

EXTENSION OF FLASH EXCHANGE GAS CHROMATOGRAPHY TO ETHYL ESTERS OF HIGHER ORGANIC ACIDS

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Analytical separations of most volatile organic compounds are rapidly accomplished by gas chromatography, but before a compound can be positively identified confirmatory information is necessary. Where possible, this confirmation is obtained by infrared or mass spectrometric procedures. However, as the separating efficiency of gas chromatographic methods has increased sample sizes have also decreased, and it has become increasingly difficult to confirm, by conventional methods, the identity of components separated by the newer gas chromatographic technique.

With these limitations in mind the flash exchange gas chromatographic technique of RALLS¹ was investigated for its possible use as a method for obtaining additional evidence of identity for organic acids previously detected directly as the free acid by gas chromatography².

In the RALLS method the potassium salts of C₁ to C₆ monobasic, aliphatic carboxylic acids were reacted with potassium ethyl sulfate at 300° to produce ethyl esters. The products were volatilized directly into a gas chromatographic column connected to thermal conductivity detectors. In order to use the method with the more sensitive argon ionization detectors, it was modified so that smaller quantities of starting material could be used. This paper describes changes in technique that are necessary to accomplish this objective and to extend the method to the preparation and analysis of ethyl esters of organic acids of carbon content up to and including C₁₈. Use of the method to confirm the identity of organic acids in fermented liquors used in bread production is also described.

APPARATUS

Chromatograph

A Barber-Colman Model 10 gas chromatographic unit** employing an ionization type detection cell containing 56 μ C of radium-226 was used in these experiments. The cell was operated at 250° and a potential of 1000 V. The U-shaped column

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consisted of heavy-walled borosilicate glass tubing, 4 mm inside diameter, 8 feet long and packed with Lac-I-R 296 (glycol-adipate polymer) (5 parts) on fire-brick acid washed (40- to 60-mesh) (95 parts). The column temperature was maintained at 60°, 125°, or 175°, depending upon the volatility of the fraction examined. A preheater maintained at 275° ± 5° surrounded the inlet. Gas (argon) flowrate was 100 ml/min with an inlet pressure of 10 p.s.i.g. and atmospheric outlet pressure.

Hypodermic needles

22 gauge, 1½ and 2 in. in length.

REAGENTS

Organic acids

The carboxylic acids used were commercial samples, except for dimethylacrylic acid which was synthesized³. All yielded single peaks when tested individually in the gas chromatographic apparatus. Stock solutions of sodium and potassium salts were prepared from purchased and synthesized acids.

The potassium ethyl sulfate was prepared following the directions of EVANS AND ALBERTSON⁴.

Standard solutions

Standard solutions of sodium or potassium salts of the more volatile organic acids were made up to contain approximately 10 mg of salt/ml of solution. Salts of the higher-boiling acids in the C₁₀ to C₁₈ range were prepared to contain about 20 mg of salt/ml of solution.

A 0.1 ml aliquot of each acid was placed in a 3-ml centrifuge tube, and an equal weight of potassium ethyl sulfate in approximately 0.1 ml solution was added. To the colorless mixture 0.01 ml of ordinary red ink was added as a marker. Where a synthetic mixture of 6 or more acids was prepared, the salts were added in solution and evaporated to dryness on a steam bath prior to the addition of the potassium ethyl sulfate, red ink, and water.

Diatomaceous earth

"Celite", Johns Manville analytical grade.

Fermentation liquor

A pre-ferment, such as that used in certain methods of bread production, was extracted with solvent and alkali as described in a previous publication².

EXPERIMENTAL PROCEDURE AND RESULTS

Samples were prepared for injection into the chromatographic column in the following manner. Celite was introduced into hypodermic needles by partially stuffing the hubs with absorbent cotton, attaching the needles to a vacuum source (water aspirator) and then dipping them briefly into Celite. After dipping they were quickly

removed to avoid compacting the filler. Approximately $1\frac{1}{2}$ mg of Celite was picked up by a $1\frac{1}{2}$ -in. needle and 2 mg by a 2-in. needle. The plug of absorbent cotton was removed before using the needles.

A needle containing Celite was then placed into a 3-ml centrifuge tube containing the mixture to be analyzed. The needles usually filled satisfactorily by capillary action but, in some cases, slight aspiration was applied by means of a hypodermic syringe. The red ink in the mixture colored the Celite and served to indicate when the needle was filled. The ink apparently in no way interfered with the reaction. The Celite in the needle tended to pick up about twice its weight of solution. The needle and its contents were then dried in a convection oven (105°) for 25 min. The needle containing the dry mixture was next connected to a source of argon (25 p.s.i.g.), inserted through a rubber septum into the preheated section of the chromatographic column and left in place for 25 sec. As the exchange reaction to form the ethyl esters took place, the products were swept into the column by the argon gas stream.

