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Note

Reversed-phase and soap thin-layer chromatography of dipeptides

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In a previous paper[1], reversed-phase thin-layer chromatography on silanized silica gel alone and impregnated with anionic and cationic detergents (soap TLC) was shown to be a powerful technique for the study of the separation of di-, tri-, tetra- and pentapeptides. Alkaline eluents, which cannot be employed in reversed-phase column chromatography[2,3], can be used here. The separations that can be carried out are correlated with the type and concentration of detergent on the layer and with the type and pH of the eluent.

We subsequently tried to build up a reference map of R_F values for the identification and the separation of dipeptides. For this purpose, we studied the chromatographic behaviour of 61 dipeptides under the same experimental conditions used for the above-mentioned peptides[1].

EXPERIMENTAL

The solutions of the test compounds and the layers of silanized silica gel alone and impregnated with detergents were prepared according to the previous work[1]; the method of detection and the materials used were also as described previously[1]. All the measurements were carried out at 25°C. The migration distance was 11 cm unless stated otherwise.

The following abbreviations are used: Gly = glycine; Ala = alanine; β-Ala = β-alanine; Ser = serine; Thr = threonine; Val = valine; Met = methionine; Trp = tryptophan; Glu = glutamic acid; His = histidine; Leu = leucine; Phe = phenylalanine; Tyr = trysosine; Pro = proline; Ile = isoleucine.

RESULTS AND DISCUSSION

Table I lists the R_F values of 61 dipeptides on layers of silanized silica gel alone and impregnated with increasing amounts of H-DBS, using as the eluent aqueous–organic mixture containing water, methanol (30%) and glacial acetic acid (5.7%) (apparent pH = 2.75). The percentages of H-DBS refer to the alcoholic solution in which the silanized silica gel was suspended.

As previously observed[1], on layers of silanized silica gel alone the dipeptides with hydrophilic amino acids (Gly, Ala, β-Ala, Ser, Thr, Pro, His and Glu) run practically with the solvent front. Those containing one or two hydrophobic amino

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TABLE I

R_F VALUES OF DIPEPTIDES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE AND IMPREGNATED WITH INCREASING AMOUNTS OF H-DBS

Eluent: water–methanol–acetic acid (64.3:30:5.7).

