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Identification of Rhodamine 6g and Rhodamine B dyes present in ballpoint pen inks using high-performance liquid chromatography and UV-Vis spectrometry

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Abstract

Ballpoint pen inks contain one or more dyes. Characterization of dyes present in ballpoint pen inks is very important in forensic document examination. The condition of high-performance liquid chromatographic separation in this work has been optimized. HPLC in this work provides good separation of two dyes of rhodamine 6g, rhodamine B, and four ballpoint pen inks of Staedtler 430M, Corvina 81, Bic and Micron red. Photodiode array and fluorescence and VIS/UV detections after HPLC separation provide specific results of different components in dyes and inks, and also show that this analytical method becomes reliable and time-saving. The method used in this work has strong potential for the routine characterization and identification of ballpoint pen inks in most laboratories.

Keywords: ballpoint pen inks, dyes, Rhodamine 6G, Rhodamine B, high-performance liquid chromatography, photodiode array spectroscopy, fluorescent spectroscopy, VIS/UV spectroscopy, forensic science.

Introduction

Inks are complex mixtures of a number of different compounds. Depending on the color and writing or printing instrument, a wide variety of different formulations can be encountered. Modern inks contain synthetic acid or base dyes, inorganic and organic color pigments, surfactants, antioxidants, resins, viscosity adjusters, lubricants, glycol and glycerol all in varying amounts. Formulations of ballpoint pen inks may contain very fine pigment dispersion, salts of synthetic base dyes, neutral or others such as direct dyes.

Although the analysis of inks is largely reduced to the analysis of dried inks, their complex composition remains a challenge to the forensic scientists[1,2]Many highly sophisticated instruments prove to offer definite answer to identification of compounds, however, many laboratories are not financially strong to equip those instruments[3-8]. High performance liquid chromatography (HPLC) which most laboratories have has been shown to be an efficient method for the separation of dyestuffs in inks, since it provides high sensitivities and resolutions. It is even more attractive when coupled with a diode array detector (PDA). This detector is capable of gathering spectra at any point during the separation[9]. With this additional spectral information, components of the colored as well as the non-colored fraction of inks can be identified.

Experimental

Materials and apparatus

Four red ballpoint pens, Staedtler 430 M, Corvina 81, Bic red and Micron red were bought in Great Britain.

Two kinds of paper, Chapman carbonless paper and Chapman refilled paper pad were used in this work. A syringe with the pointed end filed flat was used for punching microplugs from the paper on which ink had been deposited. A wired plunger was used to push the microplugs out from the needle of the punch into a Wheaton cone-shaped microvial (1 mL).

Methanol, tetrahydrofuran, ethyl acetate, ethyl alcohol

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and acetonitrile of LC grade were purchased from Taiwan Alps Chem. Co., Ltd., and used throughout this work.

Water used to prepare the HPLC eluent was filtered and deionized using Milli-Q-Plus.

A 5 mM Pic B-7 water solution was prepared by weighing 0.101 g sodium-1-heptanesulphonate (TCI, Tokyo, Kasei Kogyo, Co., Ltd.) into a 1000 mL volumetric flask and diluting to volume with water where appropriate. This was used for the aqueous part of the HPLC solvent mixture. Aqueous solution of 5 mM Pic B-7 at pH values of 4, 4.5, 5.5, 6.5, 7.5 and 8 were prepared by adjusting the original solution either with concentrated hydrochloric acid or ammonia solution.

The high-performance-liquid-chromatographic system employed for the analytical work consisted of a Water 600 pump with a 717 autosampler. The analytical column was a Waters Novapak C-18 column (150*3.9 mm, i.d.) and samples of 10 μL were injected into the HPLC. The eluent used for ink samples Staedtler 430 M and Corvina 81 was tetrahydrofuran: acetonitrile: 5 mM Pic B-7 water solution (10:70:20 v/v) at a flow rate of 1.2 mL/min, while the eluent for Bic red and Micron red inks was acetonitrile: methanol: 5 mM Pic B-7 water solution (45:35:20 v/v) at a flow rate of 0.8 mL/min.

A Waters 996 photodiode array detector was used for UV/VIS detection scanning from 200 nm to 800 nm.

A Waters 474 fluorescence detector in series placed after the photodiode array detection was used for fluorescence analysis. Excitation and emission wavelengths were set at 350 nm and 550 nm respectively for ink samples Staedtler 430 M and Corvina 81, while 545 nm and 555 nm were used for ink samples Bic red and Micron red. All spectra of PDA and fluorescence were recorded with a Millenium software package.

A Merck in-line filter (Merck Lincho Cart Manu-fix 25-4) was positioned before the Novapak C-18 column.

Rhodamine 6g and rhodamine B dyes of reagent grade were purchased from TCI, Tokyo, Kaisei Kogyo, Co., Ltd.

All thin layer chromatography (TLC) plates used were aluminum backed silica gel 60 purchased from Merck, Taiwan.

A Branson 8210 ultrasonic bath was employed for the solvent extraction step.

Sample preparation for the optimization of HPLC separation of Staedtler 430 M, Corvina 81, Bic red and Micron red inks

Separate lines of Staedtler 430 M, Corvina 81, Bic and Micron inks were drawn on Chapman carbonless paper. Microplugs from the lines of each ink were punched out of the paper by a self-made needle puncher with a plunger to push the 15 paper discs out of the needle. The 15 paper discs were put in a microvial and stirred with a long needle to separate the discs. An aliquot of 20 μL LC grade methanol was added into the microvial for extraction for 6 minutes at ambient temperature in a ultrasonic bath. After 6 minutes extraction, the methanolic extract was transferred into another clean microvial for the subsequent procedures.

Effect of Different Extraction Times on the Appearance of the Separated Dye Mixtures in the Inks under Examination

From written lines of the Staedtler 430 M ink on Chapman carbonless paper were punched out five sets, each of 15 discs (each 0.1 cm diameter), and placed in five respective microvials. To each microvial was added an aliquot of 20 μL LC grade methanol and the microvial tightly capped and placed in an ultrasonic bath at ambient temperature of 26℃ for different extraction times of 1, 3, 6, 9 and 12 minutes. The methanolic extract from each extraction was then transferred into another clean microvial for HPLC separation with photodiode array and fluorescence detection. The results were recorded and later used for peak ratio calculation.

All these steps were repeated for Bic red ink and the results were recorded for peak ratio calculation and comparison analysis.

Effect of Quantity of Dyes Extracted from Chapman Carbonless Paper on the Appearance of the Separated Dye Mixtures Contained in the Inks under Examination

Written lines of Staedtler 430 M ink on carbonless paper were punched out of the paper in a series of 2, 5, 10, 15 and 20 discs and placed in respective microvials. The discs in each microvial were stirred and separated with a long needle. An aliquot of 20 μL LC grade methanol was added into each microvial which was tightly capped and placed in an ultrasonic bath at ambient temperature for 6 minutes. Each methanolic extract was

then transferred to another clean microvial and tightly capped for subsequent procedures of HPLC separation with photodiode array and fluorescence detection. The results were recorded by calculating peak ratios.

All these steps were repeated for another Bic red ink and the results were recorded by calculating peak ratios for comparison analysis.

