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COMPARATIVE EVALUATION OF THE PERFORMANCE OF SILICA GEL TLC PLATES AND IRREGULAR AND SPHERICAL-PARTICLE HPTLC PLATES

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SUMMARY

Commercial precoated thin-layer chromatography (TLC) and high-performance TLC (HPTLC) plates with spherical or irregular particles have been compared on the basis of theoretical plate number (*N*), resolution (*R*), linearity, development time, and limit of sensitivity for analysis of a multicomponent analgesic tablet in the fluorescence quenching mode and analysis of a five-component dye mixture in the visible mode. Standardized plate preparation, sample application, and development procedures were used, and separations were conducted over the usual development distances of 12.0 cm for TLC and 6.0 cm for HPTLC. It was found that TLC often gave the best *N* and *R* values, but that development time, sensitivity, and linearity were better for HPTLC. Spherical-particle HPTLC plates performed better for the drug mixture and irregular HPTLC plates performed better for the dye mixture. Some results are also given for preadsorbent irregular-particle HPTLC and ultra-TLC plates.

INTRODUCTION

The literature contains many statements that high-performance thin-layer chromatography (HPTLC) is superior to classical TLC. For example, Wall [1] wrote that the performance of HPTLC is an order of magnitude greater than that of TLC, that it is possible to perform separations on HPTLC plates that are impossible on TLC plates, and that HPTLC layers are used for the most reliable results and are a substantial advance in the practice of TLC. Rabel [2] described HPTLC layers made with small-particle spherical silica gel and stated that advantages over conventional HPTLC plates with irregular particles include more rapid separations and more compact spots (with, presumably, better resolution).

The purpose of this research was to compare the three types of layer, to assess the validity of statements such as these, by using standardized TLC and HPTLC procedures and detection in visible and fluorescence-quenching modes. The characteristics evaluated were efficiency (number of theoretical plates, *N*), resolution (*R*), development time, linearity of calibration plots for quantitative analysis with a densitometer, and limit of sensitivity (LOS).

EXPERIMENTAL

Preparation of Solutions

A test solution of an extra-strength analgesic tablet with a label declaration of 65 mg caffeine, 250 mg acetaminophen, and 250 mg acetylsalicylic acid was prepared in methanol as described elsewhere [3]. The final concentrations of the components were 0.065, 0.250, and 0.250 mg mL^{-1}, respectively. A standard caffeine solution was prepared at a concentration of 0.500 mg mL^{-1} in methanol. Test Dye Mixture IV (Analtech, Newark, DE, USA; catalog No. 30-01) containing 8.0 mg mL^{-1} each of Sudan orange G (yellow, gives two zones), solvent blue 35 (blue), Sudan II (orange), solvent green 3 (green), and fast red 7B (purple) was diluted with toluene to a concentration of 2 mg mL^{-1}.

Chromatographic Procedures

TLC and HPTLC were performed using the standardized approach described by Reich and Schibli [4]. The following glass-backed precoated plates from EMD Chemicals (Gibbstown, NJ, USA; an affiliate of Merck, Darmstadt, Germany) were tested:

- 20 cm × 20 cm silica gel 60 TLC plates (Catalog no. 5715-7) with a 0.25 mm layer of irregular particles of mean size 10–12 μm and distribution 5–20 μm, with manganese-activated zinc silicate ultraviolet (UV) indicator (green);
- 10 cm × 20 cm silica gel 60 HPTLC plates (Catalog no. 1.15696.0001) with a 0.2 mm layer of irregular particles of mean size 5–6 μm and distribution 4–8 μm, with magnesium tungstate (acid-stable) UV indicator (blue); and
- 10 cm × 20 cm silica gel 60 HPTLC plates (Catalog no. 1.15445.0001) with a 0.2 mm layer of spherical particles of mean size 7 μm and distribution 6–8 μm, with blue UV indicator.

Plates used for separation of the tablet components were precleaned by development to the top with methanol, then dried on a Camag (Wilmington, NC, USA) plate heater at 120°C for 30 min. For dye separations the plates were used as received.

Initial zones were applied as bands by spraying with a Camag Linomat IV fitted with a 100-µL syringe and operated with the settings: band length 6 mm, rate of application 4 s μL^{-1}, table speed 10 mm s^{-1}, distance between bands 4 mm, distance from the plate edge 7 mm, distance from the bottom of the plate 1.0 cm.

Plates were developed with ethyl acetate–glacial acetic acid, 95:5, as mobile phase for the tablet solution and toluene as mobile phase for the dye mixture, in a Camag TLC or HPTLC twin-trough chamber containing an Analtech saturation pad in the back trough. The tank was left to equilibrate for 20 min before insertion of the spotted plate into the front trough with the layer facing the saturation pad. The development distance was 6.0 cm for HPTLC and 12.0 cm for TLC.

