ORIGINAL RESEARCH PAPER

The discrimination potential of VSC, TLC and HPLC for ballpoint inks

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ABSTRACT

Introduction: The necessity arises to analyse blue and black ballpoint pen inks frequently encountered in forensic documents.

Objective: To evaluate the discrimination potential of Video Spectral Comparator (VSC), Thin Layer Chromatography (TLC), and High Performance Liquid Chromatography (HPLC) methods in analysing blue and black ballpoint pen inks in documents.

Methods: A selection of 10 blue and 10 black ballpoint inks were examined by a Video Spectral Comparator (VSC), Thin Layer Chromatography (TLC), and High Performance Liquid Chromatography (HPLC) methods.

Results: The discriminating powers for VSC, TLC, and HPLC methods were determined to be 0.73, 0.82, and 0.84 for the blue inks and 0.56, 0.78, and 0.84 for the black inks. The overall discrimination powers of three methods were 0.91 and 0.84 for the black and blue inks, respectively.

HPLC method differentiated the highest number of blue and black pairs and its discrimination power is slightly higher than TLC. The VSC showed a significant discrimination power improvement of blue over black inks. Statistical analysis confirmed that the VSC has higher discrimination power to differentiate blue ballpoint inks than black ballpoint inks. None of the techniques discriminated the full range of ink pairs studied.

Conclusion: The power of the individual techniques to discriminate between inks varied. The results show that these three techniques are complementary to some extent. A combination of all three techniques provides the greatest discrimination power.

Keywords: Destructive and non- destructive, ink analysis, questioned documents, discrimination powers

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INTRODUCTION

Forensic Science is the application of scientific knowledge to the law. It provides impartial, reliable, pertinent and often definitive evidence about a given case for use in the courts of law¹. Document examination is a branch of forensic science that includes the analysis of handwriting as well as the detection of forged documents. Documents such as cheques, contracts, vouchers, bills and various other documents are frequently entrusted to the laboratory for analysis. These documents may contain one or more forgeries commonly encountered such as alterations, additions, substitutions, counterfeiting or obliterations. The detection of forgeries on a document is a key function within document examination, since valuable evidence can be obtained by using a series

of different analytical techniques². In this research, it is necessary to discriminate pen inks on questioned documents to determine authenticity or to decipher fraud³. In some cases, two or more entries are compared to determine similarities or differences in the inks. Sometimes, the suspected writing implement is submitted to compare with the writing on a paper. As a consequence of this, many techniques have been researched to discriminate between ink formulations on paper and to improve the discrimination power of ink analysis methods^{4,5}. Although a number of techniques have been developed to discriminate ballpoint ink samples, most forensic laboratories, including developing countries, widely use Video Spectral Comparator (VSC), Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as discriminating techniques because of their favourable combination of performance, economy, and simplicity⁶. Therefore, any research on the discrimination potential of these techniques for the analysis of black and blue ballpoint inks is important for the future of forensic document examination. Therefore, the knowledge on ink analysis techniques and their discriminatory powers is essential for correct opinions.

OBJECTIVE

To evaluate the discrimination potential of Video Spectral Comparator (VSC), Thin Layer Chromatography (TLC), and High Performance Liquid Chromatography (HPLC) methods in analysing blue and black ballpoint pen inks in documents.

MATERIAL AND METHOD

A total of 20 ballpoint pens (10 black and 10 blue varieties) belonging to different brands were purchased from a local market in order to form a broad collection of pens available at the time of study. The blue ballpoint pens were marked from P1 to P10 and black pens were marked from P11 to P20. For the ease of analysis, the pen numbers were written on white copy papers to simulate casework as shown in Figure 1. Two sets of writings were performed to determine the reproducibility of each technique.

Fig. 1: Ink samples used for the analysis

Ballpoint inks were analysed using a stereo microscope, a Video Spectral Comparator, TLC, and HPLC methods in order to determine the capability of each technique to discriminate entries produced by blue and black ballpoint pens. The ballpoint pen inks of each color were inter-compared with each other forming 45 blue pairs and 45 black pairs.

A. Video Spectral Analysis

The P1 to P20 inks were examined using infrared and ultraviolet lights integrated into the Video Spectral Comparator (VSC 5000, Foster+Freeman Ltd, England). While examining inks under infrared radiation, three functions of the VSC were utilized: (1) flood lighting, (2) spot (Luminescence) and (3) Plot spectrum. UV reactions of inks were examined using UV 365 nm energy. Each ink was examined on one paper at the same time using the same wavelength to avoid any change of light examination factors.

