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TECHNICAL NOTE

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Differentiation of Blue Ballpoint Pen Inks by Laser Desorption Ionization Mass Spectrometry and High-Performance Thin-Layer Chromatography

ABSTRACT: The differentiation of inks on a questioned document can highlight a fraudulent insertion and is usually carried out by optical comparison and thin-layer chromatography (TLC). Laser desorption ionization mass spectrometry (LDI-MS) may also be used for the analysis of dyes from ink. This analytical technique was compared with a standard method of high-performance TLC (HPTLC) according to their capacity to differentiate blue ballpoint inks. Ink entries on paper from 31 blue ballpoint pens have been analyzed and their dye ink formulations compared. The pens were classified into 26 classes by LDI-MS against 18 for HPTLC. LDI-MS proved to be a more powerful method for differentiating ink formulations because it provides information about dye structures (molecular weights) and relative quantification of dye classes (peak areas). Sample preparation was minimal and analysis time was short in contrast to the more complex extraction, application, and development steps of the HPTLC method. However, only basic dyes and pigments were identified using positive mode LDI-MS, while HPTLC did yield additional information about acid dyes.

KEYWORDS: forensic science, questioned document, ballpoint ink, high-performance thin-layer chromatography, laser desorption ionization mass spectrometry, discriminating power

Two goals of chemically analyzing ink are to classify different inks available on the market and to determine whether more than one writing ink is present on a suspect document. The presence of two or more different inks can raise the suspicion of a fraudulent insertion as possible cause. Therefore, the objective of this study is not to identify a particular ink formulation but rather to discriminate different writing inks on a document.

Standard procedures used routinely in the forensic examination of ink include optical methods (1,2) and thin-layer chromatography (TLC) (3). Most analytical methods focus on colorant determination because ink formulations tend to have unique organic dye and pigments (4–7). During the 1970s and 1980s, mass spectrometry (MS) was evaluated as a means of analyzing fiber dyes (8,9) and was later found to be effective for analyzing ballpoint dyes (10). Some methods may be more sensitive, precise, and reproducible than others, but all of them give a qualitative measurement of the dyes present in an ink sample.

Laser desorption ionization MS (LDI-MS) has the advantages of being nondestructive and rapid (11,12), but has rarely been used in forensic laboratories. The purpose of this work was to determine whether LDI-MS can supplement the routine high-performance TLC (HPTLC) method for discriminating blue ballpoint inks according to their dye formulations. The respective discrimination powers of these two methods were compared in this study.

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Experimental

Materials

Thirty-one blue ballpoint pens were randomly chosen from the German market (23 were purchased from shops and eight were provided by the BKA, Bundeskriminalamt, Wiesbaden, Germany). These pens were used to prepare entries on multifunction bright-white, wood-free, and chlorine-free paper from Igepa Plus (80 g/m^2, DINA4, number 806 A 80, Reinbeck, Germany). The straight ink lines were immediately sampled for analysis after their application onto the paper.

LDI-MS Method

Small paper pieces measuring about 5 mm × 8 mm each, bearing three strokes from the same pen running parallel to the long edge, were cut, fixed to a metallic sample holder with carbon tape, and introduced into the MS. Mass analyses were conducted on a home-built matrix assisted laser-desorption/ionization (and LDI) reflector time-of-flight (TOF) mass spectrometer. The desorption/ionization was performed with a pulsed nitrogen laser (337 nm, 3 nsec, ~ 20 µm focus diameter). Delayed extraction was used and only positive ions were recorded. Mass spectra were generated by summing 100 individual laser pulses typically yielding a mass resolving power ($\Delta m/m$) between 3000 and 6000. The laser irradiance was regulated with an attenuator situated between the

laser and the sample. This provided a means of controlling the operative threshold irradiance at which analytes were desorbed and ionized adequately. Six summed MS spectra were recorded along the three lines produced by each ballpoint pen. The instrument was calibrated using the three signals generated by pure dye basic violet 3 (BV3, Fig. 3). Blank paper samples were analyzed to ensure they did not contribute to mass signals in the spectra.

