J Forensic Sci, Mar. 2004, Vol. 49, No. 2
Paper ID JFS2003295
Available online at: www.astm.org

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Forensic Identification of Dyes Extracted from Textile Fibers by Liquid Chromatography Mass Spectrometry (LC-MS)*

ABSTRACT: LC-MS is used for the identification of dyes extracted from textile fibers and the utility of the method for forensic trace analysis is demonstrated. The technique is shown to provide a high degree of chemical structural information, making dye identification highly specific in comparison to optical and/or chromatographic methods of dye analysis. A UV-visible absorbance detector, placed in series before the MS detector, facilitates monitoring the elution of dyes in the presence of other non-dye components extracted from colored textile fibers. In this way, dye identification becomes practical, even when a dye standard is not available for comparison. A set of 22 reference dyestuffs and 10 dyes extracted from textile fibers were analyzed to demonstrate the utility of the method. Six of the extracted dyes corresponded to dyes also contained in the set of 22 reference dyestuffs. Reference dyestuffs were not available for four of the extracted dyes. Triethylamine (TEA) was shown to increase the electrospray ionization-mass spectrometry (ESI-MS) response of dyes containing multiple sulfonated groups.

KEYWORDS: forensic science, textile dye, LC-MS, trace analysis

Textile fibers are frequently encountered as physical evidence in criminal investigations. An important aspect of forensic fiber examination is the characterization of textile dyestuffs for the purpose of establishing the possibility of a common source of a questioned and a known fiber. There are currently several instrumental methods used for dye analysis including ultraviolet, visible, and fluorescence spectrophotometry (1), infrared spectrometry (2), and high performance liquid chromatography (HPLC) (3), along with non-instrumental methods, such as dye extraction followed by thin layer chromatography (4). The discriminating power of these techniques may be judged as low in certain instances, for example when two dyes differ only by the presence of an auxochrome in one of the dyes. Two dyes of highly similar structure may appear to be the same color, have virtually indistinguishable UV-visible absorption spectra, and may have retention times that are not easily distinguishable under HPLC analysis. These shortcomings can influence the comparison of dyes extracted from questioned and known fibers, as well as the comparison of a known standard with a dye extracted from a questioned fiber. In instances where the optical and/or chromatographic behaviors of dyes from a questioned and a known source are different, existing techniques are sufficient to disprove a common source. However, in light of the many hundreds of dyes used in the textile industry, and the common occurrence of dyes differing by a single auxochrome, an analytical method that provides molecular-level information on dye structure is needed for unambiguous forensic identification or comparison in common source determinations.

Electrospray ionization (ESI) is arguably the most universal technique for the ionization of nonvolatile and thermally-labile molecules for mass spectrometry analysis (5). Multiple charging, which often occurs in an ESI, makes it possible to determine the mass of large molecules without requiring a mass spectrometer with an extended m/z range. As a soft ionization source, molecular ions are generally produced. The combination of multiple charging and the common formation of molecular ions make ESI-MS a powerful technique for the identification of textile dyes. The use of ESI-MS, without an LC separation scheme, has recently been reported for the analysis of a small set of acidic dyes (6). In the absence of an LC separation scheme, the authors concluded that ESI-MS/MS was required for absolute verification of a dye mixture identity. Modern LC-MS methodologies are available that utilize very low flow rates (μL/min even nL/min rates) and thus require extremely low sample volumes and are highly congruent with the needs of forensic analysts (6).

The combination of liquid chromatography and mass spectrometry (LC-MS) is a highly selective and sensitive analytical method that has been shown to be useful for the characterization of dyes according to their molecular structures (7–18). Mayer and coworkers have analyzed disperse dyes and azo dyes from textile sources by LC-MS (15,16). Ballpoint pen inks have been analyzed by ESI-MS for forensic purposes (17). In previous work by Yinon and Saar (8), thermospray ionization LC-MS was used to analyze known dyestuffs extracted from textile fibers. Several dyed fiber samples were investigated by extracting and analyzing the dye from a single 5–10 mm long piece of fiber with 5 μL of organic solvent. In a related non-forensic application, a combination of LC with diode-array detection and/or ESI-MS has been utilized for the determination of oxidative hair dyes uses in commercial formulations (18). In the study reported here, we employ a UV-visible absorption detector in series with an ESI-MS(quadrupole) to produce a somewhat

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* Presented at: 55th Annual Meeting of the American Academy of Forensic Sciences, Chicago, IL, February 2003.
This work was sponsored by the National Institute of Justice, Office of Science and Technology, Investigative and Forensic Sciences Division, under grant 98-IJ-CX-K0003. The work was done at the National Center for Forensic Science, a National Institute of Justice program hosted by the University of Central Florida, and a member of the Forensic Science Resource Network.
Received 1 Sept. 2003; and in revised form 16 Nov. 2003; accepted 16 Nov. 2003; published 19 Feb. 2004.

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generalized method for the analysis of dyes extracted from forensic samples.

Spectral libraries have found considerable application for the identification of electron ionization mass spectra. Similarly, the application of LC-MS to the forensic analysis of dyestuffs could benefit from a reference spectral database; however, before beginning to develop a dyestuffs database, it is important to demonstrate the general applicability and utility of the analytical method. The study reported here represents the first step in the development of a generalized method of high forensic value and the eventual compilation of a reference database for forensic purposes. Extraction of textile dyes almost invariably leads to the recovery of additional, often UV-absorbing, components such as fluorescent brighteners. These fiber components would also be important to include in a database designed to meet the needs of the forensic community. Although the importance of a database for dye identification with molecular specificity is readily recognized, it is also important to note that structural identification is not strictly necessary in comparing questioned and known items. A rigorous comparison of the chromatographic behavior of a fiber extraction solution, complemented by molecule-related MS data for each eluting component, has the potential of greatly increasing the analyst's confidence in a probable common source determination.

In this paper, dyestuffs from several classes (acid, direct, disperse. . .) are analyzed by LC-ESI-MS. Reagent dyestuffs and dyes extracted from textile fibers are analyzed to demonstrate the utility of the methodology. Several dyes that are insensitive to ESI-MS detection, especially those containing multiple sulfonated groups, are shown to be readily analyzed by the addition of triethylamine (TEA) to enhance the signal intensity. The use of UV-visible absorption coupled in series with ESI-MS detection is also demonstrated for monitoring dyes separated by HPLC.

Experimental

Instrumentation

An Agilent 1100 MSD quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and interfaced to an Agilent 1100 HPLC are used for this study. The instrument can be conveniently switched between positive and negative ion modes for the detection of dyes that form either negative or positive ions. Mass fragmentation ions are obtained by changing the "fragmentor" voltage to add additional energy to induce the decomposition of molecular ions formed in the ESI source. The fragmentor voltage is a key parameter in the Agilent 1100 MSD. The mass spectrum pattern obtained by applying a fragmentor voltage should be compared to those obtained by MS/MS in ion trap instruments. A syringe pump (KDS-100 Infus-pump) was used to introduce TEA post-column.

