filled by injector or detector or detector volume:

$$\frac{\tau_i}{\tau_c} = \frac{N_i}{N}\left(\frac{n}{k}\right)^{1/2} \quad [21]$$

From eqn [21], an expression has been derived linking fractional loss of resolution, ΔR_s, due to the injector or detector volume to column and retention characteristics:

$$v_i = 0.866\pi d_c^2 (LH)^{1/2}\left[\frac{1}{(1-\Delta R_s)^2} - 1\right]^{1/2}(1+k) \quad [22]$$

where v_i is the injection volume and L is column length. Since H is a function of d_c, v_i is proportional to $d_c^{5/2}$ and for a 10 m × 50 μm column operated under practical conditions, a 1% loss of resolution requires an injection volume of only 50 nL.

For equal distribution coefficients, K_D, an equation relating the maximum loadabilities, L_p and L_c, for packed and open tubular columns has been derived:

$$\frac{L_p}{L_c} = 1.24\left(\frac{d_{pc}}{d_c}\right)^2\left[\frac{1+\dfrac{K_D\varepsilon_i(1-\varepsilon_e)}{\varepsilon_T}}{1+\dfrac{4K_D d_F}{d_c}}\right]\left(\frac{L_p}{L_c}\right)^{1/2}\left(\frac{d_p}{d_c}\right)^{1/2} \quad [23]$$

where d_{pc} is the diameter of the packed column; ε_i, ε_e and ε_T are intraparticle, interstitial and total porosities, respectively; and L_p and L_c are the lengths of the packed and open tubular columns, respectively.

Table 1 lists the maximum sample loadability ratios for different K_D values for typical packed and open tubular columns; as might be expected, sample loadabilities are much greater on packed columns.

Table 1 Sample loadability ratios for different distribution coefficients

K_D	$\frac{L_p}{L_c}$
1	31
10	101
100	373

Packed column: $L_f = 20$ cm; $d_p = 5$ μm; $d_{pc} = 1$ mm; $\varepsilon_i = 0.5$; $\varepsilon_e = 0.4$; $\varepsilon_T = 0.7$. Open tubular column: L_c10 m; $d_c = 50$ μm; $d_F = 0.2$ μm.

Increasing the column diameter is the simplest approach to increasing the sample capacity of packed columns, since the surface area of stationary-phase support particles is generally maximized. However, for capillary columns, sample capacity can be increased by increasing the stationary phase film thickness, d_F. This should also lead, in theory, to a reduction in column efficiency but in practice such a reduction is only small, with film thickness up to 1 μm.

Further Reading

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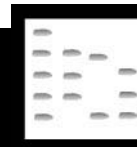
CHROMATOGRAPHY: THIN-LAYER (PLANAR)



Densitometry and Image Analysis

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Introduction

Densitometry is a means of measuring the concentration of chromatographic zones on the developed thin-layer chromatography high performance thin-layer chromatography (TLC in HPTLC) layer. The instrument does this without disturbing the substance in the chromatogram. The method and computer-controlled instrumentation produces results that are not only

reproducible, but also highly accurate (~1% standard deviation). Scanning is a fast process (up to a scan speed of 100 mm s^{-1}) with a spatial resolution in steps from 25 to 200 μm. Full UV/visible spectra (190–800 nm wavelength) of separated analytes can be recorded at high speed and peaks can be checked for purity by obtaining and comparing spectra from the start, middle and end of the peaks. With the use of highly sensitive charged coupled device (CCD) cameras, the photographic image of the developed TLC/HPTLC plate can be stored as a video image. This can be video-scanned to determine the concentration of separated components or can be printed when required as part of a document for a permanent record of the results. Many images can be stored on the computer hard drive and archived whenever required.

The Development of Modern Scanning Densitometry

The results of a developed TLC/HPTLC plate or sheet can be quantified in a number of ways. Visually, an estimate of concentration can be made. Many related substance tests in the pharmacopoeias rely on the concentration of the sample impurities being less than the standard concentration as seen visually. These are limit tests which depend on the eye of the observer determining that the concentration of the unknown is less than that of the standard. It has been estimated that the human eye can detect down to about 1 μg of a coloured spot on a TLC plate with a reproducibility of about 10–30%.

Better quantification can be obtained by eluting the relevant chromatographic zone from the adsorbent followed by spectrophotometry. The position of the zone can be marked out with a sharp bradawl and a microspatula used to scrape away the zone. These scrapings are then transferred to a container where a suitable solvent can be used to dissolve the compounds of interest from the particles of the adsorbent. The mixture is filtered and the concentration of the analyte in solution determined by transmittance/absorption spectrophotometry. There is little to recommend in this procedure as it is both tedious and time-consuming. It also requires meticulous care as errors can easily creep into the procedure. It is difficult to ensure that all the sample is completely removed from the TLC layer and is transferred from the chromatographic zone for further work-up if it is not easily seen in the visible or UV parts of the spectrum.

The technique of scanning densitometry determines the concentration *in situ*. It scans at set spectral wavelengths and does not rely on removal of any of the chromatographic zones from the TLC/HPTLC plate. Hence the previous problems and errors are eliminated. Scanning densitometry dates back to the 1950s when it was used to scan thin strips of paper chromatograms containing separated amino acids. Since then these primitive instruments have undergone considerable change, to the extent that they are now advanced analytical tools of similar capabilities to modern HPLC instrumentation.

