J Forensic Sci, Nov. 2002, Vol. 47, No. 6
Paper ID JFS2002052_476
Available online at: www.astm.org

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Ballpoint Pen Inks: Characterization by Positive and Negative Ion-Electrospray Ionization Mass Spectrometry for the Forensic Examination of Writing Inks*

ABSTRACT: A method based on profiling of dye components by electrospray ionization mass spectrometry (ESI/MS) is described for the characterization of ballpoint pen inks. The method involves benzyl alcohol (30 μL) extraction of ink from paper. The extracts of ink lines 1 and 5 mm in length are used for direct ESI/MS analysis in positive and negative modes, respectively. The instrumental analysis takes 3 min. Basic and acid dyes in the inks are detected in the positive and negative modes, respectively, with each dye yielding one or two characteristic ion peaks. The mass spectrum, which is mainly a compositional signature of the dyes in the ink, was not affected by the type of paper from which the ink was extracted, or by natural ageing of the ink on document in the absence of light. However, exposure to fluorescent illumination caused dealkylation of polyalkylated basic dyes and resulted in changes in the homologous distribution of the dyes. In this study, a total of 44 blue inks, 23 black inks, and 10 red inks have been analyzed, and the mass spectra were used to establish a searchable library. ESI/MS analysis provides a simple and fast way to compare ink specimens and in combination with on-line library search permits rapid screening of inks for forensic document investigations.

KEYWORDS: forensic science, questioned documents, ballpoint inks, electrospray ionization, mass spectrometry, ink library

Altered or falsified documents are often encountered during investigations regarding tax evasion and criminal activities. In combination with other physical methods, chemical analysis of writing inks is often used to establish the authentic or fraudulent nature of questioned documents. In many cases, it involves the comparison of two or more ink entries on one or more documents to determine if they have been written with similar inks. In some cases, the ability to determine the source of a questioned ink could be used as an investigative aid. The most common type of inks in these investigations is ballpoint pen inks (1), which are composed mainly of solvents, ionic basic and acid dyes, resins, additives, and other components. Characterization of whole inks by diffuse reflectance Fourier transform infrared spectrometry (2) has been attempted. The procedure, however, requires a relatively large extracted ink sample, which may not always be available from the questioned document. In addition, most of the components of ink absorb over the same infrared range, resulting in a complex spectrum that shows only subtle differences from those of other inks. To further complicate the interpretation, the spectra are dependent on the amount of ink solvents. Unlike solvents that disappear slowly with time, dyes are non-volatiles and are more suitable for ink characterization. Separation techniques such as thin layer chromatography (TLC) (3,4), high-performance liquid chromatography (HPLC) (5,6), and capillary electrophoresis (CE) (7) have been applied to provide information on the identity of writing inks based on their dye composition. Direct analysis of ink dyes by mass spectrometry based on field desorption (FD) (8) and laser desorption (LD)(9) has also been reported. These soft ionization techniques provide molecular information of dyes and as a result yield a mass spectrum of the composite dyes in the ink. In general, these spectrometric techniques are less time consuming than the chromatographic approaches since separation of components by time is not required. Furthermore, unlike elution or migration times that tend to shift over time, mass-to-charge ratio measurements are absolute values and are more desirable for use as characterization parameters, particularly in building a databank. The FDMS approach (8) depends on manual deposition of the ink extract on the FD emitter where analytes are ionized and desorbed when the surface is heated in the presence of a high electric field. In the report, only results obtained in positive mode for basic dyes have been reported. A search and match technique for ink classification has not been provided except by visual comparison of spectra that becomes difficult for an extensive standard reference database. Moreover, the applicability of the method to document analysis has not been well validated, which includes reproducibility of the method, as well as the effects of paper type, light exposure, and ageing on the interpretation of mass spectra. The LDMS technique (9) uses pulsed laser to desorb and ionize ink dyes directly from paper without the need of extraction. However, the study was focused on the degradation of dyes for the purpose of ink dating, and little discussion was devoted to establishing the technique as a reliable method for profiling inks. The objective of this work was to develop a simple and reliable characterization method that allows automation for MS analysis in

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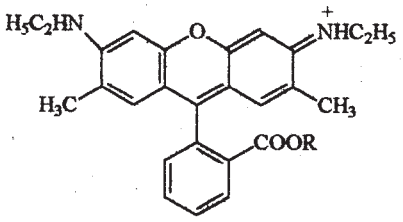
* This work was partly funded by the Technical Support Working Group under the Memorandum of Understanding for Counter-Terrorism Research and Development.

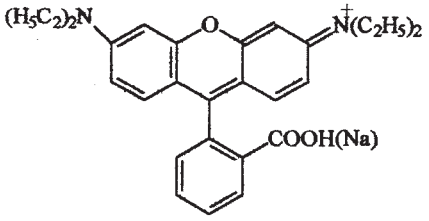
Received 7 Feb. 2002; and in revised form 24 April 2002; accepted 4 May 2002; published 2 Oct. 2002.

positive and negative modes and ink identification through computerized on-line library search.

