

Figure 1. Microscopic images of handwriting samples showing special characteristics

Table 2. Luminescence characteristic of 20 ink samples

Sample	Wavelength of Filter Barrier		
	400 – 535 nm	485 – 610 nm	605 – 730 nm
BP01	Yellowish glow	Yellowish glow	Whitish glow
BP02	Yellowish glow	Yellowish glow	Whitish glow
BP03	ND	ND	ND
BP04	ND	ND	ND
BP05	Bluish glow	Bluish glow	Whitish glow
BP06	Bluish glow	Bluish glow	Whitish glow
BP07	ND	ND	ND
BP08	Bluish glow	Bluish glow	Whitish glow
BP09	Yellowish glow	Yellowish glow	Whitish glow
BP10	Bluish glow	Bluish glow	Whitish glow
GP01	ND	ND	ND
GP02	ND	Bluish glow	Bluish glow
GP03	ND	Bluish glow	Bluish glow
GP04	ND	Bluish glow	Bluish glow
GP05	ND	ND	ND
GP06	ND	ND	ND
GP07	ND	ND	ND
GP08	ND	ND	ND
GP09	ND	ND	ND
GP10	ND	ND	ND

*ND-Not detected

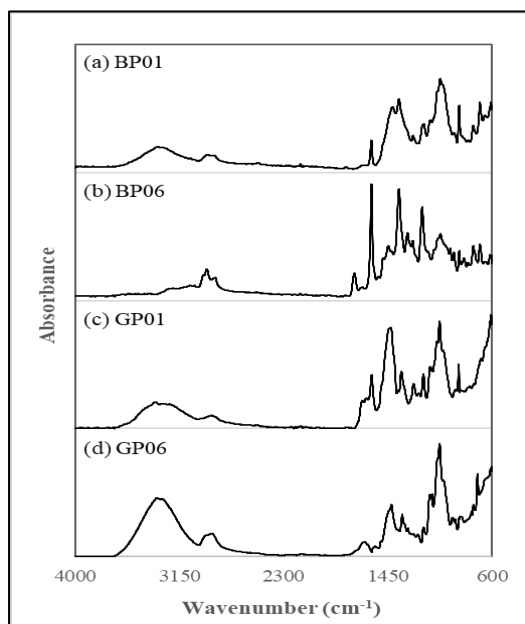


Figure 2. Representative ATR-FTIR profiles of ballpoint pen inks (a-b) and gel pen inks (c-d)

