Statistical discrimination of black gel pens inks analyzed by laser desorption/ionization mass spectrometry

Abstract

Pearson correlation coefficients were applied for the objective comparison of 30 black gel pen inks analysed by laser desorption ionisation mass spectrometry (LDI-MS). The mass spectra were obtained for ink lines directly on paper using positive and negative ion modes at several laser intensities. This methodology has the advantage of taking into account the reproducibility of the results as well as the variability between spectra of different inks. A differentiation threshold could thus be selected in order to avoid the risk of false differentiation. Combining results from positive and negative mode yielded a discriminating power up to 85%, which was better than the one obtained previously with other optical comparison methodologies. The influence of brands was also found to be minimal.

Keywords: Gel pens, Ink comparison, discriminating power, statistical treatment, LDI-MS

1. Introduction

Discrimination of inks on questioned documents is a particularly important issue of forensic document examination. The analysis of ink entries may indeed highlight a fraud, for example in the form of an addition with a different ink on the examined questioned documents. For this reason, recent developments in this field primarily focused on improving the discrimination power of ink analysis methods. In recent years, new techniques have been investigated for the analysis of inks, such as Raman Spectroscopy [1-4], X-Ray Fluorescence (XRF) Spectroscopy [3], Scanning Electron Microscopy (SEM) [2], High Performance Liquid Chromatography (HPLC) [5-8], Mass Spectrometry (MS) [9-15,6], Capillary Electrophoresis (CE) [16], and Inductively Coupled Plasma (ICP) -MS [17,18]. Furthermore, statistical treatments of the data have been additionally proposed in order to improve the analysis and the interpretation of the results [19-22,10,23,8].

While many analytical methods were found appropriate for dye-based inks discrimination, few practical solutions were investigated for pigmented inks, such as gel pens [17,5,2,18,10,24,25,3]. Gel pens were first marketed in Japan in the mid 1980s and only recently became widely used in Europe [26]. While gel pen inks sometimes also contain dyes, they are mostly composed of pigments and, as a result, cannot be usually analyzed by Thin Layer Chromatography (TLC). It was also previously observed that gel pens inks were relatively difficult to differentiate in comparison to ballpoint pen inks [17,18,3].

Positive and negative mode laser desorption ionisation (LDI) - MS were previously used to efficiently discriminate blue gel pens inks [10] and was therefore selected in this work for the analysis of 30 black gel pens inks. The data extracted from the LDI-MS spectra were then compared using a statistical method based on Pearson correlation measurements allowing for an objective comparison and ensuring that no false differentiation occurred [10,21]. As most of the samples had already been analysed in a previous study, the discriminating power (DP) was additionally compared to that obtained using optical methods (such as Video Spectral Comparison (VSC) and Micro Spectrophotometry (MSP) [17]). The issue of brands (i.e., different gel pen manufacturer) and reproducibility of the results were also particularly investigated in order to evaluate the practical potential of the proposed method.

2. Experimental

2.1. Samples

Among the 30 black gel pen inks used in this study, 25 were bought in Australia in 2007 for a previous study [17] and 5 were bought in Switzerland in 2009. A solubility test using methanol showed that most pens contained pigments (Table 1).

The gel pens were used to draw lines on white sheets of copy paper Xerox (80g/m^2). For each sample a small piece of paper containing the ink line was cut and fixed to a solid steel sample plate using a solvent free glue (UHU® stic, Bühl, Switzerland).

2.2. LDI-MS

Mass analyses were carried out on a Bruker Daltonics AutoFlex matrix assisted laser-desorption/ionization reflector time-of-flight (MALDI-TOF) mass spectrometer equipped with a pulsed nitrogen laser (337 nm). Samples were analysed directly without addition of matrix. Mass spectra of the samples were recorded in positive and negative ion modes. Mass spectra were generated by averaging 50 laser pulses along the ink strokes in positive mode (m/z from 50 to 1000 u.), and 100 laser pulses in negative mode (m/z from 25 to 750 u.).

The instrument was calibrated using an ink line of a blue ballpoint pen (BIC Cristal) with known composition of dyes determined in previous studies [27,15]. The signals used for calibration in positive mode were the ions at 372.2 and 358.2 m/z generated by the dye basic violet 3 (BV3) and the peaks at 456.3 and 428.3 m/z of the dye basic violet 4 (BV4). In negative mode the ink of another blue ballpoint pen (Faber-Castell Graf) was used to calibrate the instrument by taking into account the mass signals at 814.0, 734.0 and 654.0 m/z produced by the dye solvent blue 38 (SB38).

Signal intensity and peak resolution were used to determine the optimal laser intensities. This was more difficult to achieve for the gel pen inks in the present study than it was for ballpoint inks in previous studies [28,29]. Therefore three laser intensities were selected in order to cover the best conditions for all gel pen inks analyzed (i.e., for the instrument used in this study 40, 60 and 80% in positive and 40, 65 and 90% in negative mode respectively). In a first step, this enabled to comprehensively study the mass spectra of each gel pen inks in the given optimal laser intensity for the specific ink. In a second step, it was then possible to compare the mass spectra from different gel pen inks using the same analysis conditions. For each of the laser intensities three sets of measurements were performed along the ink lines in positive and negative ion modes (i.e. 3 replicate analyses x 3 laser intensities x 2 modes = 18 mass

spectra per gel pen inks). Blank measurements were performed as well to determine if there was any contribution of the paper or glue interfering with the mass spectra of the ink samples.

