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Effects of ion-pairing reagents on the electrospray signal suppression of sulphonated dyes and intermediates

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High-performance liquid chromatography/mass spectrometry (HPLC/MS) analysis of anionic species such as sulphonic acid dyes and intermediates requires volatile ion-pairing mobile phase additives. Six di- and trialkylammonium acetates were compared with tetraalkylammonium salts and ammonium acetate in the concentration range 0–20 mmol l^{-1} as mobile phase additives for HPLC/MS of polysulphonated compounds. The effects of the structure and concentration of the ion-pairing reagents on the electrospray response of mono-, di- and tetrasulphonic aromatic acids and acid dyes were studied in detail. Further, five different mass analysers and instrument geometries were compared. A higher signal decrease is observed with linear geometry instruments in comparison to orthogonal or even Z-spray geometry mass spectrometers. The concentration of mobile phase additives has a significant influence on the abundance ratios of multiply charged ions in the mass spectra of polysulphonated compounds. The competing ions of sulphonic acids may also cause significant signal suppression. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: electrospray ionization; liquid chromatography/mass spectrometry; ion suppression; sulphonic acids; dyes

INTRODUCTION

High-performance liquid chromatography/mass spectrometry (HPLC/MS) with atmospheric pressure ionization techniques is routinely applied for the analysis of various organic and organometallic compounds with wide ranges of polarities and molecular masses.[1–3] From among the available ionization techniques, electrospray ionization (ESI) in the negative-ion mode is the most suitable for (poly)sulphonic acids.[4–17] Negative-ion atmospheric pressure chemical ionization (APCI) can be also applied for the MS of some mono- and disulphonic acids,[4–7] but the sensitivity is usually worse than with the ESI technique. Negative-ion thermospray ionization[4,18] was used in early work before the introduction of ESI. Matrix-assisted laser desorption/ionization (MALDI) has also been used for the analysis of sulphonated azo dyes[8–10] and phthalocyanines.[9]

Many ionic compounds are weakly retained, if at all, in reversed-phase HPLC with common aqueous–organic mobile phases without ionic additives. Two approaches can be used to increase the retention and improve the separation selectivity of completely ionized polysulphonic acids:[19,20] (a) 'salting-out' chromatography with mobile phases containing inorganic salts, e.g. 0.1–1 mol l^{-1} sodium sulphate, which, however, is not compatible with MS detection; (b) ion-pairing chromatography with alkylammonium ionic additives in aqueous–organic mobile phases. Non-volatile tetraalkylammonium salts generally provide the best chromatographic selectivity, but are incompatible with MS detection. More volatile di- and trialkylammonium acetates or formates as ion-pairing reagents are more suitable for the HPLC/MS analysis of polysulphonated dyes and dyestuffs intermediates,[11–13] as they offer a good compromise between chromatographic retention and selectivity on the one hand and the electrospray response on the other. Addition of 5–10 mmol l^{-1} ammonium acetate to aqueous–organic mobile phases with a high water content can yield satisfactory separations of some mono- and disulphonic acids[5,11] and other anionic dyes,[14] but often fails to provide sufficient retention and separation selectivity for compounds with more than two sulphonic acid groups or for complex dyes with several polar and ionic functional groups.

It is well known that ionic species (e.g. ion-pairing reagents, inorganic salts and other competing ions) in the electrosprayed liquid can influence the signal of the target compound. The signal suppression effects in ESI mass spectrometry have recently attracted considerable

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attention.[12,21–24] For example, the relative ESI responses of eight amines were measured in the presence of fluorinated carboxylic acids and compared with the signal intensity when using only formic acid–ammonium formate buffer.[21] The signal dropped to 45–60% with trifluoroacetic acid and to 15–30% with heptafluorobutanoic or perfluoroheptanoic acids. Gangl *et al*.[24] suggested the use of a nanosplitting device with high splitting ratios (2000 : 1) to reduce the signal suppression effects in mobile phases with ionic additives and to improve the mass sensitivity. Nanoelectrospray is less sensitive to the concentration of non-volatile salts in the electrosprayed liquid, because the initial droplets have a smaller diameter, and therefore less Coulombic explosions are needed for ion evaporation than in conventional ESI.[25] Socher *et al*.[6] introduced a promising approach for HPLC/MS of sulphonic acids based on using conventional ion-pairing HPLC with 30 mmol l^{-1} tetrabutylammonium acetate in combination with a cation-exchange suppressor cartridge placed between the chromatographic column and the mass spectrometer. However, the suppressor columns designed for aqueous ion chromatography may cause problems when used with mobile phases containing an organic solvent. They protect only the MS part of the system, but the HPLC system has to be carefully cleaned anyway after the use of alkylamines. A micro-membrane suppressor placed between a UV and a particle beam electron ionization mass spectrometer can be used instead, as described by Escott *et al*.[26] for the analysis of both cationic and anionic species with ion-pairing HPLC/MS. Ion-exchange HPLC on aminopropyl columns can also be used for the separation of sulphonic acids,[7] but very high concentrations of mobile phase additives are required (up to 120 mmol l^{-1} of ammonium acetate and 762 mmol l^{-1} of acetic acid in 65% aqueous acetonitrile), which negatively affects the ESI signal.

