

Ibuprofen, Carbamazepine and β -Estradiol Determination Using Thin-Film Microextraction and Gas Chromatography-Mass Spectrometry

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The use of thin-film microextraction for the extraction of selected pharmaceutical compounds followed by gas chromatography-mass spectrometry detection was evaluated. A segment of polysiloxanes polymer sheet was used as low cost, single use, disposable extraction phase, while Milli-Q water spiked at 20 $\mu\text{g L}^{-1}$ with the analytes was used for the optimization assays. The controlling parameters for the extraction were optimized via experimental design and it was found that an extraction time of 3 h using a sample volume of 1000 mL at pH 4 with the addition of 20% methanol and 20% sodium chloride provided the greatest extraction efficiency. Recoveries between 67.1 and 85.0% were achieved, with a repeatability lower than 20% (expressed as coefficient of variation) and limit of detection ranged from 0.41 and 0.92 $\mu\text{g L}^{-1}$. The proposed method show similar analytical performance when compared to the determination of the analytes using stir bar sorptive extraction.

Keywords: thin-film extraction, β -estradiol, carbamazepine, ibuprofen

Introduction

A wide variety of pharmaceutical and personal care products (PPCPs) and their metabolites are continuously introduced in the environment through human excretion, washing and manufacturing.¹ In recent years, the interest in these compounds has increased, with special attention paid to their presence in the aquatic environment.² However, there is little information about the adverse effects of human pharmaceuticals on aquatic organisms.³ Indeed it is known that even though the concentrations found in surface water are very low, the constant introduction of low concentrations represents a risk; compounds such as β -estradiol, carbamazepine and ibuprofen have been shown to have adverse effects on aquatic ecosystems.²

A wide range of analytical methods have been developed for the analysis of PPCPs in aqueous samples. High-performance liquid chromatography (HPLC) with ultraviolet (UV) detection methods are commonly used for the determination of PPCPs in several matrices;⁴ gas

chromatography-mass spectrometry (GC-MS) is another good alternative, because it can be used to identify these analytes with low limits of detection (LOD).⁵ However, in aqueous environmental samples the concentrations of PPCPs are very low and extraction and preconcentration steps are required for their determination. Commonly used PPCPs can be easily extracted via solid-phase extraction using a water-wettable, hydrophilic/lipophilic balance, reversed-phase sorbent with proper pH adjustment,⁶⁻⁹ with recoveries up to 107%⁶ on optimum experimental conditions and LOD as low as 1 ng L⁻¹ when coupled with ultra high performance liquid chromatographic system attached to high-resolution mass spectrometry (UHPLC-MS/MS).¹⁰ Solid-phase microextraction (SPME)^{11,12} and stir bar sorptive extraction (SBSE)^{13,14} have also been proven to be effective for the determination of these analytes in aqueous sample. Recoveries for estrogen compounds range from 50 to 120% of the spiked concentration when using SPME⁶ and, for SBSE average recoveries of 93.1% were achieved for selected PPCPs.¹⁴ Both of these techniques have the advantages of isolate the analytes from potential interfering matrix components and being environmentally friendly;

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since they use little or no solvent during the extraction and analytes can be thermally desorbed. However, SPME commercial fiber is expensive and has a small quantity of polydimethylsiloxane (PDMS) in the coating which limits analyte adsorption. Commercial SBSE devices afford better recovery values through the use of higher PDMS volumes, but require a special thermal desorption unit for fully solvent-free operation.

