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A comparison between DART-MS and DSA-MS in the forensic analysis of writing inks

Nicholas Drury^a, Robert Ramotowski^b, and Mehdi Moini^{a,*}

^aDepartment of Forensic Sciences, George Washington University, 2100 Foxhall Rd, Washington, D.C., United States

^bU.S. Secret Service, Forensic Services Division, 950 H Street, NW Suite 4200, Washington, D.C. 20223, United States

Abstract

Ambient ionization mass spectrometry is gaining momentum in forensic science laboratories because of its high speed of analysis, minimal sample preparation, and information-rich results. One such application of ambient ionization methodology includes the analysis of writing inks from questioned documents where colorants of interest may not be soluble in common solvents, rendering thin layer chromatography (TLC) and separation–mass spectrometry methods such as LC/MS (-MS) impractical. Ambient ionization mass spectrometry uses a variety of ionization techniques such as penning ionization in Direct Analysis in Real Time (DART), and atmospheric pressure chemical ionization in Direct Sample Analysis (DSA), and electrospray ionization in Desorption Electrospray Ionization (DESI). In this manuscript, two of the commonly used ambient ionization techniques are compared: Perkin Elmer DSA-MS and IonSense DART in conjunction with a JEOL AccuTOF MS. Both technologies were equally successful in analyzing writing inks and produced similar spectra. DSA-MS produced less background signal likely because of its closed source configuration; however, the open source configuration of DART-MS provided more flexibility for sample positioning for optimum sensitivity and thereby allowing smaller piece of paper containing writing ink to be analyzed. Under these conditions, the minimum sample required for DART-MS was 1 mm strokes of ink on paper, whereas DSA-MS required a minimum of 3 mm. Moreover, both techniques showed comparable repeatability. Evaluation of the analytical figures of merit, including sensitivity, linear dynamic range, and repeatability, for DSA-MS and DART-MS analysis is provided. To the forensic context of the technique, DART-MS was applied to the analysis of United States Secret Service ink samples directly on a sampling mesh, and the results were compared with DSA-MS of the same inks on paper. Unlike analysis using separation mass spectrometry, which requires sample preparation, both DART-MS and DSA-MS successfully analyzed writing inks with minimal sample preparation.

Keywords

DART/TOF-MS; DSA/TOF-MS; Forensic science; Ink analysis

*Corresponding author. moini.mehdi@gmail.com (M. Moini).

1. Introduction

Forensic science focuses on the application of science to the law, and has many sub-disciplines, including questioned document examination. Questioned document examination includes a thorough characterization of the composition of inks used in questioned documents, such as forged checks or business contracts [1–3]. Questioned document examiners may be able to determine, based on the ink, what type of pen was used; if more than one ink is present on the same document; the potential age of the ink; and the geographical distribution of the ink (i.e., where the ink is produced), to trace the original document back to a potential location [2]. Inks have different chemical compositions based on the type of writing instrument used. Several types of writing instruments include ballpoint pens, gel pens, fountain pens, and felt-tip pens or “markers” [4]. Moreover, multiple inks with the same color may be present on the same document; however, they can be differentiated by their chemical composition (profiles) [3].

Inks are a complex mixture consisting of a liquid vehicle, which is the liquid portion of the ink which transports colorants onto a surface; a colorant (a dye or a pigment); and additives [5–7]. Colorants impart color to the ink, and may include dyes or pigments such as carbon black, Michler’s ketone, crystal violet, or their combinations. Dyes are soluble within the vehicle whereas pigments are insoluble, solid, ground-up material suspended in the vehicle. Whether a colorant is a dye or a pigment depends upon the vehicle, and therefore, the type of ink. Lastly, additives include a wide variety of materials, including *pH* modifiers, emulsifiers, and buffers [8]. Pigments, being insoluble in the vehicle, may be insoluble in common solvents used in gas or liquid chromatography mass spectrometry (GC–MS or LC–MS) or electrospray-based mass spectrometry (ESI–MS), limiting the information which can be obtained.

