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Chromatographic Separation of α-Amino Acids on Antimony(V) Phosphate-Silica Gel 'G' Plates from Some Synthetic Mixtures and Drug Samples

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Thin-layer chromatography of α-amino acids has been performed on the antimony(V) phosphate-silica gel 'G' plates in different aqueous, nonaqueous, and mixed solvent systems. The study has resulted into the separation and determination of amino acids from their mixtures of the same type such as acidic from acidic, and neutral from neutral. Separations of basic amino acids from neutral and acidic amino acids have also been made. The method has been utilized for the quantitative separation of amino acids from two drugs namely 'Astymin Forte' and 'Santevini (Plus)'.

Silica gel-G has been used earlier in the thin-layer chromatographic studies of amino acids by Brenner,[1,2] Neher,[3] Opienska-Blauth,[4] and many others[5,6] in various solvent systems. Attempts have also been made for their detection using resin beads.[7—9] Synthetic inorganic ion exchangers have great promise in column,[10] paper,[11] and thin-layer[12] chromatographic separations of amino acids. However, most of these studies relate only to the qualitative aspects and very few quantitative studies have been reported so far using the materials. Moreover, the separations achieved are amongst different categories of amino acids like basic-acidic, basic-neutral, and neutral-acidic.[13] The present study is an attempt to explore the possibility of using inorganic ion exchangers for the quantitative separation of amino acids of the same type. This paper reports the results of the thin-layer chromatographic study on silica gel 'G' mixed with antimony(V) phosphate cation exchanger in varying ratios, as the latter has been found to modify the separation ability of silica gel 'G'.

Experimental

Reagents and Chemicals: Antimony(V) chloride used in this study was of 98% purity obtained from Fluka (Switzerland) and the trisodium orthophosphate was a B. D. H. Poole (England) product. All other reagents and chemicals were of analytical grade.

Apparatus: A TLC applicator of M/S Toshniwal (India) was used to prepare thin layers of 0.1 mm thickness on glass plates (20×3 cm). These thin layers were developed by the ascending technique in ordinary 24×6 cm glass jars containing the developer.

A micropipette (1—100 μl capacity) of "Gilson Medical Electronic (France)" was used to put a spot of known amount on the TLC plates for quantitative separations.

A Bausch and Lomb Spectronic 20 spectrophotometer was used for colorimetric studies.

Preparation of the Thin-Layer Plates: Thin-layer plates were prepared either of the pure silica gel-G or the mixtures of antinony(V) phosphate (AP), prepared by an earlier method,[14] and silica gel-G of the following compositions (w/w):

5 : 95, 10 : 90, 15 : 85, 20 : 80, 25 : 75, 30 : 70, 40 : 60, and 50 : 50.

The method adopted to prepare the plates was as follows:

Twenty-five grams of the adsorbent were mixed with 60 ml of glass distilled water (GDW) and shaken well for 10 min to make a slurry, which was then spread over the glass plates with the help of an applicator to obtain a uniform thin layer of 0.1 mm thickness. The plates were dried, first at room temperature and then at 100 °C for 1 h before use.

Test Solutions: One tenth percent aqueous solutions of 24 α-amino acids, symbolized below, were prepared in GDW.

Neutral Amino Acids; N_1: Glycine, N_2: L-Hydroxyproline, N_3: DL-Alanine, N_4: DL-2-Aminobutyric acid, N_5: DL-Serine, N_6: Proline, N_7: DL-Valine, N_8: DL-Threonine, N_9: DL-Cysteine·HCl, N_{10}: L-Leucine, N_{11}: DL-Isoleucine, N_{12}: DL-Norleucine, N_{13}: DL-Methionine, N_{14}: Tyrosine, N_{15}: DL-Phenylalanine, N_{16}: DL-Tryptophan, N_{17}: DL-(3,4-Dihydroxyphenyl) alanine, N_{18}: L-Cystine.

Basic Amino Acids; B_1: L-Ornithine, B_2: L-Lysine·HCl, B_3: L-Histidine, B_4: L-Arginine.

Acidic Amino Acids; A_1: Aspartic acid, A_2: Glutamic acid.

Ninhydrin Reagent; A 2% alcoholic solution of ninhydrin was used for the detection of amino acids.

