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RESEARCH ARTICLE

Paper Spray Mass Spectrometry for the Forensic Analysis of Black Ballpoint Pen Inks

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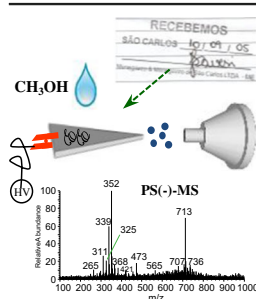
Abstract. This article describes the use of paper spray mass spectrometry (PS-MS) for the direct analysis of black ink writings made with ballpoint pens. The novel approach was developed in a forensic context by first performing the classification of commercially available ballpoint pens according to their brands. Six of the most commonly worldwide utilized brands (Bic, Paper Mate, Faber Castell, Pentel, Compactor, and Pilot) were differentiated according to their characteristic chemical patterns obtained by PS-MS. MS on the negative ion mode at a mass range of m/z 100–1000 allowed prompt discrimination just by visual inspection. On the other hand, the concept of relative ion intensity (RII) and the analysis at other mass ranges were necessary for the differentiation using the positive ion mode. PS-MS combined with partial least squares (PLS) was utilized to monitor changes on the ink chemical composition after light exposure (artificial aging studies). The PLS model was optimized by variable selection, which allowed the identification of the most influencing ions on the degradation process. The feasibility of the method on forensic investigations was also demonstrated in three different applications: (1) analysis of overlapped fresh ink lines, (2) analysis of old inks from archived documents, and (3) detection of alterations (simulated forgeries) performed on archived documents.

Keywords: Questioned documents, Paper spray mass spectrometry, Ambient mass spectrometry, Black ballpoint pen inks, Forensic analysis, Chemometrics

Introduction

Demands for the investigation of questioned documents are enormous in forensic laboratories. Particularly, the deliberate modification of hand-written ink entries in wills, contracts, certifications, invoices, and deeds is one of the main types of frauds in the forgery of documents. Examining the optical properties of the inks using microscopy techniques is sometimes sufficient to discriminate the entries on a paper [1]. However, the chemical analysis at the molecular level may provide undeniable evidence of the ink origin and age. Thus, the field of chemical analysis of inks in questioned documents is constantly advancing by increase in the development of new, fast, reliable, and non- or semidestructive methodologies [1–5].

Several methods based on ambient ionization mass spectrometry (ambient MS) have been proposed for the analysis of blue inks in ballpoint and marker pens [5–10]. This includes easy ambient sonic-spray ionization (EASI) [5], desorption electrospray ionization (DESI) [6], direct analysis in real time (DART) [7], electrospray-assisted laser desorption/ionization (ELDI) [8], desorption atmospheric pressure chemical ionization (DAPCI) [9], paper spray ionization (PS) [10], and direct analyte-probed nanoextraction (DAPNe) coupled to matrix-assisted laser desorption ionization (MALDI) [11]. However, only three ambient MS techniques, namely DART [7, 12], ELDI [13], and DAPNe-MALDI [11], have been explored in the study of black pen inks, and only one of these studies [12] has discriminated black pen inks of different brands. This lower number of reports on black ink analysis has also been observed for other analytical techniques, such as infrared spectroscopy, Raman, and chromatography. This is certainly due to the lower

variability found in the black ink composition used in different brands, which makes their differentiation a more difficult task from the analytical point of view.

Parallel to ambient MS techniques, Raman [14–16], near infrared hyperspectral imaging [4], and visible reflectance spectroscopy [17] have also been used in semi- or nondestructive chemical methods for forensic studies of black pens. Minimal sample destruction is highly desirable in forensic studies in which the preservation of proofs is very important. Furthermore, ambient MS, Raman, and infrared spectroscopy do not require any prior steps of sample preparation/extraction that are usually laborious and time-consuming. In particular, the high degree of selectivity of mass spectrometry, which usually allows structural identification, makes this technique very appealing for classification and differentiation analysis in forensic studies. Examples of other techniques already used for analyzing black pen inks, and that demand sample extraction, include direct infusion electrospray mass spectrometry (ESI-MS) [18], UV-vis spectrometry [19], high-performance liquid chromatography [20], thin layer chromatography [21], capillary electrophoresis [22], and gas chromatography [23].

The development of tools and analytical methodologies using paper spray mass spectrometry (PS-MS) is growing fast since its introduction by Cooks' group in 2010 [24]. Together with the typical appealing features of ambient ionization techniques, such as the possibility to perform rapid qualitative analysis with minimum or no sample preparation, PS-MS on chromatographic paper allows quantitative chemical analysis and requires a simpler instrumentation setup, being more compatible with miniature mass spectrometers [24, 25]. PS-MS has been used for a diverse array of analytical applications, which vary from the determination of therapeutic drugs [26, 27] and proteins [28] in whole blood to the analysis of food additives and contaminants [29, 30], discrimination of bacteria [31], and chemical reaction monitoring [32, 33]. In forensic applications, PS-MS has been used for the analysis of documents, specifically in the detection of forgeries using blue ballpoint pen inks [10] and erasable pens [34], for the discrimination and detection of frauds in beers [35], coffees [36], teas [37], essential oils [38], and for the analysis of drugs of abuse [39–43].

Driven by the appealing features of the PS-MS technology and its successful application on the forensic discrimination of blue ballpoint pen inks recently proved by our group [10], we report here a study on applying PS-MS to the rapid analysis of black ballpoint pen inks. For this, we first evaluated the capability of the technique to differentiate black inks according to their brands and to monitor changes on their composition after light exposure (assessment of fading patterns). This monitoring was accomplished by multivariate calibration, generating a partial least squares (PLS) [44] model to predict the black pen ink degradation. Then, this model was optimized by variable selection in order to detect the most important ions related to this degradation process. In addition, the method was employed in the analysis of naturally aged documents from our personal archives. Finally, simulated counterfeit documents obtained by overlapping ink lines on original entries were successfully characterized by the developed method.

