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Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases

Karin Balmér*, Bengt-Arne Persson and Per-Olof Lagerström

Bioanalytical Chemistry, Astra Hässle AB, S-431 83 Mölndal (Sweden)

ABSTRACT

The enantioselective separation of omeprazole on different chiral stationary phases was investigated. The two enantiomers could be resolved on three different phases with immobilized protein, Chiral-AGP, Ultron ES-OVM and BSA-DSC, employing aqueous mobile phases with 2-propanol as organic modifier. On Chiralpak AD, an amylose-based chiral stationary phase, the enantiomers of omeprazole and three analogues could be separated using a non-polar hexane–ethanol mobile phase. For omeprazole the retention order was reversed when 2-propanol was replaced with ethanol or methanol as the modifier of hexane in the mobile phase.

INTRODUCTION

The development of new chiral stationary phases for liquid chromatography has enormously enhanced the tools for the separation of stereoisomers in biomedical analysis. The interest in and the demand for studies on the pharmacokinetics of individual enantiomers after administration of drugs as racemates have rapidly increased. We have previously reported the enantioselective determination of metoprolol by liquid chromatography on Chiral-AGP [1] and Chiralcel OD [2] and also of almokalant [3], which in addition to an asymmetric carbon in the amino alcohol structure contains a sulphoxide group, giving a total of four isomers.

Omeprazole is an inhibitor of gastric acid secretion and is widely used as an antiulcer drug and against other acid-related diseases. Omeprazole is a substituted benzimidazole containing a sulphoxide group and is administered as a racemate. The two enantiomers of omeprazole and related benzimidazoles were separated by Allenmark *et al.* [4] using a stationary phase of bovine serum albumin (BSA) immobilized on silica. More recently, Erlandsson *et al.* [5] used trisphenylcarbamoylcellulose coated on 3-aminopropylsilica, Marle *et al.* [6] cellulase immobilized on diol silica and Vandenbosch *et al.* [7] three different protein-based phases in a comparative study. Gaffney *et al.* [8] resolved phenyl vinyl sulphoxide by liquid chromatography on a Chiralcel OB column and studied different alcohols as modifiers in the non-polar mobile phase.

In this study we examined the separation of the enantiomers of omeprazole on different chiral stationary phases and also of three structural analogues on one of the columns. We used three phases with immobilized protein: Chiral-AGP with α_1-acid glycoprotein, Ultron ES-OVM with ovomucoid and BSA-DSC with bovine serum albumin cross-linked into 3-aminopropylsilica using N-succinimidyl carbonate [9]. We could separate the enantiomers on all three columns and our studies were limited to the effect of pH and a comparison of two organic

* Corresponding author.

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modifiers in the aqueous mobile phase. On Chiralcel OD and Chiralpak AD, which contain cellulose and amylose tris(3,5-dimethylphenylcarbamate), respectively, coated on macroporous silica we also achieved the resolution of the enantiomers of omeprazole. The best enantioselectivity was achieved with Chiralpak AD, where interesting selectivity effects were observed by variation of the nature of the alcohol as modifier in the non-polar mobile phase. We also examined the enantioselectivity of this chiral stationary phase for a number of other benzimidazoles with different substitution patterns.

EXPERIMENTAL

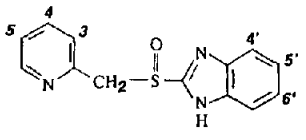
Chemicals

Omeprazole, lansoprazole, pantoprazole and timoprazole (Fig. 1) were obtained from Medicinal Chemistry, Astra Hässle, acetonitrile, hexane, methanol and 2-propanol (HPLC grade) from Rathburn (Walkerburn, UK), ethanol from Kemetyl (Stockholm, Sweden) and 1-propanol and buffer substances of analytical-reagent grade from Merck (Darmstadt, Germany). A Milli-Q system (Millipore, Molsheim, France) was used to supply purified water.

Instrumentation

The liquid chromatograph was composed of a Gynkotek 480 pump (Germering, Munich, Germany), a Kontron 460 autosampler (Tegimenta, Rotkreuz, Switzerland) and a Kratos (Ramsey, NJ, USA) Spectroflow 783 UV detector operated at 302 mn. A thermostated bath (Lauda RMS, Königshofen, Germany) was used to control the water temperature in the column jacket to 35°C if not stated otherwise. The chromatograms were processed by an SP 4400 integrator (Spectra-Physics, San José, CA, USA).

