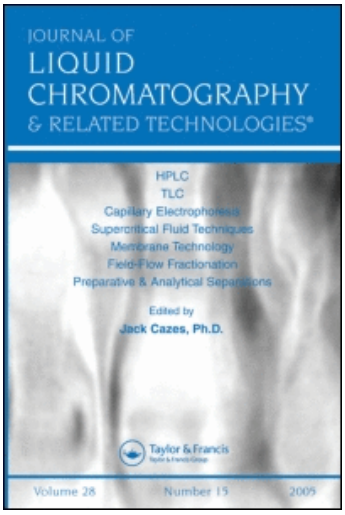

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Separation of Volatile Fatty Acids by High-Pressure Liquid Chromatography

Kathryn J. Bush[a]; R. W. Russell[a]; J. W. Young[a]
[a] Nutritional Physiology Group Department of Animal Science, Iowa State University, Ames, Iowa

To cite this Article Bush, Kathryn J. , Russell, R. W. and Young, J. W.(1979) 'Quantitative Separation of Volatile Fatty Acids by High-Pressure Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 2: 9, 1367 — 1374
To link to this Article: DOI: 10.1080/01483917908060143
URL: http://dx.doi.org/10.1080/01483917908060143

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(9), 1367-1374 (1979)

QUANTITATIVE SEPARATION OF VOLATILE FATTY ACIDS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Kathryn J. Bush, R. W. Russell, and J. W. Young
Nutritional Physiology Group
Department of Animal Science
Iowa State University
Ames, Iowa 50011

ABSTRACT

Volatile fatty acids (acetic, propionic, butyric, isovaleric, and valeric) are separated isocratically on a reverse phase C_{18} μBONDAPAK column in less than 20 min. The eluent was 0.01 M NaH_2PO_4 buffer, pH 3.5, containing 10% methanol. Separations were monitored by UV absorption at 210 nm. Peak height measurements gave quantitative linear responses from 0.25 μmole to 2.50 μmole of each acid.

INTRODUCTION

There is considerable interest in measuring volatile fatty acid concentrations in a variety of samples. Concentrations in blood and in intestinal contents are important in metabolic studies in animals, especially in ruminants where acetate, propionate, and butyrate constitute the major portion of absorbed energy. Volatile fatty acids are of interest also in the food industry because they influence flavor and aroma in cheese, wine, and fruit.

Chromatographic methods have been developed for identification and quantitation of short-chain organic acids, which include volatile fatty acids. Partition chromatography on silica gel (1) and anion exchange chromatography (2) have been successful for numerous applications. These methods are slow and cumbersome, however, and require a complex system of solvents. Low-pressure partition (3) and anion exchange chromatography (4) methods give adequate resolution only of nanoequivalent quantities of acids and require complicated photometric detection systems.

Gas-liquid chromatography frequently is used for volatile fatty acid separations but has the disadvantage of small sample size, and the component of interest generally is destroyed during detection (5). Reverse phase high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection has been used for separation of long- and short-chain fatty acid derivatives, such as naphthacyl and substituted phenacyl esters (6), but quantitation of esters of fatty acids shorter than C_{10} has not been reported (7). Our paper reports the separation and quantitation of volatile fatty acids by reverse phase HPLC.

MATERIALS AND METHODS

Analyses were performed with a Waters Associates Model ALC/GPC 501 liquid chromatograph equipped with a Rheodyne Model 7120 sample injector. Detection of the acids was at 210 nm with a Perkin-Elmer model 55 variable-wavelength spectrophotometer. The response was monitored with a Houston Instruments Omniscribe recorder set at 10 mV providing 0.02 absorbance units full scale. All results were obtained by using a 3.9 mm X 30 cm μBONDAPAK C_{18} column (Waters Associates). Eluents were 0.01 M NaH_2PO_4 buffers, pH 3.5, containing different percentages of methanol. Solvents were mixed, filtered through a 0.45 μm Millipore filter, and

degassed by vacuum filtration just before use. Eluent flow rate was 1 to 2 ml/min, requiring a pressure of 1000 to 2000 psi.

Standards of acetic, propionic, and butyric acids were prepared from sodium salts with a purity of at least 99% from Fisher Chemical Co. Valeric and isovaleric standards were prepared by using the free acids with a purity of at least 99% from Sigma Chemical Co. Samples were adjusted to pH 2.0 with H_3PO_4, and volumes of 50 µl were injected. Quantitation was by peak height measurements.

