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ARTICLES

# Statistical Discrimination of Black Ballpoint Pen Inks Using Ultra-Performance Liquid Chromatography with Principal Component Analysis[1]

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**Abstract**—The aim of this study is to propose an approach for the analysis of black ballpoint pen writing inks based on ultra-performance liquid chromatography (**UPLC**) combined with principal component analysis (**PCA**). A total of twelve varieties of black ballpoint pens available in the Malaysian market were examined by an UPLC that coupled with a photodiode array detection (**PDA**). Chromatograms of ink samples were extracted at 279, 370 and 400 nm. Chromatographic data obtained were subjected to PCA after normalization. Seven principal components were produced from a total of 15 raw peaks. The new set of variables was then used for running one-way ANOVA to differentiate 66 pen-pair formed from twelve varieties of black ballpoint pen. The approach proposed here has successfully differentiated all pen-pair thus achieving 100% discrimination power.

*Keywords*: forensic science, ink analysis, principal component analysis, ballpoint pen ink

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Forensic document examination includes handwriting comparison, determination of document alterations as well as ink analysis. The ultimate aim of the forensic document examiner is to investigate the authenticity or validity of questioned documents [1]. It is to determine whether the questioned documents have undergone any form of alterations, erasures or additions. Documents like wills, contracts and medical reports are the most common kinds of questioned documents encountered by forensic document examiners [2].

In brief, methods of ink analysis can be categorized as destructive and non-destructive. Non-destructive methods should be carried out first, as they do not affect the integrity of samples [3]. Examples of non-destructive methods that can be used to analyze inks include Fourier transform infrared spectroscopy (FTIR) [4–7], Raman spectroscopy and micro-spectrophotometry [4]. However, non-destructive methods provide limited information about the composition of inks. As a result, questioned documents should be further analyzed by destructive methods such as chromatographic [8, 9] and electrophoresis [10] in order to obtain more substantive information.

Recently, several studies reported the application of multivariate analysis to assist in forensic evidence interpretation. Well known clustering methods in the form of PCA and hierarchial cluster analysis (HCA) were explored to discriminate soil, explosive residues, hair fiber, document paper and identification of pen inks [3, 11–15]. Statistical evaluation of data ensures an objective analysis of the data set.

As such, this study aims to apply PCA on the chromatographic data of black ballpoint pen inks obtained by using UPLC for discrimination analysis. On one hand, PCA was applied to provide an objective means of discrimination analysis. On the other hand, UPLC has comparable advantages to conventional HPLC because it has faster analysis time and produces chromatogram with superior resolution and sensitivity [16]. As such, the chromatogram may yield additional information that would assist in the discrimination of pens inks.

## EXPERIMENTAL

Samples composed of twelve varieties of black ballpoint pen inks are listed in the table. Each variety is composed of four different individual pens. A sheet of A4 white copy paper (Double A, 80 g/cm$^2$) made in Thailand was used as substrate for depositing inks.

Each pen was used to write 'HUNDRED THOUNSAND ONLY' three times on white sheets of

[1] The article is published in the original.

A4 paper to prepare three specimens. For each specimen, a small piece of ink entries measuring about 5 × 20 mm was cut and put into an extraction tube containing 1.5 mL of 80% (v/v) acetonitrile. The extraction tube was then left at room temperature for 30 min. After that, the extracted ink solution was filtered with a Nalgene™ filter (0.2 μm nylon membrane).

A reversed-phase UPLC was used to separate the inks components. The UPLC system was constructed from the following components: Waters® Acquity UPLC™ system that consist of the Acquity UPLC Binary Solvent Manager, the Acquity UPLC Sample Manager and the Waters 2996 Photodiode Array Detector with a low volume flow cell. Analytes were separated by an Acquity BEH $C_{18}$ (2.1 × 150 mm) with 1.7 μm particle size from the Waters. Total analysis time was 8 min and the flow rate was set at 0.20 mL/min. Samples were eluted with a gradient elution system. Two types of mobile phases used were acetonitrile (HPLC grade, Fisher Scientific UK Ltd.) (solvent A) and distilled water (solvent B). Chromatograms of all samples were extracted at 279, 370 and 400 nm. All data collection and processing was carried out by Waters® Empower™ chromatography data software.