The analytical columns were Chiral-AGP, 5 μm (100 × 4.0 mm I.D.) from ChromTech (Stockholm, Sweden), Ultron ES-OVM (150 × 4.6 mm I.D.) from Rockland Technologies (Newport, DE, USA), BSA-DSC (100 × 4.6 mm I.D.), kindly supplied by Professor S. Allenmark (Göteborg, Sweden) and Chiralpak AD and Chiralcel OD (250 × 4.6 mm I.D.) from Daicel Chemical Industries (Tokyo, Japan).



	3	4	5	4'	5'	6'
I	-	-	-	-	-	-
II	CH_3	OCH_3	CH_3	-	OCH_3	-
III	CH_3	OCH_2CF_3	-	-	-	-
IV	OCH_3	OCH_3	-	-	$OCHF_2$	-

Fig. 1. Structure of analytes. I = Timoprazole: II = omeprazole; III = lansoprazole; IV = pantoprazole.

Test solutions

Standard solutions were prepared in the respective aqueous mobile phase for chromatography on the columns with a protein-bonded phase. With the Daicel columns and non-polar mobile phases the substituted benzimidazoles were dissolved in ethanol, which could be injected in small volumes (10–20 μl) without causing any peak distortion. Larger volumes, 50–100 μl, were injected after dilution with mobile phase.

RESULTS AND DISCUSSION

Most of the experiments were performed with omeprazole as the solute, as the two enantiomers of this substance were available and the elution order could be estimated. The other substituted benzimidazoles studied were included in the separation on the Chiralpak AD column in order to illustrate the resolution of compounds with different substituent patterns and to indicate the complexity but also the possibilities. The structures are shown in Fig. 1.

Protein stationary phases

The enantiomers of omeprazole could be separated on both the Chiral-AGP and the BSA-DSC column with the (+)-isomer eluting first. 2-Propanol was the organic modifier in phos-

phate buffer solution, pH 6.0–7.0 ($I = 0.1$), and the separation factor α was about 1.3 and 1.9, respectively. There was a decrease in α when the pH was increased from 6.0 to 7.0, in particular on the BSA-DSC phase. The Ultron ES-OVM column also resolved the two enantiomers, but they eluted in the reverse order, the (−)-enantiomer first, with a separation factor of about 1.2. With the Chiral-AGP stationary phase there was a major increase in α when 4% 2-propanol was replaced with 10% acetonitrile as the organic modifier in the aqueous mobile phase; α ranged from 2.0 to 2.4 and increased with increasing pH. For the analytical columns with a protein stationary phase the plate number was in the range 6000–10 000 per metre. The three stationary phases retained omeprazole to various extents, which was compensated for by the content of organic modifier as exemplified in the chromatograms in Fig. 2.

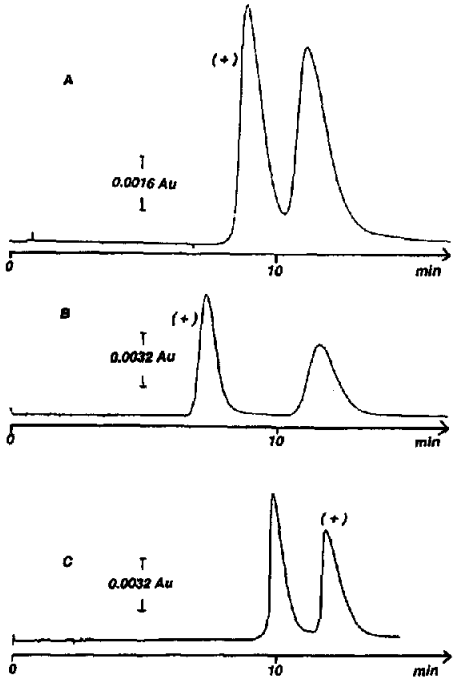


Fig. 2. Enantioselective separation of omeprazole on protein-based columns at pH 7.0 ($I = 0.1$), flow-rate 1.0 ml/min and ambient temperature. (A) Chiral-AGP, 3% 2-propanol, $\alpha = 1.26$; (B) BSA-DSC, 5% 2-propanol, $\alpha = 1.63$; (C) Ultron ES-OVM, 10% 2-propanol, $\alpha = 1.22$.

Amylose-based stationary phase

Previous attempts to achieve a baseline resolution of the enantiomers of omeprazole were not successful using cellulose-based chiral stationary phases, Chiralcel OD giving the best results of those tested and an α value of about 1.1. The corresponding amylose-based phase, Chiralpak AD, showed superior enantioselectivity for omeprazole, however, but also resolved racemates of the other sulphoxide-substituted benzimidazoles to different extents. The nonpolar mobile phase consisted of ethanol–hexane (20:80, v/v) and the capacity and separation factors are given in Table I. With this mobile phase omeprazole is resolved with an α value of 1.8 and the (−)-enantiomer is eluted first. The separation factor is even larger (3.6) for timoprazole, a compound without substituents (Fig. 1). The other two substances, drugs commercially available or in the clinical phase, with different kinds of substituents show much lower separation factors in this system.

