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High-Performance Liquid Chromatography–Ultraviolet–Visible Spectroscopy–Electrospray Ionization Mass Spectrometry Method for Acrylic and Polyester Forensic Fiber Dye Analysis*

ABSTRACT: A critical point of comparison between a fiber collected from a crime scene and a fiber from a known source is the color. Fiber dye analysis using thin-layer chromatography or ultraviolet (UV)–visible (Vis) microspectrophotometry provides useful, although limited, data for comparison. High-performance liquid chromatography–electrospray ionization mass spectrometry (LC/MS) overcomes these limitations by integrating chromatography, ultraviolet–visible spectroscopy, and mass spectrometry into a single instrument. In order to evaluate the applicability of the LC/MS to forensic fiber dye analysis, a multi-stage chromatographic method using acidified water and acidified acetonitrile was developed that separated and identified a mixture of 15 basic and 13 disperse dye standards. The LC/MS also detected and analyzed dyes extracted from individual 0.5 cm acrylic and polyester fibers, demonstrating its applicability to this type of analysis. With regard to the analysis of disperse dyes in polyester fibers, the replacement of pyridine with acetonitrile in the extraction system allowed direct injection of the extracts into the LC/MS. The advantage of the LC/MS over other instrumental methods of textile dye analysis is demonstrated by the analysis and differentiation of three black acrylic fibers: two fibers had similar UV–Vis spectra but were differentiated with chromatography and two had similar UV–Vis spectra and chromatograms but were differentiated using the mass spectrometer.

KEYWORDS: forensic science, trace evidence, fiber, textile dye, high-performance liquid chromatography-mass spectrometry, basic dyes, disperse dyes

The forensic analysis of fiber evidence involves the evaluation and comparison of the physical and chemical characteristics of the submitted fibers, one of which is color. The color of a fiber is imparted by dyes that are added during the manufacturing process. Each type of fiber, such as acrylic or polyester, differs in the chemical structure of its polymer chain. Therefore, different dye classes have been developed to maximize the dyeability (ability to accept the dye) and color fastness (resistance to fading) of the fiber. The main difference between these dye classes is the chemical processes used to bind the dye molecules to the polymer, resulting in covalent bonds, ionic bonds, or Van der Waals attractions. By virtue of their application, all dyes within a particular class are removed from the fiber using the same general extraction system that typically has no effect on other classes (e.g., the solvent system for basic dyes does not remove disperse dyes). These resulting extraction solutions are then analyzed and compared to determine whether or not the dyes are similar.

Current methods of analyzing the dye in a fiber, such as ultraviolet (UV)–visual (Vis) microspectrophotometry and thin-layer chromatography (TLC), have demonstrated their ability to be highly discriminating. However, both of these methods have some limitations that the high-performance liquid chromatograph (HPLC) coupled with a mass spectrometer overcomes. The UV–Vis microspectrophotometer objectively characterizes the color of a fiber by generating an absorbance spectrum. The main advantage of the microspectrophotometer is that analysis can be performed directly on the fiber—no extraction is required. UV–Vis microspectrophotometry alone fails in its ability to differentiate dye isomers, discriminate between fibers of the same color but comprised of different dye mixtures (metamerism), or to analyze deeply colored items (1). Unlike the microspectrophotometer, TLC requires extraction of the dye from the fiber and selection of an appropriate mobile phase for chromatography. The dyeing mechanism (viz. dye class) is determined using a system of extraction solvents and evaluating which one successfully removes the color from the fiber. TLC suffers from a lack of reproducibility between laboratories and difficulty in comparing lightly colored items (including shades of yellow) (1). High-performance liquid chromatography–electrospray ionization (ESI) mass spectrometry (LC/MS) offers the same dye class information as TLC (via extraction and chromatography) and yet the spectroscopic information of UV–Vis microspectrophotometry is not restricted by either lightly or deeply colored fibers. LC/MS is sufficiently sensitive in detecting all dyes, even those in relatively low concentrations found in lightly colored fibers. Likewise, LC/MS is sufficiently selective in detecting dyes in low concentrations where they might ordinarily be masked in deeply colored fibers by dyes in high concentrations. Additionally, the LC/MS generates mass information that discriminates between coeluting compounds and can be used to confirm the identity of the dye. This means that the instrument can compare all colors (including custom blending), tints, and hues present in fiber evidence and differentiate between dyes of similar color, including isomers. The only limitation of the LC/MS is that the dye must be able to be extracted from the fiber

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in order to be analyzed. Forensic applications of LC/MS exist for the analysis of controlled substances (2), explosives, poisons, toxins, biologicals (http://www.lcgceurope.com/lcgceurope/article/articleDetail.jsp?id=36,589), and inks (3,4). However, the application of this instrumental technique to textile dye identification and characterization has not been investigated until recently.

The advantage LC/MS has over traditional fiber dye analysis techniques is the identification capability of the mass spectrometer, which had to wait for technology to solve the problem of creating gas phase ions of nonvolatile analytes in a liquid medium. The conversion of non-volatile analytes, such as textile dyes, from the HPLC into gas phase ions appropriate for the mass spectrometer is accomplished using an ESI interface. ESI forms ions using an electrical field in lieu of the electron beam associated with impact ionization methods, resulting in a "soft ionization." In order to be affected by the electric field and subsequently analyzed, the target molecule needs to be ionized. Analyte ions are formed in the mobile phase by adjusting the pH or adding ion-pairing reagents. As the mobile phase containing the ionized analyte enters the interface of the mass spectrometer, it is nebulized using a heated stream of nitrogen gas. The mist then passes through an electrical field that orients the ions in each droplet. As the solvent evaporates in the heated chamber, a point is reached where the droplets become so small that the oriented charges begin to repel each other. A "Coulombic explosion" of the droplet ensues, resulting in gas phase ions that are then drawn into the mass analyzer via a capillary under low pressure. As an electrical field is used to produce ions, the fragmentation of the analyte can be controlled and the resultant spectrum is typically the mass of the molecular ion (http://www.waters.com/watersdivison/waters_website/applications/lcms_itq.asp). The use of an ion trap detector provides the added advantage of MS^n capabilities, allowing for further characterization of the analyte. Widely used for the analysis of large molecules, ESI appears to be well suited for the analysis of textile dyes.

