Discrimination of Ballpoint Pen Inks by High Performance Capillary Electrophoresis and High Performance Liquid Chromatography

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Previous research demonstrates that Capillary Zone Electrophoresis has been successful in discriminating between various writing inks, including those found in felt tip and fountain pens. This preliminary study demonstrates ballpoint pen inks being discriminated using High Performance Capillary Electrophoresis (HPCE). Ballpoint pen inks were also tested using High Performance Liquid Chromatography (HPLC) and the results compared with those obtained from HPCE. Methodologies for sample preparation of four black ballpoint pen inks were developed to test a Beckman P/ACE System 5000 for HPCE and a Waters 712 WISP millepore injector system for HPLC. The initial data from HPCE and HPLC consistently differentiated between the four black ballpoint pen ink samples. The advantages of HPCE over HPLC include lower operating costs and the use of buffers rather than solvents. Further research in HPCE and ballpoint pen inks should provide an effective document examination tool for the discrimination of ballpoint inks.

Electrophoresis has been applied to ink analysis for the purposes of document examination extending at least as far back as the 1950s (Brown and Kirk, 1954 and Harrison, 1958). This analytical technique has undergone a dramatic evolution and improvement to become what is generally referred to as capillary electrophoresis (CE). It is currently being applied to many diverse fields including those in the forensic sciences (Northrup, et al., 1994). CE offers the document examiner an automated method for ink analysis which provides quick analysis times, great separation capability, efficiency (Fanali and Schudel, 1991), and small sample size requirements.

The main purpose of this paper was to assess the application of High Performance Capillary Electrophoresis (HPCE) to the separation of four black coloured ballpoint pen inks. High Performance Liquid Chromatography (HPLC) was also used to separate the same four ballpoint pen inks with the intention of comparing this data with the HPCE results. HPLC is well documented in the literature (Tebbett, 1991) for its usefulness in ballpoint pen ink separation. It is a sophisticated analytical technique providing improved sensitivity and resolution in ink separations relative to conventional thin-layer chromatography (TLC). HPLC is accomplished by injecting ink samples onto a silica column with a mobile phase consisting of a solvent/buffer mixture flowing through it. As the ink sample travels along the column, the components which separate are detected by an ultra violet (UV) detector and recorded in the form of chromatograms (Figure 1).

Capillary electrophoresis was developed by combining the strong separation processes associated with electrophoresis with the automated instrumentation of chromatography. CE samples are separated within a buffer-filled, narrow-bore capillary by the application of an electrical field. Because various compounds have different mobilities under an applied voltage, the separation of a sample's basic components can be achieved in the buffer filled capillary. Fundamental to CE is the bulk flow of liquid in the capillary which is referred to as electro-osmotic or electroendosmotic flow (EOF). This movement of liquid from the anode to the cathode in HPCE is created by the effect of the applied electrical field on the inner wall of the buffer filled capillary tube (Heiger, 1992). The type of buffer solution and its pH will directly reflect on how effectively a sample separates under CE conditions (Figure 2). CE also depends on a UV detector

Table 1.

Ink	Source
A	**Formulabs, made in California**, United States, dated 1989
B	Formulabs, made in California, United States, dated 1988
C	Paper-Mate Flexgrip, fine tip ballpoint pen, dated 1995
D	PM-39 Bic, medium tip ballpoint pen, dated 1995

