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Comparison of Dyes from Transferred Fibers by Scanning Densitometry

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ABSTRACT: The application of scanning densitometry to the analysis of dyes extracted from transferred fibers is examined. The Camag high-performance thin-layer chromatography (HPTLC)/thin-layer chromatography (TLC) scanner can provide all of the information normally available from visual examination of the TLC plates. The position, color, and relative proportions of the various components are obtained in a semiquantitative form. The instrument can provide useful data in both the visible and fluorescence modes with greater sensitivity than is available from visual observations. The potential of the instrument for examination of dyes from pale-colored fibers is demonstrated.

The data obtained are objective and do not suffer from the variations that can occur when using subjective visual observations.

KEYWORDS: criminalistics, dyes, fibers

Thin-layer chromatography (TLC) has been applied to the separation of dyes extracted from textiles for many years [*1*]. It has been used more recently to compare dyes extracted from fiber monofilaments that may have been transferred during a criminal contact.[3,4]

When interpreting a TLC plate, the analyst should take into account the color, retention factor (Rf), and relative proportions of various components. All of these properties can assist the analyst in assessing the similarity or lack of similarity of the dye mix from the transferred fibers to the dye mix from fibers from the suspected source garment.

Microspectrophotometry has been suggested for objective determination of the colors of dyes on fibers prior to extraction [*2*][5,6] and has also been used for comparison of the colors of spots on TLC plates [*3*]. The determination of the Rf is a simple matter of

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determining the ratio of the distance traveled by the dye spot to the distance traveled by the solvent front. However, experience suggests that, in practice, determination of the relative proportion of the various dye components is usually carried out by visual observation. The application of instrumental techniques to the interpretation of the relative proportion of textile dye components on TLC plates has not been discussed in the forensic literature surveyed by the authors. Peak ratioing techniques have, however, been applied to the interpretation of high-performance liquid chromatography (HPLC) information [*4*].

A scanning densitometer can be used to produce a graphical representation of the spots on a TLC plate in the form of peaks of a certain height. The peak height ratios for the various components can then be determined. By calculating these ratios for the visible, ultraviolet, and fluorescence modes, it may be possible to increase and improve the comparative data obtained from the interpretation of a TLC plate, beyond those normally available by visual examination.

This paper is concerned with the results of studies which investigated the following:

(*a*) the possibility of using scanning densitometry to obtain dye ratios and hence of attempting to improve the comparative data obtained by utilizing a semiquantitative technique, and
(*b*) the potential of scanning densitometry for the detection of pale-colored dyes, such as yellow ones [*5*].

To facilitate these aims, a series of investigations was performed on bulk dye extracts and on dyes from monofilaments extracted in capillary tubes.

Experimental Procedure

The fibers used in these investigations were obtained from used clothing and from manufacturer's pattern cards supplied by Hoescht Pty Ltd. (Australia). The extractions of dyes from single monofilaments and small groups of fibers were carried out in sealed Micro-Hematocrit tubes (Clay Adams, a division of Becton, Dickson, and Co., USA). These capillary tubes were 75 mm in length, 1.5 mm in outside diameter, and 1.10 mm in inside diameter.

A Carl Zeiss stereomicroscope was used to facilitate the manipulation of short lengths of monofilament. In Cases 1, 2, and 4 (below), aqueous pyridine was used as the extraction solvent. In all other cases chlorobenzene was used.

The following thin-layer plates were used during the various experiments:

(*a*) Merck DC-Plastikfolien Kieselgel 60 F254, 20 by 20 cm, 0.2-mm layer;
(*b*) Merck DC-Plastikfolien Kieselgel 60, 20 by 20 cm, 0.2-mm layer (without fluorescent indicator); and
(*c*) Merck HPTLC Fertigplatten Kieselgel 60, 10 by 10 cm (without fluorescent indicator).

Unless otherwise stated, the chromatograms were developed once in a Camag TLC tank by normal ascending chromatography employing chloroform as the mobile phase. The high-performance thin-layer chromatography (HPTLC) plates in Cases 5 and 6 were developed in a Camag linear TLC tank.

