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Comparison of different sorbent materials for *on-line* solid-phase extraction with liquid chromatography–atmospheric pressure chemical ionization mass spectrometry of phenols

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Abstract

On-line solid-phase extraction (SPE) was interfaced to liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (HPLC–APCI-MS) for the determination of US Environmental Protection Agency (EPA) phenols. The system, allowing fully automated operation, was used to evaluate different SPE cartridge materials and dimensions. Six different SPE materials (C_{18} HD, Polymer Labs PLRP-s, Hamilton PRP-1, Hysphere GP, Hysphere SH and Waters Oasis) were tested. Criteria for their comparison were first the recovery for the different phenols and its reproducibility, but also chromatographically relevant items like peakshape in the *on-line* elution mode. High recoveries and good relative standard deviations were obtained particularly for the newer, strongly retaining SPE materials that have become commercially available recently (the Hysphere materials and Waters Oasis) compared to the well known silica-based and weaker polymeric adsorbents like PLRP-s and PRP-1. These advantages are, however, traded in for good chromatographic peakshape, since the stronger adsorbents give rise to notable peak broadening in *on-line* elution. This is particularly true when using APCI-MS detection which on the one hand offers excellent selectivity and sensitivity, but imposes additional restrictions on the mobile phase composition in order not to suppress the response significantly. The influence of these parameters on the on-line-SPE–HPLC–MS determination of EPA phenols is discussed and present limitations are pointed out.

Keywords: Sorbents; Solid-phase extraction; Water analysis; Phenols; Chlorophenols; Nitrophenols

1. Introduction

Phenols, and particularly chlorinated phenols, are toxic at concentrations in the low μg/l range and also persistent in the environment. As a consequence, phenolic compounds are listed in the US Environmental Protection Agency (EPA) priority list of pollutants and in Directive 76/464/EEC of the European Union, related to dangerous substances discharged into the aquatic environment, and therefore require regular monitoring.

The occurrence of phenols as industrial pollutants in surface waters is quite common as by-product in the production of plastics and dyes and in the pulp industry [1]. This contamination source by far overweighs natural and biogeochemical formation pathways (e.g. farm waste, degradation of humic substances, tannins and lignins) and the contribution of pesticide degradation products [2].

Among the various methods developed for the analysis of phenols in wastewater samples (e.g. GC–MS [3] or LC–diode array detection (DAD) [4,5] or

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LC–electrochemical detection (ED) [6,7]), HPLC–atmospheric pressure chemical ionization (APCI) MS [8–11] and HPLC–particle beam (PB) MS [12] are particularly favorable due to the inherent selectivity of mass spectrometric detection. Nevertheless the detection limits imposed by environmental quality legislation can only be achieved by sample preparation techniques, which provide high enrichment factors of the investigated analytes. Solid phase extraction represents the nowadays most commonly used sample preparation technique for clean-up and sample pre-concentration. Most of the work reported so far for the enrichment of phenols was performed with solid-phase extraction (SPE) materials exhibiting common reversed-phase retention properties. In order to overcome the problem of low breakthrough volumes with low molecular weight, highly polar analytes such as phenol or 4-nitrophenol, the use of strong polymeric phase adsorbents or particularly modified polymeric phases [13–17] was investigated. Totally different approaches like solid-phase extraction with the use of an ion-pairing reagent were also reported by Marcé et al. [18–20] or column switching techniques [4] by Brouwer and Brinkman. A complementary approach to the derivatization of the stationary phase in order to increase the retention of the analytes is to derivatize the analytes before enrichment. This approach, achieved by acetylation of the acidic phenolic hydroxyl group was reported by Bao et al. [21].

The use of mass spectrometric detection for liquid chromatography imposes additional limitations to the liquid chromatographic separation of phenols and the solid-phase enrichment. The HPLC mobile phase used in the negative APCI mode can have a profound effect on sensitivity [22,23]. The application of organic acids or volatile buffers as liquid phase modifier can dramatically affect ionization or even quench ionization completely. In contrast to this, signal enhancement was observed in the atmospheric pressure ionization (API) electrospray (ESI) negative mode detection by post-column addition of bases [11], but only chlorinated phenols were studied. HPLC–ESI-MS detection of weakly acidic phenols like phenol or dimethylphenol was only performed under atypical conditions with pure methanol as eluent and ammonium acetate buffer as additive [8]. This approach however lacked general applicability since the stronger retained phenols did not elute from the column.

In general non-volatile salts have to be avoided as mobile phase additives and also the use of ion-pairing reagents is strongly discouraged. Both contamination of the atmospheric pressure ionization interface and signal suppression for the phenols of weaker acidity would occur. On the other hand, the use of particular mobile phase additives (acids, volatile/involatile buffers, etc.) would greatly improve chromatographic performance of the very acidic nitrophenols or pentachlorophenol.