Chromatograms of the ethyl esters of various acids, prepared by the above method, are illustrated in Figs. 1 and 2. If a large excess of potassium ethyl sulfate is

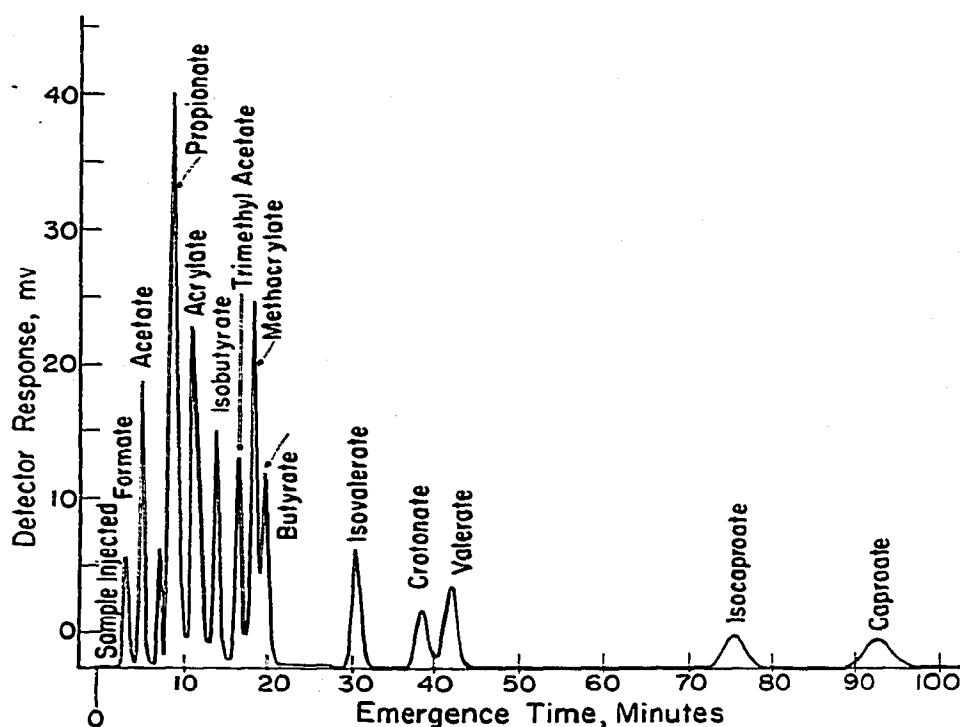


Fig. 1. Chromatogram of a known mixture of ethyl esters prepared from organic acids by flash exchange gas chromatography. Column temperature 60° . Each peak represents between 1 and 10 μg of material.

used, an extraneous peak may appear early in the chromatogram of the lower-boiling esters (third peak, Fig. 1). However, this component does not seem to interfere with separation of the acids that have been investigated; these include most of those commonly encountered.

