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Separation of Blue Ballpoint Pen Inks- A Comparison of Solvent Systems on Thin Layer Chromatography Techniques

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Abstract: This article is chromatographic analysis of inks for forensic science. Thin layer chromatographic technique (TLC) is often used in separation of writing inks because it is rapid and requires no sophisticated instrumentation. The repeatability and reproducibility of TLC analyses of inks depend on several factors. However, the critical importance is the use of solvent for the extraction process and for mobile phase. The purpose of the present study is to compare the effectiveness of blue ballpoint pen ink separation by TLC between different solvent extraction and variant mobile phase systems. Thirty blue ballpoint pens commonly used in Thailand were extracted from document then three solvents (ethanol, acetone or dichloromethane) and five solvent systems as mobile phase were used to separate pigment compounds in each sample. The R_f values were calculated for discrimination analysis of all blue pens via two-way ANOVA. The results showed that the most important factor affecting ink classification was solvent extraction. The ethanol was the best solvent for extraction and the optimal mobile phase was n-butanol: ethanol: water (50:15:10 v/v/v) having statistically significant level of 0.05. This mobile phase system could be used to classify all 30 inks into 12 different groups with the discrimination power (DP) of 89.20%. In the future, the qualitative data from TLC plates will more reliable by multivariate statistical techniques can also be applied on effectively interpretation.

Keywords: forensic, ballpoint pen inks, thin-layer chromatography, ink analysis

1. Introduction

The complexity and globalization of today's technological society makes it necessary for people to relate or convey important documents. As such, document forgery is a common problem, especially using pen or ink in writing to forge or edit documents and signatures. Most questionable documents consist of forged bank checks, bills, handwritten correspondence, contracts and others, which require analysis of ballpoint pen inks. Ink analysis is an important forensic procedure because it can reveal useful information for an investigation. Modern inks contain mixtures of various substances that are meant to improve ink characteristics (Roux *et al.,* 1999; Valia, 2017; Vogt, 1997). The most important component of coloring material comes in the form of dyes, pigments, and various combinations. Dyes are soluble in the vehicle that is a mixture of solvents, oils and resins. This carrier is an important component of the ink, which affects its flowing and drying characteristics. The solvent mixes a variety of types of inorganic materials such as glycol, glycol ether or allopathic alcohol, which have a boiling point higher than 180 °C so they can be stable at room temperature. The evaporation of the solvent and prevention of fouling at the tip of the pen results in self-locking by default. This process does not affect the flow of ink when it is written on paper. Pigments are solid, opaque particles that have molecules linked together in crystalline structures, which provide color for the ink. The color depends on the raw materials used in production. Mostly, blue can be obtained with substituted triphenylmethane pigment. Other substances used to improve certain properties will have different characteristics in accordance with the particular purpose of the pen (Thanasoulias and Parisis, 2003). The aim of most analyses is comparison of different writing inks on a document, which is the primary goal of investigations (Djozan *et al.,* 2008).

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Forensic document examination, especially the analysis of inks, can be divided into two approaches including non-destructive document and destructive document. Non-destructive analytical methods will choose specific characteristics of ink to serve as parameters, such as its colors, luminescence and radiation absorption. Questionable documents may be differentiated by properties of transmission, reflection and fluorescence spectra obtained for inks deposited on the paper surface. However, the methods of physic-chemical analysis can determine the type and composition of ink, leading to ink identification (Feraru and Meghea, 2014). Destructive document analysis starts by removing a small section from the ink line with extraction solvent to open up more avenues of analysis. In particular, the chromatographic separation of colored pigments from component dyes can be useful. Even though a blue ballpoint pen can only write in one color, the ink is actually made from a mixture of different colored pigments. This method has proven highly productive for the comparison and matching of ink with the database of chromatograms (Ismail *et al.,* 2014; Lewie, 1996; Zlotnick and Smoth, 1999; Samanidou *et al.,* 2004).