The instrument used was a Camag TLC/HPTLC scanner, Model No. 76510. It was equipped with a tungsten light source for analysis in the visible range, a deuterium light source for analysis in the ultraviolet range, and a mercury vapor lamp for analysis using fluoresence techniques.

In this instrument, light from a source passes through a monochromator, and an image of a slit of 10 or 30-nm spectral bandwidth is formed on the TLC plate. The TLC plate

is mounted on a moving stage. During an analysis, the stage moves past the slit image, and the scattered radiation from the plate is detected by a photomultiplier. The signal from the photomultiplier is fed to a chart recorder, where peaks are formed which correspond to spots on the plate.

The TLC plates were prepared as described in the following sections, developed in chloroform, and analyzed using the Camag densitometer. The following instrumental conditions were used for Cases 1 through 6:

Sensitivity, 9
Scan speed, 1 mm/s
Detection wavelength, 525 nm
Spectral bandwidth, 10 nm
Slit, 0.3 mm

As discussed later in the paper, in Cases 7 and 8 the detection wavelength was varied to increase sensitivity.

Ratio Determinations on Similar Aliquots (Case 1)

This plate was prepared using aliquots of a dilute dye extract. Thirty identical volumes of dye mix were placed onto the plate using a Camag Microapplicator.

Ratio Determinations for Different Aliquots (Case 2)

A plate was prepared in a manner similar to that used for Case 1. Different volumes of dye mix were placed on the plate using an adjustable micropipette. The volumes ranged from 0.5 to 2.5 μL.

Relative Ratios of Dye Components from Monofilaments from Unused Cloth (Case 3)

Eleven polyester monofilaments dyed with Hoescht Samaron Red SL were placed in capillary tubes with 4 μL of chlorobenzene. The tubes were sealed and placed in an oven at 110°C for approximately 10 min. The dye extracts were then spotted onto a TLC plate. The chromatogram consisted of a red and a yellow spot.

Relative Ratios of Dye Components from Monofilaments from Used Clothing (Case 4)

Ten monofilaments, each 30 mm in length, taken from a pair of brown polyester trousers, were extracted with aqueous pyridine in separate sealed capillary tubes in an oven at 100°C. The dye solutions were then spotted onto a TLC plate.

Relative Ratios of Components Separated by HPTLC (Case 5)

Twenty 100-nL and twenty 200-nL aliquots of a dye solution, prepared by extracting a number of fibers from the brown polyester trousers used in Case 4 in 200 μL of chlorobenzene, were placed onto a Merck high-performance thin-layer chromatography (HPTLC) plate. The plate containing the dye mix was then developed in a Camag linear TLC tank, employing chloroform as the solvent system.

Relative Ratios of Dye Components Extracted from Monofilaments and Separated by HPTLC (Case 6)

Ten 40-mm lengths of polyester monofilaments dyed with Hoescht Samaron Red SL were extracted with chlorobenzene in capillary tubes. The extracts were then spotted onto a Merck HPTLC plate, which was then developed in a Camag linear TLC tank, employing chloroform as the solvent system.

Relative Ratios of Fluorescent Components (Case 7)

In order to produce a dye mix containing three fluorescent components, three different red polyester fibers were obtained from a Hoescht Samaron disperse dye pattern card. The fluorescent properties of each of these dyed fibers had previously been determined by viewing the pattern card under an ultraviolet (UV) light source.

These fibers were placed in the same vial, and the dyes were extracted with 200 μL of chlorobenzene. Fourteen 2-μL aliquots of this dye mix were placed onto a TLC plate using a Camag Microapplicator, and the plate was developed in chloroform. The plate was then placed into the scanning densitometer, and the spots were scanned using excitation radiation from the mercury vapor lamp. The 366 and 254-nm lines were selected for excitation. In order to prevent this excitation radiation from entering the photomultiplier, an ultraviolet cutoff filter was used.

