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On-line preconcentration and separation of neutral and charged aromatic compounds by ion interaction chromatography

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Abstract

An ion-interaction based chromatographic procedure was developed for the on-line preconcentration, separation and UV detection of benzene and naphthalene sulphonic derivatives having different charges. The retention behaviour of the species was studied on an octylsilica stationary phase, using cetyltrimethylammonium chloride (CTACl) as ion pair reagent and methanol as organic modifier in the presence of sodium chloride. The effect of CTACl, CH_3OH and NaCl concentrations was evaluated for the separation of neutral compounds (benzene and naphthalene), monosulphonic acids (benzenesulphonic, toluene-4-sulphonic and 1-naphthalenesulphonic acids), disulphonic acids (benzene-1,3-disulphonic and naphthalene-1,5-disulphonic acids) and naphthalene-1,3,6-trisulphonic acid. The preconcentration recoveries, after the optimization of CTACl concentration added to the sample, made it possible to reduce the detection limits from ppm to sub-ppb levels for some analytes.

Keywords: Sample preparation; Aromatic compounds; Benzene; Naphthalene; Sulfonic acids, aromatic

1. Introduction

Aromatic sulphonic acids derived from benzene and naphthalene are a group of polar water-soluble compounds widely used as raw materials in the manufacture of dyestuffs, pharmaceuticals, pesticides, wetting agents and dispersants. The determination of such compounds is therefore important not only for quality control of industrial products, but also for monitoring residual amounts in environmental samples, e.g., natural waters. For these purposes, the analytical procedure can be performed by direct injection of the sample or, when lower detection limits are required, by preconcentration techniques.

The chromatographic systems investigated for the determination of benzene and naphthalene derivatives include reversed-phase HPLC [1–9], ion interaction chromatography [10–15], ion chromatography [16,17], capillary electrophoresis [18] and gas chromatography [19]. Analytes can be detected by classical UV–Vis spectrophotometry [1–6,12–15,18] and with diode-array [7,10] or fluorescence detection [9,11]. Some coupled techniques have also been used: ion chromatography–MS [17] and capillary electrophoresis–MS [18]. Off-line enrichment procedures have been studied in order to achieve lower detection

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limits. They are usually based on anion exchangers [9] or RP-18 silica sorbents [10,11]. Few studies include the on-line preconcentration technique [6,16] and in no case has the method allowed the on-line preconcentration, followed by chromatographic separation, of both neutral aromatic compounds (e.g. naphthalene) and charged sulphonate derivatives.

The aim of this work was the development of a chromatographic procedure, based on the ion interaction mechanism, for the on-line preconcentration, separation and UV detection of several compounds with different charges. The system was optimized by a detailed study of the effect of eluent composition on the capacity factors. Such a procedure shows the possibility of the simultaneous determination of neutral and mono-, di- and tri-negatively charged compounds.

2. Experimental

2.1. Instrumentation

The chromatographic apparatus consisted of a Varian (Walnut Creek, CA, USA) Model 9010 pump equipped with a Rheodyne injector and a Model 332 UV–Vis variable-wavelength detector (Kontron Instruments, Milan, Italy). The chromatograms were recorded with an Axxiom Chromatography (Calabasas, CA, USA) Model 727 data station. The sample loading for the preconcentration procedure was performed with a Gilson (Middleton, WI, USA) Model 302 pump. The separation and preconcentration columns were a Merck LiChrospher 100 CH-8, 10 μm (250 × 40 mm I.D.) and a Merck LiChrospher 100 RP-18, 5 μm (4 × 4 mm I.D.), respectively. An Orion (Cambridge, MA, USA) digital pH meter was used for pH measurements.

All chromatograms were obtained at room temperature. The flow-rate was 1.0 ml/min for the separation column and 4.0 ml/min for sample loading during the preconcentration procedure. UV absorbance detection was performed at 210 nm. Retention times were the means from triplicate injections. The dead volume was measured by the injection of water.

