FORENSIC SCIENCE JOURNAL SINCE 2002

Forensic Science Journal
2002;1:1-14

A survey of methods used for the identification and characterization of inks

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Received: November 2, 2001/ Received in revised form: Decemberv 7, 2001 / Accepted: December 7, 2001

ABSTRACT

Over the past 40 years, document examiners have strived on the scientific examination and identification of writing inks. Government agencies at all levels and lawyers in the private sector are using the examination of inks as a means of establishing the authenticity or fraudulent nature of questioned documents. This survey traces the development of ink examination and identification techniques from the 1950s to the 1990s. These techniques include paper chromatography, paper electrophoresis, luminescence, microspectrometry, diffuse reflectance Fourier transform infrared, luminescence photography, laser excitation and spectroscopy, thin-layer chromatography, high-performance liquid chromatography, and capillary electrophoresis. This survey can help document examiners in governmental and private sectors to find necessary and useful methods in ink examination and identification.

Keywords: Writing ink, Ink analysis, Document examination, Thin-layer chromatography, High-performance liquid chromatography, Capillary electrophoresis, Forensic documents.

Introduction

The introduction of chromatographic methods of comparison of writing inks has had a major impact on the detection of fraudulent documents. Subtle alterations to documents such as tax returns, wills, and insurance claims, can have significant financial implications. The detection of alterations or additions to a document and an assessment of when the document was written have become a prime concern of document examiners and ink chemists. The comparison of two inks involves both chemical and physical examinations, making use of optical microscopy, infrared reflectance and luminescence, ultraviolet, fluorescence, solubility tests, and thin-layer chromatography [1]. Thin-layer chromatography is the most successful method presently used for the separation and subsequent comparison of ink components, being rapid and relatively simple to use. Thin-layer chromatography (TLC) has succeeded paper chromatography as a means of ink comparison and high-performance liquid chromatography (HPLC) promises to offer even greater capabilities in the analysis of inks. In addition, several chromatographic and spectroscopic techniques are presently being evaluated for their application to ink examination.

Methods for the detection of fraudulent documents developed slowly up to about 1950. Document examiners traditionally would not considered any test that would destroy the original condition of the document. Document examiners, with few exceptions, did not have the necessary scientific background to consider chemical or physical analysis of inks and paper, and chemical and physical examination of inks will inevitably cause some, although slight, destruction to a questioned document. Therefore, improvements in this field were slow.

Before 1950, inks on questioned documents were primarily observed and examined by photography, using filters to enhance different contrasts between different inks [2]. Also the documents were additionally examined by observing the colours of inks under various wavelengths of light ranging from ultraviolet to infrared. Occasionally, chemical tests were also used to detect metals such as copper, vanadium and chromium in fountain pen inks. These procedures were sometimes useful to distinguish among different types of inks. But they did not provide individualization information to characterize the various formulations.

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Experimentals

Paper chromatography and paper electrophoresis

In 1954, Professor Kirk investigated paper electrophoresis and evaluated and compared this technique with paper chromatography [3]. Chromatography depends primarily, thought not entirely on slight solubility differences and resulting partition ratios between different solvents. However, paper electrophoresis depends on an entirely different principle [4,5]. Materials that are ionic or can be rendered ionic by adjustment of the environment will move in an electric field toward the electrode of opposite sign. In his electrophoretic analysis, 36 blue black inks, 18 black inks were tested. Barbital and acetate buffers were used to contest the pH and hence the ionization state of the dyes. He concluded that paper chromatography and paper electrophoresis should be considered to be mutually supplementary to each other in the identification of inks. Paper electrophoresis was considered more valuable and efficient because it appeared to separate and utilize constituents that did not separate well with paper chromatography.

In 1961, Crown et al [6] introduced a method of paper chromatography. Solvents used were single solvent or solvent mixture such as ethyl acetate, lacquer thinner and ethyl alcohol (10:1 v/v), nitroethane and nitroethane with ethyl alcohol and lacquer thinner. It was found that no single solvent or combination of solvents separated all dyestuffs in the inks. They also introduced preliminary tests for ink dyestuffs. Dyes were grouped into types, varieties, or groups according to their dyeing, and/or chemical properties. Some dyestuffs were able to be grouped according to their reaction to certain chemicals. For example, phthalocyanines yielded a bright green colour with hydrochloric and sulfuric acids, while ignition of the dyestuff gives a green flame indicative of copper. Victoria blue gives a brown reaction with both acids and bases, while Rhodamine B was unaffected by sodium hydroxide and decolourized to a faint orange yellow with hydrochloric acid. The dye also showed bright pink fluorescence under long or short wavelength ultraviolet light. Eosines gives an orange colouration with sodium hydroxide and decolourizes to a faint yellow with hydrochloric acid. From all the results of these experiments, these workers were able to construct a scheme for the identification of ballpoint ink formulations. The scheme may be summarized as

(a) The ink line is spotted with concentrated hydrochloric acid, using a pipette and the colour of the ink line itself and the colour of the "bleed," if any, observed.

(b) When necessary, the hydrochloric acid spot should be absorbed onto filter paper, neutralized with saturated sodium bicarbonate solution and the resultant colour observed.

(c) A second spot should be made on the ink line with N,N-dimethyl formamide and any reaction observed under short and long wavelength ultraviolet light. All hydrochloric acid spots on the document itself must be neutralized afterwards, to prevent damage to the document.

Through this scheme, non-phthalocyanine inks could be differentiated from phthalocyanine inks. Additionally the presence or absence of Rhodamine B dye could be determined.

Luminescence

In 1978, Hardcastle et al [7] introduced a method to detect enhanced luminescence of ink components thereby differentiating many kinds of ink. The inks under investigated were illuminated with blue/green light, and any infrared luminescence recorded photographically on an infrared sensitive film via a filter, which only permitted the passage of infrared light. The theory associated with investigation is based on promotion of the dye molecules to a higher energy state by the absorption of a photon of energy. The excited molecule can then return to the ground state in one or two ways. The more common mode is by thermal decay whilst the other involves the emission of radiation. At normal temperatures the intensity of luminescence was low, but by lowering the temperature, the frequency and energy of molecular collisions were reduced and the proportion of excited molecules decaying by radiation emission increases. It was clear that luminescence, when present, was enhanced by cooling. They used liquid nitrogen to cool the inks on documents and found that the luminescence was greatly enhanced and the original ink on documents becomes readily decipherable.

