
**FUNCTIONALIZED POLYMERIC SORBENTS FOR SOLID-PHASE
EXTRACTION OF POLAR POLLUTANTS**

ABSTRACT

This study assesses how the physical characteristics of styrene-divinylbenzene (PS-DVB) resins affect the extent to which they are modified when they are functionalized, and how they affect recoveries in the on-line solid-phase extraction (SPE) of some polar phenolic compounds, pesticides and metabolites from water samples which were analyzed by liquid chromatography and UV detection. For this purpose, three commercial PS-DVB resins with different physical characteristics (Amberchrom GC-161m and two different PLRP-S resins) were chemically modified by placing a hydrophilic group, an *o*-carboxybenzoyl moiety, on their surface; although the physical characteristics are different, the extent of the modification did not vary significantly. The results from the SPE process with each sorbent were related to their physical and chemical properties. The polymers with higher surface area provided better recoveries. Moreover, the recoveries for these analytes were better with the chemically-modified polymers due to the higher polarity of these sorbents. The best recovery values were with the sorbent obtained from the chemical modification of Amberchrom GC-161m, the commercial sorbent with higher surface area. For instance, in the analysis of 50 ml of a sample of $2 \mu\text{g l}^{-1}$, the recovery of phenol was 40% with Amberchrom GC-161m and 65% with the chemically-modified Amberchrom GC-161m.

Keywords: Water analysis; Polar pollutants; Solid-phase extraction; Chemically-modified polymeric resins.

INTRODUCTION

Solid-phase extraction (SPE) is the most important technique for sample enrichment in water analysis. There are many applications in off-line and on-line SPE methods but, at present, on-line methods are preferred because of their advantages over off-line procedures [1,2].

Several types of sorbents have been developed, such as chemically bonded silica, carbon blacks and styrene-divinylbenzene copolymers (PS-DVB). The most problematic compounds are the polar ones, whose breakthrough volumes are low in most of the sorbents mentioned. On using recently developed sorbents, such as highly crosslinked polymers and chemically-modified polymeric resins, the breakthrough volumes are usually higher [3].

Highly crosslinked polymers have an open structure (high-porosity materials) which increases their specific surface area and allows greater π - π interactions between analytes and sorbents [3,4]. The breakthrough volumes will therefore be higher than those obtained when less crosslinked sorbents are used. Some highly crosslinked polymers are Lichrolut EN, Envi-Chrom P, Styrosorb, Isolute ENV and HYSphere-1 which have been found to give higher recoveries than the classical SPE sorbents in the trace enrichment of polar analytes from aqueous samples [3, 5-11].

Chemically-modified polymeric resins of PS-DVB have a higher surface polarity than underivatized PS-DVB, and for this reason polar analytes are retained more in these sorbents (π - π and polar interactions) [3]. Different polar functional groups such as acetyl [5,12-15], hydroxymethyl [13,14], benzoyl [6] and *o*-carboxybenzoyl [16-18] have been introduced into polymeric resins. There are also some commercially available functionalized PS-DVB such as Bond Elut PPL [7,17]. Due to their hydrophilicity, these sorbents have given higher recoveries than their

unmodified analogues, mainly for the most polar analytes [5,12-14].

As well as the functionalization and the crosslinking degree of the polymeric SPE sorbents, their physical characteristics (pore volume, pore size and surface area) also exert a considerable effect on the recovery of the analytes. Particle size can also affect the retention, but in porous materials this effect is very small in comparison to the increase in retention observed with an increase in specific surface area and/or a decrease in pore size (which are strongly inter-related). Nevertheless, some authors [8] obtained a higher breakthrough volume of phenol when a highly crosslinked sorbent (HYSphere-1) with smaller particles was used. A lower surface area generally means that the sorbent will have a somewhat less capacity. This was demonstrated by some authors [9,19,20] and the explanation given was that more interactions were possible between the analytes and the sorbent. Similarly, pore size and surface area can also affect the degree of functionalization in the chemical modification of the polymeric resins. The highly porous materials have a higher real active surface available which enables easier accessibility to the reactive sites, thus allowing the degree of functionalization to be increased.

