Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 837–842, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
© 1990 Pergamon Press plc

α_1-Acid glycoprotein column in the high-performance liquid chromatographic analysis of some groups of chiral drugs*

SÁNDOR GÖRÖG† and BULCSU HERÉNYI

Chemical Works Gedeon Richter Ltd, P.O. Box 27, H-1475 Budapest, Hungary

Abstract: The usefulness of the α_1-acid glycoprotein (Chiral–AGP) column in the analysis of chiral drugs and related materials is illustrated in three different fields. Excellent separations of the enantiomers of pharmacologically active α-ethyl benzhydrols such as 3-trifluoromethyl-α-ethyl benzhydrol (flumecinol) and 11 other derivatives are described. The enantiomers of *N*-protected amino-acid esters or amides can also be separated. The effect of the derivatization of the free α-amino group on the resolution of the enantiomers is exemplified by the formylation of alanine benzyl ester. As an example of the use of the method for the estimation of the optical purity of chiral drugs, the determination of the "*cis*(−)" impurity in "*cis*(+)" diltiazem is presented.

Keywords: *α_1-Acid glycoprotein; chiral HPLC; enantiomeric separation; α-ethyl benzhydrols; amino acid derivatives; diltiazem.*

Introduction

The increasing importance of the chromatographic separation of the enantiomers of chiral drugs and related materials in biochemistry, pharmacology and drug analysis, and the rapidly expanding methodological possibilities (mainly HPLC) to achieve separation are well reflected by the publication in the last few years of no less than seven books on chiral chromatography [1–7]; a special issue of the *Journal of Liquid Chromatography* [8], chapters in books, e.g. [9–11] and innumerable reviews and other papers have also been devoted to this topic.

The three main approaches to chiral HPLC are: separation on achiral stationary phases after diastereomeric derivatization; the formation of diastereomeric adducts with chiral additives in the eluent; and direct separation on chiral stationary phases. The third approach seems to have developed most rapidly. One important achievement in this field has been Hermansson's introduction [12] of α_1-glycoprotein chemically bonded to silica as the chiral stationary phase. This phase, especially the second-generation column, Chiral–AGP, is eminently suitable for the separation of the enantiomers of acidic [12–14] and basic [12, 13, 15–17] drugs; the enantiomers of non-protolytic compounds such as warfarin, methylphenylcyano-acetic acid methyl ester [12] have also been successfully separated.

The aim of the present paper is to report on the use of the Chiral–AGP column for the enantiomeric separation of further groups of drugs and related materials which have not yet been investigated by this technique, such as α-ethyl-benzhydrol derivatives and amino acid derivatives; in addition the estimation of the enantiomeric purity of diltiazem is demonstrated.

Experimental

Apparatus

A Hewlett–Packard 1090 high-performance liquid chromatograph equipped with a 1040 linear photodiode array UV-detector was used. The identity of the UV spectra of the separated enantiomers was checked in all cases.

Stationary phase

The 100 × 4.6 mm Chiral–AGP column was purchased from ChromTech (Norsborg, Sweden).

* Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

† Author to whom correspondence should be addressed.

Eluents

0.01 M Phosphate buffer containing various amounts of 2-propanol at various pH values was used.

Chemicals and test materials

The chemicals and solvents used in this study were of analytical reagent grade and were purchased from Aldrich (Beerse, Belgium) or Reanal (Budapest, Hungary). The test samples were from the laboratories of the Chemical Works of Gedeon Richter Ltd (Budapest, Hungary).

Reagent

Acetic–formic mixed anhydride was prepared by mixing 7.5 ml of anhydrous formic acid ($\nless$99%; with 17.5 ml of acetic anhydride. The mixture was used as the reagent after allowing it to stand overnight at room temperature [22, 23].

Formylation of alanine benzyl ester

In the case of salts with organic acids, 5 mg of the test substance was dissolved in 50 μl of the mixed anhydride reagent. For the hydrochloride 5 μl of triethylamine was also added. The mixture was allowed to stand at room temperature for 30 min.

Chromatography

Test solutions of 0.1% were prepared using the mixture of 0.01 M phosphate buffer with as much 2-propanol as necessary for the dissolution of the test material. In the case of *Z*-phenylglycineamide the test solution contained 10% (v/v) of dimethylformamide. If the formylation reaction was used an aliquot of 100 μl was neutralized before dilution with the eluent.

