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Thermospray, particle beam and electrospray liquid chromatography–mass spectrometry of azo dyes

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ABSTRACT

A high-performance liquid chromatographic–mass spectrometric (HPLC–MS) method was used for the analysis and characterization of fourteen commercial azo and diazo dyes. Thermospray analysis of these dyes using the "filament-on" chemical ionization operation mode produced mass spectra consisting primarily of $[M + H]^+$ ions with very few fragments. Particle beam electron impact ionization mass spectrometric analysis resulted in molecular ion information and various fragment ions. Characterization of the azo dyes could be achieved by observing typical fragment ions formed by cleavage of the C–N bonds on either side of the azo linkage, and cleavage of the N=N double bond with transfer of one or two hydrogen atoms to form an imine or amine. Many commercial azo dyes contain precursors, by-products of the synthesis, degradation products or other impurities. Some of these components were tentatively identified by their mass spectra and by review of the chemistry of dye manufacture. Electrospray negative ion operation was suitable for the characterization of a disulfonated azo dye and showed singly and doubly charged molecular ions and fragment ions at higher capillary–skimmer potential differences. The base peak was attributed to a doubly charged naphthol cleavage product.

INTRODUCTION

Azo dyes have been manufactured in the USA since the early 1900s and are still a very important class of synthetic chemicals. They are widely used as colorants in a variety of products, such as textiles, paper, leather, gasoline and foodstuffs [1]. These widely distributed chemicals represent a potential human health risk, as some have been shown to be carcinogenic [2–7]. It has also been shown that synthetic precursors, intermediates, by-products and degradation products of these dyes could be potential health hazards owing to both their toxicity and their carcinogenicity.

Commercial dyes are not single chemical compounds but instead are mixtures of compounds including dyes and impurities that have been standardized for their end use. Some azo dyes are of high purity (except for colorless diluents or dispersing agents) or can be readily purified further by appropriate isolation procedures and crystallization. However, other dyes, especially those prepared by several coupling reactions and those containing several sulfonic groups, are difficult to isolate in the pure state [8–10]. Sometimes manufacturers have refused to divulge structural details of their dyes [11]; also, some organic dyes have occasionally been sold under false labeling [12]. These are extreme examples, but they illustrate the need for comprehensive analysis of commercial dyes by methods designed for specific purposes. Analytical methods such as thin-layer (TLC), paper, high-performance liquid (HPLC) and gas-liquid chromatography, infrared and nuclear magnetic resonance spectroscopy, X-ray powder diffraction, chemical degradation and mass spectrometry (MS) are mainly used to detect and identify dyestuffs in various matrices [13]. However, these techniques have several disadvantages stemming from non-specific detection [14], lack of sensitivity or incompatibility with non-vola-

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tile, thermally unstable organic compounds. On-line HPLC–MS using thermospray (TSP) has been used to overcome some of these disadvantages for the analysis of environmental hazardous compounds [15,16] and has proved to be a suitable technique for many azo dyes [17–28]. Further HPLC–particle beam (PB) MS [29–31] has been demonstrated to generate electron impact ionization (EI) mass spectra from a series of commercial dyes [32]. A technique termed "ion spray" [33] was shown to generate ions of mono- and disulfonated azo dyes amenable to mass spectrometry [34,35].

This paper describes the analysis of fourteen commercial dyes, some of which are on the US Environmental Protection Agency list of toxic substances (Fig. 1), by HPLC–TSP-MS and HPLC–PB-MS. Both methods were compared and used to identify the parent azo dyes and their synthetic intermediates, by-products, additives or degradation products in these particular samples of interest. In addition, electrospray (ESP)-MS [36,37] was investigated for the mass spectrometric characterization of the acid dye.

EXPERIMENTAL

Materials

The following fourteen dyestuffs, listed in Fig. 1 and identified by their Color Index (C.I.), name and number, were obtained from the sources indicated: Nos. **1**–**7** and **14** (Aldrich, Milwaukee, WI, USA); **8** (Ciba-Geigy, Dyestuffs and Chemicals Division, Greensboro, NC, USA); **9** (BASF, Charlotte, NC, USA); **10** (Eastman Chemicals, Kingsport, TN, USA); **11** (Sandoz Colors and Chemicals, Charlotte, NC, USA); and **12** and **13** (Cromton & Knowles, Charlotte, NC, USA). All other chemicals were purchased from Aldrich. Some products with low dye contents were used after further extraction; the other commercial dyes were used without further purification. The solvents used for extraction, reaction or liquid chromatography were of HPLC/GC grade (Baxter Healthcare, Muskegon, MI, USA), and the water was doubly distilled and filtered through a Milli-Q water purification system (Millipore, Bedford, MA, USA) prior to use.

TLC was performed on normal-phase Macherey–Nagel SIL G/UV_{254} plates (Alltech, Avondale, PA, USA) with the mobile phase toluene–ethyl acetate (EtOAc) (4:1) for solvent dyes. For all other dyes, the TLC analysis was conducted using toluene–EtOAc (1:4). The use of a more polar eluent to study the product of the disperse dyes was essential to resolving components that would otherwise have remained at the origin. The acid dye was eluted with the mobile phase 96–99% ethanol–EtOAc (4:1).

Purification

HPLC–MS analysis was usually performed on the commercial dyes without purification. Because some of the commercial dyes had a diluent content of over 50% (**5**, **6**, **7**, **8**, and **13** in Fig. 1), they were first extracted with pure dichloromethane to separate them from material such as salts and surfactants. These extracts were then evaporated to dryness and dissolved in acetonitrile or methanol for analysis by HPLC–MS. To obtain highly pure azo dye standards, the pure (96–99%) or previously purified commercial dyes were additionally recrystallized from toluene–light petroleum (1:1) and dried. These standards were normally used at a concentration of 500 ng/μl for comparison purposes and further diluted to estimate detection limits.

Analysis

Prior to the HPLC–MS analysis, the melting points (m.p.) of all azo dyes were determined and TLC analysis was performed to screen for major visible organic impurities. The m.p. in °C (retardation factor, R_F) were as follows: **1**, 111, decomp. (0.97); **2**, 101–102 (0.87); **3**, 132–135 (0.98); **4**, 156–157 (0.98); **5**, 159–160 (0.65); **6**, 153–154 (0.90); **7**, 172–173 (0.89); **8**, 194–195 (0.80); **9**, 121–124 (0.54); **10**, 149–152 (0.1); **11**, 138–140 (0.35); **12**, 141–143 (0.65); **13**, 154–156 (0.31); **14**, 319–321 (0.0).

Equipment for HPLC–TSP-MS

The HPLC mobile phases were delivered by two Waters Series 6000A solvent-delivery systems (Waters, Milford, MA, USA) controlled by a Chem-Research chromatographic data management/system controller, Version 2.4.5 (ISCO, Lincoln, NE, USA). The samples were injected with a Waters U6K injector and separated on a Spherisorb ODS II, 5-μm particle size, 25 cm × 4.6 mm I.D. column (Regis Chemical, Morton Grove, IL, USA). A Waters Model 440 absorbance detector set at 254 nm was connected in-line before the TSP interface.