Thin layer chromatography (TLC) is a solid-liquid from chromatography, which can separate the composition of the sample for the components distributed between two phases. The stationary phase is normally a polar adsorbent and a single or combination of solvents that is a mobile phase to dissolve the substance from the stationary phase. Different substances have different adsorption and movement properties. TLC is a simple approach to use, as well as being rapid, inexpensive, and minimally destructive to the document because it requires only a small amount of sample for examination. At the same time, it can show a high degree of characterized selectivity and repeatability for results. Accordingly, this study focuses on destructive document analysis using the chemometrics approach for data analyses that use both mathematical and statistical methods to improve the accuracy of identification and the discrimination of pen inks (Loong Chuen, 2015; Senior *et al.,* 2012).

The TLC plate gaining popularity is alumina adsorbent. This method is often performed using the retention factor (R_f) values for the qualitative evaluation of chromatograms. These values are used to compare the TLC results from all study inks. These comparisons help to find the characteristics of inks, resulting in estimating the source of questionable ink. Ink analysis by TLC is a powerful forensic tool and has provided important evidence that had a significant impact on the outcome of several high profile cases (Julia *et al.,* 2016). This is an important part of creating a reference ink library by using the same type of TLC plate for searching and comparing. Minor changes in the mobile phase system could generate more significant changes in the colorants R_f values and expand to potential false negative. One of several factors that especially critical regard to the repeatability and reproducibility of the results that is the use substance suitable for isolation of sample from the substrate of document (i.e. paper) and chose appropriate mobile phase system. The purpose of this research focused on investigating the effects of ink separation by TLC comparison of different solvents for extraction in conjunction with different mobile phase systems.

2. Materials and methods

2.1 Equipment and Chemicals

Random sampling a number of blue ballpoint pen inks from different 30 from 90 blue ballpoint pen inks have been purchased from local markets in Bangkok, Thailand (at the time of study) show in Table 1.

Table 1. The list of studied blue ballpoint pens

No.	Commercial characteristics
1	GRIP X P5 FABER-CASTELL (0.5 mm)
2	Pentel ENERGEL BL107 (0.7 mm)
3	XF STAEDTLER LUNA Ball
4	REBNOK Hi SPIRIT
5	Semi Gel WIN pen (0.7 mm)
6	QuanTumGeloPlus^{+}Power 1248 (0.7 mm)
7	Uni-ball Signo DX (0.38 mm) MITSUBISHI UM-151
8	M&G 0.5 mm Gel Pen
9	REBNOK Ultra Grip
10	PAPER:MATE REYNOLDS 045 (0.8 mm)
11	LANCER Wave 0.5mm. 825 W
12	QuanTumGeloPlus^{+} Curve 125
13	Orange FOR MEN
14	Pentel ENERGEL Loquid Gel Ink Needle Tip 0.5 mm
15	YAYA HANS&JANE@2006 BIN's
16	FABER-CASTELL TRUE GELL (0.7 mm)
17	PAPER:MATE InkJoy 100 XF
18	g'soft SUPER GRIP 0.28
19	Java e-office ball (0.7 mm)
20	UCAN 0.5 GP-007
21	BIC Xtra EZ+ 0.7 BLU
22	Horse Hand-Cuptal N500
23	UD Intense Gel 0.5 mm

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24	g'soft GS007 0.38-BLUE
25	QuanTum SKATE 114 CANDLE
26	Uni JETATREM 101 0.7
27	PAPER:MATE InkJoy 500 RT XF
28	Uni JETATREM SX-210
29	M&G AGP12371 0.7 mm
30	FABER-CASTELL BALL PEN 1423 0.7

Purchased from local markets (May-July 2017)

All pens were allocated reference number during this study. Each pen was used to write the author's name two times on a piece of A4 white paper (Double A, 80 gram.) to be consistent with the documents at crime scene. After that, the inks entry was punch a size circle 5 mm diameter 5 holes from each sample that was used as substrate for depositing inks.

The following chemicals were used in this study: ethanol, acetone, dichloromethane, *n*-butanol, water, ethyl acetate, cyclohexane, methanol, ammonia and toluene. All chemicals were used without any further modifications.