2.2. Reagents and solutions

Eluents were prepared by dissolving analytical-reagent grade chemicals in high-purity water obtained using a Milli-Q system (Millipore, Bedford, MA, USA) and filtering through a 0.45-μm filter. The eluent components were methanol for chromatography and sodium chloride from Merck (Darmstadt, Germany) and cetyltrimethylammonium chloride (CTACl) from Fluka (Buchs, Switzerland). Before mixing the organic modifier, the eluent pH was adjusted to 7.5 with ammonia.

Concentrated stock solutions of the analytes were prepared by dissolving in water–methanol (40:60, v/v) benzene, benzyldimethylhexadecylammonium chloride, naphthalene, naphthalene-1-sulphonic acid sodium salt, naphthalene-1,5-disulphonic acid disodium salt, naphthalene-1,3,6-trisulphonic acid trisodium salt, propionic acid sodium salt, sodium nitrate, benzenesulphonic acid, benzene-1,3-disulphonic acid disodium salt, oxalic acid dihydrate and toluene-4-sulphonic acid sodium salt (Fluka). Benzene, benzyldimethylhexadecylammonium, propionate and oxalate solutions were $1.0 \cdot 10^{-2}$ M and all the others were $1.0 \cdot 10^{-4}$ M.

3. Results and discussion

3.1. Eluent

The ion interaction chromatographic technique is useful for the simultaneous separation of compounds with very different charges and hydrophobicities. The selection of the optimum eluent composition and stationary phase is of great importance in order to achieve the best compromise between the different classes of analytes in the separation. In this work, the eluent contained, after optimization, methanol as organic modifier, cetyltrimethylammonium chloride (CTACl) as ion interaction reagent and sodium chloride as ion strength modifier (com-

peting ion). A reversed-phase silica C_8 separation column was selected. The reduced hydrophobicity of such a stationary phase, in comparison with the more commonly used C_{18}, allows us to separate neutral compounds with a lower percentage of organic modifier. Therefore, it is possible to change the eluent composition, avoiding precipitation phenomena, over wider ranges of concentration of ion interaction reagent and salt, both important parameters for optimizing the separation of charged compounds.

Some attempts were first made to select an organic modifier and an ion interaction reagent, methanol and CTACl respectively, after a detailed evaluation of the concentrations required for the solubility limits of analytes and ion pairs in the eluent. As regards the system investigated, the concentration ranges were fixed at 55–65% methanol, 0.44–5.0 m*M* CTACl and 0–80 m*M* NaCl.

3.2. Separation

Our main interest was the study and characterization of the chromatographic behaviour of the different classes of compounds, with different charges, in order to develop analytical methods and model the system. A theoretical discussion has been reported in a previous paper [20] and, as regards this plan, a cation species, namely benzyldimethylhexadecylammonium chloride, was also considered. Table 1 shows the charge and nature of the analytes considered. It must be noted that samples containing both a hydrophobic anion and cation may precipitate neutral ion pairs. The precipitation takes place as a function of analyte concentration and matrix composition. In any case, the simultaneous analysis of neutral, anionic and cationic species could have an important role, e.g., in the quality control of industrial processes.

Table 1
Analytes as a function their charge and hydrophobicity

Charge	Analyte	
	Benzene structure	Naphthalene structure
0	Benzene	Naphthalene
+1	Benzyldimethylhexadecyl ammonium chloride	
−1	Benzenesulphonic acid Toluene-4-sulphonic acid	Naphthalene-1-sulphonic acid
−2	Benzene-1,3-disulphonic acid	Naphthalene-1,5-disulphonic acid
−3	–	Naphthalene-1,3,6-trisulphonic acid

The separation of the analytes was optimized by evaluating the effect of eluent composition on retention. The study of retention behaviour as a function of percentage of methanol in the eluent showed the typical exponential dependence for all the analytes investigated. These exponential curves are almost parallel when the analytes have similar hydrophobicity.

