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Differentiation of blue ballpoint pen inks by positive and negative mode LDI-MS

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Abstract

Usually, the differentiation of inks on questioned documents is carried out by optical methods and thin layer chromatography (TLC). Therefore, spectrometric methods were also proposed in forensic literature for the analysis of dyes. Between these techniques, laser desorption/ionization mass spectrometry (LDI-MS) has demonstrated a great versatility thanks to its sensitivity to blue ballpoint ink dyes and minimal sample destruction. Previous researches concentrated mostly on the LDI-MS positive mode and have shown that this analytical tool offers higher discrimination power than high performance TLC (HPTLC) for the differentiation of blue ballpoint inks. Although LDI-MS negative mode has already been applied in numerous forensic domains like the studies of works of art, automotive paints or rollerball pens, its potential for the discrimination of ballpoint pens was never studied before. The aim of the present paper is therefore to evaluate its potential for the discrimination of blue ballpoint inks. After optimization of the method, ink entries from 33 blue ballpoint pens were analyzed directly on paper in both positive and negative modes by LDI-MS. Several cationic and anionic ink components were identified in inks; therefore, pens were classified and compared according to their formulations. Results show that additional information provided by anionic dyes and pigments significantly increases the discrimination power of positive mode. In fact, it was demonstrated that classifications obtained by the two modes were, to some extent, complementary (i.e., inks with specific cationic dyes not necessarily contained the same anionic components).

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1. Introduction

The main goal of the forensic examination of inks is to highlight a material heterogeneity on a questioned document. In fact, the presence on a single document of two or more different inks (i.e., the use of different writing instruments) may indicate a forgery. Ballpoint pens are certainly wide used by people and many analytical methods were proposed to discriminate between different ballpoint ink formulations on paper [1-10]. The standard examination procedure for these inks includes first a study of the optical properties of the ink (e.g., absorption and luminescence) [11] followed by the separation of the dyes by thin layer chromatography (TLC) [1], a technique showing a good discrimination power [12-14]. The problem of this methodology is that it requires a local destruction of the questioned document to extract ink samples. Moreover, the sample preparation and analysis are time consuming procedures. Alternative non-destructive optical methods such as microspectrophotometry (MSP) were also proposed [2, 3]; these techniques are however less discriminating [14].

Lately, mass spectrometric methods were evaluated because they seemed more promising in terms of sample destructivity, analysis time and discrimination power than previous analytical tools. Like TLC, mass spectrometric methods focus on the analysis of colored compounds. This ensures high discrimination power considering that combinations of organic dyes and inorganic pigments are highly variables in ink formulations [15]. Secondary ion mass spectrometry (SIMS) [4], field desorption (FD) [5], electrospray ionization (ESI) [7], laser desorption/ionization (LDI) [6, 12, 16-20], matrix-assisted laser desorption/ionization (MALDI) [19, 21], direct analysis in real time (DART) [8] and desorption electrospray ionization (DESI) [22, 23] were proposed for ballpoint inks examination. In addition to classical methods, mass spectrometry may yield information on the structure of the analyzed molecules (i.e., mass or/and fragmentation pattern). More specifically, the interest regarding the application of LDI-MS to ballpoint ink examination is high, because it is able to guarantee minimal sample destruction and to ionize ink dyes and pigments directly from paper with very little fragmentation and without sample preparation.

With LDI, both positive and negative ions can be obtained from a complex mixture. Previous researches in the domain of questioned documents concentrated mostly on the LDI-MS positive mode and have demonstrated that this analytical tool offers higher discrimination power than high performance TLC (HPTLC) for the differentiation of blue ballpoint inks [12]. Regarding LDI-MS negative mode, forensic literature reports numerous applications in different domains to extend the capability of positive mode; examples are identification of pigments used in works of arts [24, 25], in illuminated manuscripts [26], in automotive paints [27], in rollerball pens [28] and in inkjet

printers [29]. Nevertheless, its potential for the identification of pigments and dyes used in ballpoint inks and for the discrimination of ballpoint pens was never studied before. Application of LDI-MS negative mode should allow the detection of anionic dyes used in ink formulations in addition to cationic ones, providing in this way new information for ballpoint pen discrimination. The purpose of this study is to evaluate the potential gain of discrimination power in blue ballpoint inks analysis by considering both LDI-MS positive and negative modes. After optimization of the method in both modes, reference substances were analyzed in order to identify signals in the mass spectra originating from dyes. Additionally, the use of relative peak areas allowed to further optimize classification. HPTLC analyses of reference substances and representative inks were also performed to confirm the identifications obtained by LDI-MS.

2. Experimental

2.1 Materials

33 blue ballpoint pens were randomly chosen from the German and Swiss market between 2001 and 2006. These pens were used to prepare entries on multifunction white paper from Xerox© (Business Laser-Copy-Inkjet A4 80 g/m2, Rochester, NY USA). The straight lines were made with a ruler applying normal hand pressure. Reference solutions of organic dyes and inorganic pigments were also prepared to identify peaks in mass spectra of inks. Each colorant was dissolved in ethanol from Fluka© (Absolute Ethanol, Buchs, Switzerland) at a concentration of about 1 mg/mL inside polypropylene laboratory tubes from BD Falcon© (Blue Max© Conical Tubes 50 mL, Franklin Lakes, NJ, USA). These solutions were stocked inside a refrigerator at a temperature of 5°C, safe from direct light. In total 33 reference solutions were prepared (Table 1).

2.2 LDI-MS method

Sample preparation – Concerning the pen entries, small pieces of paper measuring about 4 x 10 mm each, bearing a single fresh stroke running parallel to the longer edge, were cut and fixed directly on the metallic sample plate using an adhesive roller from Henkel KGaA© (Pritt© Glue-it© Permanent, Düsseldorf, Germany). For the reference solutions, an aliquot of 30 µL of each solution was simply deposed on the metallic sample plate.

Instrumentation – Positive and negative mass analyses were conducted on a commercial Axima-CFR© Plus matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometer from Kratos Analytical Ltd© (Kyoto, Japan) outfitted with a pulsed nitrogen laser (337 nm, 3 ns, with an operating spot size diameter of about 0.1 mm). This laser delivered pulses

with maximal energy of 300 μJ per shot. The beam energy reaching the sample was adjusted by a step motor interposing different portions of a gradient mirrored disc (position from 0 to 180). Spectra were generated by averaging 150 scans in order to generate a single averaged mass spectrum; scans were performed by moving continuously the laser on the sample in order to avoid local dye degradations. The ion gate was adjusted so that only the spectral range comprised from m/z = 100 and m/z = 1500 were considered. External mass calibration was performed using known molecular peaks of specific dyes in pure solutions; these dyes were BV3 ($[C_{25}H_{30}N_3]^+$; m/z = 372.243) and BB26 ($[C_{33}H_{32}N_3]^+$; m/z = 470.259) for positive mode and AY36 ($[C_{18}H_{14}N_3SO_3]^-$; m/z = 352.080) for negative mode. Samples were immediately introduced into the MS after their preparation and 3 averaged mass spectra were acquired in different locations of the sample for each analytical mode (positive and negative) in order to take into account the heterogeneity of the ink strokes and allow error measurements. Paper and glue blank analyses were performed to insure that no signal interference was produced by other sources than the ballpoint ink lines.

Optimal laser irradiance – The effect of the ionization process was evaluated for positive and negative modes by studying the effects on mass spectra of increasing laser irradiance, such as induced photodegradation of the dyes or loss of resolution of the mass signals. It was previously demonstrated that too high laser irradiance can cause photodegradation of cationic dyes, while too low laser irradiance is insufficient to provide exploitable spectrum intensity [19]. Therefore, the use of optimal laser irradiance is necessary to prevent these problems and insure reproducibility. It is important that the laser irradiance be kept the same for each acquisition of different samples in order to ensure results comparability. Empirically, optimal laser irradiance was evaluated by testing the effects of increasing laser nominal values on the spectra recorded from the analysis of strokes prepared with a reference ballpoint pen (BIC© Cristal Medium) that contained two cationic dyes, BV3 and BV4, and one anionic dye, SB38. Criterions were spectrum maximal intensity, peak resolutions (i.e., the peak averaged mass divided by the half-height peak width, $m/\Delta m$) and dye relative peak areas (see below for definition).