The aim of this paper is to assess the effect of the different physical characteristics of PS-DVB resins on the recoveries in the SPE process and on the extent of the modification in the functionalization of these sorbents. Three polymeric resins modified with an *o*-carboxybenzoyl moiety were synthesized from various commercial PS-DVB resins with different physical characteristics. They were tested for the on-line SPE of some polar phenolic compounds, pesticides and metabolites which were determined by high-performance liquid chromatography (HPLC) with UV detection. These sorbents were compared one another and to their unmodified analogues, and the differences in sorbent behaviour were correlated with their physical characteristics.

EXPERIMENTAL

Reagents and Standards

The eight target compounds can be divided into three groups: phenolic compounds (phenol, 4-nitrophenol and resorcinol), triazine pesticides and metabolites (atrazine, desisopropylatrazine (DIA), and desethylatrazine (DEA)) and carbamate pesticides (oxamyl and methomyl). The chemical structures of these compounds are shown in Fig. 1. The phenolic compounds were obtained from Aldrich-Chemie (Steinheim, Germany) and the pesticides and metabolites from Riedel-de Haën (Seelze, Germany). They were all 95-99% purity. Stock solutions of each compound (2000 mg l⁻¹) were prepared in methanol. Standard solutions of 100 mg l⁻¹ were prepared weekly by diluting the stock solutions with Milli-Q quality water (Millipore, Bedford, MA, USA) and they were used daily to spike water samples. Stock solutions were stored under refrigeration at 4°C for three months.

Acetonitrile (Lichrosolv) for chromatography (Merck, Darmstadt, Germany) and Milli-Q quality water, adjusted to pH 3 with H₂SO₄ (Probus, Badalona, Spain), were used to prepare the mobile phase.

HCl (Probus) was added to adjust the pH of the sample to 2.5 before the SPE process in order to prevent the phenolic compounds from occurring in ionic form. Real water samples were filtered through a 0.45 µm nylon membrane (Whatman, Maidstone, UK) before the SPE to eliminate particulate matter.

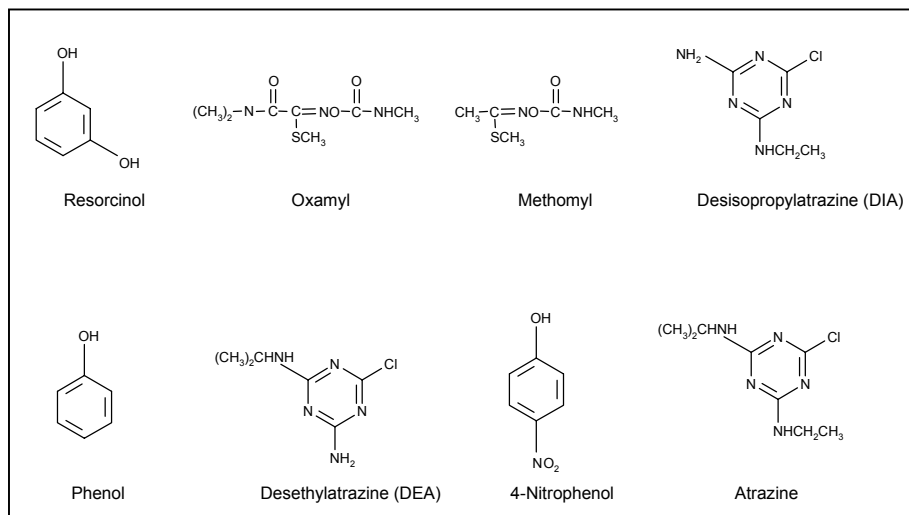


Fig. 1. Chemical structures of the target compounds

A volume of 500 μl of a 10% (w/v) Na_2SO_3 solution (Panreac, Barcelona, Spain) was added to 50 ml of Ebro river water samples to reduce frontal signals in the chromatograms from the coextracted humic substances [16,18].

