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Enhanced Trace-Fiber Color Discrimination by Electrospray Ionization Mass Spectrometry: A Quantitative and Qualitative Tool for the Analysis of Dyes Extracted from Sub-millimeter Nylon Fibers

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Enhanced Trace-Fiber Color Discrimination by Electrospray Ionization Mass Spectrometry: A Quantitative and Qualitative Tool for the Analysis of Dyes Extracted from Sub-millimeter Nylon Fibers

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The application of electrospray-ionization mass spectrometry (ESI-MS) to trace-fiber color analysis is explored using acidic dyes commonly employed to color nylon-based fibers, as well as extracts from dyed nylon fibers. Qualitative information about constituent dyes and quantitative information about the relative amounts of those dyes present on a single fiber become readily available using this technique. Sample requirements for establishing the color-identity of different samples (*i.e.*, comparative trace-fiber analysis) are shown to be sub-millimeter. Absolute verification of dye-mixture identity (beyond the comparison of molecular weights derived from ESI-MS) can be obtained by expanding the technique to include tandem mass spectrometry (ESI-MS/MS). For dyes of unknown origin, the ESI-MS/MS analyses may offer insights into the chemical structure of the compound – information not available from chromatographic techniques alone. This research demonstrates that ESI-MS is viable as a sensitive technique for distinguishing dye constituents extracted from a minute amount of trace fiber evidence. A protocol is suggested to establish/refute the proposition that two fibers – one of which is available in minute quantity only – are of the same origin.

An important aspect of forensic fiber examinations involves the comparison of dyestuffs used to impart color on or in textile fibers. Information obtained from dyes used to color fibers can provide supporting evidence in forensic casework when comparing two fibers obtained from different locations. To determine that two fibers are of the same origin, it is necessary that they be shown to have the same dye components and that the ratio in which these components are present should be identical. Comparisons of absolute dye concentrations (i.e. nano-grams dye per mm fiber) may not be necessary – or even advisable. Dye intensity may not be distributed uniformly along different fibers from the same coloring batch, or even along the length of a particular fiber. Thus, a forensic evaluation should comprise a qualitative evaluation of dye content and a quantitative determination of the relative amounts in which those dyes are present.

A review of current textile dying techniques found that, in most cases, manufacturers use the same dye constituents in differing ratios to impart different colors to their products. This practice facilitates computer-assisted production control. Most textile dyers use three dyes; a yellow, a red, and a blue to produce the desired effect. Although there are numerous yellow-, red-, and blue dyes from which to choose, an individual textile manufacturer may use only a small selection to produce the myriad hues in his product line. Thus, the ability to determine dye-constituent ratios, as well as the actual dyes used in a coloring process, is virtually essential to definitively compare dyed fibers.

The predominant techniques currently employed in forensic fiber-color examinations include

microspectrophotometry[1,2] and thin-layer chromatography (TLC).[3] Microspectrophotometry is the most widely utilized color comparison technique in federal, state, and local forensic laboratories. To the forensic scientist, nondestructive analysis of evidence and application to extremely small sample sizes are the most attractive characteristics of this method. However, the lack of discriminatory power is an inherent limitation. Microspectrophotometry evaluates the spectral characteristics of the composite-dye mixture, but says nothing about the individual dye components. Considerable diagnostic information is therefore left unexamined.

In contrast, TLC is a destructive method in which dyes are extracted from the fiber and subsequently separated and qualitatively compared. The method is relatively insensitive, and may require more fiber for an analysis than the forensic scientist is willing to sacrifice. Furthermore, TLC is generally able to distinguish only the dyes present, but not the ratios in which they are present.

High-performance liquid chromatography (HPLC)[3] is a third analytical method that has been employed to a limited extent in the forensics laboratory for dye analysis. HPLC has been applied to acid-[4], disperse-[5,6], and basic-[7] forensic dye analysis. HPLC offers better separation than TLC, and

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1 Erying, M. B. *Anal. Chim. Acta* **1994**, *288*, 25-34.

2 Robertson, J. Forensic Fiber Examination of Fibers; Ellis Horwood: New York, **1992**; Chapter 4.

3 Robertson, J.; Forensic Fiber Examination of Fibers; Ellis Horwood: New York, **1992**; Chapter 5.

4 Laing, D. K.; Gill, R.; Blacklaws, C.; Bickley, H. M. *J. Chromatogr.* **1988**, *442*, 187-208.

5 West, J. C. *J. Chromatogr.* **1981**, *208*, 47-54.

6 Wheals, B. B.; White P.C.; Patterson M.D. *J. Chromatogr.* **1985**, *350*, 205-215.

7 Griffin, R. M. E.; Kee T. G.; Adams R.W. *J. Chromatogr.* **1988**, *445*, 441-448.

provides quantitative information. However, HPLC columns and gradient- or isocratic elution systems are specific to only a limited group of similar compounds. The columns required for analysis are generally expensive, and large amounts of solvent may be needed compared to other separation methods. In addition, HPLC fiber length requirements are comparable to the lengths necessary for TLC analysis. Application of modern techniques of micro-HPLC may alleviate one or more of these shortcomings, but we have not found literature references to such application in the field of forensic fiber comparison.

Capillary electrophoresis is another method that shows significant promise as a forensic screening tool for trace-fiber dye analysis.[8,9] In a recent study, the ability to detect acid dyes extracted from nylon fibers between 1- to 3 mm, for dark and light colored fibers respectively, was achieved using large volume stacking with polarity switching.[8] This technique was sensitive to dye constituents as well as manufacturing additives and impurities yielding unique "fingerprint" electropherograms that could be compared to a potential source material. However, quantitative results for this method indicated that the spectral-based detection lacked the sensitivity required for precise comparative measurements.

