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Determination of the enantiomers of omeprazole in blood plasma by normal-phase liquid chromatography and detection by atmospheric pressure ionization tandem mass spectrometry

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Abstract

An enantioselective assay of omeprazole in blood plasma using normal-phase liquid chromatography on a Chiralpak AD column and detection by mass spectrometry is described. Omeprazole is extracted by a mixture of dichloromethane and hexane and, after evaporation, redissolution and injection, separated into its enantiomers on the chiral stationary phase. Detection is made by a triple quadrupole mass spectrometer, using deuterated analogues as internal standards. The method enables determination in plasma down to 10 nmol/l (LOQ) and shows excellent consistency suited for pharmacokinetic studies in man. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Omeprazole

1. Introduction

Omeprazole is an inhibitor of gastric acid secretion widely used as an antiulcer drug and against other acid-related diseases. It is a substituted benzimidazole containing a sulphoxide group and is administered as a racemate. The two enantiomers of omeprazole and related benzimidazoles have been separated by Allenmark et al. [1] by liquid chromatography (LC) on a chiral stationary phase of bovine serum albumin (BSA) immobilized on silica, by Erlandsson et al. [2] on trisphenylcarbamoyl-cellulose coated on 3-aminopropylsilica and by Marle et al. [3] on cellulose immobilized on diol silica. Vandenbosch et al. [4] compared three different protein-based phases, Persson and co-workers [5] investigated protein- and cellulose-based chiral phases, as did Tanaka and co-workers [6,7] for resolution of pantoprazole enantiomers and Nakano and co-workers [8] for lansoprazole enantiomers in blood plasma.

Enantioselective measurement of omeprazole in blood plasma was demonstrated by Cairns et al. [9] using Chiral-AGP as chiral stationary phase, and Karlsson and Hermansson [10] showed a chromatogram from a plasma sample after separation on the same phase. In a study on the stereoselective disposition of omeprazole by Tybring et al. the enantioselective separation was preceded by separation on a C_{18} column [11]. However, although enantioselectivity can be achieved on different chiral phases, it seems as if the overall selectivity towards metabolites and even more endogenous compounds in the plasma sample using UV detection is not sufficient

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for pharmacokinetic studies. We therefore present a method based on separation on Chiralpak AD with the much more powerful mass spectrometer as detector, enabling the determination of at least as low concentrations as for a previous racemic method [12]. The conclusion is that this ionspray LC–MS–MS method has advantages in providing shorter analytical run time, higher selectivity and much lower limit of quantification compared with previous analytical methods.

2. Experimental

2.1. Chemicals and reagents

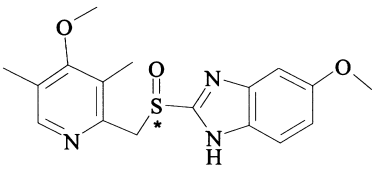
Omeprazole and [D_4]-omeprazole (Fig. 1) were obtained within Astra Hässle. (The position of the fourth D is not known.) Acetonitrile, dichloromethane, isohexane, hexane and methanol were of HPLC grade from Rathburn (Walkerburn, UK). 2-Butanol, acetic acid, formic acid and buffer substances were of analytical grade from E. Merck (Darmstadt, Germany) and ethanol (99.5%) and ethanol (95%) from Kemetyl (Stockholm, Sweden). Water was from an ELGA purification system (High Wycombe, UK).

2.2. Liquid chromatographic system

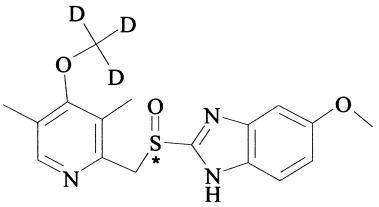
The LC system consisted of a pump, Perkin-Elmer 200 series and an autosampler, Perkin-Elmer 200 series (Überlingen, Germany) with a 100-μl variable loop. Make-up pump was a Shimadzu LC10AD (Kyoto, Japan). The mass spectrometer was a Perkin-Elmer Sciex API-365 (Foster City, CA, USA), triple quadrupole mass spectrometer with pneumatically assisted electrospray (ionspray) interface.

The chiral column was a Chiralpak AD (50×4.6 mm, 10 μm) from Daicel Chemical Industries (Tokyo, Japan). An Optiguard CN (15 mm×1 mm, 10 μm) was used as guard column Optimize Technologies (Oregon City, OR, USA). The mobile phase consisted of 0.004% concentrated acetic acid, 30% ethanol (99.5%) and 1% acetonitrile in isohexane (by volume). Flow-rate was 1.0 ml/min for the separation column. The make-up liquid was 0.2% formic acid in ethanol (95%) with a flow-rate of 0.25 ml/min, before split. The retention time for *S*-omeprazole was about 3.0 min and for *R*-omeprazole 4.5 min at ambient temperature with the same retention times for their deuterated analogues.