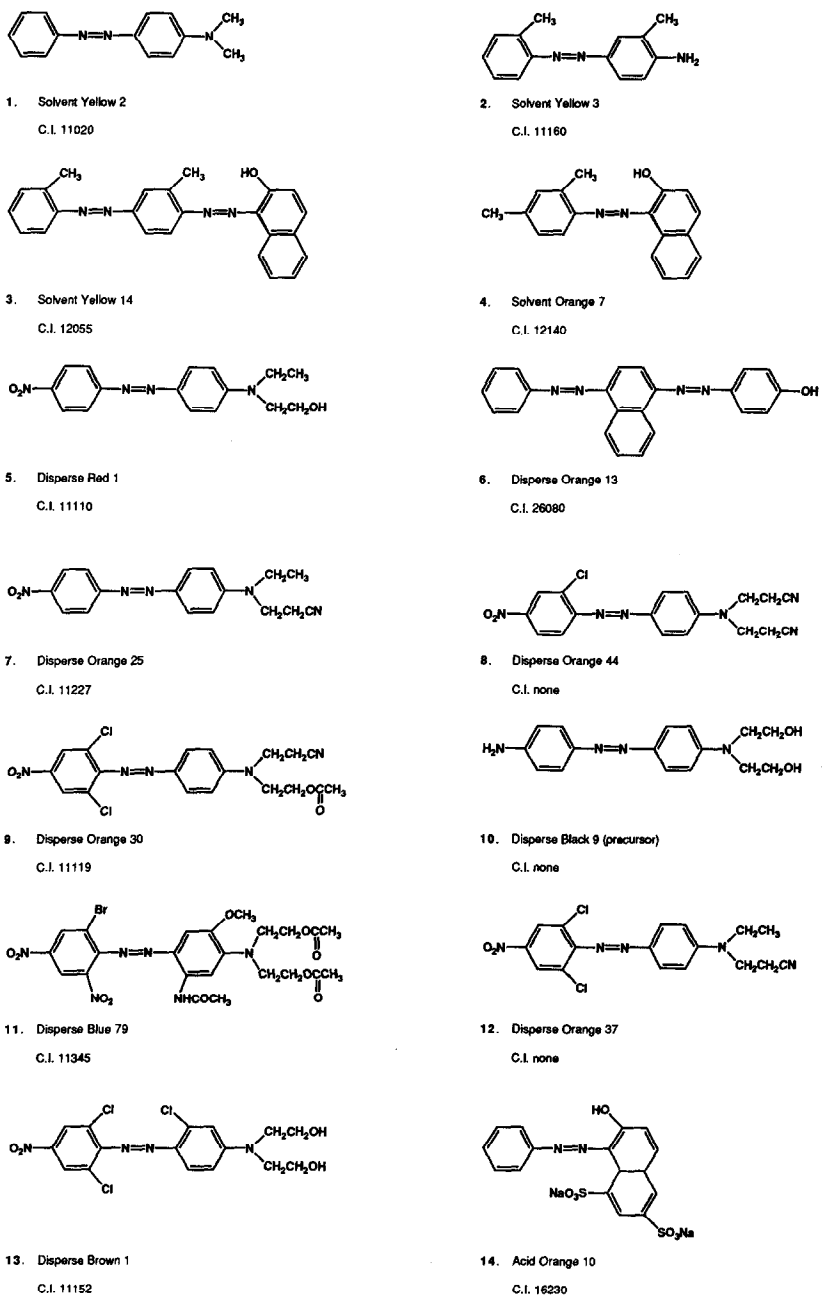

Fig. 1. Structures, names and Colour Index (C.I.) numbers of the azo dye samples used.

Postcolumn buffer addition was performed using a Waters Model 6000 pump to deliver an ammonium acetate solution to a coaxial mixing tee [38].

A Vestec HPLC–TSP-MS Interface Model 701C [39] (Vestec, Houston, TX, USA) was installed on a Finnigan MAT (San Jose, CA, USA) Model 4500

quadrupole mass spectrometer. The solvent was pumped out of the source through a liquid nitrogen cryogenic trap prior to a mechanical rough pump.

HPLC–TSP-MS conditions

The injection volume was 75 μl and the measurements were performed with a methanol–water gradient (from 40% to 100% methanol in 15 min) at a flow-rate of 0.5 ml/min. Ammonium acetate buffer (0.2 *M*) was added postcolumn at 0.5 ml/min to improve sensitivity [40]. The vaporizer temperature was 210–220°C, the tip heater was 290°C, the source block temperature was 260°C and the instrument was operated in the "filament-on" chemical ionization (CI) mode with the repeller set to 0 V. The mass spectrometer instrument scanned from *m/z* 150 to 650 at a rate of 1 s per scan in the pulsed positive/negative-ion detection mode.

Equipment for HPLC–PB-MS

HPLC was performed using a Waters Series 600 multi-solvent delivery system controlled by a Waters 600-MS system controller. The samples were injected with a Waters U6K injector and separated on a Spherisorb ODS II column. A Waters 484 MS tunable absorbance detector set at 254 nm was placed in-line before the Model 59980A PB interface (Hewlett-Packard, Palo Alto, CA, USA). The PB interface was connected to a Hewlett-Packard Model 5988A single quadrupole mass spectrometer.

HPLC-PB-MS conditions

The injection volume was 75 μl and the measurements were performed with a methanol–water gradient (from 40% to 100% methanol in 15 min) at a flow-rate of 0.5 ml/min. The PB desolvation chamber temperature was set to 60°C and a helium inlet pressure of 2.75 bar was maintained. The ion source was operated at 200°C. The filament emission current was 0.3 mA, the electron energy was set at 70 eV and the electron multiplier voltage at 2230 V. The manifold temperatures were set to 90°C (front) and 45°C (rear). The sensitivity of the system was checked by analyzing caffeine.

Equipment for ESP-MS

A Sage Model 341B (Orion Research, Boston, MA, USA) microsyringe pump was used for the flow-injection (FI) analysis of the acid dye **14**. A 50-μl PS C-160 FN syringe for a Waters U6K injector (Dynatech, Baton Rouge, LA, USA) was used for the infusion into the Analytica HPLC–ESP-MS Model 100547-3 interface (Analytica of Branford, Branford, CT, USA). The ESP interface was connected to a Hewlett-Packard Engine Model HP 5989A quadrupole mass spectrometer. The solvent was pumped out of the source in two different pumping stages by two auxiliary mechanical rough pumps.

ESP-MS conditions

The dye was dissolved at a concentration of 100 ng/μl in 2-propanol–water (1:1) and infused at a flow-rate of about 1.2–3.6 μl/min into the ESP interface. The adjustable ESP needle used a 50 μm I.D. stainless-steel tube to deliver solvent and an additional outer concentric tube to add dry oxygen gas to prevent coronal discharges in the negative-ion operation mode (oxygen acts as an electron scavenger). In this ESP interface, the needle is grounded and charging occurs by keeping the cylindrical electrode (V_1) at about 2.8 kV, the end plate (V_2) at 2.6 kV and the capillary (V_3) at 3 kV (positive-ion operation required reverse polarity, and V_{1-3} operated about 1 kV higher than in negative-ion operation). The best results for negative ions were obtained with skimmer (S) and lens (L) voltage of $S_1 = -40$ V, $S_2 = -16$ V, $L_1 = 38$ V, $L_2 = 83$ V and $L_3 = 36$ V. The potential difference between the skimmer and the end of the capillary controlled the extent of fragmentation through collisional-activated dissociation (CAD). The potential difference was varied between −40 and −400 V. The mass spectrometer scanned from *m/z* 40 to 500 at a step size of 0.1 unit and a rate of 0.5 scan per second. The threshold value was set to 100. The system was checked by analyzing adenosine-5′-monophosphate in 2-propanol–water (1:1) for negative-ion and arginine–gramicidin S in methanol–water (1:1) for positive-ion operation.

