Amino Acids (1991) 1: 279–281

Amino
Acids

The use of a new silica gel separation system in thin-layer chromatography

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Summary. A new method for the chromatography of amino acids is described in which D- or L-amino acids are separated on ICT-Empore thin-layers. The compounds are developed ascending by means of normally used solvent systems. An overloading of the plates is nearly impossible. On the other hand, hydrophilic amino acids are well separated. A second front, moving with these amino acids and emerging with ninhydrin stain, was not detectable.

Keywords: Amino acids – Thin-layer chromatography – Ascending method

Introduction

Thin-layer chromatography is used extensively in amino acid-, peptide- and protein research, although HPLC is now well established. The technique is easy to handle and its reproducibility makes it an ideal method for the analysis and separation of amino acid classes on a microscale [1]. However, in silica gel thin-layer systems available, subsequent analysis of amino acids after resolution by chromatography or electrophoresis may be hampered by binding material present in silica gel plates. This material moves with the same speed as some hydrophilic amino acids and shows the same colour with ninhydrin.

Materials and methods

Empore silica gel TLC sheets, whithout fluorescence indicator, were obtained from ICT Frankfurt, and silica gel (0.2 mm), No. 37346, from Riedel-de Haen, Seelze. DC plastic folie silica gel 60 (0.2 mm), No. 5748, was purchased from E. Merck, Darmstadt. All solvents were analytical grade from E. Merck.

1. Chromatography

Empore thin-layer material is silica gel in a flexible teflon sheet. Neither a supporting plate nor any binding material is necessary. The sheet is placed in a little frame (stainless steel) supporting rods for two sheets. The frame is placed in a chromatography tank quickly to

avoid unsaturation of the atmosphere in the tank. 30 min before chromatography started, the tank was lined with Whatman No. 1 filter paper and the solvent was poured into the tank, wetting the filter paper at the same time. The volume of solvent should be such that there is about 0.5 cm of solvent at the bottom of the tank.

2. Spotting

Amino acids, dissolved in water, are spotted onto the silica gel with thin capillary tubings (1 mm internal diameter) drawn out to a fine point. The spots should be as small as possible and the amount of the compound should not exceed 10 μg per spot; the volume is usually 0.5–2 μl per spot. The spots should be about 1 cm from the bottom of the sheet to avoid immersion of the amino acids in the solvent and subsequent loss of the acids. Seven spots can be run on one plate. Care should be taken to ensure that each of the applications to the spot is quite dry before the next application; if this is not adhered to, the spot will spread and the resolution will be lowered.

3. Amino acid stock solutions

The following amino acids were tested: glycine, phenylalanine, tyrosine, histidine, tryptophane, valine, isoleucine, serine, threonine, lysine, arginine, aspartic acid and glutamic acid. Approximately 2–3 mg of amino acid were dissolved in 1–2 mL of water stabilized with methanol.

4. Solvents

Chromatograms were developed ascending in two different solvent systems: n-butanol: acetic acid: water (60:20:20, v/v/v) and *n*-butanol: acetic acid: water (20:5:25, v/v/v).

5. Detection

After a chromatographic run (running time 1–2 h) the sheet was dried at 105° for 1–2 min. The spots were visualized with ninhydrin for all amino acids and by specific spray reagents (2, 3): Pauly's reagent for histidine and tyrosine, Ehrlich's reagent for tryptophane and Sakaguchi reagent for arginine.

Results and discussion

The Empore silica gel TLC used separates amino acids which are not easy separable with other silica gel preparations [4], for example glutamic acid and aspartic acid or serine and glycine. A comparison with commercially available silica gel plates reveals the reason: although Empore sheets show spots with usual diameters, silica gel plate Nol 37346 seems to be overloaded with the amino acid concentration used (Fig. 1 and 2).

In contrast to other thin-layer plates Empore sheets contain no organic binder; the sheets remain completely white after the ninhydrin stain.

A disadvantage of silica gel thin-layer chromatography of amino acids and peptides, compared with paper chromatography, is the loss of silica gel material from the plate by means of elution of the adsorbed compounds. Thus elution experiments with Empore sheets are in progress.

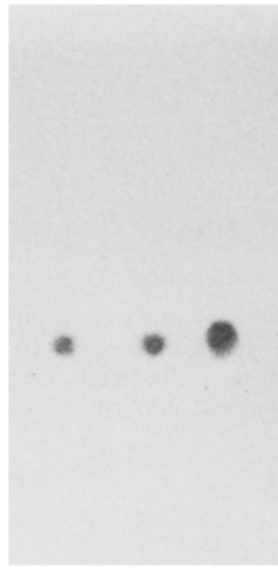

Fig. 1. Thin-layer chromatogram on Empore silica gel TLC. Solvent system n-butanol : acetic acid : water (60 : 20 : 20). Two blue spots are obtained with 1 μl isoleucine solution and one spot with 2 μl after the ninhydrin stain

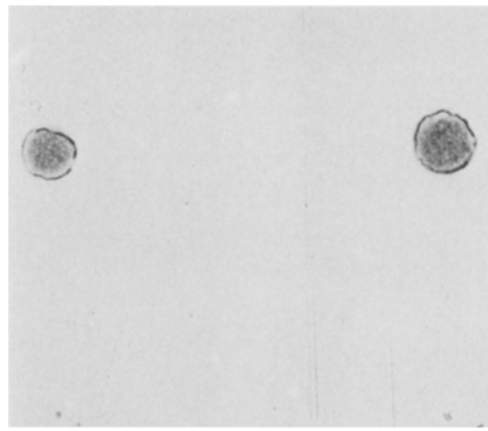

Fig. 2. Thin-layer chromatogram on Silica gel No. 37346. The same solvent as in Fig. 1 was used. One red spot was obtained with 2 μl isoleucine and one spot with 5 μl

References

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