2.3. Statistical treatment

The data was treated with PASW statistics 18 (Mathsoft, Inc.), Microsoft Excel (Microsoft Corporation) and OriginPro 8.1 SR3 (OriginLab Corporation). The raw data of the mass spectra were extracted in text files (corresponding to more approximately 60'000 data point per mass spectra covering mass from 0 to 1000 kDa). Standardization (subtracting by the mean and dividing by the standard deviation) was used as pre-treatment to reduce the influence of different absolute scales from one analysis to another [10]. The similarity between the different spectra has been measured using Pearson correlation coefficient [30,31,10] (Figure 1). The Pearson values range from -1 (anti-correlated) to 1 (correlated). Calculations yield two sets of results for each configuration (hypothetical example in Figure 1):

- the intra-variability distribution; i.e., correlation values between the 3 replicate analyses from the same ink sample = 90 Pearson values (see plain bars in Figure 1)
- the inter-variability distribution; i.e., correlation values between ink analyses from 30 different pens = 435 Pearson values (see empty bars in Figure 1).

A Pearson decision threshold could then be estimated in order to minimize the number of false negative (FN) or/and false positive (FP) (see threshold line on Figure 1).

In order to compare the separations obtained between the two sets of results, Receiver Operating Characteristic (ROC) curves were used. A ROC curve is represented by the sensitivity (true positive fraction) as a function of 1-specificity (false positive fraction). The area under the curve (AUC) quantifies the overlapping degree of the distribution of the two populations. Ideally, if the two distributions do not overlap and a ROC value of 1 is obtained [31-33]. The ROC curves also help choosing a decision threshold minimizing the false negative rate and enables to calculate the discriminating power (DP) for the given threshold (Table 2) The DP of the technique is calculated according to the following equation [15]:

$$DP = 1 - \frac{2M}{n(n-1)} \qquad (1)$$

where *M* is the number of non-discriminated pairs of samples and *n* is the total number of samples. The DP is a measurement of the selectivity of the method to differentiate the gel pen inks analyzed.

3. Results and discussion

LDI-MS spectra from the black gel pen inks were acquired both in the positive and negative ion mode in three different laser intensities (see examples Figure 2). While some inks yielded good spectra with a low intensity (e.g., 40%), other inks necessitated a higher intensity to yield any signal (e.g, 90%). In order to compare these spectra objectively and based on the whole data set, Pearson correlation calculations were calculated between spectra from the same pen (i.e., intra-variability, 90 values) and from different pens (i.e., inter-variability, 435 values).

The respective couple of distributions (see example in Figure 3) were then compared using ROC analyses in order to evaluate their overlapping areas (see example in Figure 4): the closer to 1 the area under the curve (AUC), the better the separation. Note that only data acquired in the same analytical conditions (e.g., positive mode using laser intensity of 60%) were compared. To decrease the influence of large peaks standardization was applied to the data, which led to a significant improvement of the discrimination both in the negative and positive ion mode (Figure 4).

The best separation was actually obtained for standardised data acquired in the positive ion mode with a laser intensity of 60% (AUC of 0.972), followed by standardised data acquired in the negative ion mode with a laser intensity of 65% (AUC of 0.964) (see Table 3). ROC analyses also yielded information on the discriminating power (DP) and the false negative rate as a function of Pearson correlation values (Figure 5).

The main objective in the comparison of ink samples was to minimize the number of false negatives (FN), i.e. avoiding a false differentiation of questioned ink samples (Table 2). In fact, false differentiations yield the risk of judicial errors (e.g. false conclusion that an ink entry was added with a different pen on a document). On the other hand, it is also important to minimize the false positive (FP) rate in order to obtain an optimal DP for the method, i.e. minimizing the non differentiation of different ink samples (Table 2). However, false non differentiations are relatively normal in ink analysis and its consequence is not so significant (e.g. ink formulation is not so variable and many different pens are actually filled with the

same ink preparation). Thus from the data of the ROC curves, it was possible to extrapolate the DP (or the specificity of the method) for a 0% FN rate (or 1-sensitivity) (Table 3). For example, in the positive mode the best separation yielded a minimum Pearson value of 0.135 for the overlapping area with a DP of 71%. This also meant that 29% FP were recorded (i.e. different samples that were not differentiated). In the negative mode the best separation gave a minimum Pearson value of 0.158 with a DP of 60%. When attempting to increase the DP, the risk of false differentiation increased rapidly (see increase of false negative in Figure 5).

Combining the information yielded by the two analysis modes led to a significantly increased discrimination, thus showing the complementary of the data. In fact 60 pairs differentiated in the negative ion mode were not differentiated in the positive mode, while 109 pairs of ink samples were only differentiated in the positive mode. This meant that a total of 368 pairs could be differentiated when combining the results obtained in the two analysis mode for a total DP of 85% (Table 4). For example comparison of the pair of samples 2 and 25 (Figure 1) yielded a Pearson value above the differentiation threshold (i.e., 0.372 > 0.158 in Table 3) and was thus not differentiated in the negative ion mode, while the Pearson value obtained in the positive mode allowed clear differentiation of the sample (i.e., -0.173 < 0.135 in Table 3).

