Journal of Chromatography, 82 (1973) 382–389

CHROM. 6753

Note

Gas chromatographic separation of metabolic acids on surface-modified Chromosorb*

KLAUS O. GERHARDT and WALTER A. AUE**

Department of Agricultural Chemistry, University of Missouri, Columbia, Mo. 65201 (U.S.A.)

(Received February 12th, 1973)

In some recent studies[1–3] we have shown that the heat treatment of Carbowax 20M-coated support, followed by exhaustive extraction with methanol or other organic solvents, yielded very efficient chromatographic packings. Several types of polar compounds separated with sharp, symmetrical peaks. Furthermore, there was little indication of the decomposition, which labile compounds commonly suffer on supports coated with very thin polymer layers.

The layer of Carbowax 20M on Chromosorb W obtained by heat treatment and exhaustive extraction was quite thin —most likely below 15 Å. Under these conditions, retention times were short, and high carrier gas flows could be used with little loss in chromatographic efficiency.

An additional advantage of our phase was its ability to separate closely related substances, most notably some which could not be resolved on a regular Carbowax 20M packing of higher load. The successful separation of the butyl esters of nitrilotriacetic and citric acids[1], in particular, encouraged us to investigate the potential of this phase for resolving a number of metabolic acids. The selection of acids for this study was guided by their commercial availability, their biochemical importance, and their expected chromatographic behavior. Nineteen acids were combined in one mixture and sixteen in another, grouped for easy separation by temperature-programmed gas chromatography (GC). Their esterification with *n*-butanol/HCl (the hydroxyl group of hydroxy-acids was not derivatized) followed the procedure reported for nitrilotriacetic and citric acids[1].

Numerous analytical methods have of course been developed for the determination of organic acids, and the literature on this subject appears far too extensive to cite. Refs. 4–13 in this report as well as some references on citric acid in our earlier paper[1] deal with GC techniques. However, this list is by no means exhaustive.

In addition to exploring whether or not our phase would permit satisfactory chromatography of various metabolic acids, we wanted to investigate the correlation of chromatographic efficiency to the carrier gas flow, *i.e.* by measuring HETPs at isothermal conditions; and resolution in a temperature program. Gas–liquid

* Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 6632. Supported by the Environmental Trace Substances Center, Columbia, Mo., U.S.A.

** Contact for reprint requests at: Department of Chemistry, Dalhousie University, Halifax, N.S., Canada.

(GLC) and gas–solid (GSC) chromatographies differ, among other aspects, in their Van Deemter plots: The minimum of GSC occurs at higher linear gas velocities, and the subsequent rise of HETP at higher flow-rates has a much gentler slope. Although our phase has not yet been adequately characterized in a "GSC *versus* GLC" context, it is safe to assume that it bears traits of both concepts. Consequently, it was interesting for us to define closer the range of carrier gas flow suitable for analytical conditions.

EXPERIMENTAL

Acid mixtures were prepared in water, ether or pyridine and aliquots placed into 16 mm × 75 mm culture tubes with PTFE-lined screw caps. Prior to use, these tubes had been boiled for 2 h in concentrated hydrochloric acid and rinsed with deionized water.

After the solvent had been evaporated under a stream of nitrogen, 2 ml of dry 3 *N* HCl–butanol[7] were added to the dry sample. The tightly-capped culture tube was stirred ultrasonically for 2 min at room temperature, and transferred for esterification to a water-bath kept at 75°. After 30 min it was allowed to cool, and the excess reagent was removed by a slow stream of nitrogen in a tube-heating block. Although the evaporation was started initially at 85°, the heater was turned off after 2 min and the tubes were withdrawn and closed immediately when they appeared dry. In some cases, *n*-hexadecane was added prior to the esterification step as a "keeper".

For GC, 100 μl dry acetone were added to the esterified acids and 1 μl of the solution was injected into the instrument. The butyl esters were separated on a 175 cm × 2 mm I.D. borosilicate glass column packed with Chromosorb W, 60–80 mesh, surface-modified with Carbowax 20M; and were determined by a hydrogen flame ionization detector (FID).

