

FORENSIC ANALYSIS OF BLUE BALLPOINT PEN INKS ON QUESTIONED DOCUMENTS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY TECHNIQUE (HPTLC)

(Analisis Forensik Dakwat Biru Pen Mata Bulat Atas Dokumen Yang Dipertikai Dengan Kromatografi Lapisan Nipis Berprestasi Tinggi (HPTLC))

Loong Chuen Lee*, Sitti Mariam Nunurung, Ab Aziz Ishak

*Forensic Science Programme, Faculty of Health Sciences,
Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*

**Corresponding author: lc_lee@ukm.edu.my*

Abstract

Nowadays, crimes related to forged documents are increasing. Any erasure, addition or modification in the document content always involves the use of writing instrument such as ballpoint pens. Hence, there is an evident need to develop a fast and accurate ink analysis protocol to solve this problem. This study is aimed to determine the discrimination power of high performance thin layer chromatography (HPTLC) technique for analyzing a set of blue ballpoint pen inks. Ink samples deposited on paper were extracted using methanol and separated via a solvent mixture of ethyl acetate, methanol and distilled water (70:35:30, v/v/v). In this method, the discrimination power of 89.40% was achieved, which confirm that the proposed method was able to differentiate a significant number of pen-pair samples. In addition, composition of blue pen inks was found to be homogeneous (RSD <2.5%) and the proposed method showed good repeatability and reproducibility (RSD <3.0%). As a conclusion, HPTLC is an effective tool to separate blue ballpoint pen inks.

Keywords: forensic science, ink analysis, High Performance Thin Layer Chromatography, discrimination power, ballpoint pen

Abstrak

Baru-baru ini, jenayah yang berkaitan dengan dokumen palsu semakin meningkat. Sebarang pemadaman, penambahan atau pengubahsuaian dalam kandungan dokumen sering melibatkan penggunaan alatan tulis seperti pen mata bulat. Oleh itu, ia terdapat satu keperluan yang nyata untuk membangunkan satu protokol analisis dakwat yang cepat dan tepat untuk menyelesaikan masalah ini. Kajian ini bertujuan untuk menentukan kuasa diskriminasi teknik lapisan nipis berprestasi tinggi (HPTLC) menganalisis satu set dakwat biru pen mata bulat. Sampel dakwat yang termendap pada kertas diekstrak mengguna metanol dan dipisahkan dengan campuran pelarut yang mengandungi etil asetat, etanol dan air suling (70:35:30). Dalam kaedah ini, 89.40% kuasa diskriminasi tercapai, ini mengesahkan kaedah yang dicadangkan berupaya membeza bilangan pasangan pen yang signifikan. Sebagai tambahan, komposisi dakwat biru pen mata bulat didapati homogen (RSD < 2.5%) dan kaedah yang dicadangkan menunjukkan kebolehulangan dan keterulangan yang baik (RSD<3.0%). Sebagai kesimpulan, HPTLC adalah satu alatan yang efektif untuk memisahkan dakwat biru pen mata bulat.

Kata kunci: sains forensik, analisis dakwat, kromatografi lapisan nipis berprestasi tinggi, kuasa diskriminasi, pen mata bulat

Introduction

Forensic document examination always involves the analysis of blue ballpoint pen inks. Chemical analysis of inks is therefore an important procedure aimed at detecting any kind of frauds. There are quite a number of articles

reporting the examination of blue ballpoint pen inks using chromatography techniques [1-10]. Each of them reported discrimination power (DP) above 90% for blue ballpoint pen inks.

In 1998, Tsutsumi & Ohga [1] described a preliminary preparation of a standard thin layer chromatography (TLC) library for a total of 35 ballpoint pens composing of black, red and blue inks. Authors found that Merck normal phase plate using ethyl acetate/ethanol/water (14:7:6) [1] gave higher degrees of dye separation if compared to the one using ethyl acetate/methanol/28% aqueous ammonia (5:2:1) and trichloroethylene/1,1,1-trichloroethane/ethyl acetate (10:1:1). On the other hand, Roux et al. [2] stated that TLC was able to discriminate inks between and within brands, models, and batches of blue ballpoint pen inks in Australia with DP of 98%.

