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PARTICLE BEAM LIQUID CHROMATOGRAPHY–ELECTRON IMPACT MASS SPECTROMETRY OF DYES[a]

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SUMMARY

A liquid chromatograph was interfaced with a triple quadrupole mass spectrometer by means of a particle beam-type interface. The system was used for the analysis and characterization by electron impact mass spectra of a series of commercial dyes. The pure dyes were separated from their impurities with a reversed-phase C_{18} column using methanol–water as the mobile phase. Detection limits were determined using the system as a single quadrupole mass spectrometer. Sensitivity for dyes was found to be two to three orders of magnitude worse than with thermospray ionization using a wire repeller. Characterization of the azo dyes could be achieved by observing typical fragment ions formed by cleavage of the N–C and C–N bond on either side of the azo linkage and/or cleavage of the N = N double bond with transfer of two hydrogen atoms to form an amine.

INTRODUCTION

Dyestuffs are of major environmental interest because of their widespread use as colorants in a variety of products, such as textiles, paper, leather, gasoline and foodstuffs. Synthetic intermediates, byproducts and degradation products of these dyes could be potential health hazards owing to their toxicity and/or carcinogenicity.

Several methods have been developed for the identification and determination of these dyes in order to monitor environmental contamination. Thermospray high-performance liquid chromatography–mass spectrometry (TSP-LC–MS) has been found to be a suitable technique for the analysis of the non-volatile dyes[1–7]. It is sensitive, specific and the ionization process is soft. One of the drawbacks of TSP-LC–MS is that one obtains mainly molecular and adduct ions and this information

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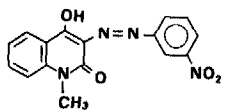
AZO CLASS



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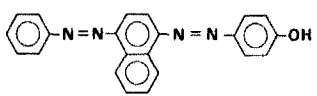

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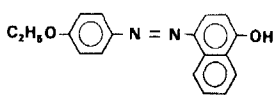

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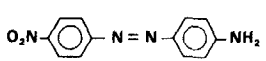

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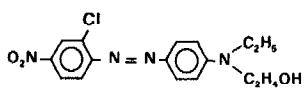

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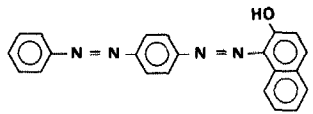

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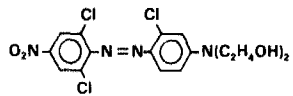

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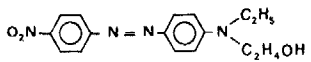

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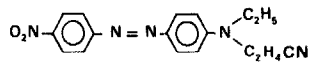

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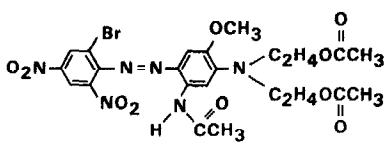
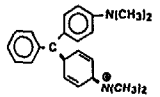


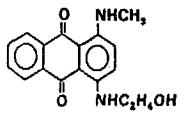
10. Disperse Blue 79
C.I. None

ARYLMETHANE CLASS



11. Basic Green 4
C.I. 42000

ANTHRAQUINONE CLASS



12. Disperse Blue 3
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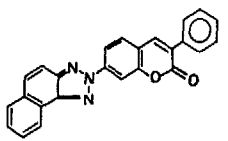
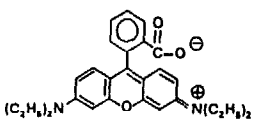
COUMARIN CLASS



13. Fluorescent Brightener 236
C.I. None

XANTHENE CLASS



14. Solvent Red 49
C.I. 45170.1

may not be sufficient for structural elucidation of dyes of unknown structure. While tandem mass spectrometry (MS–MS) has been used to deconvolute fully the structural information contained in the TSP mass spectra of dyes[1,3], it would be of great utility to analyze dyes with a single quadrupole LC–MS system and obtain structural information.