Peptide	*Layer*				
	SiO_2	*SiO_2 + 1% H-DBS*	*SiO_2 + 2% H-DBS*	*SiO_2 + 3% H-DBS*	*SiO_2 + 4% H-DBS*
Gly–Thr	0.96	0.86	0.69	0.59	0.46
Gly–Val	0.91	0.51	0.37	0.27	0.17
Gly–Met	0.93	0.51	0.36	0.25	0.15
Gly–Trp	0.70	0.22	0.12	0.06	0.05
Gly–Glu	0.96	0.93	0.74	0.65	0.48
Gly–His	0.96	0.47	0.18	0.08	0.03
Ala–Thr	0.96	0.90	0.70	0.58	0.44
Ala–Trp	0.68	0.20	0.12	0.07	0.05
β-Ala–Ala	0.96	0.75	0.56	0.45	0.30
β-Ala–His	0.96	0.44	0.16	0.06	0.02
Ser–Gly	0.96	0.90	0.72	0.62	0.45
Ser–Ala	0.96	0.78	0.63	0.50	0.34
Ser–Leu	0.84	0.31	0.19	0.14	0.07
Ser–Phe	0.78	0.28	0.17	0.13	0.06
Ser–His	0.96	0.44	0.16	0.07	0.02
Thr–Gly	0.96	0.89	0.72	0.60	0.43
Val–Gly	0.94	0.63	0.49	0.40	0.25
Val–Ala	0.94	0.57	0.43	0.37	0.24
Val–Val	0.84	0.34	0.22	0.17	0.11
Val–Leu	0.69	0.16	0.09	0.08	0.05
Val–Phe	0.64	0.14	0.08	0.07	0.04
Val–Tyr	0.83	0.40	0.27	0.22	0.16
Leu–Gly	0.90	0.45	0.30	0.22	0.15
Leu–Ala	0.89	0.40	0.28	0.21	0.15
Leu–β-Ala	0.85	0.35	0.24	0.18	0.13
Leu–Ser	0.95	0.55	0.39	0.30	0.18
Leu–Ile	0.60	0.10	0.07	0.06	0.04
Leu–Trp	0.47	0.06	0.03	0.02	0.02
Leu–Phe	0.48	0.06	0.03	0.03	0.02
Pro–Gly	0.96	0.71	0.55	0.44	0.27
Pro–Ala	0.94	0.65	0.50	0.40	0.24
Pro–Leu	0.74	0.19	0.11	0.08	0.04
Pro–Phe	0.66	0.17	0.09	0.07	0.04
Met–Gly	0.95	0.54	0.40	0.30	0.17
Met–Val	0.80	0.25	0.16	0.12	0.07
Met–Leu	0.62	0.13	0.07	0.05	0.04
Met–Met	0.78	0.23	0.16	0.13	0.08
Met–Phe	0.55	0.09	0.06	0.05	0.03
Met–Tyr	0.77	0.30	0.22	0.17	0.11
Met–His	0.96	0.15	0.05	0.03	0.00
Trp–Gly	0.77	0.24	0.14	0.08	0.05
Trp–Ala	0.74	0.22	0.13	0.08	0.05
Trp–Leu	0.39	0.04	0.03	0.02	0.02
Trp–Phe	0.36	0.03	0.03	0.02	0.02
Phe–Ser	0.88	0.40	0.25	0.19	0.10
Phe–Val	0.65	0.13	0.07	0.06	0.04
Phe–Pro	0.57	0.16	0.07	0.06	0.04

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TABLE I *(continued)*

Peptide	*Layer*				
	SiO_2	*SiO_2 + 1% H-DBS*	*SiO_2 + 2% H-DBS*	*SiO_2 + 3% H-DBS*	*SiO_2 + 4% H-DBS*
Phe–Trp	0.40	0.03	0.03	0.02	0.02
Phe–Phe	0.42	0.04	0.03	0.02	0.02
Phe–Tyr	0.64	0.17	0.10	0.07	0.04
Tyr–Gly	0.93	0.61	0.43	0.34	0.20
Tyr–Ala	0.93	0.54	0.39	0.31	0.19
Tyr–Phe	0.59	0.12	0.09	0.06	0.04
His–Gly	0.96	0.43	0.14	0.07	0.02
His–Ala	0.96	0.35	0.10	0.05	0.02
His–Ser	0.96	0.47	0.18	0.10	0.03
His–Leu	0.90	0.04	0.02	0.02	0.00
His–Pro	0.95	0.31	0.09	0.04	0.01
His–Met	0.93	0.10	0.04	0.03	0.00
His–Phe	0.82	0.03	0.02	0.02	0.00
His–Tyr	0.93	0.13	0.05	0.03	0.00

acids residues (Val, Ile, Leu, Tyr, Phe and Trp) are more retained. The position of the hydrophobic amino acid residue in the molecule has a considerable effect on the retention; in fact, considering pairs of isomeric dipeptides (Gly–Trp/Trp–Gly; Ala–Trp/Trp–Ala; Ser–Leu/Leu–Ser), the compound with the hydrophobic amino acid as a terminal residue is the more retained.

Such differences in the R_F values, however, are not sufficient to effect a complete separation of the isomers on silanized silica gel alone as the spots obtained are not compact. The same occurrence, even if to a lesser extent, is observed on layers impregnated with small percentages of detergent (1%). At higher H-DBS concentrations, the spots are compact and therefore suitable for analytical purposes.

As regards the influence of the detergent on the chromatographic characteristics of the dipeptides, it should be noted that the presence of H-DBS on the layer results in a considerable increase in the retention of all compounds, particularly those formed by hydrophobic or basic amino acids.

