EPA/600/A-95/072

LC/MS TECHNIQUES FOR THE ANALYSIS OF DYES

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1. 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, by-products, and degradation products of these dyes could be potential health hazards because of their toxicity or carcinogenicity.

The analysis of dyes poses special problems for the chemist. The dyes do not belong to one group of chemical compounds, but encompass many chemical functionalities, ranging from mostly ionic to purely covalent. The analysis of such a large variety of compounds poses difficulties because of large differences in solubility, volatility, ionization efficiency, etc. A semantic problem often leading to confusion in the analysis of dyes is the difference between dye classification and dye use. Dye classification is based on the major functionality of the dye: azo, anthraquinone, polymethine, phthalocyanine, sulfur, arylmethane, stilbene, and coumarin being the main classes. The use of a dye generally refers to the manner in which the dye is applied. Some of the more common applications are in acidic or basic media, as mordants, lakes, pigments, solvents, or dispersants.

As an additional complication, some of the manufacturing precursors to dyes are carried over to, and are not removed from, the final dye product. The result is a complex mixture characterized not only by the dye itself, but also by several other compounds. Most dyes, including sulfonated azo dyes, are nonvolatile or thermally unstable, and therefore are not amenable to gas chromatography (GC) or gas phase ionization processes. Therefore, GC/MS techniques cannot be used.

Several desorption ionization methods have been used for the analysis of dyes:

a. Field Desorption (FD) Mass Spectrometry [1-3].

b. Secondary Ion Mass Spectrometry (SIMS) [4-7]

c. Californium-252 Plasma Desorption Mass Spectrometry (PDMS) [8,9].

d. Fast Atom Bombardment (FAB) Mass Spectrometry [10-14].

e. Laser Desorption Mass Spectrometry [15].

Because of low sample purity, modern complex dyes cannot be analyzed using the above mentioned direct probe techniques. However, the combination of liquid chromatography with mass spectrometry (LC/MS) enables the separation of nonvolatile, thermally unstable, and polar dyes for introduction into the mass spectrometer for identification. Significant advances in combining LC and MS have occurred in recent years and have been extensively reviewed [16- 19].

Most of the earlier work on LC/MS focussed on the incompatibility of LC mobile phase flows and the vacuum requirements of the mass spectrometer. An aqueous reversed-phase LC mobile phase at a flow rate of 1 mL/min can generate 1- 4 liters of gas when introduced into a mass spectrometer at 10^{-6} torr. This exceeds the operational requirements of most MS systems. In addition, the thermal lability or low volatility of the analytes may impede their transformation into the vapor state and subsequent ionization by electron ionization (EI) or chemical ionization (CI). As a result of research in interfacing LC with MS, three major types of interfaces and LC/MS techniques have been developed:

a. Thermospray (TS).

b. Particle Beam (PB).

c. Ion spray and Electrospray (ES).

These LC/MS methods have been applied in a large variety of analytical problems. This chapter will describe the application of these LC/MS techniques in the analysis of dyes.

2. Thermospray (TS) - LC/MS

2.1. Principles of Operation

Thermospray is a widely accepted technique because it can handle most conventional LC solvents and flow rates, as well as provide a means to gently ionize most nonvolatile or thermally unstable samples. It has good sensitivity, within a factor of 10 to that of GC/MS. The interface is commercially available for most mass spectrometers and is simple to use.

The techniques and mechanisms of thermospray have been reviewed [19]. The aqueous solution of the sample contains a volatile electrolyte (typically, ammonium acetate) at concentrations near 10^{-1} M. The TS interface consists of a vaporizer, where the mobile phase is heated to form a high-velocity spray. As a result of the statistical distribution of ions in this spray, some of the micrometer-size droplets of the spray are electrically charged. The high electric field induces desorption of performed ions from the liquid solution into the gas phase. These ions could result from the solute molecule by protonation or addition of solvent cluster ions. The primary ions produced in the TS process are identical with those produced in solution: in ammonium acetate solution, the ions are NH_4^+ and $CH_3CO_2^-$, and clusters of these ions with water, ammonia, and acetic acid. Equal amounts of positive and negative ions are produced. The droplets enter the source, where the ions are extracted through the ion exit cone, while neutral molecules go to a cold trap connected to

a mechanical vacuum pump. This extraction process allows the introduction of a total of 1-2 mL/min of LC effluent, while maintaining a pressure of 10^{-5} torr in the mass spectrometer.

The TS interface can accept flow rates as low as 0.1 mL/min through the addition of solvent post-column to result in a total flow rate of 1 mL/min. It can accommodate most solvents used in normal or reversed-phase LC and any volatile buffer. This is the only interface that operates optimally under highly aqueous conditions, with best sample ion currents at 100% water [20]. The interface can be operated smoothly through a solvent gradient LC analysis if the vaporizer temperature is adjusted to compensate for changes in the heat of vaporization of the changing LC solvent. Buffers necessary for thermospray ion formation do not have to interfere with the LC chromatographic separation because they can be added post-column [21], resulting in optimal LC and MS operation.

Thermospray is both an ionization and enrichment technique. Ions may be produced by CI, initiated by a filament or discharge, or through ion evaporation. The major disadvantage of thermospray is that the ionization occurs in the solvent at a relatively high source pressure of at least 1 torr. As a result, electron ionization (EI) cannot be used. The spectra, therefore, cannot be compared with those of the readily accessible, commercially available EI libraries.

2.2. Applications to Dye Analysis

Betowski and Ballard [22] used tandem mass spectrometry in conjunction with a TS interface to elucidate the structure of Basic Red 14. The instrument was a Finnigan MAT Triple Stage Quadrupole modified for thermospray ionization. The HPLC consisted of a Rheodyne Model 7125 injector valve and a Waters 6000A solvent delivery system. The column was a Brownlee RP-2, 10cm x 4.6mm I.D.,

analytical cartridge column. Aqueous 0.1 M ammonium acetate/methanol (93:3 v/v) at a flow rate of 1.3 mL/min was used as TS buffer

The TS positive ion mass spectrum of Basic Red 14 generated a base peak of m/z 344 that was construed to be the parent ion. However, in addition to this ion, there were peaks of significant abundances at m/z 174 and 189. There was also a peak (ca. 35-40 percent relative abundance) at m/z 346. Since TS ionization is a soft technique, these ions were attributed to impurities in the Basic Red 14. This assumption was strengthened by the MS/MS collisional induced dissociation (CID) spectrum of m/z 344. The ions at m/z 174 and 189 were not present in this spectrum. The CID spectra of the ions at m/z 174 and 189 suggested these were the ions of an indoline and a benzaldehyde, respectively, and the structures were confirmed with the authentic standards. An important class of cationic dyes, the methines, is prepared by the condensation of an aldehyde with an indoline. When the condensation reaction of the identified indoline and benzaldehyde was performed, the mixture turned red, and the thermospray spectrum of the product showed a m/z 344, which, under CID conditions generated a spectrum identical to the initial spectrum. The m/z 346 ion proved to have originated from a compound formed as a result of a reduction of the parent dye.

Ballard and Betowski [23] continued their work on TS of dyes, using the same instrument, with a study of 16 dyes belonging to six different classes. They reported detections limits from 15 to 200 ng for a variety of dyes. Thermospray ionization worked well for the representative dyes of the azo, methine, arylmethane, anthraquinone, coumarin, and xanthene classes. However, this technique worked less well for the sulfonated dyes. For example, Acid Orange 6 (Fig. 1) is a sodium salt of a monosulfonated, monoazo dye having a molecular weight of 316 daltons. In the positive ion TS spectrum, the $(M + H)^+$ and the $(M + Na)^+$ ions are observed at m/z 317 and 339, respectively. The base peak in this spectrum, however, is the ion at m/z 295, which corresponds to the protonated sulfonic acid form of this dye.

The negative ion TS spectrum shows an ion at m/z 293, which corresponds to the anion of this dye, $(M - Na)^-$. When the auxiliary filament was turned on, m/z 294 appeared. This ion was attributed to the electron capture product of the free sulfonic acid, which may be present as an impurity. The negative ion TS mode was found to be less sensitive than the positive ion TS mode by at least a factor of ten.

Covey and Henion [24] used a dual purpose DLI/TS LC/MS interface, which was introduced into a Hewlett-Packard 5985B GC/MS via the standard, direct insertion probe inlet. A variety of compounds were analyzed with this instrument, including an industrial dye, sulforhodamine B. The TS mass spectrum included an MH^+ ion at m/z 559 and a fragment ion at m/z 467.

Voyksner [25] demonstrated the use of TS-LC/MS to characterize azo, diazo, and anthraquinone dyes in wastewater, soil, and gasoline. TS mass spectra of the analyzed dyes produced mainly MH^+ ions with few fragments. Switching the filament "on", produced additional fragment ions which helped in the structural elucidation of these dyes. The commercial diazo and anthraquinone dyes proved to be very complex mixtures of nearly 40 alkyl-substituted dye components, making monitoring and identification of a particular dyestuff difficult. The detection limits were found to be 10 ppt in wastewater, 100 ppb in soil, and 1 ppm in gasoline. Fig. 2 shows the mass chromatograms of a commercial red dye spiked into gasoline.

Thermospray LC/MS/MS was found to be effective in the analysis of wastewater for disperse azo dyes [26]. In this study, Disperse Red 1 was used to test the effectiveness of an activated sludge process. Primary effluent from a municipal wastewater treatment plant was used as the feed for the system. The system was spiked with two concentrations of the dye. The samples were analysed by a combination of HPLC/UV-visible, TS-LC/MS, and TS-LC/MS/MS. The results from the mass spectrometric methods for various samples agreed with the HPLC/UV-visible results within 5 to 18 percent. The average precision for

the mass spectrometric methods was 12 percent. The TS- LC/MS and TS-LC/MS/MS systems were important for not only monitoring the disappearance of Disperse Red 1, but also for identifying breakdown products from the activated waste process. A major degradation product had the same nominal mass as a major interference ion. Tandem mass spectrometry was required to differentiate between the background ion and the degradation product ion. Another complication in identifying this product was that the initial mobile phase gradient did not cause this compound to elute. It took the addition of 0.1 M ammonium acetate to the mobile phase to help elute this breakdown product.

Flory et al. [27] investigated various factors that affected the thermospray response for nine sulfonated azo dyes. They used a modified Hewlett-Packard 5988A mass spectrometer, connected to a Scientific Systems Model GS400 HPLC gradient system via a Vestec TS interface. Their major finding was that too high a concentration of ammonium acetate buffer suppresses the ionization of these anionic dyes. They suggested that the major ionization processes in these dyes is anion evaporation directly from the droplet. If too high a concentration of ammonium acetate is added, ejection of the more volatile acetate ion will compete with the evaporation of the dye anion.

Yinon et al. [28, 29] worked on increasing the sensitivity of the TS technique by use of a wire repeller. A series of dyes belonging to different chemical classes (Fig. 3) were analyzed by TS-LC/MS using a modified source containing a wire repeller. The instrument used was a Finnigan MAT triple-stage quadrupole (TSQ) equipped with a Vestec ion source and thermospray interface. This ion source was originally not provided with a repeller. A hole was drilled exactly opposite the ion-extraction funnel and a 0.025 in. diameter copper wire was introduced, insulated with a ceramic tube. The wire faces the funnel but does not protrude into the ion chamber volume. The repeller was operated at a voltage range of 220-250 Volts. The HPLC consisted of a Rheodyne Model 7125 injector valve and a Spectra-Physics SP8700XR

solvent delivery system. A syringe pump (ISCO LC-5000) was connected to the system to deliver the buffer (0.1 M ammonium acetate) postcolumn-via the TSP interface into the source. Methanol-water was used as mobile phase. An increase of about two orders of magnitude was obtained with the wire-repeller, as well as an increase in the relative intensity of the molecular ion versus the fragment ions. Figure 4 shows the mass spectra of Disperse Blue 79 at repeller 0 and 250 Volts, respectively.

The TS positive ion mass spectra of several sulfonated dyes were recorded for the first time because of the increased sensitivity. For example, the mass spectrum of Acid Blue 40 has a base peak at m/z 372, probably due to loss of $NaSO_3$ from the protonated molecule and replacement of this $NaSO_3$ group by a hydrogen atom. Lower-abundance ions in the mass spectrum included the MH^+ ion at m/z 474, an $[M+Na]^+$ ion at m/z 496, $[M+Na-HCOCH_3]^+$ at m/z 452, $[MH-H_2COCH_3]^+$ at m/z 429, $[MH-NH_2-H_2COCH_3]^+$ at m/z 412, $[MH-SO_3]^+$ at m/z 394, and $[MH-NaSO_3+H-CH_2CO]^+$ at m/z 330.

Losses of SO_3Na and $2SO_3Na$ as well as losses of Na and 2Na, from the protonated molecule, were observed in the sulfonated dyes. The loss of each one of these groups involved the replacement by a hydrogen atom. Acid Blue 40, which has only one SO_3Na group, and Acid Red 114, which has two SO_3Na groups attached to the same ring, lose one and two SO_3Na groups, respectively. Direct Red 81 and Acid Blue 113, each having two SO_3Na groups on different rings, lose both one and two Na atoms, but do not lose SO_3 groups, which seem to be more strongly attached to the molecule.

The same instrument was used to acquire repeller-activated collisionally induced dissociation (repeller-CID) mass spectra of dyes [30]. These were obtained by applying a voltage of 400 V on the wire-repeller. The mass spectra contained a large number of fragment ions that were useful for structural elucidation. Some of these fragment ions were found to be similar to those obtained by thermospray tandem

mass spectrometry with CID, and some of them were similar to those obtained by EI, using a particle beam-LC/MS. Fig. 5 shows a comparison of three mass spectra of Disperse Yellow 5: TS-repeller-CID, TS-MS/MS-CID and EI.

A TS-LC/MS system was modified for the analysis of dyes by restricting the TS vaporizer exit orifice and adding a needle-tip repeller to the ion source [31]. An increase in signal response for disulfonated azo dyes was observed in the negative-ion mode. TS mass spectra contained mainly $[M-Na]^-$, $[M-2Na]^{2-}$, and $[M-2Na+H]^-$ ions.

The red dyes of woven fabrics from the Greco-Roman period, excavated at Akoris in middle Egypt, were investigated by a series of analytical techniques, including TS-LC/MS with selected ion monitoring [32]. The instrument used was a Shimadzu QP-1000 LC/MS. The HPLC consisted of a LC 6-A pump and a Shim-pack CLC-ODS(M) 15cm x 4.6 mm I.D. column. The solvent was methanol: 0.1M ammonium acetate: acetic acid (100:40:7) at a flow rate of 1 mL/min. Results showed the presence of Alizarin (MH^- at m/z 240), suggesting that the fabrics were dyed with madder.

TS-LC/MS together with GC/MS and particle beam-LC/MS were used to study photodegradation products of Disperse Red 167 [33]. This dye is a widely used dye especially for automobile cloth interiors. The instrument used was a Finnigan MAT 4500 quadrupole mass spectrometer equipped with a Vestec TS interface. The chromatographic separation was performed on a Waters 600 MS HPLC pump with a Supelcosil LC-1 25cm x 4.6 mm I.D. column. The flow rate was 1 mL/min through the column with post-column addition of 0.3 M ammonium acetate at 0.2 mL/min. The TS data provided insight into a great number of photodegradation products but was less helpful in structure elucidation.

TS-LC/MS was used to analyze a series of disperse dyes extracted from polyester and cellulose acetate fibers, a basic dye from orlon fiber, and a vat dye from denim (Table 1) [34]. Molecular characterization of each dye was obtained from the extract of a single fiber, 5-10 mm long. The instrument used was a 4501B Finnigan MAT quadrupole mass spectrometer with a TS interface and ion source. The HPLC system consisted of CM4000 LDC Milton Roy solvent-delivery system with a Rheodyne 7125 injector valve and a Merck C_{18}, 15 cm x 4mm I.D. column. The mobile phase, at a flow rate of 0.9 mL/min, was methanol-water, starting at 50:50 and changing within 5 min. to 100% methanol. The buffer, 0.1 M ammonium acetate, was delivered post-column via the TS interface into the ion source by a Constametric Bio 3000 Milton Roy delivery pump. The ion source repeller was operated at a voltage of 100 V. Figure 6 shows the reconstructed ion chromatogram and TS mass spectrum of Indigo (Vat Blue 1).

