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Discrimination of Blue and Black Ballpoint Pen Inks for Forensic Investigations

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ABSTRACT

Background: Great attempts have been made to fight fraud and falsification of crucial documents such as travel documents. However, document fraud and falsification are still rampant. This study aimed to determine the profile of Kenya's blue and black ballpoint pens inks. A total of ten ballpoint pen inks were included in the study. Of this, six were blue ballpoint pen inks, and the remaining four were black from distinct manufacturers.

Methods: The techniques used for pens ink analysis were thin-layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-vis) spectroscopy.

Results: TLC showed a variation in the number of pigments in pen inks with a discriminating power (DP) of 0.87 and 0.83 for blue and black ballpoint pens, respectively. UV-Vis spectroscopy had a DP of 0.93 for blue pen inks and 0.67 for black pen inks, whereas FTIR spectroscopy had a DP of 1.0 for both blue and black pen inks. Hydroxyl, carboxyl, amino, nitro, and ester/ether functional groups were detected in blue and black pen inks. Lastly, multivariate analysis revealed that absorption of the blue and black pen inks in either methanol or ethanol was not different ($P > 0.05$).

Conclusion: Taken together, findings from this study demonstrate the qualitative characteristics and functional group profile of blue and black ballpoint pen inks. However, more studies are needed to establish a reference for pen ink analysis for forensic investigations in Africa and beyond.

Keywords: ballpoint ink pen, TLC, FTIR, UV-Vis spectroscopy, discrimination power, Kenya

BACKGROUND

Document verification/analysis is an important pillar in forensic investigations. Despite the adopting of digital signature and biometric systems, handwritten signatures and documents remain crucial for ensuring authenticity. Today, falsification and forgery of documents are rampant across the globe, including in Kenya [1]. The type of pen likely to be linked to cases of document falsification and forgery is ballpoint ink pens since it is one of the most preferred types of pens worldwide [2,3]. Ballpoint ink pens comprise several parts, namely the ink chamber, ballpoint tip, and barrel [4]. Thus, the tip of the ballpoint ink pen can distinguish one type. The ink chamber contains an ink reservoir for ballpoint ink pens. Over the past decades, there has been a tremendous increase in the number of ink hues found in pens owing to advancements in ink manufacturing technology and the need to improve the quality of pens [5,6]. The composition of ballpoint pen ink hues varies among manufacturers. Furthermore, pens have a unique ink composition for pen manufacturers, akin to DNA and fingerprints for humans [7].

Various analytical techniques have so far been used to analyze pens inks. Spectroscopic techniques have been used widely to analyze contested documents since the 1990s [8,9]. This is because sample processing before analysis is not necessary, and thus, a sample of a document can be analyzed with no or minimal damage to evidence. The spectroscopic methods include infrared spectroscopy, Raman Spectroscopy, mass spectroscopy, luminescence spectroscopy, Fourier transform infrared spectroscopy (FTIR) spectroscopy, and ultraviolet-visible (UV-Vis) spectroscopy [6,10,11]. Besides spectroscopy, chromatographic techniques such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) can also be used to analyze pen inks [12–14].

Analysis of pen inks offers a valuable opportunity for robust forensic investigation of disputed documents. It helps identify pen inks based on their traits and chemical composition. Differences in the chemical composition of the hue of pen ink among different manufacturers arise from the inclusion or omission of chemical components of ink [7]. Most pen ink analysis studies published to date have been conducted in the United States of America (USA) and Europe. Furthermore, the USA successfully prosecuted different cases based on evidence obtained from analyses of pen inks [15–17]. However, African countries are not yet published data or databases on composition pens inks, thus undermining efforts to resolve cases involving disputed documents.

African countries rely on adding costly security measures in vital documents to avert forgery or falsification. This approach diverts much-needed resources for development and other competing priories such as health, thus delaying, in part, the attainment of sustainable development goals. This study aimed

to analyze pen inks to discriminate between blue and black ballpoint pen inks from Kenya for forensic investigations. Blue and black point pens are widely used across the globe, including Kenya. To the best of our knowledge, this is the first study to analyze pen inks from Kenya and thus serves as a baseline for future studies in the region. It also provides valuable information for establishing a database that can act as a reference for forensic investigations.

