

The Analysis of Dyes in Black Ballpoint Pen Inks using High Performance Thin Layer Chromatography

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ABSTRACT: Forensic document examination always aimed to solve criminal activity associated to dispute sensitive documents with significant financial values such as insurance claims, wills, and contracts. Twelve varieties of black ballpoint pen inks were analysed using high performance thin layer chromatography (HPTLC). 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). Direct visual comparison analysis enables discrimination between models coming from a single brand for a few selected pen brands. Simple sample preparation and minimal analysis time, and high discrimination power gives this technique advantages over others currently utilised in the forensic field. In addition, composition of black pen inks was found to be homogeneous (RSD <1%) and the proposed method showed good repeatability and reproducibility (RSD <3.0%). As a conclusion, the proposed HPTLC technique is useful for the precise discrimination of black ballpoint pen inks.

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

Introduction

Forensic document examination always aims to solve criminal activity associated to dispute sensitive documents with significant financial values such as insurance claims, wills, and contracts. Chemical analysis of writing inks is to identify its source and detecting any kind of frauds. Most of the available literatures concentrated on examination of ballpoint pen inks using thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC) that focused on blue ballpoint pen inks [1-7]. There is lack of studies reporting the discrimination power of HPTLC techniques for analysing black ballpoint pen inks, especially those pens that collected from the market in Malaysia. The aim of this paper was to determine the discrimination power of HPTLC technique to analyse black 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 (MeOH), 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 (Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Malaysia).

Samples

A set of black ballpoint pen consists of 12 varieties were purchased from markets in Malaysia. All pens were allocated a reference number during this study, as shown by 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.

Sample Preparation

Each of the pens was used to write "UKM" 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 μ L 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.

Table 1: ID number of all 12 varieties of pen samples

Brand	Model	ID number
Pepermate	Kilometrico M	A1
	KV2 M	A2
Faber-castel	Click ball 1422 (TRANSPARENT)	B1
	Ball pen 1423 (TT-GREEN)	B2
G' soft	PDA 2 Delta 0.5 Semi F	C1
	R-100 F	C2
BIC	RS2 F	D1
	Bu3 F	D2
Faster	CX444 F/Super Smooth	E1
	CX1006	E2
MGM	E-Rite 716	F1
	FINO 0.7	F2

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 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 fresh daily. They were 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 during all the HPTLC analyses to ensure high reproducibility.

Ink homogeneity, repeatability and reproducibility of the method

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 black pen inks were shown by Figure 1. In general, the HPTLC profiles of most ink were very similar. The HPTLC results reflected that all pen inks contained CV as well as another dye (indicated by yellow-coloured band). The yellow-coloured band most probably C.I. Acid Yellow 42 or Metanil Yellow [1].

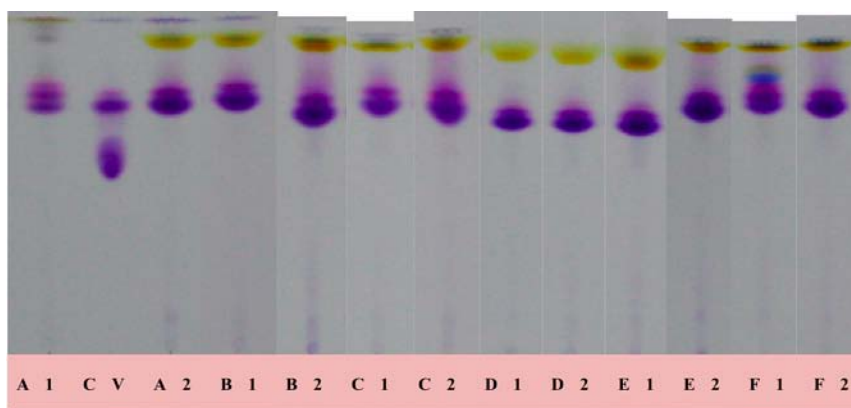


Figure 1: HPTLC plate showing dye profile of 12 black ballpoint pen inks (A1-F2). Second track from the left was standard dye CV. (Note: Figure was reconstructed from several HPTLC chromatograms to ease the visual comparison between ink spots of different pen varieties)

On the other hand, solvent blank and paper blank did not give any observable band under normal white light and UV light. The 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) indicating that the inks were quite homogeneous across different individual pens that produced within a single batch of production for a selected pen variety.