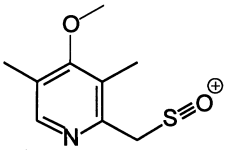
The effluent from the chromatographic column, together with the post-column addition of ethanol containing formic acid, was split in a Valco tee



S- and R-omeprazole



[D_4]- S- and R-omeprazole



Product ion of omeprazole

Fig. 1. Structure of analytes and the daughter ion (m/e 198) of *S*- and *R*-omeprazole.

connection, and the flow to the ionspray interface was about 35 μl/min. The orifice voltage was set at 40 V, the collision energy (Q0–R02) at 12 V and ionspray voltage at 3800 V. The collision gas thickness was set at 260×10^{13} (molecules/cm^2). Nitrogen was used as nebulizer gas and the sample pump was switched on to avoid risk of explosion. Other settings were used as obtained during routine optimization of the instrument. *S*- and *R*-omeprazole were monitored at *m*/*z* 198 product ion of 346. [D_4]-*S*- and -*R*-omeprazole were monitored at *m*/*z* 202 product ion of 350. The dwell time was 1000 ms for each mass transition.

2.3. Sample preparation procedure

The standard solution was prepared by dissolving omeprazole in carbonate buffer, pH 9.3, $I=0.1$ also containing 5% of methanol. The solution was stored frozen in 5-ml portions for up to 3 months. Omeprazole in solution should be protected against direct sunshine. Calibration samples (at least six replicates per day) were prepared by adding 50 μ1 of a standard solution of omeprazole, 9.6 pmol/l, to 300 μ1 of drug-free blank plasma, giving a concentration of 800 nmol/l of each enantiomer. These samples were run in parallel to the unknown study plasma samples. The internal standard solution of [D_4]-omeprazole was prepared and stored in the same way as the standard solution.

Venous blood samples were collected into heparin tubes and separated by centrifugation for 5 min at 1500 *g*. The plasma was transferred to disposable polypropylene tubes and frozen at −18°C. The frozen blood plasma samples was allowed to thaw at room temperature mixed and centrifuged. Three hundred μl of the samples were transferred to a 10-ml centrifuge tube. Twenty-five μl of NaH_2PO_4 (1 *M*), 50 μl of internal standard solution (8 μmol/l [D_4]-omeprazole in carbonate buffer, pH 9.3, $I=0.1$ containing 5% methanol) and 4.00 ml of extraction solvent, 1% 2-butanol in dichloromethane–hexane (1:1, v/v), were added, and the tubes were shaken for 10 min. After centrifugation for 5 min the aqueous phase was frozen in a dry-ice ethanol bath and the organic phase transferred to glass tubes with conical bottom. After evaporation under nitrogen the residue was dissolved in 250 μl of redissolution solvent, 1% acetonitrile in ethanol–hexane (3:7, v/v), and then ultrasonicated for 2 min and vortexed for 2 min, and 40 μl was injected with the autosampler onto the chromatographic column.

3. Results and discussion

3.1. Extraction

The absolute recoveries from plasma were calculated by comparing peak heights of *S*-omeprazole, *R*-omeprazole, [D_4]-*S*-omeprazole and [D_4]-*R*-ome-

Table 1
Within-day precision, absolute and theoretical recoveries

Compound	Concentration (nmol/l)	Absolute recovery (%)	Theoretical recovery (%)	Coefficient of variation ($n=8$)
S-Omeprazole	2780	100.0	97.5	2.4
	309	103.5		1.5
	34	107.7		5.9
	11	106.8		4.9
R-Omeprazole	2780	101.1	97.5	3.3
	309	96.5		1.4
	34	99.4		4.9
	11	97.0		11.6
[D_4]-*S*-Omeprazole	906	100.1	97.5	
[D_4]-*R*-Omeprazole	906	98.3	97.5	

prazole with freshly prepared mobile phase containing the same amounts of respective compound injected directly onto the chromatographic system. The absolute and the theoretical recoveries are presented in Table 1. Dichloromethane alone has been used for solvent extraction from blood plasma enabling direct injection of an aliquot on to a silica column and a nonpolar organic mobile phase with dichloromethane as dominating component [12]. This was used for reason of easiness, but was not adaptable here owing to lack of compatibility with the mobile phase causing distortion of the chromatogram. A mixture of dichloromethane–hexane and favourable volume ratio gave an extraction recovery around the theoretical one, 97.5%. One percent 2-butanol was present in the extractant which we believe is favourable to prevent losses of omeprazole by degradation and adsorption during evaporation of the organic solvent extract. The liquid used for redissolution of the extract residue was the same as the mobile phase apart from acetic acid being excluded, since omeprazole degrades by storage in acidic solution.

3.2. Separation

A couple of chiral stationary phases were tested, both protein-based phases and cellulose- and amylosed-based ones, in terms of enantioselectivity, peak sharpness and selectivity versus metabolites or rather unknown co-eluting endogenous compounds. The challenge from the latter ones was a major obstacle in our efforts, also reflected in previously published enantioselective assays. The obvious approach to overcome this is, of course, to turn from the UV detector, quite useful for nonchiral LC analysis of this group of compounds, to the mass spectrometer.

The composition of the mobile phase was not critical in terms of enantioseparation and neither was temperature. Since deuterated internal standards are used, those are eluting at the same retention times.