HPLC techniques have been established for the separation of various dye classes; however, they are not necessarily compatible with the mass spectrometer or they have not been applied to single fibers. Griffen et al. (5) developed an isocratic solvent system for the separation of an unknown mixture of basic dyes. Azo sulfonated acid dyes were separated by Chen et al. (6) using a methanol and aqueous ammonium acetate mobile phase, while azo and anthraquinone acid dyes were separated by Holčapek et al. (7) with a similar gradient. Acid dyes were also separated by a complex mobile phase consisting of methanol, water, tetramethylammonium chloride, and ammonium dihydrogenphosphate by Laing et al. (8). Speers et al. (9) used acidified acetonitrile and water for the separation of acid, basic, and disperse dyes. Success was also reported using a methanol and water mobile phase to separate disperse, acid, basic, vat, and direct dyes (10–12). Many of the aforementioned methods have been applied to either wastewater analysis or the extraction of bulk fabric samples, not to single fibers. Additionally, many of the HPLC solvents and reagents used are nonvolatile and therefore inappropriate for integration into the mass spectrometer. Thus, in order to apply the LC/MS in a forensic setting, existing HPLC methods for dye analysis must be modified and the analysis of single fibers must be viable.

Any method applied to the analysis of dyes extracted from fibers must be adequately sensitive for forensic applications. The amount of dye in a fiber length of 2–10 mm is *c.* 2–200 ng, with some of the lightest shades containing significantly less (8). Speers et al. (9) determined the limits of detection (LOD) for the HPLC analysis of basic, disperse, and acid dye using 5-μL injections. An LOD range was found of 40–800 pg for basic dyes, 230–1250 pg for acid dyes, and from 80 to 1280 pg for disperse dyes. Thus, the LC/MS meets the criteria for analyzing textile dyes from single fibers.

The focus of this study was to develop a methodology for LC/MS analysis of textile dyes in forensic applications that is both appropriate for MS detection and casework-sized samples. The development of a single HPLC method for the separation of both basic and disperse dye classes, as well as methods that can be used on dyes extracted from fibers ≤ 0.5 cm in length was investigated. Existing HPLC methods were adapted for the LC/MS and verified by extracting single fibers.

Experimental Procedure

HPLC/UV–Vis/ESI MS

The instrument was an 1100 series Agilent HPLC/MS (Fremont, CA) with an atmospheric pressure electrospray interface (AP-ESI). The HPLC system consisted of a binary pump solvent-delivery system with a 100 μL sample loop. The column was a narrow bore Agilent Zorbax SB-C18 column (2.1 × 150 mm) with an Agilent Eclipse XDBC8 narrow bore guard column (2.1 × 2.5 mm). Chromatography was developed using a gradient of acidified acetonitrile (pH 2.5) (HPLC Grade Acetonitrile; Burdick and Jackson, Muskegon, MI) and acidified nanopure water (pH 2.5) (Barnstead Thermolyne, Dubuque, IA). The pH was adjusted using formic acid (Formic Acid, EM Science, Darmstadt, Germany). The flow rate was 0.225 mL/min for a 1-μL injection sample with a column oven temperature of 35°C for basic dyes and 45°C for disperse dyes, with an 8 min postrun. The MS trap (Bruker Esquire 3000 Plus, Fremont, CA) acquired data in the range 50–1000 mass-to-charge (*m/z*) at a maximum accumulation time of 200 ms with a target of 30,000 ions in the positive-ion ESI mode. Nebulizing gas was set at 35.0 psi, with the drying gas being pumped at 12.0 L/min, and the temperature in the ion source was held at 350°C. The diode array detector (DAD) was set to collect the highest signal in the visible region.

Spectrophotometer

Analysis was performed using an A U-3000 Hitachi spectrophotometer (Hitachi Instruments Inc., Danbury, CT). Dyed and undyed extracts were placed in cuvettes, with the latter used as the blank. Scans were collected from 350 to 800 nm at a rate of 300 nm/min with a slit width of 2.0 nm.

Dye Standards

Dyes were obtained from Clarient Corporation (Charlotte, NC), Dyestar (Charlotte NC), Classic Dyestuff (High Point, NC), and Sigma-Aldrich (St. Louis, MO). Standard solutions were made by dissolving 0.005 g of dyestuff in 50 mL of the solvent system (1.0×10^{-4} g/mL). Basic dye standards were dissolved in a 50:50 solution of acidified acetonitrile (pH 2.5) and water (pH 2.5), while the disperse dye standards were dissolved in a 45:45:10 solution of acidified acetonitrile (pH 2.5), water (pH 2.5), and methanol (HPLC Grade Methanol, Burdick and Jackson). Additional information on dye standards is summarized in Table 1.

