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Short Communication

Comparison of commercially available thin-layer chromatography plates with mixtures of dyes, analgesics and phenols

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ABSTRACT

A series of five commercially available thin-layer chromatography plates has been compared using three different mixtures. The plates examined were Analtech, Baker, Merck, Whatman and Eastman Kodak silica gel plates. The first four are layers on glass while the last is a layer on a polyester sheet. All have a F_{254} fluorescent indicator. The Kodak plate is not strictly comparable to the others but was included since it is quite widely used. Some very significant differences were noted in R_F values as well as in the relative order in which the components were eluted. The highest R_F values on glass plates were generally obtained either using Analtech or Whatman plates depending on the solvent. The lowest R_F values were nearly always obtained using Merck plates. Screening of chromatographic plates is particularly important when analyzing unknown mixtures.

INTRODUCTION

Thin-layer chromatography (TLC) as an analytical tool has been in use for many years. Although it can be used as a method of quantitative analysis, the major uses are for qualitative purposes. Identification of the components of a mixture is a primary use. Another practical use is in screening reaction mixtures for both major and minor products – either known or unknown.

Industrial as well as academic laboratories frequently have on hand several types of commercially available TLC plates. However, it has been our experience that screening of the plates themselves is seldom done. Many investigators use a "favorite" plate or one that has worked well on a previous project or has been recommended by a colleague. The purpose of the present work was to analyze several mixtures in order to compare commercially available TLC plates under identical conditions and solvent systems to determine whether differences in the plates would give varying results.

A number of references have been found comparing reversed-phase TLC plates under various conditions [1–6]. Work has been done on toxicologically interesting substances comparing silica gel coated films and sheets with silica gel coated glass plates [7]. Work has also been done with varying solvent systems since these play a major role in analyses by TLC. It is believed, however, that the majority of investigators tend to screen solvent systems much more extensively than the plates themselves [8–11].

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EXPERIMENTAL

Mixtures analyzed

Three different mixtures have been analyzed in order to determine if differences exist between various commercial TLC plates. The first mixture examined was a solution of six major dyes in toluene and is available as Test Dye Mixture IV (30-04) from Analtech. A number of minor unknown dyes were present. The major dyes are listed in Table I with the color and a "typical" R_F value on silica gel with toluene as the mobile phase as reported by Analtech in an unnumbered bulletin accompanying the dye mixture. No details are given on plate or experimental conditions.

The second mixture examined consisted of four compounds found in various commercial analgesic products such as Advil, Tylenol, Anacin and Excedrin. The compounds are acetaminophen, ibuprofen, acetylsalicylic acid and caffeine and were dissolved in chloroform–ethanol (1:1).

The third mixture tested was composed of phenol, *o*-hydroxymethylphenol (o-MP), *p*-hydroxymethylphenol (p-MP), *o,o'*-dihydroxydiphenylmethane (o,o'-DHDPM) and *p,p'*-dihydroxydiphenylmethane (p,p'-DHDPM) dissolved in methanol. This mixture is typical of those found in commercial water soluble phenol-formaldehyde resins [12].

Each of the three mixtures was run on the five TLC plates with different solvents. The R_F values reported are the average of three separate runs.

TLC plates

Five commercially available TLC plates were used in obtaining data. These plates are identified as Analtech 47511 (Newark, DE, USA), Baker 7001-04 (Phillipsburg, NJ, USA), Eastman Kodak 13181 (Rochester, NY, USA), Merck 5715-7 (Darmstadt, Germany) and Whatman 4500-105 (Maidstone, UK). With the exception of the Kodak plate all were 250-μm silica gel layers on glass with a fluorescent indicator. The Kodak plate is a 100-μm layer on a polyester sheet and although not directly comparable was included in the study because it is widely used in academic and industrial laboratories. All plates have an average pore diameter of 60 nm. The Baker plate contains an inorganic binder while all others have an organic binder. This information was obtained from technical bulletins or direct contact with the manufacturer.

