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The Use of HPLC with Multiwavelength Detection for the Differentiation of Non Ball Pen Inks

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ABSTRACT: High performance liquid chromatographic (HPLC) systems were developed for the examination of blue non ball pen inks. Using an isocratic chromatographic system, together with a Spectra Physics multiwavelength detection system, over 100 blue non ball pen inks were distinguished. The majority of these had not previously been separated by traditional methods. This approach offers a number of advantages over thin layer chromatography. Sensitivity is greatly improved, allowing the detection of picograms of sample, and the increased resolution results in far more information being produced, allowing a more meaningful comparison of samples.

KEYWORDS: questioned documents, ink analysis, HPLC, multiwavelength detection

The forensic-science examination of writing inks attempts to accomplish two purposes: to determine whether two or more entries were made with the same formula of ink, or to determine the time frame in which an entry was made, or both. The first problem is usually solved through a comparison of the physical and chemical properties of the inks. Although some progress towards the absolute aging of inks has been made [*1,2*], the technique known as relative dating is the technique more commonly used to determine whether a document was written when it was purported to have been. Relative dating is approached by comparing the properties (particularly the thin layer chromatograms) of a questioned sample against a library of samples of known age and origin.

Thin layer chromatography is the most successful method presently used for the separation and subsequent comparison of ink components, being quick and relatively simple to use. It does, however, lack the sensitivity and resolving power of some of the more sophisticated analytical techniques. High performance liquid chromatography (HPLC), has the potential of giving greater separation of dyestuffs than TLC, with an increase in the sensitivity of detection. It therefore follows that liquid chromatography should be applied to the analysis of inks.

The majority of work in the area of HPLC of inks has been limited to the examination of ball pen inks. Early investigations were carried out by Colwell and Karger [*3*] who

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attempted to separate ball pen inks using a normal phase silica column with an eluent consisting of methanol:formamide (98:2) and with detection at both 254 and 580 nm. In this study, ratios were made between peak heights at both wavelengths. The assumption was that inks having similar dye composition (that is, having similar chromatograms at 580 nm) may differ in their resin content and show differences in the chromatograms at 254 nm. In fact, the dyes themselves also show some absorbance at 254 nm, which may interfere with these calculations. Nonetheless, 25 different ball pen inks could be distinguished by this method.

The use of reversed phase HPLC for ink analysis was first described by Lyter [*4*]. Using a C18 column and a mobile phase of acetonitrile:water (80:20) together with 0.005M heptanesulphonic acid and 0.02% acetic acid to suppress ionization of the dyes, several ball pen inks could be separated using absorbance detection at 580 nm. The inks were extracted from the paper with 40 μl of mobile phase and injected directly onto the HPLC column. Subsequent work by Lyter [*5*] was able to distinguish 10 ball pen inks. However the resins could not be detected and the sensitivity was poor requiring the use of about 1 cm of an inked line for each analysis.

Very little work has been done on HPLC analysis for non ball pen inks, since the majority of questioned document cases have, until recently, involved ball pen inks. However with the development of the "rolling ball" pen, interest in non ball pen inks has increased. As with ball pen ink, non ball pen inks contain two fractions, colored and non colored. The colored fraction consists of many different types of acidic and basic dyes adapted from the textile industry. Lyter has suggested that the HPLC analysis for non ball pen inks should be directed solely at the dye fraction of the ink, since the solvents present are unlikely to persist on the paper once the ink is dry. Normal phase conditions for the examination of non ball pen inks have been suggested by Colwell and Karger. Analysis was however only on a limited number of inks and at this time no chromatographic conditions have been adequately examined as to allow the routine examination of non ball pen inks by HPLC.

There are two major problems associated with the use of HPLC analysis for inks. These are the lack of sensitivity, necessitating the use of about 1 cm of an inked line, and the fact that when a single wavelength is monitored it is necessary to replicate analyses at different wavelengths in order to adequately detect the different dye components. We have attempted to circumvent both of these problems using a multiwavelength detector. This study has concentrated on a family of 113 blue non ball pen inks most of which could not be differentiated by thin layer chromatography.