The total number of sulphonic acid groups in polysulphonated dyes can be determined, as described by Ballantine *et al*.[27] When diethylamine (DEA) or triethylamine is added to the electrosprayed solution, characteristic adducts of deprotonated molecules of polysulphonic acids with DEA are formed. The maximum number of DEA molecules present in the adducts is equal to the total number of sulphonic acid groups. In a similar approach,[5] the total number of sulphonic acid groups is determined using the $[M - (x + y)H + yNa]^{x-}$ ions with the highest charge or the highest number of protons replaceable by sodium ions.

Volatile ion-pairing mobile phase additives have often been used for the HPLC/MS of polysulphonic acids, but little is known about their effects on the ESI signal of the analyte ions. In the present work, we investigated the effects of various mobile phase additives, of competing ions and of the instrumental geometry on the signal suppression in the negative-ion ESI-MS of sulphonic acids. For this purpose, a robust, reproducible and fast method was developed with low instrument contamination. We studied the effects of dipropylammonium acetate (DPAA), triethylammonium acetate (TEAA), dibutylammonium acetate (DBAA), tripropylammonium acetate (TPAA), dihexylammonium acetate (DHAA), tributylammonium acetate (TBAA), tetrabutylammonium acetate (TeBAA), tetrabutylammonium hydrogensulfate (TeBAS) and ammonium acetate on the ESI signals of three test sulphonic acids: a simple aromatic monosulphonic acid (A), a substituted disulphonated naphthalene acid (B) and a complex polysulphonated commercial dye with four sulphonic acid groups (C). Finally, the effect of competing co-ions on the signal decrease was investigated.

EXPERIMENTAL

Chemicals

Acetonitrile (HPLC grade) was purchased from Baker (Deventer, The Netherlands) and acetic acid and ammonium acetate (reagent grade) from Lachema (Brno, Czech Republic). Distilled water was deionized and all solvents were filtered through a 0.22 μm Millipore filter. The samples of sulphonated dyes and intermediates were obtained from Synthesia (Pardubice, Czech Republic): naphthalene-2-sulphonic acid (2-NSA), 1-amino-8-hydroxynaphthalene-3,6-disulphonic acid (H-acid), Saturn Blue L4G (Color Index (CI) Direct Blue 78), Egacid Yellow M (CI Acid Yellow 36), Egacid Blue A2G (CI Acid Blue 40), Saturn Yellow LFF (CI Direct Yellow 28), Saturn Green L5G (CI Direct Green 28) and Rylan Bordeaux B (CI Acid Violet 90). Stock solutions of 300 mg l^{-1} naphthalene-2-sulphonic acid (A), H-acid (B) and Saturn Blue L4G (C) in 50% aqueous acetonitrile were diluted with the same eluents, yielding working solutions containing 3 mg l^{-1} of A, 30 mg l^{-1} of B or 30 mg l^{-1} of C. One of the following ion-pairing ammonium acetates was added to the working solution at appropriate concentrations during the dilution step: dipropylamine (DPAA) and dihexylamine (DHAA), both from Sigma (St. Louis, MO, USA), triethylamine (TEAA) from Cruachen (Glasgow, UK), dibutylamine (DBAA) from Janssen Chimica (Beerse, Belgium), tripropylamine (TPAA) from Aldrich (Milwaukee, WI, USA), tributylamine (TBAA) and tetrabutylammonium acetate (TeBAA) from Fluka (Buchs, Switzerland) and tetrabutylammonium hydrogensulfate (TeBAS) from Serva (Heidelberg, Germany).

Mass spectrometry

An Esquire 3000 ion trap mass analyser (Bruker Daltonics, Bremen, Germany) was used in most experiments. The mass spectra were recorded in the range m/z 50–600 in the negative-ion ESI mode. The optimization of tuning parameters had no significant effect on the distribution of particular ions, hence the mass spectrometer was tuned to give a maximum response for the test compounds, i.e. the 'target mass' tuning parameter was set to m/z 300. Samples were injected using a 10 μl sample loop directly into 50% aqueous acetonitrile delivered continuously by an infusion pump at 10 μl min^{-1} into the ion source. The ESI responses were determined using the signals of the $[M - H]^-$ ions (m/z 207) for 2-NSA, the sum of the signals of $[M - H]^-$ (m/z 318), $[M - H - HSO_3]^{-\bullet}$ (m/z 237) and $[M - 2H]^{2-}$ (m/z 158.5) ions was used for H-acid and the signals of $[M - 2H]^{2-}$ (m/z 482.5) and $[M - 3H]^{3-}$ (m/z 321.3) ions for Saturn Blue L4G.

