JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 335–340

Received September 12, 2001
Accepted November 12, 2001
ISSN 0022-3573

Stereoselective distribution and stereoconversion of zopiclone enantiomers in plasma and brain tissues in rats

Christine Fernandez, Patrice Alet, Catherine Davrinche, Joelle Adrien, Alain Thuillier, Robert Farinotti and François Gimenez

Abstract

Concentrations of (−)-zopiclone and (+)-zopiclone were determined in plasma and brain after oral administration, to investigate the stereoselectivity of distribution in rats. Zopiclone enantiomers were administered separately to rats and concentrations were determined by chiral HPLC in plasma and brain. In initial experiments, rats were treated with urethane before cannulation for blood sampling but as this drug modified zopiclone pharmacokinetics, it was not used in subsequent studies. This study showed that zopiclone pharmacokinetics after oral gavage in rats are stereoselective. After oral administration of (+)-zopiclone, no stereoconversion was observed in plasma. Conversely, after administration of (−)-zopiclone, both enantiomers were found in plasma and brain with (+)-zopiclone/(−)-zopiclone ratios of 1 and 8.4 in plasma and brain, respectively. Our findings suggest that zopiclone undergoes stereoconversion and that it is stereospecifically distributed to the brain.

Faculty of Pharmacy, Clinical Pharmacy Department, UPRES EA 2706, Chatenay-Malabry, France

Christine Fernandez, Catherine Davrinche, Robert Farinotti, François Gimenez

Pitié Salpetrière Hospital, Pharmacy Department, Paris, France

Christine Fernandez, Patrice Alet, Alain Thuillier

Necker – Enfants Malades Hospital, Pharmacy Department, Paris, France

François Gimenez

Faculty of Medicine, Pitié Salpetrière, INSERM U288, Paris, France

Joelle Adrien

Correspondence: C. Fernandez, Pitié Salpetrière Hospital, Pharmacy Department, 47, Boulevard de l'Hopital, 75013 Paris – France. E-mail: christine.fernandez@psl.ap-hop-paris.fr

Introduction

Zopiclone (Figure 1) is a chiral cyclopyrrolone used as a short-acting hypnotic. It is usually well tolerated, with no major adverse reactions compared with those of benzodiazepines. After oral administration in humans, zopiclone is rapidly absorbed and has a bioavailability of about 80%. It is extensively metabolized, mainly through *N*-demethylation and *N*-oxidation. It has also been reported that zopiclone pharmacokinetics are stereoselective after administration to humans (Julou et al 1983; Fernandez et al 1993b; Foster et al 1994). Blaschke et al (1993) showed that, in-vitro, binding to benzodiazepine sites is stereoselective.

Several analytical methods have been reported in the literature, most of which are non-stereospecific (Otsuko et al 1995; Stanke et al 1996). Two methodologies were described for the determination of zopiclone enantiomers: a capillary electrophoresis assay and several HPLC methods involving either sequential techniques or automated column switching (Fernandez et al 1991, 1993a; Blaschke et al 1993; Koppenhofer et al 1996).

As the brain is zopiclone's site of action, we investigated the distribution of zopiclone enantiomers in the brain after administration of zopiclone enantiomers to rats.

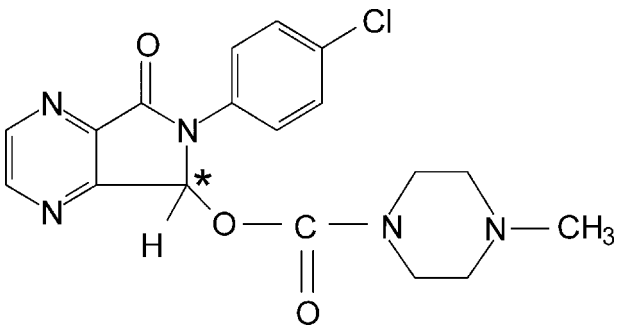

Figure 1 Chemical structure of the enantiomers of zopiclone (*position of the asymmetric carbon).