2.3 HPTLC Method

Sample preparation – Ink entries of about 1 cm length were scraped from the paper with a scalpel. A sample of blank paper of equal dimensions was also analyzed. All solvents were purchased from Fluka© and their purity was of ACS grade. The extractions were accomplished in a conical vial from Supelco© (Buchs, Switzerland) using 15 μL of pure methanol. The vial was hermetically sealed and kept in the dark for 24 hours at room temperature.

Instrumentation – 2.5 and 5 µL aliquots were applied to a 10 x 20 cm HPTLC silica gel plate form Merck© (Switzerland) by using a Camag© Linomat IV© spot applicator (line application of 5 mm length). It must be noted that every plate included a standard of BV1. The HPTLC plate was then developed in a horizontal developing chamber using the following solvent system: 1-butanol, 2-propanol, bi-distilled water and acetic acid (10 : 5 : 5 : 0.5). The developing time was 15 minutes. The spots on the TLC plates were then compared with respect to their relative retention time values and color.

2.4 Discrimination power (DP)

The ability of the technique to differentiate entries produced by 33 blue different ballpoint pens was evaluated by calculating the DP according to the following equation [12, 30]:

$$DP = 100 \cdot \left[1 - \frac{2 \cdot m}{n \cdot (n-1)}\right] \quad (1)$$

where *m* is the number of undiscriminated stroke pairs after comparisons and *n* is the total number of pens. For each ink, both positive and negative spectra were registered. By comparison with reference substances, several mass peaks could be identified and a preliminary discrimination based on qualitative information (absence/presence of dyes, pigments and their related compounds) was performed. Then, a further quantitative discrimination was performed on the basis of relative peak areas (RPAs). RPA is an indicator introduced by Weyermann et al. [19] to describe relative signal intensities of dyes; mathematically, it is defined as follows:

$$RPA_i = \frac{A_i}{A_{tot}} \quad (2)$$

where A_i is the area of the peak at m/z = i and A_{tot} is the total area of a group of related peaks in the same mass spectrum. RPA definitions used in this paper are given in Table 2. Previous research has demonstrated that RPAs of cationic dyes vary greatly among different fresh ballpoint inks [12] revealing the utility of these indicators for discrimination. For each analytical mode, DP was calculated separately and then combined to obtain the total DP of the technique in order to evaluate the added value of negative mode compared to the positive mode. Tests were performed to ensure that RPAs were reproducible within each ink stroke.

3. Results and discussions

3.1 Optimization of the method

Optimization is an essential step in every analytical method. In forensic context, this procedure is particularly important because comparability of measurements (e.g., RPAs) taken from various specimens or samples must be guarantee by adopting optimal and homogeneous analytical conditions. In this paragraph we illustrate some important effects on properties of spectra caused by irradiance variation in order to find the best conditions. Optimization procedure described here is clearly instrumentation-dependent and should be performed every time a new set of analysis is performed (particularly when the aim is feeding data in a library).

Irradiance is the most sensible parameter to consider in LDI analyses. On one hand, the quantity of ions produced per laser shot, and consequently the sensitivity of the technique strongly depends on the used laser irradiance. On the other hand a too high laser power causes both dyes fragmentation (i.e., the surplus of energy is sufficient to break weaker molecular bonds), and the loss of peak resolution (i.e., the high concentration of ions reaching the mass detector causes its saturation). Through analyses of reference ballpoint pen entries, these effects were empirically observed, although some differences were noticed between positive and negative modes. As expected, in both analytical modes, peak resolution decreased proportionally to laser nominal values (Fig. 1) while spectrum maximal intensity increased (Fig. 2). It was however observed that the irradiance needed to generate spectra of comparable maximal intensity were rather different between positive and negative mode (Fig. 2): e.g., for reference ink a nominal value of 70 was necessary in positive mode to obtain maximal intensity of about 10’000 mV, while a nominal laser value of 95 was necessary in negative mode to obtain the same maximal intensity. Experimentation confirmed that this result could be generalized to all analyzed ballpoint pens and thus highlighted the lower sensitivity of negative mode.

It is of forensic importance to note that increasing laser irradiance also provoked the fragmentation of cationic dyes. Since RPA are defined as the proportion of a compound in regard to its correlated molecules (Table 2), it must be deduced from this observation that RPA values for cationic dyes are influenced by the laser irradiance. An example is given in Figure 3a for reference ink. The graph confirms that the molecular ion RPA values of the triphenylmethane cationic dye BV3 (RPA_{372}) decreased in correlation to nominal laser irradiance, while degradation products were formed (i.e., increasing values of RPA_{358} and RPA_{344}). Similar observations were previously reported in the literature [19, 20] and demonstrate that dependence between laser irradiance and degradation of cationic dyes is a consequence that have to be considered if LDI-MS is used to discriminate inks.

No information of this type was yet proposed for negative ions. An evaluation of laser irradiance effects on RPA values of anionic dyes was then performed with the reference ink. Molecular ion RPA value of the copper phthalocyanine sulfonic acid derivative SB38 (RPA_{735}) identified in the reference ballpoint pen was monitored over selected irradiance values (Fig 3b). Results seem to indicate that SB38 also underwent chemical degradation when laser energy increased and that RPA values for the anionic dyes are as well a function of the laser irradiance. This confirm that the ink strokes must be analyzed using the same laser irradiance.

Based on the collected data, the choice of an optimal laser power for positive mode was straightforward considering the good global affinity of the ink cationic components to the laser. An optimal irradiance minimizing dye fragmentation and loss of peak resolution can be selected without decreasing too much signal intensity: a nominal value of 60 was therefore selected to acquire positive spectra of 33 ballpoint pens in this work. In comparison, the choice of the optimal laser irradiance for negative mode is more difficult because of the lower sensitivity: the highest irradiance level minimizing loss of resolution was not generally sufficient to provide qualitative spectra. Therefore a compromise between a decrease in resolution and an increase in laser irradiance was carefully selected. The main problem was that poor resolution can result in the loss of information about the isotopic distribution of some compounds and as a consequence only the average mass could then be determined (Fig. 4). From these observations, a nominal irradiance of 80 was selected to record negative spectra of ballpoint pens.

Using optimal laser irradiances, reproducibility of spectra was evaluated on fresh and old samples made with the reference pen before analyzing other ink entries. It was observed that positive spectra were slightly more reproducible than negative ones. In fact, the detection of lower intensity peaks was less reproducible in negative mass spectra and spectral noise was generally more intense. Relative peak intensities in negative mode showed more variability than in positive mode. For the molecular mass peaks of identified compounds, mass accuracy was also good in both modes. For example, molecular peak of BV3 (exact mass: 372.243 u) gave an average m/z ratio of 372.285±0.049 (RSD ≈ 0.01%) in positive mode and molecular peak of SB38 (exact mass: 734.993 u) gave an average m/z ratio of 734.805±0.216 (RSD ≈ 0.03%) in negative mode.

3.2 Identification of dyes and pigments

Positive and negative spectra were registered from ink entries made with 33 ballpoint pens. Principal dyes and pigments were identified by comparison with spectra acquired from reference solutions (Tables 3 and 4).

For positive spectra (Table 3), characterization of cationic compounds was previously reported in literature about LDI-MS analyses on ballpoint inks [6, 12, 16, 17, 31]. Amongst identified dyes in positive spectra (Table 3), the most common were BV3 (94% of the analyzed pens), BB7 (39%) and BB26 (33%), while BV4 and SB2 were found in less than 10% of the analyzed inks. These cationic species were always detected with a low amount of derivated species. The pigment PB15 was also identified in some positive spectra (less than 10%).