RESULTS AND DISCUSSION

The main purpose of this study, *i.e.* to demonstrate the analytical utility of a surface-modified support for the separation of the *n*-butyl esters of various metabolic acids, has been essentially achieved. Figs. 1 and 2 show temperature-programmed separations of 35 of these compounds. The traces of separations at the 10-ng level have been included to demonstrate the relatively low bleed-rate of the polymer used to deactivate the support, and the fact that some, if not all, of the esters could be detected with relative ease.

It is obvious from the chromatographies that a few of the acid derivatives were present at much lower levels than commensurate with the initial sample composition. This point is made again by the calibration curves shown in Figs. 3–5. A number of conditions could be reasonably suspected of producing these adverse effects. Since we were interested in demonstrating the analytical potential of this GC phase for a group of compounds, rather than to devise analytical methods for individual members, all acids were derivatized under the same conditions. Although these conditions represent a reasonable choice for esterifying 35 acids in two mixtures, they are by no means optimal for individual compounds. Some

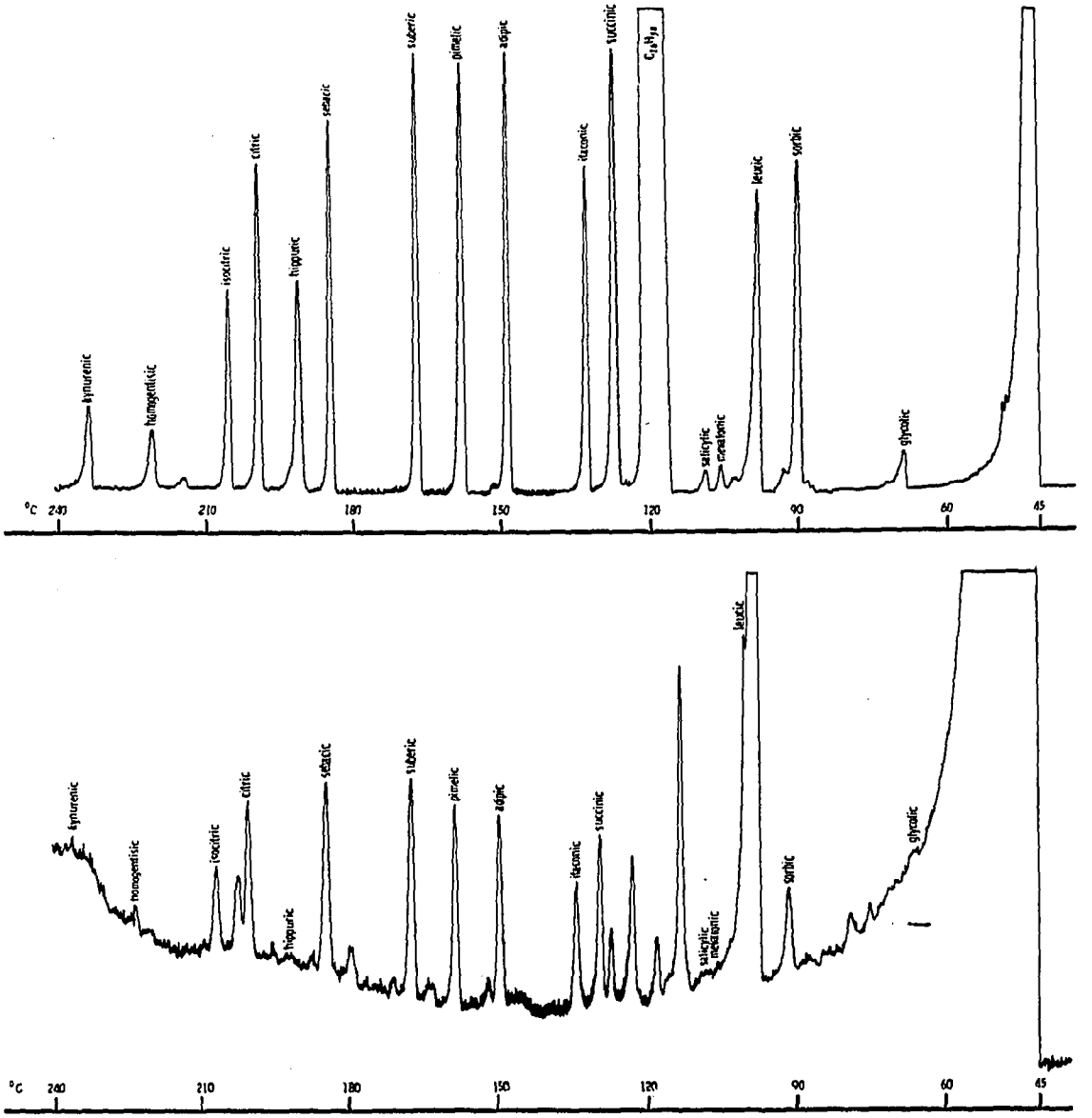

Fig. 1. Separation of sixteen acids at the 1.0-μg (upper trace) and 10-ng levels. Column: Chromosorb W, AW, 60–80 mesh, surface-modified with Carbowax 20M, in a 175 cm × 2 mm I.D. Pyrex U-tube. Programmed at 3 °/min from 45° to 240°. Nitrogen flow-rate, 30 ml/min; sample size, 1 μl, containing 1.0 μg or 10 ng of each acid, respectively. MT-220, FID.