Methods

Samples of 113 different non ball pen inks in the form of 1 cm by 0.5 cm pieces of paper soaked in the ink were obtained from the ink library maintained by the Internal Revenue Service Forensic Laboratory, Chicago. Unfortunately, at the manufacturers request, specific information concerning the identity of these inks cannot be disclosed. This family of inks was subdivided into 17 subgroups based on preliminary thin layer chromatographic examination. Further thin layer chromatographic examination of this family of inks with both regular TLC plates (Eastman) and High Performance TLC (HPTLC) plates failed to differentiate the majority of the samples. HPLC was therefore employed in an attempt to separate the inks further.

Sample Preparation

A sample consisting of 5 mm by 1 mm was cut from this reference material and placed in a tapered tube with 50 μl of HPLC mobile phase. The suspension was agitated in a

sonic bath for 20 min to extract the dye, and 20 μl of the supernatant was then injected directly onto the HPLC column. Each analysis was performed in duplicate. The eluent from the column was monitored between 200 and 800 nm in order to detect those components that absorbed in either the visible or UV range. Each sample was extracted 5 times in order to ascertain the reproducibility of the technique. Samples of the same ink but of different batch numbers were also examined as were extracts from different types of paper, to ensure that chemicals present in the paper did not interfere with the analysis.

Instrumentation

A Perkin Elmer Series 3B liquid chromatographic pump was used to deliver mobile phase to the analytical column. Samples were injected onto the system via a Rheodyne injection valve incorporating a 20 μl loop. Detection of the eluting components was achieved with a Spectra Physics Focus detection system and integration and data manipulation was with an IBM Personal System/2 Model 70 computer.

HPLC

The following HPLC systems were evaluated for their ability to differentiate 17 non ball pen inks representing examples of each of the subgroups of the inks under investigation:

1. Column : Spherisorb 5 μm ODS
Eluent : Acetonitrile : Water (80:20) with 0.005 M heptanesulfonic acid and 0.02% acetic acid [*4*].
2. Column : Spherisorb 5 μm
Eluent : Dichloroethane : Ethanol : Formamide (89:10:1) [*3*].
3. Column : Spherisorb 5 μm ODS
Eluent : Acetonitrile : Tetrahydrofuran : Water (924:432:644) with citric acid (1.75 g/L) and hexane sulfonic acid (0.75 g/L) [*6*].
4. Column : Spherisorb 5 μm ODS
Eluent : Methanol : Water (60:40) with 0.005 M tetra-n-butylammonium phosphate at pH 7.2 [*7*].
5. Column : Spherisorb 5 μm
Eluent : Methanol : Ammonium acetate solution (pH 9.7) (9:1) [*8*].

In each case, the flow rate was maintained at between 1 to 2 mL/min.

Data Manipulation

The chromatogram of each ink was obtained at all wavelengths in the ultraviolet and visible regions, 200 to 800 nm (resolution 1 nm, wavelength precision 0.01 nm). Peak purity was determined by examination of the ultraviolet spectrum of each eluting peak in the chromatogram. If the compound is fully separated from other ink components, then the UV spectrum obtained will be the same throughout the width of the peak. Comparison of peaks in different chromatograms having similar retention times, was achieved by obtaining the absorbance spectrum of the peak, together with its first and second derivatives. Subtle differences in absorbance spectra of different compounds are enhanced by examination of their derivative spectra. Comparison of derivative spectra can only be performed once the peak purity has been ascertained.