Concerning negative mass spectra (Table 4), the identification of anionic compounds present in blue ballpoint inks is particularly interesting because it was never detailed before in the literature for direct LDI-MS analysis of ballpoint pen strokes. Generally, in negative spectra three dyes and pigments were identified: SB38 was the most common compound (64% of the analyzed pens), while PB15 and AB92 were found in less than 10% of the analyzed inks. Another dye, AB9, was also found in one ink (pen #2) by comparison with pure dye spectrum, but structures related to correlated peaks were not identified. So, this dye wasn't used for discrimination.

PB15 ($C_{32}H_{16}N_8Cu$; m/z = 575.1) is the commercial name of the pigment copper phthalocyanine (CuPc); it is widely used in industrial applications because of its remarkable chemical stability and excellent fastness to light [32, 33]. MALDI-MS literature reported previously that neutral metallophthalocyanine pigments are usually detected in both ionization modes because of the formation of M^+ and M^- molecular ions [34-36]. For PB15, these observations were lately supported by some forensic studies using LDI-MS that identified this pigment in applications like artist's paints [24], paper additives [24], security inks [37], automotive coating paints [27, 38], rollerball pen inks [28] and inkjet printer inks [29]. Analysis of blue ballpoint inks confirmed these results. In fact, PB15 is recordable in both ionization modes with intense signals at m/z = 575.1 (which correspond respectively to radical ions $[CuPc]^+$ and $[CuPc]^-$) and by some isotopic peaks at higher m/z.

Additionally, data show that many inks gave a variety of signals over m/z = 580 when using negative mode. Major signals were detected at m/z = 655.0, 735.0, 815.0 and 894.9. In some inks, peaks at m/z = 654.0, 734.0, 813.9 and 893.9 were also very intense. However, none of these signals was simultaneously detected in positive mode. It was observed that, between this peaks, a difference of 80 m/z existed. Since sulfonic acid group (SO_3H) has an exact mass of about 81 u, this observation suggests that, between successive ion pairs, a hydrogen atom was substituted by a sulfonic acid group (Fig. 5 and Table 4).

Functionalization of copper phthalocyanine is a common practice in industrial chemistry. In fact, functionalization can change chemical and physical characteristics of copper phthalocyanine in

order to adapt its properties to specific commercial applications [32]. Particularly, sulfonation produces sulfonic acid derivatives that are water-soluble and can be used as direct dyes [32, 39] or to increase the stability [28]. Amongst derivatives of PB15, the di-sulfonic acid derivative $CuPc(SO_3H)_2$ is commercially sold as SB38 ($C_{32}H_{16}N_8S_2O_6Cu$; m/z = 735.0) (Fig. 5). SB38 is the most common anionic dye derived from PB15 and it was already identified in blue ballpoint inks using electrospray ionization (ESI) mass spectrometry [7]. Others anionic dyes that could be derived from PB15 are the mono-sulfonic acid derivative $CuPc(SO_3H)$, the tri-sulfonic acid derivative $CuPc(SO_3H)_3$ (i.e., mono-sulfonic acid derivative of SB38) and tetra-sulfonic acid derivative $CuPc(SO_3H)_4$ (i.e., di-sulfonic acid derivative of SB38) (Fig. 5).

Thus, peak at m/z = 655.0 corresponds to radical anion of the mono-sulfonic acid derivative of PB15, namely $[CuPc(SO_3H)]^-$; peaks at m/z = 735.0, 815.0 and 894.9 correspond respectively to radical anions of simple SB38, mono- and di-sulfonic acid derivatives of SB38, namely $[CuPc(SO_3H)_2]^-$, $[CuPc(SO_3H)_3]^-$ and $[CuPc(SO_3H)_4]^-$. The second set of signals composed by the peaks at m/z = 654.0, 734.0, 813.9 and 893.9 correspond to respective anionic species $CuPc(SO_3^-)$, $CuPc(SO_3H)(SO_3^-)$, $CuPc(SO_3H)_2(SO_3^-)$ and $CuPc(SO_3H)_3(SO_3^-)$ (Fig. 5). This is in agreement with previous published analysis of sulfonic acid derivatives of PB15 using MALDI-MS [36]. To notice that for each derivative, radical anion and respective anionic species were always co-present in negative spectra. Since each of these two forms was accompanied by a series of isotopic peaks at higher m/z (with an isotopic distribution similar to that of PB15), the total isotopic mass pattern resulted always as a sum of this two isotopic distributions (Fig. 4).

Nevertheless, the different PB15 and SB38 derivatives were not always co-detected in each analyzed blue ballpoint ink. Empirically, data showed that mono-sulfonic acid derivative was detected only if PB15 basic form is present in the ink. On the other hand, S38 mono- and di-sulfonic acid derivatives were solely detected in presence of SB38 basic form. From these observations, PB15 basic form and mono-sulfonic acid form were considered related species in RPA definitions; the same was done for SB38 basic form and its two sulfonic acid derivatives (Table 2).

Other two anionic compounds were identified in negative spectra: AB9 ($C_{37}H_{37}N_2S_3O_9$) and AB92 ($C_{26}H_{19}N_3S_3O_{10}$). AB9 and AB92 are sulfonic acid derivative of triphenylmethane and azobenzene respectively. Thanks to their sulfonic acid groups, both are potentially multiply negatively charged (Fig. 6). For this reason, they rarely yield molecular ions in LDI-MS experiments [21]. However, comparison of ink and pure dye spectra, as well as HPTLC confirmation, permitted to confirm their presence and to identify some characteristic peaks.

In LDI-MS negative spectra of inks, AB92 was characterized in negative spectra by the presence of two signals at m/z = 313.5 and 628.0. The peak at m/z = 628.0 is produced by the loss of a proton from a sulfonic acid group and the formation of a mono-anionic species; namely, $R(SO_3H)_2(SO_3^-)$. The second peak at m/z = 313.5 is probably produced by the lost of two protons from two sulfonic acid groups and the formation of a di-anion; namely, $R(SO_3H)(SO_3^-)_2$ (Fig. 6). Identification was also confirmed by HPTLC. Similar observations were previously made by ESI-MS analysis of blue ballpoint pen [7].

AB9 was characterized by the presence of two intense signals at m/z = 170.2 and 185.2. Molecular structures of ions behind these peaks are actually unknown but presence of this dye could be confirmed by HPTLC. It was anyway found in only one of the analyzed ballpoint inks. Previously, it was identified in both blue ballpoint [7] and blue gel pens [40] by ESI-MS, a method causing less fragmentation than LDI-MS.

3.3 Discrimination power (DP)

To discriminate different ballpoint pen inks, in a first step qualitative comparison of the mass spectra was made considering only the dyes and pigments composition. In positive mode, this procedure permitted to distinguish 7 different groups of ink formulations, where two large groups included 64% of the ballpoint pens. On the other hand, the same procedure applied in negative mode permitted to form 4 different groups, where two large groups included 85% of the pens. Cationic dyes/pigments obviously offered a better DP in comparison to anionic compounds (78.4% versus 64.0%). Nevertheless, the results indicated that qualitative discrimination of blue ballpoint pens based on a single ionization mode was not so high. Some cationic or anionic dye combinations seem to be very frequent in blue ballpoint inks (an example is the combined presence of BV3 and BB26). It is interesting to note that DP rose up to 87.9% when qualitative information of both modes were combined. This demonstrates that blue ballpoint formulations having a specific combination of cationic compounds do not have necessarily an identical combination of anionic dyes and pigments (e.g., inks composed by cationic dyes BV3 and BB26 didn't necessarily have SB38 and all its sulfonic acid forms). The two ionization modes are therefore complementary. For example, the LDI-MS analysis in positive mode didn't allow the differentiation of ballpoint pens #2, #15, #23 and #28 (Table 3). These three inks all contained BV3. Their analysis in negative mode brought however the differentiation of pens #2 and #15 from the others (while pens #23 and #28 remained undiscriminated) (Table 4). Actually, several additional anionic dyes could be identified in the negative spectrum of the ink from pen #2 (Fig. 7): AB9 (m/z = 170.2 and 185.2),

AB92 (m/z = 313.5 and 628.0), SB38 (m/z = 735.0) and SB38 mono-sulfonic acid derivative (m/z = 815.0).