As a consequence, the performance of *on-line* solid-phase extraction had to be optimized particularly for mass spectrometric detection. Whereas solid-phase extraction was carried out under common conditions for phenols, liquid chromatography with mass selective detection had to be optimized to possibly achieve a sensitivity in the same order of magnitude for the whole range of EPA phenols within a single HPLC run and with the exclusive use of one MS interface. Parameters to be considered were the breakthrough volume of the analytes and the resolution and peak shapes obtained with *on-line* elution onto the analytical column. This work reports the investigation of the suitability of various solid-phase extraction materials for the enrichment and *on-line* analysis of selected phenols and the optimum conditions under which they are to be used with the commercially available Spark Prospekt *on-line* SPE unit.

2. Experimental conditions

2.1. Chemicals

The phenol standards used in this study had a purity of at least 99% and were obtained from Fluka, Vienna, Austria (2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol and pentachlorophenol), from Aldrich, Vienna, Austria (2-chlorophenol, 2,4-dichlorophenol, 4-nitrophenol, 4-chlorophenol, phenol and 4-chloro-3-methylphenol) and from Riedel-de Haen, Vienna, Austria (2,4,6-trichlorophenol and 2,4-dimethylphenol). All standards were dissolved in methanol, stored in the dark at 4°C and

a working solution was daily prepared by diluting the mixed stock solution with water. Solvents for reversed-phase liquid chromatography, like methanol G Chromasolv and acetonitrile Chromasolv were purchased from Riedel-de Haen. The high-purity water was taken from a Milli-Q water system (Millipore, Eschborn, Germany).

2.2. LC–MS analysis

Liquid chromatographic separation and mass spectrometric detection were performed on a HPLC–MS system (HP series 1100, Hewlett-Packard, Waldbronn, Germany, and Palo Alto, CA, USA) with the HP Chemstation software version A.07.01. The applied system consisted of a quaternary pump (G1311A), a column thermostat (G1316A), a degasser unit (G1322A), an auto sampler (G1313A) and a mass-selective detector (G1946A) with an APCI interface (G1947A). The column thermostat was set to 25°C and the chromatography was performed with a methanol water gradient without acid addition to the liquid phase to avoid signal suppression of phenol, monochlorinated and methylated phenols. The LC eluent conditions varied from 40% organic solvent (6 min isocratic conditions) to 100% organic solvent (6 to 20 min linear gradient) in 20 min. All chromatographic separations were performed with a Kromasil C_{18} analytical column (250 mm×4 mm I.D., 5 μm spherical particles) which contains very low metal impurities (Na, Al and Fe<10 ppm) obtained through the Austrian Research Center, Seibersdorf, Austria) at a flow-rate of 0.8 ml min^{-1}.

Concentrations of single phenols ranged from 100 to 300 ng (absolute), except for phenol where an absolute amount of 700 ng was used to obtain sufficiently good signal-to-noise ratios for the comparison of different cartridges.

Analytes were detected in negative ion mode at 400°C vaporizer temperature, 350°C drying gas temperature, 5 l min^{-1} drying gas flow for APCI, 50 p.s.i. vaporizer gas pressure (1 p.s.i.=6894.76 Pa), and 1500 V capillary voltage. The corona current was set to 10 μA in the negative ion mode. Full scan data were acquired by scanning from m/z 80 to 280 using a cycle time of 1 s and a peak width of 0.1 s.

2.3. On-line solid-phase extraction

A Spark Prospekt (Emmen, Netherlands) solid-phase extraction system consisting of a solvent delivery unit (SDU; model 796) and a Prospekt sample preparation unit (model 795) was used for the *on-line* solid-phase extraction sample pre-concentration experiments. Sequences and single runs were programmed with the Spark Sequence program version A.01.21. Fully automated sample preparation sequences were performed by running in parallel sequences on the Spark Prospekt system and on the HP 1100 system where the two systems communicated in handshake mode. Commercially available extraction cartridges (from Spark, except where noted) with 10 mm length and 2 mm respectively 3 mm inner diameter were used. Mainly polymer based phases, namely Hysphere SH (modified polystyrene–divinylbenzene phase, 15–25 μm particles, irregular shape), Hysphere GP (polydivinylbenzene, 5–15 μm particle size, spherical shape), Hamilton PRP-1 (crosslinked styrene–divinylbenzene polymer phases, 12–20 μm particles, spherical shape) and Polymer Labs. PLRP-s (crosslinked styrene–divinylbenzene polymer, 15–25 μm particle size, spherical shape), were used. Hysphere C_{18} HD (end-capped, C_{18} phase with high density of octadecyl chains, 7 μm particle size, spherical shape) was the only silica-based phase used in this study. Additionally, the recently introduced Oasis material (macroporous polydivinylbenzene–*N*-vinylpyrrolidone copolymer) from Waters, Vienna, Austria was evaluated.

