Development and application of a polar coating for stir bar sorptive extraction of emerging pollutants from environmental water samples

D. Bratkowska[1], R.M. Marcé[1]*, P.A.G. Cormack[2], F. Borrull[1], N. Fontanals[1]

[1] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili
Campus Sescelades Marcel·lí Domingo, s/n, 43007 Tarragona, Spain
Phone + 34 977 55 81 70
Fax +34 977 55 84 46
*e-mail: rosamaria.marce@urv.cat
[2] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Keywords: stir bar sorptive extraction (SBSE); monolithic polar material; pharmaceuticals and personal care products (PPCPs); environmental water samples

Abstract

In the present study, a stir bar coated with hydrophilic polymer based on poly(N-vinylpyrrolidone-co-divinylbenzene) was prepared for the sorptive extraction of polar compounds. The main parameters affecting the polymerisation of the coating were investigated.

The new stir bar was applied successfully in stir bar sorptive extraction with liquid desorption followed by liquid chromatography-mass spectrometry in tandem with a triple quadrupole for the determination of a group of polar pharmaceuticals and personal care products (PPCPs) in environmental water matrices. Different variables affecting extraction and desorption such as agitation speed, temperature, ionic strength and extraction time were optimised. The results showed that the stir bar is able to enrich the selected analytes effectively.

The developed method was applied to determine a group of PPCPs in different complex environmental samples, including river, effluent and influent waste water.

1. Introduction

One of the research fields in analytical chemistry is the development of methods to determine contaminants in environmental samples. Due to the low concentration of these contaminants in these kinds of samples, a sample preparation technique together with a high sensitive instrumental technique is required.

For aqueous sample preparation, commonly used techniques include solid-phase extraction (SPE), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE), among others [1-3]. SPE is still the most commonly used technique due to the variety of sorbents commercially available which cover the extraction of compounds with a wide range of physico-chemical properties. In the recent years, SBSE has been proven to be a promising technique for the extraction of liquid samples [1,4] due to the larger coating compared to the one in SPME [3].

In SBSE, the magnetic stir bar encapsulated in a polymeric coat is introduced in the aqueous samples and after stirring for a certain time, the analytes are desorbed thermally or with a solvent. The main factor that determines the extraction efficiency is the partition coefficient of analytes between the phases. Since the stir bar has a larger volume of polymeric phase than SPME fibres, higher extraction efficiency can be obtained [4]. During the last few years, it has been clearly demonstrated that a wide range of compounds, such as pesticides, steroids, fatty acids, drugs, and so on can be extracted by SBSE [4-6], and this technique has been applied successfully to trace analysis in biomedical [5], pharmaceutical [6] and environmental samples

[1]. However, the application of these techniques to polar compounds is still a challenge due to the characteristics of the extraction materials.The only commercially available sorptive extraction phase is poly(dimethylsiloxane) (PDMS). Nevertheless, since PDMS is a non-polar phase, it is suitable for extracting less polar compounds. Correspondingly, polar analytes are not well recovered and the applicability of PDMS-coated stir bars is reduced to the extraction of the non-polar or semi-polar compounds [6-9]. To overcome this limitation, polar coatings based on different materials prepared by sol-gel technology [10], monolithic approach [11], polyurethane foam [12] or activated carbons [13] have been tested as SBSE coating. Recently, some monolithic polar coatings have been developed to enrich different analytes by SBSE [11,14-17]. For instance, poly(vinylimidazole-divinylbenzene) was successfully applied to extract polar aromatic amines from water samples [17]or vinylpyrrolidone-divinylbenzene (VPD-co-DVB) was applied to the extraction of metals, phenols, anilines, hormones and polycyclic aromatic hydrocarbons (PAHs) [14,15] .

The objective of the present work was the preparation of an improved stir bar with polar coating based on poly(VPD-co-DVB) for SBSE and its application followed by liquid chromatography-mass spectrometry in tandem (LC-MS-MS) for the determination of a broad range of compounds that include polar pharmaceuticals and personal care products (PPCPs) from complex environmental matrices.

2. Experimental

2.1 Reagents and standards

The reagents used for the polymer coating syntheses were divinylbenzene (DVB) (80% grade) supplied by Aldrich (Steinheim, Germany) and N-vinylpyrrolidone (VPD) (99% grade) supplied by Fluka (Buchs, Switzerland). DVB and VPD were freed of polymerisation inhibitors by passing them through short columns packed with neutral alumina. The cyclohexanol (99%) and 1-dodecanol (98%) used as porogens were purchased from Aldrich. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck, Darmstadt, Germany).

The analytes selected to evaluate the sorption characteristics of the stir bars were: paracetamol, caffeine, antipyrine, propranolol, carbamazepine, ibuprofen, diclofenac, methylparaben, ethylparaben, propylparaben, triclocarban, 2,4-dihydroxybenzophenone (DHB), 2,2´-dihydroxy-4-methoxybenzophenone (DHMB) and benzophenone-3 (BP3); all were obtained from Aldrich. The chemical structure, pK_a and log K_{ow} of each analyte are shown in Fig.1.

Standard solutions for each compound at 1000 mg L^{-1} were prepared in methanol (SDS, Peypin, France). The mixture of all the compounds was prepared by diluting the standard solution in Milli-Q water (Millipore, Bedford, MA, USA).

LC-grade acetonitrile (SDS) and Milli-Q water were used to prepare the mobile phase. Formic acid (Prolabo, Bois, France), hydrochloric acid (Probus, Barcelona, Spain) and sodium hydroxide (Panreac, Barcelona, Spain) were used to adjust the pH of the mobile phase or of the sample. Sodium chloride was obtained from Aldrich.

2.2 Stir bar preparation

In a typical synthesis of a monolith, the monomers DVB (85%, w/w) and VPD (15%, w/w) (40% w/w total monomer in feed relative to solvent) and AIBN (1% mol relative to polymerisable double bonds) were added to the porogen (10% w/v 1-dodecanol in cyclohexanol). The monomer mixture, initiator and porogen were mixed ultrasonically, and the homogenous solution poured into a glass tube with a defined diameter (4 mm i.d.). A stir bar (10 x 3 mm i.d.) was inserted into the middle of a spring (3 mm i.d.) and then immersed vertically into the polymerisation solution. Before polymerisation, the monomer solution was placed on an ice-bath, sparged with oxygen-free nitrogen for 5 min to remove oxygen and then sealed under a nitrogen atmosphere. The sealed glass tube with the stir bar inside was then left in a water bath set at 60 °C for 36 h. After this time, the glass tube was crushed carefully and the glass removed to reveal the polymer monolith. A smooth, solid monolith with good dimensional stability was obtained. Fig. 2 schematises the obtained stir bar assembly. The monolithic material on the bar was Soxhlet-extracted with MeOH for 24 h to remove residual monomers and initiator, soluble oligomers and porogen. The final dimensions of the poly(VPD-co-DVB) stir bar was as follows: length: 10 mm; polymer thickness: 0.5 mm corresponding to a volume of polymer of about 70 µl.

The monolithic poly(VPD-co-DVB) were then characterised. The specific surface area of the monolith was measured using a BET treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The carbon, hydrogen and nitrogen content of the polymer were determined with elemental microanalysis using a Carlo-Erba EA 1106 Instrument. FTIR spectroscopy measurements were performed using a Perkin-Elmer Spectrum One FTIR Spectrometer.

2.3 LC-(ESI)MS-MS analysis

The chromatographic experiments were performed with an Agilent HP 1200 liquid chromatograph coupled to a triple quadrupole mass spectrometer with electrospray interface (ESI) from Agilent Technologies (Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain) and the volume injected was 50 µl. The mobile phase was acetonitrile and Milli-Q water adjusted to pH 3 with formic acid. The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 30 ºC. The gradient profile was initially from 20% to 100% ACN in 15 min (held 5 min), after which time the mobile phase was returned to the initial conditions (20% ACN) in 3 min.

The (ESI)MS-MS parameters were optimised by direct injection of each analyte. Nitrogen was used as collision, nebulising and desolvation gas. The ESI interface conditions were optimised for each analyte and were: a nebuliser pressure of 45 psi, capillary voltage of 4000 V, desolvation (drying gas) N_2 flow was set at 12 l min^{-1} and desolvation temperature was 350 ºC. Analyses were performed under multiple reaction monitoring (MRM) mode and MRM transitions for each analyte are summarised in Table 1.

2.4 SBSE assays

The procedure with home-made poly(VPD-co-DVB) stir bar was as follows: the stir bar was activated with 1 mL of MeOH and stirred for 5 min. After drying with lint-free tissue, the stir bar was placed into a flask with 50 mL of water sample at pH 5.0, with 10% of NaCl and then the sample was stirred at 900 rpm for 4 hours at room temperature. After the extraction, the stir bar was removed with tweezers, dipped briefly in Milli-Q water and dried with a lint-free tissue. In order to desorb the analytes with a solvent, stir bar was placed into a vial with 5 mL of MeOH stirred at 900 rpm for 15 minutes. Then, the stir bar was magnetically removed and the extract was evaporated to dryness under gentle stream of nitrogen. The dry residues were redissolved in 1 mL MeOH:water (1:1) and transferred to a vial, closed with a seal using a hand crimper, and placed into the automatic liquid sampler tray for LC-(ESI)MS-MS analysis.

The retention capability of the poly(VPD-co-DVB) stir bar was compared to the commercially available ~~stir bar~~ PDMS-coated stir bar (Twister), obtained from Gerstel (Mulheim Ruhr, Germany). It consists of a 10 mm long glass-encapsulated magnetic stir bar, externally coated with a layer of 0.5 mm thick, corresponding to a volume of 24 µL of PDMS. Prior to the first use, the stir bar was placed in a vial containing ACN and conditioned for 24 h. Although there are two sizes of PDMS-coated stir bar commercially available, this smaller size was selected, since it provided high sorptive capacity for water sample volumes range from 1 to 50 mL [4].

Reconditioning of stir bars was done after the extraction in MeOH for poly(VPD-co-DVB) stir bar or methylene chloride:MeOH (1:1) for PDMS.coated ~~sitr~~ stir bar [18]. Stir bars were conditioned by placing them in vials with the solvent for 20 min. Then the solvents were refreshed and the procedure was repeated three times. Subsequently, the stir bars were dried with a lint-free tissue, and kept in a vial for the next analysis.

2.5 Sample collection

The river water samples were collected from Ebre River. The waste water samples were collected from the effluent and influent of two sewage treatment plants (STPs).

To ensure proper conditions for keeping the samples, all the real water samples (Ebre river, effluent and influent waste water from STPs) were adjusted to pH~3 with HCl and stored at 4 ºC before analysis. They were filtered through 0.45 μm nylon membranes (Supleco, Bellefont, PA, USA) before the stir bar extraction to eliminate the particulate matter of real samples.

3. Results and discussion

3.1 Preparation and characterisation of the stir bars

With the aim of improving the efficiency of polar compounds by SBSE with commercially available PDMS, a polar group should be introduced in the monolithic coating of the stir bar. Due to the good results provided by vinylpyrrolidone as polar group in the commercial OASIS HLB sorbent for SPE [19] we selected it as a potential polar monomer. In fact, in a recent study vinylpyrrolidone was already tested as polar monomer in the stir bar coating and results for phenols were better than those obtained with PDMS stir bar [14].