After the completion of the fluorescence scans, the plate was scanned in the visible absorption mode using a wavelength of 550 nm.

The relative standard deviation (RSD%) of the peak height ratios for each combination of the three dye components was then calculated for analysis in the fluorescence and visible absorption modes.

An attempt was made to analyze the plate in the ultraviolet absorbance mode using a range of detection wavelengths, but the dyes did not absorb sufficiently in this region to produce satisfactory peaks on the chart recorder.

Detection of Yellow Dyes (Case 8)

Fibers dyed with Color Index (CI) Disperse Yellow 114, and CI Disperse Yellow 54 were obtained from pattern cards. The dye was extracted from the fibers using chlorobenzene (0.2 mL).

Aliquots of this extract were placed onto a plastic-backed Merck Keiselgel 60 F254 DC-Plastikfolien thin-layer plate. The volumes of dye used ranged from 2 to 0.025 μL. These volumes were dispensed from Camag Microapplicator and Camag Nanoapplicator adjustable pipettes.

The plate was developed in chloroform, through a distance of 5 cm. The visual detection limit was determined using both reflected and transmitted light from a fluorescent desk lamp.

The developed plate was then attached to the sample tray of the Camag scanning densitiometer. The wavelength that produced the maximum sensitivity was determined by repeated scan of a spot of each dye over a range of wavelengths. The instrumental conditions were the same as those described above, except that a detection wavelength of 440 nm was chosen to optimize sensitivity. The chart recorder range was varied to produced a noise level 3 to 5 mm in height. The spots were then scanned sequentially across the plate.

Results and Discussion

In each of the cases, the standard deviation (SD), the mean value for n measurements, and the relative standard deviation (RSD%) of the ratio of two dye components were calculated.

After development, each plate contained several individual chromatograms. In some experiments one of these chromatograms was analyzed a number of times to obtain an indication of the variation inherent in the scanning process. The individual chromatograms were then analyzed once in order to obtain an indication of the variability due to the scanning process and to chromatography. Where applicable, Bartlett's test [6] was applied to compare the various standard deviation values. Such calculations produced a value "B," which was compared with the critical value obtained from chi-square tables. If "B" was less than the critical value, the null hypothesis was accepted and the two standard deviation values were said to be equal at a 95% confidence level.

Variations due to concentration effects were then examined by placing different volumes of dye extract onto the plate. The results of this test were expected to give an indication of the need to compare identical lengths of homogeneously dyed fibers.

The dye extracts from monofilaments were then examined, thus extending the technique to approximate casework conditions. The RSD% so obtained included effects due to instrumental variation, chromatographic variation, and variation within the sample.

Ratios of Dye Components for Similar Aliquots of Dye (Case 1)

Care was taken during the tests described above to ensure that the edge effect was reduced to a minimum [7]. The tests were carried out to determine the contribution to the RSD% of the peak height ratios and the contribution of variations due to the instrument and to chromatography. The possible effect of variation in the dye concentration was minimized by using the same quantities of dye on each chromatogram. Thus, 30 similar aliquots of a brown dye extract were placed on the plate with an adjustable-volume pipette. The plate was then developed and analyzed as previously described. A single chromatogram was analyzed 20 times and the 30 individual chromatograms on the same plate were each analyzed once. The peak height ratios were determined for two components (red Rf 0.1 and yellow Rf 0.2). The calculations based on these determinations are shown in Table 1.

The RSD% for the analysis of different chromatograms (4.7%) compared with that obtained for the measurement of the single chromatogram (0.46%) indicates that chromatographic variation is a major contributor to the variation in the ratio value obtained.

Bartlett's test was carried out on the two standard deviation values in Table 1. A "B" value of 59.7 was obtained (critical value = 3.84; $P = 0.05$). Thus, there is a significant difference between the two standard deviation values.

