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Chiral high-performance liquid chromatographic separations on an α_1-acid glycoprotein column

II☆. Separation of the diastereomeric and enantiomeric analogues of vinpocetine (Cavinton)

Bulcsu Herényi and Sándor Görög*

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

ABSTRACT

A chiral α_1-acid glycoprotein column (Chiral-AGP) was used for the separation of vinpocetine and its three diastereomeric and enantiomeric analogues. As a consequence of the extremely large differences between the affinities of the isomers to the chiral stationary phase, gradient elution [up to 35% (v/v) 2-propanol] and a relatively high pH (7.73) were necessary to achieve a good separation within a reasonable time. The elution order of the isomers was *cis*-(+)- (vinpocetine), *trans*-(−)-, *cis*-(−)-, *trans*-(+)-. Because of the unfavourable elution order the method does not seem to be suitable for the determination of the optical purity of vinpocetine.

INTRODUCTION

One of the most succesful chiral high-performance liquid chromatographic columns for the enantioseparation of drugs and related materials is the Chiral-AGP column introduced by Hermansson [2]. This contains the second generation of the chiral stationary phase consisting of α_1-acid glycoprotein chemically bonded to silica. The enantioseparation of a large variety of acidic, basic and non-protolytic drugs and related materials using the Chiral-AGP column was described by Hermansson (2). In our laboratory the enantioseparation of various α-ethylbenzhydrols, amino acid derivatives and heterocycles has been achieved [1].

The aim of this study was to check the possibility of using the Chiral-AGP column for the solution of a more complicated problem, namely the separation of the four enantiomers of the two diastereomers of a synthetic vincamine analogue, vinpocetine (Cavinton).

EXPERIMENTAL

A Hewlett-Packard Model 1090A high-performance liquid chromatograph equipped with a Model 1040 diode-array UV detector was used. The column (100 × 4.6 mm I.D.) containing Chiral-AGP was purchased from ChromTech (Norsborg, Sweden). The eluent components were (A) 0.1 *M* phosphate buffer (pH 4.5–7.73) and (B) 2-propanol.

The over-all concentration of the isomers of vinpocetine in the test solution was about 0.1%, the solvent being water–2-propanol (1:1). Aliquots of 20 μl of this solution were injected into the chromatograph. The following gradient programme was used: 0–10 min, 18% (v/v) B, isocratic; 10–25 min, from 18 to 25% (v/v) B; 25–30 min, 25% B, isocratic; 30–40 min, from 25 to 35% (v/v) B; 40 min, 35%

☆ For Part I, see ref. 1.

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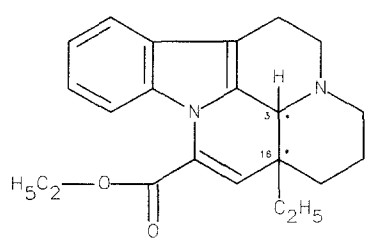
B, isocratic (see Fig. 3). The flow rate was 0.8 ml/min. The separations were performed at ambient temperature. The chromatograms were monitored at the maximum of the UV spectrum of vinpocetine at 315 nm.

RESULTS AND DISCUSSION

Vinpocetine (Cavinton) is a cerebral oxygenation enhancer made by Chemical Works Gedeon Richter (Budapest, Hungary). The structures of this synthetic eburnane derivative together with its three isomers (enantiomers of the two diastereomeric forms) are shown in Fig. 1.

The chiral chromatography of pharmacologically active eburnane derivatives has been described by Szepesi *et al.* [3]. Using cyanopropylsilica as the stationary phase and camphorsulphonic acid as a chiral mobile phase additive, they separated the eight enantiomers of the four diastereomers of vincamine. Making use of the advantageous feature of dinamic chiral chromatography, namely that the elution order can be regulated by the polarity of the chiral additive, they solved the problem of the determination of the optical purity of vinpocetine. Using (+)-camphorsulphonic acid, the peak of the *cis*-(−)-enantiomeric impurity is eluted first, allowing its determination down to the 0.2% level.



	3-H	16-C_2H_5
cis(+)	α	α
cis(-)	β	β
trans(+)	α	β
trans(-)	β	α

Fig. 1. Structures of vinpocetine [*cis*-(+)-; 3 (*S*), 16 (*S*] and its diastereomeric and enantiomeric analogues.

In the course of investigations using a Chiral-AGP column for the separation of the four enantiomers, the main problems were the separation of the *trans*-(−)-isomer from vinpocetine, which is the *cis*-(+)-isomer, and the elution of the other two isomers [especially the *trans*-(+)-isomer] from the column, as they are extremely strongly bound to the α_1-acid glycoprotein.

