

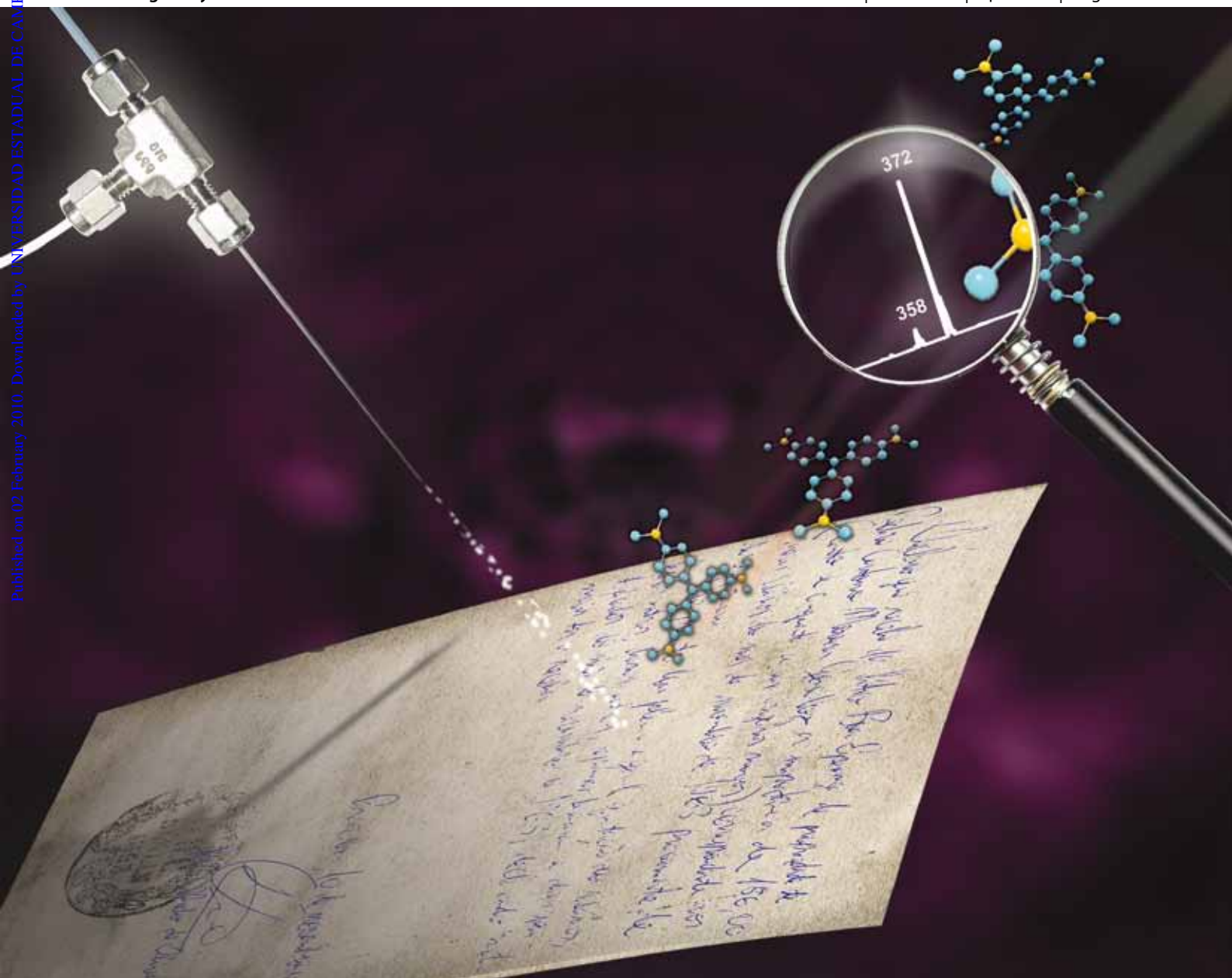
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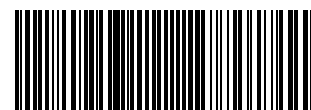
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CRITICAL REVIEW

Daniel J. Weston
Ambient ionization mass spectrometry: current understanding of mechanistic theory; analytical performance and application areas

PAPER

Marcos N. Eberlin *et al.*
Fingerprinting and aging of ink by easy ambient sonic-spray ionization mass spectrometry



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Fingerprinting and aging of ink by easy ambient sonic-spray ionization mass spectrometry†

Priscila M. Lalli,^a Gustavo B. Sanvido,^a Jerusa S. Garcia,^a Renato Haddad,^a Ricardo G. Cosso,^b Denison R. J. Maia,^b Jorge J. Zacca,^c Adriano O. Maldaner^c and Marcos N. Eberlin^{*a}