Equipment and conditions for high-resolution measurements

The VG ZAB-E high-resolution mass spectrometer (Fisons Instruments, Manchester, UK) scanned from *m/z* 50 to 500 at a rate of 10 s per scan with a reset time of 2 s and a response time of 0.01 ms. The ion source was operated at 200°C. The trap

current was 100 μA and electron energy was set to 70 eV.

RESULTS AND DISCUSSION

The TLC analysis of the fourteen azo dyes indicated no significant visible impurities, and the melting point determination indicated no major difference between the tested samples and additionally purified standards or reported values [41]. Both methods are simple and fast but are not suitable for the detection and characterization of trace components. Therefore, a more sensitive and specific procedure such as HPLC–MS was needed for the separation, detection and characterization of non-volatile azo dyes and their by-products. Table I lists the retention times, the major fragments observed under TSP and PB conditions and the percentage of UV absorption[a] of the corresponding component peak for the fourteen commercial dyes and their detectable impurities; Figs. 2–8 show mass spectra of seven selected solvent and disperse azo dyes.

HPLC–TSP-MS

In TSP chemical ionization (CI) mass spectrometry, most of the ion current is concentrated in a single ion, usually the protonated molecule $[M+H]^+$ of the parent azo dye. All solvent and disperse dyes tested remained very stable, and lower molecular mass fragment ions with a relative intensity of over 25% were not detected. Fig. 2A and 3A show the TSP mass spectra of the isomeric azo dyes Solvent Yellow 2 and 3 (**1** and **2**). Both mass spectra are very similar and provide only an $[M+H]^+$ ion peak without any structural information. As shown in Figs. 4A and 5A, Solvent Yellow 14 (**3**) and Disperse Orange 13 (**6**) show an intense $[M+H]^+$ ion and little or no fragmentation in the CI process. Only Disperse Orange 44 (**8**) shows an additional $[M+NH_4]^+$ adduct ion at *m/z* 400, but with a low relative intensity of only 1%. The most difficult dyes to study by mass spectrometry are the acid dyes. The salts of sulfonic acid dyes cannot be volatilized without thermal degradation. Even the free acids are extremely non-volatile and their $[M+H]^+$ ions are usually not very intense. Only an $[MH-2Na+2H]^+$ ion at *m/z* 409 of Acid Orange 10 (**14**) has been tentatively identified. A similar fragment ion has been identified in the TSP mass spectrum of Acid Blue 113 as base peak [22]. The base peak in the mass spectrum of dye **14** is at *m/z* 326 and is probably due to a partially desulfonated $[M-Na-NaSO_3]^+$ ion.

In some of the commercial azo dyes, by-products were separated and characterized by mass spectrometry. Solvent Yellow 2 (**1**) contained a dye residue with a partially demethylated amino group (*m/z* 211) and Solvent Yellow 3 (**2**) contained a small amount of isomer with the methyl group in the 2′-position (instead of the more favorable 3′-position) on the phenyl ring. Neither of these by-products was detected by HPLC–PB-MS (possibly owing to the non-volatility or low concentration of these products) and are therefore not fully confirmed. In addition, Disperse Orange 30 (**9**) showed the protonated parent dye at *m/z* 450 and an ion at *m/z* 416 that was attributed to a substitution of one chlorine ($[M+H-Cl+H]^+$). Fig. 6 shows the total ion current (TIC) chromatogram, the corresponding TSP mass spectrum of the parent azo dye (mass peak number 2) and the TSP spectrum of its hydrolyzed by-product (mass peak number 1). Disperse Blue 79 (**11**) contained at least three degradation products (*m/z* 582, 325 and 310), but only in small amounts and not fully resolved by the chromatographic system. The peak at *m/z* 582 can probably be attributed to the hydrolysis product $[M-COCH_3+H]^+$; the peak at *m/z* 325 resulted from the amine formed after a reductive cleavage of the azo linkage; *m/z* 310 is consistent with the partially hydrolyzed compound produced under heterolytic azo bond cleavage conditions seen as R^3 in the footnote of Table I. Disperse Orange 37 (**12**) possessed a structurally unknown impurity with the base peak at *m/z* 105; it was not detectable by HPLC–PB-MS.

HPLC–PB-MS

From most parent azo dyes it was possible to obtain molecular ion information and characteristic fragmentation patterns for structural elucidation. Figs. 2B–4B list EI mass spectra of solvent dyes, and Figs. 5B, 7 and 8 list mass spectra of more com-

[a] The UV absorption chromatograms of the azo dyes are not reported, and only the percentage of UV absorption is listed to estimate the relative content of the detected components under the assumption of similar molar absorptivities.

TABLE I

THERMOSPRAY AND PARTICLE BEAM MASS SPECTRA OF AZO DYES

t_R = Mean retention time in TIC chromatograms (min); M_r = calculated molecular mass; UV (%) = UV peak area based on chromatogram obtained with UV detection at 254 nm used for independent detection of the components and to estimate their approximate content; Unknown = structure unknown; N.D. = not detected by this method. Amount injected on HPLC column, 37 μg; solvent, methanol–water gradient.

No.	Dye	By-product or contamination[a]	M_r	t_R (min)	m/z (relative intensity, %)		UV (%)
					Thermospray	Particle beam	
1	Solvent Yellow 2		225	25.1	226(100); 227(15)	225(58); 148(11); 120(67); 77(100); 51(37)	95
		$C_6H_5N_2C_6H_4NHCH_3$	211	20.5	229(1); 212(100)	N.D.	4
2	Solvent Yellow 3		225	24.8	234(1); 226(100)	225(59); 134(14); 106(100); 91(21); 77(22); 75(14); 51(3)	90
		$C_6H_5N_2C_6H_4N(CH_3)_2$	225	20.4	234(1); 226(100)	N.D.	8
3	Solvent Yellow 14		248	29.8	252(1); 251(5); 250(32); 249(100)	248(90); 220(4); 219(8); 171(19); 143(100); 115(97); 105(2); 89(14); 77(40); 51(17)	97
4	Solvent Orange 7		276	41.6	279(11); 278(37); 277(100)	276(100); 248(12); 247(36); 171(10); 143(94); 115(83); 105(86); 77(43); 65(12)	90
		Unknown	281	25.0	282(100); 281(16)	N.D.	10
5	Disperse Red 1		314	30.4	315(100); 285(6)	314(15); 283(100); 255(4); 237(6); 133(30); 105(8); 76(6)	85
6	Disperse Orange 13		352	39.0	353(100); 264(5); 248(4)	352(98); 326(2); 263(9); 247(54); 231(32); 142(31); 121(54); 105(20); 93(100); 77(95); 65(44)	70[b]
7	Disperse Orange 25		323	30.4	324(100); 294(13); 181(4)	323(34); 308(4); 283(100); 253(10); 173(17); 133(38); 105(12); 104(18); 76(14)	84[b]
		Unknown	338	28.3	N.D.	338(5); 310(59); 243(8); 180(1); 158(100); 120(29)	2
8	Disperse Orange 44		382	26.2	400(1); 383(100); 353(17); 300(6)	382(14); 352(7); 342(29); 329(31); 299(33); 198(32); 158(29); 145(100); 105(62); 92(30); 65(28)	90[b]
9	Disperse Orange 30		449	29.9	452(63); 450(100); 416(19); 118(10); 90(11)	220(11); 218(22); 192(28); 190(36); 158(100); 144(64); 118(69); 109(95); 87(68); 77(90); 54(48)	60
		$RN(C_2H_4CN)(C_2H_4OH)$	407	26.4	412(18); 410(74); 408(100); 374(15); 227(18)	220(13); 218(26); 192(18); 190(26); 158(100); 144(96); 118(44); 109(65); 74(55); 54(44)	36