For quantitative determination the reagent was prepared as follows:

Four hundred milligrams of the hydrated tin(II) chloride ($SnCl_2 \cdot 2H_2O$) were dissolved in 250 ml of the citrate buffer of pH 5.0, prepared by dissolving 21 g of citric acid in 200 ml of 1 M NaOH (1 M=1 mol dm^{-3}) and further diluting to 500 ml with GDW. It was termed as solution "A".

In another flask 4% ninhydrin solution was prepared in methyl cellosolve (ethylene glycol monomethyl ether). It was termed as solution "B".

Freshly prepared equivolume mixtures of these two solutions were used in all the determinations.

Solvent Systems Used as Developers: The following solvent systems were used as developers:

Demineralized water (DMW), acetic acid (HAC), methanol (Me), ethanol (Et), 1-butanol (Bu), benzene (Bz), acetone, diethyl ether, 1-butanol+methanol (1 : 1), ethanol+benzene (1 : 1), methanol+benzene+DMW (1 : 1 : 1), methanol+1-butanol+DMW (1 : 1 : 1), 1-butanol+acetic acid+DMW

(4:1:5), ethanol+acetic acid+DMW (4:1:5), acetone+benzene+acetic acid (4:5:1).

Procedure. Thin glass capillaries were used to spot the test solutions. The solvent systems were allowed to ascend 10 cm on the plates. After drying at room temperature the spots were detected by spraying with alcoholic ninhydrin solution, warming for a few minutes. Figure 1 gives the R_f values of all the 24 α-amino acids in different developers.

Separations Achieved. (A) Synthetic Mixtures: Binary, ternary, and multinary mixtures were prepared by mixing equal volumes of the amino acid solutions. The mixtures were spotted on the plates with a micropipette and the development was made as usual. Pilot chromatograms were run under similar conditions to ascertain the actual position of the spots on the experimental plates. After the development, the spots on pilot plates were detected using the ninhydrin reagent. The same portions of the experimental plates were then scratched out and the amino acids present in these portions were extracted with small portions of the absolute alcohol, total 5 ml being used for complete elution.

Determination was carried out by the standard ninhydrin method[15] as follows:

One milliliter each of the sample solution and ninhydrin reagent were mixed thoroughly in a test tube and heated in a boiling water bath for about 20 min. Five milliliters of the diluting solution (equal volumes of GDW and 1-propanol) was