Ambient ionization techniques are becoming increasingly popular in laboratories due to their short analysis time and minimal sample preparation enabling high throughput analysis. Ambient ionization mass spectrometry uses a variety of ionization techniques such as penning ionization in Direct Analysis in Real Time (DART), and atmospheric pressure chemical ionization in Direct Sample Analysis (DSA), and electrospray ionization in Desorption Electrospray Ionization (DESI). While the scope of DSA–MS in forensic casework is limited, DSA–MS has been used in forensic drug analysis for the examination of phenethylamines, and in ink analysis [6]. On the other hand, DART–MS has been extensively used for the analysis of explosives, paints, gunshot residue, and drug analysis [9–13]. Current DESI–MS techniques are only capable of detecting dyes and additives in questioned documents [6,14]. Current techniques of ink analysis in questioned document cases also include LC/MS(–MS), GC/MS, and MALDI–MS [15–17]. Recently, we compared analysis of writing inks using DSA–MS, GC–MS, and LC–MS. Briefly, GC–MS was shown to be the least informative analysis method for ink compositions, since colorants were mostly not detected and solvents and volatile components detectable by GC–MS tend to disappear very rapidly. Both DSA–MS and LC–MS were able to detect colorants; however, the DSA–MS results were obtained within seconds of mounting the sample while LC–MS analysis took several minutes. In addition to longer analysis time, solubility issues and the elution of small highly charged compounds with the void volume, and longer sample preparation time were

other main drawbacks of LC–MS. DSA-MS detected more ink-related compounds and in more samples than LC–MS. In this article, we compare DSA-MS with another commonly used ambient ionization technique, DART-MS.

DART-MS and DSA-MS techniques are ideal for the analysis of ink since ink samples can be introduced into the ionization region using the same medium that they are dispersed in. DSA-MS is an ambient ionization technique that utilizes atmospheric pressure chemical ionization (APCI) in which heated nitrogen gas is ionized by an electrical discharge, which initiates the desorption and ionization process [18]. Nitrogen ions ionize water molecules in the source and form water cluster ions that will ultimately ionize the analytes of interest. DSA-MS utilizes a closed system in which samples are introduced via a sample holder containing 13 sampling spots inside a closed housing. The housing is continuously swept with the flow of nitrogen gas from the APCI source that minimizes the ambient air entering the housing, thereby reducing chemical background noise. DART-MS on the other hand uses a penning ionization technique to initiate the ionization process, relying upon the formation of metastable helium atoms to generate protonated water clusters, which then ionize the analyte of interest [6,19]. DART-MS uses an open source in which individual samples on a variety of media are introduced into the ionization region as individual samples or placed on sampling trains for automated analysis. Both DSA-MS and DART-MS can be used to analyze ink samples taken directly from questioned documents. The purpose of this Technical Note is to report an evaluation of the analytical figures of merit including sensitivity, linear dynamic range, and repeatability, for DSA-MS and DART-MS analysis of writing inks, with specific regard to the analysis of colorants, and demonstrate the application of DART-MS to the analysis of United States Secret Service ink samples directly on a sampling mesh.

2. Materials and methods

2.1. Sample preparation

Details on the methodology for analyzing writing inks for DSA-MS and DART-MS have been previously published [6–8]. To compare the performance of the two techniques for the analysis of writing inks, three pens were used: a Paper Mate Stick blue ballpoint pen, a Bic Atlantis blue ballpoint pen, and a Zebra black gel pen. These were unaged ink samples. To use an identical sample introduction, the DSA-MS sample mesh holder was used. The DSA-MS mesh holder directly fits on the DSA-MS system. To use the same mesh holder on DART-MS, a sampling train was fabricated in house (Fig. 1). All mesh screens were burned prior to analyzing ink samples.

The minimum sample length required for detection was determined by placing single 7 mm, 5 mm, 3 mm, and 1 mm stroke lines of a Paper Mate stick blue ballpoint pen on Staples® printer paper. These paper samples were cut to the width of the ink stroke, placed onto a DSA-MS mesh holder screen, clamped into the DSA-MS mesh holder assemblies, and mounted on either the DSA-MS linear rail or the fabricated sampling train for DSA-MS and DART-MS analysis, respectively. Care was taken to make the strokes as close to the center of the region of interest as possible. Repeatability was determined by placing ten single-stroke 5 mm lines of a Bic Atlantis blue ballpoint pen on Staples® printer paper, and

analyzing by DSA-MS or DART-MS. The 5 mm ink stroke was chosen because it was the minimum amount of sample that produced the most consistent spectra. The ten samples were analyzed in sequence. Linear dynamic range of the two sources was determined by placing single-stroke 1 mm, 3 mm, 5 mm, and 7 mm ink lines from a Bic Atlantis blue ballpoint pen on Staples® printer paper, and mounting them into the mesh holder assembly. Finally, to demonstrate the application of DART-MS to the analysis of ink (DSA-MS data was previously published — see Ref. [6]), 12 black and 20 blue ballpoint ink samples, as well as 16 black and 20 blue non-ballpoint ink samples obtained from the United States Secret Service were analyzed by writing directly on mesh (PE part # MZ305382 Rev B). The samples used are listed in Table S1 (Supplemental material). Samples 1–12 were not analyzed because too little ink was available on the mesh for sampling. The ink samples were approximately two years old. The mesh was clamped into a DSA-MS mesh holder assembly. Samples were introduced one by one into the DART-MS ionization region as shown in Fig. 1.