The reagents used in the synthetic procedures were nitrobenzene from Probus, and aluminium chloride and phthalic anhydride, both from Fluka (Buchs, Switzerland).

SPE Materials and Synthetic Procedure

Various porous crosslinked PS-DVB beads, such as Amberchrom GC-161m (Tosohaas, Montgomeryville, PA, USA) and two PLRP-S resins with different physical properties (PLRP-S 10 and PLRP-S 30) (Polymer Laboratories, Shropshire, UK) were chemically modified by placing an *o*-carboxybenzoyl on their surface. Some physical characteristics of these commercial resins are shown in

Table 1.

Table 1
Physical characteristics of the polymeric resins

Resin	Surface area (m ² g ⁻¹)	Pore size (nm)	Particle size (μm)
Amberchrom	900	15	50-100
PLRP-S 10	500	10	10-15
PLRP-S 30	375	30	10-15

The *o*-carboxybenzoyl derivatives of each resin were prepared by aromatic electrophilic substitution with phthalic acid anhydride, using nitrobenzene as the solvent and aluminium chloride as the catalyst. 2.5 g of the divinylbenzene-crosslinked polystyrene was swollen in 70 ml of nitrobenzene at room temperature under mechanical stirring for 8 h. Mechanical stirring was used instead of magnetic stirring in order to prevent degradation of the beads. Afterwards, 4.5 g of AlCl₃ was added slowly and under mechanical stirring. Subsequently, 5.2 g of phthalic anhydride was added. The reaction mixture was stirred for 15 h at room temperature. The reaction was quenched by adding acetone with 1% HCl. The filtered polymers were washed twice with methanol and dried under vacuum at 60 °C. The chemically-modified polymeric resins from Amberchrom, PLRP-S 30 and PLRP-S 10 were named FC1, FC4 and FC5, respectively.

HPLC Conditions and On-Line SPE

Two Shimadzu LC-10AD pumps (Tokyo, Japan) were used to deliver the mobile

phase which was degassed with a Shimadzu DGU-4A degasser. The compounds were separated using a 25 x 0.46 cm i.d. Kromasil 100 C₁₈ column, particle size 5 µm, supplied by Teknokroma (Barcelona, Spain) and the temperature of this column was controlled by a Shimadzu CTO-10A oven. The analytes were detected by a Shimadzu SPD-10A UV spectrophotometric detector. A Hewlett-Packard (Waldbronn, Germany) dual-channel interface module HP-35900C connected the analytical system to the HP-3365 Series II Chemstation which collected and recorded the chromatographic data.

To check the response of the instrument, standard solutions were injected using a Rheodyne valve (Cotati, CA, USA) with a 20 µl loop, and an automatic Must column-switching device (Spark Holland, Emmen, The Netherlands) was used for on-line SPE.

The analytes were separated using a gradient of Milli-Q water (adjusted at pH 3 with sulphuric acid) (solvent A)-acetonitrile (solvent B) from 15 to 25% B in 9.30 min, to 50% B in 4.30 min, to 100% B in 6 min and then 2 min isocratic elution at 100% B. Afterwards, the mobile phase returned to the initial conditions in 2 min. The post-run time was 10 min. The flow rate of the mobile phase was 1 ml min⁻¹ and the temperature of the column oven was set at 65°C. The wavelength program used allowed to detect each compound at its maximum absorbance. The detection was performed at 280 nm for the phenolic compounds and at 240 nm for the pesticides and metabolites.