The affinity sequence of the different dipeptides changes greatly on changing from the layers of silanized silica gel to impregnated layers, whereas it remains almost constant as the detergent concentration is increased.

Influence of the acidity of the eluent and the concentration of the organic solvent

As previously observed[1], the chromatographic behaviour of dipeptides is considerably affected by the apparent pH of the eluent and by the counter ion (Na^+) concentration (Table II). The cationic form is more retained than the zwitterionic form and the latter more than the anionic form. In the pH range where the cationic form prevails (1.25–2.25), curvilinear R_M *versus* pH trends are obtained with dipeptides formed by hydrophobic amino acids and straight lines for the other compounds. The ΔR_M *versus* ΔpH slopes (0.3–0.5) agree with those found for the peptides[1] and, although much smaller than the theoretical values, indicate the presence of an ion-exchange process in the retention of hydrophilic dipeptides.

The affinity sequence of the different dipeptides can be predicted from that of

TABLE II

R_F VALUES OF DIPEPTIDES ON THIN LAYERS OF SILANIZED SILICA GEL IMPREGNATED WITH 4% H-DBS

Eluents: (1) 0.1 *M* HCl + 1 *M* CH_3COOH in 30% CH_3OH (pH = 1.25); (2) 0.05 *M* HCl + 1 *M* CH_3COOH in 30% CH_3OH (pH = 1.55); (3) 0.1 *M* NaCl + 1 *M* CH_3COOH in 30% CH_3OH (pH = 2.75); (4) 0.1 *M* NaCl + 0.1 *M* CH_3COOH in 30% CH_3OH (pH = 3.30); (5) 0.1 *M* CH_3COONa + 0.1 *M* CH_3COOH in 30% CH_3OH (pH = 5.10); (6) 1 *M* CH_3COONa in 30% CH_3OH (pH = 8.15).

Peptide	*Eluent*					
	1	*2*	*3*	*4*	*5*	*6*
Gly–Thr	0.71	0.64	0.73	0.72	0.73	0.83
Gly–Val	0.30	0.28	0.34	0.33	0.55	0.81
Gly–Met	0.25	0.24	0.28	0.28	0.49	0.78
Gly–Trp	0.07	0.07	0.08	0.06	0.16	0.45
Gly–Glu	0.74	0.69	0.75	0.74	0.77	0.90
Gly–His	0.08	0.05	0.12	0.15	0.18	0.81
Ala–Thr	0.71	0.64	0.72	0.71	0.73	0.85
Ala–Trp	0.07	0.07	0.08	0.06	0.17	0.45
β-Ala–Ala	0.56	0.52	0.56	0.56	0.66	0.83
β-Ala–His	0.08	0.05	0.12	0.16	0.19	0.79
Ser–Gly	0.74	0.67	0.73	0.72	0.77	0.85
Ser–Ala	0.64	0.58	0.63	0.62	0.72	0.86
Ser–Leu	0.15	0.14	0.15	0.11	0.30	0.66
Ser–Phe	0.14	0.13	0.13	0.10	0.25	0.57
Ser–His	0.08	0.05	0.10	0.11	0.16	0.81
Thr–Gly	0.73	0.67	0.72	0.70	0.73	0.85
Val–Gly	0.45	0.42	0.45	0.42	0.50	0.76
Val–Ala	0.42	0.40	0.44	0.43	0.56	0.81
Val–Val	0.21	0.20	0.22	0.22	0.43	0.77
Val–Leu	0.12	0.11	0.12	0.10	0.20	0.55
Val–Phe	0.09	0.07	0.09	0.05	0.14	0.37
Val–Tyr	0.25	0.22	0.27	0.21	0.43	0.75
Leu–Gly	0.23	0.20	0.25	0.21	0.31	0.54
Leu–Ala	0.23	0.20	0.25	0.21	0.36	0.70
Leu–β-Ala	0.22	0.18	0.25	0.16	0.21	0.59
Leu–Ser	0.33	0.31	0.37	0.32	0.41	0.73
Leu–Ile	0.08	0.06	0.06	0.04	0.09	0.48
Leu–Trp	0.03	0.03	0.03	0.02	0.05	0.19
Leu–Phe	0.03	0.03	0.03	0.02	0.04	0.16
Pro–Gly	0.54	0.47	0.55	0.53	0.59	0.79
Pro–Ala	0.46	0.39	0.49	0.47	0.58	0.81
Pro–Leu	0.09	0.07	0.11	0.07	0.22	0.58
Pro–Phe	0.07	0.06	0.09	0.06	0.16	0.43
Met–Gly	0.34	0.31	0.37	0.32	0.41	0.68
Met–Val	0.14	0.12	0.17	0.12	0.30	0.70
Met–Leu	0.07	0.06	0.07	0.05	0.13	0.46
Met–Met	0.13	0.11	0.13	0.10	0.24	0.58
Met–Phe	0.05	0.04	0.05	0.03	0.09	0.27
Met–Tyr	0.17	0.16	0.17	0.14	0.31	0.67
Met–His	0.02	0.02	0.03	0.04	0.05	0.54
Trp–Gly	0.11	0.09	0.10	0.07	0.12	0.30
Trp–Ala	0.11	0.09	0.11	0.07	0.17	0.40
Trp–Leu	0.03	0.02	0.03	0.02	0.03	0.15
Trp–Phe	0.02	0.02	0.02	0.02	0.03	0.09
Phe–Ser	0.18	0.17	0.23	0.17	0.26	0.56