Comparison with other geometries of mass spectrometers

Five mass spectrometers were used in the inter-laboratory comparison of the effects of the ion source and instrument geometry on the signal decrease of sulphonic acids caused by ion-pairing reagents. The experimental conditions were the same as for the Esquire 3000 analyser, except for the following parameters specific for particular instruments: a Platform quadrupole mass analyser (Micromass, UK) was used with a sample cone voltage of 30 V and a source temperature of 80 °C; an LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) with a spray voltage of −4.2 kV, a flow-rate of sheath gas of 45 arbitrary units, a capillary temperature of 200 °C and a voltage of −11.0 V; a ZQ 2000 quadrupole mass analyser (Waters, Milford, MA, USA) with a sample cone voltage of 45 V, an extraction cone voltage of 3 V, a capillary voltage of 3.5 kV, a source temperature of 100 °C and a desolvation temperature of 250 °C; and an Automass Multi quadrupole mass analyser (Thermo Finnigan) with a fragmentation voltage of −29 V and a pressure and temperature of drying gas of 4 bar and 280 °C, respectively.

Evaluation of the signal suppression effects

The eluent consisting of 50% acetonitrile–water was delivered into the electrospray ion source at a flow-rate of 10 μl min^{-1} using an infusion pump (Series 74 900, Cole Parmer, Vernon Hills, USA). First, a solution of each of the test compounds A, B or C without any additive was injected using a 10 μl sample loop. The signal should last for 1 min at a flow-rate of 10 μl min, but the signal plug was wider and tailed owing to the diffusion. Thirty scans from the top plateau were averaged and used for the calculation of the ESI-MS response, as shown in Fig. 1(a). The response for each target compound in 50% aqueous acetonitrile was evaluated as the mean value from triplicate injections. The same procedure was repeated with solutions containing individual mobile phase additives (Fig. 1(b)) and the mean signal value was related to the response in 50% aqueous acetonitrile without additives from previous triplicate injections (100%, Fig. 1(a)) to determine the relative signal decrease that can be attributed to the mobile phase additive. Thereafter, the ion source was cleaned and the procedure was repeated with another additive.

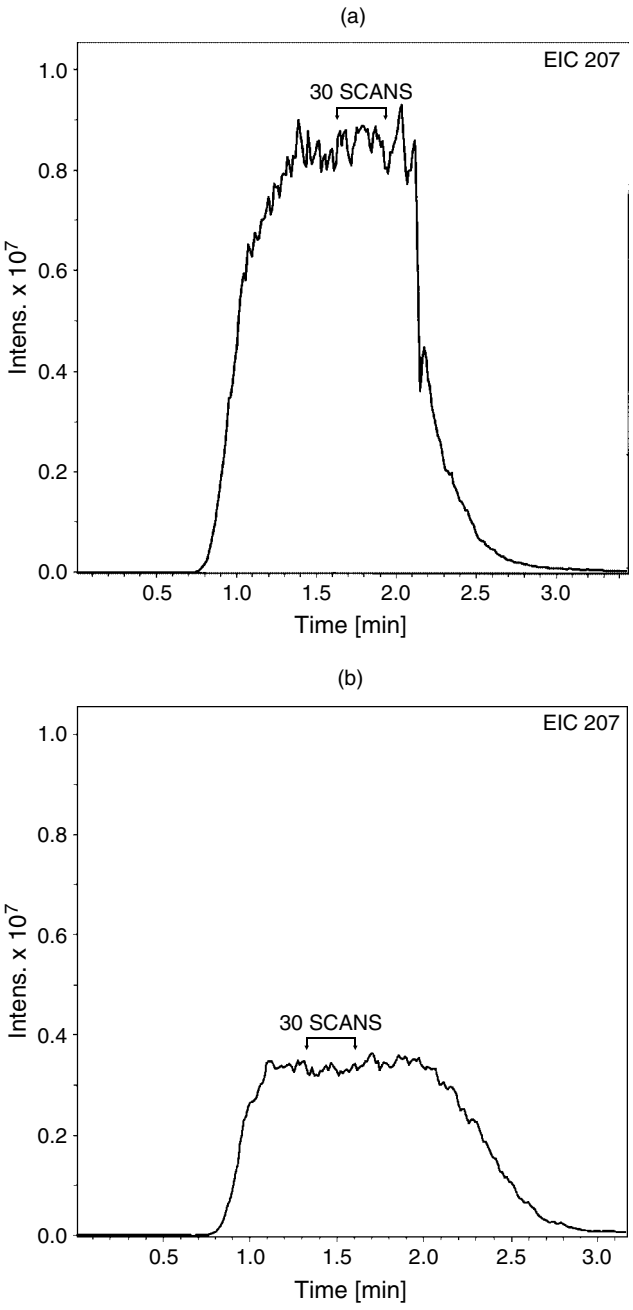

Figure 1. Extracted ion current (EIC) records of *m/z* 207 in the flow-injection analysis of naphthalene-2-sulphonic acid in 50% aqueous acetonitrile at a concentration of 3 mg l^{-1}: (a) without any additive; (b) with 2.5 mmol l^{-1} dihexylammonium acetate (DHAA).