In a second step, the variability of RPA values of identified cationic and anionic compounds was evaluated to further discrimination. The standard deviation (SD) of RPA values of cationic and anionic dyes and pigment was evaluated in fresh (immediately analyzed after deposition) and old inks (analyzed after 4-months safe from direct light). For positive RPAs, SD was found to be below 0.020; for negative RPAs, it was found to be below of 0.050. Two strokes were therefore considered discriminated when one of their RPA values differed at least of 0.050 and 0.125 respectively (i.e., over 2.5 times the SD, which corresponds to a confidence interval of 99%). RPA values permitted to discriminate an additional 50 pairs of pens in positive mode, increasing the DP from 78.4% to 87.9%. This improvement was primarily due to the large variability of initial RPA values of BV3 molecular ion (RPA_{372}) in ballpoint inks which allowed further discrimination of the ballpoint pens containing this dye (i.e., 94% of the ballpoint pens contained BV3). In negative mode, RPA values allowed to discriminate an additional 80 pairs, increasing the DP from 64.0% to 79.2%. This improvement was mainly due to the high variability of RPA values of SB38 and its sulfonic acid derivatives. When combining the qualitative information and RPA values of both modes, the total DP was thus significantly improved up to 96.0%.

Unknown mass signals permitted to appreciate further differences into mass spectra of ballpoint pens. 76% of the positive spectra contained additional signals that could not be identified, while each negative spectrum showed unknown mass peaks. In each case, these signals would allow the discrimination of additional pairs. It is interesting to note that the contribution of unidentified peaks was more important for anionic spectra. In fact, considering only the negative mode, they would even permit to discriminate an additional 106 pairs of pens and, therefore, to increase the negative mode DP from 79.2% to 99.2%.

Including all information from the positive and negative spectra, 2 groups of pens remained undiscriminated (i.e., pens 24-25 and 32-33) for a final DP of 99.6%, which represents a slight improvement compared to the use of positive mode only (Table 5). The undiscriminated ballpoint pens ink might actually be of the same formulation. For example, pens 32 and 33 were of the same brand and differed only by the size of the ballpoint (fine versus medium), while pens 24 and 25 were produced by the same manufacturer in the same year.

These results showed that acquisition of ballpoint inks LDI-MS negative spectra can improve the differentiation obtained by LDI-MS analyses in positive mode (Table 5). Information about the anionic composition of ink may in some case be useful to differentiate blue ballpoint pens that

remained undiscriminated considering only cationic compounds. Discrimination potential of unknown mass signals was very high. Nevertheless, 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. This observation is particularly valid for mass peaks of low intensity in negative spectra. Therefore differentiation of two ink formulations based solely on unidentified signals should further be studied before being applied in real casework.

4. Conclusion

This study has confirmed the high discrimination potential of LDI-MS combined to minimal preparation and analysis time. The optimization of the method, particularly the selection of appropriate and constant laser irradiance, was a crucial step to ensure the reproducibility and comparability of the results. This step should not be underestimated in order to minimize the influence of the technique on the results.

Many dyes, pigments and related products were identified among the analyzed ballpoint pen inks. Negative mode allowed the identification of additional anionic dyes that were not recorded in positive mode (i.e., Acid Blue 9, Acid Blue 92 and several sulfonic acid derivatives of Pigment Blue 15) and brought therefore further discrimination.

Results moreover demonstrated the possibility to increase the discriminating power of the technique using additional mass information supplied by the negative mode analysis in the same manner than for the positive mode. The potential of relative peak area (RPA) definitions was thus demonstrated in both modes.

An important number of mass signals remained unidentified, particularly for the negative mode spectra. These signals brought a large quantity of additional information that proved very useful to improve the discrimination of inks up to 99%. Further researches should now endeavor to comprehensively study the cationic and anionic composition of blue ballpoint inks in order to improve the discrimination process reliably by identifying unknown signals.

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C. I. name	C. I. number	Abbreviation	Commercial name	Furnisher
Unknown	-	-	Fast Black K Salt	Fluka®
Acid Black 2	50420	AB2	Nigrosin Water Soluble	Fluka®
Acid Blue 9	42090	AB9	Duasyn Acid Bue AE02	Hoechst®
Acid Blue 92	13390	AB92	Cyaninsäure Blau R	Chroma®
Acid Orange 7	15510	AO7	Duasyn Acid Orange P01	Hoechst®
Acid Orange 10	16230	AO10	Orange G	Fluka®
Acid Red 52	45100	AR52	Duasyn Acid Rhodamin B-SF	Hoechts®
Acid Violet 17	42650	AV17	Acid Violet 17	Aldrich®
Acid Yellow 36	13065	AY36	Metanil Yellow	Fluka®
Basic Blue 7	42595	BB7	Victoria Pure Blue BO	Sigma-Aldrich®
Basic Blue 11	44040	BB11	Victoria Blue R	Aldrich®
Basic Blue 26	44045	BB26	Victoria Blue B	Aldrich®
Basic Green 1	42040	BG1	Malachtgrün	Chroma®
Basic Green 4	42000	BG4	Malachite Green Oxalate Salt	Sigma®
Basic Red 1	45160	BR1	Rhodamine 6G	Fluka®
Basic Violet 1	42535	BV1	Methyl Violet	Fluka®
Basic Violet 3	42555	BV3	Crystal Violet	Fluka®
Basic Violet 4	42600	BV4	Ethyl Violet	Fluka®
Basic Violet 10	45170	BV10	Rhodamine B	Sigma®
Pigment Blue 15	74160 :4	PB15	Irgalite Blue GLVO	Labor Dr. Ph. Bugnon
Reactive Red 106	-	RR106	Reactive Red 106	Town End®
Reactive Yellow 145	-	RY145	Reactive Yellow 145	Helion Chemie®
Solvent Black 3	26150	SB3	Neptun Schwarz X60	BASF®
Solvent Black 7	50415 B	SB7	Nigrosin B Alcohol Soluble	Fluka®
Solvent Blue 2	42563:1	SB2	Neptun Blaubase 634	BASF®
Solvent Blue 38	74180	SB38	Luxolechtblau MBS	Chroma®
Solvent Blue 136	-	SB136	Orasol Blau BL	Cibe Speciality Chemicals Inc®
Solvent Brown 1	11285	SB1	Fat Brown RR	Aldrich®
Solvent Green 1	42000:1	SG1	Malachite Green Carbinol Base	Adlrich®
Solvent Orange 3	11270	SO3	4-phenylazo-M-phenylenediamine	Sigma-Aldrich®
Solvent Red 49	45170	SR49	Rhodamine B Base	Aldrich®

Table 1 - Reference substances analyzed by LDI-MS positive and negative mode, and HPTLC, in order to identify the compounds detected in ballpoint pen entries.

