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Forensic classification of ballpoint pen inks using high performance liquid chromatography and infrared spectroscopy with principal components analysis and linear discriminant analysis

Ashwini Kher [a,*], Mary Mulholland [b], Elinore Green [c], Brian Reedy [c]

[a] School of Chemistry, Monash University, P.O. Box 23, Vic. 3800, Australia
[b] School of Chemistry, UNSW, Sydney, NSW 2052, Australia
[c] Department of Chemistry, Materials and Forensic Science, University of Technology, P.O. Box 123, Broadway, Sydney, NSW 2007, Australia

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Abstract

Several varieties of blue ballpoint pen inks were analyzed by high performance liquid chromatography (HPLC) and infrared spectroscopy (IR). The chromatographic data extracted at four wavelengths (254, 279, 370 and 400 nm) was analyzed individually and at a combination of these wavelengths by the soft independent modeling of class analogies (SIMCA) technique using principal components analysis (PCA) to estimate the separation between the pen samples. Linear discriminant analysis (LDA) measured the probability with which an observation could be assigned to a pen class. The best resolution was obtained by HPLC using data from all four wavelengths together, differentiating 96.4% pen pairs successfully using PCA and 97.9% pen samples by LDA. PCA separated 60.7% of the pen pairs and LDA provided a correct classification of 62.5% of the pens analyzed by IR. The results of this study indicate that HPLC coupled with chemometrics provided a better discrimination of ballpoint pen inks compared to IR. The need to develop a suitable IR method for analysing blue ballpoint pen inks has been emphasized and it is hoped that the development of such a method would indeed provide a valuable tool for the non-destructive analysis of blue ballpoint pen ink samples for forensic purposes.

Keywords: High performance liquid chromatography; Infrared spectroscopy; Blue ballpoint pens; SIMCA; Discriminant analysis

1. Introduction

Ballpoint pen inks contain a variety of components including dyes, additives and solvents. These components can be assayed by a number of analytical chemistry techniques including high performance liquid chromatography (HPLC) [1–3] and infrared spectroscopy (IR) [4,5]. This work evaluated and compared these two techniques for a variety of blue ballpoint pens. The ability to differentiate between inks can allow the forensic scientist to evaluate the authenticity of a suspicious document [6]. Inks are considered as class evidence in forensic cases, as the manufacturing involves a large-scale blend of chemicals [7]. Inks contain about 45% dyes together with solvents and additives such as resins and organic acids [8]. Although it is difficult to determine whether an individual pen was used to write a document, it is feasible to identify the brand of pen. This requires some kind of classification or discrimination between the different brands of inks.

Other analytical techniques such as thin layer chromatography, infrared spectroscopy (IR), capillary electrophoresis, microspectrophotometry and X-ray emission analysis have been applied to the analysis of inks [6,7,9–12]. From a forensic perspective, although HPLC is a destructive method, it offers detailed information regarding the concentration of the different components of inks. Alternatively, IR can be applied because of its non-destructive nature with minimal sample preparation, rapid analysis times and cost-effectiveness. This research has evaluated and compared the application of HPLC and IR for the characterization of blue ballpoint pen ink

* Corresponding author. Tel.: +61 3 9905 3878; fax: +61 3 9905 4597.
E-mail addresses: akher101@bigpond.net.au, Ashwini.Kher@sci.monash.edu.au (A. Kher).

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samples followed by their subsequent data analysis for classification purposes.

The HPLC method used in this work is a modification of Lyter's method [1], with the added advantage of using a photodiode array detector, as pen inks contain compounds detectable at different wavelengths. Attenuated total reflectance (ATR) was chosen as a simple mode for acquiring the IR spectrum of the ballpoint pen ink samples on the same laser-quality paper in the mid infrared region (4000–650 cm^{-1}), well known for its strong characteristic fundamental vibrations.

A chromatogram or a spectrum may be visualized as a pattern in multivariate space. Samples displaying similar patterns cluster closer together and those displaying dissimilar patterns are located away from each other in multivariate space. This can be demonstrated by the application of chemometric techniques such as soft independent modeling of class analogies (SIMCA) and discriminant analysis (DA) to the chromatographic or spectral data.