Thermospray, particle beam, and electrospray LC/MS were used for the analysis of a series of 14 commercial azo and diazo dyes (Fig. 7) [35]. The HPLC consisted of Waters Series 600 multi-solvent delivery system, a Waters U6K injector and a Spherisorb ODS II, 25cm x 4.6 mm I.D., 5-μm particle size, column. Postcolumn ammonium acetate buffer addition was done with a Waters Model 6000 pump. The HPLC was connected to a Finnigan MAT 4500 quadrupole mass spectrometer via a Vestec Model 701C TS interface. TS analyses of the investigated dyes produced mass spectra consisting primarily of MH^+ ions with very few fragments.

3. Particle Beam(PB) - LC/MS

3.1. Principle of Operation

Particle beam (PB) LC/MS provides a means of obtaining both EI and CI mass spectra for

compounds that can be brought into the gas phase but may not be amenable to GC due to thermal lability or lack of volatility. EI mass spectra are more easily referenced to mass spectral data bases, which can assist in the identification of unknown components in various matrices. In addition, EI fragmentation patterns offer valuable information in structure elucidation. The ability to obtain CI spectra with the same interface makes it possible to also obtain molecular weight information on the analyzed compound.

In the PB interface, the transfer of the neutral analyte from the column to the ion source of the mass spectrometer is accomplished only by aerodynamic means. The PB interface [36-38] has three basic functions: (1) aerosol formation, (2) desolvation, and (3) momentum separation of analyte from solvent. Following LC chromatographic separation, the column eluate is passed to a nebulizer, where the eluent (solvent + analyte) is dispersed into a fine mist of droplets. Aerosol formation is done by a coaxial flow of helium, which nebulizes the LC effluent. The resulting aerosol is then desolvated in a heated desolvation chamber, where the volatile solvent evaporates, while the dissolved analyte condenses to form solid micrometer sized particles. The resulting mixture of particles, solvent molecules and helium atoms is drawn through a small nozzle into a pumped chamber, causing a rapid expansion to occur. The relatively high-mass solute particles will gain high momentum from the expansion, enabling them to traverse the separator as a linear beam. Separation is achieved by sampling the particle beam through a small orifice (skimmer), leaving the gaseous solvent molecules and helium atoms to be pumped away. The process is repeated in the second stage of the separator, with a resulting enrichment of 10^{4} - 10^{5} particles relative to solvent. Finally, the particles pass through a short transfer line into the ion source. The heated walls of the source flash-volatilize the particles into the gas phase for EI or CI ionization. The PB interface can handle common solvents for normal or reversed phase separations, volatile mobile phase additives, and flow rates up to 1 mL/min.

The use of EI or CI is limited to compounds that can be brought into the gas phase. This is a severe

limitation when dealing with the nonvolatile and thermally unstable dyes that are often found in LC separations.

3.2. Applications to Dye Analysis

Marbury et al. [39] used PB-LC/MS for the analysis of a series of dyes, including Solvent Red 1, Disperse Red 11 and Disperse Blue 3. The instrument was an Extrel 400-1 quadrupole mass spectrometer fitted with a PB interface and standard EI/CI source. Methane was used as CI reagent. HPLC separations were done with a Waters gradient system with either a C_{18} RCM or C_2 SS column. Figure 8 shows the EI and CI spectra of Solvent Red 1. In addition to the molecular ion at m/z 278, structurally significant fragment ions are obtained.

Yinon et al. [40] interfaced an HPLC to a Finnigan-MAT Triple Stage Quadrupole equipped with a 4510 EI ion source by means of an Extrel ThermaBeam PB-LC/MS interface and used the system for the analysis of a series of commercial dyes. The HPLC consisted of a Spectra-Physics SP8700XR solvent-delivery system with a Rheodyne 7125 injector valve and a Varian MicroPak MCH-5-N-CAP C_{18} 15 cm x 4 mm I.D. column. Methanol-water, in a gradient mode, was used as mobile phase at a flow rate of 0.9 mL/min. Table 2 shows the EI mass spectra of the analyzed dyes. 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. No fragmentation resulting from ring cleavage was observed in the azo dyes.

The detection limits of the analyzed dyes ranged from 50 ng, for Disperse Orange 3, to 2.7 μg for

Disperse Brown 1. As a comparison, the LC/MS system's sensitivity was checked with caffeine, for which a detection limit of 5 ng was found.

Photodegradation products of Disperse Red 167 were determined by PB-LC/MS (together with TS-LC/MS-see section 2.2) with a Hewlett-Packard 5988A quadrupole mass spectrometer [33]. The chromatographic system used was a Waters 600 MS HPLC pump and a Supelcosil 5-μm LC-1, 25 cm x 4.6 mm I.D. column. The mobile phase was methanol-water, in a gradient mode, at a flow rate of 0.5 mL/min. The major photodegradation pathways were found to be reduction of the nitro group, free radical loss of N2 followed by coupling to form a substituted biphenyl, hydrolysis of the ester, and N-dealkylation.

The EI mass spectra of a series of 14 commercial azo and diazo dyes (Fig. 7) were studied using PB-LC/MS [35]. The HPLC was a Waters Series 600 multi-solvent delivery system controlled by a Waters 600-MS system controller. The dyes, dissolved in methanol or acetonitrile, were injected through a Waters U6K injector and separated on a Spherisorb ODS II, 25cm x 4.6 mm I.D., 5 μm particle size, column. The HPLC was connected through a PB interface to a Hewlett-Packard Model 5988A quadrupole mass spectrometer.

From most azo dyes it was possible to obtain molecular ion information and characteristic fragmentation patterns for structural elucidation. Fig. 9 shows the RIC chromatogram of Disperse Brown 1 and the corresponding EI mass spectra of two resolved components. The major peak (number 1), at a retention time of 29.1 min shows a mass spectrum with a relatively small molecular 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. The shoulder peak (number 2) is due to a decomposition product of the dye. The two smaller peaks (numbers 3 and 4) are probably due to plasticizer impurities. The detection limits were in the range of 500 ng

to 5 ng, with a signal-to-noise ratio of 3:1, at full scan

The chemical reduction of azo dyes containing industrial sludges usually forms a nearly colorless effluent. Depending on the identity of azo dyes in the waste, the aromatic reduction products are more harmful to the environment than the untreated sludge components.

The use of $Na_2S_2O_4$ or $SnCl_2$ to cleave the N=N- moiety of azo dyes followed by LC/MS analysis of the reduction products is a possible way to assess toxicity potential of complex waste sludges. Voyksner et al. [41] identified the reduction products from the reductive cleavage of 16 commercial azo dyes by PB-LC/MS. The instrumentation used was the same as in the previous application [35]. Standards of the formed reduction products were used to confirm identities. The chemical reduction methods resulted in nearly complete reduction of the azo bond to form aromatic amines. Overall, tin chloride was the more powerful reducing agent, yielding a greater number of products. Table 3 shows the chemical reduction products of the investigated dyes.

Measurements of dyes with TS-LC/MS were found to be by two to three orders of magnitude more sensitive than with PB-LC/MS. Pace and Roby [42] analyzed several dye wastes for synthetic precursors, transformation products, and selected aromatic amine target analytes associated with the manufacture of azo dyes. They used PB-LC/MS and GC/MS. The LC/MS system consisted of a Hewlett-Packard 1090 HPLC with photometric detector, coupled by a PB interface to a Hewlett-Packard 5988A quadrupole mass spectrometer. Both EI and NCI ionization modes were used in both PB-LC/MS and GC/MS systems. Table 4 shows the comparison of tentatively identified compounds in an azo dye sample by PB-LC/MS and GC/MS. Several compounds were detected by GC/MS, but not by PB-LC/MS, because of the higher sensitivity of GC/MS. In contrast, several azo compounds were detected by PB-LC/MS but not by GC/MS because the azo compounds decomposed in the heated GC.

4. Ion Spray and Electrospray (ES) - LC/MS

4.1. Principles of Operation

In a typical ES mass spectrometer, a solvent flow of 1 to 10 µL/min is introduced at atmospheric pressure into an ion source chamber through a stainless steel hypodermic needle, which is at ground potential [43]. The solvent flow forms a fine spray of charged droplets in response to an applied electric field of 2 - 4 kV. As the droplets evaporate, the analyte ions, whose polarity is opposite to the applied potential, migrate to the surface of the droplets, where Coulomb repulsion causes the droplets to break up into yet smaller droplets, thus enhancing evaporation. At some minimum droplet diameter, the analyte ions are believed to desorb from the droplets into the gas phase. This process is known as ion evaporation, and is the primary mechanism for gas phase ion formation in electrospray.

A compound will yield an observable ion if it is able to ionize in solution. Thus, basic compounds are dissolved in acidic solutions and acidic compounds in basic solutions. The resulting cations and anions are analyzed in positive and negative ion modes, respectively.

Ion spray (pneumatically assisted electrospray) uses pneumatics to assist nebulization and desolvation of liquids in electrospray. Nitrogen is added coaxially to the samples to assist in the nebulization of the liquid. A potential of 3 - 6 kV on the needle assembly charges the droplets for subsequent ion evaporation. Ion spray is effective in handling most reverse-phase solvents and mobile-phase additives, and it expands the upper flow rate (e.g., 50 μL/min), compared with unassisted nebulization in electrospray.

4.2 Applications to Dye Analysis

4.2.1. API-MS of Sulfonated Dyes

Atmospheric pressure ionization (API)-MS techniques of pneumatic assisted electrospray (ion spray) and electrospray [17, 18, 44] have been demonstrated for the detection of dyes. Electrospray achieves the best sensitivity for compounds that are precharged in solution (e.g., ionic species or compounds that can be protonated or deprotonated by the adjustment of pH) [43]. For this reason, the initial work with electrospray MS focused on ionic dyes such as sulfonated azo dyes, which have eluted analysis by particle beam or thermospray LC/MS. The ion spray or electrospray of monosulfonated azo dyes consists of an $[M-Na]^-$ anion with little fragmentation. Monsulfonated dyes including Acid Orange 7, Acid Red 337, Acid Red 151, Acid Red 88, Acid Yellow 151, and Acid Yellow 49 have been analyzed by electrospray or by ion spray [35, 45, 46].

Disulfonated azo dyes including Acid Red 1, Acid Black 1, and Acid Blue 113 could be analyzed by

API-MS [45-47]. The mass spectra of these dyes consist of $[M-2Na]^{-2}$ and $[M-Na]^{-}$ ions. However, Acid Blue 113 does not exhibit an [M-Na]- anion. Thermally assisted electrospray was demonstrated by Henion et al. to detect a hexasulfonated dye (Direct Red 80) of M.W. 1240 [48]. The mass spectrum showed only multiply charged ions ranging from $[M-6H]^{-}$ to $[M-2H]^{-2}$ [48].

API-MS techniques could also provide structurally relevant fragmentation information on the sulfonated azo dye by collision induced decomposition (CID) in the transport region of the interface. Typically this is accomplished by increasing a capillary or orifice potential resulting in the CID of molecular species generated by electrospray or ion spray. The fragment ion usually results from breakage of the azo linkage with the charge staying with the moiety containing SO_3. Also, $[SO_3]^{-}$ ion at m/z 80 is a common CID fragment for these sulfonated azo dyes. Figure 10 shows the CID spectrum of Acid Orange 10 using electrospray transport CID (200V capillary voltage) to obtain structural information for this disulfonated azo dye. Similarly, CID information could be obtained by tandem MS on the $[M-Na]^{-}$ anion.

The API technique also proved to be very sensitive for the detection of the sulfonated azo dyes in negative ion detection. Full scan detection was demonstrated on 1-10 ng quantities of these sulfonated azo dyes.

4.2.2. LC/MS of Dyes

The flexibility of API-MS permits the coupling of separation using high flow rates or very low flow rates for online LC/MS. The use of a heated pneumatic nebulizer with corona discharge (atmospheric pressure chemical ionization - APCI) permitted 1-2 mL/min operation, while electrospray or ion spray permitted operation at the 1-40 μL/min range. In either case, chromatographic conditions for the separation

of the sulfonated azo dyes must employ volatile buffers to minimize suppression of the signal. Sulfonated azo dyes were separated on a C_{18} column using a gradient of acetonitrile in water containing 0.1 M ammonium acetate at 2 mL/min using the APCI approach [45]

While APCI could tolerate higher levels of buffer, response for the polysulfonated azo dyes were usually poorer compared with the monosulfonated azo dyes. This difference in response may be attributed to the lower volatility of the polysulfonated dyes, resulting in less material in the gas phase for ionization by APCI. LC/MS using ion spray successfully detected the polysulfonated azo dyes [45]. To achieve the best sensitivity the LC conditions were changed to a 1.0 mm I.D. C_{18} column to reduce the flow rate to 40 µL/min and the ammonium acetate concentration was reduced to 0.001 M. Higher flow rates or higher levels of buffer would result in signal suppression.

Also, capillary electrophoresis (CE) coupled to electrospray-MS was demonstrated for the separation and detection of sulfonated azo dyes [46]. CE separations using acetonitrile flow rates of 1.8 µL/min permitted rapid separation of the sulfonated azo dyes. While the high resolution separation capabilities of CE resulted in high peak concentrations, permitting detection of low pmole quantities of dyes, the low injection volume of CE (2-30 nL) limited the concentration detection limits. Low ppm detection limits were reported for the sulfonated azo dyes by CE/MS [46].

Cationic dyes have been analyzed using positive ion detection electrospray-MS with great success. These pre-charged cationic dyes are well suited for ion evaporation ionization in electrospray MS, analogous to the negative ion formation for the sulfonated azo dyes previously discussed. For this reason, cationic dyes such as Basic Yellow 11 (methine class of dyes), Basic Violet 1 and Basic Green 4 (arylmethane class of dyes), and Basic Violet 10 and Solvent Red 49 (xanthene class of dyes) could be detected at sub-ng quantities

under full scan conditions using electrospray-MS [49]. The mass spectra of these cationic dyes exhibited only $[M]^+$ ions at conditions that do not cause CID (low capillary or orifice potentials) as shown in Figure 11a for Basic Violet 10. Several ways were evaluated in obtaining structural information on these dyes including:

(1) Electrospray transport CID.

(2) Triple quadrupole MS/MS.

(3) Ion trap mass spectrometry (ITMS) MS/MS.

The three CID techniques showed structural information but there were major differences in energies available for activation. The use of electrospray transport CID made use of the voltage at the capillary exit (Analytica of Branford electrospray source) to generate the CID fragment ions (Figure 12).

At a low capillary exit voltage (50-80V), only molecular ions are detected. At a high capillary exit voltage (160-240V) significant fragmentation is observed. Of the three techniques, the electrospray transport CID has the capability to place the largest quantities of internal energy (> 16 eV) into the ion to generate the most fragments [50]. This has been demonstrated for Basic Violet 10 (Figure 11b). Sensitivity of this approach was superior due to the minimal ion losses at the various capillary exit voltages (the total ion current was nearly constant over the voltage range evaluated in Figure 12) evaluated for CID. However, since there is no mass selection prior to CID, the signal/noise and specificity of the approach may be limited in complex samples or from coelution of components under LC/MS conditions.

The electrospray ion trap-MS (ITMS) [51-53] makes use of initial mass selection prior to CID of the selected ion in the trap. Molecular ions generated by electrospray, gated into the trap, are mass selected by a combination of rf and dc fields. A tickle voltage of 1-2V applied to the endcap for 30 ms increases the

kinetic energy of the selected ion for CID with helium in the trap (4×10^{-5} torr). The tickle voltage, tickle time, and rf trapping well depth (qz) can be varied to control the extent of energy transfer to the selected ion resulting in different amounts of fragmentation. Figure 13 shows the optimization of tickle voltage for the CID of the $[M]^+$ ion of Basic Yellow 11. For the cationic dyes, tickle voltages of 2-2.8V were optimal (qz 0.3, tickle time 30 ms, pressure 4×10^{-5} torr) for the generation of structurally relevant product ions. Higher tickle voltages ejected the molecular ions into the trap walls, resulting in loss of sensitivity. Lower voltages did not provide sufficient kinetic energy for CID of the selected molecular ion. The product ions were ejected from the trap to the multiplier using the mass selection instability mode of operation.