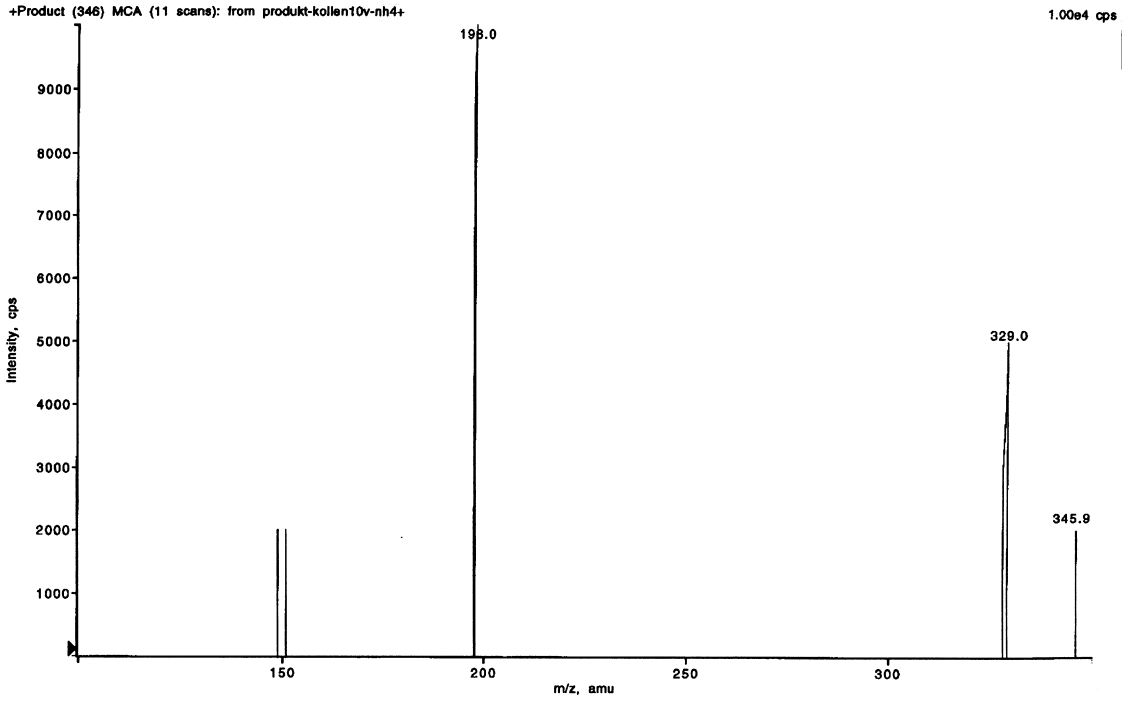


Fig. 2. Positive product ion mass spectrum of omeprazole, collision energy 10 V, showing the precursor ion at m/z 345.9 and the product ion at m/z 198.

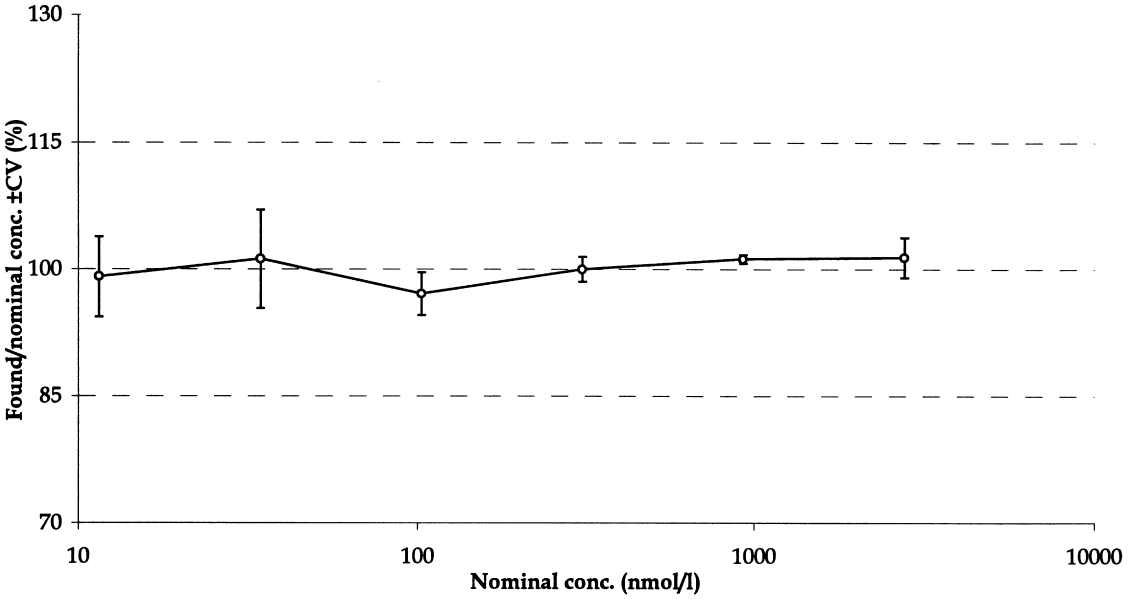


Fig. 3. Standard curve for *S*-omeprazole, 10–3000 nmol/l.