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Using easy ambient sonic-spray ionization mass spectrometry (EASI-MS), fast and non-destructive fingerprinting identification and aging of ballpoint pen ink writings have been performed directly from paper surfaces under ordinary ambient conditions. EASI-MS data obtained directly from the ink lines showed that pens from different brands provide typical ink chemical profiles. Accelerated ink aging has also been monitored by EASI-MS revealing contrasting degradation behaviors for six different common ink dyes. As demonstrated for Basic Violet 3, some dyes display a cascade of degradation products whose abundances increase linearly with time thus functioning as 'chemical clocks' for ink aging. Analysis of questionable documents has confirmed the ink aging capabilities of EASI-MS. The order of superimposition at a crossing point has also been determined by EASI-MS. For two superimposed ink lines, continuous EASI-MS analysis has also shown that the EASI spray is able to penetrate through the layers and therefore both ink layers could be characterized.

Introduction

Authenticity and dating of ink writings in questionable documents are major tasks in forensic investigations.^{1–7} Common forgery includes modifications of information, such as dates or values, and post-insertions to the original writings. This type of forgery can be identified by showing that the suspicious ink entry was done with a pen different from the first or by dating both the original and newest ink writings. Although microscopic analysis of ink line morphology is important, chemical analysis at the molecular level may provide indisputable data in regard to the type of pen and the age of the ink(s).

Several techniques have been used to analyze the chemical composition of ballpoint pen ink such as thin-layer chromatography (TLC),^{8,9} high performance liquid chromatography (HPLC),^{10,11} gas chromatography-mass spectrometry (GC-MS),^{3–6} laser desorption ionization mass spectrometry (LDI-MS)^{1,2,9} and matrix assisted laser desorption ionization (MALDI).^{1,2} These techniques suffer, however, from the inconvenience of destroying the piece of the document used for dye extraction. Recently, a new family of ionization techniques for mass spectrometric analysis has been introduced and these techniques are now known generally as ambient mass spectrometry.^{12,13} These techniques have opened a new area of chemical analysis since MS can now be performed under ordinary ambient conditions and for analytes in their native environment or placed on auxiliary holding surfaces. Ambient MS is

performed often without any sample preparation thus eliminating the need for removing a piece of the document for analyte extraction. This feature makes such techniques very attractive for forensic ink analysis. Indeed, imaging desorption electrospray ionization (DESI) has been recently tested for the non-destructive forensic analysis of inks directly from paper surfaces in some proof-of-principle experiments.¹⁴ DESI parameters were optimized for ink analysis and simulated forged writings and old written records were investigated with very promising results.

Easy ambient sonic-spray ionization (EASI)^{15,16} is one of the simplest, gentlest and most easily implemented ionization techniques for ambient mass spectrometry. An EASI source can be constructed and installed in a few minutes from a few simple MS laboratory parts (Fig. 1) and analyte pick-up from the surface

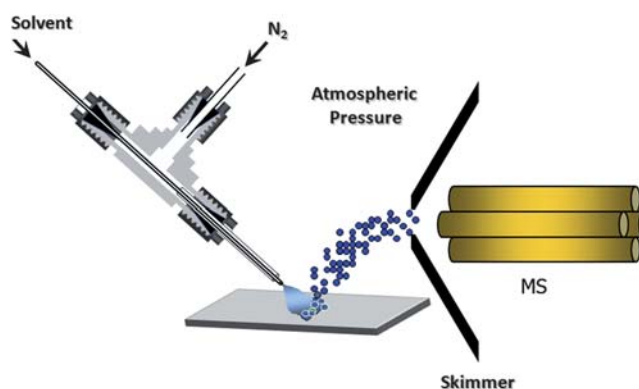


Fig. 1 Schematic of the EASI-MS system used to obtain, via a non-destructive procedure, instant chemical profiles of dyes from ink writing on documents. The mounting of an EASI source requires only a Swagelok T-element, ferrules and gas tubing, and a fused-silica capillary. For ink analysis, the EASI spray was produced using methanol as solvent and compressed nitrogen gas.

^aThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas - UNICAMP, 13084-970 Campinas, SP, Brazil

^bBrazilian Federal Police, Ministry of Justice, São Paulo Division, 05038-090 São Paulo, SP, Brazil

^cBrazilian Federal Police, Ministry of Justice, National Criminalistics Institute - INC, 70390-145 Brasília, DF, Brazil

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(desorption) plus the ionization process are assisted only by compressed nitrogen gas. EASI is based on supersonic-spray ionization (SSI),¹⁷ which creates a stream of very minute solvent droplets, both positively and negatively charged, due to the statistically unbalanced distribution of cations and anions in such droplets. Fig. 1 shows a schematic representation of the operation and the desorption and ionization steps for EASI-MS. This technique has already been successfully applied to different analytes in different matrices such as drug tablets,¹⁶ perfumes,¹⁸ surfactants,¹⁹ and biodiesel,²⁰ and has also been coupled to membrane introduction mass spectrometry¹⁵ and thin-layer chromatography.^{21,22}

In this work, we have further investigated the use of ambient mass spectrometry for forensic ink analysis. More specifically, we have studied the ability of EASI-MS to provide instant and non-destructive ink fingerprinting and aging information directly from the surface of hand-written documents. Two important tasks of ink writing analysis – line crossing and superimposition – have also been successfully performed.