This method was slightly more efficient for blue gel pens (i.e., combined DP of 92%[10]) than for black gel pens (i.e., combined DP of 85%). This may be due to the fact that black pigmented inks are generally known to be less differentiable than blue inks [34,3]. Three pens also contained dyes (#14, 15, 20 and 26 in Table 1). If there were subtracted from the dataset, the combined discriminating power decreased to 82%, which is still very efficient compared to other methods [17]. The DP obtained by LDI-MS on 25 of the black gel pens inks (AUS in Table 1) could be compared to the results obtained with routine methods such as Video Spectral Comparator (VSC) and Microspectrophotometry (MSP) performed on the same inks in a previous study [17]. DP of 49% and 74% were obtained for VSC and MSP respectively, while LDI-MS yielded a higher DP of 82%. These results also shows the influence of sample type and size on the DP value, as it is slightly lower than for the 30 black gel pens all together (see value of 85% in Table 3). This can be explained by the fact that the excluded inks (#14, 17, 20, 21 and 27) were relatively well discriminated, while the 25 selected inks included some inks that were less discriminated (e.g., # 5 or 30).

Moreover, several black gel inks analysed were actually from pens of the same brands. Comparison within inks of the same brands was therefore carried out in order to evaluate its influence on the separation. It was observed that inks from the same brand gave only slightly higher Pearson values (Figure 6) and the obtained discriminating power would not vary

significantly. When attempting to differentiate within pen distribution (black boxplots in Figure 6), the same DP values were obtained in comparison to the within brand distribution (red boxplots in Figure 6) than in comparison to the between pen distribution (blue boxplots in Figure 6).

The main issue of this method relates to the reproducibility of the results. LDI-MS spectra of the same ink may often show significant differences in the absolute and relative peak intensities. Thus, even when no important difference was visually observed between replicate spectra (see in Figure 7), the Pearson correlation values sometimes showed large variations (see examples in Table 5). As the differentiation threshold was fixed to avoid false differentiation, this poor reproducibility had a non-negligible influence on the DP.

While most comparison within spectra from the same ink yield expected high correlation values (e.g., 0.90), a few inks yield very low correlation (e.g., down to 0.134). This showed the fact that some ink yielded unreproducible spectra. One has therefore to take into account that fact before comparing spectra from different ink entries. Thus if the comparison of spectra for the same ink already yield unexpected low correlation values, the proposed method cannot be applied to further compare this ink with another ink entry. In this perspective, a higher number of replicate analyses would definitely be needed in order to identify outliers (i.e., due to a non-reproducible analysis) and/or inhomogeneous samples (i.e., incomparable spectra due to ink or paper local variations).

One should also take this into account when comparing two different gel pens inks. Instead of comparing only one spectrum from ink 1 to one spectrum from ink 2, all spectra could be compared (i.e., 3 replicate analysis for each pen each yielding 9 Pearson correlations values for the comparison of two ink entries). Ideally, the 9 obtained Pearson values should be under or above the threshold values (i.e., differentiated or undifferentiated inks, respectively). In practice, however, it happened that the values for the comparison of two samples were found both under and above the threshold (see example in Table 6).

In such cases, the decision to differentiate samples is not straightforward and several options were evaluated for the positive ion mode (see Table 7, standardised data, laser intensity 60%). Conservatively, to minimize the risk of false differentiation, one could decide that all 9 Pearson value must be above the threshold (all boxes must be coloured in Table 6). This would actually lower the DP to only 48% (instead of 71% calculated in Table 3). On the other hand, one could also accept the possibility of outliers (e.g., up to 3 outliers in Table 3) to differentiate ink samples. This option would yield a DP of 69%. Finally, it cannot be

considered acceptable to have more outliers (e.g., up to 8 outliers would actually yield a DP of 86%, however with a false differentiation rate of 3%).

These observations confirmed the importance of replicate analyses in the comparison process. It is therefore strongly advised to carry out more replicate analyses. Using LDI-MS it generally would not be a problem as the technique does not require a large amount of sample and is only semi-destructive. More replicate analysis would thus enable the detection of outliers and/or inhomogeneous ink samples, and may potentially improve overall DP of the method.

4. Conclusion

The proposed method enabled an objective comparison of LDI-MS mass spectra of 30 black gel pens inks using Pearson correlation coefficient following standardization. The statistical comparison was found to be very efficient to distinguish between the inks considered in this study. Additionally to being objective, this approach did include the intra-variability as part of the procedure and it was possible to select a decision threshold ensuring that no false differentiation occurred. The best DP of 85% was thus obtained when combining the comparison of the mass spectra acquired in the positive and negative ion modes. While the DP was slightly lower than the one obtained for blue gel pen inks (i.e., DP of 92%), it was higher than that obtained using other methods such as VSC (i.e., 49%) and MSP (i.e., 74%) for the analysis of the same black gel pen inks in a previous study. Furthermore, gel pens of the same brands could be well discriminated. The proposed statistical approach can be applied to data obtained by any analytical method, allowing more objectivity, quicker comparison of the data and fewer false negatives. The comparison can also be easily automatised. The robustness of the method should now further be evaluated by acquiring a higher number of replicate analyses to evaluate the reproducibility of the decision threshold for differentiation.

