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FAST AND SIMPLE FORENSIC RED PEN INK ANALYSIS USING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

(Analisis Forensik Cepat dan Mudah bagi Dakwat Pen Merah menggunakan Kromatografi Cecair Berprestasi Ultra)

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Abstract

Ultra-performance liquid chromatography (UPLC) is more effective than high performance liquid chromatography in terms of analysis speed and sensitivity. This paper presents a feasibility study on forensic red pen inks analysis using UPLC. A total of 12 varieties of red ballpoint pen inks were purchased from selected stationary shop. For each variety, four different individual pens were sampled to provide intra-variability within a particular variety of pen. The proposed approach is very simple that it only involved limited analysis step and chemicals. A total of 144 chromatograms were obtained from red ink entries extracted with 1.5 mL 80 % (v/v) acetonitrile. Peaks originated from pen inks were determined by comparing the chromatograms of both blank paper and blank solvent against that of ink samples. Subsequently, one-way ANOVA was conducted to discriminate all 66 possible pairs for red pen inks. Results showed that the proposed approach giving discriminating power of 95.45 %. The outcome of the study indicates that UPLC could be a fast and simple approach to red ballpoint inks analysis.

Keywords: forensic ink analysis, ballpoint pen inks, ultra-performance liquid chromatography

Abstrak

Kromatografi cecair berprestasi Ultra (UPLC) adalah lebih berkesan dari kromatografi cecair berprestasi tinggi dari segi kelajuan analisis dan kepekaan. Kertas ini mempersembahkan kajian kebolehlaksanaan pada analisis dakwat pen merah menggunakan UPLC. Sebanyak 12 jenis dakwat merah pen mata bulat dibeli dari kedai alat tulis yang terpilih. Untuk setiap jenis, empat batang individual pen telah disampel untuk memberi maklumat tentang intra-variasi bagi suatu jenis pen. Pendekatan yang dicadangkan adalah mudah kerana ia hanya melibatkan langkah analisis dan bahan kimia yang terhad. Sejumlah 144 kromatogram telah diperolehi dari dakwat tulisan merah yang diekstrak dengan 1.5 mL 80 % (v/v) asetonitril. Puncak yang berasal dari dakwat pen ditentukan melalui perbandingan antara kromatogram kertas kosong dan pelarut kosong dengan yang diperolehi dari sampel dakwat. Seterusnya, ujian ANOVA sehala telah dijalankan untuk membezalayan kesemua 66 pasangan dakwat pen merah yang terbentuk. Keputusan menunjukkan pendekatan yang dicadangkan memberikan 95.45 % kuasa pembezalayan. Hasil kajian ini menunjukkan UPLC dapat dijadikan satu pendekatan yang cepat dan mudah untuk analisis dakwat pen mata bulat merah.

Kata kunci: analisis dakwat forensik, dakwat pen mata bulat, kromatografi cecair prestasi ultra

Introduction

Over recent years, forensic document examination is an important area of forensic science and its contribution towards solving crimes is significant. On one hand, the invention of computer has resulted in reducing usage of writing instruments and paper as a form of proof, agreement and communication. Nevertheless, writing instruments like ballpoint pen and gel pen are still widely used in documentation [1]. Around 80 % of the questioned documents contain ballpoint pen ink [2]. On the other hand, the forensic analysis of ballpoint pen inks is a frequently encountered at questioned documents examination and its contribution towards solving crimes is significant. For that reason, it is vital to have a fast and simple ink analysis method capable of providing high differentiation power.

Ballpoint pen ink is a complex mixture of different types of chemicals. Inks produced by writing instrument manufacturers are specially formulated depending on their applications. Different kinds of writing instruments have their own writing mechanisms. In addition, characteristics such as color, flow capacity, viscosity and drying time are also taken into account [3]. Therefore, the final composition of inks can be quite complicated. In general, ballpoint pen ink comprises of 50 % solvent, 25 % dyes as well as 25 % resins and other additives [4]. In most cases, ink analysis always focuses on dye components because of their ability to absorb light of different wavelength [5].