(Continued on p. 416)

TABLE II *(continued)*

Peptide	*Eluent*					
	1	*2*	*3*	*4*	*5*	*6*
Phe–Val	0.07	0.07	0.09	0.06	0.15	0.44
Phe–Pro	0.06	0.04	0.07	0.05	0.13	0.32
Phe–Trp	0.02	0.00	0.02	0.02	0.03	0.12
Phe–Phe	0.02	0.02	0.03	0.02	0.03	0.11
Phe–Tyr	0.09	0.08	0.10	0.06	0.16	0.40
Tyr–Gly	0.38	0.35	0.45	0.33	0.43	0.67
Tyr–Ala	0.33	0.32	0.41	0.31	0.49	0.75
Tyr–Phe	0.06	0.05	0.08	0.04	0.12	0.33
His–Gly	0.06	0.05	0.10	0.12	0.19	0.81
His–Ala	0.03	0.03	0.08	0.09	0.18	0.81
His–Ser	0.06	0.05	0.11	0.16	0.20	0.81
His–Leu	0.01	0.00	0.02	0.02	0.04	e.s.*
His–Pro	0.03	0.02	0.06	0.07	0.15	e.s.
His–Met	0.02	0.02	0.03	0.02	0.08	e.s.
His–Phe	0.00	0.00	0.00	0.00	0.03	e.s.
His–Tyr	0.02	0.01	0.03	0.02	0.11	e.s.

* e.s. = elongated spot.

the corresponding amino acids [see curves (a), (b) and (c) in Fig. 1] and remains constant in the pH range 1.25–3.30, whereas large differences are observed at pH 5.10 and 8.15 (see Val–Gly/Val–Ala, Leu–Gly/Leu–Ala, Trp–Gly/Trp–Ala and Tyr–Gly/Tyr–Ala). With the eluent of pH 8.15 the presence of a double front, the first of which has $R_F = 0.81$, is observed (see Table II).

The study of the influence of the concentration of the organic solvent in the eluent confirms that an increase in concentration results in a smaller retention for most dipeptides and a smaller difference in their R_F values.