5. Acknowledgement

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Figure 1 – Representation of an hypothetical configuration when comparing the Pearson values obtained through comparison of replicate spectra from the **same ink (plain green bars)** with the values obtained through comparison of spectra from **different inks (empty red bars)**: usually some overlapping exists between the values and a decision threshold must be selected in order to minimize the number of **false negative (FN, plain green bars on the left of the threshold line)** and the number of **false positive (FP, empty red bars on the right of the threshold line)**.

Figure 2 –LDI-MS spectra of gel pens inks # 2, 23 and 25 in positive mode using a laser intensity of 60% (left) and negative mode using a laser intensity of 65% (right).

Figure 3 – Distribution of the Pearson values obtained when comparing spectra from different pens (above) and replicate spectra from the same pen (below). Pearson calculations were performed on standardised data obtained by LDI-MS using a laser intensity of 60% in the positive mode (right) and 65% in the negative mode (left).

Figure 4 –ROC curves of the overlapping area of four sets of distributions (summarized in table 2): raw (dots) and standardized (straight line) data for the positive mode using a laser intensity of 60% (black) and for the negative mode using a laser intensity of 65% (grey). The best separations were clearly obtained for the standardized data.

Figure 5 – False negative rate and discriminating power as a function of the Pearson value. The decision threshold was selected at 0% false negative (i.e., no false discrimination when comparing two ink entries). The false negative rate increased very rapidly with the discriminating power (black line).

Figure 6 – Distribution of correlation values obtained for the comparison of standardised spectra from the same pen, same brand, different brands and different pens analysed in the positive ion mode (left; laser intensity of 60%) and in the negative ion mode (right; laser intensity of 65%). The influence of the brands on correlation values was minimal.

Figure 7 – Replicate LDI-MS spectra of gel pens ink 25 in positive mode. While the 3 spectra look alike, the Pearson correlation coefficients were not very high: the lowest value was actually obtained for the comparison of replicate 1 and 3 (i.e., threshold of 0.135).

Table 1 - List of black gel pen inks analyzed by LDI-MS. 25 five pens where bought in Australia (AUS) in 2007 for a previous study [17], the remaining five were bought in Switzerland (CH) in 2009.

Table 2 – Explanation of the designation used to classify Pearson values obtained for the ink comparisons using ROC curves.

Table 3 – Values extrapolated from the ROC curves on Pearson values: Area under the curve (AUC), discriminating power (DP) and Pearson threshold value for a 0% false negative rate. The best discrimination was obtained for standardized data using a laser intensity of 60% (positive mode) and 65% (negative mode).

Table 4 – Number of pairs differentiated and discriminating power (DP) as a function of the analysis mode for standardised data in positive ion mode (laser intensity of 60%) and negative ion mode (laser intensity of 65%).

Table 5 – Discriminating power (DP) of VSC examination and MSP for the comparison of 25 five black gel pens [17] (AUS) compared to the DP obtained on the same pens with LDI-MS (positive and negative ion modes combined).

Table 6 –Example of Pearson correlation values obtained for comparison of replicate spectra from gel pen 25 with gel pen 2 (negative ion mode, laser intensity 65%). As expected, seven values (coloured boxes) were under the differentiation threshold (< 0.158, differentiated). Two values (bold) were however above the threshold (> 0.158, non-differentiated).

Table 7 – Number of pairs differentiated and discriminating power (DP) as a function of the number of replicate spectra differentiated between two inks (for each comparison, 9 replicate Pearson values were obtained) for standardised data obtained in positive ion mode with a laser intensity of 60%.

