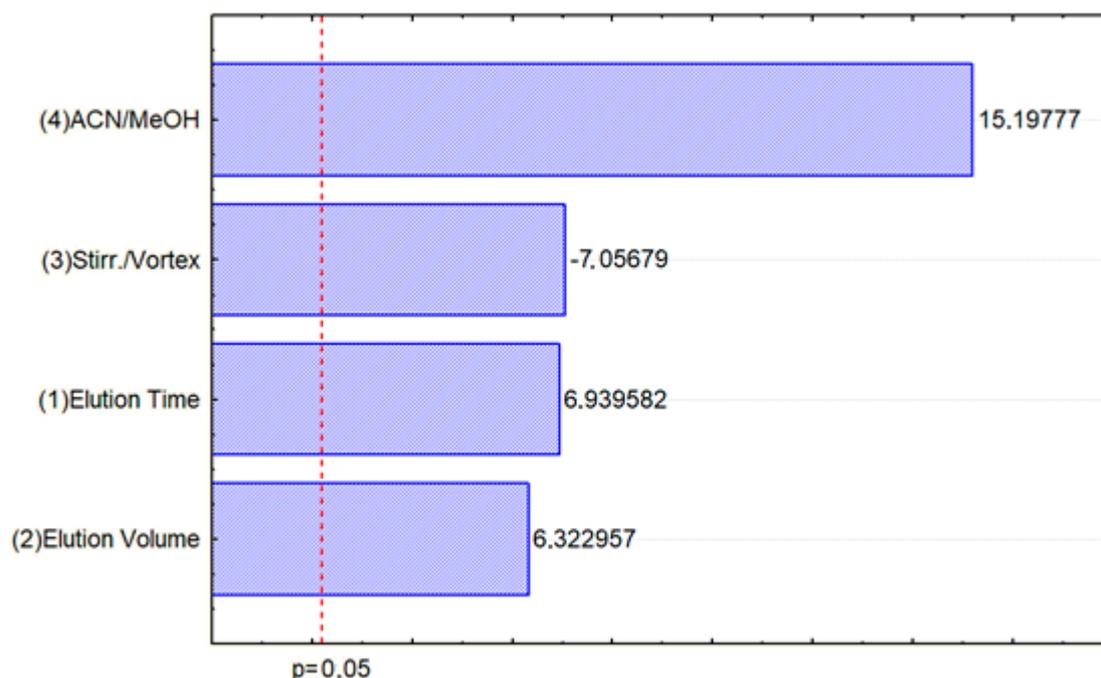


Figure 2. Pareto maps of the standardized effects (2^4 experimental configuration).

Having followed the findings of the previous design, the next task was to improve the analytical process further by employing a 3^2 experimental configuration for an elution volume of MeOH (0.75, 1.00 and 1.25 mL) and time (5, 10 and 15 min). The main effects were analyzed, as well as quadratic and interaction effects, by ANOVA (Table 2) and proved to be significant. The expressed a 3D correlation between the MeOH volume and the elution time, which is depicted in Figure 3. In particular, a higher elution time was needed when a greater amount of MeOH was used. A desirability function was used to determine the optimum extraction conditions (Figure 4).

Table 2. ANOVA results obtained by 3^2 design.

Factor	SS	df	MS	F	p
(1) Elution Time	741.745	1	747.745	48.1578	0.000025
(2) Elution Volume	615.784	1	615.784	39.9798	0.000057
(3) Stirring/Vortex mixing	767.013	1	767.013	49.7983	0.000021
(4) ACN/MeOH	3557.526	1	3557.526	230.9724	0.000000
Error	169.426	11	15.402		
Total SS	5851.495	15			

