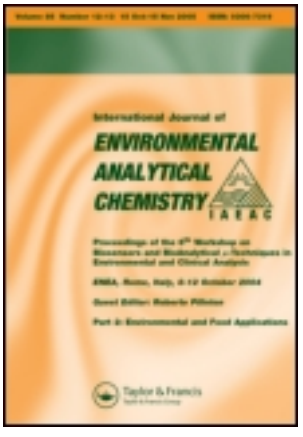

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Automatic flow system for evaluation of polystyrene-divinylbenzene sorbents applied to preconcentration of phenolic pollutants

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In this work, an evaluation of commercially available polystyrene-divinylbenzene sorbents for solid-phase extraction (SPE) of eleven phenolic compounds is intended. Considering the particle size and cross-linking degree, Amberlite XAD-4 (commercial or grounded), Macronet MN-200 and Lichrolut EN were tested. The SPE protocol was performed by an automatic system, providing repeatable experimental conditions for assessment of sorbent capacity, break-through volume and enrichment factor (EF). A positive correlation between EF and $\log K_{ow}$ was found for Amberlite XAD-4 while a negative correlation was observed between EF and molecular weight of analyte for Macronet MN-200 and for Lichrolut EN. This indicates a prevalence of hydrophobic interactions or molecular exclusion depending upon the polymer cross-linking degree. Despite the similar repeatability (RSD $< 4.7\%$, $n > 6$) and recovery values attained (97.6–102.7%, using 50 mL of sample) for all sorbents, Lichrolut EN is the best choice for analytical application as higher EF and lower LOD values (between 18 and 207 ng) were attained for this sorbent.

Keywords: automation; multisyringe flow injection analysis; phenolic pollutants; polystyrene-divinylbenzene; solid-phase extraction

1. Introduction

Phenolic compounds are ubiquitous pollutants generated in the production of plastics, dyes, drugs, pesticides, paper and also in the petrochemical industry [1]. Due to the intense and unpleasant organoleptic properties and toxicity at low concentration levels ($\mu g\,L^{-1}$), these compounds have been included in the United States Environmental Protection Agency (EPA) list of priority pollutants [2]. The determination of EPA phenolic pollutants in water samples from different sources is usually performed by separation techniques such as GC-MS [3], LC coupled to ultra-violet [4] or electrochemical detection [5]. Although GC-MS provides higher sensitivity than LC-UV, the derivatisation of the analytes prior to chromatographic run is usually unavoidable. Furthermore, a previous sample preparation step comprising the preconcentration of the target analytes and matrix removal is always required for both chromatographic methodologies.

In this context, solid-phase extraction (SPE) is a suitable technique for performing the enrichment of water samples [6] because it requires low amounts of organic solvents and allows the processing of large sample volumes. Three categories of sorbents, based on

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silica, carbon or polymeric materials, have been used to extract phenolic compounds from water samples by a reversed-phase mechanism [7]. Besides the limited pH working range, silica sorbents have the inconvenience of providing low recoveries for the more polar compounds [8]. On the other hand, when carbon sorbents were used, poor recoveries were achieved due to difficulties in removing the trapped compounds from the sorbent surface [9]. These sorbents also have poor mechanical stability, and band broadening was observed in the chromatograms [9,10]. In contrast, polymeric materials do not have limitations in the pH working range, have good mechanical properties and provide quantitative recovery of phenolic compounds [11].

Generally, polymeric sorbents comprise a polystyrene-divinylbenzene (PS-DVB) hydrophobic structure. Different particle size and cross-linking degree are available, depending on the supplier. Hence, the objective of this work was to evaluate commercially available polymeric sorbents concerning their performance for SPE of EPA priority pollutants prior to their determination. Three adsorbents were chosen: Amberlite XAD-4, Lichrolut EN and Macronet MN-200. These were chosen considering the different particle size and the cross-linking grade, which results in different surface areas. Amberlite XAD-4 was used in the commercial form (300–850 μm) and as the grounded product (125–250 μm) [12]. The commercial product has a cross-linking degree lower than 16% [13] and a surface area of $750\,m^2\,g^{-1}$. Macronet MN-200 is classified as a hyper cross-linking degree sorbent with particle size in the range 300–1200 μm and a surface area of $1000\,m^2\,g^{-1}$ [14]. Finally, Lichrolut EN particle distribution is in the range 40–120 μm, and a surface area of $1200\,m^2$ was achieved for 1 g of this PS-DVB sorbent with high cross-linking degree [15]. Amberlite XAD-4 and Lichrolut EN are currently applied to SPE of phenolic compounds [16,17]. Although generally used for wastewater treatment through sorption and removal of phenolics from waste waters, Macronet MN-200 has only once been applied for analytical purposes, to the best of our knowledge [18].

In order to attain repeatable experimental conditions, the comparison was established using a multisyringe flow injection analysis (MSFIA) system [19] to perform the concentration and elution steps of the SPE protocol in an automatic fashion. This type of automatic flow system enables the assembly of a flow network, where solutions from the different syringes can be either delivered to the flow system or returned to its own vessel, without interfering with the other channels [20]. Several elements may be incorporated into the flow network, including solid-phase reactors or columns [21,22]. In the present work, the outlet of the SPE column was connected to the LC sample loop [23], which was automatically filled with eluate before each chromatographic determination, assuring repeatable conditions.