However, a limitation of monolithic stir bars is the mechanical stability along the extractions. In order to overcome this limitation, we proposed a new design which includes a spring covering the stir (figure 2) in order to increase the mechanical resistance, the number of extractions to be done with the same stir bar, and therefore the robustness of the analytical method. To do this, a stir of 10 x 3 mm was covered by the spring (3 mm i.d.) and introduced in the glass vial (4 mm i.d.) in which the polymerisation mixture was then poured. In this case, the presence of the spring made it possible to obtain a thinner coating (0.5 mm) with similar mechanical properties. To test this new design, the starting polymerisation conditions were based on those previously reported for polar monolithic coatings [11,14], and we optimised some parameters in the polymerisation procedure in order to further enhance the properties of the final stir bar.

Some important parameters to take into account in the synthesis of a monolith are: the porogen, that is responsible for the porous formation during the polymerisation; the crosslinker monomer/polar monomer ratio in order to introduce polarity on the coating (via monomer) and rigidity (via crosslinker); the ratio between total monomers and porogen, and polymerisation temperature and the type of polymerisation initiation.

As porogen, a mixture of 1-dodecanol and cyclohexanol was used since this mixture of solvents had been demonstrated to be useful for imparting porosity into such type of monolithic materials [11,20], because the types of porous formed allow the analytes to penetrate the polymer and also provide enough surface area. From the different ratio tested, 10/90 (w/v) proved to be a convenient porogen mixture.

Regarding the monomers ratio, it was fixed at 85/15 w/w of DVB to VPD (this led to a polymer monolith with a specific surface area of 600 m^2 g^{-1}), since it was found that the use of lower amounts of DVB (75%) in the monomer feed led to a slight decrease in the specific surface areas of the product (560 m^2 g^{-1}). These specific surface areas are as expected, taking into account the high % of DVB and the non-porogen solvent used [19].

The ratio of total monomer to porogenic solvent was set at 40/60 (w/w), because higher monomer ratio led to soft polymers which were broken easily during later stirring operations.

A further variable which was examined was the nature of the initiation step in the polymerisation (thermal *versus* photochemical initiation). With this in mind, thermally-initiated and photochemically-initiated polymerisations were compared. The UV-initiated polymerisations, which were carried out at room temperature, yielded soft and fragile materials consistent with incomplete cure, whereas those materials produced by thermally-initiated polymerisations were hard and had good dimensional stability, consistent with efficient cure. As a consequence, thermal initiation was the method of choice in all subsequent polymerisations.

Finally, it was found that the polymerisation temperature had some influence upon the mechanical properties of the monolithic material. Polymers synthesised at 70 ºC were less mechanically stable than polymers synthesised at 60 ºC, therefore a polymerisation temperature of 60 ºC was employed in all subsequent polymerizations, which were run for 36 hours.

Although the optimum polymerisation conditions were quite similar to those found by Huang *et al.* [15], there are some differences as regards the total monomer/porogen ratio, polymerisation time and temperature, the format and size.

FTIR spectroscopic analyses of the poly(VPD-co-DVB) coatings confirmed that DVB and VPD had been copolymerised effectively into the monoliths. Diagnostic bands consistent with aromatic residues (from DVB) and amide groups (1690 cm^{-1}, from VPD residues) were observed.

Elemental microanalysis of the poly(VPD-co-DVB) monolith gave the following results: carbon (83.0%), hydrogen (7.7%) and nitrogen (2.8%), which are similar to the theoretical values, and provides evidence that both monomers were incorporated into the monolith.

3.2 LC-(ESI)MS-MS optimisation

Once the chromatographic separation was optimised, the best MS-MS conditions were selected and they are shown in section 2.3 and detailed in Table 1. For each analyte, two fragmentations of $[M-H]^+$ or $[M-H]^-$ were acquired. The most intense transition was selected to quantify the analytes. Paracetamol, caffeine, antipyrine, propranolol, carbamazepine and BP3 were determined under positive ionisation mode; whereas ibuprofen, diclofenac, parabens, DHB, DHMB and triclocarban exhibited a higher response in the negative mode.

All selected analytes showed good linearity ($r^2 > 0.997$) by direct injection of standards and the linear range was 0.5-100 µg L^{-1} for all the analytes, except for methylparaben (0.1-100 µg L^{-1}) and for antipyrine (1-100 µg L^{-1}). The limits of detection (LODs) calculated as a signal-noise ratio (S/N) ≥3 were 50 ng L^{-1}

for the majority of compounds, apart from paracetamol, antipyrine, ethylparaben, DHMB and ibuprofen with a LOD of 100 ng L^{-1}.

3.3 Optimisation of SBSE procedure

In order to test the extraction efficiency of the novel polar stir bar, a group of PPCPs with different polarity were selected.

During the development of the presented procedure, several parameters that influence the SBSE process were evaluated. Thus, systematic assays were performed to optimise parameters which can influence the SBSE procedure during both extraction and liquid desorption steps.

3.3.1 Liquid desorption conditions

In the first approach, we tested the best liquid desorption conditions, which ensured complete back extraction of selected analytes from the stir bars. Thus, two organic solvents: MeOH and ACN were examined as a desorption solvent. To do this, we initially used the following experimental conditions: 50 mL of the sample spiked at 1 μg L^{-1}, 1 hour of extraction time, 750 rpm of agitation speed at room temperature, and for desorption, 5 mL of organic solvent for 15 min at the same agitation speed, based on previous experience from our group [18]. From the data obtained, MeOH was chosen as the back extraction solvent due to the slightly higher ability to desorb polar analytes from the stir bars (recoveries increase in ~10%). As for desorption volume, we tested 5 mL of solvent in order to immerse the stir bar completely and ensure proper desorption. Further increasing of MeOH volume (10 mL) did not improve the desorption results.

The influence of temperature (25 ºC, 35 ºC, 40 ºC) on desorption in SBSE process was also investigated. The results obtained seem to indicate that desorption was not significantly affected by temperature. Consequently, room temperature was selected for the further experiments.

In order to investigate the time necessary to ensure complete desorption of the analytes from the stir bar, a series of experiments were performed by comparing several periods of time (10, 15, 30 and 60 min) to achieve the best liquid desorption conditions. The results indicated that stirring for 15 min is enough to desorb all analytes of interest from the stir bar and additional desorption did not show presence of the analytes. Consequently, a period of 15 min was selected for desorption process. This desoption time is quite shorter than the one previously used (i.e. 2 h) for a similar stir bar [14,15], although in that case, the speed was lower (400 rpm) and stir bar was thicker (1 mm thickness).

3.3.2 Extraction conditions

SBSE is a balanced technique and the analytical process is considerably influenced by agitation speed, temperature, ionic strength, pH and extraction time involved. Starting with the stirring rate, this factor may influence the mass transfer of the analytes during the extraction process. In the present paper, three agitation levels (600, 900 and 1250 rpm) were tested to achieve the best stirring conditions. The response obtained with 900 rpm was higher than that obtained with 600 rpm, and further increases in the stirring rate may damage the monolithic phase. As the agitation speed of 900 rpm provided better recovery of all analytes under study, it was selected for further experiments. This agitation speed was also tested for liquid desorption step and since no significant differences were observed with the initial 750 rpm, 900 rpm was the selected speed in both extraction and desorption steps.

It is noteworthy that higher stirrer speeds could be applied in the present work than were used in previous studies (400 rpm) [14,15]; in the present case, the metal spring acts to reinforce and strengthen the stir bar coating and allow higher stirrer speeds to be realised without attrition of the coating." ~~The use of this possible higher speed compared to that used in previous studies (400 rpm) [14,15] is probably due to the presence of the spring, which confers mechanically stability to the stir bar.~~

The influence of temperature (25 ºC, 35 ºC, 40 ºC) in SBSE process was also studied. It was found that the recoveries of the extractions at 25 ºC and 35ºC were very similar for most of analytes and recoveries slightly decreased (~10%) at 40ºC. As a result, 25 ºC was selected for the further experiments.

The effect of sample pH on the extraction efficiency was investigated by adjusting Milli-Q water at the pH values in the range from 3.0 to 11.0. Fig. 3 shows the influence of the sample pH on SBSE efficiency of a representative group of analytes, and it can be seen that the pH value significantly affects the extraction efficiency of some analytes. The results showed that the extraction recoveries for most of analytes improved with increasing the pH from 3.0 to 5.0 and slowly decreased (except for propranolol) at pH 7. At higher pHs the effect of pH depends on the pK_a (included in fig. 1) of the studied analytes. For example, the recovery for the analytes with pK_a values lower than 8.3 decreases at the highest pHs tested; however, compounds with high pK_a values such as carbamazepine, caffeine or antipyrine showed no significant changes. In the case of propranolol, the recovery improved under basic conditions (maximum at pH 9.0), due to its pK_a value. Considering these results, pH 5.0 was chosen for further experiments.As it is well known, the extraction efficiency in SBSE might depend on the ionic strength in the sample matrix [11]. In the present study, the effect of ionic strength in the recovery of the compounds under study was performed by addition of NaCl from 0 to 15 % (w/v) to the aqueous samples. Fig. 4 shows the effect of salt addition on extraction efficiency for the representative group of analytes. It can be seen that for the same analytes, the recoveries decreased with 5% NaCl addition, and then increased to the maximum with 10% NaCl addition. This effect may be attributed to the salting-out effect and the electrostatic interaction between polar molecules and salt ions in the solution [21]. With a higher percentage (i.e. 15%) of NaCl, the recoveries decreased. As 10% addition of NaCl in the samples provided the best recoveries, this addition was selected in the following studies.

The effect of sample volume on the recovery was also investigated. In order to select the optimum sample volume, different sample volumes (25, 50 and 100 mL) of standard solution of the analyte mixtures were extracted. The results were similar when both 25 mL and 50 mL of the Milli-Q water sample were analysed. When 100 mL of a standard solution was extracted, the recoveries decreased significantly (10-30 % recovery decrease). This result was expected because the higher the volume, the higher the extraction time needed to reach the equilibrium [22]. For this reason, 50 mL of sample was selected for further analysis.

The extraction time is also a very significant parameter. In order to select the best conditions, different extraction periods (1, 2, 4, 6 and 12 hours) were tested. Fig. 5 shows representative time extraction profiles for a representative group of analytes, where it can be seen that the extraction efficiency increased when the extraction time increased from 1 to 12 hours. However, in order to reach a balance between analysis time and extraction efficiency, 4 hours was selected as the extraction time.

After the optimisation procedure, the conditions used to investigate recoveries from environmental samples were as follows: 50 mL of the sample adjusted at pH 5.0 with 10 % NaCl addition and agitated at 900 rpm at room temperature for 4 hours for the extraction and 5 mL of MeOH stirred at the same speed for 15 min for liquid desorption

With the optimum conditions, the recovery for each analyte from the standard solution, including the evaporation step, is shown in Table 2. These recoveries were calculated from the calibration curve by direct injection. The poly(VPD-co-DVB) stir bar method attained very good performance and presented high recoveries, between 80% and 100%, for most analytes and only presented lower recovery for paracetamol, caffeine and antipyrine, whose results were expected since they are very polar compounds (see $\log K_{o/w}$ in fig. 1). These results are very satisfactory, taking into account the polarity of these compounds and the results achieved with a similar stir bar [14,15] for other compounds with a broad range of polarity, such as phenols, aromatic amines, and PAHs.

