PHARMACEUTICAL COMPOUNDS DETERMINATION IN WATER SAMPLES: COMPARISON BETWEEN SOLID PHASE EXTRACTION AND STIR BAR SORPTIVE EXTRACTION

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ABSTRACT

A variety of organic compounds and their metabolites used in pharmaceutical and personal care products (PPCP´s) are continuously introduced into the environment by domestic or industrial wastewater. Solid phase extraction (polymeric dinivylbenzene cartridge) and stir bar sorptive extraction (polydimethylsiloxane phase) methodologies were optimized for the determination of some selected PPCP´s in aqueous matrices. Carbamazepine, β estradiol, 3-(4-methylbenzylidene) champhor, benzophenone-1 and ibuprofen were extracted from aqueous samples and methanol was used as conditioning/eluting solvent. The variables involved in the extraction of the analytes in the original sample were studied, pH between 3 and 6 and sample volume between 50 and 500 mL were considered. Three concentration levels were extracted at the optimal conditions of pH 5 and 500 mL of sample volume with a recovery up to 89%. The determination was performed in a GC-MS, and a derivatization step using BSTFA+TMCS (99:1) was needed previous to the injection. Real samples from Maipo River and Villarrica Lake were analyzed with the optimized method, and concentrations below the detection limit were detected.

Keywords: SPE,SBSE, pharmaceutical and personal care products.

INTRODUCTION

The Environmental Protection Agency of the United States (USEPA) has defined as endocrine disrupters compounds to exogenous agents that interfere with the synthesis, secretion, transport, association, action or elimination of natural hormones, responsible for maintaining the homeostasis and reproduction in living organisms. Special attention has been paid to a series of compounds used in everyday products such as shampoos and facial creams, and are classified as pharmaceuticals and personal care products (PPCP, Pharmaceutical and personal care products) [1]

The PPCP are a heterogeneous group of compounds that include human and veterinary drugs and other consumer chemicals found in cosmetics [2]. In 2004, about 6 million commercial products containing PPCPs were sell worldwide and their uses have increasing 4% per year [3] was performed. This led to his study in different environmental matrices.

Most PPCPs are potential contamination chemical markers because they are persistent and bioaccumulate in living organisms and through the food chain; especially those which are more lipophilic. [4,5]. The main anthropogenic source of these chemicals is the direct introduction into domestic or industrial wastewater, and its incidence is related to manufacturing and the consumption of the final product [6]. While wastewater are treated in treatment water plants, depending on their chemical structure these chemicals are not completely eliminate or a new compound (metabolites) is generated, some with more toxicity than the original chemical [7]. Low concentrations of PPCPs have been found in groundwater and surface water intended for consumption [8]. For example carbamazepine, a common antiepileptic drug, it is not removed during wastewater treatment and therefore, ends in surface water. Since carbamazepine is used exclusively by humans their presence in natural waters can be used as an indicator of human urine and fecal contamination. Studies have examined the routes of excretion of 212 drugs coming to the conclusion that on average 64% (± 27%) of each drug is excreted via urine and 35% (± 26%) via the feces. In turn, the urine within 42% (± 28%) is excreted in its metabolized form [9].

Pharmaceutical active compound are often not biodegradable, designed to be lipophilic and biologically persistent, in order to maintain therapeutic activity until its specific function has been developed [10]. Many drugs are characterized as highly polar, which makes it necessary to developed sample preparation methods for subsequently analysis. Most analytes in water samples have been determine using solid phase extraction (SPE) in which an analyte is isolated and concentrate prior to quantification by chromatography [11]. Most recently, stir bar sorptive extraction (SBSE) has been use for determination of PPCPs in water samples [12]. In these work a comparison between a solid phase extraction and SBSE for several PPCPs such as carbamazepine (CBZ), β-estradiol (EST), ibuprofen (IB), 2,4-dihydroxybenzophenone (BP-1), 3-(4-methylbenzylidene) camphor (4MBC) was made. In both cases a GC-MS determinations with a prior derivatization step was employed.