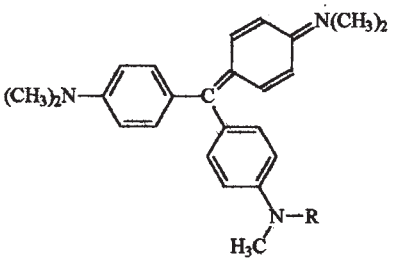
The dyes used in ballpoint pens are mainly basic dyes based on triarylmethane and rhodamine (Fig. 1) and acid dyes derived from diazo compounds or phthalocyanine (Fig. 2). These dyes are ionic in nature, with the basic and acid dyes generally containing iminium and sulfonate groups, respectively. Intuitively, they are well suited for analysis by electrospray ionization mass spectrometry (ESI/MS), which is a powerful technique for analyzing compounds preformed as charged species in the sample solution. Previous reports have also demonstrated that it is highly feasible to analyze cationic nitrogen compounds (10) and sulfonated azo dyes (11) by positive and negative ESI/MS, respectively. ESI is a soft ionization technique that usually yields an ion of characteristic



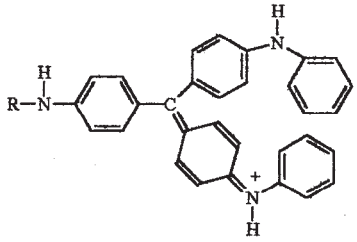
R = CH_3, Basic Red 1:1 (m/z = 429)
R = C_2H_5, Basic Red 1 (m/z = 443)



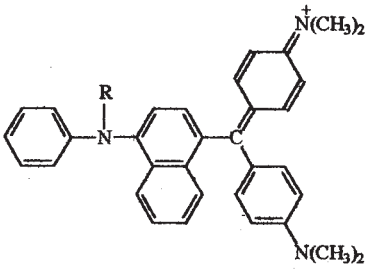
Basic Violet 10
COOH (m/z = 443)
COONa (m/z = 465)



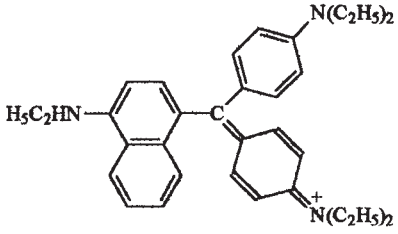
R = CH_3, Basic Violet 3 (m/z =372)
R = H, Basic Violet 1(m/z = 358)



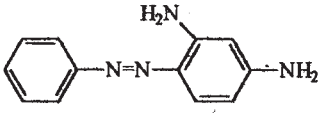
R=Ph, Solvent Blue 23 (m/z = 516)
R=H, (m/z 440)



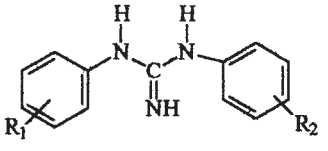
R = H, Basic Blue 26 (m/z = 470)
R = CH_3, Solvent Blue 2 (m/z = 484)



Basic Blue 7 (m/z = 478)

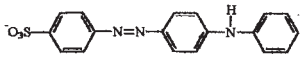


Solvent Orange 3
($[M+H]^+$ at m/z 213)

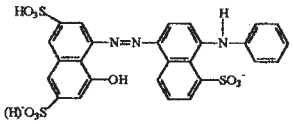


Aryl guanidines, e.g.
R_1=H, R_2=CH_3 ($[M+H]^+$ at m/z 226)
R_1=R_2=CH_3 ($[M+H]^+$ at m/z 240)

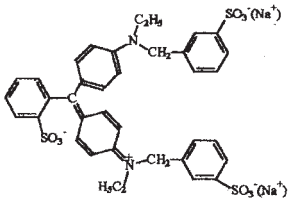
FIG. 1—*Structures of basic dyes, Solvent Orange 3 and aryl quanidines, together with m/z values of the corresponding ions detected in the positive mode.*



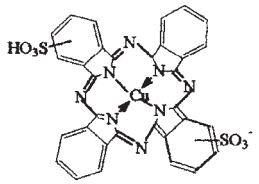
Acid Yellow 36 (m/z =352)



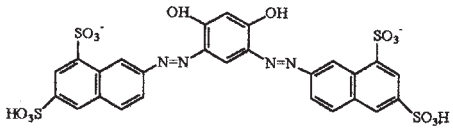
Acid Blue 92
Mono-anion (m/z = 628)
Di-anion (m/z = 313.6)



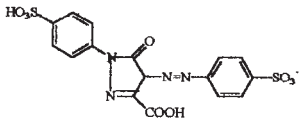
Acid Blue 9
Mono-anion (m/z = 769)
Di-anion [m/z = 373= (769-Na)/2]



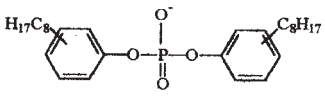
Solvent Blue 38 (m/z = 734)



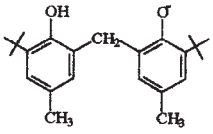
Solvent Brown 20
Di-anion (m/z 368)



Sepisol Fast Yellow TN
Mono-anion (m/z 467)



a corrosion inhibitor
(m/z 473)



an antioxidant
(m/z 339)

FIG. 2—*Structures and m/z values of negative ions derived from acid dyes, 2,2′-methylene-bis-(4 methyl-6-tert-butylphenol) (MBP), and bis[(1,1,3,3-tetramethylbutyl)phenyl] hydrogen phosphate (TMBPHP).*

mass-to-charge ratio (m/z) for each compound, with little or no fragmentation. Thus, like the FDMS and LDMS techniques, direct injection of an ink extract into an ESI mass spectrometer gives a mass spectrum that is mainly a compositional fingerprint of the dyes in the ink. This paper describes an ESI/MS method developed on an ion trap mass spectrometer, which allows automation of direct-injection, on-line library search and MS/MS operation for differentiation of dyes that yield the same parent ions. The value and limitations of the approach for evaluation of ink specimens extracted from documents will also be discussed.