This paper discusses the application of electrospray ionization mass spectrometry (ESI-MS) to the qualitative- and quantitative aspects of comparative fiber-dye analysis. Qualitative identity is primarily established by comparison of the observed masses for each peak in the ESI-MS of each fiber extract. This may be further verified by comparing the tandem mass spectra (ESI-MS/MS) for each of the dye peaks observed in the ESI-MS. Comparison of the relative intensities of the individual dye peaks in the

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[8] L. Lewis *et al.*, manuscript in preparation for submission to *Forensic Science.*

[9] Xu, X.; Leijenhorst, H.; Van Den Horen, P.; Koeijar, J. D.; Logtenberg, H. *Sci. Justice* **2001**, *41*, 93-105.

ESI-MS provides a measure of the concentration of each dye in each of the fibers being compared. In the modern forensics laboratory nylon fibers are frequently encountered,[10] and this study has accordingly concentrated on such fibers and the dyes (usually acidic) used to color them. It is anticipated however that the methods described below may be equally applicable to other fiber types and to inks.

EXPERIMENTAL SECTION

Materials: To undertake this study, a supply of real-world nylon fibers, and information regarding the dyes used to color them, was required. Shaw Industries, Inc. (Dalton, GA) supplied numerous colored nylon carpet samples with the associated dyes used to color the samples, and Collins & Aikman (Dalton, GA) provided many colored nylon windings and information regarding the dyes used to color the fibers. Ciba Specialty Chemicals (High Point, NC) provided the dyes used to color those nylon windings. For both carpet- and winding samples, the manufacturers often used identical dye components to produce different colors by varying the ratio of dyes mixed. Only a small selection of the fiber and dye samples kindly provided by these manufacturers were used in this study. They are listed in Table 1.

Fiber Extraction: Known lengths of fiber were cut and placed inside glass Wheaton 0.3-mL "v-vials" (1-mL total volume). 400 µL of 4:3(v/v) pyridine/water was added to each vial, and the caps were tightly sealed. The vials were heated at 100 ?C for 30 to 35 min. The fibers were immediately removed from the vials, and the vials were placed in a KD-Tube-Evaporator pre-heated to 90-95 ?C with air blow-down set on low for 15 min., followed by air at high for 15 to 30 min. The vials were removed,

[10] Private communication from Ron Menold, Federal Bureau of Investigation, January 1999.

allowed to cool, and solvent (i-propanol/water 4/1 containing 0.1% ammonia) was added to the equivalent of 60 µL per mm original fiber length.

Mass Spectometry: Negative-ion electrospray mass spectrometry was conducted on a Quattro-II (Micromass, Manchester, UK) instrument by direct infusion of the solutions at 5 µL/min. Dye samples were prepared for infusion at 500 ppb (~ 1µM) in acetonitrile/water (1/1). Fiber extracts were diluted to the equivalent of 60µL solvent (*i*-Propanol/Water (4/1) containing 0.1% NH_3) per mm of fiber extracted. Scans were accumulated over two minutes, and averaged to produce one spectrum. Source temperature was maintained at 90 ?C, and the

nebulizing- and drying-gas flow rates were 30 and 300 L/hr of N_2 respectively. The capillary, and one (nozzle to skimmer) voltages were 2,5 kV and 45 V, respectively. For collision induced dissociation (CID) experiments, the precursor ion was selected in the first analyzer of the triple quadrupole instrument, then allowed to collide with argon (3.4×10^{-3} mBar) in the rf-only collision quadrupole, and the resulting fragments were analyzed in the last quadrupole. The collision energy (or collision energy ramp as appropriate) for each experiment is presented in the relevant text, Figure, or Table.

RESULTS AND DISCUSSION

ESI Spectra of Individual Dyes. A negative ion electrospray spectrum of dye #4 (500 ppb in acetonitrile/water 1/1) infused into the ESI source is presented in Figure 1, and is representative of all the dye spectra in that: a) The cluster at m/z 410 corresponds to the expected anion for this dye, (see Figure 2 for known dye structures) and the single-mass spacing of the ^{13}C isotope peaks (410, 411, 412) indicates a monoanion. b) Peaks between m/z 200 and 300 are impurities presumed to be present in the solvent. They appear in the spectra of all the individual dyes, and in the "blank" spectra of

solvent without the addition of dyes. They do not interfere with dye analysis because they lie outside the range of dye peaks encountered in this study (m/z range 308-544). c) There are no dye-fragment ions discernable in the spectrum, indicative of the "gentle" nature of the ionization technique. For the type of aromatic azo dyes examined here, fragmentation is not expected in the electrospray process, and multiple peaks found within a spectrum will most probably represent a mixture of analyte types rather than fragmentation.