Results and Discussion

Of the five liquid chromatographic methods evaluated for their ability to distinguish and identify each of 17 groups of blue non ball pen inks, optimal separation was achieved with a Spherisorb 5 μm ODS column and a mobile phase of acetonitrile:water (80:20) with 0.005 M heptane sulphonic acid at a pH of 4.7. The flow rate was 1 mL/min and the eluent was monitored at all wavelengths in the UV and visible regions (200 to 800 nm). Each ink sample was extracted as previously described with 50 μl of mobile phase, and 20 μl was injected onto the HPLC column. The process of sonicating the samples

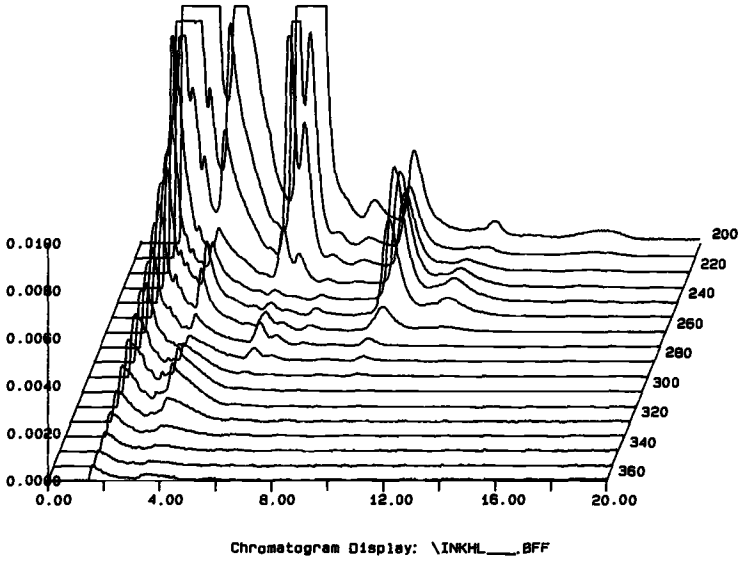

FIG. 1

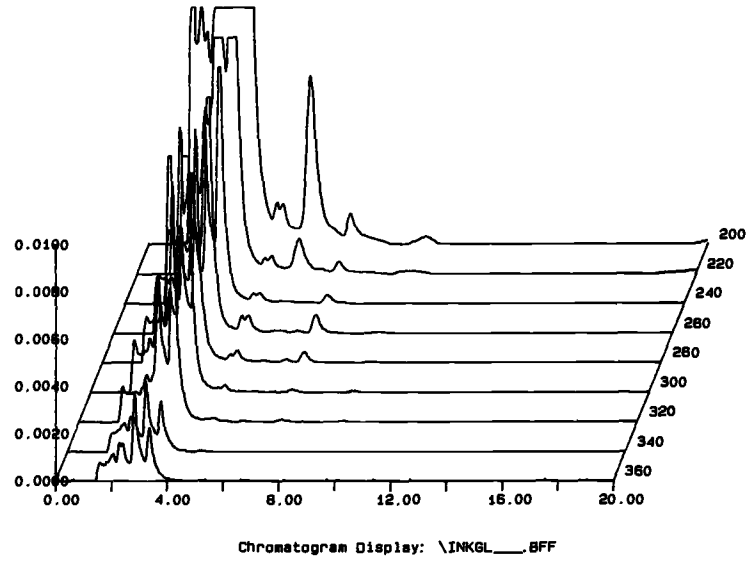

FIG. 2

with HPLC eluent for 20 min was found to be the optimum method for complete extraction of the inks from the paper. It is conceivable that other inks would not be fully extracted by this mobile phase. In such a situation, it would be necessary to extract the ink with a more suitable solvent, for example, pyridine, evaporate to dryness and reconstitute the residue in mobile phase before injection onto the HPLC. Each of the 17 groups of ink were readily separated by this method and representative chromatograms are shown in Figs. 1–4.

