CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ENANTIOSELECTIVE ANALYSIS OF ZALTOPROFEN

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SUMMARY

A simple reversed-phase chiral HPLC method has been developed and validated for direct separation of the enantiomers of zaltoprofen. Separation of the enantiomers was tested on numerous commercial chiral HPLC columns. Separation was best (resolution, $R_S = 3$) on a Chiralcel OJ-RH stationary phase. Typical retention times of the S and R enantiomers were approximately 14 and 16 min, respectively. Mobile phase composition was systematically studied to find the optimum chromatographic conditions. Validation of the method under the conditions selected showed it was selective and precise and the detector response was a linear function of zaltoprofen enantiomer concentration.

INTRODUCTION

Zaltoprofen, 2-(10,11-dihydro-10-oxodibenzo[*b,f*]thiepin-2-yl)propionic acid is a potent non-steroidal anti-inflammatory drug (NSAID) [1]. It has been used clinically for treatment of post-operative pain and low back pain for more than ten years, and has recently been reported to cause potent inhibition of cyclooxygenase-2 with fewer side effects on the gastro-intestinal tract [2–4], and to induce apoptosis in a variety of cell lines [5]. Zaltoprofen is a unique compound that also has anti-bradykinin activity [6]. Its analgesic effects may be a result of inhibition of bradykinin B_2 receptor-mediated bradykinin responses not only of cyclooxygenases but also of bradykinin-induced 12-lipoxygenase inhibitors [7].

The 2-arylpropionic acids are an important group of chiral NSAIDs, most of which are marketed as racemates, even though they are known to be stereoselective in both action and disposition [8–10]. Although the prostaglandin synthetase-inhibiting effect of zaltoprofen is attributable to the S antipode, in therapeutics it continues to be used in the racemic form

[10–12]. Enantioselective quantitation of *R* and *S*-zaltoprofen in pharmaceutical formulations is, therefore, required pharmacologically and is of potential importance for clinical and pharmaceutical quality control.

EXPERIMENTAL

Chemicals

Zaltoprofen, (±)-2-(10,11-dihydro-10-oxodibenzo[*b,f*]thiepin-2-yl)propionic acid, was obtained from our research and development department (Hyderabad, India). (The structure of the compound is shown in Fig. 1) HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Sodium perchlorate and perchloric acid (70%) were purchased from Merck (Mumbai, India). HPLC water was from a Milli-Q system (Millipore, Bedford, MA, USA). Other chemicals were of analytical grade.

Instrumentation

Chromatography was performed with an 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a G1311A quaternary pump, a G1313A degasser, a G1313A autosampler, a G1316A thermostatted column compartment, and a G1314A UV-detector; data were processed by use of Chemstation software.

Chromatographic Conditions

Compounds were separated on a 150 mm × 4.6 mm, 5 μm particle size, Chiralcel OJ-RH column. The isocratic mobile phase was a 20:80 (*v/v*) mixture of 150 mM sodium perchlorate buffer (pH adjusted to 2.5 with perchloric acid) and methanol; the flow rate was 0.6 mL min^{-1}. The column was maintained at ambient temperature and the eluent was monitored at 220 nm. The injection volume was 10 μL. Different chiral columns were investigated during method development: Chiralcel OD-RH (150 mm × 4.6 mm i.d.), Chiralcel OJ-RH (150 mm × 4.6 mm i.d.), and Chiral AD-RH (150 mm × 4.6 mm i.d.) all produced by Daicel (Japan), and Chiral AGP (150 mm × 4.0 mm i.d.), Chiral HSA (150 mm × 4.0 mm i.d.), and Chiral CBH (150 mm × 4.0 mm i.d.), all produced by Chromtech (UK).

Sample Preparation and HPLC Analysis

A stock solution (1 mg mL^{-1}) of zaltoprofen was prepared in methanol. Samples needed for validation were prepared by diluting the stock solution with mobile phase.