In 1982, Sensi et al [8] evaluated infrared luminescence as a method for differentiating inks. Infrared luminescence was found to be the most useful for the nondestructive examination of inks and was dramatically effective in differentiating some inks. They reported one case which occurred in June 1980 in which the examination of a questioned signature which on first appearance to be written with more than one ink. However, further examination showed this not to be the case and the signature was confirmed to have been written with a single ink. The reasons for the confusion in the luminescence analysis were that the written ink line had been subjected to some interferences by some substances probably perspiration.

With regard to their infrared luminescent components, they categorized inks into three classes namely ink that contain components which luminesce, inks that con-

tain no luminescent components and inks that contain some components that luminesced and other components that did not. The third class encompassed about half of all ballpoint inks. Infrared luminescence has been accepted as a valid method to differentiate among inks since 1963 [9]. Many ink formulations contain one or more components that luminesce under infrared irradiation. In about 50% of all ink formulations, these components cannot be seen under luminescence. The masking effect can be diminished in one of these inks by treating the ink lines with body oil, perspiration, acetone, acetic acid, hand lotion, milk, and twelve other solutions found in a household, allowing the luminescent properties not previously visible to be readily discerned. It was difficult to differentiate among inks using solely the nondestructive technique of luminescence because inks in the third class could range from non-luminescent to highly luminescent depending upon their content of masking components, and thus can become indistinguishable from inks in the first and second classes. In their conclusion, they suggested that examiners should be extremely cautious in using infrared luminescence method to differentiate among inks. Before any definite determination is made, some testing is required to determine if the suspect inks contain both luminescent and masking components.

In 1983, Laing et al [10] investigated a means of differentiating writing inks of similar colour by thin-layer chromatographic separation of the ink dyes and recording the visible transmission or reflectance spectra of the separated components. They studied the effect on analysis of different paper. Instrument used for recording the spectra was a microspectrometer. For transmission measurements, a small disc of paper bearing ink deposits (0.5 mm in diameter) was punched out of the paper substrate with a sharpened hypodermic needle and placed on a glass slide with a drop of xylene-based mounting medium. The ink-stained paper fibres were separated under the microscope with a scalpel blade and a cover slip applied. Visible spectra were recorded from 390 nm to 590 nm in transmission and in reflectance modes. Spectra were compared using, differences in the number of absorbance maxima, differences in the wavelength at which maxima occur, and differences in the relative intensities of absorbances where spectra showed more than one maxima.

They found that initially, the reflectance method appeared to be attractive as little sample preparation was required. However, the reproducibility of spectra obtained in the reflectance mode was poor because of "bronzing." Bronzing is the appearance of a reddish metallic sheen on the ink line. The problem of bronzing was eliminated using a mounting medium and operating transmission mode. From these results, they demonstrated that no significant differences were detected between spectra which arose from the various papers used.

Microspectrometry

Spectra of inks on paper by microspectrometric analysis deviated from the Beer-Lambert law as a result of scattering and variations in the opacity of the paper both in the transmission and the reflectance modes and also as a result of bronzing in the reflection mode [11,12,13]. In 1983, Laing et al and Hausfrog and Pfefferli both recommended that spectra obtained by transmission microspectrophotometry are better processed on small samples of ink stained paper fibres in mount medium rather than directly on the paper [14]. However, in 1988, Zeichner reported that even using this method, the reproducibility was still poor when the ink strokes were presented on tinted paper [15].

In 1992, Zeichner et al tried to improve the discriminating power of microspectrophotometry [16] for the examination of ink traces on paper on transferring a small area of inked fibres on a slide and smearing (crushing) using an engraving tool or alternatively immersing in a mounting medium. Any pressure applied while smearing must not be excessive in order to prevent the glass slide from breaking. Ten blue and ten black pens (ballpoint pen, roller pen and fibre tip pen) were tested. All the pen inks were deposited on glass slides by writing directly on glass slides or making many strokes with a pen or a polyethylene plastic sheet and pressing it on a slide. All spectra of inks were recorded by Docuspec TM/1 computerized microspectrophotometer (Nanometrics, Inc.) which includes Olympus BHT microscope with quartz halogen lamps. The instrument is equipped with a variable measuring aperture and its wavelength range is from 380 to 764 nm. The positive influence of the type of the paper on the spectra obtained was also studied by comparing their spectra on a brown cover paper to those on a white paper. The results demonstrated that all the examined blue and black inks in the study practically obeyed the Beer-Lambert law (good reproducibility) when their traces on white or brown paper were smeared on glass slides. The spectra reproducibility obtained by mounting inked fibres in Permount was significantly less especially in the case of black inks on white or brown paper and in the case of blue inks on brown paper. It was observed that the spectra of smeared inked fibres differed from spectra of respective unsmeared in deposits on glass. The changes in the spectra caused by smearing depend on the pressure applied during smearing. A similar phenomenon of a change in a spectrum upon smearing was observed in the deposits of copper phthalocyanine tetrasulphonic acid tetrasodium salt. The change was reversed upon dissolving the smeared area in water and drying. They concluded that

the transmission spectra of small samples of inked paper film smeared (crushed) on glass slide resemble spectra of smeared ink deposits and are more reproducible than spectra of ink fibres in a mounting medium. This advantage is especially significant in the case of examining ink traces on tinted paper.

Diffuse reflectance fourier transform infrared

Fourier Transform Infrared (FTIR) spectrometry has been used in the past to study characteristics of inks [17,18]. In 1991, Harris showed its use of diamond cell transmission and micro-reflectance spectroscopy to be unsuccessful for the analysis of ink-saturated paper fibres [19]. It was expected that diffuse reflectance (DR)FTIR might be a viable method for the nondestructive analysis of ink on paper, but the spectra he obtained from ink on paper did not compare with known reference spectra of the same pure pink inks.

Other attempts to analyze nondestructively ballpoint ink on paper by both reflectance and diffuse reflectance using an FTIR microscope were also unsuccessful [20]. Strong absorption from the paper tended to mask any absorption contributed by the ink. Efforts to analyze extracted ink sample by transmission on potassium bromide (KBr) window using an FTIR microscope proved partially successful. The process of casting a film of the ink extract resulted in the formation of a ring of dye components. However, the dye components tended to separate on the KBr window, and the resulting spectrum was different from a reference spectrum of the pink ink, indicating that analysis of a small segment of the dye ring might not always be representative of the ink sample as a whole.