2.2 Extraction of ink from papers

In this experiment, three different solution including ethanol, acetone and dichloromethane were extract ink from paper. Take 5 pieces of one sample per 1 hole, put into spot plate porcelain and write the number on the hole. Add 0.2 ml extract solution into the hole waiting about 20 seconds. Observe the color and concentration of the solution. The extracts were used for further study.

2.3 Thin-layer chromatography

TLC was carried out using TLC-cards with layer thickness 0.2 mm and 2×5 cm aluminum cards. Each extracts was placed in the origins point at 1 cm from the bases of the plates which was marked with pencil. The distance between samples was 3 mm. Five solvent systems were used as mobile phase as shown in Table 2. The distance of each appearance spot in each sample as well as solvent front were recorded for R_f values calculation.

Table 2. 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

*The chosen solvent system from Djozan *et al.,* 2008.

2.4 Data Analysis

All the statistical analysis was carried out using statistical package for the social sciences (SPSS). Discrimination analysis was conducted based on the data obtained from 3 extractions and 5 mobile phase systems. Two-way ANOVA was conducted to determine the effect from three solution (ethanol, acetone and dichloromethane) for ink extraction and five different mobile phase systems for inks classification. The number of pair was achieved as follows (Zlotnick and Smoth, 1999).

$$Number\ of\ pairs = n\ (n-1)/2$$

An observational study was designed so that 30 varieties of ballpoint pens, there were 435 possible pen pairs. Any pair would be labeled as distinguish by discriminating power (DP) which was defined as a ratio of number of differentiated pairs of samples with respect to the total number of all calculated (Smalldon and Moffat, 1973), using the following equation:

$$DP = \frac{Number\ of\ discriminated\ pairs}{Number\ of\ possible\ pairs}$$

3. Results and Discussion

3.1 Thin-layer chromatography analysis

Retention factor (R_f) and color tones of the bands separated by different mobile phase system were used to discriminate inks in blue ballpoint pen. The technique described in this paper was effective in identifying type of the ink used in commercial pen. Fig. 1 and 2 show the results of TLC that using ethanol as extract solvent with different mobile phase systems.

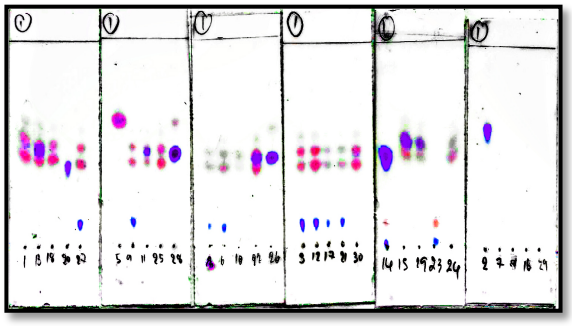

Figure 1. TLC plate containing 30 blue inks extracted by ethanol using system 1 (n-butanol: ethanol: H_2O) as mobile phase.

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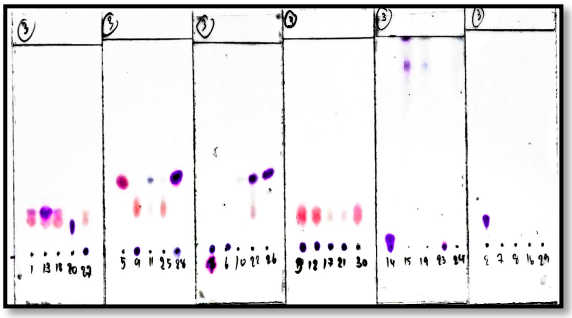

Figure 2. TLC plate containing 30 blue inks extracted by ethanol using system 3 (ethyl acetate: n-butanol: NH_3) as mobile phase.

Descriptive statistics are commonly used for summarizing data frequency or measures of central tendency (Mean and Median). Frequency analysis is a descriptive statistical method that shows the number of occurrences groups of each different extraction and mobile phase systems in TLC techniques. Based on the statistical analysis, each experiment can classify 30 inks into several groups as shown in Table 3.