Before use, the plates were cut to *ca.* 5 cm wide by 10 cm long. The test mixtures were applied from a microliter syringe and allowed to dry. The sample volume was 4 μl and the spot diameter was 4–5 mm. The plates were not pre-dried but used directly so that temperature and humidity conditions would be equal.

Procedure

The solvent to be used was placed in a chromatography tank (28 × 25 × 7.5 cm) and allowed to stand 1 h at room temperature (23–24°C). The spotted plates were all placed in the solvent at the same time and the solvent allowed to rise about 9 cm before removing and drying. The plates run with the dye mixture were examined directly. The plates run with the analgesic mixture and the phenols were examined under a 254 nm ultraviolet lamp and the spots marked. As a secondary detection method the phenol plates were sprayed with a ceric ammonium nitrate solution since any overlapping or unknown spots can often be identified by the color [12].

TABLE I

COMPOSITION OF ANALTECH DYE MIXTURE (MAJOR DYES ONLY)

From Analtech Bulletin.

Dye	Color	Typical R_F
Fat Red 7B	Purple	0.64
Solvent Green 3	Green	0.38
Sudan II	Peach	0.34
Sudan II	Orange	0.28
Sudan Blue	Blue	0.16
Sudan Orange G	Yellow	0.03

RESULTS AND DISCUSSION

Dye mixture

There is an obvious difference in the R_F values reported by Analtech in Table I using toluene with a silica gel plate and those obtained in our data. Even using a commercially available Analtech plate our data were from 44 to 81% higher for the various major spots. All of the other layers on glass showed similar but smaller differences. The Kodak plate showed differences even larger – from 53 to 243%.

