Journal of Chromatography, 104 (1975) 176–177

CHROM. 7918

Note

Analysis of hydroxyproline and hydroxylysine

Improved gas chromatographic method*

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(Received September 3rd, 1974)

The presence of abnormal amounts of hydroxyproline and hydroxylysine is a useful indicator of diseases associated with collagen metabolism[1,2]. These amino acids have most commonly been assayed by colorimetric methods which lack specificity. Recently Moss and Lambert[3] developed a procedure for the analysis of hydroxyproline and hydroxylysine in the presence of other protein amino acids by gas–liquid chromatography (GLC). This procedure was rapid and sensitive and all the protein amino acids were well separated with the exception of serine and valine. A separate analysis[4] would be required if accurate quantitation of these two amino acids was required at the same time as hydroxyproline and hydroxylysine were being assayed. We now present a GLC method by which hydroxyproline and hydroxylysine can be measured simultaneously while retaining excellent separation of all the other protein amino acids.

Hydroxyproline and hydroxylysine were obtained from Sigma (St. Louis, Mo., U.S.A.). All analyses were performed using a Hewlett-Packard Model 7611 gas chromatograph equipped with dual flame ionisation detectors. The column packing (3% SE-30 on Gas-Chrom Q, 100–200 mesh) was obtained from Applied Science Labs. (State College, Pa., U.S.A.). Pyrex columns (11–12 ft. × 2.5 mm I.D., thin walled) were filled with the stationary phase by gentle tapping under suction and conditioned overnight with a carrier gas (nitrogen) flow-rate of 30 ml/min. The chromatographic conditions were as follows: temperature programme, 90°–240° at 4 or 6°/min; injector temperature 250°; detector temperature, 280°; air flow-rate, 300 ml/min; hydrogen flow-rate, 30 ml/min; nitrogen flow-rate, 25 ml/min.

The N-heptafluorobutyryl isobutyl esters of the amino acids were prepared as previously described[5].

A typical chromatogram showing the separation of hydroxyproline and hydroxylysine from the other protein amino acids is shown in Fig. 1. As in other methods[3–6], hydroxyproline was completely separated. Hydroxylysine gave two partially resolved peaks presumably representing the DL isomers and the DL-*allo* isomers[3]. These were well resolved from the glutamic acid and lysine peaks and readily quantitated using electronic integration. The excellent separation of the other protein amino acids enabled them to be accurately quantitated simultaneously with hydroxyproline and

*NRCC No. 14388.

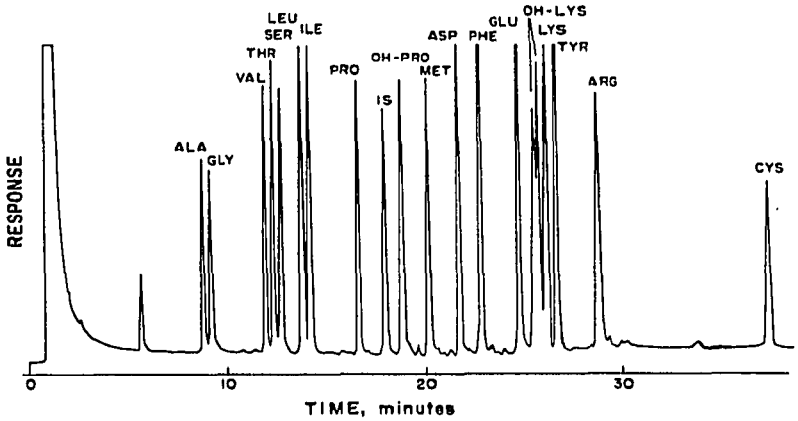

Fig. 1. Gas chromatogram showing separation of hydroxyproline and hydroxylysine from other protein amino acids. IS = Internal standard; temperature programme, 4°/min.

hydroxylysine. The separation of hydroxylysine from lysine was better using a temperature programme rate of 6° or 8° than when using 4° and thus a single determination could be completed in less than 20 min.

ACKNOWLEDGEMENT

The authors thank Mr. R. T. Scales for technical assistance.

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