In order to assess the characteristics of such a system, we have interfaced a liquid chromatograph by means of an Extrel ThermaBeam LC–MS interface to a Finnigan quadrupole mass spectrometer. The ThermaBeam is a particle beam-type interface, using a solute transport–enrichment technique, between the chromatograph and the mass spectrometer (PB-LC–MS)[8]. In the ThermaBeam interface, a thermal concentric nebulizer is used which combines nebulization and desolvation. While the nebulization process serves to increase the surface area per unit mass of the liquid effluent, the desolvation process serves to evaporate the volatile solvent components in the effluent, while leaving the solute components in the particulate state. The resulting aerosol is accelerated through two stages of pressure reduction and axial nozzles, to produce a solvent-depleted particle beam, exploiting the momentum differences in the expanding aerosol beam.

The PB-LC–MS system was used for the analysis of a series of commercial dyes, including detection limits and electron impact (EI) mass spectra. The system was mostly used as a single quadrupole mass spectrometer.

EXPERIMENTAL

Materials

The following dyestuffs, identified by their Color Index (C.I.), name and number, were obtained from the sources indicated and were used without further purification: 1–6, 8, 9, 11 and 14 (Aldrich, Milwaukee, WI, U.S.A.); 7, 10 and 13 (Sandoz Colors and Chemicals, Charlotte, NC, U.S.A.); and 12 (Ciba Geigy Dyestuffs and Chemicals Division, Greensboro, NC, U.S.A.).

Dyes were dissolved in an appropriate solvent prior to analysis: dyes 1, 3–5, 7, 9 and 14 in acetonitrile–water (50:50), 2, 8, 10 and 11 in methanol, 6 and 12 in acetonitrile and 13 in methylene chloride–acetonitrile (50:50).

Instrumentation

The mass spectrometer was a Finnigan-MAT Triple Stage Quadrupole (TSQ) equipped with a 4510 EI source. The PB-LC–MS interface was an Extrel Corporation ThermaBeam interface, fitted to the ion source by a laboratory-made adaptor as shown in Fig. 1. This heatable adaptor, made mainly of Vespel (DuPont) and partly

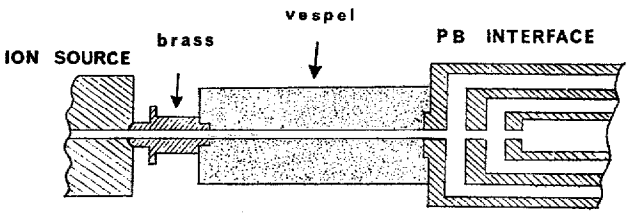


Fig. 1. PB-LC–MS interface adapter.

of brass, was heated to 200–250°C. The ion source was operated at 240°C. The filament emission current was 0.3 mA, electron energy 70 eV and electron multiplier voltage 1600 V. The preamplifier sensitivity was set at 10^{-8} A/V. The ThermaBeam nebulizer temperature was 210–240°C; this temperature varied with the composition of the mobile phase gradient. The ThermaBeam expansion chamber temperature was 95°C. The HPLC system consisted of a Spectra-Physics SP8700XR solvent-delivery system with a Rheodyne Model 7125 injector valve fitted with a 10-μl sample loop. A Varian MicroPak MCH-5-N-CAP C_{18} column (15 cm × 4 mm I.D.) was used. The chromatograph was operated in the gradient mode, starting at a mobile phase of methanol–water (50:50), changing within 5 min to 100% methanol and staying at that level for 10 min. The flow-rate was 0.9 ml/min.

RESULTS AND DISCUSSION

Because the commercial dyes were not pure, each was injected separately into the HPLC system in order to separate the pure dye from the impurities. The limits of detection were obtained by recording the mass chromatograms of characteristic ions of the dyes under full-mass scan, with a signal-to-noise ratio of at least 3:1. Table I presents the detection limits for each dye and their reported commercial purity levels. These detection limits were not corrected for the pure dye content of the materials analyzed and should therefore be regarded as an upper limit that can be obtained for commercial dyestuffs.

