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High performance liquid chromatography–mass spectrometric analysis of sulphonated dyes and intermediates

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Abstract

This paper reports our results in the analysis of polysulphonated anionic dyes and their intermediates using high-performance liquid chromatography–mass spectrometry (HPLC–MS). Negative-ion electrospray ionization is the most suitable ionization technique for the molecular mass determination of polysulphonated dyes or other dyes carrying a negative charge. From the series of $[M–xH]^{x-}$ ions and their sodiated adducts $[M–(x+y)H+yNa]^{x-}$, the molecular mass and the number of sulphonic and carboxylic groups can be determined. The mobile phase should be compatible with the mass spectrometric detection, which rules out non-volatile tetraalkylammonium salts usually used as ion-pair mobile phase additives for the HPLC of sulphonated compounds. Some mono- and disulphonated dyes and intermediates can be separated with aqueous–organic mobile phases containing 5 m*M* ammonium acetate, which is the most suitable additive as far as compatibility with MS detection is concerned. However, the retention of compounds with two or more sulphonic groups is too low for a successful separation both with this mobile phase additive and with ion-pair additives with short alkyl chains. The dihexylammonium acetate ion-pairing reagent offers a reasonable compromise in terms of sufficient volatility and adequate retention and separation selectivity for the HPLC–MS analysis of polysulphonated dyes.

Keywords: Dyes; Sulphonic acids

1. Introduction

Organic aromatic dyes belong to various classes and the overall dye production in the world was about $1 \cdot 10^9$ kg in 1994 and rises more than 10% annually [1]. Many organic dyes and intermediates used in their production contain sulphonic acid groups because of their better solubility in water. Consequently, acid dyes and aromatic sulphonates are often found as pollutants in wastewater and in surface water. Hence adequate methods for dye analysis are needed not only in production quality assessment, but are also of concern in environmental pollution control.

Reversed-phase ion-pair chromatography with UV detection has been widely used for the analysis of sulphonated dyes and intermediates. The most common ion-pairing reagents are tetraalkylammonium salts such as tetrabutylammonium [2,3] or hexadecyltrimethylammonium [4] salts.

Sometimes the separation selectivity for isomeric compounds can be improved by using hydrophobic interactions (salting-out effects) in mobile phases containing high concentrations of electrolytes, e.g. sodium sulphate [2,3,5], ammonium sulphate [6], or various other salts [7]. Reversed-phase techniques

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for the separation of ionic solutes employing ionic additives in the mobile phase are often called ''ion-interaction reversed-phase chromatography''.

In the past few years, capillary electrophoresis (CE) has become an increasingly popular technique for the analysis of aromatic sulphonates [3,8]. The selectivity of electrophoretic separation of some sulphonated dyes or of the isomers of their intermediates can be improved by adding tetraalkylammonium salts as electroosmotic flow modifiers [9,10] or cyclodextrin selectors [3,11] to the running buffers. Even though coupling of CE to MS is more demanding than the coupling of HPLC to MS, the successful separations of ionic dyes by CE–MS have been reported [12–17]. A lower-concentration volatile ammonium acetate buffer was found better suited for CE–MS separations than conventionally used borate or phosphate CE running buffers [18].

HPLC directly coupled to mass spectrometry (HPLC–MS) is the technique of choice for environmental monitoring of dyes because of its high sensitivity and ability to obtain structural information on unknown compounds [19]. For successful application of HPLC–MS to the analysis of sulphonated dyes and intermediates, the volatility of all mobile phase components is more critical than the volatility of running buffers in CE–MS, because of the higher flow-rates used in the former technique. Neither tetraalkylammonium nor inorganic salts are suitable for mass spectrometric detection in HPLC because of the signal suppression and contamination of the ion source caused by involatile species. Hence, more volatile additives to the mobile phase should be used.

Reversed-phase HPLC with aqueous–organic mobile phases containing ammonium acetate can be used for the analysis of some mono- and disulphonic acids [20–22], but the method fails if it is applied to the separation of compounds with more than two sulphonic acid groups because of too low a retention of such compounds. This is possibly the reason why the HPLC–MS technique has so far been used only for the analysis of mono- and disulphonic dyes.

A promising approach to increasing the retention and improving the separation of polysulphonated compounds in HPLC–MS is using volatile (acetate or formiate) salts of mono-, di- or trialkylamines as ion-pair reagents [23]. The retention depends on the number and the length of the alkyl chains in mono-, di- and trialkylammonium salts and on the concentration of the ion-pairing reagent [24,25]. Butylamine [26] or monoalkylamines with other alkyl chains [24,25] were tested for this purpose, but triethylamine [27] or triethylammonium acetate [28–30] have been most often used for the separation of aromatic monosulphonic acids. The retention and separation of simple naphthalene-, benzene- and anthraquinonesulphonic acids including two naphthalenetrisulphonic acids improves when dimethylbutylammonium acetate or tributylammonium acetate are used as ion-pairing additives instead of triethylammonium acetate [23]. In the mobile phases with 2.5 m*M* tributylammonium acetate (TBAA), a reasonable compromise was obtained between the chromatographic performance, which improves with increasing concentration of TBAA, and undesired electrospray ionization (ESI)-MS signal suppression occurring at higher concentrations of TBAA. Enhancement of the ionization efficiency for polysulphonated dyes in the presence of diethylamine was observed [31].