Another difference observed was the relative lo-

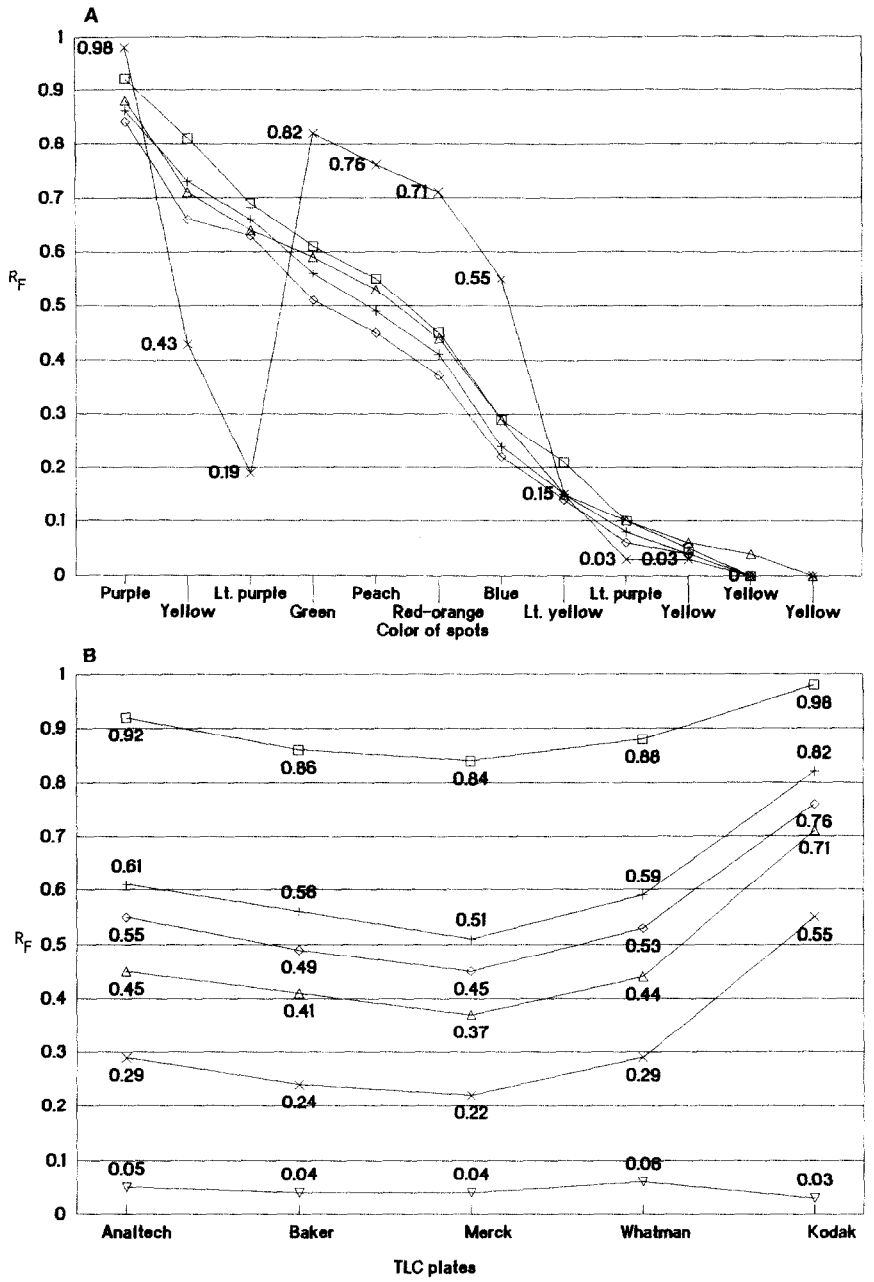

Fig. 1. (A) R_F values of dye mixture with toluene mobile phase; □ = Analtech; + = Baker; ◇ = Merck; △ = Whatman; × = Kodak. (B) R_F values of major dyes only with toluene mobile phase; □ = purple; + = green; ◇ = peach; △ = red-orange; × = blue; ▽ = yellow; Lt. = light.

cation of various spots obtained with the Kodak plate as compared to the layers on glass. On the Kodak plate as shown in Fig. 1A, two minor spots (yellow and light purple) which had the second and third highest R_F value on glass plates with toluene were displaced to sixth and seventh place. A second light purple spot appeared to overlap with the major yellow spot at R_F 0.03. For the major spots the

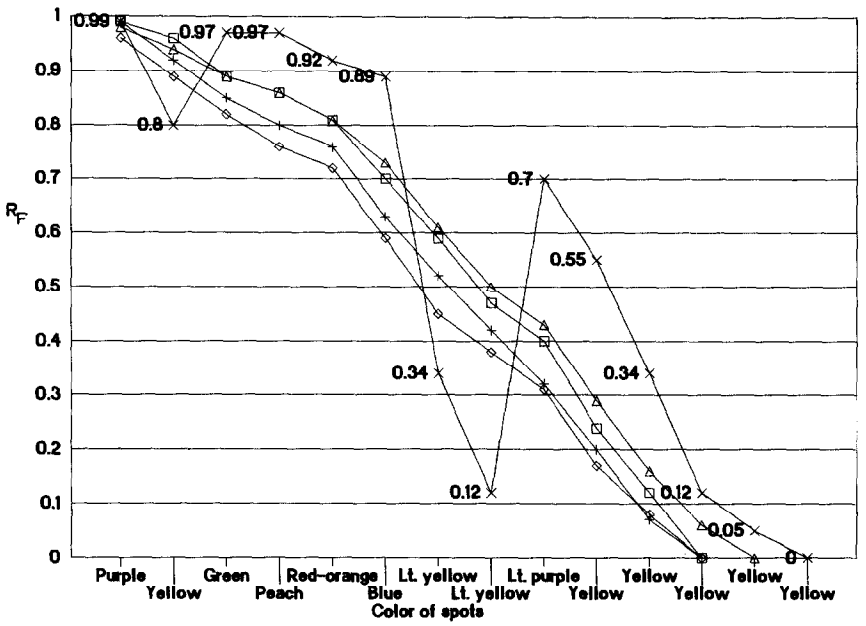

Fig. 2. R_F values of dye mixture with methylene chloride mobile phase. □ = Analtech; + = Baker; ◇ = Merck; △ = Whatman; × = Kodak.