The mass signals (m/z) of the spectra were compared to perform a preliminary classification based on qualitative information (i.e., absence/presence of peaks). The relative signal intensities of dyes were then used for further discrimination. For example, LDI mass spectra of triaryl dyes were characterized by the presence of the molecular ion and of the ions derived from dye molecules missing one or two CH_2 groups (mass difference of 14 u). Weyermann et al. (12) introduced a definition for the relative peak area (RPA) as follows:

$$\mathrm{RPA}_i = \frac{A_i}{A_{\mathrm{tot}}} \tag{1}$$

where A_i is the area of the signal at $m/z = i$ and A_{tot} is the total area of all the signals (molecular ion and related degradation products) of a particular dye. With this definition, it is possible to define a given RPA_i for the ink entries of a given ballpoint pen. BV3, for example, is characterized by the presence of the ion $M^+ = 372.2$ u and, to a lesser extent by the 358.2 and 344.2 u ions. The equation

$$\mathrm{RPA}_{372} = A_{372}/(A_{372} + A_{358} + A_{344})$$

characterizes the RPA value of the molecular ion $M^+ = 372.2$ u. These values vary greatly among different fresh ballpoint inks and can be used to discriminate different writing inks.

HPTLC Method

Ink entries about 1 cm in length were scraped from the paper with a scalpel. A sample of blank paper of equal dimensions was also analyzed. All solvents were purchased from Fluka™ (Buchs, Switzerland) and meets American Chemical Society (ACS) standards for purity. The extractions were accomplished in a conical vial (Supelco™, Buchs, Switzerland) using 15 µL of methanol. The vial was hermetically sealed and kept in the dark for 24 h at room temperature. Then, 2.5 and 5 µL aliquots were applied to a 10 cm × 20 cm HPTLC silica gel plate (Merck, Switzerland) with the aid of a Camag Linomat IV spot applicator (Camag, Mutlenz, Switzerland) (line application of 5 mm length). It must be noted that every plate included a standard of methyl violet.

The HPTLC plate was then developed in a horizontal developing chamber and two sequential solvent systems were used (3). The first system was a mixture of 1-butanol, 2-propanol, bidistilled water, and acetic acid (10:5:5:0.5); the second system was a mixture of 1-butanol, ethanol, double-distilled water, and acetic acid (15:3:3.9:0.45). The developing time was 15 min for system one and 30 min for system two. The TLC plates were then scanned at 590 nm with a Camag TLC Scanner III (Camag). The detection and semiquantitative analyses of recorded spectra were accomplished using the winCATS™® software with the Savitsky-Golay 7 filter factor. Chromatograms were compared with respect to

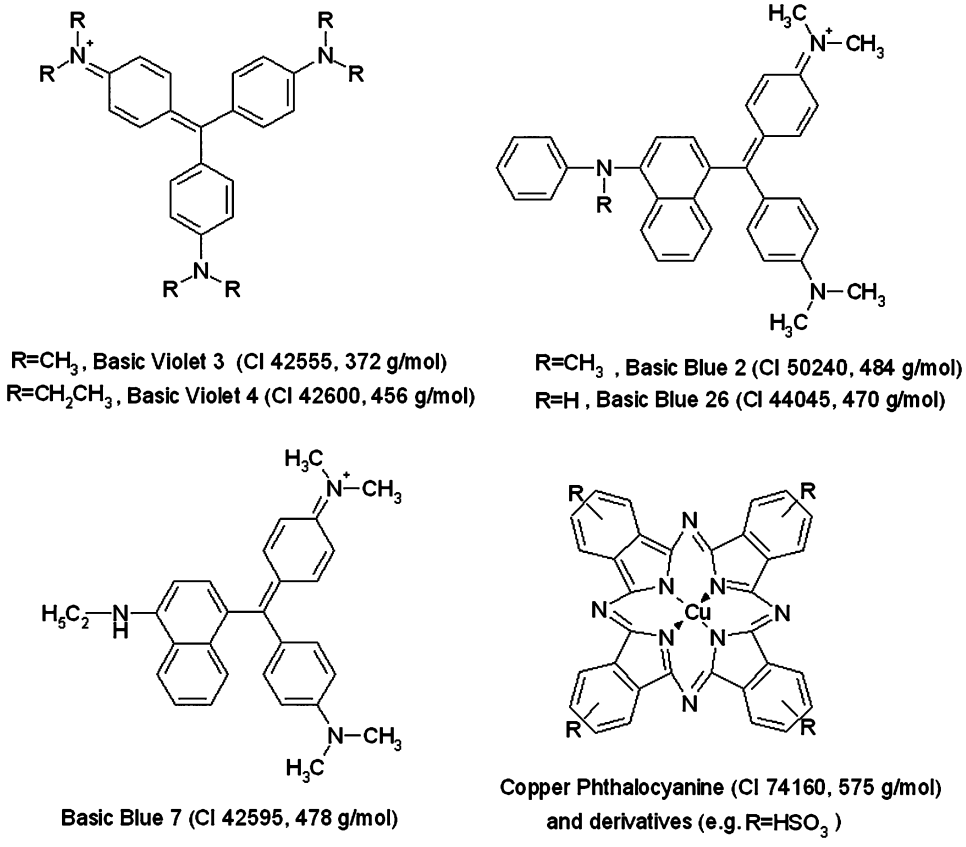

FIG. 1—*Structure of cationic dyes (with their color index, CI) typically used in ballpoint inks: basic violet 3, basic violet 4, basic blue 2, basic blue 26, basic blue 7; and copper phthalocyanine pigments.*

their relative retention time values, color and data resulting from the semi-quantitative analysis of each spot-line.