Similar spectra were obtained for each of the dyes in Table 1, and the observed m/z values are listed in Table 2. Points of interest from that Table:

1. In those instances where the chemical structure is known (dyes 4, 7, and 8) the observed mass values correspond to expectation .
2. Several of the dyes (1, 2, 3, 6, and 9) show multiple peaks indicative of mixtures. (Indeed #s 1, 2, and 3 are stated by the manufacturer to be "Proprietary Mixes"; see Table 1.)
3. Dyes #2 and 3 appear to have the same major component (#4) as well as one or two "additives". (The identical structure of the m/z 410 component of #s 2, 3, and 4 is borne out by comparison of their CID spectra; see section "Collision Induced Dissociation..." below).
4. All dyes examined are singly charged (indicated by superscript "-1" in Table 2) indicating mono-acidic functionality *except* #9 (superscripts "-2" and "-3" for two and three charges, respectively).
5. Several of the peaks listed for #9 are closely related and apparently represent the same underlying tri-acidic structure. Thus, triply charged m/z 308.1 represents a trianion with mass 924.3 Da. M/z 462.7 corresponds to the protonated version of the same structure [(924.3+1)/2 = 462.7]. Likewise 473.7 and 481.7 correspond to the sodiated and potasiated structures, respectively. Thus, 308, 462.7, 473,7 and 481.7 do not represent different dyes in the mixture, but rather

differently cationized versions of the same tri-acidic dye. However, the singly charged anion at m/z 424, and the doubly charged one at m/z 430 cannot be accommodated similarly, and seem to be different dyes in the mixture. The peaks at 408, 410, and 488 are relatively too small to be used as markers in the ESI-MS fingerprint of dye 9. At somewhat lower signal/noise ratios (S/N) than those attained here, they may become indistinguishable from the background.

6. Each of the dyes examined has at least one peak which is distinct from every other dye in this particular set. If the question were asked "Which dyes are present in a particular fiber extract?", and the choice were limited to this particular set, the answer could be provided by examining the ESI-MS spectrum of the dye mixture *without* any prior separation into the individual components. Of course, the set of possibilities examined here is extremely limited. If the set were expanded to include the hundreds of acidic dyes used in the coloring of nylon fibers, there would probably be numerous overlaps. In that case, resort to tandem mass spectrometry would provide the needed specificity (see section "Tandem Mass Spectrometry" below).

ESI Spectra from Winding Extracts. The extracts of three windings of dyed nylon threads were examined by ESI-MS. The extracts were generated by the standard procedure (see Experimental) using 50 mm each of windings 38920, 38011, and 9900 (see Table 1), and labeled I, II, and III, respectively. Each extract was diluted to the equivalent of a 1 mm sample dissolved in 60 µL of the solvent used for ESI-MS. A representative example of a windings spectrum is presented in Figure 3. Notable observations from that spectrum apply to the other two which are not separately illustrated: a) The peaks expected for the component dyes of the brown winding (m/z 464 and 544 for dye # 6, m/z 439 for dye #7, and m/z 517 for dye #8) are clearly discernable, as are the "background" peaks (*e.g.*, m/z 253, 255, 281) previously observed for in the spectra of the individual dyes (*cf* Figure 1). b) A set of peaks

apparently representing a homologous series of analytes occurs at m/z 283, 297,...367. These do not obstruct that region of the spectrum which is most informative for dye analysis (m/z 400-560), therefore they are not considered detrimental to the dye analysis. c) Peaks not attributable to the known dye components are also observed in the "dye region" of the spectrum: viz m/z 417, 451, 487/489. They appear, albeit in varying relative abundances, in all of the winding extracts examined during this study, and may represent degradation products of the nylon fiber generated during the extraction process or compounds which have been added during the dye-fixing process. The "extraneous" peaks which lie within the dye-region of the spectrum have been marked with brackets ( ) in Figure 3, and the recognized dye peaks have been marked with a rectangle.

Reproducibility of ESI-MS Dye-Peak Intensities. The data in Table 3 was gathered by infusing the three winding extracts (I, II, and III) sequentially into the mass spectrometer via a 10 µL Rheodine injection loop. The 10 µL infusion is equivalent to the extract from 170 µm of the winding. The sequence of three injections was performed four times producing a total of twelve acquired spectra within two hours. Instrumental parameters such as capillary- and cone voltages, source temperature, and nebulizing- and drying gas flowrates were held constant during the full course of these analyses. In Table 3, individual peak intensities have been normalized to the sum of the dye-peak intensities for each spectrum, and then gathered together in groups according to the color of the extracted winding. The average and standard deviation of the observed intensities for each dye component are listed. The calculated standard deviations are certainly adequate to unambiguously differentiate windings I and II (which differ only by the proportions of the dyes) from one another. Whereas winding I ("brown")

consists of dyes 6 (blue), 7 (orange), and 8 (red) in the proportion[11] 10/10/3, the proportion for winding II ("olive green") is 16/10/3. It is important to note that the *differentiation of these winding extracts, containing identical dye components in somewhat differing ratios, has been accomplished without the need to physically separate the components by chromatography*.

Tandem Mass Spectrometry. Molecular- ions (or quasi-molecular ions) observed in mass spectra only provide information regarding the mass of the analyte molecule. In the absence of fragmentation there is no structural information. Collision Induced Dissociation (CID) spectra on the other hand can provide significant structural information about the precursor ions. Even if the CID information cannot always be interpreted to draw a definitive structure, the pattern of fragment peaks derived from a particular precursor under defined dissociation conditions does provide a "fingerprint" of that precursor. It is thus particularly useful in establishing that the same analyte mass, observed in separate samples, represent the same structure.

The appearance of a CID spectrum can vary significantly depending on the collision energy (CE) applied. For a "fingerprinting" experiment it is desirable to encompass as many product ions in a single CID spectrum as possible. Figure 4 shows the products of precursor m/z 410 (dye # 4) acquired at four different CEs. At low CE (20 eV; Figure 4a) the precursor ion predominates, and m/z 80 is weak. At higher CE (35 eV; Figure 4d) m/z 80 is of moderate intensity, but m/z 410 has disappeared. In order to incorporate as much information as possible into a singe scan, the CE may be ramped as a function of m/z. Based on the results of Figures 4a-d, a CE-ramp was applied to produce the spectrum displayed

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[11] These "proportions" do not refer to the amount of dye in terms of weight or molarity, but rather in terms of the ESI-MS signal intensity they cause. Because the sensitivity of this ionization technique differs with various properties of the analyte (*e.g.*, pKa and surface activity) the molar ratios may differ considerably from the peak-intensity ratios. However, for purposes of characterization the peak-intensity ratios are equally appropriate.

in Figure 4e. The ramp ran from 30 eV (at m/z 60) to 20 eV (at m/z 430).