Today's scanning densitometer measures reflectance, quenched fluorescence or fluorescence induced by electromagnetic radiation. For this reason, the instrument is now described as a spectrodensitometer. Although all three detection modes are commonly used, fluorescence is limited by the fact that fewer substances can be induced to fluoresce. Many spectrodensitometers also have an attachment for scanning electrophoresis gels by transmission.

The principle of operation is based on light of a predetermined beam size and wavelength striking the thin-layer surface perpendicularly whilst the TLC plate moves at a set speed under the stationary beam, or alternatively the beam traverses the stationary plate. Some of the electromagnetic radiation passes into and through the layer (transmitted light) whilst the remainder, due to the opaqueness of the layer, is reflected back from the surface. When the light beam passes over an absorbing chromatographic zone, there is a difference in optical response and less of the light is reflected (or transmitted). A photoelectric cell is used to measure the reflected light. When this receives a reduced amount of reflected light due to the presence of an absorbing chromatographic zone, a means is provided of detecting and quantifying the analyte.

Fluorescence quenching mode is really a variation on absorption methods. An inorganic phosphorescent indicator or organic fluorescent indicator is incorporated into the adsorbent layer. The inorganic phosphors give either a bright green or pale blue phosphorescence depending on the compound used. The phosphorescence is very short-lived. Hence it is best observed by continual exposure to UV light at 254 nm. Most HPTLC plates contain the indicator which exhibits the pale blue phosphorescence. This indicator is acid-resistant and allows higher sensitivity of detection of separated analytes due to less intense and less 'noisy' backgrounds. TLC plates containing organic fluorescers which give a bright blue fluorescence when excited by UV light of 366 nm are not as popular. Following chromatographic development, the plates incorporating the fluorophore are scanned at 254 or 366 nm in the absorbance mode. As the sample components absorb the excited radiation, the intensity of the phosphorescence or fluorescence is diminished. Consequently a variation occurs in the reflected light detected by the photomultiplier (or photoelectric cell).

When separated analytes naturally fluoresce under UV light, the spectrodensitometer can be used to scan in the fluorescence mode. The UV light provides the energy in these instances to excite electrons in molecules of the analytes from a ground state to an excited singlet state. As the excited electrons return to the ground state, energy is emitted as radiation at a longer wavelength, usually in the visible range. For best results in using this technique, it is important to use TLC/HPTLC plates which do not contain a phosphorescent of fluorescent indicator to minimize background inteference.

Theory of Spectrodensitometry

In spectrophotometric measurements where the absorbance is measured as a result of a beam of light of set wavelength passing through a fixed pathlength of solution, a direct relationship exists between the observed absorbance and the concentration of the solution. This is known as Beer's law. However, it should be pointed out that this relationship is not linear over the whole range of concentration, and it depends on the sample solution being transparent.

As TLC/HPTLC plates are opaque, a somewhat different approach is required. In the 1930s Kubelka and Munk investigated the relationship between absorbance, transmission and reflectance, deriving mathematical expressions to explain the effects of absorbance and reflectance. When a ray of incident light comes into contact with the surface of the opaque TLC layer, some light is transmitted, some is reflected in all directions at the surface and some rays are propagated in all directions inside the adsorbent. The theory which explains to a large degree what is happening in this process is known as the Kubelka–Munk theory. Certain assumptions can be made which simplify the mathematical equations derived. The theory assumes that both the transmitted and reflected components of incident light are made up only of rays propagated inside the sorbent in a direction perpendicular to the plane of the surface. All other directions will lead to longer pathways and hence stronger absorption. These rays therefore contribute little to either the transmitted or reflected light and their contribution can be treated as negligible. When light exits from the sorbent at the layer–air boundary, light scattering occurs, and it is distributed over all possible angles with the surface.

The coefficient of light scatter (S), can therefore be proposed; this depends on the layer thickness. If we assume that this is unchanged in the presence of a chromatographic zone, the following equation can be derived for an infinitely thick opaque layer:

$$\frac{(1 - R_\infty)^2}{2R_\infty} = \frac{2.303}{S} \cdot a_m \cdot C \qquad [1]$$

where R_∞ is the reflectance for an infinitely thick opaque layer, a_m is the molar absorptivity of the sample, c is the molar concentration of the sample and S is the coefficient of scatter per unit thickness.

This equation is clearly less than ideal as the layer has a finite thickness. More meaningful expressions for the intensity of the reflected light, I_R, and the transmitted light, I_T, for a layer of thickness (l) are given by the following hyperbolic solutions:

$$I_R = \frac{\sinh(b \cdot S \cdot l)}{a \cdot \sinh(b \cdot S \cdot l) + b \cdot \cosh(b \cdot S \cdot l)} \qquad [2]$$

$$I_T = \frac{b}{a \cdot \sinh(b \cdot S \cdot l) + b \cdot \cosh(b \cdot S \cdot l)} \qquad [3]$$

where

$$a = \frac{S \cdot l + K_A \cdot l}{S \cdot l}$$

and

$$b = (a^2 - 1)^{1/2}$$

K_A is the coefficient of absorption per unit thickness.

The application of the equations to quantitative analysis in TLC is quite complex, but it can be greatly simplified by making a number of reasonable assumptions that would hold true for TLC. One thing that eqn [2] immediately reveals is that the relationship between the reflected light and the concentration of the chromatographic zone is nonlinear. This is what is found in practice over the full range of concentrations. The data when graphically displayed fit a polynomial curve (eqn [4]).

$$y = a_0 + a_1 \cdot x + a_2 \cdot x^2 \qquad [4]$$

However, over a narrow concentration range the relationship is seen to be linear. This means that if it is necessary to have a calibration curve over the whole range of concentrations, at least four but no more than six standards will be required for the determination of one separated analyte. Of course, only two standards may be needed if the concentrations are close to that of the analyte, because it can be assumed that the curve is linear over a small range.

