Journal of Chromatography, 330 (1985) 375–378
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 853

Note

Rapid separation of enantiomers by thin-layer chromatography on a chiral stationary phase

U. A. Th. BRINKMAN* and D. KAMMINGA
Free University, Department of Analytical Chemistry, de Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)
(Received May 1st, 1985)

In the past decade, thin-layer chromatography (TLC) on chemically bonded phases has attracted increasing attention[1,2]. Most papers have dealt with TLC on apolar bonded phases (C_1–C_{18}-modified silicas). Recently, several studies have been published on the potential of more polar and therefore more selective phases such as amino-, nitrile- and phenyl-bonded phases. As a further development in this direction, attempts have been made to use TLC on chiral stationary phases for the separation of enantiomers. In 1983, Wainer *et al.*[3] reported the application of a γ-aminopropyl silanized silica layer dynamically coated with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine for the separation of racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol into its isomers. At the 1985 Pittsburgh Conference, Armstrong and Alak[4] described the use of cyclodextrin-bonded phases for the separation of enantiomers of many dansylated amino acids, amino acid β-naphthylamides and ferrocenes and other metallocenes. In addition, Günther *et al.*[5] described a chiral phase made by treating an octadecyl-modified silica TLC plate with a solution of copper acetate followed by a solution of (2*S*,4*R*,2′*RS*)-4-hydroxy-1-(2-hydroxydodecyl)proline. These plates, which are now commercially available as Chiralplates from Macherey-Nagel (Düren, F.R.G.; Cat. No. 811056) were used successfully for the separation of the enantiomers of underivatized amino acids.

In this paper, we report more detailed information on the potential of Chiralplates for separations of enantiomers.

EXPERIMENTAL

Materials

The amino acids, solvents and chemicals all were of normal analytical-reagent grade. D-Glutamine and D-tryptophan were gifts from Organon (Oss, The Netherlands) and DL-serine and DL-threonine from Duphar (Weesp, The Netherlands). Approximately 0.5% solutions of the amino acids in methanol–water–formic acid (4:1:1) were used for spotting the TLC plates.

TLC

Chiralplates (10 × 20 cm) were cut into rectangular plates of about 4 × 6 cm

0021-9673/85/$03.30

with an ordinary glass cutter. After the application of about 1-mm diameter spots using a pointed paper wick partly impregnated with the sample solution, ascending development over a distance of 5 cm was carried out in Hellendahl staining jars. The plates were then left in the air to dry and subsequently sprayed with a 0.1% solution of ninhydrin in methanol. Colours were revealed after heating at 100°C for about 10 min.

RESULTS AND DISCUSSION

Plate characteristics

The faintly bluish pre-coated layer of the Chiralplate has a smooth surface and adheres well to the glass backing. Cutting of the Chiralplate into smaller plates of appropriate size can easily be achieved without any loss of material.

According to the manufacturer's instructions, the Chiralplates must be activated by heating at 100°C for 15 min. After spot application, development takes place in a saturated chamber with methanol–water–acetonitrile (50:50:200) as the eluent. This is the solvent mixture mentioned by Günther *et al.*[5] for the separation of amino acid enantiomers. For a 13-cm run, the time of development is stated to be about 25 min, which agrees with our findings.

Experimental results

In an attempt to simplify the procedure outlined above, three modifications were introduced: the heat treatment was omitted, development was carried out in an unsaturated chamber and the length of the run was reduced from 13 to 5 cm. Results for three amino acids, *viz.*, tryptophan, glutamine and valine, are shown in Fig. 1; the time of the run was about 5 min. It is evident that neither heat treatment nor chamber saturation is necessary in order to obtain a satisfactory separation of the enantiomers. Although it may be argued that chamber saturation after heat treatment may improve the spot shapes and therefore the resolution, this alternative was not tested as it is too time consuming.

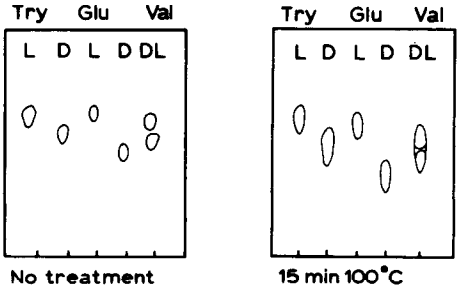

Fig. 1. TLC separation of D- and L-tryptophan, D- and L-glutamine and D- and L-valine on a Chiralplate with methanol–water–acetonitrile (50:50:200) as the eluent, using a 5-cm run in an unsaturated chamber. Right, TLC plate heated at 100°C for 15 min; left, no treatment.