Materials and Methods

Samples

All the chemicals used were of analytical reagent grade quality and were employed as received. HPLC grade methanol and acetonitrile were purchased from J.T. Baker Chemicals (Center Valley, PA, USA). Ultrapure water purified with a Milli-Q system (Millipore, Milford, MA, USA) was used in the studies. A total of 36 black ballpoint pens from six different brands utilized worldwide (Bic, Paper Mate, Faber Castell, Pentel, Compactor, and Pilot) were purchased at local stores. For each brand, six pens were purchased from different batches. The analyses were essentially conducted using ordinary A4 paper (Chamex, Memphis, TN, USA). For comparison purposes, we conducted some experiments with fresh inks in other papers, such as ordinary A4 brand One (Suzano, São Paulo, SP, Brazil), brown envelope paper (Foroni, São Paulo, SP, Brazil), cardboard paper (Horlle, Campo Largo, PR, Brazil), white letterhead envelope purchased at a local post office, and yellow Post-It Note (3M, São Paulo, Brazil). In all analysis, all papers were cut into triangles (0.5 cm of base and 1.0 cm of height) using conventional steel scissors.

Instrumentation

A Thermo LCQ Fleet mass spectrometer (Thermo Scientific, San Jose, CA, USA) was utilized for all PS-MS experiments. The analyses were performed on positive and negative ionization modes. For the positive ion mode, the optimized instrumental conditions were as follows: paper spray voltage, 4.0 kV; capillary temperature, 275 °C; capillary voltage, 55 V; tube lens voltage, 25V. For the negative ion mode, the optimized instrumental conditions were as follows: paper spray voltage, –3.5 kV; capillary temperature, 275 °C; capillary voltage, –3 V; tube lens voltage, –90 V.

Paper Spray

Aiming to perform the experiments involving the differentiation and artificial aging studies, the ballpoint pens were used to draw straight lines on the paper triangles. The triangles were positioned 5 mm away from the MS inlet using a copper clip fixed on a three-dimensional moving stage. An optimized high voltage was applied to the paper through the copper clip. Subsequently, 15 μL of the spray solvent (methanol) was added to the paper triangle to generate the MS.

The accelerated aging studies were carried out by exposing the fresh inks to a 32 W fluorescent lamp for 48, 96, 144, and 192 h, under controlled conditions. For the analysis of archived documents and documents submitted to simulated forgeries, the portions of paper containing ink lines were directly cut into triangles using steel scissors.

Multivariate Analysis

Multivariate calibration was used to monitor the variations in ink components as a function of the time of light exposure. PLS was performed using the software MATLAB 7.9.0.529 (MathWorks, Natick, MA, USA) supported by the PLS Toolbox 5.2.2 (Eigenvectors Research Inc., Manson, WA, USA). MATLAB routines for the Kennard Stone algorithm, written by M. Daszykowski (University of Selesia, Poland), and OPS (ordered predictors selection), written by R. F. Teófilo and M. M. C. Ferreira (University of Campinas, Brazil), were also used to select the most representative calibration samples and to perform variable selection, respectively.

The six pens of Paper Mate brand were exposed to light at fixed time intervals (48, 96, 144, and 192 h). Mass spectra obtained for this degradation process were organized in a matrix X and their respective time of exposure to light in a vector $\mathbf{y}$. The model was built by correlating the matrix of independent variables ($\mathbf{X}$) with the vector of dependent variables ($\mathbf{y}$). From the total of 24 samples (six pens at four time intervals), 16 were selected for the calibration and eight for the validation sets by using the Kennard-Stone algorithm [45] at each level of time.

Ordered predictors selection (OPS) [46] was used for variable selection in this study. OPS is a recently proposed method that screens the most predictive variables using a cross-validation procedure and a window search starting from the informative vectors of a PLS model, such as regression coefficients, variable importance in projection (VIP) scores, residuals, and/or signal-to-noise. The purpose of variable selection methods in multivariate calibration is to significantly reduce the number of spectral variables, producing simpler models, more robust and accurate than full spectra models. The variables that better describe the samples are selected and irrelevant information, such as noise, are excluded. Considering the high number of variables measured in a mass spectrum, the use of this tool is even more appropriate. In addition, variable selection applied to discrete spectra, such as in MS, allows detecting the ions/variables that contribute most to the monitored process. In the present study, the monitoring and characterization of the black ink degradation process was performed and, after applying PLS and OPS, the results were refined by selecting the ions with the highest variable importance in projection (VIP) scores [47].

Results and Discussion

Characterization and Differentiation of Brands of Black Ballpoint Pen Inks

First studies were carried out for evaluating the performance of PS-MS for the differentiation of fresh black inks from ballpoint pens of different commercially available brands. The operating conditions for both positive and negative ion modes were optimized using univariate approaches, aiming at generating MS signals with adequate stability and intensity. The studied and selected PS-MS parameters are summarized in Table 1.