Discrimination Power (DP)

The ability of both techniques to differentiate entries produced by 31 blue ballpoint pens was evaluated by comparing the DP of the methods according to the following equation (13):

$$DP = \text{number of discriminated pairs}/\text{number of possible pairs}$$

where the number of pairs for a sample number of n is defined as follows:

$$\text{Number of pairs} = n(n-1)/2$$

Results and Discussion

LDI-MS

Ink entries made with the 31 ballpoint pens chosen for the present work were analyzed by LDI-MS. As a first step, qualitative comparison of the mass spectra made it possible to distinguish 19 different types of inks. The following dyes were detected in the mass spectra of the 31 blue pens (reported in order of increasing *m/z*, Fig. 1): basic violet 3 (BV3, in 87% of the pens), basic blue 26 (BB26, 58% of the pens), basic blue 7 (BB7, 26% of the pens), basic blue 2 (BB2, 10% of the pens), and basic violet 4 (BV4, 3% of the pens). Moreover, Pigment Blue 15 (PG15, 13% of the pens) was also recorded.

Also, 48% of the ballpoint pen inks gave rise to additional signals that could not be identified. Some signals were probably due to pigment derivates of copper phthalocyanine; other methods, for example MS/MS yielding fragmentation patterns, have to be used in order to elucidate their exact structures. These results are valid for fresh inks and are summarized in Table 1.

In a second step, the variability of the RPA value of the dyes in ballpoint inks was evaluated to refine the discrimination into 26 classes. For example, when BV3 was identified, RPA_{372} values were found to vary between 53% and 92%. Only four pairs of ballpoint pens entries could not be discriminated by LDI-MS (Table 2). The reproducibility of the RPA values was evaluated on several ink entries from ballpoint pen 1 on fresh and old samples. The relative standard deviation (RSD) was found to be below 10%.

HPTLC

On the basis of the results provided by HPTLC, 12 classes of ballpoint inks were distinguished on a qualitative basis based on the number of spots, their retention times, and color. The tested samples could be further discriminated into 18 classes by calculating their relative peak intensities. Thirty-seven pairs were not discriminated (Table 2).

TABLE 1—*Dye composition of 31 ballpoint pens determined by LDI-MS: the dye methyl violet (basic violet 3 [BV3]), basic blue 26 (BB26), basic blue 7 (BB7), basic blue 2 (BB2), and ethyl violet (EV) have been found in several ballpoint pens.*

	Identified dyes/RPA						
Pen number	BV3 (%)	BV4	BB26 (%)	BB7 (%)	BB2 (%)	PG15 (%)	Unidentified signals (g/mol)
1	92	91%	–	–	–	–	–
2	62	–	–	–	–	–	–
3	80	–	–	93	–	100	594, 608
4	75	–	93	–	–	–	580, 594, 608, 720
5	–	–	100	33	69	–	512, 498, 720
6	84	–	91	–	–	–	515
7	85	–	–	–	–	–	515
8	77	–	91	–	–	–	515, 594, 608, 720, 770
9	80	–	91	–	–	–	515, 594, 608, 720, 770
10	–	–	78	91	–	–	176, 653, 720
11	77	–	–	91	–	–	–
12	82	–	91	–	–	–	–
13	60	–	–	94	–	–	–
14	70	–	91	–	–	–	219, 241, 515, 608, 720, 770, 951
15	78	–	91	–	–	–	720
16	73	–	94	–	–	–	503
17	72	–	95	–	–	–	–
18	76	–	–	92	–	–	–
19	53	–	91	–	–	–	594, 608
20	–	–	–	35	50	100	498, 736
21	83	–	–	–	–	–	–
22	83	–	91	–	–	–	252, 492, 580, 594, 608, 624, 727
23	86	–	100	–	–	100	498
24	87	–	100	–	–	–	–
25	83	–	–	–	–	–	–
26	–	–	–	88	88	–	–
27	72	–	–	–	–	–	–
28	63	–	77	–	–	100	492
29	85	–	85	–	–	–	264, 594, 608, 720, 770
30	61	–	–	–	–	–	–
31	75	–	100	–	–	–	515, 580, 594, 608

The relative peak area (RPA) of their molecular ions given in % allows further discrimination. Additional signals present in the mass spectra could not be identified. LDI-MS, laser desorption ionization mass spectra.