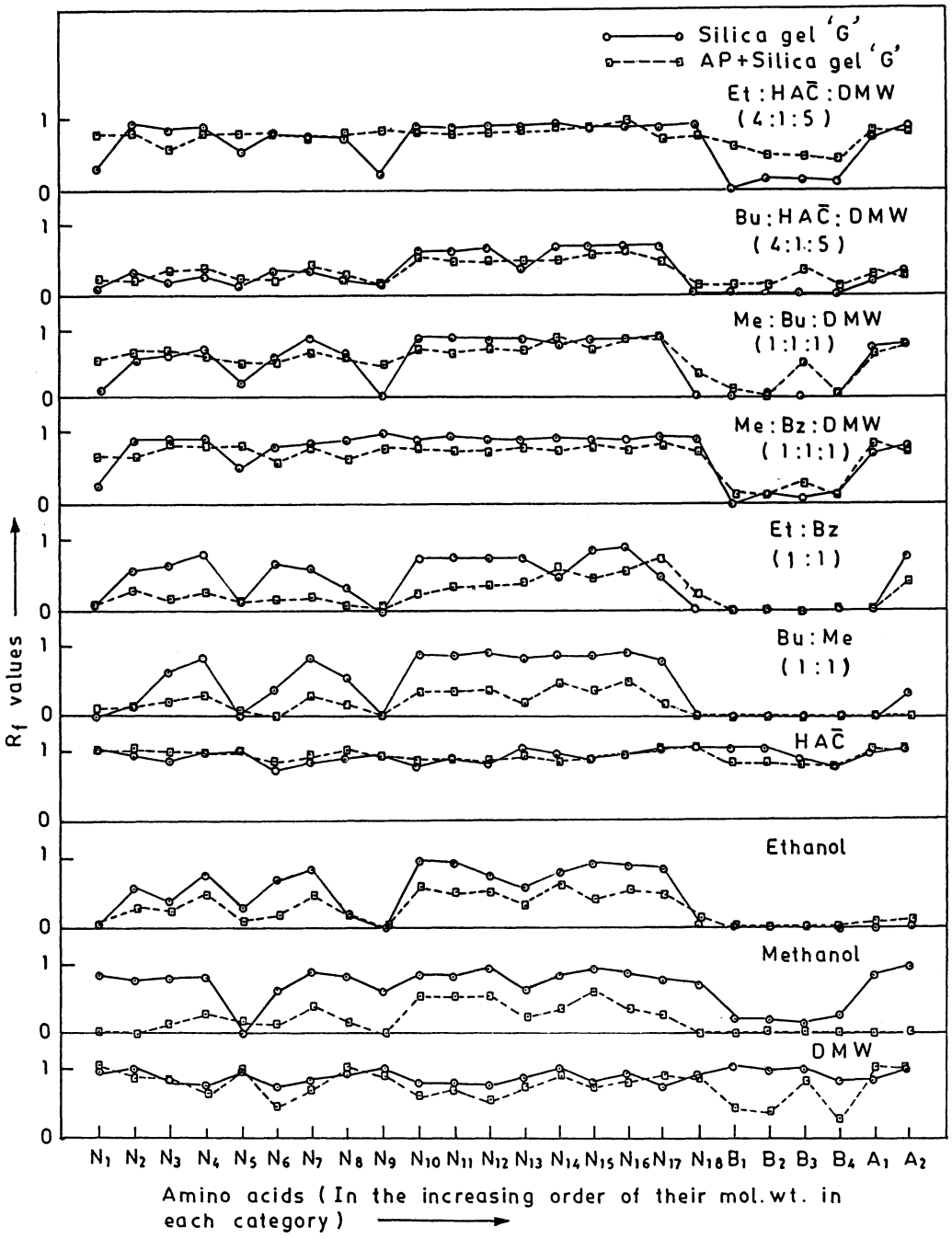

Fig. 1. Plots of R_f values for the amino acids in various solvent systems on the TLC plates of silica gel 'G' & AP+silica gel 'G' (20:80).

Table 1. Quantitative Separations of α-Amino Acids in Synthetic Mixtures on AP-Silica Gel "G" Thin-Layer Plates