In HPLC–MS of sulphonated dyes and intermediates, only the particle beam interface with electron ionization can provide detailed structural information. However, this ionization can be used only for azo dyes of low polarity [32,33]. Mass spectra obtained using soft ionisation techniques including thermospray, ESI and atmospheric pressure chemical ionization (APCI) yield information on the molecular mass only [34] and — in some instances — on the number of acid groups per molecule of sulphonated dyes [22] for a wide polarity range of compounds, ranging from non-polar dyes to ionic ones [19,22]. For ionic [19,20,22,31,35–37] and metal complex azo dyes [22,35,38], ESI is preferred to APCI. In connection with soft ionization techniques, in-source collision-induced dissociation (CID) or multiple MS (MS^n) using an ion-trap analyser [34,35] are required for structural characterisation of dyes.

To our knowledge, a generally useful separation method is still not available for sulphonated compounds with more than two sulphonic acid groups, which enables coupling with mass spectrometric detection without significant difficulties. Ion-pairing HPLC with di- or trialkylammonium acetate additives is potentially suitable for this purpose, but it is

necessary to find out a useful compromise between the effects of the alkyl length on the chromatographic properties and on the mass spectrometric performance.

In this work, dihexylammonium acetate was found to offer at the same time good chromatographic properties and still sufficient volatility for compatibility with mass spectrometric detection. We compare the mobile phases containing ammonium acetate and dihexylammonium acetate as additives for HPLC–ESI-MS analysis of sulphonated dyes and their intermediates derived from naphthalene and anthraquinone.

2. Experimental

2.1. Materials

Acetonitrile for HPLC was purchased from Baker (Deventer, Netherlands) and methanol for HPLC from Merck (Darmstadt, Germany). Water was doubly-distilled in glass with the addition of potassium permanganate. Ammonium acetate, acetic acid and 25% ammonium hydroxide solution were purchased from Sigma (St. Louis, MO, USA) and dihexylamine from Aldrich (Milwaukee, WI, USA). The samples of aromatic acids and of dyes were obtained from the Research Institute of Organic Synthesis (Pardubice, Czech Republic).

2.2. HPLC–MS

The chromatographic apparatus consisted of a Waters 616 pump, a Waters 996 diode-array UV detector and a Waters 717+ autosampler (all from Waters, Milford, MA, USA). The quadrupole mass spectrometer Platform (Micromass, Manchester, UK) equipped with ESI and APCI probes was used for all measurements. The ESI ion source temperature was set at 100°C and the negative-ion ESI mode and a cone voltage of 30 V were used in all experiments.

Dihexylammonium acetate was prepared by mixing equimolar amounts of dihexylamine and acetic acid. The mobile phases were prepared by pre-mixing the components in appropriate volume ratios and were filtered through a 0.45-μm Millipore filter prior to use. During chromatographic experiments, the mobile phase was degassed by continuous stripping by a stream of helium. Samples were dissolved in the mobile phase in appropriate concentrations for UV and MS detection. Injection volumes of 10 μl, a flow-rate of 1 ml/min and column temperature of 40°C were used in all analysis.

The separation was performed in two chromatographic systems:

1. An octadecyl silica glass cartridge column, Separon SGX C_{18} (150×3 mm I.D., 7 μm particle size) purchased from Tessek (Prague, Czech Republic) was used for the separation of aromatic sulphonic acids in a mobile phase containing methanol and 5 m*M* aqueous ammonium acetate (10:90). The splitting ratio of 1:20 was used to introduce the effluent into the electrospray ion source.
2. A Luna C_{18} column, (150×4.6 mm, 5 μm particle size) purchased from Phenomenex (Torrance, CA, USA) was used for gradient-elution ion-pair separations of aromatic sulphonic acids. Two gradients were employed, both with 2.5 m*M* dihexylamnonium acetate in water as solvent A and 2.5 m*M* dihexylammonium acetate in methanol as solvent B: (a) from 50% B in 0 min to 80% B in 35 min for naphthalenesulphonic acids and (b) from 60% B in 0 min to 100% B in 20 min for commercial sulphonated dyes. For coupling with the ESI ion source, the HPLC effluent was split at the ratio 1:50, yielding the flow of 20 μl/min to the mass spectrometer.

3. Results and discussion

As it is shown in Section 1, a wide variety of alkylammonium ions have been used previously as ion-pairing additives in the HPLC analysis of sulphonic acids and acid dyes, but only few have been applied in HPLC–MS analysis. We have not found any earlier attempts for the HPLC–MS analysis of compounds containing more than two sulphonic acid groups in the available literature. However, with new developments in dye technology more complex new dyes have appeared, some of which contain three or more $-SO_3^-$ groups. HPLC–MS would be a valuable technique for confirmation of their structure provided a suitable mobile phase additive is available for

negative-ion HPLC–ESI-MS of sulphonated dyes and intermediates. The problem consists in the opposite requirements imposed on the mobile phase by the chromatographic separation and by the on-line mass spectrometric detection. While the volatility and hence the compatibility with on-line mass spectrometry decreases as the alkyl length in the molecules of ion-pair additives increase, the retention of polysulphonated compounds in mobile phases containing ion-pair additives with short alkyl chains is usually not sufficient for their successful separation. Hence, an adequate ion-pair reagent for HPLC–MS of polysulphonated dyes should be selected as a compromise providing acceptable chromatographic retention and separation selectivity whereas its volatility should be sufficient enough not to interfere with the performance of the MS instrument.