METHODS

Study site and sample collection: A purposive and stratified sampling approach was utilized to select ballpoint blue and black pens from distinct brands that were available in June 2023 in supermarkets in the central business district of Nairobi, the capital city of Kenya. Nairobi also serves as an important commercial district of the East African Community. Nairobi is home to most industries and manufacturing companies in Kenya, including major pen manufacturing companies. In 2019, the estimated population of Nairobi was 4.3 million people [18].

Sample Analysis: Ink samples isolated from ballpoint pens included in this study were prepared using different methods depending on the analytical method for ink analyses. For thin-layer chromatography (TLC), ink was extracted from the ink cartridges and applied to individual watch glasses. About 1 mg/ml of ink was drawn from a watch glass using a disposable micropipette and dissolved in 10 ml of ethanol. The ethanol-soluble ink samples were then subjected to TLC, as described previously [13]. Three different TLC solvents/mobile systems were used for ink analyses. This included solvent 1, comprising butanol, acetic acid, and water at a ratio of 60:15: 25, solvent 2, comprising methanol, acetone, and distilled water at a ratio of 45:40:19, and solvent 3, comprising ethyl acetate, ethanol: water at a ratio of 70: 35:30.

Functional group analysis: To determine the functional groups in ink isolated from blue and black ballpoint pens, neat ink samples were analyzed using Aten Attenuated Total Reflectance -Fourier Transform Infrared (ATR-FTIR) spectroscopy (Shimadzu Europe's). Analysis was conducted using liquid ink samples only. This is because it was observed that some functional groups could not be detected in dry ink samples during the optimization of ATR FTIR spectroscopy. Ink samples were analyzed using 32 scans at a resolution of 4 cm^{-1} in an air background. The spectral range used was between 4000 cm^{-1} and 1000 cm^{-1}. After each analysis, the diamond of the ATR-FTIR spectroscope was cleaned using ethanol, acetone, isopropanol, and water. Subsequent analyses were conducted after the ATR-FTIR diamond was dry. In addition to characterizing ink based on functional groups, the ability to distinguish between pairs of ink samples was also investigated. This was accomplished by calculating the discriminating power based on peaks at a specific wavenumber and comparing each ink sample with the others [13].

$$DP = \frac{\text{number of discriminating sample pair}}{number\ of\ possible\ sample\ pairs}$$

Analysis of pen ink in different solvents: Absorption spectroscopy using an Ultraviolet-visible (UV-Vis) spectrometer (Shimadzu Bara Scientific Co., Ltd.) was utilized to determine whether the analysis of ink samples could be influenced by the solvent used. An ink sample (10.0mL) in 30.0mL of either ethanol or methanol was added into a quartz cuvette with a route length of 10 mm. A chamber volume of 1.5 ml was used to conduct the UV-Vis spectroscopy. The background was established after measurements between 220 nm and 900 nm spectra range and absorbance values between 0.05 -1.5 optical density (OD). Absolute pure ethanol and pure methanol were used as controls.

Data Analysis: As described previously, the retention factor (Rf) values were calculated for each ink pigment detected by the TLC [13]. Discrimination power was calculated using a pairwise comparison of ink samples[13]. Absorbance was recorded as optical density (OD). Multivariate analysis was applied to determine the difference between ink analyses in different solvents with a significance level was $P<0.05$

RESULTS

A total of ten distinct ballpoint pens from major ballpoint ink pen manufacturers were included in this study. Six were blue ballpoint ink pens, and the remaining four were black (Table 1). All ballpoint ink pens were assigned a unique sample identification number (ID), as shown in Table 1.

TLC analyses of ink isolated from ballpoint ink pens: Qualitative TLC ink analyses from blue and black ballpoint pens identified different components of ink that were detected with an open eye as distinct pigment bands. The pigments detected varied among ballpoint ink pens (Figure 1).