Experimental

Reagents and samples

HPLC-grade methanol and formic acid were purchased from Burdick & Jackson (Muskegon, MI, USA). The blue and red

ballpoint pens were purchased locally in Brazil. Real cases of questionable documents dated from different years were provided by the Brazilian Federal Police.

General experimental procedures

Experiments were performed on a single quadrupole mass spectrometer (LCMS-2010EV – Shimadzu Corp., Japan) equipped with a home-made EASI source described in detail elsewhere.¹⁶ A flow rate of methanol at 20 $\mu\text{L min}^{-1}$ and compressed N_2 at a pressure of 100 psi were used to form the EASI spray. The capillary-paper and surface-entrance angles were *ca.* 30°.

Ink lines were made on ordinary print paper (Chamex high white sheets from International Paper Co.) and analyzed by EASI-MS directly from the paper surface without any sample preparation or extraction. For the accelerated aging study, ink samples were exposed to a 60 W incandescent lamp for variable periods of time.

Results

Ink fingerprinting

Ballpoint pens usually contain a combination of several common dyes that vary from brand to brand.¹⁻⁷ Five dyes frequently used

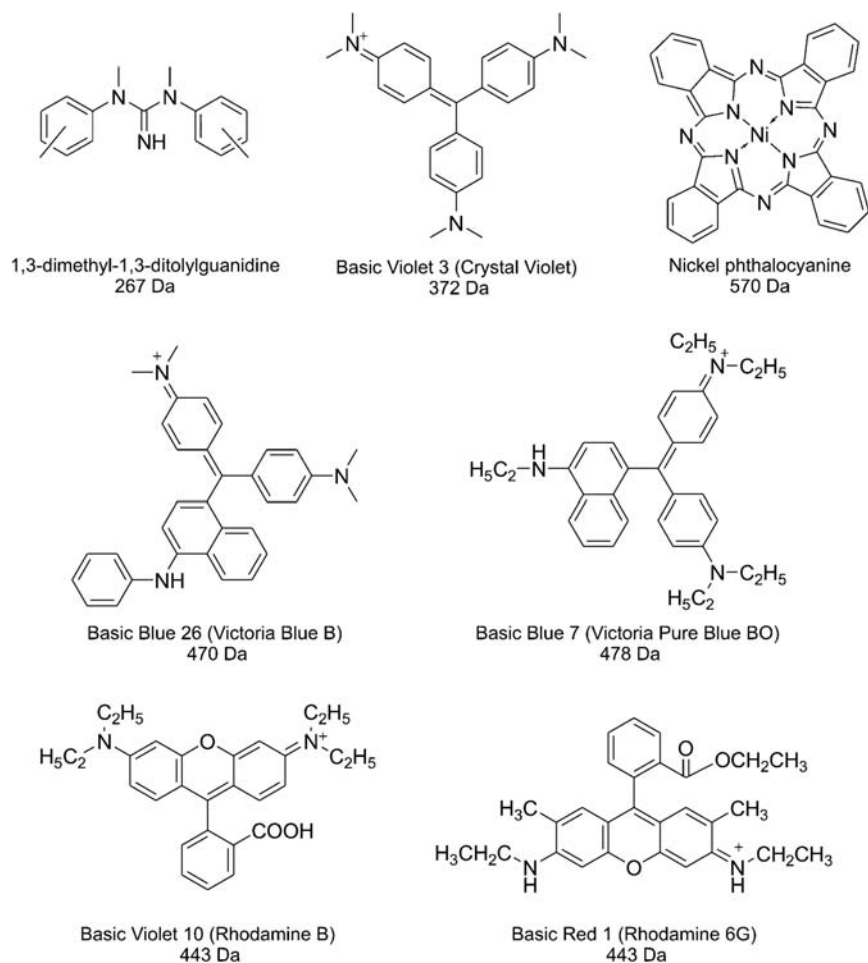


Fig. 2 Structures of the dyes detected by EASI-MS in the ink samples analyzed.