Experimental

Chemicals and Samples

Benzyl alcohol 99+%, A.C.S. reagent, methanol 99.93% Biotech solvent, and acetic acid, double-distilled PPB/Teflon grade, were purchased from Sigma-Aldrich Canada (Oakville, ON). Deionized water used was of 18 megohms. Dyes and ink formulations were supplied by Kimberly-Clark (Escondido, CA) and by Spectra Colors Corp. (Kearny, NJ). The 77 ballpoint pens listed in Table 1 were obtained from local stores. The U.S. Secret Service supplied fresh inks and aged ink-on-paper samples for the study of age effects. Six different types of off-white and white papers were supplied by Buntin Read (Ottawa, ON). They are (1) Plainfield Plus Multipurpose, 48M—24 lb–90 g/m^2, brite white, smooth; (2) Photocopy Paper 10M P4, 75 g/m^2, long grain; (3) News Print, white Hi Brite, 82M; (4) Colonial Bond Glowhite, 25% cotton; (5) Colonial Bond White, 25% cotton; and (6) Super fine linen Record Bond, 100% cotton.

Sample Preparation

Each ballpoint pen was used to draw straight lines as evenly as possible on white photocopy paper, which was then stored in a mail

TABLE 1—*List of ballpoint pens.*

	Number of Pens		
Brand*	Blue Ink	Black Ink	Red Ink
A	4	2	2
B	2	1	1
C	3	1	1
D	2	1	1
E	2	0	0
F	9	6	3
G	1	1	0
H	2	2	1
I	6	3	1
J	2	0	0
K	2	2	0
L	1	0	0
M	5	1	0
N	1	1	0
O	1	1	0
P	1	1	0

* Each letter under the heading "Brand" indicates a different manufacturer of commercially available pens.

slot shelf for at least a week away from light to let the ink dry. A Harris micro punch (Whatman Bioscience) was used to cut one and five plugs (1.2 mm in diameter/plug) from the ink line. The plugs were put in a 100-μL conical vial insert, which was placed in a 2-mL autosampler vial. Benzyl alcohol (30 μL) was added to the insert and the mixture was periodically stirred. After half an hour the solution was mixed thoroughly using a Wig-L-Bug mixer (Reflex Analytical Corporation, Ridgewood, NJ). Two and 5 μL were used for ESI/MS analysis in positive and negative modes, respectively. A paper blank was obtained by removing two plugs of blank paper near the sampling location of the ink. The blank was extracted and analyzed in the same manner as the ink sample.

Dyes and bulk ink formulations were weighed and dissolved in benzyl alcohol to give a concentration of 6 to 10 ppm for instrument analysis.

Instrumentation

The ink extracts were analyzed without chromatographic separation by flow injection into a Finnigan LCQ Duo system (Thermo Finnigan, San Jose, CA). The instrument is equipped with an electrospray interface, a Spectra-SYSTEM P4000 gradient pump, a SpectraSYSTEM A3000 autosampler, and a Finnigan Xcalibur data system revision 1.2. The autosampler injector valve was connected to the inlet valve of the interface via a red PEEK tube. The isocratic mobile phase, which was composed of 75% MeOH, 24.5% deionized H_2O, and 0.5% CH_3COOH, was adjusted to a flow rate of 200 μL/min. Typically 2 and 5 μL of ink extracts were injected for analysis in positive and negative modes, respectively. The instrument was calibrated using caffeine, MRFA, and Ultramark 1621 (Thermo Finnigan). Tuning of the instrument was achieved by optimizing ion m/z 372 in positive mode and ion m/z 352 in negative mode using a benzyl alcohol solution of a black ink (8 ppm). The capillary voltage and the tube lens offset were set to 10 and 0 V, respectively. The LCQ Duo was scanned at a rate of 5,555 amu/s. In positive mode, it was scanned from 135 to 238.3 amu and from m/z 239.3 to 900 amu. The acquisition was suspended from 238.3 to 239.3 amu to remove the strong background peak at m/z 238.8 produced by benzyl alcohol. In the negative mode, the spectrometer was scanned over the range 150 to 1500 amu. The acquisition time was 1 min with a delay of 1 min after the acquisition period. The total cycle time was about 3 min per sample.

Data Processing

The Qual Browser data processing program was used to subtract the total ion chromatogram (TIC) of each paper blank from that of the corresponding ink sample. The blank was always analyzed before the sample so that any residual carryover from the preceding sample analyzed in a sequence was removed by the subtraction. The final ink profile was obtained by averaging all the spectra across the sample peak in the subtracted TIC.

Creation of Libraries

Custom libraries of processed MS spectral data acquired in positive and negative modes were created separately on the Xcalibur data system. The spectra were obtained from the 77 pen inks shown in Table 1.

Effects of Paper and 2-h Exposure to Heat and Light (Accelerated Aging)

A series of lines were made from three different ballpoint pens on each of the six different types of white papers. Each paper sample was cut into two halves. One half was kept at room temperature away from light, while the inks on the other half were artificially aged by placing the paper on a hot plate at 105°C with exposure to a desktop fluorescent lamp (about 10 cm above) for 2 h. Untreated and accelerated aged ink counterparts on each type of paper were extracted and analyzed according to the procedure described above.

Effects of Light and 4.5-Day Exposure to Light

Lines of four individual inks were made on a sheet of white photocopy paper that was cut into two halves. One half of the sheet was kept at room temperature in the absence of light, while the other half was exposed to a desktop fluorescent lamp situated at about 10 cm above the surface of the sheet for a period of 4.5 days. Samples on both halves were extracted and analyzed as described before.