Figure 1 displays the positive and negative ion PS mass spectra obtained for typical samples of pens from the brands Bic, Paper Mate, Faber Castel, Pentel, Compactor, and Pilot (a–f), at the 100–1000 m/z range. As can be observed, for the PS(+)-MS, the ion of m/z 372, which corresponds to the purple dye Basic Violet 3 (BV3), is the most abundant in all the studied pens. This dye usually forms demethylated homologues separated by 14 m/z units as the result of a characteristic serial degradation with the replacement of methyl groups by hydrogen [5, 6]. Thus, as can be seen, the ion of m/z 358 was also detected in all the pens, and the ion of m/z 344 was observed only for the pen brands e and f. It clearly appears that the visual analysis of PS(+) mass spectra just allow the distinction of the pens e and f from the pens a, b, c, and d. Indeed, only Basic Violet 3 (m/z 372) and its demethylated homologues were detected in pens a–d while working in the mass range of m/z 100–1000. Ions of m/z 685 and 949 present in pen e, and ions of m/z 230, 274, 320, 358, and 429 present in pen f allow prompt distinction of these brands. Most of these ions could not be attributed by literature comparison or MS/MS experiments. The ion of m/z 429 is ascribed to the dye Basic Red 1:1, which was already observed in previous MS studies of black inks from ballpoint pens [18]. Together with the visual inspection, the concept of relative ion intensity (RII, proportion of a given ion in regard to correlated compounds) have been used for discriminating fresh pen inks [10, 48]. The RII determined for the extension of demethylation of the amino groups from BV3 [$RII_{372} = I_{372}/(I_{372} + I_{358} + I_{344})$] provided values of 0.97 ± 0.01, 0.92 ± 0.01, 0.90 ± 0.02, and 0.88 ± 0.02 ($n = 6$), for pens a, b, c, and d, respectively, which allowed discrimination of pen a from b, b, and d.

Table 1. Summary of the PS-MS Parameters Studied and Optimized for Both Positive and Negative Ion Modes

Parameter	Studied	Selected for PS(+)	Selected for PS(−)
Spray solvent	Acetonitrile, methanol and methanol/water (9:1 v/v)	Methanol	Methanol
Spray voltage	3.0, 3.5, 4.0, 4.5, 5.0 kV	4.0 kV	3.5 kV
Spray solvent volume	5, 10, 15, 20, 25 μL	15 μL	15 μL
Distance between paper tip and MS inlet	5, 10, 15, 20 mm	5 mm	5 mm
Capillary temperature	250, 275, 300, 325 °C	275 °C	275 °C
Capillary voltage	Set by instrument tuning	55 V	−3 V
Lens voltage	Set by instrument tuning	25 V	−90 V

PS(+): Positive ion mode, PS(−): Negative ion mode.
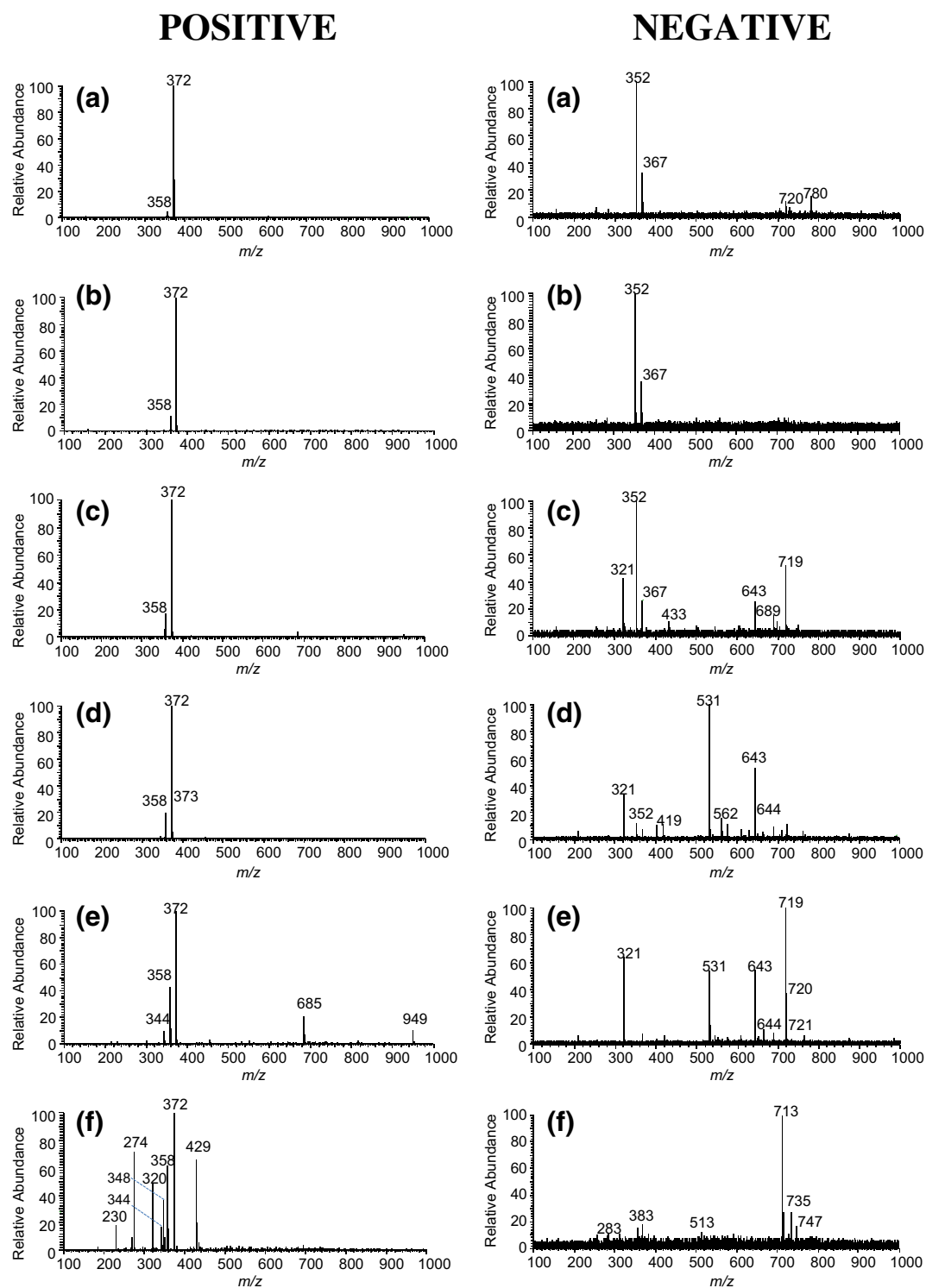
POSITIVE   **NEGATIVE**