Thin-film microextraction (TFME) was introduced by Bruheim *et al.*¹⁵ and comprise cheap commercial thin sheets of PDMS cut into different geometries, which increases the surface area-to-volume ratio to improve the recovery of analytes when using PDMS as the extraction phase.¹⁶ TFME exhibits the same extraction phenomena and extraction selectivities as SPME and SBSE,¹⁷ but with increased recovery because a higher volume of PDMS comes into contact with the sample. This configuration allows to remain the thickness of the coating or even decreases it, while the volume of the extraction phase increases, which according to the extraction principle will result in lower equilibrium times and higher extraction rates.¹⁸ TFME has been widely used for the determination of polyaromatic hydrocarbons,^{19,20} pesticides²¹ and endocrine disruptor compounds.²²

The aim of this study was to evaluate the use of TFME as a cheap and simple extraction technique for the determination of selected PPCPs with acidic, basic and neutral characteristics. An experimental design matrix was used to optimize the variables that determine TFME extraction performance: pH, extraction time, sample volume and the addition of matrix modifiers, such as methanol (MeOH) and sodium chloride (NaCl).

Experimental

Reagents

The mixture *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + chlorotrimethylsilane (TCMS), 99:1 was obtained from Supelco (Bellefonte, PA, USA). Pyridine, carbamazepine, ibuprofen and β -estradiol (purity ca. 94-99.5%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and reagents were purchased from Merck (Darmstadt, Germany) in their highest purity. Ultrapure water was obtained using a Direct-Q 3 system from Millipore (Billerica, MA, USA). Nitrogen and helium were purchased from Indura (Santiago, Chile). The PDMS silicone elastomer sheet was obtained from Goodfellow (Oakdale, PA, USA). Stock solutions (1000 mg L⁻¹) for each analyte were prepared monthly and maintained at 4 °C, and all calibration curves and extraction solutions were prepared daily.

GC-MS analysis

GC-MS was performed on a Clarus 680 GC (PerkinElmer, Waltham, MA, USA) coupled to a Clarus SQ 8T mass detector under electron impact ionization (70 eV) with a 4 min solvent delay and an interface temperature of 230 °C. Samples were separated on an HP-5ms fused-silica capillary column (Agilent Technologies, Santa Clara, CA, USA) with 0.25 μ m film and 30 m \times 0.25 mm i.d., using helium 6.0 as the carrier gas (flow rate 1.0 mL min⁻¹). The column temperature was initially held at 100 °C for 1 min, then programmed to reach 280 °C at a rate of 20 °C min⁻¹ with a final hold time of 15 min. The injector temperature was maintained at 280 °C and the injection volume was 1 μ L in the splitless mode. Table 1 lists the analyte ions used for the chromatographic analysis; a tolerance of 0.5% for retention time and a \pm 10% tolerance for relative ion intensities were employed.

Table 1. Main features of the gas chromatography-mass spectrometry (GC-MS) method

Analyte	Retention time / min	Target ion / (<i>m/z</i>)	Qualifier ion / (<i>m/z</i>)
Ibuprofen	5.87	263	117, 160
Carbamazepine	9.14	193	165, 221
β -Estradiol	10.86	285	232, 416

TFME procedure

An aliquot of Milli-Q water was spiked with each analyte at a concentration of 20 μ g L⁻¹ and then the pH of the solution was adjusted to 4 as measured using a WTW pMX 300 pH meter (Weilheim, Germany) by adding HCl. NaCl and MeOH were then each added at a concentration of 20%, followed by a 1 cm² PDMS polymer sheet. The mixture was then stirred using a Heidolph MR 3002 magnetic stirrer (Schwabach, Germany) for 2 h at 1500 rpm. For the desorption step, the polymer film was placed in MeOH (2 mL) and stirred for 30 min. The MeOH was then taken to dryness under nitrogen. Extraction were conducted in duplicate, except for reproducibility and recovery experiments (n = 5).

SBSE procedure

Stir bars coated with PDMS (0.5 mm film thickness, 10 mm length, 24 μ L PDMS, 0.94 cm² surface area) were obtained from Gerstel (Mülheim and der Ruhr, Germany) and were used to compare extraction efficiency.