10	Disperse Black 9		300	19.8	301(100)	300(35); 282(1); 269(100); 225(13); 194(3); 192(17); 190(26); 149(12); 120(10); 92(24); 77(9); 65(12)	97
11	Disperse Blue 79		624	30.2	625(50); 547(24); 368(100); 353(36)	188(1); 87(15); 71(7); 57(100)	95
		R^1-$N(C_2H_4OCOCH_3)(C_2H_4OH)$ or R^2-NH_2	582	28.1[c]	600(1); 583(100)	N.D.	4
		R^3-NH_2	325		343(< 1); 326(100)	N.D.	
		R^3-H	310		328(5); 311(100)	N.D.	
12	Disperse Orange 37		391	33.6	394(71); 392(100)	391(24); 351(56); 194(< 1); 192(1); 190(2); 173(77); 133(100); 104(32); 77(20)	94
		Unknown		21.0	122(2); 105(100)	N.D.	5
13	Disperse Brown 1		432	29.3[c]	435(99); 433(100); 321(7)	434(15); 432(15); 403(100); 401(99); 357(20); 194(1); 192(7); 190(9); 183(81); 139(76); 138(27); 104(31)	87[b]
		Unknown		30.3[c]	N.D.	390(4); 367(19); 323(4); 239(6); 198(20); 170(55); 139(100); 104(31); 75(22)	N.D.
		Unknown		34.3	N.D.	239(4); 189(5); 176(14); 149(34); 140(18); 124(100); 98(54); 84(34); 74(34); 57(20)	2[b]
		Unknown		36.3		327(1); 267(8); 134(45); 112(31); 98(100); 84(68); 74(53); 57(42)	4[b]
		Unknown	186	19.2	188(29); 187(100)		4[b]
14	Acid Orange 10		452	5.0	409(8); 326(100); 239(15)	246(1); 245(2); 218(1); 167(14); 142(10); 124(16); 94(100); 93(50); 66(55)	95

[a] R = $(O_2N)Cl_2C_6H_2N_2C_6H_4$; R^1 = $(O_2N)_2BrC_6H_2N_2C_6H_2(NHCOCH_3)OCH_3$; R^2 = $(O_2N)_2BrC_6H_2N_2C_6H_2[(NHCOCH_3)_2]OCH_3$; R^3 = $C_6H_2[N(C_2H_4OCOCH_3)C_2H_5OH]OCH_3$.

[b] Dye content in commercial product below 50% (sample extracted to separate dye, and organic by-products from loading material).

[c] Not fully resolved peak in HPLC separation.

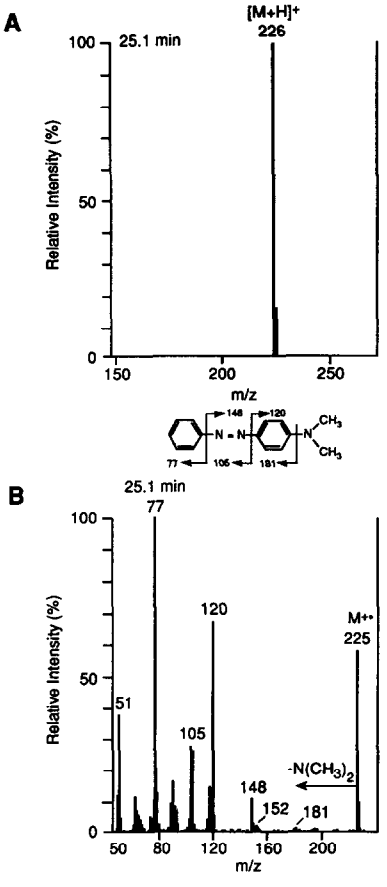

Fig. 2. (A) HPLC–TSP and (B) HPLC–PB mass spectra of Solvent Yellow 2 (**1**; M_r 225). Amount injected, 37 μg; solvent, methanol–water gradient.

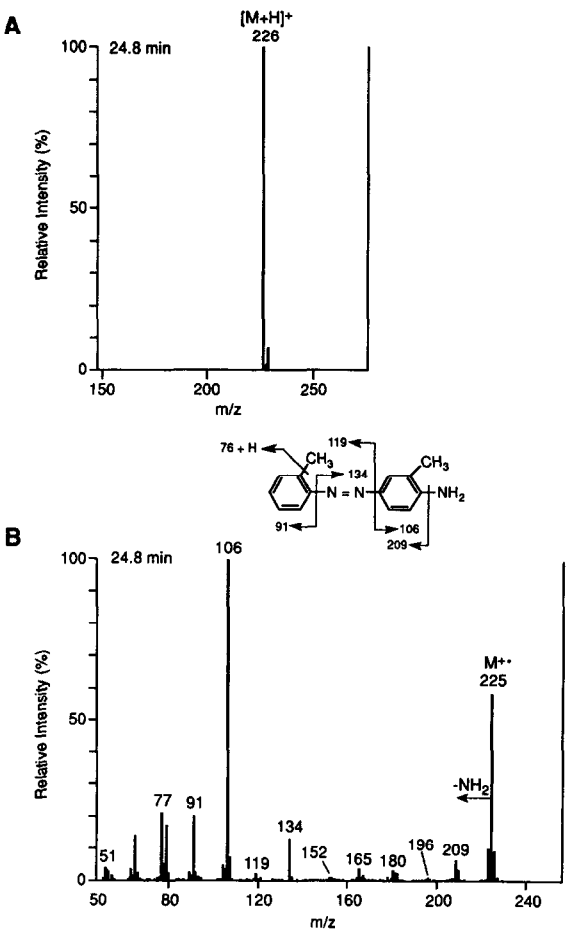

Fig. 3. (A) HPLC–TSP and (B) HPLC–PB mass spectra of Solvent Yellow 3 (**2**; M_r 225). Amount injected, 37 μg; solvent methanol–water gradient.

plex disperse dyes. The EI mass spectra of Disperse Orange 30 (**9**) and its degradation product provide no molecular ion information. Fig. 7 shows the TIC chromatogram and the fragment-rich EI mass spectra of the resolved components. Molecular mass information was only obtained by TSP-MS, as seen in Fig. 6. Disperse Blue 79 (**11**), which had the highest number of substituents of all the tested dyes, shows an easily detectable $[M+H]^+$ ion in the TSP spectrum, but the corresponding EI spectrum indicates strong fragmentation with no sign of the $M^{+\bullet}$ ion or nitro- and bromine-containing aromatic fragment ions. Only a cleaved tertiary amine fragment $[N(C_2H_4OCOCH_3)_2]^+$ with *m/z* 188 could be tentatively identified (*cf.*, Table I). A similar result was obtained on a triple-stage quadrupole instrument using a ThermaBeam EI interface [32]. The EI mass spectrum of Acid Orange 10 (**14**) indicated complete desulfonation (*m/z* 246) accomplished thermally in the mass spectrometer, as well as characteristic fragment ions of naphthol-containing azo dyes.

