Some notes on the separation of the volatile fatty acids in rumen fluid by gas–liquid chromatography

Samples of rumen fluid, taken from sheep and cattle, are analysed in this laboratory to determine total volatile fatty acids present by using a distillation procedure[1]. To determine the quantities of the individual acids present the distillates, after titration, are made alkaline with sodium hydroxide and evaporated on a hot plate. Each sample is then transferred to a small glass vial and dried at 80°. The samples are then stored at room temperature until analysed.

Method

The instrument used was a Model 800 Gas Chromatograph (Perkin-Elmer Corporation) fitted with a dual hydrogen-flame ionization detector. The columns consisted of 6 ft. lengths of stainless steel tube of $^1/_8$ in. O.D.

Column packing. The stationary phase used for the separation of the volatile fatty acids was Tween 80 and orthophosphoric acid added to the solid support in the proportions 20:2:78 (ERWIN, MARCO AND EMERY[2]). The solid support used was Embacel, 60–100 mesh (May & Baker, Ltd.) which was washed first with acetone, then with saturated alcoholic potassium hydroxide and finally with concentrated sulphuric acid. It was then washed with distilled water until the washings would no longer turn litmus paper red, dried and coated with the orthophosphoric acid in water. After drying, the Tween 80 (Applied Science Laboratories, Inc.) was added in dichloromethane. The packing was then oven dried and put through standard screens of 60 and 80 mesh. The 60/80 mesh material was packed into the precoiled columns using suction and vibration. The columns were conditioned overnight at 145° with a nitrogen flow rate of 25 ml/min.

Sample preparation. Four to 6 drops of 85% orthophosphoric acid were added to the dried sample to free the acids from their sodium salts. They were then dissolved in 1 ml of acetone, the sodium phosphates were allowed to settle and 1 to 4 μl of the supernatant solution was injected onto the column using a microlitre syringe.

Operating parameters. The injection block was maintained at 180°. The carrier gas was nitrogen and the flow rate was 50 ml/min. The oven temperature was programmed to rise from 100° when the sample was injected to 140° at 3.3°/min. The temperature was then maintained at 140° until the analysis was completed. Hydrogen flow to the detector was 32 ml/min. Air flow to the detector was 400 ml/min.

Discussion

Initially samples were prepared for analysis by dissolving in aqueous acid solution. Sulphuric acid was not satisfactory as it eventually found its way onto the column where it reacted with the liquid phase. Orthophosphoric acid (5% v/v) was tried but this method gave double peaks for acetic acid and excessive tailing of propionic acid which almost hid the isobutyric acid peak (Fig. 1). It was suspected that this effect was due to dimer formation in the sample. To suppress dimer formation the acids were dissolved in acetone after treatment of the dried sodium salts with concentrated orthophosphoric acid. When samples prepared in this way were analysed tailing was virtually eliminated and isobutyric acid was almost completely separated from propionic acid (Fig. 2). From these results it is concluded that much of the tailing

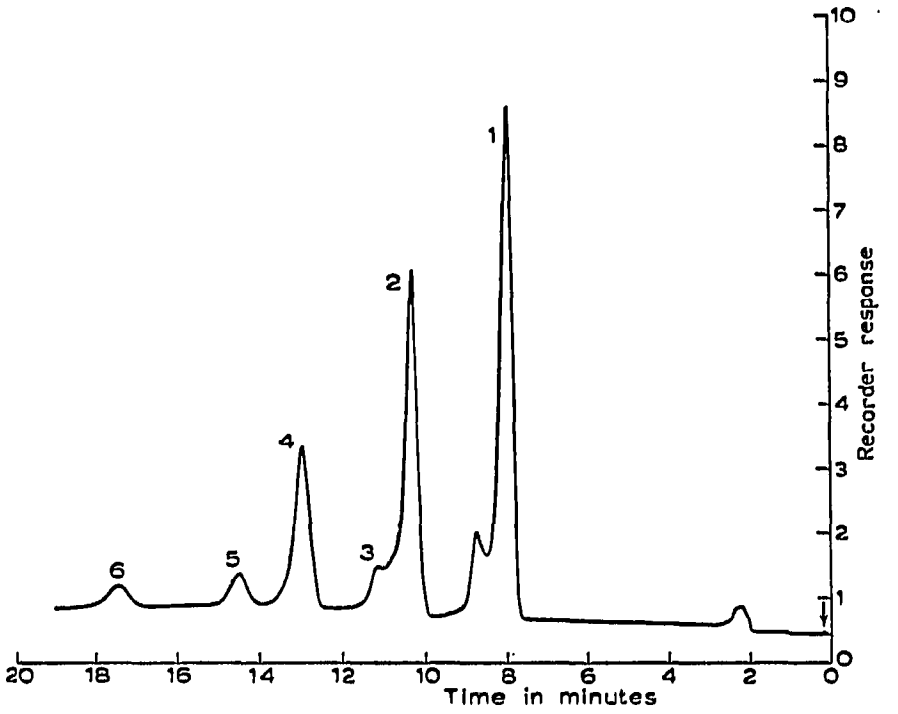

Fig. 1. Separation of a sample of 5.0 μl mixed acids C_2 to C_5 (300 μmole/ml) in 5% (v/v) orthophosphoric acid. Attenuation × 100. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = butyric acid; 5 = isovaleric acid; 6 = valeric acid.

