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Journal of Chromatography A, 671 (1994) 217–223

JOURNAL OF CHROMATOGRAPHY A

Determination of aromatic sulphonates in surface waters by high-performance liquid chromatography with coupled fluorescence and UV detection

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Abstract

An HPLC procedure for determining anthraquinonesulphonates in the presence of both benzene- and naphthalensulphonates in river water was developed. Enrichment of analytes was accomplished by means of solid-phase extraction, based on an RP-18 reversed-phase silica cartridge coated with an aliphatic amine. The analytes were separated by ion-pair chromatography with coupled fluorescence and UV detection, which resulted in a convenient way to discriminate derivatives of benzene and of naphthalene from those of anthraquinone. The peaks of anthraquinonesulphonates were identified by measuring the ratio of their absorbances at two different wavelengths. Four of the target analytes were found in water from the river Bormida (Italy).

1. Introduction

Aromatic sulphonates have been found in some European rivers (Rhine [1,2], Elba [1], Chriesbach [3] and Bormida [4]) and even in the tap water of Amsterdam [5]. These compounds are of anthropic origin, and therefore their presence in environmental and drinking water should be avoided. They are hydrophilic, stable and not readily biodegradable. Biological water purification plants have low efficiency in removing them from wastes [3]. Moreover, aromatic sulphonates can persist in the environment and can propagate easily owing to their hydrophilic characteristics. They have a wide application range in the dye, detergent and cement industries. Benzene-, naphthalene- and anthraquinonesulphonates (BZS, NS and AQS, respectively), together with their amino and hydroxy derivatives, are byproducts of the synthesis and application of dyes. Complex mixtures of these substances can pollute rivers which receive wastes from dye industries.

Analytical methods based on TLC [6], ion-exchange electrokinetic chromatography [7], ion-pair chromatography (IPC) on RP-8 or RP-18 silica [8–14], on-line enrichment on PLRP-S sorbent followed by either ion chromatography or IPC with diode-array detection [2,5], on-line enrichment on Carbopack B and subsequent IPC [3] and enrichment on a modified RP-18 silica and IPC with fluorescence detection [4] have been proposed. Preconcentration on a Dowex WGR weak anion exchanger [15] or ion-pair extraction [16] followed by derivatization and GC–MS analysis, high-performance capillary electrophoresis–MS [17] and ion chromatography–MS [18] have also been proposed. None of these studies combined a pretreatment capable of a good extraction yield for AQS

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0021-9673/94/$07.00
SSDI 0021-9673(93)E1224-N

together with a chromatographic analysis suitable for the identification of singly- and doubly-charged AQS in the presence of NS and BZS in environmental water samples.

In this work, a procedure for the preconcentration of aromatic sulphonates and an IPC method for the separation and identification by serial UV–fluorimetric detection of BZS and singly- and doubly-charged NS and AQS are described.

2. Experimental

2.1. Reagents

All aqueous solutions were prepared with ultra-high-quality (UHQ) water obtained by passing deionized water through an Elga-Stat water purification apparatus (Elga, High Wycombe, UK). Methanol and acetonitrile, both of HPLC grade with low UV absorption, and cetyltrimethylammonium bromide (CTAB) were obtained from Aldrich (Milan, Italy). The compounds listed in Table 1 were obtained from Aldrich or Kodak (Prodotti Gianni, Milan, Italy). Inorganic reagents were obtained from Carlo Erba (Milan, Italy). Solid-phase extraction (SPE) columns were obtained from Merck (Bracco, Milan, Italy).

Stock standard solutions of the investigated sulphonates were prepared with UHQ water, kept in dark-glass flasks, stored in a refrigerator (4°C) and were used within 1 week.

