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Discrimination of pen inks using thin layer chromatography and UV-visible spectroscopy

İnce katman kromatografisi ve UV-görünür spektroskopinin mürekkep ayrımında kullanımı

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ABSTRACT

In present days, crimes related to forgery and alterations are increasing day by day. In these cases, questioned document examiners are appointed to clarify the doubts arising in writing. Various writing instruments that have different ink constituents are used to produce numerous documents. When the ink is under suspicion, multiple techniques are used to differentiate the ink and its components. Among them, thin-layer chromatography (TLC) and UV-visible spectrometry were used in this study to discriminate various pen ink writings. Thin-layer chromatography was used to separate the components of pen inks and differentiate these based on colour, hRf value and number of spots. On the other hand, UV-visible spectrometry was used to discriminate inks based on the peaks of the UV-visible spectra. The percentage of total differentiation of inks with combined analysis by TLC and UV spectrometry for blue, red, black and green ballpoint pens was 89.58%, 80%, 66.67% and 71.43%, respectively and for blue, red, black and green pilot pens, a total discrimination percentage of 44.45%, 100%, 57.14% and 100% respectively was achieved. The combined results of TLC and UV-visible spectrophotometer were capable of individualising most of the pen ink samples. The analysis using a UV-visible spectrophotometer also helped support the TLC examination results.

Keywords: *Thin layer chromatography, UV-visible spectrometry, differentiation of pens, ballpoint pens, pilot pens.*

ÖZET

Günümüzde sahtecilik ve tahrifata ilişkin suçlar her geçen gün artmaktadır. Bu durumlarda, yazıyla ilgili ortaya çıkan şüpheleri açıklığa kavuşturmak için sorgulanan belge incelemecileri görevlendirilir. Farklı mürekkep bileşenlerine sahip çok sayıda belgenin üretilmesi için çeşitli yazı araçları kullanılır. Mürekkep şüphesi oluştuğunda mürekkebi ve bileşenlerini ayırt etmek için çeşitli teknikler kullanılır. Bu çalışmada çeşitli mürekkep bileşenlerini ayırt etmek için bunların arasında ince tabaka kromatografisi (TLC) ve UV-görünür spektroskopi kullanıldı. TLC ve UV spektroskopisi ile kombine analiz ile mürekkeplerin toplam farklılaşma yüzdesi mavi, kırmızı, siyah ve yeşil tükenmez kalemler için sırasıyla %89,58, %80, %66,67 ve %71,43 olurken, mavi, kırmızı, siyah ve yeşil pilot kalemler için sırasıyla %89,58, %80, %66,67 ve %71,43 oldu. sırasıyla %44,45, %100, %57,14 ve %100'lük toplam ayrımcılık yüzdesine ulaşıldı. TLC ve UV-görünür spektrofotometrenin birleştirilmiş sonuçları, kalem mürekkebi örneklerinin çoğunu bireyselleştirme kapasitesine sahipti. UV görünür spektrofotometre kullanılarak yapılan analiz aynı zamanda TLC incelemesinin sonuçlarının desteklenmesine de yardımcı oldu.

Anahtar Kelimeler: *İnce tabaka kromatografisi, UV-Görünür bölge spektroskopisi, kalemlerin ayrımı, tükenmez kalem, pilot kalem.*

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INTRODUCTION

It is usually said that a piece of paper cannot decide someone's future. However, if that piece of paper is a will, marks card or other important document, it may decide. Especially when the document is altered. Documents may be altered by means of forgery, disguise, erasures, and other methods. An ordinary person cannot know whether a document has been altered, but suspicions may be raised about its authenticity, originality, or authorship (1,2). Hence, forensic document examination plays a vital role by giving an opinion on whether the document has been altered. Inks have been used for decades to write and sign important documents. Different types of inks with distinct writing instruments and contrasting colours were used for writing.

Several methods to distinguish the ink can be applied in the cases where the ink is in question. These methods include microscopy, thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), Fourier transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GCMS), ultraviolet-visible spectrometry, etc. With TLC, dyes and a few of the pigments can be separated by comparing their retardation factor (R_f) values. Ultraviolet-visible spectrometry is a technique which works on the principle of Beer-Lambert law (3).

Crown *et al.* (4); Nakamura and Shimoda (5); Lewis (6); Brunelle (7); Djozan *et al.* (8); Houlgrave *et al.* (9); Barker *et al.* (10); Yadav (11); Aginsky (12) examined the inks by TLC and paper chromatography. Tsutsumi and Ohga (13); and Bansinge *et al.* (14) worked on the analysis of writing ink dyes by TLC and FTIR and its applications to forensic science. Roux *et al.* (15) investigated the blue and black ballpoint pen inks using filtered light examination, microspectrophotometry, and TLC. LaPorte *et al.* (16); Glover *et al.* (17); Kaluarachchi (18) compared unknown inks with the inks at the United States International Library using TLC and video spectral comparator. Cousin *et al.* (19); Sharif *et al.* (20) discussed the discriminating potential of UV-visible spectrometry, TLC, and FTIR spectroscopy for the forensic analysis of inks. Saini *et al.* (21) used TLC and visible spectrometry on the blue gel pen inks. Sombut *et al.* (22) separated pen inks using TLC. Poon *et al.* (23); Saini *et al.* (24) differentiated the inkjet printer inks by TLC and HPTLC.