Ink analysis aimed at determining the age of a questioned document and identifying the kind of writing instrument/inks used to produce the questioned document. Methods of ink analysis can be divided into destructive and non-destructive. Zieba-Palus and Kunicki reported that 95 % of ballpoint pen can be classified according their varieties via Fourier-transform infrared (FTIR) and Raman spectroscopy [6]. Recently, Lee et al. have proposed a new and non-destructive method for systematic analysis black ballpoint pen inks using micro-ATR-FTIR spectroscopy [7-9]. Nevertheless, non-destructive methods seldom provide satisfactory results. Most of the time, chromatographic techniques such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) could assist in further identification of pen ink's source. For example, Djozan et al. successfully differentiate 41 blue ballpoint pens via TLC coupled with image analysis [10]. Feasibility of HPLC for blue ballpoint pen ink classification has been studied as well [11, 12]. While Neumann and Margot have investigated the feasibility of high performance thin layer chromatography (HPTLC) for blue ballpoint pen inks [13, 14].

This study was aimed at exploring advantages of ultra-performance chromatography (UPLC) for forensic ink analysis purpose in terms of simplicity. There were two differences between this paper against two other similar papers published recently [15, 16]. Firstly, this paper present analysis of red ballpoint pen inks that is still very rare being studied. Secondly, the approach proposed here give emphasis on simplicity that we only use chemicals that are readily available in any analytical laboratory and do not apply any advanced statistical techniques such as Principal Component Analysis. In addition, UPLC is well known for its high resolution and faster separation speed [17].

Materials and Methods

Sample preparation

A total of 12 different varieties of red ballpoint pens were selected to be studied. Four different individual pens were sampled for each variety of pens. Table 1 shows the details and identification number of the pen samples. White paper template (Double A premium copy paper, A4 size, 80 gsm, Thailand) was prepared as well. Both pens and paper were brought from a selected stationary shop. Sample pen was prepared using similar methods as described in [16]. Every individual pen was used to write "HUNDRED THOUSAND ONLY" (measured approx. 50 mm x 5 mm) three times on piece of white copy papers. Ink was extracted right after 2 hours of its preparation in the same day to avoid variations introduced by ink ageing process.

UPLC analysis

Ink entry prepared with the red ballpoint pen inks were cut out and eluted with acetonitrile (HPLC grade, Fisher Scientific UK Ltd) and distilled water. All analysis was carried out on Waters® ACQUITY UPLCTM system that consisted of the ACQUITY UPLC Binary Solvent Manager, the ACQUITY UPLC Sample Manager and the Waters 2996 Photodiode Array Detector (PDA) with a low volume flow cell. The ACQUITY UPLC column BEH C18 (2.1 x 50 mm) with 1.7 mm particle size was used to separate the ink components. Injection volumes for all samples

were set to 7.5 uL. Two types of mobile phases used were acetonitrile (HPLC grade, Fisher Scientific UK Ltd) and distilled water (solvent B). After several times of trial and error, the time-dependent, gradient elution protocol was established. The mobile phases were run with a flow rate of 0.20 mL/min for a period of eight minutes. Details of gradient elution system were listed in Table 2. To collect as much information as possible, the chromatogram of red inks were recorded at 205 and 279 nm. All data were acquired and processed using Waters® Empower™ 2 chromatography data software. Chromatograms of the blank paper and blank solvent were also prepared in the same manner as the pen ink samples.

Table 1. Details and identification numbers of each selected varieties of red ballpoint pens. Each variety of pens was assigned with an identification numbers (id no.)

ID No.	Model/Brand	No. of Sample
A1	Bob/Carena	4
B1	XP 243 soft ink xtra fine/Reynolds	4
B2	Popular 240 medium/Reynolds	4
C1	R100 Fine/G'soft	4
C2	BP-GS-56 0.7/G'soft	4
D1	ExtraFinePoint/e-write	4
E1	Fornine 88	4
F1	Round stic Grip fine USA/Bic	4
G1	Kilometrico M/Papermate	4
G2	Kilometrico Fine PT/Papermate	4
G3	Kilometrico 100 LV ink med/Papermate	4
G4	KV2 Fine/Papermate	4

Table 2. Gradient elution system for red ballpoint pen inks; A=80% ACN; B=H_2O

Run Time (min)	Flow rate (mL/min)	% A	% B
Initial	0.20	50.0	50.0
1.00	0.20	80.00	20.0
3.00	0.20	100.00	0.00
4.00	0.20	100.00	0.00
4.50	0.20	50.0	50.00
8.00	0.20	50.0	50.00

Data analysis

As this paper aimed to propose simple analysis protocol to discriminate pen inks, no statistical technique is introduced to the data except one-way ANOVA was applied to determine the discrimination status of pen pairs based on p-value. Later, discriminating power (DP) was calculated using the following Equation 1[9]:

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

where M is the number of non-discriminated pairs of samples and n denotes the total number of samples. The DP is a measurement of the selectivity of the method to differentiate the pen inks analyzed [18].