While the ion trap imparts lower energies compared with CID in the electrospray transport, significant structural information could be obtained for these dyes such as Basic Violet 10 (Figure 11c). Furthermore, increasing the qz value enables more energy to be imported to the ions resulting in near equivalent fragmentation to electrospray transport CID. Also, MS^n capabilities of the ITMS could generate additional structural information [53]. Sensitivity of the ITMS approach was comparable to electrospray transport CID by the ITMS approach, offering superior signal/noise and specificity due to mass selection prior to CID of the ions.

Triple quadrupole [54, 55] was also evaluated to obtain CID information on a mass selected ion. In this instance, the molecular ion of interest was selected by quadrupole 1, the CID of the ion occurred in quadrupole 2 (30 eV lab energy with Argon target gas at 1 mtorr), and the third quadrupole was scanned to detect the product ions formed. Although the triple quadrupole offered good specificity due to mass selection prior to CID, the extent of fragmentation was limited due to a maximum of 30 eV collision energy

available on the instrument. Figure 11d displays the triple quadrupole CID mass spectrum of Basic Violet 10 for comparison to the other CID techniques discussed

Electrospray has been also successful for numerous azo dyes that are not ionic salts. Azo dyes consisting of both disperse and solvent classes of dyes have been analyzed by electrospray MS [48, 49, 52]. Specifically, disperse dyes including Blue 79, Yellow 5, Bronze 2, Orange 13, Orange 3, Red 1, Red 13, Brown 1, Solvent Red 3, Solvent Red 23 and 24, Pigment Red 3, and Methyl Red have been analyzed by electrospray or ion spray. Optimal sensitivity was usually observed at low pH conditions (e.g., 1% acetic acid) that promote the protonation of a basic site on the dye to form a cation in solution, under positive ion detection. The sensitivity for negative ion detection (high pH using 0.1% ammonium hydroxide) did not compare with positive ion detection, possibly due to the lack of sites for deprotonation to form anions in solutions. All these dyes exhibited $[M+H]^+$ ions and fragmentation under CID conditions in the electrospray transport. Examples of electrospray MS spectra of two azo dyes are presented in Figures 14 and 15. Figure 14 shows the electrospray mass spectrum of Solvent Red 24. The fragmentation was generated by CID in the electrospray transport (capillary voltage of 160V). Figure 15 shows the electrospray transport CID spectrum of Bronze 2 obtained on a Vestec electrospray source.

The electrospray MS analysis of the azo dye was usually more sensitive compared to particle beam or thermospray. However, the response did not compare to the signals generated by electrospray for the cation or sulfonated dyes.

Several anthraquinone dyes have been analyzed by electrospray MS [52]. These dyes include Disperse Blue 1 and 3. Analogous electrospray-MS conditions mentioned for the azo dyes (low pH, positive ion detection) were optimal for the detection of these anthraquinone dyes. The basic nitrogens on the anthraquine ring serve as the site of protonation to generate a cation to obtain optimal electrospray- MS

sensitivity. The electrospray spectra of these dyes consisted of only $[M+H]^+$ ion under non-CID conditions. Structural information could be obtained by CID in the electrospray transport as demonstrated for Disperse Blue I (Figure 16)

Research in new methodologies such as electrospray LC/MS have greatly enhanced the ability to characterize increasingly complex, polar and nonvolatile dyestuffs. The sensitivity and specificity (by CID in the electrospray transport or by MS/MS) achieved with electrospray LC/MS enables the monitoring for trace levels of dyes in mixtures, a necessity for environmental monitoring or production process control.

5. Summary

Table 5 attempts to compare the various LC/MS techniques, according to class of dye to be analyzed, in terms of sensitivity and specificity. Techniques like particle beam-LC/MS,which is based on gas phase ionization process, are not suitable for nonvolatile components such as sulfonated azo dyes. Thermospray-LC/MS on a single quadrupole system usually results in single ion spectra, lacking structural information for compound confirmation. Electrospray LC/MS probably offers the best combination of sensitivity and specificity (CID in electrospray transport region). However, electrospray sensitivity is often reduced for non-polar dyes that do not have sites of protonation or deprotonation to form cations or anions for their respective positive or negative ion detection

Notice

This review has been funded, in part, by the U.S. Environmental Protection Agency, through its Office of Research and Development (ORD), and conducted through a collaboration of the Environmental Monitoring Systems Laboratory in Las Vegas with the Research Triangle Institute and the Weizmann Institute of Science.

References

1. C. N. McEwen, S. F. Layton, and S. K. Taylor, *Anal. Chem.*, 49 (1977) 922.

2. A. Mathias, A. E. Williams, D. E. Games, and A. H. Jackson, *Org. Mass Spectrom.*, 11 (1976) 266.

3. H. -R. Schulten and D. Z. Kummler, *Anal. Chem.*, 278 (1976) 13.

4. S. M. Scheifers, S. Verma, and R. G. Cooks, *Anal. Chem.*, 55 (1983) 2260.

5. S. D. Richardson, A. D. Thruston, Jr., J. M. McGuire, and G. L. Baughman, *Org. Mass Spectrom.*, 26 (1991) 826.

6. S. D. Richardson, J. M. McGuire, A. D. Thruston, Jr., and G. L. Baughman, *Org. Mass Spectrom.*, 27 (1992) 289.

7. S. D. Richardson, A. D. Thruston, Jr., J. M. McGuire, and E. J. Weber, *Org. Mass Spectrom.*, 28 (1993) 619.

8. L. K. Pannell, E. A. Sokoloski, H. M. Fales, and R. L. Tate, *Anal. Chem.*, 57 (1985) 1060.

9. M. U. D. Beug-Deeb, J. A. Bennett, M. E. Inman, and E. A. Schweikert, *Anal. Chim. Acta*, 218 (1989) 85.

10. J. J. Monaghan, M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler, *Org. Mass Spectrom.*, 17 (1982) 569.

11. J. J. Monaghan, M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler, *Org. Mass Spectrom.*, 18 (1985) 75.

12. R. M. Brown, C. S. Creaser, and H. J. Wright, *Org. Mass Spectrom.*, 19 (1984) 311.

13. F. Ventura, A. Figueras, J. Caixach, D. Fraisse, and J. Rivera, *Fres. Z. Anal. Chem.*, 335 (1989) 272.

14. H. S. Freeman, R. B. Van Breemen, J. F. Esancy, D. O. Ukpommwan, Z. Hao, and W.-N. Hsu, *Text. Chem. Color.*, 22 (1990) 23.

15. M. J. Dale, A. C. Jones, P. R. R. Langridge-Smith, K. F. Costello, and P. G. Cummins, *Anal. Chem.*, 65 (1993) 793.

16. T. Covey, E. Lee, A. Bruins, and J. Henion, *Anal. Chem.*, 58 (1986) 1451A.

17. E. C. Huang, T. Wachs, J. J. Conboy, and J. D. Henion, *Anal. Chem.*, 62 (1990) 713A.

18. W. M. A. Niessen, U. R. Tjaden, and J. van der Greef, *J. Chromatogr.*, 554 (1991) 3.

19. P. Arpino, *Mass Spectr. Rev.*, 9 (1990) 631.

20. R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991

21. R. D. Voyksner, J. Bursey, and E. Pellizzari, *Anal. Chem.*, 56 (1984) 1507

22. L. D. Betowski and J. M. Ballard, *Anal. Chem.*, 56 (1984) 2604.

23. J. M. Ballard and L. D. Betowski, *Org. Mass Spectrom.*, 21 (1986) 575.

24. T. Covey and J. Henion, *Anal. Chem.*, 55 (1983) 2275.

25. R. D. Voyksner, *Anal. Chem.*, 57 (1985) 2600

26. L. D. Betowski, S. M. Pyle, J. M. Ballard, and G. M. Shaul, *Biomed. Environ. Mass Spectrom.*, 14 (1987) 343.

27. D. A. Flory, M. M. McLean, M. L. Vestal, and L. D. Betowski, *Rap. Comm. Mass Spectrom.*, 1 (1987) 48.

28. J. Yinon, T. L. Jones, and L. D. Betowski, *Rap. Comm. Mass Spectrom.*, 3 (1989) 38.

29. J. Yinon, T. L. Jones, and L. D. Betowski, *Biomed Environ. Mass Spectrom.*, 18 (1989) 445.

30. J. Yinon, T. L. Jones, and L. D. Betowski, *Rap. Comm. Mass Spectrom.*, 4 (1990) 245.

31. M. A. McLean and R. B. Freas, *Anal. Chem.*, 61 (1989) 2054

32. R. Yamaoka, N. Shibayama, T. Yamada, and M. Sato, *Mass Spectroscopy*, 37 (1989) 249

33. C. A. Haney, D. J. Grindstaff, W. N. Hsu, H. S. Freeman, and R. D. Voyksner, Proc. of the 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, Arizona, 1990, p. 1069

34. J. Yinon and J. Saar, *J. Chromatogr.*, 586 (1991) 73

35. R. Straub, R. D. Voyksner, and J. T. Keever, *J. Chromatogr.*, 627 (1992) 173

36. R. Willoughby and R. Browner *Anal. Chem.*, 56 (1984) 2626

37. P. Winkler, D. Perkins, W. Williams, and R. Browner, *Anal. Chem.*, 60 (1988) 489

38. C. S. Creaser and J. W. Stygall, *Analyst*, 118 (1993) 1467

39. D. Marbury, J. Tuschall, B. Lynn, C. Haney, and R. Voyksner, *Adv. Mass Spectrom.*, 11A (1989) 206.

40. J. Yinon, T. L. Jones, and L. D. Betowski, *J. Chromatogr.*, 482 (1989) 75.

41. R. D. Voyksner, R. Straub, J. T. Keever, H. S. Freeman, and W. -N. Hsu, *Environ. Sci. Technol.*, 27 (1993) 1665.

42. C. Pace and M. Roby. Characterization and Analysis of the Base/Neutral Fraction From Azo Dye Waste Samples. EPA Report 600/X-91/147. EMSL. Las Vegas. NV 89193. USA. 1991.

43. C. M. Whitehouse. R. N. Dreyes. M. Yamashita. and J. B. Fenn. *Anal. Chem.*, 57 (1985) 675.

44. R. D. Voyksner. *Environ. Sci. Technol.*, 28 (1994) 118A.

45. A. P. Bruins. L. O. G. Weidolf. J. D. Henion. and W. L. Budde. *Anal. Chem.*, 59 (1987) 2647.

46. E. D. Lee. W. Mück. J. D. Henion. and T. R. Covey. *Biomed. Environ. Mass Spectrom.*, 18 (1989) 253.

47. T. R. Covey. A. P. Bruins. and J. D. Henion. *Org. Mass Spectrom.*, 23 (1988) 178.

48. E. D. Lee. and J. D. Henion. *Rap. Comm. Mass Spectrom.*, 6 (1992) 727.

49. R. D. Voyksner. L. D. Betowski. and H. -Y. Lin. Proc. of the 42nd ASMS Conference on Mass Spectrometry and Allied Topics. Chicago. Illinois. 1994. p. 817.

50. R. D. Voyksner and T. Pack. *Rap. Comm. Mass Spectrom.*, 5 (1991) 263.

51. S. A. McLuckey. G. J. Van Berkel. G. L. Glish. E. C. Huang. and J. D. Henion. *Anal. Chem.*, 63 (1991) 375.

52. H.-Y. Lin and R. D. Voyksner, *Anal. Chem.*, 65 (1993) 451

53. B. D. Nourse and R. G. Cooks, *Anal. Chim. Acta*, 228 (1990) 1

54. J. Johnson and R. A. Yost, *Anal. Chem.*, 57 (1985) 758A.

55. R. A. Yost and C. G. Enke, *Anal. Chem.*, 51 (1979) 1251A

Figure Captions

Figure 1. Chemical Structure of Acid Orange 6.

Figure 2. Mass chromatograms of 1 ppm of a commercial red dye spiked into gasoline. The major red component (A) at m/z 381 and the major orange component (B) at m/z 249 are displayed.

Figure 3. Chemical structures of dyes analyzed by TS-LC/MS.

Figure 4 Comparison of three mass spectra of Disperse Yellow 5, obtained by (a) TS-repeller-CID, (b) TS-MS/MS-CID, and (c) EI.

Figure 5. TS-LC/MS (with wire-repeller) mass spectra of Disperse Blue 79 at repeller 0 and 250 Volts, respectively.

Figure 6. Reconstructed ion chromatogram (RIC) to TS mass spectrum of Indigo (Vat Blue 1).

Figure 7. Chemical structures of commercial dyes analyzed by TS-, PB-, and ES-LC/MS.

Figure 8 EI- and CI-PB-LC/MS mass spectra of Solvent Red 1.

Figure 9. RIC chromatogram of Disperse Brown 1 and the corresponding PB-EI mass spectra of two resolved components, peaks 1 and 2.

Figure 10. Electrospray mass spectrum of 100 ng/μL solution (2-propanol:water, 1:1) of Acid Orange 10 at a capillary voltage of -200V

Figure 11. Positive ion mass spectra of Basic Violet 10

a) Electrospray-MS non-CID conditions

b) Electrospray-MS CID conditions (350V capillary voltage)

c) Electrospray ITMS CID mass spectra of m/z 443 at a tickle voltage of 2.8V, qz 0.3, tickle time 30 ms and kap pressure of 4×10^{-4} torr

d) Triple quadrupole CID mass spectrum of m/z 443 at an argon pressure of 3 mtorr, collision energy 30 eV

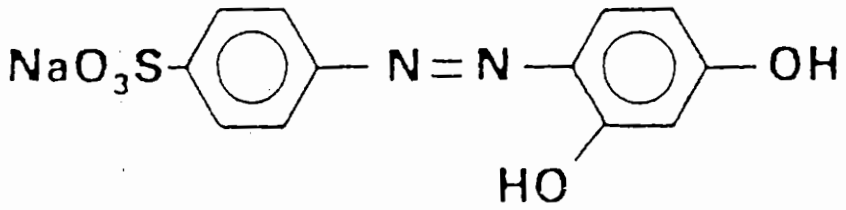
Figure 12. Electrospray transport CID of Basic Yellow 11 (mw 337) over the capillary exit voltage range of 40-240V. The relative intensities of the molecular ion, product ions, and total ion current are presented.

Figure 13. Optimization of the tickle voltage applied to the endcaps for CID of the $[M]^+$ ion to product ions for Basic Yellow 11 in the electrospray ITMS system. The total pressure in the trap was 4×10^{-4} torr, tickle voltage was applied for 30 ms at a qz of 0.3.