EXPERIMENTAL

Reagents

Carbamazepine, β-estradiol, ibuprofen, 2,4-dihydroxybenzophenone and 3- (4-methylbenzylidene) camphor were obtained from Sigma-Aldrich (Germany)

The mixture N,O-bis(trimethylsilyl)trifluoroacetamide+chlorotrimethylsilane) (BSTFA:TCMS, 99:1) was obtained from Supelco (USA). Pyridine (99%) was obtained from Sigma-Aldrich (Germany). All other solvents and reagents were purchased from Merck (Germany) in their highest purity. Ultrapure water was obtained using a Direct Q3 system from Millipore (France). Nitrogen and helium were purchased from Indura (Chile).

Stock solutions were prepared monthly and maintained at 4 °C, and all extraction solutions were prepared daily.

Solid phase extraction procedure

A 250 mL aliquot of water was spiked with each analyte at a concentration of 500 ng L^{-1}, and then the pH of the solution was adjusted to 5 as measured using a WTW pMX 300 pH meter (Germany) by adding HCl. The mixture was then stirred using a Heildoph MR3002 magnetic stirrer (Germany) for 2 hours at 1250 rpm. Analytes were extracted using a new 50 mg polymeric divynilbenzene cartridge from STYRE SCREEN UCT. Cartridges were preconditioned with 5 mL methanol, 5 mL acidic Milli-Q water a pH 4. Samples then were passes through at 1 mL min^{-1}. Cartridges were rinsed with 1 mL acidic water and dry for 20 min. The analytes were eluted using 5 mL methanol.

Stir bar sorptive extraction procedure

Stir bars (SBSE) coated with PDMS (0.5 mm film thickness, 10 mm length) were obtained from Gerstel (Műlheim and der Ruhr, Germany) and were used to compare extraction efficiency. Prior to use, the stir bars were conditioned into a vial containing 10 mL of methanol. To perform the extraction, the bar was placed into a vial containing 100 mL of spiked water sample at 500 ng L^{-1} concentration for each analyte. After the extraction the bar was dried with free lint tissue and placed in a vial with 2 mL of methanol for 30 min for desorption. After every extraction the bar was cleaned with additional 20.0 mL of methanol for 30 minutes.

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GC-MS analysis

The eluate was taken to dryness under a flow of N_2. To derivatize the residue, pyridine (25 µL) and BSTFA+TMCS (99:1; 50 µL) were added, and the solution was heated at 75 °C in a sealed mini-vial for 40 min. Ethyl acetate (100 µL) was then added after the silylation reaction was completed. Gas chromatography-mass spectrometry was performed on a Clarus 680 gas chromatograph (Perkin Elmer, 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 (0.25 µm film, 30 m x 0.25 mm id) 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 µL in the splitless mode. For each compound one quantifier and two qualifiers ions were recorded. Calibration curves between 10 and 100 µg L^{-1} were performed. Table 1 lists the analytical features of the chromatographic method.

Table 1. Chromatographic features of the proposed method.

Analyte	Observed ions (m/z)	Slope	Intercept	Correlation coeficient	Estándar error	Detection limit (µg L^{-1})
β-EST	232, 285*, 416	5,04E4	2,44E4	0,997	2,89E4	1,6
4-MBC	211, 239, 254*	4,11E4	-4,58E4	0,995	3,34E4	2,3
CBZ	165, 147, 193*, 221	1,18E5	-1,82E5	0,993	1,21E5	2,9
IB	117, 160*, 263	9,02E5	1,32E6	0,994	7,41E5	2,3
BP-1	105, 164, 343*	2,58E5	-3,62E5	0,993	2,51E5	2,8

**Quantification ion*

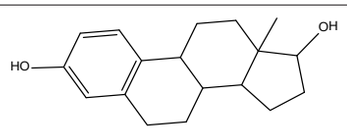
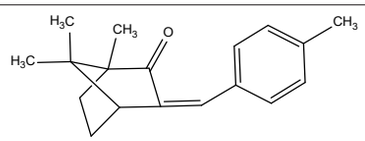
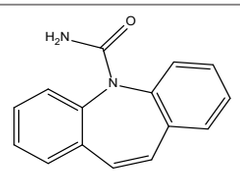
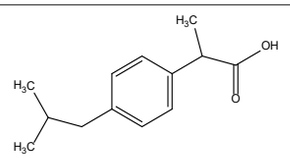
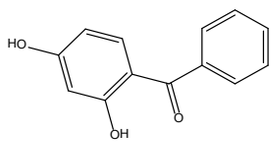
Determination water samples

Water from Maipo River (Metropolitan Region, Chile) and Villarica Lake (IX Region, Chile) was used to apply the proposed methodology. Samples were collected from selected sampling points and filtered with 0.45µm membrane filters and kept at -18 °C until extraction. Samples were subject to the extraction/desorptive procedure in optimum conditions. Samples were then enriched with the analytes at 500 ng L^{-1} concentration and extracted again to determine recoveries (n=3).