Although it may seem that errors could easily creep into the determination procedure, this is not the case. The assumptions made have only a negligible effect on the final result. Hence, even including any errors which may originate from the scanning spectrodensitometer, the percentage relative standard

deviation is normally below 2% and quite often well below 1%.

For a wide concentration range, the Michaelis–Menten regression curve can be used. The calibration is calculated as a saturation curve:

$$y = \left(\frac{a_1 \cdot x}{a_2 + x}\right) \quad [5]$$

and is theoretically only permitted within the calibration range (between the largest and the smallest standard amounts applied). This regression always passes through the origin.

In some cases there is a better curve fit to the data if the Michaelis–Menten regression does not pass through the origin. Better resolution is therefore obtained if the data produce a function that does not tend towards zero:

$$y = a_0 + \left(\frac{a_1 \cdot x}{a_2 + x}\right) \quad [6]$$

As before, this is theoretically admissible only within the calibration range.

It is also possible to linearize the data graphically. The simplest transformation procedures involve converting the data on reflectance and concentration into reciprocals, logarithms or squared terms. The following equations can thus be proposed:

$$\log R_e = a_0 + a_1 \cdot \log c \quad [7]$$

$$\frac{1}{R_e} = a_0 + a_1\left(\frac{1}{c}\right) \quad [8]$$

$$R_e^2 = a_0 + a_1 \cdot c \quad [9]$$

where R_e is the reflectance signal and c is the sample concentration.

Eqns [7] and [9] result in linearization over the middle of the concentration range, whereas eqn [8] showed better linearization, but even this fails at very low concentration. None of these methods is able to linearize the data over the whole concentration range.

A solution to the above is to use nonlinear regression analysis based on second-order polynomials. These can be described by the following equations:

$$\ln R_e = a_0 + a_1 \cdot \ln c + a_2 \cdot (\ln c)^2 \quad [10]$$

$$R_e = a_0 + a_1 \cdot c + a_2 \cdot c^2 \quad [11]$$

Over the whole concentration range, eqn [10] gives the best results. In fact, it has been shown that the data fit is not compromised when as few as three standards are used over the whole concentration range.

The mathematical treatment of the data for fluorescence intensity can be expressed according to the well-known Beer–Lambert law. The fluorescence emission (F) is given by the equation:

$$F = \theta \cdot I_0(1 - e^{-a_m \cdot l \cdot c}) \quad [12]$$

where F is the fluorescence emission and θ is the quantum yield.

For low sample concentrations the following assumption can be made:

$$e^{-a_m \cdot l \cdot c} = 1 - a_m \cdot l \cdot c \quad [13]$$

Therefore:

$$F = \theta \cdot I_0 \cdot a_m \cdot l \cdot c \quad [14]$$

It follows that, for low concentrations, the fluorescence emission is linearly dependent on the sample concentration. In practice this proves to be the case even though this equation was derived without taking into consideration the influence of absorption or scatter.

Pre-Scanning Considerations

For quantification by reflectance scanning, there is no limitation on the backing used for the chromatographic layer, whether it be glass, aluminium or plastic. However, it must be said that quality of the scanned results, reproducibility and quantitative accuracy mainly depend on the quality of the spot or band application of the sample and the choice of developing solvents. The use of automated spot and band application equipment results in a noticeable improvement in relative standard deviation. In practical terms, band application gives even greater accuracy than spot application. This is to be expected since a scanning slit length on the spectrodensitometer has to be chosen for a spot such that it covers the whole length, as the concentration of the analyte will vary across the spot, with the highest concentration being in the centre. The slit length also has to allow for any variation caused by migration of spots not occurring in a precisely vertical direction (usually due to solvent vapour not being saturated in the developing chamber). For band development, the concentration of the analyte is the same across the length of the band. Hence, there is more latitude on the choice of slit length. Small slit lengths can be chosen which tend to higher sensitivity. Under these conditions with many separations, coefficient of variation (CV) below 1% can be achieved.

Instrumentation

A number of different types of scanning spectrodensitometers are available. Most are now either partially or fully computer-controlled. The parameters such as track length, number of tracks, distance between tracks, slit length and width, scanning wavelength and speed can all be programmed into the computer. Some spectrodensitometers can perform a pre-scanning run to determine the position of maximum absorption for the separated components on the track: this is particularly useful where spot application has been used. After scanning, the spectrodensitometer generates massive amounts of data from all the tracks, including peak height and area and position of zones (start, middle and end), for every component. Usually a chromatogram can be displayed for all tracks. This can be baseline-adjusted and excess noise from the background of the layer can be subtracted. All peaks can be integrated, ready for possible quantification. Although a number of scanning modes are available, such as linear, radial (scanning from the centre for circular chromatograms) and circular scanning around a ring (circular development), by far the most popular is the linear mode, as shown in **Figure 1**.

Normally, three light sources are used in scanning densitometry: a deuterium lamp (190–400 nm), a tungsten halogen lamp (350–800 nm), and a high pressure mercury vapour or xenon lamp for intense line spectra (254–578 nm), usually required for fluorescence determinations.