The sensitivity of the system was checked by analyzing caffeine. The detection limit was found to be 5 ng, which is in accordance with the Extrel ThermaBeam LC–MS specifications.

Fig. 2–7 show examples of mass chromatograms of some of the dyes. Table II presents a list of the ions observed in the EI mass spectra of the pure dues. Several fragmentation patterns were observed, thus forming characteristic ions: cleavage of

TABLE I

DETECTION LIMITS FOR COMMERCIAL DYES

Dye	*Structure*	*Mol.wt.*	*Dye content (%)*	*Detection limit (μg)*
Disperse Yellow 5	1	324	~30	0.5
Disperse Orange 13	2	352	~15	5.5
Solvent Red 3	3	292	100	0.2
Disperse Orange 3	4	242	~20	0.05
Disperse Red 13	5	348	~25	4.4
Solvent Red 23	6	352	~85	0.5
Disperse Brown 1	7	432	~25	2.7
Disperse Red 1	8	314	~30	0.25
Disperse Orange 25	9	323	~20	0.5
Disperse Blue 79	10	624	100	0.3
Basic Green 4	11	329	~98	0.7
Disperse Blue 3	12	296	~20	0.8
Fluorescent Brightener 236	13	389	100	0.6
Solvent Red 49	14	442	~97	0.5

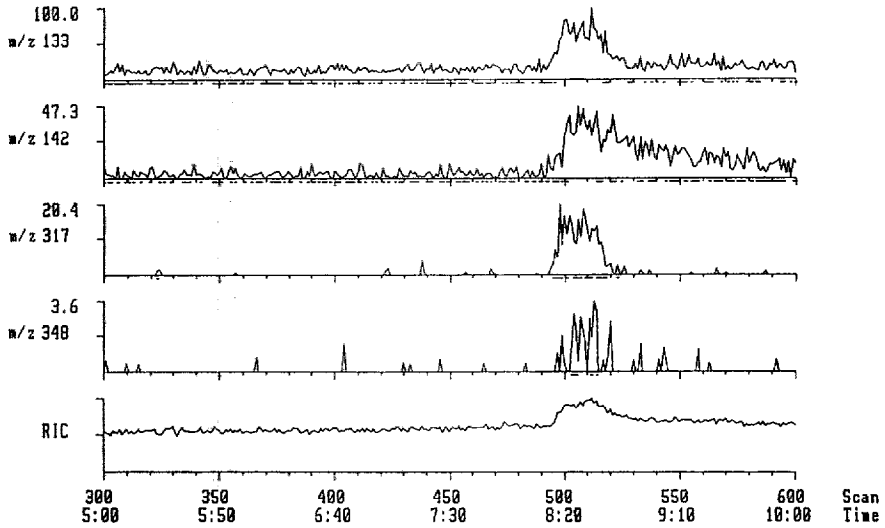


Fig. 2. Reconstructed mass chromatograms and reconstructed total ion current chromatogram (RIC) of Disperse Red 13. Amount injected into the LC–MS system, 4.4 μg. Solvent, acetonitrile–water (50:50).

the double bond between the two N atoms of the azo linkage with transfer of two hydrogen atoms to form an amine. Such cleavage with transfer of one hydrogen atom was reported previously in the secondary ion mass spectra (SIMS) of azo dyes[9], and with transfer of two hydrogen atoms in the EI mass spectrum of 2-methoxyazoben-

