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Gas–liquid chromatography of short-chain fatty acids on Dexsil 300GC

Measurement of short-chain fatty acids in various biological materials is becoming increasingly important in the physiology and taxonomy of microorganisms[1] and in the dairy, food, and beverage industries[2]. These acids have been analyzed in the free state by gas–liquid chromatography (GLC) on columns packed with polar stationary phases[2–4]. With this type of column, however, temperature programming is restricted, long analysis times are required, and high bleed rates are encountered. Furthermore, quantitation is difficult because the free acids tend to dimerize and/or adsorb on the column[2–4] and some acids, especially formic acid, tend to volatilize quite readily. In addition, formic acid is not detected by flame ionization detectors used in many instruments[4]. There are also reports that injection of free acids may damage the metallic parts of the gas chromatograph[2].

For these reasons, many workers have converted the free acids to various ester derivatives for subsequent analysis by GLC[2–4]. BROOKS *et al.*[5,6] have successfully analyzed the butyl ester (BE) and the trifluoroacetylbutyl ester (TFA-BE) derivatives of organic acids produced by several genera of bacteria by using dual 7.3 m (24 ft.) × 0.63 cm O.D. glass columns packed with 3% OV-1. However, they were not able to completely resolve the ester of formic acid from the solvent peaks.

Although this technique[5,6] has been very useful in our laboratory, there are some limitations in the GLC parameters. First, some gas chromatographs cannot accommodate dual 7.3 m × 0.63 cm O.D. columns because of instrument design. In addition, long glass columns require large amounts of packing material and are difficult to handle. In an effort to solve these problems and to improve the separation of short-chain acids, we investigated other GLC stationary phase materials, including Dexsil 300GC* (Analabs, New Haven, Conn.). In this report, we describe the use of a 3.66 m (12 ft.) 15% Dexsil column for analysis of the ester derivatives of twelve short-chain fatty acids.

Materials and methods

The acids were obtained from Chem Service, Inc. (Media, Pa.) and Eastman Organic Chemical (Rochester, N.Y.). A 1:100 dilution of formic (C_1), acetic (C_2), propionic (C_3), isobutyric (iC_4), butyric (C_4), isovaleric (iC_5), valeric (C_5), 4-methylvaleric (iC_6), caproic (C_6), heptanoic (C_7) ,and lactic (Lac) acids was made by adding 0.1 ml of each acid to 9.9 ml of anhydrous ether (Fisher Scientific Company, Fair Lawn, N.J.). The succinic acid (Suc) standard was made by dissolving 0.1 g of succinic acid in distilled water, extracting three times with diethyl ether, and concentrating the diethyl ether to 5 ml. A mixture containing 0.05 ml of each of the C_1 through C_7 standards and 0.1 ml of the Lac and Suc standards was made in a 13 × 100 screw-cap test tube. The volume of the mixture was gently reduced to 0.2 ml by evaporation with dry nitrogen, and 0.1 ml of 14% w/v boron trifluoride butanol (Applied Science, State College, Pa.) reagent was added to the sample. The mixture was left under a

* Use of trade names is for identification only and does not constitute endorsement by the Health Services and Mental Health Administration or by the U.S. Department of Health, Education, and Welfare.

fume hood for a few minutes to evaporate any remaining ether. The tube was tightly capped and placed in a 100° water-bath for 5–10 min, and cooled to room temperature. Appreximately 0.1 ml of chloroform (Matheson, Coleman, and Bell, East Rutherford, N.J.) and 0.1 ml of trifluoroacetic anhydride (TFAA, Regis Chemical Co., Chicago, Ill.) were added, the tube was tightly capped, and the content was gently mixed. After 10 min at room temperature, the sample was washed with 0.2 ml of distilled water to remove excess reagents. The chloroform layer was removed to a clean, dry test tube, and the final volume was adjusted to 0.2 ml. 1 μl of the ester derivatives was injected into the gas chromatograph.

A Perkin-Elmer Model 990 gas chromatograph equipped with flame ionization detectors and a disc integrator recorder was used for all analyses. The instrument contained a 3.66 m (12 ft.) × 0.63 cm O.D. (0.32 cm I.D.) coiled glass column packed with 15% Dexsil 300GC coated on 80–100 mesh, acid washed, DMCS treated Chromosorb W. The temperature of the injector port was 235° and the temperature of the detector was 275°. The column bath was maintained at 90° for 6 min after injection of the sample and then temperature programmed to 250° at 6°/min. The carrier gas was prepurified nitrogen with a flow-rate of 50 ml/min. The electrometer range was 10, with an attenuation of 32.