Positive mode			
Ions			**RPA definition**
BV3	M^+	m/z = 372.2	$RPA_{372} = A_{372} / (A_{372} + A_{358} + A_{344})$
	$[M - Me]^+$	m/z = 358.2	$RPA_{358} = A_{358} / (A_{372} + A_{358} + A_{344})$
	$[M - 2Me]^+$	m/z = 344.2	$RPA_{344} = A_{358} / (A_{372} + A_{358} + A_{344})$
BV4	M^+	m/z = 456.3	$RPA_{456} = A_{456} / (A_{456} + A_{428})$
	$[M - Et]^+$	m/z = 428.3	$RPA_{428} = A_{428} / (A_{456} + A_{428})$
BB26	M^+	m/z = 470.3	$RPA_{470} = A_{470} / (A_{470} + A_{456})$
	$[M - Me]^+$	m/z = 456.2	$RPA_{456} = A_{456} / (A_{470} + A_{456})$
BB7	M^+	m/z = 478.3	$RPA_{478} = A_{478} / (A_{478} + A_{450})$
	$[M - Et]^+$	m/z = 450.3	$RPA_{450} = A_{450} / (A_{478} + A_{450})$
SB2	M^+	m/z = 484.3	$RPA_{484} = A_{484} / (A_{484} + A_{470})$
	$[M - Me]^+$	m/z = 470.3	$RPA_{470} = A_{470} / (A_{484} + A_{470})$
PB15	M^+	m/z = 575.1	$RPA_{475} = A_{575} / A_{575}$

Negative mode			
Ions			**RPA definition**
AB92	$[M - 2H]^{2-}$	m/z = 313.5	$RPA_{314} = A_{314} / (A_{314} + A_{628})$
	$[M - H]^-$	m/z = 628.0	$RPA_{628} = A_{628} / (A_{314} + A_{628})$
PB15	M^-	m/z = 575.1	$RPA_{575} = A_{575} / (A_{575} + A_{655})$
	$[M + SO_3H]^-$	m/z = 655.0	$RPA_{655} = A_{655} / (A_{575} + A_{655})$
SB38	M^-	m/z = 735.0	$RPA_{735} = A_{735} / (A_{735} + A_{815} + A_{895})$
	$[M + SO_3H]^-$	m/z = 815.0	$RPA_{815} = A_{815} / (A_{735} + A_{815} + A_{895})$
	$[M + 2SO_3H]^-$	m/z = 894.9	$RPA_{895} = A_{895} / (A_{735} + A_{815} + A_{895})$

Table 2 – Relative Peak Area (RPA) defined for dyes and pigments detected in the positive and in negative mode LDI-MS (A_i is the area of the peak at m/z = i*)*. These definitions describe the proportions of those molecules in the inks in regard to correlated compounds and were used to improve discrimination between different inks.

Pen nb.	BV3			BV4		BB26		BB7		SB2		PB15
	RPA_{372}	RPA_{358}	RPA_{344}	RPA_{456}	RPA_{428}	RPA_{470}	RPA_{456}	RPA_{478}	RPA_{450}	RPA_{484}	RPA_{470}	RPA_{575}
1	0.85	0.14	0.01	0.96	0.04	-	-	-	-	-	-	-
2	0.55	0.38	0.07	-	-	-	-	-	-	-	-	-
3	0.76	0.22	0.02	-	-	-	-	0.99	0.01	-	-	-
4	0.68	0.28	0.04	-	-	0.95	0.05	-	-	-	-	-
5	-	-	-	-	-	-	-	0.97	0.03	0.95	0.05	-
6	0.74	0.23	0.02	-	-	0.92	0.08	-	-	-	-	-
7	0.74	0.24	0.02	-	-	0.95	0.05	-	-	-	-	-
8	0.68	0.28	0.04	-	-	0.93	0.07	-	-	-	-	-
9	0.68	0.28	0.04	-	-	0.91	0.09	-	-	-	-	-
10	-	-	-	-	-	-	-	0.96	0.04	0.90	0.10	-
11	0.69	0.27	0.04	-	-	0.90	0.10	-	-	-	-	-
12	0.53	0.39	0.08	0.95	0.05	-	-	0.97	0.03	-	-	-
13	0.75	0.23	0.03	-	-	0.95	0.05	-	-	-	-	-
14	0.70	0.27	0.03	-	-	0.94	0.06	-	-	-	-	-
15	0.75	0.23	0.01	-	-	-	-	-	-	-	-	-
16	0.75	0.22	0.03	-	-	0.98	0.02	-	-	-	-	-
17	0.68	0.28	0.04	-	-	-	-	0.99	0.01	-	-	-
18	0.57	0.33	0.11	-	-	-	-	-	-	-	-	1.00
19	0.67	0.29	0.04	-	-	-	-	0.98	0.02	-	-	-
20	0.66	0.30	0.03	-	-	-	-	0.99	0.01	-	-	-
21	0.95	0.04	0.00	-	-	-	-	-	-	-	-	-
22	0.64	0.31	0.05	-	-	-	-	0.98	0.02	-	-	-
23	0.78	0.21	0.01	-	-	-	-	-	-	-	-	-
24	0.58	0.33	0.09	-	-	-	-	-	-	-	-	1.00
25	0.57	0.31	0.12	-	-	-	-	-	-	-	-	1.00
26	0.70	0.27	0.04	-	-	0.96	0.04	-	-	-	-	-
27	0.71	0.26	0.03	-	-	0.95	0.05	-	-	-	-	-
28	0.61	0.32	0.07	-	-	-	-	-	-	-	-	-
29	0.72	0.25	0.04	-	-	-	-	0.97	0.03	-	-	-
30	0.61	0.33	0.06	-	-	-	-	0.98	0.02	-	-	-
31	0.59	0.35	0.06	-	-	-	-	0.99	0.01	-	-	-
32	0.70	0.26	0.03	-	-	-	-	0.97	0.03	-	-	-
33	0.74	0.24	0.01	-	-	-	-	0.98	0.02	-	-	-
%	94%			6%		33%		39%		6%		9%

Table 3 – Identified species and corresponding RPA values in the 33 ballpoint inks analyzed by LDI-MS positive mode. 5 cationic dyes and 1 pigment were identified in ink positive spectra: Basic Violet 3 (BV3), Basic Violet 4 (BV4), Basic Blue 26 (BB26), Basic Blue 7 (BB7), Solvent Blue 2 (SB2) and Pigment Blue 15 (PB15). For the 5 cationic dyes, related species were also detected and identified. Numbers indicate the presence of dye/pigment peaks while the values represent the corresponding RPAs according to definitions in table 2.

Pen nb.	AB92		PB15		SB38		
	RPA_{314}	RPA_{628}	RPA_{575}	RPA_{655}	RPA_{735}	RPA_{815}	RPA_{895}
1	0.00	1.00	-	-	0.14	0.82	0.04
2	0.13	0.87	-	-	0.24	0.76	0.00
3	-	-	-	-	-	-	-
4	-	-	-	-	0.19	0.67	0.13
5	-	-	-	-	-	-	-
6	-	-	-	-	0.48	0.52	0.00
7	-	-	-	-	0.23	0.65	0.12
8	-	-	-	-	0.22	0.68	0.11
9	-	-	-	-	0.30	0.65	0.05
10	-	-	-	-	-	-	-
11	-	-	-	-	0.34	0.62	0.04
12	-	-	-	-	0.05	0.45	0.50
13	-	-	-	-	-	-	-
14	-	-	-	-	0.75	0.25	0.00
15	-	-	-	-	-	-	-
16	-	-	-	-	0.03	0.56	0.40
17	-	-	-	-	0.31	0.61	0.08
18	-	-	0.34	0.66	0.67	0.33	0.00
19	-	-	-	-	0.14	0.76	0.10
20	-	-	-	-	0.64	0.36	0.00
21	-	-	-	-	-	-	-
22	-	-	-	-	0.26	0.62	0.12
23	-	-	-	-	0.12	0.66	0.23
24	-	-	0.51	0.49	0.64	0.36	0.00
25	-	-	0.50	0.50	0.67	0.33	0.00
26	-	-	-	-	-	-	-
27	-	-	-	-	0.29	0.66	0.05
28	-	-	-	-	0.27	0.71	0.03
29	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-
%	6%		9%		64%		

Table 4 – Identified species and corresponding RPA values in the 33 ballpoint inks analyzed by LDI-MS negative mode. Generally, 2 anionic dyes and 1 pigment were identified in ink negative spectra: Acid Blue 92 (AB92), Pigment Blue 15 (PB15) and Solvent blue 38 (SB38). For PB15 and SB38, derivated products were sometimes detected and identified. Numbers indicate the presence of dye/pigment peaks while the values represent the corresponding RPAs according to definitions in table 2.