Extraction of Dyes from Textile Fibers

Dyes were extracted from fibers into 1:1 methanol/water mixtures in a sealed glass capillary under heating at 100°C. A 20 μL aliquot of the methanol/water mixture was used to extract a 5 mm piece of thread. HPLC-MS analysis was made on 5 μL injections. Textile fibers were provided by TESTFABRICS, INC, 415 Delaware Ave., P.O. Box 26, West Pittston, PA.

HPLC Separation and Mass Spectrometry

Separation was carried out on a ZORBAX Eclipse XDB-C18 (2.1 × 150 mm) HPLC column at a mobile phase flow rate of 0.20 mL/min. A programmed solvent gradient (methanol/water) was used to achieve better separation. A 1:1 methanol/water mixture was held constant for the first 5 min of analysis, followed by a steady increase in the methanol composition to 95% at 25 min. The methanol composition was held constant at 95% until the analysis ended at 40 min. Long elution times for each run are necessary to elute all of the co-extracted components and keep the column clean to ensure reproducible performance. Mass spectrometer parameters were optimized for maximum sensitivity. The drying gas for the ESI was held at 12.0 L/min and the spray chamber temperature was set at 350°C for all analytes, unless otherwise specified.

Methanol (HPLC grade, AlliedSignal Inc., Burdick & Jackson, Muskegon, MI) was used as received. Water was prepared from Barnstead E-pure (Barnstead/Thermolyne, Iowa), with a resistance of 18.2 megohm-cm. All the dye reagents are from Aldrich Chemical Company, Inc. (Milwaukee WI), and Sigma Chemical Co. (P.O. Box 14508, St Louis, MO). Stock solutions of dyestuffs were prepared in 100% methanol at concentration of 50 μg/mL. The generic names and chemical structures for the dyestuffs are given in Table 1. The fiber composition and dye content of the fiber samples are given in Table 2.

In a generalized method, a dye-containing sample (reference dye or extracted dye) is first analyzed at a low fragmentor voltage to optimize molecular ion detection. A second analysis is conducted at a higher fragmentor voltage to induce ion fragmentation and enhance structural elucidation.

Results and Discussion

Dyes widely used in textile industry are highly varied in chemical structure and as such, their ionization efficiencies and thus MS sensitivities are also highly varied. Different ion formation pathways and different fragmentation patterns are expected for dyes of different classes under ESI conditions. Some dyes tend to produce positive ions, while others favor negative ions. Impurities from the dyestuffs and reagents added for stabilization during dyeing process may be taken into the sample solution during extraction. As a result, the HPLC-MS of the extracted dye solution could be complicated, thereby increasing the challenge of identifying textile dyes in the absence of a dye standard.

A major challenge for the analysis of textile dyes by HPLC-MS is the development of a generalized procedure for the efficient analysis of very small samples of the type commonly encountered in forensic science. If a generalized instrumental method is to be developed, it must accept extraction samples prepared in a series of solvent systems, as employed in the FBI guidelines for fiber analysis (4); otherwise, a generalized extraction method must be developed. Ideally, a standard HPLC separation protocol would be applicable across a wide range of dyestuffs. The molecular diversity encountered in industrial dyes makes the development of a completely generalized analytical protocol very challenging. As a first step toward the development of an analytical protocol for dye analysis that is somewhat generic and useful in forensic investigations, a UV-visible absorbance detector has been coupled in series with an ESI-MS detector to facilitate the identification of visible-absorbing dyes as they elute from an HPLC.

Monitoring the Dye Components Elution in HPLC Separation by UV/Visible Spectrophotometry Detector

Generally speaking, the ionization efficiency for many dyes is low, especially for the sulfonated group containing dyes. In many

TABLE 1—*Reference dyes used in this study and their chemical structures.*

	Dye	Formula Wt.	Chemical Structure
1	Acid Blue 40	473	
2	Acid Violet 7	566	
3	Direct Blue 71	1029	
4	Direct Blue 75	1055	
5	Direct Red 1	627	
6	Disperse Blue 3	296	
7	Disperse Blue 56	349	
8	Disperse Orange 3	242	
9	Disperse Orange 13	352	

TABLE 1—*Continued.*

	Dye	Formula Wt.	Chemical Structure
10	Disperse Orange 25	323	O_2N—C6H4—N=N—C6H4—N(CH_2CH_3)(CH_2CH_2CN)
11	Disperse Red 1	314	O_2N—C6H4—N=N—C6H4—N(CH_2CH_3)(CH_2CH_2OH)
12	Disperse Red 13	348	O_2N—C6H3(Cl)—N=N—C6H4—N(CH_2CH_3)(CH_2CH_2OH)
13	Disperse Yellow 3	269	CH_3—C(=O)—NH—C6H4—N=N—C6H3(CH_3)(HO)
14	Disperse Yellow 9	274	O_2N—C6H3(NO_2)—NH—C6H4—NH_2
15	Fat Brown	292	CH_3CH_2O—C6H4—N=N—C10H6—OH
16	Indigo	262	O, H, N, N, H, O
17	Malachite green	382	$N(CH_3)_2$; OH • HCl; $N(CH_3)_2$
18	Malachite green oxalate	927	[$N(CH_3)_2$+; $N(CH_3)_2$]$_2$ 2 $HC_2O_4^-$ • 2 $H_2C_2O_4$
19	Orange G	452	HO; N=N; NaO_3S; SO_3Na

TABLE 1—*Continued.*

	Dye	Formula Wt.	Chemical Structure
20	Rhodamine B base	442	$(CH_3CH_2)_2N$, $N(CH_2CH_3)_2$, O
21	Sudan III	352	HO, N=N, N=N
22	Tartrazine	534	NaO_3S, N=N, OH, N, N, NaO, O, SO_3Na

TABLE 2—*Dyes extracted from textile fibers. The number of reference dyes from Table 1 are given where the corresponding dye was available.*

Fiber sample	Dyestuff	Ref. Dye	Fiber	Chemical Structure
A	Acid Red 151	NA‡	nylon	NaO_3S, N=N, N=N, HO
B	Basic Green 4	17†	acrylic	
C	Disperse Blue 56	7*	polyester	
D	Disperse Red 1	11*	acetate	
E	Disperse Red 4	NA‡	polyester	O, NH_2, OCH_3, O, OH
F	Direct Red1	5*	cotton	
G	Direct Blue 1	NA‡	cotton	NaO_3S, N, N, H, O, NaO_3S, NH_2, H_3OC, OCH_3, N, N, H, O, SO_3Na, H_2N, SO_3Na
H	Direct Blue 71	3*	cotton	
I	Direct Blue 75	4*	cotton	
J	Direct Blue 90	NA‡	cotton	SO_3Na, N, N, Cu^{2+}, O-, O-, NaO_3S, SO_3Na, NaO_3S, N, N, -O, Cu^{2+}, -O, N, N

* See Table 1 for dye structure.
† Malachite green, see Table 1 for dye structure.
‡ Reference dye not in Table 1.