In this study, TLC and UV-visible spectrometry were used to differentiate ballpoint pen inks from pilot pen inks. They were used because they are readily available in almost all forensic laboratories, economical, easy to use, low time consumption, and easy to interpret results. Thus, the study was designed to discern the power of TLC and UV-visible spectrometry to differentiate writing inks based on their colourants.

MATERIALS AND METHODS

Collection of materials

For the study, 110 pens were collected, including 86 ballpoint pens and 24 pilot pens in blue, black, red, and green of different makes and models. The ballpoint pen included 48 blue pens, 21 black pens, 10 red pens, and 7 green pens, and the pilot pen included 9 blue pens, 7 black pens, 4 red pens, and 4 green pens.

Each pen was given a code, which was unique for each pen. B at the initial position stands for ballpoint pens, and P at the initial position stands for pilot pens. In the medial position, B, K, G and R were used for blue, black, green and red coloured pens, respectively. Each code was then given a number at its terminal position to maintain its uniqueness (Refer to Table 1-5).

Based on the literature survey, nine reference dyes were also procured, including methylene blue, victoria blue, tartrazine, malachite green, crystal violet, coomassie brilliant blue, methyl orange indicator, metanil yellow GR, and nigrosine. Pen ink writing samples were made on non-fluorescence A4 size sheets of white paper (70GSM) of the SPECTRA TRIDENT GROUP company. Ethyl acetate (pure chems), butanol (chemical enterprise), ethanol (analytical reagent), distilled water, methanol (alpha chemika), and acetic acid (pure chems) of chromatographic grade were used.

Preparation of writing sample

For the preparation of samples, various lines of approximately 5 cm each were made using each type of pen on the sheet of paper. These sheets of paper were stacked by placing three blank sheets of paper in between them to prevent cross-contamination due to migration, diffusion, etc., and were kept in a file. The file was further stored in a cupboard in a laboratory, where the humidity level and temperature were controlled at 45% humidity and 27°C.

Sample extraction

From each of the line strokes, 6 punches of 5mm diameter were punched out with the help of a puncture. The punches were then taken into a test tube, into which 1 ml of each extraction solvent was added individually. The solution was covered and kept undisturbed for 1 hour. The extraction solvent which gave the best results was chosen for the study.

Standardisation of working conditions for the TLC Method

Standardisation of the solvent system (mobile phase), extraction solvent, number of spots to be applied on the TLC plate, and number of strokes to be extracted from the paper was performed. Different types of extraction solvents, namely, acetone, ethanol, methanol, chloroform, acetic acid, ethanol and water in the ratio of 1:1, benzene, water, and n-hexane were used to extract inks.

Three different solvent systems were used, namely, butanol, ethanol, distilled water, and acetic acid in the ratio of 60:30:10:1 (solvent system A); ethyl acetate, ethanol, and distilled water in the ratio of 70:35:30 (solvent system B); and chloroform, methanol, and hexane in the ratio of 30:15:10 (solvent system C).

For the standardisation of the number of strokes, 4, 6, 8, and 10 punches of 5mm diameter were taken, and the extract was made with these pen ink strokes and spotted on TLC plates. To standardise the number of spots, the same extracts were used. Each extract was spotted 4 times, 6 times, 8 times and 10 times. The second spot was applied once the first spot dried completely.

TLC examination

The extracted samples were spotted on the pre-coated silica gel G plates (Merck KGaA) by using capillary tubes. The spots were applied by leaving 1 cm of space from the base of the TLC plates. The sample was allowed to run 5 cm from the spotting point. The developed plates were visualised under visible light and long UV light. The photographs of the plates were made under visible light as well as UV light (100-400nm) (Figure 1). The hR_f value of each spot was calculated, where hR_f is the distance travelled by a spot to the distance travelled by the solvent front multiplied by 100.

UV-visible spectrometry

Analytik Jena Specord®210 Plus model of UV-visible spectrometer (Germany) was used for analysis. ASpect UV 1.5 software (Analytik Jena, 2020) was used. The analysis was carried out in the range of 200 nm-700 nm wavelength. The pen ink writings were extracted using methanol. For this, 6 punches of 5mm diameter were taken from the pen ink writings made on a white sheet of paper and were dissolved in 1 ml methanol. A reference sample of methanol, along with blank paper punches, was analysed to remove the background spectrum. The graphs were generated for each reference sample. Figure 2 represents the graphs obtained for methyl yellow and crystal violet.

Discrimination percentage

The discrimination percentage for both techniques, separately as well as collectively, for all samples was calculated. Discrimination percentage is the ratio of the number of groups formed after analysis

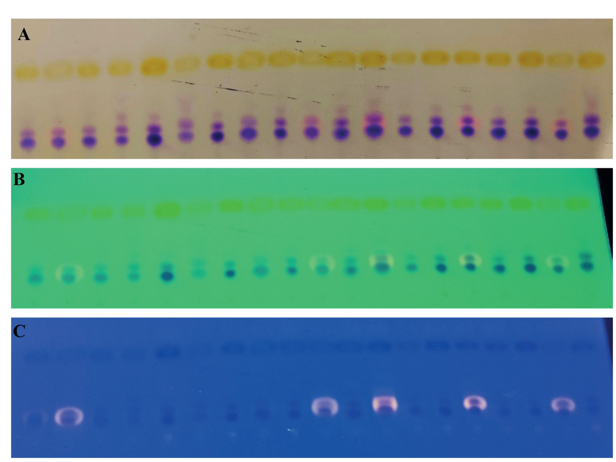

Figure 1. Showing results of TLC analysis for the differentiation of black ballpoint pens using solvent system A based on the number of sports, their colour, and hRf value: (a) under visible light, (b) under short UV light, (c) under long UV light.