RESULTS AND DISCUSSION

The research was conducted with a defined number of compounds considering that this method of extraction and subsequent detection could be applied to other compounds having respective retention times and affinity with the derivatizing used. The compounds were selected as representatives of the areas of interest, given its widespread use, Table 2 presented most important physicochemical properties of the selected analytes.

Table 2. Physicochemical properties from selected PPCP´s.

Compound	pka	Log $K_{0/W}$	Molecular weight (g/mol)	Chemical structure
β-estradiol	10,27	4,13	272.38	
3-(4-metylbenzylidene) camphor	**	5.8	254.37	
Carbamazepine	7,00	2,47	236.27	
Ibuprofen	4,91	3,97	206.28	
2,4 dihidroxybenzophenone	**	4.7	214.22	

***Non-reported value.*

SPE optimization

The first parameter considered for the solid phase extraction of the selected PPCPs from water samples studied variables correspond to sorbent material. In these case due to the characteristics of the analytes a DVB polymeric phase was selected, as an alternative to the most common cartridge Oasis HLB [13]. Samples as pH and volume sample were optimized. For pH of the sample considering that most of the compound were from acidic nature, pH 7 and lower were tested since acidification of an aqueous solution is likely to reduce the dissociation of the weakly acidic analytes, which can improve the efficiency of extraction [14,15]. As can been seen in Figure 1, only IB show an increase in the chromatographic response when pH 5, while the other analytes have no significant difference at the pH interval. For further test pH 5 was used. For sample volume water samples enriched with β-estradiol, 4-MBC, carbamazepine, ibuprofen, BP1 to concentration 500ng L^{-1} between 50 mL and 500 mL were employed. As shown in Figure 1 when increasing the sample volume there is an increased in the chromatographic response due to higher preconcentration of the analytes that will lower the detection limits of the methodology. However when using real samples higher volume will increase cartridges saturation given by interference in the matrix [16] and 500 mL were selected for further analysis.

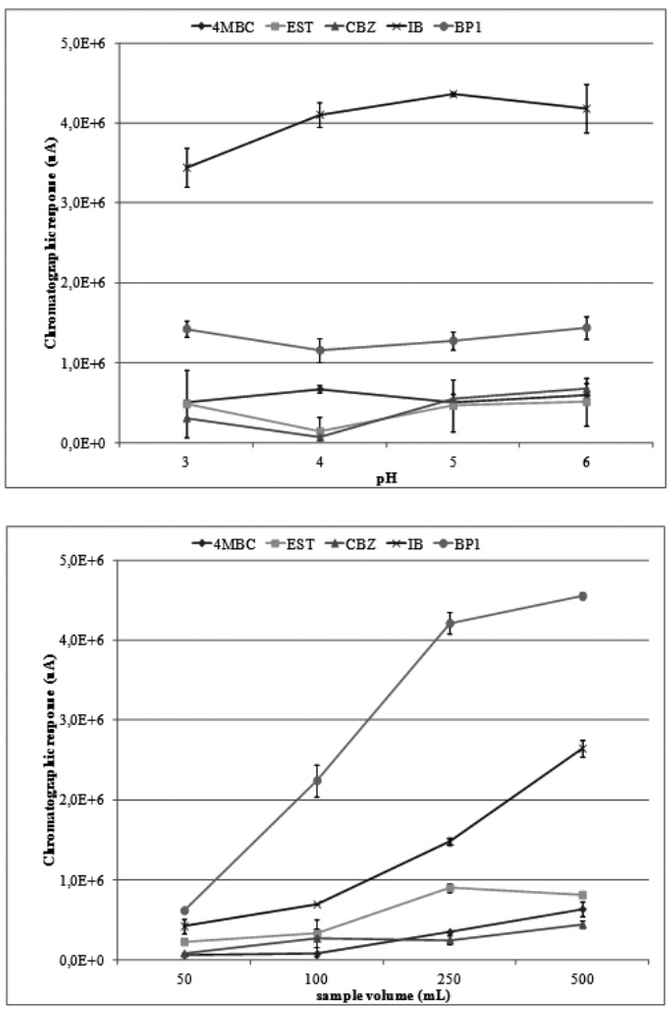


Figure 1. Influence of a) pH and b) sample volume, during SPE extraction of selected PPCPs