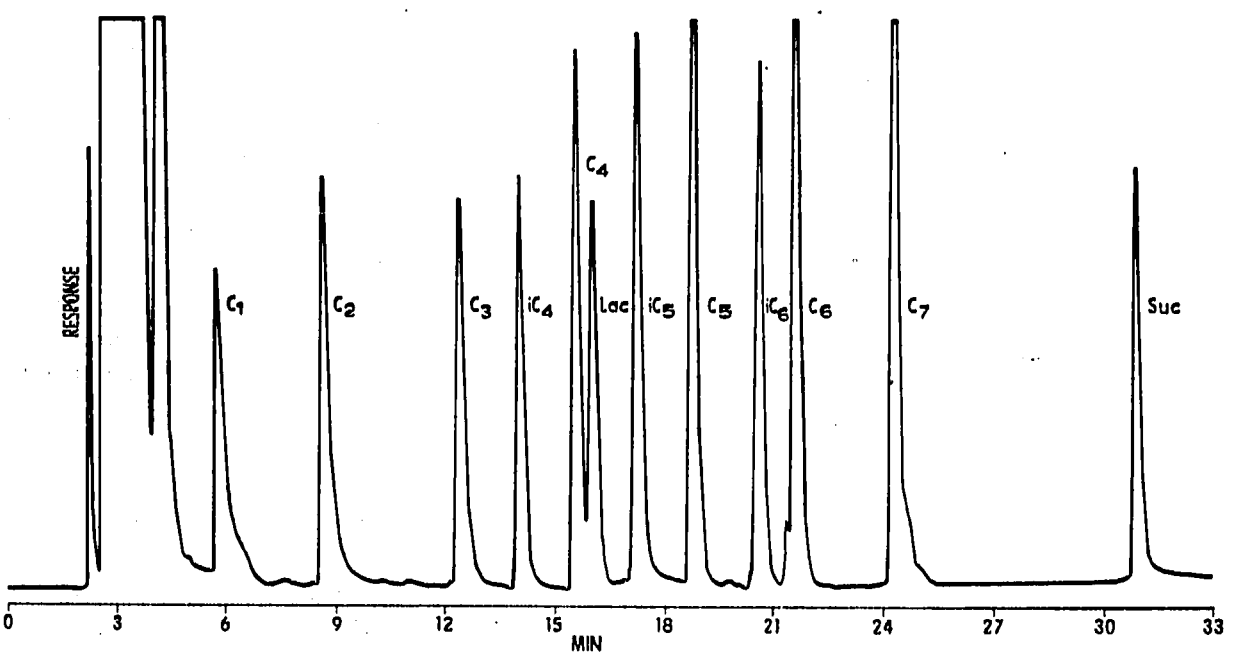

Fig. 1. Gas chromatogram of ester derivatives of short-chain fatty acids on a 15% Dexsil 300GC column. Each peak represents the following amounts: C_1 = 2.7 μg; C_2 = 2.6 μg; C_3 = 2.5 μg; iC_4 = 2.4 μg; C_4 = 2.4 μg; Lac = 5.2 μg; iC_5 = 2.3 μg; C_5 = 2.4 μg; iC_6 = 2.3 μg; C_6 = 2.4 μg; C_7 = 2.3 μg; and Suc = *ca.* 2 μg.

Results and discussion

Shown in Fig. 1 is the chromatogram of the ester derivatives of C_1 through C_7, Lac, and Suc acids which were prepared and injected into the Dexsil column as described in *Materials and methods*. All twelve acid esters produced sharp, symmetrical peaks which were well separated from each other. Column bleed was negligible, and quantitation of the acids could be accomplished without difficulty. The

first peak, formic acid, was well separated from the solvent peaks. The last peak, succinic acid, eluted at 31 min. Reproducible retention times were obtained upon repeated analyses.

In the course of this study we have observed that certain precautions must be taken for accurate quantitative measurements. First, care must be taken during solvent evaporation to insure that the sample volume remains greater than 0.1 ml. When the sample volume was reduced to less than this amount, significant loss of formic, acetic, and propionic acids occurred (approximately 30% for each). Some loss of these same three acids (approximately 10%), as well as of lactic acid, was also observed when the ester derivatives were washed more than twice. Extra washing steps may be necessary, however, for some biological samples in order to remove excess reagents and other substances which could interfere with GLC analysis. In this case, an accurate determination of the amount of acid lost during the washing procedure will be required for quantitative data to be meaningful. Some hydroxy acids may require the addition of pyridine or another catalyst to effect a complete reaction at the acylation step[6,7].

Use of Dexsil as a stationary phase eliminates the need for a dual 7.3 m (24 ft.) column system since there was no column bleed throughout the entire programmed run. An important additional advantage in using the 15% Dexsil column is the baseline separation of the formic acid ester from the solvent peaks. This facilitates an accurate quantitative measurement of the acid. The response of the flame detector to the acid esters on Dexsil is similar to the response obtained on non-polar columns (OV-1, SE-30) and indicates that these compounds have little affinity for the stationary phase. An initial temperature of 90° is quite reproducible with our instrument and should be easily attained with other gas chromatographs after a normal cooling period. In some instances, temperatures of less than 85° are difficult to reproduce without excessive cooling of the column bath or use of a subambient accessory.

Dexsil 300GC is a polycarboranesiloxane stationary phase developed by the Olin Corporation[8]. It has a temperature range of 50 to 500° and a low bleed rate. Consequently, it could serve as an additional GLC column for determination of many compounds which are presently being analyzed on non-polar silicone phases such as OV-1 and SE-30. With the 15% Dexsil column, we have obtained excellent separation of amino acid derivatives and fatty acid methyl esters of up to twenty-four carbon atoms in length. There was no significant bleed from the column even though the final temperature reached 300°. Because of this high-temperature stability, Dexsil may be useful for analysis of high-boiling compounds which previously could not be studied by GLC because of temperature limitations of the stationary phase. It should also prove useful in the combined gas chromatograph–mass spectrometer instruments which require column packing materials with the characteristics of Dexsil 300GC.

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