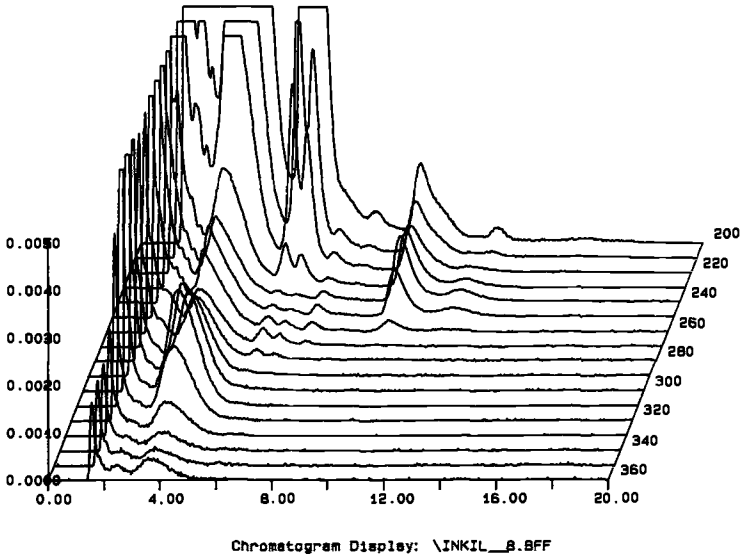

FIG. 3

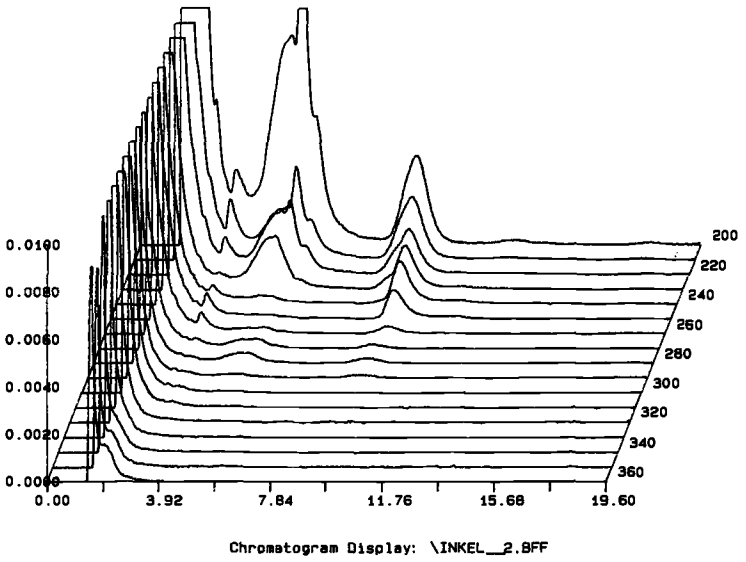

FIG. 4

FIGS. 1–4—*Representative HPLC chromatograms of blue non ball pen inks with multiwavelength detection.*

The technique was shown to be reproducible by repeated extraction and analysis of each sample (5 times). In one case however, two different batches of the same ink could be distinguished based on differences in their peak height ratios. All samples in our collection were then examined by this method, amounting to over 113 different inks. Only group A, the largest group, consisting of 32 samples, produced some chromatograms which could not be immediately distinguished from other inks in the group. With this group it was necessary to examine the ultraviolet and visible spectra of each eluting peak in the chromatogram in order to determine differences in the composition of the inks. Figures 5, 6, and 7, show the ultraviolet and first and second derivative spectra of a peak eluting after 1.48 min in three chromatograms, which on initial inspection could not be distinguished. The spectra obtained, indicates that the inks consist of different components, which could not be separated chromatographically. Using this approach, 108 of the 113 inks in the collection could be distinguished.

While we initially expected to be able to separate the inks based on their absorbance characteristics in the visible spectrum, this was not found to be the case, the chromatograms in the visible range being virtually identical for most of the inks examined. However, major differences were seen in the chromatograms in the ultraviolet region. In retrospect this is not surprising, as all the samples examined were blue inks, it is likely that the manufacturers employ similar dyestuffs, but with different vehicles (the non colored fraction) that absorb in the UV region of the spectrum.

The great advantage that this type of detection system has over traditional HPLC detectors is that complete chromatographic and spectral data can be collected simultaneously using a sample size of a few nanograms. Those inks which cannot be immediately differentiated based on their chromatographic data can be further examined by comparison of the UV or visible spectra of individual peaks and, if required, their derivative spectra.