In order to considered possible saturation of the cartridge [17], as a result of the limited active sites of the sorbent material, which are available for any interference as well as the analytes of interest, extraction using cartridge with 30 mg DVB were used but no significance difference was observed when using spiked water samples. Also extraction were made at three concentration levels 200, 500, 800 ng L^{-1} but as can be observed in Figure 2 no saturation of the cartridge was observed and a linear relation between concentration and normalize chromatographic response is observed.

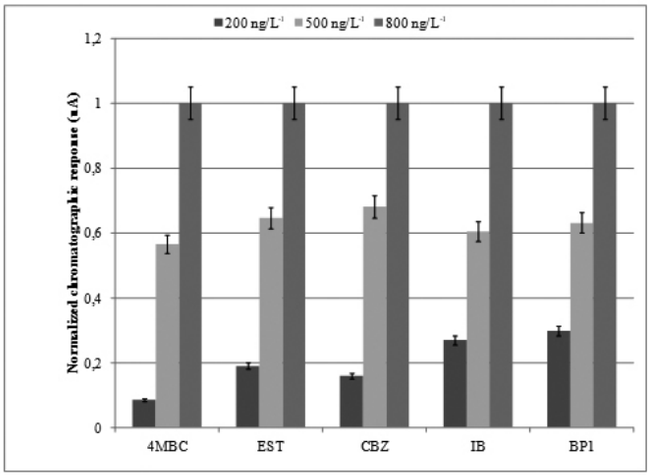


Figure 2. SPE extraction of selected PPCPs at three concentration levels.

SBSE extraction

Variables involved in the extraction were optimized in order to increase the analytes recoveries and preconcentration factor with minimum solvent [18]. pH show the same influence for SBSE extraction as in SPE extraction and all following experiments were performed a pH 5. Figure 3 shows the studies conducted in spiked water to optimize the extraction time, sample volume and salting out effect. A study of the influence of the sample volume in the extraction was performed at 1 hour extraction by spiking the same amount of analytes in different volumes of aqueous sample. The results show no significant difference between extraction efficiency at lower samples volume (due to better mass transport) but in order to increased preconcentration factor 250 mL was selected. The extraction time was also optimized, but in an effort to diminish the time for the extraction procedure and to equal it to the time employed in the SPE extraction a maximum of 120 min was choosen. Considering a 250 mL of aqueous sample a higher response was observed a 120 minutes, which is consequent with this type of extraction when higher extraction time increase the response since the extraction equilibrium could be achieved [19]. The salting out effect was studied by adding up to 30% of sodium chloride to the sample in order to increase the ionic force and improve the analyte recoveries for those analytes with lower K_{ow} [20] As can been seen in Figure 3 most of the compound increases their response when adding up to 30% of NaCl, however 4MBC show a decrease in the chromatographic response when adding a higher % of NaCl, this relies in the difference of polarity of the 4MBC (Log K_{ow} 5,4) showed in Table 2.

Previously reported desorption´s conditions were used [21], 2 mL methanol with 30 min back-extraction time. There was no carry over effect and a cleaning step pervious to the reuse of the stir bar consistent in 1 hour stirring with 10 mL of fresh methanol.

Real water samples

Real water samples from Maipo River and Villarica Lake were analized at optimum condition, with all concentration´s samples under the LOD. To verify the accuracy of the developed methodologies, the recovery experiments were carried out the analysis of spikes samples. Results obtained are summarized in Table 3. Due to the characteristics of compounds better recoveries are found with SPE methodology. A chromatogram of sample before and after spiking using SPE is presented in Figure 4, similar chormatograms when using SBSE were obtained indicating that both methods exhibited similar matrix effect.