Samples were pre-concentrated on Spark Prospekt cartridges, using the following protocol: (1) cleaning and conditioning of the SPE cartridge with 3 ml of methanol and 3 ml of acidified water (sulphuric acid, pH 2.5) at a flow-rate of 2 ml min^{-1}; (2) enrichment of 10, 20, 50 or 100 ml of the acidified sample (sulphuric acid, pH 2.5) at a flow-rate of 4 ml min^{-1}; (3) rinsing of the cartridge with a volume of 2 ml Milli-Q water at a flow-rate of 2 ml min^{-1}; (4) switching of the electronic valve of the automated SPE unit and on-line elution to the analytical column with the gradient used for chromatographic separation. Except for the application of the sample at 4 ml min^{-1} the flow-rate was generally kept at 2 ml min^{-1} in order to avoid excessive pressure build-up with the SPE-cartridge in line due to an unfavorable

composition of the water/methanol mixture. Sample and solvents were pumped with the solvent delivery unit of the Spark system. The eluent flow was directed over the solid-phase extraction cartridge during the entire chromatographic run.

Breakthrough volumes were determined by enriching different volumes of Milli-Q water spiked with the mixed phenol standard. In order to eliminate the effect of different analyte masses being enriched on the breakthrough volume, the same absolute amount of each phenol was spiked to the different volumes of sample being processed. Recoveries were calculated by the comparison of the peak areas of the individual compounds. For the calculation of the recoveries three replicates were analyzed for each investigated volume and cartridge material.

3. Results and discussion

3.1. Conditions for chromatographic analysis of phenols with MS detection

The chromatographic separation of phenols requires in most cases an acidic effluent to be used to obtain acceptable peak shapes with most of the common stationary phases. We have, however, observed that the response for some of the weakly acidic phenols (e.g. phenol, dimethylphenol and chloromethylphenol) is suppressed in API-negative ion (NI) mode when acids or salts are used as mobile phase additives. Puig and Barceló reported that phenol and methylated phenols gave no response in negative thermospray ionization (TSP) MS [17]. For this reason, electrospray MS detection of particularly this group of phenols has been suggested using pure methanol as eluent and post-column addition of ammonium acetate in methanol [8]. Therefore a graphitized carbon black column that provided very strong retention even with pure organic phases had to be used, with the disadvantage that the other phenols do not elute from the column.

The intention of this work was to enable *on-line* SPE enrichment, chromatographic separation and MS detection of a wider range of phenols with only one system and under a single set of conditions. This was achieved by using the APCI interface and a water/methanol gradient as mobile phase without the addition of acid or salts. By this means, a satisfactory response of even relatively difficult ionizable phenols was obtained and allowed their determination together with similar sensitivity as the other, highly responsive phenols.

However, extensive peak broadening with *on-line* elution under these conditions for both dinitrophenols was observed, particularly when using polymeric phases and pre-concentrating large sample volumes.

The choice of analytical column is of primary concern in combination with *on-line* SPE [4,24–27]. Ideally, the analytical column would provide a comparable or even higher retention for the analytes in order to refocus them on the column head after elution from the SPE cartridges. On the other hand a higher organic phase content at the initial conditions of the gradient could be applied to perform peak compression [16,27].

This requirement is difficult to fulfill for polymeric SPE materials that exhibit strong retention for phenols. A possibility to circumvent in part this difficulty is by matching the physical dimensions of the SPE pre-column and the analytical column: This is the case when both the diameter and the length of the analytical column are significantly larger than that of the SPE cartridge. For this reason, 2 and 3 mm diameter SPE cartridges should preferably be coupled with 4 or 4.6 mm I.D. analytical columns. Concerning the column length, it is not generally advisable to couple SPE cartridges with analytical columns of less than 15 cm length. At shorter column lengths, separation is not sufficient to achieve a satisfactory resolution, since the initial peakwidth of the analyte zones with *on-line* SPE is much larger as compared to autosampler injections (roughly by a factor of 10 or more, when one assumes that the analytes are eluted within a volume of 100 μl from the SPE cartridge in comparison to an injection volume of typically 10 μl with autosampler injection).

An additional point of consideration is that the larger the practical flow-rate for the analytical column is (which increases with the square of the column diameter), the faster is the transfer of the analytes from the SPE cartridge to the analytical column, and the narrower are the analyte peaks.

The considerations referred above indicate that the

choice of column dimensions is equally important as the stationary phase material itself. Of the different phases considered the Kromasil C_{18} columns came closest to the requirements, exhibiting a stronger retention than the other investigated phases. Additionally due to the high purity of the base material (metal traces specified to be less than 10 ppm) and the low residual silanol activity, peak tailing of the more acidic phenols like the nitrophenols and pentachlorophenol was significantly reduced. For the reasons outlined above, only columns with 250×4 mm dimensions were tested. Of these, namely Nucleosil C_{18} HD 5 μm, Nucleosil C_{18} 5 μm and LiChrospher RP C_8 5 μm proved to be suitable for *on-line* SPE and analysis, whereas columns like Nucleosil 120-7C_6H_5 (phenylpropyl stationary phase, 7 μm particle size) or Nucleosil 120-7CN (cyanopropyl stationary phase, 7 μm particle size) appeared unsuitable for this task due to their weak retention and their unsatisfactory selectivity.