2. Experimental

2.1 *Reagents and solutions*

All chemicals used were of analytical-reagent grade and used with no further purification. A MilliQ system was used to obtain ultra-pure water (resistivity $> 18\,M\Omega\,cm$), used for the preparation of all aqueous solution. Methanol HPLC grade (Merck, Darmstadt, Germany) was used for the preparation of all methanolic solutions and the same solvent was also used as eluent. The sorbents Amberlite XAD-4, Macronet MN-200 and Lichrolut EN were obtained from Fluka (Buchs, Switzerland), Purolite (Brasov, Romania) and

Table 1. Detection wavelength (λ), molecular weight, partitioning coefficients octanol/water ($\log K_{ow}$) and dissociation constants (p*Ka*) of EPA phenolic priority pollutants [25,26].

Phenolic compound	λ (nm)	MW (u)	$\log K_{ow}$	p*Ka*
2,4-Dinitrophenol (24DNP)	360	184.1	1.53	4.09
2-Methyl-4,6-dinitrophenol (46DNOC)	375	198.1	2.12	4.34
Phenol (P)	215	94.1	1.50	9.99
4-Nitrophenol (4NP)	315	139.1	1.90	7.16
2-Chlorophenol (2CP)	195	128.6	2.15	8.55
2-Nitrophenol (2NP)	210	139.1	1.78	7.21
2,4-Dimethylphenol (24DMP)	195	122.7	2.42	10.6
4-Chloro-3-methylphenol (4C3MP)	195	142.6	3.10	9.55
2,4-Dicholorophenol (24DCP)	200	163.0	2.08	7.85
Pentachlorophenol (PCP)	220	266.3	5.01	4.93
2,4,6-Trichlorophenol (246TCP)	200	197.5	3.69	7.42

Merck (Darmstadt, Germany), respectively. Amberlite XAD-4 with particle size in the range 125–250 μm was prepared as described in a previous work [24].

The phenolic compounds listed in Table 1 were obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of each compound was prepared by weighing 100 mg of the respective compound followed by dissolution in methanol in order to obtain a final concentration of 1000 mg L^{-1}. Stock solutions were rigorous diluted in methanol in order to obtain standard solutions of each individual compound or mixtures of them. For the standard solutions submitted to the preconcentration step, the dilution was performed by using 10 mmol L^{-1} aqueous HCl as solvent to match the pH of water samples.

The aqueous component of the mobile phase for the chromatographic method was a 50 mmol L^{-1} sodium dihydrogen phosphate (Fluka) solution with pH adjusted to 5.25 using a sodium hydroxide solution (6 mol L^{-1}). The organic component of the mobile phase was acetonitrile HPLC grade, obtained from Merck. The mobile phase was prepared from a 36:64 solution of the organic and aqueous components, filtered through a 0.45 μm porous membrane and degassed by ultrasounds before use.

2.2 *Chromatographic determination of phenolics*

The chromatographic method was performed by using a Merck/Hitachi LaChrom 7000 series (Hitachi Ltd., Tokio, Japan) equipped with a pump (L-7100), a diode array detector (L-7455) and an interface (D-7000). The monolithic analytical column used for the reversed-phase separation of the studied compounds was a Chromolith RP-18e (100×4.6 mm id) coupled to a pre-column (5×4.6 mm id) of the same material (Merck). The interface between the automatic SPE system and the chromatograph was a Rheodyne 7725i high pressure manual injector (Rheodyne, Rohnert Park, CA, USA), containing a loop with an internal volume of 20 μL. This loop was filled automatically at the end of each SPE cycle and its content was injected into the HPLC system by the same device.

The method used in this work was proposed by Cledera-Castro *et al.* [25], allowing the isocratic separation of eleven EPA phenolic priority pollutants in less than

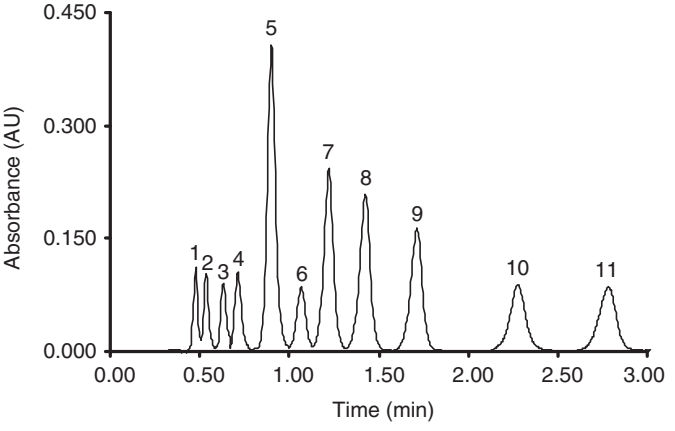

Figure 1. Typical chromatogram obtained under the conditions described in the text by direct injection of the compounds. Concentration of each phenolic compound: $10\,\mu g\,mL^{-1}$. Peak labels: (1) 24DNP, (2) 46DNOC, (3) P, (4) 4NP, (5) 2CP, (6) 2NP, (7) 24DMP, (8) 4C3MP, (9) 24DCP, (10) PCP, (11) 246TCP. Mobile phase; acetonitrile : phosphate buffer (36 : 64 vol.); flow rate, $4.00\,mL\,min^{-1}$.

three minutes (Figure 1). The only modification to the original method was the temperature. Room temperature (approximately 23°C) was adopted because no significant differences in the chromatogram were observed when this temperature was compared with the original value of 36°C.