During chromatographic analysis the properties measured were the dead time, t_0, the retention times of the first and second-eluted enantiomers, t_{R1}, and t_{R2}, respectively, the capacity factors of the first and second eluted enantiomers, k_1 and k_2, respectively, where $k = [(t_R - t_0)/t_0]$, the selectivity factor, α, where $\alpha = k_2/k_1$, and the resolution, R_S, where $R_S = 2 \times (t_{R2} - t_{R1})/(w_1 + w_2)$ where w_1 and w_2 are the widths of the two peaks.

RESULTS AND DISCUSSION

Recent global advances in new regulatory guidelines for racemic or 'pure' pharmaceutical products necessitate development of rapid, sensitive, and reproducible methods for quality control of the optical antipodes present in commercial formulations and drug substances. Liquid chromatography with chiral stationary phases is an efficient technique for quantitative separation of optical isomers. Yamamoto et al. [12] reported a normal-phase chiral HPLC method for determination of the enantiomer composition of zaltoprofen by use of a chiral cellulose column. The enantiomers were resolved ($R_S = 2.46$) by use of hexane–2-propanol–acetic acid, 90:10:2 (v/v), as mobile phase. Analysis time was over 30 min, however. The objective of this investigation was to develop and validate a simple and reliable reversed-phase HPLC method for enantioselective pharmaceutical analysis of zaltoprofen.

Van Overbeke et al. [13] reported a reversed-phase chiral HPLC method for determination of the R and S enantiomers of ibuprofen, flurbiprofen, ketoprofen, and triprofenic acid. Separation was achieved on a tris(4-methylbenzoate)cellulose column after derivatization of the compounds to form their amides. Flurbiprofen could be partially resolved into its enantiomers without derivatization. In this paper we report a reversed-phase HPLC method for determination of the R and S enantiomers of zaltoprofen without derivatization. Separation of the enantiomers of zaltoprofen was attempted on a wide range of chiral columns (listed above). The investigation was subsequently continued only with columns on which suitable separation of the enantiomers seemed possible.

Method Development

Chiral AGP (α_1-acid glycoprotein) is a plasma protein containing 181 amino acids and 14 sialic acid residues. The carbohydrate moiety of the AGP is believed to be involved in binding of basic compounds at physiological pH. Chiral separation with AGP is conducted under reversed-

phase conditions and the mobile phase is usually a mixture of aqueous buffer and organic modifier. Different buffer solutions, for example $NaClO_4$, KPF_6, KH_2PO_4, or CH_3COONH_4 can be used as mobile phases and in our work better results were achieved by using CH_3COONH_4 buffer with organic modifiers. Because tailing and broadening of peaks was observed, separations were performed with mobile phases containing different amounts of N,N-dimethyloctylamine and triethylamine. These mobile-phase modifiers did not result in improvement of the peak shapes of the zaltoprofen enantiomers. Although separation was substantial the AGP column was not considered further because of poor peak shape and the long run time (more than 65 min).

The Chiralcel OJ-RH column has a 4-methylbenzoate derivative of cellulose adsorbed on silica gel. In this work the best results were obtained by use of $NaClO_4$ mobile phases. The identity of the organic mobile phase modifier has large effect on the retention times of the enantiomers and their separation. Because small variations of the concentration of acetonitrile in the mobile phase effected both retention time and enantioselectivity, methanol was selected as organic modifier. The effect of mobile phase pH was studied in the range of 2.0–4.0 and mobile phase pH was shown to have a negligible effect on retention and selectivity. Separation of zaltoprofen enantiomers was studied using sodium perchlorate solutions of different concentration at the same pH. Although retention times of the zaltoprofen enantiomers were slightly shorter when higher concentrations of sodium perchlorate were used, increasing the ionic strength of the mobile phase had no effect on enantioselectivity.

The best separation of the enantiomers of zaltoprofen was achieved on the Chiralcel OJ-RH column with 150 mM sodium perchlorate (pH 2.5)–methanol, 20:80 (v/v), as mobile phase (Table I). A chromatogram obtained from analysis performed under these optimum conditions is shown in Fig. 1. Baseline separation of S-zaltoprofen ($k_1 = 4.21$) and R-zaltoprofen ($k_2 = 5.13$) was achieved with a total run time of 18 min. The order of elution was established by determining the optical rotation of the eluent at the respective retention times.