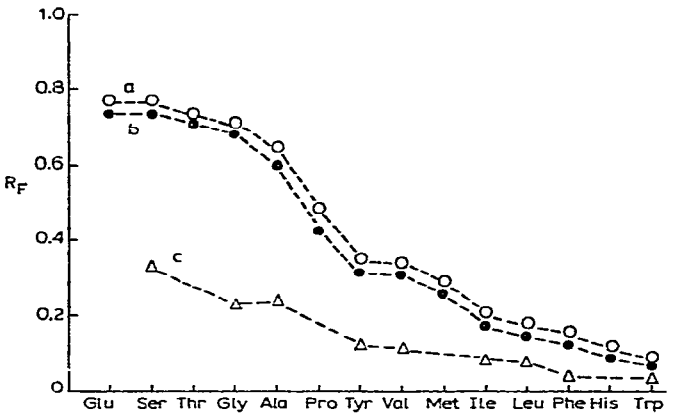

Fig. 1. Affinity sequence of amino acids (a), glycyl dipeptides (b) and leucyl dipeptides (c) on layers of silanized silica gel impregnated with 4% H-DBS. Eluent: 0.1 *M* HCl + 1 *M* CH_3COOH in 30% CH_3OH (apparent pH = 1.25). The R_F values of amino acids and of missing dipeptides were reported previously[1,4].

TABLE III

R_F VALUES OF DIPEPTIDES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE AND IMPREGNATED WITH ANIONIC AND CATIONIC DETERGENTS

Eluents: (1) water–acetic acid (7:3); (2) water–acetic acid (1:1); (3) 0.1 *M* CH_3COOH + 0.1 *M* CH_3COONa in 30% CH_3OH; (4) 1 *M* CH_3COOH in 30% CH_3OH; (5) 1 *M* CH_3COONa in 30% CH_3OH.

Peptide	*SiO_2 + 4% H-DBS*		*SiO_2, eluent 3*	*SiO_2 + 4% N-DPC*		
	Eluent 1	*Eluent 2*		*Eluent 4*	*Eluent 3*	*Eluent 5*
Gly–Thr	0.46	0.67	0.96	0.97	0.96	0.96
Gly–Val	0.19	0.46	0.96	0.97	0.95	0.93
Gly–Met	0.18	0.56	0.96	0.96	0.90	0.89
Gly–Trp	0.07	0.36	0.75	0.68	0.39	0.32
Gly–Glu	0.56	0.75	0.96	0.97	0.96	0.96
Gly–His	0.02	0.13	0.96	0.97	0.96	0.81
Ala–Thr	0.46	0.66	0.96	0.97	0.96	0.94
Ala–Trp	0.07	0.35	0.75	0.68	0.41	0.35
β-Ala–Ala	0.34	0.57	0.96	0.97	0.96	0.94
β-Ala–His	0.02	0.13	0.96	0.97	0.96	0.94
Ser–Gly	0.49	0.68	0.96	0.97	0.96	0.95
Ser–Ala	0.41	0.62	0.96	0.97	0.96	0.95
Ser–Leu	0.08	0.32	0.91	0.91	0.85	0.78
Ser–Phe	0.09	0.36	0.83	0.82	0.70	0.56
Ser–His	0.02	0.13	0.96	0.97	0.96	0.81
Thr–Gly	0.49	0.65	0.96	0.97	0.96	0.95
Val–Gly	0.26	0.52	0.96	0.97	0.96	0.95
Val–Ala	0.25	0.50	0.96	0.97	0.96	0.95
Val–Val	0.14	0.40	0.93	0.96	0.92	0.86
Val–Leu	0.06	0.28	0.84	0.90	0.82	0.69
Val–Phe	0.06	0.33	0.71	0.81	0.70	0.42
Val–Tyr	0.18	0.60	0.90	0.87	0.77	0.63
Leu–Gly	0.11	0.44	0.96	0.96	0.94	0.86
Leu–Ala	0.10	0.43	0.94	0.96	0.96	0.90
Leu–β-Ala	0.10	0.40	0.95	0.96	0.96	0.86
Leu–Ser	0.17	0.50	0.96	0.96	0.96	0.92
Leu–Ile	0.04	0.23	0.69	0.85	0.75	0.46
Leu–Trp	0.03	0.20	0.47	0.58	0.25	0.07
Leu–Phe	0.03	0.18	0.48	0.74	0.48	0.18
Pro–Gly	0.27	0.56	0.96	0.97	0.96	0.94
Pro–Ala	0.25	0.53	0.96	0.97	0.96	0.94
Pro–Leu	0.05	0.28	0.88	0.90	0.84	0.76
Pro–Phe	0.05	0.29	0.74	0.82	0.71	0.58
Met–Gly	0.20	0.51	0.96	0.96	0.96	0.92
Met–Val	0.10	0.37	0.88	0.92	0.90*	0.76
Met–Leu	0.04	0.26	0.74	0.82	0.73	0.49
Met–Met	0.09	0.37	0.85	0.87	0.78	0.63
Met–Phe	0.04	0.25	0.58	0.77	0.56	0.24
Met–Tyr	0.15	0.53	0.81	0.84	0.72	0.48
Met–His	0.00	0.09	0.96	0.97	0.95	0.70
Trp–Gly	0.09	0.41	0.73	0.77	0.59	0.44
Trp–Ala	0.08	0.39	0.79	0.81	0.62	0.38
Trp–Leu	0.03	0.21	0.43	0.57	0.26	0.10
Trp–Phe	0.03	0.22	0.35	0.48	0.15	0.04
Phe–Ser	0.14	0.44	0.89	0.94	0.85	0.72
Phe–Val	0.06	0.27	0.75	0.84	0.75	0.34*
Phe–Pro	0.05	0.23	0.58	0.82	0.68	0.53