The "ideal" CE ramp for any given precursor ion will depend on its fragmentation characteristics. However, it can always be determined empirically. The same sequence of CID experiments described for dye #4 above was implemented for each of the precursor ions enumerated in Table 2, as well as those identified as "extraneous" in Figure 3. The results – indicating the mass range to scan, the CE-ramp to apply over that mass range, and the product ions observed from such a ramped experiment – are listed in Table 4. Thus, each row in Table 4 describes a CID method and the resulting "fingerprint" for one of the dyes under investigation. Within the context of the present study, this information may seem superfluous because each of the dyes already displays a unique pattern in its ESI-MS spectrum (*cf* Table 2) without the necessity for further fingerprinting. However, if a much larger database is eventually employed incorporating hundreds of dyes, some or many of them will almost certainly display equivalent m/z values in their ESI-MS spectra. CID tables such as Table 4 may then be used as a means of differentiating those isobaric compounds.

Collision Induced Dissociation and Chemical Structure. Of the nine dyes examined in this study the chemical structures of only three (#s 4, 7, and 8 see Figure 2) are known to us, either via information from the manufacturer or from the literature. For dye # 7 the structure and the CID spectrum are readily correlatable as depicted by arrows in Figure 2 showing fragmentation of the most labile bonds. Dyes 4 and 8 possess identically substituted naphthalene moieties bonded by a diazo group to a substituted benzene ring. Not coincidentally they also exhibit the same CID fragmentation pattern (product ions at m/z 80, 168, 249/250; *cf* Table 4). The 249/250 peaks can be assigned to fragmentation between the nitrogen atoms with concomitant migration of hydrogen(s) by analogy to a

similar fragmentation investigated by Brumley *et al.*[12] The m/z 168 peak may result from the loss of an HSO_3 radical from 249, but the driving force for such a loss is not clear at this time. The CID spectra of the m/z 410 precursor ions derived from dyes 2, 3, and 4 are identical. This strongly supports the supposition that this m/z 410 component is identical in each of those dyes. The additional components of dyes 2 and 3 (additional to the main component of m/z 410) display the same fragments as those of 4 and 8, implying a similar moiety is present. It is therefore postulated that m/z 467 (of #2) and m/z 444/446 and 525 (of #3) each contain the 2-amino-8-hydroxy-3-sulfonate-2-azo configuration. Furthermore, the ~1/3 ratio of the 444/446 peaks in #3, combined with the 34/36 Da mass difference to #4 strongly implies that 444/446 is identical to the structure of 4, but with a chlorine atom attached to the phenyl ring (*ortho*- or *para* position cannot be determined from these spectra).

At this point, it should be re-emphasized that, although it is intellectually pleasing to correlate the observed CID spectrum with the expected fragments of a known structure, it is not necessary to know the chemical structure of a compound in order for its CID spectrum to serve as a fingerprint. Observing two compounds (in this case dye anions) with identical ESI-MS peaks – which also produce identical CID spectra from those peaks – essentially ensures that the compounds are identical or at least very closely related in structure. Rare exceptions could arise *e.g.*, in cases of positional isomerism such as the *ortho* or *para* substitution pattern alluded to above for the extra chlorine in dye #3.

Proposed Protocol to Compare Dye Extracts from Carpet Fiber Samples.

Proposition: Two fiber samples are believed to be identical in their dye content. Sample "A" is available in large quantity (50 mm or more). Sample "B" is available in small quantity only (one mm can

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[12] Brumley, W. C.; Brulis, G. M.; Calvey, R. J.; Sphon, J. A. *Biomed. Environm. Mass Spectrom.* **1989**, *18*, 394-400.

be spared for destructive analysis). They appear identical in color by microscopic examination.

Objective: Prove/disprove the proposition that the dye contents of the two fibers are identical using ESI-MS and CID.

Proposed Procedure:

1. Extract a 5 mm long sample of "A" as well as a 1 mm samples of both "A" and "B", and dissolve the extracts in 500, 100, and 100 µL, respectively, of the appropriate solvent mixture. Using a 10 µL loop injector, sequentially infuse samples of the 1 mm extracts and obtain the ESI-MS. If the ESI-MS are not essentially identical the proposition is refuted and the experiment is complete. If the ESI-MS spectra are essentially identical in m/z content and relative intensity, proceed to 2.
2. If the ESI-MS of dye ions from 1. above are represented in a "product ion" database (such as Table 4 above) confirm the assignments by running CID experiments under the previously established "ideal" conditions as indicated in the database.
3. If one or more of the major peaks in the ESI-MS are not represented in a "product ion" database, use the 5 mm extract to establish the "ideal" conditions for obtaining a fingerprint CID of that (those) precursor ions (as described in Section "Tandem Mass Spectrometry").
4. Using the CID conditions established above for each of the major peaks in the ESI-MS, do identical multifunction (one function per dye precursor mass) CID experiments on the 1 mm extracts of "A" and "B". If the CID spectra show identical fragmentation patterns for each pair (one from extract "A", and one from extract "B") of precursor ions, the two fibers can be deemed to be identical.
5. Even if as many as eight precursor ions need to be examined by CID, with an analysis time of 1 min each, and at a flow-rate of 5 µL/min, this will consume only 40 µL of solution. Together with the 10 µL consumed in 1. above this accounts for 50 µL of the original 100, leaving 50 µL for other

methodologies, or for a repeat of this one as necessary.