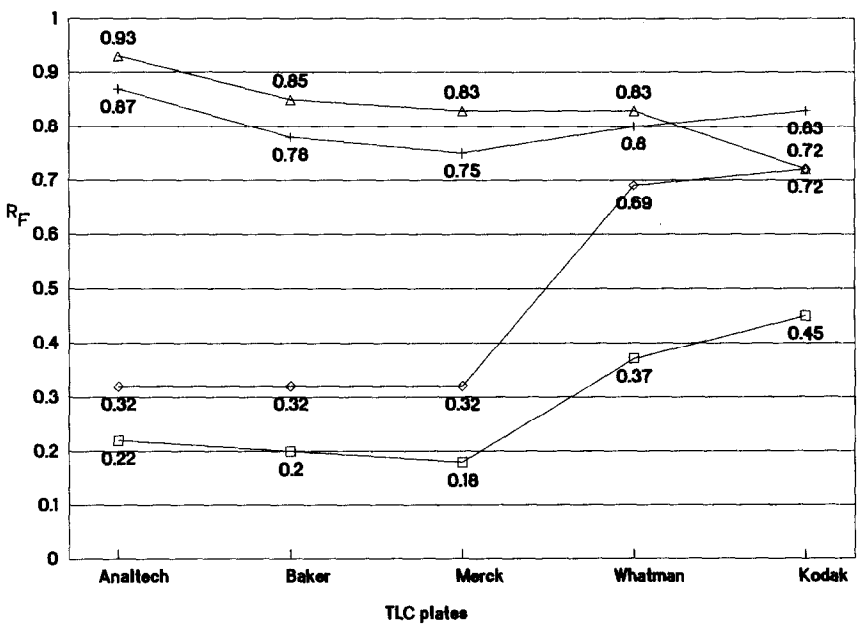

Fig. 3. R_F values of analgesic mixture. Mobile phase 1,2-dichloroethane–acetic acid (12:1); □ = Acetaminophen; + = acetylsalicylic acid; ◇ = caffeine; △ = ibuprofen.

R_F values were much higher on the Kodak plate than on the glass plates as shown in Fig. 1B with the exception of the yellow dye. Fig. 1B shows values plotted for the six major dyes only, with spot separations being nearly equal on all glass plates. The R_F values follow a pattern with Analtech and Whatman having the highest values and Merck the lowest.

With methylene chloride as the mobile phase the order in decreasing R_F values was somewhat different as shown in Fig. 2. With the glass plates the yellow minor spot had the second highest R_F and the light purple spot was ninth. With the Kodak plate the yellow minor spot was fifth and the light purple was sixth. On this plate the major peach-colored spot was not detected, possibly due to overlapping with the major green spot at R_F 0.97. The Whatman plate with toluene gave the best separation of twelve spots observed. Some overlapping of spots occurred with methylene chloride.

Analgesic mixture

The mixture of analgesics also showed some wide variations in R_F values for a given compound in a mixture. With glass plates only, caffeine with 1,2-dichloroethane–acetic acid as the mobile phase had R_F values ranging from 0.32 to 0.69; with acetaminophen the values ranged from 0.18 to 0.37 [13]. With ethyl acetate the values for acetylsalicylic acid ranged from 0.43 to 0.85. The R_F values for 1,2-dichloroethane–acetic acid were on average highest with Analtech, followed by Whatman, Baker and Merck with exceptions being caffeine and acetaminophen with the Whatman plate as shown in Fig. 3.

With ethyl acetate Analtech and Whatman gave the highest values for glass plates while Merck was lowest with the exception of acetylsalicylic acid. The best separation of analgesics as shown by the data in Fig. 4 was on the Whatman plate with ethyl acetate as the solvent. Actually all four glass plates gave good separations with differences being slight when ethyl acetate was the solvent. The Kodak plates in both solvent systems gave good separation of two components but overlapping of spots resulted in poor overall separation of all four components.

An interesting difference was also noted in the relative spot locations on the glass plates with ethyl acetate. The Analtech, Baker and Merck plates gave ibuprofen, acetylsalicylic acid, acetaminophen and caffeine in descending order of R_F values. On the Whatman plate acetaminophen and acetylsalicylic acid were reversed.