Stainless-steel precolumns 10 x 3 mm i.d.), laboratory-packed with the commercial polymeric sorbents and the respective sorbents modified with the *o*-carboxybenzoyl moiety, were used to perform the on-line trace enrichment. As in previous papers [7,17,18], the sorbents were activated with 2 ml of acetonitrile and 2 ml of Milli-Q water at pH 2.5 in sequence. Once activated, the water sample was

passed through the sorbent at a flow-rate of 4 ml min⁻¹. In the next step, the analytes trapped on the precolumn were desorbed, using only the organic solvent (acetonitrile) of the mobile phase, in the forwardflush mode for 1 min (approx. 0.16 ml acetonitrile), and transferred on-line to the analytical column. Both solvents of the mobile phase were mixed before they reached the analytical column. A Waters M45 pump (Milford, MA, USA) was used to deliver the sample and the conditioning and cleaning solvents.

RESULTS AND DISCUSSION

Characterization of the Polymers

The PS-DVB resins were electrophilically substituted with phthalic acid anhydride by the Friedel-Crafts reaction. Nitrobenzene was used as solvent and aluminum chloride as catalyst at room temperature. Higher reaction temperatures (40 and 70 °C) did not increase the extent of the reaction. Thus, the reaction temperature does not have significant influence on the modification degree.

Modifications were carried out in triplicate and elemental analysis showed that the extent of the modifications, expressed as the percentage of functionalized styrene moieties *versus* the total aromatic monomer units, was 11% for Amberchrom (FC1), 5% for PLRP-S 10 (FC5) and 10% for PLRP-S 30 (FC4). Despite the different physical characteristics of the three commercial polymers, the modification degree did not show noteworthy differences. These low degrees of substitution may be due to the restricted accessibility to the reactive sites or to the fact that phthalic acid anhydride is not as reactive as other reagents, such as the benzoyl chloride which was used to modify Amberchrom with a benzoyl moiety and the extent of the modification was 60% [6].

The modified polymers were finally sieved (sieves of 50 and 10 μm) and it could be verified that the particle size ranges had not changed in the reactions and they had the same ranges than their analogues. Their pore size and surface area were not determined because it is supposed that they had suffered small changes since the extent of modifications were low. Moreover, due to the extent of modifications did not vary significantly between the three polymers, these small changes in the physical properties were similar in all sorbents.

The polymers were also characterized by IR spectroscopy. The salient features of the infrared spectra of the resins are a broad H-bonded νOH at ca. 3500 cm^{-1} , $\nu\text{C=O}$ of the ketonic carbonyl at 1670 cm^{-1} , and COOH absorption at ca. 1700 cm^{-1} .

On-Line Trace Enrichment

Peak broadening, due to the different nature of the analytical column and the precolumn sorbent, was effectively suppressed by on-line desorbing with only the acetonitrile of the mobile phase [3,11]. Desorptions of more than 1 min were tested, but the shape of the peaks and the recoveries of all analytes were similar to those obtained in the selected conditions (forwardflush mode for 1 min).

The breakthrough volumes of the analytes with the on-line SPE sorbents were obtained according to the following method [21]. Different sample volumes (2, 4, 8, 20, 50, 100, 200 and 500 ml) of standard solutions spiked with different concentrations of analytes, so that the amount of each analyte in the samples was constant (0.1 μg injected), were percolated through the different precolumns. The recoveries were calculated by dividing the area obtained for a given sample volume by the peak area of direct loop injection of the same amount and the breakthrough

occurred when the recovery began to decrease. Table 2 and Table 3 show some of the recoveries obtained with the different sorbents when this method was used.

Table 2 shows that the recoveries with Amberchrom were the highest, mainly for the most polar analytes at high sample volumes. The recoveries with PLRP-S 30 were the lowest. For example, in the SPE of 200 ml of sample ($0.5 \mu\text{g l}^{-1}$) the recovery of oxamyl was 46%, 14% and 7% with Amberchrom, PLRP-S 10 and PLRP-S 30, respectively. Relating the physical properties of these polymers (see Table 1) to the recoveries obtained, indicated that the differences in the data obtained with the different sorbents were mainly due to the surface area. Amberchrom is the sorbent with the highest surface area ($900 \text{ m}^2 \text{ g}^{-1}$), so there were more interactions between sorbents and analytes and the retention and the breakthrough volumes increased. Furthermore, when the two sorbents with the same particle size (PLRP-S 10 and PLRP-S 30, 10-15 μm) were compared, the recoveries were higher with the sorbent which had larger surface area (PLRP-S 10).

