DISCRIMINATION STUDY BETWEEN BLUE BALLPOINT PEN INKS USING THIN LAYER CHROMATOGRAPHY AND UV-VIS SPECTROSCOPY TECHNIQUES.

Vasinee Sombut[1], Ploysai Ohama [2], Saowanee Kumpun[3], Narong Kulnides[4]
& Boon-ek Yingyongnarongkul [5]
[1-4]Department of forensic science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand,
[5]Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand,
E-Mail: [1]s61584938002@ssru.ac.th, [2]ploysai.oh@ssru.ac.th

ABSTRACT

Scientific analysis of ink is often required as evidence in investigations involving questioned document examination (QDE) in a court of law relating to signatures and handwriting. Ink becomes important forensic evidence. Blue ballpoint pen inks commonly found in Thailand, were discriminated using two techniques of thin-layer chromatography (TLC) and UV-Vis spectroscopy. Results were assessed, calculated and compared in terms of discriminatory power (DP) of ink obtained as a common writing instrument. The ink was extracted from the documents using three different solvents as ethanol, acetone and dichloromethane by both techniques. The TLC method employed five different mobile phases to separate pigment and compounds in each sample, while UV-Vis spectroscopy recorded four types of data including wavelength of λ_{max}, amplitude of λ_{max}, shoulder and minor absorption peaks. Total of 435 possible pen-pairs were distinguished based on results of the chromatograms and UV-Vis spectra. The TLC method discriminated the ink samples into 12 groups by ethanol extraction. The highest classification power was the TLC method using ethanol as the solvent the mobile phase system 1 (*n*-butanol: ethanol: H_2O in a ratio 50:15:10 by volume). The UV-Vis spectroscopy method recorded optimal DP at 77.24% for ethanol extraction. Results indicated that the TLC technique was a more effective tool for blue ballpoint pen ink analysis than UV-Vis spectroscopy technique. In the future, higher DP can be achieved. Multivariate statistical techniques applied for data interpretation may lead to the development of blue ballpoint pen ink test kits for real-time use at crime scenes.

Keywords: Blue ballpoint pen inks, Thin-layer chromatography, UV/Vis spectroscopy

INTRODUCTION

We are all aware of the important documents in our lives such as identity documents, educational documents, insurance agreements, financial documents and contracts. These important documents often require the addition of a personal and identifying signature. For this reason, many transactions or agreements still legally require the application of ink. Blue ballpoint pen ink is the most popular for writing or signing documents, and this ink variety is frequently encountered in forensic questioned document analyses. The document verification and analysis department of the Forensic Science Institute in Thailand reported a total of 480 issues of questioned documents in the year 2017, representing 12,777 items and the annual trend is increasing [1]. The evidence of suspicious documents that is analyzed and examined by using can increase the reliability of investigation. Forged documents usually comprise of two forms as falsifying a genuine document and making forgeries as false documents.

Falsifying a document involves changing or modifying the original, while forging a document is the process of creating a new document very similar to the original [2]. Currently, the forensic examination of ink can resolve three major problems which include identifying the ink source, comparing different types of ink and determining the aging processes of writing ink [3]. The aim of most analyses involves the comparison of different writing inks on a document as the primary goal of the investigations [4].

The components of particular inks are indications of specific characteristics, especially involving the coloring materials. Ink coloring materials come in the form of pigments, and components of ink also include dyes and other various combinations. Pigments are particles which provide opacity through molecules that are linked together in crystalline structures and reflect color depending on the raw materials used in their production. Blue color can be obtained using dyes with methyl violet, Victoria blue and triphenylmethane pigment. Dyes are a mixture of solvents, oils and resins, sometimes referred to as different parts of the vehicle carrier, as an important characteristic which directly affects the flow and drying properties of inks. The solvent mixture is a variety of organic and inorganic materials providing the writing instrument with the desired characteristics and made up of glycol or glycol ether. This has a boiling point very high so inks are stable at room temperature. Evaporation of the solvent and prevention of fouling by the small ball at the tip of the pen do not affect the ink flow when written on paper. Other substances are used to improve certain properties to adapt to different characteristics in accordance with the particular purpose and usage of the writing pen [5-7].