3.2. Influence of sample pH on the extraction efficiency

While the analysis of phenols by *on-line* SPE coupled to HPLC–MS separation and detection will always be carried out in the laboratory, their sampling onto the SPE cartridges is a procedure that is foreseen to be carried out in the field. For field sampling, a minimum of sample handling steps is desirable, and thus — with the perspective of an *on-site, on-line* sampling unit in mind — it is evident that no sample manipulation steps can be done before enriching the analytes on the SPE cartridges. The most critical parameter in this context is the sample pH, since it is generally known [13,16,28] that sample pH greatly affects extraction efficiencies in solid-phase extraction. Since the greatest influence on the extraction efficiency was expected to be observed when moving from the acidic range to near-neutral pH, the pH of the dilute aqueous phenol standard solutions was varied between 2.5, 4.0 and 7.5, respectively, and the recoveries were determined. The sample was acidified with concentrated sulphuric acid or brought to a near-neutral pH with triethylamine. While an increase of pH from 2.5 to 4.0 hardly influenced the recoveries, these decreased significantly for all investigated phenols at near-neutral pH (Table 1), when 20 ml of sample were pre-concentrated on a Waters Oasis cartridge. While SPE materials such as C_{18} HD, PLRP-s, PRP-1 showed low breakthrough volumes already at optimum pH, only the group of the strongly retaining adsorbents (Hysphere SH, Hysphere GP and Waters Oasis for which even phenol and 4-nitrophenol hardly show any breakthrough) was considered for the investigation of the influence of the pH. Out of these three materials that principally show very similar retention behavior, the Waters Oasis material was chosen as representative for this study.

Table 1
Recoveries of selected phenols and relative standard deviations at different sample pH values (Waters Oasis, 2 mm I.D. cartridges, 20 ml pre-concentrated)

Substance (*Nr*)	Mean value RSD (%)		
	pH 2.5	pH 4	pH 7.5
Phenol (1)	96 (4)	101 (4)	60 (3)
2,4-Dimethylphenol (2)	96 (15)	93 (4)	103 (4)
2-Chlorophenol (3)	97 (7)	99 (3)	29 (3)
4-Chlorophenol (4)	98 (3)	99 (2)	62 (4)
4-Nitrophenol (5)	92 (5)	97 (2)	3 (10)
2-Nitrophenol (6)	119 (9)	111 (6)	1 (17)
4-Chloro-3-methylphenol (7)	100 (8)	105 (2)	60 (4)
2,4-Dichlorophenol (8)	101 (5)	101 (3)	62 (4)
2,4-Dinitrophenol (9)	98 (8)	97 (7)	49 (8)
2,4,6-Trichlorophenol (10)	99 (4)	98 (3)	86 (3)
2-Methyl-4,6-dinitrophenol (11)	93 (12)	97 (3)	80 (2)
Pentachlorophenol (12)	87 (3)	89 (2)	76 (6)

As a consequence, acidification of the sample for the *on-line* SPE step could still not be avoided in order to prevent sample losses while the addition of acid to the chromatographic eluent was obsolete under the used conditions which is principally desirable for the reasons outlined above.

3.3. Recoveries of phenols for different adsorbent materials

In this study, Hysphere C_{18} HD was the only silica-based C_{18} material that was evaluated for the *on-line* enrichment of phenols. In the course of the optimisation experiments, it turned out that the nitrophenols, the monochlorphenols and phenol itself cannot be pre-concentrated on this material since break through of these analytes occurs even when only small sample volumes (10 ml) are pre-concen-

Table 2
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×2 mm I.D. C_{18} HD cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	n.d.	n.d.	n.d.	n.d.
2,4-Dimethylphenol	90 (1)	61 (5)	23 (10)	11 (9)
2-Chlorophenol	22 (3)	12 (4)	5 (7)	2 (4)
4-Chlorophenol	33 (2)	16 (2)	6 (3)	3 (4)
4-Nitrophenol	8 (8)	4 (5)	2 (10)	1 (10)
2-Nitrophenol	29 (1)	14 (5)	6 (8)	3 (5)
4-Chloro-3-methylphenol	100 (1)	97 (1)	45 (1)	23 (3)
2,4-Dichlorophenol	100 (4)	93 (6)	49 (2)	23 (2)
2,4-Dinitrophenol	39 (7)	23 (5)	8 (3)	4 (4)
2,4,6-Trichlorophenol	102 (4)	99 (6)	106 (8)	76 (5)
2-Methyl-4,6-dinitrophenol	130 (2)	121 (6)	53 (11)	30 (15)
Pentachlorophenol	95 (6)	97 (1)	105 (3)	103 (2)

trated (Table 2). With the exception of the highly chlorinated phenols all analytes break through when 50 ml of sample are pre-concentrated. As a consequence only polymeric phases were tested for further experiments.