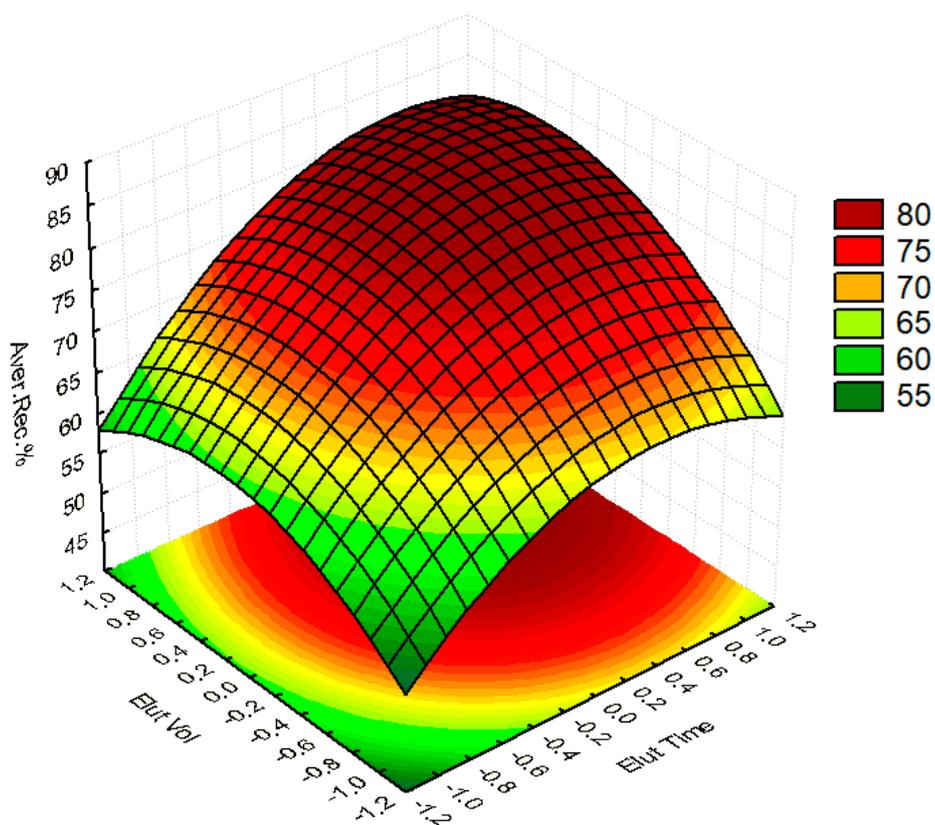


Figure 3. 3D Response Surface representation of elution time and solvent volume (coded values).

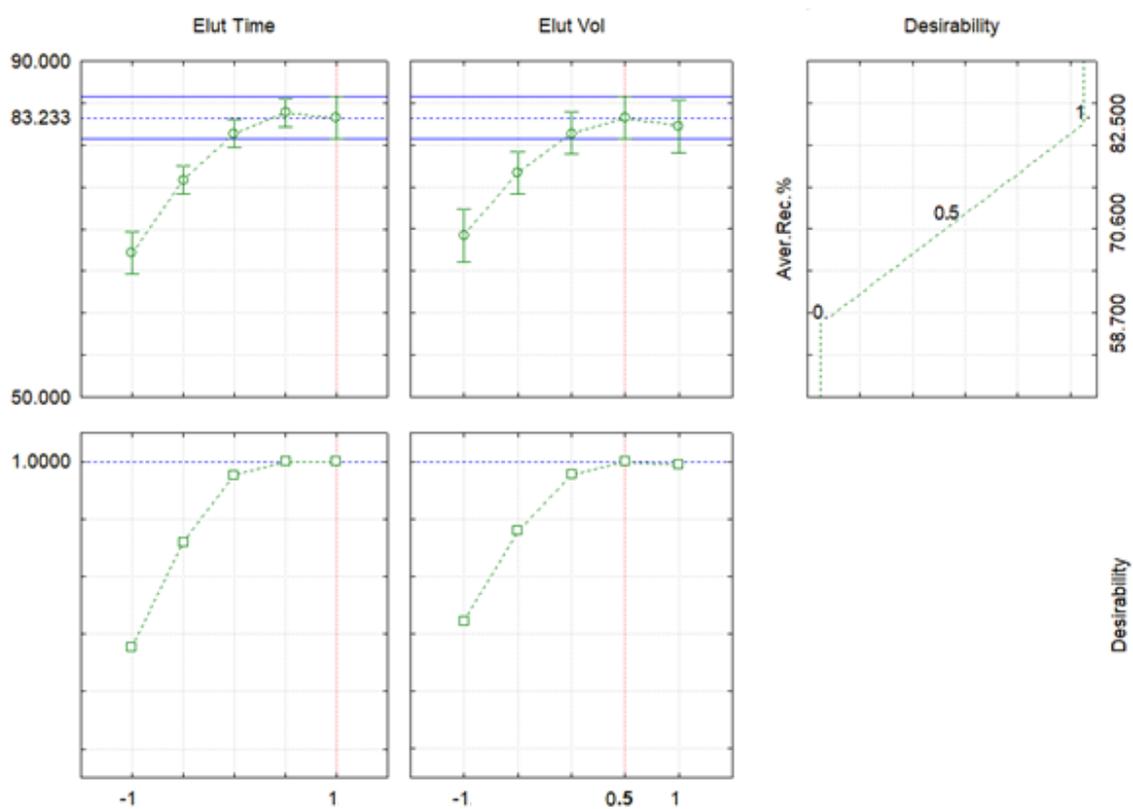


Figure 4. Desirability profiles and predict levels (coded values).