2.2. Apparatus

The HPLC system consisted of a Pye Unicam (Cambridge, UK) PU 4015 pump, a Rheodyne (Cotati, CA, USA) valve fitted with a 20-μl loop and a 250 × 4.6 mm I.D. Adsorbosphere C_8, (5 μm) column (Alltech, Deerfield, IL, USA). The

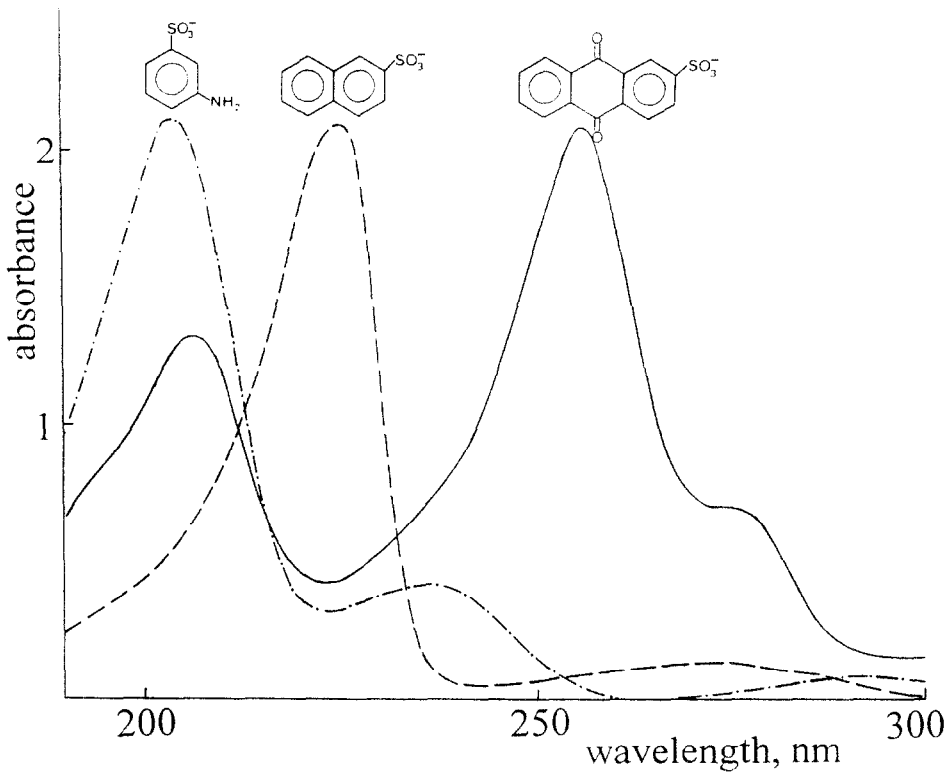

Fig. 1. UV spectra of sulphanilate, 2-naphthalenesulphonate and 2-anthraquinonesulphonate.

eluent was degassed by means of a stream of helium.

The fluorimetric detector was a Hitachi (Tokyo, Japan) F-400 computerized spectrofluorimeter, equipped with an 18-μl flow cell and an analogue output. The UV chromatograms were obtained with a Kontron Instruments (Milan, Italy) Model 430 dual-wavelength detector. A Softron (Gräfeling, Germany) PC Integration Pack was adopted for data acquisition and analysis.

3. Results

3.1. Chromatography

Detector conditions

AQS strongly absorbs UV radiation at longer wavelengths than NS and BZS. Typical UV spectra of AQS, NS and BZS are shown in Fig. 1. It can be seen that they are well differentiated; a wavelength of 258 nm can be chosen for the UV detection of AQS, because at this wavelength the intensity of the signals produced by NS and BZS is much smaller than that produced by AQS. The fluorescence detection of BZS and NS and the collection of their fluorescence spectra were performed as reported previously [10].