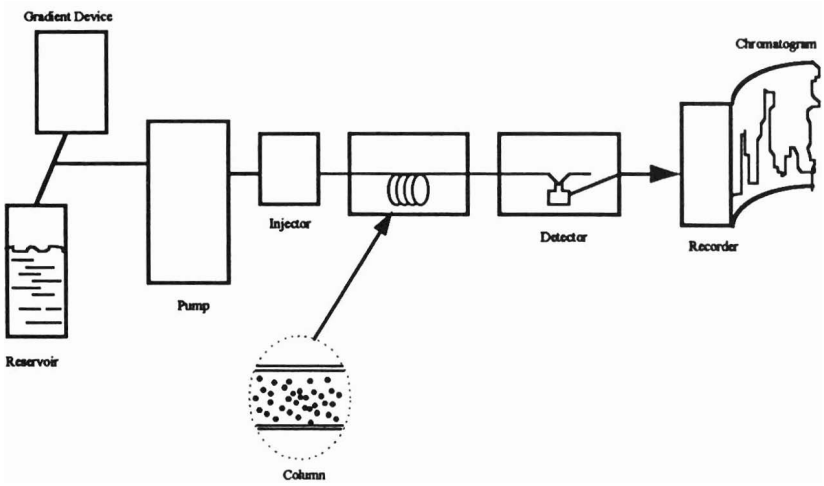

Figure 1. Schematic of HPLC setup. The pump sends the solvent through the column toward the detector. The sample is loaded onto the column through the injector port and is carried through the column by the solvent. The sample is separated into its various components on the column which are detected by the by ultraviolet detector and recorded in the form of a chromatogram.

to record the various separations brought about by this process. Results are represented by peak patterns on charts called electropherograms.

Methods and Materials

HPCE was accomplished using the Beckman P/ACE System 5500 with a UV detector set at a detection wavelength of 200 nm. This system was operated at 25°C with the voltage set at 25 kV. Samples were pressure injected onto a Beckman Electrophoresis uncoated fused silica capillary cartridge, with the capillary tube having a 60 cm length and 50 micron inside diameter. A 100 mM, 1:1 Acetonitrile/Potassium Phosphate buffer mixture with a pH of 3.2 was used.

HPLC was performed with a Waters 712 Wisp millepore Injector system using a UV 490 multi-wave detector and model 510 pumps. The wavelength was set at 200 nm. A Waters Nova Pak® C-18 column (2 mm × 150 mm) was used. The mobile phase consisted of a 100 mM Acetonitrile/Potassium Phosphate buffer and employed a gradient from (50:50) to (95:5). The pH was set at 3.2.

The four black inks were obtained from several sources and are listed in Table 1.

The four ballpoint pen inks were sampled and analysed by HPLC. In this part of the testing, samples of raw ink, paper alone, and ballpoint pen ink written on paper were taken to insure that components in the paper were not a source of contamination. Sampling involved cutting out a 40 mm line of ink from plain white bond paper and removing the ink with 100

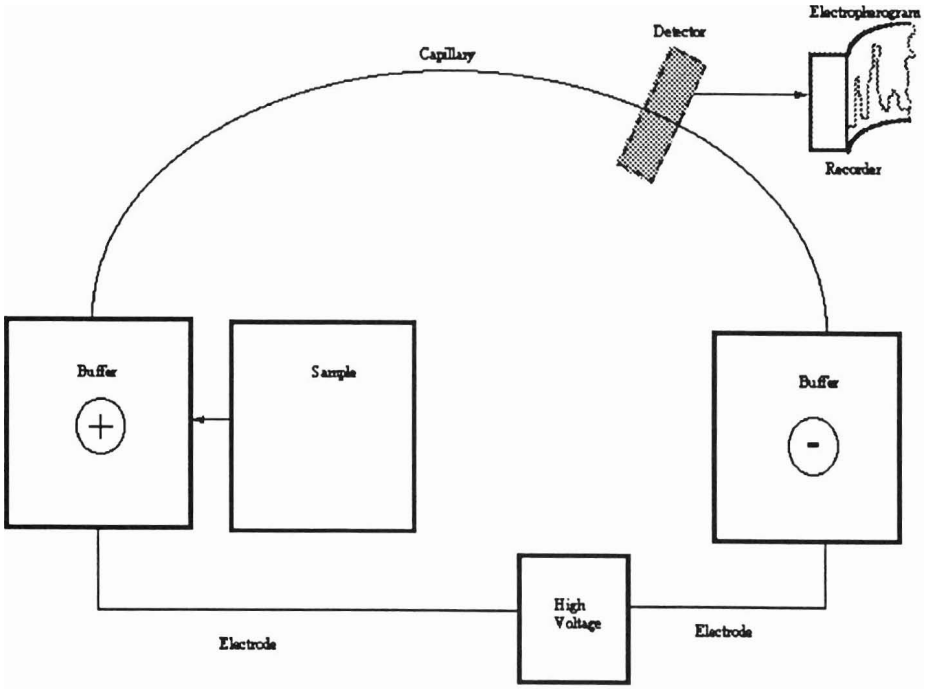

Figure 2. Schematic of HPCE setup. The sample is pressure injected on to the buffer filled capillary. The buffer and sample move toward the cathode by the application of an applied voltage where sample separations are detected using an ultraviolet light source and recorded in the form of an electropherogram.