To conclude, the optimal parameters for the FPSE process in relation to the extraction of the selected antidepressants were as follows: a circle-shaped 100% glass microfiber filter (FG) with a diameter of 1 cm, with PEG300, as the FPSE device. The extraction of the aqueous sample (1 mL, adjusted to pH 12) was carried out for 30 min and stirred at 300 rpm. Methanol was used as the desorption solvent at 1.0 mL for 10 min under stirring at 300 rpm. The final extract was adjusted to 0.1 mL before analysis to have a preconcentration factor of 10 (from 1 mL to 0.1 mL).

3.3. Analytical Performance

The figures of merit of the proposed method were also assessed. A seven-point curve was developed among the LOQ of each analyte up to 500 $\mu\text{g}\cdot\text{L}^{-1}$. The method displays linearity at the specified range with coefficients of determination (R^2) greater than 0.9989. An examination of accuracy and precision was carried out at two concentration levels (LOQ and $10 \times \text{LOQ}$) in Milli-Q water, spiked with the target analytes. The intraday precision of the process was measured through three assessments, while the interday precision was measured over three days by a triplet of measures. As depicted at Table 3, satisfactory absolute recoveries (the % concentration of target analytes recovered from Milli-Q water) in the range of 60–98% were observed. The % RSDs were less than 13% in both cases.

Table 3. Analytical parameters of Milli-Q water samples (n = 3).

Analyte	LOD $\mu\text{g}\cdot\text{L}^{-1}$	LOQ $\mu\text{g}\cdot\text{L}^{-1}$	Recovery (%)		Intraday RSD (%)		Interday RSD (%)	
			LOQ	$10 \times \text{LOQ}$	LOQ	$10 \times \text{LOQ}$	LOQ	$10 \times \text{LOQ}$
Citalopram (CIT)	1.13	3.39	61.94	69.31	2.91	2.40	5.49	3.99
Clozapine (CLO)	0.64	1.92	70.70	77.50	2.94	2.41	5.40	3.96
Mirtazapine (MIRT)	3.22	10.71	76.34	83.08	6.92	5.87	8.39	12.63
Bupropion (BUP)	2.05	6.82	71.77	75.70	4.22	4.08	10.79	7.41
Sertraline (SERT)	1.12	3.36	79.71	98.10	2.38	4.34	4.28	6.76

The method's LOD and LOQ were determined using a signal-to-noise ratio of three and 10, respectively. LODs ranged between 0.64 to 3.22 $\mu\text{g}\cdot\text{L}^{-1}$ (Table 3).

3.4. Applications in Environmental Samples

Two wastewater samples from hospital and municipal WWTP effluents and a surface water sample from Pamvotis Lake were investigated to assess the applicability of the proposed method. However, their concentration levels of the target analytes were lower than the established LODs, or antidepressants were absent during this sampling campaign. These water samples were spiked with the selected ADs at 50.0 $\mu\text{g}\cdot\text{L}^{-1}$, to confirm the efficiency of the process. A representative chromatogram is shown in Figure 5. Relative recoveries in real water samples were estimated from absolute recoveries of the Milli-Q water samples (relative recovery is defined as the % concentration of target analytes recovered from the wastewater and surface water with reference to the concentration found in the spiked Milli-Q water). Depending on the water matrix and the analyte, relative recoveries varied between 69 and 121% (Table 4).