Results and Discussion

Selection of Extracting Solvent

The dyes are known to be readily extracted by benzyl alcohol, methoxyethanol, and pyridine (12). Compared to methoxyethanol and pyridine, two- to four-fold higher responses were consistently observed in positive and negative modes when benzyl alcohol was used to extract six different inks from paper. High sensitivity is required for the analysis since ink samples are usually small in forensic examinations to minimize destruction of the documents. Benzyl alcohol was, therefore, the solvent of choice for the study. In general, much higher sensitivity was obtained in the positive mode than in the negative mode. For a single ink line on paper, one plug of ~1 mm in diameter was more than sufficient to give a good MS profile in positive mode. For dyes to be detected in the negative mode, five times more sample is required.

Selection of Mobile Phase

Carryover is a major concern when multiple samples are analyzed in sequence. It is most likely caused by residual analytes that

remain after the analysis on the walls of the PEEK tubing situated between the injector and the ESI interface. To solve the problem, it is necessary to choose the proper solvent used for carrying the sample solution through the system. Judging by the nature of the analytes that are both organic and ionic, highly polar organic solvent would be required to keep them from being adsorbed on the plastic tubing. It was found that increasing the organic content of the carrier solvent from 50:50 methanol:water (v/v) to 75:24.5:0.5 methanol:water:acetic acid (v/v) significantly reduced the carryover. Any residual carryover was removed by spectra subtraction as described in the Experimental section.

Mass Spectra of Individual Dyes

Dyes were analyzed individually. Each basic dye yielded a positive ion corresponding to the cationic moiety of the dye molecule (Fig. 1). Additional smaller peaks due to the presence of homologues were observed in some dyes. For example, Basic Blue 26 (m/z 470) contained a small amount of the trimethyl homologue at m/z 456. Similarly, the penta-ethyl Basic Blue 7 (m/z 478) was contaminated by the presence of the tetraethyl homologue at m/z 450. The homologues showed similar fragmentation pattern in their MS/MS spectra.

Basic Violet 10 and Basic Red 1 both yielded the same ions at m/z 443 (Fig. 1). However, they can be readily distinguished by their MS/MS spectra. Basic Violet 10 gave a strong daughter ion at m/z 399 due to loss of CO_2 from the acid function on the aromatic ring, while Basic Red 1 formed a fragment at m/z 415 after losing an ethylene group. The presence of Basic Violet 10 is also indicated by a small peak at m/z 465 that is due to the sodiated form of the dye, which is formed by replacement of the labile proton on the carboxyl function by sodium ion. The m/z 465 ion was not detected when sodium-free methanol from two commercial sources was used. Generally, 300 ppb of sodium ion in the mobile phase would yield a discernible ion peak at m/z 465 (25% of the ion abundance at m/z 443). To obtain reproducible spectra of samples containing Basic Violet 10, it is advisable to incorporate the dye as a control sample in the analysis sequence to check for sodium in the mobile phase. If necessary, sodium acetate is added to adjust the sodium level.

In the negative mode, the number of ion peaks observed was dependent on the number of sulfonate group on the dye molecule. For example, Acid Yellow 36, a mono-sulfonated azo dye as shown in Fig. 2, gave a single peak at m/z 352 corresponding to the organic sulfonate ion. On the other hand, for Acid Blue 9 that contains two ionizable sulfonate groups, the singly charged ion at m/z 769 was detected along with the much stronger doubly charged ion at m/z 373. Triply or higher-charged ions were either very weak or not observed in polysulfonated dyes including Solvent Brown 20 and Acid Blue 92.

Other dyes, such as Solvent Orange 3 and 25, are also used in black inks. Solvent Orange 3, chemically known as 2,4-diaminoazobenzene (Fig. 1), is a weak base but not an ionic compound. It only gave a weak $[M+1]^+$ ion at m/z 213 due to partial protonation of one of the amino groups in solution. Solvent Orange 25, of which the structure is unknown, yielded a series of negative ions at m/z 304, m/z 312, m/z 457, m/z 468, and m/z 479. No ion was observed for Solvent Yellow 19, a chromium complex without a discrete positive or negative moiety. Solvent Black 7 is a mixture of nigrosines of which the chemical structures are not known (13). The positive mass spectrum of the dye showed a predominant peak at m/z 530. The detection of this ion was used as an indication of the presence of Solvent Black 7 in ink samples.

Mass Spectra of Inks

A blue writing ink sample was extracted and analyzed on three different days. Each ESI-MS analysis lasted 3 min. Figure 3 demonstrates the day-to-day reproducibility of the spectra acquired in the positive and negative modes. Although the same ions were always detected, the relative abundances of negative ions were less reproducible than that of positive ions, a result caused by the lower sensitivity obtained in the negative mode. No significant changes in the ink spectra were observed after the benzyl alcohol solutions of six different ink formulations were allowed to stand at room temperature over a 48-h period in the autosampler tray, suggesting that the extracts are stable enough for delayed analysis in a sequence.

Seventy-seven ballpoint pens representing 16 manufacturers (Table 1) were analyzed. The inks were deposited as lines on paper and then recovered by extraction. Eighty percent of the blue and black inks analyzed contain the polymethylated Basic Violet 3 (m/z 372) and its homologues, which were detected at m/z 330, m/z 344, and m/z 358 in the positive ion spectra. The blue inks were usually characterized by the predominant presence of one or more of the cationic dyes shown in Fig. 1: Basic Blue 26 (m/z 470), Basic Blue 7 (m/z 478), Solvent Blue 2 (m/z 484), and Solvent Blue 23 (m/z 516). Solvent Blue 38, detected in the negative mode predominantly at m/z 734 as $(M—Na)^-$, was a common component found in the blue inks. Acid Blue 9, which appeared at m/z 769 and m/z 373 as singly and doubly charged negative ions, had also been found in the blue inks. The cationic dyes, Basic Red 1:1 (m/z 429), Basic Blue 26 (m/z 470), and Solvent Black 7 (m/z 530), were found in some black inks. The most commonly detected negative ion in black inks was due to Acid Yellow 36 (m/z 352). The predominant presence of positive ion m/z 443, due to Basic Red 1 and/or Basic Violet 10, characterized red inks.