Optimization of chromatographic conditions

As chromatographic conditions suitable for the separation of both mono- and disulphonate anions of benzene, naphthalene and anthraquinone were needed, the four compounds mentioned in Fig. 2 were chosen to test the influence on capacity factors of pH, organic content of mobile phase and ion-pair reagent concentration. The results are shown in Fig. 2. The hold-up time was determined from the deflection of the baseline that followed injection. Unless specified other-

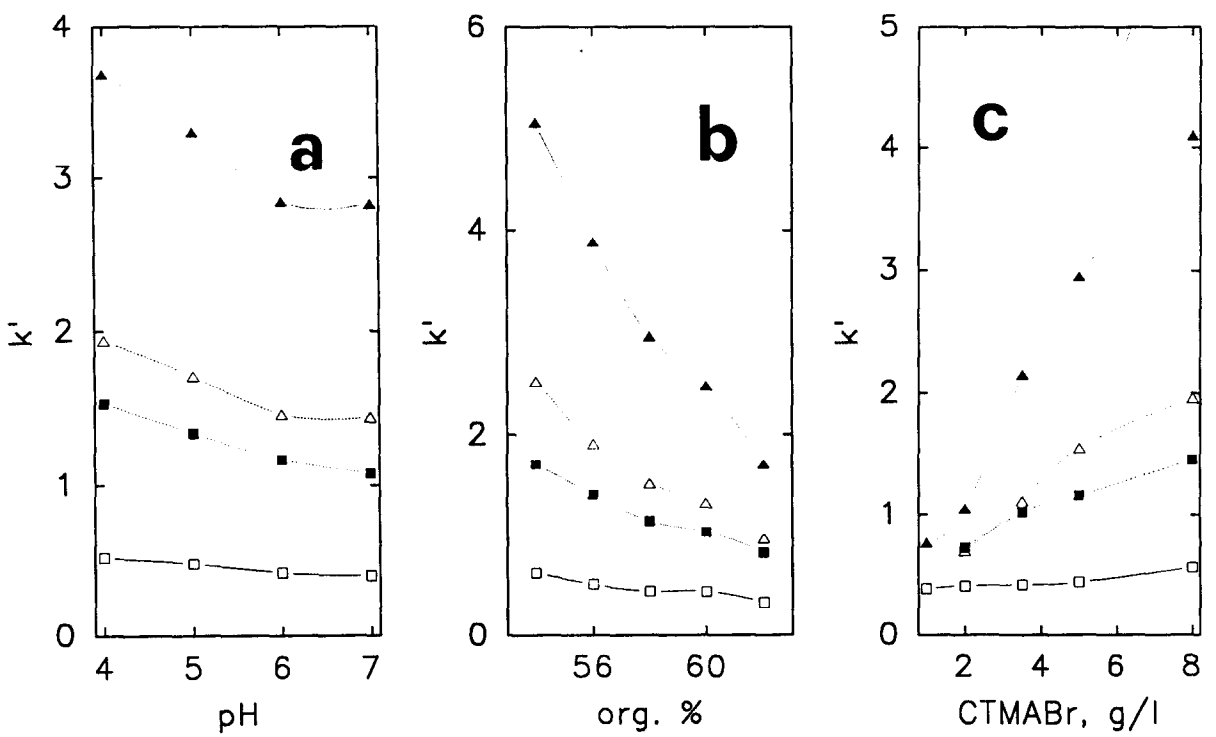

Fig. 2. Effects on k' of (a) pH, (b) organic content and (c) ion-pair reagent concentration. □ = Sulphanilate; ■ = 1-anthraquinonesulphonate; △ = 1,5-anthraquinonesulphonate; ▲ = 2-hydroxy-3,6-naphthalenesulphonate.

wise, the composition of the mobile phase was 1 g l^{-1} of ammonium phosphate (pH 7.0), acetonitrile–water (58:42) and 5 g l^{-1} of CTAB.

The same eluent composition (with a flow-rate of 1 ml min^{-1}) was adopted for the analysis of real samples, as an acidic pH produced poor peak shapes for most of the investigated compounds and both the organic solvent content and the ion-pair reagent concentration adopted gave an acceptable resolution with a short analysis time. Gradient elution was also tried, but longer times were required, as column conditioning was needed after each analysis. Isocratic conditions were preferred, with the additional advantage of obtaining more reproducible retention times.