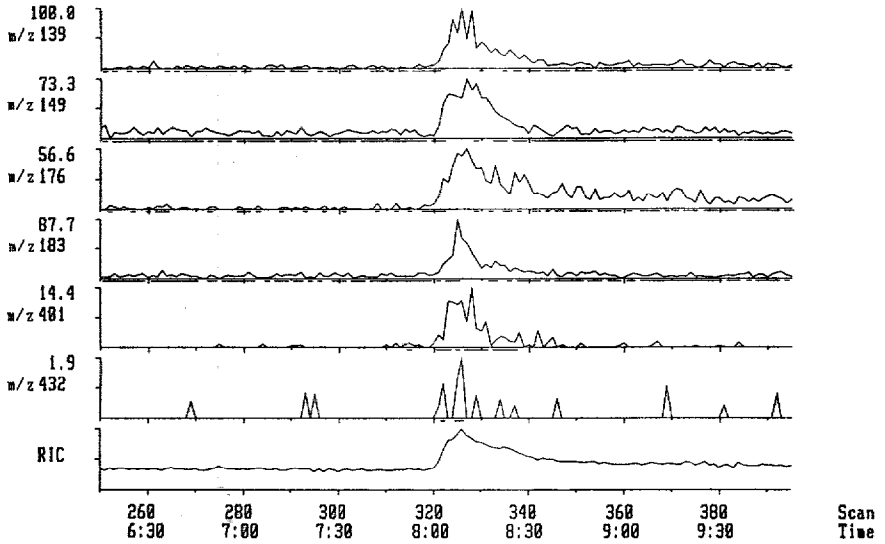


Fig. 3. Reconstructed mass chromatograms and RIC of Disperse Brown 1. Amount injected into the LC–MS system, 10 μg. Solvent, acetonitrile–water (50:50).

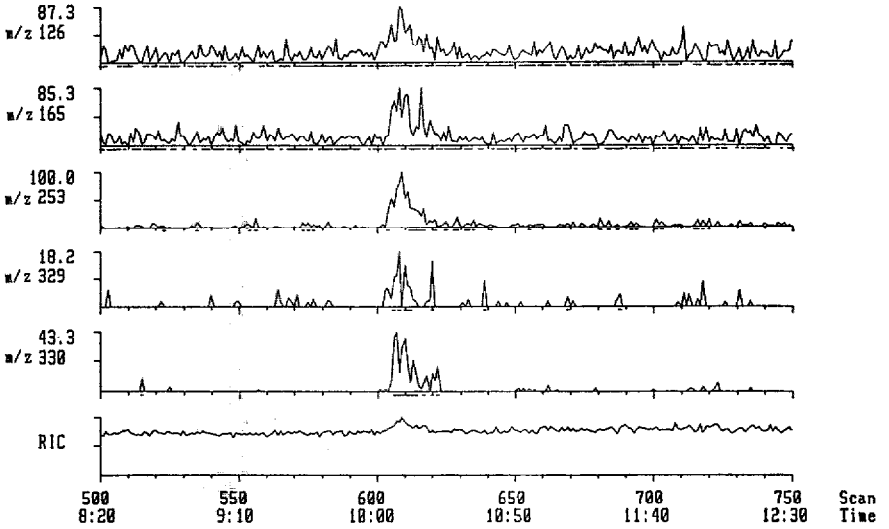


Fig. 4. Reconstructed mass chromatograms and RIC of Basic Green 4. Amount injected into the LC–MS system, 1.4 μg. Solvent, methanol.

zene[10] and in the EI mass spectra of azo dyes[11]. Formed by this process are the ion at *m/z* 138 in Disperse Yellow 5, the ion at *m/z* 93 in Disperse Orange 13, the ion at *m/z* 142 in Disperse Red 13, the ions at *m/z* 197 and 120 in Solvent Red 23, the ions at *m/z* 206 and 176 in Disperse Brown 1, the ions at *m/z* 149 and 108 in Disperse Red 1 and the ions at *m/z* 189, 149, and 108 in Disperse Orange 25. Other characteristic frag-