We compared several types of ion-pair reagents for the analysis of polysulphonated dyes. Dihexylammonium acetate (DHAA) provided acceptable retention and selectivity for polysulphonated dyes, while these solutes were only little retained (if at all) in mobile phases containing additives with shorter alkyl chains. The compatibility of DHAA with mass spectrometric detection was satisfactory, whereas the volatility of dialkyl-, trialkyl- and tetraalkylammonium salts with longer alkyl chains was too low leading to unacceptable signal noise and memory effects in on-line mass spectrometric records. The performance of several other potentially useful ion-pairing reagents for HPLC–MS of sulphonated dyes and intermediates is presently being investigated in detail [39]. In this study we compare the results obtained with volatile ammonium acetate employed earlier [22] and with DHAA as mobile phase additives for the analysis of various sulphonated dyes and intermediates. DHAA is likely to provide similar retention and separation selectivity for aromatic sulphonic acids as tetrabutylammonium sulphate (TBAS) used in our earlier experiments [2] (12 carbon atoms in DHAA alkyls versus 16 in TBAS).

The separation conditions were optimised with respect to the separation selectivity and the analysis time, so that two different gradient elution profiles were used for chromatographic separation of non-substituted and substituted naphthalene sulphonic acids and sulphonated azo naphthalene-, anthraquinone- and other commercial dyes. The experimental retention times are listed in Tables 1 and 2, respectively. As the retention factors cannot be calculated directly from the retention times in gradient elution experiments, instead, the elution concentration of methanol at the column outlet at the time of elution of the peak maximum, c_f, is given for each sample compound in Tables 1 and 2. c_f is much less affected by the starting composition of the mobile phase and by the gradient steepness than the retention times so that its values facilitate the comparison of the results obtained with different gradient profiles.

Various naphthalene mono- to tetrasulphonic acids were eluted at c_f in between 50 and 67.5% methanol (Table 1), whereas mono- to tetrasulphonated dyes with bulkier molecules were eluted at c_f in between 67.5 and 99% methanol (Table 2) when keeping a constant concentration of 0.0025 *M* DHAA during the gradient run.

In Table 1, the retention data for the chromatographic system with DHAA are compared with the data measured in mobile phases containing ammonium acetate. Fig. 1 illustrates that mobile phases with ammonium acetate principally can be used in connection with ESI-MS detection of sulphonic acids. The retention times in the mobile phase containing ammonium acetate and corresponding retention factors (Table 1) generally increase with decreasing number of sulphonic groups and decrease with increasing number of amino and hydroxy groups in the acid molecules. Differences were observed also in the retention data of some isomeric acids. For example, the retention times of isomeric 1-aminonaphthalenesulphonic acids increase in the order: 5-sulphonic<6-sulphonic<7-sulphonic<8-sulphonic acids; 6-amino-1-hydroxynaphthalene-3-sulphonic acid elutes before 7-amino-1-hydroxynaphthalene-3-sulphonic acid and naphthalene-1-sulphonic acid before naphthalene-2-sulphonic acid. However, naphthalenedi- and trisulphonic acids are either not retained at all or their elution times are low and the solutes cannot be separated from one another. Further, the bands of more strongly retained compounds are very broad (Fig. 1; note that the chromatogram was recorded at the flow-rate of 0.6 ml/min, whilst the data in Table 1 was recorded at 1 ml/min). Hence, the chromatographic systems with ammonium acetate cannot be used for the analysis of polysulphonated dyes.

Table 1
Retention times, t_R (min), retention factors, k, and elution concentrations, c_f (%), of solvent B in reversed-phase HPLC of naphthalene sulphonic acids[a]

No.	Compound	Acid groups	a		b	
			t_R (min)	c_f	t_R (min)	k
1	7-Amino-1-hydroxynaphthalene-3-sulphonic acid	1	4.2	50.52	4.6	4.26
2	5-Aminonaphthalene-1-sulphonic acid	1	4.6	50.87	3.0	2.43
3	6-Amino-1-hydroxynaphthalene-3-sulphonic acid	1	5.4	51.55	3.4	2.89
4	2-Aminonaphthalene-7-sulphonic acid	1	5.4	51.55	8.5	8.73
5	1,6-Dihydroxynaphthalene-3-sulphonic acid	1	5.6	51.72	5.1	4.84
6	1-Aminonaphthalene-6-sulphonic acid	1	5.7	51.81	6.9	6.90
7	1-Aminonaphthalene-7-sulphonic acid	1	8.1	53.87	9.3	9.64
8	1-Amino-8-hydroxynaphthalene-3,6-disulphonic acid	2	9.2	54.81	2.5	1.86
9	6-Aminonaphthalene-1,3-disulphonic acid	2	9.8	55.32	1.8	1.06
10	Naphthalene-1,5-disulphonic acid	2	10.2	55.67	–	–
11	Naphthalene-1,6-disulphonic acid	2	11.0	56.35	–	–
12	Naphthalene-1-sulphonic acid	1	11.4	56.69	13.4	14.33
13	8-Aminonaphthalene-1-sulphonic acid	1	11.5	56.78	13.1	13.99
14	Naphthalene-2-sulphonic acid	1	11.7	56.95	15.2	16.39
15	Naphthalene-1,3-disulphonic acid	2	13.0	58.07	–	–
16	Naphthalene-1,7-disulphonic acid	2	14.0	58.92	–	–
17	1,8-Dihydroxynaphthalene-3,6-disulphonic acid	2	15.8	60.47	1.6	0.83
18	Naphthalene-1,3,7-trisulphonic acid	3	16.7	61.24	1.7	0.95
19	8-Aminonaphthalene-1,3,6-trisulphonic acid	3	16.9	61.41	–	–
20	Naphthalene-1,3,6-trisulphonic acid	3	17.4	61.84	–	–
21	Naphthalene-1,3,5-trisulphonic acid	3	18.0	62.35	–	–
22	Naphthalene-1,3,5,7-tetrasulphonic acid	4	23.8	67.32	–	–