Djozan et al [3] proposed a simple and easy method for discrimination of blue ballpoint pen inks on a document. The proposed method includes a new and fast data acquisition by designing new and specific image analysis software for evaluating TLC chromatograms after scanning with ordinary office scanner, without in need of expensive instruments. This method allows one to compare two inks by considering the retention factor (R_f), color range and intensity of the separated ink components. They reported the DP of the proposed method as 92.8% [3].

An improved TLC technique, i.e. high performance thin layer chromatography (HPTLC), was introduced in the early 2010s. Senior et al [4], Neumann and Margot [5-7], Neumann et al [8] demonstrated the possibility of retrieving quantitative data from HPTLC chromatograms. Thus, statistical techniques can be applied on data interpretation and this greatly improves the accuracy of identification and discrimination of pen inks based on HPTLC technique.

To date, there has been no report presenting results of HPTLC analysis of blue ballpoint pen that collected from the market in Malaysia. Thus, taking into account the above-mentioned successful application of TLC and HPTLC techniques to the analysis of blue pen inks, we decided to make an effort to fill in the gap in current analytical methodology of forensic questioned document examination. Therefore, the aim of this paper was to determine the discrimination power of HPTLC technique to analyze blue ballpoint pen inks. Furthermore, homogeneity of inks and reproducibility as well as repeatability of HPTLC technique was also determined.

Materials and Methods

Chemical and reagents

The chemicals used throughout the experiments were methanol, ethanol and ethyl acetate produced by Fisher Scientific (UK). Crystal violet (CV) dye was purchased from Acros Organics, USA. Distilled water was used to prepare all solutions. The HPTLC silica gel plate 60 with layer thickness 0.2 mm and 20 X 20 cm aluminum cards (Merck, Germany) and horizontal developing chambers were used at the forensic science laboratory, Universiti Kebangsaan Malaysia, for chromatographic separation of dyes in ink entries.

Samples

A set of blue ballpoint pen consists of 12 varieties were purchased from stationary shops in Malaysia. All pens were allocated a reference number during this study, as shown in Table 1. Four different individual pens were sampled for each of the pen variety. Experimental work was carried out on inks extracted from ink entries produced on white photocopier paper. All papers used for written were of the same batch to avoid substrate variability.

Table 1. Reference (ID) number of all 12 varieties of pen samples

Brand	Model	ID number
Faber Castell	Click Ball 1422 Fine	A1
	Ball Point 1432 Medium	A2
G'soft	R100 Fine	B1
	PDA2 Delta Semi Fine	B2
Faster	Ball Pen CX1006 Super Smooth Semi Fine	C1
	Ball Pen CX444 Super Smooth Fine	C2
Stabilo	Performer Fine	D1
	Performer X-Fine	D2
LINC	Glycer Ball Pen	E1
	Ultra Ball Pen	E2
Paper Mate®	KV2 Medium	F1
	InkJoy 100 RT	F2

Sample Preparation

Each of the pens was used to write “BMW” on a piece of white paper. After ink deposited on a paper, the absorbed ink was extracted from the ink entry of about 1 cm X 0.5 cm with 200 uL of methanol in a polypropylene micro vial. A blank sample was prepared by treating a blank white paper with methanol. CV was prepared as standard reference dye by dissolving the dye with methanol.

High Performance Thin Layer Chromatography

The origins were at 1.5 cm from the base of the plates. HPTLC analyses were performed using ethyl acetate, ethanol and distilled water (7:3:2) as an eluent mixture. Each extracted ink sample was spotted manually using capillary tube manually. CV used as standard dye and a paper blank as well solvent blank was included in each run. To check the role of sampling on analytical results, another extraction with methanol was performed directly from the pen ink reservoir, obtaining indistinguishable data if compared with the samples extracted from the lines of paper. The mobile phase was prepared freshly daily and replaced after every two runs. Before daily operation, the tank was conditioned with mobile phase for at least 20 min. The uniform origin position on the plates, proper loading at the origin, and the solvent saturation in the developing tank were kept constant during all the HPTLC analyses to ensure high reproducibility.

