Sample introduction in analyses of volatile fatty acids in rumen fluid by gas chromatography

The procedure for gas–liquid chromatographic analysis of volatile fatty acids first described by JAMES AND MARTIN[1] has the advantage over other procedures in that the method of detection used gives an absolute determination of the amount of each acid by automatic titration, and is insensitive to other non-acidic components which may be present in complex natural mixtures. It also separates the acids very effectively.

However, the procedure also has the considerable disadvantage that, while the samples on which determinations must be carried out are usually aqueous solutions, the column packing is adversely affected by moisture, so that it is necessary to carry out quite elaborate initial preparation of the sample to remove moisture and other possible contaminants. The common practice is to prepare an ethereal solution[2] which can be injected into the columns; this process of injection of an ethereal solution also has difficulties, so that a number of methods have been proposed to overcome the problem[3].

A simpler method which has proved entirely satisfactory over several years' use here, is not to pretreat the sample but to inject it directly into a separate "cap" section of column packing which is located within the vapour jacket of the apparatus at the head of the column of the normal type described by JAMES AND MARTIN[1]. This cap performs the functions of liberating the acids but retaining water vapour.

Sample of rumen fluid have been routinely assayed by this method, with the only pretreatment being clarification by low-speed centrifugation. The samples (50 μl) as injected have a pH of about 6.5, are approximately 0.1 *M* in total fatty acids, and contain plant debris and bacteria in suspension, as well as numerous compounds of high molecular weight in low concentration. The cap packing consists of equal parts (w/w) of anhydrous sodium tetraborate, $NaHSO_4$ and Celite. Injection is *via* a septum in the usual manner with a chromatographic syringe, directing the sample into the middle of the cap packing. Dimensions used are: diameter of column 8 mm, gas flow rate 30 ml/minute, length of cap packing 10 cm. To remove the high molecular weight residues left in the cap, it is necessary to repack the cap section after every 20 samples, but the cap is arranged as an interchangeable section separate from the main column to facilitate this operation. It is necessary to dehydrate the sodium tetraborate before use by heating at 130° overnight.

The results obtained have given recoveries of 98 $\pm$ 2.5 % compared with the titration of total volatile fatty acids obtained by distillation of samples of rumen fluid; this is essentially the same as the best accuracy of the normal technique. The columns used have shown no evidence of contamination of the main packing.

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