Table 3. Classification result from each experiment, with different extract solvent and mobile phase system.

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

From Table 3, it is obvious that the TLC method using ethanol as the extract solvent and developed with mobile phase system 1 got the most separated pattern from thirty different individual pens. The collected data was able to classify 30 blue ballpoint pens into 12 different groups as shown in Table 4.

Table 4. TLC results of blue inks, with ethanol extracts and mobile phase system 1

Group	Color	R_f	Pen
1	Pale Blue	0.11	3,4,6,9,12,
	Purple	0.40	17,21,27
	Purple	0.46	
	Pale Purple	0.53	
2	Blue	0.43	26,28
	Purple	0.57	
3	Purple	0.43	15,19,24
	Blue	0.49	
	Purple	0.53	
	Pale Purple	0.60	
4	Purple	0.43	1
	Purple	0.53	
	Pale Purple	0.64	
5	Pink	0.21	5
	Blue	0.57	
6	Pale Pink	0.23	20
	Blue	0.57	
7	Pale Blue	0.03	14
	Pink	0.14	
	Blue	0.46	
8	Pale Blue	0.03	23
	Pink	0.14	
9	Pale Purple	0.43	9
	Pale Purple	0.49	
	Purple	0.60	
10	None	0.00	7,8,16,29
11	Pale Purple	0.37	10,1,25,30
	Pale Blue	0.46	
	Pale Purple	0.53	
12	Purple	0.37	11,13,22
	Blue	0.41	
	Pale Purple	0.46	
	Pale Purple	0.53	

3.2 Evaluation results of analysis

In statistical tests, the two-way analysis of variance (ANOVA) was used to compare the effect of TLC results between different extract solvent and variant mobile phase systems. In this study, the independent variable was extract solvent and mobile phase system and dependent variable was the TLC results. Each R_f value from 30 blue ballpoint pen inks of each experiment was calculated to determine whether the interaction effect between three solvents and five mobile phase systems was statistically significant at a level of 0.05.

In addition, this study also concludes the following, based on the *p*-values:

- The *p*-value for extract solvent was .224, which indicates that different of extract solvent were not associated with TLC results.
- The *p*-value for mobile phase systems was .000, which indicates that variant solvent were associated with TLC results.
- The *p*-value for the interaction between extract solvent and mobile phase systems was .000, which indicates that the relationship between extract solvent and mobile phase system depends on the variant of mobile phase system.

From profile plots (Fig. 3.), except the mobile phase system 1, there are no intersection between graphs. It indicates that there was a difference in Mean of TLC results in all groups of extraction and no interaction between extraction and mobile phase systems. However, further subgroup testing is required.

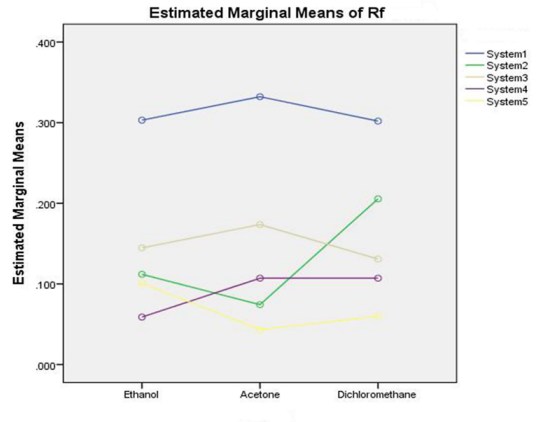

Figure 3. Estimated Marginal Means of Sum R_f

The results before subgroup testing provided information that the difference mobile phase systems influenced the effect of ink separation. The effect between groups of mobile phase systems was determined by one-way ANOVA ($p<.05$). First, subgroups were created by isolation of the extract solvent (ethanol, acetone and dichloromethane) then ANOVA analysis for each mobile phase system was done.

