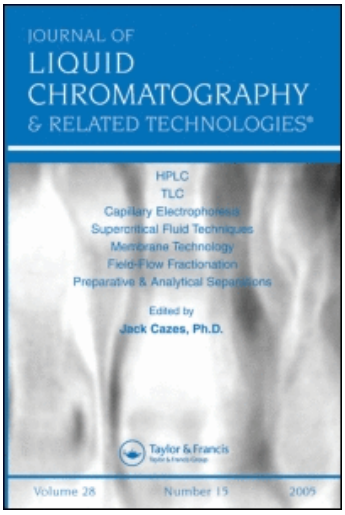

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Motofumi Ito[a]; Takeo Kuriki[a]; Junichi Goto[b]; Toshio Nambara[b]
[a] Pharma Research Laboratories Hoechst Japan Ltd., Saitama, Japan [b] Pharmaceutical Institute Tohoku University, Sendai, Japan

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SEPARATION OF RAMIPRIL OPTICAL ISOMERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

MOTOFUMI ITO[1], TAKEO KURIKI[1], JUNICHI GOTO[2], AND TOSHIO NAMBARA[2]

[1]*Pharma Research Laboratories*
Hoechst Japan Ltd.
Minamidai, Kawagoe, Saitama 350, Japan
[2]*Pharmaceutical Institute*
Tohoku University
Aobayama, Sendai 980, Japan

ABSTRACT

High-performance liquid chromatographic methods for determining the optical purities of ramipril, a novel angiotensin converting enzyme inhibitor, and its synthetic intermediates have been developed. Analytical procedures were established for the resolution of optical isomers of two moieties, precursor I and precursor II, used for the preparation of ramipril. No occurrence of racemization during condensation reaction of the two precursors could be unambiguously confirmed by the proposed methods. On the basis of analytical data ramipril bulk material proved to be optically pure.

INTRODUCTION

Ramipril, 2-[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3.3.0]octane-3-carboxylic acid, is a novel angiotensin converting enzyme inhibitor. It contains five asymmetric carbon atoms and therefore thirty-two optical isomers are theoretically possible. Ramipril is prepared by condensation of two moieties, precursor I, N-[(S)-1-ethoxy-

carbonyl-3-phenylpropyl]-L-alanine, and precursor II, (1S,3S, 5S)-2-azabicyclo[3.3.0]octane-3-carboxylic acid benzyl ester. For the clinical use, contamination with impurities, i.e., optical isomers, should be checked strictly during the synthetic process.

The present paper deals with high-performance liquid chromatographic methods for determining the optical purities of both synthetic intermediates and the final product, ramipril.

EXPERIMENTAL

Materials

Precursor I (SS and RS isomers) and precursor II were generously donated by Hoechst AG (Frankfurt, FRG). Precursor I (SR and RR isomers) and (-)-1-(1-anthryl)ethylamine were synthesized in these laboratories by the methods of Teetz et al. [1] and Goto et al. [2], respectively. Ramipril and its RS-SSS isomer were also kindly supplied from Hoechst AG. The SS-RRR, RS-RRR, and SR-SSS isomers were prepared from corresponding precursors according to the method used for the synthesis of ramipril. Trifluoroacetic anhydride and other reagents were of analytical-reagent grade. Solvents were purified by distillation prior to use and degassed by sonication.

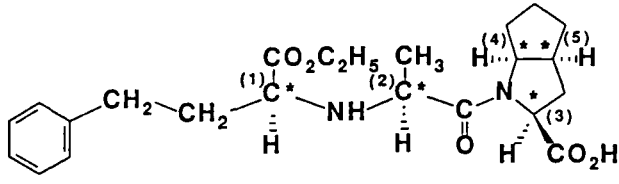
Instruments

The apparatus used for high-performance liquid chromatography (HPLC) was a JASCO Model BIP-1 chromatograph (JASCO, Tokyo, Japan) equipped with a Model VL-614 injector and a Model UVIDEC-100-IV variable detector. HPLC was carried out on Nucleosil 5 C_{18}, 50-5, SA 5 μm (M. Nagel, Düren, FRG), Spherisorb Silica 5μ (Perkin-Elmer, Norwalk, CT, USA), Sumipax OA-1004 (Sumitomo Chemical Co., Osaka, Japan) and Chiralpak OT(+)(Daicel Chemical Ind., Osaka, Japan).