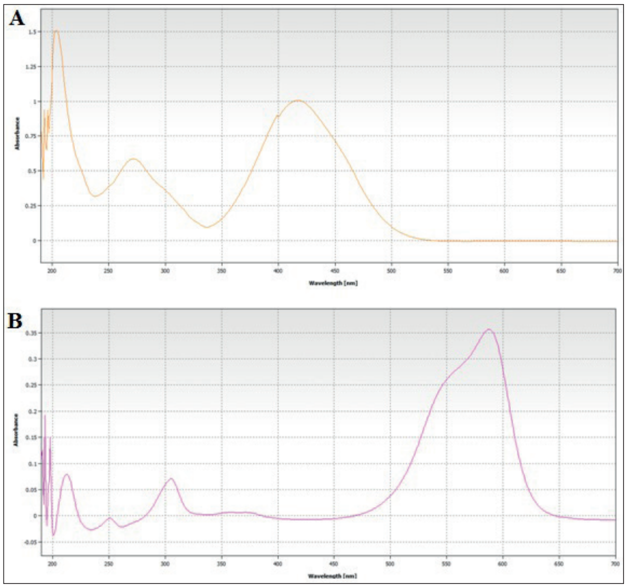

Figure 2. UV-Visible spectrometry analysis showing graph of reference dye: (a) methyl yellow and (b) Crystal Violet.

(using thin-layer chromatography and UV-visible spectrometry) to the total number of samples multiplied by 100.

Repeatability and reproducibility

Each sample was tested thrice to check the reproducibility and repeatability of the method used in the study. Hence, triplicate spotting of each ink sample was done in the case of TLC analysis. Standards were also analysed thrice.

RESULTS

Sample extraction

For sample extraction, methanol gave the best results. Out of 110 pens, 96 pen ink samples dissolved in methanol, which included 86 ballpoint pens, and 10 pilot pens (Table 1).

Standardisation of the TLC method

After standardisation, the best results were obtained when four punches of 5mm diameter were dissolved in 0.5 ml extraction solvent (methanol). Using a capillary tube, each sample was spotted 6 times on the TLC plate. The samples were spotted one over the other when the previous spot was completely dried. Out of the three solvent systems, solvent systems A and B were capable of separating the contents of ink samples.

TLC plate examination under visible light and UV-visible light

The pen inks were classified based on the number of spots, colours of spots and their hR_f values. In solvent system A, blue, red, black and green ballpoint pens were classified into 6, 4, 2 and 2 groups, respectively. Blue, red, black and green pilot pens were classified into 2, 2, 2 and 2 groups, respectively (Table 2). However, in the solvent system B, blue, red, black and green ballpoint pens were classified into 5, 5, 5 and 2 groups, respectively. Blue, red, black and green pilot pens were classified into 2, 2, 2 and 2 groups, respectively (Table 3). The hR_f value and colour of each spot for reference dyes were also calculated and analysed under visible light and UV light (Table 4). During the analysis, spots were observed under UV light for certain pen inks in both solvent system A and solvent system B.

The discrimination percentage of blue, red, black and green ballpoint pens using solvent system A was achieved to be 12.5%, 40%, 9.53% and 28.57%, respectively and using solvent system B, it was 10.41%, 50%, 23.8% and 28.57%, respectively. The percentage of total differentiation with combined analysis using both solvent systems A and B for blue, red, black and green ballpoint pens was 27.08%, 60%, 23.81% and 28.57%, respectively. For blue, red, black and green pilot pens, the discrimination percentage was 44.45%, 75%, 42.86% and 100% with solvent system A and 44.45%, 75%, 42.86% and 100% with solvent system B. Combined analysis using solvent systems A and B for blue, red, black and green pilot pens achieved a total discrimination percentage of 44.45%, 100%, 57.14% and 100%, respectively. Hence, TLC alone was not able to differentiate the pen inks satisfactorily.

UV-Visible spectrometry

All pens were then subjected to UV-visible spectrometry. The pen ink writings were extracted using methanol and were directly analysed. The blue, red, black and green ballpoint pens were classified into 23, 5, 5 and 4 groups, respectively. Blue, red, black and green pilot pens were classified into 2, 2, 2 and 2 groups, respectively (Table 5). UV-visible spectrometry alone was also not able to differentiate the pen inks sufficiently. For blue, red, black and green ballpoint pens, a total discrimination

Table 1. Representing the results of the solubility test using methanol as extraction solvent and the number of groups formed.