Mobile phase system 3 and 5 showed Sig. value of .372 and .073, respectively. The *p*-value in there groups was more than .05, conclude that there were not statistically significant difference between subgroups of extract solvent. In the other hands, mobile phase system 1, 2 and 4 shows Sig. value were .000, .000 and .002. The *p*-value in there groups were less than .05; indicates that there was a statistically significant difference between subgroups of extract solvent. The effect of ink separation in mobile phase system 1 was a statistically significant difference between ethanol /acetone extraction, and ethanol /dichloromethane extraction. The mobile phase system 2 was a statistically significant difference between ethanol / dichloromethane extraction, and acetone /dichloromethane extraction. And the mobile phase system 4 was a statistically significant difference between ethanol / acetone extraction, and acetone /dichloromethane extraction. Next, subgroups were created by isolation of the mobile phase systems then ANOVA analysis for each extract solvent was done. The results were a statistically significant difference in all groups. Descriptive by Post Hoc multiple comparisons were concluding as follows:

- A group of ethanol extracts, the mobile phase system 3 was difference from system 4, and system 1 was difference from other systems at statistically significant level of 0.05.
- A group of acetone extracts, no statistically significant difference between mobile phase system 2 and 5, but the mobile phase system 1 difference from other systems.
- A group of dichloromethane extract, the mobile phase system 1 difference from other systems but no statistically significant difference between mobile phase system 3, 4 and 5.

In addition, the standard deviation plot was checked for shifts in scale by using Mean plots, showing Mean varies between different groups of data. In the sample plot below, different extraction groups in all mobile phase systems were shown.

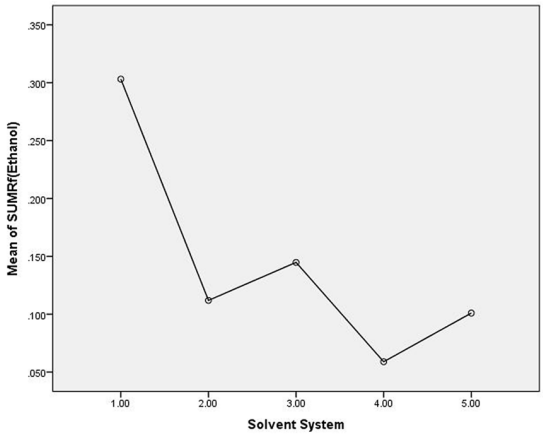

Figure 4. Mean plot of results from ethanol extract all mobile phase systems.

Experiments of ethanol extract with 5 mobile phase systems found difference pattern of ink

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separation as shown in Fig. 4. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 3, 2, 5 and 4, respectively

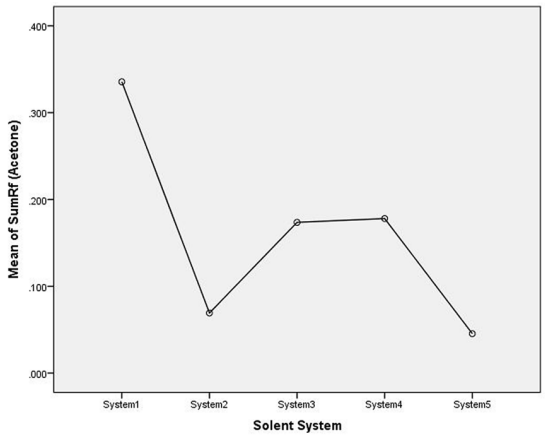

Figure 5. Mean plot of results from acetone extract all mobile phase systems.

Experiments of acetone extract with 5 mobile phase systems found difference pattern of ink separation as shown in Fig. 5. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 3, 4, 2 and 5, respectively.

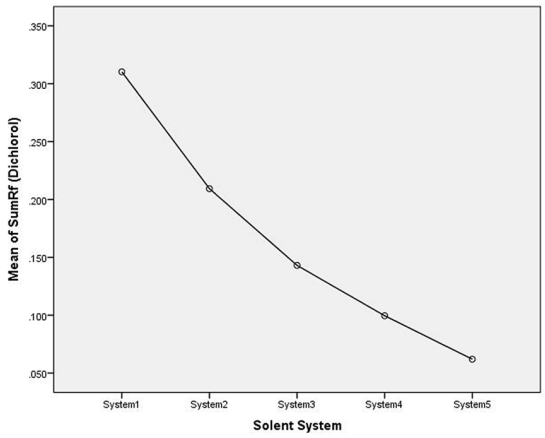

Figure 6. Mean plot of results from dichloromethane extract all mobile phase systems.