Three optical methods (**Figure 2**) have been used in the construction of scanning densitometers:

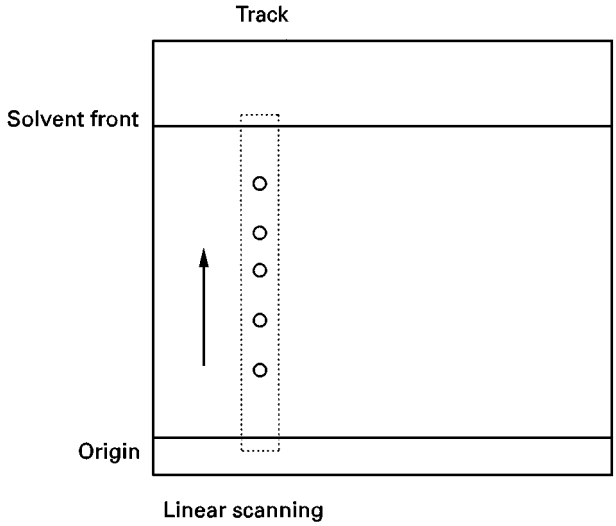


Figure 1 Linear scan of individual tracks using a scanning densitometer. Slit length and width, track length and speed of scan are all pre-selected.

1. single wavelength, single beam
2. single wavelength, double beam
3. dual wavelength, single beam

Construction 1 requires little explanation and is the type manufactured by most commercial TLC companies. Construction 2 divides the single beam into two by means of a beam splitter, so that one half scans over the chromatographic zone whilst the other scans over the background. Both beams are detected by matched photomultipliers and the difference in the signal measured. In construction 3, two wavelengths as close together as possible are chosen, such that fluctuations caused by light scattering at the light-absorbing wavelength are compensated for by subtracting the fluctuations at the different wavelength at which there is no absorption by the chromatographic zone.

In fixed-beam spectrodensitometers, the stage holding the TLC plate under the light beam moves at a constant rate, propelled by stepping motors. Where the light beam moves, it does so in a zigzag fashion over the surface of the stationary plate. Usually the zigzag scanners incorporate a curve linearization technique for absorption measurements. This uses the hyperbolic solution in eqn [2].

Applications

As the chromatogram is permanently or semipermanently held in the layer after development is complete, a number of useful techniques can be used with a scanning spectrodensitometer both to improve the evaluation of the chromatogram and to collect more important data on the separated analytes.

1. The TLC plate can be scanned at a range of different wavelengths. The optimum wavelength can therefore be chosen for maximum absorption of individual sample components. Of course, if two analytes are not completely resolved, but absorb at different wavelengths, then it is possible to quantify the results without further resolution.
2. UV/visible absorption spectra can be obtained for each separated component. Some commercial software then allows the comparison of such spectra with a library of spectra in order to identify unknowns.
3. Spectra can also be obtained for different parts of the chromatographic zone. Hence, spectra can be obtained for the upslope, apex and downslope of the peak to assist the analyst in looking for any peak impurities. Any changes in the spectrum as the light beam traverses the zone would indicate nonhomogeneity.

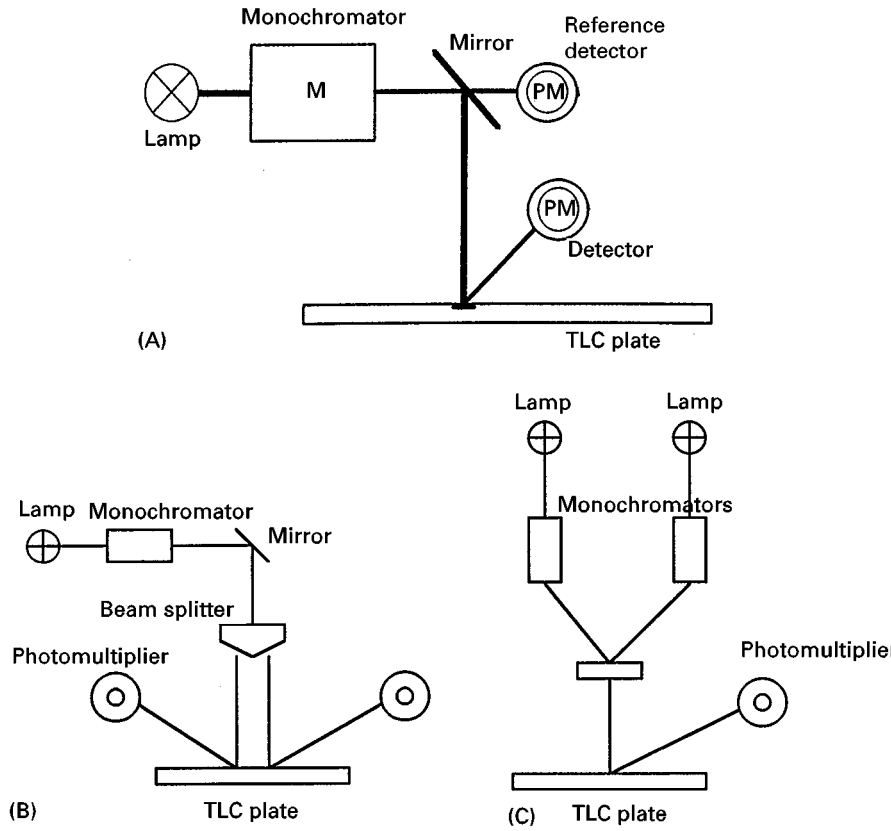


Figure 2 Scanning modes: (A) single beam; (B) single wavelength, double beam in space; (C) dual wavelength, single beam in time. PM, photomultiplier.

4. Background subtraction is another useful feature of most spectrodensitometers. Some background noise will always be present, hence the scanner software can subtract a background scan of the TLC plate before quantification.
5. Some instruments can scan and image an entire plate, enabling two-dimensional chromatograms to be evaluated (scan time less than 5 min).