Group number	Pen Type	Pen ID	Solubility
1	Ballpoint Pens	BB01-BB48	Soluble
2		BR01-BR10	Soluble
3		BK01-BK21	Soluble
4		BG01-BG07	Soluble
1	Pilot pens	PB01, PB02, PB03, PB09	Soluble
2		PB04, PB05, PB06, PB07, PB08	Insoluble
3		PR02-PR04	Soluble
4		PR01	Insoluble
5		PK02, PK03, PK05	Soluble
6		PK01, PK04, PK06, PK07	Insoluble
7		PG02-PG04	Soluble
8		PG01	Insoluble

Table 2. Groups of pen inks formed after TLC examination using solvent system A and their hR_f value under visible and UV light.

Group number	Pen ID	hRf value under visible light	Spot colour	hRf under UV light	Spot colour
1.	BB2, BB5	10.9	Sky blue	-	-
2.	BB3, BB4	18.1	Blue	-	-
3.	BB6, BB7,BB 9, BB11, BB12, BB18, BB27, BB28, BB31, BB38, BB40, BB44, BB45	18.1, 27.2	Blue, violet	-	-
4.	BB15, BB19, BB20, BB21, BB22, BB23, BB24, BB29, BB32, BB34, BB36, BB39, BB43, BB46	18.1, 27.2, 36.3	Sky blue, blue, violet	-	-
5.	BB8, BB10, BB13, BB14, BB16, BB17, BB25, BB26, BB30, BB33, BB35, BB37, BB41, BB42, BB47, BB48	20.0, 23.6, 29.0	Blue, skyblue, violet	-	-
6.	BB1	54.5	Blue	-	-
1.	BR1, BR4, BR5, BR6, BR7, BR8	18.1, 36.3, 72.7	Pink, vibrant yellow, yellow	18.1, 36.3, 72.7	Pink, lemon, fluorescent white
2.	BR2, BR3	18.1	Pink	18.1	Pink
3.	BR9	36.3	Yellow	18.1, 36.3, 72.2	Fluorescent white, lemon, Fluorescent white
4.	BR10	36.6	yellow	18.1, 36.3	Fluorescent white, lemon
1.	BK1, BK3, BK4, BK5, BK6, BK7, BK8, BK10, BK12, BK13, BK15, BK16, BK18, BK19, BK20, BK21	14.5, 20.0, 54.5	Blue, violet, yellow	-	-
2.	BK2, BK9, BK11, BK14, BK17	14.5, 16.3, 20.0, 54.5	Blue, pink, violet, yellow	16.3	Fluorescent yellow
1.	BG1	27.2	Pink	27.2	Fluorescent white
2.	BG2, BG3, BG4, BG5, BG6, BG7	-	-	-	-
1.	PB1	30.9	Pink		
2.	PB3, PB2	30.9, 54.5	Pink	30.9, 54.5	Fluorescent pink
3.	PB9	21.8, 30.9	Sky blue, pink	30.9, 41.0	Fluorescent yellow, Fluorescent yellow
1.	PR2, PR3	78.1	Pink	78.1	Fluorescent pink
2.	PR4	10.9	Brick red	-	-
1.	PK2, PK3	23.6	Pink	23.6	Pink
2.	PK5	56.3	Black	-	-
1	PG2	54.5, 21.8	Blue, Sky blue	-	-
2.	PG3	21.8	Sky blue	-	-
3.	PG4	54.1	Blue		

Table 3. Groups of pen inks formed after TLC examination using solvent system B and their hRf value under visible and UV light

Group number	Pen ID	hR_f value under visible light	Spot colour	hR_f under UV	Spot colour
1.	BB1, BB3, BB4, BB6, BB28, BB31, BB33	41.8, 45.5, 49.0, 52.7	Violet, blue, sky blue, blue	-	-
2.	BB2, BB5, BB9, BB15, BB19, BB24, BB32, BB39, BB43	10.9, 4.8, 45.5	Sky blue, violet, blue	-	-
3.	BB21, BB22, BB23, BB29, BB34, BB36	18.1, 27.2, 54.5	Sky blue, blue, violet	-	-
4.	BB7, BB8, BB12, BB13, BB14, BB16, BB17, BB25, BB27, BB30, BB38	29.0, 34.5, 56.3	Violet, violet, blue	-	-
5.	BB10, BB11, BB18, BB20, BB26, BB35, BB37, BB40, BB41, BB42, BB44, BB45, BB46, BB47, BB48	43.6, 54.5	Violet, violet	-	-
1.	BR1, BR8, BR10	16.3, 40.0, 54.5	Yellow, pink, orange neon	40.0, 54.5	Pink and yellow, yellow
2.	BR2, BR3	16.3, 40.0	Yellow, pink	16.3, 40.0	Pink and yellow, fluorescent white
3.	BR5, BR6, BR7	40.0, 54.5	Pink, orange neon	40.0, 54.5	Pink and yellow, yellow
4.	BR4	40.0, 54.5	Pink, orange neon	40.0, 72.7	Pink and yellow, yellow
5.	BR9	16.3, 40.0, 54.5	Yellow, orange neon, orange neon	32.7, 40.0, 54.5	Yellow, pink and yellow, yellow
1.	BK19, BK20, BK21	18.1	Blue	-	-
2.	BK2, BK9, BK14, BK17	21.8, 25.4, 40.0	Blue, violet, pink	40.0	Fluorescent yellow
3.	BK1, BK3, BK4, BK5, BK6, BK8, BK10, BK12, BK13, BK15, BK16	20.0, 22.2	Blue, violet	-	-
4.	BK7, BK18	24.4, 30.9, 50.9	Violet, violet, violet	-	-
5.	BK11	21.8, 40.0, 50.9	Blue, pink, violet	40.0, 50.9	Fluorescent yellow, fluorescent yellow
1.	BG1	63.6	Pink	41.8	Fluorescent white
2.	BG2, BG3, BG4, BG5, BG6, BG7	-	-	-	-
1.	PB1	23.6	Sky blue	-	-
2.	PB3, PB2	23.6, 41.8, 72.7	Sky blue, pink, blue	23.6	Fluorescent pink
3.	PB9	23.6, 41.8	Blue, pink	23.6, 52.7	Fluorescent yellow, Fluorescent yellow
1.	PR2, PR3	94.5	Pink	94.5	Fluorescent pink
2.	PR4	20.0	Brick red	-	-
1.	PK2, PK3	41.8	Pink	41.8	Fluorescent pink
2.	PK5	69.0	Black	-	-
1.	PG2	60.0	Blue	-	-
2.	PG3	36.3, 67.2	Sky blue, yellow	-	-
3.	PG4	60.0	Blue	-	-