3.3.3 Comparison to a commercial stir bar

The SBSE performance of the poly(VPD-co-DVB) stir bar was compared to the commercially available stir bar based on PDMS. The SBSE results arising from the use of the PDMS-coated stir bar are also included in Table 2. It can be seen that under the same conditions, the analyte recoveries were higher for most of the compounds with poly(VPD-co-DVB) than with PDMS. Moreover, PDMS was not able to retain any of the more polar compounds while the poly(VPD-co-DVB) stir bar showed good retention, with higher recoveries for most of the analytes. Those results clearly indicate that the selected analytes, having different polarity, presented better affinity for poly(VPD-co-DVB) phase rather than for PDMS. These results can be easily explained by the polar character of the monomer used in the synthesis of the new stir bar coating, while PDMS is an apolar phase. Another significant contribution was that the specific surface area of poly(VPD-co-DVB) monolith was 600 $m^2 g^{-1}$ whereas for PDMS-coated stir bar, the specific surface area is expected to be low from its chemical structure. In any case, it is evident that the poly(VPD-co-DVB) stir bar gives higher recoveries for all of the target analytes than the commercially available PDMS stir bar.

3.4 Analysis of real samples

Given the promising SBSE data obtained with poly(VPD-co-DVB) stir bar when the procedure was applied to Milli-Q water, the same protocol was applied to the analysis of water from the Ebre river and effluent and influent water from a waste water treatment plant (WWTP), where PPCPs had already been detected [23-25].

It is known that electrospray ionisation may give ion suppression/enhancement when complex samples are analysed and this effect was studied by spiking with the analytes the SBSE extracts of river water, effluent and influent WWTP water samples and quantifying the recoveries taking into account the unspiked SBSE extracts. The resulting ion suppression/enhancement ranged between 0 and 22%. Therefore, this effect was not significant.

To calculate the recoveries, the different water samples spiked at different levels of each analyte and the unspiked samples were analysed. Table 3 shows the SBSE results for the different water samples. The data obtained for river water samples were good, with recovery values ranging from 50 to 100% for most of the compounds and similar to the values obtained for extractions from Milli-Q water taking into account the ion suppression and thefore demonstrating the satisfactory ability of this monolithic material to retain both polar and semi-polar compounds even in the presence of the matrix. As can be seen, the data for effluent and influent WWTP water is also good, with recovery values ranging from 50% to 100% for the effluent WWTP sample and 40% to 100% for the influent WWTP sample for most of the compounds.

Then, the method was validated with river water. When the linear range was tested in a 6 concentration levels by duplicate, the analytes exhibited good linearity ($r^2 > 0.993$): between 20 and 2000 ng L^{-1} for carbamazepine and diclofenac; between 100 and 2000 ng l^{-1} for paracetamol and DHMB; and between 50 and 2000 ng L^{-1} for the rest of the compounds. The LODs, calculated using S/N≥3, were between 10 and 20 ng L^{-1} for all the compounds, with the exception of paracetamol and DHMB (50 ng L^{-1}). The repeatability and reproducibility between days of the method, expressed as the relative standard deviation (%RSD) of three analyses of 50 mL of water from the Ebro river spiked at 100 ng L^{-1} were lower than 7% and 15%, respectively, for all compounds. In any case, the good performance of the poly(VPD-co-DVB) stir bar has been demonstrated.

It should be highlighted that between 30 and 40 samples can be extracted with the same stir bar without changes in the recovery, which is quite satisfactory taking into account the thickness (0.5 mm) of the bar. The bar-to-bar reproducibility (%RSD, n=3) were lower than 10% for all the analytes.

In the river samples analysed, propylparaben (88-129 ng L^{-1}), BP3 (52-103 ng L^{-1}) and ibuprofen (LOQ-72 ng L^{-1}) were present. In one sample, caffeine and methylparaben were also found at 61 ng L^{-1} and 99 ng L^{-1}, respectively. These values are comparable to those found in the samples from the same river [18,23].

Several samples of effluent WWTPs were also analysed. Some analytes such as antipyrine, propanolol, carbamazepine, propylparaben, diclofenac and BP3 were present in the effluent WWTP water samples analysed. Table 4 includes the concentrations found in one effluent sample analysed in triplicate as an example. In another effluent samples we also found caffeine, methylparaben and DHB, whose chromatogram is shown in Figure 6, as an example. Several papers [23,26,27] also reported the presence of these analytes in this kind of sample.

Table 4 also shows the concentrations of the analytes found in an influent water sample analysed. As can be seen, analytes such as paracetamol, caffeine, methylparaben and ibuprofen were found at high concentration and therefore these samples had to be diluted to be quantified. As expected, most of these analytes were found at higher concentration than in effluent due to the waste water treatment.

Conclusions

In this study, a stir bar coated with poly(VPD-co-DVB) monolithic material was developed by optimising the conditions of the polymerisation to obtain good chemical and mechanical properties of the stir bar.

The poly(VPD-co-DVB) stir bar was successfully applied for extraction of group of analytes with polar characteristics and showed significantly higher retention for the polar analytes compared with the commercially available PDMS stir bar.

The combination of SBSE and liquid desorption with LC-(ESI)MS-MS provided an efficient, simple and sensitive method for determination of PPCPs in complex environmental samples at low levels.

Acknowledgements

The authors thank for the financial support the *Ministry of Science and Innovation* (Projects CTQ 2008-0825 and CTM 2008-06847-CO2-01) and the *Department* of *Innovation, Universities and Enterprise* (Project 2009 SGR 223). N. Fontanals also acknowledges personal funding from the Juan de la Cierva program of the the *Ministry of Science and Innovation*.

References

[1] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642-2656.

[2] F.M. Lancas, M.E. Costa, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813-824.

[3] C. Dietz, J. Sanz, C. Cámara, J. Chromatogr. A 1103 (2006) 183-192.

[4] E. Baltussen, C.A. Cramers, P. Sandra, Anal. Bioanal. Chem. 373 (2002) 3-22.

[5] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 40 (2006) 500-508.

[6] F. Sánchez-Rojas, C. Bosch-Ojeda, J.M. Cano-Pavon, Chromatographia 69 (2009) 79-94.

[7] L. Badoil, D. Benanou, Anal. Bioanal. Chem. 393 (2009) 1043-1054.

[8] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54-69.

[9] J. Regueiro, M. Llompart, E. Psillakis, J. Garcia-Monteagudo, C. Garcia-Jares, Talanta 79 (2009) 1387-1397.

[10] W.A.W. Ibrahim, W. Norfazilah, W. Ismail, A. Syazwani, A. Keyon, M.M. Sagani, J. Sol-Gel Sci. Technol. 58 (2011) 602-611.

[11] X. Huang, N. Qiu, D. Yuan, Q.M. Lin, J. Chromatogr. A 1216 (2009) 4354-4360.

[12] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, J. Chromatogr. A 1209 (2008) 10-16.

[13] N.R. Neng, A.R.M. Silva, J.M.F. Nogueira, J. Chromatogr. A 1217 (2010) 7303-7310.

[14] X. Huang, N. Qiu, D. Yuan, B. Huang, Talanta 78 (2009) 101-106.

[15] X. Huang, N. Qiu, D. Yuan, J. Sep. Sci. 32 (2009) 1407-1414.

[16] Y. Luo, Q. Ma, Y. Feng, J. Chromatogr. 1217 (2010) 2667-2673.

[17] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1217 (2010) 2667-2673.[18] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chem. 397 (2010) 2833-3839.

[19] N. Fontanals, R.M. Marcé, F. Borrull, J. Chromatogr. A 1152 (2007) 14-31.

[20] F. Svec, T.B. Tennikova, Z. Deyl, Monolithic Materials - Preparation, Properties and Applications, Elsevier, Amsterdam, 2003.

[21] X.J. Huang, J.B. Lin, D.X. Yuan, R.Z. Hu, J. Chromatogr. A 1216 (2009) 3508-3511.

[22] C. Bicchi, C. Cordero, P. Rubiolo, P. Sandra, J. Sep. Sci. 26 (2003) 1650-1656.

[23] M. Pedrouzo, S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 297-303.

[24] A. Togola, H. Budzinski, J. Chromatogr. A 1177 (2008) 150-158.

[25] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225-1234.

[26] M. Pedrouzo, S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 297-303.

[27] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1174 (2007) 27-39.

Table 1. ESI mode and MRM conditions used for LC-(ESI)MS-MS of target analytes.

Analyte	ESI Mode	Cone Voltage (V)	Precursor Ion (m/z)	Product Ions (m/z)*	Collision Energy (V)
Paracetamol	+	100	152	**110** 93	15 25
Caffeine	+	125	195	**138** 110	15 25
Antipyrine	+	100	189	**145** 115	30 30
Propranolol	+	125	260	**116** 183	15 15
Methylparaben	-	80	151	**92** 136	15 5
Carbamazepine	+	150	237	**193** 179	35 35
Ethylparaben	-	100	165	**137** 92	15 5
Propylparaben	-	100	179	**92** 136	15 5
DHB	-	130	213	**135** 169	15 15
DHMB	-	80	243	**93** 123	15 5
Diclofenac	-	75	294	**250** 214	10 20
Ibuprofen	-	75	205	**161** 69	5 5
BP3	+	130	229	**151** 105	15 15
Triclocarban	-	130	313	**160** 126	5 15

* Bold values denotated those ions used for quantification

Table 2. Recovery (%) obtained when the poly(VPD-co-DVB) and PDMS stir bars were used in SBSE of 50 mL of Milli-Q water spiked at 50 ng L^{-1} of each analyte.

Analyte	Recovery (%)	
	poly(VPD-co-DVB)	PDMS
Paracetamol	9	-
Caffeine	20	-
Antipyrine	42	-
Propranolol	87	-
Methylparaben	91	-
Carbamazepine	83	-
Ethylparaben	79	2
Propylparaben	89	10
DHB	50	-
DHMB	93	43
Diclofenac	80	4
Ibuprofen	110	11
BP3	92	85
Triclocarban	81	95

% Relative standard deviations (RSDs) (*n* = 3) were lower than 6% for %*R* >20%.
For the experimental conditions, see text.

Table 3. Recoveries (%) obtained when 50 mL of river water sample spiked at 100 ng L^{-1} with the analyte mixture and 50 mL of effluent and influent WWTP samples spiked at 500 ng L^{-1} with the analyte mixture were SBSE using the poly(VPD-co-DVB) stir bar.

Analyte	Recovery (%)		
	River	Effluent WWTP	Influent WWTP
Paracetamol	8	8	n.d.
Caffeine	14	15	n.d.
Antipyrine	34	35	29
Propranolol	56	64	49
Methylparaben	66	65	23
Carbamazepine	99	85	74
Ethylparaben	86	87	58
Propylparaben	105	103	72
DHB	45	51	40
DHMB	60	65	55
Diclofenac	75	74	67
Ibuprofen	70	73	n.d.
BP3	72	55	29
Triclocarban	50	60	55

% RSD (n=3) were lower than 7% for river water, 13% for effluent water and 17% for influent water when %R> 20%.

n.d. not determined because of the high concentration in the blank sample.

Table 4. Concentrations (ng L^{-1}) of analytes found in effluent and influent WWTP samples (n=3, RSD<17%).

Analyte	Concentration (ng L^{-1})	
	Effluent WWTP	Influent WWTP
Paracetamol	-	21650
Caffeine	-	15803
Antipyrine	118	50
Propranolol	53	<LOQ
Methylparaben	<LOQ	3883
Carbamazepine	232	173
Ethylparaben	-	775
Propylparaben	79	1889
DHB	-	<LOQ
DHMB	-	-
Diclofenac	501	238
Ibuprofen	<LOQ	15005
BP3	50	735
Triclocarban	-	-

Figure captions

Figure 1. Chemical structures, pK_a and $logK_{o/w}$ of the analytes studied.