After development plates were dried in a stream of cold air for 2–3 min. Chromatograms were viewed in daylight (dyes) or under 254-nm UV light in a Camag viewing box (tablet components). Chromatograms of the tablet components were scanned at 254 nm in the single-wavelength, single-beam mode with a Shimadzu (Columbia, MD, USA) CS-930 densitometer with a slit setting of 7 mm length and 1 mm height. Calibration plots for caffeine were established by using a ChromImage flatbed scanner densitometer with Galaxie-TLC software (AR2i Company, LePlessis Robinson, France) and the 254-nm source; the same instrument was used to record dye densitograms using the visible light source. The ChromImage was operated as described elsewhere [5].

Evaluation and Calculations

Zones on the layer and densitograms were measured with a millimeter rule to obtain data for calculation of N and R values. Replicate samples were spotted ($n = 6$ for the tablet components and $n = 3$ for the dyes), and individual measurements were averaged. The equations below, specified by Kowalska et al. [6], were used to calculate N and R, respectively, from direct measurement of dye zones on the layer or densitograms:

$$N = \frac{16 \times l \times z}{w^2}$$

$$R = \frac{z_1 - z_2}{0.5(w_1 + w_2)}$$

where l is the migration distance from the origin to the mobile phase front, z is the migration distance from the origin to the center of the solute zone, w is the chromatographic zone width in the direction of mobile phase migration, and 1 and 2 are the upper and the lower adjacent zones, respectively.

The linearity of the caffeine calibration plot was evaluated by spotting increasing amounts of the caffeine standard solution, starting with 1.00 μL (0.5 μg), and finding the range that gave a correlation factor of at least 0.99. N and R values were evaluated from densitograms of the minimum weight of applied samples required to obtain the smallest measurable peak for the most poorly detected compound (caffeine for the tablet mixture and Fast Red 7B for the dye mixture), which was defined as the limit of sensitivity (LOS).

RESULTS

Tablet Components

Although the spherical-particle HPTLC plate gave slightly better N and R values than that coated with irregular particles, when measured both on the densitograms and on the plate, efficiency and resolution of the TLC plate were significantly superior to those of both HPTLC plates (Table I).

Table I

Results obtained for the tablet components on spherical-particle HPTLC, irregular-particle HPTLC, and TLC plates. R values were calculated for the acetaminophen and acetylsalicylic acid zones and N for the caffeine zone from measurements made on densitograms and directly on the plate

Plate	HPTLC		TLC
	Spherical particles	Irregular particles	
LOS (μg)	0.13	0.26	0.39
Linearity range (μg)	0.13–6.5	0.26–6.0	0.39–4.5
Development time (min)	7–8	11–13	22–25
R (densitograms)	2.00 ± 0.054	1.87 ± 0.12	2.63 ± 0.090
N (densitograms)	352 ± 15	346 ± 26	730 ± 33
R (plate)	4.62 ± 0.19	4.08 ± 0.18	5.37 ± 0.42
N (plate)	3071 ± 92	3025 ± 190	3510 ± 361

It took longer to develop the TLC plate than the HPTLC plates, however, and the spherical-particle HPTLC plate required even less time to develop than the irregular-particle HPTLC plate. The LOS and linearity range were highest for the HPTLC plate with spherical particles, and lowest for the TLC plate. The minimum amounts of caffeine (different for each plate) gave approximately the same area counts (~400). As an example, Fig. 1 shows the calibration plot obtained for caffeine on a spherical-particle HPTLC plate.