Fabric Dyeing

White polyester satin was donated by Jo-Ann Fabrics (Woodland, CA) and dyed with disperse dyes. A stovetop dyeing recipe

TABLE 1—*Dyes used for LC/MS method development.*

Dye name	Color index constitution	Chemical dye class
Basic Black (BBlk)	—	—
Basic Blue 22 (BB22)[1]	61512	Anthraquinone
Basic Blue 45 (BB45)	—	—
Basic Blue 47 (BB47)[1]	61111	Anthraquinone
Basic Blue 69 (BB69)	—	—
Basic Blue 77 (BB77)	—	—
Basic Green 4 (BG4)[1]	42000	Triphenylmethane
Basic Orange 21 (BO21)[2]	—	Methine
Basic Orange 42 (BO42)	—	—
Basic Orange 48 (BO48)	—	—
Basic Red 14 (BR14)[1]	48016	Methine
Basic Violet 16 (BV16)[1]	48013	Methine
Basic Yellow 15 (BY15)[1]	11087	Azo
Basic Yellow 28 (BY28)[2]	19555	Methine
Basic Yellow 29 (BY29)[2]	19556	Azo
Disperse Black a (Dblka)	—	—
Disperse Black b (DBlkb)	—	—
Disperse Blue 14 (DB14)[2]	61500	Anthraquinone
Disperse Blue 60 (DB60)[1]*	61104	Anthraquinone
Disperse Brown 1 (DBr1)[1†]	11152	Azo
Disperse Green 9 (DG9)[1]	110795	Azo
Disperse Orange 1 (DO1)[1]	11080	Azo
Disperse Orange 3 (DO3)[1]	11005	Azo
Disperse Orange 25 (DO25)[1]*	11227	Azo
Disperse Red 60 (DR60)[1†]	60756	Anthraquinone
Disperse Red 91 (DR91)[1]	60753	Anthraquinone
Disperse Red 343 (DR343)	—	—
Disperse Yellow 42 (DY42)[1]*	10338	Nitro
Disperse Yellow 114 (DY114)[1]	128455	Azo

[1]Dye information obtained from Society of Dyers And Colorists. Color index. Bradford: Yorkshire, 1956–1992.

[2]Dye information obtained from Hunger K, editor. Industrial dyes: chemistry, properties, applications. Weinheim: Wiley-VCH, 2003;185:272–273.

*Dyes used for the spectrophotometer evaluation of acetonitrile extraction solvent system.

†Dyes used for the DAD evaluation of acetonitrile extraction solvent system.

LC/MS, high-performance liquid chromatography–electrospray ionization mass spectrometry; DAD, diode array detector.

was obtained (ProChemical and Dye, Somerset, MA; http://www.prochemical.com/directions/Prosperse_ImmersionPolyester.htm) and used with minimal alterations. Approximately 0.2 g swatches of fabric were used in the dyeing of all samples. The fabric was added to 100 mL water at 60°C with 0.043 g Na_2CO_3 and 50 μL of dish soap (Dawn, Procter and Gamble, Cincinnati, OH). This was stirred for 2 min, followed by rinsing the fabric with water. A solution of 200 mL water at 50°C, 50 μL of 30% acetic acid (EM Science), and 0.075 g of dyestuff filtered with 50 mL of boiling water was stirred, and the fabric was added. The dyebath temperature was raised to boiling and held for 30 min. The fabric was removed and plunged in 20°C water. This was stirred for 30 sec. The fabric was then removed and plunged in 70°C water with 50-μL dish soap and stirred for 30 sec. The fabric swatch was removed and rinsed with water. Finally, any unfixed dye adsorbed on the fabric was removed using a 50:50 methanol and acetone rinse at 40°C for 30 sec.

Various colors of acrylic fibers with unknown dyes were obtained. TLC was performed on the fibers (13) to confirm that the dyes were basic.

Dye Extraction

Single acrylic fibers ($\leq$ 0.5 cm) were extracted at 100°C for 45 min with 50 μL of a 1:1 formic acid and water solution for LC/MS analysis (13).

Disperse dyes were extracted from polyester fabric swatches with a mass of 0.005 g each. Samples to be analyzed by the LC/MS method were extracted with 100 μL, while samples for the spectrophotometer were extracted with 250 μL. All swatches were extracted at 120°C for 60 min using acetonitrile and water (4:3) or pyridine and water (4:3) (pyridine from Pierce Chemicals Co., Dallas, TX). Swatches were then removed and the extracts were diluted with 400 μL of methanol for HPLC/UV-Vis and 2250 μL for spectrophotometer analysis.

Basic Dye Chromatography Optimization

All basic dye standards were initially run on an un optimized gradient of 2–98% acetonitrile for 22 min (9) to determine each dye's base ion and absorption spectrum. Then, the mass spectrometer was optimized to maximize the abundance of each dye's base ion. Finally, the HPLC gradient was optimized for separation of all 15 basic dyes.

HPLC parameters (flow rate, solvent composition, and column temperature) were optimized using a mix (50 μL each) of 15 basic dye standards. This mix was then injected, and dye peaks were located using mass spectral and UV–Vis data.

Finally, the UV–Vis detector was optimized by finding the λ_{max} of each dye peak. The Agilent Chemstation™ software is limited in that it can only monitor five wavelengths for data manipulation. Therefore, the λ_{max} values of each dye standard within a 100 nm range were averaged together. For example, all dyes that absorbed in the 400 nm range were averaged, the 500 nm range averaged, and the 600 nm averaged. These were then selected as monitoring wavelengths.

Disperse Dye Chromatography Optimization

The MS, HPLC, and UV–Vis, optimization process was repeated for the disperse dye class. A toluene/water extraction was performed on each disperse dye standard in order to isolate the dye molecules from other interfering components of the dyestuff. The aqueous layer was then evaporated to concentrate the dye, and the residue was reconstituted with the 1:1 acidified acetonitrile and water system. The reconstituted dyestuffs, after extraction, contained the dye molecules as the primary components.

Mass spectrometer parameters were optimized with the purified dye, and a disperse dye mix was made by combining the 13 purified dye standards. The HPLC was then optimized, in the same manner as the basic mixture, for separation of these dyes, followed by UV–Vis optimization. The parameters for the disperse dye class are summarized in Table 2.

Disperse Dye Extraction System Development and Evaluation

As the classical extraction system for disperse dyes contains pyridine, a solvent not on Agilent's list for use in the ion trap (14), a novel extraction system was developed. The pH of the pyridine and water system was determined to be 8.9. Other low-molecular-weight polar solvents were selected from the Agilent list of compatible solvents (14), including acetonitrile and triethylamine. The acetonitrile and water system was found to have a pH of 8.9 at a volume ratio of 4:3. In the case of the triethylamine solution, the pH had to be adjusted to 8.9 using formic acid. Both systems were used to extract fiber swatches at 120°C for 60 min. The extracts were visually compared, and it was determined that the acetonitrile and water system yielded more color.