Solvent system	Separations achieved	Amount taken	Amount found[a)]	% Error
		μg	μg	
Ethanol : Benzene (1 : 1)	$A_1(0.08)$-$A_2(0.70)$	A_1=50.00	A_1=50.50	+1.00
		A_2=50.00	A_2=51.25	+2.50
1-Butanol : Methanol (1 : 1)	$N_1(0.00)$-$N_4(0.81)$	N_1=50.00	N_1=49.50	−1.00
		N_4=50.00	N_4=50.50	+1.00
	$N_1(0.00)$-$N_7(0.84)$	N_1=50.00	N_1=50.25	+0.50
		N_7=50.00	N_7=50.75	+1.50
	$N_1(0.00)$-$N_{10}(0.90)$	N_1=50.00	N_1=49.75	−0.50
		N_{10}=50.00	N_{10}=50.30	+0.60
	$N_1(0.00)$-$N_{11}(0.83)$	N_1=50.00	N_1=50.15	+0.30
		N_{11}=50.00	N_{11}=50.40	+0.80
	$N_1(0.00)$-$N_{12}(0.87)$	N_1=50.00	N_1=50.25	+0.50
		N_{12}=50.00	N_{12}=50.50	+1.00
	$N_1(0.00)$-$N_{13}(0.86)$	N_1=50.00	N_1=50.20	+0.40
		N_{13}=50.00	N_{13}=50.60	+1.20
	$N_1(0.00)$-$N_{14}(0.88)$	N_1=50.00	N_1=49.50	−1.00
		N_{14}=50.00	N_{14}=50.75	+1.50
	$N_1(0.00)$-$N_{15}(0.85)$	N_1=50.00	N_1=50.25	+0.50
		N_{15}=50.00	N_{15}=50.50	+1.00
	$N_1(0.00)$-$N_{16}(0.85)$	N_1=50.00	N_1=50.25	+0.50
		N_{16}=50.00	N_{16}=50.40	+0.80
	$N_5(0.00)$-$N_7(0.83)$	N_5=50.00	N_5=50.30	+0.60
		N_7=50.00	N_7=50.50	+1.00
	$N_5(0.00)$-$N_{10}(0.90)$	N_5=50.00	N_5=49.70	−0.60
		N_{10}=50.00	N_{10}=50.25	+0.50
	$N_5(0.00)$-$N_{11}(0.85)$	N_5=50.00	N_5=50.30	+0.60
		N_{11}=50.00	N_{11}=50.40	+0.80
	$N_5(0.00)$-$N_{12}(0.90)$	N_5=50.00	N_5=50.10	+0.20
		N_{12}=50.00	N_{12}=50.40	+0.80
	$N_5(0.00)$-$N_{13}(0.82)$	N_5=50.00	N_5=49.80	−0.40
		N_{13}=50.00	N_{13}=50.10	+0.20
	$N_5(0.00)$-$N_{14}(0.86)$	N_5=50.00	N_5=50.20	+0.40
		N_{14}=50.00	N_{14}=50.30	+0.60
	$N_5(0.00)$-$N_{15}(0.84)$	N_5=50.00	N_5=50.15	+0.30
		N_{15}=50.00	N_{15}=50.25	+0.50
	$N_5(0.00)$-$N_{16}(0.87)$	N_5=50.00	N_5=50.30	+0.60
		N_{16}=50.00	N_{16}=50.50	+1.00
	$N_{18}(0.00)$-$N_4(0.81)$	N_{18}=50.00	N_{18}=50.20	+0.40
		N_4=50.00	N_4=50.60	+1.20
	$N_{18}(0.00)$-$N_7(0.84)$	N_{18}=50.00	N_{18}=50.25	+0.50
		N_7=50.00	N_7=50.75	+1.50
	$N_{18}(0.00)$-$N_{10}(0.90)$	N_{18}=50.00	N_{18}=50.15	+0.30
		N_{10}=50.00	N_{10}=50.60	+1.20
	$N_{18}(0.00)$-$N_{11}(0.86)$	N_{18}=50.00	N_{18}=50.20	+0.40
		N_{11}=50.00	N_{11}=50.30	+0.60
	$N_{18}(0.00)$-$N_{12}(0.86)$	N_{18}=50.00	N_{18}=49.90	−0.20
		N_{12}=50.00	N_{12}=50.30	+0.60
	$N_{18}(0.00)$-$N_{13}(0.84)$	N_{18}=50.00	N_{18}=50.25	+0.50
		N_{13}=50.00	N_{13}=50.50	+1.00
	$N_{18}(0.00)$-$N_{14}(0.9)$	N_{18}=50.00	N_{18}=50.25	+0.50
		N_{14}=50.00	N_{14}=50.30	+0.60
	$N_{18}(0.00)$-$N_{15}(0.86)$	N_{18}=50.00	N_{18}=50.30	+0.60
		N_{15}=50.00	N_{15}=50.30	+0.60
	$N_{18}(0.00)$-$N_{16}(0.83)$	N_{18}=50.00	N_{18}=50.25	+0.50
		N_{16}=50.00	N_{16}=50.40	+0.80
	$B_1(0.00)$-$N_7(0.83)$	B_1=50.00	B_1=50.30	+0.60
		N_7=50.00	N_7=50.40	+0.80
	$B_1(0.00)$-$N_{10}(0.90)$	B_1=50.00	B_1=50.25	+0.50
		N_{10}=50.00	N_{10}=50.25	+0.50
	$B_1(0.00)$-$N_{11}(0.85)$	B_1=50.00	B_1=50.30	+0.60
		N_{11}=50.00	N_{11}=50.50	+1.00
	$B_1(0.00)$-$N_{12}(0.87)$	B_1=50.00	B_1=50.20	+0.40
		N_{12}=50.00	N_{12}=50.25	+0.50

Table 1. (Continued)