2.3 *Flow injection apparatus*

The manipulation of solutions inside the flow conduits of the automatic SPE system was performed by a BU4S multisyringe burette (Crison Instruments, Alella, Spain) equipped with a syringe of 10 mL in position 2 and a syringe of 5 mL in position 3 and, a Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France) equipped with polyvinylchloride pumping tubes. A three-way commutation valve (NResearch, Caldwell, NJ, USA) connected to the head of each syringe of the multisyringe module allowed the optional coupling to the manifold lines or to the solution vessel. An array of four extra commutation valves controlled by the same module completed the basis of the flow network of the present system. The commutation valves options were codified in on/off lines. For the valves placed at the head of the multisyringe the 'on' line indicated the communication of the solution to the flow network whereas the 'off' line was assigned to the solution reservoir.

The control of the propulsion devices was performed by a personal computer running lab-made software written in QuickBasic 4.5 (Microsoft, Redmond, WA, USA). Two different hardware channels were used. The multisyringe module control (number of steps, the direction of piston displacement and the position of the commutation valves) was performed through a serial port and the peristaltic pump control (flow direction and rotation speed) was mediated by an interface card (PCL-711, Advantech, Taipei, Taiwan).

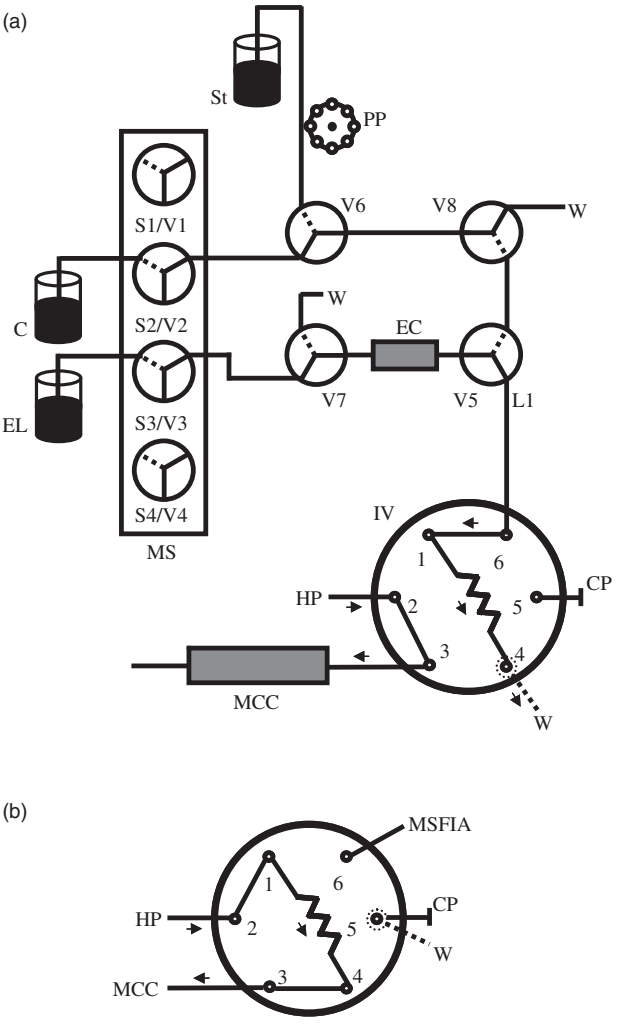

Figure 2. (a) MSFIA-SPE manifold for the automatic preconcentration of phenolic compounds, connected to the chromatographic system through the injection valve, represented on 'load' position. (b) Schematic representation of the injection valve configuration on 'inject' position. MS, muiltisyringe; S*i*, syringe; V*i*, commutation valves; EC, SPE column; IV, injection valve; PP, peristaltic pump; W, waste; L1, connection tubing (300 × 0.8 mm id); S2, syringe 10 mL; S3, syringe 5 mL; C, carrier (HCl 10 mmol L^{-1}); EL, eluent (methanol); St, sample or standard solution; HP, high-pressure pump; MC monolithic chromatographic column; MSFIA, preconcentration flow system; CP, closed port. In the commutation valves, the position 'on' is represented by a solid line while the position 'off' is represented by a dotted line. The needle port and connection to waste in IV are also represented by dotted lines.

2.4 *Manifold and automatic SPE procedure*

The automatic flow injection system components were arranged as shown schematically in Figure 2. PTFE tubing with 0.8 mm id (Omnifit, Cambridge, UK) was used for all connections. The different sorbents used in this work were packed in two different columns. For trapping Amberlite XAD-4 commercial product (213 mg), Amberlite XAD-4

grounded product (194 mg) and Macronet MN-200 (188 mg), a stainless steel column 20 mm of length and 7 mm of internal diameter was used. A polyetheretherketone (PEEK) device presenting the same tubular configuration with 24 mm length and 3 mm of internal diameter was used for packing Lichrolut EN (87 mg). In both cases, the sorbents were trapped inside the columns by using polypropylene filter disks supplied by MoBiTec (Goettingen, Germany) with a pore diameter of 35 μm (stainless steel column) or 10 μm (PEEK column).