2.2. Instrument parameters

DART-MS analyses were conducted on a DART 100 ion source (IonSense, Saugus, MA) coupled to an AccuTOF JMST100LC mass spectrometer (JEOL, Tokyo, Japan). Table 1 summarizes instrumental parameters used for DART-MS analysis. Analyses were done in positive ionization mode in the m/z range of 100–1000. Analysis time for each spot on the 13-spot mesh holder was ~20 s. The data for each spot was saved in a separate acquisition file. The mass spectrometer was calibrated before each run by placing FC-43 (perfluorotributylamine) calibration solution (Sigma-Aldrich, St. Louis, MO) on spot #1. The other 12 spots were analyzed immediately after the instrument calibration using external calibration with FC-43. For DART-MS, all data analysis was performed using the JEOL MassCenter Main and MS Tune Manager software as well as the IonSense DART Control software. The DSA-MS analyses utilized the same parameters as published in Ref. [6].

3. Results and discussion

DSA-MS and DART-MS were applied to the analysis of various writing inks and the detected colorant components are summarized in Fig. 2. As shown in Fig. 2, many colorants were base peaks in both DART-MS and DSA-MS spectra (overlapping area of Fig. 2). Some colorants were only base peaks for one of the two techniques (individual zones of Fig. 2) and had lower relative peak intensities in the other technique. Accurate monoisotopic masses of common ink colorants were aggregated and compared to peaks obtained by DART-MS and DSA-MS for purposes of identification.

Fig. 3 shows the comparison between the DSA-MS (left) and DART-MS (right) analyses of 7, 5, 3, and 1 mm pen strokes from the blue Paper Mate stick ballpoint pen. As shown Michler's ketone was detectable with DART-MS at the 1 mm level (m/z 372 and 374) on the lower right panel, which is absent on the DSA-MS analysis of the 1 mm stroke (lower left panel). However, DART-MS had more apparent background signal versus DSA-MS, which was attributed to its open source configuration. Open source configuration allows chemicals in the lab and in the vicinity of the ionization source to enter the ionization region and

produce background chemical noise, thus giving higher baseline noise for DART-MS spectra versus DSA-MS spectra. Another contributing factor to higher noise levels observed with our DART MS is its older age when compared to our DSA-MS (10 years old vs. 4 years old, respectively). For comparison, Figs. S1 and S2, respectively, show a spectrum of a blank 5 mm length Staples® printer paper sample, and a blank mesh screen illustrating that the 372 and 374 m/z peaks were not attributed to the paper or the mesh itself. Analyzing the blank mesh screens only showed typical DART-MS background ions.

On the other hand, the open source configuration of the DART-MS has important implications for sensitivity of ink detection on paper, since it allows for visual observation and precise manual positioning of smaller paper sizes within the ionization source. This effectively lowered the detection limit for DART-MS analyses to 1 mm ink strokes. At the 1 mm sample length, DSA-MS only identified components of the Staples® printer paper such as PEG (poly(ethylene glycol), m/z 283.1752 and 327.2012), which was consistent with the PEG observed when the blank Staples® printer paper itself was analyzed; however, no ink components were detected. The fully automated sample positioning system used by the DSA-MS does not allow manual positioning, which makes it difficult to position the small paper pieces at the center of the ionization region of the DSA-MS. Moreover, mounting of multiple pieces of small writing samples exactly in the center of the 12 mesh spots was tedious. In addition, because there is no camera to see the sample once positioned inside the DSA-MS ionization region, there is no way of knowing if the sample had moved during mounting on the mesh, placing mesh within the sample holder, or during the installation of sample holder in the sample housing, which affects repeatability of the DSA-MS sample analysis; mounting and analyzing samples one by one may allow better positioning of samples, however the efficiency of analysis would be much lower. On the other hand, the open source of DART-MS allows precise positioning of the sample in the middle of the ionization region thereby improving repeatability. DART-MS was found to have a percent relative standard deviation of 13% for the peak intensity of crystal violet, whereas DSA-MS had a percent relative standard deviation of 23% for the same component (Supplementary material, Fig. S3). Linear dynamic range was also impacted (Supplementary material, Figs. S4 and S5). To demonstrate the utility of the DART-MS to the analysis of the USSS writing inks, Fig. 4 shows the DART-MS spectra for 4 black and blue ballpoint and non-ballpoint inks (samples 27, 29, 45, and 65)