Results and Discussion

Previous studies used to discriminate ballpoint pen inks based on profiling of dye components by chromatographic techniques [13, 14, 19]. Most of the dye components of red ballpoint pen inks have maximum UV-absorption above 500 nm, i.e. rhodamine 6G at 524 nm [20]. However, this study intended to investigate the possibility of discriminating red ballpoint pen inks using information obtained from components other than dyes. Therefore, all of the chromatogram of inks was only scanned between 200 and 500 nm.

Prior to discrimination analysis, all of the chromatograms of ink sample were compared against the chromatogram of blank solvent and blank paper (Figure 1) in order to identify peaks originated from pen inks only. The representative chromatograms for two red pens inks are as shown in Figure 2. A total of 39 peaks obtained from chromatogram scanned at 205 and 279 nm were determined as coming from red ballpoint pen inks. Those identified peaks were most likely due to ink components other than dyes, such as additives that included driers, plasticizers and detergents which are present at very minute amount [16].

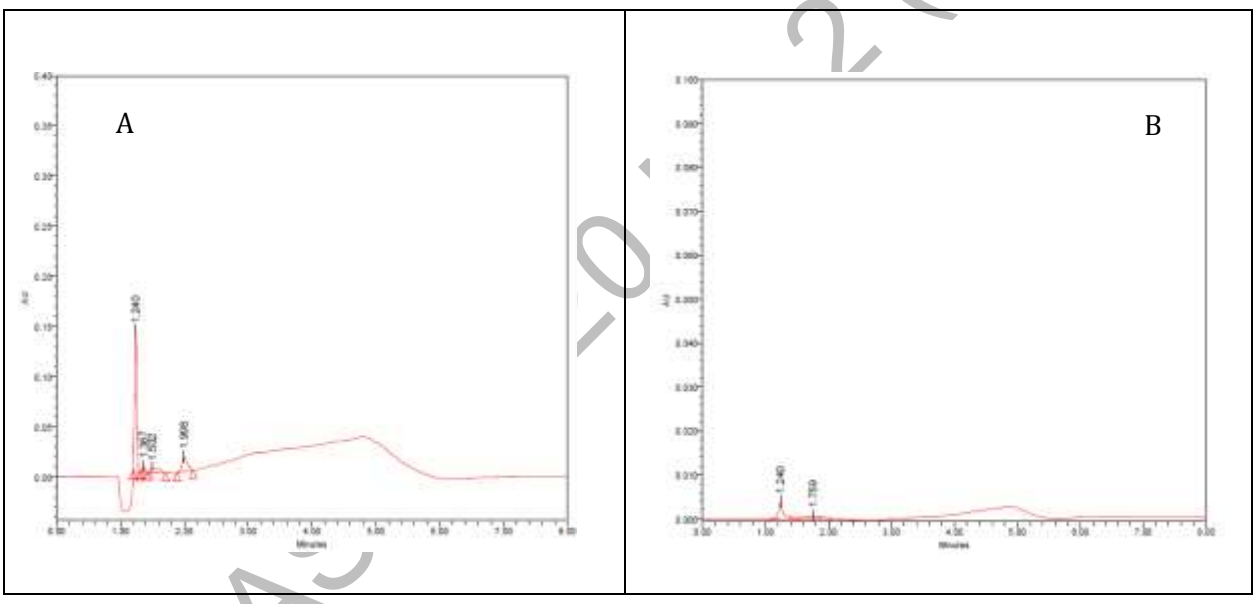

Figure 1. Chromatogram of blank. Solvent blank scanned at 205nm (A) and paper blank scanned at 279nm (B).