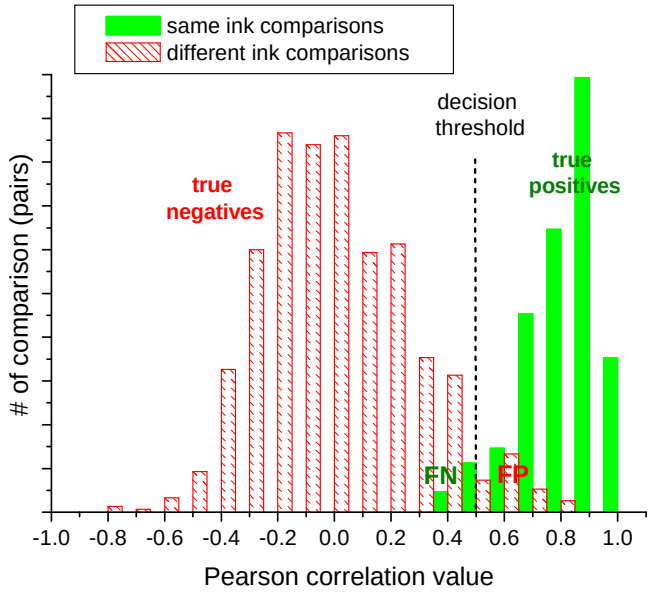
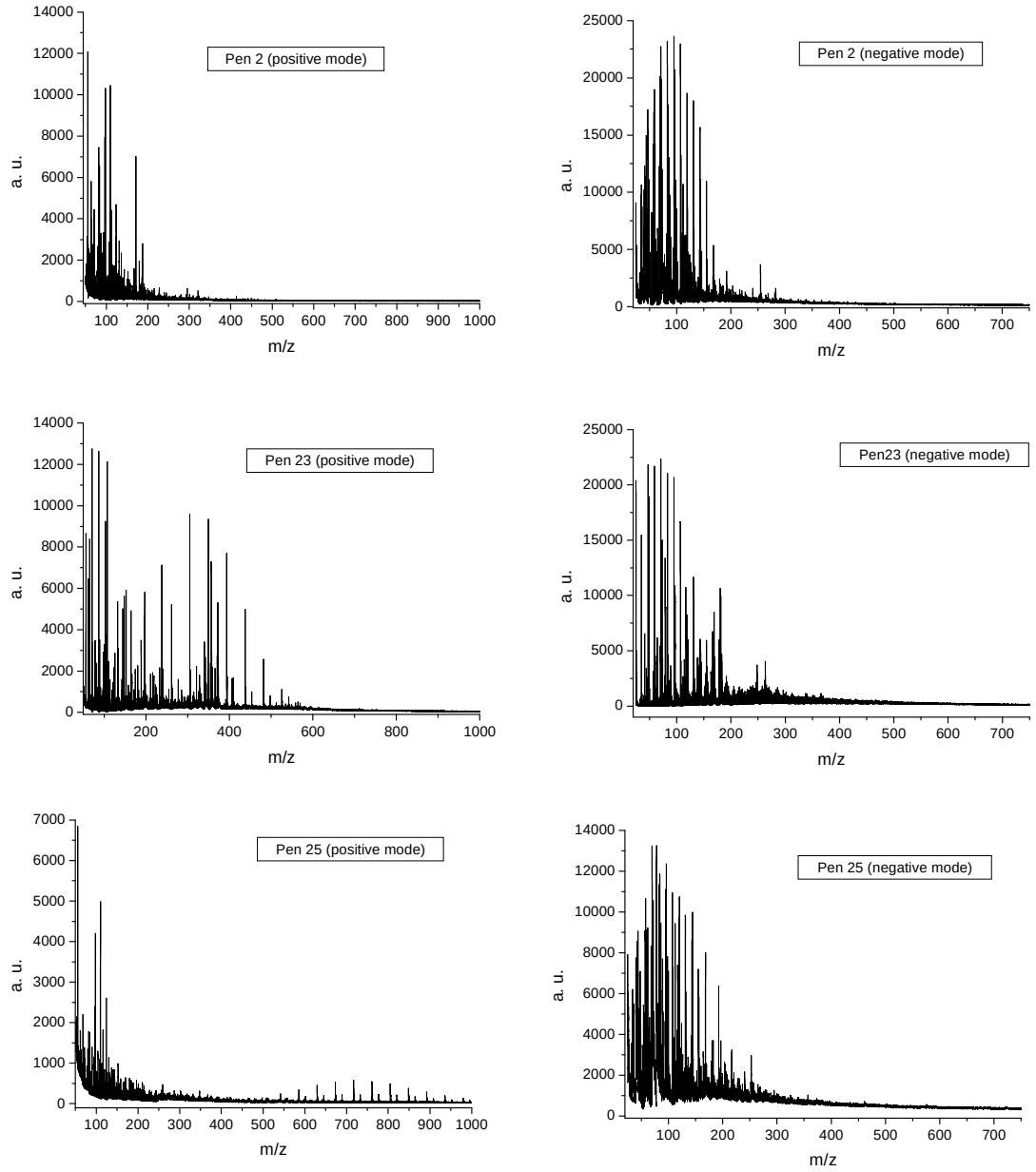


Figure 1 – Representation of an hypothetical configuration when comparing the Pearson values obtained through comparison of replicate spectra from the **same ink (plain green bars)** with the values obtained through comparison of spectra from **different inks (empty red bars)**: usually some overlapping exists between the values and a decision threshold must be selected in order to minimize the number of **false negative (FN, plain green bars on the left of the threshold line)** and the number of **false positive (FP, empty red bars on the right of the threshold line)**.