Figure 2. Assembly of the synthesised stir bar.

Figure 3. The effect of sample pH on extraction recovery for a representative group of analytes.

Figure 4. The effect of % NaCl addition to the sample on extraction recovery for a representative group of analytes .

Figure 5. The effect of extraction time on extraction recovery . for a representative group of analytes

Figure 6. MRM chromatograms of an effluent WWTP sample. For experimental conditions see the text.

*Manuscript
Click here to view linked References

Development and application of a polar coating for stir bar sorptive extraction of emerging pollutants from environmental water samples

D. Bratkowska[1], R.M. Marcé[1]*, P.A.G. Cormack[2], F. Borrull[1], N. Fontanals[1]

[1] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili
Campus Sescelades Marcel·lí Domingo, s/n, 43007 Tarragona, Spain
Phone + 34 977 55 81 70
Fax +34 977 55 84 46
*e-mail: rosamaria.marce@urv.cat

[2] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Keywords: stir bar sorptive extraction (SBSE); monolithic polar material; pharmaceuticals and personal care products (PPCPs); environmental water samples

Abstract

In the present study, a stir bar coated with hydrophilic polymer based on poly(N-vinylpyrrolidone-co-divinylbenzene) was prepared for the sorptive extraction of polar compounds. The main parameters affecting the polymerisation of the coating were investigated.

The new stir bar was applied successfully in stir bar sorptive extraction with liquid desorption followed by liquid chromatography-mass spectrometry in tandem with a triple quadrupole for the determination of a group of polar pharmaceuticals and personal care products (PPCPs) in environmental water matrices. Different variables affecting extraction and desorption such as agitation speed, temperature, ionic strength and extraction time were optimised. The results showed that the stir bar is able to enrich the selected analytes effectively.

The developed method was applied to determine a group of PPCPs in different complex environmental samples, including river, effluent and influent waste water.

1. Introduction

One of the research fields in analytical chemistry is the development of methods to determine contaminants in environmental samples. Due to the low concentration of these contaminants in these kinds of samples, a sample preparation technique together with a high sensitive instrumental technique is required.

For aqueous sample preparation, commonly used techniques include solid-phase extraction (SPE), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE), among others [1-3]. SPE is still the most commonly used technique due to the variety of sorbents commercially available which cover the extraction of compounds with a wide range of physico-chemical properties. In the recent years, SBSE has been proven to be a promising technique for the extraction of liquid samples [1,4] due to the larger coating compared to the one in SPME [3].

In SBSE, the magnetic stir bar encapsulated in a polymeric coat is introduced in the aqueous samples and after stirring for a certain time, the analytes are desorbed thermally or with a solvent. The main factor that determines the extraction efficiency is the partition coefficient of analytes between the phases. Since the stir bar has a larger volume of polymeric phase than SPME fibres, higher extraction efficiency can be obtained [4]. During the last few years, it has been clearly demonstrated that a wide range of compounds, such as pesticides, steroids, fatty acids, drugs, and so on can be extracted by SBSE [4-6], and this technique has been applied successfully to trace analysis in biomedical [5], pharmaceutical [6] and environmental samples

[1]. However, the application of these techniques to polar compounds is still a challenge due to the characteristics of the extraction materials.The only commercially available sorptive extraction phase is poly(dimethylsiloxane) (PDMS). Nevertheless, since PDMS is a non-polar phase, it is suitable for extracting less polar compounds. Correspondingly, polar analytes are not well recovered and the applicability of PDMS-coated stir bars is reduced to the extraction of the non-polar or semi-polar compounds [6-9]. To overcome this limitation, polar coatings based on different materials prepared by sol-gel technology [10], monolithic approach [11], polyurethane foam [12] or activated carbons [13] have been tested as SBSE coating. Recently, some monolithic polar coatings have been developed to enrich different analytes by SBSE [11,14-17]. For instance, poly(vinylimidazole-divinylbenzene) was successfully applied to extract polar aromatic amines from water samples [17]or vinylpyrrolidone-divinylbenzene (VPD-co-DVB) was applied to the extraction of metals, phenols, anilines, hormones and polycyclic aromatic hydrocarbons (PAHs) [14,15] .

The objective of the present work was the preparation of an improved stir bar with polar coating based on poly(VPD-co-DVB) for SBSE and its application followed by liquid chromatography-mass spectrometry in tandem (LC-MS-MS) for the determination of a broad range of compounds that include polar pharmaceuticals and personal care products (PPCPs) from complex environmental matrices.

2. Experimental

2.1 Reagents and standards

The reagents used for the polymer coating syntheses were divinylbenzene (DVB) (80% grade) supplied by Aldrich (Steinheim, Germany) and N-vinylpyrrolidone (VPD) (99% grade) supplied by Fluka (Buchs, Switzerland). DVB and VPD were freed of polymerisation inhibitors by passing them through short columns packed with neutral alumina. The cyclohexanol (99%) and 1-dodecanol (98%) used as porogens were purchased from Aldrich. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck, Darmstadt, Germany).

The analytes selected to evaluate the sorption characteristics of the stir bars were: paracetamol, caffeine, antipyrine, propranolol, carbamazepine, ibuprofen, diclofenac, methylparaben, ethylparaben, propylparaben, triclocarban, 2,4-dihydroxybenzophenone (DHB), 2,2´-dihydroxy-4-methoxybenzophenone (DHMB) and benzophenone-3 (BP3); all were obtained from Aldrich. The chemical structure, pK_a and log K_{ow} of each analyte are shown in Fig.1.

Standard solutions for each compound at 1000 mg L^{-1} were prepared in methanol (SDS, Peypin, France). The mixture of all the compounds was prepared by diluting the standard solution in Milli-Q water (Millipore, Bedford, MA, USA).

LC-grade acetonitrile (SDS) and Milli-Q water were used to prepare the mobile phase. Formic acid (Prolabo, Bois, France), hydrochloric acid (Probus, Barcelona, Spain) and sodium hydroxide (Panreac, Barcelona, Spain) were used to adjust the pH of the mobile phase or of the sample. Sodium chloride was obtained from Aldrich.

2.2 Stir bar preparation

In a typical synthesis of a monolith, the monomers DVB (85%, w/w) and VPD (15%, w/w) (40% w/w total monomer in feed relative to solvent) and AIBN (1% mol relative to polymerisable double bonds) were added to the porogen (10% w/v 1-dodecanol in cyclohexanol). The monomer mixture, initiator and porogen were mixed ultrasonically, and the homogenous solution poured into a glass tube with a defined diameter (4 mm i.d.). A stir bar (10 x 3 mm i.d.) was inserted into the middle of a spring (3 mm i.d.) and then immersed vertically into the polymerisation solution. Before polymerisation, the monomer solution was placed on an ice-bath, sparged with oxygen-free nitrogen for 5 min to remove oxygen and then sealed under a nitrogen atmosphere. The sealed glass tube with the stir bar inside was then left in a water bath set at 60 °C for 36 h. After this time, the glass tube was crushed carefully and the glass removed to reveal the polymer monolith. A smooth, solid monolith with good dimensional stability was obtained. Fig. 2 schematises the obtained stir bar assembly. The monolithic material on the bar was Soxhlet-extracted with MeOH for 24 h to remove residual monomers and initiator, soluble oligomers and porogen. The final dimensions of the poly(VPD-co-DVB) stir bar was as follows: length: 10 mm; polymer thickness: 0.5 mm corresponding to a volume of polymer of about 70 µl.

The monolithic poly(VPD-co-DVB) were then characterised. The specific surface area of the monolith was measured using a BET treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The carbon, hydrogen and nitrogen content of the polymer were determined with elemental microanalysis using a Carlo-Erba EA 1106 Instrument. FTIR spectroscopy measurements were performed using a Perkin-Elmer Spectrum One FTIR Spectrometer.

2.3 LC-(ESI)MS-MS analysis

The chromatographic experiments were performed with an Agilent HP 1200 liquid chromatograph coupled to a triple quadrupole mass spectrometer with electrospray interface (ESI) from Agilent Technologies (Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain) and the volume injected was 50 µl. The mobile phase was acetonitrile and Milli-Q water adjusted to pH 3 with formic acid. The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 30 ºC. The gradient profile was initially from 20% to 100% ACN in 15 min (held 5 min), after which time the mobile phase was returned to the initial conditions (20% ACN) in 3 min.

The (ESI)MS-MS parameters were optimised by direct injection of each analyte. Nitrogen was used as collision, nebulising and desolvation gas. The ESI interface conditions were optimised for each analyte and were: a nebuliser pressure of 45 psi, capillary voltage of 4000 V, desolvation (drying gas) N_2 flow was set at 12 l min^{-1} and desolvation temperature was 350 ºC. Analyses were performed under multiple reaction monitoring (MRM) mode and MRM transitions for each analyte are summarised in Table 1.

2.4 SBSE assays

The procedure with home-made poly(VPD-co-DVB) stir bar was as follows: the stir bar was activated with 1 mL of MeOH and stirred for 5 min. After drying with lint-free tissue, the stir bar was placed into a flask with 50 mL of water sample at pH 5.0, with 10% of NaCl and then the sample was stirred at 900 rpm for 4 hours at room temperature. After the extraction, the stir bar was removed with tweezers, dipped briefly in Milli-Q water and dried with a lint-free tissue. In order to desorb the analytes with a solvent, stir bar was placed into a vial with 5 mL of MeOH stirred at 900 rpm for 15 minutes. Then, the stir bar was magnetically removed and the extract was evaporated to dryness under gentle stream of nitrogen. The dry residues were redissolved in 1 mL MeOH:water (1:1) and transferred to a vial, closed with a seal using a hand crimper, and placed into the automatic liquid sampler tray for LC-(ESI)MS-MS analysis.

The retention capability of the poly(VPD-co-DVB) stir bar was compared to the commercially available PDMS-coated stir bar (Twister), obtained from Gerstel (Mulheim Ruhr, Germany). It consists of a 10 mm long glass-encapsulated magnetic stir bar, externally coated with a layer of 0.5 mm thick, corresponding to a volume of 24 µL of PDMS. Prior to the first use, the stir bar was placed in a vial containing ACN and conditioned for 24 h. Although there are two sizes of PDMS-coated stir bar commercially available, this smaller size was selected, since it provided high sorptive capacity for water sample volumes range from 1 to 50 mL [4].

Reconditioning of stir bars was done after the extraction in MeOH for poly(VPD-co-DVB) stir bar or methylene chloride:MeOH (1:1) for PDMS.coated stir bar [18]. Stir bars were conditioned by placing them in vials with the solvent for 20 min. Then the solvents were refreshed and the procedure was repeated three times. Subsequently, the stir bars were dried with a lint-free tissue, and kept in a vial for the next analysis.

2.5 Sample collection

The river water samples were collected from Ebre River. The waste water samples were collected from the effluent and influent of two sewage treatment plants (STPs).

To ensure proper conditions for keeping the samples, all the real water samples (Ebre river, effluent and influent waste water from STPs) were adjusted to pH~3 with HCl and stored at 4 ºC before analysis. They were filtered through 0.45 µm nylon membranes (Supleco, Bellefont, PA, USA) before the stir bar extraction to eliminate the particulate matter of real samples.

3. Results and discussion

3.1 Preparation and characterisation of the stir bars

With the aim of improving the efficiency of polar compounds by SBSE with commercially available PDMS, a polar group should be introduced in the monolithic coating of the stir bar. Due to the good results provided by vinylpyrrolidone as polar group in the commercial OASIS HLB sorbent for SPE [19] we selected it as a potential polar monomer. In fact, in a recent study vinylpyrrolidone was already tested as polar monomer in the stir bar coating and results for phenols were better than those obtained with PDMS stir bar [14].

