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Note

Analysis of short-chain acids by gas–liquid chromatography on SP-1220

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In our laboratory, we have found that the analysis of short-chain acids by gas–liquid chromatograpyy (GLC) is a useful means of identifying certain species of *Pseudomonas*[1]. Separating these acids by gas chromatography is often difficult because their boiling points are within a close range and their resolution requires much lower column temperatures than do longer chain, less volatile acids. In order to separate short-chain acids and their branched isomers successfully, we formed the butyl esters and analyzed them on a Dexsil (polycarborane siloxane) 300GC* stationary phase[2]. However, recent studies of bacterial groups other than *Pseudomonas* have shown that the former produce keto acids, some of which cannot be separated from certain other short-chain acids on Dexsil[3]. Therefore, means of separating these acids were investigated on several GLC phases, using pure reference standards. Our data show that certain keto and other short-chain acids can be readily resolved by the use of a second stationary phase in conjunction with Dexsil. A phase which is well-suited for this purpose is Supelco's SP-1220/H_3PO_4 (Supelco, Bellefonte, Pa., U.S.A.). This phase is polyester (15%) and acid (1%) and allows the resolution of "free" or underivatized acids of low molecular weight.

EXPERIMENTAL AND RESULTS

The chromatogram in Fig. 1 shows the separation of a mixture of free acids ranging from acetic (C_2) to heptanoic (C_7) on 15% SP-1220/1% H_3PO_4. For analysis, we used a Perkin-Elmer instrument (Model 900) equipped with a coiled glass column (3.04 m × 0.002 m I.D.) in which the SP-1220 phase was packed. The initial temperature of the column bath was 90° and, 4 min after the sample was injected, the temperature was increased to 200° at a rate of 5°/min. Under these conditions, all acids eluted from the column within 20 min. The identities of peaks 1–12 in order of elution (Fig. 1) are acetic (C_2), propionic (C_3), isobutyric (iC_4), butyric (C_4), isovaleric (iC_5), valeric (C_5), 2-ketoisovaleric, isocaproic (iC_6), 2-ketovaleric, caproic (C_6), 2-ketoisocaproic, and heptanoic (C_7) acids. With the exception of 2-ketobutyric acid, we were able to separate several short-chain keto acids, including pyruvic acid (not shown in Fig. 1), from other short-chain acids on SP-1220.

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

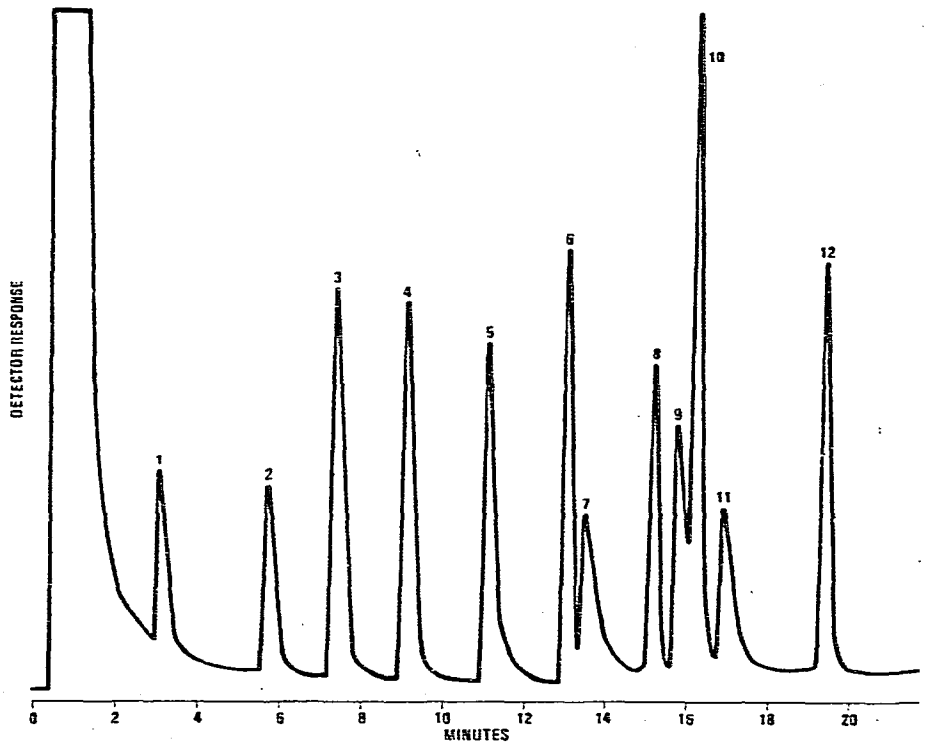

Fig. 1. Gas chromatogram of reference standards of short-chain acids on a 15% SP-1220/1% H_3PO_4 column. The peaks represent the following acids: 1 = acetic; 2 = propionic; 3 = isobutyric; 4 = butyric; 5 = isovaleric; 6 = valeric; 7 = 2-ketoisovaleric; 8 = isocaproic; 9 = 2-ketovaleric; 10 = caproic; 11 = 2-ketoisocaproic; 12 = heptanoic.