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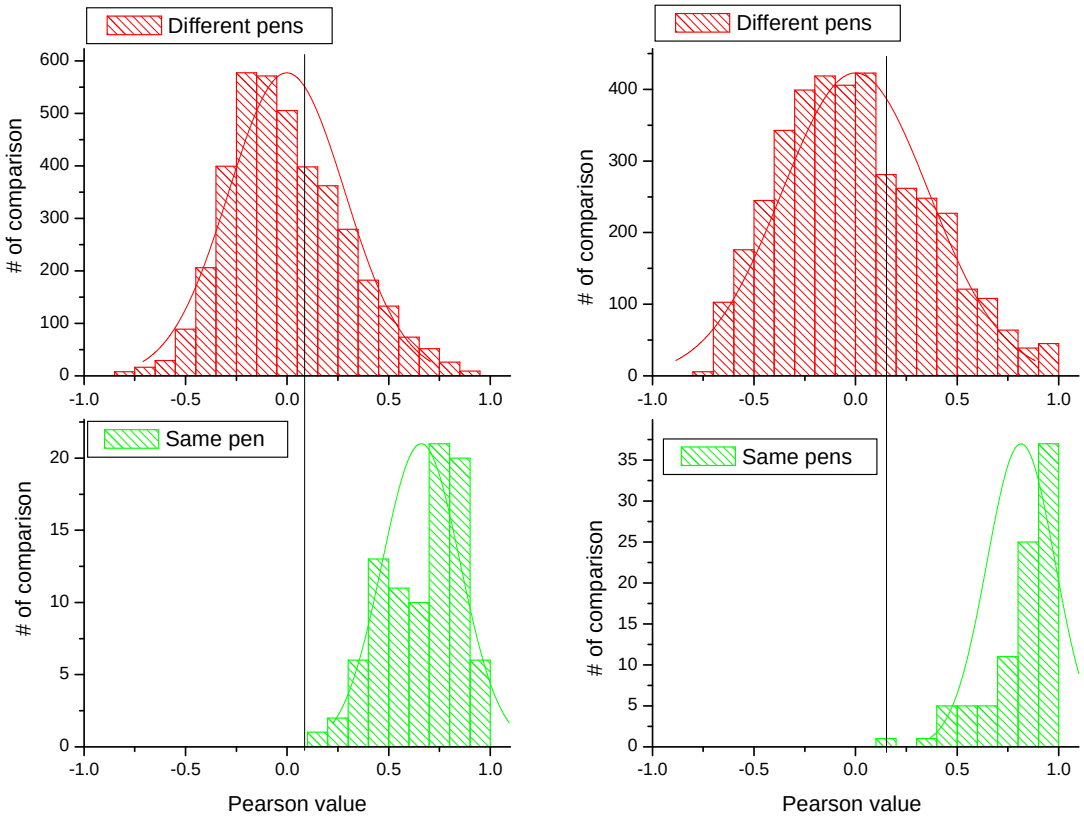


Figure 2 – Distribution of the Pearson values obtained when comparing spectra from different pens (above) and replicate spectra from the same pen (below). Pearson calculations were performed on standardised data obtained by LDI-MS using a laser intensity of 60% in the positive mode (right) and 65% in the negative mode (left).

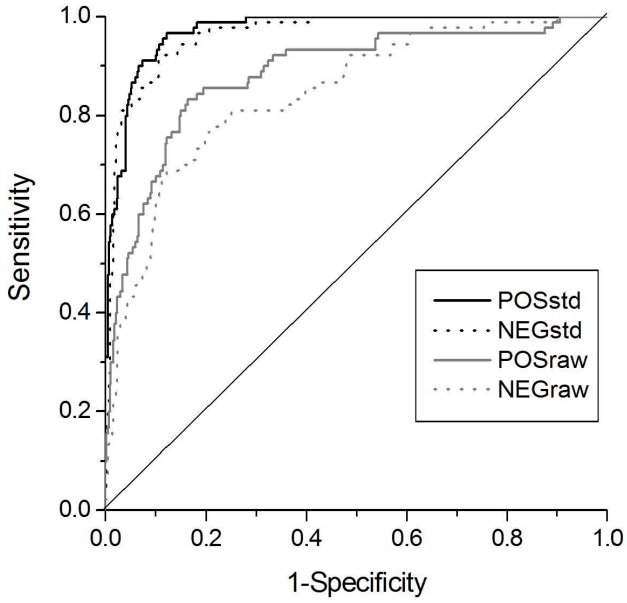


Figure 4 –ROC curves of the overlapping area of four sets of distributions (summarized in table 2): raw (dots) and standardized (straight line) data for the positive mode using a laser intensity of 60% (black) and for the negative mode using a laser intensity of 65% (grey). The best separations were clearly obtained for the standardized data.

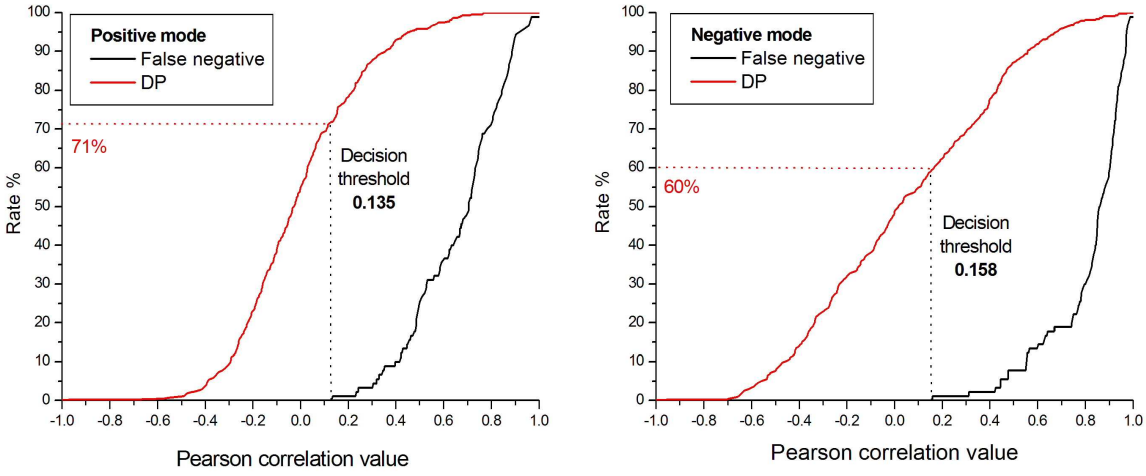


Figure 5 – False negative rate and discriminating power as a function of the Pearson value. The decision threshold was selected at 0% false negative (i.e., no false discrimination when comparing two ink entries). The false negative rate increased very rapidly with the discriminating power (black line).