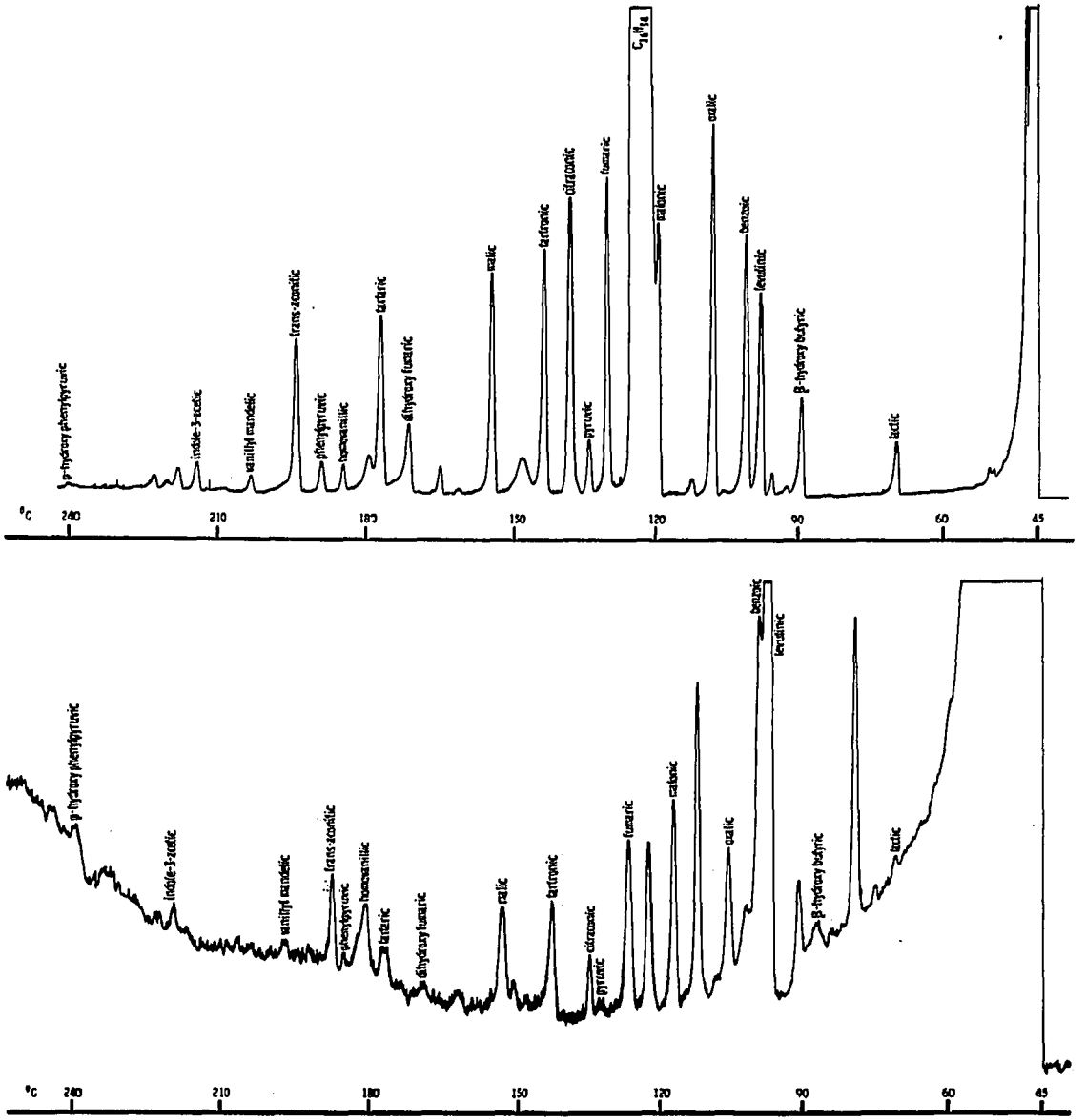

Fig. 2. Separation of nineteen acids at the 1.0-μg (upper trace) and 10-ng levels. Conditions as in Fig. 1.