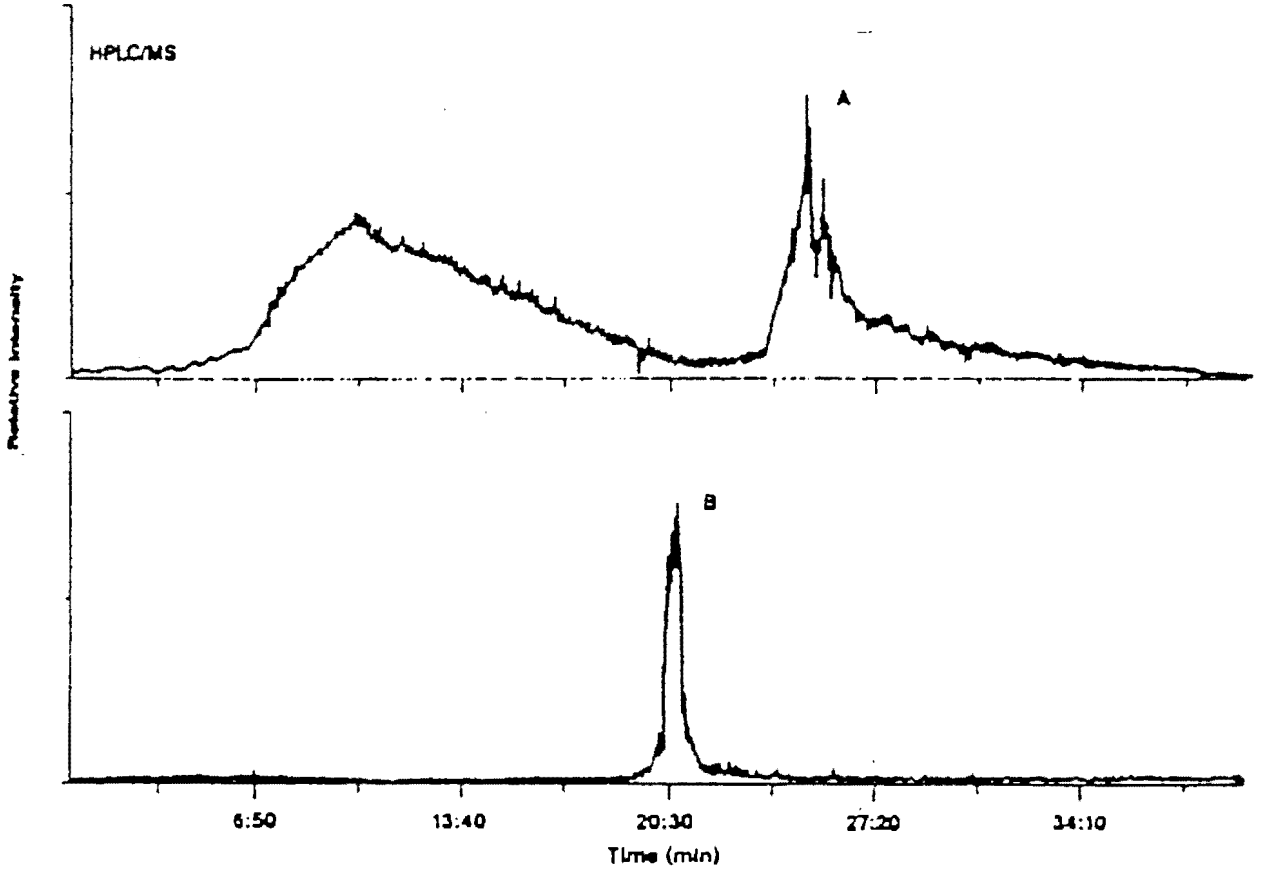
Figure 14. Electrospray-MS mass spectrum of Solvent red 24 at a capillary voltage of 160V to generate CID fragmentation. The sample (20 ng/μL) was infused in 50/50 ACN/H_2O with 1% acetic acid at 4 μL/min.

Figure 15. Electrospray mass spectrum of Bronze 2 at a repeller voltage of 40V (Vestec electrospray source) to generate structurally relevant CID ions. Conditions: 20 ng/μL solution in 80% MeOH, 20% H_2O with 1% acetic acid infused at 5 μL/min

Figure 16. Electrospray mass spectrum of Disperse Blue 1 at a capillary voltage of 160V to generate structurally relevant CID fragment ions. Conditions: 50 ng/μL solution (75/25 MeOH/H_2O, 1% acetic acid) infused at 4 μL/min.



Acid Orange 6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A
B
Relative Intensity
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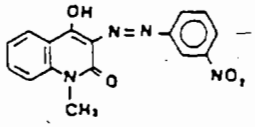
13:40
20:30
27:20
34:10
Time (min)

AZO CLASS



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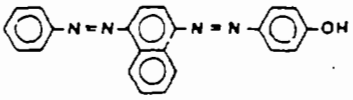

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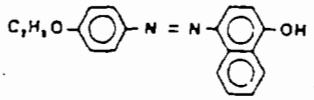

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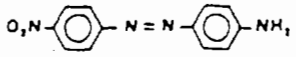

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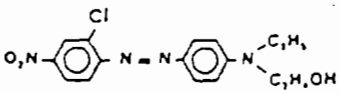

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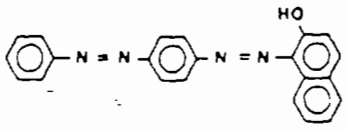

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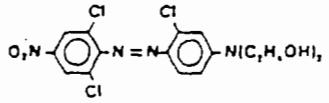

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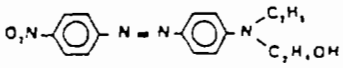

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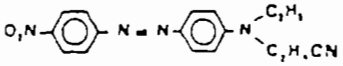

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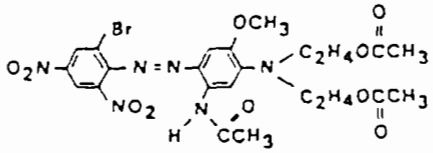

10. Disperse Blue 79
C.I. None

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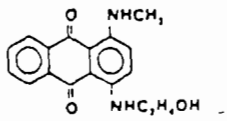

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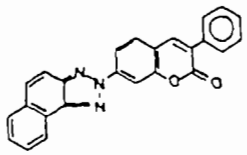
COUMARIN CLASS



19 Fluorescent Brightener 236
C.I. None

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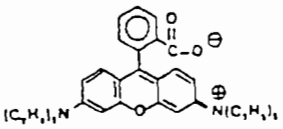

20 Solvent Red 49
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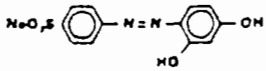
SULFONATED AZO CLASS



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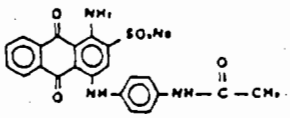

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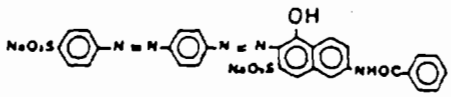

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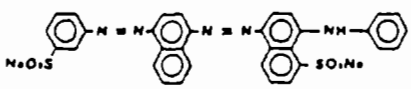

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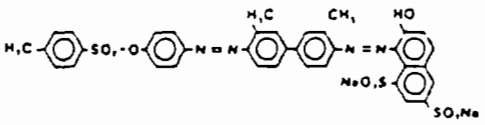

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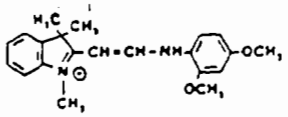
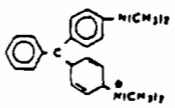
METHINE CLASS