RESULTS AND DISCUSSION

A typical chromatogram of volatile fatty acid standards is shown in Figure 1. Valeric and isovaleric are resolved as shown, but butyric and isobutyric have not been separated consistently with the reported conditions. The small peak eluting just before valeric acid is an impurity from the isovaleric standard.

Adjusting samples to pH 2.0 or below is critical, particularly for accurate determination of acetic acid, as shown in Figure 2. Standards of 60 mM acetic, 15 mM propionic, and 10 mM butyric acids were adjusted to various pH values with concentrated H_3PO_4. At sample pH values above 2.0, peak tailing occurs. Tailing could be caused by an equilibrium between ionic and non-ionic species. Only the non-ionized form is retained on the hydrophobic stationary phase. Ionic components elute with the solvent front and cause errors in quantitation. Ionization also is suppressed by the ionic mobile phase buffered at pH 3.5. This pH value is greater than one pH unit below the pKa of the volatile fatty acids. Samples of the acids can be stored at pH 2.0 without loss for 2 to 3 days at 4°C in tightly capped glass vials. Addition of ^{14}C-acetate and ^{14}C-propionate verified 100% recovery.

A linear response from 0.25 µmoles to 2.50 µmoles of each acid is illustrated in Figure 3. Correlation coefficients for

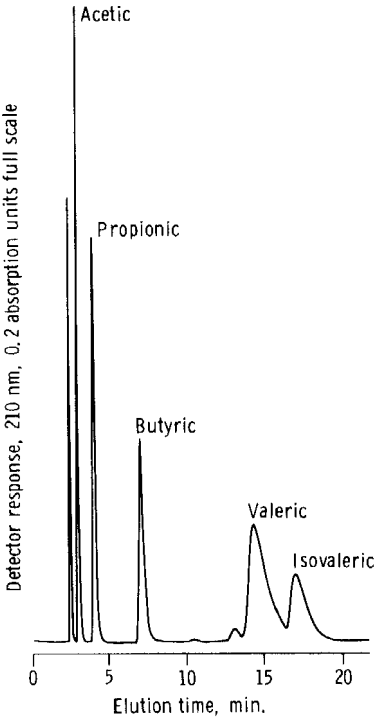

Figure 1. Separation of volatile fatty acids by HPLC. Column: 30 cm X 3.9 mm μBONDAPAK C_{18}, eluent 0.01 M NaH_2PO_4, pH 3.5/10% methanol, flow rate of 2 ml/min, injection of 2.0 μmole in 50 μl, sample pH 2.

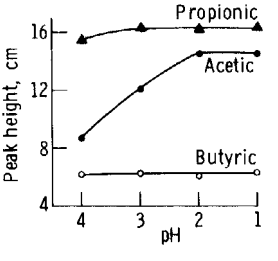

Figure 2. Effect of sample pH upon peak height of the volatile fatty acids. Acetic was detected at 100 mV whereas propionic and butyric were detected at 10 mV. Other conditions were identical to those used for Figure 1.

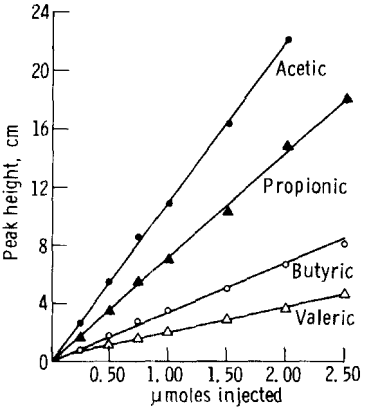

Figure 3. Peak height measurements from different amounts of volatile fatty acids. Each point represents duplicate 50 μl injections.

the calibration curves are: acetic, 0.9995; propionic, 0.9992; butyric, 0.9986; valeric, 0.9796. Because increasing chain length increases retention time, there is an increase in peak width and a decrease in peak height, which leads to a loss in sensitivity and precision of detection from acetic to valeric acids. Initial studies showed that gradient elution, with increasing methanol percentages, sharpens the valeric peak. Quantitation by peak area also will improve quantitation of valeric acid.

The present method was developed for and is being used for metabolic studies of isotopically labeled volatile fatty acids in cattle. The method is an expansion and improvement of an earlier method for isolating propionic acid from rumen contents of cattle (8). The relatively low concentrations of propionate in portal vein blood, approximately 0.25 mM, are detected after a protein-free filtrate of plasma is concentrated 10-fold by freeze-drying. The capacity of our system allows separations of quantities necessary to accurately measure radioactivity in collected volatile fatty acid fractions.