Two- to 5-μl aliquots of the above test solutions were injected into the chromatograph. The text and figure legends in the Results and Discussion section gives details of the pH and 2-propanol content of the eluent. The flow rate was 0.8 ml min^{-1}. All separations were performed at ambient temperature. The chromatograms were monitored at the maxima of their spectra or at 210 nm, if increased sensitivity was desirable.

Results and Discussion

Enantiomeric separation of α-ethyl benzhydrol derivatives

Numerous pharmacologically active racemic α-ethyl benzydrol derivatives have been prepared and investigated at the Chemical Works of Gedeon Richter Ltd (Budapest, Hungary) [18, 19]. Several attempts have been made to separate the enantiomers of the new enzyme-inducing agent of this family, flumecinol (Zixoryn[R]). The gas chromatographic separation on the Chirasil–Val phase was unsuccessful (Laukó and Görög, unpublished results); only poor resolutions were obtained by HPLC using various cyclodextrines as mobile phase additives [20] and by using the chiral phase of chemically bonded bovine serum albumin [21].

Baseline separation can easily be achieved using the Chiral–AGP column (Fig. 1). The reason for this is probably the relatively hydrophobic character of some areas of the α_1-AGP molecule which may play an important rôle in the separation mechanism [12]. Since the chirality of flumecinol is closely related to the presence of bulky apolar (phenyl and CF_3-phenyl) groups it is evident that the basis of the separation is the hydrophobic interaction with the apolar parts of the stationary phase.

The separation data of several other α-ethyl benzhydrol derivatives are shown in Table 1. It has to be emphasized that the chromatographic conditions have not been optimized for the individual derivatives; the same conditions (pH 7 and 10% (v/v) of 2-propanol in the eluent) were used leading to too short or too long retention times in some cases but the resolution of the enantiomers was good to excellent even under these conditions. The complexity of the retention and separation mechanism is well illustrated by comparing the data of flumecinol and its hydroxylated derivatives; the introduction of a phenolic hydroxyl into the 4′ position greatly improves the separation while the same in position 2′ decreases it.* Another example is the 2,5-dimethyl derivative (RGH-3395) where the separation of the enantiomers is only moderately successful; if, however, the α-ethyl group is replaced by an ethinyl group, excellent separation is achieved (α = 4.58; R_s = 4.78).

*The complexity of the retention and separation mechanism, i.e. the fact that different areas of AGP are involved in the retention of various groups of chiral drugs is certainly the reason for the different *h* values in the chromatograms as seen in Figs 1–3.

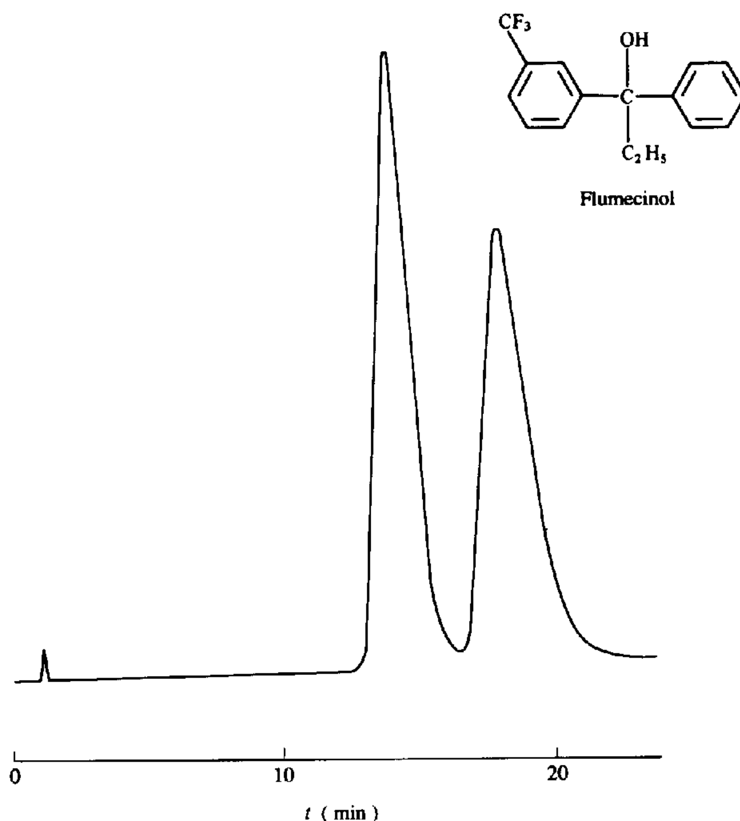
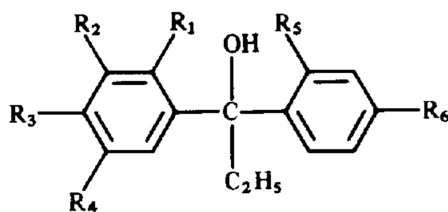