The main factors influencing the retention of the analytes on the Chiral-AGP column are the nature and proportion of the organic modifier, the pH of the buffer and temperature. In most instances described by Hermansson [2], 2-propanol was used as the organic modifier at concentrations below 20% (v/v). By using the above concentration of 2-propanol at pH 6.5, poor resolution of the *cis*-(+)- and *trans*-(−)-isomers was found and the other two isomers were not eluted at all within a reasonable time. For the elution of the *cis*-(−)- and *trans*-(+)-isomers, high 2-propanol concentrations [up to 35% (v/v)] were necessary and the resolution of the four isomers could be achieved by gradient elution only, which is unusual in chiral chromatography. The replacement of 2-propanol with other organic solvents such as methanol and acetonitrile did not afford better results. The pH profile of the separation of the four isomers is illustrated in Fig. 2. Acceptable resoltuion of the first two peaks can only be

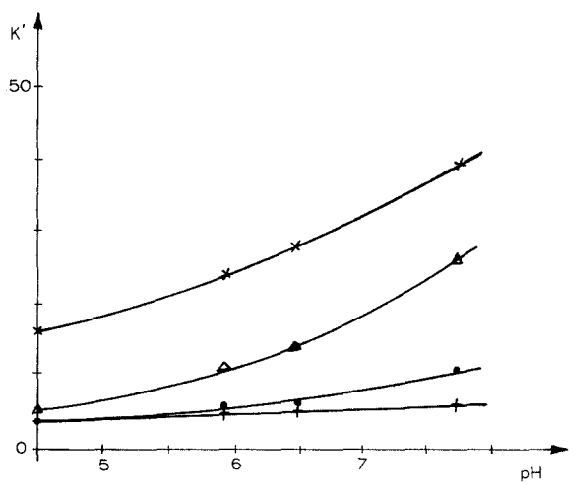


Fig. 2. pH profile of the separation of vinpocetine [*cis*-(+)-] and its diastereomeric and enantiomeric analogues. + = *cis*-(+)-; ● = *trans*-(−)-; △ = *cis*-(−)-; × = *trans*-(+)-. k' = capacity factor.

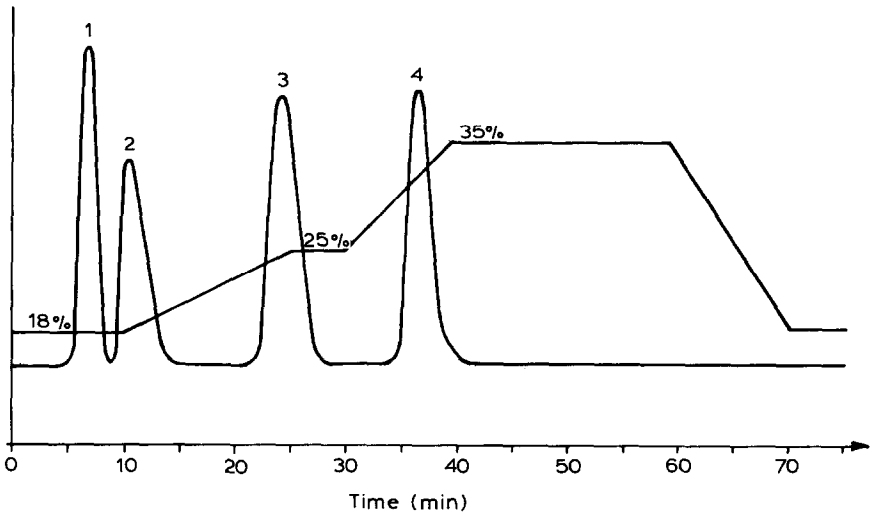


Fig. 3. Separation of vinpocetine [*cis*-(+)-] and its diastereomeric and enantiomeric analogues on the Chiral-AGP column. Peaks: 1 = *cis*-(+)-; 2 = *trans*-(−)-; 3 = *cis*-(−)-; 4 = *trans*-(+).

achieved at a pH value close to the highest value of the recommended range: a pH of 7.73 for the buffer component of the eluent was selected for the separation, which was carried out at ambient temperature. The chromatogram is shown in Fig. 3.

Unlike the above-mentioned method of Szepesi *et al.* [3], this method does not seem to be suitable for the determination of the optical purity of vinpocetine (first peak): the enantiomeric *cis*-(−)-isomer impurity (third peak) is eluted far after the peak of the drug substance.

It is interesting to compare the elution order of the diastereomers and enantiomers found in this study and the equilibrium constants (K) found by Fitos *et al.* [4] in a non-chromatographic equilibrium binding study between α_1-acid glycoprotein and various vinca alkaloid analogues. Of the four isomers in this study two were investigated in the equilibrium study: a ratio of $K_{trans\text{-}(+)\text{-}}/K_{cis\text{-}(+)\text{-}} = 30$ was found, which is in good agreement with the chromatographic results. As a consequence of the complexity of binding mechanism in which both nitrogens and the aromatic ring system are involved [4], the elution order of the enantiomers of the individual diastereomers is not easily predictable: it is +,− for the *cis* and −,+ for the *trans* derivatives. Similar observations were made among the derivatives investigated by Fitos *et al.* [4].

REFERENCES

1 S. Görög and B. Herényi, *J. Pharm. Biomed. Anal.*, 8 (1990) 837.
2 J. Hermansson, *Trends Anal. Chem.*, 8 (1989) 251; and references cited therein.
3 G. Szepesi, M. Gazdag and R. Iváncsics, *J. Chromatogr.*, 244 (1982) 33.
4 I. Fitos, J. Visy and M. Simonyi, *Biochem. Pharmacol.*, 41 (1991) 377.