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Note

Two-dimensional TLC of lipophilic compounds

Characterization of a mixed stationary phase permitting both adsorption TLC and reversed-partition TLC on one plate

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Appropriate choice of solvents for two-dimensional thin-layer chromatography (TLC) of polar substances can allow unique separations in each dimension on a single stationary phase by varying the relative contributions of adsorption, partition, pH, and ionophilic character. No such flexibility exists for non-polar substances, which separate on an essentially unidimensional polarity basis with a hydrophilic stationary phase, or on a reversed-partition basis with a hydrophobic stationary phase.

Previous two-dimensional TLC of non-polar lipids has involved adding $AgNO_3$, paraffin, or silicone oil to change the stationary phase characteristics before the second elution[1–4]. Now a method is reported of achieving adsorption or partition TLC in successive developments on the same chromatoplate without changing stationary phase composition.

MATERIALS AND METHODS

Silica gels G, H, and "H (silanized)" were obtained from E. Merck (Darmstadt, G.F.R.). Calcium sulfate dihydrate (AR) (Mallinckrodt, St. Louis, Mo., U.S.A.) was converted to the hemihydrate by heating at 110 °. Standards from commercial sources were purified by crystallization or conventional TLC. All solvents were distilled before use. Dimethyldichlorosilane (DMCS) was obtained from Applied Science Labs., State College, Pa., U.S.A.

Silica gel H was silanized by slurrying in anhydrous toluene, and adding DMCS in 1-ml portions with stirring until further addition did not produce ebullition (caution: HCl gas evolved.) If the required excess of DMCS is present, addition of a few g of fresh silica gel H will produce rapid bubbling. The slurry was allowed to stand overnight at room temperature, loosely covered. The preparation was completed by vacuum-assisted filtration in a büchner funnel, rinsing with 3 volumes of anhydrous benzene, followed by 3 volumes of anhydrous methanol. The büchner funnel was loosely covered, and vacuum-assist maintained for 4 h. Drying was completed at 70° in a tray[5]. The product was designated silica gel "HS".

The mixed sorbent, termed silica gel "G+HS", was prepared by carefully mixing the following weight percentages: 50% silica gel G, 43% silica gel HS, and 7% $CaSO_4 \cdot \frac{1}{2}H_2O$. No significant differences were found upon substitution of silica gel "H" for "G", or commercial silica gel "H (silanized)" for "HS". Optimum layer integrity was obtained with $>10\%$ $CaSO_4 \cdot \frac{1}{2}H_2O$ (including the amount found in silica gel G). Layers were spread on 20×20 cm glass plates as a 70% aqueous ethanol slurry. Plates were dried overnight at room temperature. Activation, when desired, was accomplished at 70° overnight. Non-activated plates were found suitable for our purposes, and were used throughout. TLC was accomplished by the ascending technique in a vapor-saturated tank. If solvents were carefully removed from the plate between developments, the R_F values in the second dimension were equivalent to those obtained by unidimensional TLC in that solvent. Visualization of spots was accomplished with iodine vapor, or by spraying with 50% aqueous H_2SO_4 and heating at 120°. For preparative purposes, the still slightly damp plate was scraped and the scrapings eluted with ethyl acetate.

RESULTS

The solvent systems used were as follows: (I) hexane–ethyl acetate (95:5), (II) hexane–ethyl acetate (90:10), (III) hexane–ethyl acetate (80:20), (IV) methanol–water (70:30), (V) *p*-dioxane–water (75:25), and (VI) *p*-dioxane–acetic acid–water (50:30:20).

Table I shows R_F values for various standards in several different solvent systems. The major features of interest are the normal, silica gel G-type separations found with non-polar solvents (I, II, III), and the reversed-phase partition separations found with polar solvents (IV, V, VI). Trials in our laboratory have shown that methanol or acetonitrile-based solvents containing water are not suitable for reversed-partition TLC of non-polar high-molecular-weight lipids like monohydroxysteroids, as these tend to remain at the origin because of low solubility in the solvent system. This characteristic can be exploited to separate lower terpenes from steroids, as shown by solvent system IV. Otherwise, dioxane or tetrahydrofuran-based solvents are more suitable for steroid reversed-partition TLC. Even with these solvents, heavy loading (10 μg/spot) with high-molecular-weight lipids must be rigorously avoided.

The R_F values in Table I were obtained by unidimensional TLC, but the R_F values in each direction for two-dimensional TLC are quite comparable. Division of the plate into one- and two-dimensional areas, as shown in Fig. 1, has proved very useful, as standards may be run on the same plate and R_F values directly compared.

DISCUSSION

A practical method for two-dimensional TLC of non-polar lipids has been described. This method was developed for a specific application in our research, but it is a method which suggests general utility in separations of non-polar lipids, a group of compounds for which there has heretofore been no simple easily-reproducible method of two-dimensional TLC.

TABLE I

R_F VALUES FOR DIFFERENT COMPOUNDS USING NON-ACTIVATED SILICA GEL G+HS PLATES.

Elution distance was approximately 14 cm.