Solvent system	Separations achieved	Amount taken	Amount found[a)]	% Error
		μg	μg	
1-Butanol : Methanol (1 : 1)	$B_1(0.00)$–$N_{13}(0.84)$	B_1=50.00	B_1=50.25	+0.50
		N_{13}=50.00	N_{13}=50.20	+0.40
	$B_1(0.00)$–$N_{14}(0.88)$	B_1=50.00	B_1=50.20	+0.40
		N_{14}=50.00	N_{14}=50.20	+0.40
	$B_1(0.00)$–$N_{15}(0.85)$	B_1=50.00	B_1=50.25	+0.50
		N_{15}=50.00	N_{15}=50.25	+0.50
	$B_1(0.00)$–$N_{16}(0.85)$	B_1=50.00	B_1=50.15	+0.30
		N_{16}=50.00	N_{16}=50.30	+0.60
	$B_3(0.00)$–$N_4(0.85)$	B_3=50.00	B_3=50.20	+0.40
		N_4=50.00	N_4=50.30	+0.60
	$B_3(0.00)$–$N_7(0.84)$	B_3=50.00	B_3=50.25	+0.50
		N_7=50.00	N_7=50.25	+0.50
	$B_3(0.00)$–$N_{10}(0.88)$	B_3=50.00	B_3=50.30	+0.60
		N_{10}=50.00	N_{10}=50.40	+0.80
	$B_3(0.00)$–$N_{11}(0.83)$	B_3=50.00	B_3=50.15	+0.30
		N_{11}=50.00	N_{11}=50.30	+0.60
	$B_3(0.00)$–$N_{12}(0.88)$	B_3=50.00	B_3=50.25	+0.50
		N_{12}=50.00	N_{12}=50.30	+0.60
	$B_3(0.00)$–$N_{13}(0.84)$	B_3=50.00	B_3=50.40	+0.80
		N_{13}=50.00	N_{13}=50.40	+0.80
	$B_3(0.00)$–$N_{14}(0.90)$	B_3=50.00	B_3=50.40	+0.80
		N_{14}=50.00	N_{14}=50.80	+1.60
	$B_3(0.00)$–$N_{15}(0.84)$	B_3=50.00	B_3=50.30	+0.60
		N_{15}=50.00	N_{15}=50.60	+1.20
	$B_3(0.00)$–$N_{16}(0.88)$	B_3=50.00	B_3=50.20	+0.40
		N_{16}=50.00	N_{16}=50.60	+1.20
	$B_3(0.00)$–$N_{17}(0.80)$	B_3=50.00	B_3=50.20	+0.40
		N_{17}=50.00	N_{17}=50.30	+0.60
Absolute ethanol	$N_9(0.00)$–$N_{10}(0.90)$	N_9=50.00	N_9=50.20	+0.40
		N_{10}=50.00	N_{10}=50.30	+0.60
	$N_9(0.00)$–$N_4(0.74)$	N_9=50.00	N_9=49.70	−0.60
		N_4=50.00	N_4=50.25	+0.50
	$B_1(0.00)$–$N_4(0.75)$	B_1=50.00	B_1=50.25	+0.50
		N_4=50.00	N_4=50.25	+0.50
	$B_2(0.00)$–$N_4(0.74)$	B_2=50.00	B_2=50.25	+0.50
		N_4=50.00	N_4=50.35	+0.70
	$B_2(0.00)$–$N_7(0.87)$	B_2=50.00	B_2=50.20	+0.40
		N_7=50.00	N_7=50.40	+0.80
	$B_2(0.00)$–$N_{10}(0.94)$	B_2=50.00	B_2=50.30	+0.60
		N_{10}=50.00	N_{10}=50.40	+0.80
	$B_2(0.00)$–$N_{11}(0.90)$	B_2=50.00	B_2=50.30	+0.60
		N_{11}=50.00	N_{11}=50.35	+0.70
	$B_2(0.00)$–$N_{12}(0.73)$	B_2=50.00	B_2=50.30	+0.60
		N_{12}=50.00	N_{12}=50.45	+0.90
	$B_2(0.00)$–$N_{15}(0.90)$	B_2=50.00	B_2=50.20	+0.40
		N_{15}=50.00	N_{15}=50.40	+0.80
	$B_4(0.00)$–$N_4(0.76)$	B_4=50.00	B_4=50.20	+0.40
		N_4=50.00	N_4=50.30	+0.60
	$B_4(0.00)$–$N_7(0.83)$	B_4=50.00	B_4=50.15	+0.30
		N_7=50.00	N_7=50.30	+0.60
	$B_4(0.00)$–$N_{10}(0.94)$	B_4=50.00	B_4=50.25	+0.50
		N_{10}=50.00	N_{10}=50.35	+0.70
	$B_4(0.00)$–$N_{11}(0.90)$	B_4=50.00	B_4=50.25	+0.50
		N_{11}=50.00	N_{11}=50.25	+0.50
	$B_4(0.00)$–$N_{12}(0.73)$	B_4=50.00	B_4=50.35	+0.70
		N_{12}=50.00	N_{12}=50.35	+0.70
	$B_4(0.00)$–$N_{14}(0.78)$	B_4=50.00	B_4=50.25	+0.50
		N_{14}=50.00	N_{14}=50.35	+0.70
	$B_4(0.00)$–$N_{15}(0.90)$	B_4=50.00	B_4=50.30	+0.60
		N_{15}=50.00	N_{15}=50.45	+0.90
	$B_4(0.00)$–$N_{16}(0.88)$	B_4=50.00	B_4=50.25	+0.50
		N_{16}=50.00	N_{16}=50.25	+0.50
Absolute methanol	$B_1(0.23)$–$A_1(0.79)$	B_1=50.00	B_1=50.20	+0.40
		A_1=50.00	A_1=50.20	+0.40