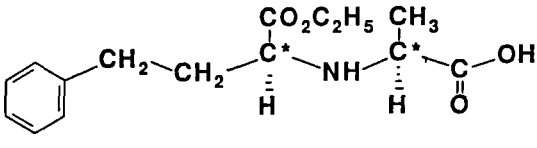
RESULTS AND DISCUSSION

Ramipril is prepared by condesation of two moieties, precursor I and precursr II (Fig. 1). Initially, examinations were made on the optical purity of precursor I which is obtainable from L-alanine benzyl ester and ethyl 3-benzoylacrylate by the Michael reaction, followed by catalytic debenzylation. Precursor I (SS) and its optical isomers, after methylation with diazomethane, were resolved into two peaks (SR + RS and SS + RR), when chromatographed on a Nucleosil 50-5 column with 0.4% ethanol in hexane (Fig. 2). On the other hand, derivatization of a diastereomeric mixture with (-)-1-(1-anthryl)ethylamine [2,3] and subsequent HPLC on a normal phase column provided three peaks (SR + RR, SS and RS) as illustrated in Fig. 3.

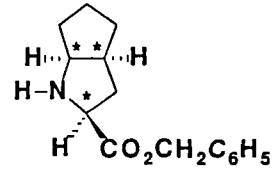
The analytical results of precursor I bulk material by the combined use of the above two procedures (Method I and Method II) are listed in Table 1. It is evident from the data that



Ramipril



Precursor I



Precursor II

FIGURE 1. Structures of ramipril, precursor I and precursor II.

TABLE 1

Contents of Optical Isomers in Precursor I

Method*	Optical isomer	Content (%)
I	SS+RR	98.9
	RS+SR	1.1
II	SS	98.6
	RS	1.4
	SR+RR	N.D.

*Details are described in captions of Fig. 2 and Fig. 3, respectively

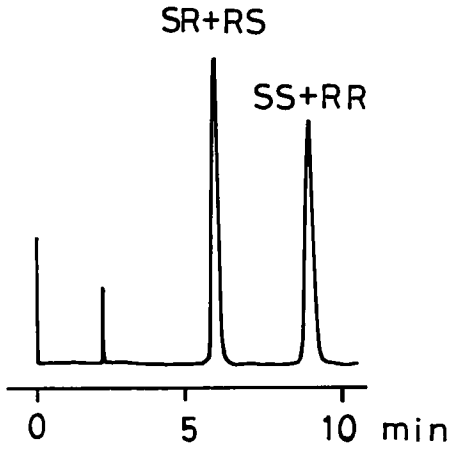


FIGURE 2. Chromatogram of precursor I and its optical isomers derivatized with diazomethane on Nucleosil 50-5. Conditions: mobile phase, 0.4% ethanol in hexane, 2 ml/min; detection, UV 210 nm.

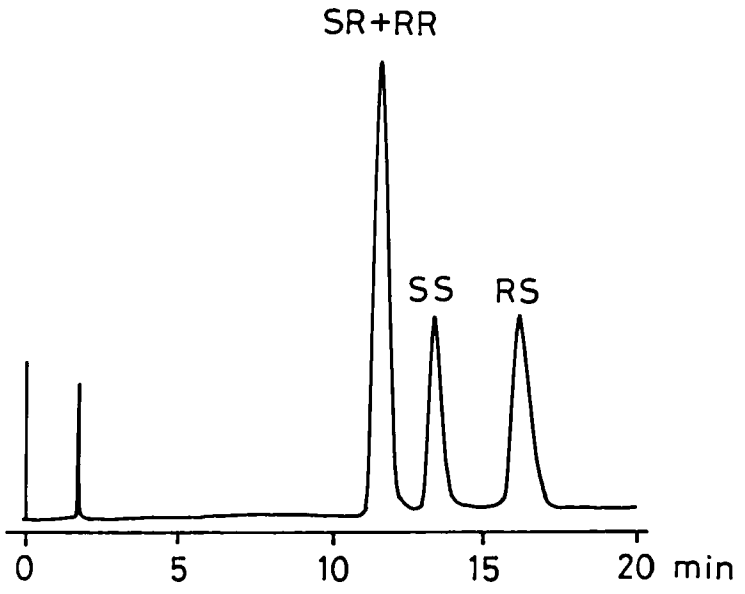


FIGURE 3. Chromatogram of precursor I and its optical isomers derivatized with (-)-1-(1-anthryl)ethylamine on Nucleosil 50-5.
Conditions: mobile phase, hexane/tetrahydrofuran (4:1), 2 ml/min; detection, UV 254 nm.

precursor I was contaminated by the RS isomer in 1.1-1.4% while the SR and RR isomers were below the detection limit (0.5%).