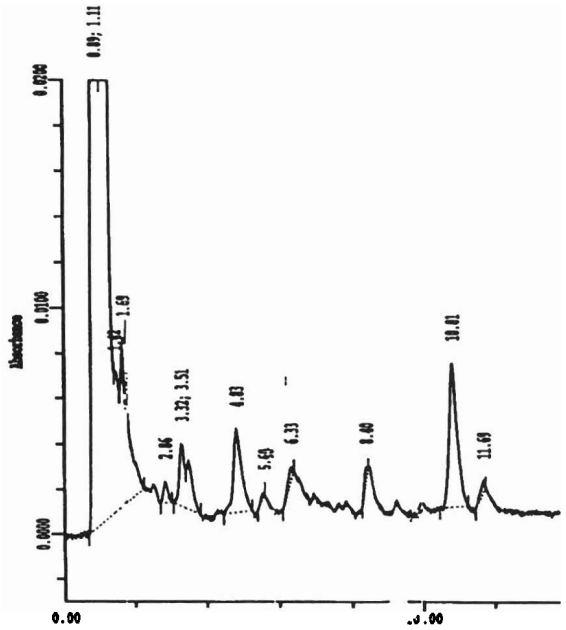

Fig 3a. Chromatogram of Ink A

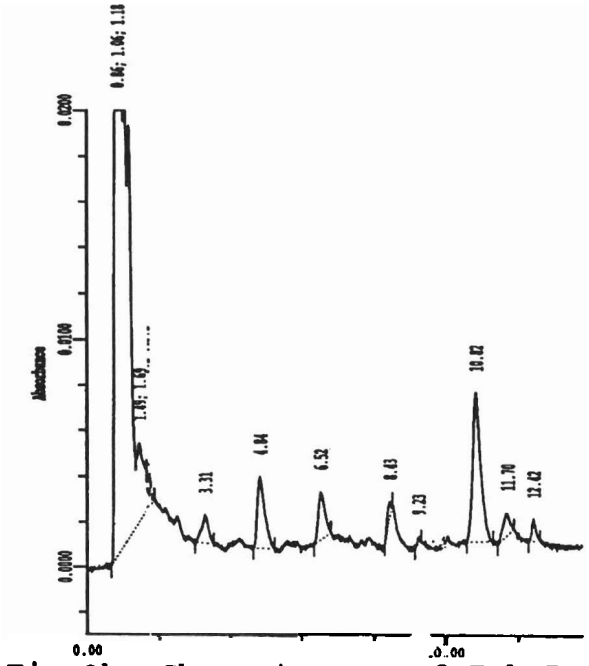

Fig 3b. Chromatogram of Ink B

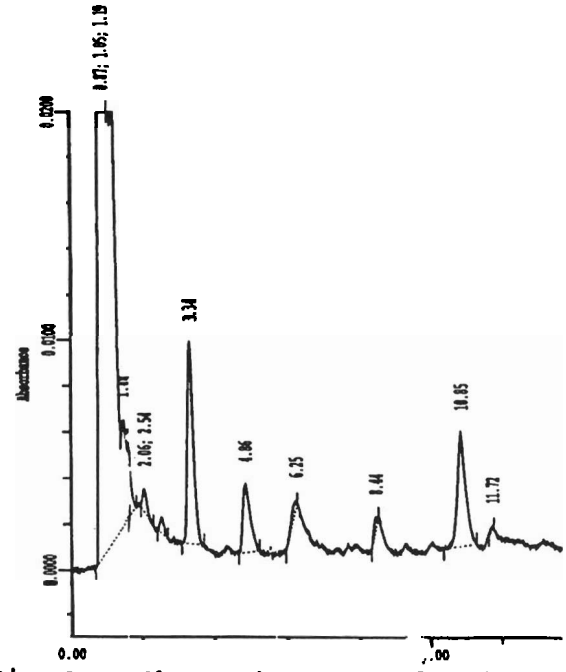

Fig 3c. Chromatogram of Ink C

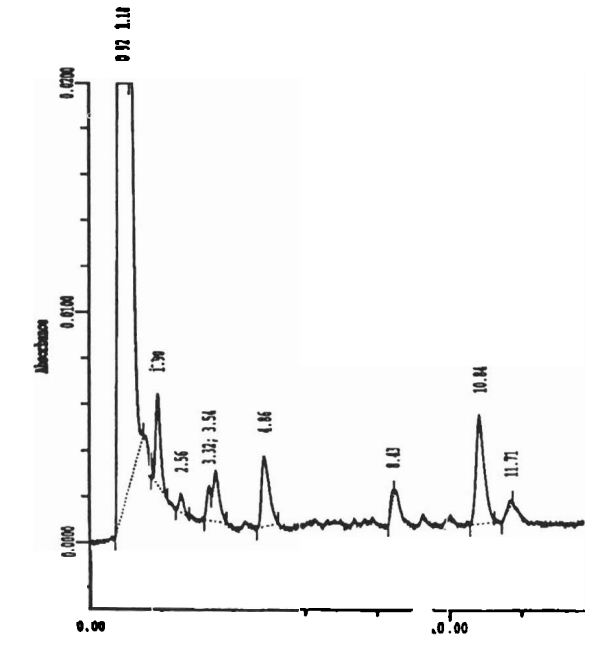

Fig 3d. Chromatogram of Ink D

Figure 3. HPLC chromatograms of the four inks, A through D. The chromatogram x-axis indicates time in 2-minute intervals and the y-axis is absorbance.

microlitres of (1:1) Acetonitrile/Potassium Phosphate buffer, (pH - 3.2, 100 mM). All samples were placed in an ultrasonic bath for 25 minutes and then centrifuged for 30 minutes. See Figure 3 for the chromatograms of inks A through D.