When ethanol was replaced with 1- or 2-propanol as the modifier of hexane in the mobile phase, the retention order for omeprazole was changed and the (+)-isomer eluted first, as shown in Fig. 3. The separation factor was in the range 1.1–1.3. The chromatographic performance was lower than that with ethanol but was slightly improved by addition of water (1 g/l). An interesting effect was that the addition of water decreased the capacity factors, k', with 1-propanol as modifier but increased k' with 2-propanol as modifier. As the retention order

TABLE I

CAPACITY AND SEPARATION FACTORS FOR ENANTIOSELECTIVE SEPARATION OF SULPHOXIDE COMPOUNDS ON THE CHIRALPAK AD COLUMN

Mobile phase: ethanol–hexane (20:80, v/v).

Substance	k'_1	α
Timoprazole	3.98	3.63
Omeprazole	4.86	1.81
Lansoprazole	2.91	1.15
Pantoprazole	6.10	1.19

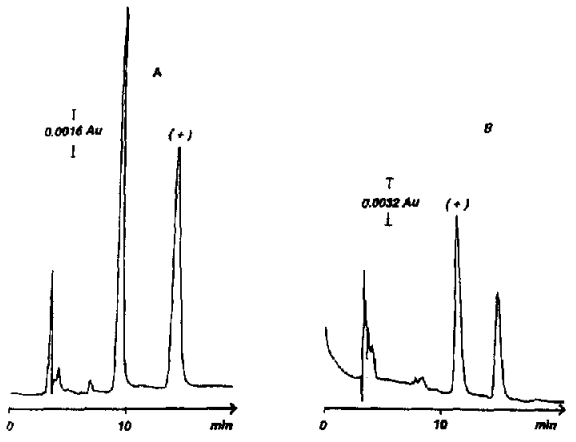


Fig. 3. Enantioselective separation of omeprazole on Chiralpak AD column, flow-rate 1.0 ml/min. (A) Mobile phase ethanol–hexane (35:65, v/v), $k'_1 = k'_{(-)} = 2.66$, $\alpha = 1.72$; (B) mobile phase 2-propanol–hexane, (14:86, v/v) $k'_1 = k'_{(+)} = 4.16$, $\alpha = 1.20$.

relative to the propanols is the opposite of that with ethanol, mixtures of these solvents in certain proportions will result into a single peak; $\alpha = 1.0$ was obtained with 4% of ethanol and 10% of one of the propanols. The addition of methanol gave the same retention order as ethanol but was only used together with 2-propanol owing to its poor solubility in hexane.

A chemometric approach was employed to study the influence of methanol on k' for the omeprazole enantiomers and to examine the retention mechanism. The design was a full factor system with the content of methanol and 2-propanol as factors and the quadratic terms were taken into consideration in the MODDE program version 2.0 from Umetri (Umeå, Sweden). The response factors were the logarithms of the k' values and the corresponding α value determined for the isomers of omeprazole. The influence of methanol and 2-propanol on $k'(+)$, $k'(-)$ and α is illustrated in Fig. 4. As can be seen, the addition of methanol reversed the retention order and α increased from 0.85 to 2.5. This enhancement of α was caused by the different influences on $k'(+)$ and $k'(-)$ by methanol; $k'(+)$ remains fairly constant with methanol addition whereas $k'(-)$ is influenced by both 2-propanol and methanol. A much more advanced study on the chiral resolution of benzimidazole sulphoxides using a multivariate statistical technique was presented recently [10].

The unsubstituted compound timoprazole showed the highest enantioselectivity in the ethanol–hexane phase (Table I). It was then of

