GAS–LIQUID CHROMATOGRAPHY OF VOLATILE FATTY ACIDS FROM FORMIC ACID TO VALERIC ACID

III. ANALYSIS OF DILUTE ETHEREAL SOLUTIONS USING A THERMAL CONDUCTIVITY DETECTOR

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INTRODUCTION

The work reported in this paper arose out of difficulties that were experienced using a commercial gas chromatograph equipped with a thermal conductivity detector, for the quantitative analysis of volatile fatty acids isolated from biological materials. The volatile fatty acids present in biological materials are usually isolated by steam distillation, followed by titration to give an aqueous solution of sodium or potassium salts. For estimation by gas–liquid chromatography the free acids must be prepared, usually in an anhydrous state. JAMES AND MARTIN[1] described a method for preparing an anhydrous ethereal solution of the free acids from their sodium salts. Other workers, however, have been unable to obtain quantitative recoveries with the method and various alternative procedures for obtaining the free acids and applying them to columns[2–6] have been described. Most of these involve acidification and subsequent extraction into ether. To ensure complete recovery of the acids, comparatively large volumes of ether are required and the combined extracts are correspondingly dilute. With the sensitive hydrogen flame and argon ionization detectors the volume of such extracts required to give adequate detector response is unlikely to be excessive. By contrast, the low sensitivity of thermal conductivity detectors necessitates the injection of comparatively large volumes of dilute ethereal solutions into the column system.

SMITH *et al.*[7] used "a 10 ft. Ucon (polar) column" to determine the C_2 to C_4 acids in 0.1 ml samples of ethereal solutions. However, with most of the packings that have been described for separating volatile fatty acids, samples of ethereal solutions greater than about 50 μl give ether peaks which seriously overlap those of the lower fatty acids.

Ethereal solutions of volatile fatty acids cannot be concentrated without loss, except by elaborate and time consuming methods[5,8]. JAMES AND MARTIN[1] applied acids, in ethereal extracts, to columns by evaporating the ether and acids in a stream of air drawn through the columns, which were at room temperature. VAN DE KAMER *et al.*[2] and MCINNES[3] modified the end of the column so that a solution of the acids

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in ether could be placed in a bulb and then distilled on to the column in the carrier gas stream. LANIGAN AND JACKSON[9] acidified the dried sodium salts of the acids in the carrier gas stream which then carried the free acids on to the column. Many of the workers mentioned above[1–4,9] used acid–base titration to detect the acids, so that ether and water in the sample did not interfere, provided they were insufficient to upset the separation of acids on the column.

EXPERIMENTAL AND RESULTS

Apparatus and materials

The instrument, some of the materials, the columns, and the method for preparing the column packings have been described previously[10]. Haloport F, Ucon LB-550-X (a polypropylene glycol) and diethylene glycol succinate were purchased from F & M Scientific Corporation and Tween 80 (a product of Honeywell-Atlas Ltd.) from a local distributor.

The columns were conditioned at 150° for 16 h with a carrier gas flow rate of 5 ml/min.

Sample application

In seeking a solution to the problem discussed above, 0.5 ml of an ethereal solution was adopted as the maximum sample size.

Various attempts were made to distil acids on to columns by methods similar to those used by other authors[1–4,9]. Although these experiments indicated that the acids could be separated from 0.5 ml ether on some columns using an appropriate application procedure, it became apparent that the separation of water from the acids would be a much more difficult task. Therefore, a search was undertaken for a column packing which would not only resolve a mixture of the volatile fatty acids from formic to *n*-valeric, but also separate the acids from 0.5 ml of ether and the small amount of water present in ether extracts dried with sodium sulphate.

Column packings

Liquid phases of the silicone oil–fatty acid type were unsuitable for reasons discussed earlier[10,11]. Exploratory experiments indicated that diethylene glycol succinate–phosphoric acid[12] might be suitable for separating ether and water from the acids. However, this phase did not adequately separate formic acid from acetic and propionic acids. The stationary phases described previously[10] did not give a satisfactory separation of the lower acids from water and the large volume of diethyl ether.

On Tween 80 columns (SMITH[13]) formic acid had a retention time between that of acetic and propionic acids; because of the excessive tailing of the acids, formic acid seriously overlapped both of the higher ones. Furthermore, the separation of water from the acids was not adequate for accurate work.

Addition of 10% orthophosphoric acid to the Tween 80 reversed the elution order of acetic and formic acids, but formic acid tailed to an even greater extent than in the absence of phosphoric acid.

The stationary phase of dioctyl sebacate and sebacic acid (RAUPP[14]) gave satisfactory separations of the C_1 to C_5 acids, but the retention times in the tempera-

ture range 125 to 145° were too short to permit adequate separation from water and a large volume of ether. Some improvement was effected by operating the column at 65° for the first ten minutes and then raising the temperature to 135°, but separation of ether and water from the acids was still unsatisfactory.

4 ft columns packed with 20% Ucon LB-550-X (SMITH *et al.*[7]) on acid-washed Chromosorb W did not separate formic and acetic acids. Furthermore, the peaks of all the acids were asymmetrical and tailed into the peaks of the following acids. A notable feature of this packing was the long retention times of the acids at lower temperatures, *e.g.* 65°. Replacement of 20% of the Ucon with behenic acid practically eliminated tailing, but formic and acetic acids were still not resolved. A mixture of equal parts of Ucon and behenic acid gave a partial separation of formic from acetic acid. When behenic acid was replaced by sebacic acid, the separation of formic from acetic acid was improved considerably (Fig. 1); a better separation of the butyric acid isomers was also obtained.