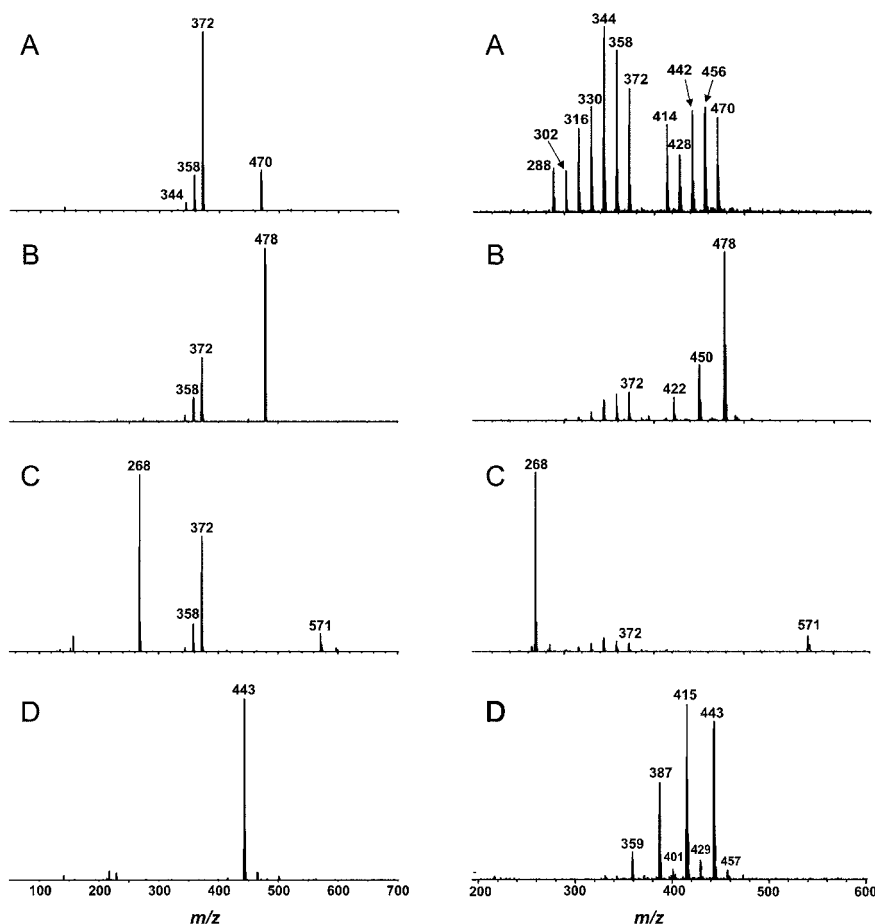


Fig. 3 EASI(+)-MS fingerprints of fresh ink writings from blue (A–C) and red (D) pens (left) and after accelerated aging performed with a 60 W incandescent light for 72 h (right).

in blue ballpoint pen ink composition were detected in the samples analyzed (Fig. 2): Basic Violet 3 (m/z 372), Basic Blue 26 (m/z 470), Basic Blue 7 (m/z 478), nickel phthalocyanine (m/z 571) and 1,3-dimethyl-1,3-ditolyguanidine (m/z 268). In red pens, ions of m/z 443 were detected, which may correspond to either Basic Violet 10 or Basic Red 1, or both. Basic Violet 3 is the most common dye used in blue pens (low cost and intense blue color) and was detected in all of the blue samples analyzed.

Fig. 3 (left) presents EASI(+)-MS fingerprints for three blue pens and one red pen, which characterize the fingerprints obtained from various pens. Acidic methanol was used as the solvent due to its improved ability to selectively desorb ink dyes from paper surfaces.¹⁴ Note that EASI-MS reveals characteristic chemical profiles for each ink owing to varying dye composition. These fingerprints may reveal that different pens have been used to produce the writings in a questionable document, particularly when comparing the original writing with suspicious writing.

Ink aging

Degradation profiles of all of the dyes were studied by accelerated aging. Initially, the ink writings on the paper surface were exposed to light for 72 h. Fig. 3 (right) shows EASI-MS of the degraded inks. Note that Basic Violet 3 (m/z 372) and Basic Blue 26 (m/z 470) form a serial cascade of very typical degradation

products separated by 14 m/z units: m/z 372, 358, 344, 330, 316, 302 and 288 for Basic Violet 3 and m/z 470, 456, 442, 428, 414 for Basic Blue 26 owing to replacement of methyl groups by hydrogen.

Basic Blue 7 (m/z 478) also displays a unique cascade of ‘chemical clocks’ and structurally diagnostic ions separated by 28 m/z units: m/z 478, 450 and 422 (Fig. 3B) owing to degradation by successive replacements of ethyl groups by hydrogen. The EASI-MS fingerprints of Fig. 3C are interesting since they show no detectable degradation products for two dyes: 1,3-dimethyl-1,3-ditolyguanidine (m/z 268) and nickel phthalocyanine (m/z 571). This is an important finding for aging studies since these dyes seem to be unaltered during ink aging, so their presence among other degradable dyes may serve as internal standards for ink aging.

EASI-MS for the red ink (Fig. 3D) also shows the typical cascade of degradation product ions: m/z 443, 415, 387 and 359 (as well as a likely minor homologous series: m/z 457, 429, 401) from ethyl/H replacements from one of the isomeric dyes Basic Violet 10 or Basic Red 1, or from both.