Non-dye components were also detected at m/z 339 and m/z 473 in negative mode (Fig. 2), which are the $[M-1]^-$ ions of the antioxidant, 2,2′-methylene-bis-(4 methyl-6-tert-butylphenol) (MBP), and the corrosion inhibitor, bis[(1,1,3,3-tetramethylbutyl)phenyl] hydrogen phosphate (TMBPHP), respectively. These compounds are common additives for ballpoint pen inks. Positive ions at m/z 524 and m/z 546, and negative ions at m/z 321, m/z 325, m/z 407, m/z 434, and m/z 735, were among the peaks detected in some inks but could not be identified. The peak at m/z 407 was consistently observed with the negative ion m/z 734 that corresponds to Solvent Blue 38 (Fig. 3). Others ions could be derived from non-dye components in the inks. Aryl guanidines (Fig. 1) are not ink dyes but, as bases, are often used to form salts with acid dyes or are added to raise the pH of the ink (personal communication with Dr. Ben Fabian at Formulabs, Inc.). Detection of the homologous ions at m/z 212, m/z 226, m/z 240, m/z 254, and m/z 268 indicates the presence of a mixture of phenyl-, tolyl- and xylol-guanidines. In some ink formulations, only ditolyl-guanidine (m/z 240) was detected. It should be noted that while all ballpoint inks gave positive ions, not all of them yielded detectable negative ions.

The analysis of 23 black inks representing 13 different pen brands showed that pens of the same brand but of different shapes or grips did not always give the same ink profiles. For example, for six black pens of the same brand investigated, only three yielded the same ink profiles in positive and negative mode. In one case, two inks from different branded pens were found to give similar positive and negative ion profiles; while, in another case, the same positive spectra but different negative ion spectra were obtained. Similar observations were made for the blue pens. The results clearly demonstrate that same MS profiles may, at best, indicate

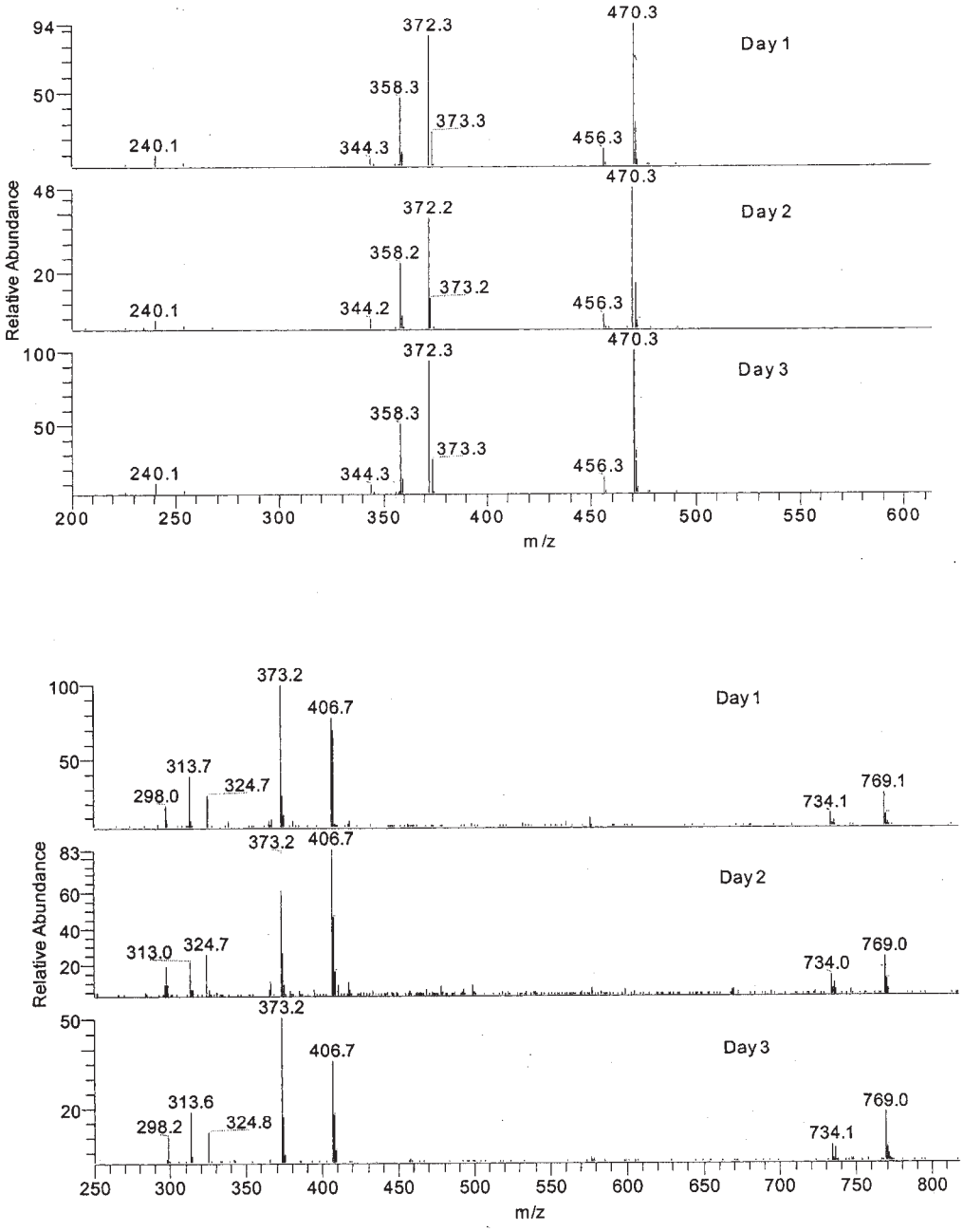


FIG. 3—*Day-to-day reproducibility of mass spectral dye profiles. The top three traces and the last three traces were acquired in positive and negative modes, respectively.*