LDI-MS mode	**Identification dyes and pigments**	**RPA values**	**Additional unidentified signals**
Positive mode	78.4% [114]	87.9% [64]	98.9% [6]
Negative mode	64.0% [190]	79.2% [110]	99.2% [4]
Both modes	87.9% [64]	96.0% [21]	99.6% [2]

Table 5 – Discrimination power (DP) calculated in positive and negative mode using different levels of information (numbers in brackets are the pairs of pens that remain undiscriminated). Combining information of both modes did increase the DP values. The information yielded by additional signals should not be used in casework before they were identified.

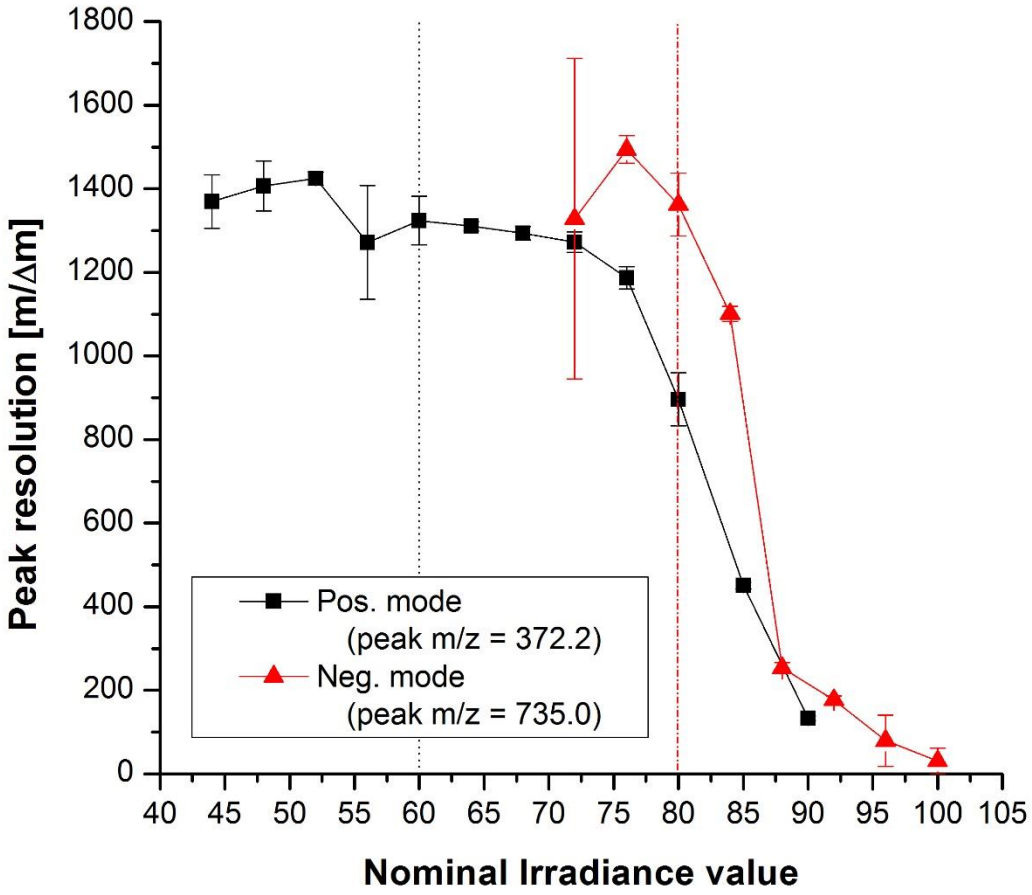


Figure 1 – Examples of irradiance effects on resolutions (m/Δm) of selected peaks. Peak resolution is decreasing as a function of nominal laser irradiance in LDI-MS positive and negative modes, represented here by molecular ions of dyes BV3 and SB38 respectively. The resolution was observed to decrease importantly when using laser irradiance values over 72 for positive and over 80 for negative mode respectively. Right dotted and left dash-dotted lines represent nominal irradiance values chosen to acquire ballpoint inks spectra in positive and negative modes respectively.

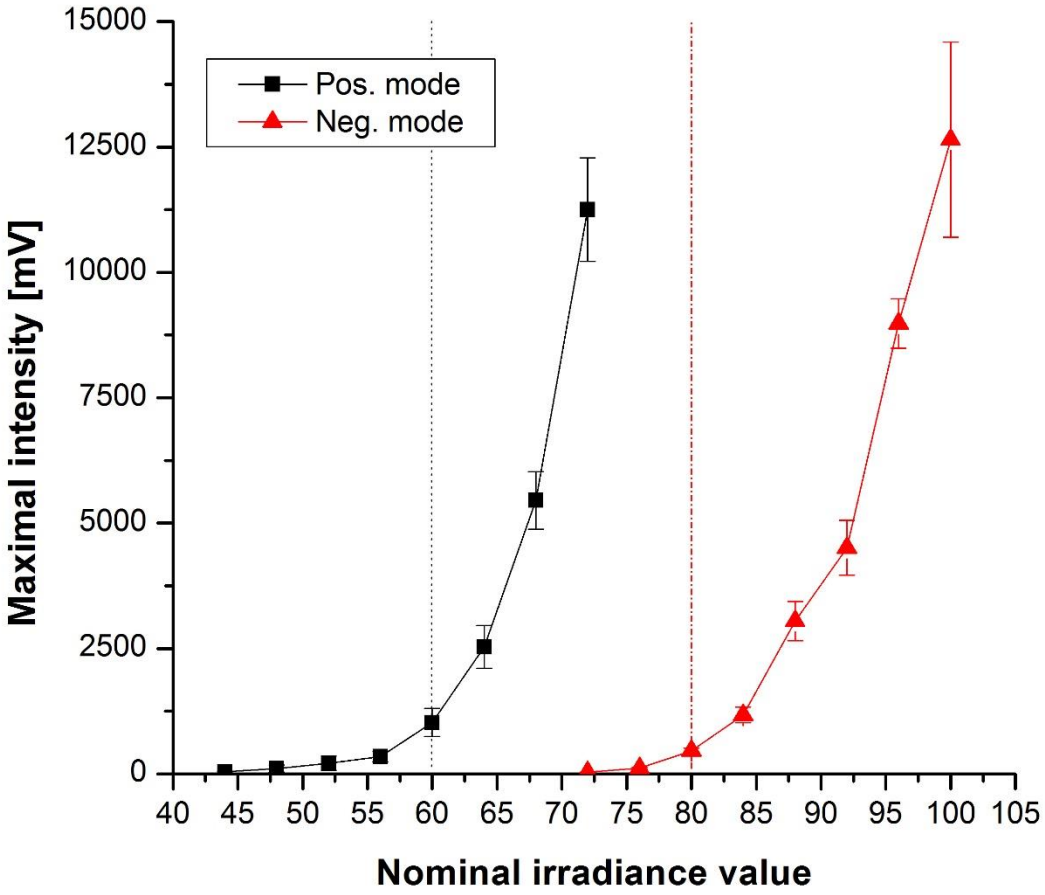


Figure 2 – Representation of irradiance effect on spectrum maximal intensity. Signal maximal intensity (mV) increases in function of nominal laser irradiance in LDI-MS positive and negative modes. Right dotted and left dash-dotted lines represent nominal irradiance values chosen to acquire ballpoint inks spectra in positive and negative modes respectively.

