

CHROM. 6939

Note

Variations in R_F sequence in thin-layer chromatography on precoated plates

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(Received June 14th, 1973)

Rábek¹ reported inversions in the elution order of vitamins during thin-layer chromatography (TLC) when silica gel plates from various suppliers were utilized. In a discussion following the presentation of his work, he noted that the observed phenomenon could have been due to changed composition of the solvent and/or chamber saturation. Kananen *et al.*, in their extensive TLC comparison study², recorded apparent elution order inversions among plates from different suppliers, but made no comment. In attempting to utilize a published method for the TLC separation of antimicrobial agents³, we encountered a clear example of elution order inversion between plates from different suppliers. This appears to be due to interaction between the compounds and the binder and/or silica gel utilized by the suppliers.

Merck silica gel F₂₅₄ (E. Merck, Darmstadt, G.F.R.) precoated layers developed in benzene-ether (80:20) have been reported to resolve mixtures of hexachlorophene (HCP), trichlorocarbanilide (TCC) and tribromosalicylanilide (TBS) with increasing R_F values, respectively³ (Table I). In attempting the same separation using the stated system in a saturated chamber, and spotting components at levels of 1-5 μ g, it was found that the elution order was altered to TCC-HCP-TBS. Other brands of ready-made and self-prepared layers were examined. The inverted elution sequence was also observed with most of these (Table I). However, silica gel GF/MH (medium hard) and silica gel G/HLF (hard) layers, both prepared by Analtech (Newark, Del., U.S.A.), afforded the published elution order (HCP-TCC-TBS)³.

In order to confirm that the observed elution sequence inversions were due to the layers rather than variations in the TLC conditions, layers exhibiting both elution orders were chromatographed simultaneously in the same chamber. Elution orders were unchanged.

Other solvent systems were examined for separating the three antimicrobial agents. A number of these appeared to give rise to elution order inversions compared with a majority of the layers. However, the variations were not as dramatic as those found with benzene-ether, and could be due to normal R_F variations. Typical of these was the hexane-ethyl acetate (70:30) system (Table I).

In the benzene-ether (80:20) solvent system, the R_F values for HCP on plates

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TABLE I
R_F* VALUES FOR LAYERS

Layer description**	Benzene-ether (80:20)			Hexane-ethyl acetate (70:30)		
	Hexachloro- phene (HCP)	Trichlorocarb- anilide (TCC)	Tribromosalicyl- anilide (TBS)	Hexachloro- phene (HCP)	Trichlorocarb- anilide (TCC)	Tribromosalicyl- anilide (TBS)
Silica gel F ₂₅₄ Merck precoat (ref. 3)	0.25-0.36	0.35-0.46	0.78-0.86	—	—	—
Silica gel G Merck self-made	0.23	0.17	0.49	0.12	0.23	0.55
Silica gel F ₂₅₄ Merck precoat	0.29	0.14	0.54	0.09	0.11	0.39
Silica gel F ₂₅₄ Merck precoat on aluminum	0.25	0.12	0.51	0.09	0.09	0.39
Silica gel 60 Merck precoat	0.26	0.13	0.52	0.13	0.11	0.45
Silica gel H Merck self-made	0.26	0.18	0.39	0.08	0.16	0.39
Silica gel G Woelm-Analtech precoat	0.27	0.16	0.64	0.11	0.15	0.45
Silica gel GF Macherey, Nagel & Co.*** precoat on plastic	0.26	0.12	0.47	0.14	0.10	0.39
Sil G (without CaSO ₄) Macherey, Nagel & Co. precoat on plastic	0.17	0.15	0.42	0.05	0.13	0.38
Silica gel GF/MH Analtech precoat	0.07	0.24	0.43	0.02	0.21	0.30
Silica gel G/HLF Analtech precoat	0.07	0.13	0.41	0.03	0.09	0.18

* Mean for three plates.

** Unless otherwise noted carrier plate is glass; layers 250 μm; precoated layers used as supplied; non-fluorescent layers visualized by spraying with 0.005% fluorescein in 0.5 N ammonia and viewing under short-wave UV light[†].

*** Macherey, Nagel & Co., Düren, G.F.R.

exhibiting the elution order inversion were similar to those reported earlier³. Values for TCC and TBS appeared to be markedly suppressed (Table I). On the two layers which were found to afford the published elution order (Analtech silica gel GF/MH and G/HLF), R_F data for TCC and TBS were similar to those found with the other layers examined by us, but the R_F of HCP was clearly depressed.

The dearth of information regarding the preparation of the various brands of silica gel layers and the nature of binders utilized in preparing ready-made layers, makes it difficult to rationalize the elution order inversions reported here and those found previously³. Indeed changes in the composition of any one of these, instituted presumably to produce a superior layer, would result in the reported effects on R_F values.

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