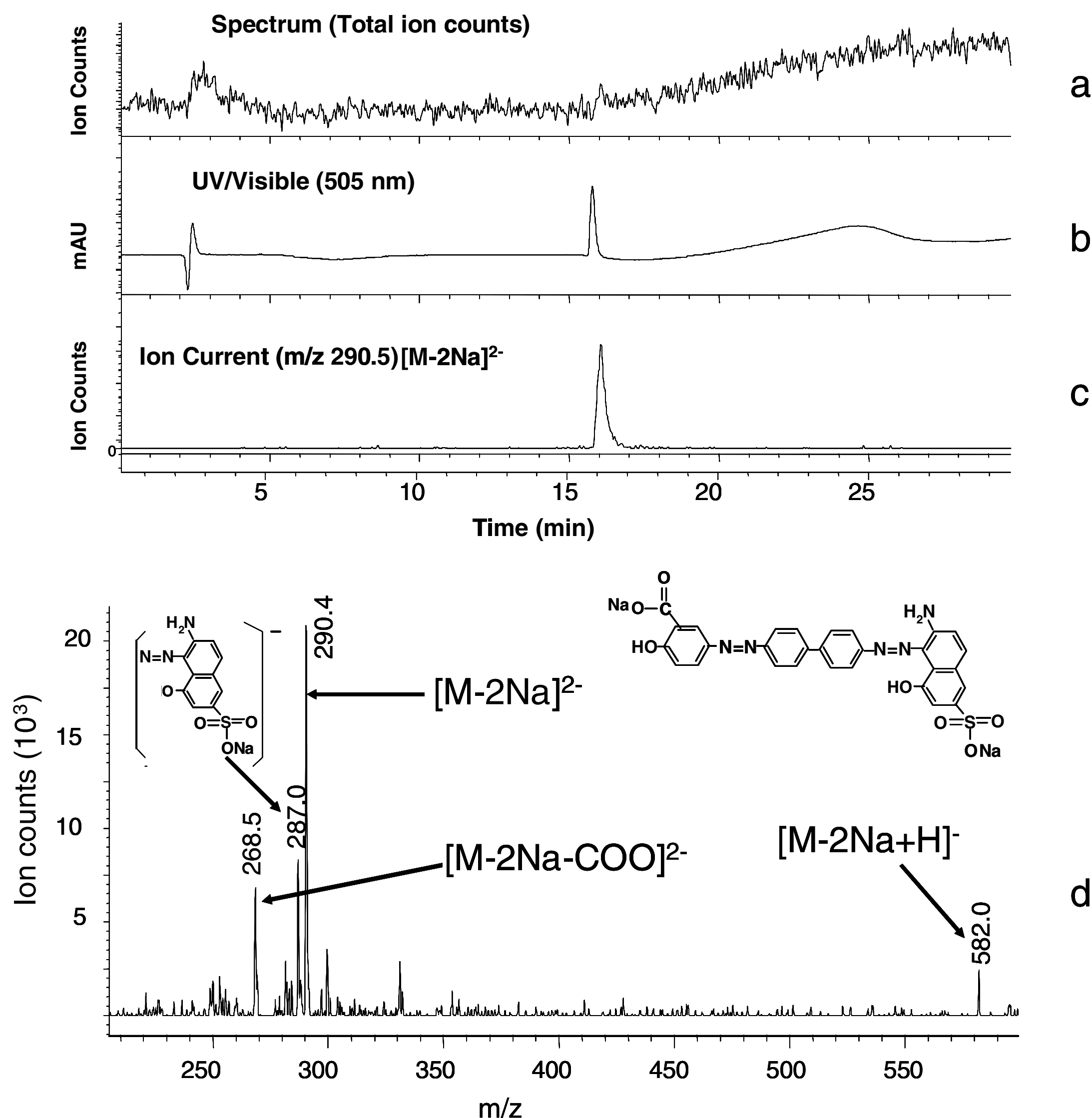

FIG. 1—*HPLC-MS data for direct red 1 extracted from a cotton fiber (a) total ion chromatogram, (b) UV-Visible signal monitored at 505 nm, (c) extracted ion current for m/z 290.5, (d) mass spectrum corresponding to the peak observed in the m/z 290.5 extracted ion current.*

cases, it is difficult to determine when an analyte elutes from a HPLC, due to the weak analyte signal in the presence of other easily ionized components in the sample. Without a method to identify the analyte elution peak, or at least to minimize the possible candidate elution peaks, it becomes necessary to analyze every eluting compound. As a result, the methodology is not practical, if not impossible, for routine dye identification. An example of the challenge is the identification of the dye direct red 1 extracted from acetate fiber, as shown in Fig. 1. From the total ion current (TIC) chromatogram (top trace in Fig. 1*a*), it is difficult to determine the elution time for the dye. A standard of direct Red 1 was available for independent analysis and was shown to produce an ion $(M\text{-}2Na)^{2-}$ with an m/z 290.5. The extracted ion current for m/z 290.5 (Fig. 1*c*) revealed a strong peak in the chromatogram at a retention time of c.a. 15.9 min, corresponding to the elution of direct Red 1. The mass spectrum associated with the analyte peak in Fig. 1*c* is shown in Fig. 1*d*, along with the structures of the major ions. In the absence of the dye standard and the associated MS fragment information, it is clear that it would be difficult or impossible to identify the dye in the TIC. The chromatogram obtained by monitoring the UV-visible absorbance at 505 nm is shown in Fig. 1*b*. A single sharp peak is observed at a slightly shorter retention time than the peak in the extracted ion current chromatogram. Unknown dye identification for forensic or other purposes, employing HPLC-MS without the aid of a UV-visible absorbance detector could be virtually impossible in cases where the dye responds weakly under the ionizing conditions. It is for this reason that we have sought to use the light absorbing characteristics of dyes coupled with the high information content associated with mass spectrometry to produce a generally applicable method of dye analysis.