The effect of the concentration of the ion interaction reagent (IIR) in the eluent on the capacity factors was evaluated without addition of and for different concentrations of sodium chloride. When the eluent does not contain NaCl (Fig. 1), the capacity factors increase up to a constant value corresponding to 1 m*M* CTACl;

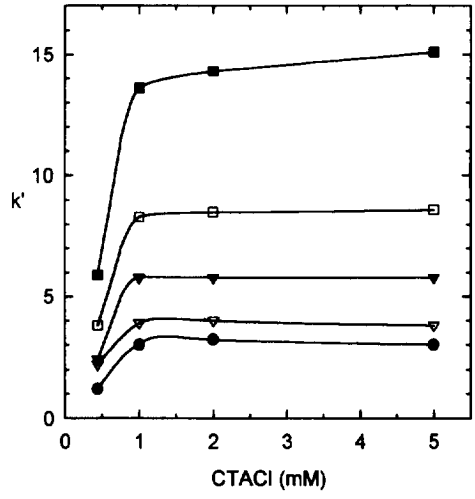

Fig. 1. Effect of CTACl concentration in eluents which do not contain salt. ● = $1.0 \cdot 10^{-2}$ *M* propionate; ▽ = $1.0 \cdot 10^{-4}$ *M* nitrate; ▼ = $1.0 \cdot 10^{-2}$ *M* benzenesulphonate; □ = $1.0 \cdot 10^{-2}$ *M* toluene-4-sulphonate; ■ = $1.0 \cdot 10^{-4}$ *M* naphthalene-1-sulphonate. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250 × 4 mm I.D.); eluent composition, CH_3OH–water (65:35, v/v) and CTACl as shown.

no significant retention difference occurs for higher concentrations of CTACl in the eluent. Moreover, the different hydrophobicities of the analytes are reflected in the different capacity factors shown at every CTACl concentration.

Fig. 2 shows the effect of IIR concentration on the capacity factor, at 80 m*M* NaCl, for naphthalene-1,3,6-trisulphonate, naphthalene-1,5-disulphonate, naphthalene-1-sulphonate, naphthalene and benzyldimetyl hexadecylammonium. The retention behaviour differs considerably as a function of analyte charge. The capacity factor of benzyldimethylhexadecylammonium cation decreases when the concentration of the IIR increases, according to the electrostatic repulsion. For the same reason, the capacity factor of naphthalene, which has no electrostatic interaction, is unaffected by the concentration of the IIR. The curves for mono-, di- and tri-charge naphthalene show a different and increasing slope, as predicted by the ion interaction mechanism. It is clear (Fig. 2) that a separation of neutral and charged compounds requires particular attention to the selection of a suitable concentration of CTACl in order to avoid peak overlap.

Fig. 3 shows the retention behaviour of analytes as a function of salt concentration in the eluent. The capacity factors of benzene are not affected by sodium chloride concentration owing to the uncharged nature of the analyte, while ionic strength does not play a significant role in the liquid–liquid partitioning for the range of salt concentrations investigated. In contrast, the trends for charged analytes are opposite to those described above for the study of the effect of CTACl. The capacity factors of anionic compounds (e.g., benzenesulphonate) decrease when the concentration of chloride, acting as a competing ion, increases. On the other hand, the capacity factors of hydrophobic cation species increase with increase in concentration of chloride ions. In this case the chloride ion acts as a counter ion.

The study also involved the evaluation of the chromatographic behaviour of anions usually occurring in natural samples (e.g., nitrate, propionate and oxalate) in order to avoid peak