Optimization of the HPLC Separation of Staedtler 430 M, Corvina 81, Bic and Micron Inks

Written lines (3 cm) of each ink sample of Staedtler 430 M, Corvina 81, Bic and Micron were cut into small squares (0.1*0.2 cm) and placed in a microvial. An aliquot of 30 µL LC grade methanol was added into the microvial for extraction for 6 minutes in an ultrasonic bath. The methanolic extract was then transferred into a clean microvial and tightly capped for HPLC separation. Water solution and organic solvents used in the eluent mixture were acetonitrile, tetrahydrofuran and H_2O + 0.005 M Pic B-7, for Staedtler 430 M and Corvina 81 inks. The optimization of the separation of the components in both inks were obtained by varying the eluent composition and by changing the pH values of the aqueous solution from 4 to 8 using either concentrated hydrochloric acid or ammonia water. Water solution and organic solvents used for the separation of Bic red and Micron red inks were acetonitrile, methanol and 0.005 M Pic B-7 water solution. Optimization of separation was obtained by varying the eluent composition and by adjusting the pH values of the aqueous solution from 4 to 8 using either concentrated hydrochloric acid or ammonia water.

High Performance Liquid Chromatographic Separation with Photodiode Array and Fluorescence Detection

An aliquot of 10 µL methanolic extract of each ink sample obtained from the steps as described in sample preparation and extraction was injected onto the HPLC system for separation and photodiode array and fluorescence detection. The HPLC system consisted of a Waters pump, 667 autosampler used to inject the methanolic extract into the system, a C-18 column (150*3.9 mm i.d.) used for separation, a Waters photodiode array detector and a fluorescence detector used for VIS/UV and fluorescence detection respectively. The photodiode array detection used a scanning range from 200 nm to 800 nm. The excitation and emission wavelengths were set according to the excitation and emission maxima of each sample. The mobile phase composition used in the separation was changed to find the optimal eluent for each ink sample. The mobile phase was optimized to an isocratic eluent composition of tetrahydrofuran: acetonitrile : H_2O +0.005 M Pic B-7 (10:70:20 v/v) for ink samples of Staedtler 430 M and Corvina 81, while another isocratic eluent composition of methanol: acetonitrile : H_2O +0.005 M Pic B-7 (35:45:20 v/v) was optimum for Bic and Micron inks. The results of photodiode array and fluorescence detection were recorded using a Waters Millenium software capable of data acquisition, calculation, integration, and calibration of the results.

For each ink sample analyzed by HPLC with both photodiode array and fluorescence detectors, fluorescent components were shown to be present in each chromatogram of each sample. The peak heights were calculated. Their heights were divided by each other to obtain their ratios. For example, for each of the 4 major components, the ratios calculated were peak 1 / peak 2, peak 2 / peak 3…, peak 3/ peak 4.

TLC Development

A TLC plate was prepared (6 * 5 cm) with a pencil baseline drawn 1 cm from the bottom of the plate. Each methanolic extract of ink and dye samples was separately applied onto the plate using capillary tubes. Each spot was 1.5 cm from the other. The plate was developed with a solvent mixture of ethyl acetate: ethanol: H_20 (70:30:20 V/V). When the solvent front reached the point 1 cm from the baseline, the plate was taken out and dried. When the plate was completely dry, it was then returned to the development tank again for another development over a distance of 5 cm. The plate was taken out and dried. The separated color spots of the sample on the TLC plate were measured and their Rf values and visual colors recorded.

High Performance Liquid Chromatographic Separation with Photodiode Array and Fluorescence Detection

Each of the components of the dye and ink samples resolved by TLC and which were colored or fluoresced under UV 366 nm, were scraped off the plate, extracted with methanol in an Eppendorf tube using an ultrasonic bath for 6 minutes. The extracts were all centrifuged

at 1000 g and the clear supernatant transferred to microvials. These extracts were subjected to HPLC separation with photodiode array and fluorescence detection. The components of Staedtler 430 M, and Corvina 81 inks and rhodamine 6g dye were eluted using an isocratic eluent of acetonitrile: tetrahydrofuran: H_2O+0.005 M Pic B-7(70:10:20 v/v) at a flow rate 1.2 mL/min, while the components of Bic and Micron inks and rhodamine B dye were eluted with an isocratic eluent of acetonitrile: methanol:H_2O+0.005 M Pic B-7 (45:35:20 v/v) at a flow rate of 0.8 mL/min. The separation column was a C-18 phase. The column temperature was ambient. Detectors linking after the separation column were first a photodiode array detector with a scanning range from 200 nm to 800 nm, and secondly a fluorescence spectrophotometer with set excitation and emission wavelengths of 350 nm, 550 nm respectively for Staedtler 430 M, Corvina 81 inks and rhodamine 6g dye, and 545 nm and 555 nm for Bic red, Micron red inks and rhodamine B dye. All retention times of the peaks of the components both in their photodiode array and fluorescence spectra were recorded. All spectral absorbance maxima and other minor absorbance of all the peaks of the components were recorded.

Identification of the Rhodamine 6g Dye with a Dye Component of Staedtler 430 M Ink

The Rhodamine 6g dye and the Staedtler 430 M ink were processed through the TLC separation process described above. The Rf values, visual colors, and fluorescences under UV at 254 nm and 366 nm, of the separated components of the rhodamine 6g dye and Staedtler 430 M inks were measured, observed and compared with each other.

Components with equivalent Rf values, visual colors, fluorescences were processed through the photodiode array and fluorescence detection. Their absorption maxima, excitation and emission wavelength maxima and retention data were recorded and compared with each other.

The absorbance maxima and other minor absorbance of each component were also compared with each other. The absorbance values for each separated compound at 520 nm and 530 nm were selected from the photodiode array spectra and their peak heights were measured, recorded and ratioed to each other. The ratios of all components with equivalent Rf values on the TLC plate and their retention times on photodiode array spectra were compared.

Identification of Rhodamine 6g Dye with the Dye Components Contained within Corvina 81 Ink

Dye components in rhodamine 6g dye and in Corvina 81 ink were separated by TLC, scraped off the plate, extracted into methanol and detected by HPLC with photodiode array, fluorescence detection. Results from all analytical stages and detection were recorded and compared.

Identification of Rhodamine B Dye with the Dye Components Contained within Bic Ink

Dye components in rhodamine B dye and Bic ink were separated by TLC, scraped off the plate, extracted into methanol and analysed by HPLC using photodiode array and fluorescence detection. Results from all analytical stages were recorded and compared.

Identification of Rhodamine B Dye with the Dyes in Micron Ink

The components of rhodamine B dye and Micron ink were separated by TLC, scraped off the plate, extracted into methanol, and analyzed by HPLC with photodiode array, fluorescence detection. Results from all analytical stages and detection were recorded, tabulated and compared with each other.

Results and Discussion

Optimization of the HPLC Separation of Staedtler 430 M, Corvina 81, Bic and Micron Inks

The Staedtler 430 M and Corvina 81 Inks

Lyter [10] suggested that the best mobile phase for basic dyes using C-18 as the separation column was a composition of acetonitrile: H_2O in a ratio of 70:30 v/v. The eluent mixture suggested by Lyter was tried for Staedtler 430 M and Corvina 81 inks at a flow rate of 1.2 mL/min. Only two components were eluted within 15 minutes and this was not consistent with the number of components shown as four spots for both inks on the TLC plate using a solvent mixture of ethyl acetate: ethanol: water (70:30:20 v/v) for separation.