The complete protocol sequence for the automatic SPE procedure, including nine steps, is described in Table 2. The first command comprises the system cleaning (including the extraction column) from the previous cycle with 3500 μL of methanol followed by the sorbent bed conditioning with 2500 μl of HCl 10 mmol L^{-1} (step 2). After this, the peristaltic pump was activated at a flow rate of 4 mL min^{-1}, and a variable volume of sample was loaded into the extraction column (step 3). Afterwards, the syringes were refilled with the volume of methanol and HCl solution necessary for the next two steps (step 4). Subsequently, 2500 μL of HCl solution were propelled through the sample channels and the extraction column in order to remove residues of the compounds (step 5). After the commutation of the appropriate valves, a variable volume of methanol (dependent of the sorbent used) was sent in the direction of the sorbent bed to elute the analytes (step 6). During this step the injection valve loop was filled and 20 μL of the eluate were injected into the chromatographic system by rotating the HPLC injection valve to the 'inject' position (step 7), beginning the chromatographic run. At last, the syringes were refilled (step 8) and the injection valve was turned to the 'load' position (step 9). Thereafter, the system was ready for the next SPE cycle.

3. Results and discussion

3.1 *Design of the automatic SPE method*

The SPE automatic manifold was based on a multisyringe burette that allowed a high precision in controlling the flow rates and volumes of solvents/solutions used for sorbent elution and conditioning. Furthermore, the assembling of a peristaltic pump into the manifold for propelling the sample through the extraction column enabled drastic reduction of the time necessary for the sampling step. Otherwise, the sample would be propelled by one of the burette syringes, but it would require several washing steps to prevent carryover between samples.

The inclusion of the extraction column between two commutation valves (V5 and V7) allowed that the operations of loading and elution were performed in opposite ways, avoiding the possible compaction of the sorbent particles and the consequent creation of back pressure which could result in the modification of the flow rate or in the column clogging. The commutation valve V8 provided an alternative flow path for the sample exchange without contamination of the extraction column.

The performance of the different sorbents was assessed by considering the sorbent capacity, the breakthrough volume and the enrichment factor obtained after the preconcentration step. The evaluation of the elution volume, breakthrough volume and the sorbent capacity was performed using P and 246TCP as model compounds. These compounds were chosen because they represented the extreme zones of polarity and molecular weight (Table 1) and they were also located in the extreme zones of retention time in the chromatographic run (Figure 1).

Table 2. Procedure for the automatic solid-phase extraction of phenolic compounds prior to its chromatographic analysis.

Step	Instrumentation	Protocol	Description
1	Multisyringe piston pump	Dispense 3500 μL at 3 mL min^{-1} with heads V2 off, V3 on, V5 on, V6 off, V7 on, V8 off, IV load	Washing of sorbent bed and injection valve loop with methanol
2	Multisyringe piston pump	Dispense 2500 μL at 5 mL min^{-1} with heads V2 on, V3 off, V5 off, V6 on, V7 off, V8 off, IV load	Sorbent conditioning with HCl, pH 2.0
3	Peristaltic pump	Propel X μL at 4 mL min^{-1} with heads V2 off, V3 off, V5 off, V6 off, V7 off, V8 off, IV load	Sample loading (variable volume up to 100 mL)
4	Multisyringe piston pump	Pickup 3720–4350 μL at 15 mL min^{-1} with heads V2 off, V3 off, V5 off, V6 off, V7 off, V8 off, IV load	Piston bar adjustment
5	Multisyringe piston pump	Dispense 2500 μL at 5 mL min^{-1} with heads V2 on, V3 off, V5 off, V6 on, V7 off, V8 off, IV load	Matrix removal and tubing cleanup with HCl, pH 2.0
6	Multisyringe piston pump	Dispense 610–925 μL at 1.5 mL min^{-1} with heads V2 off, V3 on, V5 on, V6 off, V7 on, V8 off, IV load	Elution of the analytes using methanol
7	HPLC injection valve	Rotate IV to inject position	Start of chromatographic run
8	Multisyringe piston pump	Pickup 9500 μL at 15 mL min^{-1} with heads V2 off, V3 off, V5 off, V6 off, V7 off, V8 off, IV inject	Piston bar adjustment
9	HPLC injection valve	Rotate IV to load position	Connect IV loop to MSFIA-SPE system

The initial conditions for the operation of the automatic SPE system were fixed according to previous work describing on-line coupling of the PS-DVB sorbents with liquid chromatographic systems [26]. The flow rates used during the SPE cycle were chosen considering the time of contact between the solutions (washing/conditioning solution, sample and eluent) and the sorbent, but also the existence of back pressure during the operation and the dispersion of the segment of eluate before filling the loop of the chromatographic injection valve. For these reasons flow rates of $4\,mL\,min^{-1}$ for sample loading, up to $5\,mL\,min^{-1}$ for sorbent system washing/conditioning and $1.5\,mL\,min^{-1}$ for the elution step were used in all experiments.

The volumes used for system washing/conditioning were selected as the minimum values that avoided 'memory effect' between samples and a correct conditioning of the extraction column before the sample loading step. A volume of 3500 μL of methanol was sent through the extraction column to perform the removal of all analytes which could be present into the flow line and two portions of 2500 μL of HCl 10 mmol L^{-1} were sent by S2 to the SPE device immediately before and after the sample loading in order to conditioning the sorbent and for removing sample residues before elution, respectively.

The elution volume was fixed after obtaining the elution profile of the model compounds. The experiment consisted in the extraction of 5 μg of P and 246TCP and subsequent evaluation of the peak area obtained with different elution volumes in the range 550–1250 μL. Similar elution profiles were obtained for both compounds in each sorbent, indicating methanol as a suitable solvent for desorption of the analytes. The volume for which the maximum peak areas were obtained varied from 610 μL (for Lichrolut EN) to 925 μL (for Amberlite XAD-4 125–250 μm). These differences were observed due to the difference of mass of sorbent used and the bed volume of the extraction columns. The length of the connection tubing between the extraction column and the chromatograph injection valve loop (L1, Figure 2) was the minimum possible in order to minimise the dispersion and consequently the dilution of the segment of eluate.