Selection of the monitoring wavelength when using a single wavelength detector, as employed here, is straightforward with the aid of a color-wheel or C.I.E. diagram. Selection of a monitoring wavelength that exactly corresponds to the dye absorption maximum is not critical since UV-Visible absorption bands are typically tens of nanometers, or more, wide at half maximum. When multiple wavelength detectors are available, i.e., diode arrays, the same color information can be used to select the peak of interest. It is important to point out that the absorption maximum (color) of some textile dyes are pH-dependant and/or solvatochromic (undergo a wavelength shift as a function of solvent polarity). If the color of the dye extraction solution does not correspond to the

original color of the extracted fiber, the monitoring wavelength should be chosen based on the color of the extraction solution. Since the elution passed the UV/visible spectroscopy detector before entering the mass spectrometer, the mass detection peak was delayed by approximately 13 s relative to the absorbance detector at a flow rate of 0.2 mL/min. We have found this delay to be highly reproducible (<1 s variability for 20 s wide peaks), although dependent on flow conditions and separation distance between the two detectors. With the assistance of the UV-visible absorbance detector, identification of totally unknown dyes is facilitated, making dye identification by LC-MS practical.

Optimization of the Parameters for LC-MS

The limits of detection of our LC-MS for a set dyes from Table 1 was evaluated at a signal to noise ratio of three for the molecular ion, by introducing standard solutions with a syringe pump and bypassing the chromatography column. Among the dyestuffs evaluated, the limits of detection for rhodamine B base, malachite green oxalate and malachite green were determined to be in the range of 1–5 ng/mL. The detection limits for disperse dyes, such as disperse Red 1 and disperse Yellow 3, were determined to be in the range of 10–100 ng/mL. Limits of detection for direct dyes containing multiple sulfonated groups were found to be greater than 100 ng/mL. In order to optimize MS sensitivity for dye detection, several parameters were considered.

In the ESI interface, the drying gas temperature directly affects the ionization efficiency of the analyte. To maximize the production of gas phase analyte ions, it is necessary to efficiently remove the solvent from the small liquid drops produced after a sample solution is sprayed by the ionization source. A typical temperature range is 330–350°C for most of dyes. The variation in signal intensity with temperature for acid blue 40 increased rapidly in the range of 200–250°C, then slowly increased by an additional 25% up to a temperature of 350°C, corresponding to the maximum temperature limit for our instrument. Since most commercial dyes used for textile fiber are chemically quite stable, a high temperature can be employed for the drying gas, making the solvent removal efficient. In the analyses reported here, the temperature was set at 350°C, to optimize sensitivity.

The "fragmentor" voltage is another parameter that affects analyte signal intensity and fragmentation pattern. The fragmentor provides additional energy to assist in the decomposition of ions, resulting in a change in analyte signal intensity. A graph of molecular ion intensity as a function of applied fragmentor voltage is shown in Fig. 2*a* for a series of disperse dyes analyzed in the positive ion

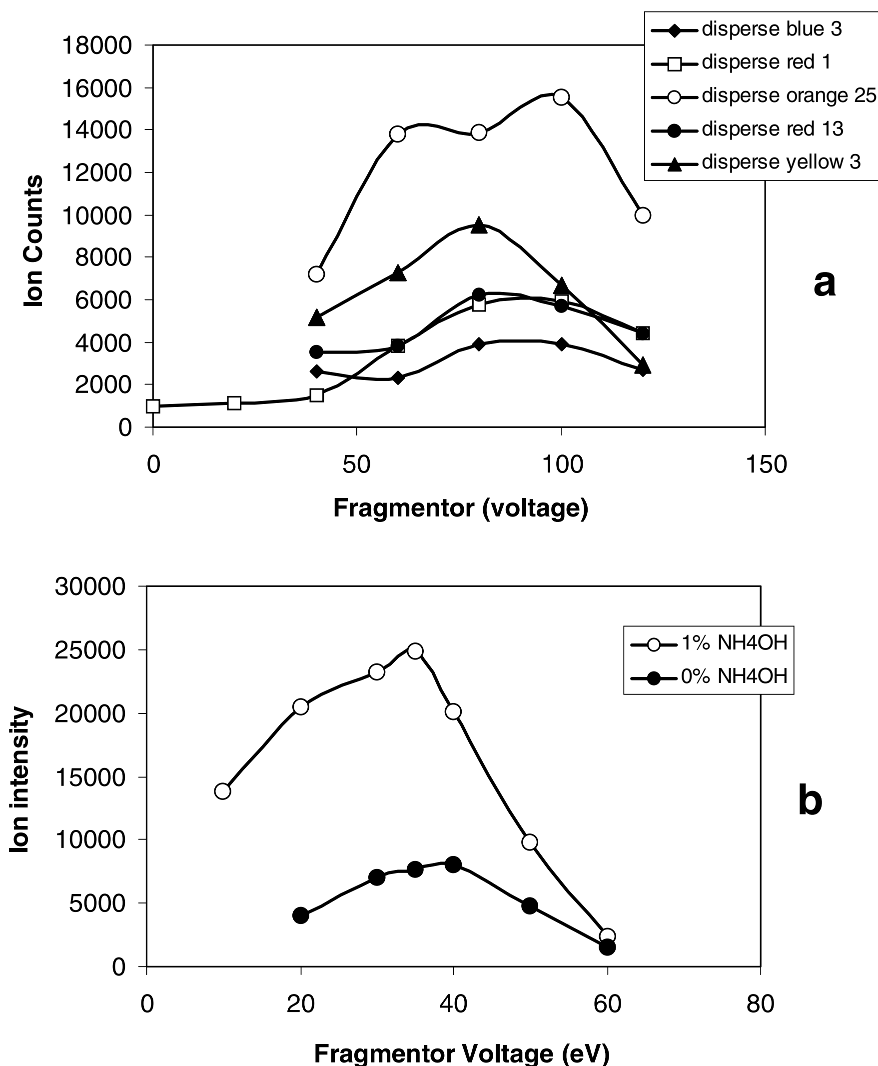

FIG. 2—*(a) Total ion count determined as a function of the fragmentor voltage for a series of disperse dyes analyzed in the positive ion mode. (b) Total ion count for orange G analyzed in the negative ion mode at varying fragmentor voltages.*