TABLE 2—*Discrimination power of LDI-MS and HPTLC.*

	Method			
	LDI-MS		HPTLC	
Not discriminated	Pens	Pairs	Pens	Pairs
Group 1	12, 24	1	6, 8, 9, 12, 14, 19, 22, 31	28
Group 2	21, 25	1	21, 25, 27, 30	6
Group 3	11, 18	1	11, 18	1
Group 4	2, 30	1	4, 5	1
Group 5	8, 9	1	23, 24	1
# of not discriminated pairs		5		37
# of possible pairs		465		465
# of discriminated pairs		460		428
DP		0.99		0.92

LDI-MS, laser desorption ionization mass spectra; HPTLC, high-performance thin-layer chromatography; DP, discrimination power.

DP

As the above results indicate, the DP is slightly higher for LDI-MS (99%) than for HPTLC (92%; Table 2). This difference is primarily due to the ability of LDI-MS to discriminate two large groups of ballpoint pens containing BV3 and BB26 (seven pens) and inks containing only BV3 (four pens), whereas these samples could not be differentiated using the HPTLC method. Moreover, PG15 was not extracted by methanol and was therefore not analyzed by HPTLC.

It is interesting to note that the classifications obtained by the two different methods were, to some extent, complementary. Mass spectra of ink from ballpoint pen 2 only showed the dye BV3, while five unidentified spots on the HPTLC plate indicated the presence of additional dyes (Fig. 2). In eight ballpoint pen inks, a light blue spot was visible on the HPTLC plate where the ink sample was deposited; however, this dye did not appear in the LDI mass spectra. This can be explained by the fact that anionic dyes were not detected by LDI-MS in the positive detection mode, although they were clearly revealed by HPTLC.

The presence of additional peaks in the mass spectra and the determination of the RPA values of the identified dyes allow the LDI-MS technique to discriminate additional pairs. For example, ballpoint pens 12 and 19 were not discriminated by HPTLC, because their chromatography yielded very similar results. The intensities of the peaks were different (Fig. 3), but this can be explained by slightly different quantities of ink that were applied onto the plate. They were discriminated by LDI-MS because of the large difference in the RPA value of the dye BV3 (82% for pen 12 against 53% for pen 19). Moreover, the proposed HPTLC method was not able to separate clearly the dyes BV3 and BB26, due to the fact that they had nearly identical retention times. As BB26 was markedly less concentrated than BV3, it was much more difficult to detect (Fig. 3).

Four pairs of pens (8 and 9, 11 and 18, 12 and 24, 21 and 25) were not discriminated by any of the methods used. Also, pens 8 and 9 were produced by the same manufacturer; the six remaining ink formulations were distinguished on the basis of gas chromatography MS (GC/MS) analysis of ballpoint solvents in an earlier publication (14).

The major advantages of LDI-MS over HPTLC are the minimal sample preparation and destruction due to the fact that the analysis can be conducted directly on the paper. The method is therefore rapid in comparison with the long preparation and elution time needed for the chromatography. Moreover, the mass spectra contain information that can assist in the identification of the dyes. Finally, additional signals in the mass spectra (from pigments and additives), as well as the RPA definition used to calculate the ratio of a dye and its *N*-demethylated products allow for better discrimination of the inks. Conversely, HPTLC detected signals from anionic dyes that were not evident in the LDI mass spectra. The negative mode detection may be sufficient to resolve this problem. Modifying the preparation method by adding a matrix to the ink strokes on paper (15,16) may allow additional detection of low concentrations of pigments and resins in the mass spectra.