The effect of changes in mobile phase composition on the resolution of the enantiomers was studied by changing the methanol content in increments of 2% from 18:82 to 22:78 (v/v) 150 mM sodium perchlorate–methanol. The effect of flow rate in increments 0.1 mL min^{-1} from 0.7 to 0.5 mL min^{-1} was also studied. These studies showed the method was robust, because small changes in mobile phase composition and flow rate did not

Table I

Chromatographic data determined for separation of the enantiomers of zaltoprofen on a 150 mm × 4.6 mm i.d. Chiralcel OJ-RH column with different mobile phases. The flow rate was 0.6 mL min^{-1} except where indicated

Mobile phase[a]	k_1	α	R_S
150 mM NaClO$_4$–methanol, 20:80 (v/v), pH 2.5	4.21	1.22	2.98
150 mM NaClO$_4$–methanol, 18:82 (v/v), pH 2.5	3.43	1.22	2.99
150 mM NaClO$_4$–methanol, 22:78 (v/v), pH 2.5	4.97	1.22	3.12
150 mM NaClO$_4$–methanol, 20:80 (v/v), pH 2.4	4.07	1.21	2.90
150 mM NaClO$_4$–methanol, 20:80 (v/v), pH 2.6	4.67	1.21	3.02
150 mM NaClO$_4$–methanol, 20:80 (v/v), pH 2.5, 0.5 mL min^{-1}	3.91	1.23	3.13
150 mM NaClO$_4$–methanol, 20:80 (v/v), pH 2.5, 0.7 mL min^{-1}	3.96	1.22	2.82

[a]The pH of the sodium perchlorate buffer was adjusted with perchloric acid
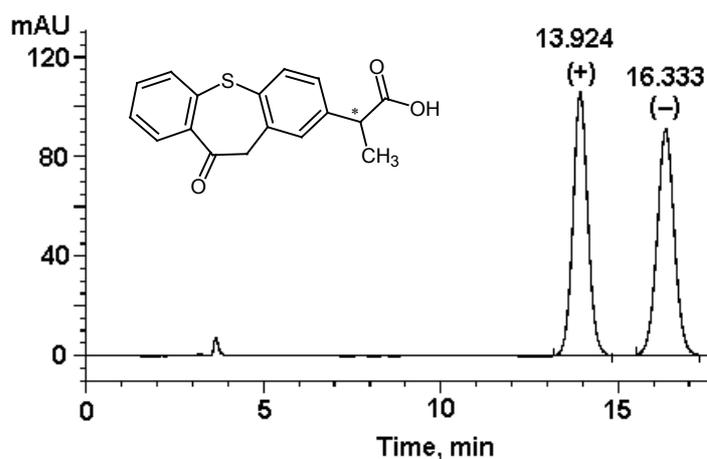

Fig. 1

Chromatogram obtained from analysis of the enantiomers of zaltoprofen on a Chiralcel OJ-RH column under the optimum conditions

significantly affect separation of the enantiomers of zaltoprofen (Table I). The selectivity of the method was also satisfactory, because baseline separation of the enantiomers was achieved. The method is, therefore, suitable for routine, accurate analysis of the enantiomer composition of zaltoprofen samples.

Method Validation

During development of the method on the Chiralcel OJ-RH column it was shown that the method was selective and robust, because the enantiomers were separated to baseline ($R_S = 2.98$) and small changes in mobile phase composition did not significantly effect the separation. The detection limit (DL), determined as the amount for which the signal-to-noise ratio was ~3:1 for both enantiomers of zaltoprofen, was 0.2 μg mL^{-1}. The quantification limit (QL) for both enantiomers was 0.6 μg mL^{-1}; this was confirmed by determination of the relative standard deviation (RSD)