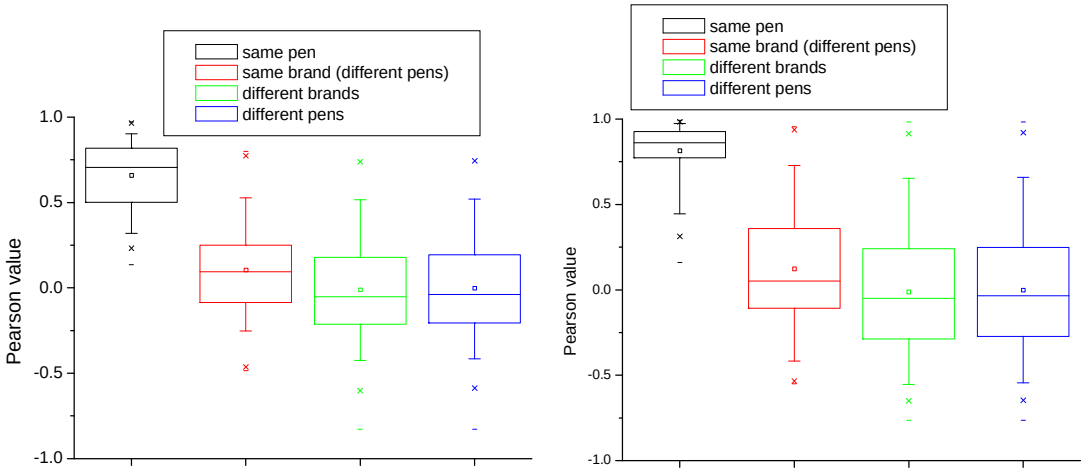
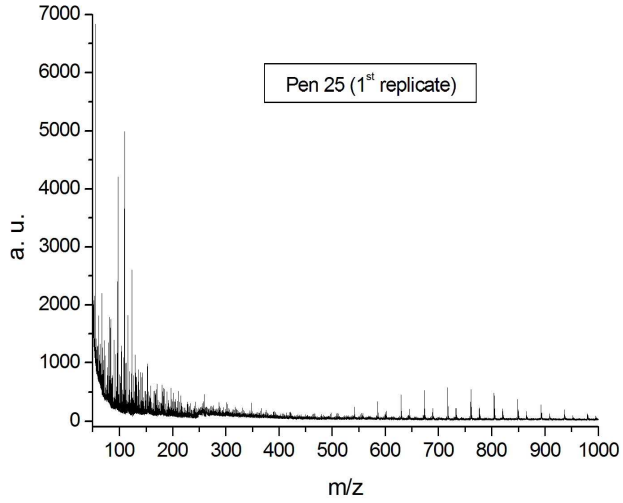
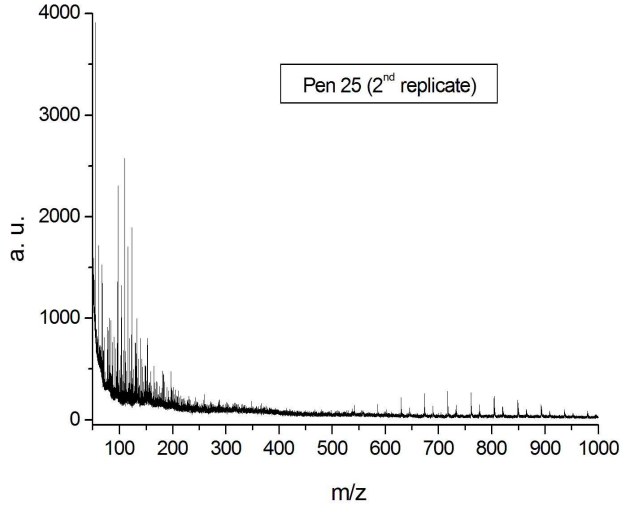


Figure 6 – Distribution of correlation values obtained for the comparison of standardised spectra from the same pen, same brand, different brands and different pens analysed in the positive ion mode (left; laser intensity of 60%) and in the negative ion mode (right; laser intensity of 65%). The influence of the brands on correlation values was minimal.





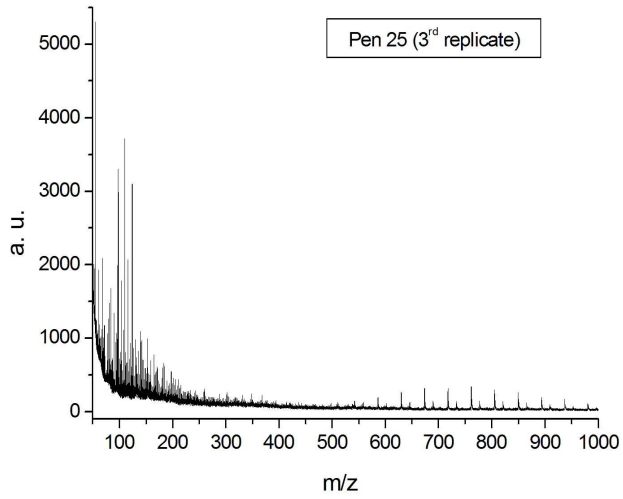


Figure 6 – Replicate LDI-MS spectra of gel pens ink 25 in positive mode. While the 3 spectra look alike, the Pearson correlation coefficients were not very high: the lowest value was actually obtained for the comparison of replicate 1 and 3 (i.e., threshold of 0.135).