that the compared specimens are derived from the same ink formulation, but not necessarily from the same pen. On the other hand, if the dye profiles are distinctly different, the specimens are definitely not derived from the same ink or pen.

Finding Dyes in Known Ink Formulation

Fourteen inks of known dye composition formulated for ballpoint pens were obtained and analyzed. The results are summarized in Table 2. In general, all of the ionic dyes, the antioxidant MBP, and the corrosion inhibitor TMBPHP present in the samples were observed. As discussed earlier, Solvent Orange 3 and Solvent Yellow 19 could not be detected by ESI/MS due to their chemical nature. The conventional ink identification technique, TLC (3), was used to confirm the presence of these dyes. The three red ink formulations, Red 1, Red 2 and Red 3, gave the same MS profile. They were formulations of different viscosity but were composed of the same dye mixture. These inks served to demonstrate that the same mass spectral characteristics do not necessarily indicate the same ink formulation.

Effects of Age on Profiles

Some dye components in an ink formulation might undergo degradation during storage. These changes will alter the mass spectrum acquired and affect the evaluation of the suspected ink sam-

ple. To study the effects of aging, two sets of ink samples of different ink formulations were analyzed. In each set, there were five ink-on-paper samples of different ages, i.e., 1, 2, 3, 4, and 6 years. These samples were prepared using five pens manufactured from different batches of the same ink formulation. They were kept in brown envelopes away from light to simulate natural aging. The ink in Set 1 was formulated predominantly with Basic Violet 1, Basic Violet 3, and Acid Yellow 36, while that in Set 2 contained Basic Violet 10 as the major dye component, with minor amounts of Basic Violet 1 and 3. For each set, the MS pattern obtained from each of the five aged samples was compared with that of the fresh ink from the same pen used to prepare the aged sample. In both sets, no significant differences in the MS profiles between the aged and fresh inks were observed (Fig. 4). This finding is consistent with the observations made by Andrasko (1): for an ink consisting of Basic Violet 3 and its homologs, considerable changes in the homologous profile were detected after the ink-on-paper sample had been exposed to daylight near a window for several hours, but no significant changes were observed when the same ink sample was stored in darkness for three weeks. These observations and ours do not support the suggestion that degradation of the triarylmethane dyes could occur in the dark (9). It is not clear, however, if natural ageing in the absence of light would modify the profile of other dye composition not investigated in this study.

Basic Violet 1 (m/z 358) and Basic Violet 3 (m/z 372), which are homologues, have been reported to undergo oxidative demethylation on exposure to daylight, fluorescent light (1), or ultraviolet light (9), resulting in change in the peak ratios of the two dyes. The phenomenon of progressive dealkylation was also observed in our study when two fresh ink samples containing these dyes were exposed for 4.5 days to light from a fluorescent tube situated at a distance of 10 cm from the ink samples. As shown in Fig. 5, for both Ink A and Ink B, the intensity of m/z 358 ion increased relative to that of m/z 372 ion. Similarly, m/z 344 ion, which was the demethylated product of Basic Violet 1, became stronger. In this study, it was found that other triarylmethane dyes, including Basic Blue 26 (m/z 470, Fig. 5), Solvent Blue 2 (m/z 484), and Basic Blue 7 (m/z 478), also underwent demethylation. Photodealkylation of the amino groups is not limited to triarylmethanes. The occurrence in rhodamine dyes has been reported previously (14) and

TABLE 2—*Analysis results on dye composition in known ink formulations.*

	Found Components (m/z of Major Ions)		
Sample	Positive Mode	Negative Mode	Components Not Found
Blue1	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Ditoyl guanidine (m/z 240)	Solvent Blue 38 (m/z 734)	
Blue 2	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Basic Violet 10 (m/z 443) Aryl guanidines (m/z 226, m/z 240, m/z 254, m/z 268)	Solvent Blue 38 (m/z 734) Acid Blue 9 (m/z 373.5, m/z 769) Antioxidant (m/z 339) Corrosion inhibitor (m/z 473)	Solvent Yellow 19
Blue 3	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Solvent Blue 23 (m/z 516) Aryl guanidines (m/z 226, m/z 240, m/z 254, m/z 268)	Solvent Blue 38 (m/z 734)	
Blue 4	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Solvent Blue 23 (m/z 516)	Solvent Blue 38 (m/z 734) Antioxidant (m/z 339) Corrosion inhibitor (m/z 473)	
Blue 5	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Solvent Blue 23 (m/z 516)	Solvent Blue 38 (m/z 734)	
Black 1	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Nigrosine (m/z 530)		Solvent Orange 3
Black 2	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Nigrosine (m/z 530)	Acid Blue 9 (m/z 373.5, m/z 769)	Solvent Orange 3
Black 3	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Basic Red 1 (m/z 443) Nigrosine (m/z 530)	Acid Yellow 36 (m/z 352)	
Black 4	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Ditoyl guanidine (m/z 240)	Solvent Blue 38 (m/z 734)	Solvent Orange 3
Red 1	Basic violet 10 (m/z 443)	Acid Yellow 36 (m/z 352)	Solvent Yellow 19
Red 2	Basic Red 1 (m/z 443)	Solvent Orange 25 (m/z 304, m/z 312, m/z 457, m/z 468)	
Red 3			
Purple 1	Basic Violet 1 (m/z 372) Basic Violet 3 (m/z 358) Basic violet 10 (m/z 443)	Acid Blue 9 (m/z 373.5, m/z 769)	
Green 1	Solvent Green 1 (m/z 329) Aryl guanidines (m/z 226, m/z 240, m/z 254, m/z 268)	Solvent Blue 38 (m/z 734) Sepisol Fast Yellow TN (467)	