By changing the eluent composition to acetonitrile: 5 mM Pic B-7 water solution (80:20 v/v), the two components were eluted faster, but there were still only two components eluting within 15 minutes. The addition of tetrahydrofuran into the eluent composition in the ratio of tetrahydrofuran: acetonitrile: 5 mM Pic B-7 water solution (15:65:20 v/v) reduced the polarity and resulted in four major peaks eluting within 6 minutes. However, the resolutions between pairs of peaks were poor, their values being calculated according to the following equation.

$$R_s = \frac{2\Delta t}{W_{b1}+W_{b2}}$$

Where Rs is resolution, Δt = t2- t1 ; t1 is the retention time of peak 1; Wb1 is the width of the peak base of peak 1. The affect of peak resolution using different eluent composition for the separation of Staedtler 430 M ink is shown in Table 1.

Table 1 Resolutions of peak pairs of Staedtler 430 M ink achieved by varying the eluent composition

Resolution of each two peaks	**Eluent Composition** (Tetrahydrofuran:acetonitrile:5mM Pic B-7 water solution v/v)				
	0:75:25	0:80:20	5:75:20	10:70:20	15:65:20
p1[a] and p2	---	3.96	4.53	3.95	2.72
p2 and p3	---	---	2.93	1.95	1.90
p3 and p4	---	---	1.08	1.34	1.30

[a] means peak 1 on the fluorescence chromatogram, etc.

The eluent with increasing amount of water solution needed more time to elute all dye components in both Staedtler 430 M and Corvina 81 inks. However, the addition of tetrahydrofuran to the eluent composition caused shorter elution time for all components, but at the same time decreased resolution between peak pairs. The eluent composition of tetrahydrofuran: acetonitrile: 5 mM Pic B-7 water solution (10+70+20 v/v) seemed to be optimal for the separation of Staedtler 430 M and Corvina 81 inks.

The precision of the retention times of the peaks of both Staedtler 430 M and Corvina 81 inks using this eluent composition at a flow rate of 1.2 mL/min is shown in Tables 2 and 3. An exemplary fluorescence chromatogram of Staedtler 430 M ink is shown in Fig. 1.

Table 2 Precision of the retention times of the peaks in the fluorescence chromatogram of Staedtler 430 M ink (N=12)

Peak	1 [a]	2	3	4
Retention times in mins	1.217±0.0%	3.887±0.1%	5.789±0.2%	7.031±0.2%

[a] Peak 1 means No.1 peak in the fluorescence chromatogram etc.

Table 3 Precision of the retention times of the peaks in the fluorescence chromatogram of Corvina 81 ink (N=12)

Peak	1 [a]	2	3	4
Retention times in mins	1.217±0.0%	3.861±0.1%	5.781±1.4%	7.013±0.9%

[a] means No.1 peak in the fluorescence chromatogram etc.

The results showed small changes in retention times of peaks that indicated good precision. Their relative deviation ranged from 0.0% to 0.2% for Staedtler 430 M ink and 0.0% to 1.4% for Corvina 81 ink respectively.

The effect of pH values of the aqueous part of the eluent on ratios was studied. The pH values chosen were those that a C-18 column should tolerate. Samples of Staedtler 430 M were processed with eluents in which pH values of the aqueous part of the eluent were prepared as mentioned. The results of peak ratios are shown in Table 4 and those peaks chosen for calculating peak ratios are illustrated in Fig.1.

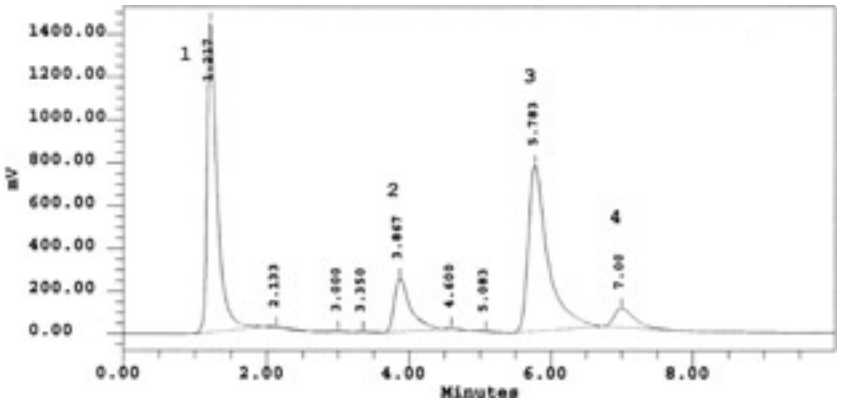

Fig.1 Fluorescence chromatogram of Staedtler 430 M ink

Table 4 Resolutions in the peak pairs of Staedtler 430 M ink by adjusting the pH values of the eluent aqueous portion

pH values in eluent aqueous portion	Resolution		
	Peaks 1[a] to 2	Peaks 2 to 3	Peaks 3 to 4
4.0	4.20	1.87	1.50
4.5	4.19	1.90	1.35
5.5	3.95	1.95	1.20
6.5	3.92	2.50	1.12
7.5	3.90	2.80	1.14
8.0	3.90	2.80	1.14

[a] means No. 1 peak in the fluorescence chromatogram etc.

The pH value of the 5 mM Pic B-7 water solution was 4.8. Eluents of different pH values affected the resolution of each peak pair. The eluent with higher pH values decreased the resolutions of peaks 1 and 2, but increased the resolutions of peaks 2 and 3 while the resolutions of peaks 3 to 4 were slightly improved. The eluent with lower pH values prolonged the elution time. Since pH values did not have a decisive influence on the peak resolution, an optimal pH value 4.8 for the 5 mM Pic B-7 water solution without any adjustment, was chosen for all subsequent work for Staedtler 430 M and Corvina 81 inks. Higher resolution between peaks 1 and 2 allowed for easy observation of changes in relative peak sizes arising from the effect of ink aging.

The effect of different extraction times on peak ratios was studied using the optimal eluent. The results of peak ratios for all samples of different extraction time are shown in Table 5.

Table 5 Peak ratios of the methanolic extracts of Staedtler 430 M ink for different extraction time (N＝10)

Extraction time (mins)	Ratios		
	Peak 1 [a] /peak 2	Peak 2/peak 3	Peak 3/peak 4
1	5.652±3.0%	0.252±2.1%	7.950±0.2%
3	5.812±1.8%	0.257±1.9%	7.958±0.1%
6	5.732±2.0%	0.264±2.2%	7.975±0.6%
9	5.917±4.0%	0.259±3.8%	7.944±0.2%
12	5.483±3.3%	0.290±2.4%	7.980±0.6%

[a] means No.1 peak on the fluorescence chromatogram etc.

The results showed that different extraction times had little effect on the ratios of the fluorescence intensities of each peak pair on the chromatograms. This suggested that all dyes of the ink samples were fully extracted by methanol from the paper. The relative deviations of all peak ratios were considered to be acceptable ranging from 0.1% to 4.0%, peak ratios of 3 to 4 being the smallest from 0.1% to 0.6%. Considering their easy preparation and time recording in extraction, we selected an extraction time in methanol for 6 minutes and employed this extraction time in all subsequent work.

A study as to whether the amount of inked discs removed from the paper might affect the ratio values was undertaken. The results of fluorescence intensities of all samples were obtained according to the steps as described above. The results of peak ratios of all sets for the different numbers of discs are shown in Table 6.

Table 6: Peak ratios of the methanolic extracts of different numbers of discs obtained from Staedtler 430 M ink (N=10)

No. of discs in extraction	Ratios		
	Peak 1 [a] /peak 2	Peak 2/peak 3	Peak 3/peak 4
2	5.428±2.4%	0.288±3.4%	8.049±0.5%
5	5.595±2.6%	0.284±4.2%	8.082±1.9%
10	6.030±1.9%	0.277±1.8%	7.960±0.2%
15	6.341±3.3%	0.270±2.9%	7.932±0.4%
20	6.350±3.7%	0.266±2.6%	7.865±0.1%

[a] means No.1 peak on the fluorescence chromatogram etc.