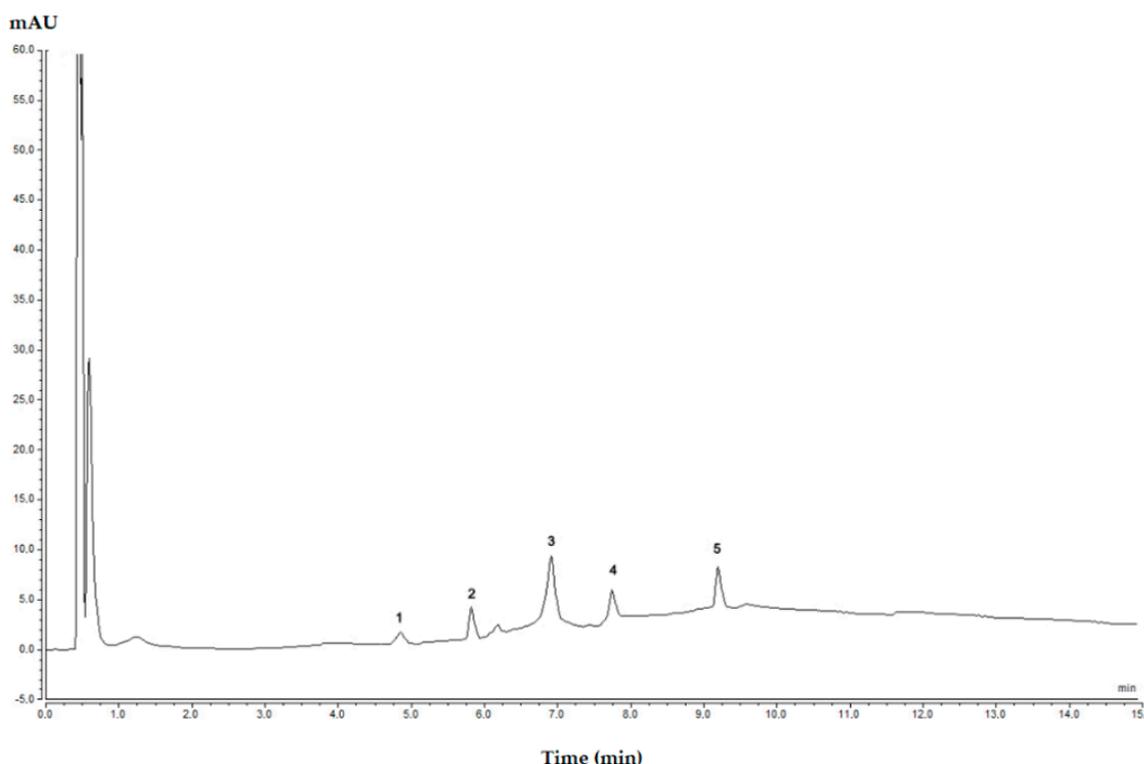


Figure 5. A representative chromatogram obtained from Lake water spiked at $50.0 \mu\text{g}\cdot\text{L}^{-1}$, after FPSE procedure coupled with High-Performance Liquid-Chromatography–Photodiode Array Detection (FPSE-HPLC–DAD) (1. MIRT, 2. BUP, 3. CLO, 4. CIT, 5. SERT).

Table 4. Analytical parameters of hospital and urban effluents and surface water samples (n = 3).

Analyte	Lake Water		Hospital Effluent		Urban Effluent	
	$50.0 \mu\text{g}\cdot\text{L}^{-1}$		$50.0 \mu\text{g}\cdot\text{L}^{-1}$		$50.0 \mu\text{g}\cdot\text{L}^{-1}$	
	Absolute Recovery (%)	Relative Recovery (%)	Absolute Recovery (%)	Relative Recovery (%)	Absolute Recovery (%)	Relative Recovery (%)
Citalopram (CIT)	65.37	100.57	81.94	116.06	72.92	112.18
Clozapine (CLO)	74.99	111.36	56.18	86.43	60.04	92.37
Mirtazapine (MIRT)	101.34	104.07	99.01	120.75	107.86	113.34
Bupropion (BUP)	65.93	93.16	53.46	75.54	49.18	69.49
Sertraline (SERT)	69.07	107.60	61.22	94.20	56.14	86.38

4. Conclusions

The study presented above is the first attempt to introduce the procedure of Fabric Phase Sorptive Extraction to extract and pre-concentrate five ADs contained in aqueous environmental samples. The aim of our study was not to reach the highest sensitivity of the methodology, otherwise different preconcentration factors and more sensitive analytical techniques (ultra-high performance liquid chromatography coupled to high resolution mass spectrometry, UPLC–HRMS) would have been employed. FPSE proved to be an easy, green and fast analytical method for the determination of the selected psychoactive drugs by employing HPLC-UV-Vis/DAD at concentration levels that may occur in natural aquatic systems ($\mu\text{g}\cdot\text{L}^{-1}$). The most significant variables affecting the efficiency of the process were optimized by employing different experimental configurations. The proposed method is reproducible with good RSDs and acceptable recoveries in Milli-Q and real water samples, respectively. In addition, the method was applied in real water samples (hospital and urban effluents and Pamvotis Lake samples), but no ADs were detected. The future aim of the research team is to investigate different substrate/sorbent combinations in order to produce a FPSE device that would extract a broader

number of pharmaceuticals and employ a more sensitive analytical technique (UPLC–HRMS) for their determination.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2297-8739/7/3/39/s1>, Figure S1: FT-IR spectra of different components in (a) Whatman uncoated material, (b) PEG-PPG-PEG polymer, (c) MTMS precursor and (d) Whatman PEG-PPG-PEG coated media, Figure S2: General procedure applied in FPSE extraction.

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