However, in 1978, Fuller and Griffiths discussed chromatographic separating by TLC followed by analysis using DR with FTIR [21]. In 1986, Suzuki and Gresham also studied the analysis of solids in solution using DR with FTIR [22]. They found that good spectra could be obtained from samples prepared by direct deposition of a solution onto KBr, pre-packed into a microsample cup, this being followed by evaporation of the solution. In 1992, Merrill et al utilized FTIR software that provided the ability to create a customized computer searchable spectral library to be prepared from diffusion reflectance FTIR spectra of inks. They investigated dye components, resins and other additives in situ, but also extracts of ballpoint pen inks from writing samples. Ink extracts were deposited directly onto the KBr filled DR microsampler cup. The samples were analyzed by DR using a full aperture and 256 scans. They found that the inks analyzed by their technique showed considerable differences between different manufacturers. Because the software had the ability to subtract spectra, they found that this could be used as a major advantage to detect resins not visible by TLC [23].

Luminescence photography

In 1973, Kevern [24] investigated the identification of inks by combining the techniques of thin-layer chromatography and luminescence photography. The luminescence photography offered a very sensitive method of spot detection from thin-layer chromatograms. In this work, more than a hundred different ink samples were collected and examined by thin-layer chromatography using a number of different eluents. Some of these eluents were modifications of those suggested by other workers [25,26,27,28] and which lead to a more satisfactory resolution of the ink dyes. For ballpoint pen inks, an eluent of acetone/ distilled water (85:15 v/v) was optimal, but for black inks the eluent of ethyl alcohol (absolute)/ 0.88 ammonia water solution (99:1 v/v) was found to be best.

Spots on the thin-layer chromatography, like ink lines, were characterized, from infrared luminescence photography according to whether the luminescent lines appeared white, or black infre-red absorbant, or were transparent to infrared radiation and not visible.

He found that not all visible spots were luminescent which some spots that were not visible were luminescent. Certain inks that were not luminescent as a whole had luminescent components. Some solvents caused an ink or spot on a chromatogram to change colour or even became invisible. Each change could either be permanent or temporary and in the latter instance, the colour could return on warming.

He concluded that most of the inks could be identified for their colours and thin-layer chromatograms. Infrared luminescence photography increased the sensitivity of the technique and showed that some ink components, when illuminated, emitted infrared radiation.

Laser excitation and spectroscopy

In 1986, Sinor et al [29] used lasers and optical spectroscopy for questioned document examination. In ink examination, inks could be distinguished visually via laser-induced fluorescence. In such situations, photographic documentation of the case suffices and there is no need to contemplate spectroscopic measurements. They are, however, primarily concerned with instances in which visual inspection is not able to discriminate between similar inks even under laser excitation. Three techniques used are absorption microspectrometry, thin-layer chromatography and infrared luminescence. Sinor and coworkers investigated 30 black, blue and red ballpoint and porous tip pens. Instrument used for absorp-

tion spectra was a Perkin-Elmer 356 spectrometer. IR luminescence photographs of samples tracings were undertaken using 5145 An Ar-laser excitation. The thin-layer chromatography used 100 % C-18 silanlized silica gel glass plate. The solvent used for separation was a mixture of equal parts (by volume) of acetone, methanol and distilled water. Ink samples were applied by spotting.

It is clear from their results that laser excitation to the separated dyes on TLC plates could substantially improve sensitivity. These results demonstrated that luminescence efficiencies were increased by reducing the sample temperature using liquid N_2. They also found that ballpoint inks tended to be suspensions, rather than true solution, one could anticipate small composition fluctuations within a given sample. Accordingly, minor spectral differences should not be taken as a basis for concluding ink differences.

In 1988, Cantu et al [30] worked on spectral recording of luminescence observation on inks separated by TLC. They advocated the use of an Argon ion laser as an energy source to illuminate the separated dyes. All results were recorded with IR sensitive camera system the heart of which was a silicon-viticon IR-sensitive cathode ray tube (CRT) and a fluorescence spectrophotometer. They concluded that the excitation or emission spectra of an ink known to exhibit IR luminescence predicted how this ink behaved when observed with the different viewing systems. Their work showed that the different observational recording system on the same ink did not produce independent results but were part of the same fluorescence emission.

Thin-layer chromatography

Thin-layer chromatography (TLC) is one of the simplest and most widely used chromatographic techniques. In TLC the stationary phase is a layer of powdered materials adhering to a smooth support such as a glass plate, aluminum or plastic sheet.

The samples are spotted on a line (the origin) which is drawn near the bottom of the plate. The plate is then placed in a nearly vertical position in a chamber contained a liquid or a mixture of liquids called the mobile phase. The mobile phase is allowed to migrate up the plate to a certain height. As it does so it moves the applied samples characteristic distances. The plate is then removed, dried, and observed under visible light, exposed to a UV light, or visualised by spraying with a chromatogenic agent which reacts with the separated samples to form coloured products. The retardation factor (Rf) of a compound under a certain set of chromatographic conditions is defined as the ratio of distance traveled by the compound to the distance traveled by the solvent front from the original spotted position. The Rf of a compound depends on the type of adsorbent and developing solvent and can be used to identify a compound. The density of the separated spot may be used to estimate the quantity or concentration of that compound [31-33].

In 1974, Kuranz [34] proposed a technique to optimize the separation of ink dyestuffs with similar Rf values. This technique allowed better separations when used on these difficult mixtures. The usual procedure was to place the spotted plate/strip (10 cm $\times$ 1.6 cm) in the glass vial or solvent tank and to remove it after the elution had risen up the plate/strip to an appropriate height. One problem with this procedure arose when the dyestuffs involved had similar Rf values and thus were not completely separated. To overcome this problem, the following modification to the standard single pass technique was developed. Instead of placing the spotted plate/strip into the developing chamber after drying, certain areas of the silica gel or cellulose layers were carefully removed from the underlying support sheet as shown in Figure 1.

After the active layer had been removed, the plate was placed in the developing chamber and run in the usual fashion. The removal of the active layer in the pattern shown in Figure 1 served to channel all the eluent through the spot and produced a different result than the standard technique. Instead of elliptical overlapping spots, a series of discrete bands was generated. This technique was proved to be effective and useful for the separation of ink dyestuffs, especially those which were difficult to separate using the standard method [35,36].