Table 4. TLC examination of reference dyes using solvent system A and solvent system B and their hRf value under visible and UV light

Reference dyes									
				Visible light			UV Light		
S. no.	Reference dyes	dyes code	Solvent system	No. of spots	hR_f value	colour	No. of spots	hR_f value	colour
1.	Methylene blue	RD1	A	2	9.0	Sky blue	-	-	-
					52.7	Blue			
			B	3	9.0	Sky blue	-	-	-
					52.7	Blue			
					56.3	Blue			
2.	Victoria blue	RD2	A	3	20.0	Blue	1	56.3	Fluorescent white
					50.9	Blue			
					76.3	Violet			
			B	3	14.5	Blue	1	1	Fluorescent white
					58.1	Blue			
					65.4	Blue			
3.	Tartrazine	RD3	A	-	-	-	-	-	-
			B	2	27.2	Yellow	-	-	-
					87.2	Red			
4.	Malachite green	RD4	A	3	9.0	Sky blue	-	-	-
					18.1	Violet			
					76.3	Violet			
			B	1	7.2	Sky blue	-	-	-
5.	Crystal violet	RD5	A	2	18.1	Purple	-	-	-
					40.0	Purple			
			B	2	9.0	Purple	-	-	-
					58.1	Purple			
6.	coomassie brilliant blue	RD6	A	3	27.2	Blue	1	21.8	Fluorescent white
					45.4	Blue			
					58.1	Blue			
			B	2	45.4	Blue	2	34.5	Fluorescent white
					83.6	Violet		40.0	Fluorescent white
7.	Methyl orange indicator	RD7	A	2	40.0	Yellow	-	-	-
					65.4	Yellow			
			B	1	76.3	Yellow	-	-	-
8.	Metanil yellow GR	RD8	A	1	63.6	Yellow	-	-	-
			B	1	81.8	Yellow	-	-	-
9.	Nigrosine	RD9	A	1	63.6	Reddish yellow	1	58.1	Fluorescent pink
			B	2	52.7	Brown	-	-	-
					81.8	Orange			

percentage of 47.92%, 50%, 23.81% and 28.57% were achieved with UV-visible spectrometry, and a discrimination percentage of 22.22%, 75%, 28.57% and 50% was achieved for pilot pens. The method was dependent on the concentration of the pen ink writing sample. Reference dyes were also analysed, and their UV-visible spectra were generated (Table 5). The UV-visible spectrometry data was used to know the type of dye or pigment used in the constituents of pen inks based on the comparison of the peaks with reference dyes. Hence, it helped to support the results of the TLC examination.

However, when the results of all three techniques, that are, solubility test, TLC analysis with two types of solvent systems and UV-visible spectrometry, were combined for blue, red, black and green ballpoint

Table 5. Groups of pen inks formed after UV-visible spectrometry along with their wavelengths