[a] Flow rate = 1 ml/min at 40°C. a = Linear gradient, 50–80% B in 35 min; A: 2.5 m*M* dihexylammonium acetate (DHAA) in water, B: 2.5 m*M* DHAA in methanol, column Luna C_{18}, 5 μm (150×4.6 mm I.D., V_0 = 1.66 ml). b = Isocratic elution, 4.5 m*M* ammonium acetate in 90% aqueous methanol, column Separon SGX C_{18} (150×3.3 mm I.D., V_0 = 0.87 ml).

Using gradient elution with increasing concentration of methanol at a constant concentration of 2.5 m*M* DHAA, good resolution was obtained for four positional isomers of naphthalenedisulphonic acids and three isomers of naphthalenetrisulphonic acids (Fig. 2 and Table 1). The dihexylammonium acetate mobile phase ion-pair reagent is compatible with ESI-MS detection and the band broadening of all acids was acceptable (Fig. 2).

The retention behaviour of aromatic sulphonic acids in aqueous–methanolic DHAA was very similar to ion-pairing chromatography with tetraalkylammonium salts [2], i.e., the retention of naphthalenepolysulphonic acids increased in the order of increasing number of sulphonic groups (mono-≤di-<tri-<tetra-), opposite to the behaviour in the mobile phase with ammonium acetate. The elution order of isomeric acids was the same as in mobile phases containing TBA ion-pair reagents or ammonium acetate: naphthalene 1,5-di-<1,6-di-<1,3-di-<1,7-disulphonic acids. Strong retention of 8-aminonaphthalene-1-sulphonic acid can be attributed to its decreased polarity by the formation of an intramolecular hydrogen bond between its NH_3^+ and SO_3^- groups. The elution order of naphthalene trisulphonic acids is difficult to compare directly, as it strongly depends on the character of the support of the bonded stationary phase [3]. As illustrated by the retention data in Table 2, gradient elution with mobile phases containing 2.5 m*M* DHAA is suitable for separation of a range of sulphonated dyes containing 1–5 sulphonic acid groups.

Mobile phases containing DHAA provide good resolution of Rylan metal complex disulphonated dyes, whilst these dyes either are not retained or often show strange peak splitting in the mobile phases with 5 m*M* ammonium acetate. This was the case also with most Egacid and Saturn type dyes containing 2–5 sulphonic acid groups.

Some compounds are not fully resolved even in

Table 2
Retention times, t_R (min), and elution concentrations, c_f (%), of solvent B in gradient elution reversed-phase HPLC of sulphonated dyes[a]

No.	Colour index name	Trade name	M_r	Acid groups	t_R (min)	c_f
1	Acid violet 7	Egacid red 6B	522.1	2	7.4	67.62
2	Acid yellow 23	Egacid yellow T	468.0	3	8.7	70.22
3	Acid orange 10	Egacid orange GG	408.0	2	10.0	72.82
4	Acid yellow 36	Egacid yellow M	353.1	1	13.8	80.42
5	Direct red 79	Saturn red L4B	960.0	4	13.9	80.62
6	Acid blue 40	Egacid blue A2G	451.0	1	14.1	81.02
7	Acid red 118	Midlon red E	540.1	1	14.5	81.82
8	Direct blue 106	Saturn blue LB	696.0	2	14.5	81.82
9	Mordant yellow 8	Alizarin chrome Yellow R	402.1	2	15.2	83.22
10	Reactive green 8	Ostazin olive H-G	1145.0	5	15.8	84.42
11	Acid red 357	Rylan red 3G	887.0	2	16.1	85.02
12	Direct green 26	Saturn green LB	1222.1	5	17.8	88.42
13	Reactive blue 109	Procion blue MX-26	928.9	4	18.3	89.42
14	Direct blue 78	Saturn blue L4G	967.0	4	18.4	89.62
15	Direct green 28	Saturn green L5G	914.1	3	20.3	93.42
16	Acid violet 90	Rylan bordeaux B	897.0	2	20.5	93.82
17	Acid yellow 194	Rylan yellow 3R	900.0	2	21.9	96.62
18	Acid orange 142	Rylan orange R	887.0	2	22.7	98.22

[a] Linear gradient, 60–100% B in 20 min. Solvents A, B, column and conditions as in Table 1a.

gradient elution with DHAA containing mobile phases, but chromatograms of the coeluting dyes can be reconstructed by monitoring chromatograms using appropriate selected ions. If necessary, the resolution can be enhanced by using a less steep gradient. Applications of the present HPLC method to a wider range of polysulphonated dyes are the subject of our current research [39].