PLRP-s and PRP-1 showed very similar results for the recoveries of phenols (Tables 3 and 4, respectively). Phenol is not effectively trapped resulting in break through when less than 10 ml of sample is pre-concentrated. Also, 4-nitrophenol shows a significant break through on the PRP-1 material when more than 50 ml of water sample are enriched on the cartridge. All other analytes are recovered without significant losses even if 100 ml of sample are pre-concentrated.

Hysphere SH (formerly Hysphere-1) is the sorbent with the strongest retention among the materials investigated. Two cartridge sizes were available and have been compared: 10 mm×2 mm I.D. and 10 mm×3 mm I.D. Both of them do not show a

Table 3
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×2 mm I.D. PLRP-s cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	43 (18)	22 (4)	8 (6)	5 (13)
2,4-Dimethylphenol	90 (4)	109 (1)	102 (2)	102 (4)
2-Chlorophenol	104 (3)	114 (3)	110 (3)	95 (4)
4-Chlorophenol	102 (3)	108 (2)	105 (2)	93 (2)
4-Nitrophenol	104 (4)	112 (4)	62 (6)	34 (6)
2-Nitrophenol	107 (3)	118 (2)	118 (1)	114 (2)
4-Chloro-3-methylphenol	101 (1)	107 (4)	97 (1)	105 (2)
2,4-Dichlorophenol	100 (4)	104 (3)	99 (3)	99 (5)
2,4-Dinitrophenol	85 (14)	118 (4)	89 (11)	96 (3)
2,4,6-Trichlorophenol	100 (2)	111 (6)	98 (5)	103 (3)
2-Methyl-4,6-dinitrophenol	109 (4)	108 (2)	90 (14)	95 (5)
Pentachlorophenol	97 (5)	102 (5)	88 (7)	97 (3)

Table 4
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×2 mm I.D. PRP-1 cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	41 (5)	21 (5)	8 (5)	4 (8)
2,4-Dimethylphenol	98 (5)	111 (5)	102 (1)	98 (4)
2-Chlorophenol	107 (3)	111 (2)	101 (6)	77 (2)
4-Chlorophenol	105 (3)	107 (6)	97 (2)	83 (6)
4-Nitrophenol	100 (3)	110 (1)	61 (1)	28 (7)
2-Nitrophenol	108 (2)	121 (3)	122 (1)	114 (1)
4-Chloro-3-methylphenol	104 (2)	103 (2)	100 (3)	106 (4)
2,4-Dichlorophenol	101 (1)	109 (2)	100 (4)	104 (2)
2,4-Dinitrophenol	90 (2)	121 (4)	111 (3)	110 (3)
2,4,6-Trichlorophenol	103 (5)	102 (2)	102 (1)	107 (1)
2-Methyl-4,6-dinitrophenol	116 (11)	109 (2)	115 (3)	106 (2)
Pentachlorophenol	95 (5)	101 (3)	97 (3)	108 (6)

significant breakthrough of phenols if up to 100 ml sample is enriched. The results obtained with the 2 mm I.D. cartridges (Table 5) are generally comparable with those from the 3 mm I.D. cartridges (Table 6). Only phenol itself shows a slightly better recovery for the 3 mm I.D. cartridge compared to the smaller inner diameter, but due to the less pronounced peak broadening that is obtained with the smaller inner diameter cartridges, the 2 mm I.D. cartridges have to be preferred (Fig. 1). The advantage of large breakthrough volumes of the Hysphere SH cartridges in contrast to the PLRP-s and PRP-1 materials is however counterbalanced by a significant peak broadening.

Both the Hysphere GP and Waters Oasis SPE materials provided excellent recoveries for phenols when 50 ml sample were enriched (Tables 7 and 8, respectively). Except for phenol the recoveries were on both materials quantitative even with 100 ml sample volume. The recovery of phenol drops to

Table 5
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×2 mm I.D. Hysphere SH cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	102 (6)	111 (3)	91 (4)	71 (8)
2,4-Dimethylphenol	97 (2)	108 (10)	97 (2)	103 (3)
2-Chlorophenol	105 (2)	115 (2)	105 (2)	105 (4)
4-Chlorophenol	106 (1)	112 (1)	101 (3)	103 (5)
4-Nitrophenol	104 (1)	118 (2)	113 (1)	109 (3)
2-Nitrophenol	111 (5)	122 (3)	123 (4)	112 (2)
4-Chloro-3-methylphenol	104 (2)	110 (4)	102 (2)	104 (6)
2,4-Dichlorophenol	102 (5)	113 (5)	108 (2)	99 (3)
2,4-Dinitrophenol	94 (3)	116 (1)	103 (1)	112 (4)
2,4,6-Trichlorophenol	102 (1)	104 (1)	107 (6)	99 (7)
2-Methyl-4,6-dinitrophenol	116 (7)	103 (8)	110 (9)	100 (8)
Pentachlorophenol	96 (3)	105 (3)	94 (3)	103 (2)