One type of modern writing ink that is troublesome to analyze are the gel inks. Gel inks use carbon-based dyes and pigments. Moreover, the ink is much more viscous than traditional rollerball or ballpoint pen inks, due to the incorporation of resins and thickening agents [20]. However, a black Zebra gel pen was successfully analyzed by both DSA-MS and DART-MS. Several 5 mm strokes of this ink were analyzed by DSA-MS and DART-MS, and a pigment commonly found in black gel inks, Rhodamine B, was easily identifiable by both techniques; however, the protonated ion of Rhodamine B was the base peak in the DART-MS spectrum, while it was only 40% of the base peak under DSA-MS. Fig. 5 shows a comparison of the DART-MS and DSA-MS analysis of the Zebra black gel pen and a blue Paper Mate ballpoint pen. Figs. S4 and S5 show the linear dynamic range for both methods, using the Bic Atlantis blue ballpoint pen. The correlation coefficients are different, being

0.8802 for DSA-MS and 0.9329 for DART-MS, respectively. The linearity for DART-MS is only slightly better than for DSA-MS, but this could again be due to a positioning issue.

4. Conclusion

Both DART-MS and DSA-MS techniques were able to detect a variety of writing inks. Also, both detect similar colorants in writing inks, with similar sensitivities, and are repeatable. The open source DART-MS allowed for manual positioning of samples for more accurate positioning of small ink writing on very small pieces of paper; however, DSA-MS provided “cleaner” mass spectra with less background noise. Therefore, the choice between the two techniques depends on the sample size available for analysis, as well as limit of detection, ease of analysis, and specificity. Also, it was demonstrated that components of gel inks including their colorants and other components in the vehicle were readily analyzed by DART-MS and DSA-MS; however, the relative intensity of the colorant (Rhodamine B) was higher using DART-MS. In a questioned document examination, DART-MS or DSA-MS would require cutting a small portion of the document to be analyzed. Neither DART-MS nor DSA-MS visibly alter the ink, making future optical comparison possible. Additionally, as shown in Fig. 3, results of a questioned document examination using DART-MS and DSA-MS do not deviate significantly from one another especially when the 7 and the 5 mm pen strokes were used. This is despite the instruments belonging to two different manufacturers. Therefore, questioned document examiners are not hindered by variability in results in rendering their conclusions. To determine if two inks can or cannot be distinguished, their DSA-MS or DART-MS spectra must be compared. If the two inks do not contain the same colorants (as determined through the m/z values observed in the spectra), or if the same colorants are deemed present with large differences in relative intensities, then it can be concluded the two inks are not the same, meaning they are distinguishable. If the same colorants are observed, with similar relative intensities, one may conclude they could be the same (indistinguishable). To be more certain, it is necessary to statistically analyze multiple samples of the same ink to determine whether two inks are indistinguishable or not at a given level of confidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.forsciint.2018.05.009>.