The widespread use of planar chromatography means that the applications of spectrodensitometry are almost limitless. Hence, there are extensive publications on the use of scanning densitometry in all types of industry and research. Many of the instrument and plate manufacturers also provide application methods and extensive bibliographies. For example, in all of the following areas scanning densitometry has been used for quantification.

1. Biomedical: organic acids, lipids, steroids, carbohydrates, amino acids
2. Pharmaceutical: stability and impurities of synthetic drugs, antibiotics, drug monitoring, alkaloids
3. Food science: mycotoxins (including aflatoxins), drug residues, antioxidants, preservatives, natural pigments, food colours, spices, flavonoids
4. Forensic: drugs of abuse, poisons, alkaloids, inks
5. Clinical: therapeutic drug monitoring, identification of metabolic drug disorders
6. Environmental: pesticide residues in crops, crop protection agents in drinking water, industrial hygiene
7. Industrial: product uniformity, impurity profile, surfactants, synthetic dyes

To give a flavour of the capability of the technique, the following examples can be considered. **Figure 3** shows the scan obtained from the separation of a number of sulfonamides and antibiotics from a complex animal feed matrix on an HPTLC silica gel plate. Scanning at five different wavelengths allows each of the components to be quantified by measurement at its absorption maximum. The three-dimensional presentation also allows the minor impurities to be more clearly identified. Multi-wavelength scanning is also illustrated in **Figure 4** with an automated multiple development (AMD) separation of pesticides in tap water on HPTLC silica gel plates. **Figure 5** illustrates the fluorescence scan of a range of saturated fatty acids from C_6 to C_{24}, an important food application in fats and vegetable oils. The fatty

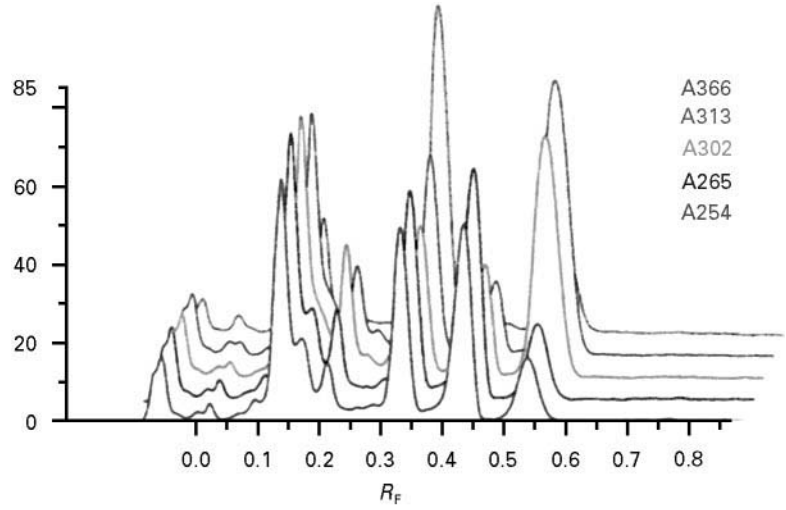


Figure 3 (See Colour Plate 26). Separation of sulfonamides in a complex animal feed matrix on an HPTLC silica gel plate. The plate has been scanned at five different wavelengths and the chromatogram overlaid in a three-dimensional presentation. Reprinted from Camag literature, CAMAG, Muttenz, Switzerland.

acids were derivatized before separation by a unique on-layer technique. The acids are resolved as their dansylcadaverine derivatives on an HPTLC RP18 layer.

The use of spectral identification of an unknown is demonstrated in **Figure 6**. The unknown was eventually identified as morphine, but because ethylmorphine and codeine have such similar spectra (as shown in the overlay), it was necessary to search the spectrum library for the best-fit recorded spectra, and also to check the correlation with the R_F value. This enabled a correlation with morphine of 98.4% to be obtained for the unknown. This example illustrates the need for the analyst not only to search for the best fit, but also to check the correlation with the R_F value. Had the search been limited to the spectrum library, ethylmorphine could well have been chosen as the unknown.

Video Densitometry

Video densitometry has been developed in the last few years and is now being deployed throughout industry and research. Such instruments use an imag-

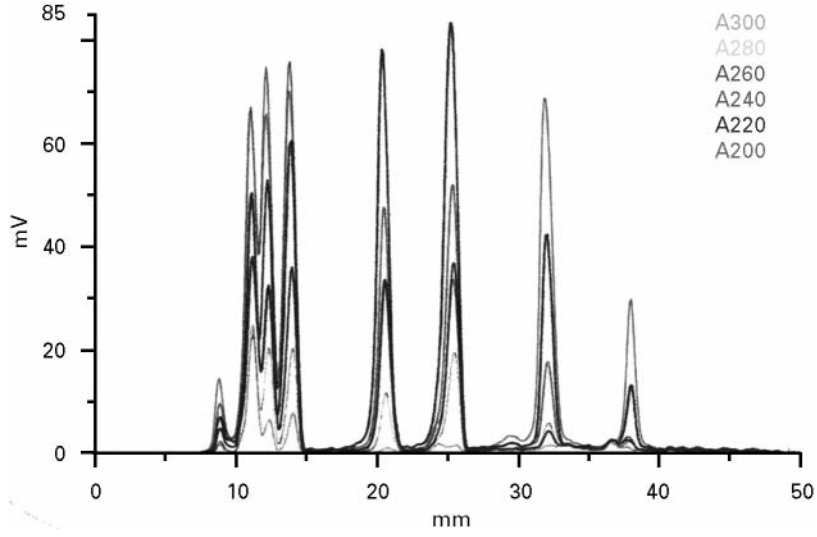


Figure 4 (See Colour Plate 27). Separation of pesticides in tap water on an HPTLC silica gel plate by AMD. Multi-wavelength (six wavelengths) evaluation permits resolution by optical means of fractions insufficiently separated. Reprinted from Camag literature, CAMAG, Muttenz, Switzerland.