In 1975, Kelly and Cantu proposed a TLC method for ink analysis [37] that required the samples of inks to be first extracted with any one of reagent grade of n-butanol, ethanol, ethyl acetate and secondly pyridine. The TLC plate used was silica gel without fluorescent

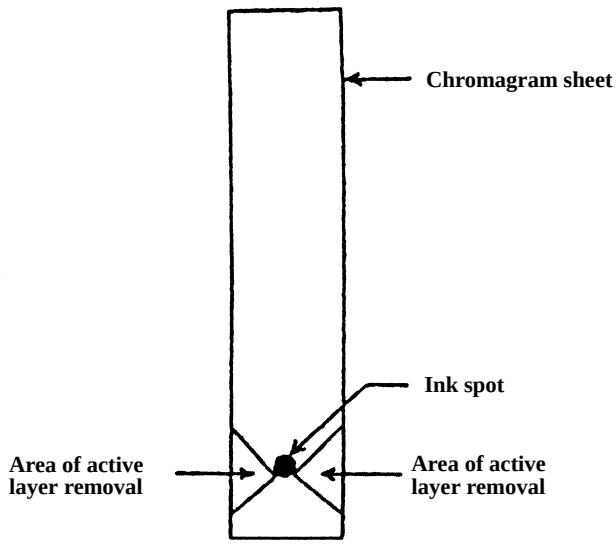

Fig. 1 Pattern for active layer removal

indicator and the solvent systems were either ethyl acetate/absolute ethanol/ distilled water (75:35:30 v/v), or n-butanol/ethanol/ distilled water (50:10:15 v/v). They concluded that these two solvent mixtures were sufficient for the separation of the components of most inks. Normally, extraction of 8 to 10 microdots (0.5 mm each) removed from the inked paper was adequate for identification of the ink.

In 1976, Crown et al reported that in the Bureau of Alcohol, Tobacco, and Firearms of the United States of America a single thin-layer chromatographic system was set up and being evaluated as a useful means for identifying inks [38,39]. Ink samples were all dissolved in pyridine, and applied on silica gel plate. The eluent for separation was ethyl acetate/ ethanol/ distilled water (70:35:30 v/v). They found that this solvent system was usually sufficient for distinguishing most ink formulations but if necessary, another TLC system was performed using Merck silica gel plates and a solvent system composed of n-butanol/ ethanol/ distilled water (50:10:50 v/v). A selection of 720 different ink formulations was examined using these two TLC system separations which were found to be effective and successful.

In 1977, Brunelle et al compared typewriter ribbon inks employing thin-layer chromatography [40]. Over 150 typewriter ribbons obtained from seven major manufacturers of typewriter ribbon inks in the United States of America were examined. The ribbons were cut with scissors into 1-cm^2 pieces that were extracted with pyridine for 30 minutes. A two-step TLC development was employed using eluent mixtures of ethyl acetate/ ethanol/ water (75:35:30 v/v), and n-butanol/ ethanol/ water (50:10:15 v/v). The chromatographic plates were developed 30 minutes in the first solvent system A and for sixty minutes with the second with drying in between. They found in their results that in most instances typewriter ribbon inks of the same colour produced by different manufacturers could be easily distinguished after running in just the first solvent system. All inks of the same colour from different manufacturers could be distinguished when the two solvent systems were used. They also noted that some inks showed insignificant batch variation while some others showed dramatic differences from batch to batch. Some manufacturers of the same typewriter ribbon inks produced several different formulations of the same coloured inks. Results of an experiment conducted to determine the effects of paper and ribbon composition on typewriter ink showed that these parameters have observable effect on the pure ink.

In 1979, Verma et al [41] worked on the analysis of fibre-tip pen inks using thin-layer chromatography. They made use of 12 sign-pen inks of three brands purchased from the local market. Silica gel plate was used for the dye mixtures and the ink samples were dissolved in ethanol for application to the plates. Two eluent compositions were employed one eluent composition being a mixture of butan-1-ol/ acetic acid/ water (6:1:2 v/v), and the other butan-1-ol/ acetic acid/ water/ 1,4-doxane (6:2:2:1 v/v). They found that the 12 inks were successfully differentiated using these two solvent mixtures when their Rf values of the separated spots on the TLC plates were compared. Some inks of different brands contained the same dye components and consequently showed no difference at all on the TLC plate. Orange, pink, red and crimson inks fluoresced under ultraviolet, and this provided more information for identification of these inks when their components were separated on TLC.

In 1982, Tappolet et al [42] studied the application of High-Performance TLC (HPTLC) for the characterization of writing inks. They tried to determine the most reliable mode of operation and the most suitable solvent mixtures for each type and colour of writing ink. The Merck silica gel plates used were put in a drying place for one hour at 60˚C in order to eliminate the water molecules from the sorbent. In relation to the application of HPTLC to fountain pen inks, they found that a 1:5 (v/v) dilution of fountain pen ink was necessary before application to the TLC plates. For each type and colour of ink, they found the best solvent mixture for a good separation of the dyes and an acceptable reproducibility of the chromatograms to be ethyl acetate/ ethanol/ distilled water (70:35:30 v/v) for blue and red ballpen inks, whilst the solvent mixture of iso-butanol/ ethanol/ acetic acid 99%/ distilled water (20:5:5:10 v/v) was the best for black and black blue fountain pen inks. They also made an evaluation of the relative values of HPTLC and TLC. HPTLC had three advantages over the TLC including rapidity of development, increased sensitivity (smaller sample) and better quality of separation with greater reproducibility.

Kelly and Brunelle [43] proposed a standard procedure for the identification of the ink in which they recommended that 10 microplugs (0.5 mm diameter) from a written ink strokes were punched out and extracting ink solven for TLC analysis. Tappolet's tests [42] showed that the average quantity removed by the procedure was equivalent to 2 µl of the liquid ink sample. This is about 65 times the quantity needed for HPTLC.