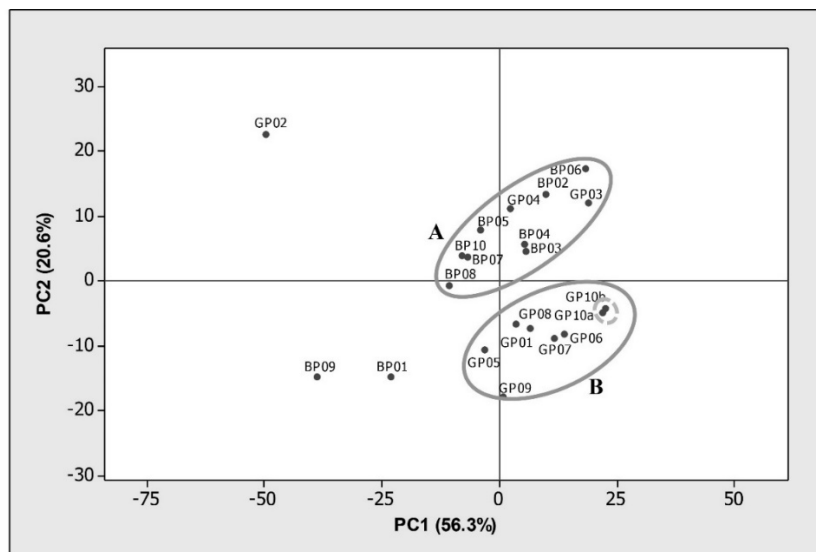


Figure 3. Score plot of 20 ink samples upon PCA

Table 3. Separation of ink components with respective R_f values

Solvent System	Methanolic Sample												
	BP01	BP02	BP03	BP04	BP05	BP06	BP07	BP08	BP09	BP10	GP02	GP03	GP04
A	0.59	0.73	0.62	0.73	0.57	0.57	0.57	0.58	0.58	0.53	0.57	0.58	0.58
	0.73	0.77	0.72	0.77	0.75	0.73	0.78	0.75	0.73	0.62	0.77	0.74	0.73
	0.78	0.82	0.77	0.81	0.79	0.78	0.82	0.78	0.78	0.76	0.81	0.84	0.83
	0.83	0.83	0.81	0.85	0.82	0.82	0.85	0.82	0.82	0.81	0.83	0.86	0.85
	0.85	0.87	0.85	0.89	0.85	0.87		0.84	0.85	0.83	0.87	0.89	0.88
	0.89		0.89		0.88	0.90		0.87	0.88	0.88		0.92	0.91
B	0.19	0.27	0.20	0.28	0.28	0.28	0.28	0.19	0.19	0.29	0.28	0.28	0.29
	0.26	0.49	0.26	0.51	0.49	0.49	0.49	0.24	0.26	0.35	0.49	0.50	0.49
	0.49	0.55	0.49	0.57	0.52	0.52	0.52	0.47	0.50	0.39	0.51	0.52	0.52
	0.55	0.64	0.54	0.65	0.58	0.58	0.58	0.53	0.55	0.56	0.57	0.60	0.60
	0.60	0.71	0.60	0.72	0.61	0.60		0.58	0.60	0.60	0.59	0.61	0.62
	0.65		0.66		0.67	0.67		0.64	0.65	0.64		0.67	0.68

From the 13 methanolic soluble samples subjected to TLC analysis, the solvent system consisting of ethyl acetate: ethanol: distilled water in a ratio of 14:7:6 allowed for the grouping of 13 samples into seven different groups. The number and colour of bands from these samples were observed to vary from four to six in red and blue shades in addition to several spots which fluoresced under UV light. The result suggested the possible utilisation of different or a combination of different colouring agents by manufacturers to give the desired colours that appear hardly distinguishable under naked-eye visualisation. On the other hand, the solvent system consisting of n-butanol: ethanol: distilled water in a ratio of 4:1:1 needed a longer separation time. One ink sample (BP08) exhibited the same band number and band pattern as the other two ink samples (i.e., BP05 and BP06) with the former solvent system, was successfully distinguished by this solvent system.

The separation of a compound or ink mixture highly depends on the type of absorbent and solvent system used [16]. Hence, a particular solvent system is not adequate to separate all dye substance [16]; therefore, different solvent systems could result in varying dye

separation. Despite resulting in relatively poor separation, the second solvent system still helped in discriminating the inks which are indistinguishable by the first solvent system and vice versa. Therefore, it is essential to employ different solvent systems for better discrimination of pen inks, if a sufficient sample is available for such analysis.

Comparison of ink samples

Under most circumstances, non-destructive visual microscopic examination and comparison against respective ink samples are preferred to avoid any further damage on the disputed documents, particularly for those stand exhibit in court. Undoubtedly, destructive methods, including TLC analysis in this study, provided much detailed information although it could impose irreversible damage to the documents, but shall only be attempted whenever sufficient samples are available and allowed for such analysis. In this study, a series of different analytical techniques, initiated by microscopic examination, VSC observation, ATR-FTIR spectroscopy, and TLC analysis has successfully created possible discrimination among ink samples. A schematic diagram summarising how the samples were

successively distinguished by each technique is presented in Figure 4.

In many instances, microscopic examination alone allowed the differentiation of two main types of pen inks, namely the ballpoint pen inks and gel pen inks, in line with the label on each pen. Based on luminescence behaviour, not all ink samples luminescence to establish sample-to-sample linkage but could assist in the exclusion of an ink sample from a source of origin. ATR-FTIR showed that ink samples from the same types tend to locate closer in the score plot, as they could have contained very similar compositional profiles. However, it was also found that two ink samples labelled as gel pens in the study were also clustered together with other ballpoint pen ink samples, and thus required careful interpretation during forensic document examination on their ATR-FTIR profiles. The use of ATR-FTIR data point and PCA score plot clustering is useful to include or exclude a group of pen samples suspected to have been used in an alteration.

Dye-based ballpoint pen inks were found soluble in methanol, supporting the microscopic examination demonstrating ballpoint pen morphological features. The three ink samples, namely GP02, GP03 and GP04, that showed gel pen ink characteristics through microscopic examination were also found to be

dissolved in methanol and luminesced under 485-610 and 605-730 nm light sources. These samples exhibited hybrid characteristics from both ballpoint pen and gel pen inks. By TLC, inks samples could be further discriminated from the rest, given that they were priorly dissolved in methanol. It was worth noting that TLC analysis was only restricted to ballpoint pen and hybrid pen inks. The technique is necessary in some cases where samples are sufficient and other non-destructive techniques were not able to discriminate among the samples.