6. Note that application of the above procedure does not require physical separation of the component dyes prior to spectroscopic analysis. Nevertheless, the procedure is "two dimensional" because separation of the ESI-MS ions does occur within the first mass filter of the triple quadrupole mass spectrometer. The use of a tandem mass spectrometer affords the advantages of a two dimensional analysis, without some of the disadvantages associated with physically separating a mixture of compounds into its individual components.

FUTURE DEVELOPMENTS.

Use of other, more sophisticated mass spectrometry equipment for these dye-comparison problems is likely to yield even greater specificity and greater sensitivity. The analysis of dye extracts described above (Figure 3 and Table 3) used infusion of a solution at 5 µL/min for a period of two minutes to acquire each spectrum, *i.e.*, 10µL of solution, representing the extract of 170 µm of fiber. Using microspray technology rather than standard electrospray technology would allow slower infusion (< 500 nL/min) without loss of signal intensity and without deterioration of the signal/noise ratio.[13] Thus, in a microspray variation of the *Proposed Protocol* the total amount of solution needed would be reduced from 50 µL (*cf* step 5 of the Protocol in the preceding paragraphs) to less than 5 µL. The "extra" solution would be available for repeat analyses or for the application of other methods. Alternately, longer acquisition times could be accommodated for the analysis of very lightly colored fibers, which

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[13] Caprioli, R.M.; Emmett, M. E.; Andren, P. *Proceedings of the 42nd ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, IL, May 29-June 3, 1994; p754.

contain less dye material per mm fiber. The application of nanospray techniques[14] (flowrates down to 20 nL/min) would provide a further incremental reduction in the amount of solution required for the mass spectrometry analyses. Unfortunately, our instrumentation is not currently equipped with either a microspray- or a nanospray accessory, and we have not been able to test these low-flow variations.

CONCLUSIONS.

In order to definitively establish that two fiber samples are of identical origin, it is *i.a.* necessary to demonstrate that their dye components are identical, and that those dyes are present in the same proportions in each fiber. The qualitative comparison is necessary because fiber manufacturers often use identical dyes in different proportions to create differently colored fibers. The combination of electrospray ionization mass spectrometry and tandem mass spectrometry has been shown to provide both the qualitative and quantitative information required such comparisons. The technique is sufficiently specific and sensitive to allow comparison of two fibers, one of which is available lengths of as little as one millimeter. The use of more sophisticated electrospray techniques (microspray and nanospray) would further enhance both specificity and sensitivity.

ACKNOWLEDGEMENTS.

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[14] Wilm, M; Mann, M. *Anal. Chem.* **1996**, *68*, 1-8.

who recently turned in his labcoat to pursue a career as an FBI agent.

This work was funded by the FBI under DOE Project Number 2051-1119-Y1 with BWXT Y-12 L.L.C., U.S. Department of Energy, under contract number DE-AC05-00OR22800.

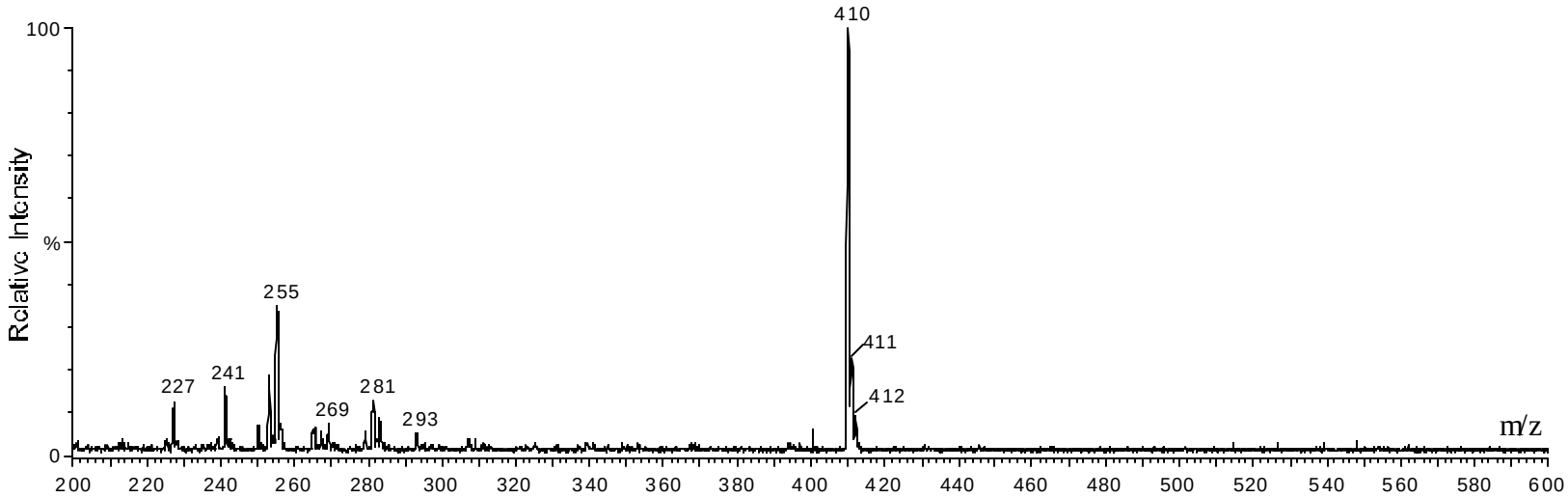

Figure 1: ESI-MS of Dye #4 (500 ppb in CH_3CN/H_2O 1:1).

