

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

I. CARBOXYLIC ACIDS AS STATIONARY PHASES

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In their classical paper on gas-liquid chromatography JAMES AND MARTIN¹ described the separation and micro-estimation of volatile fatty acids, using columns of Celite impregnated with a mixture of a silicone oil and stearic acid.

In spite of the excellent performance and stability of these columns reported by JAMES AND MARTIN, other workers have since observed a rapid loss of efficiency when they have been used at temperatures in the region of 135°. Some workers have attributed the loss of efficiency to bleeding of stearic acid from the column^{2,3}. To prevent this, MCINNES² and BOER³ replaced stearic acid with the less volatile behenic acid, but this did not prolong the life of the columns to any appreciable extent. In this laboratory too, columns containing silicone oil-stearic acid and silicone oil-behenic acid as liquid phases have become useless after running at 135° for 16 h.

If the loss of efficiency were due to some change in the liquid phase, it seemed more likely that the silicone oil would be involved rather than the behenic acid, since behenic acid is unlikely to undergo any chemical change at 135° in an atmosphere of nitrogen, whereas lack of information about the composition, stability and purity of the silicone oil leaves some doubt concerning its behaviour. Furthermore there appeared to be no reason why behenic acid alone should not be satisfactory as a liquid phase.

This communication reports some of the observations which have been made in this laboratory on the use of behenic acid and sebacic acid as liquid phases for the separation of C₁ to C₅ saturated monocarboxylic acids.

EXPERIMENTAL

Apparatus and materials

The instrument used in this work was an F & M Model 300 gas chromatograph (F & M Scientific Corporation) which employs a hot-wire thermal-conductivity detector. The detector was operated at 200° with a filament current of 150 mA. Acid-washed Chromosorb W, 80-100 mesh (purchased from F & M Scientific Corporation) was used as the solid support. Behenic acid (Hopkin & Williams) was recrystallized twice from chloroform and sebacic acid (British Drug Houses) was recrystallized twice from acetone. The stainless steel columns were 122 cm long and 4 mm internal diameter. The column packings were prepared by dissolving the liquid phase

in acetone, adding acid-washed Chromosorb W (80–100 mesh) and removing the acetone at reduced pressure in a rotary evaporator. When the acetone had been removed, the temperature was raised above the melting point of the liquid phase and maintained for about 10 min to ensure even distribution of the liquid phase. The columns were conditioned at 135° for 16 h with a carrier gas flow rate of 5 ml/min. 1.5 μ l samples of a mixture of approximately equal weights of formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids were used for testing column performance.

RESULTS

Fig. 1 shows a typical separation of a mixture of C₁ to C₆ acids on a column packed with 20% (w/w) behenic acid on acid-washed Chromosorb W. This column showed no loss of efficiency when operated at 135° for a week with a carrier gas flow of 30 ml/min. At 115° a complete separation of formic from acetic acid and of isobutyric from *n*-butyric acid was obtained. In an attempt to improve the symmetry of the formic

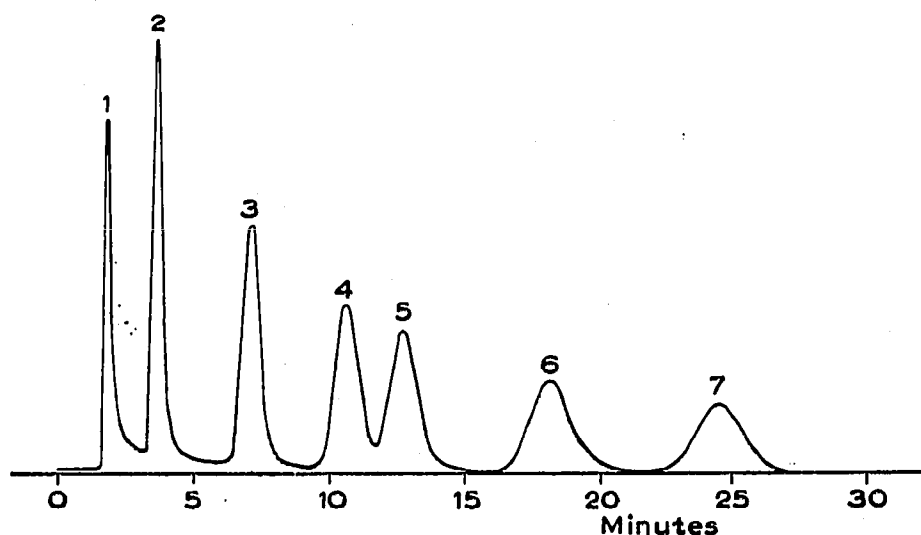


Fig. 1. Separation of a mixture of C₁ to C₆ acids on a column of 20% (w/w) behenic acid on acid-washed Chromosorb W. Column temperature 135°. Helium flow rate 30 ml/min. Attenuation \times 8. Peaks: 1 = formic acid; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = *n*-butyric acid; 6 = isovaleric acid; 7 = *n*-valeric acid.