In brief, the 20 ink samples were categorised into 11 different groups upon analyses. The presence of hybrid pen ink possessing both ink characteristics of ballpoint and gel pens as found in our study demonstrated that careful interpretation during the forensic investigation against written document related to ink is required, especially when alteration involves gel pen inks. Under one analytical technique, a hybrid pen may resemble the characteristics of either gel pen ink or a ballpoint pen ink and could lead to false positive or false negative results. This may make even an unauthentic document seem authentic if not carefully interpreted. Nonetheless, further analytical work is necessary for defensible characterisation of the source of pens, including identification of specific compounds in the ink composition.

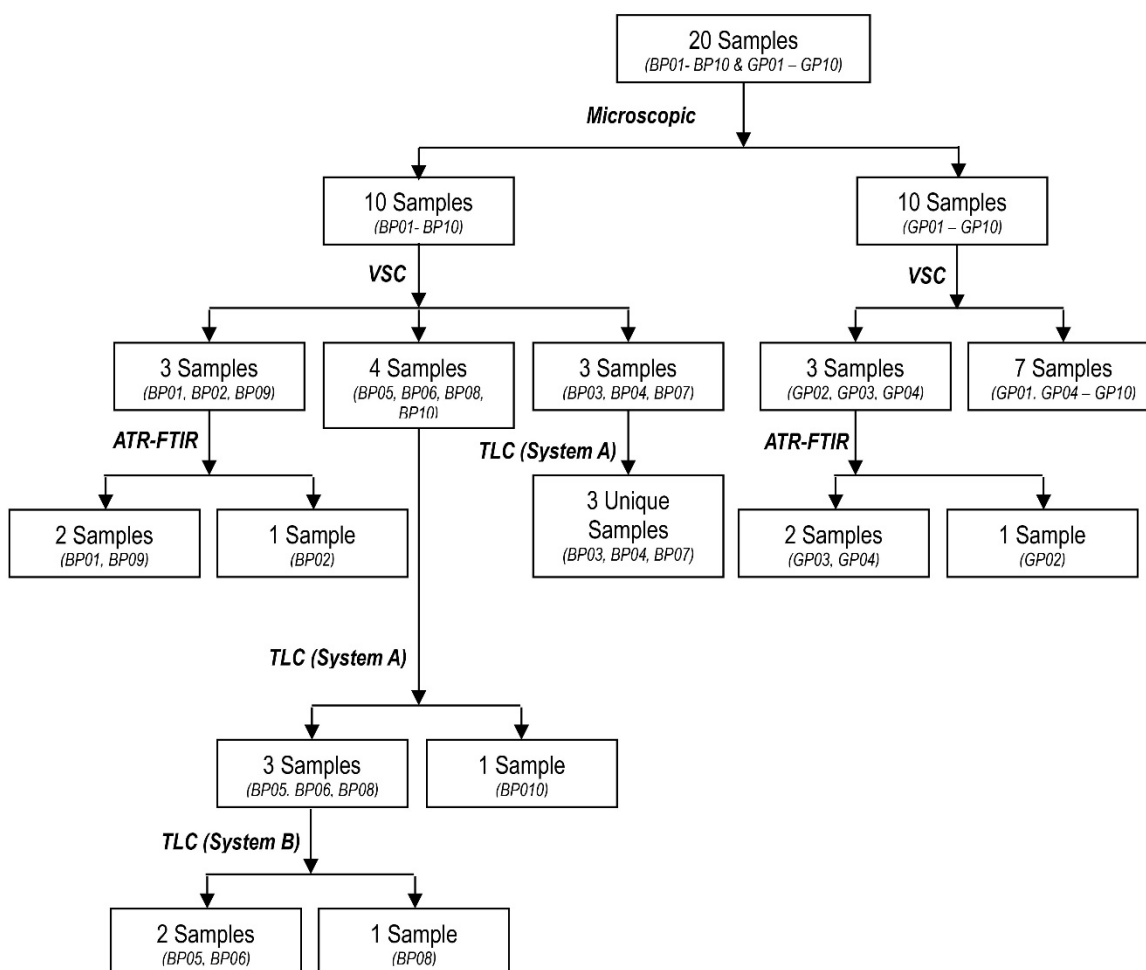


Figure 4. Discrimination of pen samples by a series of analytical techniques

Conclusion

Based on a combination of analytical techniques, the ink from pen samples tested in this study could be discriminated from the others, especially between a ballpoint pen and gel pen inks. Marked differences between the characteristics of these inks were evidently making them readily distinguishable. Nevertheless, a conclusion should not be made solely based on microscopic findings as gel pen inks could show characteristics similar to ballpoint pen inks according to their chemical compositions. The experimental protocol used in this study can be used for the exclusion of different sources of pen inks by comparing the profiles on suspected samples and crime

scene samples. We also highlighted the characteristics of hybrid pen that worth serious attention during a forensic investigation, especially when it is widely used in the future.

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