The columns finally adopted for the analytical procedure described below, were of stainless steel, 122 cm long and 4 mm internal diameter, packed with a

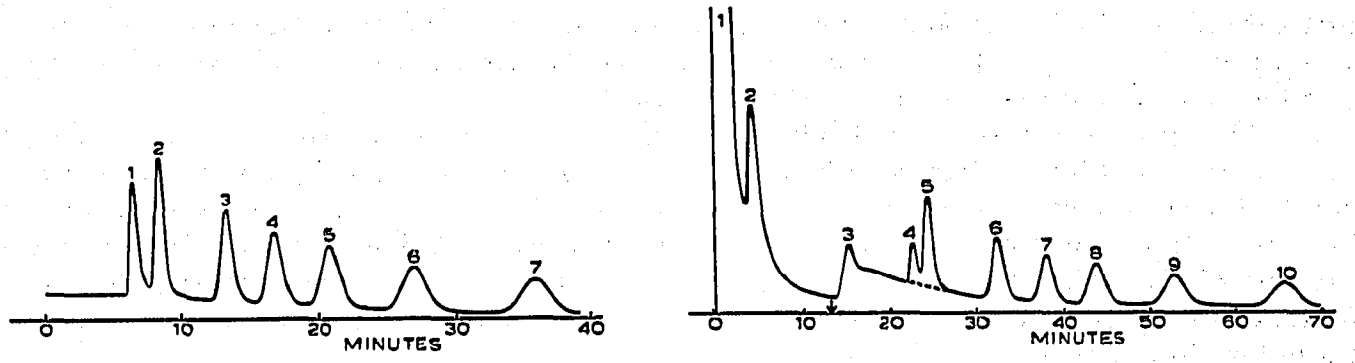

Fig. 1. Separation of a mixture of C_1 to C_5 acids on a column of 10% (w/w) Ucon LB-550-X and 10% (w/w) sebacic acid on acid-washed Chromosorb W. Sample, 2 μl mixed acids. Column temperature 145°. Helium flow-rate 30 ml/min. Attenuation × 8. Peaks: 1 = formic acid; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = *n*-butyric acid; 6 = isovaleric acid; 7 = *n*-valeric acid.

Fig. 2. Separation of acids in an ethereal solution prepared from a mixture of sodium salts of C_1 to C_5 acids on the column described in Fig. 1. Sample, 0.5 ml ethereal solution containing 1 mg mixed acids. Column temperature 65°, then raised to 135° where indicated ↓. Helium flow-rate 30 ml/min. Attenuation × 4. Peaks: 1 = ether, 2 and 3 = water; 4 = formic acid; 5 = acetic acid; 6 = propionic acid; 7 = isobutyric acid; 8 = *n*-butyric acid; 9 = isovaleric acid; 10 = *n*-valeric acid. Broken line represents true base-line of peaks 4 and 5.

mixture of Ucon LB-550-X (1 part), sebacic acid (1 part) and acid-washed Chromosorb W, 80–100 mesh (F & M Scientific Corporation) (10 parts). This column gave complete separation of acids from acetic to *n*-valeric; when the acids were applied as a solvent-free mixture the separation of formic from acetic acid was almost complete too (Fig. 1), but resolution was poorer when the acids were dissolved in 0.5 ml of ether. Water also had a deleterious effect, but the separations obtained with ethereal extracts dried with sodium sulphate (Fig. 2), were adequate for estimation of peak-areas by triangulation. Further drying with calcium sulphate might be worthwhile in some circumstances.

Analytical procedure

Ethereal solutions of volatile fatty acids were prepared from aqueous solutions of sodium salts by the method of McINNES[3].

The carrier-gas flow rate was 30 ml/min and the injection port was maintained at about 160°. With the column at 65°, up to 0.5 ml of an ethereal solution of volatile fatty acids was introduced into the injection port with a gas-tight syringe (Hamilton Co., Inc.).

10 to 15 min after injection of the sample the column temperature was raised rapidly (at about 80°/min) to 135°. A typical chromatogram is shown in Fig. 2; with large samples the water peak still overlapped the formic and acetic acid peaks. In such cases the broken line shown in Fig. 2 was used as a baseline for determining peak-areas for formic and acetic acids.

DISCUSSION

Although thermal conductivity detectors have been superceded to a considerable extent by more sensitive detectors, gas chromatographs employing the former are still used in many laboratories. In the field of volatile acid analysis they have an advantage over hydrogen flame detectors in that they respond to formic acid. However, when the solutions available for analysis are very dilute the low sensitivity of thermal conductivity detectors is a serious disadvantage.

The procedure described above permits the determination of the volatile fatty acids in dilute ethereal solutions using a gas chromatograph with a thermal conductivity detector. The success of the method depends, to a large extent, on the long retention times of the acids compared with ether and water, on columns containing Ucon LB-550-X at 65°. The stability of the column described was satisfactory; more than sixty separations were carried out on one column without detectable loss in efficiency.

For quantitative work without an internal standard, it is desirable to use a gas-tight syringe, since the pressure developed on injecting a large volume of ether into the injection port, may force some of the sample past the plunger of the syringe.

The method described above has been used in this laboratory for the determination of volatile fatty acids isolated from rumen contents and silages.

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SUMMARY

Some of the difficulties associated with gas–liquid chromatography of C_1 to C_5 fatty acids isolated from biological materials are discussed. Attention is directed particularly to the problems which arise when thermal conductivity detectors are used.

Column packings described previously for the separation of volatile fatty acids were unsatisfactory for analysing dilute ethereal solutions of the acids, especially if they contained small quantities of water.

A new stationary phase which adequately separated the acids from water and up to 0.5 ml of ether is described.

A procedure is described for the estimation of C_1 to C_5 fatty acids, isolated from biological material, using a gas chromatograph with a thermal conductivity detector.

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