Experiments of dichloromethane extract with 5 mobile phase systems found difference pattern of ink separation as shown in Fig. 4. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 2, 3, 4 and 5, respectively.

3.3 Definite discrimination power (DP)

The other way to differentiate various blue ballpoint pens was evaluated by comparing the couple of different inks. The comparison between all possible binary combinations of 30 studied inks, for 450 cases, was done. As a result for ethanol extract using mobile phase system 1, 2, 3, 4 and 5, 388, 324, 381, 316 and 349 pairs were differentiated (Fig.7). The results for acetone extract using mobile phase system 1, 2, 3, 4 and 5, 385, 383, 355, 294 and 320 pairs were differentiated. The last group was a result for dichloromethane extract using mobile phase system 1, 2, 3, 4 and 5, 371, 359, 328, 318 and 353 pairs were differentiated.

In order to show the possibility to differentiate the examined inks by this method the discriminating power (DP) was calculated according to Eq. (2). In this method, the DP was achieved as follows in table 5.

Table 5. DP of groups in each different extract solvents and mobile phase system (unit: percent)

Extract	Mobile phase system				
	1	2	3	4	5
Ethanol	89.20	74.48	87.59	72.64	80.23
Acetone	88.51	88.05	81.61	67.59	73.56
Dichloro-methane	85.29	82.53	75.40	73.10	81.15

The result DP showed that ethanol extraction with mobile phase system 1 successfully differentiated 47 pen-pair formed from 30 varieties of blue ballpoint pen. The approach proposed here is the effective tool for ink separation and discrimination, consistent with Loong Chuen, 2015.

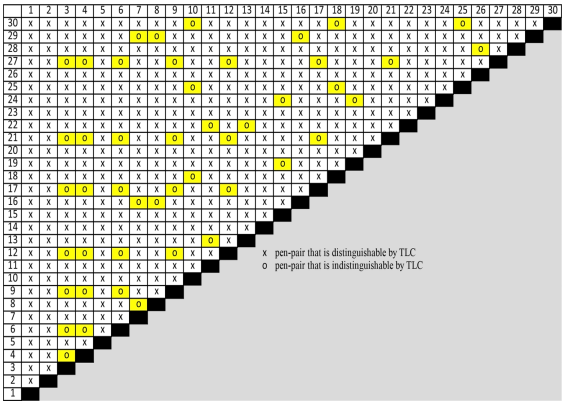

Figure 7. All possible combination of comparing inks with TLC using ethanol extract with mobile phase system 1 as a mobile phase.

4. Conclusions

The aim of this study is to compare the effectiveness of blue ballpoint pen ink separation by TLC between different solvent extraction and variant mobile phase systems. Two-way ANOVA analysis found that both extract solvent and mobile phase systems were influence to TLC results. The relationship between extract solvents and mobile phase systems depends on the variant of mobile phase systems. The estimated marginal means graph, one-way ANOVA analysis and Mean plot graphs, proved that the most distinguishable TLC result of blue ballpoint inks was that using ethanol as the extract solvent and using mobile phase system 1, which consist of n-butanol: ethanol: water (50:15:10 v/v/v), as the developing agent. This combination could classify 30 blue ballpoint pens into 12 different groups with the discrimination power (DP) of 89.20%. This experiment was found to be useful in classification and individualization of a questioned ink from a database through calculating R_f value. In the future, the qualitative data from TLC plates will be converted into quantitative data by using certain analysis software. Therefore, higher DP could be achieved while multivariate statistical techniques can also be applied on data interpretation and lead to development of blue ballpoint pen ink test kit for used in the crime scene.

5. Acknowledgement

A greatly thankful to all the staffs both faculty of science and technology and department of forensic science, graduated school, SSRU, for support, equipment and facilities provided.

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