The negative-ion ESI mass spectra of polysulphonic acids can be easily interpreted as described previously [22]. The molecular mass and the total number of sulphonic and carboxylic acid groups can be determined from the series of $[M–xH]^{x-}$ ions and their sodiated adducts $[M–(x+y)H+yNa]^{x-}$, where the maximum value of x or $(x+y)$ is equal to the total number of acidic groups [22]. The charge of the series of sodiated adducts can be easily determined from the mass difference between, for examples, $[M–xH]^{x-}$ and $[M–(x+1)H+Na]^{x-}$ ions, which is $\Delta m/z = 22/1$ for singly charged, $\Delta m/z = 22/2 = 11$ for doubly charged or in general $\Delta m/z = 22/x$ for negative ions with x charge.

The determination of the total number of sulphonic and carboxylic acid groups is illustrated in Fig. 3, where the spectra of three dyes with 4, 6 and 8 sulphonic acid groups are shown. (The structures of the dyes are shown in Fig. 4.) The spectra were measured by the direct infusion of samples containing very high concentrations of inorganic salts (approximately 5–20%, w/w), where adducts of deprotonated molecules with sodium atoms were observed. In all examples, characteristic series of sodiated adducts $[M–(x+y)H+yNa]^{x-}$ differing by one or two sodium atoms are observed, as indicated. The charges of the particular ion series were determined from the differences $22/x$, where x is equal to the charge state. The number of acidic groups is equal to the highest observed charge state and/or the highest number of protons replaced by sodium ions. The molecular mass and the number of acidic groups of unknown impurities can be determined even without chromatographic separation, as illustrated in Fig. 3c, where the unknown compound with six sulphonic acid groups and the molecular mass $M_r = 1688$ was unambiguously identified from the ions m/z 280.7 ($[M–6H]^{6-}$), m/z 341.4 ($[M–6H+Na]^{5-}$) m/z 432.4 ($[M–6H+2Na]^{4-}$) and other less abundant ions (not labelled), e.g. $[M–6H+2Na]^{5-}$ and $[M–5H+2Na]^{4-}$.