Ratio comparison among the sets of different numbers of punched discs indicated that the different disc numbers extracted in methanol did not significantly affect the ratios. Ratios of peaks 1 to 2 almost did not change when the number of discs for extraction exceeded 15. The ratios of peaks 1 to 2 and 2 to 3 had relative deviations from 1.8% to 3.7%. The ratios of peaks 3 to 4 were much stable when the number of discs for extraction exceeded 10. The less destruction there is of the ink sample in case work the more acceptable, for this reason 10 discs was considered to be optimal. However, in this work, in order to achieve more stable ratios and because of the easy access to a sufficiency of prepared samples, the number of discs chosen for subsequent work was 15.

The Bic and Micron Inks

When using the same eluent as was used in the separation of the components of the Staedtler 430 M and Corvina 81 inks, all dye components of the Bic and Micron inks were eluted within two minutes, but the resolution of the peaks present in the chromatogram

was poor. Any increase of tetrahydrofuran added to the eluent shortened the elution time for all components. For this reason the solvent tetrahydrofuran was considered unsuitable for the separation of the components of the Bic and Micron inks. In order to improve the selectivity of the eluent to these two inks, methanol was used in place of tetrahydrofuran. The results of separation with eluents of different composition are shown in Table 7. And one exemplary fluorescence chromatogram of Bic red ink is illustrated in Fig. 2.

Table 7 Resolutions of peak pairs present in the fluorescence chromatogram of Bic ink by varying the eluent composition

Rs [a]	**Eluent composition**						
	Methanol:acetonitrile:5 mM Pic B-7 water solution (v/v)						
	0:70:30	0:75:25	0:80:20	10:70:20	15:65:20	25:55:20	35:45:20
p1 [b] /p2	0.76	0.86	1.23	1.23	1.23	1.30	1.50
p2/p3	0.91	0.96	0.97	1.15	1.15	1.22	1.02
p3/p4	0.96	1.03	1.09	1.11	1.11	1.09	1.02
p4/p5	0.69	0.71	0.78	0.76	0.76	0.77	0.98

[a] means resolution.
[b] means peak 1 on the fluorescence chromatogram etc.

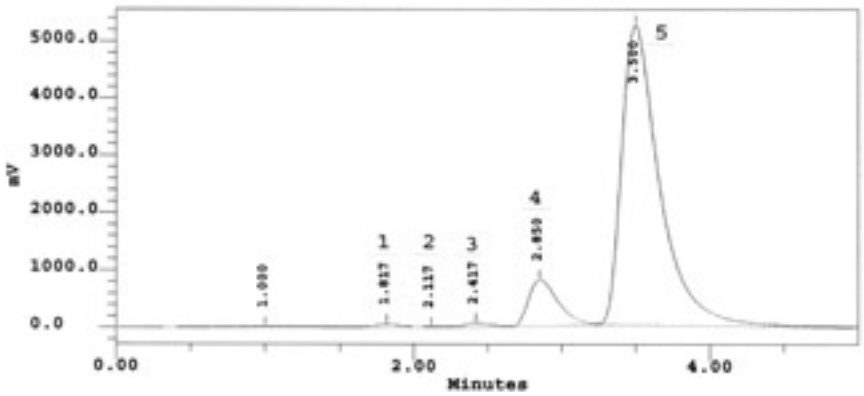

Fig. 2 Fluorescence chromatogram of Bic ink

Since the resolutions of peaks 4 and 5 obtained in Table 7 varied from 0.69 to 0.98, these two peaks were considered to be not well separated. The best separation was obtained using the eluent composition of methanol: acetonitrile: 5 mM Pic B-7 water solution (20:45:35 v/v) with which the resolution of peaks 4 to 5 was 0.98. The effect of pH values on separation was studied to see if this would provide any improvement. The results of the resolutions of peak 4 to 5 are shown in Table 8.

Table 8 Resolutions of peaks 4 to 5 of Bic ink by adjusting the pH values of the eluent aqueous portion (N=8)

pH value in the aqueous part of the eluent	**Resolution**
	Peaks 4 and 5
4.0	0.98
4.5	0.98
5.5	0.86
6.5	0.81
7.5	0.78
8.0	0.72

The eluent with pH values for the aqueous part of the eluting solvent which ranged from 4.0 to 8.0, decreased the resolution of peaks 4 and 5. The resolution of these two peaks could not be further improved when pH values exceeded 4.5. The best separation for peaks 4 and 5 was obtained using the eluent composition of methanol: acetonitrile: 5 mM Pic B-7 water solution (35:45:20 v/v) for which the resolution of peaks 4 and 5 were 0.98. The eluent with a composition of methanol: acetonitrile: 5 mM Pic B-7 water solution (15:65:20 v/v) was considered better for the resolution of peaks 1 and 2, and peaks 2 and 3 and also peaks 3 and 4 with values of 1.23, 1.15 and 1.11 respectively, but under this condition, the resolution of peak 4 and 5 was unacceptable at just 0.76. For these reasons, there was considered to be no need to adjust the pH values for the separation of Bic and Micron inks.

Bic and Micron inks were separated using a C-18 column with an eluent composition of methanol: acetonitrile: 5 mM Pic B-7 water solution (35:45:20 v/v) at a flow rate of 0.8 mL/min. The precision in retention times of all peaks present on the fluorescence chromatograms are shown in Table 9.

Table 9 Precision of the retention times of the peaks on the fluorescence chromatograms of Bic and Micron red inks (N=5)

Ink name	Retention times of peaks				
	1 [a]	2	3	4	5
Bic red	1.889±0.4%	2.119±0.9%	2.475±1.6%	2.892±0.3%	3.557±0.3%
Micron red	1.906±0.6%	2.119±0.5%	2.477±0.6%	2.886±0.5%	3.540±0.5%

[a] means peak 1 in the fluorescence chromatogram etc.

The results demonstrated that the precision of each peak present in the chromatograms were good in that the relative deviations for Bic ink varied from 0.3% to 1.6%, while those for Micron ink were 0.5% to 0.6%. When the concentration of the methanolic extracts of Bic and Micron inks was low, the fluorescence signals of peaks 1 and 2 on the chromatograms were too small to be measurable. These two peaks were therefore not considered appropriate to be used for comparison purposes. The effect of different numbers of discs on peak ratios was studied and the results are shown in Table 10.

Table 10 Peak ratios of the methanolic extracts of different numbers of discs obtained from Bic ink (N=5)

No. of discs in extraction	Ratio
	Peak 4 [a] /peak 5
5	0.054±3.2%
10	0.053±2.1%
15	0.051±0.5%
20	0.049±1.8%

[a] means the No. 4 peak in the chromatogram etc.

The methanolic extracts of 5 discs had the highest relative deviation of 3.2%. Fifteen discs had the lowest relative deviation of 0.5%. Either 10 or 15 discs would be considered suitable for extraction.

The effect of different extraction times on peak ratios was also studied and the results of peak ratios of peaks 4 to 5 for all samples of different extraction time are shown in Table 11.