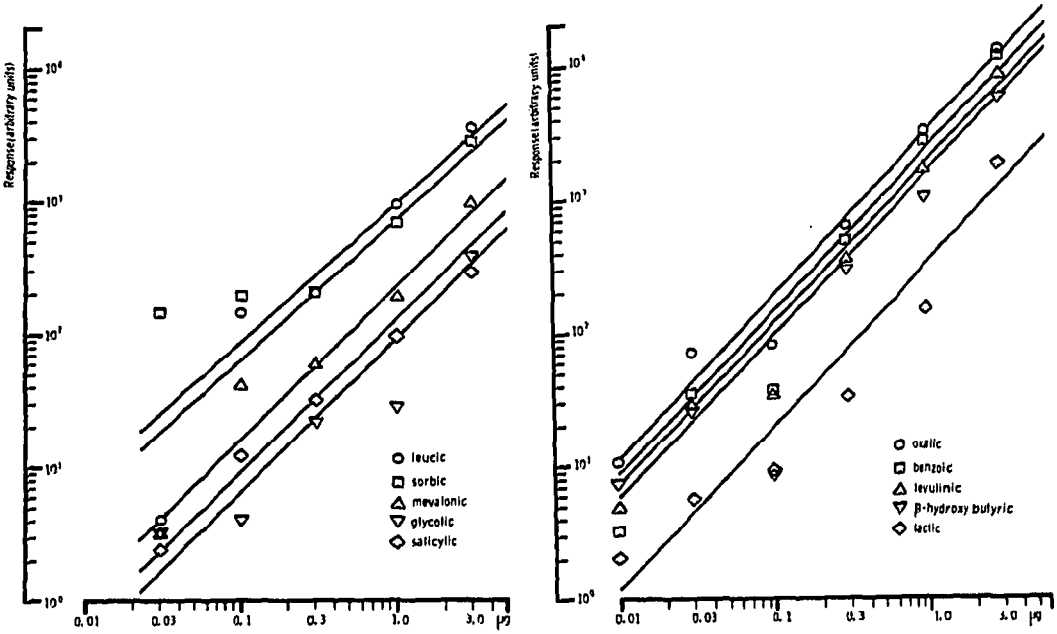

Fig. 3. Calibration curve of acids eluting in lower temperature range.