The forensic analysis of ink can be divided into non-destructive and destructive approaches. Non-destructive analytical methods involve the specific characteristics of inks as parameters such as colors, visual luminescence and radiation absorption. Inks deposited on the paper surface of questionable documents may be differentiated by properties of transmission, reflection and fluorescence spectra [8]. Destructive analytical methods start by extracting the ink from the paper and offer a variety of analytical techniques including thin layer chromatography (TLC), high performance liquid chromatography (HPLC), chemical analyses using separation mixture techniques and UV-Vis spectroscopy through absorbance of compounds that can be detected in both ultraviolet and visible wave ranges. Even though a blue ballpoint pen presents as only one color, the ink is actually made from a mixture of various chemicals. Physico-chemical analytical methods can be used to determine the type and composition of ink, leading to successful identification [9].

Among these options, TLC techniques are applied most often because this method is rapid and convenient with inexpensive material composition and requires no sophisticated instrumentation. TLC techniques are commonly used to check the purity of a substance, confirm the type of substance and determine the number of components in the mixture. An important factor in TLC testing is the process of separating ink samples from the surface of the document. A sufficient amount of ink is required for verification while rendering minimal damages to the inspected documents. Another factor is the repeatability and reproducibility of ink analysis results depending on the selection of the appropriate solvent system [10]. Meanwhile, the principle of the UV-Vis spectroscopy technique is that different chemicals absorb ultraviolet and visible radiation at disparate intensities and wavelengths, therefore allowing detection through adsorption to produce a “chemical fingerprint” [11].

This research focused on destructive document analysis using thin layer chromatography and UV-Visible spectroscopy techniques to distinguish blue ballpoint pen ink. Before proceeding with the chemical analysis, the ink was removed from the substrate, taking great care to preserve the integrity of the documents. Data analyses used both mathematical and statistical methods to improve the accuracy of discrimination of pen inks. This study used discrimination power (DP) to explain the ability to eliminate ink differences for each of the methods tested.

OBJECTIVES

1. To determine discrimination power (DP) for comparison of blue ballpoint pen ink analysis between thin-layer chromatography and UV-Vis spectroscopy techniques.
2. To study the influence of different solvents and mobile phases on the separation of each blue ballpoint pen using thin layer chromatography.
3. To compare the effect of ink separation from each blue ballpoint pen by the UV-Vis spectroscopy method when using different solvents.

METHODOLOGY

Thirty blue ballpoint pens were purchased from a market in Bangkok, Thailand and subjected to systematic random sampling. All pens were allocated reference numbers as listed in Table 1. Each pen was used to write the author's name two times on a piece of A4 white paper (Double A, 80 gram) to replicate consistency with documents at a crime scene. The sample setting size was determined as a punched hole of 5 mm diameter and five holes were collected from each sample and used as a substrate for the deposited inks.

Extraction of ink from paper

Five pieces of each sample of ink deposited on the paper were placed in a ceramic tray and the number of the ink was recorded. Then, 0.2 ml of extract solution was added and left for about 20 seconds at room temperature. In this experiment, ethanol, acetone and dichloromethane were used as the three different chemicals for ink extraction. The solutions obtained after extraction of the ink were subjected to both TLC and UV-Vis spectroscopy examination methods.