The order was the same for the modified resins (see Table 3). Since the extent of the modification by the three sorbents did not vary significantly, the higher retention may also be due to the larger surface area of the sorbent.

Table 2
Recovery values (% R.) obtained by preconcentrating different sample volumes with the three commercial sorbents

Compound	20 mL			50 mL			200 mL		
	AMB (*)	PLRP-S 10	PLRP-S 30	AMB (*)	PLRP-S 10	PLRP-S 30	AMB (*)	PLRP-S 10	PLRP-S 30
Resorcinol	14	5	—	6	—	—	—	—	—
Oxamyl	74	68	52	65	54	25	46	14	7
Methomyl	77	70	62	65	42	29	31	10	8
DA	73	73	68	67	46	36	32	11	9
Phenol	69	43	42	40	20	23	26	8	14
DEA	73	72	75	73	77	75	73	60	48
4-Nitrophenol	72	70	72	70	72	69	55	22	24
Atrazine	74	69	71	78	73	77	79	85	76

a) 5 µg l⁻¹, b) 2 µg l⁻¹ and c) 0.5 µg l⁻¹. — not detected

(*) AM berchrom GC-161m

% RSDs are lower than 10% in all instances (n= 4).

Table 3
Recovery values (% R) obtained by preconcentrating different sample volumes with the three modified sorbents

Compound	20 mL			50 mL			200 mL		
	FC1	FC5	FC4	FC1	FC5	FC4	FC1	FC5	FC4
Resorcinol	24	13	8	15	—	—	—	—	—
Oxamyl	70	72	71	71	72	63	62	42	21
Methomyl	74	73	75	74	76	69	59	29	29
DA	76	75	75	75	76	69	64	30	22
Phenol	73	79	70	65	45	33	31	10	10
DEA	72	75	76	71	75	76	70	79	77
4-Nitrophenol	69	72	72	70	73	73	68	53	43
Atrazine	80	71	72	87	74	74	83	83	85

a) 5 µg L⁻¹, b) 2 µg L⁻¹ and c) 0.5 µg L⁻¹. — not detected
% RSDs are lower than 10% in all instances (n= 4)

By comparing each commercial sorbent with its respective chemically-modified polymer, it was seen that the introduction of a hydrophilic group, an *o*-carboxybenzoyl moiety, in the PS-DVB increased the recoveries of the analytes (compare Table 2 with Table 3). This is because the polarity of the polymer surface increased, so more polar interactions (hydrogen bonds and dipolar interactions) can be formed between the polar analytes and the SPE sorbent [22]. Consequently, the capacity of the modified polymers is increased and the breakthrough volumes of these analytes are larger.

According to these results, FC1 was selected as the best of the sorbents studied for the trace enrichment of polar compounds. The polarity of its surface and its larger surface area allowed a greater number of the two types of interaction (π - π and polar interactions) with the analytes and consequently higher recoveries.

It should be pointed out that with the instrumental design used the coupling of SPE sorbents with different particle sizes to the sorbent of the analytical column (5 μm) (see Table 1) no peak broadening was observed. This is shown in Fig. 2 which shows chromatograms obtained with some of the sorbents studied. It should be pointed out that with these polymers the particle size seems not to be so influential on the breakthrough volume as is mentioned in other studies where HYSphere-1 was used as SPE sorbent [8].