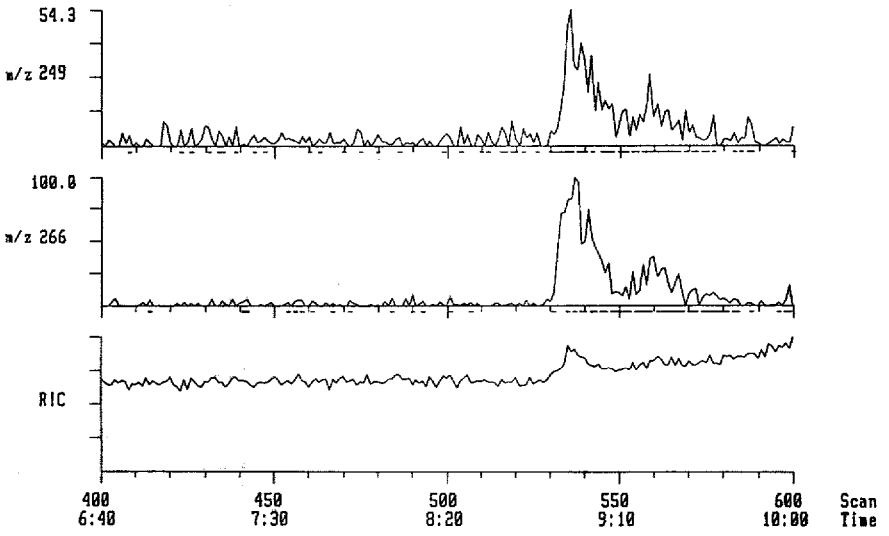


Fig. 5. Reconstructed mass chromatograms and RIC of Disperse Blue 3. Amount injected into the LC–MS system, 0.8 μg. Solvent, acetonitrile.

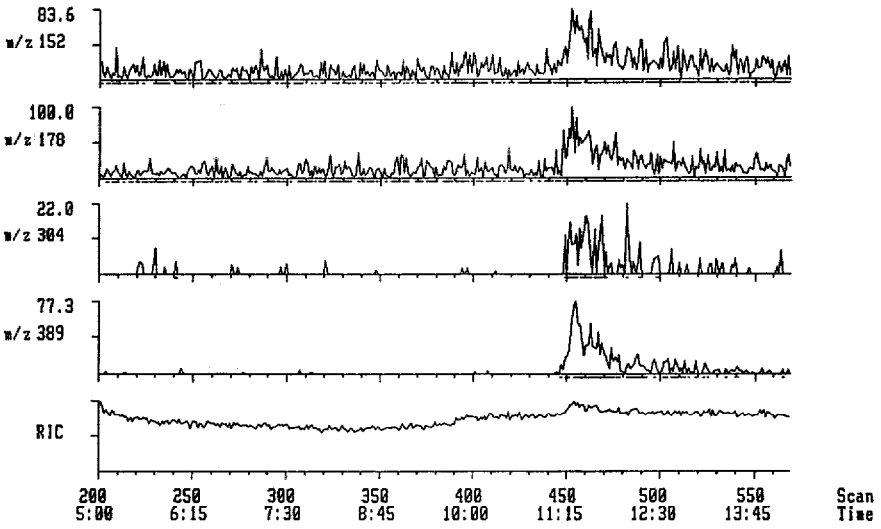


Fig. 6. Reconstructed mass chromatograms and RIC of Fluorescent Brightner 236. Amount injected into the LC–MS system, 0.6 μg. Solvent, methylene chloride–acetonitrile (50:50).

mentations observed in azo dyes were cleavage of the azo C–N bond and the N–C bond on either side of the azo linkage. Both these cleavages were previously observed in azo dyes[11] and in substituted azobenzenes[11,12]

An interesting mass spectrum was observed for Basic Green 4, a cationic dye (Fig. 7). The EI mass spectrum includes an ion at m/z 329 which is the molecular