RESULTS

Test procedure and flow-rate effects

The reproducibility of the simple procedure for the determination of ESI signal suppression described in the Experimental section and illustrated by Fig. 1 was checked by 25 injections of a sample of naphthalene-2-sulphonic acid without any additive at a flow-rate of 10 μl min^{-1}, with a relative standard deviation of 2.8% without any cleaning of the system. Hence, the method was found suitable for the investigation of suppression of the electrospray response by mobile phase additives and was used for the tests performed with five different instruments in four laboratories. The direct infusion procedure is fast, reproducible and requires little ion-pairing additives, causing only low ion source contamination. Eliminating a chromatographic column avoids problems connected with the dependence of the ESI signal on the chromatographic retention, which is governed mainly by the type of HPLC column and ion-pairing reagent used.

The effects of the flow-rate on the signal in the direct infusion approach are shown in Table 1 for the ESI signal of 2-NSA. The response generally decreases at higher flow-rates, but the decrease is relatively non-significant in the range between 10 and 100 μl min^{-1}. Increasing the flow-rate affects more significantly the response in mobile phases without

Table 1. Dependences of the total and relative response decreases of 2-NSA in 50% aqueous acetonitrile on the flow-rate and the presence of the additive

	Without additive		With 2.5 mmol l^{-1} DHAA			With 2.5 mmol l^{-1} ammonium acetate		
Flow-rate (μl min^{-1})	Total response ($\times10^{-5}$)	Relative response (%)[a]	Total response ($\times10^{-5}$)	Relative response (%)[a]	Response decrease (%)[b]	Total response ($\times10^{-5}$)	Relative response (%)[a]	Response decrease (%)[b]
10	12.45	100	3.23	100	25.9	3.7	100	29.7
100	11.26	90.4	2.99	92.6	26.6	4.39	118.6	39.0
500	7.17	57.6	2.51	77.7	35.0	3.57	96.5	49.8
1000	5.02	40.3	2.07	64.1	41.2	2.84	76.8	56.6

[a] Relative to the total response at the flow-rate of 10 μl min^{-1}.

[b] Relative to the total response at the corresponding flow-rate in the mobile phase without additives.

additives than in mobile phases with DHAA or ammonium acetate at flow rates of 500–1000 μl min^{-1}, commonly used with conventional analytical HPLC columns of 3–4 mm i.d. However, the results at a flow-rate of 10 μl min^{-1} in this work were almost identical with those at of 100 μl min^{-1}, commonly used with 1–2 mm i.d. microbore columns typically used in ion-pairing HPLC/MS, and hence provide a realistic picture of the additive effects on signal suppression under real experimental conditions.

Mobile phase and instrumental effects on the electrospray signal intensity

The ESI signal of naphthalenesulphonic acids and acid dyes depends on many factors. In addition to instrumental effects such as the flow-rate of the solution entering the ion source, the type and tuning of the mass spectrometer, the competition for charges between the individual molecules in the ion source can also affect the signal intensity. We investigated the following phenomena in a systematic way: (a) the influence of the type and concentration of ion-pairing mobile phase additives on the ESI signal; (b) the ESI signal suppression caused by another analyte with the same charge; (c) the effects of the ion-pairing reagent on the relative abundances of multiply charged ions formed during the ionization of polysulphonic acids; and (d) the effects of the instrument type and geometry on the ESI signal suppression.

Monosulphonic aromatic acids and dyes predominantly form $[M - H]^-$ ions in the negative-ion ESI mode, whereas polysulphonic acids form a series of multiply charged $[M - xH]^{x-}$ ions. The multiply charged ions can be used for the determination of the total number of sulphonic acid groups in a dye.[5] Hence we did not attempt to suppress the multiply charged ions; instead, we used the sum of the signal intensities of all the ions.

Influence of various ionic mobile phase additives on the electrospray response

Figure 2 illustrates the suppression of the electrospray response of naphthalene-2-sulphonic acid (A), H-acid (B) and