TABLE 3—*Characteristic ions observed for reference dyestuffs in Table 1.*

Dye No.	m/z	Fragmentor Voltage	Ion	Dye No.	m/z	Fragmentor Voltage	Ion
1	472.0	>20	$[M\text{-}H]^-$		318.0	120–160	$[M\text{-}CH_2OH+H]^+$
	450.0	>20	$[M\text{-}Na]^-$		289.0	120–160	$[M\text{-}CH_2OH\text{-}C_2H_5+H]^+$
	407.0	>200	$[M\text{-}COCH_3\text{-}Na]^-$		178.0	120–160	$[M\text{-}C_6H_3(N)(NO_2)Cl]^+$
	386.0	>140	$[M\text{-}SO_2\text{-}Na]^-$		147.0	120–160	$[NC_6H_4N(C_2H_5)CH_2]^+$
	343.0	>180	$[M\text{-}SO_2\text{-}COCH_3\text{-}Na]^-$	13	270.0	40–100	$[M+H]^+$
2	260.0	50–80	$[M\text{-}2Na]^{2-}$		292.0	40–120	$[M+Na]^+$
	543.0	50–80	$[M\text{-}Na]^-$		561.0	40–80	$[2M+Na]^+$
	271.0	50–80	$[M\text{-}Na\text{-}H]^{2-}$	14	273.0	100–160	$[M\text{-}H]^-$
3	312.7	20–70	$[M+H\text{-}4Na]^{3-}$		242.0	100–160	$[M\text{-}NH_2\text{-}O]^-$
	234.3	20–70	$[M\text{-}4Na]^{4-}$		226.0	100–180	$[M\text{-}NH_2\text{-}2O]^-$
	469.5	20–70	$[M+2H\text{-}4Na]^{2-}$		213.0	100–160	$[M\text{-}NO_2\text{-}NH_2+H]^-$
	940.0	20–70	$[M+3H\text{-}4Na]^-$		168.0	100–160	$[M\text{-}NHC_6H_4NH_2+H]^-$
4	240.8	40–60	$[M\text{-}4Na]^{4-}$		167.0	100–160	$[M\text{-}NHC_6H_4NH_2]^-$
	321.3	40–60	$[M\text{-}4Na+H]^{3-}$	15	291.0	100–180	$[M\text{-}H]^-$
5	290.5	20–110	$[M\text{-}2Na]^{2-}$		262.0	100–140	$[M\text{-}C_2H_5\text{-}H]^-$
	287.0	100–170	$[M\text{-}C_6H_3(OH)(CO_2Na)N_2$		142.0	140–180	$[C_{10}H_6O]^-$
			$(C_{12}H_8)\text{-}H]^-$		92.0	140–180	$[C_6H_4O]^-$
	268.5	100–150	$[M\text{-}COO\text{-}2Na]^{2-}$	16	263.0	40–360	$[M+H]^+$
	582.0	20–170	$[M\text{-}2Na+H]^-$	17	329.0	20–220	$[M\text{-}HO\cdot HCl]^+$
6	297.0	70–140	$[M+H]^+$		383.0	60–100	$[M+H]^+$
	319.0	70–140	$[M+Na]^+$		353.0	60–100	$[M+H\text{-}2CH_3]^+$
	252.0	100–150	$[M\text{-}C_2H_4OH+H]^+$	18	928.0	150–220	$[M+H]^+$
7	349.0	40–60	$[M\text{-}H]^-$ for ^{81}Br		972.0	150–220	$[M+2Na\text{-}H]^+$
	347.0	40–60	$[M\text{-}H]^-$ for ^{79}Br		884.0	150–220	$[M\text{-}COO+H]^+$
8	265.0	60–90	$[M+Na]^+$		840.0	150–220	$[M\text{-}2COO+H]^+$
	243.0	60–120	$[M+H]^+$		329.0	20–220	$[(C_6H_5)C(C_6H_4N\ CH_3CH_3)_2]^+$
	150.0	70–120	$[M\text{-}C_6H_4NH_2]^+$		313.0	150–220	$[(C_6H_5)C(C_6H_4N\ CH_2CH_3)$
9	351.0	60–200	$[M\text{-}H]^-$				$(C_6H_4\ CH_2CH_3)]^+$
	246.0	100–220	$[M\text{-}C_6H_5N_2\text{-}H]^-$	19	203.0	20–60	$[M\text{-}2Na]^{2-}$
	231.0	140–180	$[M\text{-}C_6H_4(N_2)OH]^-$		429.0	30–70	$[M\text{-}Na]^-$
	92.0	140–220	$[C_6H_4O]^-$		150.5	30–70	$[M\text{-}C_6H_5N_2\text{-}2Na]^{2-}$
10	324.0	40–160	$[M+H]^+$	20	443.0	60–180	$[M+H]^+$
	346.0	60–10	$[M+Na]^+$		465.0	80–180	$[M+Na]^+$
	283.0	80–160	$[M\text{-}CH_2CN]^+$		399.0	120–180	$[M+H\text{-}CO_2]^+$
	255.0	100–160	$[M\text{-}CH_2CN\text{-}C_2H_4]^+$		241.0	60–100	$[C_6H_4OC_6H_4N(C_2H_5)_2+H]^+$
	237.0	120–160	$[M\text{-}CH_2CN\text{-}NO_2]^+$	21	373.0	100–140	$[M+Na\text{-}2H]^-$
11	315.0	60–160	$[M+H]^+$		351.0	100–200	$[M\text{-}H]^-$
	337.0	60–160	$[M+Na]^+$		246.0	100–140	$[M\text{-}H\text{-}C_6H_5N_2]^-$
	284.0	100–160	$[M\text{-}CH_2OH+H]^+$		197.0	100–200	$[C_6H_5N_2C_6H_4NH_2]^-$
	255.0	120–160	$[M\text{-}CH_2OH\text{-}C_2H_5+H]^+$	22	233.0	30–50	$[M\text{-}3Na+H]^{2-}$
	134.0	100–160	$[M\text{-}C_2H_5\text{-}OH]^{2+}$		155.0	20–45	$[M\text{-}3Na]^{3-}$
12	349.0	80–160	$[M+H]^+$		140.3	20–50	$[M\text{-}3Na\text{-}COO]^{3-}$

mode. The optimal range of applied fragmentor voltage was determined to be in the 80–100 V range. The optimal applied fragmentor voltage range for observing the molecular ion in the negative ion mode was determined to be in the 30–60 V range for the dye orange G, as shown in Fig. 2*b*. Although the optimal applied voltage varies with dye structure, a compromise voltage can be identified for the purpose of unknown sample analysis. We have employed fragmentor voltages of 100 V in the positive ion mode and 40 V in the negative ion mode for optimal molecular ion detection. At these fragmentor voltage settings, molecular ions $(M+H)^+$ or $(M+Na)^+$ can be expected in the positive ion mode, and $(M\text{-}H)^-$, $(M\text{-}xNa)^{x-}$, $(M\text{-}xNa+yH)^{-(x-y)}$ ($x=1-4$, $y=0-3$, sodium salt dyes), or $(M\text{-}xH+yNa)^{-(x-y)}$ ($x=1-4$, $y=0-3$, acid form dyes) can be expected in the negative ion mode, where M represents the dye's molecular formula. Table 3 lists the major ions observed for the reference dyes used in this work. An adjustment of the fragmentor voltage induces fragmentation for dye structure elucidation. A compromise fragmentor voltage is also required to facilitate reproducible fragmentation and database development; however, instrument-to-instrument spectral reproducibility of ion relative intensity can still provide a challenge. Dye identification based on a small number of ions may lead to cross-platform uncertainties and limit database utility. On the other hand, MS/MS analysis of the fragmentation of a limited number of ions can greatly reduce the uncertainty of dye identification, even in under conditions of variable ion relative intensity. The challenge of generating reproducible fragmentation spectra has recently been addressed (19,20).