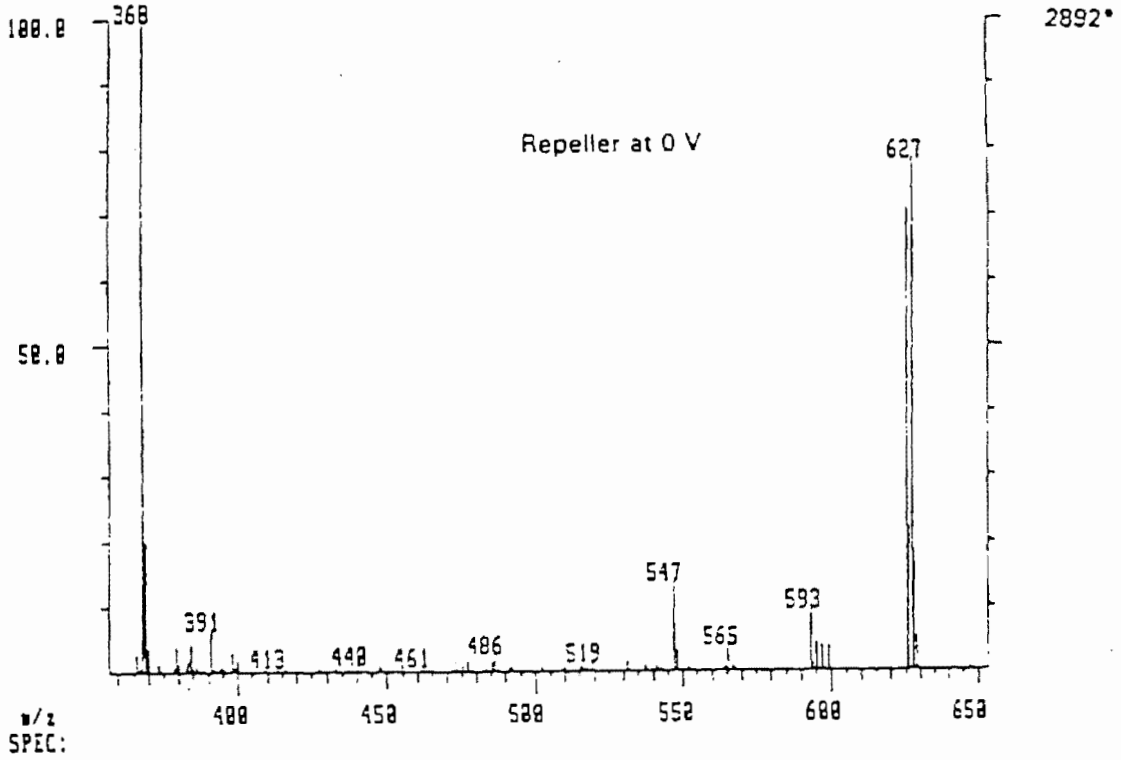


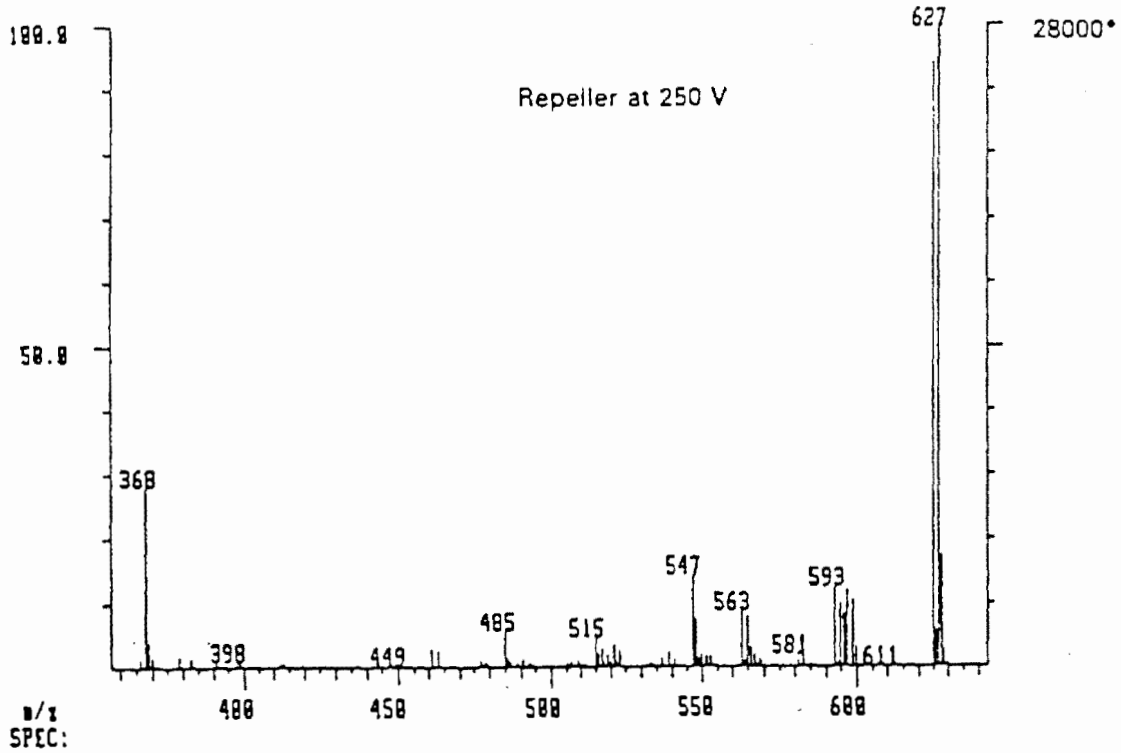
16. Basic Yellow 11
C.I. 48055

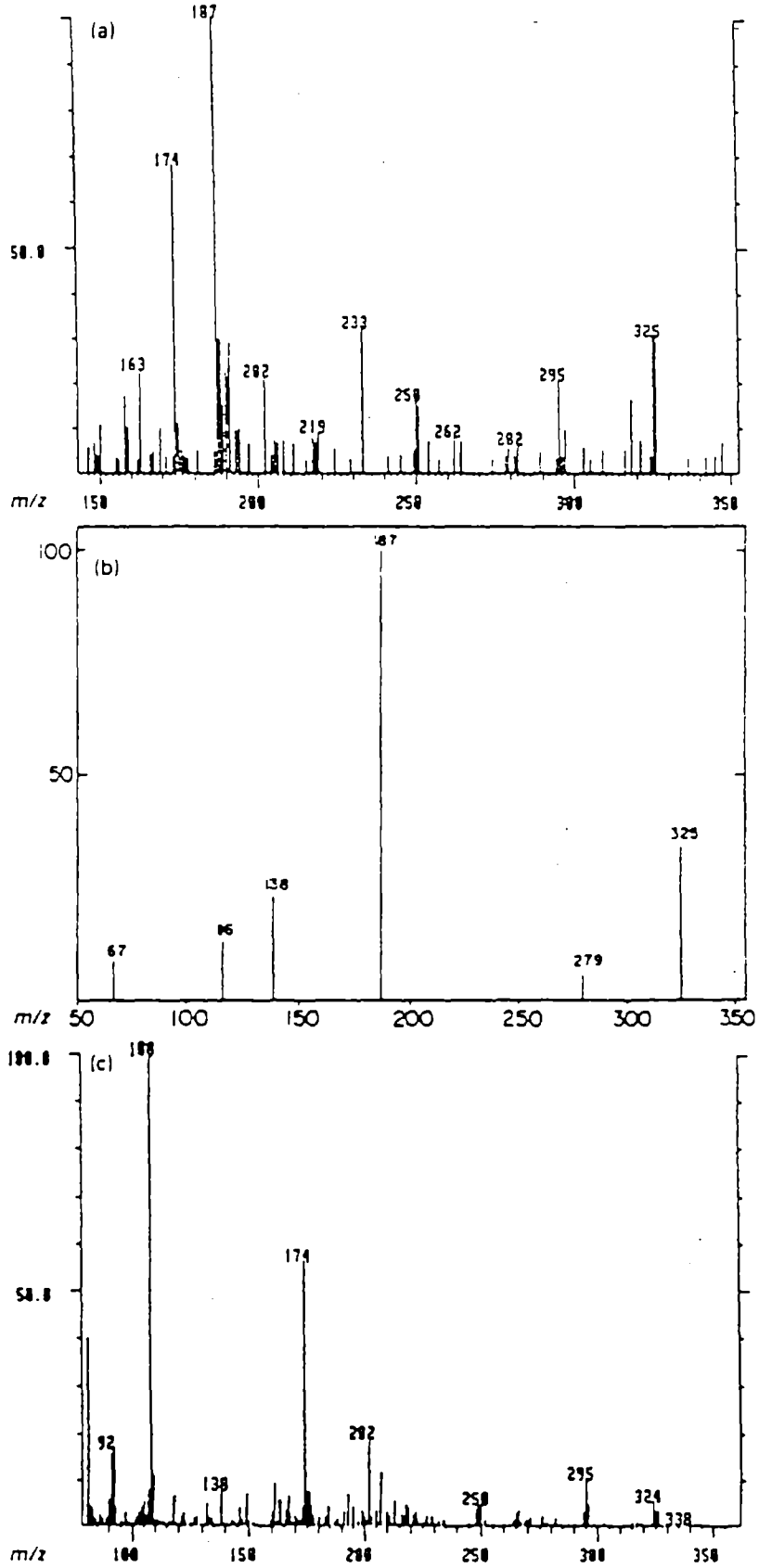
ARYLMETHANE CLASS

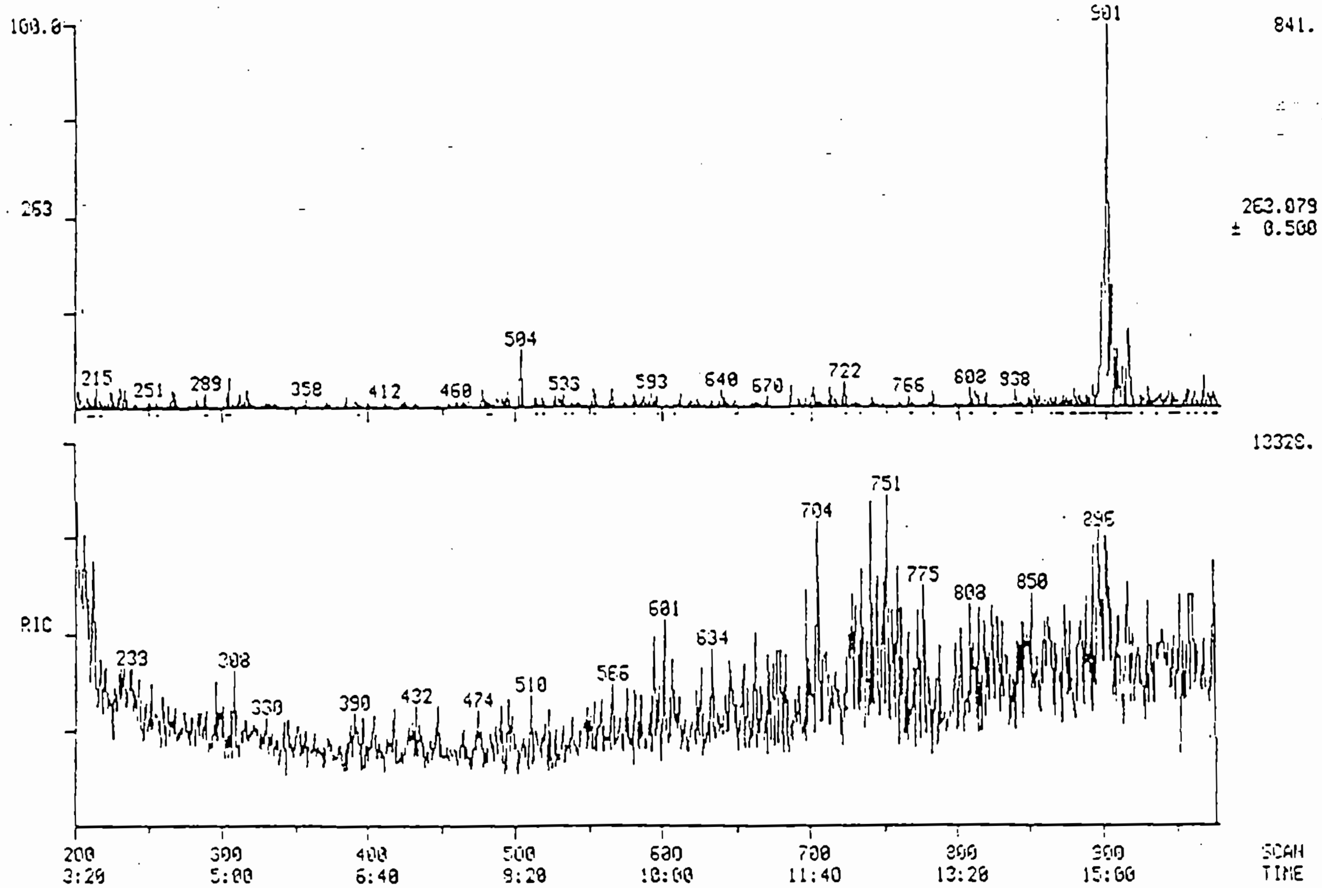


17. Basic Green 4
C.I. 42000

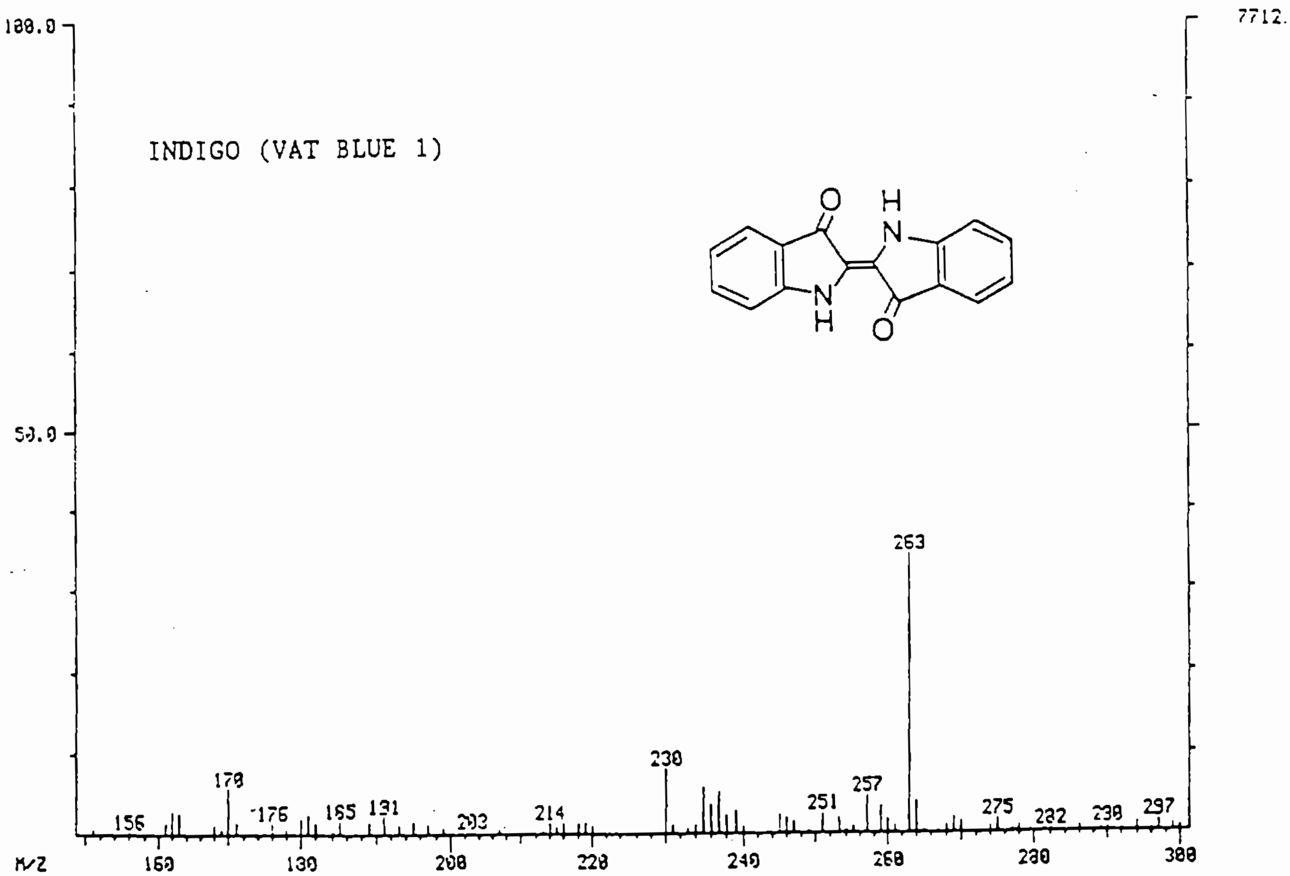

100.0
50.0
368
391
413
440
461
486
519
547
565
593
627
2892*
Repeller at 0 V
m/z
SPEC:
400
450
500
550
600
650


100.0
50.0
368
398
449
485
515
547
563
581
593
611
627
28000*
Repeller at 250 V
m/z
SPEC:
400
450
500
550
600

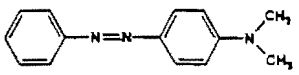

(a)
187
174
163
282
233
219
258
262
282
295
325
m/z
(b)
100
50
187
138
96
67
279
325
m/z
50
100
150
200
250
300
350
(c)
174
92
282
138
295
324
338
m/z



100.0
263
RIC
841.
263.079
± 0.500
13328.
901
504
215 251 289 358 412 460 533 593 640 670 722 766 802 838
751
704
895
775
808
850
681
634
233 308 330 390 432 474 510 566
200 3:20
300 5:00
400 6:40
500 8:20
600 10:00
700 11:40
800 13:20
900 15:00
SCAN
TIME

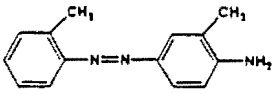


100.0
50.0
7712.
INDIGO (VAT BLUE 1)
263
230
170
257
251
156 176 185 191 203 214 275 282 290 297
M/Z
160 180 200 220 240 260 280 300



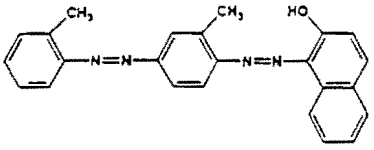
1. Solvent Yellow 2

C.I. 11020



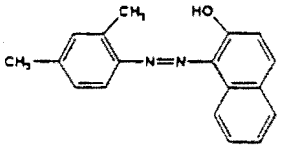
2. Solvent Yellow 3

C.I. 11160



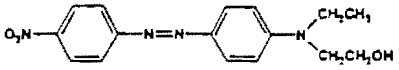
3. Solvent Yellow 14

C.I. 12055



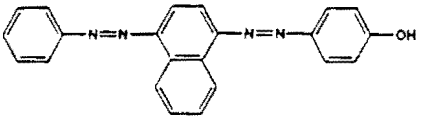
4. Solvent Orange 7

C.I. 12140



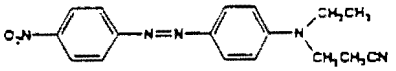
5. Disperse Red 1

C.I. 11110



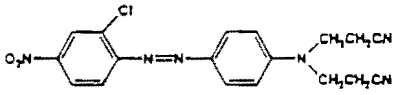
6. Disperse Orange 13

C.I. 26080



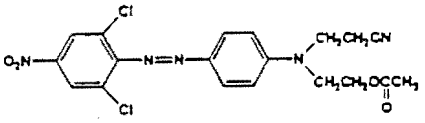
7. Disperse Orange 25

C.I. 11227



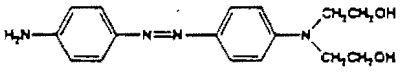
8. Disperse Orange 44

C.I. none



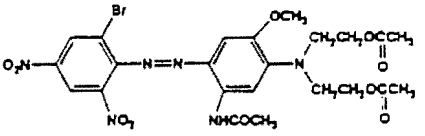
9. Disperse Orange 30

C.I. 11119



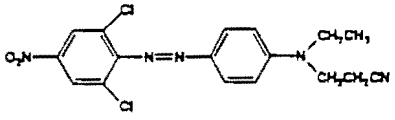
10. Disperse Black 9 (precursor)