TABLE 1—*Statistical results of the ratio values obtained from chromatograms from Case 1.*

	Same Chromatogram	Different Chromatograms
Mean	0.65	0.59
SD	0.003	0.028
RSD%	0.46	4.7
n	20	30
Peak height range, mm		
Red spot	. . .	27–50
Yellow spot	. . .	46–96
Noise, mm	<1	<1

Ratio Determinations for Different Concentrations of a Dye (Case 2)

This plate contained a range of dye concentrations which were obtained by spotting different aliquots of a brown dye solution onto the TLC plate. The standard deviations of the ratios of peak heights obtained for two dye components (red Rf 0.14 and yellow Rf 0.30) from each chromatogram were calculated and are shown in Table 2.

The RSD% value for the various concentrations of dyes (4.3%) was of the same order as that obtained for the identical volumes of dye extract (4.7%) in Case 1.

Common practice is to examine fibers of identical lengths so that the determination of the relative proportions of components can be made visually. However, the above results indicate that the instrumental technique for determination of the ratios of peak heights of dye components may be suitable for comparison of different concentrations of the same dye mix on the plate. It may be possible, therefore, to compare two fibers of different length. This is important in forensic science since it reduces the need to obtain a control fiber the same length as the transferred fiber of interest. The tedious microscopic manipulation of short fiber monofilaments necessary in straightening the fibers and measuring their respective lengths and the cutting of a control fiber to match each transferred fiber being examined are eliminated.

Another reason commonly advanced for comparison of identical lengths of fiber monofilaments is that trace components may become visible in the chromatogram of the larger sample. The presence of these additional components may lead to doubt as to the similarity of the two samples. When scanning densitometry is applied to different lengths of fiber, and additional components are apparent from visual observation of the larger sample, the possibility that these components are an artifact of the quantity of dye placed onto the plate should be considered. This difficulty in visual interpretation will be overcome by the tenfold increased sensitivity available to the analyst by the use of densitometry (Case 8). This will mean that trace components should be detectable in the smaller sample, provided that no more than a tenfold variation in the lengths of the two fibers is being employed. Thus the technique described is suitable for determination of the ratios of dye components from different lengths of fibers, provided the variation in the length is maintained within the limits described.

Ratios of Dye Components in Monofilaments from Unused Cloth (Case 3)

This experiment approximated the real situation and examined the ratios obtained for the separated dye components extracted from monofilaments. To eliminate possible variation in the RSD% caused by heterogeneity of a sample, the fibers were taken from a manufacturer's pattern card. These fibers had not been subjected to laundering or

TABLE 2—*The statistical analysis of peak height ratios of two components obtained from various concentrations of a dye mix (Case 2).*

	Different Concentrations of Dye
Mean	2.3
SD	0.10
RSD%	4.3
n	12
Peak height range, mm	
Red spot	61–153
Yellow spot	27–69
Noise, mm	<1

excessive exposure to sunlight. Table 3 shows the results of the analysis of dye extracts from eleven fiber monofilaments, which separated into an orange component and a red component.

The RSD% for homogeneously dyed monofilaments was 11%. This was considerably higher than the 4.7% RSD obtained when sampling error was eliminated by the use of aliquots of the same dye solution. The signal-to-noise ratio (a minimum of six) indicated that, even when working close to the instrumental limit of detection, the absorbance ratio technique can be used to provide data on dye mixes extracted from fiber monofilaments and thus has potential for comparison of the dye components on transferred fibers originating from criminal cases.

Ratios of Dye Components in Monofilaments Obtained from Used Clothing (Case 4)

This experiment approximated the casework situation and examined the variation in results that may occur as a result of sample variability. Pale brown fibers were taken from a pair of polyester trousers that had been subjected to extensive laundering and exposure to sunlight. The dye mix separated into two yellow components, Rf 0.04 (A) and 0.08 Rf (B), and a red component, Rf 0.12 (C). The calculations based on the peak height ratio determinations for Components A, B, and C are shown in Table 4.

