Improved Two-dimensional Paper Chromatography of Amino Acids and Some Related Compounds

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A two-dimensional paper chromatographic procedure has been devised in our laboratory for the separation and identification of most of the common, naturally occurring amino acids and related compounds. The purpose of the present work is to provide a procedure in conjunction with the column amino acid analysis, which we carry out on a Beckman Amino Acid Analyzer.

We shall here describe a new combination of solvent systems, which is extremely efficient, easier to prepare, and above all far less hazardous than the usual phenol, collidine and lutidine systems.

After a series of experiments with different chromatographic papers, most satisfactory separation of individual amino acids was obtained with the paper EDEROL 202 (Binzer, Hatzfeld/Eder). With this paper, the resulting amino acid spots were more uniform and compact, less irregular and with excellent resolution.

In our system leucine and isoleucine are not always separated, and lysine and methylhistidine are overlapped by the cysteic acids (spots 5 and 6, Fig. 1) and diaminobutyric acid (spot 7). This problem is overcome by separating the amino acid mixture with ion-exchange column operations, into a basic fraction and an acidic and neutral fraction. This preliminary separation also purifies an amino acid extract from extraneous materials which is necessary for high resolution.

The apparatus used in our investigations were two Universal Chromatanks from Shandon Scientific Co., London.

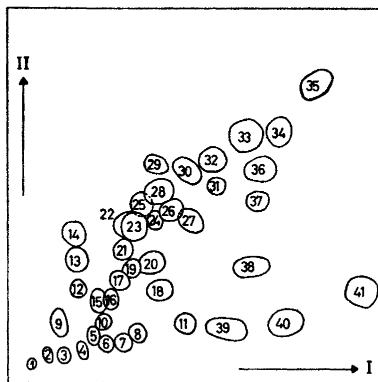

Fig. 1. Two-dimensional paper chromatogram of the various amino acids and related compounds. (For identification of numbered spots see Table 1).

Table 1. Amino acids and related compounds corresponding to numbered spots shown in Fig. 1.

1	DL-3,4-Dihydroxyphenylalanine
2	L-Glutathione
3	DL-Lanthionine
4	L-Cystine
5	DL-Homocysteic acid
6	L-Cysteic acid
7	L-α-γ-Diaminobutyric acid
8	L-Histidine
9	L-Arginine
10	DL-Asparagine
11	Taurine
12	DL-Aspartic acid
13	L-Glutamic acid
14	α-Aminoadipic acid
15	DL-Citrulline
16	L-Glutamine
17	Glycine
18	DL-Serine
19	L-Azetidine-2-carboxylic acid
20	DL-Homoserine
21	β-Alanine
22	γ-Aminobutyric acid
23	DL-α-Alanine
24	L-Proline
25	β-Aminoisobutyric acid
26	L-Tyrosine
27	S-Methylcysteine
28	DL-α-Aminobutyric acid
29	DL-α-Aminoisobutyric acid
30	DL-Valine
31	DL-Methionine
32	DL-Norvaline
33	L-Leucine + Isoleucine
34	DL-Thyronine
35	α-Aminocaprylic acid
36	DL-β-Phenylalanine
37	DL-Tryptophan
38	DL-Threonine
99	1,4-Diaminobutane
40	1,5-Diaminopentane
41	1,7-Diaminoheptane

The tanks are especially suitable in routine and control work, and enable five 10 inches square papers to be run simultaneously with a high degree of reproducibility.

When the spotting of the amino acid mixture was completed, the chromatograms were inserted in an aluminium frame and placed in the first chromatographic cabinet, arranged for ascending chromatography. The solvent for the first dimension was then prepared by mixing together *sec*-butanol, *tert*-butanol, 2-butanone, water and diethylamine in the proportion 40:40:80:50:1, (v/v), Ref. 1. When the mixture was shaken, a clear, monophasic unsaturated solution resulted. A portion of this solvent was placed in the bottom of the chromatographic cabinet, and complete equilibration of the tank atmosphere with the solvent vapors was allowed to occur. The development took place at a temperature of 24° ± 1°, and the analysis was run for 18 h.

After development in the first dimension, the frame with the chromatograms was removed from the tank and dried as directed by Ambe and Tappel.[1]

Development in the second dimension was also carried out by way of the ascending technique, using a mixture of *sec*-butanol, formic acid (88 %) and water in the proportion 75:15:10, (v/v), Ref. 2. About a 20 h run at the same temperature was found to be expedient. The chromatograms were air-dried and then sprayed with ninhydrin, (0.5 g ninhydrin dissolved in 100 ml butanol saturated with water and containing 7 ml acetic acid). The spots were observed after 3 h, 24 h and then heated at 60° for 20 min.

The two-dimensional chromatogram in Fig. 1, (a tracing from contact photograph) demonstrates the excellent resolution of a mixture of 41 amino acids and related compounds. The spots are designated by numbers, which refer to the numbers in Table 1.

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2. Cramer, F. *Papierchromatographie*, 2. Auflage, Verlag Chemie, GMBH., Weinheim 1953, 0. 55.

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