Table 1. (Continued)

Solvent system	Separations achieved	Amount taken	Amount found[a)]	% Error
		μg	μg	
Absolute methanol	B_1(0.26)–A_2(0.79)	B_1=50.00	B_1=50.25	+0.50
		A_2=50.00	A_2=50.35	+0.70
	B_2(0.20)–A_1(0.81)	B_2=50.00	B_2=50.20	+0.40
		A_1=50.00	A_1=50.40	+0.80
	B_2(0.25)–A_2(0.79)	B_2=50.00	B_2=50.25	+0.50
		A_2=50.00	A_2=50.35	+0.70
	B_3(0.14)–A_1(0.83)	B_3=50.00	B_3=50.30	+0.60
		A_1=50.00	A_1=50.75	+1.50
	B_3(0.15)–A_2(0.79)	B_3=50.00	B_3=50.30	+0.60
		A_2=50.00	A_2=50.60	+1.20
	B_4(0.22)–A_1(0.80)	B_4=50.00	B_4=50.20	+0.40
		A_1=50.00	A_1=50.30	+0.60
	B_4(0.25)–A_2(0.79)	B_4=50.00	B_4=50.25	+0.50
		A_2=50,00	A_2=50.45	+0.90
Me : Bu : DMW (1 : 1 : 1)	N_1(0.16)–N_8(0.70)	N_1=50.00	N_1=50.20	+0.40
		N_8=50.00	N_8=50.30	+0.60
1-Butanol : Methanol (1 : 1)	N_{13}(0.85) from N_1(0.00)+B_2(0.00)	N_{13}=30.00	N_{13}=30.70	+2.33
	B_2(0.00) from N_7(0.85)+N_8(0.7)+ N_{10}(0.85)+N_{11}(0.85) +N_{13}(0.85)+N_{15}(0.85) +N_{16}(0.90)	B_2=20.00	B_2=19.65	−1.75

Parentheses show the R_f values of amino acids.
a) Each result is a mean of five replicates.

Table 2. Some Ternary Separations of α-Amino Acids in Synthetic Mixtures on AP–Silica Gel "G" Thin-Layer Plates

Solvent system	Separations achieved
Ethanol : Benzene (1 : 1)	N_9–N_8–N_{16} (0.00)(0.60)(0.80)
	N_9–N_3–N_{16} (0.00)(0.65)(0.83)
1-Butanol : Methanol (1 : 1)	N_{18}–N_8–N_{12} (0.00)(0.8)(0.93)
	N_1–N_8–N_{14} (0.08)(0.65)(0.88)

Parentheses show the R_f values of amino acids.

then mixed to this solution while still on the water bath. A blank was also prepared in a similar manner. After cooling the solution was further diluted to 10 ml with the diluting solution. The purple color so developed has its λ_{max} at 570 nm. A standard curve was prepared for glycine. Tables 1 and 2 summarize the determination and separation results.