3.2 *Sorbent loading capacity*

For each sorbent a calibration curve was established by extracting 5 mL of individual standard solutions of P and 246TCP in the range 1–10 mg L^{-1}. The volume loaded was selected in order to guarantee that no breakthrough occurs. In all cases, no deviations in the linearity of the calibration curve were observed up to the maximum amount of P and 246TCP loaded, which corresponded to 35 μg of each compound for Lichrolut EN and 50 μg for the remaining sorbents. These results demonstrated that no overloading occurred up to the maximum amounts of the compounds extracted. The amount of 50 μg of P and 246 TCP was not tested with Lichrolut EN due to the high value of peak area (maximum peak height > 1 AU) obtained for concentrations above 7 mg L^{-1}.

3.3 *Breakthrough volume*

The breakthrough volume corresponds to the largest sample volume that can be processed without loss of analyte and for which recovery for all sample volumes lower than the breakthrough volume will be 100% [27]. This value defines the maximum volume which allows an exhaustive extraction of the analyte from the matrix. Two classical methods have been described for the experimental measurement of the breakthrough volume [6,27].

The frontal analysis method is based on the continuous feeding of the SPE column with analyte, accompanied by continuous or discrete monitoring of the non-retained analyte by detecting the UV signal at the outlet of the sorbent bed. Another common approach to estimate the breakthrough volume is the preconcentration of different sample volumes, each containing the same amount of analytes and then measuring the analytical signal obtained after elution of the compounds. Both methods are time-consuming and may not reflect the real working conditions, especially the concentration levels used when sample analysis is performed. For these reasons, in the present work, a different experimental approach based on the enrichment of different sample volumes with a constant concentration of analyte is proposed, by taking advantage of the time based sampling of the automatic flow system. Therefore, the loading volume was defined (and changed) by computer control, after fixing the flow rate and the time during which the peristaltic pump was activated (Table 2, step 3). A mass calibration curve was established by plotting the peak area against the mass of analyte loaded into the column [28]. This experiment consisted in the extraction of sample volumes between 1 and 100 mL of a standard solution containing 500 μg L^{-1} of the model compound. This concentration was chosen in order to guarantee a maximum amount of compound that did not cause overloading of the extraction column. In the calibration curve, when a deviation from the linearity of the calibration curve was observed, it was considered that breakthrough occurred [29]. The results obtained for all sorbents (Table 3) are expressed as the volume range, defined by the two experimental points between which breakthrough occurred, or as the maximum volume loaded without breakthrough.

Due to the hydrophobic character of the sorbents used, the values of breakthrough volume were lower for the more polar compound tested (P). For the sorbents with high cross-linking degree, as Macronet MN-200 and Lichrolut EN, no breakthrough was observed up to the maximum volume of 246TCP loaded (100 mL). The best performance for P was achieved when Amberlite XAD-4 (grounded product) and Lichrolut EN were used, with breakthrough volumes above 75 mL. Considering the results obtained for both model compounds, the highest breakthrough volumes were achieved for Lichrolut EN.

3.4 *Enrichment factors and analytical figures of merit*

The enrichment factors (EF) were calculated for each sorbent by using the ratio between the slope of the calibration curves obtained after extraction of 50 mL of standard mixtures and the slope obtained by injecting directly the analytes into the chromatographic system [30]. The concentration range used was 20–100 μg L^{-1} for each compound in the extracted

Table 3. Breakthrough volume range (mL) obtained for the different sorbents after extraction of volumes between 1–100 mL of P or 246TCP standard solutions with a concentration of 500 μg L^{-1}.

Sorbent	P	246TCP
Amberlite XAD-4 (commercial product)	$40 < V < 50$	$75 < V < 100$
Amberlite XAD-4 (grounded particles)	$75 < V < 100$	$50 < V < 100$
Macronet MN-200	$25 < V < 50$	$V > 100$
Lichrolut EN	$V > 75$	$V > 100$

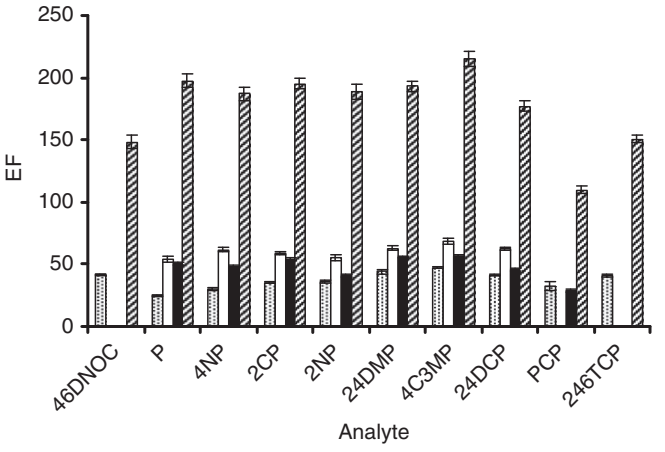

Figure 3. Enrichment factors (EF) for 50 mL of standard mixtures with concentrations in the range 6–80 μg L^{-1} using the different sorbents (dotted bar, Amberlite XAD-4 (commercial product); white bar, Amberlite XAD-4 (125–250 μm); black bar, Macronet MN-200; striped bar, Lichrolut EN).