An additional complicating factor in optimizing the quadrupole MS responses is the variable stability of ions in the solvated and vapor states. For example, we have examined the stabilities of $(M+Na)^+$ and $(M+H)^+$ formed from Disperse Orange 3 in the positive ion mode. The ratio of these two ions is influenced by the concentration of sodium ions in the system. The adduction of cations is frequently observed in ESI-MS analysis, especially when alkali metal ions are present as additives or impurities in submillimolar concentrations (21). Alkali metal adduct formation with acetamide, acetic acid and acetate anion have been calculated to have exothermic or slightly endothermic enthalpies (22). The formation of $(M+H)^+$ generally exceeds that of $(M+Na)^+$, except in the presence of high sodium ion concentration. The MS signal

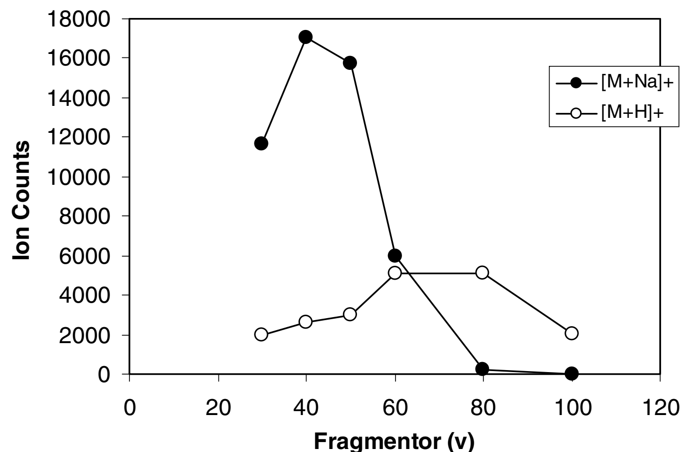

FIG. 3—*Ion counts for* $(M+Na)^+$ *and* $(M+H)^+$ *formed from disperse orange 3 in the positive ion mode.*

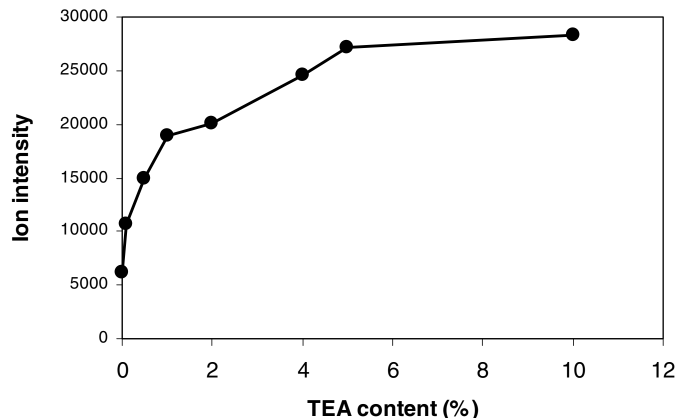

FIG. 5—*Signal for tartrazine as a function of TEA concentration.*

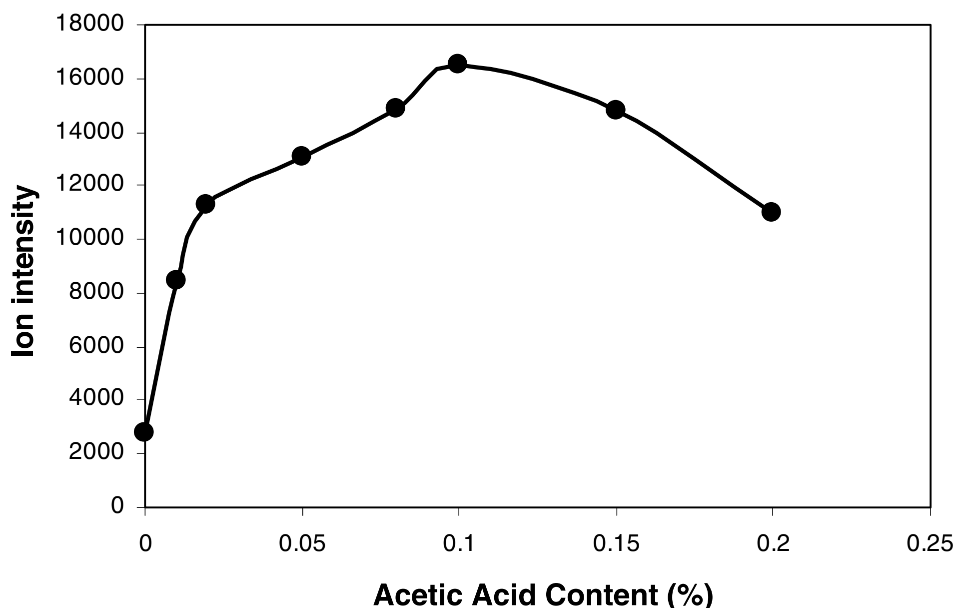

FIG. 4—*Optimal concentration of acetic acid for disperse red 1 analysis in the positive ion mode.*

from these ions also depends on the fragmentor voltage. Figure 3 shows a plot of the detector response for each of these ions as a function of the fragmentor voltage. From the results shown in Fig. 3, it can be seen that low fragmentor voltage yields a stronger signal for $(M+Na)^+$ relative to $(M+H)^+$. However, increasing the fragmentor voltage results in a dramatic decrease in the signal due to $(M+Na)^+$. The observed ion decomposition occurred in the gas phase after the analytes in solution were converted to gas phase ions for introduction into the mass analyzer. The detection of higher levels of $(M+H)^+$ at a higher fragmentor voltage reflects the higher stability of $(M+H)^+$ in the gas phase, as compared with that of $(M+Na)^+$. This result also demonstrates the challenge of finding a compromise fragmentor voltage for library development.

Sensitivity Enhacement by Using Additives

The ionization efficiency of some dyes can be enhanced by the addition of complexing or ion-pairing components in the solvent system. Instrument response in ESI-MS closely relates with the dissociation of the analyte in solution. Therefore, additives that increase the dissociation of analytes will enhance their signal intensity. Addition of acetic acid was investigated as a means of promoting positive ion production for disperse Red 1. An optimal concentration of acetic acid was found to be approximately 0.1%, as shown in Fig. 4. Similarly, basic reagents, such as triethylamine (TEA) or ammonium hydroxide can be used to enhance negative ion production. In both cases, the reagent is simply undergoing an acid-base reaction with the analyte.

