Forensic Science International 261 (2016) 71–81

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Detection and identification of dyes in blue writing inks by LC-DAD-orbitrap MS

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ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form 17 December 2015
Accepted 21 January 2016
Available online 3 February 2016

Keywords:
Questioned document examination
LC-DAD-Orbitrap MS
Identification
Dye
Blue writing ink

ABSTRACT

In the field of forensic questioned document examination, to identify dyes detected in inks not only provides a solid foundation for ink discrimination in forged contents identification, but also facilitates the investigation of ink origin or the study regarding ink dating. To detect and identify potential acid and basic dyes in blue writing inks, a liquid chromatography-diode array detection-Orbitrap mass spectrometry (LC-DAD-Orbitrap MS) method was established. Three sulfonic acid dyes (Acid blue 1, Acid blue 9 and Acid red 52) and six triphenylmethane basic dyes (Ethyl violet, Crystal violet, Methyl violet 2B, Basic blue 7, Victoria blue B and Victoria blue R) were employed as reference dyes for method development. Determination of the nine dyes was validated to evaluate the instrument performance, and it turned out to be sensitive and stable enough for quantification. The method was then applied in the screening analysis of ten blue roller ball pen inks and twenty blue ballpoint pen inks. As a result, including TPR (a de-methylated product of Crystal violet), ten known dyes and four unknown dyes were detected in the inks. The latter were further identified as a de-methylated product of Victoria blue B, Acid blue 104, Acid violet 49 and Acid blue 90, through analyzing their characteristic precursor and product ions acquired by Orbitrap MS with good mass accuracy. The results showed that the established method is capable of detecting and identifying potential dyes in blue writing inks.

1. Introduction

Ink analysis is an essential part of forensic examination of questioned documents. The differentiation of ink composition helps to detect forged contents on a questioned document. Generally speaking, writing inks consist of colorants (dyes or pigments), solvents, resins and other additives [1]. Among the components that could be easily analyzed, dyes are the characteristic and relatively stable ingredients in inks. Many methods have been applied in the discrimination of writing inks from the aspect of dyes, such as ultraviolet-visible spectrophotometry [2], Raman spectroscopy [3,4], thin layer chromatography (TLC) [2,5,6], capillary electrophoresis [7] and high performance-liquid chromatography (HPLC) [8]. Usually, identification of dyes is not necessary in these discrimination methods. To compare the difference in spectrum or chromatogram of samples can efficiently discriminate inks to a certain extent.

However, forensic ink analysis should go further than mere comparison. First of all, to identify dyes that are different in analyzed inks interprets the difference observed in spectra or chromatograms, which would improve the precision and reliability of forensic reports. Otherwise, as Gallidabino et al. once put forward in the ink discrimination study using laser desorption ionization - time of flight mass spectrometry (LDI-TOFMS), "the accuracy of discriminations based on unidentified peaks must be carefully considered, because their reproducibility was observed to be less reliable than that of identified signals."[9]. Secondly, to identify dyes provides information of the ink composition. In forensic investigation, additional knowledge regarding ink composition facilitates the search of ink origin, and it is important information for forensic ink library like the US Secret Service Digital Ink Library [6]. In forensic research, such knowledge is also the foundation of ink dating study, e.g., finding new dyes in ink formulas that degrades as the ink entries age or knowing the approximate date when a certain ink formula first appeared on the market [1].

Mass spectrometry is a key technique for identification, for it yields information on the structure of the analyzed molecules. With the developments of analytical techniques, mass spectrometry with

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http://dx.doi.org/10.1016/j.forsciint.2016.01.038
0379-0738/

different ionization sources, such as electrospray ionization mass spectrometry [10,11], time of flight secondary ion mass spectrometry (TOF-SIMS) [12,13], matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOFMS) or LDI-TOFMS[9,14,15] and direct analysis in real time ionization- time of flight mass spectrometry (DART-TOFMS) [16], has been applied in forensic ink analysis. While discrimination power is still the focus of these studies, more attentions have been paid to the identification of detected dyes [9,11,16]. In these studies, the prepared ink samples were directly analyzed by mass spectrometry. Unlike the straightforward manner that TLC presents dyes, the mass spectrum information is "colorless" and needs interpretation. For instance, the mass spectrum acquired by LDI-TOFMS or DART-TOFMS (both are in-situ analyzing techniques and induce only minor or no destruction of document) includes the molecular information of all ionized chemicals. Without reference substances, it is difficult to identify the ions of dyes from a cluster of signals, especially for those at less sufficient concentrations. Since the chemical composition of ink entries in real cases is unpredictable, how to detect and identify the potential dyes, i.e. perform a non-target identification of dyes [17], in an ink sample more efficiently?

Liquid chromatography- diode array detector-Orbitrap mass spectrometry (LC-DAD-Orbitrap MS) system is a promising answer to this question. LC-DAD is a classical combination for dye analysis, as DAD can detect colorants following adequate LC separation [18]. Orbitrap mass spectrometer is a new generation of high resolution mass spectrometer. It can measure the accurate masses of ions at a rapid speed with high resolution, well appropriate for non-target identification [17,19]. It has been applied in the field of environment monitoring [20], food administration [21], clinical and forensic toxicology [19,22], and forensic trace evidence [23,24], but not in the field of forensic ink analysis yet. The main concern might be the destructive sampling. Fortunately, due to the high selectivity and sensitivity of Orbitrap MS, as well as the concentrating effect of LC, the sample amount required for LC-DAD-Orbitrap MS analysis will only induce minor destruction of document. In the meantime, a more efficient and stable analysis of ink dyes can be performed with the LC-DAD Orbitrap MS system.