acid peak, 4% (w/w) orthophosphoric acid¹ was added to acid-washed Chromosorb W before the behenic acid. A typical separation is shown in Fig. 2. Although the tailing of formic acid was slightly reduced by adding phosphoric acid to the stationary phase, a new peak (Fig. 2, peak 1) appeared before formic acid. This was probably due to decomposition of formic acid as it only appeared when formic acid was present in the sample.

The success achieved with behenic acid prompted the investigation of sebacic acid as a liquid phase. 20% (w/w) sebacic acid was deposited on acid-washed Chromosorb W.

The retention times on the sebacic acid column were more than double those on the behenic acid column when both columns were operated at 135° and a flow-rate of 30 ml/min. This was probably due to the sebacic acid column containing about twice

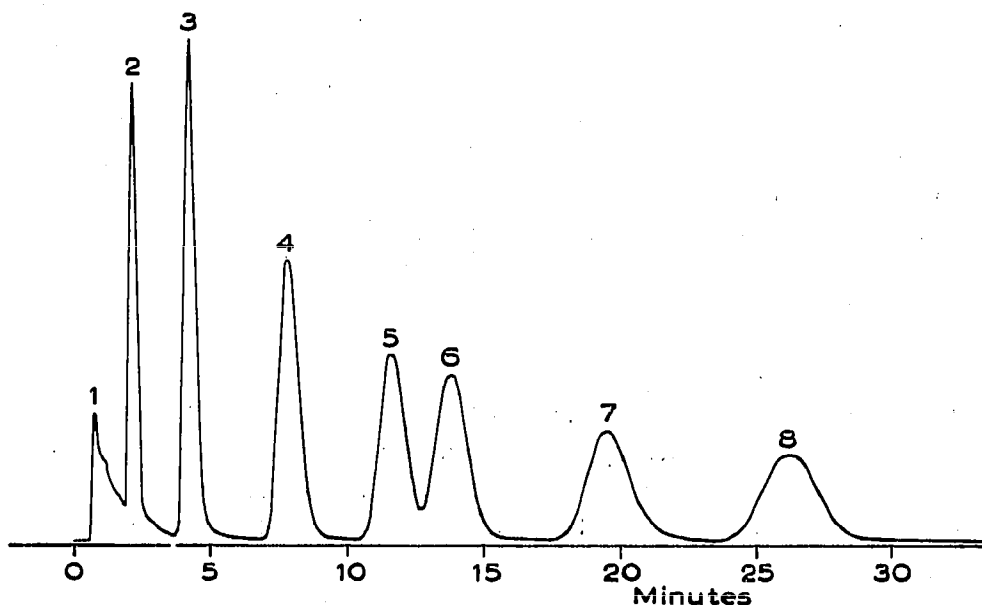


Fig. 2. Separation of a mixture of C_1 to C_5 acids on a column of 20% (w/w) behenic and 4% (w/w) orthophosphoric acids on acid-washed Chromosorb W. Column temperature 135° . Helium flow-rate 30 ml/min. Attenuation $\times 8$. Peaks: 1 = formic acid decomposition product; 2 = formic acid; 3 = acetic acid; 4 = propionic acid; 5 = isobutyric acid; 6 = *n*-butyric acid; 7 = isovaleric acid; 8 = *n*-valeric acid.