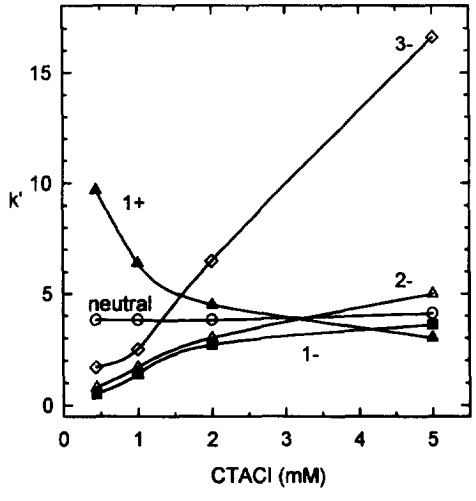

Fig. 2. Effect of CTACl concentration in the eluent on the capacity factors (k') of (◇) $1.0 \cdot 10^{-4}$ *M* naphthalene-1,3,6-trisulphonate (△) $1.0 \cdot 10^{-4}$ *M* naphthalene-1,5-disulphonate, (■) $1.0 \cdot 10^{-4}$ *M* naphthalene-1-sulphonate, (○) $1.0 \cdot 10^{-4}$ *M* naphthalene and (▲) $1.0 \cdot 10^{-2}$ *M* benzyldimethylhexadecylammonium. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250 × 4 mm I.D.); eluent composition, CH_3OH–water (65:35, v/v) containing 80 m*M* NaCl and CTACl as shown.

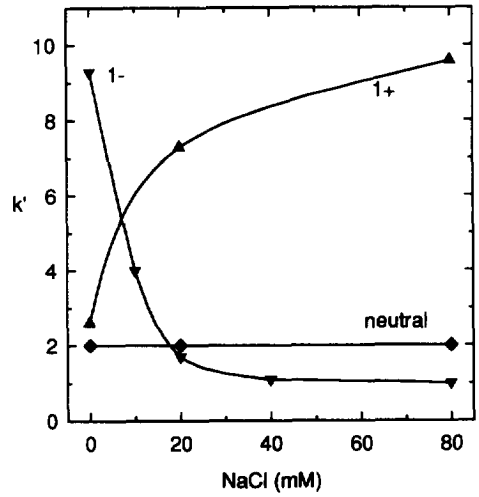

Fig. 3. Effect of sodium chloride concentration in the eluent on the capacity factors (k') of (▼) $1.0 \cdot 10^{-4}$ *M* benzenesulphonate, (◆) $1.0 \cdot 10^{-2}$ *M* benzene and (▲) $1.0 \cdot 10^{-2}$ *M* benzyldimethylhexadecylammonium. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250 × 4 mm I.D.); eluent composition, CH_3OH–water (60:40, v/v) containing 1.0 m*M* CTACl and NaCl as shown.

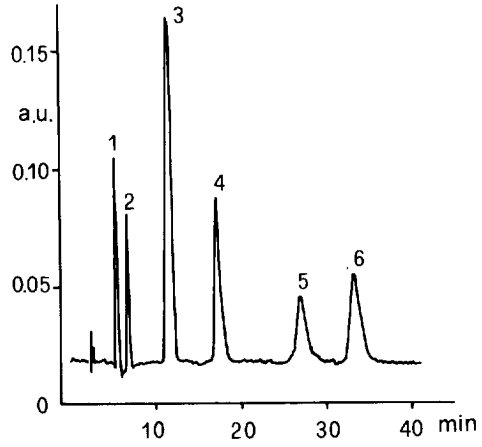

Fig. 4. Isocratic separation of analytes with negative, neutral and positive charges in the same chromatographic run: (1) $6.0 \cdot 10^{-3}$ *M* propionate; (2) $1.0 \cdot 10^{-5}$ *M* nitrate; (3) $2.0 \cdot 10^{-3}$ *M* benzene; (4) $1.0 \cdot 10^{-4}$ *M* toluene-4-sulphonate; (5) $6.0 \cdot 10^{-3}$ *M* benzyldimethylhexadecylammonium; (6) $2.0 \cdot 10^{-5}$ *M* naphthalene. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250×4 mm I.D.); eluent composition, CH_3OH–water (55:45, v/v) containing 2.0 m*M* CTACl and 80 m*M* NaCl.