In this study, a method based on LC-DAD-Orbitrap MS system was established to evaluate its potential for detecting and identifying acid and basic dyes in blue writing inks. Three acid and six basic reference dyes were employed in the method development. Determination of the nine dyes was validated to assess the instrument performance. Afterwards, the established method was applied in the analysis of ink samples from ten blue roller ball pens and twenty blue ballpoint pens. As a result, in addition to nine reference dyes and Tetramethyl Para Rosaniline (TPR, a de-methylated product of Crystal violet), four unknown dyes were detected by LC-DAD. They were identified as three acid dyes and one de-methylated product of Victoria blue B, through analyzing their characteristic precursor and product ions acquired by Orbitrap MS with high resolution and mass accuracy.

2. Material and methods

2.1. Reagents and chemicals

Acid blue 1 (CAS: 129-17-9, 100.0%) and Acid blue 9 (CAS: 3844-45-9, 90.0%) were acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acid red 52 (CAS: 3450-42-1) and Basic blue 7 (CAS: 2390-60-5) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Victoria blue R (CAS: 2185-86-6) was obtained from CHEM SERVICE (Pennsylvania, USA). Ethyl violet (CAS: 2390-59-2, 80%) was obtained from Sigma-Aldrich Chemistry (Missouri, USA). Crystal violet (CAS: 548-62-9), Methyl violet 2B (CAS: 8004-87-3) and Victoria blue B (CAS: 2580-56-5) were purchased from ACROS ORGANICS (New Jersey, USA).

Methanol and ammonium acetate (≥99.0%) of HPLC grade were bought from Sigma-Aldrich Chemistry (Missouri, USA). De-ionized water was produced by a Milli-Q system (Millipore, Massachusetts, USA).

A 10 mM ammonium acetate solution was prepared by dissolving 0.385 g of ammonium acetate in 500 mL of de-ionized water.

2.2. Ink samples

Ink samples from ten blue roller ball pens and twenty blue ballpoint pens analyzed in this study were listed in Table 1.

2.3. Sample preparation

Ink lines of roller ball pens and ballpoint pens were drawn on two separate sheets of copy paper (80 g/m^2, Double A, Thailand) with a ruler. The ink samples were removed from the paper using a Harris micro-punch (0.5 mm i.d., Ted Pella, Inc., Redding, CA, USA). Four hole punches of each ink sample ($n = 2$) were extracted and transferred into a 1.5-mL Eppendorf tube. Next, 100 μL of extracting solvent, i.e., 50% methanol/water (v/v) for roller ball pen ink and methanol for ballpoint pen ink, was added to each tube. The extraction procedure was conducted for 20 min at room temperature on a shaker (Xinkang, Taizhou, China) at a frequency of 30 HZ. Then, 5 μL of the extracted liquid was injected into the LC-DAD-Orbitrap MS system.

The dyes identified in the ink samples with reference substances were quantified using the calibration curves built up with standard solutions. For those dyes at concentrations higher than the upper limits of the calibration ranges, the ink samples were diluted to one-tenth of the original concentration with corresponding extracting solvents and reanalyzed.

Table 1
The blue writing inks analyzed in this study.

Sample ID.	Brand	Origin	Type
RB1	PELIKAN® roller ball 338	Germany	Roller ball pen
RB2	A.G. Spalding & Bros®	Japan	Roller ball pen
RB3	PARKER® roller ball refill	Britain	Roller ball pen
RB4	SCHNEIDER® maxima 885	Germany	Roller ball pen
RB5	Artline® Lite Touch	Japan	Roller ball pen
RB6	STAEDTLER® Noris 333	Germany	Roller ball pen
RB7	Artline®softline 1700	Japan	Roller ball pen
RB8	STABILO® fluid 5	Germany	Roller ball pen
RB9	BIC® soft feel	France	Roller ball pen
RB10	BIC® Skater	France	Roller ball pen
BP1	Snow White® J-105	China	Ballpoint pen
BP2	PARKER®	USA	Ballpoint pen
BP3	CROSS®	USA	Ballpoint pen
BP4	Pilot®	Japan	Ballpoint pen
BP5	TOMBO®	Japan	Ballpoint pen
BP6	SHEAFFER®	USA	Ballpoint pen
BP7	SCHNEIDER® 740 M	Germany	Ballpoint pen
BP8	SCHNEIDER® 75F	Germany	Ballpoint pen
BP9	Morning Glory® VBP-8109	China	Ballpoint pen
BP10	HuiBoJia® 1445(0.7)	China	Ballpoint pen
BP11	BeiFa® 0.5 mm	China	Ballpoint pen
BP12	Duke® 0.7 mm	China	Ballpoint pen
BP13	Mitsubishi® SA-A	Japan	Ballpoint pen
BP14	Zebra® R-8000	Japan	Ballpoint pen
BP15	BIC® cristal grip assorted	France	Ballpoint pen
BP16	WHS SMITH®	Britain	Ballpoint pen
BP17	Staedtler® stick 430 M	Germany	Ballpoint pen
BP18	BaiNeng®	China	Ballpoint pen
BP19	Fenghua®	China	Ballpoint pen
BP20	Staples®	China	Ballpoint pen