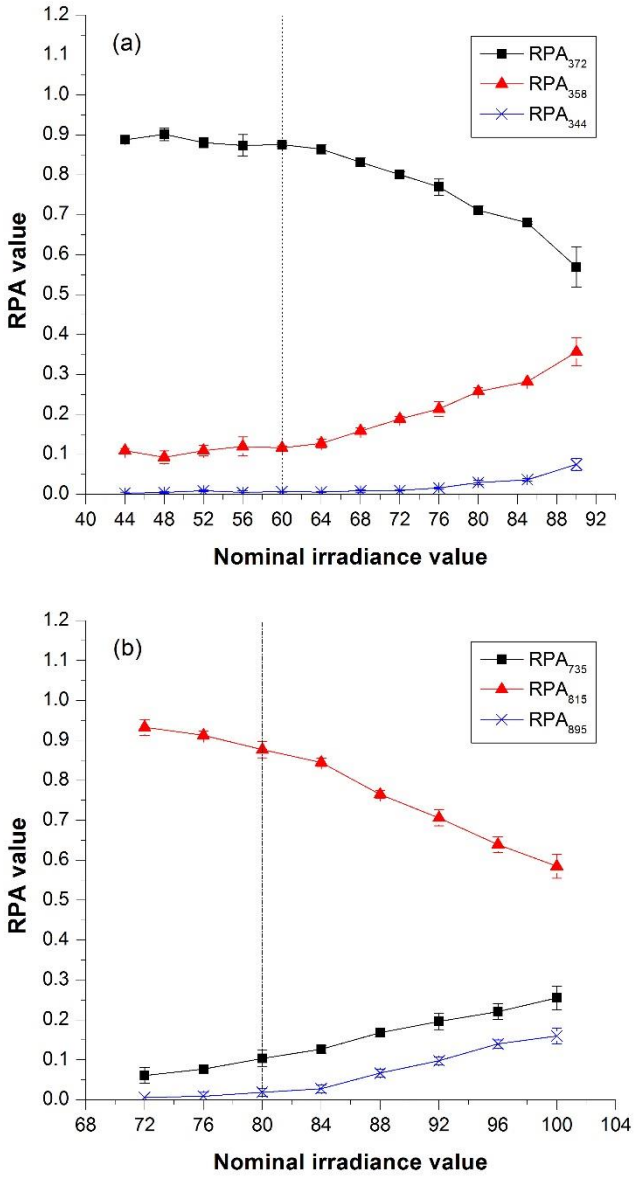


Figure 3 – Examples of irradiance effects on RPA values. Fig. (a) shows effects on BV3 in positive mode: RPA_{372} (BV3) decreased as a function of nominal laser irradiance, while RPA_{358} (BV3 – Me) and RPA_{344} (BV3 – 2Me) increased. This effect is particularly marked over a laser irradiance value of 64. Fig. (b) shows similar effects on SB38 in negative mode: RPA_{815} (SB38 + SO_3H) decreased as a function of nominal laser irradiance, while RPA_{735} (SB38) and RPA_{895} (SB38 + $2SO_3H$) increased. This effect is particularly marked over a laser irradiance value of 84. Dotted line in fig. (a) and dash-dotted line in fig. (b) represent nominal irradiance values chosen to acquire ballpoint inks spectra in positive and negative modes respectively.

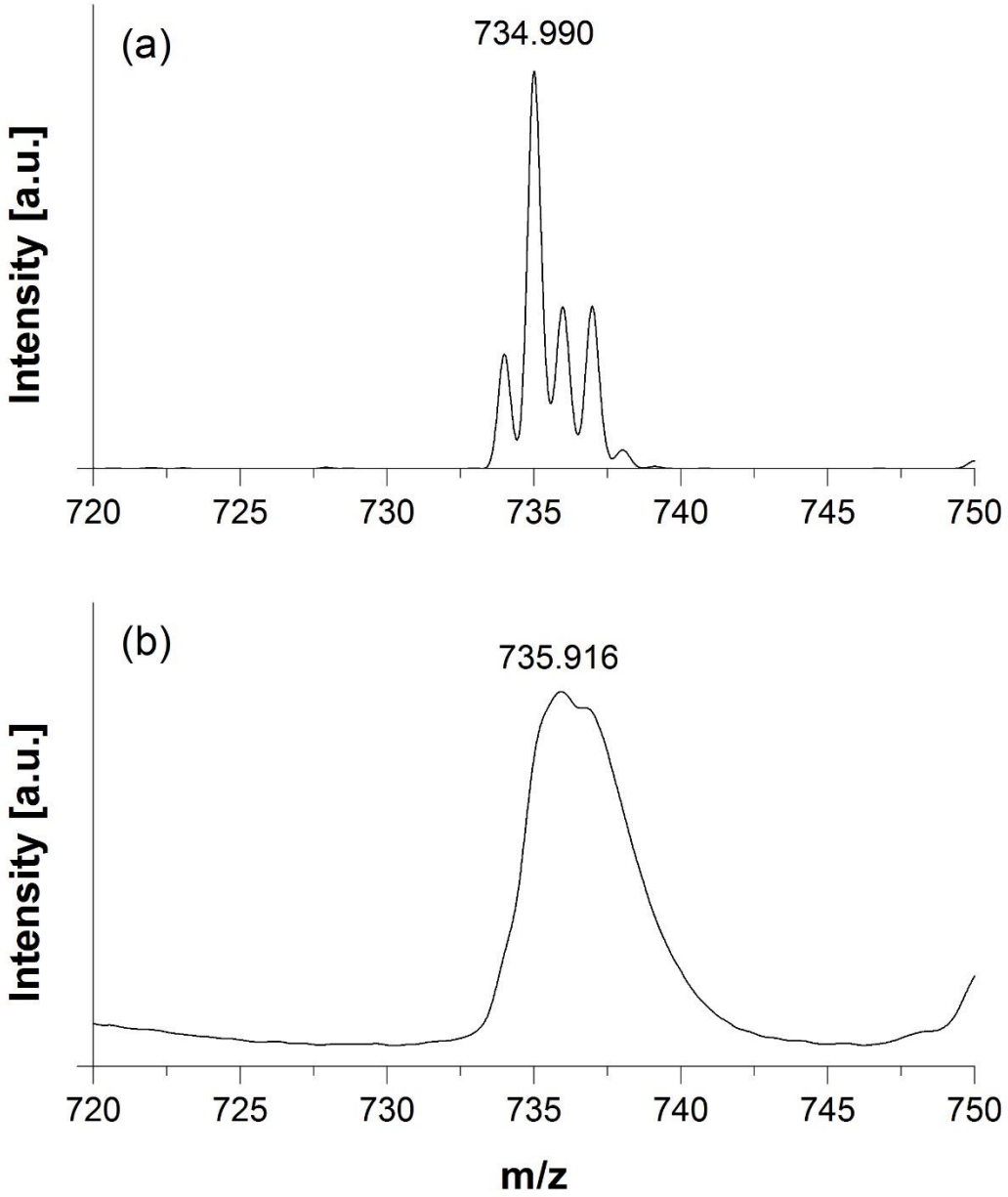
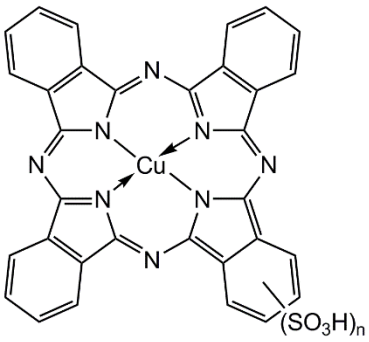
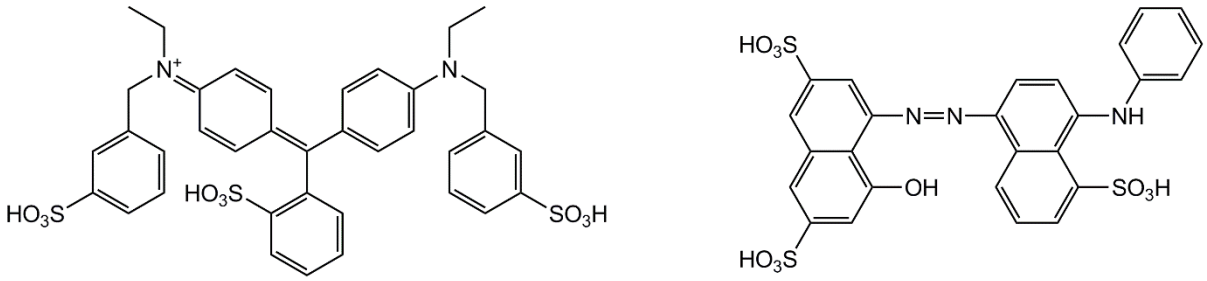


