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Haleem J. Issaq^a

^a Chemical Carcinogenesis Program Frederick Cancer Research Center, Frederick, Maryland

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Two-Phase Thin-Layer Chromatography^{1,2}

Haleem J. Issaq

Chemical Carcinogenesis Program
Frederick Cancer Research Center
Frederick, Maryland 21701

Thin layer chromatography (TLC) is normally carried out on single-phase (adsorbent) plates. However, it may be necessary to use more than one adsorbent to achieve the separation of complex mixtures. Literature reports indicate that the use of mixed phases in TLC is not very common, possibly because the plates are difficult to prepare and precoated single-phase plates of different adsorbents are readily available (silica gel, reversed phase, cellulose, alumina, etc.).

Our experiments have shown that a two-phase TLC plate offers a viable solution for work with mixed adsorbents. The silanized part of the plate is prepared according to the method of Aringer and Eneroth (1), namely by developing a 20 x 20 cm commercially available precoated silica gel plate in hexa-

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methylidisilazane (HMDS) for 10 cm. It is removed from the developing tank, and dried in an oven at 100°C for 2 h to give a two-phase TLC plate having silanized and unsilanized silica gel phases side-by-side. Such a plate offers the benefits of side-by-side positioning of polar and non-polar adsorbents on the same plate and of two-dimensional development on the underivatized silica gel side of the plate. It is also simple to prepare and requires no special apparatus.

To illustrate its use, a mixture of 7 α -, 7 β -hydroxycholesterol, and 5 α , 6 α - and 5 β , 6 β -epoxycholesterol (prepared by derivatization of the epoxycholesterols with tetrasilylimidazole) (Figure 1) was separated. The sample mixture was spotted on the lower left corner of the TLC plate which was coated with underivatized silica gel. The plate was developed in diethylether.

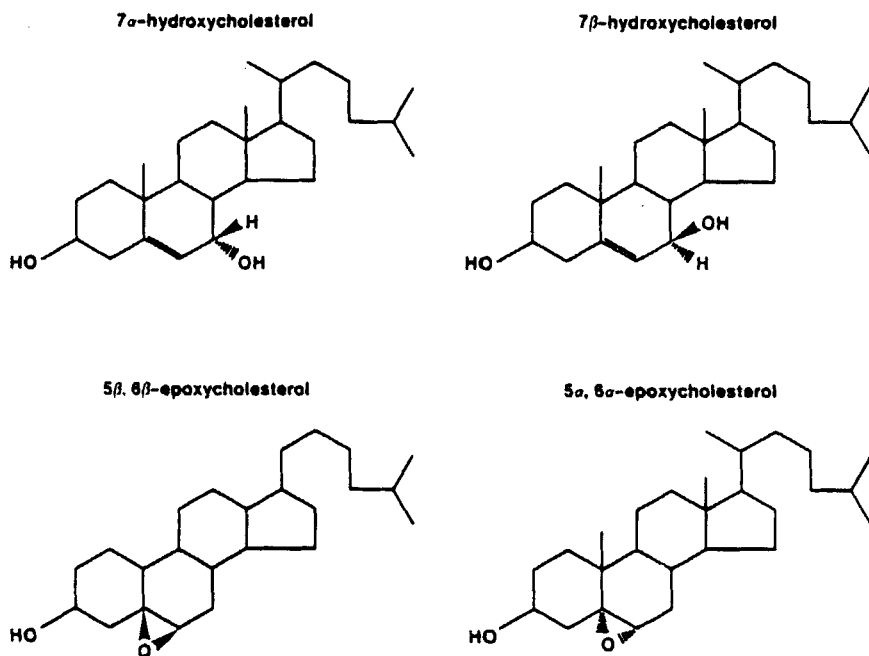
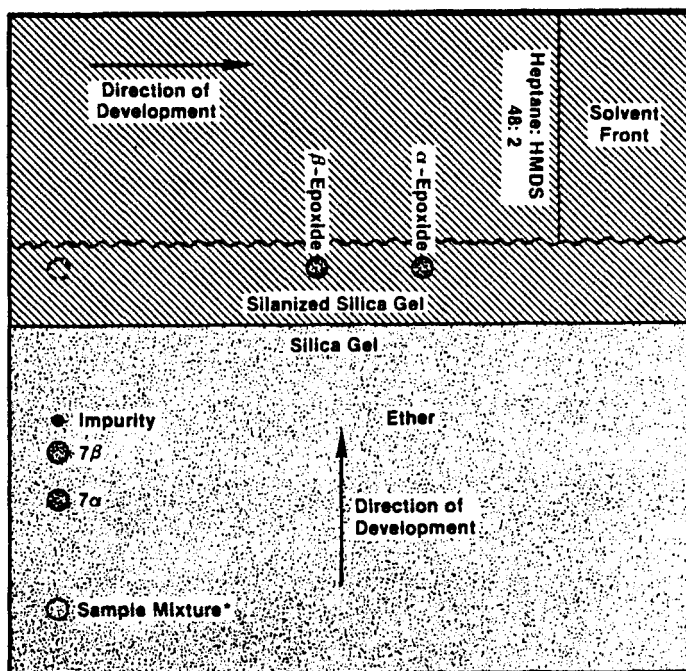


Figure 1. Structural formulae of cholesterol separated by two-phase TLC.

After the solvent front had traveled 2-3 cm into the silanized side, the plate was removed, dried, turned 90°, and developed in heptane:HMDS (48:2). Figure 2 shows the separation of the mixture of cholesterol, in which resolution of the isomeric epoxide mixture was subsequently obtained on the silanized absorbent side of the plate.

The two-phase TLC plate may be used for cleanup of a sample on one phase and separation on the other, and for the separation of complex mixtures of

2-Phase TLC



*Sample Contains: 7α -hydroxycholesterol
 7β -hydroxycholesterol
 TMS derivatives of 5α , 6α -
 and 5β , 6β -epoxycholesterol

Figure 2. Separation of 7α -, 7β -hydroxycholesterol, and TMS derivatives of 5α , 6α - and 5β , 6β -epoxycholesterol on two-phase TLC plates.

varying polarities. The sample can be spotted on either phase of the plate and the width of the silanized part of the plate may be adjusted as needed. Other silylating agents and phases can be prepared as needed.

REFERENCES

1. L. Aringer and P. Eneroth, *J. Lipid Res.* 15, 389 (1975).