Figure 1. Representative PS(+)-MS (left) and PS(–)-MS (right) obtained for the black ballpoint pens studied in this work, at the 100–1000 *m/z* range. (**a**) Bic, (**b**) Paper Mate, (**c**) Faber Castel, (**d**) Pentel, (**e**) Compactor, and (**f**) Pilot

Owing to the poor discrimination provided by the information arising from the PS(+)-MS at the 100–1000 *m/z* range, we restricted the mass region to *m/z* 100–320 to cover small molecules (not only dyes, but also ink additives and solvents), useful for differentiation, thus eliminating the influence of the intense ion of *m/z* 372. The MS was acquired separated with

different instrumental conditions (i.e., using a different instrument tune adjusted for the mass range of *m/z* 100–300, but keeping the same parameters for the PS ionization). Typical spectra for each brand are shown in Figure 2. As can be observed, a visual inspection is now enough for distinguishing the different brands. The ions with *m/z* 157 (present in pens a
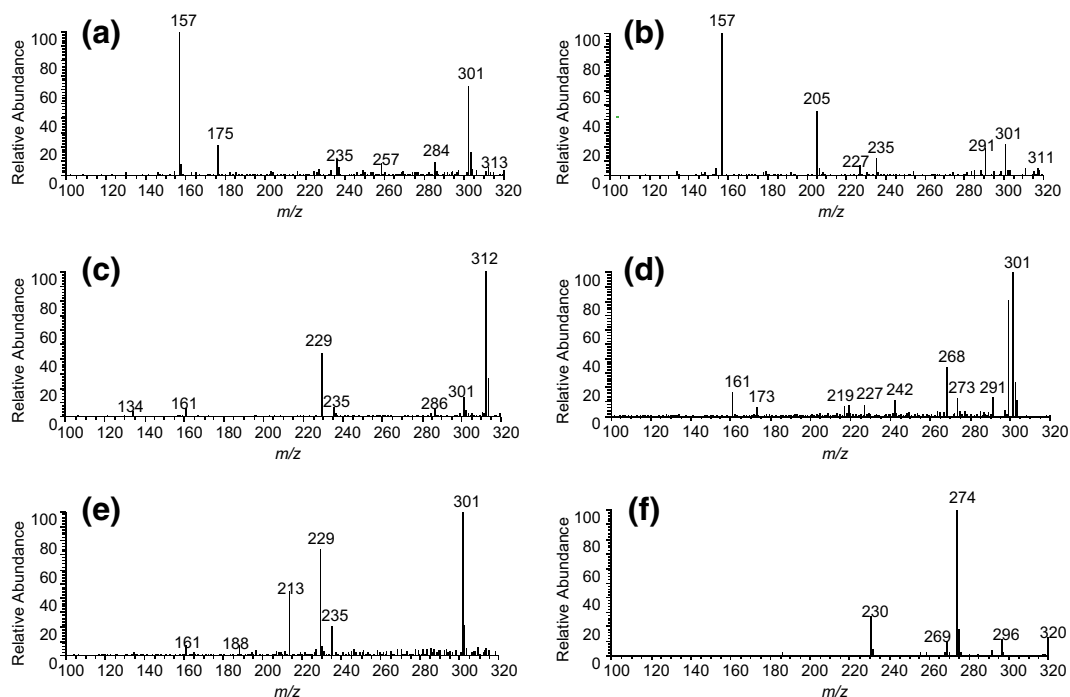
Figure 2. Typical PS(+)-MS recorded for the black ballpoint pens studied in this work (a to f) at the 100–320 m/z range. (**a**) Bic, (**b**) Paper Mate, (**c**) Faber Castel, (**d**) Pentel, (**e**) Compactor, and (**f**) Pilot

and b), m/z 161 (pens c, d, and e), m/z 213 (pen e), m/z 268 (pen d), and m/z 284 (pen a), were attributed by literature comparison to ethoxydiglycol, phenoxyethanol, Solvent Orange 3, Basic Yellow 2, and Basic Blue 9, respectively [18, 49]. The ion with m/z 301 noticed for all inks (and black papers, see Supplementary Figure S1) probably refers to dibutylphthalate (plasticiser), a background compound commonly detected in MS analysis [50].

Analyzing the negative PS mass spectra (Figure 1), on the other hand, it is evident that a simple visual inspection on the full mass range (m/z 100–1000) allows prompt discrimination of the different studied brands. Note that pens a and b presented very similar profiles, except for the presence of ions with m/z 720 and m/z 780 in pen a. Acid Yellow 36 (m/z 352) [48], a mono-sulfonated azo dye, was the most common ion detected in the negative mode, being present in pens a, b, c, and d.

The chemical structures of the main ink components identified by PS-MS in the samples analyzed are presented on Table 2.