Materials and Methods

Drugs and chemicals

Racemic zopiclone was donated by Rhone-Poulenc Rorer (Vitry/Seine, France). Prazosin, the internal standard, was purchased from Sigma (Saint Quentin Fallavier, France). Sodium hydrogenophosphate and ethanol were from Merck (Nogent/Marne, France), n-hexane from Carlo Erba (Rueil Malmaison, France), isopropanol, isopentane, methylene chloride and acetic acid were from local suppliers and were all of analytical grade.

Zopiclone enantiomers were obtained by semi-preparative liquid chromatography using a Chiralcel OD column (250 × 10 mm; Daicel Chemical Industries Ltd). The mobile phase was ethanol–n-hexane (60:40, v/v) at a flow rate of 2 mL min^{-1}. The wavelength was set at 305 nm and 300 μL of 1 μg mL^{-1} racemic zopiclone was injected onto the column at each run. The fractions collected were evaporated to dryness under nitrogen and stored at −20°C up to reconstitution with acetonitrile and quantification. Enantiomer purity was assessed by liquid chromatography using an analytical chiral column (Chiralcel OD; Daicel). Enantiomeric purity was found to be 99.5% and 99.3% for (+)-zopiclone and (−)-zopiclone, respectively (Fernandez et al 1995).

Stereoconversion of zopiclone enantiomers

Stability and stereoconversion of zopiclone enantiomers were investigated in the two vehicles used, arabic gum and Tween 80, and in the two biomedia, plasma and brain. Zopiclone solutions were allowed to stand for 24 h at room temperature and at 37°C. Each solution (1 mL) was then analysed using the method described in the analytical section.

Plasma and brain pharmacokinetics of zopiclone enantiomers in rats

Adult male Sprague Dawley rats (270–330 g) were kept under standardised conditions with free access to food and water. Experiments began one week after their arrival at the laboratory.

First, to enable the collection of more than one blood sample per rat, rats were anaesthetized with urethane and a catheter was inserted in the left common jugular vein cannulated with polyethylene tubing, internal diameter 0.58 mm. The influence of urethane on zopiclone pharmacokinetics was investigated in a pilot study by analysing zopiclone concentrations after administration of 3 mg kg^{-1} of racemic zopiclone by the oral route with or without pre-treatment with urethane. Two blood samples were collected per rat (4 mL from each rat). In each group 12 rats were used, representing 24 samples taken at 8 time points: 0, 15, 30, 45, 60, 90, 150 and 180 min after treatment.

Because this pilot study indicated that the pharmacokinetics of zopiclone were modified by the anaesthetic agent, another study was conducted in which rats were not pre-treated. These rats were killed by decapitation to collect the blood and brain. Zopiclone (3 mg kg^{-1}) was administered as a solution (600 μg mL^{-1}) in arabic gum (5%) and Tween 80 (0.01%). Twenty-four rats were used for the study. Three rats were killed at each point of the kinetics and blood was collected after decapitation (15, 30, 45, 75, 90, 120, 180 and 240 min after drug administration). After decapitation, the brain was quickly removed and washed in sodium chloride 0.9%. Plasma was separated by centrifugation at 4°C. Plasma and brain samples were stored at −20°C until analysis.

In further studies, 48 rats (weighing 270–330 g) received 1.5 mg kg^{-1} of the separated enantiomers by the oral route according to the same protocol.

Analytical methods

Plasma

To 1 mL of rat plasma sample were added: 100 μL of the internal standard (1 μg mL^{-1} in water), 1 mL of 0.05 M phosphate buffer (pH 8) and 10 mL of methylene chloride–isopentane–isopropanol (50:47.5:2.5, v/v). The sample was mixed thoroughly by horizontal shaking and centrifuged at 1000 *g* for 10 min. The supernatant (organic phase) was then removed and evaporated to dryness under nitrogen at 40°C. The residue was reconstituted in 120 μL of mobile phase and injected onto the chromatographic system.

Standard concentration curves were obtained by adding known amounts of racemic zopiclone (1 μg mL^{-1} in water) in the range 0–250 ng mL^{-1} to normal plasma.

Brain

Frozen tissues were thawed at room temperature and weighed in polypropylene tubes. A volume of 100 μL of internal standard was added to each sample (whole brain). Acetic acid (0.1 N) was added, followed by homogenisation for 30 s using an Ultra Turrax homogeniser. The volume of acetic acid was calculated according to the weight of tissue and in accordance with the formula : volume of acetic acid (mL) = 3 × weight of brain (g). Then, tubes were centrifuged for 10 min at 1000 *g*. The supernatant was transferred to a glass tube and 1 mL of 1 N potassium hydrogen carbonate was added. After vortex mixing, zopiclone and internal standard were extracted with 10 mL of methylene chloride–isopentane–isopropanol (50:47.5:2.5, v/v) by horizontal shaking for 10 min and centrifuging for 10 min at 1000 *g*. The supernatant was collected and treated as for plasma.

Due to the difficulty in obtaining enough rat brain samples for standard brain curves, extraction of zopiclone was compared using calf brains and rat brains. As a pilot study has shown low concentrations in rat brain, whole brain was necessary for the quantification of zopiclone. Standard brain samples were obtained by spiking parts of the brain (1.5 g of calf brain or a whole rat brain) with a standard solution of zopiclone (1 μg mL^{-1}) to obtain concentrations ranging from 0 to 500 ng/brain sample.

Liquid chromatography

The fully automated chromatographic system consisted of an autosampler (Waters 717 plus, Waters, Milford, USA), 2 isocratic pumps (TSP P100, ThermoQuest Products, Les Ulis, France), a fluorescence detector (RF 535, Shimadzu, Touzart et Matignon, Les Ulis, France) and a switching valve (LEA, Labmetrix Technologies, Roissy, France). Data acquisition was performed using the Borwin software (JMBS Developements, Le Fontanil, France).

All analyses were carried out under isocratic conditions on a Nucleosil cyanopropyl (150 × 4.6 mm, Touzart et Matignon, Vitry/Seine, France) and a Chiralcel OD (250 × 4.6 mm, Daicel, JT Baker France, Paris, France) column.

The analytical method was similar to the one previously described by Fernandez et al (1993a). The mobile phase was prepared by adding 50 mL of n-hexane to 50 mL of ethanol and 2 mL of distilled water. The system was run at room temperature at a flow rate of 0.7 mL min^{-1} on both columns. Detection was by fluorescence with excitation at 305 nm and emission at 470 nm.

A switching system was used where the sample was injected onto the cyano-column to separate zopiclone and the internal standard from the matrix. After eluting interfering compounds, the valve was switched and zopiclone and the internal standard were eluted to the chiral column where each enantiomer and the internal standard were resolved.

Calibration curves ranged from 0 to 250 ng mL^{-1} and 0 to 500 ng g^{-1} in plasma and brain, respectively. For validation procedures, 3 control samples were analysed in plasma (25, 87.5 and 200 ng mL^{-1}) and 2 in brain (25 and 250 ng/brain). For each concentration, intraday and interday precisions were calculated from 10 and 20 samples, respectively.

Pharmacokinetic parameters

The AUCs (areas under curves) from zero to infinity were calculated by the linear trapezoidal method with extrapolation by use of the last-measured concentration and the terminal-disposition constant.

The extrapolated area was always under 20% of the total AUC.

Results and Discussion

HPLC determination of zopiclone enantiomers in plasma and brain tissue

Since zopiclone extraction was the same from calf brain and rat brain, calf brain was used for standard curves for zopiclone determinations in rat brain.

In the chromatographic conditions described previously, retention times were about 12, 15 and 18 min for prazosin (internal standard), (−)-zopiclone and (+)-zopiclone, respectively. In plasma and brain, the method was linear over the range investigated (0–250 ng mL^{-1} and 0–500 ng g^{-1}, respectively).

Plasma recoveries were 87.9 ± 3.4% and 94.4 ± 7.4% at low concentrations (25 ng mL^{-1}) for (−)-zopiclone and (+)-zopiclone, respectively and 96.5 ± 3.4% and 92.9 ± 7.5% at high concentrations (87.5 ng mL^{-1}) for (−)-zopiclone and (+)-zopiclone, respectively. Brain recoveries were 75.4 ± 6.0% and 73.3 ± 7.3% at low concentrations (25 ng g^{-1}) for (−)-zopiclone and (+)-zopiclone, respectively and 85.9 ± 5.8% and 85.3 ± 2.8% at high concentrations (125 ng g^{-1}) for (−)-zopiclone and (+)-zopiclone, respectively. For intraday and

interday precisions, mean error ranged from −12.8% to +4.4% in plasma and −12.8% to +0.2% in brain. Limits of quantification were 4 ng mL^{-1} (n = 5, CV < 12%) and 9 ng g^{-1} (n = 5, CV < 15%) in plasma and brain, respectively.

Investigation of stereoconversion of zopiclone enantiomers

No stereoconversion was observed in arabic gum–Tween solution, in plasma samples or in brain samples after one month's storage at −20°C. Similar results were previously obtained for human plasma and urine (personal data).

Influence of urethane on zopiclone pharmacokinetics

The profiles of zopiclone concentrations versus time, without and with pre-treatment with urethane, are presented in Figures 2 and 3. There was a large decrease in AUCs after urethane pre-treatment (49905 μg mL^{-1} min vs 25245 μg mL^{-1} min). This decrease could be due to a reduction of zopiclone absorption after oral administration of the drug. This phenomenon was previously described in the literature by Yuasa et al (1995) who showed that urethane can affect intestinal absorption of 5-fluorouracil by reducing transport (carrier-mediated as well as passive transport).

The stereospecificity observed after administration of racemic zopiclone, showing (+)-zopiclone concentrations to be higher than those of (−)-zopiclone, is similar that previously described in humans (Fernandez et al 1993b).

As urethane modified zopiclone pharmacokinetics, rats were not anaesthetized for further experiments and rats were treated with zopiclone and three sacrificed at appropriate times of the pharmacokinetic study (24 rats).

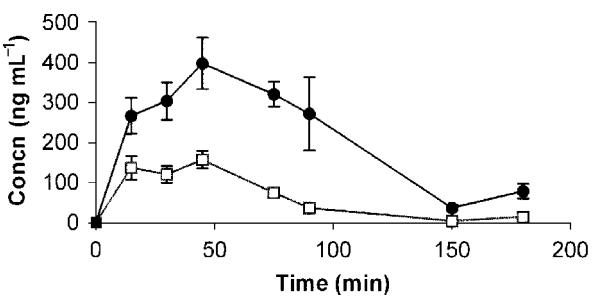

Figure 2 Plasma concentration (mean±s.d., n = 3) of zopiclone enantiomers versus time after oral administration of 3 mg kg^{-1} of racemic zopiclone to rats without pre-treatment with urethane (●, (−)-zopiclone; □, (+)-zopiclone).

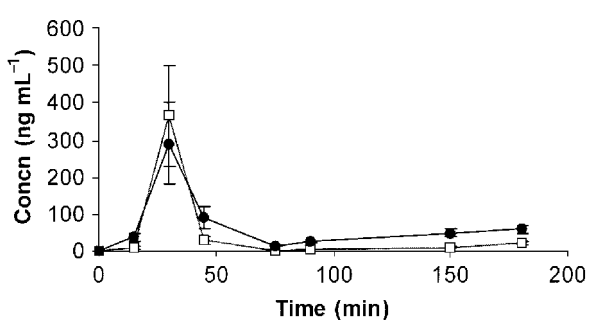

Figure 3 Plasma concentration (mean±s.d., n = 3) of zopiclone enantiomers versus time after oral administration of 3 mg kg^{-1} of racemic zopiclone to rats after pre-treatment with urethane (●, (−)-zopiclone; □, (+)-zopiclone).

Pharmacokinetics of zopiclone enantiomers in plasma

The plasma concentration versus time profiles of zopiclone enantiomers after oral administration of (−)- and (+)-zopiclone are presented in Figure 4.

Three rats were killed at each sampling time. The curve was built using the mean value of these concentrations. Hence, pharmacokinetic parameters were not calculated from this single curve and did not allow us to perform a statistical analysis.

The literature does not report any assay on zopiclone enantiomer pharmacokinetics in rats after administration of the separated enantiomers. Our results show that zopiclone pharmacokinetics are stereoselective after oral administration in rats. Furthermore, oral administra-

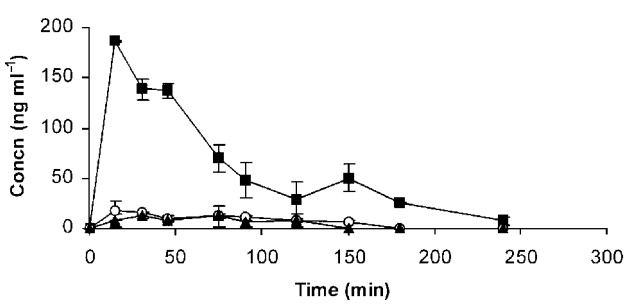

Figure 4 Plasma concentration (mean±s.d., n = 3) of zopiclone enantiomers after oral administration of zopiclone to rats (■, (+)-zopiclone concentration after administration of (+)-zopiclone; ○, (−)-zopiclone concentration after administration of (−)-zopiclone; ▲, (+)-zopiclone concentration after administration of (−)-zopiclone).

tion of (−)-zopiclone led to the appearance in blood of both (+)- and (−)-enantiomers, whereas oral dosing with (+)-zopiclone resulted in the presence of (+)-zopiclone only.

(−)-Zopiclone undergoes a chiral inversion process which does not occur with (+)-zopiclone. Previous investigations showed that zopiclone is susceptible to degradation and racemization which could occur in ethanolic solution (Fernandez et al 1995). This racemization never exceeds 10% and is similar for both enantiomers. We demonstrated that zopiclone does not stereoconvert in the mixture of arabic gum and Tween used for the oral administration in rats and does not racemize in plasma or brain tissue ex vivo, during storage. The racemization observed in our experiment is therefore an enzymatic conversion occurring in-vivo for a specific enantiomer. Disposition of (−)-zopiclone is probably influenced by one or more enantioselective processes.

Stereoconversion can occur during the absorption or the distribution phase. Such a phenomenon was described for 2-arylpropionic acids (ibuprofen (Lee et al 1985) and fenoprofen (Berry & Jamali 1991; Hall et al 1993)), stiripentol (Zhang et al 1994) and oxazepam (Yang & Lu 1989).

Hall et al (1993) demonstrated that no presystemic inversion of (*R*)- to (*S*)-ibuprofen occurred in humans. Their experiments used a rapidly absorbed dosage form. Conversely, Jamali et al (1992) evidenced that presystemic inversion could be related to the residence time in the intestine; the inversion was observed with slowly absorbed tablets but not with rapidly absorbed forms. Incubation of ibuprofen enantiomers with gastrointestinal tissue also showed a stereoconversion. The site of inversion for 2-arylpropionic acids is still a subject of debate as, for instance, Foster & Jamali (1988) demonstrated that (*R*)-ketoprofen undergoes inversion via the gastrointestinal tract. For stiripentol, the (*R*)-enantiomer is subject to a metabolic chiral inversion, leading to the (*R*)- and the (*S*)-forms when the (*R*)-enantiomer is administered by oral route to rats while administration of the (*S*)-enantiomer resulted in detection of only (*S*)-stiripentol in plasma (Zhang et al 1994).

In the case of zopiclone, a difference between zopiclone AUC values was observed in relation to the enantiomer administered. After administration of (+)-zopiclone, its concentrations were always higher than cumulative (+)- and (−)-zopiclone concentrations obtained after administration of (−)-zopiclone. In the same way, after administration of (−)-zopiclone, cumulative values of (−)-zopiclone AUC and (+)-zopiclone AUC (4.4 and 2.2 μg min mL^{-1}, respectively) were lower than that of (+)-zopiclone after administration of this enantiomer (14.4 μg min mL^{-1}).

This difference could be due to a difference between (+)- and (−)-zopiclone absorption, which could be lower for (−)-zopiclone. It could also be explained by a more extensive metabolism of (−)-zopiclone, occurring before the stereoconversion step. Liver metabolism is probably not involved in stereoconversion, as metabolic studies performed on rat isolated hepatocytes did not provide evidence of this: (−)-zopiclone and (+)-zopiclone solutions (0–388 mg L^{-1}) incubated for 6 h with rat isolated hepatocytes were metabolised into (−)-*N*-oxide zopiclone, (−)-*N*-demethyl zopiclone and (+)-*N*-oxide zopiclone, (+)-*N*-demethyl zopiclone, respectively. No stereoconversion was noted during these experiments: (−)-zopiclone and (+)-zopiclone were not converted into their antipode (personal data). Spontaneous racemization of zopiclone in the gastrointestinal tract can not be involved in this stereoselectivity because it would have affected both isomers to the same extent.

Brain–plasma partition

The brain concentration versus time profiles of zopiclone enantiomers after administration of the racemic mixture or the separated enantiomers are presented in Figure 5.

When (+)-zopiclone is administered, no (−)-zopiclone was found in the brain. Conversely, after administration of (−)-zopiclone, both enantiomers were detected in brain tissues. (+)-Zopiclone concentrations were always higher after administration of (−)-zopiclone (AUCs: 22.8 μg min g^{-1} and 2.7 μg min g^{-1} for (+)-zopiclone and (−)-zopiclone, respectively) than those obtained after administration of (+)-zopiclone

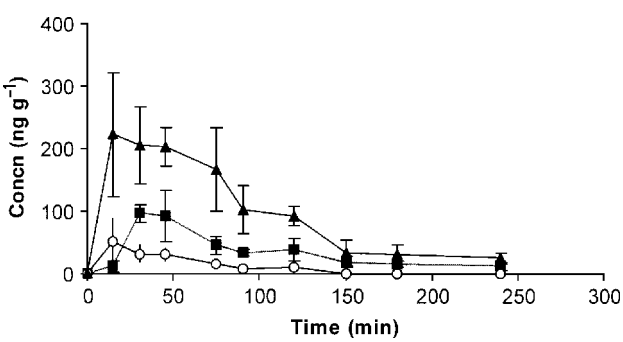

Figure 5 Brain concentration (mean±s.d., n = 3) of zopiclone enantiomers after oral administration of zopiclone to rats (■, (+)-zopiclone concentration after administration of (+)-zopiclone; ○, (−)-zopiclone concentration after administration of (−)-zopiclone; ▲, (+)-zopiclone concentration after administration of (−)-zopiclone).

(AUC: 8.0 μg min g^{-1} for (+)-zopiclone). This can only be explained by a higher (−)-zopiclone brain uptake followed by a high stereoconversion rate in-situ. Sam et al (1997) reported that *R*(−)- and *S*(−)-apomorphine concentrate in cerebrospinal fluid compared with plasma and that *R*(−)-apomorphine follows a stereoselective uptake. Van Bree et al (1990) also provided evidence of a stereoselective transport mechanism for baclofen across the brain barrier, resulting in higher brain concentrations of (*R*)-baclofen than its antipode. Yang & Lu (1989) demonstrated a stereoselectivity in hydrolysis of 3-*O*-acyl-oxazepam enantiomers by esterases in rat brain homogenates, the (*S*)-enantiomer being more rapidly hydrolysed in brain whereas its antipode is more rapidly hydrolysed in liver.

Conclusion

In conclusion, we have shown there to be a clearly defined stereospecificity and stereoconversion during brain distribution of zopiclone: (+)-zopiclone concentrations obtained after administration of (−)-zopiclone are higher than after direct administration of (+)-zopiclone. Blaschke et al (1993) have demonstrated that (+)-zopiclone has more affinity for benzodiazepine sites than its antipode. Hence, if no interspecies difference exists between rats and humans, administration of (−)-zopiclone, leading to higher brain (+)-zopiclone concentrations, would justify the use of only (−)-zopiclone in therapeutics. However, the pharmacological significance of these findings is still not clear.

References

Berry, B. W., Jamali, F. (1991) Presystemic and systemic chiral inversion of R(−)Fenoprofen in the rat. *J. Pharmacol. Exp. Ther.* **258**: 695–701

Blaschke, G., Hempel, G., Muller, W. E. (1993) Preparative and analytical separation of the zopiclone enantiomers and determination of their affinity to the benzodiazepine receptor binding site. *Chirality* **5**: 419–421

Fernandez, C., Baune, B., Gimenez, F., Thuillier, A., Farinotti, R. (1991) Determination of zopiclone enantiomers in plasma by liquid chromatography using a chiral cellulose carbamate column. *J. Chromatogr. B Biomed. Sci. Appl.* **572**: 195–202

Fernandez, C., Gimenez, F., Baune, B., Maradeix, V., Thuillier, A. (1993a) Determination of the enantiomers of zopiclone and its two chiral metabolites in urine using an automated coupled achiral-chiral chromatographic system. *J. Chromatogr. B Biomed. Sci. Appl.* **617**: 271–278

Fernandez, C., Maradeix, V., Gimenez, F., Thuillier, A., Farinotti, R. (1993b) Pharmacokinetics of zopiclone and its enantiomers in Caucasian young healthy volunteers. *Drug Metab. Dispos.* **21**: 1125–1128

Fernandez, C., Gimenez, F., Mayrargue, J., Thuillier, A., Farinotti, R. (1995) Degradation and racemization of zopiclone enantiomers in plasma and partially aqueous solutions. *Chirality* **7**: 267–271

Foster, R. T., Jamali, F. (1988) Stereoselective pharmacokinetics of ketoprofen in the rat: influence of route of administration. *Drug Metab. Dispos.* **16**: 623–626

Foster, R. T., Caille, G., Ngoc, A. H., Lemko, C. H., Kherani, R., Pasutto, F. M. (1994) Pharmacokinetics of zopiclone enantiomers in humans following oral dose administration of racemate. *Pharm. Res.* **11**: 402

Hall, S. D., Rudy, A. C., Knight, P. M., Brater, D. C. (1993) Lack of presystemic inversion of R-ibuprofen to S-ibuprofen in humans. *Clin. Pharmacol. Ther.* **53**: 393–400

Jamali, F., Mehvar, R., Russell, A. S., Sattari. S., Yakimets, W. W., Koo, J. (1992) Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: intestinal chiral inversion. *J. Pharm. Sci.* **81**: 221–225

Julou, L., Bardone, M. C., Blanchart, J. C., Garret, C., Stutzmann, J. M. (1983) Pharmacological studies on zopiclone. *Pharmacology* **27**: 46–58

Koppenhofer, B., Epperleim, U., Christian, B., Lin, B., Ji, Y., Chen, Y. (1996) Separation of enantiomers of drugs by capillary electrophoresis III beta-cyclodextrin as chiral solvating agent. *J. Chromatogr. A.* **735**: 333–343

Lee, E. J. D., Williams, K., Day, R., Graham, G., Champion, D. (1985) Stereoselective disposition of ibuprofen enantiomers in man. *Br. J. Clin. Pharmacol.* **19**: 669–674

Otsuko, K., Seto, H., Futagami, K., Oishi, R. (1995) Rapid and sensitive detection of benzodiazepines and zopiclone in serum using high-performance thin layer chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **669**: 408–412

Sam, E., Sarre, S., Michotte, Y., Verbeke, N. (1997) Distribution of apomorphine enantiomers in plasma, brain tissue and striatal extracellular fluid. *Eur. J. Pharmacol.* **329**: 9–15

Stanke, F., Jourdil, N., Lauby, V., Bessard, G. (1996) Zopiclone and zolpidem quantification in human by high liquid chromatography with photodiode-array detection. *J. Liq. Chromatogr. Relat. Technol.* **19**: 2623–2633

Van Bree, J. B. M. M., Heijligers-Feijen, C. D., de Boer, A. G., Danhof, M., Breimer, D. D. (1990) Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology. *Pharm. Res.* **8**: 259–262

Yang, S. K., Lu, X. L. (1989) Racemisation kinetics of enantiomeric oxazepams and stereoselective hydrolysis of enantiomeric oxazepam 3-acetates in rat liver microsomes and brain homogenate. *J. Pharm. Sci.* **78**: 789–795

Yuasa, H., Matsuda, K., Watanabe, J. (1995) Influence of anesthetic regimens on the intestinal absorption of 5-fluorouracil in rats. *Biol. Pharm. Bull.* **18**: 747–752

Zhang, K., Tang, C., Rashed, M., Cui, D., Tombret, F., Botte, H., Lepage, F., Levy, R. H., Baillie, T. A. (1994) Metaboic chiral inversion of stiripentol in the rat. *Drug Metab. Dispos.* **22**: 544–560