Prior to use, the stir bars were conditioned into a vial containing 20 mL of methanol. To perform the extraction, the same procedure as the PDMS polymer sheet was

followed for both extraction and elution of analytes using optimum conditions of TFME method. Between each extraction, the bar was cleaned with additional 20 mL of methanol for 30 min.

Derivatization procedure

To derivatize the analytes, the residue was redissolved in pyridine (25 μ L) and BSTFA + TMCS (99:1; 50 μ L) were added. The solution was heated at 75 $^{\circ}$ C in a sealed mini-vial for 40 min. Ethyl acetate (100 μ L) was then added after the silylation reaction be completed.

Sample procedure

Water samples were collected from the Maipo River (Santiago, Chile). The collected water samples were filtered through 0.45 μ m nylon filters and stored at 4 $^{\circ}$ C in dark bottles until analysis.

Results and Discussion

β -Estradiol, carbamazepine and ibuprofen were selected as model compound due to their neutral, basic and acidic characteristic, respectively, in order to study the applicability of the method for PPCPs determination.

For the TFME, a 1 cm^2 polymer phase was used in Milli-Q water spiked with the analytes at 20 $\mu\text{g L}^{-1}$. Certain variables that affect the extraction performance, such as the temperature and agitation velocity, were not investigated in the present study, with the extraction being performed at ambient temperature, even when increasing the temperature results in a higher extraction efficiency due to an increase in the diffusion coefficient, as has been reported.²² With respect to agitation, a speed of 1500 rpm was chosen because more efficient extraction has been observed with greater agitation.²³ The effect of the sample volume, extraction time and pH were optimized using a Box-Behnken design with a total of 15 experiments. Table 2 contains the encoded values and their corresponding natural values.

As can be seen in the total response surface in Figure 1, when grouping the responses of all the compounds, the results indicated that increasing the extraction time increased the extraction efficiency, and this result is comparable to

results obtained when using single variable optimization.²² It is possible that the response could increase beyond the selected parameters and an extraction time higher than 180 min could become an optimum condition. In addition, the relationship between the sample volume and extraction time can also be seen in the Pareto chart in Figure 1 as another important factor. This result is reasonable given that extraction is controlled mainly by mass transfer in the solution. When a higher sample volume was used, the preconcentration factor was increased; therefore, the extraction sensitivity was improved. A lower solution pH was found to enable increased interaction between the acidic analytes and the polymer. However, the PDMS polymer is known to deteriorate at pH values less than 2.²⁴ Table 3 lists the optimum values determined for each variable.

Since the analytes are mid-polar compounds, the use of sample modifiers was considered. The salting out effect was studied because the addition of a salt is known to enhance the interaction of the analytes with the polymer by

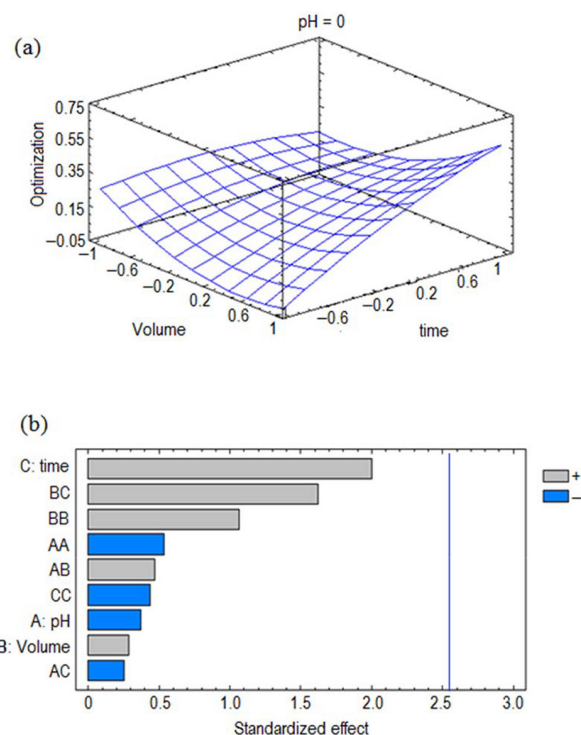


Figure 1. Response surface (a); and Pareto chart (b) for the volume, pH and extraction time factors.