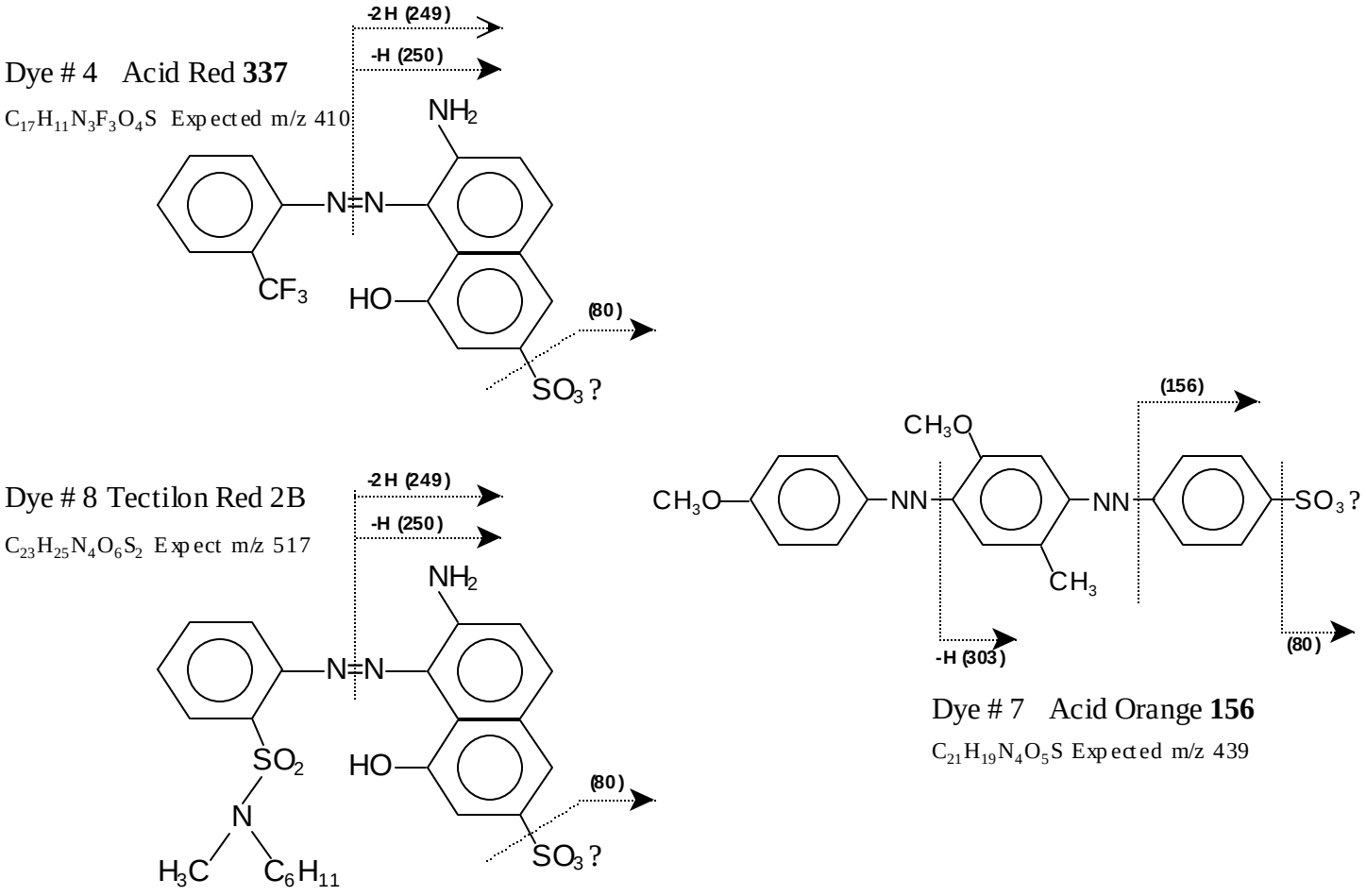

Figure 2: Structures and observed CID fragmentations of the known dyes.