However, a limitation of monolithic stir bars is the mechanical stability along the extractions. In order to overcome this limitation, we proposed a new design which includes a spring covering the stir (figure 2) in order to increase the mechanical resistance, the number of extractions to be done with the same stir bar, and therefore the robustness of the analytical method. To do this, a stir of 10 x 3 mm was covered by the spring (3 mm i.d.) and introduced in the glass vial (4 mm i.d.) in which the polymerisation mixture was then poured. In this case, the presence of the spring made it possible to obtain a thinner coating (0.5 mm) with similar mechanical properties. To test this new design, the starting polymerisation conditions were based on those previously reported for polar monolithic coatings [11,14], and we optimised some parameters in the polymerisation procedure in order to further enhance the properties of the final stir bar.

Some important parameters to take into account in the synthesis of a monolith are: the porogen, that is responsible for the porous formation during the polymerisation; the crosslinker monomer/polar monomer ratio in order to introduce polarity on the coating (via monomer) and rigidity (via crosslinker); the ratio between total monomers and porogen, and polymerisation temperature and the type of polymerisation initiation.

As porogen, a mixture of 1-dodecanol and cyclohexanol was used since this mixture of solvents had been demonstrated to be useful for imparting porosity into such type of monolithic materials [11,20], because the types of porous formed allow the analytes to penetrate the polymer and also provide enough surface area. From the different ratio tested, 10/90 (w/v) proved to be a convenient porogen mixture.

Regarding the monomers ratio, it was fixed at 85/15 w/w of DVB to VPD (this led to a polymer monolith with a specific surface area of 600 m^2 g^{-1}), since it was found that the use of lower amounts of DVB (75%) in the monomer feed led to a slight decrease in the specific surface areas of the product (560 m^2 g^{-1}). These specific surface areas are as expected, taking into account the high % of DVB and the non-porogen solvent used [19].

The ratio of total monomer to porogenic solvent was set at 40/60 (w/w), because higher monomer ratio led to soft polymers which were broken easily during later stirring operations.

A further variable which was examined was the nature of the initiation step in the polymerisation (thermal *versus* photochemical initiation). With this in mind, thermally-initiated and photochemically-initiated polymerisations were compared. The UV-initiated polymerisations, which were carried out at room temperature, yielded soft and fragile materials consistent with incomplete cure, whereas those materials produced by thermally-initiated polymerisations were hard and had good dimensional stability, consistent with efficient cure. As a consequence, thermal initiation was the method of choice in all subsequent polymerisations.

Finally, it was found that the polymerisation temperature had some influence upon the mechanical properties of the monolithic material. Polymers synthesised at 70 ºC were less mechanically stable than polymers synthesised at 60 ºC, therefore a polymerisation temperature of 60 ºC was employed in all subsequent polymerizations, which were run for 36 hours.

Although the optimum polymerisation conditions were quite similar to those found by Huang *et al.* [15], there are some differences as regards the total monomer/porogen ratio, polymerisation time and temperature, the format and size.

FTIR spectroscopic analyses of the poly(VPD-co-DVB) coatings confirmed that DVB and VPD had been copolymerised effectively into the monoliths. Diagnostic bands consistent with aromatic residues (from DVB) and amide groups (1690 cm^{-1}, from VPD residues) were observed.

Elemental microanalysis of the poly(VPD-co-DVB) monolith gave the following results: carbon (83.0%), hydrogen (7.7%) and nitrogen (2.8%), which are similar to the theoretical values, and provides evidence that both monomers were incorporated into the monolith.

3.2 LC-(ESI)MS-MS optimisation

Once the chromatographic separation was optimised, the best MS-MS conditions were selected and they are shown in section 2.3 and detailed in Table 1. For each analyte, two fragmentations of $[M-H]^+$ or $[M-H]^-$ were acquired. The most intense transition was selected to quantify the analytes. Paracetamol, caffeine, antipyrine, propranolol, carbamazepine and BP3 were determined under positive ionisation mode; whereas ibuprofen, diclofenac, parabens, DHB, DHMB and triclocarban exhibited a higher response in the negative mode.

All selected analytes showed good linearity ($r^2 > 0.997$) by direct injection of standards and the linear range was 0.5-100 µg L^{-1} for all the analytes, except for methylparaben (0.1-100 µg L^{-1}) and for antipyrine (1-100 µg L^{-1}). The limits of detection (LODs) calculated as a signal-noise ratio (S/N) ≥3 were 50 ng L^{-1}

for the majority of compounds, apart from paracetamol, antipyrine, ethylparaben, DHMB and ibuprofen with a LOD of 100 ng L^{-1}.

3.3 Optimisation of SBSE procedure

In order to test the extraction efficiency of the novel polar stir bar, a group of PPCPs with different polarity were selected.

During the development of the presented procedure, several parameters that influence the SBSE process were evaluated. Thus, systematic assays were performed to optimise parameters which can influence the SBSE procedure during both extraction and liquid desorption steps.

3.3.1 Liquid desorption conditions

In the first approach, we tested the best liquid desorption conditions, which ensured complete back extraction of selected analytes from the stir bars. Thus, two organic solvents: MeOH and ACN were examined as a desorption solvent. To do this, we initially used the following experimental conditions: 50 mL of the sample spiked at 1 μg L^{-1}, 1 hour of extraction time, 750 rpm of agitation speed at room temperature, and for desorption, 5 mL of organic solvent for 15 min at the same agitation speed, based on previous experience from our group [18]. From the data obtained, MeOH was chosen as the back extraction solvent due to the slightly higher ability to desorb polar analytes from the stir bars (recoveries increase in ~10%). As for desorption volume, we tested 5 mL of solvent in order to immerse the stir bar completely and ensure proper desorption. Further increasing of MeOH volume (10 mL) did not improve the desorption results.

The influence of temperature (25 ºC, 35 ºC, 40 ºC) on desorption in SBSE process was also investigated. The results obtained seem to indicate that desorption was not significantly affected by temperature. Consequently, room temperature was selected for the further experiments.

In order to investigate the time necessary to ensure complete desorption of the analytes from the stir bar, a series of experiments were performed by comparing several periods of time (10, 15, 30 and 60 min) to achieve the best liquid desorption conditions. The results indicated that stirring for 15 min is enough to desorb all analytes of interest from the stir bar and additional desorption did not show presence of the analytes. Consequently, a period of 15 min was selected for desorption process. This desoption time is quite shorter than the one previously used (i.e. 2 h) for a similar stir bar [14,15], although in that case, the speed was lower (400 rpm) and stir bar was thicker (1 mm thickness).

3.3.2 Extraction conditions

SBSE is a balanced technique and the analytical process is considerably influenced by agitation speed, temperature, ionic strength, pH and extraction time involved. Starting with the stirring rate, this factor may influence the mass transfer of the analytes during the extraction process. In the present paper, three agitation levels (600, 900 and 1250 rpm) were tested to achieve the best stirring conditions. The response obtained with 900 rpm was higher than that obtained with 600 rpm, and further increases in the stirring rate may damage the monolithic phase. As the agitation speed of 900 rpm provided better recovery of all analytes under study, it was selected for further experiments. This agitation speed was also tested for liquid desorption step and since no significant differences were observed with the initial 750 rpm, 900 rpm was the selected speed in both extraction and desorption steps.

It is noteworthy that higher stirrer speeds could be applied in the present work than were used in previous studies (400 rpm) [14,15]; in the present case, the metal spring acts to reinforce and strengthen the stir bar coating and allow higher stirrer speeds to be realised without attrition of the coating."

The influence of temperature (25 ºC, 35 ºC, 40 ºC) in SBSE process was also studied. It was found that the recoveries of the extractions at 25 ºC and 35ºC were very similar for most of analytes and recoveries slightly decreased (~10%) at 40ºC. As a result, 25 ºC was selected for the further experiments.

The effect of sample pH on the extraction efficiency was investigated by adjusting Milli-Q water at the pH values in the range from 3.0 to 11.0. Fig. 3 shows the influence of the sample pH on SBSE efficiency of a representative group of analytes, and it can be seen that the pH value significantly affects the extraction efficiency of some analytes. The results showed that the extraction recoveries for most of analytes improved with increasing the pH from 3.0 to 5.0 and slowly decreased (except for propranolol) at pH 7. At higher pHs the effect of pH depends on the pK_a (included in fig. 1) of the studied analytes. For example, the recovery for the analytes with pK_a values lower than 8.3 decreases at the highest pHs tested; however, compounds with high pK_a values such as carbamazepine, caffeine or antipyrine showed no significant changes. In the case of propranolol, the recovery improved under basic conditions (maximum at pH 9.0), due to its pK_a value. Considering these results, pH 5.0 was chosen for further experiments.As it is well known, the extraction efficiency in SBSE might depend on the ionic strength in the sample matrix [11]. In the present study, the effect of ionic strength in the recovery of the compounds under study was performed by addition of NaCl from 0 to 15 % (w/v) to the aqueous samples. Fig. 4 shows the effect of salt addition on extraction efficiency for the representative group of analytes. It can be seen that for the same analytes, the recoveries decreased with 5% NaCl addition, and then increased to the maximum with 10% NaCl addition. This effect may be attributed to the salting-out effect and the electrostatic interaction between polar molecules and salt ions in the solution [21]. With a higher percentage (i.e. 15%) of NaCl, the recoveries decreased. As 10% addition of NaCl in the samples provided the best recoveries, this addition was selected in the following studies.

The effect of sample volume on the recovery was also investigated. In order to select the optimum sample volume, different sample volumes (25, 50 and 100 mL) of standard solution of the analyte mixtures were extracted. The results were similar when both 25 mL and 50 mL of the Milli-Q water sample were analysed. When 100 mL of a standard solution was extracted, the recoveries decreased significantly (10-30 % recovery decrease). This result was expected because the higher the volume, the higher the extraction time needed to reach the equilibrium [22]. For this reason, 50 mL of sample was selected for further analysis.

The extraction time is also a very significant parameter. In order to select the best conditions, different extraction periods (1, 2, 4, 6 and 12 hours) were tested. Fig. 5 shows representative time extraction profiles for a representative group of analytes, where it can be seen that the extraction efficiency increased when the extraction time increased from 1 to 12 hours. However, in order to reach a balance between analysis time and extraction efficiency, 4 hours was selected as the extraction time.

After the optimisation procedure, the conditions used to investigate recoveries from environmental samples were as follows: 50 mL of the sample adjusted at pH 5.0 with 10 % NaCl addition and agitated at 900 rpm at room temperature for 4 hours for the extraction and 5 mL of MeOH stirred at the same speed for 15 min for liquid desorption

With the optimum conditions, the recovery for each analyte from the standard solution, including the evaporation step, is shown in Table 2. These recoveries were calculated from the calibration curve by direct injection. The poly(VPD-co-DVB) stir bar method attained very good performance and presented high recoveries, between 80% and 100%, for most analytes and only presented lower recovery for paracetamol, caffeine and antipyrine, whose results were expected since they are very polar compounds (see $\log K_{o/w}$ in fig. 1). These results are very satisfactory, taking into account the polarity of these compounds and the results achieved with a similar stir bar [14,15] for other compounds with a broad range of polarity, such as phenols, aromatic amines, and PAHs.