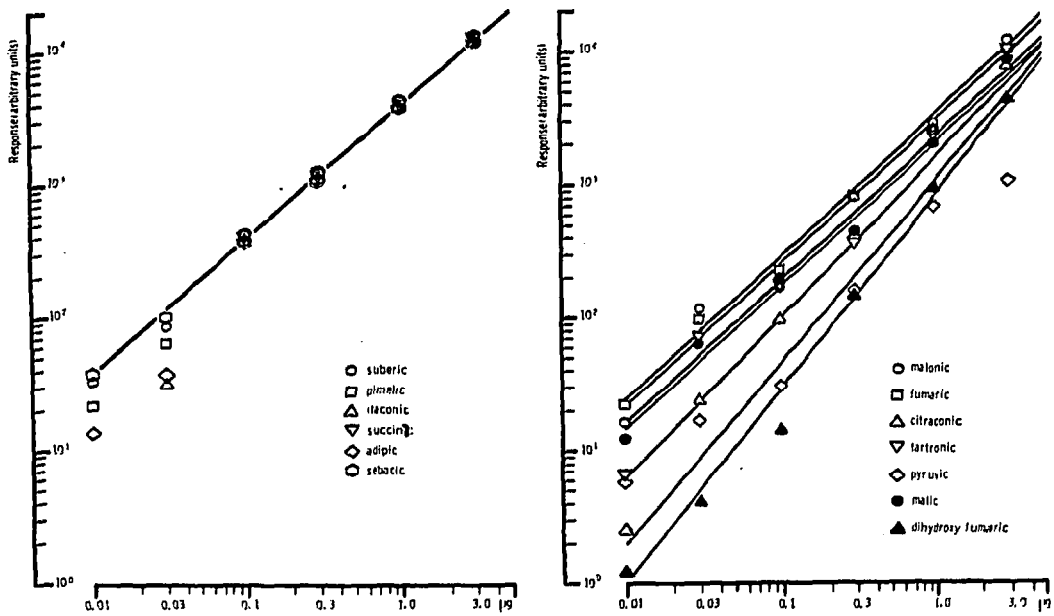

Fig. 4. Calibration curve of acids eluting in medium temperature range.

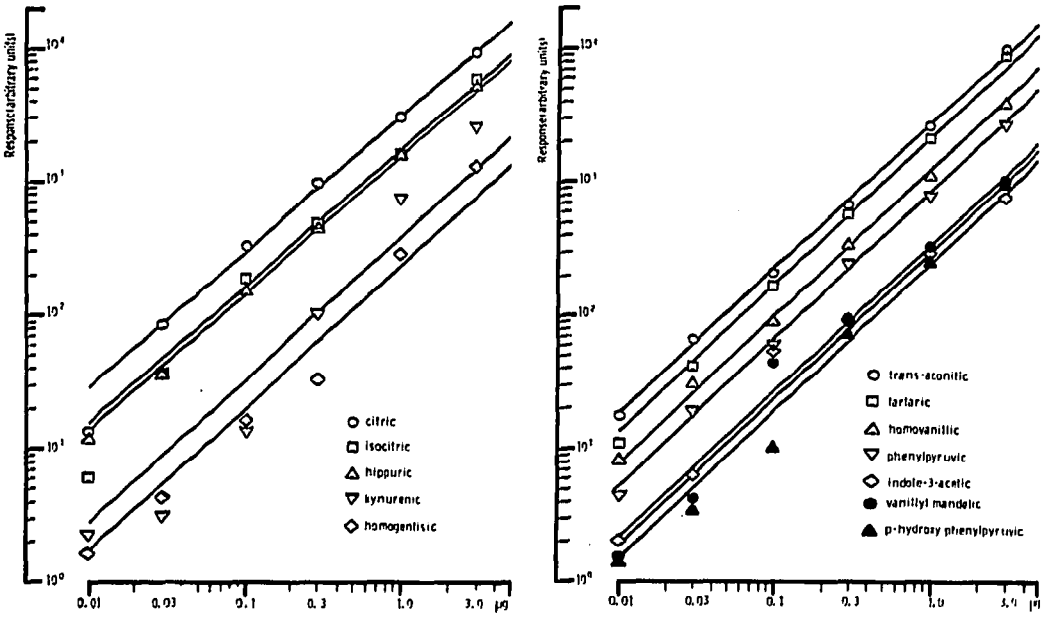

Fig. 5. Calibration curve of acids eluting in higher temperature range.

acids or their derivatives are not too stable and could have decomposed during either the esterification, or the solvent removal, or the GC separation.

It is also possible that some low-boiling esters were lost by evaporation while removing the reagent. In order to reduce their loss, *n*-hexadecane was added as a "keeper" prior to esterification. The upper traces of Figs. 1 and 2 show its presence.

Calibration curves, however, were determined without the aid of a keeper. The *n*-hexadecane used was unfortunately not free of impurities which interfered in the low concentration ranges.

While it is obvious that the described technique represents but a general approach, strongly in need of modification if particular acids of interest are to be determined, the overall method and especially the GC separation hold considerable promise for the analysis of some metabolic acids at trace levels. Retention temperatures are low and could be further reduced if the methyl instead of the butyl ester were employed. Phase bleed is minimal. Although some decomposition of derivatives on the column is probable, its extent could be tolerated in some, if not all cases. It should be noted that the slight discrepancies in retention temperatures evident in Figs. 1 and 2 are due to imprecise reproduction of the chosen temperature program in the chromatograph we used.