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TABLE III *(continued)*

Peptide	*SiO_2 + 4% H-DBS*		*SiO_2, eluent 3*	*SiO_2 + 4% N-DPC*		
	Eluent 1	*Eluent 2*		*Eluent 4*	*Eluent 3*	*Eluent 5*
Phe–Trp	0.02	0.20	0.41	0.47	0.14	0.02
Phe–Phe	0.02	0.19	0.43	0.66	0.39	0.06
Phe–Tyr	0.09	0.43	0.69	0.79	0.54	0.15
Tyr–Gly	0.28	0.64	0.91	0.94	0.85	0.81
Tyr–Ala	0.26	0.62	0.93	0.96	0.89	0.81
Tyr–Phe	0.08	0.40	0.62	0.75	0.44	0.16
His–Gly	0.03	0.13	0.96	0.97	0.96	0.81
His–Ala	0.02	0.11	0.96	0.97	0.96	0.78
His–Ser	0.03	0.13	0.96	0.97	0.96	0.81
His–Leu	0.00	0.03	0.88	0.97	0.96	e.s.
His–Pro	0.01	0.11	0.96	0.97	0.96	0.74
His–Met	0.00	0.04	0.96	0.97	0.96	0.50*
His–Phe	0.00	0.02	0.76	0.93	0.89	e.s.**
His–Tyr	0.00	0.08	0.96	0.97	0.96	e.s.

* Slightly elongated spot.
** e.s. = elongated spot.

Aqueous–organic mixtures as eluents

The use of mixtures containing 30% and 50% of acetic acid[1] gave interesting results for the dipeptides. From the data in Table III (column 1), the separation between Gly–Glu and Gly–Thr, which cannot be effected with the eluents in Tables I and II, should be noted. The dipeptides containing tyrosine exhibit a very small affinity towards the exchanger on eluting with water–acetic acid (1:1) (see column 2 in Table III) and can therefore be separated from the other dipeptides which show similar chromatographic characteristics under different elution conditions (*i.e.*, Met–Tyr from Met–Val; Val–Tyr from Val–Val; Phe–Tyr and Tyr–Phe from Phe–Val). With such an eluent tyrosine behaves in the same way[4].