Table 11 Peak ratios of the methanolic extracts of Bic ink subjected to different extraction times

Extraction time (mins)	Ratio
	Peak 4 [a] /peak 5
1	0.053±0.2%
3	0.054±2.7%
6	0.052±2.4%
9	0.051±1.9%
12	0.050±4.0%

[a] means the No. 4 peak in the chromatogram etc.

The results showed that different extraction time did not significantly affect the peak ratios. The relative deviations of all peak ratios were considered to be acceptable ranging from 0.2% to 4.0%. Ratios of extraction time of one minute had the lowest relative deviation of 0.2% in comparison with those ratios of 3, 6, 9 and 12 minutes which had relative deviations ranging from 1.9% to 4.0%. However, extraction time for one minute was relatively difficult to record, therefore, an extraction time in methanol of 3 or 6 minutes was decided to be appropriate to be employed in the separation work for Bic red and Micron red inks.

In order to obtain stable results, the instrument used required well prepared and "warmed-up." Prior to each HPLC analysis, the HPLC system needed to be stabilized to complete the instrument warm-up steps. These latter include degassing the solvent mixture with helium gas at a flow rate of 50 ml/min for half an hour and using the degassing solvent to equilibrate the analytical column at a flow rate of 0.8 ml/min for an hour before use. After all warm-up preparations, the column maintained a pressure of between 900 psi and 1000 psi.

In conclusion for the HPLC separation and analysis of the Staedtler 430 M, Corvina 81, Bic red and Micron red inks, the eluent composition of tetrahydrofuran: acetonitrile: 5 mM Pic B-7 water solution (70:10:20 v/v) was considered to be optimal for the Staedtler 430 M and Corvina 81 inks, while the composition of methanol: acetonitrile: 5 mM Pic B-7 water solution (35:45:20 v/v) was optimal for the Bic red and Micron red inks. No single eluent composition was found to be suitable for these four inks. The precision of peaks appearing on the chromatograms was acceptable with their relative deviations ranging from 0.0% to 1.6%. The peak ratio calculation method proved to be mass independent, since the methanolic extracts of different numbers of discs did not affect the peak ratios of the two groups of inks. This seemed to demonstrate that after 6 minutes extraction in methanol, all ink dye components recovered from Chapman carbonless paper were fully extracted or at least, the extraction rate of each ink dye component on paper was nearly same. The methanolic extract of 5 discs did not have much difference in peak ratio values as those of the methanolic extracts of 10, 15 and 20 discs. However, its relative deviations were higher than all other ratios. This was true for both groups of inks. From this, it was concluded that 10 discs punched out of the paper for extraction would be the minimal numbers for HPLC analysis. Extraction time of 1, 3 and 9 minutes in methanol had relatively high deviations for the ratios of peaks 1 to 2 of Staedtler 430 M ink, but this was not so for peak pairs 2 and 3, and 3 and 4. For the extraction time of 6 minutes, the relative deviations of all ratios varied from between 0.6% to 2.2% for Staedtler 430 M ink, while Bic red ink had a relatively smaller deviation of 2.4%. Because of their relatively smaller percent deviations for all peak ratios, an extraction time of 6 minutes in methanol was considered to be the appropriate extraction time for all inks employed in this work. The pH values of the aqueous part of the eluent had an effect on resolutions of peak pairs. Higher pH values increased the resolutions of one peak ratio, but at the same time decreased another. Two peaks on the chromatogram of Bic red were not well separated; moreover, lower pH values of the aqueous part of the eluent could still not separate them. An optimal pH value for the 5 mM Pic B-7 water solution of 4.8 was arrived at. This pH value was used in all subsequent work.

HPLC Separation with Photodiode Array Detection of Staedtler 430 M, Corvina 81, Bic red and Micron Red Inks and Rhodamine 6G and Rhodamine B Dyes

All the ink samples and dyes were processed through HPLC separation with photodiode array detection

according to the procedures described. Results of the photodiode array detection were employed to compare the retention times and photodiode array spectral absorbance of each HPLC separated component of all the ink and dye samples. Results of the photodiode array scanning for each HPLC separated component present in all ink and dye samples which ranged from 200 nm to 800 nm were stored in the database of the Waters Millenium software package. Two kinds of data were extracted from the database for comparisons. One datum was the absorption maxima from scanned photodiode array absorption spectra ranging from 200 nm to 800 nm for each component present in an ink or dye. These absorbance maxima and other maxima of absorbance minors arising from those HPLC separated components of the inks or dyes that showed equivalent retention times in the PDA chromatograms were compared. The other datum referred to as absorbance ratio, required that the absorbance ratios were determined from spectral data of PDA absorption of eluting components. By selecting a wavelength of 530 nm as a reference wavelength, absorbance of each component were devided by the absorbance at the reference wavelength.

The results of Rf values of all TLC separated components of ink and dye samples on TLC plate, retention times in PDA chromatograms, absorbance maxima and other maxima of absorbance minors ranging from 200 nm to 800 nm for all HPLC separated components of ink and dye samples are shown in Table 12. Two typical PDA chromatograms of Staedtler 430 M ink and rhodamine 6g dye are illustrated in Figs. 3 and 4.

Table 12 Absorbance maxima and other maxima of absorbance minors for all resolved components of ink and dye samples analysed by TLC and HPLC with photodiode array detection

Ink name	Spot No [a].	Rf values [b]	Retention time in mins[c]	(max) in nm [d]	(minor) in nm [e]
Staedtler 430 M	1	0.57±1.7%	1.176±0.2%	520.7	243,295,348,394,593
	2	0.62±3.2%	3.826±0.3%	530.4	248,423,467,579,686
	3	0.66±1.5%	5.733±0.2%	530.4	248,276,348,593
	4	0.73±1.3%	6.970±0.2%	530.4	243,348,467,686
Corvina 81	1	0.57±1.7%	1.169±0.5%	520.7	243,295,348,394,593
	2	0.63±3.1%	3.816±0.3%	530.4	248,423,467,579,686
	3	0.68±2.9%	5.698±0.6%	530.4	248,276,348,593
	4	0.74±1.3%	6.891±0.7%	530.4	243,348,467,686
Rhodamine 6g	1	0.57±1.7%	1.171±0.3%	520.7	243,295,348,394,593
	2	0.63±3.1%	3.850±0.7%	530.4	248,423,467,579,686
	3	0.67±1.4%	5.550±0.1%	530.4	248,276,348,593
Bic red	1	0.74±1.3%	2.918±0.2%	530.4	348,399,423
	2	0.77±1.2%	3.648±0.3%	549.9	257,305,352
Micron red	1	0.74±1.3%	2.883±0.1%	530.4	348,399,423
	2	0.77±1.2%	3.586±0.2%	549.9	257,305,352
Rhodamine B	1	0.74±1.3%	2.853±0.2%	530.4	348,399,423
	2	0.77±1.2%	3.587±0.3%	549.9	257,305,352

[a] and [b] mean the number and the Rf values of the separated spots on the TLC plate.
[c] means the retention time of each spot eluted by HPLC on the pda chromatograms.
[d] means the maxima of pda absorbance.
[e] means maxima of other minor pda absorbances.