Table 6
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×3 mm I.D. Hysphere SH cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	104 (6)	111 (7)	111 (2)	91 (6)
2,4-Dimethylphenol	95 (3)	103 (1)	117 (8)	114 (7)
2-Chlorophenol	107 (4)	115 (5)	118 (3)	113 (1)
4-Chlorophenol	111 (6)	112 (3)	112 (2)	113 (1)
4-Nitrophenol	113 (4)	122 (1)	122 (1)	115 (1)
2-Nitrophenol	124 (2)	122 (1)	129 (4)	131 (9)
4-Chloro-3-methylphenol	109 (7)	114 (4)	121 (1)	116 (2)
2,4-Dichlorophenol	106 (2)	110 (2)	116 (3)	111 (1)
2,4-Dinitrophenol	116 (7)	136 (8)	134 (2)	116 (3)
2,4,6-Trichlorophenol	108 (7)	108 (4)	109 (2)	109 (1)
2-Methyl-4,6-dinitrophenol	150 (7)	142 (9)	139 (4)	130 (8)
Pentachlorophenol	100 (3)	103 (2)	112 (4)	111 (1)

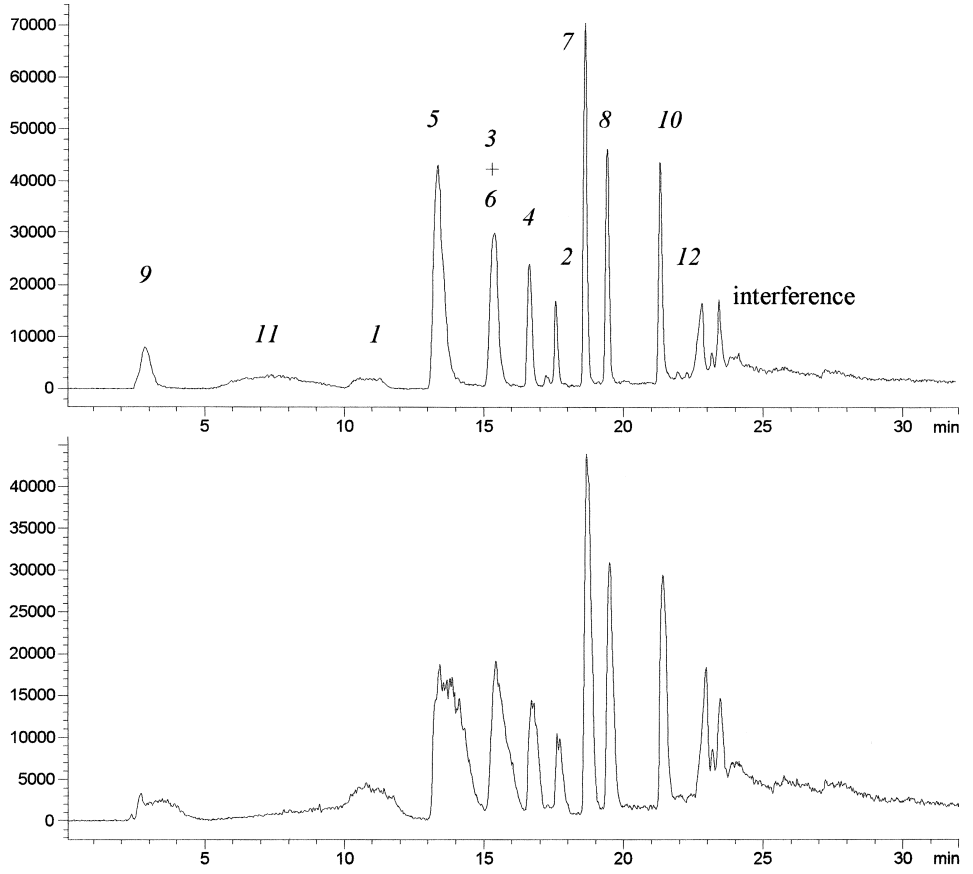

Fig. 1. Comparison of the *on-line* elution from Hysphere SH solid-phase extraction cartridges with different inner diameter [2 mm (top) and 3 mm (bottom), respectively] and its effect on the peak shape. Peak numbers correspond to the compound names given in Table 1.