Particle beam EI spectral fragmentation of these azo dyes centers around cleavage of the azo linkage. Cleavage of the azo linkage under mass spectrometric conditions can occur in three ways: at the N–C bond on the coupler side of the azo linkage (the positive charge often remains with the coupler component) [13,17], at the C–N of the remaining aromatic fragment or at the double bond between

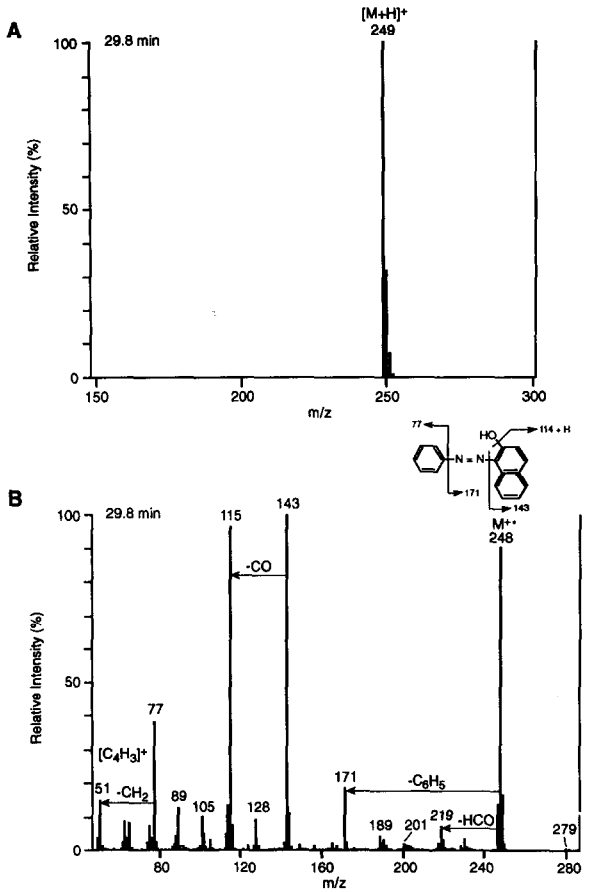

Fig. 4. (A) HPLC–TSP and (B) HPLC–PB mass spectra of Solvent Yellow 14 (**3**; M_r 248). Amount injected, 37 μg; solvent, methanol–water gradient.

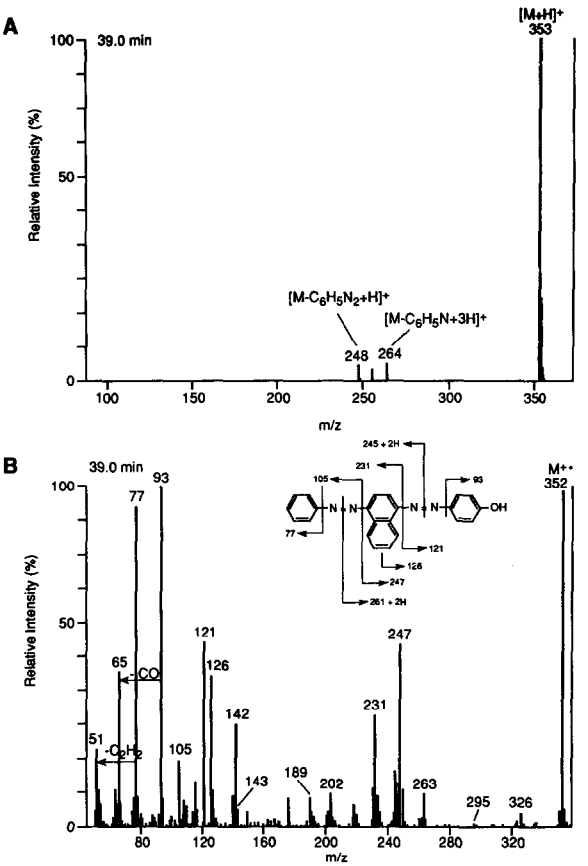

Fig. 5. (A) HPLC–TSP and (B) HPLC–PB mass spectra of Disperse Orange 13 (**6**; M_r 352). Amount injected, 37 μg; solvent, methanol–water gradient.

the two N atoms of the azo linkage. Cleavage of the C–N and N–C bonds on either side of the azo linkage has been observed previously in azo dyes [13,32] and in substituted azobenzene [13,42].

The EI mass spectrum of Solvent Yellow 2 (Fig. 2B) shows an $M^{+\bullet}$ ion at *m/z* 225, an N–C cleavage product at *m/z* 148 and a C–N product at *m/z* 120. The base peak at *m/z* 77 has been attributed to $[C_6H_5]^+$ and *m/z* 51 to the following fragment $[C_4H_3]^+$. The isomeric dye Solvent Yellow 3 shows an EI spectrum (Fig. 3B) with an $M^{+\bullet}$ ion at *m/z* 225, a $[CH_3H_2NC_6H_3N_2]^+$ ion at *m/z* 134 and a $[CH_3C_6H_4]^+$ ion at *m/z* 91. The base peak at *m/z* 106 has been tentatively proposed as $[CH_3C_6H_4NH]^+$. Such transfer of one hydrogen atom was previously reported in the secondary ion mass spectra of azo dyes [43]. The ions $[C_6H_5]^+$ and $[C_4H_3]^+$ at *m/z* 77 and 51 again indicate the presence of a phenyl group. The naphthol-containing azo dye Solvent Yellow 14 gives a very intense $M^{+\bullet}$ ion peak at *m/z* 248 in the EI spectrum (Fig. 4B). The spectrum illustrates a number of fragmentations characteristic of a hydroxyl group on an aromatic ring. The odd-electron $[M-CO]^+$ peak at *m/z* 220, which is accompanied by $[M-CHO]^+$ at *m/z* 219, is especially useful in recognizing this functional group. The peaks of N–C cleavage products $[HOC_{10}H_6N_2]^+$ and $[C_6H_5N_2]^+$ at *m/z* 171 and 105, respectively, are not very intense. The base peak at *m/z* 143 has been tentatively attributed to the C–N cleavage product $[HOC_{10}H_6]^+$. Again,