mixtures and 500–8000 μg L^{-1} in the standard mixtures used for direct injection. When Lichrolut EN was used, the concentration of the compounds in the extracted mixtures was adjusted for the range 6–30 μg L^{-1}. The results obtained in this experiment (Figure 3) demonstrated that Lichrolut EN provided the higher values of enrichment factor (between 110 and 215), with a mean value of 176. The lower values of EF were obtained for Amberlite XAD-4 with a range between 24 and 47 and an average value of 37. These results could be explained for the differences between the two sorbents in the cross-linking degree and consequently, in the surface area available for the interactions between the analytes and the sorbent. However, for Amberlite XAD-4 with small particle size (125–250 μm) the values of EF increased (values between 54 and 67) and were higher than those obtained when using Macronet MN-200 (values between 29 and 57) that is a polymer with a very high cross-linking degree. These results show that an enhancement of the surface area by decreasing the particle size improved the capacity of the PS-DVB sorbents with low cross-linking for the retention of these phenolic compounds, resulting in performances comparable with sorbents with high cross-linking degree that by the nature of their structure have higher surface areas.

Phenol and its primary derivatives listed as priority pollutants by the EPA (Table 1) have distinct physical properties according to the different groups present in the aromatic ring. Thus, the values obtained for EF were compared with the partition coefficient and the molecular weight. In the case of Amberlite XAD-4, a positive correlation ($R = 0.867$) between the enrichment factor and the $\log K_{ow}$ was observed (Figure 4). A linear relationship was established: $EF = (10.96 \pm 7.24) \times \log K_{ow} + 12.0 \pm 17.0$, where limits of confidence are indicated for $\alpha = 0.05$, d.f. $= 5$. Thus, the interval of confidence for the slope value did not include the zero value and the slope value obtained was significantly different from zero ($t_{calc} = 8.705$, $t_{tab} = 2.571$, $\alpha = 0.05$, d.f. $= 5$), providing evidence about the linear relationship between these two variables [31]. This relationship may be explained by the reversed-phase mechanism of interaction between the phenolic compounds and the polymer, which promotes the adsorption of the more hydrophobic compounds. A similar behaviour was found for the grounded

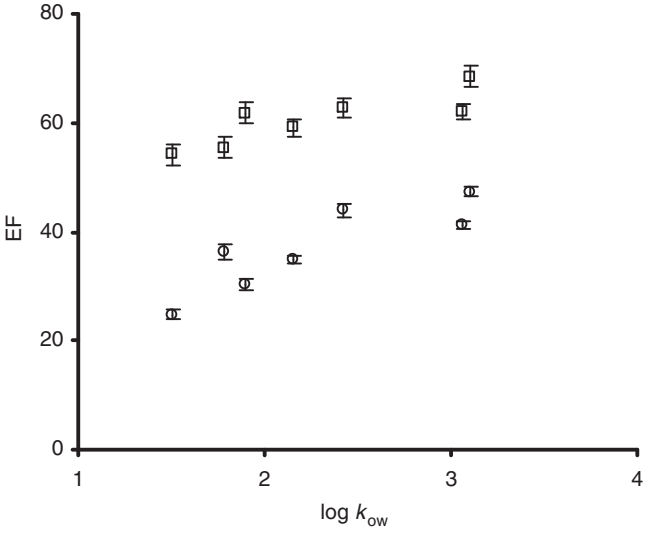

Figure 4. Relation between enrichment factor (EF) and the partitioning coefficient ($\log K_{ow}$) of phenolic compounds after SPE using Amberlite XAD-4 (commercial product (○) and grounded particles (□)).

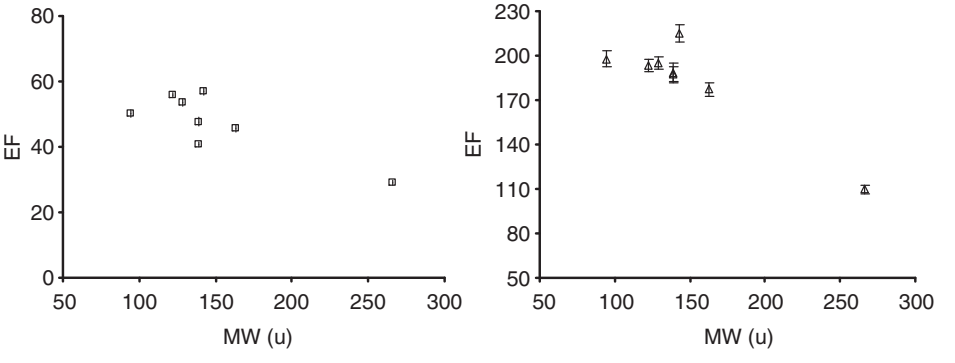

Figure 5. Relation between enrichment factor (EF) and the molecular weight (MW) of phenolic compounds obtained after SPE using the solid-phases Lichrolut EN (Δ) and Macronet MN-200 (□).

product: $EF = (6.64 \pm 4.81) \times \log K_{ow} + 45.4 \pm 11.3$, $R = 0.846$, $\alpha = 0.05$, d.f. = 5. The slope value was also significantly different from zero ($t_{calc} = 7.930$, $t_{tab} = 2.571$, $\alpha = 0.05$, d.f. = 5). Nevertheless, for grounded Amberlite XAD-4, the values of EF were higher due to the enhancement of the total surface area available for the extraction process.