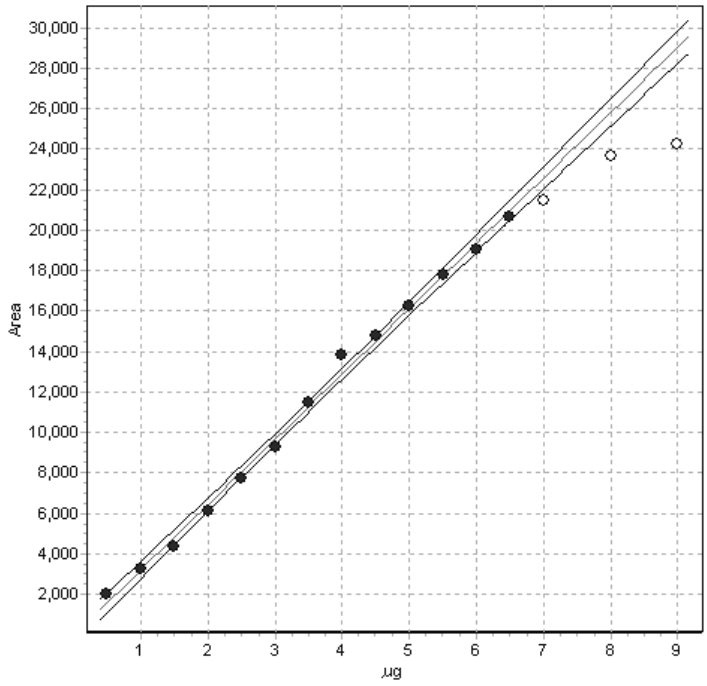

Fig. 1

Calibration plot for caffeine on a spherical-particle HPTLC plate. The *y* axis corresponds to peak area and the *x* axis to the weight in µg. The black spots are those included in the calibration plot (correlation coefficient 0.9958). The gray spots occur after the onset of non-linearity and are therefore not included in the calibration plot

Dye Mixture

Better *N* and *R* values were again obtained by use of the TLC plate. Because there were six main dye zones in the mixture and some impurity zones that gave measurable peaks, six *R* values were calculated for each

plate, and one *N* value for the zone of Sudan II dye. The highest value of *N* was obtained for the TLC plate and the lowest value was obtained for the spherical-particle HPTLC plate. The highest first three *R* values (for the four bottom zones) were obtained on the TLC plate and the lowest *R* values for those zones were obtained on the spherical-particle HPTLC plate. For the fourth and fifth *R* values the values were highest on the spherical-particle HPTLC plate and lowest for the HPTLC plate with irregular particles. The sixth *R* value was for separation of the top main dye, fast red 7B, from the yellow-colored zone of an impurity. Complete separation of these two zones occurred on the TLC plate only; partial separation only was achieved on the spherical-particle HPTLC plate and no separation on the irregular-particle HPTLC plate. Development times on all plates were the same as for analysis of tablet components.

Data from and illustrations of the separations are given in Table II and Figs 2–4.

Table II

Results for the dye components on spherical-particle HPTLC, irregular-particle HPTLC, and TLC plates

Plate	HPTLC		TLC
	Spherical particles	Irregular particles	
LOS (μg)	4.0	8.0	12.0
R_1 [a]	1.57 ± 0.015	2.53 ± 0.026	2.99 ± 0.11
R_2 [b]	1.91 ± 0.032	2.23 ± 0.066	2.52 ± 0.070
R_3 [c]	1.14 ± 0.031	1.35 ± 0.040	1.42 ± 0.035
R_4 [d]	1.68 ± 0.025	1.54 ± 0.025	1.57 ± 0.015
R_5 [e]	1.28 ± 0.021	1.07 ± 0.047 [h]	1.10 ± 0.12
R_6 [f]	0.51 ± 0.035	0.0	1.07 ± 0.082
N [g]	1048 ± 9	1766 ± 196	1997 ± 17

[a]Resolution between the Sudan orange G (1) and the solvent blue 35 zones
[b]Resolution between the solvent blue 35 and the Sudan II zones
[c]Resolution between the Sudan II and the solvent green 3 zones
[d]Resolution between the solvent green 3 and the Sudan orange G (2) zones
[e]Resolution between the Sudan orange G (2) and the impurity zones
[f]Resolution between the impurity and the fast red 7B zones
[g]Number of theoretical plates for the Sudan II zone
[h]Resolution between the Sudan orange G (2) zone and the single peak for both the impurity and the fast red 7B zones (because the last two were not separated on the irregular-particle HPTLC plate)