In order to analyse real water samples, 50 ml of samples were selected as the volume to preconcentrate in FC1, because the recovery values for all compounds were higher than 65%, except for resorcinol (15%). With higher sample volumes, the recoveries decreased for most polar analytes, mainly for phenol whose recovery was 31% for 200 ml of sample (see Table 3). These recoveries were higher than the obtained in previous works [5,6] with Envi-Chrom P (a highly crosslinked polymer) for oxamyl, methomyl and phenol; in the preconcentration of 25 ml of

sample with Envi-Chrom P the recoveries were 65, 64 and 47%, respectively. The recoveries with FC1 were higher than those obtained by other authors [20] for oxamyl and methomyl (less than 25% recovered preconcentrating 25 ml sample) using standard C₁₈ and C₁₈/OH (specifically designed for polar compounds) sorbents (precolumn size 10 x 2 mm i.d.). With the other SPE sorbents used in this study smaller sample volumes have to be selected to obtain good recoveries. Consequently, a significant improvement from classical SPE sorbents is that FC1 enables higher sample volumes to be preconcentrated and lower concentrations to be determined.

Analysis of Real Water Samples

When the sample volume had been established (50 ml), the linear range, detection limits, repeatability and reproducibility between days of the method were determined (except for resorcinol which was eluted at this sample volume) by fortifying Ebro river water samples. The linear range of the method was between 0.8 and 50 µg l⁻¹, for most of the analytes, and 0.3 and 50 µg l⁻¹ for atrazine. The correlation coefficients were good, with values between 0.9990 for DEA and 0.9998 for 4-NP. Detection limits, as results of fortification experiments, were calculated by the method of Winefordner and Long using the statistical program ULC (Univariate Linear Calibration) with *k* equal to 6 [23,24]. They were between 0.1 µg l⁻¹ for atrazine and 0.4 µg l⁻¹ for phenol, except for DEA which had a higher limit of detection, 0.6 µg l⁻¹, because it eluted at the same retention time as an interference of the sample matrix.

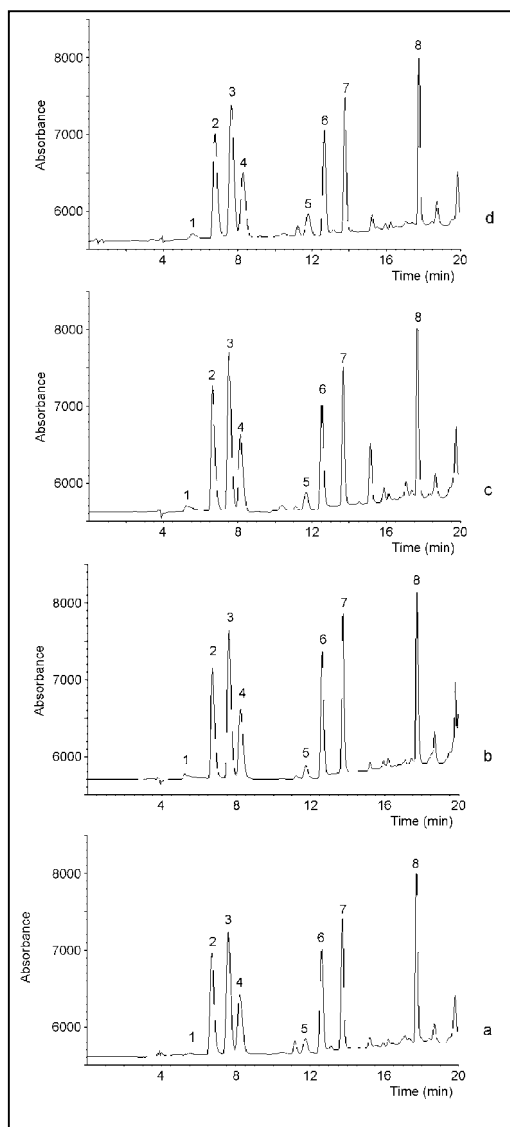


Fig. 2. Chromatograms obtained by on-line trace enrichment of 50 ml of standard solution of $2 \mu\text{g l}^{-1}$ using as a precolumn sorbent (a) Amberchrom, (b) FC4, (c) FC5 and (d) FC1. Peak assignments: (1) resorcinol, (2) oxamyl, (3) methomyl, (4) DIA, (5) phenol, (6) DEA, (7) 4-nitrophenol, and (8) atrazine.