3.3.3 Comparison to a commercial stir bar

The SBSE performance of the poly(VPD-co-DVB) stir bar was compared to the commercially available stir bar based on PDMS. The SBSE results arising from the use of the PDMS-coated stir bar are also included in Table 2. It can be seen that under the same conditions, the analyte recoveries were higher for most of the compounds with poly(VPD-co-DVB) than with PDMS. Moreover, PDMS was not able to retain any of the more polar compounds while the poly(VPD-co-DVB) stir bar showed good retention, with higher recoveries for most of the analytes. Those results clearly indicate that the selected analytes, having different polarity, presented better affinity for poly(VPD-co-DVB) phase rather than for PDMS. These results can be easily explained by the polar character of the monomer used in the synthesis of the new stir bar coating, while PDMS is an apolar phase. Another significant contribution was that the specific surface area of poly(VPD-co-DVB) monolith was 600 $m^2 g^{-1}$ whereas for PDMS-coated stir bar, the specific surface area is expected to be low from its chemical structure. In any case, it is evident that the poly(VPD-co-DVB) stir bar gives higher recoveries for all of the target analytes than the commercially available PDMS stir bar.

3.4 Analysis of real samples

Given the promising SBSE data obtained with poly(VPD-co-DVB) stir bar when the procedure was applied to Milli-Q water, the same protocol was applied to the analysis of water from the Ebre river and effluent and influent water from a waste water treatment plant (WWTP), where PPCPs had already been detected [23-25].

It is known that electrospray ionisation may give ion suppression/enhancement when complex samples are analysed and this effect was studied by spiking with the analytes the SBSE extracts of river water, effluent and influent WWTP water samples and quantifying the recoveries taking into account the unspiked SBSE extracts. The resulting ion suppression/enhancement ranged between 0 and 22%. Therefore, this effect was not significant.

To calculate the recoveries, the different water samples spiked at different levels of each analyte and the unspiked samples were analysed. Table 3 shows the SBSE results for the different water samples. The data obtained for river water samples were good, with recovery values ranging from 50 to 100% for most of the compounds and similar to the values obtained for extractions from Milli-Q water taking into account the ion suppression and thefore demonstrating the satisfactory ability of this monolithic material to retain both polar and semi-polar compounds even in the presence of the matrix. As can be seen, the data for effluent and influent WWTP water is also good, with recovery values ranging from 50% to 100% for the effluent WWTP sample and 40% to 100% for the influent WWTP sample for most of the compounds.

Then, the method was validated with river water. When the linear range was tested in a 6 concentration levels by duplicate, the analytes exhibited good linearity ($r^2 > 0.993$): between 20 and 2000 ng L^{-1} for carbamazepine and diclofenac; between 100 and 2000 ng l^{-1} for paracetamol and DHMB; and between 50 and 2000 ng L^{-1} for the rest of the compounds. The LODs, calculated using S/N≥3, were between 10 and 20 ng L^{-1} for all the compounds, with the exception of paracetamol and DHMB (50 ng L^{-1}). The repeatability and reproducibility between days of the method, expressed as the relative standard deviation (%RSD) of three analyses of 50 mL of water from the Ebro river spiked at 100 ng L^{-1} were lower than 7% and 15%, respectively, for all compounds. In any case, the good performance of the poly(VPD-co-DVB) stir bar has been demonstrated.

It should be highlighted that between 30 and 40 samples can be extracted with the same stir bar without changes in the recovery, which is quite satisfactory taking into account the thickness (0.5 mm) of the bar. The bar-to-bar reproducibility (%RSD, n=3) were lower than 10% for all the analytes.

In the river samples analysed, propylparaben (88-129 ng L^{-1}), BP3 (52-103 ng L^{-1}) and ibuprofen (LOQ-72 ng L^{-1}) were present. In one sample, caffeine and methylparaben were also found at 61 ng L^{-1} and 99 ng L^{-1}, respectively. These values are comparable to those found in the samples from the same river [18,23].

Several samples of effluent WWTPs were also analysed. Some analytes such as antipyrine, propanolol, carbamazepine, propylparaben, diclofenac and BP3 were present in the effluent WWTP water samples analysed. Table 4 includes the concentrations found in one effluent sample analysed in triplicate as an example. In another effluent samples we also found caffeine, methylparaben and DHB, whose chromatogram is shown in Figure 6, as an example. Several papers [23,26,27] also reported the presence of these analytes in this kind of sample.

Table 4 also shows the concentrations of the analytes found in an influent water sample analysed. As can be seen, analytes such as paracetamol, caffeine, methylparaben and ibuprofen were found at high concentration and therefore these samples had to be diluted to be quantified. As expected, most of these analytes were found at higher concentration than in effluent due to the waste water treatment.

Conclusions

In this study, a stir bar coated with poly(VPD-co-DVB) monolithic material was developed by optimising the conditions of the polymerisation to obtain good chemical and mechanical properties of the stir bar.

The poly(VPD-co-DVB) stir bar was successfully applied for extraction of group of analytes with polar characteristics and showed significantly higher retention for the polar analytes compared with the commercially available PDMS stir bar.

The combination of SBSE and liquid desorption with LC-(ESI)MS-MS provided an efficient, simple and sensitive method for determination of PPCPs in complex environmental samples at low levels.

Acknowledgements

The authors thank for the financial support the *Ministry of Science and Innovation* (Projects CTQ 2008-0825 and CTM 2008-06847-CO2-01) and the *Department* of *Innovation, Universities and Enterprise* (Project 2009 SGR 223). N. Fontanals also acknowledges personal funding from the Juan de la Cierva program of the the *Ministry of Science and Innovation*.

References

[1] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642-2656.

[2] F.M. Lancas, M.E. Costa, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813-824.
[3] C. Dietz, J. Sanz, C. Cámara, J. Chromatogr. A 1103 (2006) 183-192.
[4] E. Baltussen, C.A. Cramers, P. Sandra, Anal. Bioanal. Chem. 373 (2002) 3-22.
[5] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 40 (2006) 500-508.
[6] F. Sánchez-Rojas, C. Bosch-Ojeda, J.M. Cano-Pavon, Chromatographia 69 (2009) 79-94.
[7] L. Badoil, D. Benanou, Anal. Bioanal. Chem. 393 (2009) 1043-1054.
[8] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54-69.
[9] J. Regueiro, M. Llompart, E. Psillakis, J. Garcia-Monteagudo, C. Garcia-Jares, Talanta 79 (2009) 1387-1397.
[10] W.A.W. Ibrahim, W. Norfazilah, W. Ismail, A. Syazwani, A. Keyon, M.M. Sagani, J. Sol-Gel Sci. Technol. 58 (2011) 602-611.
[11] X. Huang, N. Qiu, D. Yuan, Q.M. Lin, J. Chromatogr. A 1216 (2009) 4354-4360.
[12] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, J. Chromatogr. A 1209 (2008) 10-16.
[13] N.R. Neng, A.R.M. Silva, J.M.F. Nogueira, J. Chromatogr. A 1217 (2010) 7303-7310.
[14] X. Huang, N. Qiu, D. Yuan, B. Huang, Talanta 78 (2009) 101-106.
[15] X. Huang, N. Qiu, D. Yuan, J. Sep. Sci. 32 (2009) 1407-1414.
[16] Y. Luo, Q. Ma, Y. Feng, J. Chromatogr. 1217 (2010) 2667-2673.
[17] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1217 (2010) 2667-2673.[18] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chem. 397 (2010) 2833-3839.
[19] N. Fontanals, R.M. Marcé, F. Borrull, J. Chromatogr. A 1152 (2007) 14-31.
[20] F. Svec, T.B. Tennikova, Z. Deyl, Monolithic Materials - Preparation, Properties and Applications, Elsevier, Amsterdam, 2003.
[21] X.J. Huang, J.B. Lin, D.X. Yuan, R.Z. Hu, J. Chromatogr. A 1216 (2009) 3508-3511.

[22] C. Bicchi, C. Cordero, P. Rubiolo, P. Sandra, J. Sep. Sci. 26 (2003) 1650-1656.

[23] M. Pedrouzo, S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 297-303.

[24] A. Togola, H. Budzinski, J. Chromatogr. A 1177 (2008) 150-158.

[25] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225-1234.

[26] M. Pedrouzo, S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 297-303.

[27] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1174 (2007) 27-39.

Table 1. ESI mode and MRM conditions used for LC-(ESI)MS-MS of target analytes.

Analyte	ESI Mode	Cone Voltage (V)	Precursor Ion (m/z)	Product Ions (m/z)[*]	Collision Energy (V)
Paracetamol	+	100	152	**110** 93	15 25
Caffeine	+	125	195	**138** 110	15 25
Antipyrine	+	100	189	**145** 115	30 30
Propranolol	+	125	260	**116** 183	15 15
Methylparaben	-	80	151	**92** 136	15 5
Carbamazepine	+	150	237	**193** 179	35 35
Ethylparaben	-	100	165	**137** 92	15 5
Propylparaben	-	100	179	**92** 136	15 5
DHB	-	130	213	**135** 169	15 15
DHMB	-	80	243	**93** 123	15 5
Diclofenac	-	75	294	**250** 214	10 20
Ibuprofen	-	75	205	**161** 69	5 5
BP3	+	130	229	**151** 105	15 15
Triclocarban	-	130	313	**160** 126	5 15

[*] Bold values denotated those ions used for quantification

Table 2. Recovery (%) obtained when the poly(VPD-co-DVB) and PDMS stir bars were used in SBSE of 50 mL of Milli-Q water spiked at 50 ng L^{-1} of each analyte.

Analyte	Recovery (%)	
	poly(VPD-co-DVB)	PDMS
Paracetamol	9	-
Caffeine	20	-
Antipyrine	42	-
Propranolol	87	-
Methylparaben	91	-
Carbamazepine	83	-
Ethylparaben	79	2
Propylparaben	89	10
DHB	50	-
DHMB	93	43
Diclofenac	80	4
Ibuprofen	110	11
BP3	92	85
Triclocarban	81	95

% Relative standard deviations (RSDs) (*n* = 3) were lower than 6% for %*R* >20%.
For the experimental conditions, see text.

Table 3. Recoveries (%) obtained when 50 mL of river water sample spiked at 100 ng L^{-1} with the analyte mixture and 50 mL of effluent and influent WWTP samples spiked at 500 ng L^{-1} with the analyte mixture were SBSE using the poly(VPD-co-DVB) stir bar.

Analyte	Recovery (%)		
	River	Effluent WWTP	Influent WWTP
Paracetamol	8	8	n.d.
Caffeine	14	15	n.d.
Antipyrine	34	35	29
Propranolol	56	64	49
Methylparaben	66	65	23
Carbamazepine	99	85	74
Ethylparaben	86	87	58
Propylparaben	105	103	72
DHB	45	51	40
DHMB	60	65	55
Diclofenac	75	74	67
Ibuprofen	70	73	n.d.
BP3	72	55	29
Triclocarban	50	60	55

% RSD (n=3) were lower than 7% for river water, 13% for effluent water and 17% for influent water when %R> 20%.

n.d. not determined because of the high concentration in the blank sample.

Table 4. Concentrations (ng L^{-1}) of analytes found in effluent and influent WWTP samples (n=3, RSD<17%).

Analyte	Concentration (ng L^{-1})	
	Effluent WWTP	Influent WWTP
Paracetamol	-	21650
Caffeine	-	15803
Antipyrine	118	50
Propranolol	53	<LOQ
Methylparaben	<LOQ	3883
Carbamazepine	232	173
Ethylparaben	-	775
Propylparaben	79	1889
DHB	-	<LOQ
DHMB	-	-
Diclofenac	501	238
Ibuprofen	<LOQ	15005
BP3	50	735
Triclocarban	-	-

Figure captions

Figure 1. Chemical structures, pK_a and $\log K_{o/w}$ of the analytes studied.

