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Direct HPLC Separation of Enantiomers of Pantoprazole and Other Benzimidazole Sulfoxides Using Cellulose-Based Chiral Stationary Phases in Reversed-Phase Mode

MAKOTO TANAKA, HIDEKI YAMAZAKI, AND HIDEO HAKUSUI
Drug Metabolism and Analytical Chemistry Research Center, Developmental Research Laboratories, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan

ABSTRACT A direct, isocratic, and simple reversed-phase HPLC method was described for the separation of enantiomers of the proton pump inhibitor, rac-pantoprazole (PAN) using cellulose-based chiral stationary phases (Chiralcel OD-R and Chiralcel OJ-R). Some structurally related chiral benzimidazole sulfoxides, rac-omeprazole (OME) and rac-lansoprazole (LAN), were also studied. Chiralcel OJ-R was successful in the resolution of enantiomers of rac-PAN and rac-OME, while Chiralcel OD-R was most suitable for resolving the enantiomers of rac-LAN. Highest enantioselectivity to rac-PAN and rac-OME was achieved on Chiralcel OJ-R by using acetonitrile as an organic modifier, whereas methanol afforded better resolution of rac-LAN on Chiralcel OD-R than acetonitrile. Increases in buffer concentration and column temperature decreased retention and did not improve the resolution of the enantiomers on both columns. Using a mixture of 50 m*M* sodium perchlorate solution and acetonitrile as a mobile phase at a flow rate of 0.5 ml/min, maximum separation factors of 1.26 and 1.13 were obtained for the enantiomers of rac-PAN and rac-OME using a Chiralcel OJ-R column, while maximum separation factor of 1.16 was obtained for the enantiomers of rac-LAN using a Chiralcel OD-R column.

KEY WORDS: enantiomeric separation, Chiralcel OD-R, Chiralcel OJ-R, reversed-phase HPLC, proton pump inhibitor, pantoprazole, omeprazole, lansoprazole

INTRODUCTION

Pantoprazole, 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (rac-PAN) is a selective and long acting inhibitor of gastric H^+/K^+-ATPase (proton pump).[1] Clinical studies in healthy subjects have demonstrated that rac-PAN was well tolerated after single and multiple intravenous and oral administration and produced a dose-dependent reduction in gastric acid output.[2–5] Rac-PAN is currently under phase 2 clinical trials as an antiulcer drug in Japan.

Compounds which contain tricoordinated sulfur atoms in a pyramidal structure can exist in different optically active forms, as does rac-PAN, which is used clinically as a racemate (Fig. 1). Omeprazole (rac-OME), the first registered substance of this class, and lansoprazole (rac-LAN) are also chiral benzimidazole sulfoxides and administered as racemates (Fig. 1). The enantiomers of rac-OME and related sulfoxides were separated by HPLC on protein-based phases[6,8] and using albumin as a mobile phase additive.[9] More recently, HPLC separation of enantiomers of rac-OME,[10–11] rac-PAN,[11] and rac-LAN[11] using cellulose or amylose-based chiral stationary phases in the normal-phase mode has been reported. Balmer et al. reported that much higher enantioselectivity was achieved with Chiralpak AD than with Chiralcel OD and protein-based columns.[11] The normal-phase mode, however, has some disadvantages over the reversed-phase mode. First, normal-phase HPLC utilizes a nonpolar mobile phase such as mixtures of hexane and alcohol, which are highly inflammable and expensive. Second, extracts of biological fluids including serum and urine contain a lot of endogenous polar substances, which are strongly retained on normal-phase columns and would shorten the lifetime of the column. Thus, complex and time consuming clean up procedures would be required to remove these interfering substances.

On the other hand, in reversed-phase HPLC, polar substances are only weakly retained on the column and are eluted from it rapidly. Furthermore, the aqueous buffers used as component of a mobile phase will permit direct introduction of aqueous samples onto the column as well as regulation of the retention by changing the mobile phase composition (pH, ionic strength, or content of organic modifier).

Recently, a new reversed phase cellulose-based chiral stationary phase (Chiralcel OJ-R) has become available in addition

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Address reprint requests to Makoto Tanaka, Ph.D., Drug Metabolism and Analytical Chemistry Research Center, Developmental Research Laboratories, Daiichi Pharmaceutical Co. Ltd., 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134, Japan.