In 1983, Blackledge et al [44] worked on the differentiation of inks of the same brand by infrared luminescence photography of their TLC chromatograms. Three TLC plates and four solvent mixtures were tested. Three types of commercial TLC plates were used without any treatment. They are Merck, Type 5719, TLC plates (glass backed), silica gel 60 F 254 precoated, 5 cm × 10 cm, 0.25 mm layer thickness; Merck, Type 5549, TLC aluminium sheets, silica gel 60 F 254 precoated, 5 cm × 7.5 cm, 0.2 mm layer thickness and Merck, Type

Table 1 Different solvent systems for TLC separation

No.	Solvent mixtures
1	Ethyl acetate/ ethanol/ water (70:35:30 v/v)
2	Butan-1-ol/ ethanol/ water (50:10:15 v/v)
3	Acetone/ water (2:1 v/v)
4	Butan-1-ol/ethanol/ water/ acetic acid(18:2:2:1 v/v)

5556, HPTLC aluminium sheets, silica gel F 254 precoated for nano-TLC, 5 cm × 7.5 cm, 0.2 mm layer thickness. Four solvent systems were tried and they are shown in Table 1.

From their results, they found all four solvent mixtures achieved a reasonable separation of the visible components, but none of the systems showed any differences between the inks other than slight quantitative differences that could have been due to variations in the quantity of ink spotted at the origin. However, the four TLC plates were photographed using panchromatic film and infrared film for luminescence showing an extra luminescent band for one ink sample developed by solvent mixture of ethyl acetate/ ethanol/ water (70:35:30 v/v). Infrared luminescence photography showed that foil backed plates produced more compact bands and even revealed differences between two pen inks. In their conclusion, they stated that infrared luminescence photography of thin-layer chromatography is an extremely sensitive technique and may reveal difference between inks that are not evident by other methods. TLC systems which do an excellent job of separating the visible components of an ink may not be so successful in separating those components which exhibit infrared luminescence. It is, therefore, in a given case, it may be necessary to try several different solvent systems or even different types of TLC plates.

In 1984, Ordidge et al [45] employed thin-layer chromatography for the analysis of stamp inks on passports. Photographs (black and white) were prepared using photographic paper (Kodak Bromide), developed (Kodak D-163) and washed in the recommended manner. They were mounted on white card and ink lines drawn across the card and photograph using fluid and fibre-tipped inks. It is difficult to write on photographic emulsions using ballpoint pens. The photographs were left for 12 months partly exposed to light, but kept mainly in the dark. After this time period, the samples were examined. Colour was assessed by microscopic examination. Infrared luminescence and reflectance were recorded photographically. Small samples of ink were removed from the paper for analysis by thin-layer chromatography and the results were observed using closed-circuit television systems. Any stamp ink on the passport was extracted by a solvent mixture of pyridine and water (1:1 v/v). Merck HPTLC plates were used and the separation eluent was a mixture of butan-1-ol/ ethanol/ water (4:1:1 v/v). They found that this TLC system was successful for the separation of dyes contained within 5 blue-tipped pen inks, five black fibre-tipped pen inks, five fluid pen inks and two black fluid pen inks. However, when they repeated the analysis on 12 ink line pairs of the new and 12 months old, four of them were erroneously concluded to be different where originally they were the same. These involved two blue fibre-tipped pen inks, one black fibre-tip and one blue fluid-tip pen ink. They suggested in conclusion that extreme care must be taken in the assessment of results from thin-layer chromatography of ink samples on photographic paper, and samples could only be assessed to be different if gross differences were observed in the number, colour and Rf values of the dye components.

In 1993, Lyter et al [46] worked on the assessment of instrumentation requirements for ink identification that could be used as adjuncts to TLC. Their evaluations included video scanners, CCD detection, and also reflectance spectrophotometry. Ink samples were first dissolved in methanol and chromatographied using a TLC eluent of ethyl acetate: ethanol: water (14:7:6 v/v). The separated spots on the TLC plate were examined by the adjunct instrumentation. They indicated that the factors examined in the evaluation were colour specificity and resolution. Densitometry was acceptable for the analysis of different coloured components but video scanners were more colour dependent. Scanners did not provide sufficient resolution, but this could be improved by increasing the scan time.

In 1993, Aginsky [47] using TLC studied those pigments that demonstrated only slight solubility in some solvents. He chose 120 synthetic pigments and dyes, toners for copying machines, and writing and printing inks for the study. Approximately 2 to 3 mm^2 of these ink samples were removed by scratching (writing inks-by cutting) with help of a safety razor. The ink samples were placed into small vials for extraction. Most samples were dissolved in the very polar solvent, dimethylformamide, but those pigments that were insoluble in dimethylformamide were dissolved in concentrated sulfuric acid. The TLC separation employed was multiple development. The eluent chloroform chromatographed basic and acidic dyes but not oil soluble and ethanol soluble dyes, while the eluent mixture, ethyl acetate/ isopropanol/ water/ acetic acid (20:15:10:1 v/v) was used to developed basic, acidic dyes and oil soluble, ethanol soluble, and water soluble dyes. An eluent of concentrated sulfuric acid was shown to be applicable to most phthalocyanine and other slightly soluble organic pigments. He concluded that this three-step TLC separation was very successful, and proved this system to be the most applicable one for separating dye components of writing inks found in Russia.

High-performance liquid chromatography

In high-performance liquid chromatography (HPLC) the mobile phase is a liquid or a mixture of liquids which is moved through a column by a pump. The Stationary phase is a micro-particulate packing commonly uniform porous silica particles, with spherical or irregular shape, and several µm in diameter. This stationary phase is packed in a stainless steel tube or a glass column. After passing through the column the eluent passes through a detector system to monitor the separated compounds. The bonding of different chemical groups to the surface of the silica particles determines the different separation mechanisms. In normal phase LC, the stationary phase is relatively polar and the mobile phase relatively non-polar, whilst for reversed phase liquid chromatography a non-polar bonded stationary phase and a polar mobile phase is employed. Separation is due to differences in the partition coefficients of solutes between the stationary and mobile phases. The mobile phase may be a pure solvent or a mixture of solvents, but its polarity must be markedly different from that of the stationary liquid so that the two are immiscible. The choice of liquid pairs is largely empirical. A separation that employs a single solvent system is termed isocratic elution. Frequently, separation efficiency is greatly enhanced by gradient elution. Here two or more solvent systems that differ significantly in polarity are employed. After elution is begun, the ratio of the two solvents is varied in a programmed way, sometimes continuously and sometimes in a series of steps. In ion exchange chromatography the stationary phase is an ion exchange resin, and separation is governed by the strength of the interactions between solute ions and the ionic exchange sites on the resin. In size exclusion chromatography the stationary is a wide pore gel that can separate molecules on the basis of their sizes and shape, the largest molecules travelling most rapidly through the system.