#	Brand	Colorant type	Origin
1	Artline Geltrac	pigments	AUS
2	Artline Drawing System	pigments	AUS
3	Artrite Black Gel Pen	pigments	AUS
4	BIC Cristal Gel Roller	pigments	AUS
5	BIC Intensity Clic	pigments	AUS
6	BIC Velocity Gel	pigments	AUS
7	Gelerations	pigments	AUS
8	Papermate Gel Glide	pigments	AUS
9	Papermate Gel Grip	pigments	AUS
10	Papermate Gel Roller II	pigments	AUS
11	Parker Black Gel	pigments	AUS
12	Pelikan Candy-Gel	pigments	AUS
13	Penline Gel	pigments	AUS
14	Pentel Energel BL17-A	dyes	CH
15	Pentel Energel BL77-A	dyes	AUS
16	Pentel K116-A	pigments	AUS
17	Pentel K118-AO	pigments	CH
18	Pentel K160	pigments	AUS
19	Pentel K227	pigments	CH
20	Pilot Frixion Ball	dyes	CH
21	Pilot P-700	pigments	AUS
22	Pilot Super Gel	pigments	AUS
23	SchoolZone	pigments	AUS
24	Staedtler Pigment Liner	pigments	AUS
25	Uniball Gel Impact	pigments	AUS
26	Uniball Jetstream	dyes	AUS
27	Uniball Signo Broad	pigments	CH
28	Uniball Signo UM-151	pigments	AUS
29	Uniball Signo UM-170	pigments	AUS
30	Zebra Jimnie Gel	pigments	AUS

Table 1 - List of black gel pen inks analyzed by LDI-MS. 25 five pens where bought in Australia (AUS) in 2007 for a previous study [17], the remaining five were bought in Switzerland (CH) in 2009.

Classification	Pearson threshold	Values designation
same ink comparisons	below and above	positive (P)
	above	true positive (TP)
	below	false negative (FN)
different ink comparisons	below and above	negative (N)
	below	true negative (TN)
	above	false positive (FP)

Table 2 – Explanation of the designation used to classify Pearson values obtained for the ink comparisons using ROC curves.

Analytical settings		Data	ROC		
Mode	**Laser %**	**Pre-treatment**	**AUC**	**DP** (0% false negative)	**Pearson threshold**
Positive	40	raw	0.816	21%	0.063
	60		0.884	9%	0.116
	80		0.841	0%	0.386
	40	standardised	0.932	37%	-0.079
	60		**0.972**	**71%**	**0.135**
	80		0.922	48%	-0.081
Negative	40	raw	0.787	18%	0.009
	65		0.845	10%	0.159
	90		0.841	23%	0.106
	40	standardised	0.983	50%	0.008
	65		**0.964**	**60%**	**0.158**
	90		0.913	3%	-0.588

Table 3 – Values extrapolated from the ROC curves on Pearson values: Area under the curve (AUC), discriminating power (DP) and Pearson threshold value for a 0% false negative rate. The best discrimination was obtained for standardized data using a laser intensity of 60% (positive mode) and 65% (negative mode).

	# pairs discriminated (total 435)	DP
Positive mode	308	71%
Negative mode	259	60%
Combined	368	**85%**

Table 4 – Number of pairs differentiated and discriminating power (DP) as a function of the analysis mode for standardised data in positive ion mode (laser intensity of 60%) and negative ion mode (laser intensity of 65%).

Comparison method	# pairs discriminated (total 300)	DP
VSC	146	49 %
MSP	223	74%
LDI-MS	246	**82%**

Table 5 – Discriminating power (DP) of VSC examination and MSP for the comparison of 25 five black gel pens [17] (AUS) compared to the DP obtained on the same pens with LDI-MS (positive and negative ion modes combined).

	Pen 25 a	Pen 25 b	Pen 25 c
Pen 2a	**0.372**	-0.446	-0.347
Pen 2b	**0.528**	- 0.245	-0.296
Pen 2c	0.020	-0.237	-0.117

Table 6 –Example of Pearson correlation values obtained for comparison of replicate spectra from gel pen 25 with gel pen 2 (negative ion mode, laser intensity 65%). As expected, seven values (coloured boxes) were under the differentiation threshold (< 0.158, differentiated). Two values (bold) were however above the threshold (> 0.158, non-differentiated).

# replicate spectra discriminated (total 9 replicate)	# Outliers	# pairs discriminated (total 435 pairs)	DP (%)	False negative (%)
9	0	208	48	0
6-9	up to 3	299	69	0
1-9	up to 8	375	86	3

Table 7 – Number of pairs differentiated and discriminating power (DP) as a function of the number of replicate spectra differentiated between two inks (for each comparison, 9 replicate Pearson values were obtained) for standardised data obtained in positive ion mode with a laser intensity of 60%.