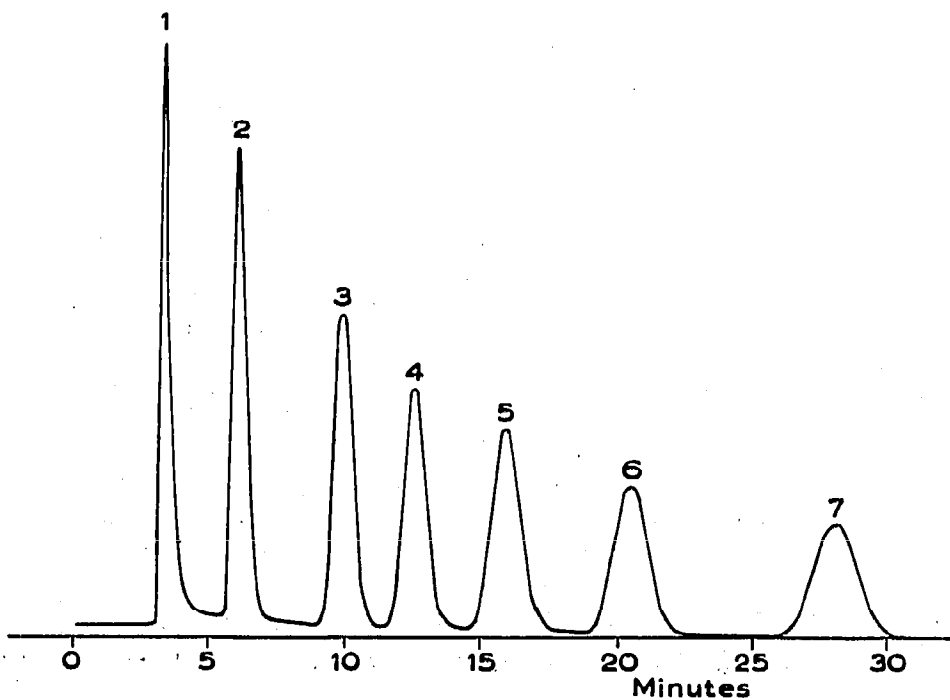


Fig. 3. Separation of a mixture of C_1 to C_6 acids on a column of 20% (w/w) sebacic acid on acid-washed Chromosorb W. Column temperature 135° . Helium flow-rate 60 ml/min. Attenuation $\times 4$. Peaks: 1 = formic acid; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; 5 = *n*-butyric acid; 6 = isovaleric acid; 7 = *n*-valeric acid.

the number of carboxyl groups in the behenic acid column, thus allowing greater association in the liquid phase. When the flow rate was increased to give retention times roughly comparable with those on the behenic acid column, the separation shown in Fig. 3 was obtained. It is apparent that the sebacic acid column gave a better separation of formic from acetic acid and also of isobutyric from *n*-butyric acid, than did the behenic acid column.

The stabilities of the packings described above appear to be quite satisfactory. The behenic and sebacic acid columns have been run continuously at temperatures between 135° and 150° with a carrier gas flow-rate of 30 ml/min for more than a week without any apparent loss of resolving power.

DISCUSSION

The behenic and sebacic acid packings described above, appear to be superior to packings which have been described previously for the separation of C₁-C₅ volatile fatty acids. Polyester type stationary phases have been widely used for separating mixtures of volatile fatty acids, but the retention time of formic acid on these columns usually lies somewhere between those of acetic and propionic acids and seriously overlaps the peak of either or both of these acids. Similar results are obtained with Carbowax and Tween 80 as liquid phases. The stationary phase of dioctyl sebacate containing 15 % of sebacic acid described by RAUPP⁴, gives excellent separations of the C₁ to C₅ acids, but the separation of formic from acetic acid is inferior to those given by behenic and sebacic acids.

Behenic acid and sebacic acid can only be used as stationary phases at temperatures above their melting points, namely, 80° and 133° respectively. This limitation may preclude their use in some circumstances. In this connection it would be of interest to investigate the use of high molecular weight unsaturated acids as liquid phases, since these have much lower melting points than the corresponding saturated acids. The excellent separations obtained with behenic acid as a liquid phase suggest that the instability of the silicone oil-stearic acid phase may be associated with the silicone oil; an investigation of this problem is in progress in this laboratory and will be the subject of a latter communication.

ACKNOWLEDGEMENTS

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SUMMARY

The use of behenic acid and sebacic acid as liquid phases for the gas-liquid chromatography of C₁ to C₅ fatty acids has been described. Complete resolution of all normal and iso-acids was obtained on 122 cm columns containing sebacic acid as the liquid phase at 135°. With behenic acid as the liquid phase the separations of formic from acetic acid and isobutyric from *n*-butyric acid were not quite complete at 135°, however, complete separation was obtained at 115°.

Both the behenic and sebacic acid columns showed satisfactory stability at 135°.

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