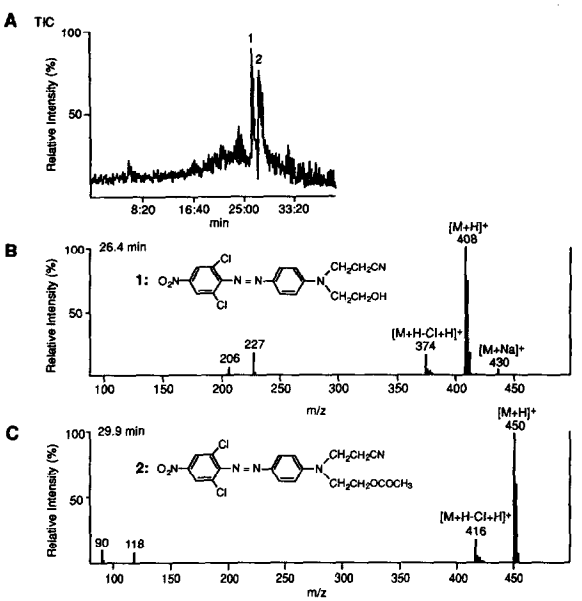

Fig. 6. (A) HPLC–TSP-MS TIC chromatogram of Disperse Orange 30 (**9**; M_r 449) and (B and C) corresponding TSP CI mass spectra. (B) Peak 1 = hydrolyzed decomposition product and (C) peak 2 = parent dye. Amount injected, 37 μg; solvent, methanol–water gradient.

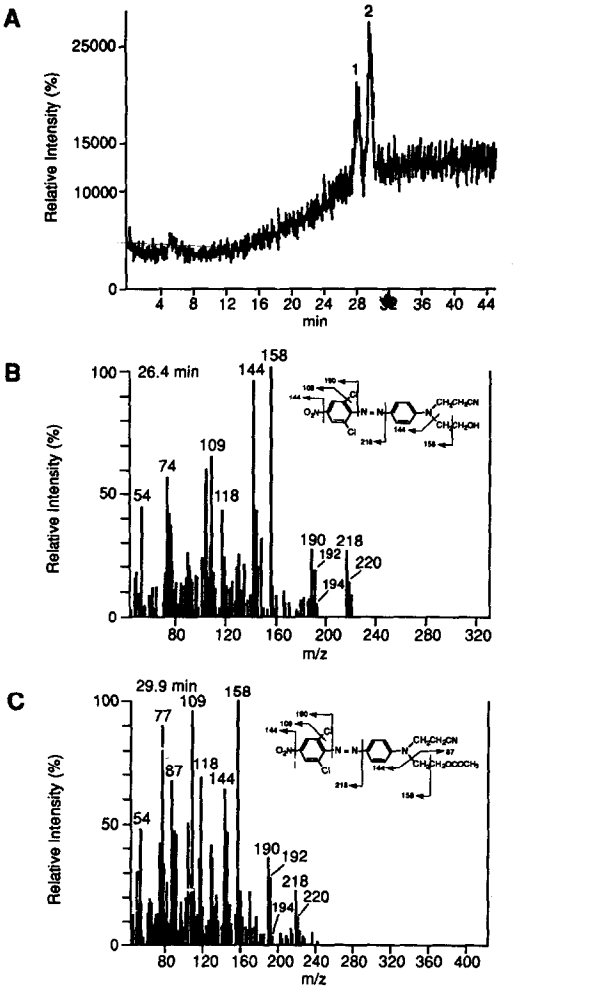

Fig. 7. (A) HPLC–PB-MS TIC chromatogram of Disperse Orange 30 (**9**; M_r 449) and (B and C) corresponding PB EI mass spectra. (B) Peak 1 = hydrolyzed decomposition product and (C) peak 2 = parent dye (no molecular ion information obtained). Amount injected, 37 μg; solvent, methanol–water gradient.

ions at *m/z* 77 and 51 indicate a phenyl fragment.

The $M^{+\bullet}$ ion at *m/z* 276 is the prominent ion of the naphthol-containing azo dye Solvent Orange 7 (**4**), followed by the characteristic C–N cleavage products $[HOC_{10}H_6]^+$ at *m/z* 143 and $[(CH_3)_2C_6H_3]^+$ at *m/z* 105. The charged N–C cleaved fragment $[HOC_{10}H_6N_2]^+$ with low intensity can be seen at *m/z* 171. Also, the characteristic loss of CO and HCO can be registered at *m/z* 348 and 347.

The EI mass spectrum (Fig. 5B) of the more complex diazo dye Disperse Orange 13 (**6**) presents fragment ions that occurred from cleavage of both azo linkages. Besides the C–N and N–C cleavage products ions at *m/z* 77, 93, 105, 121, 126 and 231, an ion at *m/z* 263 appears to come from cleavage between the two azo nitrogen atoms, with transfer of two hydrogen atoms to form the amine. This is an example of the fourth possibility to cleave a dye's azo bond under mass spectrometric conditions. Such a cleavage, with transfer of two hydrogen atoms, was previously reported in the EI mass spectra of 2-methoxyazobenzene [44] and diverse azo dyes [13,32]. The low-resolution measurements did not allow a clear distinction of isomeric fragment ions. The ion at *m/z* 247 could be formed either by a C–N cleavage reaction or by cleavage of the azo linkage with transfer of two hydrogen atoms. Additional high-resolution measurements confirmed the C–N cleavage product $[C_{10}H_6N_2C_6H_4OH]^+$ (deflection 1.1 ppm) seen in Fig. 5B.

Fragmentation β to the amine nitrogen usually leaves the charge with the amine moiety. If the amine substituent is $-CH_2CH_2X$, where X is OH, OR, OCOR or CN, β fragmentation is very common [13]. The mass spectra of dyes **5**, **10** and **13** show intense $[M-CH_2OH]^+$ ions. A similar β fragmentation mechanism forming $[M-CH_2CN]^+$ ions can be observed with dyes **7**, **8** and **12** (Table I). Charged fragments with strong electron-withdrawing substituents are not very stable and appear only with weak intensity in the mass spectrum. Usually, these peaks are not very characteristic of identification. In the PB mass spectrum of Disperse Orange 30 (**9**), the peak at *m/z* 190 implies the presence of the C–N cleavage product $[O_2N(Cl_2)C_6H_2]^+$. The fragmentation pattern *m/z* 190, 192 and 194 in the two mass spectra of Fig. 7 is consistent with the isotopic abundances for combinations of two chlorine atoms. Disperse Orange 37 (**12**) and Disperse Brown 1 (**13**), with the same phenyl substituent in the molecule, show a similar fragmentation pattern for this C–N cleavage product but with weaker intensity. In Disperse Orange 25 (**7**), a small amount of a structurally unknown impurity with $M^{+\bullet}$ at *m/z* 338 has been separated and detected. Disperse Orange 30 (**9**) contained a considerable amount (about one third of the content) of hydrolyzed by-product (mass peak number 1 in Fig. 7) with $M^{+\bullet}$ at *m/z* 582.