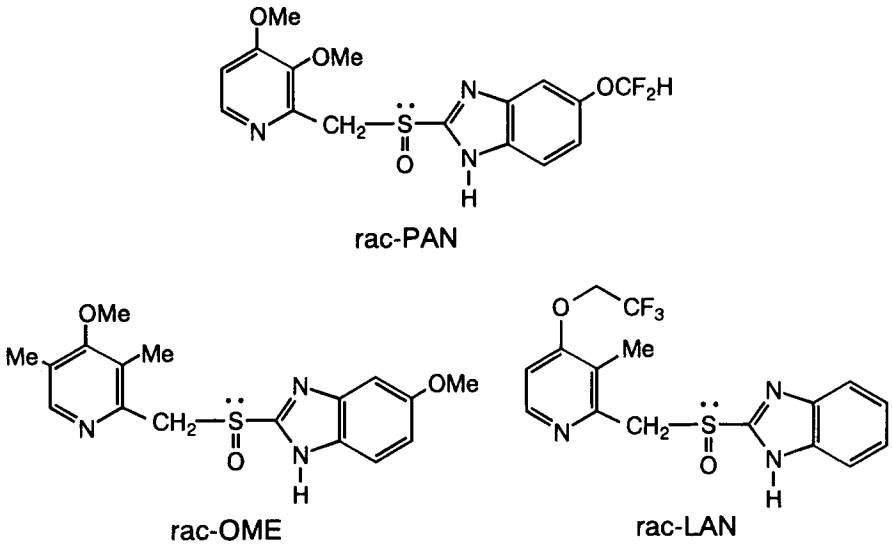

Fig. 1. Chemical structures of rac-pantoprazole (rac-PAN), rac-omeprazole (rac-OME), and rac-lansoprazole (rac-LAN).

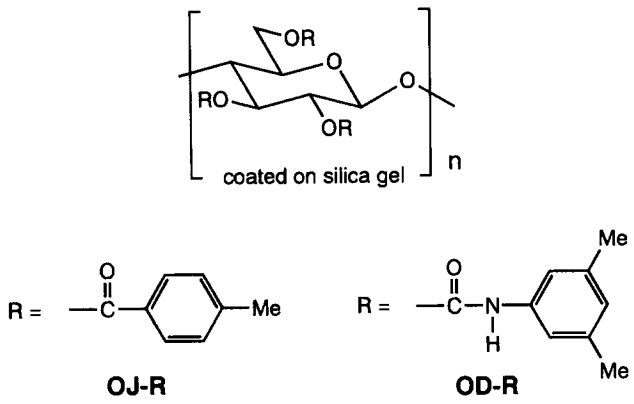

Fig. 2. Structure of Chiralcel OD-R and OJ-R chiral stationary phases.

to the already available Chiralcel OD-R (Fig. 2).[12,13] In this paper a direct method for the separation of the enantiomers of rac-PAN and other proton pump inhibitors using reversed-phase HPLC on chiral stationary phases (Chiralcel OD-R and Chiralcel OJ-R) is described.

EXPERIMENTAL

Chemicals and Reagents

Rac-pantoprazole sodium sesquihydrate, sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazolide sesquihydrate and (+)- and (−)-pantoprazole were obtained from Byk Gulden (Konstanz, Germany). The absolute configurations of (+)- and (−)-PAN have not been established. Rac-omeprazole and rac-lansoprazole (Fig. 1) were obtained from Daiichi Pharmaceutical Co. Ltd. Acetonitrile and methanol were an HPLC-grade solvent (Kanto Chemical, Tokyo, Japan). All other chemicals were of analytical reagent grade and used without further purification. Purified water from a Milli-Q system (Waters Assoc., Millipore, Milford, MA) was used.

Instruments and Chromatographic Conditions

The HPLC system consisted of two pumps (Model L-6200 and L-6000, Hitachi, Tokyo, Japan) and a variable-wavelength UV detector (Model L-4000, Hitachi). The UV detector was operated at 286 nm. Autosampler (Model AS-4000, Hitachi) and column switching module (Model E1E010, Senshu Scientific Co., Tokyo, Japan) were used for the introduction of samples. Test compounds were injected into precolumn (LiChroprep PR-2, 25–40 μm particle size, 10 × 6.0 mm i.d.) (E. Merk, Darmstadt, Germany), which was washed with water at a flow rate of 0.5 ml/min for 2 min. Then by valve operation, the mobile phase was directed in back-flush mode to the precolumn, which was in line with the analytical column.

A LiChrospher 100, RP-18 (4 × 4 mm i.d., 5 μm particle size) (E. Merk, Darmstadt, Germany) was used as a guard column and attached ahead of the analytical column. The column temperature was kept at 40°C in a column oven (Model RE-8010, Tosoh, Tokyo, Japan) if not stated otherwise.