Chromatograms

The chromatograms of standard mixtures of fluorescent and non-fluorescent aromatic sulphonates are shown in Fig. 3. The absorbance of NS and BZS at 258 nm is much lower than that of AQS, and consequently it was observed that the UV peaks of NS and BZS became comparable in height to those of AQS only if the concentrations of the substances belonging to the former two groups were from one to two orders of magnitude larger than those of AQS. The fluorescent analytes contained in the standard solution employed for running the chromatogram showed in Fig. 3c gave a UV signal that was not distinguishable from the baseline. This was due both to the higher sensitivity of fluorimetric compared with UV detection (which allowed the use of more dilute standards) and to the lower molar absorptivity (at 258 nm) of NS and BZS compared with that of AQS.

Table 1 gives the retention times of the investigated substances, together with the ratio between the absorbances measured at 258 and 275 nm. Those ratios, together with retention times, were used for the identification of non-fluorescent UV absorbers.

Quantitative analysis was based on peak-height measurements. For real samples, standard additions of the identified substances were necessary in order to determine their concentrations.

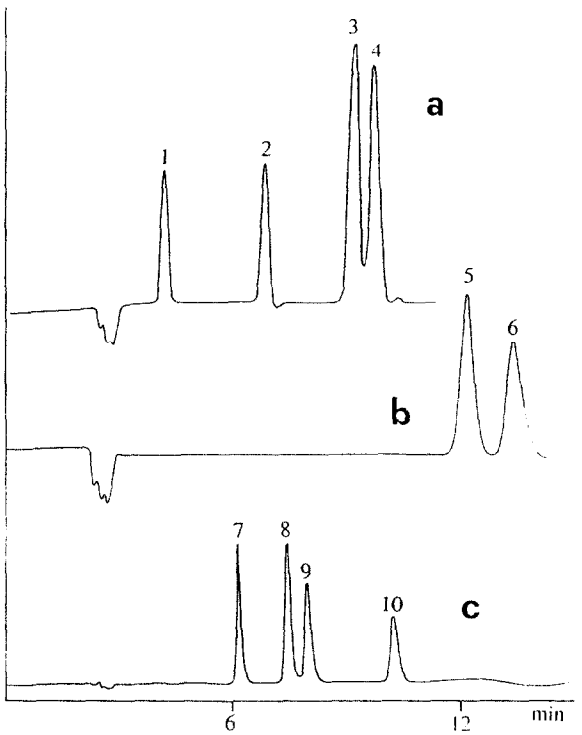

Fig. 3. Chromatograms of a standard mixture. NS = naphthalenesulphonate; NDS = naphthalenedisulphonate; AQS = anthraquinonesulphonate; AQDS = anthraquinonedisulphonate. (a) Fluorescence detection λ_{ex} = 240 nm, λ_{em} = 660 nm; (b) fluorescence detection, λ_{ex} = 250 nm, λ_{em} = 455 nm; (c) UV detection (258 nm). Peaks: 1 = sulphanilate, 0.25 mg l^{-1}; 2 = 2-NS, 0.25 mg l^{-1}; 3 = 2,7-NS, 0.5 mg l^{-1}; 4 = 1,5-NS, 1 mg l^{-1}; 5 = 2-hydroxy-6,8-NDS, 0.1 mg l^{-1}; 6 = 2-hydroxy-3,6-NDS, 0.1 mg l^{-1}; 7 = 1-AQS, 10 mg l^{-1}; 8 = 1,5-AQDS, 30 mg l^{-1}; 9 = 2-AQS, 10 mg l^{-1}; 10 = 2,6-AQDS, 10 mg l^{-1}.