TABLE 3—*The statistical results of the peak height ratios obtained from separated dye components extracted from monofilaments of polyester that had not been washed or exposed to normal wear (Case 3).*

	Different Chromatograms
Mean B/A	1.2
SD	0.13
RSD%	11
n	11
Peak height range, mm	
A	13–42
B	12–55
Signal-to-noise ratio, minimum	6
Noise, mm	2

TABLE 4—*The mean and standard deviation values for the ratio of two dye components on monofilaments of polyester which had been subjected to several years of wear (Case 4).*

	Same Chromatogram,	Different Chromatograms	
	C/B	A/C	C/B
Length, mm	30	30	30
Mean	2.1	0.90	1.9
SD	0.032	0.22	0.17
RSD%	1.5	24	8.9
n	19	10	9
Peak height range, mm			
A	. . .	51–135	
B	. . .	0–77	
C	. . .	70–166	
Noise, mm		3	

The RSD% for the repeated analysis of the same chromatogram (1.5%) is of the same order as that obtained previously (0.46%) (Case 1). These values represent the variation due to the instrument and to the measuring technique. Results from Case 1 have shown that the variations due to chromatography gave an RSD% of about 5%. In the present experiment (Case 4), the variation due to the sample has led to the following results:

(*a*) a modest increase in RSD% of the C/B ratio (8.9%) when the samples contained all of the dye components and were unaffected by wear or processing variations, and

(*b*) a considerable increase in RSD% of the A/C ratio (24%) when the samples were apparently affected by one or more of the factors described below.

The yellow component, B (Rf 0.07), was absent from one of the chromatograms of a 30-mm length of fiber. This chromatogram was not included in the calculations. It became apparent that some components were not evenly distributed on the fibers. This may have been due to the dyeing process or have occurred because some of the components may, in fact, have been breakdown products produced after the dyeing of the garment as a result of prolonged laundering or exposure to sunlight. Another possibility could be that the spinning of the original fabric involved the use of fibers dyed with different dye batches. Grieve [*8*] reported such a variation in dye composition between fibers from a given garment.

The variation in the dye content of this real sample would indicate that, in a forensic science examination, a number of fibers from the source garment should be examined. Possible mismatch of two samples through a variation in the composition of the dyes on the source garment can thus be reduced.

Ratios of Dye Components Separated by HPTLC (Case 5)

An HPTLC plate was prepared and 100 and 200-nL aliquots of a brown dye extract were placed on the plate.

The 100-nL aliquots of dye produced spots that were near to the visual limit of detection. These spots produced peaks with a signal-to-noise ratio of 5:1. No attempt was made to determine the standard deviation of the ratio, because the noise level was so high and it was difficult to determine the peak maximum or the baseline points.

Table 5 shows the statistical analysis of the peak height data obtained for the 200-nL chromatograms.

Bartlett's test was applied to the two standard deviation values shown in Table 5. A "*B*" value of 21.8 was obtained (critical value = 3.84; $P = 0.05$). Thus, a significant difference was found between the two standard deviation values. Once again, the standard deviation of the measurement from the different chromatograms reflected the effect of chromatographic variations.

These RSD% values are of the same order as those obtained from conventional TLC

TABLE 5—*The statistical results for the ratio of two dye components in 200-nL aliquots of dye extract separated using HPTLC plates (Case 5).*

	Same Chromatogram	Different Chromatograms
Mean	0.85	0.84
SD	0.014	0.046
RSD%	1.6	5.5
n	20	21

plates (Case 2). It would therefore appear that, for ratio determinations, no advantage is obtained by using HPTLC plates. However, it should be remembered that HPTLC plates provide greater sensitivity than conventional TLC plates [9].

Ratios of Dye Components Separated by HPTLC from a Number of Monofilaments (Case 6)

The HPTLC plate that contained the separated dye components from 40-mm lengths of monofilament dyed with the Hoescht dye Red SL were developed. This dye separated into two components, A and B. The dyes extracted from these monofilaments produced spots on the HPTLC plate which were close to the visual detection limit. Table 6 gives the obtained results.

Bartlett's test ("B" = 53.8; critical value = 3.84; P = 0.05) indicated that there is a significant difference between the two standard deviation values shown in Table 6. This reflects variations due to the method and to the variations in the dye composition of the monofilament.