The repeatability and the reproducibility between days of the method, expressed as the relative standard deviation of 4 analyses of 50 ml of Ebro river water spiked at $1 \mu\text{g l}^{-1}$, were lower than 10% for all compounds.

Fig. 3 shows the chromatograms obtained when preconcentrating in FC1 50 ml of Ebro river water (April 1998) with and without standard addition of $1 \mu\text{g l}^{-1}$ of each target analyte. In the blank chromatogram, three peaks at the same retention time as DIA, DEA and atrazine appear, which indicates that these three substances might be present, whereas there were no peaks corresponding to the other analytes. For DIA and DEA the signal was near the detection limits and they could not be quantified. Nevertheless, a concentration of $0.35 \mu\text{g l}^{-1}$ was found for atrazine. These pesticides have already been found in samples of the same origin in our laboratory using HPLC-APCI-MS [25].

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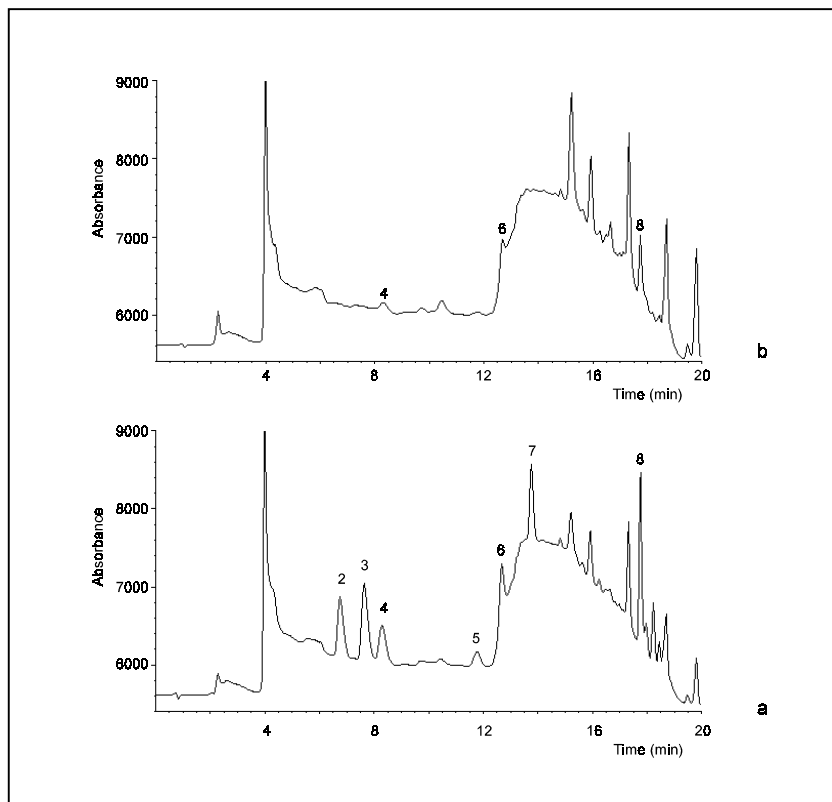


Fig. 3. Chromatograms obtained by on-line solid-phase extraction of (a) 50 ml of Ebro river water with standard addition of $1 \mu\text{g l}^{-1}$ of each target analyte and $500 \mu\text{l}$ of sulphite solution, (b) 50 ml of Ebro river water with addition of $500 \mu\text{l}$ of sulphite solution. Peak assignments: (4) DIA, (6) DEA and (8) atrazine.

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