The advantage of using the two GLC phases in combination can be readily demonstrated in the analysis for metabolic products of several bacterial species. For example, *Flavobacterium meningosepticum* produces a number of short-chain acids, all of which can be identified by the combined use of SP-1220 and Dexsil. A typical profile of these acids analyzed on SP-1220 is shown in Fig. 2. Peaks 1, 3, 6, and 7 were identified on both columns and by mass spectrometry[4] as propionic, isovaleric, phenylacetic (PA), and 2-ketoglutaric acids. Identities of the remaining three acids were established on one of the two columns. Retention time data from the Dexsil column indicated that peaks 4 and 5 (Fig. 2) were normal acids (isocaproic and isoheptanoic, respectively). Since we had previously had trouble separating normal and keto acids on Dexsil[3], we considered the possibility that these could also be keto acids. A comparison of retention times of reference standards on SP-1220 (Fig. 1) showed that peak 4 represented 2-ketoisovaleric acid and that peak 5 represented 2-ketoisocaproic acid. Conversely, the identity of iC_4 (peak 2, Fig. 2) was established on Dexsil, because iC_4 and 2-ketobutyric acids elute together on SP-1220. Although peaks for pyruvic and phenylpyruvic acids are not shown in Fig. 2, trace amounts were detected in the growth medium of some strains of *F. meningosepticum*.

It is clear from these data that the Dexsil and SP-1220 columns must be used in combination in order to separate and identify all the acids present in the growth

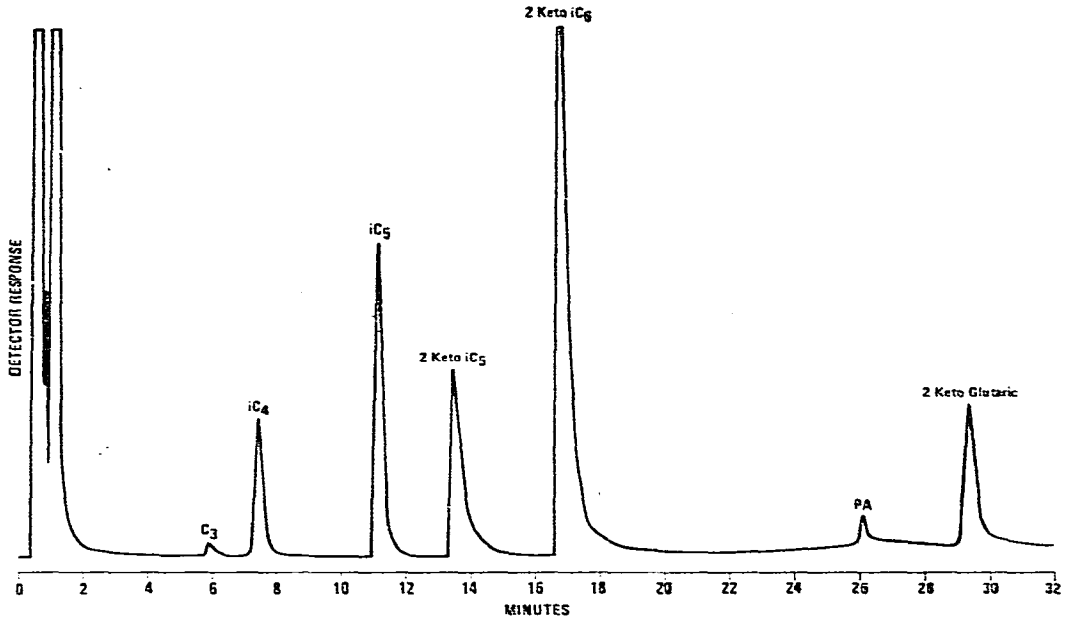

Fig. 2. Gas chromatogram of the short-chain acids produced by *Flavobacterium meningosepticum*. Acids are identified as 1 (C_3) = propionic; 2 (iC_4) = isobutyric; 3 (iC_5) = isovaleric; 4 (2 Keto iC_5) = 2-ketoisovaleric; 5 (2 Keto iC_6) = 2-ketoisocaproic; 6 (PA) = phenylacetic; and 7 (2 Keto Glutaric) = 2-ketoglutaric. Analysis was made on a 15% SP-1220/1% H_3PO_4 column.

medium of *F. meningosepticum*. Based on retention time data from both columns, strains of *F. meningosepticum* were readily distinguished from those of *Achromobacter* sp., which also produce keto acids[3]. Because of the short analysis time and commercial availability of the GLC phases and reference standards, this method of examining spent media extracts on two columns can be applied as a rapid identification procedure for routine clinical isolates.

REFERENCES

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