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## Note

# Long-chain acetates as internal standards in the gas–liquid chromatography of volatile fatty acids

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Volatile fatty acids (VFA) are responsible for the flavours of many food products. The ordinary gas–liquid chromatographic (GLC) method of VFA analysis could be improved by using internal standards and the purpose of this work was to examine the use of some acetates for this purpose.

## EXPERIMENTAL

$C_2$–$C_7$ volatile fatty acids and $C_2$–$C_{12}$ alcohols were obtained from Polyscience Corp. and dichloroacetic acid (AnalaR grade from POCH) was used as a 10% (w/v) solution in distilled water[1]. Acetates of $C_6$–$C_{12}$ alcohols were synthesized in our laboratory from the above alcohols and acetyl chloride (AnalaR grade, POCH).

Five microlitres of VFA solution in 10% dichloroacetic acid[1] containing 0.1 nl of each acid and acetate were injected onto the chromatographic column.

A Beckman GCM gas chromatograph was used, equipped with a dual column system, flame-ionization detectors and a Beckman recorder. The chromatographic conditions were: stainless-steel columns, 1.8 m × 4 mm I.D., filled with 20% EGA on Chromosorb W HP, 100–120 mesh; nitrogen flow-rate, 53 ml/min (1.8 atm); hydrogen, 1.5 atm; air, 2 atm; recorder chart speed, 0.2 in./min; column temperature, constant at 140°, 150° or 180° or programmed from 130° to 210° at the rate of 10°/min; injection block temperature, 250°; detector temperature, 300°.

## RESULTS AND DISCUSSION

A typical chromatogram of VFA and long-chain acetates is shown in Fig. 1. The order of the various VFA on the chromatogram, depending on the carbon number, is typical for the conditions of analysis[2,3].

Of the internal standards studied, the most promising were heptyl acetate, which appears between acetic and propionic acids, and decyl acetate, which appears just after valeric acid. Hexyl acetate appears on the solvent peak, octyl acetate in the position of isobutyric acid, nonyl acetate could interfere with the two possible isomers of valeric acid (2-methylbutyric and 3-methylbutyric acid), while undecyl and dodecyl

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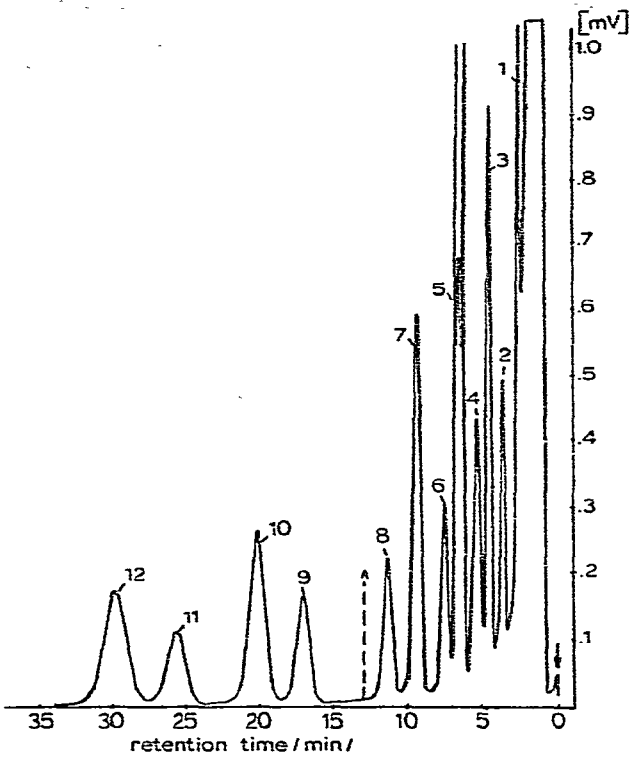


Fig. 1. Typical chromatogram of volatile fatty acids and some long-chain acetates. Constant column temperature, 150°. Peaks: 1 = hexyl acetate; 2 = acetic acid; 3 = heptyl acetate; 4 = propionic acid; 5 = octyl acetate; 6 = butyric acid; 7 = nonyl acetate; 8 = valeric acid; A = decyl acetate (position calculated from Fig. 3); 9 = caproic acid; 10 = undecyl acetate; 11 = enanthic acid; 12 = dodecyl acetate. The amount of each compound injected into the chromatographic column was 0.1 nl in 5 $\mu$l of 10% dichloroacetic acid solution.