3.3. Mass spectrometric conditions

The nonpolar mobile phase was combined with a make-up liquid of 0.2% formic acid in ethanol (95%), post-column. The flow was thereafter split so that about 35 μl/min entered the ionspray. The make-up liquid is needed to induce sufficient ionization in the API source. Nitrogen was used as nebulizer gas to lower the risk of explosion. The combination of normal-phase LC and API-MS–MS

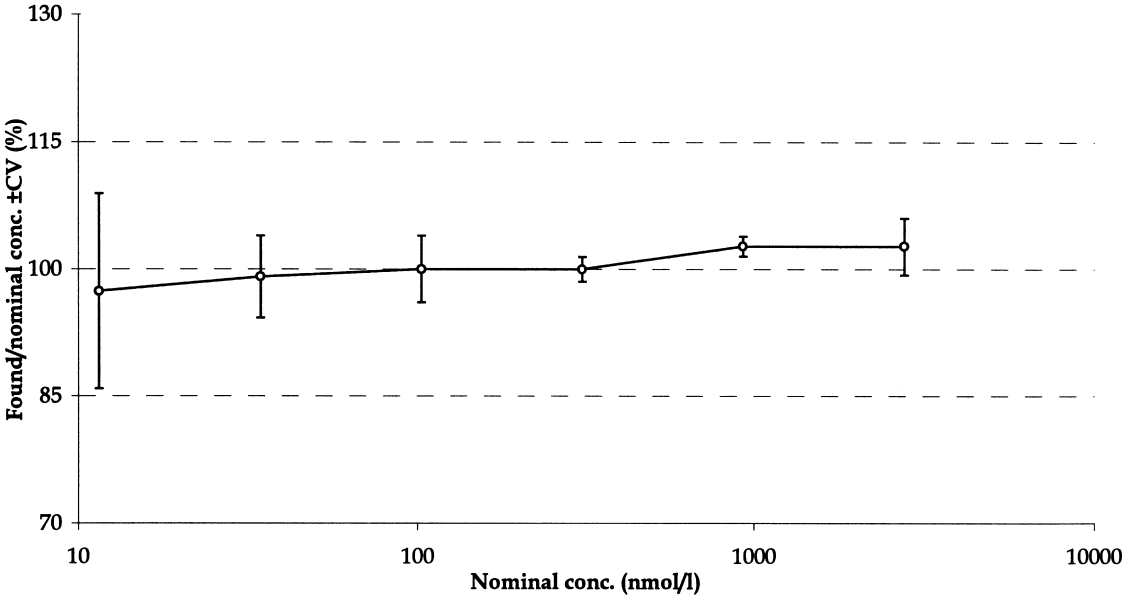


Fig. 4. Standard curve for *R*-omeprazole, 10–3000 nmol/l.