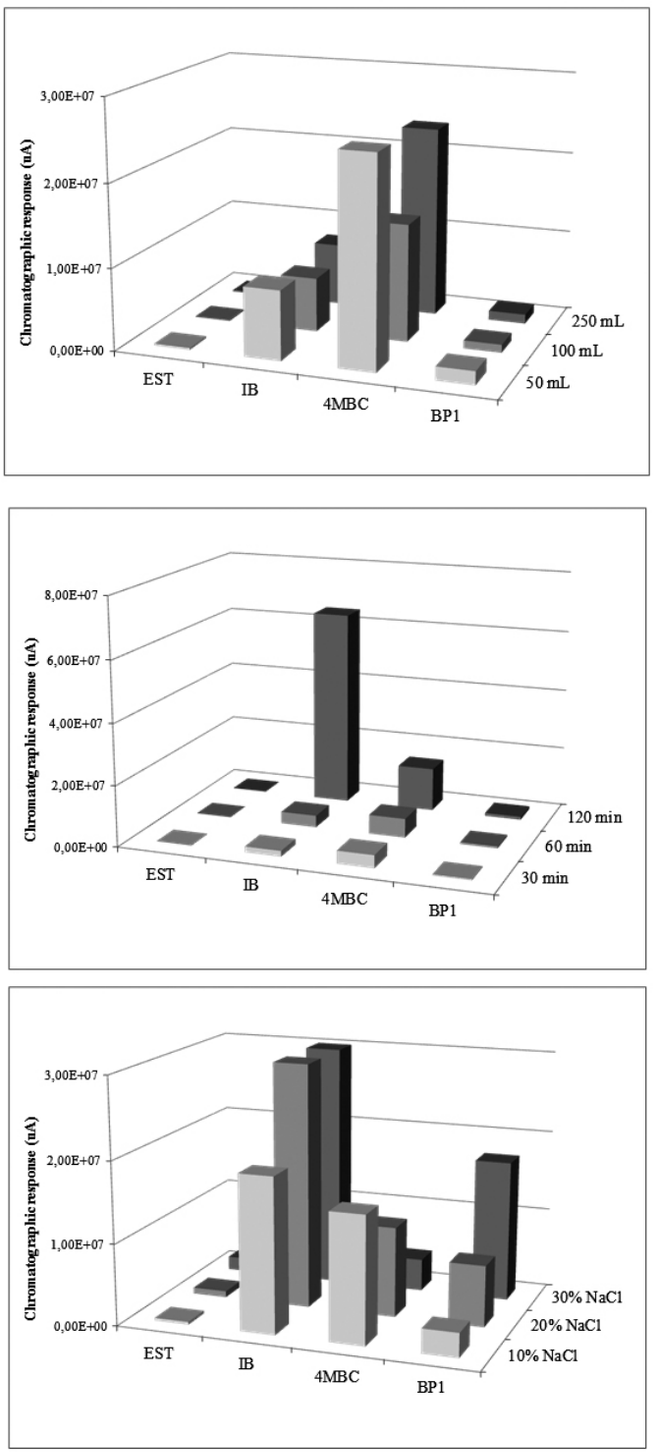


Figure 3. Influence of a) volume sample, b) extraction time and c) %NaCl during SBSE extraction of selected PPCPs

Table 3. Recovery obtained at optimum condition.

	SPE		SBSE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
IB	88.6	9.6	75.0	12.4
BP1	90.0	11.5	81.2	14.2
4MBC	82.5	14.0	83.2	9.4
CBZ	86.6	11.0	77.5	9.1
EST	83.1	6.3	71.4	10.7

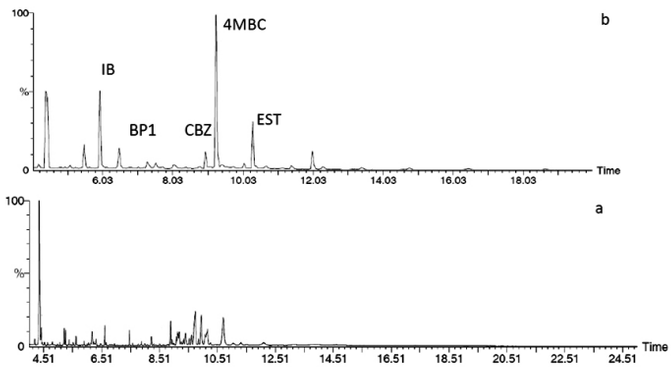


Figure 4. Chromatogram of a) unspiked sample and b) spiked sample.