B. Thin Layer Chromatography

Twenty one plastic vials (eppendorfs –Fisher scientific) were marked as P1 to P20 and C (C for control). Six plugs from each pen line and the same number of plugs of paper from a control area were punched out using a micro punch (Harris micro-punch 1.20 mm) and samples were placed into the relevant plastic vials. 10 µl of absolute ethanol was used as the extraction solvent. The samples were agitated using the ultrasonic bath for 10 minutes to maximize the amount of ink extracted. TLC analysis was performed in accordance with ASTM international standard guide E1422-057. The spotted (Whatman glass-backed silica) plates were placed in developing tanks previously equilibrated for 15 minutes with solvent system: ethyl acetate : absolute ethanol : water (70:35:30, v/v). The distance travelled by the solvent and the distance travelled by each spot were measured and the retardation factor (R_f value) was determined for each spot. Each TLC separation was performed three times using the same conditions.

C. High Performance Liquid Chromatography

In this study ten plugs from each pen line were punched out using a micro punch (Harris micro-punch 1.20 mm) and samples were placed into the plastic vials marked as P1 to P20. 40 µl of HPLC grade methanol (Fisher scientific) was added to the samples. The samples were sonicated for 10 minutes to maximize the amount of ink extracted. The HPLC system employed for the analytical work consisted of a GILSON separation pump with a UVD340S multi-

diode array detector (scanning range from 200 nm to 600 nm). The software used to operate, collect and manipulate data was Chromeleon Client 6.10. The analytical columns used for this work were a SHANDON 4mm 15 cm C-18 column and a BECKMAN 4.6mm x 15 cm C-18 column. The sample injection volume was 20 µl. Six different mobile phases were tested to achieve optimal separation: (1) acetonitrile: methanol: water (30:45:25 v/v), (2) 100% acetonitrile, (3) acetonitrile:water (70:30 v/v), (4) acetonitrile:water (60:40 v/v), (5) acetonitrile:water (50:50 v/v) and (6) acetonitrile:water (50:50 v/v) with 1 ml acetic acid per litre of solvent. Each mobile phase was eluted for 10 min at a flow rate of 1 ml/min. Each HPLC separation was repeated using the same conditions so as to test the reproducibility.

D. Discrimination Power (DP) and Statistical Analysis

The discriminating powers of each method for blue and black ballpoint pens were arranged in a contingency table. The Chi-squared test for association was conducted by means of “R” to determine if there was association between techniques and colour of pens. The association plot was also drawn to observe the relationship between methods and pens graphically. The discrimination power of three techniques, VSC, TLC, and HPLC was calculated in accordance with the equation introduced by Smalldon and Moffat⁸:

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

RESULTS

A. Video Spectral Comparison

The results obtained for all the VSC functions, flood (IR reflectance mode), spot (Luminescence), plot spectrum and UV fluorescence, were arranged in pairs so as to evaluate the discrimination potential of each function of VSC. Table 1 shows the discrimination powers of each VSC function and total discrimination power of VSC when combined. All four VSC functions failed to discriminate twenty pairs of black ink out of 45 pairs studied. Although none of the functions showed any difference for black ballpoint inks, the discrimination powers for blue ballpoint inks were varied. IR Luminescence and absorption spectrometry showed the highest discrimination power for blue inks while IR reflectance showed the lowest. The combination of four VSC functions increased the discrimination power of VSC technique.

Table 1: Discrimination Powers of each VSC function

Technique	Discrimination Power	
	Blue	Black
IR Reflectance	0.53 (24)	0.56 (25)
IR Luminescence	0.64 (29)	0.56 (25)
Absorption spectrometry	0.64 (29)	0.56 (25)
UV Fluorescence	0.60 (27)	0.56 (25)
Combination of 4 VSC functions	0.73 (33)	0.56 (25)

B. Thin Layer Chromatography

Based on the number of bands, R_f values and colour of the spots, TLC was able to classify blue inks into 6 groups and black inks into 4 groups. Also TLC could completely discriminate 4 blue inks (P4, P5, P7, P10) from the 10 samples and 1 black ink (P15) from the 10 black samples.

C. High Performance Liquid Chromatography

High performance liquid chromatography was conducted using different eluent systems [Six different mobile phases were tested to achieve optimal separation: (1) acetonitrile: methanol: water (30:45:25 v/v), (2) 100% acetonitrile, (3) acetonitrile:water (70:30 v/v), (4) acetonitrile:water (60:40 v/v), (5) acetonitrile:water (50:50 v/v) and (6) acetonitrile:water (50:50 v/v) with 1 ml acetic acid per litre of solvent]. Since the BECKMAN C-18 column with acetonitrile:water (50:50 v/v) mobile phase yielded the best chromatograms at 205 nm wavelength, resultant chromatograms were selected for analysis. HPLC classified blue inks into 6 groups and black inks into 5 groups based on the retention times. HPLC could completely discriminate 4 blue inks (P4, P5, P7, P9) from the 10 samples and 2 black inks (P15, P18) from the 10 samples.