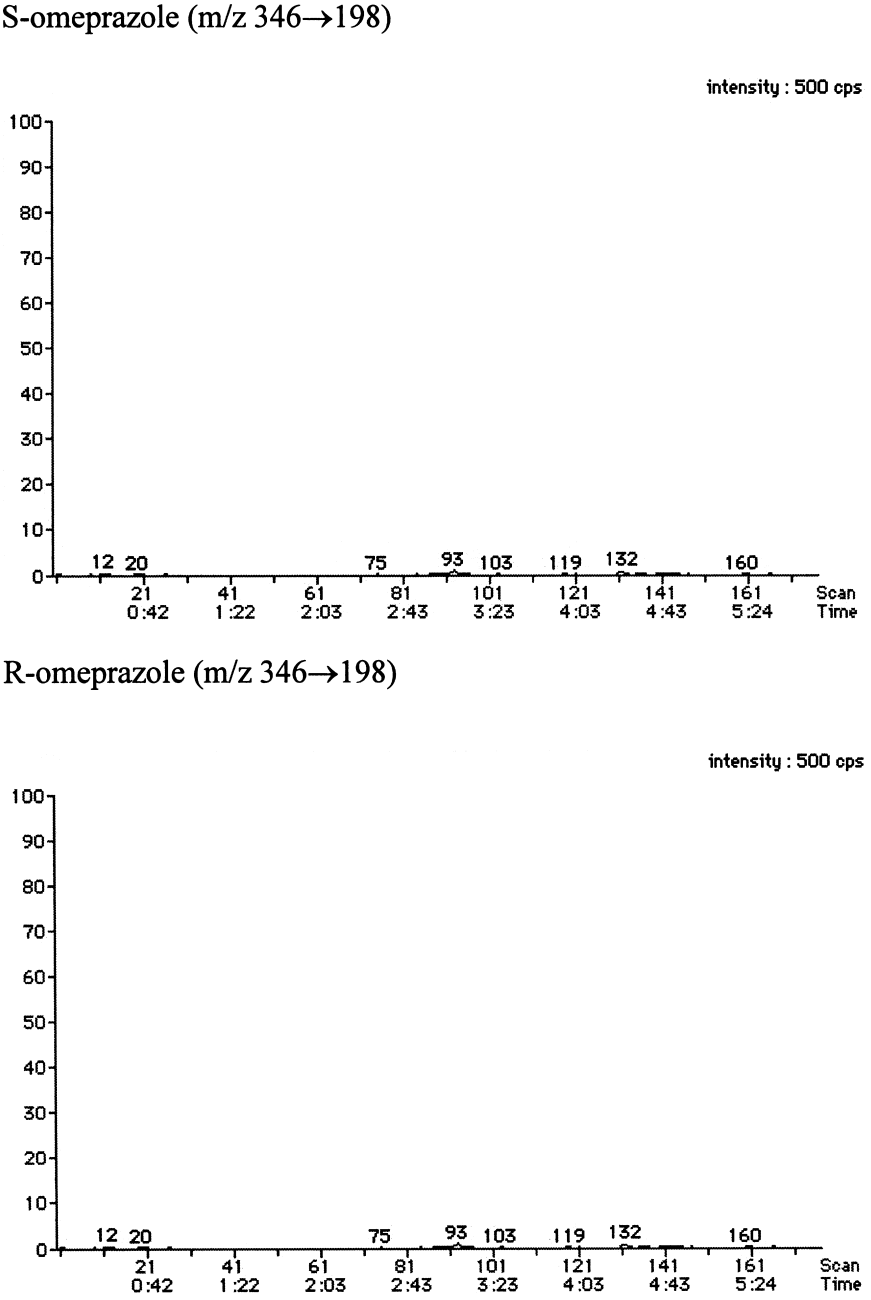


Fig. 5. Chromatogram from a blank plasma sample containing [D_4]-*S*-omeprazole and [D_4]-*R*-omeprazole, 679 nmol/l of each. Stationary phase, Chiralpak AD; mobile phase, 0.004% acetic acid in acetonitrile–ethanol–isohexane (1:30:69, v/v).

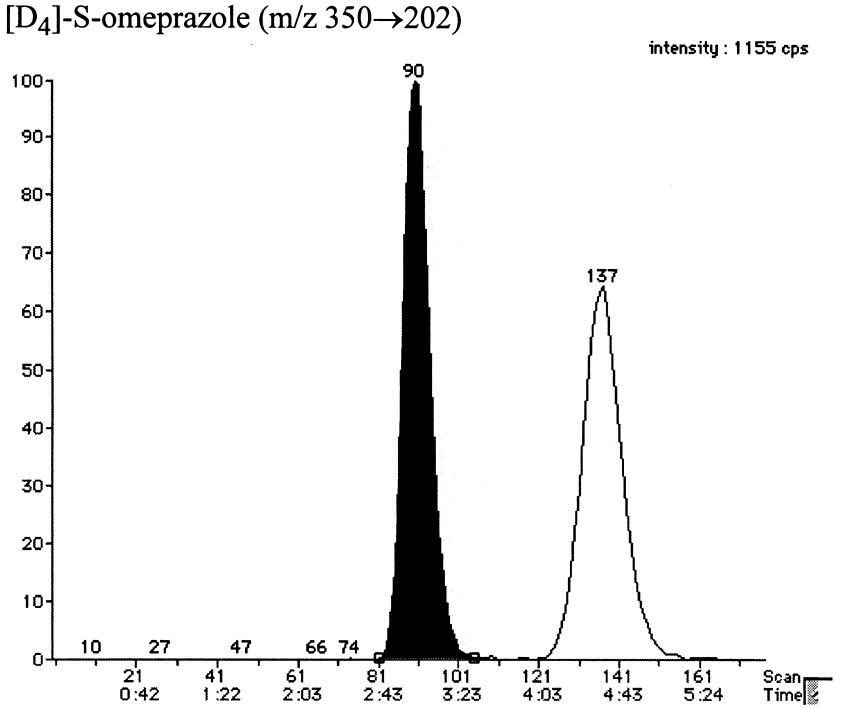


Fig. 5. (*continued*)

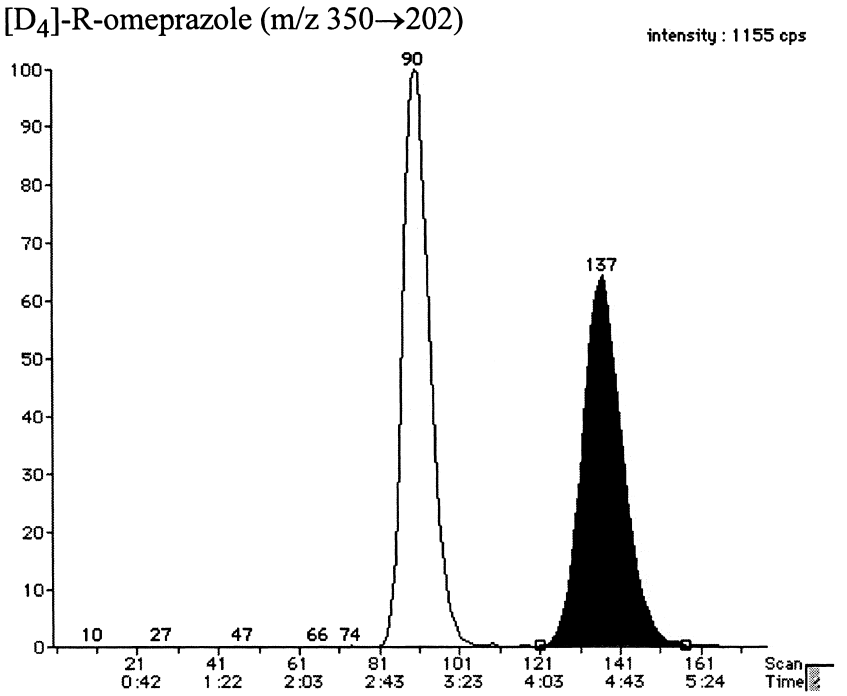
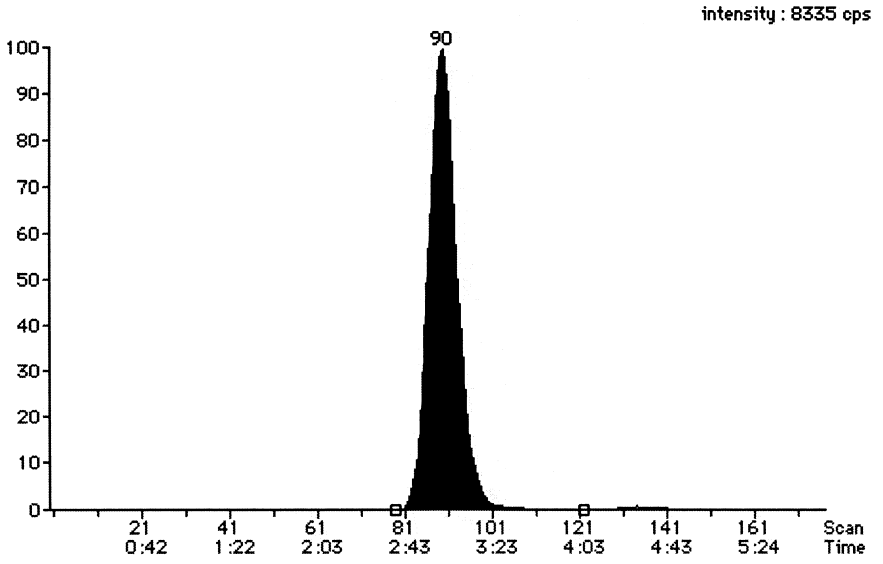