Figure 4 – Effects on signal resolution of SB38 peaks in LDI-MS negative mode when the nominal laser irradiance was increased from 80 (a) to 92 (b). In negative spectra, signal corresponding to SB38 is a mixture of its radical anion $[CuPc(SO_3H)_2]^-$ and the respective anionic species $CuPc(SO_3H)(SO_3^-)$. The summed isotopic pattern between this species is visible when laser irradiance is suitable (a). However, the isotopic information was lost when the laser irradiance was set too high and as a consequence only the average mass could then be determined (b).



Number of substitutions	Species names	Adopted abbreviation	Related peaks	Corresponding ions
n = 0	Pigment Blue 15	PB15	m/z = 575.1^{+} m/z = 575.1^{-}	$[CuPc]^{+}$ $[CuPc]^{-}$
n = 1	Mono-sulfonic acid derivative of PB15	PB15 + SO_3H	m/z = 655.0^{-} m/z = 654.0^{-}	$[CuPc(SO_3H)]^{-}$ $CuPc(SO_3^{-})$
n = 2	Di-sulfonic acid derivative of PB15 or Solvent Blue 38	SB38	m/z = 735.0^{-} m/z = 734.0^{-}	$[CuPc(SO_3H)_2]^{-}$ $CuPc(SO_3H)(SO_3^{-})$
n = 3	Tri-sulfonic acid derivative of PB15 or Mono-sulfonic acid derivative of SB38	SB38 + SO_3H	m/z = 815.0^{-} m/z = 813.9^{-}	$[CuPc(SO_3H)_3]^{-}$ $CuPc(SO_3H)_2(SO_3^{-})$
n = 4	Tetra-sulfonic acid derivative of PB15 or Di-sulfonic acid derivative of SB38	SB38 + $2SO_3H$	m/z = 894.9^{-} m/z = 893.9^{-}	$[CuPc(SO_3H)_4]^{-}$ $CuPc(SO_3H)_3(SO_3^{-})$

Figure 5 – Structure of copper phthalocyanines (CuPc) derivatives identified in blue ballpoint inks together with m/z values of corresponding ions. The difference between these molecules lies in the number of sulfonic acid functional groups added to the basic structure.



Acid Blue 9 (AB9)
mono-anionic species: m/z = 748.2
di-anionic species: m/z = 373.6

Acid Blue 92 (AB92)
mono-anionic species: m/z = 628.0
di-anionic species: m/z = 313.5

Figure 6 – Structure of two dyes detected in inks using LDI-MS negative mode, together with theoretical m/z values of corresponding negative ions.

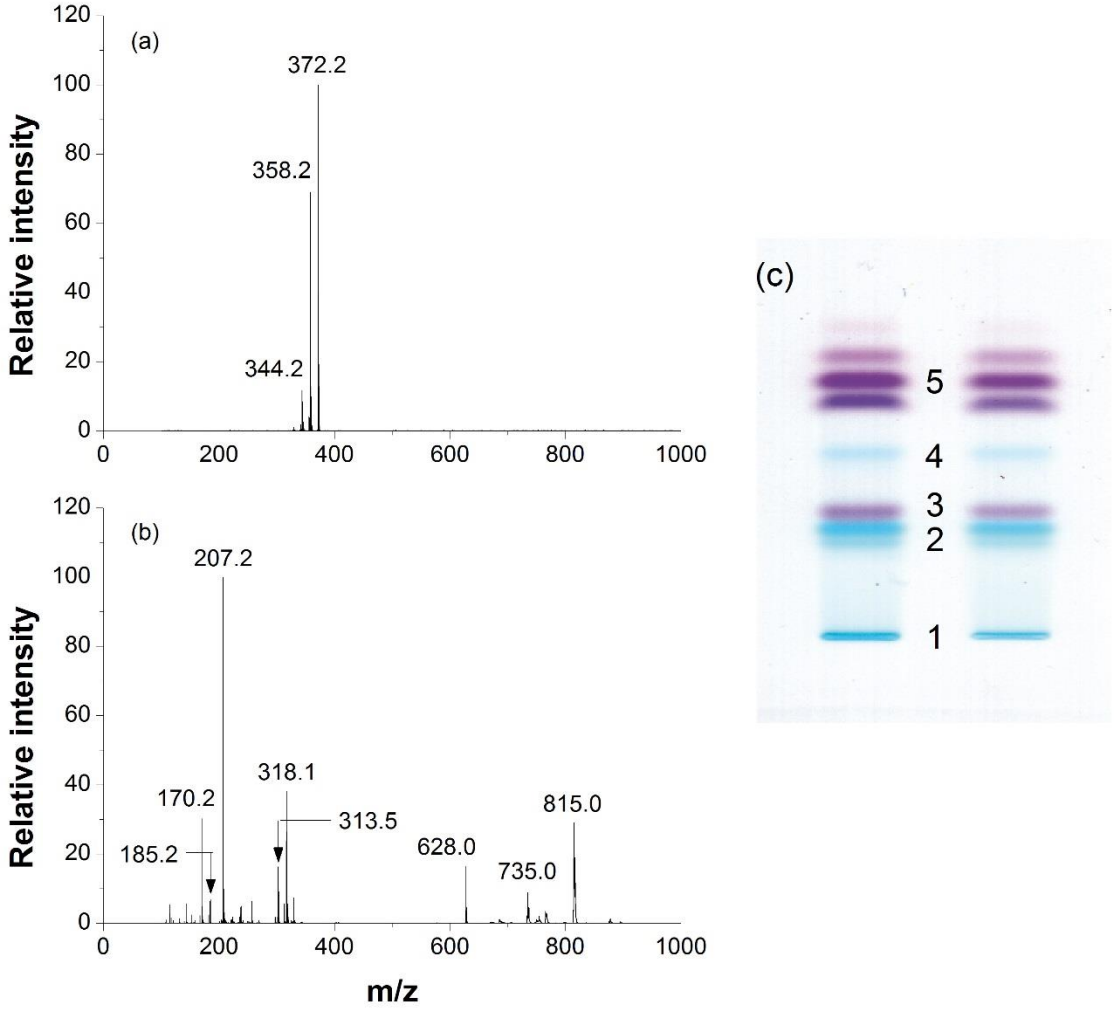


Figure 7 – LDI positive (a) and negative (b) mass spectra of ballpoint pen #2. In positive mass spectrum, only the dye BV3 and its fragmentation products were identified (m/z = 372.2, 358.2 and 344.2). On the other hand, in negative mass spectrum numerous dyes were present: AB9 (m/z = 170.2 and 185.2), AB92 (m/z = 313.5 and 628.0), SB38 (m/z = 735.0) and its mono-sulfonic acid derivative (m/z = 815.0). Fig (c) shows the HPTLC plate of the same ink. The dye SB38 (spot 1), AB 9 (two spots 2), AB 92 (spot 3) and BV3 (three spots 5) were identified by comparison of the retention times and colors with reference substances. While the retention time of spot 4 did correspond to the reference substance BG4, the identification could not be confirmed by LDI-MS results.