The titration values showed that quantitative recoveries of the bile acids were obtained.

The present study indicates that methylated Sephadex is a useful support in reversed phase chromatography with solvent systems of medium polarity. It is probably not suitable for systems less polar than F 2 used in this investigation but further experiments may show that it is of value in more polar solvents. Since the partially methylated Sephadex swells in water it might also be useful as a support for aqueous stationary phases in "straight" partition chromatography. The high capacity and the ease with which it can be regenerated are important advantages of this support.

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Department of Chemistry, Karolinska Institutet, Stockholm (Sweden)

ERNST NYSTRÖM
JAN SJÖVALL

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Notes

Separation of amino acid *n*-butyl esters by means of thin-layer chromatography

During previous work concerning the gas-chromatographic separation of amino acid derivatives, the purity of amino acid *n*-butyl esters was checked by means of thin-layer chromatography. The method used for the preparation of these esters is described elsewhere.[1]

Glass plates (20 × 20 cm) were covered with Kieselgel G (Merck) in layers of 0.25 mm. The solvent used for the separation of the butyl esters was a mixture of

benzene and *n*-butanol (75:25). Under these conditions only the esters migrate with a characteristic R_F value, while the amino acids do not move from the point of deposition.

The solvent system has been found useful for the separation of the following amino acid butyl esters: alanine, valine, leucine, isoleucine, norleucine, alloisoleucine, proline, allo-hydroxyproline, 4-hydroxyproline, threonine, glycine, methionine, aspartic, glutamic and α-aminobutyric acid.

TABLE I

R_F VALUES OF AMINO ACID BUTYL ESTERS IN THIN-LAYER CHROMATOGRAPHY

n-Butyl ester of	R_F		*Colours, after spraying with ninhydrin in 0.2% butanol*
	A *	*B* *	
Glycine	0.580	—	Orange
Alanine	0.540	—	Yellow
Allo-hydroxyproline	0.310	—	Yellow
α-Aminobutyric acid	0.300	—	Red
4-Hydroxyproline	0.271	—	Yellow
Norvaline	0.242	—	Purple-pink
Valine	0.200	—	Purple-pink
Norleucine	0.197	—	Red
Leucine	0.186	—	Red
Methionine	1.091	—	Pink
Isoleucine	0.188	—	Purple-pink
Glutamic acid	0.150	—	Orange
Phenylalanine	0.140	—	Pink-red
Aspartic acid	0.130	—	Orange
Citrulline	0.080	—	Orange
Lysine	0	0.340	Purple-pink
Histidine	0	0.360	Orange
Ornithine	0	0.355	Purple-pink

* Solvents: A = benzene–*n*-butyl alcohol (75:25, v/v). Running time: 1 h. B = *n*-butyl alcohol–acetic acid–water (120:30:50, v/v/v). Running time: 3h.

The esters of basic amino acids do not move with the above-mentioned solvent although their separation can be achieved using the common solvent system: *n*-butanol–acetic acid–water (120:30:50).

The R_F values of amino acid butyl esters on thin layers are reported in Table I.

Istituto di Ricerche Farmacologiche "Mario Negri", Milan (Italy)* E. MUSSINI
F. MARCUCCI

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* Via Eritrea 62, Milan.