Group no	Pen ID	Peak wavelength	Group no	Pen ID	Peak wavelength
1.	BB12, BB14, BB16, BB20, BB23, BB30, BB35, BB37, BB45	210, 272, 278, 304, 584	1.	BK1, BK4, BK6, BK14	207, 251, 278, 304, 417, 553, 586
2.	BB5, BB39, BB46	202, 210, 272, 278, 304, 584, 625	2.	BK2, BK5, BK7, BK9, BK12, BK16, BK18, BK19	202, 207, 251, 265, 272, 278, 304, 417, 586, 553
3.	BB4, BB15, BB17	202, 210, 272, 278, 304, 408, 584, 625	3.	BK15	202, 251, 265, 304, 417, 586, 553
4.	BB1, BB6, BB21	202, 251, 304, 584	4.	BK3, BK11, BK13, BK17, BK21	207, 251, 265, 272, 278, 304, 417, 586, 553
5.	BB25, BB47	212, 251, 272, 278, 304, 584	5.	BK8, BK10, BK20	202, 207, 251, 265, 272, 278, 304, 417, 553, 586
6.	BB31	208, 253, 303, 357, 584	1.	BR1	212, 216, 252, 260, 272, 278, 350, 531
7.	BB19, BB32	208, 253, 272, 278, 303, 357, 384	2.	BR10	212, 216, 252, 266, 272, 278, 350, 531
8.	BB7, BB10	202, 251, 272, 278, 304, 584	3.	BR3	203, 264, 272, 278, 546
9.	BB24	202, 210, 251, 272, 278, 408, 584, 625	4.	BR2	203, 259, 546
10.	BB29	202, 251, 272, 278, 304, 584, 625	5.	BR4, BR5, BR6, BR7, BR8, BR9	203, 264, 272, 278, 350, 531
11.	BB13	210, 251, 304, 320, 584	1.	BG1, BG2, BG4, BG5	209, 265, 272, 278, 345, 600, 666
12.	BB44	209, 267, 305, 584	2.	BG3	209, 265, 272, 278, 446, 660
13.	BB3, BB8	213, 272, 278, 304, 584	3.	BG6	202, 272, 278, 666
14.	BB11	212, 272, 304, 320, 584	4.	BG7	202, 272, 278, 345, 600, 666
15.	BB38	212, 272, 278, 304, 584	1.	PB2, PB3	226, 307, 562, 617
16.	BB43	202, 251, 304, 584, 625	2.	PB9	202, 260, 307, 356, 410, 557, 625
17.	BB48	202, 251, 272, 278, 304, 584	1.	PK2	202, 405, 572
18.	BB2, BB22	210, 272, 304, 584, 625	2.	PK3	202, 558
19.	BB18, BB26, BB27, BB28, BB41	202, 210, 272, 278, 304, 584	1.	PR2, PR4	203, 310, 343, 409, 526
20.	BB33, BB36	210, 272, 278, 304, 584, 625	2.	PR3	202, 215, 335, 509
21.	BB42, BB40	210, 272, 278, 304, 320, 584	1.	PG2	201, 402, 631
22.	BB34	208, 272, 584, 625	2.	PG3	207, 292, 345, 418, 441, 598, 665
23.	BB9	210, 272, 278, 304, 320, 584, 625			

pens, a total discrimination percentage of 89.58%, 80%, 66.67% and 71.43% respectively was achieved. For blue, red, black and green pilot pens, a total discrimination percentage of 44.45%, 100%, 57.14% and 100%, respectively, was achieved. Schematic flow charts of differentiating the pen inks using all the techniques were made for easy understanding (Figure 3-10).

DISCUSSION

The objective of the study was to differentiate ballpoint and pilot pen ink writings by using thin-layer chromatography and UV-visible spectrometry. The ink consists of dyes and pigments as a colouring agent. Methanol was used to extract the pen inks for both types of pens. Methanol is a colourless

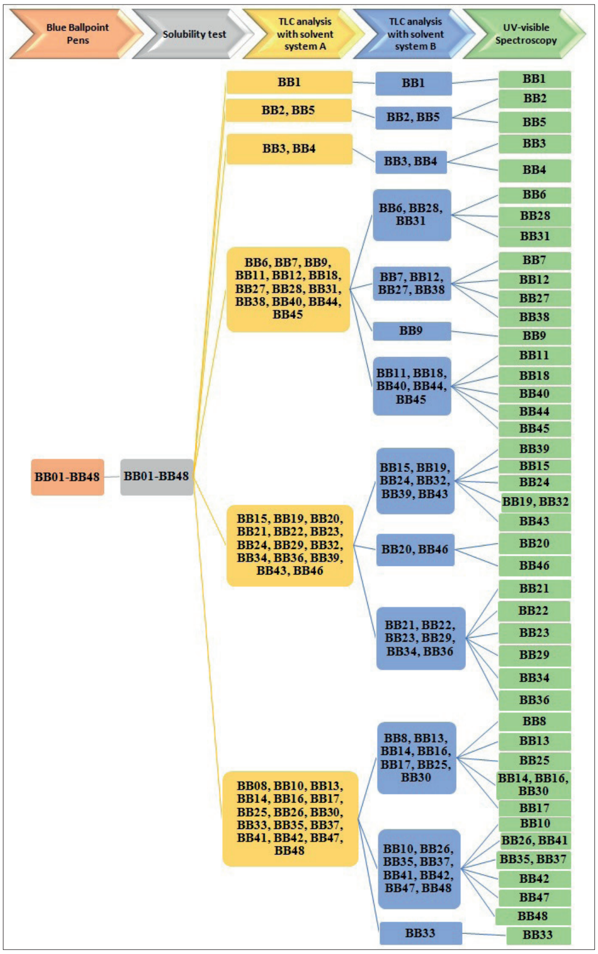

Figure 3. Schematic flow chart showing the differentiation of blue ballpoint pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