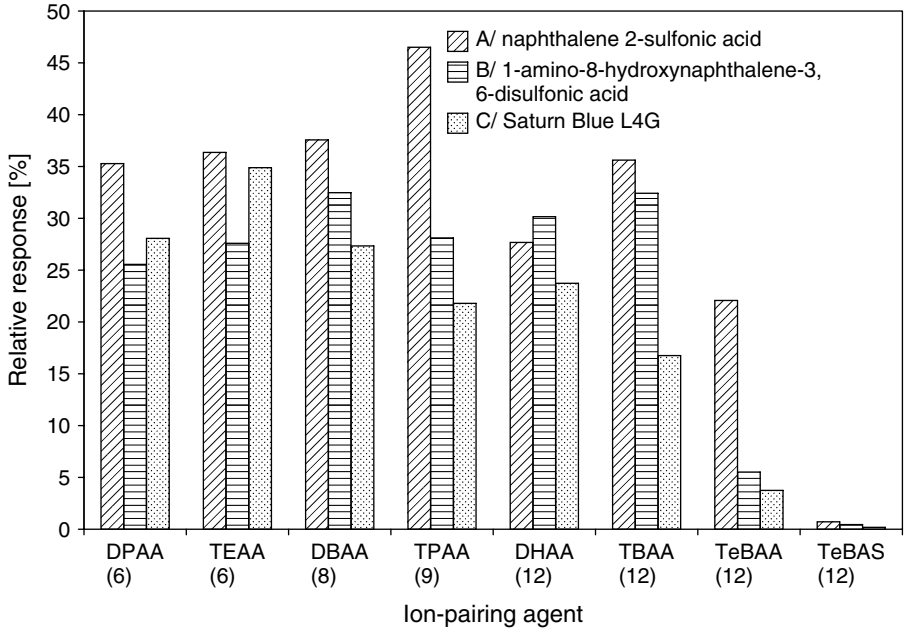

Figure 2. Relative electrospray responses of naphthalene-2-sulphonic acid (A), 1-amino-8-hydroxynaphthalene-3,6-disulphonic acid (H-acid) (B) and Saturn Blue L4G (C) in 50% aqueous acetonitrile containing 2.5 mmol l^{-1} of various ion-pairing reagents with different numbers of carbon atoms in alkylammonium cations (in parentheses). For abbreviations, see the Experimental section.

Saturn Blue L4G (C) by seven di-, tri- and tetraalkylammonium acetate ion-pairing reagents in comparison with a conventional ion-pairing reagent, tetrabutylammonium hydrogensulfate (TeBAS), which almost completely suppresses the electrospray response. More volatile di- and trialkylammonium reagents, typically used in ion-pairing HPLC/MS, decrease the signal intensity less significantly than tetrabutylammonium acetate. The signal decrease depends on the number of sulphonic acid groups in the molecule, being most significant with the commercial dye Saturn Blue L4G containing four sulphonic acid groups, for six reagents from among the eight studied (see Fig. 2). The signal intensity decrease is the least significant for monosulphonic acid with all ion-pairing reagents, except for DHAA. The signals of the other eight test mono- to tetrasulphonic acids decrease to 18–37% in a solution containing 2.5 mmol l^{-1} DHAA: naphthalene-2-sulphonic acid (decrease to 27.7%), H-acid (30.2%, two sulphonic groups), Saturn Blue L4G (23.7%, four sulphonic groups), Egacid Yellow M (31.5%, one sulphonic group), Egacid Blue A2G (37.0%, one sulphonic group), Saturn Yellow LFF (22.0%, two sulphonic groups), Saturn Green L5G (30.7%, three sulphonic groups) and Rylan Bordeaux B (17.5%, two sulphonic groups). The relative signal decrease is more significant for polysulphonic acids than for monosulphonic acids, such as Egacid Blue A2G and Egacid Yellow M, and probably depends also on the molecular mass and the presence of other functional groups.

The volatility of alkylammonium ion-pairing reagents is principally controlled by the total length of the alkyl chains. No clear correlation was found between the boiling-points of the particular ion-pairing reagents and the signal decrease. The differences among the individual di- and trialkylammonium acetates are not very significant (Fig. 2), but all these reagents provide better responses than tetraalkylammonium salts.

Other factors affecting the operation of the HPLC/MS system must be also considered. The ion-pairing reagents with longer alkyl chains provide better HPLC retention and selectivity, but on the other hand with bulkier alkylammonium ions spectral interferences are more likely to occur (e.g., m/z 102 for TEAA, 186 for DHAA or TBAA and 242 for TeBAA reagents). The spectral interferences are less critical in the lower mass range, where various mobile phase adducts are expected anyway. It was shown in our previous work[11] that DHAA provides a reasonable compromise between the HPLC selectivity and the ESI-MS performance for polysulphonated dyes. TEAA provides adequate retention and separation selectivity for many mono- and disulphonated compounds,[4,12] and it should be preferred to DHAA, where possible.