Next effort was directed to elucidation of optical purity of precursor II, which has three asymmetric carbon atoms in a molecule. For the preparation of ramipril, the SSS form among eight possible optical isomers is required as precursor II. This compound is synthesized by the enamine reaction. Methyl 2-acetylamino-3-(2-oxocyclopentyl)propionate is prepared by condensation of cyclopentenopyrrolidine with methyl 2-acetylamino-3-chloropropionate. Subsequent cyclization under acidic condition and catalytic hydrogenation, followed by benzylation provide racemic cis-endo-2-azabicyclo[3.3.0]octane-3-carboxylic acid benzyl ester in nearly quantitative yield. Fractional

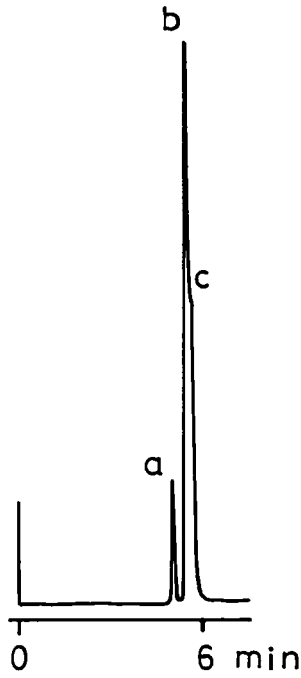


FIGURE 4. Chromatogram of precursor II and its optical isomers derivatized with trifluoroacetic anhydride on Spherisorb Silica.
Conditions: mobile phase, 2% ethanol in hexane, 1 ml/min; detection, UV 210 nm.
a: cis-exo form, b: cis-endo form, c: trans-1 form

crystallization of the salt formed from N-benzyloxycarbonyl-L-phenylalanine affords the desired cis-endo SSS compound (precursor II).

A mixture of precursor II (cis-endo, SSS) and its optical isomers, after treatment with trifluoroacetic anhydride, was subjected to HPLC on Spherisorb Silica with 2% ethanol in hexane, providing only two peaks, cis-exo form and cis-endo + trans-1 forms on the chromatogram (Fig. 4). The use of a chiral column, Sumipax OA-1004, favored the separation of cis form and trans-1 form (Fig. 5). Thus, the combined use of both chromato-

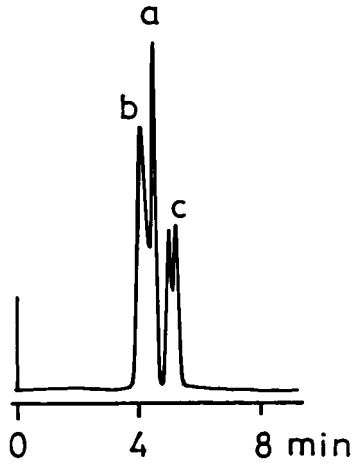


FIGURE 5. Chromatogram of precursor II and its optical isomers derivatized with trifluoroacetic anhydride on Sumipax OA-1004.
Conditions: mobile phase, 0.5% ethanol in hexane, 2 ml/min; detection, UV 210 nm.
a: cis-exo form, b: cis-endo form, c: trans-1 form

graphic methods could check the contaminated cis-exo and trans-1 forms in the cis-endo form product. The trifluoroacetylated cis-endo forms were resolved into two compounds (SSS and RRR forms) on a Chiralpak OT(+) column with methanol/water (10:1) as a mobile phase as shown in Fig. 6. The availability of these methods could serve for determining the optical purity of precursor II bulk material consisting of the desired cis-endo SSS form in 98.2% and a trace amount of contaminants having the R-configuration at the 3-, 4-, and/or 5-positions.