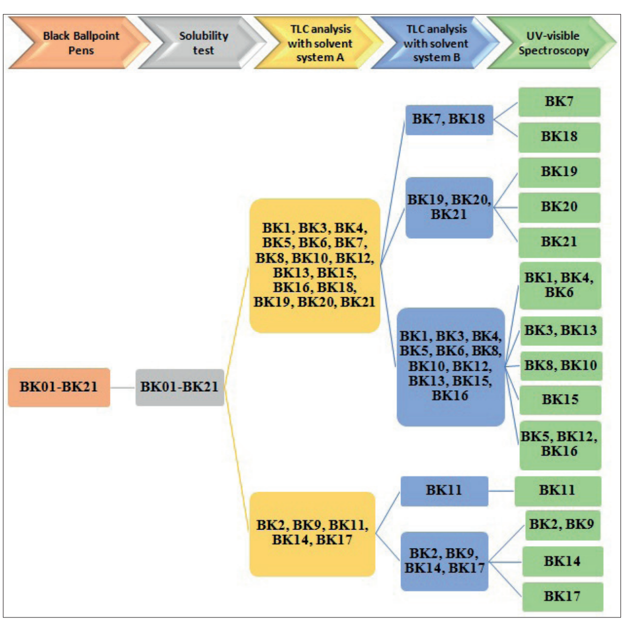

Figure 4. Schematic flow chart showing the differentiation of black ballpoint pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

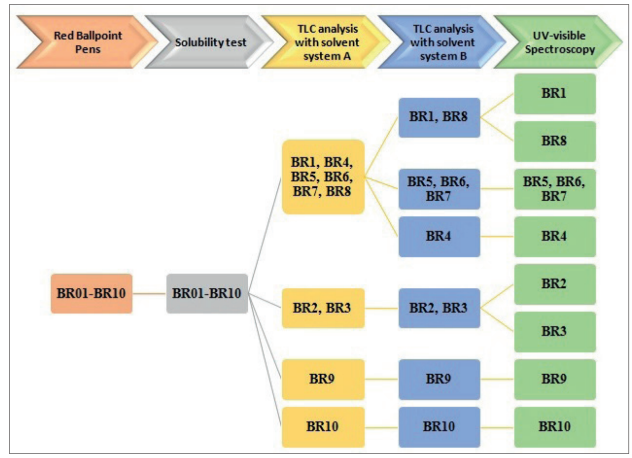

Figure 5. Schematic flow chart showing the differentiation of red ballpoint pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

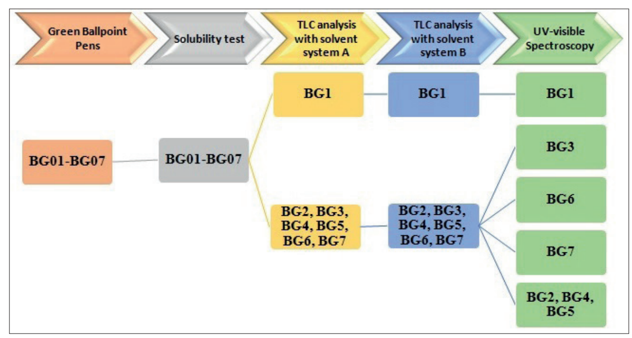

Figure 6. Schematic flow chart showing the differentiation of green ballpoint pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

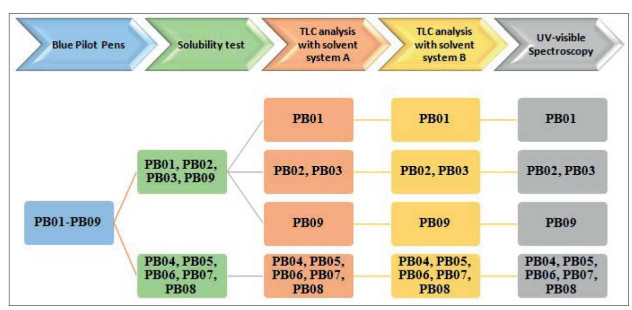

Figure 7. Schematic flow chart showing the differentiation of blue pilot pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

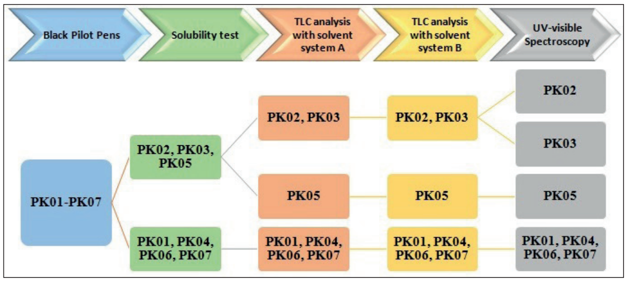

Figure 8. Schematic flow chart showing the differentiation of black pilot pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

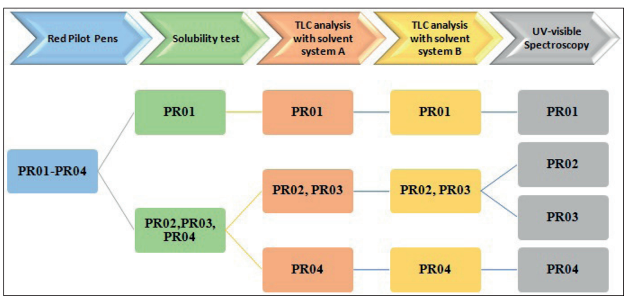

Figure 9. Schematic flow chart showing the differentiation of red pilot pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