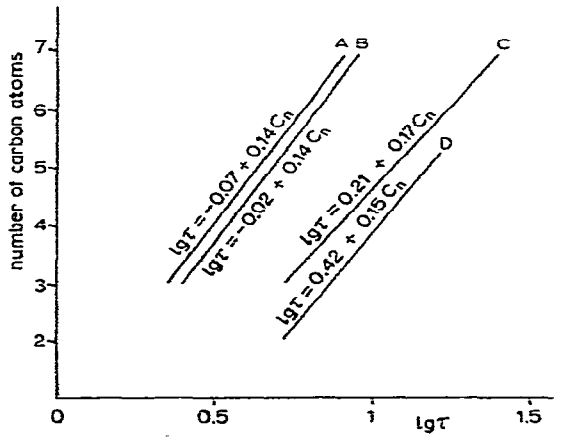


Fig. 2. Correlations between the logarithm of retention time and number of carbon atoms for different column temperatures. A, programmed temperature from 130° to 210° at the rate of 10°/min; B, isothermal at 160°; C, 150°; D, 140°.

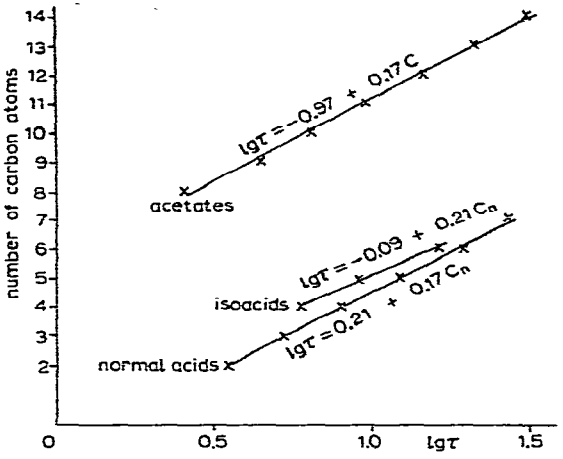


Fig. 3. Correlations between the logarithm of retention time and number of carbon atoms for acetates, iso-acids and normal volatile fatty acids for a constant column temperature of 150°.

acetates appear too late, so that these compounds cannot be good internal standards.

Long-chain normal alcohols have the same retention times as normal fatty acids with a carbon chain shorter by four carbon atoms; hence *n*-octanol appears in the place of butyric acid, *n*-nonanol in place of valeric acid and *n*-decanol in place of caproic acid.

Relationships between the number of carbon atoms in the molecules of fatty acids and the ogarithm of retention time for different conditions of analysis are shown in Fig. 2. Very similar results were obtained for a programmed temperature from 130° to 210° at the rate of 10°/min and for a constant column temperature of 160°, but in both instances the separation of some isomers was not satisfactory. We therefore chose for use in subsequent analyses a constant column temperature of 150°, which gave good separations and a time of analysis that was not too long. For these conditions there were linear relationships between the logarithm of the retention time and number of carbon atoms for normal acids, iso-acids and acetates of long-chain alcohols (Fig. 3). The lines for acetates and acids are exactly parallel. These relationships enable one to identify unknown compounds and to choose a suitable internal standard for each group of acids or acetates to be determined quantitatively.

## REFERENCES

1 J. E. Steinhauer and L. E. Dawson, *J. Food Sci.*, 34 (1969) 359.
2 G. W. Lanigan and R. B. Jackson, *J. Chromatogr.*, 17 (1965) 238.
3 A. D. Corica and R. Samperi, *Anal. Chem.*, 46 (1974) 140.