Using the simplified procedure, and with methanol–water–acetonitrile (50:50:200) as the eluent, hR_F data were collected for eleven pairs of amino acid enantiomers. As can be seen from Table I, useful separations were obtained in eight instances; no resolution of enantiomers was observed for threonine, serine and ci-

TABLE I

hR_F DATA FOR D- AND L-AMINO ACIDS OBTAINED ON CHIRALPLATES WITH METHANOL–WATER–ACETONITRILE (50:50:200) AS THE ELUENT

Amino acid	hR_F	
	D-*Enantiomer*	L-*Enantiomer*
Citrulline	36	36
Glutamine	43	62
Isoleucine	51	63
Norleucine	50	61
Methionine	52	60
Phenylalanine	53	65
Serine	50	50
Threonine	54	54
Tryptophan	52	62
Valine	49	58
Norvaline	52	60

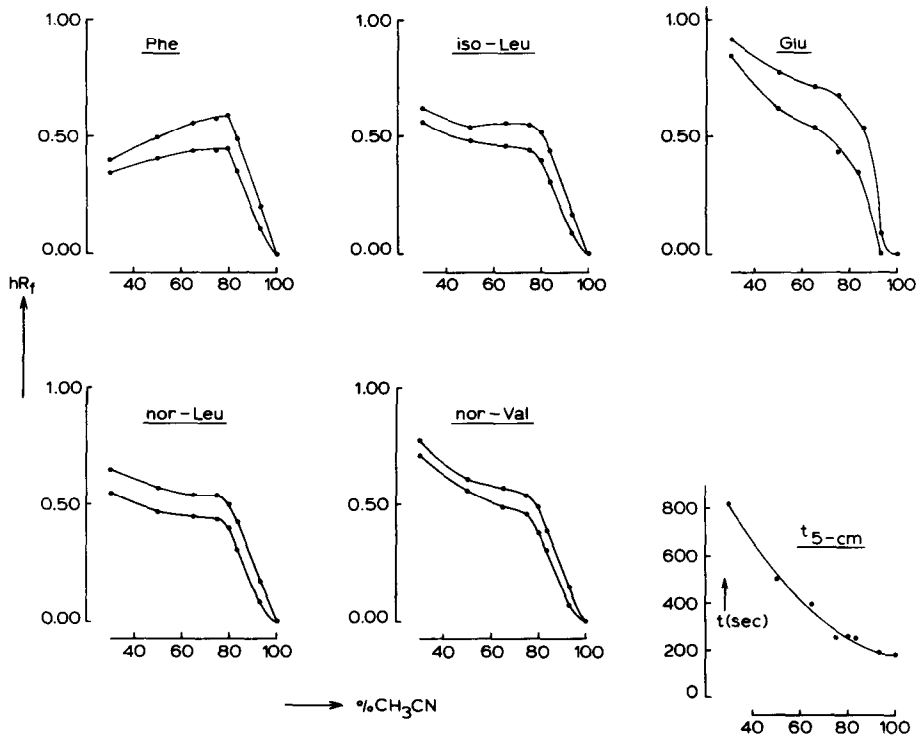

Fig. 2. Plots of hR_F *versus* acetonitrile concentration for the L- and D-enantiomers of glutamine, isoleucine, norleucine, phenylalanine and norvaline on Chiralplates, and the corresponding plot of migration time *versus* acetonitrile concentration for a 5-cm run. The L-amino acids invariably have the higher hR_F values.

trullline. The results are in very good agreement with those of Günther *et al.*[5], who separated six of the enantiomer pairs tested in our work.

Finally, the solvent mixture used as the eluent was changed to acetonitrile–water and plots of hR_F against the percentage of organic modifier were constructed for five selected enantiomer pairs. Results for 30–100% of acetonitrile are shown in Fig. 2, which includes a plot of migration time (for a 5-cm run) *versus* solvent composition. It can be seen that good separations were invariably obtained with an eluent containing about 80% of acetonitrile, which sets the time of development at only about 4 min.

CONCLUSION

Use of Chiralplates allows very rapid separations of enantiomers of various D- and L-amino acids. The original procedure, which requires about 1 h per TLC plate, has been simplified. About 5 min is sufficient for a satisfactory resolution and to control the optical purity of the compounds. Further work in this field is in progress.

ACKNOWLEDGEMENT

We thank Macherey-Nagel for the gift of several Chiralplates.

REFERENCES

1 U. A. Th. Brinkman and G. de Vries, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1982) 476.
2 U. A. Th. Brinkman, *Proceedings of the 3rd International Symposium on Instrumental HPTLC, Würzburg, 1985*, Institute for Chromatography, Bad Dürkheim, 1985, p. 25.
3 I. W. Wainer, C. A. Brunner and T. D. Doyle, *J. Chromatogr.*, 264 (1983) 154.
4 D. W. Armstrong and A. Alak, Paper presented at the *36th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, February/March 1985*, paper no. 1138.
5 K. Günther, J. Martens and M. Schickedanz, *Angew. Chem.*, 96 (1984) 514.