Discriminating Power (DP)

The discriminating powers (DP) of the different brands and different models of the same brand were calculated according to Smalldon and Moffat [11], where Equation 1:

$$DP = (\text{Number of discriminated pairs}) / (\text{number of possible pairs}) \quad (\text{Eq. 1})$$

Ink homogeneity, repeatability and reproducibility

Two varieties of pen inks (choose from the 12 studied ink varieties) presented the most versatile HPTLC profile were selected to determine the homogeneity of ink as well as the precision of the HPTLC methods. Relative standard deviation (RSD) was determined for each test.

For determination of ink homogeneity, each of the two selected inks was used to prepare nine different ink entries on white paper. After then, each of the nine extracted inks were spotted once on the plate and were analyzed according to procedures explained above on a single HPTLC plate.

Repeatability of method was determined by preparing one single ink entries from each of the selected pen ink varieties. Later, nine different ink spots were deposited on HPTLC plate from each of the two prepared ink extracts on a single HPTLC plate and analyzed according to procedures explained above. In order to examine the reproducibility of the method, the procedures described for determination of repeatability were repeated at four different HPTLC plates in four different days.

Results and Discussion

HPTLC Analysis

HPTLC profiles of the 12 studied blue pen inks were shown in Figure 1. In general, the HPTLC profiles of most inks were very similar. The HPTLC results reflected that CV was the major dye component present in the blue ballpoint pen inks. Based on Figure 1, all the pen inks contained CV as their main coloring agent, except sample pen D1 and D2, i.e. models of Stabilo. Previous literature reports also agreed with our observation that CV is one of the main coloring agents in blue pen inks [12-14].

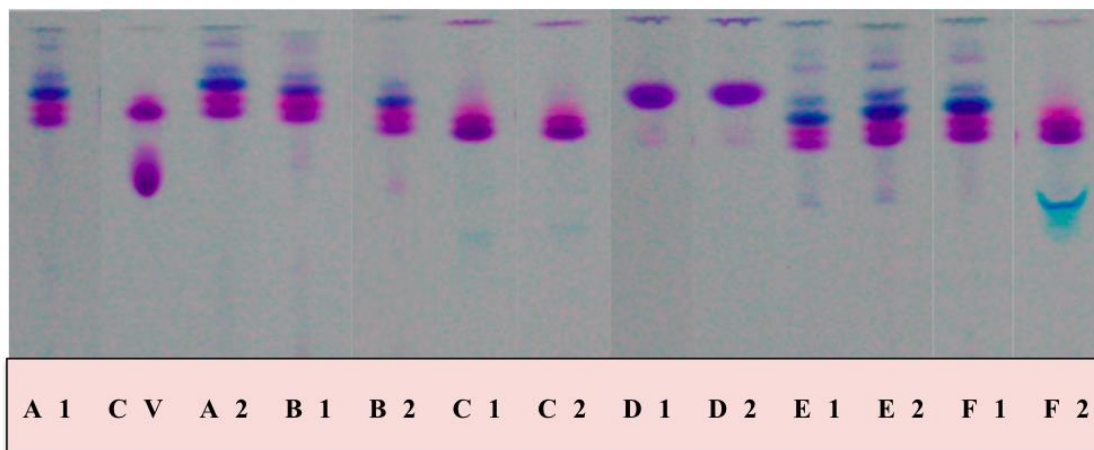


Figure 1. HPTLC plate showing dye profile of 12 blue ballpoint pen inks (A1-F2).

Second track from the left was standard dye CV. The figure was reconstructed from several HPTLC chromatograms for easy comparison between ink spots of different pen varieties. On the other hand, solvent blank and paper blank did not give any observable band under both normal white light and UV light. The retention factor (R_f) values recorded for each bands of each pen variety were the average product of dye profiles separated from four different individual pens of a particular pen variety. Analyzing of four different individual pens for each pen variety aimed to average out and minimize any errors. It was observed that ink profiles as well as the associated R_f values of a single pen ink variety extracted from four different individual pens were highly similar (Figure 2). This means the inks were quite homogeneous across different individual pens that produced within a single batch of production for a selected pen variety.