Table 7
Recoveries and relative standard deviations for *on-line* SPE of selected phenols using a 10 mm×2 mm I.D. Hysphere GP cartridge with different sample volumes

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	89 (2)	95 (6)	87 (1)	47 (5)
2,4-Dimethylphenol	84 (14)	100 (4)	105 (2)	90 (3)
2-Chlorophenol	99 (1)	97 (2)	102 (1)	99 (7)
4-Chlorophenol	95 (1)	97 (1)	103 (3)	94 (5)
4-Nitrophenol	94 (1)	97 (2)	101 (2)	103 (1)
2-Nitrophenol	102 (3)	101 (1)	119 (5)	117 (5)
4-Chloro-3-methylphenol	96 (5)	96 (2)	103 (1)	95 (3)
2,4-Dichlorophenol	95 (2)	92 (2)	100 (3)	97 (3)
2,4-Dinitrophenol	97 (7)	97 (5)	100 (1)	103 (3)
2,4,6-Trichlorophenol	97 (2)	94 (4)	110 (3)	99 (3)
2-Methyl-4,6-dinitrophenol	92 (6)	97 (4)	96 (1)	100 (3)
Pentachlorophenol	86 (3)	88 (3)	105 (3)	100 (1)

47±5% on Hysphere GP and to 81±2% on Waters Oasis. These values are somewhat lower than for the Hysphere SH cartridges, but at the same time the chromatographic peak broadening is significantly reduced.

3.4. Further considerations on the choice of on-line SPE cartridge material

Whereas the results obtained for the silica-based C_{18} phase hardly showed overestimation (only 2-methyl-4,6-dinitrophenol shows recoveries higher than 100% due to peak broadening caused by the ionic character of the analyte under chromatographic conditions), higher recovery values than 100% were often observed when polymeric phases were used and if large volumes were pre-concentrated. Since the relative standard deviations essentially ranged from 1 to 8% the experimental error may not serve to explain these values. On the contrary, the difficulties

Table 8
Recoveries and relative standard deviations for *on-line* solid-phase extraction of selected phenols using a 10 mm×2 mm I.D. Oasis cartridge with different sample volumes/recoveries (relative standard deviation) of Waters Oasis with different volumes pre-concentrated

Substance	Mean value RSD (%)			
	Sample volume			
	10 ml	20 ml	50 ml	100 ml
Phenol	97 (3)	96 (4)	92 (1)	81 (2)
2,4-Dimethylphenol	104 (9)	96 (15)	100 (4)	92 (7)
2-Chlorophenol	99 (5)	97 (7)	99 (2)	96 (3)
4-Chlorophenol	97 (3)	98 (3)	101 (4)	100 (4)
4-Nitrophenol	94 (1)	92 (5)	99 (1)	99 (2)
2-Nitrophenol	119 (1)	119 (9)	136 (1)	132 (2)
4-Chloro-3-methylphenol	96 (1)	100 (8)	108 (1)	105 (2)
2,4-Dichlorophenol	101 (3)	101 (5)	109 (2)	101 (1)
2,4-Dinitrophenol	96 (7)	98 (8)	103 (4)	112 (1)
2,4,6-Trichlorophenol	99 (3)	99 (4)	111 (4)	103 (1)
2-Methyl-4,6-dinitrophenol	96 (5)	93 (12)	97 (3)	102 (3)
Pentachlorophenol	88 (4)	87 (3)	103 (4)	99 (1)

and errors of data evaluation significantly increased due to integration problems caused by peak broadening and alteration of chromatographic behavior when *on-line* elution was performed.

Compared to autosampler injections where analytes are injected in a small solvent volume (10 μl) and refocused in a narrow zone at the head of the analytical column, *on-line* elution negatively affects the chromatographic separation due to required larger elution volumes and partially insufficient refocusing on the analytical column. Additionally, the dispersion of the analytes on the enrichment