Overall, the number of pigments detected varied from two to seven pigments. The purple pigment was commonly observed in the ink of both blue and black ballpoint ink pens. Interestingly, some pigments were only present in blue ballpoint ink pens and absent in black and vice versa. For instance, the light blue pigment was detected in blue ballpoint ink only, whereas blue, black, and pink pigments were detected only in black ballpoint ink pens (Figure 1). The retention factors (R_f) for different pigments are shown in Table 2 and Table 3. The Rf and number of pigment bands were used to calculate the discrimination power of blue and black ballpoint ink pens (Table 3) as previously described [13]. The discrimination factor for black and blue ballpoint pens was determined to be 0.87 and 0.83, respectively (Table 4).

Spectroscopic analysis of ink isolated from ballpoint pens: FTIR spectroscopy was used to identify the functional groups in inks isolated from blue and black pens. In so doing, peaks were observed in three spectra ranges, namely from 1000 cm^{-1} to 1700 cm^{-1}, 1000 to 1400 cm^{-1}, and 1500 cm^{-1} to 1700 cm^{-1}. This study defined the fingerprint region as a peak below 1500 cm^{-1} in the infrared spectrum to identify functional groups in the ink samples, in contrast, peaks above 1500 cm^{-1} were used to distinguish functional groups. The fingerprint region also provided information on the chemical bonds present in the ink.

Five primary functional groups were identified in blue and black ballpoint pen inks hydroxyl, carboxyl, amino, nitro, and ester/ether groups. Considering that FTIR spectra (Figure 2a) for different inks samples were similar and visual inspection of the spectra could not distinguish ink isolates analyzed in this study, FTIR absorption patterns of the highest peaks for each sample were therefore analyzed at four different wavelengths i.e., form 3200 cm^{-1} to 3600 cm^{-1}, from 2500 to 3500 cm^{-1} from 1680 cm^{-1} to 1750 cm^{-1} and 1000 cm^{-1} to 1300 cm^{-1} (Figure 2a). Ink samples from blue and black ballpoint pens had a discrimination power 1. Noteworthy, the spectra peak pattern of ink samples analyzed using UV-Vis could also not be distinguished by visual inspection (Figure 2b). The discrimination power for blue and black ink in UV-Vis spectroscopy was 0.93 and 0.67, respectively, with the peak absorbance recorded at a wavelength of 582-669 cm^{-1} and 248 - 305 cm^{-1}. Further multivariate analysis did not reveal any significant difference between the analysis of ink samples when either methanol or ethanol was used (P>0.05).

DISCUSSIONS

Analysis of pen inks is essential for forensic investigations of disputed documents. This study shows that Kenya's blue and black ballpoint pen inks comprise different dyes, additives vehicles and pigments. These findings are consistent with earlier studies, particularly about violet dye band characteristics and the existence of various multiple dye components combined in a specific ratio [12]. Based on their band patterns, the ink samples were divided into many classes, and some of the color families in black ballpoint pen inks (pink, yellow, bluish-greenish, bright blue, dark blue, dark purple, and light purple) classifications agreed with the results of earlier studies carried out in Pakistan [13]. Furthermore, the number of bands among ink classes in this study was slightly different compared to previous studies. A study published in 2019 observed that the number of bands in inks from pens mostly ranged between three and four bands, unlike the present study, where the number of bands ranges from three to five [13]. Moreover, this study categorized ink samples by separating various components and Rf values like in an earlier study [12]. Similarly, studies conducted in Asia (Pakistan and India) and Australia used discriminating Power (DP) for the pairwise differentiation to differentiate inks based on variations in the number of bands they produced, as undertaken in the present study [13,19,20].

In this study, two ink samples from blue ballpoint pens (B4 and B3) belonging to Class I and IV were non-discriminating pairs. Therefore, DP cannot be determined for this class. Class II and Class III are differentiated based on the bands they display; as seen in the picture, each contained two samples (discriminating pairs), thus making it possible to calculate the DP for this class. The obtained TLC chromatogram bands divided six blue ballpoint pen inks into three main classes. B1 and B2 were grouped under class II owing to the presence of three bands, namely, dark purple, light purple, and light blue. This was congruent with the previous study conducted by Sharif [13].

