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Examination of Ball Pen Ink by High Pressure Liquid Chromatography

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ABSTRACT: High pressure liquid chromatography (HPLC) was used to analyze several samples of ball pen ink. Analytical parameters were developed to analyze the various constituents of ball pen ink, both qualitatively and quantitatively. Different ball pen ink formulations were examined, as were batch samples of a single formulation. HPLC has proved to be useful in the examination of ball pen ink, allowing for differentiation of formulations and batches of the same formulation.

KEYWORDS: questioned documents, inks, chromatographic analysis

The potential value of ink analysis to the detection of fraudulent documents has been recognized for many years by the forensic science community. Early attempts at ink differentiation were confined to analysis by nondestructive techniques, and although useful data were obtained, they were often not adequate to differentiate two inks [*1-3*]. The willingness of the courts to allow semidestructive techniques provided the forensic chemist the opportunity to use chemical analyses such as thin-layer chromatography (TLC) [*4-7*] and high pressure liquid chromatography (HPLC)[2] [*8*]. The use of TLC allowed improved differentiation between ink samples and even encouraged the amassing of a collection of ink samples known as the Standard Ink Library [*6*]. This investigation is a preliminary effort to analyze completely small samples of ball pen ink by HPLC.

Reagents and Apparatus

Solvents and reagents included acetonitrile (HPLC), pyridine (spectral grade), and PIC B-7 reagent (Waters Associates, Inc.). Ink samples were obtained from various ink manufacturers and are part of the Bureau of Alcohol, Tobacco and Firearms' Standard Ink Library (Table 1). The apparatus included:

(1) A high pressure liquid chromatograph, Waters Associates, Inc.; Model 6000 solvent delivery system, Model U6K injector, Model 660 solvent programmer, and Model 440 dual wavelength ultraviolet (UV)/visible detector. The Omniscribe dual pen recorder was from Houston Instruments;

(2) μBondapak C-18 column, 30 cm by 3.9 mm inside diameter (Waters Associates) consisting of 10-μm particles of silica with a bonded phase of C-18;

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(3) 25 μL syringe (Precision Sampling); and
(4) Bausch & Lomb Spectronic 20, UV/visible spectrometer.

Supplies consisted of a blunt hypodermic needle (20 gauge), Whatman chromatographic paper (1 M), 50% cotton fiber bond paper, and unbleached wood fiber tablet paper.

Experimental Procedures

Samples of dyes, resins, and organic acids (the major components of ball pen ink) were obtained from an ink manufacturer and were examined by reversed phase paired-ion chromatography. The parameters used were mobile phase, 80% acetonitrile/20% water with 0.005*M* Pic B-7 reagent; flow rate, 2 mL/min; chart speed, 0.5 cm/min; detector wavelengths, 546 and 254 nm; and attenuation, 0.05 absorbance units, full scale. The wavelength of choice was 546 nm owing to the complexity of the chromatogram at 254 nm.

The dye, resin, and organic acid samples were examined by extracting the components with pyridine and with acetonitrile/water (80:20) from the paper onto which they had been dried. Acetonitrile/water was unacceptable as an extraction solvent and thereafter pyridine was used exclusively. The concentration of the samples varied from micrograms per millilitre to milligrams per millilitre, while the injection size remained 10 μL. The resins and organic acids could not be detected with this system at concentrations corresponding to their levels in ball pen ink, whereas the dyes were sufficiently detectable and differentiable. It was therefore decided to limit this investigation to the analysis of the dye components of ball pen ink as they appear in a dried-ink sample.

Ten different ink formulations (A to J), which are difficult to differentiate by TLC, were applied to Whatman chromatography paper. Three samples of ten plugs each were taken from each paper by using a blunt 20-gauge hypodermic needle. This is approximately 0.5 μg of ink since 25 mm (1 in.) of line writing equals approximately 1 μg and there are 20 plugs per inch. Each ten-plug sample was extracted with 20 μL of pyridine, and a 10-μL aliquot of the extract was injected into the HPLC system. The qualitative differences were observed, (Fig. 1), and the quantitative differences were calculated by normalizing peak heights with the largest peak assigned a value of 100%. A maximum deviation from the mean of 2% was found between the three injections of each sample.

