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Note

Modified pre-column for the preparative-scale separation of volatile fatty acids

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Many techniques, based on gas–liquid chromatography (GLC), are used for separation of short-chain, volatile fatty acids (VFA) found in rumen liquor and other biological fluids, and these techniques are generally carried out on a microanalytical scale. With many of the techniques, complicated sample preparation is necessary to remove water from the solution prior to injection. On the other hand, methods have been sought to reduce the effects of water (*e.g.*, azeotrope formation and ghosting) on the resolution of the VFA by the GLC columns. Ottenstein and Bartley[1] have discussed the problem. Monk and Forrest[2] described a pre-column which retained water and released the VFA as free acids from a solution of their sodium salts. This pre-column was adapted by Henning and Hird[3] for more dilute solutions.

In cases where yields of VFA sufficient for subsequent analysis of radioactivity are required, a larger-scale process is necessary. This has often taken the form of silica gel columns, *e.g.* Leng and Leonard[4], although Annison[5] and Bergman and Wolff[6] have described preparative-scale GLC techniques.

The present communication describes modifications, suitable for preparative-scale GLC, of the pre-column originally devised by Monk and Forrest[2].

EXPERIMENTAL AND RESULTS

The pre-column consisted of 15 cm of stainless-steel tubing (6 mm O.D.) joined to the main GLC column with a Swagelok union. The pre-column contained a mixture of PTFE T6 granules and sodium hydrogen sulphate (5:4, w/w). The PTFE granules and the inner walls of the pre-column were coated with Trimer acid (1 %, w/w). The sodium hydrogen sulphate had previously been ground finely (120 mesh) and dried at 200° overnight.

The pre-column described above was compared with that described by Monk and Forrest, which contained a mixture of Celite, sodium hydrogen sulphate and anhydrous sodium tetraborate (1:1:1, w/w). Each pre-column was fitted to a main column, 2 m × 2 mm I.D., packed with 5% FFAP* on Chromosorb G AW DMCS (80–100 mesh) and 40 μl of a solution containing sodium acetate (0.3 M), sodium

* FFAP is Carbowax 20M cross-linked with 2-nitroteraphthalic acid.

propionate (0.1 M) and sodium butyrate (0.1 M) in 5% (w/w) orthophosphoric acid was repeatedly injected on to each pre-column.

In each case the detector response was plotted against the number of injections, and the results are given in Figs. 1 and 2. The peak sizes decreased markedly after eight to ten injections with the Monk and Forrest design of pre-column, whereas the modified design maintained a more even response for eighteen to twenty-two injections. The shape of the peak on the GLC trace also deteriorated after similar numbers of injections.

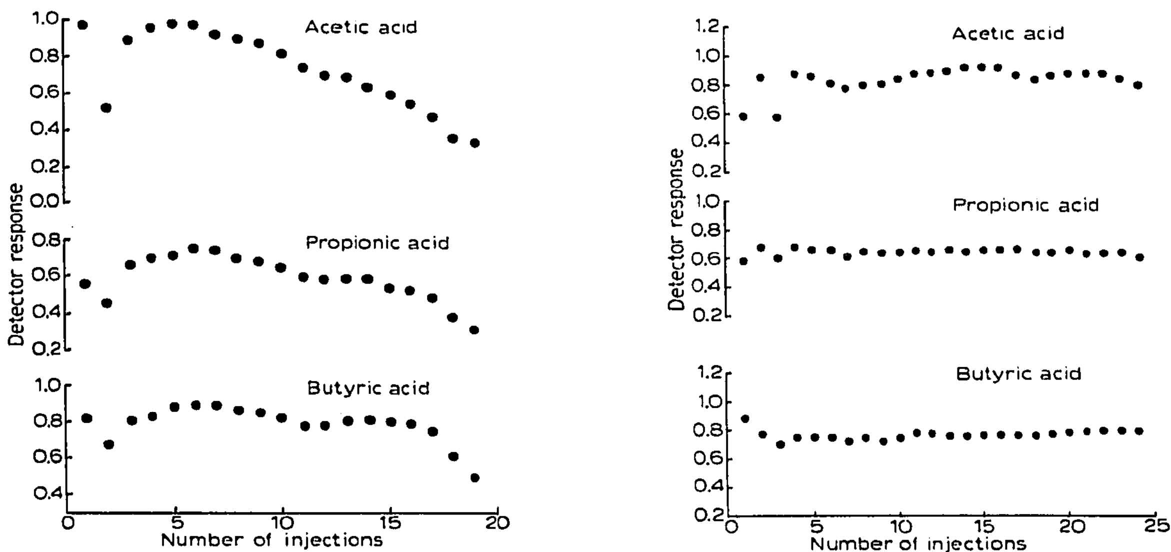

Fig. 1. Detector response following repeated injections of a standard mixture of three fatty acids to a GLC column fitted with a pre-column containing equal parts of celite, sodium hydrogen sulphate and anhydrous sodium tetraborate as described by Monk and Forrest[2].

Fig. 2. Detector response following repeated injections of a standard mixture of three fatty acids to a GLC column fitted with a pre-column containing five parts of PTFE T6 granules coated with 1% (w/w) Trimer acid and four parts of sodium hydrogen sulphate.

DISCUSSION

Because of the difficulty in obtaining either a sufficiently concentrated solution of the VFA in ether for column injection, or adequate resolution of the VFA on the column after injection of an aqueous solution, a pre-column was found to be essential for VFA separation on the preparative scale. The use of Celite, as suggested by Monk and Forrest, even when coated in orthophosphoric acid and/or Trimer acid, as a substitute for the PTFE T6 granules only gave a useful pre-column life of twelve to fourteen injections. Tailing of the VFA, especially acetic acid, was greatly reduced by coating the inner wall of the pre-column with Trimer acid. Because Monk and Forrest and later Henning and Hird all injected aqueous solutions at near neutral pH, they included reagents in the pre-column to liberate the VFA from their sodium salts. The use, in the present work, of 5% orthophosphoric acid to dissolve the VFA

obviated the need for such substances. Erwin *et al.*[7], working on an analytical scale, were able to deproteinise rumen fluid with metaphosphoric acid and inject this solution directly. However, in our experience, direct injection of such a solution on a preparative scale did not meet with success, and use of a pre-column containing coated PTFE granules and sodium hydrogen sulphate was necessary.

Ghosting was not a serious problem, even at the end of the useful life of the pre-column. Injection of either water or 5% orthophosphoric acid after an injection of the VFA resulted in only a very small peak for each acid. The amount of each acid released as a result of ghosting was calculated to be less than 1% of the previous injection. This was confirmed with the use of radioactive VFA.

It should be noted that the pre-column was not required to give an accurate measurement of each VFA present. Each acid was collected by stream splitting and trapping in toluene to enable radioactivity to be measured.

The amount of each VFA present was measured by applying an aliquot of the trapped sample to an analytical column without the use of a pre-column.

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