C.I. none



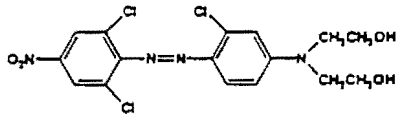
11. Disperse Blue 79

C.I. 11345



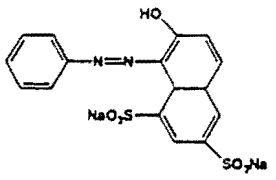
12. Disperse Orange 37

C.I. none



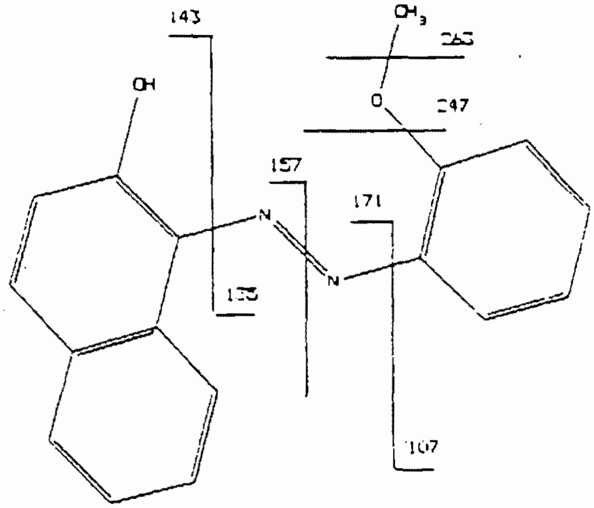
13. Disperse Brown 1

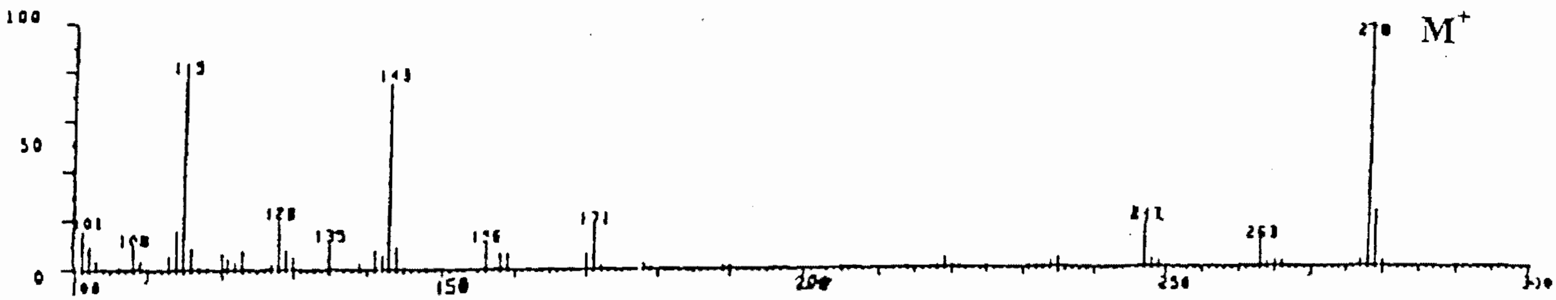
C.I. 11152

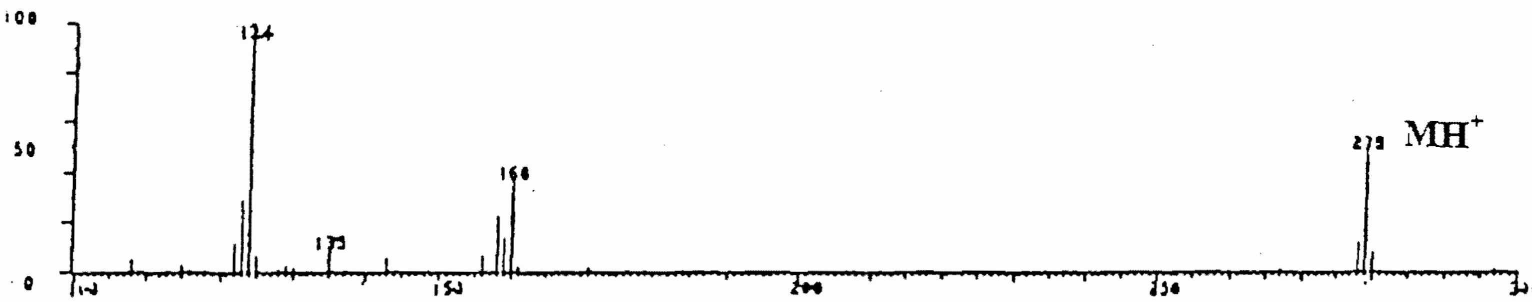


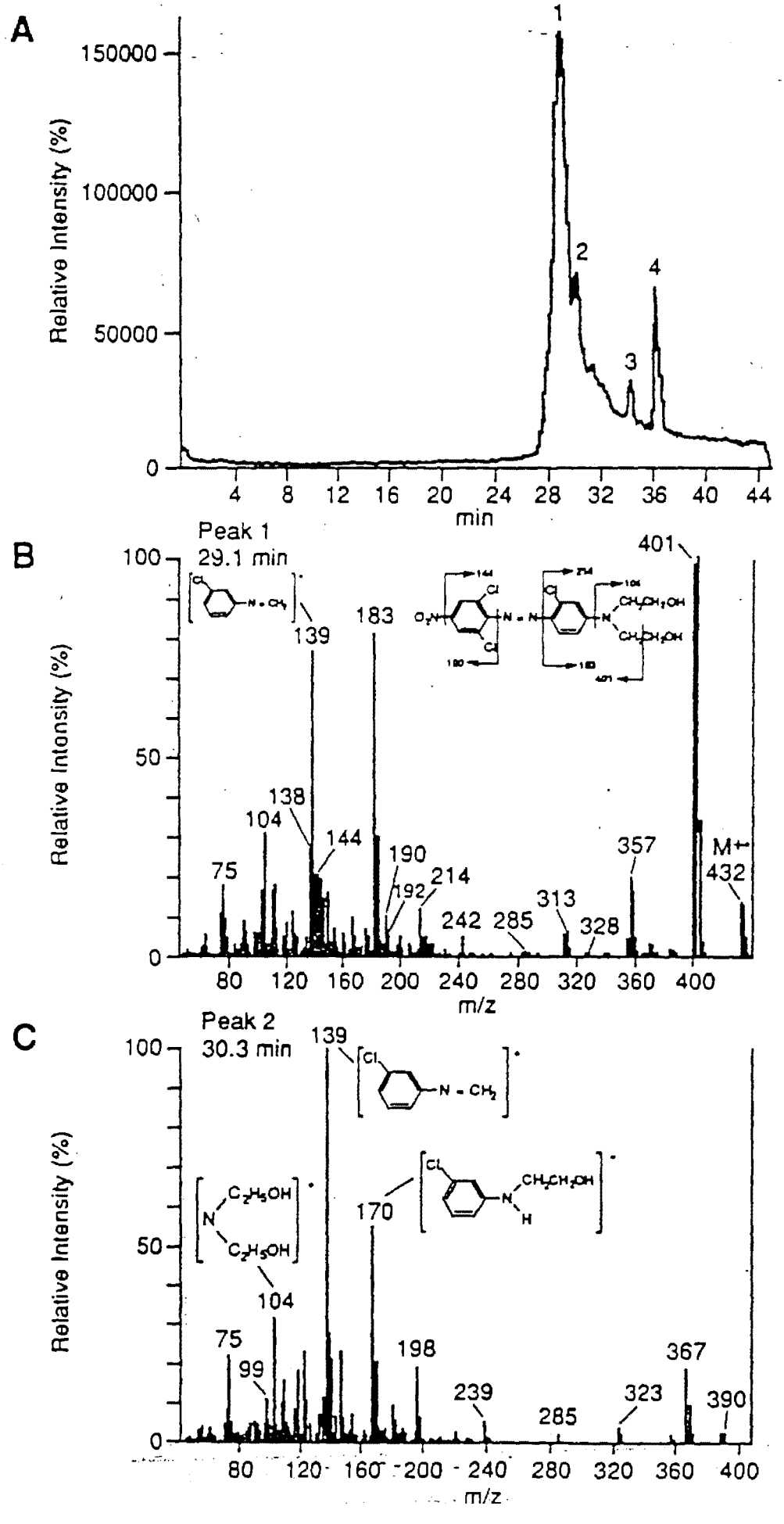
14. Acid Orange 10

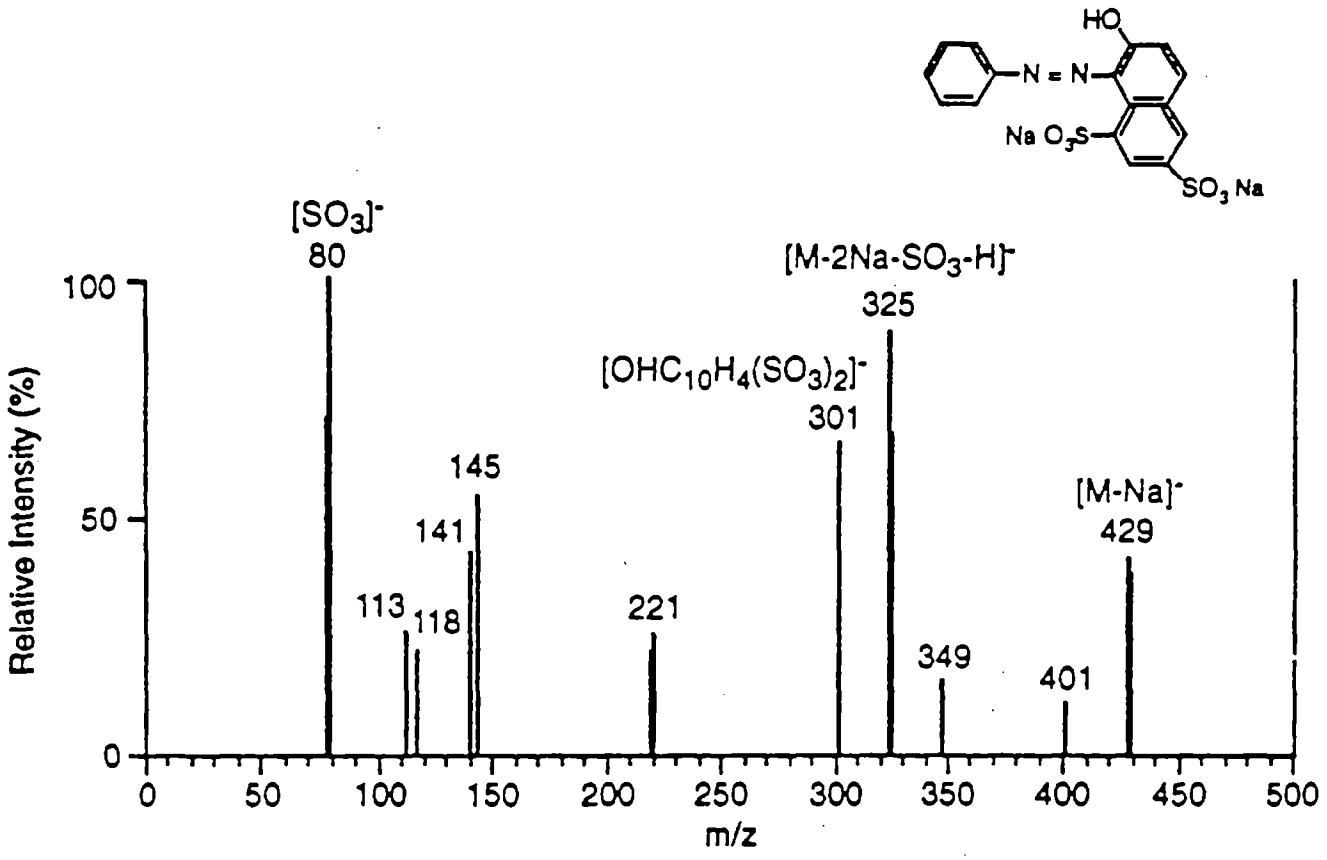
C.I. 16230

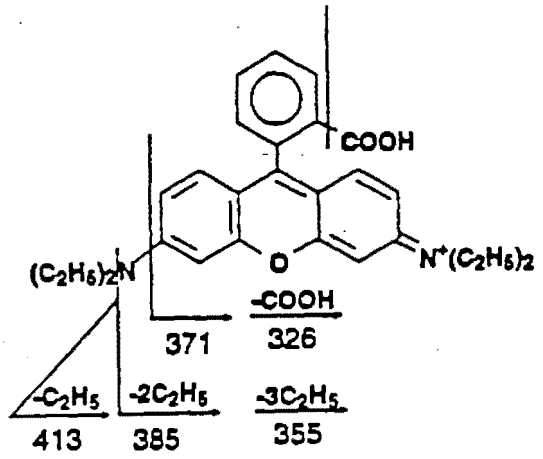

143
CH3
263
OH
O
247
157
N
171
N
135
107


100
50
0
101
108
115
128
135
143
156
171
247
263
278
M+
150
200
250

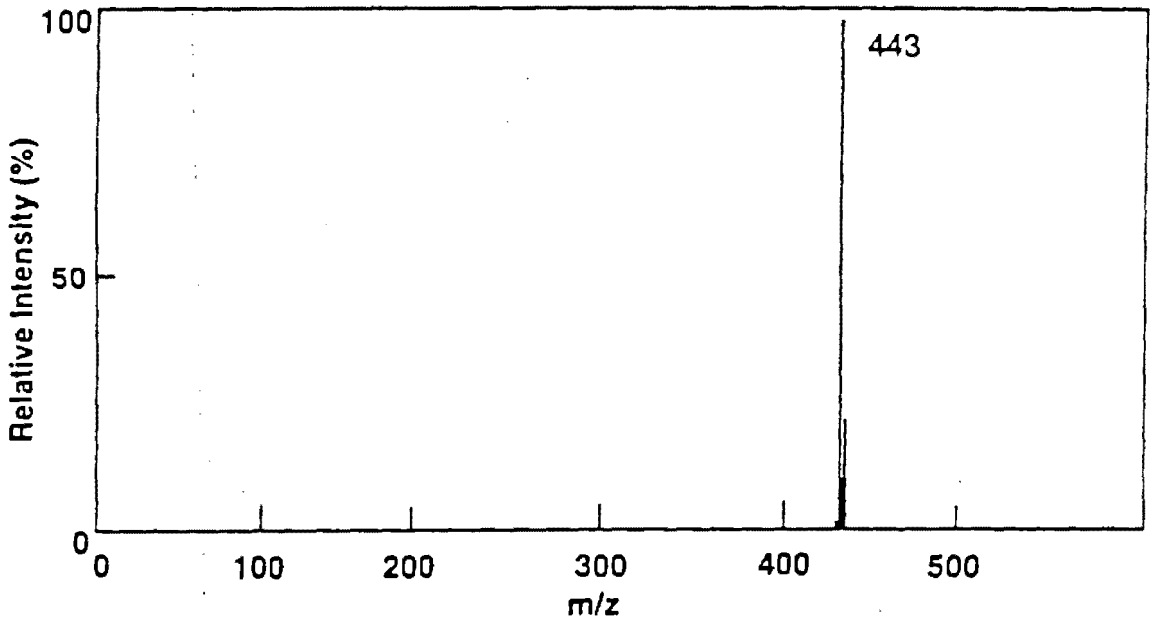

100
50
0
124
135
166
279
MH+
200
250


A
Relative Intensity (%)
150000
100000
50000
0
1
2
3
4
4 8 12 16 20 24 28 32 36 40 44
min
B
Peak 1
29.1 min
Relative Intonsity (%)
100
50
0
139
183
401
138
104
144
75
190
192
214
357
M+
432
313
242 285
328
80 120 160 200 240 280 320 360 400
m/z
C
Peak 2
30.3 min
Relative Intensity (%)
100
50
0
139
170
104
75
99
198
239
285
323
367
390
80 120 160 200 240 280 320 360 400
m/z

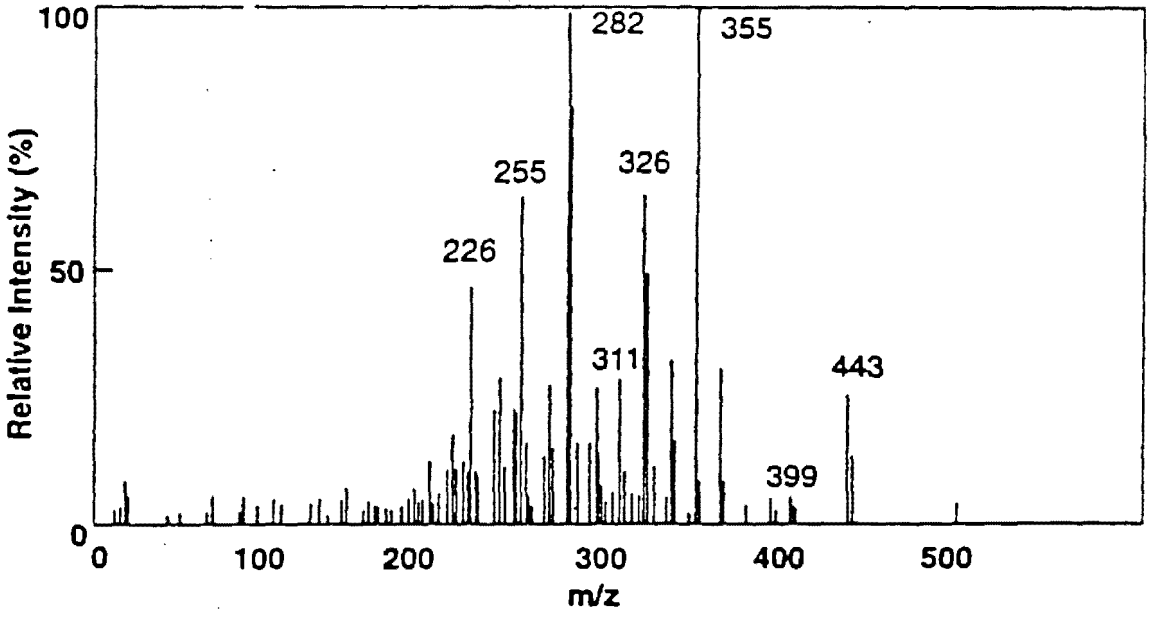

HO
N = N
Na O3S
SO3 Na
[SO3]-
80
[M-2Na-SO3-H]-
325
[OHC10H4(SO3)2]-
301
145
141
113
118
221
[M-Na]-
429
349
401
100
50
0
Relative Intensity (%)
0
50
100
150
200
250
300
350
400
450
500
m/z