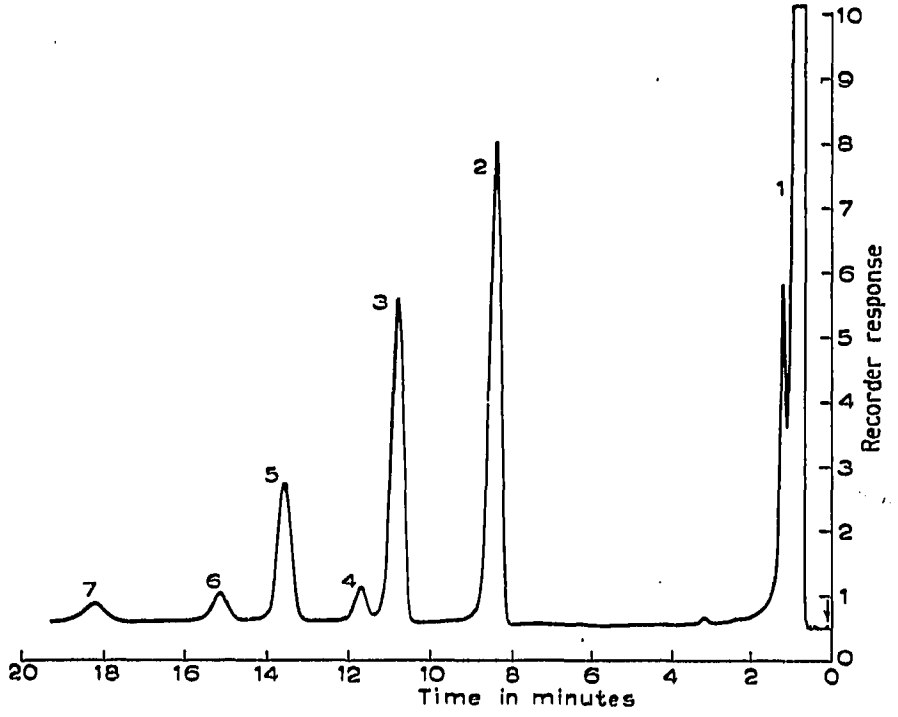

Fig. 2. Separation of a sample of 2.5 μl mixed acids C_2 to C_5 (300 μmole/ml) in acetone. Attenuation × 100. Peaks: 1 = acetone; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = butyric acid; 6 = isovaleric acid; 7 = valeric acid.

observed when aqueous solutions of the acids were analysed (Fig. 1) was due to dimer formation and that the orthophosphoric acid used in the column packing and sample preparation was insufficient to prevent it.

After 20 to 30 samples had been analysed, depending on the volume of sample injected, a long series of small peaks appeared throughout the chromatogram (Fig. 3). This was due to the accumulation in the injection block of the indicator used in the titration and its eventual pyrolysis. This problem was eliminated by raising the temperature of the injection block to 350° and backflushing it with compressed air after each run of 10 to 15 samples per column.

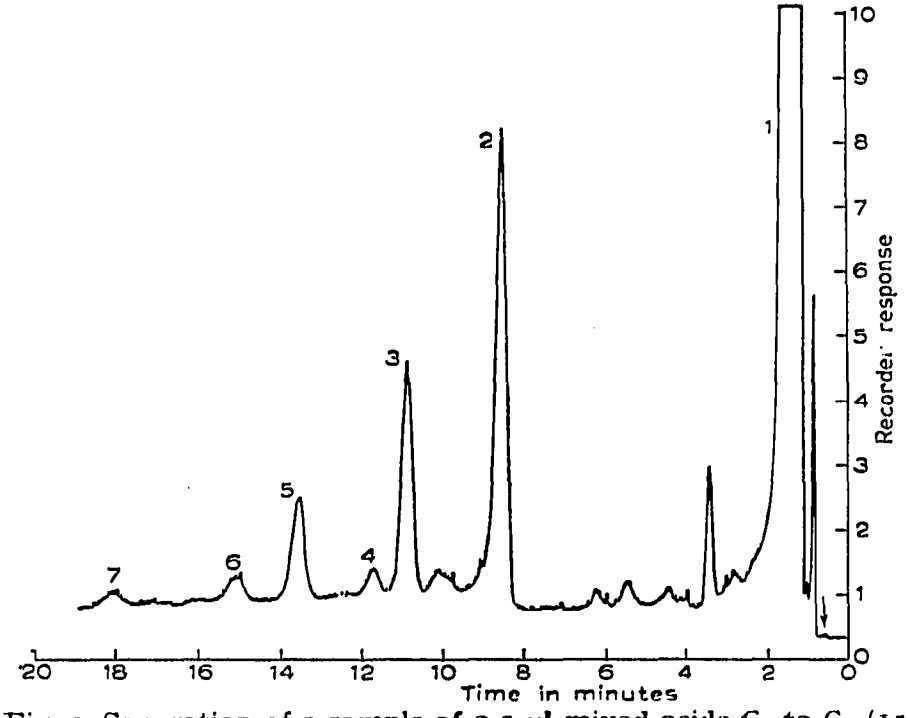

Fig. 3. Separation of a sample of 2.5 μl mixed acids C_2 to C_5 (150 μmole/ml) in acetone. Attenuation × 50. Peaks: 1 = acetone; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = butyric acid; 6 = isovaleric acid; 7 = valeric acid.

A comparison of Figs. 1 and 2 indicates that the response of the hydrogen-flame ionization detector was apparently depressed when aqueous samples were analysed. In each case a solution containing 300 μmole per ml was used and the detector attenuation was the same. An injection of 5.0 μl of the aqueous solution gave a similar recorder response to an injection of 2.5 μl of the acetone solution. This finding agrees with the report of FOSTER AND MURFIN[3].

Repeatability estimates showed that the method was accurate to ± 1 %.

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2 E. S. ERWIN, G. J. MARCO AND E. M. EMERY, *J. Dairy Sci.*, 44 (1961) 1768.
3 J. S. FOSTER AND J. W. MURFIN, *Analyst*, 90 (1965) 118.

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