Further, ink samples B5 and B6 were categorized in class III because they had four bands. This class displayed color bands observed in B1 and B2 except for the extra bluish-green band. Class IV consists of only B3. Sample (B3) was unique because it was the only sample with an extra dark blue-brown band compared to other samples in this study. Although B4 could have been grouped as class I, this was not done because this ink sample had a light blue band. Nevertheless, as reported previously, all ink dyes observed in the samples analyzed here had mainly purple and blue dyes [21].

Using TLC, this study determined the discriminating abilities of blue and black ballpoint pen inks to be 0.86 and 0.83, respectively. However, higher DP values than those observed in this study have been reported, such as, 0.263 [13], 0.94 [20], and 0.92 [22]. Compared to previous forensic studies, the DP of black ballpoint inks in the present study was higher than in the previous study, 0.5[13]. High DP values than those observed in this study have, however, been reported, such as 0.89 [23], 0.9 [24], 0.95 [21], and 0.99 [19]. The study findings and those of others suggest that TLC used in the study discriminated between ballpoint ink samples of different brands.

To identify functional groups, present in the ballpoint pen ink sample, FTIR spectroscopy was used. The findings showed that hydroxyl, carboxyl, amino, nitro, and ester/ether are present in the inks. These functional groups showed that the inks comprise dyes, pigments, solvents, resins, and additives. The study identified clear peaks for all the ballpoint pen inks at IR spectra between 3200 and 3600 cm^{-1} regions. This indicates the presence of linked OH groups in the ballpoint ink formulations. Consisted with this study a previous study reported that the most critical region of ballpoint pen ink analysis is between 650 cm^{-1} and 1800 cm^{-1} [25]. Moreover, this study utilized a different FTIR spectral range between 1000 and 1800 cm^{-1}, considered the primary imperative region for ink analysis [25].

Both blue and black ballpoint pen inks had features maxima at 1585 cm^{-1} and 1170 cm^{-1} [26]. This could be attributed to triaryl methane dye in the inks, as reported previously [27]. The strong band, which appeared between 1580-1600 cm^{-1}, in all the ballpoint (blue and black) suggests the presence of amino groups (N-H) in the ink of ballpoint pens in Kenya. The spectra also exhibited peaks at 2900 cm^{-1}, indicating the presence of CH_3 and CH_2 stretching bond vibrations in all the sample inks. Another observation regarding the functional group of ballpoint pen inks is that all ten have strong carbonyl (C=O) bands between 1520 and 1700 cm^{-1}. The peak at about 1560 cm^{-1} showed the asymmetrical bending vibration of C-O-C bonds, confirming the presence of dyes and resins. Observation of a strong C-O stretching at about 1100 cm^{-1} suggested the inclusion of saturated ethers in the ink ballpoint pens. This also suggested that additives were present in ink samples of blue and black ballpoint ink pens from Kenya. Previous studies have reported similar results, with silica having a characteristic absorption band at around 1100-950 cm^{-1}, while polyethylene glycol (PEG) has one at 1100-1300 cm^{-1} (C-O stretching) [28]. Furthermore, the peak from 1380 cm^{-1} was associated with the N-O groups, which is visible in almost all the samples in the current study. This peak is usually associated with a component of the ink vehicle [29]. Therefore, results from this study show that the ink of black and blue ballpoint pens in Kenya contains dye, solvent, resins, and additives in congruence with a previous study [30].

In addition to characterizing ink based on functional groups, the ability to distinguish between pairs of ink samples was also investigated. This was accomplished by calculating the discriminating power based on peaks at a specific wavenumber and comparing each ink sample to another sample as described [13]. Sample pairs that show absorption peaks in the FTIR spectrum in the same absorption peaks were considered non-discrimination sample

pairs. In contrast, sample pairs that showed absorption peaks in the FTIR spectrum were considered discriminating sample pairs. So, if the two inks indicate absorption peaks at the same wavelength, they are similarly said to be non-discrimination pairs and vice versa.