Figure 1
Separation of the enantiomers of flumecinol. 2-Propanol content in the eluent: 10% (v/v); pH = 7.

Table 1
Separation of the enantiomers of some α-ethyl benzhydrol derivatives

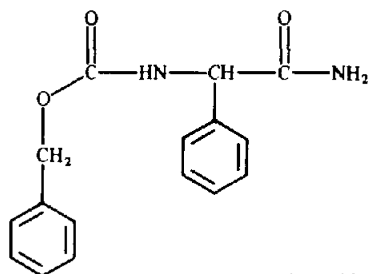
	R_1	R_2	R_3	R_4	R_5	R_6	k_2'	α	R_s
Flumecinol	H	CF_3	H	H	H	H	11.28	1.34	1.30
	H	H	CF_3	H	H	H	12.68	1.54	2.11
	H	CF_3	H	H	H	OH	6.16	1.80	2.91
	H	CF_3	H	H	OH	H	11.32	1.14	—
	H	CF_3	H	H	H	O—CO—NH—CH_3	5.26	1.68	1.70
	H	CF_3	H	H	H	O—CO—NH—C_2H_5	6.31	1.92	2.32
RGH-2801	H	CF_3	H	H	H	O—CO—NH—*n*—C_3H_7	10.40	1.80	1.97
	H	CF_3	H	H	H	O—CO—NH—*n*—C_4H_9	9.27	1.74	2.09
RGH-6201	H	CF_3	H	H	H	O—$(CH_2)_2$—N $(C_2H_5)_2$	6.50	1.06	—
RGH-3375	H	OCH_3	OCH_3	OCH_3	H	H	0.96	1.24	—
RGH-3395	CH_3	H	H	CH_3	H	H	7.23	1.20	0.95



Preliminary results with amino acid derivatives

Taking into consideration the structural and steric characteristics necessary for a good enantiomeric separation with the Chiral–AGP column, amino acids do not seem to be promising candidates. The reason for this is that in the majority of cases they do not contain ring systems which would be necessary for the separation but they do contain primary amino-groups which definitely decrease the separation efficiency [12].

In some cases, however, enantiomeric separation of amino acid derivatives (important intermediates of peptide syntheses) can be achieved. This is the case, for example, with *N*-carbobenzyloxy-phenylglycinamide where on the one hand the bulkiness of the protecting group on the α-amino group, and on the other hand the presence of the phenyl rings in the molecule create favourable conditions for the separation. Using a pH of 8 and 1% (v/v) of 2-propanol as the uncharged modifier good separation (α = 3.28) and resolution (R_s = 2.48) was achieved.



k'_2 = 19.7
α = 3.28
R_s = 2.48

The importance of the derivatization of the primary α-amino group is exemplified by the separation of the enantiomers of alanine benzyl ester. Inspection of curve (a) of Fig. 2 shows that the separation and resolution is very poor even after careful optimization of the pH and the concentration of the organic modifier. Curve (b) shows the same separation after the formylation of the α-amino group. The result of derivatization is a sufficient separation of the enantiomers. The derivatizing reagent, acetic–formic mixed anhydride, was introduced into the non-aqueous titrimetric analysis of amines by Görög and Szepesi [22, 23]. The reagent can be prepared by simple mixing of equivalent quantities of anhydrous formic acid and acetic anhydride. The formylation of even the sterically hindered secondary amines takes place quantitatively at room temperature; the primary α-amino group reacts almost instantaneously. After the completion of the reaction the anhydride is easily decomposed by simply neutralizing the solution prior to injecting it into the chromatograph. It is worth mentioning