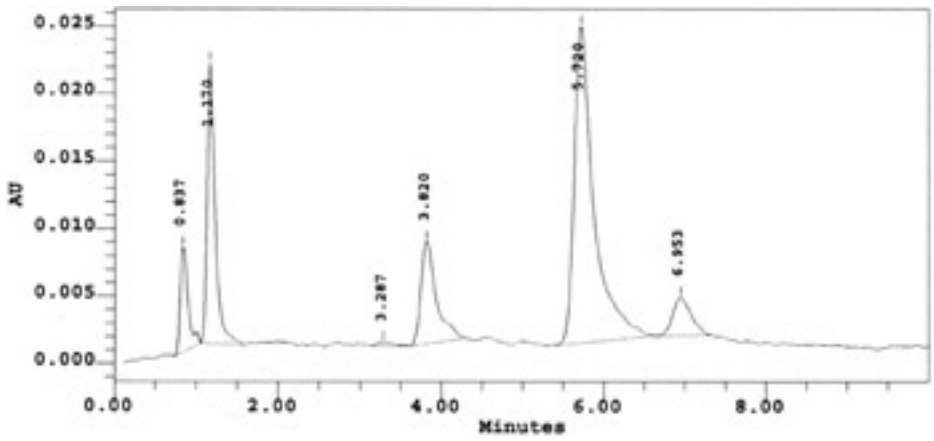

Fig. 3 Photodiode array chromatogram of Staedtler 430 M ink at 530 nm

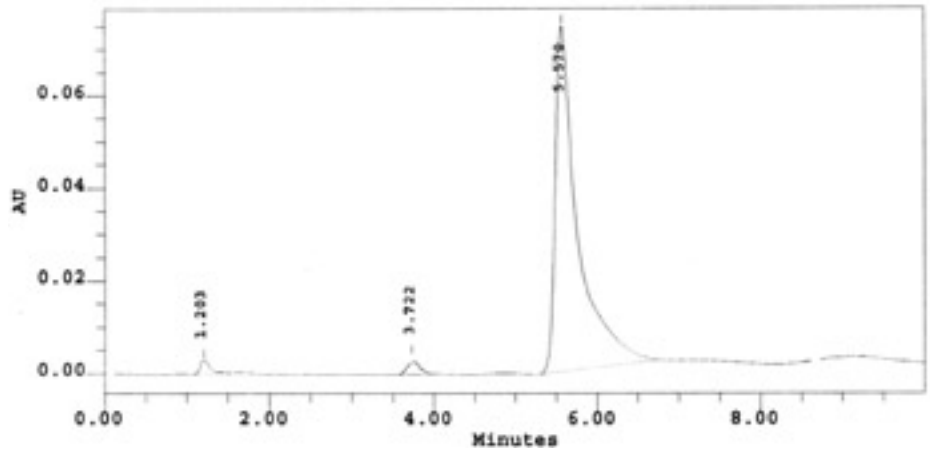

Fig.4 PDA chromatogram of Rhodamine 6g dye at 530 nm

By TLC, four spots for both Staedtler 430 M and Corvina 81 inks and three spots for rhodamine 6g dye were present on the TLC plate after separation. All the separated spots on the TLC plate were scraped off the plate and extracted into methanol. The methanolic extracts were all separately injected into the HPLC system for analysis. Each methanolic extract gave clear and large sharp peaks on the PDA chormatogram, but there were some small peaks appearing on the chromatograms for some methanolic extracts. The average PDA spectral absorbance of these small peaks were about 2% of those of the large sharp peaks representing the main components. This indicated that the TLC separation resolved most of the components into different spots.

The methanolic extract of No.1 spot of Staedtler 430 M ink on the TLC plate gave a clear and sharp peak at a retention time of 1.176±0.2% min, but the extract of No.4 spot on the TLC plate had one sharp peak representing the major component and four small peaks representing other components with a small PDA absorbance. This result was consistent with both Corvina 81 ink and rhodamine 6g dye. No.1 spot with the smaller Rf value seemed to be well resolved, but No.4 spot with the larger Rf value had some small traces from former spots eluting with the eluent. This seems to indicate that the longer the solvent mixture elutes on the TLC plate, the weaker the ability of separation becomes. These small peaks in the HPLC PDA chromatogramsfor some methanolic extracts. The average PDA spectral absorbance of these small peaks were about 2% of those of the large sharp peaks representing the main components. This indicated that the TLC separation resolved most of the components into different spots.

The methanolic extract of No.1 spot of Staedtler 430 M ink on the TLC plate gave a clear and sharp peak at a

retention time of 1.176±0.2% min, but the extract of No.4 spot on the TLC plate had one sharp peak representing the major component and four small peaks representing other components with a small PDA absorbance. This result was consistent with both Corvina 81 ink and rhodamine 6g dye. No.1 spot with the smaller Rf value seemed to be well resolved, but No.4 spot with the larger Rf value had some small traces from former spots eluting with the eluent. This seems to indicate that the longer the solvent mixture elutes on the TLC plate, the weaker the ability of separation becomes. These small peaks in the HPLC PDA chromatograms, representing other components, seem not to interfere with the major peaks on the PDA chromatograms because their absorbance were small. This interference of the small peaks on HPLC PDA chromatograms representing other components could be eliminated only by scraping off the TLC layer of the centre of each spot.

No.1, No. 2 and No.3 spots of Staedtler 430 M and Corvina 81 inks had equivalent Rf values with No.1, No.2 and No.3 spots of rhodamine 6g dye on the TLC plate. No.1 spots of Staedtler 430 M and Corvina 81 inks and rhodamine 6g dye were all eluted in HPLC at about 1.176 min on the PDA chromatograms. One exemplary PDA chromatogram of Staedtler 430 M ink is illustrated in Fig. 5.

No. 2 and No. 3 spots of Staedtler 430 M, Corvina, rhodamine 6g on the TLC plates were eluted in HPLC at 3.826±0.3% min and 5.733±0.3% min, 3.816±0.3% min and 5.698±0.6% min, and 3.850±0.7% min and 5.550± 0.1% min respectively on the PDA chromatograms. An exemplary PDA chromatogram of the No.3 spot of Staedtler 430 M ink recovered from the TLC plate was illustrated in Fig. 6. No. 4 spots of Staedtler 430 M, Corvina 81 on the TLC plates were eluted in HPLC at 6.972±0.2% min and 6.891±0.7% min respectively.

The results of ratios of the PDA absorbance at 530 nm of all these three peaks representing No.1, No.2 and No.3 spots on the TLC plates present in Staedtler 430 M and Corvina 81 inks and rhodamine 6g dye obtaining by comparing the absorbance of the peak eluting at 5.723±0.2% min as the reference PDA absorbance are reported in Table 13.

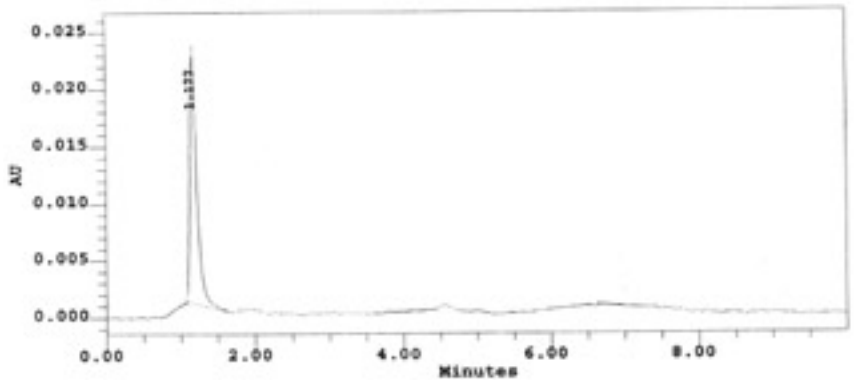

Fig. 5 HPLC PDA chromatogram of No.1 spot of Staedtler 430 M ink recovered from the TLC plate and eluting at 1.176±0.2% min