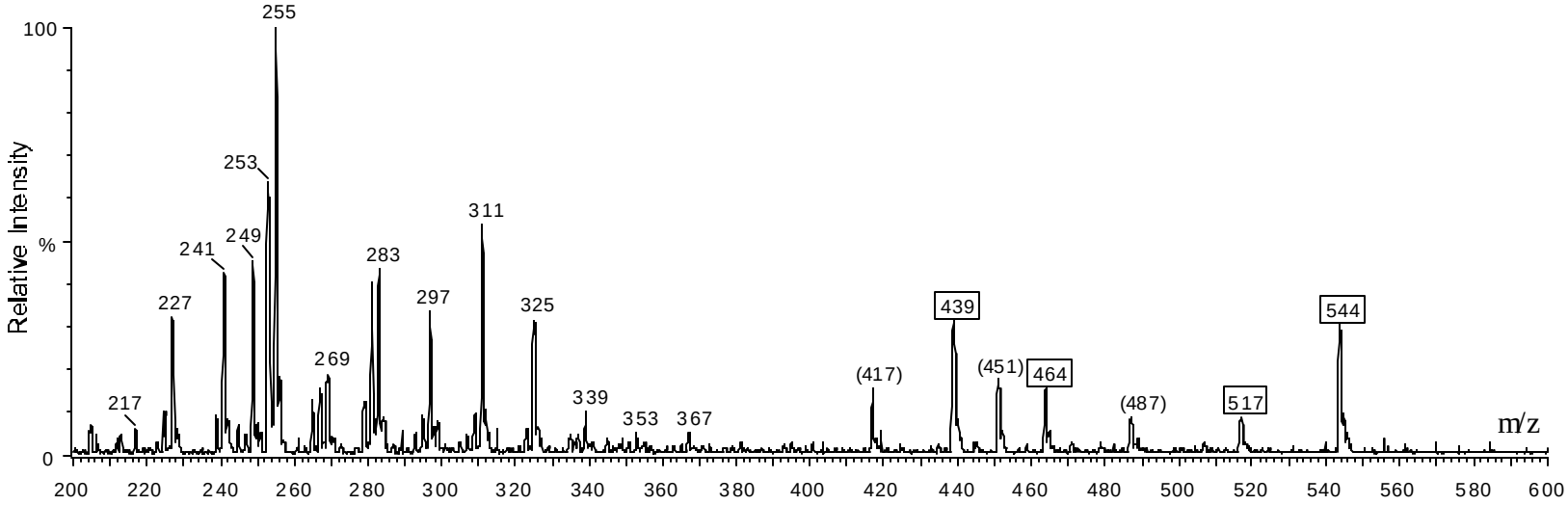

Figure 3: ESI-MS of the extract from Winding I ("brown") at the equivalent of 1 mm/60 µL of i-Prop/H_2O/0.1% NH_3. Dye peaks are marked with rectangles and extraneous peaks within the dye-region marked with brackets ( ).

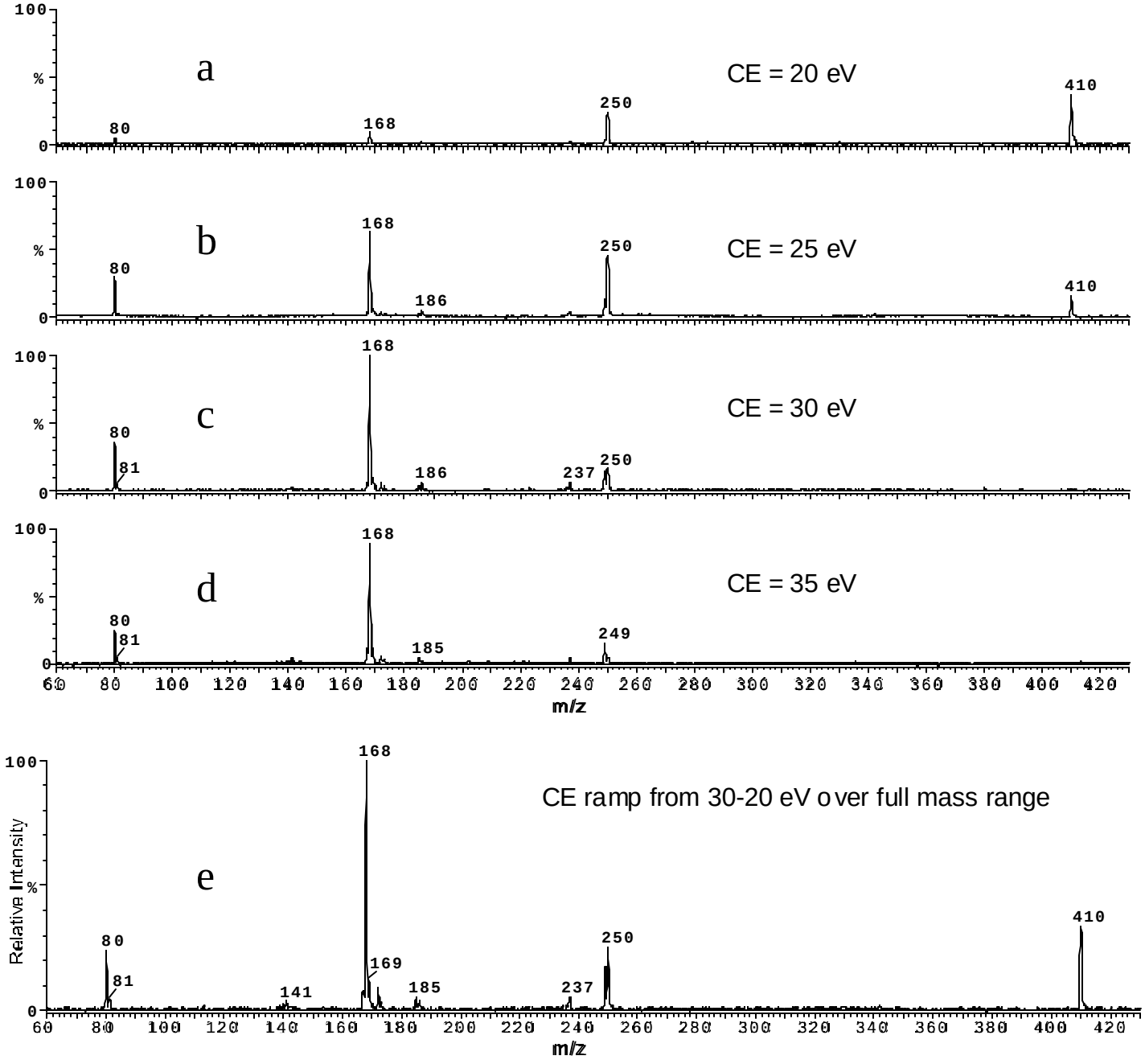

Figure 4: a-d) CID spectra of precursor anion m/z 410 from dye # 4, obtained with the collision energies indicated, and **e**) obtained with a collision energy ramp.

