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Citation Classics

Skipski V P, Peterson R F & Barclay M. Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J.* **90**:374-8, 1964.

The authors describe the conversion of their previously-developed procedure for qualitative separation of phospholipids by thin-layer chromatography to a quantitative analysis which permitted the determination of the main known phospholipids in animal tissues. [The *SCI*® indicates that this paper was cited 696 times in the period 1964-1976.]

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"In the beginning of the sixties, we were faced with the problem of characterizing the phospholipid profiles in different human serum lipoprotein classes, as well as the phospholipid composition of animal cell surface membranes. The attempt to use silicic acid column chromatography along with paper chromatography for phospholipid determination had only moderate success at our hands. At that time, the first papers concerning the separation of phospholipids by thin-layer chromatography began to appear in different journals. Wagner *et al.*[1] described the separation of phospholipids by a one-dimensional thin-layer chromatographic procedure. However, this system did not give reliable separation of phosphatidylserine and phosphatidylinositol and some other non-nitrogen containing phospholipids. We tried to modify the procedure of Wagner *et al*. for the goal of separating all phospholipid classes However, our attempt never became reality.

"In our first attempt to modify the Wagner *et al*. system, experiments revealed that the position of phosphatidylserine on chromatoplates prepared from silica gel G (containing calcium sulfate as a binder) depended not only upon the type of compound (phosphatidylserine) but also upon the amount. This 'load effect' was due to the presence of calcium sulfate and was eliminated empirically by using the basic silica gel G chromatoplates. However, this sysem still did not permit separation of even the most common phospholipids present in animal tissues. Therefore, as soon as silica gel without calcium sulfate appeared on the market, we worked out another system of thin-layer chromatography for separation of phospholipids using this type of adsorbent and converted it to a quantitative procedure. This one-dimensional chromatographic system permitted the separation of most common phospholipids present in mammalian tissues including phosphatidylserine and phosphatidylinositol.

The limitations of this procedure, some realized only retrospectively, are as follows:

1. Phosphatidylgiycerol has a mobility similar to phosphatidylethanolamine.
2. With changes in both air humidity and batch number of silica gel, it was necessary to alter the proportion of ingredients in the developing solvent; e.g., if the phosphatidylethanolamine spot has an Rf value lower than 0.70, it is advisable to increase the content of water or acetic acid in the developing solvent and *vice versa*. The amount of methanol in the developing solvent apparently determines the distance between phosphatidylinositol and phosphatidylcholine.
3. Some non-nitrogen-containing phospho lipids did not separate in the system described in the Abstract.

Problems 1 and 3 were solved by developing additional thin-layer chromatographic systems which permitted the separation of phosphatidic acid, phosphatidylglycerol, cardiolipin and phosphatidyl ethanolamine. All these systems are described in *The Methods in Enzymology*.[2]

The authors are very happy that the procedure worked out by them found a wide application in many laboratories.

REFERENCES

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2. **Lowenstein J M,** ed. *The methods in enzymology.* Vol. 14. *Lipids*. New York: Academic Press, 1969. 771 pp.