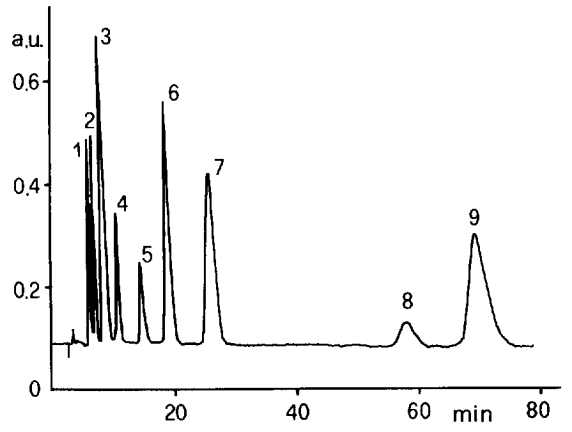

Fig. 5. Isocratic separation of (1) $1.0 \cdot 10^{-3}$ *M* oxalate, (2) $5.0 \cdot 10^{-5}$ *M* nitrate, (3) $1.0 \cdot 10^{-2}$ *M* benzene, (4) $1.0 \cdot 10^{-4}$ *M* benzenesulphonate, (5) $1.5 \cdot 10^{-4}$ *M* toluene-4-sulphonate, (6) $1.0 \cdot 10^{-4}$ *M* naphthalene, (7) $1.0 \cdot 10^{-4}$ *M* naphthalene-1-sulphonate, (8) $1.0 \cdot 10^{-4}$ *M* benzene-1,3-disulphonate and (9) $1.0 \cdot 10^{-4}$ *M* naphthalene-1,5-disulphonate. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250×4 mm I.D.); eluent composition, CH_3OH–water (60:40, v/v) containing 2.0 m*M* CTACl and 20 m*M* NaCl.

overlap during the analysis of sulphonic acids. As shown in Fig. 4, in the same chromatographic run, the separation of anionic, neutral and cationic analytes was therefore possible by selecting a suitable eluent composition. Fig. 5 shows an example of the isocratic separation of benzene, naphthalene, oxalate, nitrate and several sulphonate derivatives after the eluent optimization. Well resolved peaks are also obtained for disulphonate compounds and naphthalene-1,3,6-trisulphonate but, for analytical purposes, the separation is too time consuming and a gradient separation was required.

From the above results, the most efficient parameter to be manipulated in order to obtain a suitable separation is NaCl concentration. Fig. 6 shows the simultaneous elution of neutral, mono-, di- and tri-charged analytes with a gradient of salt. The concentration of NaCl was increased linearly from 20 to 80 m*M* between elution times of 18 and 25 min and then kept constant until the elution of all the species. The advantage of such a method is that it allows a very short equilibration time (a few minutes) after elution, while

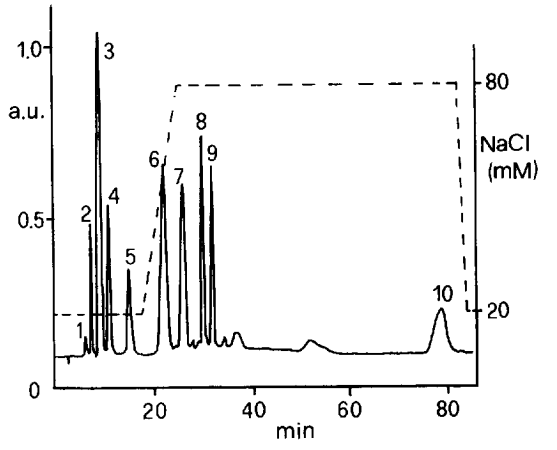

Fig. 6. Gradient separation of (1) $1.0 \cdot 10^{-4}$ *M* oxalate, (2) $3.6 \cdot 10^{-5}$ *M* nitrate, (3) $1.8 \cdot 10^{-2}$ *M* benzene, (4) $7.1 \cdot 10^{-5}$ *M* benzenesulphonate, (5) $3.0 \cdot 10^{-4}$ *M* toluene-4-sulphonate, (6) $3.6 \cdot 10^{-5}$ *M* naphthalene, (7) $3.6 \cdot 10^{-5}$ *M* naphthalene-1-sulphonate, (8) $1.8 \cdot 10^{-5}$ *M* benzene-1,3-disulphonate, (9) $1.8 \cdot 10^{-5}$ *M* naphthalene-1,5-disulphonate and (10) $7.1 \cdot 10^{-5}$ *M* naphthalene-1,3,6-trisulphonate. Sample loop, 100 μl; column, LiChrospher 100 CH-8, 10 μm (250×4 mm I.D.); eluent composition, CH_3OH–water (60:40, v/v) containing 2.0 m*M* CTACl and gradient of NaCl as shown.