Our procedure is quite flexible. Increasing the methanol percentage of the mobile phase decreases the retention time of the acids. Adjustment of the methanol percentage has been used to separate acids of interest. For example, in cattle blood, lactate can be resolved from acetate by using 0.01 M NaH_2PO_4, pH 3.5, without methanol, as the mobile phase, as shown in Figure 4A. Addition of 3% methanol to the mobile phase allows separation of propionic acid from interfering peaks present in

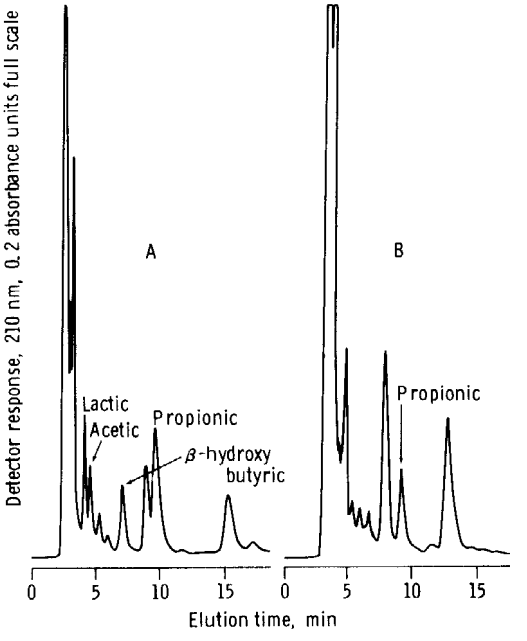

Figure 4. HPLC separation of volatile fatty acids present in cattle blood. Protein-free arterial blood plasma was concentrated 10-fold and spiked with 1.0 μmole of propionic acid. Column: 30cm X 3.9mm μBONDAPAK C_{18}, flow rate of 1 ml/min, injection 50 μl, sample pH 2. (A) eluent of 0.01 M NaH_2PO_4, pH 3.5. (B) eluent of 0.01 M NaH_2PO_4/3% methanol.

protein-free filtrates of plasma (Figure 43). Such an addition of methanol to the mobile phase causes co-elution of lactic with acetic acid, and also shifts the elution pattern of the other acids as seen by comparing figure 4A to 4B.

ACKNOWLEDGMENTS

This is Journal Paper No. J-9574 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2186. Supported in part by funds provided by PHS/NIH Grants No. AM-10706 and 5 S05RR 07034-09. The authors thank Dick Cotter of Waters Associates for his original suggestion to use acidic phosphate buffers to improve resolution of short-chain organic acids.

REFERENCES

1. Ramsey, H. A., Separation of Organic Acids in Blood by Partition Chromatography, J. Dairy Sci. 46, 480 (1963).

2. Von Korff, R. W., Ion-Exchange Chromatography of Citric Acid Cycle Components and Related Compounds, Methods Enzymol. 13, 425 (1969).

3. Stahl, K. W., Schafer, G., Lamprecht, W., Design of a High Efficiency Liquid Chromatograph Using Specific Detection and its Evaluation for Analysis of Tricarboxylic Acid Cycle (TCA) Intermediates and Related Compounds on a Nano-Equivalent Scale, J. Chromatogr. Sci. 10, 95 (1972).

4. Bengtssen, L., Samuelson, O., Separation of Microgram Quantities of Dicarboxylic Acids by Anion-Exchange Chromatography, Anal. Chim. Acta 57, 93 (1971).

5. DiCorcia, A., Samperi, R., Determination of Trace Amounts of C_2-C_5 Acids in Aqueous Solutions by Gas Chromatography, Anal. Chem. 46, 140 (1974).

6. Jordi, H. C., Separation of Long and Short Chain Fatty Acids as Naphthacyl and Substituted Phenacyl Esters by High Pressure Liquid Chromatography, J. Liq. Chromatogr. 1, 215 (1978).

7. Miller, R. A., Bussell, N. E., Ricketts, C., Quantitation of Long Chain Fatty Acids as the Methoxphenacyl Esters, J. Liq. Chromatogr. 1, 291 (1978).

8. Herbein, J. H., Van Maanen, A. D., McGilliard, A. D., Young, J. W., Rumen Propionate and Blood Glucose Kinetics in Growing Cattle Fed Isoenergetic Diets, J. Nutr. 108, 994 (1978).