References

1. Wang X, Yu J, Xie M, Yao Y, Han J. Identification and dating of the fountain pen ink entries on documents by ion-pairing high-performance liquid chromatography. *Forensic Sci Int.* 2008; 180(1): 43–49. [PubMed: 18703295]
2. Jones RW, McClelland JF. Analysis of writing inks on paper using direct analysis in real time mass spectrometry. *Forensic Sci Int.* 2013; 231(1–3):73–81. [PubMed: 23890618]
3. Neumann C, Ramotowski R, Genessay T. Forensic examination of ink by high-performance thin layer chromatography — the United States Secret Service Digital Ink Library. *J Chromatogr A.* 2010; 13:2793–2811.
4. Kalantzis N. Understanding the nature of document evidence. In: Bailey D, editor *Practical Veterinary Forensics* CABI; 2016 138
5. Djozan D, Baheri T, Karimian G, Shahidi M. Forensic discrimination of blue ballpoint pen inks based on thin layer chromatography and image analysis. *Forensic Sci Int.* 2008; 179(2):199–205. [PubMed: 18639403]
6. Nguyen L, Moini M. Direct sample analysis-mass spectrometry vs separation mass spectrometry techniques for the analysis of writing inks. *Forensic Chem.* 2016; 1:78–85.
7. Grechukha N, Gorshkova K, Panov M, Tumkin I, Kirillova E, Lukianov V, Kochemirovsky V. Analysis of the aging processes of writing ink: Raman spectroscopy versus gas chromatography aspects. *Appl Sci.* 2017; 7(10):991.
8. Jones RW, Cody RB, McClelland JF. Differentiating writing inks using direct analysis in real time mass spectrometry. *J Forensic Sci.* 2006; 51(4):915–918. [PubMed: 16882239]
9. Lesiak AD, Adams KJ, Domin MA, Henck C, Shepard JRE. DART-MS for rapid, preliminary screening of urine for DMAA. *Drug Test Anal.* 2014; 6:788–796. DOI: 10.1002/dta.1540 [PubMed: 24167149]
10. Lesiak AD, Musah RA, Cody RB, Domin MA, Dane AJ, Shepard JRE. Direct analysis in real time mass spectrometry (DART-MS) of “bath salt” cathinone drug mixtures. *Analyst.* 2013; 138:3424.doi: 10.1039/c3an00360d [PubMed: 23636110]
11. Habala L, Valentová J, Pechová I, Fuknová M, Devínský F. DART–LTQ ORBITRAP as an expedient tool for the identification of synthetic cannabinoids. *Leg Med.* 2016; 20:27–31. DOI: 10.1016/j.legalmed.2016.03.006
12. Forbes TP, Sisco E, Staymates M, Gillen G. DART-MS analysis of inorganic explosives using high temperature thermal desorption. *Anal Methods.* 2017; 9:4988–4996. DOI: 10.1039/C7AY00867H [PubMed: 29651308]
13. Cody R, Edwards D, Black O, Cizdziel J. Identification of polymers and organic gunshot residue in evidence from 3D-printed firearms using DART-mass spectrometry: a feasibility study. *Forensic Chem.* 2017; 5:26–32. <https://www.sciencedirect.com/science/article/pii/S2468170917300140>.
14. Ifa DR, Gumaelius LM, Eberlin LS, Manicke NE, Cooks RG. Forensic analysis of inks by imaging desorption electrospray ionization (DESI) mass spectrometry. *Analyst.* 2007; 132(5):461–467. [PubMed: 17471393]
15. Jian Xu Y, Xin Zhou X, Fan Shi X. HPLC and HPLC/MS analysis of red ink on counterfeit 100-yuan notes. *Forensic Sci Int.* 2016; 259:47–52. DOI: 10.1016/j.forsciint.2015.11.018 [PubMed: 26750988]
16. Weyermann C, Kirsch D, Vera CC, Spengler B. A GC/MS study of the drying of ballpoint pen ink on paper. *Forensic Sci Int.* 2007; 168:119–127. DOI: 10.1016/j.forsciint.2006.06.076 [PubMed: 16901668]
17. Siegel J, Allison J, Mohr D, Dunn J. The use of laser desorption/ionization mass spectrometry in the analysis of inks in questioned documents. *Talanta.* 2005; 67:425–429. DOI: 10.1016/j.talanta.2005.03.028 [PubMed: 18970184]
18. Moini M. APCI: principals, theory, and instrumentation. In: Gross M, editor *Encyclopedia of Mass Spectrometry* Elsevier; 2007
19. Huang M, Cheng S, Cho Y, Shiea J. Ambient ionization mass spectrometry: a tutorial. *Anal Chim Acta.* 2011; 702(1):1–15. [PubMed: 21819855]
20. Gernandt M, Urlaub J. An introduction to the gel pen. *J Forensic Sci.* 1996; 41(3):503–504.

Fig. 1.
In-house fabricated sampling train.

Fig. 2.

Ink components identified by DART-MS and DSA-MS. Ink components in the overlapping zones were base peaks for both techniques; ink components in individual zones were base peaks only for that specific technique and had lower relative peak intensity for the other technique.

Fig. 3.
Limit of detection of DSA-MS (left) and DART-MS (right) of blue ballpoint pen ink from Paper Mate stick blue ballpoint pen.

Fig. 4.
DART-MS analysis of black and blue non-ballpoint (NBP) and ballpoint (BP) pen inks.

Fig. 5.

Analysis of gel pen and ballpoint pen by DSA-MS and DART-MS. (A): DSA-MS spectrum of gel pen; (B): DART-MS spectrum of Zebra gel pen; (C): DSA-MS spectrum of blue ballpoint Paper Mate stick pen; (D): DART-MS spectrum of blue ballpoint Paper Mate stick pen.

Table 1

DART-MS instrument parameters.

Parameter	Value
Helium flow	10.00 L/min
Heater temperature	350 °C
Needle voltage	800 V
Ring lens voltage	7–10 V
Orifice 1 voltage	110 V–127 V
Orifice 2 voltage	10 V
Detector voltage	2700 V
Peaks voltage	900 V
Bias voltage	25 V
Pusher bias voltage	0.45 V
R/L voltage	10 V
Top/bottom voltage	1 V