Fig. 6. Chromatogram from an authentic plasma sample containing *S*-omeprazole, *R*-omeprazole, [D_4]-*S*-omeprazole and [D_4]-*R*-omeprazole, 2360, 17, 679 and 679 nmol/l, respectively. Stationary and mobile phases as in Fig. 5.

S-omeprazole (m/z 346→198)



R-omeprazole (m/z 346→198)

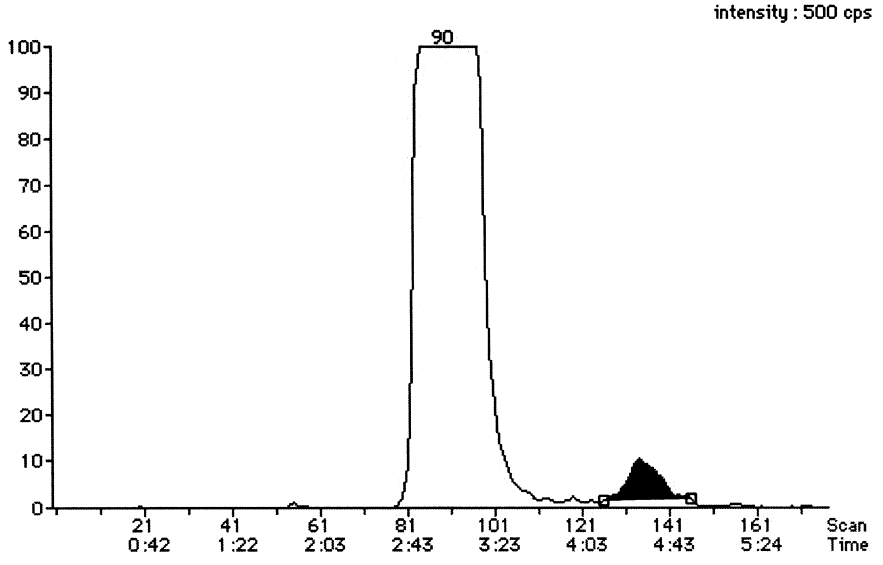


Fig. 6. (*continued*)