An alternative approach can be used for the

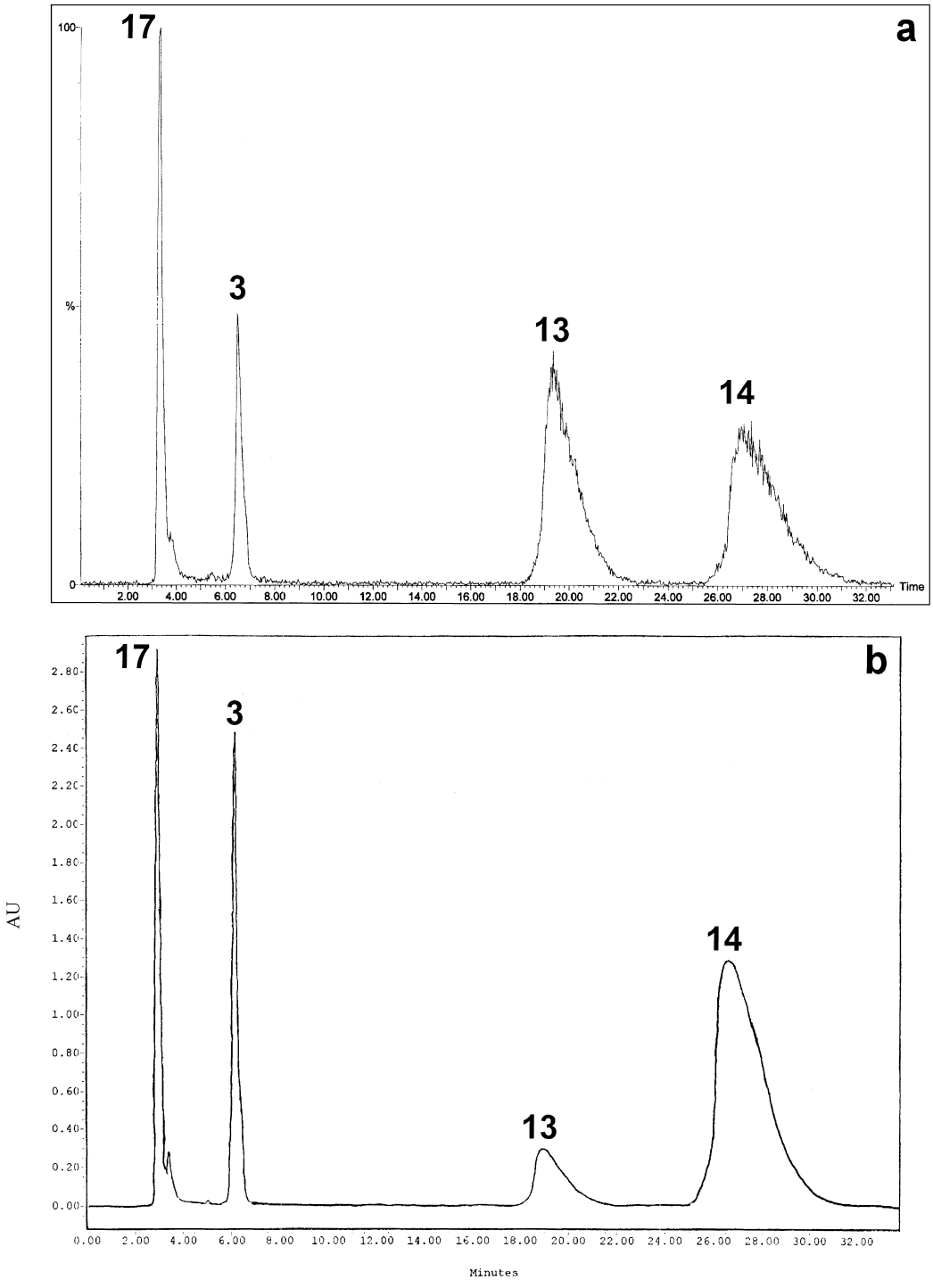

Fig. 1. HPLC separation of four naphthalenesulphonic acids on a C_{18} column with a mobile phase containing ammonium acetate as a volatile ion-pairing reagent. (a) Total ion current record using negative-ion ESI-MS detection, (b) UV chromatogram at 230 nm. Isocratic elution and a flow-rate of 0.6 ml/min, for other details see Section 2. Peak numbers as in Table 1.

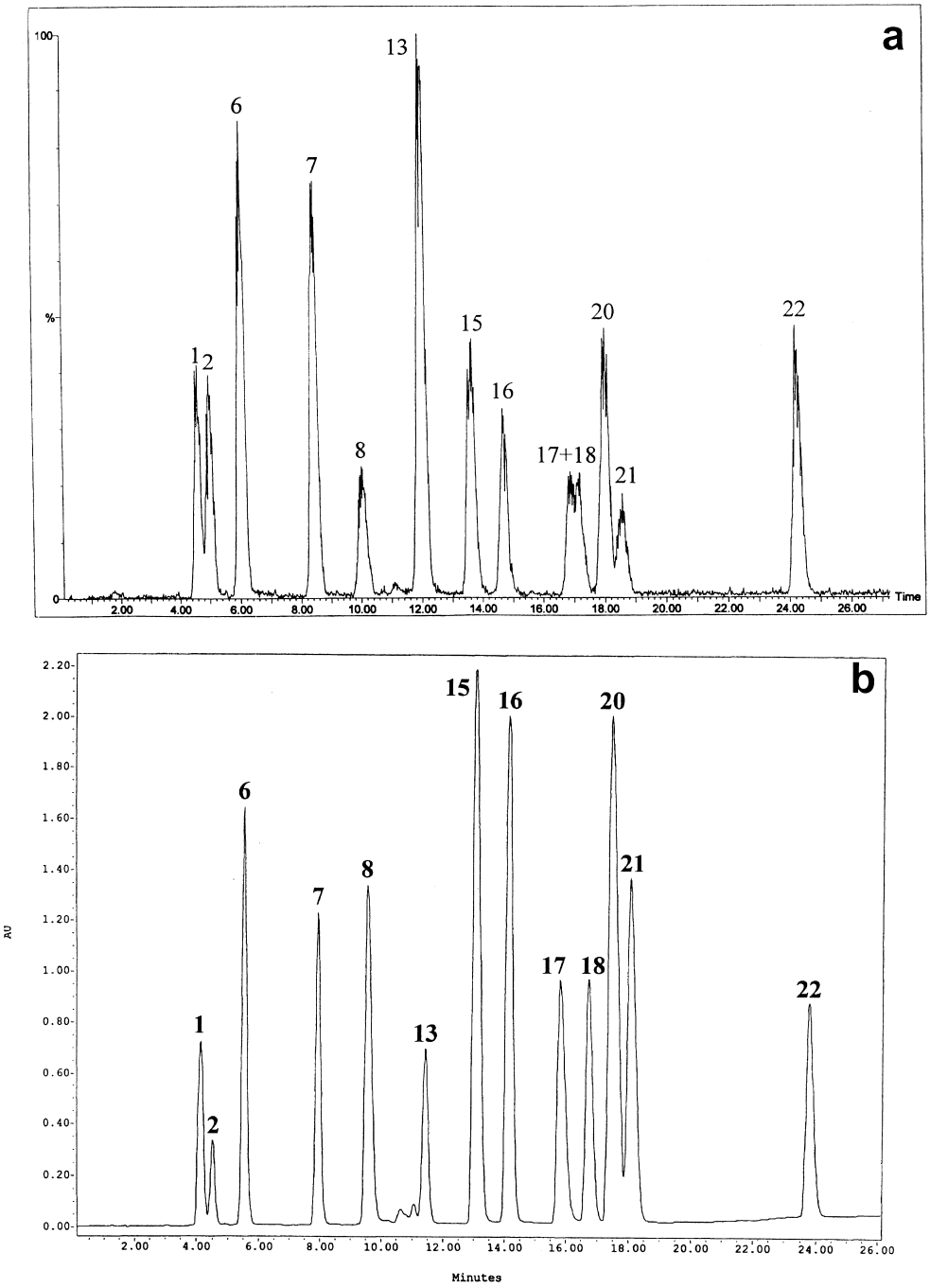

Fig. 2. HPLC separation of 13 naphthalenesulphonic acids on a C_{18} column with a mobile phase containing dihexylammonium acetate as a volatile ion-pairing reagent. (a) Total ion current record using negative ESI-MS detection, (b) UV chromatogram at 230 nm. Gradient elution, for details see Section 2. Peak numbers as in Table 1.