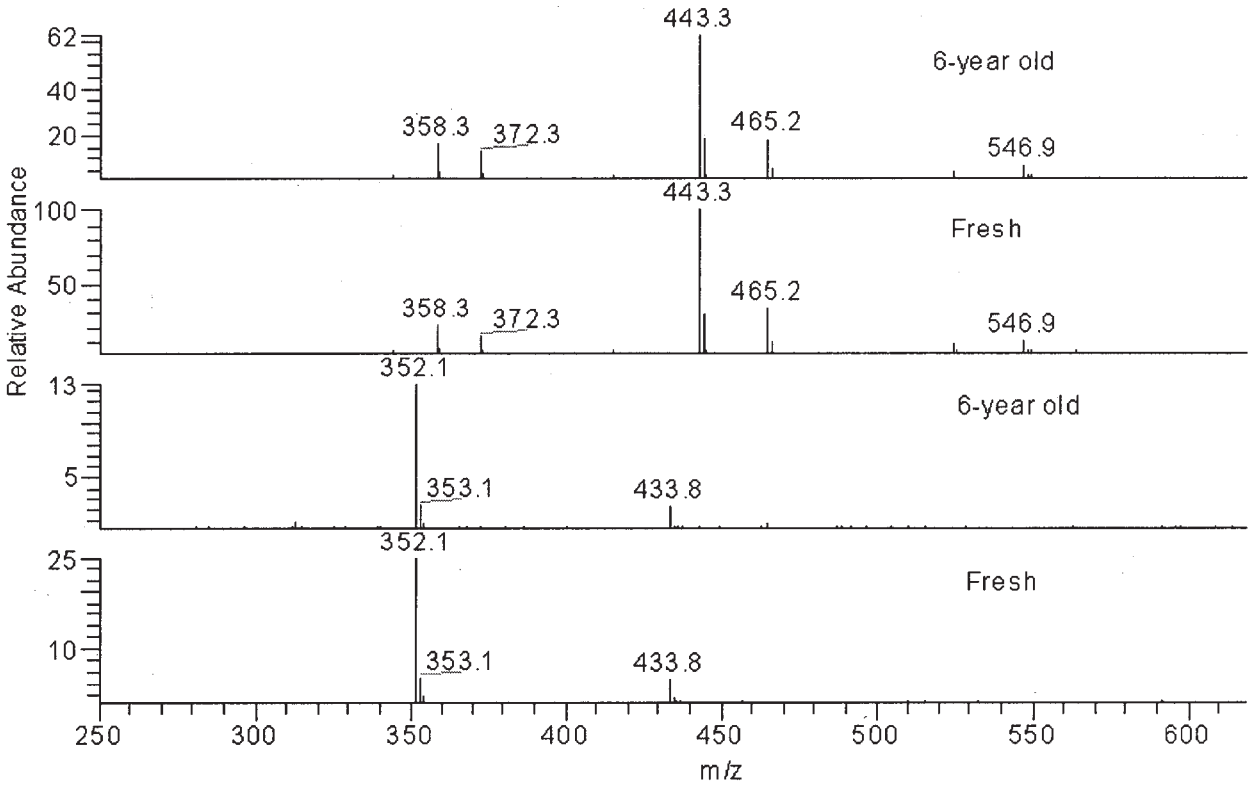


FIG. 4—*Comparison of mass spectra of a fresh ink and the ink aged for six years on paper in the absence of light. The top two traces and the last two traces were acquired in positive and negative modes, respectively.*

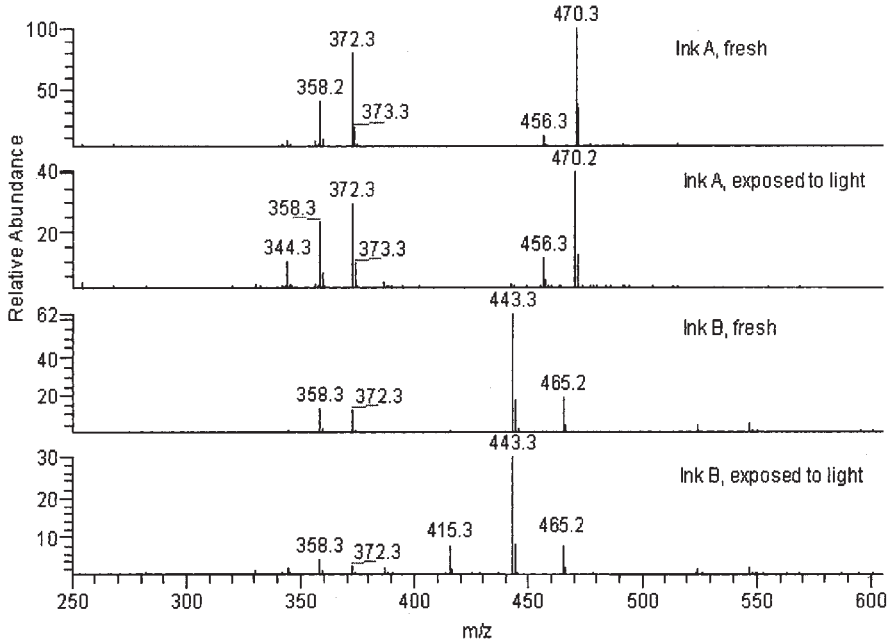


FIG. 5—*Effects of light on the composition of basic dyes. The second and last traces were obtained from inks that have been exposed to light from a fluorescent tube situated at a distance of about 10 cm for 4.5 days.*