All statistical analyses were run on the Statistical Package for the Social Sciences, SPSS (Statistical Package for the Social Sciences, Window version 15.0, SPSS Inc., Chicago, USA). The data extracted from chromatogram were then compared using one-way ANOVA allowing for an objective comparison. With 12 varieties of ballpoint pens, there are 66 possible pen pairs ([12(11)]/2 = 66). One-way ANOVA was conducted to determine pen-pair that can be discriminated. Any pair that gives $p$-value less than 0.05 would be labeled as discriminated or vice versa. After that, discriminating power (**DP**) was calculated using the following equation:

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

where $M$ is the number of non-discriminated pairs of samples and $n$ is the total number of samples. The DP indicates the selectivity of the UPLC technique used to differentiate the black ballpoint pen inks tested [9].

## RESULTS AND DISCUSSION

A specific and sensitive HPLC–PDA method for separating inks components was described. The sample volume required is only 7.5 μL, compared with 20 μL for HPLC methods. In addition, total run time is also significantly shortened (8 min) compared with the HPLC method that requires 15 min for full separation of pen inks [8].

In order to identify peaks originating only from pen inks, every chromatogram of inks was compared against that of blank paper and blank solvent. As a result, a total of 15 peaks were selected within which wavelengths 279, 370 and 400 nm contributed seven, four and four retention times, respectively. All peak area values were then transformed into the form of percentages in order to rule out the bias from the unequal amount of ink deposited on paper as well as different ink extraction efficiency.

Details and identification number of selected varieties of black ballpoint pens. Each variety of pens was assigned with an identification number (ID no.)

| ID no. | Pen model |
|---|---|
| A1 | MGM e-Rite 716 0.7 mm |
| A2 | MGM Fino Japanese Fluid Ink 0.7 mm |
| B1 | Faster Super Smooth Semi Fine 0.6 mm |
| B2 | Faster Cx444 Super Smooth Fine |
| C1 | Paper Mate Kilometrico M 1.0 mm |
| C2 | Paper Mate KV2 M 1.0 mm |
| D1 | Bic $Bu^3$ Fine |
| D2 | Bic RS2 Fine |
| E1 | g'softR100 Fine |
| E2 | g'soft PDA 2 Delta Semi Fine 0.5 mm |
| F1 | Faber-Castell Click Ball 1422 Fine 0.7 mm |
| F2 | Faber-Castell Ball Pen 1423 Medium 1.0 mm |

In this study, the chromatograms were extracted at wavelength below 500 nm. It is well known that the maximum absorption of major components dyes of black ballpoint pen inks is in the range of 570–600 nm [15]. In other words, the components eluted from the list of studied pen inks were mainly from the minor constituents of inks, i.e. additives. Those substances are used to finely tune the characteristics of the ink, (viscosity adjusters, antioxidants, surfactants, softeners and plasticizers) [3]. A typical raw UPLC chromatogram obtained from the black ballpoint pen inks is shown in Fig. 1. On the other hand, UPLC chromatogram of paper blank did not present any signifcant peaks, as shown by Fig. 2.

Prior to differentiation analysis, the raw variables were processed by PCA first. PCA is a multivariate technique that reduces the large number of raw data to fewer latent variables which are also known as principal components (**PC**). PC is a linear combination of several inter-correlated raw variables [17]. The chosen 15 retention times were reduced to seven principal components by PCA.