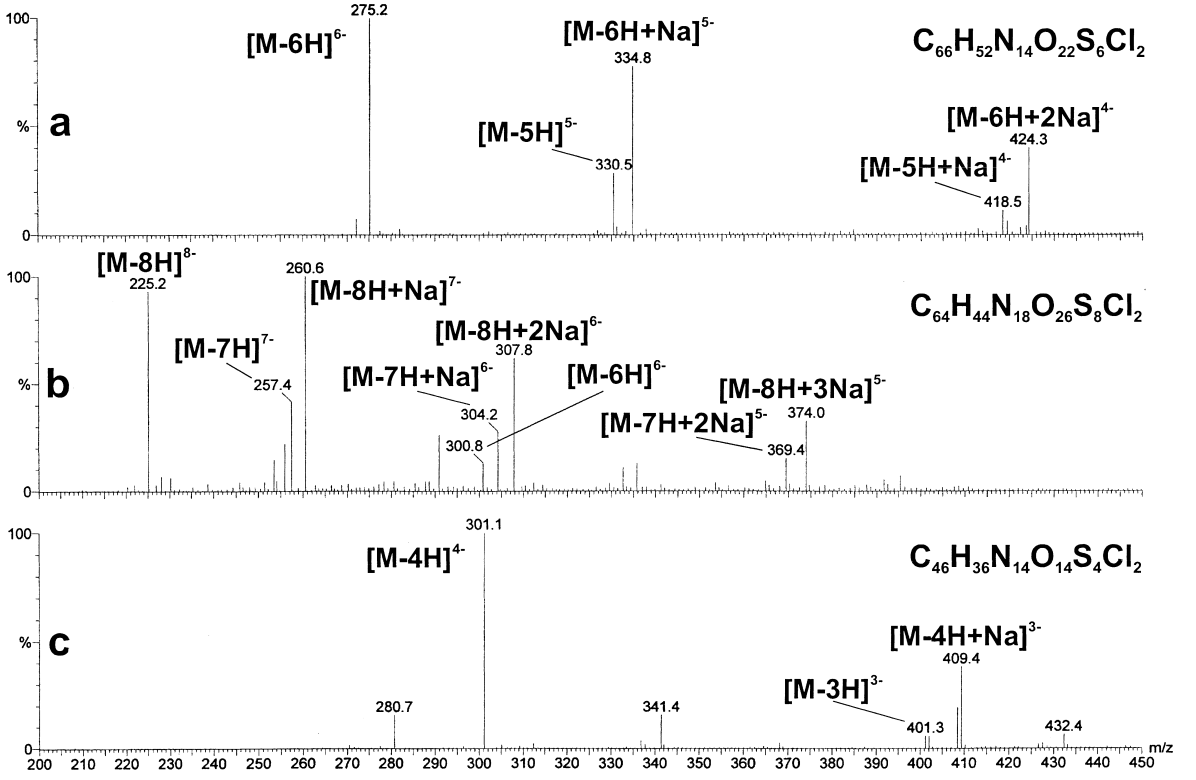

Fig. 3. Negative-ion ESI mass spectra of three polysulphonated dyes measured by direct infusion of their solutions with high salt concentrations. (a) $M_r = 1654.1$ and six sulphonic groups, (b) $M_r = 1806.0$ and eight sulphonic groups, (c) $M_r = 1206.1$ and four sulphonic groups. The structures of the dyes are shown in Fig. 4.

interpretation of the ESI mass spectra of unknown polysulphonated acids in highly salted solutions. If the sulphonic acid groups are considered as corresponding sodium salts, the compound shown in Fig. 3a has the molecular formula $C_{66}H_{46}N_{14}O_{22}Cl_2S_6Na_6$ and the molecular mass $M_r = 1786.1$. In such case, the most abundant ions in the particular ion series correspond to $[M-xNa]^{x-}$ ions. Actually, the approaches described in this and in the preceding paragraphs differ only in the notation of the ions.

When the charges of the ions are known it is possible to infer the molecular mass using each multiply charged molecular ion species and the precision of molecular mass determination can be improved by averaging. The ESI mass spectra measured by HPLC–MS show decreased relative abundance of sodiated adducts in comparison to the direct infusion measurements, hence the $[M-xH]^{x-}$ ions predominate, which simplifies the interpretation of the mass spectra. The charges can be also determined from the mass differences of the isotopic ions determined by higher resolution scans using the ion trap analyser, which can distinguish isotopes at least for ions with up to five charges, i.e., differences as low as $\Delta m/z = 0.2$.

The negative-ion ESI mass spectra of the Rylan type disulphonated trivalent metal complex dyes (compounds 11, 16, 17 and 18 in Table 2) are similar to those of other sulphonated dyes, but the maximum negative charge or the maximum number of protons replaceable by sodium ions is equal to the number of acidic groups (two) plus one, because these complexes are anions with one negative charge.