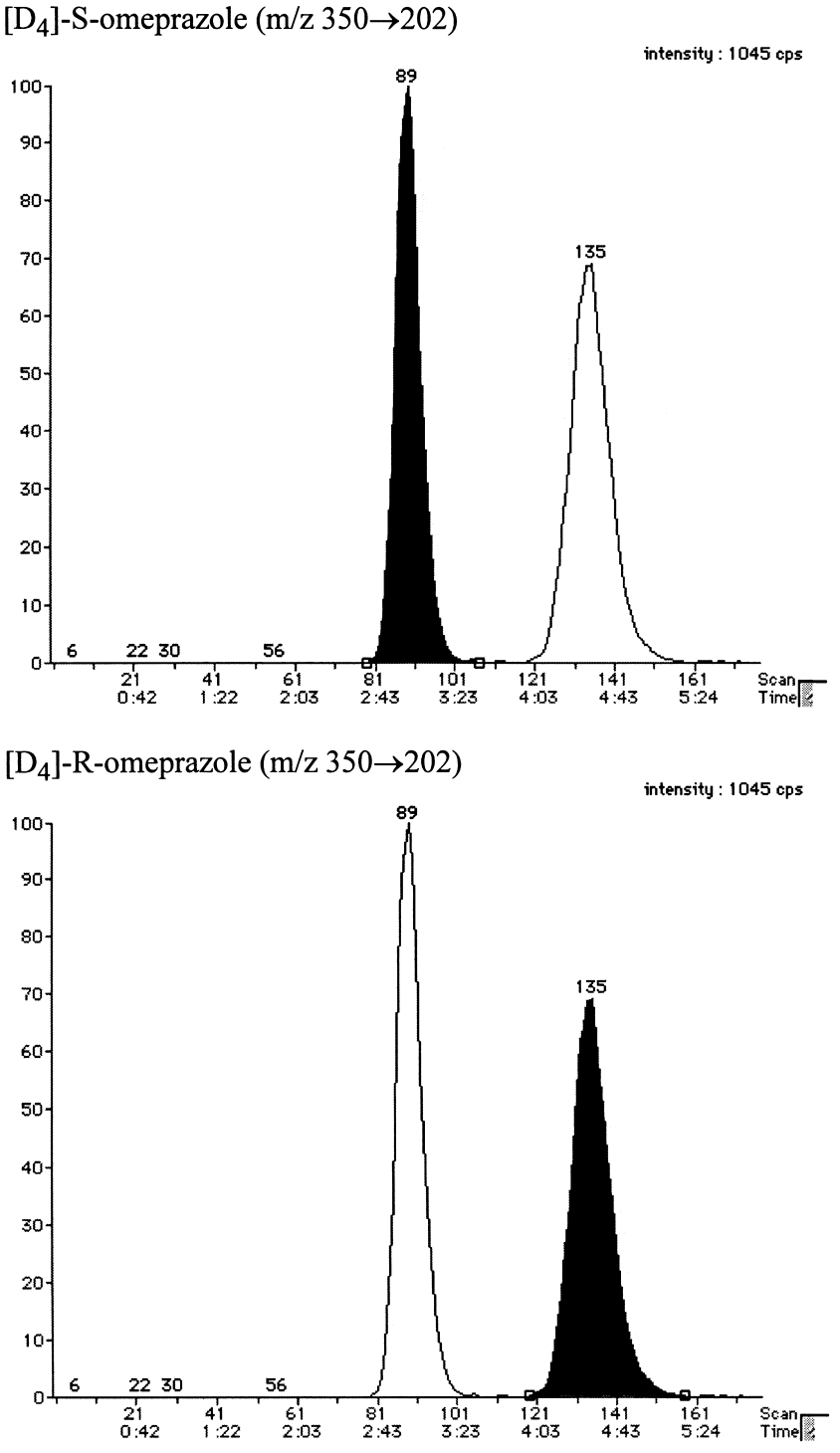


Fig. 6. (*continued*)

for drug analysis was discussed in a recent publication as regards post-column reagent addition and other aspects [13]. The mass spectra of omeprazole and its deuterated internal standard showed precursor ions at m/z 346 and 350 and product ions at m/z 198 and 202, respectively, which were used for monitoring (Fig. 2).

3.4. Quantification and accuracy

Peak areas (heights) of *S*- and *R*-omeprazole relative to those of the respective internal standards were determined by peak integration using the API standard software (MacQuan). The concentration in the unknown samples was estimated from the simultaneously analysed calibration plasma samples used for the daily calibration. Within-day precision of the analytical method was estimated using plasma samples spiked at four different concentration levels. The coefficient of variation ranged between 1.5 and 5.9% for the *S*-enantiomer and 1.4 and 11.6% for the *R*-enantiomer in the concentration range 10–3000 nmol/l. The between-day precision was estimated at 3.1 and 3.6%, respectively, for the *S*- and *R*-enantio-