During this study, the same column served for several months without showing any significant deterioration of performance. On first use, it was ready for trace analyses after but a few hours of conditioning.

A further advantage of the column, attributable to the extremely thin polymer layer, is the relatively high linear velocity of carrier gas which can be employed without loss of efficiency and resolution. The Van Deemter plot shown in Fig. 6

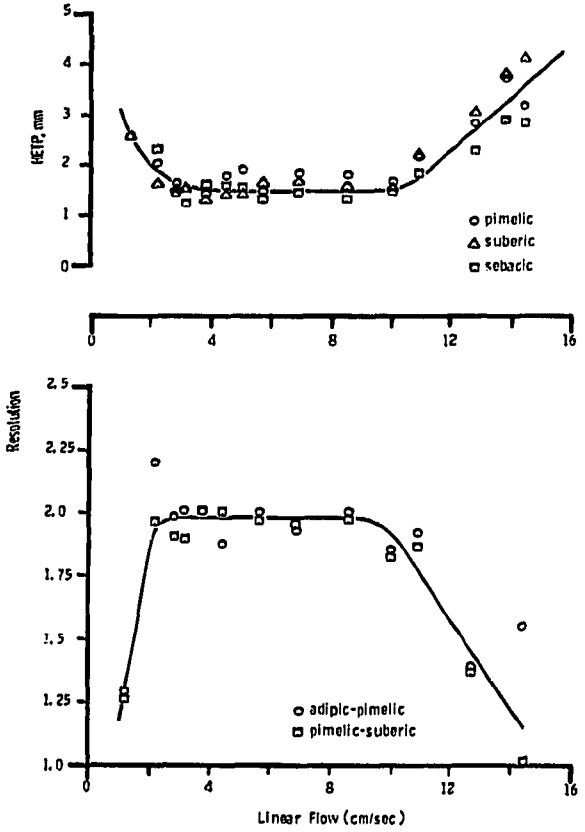

Fig. 6. GC efficiency (isothermal) and resolution (temperature programmed) as a function of carrier gas flow. Chromosorb W, AW, 100–120 mesh, surface-modified with Carbowax 20M in 175 cm × 2 mm I.D. Pyrex U-tube. HETP, isothermal at 165°; resolution curve programmed at 3° min from 105° to 210°. Carrier gas, nitrogen.

is somewhat peculiar, exhibiting characteristics of both GSC and GLC. Close to optimum conditions prevail from approximately 2 to 10 cm/sec of nitrogen flow.

ACKNOWLEDGEMENTS

We are thankful to Mr. Shubhender Kapila, who prepared the surface-modified support used in this study, and to Mr. David P. Pozniak, for competent technical assistance.

REFERENCES

1 W. A. Aue, C. R. Hastings, K. O. Gerhardt, J. O. Pierce, II, H. H. Hill and R. F. Moseman, *J. Chromatogr.*, 72 (1972) 259.
2 W. A. Aue, C. R. Hastings and S. Kapila, *J. Chromatogr.*, 77 (1973) 299.
3 W. A. Aue, C. R. Hastings and S. Kapila, *Anal. Chem.*, 45 (1973) 725.
4 E. C. Horning and M. G. Horning, *J. Chromatogr. Sci.*, 9 (1972) 129.

5 B. S. Middleditch and D. M. Desiderio, *Anal. Lett.*, 5 (1972) 605.
6 H. Rosenquist, H. Kallio and V. Nurmikko, *Anal. Biochem.*, 46 (1972) 224.
7 R. W. Zumwalt, K. Kuo and C. W. Gehrke, *J. Chromatogr.*, 57 (1972) 193.
8 B. Bartoníček and S. Lukáč, *Chromatographia*, 3 (1970) 108.
9 P. G. Robinson, *J. Chromatogr.*, 74 (1972) 13.
10 P. G. Simmonds, B. C. Pettitt and A. Zlatkis, *Anal. Chem.*, 39 (1967) 163.
11 R. Boland, *Ph. D. Thesis*, University of Missouri, Columbia, 1971.
12 D. W. Baker, *Amer. Offic. Anal. Chem. Meet., 86th, Washington, D.C., October, 1972.*
13 E. G. C. Clarke, D. J. Humphreys and E. Stoilis, *Analyst (London)*, 97 (1972) 433.