Some detectors used for HPLC employ UV absorbance, refractometry, fluorescence, electrochemical, infrared absorbance, and mass spectrometry responses. For ink analysis, the use of UV absorbance, infrared absorbance, fluorescence and mass spectrometric detectors have been reported [48-51].

In 1977, Colwell et al [52] investigated ballpoint pen inks using an HPLC system. A written line was sampled by solvent extraction of 10 microplugs punched from the paper with a syringe needle. All separations were performed on a 25 cm silica gel (10 µm) column with a mobile phase of 2% formamide in methanol. Differences in Ballpoint pen inks, based on the different dyes present or sometimes on the relative dye amounts, were easily established. They also mentioned the special ability of a method based on recording the ratio of vehicle (resins, viscosity adjusters etc.) to dye or on the types of vehicles present. For direct analysis of vehicle components, the solvents used for sample extraction was a mixture of 2% isopropanol in heptane and the detector monitoring wavelength was 254 nm. They also investigated a variety of papers on which inks could be deposited to see if these interfered in detection when employing visible or UV monitoring. For this investigation, 10 microplugs were punched from each paper with a syringe needle and extracted with 2% formamide in methanol. No interference was found on the chromatograms for any of the papers in either the visible or UV region. Even using the weaker solvent extracting condition of 2% iospropanol in heptane, no interferences in the chromatograms were observed. Three different samples of punched-out plugs from written lines each of several inks were individually extracted and 10 µl of each sample was chromatographed for the test of reproducibility. The retention times and relative peak heights of each of the dye peaks were reproducible within 2% which is more than adequate for analysis. It was somewhat more difficult to distinguish two inks that contained the same dyes but in different relative amounts than for those which had different dyes but the relative peak heights of the bands in each pair clearly permitted characterization of the differences in the inks. Some preliminary work demonstrated that the solvent conditions used for ballpoint pen inks were too strong for felt-tip pen inks and as consequence a weaker solvent system consisting of dichloromethane/ ethanol/ formamide (89:10:1 v/v) was considered more suitable for felt-tip pen inks.

In 1982, Lyter et al [53] examined ballpoint pen inks by HPLC. Ten different ink formulations, which are difficult to differentiate by TLC, were applied to Whatman chromatographic paper. Three samples of ten plugs each were taken from each paper by using 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 mobile phase employed for the HPLC separation was acetonitrile/ 0.005 M Pic B-7 water solution (80:20 v/v) at a flow rate of 2 ml/min, and the column was µ Bondapak C-18, 30 cm*3.9 mm i.d. Detection was employed by a dual wavelength ultra/visible monitor monitoring at 254 and 546 nm. The wavelength of choice for comparison was 546 nm owing to the complexity of chromatograms at 254 nm.

The work demonstrated that the HPLC system used was capable of quantitative as well as qualitative differentiation of all ten different ink formulations that were difficult to differentiate by other means including TLC. The quantitative differences were calculated by ratioing

peaks with the largest peak assigned a value of 100%. A maximum deviation from the mean of 2.0% was found between three injections of each sample.

Lyter et al also found that five plugs of an ink sample in one drop of pyridine were more than sufficient to obtain a usable chromatogram regardless of paper type. Paper type did have an effect on the extractability of a given ink formulation while an increase in sample size from five to ten microplugs, resulted in greater peak heights, with the increase in peak height equivalent to the corresponding increase in sample size. His investigation of the analysis of four different batch samples of a single ink showed that they were qualitatively similar but quantitatively different. The sources of the quantitative differences could be associated with both the reproducibility variation and differences paper type.

In 1984, Keto [54] investigated the characterization of alkali blue pigment in counterfeit currency using high-performance liquid chromatography. The alkali blue pigment (Pigment Blue 19, CI No. 42750:1) is a synthetic organic component that was generally incorporated into black letter press and offset inks to reduce the brown undertone of the primary pigment, carbon black. Ten samples of pigment were taken from a single pigment batch from different manufacturers and all samples dissolved in methanol. The column used for separation was a micro Bondapak C-18 reverse phase 30 cm*4 mm i.d. with eluent composed of methanol/ water (1:2 v/v) (A) and 100% methanol (B). A linear gradient elution program of 10% B/90% A to 100% B in 15 minutes was used. He also used F and T tests to statistically evaluate the data. From the F test results, he found that different manufacturers showed greater variability within samples than between. A T test was employed to the retention times of each eluted peak on the chromatogram. At 95% confidence level, two-sided retention time tolerance intervals contain the given chromatographic components in 95 out of 100 separations of alkali blue. These tolerance intervals can be considered as a measure of the chromatographic separation, including solvent preparation, gradient formation and column degeneration. All retention times of eluted peaks on the chromatograms were found to fall in the tolerance intervals of retention times.

A generalized manufacturing process for alkali blue begins with aniline and formaldehyde and proceeds through five different stages. The final product is therefore a mixture of different structures, depending on the degree to which the carbinol base is phenylated. Manufacturers strive to control the degree of phenylation, the amount of which is affected by variations in time and temperature and the amount and type of catalyst used. Taken this into account Keto was able to point out that forensically significant differences could be detected between samples of alkali blue from three different sources.

In 1988, Griffin et al [55] developed an HPLC system for the separation of basic dyes. Standard dyes were obtained from Ciba Geigy company and the column used was packed with Phase Sep Spherisorb silica (5 µm). The monitor used was a photodiode array detector and eluent was ammonia acetate prepared by mixing a volume of 94 ml of concentrated ammonia and 21.6 ml of concentrated acetic acid with 884 ml of distilled water and adjusting the pH to 9.76 using either concentrated ammonia or concentrated acetic acid. They found that a silica column with a buffered (pH 9.7) water-methanol eluent was acceptable for separation of 21 commercial basic dyes. A gradient elution program which varied the buffer concentration provided even better results. The photodiode array detector coupled with a NEC-APC III computer could identify the different components even when some of the components were not well resolved during HPLC separation.