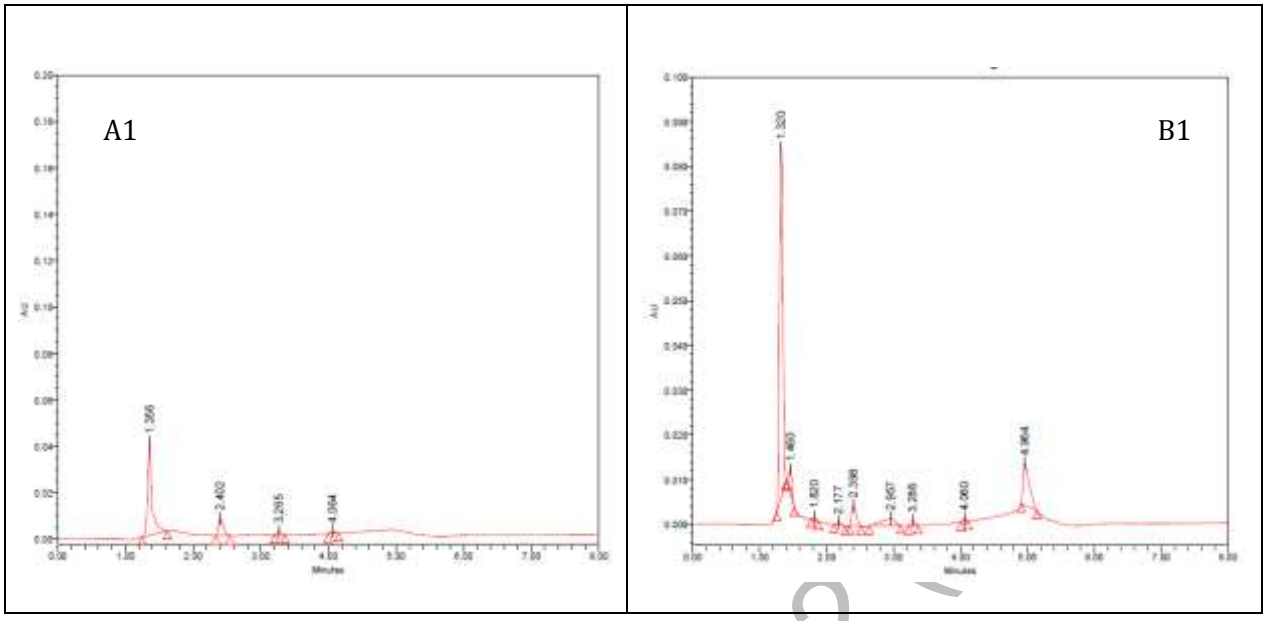

Figure 2. The representative chromatogram of two red ballpoint pen inks, i.e. A1 and B1 scanned at 279 nm.

Since we could not standardize the amount of ink deposited on paper for every ink entry, bias could be introduced if the obtained peak area value is compared directly. To ensure more reliable comparative analysis, the data was normalizing towards a single selected peak to eliminate variations occurred due to uneven initial ink deposition on paper [15]. After careful inspection on all the chromatograms, the first peak (i.e. retention time 1.280 – 1.399) in both chromatograms scanned at 205 and 279 nm was selected to be the normalization factor. It was chosen for the purpose of normalization as its peak is sharp and is the highest in the chromatogram. The peak area value of the other peaks is then divided with the normalization factor.

One-way between groups ANOVA was conducted based on the normalized peak area values. Pen pair that gives p-value less than 0.05 is determined to be discriminated pair and pair that give p-value more than 0.05 will be labelled as non-discriminated pair. The summary result of the comparative analysis between 66 possible pen-pair is shown by Figure 3. Only three pen-pair cannot be discriminated based on their chromatograms, i.e. B1-F1, B1-E1 and E1-F1. Pen-pairs that cannot be differentiated from each other may have the same or very similar formulation. In other words, this indicates the possibility of purchasing inks from a single ink manufacturer as an inks manufacturer may sell their ink to more than one instrument manufacturers [14]. In brief, the proposed approach gave discrimination power around 95.45 %.

The obtained DP is comparable to other approaches that involved much complicated analytical instrument, i.e. real time mass spectrometry (DART MS) [21] or advanced analysis tools such as image analysis [10]. In this study, differentiation of inks was based purely on the relative peak area of peaks at pre-defined wavelength range. And the objectivity of comparative analysis is secured by applying one-way ANOVA and decision is made based on the p-value. The approach proposed here is fast and simple.