3.2. Sample pretreatment

As the real samples contained non-ionic interferents, a clean-up procedure capable of removing them was required. Therefore, the real samples were eluted through an RP-18 SPE cartridge; no effect on the concentrations of standard solutions of sulphonates was observed.

Preconcentration of the analytes was also needed. An SPE procedure based on RP-18 silica coated with CTAB by absorbing it from a 0.4% aqueous solution [4], already employed for

Table 1
Chromatography of aromatic sulphonates

Substance[a]	Retention time (min)	Detection[b]	Absorbance ratio[c]
1-AQS	6.20	3	3.7 ± 0.1
2-AQS	7.56	3	3.7 ± 0.1
1,5-AQDS	8.05	3	6.7 ± 0.1
2,6-AQDS	10.31	3	3.1 ± 0.1
2-NS	6.73	1	1.0 ± 0.5[d]
2,7-NDS	9.10	1	1.0 ± 0.5[d]
1,5-NDS	9.60	1	0.3 ± 0.2[d]
2-OH-6,8-NDS	12.10	2	1.0 ± 0.7[d]
2-OH-3,6-NDS	13.30	2	1.0 ± 0.8[d]
Sulphanilate	4.08	1	1.2 ± 1[d]

[a] AQS = anthraquinonesulphonate; AQDS = anthraquinonedisulphonate; NS = naphthalenesulphonate; NDS = naphthalenedisulphonate.
[b] 1 = fluorescence detection, $\lambda_{ex} = 240$, $\lambda_{em} = 660$ nm; 2 = fluorescence detection, $\lambda_{ex} = 250$, $\lambda_{em} = 455$ nm; 3 = UV detection (258 nm).
[c] Absorbance at 258 nm/absorbance at 275 nm ± absolute error.
[d] Molar absorptivity <500.

the analysis of BZS and NS, was tried also for AQS, but poor recoveries were obtained. Standard solutions of AQS were analysed after eluting them through the treated SPE cartridges, and the amount of eluted AQS was found to be undetectable, showing that the stationary phase effectively retained these compounds. Nevertheless, elution with methanol did not recover significant amounts of adsorbed AQS, although BZS and NS were almost quantitatively recovered under the same conditions. On the other hand, the chromatographic order of elution of AQSs and NSs indicated that some NSs were retained on an apparently similar stationary phase even longer than were AQSs, but nevertheless the SPE recoveries of NSs were higher. It must be noted that CTAB and AQS were adsorbed on the solid phase for SPE under different conditions to the chromatographic ones, and that SPE cartridges were dried with an air flow after adsorption of analytes; these reasons can probably explain the different behaviour of AQS in chromatography and SPE. Therefore, a different anion exchanger, consisting of an RP-18 SPE silica cartridge coated with an aliphatic amine, was tried. A 400-mg RP-18 SPE cartridge was loaded by eluting 2 ml of an aqueous solution of 0.01 *M* octyldimethylammonium acetate adjusted to pH 4. The analytes were desorbed by 2 ml of methanol. High recoveries were obtained for all the investigated substances, except sulphanilate, which was only partially retained. The extraction yields, together with the detection limits resulting after tenfold preconcentration of the samples, are reported in Table 2.

The influences of pH and inorganic salt concentrations on the recoveries from standard solutions were investigated. A 30 g l^{-1} concentration of NaCl did not significantly influence the recoveries of AQS, whereas it halved the recoveries of NS; in contrast, 2 g l^{-1} of Na_2SO_4 did not significantly alter the recoveries of NS, whereas it reduced slightly (by 5–10%) those of AQS. A slight increase in hydrogen ion concentration (pH 4) had no observable effect.