In addition to the A4 paper (brand Chamex) utilized in these studies, for comparison purposes we also evaluated the analysis of fresh black inks (pens a and f) written on other papers, such as A4 white office (brand One), brown envelope, white letterhead envelope, cardboard, and yellow Post- t Note. The analyses were performed at the 100–1000 m/z range in positive and negative ion modes. Supplementary Figure S1 displays the full scan background mass spectra (positive and negative ion modes) obtained for each studied blank paper, and Supplementary Figures S2 and S3 show the mass spectra obtained for inks of pens a and f. For pen a (Supplementary Figure S2), it clearly appears that no significant changes can be observed in the MS
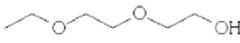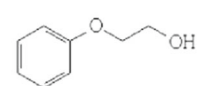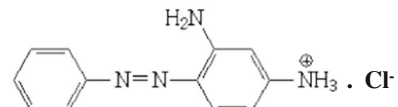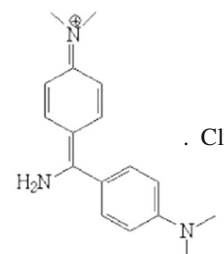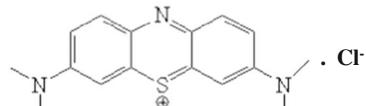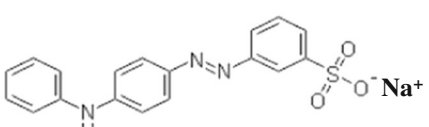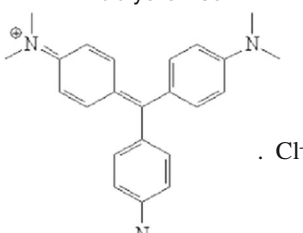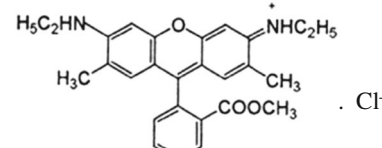
profiles when different types of papers are used. Note that the ion of m/z 367 present in the PS(–)-MS for the ink analysis on papers I, II, IV, and VI, is also present in the corresponding blanks. For pen f, some heterogeneity is observed in the relative intensity of the main peaks. However, the ions that characterized this pen are observed in all mass spectra, namely, m/z of 274, 358, 372, and 429 for PS(+)-MS and m/z of 713 for PS(–)-MS.

Degradation Studies

The characterization of the ink photodegradation was performed with the aid of a fluorescent lamp. Preliminary tests showed that no significant changes were observed for the negative mode MS of ink samples as a function of the aging. Thus, all the degradation studies were performed using only the positive ion mode. In addition, the results obtained for the PS(+)-MS at the 100–320 and 400–1000 m/z ranges also showed few changes after exposing the inks to the light. For the 100–1000 m/z range, the obtained spectra evidenced the degradation of the dye Basic Violet 3 (m/z 372) through the increase in the intensity of its demethylated homologues, especially the signals of m/z 358, 344, and 330. Thus, this m/z range was selected for conducting degradation studies.

Paper Mate was chosen as a representative brand for this study. This was because its PS(+)-MS is similar to four out of the six analyzed brands (see Figure 1). Six independent samples obtained from different batches were submitted to the same degradation conditions, in triplicates. The degradation process was monitored for 192 h. After the sample insertion in the degradation box, ink samples were removed from the

Table 2. Major Components Identified by PS-MS in the Black Ballpoint Pen Inks

m/z	Nominal Mass (Da)	Íon	Compound
157	134	[M+Na]+	Ehoxydiglycol
161	138	[M+Na]+	Phenoxyethanol
213	248	[M-Cl]+	Solvent Orange 3
268	303	[M-Cl]+	Basic Yellow 2
284	319	[M-Cl]+	Basic Blue 9
352	375	[M-Na]-	Acid yellow 36
372	407	[M-Cl]+	Basic Violet 3
429	464	[M-Cl]-	Basic Red 1:1

light exposure at each 48 h, totaling four different times of exposure: 48, 96, 144, and 192 h.

The concept of relative ion intensity (RII) was utilized to characterize the extension of demethylation of BV3. RII was utilized similarly as in previous studies involving fresh inks, with the aim to monitor the decrease of the m/z 372 signal. As BV3 has six terminal methyl groups, the relative intensities of the signals related to the demethylated derivatives correlated to this dye were used in the calculation, $RII_{372} = I_{372}/(I_{358} + I_{344} + I_{330})$. The RII values estimated for the six Paper Mate batches at the four intervals were plotted as a function of the time of light exposure (Figure 3). The determination coefficient (R^2) and the equation of a straight line were obtained through the average of the points of the six different batches.

The estimated equation for this model provides a small slope, indicating a relatively slow degradation of BV3. In addition, the results for the last point (192 h) seem to indicate the interruption of the degradation process. This observation is consistent with dating studies found in the literature, which have demonstrated that the resins of inks polymerize in contact with air, becoming so thick that they prevent the oxygen exchange of these inks with the atmosphere. This results in a significant decrease in the rate of dyes degradation. Furthermore, this polymerization causes significant changes in the solvent volatilization rate. Up to 90% of the ink solvent volatilizes in the first minutes after the deposition of the ink on the paper, and the polymerization contributes to reducing the volatilization until at a point it ceases [51]. After this stabilization, it is very difficult to monitor the ink degradation.

In this univariate calibration model, only the degradation of BV3 dye was monitored. Nevertheless, other components could be important in the monitoring of this process. With this in mind, a PLS multivariate calibration model was also built using the entire spectra. The full spectra PLS model was built with 901 variables (m/z values). The best model was chosen by random subsets cross-validation with four latent variables, accounting for 71.4% of the variance in the X block and 93.1% in the Y block.

In an attempt to improve the results and the interpretability of this full spectra model, variable selection with OPS was performed. OPS was optimized by testing seven options of informative vectors for the initialization, and a starting window containing 20 variables with increments of five. The best model was selected based on the lowest root mean square error of cross-validation (RMSECV) and the highest correlation coefficient between the reference and predicted values (r_{CV}).

The optimized OPS-PLS model was obtained starting from the regression vector, using 50 variables and four latent variables. Moreover, figures of merit shown in Table 3, such as r_{CV}, and the root mean square errors of calibration (RMSEC) and prediction (RMSEP), indicated the better performance of the OPS-PLS model.