After the new solvent system was selected, the adequacy of dye extraction relative to the pyridine system was evaluated to verify it

TABLE 2—*Optimized gradients for basic dye separation, disperse dye separation, and the master method.*

Time (min)	% ACN versus acidified water	Flow rate (mL/min)
Basic dye method		
0	34	0.225
2	34	0.225
7	42	0.225
28	48	0.225
31	98	0.225
35	98	0.400
Disperse dye method		
0	54	0.225
2	54	0.225
27	78	0.225
32	98	0.400

Time (min)	% ACN versus acidified water*	Temperature (°C)	MS (V)
Master method			
0	34	35	CV = – 3375
2	34	35	CEV = 188
7	42	35	TD = 60
28	48	35	
30	54	35	
34	54	45	CV = – 4000
59	78	45	CEV = 154
64	98	45	TD = 56

*Flow Rate was 0.225 mL/min until 98% ACN when it switched to 0.400 mL/min.

CV, capillary voltage; CEV, capillary exit voltage; TD, trap drive; MS, mass spectrometry; ACN, acetonitrile.

as a reasonable substitute. This was performed using a UV–Vis spectrophotometer. Three dyestuffs representing various colors and chemical structures (see Table 1) were selected. Dyed fabric swatches, as well as undyed fabric blanks, were extracted with both the pyridine and acetonitrile solvent systems. For each dye, a wavelength range that covered the area under the peak maximum was selected for comparison.

Relative extraction efficiency analysis was also performed using the DAD of the LC/MS. To establish a calibration curve, an estimate of dye concentration in 0.005 g of dyed fabric extracted with 0.5 mL of solvent was made assuming that a fiber contained less then 1% dye. Dyestuffs of unknown dye concentration were used as received, without the additional purification step. To enable full solvation of the dye standards, the solvent system for the calibration curves required the addition of methanol. The acetonitrile system was 45:45:10 acetonitrile, water, and methanol and the pyridine system was 45:45:10 pyridine, water, and methanol. The absorbance of each dye peak was calculated through automatic integration under the curve and manual integration for the lowest concentration dilutions. Absorbance versus concentration was plotted and linear regression was calculated using the method of least squares.

The next step involved extraction of dye from the swatches and analysis. The swatches were extracted at 120°C for 90 min with both 4:3 acetonitrile:water and pyridine:water solvent systems. The extracts were then injected, and the absorbance of each dye peak was measured. The concentration of dye in the extracts was then calculated using Beer's Law.

Single Fiber Analysis

Acrylic fibers containing unknown dyes were used for testing the optimized parameters for basic dyes. A standard 1:1 formic acid and water (13) solution was utilized as the extraction solvent system. A single fiber of 0.5 cm was placed in a vial insert and extracted. This vial was then placed in the autosampler where 10 µL injections were placed on column and analyzed using the basic dye-optimized parameters.

The disperse dye method was also evaluated using single fiber samples. Polyester fabric swatches were dyed with disperse dyestuffs, and 0.5 cm single fibers were extracted using the acetonitrile and water system. The fibers were placed in vial inserts and extracted with 50 µL of solvent at 120°C for 60 min. The fibers were then analyzed using the optimized parameters for the disperse dye method.

Results and Discussion

Chromatography Optimization

The final optimized parameters for the basic dyes can be seen in Table 2 and the chromatogram of the basic dye mix can be seen in Fig. 1. Of the 15 dyes in the mix, 11 were able to be resolved using the gradient that was developed. The other four dyes (BY28, BBlk, BB77, and BB45) could not be resolved by adjusting the HPLC parameters.

Unlike the basic dyes, the disperse dye standards contained many unidentified compounds in addition to dyes. This made monitoring the dye *m/z* ion difficult, because in many cases, the