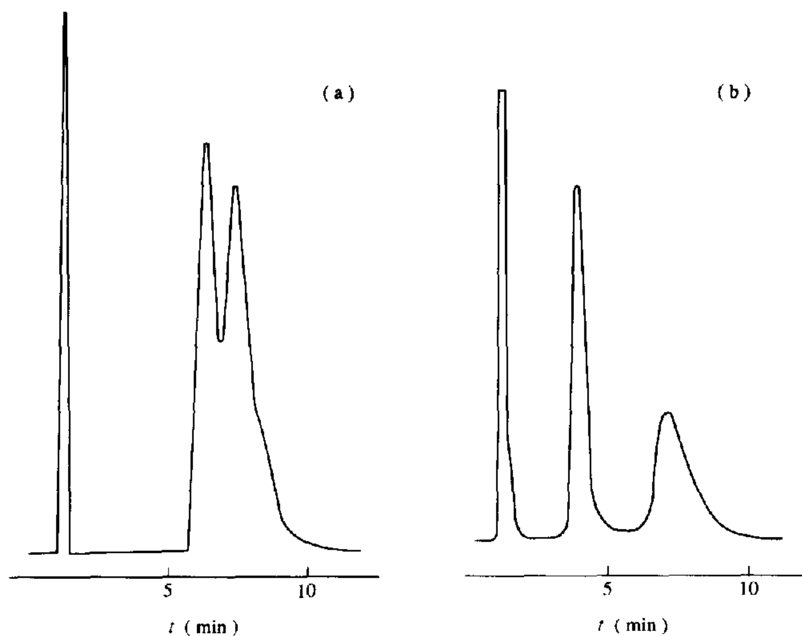
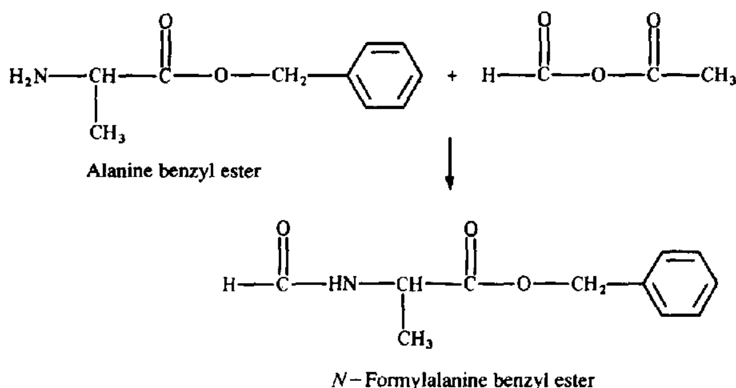


Figure 2
Separation of the enantiomers of alanine benzyl ester. (a) Separation without derivatization. (b) Separation after formylation. 2-Propanol content in the eluent: 1% (v/v); pH = 8.



that a similar derivatization concept was used by Enquist and Hermansson [24] to improve the separation of the enantiomers of atenolol from each other and from endogeneous constituents. In this case the acetylation of the secondary amine and hydroxyl groups required a reaction time of 2 h at 60°C.

Work is in progress to find the applications and limitations of the above described derivatization principles in the analysis of amino acids and their derivatives.

Estimation of optical purity

The sufficient loadability of the column should enable its use for the estimation of the optical purity of drugs which are administered as pure enantiomers. This is exemplified by the determination of the "*cis*(−)" impurity in diltiazem which is used in the therapy as the "*cis*(+)" enantiomer. Figure 3 shows the chromatogram of diltiazem spiked with 1% of the "*cis*(−)" isomer.

The excellent resolution of the two peaks is remarkable because in this case the situation is disadvantageous: the impurity peak is eluted after the main peak.

$$\%cis(-) = 7.81 \times 10^{-6} \times \text{peak area} - 0.15 \quad (r = 0.9989)$$

The regression equation for the concentration range of 0.1–5% of the "*cis*(−)" impurity with its high correlation coefficient indicates that the method is suitable for the detection and quantification of the enantiomeric impurity even below 1%. The direct estimation of the optical purity by means of chromatography on chiral stationary phases is superior to the insensitive classical method of measuring the optical rotation and is a good alternative to the HPLC method based on chiral derivatization [25].