The most common blue ink dye, Basic Violet 3 (m/z 372), was then chosen to be monitored during accelerated ink aging. Ink writings from a single pen applied on paper were exposed to the incandescent light for variable periods of time. Fig. 4 shows the EASI-MS fingerprints for the fresh ink and that after 19 h of light

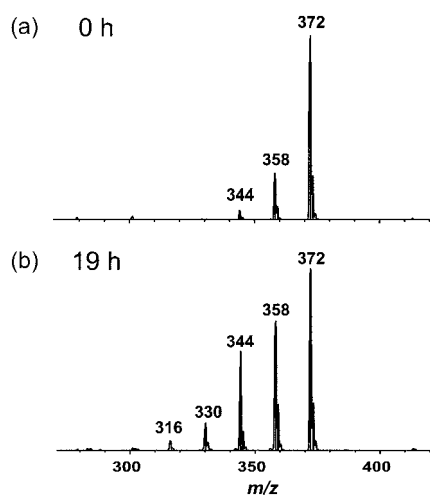


Fig. 4 EASI(+)-MS fingerprints obtained for (a) a fresh blue ink writing and (b) after accelerated aging for 19 h due to exposure to an incandescent 60 W light.

exposure. For the fresh ink, the ions of m/z 358 and 344 may already indicate some degradation but they may also represent homologous impurities common in commercial preparations of azo dyes. After 19 h of exposure to the incandescent light, the typical cascade of 'clock ions' of m/z 372, 358, 344, 330 and 316 is observed at much increased abundances.

To test whether linear dye decay occurs, the ratios between the absolute intensity of the ion of m/z 372 to that of the sum of intensities of the degradation product ions were plotted *versus* the time of light exposure (Fig. 5). A rather straight line with $r^2 = 0.992$ was obtained, indicating a good linear relationship. Indeed, such products seem to work as reliable 'chemical clocks' for ink aging as long as conditions remain somewhat undisturbed or the same page of a document is considered.

The degradation products could work as 'chemical clocks' for ink aging if inks do not degrade considerably inside the pen cartridge. If they do degrade, however, the clock would be severely disturbed by an unknown 'dead time' for old pens. To

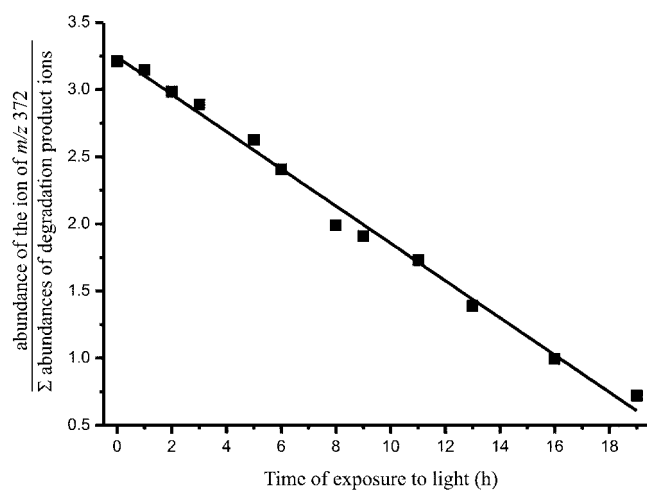


Fig. 5 Plot of the time of exposure to the incandescent 60 W light *versus* the ratio of intensity of the precursor Basic Violet 3 ion (m/z 372) to the sum of intensities of its degradation products (m/z 358, 344, 330, 316).

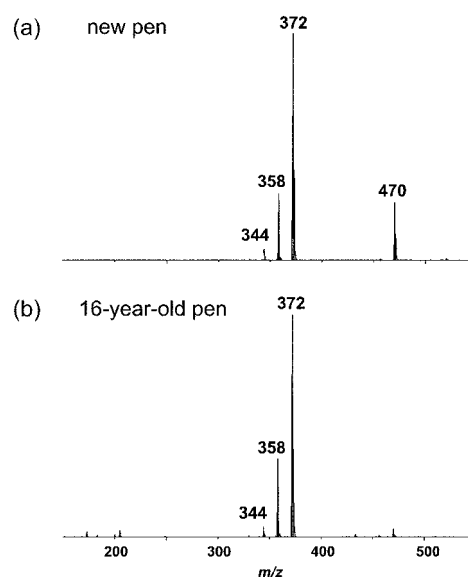


Fig. 6 EASI(+)-MS fingerprints of ink writing made using (a) a brand new ballpoint pen and (b) a 16-year-old pen. The percentage of the sum of degradation products relative to the precursor ion of m/z 372 was 34% for the new and 37% for the 16-year-old pen.

evaluate this parameter, several blue ballpoint pens with varying ages up to 16 years old were tested. Fig. 6 compares, as an illustrative example, the EASI-MS fingerprints of an ink writing from a brand new blue pen (2009) with that from a 16-year-old pen (as marked, the pen was made for a 1993 scientific congress). Note that the dye compositions are close but not identical, but both fingerprints display nearly the same 'degradation' profiles. No degradation products are observed for Basic Blue 26 (m/z 470) even after 16 years (Fig. 6b) whereas the ions of m/z 358 and 344 are likely homologous impurities rather than degradation products.