Table 1: Dyes and Colored Windings Used.

DYES

Lab-Code #	Commercial Name	CI Name
1	Telon Yellow FRL01 200	Proprietary Mix
2	Telon Red 2BN 200	Proprietary Mix
3	Nylanthrene Red CRBS 200	Proprietary Mix
4	Telon Red FRLS 175	Acid Red 337
5	Telon Blue BRL 200	Acid Blue 324
6	Tectilon Blue 4RS 200%	Unknown
7	Tectilon Orange 3G 200%	Acid Orange 156
8	Tectilon Red 2B 200%	Unknown
9	Dye-O = Lan Black RPL 150%	Acid Black 172

WINDINGS

Manufacurer's Code	Manufacurer	Dye Components	Manufacurer's Color Description
9900	Collins & Aikman	6,7,8	Brown
38920	Collins & Aikman	6,7,8	Olive Green
38011	Collins & Aikman	6,7,9	Black

Table 2: Observed Relative ESI Anion Intensities and Charge States for the Dyes Listed in Table 1.

	Observed Anion m/z Values																			
	308	*408*	*410*	*424*	*427*	*430*	*439*	*444/ 446*	*450*	*462.7*	*464*	*467*	*473.7*	*481.7*	*488*	*489/ 491*	*504*	*517*	*525*	*544*
Dye #																				
1					100^{-1}											$44/34^{-1}$	13^{-1}			
2			100^{-1}									90^{-1}								
3			100^{-1}					$56/22^{-1}$											53^{-1}	
4			100^{-1}																	
5									100^{-1}											
6											37^{-1}									100^{-1}
7							100^{-1}													
8																		100^{-1}		
9	100^{-3}	3^{-1}	3^{-1}	5^{-1}		6^{-2}				9^{-2}			16^{-2}	6^{-2}	2^{-1}					

Observed charge state indicated by superscript; thus,

XX^{-1}	**= singly charged cluster (^{13}C isotopes @ 1.0 Da)**
XX^{-2}	**= doubly charged cluster (^{13}C isotopes @ 0.5 Da)**
XX^{-3}	**= triply charged cluster (^{13}C isotopes @ 0.3 Da) (requires "high resolution" to observe isotope peaks)**

Table 3: Relative intensities, averages, and standard deviations for four repetitions of the ESI-MS spectra of three differently colored windings.

	Dye #	7	9	6	9	9	8	6
	m/z	439	462.5	464	473.7	481.7	517	544
Injection #	**winding**	**% of "total ion current"**						
1	I = "brown"	42.4		10.3			14.0	33.3
4	I = "brown"	44.0		11.4			13.4	31.2
7	I = "brown"	43.9		12.9			12.8	30.5
10	I = "brown"	42.7		14.4			12.0	30.9
	Average	**43.2**		**12.3**			**13.1**	**31.5**
	Stand. Dev.	**0.7**		**1.5**			**0.7**	**1.1**
2	II = "green"	34.7		12.8			12.3	40.1
5	II = "green"	34.2		14.3			11.8	39.6
8	II = "green"	34.1		17.0			10.3	38.6
11	II = "green"	34.3		17.9			11.0	36.8
	Average	**34.4**		**15.5**			**11.4**	**38.8**
	Stand. Dev.	**0.2**		**2.0**			**0.8**	**1.3**
3	III = "black"	19.8	29.4	9.7	15.8	4.0		21.3
6	III = "black"	20.1	29.8	10.2	15.6	3.8		20.5
9	III = "black"	20.4	28.2	12.4	16.0	2.2		20.7
12	III = "black"	21.0	27.2	12.8	14.2	4.1		20.7
	Average	**20.3**	**28.6**	**11.3**	**15.4**	**3.5**		**20.8**
	Stand. Dev.	**0.5**	**1.0**	**1.3**	**0.7**	**0.8**		**0.3**

Table 4: Mass range scanned, collision energy (ramp) employed, and fragments observed for each of the precursor ions enumerated in Table 2.

Precursor m/z	Dye # or Winding #	Scanned m/z Range	Collision Energy Ramp	Observed CID fragments [m/z]
308	9	80-550	19	203 / 308 / 316 / 352 / 408 / 424 / 430
410	2,3,4	60-430	30-20	80 / 168 / 249 / 250 / 410
417	I, II, III	60-437	40-20	83 / 189
427	1	60-447	30	292 / 409 / 427
424	9			Too small for good CID @ 500 ppb of dye #9
430	9			Too small for good CID @ 500 ppb of dye #9
439	7	60-459	40-20	80 / 156 / 303 / 395 / 423 / 439
444/446	3	60-466	30-20	80 / 168 / 249 / 250 / (444/446)
450	5	60-470	35	344 / 386 / 450
451	I, II, III	60-471	35-30	83 / 125 / 207 / 225 / 333 / 433 / 451
462.7	9	60-482	30-20	142 / 172 / 267 / 281 / 352 / 424 / 463
464	6	60-484	35	344 / 400 / 464
473.7	9			Too small for good CID @ 500 ppb of dye #9
467	2	60-487	30-20	80 / 168 / 249 / 250 / 467
481.7	9			Too small for good CID @ 500 ppb of dye #9
487/489	I, II, III	60-510	25	283 / (487/489)
489/491	1	60-510	30-15	94 / 123 / 186
504	1			Too small for good CID @ 500 ppb of dye #1
517	8	60-537	30	80 / 168 / 249 / 250 / 340 / 517
525	3	60-535	30-20	168 / 185 / 249 / 250 / 264 / 340 / 525
544	6	60-564	35	356 / 420 / 480 / 544