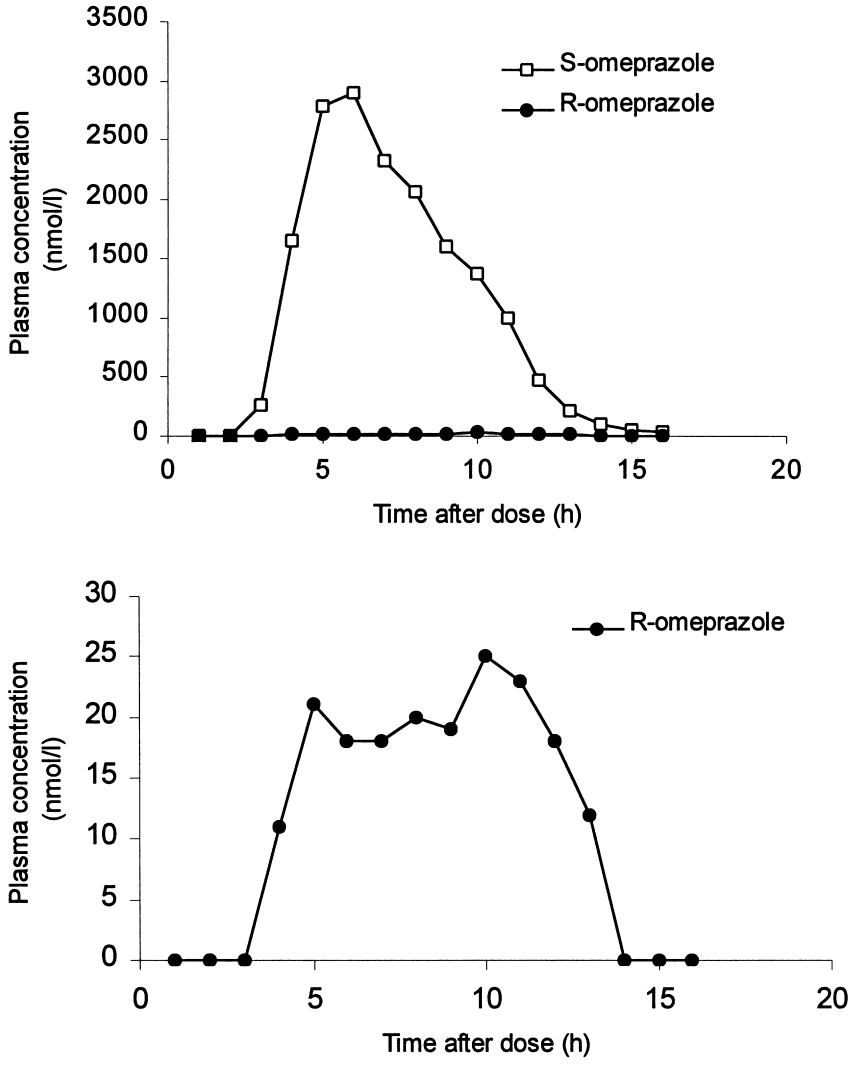


Fig. 7. Plasma curves from a volunteer given a single dose of 40 mg of *S*-omeprazole.

mers, 700 nmol/l. Limit of quantification was set at 10 nmol/l for each enantiomer (defined as the concentration where the coefficient of variation is <20%).

The daily calibration was based on at least six replicate calibration samples at one concentration besides a blank plasma sample. Full standard curves for *S*- and *R*-omeprazole in plasma comprising six different concentrations were run at least once a month during periods of routine analysis to control linearity (Figs. 3 and 4). There were three replicates at each concentration, except for the level used in daily calibration and at limit of quantification (LOQ), where eight replicates of each were assayed. The curves were found to be linear over the range of 10–3000 nmol/l for both enantiomers. Quality control samples were run daily to ensure day-to-day repeatability. No interference with the omeprazole enantiomer peaks appeared in the chromatograms and the Chiralpak AD columns showed excellent stability during the pharmacokinetic studies analysed. A chromatogram from a plasma sample taken prior to dose shows the lack of disturbances, Figs. 5 and 6 show chromatograms after a dose of *S*-omeprazole, where also a tiny peak of the R-form appears. Plasma curves from a clinical study, where *S*-omeprazole was given to volunteers, are shown in Fig. 7. A mean chiral formation obtained of the *R*-isomer was <0.5%. Besides, administered *S*-omeprazole contained 0.2% of the *R*-isomer as an impurity. As demonstrated here, the combination of the Chiralpak AD column with high enantioselectivity for omeprazole and the excellent capability of the mass spectrometer, is a most powerful tool in drug analysis.

3.5. Stability

Omeprazole in standard solution (methanol–carbonate buffer, pH 9.3, $I=0.1$ (5/95, v/v), was stable for at least 3 months at −18°C. No chiral inversion was noticed during the storage. In spiked plasma samples, omeprazole was stable for at least 96 h at +22°C and for at least 1 year at −18°C. Omeprazole in spiked plasma samples was stable during the process of repeated freezing and thawing. Omeprazole in study plasma samples was stable for at least 4 days at +22°C and in pooled, study plasma samples at −18°C for at least 11 months. Omeprazole was stable in processed study samples for at least 24 h at +22°C and for at least 7 days at −18°C.

Acknowledgements

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References

[1] S. Allenmark, B. Bomgren, H. Borén, P.O. Lagerström, Anal. Biochem. 136 (1984) 293.
[2] P. Erlandsson, R. Isaksson, P. Lorentzon, P. Lindberg, J. Chromatogr. 532 (1990) 305.
[3] I. Marle, P. Erlandsson, L. Hansson, R. Isaksson, C. Pettersson, G. Pettersson, J. Chromatogr. 586 (1991) 233.
[4] C. Vandenbosch, D. Massart, W. Lindner, J. Pharm. Biomed. Anal. 10 (1992) 895.
[5] K. Balmér, B.A. Persson, P.O. Lagerström, J. Chromatogr. A 660 (1994) 269.
[6] M. Tanaka, H. Yamazaki, H. Hakusui, Chirality 7 (1995) 612.
[7] M. Tanaka, H. Yamazaki, Anal. Chem. 68 (1996) 1513.
[8] H. Katsuki, H. Yagi, K. Arimori, C. Nakamura, M. Nakano, S. Katafuchi, Y. Fujioka, S. Fujiyama, Pharm. Res. 13 (1996) 611.
[9] A.M. Cairns, R.H.Y. Chiou, J.D. Rogers, J.L. Demetriades, J. Chromatogr. B 666 (1992) 323.
[10] A. Karlsson, S. Hermansson, Chromatographia 44 (1997) 10.
[11] G. Tybring, Y. Böttiger, J. Widen, L. Bertilsson, Clin. Pharmacol. Ther. 62 (1997) 129.
[12] P.O. Lagerström, B.A. Persson, J. Chromatogr. 309 (1984) 347.
[13] T. Alebic-Kolbali, A.P. Zavitsanos, J. Chromatogr. A 759 (1997) 65.