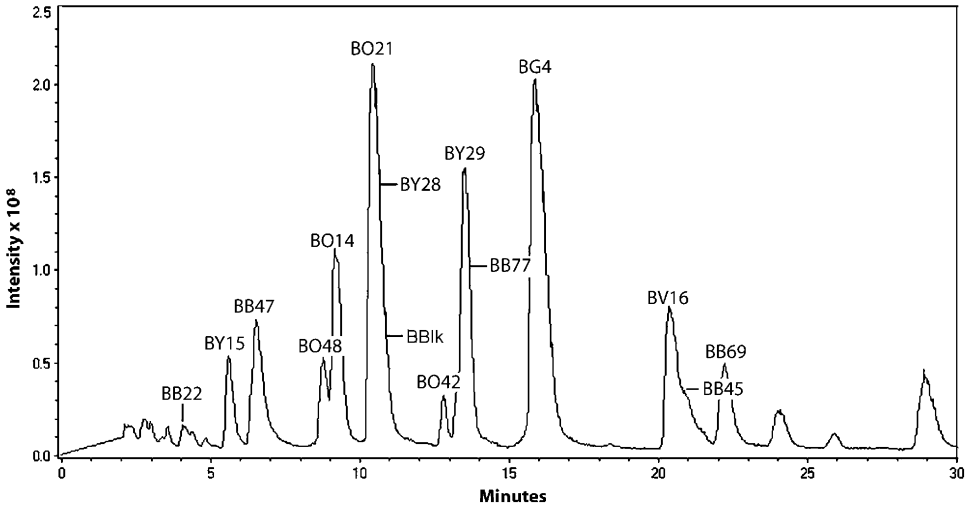

FIG. 1—*Chromatogram of separated basic dyes.*

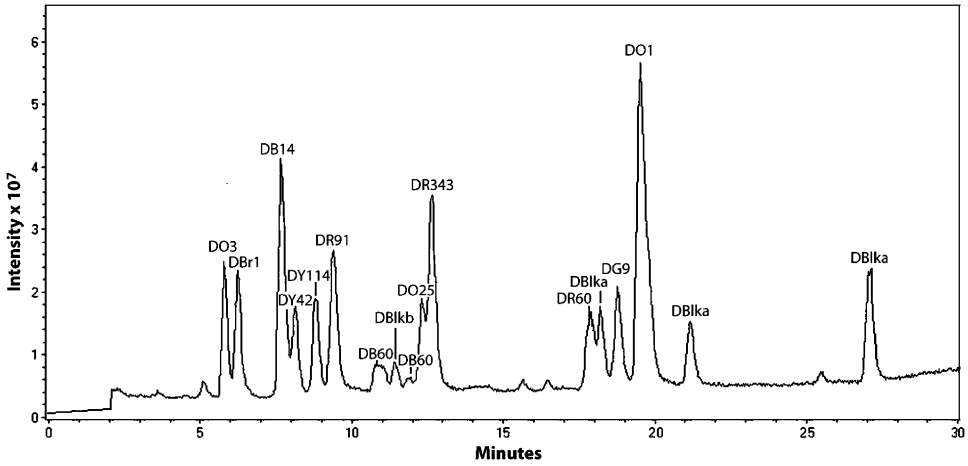

FIG. 2—*Chromatogram of separated disperse dyes.*

TABLE 3—*Efficiency of relative extraction calculated from the spectrophotometer.*

Sample	acn1/pyr1	acn1/pyr2	acn2/pyr1	acn2/pyr2	Range
DY42	1.00	1.18	0.90	1.06	0.90–1.18
DB60	0.94	1.03	0.77	0.85	0.77–1.03
DO25	1.12	1.09	1.07	1.05	1.05–1.12

acn1, first acetonitrile extract; acn2, second acetonitrile extract; pyr1, first pyridine extract; pyr2, second pyridine extract.

TABLE 4—*Efficiency of relative extraction calculated from the DAD.*

Sample	acn1/pyr1	acn1/pyr2	acn2/pyr1	acn2/pyr2	Range
DR60	0.63	0.55	0.67	0.59	0.55–0.67
DBr1	0.74	0.62	0.67	0.56	0.56–0.74

acn1, average of replicate first acetonitrile extracts; acn2, average of replicate second acetonitrile extracts; pyr1, average of replicate first pyridine extracts; pyr2, average of replicate second pyridine extracts; DAD, diode array detector.

dye was not distinguishable from the background. This is important as the mass spectrometer voltages are optimized for the dye molecule in order to maximize detector sensitivity. These dyestuffs contain additional compounds, such as auxiliaries, which are used to facilitate dyeing polyester fibers. After the dye is affixed to the fiber, these other chemicals are removed from the final product, leaving only the dye bound to the fiber. Thus, "real-life" cases involving disperse dyes may not necessarily involve the detection or classification of these other compounds, which, in the dyestuff itself, are masking the presence of the dye molecule. Therefore, it was deemed necessary to separate the dye molecule from the neat dyestuff before injection on the LC/MS.

Organic solvents were selected as possible purifying solvents, because unlike the auxiliaries, the dye molecules have polar functionalities and would be left in the aqueous phase. Several organic solvents were investigated, including toluene, hexane, and chloroform. Toluene resulted in the highest concentration of dye in the aqueous phase, thus resulting in more "purified" dye standards. The solvent–solvent extraction gave adequate results and facilitated the optimization of both the HPLC and the mass spectro-

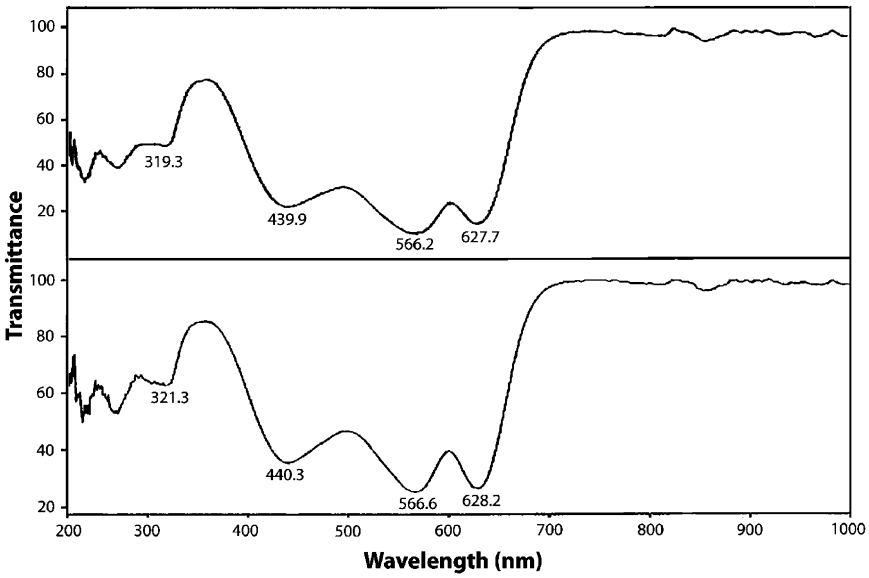

FIG. 3—*Ultraviolet/Visible microspectrophotometer spectra of black acrylic fiber extracts, Fiber A (top), Fiber B (bottom).*

meter. A chromatogram of the purified disperse dyes can be seen in Fig. 2.

Extraction Efficiency Evaluation

In order to validate the use of acetonitrile in place of the pyridine in the extraction system for disperse dyes, an efficiency evaluation was performed. The relative efficiency of the acetonitrile versus pyridine was determined by integrating the peaks over a selected wavelength range. A ratio of the area under the curve for the acetonitrile divided by that for the pyridine was calculated. One dye, C.I. Disperse Blue 60, showed anomalous variability in the calculated ratios, the cause of which was unknown. To address this anomaly, all extractions were considered individually and a range was calculated in order to compare the extraction systems. Each dye standard was extracted with both solvents and ratios of each combination were calculated (see Table 3).