In 1989, White et al [56] proposed an optimal HPLC system for differentiating acidic dyes and identifying these acid dyes with multi-wavelength detection and absorbance ratio characterization. The optimum eluent conditions for the chromatography of the acidic dyes on the 5 µm polystyrene-divinylbenzene (PSDVB) packing material were found to be acetonitrile/ water (50:50 v/v) containing 0.7 g/L of citric acid and 3.396 g/L (0.01 M) tetrabutylammonium hydrogen sulphate (TBAH) adjusted to pH 9.0 with concentrated ammonia solution. White pointed out that traditionally, with single wavelength HPLC detection, qualitative analysis of a solute was based on the measurement of its retention time. However, with a large group of compounds, the discrimination between many of the dyes was poor. Results from these experiments showed that it was possible to achieve a much higher degree of sample discrimination using relative retention times and absorbance ratioing methods. The absorbance ratioing method requires that a multi-wavelength detector is set to monitor several wavelengths simultaneously. During a chromatographic run one of the selected wavelengths is selected as the pilot wavelength. The pilot wavelength is selected according to the colour of the dye solution as follows: yellow or green, 400 nm; orange, pink or red, 500 nm; and purple, 590 nm. In order to obtain absorbance ratio data the pilot wavelength was used as the reference wavelength and the ratios of other absorbance wavelengths to this reference were determined. If, for example, the reference wavelength of 500 nm was selected for a particular dye, then several absorbance ratios were obtained, e.g., A500/A590, A500/A550, A500/A540 and etc. In White et al's experimental results, 52 dyes, apart from different salts of the same dyes, were discriminated

successfully using the absorbance ratio data, even where the samples displayed similar retention times and colours.

In 1992, Tebbett et al [57] worked on the use of HPLC with multi-wavelength detection for the differentiation of non-ballpoint pen inks. They pointed out that non-ballpoint pen inks in the same way as ballpoint pen inks contain two major fractions, coloured and non-coloured. The coloured fraction consisted of many different types of acidic and basic dyes adapted from the textile industry. Lyter [53] suggested that the HPLC analysis for non-ballpoint pen inks should be directed solely at the dye fraction of the ink, since the solvent present were unlikely to persist on the paper once the ink was dry. Tebbett also mentioned that there were two major problems associated with the use of HPLC analysis for inks. These were the lack of sensitivity, necessitating the use of about 1 cm of an ink line, and the fact that when a single wavelength was monitored it was necessary to replicate analyses at different wavelengths in order to adequately detect the different dye components. To solve these latter requirements, multiwavelength detection becomes a necessity for ink analysis.

The 113 different non-ballpoint pen inks analyzed were first subdivided into 17 subgroups based on TLC analysis. Samples of inks within each subgroup could not be further differentiated by TLC or HPTLC. These authors then used an HPLC mobile phase solvent mixture as the solvent to extract the ink dyes from the paper. A column of Spherisorb 5 µm C-18 was used for separation and the eluent from the column was monitored between 200 to 800 nm in order to detect those components that absorbed in either the visible or UV range. A selection of HPLC solvent systems were evaluated for their ability to differentiate the 17 non-ballpoint pen inks representing examples of the subgroups generated from the TLC analysis of the inks. These eluents are listed in Table 2.

A chromatogram of each ink was obtained over all wavelengths in the ultraviolet and visible regions, (200 nm to 800 nm). Peak purity was determined by examination of the ultra-violet spectrum of each eluting peak in the chromatogram. If the compound was fully separated from other ink components, then the UV spectrum obtained were the same throughout the width of the chromatographic peak. Comparison of peaks in different chromatograms having similar retention times was achieved from the obtained absorbance spectrum of the peak together with its first and second derivatives. They concluded that of the five liquid chromatographic methods evaluated for their ability to distinguish and identify each of the 17 groups of blue non-ballpoint pen inks, optimum separation was achieved with a Spherisorb 5 µm ODS column and a mobile phase of acetonitrile/water (80:20 v/v) 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. 108 of the 113 inks in the collection could be distinguished by the proposed method. A great advantage was that complete chromatographic and spectral data were collected simultaneously using a sample size of a few nanograms. From their results ink lines of 0.5 mm length were confirmed to be sufficient enough for the analysis. They suggested that it was possible to obtained complete chromatographic and spectral data from a single period or comma extracted from a hand-written document.

In 1993, Lofgren et al [58] worked on an HPLC analysis of printing inks. Extraction of printing inks was first achieved by heating for few minutes with dichloromethane, and secondly by re-extracting with 2% hydrochloric acid in methanol. They found that most samples were satisfactorily solved using these two extraction steps. Any residue of printing ink on the paper were finally extracted by heating the sample at 80˚C with pyridine for at least 5 minutes. The use of pyridine as the extraction solvent was found necessary only for blue printing inks. Some of the blue-shadowed inks could not be removed from the documents completely by the extraction procedure used. The unextractable residue was composed of various phthalocyanines, which were insoluble in solvents, resistant to heat but had excellent fastness to light. Four mobile phases employed in the study were useful for separation of printing inks into their components. Only one of the four mobile phases was less successful in separating the inks into individual components. The combination of low pH and the use of perchlorate as ion-pairing agent in the mobile phase gave the best separation with well resolved peaks and a broad variation in retention times. The use of acetonitrile instead of methanol improved the separation efficiency and also decreased the changes in baseline owing to gradient eluation. They demonstrated that the eluent of A–30% acetonitrile +70% water containing 10 mM KClO4, pH adjusted to 3.0 with perchloric acid and

Table 2 Solvent systems for HPLC separation and analysis

System	Mobile phase
1	Acetonitrile/water (80:20 v/v) with 0.005 M heptane sulphonic acid and 0.02% acetic acid
2	Dichloroethane/ethanol/formamide (89:10:1 v/v)
3	Acetonitrile/tetrahydrofuran/water(924:432:644 v/v) with citric acid(1.75g/L) and hexane sulphonic acid(0.75g/L)
4	Methanol/ water (60:40 v/v) with 0.005M tetra-n-butyl-ammonium phosphate at pH 7.2
5	Methanol/ammonium acetate solution (pH 9.7) (9:1 v/v)

B–100% acetonitrile in a gradient combination provided the best separation. The photodiode array detector was used to monitor column effluent at 254 nm while 350 nm for excitation and 550 nm for emission where employed in fluorescence detection. They indicated that the results from these two detectors possessed very good identification power for all samples used in this work. They also employed the analytical procedures on an actual case. A series of forged payments from the Social Insurance Office of Sweden and several Postal Giro payment forms were investigated. Comparison was made with the analyses of corresponding inks on the genuine documents. The composition of printing inks on the suspect documents differed qualitatively from those of the genuine ones. The analyses carried out on several suspect documents showed that the composition of inks of the same shade on the same kind of document was indistinguishable.