Table 3. Optimum values for the extraction variables

Table 2. Optimization values for the extraction variables

Factor	Volume / mL	pH	Extraction time / min	NaCl / %	MeOH / %
-1	200	3	60	0	0
0	600	4	120	10	10
+1	1000	5	180	20	20

Variable	Encoded value	Natural value
Extraction time / min	1	180
Sample volume / mL	1	1000
pH	-0.057	4
NaCl / %	1	20
Methanol / %	1	20

decreasing their solubilities.²¹ Therefore, NaCl was added at concentrations up to 20% (due to salt solubility). The addition of MeOH, a common sample modifier for mid-polar compounds, can increase the solubility of the analytes in the solution.²⁵ For optimization of these variables, a 3^2 design was used and the encoded and natural values are also listed in Table 2. It can be seen in the Pareto chart (Figure 2) that both of the modifiers were significant variables. The optimum values are listed in Table 3 and show that increasing both the NaCl and MeOH concentrations increased the extraction response; however, the response surface (Figure 2) show just a slightly increment when adding 20% MeOH.

For all of these experiments, a well-known desorption step was employed. MeOH was chosen because it does not damage the polymer phase or cause swelling of the PDMS.²³ Desorption time was optimized between 10 to 30 min of agitation at high speed and 30 min was chosen during the optimization of the desorption time agitation. Under this condition, no carryover effect was observed.

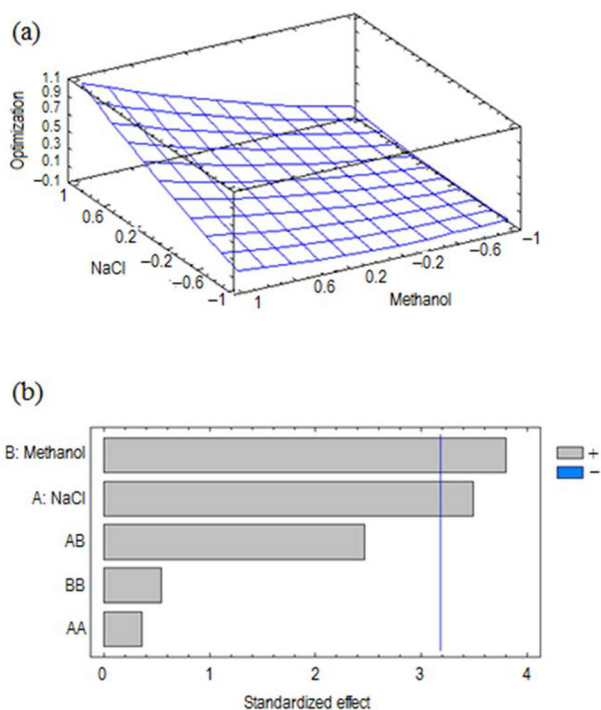


Figure 2. Response surface (a); and Pareto chart (b) for percentages of NaCl and methanol factors.

The quantity of analyte that can be extracted using thin-film extraction is determined by the volume of the extraction phase.¹⁵ Therefore, increasing the size of the polymer film used for the extraction was also evaluated. A 2 cm² polymer sheet was introduced to the analyte solution and the same two experimental designs were performed. Similar trends to those observed using the 1 cm² polymer phase were observed with the larger polymer sheet and the optimum

conditions were again those presented in Table 2. Notably, there was an increase in the responses for carbamazepine and β -estradiol, reflecting the fact that analyte recovery is also dependent on the analyte/polymer phase ratio and the octanol-water partition coefficient (K_{ow}) of the analyte.¹⁵ However, no effect on the recovery of ibuprofen when using the larger polymer phase was observed, meaning that this analyte extraction was already exhaustive when using less volume of PDMS.