longer times would be required if either the methanol percentage or the concentration of the IIR was varied during the gradient. In addition, the gradient of sodium chloride does not give appreciable baseline drifting.

3.3. Preconcentration

After the optimization of the separation procedure, an on-line preconcentration method, also based on the ion interaction mechanism, was developed and both benzene and naphthalene mono- and disulphonate compounds were chosen to evaluate its suitability. In this case the 100-μl injection loop was replaced with a LiChrospher RP-18 microcolumn and both the percentage recovery and enrichment efficiency were evaluated. Peak areas obtained by direct injection of samples (100-μl loop, analyte concentrations ten times the detection limit) were compared with those obtained by loading the preconcentration microcolumn with 50.0 ml of the sample after its dilution (1:500). With such a procedure the same nominal amount of analyte was loaded and considered for the computation. Table 2 shows the preconcentration recoveries obtained by the different procedures developed (see below). Experiments were performed by cleaning the microcolumn with 20.0 ml of methanol followed by 5.0 ml of water before every sample loading or by loading the sample into the microcolumn preliminarily washed and conditioned with the eluent; better efficiency was obtained with the last procedure. Microcolumn cleaning does not appreciably affect the recovery of naphthalene but the absence on the stationary phase of cetyltrimethylammonium resulted in a reduced recovery for mono-charged compounds, and di-charged compounds were totally unretained. The higher analyte recoveries obtained without cleaning the microcolumn are attributed to the sorption of cetyltrimethylammonium during conditioning with the eluent.

The behaviour of monosulphonic compounds is significant; the preconcentration recoveries follows the same order at the molecule hydrophobicity, naphthalene-1-sulphonate > toluene-4-sulphonate > benzenesulphonate, when the microcolumn is washed (Table 2, column 1), but the maximum values are reached when the microcolumn is loaded with the eluent (Table 2, column 2) and ion pairs have originated. Based on these results, experiments were also performed by adding the ion-pair reagent to the sample. This procedure resulted in a decrease in the recoveries (Table 2), probably owing to the formation of micelles which are not retained. Such a hypothesis is supported by the value of the critical micelle formation for cetyltrimethylammonium in aqueous solution at 25°C (critical

Table 2
Preconcentration recovery (%) as a function of cetyltrimethylammonium (CTA) concentration added to the sample

Analyte	Recovery (%)[a]			
	No CTA[b]	No CTA[c]	1.0 m*M* CTA[c]	5.0 m*M* CTA[c]
Benzene	8 ± 1	5 ± 2	0	0
Naphthalene	74 ± 4	78 ± 7	76 ± 9	0
Benzenesulphonic acid	8 ± 2	86 ± 6	0	0
Toluene-4-sulphonic acid	32 ± 2	85 ± 2	89 ± 11	0
Naphthalene-1-sulphonic acid	73 ± 4	75 ± 4	77 ± 5	0
Benzene-1,3-disulphonic acid	0	82 ± 2	60 ± 5	17 ± 2
Naphthalene-1,5-disulphonic acid	0	82 ± 3	30 ± 6	6 ± 1
Naphthalene-1,3,6-trisulphonic acid	0	5 ± 2	25 ± 4	5 ± 2

[a] $n = 3$.
[b] Preconcentration microcolumn precleaned with 20.0 ml of CH_3OH and washed with 5.0 ml of H_2O.
[c] Preconcentration microcolumn preconditioned with eluent.