The same ten ink formulations (A to J) were also applied to 50% cotton fiber bond paper and unbleached wood fiber tablet paper, and analyses using the above procedure were made with each sample on each of the three papers, Whatman, bond, and tablet, to ascertain the effect of paper type. A maximum deviation from the mean of 5% was observed (Table 2). Three samples of ink formulation D were taken from the Whatman paper in 0.6-cm diameter punches and extracted with varying amounts of pyridine to yield three different solution intensities. The absorbance of each of these solutions was then recorded with a Spectronic 20

TABLE 1—*Data for the ink samples.*

Ink Formulation	Company	Pen Name
A	Anja Engineering	cannot be determined
B	Carter Ink Co.	Carter ball-point pen
C	Chromex Co.	cannot be determined
D	Fisher Pen Co.	pressurized cartridge by Fisher
E	Hedra, Inc.	cannot be determined
F	Papermate Co.	Papermate pens and cartridges
G	Papermate Co.	Papermate pens and cartridges
H	Scripto Pen Co.	Scripto pens and refills
I	Scripto Pen Co.	Scripto pens and refills
J	Sheaffer Pen Co.	Sheaffer pens and refills

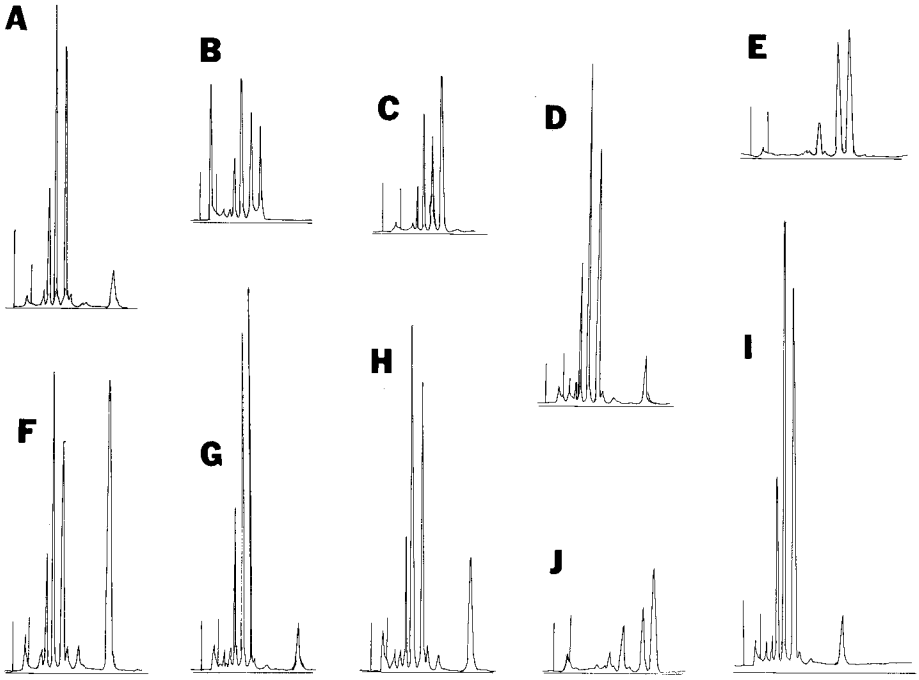

FIG. 1—*Chromatograms of ten different ink formulations (A to J).*

spectrometer; subsequently, 10-μL aliquots of each sample were injected into the chromatographic system. Figure 2 shows absorbance plotted versus peak height for the three largest peaks of each of the three samples of ink formulation D. Ink D was then placed on two different paper types, bond and Whatman. From each paper type two 5-plug and two 10-plug samples were taken. To each of these samples one drop of pyridine was added, a 10-μL aliquot of each sample was then injected into the HPLC system, and the peak heights of the three largest peaks from each of these four samples were measured. These values were plotted on the line graphs of Fig. 2, the resultant being Fig. 3.