A disadvantage of this stationary phase was the limited ion-exchange capacity, (*ca.* 1 μequiv. g^{-1}). A 400-mg cartridge was saturated with an equimolar mixture of 50 μg of the four AQSs listed in Table 2. The breakthrough volume (10 mg l^{-1} solutions) ranged from 5 to 11 ml for

Table 2
SPE of aromatic sulphonates

Substance[a]	Mean recovery ± S.D. ($n = 3$) (%)	Detection limit ($\mu g\ l^{-1}$)[b]
1-AQS	80 ± 5	20
2-AQS	80 ± 7	60
1,5-AQDS	81 ± 3	20
2,6-AQDS	81 ± 6	40
2-NS	113 ± 13	1
2,7-NDS	118 ± 16	2
1,5-NDS	111 ± 20	20
2-OH-6,8-NDS	86 ± 6	1
2-OH-3,6-NDS	89 ± 11	2
Sulphanilate	10 ± 10	200

[a] Abbreviations as in Table 1.
[b] Detection limit as measured after preconcentration (signal-to-noise ratio = 3, preconcentration factor = 10).

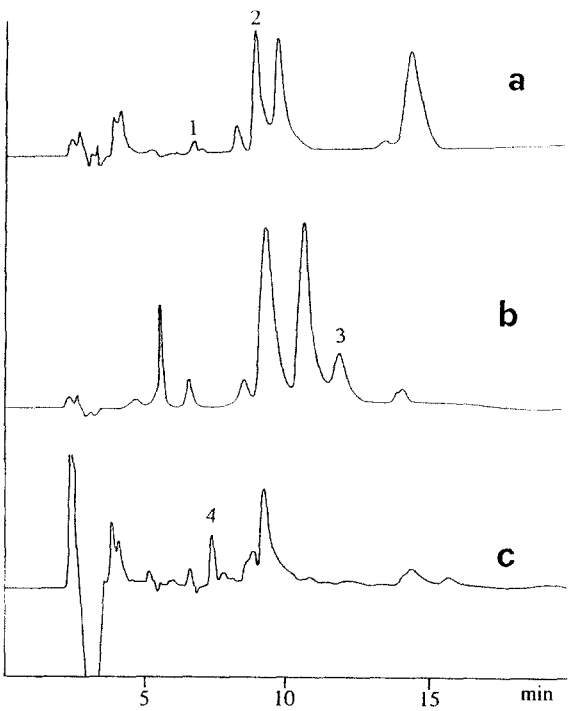

Fig. 4. Chromatograms of a water sample. (a) Fluorescence detection, $\lambda_{ex} = 240$ nm, $\lambda_{em} = 660$ nm; (b) fluorescence detection, $\lambda_{ex} = 250$ nm, $\lambda_{em} = 455$ nm; (c) UV detection (258 nm). Peaks: 1 = 2-NS, 0.32 mg l^{-1}; 2 = 2,7-NS, 3.46 mg l^{-1}; 3 = 2-hydroxy-6,8-NDS, 0.65 ml l^{-1}; 4 = 1,5-AQDS, 1.1 mg l^{-1}.

AQSs and NSs, whereas it was 0.5 ml for sulphanilate.

3.3. Analysis of river water

The river water, after being used by a factory in the production of dyes, is returned to the river. Four samples of that returned water were treated as described and then analysed. The chromatogram obtained for one of those samples is shown in Fig. 4. Four of the target analytes were found to be present. Their concentrations in the four samples ranged between 0.32 and 3.52 mg l^{-1}.

The identity of the naphthalenesulphonates was ascertained by comparing their fluorescence spectra with those of standards stored in a library, as reported previously [4]. The identity of the 1,5-anthraquinonesulphonate was confirmed by its absorbance ratio: a volume of 6.7 ± 0.1 was found, in good agreement with the expected value. The retention times of the remaining UV peaks did not coincide with any of the examined AQSs.

4. Conclusions

An HPLC procedure for the analysis of anthraquinonesulphonates in the presence of naphthalene- and benzenesulphonates in river water has been described. Sample clean-up and SPE preconcentration of the analytes were optimized. This method was successfully applied to the analysis of samples of polluted river water.

5. References

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