Figure 2. Assembly of the synthesised stir bar.

Figure 3. The effect of sample pH on extraction recovery for a representative group of analytes.

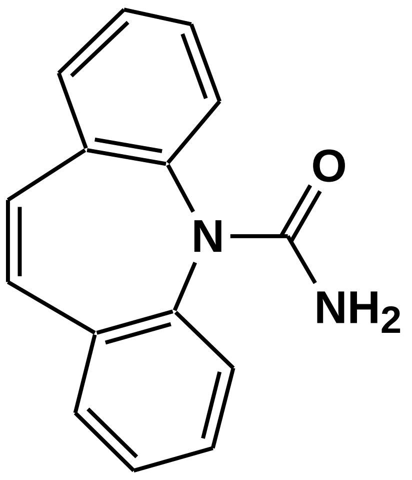
Figure 4. The effect of % NaCl addition to the sample on extraction recovery for a representative group of analytes .

Figure 5. The effect of extraction time on extraction recovery . for a representative group of analytes

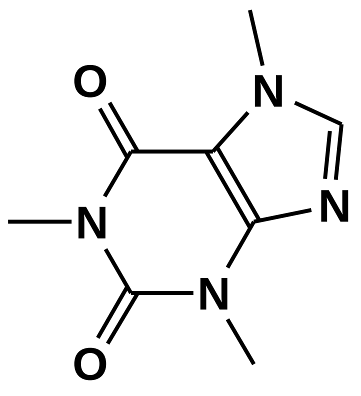
Figure 6. MRM chromatograms of an effluent WWTP sample. For experimental conditions see the text.

Figure1

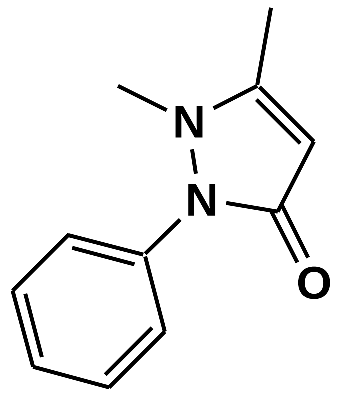
Click here to download Figure: ACA-11-589R1_Figure1.ppt



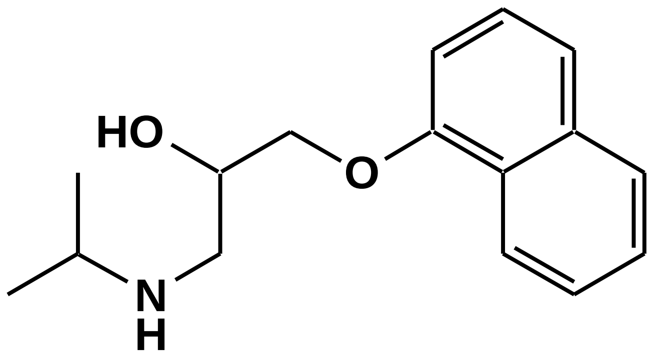
Carbamazepine
pK_a 13.7
$\log K_{ow}$ 1.9



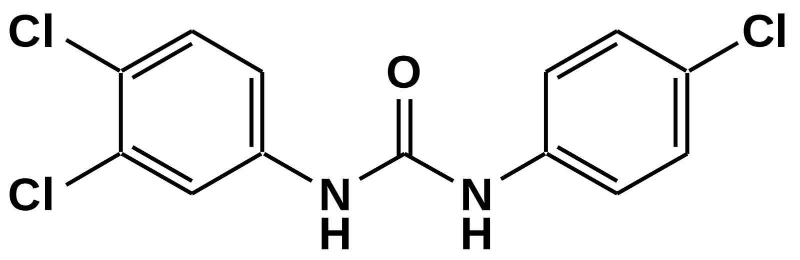
Caffeine
pK_a 13.4
$\log K_{ow}$ -0.6



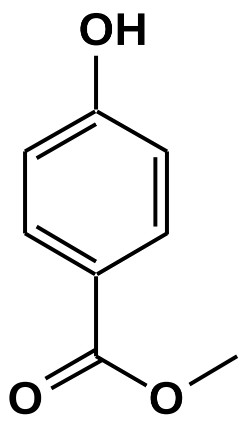
Antipyrine
pK_a 13.3
$\log K_{ow}$ 0.4



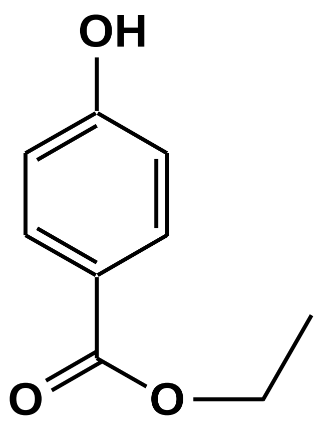
Propranolol
pK_a 9.5
$\log K_{ow}$ -0.2



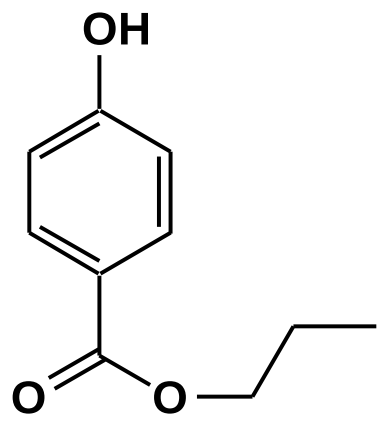
Triclocarban
pK_a 12.8
$\log K_{ow}$ 6.1



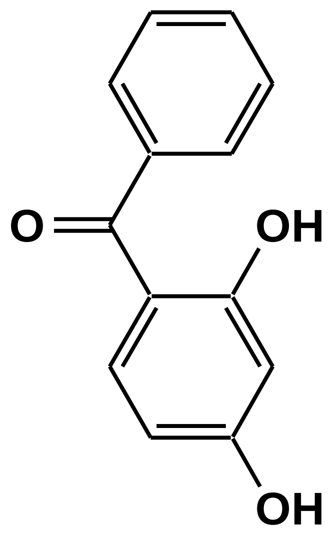
Methylparaben
pK_a 8.3
$\log K_{ow}$ 2.0



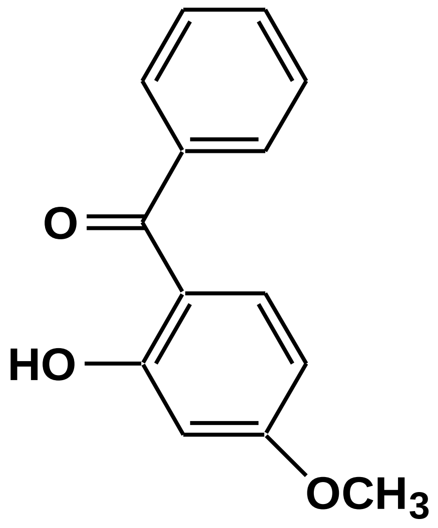
Ethylparaben
pK_a 8.3
$\log K_{ow}$ 2.4



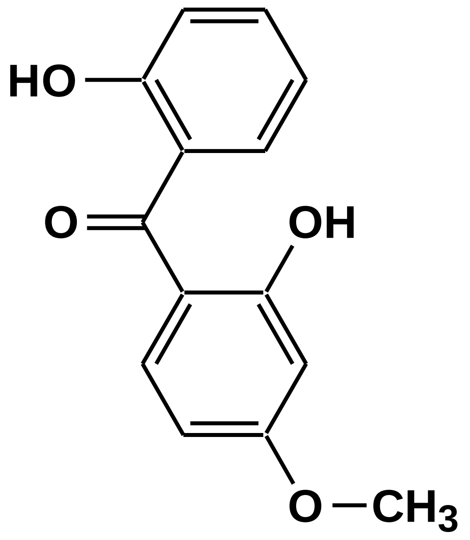
Propylparaben
pK_a 8.2
$\log K_{ow}$ 2.9