From Table 3, it can be seen that the disperse dyes, C.I. Disperse Yellow 42, C.I. Disperse Blue 60, and C.I. Disperse Orange 25, had a comparable extraction whether acetonitrile or pyridine was used. C.I. Disperse Blue 60 showed different absorbance peak shapes depending on the extraction solvent. These differences may be because of solute–solvent interactions such as hydrogen bonding and Van der Waals forces between a highly polar dye and the polar solvent. There were no shifts in λ_{max} or peak shapes for the other disperse dyes. Although the peak shape for Blue 60 changed, the wavelength range selected for integration continued to describe the absorbance. Thus, the area under the curve could still be used for calculations. The result, an average ratio of 1.01, demonstrates that the acetonitrile system is as efficient at removing the disperse dyes from polyester as the pyridine system.

The relative extraction efficiency of pyridine to acetonitrile was also evaluated using the DAD. Calculations were performed in a similar manner, using the area under the curve and calculating a ratio for the acetonitrile response versus the pyridine response.

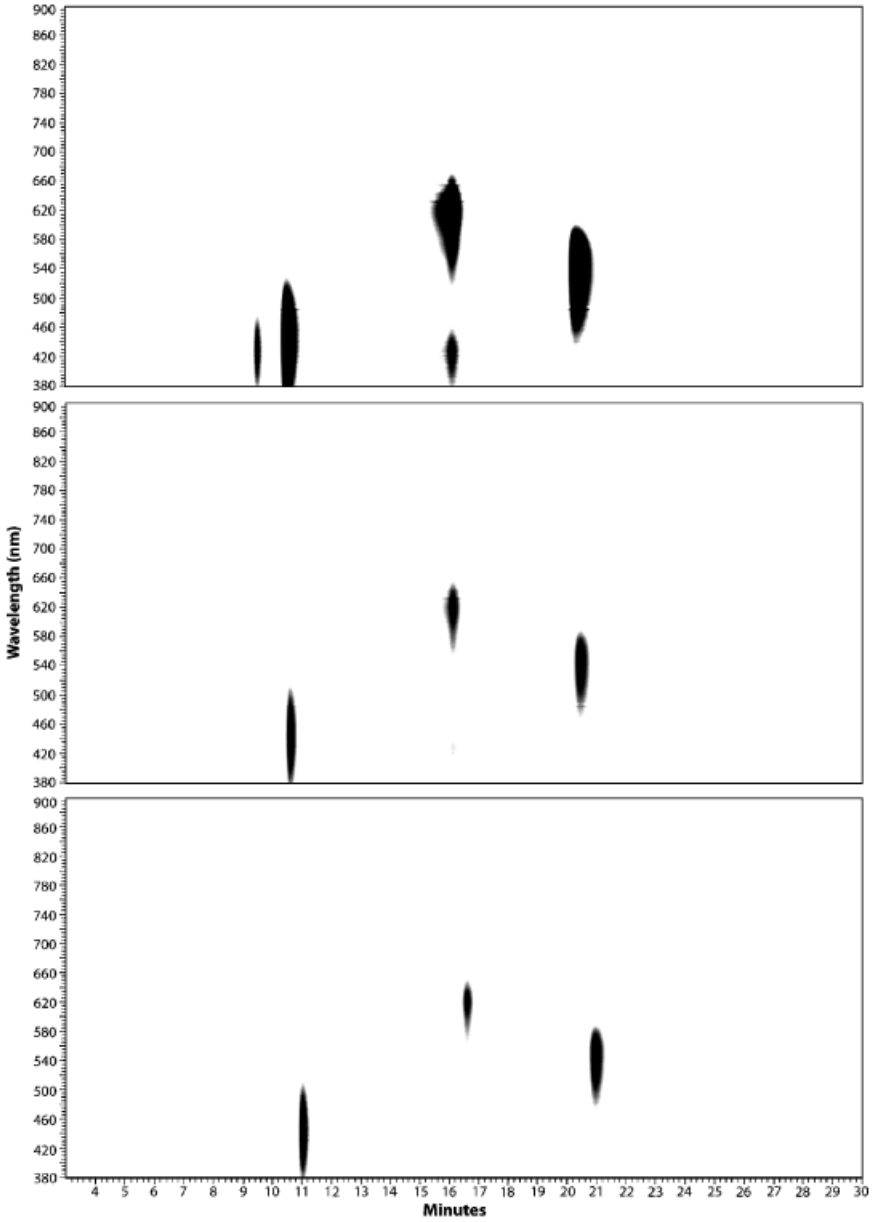

FIG. 4—*Isoabsorbance plots of three black acrylic fiber extracts. Fiber A (top), Fiber B (middle), and Fiber C (bottom).*

The diode array for the LC/MS showed an extraction efficiency of *c*. 62% (see Table 4). Again, this result demonstrates that acetonitrile is an appropriate substitute for pyridine in the extraction system.

Spectrometer and DAD Comparison

The apparent discrepancy in the relative extraction efficiency results, 100% for the spectrophotometer and 62% for the DAD, may be explained by the difference in how the data are collected by each instrument. The DAD collects absorbance data at all wavelengths for each separated component as they elute from the column. However, during the data analysis, integration is performed as a function of time for a particular wavelength, ignoring the broad absorbance range characteristic of UV–Vis spectra. Thus, when calculating an absorbance value, the result is not the sum of the absorbance over a range of wavelengths, but for a discrete wavelength as the compound elutes. Additionally, the LC/MS has the ability to separate isomers. Therefore, if a particular dye is a mixture of isomers, the DAD measures their absorbance separately. Conversely, the spectrophotometer collects its absorbance data independent of time. The integration of the spectrophotometer data involves the absorbance over a range of wavelengths and can include isomer contribution. Thus, the data from the DAD are of a single component of the fiber extraction at a particular wavelength, whereas the data from the spectrophotometer are for all absorbing compounds in the selected wavelength band. This is one possible explanation for the difference in the results of the extraction efficiency for the two instruments. A comprehensive evaluation of this difference is beyond the scope of this project.