Several parameters are known to influence ink degradation such as document storage conditions (*e.g.* humidity, temperature and exposure to light) and the type of paper used. Absolute age estimates are therefore difficult to make if storage conditions are known to have changed substantially but relative ink aging appears quite reliable based on the 'chemical clocks' from degradation. Ink writing on a document should present equal degradation profiles if they were produced by the same pen at the same time. If not, relative ink dating should demonstrate the temporal difference.

Inks in documents

Naturally aged inks were also analyzed using legal documents with different ages provided by the Brazilian Federal Police (Fig. 7). Note that different dye compositions are detected by EASI-MS due to the use of different pens, but the most common Basic Violet 3 dye and its 'chemical clock' cascade of products (m/z 372, 358, 344, 330, 316) are fortunately observed in the inks for all documents. This was an uncontrolled experiment since documents were not necessarily stored under the exact same conditions, yet the older the document more degradation product ions were indeed detected.

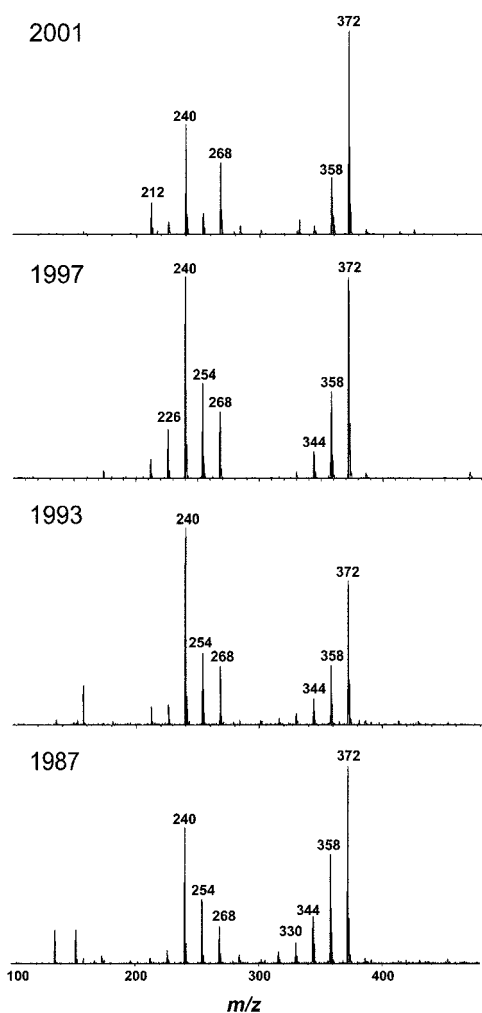


Fig. 7 EASI(+)-MS fingerprints for legal documents from different years provided by the Brazilian Federal Police. The percentage of the sum of degradation products relative to the precursor ion of m/z 372 was 32% for 2001, 59% for 1997, 72% for 1993 and 96% for 1987.

Fig. 8 shows the identification by EASI-MS of ink writing forgery. Lines were written using the same blue pen, but at different ‘simulated’ years. Using a similar test applied before for different pens,¹⁴ the number ‘3’ was hand-written on a piece of paper and the ink was subjected to accelerated aging for 6 h by exposure to the 60 W incandescent light. We estimate from Fig. 7 that 6 h of accelerated aging simulates *ca.* 10 years of natural aging. Then, number 3 was forged into number ‘8’ with fresh ink. The EASI spray was then scanned across the entire contour of the ‘8’. Fig. 8 shows characteristic EASI-MS for both fresh and ‘10 year-old’ ink writings.

Superimposition and crossing of ink lines

Fraudulent modifications of information on documents may be made by the superimposition of ink lines. To verify the capability of EASI-MS to detect the presence of more than one layer of ink in a single line, a line was written with a blue pen that contains mainly Basic Violet 3 (m/z 372) and 1,3-dimethyl-1,3-ditolyguanidine (m/z 268). This line was then overlaid by a second