Considering the analytical results that the two precursors were contaminated by the RS form in 1.1-1.4% and the RRR form in 1.8%, ramipril (SS-SSS form) bulk material would possibly contain three optical isomers, RS-SSS, SS-RRR, and RS-RRR forms. Also, in the synthesis of ramipril, racemization may possibly occur during condensation of precursor I and precursor II in the presence of dialkyl phosphinic acid anhydride and potassium

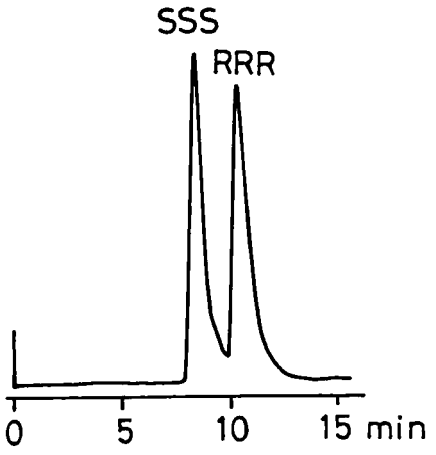


FIGURE 6. Chromatogram of precursor II and its optical isomers derivatized with trifluoroacetic anhydride on Chiralpak OT(+).
Conditions: mobile phase, methanol/water (10:1), 1 ml/min; detection, UV 254 nm.

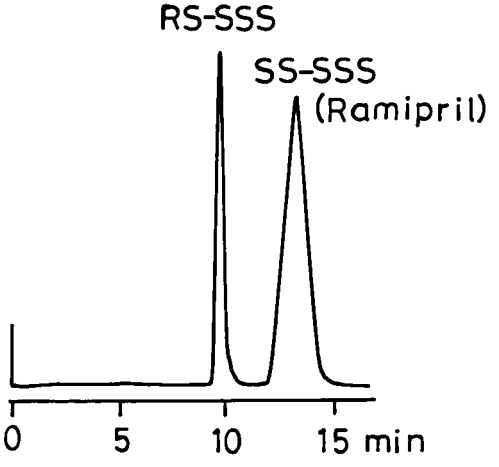


FIGURE 7. Chromatogram of ramipril and its optical isomer (RS-SSS) on Nucleosil SA.
Conditions: mobile phase, 0.15% KH_2PO_4 (pH 3.0, H_3PO_4)/acetonitrile (1:2), 1 ml/min; detection, UV 210 nm.

TABLE 2

Separation of Ramipril and Its Optical Isomers

Ramipril	Optical Isomer	k'		α	R
SS-SSS	SS-RRR	10.60	6.67	1.59	5.69
	RS-RRR SR-SSS	10.60	9.14	1.16	1.99

Conditions: column, Nucleosil 5 C_{18}; mobile phase, 0.15% KH_2PO_4 (pH 2.4, H_3PO_4)/methanol (1:1), 1 ml/min; detection, UV 210 nm.

carbonate [4]. The SR-SSS isomer which may be formed in this case, has an antipode relation to the RS-RRR form.

Ramipril and RS-SSS form were readily resolved by HPLC on a Nucleosil SA column with KH_2PO_4 (pH 3.0)/acetonitrile (1:2)(Fig. 7). Distinct separation of ramipril from other three possibly occurring optical isomers (SS-RRR, RS-RRR, and SR-SSS forms) was attained by reversed-phase HPLC on a Nucleosil 5 C_{18} using KH_2PO_4 (pH 2.4)/methanol (1:1) as a mobile phase as listed in Table 2. In actuality, contamination of ramipril by the racemization-induced optical isomers was not detected as judged by the present method.

Considering the synthetic route of ramipril, reliable procedures for determining the optical purities of ramipril as well as the two precursors were thus established by means of HPLC. Based upon the result that the two precursors contained each one optical isomer, the purity of ramipril was checked with regard to contamination by optical isomers (RS-SSS, SS-RRR, and RS-RRR forms). A careful examination by the proposed methods revealed that ramipril bulk material was optically pure.

Contaminated optical isomers would be eliminated by repeated recrystallization at the last step.

It is hoped that newly established HPLC methods will serve for the quality control of ramipril and its key intermediates.

ACKNOWLEDGEMETNTS

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