The plot of reference versus predicted values for the OPS-PLS model is shown in Figure 4. In contrast to the univariate calibration (Figure 3), less point dispersion was observed, associated to a higher determination coefficient. This better linearity suggests that there are signals besides those from the degradation of BV3 that are important to monitor the aging of the black pen inks. A way to search for the most discriminant variables, and thus diagnostic ions, is to analyze the VIP scores of PLS models. VIP scores are weighted sums of squares of the PLS weights, and values higher than 1.0 are associated to the most predictive variables [47]. Figure 5 shows the VIP scores for the OPS-PLS model. Three ions of m/z 358, 344, and 330, originated from the three first demethylation steps of BV3, are among the most predictive. The first two are the most important ones, with the highest values (10.8 and 6.0, respectively). The other eight ions were detected as discriminant and can be considered diagnostic ions for this degradation process, including signals of high m/z, such as the ions with m/z 785, 815, and
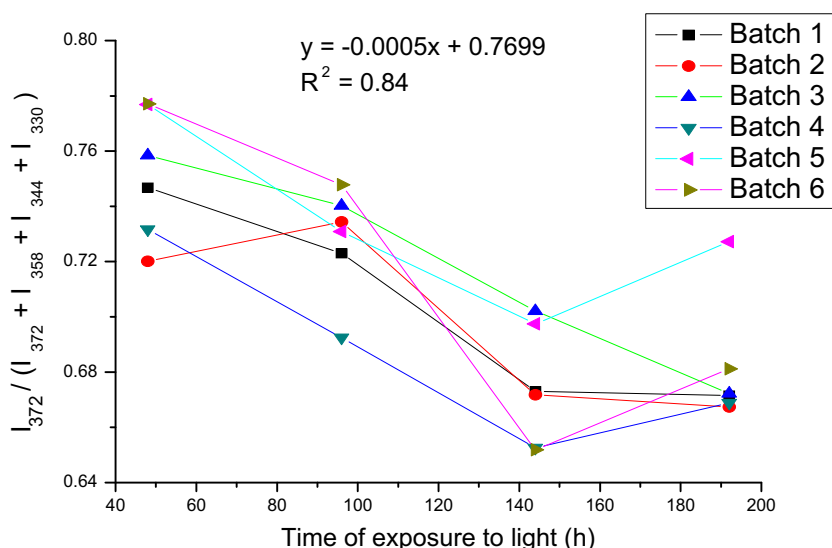

Figure 3. Plot of the relative ion intensity of dye BV3 (m/z 372) as a function of the exposure time obtained from PS(+)-MS of degradated Paper Mate pen inks

Table 3. Parameters Used to Evaluate the Full Spectra PLS and OPS-PLS Models, Obtained from PS(+)-MS in the Degradation Study

	N° LV	Variance in **X** block (%)	Variance in **y** block (%)	RMSEC (h)	RMSEP (h)	r_{CV}
Full spectra PLS	4	71.4	93.1	14.1	19.6	0.94
OPS-PLS	4	85.6	96.0	10.7	18.7	0.98

827. However, these ions were not visualized in fresh inks MS and their attributions could not be found by literature comparison.

The highest errors provided by the OPS-PLS model were estimated for the samples with the longest times of exposure to light (192 h, Figure 4). This behavior is consistent with dating studies found in the literature, as previously discussed in this subsection for the univariate model.

Analysis of Archived Documents

The applicability of the proposed PS-MS approach was first demonstrated through the analysis of black inks from documents of our personal archives. Figure 6 presents the PS(+) and PS(−) mass spectra resulted from the analysis of four documents (invoices from different products/services): three from 2005 (I, II, and IV) and one from 2015 (III). As observed, clearly distinct profiles were obtained while using the negative ion mode for the different inks, ensuring prompt discrimination of the documents. In the positive ion mode, on the other hand, while unique profiles were observed for the inks of documents III and IV, quite similar profiles were obtained for documents I and II. Nevertheless, together with the PS(−) spectra, their distinction could also be performed by PS(+) through the relative ion intensity (RII$_{372}$, 0.95, and 0.86 for documents I and II, respectively). Note that even after 11 y, the level of degradation experienced by the Basic Violet 3 (m/z 372) was very small for the inks of documents I and II, which corroborates our results on artificial aging studies, namely, the notable resistance of BV3 to the degradation while in black ink
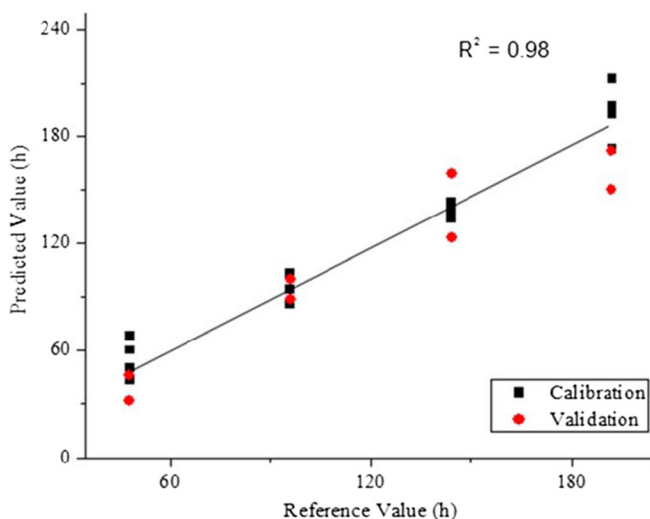

Figure 4. Plot of the reference *versus* predicted values for the OPS-PLS model obtained from PS(+)-MS in the degradation study

formulations. More interesting, the serial cascade of degradation was more intense (RII of 0.56) on the most recent document (III, dated from 2015). This probably reflects the conditions of the document storage before being archived and/or the ink composition for this specific brand of pen. The ions with m/z 478 and 443 detected in the ink of document IV were ascribed to Basic Blue 7 (m/z 478) and Basic Violet 10 (m/z 443). These two dyes have been reported as black ink components [18, 52]. However, they were not found on the brands studied in this work.

Detection of Superposition of Lines and Simulated Forgeries

The PS-MS approach was also applied on the analysis of the superposition of ink lines and detection of alterations designedly made on our archived documents (simulated forgeries). First, we studied mixtures of fresh inks through the analysis of overlapped lines made with pairs of pens studied in this work. All possible combinations of brands were tested. The results revealed the good capability of PS-MS to distinguish overlapped lines from the original single inks by providing different mass spectra patterns in both positive (100–320 m/z range) and negative ion modes (data not shown). The RII$_{372}$ could also be used to distinguish some combinations.