Although the same mechanism of interaction was present, the behaviour reported for Amberlite XAD-4 was not observed with the sorbents with high cross-linking degree (Macronet MN-200 and Lichrolut EN, data not shown). Furthermore, for these sorbents, a negative correlation between the EF and the molecular weight was found (Figure 5). A linear relationship was established for Macronet MN-200: $EF = (-0.146 \pm 0.105) \times MW + 69.3 \pm 16.4$, $R = 0.812$, $\alpha = 0.05$, d.f. = 6. Thus, the interval of confidence for the

Table 4. Analytical figures of merit obtained for each sorbent tested.

	Amberlite XAD-4 (commercial)				Amberlite XAD-4 (125–250 μm)				Macronet				Lichrolut			
Compound	LOD/ng	Tested conc./ μg L^{-1}	RSD[a]	Recovery %[b]	LOD/ng	Tested conc./ μg L^{-1}	RSD[a]	Recovery %[b]	LOD/ng	Tested conc./ μg L^{-1}	RSD[a]	Recovery %[b]	LOD/ng [23]	Tested conc./ μg L^{-1}	RSD[a]	Recovery %[b]
24DNP	289	85.0	4.1	97.6 ± 1.6	262	85.0	2.2	99.7 ± 1.8	368	85.0	2.7	98.7 ± 3.5	207	25.5	1.2	100.3 ± 1.1
46DNOC	480	81.2	1.7	100.0 ± 0.5	437	81.2	3.5	98.8 ± 1.1	321	81.2	1.9	100.4 ± 0.3	103	24.4	2.8	102.1 ± 1.1
P	358	82.4	2.9	98.5 ± 2.3	290	82.4	2.6	99.6 ± 3.8	163	82.4	1.4	100.2 ± 0.4	157	24.7	2.1	98.1 ± 1.4
4NP	286	92.8	2.3	98.9 ± 2.0	300	92.8	3.0	98.3 ± 0.3	83	92.8	1.4	99.6 ± 0.4	110	27.8	1.4	99.2 ± 0.4
2CP	226	86.2	1.9	99.2 ± 1.6	180	86.2	1.9	99.3 ± 1.2	209	86.2	1.9	99.5 ± 1.2	31	25.9	1.7	99.2 ± 1.3
2NP	317	81.1	2.6	98.1 ± 1.2	224	81.1	1.5	99.5 ± 1.2	86	81.1	1.6	99.7 ± 1.7	87	24.3	2.3	101.6 ± 1.6
24DMP	293	84.1	1.3	99.5 ± 0.1	297	84.1	2.9	98.4 ± 1.6	171	84.1	1.6	99.1 ± 0.5	30	25.2	1.6	99.1 ± 0.7
4C3MP	292	79.9	1.4	99.5 ± 0.3	224	79.9	1.2	99.8 ± 1.5	98	79.9	1.4	99.7 ± 0.4	18	24.0	2.0	100.9 ± 3.4
24DCP	305	77.4	1.3	99.1 ± 0.4	287	77.4	1.2	99.9 ± 1.6	204	77.4	1.9	100.1 ± 0.3	39	23.2	1.9	99.8 ± 3.2
PCP	381	42.9	4.5	99.3 ± 3.9	NA	NA	NA	NA	227	42.9	1.9	100.6 ± 2.4	66	12.9	2.2	100.2 ± 5.0
246TCP	244	83.9	1.6	100.6 ± 0.9	NA	NA	NA	NA	NA	83.9	1.6	102.7 ± 2.7	55	25.2	5.1	NA

Notes: [a]Calculated using the statistics $s_{y/x}$ as estimate of standard deviation [33] ($n = 6$ or $n = 12$) divided by the tested concentration.
[b]$n = 3$.
NA = not available.

Table 5. Analytical performance of on-line SPE chromatographic methods for determination of pollutant phenolics.

Methodology	Working range/$\mu g\,L^{-1}$	LOD/$\mu g\,L^{-1}$	Sorbent	Sample volume/mL	Determination frequency/h^{-1}	Organic solvent/mL	Efluent[a]/mL	Reference
LC-DAD	20–100	4.5–9.6	XAD-4 (commercial)	50	4	4.1–4.4[c]	9.5	Present work
LC-DAD	20–100	3.6–8.7	XAD (125–250 μm)	50	4	4.1–4.4[c]	9.5	Present work
LC-DAD	20–100	1.7–7.4	Macronet MN-200	50	4	4.1–4.4[c]	9.5	Present work
LC-DAD	6–30	0.4–4.1	Lichrolut EN	50	4	4.1–4.4[c]	9.5	Present work
LC-DAD	1.5–140	0.3–2	Lichrolut EN	25–100	4–10	4.1[c]	9.1	[23]
LC-ED	0.01–10	0.001–0.075	PLRP-S	1.0–4.0	2.6	16[c]	28	[33]
LC-MS	100–300	n. a.	Several[b]	≤100	2	3[c]	8	[34]
SFC-DAD	3–25	0.4–1.9	PLRP-s	20	1.5	10[c]	20	[35]
LC-UV	0.01–25	0.01–0.7	Lichrolut EN	100	1.6	5[c] + 5[d]	12.5	[9]
LC-UV-FL	1.5–100	0.2–0.6	Modified PS-DVB	≤100	0.93–2	12[c]	24	[36]
LC-UV-ED	0.05–20	0.035–0.07	Modified PS-DVB	≤20	2	12[d]	22	[10]

Notes: [a]Excluding sample volume.
[b]Comparative study between C18 HD, PLRP-S, Hamilton-PRP-1, Hysphere GP, Hysphere SH and Oasis HLB.
[c]Methanol.
[d]Acetonitrile.
SFC, supercritical fluid chromatography; LC, liquid chromatography; DAD, diode-array detection; ED, electrochemical detection; MS, mass spectrometry detection; UV, ultra-violet detection; FL, fluorimetric detection.
n.a. Not available.