Analytical features

For quantitative analysis, it is not necessary that the analytes are completely extracted, as long as the extraction is carefully timed and the experimental conditions remain constant.²⁶ Calibration curves were constructed using seven points for analytes concentrations ranging from 1 to 50 $\mu\text{g L}^{-1}$ in aqueous solutions ($n = 2$). The resulting calibration equations are presented in Table 4, along with their associated limits of detection and quantification (values were calculated using the parameters of the calibration curves).

Water samples fortified at 20 $\mu\text{g L}^{-1}$ for all analytes were used to evaluate accuracy. 5 samples were prepared and extracted the same day for recovery tests and repeatability assays. Both results are shown in Table 4. Intermediate precision was evaluated as the coefficient of variance of the analysis of water samples on three different days using five repetitions and the values obtained were between 8.4 and 20.0%. Note that the PDMS phase was not reused and the polymer sheet was not homogenous. Despite these issues, the obtained values are similar to those reported by other authors using similar extraction processes.²⁵

However, when comparing the proposed methodology with other reported in the literature, lower LOD are described. Neng *et al.*⁵ use polyethylene sorptive bars with large volume injection GC-MS, achieving lower LOD with no derivatization step. On the other hand, when using UPLC-MS/MS for quantification, LOD of 1 ng L⁻¹ are reported. However, in Celano *et al.*⁹ work is used a combination of extraction methodologies. To be able to achieve such low LOD, the developed TFME methodology needs to be coupled with a more sensitive detection method. However, the simplicity of the extraction, the cheap extraction phase and good analytical performance make the proposed methodology a suitable alternative for determinations of the selected PPCPs.

Comparison with SBSE methodology

A comparison of the extraction efficiency was made between TFME and SBSE using five extractions of water

Table 4. Analytical parameters for the analytes in aqueous samples

Analyte	Calibration curve	Correlation coefficient	LOD / ($\mu\text{g L}^{-1}$)	LOQ / ($\mu\text{g L}^{-1}$)	Accuracy and repeatability ^a	
					Coefficient of variation / %	Recovery / %
β -Estradiol	$y = 0.86E4x + 5.133$	0.9852	0.41	1.36	11.8	85.0
Carbamazepine	$y = 4.21E4x + 1.13E3$	0.9809	0.72	2.40	12.5	67.1
Ibuprofen	$y = 2.72E5x + 9.31E4$	0.9914	0.92	3.07	6.4	79.5

^aConcentration level $20 \mu\text{g L}^{-1}$ and $n = 5$. LOD: Limit of detection; LOQ: limit of quantification.

samples spiked at $20 \mu\text{g L}^{-1}$ with optimized conditions. The results are presented in Figure 3. Under similar extraction conditions, similar recoveries are obtained when using SBSE instead of a polymer sheet; however, for carbamazepine, a slightly increase in efficiency can be observed probably due to the polymer composition not being 100% PDMS as established by the manufacturer, which allows a better interaction between the analytes and the extraction phase. In addition to the recovery values, it was observed similarities between the repeatability of both methods.

Real samples

The proposed method was applied to the analysis of real aqueous environmental samples obtained from 5 sites in Maipo River. All samples showed results under LOD. To verify the accuracy of the method, recovery experiments

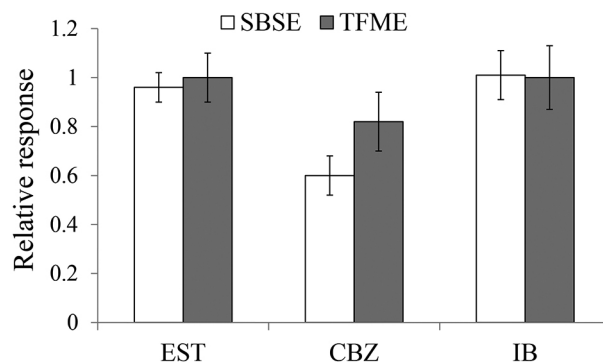


Figure 3. Relative response of analytes when determined with stir bar sorptive extraction (SBSE) and the proposed methodology. CBZ: Carbamazepine; EST: β -estradiol; IB: ibuprofen; TFME: thin-film microextraction.