Conclusion

With respect to the chemical differentiation of inks, LDI-MS has proven to be a very powerful technique to discriminate ballpoint dyes in ink formulations directly from written ink entries on paper. Sample preparation is minimal and analysis time is short in contrast to the more complex extraction, application, and devel-

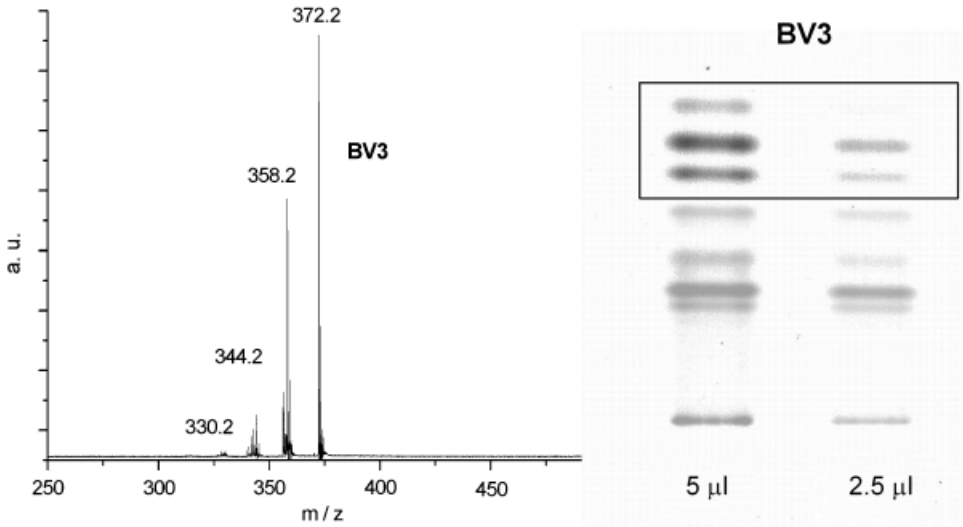

FIG. 2—*Laser desorption ionization mass spectra (LDI-MS; to the right) and high-performance thin-layer chromatography (HPTLC) plate (to the left) of the ballpoint pen 2. While only basic violet 3 was detected by LDI-MS, at least one additional dye and one pigment were revealed by HPTLC analysis.*

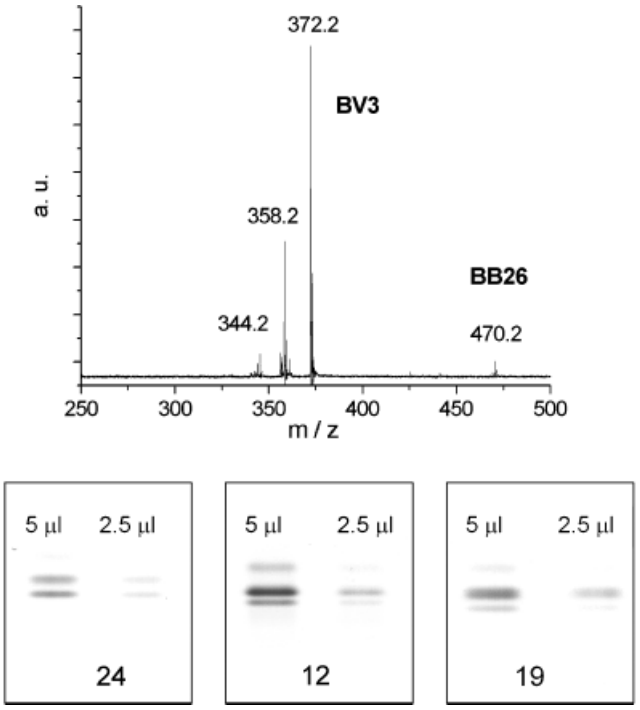

FIG. 3—*In laser desorption ionization mass spectra (LDI-MS) of the ink entries from ballpoint pen 12, signals from the dyes basic violet 3 (BV3) and basic blue 26 (BB26) have been identified. On the thin-layer chromatography plate, ink entries of the ballpoint pens 24, 12, and 19, spots from BV3 and BB26 have also been identified, but are partly superposed because they eluted together. As one dye is markedly more concentrated than the other, it is difficult to identify them.*

opment steps of the HPTLC method. MS resulted in separating the 31 blue ballpoint pens into 26 classes compared with the 18 groups obtained by using the HPTLC method.

The results for LDI-MS and HPTLC analysis have proven to be complementary to some extent. The presence of anionic dyes recorded by HPTLC, was not detected by LDI-MS in the positive mode. However, LDI-MS yielded information on the structure of the basic dyes and pigments (mass or/and fragmentation pattern), while spot identification and peak integration on the HPTLC plate were less precise.

A proposed strategy to differentiate and characterize ballpoint inks entails first using inexpensive and rapid optical comparison with light sources at different wavelengths. This preliminary step is followed by identification of the dye composition preferentially by LDI-MS. Forensic scientists should keep in mind that aging of ink dyes can substantially influence the results of this method (11,17).

Analyses of other substances present in ballpoint ink, such as solvents or resins, should be performed to differentiate pairs that cannot be discriminated by dye composition determination.

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