slope value did not include the zero value and the slope value obtained was significantly different from zero ($t_{calc} = -8.342$, $t_{tab} = 2.447$, $\alpha = 0.05$, d.f. = 6), providing evidence about the linear relationship between these two variables [31]. For Lichrolut EN, $EF = (-0.562 \pm 0.251) \times MW + 267 \pm 40$, $R = 0.913$, and $t_{calc} = -13.411$, $t_{tab} = 2.447$ for $\alpha = 0.05$, d.f. = 6. For these cases, the molecular size of the phenolic compound had an important role on the extraction process because the high cross-linking between the polymeric chains worked as a molecular sieve, promoting the sorption of the compounds with low molecular size. These results are in agreement with recent observations from Sychov *et al.* [32], indicating that the whole interior of the hypercross-linked polystyrene particled is accessible to small analytes but not to larger molecules.

Concerning the analytical figures of merit (Table 4), the determination of LOD values was based on the calibration curves established for the determination of EF and they are given as mass of compound (present in 50 mL of sample), calculated from the concentration obtained for the interception plus three times $s_{y/x}$ [31]. LOD values between 180 and 480 ng were obtained for Amberlite XAD-4, with mean values of 316 and 278 ng for commercial and grounded sorbent, respectively. Lower values were obtained for Macronet MN-200 (LOD between 83 and 368 ng, with mean value of 193 ng) and the lowest values were obtained for Lichrolut EN (LOD between 18 and 207 ng, with mean value of 82 ng). This was expected as the highest EF values were obtained for this sorbent.

The linear calibration range was 20–100 $\mu g\,L^{-1}$ for a preconcentration volume of 50 mL, except for Lichrolut EN. For this sorbent, the linear range attained was 6–30 $\mu g\,L^{-1}$ for the same sample volume. Repeatability was assessed through the statistic $s_{y/x}$, applied as an estimate of the standard deviation ($n = 6$ or 12) [31]. Values lower than 4.5% were obtained for all sorbents tested (Table 4), accounting for the repeatable conditions attained by the automation of the SPE procedure. Recovery studies were performed using 50 mL of MilliQ water fortified with 80 $\mu g\,L^{-1}$ of each phenolic compound (or 24 $\mu g\,L^{-1}$ for Lichrolut EN). Similar results were attained for all sorbents, with recovery values between 97.6 and 102.7%. For Lichrolut EN further analytical application was developed [23], providing statistically comparable results when certified reference material was analysed. In fact, for samples RTC-QCI-032 and U-QCI-076 (Promochem, LGC Standards), the total phenolics content was in agreement with the certified value [23]. Moreover no matrix effect was observed for mineral, tap and seawater samples, with mean recovery values ranging from 89 to 103% for all analytes tested.

Compared to previously described on-line SPE methods for chromatographic determination of phenolics [9,10,23,33–36], the sorbents studied here presented better or similar performance (Table 5). In fact, organic solvent consumption was similar (4–5 mL) and effluent production was lower (9.5 mL compared to 20–28 mL) in this work. The method proposed by Wissiack *et al.* [34] provided better performance regarding these aspects, but the concentration working range was higher and narrower.

4. Conclusions

In the present work, the utilisation of an automatic flow system provided repeatable conditions for comparison of different SPE sorbents. For this, the implementation of different flow directions for loading and elution operations was essential, because it prevented the compaction of the sorbent bed and avoided the creation of preferable flow paths. Compared to previous alternatives in this area, the multisyringe equipment was an

advantageous choice as it was compatible with organic solvents because only glass syringes and PTFE valves were in contact with the manipulated fluids. Moreover, the hyphenation with the LC equipment through its injection valve was successful while the computer control of the SPE protocol avoided unnecessary reagent (conditioning solution, eluent) consumption during the chromatographic run.

Concerning the performance of the sorbents, higher breakthrough volumes were obtained for Lichrolut EN for both model analytes studied (P and 246TCP). Higher values were also obtained for Lichrolut EN, followed by Amberlite XAD-4 (grounded particles), Macronet MN-200 and Amberlite XAD-4 (commercial particles), regarding the enrichment factors obtained under the same experimental conditions. As a consequence, lower values of LOD were also attained when applying Lichrolut EN. Nevertheless, repeatability and recovery values were similar for all sorbents tested. These results indicate Lichrolut EN as the best choice for analytical applications because lower concentrations can be determined by using this sorbent, fostering the determination in samples at low ng mL^{-1} levels using conventional HPLC-DAD technique [23].

Concerning the molecular interactions between the analytes and the sorbent, it was observed a positive correlation between the EF values and the hydrophobic character of the phenolic compound (expressed as $\log K_{ow}$) for Amberlite XAD-4, justified by the enhanced retention of more hydrophobic molecules due to the prevalence of the reversed-phase mechanism of interaction between the analytes and this sorbent. For the sorbents with higher cross-linking degree, this was not observed. In fact, a negative correlation between the EF values and the molecular weight of the analyte was verified, indicating that the structure of these sorbents may act like a molecular sieve, granting or restricting access to inner surfaces according to the analyte size. This fact may have consequences not only in the analytical application of these sorbents but also on their application for removal of phenolic pollutants in effluents. In both situations, larger molecules will be less retained despite the enhancement of surface area brought by the higher cross-linking degree.

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