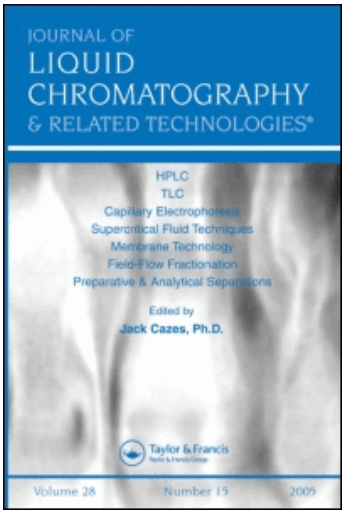

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Reversed Phase Thin Layer Chromatography of Amino Acids

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Reversed Phase Thin Layer Chromatography of Amino Acids

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The use of reversed phase layers for the thin layer chromatography of amino acids is described. Only when a modifier was added to the mobile phase was clear separation of the amino acids achieved. Ion paring with trifluoroacetic acid overcame problems with streaking and poor separation on C_2 or C_{18} reversed phase layers. All amino acids could not be separated with a single mobile phase. Thus, three different combination of acetonitrile-0.4% trifluoroacetic acid were used to separate eighteen amino acids with derivatization. No derivative was required.

The separation of the amino acids by thin layer chromatography (TLC) has been the subject of continuing investigation since the early review of Pataki (1). Biou et al., (2) recently reported the use of two dimensional separation of dansylated amino acids. This solved two problems: detection and better separation, but altogether it is time consuming. The present trend appears to be toward derivatization with dansyl chloride (3), or phenylthiohydantoin (4) for high performance liquid chromatography. Macek et al., (4) and Lepri et al., (6) used reversed phase layers for separation of derivatized amino acids.

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Sherma et al., (7) reported difficulties with the use of reversed phase layers for separation of the amino acids and reported long developing times with the mobile phases that were used.

MATERIALS

Whatman LKC18 and LKC2 layers (20 x 20 cm) were scored into 10 mm lanes with a Schoeffel scoring apparatus. The plates were used directly from the shipping container. All solvents were Omnisolve from EM Sciences (Gibbstown, N.J.). Water was deionized and filtered of organic matter. All amino acids were obtained from Sigma Chemical Co. (St. Louis, MO) and made as 1μ G/1 μL solutions in water. The detection reagent was ninhydrin (0.2% in acetone) sprayed on the chromatogram and heated in an oven at 110° for several minutes.

The mobile phases were acetonitrile with 15 to 25 percent of 0.4% trifluoroacetic acid in water made daily.

METHODS

The sample of amino acid was applied to the preadsorbent area of the plate usually in 5 μG aliquots. After linear development to 2 cm from the top of the plate, the plate was dried at ambient conditions, then dried in an oven at 170° for 2 minutes to remove solvent. Then the chromatogram was sprayed uniformly with the ninhydrin reagent. This was followed by heating at 110° in an oven for several minutes or until the reacting zones appeared.

RESULTS

Table 1 shows the R_f values for the listed amino acid in the noted mobile phases. The group in the upper part of the table would migrate with higher R_f in mobile phases of higher water content. The results obtained with the mobile phases not containing the trifluoroacetic acid were poor with tailing and in general poor resolution. The bands obtained with C_2 reversed phase layers were tighter than those shown by the C_{18} layers.

Table 1

R_f x 100 Values for Amino Acids on Reversed Phase Layers
Mobile phase (Acetonitrile-0.4% TFA)

Amino Acid	85:15		80:20		75:25	
	C_2	C_{18}	C_2	C_{18}	C_2	C_{18}
Valine	50	44	74	71		
Leucine	62	60	88	89		
Isoleucine	59	56	80	84		
Phenylalamine	68	67	90	97		
Tyrosine	56	50	78	82		
Alanine	22	12	41	35		
Proline	43	37	63	62		
Threonine	20	10	38	31		
Glutamine	13	6	30	25		
Glycine	11	4	23	15		
Glutamic Acid	8	0	25	18		
Aspartic Acid	4	0	17	12		
Serine			20	0	34	0
Cysterine			59	0	74	0
Cystine			6	0	10	0
Asparagine			23	0	37	0
Histidine			3	0	7	0
Arginine			4	0	7	0

The development time for both the C_2 and C_{18} chromatograms was 30 minutes, which is somewhat shorter than many separations previously reported. The use of C_{18} layers for separation of the group in the lower

Table II

Resolution of Pairs Difficult to Separate on C_2 layer

	85:15	80:20
Leucine	62	88
Isoleucine	59	80
Theronine	19	38
Serine	8	20
Glycine	11	23
Alanine	23	41

portion of the table did not result in any movement although there was movement in the C_2 layer.

Table II shows the resolution of the three pairs of amino acids that have been traditionally hard to separate (6). Leucine-isoleucine, threonine-serine and glycine-alanine are readily separated in the mobile phases listed. The methods described present a rapid means for separating the common amino acids.

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