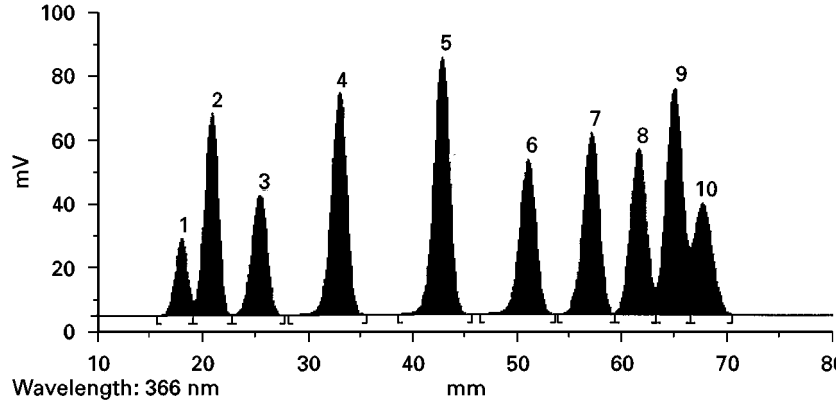
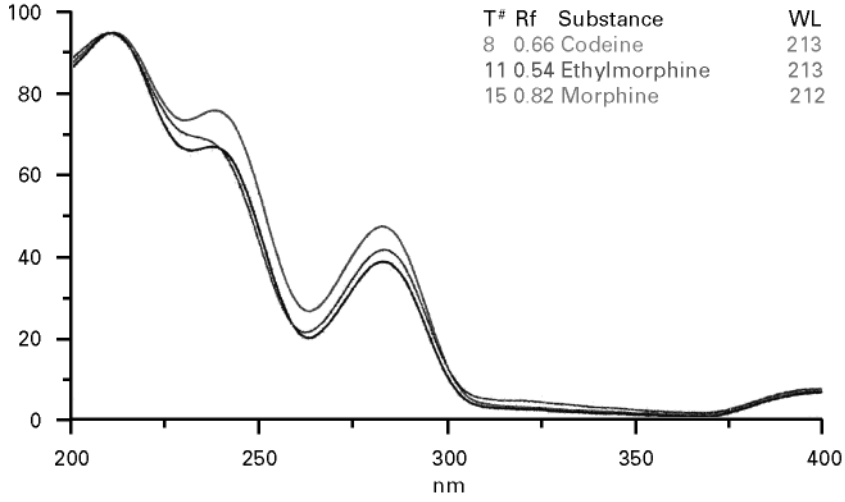


Figure 5 Fluorescence scan of dansylcadaverine derivatized fatty acids separated on an HPTLC silica RP18 plate.

ing system consisting of a high resolution CCD camera with a zoom attachment to focus and enlarge the chromatogram, if required and a suitable illumination system. The camera is linked to a computer (usually a PC) and a video printer. The software controls the camera, as well as all parameters such as brightness, contrast, colour balance and intensity. These can be saved for future use or kept as a record of the results. The chromatogram can be presented as an image on the video printer and can be quantified to obtain the concentration of analytes using the mathematical procedures used in scanning densitometry. As in spectroddensitometric scanning, the software does all the necessary calculations to determine the concentration of analytes. For weakly fluorescing analytes, a small camera aperture (F : 22) can be used with long time integration. This enables the imagining of fluorescing compounds which are often invisible to the human eye. The images can be annotated and that annotation stored separately for



Correlation of spectra without R_F value		Correlation of spectra with R_F value	
Substance	*Correlation*	*Substance*	*Correlation*
Ethylmorphine	0.9924	Morphine	0.9839
Codeine	0.9905	Atenolol	0.9223
Nalorphine	0.9848	Salbutamol	0.9174
Morphine	0.9839	Sotalol	0.8939

Figure 6 (See Colour Plate 28). UV spectra of codeine, ethylmorphine and unknown (morphine) overlaid. Spectra of codeine and ethylmorphine taken from spectral library. Spectrum of morphine taken from chromatogram. Reprinted from Camag literature, CAMAG, Muttenz, Switzerland.

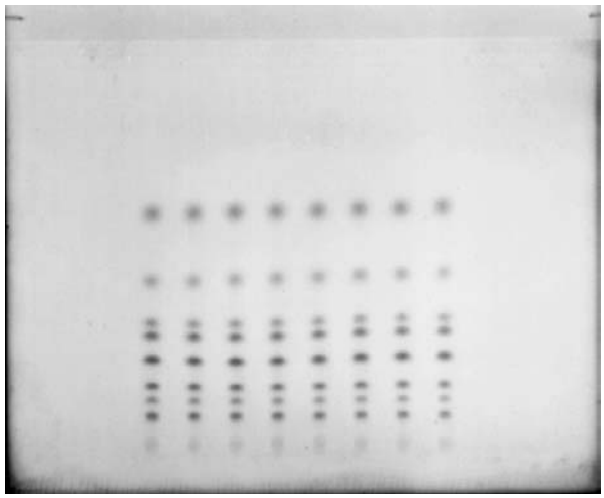


Figure 7 Video scan of separation of corticosteroids on an HPTLC silica gel plate. Detection reagent: blue tetrazolium solution. Spot application with automatic equipment.