As another proof-of-concept study, archived documents were analyzed after being altered using the pens studied in this work, following by a light exposure of 72 h. Figure 7 shows the PS-MS analysis of a hotel invoice from 2005, where some entries of the original document were modified or overlapped by using the pens (Figure 1, a–f) studied in this paper. Some of these portions are marked as red triangles in the picture (see Figure 7). One of the modifications was made on the total amount of the invoice, where the number 3 was converted into 8. First, analyzing the PS(+) mass spectra, the insertions with pens a–e could be detected by comparing the RII$_{372}$ obtained for the "counterfeited" portions (0.65, 0.63, 0.66, 0.66, and 0.62 for pens a–e) with that calculated for the original ink (RII$_{372}$ = 0.95) (see Figure 7). As observed, the PS(+)-MS obtained for the entries altered with pen f is quite different from the original pattern, thus allowing prompt discrimination. For the MS analysis using the negative ion mode, as observed, except for the adulteration with pen a, the PS(−) mass spectra of the original ink is distinguishable from those obtained after the insertions (b–f).

Another case example of ink analysis in archived documents is displayed in the Supplementary Information (Supplementary Figure S4). This document is a receipt from 2015. As can be observed for the mass spectra obtained in the negative
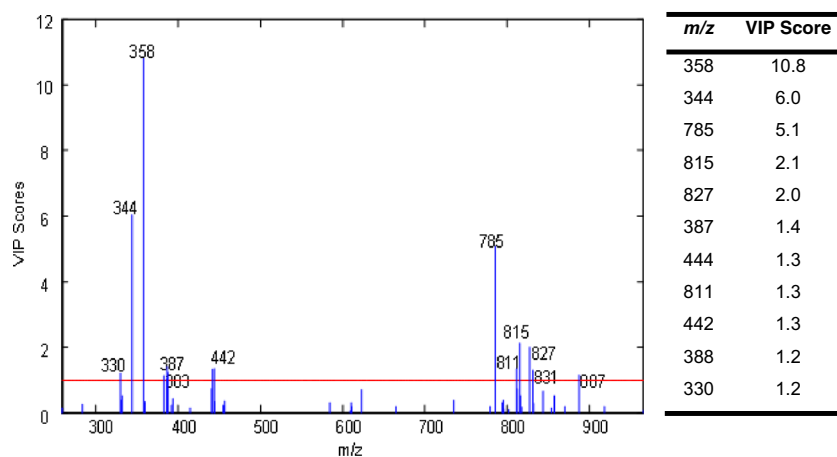
m/z	VIP Score
358	10.8
344	6.0
785	5.1
815	2.1
827	2.0
387	1.4
444	1.3
811	1.3
442	1.3
388	1.2
330	1.2

Figure 5. VIP scores versus *m/z* for the OPS-PLS model obtained from PS(+)-MS in the degradation study. The highest VIP scores are listed next to (at left) the plot

ion mode, the differentiation between the original ink and the "counterfeit" portions can be made by simple visual comparison. For the positive ion mode, on the other hand, the relative ion intensity concept is needed for the ion of *m/z* 372.

It is important to emphasize that even after performing the PS-MS analysis, a certain amount of ink is left on the paper triangle. Thus, PS-MS do not completely destroy the sample, being a semidestructive approach. This sample can therefore be saved together with the original document and stored for further analysis (by PS-MS or other analytical techniques) in cases of proof confirmation, which is often required in forensic investigations. Considering all samples studied in this work,
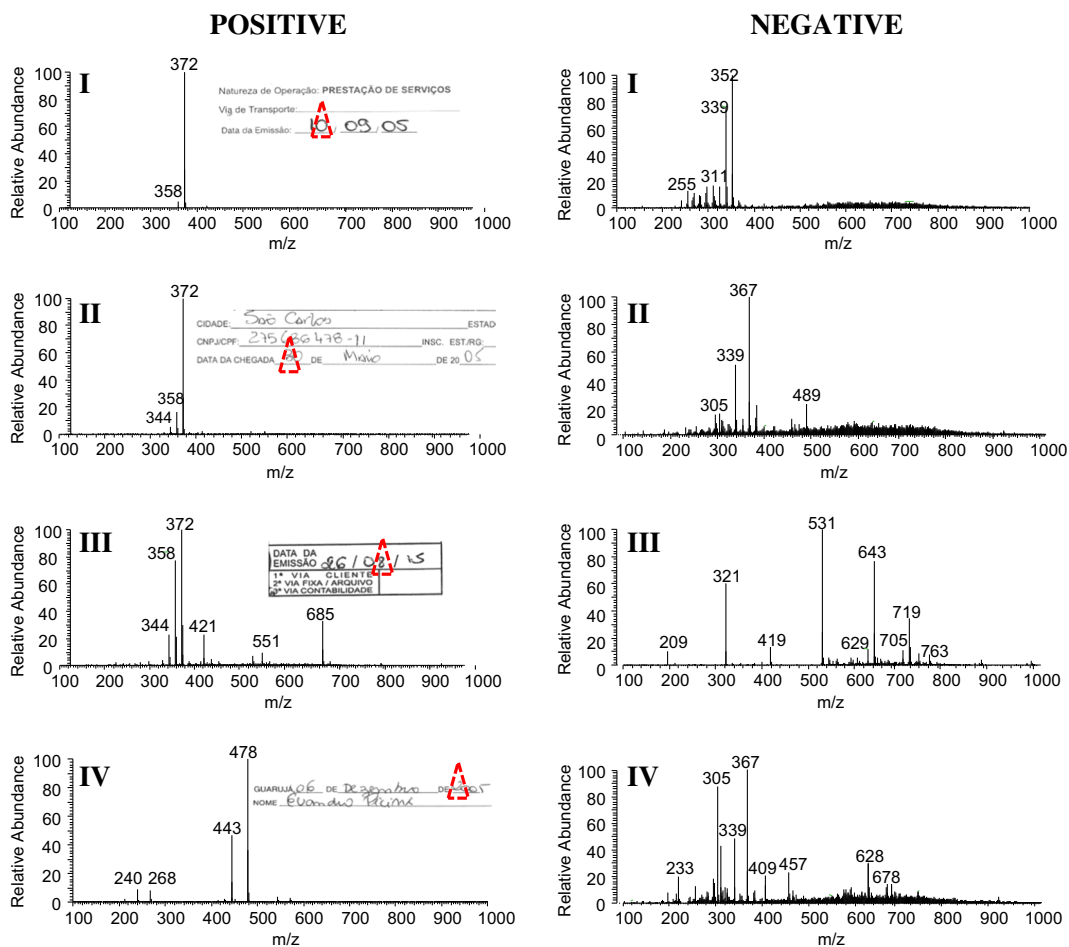
POSITIVE **NEGATIVE**