Capillary electrophoresis (micellar electrokinetic capillary electrophoresis)

Capillary electrophoresis (CE) is one of the most important analytical techniques that can provide rapid, high-resolution separations of complex mixtures [59]. In CE, separation is carried out by the two related electrokinetic effects: electrophoresis and electroosmosis. In 1984, Terabe et al introduced an important development in the use of micelles to facilitate the separation of neutral species in CE [60]. When a high voltage is applied to a capillary tube filled with sodium dodcyl sulphite (SDS) micelle solution, the negatively charged SDS micelles migrate at a velocity Vep toward the positive electrode by electrophoresis and the aqueous solution can flow at a velocity Veo toward the negative electrode by electroosmosis. $|\text{Since Veo}| > |\text{Vep}|$, the micelles will move slowly toward the negative electrode. When a neutral analyte is added into the micellar solution, some portion of the solubilizate may be solubilized into the micelles. When inside the micelles, the solubilizate will migrate with the bulk flow. Thus selective partitioning of the analytes into the micellar phase causes them to migrate at different rates from that of the bulk electroosmotic flow rate. The micelles can be considered as the "Stationary phase," and the free solution is the "mobile phase." Micellar Electrokinetic Capillary Electrophoresis (MECE) may be classified as a type of liquid-liquid partition chromatography. In conventional elution chromatography, a totally retained compound is never eluted. Conversely a compound that is totally solubilized by the micellar phase in MECE is eluted in a time that is equivalent to the effective retention time of the retarded micelles. Hence, MECE is characterized by a limited elution range. Although electrically neutral substances cannot be separated by conventional CE, micellar electrokinetic capillary electrophoresis (MECE) permits the separation of neutral substances. Capillary zone electrophoresis--with its great resolution power, high efficiency, short analysis time and other advantages--is a relatively powerful technique used for the separation of compounds with different mobility by using a buffer-filled capillary cartridge and applying a relatively high electric field. Capillary zone electrophoresis was introduced by Hjerlen, Jorgenson and Lukacs, and Mikkers et al and has been used for the separation of the dyestuffs [61-64].

In 1991, Fannili et al [65] introduced a way to identify inks using this instrument. The ink analysis was performed with a high power electrophoresis equipped with a deuterium lamp (190 to 380 nm) ultraviolet (UV) detector. The detection wavelength was set at 206 nm. Capillary cartridge filled with a background electrolyte composed of a mixture of 0.1 M ammonia acetate buffer solution (pH 4.5) and methanol (3:1 v/v) was used for the ink separation. The electric chamber near the detector was positively charged, indicating that the ink dyes were moving as anions. The samples used were water-soluble red and black fibre-tip pen inks. They found that water-soluble fibre-tip pen inks were evidently different from each other using this analytical procedure.

In a further experiment Siouffi et al performed a preliminary separation of the ink dyes using RPTLC [66] extracted the dyes from the TLC plate and analysed the extract by capillary zone electrophoresis. They predicted that this technique could be used in ink dating.

Conventionally CE had been shown to be successful for the separation of anionic and cationic dyes and charged samples, but in 1993, Burkinshaw et al [67] explained the use of MECE for the analysis of dyes and other compounds employed in the dye-manufacturing and dye-using industries. The micellar electrokinetic capillary chromatography (MECC) enabled aqueous soluble, electrical neutral dye species to be separated. Additionally, by incorporating a co-solvent into the buffer system aqueous insoluble neutral dye species could be separated. Burkinshaw found that two acid dyes of similar molecular structure and relative molecular mass were not separated at all using a conventional buffer (10 mM KH_2PO_4, pH 9), but a micellar buffer system (10 mM Na_2B4O_7 - 40 mM SDS) separated the two compounds well. The workers believed that the increased separation efficiency when using MECE indicated strongly that this technique had potential to be an excellent method for the analysis of the dyes.

Conclusions

In the past years, considerable effort has been

expended in the identification of writing inks. Initially, the methods of identification were limited to physical methods of a non-destructive nature (Infrared, ultraviolet and visible examination). In instances where it was only necessary to differentiate between inks, non-destructive analyses may suffice [68]. When further identification is required, it is often necessary to resort to chemical method (i.e., thin layer chromatography, spectrophotometry, etc.), requiring the taking of samples of the ink directly from the document [69]. Many of these have been based on the use of TLC as a means of separating and identifying extremely small quantities of writing ink dyes. Differences in the techniques are primarily in the areas of extracting solvent, chromatographic media, and eluent used.

Thin layer chromatography is a well-established technique for the comparison of inks. Thin layer chromatography procedures are rapid and have been optimized by document examiners and ink chemists. It is still plays a very important role in the routine examination of inks, although a great deal of research and development work is underway involving the evaluation of more sophisticated and sensitive instrumentation for ink analysis. High performance liquid chromatography has the potential to give greater separation of dyestuffs than TLC with an increase in sensitivity of detection. It therefore follows that liquid chromatography should be applied to the analysis of inks.

Capillary electrophoresis is a technique that has been introduced as a successor to traditional paper and gel electrophoresis. The technique is quickly generating much interest. Researches have described the uses of capillary electrophoresis for the separation of inks. The excellent performance of this separation technique proves that it has great potential in the analysis of inks in the future.

Several instrumental analytical methods have been demonstrated as having potential for ink analysis. Most of them are based on a chromatographic technique with an appropriate detection method. The use of GC was described in the examination of the volatile components of inks. However, the interfacing of GC with FTIR spectrometer and mass spectrometer [70] may be useful not only for the comparison of inks, but also for the identification of the ink components. HPLC is ideal for the separation of ink components not only for vehicles and other additives but also for dye components. Multiwavelength detectors of the diode array type are capable of rapidly scanning through the UV and visible spectrum many times per second. The UV spectra of each component in the mixture can be obtained and compared to the spectra of standard dyes as an aid to identification and the detector provides immediate qualitative assessment of peak homogeneity and a rapid differentiation of spectral differences and similarities.

The feasibility of the application of micellar electrokinetic capillary electrophoresis (MECE) has been investigated. MECE is a highly efficient separation technique and is ideally suitable for the separation of inks.

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