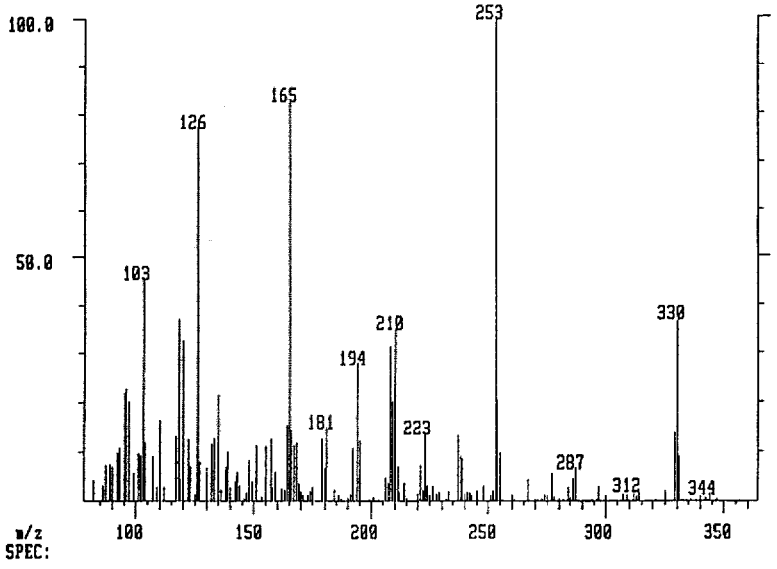


Fig. 7. EI mass spectrum of Basic Green 4, obtained by PB-LC–MS. Amount injected, 1.4 μg. Solvent, methanol. The spectrum was obtained by taking average of sums of spectra of scans 603–612 in the RIC in Fig. 4.

TABLE II

PARTICLE BEAM EI MASS SPECTRA OF DYES

Dye	*Mol.wt.*	*m/z of ions observed (% relative abundance)*
Disperse Yellow 5	324	324(1); 295(1.5); 202(3); 174(7); 138(9); 108(100); 92(17)
Disperse Orange 13	352	352(2); 247(10); 142(26); 115(22); 109(22); 93(100)
Solvent Red 3	292	292(17); 263(3); 235(4); 171(6); 149(9); 143(100); 121(48); 115 (36); 108(18)
Disperse Orange 3	242	242(3); 213(4); 212(10); 120(55); 92(100)
Disperse Red 13	348	317(22); 287(20); 154(17); 144(25); 142(28); 134(25); 133(100); 126(40); 120(30); 105(50); 104(50); 99(20); 92(32); 90(40)
Solvent Red 23	352	352(4); 267(1.5); 197(20); 143(30); 120(46); 115(32); 108(11); 93 (40); 92(100)
Disperse Brown 1	432	432(1.5); 403(15); 402(5); 401(17); 359(5.5); 357(7); 313(5); 214 (15); 208(17); 206(36); 185(40); 183(78); 176(39); 167(32); 149 (77); 139(100); 124(49); 104(82); 90(48)
Disperse Red 1	314	314(4); 297(2); 283(34); 267(11); 253(19); 237(8); 207(9); 180(15); 168(18); 149(15); 147(18); 133(100); 120(49); 108(63); 105(55); 103(47)
Disperse Orange 25	323	323(1); 293(12); 283(7); 253(26); 240(9); 224(3); 189(3); 173(10); 149(20); 133(35); 120(62); 108(31); 105(19); 104(20); 93(18); 92 (100)
Disperse Blue 79	624	87(100)
Basic Green 4	329	330(38); 329(13); 287(8); 255(10); 254(21); 253(100); 237(13); 223 (12); 210(35); 209(20); 208(32); 194(29); 181(15); 165(82); 135 (22); 126(78); 120(32); 118(37); 103(45); 95(22)
Disperse Blue 3	296	267(12); 266(100); 249(49); 234(28); 220(22); 204(11); 194(10); 181(8); 180(9); 165(17); 164(13); 152(22); 139(12); 124(15); 110 (13); 104(19)
Fluorescent Brightener 236	389	390(29); 389(100); 361(19); 333(11); 304(19); 207(75); 206(28); 195(38); 181(26); 180(18); 179(43); 178(82); 165(18); 152(78); 151 (47); 139(35); 127(35); 114(21); 105(30); 102(57)
Solvent Red 49	442	399(18); 398(34); 397(26); 327(18); 326(100); 282(18); 199(20); 184(40); 177(23); 170(23); 163(20); 162(18); 156(32); 149(48); 142 (20); 105(16); 91(19)

