DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 18(9), 1013-1022 (1992)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC IDENTIFICATION OF RAMIPRIL, AND ITS PRECURSOR ENANTIOMERS USING A CHIRALPAK OT(+)- COLUMN

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ABSTRACT

Ramipril and its precursor I enantiomers (all-S and all-R optical isomers) were identified by liquid chromatographic method using Chiralcel stationary phase OT(+) column, after derivatization of proline residue with trifloroacetic anhydride to form trifloroacetamide. Maximum resolution (R) of 1.06 was obtained for precursor I enantiomers when chromatogramed as mixture. The method can be applied for optical purity determination of these precursors which are used for ramipril synthesis and also their detection as impurities in ramipril.

INTRODUCTION

Ramipril is a new drug presently in use as angiotensin-convering enzyme (ACE) inhibitor with improved bioavailability and pharmacokinetics, (Fig.1). Ramipril is synthesized by condensation of two precursors: precursor I (1S,3S,5S-2-azabicyclo) [3.3.0] octane-3-carboxylic acid benzyl ester (Fig.1) and precursor II, (S,S)-N-[1-ethoxycarbonyl-3-phenylpropyl alanine).(1)

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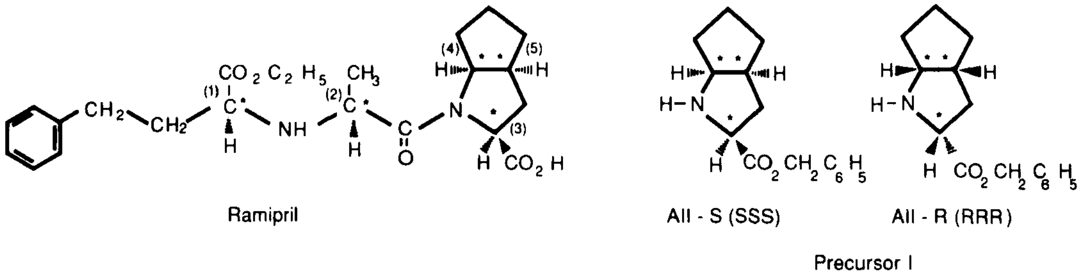

Fig. 1 The absolute configurations of ramipril and precursor I enantiomers (SSS and RRR optical isomers)

Accordingly, ramipril the active isomer, contains five chiral carbons all in S - configuration, and their correct stereochemistry is essential for its pharmacological activity.

Horvath <u>et</u> <u>al</u>(2) reported that dipetides containing L-proline may show peak splitting when reversed-phase liquid chromatography system was used. Similar observations were reported by Gustafsson <u>et</u> <u>al</u>(3) when analyzed ramipril and ramiprilate, its active metabolite using an octadecyl-bonded silica stationary phase with phosphate buffer as a mobile phase. This paper describes a method for separation of precursor I enantiomers and their determination and identification as possible impurities during the synthetic procedure of ramipril using commercially available Chiralpak OT (+) column.

EXPERIMENTAL

Apparatus

The liquid chromatography system consisted of a Waters Model M-45 pump, a U6K injector, and a Lambda-Max Model 481 LC Spectrophoto-

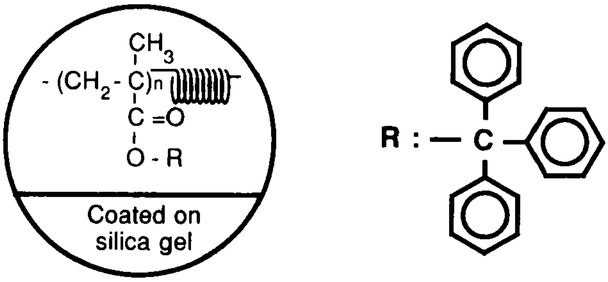

Fig. 2 The structure of the chiral stationary phase OT (+) used in this study.

meter UV detector operated at 254nm. The stationary phase of Chiralcel OT (+) (25 cm x 4.6 mm, I.D.) (Daicel Chemical Industries, Tokyo, Japan), the structure of which is shown in Fig. 2.

Chemicals

Ramipril and precursor I enantiomers (all-S and all-R) were kindly supplied by Hoechst AG, (Frankfurt am Main, Germany). HPLC Grade methanol was obtained from Alltech Associates Inc., Deerfield, Ill., USA, benzene and triethylamine were obtained from Fisher Scientific, Fairlawn, New Jersey, USA, and trifloroacetic anhydide was obtained from Fluka Chemie AG, Buchs, Switzerland.