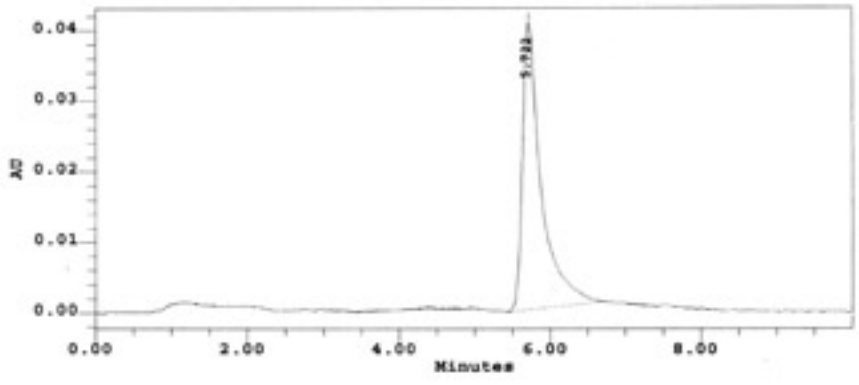

Fig. 6 HPLC PDA chromatogram of the No.3 spot of Staedtler 430 Mink recovered from the TLC plate.

Table 13 PDA absorbance ratios of three selected peaks present in Staedtler 430 M and Corvina 81 inks and Rhodamine 6g dye

Ink and dye name	Ratio = peak intensity x/peak intensity y	
	p1 [a]/p2 [b]	p2/p3 [c]
Staedtler 430 M	6.323	0.275
Corvina 81	4.936	0.404
Rhodamine 6g	28.13	0.0013

[a] means the peak eluting at 1.176 min.
[b] the peak eluting at 5.733 min.
[c] the peak eluting at 6.972 min.

Rhodamine 6g dye showed 3 peaks having retention times of 1.171±0.3% min, 3.850±0.7% min and 5.550 ±0.1% min in the PDA chromatograms. Both Staedtler 430 M and Corvina 81 have three peaks originating from No.1, No.2 and No.3 spots on the TLC plate. The retention times of these three peaks for both inks are the same. Moreover, these three peaks also have the same retention times of peaks obtained for rhodamine 6g dye. But from the results on Table 13, these peaks clearly indicate that the concentration of each component present in respective inks and dye is different. One explanation for this phenomenon is that rhodamine 6g dye used as a standard reagent in this work and the rhodamine 6g dye added in the ink pastes of Staedtler 430 M and Corvina 81 inks, might come from different sources where the concentrations of each component of rhodamine 6g dye are different. There might be another possibility for this phenomenon in that the amount of each component showing a peak intensity in the PDA chromatogram was extracted from different papers at different extraction rates owing to the complexities of other additives, such as the ink vehicles, viscosity adjusters in ink pastes and paper composition, the concentration, shown as peak intensity, may therefore be different. However, from the results of the effects of different extraction times on the appearance of the separated dye mixtures in the inks and dyes, longer extraction times did not significantly affect the peak ratios and seemed to indicate that all dyes present on the paper could all be extracted out from the paper after six minutes extraction in methanol. The first explanation might, therefore, be the more likely one.

Bic ink had two separated spots on the TLC plate. The retention times of these methanolic extracts of these two spots on the PDA chromatograms were 2.918±0.2% min for No. 1 spot and 3.648±0.3% min for No. 2 spot. Micron ink had two spots on the TLC plate fluorescing under UV irradiation at 366 nm. The retention times of the methanolic extracts of these two spots in the PDA chromatograms were 2.883±0.1% min for No. 1 spot and 3.586±0.2% min for No. 2 spot. The retention times of these spots for both Bic and Micron inks were considered to be the same. The dye rhodamine B had two spots too on the TLC plate. The retention times of the methanolic extracts of the two spots in the PDA chromatogram were also the same as those in Bic ink as reported in Table 12.

The components of all ink and dye samples were shown as separated spots by TLC separation on the TLC plate. Each component was scraped off the TLC plate, extracted in methanol and the methanolic extract was injected into HPLC for photodiode array analyses. Photodiode array detection, of which the scanning ranged from 200 nm to 800 nm, following the HPLC separation was employed to each eluting component. Photodiode array detection can provide an absorption spectrum ranging from 200 nm to 800 nm for each HPLC eluting component, but also an absorption spectrum at selected wavelengths within 200 nm and 800 nm for all HPLC eluting components in a run.

Another photodiode array investigation was also undertaken to explore the absorbance maxima and maxima of other absorbance in the photodiode array absorption spectra of each of all HPLC separated components representing the No. 1, No.2 and No.3 spots of rhodamine 6g dye and No.1, No. 2, No. 3 and No.4 spots of Staedtler 430 M and Corvina 81 inks on the TLC plate. The photodiode array absorbance maxima of the No.1 spots of Staedtler 430 M and Corvina 81 inks and No. 1 spot of rhodamine 6g dye were the same as reported in Table 12. However, variations of maxima of other absorbance were found to occur. For example, the maxima of other absorbance for the No.1 spots of the two inks and the dye were 243, 295, 394,

593 nm for Staedtler 430 M ink, 243, 295, 348 and 593 nm for Corvina 81 ink and 243, 295, 348, 394 and 593 nm for rhodamine 6g dye. These differences could be eliminated by injecting more volume of 15 µl instead of 10 µl of methanolic extracts of the No. 1 spots on the TLC plate for Staedtler 430 M and Corvina 81 inks and rhodamine 6g dye into HPLC for separation and the photodiode array detection. After this, the maxima of other absorbance became the same for Staedtler 430 M and Corvina 81 inks and rhodamine 6g dye. The photodiode array absorption spectrum obtained after the HPLC separation for the No. 1 spot of rhodamine 6g dye on the TLC plate is illustrated in Fig. 7.

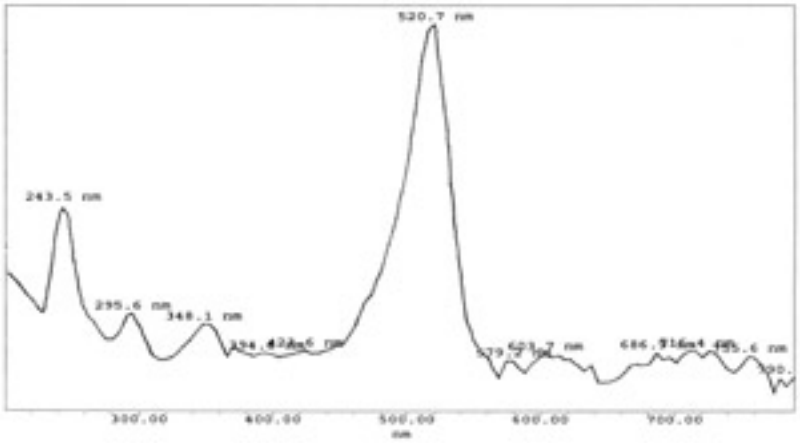

Fig. 7 Photodiode array absorption spectrum for the No.1 spot of Rhodamine 6g on the TLC plate

The photodiode array absorbance maxima and maxima of other absorbance for the methanolic extracts of No.2 spot of Staedtler 430 M were 530.4 nm and 248, 423, 467, 579 and 686 nm, for the No. 3 spot 530.4nm and 248, 276, 348 and 593 nm and for the No. 4 spot 530.4 nm and 243, 348, 467, 579 and 686 nm respectively. No. 2, 3 and 4 spots of Corvina 81 ink and No. 2 and No. 3 spots of rhodamine 6g showed similar results as reported in Table 12. From photodiode array examination, the No.1, No. 2, No. 3 and No. 4 spots of Staedtler 430 M and Corvina 81 inks and the No.1, No.2 and No. 3 spots of rhodamine 6g were considered to be the same.