These same four inks were sampled and analysed by HPCE and the results compared with those obtained by HPLC. The raw ink, paper, and ink on paper samples were prepared with 50 micro litres (1:1) Acetonitrile/Potassium Phosphate buffer, (pH 3.2, 100 mM). It was interesting to note that the minimal amount of sample actually used by the CE process is on the

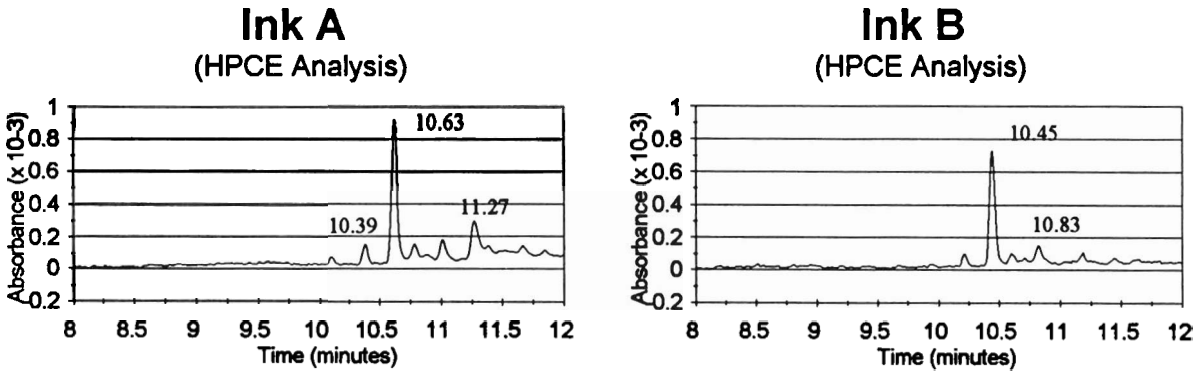

Fig 4a. Electropherogram of Ink A. (Selected portion)

Fig 4b. Electropherogram of Ink B. (Selected portion)