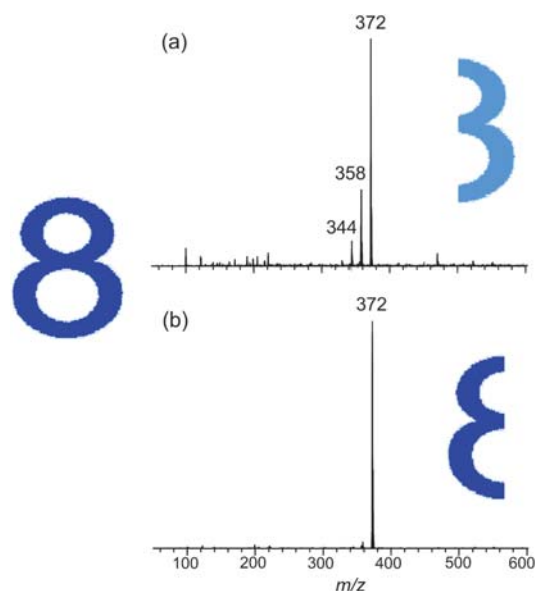


Fig. 8 Typical EASI(+)-MS fingerprints acquired by scanning across the ‘8’ line for the (a) accelerated ‘*ca.* 10 year-old’ aged ink and (b) the fresh blue ink.

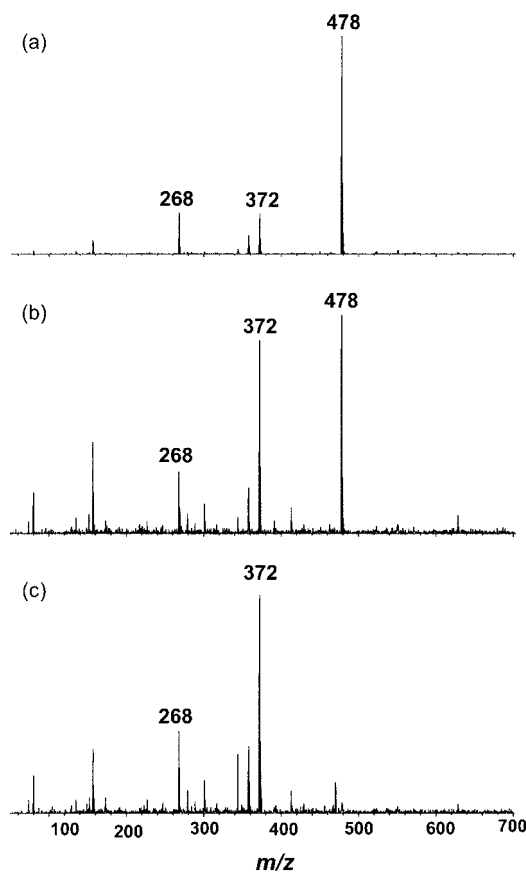


Fig. 9 EASI(+)-MS fingerprints of a double layer of superimposed blue inks obtained when the spray collected dyes from (a) the upper layer at time zero; (b) from both layers after *ca.* 5 s and (c) from the lower layer after *ca.* 10 s.

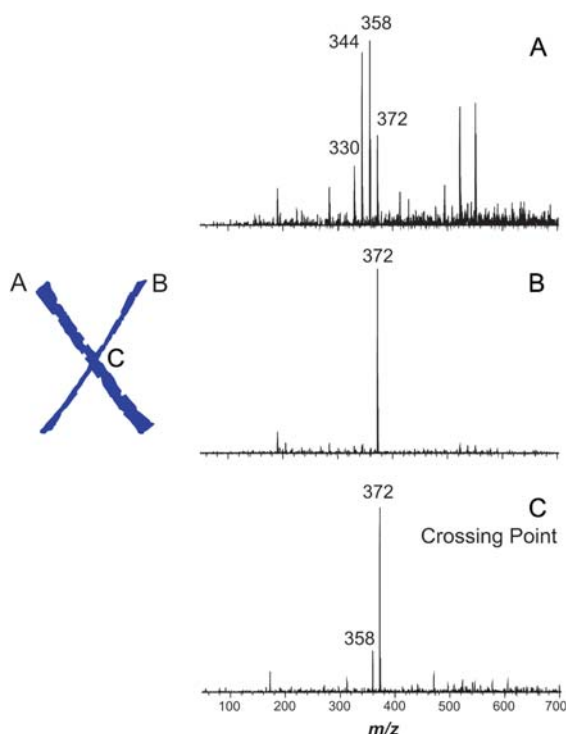


Fig. 10 EASI(+)-MS fingerprints for intersecting blue ink lines (A and B) and at the 'X' crossing point (C).

ink line written with a pen that contains mainly Basic Blue 7 (m/z 478). The resulting double line of blue ink was then analyzed by EASI-MS (Fig. 9) at a fixed point for a few seconds. Initially, Basic Blue 7 (m/z 478) dominates (Fig. 9a) but as time went by the EASI spray continuously washed the surface and the dye profile had changed already after 5 s (Fig. 9b). After nearly 10 s of spraying, a profile characteristic of the lower ink layer was detected (Fig. 9c). Therefore, the EASI spray was able to penetrate deeply enough through the double ink line so as to clearly reveal the use of two superimposed layers of ink.