Layers impregnated with cationic detergents

Table III lists the R_F values of the dipeptides on layers of silanized silica gel impregnated with 4% N-DPC on eluting with 1 *M* acetic acid in 30% methanol (apparent pH = 2.75), with 0.1 *M* acetate buffer in 30% methanol (apparent pH = 5.10) and with 1 *M* sodium acetate in 30% methanol (apparent pH = 8.15), and also on silanized silica gel alone with the eluent of pH 5.10.

On layers impregnated with N-DPC, the retention of the dipeptides with the eluent of pH 2.75 is lower than that observed on silanized silica gel alone, and very few advantages are obtained from an analytical standpoint.

With the eluent of pH 5.10, in contrast, for many dipeptides a higher retention is observed than with silanized silica gel alone (see column 3 in Table III) and many separations can be carried out on dipeptides with one or two hydrophobic amino acids. Such higher retentions can be ascribed to a decrease in the detergent–dipeptide repulsive forces and/or to the presence of an anion-exchange process between the zwitterionic form of the dipeptide and the N-DPC counter ion. Such behaviour is also observed on eluting with the alkaline solution (see column 6) where the dipeptides are prevailingly in the anionic form.

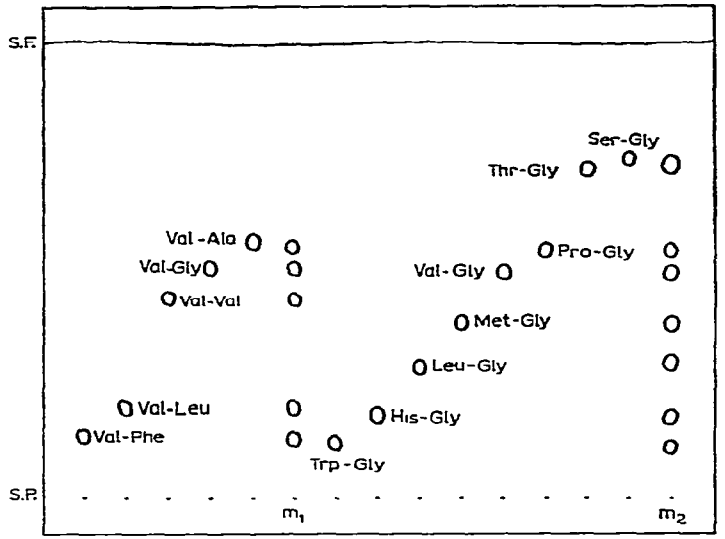

Fig. 2. Thin-layer chromatogram of dipeptides on silanized silica gel impregnated with 4% H-DBS solution. Migration distance = 12 cm. Eluent: 0.1 *M* CH_3COOH + 0.1 *M* CH_3COONa in 30% CH_3OH (apparent pH = 5.10). m_1 = Mixture of the five valyl dipeptides; m_2 = mixture of the eight dipeptides with glycine as final residue. S.P. = starting point; S.F. = solvent front.

The different affinity of the anionic form of the dipeptides towards the exchanger can be used for their separation, with the exception of those formed by hydrophilic amino acids or containing histidine as the starting residue because in the last case elongated spots are generally obtained.

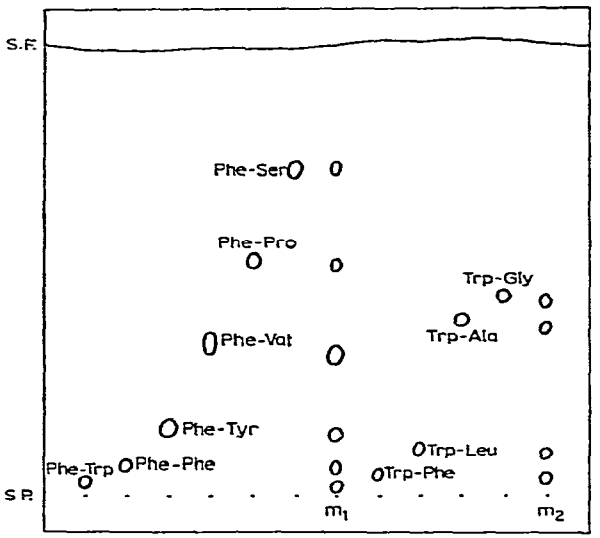

Fig. 3. Thin-layer chromatogram of dipeptides on silanized silica gel impregnated with 4% N-DPC solution. Migration distance = 12 cm. Eluent: 1 *M* CH_3COONa in 30% CH_3OH (apparent pH = 8.15). m_1 = Mixture of the six phenylalanyl dipeptides; m_2 = mixture of the four dipeptides with tryptophan as starting residue.