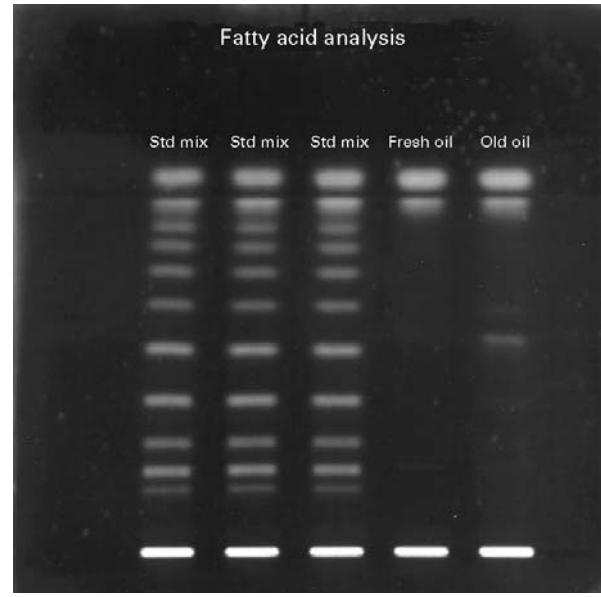
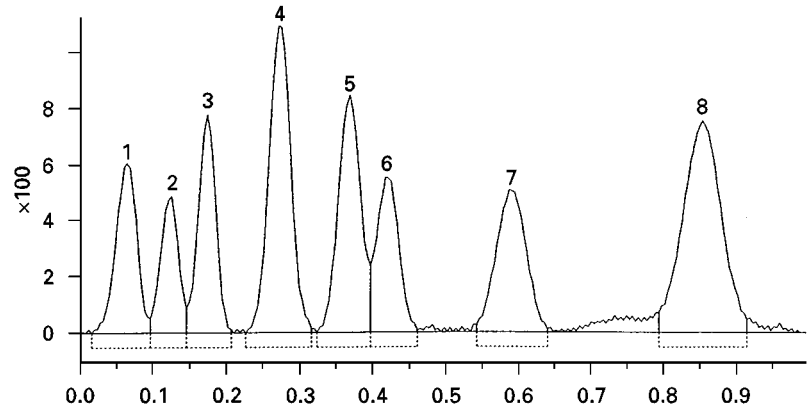


Figure 9 Video scan of separation of pre-derivatized saturated fatty acids on an HPTLC RP18 plate. The plate was scanned at 366 nm to produce fluorescent zones. Band application with automated equipment.



Track 3 Sample a

Peak no	*Start*		*Maximum*			*End*		*Area*		*Subst name*
	R_F	*H*	R_F	*H*	*[%]*	R_F	*H*	*A*	*[%]*	
1	0.016	0.0	0.065	606.4	10.76	0.097	50.9	5076.4	9.53	8
2	0.097	50.9	0.126	488.3	8.67	0.146	87.9	3432.6	6.44	7
3	0.146	87.9	0.174	780.2	13.85	0.206	12.1	5353.3	10.05	6
4	0.227	0.0	0.271	1094.6	19.42	0.316	13.8	9440.8	17.72	5
5	0.324	0.0	0.368	844.7	14.99	0.397	240.7	7487.0	14.05	4
6	0.397	240.7	0.417	555.2	9.85	0.462	6.1	4914.6	9.22	3
7	0.543	26.1	0.587	509.7	9.05	0.640	10.6	6065.0	11.38	2
8	0.794	70.3	0.854	755.8	13.41	0.915	46.6	11519.2	21.62	1

Total height, 5634.96; total area, 53288.8.

Figure 8 Video scan of separation of corticosteroids on an HPTLC silica gel plate.

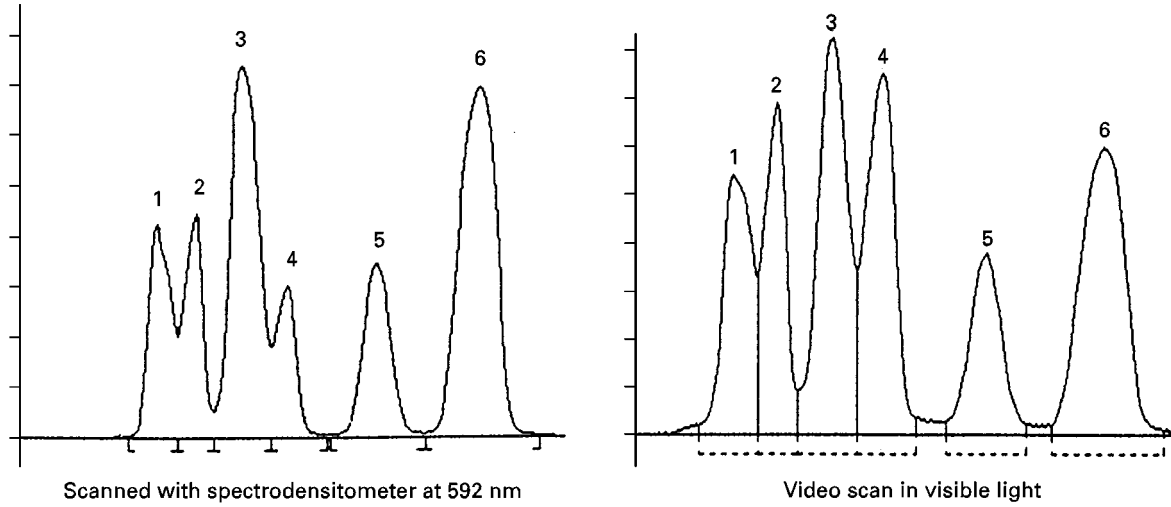


Figure 10 Separation of dye mixture developed on an HPTLC silica gel plate with toluene as mobile phase. Comparison of spectrodensitometric scan with video scanning.

readiness in annotating further images. Such images can be stored in a variety of files which can then be used in a number of well-known office programs, such as Word, and PowerPoint.