This study observed that B1, B3; B1, B4; B1, B5; B1, B6, B2, B3; B2, B5; B2, B6; B3, B5; B4, B5; B4, B6, B1, B2; B2, B4; B3, B4, B3, B6 and B5, B6 were discriminating pairs. Thus, 15 possible pairs of ink samples were formed for six blue ballpoint pen ink samples, and all pairs of ink samples were discriminated. This also implied that none of the blue ballpoint ink pens were non-discriminating pair. Furthermore, this results in a discriminating power of 1.0, higher than in previous studies; 0.605 and, 0.97 0.605 [13] and 0.97 [31]. Similarly, six possible pairs of ink samples have been formed for the discrimination of black ballpoint pen ink. The pairs of ink samples were determined to be discriminated pairs. The discriminating power was calculated at 1.0, higher than previous reports [13]. Even though ATR-FTIR analysis (Attenuated Total Reflection Fourier Transform Infrared) is frequently used for ink analysis [32], the current study demonstrates potential application in Kenya. This is supported by the ease of analysis following sample preparation and the relative sample processing speed over TLC [12]. Distinct variations between the ink samples were observed in the present study, congruent with TLC and UV-Vis spectroscopy results. TLC and UV-VIS analysis failed to address variations in some instances, whereas ATR FTIR revealed important differences. For instance, samples B1 and B5 had different R_f values and were visually similar in TLC. Still, ATR FTIR clearly showed that the samples differed because of changes in their absorption infrared spectra at different wavelength ranges.

Previous research suggested that UV-Vis spectroscopy might distinguish distinct ink samples, even if they are of the same color, based on the peak position and relative intensity in the UV-Vis absorption spectra [13,33]. UV-Vis spectroscopy was utilized in this study with little variation caused by user-dependent factors, and it provided significant advantages in reproducibility and reliability. UV-Vis spectroscopy was used to help detect dyes/pigments and other ink constituents, such as solvents and resins. UV-Vis spectroscopy was found to be useful in the characterizing ink samples, as demonstrated by a DP value of almost 100%.

When the data from UV-Vis spectroscopy and TLC were compared, it was observed that the UV-Vis spectra revealed apparent visible differences and provided more information than TLC. For instance, two distinct samples (B1 and B5) had the same peak wavelengths at 248,269,305,352 and 582 but different comparable absorption peaks in their spectra. Thus, UV-Vis absorption spectra provide additional and useful data, which enhance the ability to differentiate samples. On the other hand, the UV-Vis spectral data for samples B2 and B3 revealed a significant visual difference. This was supported by TLC data which revealed that these samples were different from one another.

Conversely, there have been cases where UV-Vis spectroscopy could not differentiate between some samples because of a lack of data in the present study. Samples B1 and B5 might have been different, although neither TLC nor UV-Vis data were sufficient to demonstrate it. As a result, it is essential to utilize UV-Vis spectroscopy as an initial method of analysis and a confirmatory test needed to increase the reliability of the results. For this study, the discriminating power calculated based on qualitative information for blue ballpoint inks 0.93 is higher than the finding from previous studies, which range from 0.563 to 0.71 [13,20,34]. For black ballpoint pen inks, the discrimination was lower at 0.67.

Analysis of the peaks in ethanol and methanol revealed no significant differences. To date, no published study describes the comparison absorption of ballpoint pens inks in different solvents using UV-Vis spectroscopy. Nonetheless, one TLC-based study has been published, which shows significant absorption differences among different ballpoint pen inks in different solvents [35]. This demonstrates that neither the solvents ethanol nor methanol impacted the absorption of ballpoint ink pens. These findings suggest that ethanol or methanol did not considerably hamper the absorption ability of ballpoint ink. The lack of significant differences could explain two factors. This includes the chemical composition of the ink and how the ink interacts with solvents or other factors that should have been considered in this investigation. The weak relationship between the solvents and absorption suggests that the two solvents might not affect the absorption of different ink ballpoint inks currently available in Kenya. Future research should investigate additional solvents or experimental configurations that could impact ink pen absorption. One of the limitations of this study was that only two solvents (alcohols) were included. Only methanol and ethanol were employed in the analysis. This may be explained by the comparable chemical structures of methanol and ethanol, including a single hydroxyl (-OH) group connected to a carbon chain. Other limitations were that the present study did not account for drying time, spreading, or the ink pen type; it only looked at absorption.