TEA undergoes acid-base reactions with acidic dyes to promote dye dissociation and ion pair formation. The pH of a solution containing 20 mM TEA was determined to be 11.3. The acid-base reaction of TEA increases the ion content in solution and leads to chemical enhancement of the dye signal intensity. The boiling point of TEA, 89°C, is low and does not suppress solvent evaporation, thereby facilitating ion formation. The signal for tartrazine, for example, is improved by a factor of five when the content of TEA in the sample solution is 5%, see Fig. 5. In the absence of TEA, the MS signal from multiply sulfonated dyes was weak or not observed at all, even at 2 μg/mL levels of dye. The analysis of dyes containing multiple (i.e three or more) sulfonate groups is enhanced by the

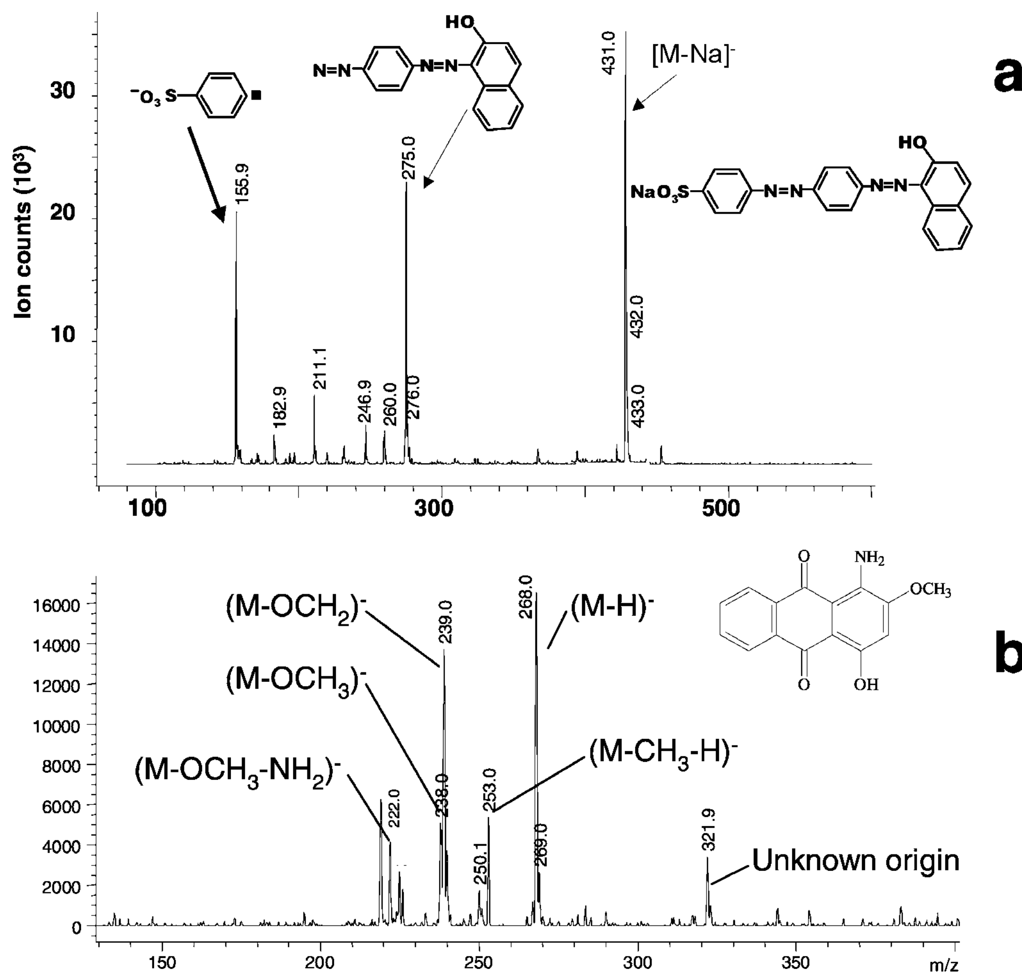

FIG. 6—*Mass spectra from extracted dyes (a) acid red 151 (b) disperse red 4 (c) basic green 4 (d) direct blue 1.*

addition of TEA into the mobile phase at a concentration of 0.3%. This was found to be especially true for Direct Blue 1, Direct Blue 71, and Direct Blue 90, each of which contains 4 sulfonic acid groups. Upon addition of 0.3% TEA in water reservoir of the mobile phase, sharp peaks emerged and these highly sulfonated dyes were readily identified by HPLC-MS.

Analysis of Dye Standards and Identification of Dyes Extracted from Textile Fibers

Dyestuff standards, 22 in total, were analyzed as a first step in establishing a methodology for the identification of dyes extracted from textile fibers. The most abundant ions observed for these dyestuffs are summarized in Table 3. Molecular ions, $(M + H)^+$ and $(M + Na)^+$, were usually observed in the positive ion mode. More complex results are observed in the negative ion mode. The ions observed in the negative ion mode depend on the number of acid groups present and their degree of dissociation. Ions such as $(M\text{-}H)^-$, $(M\text{-}xNa + yH)^{-(x-y)}$ $(x = 1 - 4, y = 0 - 3$, for dye sodium salts), $(M\text{-}xH + yNa)^{-(x-y)}$ $(x = 1 - 4, y = 0 - 3$, for acid dyes) are frequently observed.

A set of 10 dyes were extracted from textile fibers and identified by the generalized procedure discussed in this paper. Some example chromatograms and mass spectral data are shown in Fig. 6. The mass spectrum of direct Red 1, shown in Fig. 1, was also obtained from the extracted dye. Utilizing the UV-visible absorption detector served as a good monitor for the chromatographic elution of dyes of a known color. A comparison of mass spectra obtained under identical conditions for extracted and reference samples of disperse Red 1 are shown in Fig. 7*a* and 7*b* respectively. The spectra in Fig. 7 are a typical representation of the high quality of spectra match that can be obtained for reference and extracted textile dyes measured under identical conditions. The results shown in Fig. 7 demonstrate the potential for compiling spectral libraries and, perhaps more importantly, the increased degree of certainty that can be obtained in questioned and known comparisons of dyed textile fibers.

Conclusions

LC-MS with electrospray ionization (ESI) can be coupled with in-line UV-visible detection to facilitate the analysis of textile dyes extracted from "forensic-size" samples. The molecular-level information contained within the mass spectrum provides a much more reliable method of making questioned and known common source determinations. The analytical method may potentially lead to a useful database of dye mass spectra; however, challenges associated with the selection of fragmentation conditions for optimal spectral characterization remain to be overcome. In addition, it is important to test the compatibility of this LC-MS method with fiber dye extraction solvent systems and protocols outlined by the Federal Bureau of Investigation (4).

