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A Chromatographic Separation Method of Verapamil Enantiomers by HPLC

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In this work, a chromatographic HPLC method has been developed to separate enantiomers of calcium channel blocker verapamil using a narrow-bore AGP column and a weak flow rate. The method is reproducible and accurate.

Key Words: Verapamil, α-AGP column, D-7000 HSM.

INTRODUCTION

In recent years, pharmaceutical companies have placed greater importance on examining the stereoisomeric composition of drugs having a chiral centre. Previously, chiral compounds could not be separated by conventional HPLC techniques without derivatization, enantiomers into diastereoisomers. Now improved chiral stationary phases allow for separation and quantitation of these enantiomers.

Verapamil is a chiral calcium channel blocking drug which is an effective agent in the treatment of hypertension and arrhythmias. It is administered as a racemate, the two enantiomers differ considerably in their pharmacological potency with S-enantiomer being 10–20 times more potent than R-enantiomer[1–3].

High performance liquid chromatographic methods for the determination of verapamil and its metabolite, norverapamil, in pharmaceutical preparation and human plasma have been reported[4–7]. These include ultraviolet detection and fluorescence detection. But some of these techniques are less sensitive[8–10].

In this work, a chromatographic HPLC method has been developed to separate R and S-form of verapamil using a narrow-bore α-AGP column as a chiral stationary phase and a weak flow rate.

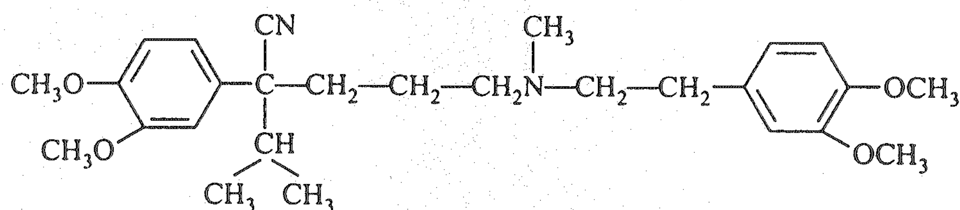

Fig. 1. Chemical structure of verapamil

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EXPERIMENTAL

The chromatographic system consisted of a Merck-Hitachi 1A chrom L-7000 pump, a diode array detector D-7000 with Hsm-7000 HPLC system manager, and a narrow-bore chiral AGP column (100 × 2 mm i.d.); the detection was at 205 nm. Racemic verapamil-HCl tablets were procured from Streuli & Cie SA; potassium hydroxide, phosphoric acid, TEA, ACN were obtained from Sigma-Aldrich.

Sample treatment procedures

Tablet formation assay: Five intact tablets of verapamil (40 mg) were weighed accurately to obtain the average tablet weight; the tablet was then crushed and triturated in a mortar until a fine powder was obtained. An amount of the powder equivalent to one tablet was weighed accurately and taken in 10 mL of ethanol.

Preparation of calibration curves:

Standard preparations: A stock solution of 4000 mg/L (4000 ppm) was prepared by dissolving 4 mg of standard verapamil in 10 mL of ethanol. From this solution, linearity over the range 10–120 ppm of verapamil was examined for both R- and S-form of verapamil and in each case, 5 μL were injected on to the column 3 times and repeated for 10 μL.

RESULTS AND DISCUSSION

The alternative approach to the direct resolution of enantiomers is to prepare diastereomeric derivatives followed by chromatography on a non-chiral column. This procedure is time-consuming and the derivatization of the diasteromeric must be carefully controlled to ensure that the enontiomers react to the same extent with the chiral reagent. However, the direct resolution procedures are preferred, especially in cases when the compound lacks a suitable group of derivatives.

Optimization of the separation

Separation on α-AGP column are very sensitive to temperature, pH, ionic strength and solvent composition. A small change in these variables causes a drastic change in retention and resolution, so that constant conditions must be maintained for reproducible results.