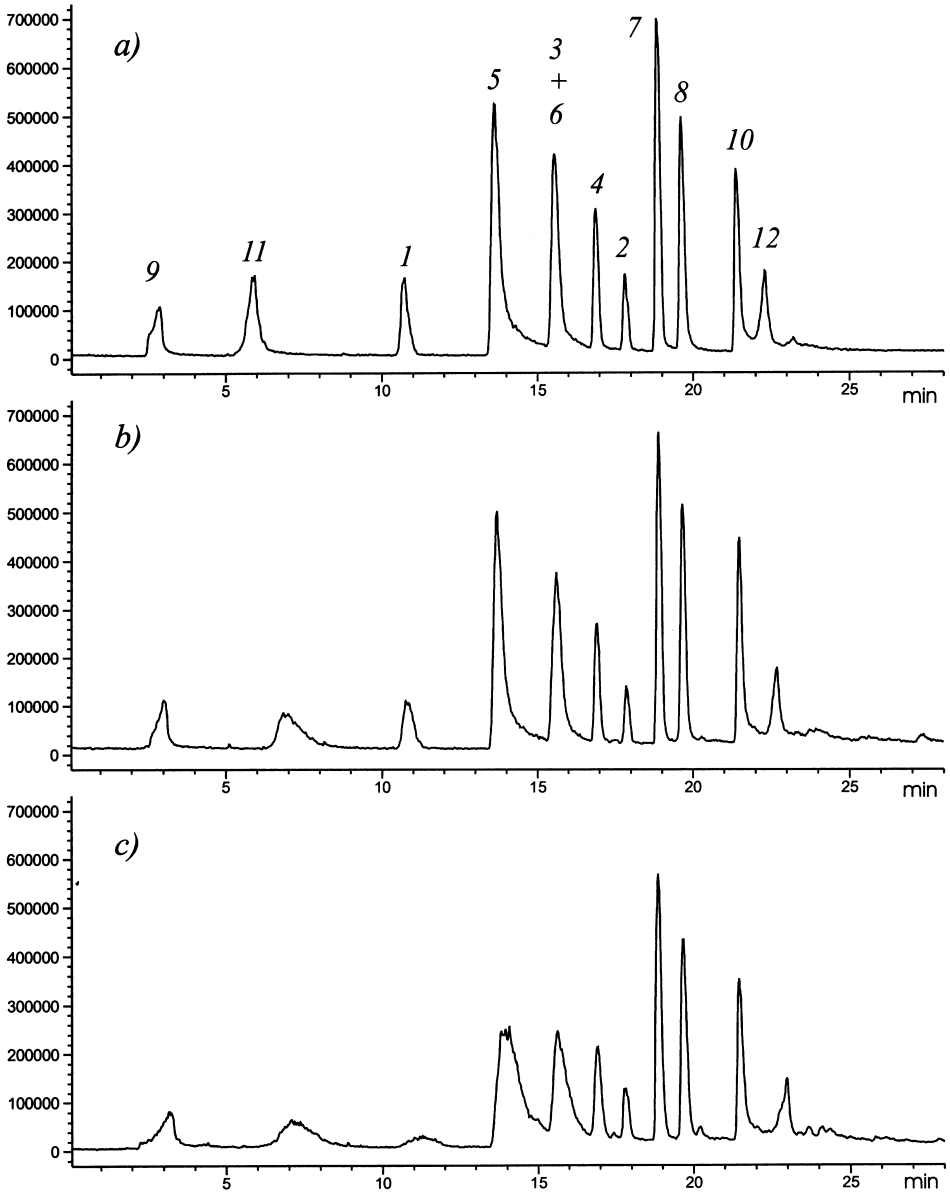

Fig. 2. Influence of different pre-concentrated sample volumes on the peak shape with a Hysphere GP 2×10 mm cartridge (a) 10 μl ALS injection; *on-line* SPE-elution of (b) 10 ml and (c) 100 ml sample volume. Peak numbers correspond to the compound names given in Table 1.

column increases when large volumes are pre-concentrated, resulting in broader elution profiles of the analytes for the analysis with *on-line* SPE (Fig. 2).

4. Conclusions

The proposed LC–MS method offers for the first time the possibility to detect the entire set of EPA phenols within a single run and with only one MS interface. This allows to take advantage of the inherent selectivity and also of the excellent sensitivity of MS detection as compared to commonly used diode array UV detection which makes this technique very well suited for screening analysis of phenols. In combination with *on-line* SPE, sample preparation is reduced to a minimum and the detection limits enforced by environmental regulation can be achieved. The major drawback of the method is that the chromatography of the polar dinitrophenols is compromised when conditions are used under which all phenols are detectable by MS with reasonable sensitivity, especially when *on-line* elution after the pre-concentration of large volume water samples (50 to 100 ml) is performed. The method can easily be adapted to improve the chromatography for dinitrophenols by the addition of acid to the mobile phase in the higher ppm range, but in this case signal suppression for phenol and dimethylphenol will occur.

As concerns the choice of cartridge format, those with 2 mm diameter have to be preferred when polymeric phases with strong retention capabilities are used, in order to avoid excessive peak-broadening during *on-line* elution. Adsorbents materials like Hysphere GP and Waters Oasis cartridges provide the best compromise between excellent recoveries and acceptable peakshapes if only 10 or 20 ml are pre-concentrated. The enrichment of larger water sample volumes (50 ml to 100 ml) generally resulted in increased peak-broadening for *on-line*-elution and separation.

It may be concluded that, although the sensitive determination of a wide range of — in their chemical properties strongly differing — phenolic compounds remains a difficult task, the use of *on-line* SPE with APCI-MS detection is attractive and highly versatile when optimum operating conditions are respected. Under these conditions, the technique clearly exceeds the diode array UV detection both in terms of sensitivity and selectivity, although only small sample volumes need to be processed, which means a significant reduction in the sample preparation time for the entire analytical procedure.

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