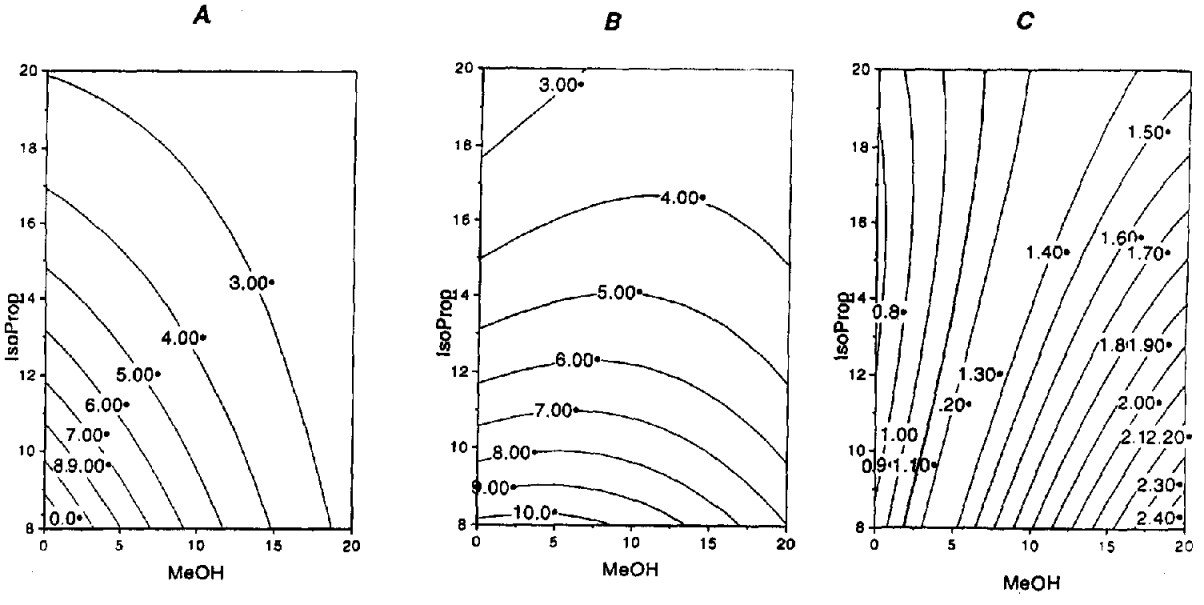


Fig. 4. Contour plots of (A) $k'_{(-)}$, (B) $k'_{(+)}$ and (C) α from the chemometric study of the isomers of omeprazole on Chiralpak AD.

interest to examine the influence of methanol addition to a mobile phase of hexane–2-propanol on the separation factor for this compound. One of the isomers of timoprazole had about the same capacity factor as the (−)-enantiomer of omeprazole and decreased in the same way when the methanol content increased. The other enantiomer of timoprazole, assumed to be the (+)-form, demonstrated a much more complex retention pattern. First it paralleled the (−)-enantiomer, giving a fairly constant α value of about 1.2, but with a further increase in methanol content the capacity factor of the (+)-isomer increased strongly. This is illustrated in Fig. 5, where the profiles of the capacity factors of timoprazole are plotted. The separation factor between the enantiomers reached a value of about 2.2 at 8% methanol in a mobile phase also containing 20% of 2-propanol in hexane. It seems that there is competition between the two alcohols as modifiers in the mobile phase. The drastic effect on the retention of one of the two enantiomers of timoprazole may be due to methanol at higher concentration displacing 2-propanol adsorbed on the chiral stationary phase. If conformational changes occurred, they were found to be completely reversible.

The different behaviours of the enantiomers of omeprazole and timoprazole show that the chiralselective stationary phase Chiralpak AD consists of different sites, as discussed in a previous paper on the separation of amino alcohols on Chiralcel OD [11]. For the (−)-antipodes of omeprazole and timoprazole there is almost no effect from methanol, which indicates that the (−)-enantiomer is not retained by the site influenced by this modifier. For the (+)-enantiomers there is a significantly different effect leading to a reversed retention order for the omeprazole isomers and a strange elution profile for timoprazole, despite the latter being an unsubstituted compound. This emphasizes the complexity of the chiral stationary phases and the difficulties of elucidating enantioselectivity effects, but also points to the possibilities of influencing the separation by components in the non-polar mobile phase. Conformational changes in the chiral stationary phase, which have been found to be reversible, mediated by low-molecular-mass alcohols, in this study and by water in previous work [11], can be considered to be responsible for the observed effects on the enantioselectivity.

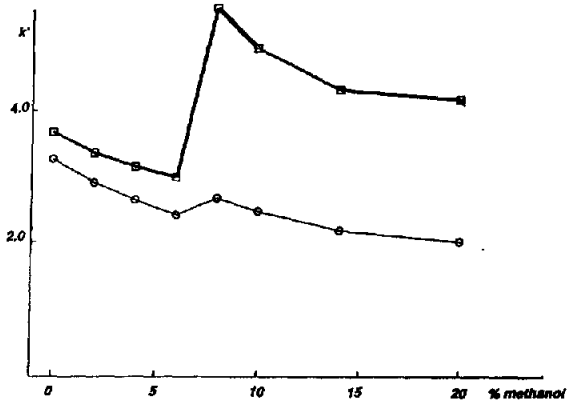


Fig. 5. Influence on the capacity factors, k', of the isomers of timoprazole of the addition of methanol to 2-propanol–hexane (20:80, v/v) mobile phase. Chiralpak AD column. □ = (+)-Isomer; ○ = (−)-isomer.

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