Finally, samples of four different batches of ink formulation A were placed on Whatman paper, and 10-plug samples were taken and eluted with one drop of pyridine. Ten-microlitre aliquots of these samples were injected into the HPLC system, and the resulting chromatograms were analyzed.

Results

The HPLC system allowed detection of quantitative and qualitative differences among the ten ink formulations examined. Figure 1 and Table 2 illustrate these differences. Note first the unretained peak with normalized height of 99.7% in Ink B to as low as 4.4% in Ink A. Note also in Inks E and J the absence of peaks at $k' = 1.00$ and 1.20, where k' is defined as distance from injection point to center of peak divided by distance from injection point to dead volume. Many other differences are discernible, knowing that the reproducibility was within 2% normalized peak height for three injections of each of the inks examined.

Table 2 also shows, by use of the deviation from the mean, the results obtained in examining the effect of paper type on each of the ten ink formulations. Note that the effect of paper type can be as much as 5% normalized peak height; for example, for Ink B the peak at $k' =$

TABLE 2—*Normalized average peak heights as percentages plus or minus the standard deviation of three 10-μL injections of each ink (A to J) on each of three papers (Whatman, bond, and tablet).*

	Ink Formulations									
k'	A	B	C	D	E	F	G	H	I	J
−0.25	4.4 ± 2	99.7 ± 0.5	8 ± 0.5	5.6 ± 0.1	5.9 ± 2	13.1 ± 0.2	11.4 ± 1	10.4 ± 0.2	5.5 ± 0.1	6.7 ± 0.5
0.35	...	6.7 ± 0.5	...	6.9 ± 0.5	...	...	3.4 ± 0.2	5.4 ± 0.2	4.7 ± 0.2	...
0.55	...	7.2 ± 1	4.6 ± 0.1	6.5 ± 0.1	...	...	...	...	...	...
0.80	6.4 ± 1	...	...	...	...	7.7 ± 0.2	5.9 ± 1	5.8 ± 0.3	5.9 ± 0.3	...
1.00	40.3 ± 2	41 ± 2	30.4 ± 0.5	41.3 ± 0.1	...	44.4 ± 0.2	38.9 ± 1.5	38.3 ± 1	38.7 ± 2.5	...
1.20	100	95.9 ± 5	77.4 ± 0.5	100	...	99.5 ± 0.5	100	100	100	...
1.60	84.5 ± 2	71 ± 1.5	64.4 ± 0.3	2.3 ± 0.3	...	73.7 ± 2	79.4 ± 3	77.4 ± 3	84.5 ± 2	...
2.05	2.6 ± 0.5	65.2 ± 2	100	2.4 ± 0.2	3.6 ± 0.5	6.2 ± 0.3	2.5 ± 0.5	6.3 ± 0.1	1.7 ± 0.2	...
2.40	1.45 ± 0.5	...	...	2 ± 0.3	23.4 ± 3	8.9 ± 0.1	4.3 ± 0.5	4.9 ± 0.2	1.2 ± 0.2	16.7 ± 01
2.80	1.45 ± 0.5	...	...	...	2.7 ± 0.5	...	...	...	...	...
3.05	...	...	...	...	...	...	...	...	...	44.4 ± 0.3
3.60	...	...	...	...	84.1 ± 5	...	...	...	...	...
4.15	13 ± 0.5	...	...	15.4 ± 0.2	100	99.3 ± 1	63.3 ± 1.5	32.8 ± 1	10.3 ± 0.2	...
4.35	...	...	...	...	...	...	...	...	...	50 ± 0.5
4.95	...	...	...	...	...	...	...	...	...	100