The influence of the temperature on the capacity factor (k′) was examined and k′ was found to increase when the temperature increased and 25°C was maintained. An increase in the pH caused a large increase in the value of k′, with a corresponding increase in resolution (R). pH = 7 was maintained and kept constant during all the experiments.

Concentration in the range 5–15% of acetonitrile was also examined and a very small increase in the concentration of ACN causes a large fall in the value of k′, as well as a loss of resolution. Therefore 12% of ACN was maintained and kept constant.

The addition of a small quantity of TEA (0.1%) was found to improve peak shape and resolution of the two enantiomers. An increase in flow rate produced a large fall in resolution. Therefore all the separations were run at 0.2 mL/min.

All these parameters are demonstrated in Fig. 2, with mobile phase consisting of 10 mM phosphate buffer containing 0.1% TEA, pH 7 and flow rate 0.2 mL/min and AGP column (100 × 2 mm i.d.).

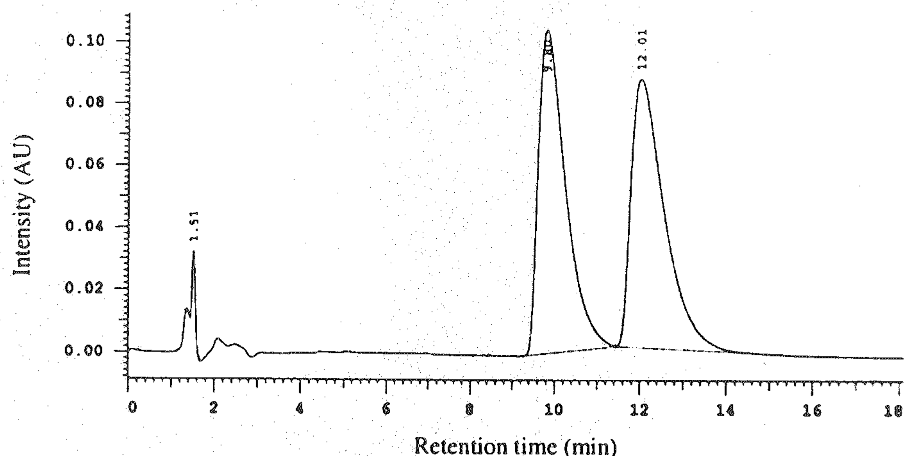

Fig. 2. Separation of the two enantiomers of verapamil (R & S forms)

Linearity over the range 10–120 mg/mL of both R- and S-form of verapamil was examined and determined by plotting the peak heights and surface area *vs.* concentration. The method was linear in accordance with Beer's law over this range and the linearity equations were

$$Y_{R\text{-ver}} = 520.83x - 1116.7, \quad R^2 = 0.9896$$

and

$$Y_{S\text{-ver}} = 414.17x + 1161.1, \quad R^2 = 0.9848$$

with regression coefficients for R-form and for S-form, respectively.

The reproducibility for replicate injection and accurate data derived from intra assay reproducibility studies are shown in Tables 1 and 2.

TABLE-1

Contents	RSD R-ver	RSD S-ver (for concentration)
80	1.65	0.99
100	2.5	2.7
120	4.9	3.13

TABLE-2

Concentration (ppm)	RSD R-vera	RSD S-vera (for retention time)
80	0.57	0.44
100	0.34	0.22
120	0.59	0.55

Recovery: The recovery of extracted verapamil was estimated by comparing peak areas of standard after extraction with the peak areas of standard solution, which corresponds to 84% recovery.

Conclusion

In conclusion, the two forms of verapamil enantiomers have been resolved by HPLC using immobilized α-acid glycoprotein (orosomucoid) as the chiral stationary phase. Base line separation was achieved and enantioselectivity of the AGP-column was regulated by adjusting the temperature, pH, concentration of organic modifier and flow rate. The method was reproducible, accurate and can be used for pharmacokinetic studies.

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