4. Conclusions

Even though reversed-phase HPLC with mobile phases containing ammonium acetate is compatible

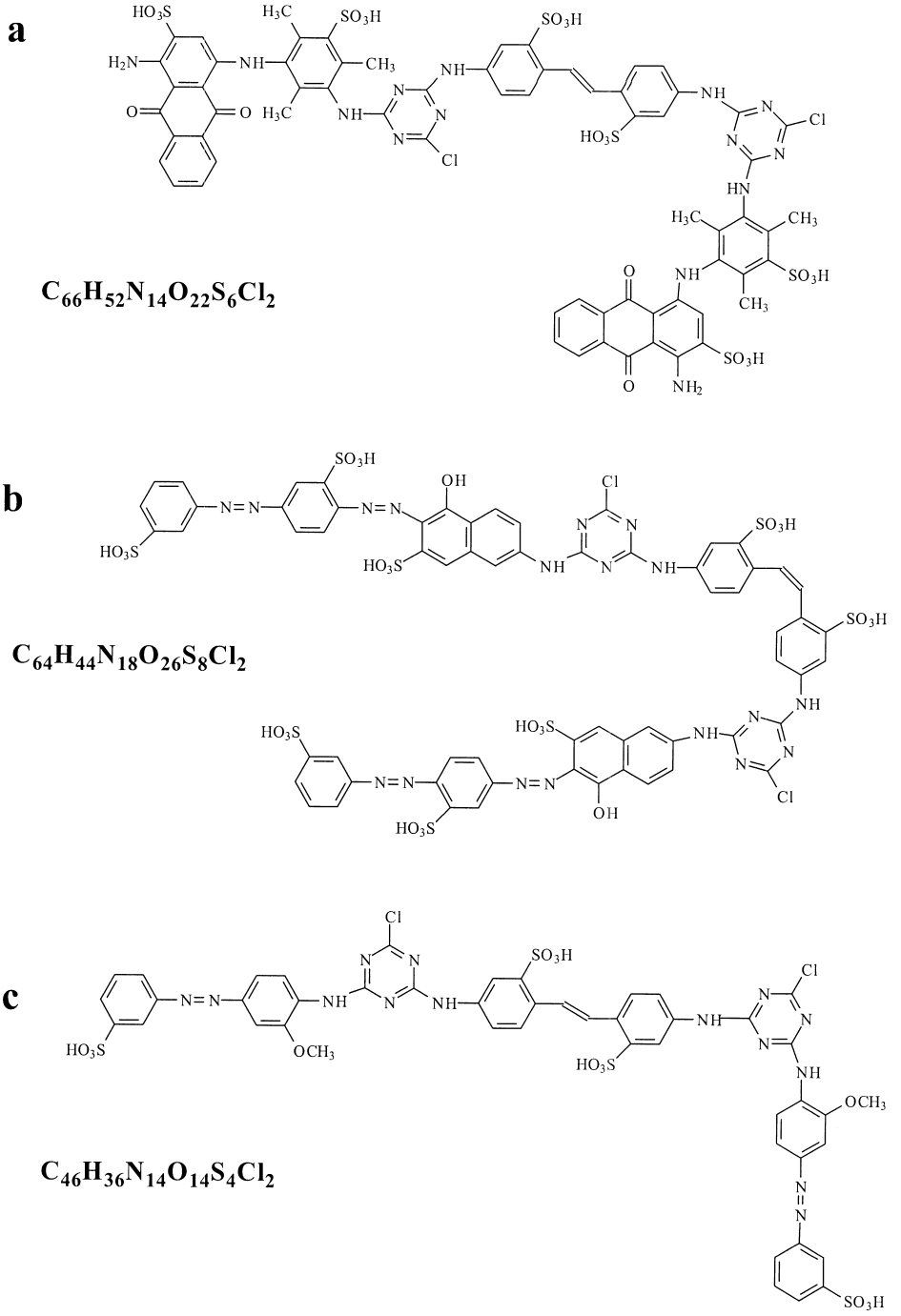

Fig. 4. Structures of polysulphonated dyes providing the mass spectra in Fig. 3. (The samples were provided by Mr. Al Sabbari Abdo Saleh.) M_r: (a) 1654.1; (b) 1806.0; (c) 1206.1.

with ESI-MS detection, compounds containing more than two sulphonic acid groups are not retained and many disulphonic acids are poorly resolved. Adequate retention of polysulphonated dyes and intermediates requires an ion-pairing reagent with sufficiently long alkyl chains, however, volatilities of ion pairing reagents decrease and possible negative effects on the performance of an on-line mass spectrometer are more likely to occur for reagents with longer alkyls. The DHAA ion-pairing reagent is volatile enough and provides separation selectivity and efficiency comparable to commonly used less volatile tetraalkylammonium salts. Hence, the DHAA mobile phase additive is a useful compromise between the chromatographic and the mass spectrometric performance for the HPLC–ESI-MS analysis of polysulphonated dyes and intermediates.

The negative-ion ESI mass spectra of polysulphonic acids provide a simple means for the determination of the molecular mass and of the number of acid groups in a molecule of a polysulphonated dye from a series of peaks of deprotonated molecules $[M-xH]^{x-}$ and of their adducts with sodium ions $[M-(x+y)H+yNa]^{x-}$ with multiple negative charges. This approach can be also applied to sulphonated metal complex azo dyes with trivalent metal ions. Application possibilities of the present method for quantitative analysis of polysulphonated dyes are presently being investigated.

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