Analytical applications

Many separations can be effected on the basis of the R_F values reported in Tables I–III on layers of silanized silica gel alone and impregnated with anionic or cationic detergents. We carried out those concerning closely related dipeptides.

Fig. 2 shows the separation of eight dipeptides containing glycine as the terminal residue and that of five valyl dipeptides on layers of silanized silica gel impregnated with 4% H-DBS, with 0.1 *M* acetic acid + 0.1 *M* sodium acetate in 30% methanol as the eluent.

The separation between Ser–Gly and Thr–Gly cannot be effected. On layers impregnated with 4% N-DPC, eluting with 1 *M* sodium acetate in 30% methanol (Fig. 3), we separated the six phenylalanyl dipeptides and the four dipeptides with tryptophan as the starting residue. These separations demonstrate the importance of the type of detergent in the resolution of hydrophobic dipeptides. Such separations, in fact, cannot be achieved either on silanized silica gel alone or impregnated with anionic detergents.

The separation of the phenylalanyl dipeptides can be effected on the same layers, also eluting with 0.1 *M* acetic acid + 0.1 *M* sodium acetate in 30% methanol, with better resolution among Phe–Tyr, Phe–Phe and Phe–Trp.

As regards the pairs of isomeric dipeptides, Table IV lists the optimal conditions for their separation. The pairs which are not reported are not well separated under the conditions used. The data in Table IV show the versatility of this technique and its dependence on the kind of eluent and the type of stationary phase employed.

TABLE IV

PAIRS OF ISOMERIC DIPEPTIDES SEPARATED ON LAYERS OF SILANIZED SILICA GEL ALONE AND IMPREGNATED WITH DETERGENTS

Isomeric dipeptides	*Layer*	*Eluent*
Trp–Phe; Phe–Trp	SiO_2	0.1 *M* CH_3COONa + 0.1 *M* CH_3COOH in 30% CH_3OH
Leu–Ser; Ser–Leu	SiO_2 + 4% H-DBS	0.1 *M* HCl + 1 *M* CH_3COOH in 30% CH_3OH or 1 *M* CH_3COONa in 30% CH_3OH
Gly–Trp; Trp–Gly	SiO_2 + 4% H-DBS	1 *M* CH_3COONa in 30% CH_3OH
Pro–Phe; Phe–Pro	SiO_2 + 4% H-DBS	1 *M* CH_3COONa in 30% CH_3OH
Phe–Ser; Ser–Phe	SiO_2 + 4% H-DBS	0.1 *M* NaCl + 1 *M* CH_3COOH in 30% CH_3OH
Val–Gly; Gly–Val	SiO_2 + 4% H-DBS	0.1 *M* HCl + 1 *M* CH_3COOH in 30% CH_3OH
Gly–Met; Met–Gly	SiO_2 + 4% H-DBS	0.1 *M* CH_3COONa + 0.1 *M* CH_3COOH in 30% CH_3OH
Met–His; His–Met	SiO_2 + 4% N-DPC	1 *M* CH_3COONa in 30% CH_3OH
Trp–Ala; Ala–Trp	SiO_2 + 4% N-DPC	0.1 *M* CH_3COONa + 0.1 *M* CH_3COOH in 30% CH_3OH
Phe–Tyr; Tyr–Phe	SiO_2 + 4% N-DPC	0.1 *M* CH_3COONa + 0.1 *M* CH_3COOH in 30% CH_3OH

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