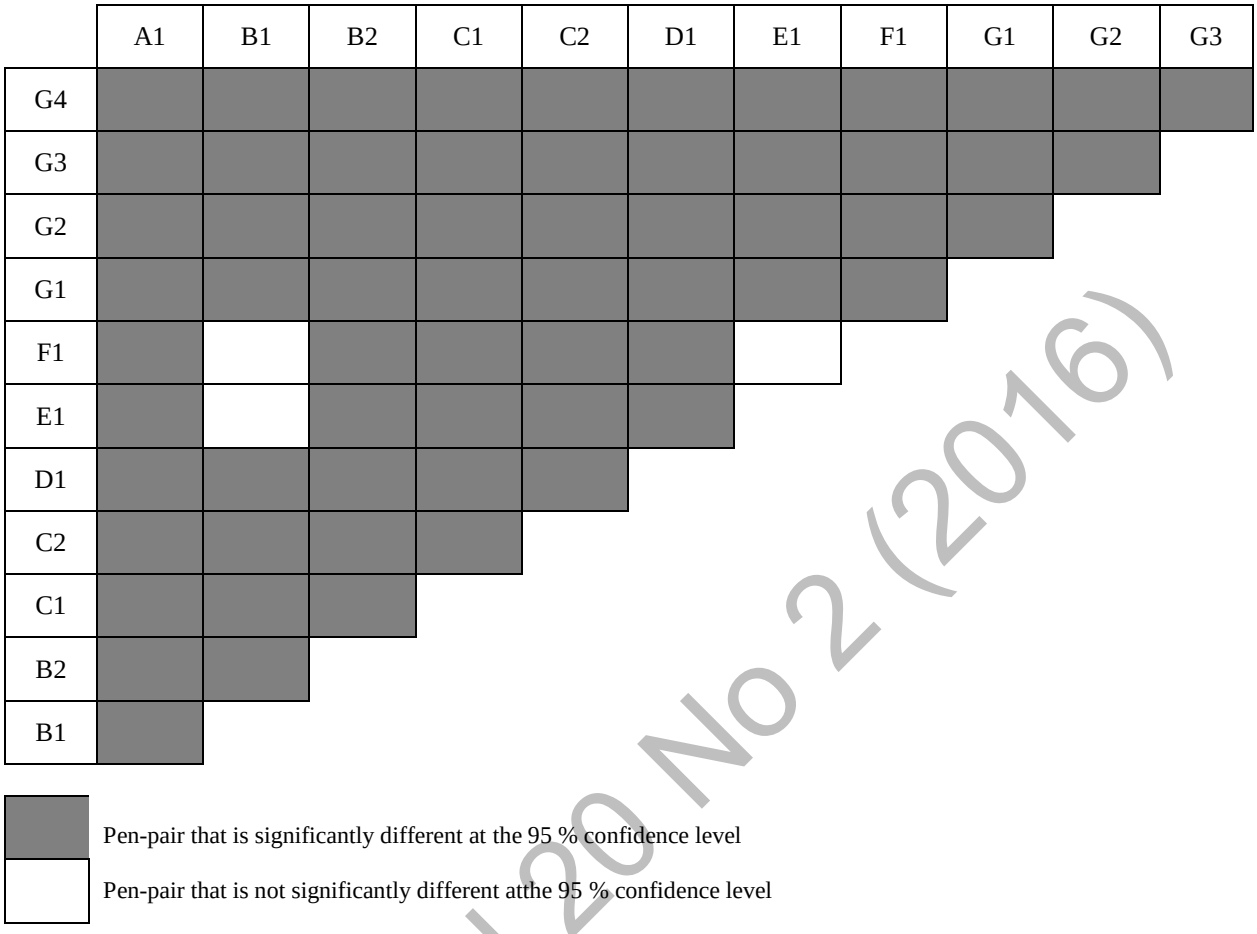

Figure 3. Matrix showing summary result of differentiation of red ballpoint pen inks based on UPLC chromatograms via one way ANOVA analysis

Conclusion

The present study showed that the written extracts from different ballpoint pens can be differentiated at satisfactory level with UPLC coupled with UV/PDA detection. The proposed technique found to be effective as it only involved common chemical, i.e. acetonitrile, and simple statistical techniques, i.e. one-way ANOVA. Besides, composition of minor components in ballpoint pen inks could be as efficient as dye components in classification of pen inks since it was supported by the high level of discriminating power calculated in this study.

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