(B) Drug Samples: Two drug samples namely 'Astymin Forte' (capsule) and 'Santevini (Plus)' (liquid) were taken for their analyses. These drugs have the following composition in mg as per the labels on them:

'Astymin Forte' manufactured by TABLETS (India) Ltd. has: L-Leucine=18.3, L-Isoleucine=5.9, L-Lysine·HCl=25.0, L-Phenylalanine=5.0, L-Threonine=4.2, L-Valine=6.7, L-Tryptophan=5.0, DL-Methionine=18.4, 5-hydroxyanthranilic acid hydrochloride=0.2, Vitamin A (as palmitate)=2500.0, Vitamin D_3=200.0, Thiamine mononitrate=5.0, Vitamin B_2=3.0, Nicotinamide=25.0, Vitamin B_6=1.5, Folic acid= 0.75, Calcium pantothenate=5.0, Vitamin B_{12}=2.5, Vitamin C=40.0, Vitamin E=7.5.

'Santevini (Plus)' manufactured by SANDOZ (India) Ltd. has: L-Lysine·HCl=100.0, Glycine=−, DL-Methionine=29.0, Peptone solide=300.0, Vitamin B_1=0.6, Vitamin B_2=0.9, Vitamin B_6=1.1 Vitamin B_{12}=1.0, Nicotinamide=10.0, Calcium=75.0, Magnesium=6.0, Manganese=1.0.

Table 3. Determination of L-Lysine and DL-Methionine in 'Astymin Forte' and 'Santevini (Plus)' on AP–Silica Gel "G" Thin-Layer Plates

Commercial name of the drug	Labelled amount of amino acids in drug	Amount taken	Amount found	% Deviation from the labelled composition
	mg[a)]	μg	μg[b)]	
1. Astymin Forte (Capsule)	L-Lysin·HCl=25.00	50.00	49.15	−1.70
2. Santevini (plus) (Liquid)	DL-Methionine=29.00	58.00	59.20	+2.07

a) Per capsule/15 ml of the drug. b) Each result is a mean of five replicates.

Procedure: 'Astymin Forte' was stirred well in 10 ml of the GDW and then filtered. The filtrate was diluted to 50 ml with GDW. Fifteen milliliters of 'Santevini (Plus)' was directly diluted to 50 ml with GDW.

Spots of the standard solutions prepared as above were put on the TLC plates with a micropipette and the rest of the method followed was the same as described above. Table 3 gives the results.

Results and Discussion

The main feature of these studies is to evolve a simple, less expensive, and accurate method for the separation of amino acids of the same type such as acidic from acidic, and neutral from neutral and also to separate basic amino acids from all other types. Mixing of the silica gel 'G' to an inorganic ion exchanger lowers its cost to a great extent and enhances its binding capacity with the glass plates in addition to the improvement of its resolution power for amino acids. The silica gel 'G' is a well-known powerful adsorbent for many molecules. The ion exchange behavior of the exchanger and the adsorption characteristics of the silica gel 'G' jointly operate in the separation process.

Figure 1 illustrates the trend of the R_f values of α-amino acids on plain silica gel 'G' plates as well as on the mixed thin layers. On plain silica gel 'G' all amino acids move appreciably in DMW, absolute methanol, ethanol, and acetic acid. It may be due to the solubility of amino acids in such solvents. In organic solvents like benzene, acetone, 1-butanol, and ether, the migration of amino acids is negligible because they are sparingly soluble in such solvents. In the mixed solvent systems the migration improves in some cases, except for the basic amino acids. Presence of an extra-NH_2 group in basic amino acids may be responsible for this behavior as it has an extra lone pair of electrons. Silica may, therefore, have a greater affinity for such a molecule as compared to the acidic or neutral amino acids.

Addition of antimony(V) phosphate to silica gel 'G' promotes a differential migration of amino acids perhaps due to the combined effect of the ion exchange behavior of AP and the adsorption behavior of the SiO_2 surface. Hence, the separations become possible. For instance, a separation of aspartic acid from glutamic acid is possible in ethanol : benzene (1 : 1) solvent system on the AP+silica gel 'G' (20 : 80) plates, whereas this separation could not be achieved on pure silica gel 'G' plates. A study of the variation in the AP : silica gel 'G' ratios reveals the fact that the most fruitful results are obtained on the thin layers consisting of 20% AP. Below this composition the results were no different from pure silica gel 'G' and above this composition the resolution power of the adsorbent is not further enhanced. This combination was, therefore, used for all the studies. Tables 1 and 2 summarize the results. The practical utility of the method has been illustrated by analyzing two drug samples, the results of which are summarized in Table 3.

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