Table 1
The list of studied blue ballpoint pens

No.	Commercial characteristics	No.	Commercial characteristics
1	GRIP X P5 FABER-CASTELL	16	FABER-CASTELL TRUE GELL
2	Pentel ENERGEL BL107	17	PAPER:MATE Ink Joy 100 XF
3	XF STAEDTLER LUNA Ball	18	g'soft SUPER GRIP
4	REBNOK Hi SPIRIT	19	Java e-office ball
5	Semi Gel WIN pen	20	UCAN GP-007
6	QuanTum GeloPlus$^+$Power 1248)	21	BIC Xtra EZ+ BLU
7	Uni-ball Signo DX MITSUBISHI UM-151	22	Horse Hand-Cuptal N500
8	M&G Gel Pen	23	UD Intense Gel
9	REBNOK Ultra Grip	24	g'soft GS007-BLUE
10	PAPER:MATE REYNOLDS 045	25	QuanTum SKATE 114 CANDLE
11	LANCER Wave 825 W	26	Uni JETATREM 101
12	QuanTum GeloPlus$^+$ Curve 125	27	PAPER:MATE InkJoy 500 RT XF
13	Orange FOR MEN	28	Uni JETATREM SX-210
14	Pentel ENERGEL Liquid Gel Ink Needle Tip ball	29	M&G AGP12371
15	YAYA HANS&JANE@2006 BIN's	30	FABER-CASTELL BALL PEN 1423

Thin-layer chromatography

TLC-cards were prepared with layer thickness of 0.2 mm and size 2×5 cm. Origins were set at 1 cm from the base of the plates, and a distance of 0.3 cm was measured between each sample and marked by a pencil. The ink samples were separated using five mobile phases following previous details in the literature [12] as shown in Table 2. Each TLC-card was placed in the tank and closed until the substance ran to the line of the solvent front. The color and distance run of each appearance spot for each sample were observed and recorded to calculate the retention factor (R_f) values.

Table 2
Different mobile phase systems used in TLC method.

No.	Mobile phase system	Ratio
1	*n*-butanol: ethanol: H_2O	50:15:10
2	ethyl acetate: cyclohexane: methanol: NH_3	70:15:10:5
3	ethyl acetate: *n*-butanol: NH_3	60:35:30
4	ethyl acetate: ethanol: H_2O	70:35:30
5	Toluene: acetone: ethanol: NH_3	30:60:7:2

UV-VIS spectroscopy

UV-Vis spectroscopy was carried out using a Shimadzu UV-Vis spectrometer (Bara Scientific Co., Ltd.) with quartz cuvettes comprising a path length of 10 mm and chamber volume of 1.5 ml. Ink was extracted from each pen utilizing ethanol, acetone and dichloromethane solution. The solution extracts were analyzed using the same instrument in absorbance mode, with same solution used as the reference solvent. Sample concentrations were optimized to provide a sample absorption maximum of around unity. All spectra were scanned from 200 to 800 nm and all ranges were used in the software analysis.

RESULTS

Extraction results from the data collected determined inks insoluble in ethanol solvent as pen numbers 7, 8, 16 and 29. When using acetone solvent, pen inks that did not dissolve included numbers 7, 8, 14, 16, 23 and 29 and with dichloromethane solvent pen ink numbers 2, 7, 8, 16, 23 and 29 did not dissolve. Therefore, ethanol was selected as the best extraction solvent for all systems since it was able to dissolve 26 out of the total of 30 pens inks, while extraction with acetone and dichloromethane recorded dissolution of only 24 pen inks.

Thin-layer chromatography analysis

Results of the analysis of 30 types of blue ballpoint pen by TLC technique using three different solvents and five mobile phase systems were recorded as retention factor (R_f) and color tones. Initial data analysis, by descriptive statistical methods considering the frequency of duplicate data, indicated the number of occurrences as groups of each different extraction and mobile phase system. Based on the statistical analysis, each experiment classified the 30 inks into several groups as shown in Table 3. Ethanol solvent combined with mobile phase system 1 divided the inks into 12 different groups.

Table 3
Classification result from each experiment, different extract solvents and mobile phase systems.

Extract Solvent	Mobile phase system 1	Mobile phase system 2	Mobile phase system 3	Mobile phase system 4	Mobile phase system 5
Ethanol	12	6	10	6	7
Acetone	9	9	9	6	5
Dichloromethane	8	8	7	5	6

For the statistical test, two-way analysis of variance (ANOVA) at a significance level of 0.05 was used to compare the effect of TLC results between the three extract solvents and the five mobile phase systems. Based on the *p*-values show in Table 4 the following conclusions were drawn.