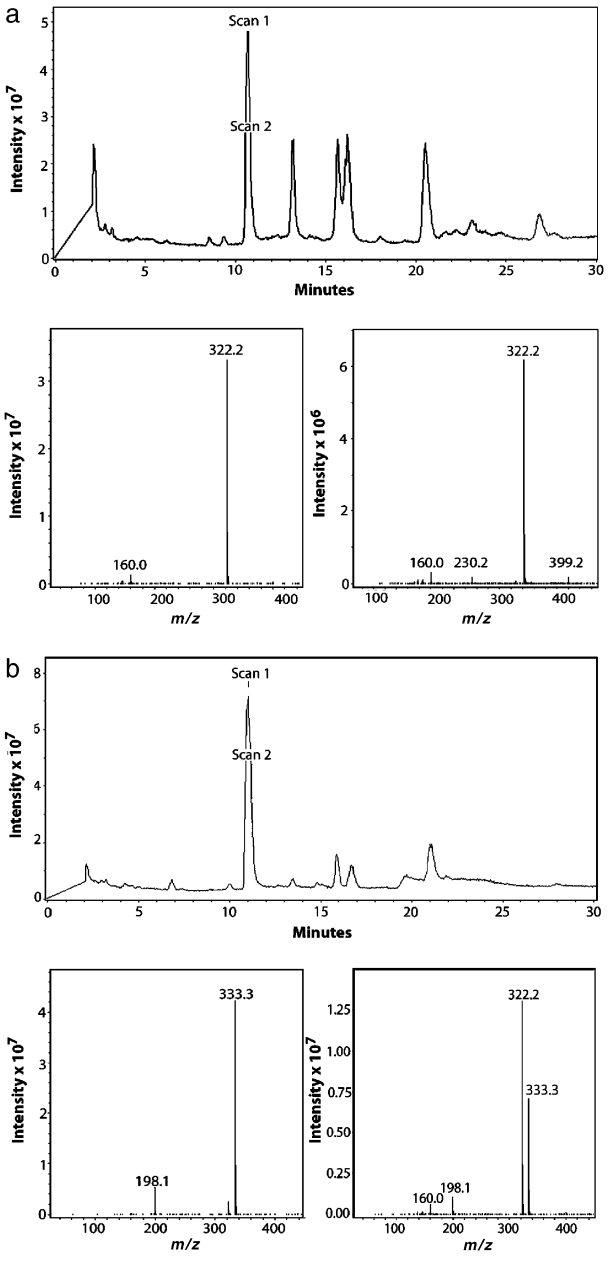

FIG. 5—*(a) Chromatogram and mass spectra of Fiber B. The chromatogram (top), mass spectrum Scan 1 with m/z value of 322.2 (bottom left), and mass spectrum Scan 2 with mass-to-charge (m/z) value of 322.2 (bottom right). (b) Chromatogram and mass spectra of Fiber C. The chromatogram (top), mass spectrum Scan 1 with m/z value of 333.3 (bottom left), and mass spectrum Scan 2 with m/z value of 322.2 (bottom right).*

Method Application

Basic Method—The optimized LC/MS parameters for fiber dye analysis were tested using the extracts of single fibers. Several fibers of different colors were analyzed in this manner, with their mass spectral and UV–Vis spectra being compared. In some instances, fibers with the same apparent color were shown to have different dye profiles by the resulting chromatograms. In other instances, differences were found only when the mass spectral data were reviewed, thereby demonstrating the additional discriminating power of the LC/MS. The examination of three black acrylic fibers shows the advantage of LC/MS over other methods of fiber dye analysis.

The comparison of Fibers A and B demonstrates the superiority of LC/MS over UV–Vis microspectrophotometry in its ability to discriminate between similarly colored items. The UV–Vis spectrum of Fiber A could not be discriminated from Fiber B (see Fig. 3). When the same fibers were extracted and the dyes were analyzed with LC/

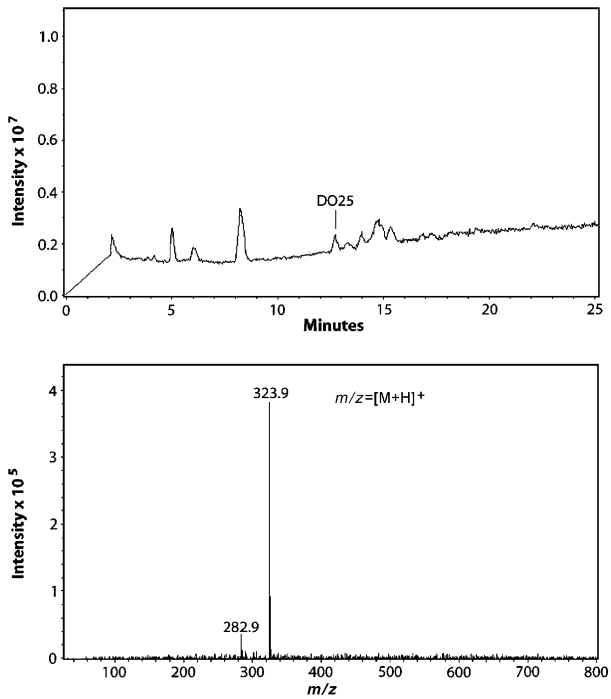

FIG. 6—*Chromatogram (top) and mass spectrum (bottom) of extract of 0.5 cm single polyester fiber dyed with DO25.*