The analytical columns were Chiralcel OD-R (250 × 4.6 mm i.d., 10 μm particle size) and Chiralcel OJ-R (150 × 4.6 mm i.d., 5 μm particle size) column from Daicel Chemical Industries (Tokyo, Japan). Acetonitrile and methanol were used as organic modifiers in mobile phase. A mixture of organic solvent and water, 50 m*M* phosphate buffer (diammonium hydrogen phosphate–ammonium dihydrogen phosphate) (pH 6.8) or 50 m*M* sodium perchlorate ($NaClO_4$) were used as the mobile phase at a flow rate of 0.5 ml/min. The mobile phase was degassed in an ultrasonic bath before use.

Preparation of Standard Solutions

Stock solutions of rac-, (+)-, and (−)-PAN, rac-OME, and rac-LAN were prepared by dissolving test compounds (ca. 1 mg each) in methanol (0.1 ml), the volume of which was adjusted with water to 10 ml in a volumetric flask after addition

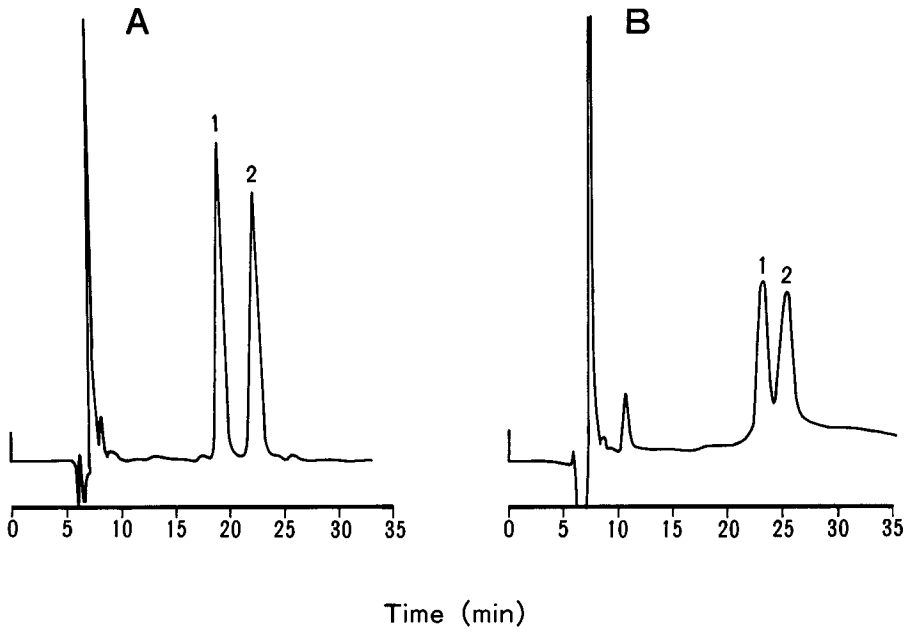

Fig. 3. Enantioselective separation of (A) rac-pantoprazole and (B) rac-omeprazole on Chiralcel OJ-R. Flow-rate: 0.5 ml/min; temperature: 40°C; detection: UV at 286 nm; mobile phase: acetonitrile/50 m*M* $NaClO_4$ (25:75) for rac-pantoprazole and acetonitrile/50 m*M* $NaClO_4$ (20:80) for rac-omeprazole; injected amount: 500 ng. The chromatograms are shown with a full scale of 20 mV.

of 1 *M* sodium hydroxide (0.01 ml), which is necessary to improve the solubility and stability of the compounds. The stock solutions were diluted with water successively to prepare the working standard solutions. The stock solutions were stored at −20°C in a freezer.

RESULTS AND DISCUSSION

Most of the investigation was performed with rac-PAN as the analyte, because the two enantiomers of rac-PAN were available. The separation of the enantiomers of other benzimidazole sulfoxides, rac-OME and rac-LAN, was also studied to evaluate the effect of different substituent patterns on the enantiomeric resolution. The chemical structures of rac-PAN, rac-OME, and rac-LAN are shown in Figure 1.

Optimization of Chromatographic Conditions

First, acetonitrile and methanol were evaluated as organic modifiers in water. A baseline resolution of the enantiomers of rac-PAN was achieved using Chiralcel OJ-R as the chiral stationary phase and acetonitrile as modifier. The use of sodium perchlorate ($NaClO_4$) solution or phosphate buffer instead of water decreased retention slightly, but had no significant influence on the resolution of the enantiomers. The effect of pH on the resolution of the enantiomers was not investigated because proton pump inhibitors are unstable under acidic conditions[14] and aqueous mobile phases with a high pH (pH > 7.0) could not be employed in order to prevent rapid column deterioration. The effect of temperature on the chiral separation was studied at 40°C and room temperature. An increase in temperature had almost no effect on resolution and resulted in decreased retention. Thus the column temperature was maintained at 40°C. The replacement of acetonitrile with methanol resulted in reduced resolution and broad peaks.