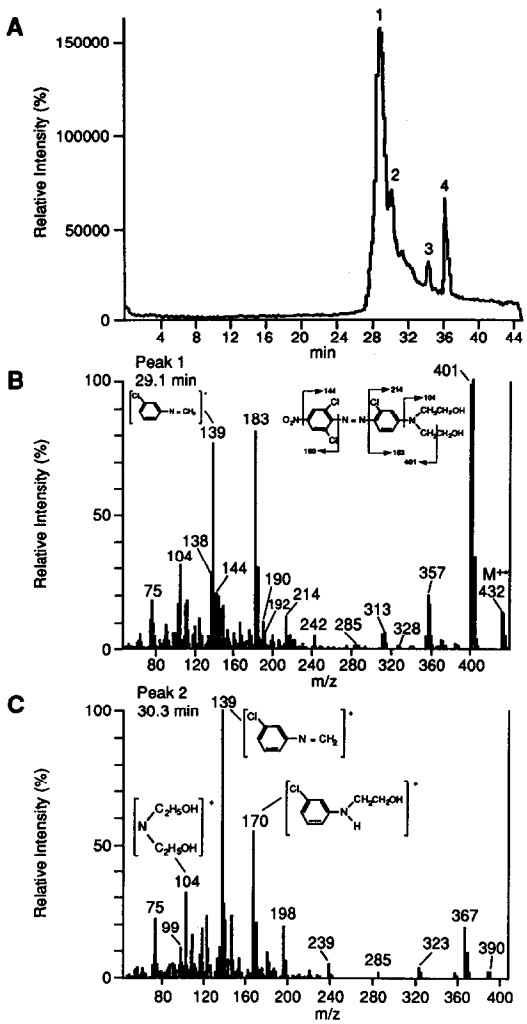

Fig. 8. (A) HPLC–PB-MS TIC chromatogram of Disperse Brown 1 (**13**; M_r 432) and (B and C) corresponding PB EI mass spectra. (B) Peak 1 = parent dye ($M^{+\bullet}$ = 432) and (C) peak 2 = decomposition product. Amount injected, 37 μg; solvent, methanol–water gradient.

Fig. 8 shows the TIC chromatogram of Disperse Brown 1 (**13**) and the corresponding PB mass spectra of two resolved components. The major peak number 1 at a retention time (t_R) of 29.1 min shows a mass spectrum with a relatively weak $M^{+\bullet}$ ion at *m/z* 432 and an intense β cleavage fragment $[M-CH_2OH]^+$ at *m/z* 401, as well as an intense C–N cleavage product at *m/z* 183. Additional high-resolution measurements confirm the molecular ion (deflection 4.0 ppm) and the structure of the *m/z* 139 ion (deflection 5.5 ppm). The mass spectrum of the shoulder peak at t_R 30.3 min could only be partially characterized. The structure of the *m/z* 139 ion was again confirmed by high-resolution measurements. The two smaller peaks, numbers 3 and 4 (mass spectra not shown), may come from plasticizer impurities. However, no accurate structural characterization was possible from the data obtained. Other dye samples analyzed in other investigations [45] showed similar structurally unidentifiable mass spectra in the same retention time range when they were stored in plastic containers for a long period.

Detection limits

The detection limits[a] of the dye standards were in

[a] The detection limits were calculated by multiplying the analyte concentration by the applied flow-rate and the total acquisition time used for the particular mass spectrum.

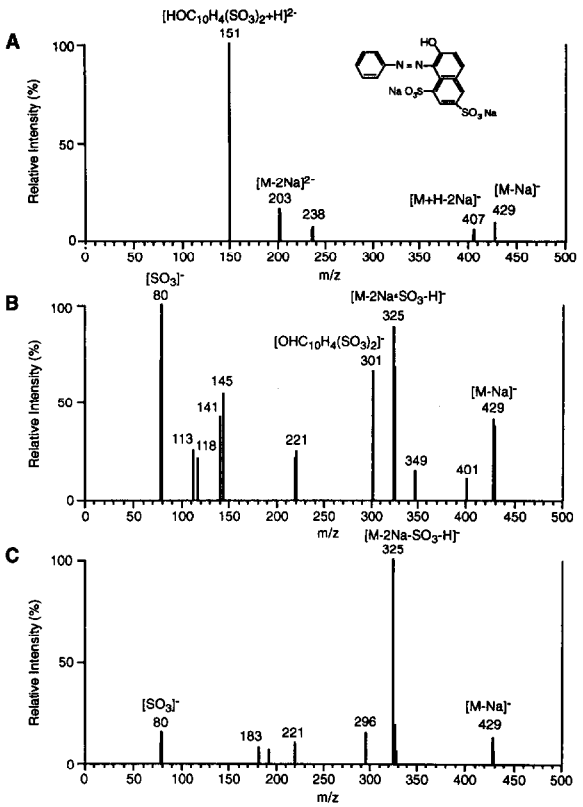

Fig. 9. Flow-injection–ESP mass spectra of 100 ng/μl Acid Orange 10 (**14**; M_r 452) in 2-propanol–water (1:1) at CAD voltages of (A) −100, (B) − 200 and (C) −300 V.

the range of 500 ng to 5 μg for HPLC–PB-MS recording the mass chromatogram of characteristic intense ions of the dyes under full mass scan and with a signal-to-noise ratio of at least 3:1. Measurements with HPLC-TSP-MS under full-scan MS conditions were at least two to three orders of magnitude more sensitive. Selected ion monitoring was not evaluated as a method of improving detection limits at this early experimental stage. More measurements will be needed to obtain more accurate information about detection limits of specific azo dyes.

ESP-MS

The disulfonated azo dye Acid Orange 10 (**14**) showed a weak $[MH-2Na+2H]^+$ ion in TSP ionization and underwent considerable thermal degradation in EI ionization (Table I). A promising alternative for the analysis of acid dyes is the relatively "soft" ESP ionization process [33–35]. Owing to the strong acid characteristics of this azo dye, ESP in the negative-ion operation mode was able to detect characteristic anions formed in the solution. The negative-ion ESP spectra in Fig. 9 were recorded under three representative capillary–skimmer potential differences of −100 V (A), −220 V (B) and −300 V (C). Lower potential differences minimized CAD energies resulting in the formation of singly charged ions $[M-Na]^-$ at *m/z* 429, $[M+H-2Na]^-$ ion at *m/z* 407 and a doubly charged $[M-2Na]^{2-}$ ion at *m/z* 203. The base peak at *m/z* 151 has been tentatively attributed to the C–N naphthol cleavage product $[HOC_{10}H_4(SO_3)_2+H]^{2-}$. Higher CAD energies (larger capillary-skimmer potential differences) resulted in more fragmentation with some losses in sensitivity. The peak at *m/z* 151 disappeared, and $[M-2Na-SO_3-H]^-$ ions at *m/z* 325 and SO_3^- ions at *m/z* 80 appeared from cleavage of the sulfonic group. At a capillary–skimmer potential difference of −300 V and higher, only the *m/z* 325 peak was present in the mass spectrum. Increasing the pH of the solution from 3.5 to 10 by adding 1% ammonia solution decreased the intensity of the base peak in the spectrum in Fig. 9A approximately fourfold. Also, a minor decrease of the *m/z* 407 peak was observed. The alkaline conditions did not affect the abundances of the other peaks in the mass spectrum. The ESP-MS detection limit[a] in negative ion operation under full-scan MS conditions was 10 ng.