Derivatization

The proline residue of ramipril and its precursor I enantiomers (all-S and all-R) were derivatized using trifloroacetic anhydride. In molar ratio, 4mg of starting material, 14µl of triethylamine, 250µl of benzene, and 20µl of trifloroacetic anhydride were added, mixed, then heated on a waterbath for 20 minutes at 70°C. The samples were then dried using nitrogen stream, the samples were then redissolved in methanol.

Chromatographic Conditions

The mobile phase consists of pure methanol, flow rate was 0.8 ml/min and chart speed was 0.5 cm/min. Temperature was maintained at 23°C and pressure was maintained 250 psi throughout the experiment. Sample amount injected was 2.5 nmole. Detection was obtained at UV 254nm with sensitivity range 0.01 AUFS.

RESULTS AND DISCUSSION

Gustafsson et al,(3) recently studied the influence of various operating conditions on the retention, peak splitting and band broadening of ramipril and its active metabolite ramiprilate using reversed-phase Nucleosil column and a mobile phase of phosphate buffer containing various amount of acetonitrile, tetrahydrofuran or methanol as organic modifier. Reported here a method for the separation, identification and detection of ramipril precursor I enantiomers as possible impurties during the synthetic procedure of ramipril using Chiralpak OT (+) column and pure methanol as a mobile phase. The enantiomeric elution order was determined by chromatographing the individual enantiomers (all-S and all-R) of precursor I, and ramipril under the same conditions. The capacity factor for ramipril was found to be (k=0.89) (Fig.3). As to the precursor I enantiomers, the peak that eluted with lower capacity factor (k'=0.54) was identified as all-S enantiomers (Fig.4) while the

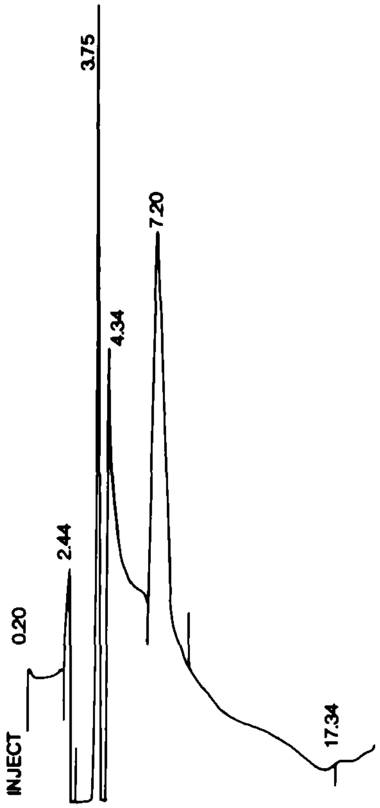

Fig. 3 Chromatogram of ramipril derivatized with trifluoroacetic anhydride on OT (+) column (250 mm x 4.6mm I.D.). Conditions: mobile phase, methanol; flow rate, 0.8ml/min.; temperature, 23°C detector, UV at 254nm; sensitivity, 0.01.

peak that eluted with a higher capacity factor (k"=0.73) was identified as all-R enantiomers (Fig.5). The derivatization reaction, without any samples, was also subjected to the same chromatographic conditions to serve as a blank is shown in Fig.6. A mixture of equal amounts all-S, all-R precursor enantiomers and ramipril was also chromatographed under the same conditions and was found that the elution order and capacity factors in agreement with that of the

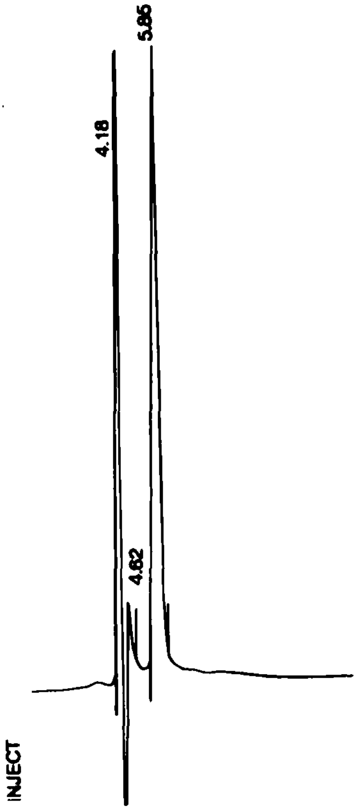

Fig.4 Chromatogram of precursor I (all-S) derivatized with trifluoroacetic anhydride. Conditions were the same as in Fig.3.

individual samples.(Fig.7) The maximum and symetrical stereochemical resolution (R) obtained for all-S and all-R enantiomers was 1.06.