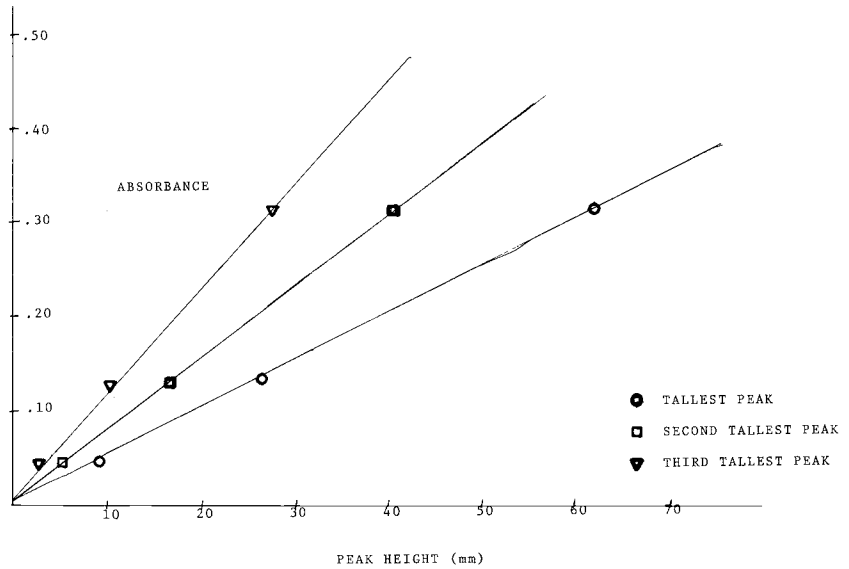

FIG. 2—*Average peak height versus absorbance for the three largest peaks of three 10-μL injections each of three solutions of Ink D.*

1.20 and for Ink E the peak at $k' = 3.60$. Although this variation because of paper type does exist, a careful examination of Table 2 will indicate that differences in excess of 5% normalized peak height still exist as in the peaks at $k' = -0.25$ (unretained), 1.00, 1.20, 1.60, 2.40, and 4.15. This, along with qualitative differences, allows for differentiation of the ten ink formulations.

Figure 2 shows absorbance plotted against peak height for the three largest peaks of each of the three samples of Ink Formulation D. As shown by Fig. 2 the line graphs for this relationship obey Beer's Law. Also note that an adequate chromatogram was obtained with a sample measuring 0.04 absorbance unit. Varying quantities of sample yielded peak heights corresponding to absorbance measurements well in excess of 0.04. The values from this study were plotted on the line graphs of Fig. 2 and show that five plugs of an ink sample in one drop of pyridine is more than sufficient to obtain a usable chromatogram, regardless of paper type (Fig. 3). It is evident, however, that paper type does have an effect on the extractability of a given ink formulation. As is expected, an increase in sample size, five to ten plugs, does result in higher peak heights, with the increase in peak height being approximately the same factor as the increase in sample size.

The analysis of four batch samples of Ink A yielded chromatograms (Fig. 4) that are qualitatively similar but quantitatively different. Table 3 shows these quantitative differences, which exceed both the 2% difference attributable to reproducibility and the possible 5% difference caused by paper type. Note this in the peaks at $k' = 1.00$, 1.20, and 1.60.

Summary

The technique permitted differentiation of ten ball pen ink formulations that are difficult to differentiate by other means. Qualitative and quantitative differences were exhibited with sample sizes as small as 0.25 μg. Although extraction solvent and paper type affect the analytical results, a valid comparison could be made which provided for differentiation even