CONCLUSION

Pharmaceuticals are one major group of emerging contaminants that are commonly found in environmental matrices. Two sample preparation methods combined with derivatization with BSTFA and GC-MS detection for the determination of selected PPCPs in aqueous samples were developed in this study, both methodologies were able to simultaneous analyze pharmaceuticals belonging to different therapeutic groups and having differences in their chemical structures. SPE showed better recoveries and RSD for river and lake water when extracted 500 mL sample at pH 5 rather than using SBSE at same sample volume and pH and adding NaCl at 30% with an extraction time of 120 min. Both methodologies were adequate for the determination of the analytes in study, with recoveries between 71 and 82% for SBSE and SPE respectively, with RSD lower than 15% for both methods, proving to be an tool to collect information about entrance, distribution and impact of pharmaceutical in the environment.

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REFERENCES

1.- S. Sauvé, M. Desrosiers, Chem. Cen. J.l **8**, 15. (2014)
2.- J.B. Ellis, Environ. Pollut. **144**; 184, (2006)
3.- D.G Daughton, Environ. Impact Assess. Rev. **24**, 7-8, 711, (2004)
4.- A. Ramirez, R. Brain, S. Usenko, M. Mottaleb, J. O´Donnell, L.Stahl, J. Wathen, B. Xnyder, J. Pitt, P. Perez-Hurtado, L. Dobbins, B. Brooks, C. Chambliss, Environ. Toxicol. Chem. **28**, 2587, (2009)
5.- M. Pedrouzo, F.Borrull, R.M. Marcé, E. Pocurull, Trends Anal. Chem. **30**, 1247, (2011)
6.- B. Halling-Sorensen, S. Nors-Nielsen, P.F. Lanzky, F. Ingerslev, H.C. Holtenlutzhoft, S.E. Jorgensen, Chemosphere **36**, 357, (1998)
7.- J.C. Duran-Alvarez, E. Becerril-Bravo, V. Silva-Castro, B. Jiménez, R. Gibson, Talanta **78**, 1159, (2009)
8.- R. Liu, J.L. Zhoun, A. Wilding,. J. Chromatogr. A, **1022**, 179, (2004)

9.- W.W. Buchberger, J. Chromatogr. A, **1218**, 603, (2011)
10.- M.S. Díaz-Cruz, M.J.L. de Alda, D. Barceló, Trends Anal. Chem, **22**, 340, (2003)
11.- T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, J. Hazard. Mater. **183**, 701, (2010)
12.- Nguyen K., Scapolla, C., Di Carro M, Magi, Talanta **85**. 2375, (2011)
13.- N. Migoswska, M. Caban, P. Stepnowski, J. Kumirska. Sci. Total Environ. **441,** 77, (2012)
14.- J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Anal. Chim. Acta **550**, 116, (2005)
15.- M. Kawaguchi, R. Ito, N. Endo, N. Sakui, N. Okanouchi, K. Saito, N. Sato, T. Shiozaki, H. Nakazawa, Anal. Chim. Acta, **557**, 272, (2006)
16.- P. Paiga, A. Lolic, F. Hellebuyck, L. Santos, M. Correia, C. Delerue-Matos, J. Pharm. Biomed. Anal., **106**, 61, (2015)
17.- S. Weigel, R. Kallenborn, H. Huhnersuff, J. Chromatogr. A, **1023**, 183, (2004)
18.- M. Fernández, A Giordano, M. Ruiz, G. Font, Y. Picó, Anal. Bioanal. Chem., **393**, 1733, (2009)
19.- I. Bruheim, X. Liu, J. Pawliszyn, Anal. Chem. **75**, 1002, (2003)
20.- P. Richter, A Giordano, C. Choque, C. Leiva, B. Sepulveda, J. Chromatogr. A., **1216**, 8598, (2009)
21.- A. Giordano, P. Richter, K. Leiva, L. Ascar, J. Chil. Chem. Soc. **58**, 2, (2013)