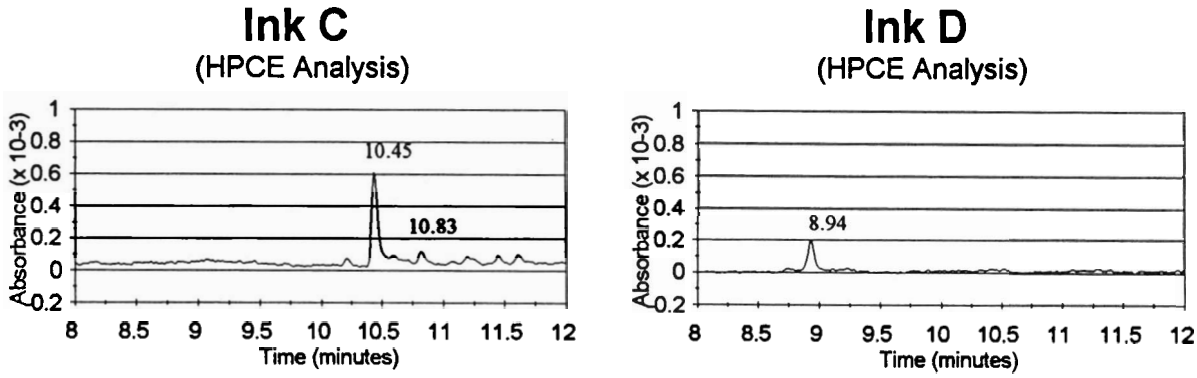

Fig 4c. Electropherogram of Ink C. (Selected portion)

Fig 4d. Electropherogram of Ink D. (Selected portion)

Figure 4. HPCE electropherograms of the four inks, A through D. The x-axis of each electropherogram indicates time in 30 second intervals, the y-axis is absorbance.

order of nanolitres, allowing for additional runs from the same sample to be completed. All samples were placed in an ultrasonic bath for 25 minutes and then centrifuged for 30 minutes. Some experimentation was employed to establish a reasonable and effective ink on paper sample size. The sample sizes tested were 40 mm, 30 mm, 15 mm, 10 mm, 5 mm, and 2 mm lines of ink on paper. The resulting electropherograms for the different sample sizes provided consistent results down to the 5 mm sample size. See Figure 4 for the electropherograms of inks A through D using 5 mm sample sizes.

It was useful to determine the effect of longer pressure injection intervals on this analysis. Accordingly, the intervals tested were 5, 10, 15, 20, and 30 seconds with various samples of the four inks. Consistent results were maintained in the electropherograms up to the 20 second interval. Pressure injections at 30 seconds showed rounding of various peaks on electropherograms indicating a loss of resolution. Sample run times of 30 minutes were used initially but it was found that each sample was effectively completed after 15 minutes. This allowed for a reduction of the HPCE operation time by 50% in the final ink on paper sample runs.

Results and Discussion

With reference to the four inks tested, it is not known if the identified peaks on HPCE electropherograms represent the same or different peaks on the related HPLC chromatograms. Because the two separation techniques are sufficiently different in how they function, it is possible that each method may be separating different components from the same ink sample. If this is the case, HPLC and HPCE might be complementary separation techniques for ink differentiation. This would potentially result in both methods being used in conjunction with each other to more completely discriminate one ink sample from another.

One way to assess whether the various peaks identified by HPLC and HPCE are the same or not is to use TLC as done by Fanali and Schudel (Fanali and Schudel, 1991). Ink samples separated on TLC plates would be further assessed by HPCE and HPLC to identify distinct ink components. This would determine which peaks are directly related to certain components in an ink sample and how HPCE and HPLC data intercompare. A second study utilizing a larger group of inks, HPCE, HPLC, and TLC would be the next step to consider.

For ink differentiation or other sample separations both HPLC and HPCE techniques offer automated instrumentation, reasonably fast separation times and small sample size requirements. However, some operational advantages were noted for HPCE over HPLC.

1. Aqueous based buffer solutions instead of chemical solvents are required for the operation of HPCE. This is an advantage from a health and safety perspective for the operator.
2. The amount of solvent waste generated by HPCE compared to HPLC is insignificant. Therefore, HPCE results in a cost savings because the cost of waste disposal is significantly reduced. HPCE is more friendly to the environment because of the reduction in solvent waste generation.
3. HPCE requires a minimal amount of sample, on the order of nanolitres, which could be very useful for examinations where a limited amount of ink is available for testing. Additional tests could be made from the same prepared sample without further destruction of the exhibit in question.
4. Finally, the cost of a new HPCE column is considerably less expensive to replace relative to HPLC columns (Personal communication with Murray Malcolm).

In conclusion, the HPCE separation technique successfully discriminated among four black ballpoint pen inks (Figure 4) using a 5 mm line of ink on paper samples. Further research in this area should establish HPCE as an effective document examination tool for the differentiation of inks.

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