cation. The ion at m/z 330, $(M')^+$, is the ion representing the leuco compound, that is, the reduced neutral form of the cationic dye generated by the addition of a hydride ion to the cation. The two ions at m/z 165 and 126 are the doubly charged ions M'^{2+} and $[M'-C_6H_6]^{2+}$, respectively. This was substantiated by recording the collision activated dissociation (CAD) spectra of these two ions. The CAD spectrum of the ion at m/z 165 produces a single ion at m/z 126, which can be explained by the loss of C_6H_6 from the doubly charged M'^+ ion. The CAD spectrum of the ion at m/z 126 is shown in Fig. 8. The most intense daughter ion is at m/z 118. The loss of eight apparent mass units can be explained only by both ions being doubly charged, and therefore the ion at m/z 118 is due to the loss of CH_4 (16 mass units), thus forming the $[M'-C_6H_6-CH_4]^{2+}$ ion.

The EI mass spectra of dyes obtained with the PB-LC–MS system are similar to the EI spectra obtained by a solid probe in the contents of the fragment ions, but not necessarily in their relative intensities. An example is shown in Figs. 9 and 10, which

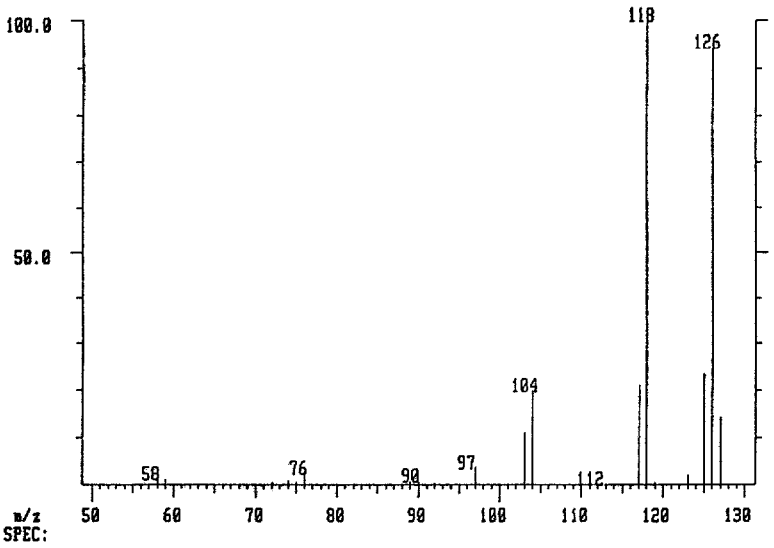


Fig. 8. CAD spectrum of the ion at m/z 126 in Basic Green 4.

show the EI mass spectra of Solvent Red 3 obtained by PB-LC–MS and with the solid probe, respectively. In PB-LC–MS the dye was injected through the HPLC column and therefore the EI mass spectrum obtained was of the pure compound. When using the solid probe, the sample included the dye in addition to the impurities. The mass spectrum therefore does not include the lower mass range because of the impurity background.

