A modified method for the paper chromatography of long-chain fatty acids

Although quantitative analysis of fatty acids is best performed by vapour-phase chromatography after conversion to the methyl esters, nevertheless paper chromatography offers certain advantages for identification and semi-quantitative estimation. Thus the preparation of samples for analysis is simplified, since the free fatty acids can be separated on paper without prior esterification; and of course, the simplicity of both apparatus and technique is an attractive feature.

Numerous methods have been described[1–4] for separating long-chain fatty acids on paper, involving reversed-phase chromatography. Similarly, many spray reagents have been developed for visualising the spots. Of these, quantitative reactions usually depend on forming a metal soap with subsequent development of a coloured complex with the metal. For qualitative and semi-quantitative work, an indicator, such as bromphenol blue is frequently employed.

The purpose of the present communication is to describe a simplified version of previous separation procedures and to suggest the use of a different spray solution that gives a clear demonstration of spots even with very small quantities of acids.

For the preparation of the papers, Whatman No. 3 paper, in strips of 23 × 5 cm, were immersed for about 5 min in a 10% (v/v) solution of liquid paraffin (B.P.) in ether, contained in a flat dish. It was considered advisable to cover the dish so as to avoid undue evaporation of ether but this point was not critical; neither was the time of impregnation, provided that at least 5 min soaking was allowed. At the end of this period, the impregnated paper strips were hung in the fume-cupboard to allow the ether to evaporate. At this stage, the papers are ready for chromatography, though it may be preferable to leave them overnight before use. Similarly, although the impregnated papers remain efficient on storing for a few days, it has been observed occasionally that storage for longer than two weeks results in poor separation of acids.

The solvent system used was a 4:1 solution (v/v) of acetone–water. It has not been found necessary to equilibrate solvent with stationary phase (liquid paraffin), but the purity of the acetone is an important factor. Analytical grade acetone may be used without redistillation, but commercial quality solvent is unsuitable.

The separation of long-chain saturated fatty acids has been achieved with the reversed-phase papers and solvent described by means of ascending chromatography. Equilibration of papers with solvent vapour for lengthy periods is unnecessary, although it may be advantageous to allow a short period (30 min). The time required for good separation is about 3 h, involving movement of the solvent front of approximately 12 cm from the starting point. In this procedure, typical R_F values are as follows:

Stearic acid	0.23
Palmitic acid	0.40
Myristic acid	0.55

With such convenient values of R_F, lower acids down to C_{10}, and slightly higher

acids can be detected on the same chromatogram. If a larger range of chain length is required, impregnation of the paper with a lower concentration of paraffin allows the acids to travel faster, and the same effect can be achieved by increasing the acetone content of the solvent.

The spray reagent is essentially an alkaline solution of Nile Blue, so that the background colour is pale red, whilst the fatty acids show up as blue spots. The reagent is prepared as follows:

Nile Blue sulphate solution (0.2 % in ethanol) 50 ml
Triethanolamine 15 ml (approximately)
Ethanol 50 ml

Triethanolamine is added to the stock solution of Nile Blue sulphate in ethanol until the blue colour changes to a clear red, untinged by blue. The solution is then diluted with ethanol.

At first ammonia was tried as the means for providing an alkaline medium, but this is volatile, and the background of the sprayed chromatogram quickly turned blue owing to CO_2 in the atmosphere. Triethanolamine is advantageous in that it remains on the paper, and the colour change of the background is therefore retarded.

In order to achieve the utmost simplicity of technique, no precautions have been taken in this method of separation to eliminate variables; and hence R_F values tend to change, most frequently because of change in temperature. Because of such considerations, it is always essential to run a control sample, preferably a known mixture of myristic, palmitic and stearic acids. Another fault, inherent in all similar techniques, is that unsaturated acids give the same R_F value as the saturated acid containing two carbon atoms less.

Nevertheless, good separations are obtained by this method, and the chromatogram can be sprayed immediately after development, without the need for lengthy treatment as in some other techniques. The method has been used successfully for the preliminary examination of fatty acids from biological tissues.

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[1] H. P. KAUFMANN AND W. H. NITSCH, *Fette u. Seifen*, 56 (1954) 154.
[2] H. WAGNER, L. ABISCH AND K. BERNHARD, *Helv. Chim. Acta*, 38 (1955) 1536.
[3] Č. MICHALEC, *Biochim. Biophys. Acta*, 28 (1958) 212.
[4] P. E. BALLANCE AND W. M. CROMBIE, *Biochem. J.*, 69 (1958) 632.

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