In addition, this study also conducted a parallel set using inks extracted directly from the reservoir. None of previous studies have done such verification step. Based on this study, differences observed between HPTLC profiles of four different individual pens were not significant for all pen varieties. This indicated extraction via methanol was efficient to extract all the dyes present in the ink entries.

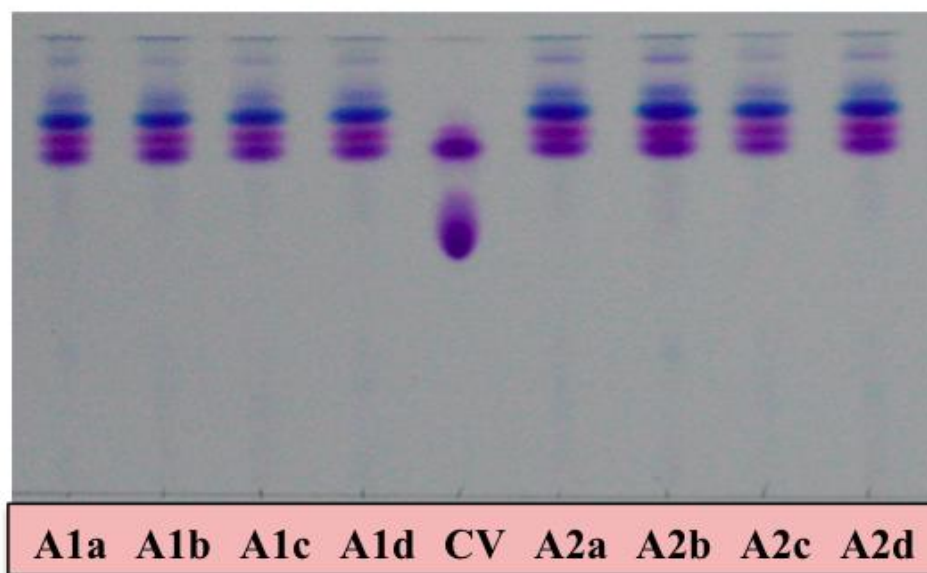


Figure 2. First four tracks (from the left) and the subsequent four tracks were extracted from pen A1 and A2, respectively. Each of the four spots were originated from four different individual pens of pen A1 and A2, respectively

Classification and differentiation

Based on Figure 1, the color tones of the bands separated by the normal-phase HPTLC analysis enable the classification of the writing pens into four different groups. Group one (named as G1) consisted of only sample D which did not have CV in its ink composition. Whereas, sample C1-2, i.e. models of Faster pens containing only CV as their major dye were grouped in the second group (named as G2). Sample F2 which contained CV as well as a light blue-colored bands at the lower position relative to the CV, named as G3. The fourth group (named as G4) consisted of the remaining sample A1-2, B1-2, E1-2, and F1 which evidenced the presence of CV and another dye (blue-colored band on the top of the band of CV) in their ink composition.

Within G1, both pen models from Stabilo, i.e., D1 and D2 refer to Performer F and Performer X-F, used dye other than CV as their major coloring agents. The color profiles, i.e. relative intensity of color and shape of the separated bands, of both models of Stabilo were appeared very similar to each other. Stabilo appears to use the same inks for all the models tested that only differed at point size.

On the other hand, both pen models from Faster were grouped in G2. They contained only one major dye, i.e. CV, as their coloring agent. Nonetheless, the appearance of another very light-blue-colored band below the CV's band supported that Faster did add in another minor dye in their formulation. Again, both pen models of Faster were models that only differed at point size, i.e. Ballpen semi fine and Ballpen fine, and thus exhibited very similar dye profiles.