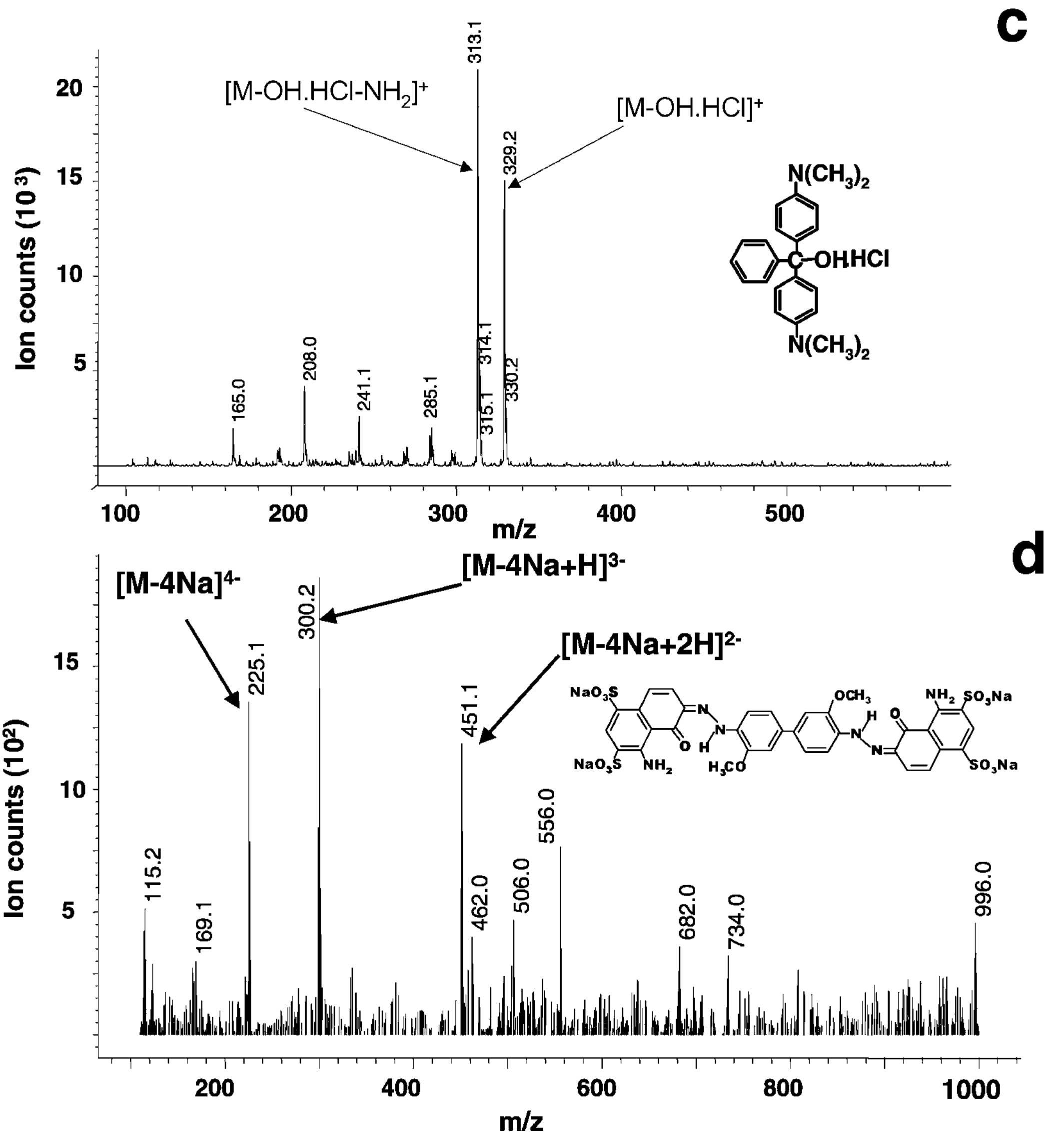

FIG. 6—*Continued.*

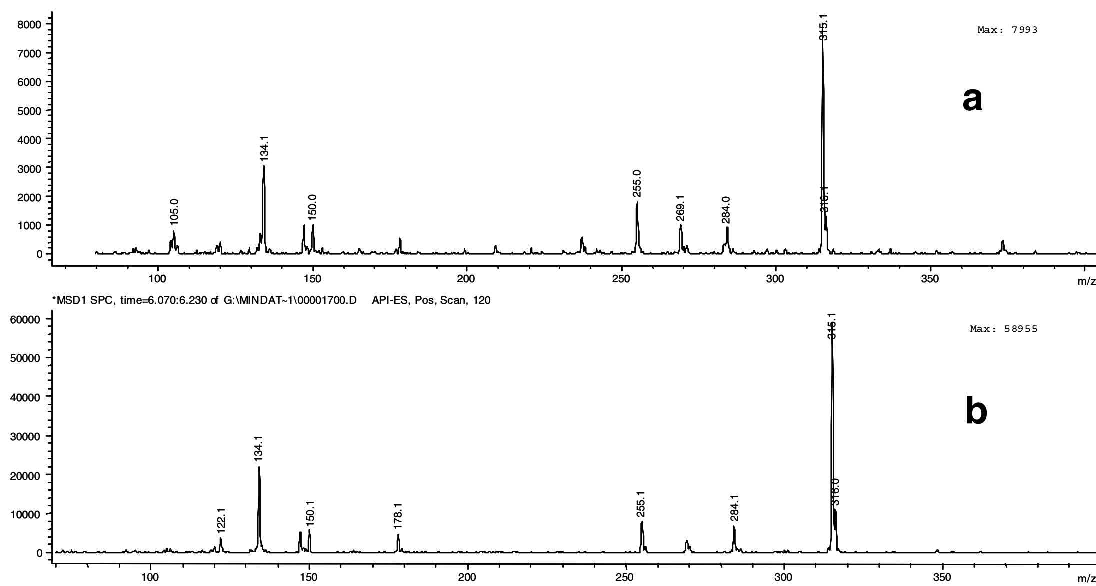

FIG. 7—*Mass spectra of (a) disperse red 1 extracted from an acetate fiber and (b) reference disperse Red 1, collected under identical conditions.*

Acknowledgment

This work was sponsored by the National Institute of Justice, Office of Science and Technology, Investigative and Forensic Sciences Division, under grant 98-IJ-CX-K0003. The work was done at the National Center for Forensic Science, a National Institute of Justice program hosted by the University of Central Florida, and a member of the Forensic Science Resource Network.

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