(A)

y = 28.6x - 91.7
R^2 = 0.9992

(B)

y = 28.6x - 82.4
R^2 = 0.9996
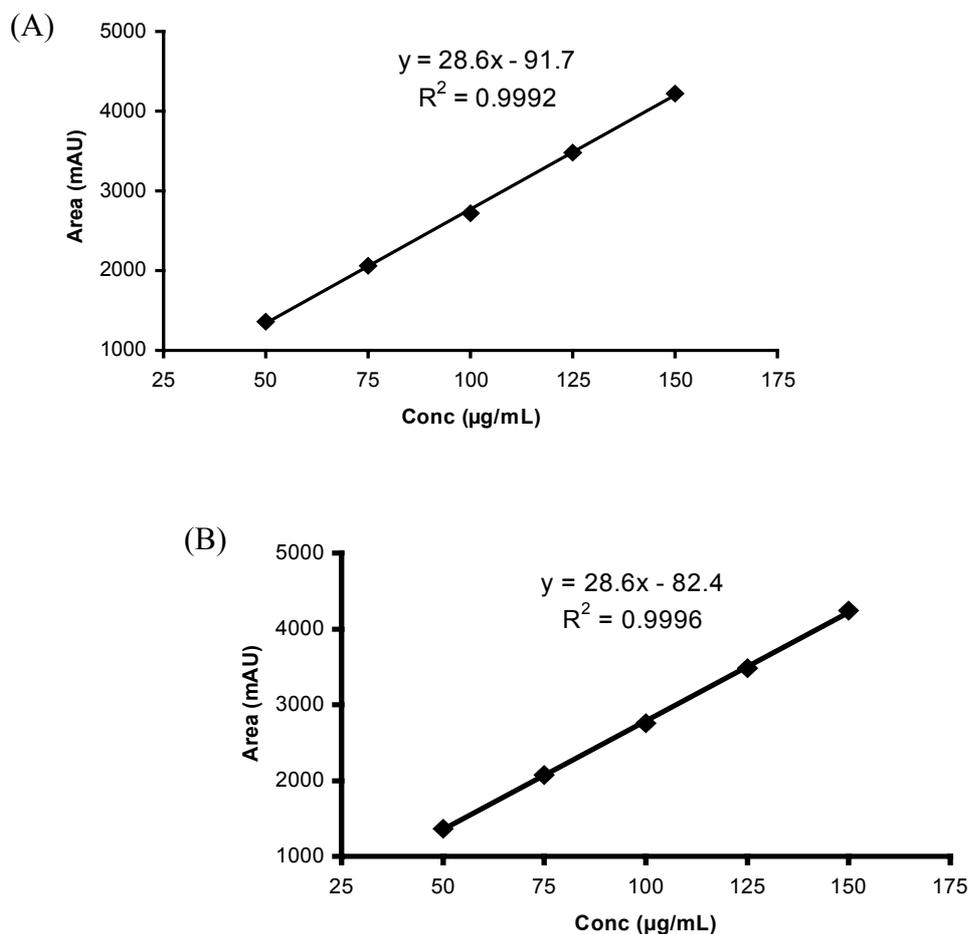
Fig. 2

Linearity of the method for determination of the isomers of zaltoprofen: (A) first-eluting enantiomer, (B) second-eluting enantiomer

for five injections of zaltoprofen solution at the previously determined quantification limit. The RSD for five injections at the QL was 0.57% for the first-eluting enantiomer and 0.69% for the second-eluting enantiomer. The repeatability (within-day precision, $n = 6$) for first and second-eluting enantiomers, respectively, as RSD, was 0.3 and 0.2%. The reproducibility (between-day precision, $n = 3$) for the first and second-eluting enantiomers, respectively, as RSD, was 1.1 and 1.5%; these results confirm the good precision of the method. The linearity of detector response was determined at five concentrations from 50–150 µg mL^{-1}. The equation of the regression line was calculated; the results are presented in Fig. 2. The correlation co-efficients for the relationships between concentration and detector response (0.9992 and 0.9996 for the first and second-eluting enantiomers, respectively) show the response was linear in the range examined.

CONCLUSION

A simple reversed-phase HPLC method for analysis of the enantiomers of zaltoprofen has been developed and validated. Use of a Chiralcel OJ-RH column with 150 mM sodium perchlorate (pH 2.5)–methanol, 20:80 (*v/v*), as mobile phase was most suitable for separation of the enantiomers. The method is linear and precise, with QL = 0.6 µg mL^{-1} for both enantiomers, and is suitable for daily direct enantioselective analysis of zaltoprofen. The method is also very robust and enables quality-control analysis of enantiomer composition and purity with large sample throughput.

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