We have shown that by using a simple isocratic HPLC system, together with the highly sensitive Spectra Physics detection system, even very closely related inks can be distin-

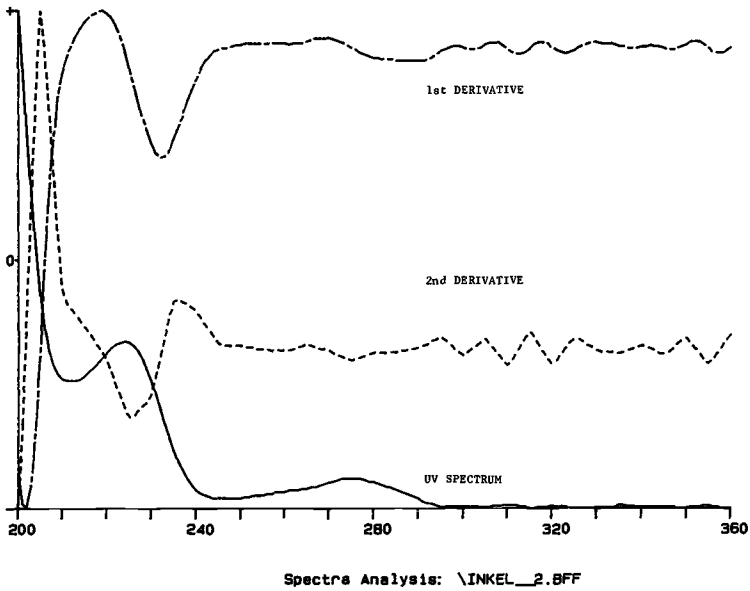

FIG. 5

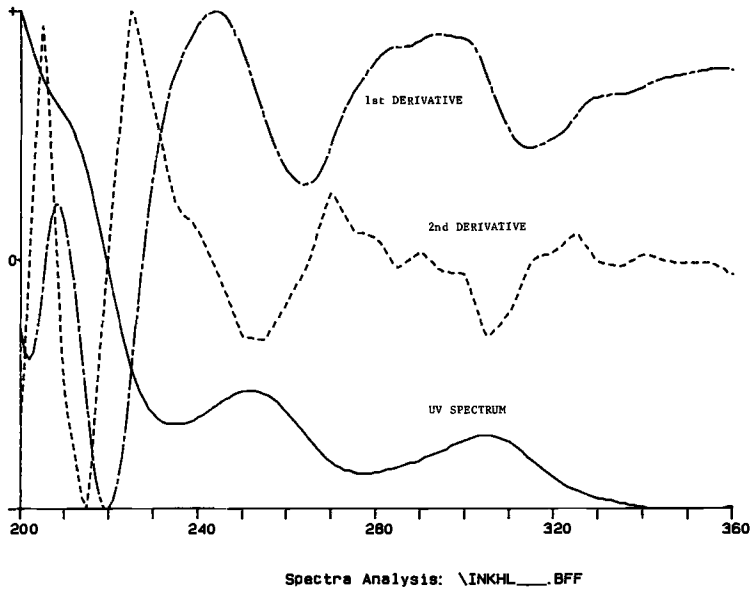

FIG. 6

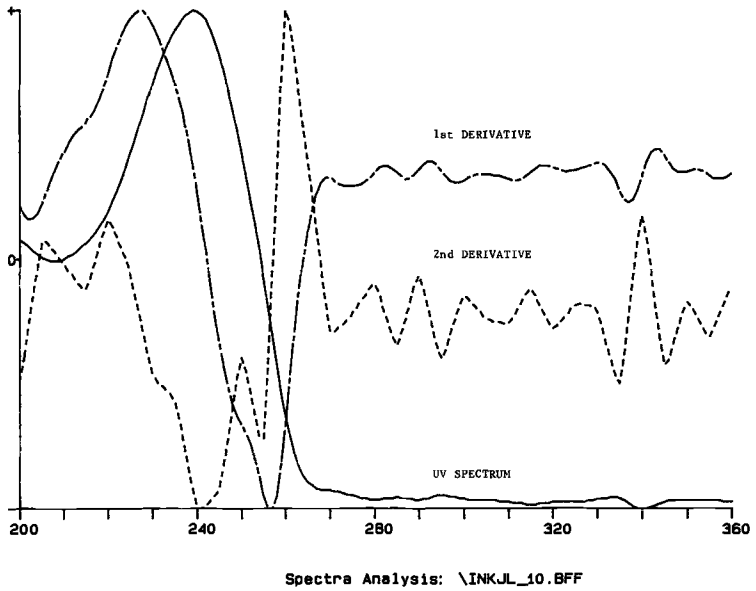

FIG. 7

FIGS. 5, 6, and 7—*Distinction of closely related inks (Nos. 8, 11, and 22, group A) by examination of the ultraviolet spectra and first and second derivatives of the chromatographic peak at 1.48 min. UV spectrum (—), 1st derivative (---), 2nd derivative (---).*

guished. In this study, a 5 mm line cut from the document was used for the analysis. However, the sensitivity of the technique suggests that meaningful data could be obtained from a much smaller sample size if required. The detector was typically operated at 0.01 Absorbance Units Full Scale for a 5 mm line of ink. A stable baseline was however possible with a sensitivity setting of 0.0005 AUFS, representing a 20 fold increase in sensitivity. In fact it was possible to obtain complete chromatographic and spectral data from a single period or comma extracted from a hand written document (Fig. 8). The extreme sensitivity of this detection system is