Journal of Chromatography, 168 (1979) 290–291

CHROM. 11,424

Note

Rapid thin-layer chromatographic separation of isoleucine, leucine, and phenylalanine

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(First received May 26th, 1978; revised manuscript received August 1st, 1978)

In the course of preparing the pentapeptide isoleucine–glutamic acid–glutamic acid–leucine–phenylalanine according to the method of Houser *et al.*[1], it was necessary to confirm its amino acid composition at each stage of its solid phase synthesis. In part, this confirmation has been accomplished by acid hydrolysis of the peptide followed by thin-layer chromatography (TLC). The separation of Ile, Leu, and Phe by TLC is a known laboratory problem since the migrations of these particular amino acids are very close in most solvent systems. Although Gatto and Borders[2] have published a method which will separate Ile and Leu to a moderate degree, we wish to report a rapid, efficient, one-dimensional TLC system based on modifications of Gatto and Border's original procedure which allows for the complete resolution of Ile, Leu, and Phe.

EXPERIMENTAL

Materials

Anasil silica gel Type GF glass chromatographic plates (250 μm thick) obtained from Analabs (Boston, Mass., U.S.A.) are used throughout the procedure. Standard amino acids and ninhydrin spray were obtained from Sigma (St. Louis, Mo., U.S.A.) while solvents were purchased from commercial sources.

Methods

Hydrolysis of the pentapeptide is accomplished with 6 *M* hydrochloric acid by heating the peptide solution to 110° for 22 h. This hydrolyzed preparation is then neutralized with sodium hydroxide and diluted to a concentration of approximately 1 μg/ml of each amino acid before spotting on the TLC plate. In addition, similar concentrations of purified amino acids are chromatographed simultaneously as reference compounds.

A solvent system consisting of methyl ethyl ketone–pyridine–water–glacial acetic acid (73:5:20:2) was developed for this procedure. This solvent system is a significant modification of Gatto and Border's[2] original solvent mixture which describes a component ratio of 73:15:15:2. A second modification is the use of precoated

TLC glass plates, thus effecting a more efficient movement of the ascending solvent front.

After development, the amino acids are visualized by spraying the air-dried plates with ninhydrin solution and heating for 3 to 5 min at 110°. By our technique, complete separation of Ile, Leu, and Phe is obtained in less than 2 h.

RESULTS AND DISCUSSION

Our solvent system, based on important modifications of a previously described procedure, permits a conclusive separation by TLC of Ile, Leu, Phe and selected other amino acids without interference in their individual mobilities. As shown in Fig. 1, the R_F value for each amino acid differs significantly from the others, thus effecting a definitive separation. Moreover, our method is superior because it permits a rapid, complete, one-dimensional separation of these three closely migrating amino acids. The TLC system described herein provides a more useful tool for the identification of Ile, Leu, and Phe during investigations which necessitate the separation of these amino acids.

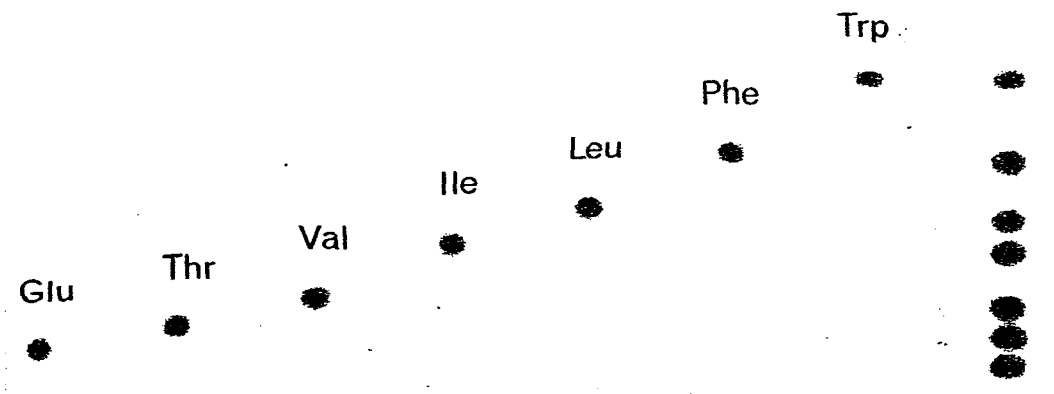

Fig. 1. A photograph of a TLC plate illustrating the mobilization of the seven amino acids utilized. The R_F values are as follows: Glu = 0.15; Thr = 0.17; Val = 0.19; Ile = 0.23; Leu = 0.26; Phe = 0.31; Trp = 0.40.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Ms. Dianne Henke and Ms. Donna Miller with the preparation of the manuscript.

This research was supported in part by a School of Dental Medicine Pilot Project Grant and a Research and Projects Grant from the Graduate School, Southern Illinois University.

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