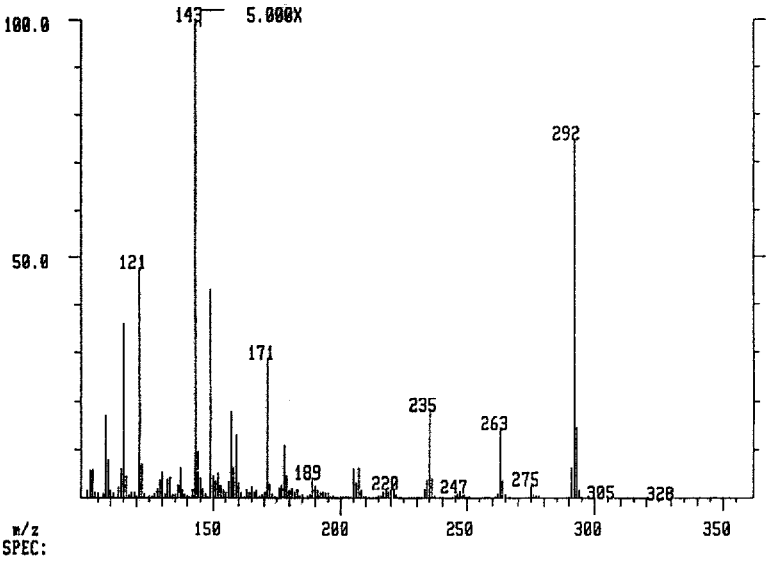


Fig. 9. PB-LC–MS EI mass spectrum of Solvent Red 3. Amount injected, 3.6 μg. Solvent, acetonitrile–water (50:50).

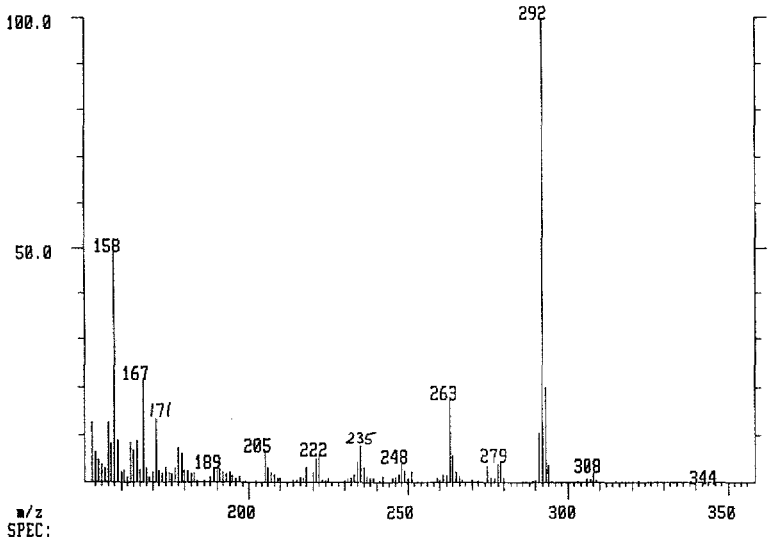


Fig. 10. Solid probe EI mass spectrum of Solvent Red 3.

CONCLUSIONS

It has been demonstrated that structural information on dyes can be obtained by recording their EI mass spectra with an LC–MS system, using a PB interface. However, the sensitivity for dyes was about two to three orders of magnitude worse than in TSP ionization with a wire repeller[7]. This difference in sensitivity is probably due to the two pumping stages in the PB interface where part of the sample is being pumped away. Also, whereas in TSP ionization most of the ion intensity is concentrated in a single ion, usually the MH^+ ion, in PB-EI ionization the ion intensity is divided amongst a large number of fragment ions.

Characteristic EI fragmentations in azo dyes included cleavages of the N–C and C–N bonds on either side of the azo linkage and cleavage of the N=N double bond with transfer of two hydrogen atoms to form an amine. The mass spectra of most azo dyes contained a small molecular ion or none at all. With all the azo dyes no fragmentation resulting from ring cleavage was observed.

The sensitivity for Disperse Orange 3 was better than that for the other dyes, probably beause of its amino group. Fragmentation of other dyes involved first a rearrangement to form such an amino group.

The mass spectrum of Basic Green 4, which is a cationic dye belonging to the arylmethane class, included a molecular ion and an $(M+H)^+$ ion, generated by the addition of a hydride ion to the cation. Two major doubly charged fragment ions were observed in the mass spectrum of this dye.

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