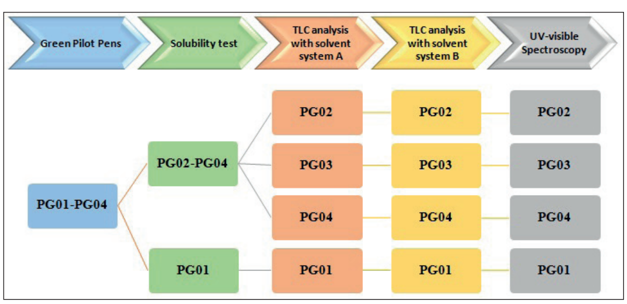

Figure 10. Schematic flow chart showing the differentiation of green pilot pens using solubility test, TLC analysis using solvent system A, TLC analysis using solvent system B, and UV-visible spectrometry.

solvent that helps differentiate between dye-based and pigment-based inks, and it extracted most of the ink samples used in the study. Out of the 110 pens, pen ink writings made by 96 pens were successfully extracted using methanol. The results were obtained quickly compared to other extraction solvents. Djozan *et al.* (8), Yadav (11), Bansinge *et al.* (14) also utilised methanol for the extraction purpose, as it gave better extraction than other solvents. On the other hand, Saini *et al.* (24) used ethanol and picric acid as the extraction solvent. Thus, the results of ink extraction were in accordance with the previous studies.

By utilizing the technique of thin-layer chromatography, it was possible to effectively differentiate and identify the dye components present in the pen ink composition. This method has proven to be highly reliable, providing valuable insights into the chemical composition of the pen inks (9). After analysis, the pen samples were grouped into several groups that had similar dye components. Various pen manufacturers might have the same source of ink or raw material. Saini and Rathore (25,26) made groups based on similarities in hR_f values using TLC, HPTLC, and GCMS. Roux *et al.* (15), Bansinge *et al.* (14), and Sharif *et al.* (20) reported that thin-layer chromatography was a reliable technique for differentiation. The results presented in this study are aligned with the previous findings, further strengthening the credibility of the research. The findings are significant, and they serve as a crucial reference point for future research in the field.

From the results of TLC, it was observed that various mobile phases have different abilities to differentiate between ink samples. The results showed that two solvent systems used in the study produced different groupings of ink samples. Different pens from the same brand were sometimes grouped together, but other times, they were separated into different groups. This could be because of variations in ink composition within specific models of the brand. Therefore, it cannot be assumed that all pens from the same brand have identical ink compositions. Djozan *et al.* (8), Houlgrave *et al.* (9), Saini and Saroa (27) and Saini *et al.* (28) also reported that there might be the use of more than one ink or toner formulation by the same brand manufacturer. Some additional spots were also observed when the TLC plates were analysed under UV light, but they did not appear under visible light. Houlgrave *et al.* (9) also reported such spots which appeared under UV light and not in visible light, which helped in better differentiation of ink samples.

Pen ink dyes or pigments were analysed using a UV-visible spectrophotometer and thin-layer chromatography to determine their composition. Reference dyes were compared to the observed peaks to ensure precise findings. The concentration of the ink sample taken for the analysis was also taken into account, and pen wavelengths were classified for straightforward comparison. However, the results were influenced by the composition of the pen inks used to write. As a result, the pens were grouped based on their wavelengths to facilitate comparison. Cousin *et al.* (19) used extracts made of ethanol for UV-visible spectrometry examination and achieved good differentiation of peaks. Sharif *et al.* (24) also used distilled water as an extraction solution for analysis.

This study successfully discriminated the pen ink samples and grouped them into several groups. The percentage of total differentiation with combined analysis for blue, red, black and green ballpoint pens was 89.58%, 80%, 66.67% and 71.43%, respectively and for blue, red, black and green pilot pens, a total discrimination percentage of 44.45%, 100%, 57.14% and 100% respectively was achieved. While analysing the samples using UV-visible spectrometry, it is suggested to take care of the concentration of the ink sample taken for the analysis in the UV-visible spectrophotometer.

CONCLUSIONS

Thin layer chromatography is an affordable technique; it helps differentiate the ink components, and several groups were made that had similar ink compositions. In TLC, methanol was used as an extraction solvent. Solvent systems A (butanol, ethanol, distilled water in the ratio of 60:35:30) and solvent system B (ethyl acetate, ethanol, distilled water, acetic acid in the ratio of 60:30:10:1) were taken as solvent systems because both the solvent systems gave variable differentiation of ink components and separation was successful. TLC differentiated all the samples into several groups using hR_f value, solvent system used, number of spots, colour of spots, and UV light examination. The percentage of total differentiation with combined analysis for blue, red, black and green ballpoint pens was 89.58%, 80%, 66.67% and 71.43%, respectively and for blue, red, black and green pilot pens, a total discrimination percentage of 44.45%, 100%, 57.14% and 100% respectively was achieved. The analysis using a UV-visible spectrophotometer helped to support the results of the TLC examination. It was suggested that the concentration of the ink sample taken for the analysis be considered while using UV-visible spectrometry. TLC and UV-visible spectrometry alone could not fully individualise all the ink samples. However, when used in combination, satisfactory results were achieved.

List of abbreviations

FTIR - Fourier Transform Infra-Red

GCMS - Gas Chromatography-Mass Spectrometry

GSM – Grams per Square Meters

HPTLC - High-Performance Thin Layer Chromatography

R_f - Retardation Factor

TLC - Thin Layer Chromatography

UV - Ultraviolet

UV-vis - Ultraviolet-visible

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