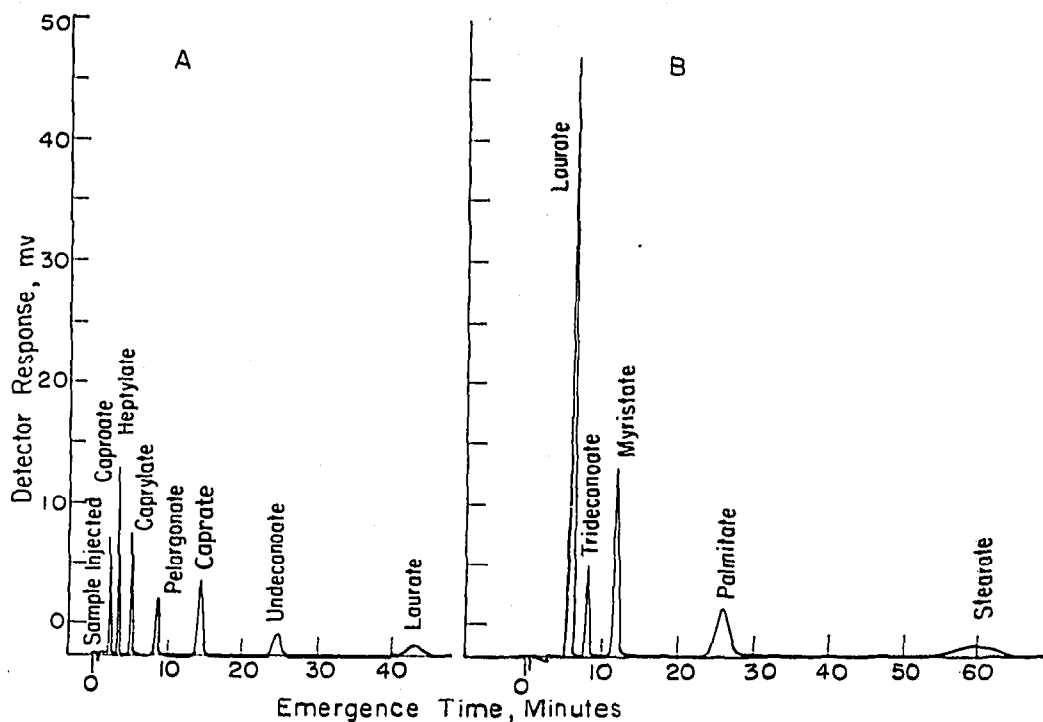


Fig. 2. Chromatograms of a mixture of higher boiling ethyl esters prepared from organic acids by flash exchange chromatography. A. Column temperature 125°. B. Column temperature 175°. Each peak represents between 1 and 10 μg of material.

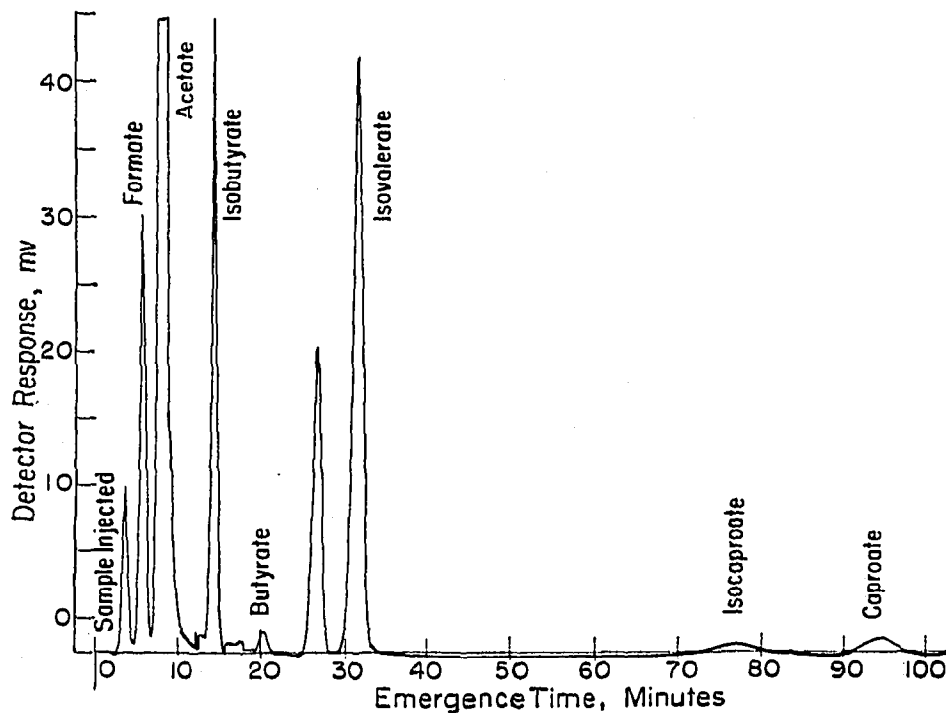


Fig. 3. Chromatogram of ethyl esters prepared by flash exchange chromatography of the organic acids extracted from a pre-ferment for bread making. Column temperature 60°.

For convenience, analytical separations of free organic acids have been conducted stepwise at three different temperatures covering the boiling-point range. However, separations of all acids C_1 to C_{18} as their ethyl esters can be satisfactorily accomplished in a single run using programmed heating techniques. No attempt has been made to quantitate results as the exchange reaction does not go to completion¹. Furthermore, the reaction mixture is unstable at room temperatures and the amount of material available for exchange slowly changes with time.

A chromatogram of the ethyl esters of organic acids found in a bread pre-ferment is shown in Fig. 3. The acids had previously been tentatively identified directly by gas chromatography².

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SUMMARY

The method of flash exchange to form esters of organic acids for gas chromatography has been modified for use with argon ionization detectors and extended to include organic acids of carbon content through C_{18} . An aliquot of a mixture of potassium (or sodium) salts of organic acids and potassium ethyl sulfate in water is drawn into a hypodermic needle containing diatomaceous earth. The water is removed by warming the mixture in the needle in a convection oven at 100° . The needle is connected to a source of argon (15 p.s.i.g.) and then inserted into a pre-heated (275°) section of the gas chromatography column. As the exchange reactions to form ethyl esters take place, the products are swept into the column by the argon gas stream. Chromatograms of the esters contain sharp, well-separated peaks. Use of the method to confirm the identity of organic acids in fermented liquors used in bread production is described.

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