The illumination system needs a number of features in order to get the best results from the CCD camera unit. Illumination from above is necessary, both visible light and UV light at 254 and 366 nm (depending on the chromatogram). However, it is essential that the light fittings do not interfere with the camera's field of view. Lighting from below the plate can in some cases also prove advantageous in giving a bright image.

Figure 7 illustrates a video print of a separation of corticosteroids developed on an HPTLC silica gel plate. The steroids were detected with blue tetrazolium reagent. **Figure 8** shows the scan taken using the software option available. R_F data is recorded in the table below. **Figure 9** illustrates a further video print, this time of fluorescent chromatographic zones, photographed under UV light (366 nm). This is a separation of derivatized saturated fatty acids from C_6 to C_{24} (conditions as in Figure 5).

Although it is possible to quantify results from the video scan, they are not as accurate as those obtained from a spectrodensitometer. **Figure 10** and **Table 1** show a comparison of the CV for a six-component dye test mixture separated on an HPTLC layer. Whereas the CVs for spectrodensitometric scan are below 2%, those for the video imaging system are typically from 2 to 4%. As most USP (United States Pharmacopoeia) and EP (European Pharmacopoeia) monographs accept CVs of $\pm$ 6% in most, if not all, cases, the use of video densitometry is acceptable. However, it should be remembered that for fluorescence quenching and absorption measurements below 254 nm, video densitometry will not show any detection. This is one of the present limitations of the technique. Some substances do require shorter wavelength UV light for their detection. In these instances spectrodensitometry is presently the only solution.

Table 1 Comparison of coefficient of variance (CV) with video scanning and spectrodensitometric scanning. Separation of dyes on an HPTLC silica gel plate using toluene as mobile phase

Dye	R_F	*Video scan with white light*		*Spectrodensitometric scan at* 592 *nm*	
		Mean value (%)	*CV* (%)	*Mean value* (%)	*CV* (%)
Black	0.04				
Grey	0.10	99.4	3.50	101.5	0.83
Red	0.17	102.8	3.10	97.8	0.31
Blue	0.23	103.0	3.52	101.2	1.90
Pink	0.36	99.7	3.46	98.3	0.96
Yellow	0.51	98.6	1.30	98.8	0.56

Future Trends

It seems unlikely that video densitometry will ever replace spectrodensitometry as both techniques have unique advantages. On the one hand spectrodensitometry allows the scanning of TLC/HPTLC plates at selectable wavelengths, the acquisition of UV/visible spectra, the determination of peak purity and high accuracy of results. On the other hand, video scanning provides a computer or printed image that can serve as a permanent record of the results obtained which can be documented at any time in a report. Also, for some requirements the accuracy of scanning is sufficient for quantitative evaluation.

With improved software, both densitometric and video scanners are likely to become still more user-friendly. However, more dramatic improvement in the accuracy and reliability of results is more likely to come from the continual improvements taking place in the quality of adsorbents making up the layer. With the introduction of smaller (4 μm) spherical particle sizes, the quality of separation will improve, hence this will be reflected in the scans and quantitative results obtained with both spectrodensitometry and video scanning.

See also: **III/In-Depth Distribution in Quantitative Thin-layer Chromatography.**

Further Reading

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Historical Development

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History

Today the term planar chromatography is commonly used as a synonym for high performance thin-layer chromatography (HPLTC) and conventional thin-layer chromatography (TLC). Originally it referred more generally to a family of techniques including TLC, some types of electrophoresis and paper chromatography, which all have in common a stationary phase in the form of a flat thin layer rather than packed into a column. Modern planar chromatography is a form of liquid chromatography and its history is closely linked to the development of chromatography as an analytical tool.

Early roots go back to Beyrinck who in 1889 separated hydrochloric and sulfuric acid by diffusion through a thin layer of gelatine on a glass plate. With the same technique, Wijsman in 1898 was able to demonstrate the presence of two enzymes in malt diastase. When at the end of the 1930s Tswett's column chromatography became successful, research focused on a faster microchromatographic method, which allowed the exact identification of adsorbed substances. This situation encouraged the transition from a regular column to an open column, a thin layer of adsorbent.

Izmailov and Shraiber are regarded as the inventors of TLC (**Table 1**). In 1938 they described a method in which microscopic slides were coated with 2 mm layers of a slurry made of chalk, talc, magnesium oxide, lime aluminium oxide or other adsorbents and water. On drying, a thin adsorbent layer was formed. The authors investigated belladonna and other plant extracts by placing a drop of the extract on to the layer. This resulted in the so-called ultra chromatogram that was visualized under ultraviolet light. The chromatogram was then developed with several drops of solvent. The most important advantage of the new method in comparison to column chromatography was the short time of analysis and the low consumption of adsorbents, solvents and samples.

Crowe reported in 1941 the use of a microchromatographic method to select suitable solvents for column chromatography. The procedure was