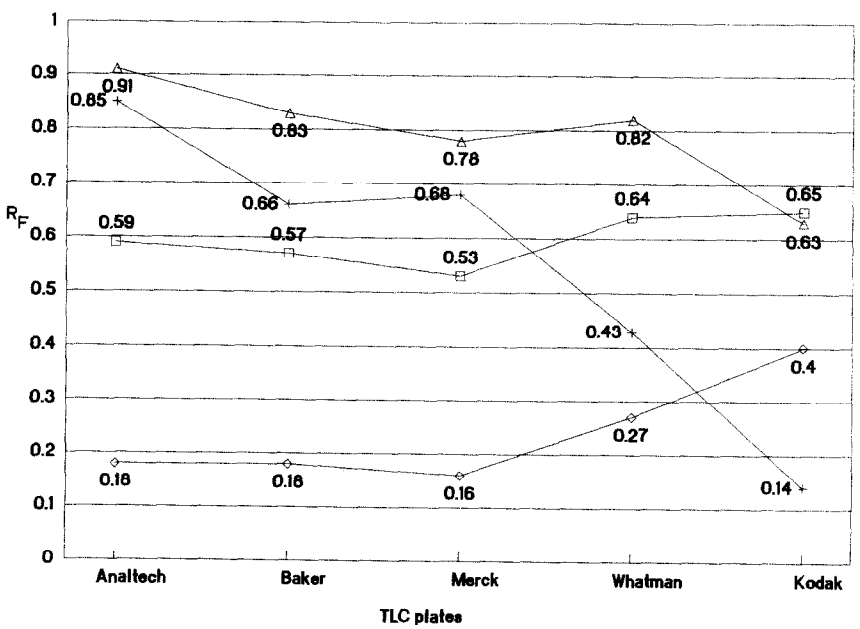

Fig. 4. R_F values of analgesic mixture. Mobile phase ethyl acetate. Symbols as in Fig. 3.

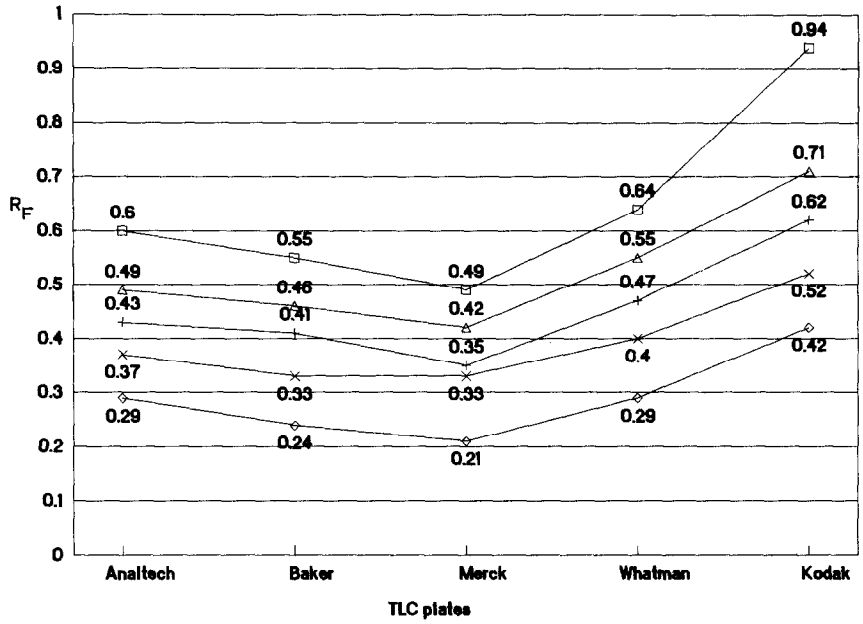

Fig. 5. R_F values of mixture of phenols. Mobile phase cyclohexane–acetone (7:3). □ = Phenol; + = o-MP; ◇ = p-MP; △ = o,o'-DHDPM; × = p,p'-DHDPM.