Figure 2. First four tracks (from the left) and the subsequent four tracks were extracted from pen A1 and A2, respectively. (Note: Each of the four spots were originated from four different individual pens of pen F1, respectively)

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.

Classification and differentiation

Based on Figure 1, the colour 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 A1 which did not show a yellow-coloured band. Whereas, sample F1, i.e. E-RITE of MGM, showed an extra light-blue-coloured band in between yellow-coloured band and CV's bands (named as G2). Sample D1-2 had yellow-coloured band which did not overlapping with G3 black-coloured bands. The fourth group (G4) consisted of remaining pens, i.e. A2, B1-2, C1-2, E1-2, and F2, which evidenced the presence of an extra dye, other than Metanil Yellow and CV, as indicated by an extra black-coloured band within the yellow-coloured band.

Within G1, sample A1 was from the brand of Papermate and was found differed from another model from Papermate (A2). The colour profiles, i.e. relative intensity of colour and shape of the separated bands, of both models of PM were appeared very different to each other. It seemed like Papermate was filled dissimilar inks for different models under its brand.

A similar scenario also presented by both models of MGM, i.e. F1 and F2, in which both of the MGM's pen models were totally different from each other. F1 showing an extra blue-coloured band which was absent in F2. It was most probably due to the presence of Solvent Blue 5 [1].

In most cases, models from the same brands could not be distinguished from each other. For instance, the scenario presented by the third group (G3) which including both models of pen BIC, was totally different from both cases mentioned before this. Both models of BIC presented highly similar HPTLC profiles that cannot be differentiated from each other (Figure 1).

The last group (G4) included the results obtained for inks of producers other than A1 of Papermate, F1 of MGM, BIC which included FC, G'soft, Faster, A2 of Papermate and F2 of MGM. HPTLC profiles of all of

them looked very similar to each other (Figure 1). Their chromatogram profiles were characterized by three bands, i.e. violet-coloured, yellow-coloured and black/dark-blue coloured. They were most probably evidenced the presence of crystal violet, metanil yellow and direct black 154 [1].

Nonetheless, there was some possibility of distinguishing between the inks extracts of the remaining pen varieties by calculating the relative colour intensity of bands. However, this study did not determine the relative colour intensity of bands due to unavailability of CAMAG TLC Scanner with WinCats software (CAMAG AG, Switzerland). Alternatively, colourless 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 of the method

Verification of the repeatability and reproducibility as well as determination of ink homogeneity was conducted with the use of two selected pen varieties, pen A1 and F1. They were chosen because both of them presenting the most diversify HPTLC profiles. RSD values were calculated for each test and summarised in Table 2.

Table 2: Ink homogeneity, repeatability and reproducibility of HPTLC technique using pen A1 and F1 as samples

Spot no.	Homogeneity RSD (%)		Repeatability RSD (%)		Reproducibility RSD (%)	
	A1	F1	A1	F1	A1	F1
1	0.4318	0.6341	1.5753	1.1046	4.3333	3.8454
2	0.9690	0.6116	1.0193	1.0521	4.1659	3.5484
3	0.8395	0.5856	0.5494	0.8639	3.5584	2.8041
4	0.8985	0.4991	0.7111	0.8353	3.2833	2.8267
5	0.7674	0.5426	0.4688	0.8866	0.3034	3.726
6	na	0.4736	na	0.5482	na	0.8031

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 are a combination of human error and the heterogeneity inherent in sample. Nonetheless, both pen samples gave very satisfy results ($RSD < 1\%$) 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 time (R_f) 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 2%). On the other hand, by referring to the column labeled as reproducibility of Table 2, the effect of different plates triggered a higher RSD values if compared to the one obtained for repeatability for both pen samples.

Conclusions

The HPTLC technique was able to differentiate certain models of a single brand

even only based on direct visual examination on the developed HPTLC plates. A better discrimination power could have achieved if quantitative output such as intensity of colour tones could be collected. Besides, the inks of two selected models were found homogenous within the ink reservoir. The HPTLC technique proved to be simple, repeatable ($RSD < 2\%$) and reproducible ($RSD < 5\%$).

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