TABLE 3—*Results of library searches of positive and negative mass spectra of 14 extracted inks. Hit numbers of the right matches are shown.*

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
Positive mode	1	1	2	5	1	1	1	3	1	5	7	1	2	3
Negative mode	3	1	1	1	1	4	1	1	9	2	1	2	1	9

observed in the present study. For example, the polyethylated Basic Violet 10 (m/z 443) yielded a de-ethylated product at m/z 415 ion (Fig. 5). In general, light exposure caused photodealkylation of basic dyes containing polymethyl or ethyl groups. As a result, the distribution of the homologous ions was modified. In normal aging conditions, the documents are not exposed to light, and the ink profile will remain intact as shown earlier in this study. In view of these findings, the homologue patterns of basic dyes must not be used unduly to assess the compared specimens from different documents, because their storage conditions are not always known.

Effects of Paper Type

The study was carried out using three different ink formulations and six different types of white writing paper. One of the papers was made of 100% cotton; two were composed of the same cotton content (25%) but differed in the amount of whitening agent; also included was a yellowish newsprint paper, an off-white photocopy paper, and a bright-white multipurpose paper. The inks were extracted about four weeks after deposition on the papers. No significant changes in the positive and negative spectra were observed for the same ink extracted from different papers. The same observations were made for the writing specimens that had been artificially aged by heating at 100°C with fluorescent light exposure for 2 h. These results point to the validity of using the ESI-MS method to compare ink samples from different documents.

Libraries of Pen Inks

Libraries composed of positive and negative spectra of ink extracts derived from the 77 ballpoint pens (Table 1) were created. Fourteen of the 77 inks were again extracted from paper and analyzed to test the search capabilities of the ink library. For each test sample, the electronic search yielded a hit list that showed the possible matches in the order of decreasing matching score, which is a measurement of matching quality. The matching scores of the positive and negative spectra of the correct matches, as calculated by the library search software, were consistently in the range of 7 to 9, and 5 to 6.5, respectively, on a scale of 1 to 10. As indicated earlier, the relative peak abundances between negative ions were less reproducible from day to day, and consequently poorer match quality was obtained for the negative spectra. As shown in Table 3, the right matches for the positive and negative mass spectra can be found in the first ten hits. For most of the test samples, the right matches appeared as the first hits. For other cases, the spectra and the matching scores of the better but the wrong hits were very similar to those of the right matches. Since each ink is characterized by a positive and a negative spectrum, the probability of a correct identification increases because the conclusions drawn from the evaluation of the two spectra should be consistent. For example, for Ink S11, the right match for the positive spectrum appeared as the seventh hit with a matching quality of 8.82, which is comparable to those of the first six hits (8.85 to 9.16), and those of the eighth, ninth, and tenth matches (7.89 to 8.81). Although all these inks have very similar positive spectra as S11, none can be the right match because their negative spectra are entirely different from that of S11. In effect, electronic search and match technique should only be taken as an aid for the identification of unknown. It reduces the number of possible matches such that evaluation by the analyst as described above becomes manageable. When further discrimination is required, other ink identification techniques such as quantitative TLC (3,4), HPLC (5,6), or CE (7) should be used to provide additional information.

Conclusions

The results of this investigation show that ESI/MS is a reliable method for the characterization of ballpoint pen inks. The method allows comparison of ink specimens in a simple and fast manner. Spectral differences between different ink formulations are usually readily discernible by visual examination. On the other hand, the same spectral characteristics do not always indicate the same ink formulation or that the compared ink specimens are derived from the same pen. Comparisons of other ink components, such as solvents and resins, of the samples by other techniques are required before drawing a conclusion. The nature of paper from which the ink was sampled has been shown to have no effect on the profile, indicating that comparison of ink samples from different documents is feasible. Furthermore, natural ageing in the absence of light did not appear to significantly modify the ESI/MS profiles. The homologous distribution of polyakylated basic dyes, however, may be altered due to dealkylation caused by light exposure. Therefore, unless the storage conditions of the compared ink specimens are known to be the same, the peak ratios of homologous ions should not be used unduly in the evaluation of ink entries from different documents. This cautionary note does not, however, apply to comparing writing specimens from the same document. With respect to the problem of ink identification that is known to be complex and not amenable to an easy solution, on-line computerized library searching adds significant value to the method by providing a rapid means to narrow the field of possibilities.

Acknowledgments

The authors thank Dr. Pierre Pilon and Mr. Marc Gaudreau at the Laboratory and Scientific Services Directorate, and Dr. Tony Cantu at the U.S. Secret Services for initiating the project and their general support. The technical help by Ms. Mireille Loyer is greatly appreciated. Special thanks are due to Dr. Ben Fabian at Formulabs, Inc. for valuable discussions and supplying various dyes and ink formulations of known composition.

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