COOH
$(C_2H_5)_2N$
O
$N^+(C_2H_5)_2$
-COOH
371
326
$-C_2H_5$
413
$-2C_2H_5$
385
$-3C_2H_5$
355

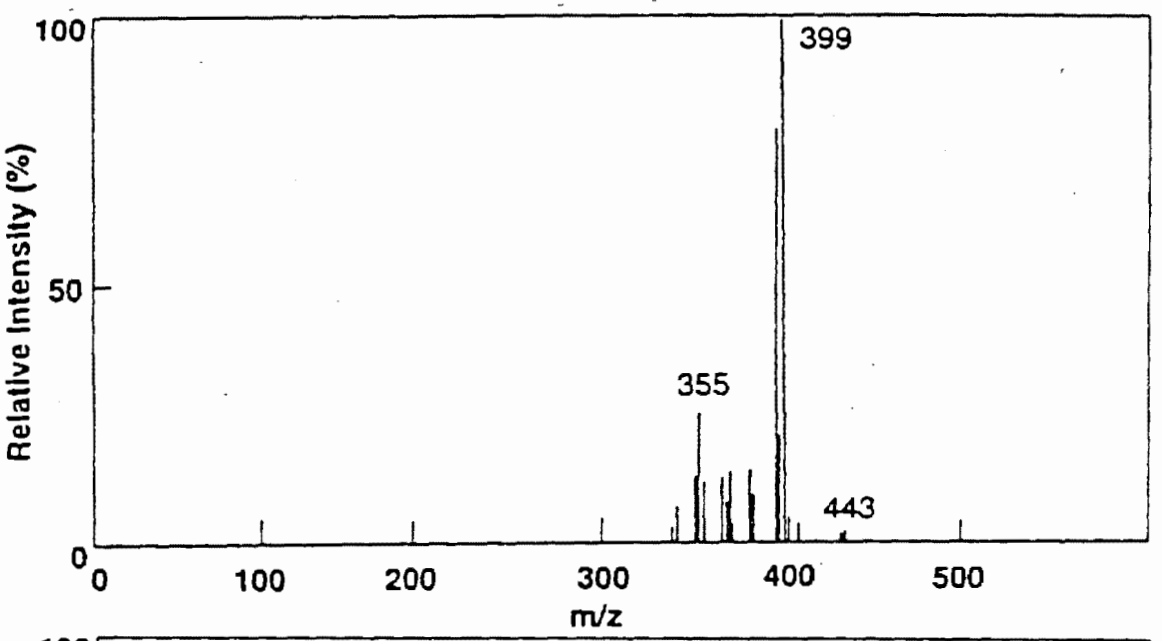
A


100
50
0
Relative Intensity (%)
443
0
100
200
300
400
500
m/z

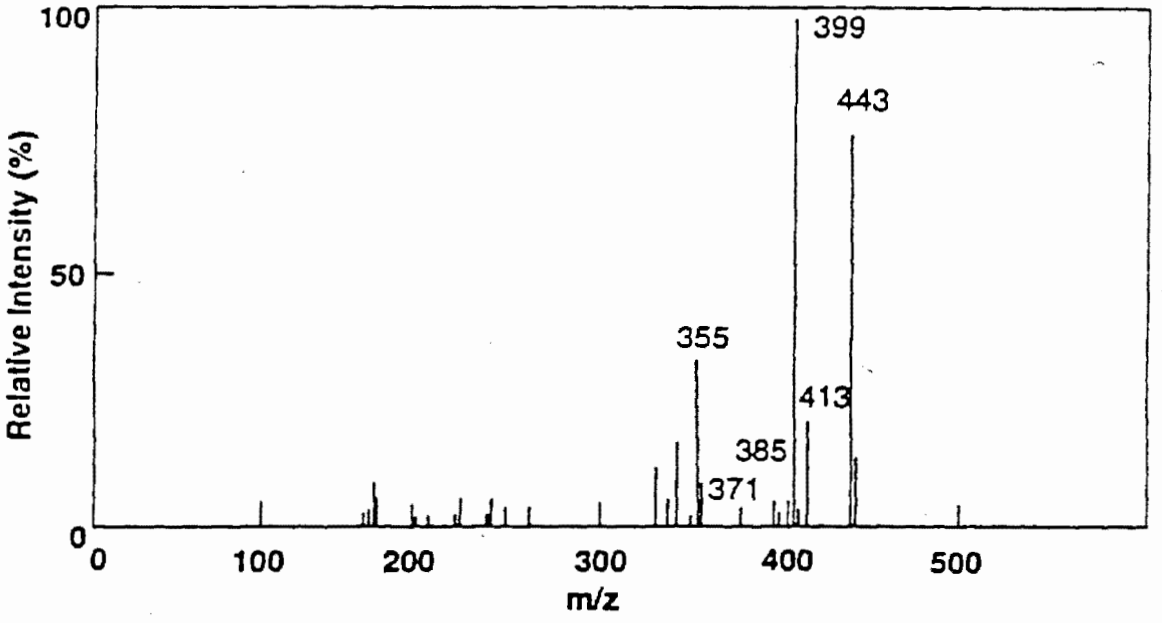
B

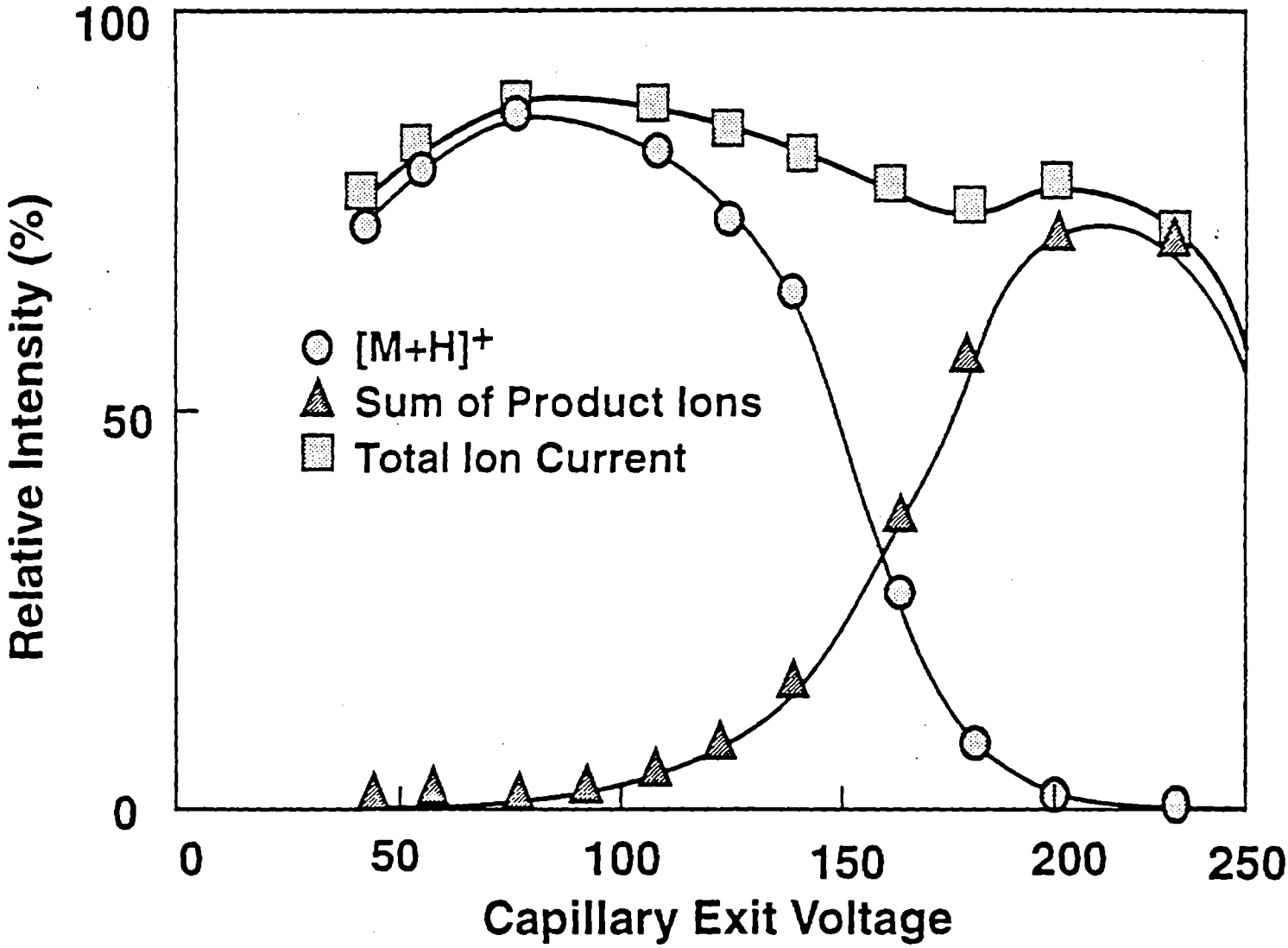

100
50
0
Relative Intensity (%)
282
355
255
326
226
311
443
399
0
100
200
300
400
500
m/z

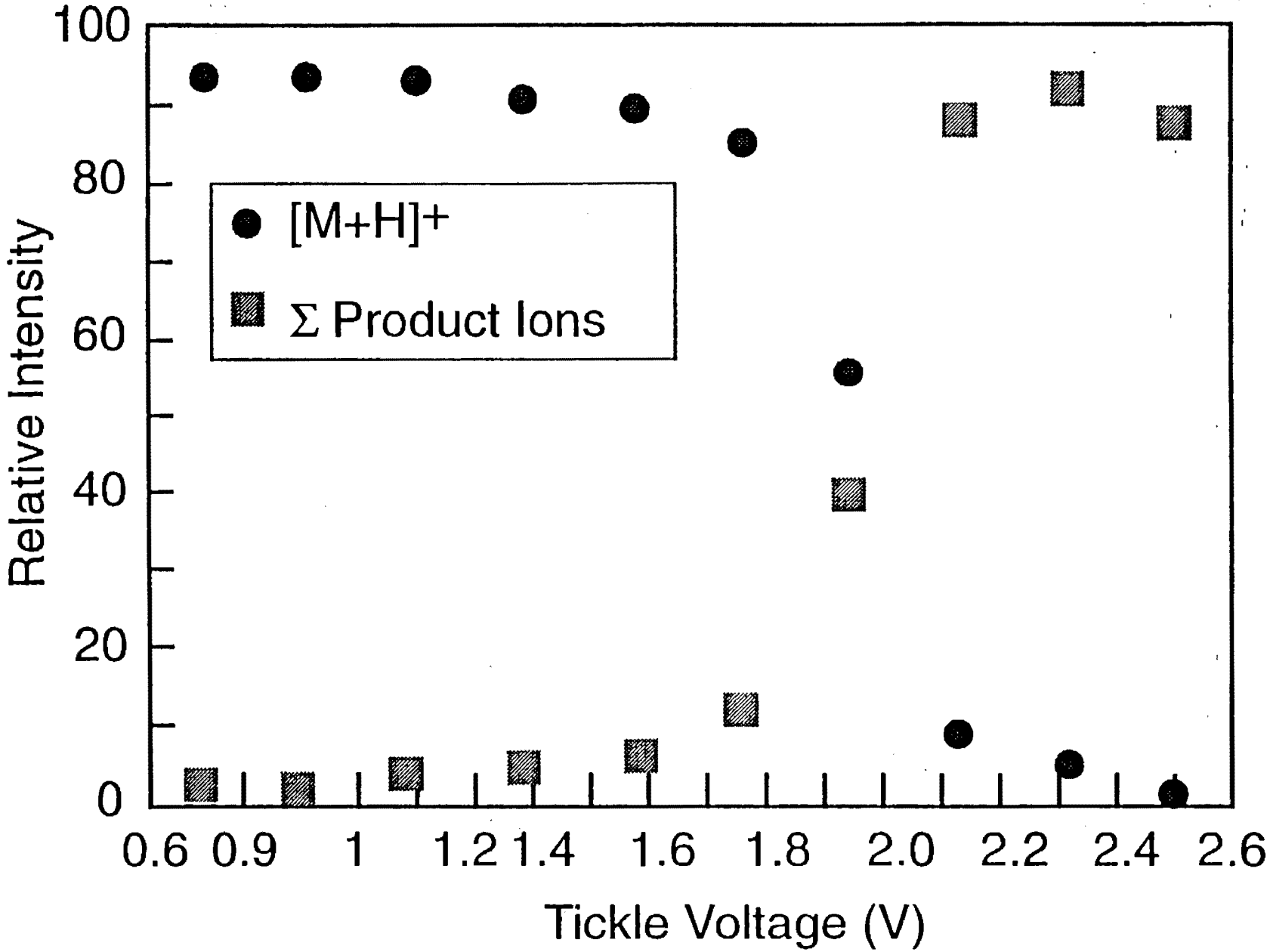
C

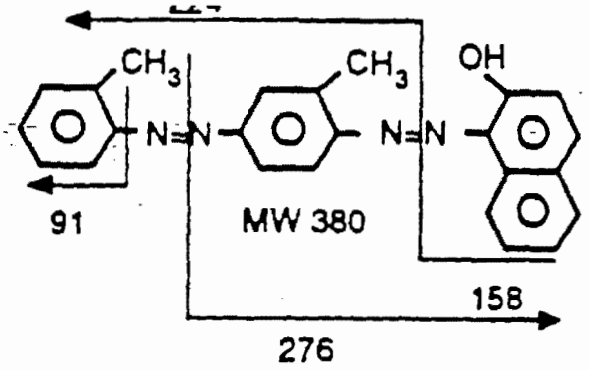

100
50
0
Relative Intensity (%)
399
355
443
0
100
200
300
400
500
m/z

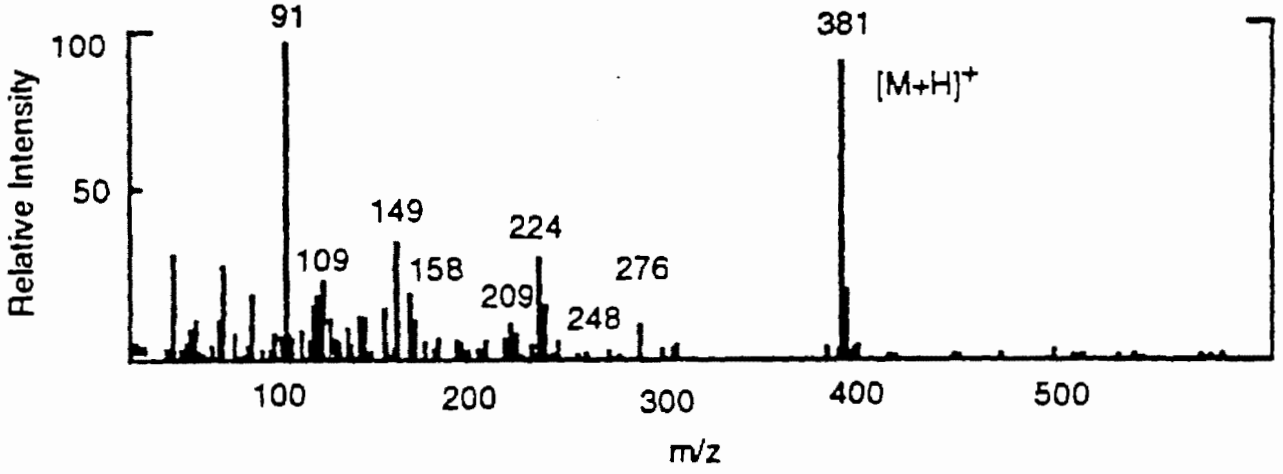
D

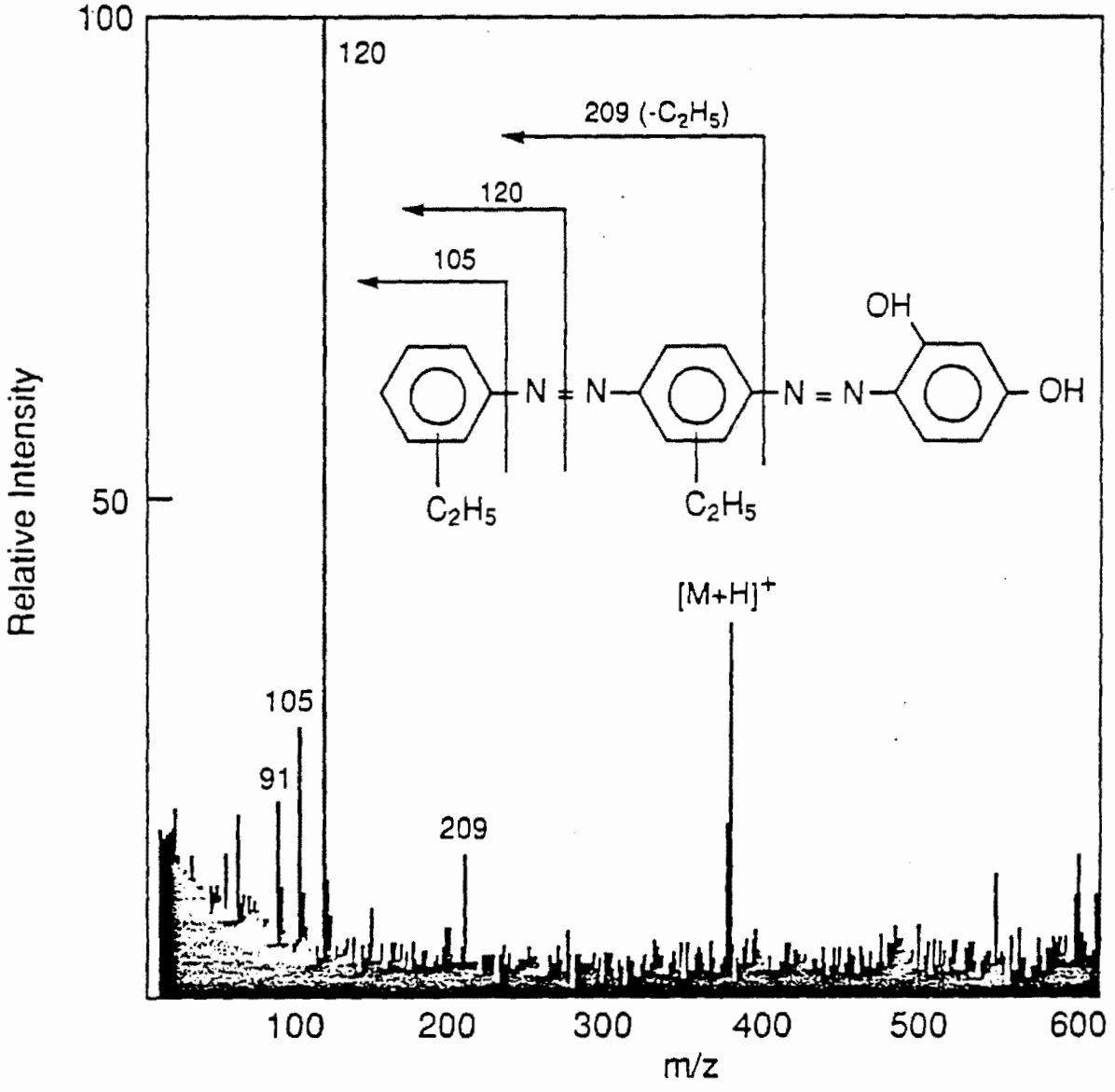

100
50
0
Relative Intensity (%)
399
443
355
413
385
371
0
100
200
300
400
500
m/z


100
50
0
Relative Intensity (%)
[M+H]+
Sum of Product Ions
Total Ion Current
0
50
100
150
200
250
Capillary Exit Voltage


100
80
60
40
20
0
Relative Intensity
[M+H]+
Σ Product Ions
0.6
0.9
1
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
Tickle Voltage (V)


CH3
CH3
OH
N=N
N=N
91
MW 380
158
276


91
381
100
[M+H]+
Relative Intensity
50
149
224
109
158
276
209
248
100
200
300
400
500
m/z