TABLE 1. Optimized parameters of capacity factor (*k*) and stereochemical separation factor (α) of pantoprazole, omeprazole, and lansoprazole on Chiralcel OD-R and Chiralcel OJ-R columns[a]

Compound	Solvent	Column	k_1	k_2	α
rac-PAN	A	OJ-R	2.11	2.65	1.26
rac-OME	B	OJ-R	2.64	2.97	1.13
rac-LAN	C	OD-R	2.55	2.95	1.16

[a]Chromatographic conditions: solvent system A = acetonitrile/50 m*M* $NaClO_4$ (25:75), solvent system B = acetonitrile/50 m*M* $NaClO_4$ (20:80), solvent system C = methanol/50 m*M* $NaClO_4$ (65:35), k_1 = first eluted peak, k_2 = second eluted peak.

By using acetonitrile–50 m*M* $NaClO_4$ as a mobile phase, Chiralcel OJ-R showed high enantioselectivity to rac-PAN and rac-OME (Fig. 3), whereas the enantiomers of rac-LAN could not be separated on this column at all. The capacity and separation factors are shown in Table 1. A baseline separation of the enantiomers of rac-PAN was achieved under these conditions with separation factor of 1.26. Compared to rac-PAN, slightly reduced resolution of enantiomers of rac-OME was achieved with separation factor of 1.13.

Recently chiral sulfoxides, flosequinan, and ML-1035 were separated on Chiralcel OD in the normal phase mode.[15,16] Thus, we attempted the separation of the enantiomers of rac-LAN using the different reversed-phase cellulose-based column (Chiralcel OD-R). Compared to Chiralcel OJ-R, reduced resolution of enantiomers of rac-PAN and rac-OME was obtained with this column, while almost baseline separa-

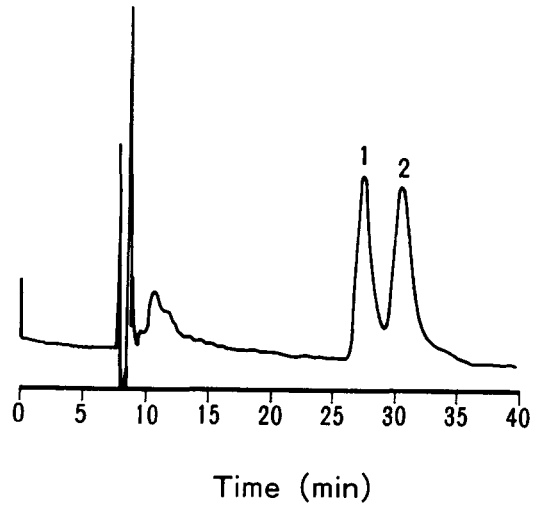

Fig. 4. Enantioselective separation of rac-lansoprazole on Chiralcel OD-R. Flow-rate: 0.5 ml/min; temperature: 40°C; detection: UV at 286 nm; mobile phase: methanol/50 m*M* $NaClO_4$ (65:35); injected amount: 500 ng. The chromatogram is shown with a full scale of 10 mV.

tion of enantiomers of rac-LAN was achieved with Chiralcel OD-R with separation factor of 1.16 (Fig. 4 and Table 1). Although the chemical structures of rac-PAN, rac-OME, and rac-LAN were very similar, Chiralcel OD-R and Chiralcel OJ-R showed different enantioselectivity toward these benzimidazole sulfoxide derivatives.

Determination of Enantiomeric Elution Order

The enantiomeric elution order was determined by chromatographing the individual enantiomers of rac-PAN under the same chromatographic conditions. On the Chiralcel OJ-R column, the peak that eluted first was identified as (−)-PAN and the second peak was identified as (+)-PAN with acetonitrile as organic modifier. Interestingly, the replacement of acetonitrile with methanol reversed the elution order. The elution order on the OD-R column with acetonitrile as modifier was the same as that on the OJ-R column with acetonitrile. The enantiomers of rac-PAN were not separated when acetonitrile was replaced with methanol. The enantiomeric elution order of rac-OME and rac-LAN was not determined due to the lack of analytical reference compounds of their enantiomers.

CONCLUSION

A baseline separation of PAN enantiomers was achieved by direct reversed-phase HPLC using newly developed Chiralcel OJ-R column. Chiralcel OJ-R and OD-R showed different enantioselectivity toward rac-PAN, rac-OME, and rac-LAN. Because of good stereochemical resolution obtained, especially for rac-PAN, the methods could be applied for the stereoselective pharmacokinetic studies of rac-PAN in experimental animals and man.

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