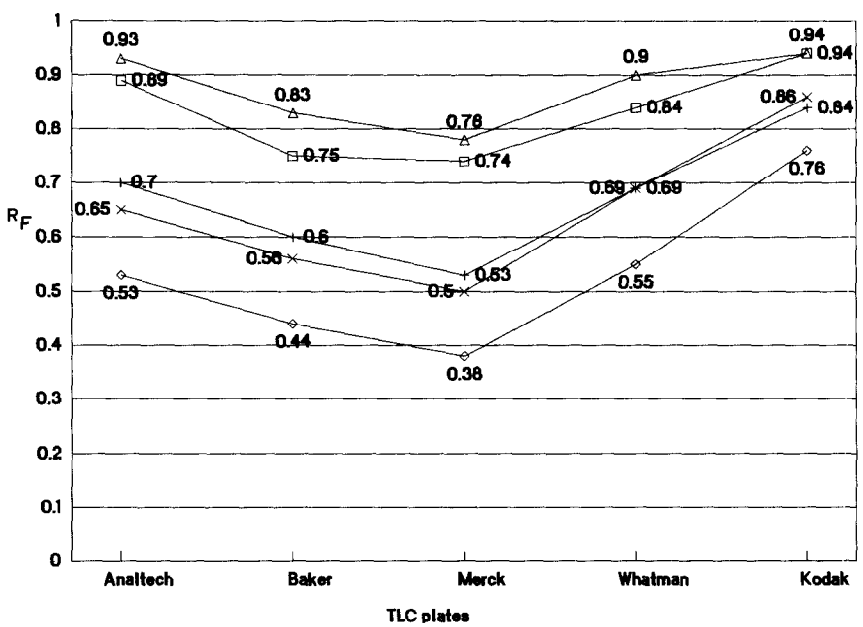

Fig. 6. R_F values of mixture of phenols. Mobile phase chloroform–methanol (92:8). Symbols as in Fig. 5.

TABLE II

RELATIVE EFFECTIVENESS OF SEPARATION

Relative separation effectiveness	Dye mixture		Analgesic mixture		Phenol mixture	
	Toluene	Methylene chloride	Dichloroethane–acetic acid	Ethyl acetate	Cyclohexane–acetone	Chloroform–methanol
Best	Analtech	Merck	Merck	Analtech	Kodak	Analtech
	Baker	Baker	Baker	Baker	Whatman	Baker
	Kodak	Analtech	Analtech	Whatman	Merck	Merck
	Merck	Whatman	Whatman	Merck	Analtech	Whatman
	Whatman	Kodak	Kodak	Kodak[b]	Baker	Kodak[b]
Worst	Analtech[a]					

[a] From Table I (Analtech data).
[b] Spots overlapped.

Phenol mixture

Data for the mixture of phenols are shown in Figs. 5 and 6. Although there were some R_F variations between the glass plates, the range of variations was not as great as with the dyes and analgesics. However, the range of R_F values was in nearly the same order as with previous mixtures. Either the Analtech or Whatman plates gave the highest values depending on the solvent used, followed by Baker and then Merck with the lowest values. The maximum range between high and low R_F values for the glass plates was 45%. It is of interest that using the two different solvent systems the relative positions of phenol and o,o'-DHDPM were reversed. With cyclohexane–acetone, phenol had the highest R_F value on all five plates while o,o'-DHDPM was second. With the chloroform–methanol solvent the positions were reversed on all the glass plates and the two spots coincided on the Kodak plate. This was determined by observation of the spot colors obtained on spraying the chromatograms with ceric ammonium nitrate after the examination under UV light.

General

(1) Several factors common to all solvents and plates have been observed. Table II shows the relative effectiveness of separation based on distances between spots on each plate.

(2) Highest R_F values on glass plates are consistently found with either Analtech or Whatman with Merck being lowest.

(3) With a few exceptions reasonable separations are obtained on all of the glass plates in spite of large variations in R_F values or reversal of relative spot locations.

(4) In comparing the Kodak flexible plate with the glass plates, the Kodak plate generally has higher R_F values probably because of the smaller layer thickness. The exception is the analgesics where acetaminophen and caffeine have lower values.

(5) Run times on the Kodak plate are 2–3 times longer than on the glass plates, all of which are consistent within 1–2 min.

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