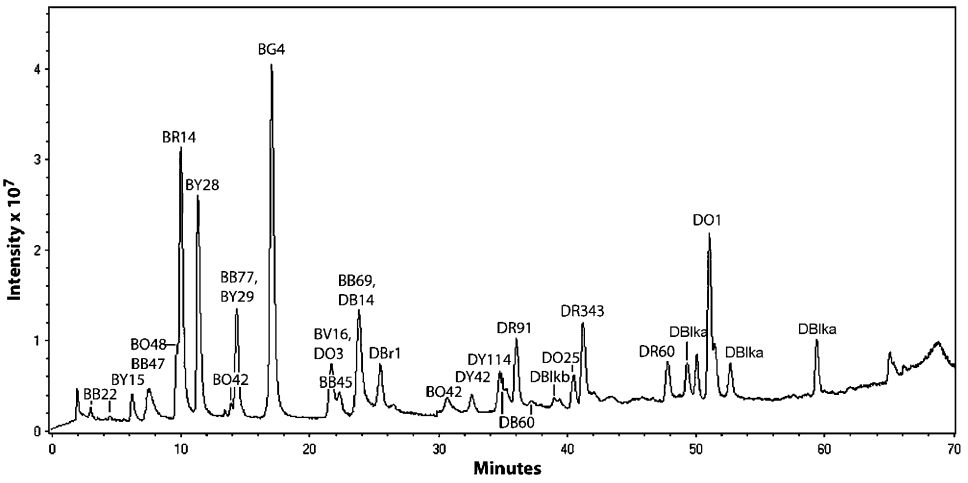

FIG. 7–*Chromatogram of 15 basic dyes and 13 disperse dyes.*

MS, the isoabsorbance plots illustrated a difference between the two dye profiles (see Fig. 4). For Fiber A, there is an additional absorbance at *c*. 9.5 min, which is absent from the spectrum of Fiber B. This shows that although Fibers A and B appeared similar from conventional UV–Vis microspectrophotometry, they are, in fact, different.

The advantage of the mass spectral data in addition to the UV–Vis data is demonstrated when comparing Fibers B and C. Fiber extracts B and C could not be discriminated by isoabsorbance plots, showing that the fiber extracts contained the same number of dye components with similar colors. These spectra are displayed in Fig. 4. The chromatograms of these extracts contained several peaks that occurred at similar retention times, requiring further evaluation using the mass spectrometer. The mass spectra at the apex and at the tail end (at half-peak maximum) of the most abundant peaks of the chromatogram (at *c*. 10 min) were compared. Figure 5 shows the chromatograms and mass spectra of these two fibers. Although the peaks appear similar from the chromatograms and isoabsorbance plots, the mass spectral profiles are quite different. Fiber B has a single species throughout the entire peak with an *m/z* value of 322.2. Fiber C contains two species coeluting: one species with an *m/z* value of 333.3 at the apex and the second species with an *m/z* value of 322.2 on the tail end of the peak. Therefore, the two dye profiles are dissimilar. The integration of the mass spectrometer with the HPLC/UV–Vis allowed discrimination between the dyes on these fibers that could not be differentiated by chromatography or UV–Vis.

Disperse Method—The optimized parameters for disperse dyes were also verified by analyzing fiber extracts. Figure 6 shows the chromatogram and mass spectra of the extract of a 0.5 cm single fiber removed from C.I. Disperse Orange 25 dyed fabric (see Table 1 for additional dye information). The formula weight of this dye is 323 amu and the chromatogram has a peak with an *m/z* value of 323.9. This $[M+H]^+$ ionization is characteristic of the electrospray positive ionization method. In addition to the dye peak, there are other unidentified peaks in the chromatogram. An undyed fiber was extracted and analyzed using the same method, yielding similar chromatographic peaks. Although these peaks have not yet been identified, they are most likely from the processing or manufacturing of the fiber in that they are consistent throughout all of the extracts for the same fiber type.

Development of Master Method—Individual basic and disperse dye instrumental methods were combined and evaluated with the dye standards. A mixture of all dye standards, basic and purified disperse dyes, was injected into the column. The optimized parameters for the master method are listed in Table 2, and the chromatogram of the dye mixture can be seen in Fig. 7. After the individual methods were optimized, it was found that there was no overlap in their solvent gradients. Thus, their gradients could simply be added together to create a "master method." Mass spectrometer parameters for the first section of the method in Table 2, average values calculated during optimization, included a capillary voltage (CV) set to −3375 V, capillary exit voltage (CEV) set to 188 V, and the trap drive (TD) set to 60 V. After 30 min, where the method changes from the parameters for the basic dyes to those for the disperse dyes, the MS parameters change to a CV of −4000 V, CEV of 154 V, and TD of 60 V. The MS scanned a mass range of 50–1000 *m/z* in 200 ms or a target of 30,000 ions for the entire run.

Evaluation of Table 2 shows that each method, Basic, Disperse, and the final "master" method, has several segments. For each method, it was necessary to adjust the slope of the gradient to resolve as many of the dyes as possible. In some instances, the slope had to be decreased to obtain adequate resolution. Lastly, the flow rate for each method was steeply increased toward the end of the run to facilitate cleaning the column of any late-eluting components.

Conclusions

HPLC/UV–Vis/ESI MS for the analysis of textile dyes was successfully applied in a forensic setting. These results demonstrate that LC/MS is a viable method for the analysis of dyes in polyester and acrylic fibers. Parameters were optimized for disperse and basic dye classes that allowed for the separation and identification of dyes through mass spectral and UV–Vis data. Single fibers were successfully extracted and analyzed using these methods, including the use of a novel acetonitrile extraction for disperse dyes from polyester fibers in lieu of pyridine. Finally, a master method was developed that could separate and identify both disperse and basic dye standards. Continuing development of LC/MS analysis for all dye classes as well as the development of an extended spectral database will provide a greater discrimination capability in forensic fiber analysis.

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