were carried out by the analysis of spiked samples, with recoveries between 62.7-81.0% (Figure 4). The signal identification was made by comparison of standards in pure

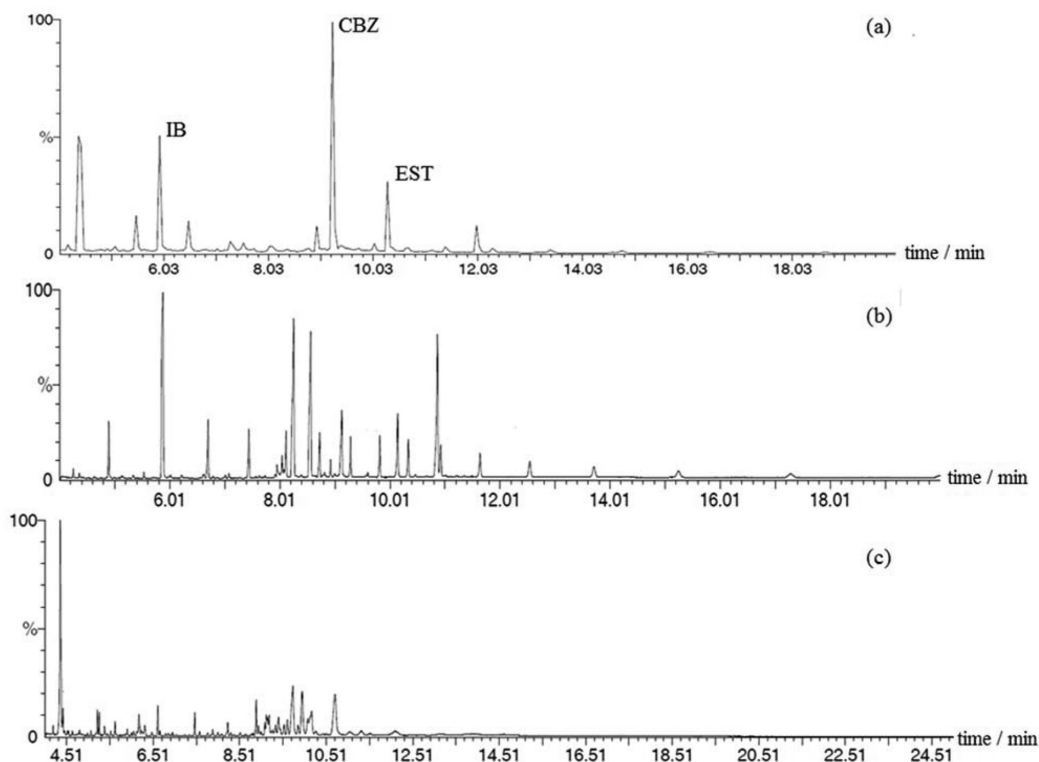


Figure 4. Chromatograms obtained from spiked sample (a); real sample (b); and blank (c). CBZ: Carbamazepine; EST: β -estradiol; IB: ibuprofen.

solvent, while the recovery of the analytes was calculated using calibration curves in sample matrix.

Conclusions

The extraction/preconcentration of selected PPCPs using TFME was demonstrated. The use of experimental design reduced the experimental work and gave reliable information regarding the parameters controlling the extraction performance. We found that the sample volume, extraction time and the quantity of added MeOH and NaCl were of relevance to the extraction efficiency and should be particularly controlled. The use of TFME for PPCP analysis has the advantages of being cheap, easy, environmental friendly and a suitable alternative approach to determine pharmaceutical compounds in water samples.

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