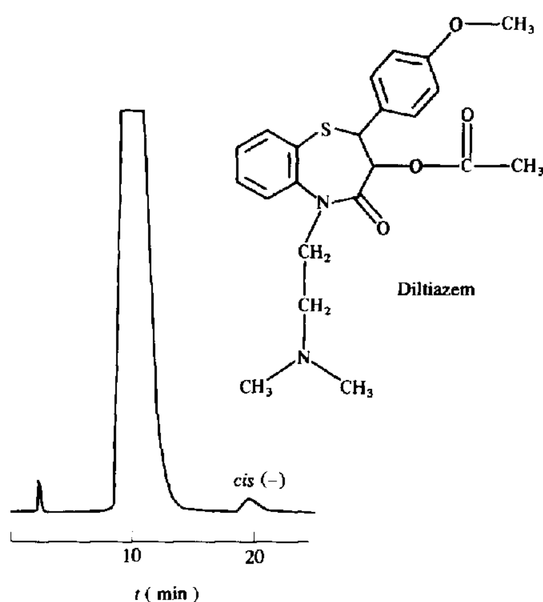


Figure 3
Chromatogram of diltiazem ("*cis*(+)") spiked with 1% of the "*cis*(−)" enantiomer. 2-propanol content in the eluent: 10% (v/v); pH = 7.

References

[1] R.W. Souter, *Chromatographic Separations of Stereoisomers*. CRC Press, Boca Raton (1985).
[2] M. Zief and L.J. Crane (Eds), *Chromatographic Chiral Separations*. Marcel Dekker, New York (1987).
[3] I.W. Wainer and D.E. Drayer (Eds), *Drug Stereochemistry: Analytical Methods and Pharmacology*. Marcel Dekker, New York (1988).
[4] S.G. Allenmark, *Chromatographic Enantioseparation: Methods and Application*. Ellis Horwood, Chichester (1988).
[5] A.M. Krstulovic (Ed.), *Chiral Separations by HPLC: Application to Pharmaceutical Compounds*. Ellis Horwood, Chichester (1989).

[6] W.J. Lough (Ed.), *Chiral Liquid Chromatography*. Blackie, Glasgow (1989).
[7] T. Stevenson and I.D. Wilson, *Chiral Separations*. Plenum Press, New York (1989).
[8] S. Hara and J. Cazes (Eds), *J. Liquid Chromatogr.* **9**(2), 3 (1986).
[9] W. Lindner, in *Chemical Derivatization in Analytical Chemistry* (R.W. Frey and J.F. Lawrence, Eds), Vol. 2, pp. 145–190. Plenum Press, New York (1982).
[10] W. Lindner and C. Petterson, in *Chromatography in Pharmaceutical Development* (I.M. Wainer, Ed.), pp. 63–110. Aster, Oregon (1985).
[11] S. Görog, in *Detection-oriented Derivatization Techniques in Liquid Chromatography* (H. Lingeman and W.J.M. Underberg, Eds), pp. 193–216. Marcel Dekker, New York (1990).
[12] J. Hermansson, *Trends Anal. Chem.* **8**, 251–256 (1989), and references from the same author therein.
[13] M. Enquist, Ph.D. Theses, University of Uppsala (1989).
[14] J. Hermansson and M. Eriksson, *J. Liquid Chromatogr.* **9**, 621–626 (1986).
[15] G. Schill, I. Wainer and S. Barkan, *J. Liquid Chromatogr.* **9**, 641–646 (1986).
[16] M. Enquist and J. Hermansson, *J. Chromatogr., Biomed. Appl.* **494**, 143–156 (1989).
[17] M. Lienne, M. Caude, R. Rosset and A. Tambute, *J. Chromatogr.* **467**, 406–413 (1989).
[18] Hung. Patent No. 167,432 (1977).
[19] Br. Patent No. 1,478,485 (1977).
[20] M. Gazdag, G. Szepesi and L. Huszár, *J. Chromatogr.* **371**, 227–234 (1986).
[21] S. Görög, A. Laukó and B. Herényi, *J. Pharm. Biomed. Anal.* **6**, 697–705 (1988).
[22] S. Görög and G. Szepesi, *Z. Anal. Chem.* **251**, 303–306 (1970).
[23] G. Szepesi and S. Görög, *Acta Pharm. Hung.* **41**, 157–163 (1971).
[24] M. Enquist and J. Hermansson, *Chirality* **1**, 209–215 (1989).
[25] T. Takaya, Y. Kishida and S. Sakakibara, *J. Chromatogr.* **215**, 279–287 (1981).

[*Received for review 4 April 1990*]