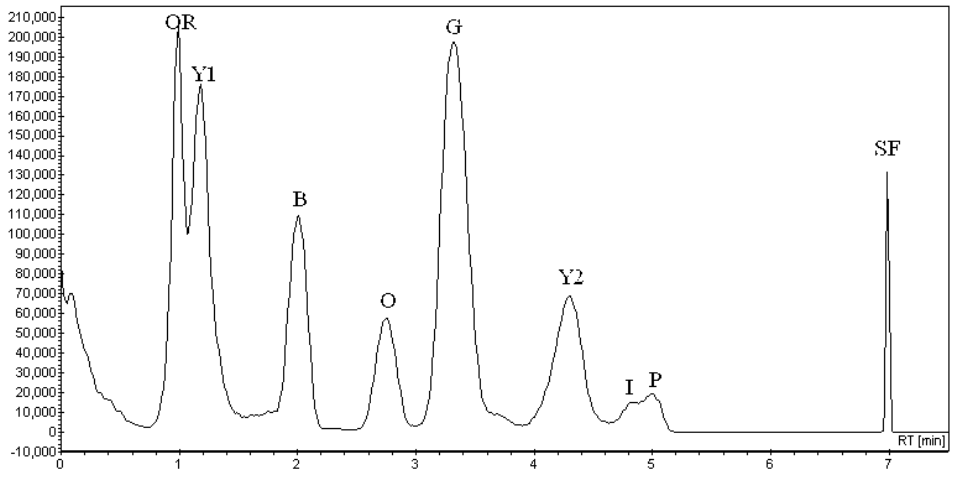

Fig. 2

Densitogram obtained from separation of the dyes in 2 µL of 2 mg mL^{-1} Test Dye Mixture IV on a spherical-particle HPTLC plate: OR, origin; Y1, Sudan orange G (1); B, solvent blue 35; O, Sudan II; G, solvent green 3; Y2, Sudan orange G (2); I, impurity; P, fast red 7B; SF, solvent front. The *x* axis is in centimeters corresponding to zone migration distances on the layer

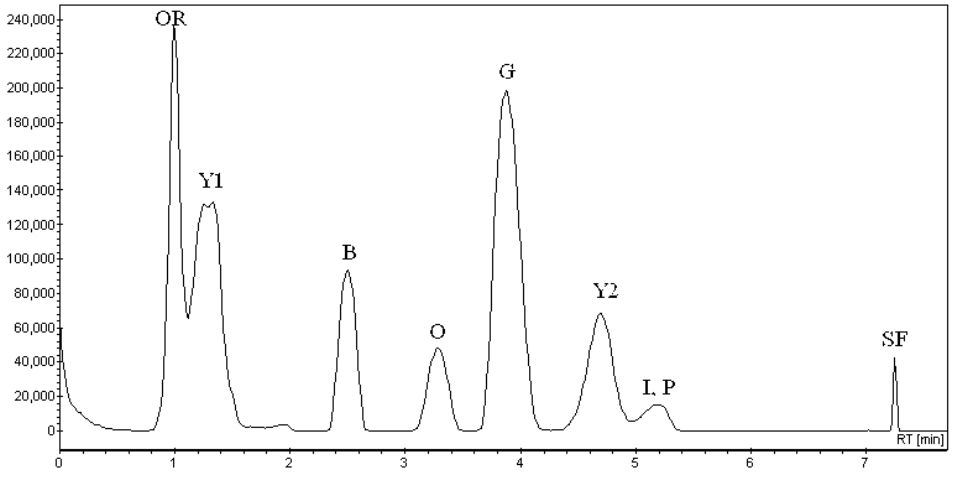

Fig. 3

Densitogram obtained from separation of the dyes in 4 µL of 2 mg mL^{-1} Test Dye Mixture IV on an irregular-particle HPTLC plate. Peak and *x* axis labeling as for Fig. 2

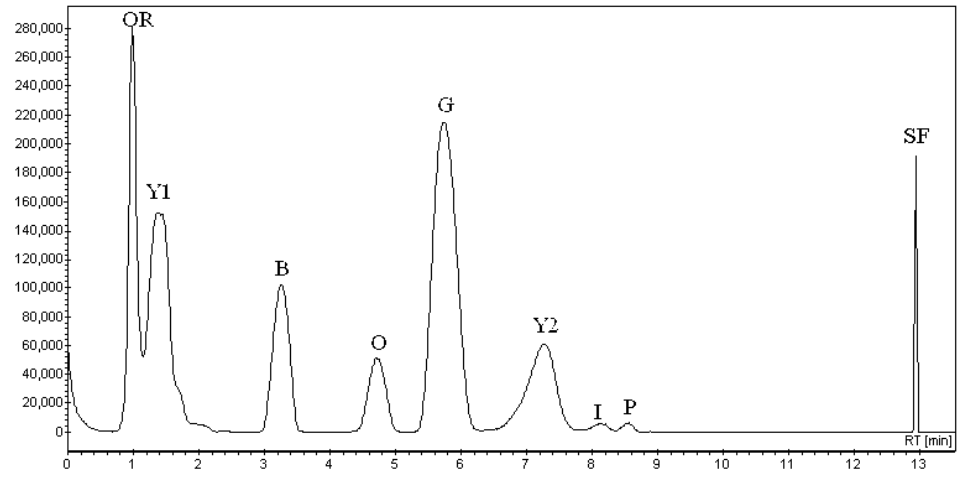

Fig. 4

Densitogram obtained from separation of the dyes in 6 μL of 2 mg mL^{-1} Test Dye Mixture IV on a TLC plate. Peak and *x* axis labeling as for Fig. 2

DISCUSSION

The lowest possible weight of sample that enabled accurate scanning of the most poorly detected compound was applied to each plate, so the plate would not be overloaded and comparisons of *N* and *R* would be valid. Plates were also developed over their generally recommended, standard distance (6.0 cm for HPTLC and 12.0 cm for TLC). Calibration plots were constructed, by linear least-squares regression starting with the lowest scannable weight, to determine the range of linearity.