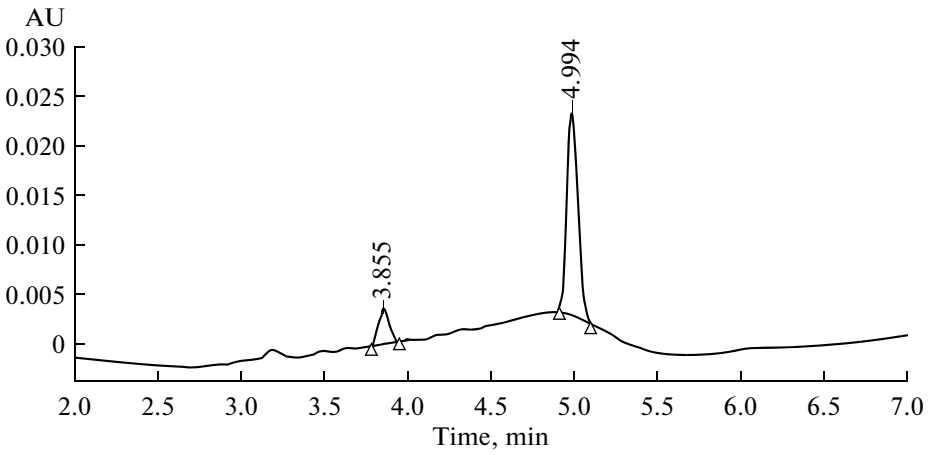


**Fig. 1.** UPLC chromatogram of A1 pen inks extracted at 370 nm.

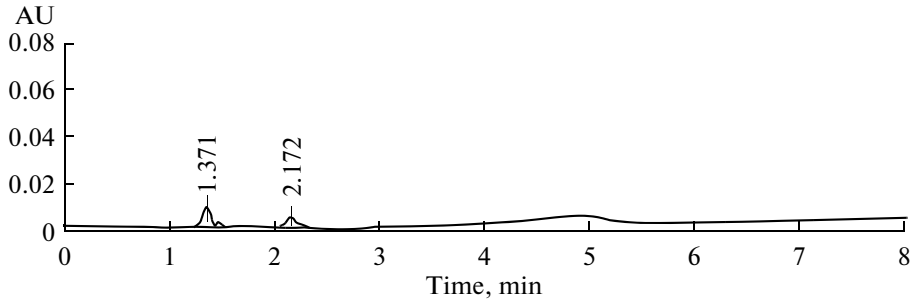


**Fig. 2.** UPLC chromatogram of paper blank extracted at 370 nm.

Subsequently, 66 possible pen pairs were formed from twelve different varieties of black ballpoint pens. One way ANOVA was conducted using all aforementioned seven principal components as variables to discriminate those 66 pairs of black ballpoint pens. The discrimination power obtained by this approach was 100%. This study illustrated the vital role of minor components of inks in differentiating inks of different models and brands. Even though the quantity of additives in inks are less than dyes but the variations of additives are greatest than that of dyes. Due to that fact, pen models from the same brand that tend to contain highly similar inks were differentiated successfully in this study.

In conclusion, UPLC coupled with PCA has been shown to be able to distinguish all twelve varieties of studied black ballpoint inks. The discrimination power calculated was 100%. The proposed approach enabled an objective comparison of UPLC chromatogram of black ballpoint pens using one-way ANOVA following PCA. With fewer numbers of raw variables, the cost of analysis is also reduced. The proposed methodology can be extended to red and blue ballpoint inks.

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## REFERENCES

1. Causion, V., Casamassima, R., Marega, C., Maida, P., Schiavone, S., Marigo, A., and Villari, A., *J. Forensic Sci.*, 2008, vol. 49, p. 1468.
2. Weyermann, C., *Doctoral (Biol.-Chem.) Dissertation*, Giessen: Justus Liebig Univ., 2005.
3. Denman, J.A., Skinner, W.M., Kirkbride, K.P., and Kempson, I.M., *Appl. Surf. Sci.*, 2010, vol. 256, p. 2155.
4. Zięba-Palus, J. and Kunicki, M., *Forensic Sci.Int.*, 2006, vol. 158, p. 164.
5. Lee, L.C., Othman, M.R., Pua, H., Ishak, A.A., and Ishar, S.M., *Probl. Forensic Sci.*, 2012, vol. 92, p. 253.
6. Lee, L.C., Othman, M.R., and Pua, H., *Malaysian J. Anal. Sci.*, 2012, vol. 16, p. 262.

7. Lee, L.C., Othman, M.R., Pua, H., and Ishak, A.A., *Malaysian J. Forensic Sci.*, 2012, vol. 3, p. 5.
8. Kher, A., Mulholland, M., Green, E., and Reedy, B., *Vib. Spectrosc.*, 2006, vol. 40, p. 270.
9. Weyermann, C., Marquis, R., Mazzella, W., and Spengler, B., *J. Forensic Sci.*, 2007, vol. 52, p. 216.
10. Zlotnick, J.A. and Smith, F.P., *J. Chromatogr. B: Biomed. Sci. Appl.*, 1999, vol. 733, p. 265.
11. Banas, K., Banas, A., Moser, H.O., Bahou, M., Li, W., Yang, P., Cholewa, M., and Lim, S.K., *Anal. Chem.*, 2010, vol. 82, p. 3038.
12. Barrett, J.A., Siegel, J.A., and Goodpaster, J.V., *J. Forensic Sci.*, 2011, vol. 56, p. 95.
13. Thanasoulias, N.C., Piliouris, E.T., Kotti, M.-S.E., and Evmiridis, N.P., *Forensic Sci. Int.*, 2002, vol. 130, p. 73.
14. Van Es, A., de Koeijer, J., and Van der Peijl, G., *Sci. Justice*, 2009, vol. 49, p. 120.
15. Thanasoulias, N.C., Parisis, N.A., and Evmiridis, N.P., *Forensic Sci. Int.*, 2003, vol. 138, p. 75.
16. Gaikwad, P.V., Sawat, S.D., Ghante, M.R., and Munot, N.M., *Pharm. Globale*, 2010, vol. 2, p. 1.
17. Abdi, H. and William, L.J., *WIREs Comput. Stat.,* 2010, vol. 2, p. 433.