CONCLUSIONS

It has been demonstrated that molecular mass information on azo dyes can be obtained by recording their CI mass spectra by using a TSP interface on a single quadrupole mass spectrometer. To gain useful structural information on these dyes, it is neces-

[a] In contrast to this, positive ESP ionization of the acid dye showed an almost 50-fold lower sensitivity. Only a weak sodium adduct $[M+Na]^+$ ion at *m/z* 475 has been tentatively identified at the corresponding positive potentials of 100, 240 and 300 V (mass spectra not shown).

sary to record their EI mass spectra with an HPLC–MS system using a PB interface. Only through the complementary application of both techniques could the azo dyes be satisfactorily characterized. In some instances, additional organic impurities could be identified by one or both of the techniques. These components were derived from decomposition of the parent dye, from the coupling reaction process or from external sources during the handling of the commercial products. Characteristic fragmentation in azo dyes included cleavage of the C–N and N–C bonds on either side of the azo linkage, and cleavage of the N=N double bond with transfer of one or two hydrogen atoms, to form an imine or amine, respectively. Most azo dyes showed a molecular ion, and fragmentation from ring cleavage was observed. Naphthol-containing azo dyes presented $[M-CO]^+$ and $[M-HCO]^+$ fragment ions, which are not very intense but characteristic of a hydroxyl group on an aromatic ring.

ESP ionization was successful in generating characteristic singly and doubly charged negative ions of the strongly acidic azo dye, Acid Orange 10 (**14**). A weak adduct between the molecule and a sodium ion $[M+Na]^+$ was the only positive ion identified for this azo dye.

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REFERENCES

1 M. S. Reisch, *Chem. Eng. News*, 66 (1988) 7.
2 W. C. Hueper, *Occupational and Environmental Cancers of the Urinary System*, Yale University Press, New Haven, CT, 1969.
3 *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 8, IARC, Lyon, 1975.
4 C. E. Searly (Editor), *Chemical Carcinogenesis (ACS Monograph No. 173)*, American Chemical Society, Washington, DC, 1976.
5 J. F. Robens, G. S. Diu, J. M. Ward, J. R. Joiner, R. A. Griesemer and J. F. Douglas, *Toxicol. Appl. Pharmacol.*, 54 (1980) 431.
6 M. Boeninger, *The Carcinogenicity and Metabolism of Azo Dyes, Especially Those Derived from Benzidine*, Publication No. 80-119, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, 1980.
7 H. S. Freeman, J. F. Esancy, K. P. Mills and W. M. Whaley, *Dyes Pigments*, 8 (1987) 417.
8 P. Rabin, *Nature (London)*, 199 (1963) 596.
9 R. L. Reeves, R. S. Kaiser and K. T. Finley, *J. Chromatogr.*, 47 (1970) 217.
10 R. L. Reeves, *J. Am. Chem. Soc.*, 97 (1975) 6019.
11 J. E. Scott, *Histochemie*, 29 (1972) 129.
12 K. H. Drexhage, in F. P. Schäfer (Editor), *Topics in Applied Physics*, Vol. 1, Springer, Berlin, 1971, p. 178.
13 K. Venkataraman (Editor), *The Analytical Chemistry of Synthetic Dyes*, Wiley, New York, 1977.
14 A. Shan, D. Harbin and C. W. Jameson, *J. Chromatogr. Sci.*, 26 (1988) 439.
15 A. L. Yergey, C. G. Edmonds, I. A. S. Lewis and M. L. Vestal, *Liquid Chromatography/Mass Spectrometry, Techniques and Applications*, Plenum Press, New York, 1990.
16 M. A. Brown (Editor), *Liquid Chromatography/Mass Spectrometry, Application in Agricultural, Pharmaceutical, and Environmental Chemistry (ACS Symposium Series*, No. 420), American Chemical Society, Washington, DC, 1990.
17 J. M. Bellard and L. D. Betowski, *Org. Mass Spectrom.*, 21 (1986) 575, and references cited therein.
18 T. Covey and J. Henion, *Anal. Chem.*, 55 (1983) 2275.
19 L. D. Betowski and J. M. Ballard, *Anal. Chem.*, 56 (1984) 2604.
20 R. D. Voyksner, *Anal. Chem.*, 57 (1985) 2600.
21 L. D. Betowski, S. M. Pyle, J. M. Ballard and G. M. Shaul, *Biomed. Environ. Mass Spectrom.*, 14 (1987) 343.
22 J. Yinon, T. L. Jones and L. D. Betowski, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 445.
23 R. D. Voyksner, T. W. Pack, C. A. Haney, H. S. Freeman and W.-N. Hsu, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 1079.
24 J. Yinon, T. L. Jones and L. D. Betowski, *Rapid Commun. Mass Spectrom.*, 4 (1990) 245.
25 A. Groepplin, M. W. Linder, K. Schellenberg and H. Moser, *Rapid Commun. Mass Spectrom.*, 5 (1991) 203.
26 C. Lindberg and J. Paulsen, *J. Chromatogr.*, 394 (1987) 117.
27 W. H. McFadden and S. A. Lammert, *J. Chromatogr.*, 385 (1987) 201.
28 W. H. McFadden, D. A. Garteiz and E. G. Siegmund, *J. Chromatogr.*, 394 (1987) 101.
29 R. C. Willoughby and R. F. Browner, *Anal. Chem.*, 56 (1984) 2626.
30 R. C. Willoughby and F. Poeppel, presented at the *35th Annual Conference on Mass Spectrometry and Allied Topics*, Denver, CO, USA, May 24–29, 1987, p. 289.

31 K. Vekey, D. Edwards and L. F. Zerelli, *J. Chromatogr.*, 488 (1989) 73.
32 J. Yinon, T. L. Jones and L. D. Betowski, *J. Chromatogr.*, 489 (1989) 75.
33 A. P. Bruins, T. R. Covey and J. D. Henion, *Anal. Chem.*, 59 (1987) 2642.
34 A. P. Bruins, L. O. G. Weidolf, J. D. Henion and W. L. Budde, *Anal. Chem.*, 59 (1987) 2647.
35 E. D. Lee, W. Mück, J. D. Henion and T. R. Covey, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 844.
36 C. M. Whitehouse, R. N. Dreyer and J. B. Fenn, *Anal. Chem.*, 57 (1985) 675.
37 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.*, 9 (1990) 37.
38 R. D. Voyksner, J. T. Bursey and E. D. Pellizzari, *Anal. Chem.*, 56 (1984) 1507.
39 *Thermospray LC–MS Instruction Manual for Model 701C Edition Finnigan 4500*, Vestec, Houston, TX, 1988.
40 R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991.
41 *Beilstein's Handbuch der Organischen Chemie*, Springer Verlag, Berlin, Vol. 16, 1993, pp. 168, 300, 312, 344; Vol. 16 (2), 1951, p. 53; Vol. 16 (4), 1986, p. 460. Chem. Abstr. Registry Nos. 3118-97-6; 1936-15-8; 60-11-7; 97-56-3; 6253-10-7; 2872-52-8; 3148-56-1; 842-07-9.
42 J. C. Gilland, Jr., and J. S. Lewis, *Org. Mass Spectrom.*, 9 (1974) 1148.
43 S. M. Scheifers, S. Verma and R. G. Cooks, *Anal. Chem.*, 55 (1983) 2260.
44 J. H. Bowie, G. E. Lewis and R. G. Cooks, *J. Chem. Soc.*, B, 621 (1961).
45 R. D. Voyksner, R. Straub and J. T. Keever, Research Triangle Institute, unpublished results.