It is important to note that user interpretation can affect visual observations in TLC. UV-Vis's examination revealed slight variations in the absorption spectra of samples B1 and B5, but the wavelength patterns remained the same. On the other hand, ATR FTIR data allowed for a distinct separation of the samples based on their absorption infrared spectra at the functional group and fingerprint region. Although the application of ATR-FTIR analyses is promising based on the finding of this study, the future application will be undermined by restrictions on sample preparation techniques and sample requirements. Therefore, ATR FTIR should not be the primary analysis method unless the material is already in a "dry" form or a better sample preparation process is used. However, ATR FTIR can be used to complement other ink analysis methods. Today, analyses for pen inks have wide applications in forensic science and investigations. These include determining the origin and source of pen inks, identifying forged or falsified documents and providing evidence for administration of justice[15–17].

CONCLUSION

Ballpoint pen inks comprised different pigments, varying according to different blue and black ballpoint ink pen brands. Despite different colors, blue and black ballpoint ink pens from different manufacturers or brands in Kenya had the same functional groups: hydroxyl, carboxyl, amino, nitro, and ester/ether. Therefore, most ballpoint ink pens used in Kenyan contain crystal violet as a dye for color providence. Using methanol or ethanol as a solvent does not impact the results obtained using UV-Vis for ink evaluation. FTIR spectroscopy is the best method for discriminating ink from ballpoint pens based on the study results.

RECOMMENDATIONS

Additional studies should be conducted with large sample sizes, including uncommon pens in Kenya and ballpoint ink pens of different colors, rather than blue and black. In addition, other solvent systems for ballpoint ink pen analysis should be evaluated since only alcohol-based systems were investigated in this study.

List of Abbreviations

DP Discriminating power

FTIR Fourier Transform Infrared

IFR Infrared Spectroscopy

Rf Retention/retardation factor

TLC Thin Layer Chromatography

UV-Vis Ultraviolet-visible

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FIGURE LEGENDS

Figure 1: TLC analysis of ballpoint ink pens: a) blue ink and b) black ink

Figure 2: Spectroscopy graphs of blue ballpoint pen ink analyses using **(a)** FTIR and **(b)** UV-Vis spectroscopy. The x-axis represents wavelength, and the y-axis represents absorption. The UV-Vis was generated using A Shimadzu spectrometer (Bara Scientific Co., Ltd.). FTIR spectrum was generated using ATR-based spectroscopy (Shimadzu Europe)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

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No competing interest

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Authors' contributions

MKK conducted experiments and drafted the manuscript. GO, DN and EM designed the experiments. DN and EM supervised the experiments as well as data analysis. DN and EM reviewed and edited the manuscript. All authors approved the final manuscript.

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Authors' information

MKK is an MSc Forensic Science Kirinyaga University student. DN, EM and GO lectures at Kirinyaga University

Tables

Table 1 Sample Blue and Black ballpoints of different brands

S/No	Ink sample	Sample ID	Manufacturer
Blue ballpoint pens			
1.	Crown	B1	Adix Plastics Ltd
2.	M&G Co-Open	B2	Shanghai M&G Stationery Inc
3.	Bic Sharp	B3	Haco Industries Kenya Ltd
4.	Bic Crystal	B4	Haco Industries Kenya Ltd
5.	Smoothline	B5	Druk Exporting Company
6.	Tepee	B6	Tepee Brush Manufactures Ltd
Black ballpoint ink pens			
7.	Smoothline	KO1	Druk Exporting Company
8.	Bic Crystal	KO2	Haco Industries Kenya Ltd
9.	Tepee	KO3	Tepee Brush Manufactures Ltd
10.	Bic Sharp	KO4	Haco Industries Kenya Ltd

Table 2: Retention factors of pigments detected after TLC analysis of ink from blue ballpoint ink pens

Sample ID	**Spot 1 Dark purple**		**Spot 2 Light Purple**		**Spot 3 Light blue**		**Spot 4 Dark blue**		**Spot 5 Bluish-green**	
	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f
B1	5	0.67	5.3	0.71	5.5	0.73	-	-	-	-
B2	4.6	0.61	5	0.67	5.3	0.71	-	-	-	-
B3	3.6	0.61	4.6	0.65	4.9	0.79	5.8	0.48	5.9	0.77
B4	4.6	0.61	5.0	0.67	-	-	-	-	-	-
B5	4.6	0.61	5.0	0.67	5.4	0.72	-	-	5.6	0.75
B6	4.7	0.63	5.1	0.68	5.4	0.72	-	-	5.6	0.75

The solvent front is 7.5 cm from the origin line; DMO: Distance moved by ink pigments from the origin (cm); R_f: retention factor.