Effects of the concentrations of DHAA and of ammonium acetate on the electrospray response

The relative responses of three test compounds (A, B and C) decrease with increasing concentration of DHAA and ammonium acetate in the concentration ranges usually used for chromatographic separations (Fig. 3). A significant signal decrease is observed at low concentrations of DHAA: the relative responses of all three test compounds in 1 mmol l^{-1} DHAA decrease to less than 50% of the signal intensity

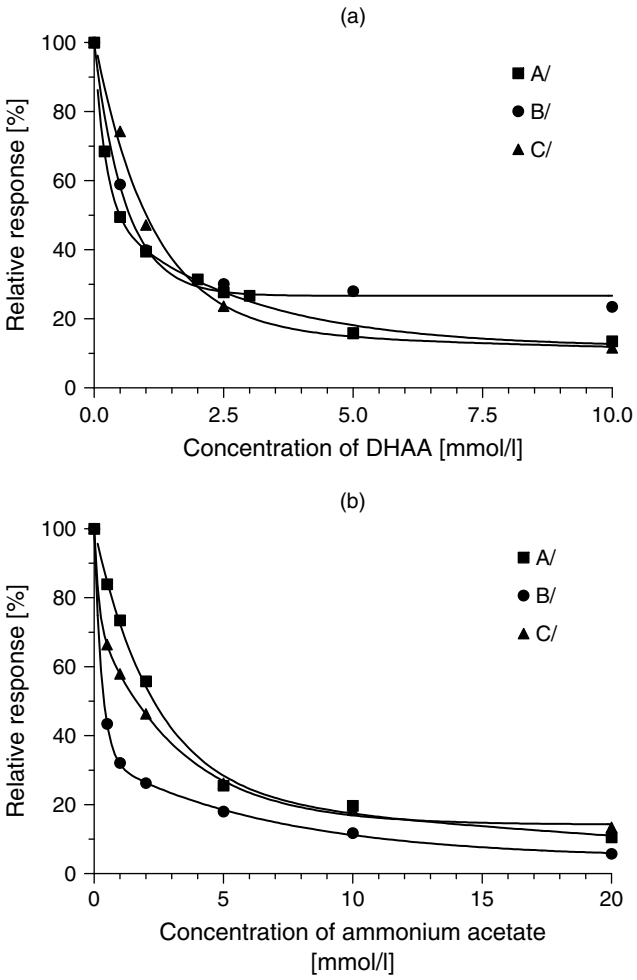

Figure 3. Dependences of the electrospray response of test compounds in 50% aqueous acetonitrile (3 mg l^{-1} of naphthalene-2-sulphonic acid (A), 30 mg l^{-1} of H-acid (B) and 30 mg l^{-1} of Saturn Blue L4G (C)) on (a) concentration of DHAA and (b) concentration of ammonium acetate.

in the mobile phase without DHAA. A similar decrease is also apparent for ammonium acetate (Fig. 3(b)), which is, surprisingly, even larger than with DHAA at concentrations of ammonium acetate higher than 5 mmol l^{-1}. Ammonium acetate is commonly considered to be one of the most suitable mobile phase additives for the HPLC/MS of ionic compounds.

Competitive ionization between two monosulphonic acids

When analysing technological samples of sulphonated dyes and sulphonic acid intermediates directly by MS, many species can be present at the same time in the ESI source and may affect the signal intensities of one another. We investigated competitive ionization effects of two monosulphonated dyes, Egacid Yellow M (M_r = 353) and Egacid Blue A2G (M_r = 451). In the first set of experiments (Fig. 4, plot A), the concentration of Egacid Yellow M as a 'target' compound in 50% aqueous acetonitrile was kept constant at 3 mg l^{-1}, and Egacid Blue A2G as an 'interference' was added to the solution at various concentration ratios (1 : 0.5, 1 : 1, 1 : 5, 1 : 10 and 1 : 50). The response of the 'target' compound without the addition

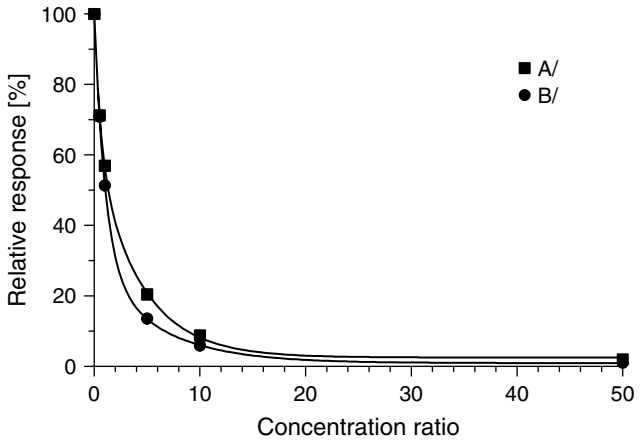

Figure 4. Competitive ionization of two monosulphonated dyes, Egacid Yellow M and Egacid Blue A2G. Concentration of the 'target' compound is 3 mg l^{-1}; the 'interfering' compound is added to 50% aqueous acetonitrile at various concentration ratios (1 : 0.5, 1 : 1, 1 : 5, 1 : 10 and 1 : 50). The target compound is Egacid Yellow M (plot A) and Egacid Blue A2G (plot B).

of 'interfering' compound was set to 100%. In the second set of experiments (Fig. 4, plot B), the 'target' and the 'interfering' compounds were interchanged. In both cases, a strong decrease in response was observed in solutions containing an excess of the 'interfering' compound. At a concentration ratio of 1 : 10, the relative responses of the 'target' compounds decrease to <10% of the original values. Hence it is often difficult to obtain meaningful ESI mass spectra of polysulphonated dyes using the direct introduction of complex samples or solutions containing high concentrations of salts into the ion source. Similar signal suppression was reported by Preisler *et al.*[28] in MALDI measurements of peptide mixtures. Most ion-pairing reagents tested decreased the ESI signal intensity less significantly than the competing sulphonic acid co-ions in the direct infusion experiments. This problem is avoided by using HPLC/MS.