- The extract solvent factor had a *p*-value of .224 which was more than 0.05, indicating that the extract solvent was not associated with the separation of each blue ballpoint ink.
- The mobile phase system factor had a *p*-value of .000 which was less than 0.05, indicating that the variant solvents were associated with separation of each blue ballpoint ink.
- Interaction between extract solvent and mobile phase system had a *p*-value of .000 which was less than 0.05, indicating that the relationship of both factors depended on the variants of the mobile phase system.

Table 4
The results of variance analysis (ANOVA)

Source	**df**	**Mean Square**	**F**	**Sig.**
Extract	2	.024	1.502	.224
Mobile phase	4	.823	50.641	.000*
Extract*Mobile phase	8	.072	4.410	.000*
Error	435	.016		
Total	450			

Another method to determine the discrimination power (DP) of the blue ballpoint pen ink analysis was by comparing pairs of different inks. The number of pair was achieved as follows:

$$\text{Number of pairs} = [n\,(n-1)\,]/2$$

All possible binary combinations of the 30 studied inks were calculated at 435 pairs. Results of ethanol extraction when using mobile phase systems 1, 2, 3, 4 and 5 indicated that 388, 324, 381, 316 and 349 pairs were differentiated, while results of acetone extraction gave 385, 383, 355, 294 and 320 pairs, and dichloromethane extraction resulted in 371, 359, 328, 318 and 353 pairs. Pairs were labeled as distinguished by discrimination power (DP) which was defined as the ratio of the number of differentiated pairs of samples with respect to the total number of all pairs and calculated according to Lawrence [13]. The DP results showed that ethanol extraction with mobile phase system 1 was the most effective tool for ink separation and discrimination. This combination differentiated 388 pen-pairs out of the total of 435 from the 30 varieties of blue ballpoint pen inks as shown in Table 9.

UV-VIS spectroscopy analysis

The blue ballpoint pen inks were readily discriminated on the basis of UV-Vis spectra. Data were recorded in four types as wavelength of λ_{max}, amplitude of λ_{max}, shoulder and minor absorption peaks. All data using the three different extraction solvents were classified into 7, 5 and 5 groups as shown in Tables 5-7, respectively.

Table 5
Classification thirty blue ballpoint pen inks using ethanol by UV-Vis spectroscopy

	G.1	G.2	G.3	G.4	G.5	G.6	G.7
Wavelength	583	585	610-612	596	none	553/626	584
Amplitude	0.15-0.24	0.30-0.41	0.13-0.14	0.36-0.38	none	0.2/0.17	0.34
Shoulder	535-549	537-552	555	627-632	none	none	537
Minor	303-305	303-307	309	307/ 347-354	none	307.5	many

Table 6
Classification thirty blue ballpoint pen inks using acetone by UV-Vis spectroscopy

	G.1	G.2	G.3	G.4	G.5
Wavelength	586	588	590	none	600
Amplitude	0.42-0.58	0.37-0.38	0.53-0.69	none	0.75-0.92
Shoulder	546-550	545-550	545-550	none	none
Minor	304-306	303-307, 350	303-309, 350	none	347, 297

Table 7
Classification thirty blue ballpoint pen inks using dichloromethane by UV-Vis spectroscopy

	G.1	G.2	G.3	G.4	G.5
Wavelength	586	593	589	none	600
Amplitude	0.17-0.29	0.48	0.32-0.47	none	0.36-0.48
Shoulder	540-550	547	546	none	none
Minor	320-327	330	325	none	345