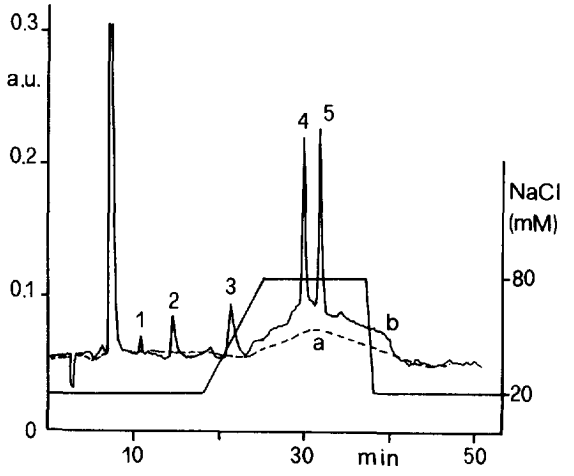

Fig. 7. On-line preconcentration followed by gradient separation of some aromatic neutral and charged compounds in tap water. Sample filtered through a 0.45-μm filter; 50.0-ml sample loading. (a) Tap water alone; (b) tap water spiked with (1) $1.0 \cdot 10^{-7}$ *M* (16 μg/l) benzenesulphonate, (2) $1.0 \cdot 10^{-7}$ *M* (17 μg/l) toluene-4-sulphonate, (3) $1.0 \cdot 10^{-8}$ *M* (1.3 μg/l) naphthalene, (4) $1.0 \cdot 10^{-7}$ *M* (24 μg/l) benzene-1,3-disulphonate and (5) $4.0 \cdot 10^{-8}$ *M* (11 μg/l) naphthalene-1,5-disulphonate. Preconcentrator, LiChrospher 100 RP-18, 5 μm (4×4 mm I.D.); column, LiChrospher 100 CH-8, 10 μm (250×4 mm I.D.); eluent composition, CH_3OH–water (60:40, v/v) containing 2.0 m*M* CTACl and gradient of NaCl as shown.

micelle concentration = 1.3 m*M*) [21]. Experiments performed in order to evaluate the maximum loading flow-rate showed 4.0 ml/min to be the best compromise for a good recovery and a low counter-pressure of the microcolumn.

From the experiments performed, it follows that the method can be applied, with the best recoveries (×400 preconcentration factor), by loading the filtered sample directly, avoiding manipulation and risk of sample contamination due to the addition of CTACl. In addition, on-line preconcentration gave no blank signals for the analytes investigated.

Fig. 7 shows the analysis of a tap water sample, after preconcentration, spiked with aromatic neutral and charged compounds at the μg/l level. The narrow peak due to the nitrate ion naturally occurring in tap water and the broad baseline hump due to the gradient (Fig. 7a) do not interfere in the determination, as shown in Fig. 7b.

3.4. Detection limits

Detection limits (three times the background signal) were evaluated for all the species (Table 3). The data also show the efficiency of on-line

Table 3
Detection limits achieved either with a 100-μl sample loop (direct injection) or with a 50.0-ml loading (on-line preconcentration)

Analyte	Detection limits[a]	
	Direct injection[b] (mg/l)	Preconcentration[c] (μg/l)
Benzene	2	–
Naphthalene	0.09	0.3
Benzenesulphonic acid	3	8
Toluene-4-sulphonic acid	1	3
Naphthalene-1-sulphonic acid	0.4	2
Benzene-1,3-disulphonic acid	0.2	0.9
Naphthalene-1,5-disulphonic acid	0.2	0.6
Naphthalene-1,3,6-trisulphonic acid	4	–

Tap water has been considered as reference matrix. Values calculated as three times the background signal.
[a] $n = 3$.
[b] Sample volume = 100 μl.
[c] Sample volume = 50.0 ml.

preconcentration procedures which permit the detection of sub-ppb concentrations. It must be noted that detection limits refer to UV absorption at 210 nm, useful for determinations of a wide range of analytes, but they could be further lowered with a different detection method, e.g. fluorescence detection.

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