Effects of ion-pairing reagent on the charge distribution of polysulphonic acids

An ion-pairing reagent influences not only the sensitivity out also the charge distribution among the series of multiply charged ions of polysulphonic acids $[M - xH]^{x-}$. The effects of DHAA and ammonium acetate on the charge distribution between $[M - 3H]^{3-}$ and $[M - 2H]^{2-}$ ions of H-acid are illustrated in Fig. 5. At increasing concentrations of DHAA (Fig. 5(a)) or ammonium acetate (Fig. 5(b)), the abundances of all ions decrease, but the $[M - 2H]^{2-}/[M - 3H]^{3-}$ ratio is changed in favour of less charged ions. Further, the addition of DHAA reduces the relative abundances of the sodiated adducts, which simplifies the interpretation of the mass spectra, but may lead to a loss of information on the total number of sulphonic acid groups which can be obtained from the ions with the highest charge or the highest number of protons replaceable by the sodium ion.[5]

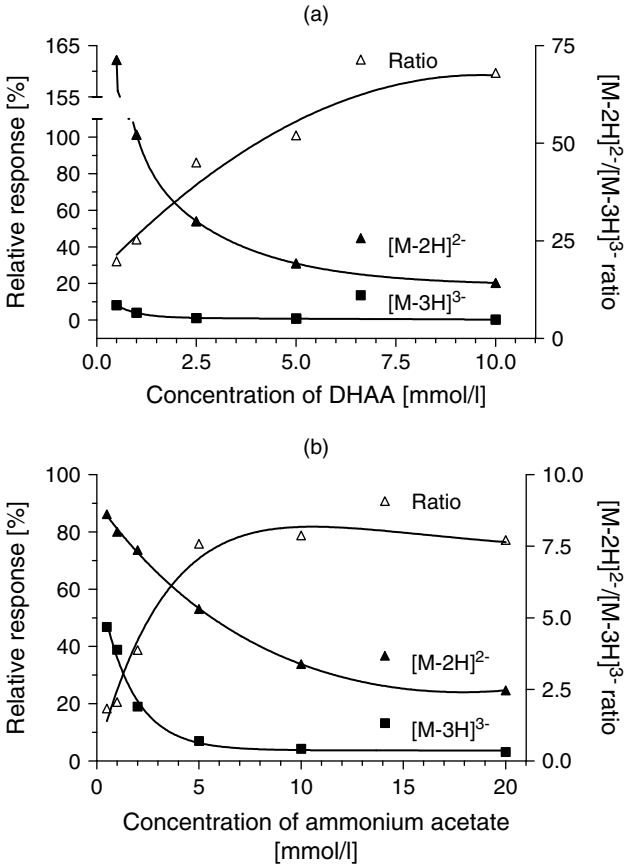

Figure 5. Dependences of the relative response decreases of $[M - 2H]^{2-}$ ions (*m/z* 482.5), $[M - 3H]^{3-}$ ions (*m/z* 321.2) and the ratio of $[M - 2H]^{2-}/[M - 3H]^{3-}$ ions of Saturn Blue L4G on the concentrations of (a) DHAA and (b) ammonium acetate.

Comparison of different ion source and instrument geometries

An orthogonal electrospray ion source geometry, with perpendicular trajectories of the ions entering the mass analyser, is considered to cause lower instrument contamination than a linear ion source geometry. The effects of five different ion sources and instrument geometries on the signal suppression in ion-pairing HPLC/MS were compared in this work. The lowest signal decrease caused by DHAA or ammonium acetate mobile phase additives was observed with the Z-spray geometry from Micromass (two 90° ion trajectories) and the relative decrease was the highest with linear geometries.