Various concentrations of 2-propanol (10%-40%) in haxane were also used as an alternative mobile phase. However, the results had shown only partial separation of the two enantiomers of precursor I of

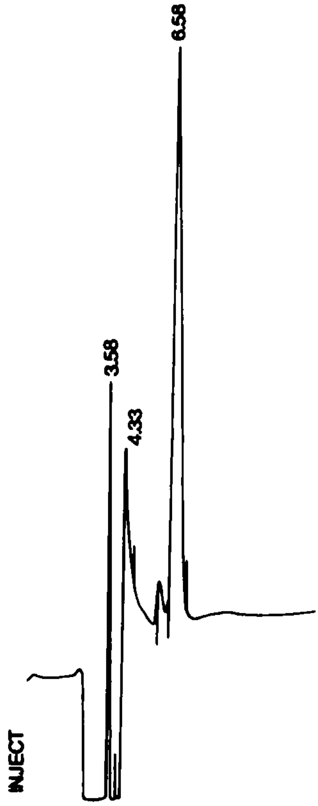

Fig. 5 Cromatogram of precursor I (all-R) derivatized with trifluoroacetic anhydride. Conditions were the same as in Fig.3.

ramipril. Effect of column temperature on the resolution of precursor I enantiomers was also studied, with methanol as mobile phase and was found that temperature has no effect on the resolution of these enantiomers.

CONCLUSION

This method could be applied for optical purity determination of precursor I enantiomers which the all-S is used in the synthesis of

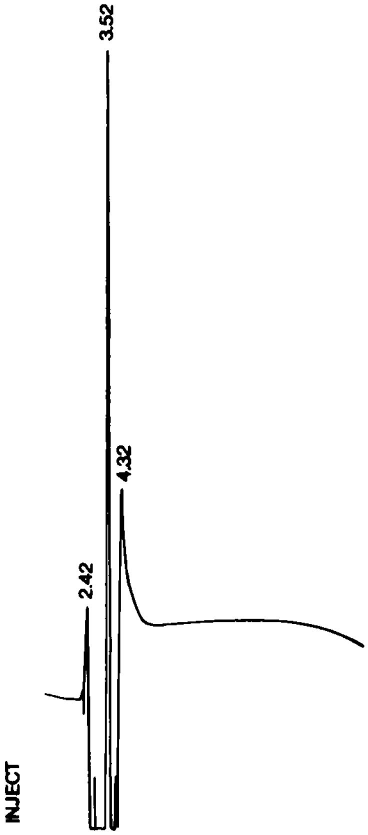

Fig. 6 Chromatogram of a blank for the derivatization reaction with trifluoroacetic anhydride on OT (+) column (250mm x 4.6nm I.D.). Conditions were the same as in Fig.3.

ramipril. Furthermore, it could be used as a reliable method for checking the purity of ramipril with regards to contamination by these precursors both in bulk material and pharmaceutical formulations.

ACKNOWLEDGMENTS

The author wish to thank Dr. H. Urbach, Hoechst Aktiengesellschaft, Leitung Pharma Forschung, Frankfurt am Main, Germany, for providing

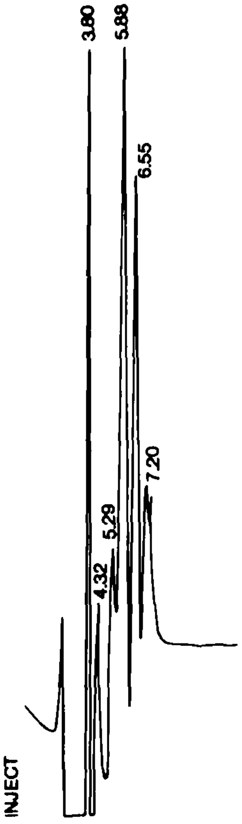

Fig. 7 Chromatogram of a mixture of ramipril, precursor I enantiomers derivatized with trifluoroacetic anhydride. Conditions were the same as in Fig. 3.

the sample of ramipril and precursors I enantiomers used in his study.

We would like to express our thanks to the Administration of the King Faisal Specialist Hospital and Research Centre for its support of the Drug Development Laboratory Research Program.

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