D. Discrimination Power and Statistical Analysis

Table 2 illustrates how the VSC, TLC, and HPLC methods discriminated each pair of blue ballpoint inks. It shows the discrimination powers (& no. of discriminated pairs) of methods examined in this study.

Table 2: Discrimination Powers of VSC, TLC, and HPLC techniques

Technique	Discrimination Power (DP)	
	Blue	Black
VSC	0.73 (33)	0.56 (25)
TLC	0.82 (37)	0.78 (35)
HPLC	0.84 (38)	0.84 (38)
Total	0.91 (41)	0.84 (38)

The obtained results showed that VSC analysis had a higher discrimination power for blue inks 0.73(33) than black inks 0.56(25). Out of 45 pairs of blue ballpoint pen inks, 37 pairs were discriminated by TLC yielding a discrimination power of 0.82. Analysis of chromatograms allowed the discrimination of 35 out of 45 pairs of black inks. The discrimination power for black ink via TLC was 0.78. The results showed that, from the 45 pairs, 41 pairs of blue ink were discriminated by all methods giving the discrimination power of 0.91 whereas these three techniques could discriminate 38 pairs of black ink giving discrimination power of 0.84. HPLC showed the highest discrimination power of the three techniques for both inks.

DISCUSSION

Ten blue and ten black ballpoint pen inks of different brands were analysed by means of VSC, TLC, and HPLC techniques. The obtained results revealed that the three techniques tested in this study did not yield identical results in discrimination of ballpoint pen inks. The discrimination powers for VSC, TLC, and HPLC methods were determined to be 0.73, 0.82, and 0.84 for the blue inks and 0.56, 0.78, and 0.84 for the black inks, respectively. The overall discrimination powers of three methods were 0.91 and 0.84 for the blue and black inks respectively (Table 2). None of the techniques discriminated the full range of ink pairs studied. All methods failed to discriminate four pairs of blue ink and seven pairs of black inks, it is possible that the same ink is used in those brands or failure to discriminate these inks by VSC, TLC, and HPLC methods. HPLC method differentiated the highest number of blue and black pairs. However, the discrimination power is only slightly higher for HPLC than for TLC. Since the discriminating abilities of TLC and HPLC techniques for both blue and black inks were high, these chromatographic methods can undoubtedly be used in cases of ballpoint ink analysis.

According to the association plot, blue inks have positive association with VSC method whereas black inks have negative association. This indicates that the VSC is more suitable for analysis of blue inks rather than the analysis of black inks. This argument is supported by the discrimination powers of blue and black inks for the VSC method. It was 0.73 for blue inks and 0.56 for black inks. Since blue inks show broader colour variation with different components than that of black inks VSC might show a significant favouring of blue over black inks. It was seen in microscopic examination and TLC analysis too. Thus, black inks have positive association with both TLC and HPLC methods while it shows negative

association for VSC method. This relationship indicates that TLC and HPLC methods are more suitable for analysis of black inks than the VSC method. Black inks showed higher discrimination powers for TLC and HPLC methods than that of the VSC method.

The VSC, TLC, and HPLC can be used for ink analysis, but as seen above not all of the techniques suitable at every time. HPLC showed the highest discrimination power of all techniques. This is because HPLC can detect not only colourants of inks but also it can detect other components: solvents, additives etc. Conversely VSC and TLC reveal the results of colourants only. In addition it should be pointed out that the VSC is a comparator and not an analytical instrument like HPLC.

CONCLUSION

The three techniques are complementary to some extent. However, all three methods failed to discriminate eleven pairs of ink studied: 4 pairs of blue ink and seven pairs of black ink. Although the VSC shows a lesser discrimination power than TLC and HPLC, it should be used first, because of its advantages over chromatographic techniques: non-destructive and no sample preparation needed. A combination of all three techniques provides the greatest discrimination power. In this study, the total discrimination powers were achieved 0.91 and 0.84 for blue and black ballpoint inks respectively, which confirm that the used techniques were able to discriminate a significant number of pairs of ink. However, the failure to discriminate eleven pairs of ink by these techniques suggests the importance of the use of other techniques other than VSC, TLC and HPLC.

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CONFLICTS OF INTEREST

The authors have no conflicts of interests to disclose

ETHICAL ISSUES

None

AUTHOR CONTRIBUTIONS

KASK: Total work done by the author

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