Figure 6 illustrates the influence of the ion source geometry on the relative response, which decreases in the order Z-spray (ZQ 2000) > orthogonal spray (Esquire 3000 and Automass Multi) > linear spray (LCQ and Platform). With the orthogonal geometries, only the selected polarity ions can enter the mass analyser, whereas the ions with opposite charges and neutral species do not enter the mass analyser, unlike with the direct path geometries, where all the species are directed against the sample orifice, where they can cause strong contamination and a subsequent signal decrease. On the other hand, the differences among some instruments are very low (such as with the two Thermo Finnigan instruments tested, curves 3 and 4 in Fig. 6),

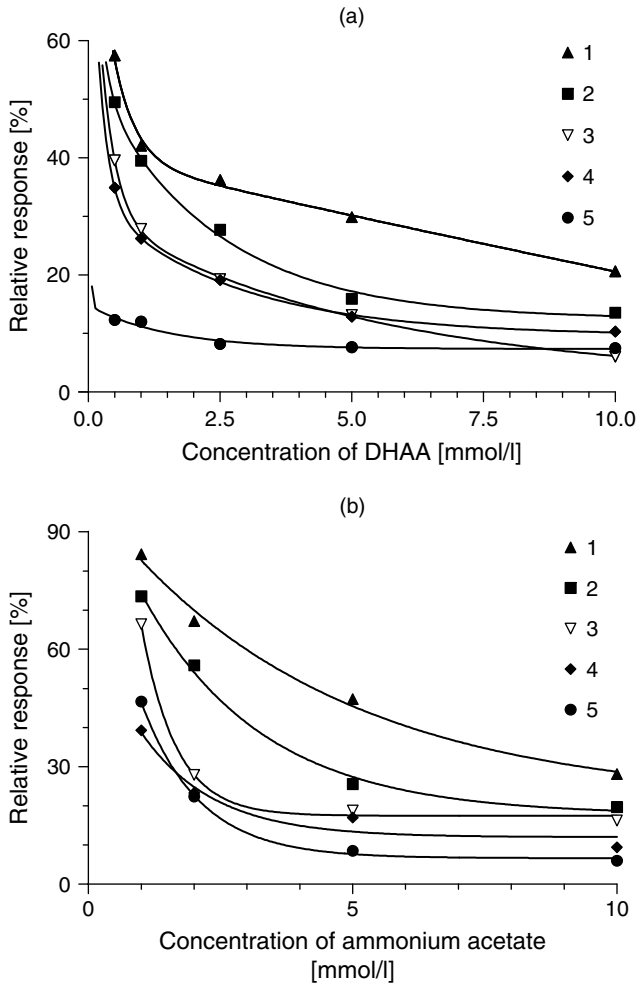

Figure 6. Effects of the ion source and instrument geometry on the electrospray response of naphthalene-2-sulphonic acid in the presence of (a) DHAA and (b) ammonium acetate. Notation: 1, a quadrupole analyser with Z-spray geometry (ZQ 2000, Waters); 2, an ion trap analyser with orthogonal geometry (Esquire 3000, Bruker Daltonics); 3, a quadrupole analyser with orthogonal geometry (Automass Multi, Thermo Finnigan); 4, anion trap analyser with direct geometry (LCQ, Thermo Finnigan); 5, a quadrupole analyser with direct geometry (Platform, Micromass).

which suggests that the ion source geometry is the most important factor affecting the signal suppression but not the only one. The similarity of the results obtained with one manufacturer's instruments indicates that the signal decrease is also influenced by the overall instrument configuration, e.g. the different method of ion desolvation by a heated capillary or by drying gas, different ion optics electrodes, etc. Regardless of small differences in specific cases, the general rule (two 90° angles > one 90° angle > direct geometry) applies in all 50 experiments except one (DHAA, 10 mmol l^{-1}, curve 3 in Fig. 6(a)). Figure 6 shows that with the different instrument geometries the highest (36.2%) and the lowest (8.2%) relative responses for 2.5 mmol l^{-1} of DHAA or the highest (47.2%) and the lowest (8.5%) relative responses for 2.5 mmol l^{-1} of ammonium acetate can differ as much as 4.4 or 5.6 times. This important factor should be taken into account, especially in trace analysis, where the orthogonal type of geometry (e.g. Z-spray) should be preferred to the linear ion path.

CONCLUSIONS

Ion-pairing mobile phase additives required for the successful HPLC separation of polysulphonic acids suppress the electrospray signal and may cause contamination of the mass spectrometer. This effect is most significant with tetraalkylammonium ions. Di- and trialkylammonium acetates offer similar separation selectivity to tetraalkylammonium salts, but cause a much lower electrospray signal decrease. They are therefore more suitable for HPLC/MS of polysulphonated dyes and intermediates. The differences between the individual di- and trialkylammonium acetates are not very significant. Ammonium acetate, which is frequently used for HPLC/MS of ionic compounds, causes similar signal suppression of (poly)sulphonic acids as di- and trialkylammonium acetates. Moreover, di- and trialkylammonium ion-pairing reagents provide a better separation selectivity than ammonium acetate and therefore are to be preferred for HPLC/MS of polysulphonic acids. The ion suppression effects follow the general rule for the five instrument geometries compared in our study: Z-spray < orthogonal spray < linear spray. Strong signal suppression effects of competitive co-ions of an 'interfering' sulphonic acids are observed. We believe that the results of this work can be useful for improving the quality of separation and the sensitivity of MS detection in the HPLC/MS analysis of anionic compounds.

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