100
50
Relative Intensity
120
209 (-C2H5)
120
105
OH
N = N
N = N
OH
C2H5
C2H5
[M+H]+
105
91
209
100
200
300
400
500
600
m/z

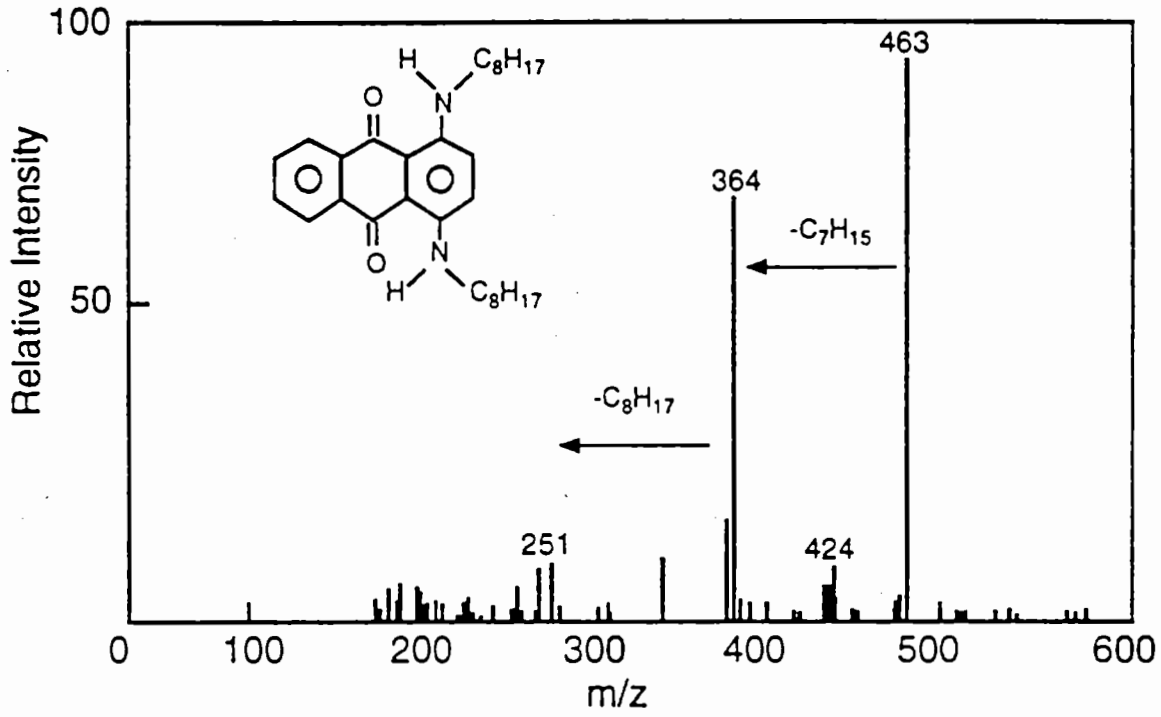

100
50
Relative Intensity
463
364
-C7H15
-C8H17
251
424
0
100
200
300
400
500
600
m/z
H
C8H17
N
O
O
N
H
C8H17

Table 1. Investigated Samples
C.I. = Color Index.

Commercial Name of Dye	C.I. Name	C.I. Number	M.W.	Type of Fiber	Manufacturer
1.5% Serisol Fast Yellow GD	Disperse Yellow 3	11855	269	Diacetate	Yorkshire
2.0% Serisol Fast Yellow PL 150	Disperse Yellow 9	10375	274	Diacetate	Yorkshire
1.2% Resolin Yellow 5GS	Disperse Yellow 5	12790	324	Polyester	Bayer
0.72% Dispersol Orange B-A Grains	Disperse Orange 1	11080	318	Polyester	ICI
0.6% Serilene Orange 5R300	Disperse Orange 1	11080	318	Polyester	Yorkshire
0.6% Serilene Orange 2RL200	Disperse Orange 25	11227	323	Polyester	Yorkshire
0.72% Dispersol Orange B-2R 200 Grains	Disperse Orange 25	11227	323	Polyester	ICI
1% Resolin Orange F3R 200%	Disperse Orange 25	11227	323	Polyester	Bayer
2.2% Resolin Orange RL	Disperse Orange 13	26080	352	Polyester	Bayer
0.6% Serilene Yellow Brown 2RL 150	Disperse Orange 37	-	391	Polyester	Yorkshire
1.5% Serisol Brilliant Red X3B 200	Disperse Red 11	62015	268	Diacetate	Yorkshire
0.6% Serisol Fast Scarlet BD 200	Disperse Red 1	11110	314	Diacetate	Yorkshire
0.6% Serisol Fast Crimson BD 150	Disperse Red 13	11115	348	Diacetate	Yorkhire
0.6% Serilene Red Brown R-FS 150	Disperse Brown 1	11152	432	Diacetate	Yorkshire
1.5% Serisol Brilliant Blue BGN 300	Disperse Blue 3	61505	296	Diacetate	Yorkshire
1.0% Resolin Blue BBLS	Disperse Blue 165	-	405	Polyester	Bayer
1.0% Yoracryl Yellow RL	Basic Yellow 28	-	309	Orlon	Yorkshire
Indigo	Vat Blue 1	73000	262	Denim	Levi Strauss

Table 2. 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)

Table 3. Identification of Chemical Reduction Products of Colorants 1-16 by HPLC/MS[a]

No.	Dye	Identified Reduction Products	Mol wt.	t_R	Particle Beam (*m/z*, relative intensity)	UV (%)
1	Solvent Yellow 2	aniline [b]	93	12.9	93(100); 66(39)	>1
		N,N-dimethyl-1,4-diaminobenzene	136	28.4	136(100); 120(85); 93(37); 81(41)	88
2	Solvent Yellow 3	2-aminotoluene [b]	107	18.2	107(74); 106(100); 77(26); 51(15)	90
		2-methyl-1,4-diaminobenzene [b]	122	30.0	122(100); 94(33); 78(26); 58(19)	3
		unchanged dye [c]	225	25.8	225(58); 134(17); 106(100); 91(28); 77(23); 75(13); 51(4)	3
3	Solvent Yellow 14	aniline [b]	93	13.1	93(100); 66(37)	7
		1-amino-2-naphthol [c]	159	19.3	159 (100); 130(89); 103(26); 77(22); 51(15)	49
		unchanged dye [c]	248	28.3	248(90); 219(7); 171(15); 143(100); 115(97); 89(10); 77(41); 51(10)	20
4	Solvent Orange 7	2,4-dimethylaniline [b]	151	22.1	151(11); 121(100); 106(78); 77(15)	30
		1-amino-2-naphthol [b]	159	20.2	159(100); 130(63); 103(13); 77(17); 51(11)	31
5	Solvent Red 24	2-aminotoluene [b]	107	22.4	107(100); 91(55); 77(49); 51(25)	18
		2-methyl-1,4-diaminobenzene [d]	122	40.4	122(33); 104(46); 71(41); 55(100)	16
		1-amino-2-naphthol [b]	159	19.1	159(100); 130(70); 103(20); 77(20)	42
6	Pigment Red 3	1-amino-2-naphthol[b]	159	19.5	159(100); 130(62); 103(15); 77(19)	70
7	Disperse Red 1	4-nitroaniline [b]	138	13.0	138(100); 108(83); 92(50); 65(75)	70
8	Disperse Orange 13	aniline [b]	93	13.1	93(100); 66(40)	>1
		4-aminophenol [b,c]	109	6.2	109(10); 108(100); 80(48); 64(7)	10
		1,4-diamino-naphthalene	158	10.7	158(100); 109(50); 80(37)	78
9	Disperse Orange 25	4-nitroaniline[b]	138	12.3	138(100); 108(83); 92(50); 65(75)	18
		N-(2cyanoethyl)-*N*-(ethyl)-1,4-diaminobenzene	189	14.8	189(25); 149(100); 120(34); 92(4)	75
10	Disperse Orange 44	*N,N*-bis(2-cyanoethyl)-1,4-diaminobenzene [b]	214	8.3	214(40); 174(100); 120(37); 106(5)	70
11	Disperse Orange 30	1,4-diamino-2,6-dichlorobenzene	176	13.8	176(27); 149(73); 124(40); 98(100); 81(56); 78(63)	35
		N-(2-cyanoethyl)-*N*-(2-hydroxyethyl)-1,4-diaminobenzene	205	6.1	205(45); 174(85); 165(80); 120(100); 92(30); 65(20)	35
		2,6-dichloro-4-nitroaniline	206	25.5	208(40); 206(60); 178(50); 176(84); 162(20); 160(30); 135(15); 133(22); 126(30); 124(100); 92(28); 90(31)	20
		N-(2-cyanoethyl)-*N*-(2-acetoxyethyl)-1,4-diaminobenzene	247	10.0	247(1); 205(32); 174(91); 165(54); 120(100); 92(20); 65(20)	45
12	Disperse Black 9	1,4-diaminobenzene [b]	108	8.3	108(100); 92(44); 80(66); 67(25); 52(64)	30
		N,N-bis(2-hydroxyethyl)-1,4-diaminobenzene	196	21.7	196(25); 165(100); 120(20); 93(14)	60
13	Disperse Blue 79	2-bromo-1,4,6-triaminobenzene [e]	202	10.4	203(15); 202(5); 201(10); 88(23); 70(83); 61(100)	25
		3-acetamido-4-[*N,N*-bis(2-acetoxyethyl)-amino]-1-amino-5-methoxybenzene	367	15.1	367(10); 294(15); 208(9); 87(100)	60

No	Dye	Identified Reduction Products	Mol wt.	t_R	Particle Beam (*m/z*, relative intensity)	UV (%)
14	Disperse Orange 37	1,4-diamino-2,6-dichlorobenzene	176	23.0	176(27); 149(73); 124(40); 98(100); 84(56); 74(63)	40
		N-cyanoethyl-*N*-ethyl-1,4-diaminobenzene	189	26.1	189(19); 149(100); 120(73); 106(8); 92(21)	40
		4-amino-2,6-dichloro-4'[(*N*-(2-cyanoethyl)-amino]azobenzene	333		334(15); 333(27); 293(100); 265(21); 229(7); 201(33); 149(49); 120(41);100(15); 92(9)	10
15	Disperse Brown 1	1,4-diamino-2,6-dichlorobenzene	176	22.3	176(56); 149(28); 134(59); 98(100); 84(53)	40
		3-chloro-*N*,*N*-bis(2-hydroxyethyl)-1,4-diaminobenzene	230	17.4	230(21); 199(100); 155(37); 127(13)	40
16	Acid Orange 10	aniline [b]	93	13.5	93(100); 66(40)	40
		8-amino-7-hydroxynaphthalene-1,3-disulfonic acid, disodium salt [b,d]	363	16.6	Not detected [f]	48

[a] t_R = retention time in TIC chromatogram (min); *m/z* (relative intensity) reports the major peaks (>5%) of each product down to *m/z* 50. A maximum of 18 ions are reported in descending *m/z* ; UV (%) = [peak area of identified reduction product]/$\sum$ [of the peak areas in the chromatogram of the reduced sample at a wavelength of 254 nm]. The identified reduction products were <0.5% of the total peak area in the unreduced HPLC/UV analysis of the parent dye.

[b] Identity confirmed with standard.

[c] Observed only after $Na_2S_2O_4$ reduction.

[d] Observed only after $SnCl_2$ reduction.

[e] Only identified by HPLC/PB-MS in sample reduced with $SnCl_2$.

[f] Identity confirmed by thermospray-MS; ions detected include 371(100), 364(5), 319(4), and 274(9).

Table 4. Comparison Of Particle Beam LC/MS and GC/MS In Tentatively Identifying Compounds In An Azo Dye Sample

		MW	PB LC/MS		GC/MS	
			EI	NCI	EI	NCI
1.	Aniline	93			X	
2.	N-phenylformamide	121			X	
3.	4-nitroaniline	138			X	
4.	N-cyanoethylaniline	146			X	
5.	4-phenylphenol	170	X	X	X	
6.	2,4,6-trimethoxy-1,3,5-triazine	171			X	
7.	2-chloro-4-nitroaniline	172		X	X	X
8.	2,6-dimethoxy-4-(N,N-dimethyl amino)-1,3,5-triazine	184			X	
9.	N,N-bis(cyanoethyl)aniline	199	X	X	X	
10.	2-bromo-4-nitroaniline	216			X	X
11.	Sulfur (S8)	256		X	X	X
12.	2-bromo-4,6-dinitroaniline	261		X	X	X
13.	4-bromo-N,N-bis(cyanoethyl)-aniline	277	X	X	X	
14.	Hexachlorobenzene	282		X		X
15.	Tribromoanisole	342		X	X	X
16.	4-(2'-chloro-4'-nitrophenylazo)-N,N-bis(cyanoethyl)aniline	382	X	X		
17.	4-(2'-bromo-4',6'-dinitrophenyl azo)-3-acetamido-N,N-diethyl aniline	478	X	X		
18.	4-(2'-chloro-4'-nitrophenylazo)-3-acetamido-N,N-bis(ethyl ethonate)aniline	505		X		
19.	4-(2'-bromo-4',6'-dinitrophenyl azo)-5-acetamido-2-methoxy-N,N- diethylaniline	508	X	X		
20.	Disperse blue 79	624	X	X		

MW = molecular weight
PB = particle beam
EI = electron ionization
NCI = negative chemical ionization (carbon dioxide)

Table 5. Predicted Success Of Varous LC/MS Techniques for The Characterization Of Selected Dye Classes

	MS Techniques		
Dye Classes	Particle Beam-LC/MS (EI/CI)	Thermospray LC/MS	Electrospray LC/MS
Sulfonated Azo	○	◐	●
Cationic	○	◐	●
Azo (disperse)	◐	●	●
Azo (solvent)	◐	●	●
Anthraquinone	◐	◐	◐

○ ◐ ●

——————> ——————>

Increasing success for MS analysis in terms of sensitivity and specificity.

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECI
EPA 600/A-95/072		

4. TITLE AND SUBTITLE
LC/MS Techniques for the Analysis of Azo Dyes

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S) Yinon, J. (1); Betowski, L.D. (2); Voyksner, R.D. (3)

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS

(1) Weizmann Institute of Sci., Dpt of Env. Sci. Rehovot, 76100 ISREAL; (2) U.S. EPA; (3) Research Triangle Institute, RTP, NC

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
CR 819555

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. EPA/ORD/NERL
Characterization Research Division
POB 93478, Las Vegas, NV 89193

13. TYPE OF REPORT AND PERIOD COVERED
Book Chapter

14. SPONSORING AGENCY CODE
EPA 600/07

15. SUPPLEMENTARY NOTES
Yinon, J.; Betowski, L.D.; Voyksner, R.D. "LC/MS Techniques for the Analysis of Azo Dyes," In *Applications of LC/MS in Environmental Chemistry*, D. Barcelo, ed. Elsevier (publisher)

16. ABSTRACT

Dyestuffs are of major environmental interest because of their widespread use as colorants for e.g., textiles, paper, leather, and foodstuffs. Synthetic intermediates, by-products and degradation products could be potential health hazards because of their toxicity or carcinogenicity. The dyes do not belong to one group of chemical compounds. The analysis of such a large variety of compounds poses difficulties because of differences in solubility, volatility, ionization efficiency, etc. Furthermore, some of the manufacturing precursors to dyes are carried over to and are not removed from the final dye product. The result is a complex mixture characterized not only by the dye itself, but also by several other compounds. Most dyes, including sulfonated azo dyes are nonvolatile or thermally unstable, and therefore, are not amenable to GC or gas phase ionization processes. Thus, GC/MS techniques cannot be used. However, the combination of LC with MS enables the separation of nonvolatile, thermally unstable, and polar dyes for introduction into the MS for identification. As a result of interfacing LC with MS, three major types of interfaces and LC/MS techniques have been developed: (1) Thermospray, (2) Particle Beam, and (3) Ion Spray and Electrospray. This chapter describes the application of these LC/MS techniques in the analysis of dyes.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Analytical Methods, Dye Analysis, Sulfonated Dyes, Thermospray		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 58
Release to the Public	20. SECURITY CLASS (This Page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE