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Short communication

Semi-preparative chiral resolution of zopiclone and *N*-desmethylzopiclone[1]

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1. Introduction

Zopiclone, a hypnotic from the cyclopyrrolone class, is commercially available as a racemate (Imovane®). Receptor binding studies have suggested that (+)-zopiclone has a 50-fold greater affinity to benzodiazepine receptors than (−)-zopiclone [1]. It is therefore of interest to measure the concentration of this hypnotic in biological species in a stereospecific way. In order to extend the radioimmunoassay (RIA) developed earlier for the racemate [2] to a stereoselective RIA procedure, the enantiomers of both the parent drug and its *N*-desmethyl metabolite were needed in a pure state (Fig. 1). Since only gram amounts of the racemates were available and since the cyclopyrrolones are very labile products in polar solvents, the preparative enantioseparation by crystallization of diastereoisomers was not considered.

Chiral resolution of enantiomers by liquid chromatography can be approached in four ways: (1) derivatization to diastereoisomers using a chiral reagent, followed by achiral chromatography; (2) addition of a chiral reagent to the mobile phase, followed by achiral chromatography; (3) direct separation of the enantiomers on a chiral stationary phase; and (4) derivatization with an achiral reagent and subsequent separation on a chiral stationary phase. A chiral stationary phase column consisting of silica gel coated with an

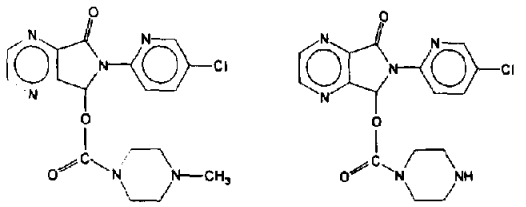

Fig. 1. Structural formulas of zopiclone and *N*-desmethylzopiclone.

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Table 1
Summary of HPLC parameters
(Retention times are given in minutes. Capacities are calculated relative to a reference peak (t_{ref}, synthesis impurity))

Parameter	Zopiclone	*N*-desmethylzopiclone
t_{ref}	7.6	8.2
$t_R(+)$	14.6	15.8
$t_R(-)$	20.1	33.7
$k'_1 = (t_R(+) - t_{ref})/t_{ref}$	0.9	0.9
α	1.74	3.44
R_s	9.8	9.8

amylose tris-methylbenzylcarbamate polymer was selected, and the enantiomeric separation was performed without prior derivatization [3].

2. Experimental

2.1. Materials and reagents

The starting compounds, zopiclone and *N*-desmethylzopiclone, were synthesized by a slightly adapted procedure as described in the patent literature for the preparation of zopiclone [4]. Diethylamine (for synthesis), 1,4-dioxane (G.R.), hydrochloric acid (32%), hexachloroplatinic (IV) acid hexahydrate (40% Pt), potassium iodide (G.R.), and the mobile phase solvents *n*-hexane and ethanol (gradient grade, LiChrosolv®) were purchased from Merck (Darmstadt, Germany).

An analytical Chiralpak AS (0.46 cm × 25 cm) column and a Chiralpak AS (0.46 cm × 5 cm) precolumn (Daicel Chemical Industries, Japan) were used. High performance liquid chromatography (HPLC) was carried out with a Merck Hitachi Model L-6002 pump, equipped with a Rheodyne injector (Model 7125; Berkeley, CA), supplied with a 500 μl sample loop. The column eluates were monitored with a Merck Hitachi L-4250 UV detector at 305 nm. Polypropylene syringe filters with a cellulose membrane (13 mm diameter, 0.2 μm pore size) were from Hewlett-Packard. Thin layer chromatography was done on Polygram Sil G/UV_{254} plates (Machery-Nagel, Düren, Germany). The acidified iodoplatinate reagent was prepared by adding 2 ml hydrochloric acid 32% to a solution of 0.25 g hexachloroplatinic (IV) acid hexahydrate (40% Pt) and 5 g potassium iodide in 100 ml of water. The specific rotation was determined on a Thorn–NPL Automatic Polarimeter Type 243 with a tungsten filament lamp and using an interference filter with a monochromatic output of 589.3 nm (sodium) and a bandwidth of 8 nm. The basic range of the instrument was $\pm 0.7°$ arc and the glass cell used had a 2 cm pathlength.

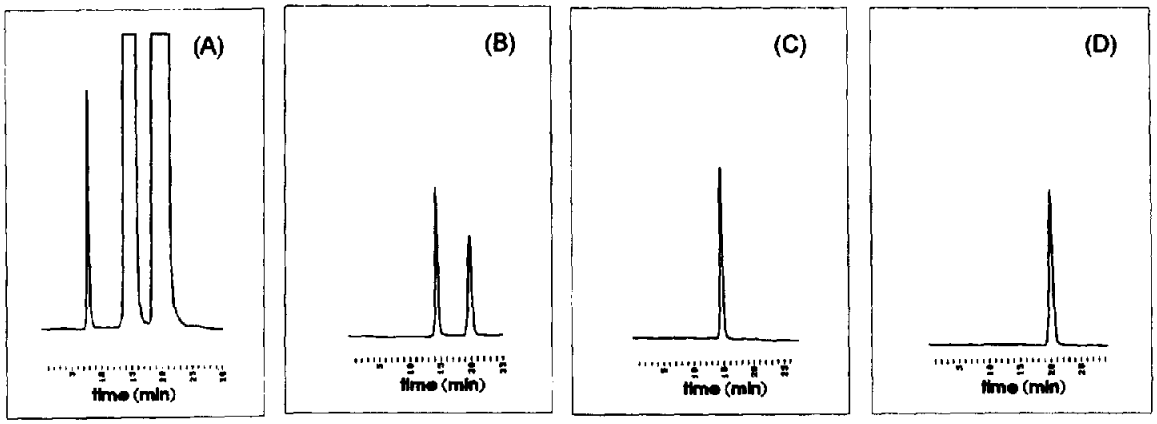

Fig. 2. The enantiomeric separation of zopiclone: typical chromatograms representing (A) a preparative load (2.4 mg of racemate per injection), (B) an analytical separation, (C) the (+)-enantiomer, and (D) the (−)-enantiomer.

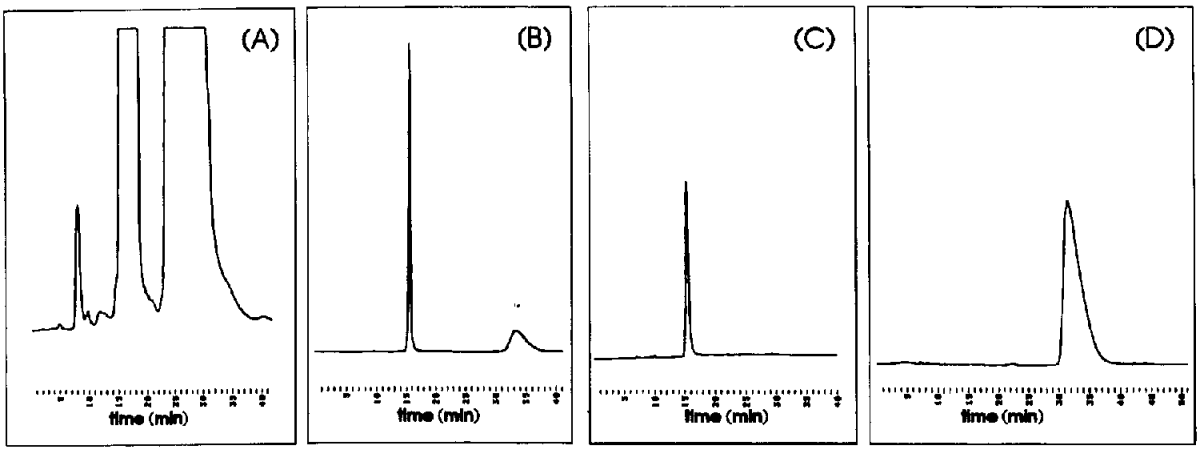

Fig. 3. The enantiomeric separation of *N*-desmethylzopiclone: typical chromatograms representing (A) a preparative load (3 mg of racemate per injection), (B) an analytical separation, (C) the (+)-enantiomer, and (D) the (−)-enantiomer.

2.2. Chiral resolution of zopiclone

A stock solution containing 1.2 mg ml^{-1} of zopiclone in dioxane was divided into 2 ml portions and evaporated to dryness under a stream of nitrogen. The racemate was dissolved in 500 μl of mobile phase [*n*-hexane/ethanol/diethylamine (60:40:0.1, v/v/v)], filtered and then immediately injected into the HPLC system. The flow rate was maintained at 0.5 ml min^{-1} and the column pressure did not exceed 33 bar. The separations were performed at ambient temperature and the column eluates were monitored at 305 nm. The peaks representing the separated enantiomers were collected and the mobile phase was evaporated immediately to prevent hydrolysis of the urethane function. When examined by thin layer chromatography on silica plates with CH_2Cl_2–MeOH (92.5:7.5, v/v) as eluent, the enantiomers were localized ($R_f = 0.3$) with longwave UV light (365 nm) and by spraying the plates with the acidified iodoplatinate reagent.

Capacity factors (k'), enantioseparation factors (α), and resolutions (R_s) were calculated and tabulated (Table 1). The t_0 value of the column was not determined, but the elution time of an impurity ($t_{ref} = 7.6$ min) was used as a reference value to calculate capacity factors. Typical chromatograms representing a preparative separation (2.4 mg load) and an analytical separation, as well as chromatograms of the separated enantiomers, are shown in Fig. 2.

To determine the specific rotation of the purified enantiomers, solutions of 2 mg ml^{-1} were made in dioxane. The first eluted compound was found to be the (+)-enantiomer ($[\alpha]_D = 129.5 \pm 2.7$, $T = 21°C$) and the second eluted peak was the (−)-enantiomer ($[\alpha]_D = -130.3 \pm 2.4$, $T = 21°C$).

2.3. Chiral resolution of N-desmethylzopiclone

A stock solution containing 1.5 mg ml^{-1} of *N*-desmethylzopiclone in dioxane was divided into 2 ml portions and evaporated to dryness under a stream of nitrogen. The racemate was dissolved in 500 μl of mobile phase [*n*-hexane/ethanol/diethylamine (55:45:0.1, v/v/v)], filtered and then immediately injected into the aforementioned HPLC system. The collected peaks were examined by thin layer chromatography on silica plates with CH_2Cl_2–MeOH (92.5:7.5, v/v) as eluent; the enantiomers were localized ($R_f = 0.08$) with longwave UV light (365 nm) and by spraying the plates with the acidified iodoplatinate reagent.

Table 1 summarizes the calculated capacity factors (k'), enantioseparation factors (α), and resolutions (R_s). Typical chromatograms representing a preparative separation (3 mg load) and an ana-

lytical separation, as well as chromatograms of the separated enantiomers, are shown in Fig. 3.

To determine the specific rotation of the purified enantiomers, solutions of 2 mg ml^{-1} were made in dioxane. The first eluted compound was found to be the (+)-enantiomer ($[\alpha]_D = 132.3 \pm 2.5$, $T = 21°C$) and the second eluted peak was the (−)-enantiomer ($[\alpha]_D = -134.5 \pm 2.4$, $T = 21°C$).

3. Conclusions

Separation of the enantiomers was accomplished by liquid chromatography using a commercially available Chiralpak AS column. The asymmetric peak shape of (−)-*N*-desmethylzopiclone in comparison with that of (−)-zopiclone shows that the interaction of stereoisomers with the chiral stationary phase is critical and often not predictable. Nonetheless, a maximum load of 3 mg per injection could be achieved for the *N*-desmethyl metabolite. In one working week, about 100 mg of the enantiomers of *N*-desmethylzopiclone and about 50 mg of the enantiomers of zopiclone were collected in a pure state.

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