Crossing sequence of lines

Microscopy is normally applied to determine the crossing sequence of lines in questionable documents but, for instance, when two similar pens are used, this approach may fail due to physical and chemical mixing at the crossing point.²³ To evaluate the performance of EASI-MS in this challenging forensic investigation, an 'X' was hand-written with intercepting lines from different types of blue pens. EASI-MS was collected at line A (Fig. 10A), line B (Fig. 10B) and then at exactly the crossing point (Fig. 10C). Clearly, line B is determined as being on top.

Conclusions

As further demonstrated in this study, ambient mass spectrometry can be applied to many areas of ink chemical analysis. Using EASI-MS performed directly on paper surfaces, we have been able to collect fingerprint spectra with characteristic dye profiles for different inks. Applications to ink typification, forgery, aging and superposition and crossing of ink lines have been

demonstrated. A linear correlation for accelerated aging and degradation products for some of the most typical ink dyes has been obtained, demonstrating that this cascade of products works as a reliable 'chemical clock' for ink aging, particularly when relative ages are compared.

Several procedures to prevent forgery when inks are used to prepare important documents considering further inspection by EASI-MS can also be proposed, such as the use of a unique (site-specific) composition of dyes, for instance, for use in legal writings as, for instance, in passport annotations by pens or stamps. This fast and non-destructive method that provides ink characterization at the molecular level directly at the surface of questionable documents seems therefore to offer a powerful tool for the forensic analysis of inks.

Acknowledgements

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References

- 1 J. Siegel, J. Allison, D. Mohr and J. Dun, *Talanta*, 2005, **67**, 425–429.
- 2 C. Weyermann, D. Kirsch, C. Costa-Vera and B. Spengler, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 297–306.
- 3 J. H. Bügler, H. Buchner and A. Dallmayer, *J. Forensic Sci.*, 2008, **53**, 982–988.
- 4 C. Berger-Karin, U. Hendriks and J. Geyer-Lippmann, *J. Forensic Sci.*, 2008, **53**, 989–992.
- 5 S. Locicero, L. Dujourdy, E. Lock, W. Mazzella and P. Margot, *Sci. Justice*, 2004, **44**, 65–171.
- 6 C. Weyermann, D. Kirsch, C. Costa-Vera and B. Spengler, *Forensic Sci. Int.*, 2007, **168**, 119–127.
- 7 R. L. Brunelle, C. H. Breedlove and C. R. Midkiff, *J. Forensic Sci.*, 1987, **32**(6), 1511–1521.
- 8 D. Djozan, T. Baheri, G. Karimian and M. Shahidi, *Forensic Sci. Int.*, 2008, **179**, 199–205.
- 9 C. Weyermann, R. Marquis, W. Mazzella and B. Spengler, *J. Forensic Sci.*, 2007, **52**, 216–220.
- 10 A. Kher, M. Mulholland, E. Green and B. Reedy, *Vib. Spectrosc.*, 2006, **40**, 270–277.
- 11 V. F. Samanidou, K. I. Nikolaidou and I. N. Papadoyannis, *J. Liq. Chromatogr. Relat. Technol.*, 2005, **27**, 215–235.
- 12 A. Venter, M. Nefliu and R. G. Cooks, *TrAC, Trends Anal. Chem.*, 2008, **27**, 284–290.
- 13 G. A. Harris, L. Nyadong and F. M. Fernandez, *Analyst*, 2008, **133**, 1297–1301.
- 14 D. R. Ifa, L. M. Gumaelius, L. S. Eberlin, N. E. Manicke and R. G. Cooks, *Analyst*, 2007, **132**, 461–467.
- 15 R. Haddad, R. Sparrapan, T. Kotiaho and M. N. Eberlin, *Anal. Chem.*, 2008, **80**, 898–903.
- 16 R. Haddad, R. Sparrapan and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2901–2905.
- 17 A. Hirabayashi, M. Sakairi and H. Koizumi, *Anal. Chem.*, 1995, **67**, 2878–2882.
- 18 R. Haddad, R. R. Catharino, L. A. Marques and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 3662–3666.
- 19 S. S. Saraiva, P. V. Abdelnur, R. R. Catharino, G. Nunes and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2009, **23**, 357–362.
- 20 P. V. Abdelnur, L. S. Eberlin, G. F. Sá, V. Souza and M. N. Eberlin, *Anal. Chem.*, 2008, **80**, 7882–7886.
- 21 R. Haddad, M. S. Milagre, R. R. Catharino and M. N. Eberlin, *Anal. Chem.*, 2008, **80**, 2744–2750.
- 22 L. S. Eberlin, P. V. Abdelnur, A. Passero, G. F. de Sa, R. J. Daroda, V. de Souza and M. N. Eberlin, *Analyst*, 2009, **134**, 1652–1657.
- 23 J. Stitt, M. Phillips, J. Brandi and C. Roux, *Forensic Sci. Int.*, 2003, **136**, 81–81.