Figure 6. PS(+)-MS and PS(−)-MS analysis of black inks from four different archived documents. The samples are invoices of different sources from 2005 (I, II, and IV) and 2015 (III)
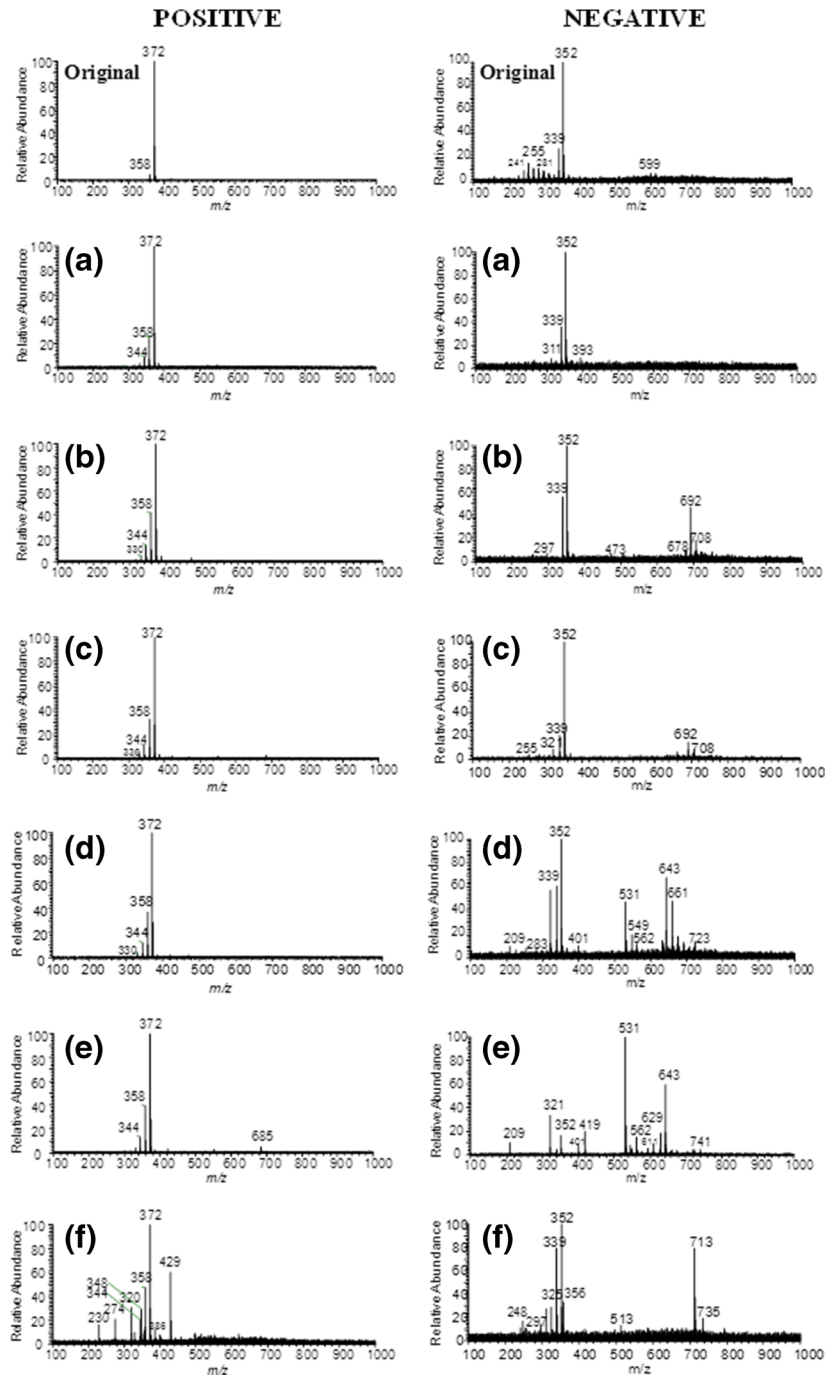
Figure 7. Comparative PS-MS analysis of a hotel invoice emitted in 2005 before and after being altered with pens a–f and subjected to light exposure during 72 h

we observed that at least three consecutive analyses could be performed without important changes in the MS profiles.

Conclusion

We have demonstrated the effectiveness of PS-MS on the forensic analysis of black ballpoint pen inks. The discrimination power for characterizing fresh, old, and artificially aged writings, together with the speed of the PS-MS approach make it attractive for the implementation in forensic laboratories. It is worth highlighting that the need of cutting the document in triangles for performing the analysis does not substantially detract its overall effectiveness as the considerable amount of ink remaining in the paper can be used for further analysis. This characteristic is very important from the forensic point of view. Moreover, the PS-MS instrumentation brings substantial simplification over existing MS ambient approaches, being suitable to be coupled/operated with mass spectrometers without the need of auxiliary gas or especial assembling.

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