Analysis of Phospholipids by One-Dimensional Thin-Layer Chromatography

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Introduction

Thin-layer chromatography (TLC) has been used widely for phospholipid analysis (1). Complete separation of the six major phospholipid groups usually requires two-dimensional TLC development (2). Although the two-dimensional technique is quite useful for complex mixtures, it suffers the disadvantage of being able to handle only one sample per plate.

A growing number of methods have been developed to attempt one-dimensional phospholipid separations. Some of these methods use TLC plates prepared with various buffer systems (3). Others specify the use of standard silica gel plates (4). Our laboratory, however, has received reports concerning difficulty in duplicating work performed elsewhere (5). Most, if not all, of these methods use a mobile phase that is based on chloroform and include polar modifiers.

This article presents a one-dimensional HPTLC phospholipid separation that minimizes the effect of relative humidity as well as that of slight variations in the mobile phase composition.

Experimental

Biological samples were supplied as whole bovine-brain, gerbil-liver, and soybean extracts. Separations were performed on preadsorbent high-efficiency silica gel, type HLF plates (Analtech, Inc., Newark Delaware). The plates were pre-washed in 1:1 chloroform/methanol, and then dried and washed again in 50:6:6 denatured ethanol/chloroform/ammonium hydroxide. Samples of 500 nL were applied with nanoliter-size disposable glass capillary tubes. The mobile phase was also 50:6:6 denatured ethanol/chloroform/ammonium hydroxide. Charring was performed at 160° C with 10% cupric sulfate in 8% phosphoric acid (6). The developed plates were scanned at 350 nm in the absorbance mode.

Results and Discussion

A large number of solvent combinations were examined for use as possible mobile phases to produce a complete separation of the major phospholipid groups. One advantage of this mobile phase is the insensitivity of the separation to small changes in mobile phase composition. Good separation of major phospholipid groups results when the amounts of either chloroform or ammonium hydroxide are varied between 5 mL and 7 mL. Figures 1 and 2 demonstrate the stability of Rf values in these regions. Other advantages as well as further details are discussed in the expanded version of this application note.

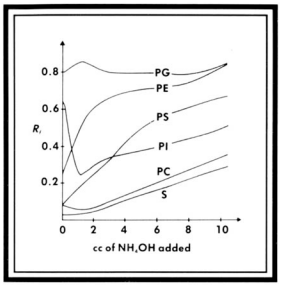

FIGURE 1: The influence of ammonium hydroxide addition to 50 ml of ethanol on the R_f values of phospholipids.

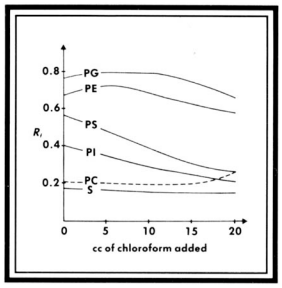

FIGURE 2: The influence of chloroform addition to 55 ml of 50:5 ethanol/ammonium hydroxide on the R_f values of phospholipids.

Conclusion

The method described here has demonstrated an ability to handle natural mixtures of phospholipids in biological extracts. Close control of relative humidity is not necessary. Small variations in the mobile phase do not adversely affect the separation. The mobile phase of 50:6:6 denatured ethanol/chloroform/ammonium hydroxide used in conjunction with preadsorbent silica gel HPTLC plates provides a good separation of the major phospholipid groups in a single development.

References

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