Bic ink had two separated spots on the TLC plate. The absorbance maxima and maxima of other absorbance for the two spots over the wavelength range 200 nm and 800 nm in photodiode array detection were 530.4 nm and 348.1, 399.5, 423.6 nm for No.1 spot and 549.9 nm and 257.7, 305.1 and 352.9 nm for No. 2 spot respectively.

Micron ink had two spots on the TLC plate fluorescing under UV irradiation at 366 nm. The absorbance maxima and maxima of other absorbance for the two spots were also the same as those in Bic ink as reported in Table 12. The rhodamine B dye also demonstrated two spots on the TLC plate. The photodiode array absorbance maxima for the major and other smaller absorbance for the two spots were the same as those found for Bic ink as reported in Table 12.

The absorbance ratio calculation method for identification was also employed. Two selected wavelengths from the photodiode array spectra were selected and comparisons made of the ratios for the ink samples and dyes. Wavelengths 520 nm and 530 nm were selected for comparing the same ratio sets for Staedtler 430 M, Corvina 81 inks and rhodamine 6g dye, and another two wavelengths, 530 nm and 550 nm, for Bic red, Micron red inks and rhodamine B dye. The results obtained for comparison are shown at Table 14 and Table 15.

Table 14 Peak ratios of the absorbance at 520 nm to 530 nm of various inks and dye (N = 10)

Ink and dye name	Ratio = 520/530			
	Peak 1[a]	Peak 2[b]	Peak 3[c]	Peak 4[d]
Staedtler 430 M	1.515±0.5%	0.874±0.5%	0.866±0.2%	0.822±2.7%
Corvina 81	1.510±0.5%	0.871±1.0%	0.863±0.9%	0.815±2.2%
Rhodamine 6g	1.520±0.6%	------[e]	0.862±2.0%	------

[a] Retention time = 1.176 min.
[b] Retention time = 3.826 min.
[c] Retention time = 6.450 min.
[d] Retention time = 7.533 min.
[e] The absorbance at 520 nm was too small to be calculated.

Table 15 Peak ratios of the absorbance at 530 nm to 550 nm of various inks and dye (N = 5)

Ink and dye name	Ratio = 530/550	
	Peak 1[a]	Peak 2[b]
Bic red	0.727±2.0%	0.691±0.4%
Micron red	0.722±4.1%	0.689±0.4%
Rhodamine B	0.717±1.0%	0.693±0.8%

[a] Retention time = 2.918 min.
[b] Retention time = 3.648 min.

Peak 1 in the first part of the Table 14 represents the No. 1 spots of Staedtler 430 M, Corvina 81 inks and rhodamine 6g dye on the TLC plate. And peaks 2, 3 and 4 represent the No. 2, No. 3 and No. 4 spots of Staedtler 430 M and Corvina 81 inks and No.2 and No. 3 spot of rhodamine 6g dye respectively. The ratios for spots No. 1 were 1.515±0.5%, 1.510±0.5% and 1.520±0.6% for peaks 1 on the PDA chromatograms respectively. The ratios for spots No.2 were 0.874±0.5%, and 0.874±1.0% for peaks 2 on the PDA chromatograms. The ratios for spots No.3 and No.4 were 0.877±0.5%, 0.878±1.0%, 0.878±1.1% for peaks 3 and 0.866±0.2%, 0.863±0.9% and 0.862±2.0% for peaks 4 on PDA chromatograms respectively. All these values in comparisons were considered to be identical to each other using a statistical T test. The results were reported in Table 16.

Rhodamine B, Bic red and Micron red inks all had two spots fluorescing under UV radiation at 366 nm on the TLC plate. The absorption ratios for No. 1 spots of the dye and the inks on the TLC plate were 0.727±2.0%, 0.722±4.1% and 0.717±1.0%. The ratios for the second spots were 0.691±0.4%, 0.689±0.4% and 0.693±0.8% respectively. All these ratios were considered to be identical using a T test. The results are reported in Table 16.

Table 16 Results of a T test undertaken on the absorption ratios of respective peaks of various inks and dye

Comparison	Peak 1	Peak 2	Peak 3	Peak 4
S[a] to C[b]	0.823[g]	0.168	0.125	0.217
S to 6g[c]	0.088		0.232	
6g to C	0.389		0.254	
B[d] to M[e]	0.371	0.146		
B to Rb[f]	0.085	0.245		
M to Rb	0.359	0.083		

[a] is Staedtler 430 M ink.
[b] is Corvina 81 ink.
[c] is rhodamine 6g.
[d] is Bic red ink.
[e] is Micron red ink.
[f] is rhodamine B dye.
[g] is p value.

All 14 p values representing each respective peak on the PDA chromatograms of Staedtler 430 M, Corvina 81 inks and rhodamine 6g dye, and Bic, Micron inks and rhodamine B dye are considered to be identical. This demonstrated that all components shown as respective peaks with corresponding peak numbers on the PDA chromatograms of Staedtler 430 M, Corvina 81 inks and rhodamine 6g dye are the same, and Bic, Micron inks and rhodamien B are also the same.

Based on the comparative examination of the four inks and two dyes, it is clear that rhodamine 6g dye is present in the red inks of Staedtler 430 M and Corvina 81 and rhodamine B in the red inks of Bic and Micron. The results of this study have demonstrated that HPLC coupled with fluorescence, photodiode array and VIS/UV detectors is a useful and reliable analytical scheme for the evidential characterization and identification of ballpoint pen inks and dyes.

Reference

1. Brunelle RL, and Pro MJA. Systematic approach to ink identification. J of the Association of Official Analytical Chemists. 1972;55:823-826.
2. Kelly J and Cantu AA. Proposed standard methods for ink identification. J of the Association of Official Analytical Chemist.1975;58:122-125.
3. Sheng LS, Covey JE, Shew SL, Winger BE and Compana JE. Matrix-assisted laser desorption ionization fourier transform spectrometry. Mass Spectrometry. 1994;8;498.
4. Starchura S and Allison J. Identification of organic pigments in automotive coatings using laser desorption mass spectrometry. J Forens Sci. 2007;52(3):595-603.
5. Bommarito CR and Szymanski D. Analysis of forensic soil samples via high-performance liquid chromatography and ion chromatography. J Forens Sci 2007;52(1):24-30.
6. Brazeau L and Gaudreau M. Ballpoint pen inks: the quantitative analysis of ink solvents on paper by solid-phase microextration. J Forens Sci 2007;52(1):209-215.
7. Weyermann C, Marquis R and Spengler B. Differentiation of blue ballpoint pen inks by laser desoption ionization mass spectrometry and high-performance thin layer chromatography. J Forens Sci 2007;51(4):216-220.
8. Jones R, Cody R and McCleland J. Differentiating writing inks using direct analysis in real time mass spectrometry. J Forens Sci 2006;51(4):915-918.
9. Xu Y, Wang J and Yao L. Dating the writing age of black roller and gels inks by gas chromatophy and UV-VIS spectrometer. Forens Sci Int 2006;162(1-3):140-143.
10. Lyter AH. Analysis of writing inks by high pressure liquid chromatography. Marcel Dekker. New York. 1983;399.