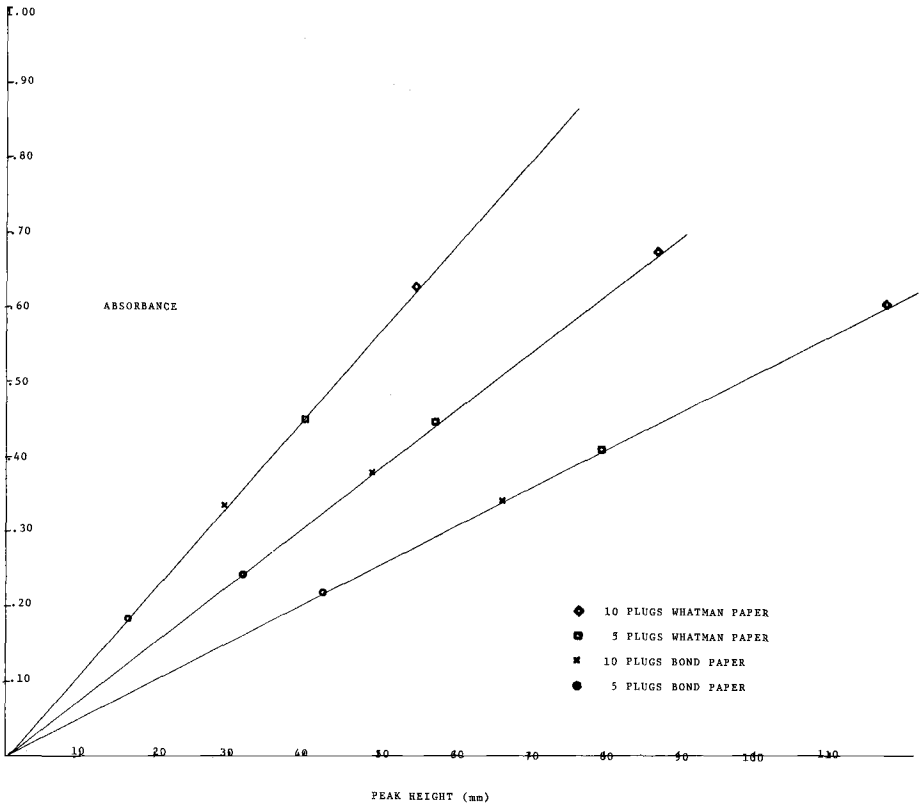

FIG. 3—*Peak heights of ten- and five-plug samples of Ink D on Whatman and bond papers plotted on line graphs of Fig. 2.*

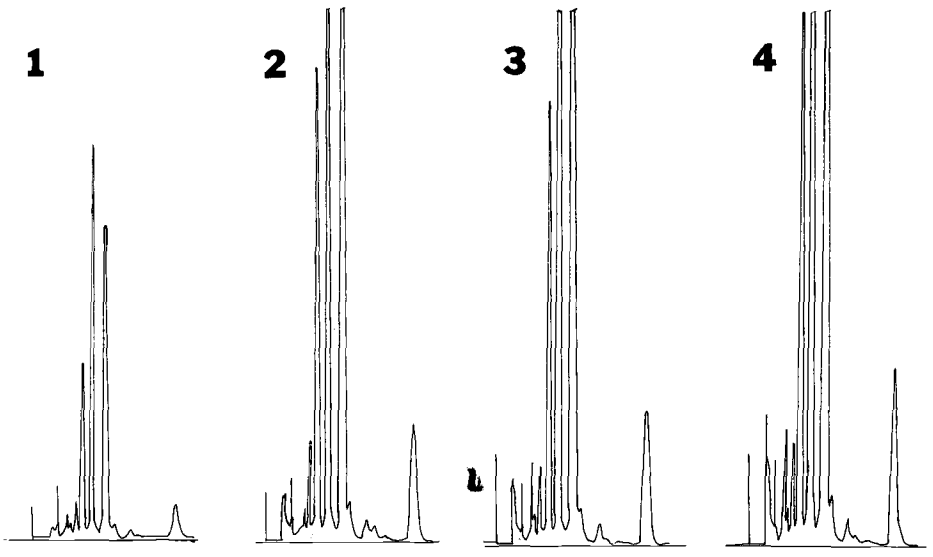

FIG. 4—*Chromatograms of four different batches of Ink D.*

TABLE 3—*Normalized average peak heights as percentages of three 10-μL injections of four batches of Ink Formulation D.*

	Batch			
k'	1	2	3	4
−0.25	2.9	6.3	7.8	8.6
0.80	8.5	12.2	8.3	5.9
1.00	42.7	63.8	51.4	43.3
1.20	100	100	82.5	93.1
1.60	77.5	86.1	100	100
2.05	1.2	2.6	1.1	1.5
2.40	2.0	2.2	2.2	1.5
2.80	...	1.3	0.4	0.2
4.15	8.8	16.8	15.8	11.6

among batches of the same formulation. A more comprehensive study incorporating more ink formulations and additional batch samples is being carried out.

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