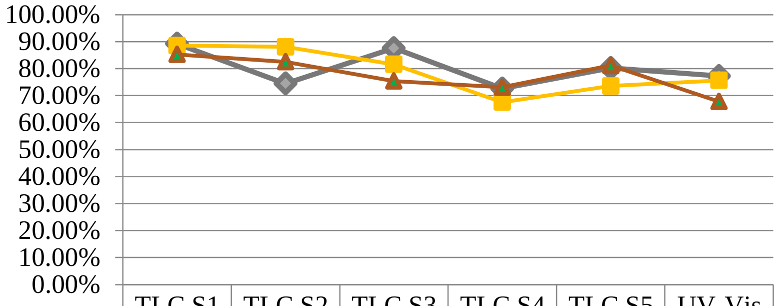
Comparative analysis results of blue ballpoint pen inks from the UV-Vis spectroscopy method were classified by one way variances. One-way ANOVA gave *p*-value of .365 as shown in Table 8. This value was more than .05 and indicated that results of ink separation by the three extraction solvents were not significantly different.

Table 8
One-way ANOVA

λ_{max}	df	SS	MS	F	Sig.
Between Groups	2	123406.867	61703.433	1.019	.365
Within Groups	87	5268977.233	60562.957		
Total	89	5392384.100			

Results were also calculated and compared in terms of discrimination power (DP) of both TLC and UV-Vis spectroscopy methods and shown as percentage values in Table 9. Highest classification power was recorded by the TLC method using ethanol solvent and mobile phase system 1 (*n*-butanol: ethanol: H_2O in ratio 50:15:10 by volume).

Table 9
Discriminating power (DP) for all techniques



	TLC S1	TLC S2	TLC S3	TLC S4	TLC S5	UV-Vis
◆ Ethanol	89.20%	74.48%	87.59%	72.64%	80.23%	77.24%
■ Acetone	88.51%	88.05%	81.61%	67.59%	73.56%	75.63%
▲ Dichloromethane	85.29%	82.53%	75.40%	73.10%	81.15%	67.81%

CONCLUSION AND FUTURE WORK

The experiments to separate blue ballpoint pen inks using thin layer chromatography and UV-Vis spectroscopy methods employed three different solvents as ethanol, acetone and dichloromethane with diverse abilities to dissolve blue ink. Ethanol proved to be the best extraction solvent in both systems and dissolved 26 out of the total of 30 blue ballpoint pen inks. Solubility principle of varying polarities can be extracted by using appropriate solvents. Results were consistent with Djavanshir's study [12] who suggested that high polarity molecules were required to dissolve pen ink. UV-Vis spectroscopy using acetone and dichloromethane showed only 22 pen inks were dissolved and the amount of ink dissolved was less than recorded by thin layer chromatography. Observations during the experiment resulted in an additional 0.8 ml. of solution to fill the chamber volume of 1.5 ml, causing diluted color before analysis by UV-Vis spectroscopy. Spectroscopy results in some inks did not present clear peaks as no substances could be extracted. Therefore, the TLC technique was able to separate the inks better than UV-Vis spectroscopy when using a small amount of substance.

The first aim of this study was to determine and compare the discrimination power (DP) of blue ballpoint pen inks using both thin-layer chromatography and UV-Vis spectroscopy techniques. For thin-layer chromatography, several factors attained DP between 67.59% and 89.20%, while UV-Vis spectroscopy achieved DP between 67.81% and 77.24% according to research by LoongChuen and Mehwish [14, 15]. The TLC experiment was found to be useful in the classification and individualization of a questioned ink from a database by calculating the R_f value. Effective blue ballpoint pen ink separation by TLC between the three different solvents and the five variant mobile phase systems was analyzed by two-way ANOVA. Results showed that variants of the mobile phase systems influenced the separation of each blue ballpoint pen ink at a significance level of 0.05. The most notable TLC results gave discrimination power (DP) of 89.20% for the method using ethanol solvent and mobile phase system 1 that consisted of n-butanol: ethanol: water (50:15:10). Conversely, the effect of ink separation from each blue ballpoint pen using UV-Vis spectroscopy for different solvents presented the highest DP value at 77.24% for the method using ethanol solvent

In the future, higher DP can be achieved. Multivariate statistical techniques applied for data interpretation may lead to the development of blue ballpoint pen ink test kits for real-time use at crime scenes.

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