due to the location of the sample cell after the diffraction grating. This forward optics design is similar to that employed for regular UV-Vis detectors, whereas diode array detectors use reversed optics with the sample cell before the diffraction grating. This detection system therefore has the multiwavelength scanning capabilities of diode array detectors but with the sensitivity of fixed wavelength detectors.

Conclusion

The increased sensitivity offered by this technique, enables meaningful data to be obtained from samples previously considered to be too small to work with. In addition, the improved resolution associated with HPLC separations, together with the data handling capabilities of this instrument, allow even closely related samples to be differentiated. This in turn gives the examiner a greater degree of certainty concerning the comparison of two samples, and may result in greater evidential value being placed on this type of analysis.

Acknowledgment

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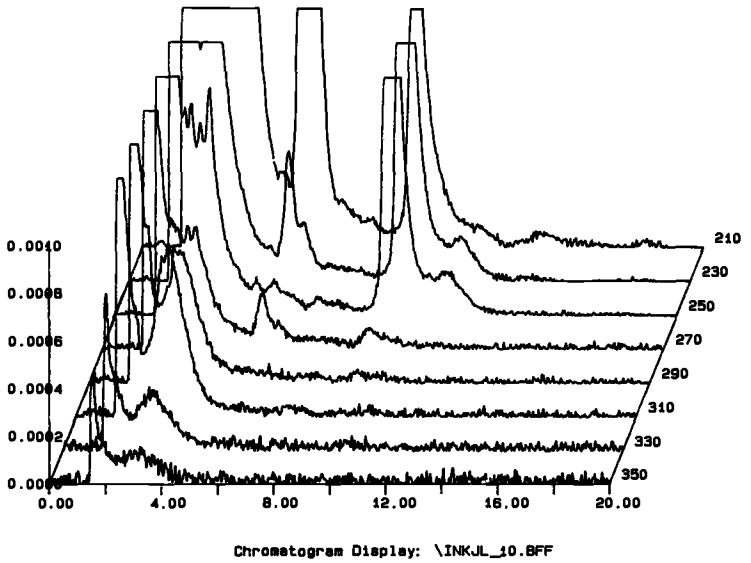

FIG. 8—*HPLC chromatogram of ink obtained from a single period, indicating the sensitivity of the technique.*

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