Compound	*Solvent systems*					
	I	*II*	*III*	*IV*	*V*	*VI*
Geraniol	0.14	0.24	0.48	0.63	0.72	0.60
Nerol	0.15	—	0.56	0.65	0.71	—
Linalool	—	—	0.81	0.46	0.71	—
Myrcene	—	—	1.0	0.64	0.42	—
α-Terpineol	—	—	0.64	0.65	0.76	—
Terpinolene	—	—	1.0	0.65	0.78	—
Eugenol	0.15	—	0.78	0.72	0.77	0.73
trans,trans-Farnesol	—	0.26	0.51	0.46	0.60	0.52
cis,trans-Farnesol	—	0.33	0.60	0.46	0.60	0.52
Pristane	1.0	1.0	1.0	—	—	—
Phytol	—	—	0.72	—	0.46	0.27
Cholesterol	0.11	0.22	0.59	0	0.45	0.22
5α-Cholestanol	0.11	—	0.57	0	0.40	0.17
Lanosterol	0.20	0.35	0.79	0	0.45	0.22
24,25-Dihydrolanosterol	0.20	—	0.79	0	0.40	0.17
Stigmasterol	0.10	—	0.51	0	0.39	—
β-Sitosterol	0.10	—	0.51	0	0.39	0.20
Cycloartenol	0.21	—	0.82	0	0.39	0.19
24-Methylenecycloartanol	0.21	—	0.82	0	0.39	0.20
Cholesteryl acetate	0.74	—	0.98	0	—	—
Lanosteryl acetate	0.77	—	0.99	0	—	—
Squalene	0.98	0.99	1.0	0	0.21	0.07
Squalane	1.0	1.0	1.0	0	—	—
2,3-Oxidosqualene	0.71	0.92	—	0	0.32	—

The principle of the method is that the two types of silica gel in the stationary phase act independently of each other. In polar solvents adsorption to silica gel G does not occur appreciably, so the major effect is reversed-phase partition with the hydrophobic silanized silica gel. Conversely, in non-polar solvents the major effect is adsorption to silica gel G. An intermediate polarity solvent, like ethyl acetate or ether, permits elution of most non-polar lipids to near R_F 1.0.

The method has the following advantages: (1) difficult-to-reproduce spraying or dipping operations are eliminated; (2) no paraffin or silicone oil contaminates non-polar lipids, so derivatization and/or subsequent TLC are rendered unnecessary; and (3) because the layer composition is uniform, stable, and reproducible, R_F values are stable and standards are easily correlated.

Further improvements may include: use of long-chain alkyl groups in silanization to improve partition properties for reversed-phase TLC[6]; addition of glass fiber or asbestos to improve layer durability; and argentation TLC by the normal method.

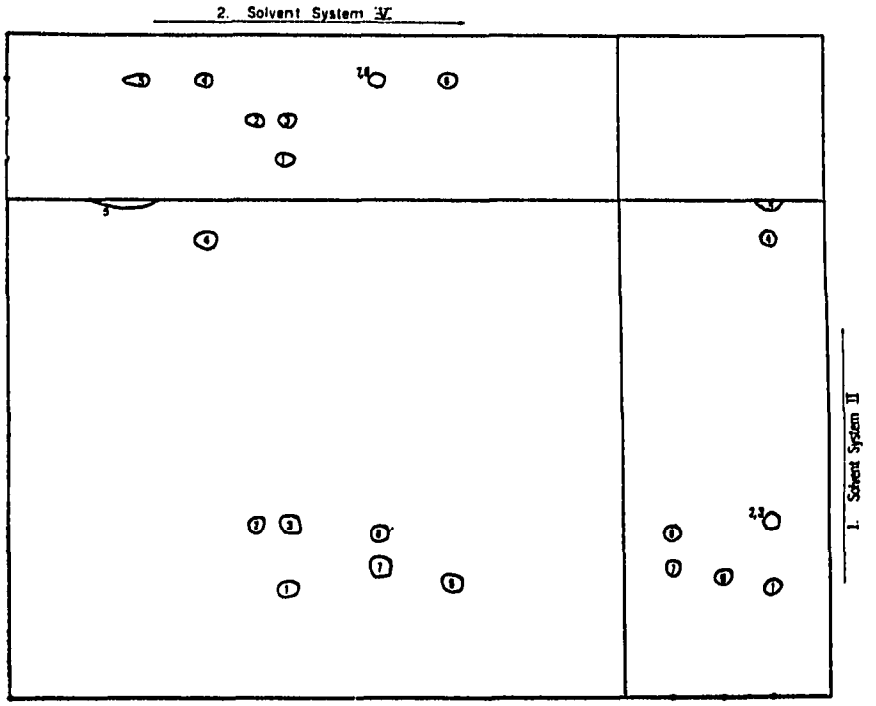

Fig. 1. Two-dimensional TLC of a synthetic mixture, with standards run in unidimensional sections of the plate in each dimension. A non-activated silica gel G+HS plate was developed to 14 cm in each dimension. Development times were 45 min in the first dimension and 2 h in the second. Compounds tested were: (1) cholesterol; (2) dihydrolanosterol; (3) lanosterol; (4) oxidosqualene; (5) squalene; (6) geraniol; (7) *trans,trans*-farnesol; (8) *cis,trans*-farnesol.

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