The third group (G3) included only a single pen model, i.e. Inkjoy 100 RT (F2), from PaperMate. The scenario presented here was totally different from both cases mentioned before this. Both models of PaperMate can be differentiated from each other in which F1 did not show an extra light-blue colored band (Fig. 1), indicating both are from different sources. This can be explained by the fact that F2 was produced in India compared to F1 was manufactured in Malaysia.

The last group (G4) included the results obtained for inks of producers other than Stabilo, Faster and F2 of PaperMate, which included FC, G'soft, LINC and F1 of PaperMate. Their chromatogram profiles were characterized predominantly by two prominent dyes, i.e. CV and another unidentified dye (dark-blue colored bands). HPTLC profiles of all of them looked very similar to each other (Fig. 1). However, detailed examination revealed that both models of G'soft (B1-2) and LINC (E1-2) could be differentiated from the rest by a hardly visible band located at the lowest R_f values for each of them. The color of that minor band of B1-2 and E1-2 exhibited pink and blue color, respectively.

Discriminating power (DP)

Figure 3 showed the summary results of differentiation of 12 blue pen inks varieties. In the group of 12 blue ballpoint pen inks, 66 possible pairs of compared samples have been created. It has been found that all samples except seven pairs can be identified based on their HPTLC chromatogram (Fig. 1). Thus the DP has been equal to about 89.40%.

$$DP = (66-7)/66 = 59/66 = 89.40\%$$

	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1
F2											
F1											
E2											
E1											
D2											
D1											
C2											
C1											
B2											
B1											
A2											

	Pair that is distinguishable by HPTLC
	Pair that is indistinguishable by HPTLC

Figure 3. Differentiation of blue ballpoint pen inks based on HPTLC technique

Nonetheless, there was some possibility of distinguishing between the inks extracts of the remaining pen varieties by calculating the relative color intensity of bands. However, this study did not determine the relative color intensity of bands due to unavailability of CAMAG TLC Scanner with WinCats software (CAMAG AG, Switzerland). Alternatively, colorless components, i.e. additives, could be used to discriminate models from a single

manufacturer. The occurrence of the same separation patterns is possibly attributed to the situation that plural products from one manufacturer contain common dyestuff components, and ink compositions depend on the limited raw dyestuffs provided by dyestuff makers, which are smaller in number compared to writing instrument makers [8].

Ink homogeneity, repeatability and reproducibility

Verification of the repeatability and reproducibility as well as determination of ink homogeneity was conducted with the use of two selected pen varieties, E2 and F2. They were chosen because both of them presenting the most diversify HPTLC profiles. RSD values were calculated for each test and summarized in Table 2.

Table 2. Ink homogeneity, repeatability and reproducibility of HPTLC technique using pen E2 and F2

Spot No.	Homogeneity		Repeatability		Reproducibility	
	RSD (%)		RSD (%)		RSD (%)	
	E2	F2	E2	F2	E2	F2
1	1.2850	2.0528	0.7917	1.4969	1.3597	1.9959
2	1.2216	1.9388	1.3321	1.7839	2.8193	2.0672
3	1.6975	0.9374	1.2318	2.2311	1.5162	2.1167
4	0.5775	1.5680	1.4608	1.8213	2.3096	2.5279
5	0.5779	1.3280	0.8302	0.8343	1.9646	2.4950
6	0.4712	na	1.0982	na	1.9247	na

na= not available

As a test of homogeneity, the highest and lowest RSD were exhibited by the first spot of F2 and spot number sixth of E2, respectively. The errors associated with the measurements were a combination of human error and the heterogeneity inherent in sample. Nonetheless, both pen samples gave very satisfy results (RSD<2%) and their inks were concluded as homogeneous.

The validation study focused on the precision of the method in terms of repeatability and reproducibility of the retention factor (Rf) of bands. By looking at the RSD values of the nine repetitions of a sample (repeatability column of Table 2), the conclusion was that different HPTLC plates did not have a great impact on the precision of the method (in all cases RSD was less than 3%). On the other hand, by referring to the reproducibility of Table 2, the effect of different plates triggered a slightly higher RSD values if compared to the one obtained for repeatability for both pen samples.