SIMCA using principal components analysis (PCA) operates by redefining the data so that a reduced number of orthogonal principal components (PCs) explain most of the variability in the data [12–14]. The residual variance for every object in every class is then determined. An unknown object is then assigned to a class on the basis of its residual variance using the *F*-test. The distance between the different samples of pen classes in multivariate space is then calculated [15]. The SIMCA technique operates on a class of objects and provides a classification for the full IR spectra. As SIMCA does not provide a quantitative probability with which an observation can be assigned to an individual class, LDA algorithm has been used in this research. LDA generates a discriminant function as a linear combination of measured variables used to discriminate between the different classes of known samples. The same discriminant function can then be applied in future to assign an unknown sample to a class with a measure of certainty called the posterior probability [16–18].

However, LDA is often facilitated by a preliminary data reduction step prior to the analysis [13,19–23]. For the spectral data PCA was applied to reduce the number of variables however, the chromatographic data did not require data reduction. PCA decomposes the original matrix into three smaller matrices: the score matrix, the loading matrix and the residual matrix [24]. The relevant information needed for classification of objects is located in the scores matrix and hence the PCA scores are popularly used as input variables for the LDA. [9,10,19–22,25].

The aim of this research was to characterize the blue ballpoint pen samples by the application of PCA and LDA to the HPLC and IR data and to compare the relative merits of each approach.

2. Experimental

2.1. HPLC analysis of blue ballpoint pen inks

HPLC grade acetonitrile and methanol were purchased from Selby-Biolabs, Victoria, Australia. Milli-Q Water from a Millipore filtration system was used.

Eight blue ballpoint pen varieties were chosen for analysis and labeled as S1–S8. Six pens of each variety were purchased and a single sample was obtained from each pen. With two injections for each sample, this resulted in a total of 12 chromatograms for each of the pen varieties, which are listed in Table 1.

The ink from these 48 pens was extracted and applied to several sheets of blank Victoria copy paper from the same ream to minimize differences arising from the paper support. Each blank sheet of paper was cut into nine equal portions on which continuous lines of words were written using an individual ballpoint pen. Acetonitrile (80%, 50 μL) was used as the extracting solvent. The sample size was chosen on the basis of previous work by Lyter [1], who used a micro-hole punch with an internal diameter of 1.8 mm. Using 10 of these plugs with 50 μL of the extracting solvent resulted in a sample size of 18 mm/50 μL. As a hole punch with an internal diameter of only 6 mm was used in this work, only three plugs were used with 50 μL of the solvent in order to generate the same sample size used by Lyter. Each ink sample was ultra-sonicated with the extraction solvent for 2 min to maximize the amount of ink extracted.

All HPLC work was carried out on a Waters™ 2690 separations module with a Waters™ 996 PDA detector. The software management system used to operate, collect and manipulate data was Waters MassLynx, version 3.3. The column used for this work was a Waters™ Nova-Pak C18 150 mm × 3.9 mm. The sample injection volume was 20 μL. A gradient of 0–100% acetonitrile was run for 15 min at a flow rate of 1.0 mL/min to separate the ink components. The Alliance HPLC machine was primed (drawing solvent through the solvent line) before each run to remove any air bubbles. Fresh solvents were collected and filtered (0.45 μm) each day, using a Duopore filtration system.

Chromatograms of blue inks were collected at four different wavelengths—254, 279, 370 and 400 nm to enable better

Table 1
Sample identification

Sample ID	Sample name
S1	UniBall Laknock
S2	Zebra Rubber 1.0
S3	Pilot BP-S
S4	Pilot BPS-GP
S5	Bic Soft-feel Rubber
S6	Bic Crystal
S7	Bic Cliptop Retractable
S8	UniBall SA-S
1	Bic
2	Bic Soft-feel Medium Retractable, USA
3	Pilot BP-S-F-L Fine
4	Pilot BP-S-M-L Medium
5	Bic Clic 2000, NZ
6	Zebra Rubber 1.0 Jimnie Retractable, Indonesia
7	Staedtler 422 Stainless Steel
8	Staedtler Stick 430 F

S1–S8, HPLC pen samples; 1–8, IR pen samples.