Comparison of the peak heights obtained on the conventional TLC plate in Case 3 (Table 3), and those obtained by HPTLC (Table 6) showed that, for analysis of similar lengths of fiber from the same dye batch, the spots on the HPTLC plate produced peaks that were three to five times higher than the peaks produced by the spots on the conventional TLC plate. The sensitivity of HPTLC is therefore three to five times that of conventional TLC. These results are generally in agreement with the increase in sensitivity reported by Zlatkis and Kaiser [9] with the use of HPTLC plates.

These results also indicate that, although the RSD% of the ratio values obtained were of an order similar to those obtained for the conventional TLC plates, the threefold to fivefold increase in sensitivity suggests that the use of HPTLC plates should be incorporated into any comprehensive procedure for comparison of dyes from microscopic quantities of textile fibers.

Ratios of Dye Components by Fluorescence and Visible Absorbance Measurements (Case 7)

Commonly, fluorescing dye components can be detected by viewing either the fiber or the developed TLC plate under an ultraviolet light source. However, such an approach does not provide numerical data.

The components studied were limited to colored dye components that exhibit fluorescence rather than optical brighteners which may be colorless but also exhibit fluo-

TABLE 6—*The statistical results for the ratio of two dye components extracted from 40-mm monofilaments separated on HPTLC plates (Case 6).*

	Same Chromatogram	Different Chromatograms
Mean B/A	2.5	2.9
SD	0.046	0.32
RSD%	1.8	11
n	30	8
Peak height range, mm		
A	. . .	58–142
B	. . .	21–58
Signal to noise ratio, minimum	18	10
Noise, mm	2	2

rescence. However, in principle, the use of the Camag TLC/HPTLC scanner in the fluorescence mode should detect the presence of any fluorescent compounds on the plate.

The dye mix used was separated into three components: Rf 0.5 (A), Rf 0.2 (B), and Rf 0.07 (C). The results, which were obtained by scanning a number of chromatograms containing equal aliquots of the dye extract in the fluorescence and visible absorbance modes, are shown in Table 7.

The mean values from Table 7 show that, for a particular group of dyes, the ratio values obtained in the fluorescence and visible absorbance modes appear, on the whole, to be unrelated. This would be expected, since visible light absorbance (color) and visible light emission (fluorescence) reflect different aspects of molecular structure [*10*].

Consequently, additional comparative information can be obtained by determination of the relative ratios of components in the visible absorbance and the fluorescence modes. This additional information will assist the analyst in forming an opinion as to the similarity, or lack of similarity, of fibers from a suspected source garment to fibers associated with a crime scene.

Detection of Yellow Dyes Extracted from Pale Textile Fibers (Case 8)

The ability of the eye to detect color is not constant over the entire visible spectrum. Billmeyer and Saltzman [*11*] produced a graph of the response of the human eye to different parts of the visible spectrum. The maximum response is in the yellow-green region around 555 nm. However, in practice, yellow-colored dyes, when viewed against the white background of the TLC plate, can be difficult to detect [*5*]. Grieve [*8*] concluded that, when dealing with such pale colors, microspectrophotometry of the whole fiber can be used for comparison, but TLC techniques should not be attempted. Microspectrophotometry of the whole fiber does not provide information on the individual dye components. Thus, failure to carry out a full analysis of the dye content by TLC must necessarily limit the significance of any conclusion drawn from the comparison of these pale-colored fibers.

However, by combining TLC and scanning densitometry, it may be possible to detect yellow-colored dyes at levels below the visual detection limit and thus produce valuable comparative information. Confirmation of the approximate color of these low levels of dye can be obtained by noting the variation in absorbance with change in wavelength.