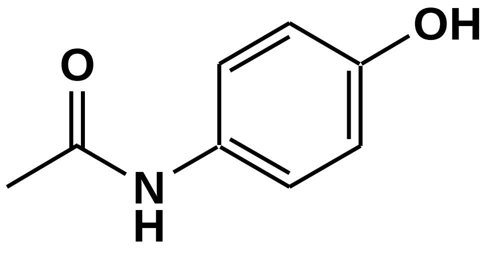
DHB
pK_a 7.7
$\log K_{ow}$ 3.2



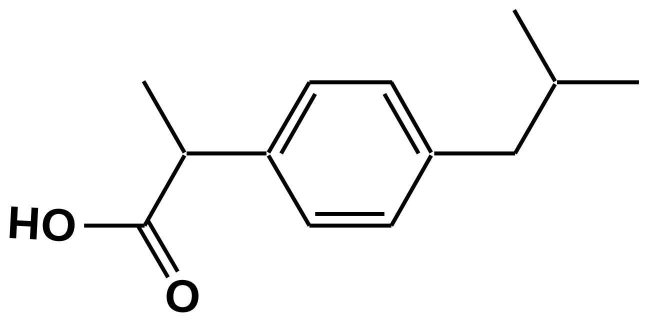
BP3
pK_a 7.6
$\log K_{ow}$ 4.0



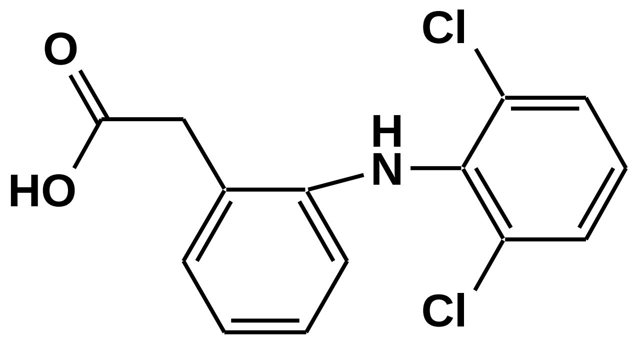
DHMB
pK_a 7.1
$\log K_{ow}$ 3.7



Paracetamol
pK_a 9.7
$\log K_{ow}$ 0.5



Ibuprofen
pK_a 4.4
$\log K_{ow}$ 2.8



Diclofenac
pK_a 4.2
$\log K_{ow}$ 3.7

Figure2

Click here to download Figure: ACA-11-589R1_Figure2.ppt

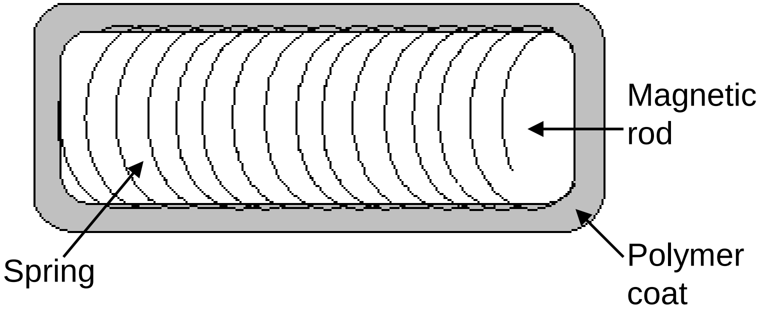


Figure3

Click here to download high resolution image

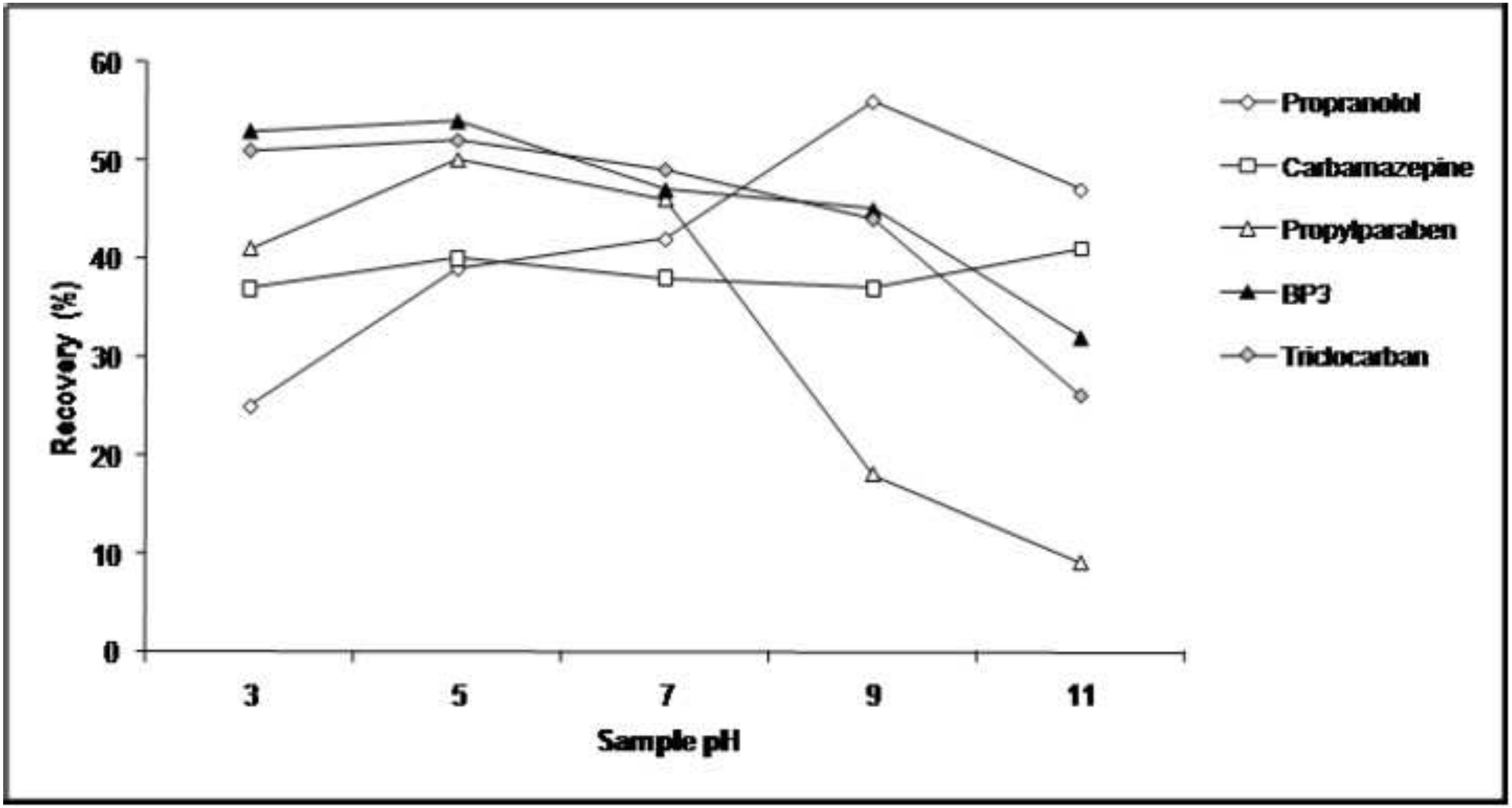


Figure4

Click here to download high resolution image

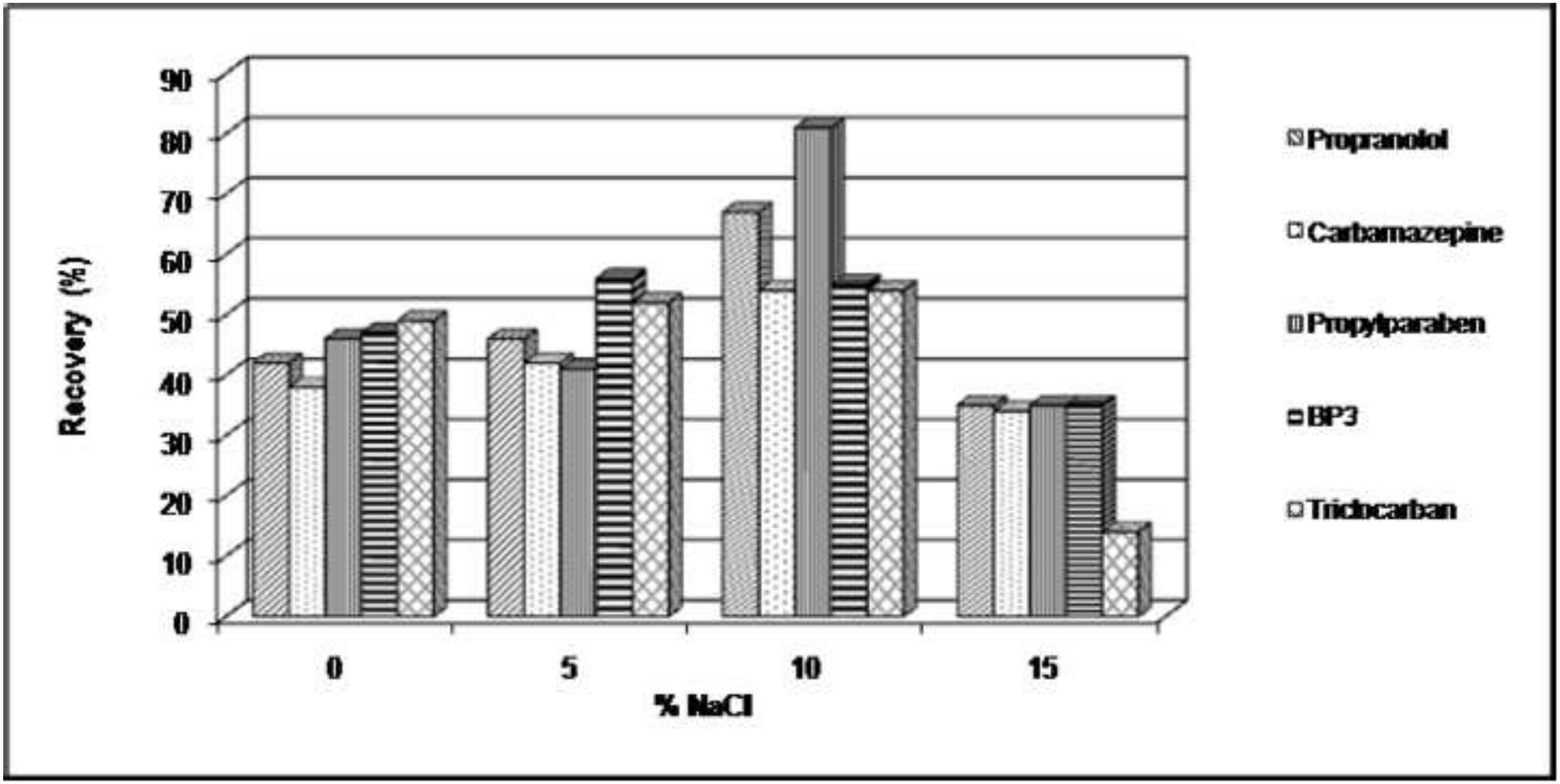


Figure5

Click here to download high resolution image

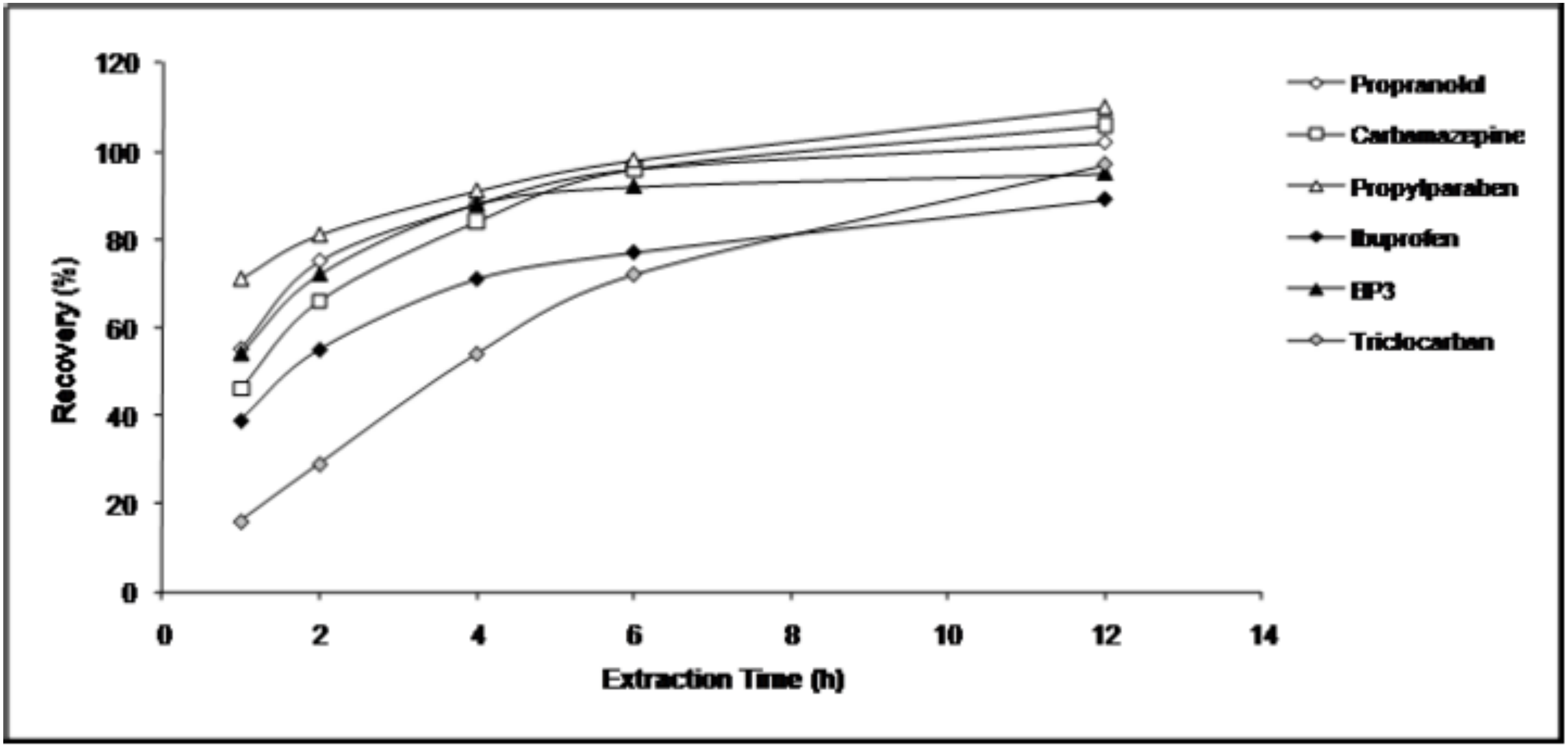


Figure6

Click here to download Figure: ACA-11-589R1_Figure 6.ppt