Table 3: Retardation factors of pigments detected after TLC analysis of ink from black ballpoint ink pens

	Spot 1 Light Purple		**Spot 2 Dark purple**		**Spot 3 Light blue**		**Spot 4 Blue**		**Spot 5 Light black**		**Spot 6 Yellow**		**Spot 7 Dark Brown**		**Spot 8 Pink**	
Sample ID	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f	DMO	R_f
KO1	5.2	0.67	4.7	0.60	-		-	-	7.1	0.91	6.5	0.83	-	-	-	-
KO2	4.6	0.59	4.5	0.58	5.3	0.68	6.1	0.78	6.3	0.81	-	-	7.3	0.94	5.2	0.67
KO3	4.0	0.51	4.7	0.60	-		-	-	-	-	6.5	0.83	-	-	-	-
KO4	6.0	0.77	4.7	0.60	-		-	-	6.6	0.85	-	-	7.2	0.92	-	-

Solvent front:7.8 cm from the origin line; DMO: Distance moved by ink pigments from the origin (cm); R_f: retardation factor

Table 4: Discriminating power of blue and black ballpoint ink pen based on TLC analysis

Type of ballpoint Pen ink	**n= total no. of samples**	**Total no. of pairs = n(n-1)/2**	**Discriminating pairs (total no.)**	**Non-discriminating pairs (total no.)**	**DP**
Black	4	4*3/2=6	KO1, KO2; KO1, KO3; KO2, KO3; KO2, KO4; KO3, KO4	KO1, KO4	5/6 = 0.83
Blue	6	6*5/2=15	B1, B3; B1, B4; B1, B5; B1, B6; B2, B3; B2, B4; B2, B5; B2, B6; B3, B4; B3. B5; B3, B6; B4, B5; B4, B6.	B2, B1; B5, B6	13/15=0.87

DP: Discriminating power (No. of discriminating pairs/ Total no. of pairs

Figures

Figure 1

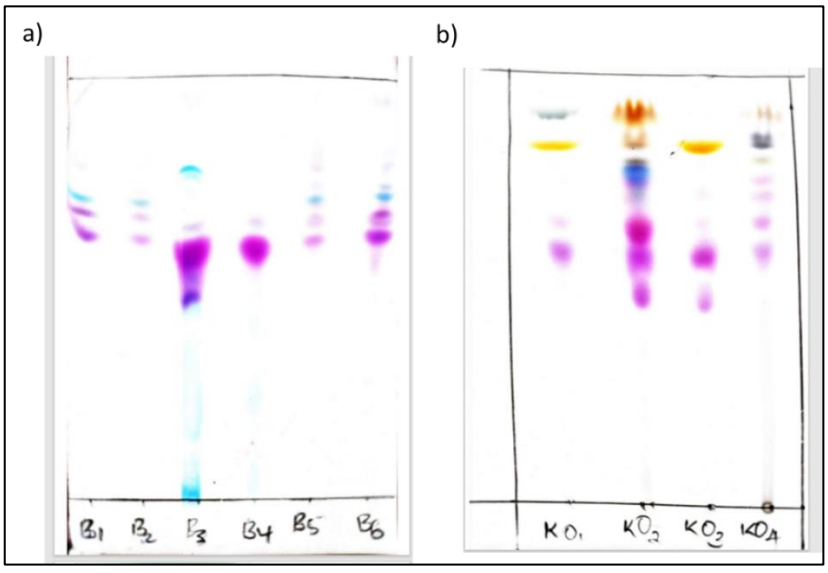

Figure 2