Conclusion

The discrimination power (DP) of HPTLC technique to analyses blue ballpoint pen inks was determined to be around 89.40%. The inks of two selected models were found homogenous within the ink reservoir. The HPTLC technique proved to be simple, reproducible (RSD<3%) and repeatable (RSD <3%). The results showed that it is worthy to expand the study by including more samples. Subsequently, the qualitative data from HPTLC plates could be converted into quantitative data by using certain image analysis software. So that, higher DP could be achieved while multivariate statistical techniques can also be applied on data interpretation.

Acknowledgement

We would like to express our deep gratitude and appreciation to all the staffs of the Forensic Science Program from UKM.

References

1. Tsutsumi, K. & Ohga, K. (1998). Analysis of writing ink dyestuffs by TLC and FTIR and its application to forensic science. *Analytical Science* 14: 269-274.

2. Roux, C., Novotny, M., Evans, I., & Lennard, C. (1999). A study to investigate the evidential value of blue and black ballpoint pen inks in Australia. *Forensic Science International* 101: 167-176.
3. Djozan, D., Baheri, T., Karimian, G. & Shahidi, M. (2008). Forensic Discrimination of Blue Ballpoint Pen Inks Based on Thin Layer Chromatography and Image Analysis. *Forensic Science International* 179(2–3): 199-205.
4. Senior, S., Hamed, E., Masoud, M. & Shehata, E. (2012). Characterization and dating of blue ballpoint pen inks using principal component analysis of UV-Vis absorption spectra, IR spectroscopy and HPTLC. *Journal of Forensic Sciences* 57:1087-1093.
5. Neumann, C. & Margot, P. (2009). New perspectives in the use of ink evidence in forensic science: Part I: Development of a quality assurance process for forensic ink analysis by HPTLC. *Forensic Science International* 185: 29-37.
6. Neumann, C. & Margot, P. (2009). New perspectives in the use of ink evidence in forensic science: Part II: Development and testing of mathematical algorithms for the automatic comparison of ink samples analysed by HPTLC. *Forensic Science International* 185: 38-50.
7. Neumann, C. & Margot, P. (2009). New perspectives in the use of ink evidence in forensic science: Part III: Operational applications and evaluation. *Forensic Science International* 192: 29-42.
8. Neumann, C., Ramotowski, R. & Genessay, T. (2011). Forensic Examination of Ink by High-Performance Thin Layer Chromatography—the United States Secret Service Digital Ink Library. *Journal of Chromatography A* 1218(19): 2793-2811.
9. Kher, A., Mulholland, M., Green, E., Reedy, B. (2006). Forensic classification of ballpoint pen inks using high performance liquid chromatography and infrared spectroscopy with principal components analysis and linear discriminant analysis. *Vibrational spectroscopy* 40:270-277.
10. Causin, V., Casamassima, R., Marega, C., Maida, P., Schiavone, S., Marigo, A., Villari, A. (2008). The discrimination potential of UV spectrophotometry, TLC, and FTIR for the forensic analysis of black and blue ballpoint inks, *Journal of Forensic Science* 53(6):1468-1473.
11. Smallldon, K.W., Moffat, A.C. (1973). The calculation of discrimination power for a series of correlated attributes. *Journal of Forensic Science* 13:291-295.
12. Seifar, R.M., Verheul, .M., Aiese, F., Brinkman, U.A. & Gooijer, C. (2001). Applicability of surface-enhanced resonance Raman scattering for the direct discrimination of ballpoint pen inks. *Analyst* 126:1418-1422.
13. Ng, L-K., Lafontaine, P. (2002). Ballpoint pen inks: Characterization by positive and negative ion-electrospray ionization mass spectrometry for the forensic examination of writing inks. *J. Forensic Science* 47: 1238-1247.
14. Zieba-Palus, J. & Kunicki, M. (2006). Application of the micro-FTIR spectroscopy, Raman spectroscopy and XRF method examination of inks. *Forensic science international* 158:164-172.