A plate was prepared which contained a range of aliquots of a dye mix. The dye mix

TABLE 7—*The statistical evaluation of peak height data obtained from measurements taken in the fluorescence and visible absorbance modes (Case 7).*

	Fluorescence Mode			Visible Mode		
	A/B	C/B	A/C	A/B	C/B	A/C
Mean	4.3	7.8	0.56	0.85	1.4	0.63
n	14	14	14	14	14	14
SD	0.27	0.44	0.019	0.045	0.059	0.033
RSD%	6.2	5.6	3.4	5.3	4.2	5.2
Peak height range, mm						
A		92–106			62–77	
B		19–25			74–88	
C		164–184			97–118	
Noise, mm		<1			2	

resolved into two yellow components, Rf 0.1 and Rf 0.5. Each chromatogram was scanned once, and the peak heights were determined.

Table 8 shows the peak heights, noise levels, and visual detection limits for yellow dye using both reflected (R) and transmitted (T) light, as well as the detection limit obtained by the Camag scanner (CS).

The plates were examined using reflected and transmitted light from a fluorescent light source. The visual detection limit was taken as the lowest volume that gave an observable yellow spot. This spot had to be observable to a degree that the analyst had no doubt as to its presence. The instrumental limit of detection was considered to be the presence of a peak with a height three times the standard deviation of the blank [*12*].

The visual limit of detection was, in this case, three times better with transmitted light observations than with reflected light observations. In the past, aluminum-backed plates have been used for fiber dye comparison [*5*].[7] Transmitted light observations cannot be made with aluminum plates and, thus, a simple technique for increasing the sensitivity of visual observations is lost if these plates are used. Recently, plastic-backed plates have become commercially available; consequently, for forensic science dye comparisons, plastic-backed or glass-backed plates should be recommended.

The detection limit for yellow dyes by scanning densitometry was 10 to 20 times lower than that obtained by visual means using reflected light. This improvement in sensitivity, obtained by the application of an instrumental technique, compares well with that reported by West [5], who found that, by using HPLC, a tenfold improvement in sensitivity over that obtained by visual examination of the TLC plate was made possible. This indicates that the combination of TLC and scanning densitometry may offer a simple and reliable multiple-wavelength technique for comparison of dyes extracted from textile

TABLE 8—*The peak height and detection limit data obtained for a range of aliquots of yellow dye after TLC. The visual detection limits by reflected light (R), transmitted light (T), and scanning densitometry (CS) are also shown. The (+) indicates that the recorder response was off the scale for the instrument settings used. The peak heights were obtained using a single scan (Case 8).*

		Peak Height, mm	
Sample No.	Aliquot, μL	CI Disperse Yellow 54	CI Disperse Yellow 114
1	2.00	+	+
2	1.50	+	+
3	1.25	+	+
4	1.00	+	175
5	0.75	185	143
6 (R)	0.50	138	116
7	0.20	71	66
8 (T)	0.15	56	42
9	0.125	43	37
10	0.100	28	32
11	0.075	28	23
12	0.050	24	20
13 (CS)	0.025	10	11
Noise	. . .	5	5

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fibers and that the results may be comparable to those obtained using HPLC equipped with a diode array detector.

Since the Camag scanner can detect the presence of dyes below the visual limit of detection, a confirmation of the color of these "invisible" spots is necessary. This same instrument can be used to produce a spectrum of a spot on a TLC plate manually. Using this spectrum, the color of the spots and the similarity or nonsimilarity of this color to the color of a control spot can be determined approximately.

Rf information can be obtained from the position of the peak on the chart paper relative to the origin.

Conclusions

The Camag scanning densitometer can thus provide all the information about a dye component on a TLC plate that is normally obtained by visual observation; that is, the position, color, and relative proportions of the various components. Most importantly, the relative proportions of the various components obtained by densitometry are semi-quantitative and thus reduce the errors caused by qualitative visual comparison. These determinations can be made at levels both above and well below the visual limits of detection. Also, determination of the relative proportions can be made in the fluorescence mode, thus providing extra numerical data with which the analyst can form an opinion regarding the similarity or nonsimilarity of the fibers in question. The data obtained by using the Camag densitometer are objective, and the variations in interpretation that can occur with subjective visual observations are significantly reduced or eliminated.

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