N and *R* values were calculated from measurements of zones detected visually directly on the layer, and from measurements of peaks from densitometric scanning. The maximum of the scanned peak was used as the center of the zone, and the zone width was measured as the width of the scanned peak at the baseline [6]. Zones on densitograms were wider than those detected visually, but comparable zone-center measurements were obtained. This led to lower calculated *N* and *R* values from direct plate measurements, but trends among the plates were the same by both methods.

Statements in the book and journal literature and in manufacturers' catalogs lead to the expectation that spherical-particle HPTLC plates would give the best results, followed by irregular-particle HPTLC and TLC plates.

As described above, the results obtained under our experimental conditions do not uniformly confirm this order of performance. *N* and *R* values were higher on TLC plates than on HPTLC plates because *z* values (zone migration distance, used to calculate *N*) and Δz values (zone separation distance, used for calculation of *R*) increased more quickly than *w* values (zone widths) for the longer development distance (*l* value) used for TLC compared with HPTLC. Although we tested only a small number of compounds on plates from one manufacturer and spray-on band application, it is a significant finding that TLC can be more efficient and enable greater resolution than HPTLC if time is taken to develop the additional 6 cm. It is our belief that similar relative results would probably be obtained with other analytes, layers, mobile phases, and detection procedures (e.g. visible detection after derivatization, or fluorescence).

Plates with a concentration (preadsorbent) zone and 19 vertical channels (lanes) can be used to achieve excellent densitometric quantification with manual zone application using a micropipet [7]. We tested an irregular particle, channeled silica gel HPTLC plate with a large-pore silica concentration zone (EMD Chemicals; catalog no. 13 153) to see if it was advantageous also with automated sample application. Most of the *R* and *N* values calculated for this plate were higher than for both the spherical and irregular-particle HPTLC plates, and were close to or even exceeded the *N* and *R* values obtained for the TLC plate. Separation of the top zone from the impurity did not, however, occur on this preadsorbent irregular-particle HPTLC plate. This plate is no longer sold by EMD Chemicals, but our results indicate that it might be valuable for some separations because of the double effects of automated Linomat band application with a concentration zone to reduce the widths of developed zones. Densitograms could be recorded on the channeled plate using the ChromImage with exactly the same operating procedure as for unchanneled plates [5], except that the plate image would have to be exported to Diamir with manual selection (the lanes should be defined manually so that the selected area does not include the channels).

Another plate with spherical silica gel particles available from EMD chemicals has a 0.1 mm layer of spherical 3–5 μm particles on an aluminum support [2]. This layer was made for Raman spectrometry and results in a tenfold increase in signal sensitivity compared with a similar layer with irregular silica gel. We did not have this layer available for inclusion in the comparative study.

Ultra-TLC (UTLC) [8,9] uses a new type of silica gel monolithic layer with no particles (EMD Chemicals; catalog no. 1.05007.0001). Its physical properties are 60 mm × 36 mm plate dimensions, 10-µm layer thickness, 30–40-Å-diameter meso pores, 1–2-µm-diameter macro pores, ca. 350 $m^2 g^{-1}$ specific surface area, and ca. 0.3 mL g^{-1} specific pore volume (meso pores). The recommended sample application volume is 5–20 nL and the recommended migration distance is 1–3 cm, but the concentrations of the solutions used are not specified [8,9]. We could not test this layer with a tablet solution because it is not sold with a fluorescent indicator. Although we did not have a 20-nL micropipet, we spotted approximately 20 nL of the most concentrated dye solution from a 100 nL Drummond Microcap micropipet (Broomall, PA, USA) and developed the UTLC plate in a twin-trough chamber sold for developing 10 cm × 10 cm HPTLC plates; only two of the zones were visible. The smallest application volume that gave visible spots for all the dye components was 200 nL; in the resulting chromatogram the bottom two zones were well separated from each other and from the third zone, but the top four zones were unresolved. UTLC plates have a very thin layer that is somewhat fragile and require a very small applied volume but, to enable detection, a minimum weight must still be applied in this volume. They seem to be an interesting prototype layer but their value in practical analysis of real samples has not yet been widely demonstrated.

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