Acta Pharm. **66** (2016) 279–287 Original research paper

DOI: 10.1515/acph-2016-0013

Pharmacokinetic comparisons of *S*-oxiracetam and *R*-oxiracetam in beagle dogs

WUSAN WANG[1]
HUI JI[1,*]
TINGTING LI[1,*]
YUANWEI JIA[2]
HAITANG XIE[2]

[1] *Department of Pharmacology*
College of Pharmacy
China Pharmaceutical University
Nanjing 210000, China

[2] *Yijishan Hospital*
Wannan Medical College
Wuhu 241002, Anhui Province
China

Accepted October 27, 2015
Published online March 24, 2016

A pharmacokinetic comparison and conformational stability study of *S*-oxiracetam (*S*-ORT) and *R*-oxiracetam (*R*-ORT) in beagle dogs was used to investigate the possible mechanism of different effects of two oxiracetam enantiomers through a random crossover design. After drug administration to beagle dogs, blood samples were collected at different time points for pharmacokinetic analysis using the UPLC-ESI-MS/MS method. Parts of plasma samples were used for conformation transformation studies using a normal phase high performance liquid chromatographic (NP HPLC) method. The study showed that oxiracetam enantiomers maintained their original conformation when administered orally to beagle dogs. Concentrations of *S*-ORT were significantly higher than *R*-ORT 1.5 and 2 h after administration; the $AUC_{0-\infty}$ of *S*-ORT after oral administration tended to be higher than that of *R*-ORT, which showed that the different effects between *S*-ORT and *R*-ORT may be partly associated with their distinctive absorption at least.

Keywords: *S*-oxiracetam, *R*-oxiracetam, conformation, pharmacokinetics, beagle dogs

Oxiracetam (4-hydroxy-2-oxo-1-pyrrolidine acetamide, ORT) is a derivative of piracetam. As a nootropic agent, oxiracetam can improve both learning and memory processes and is used in the treatment of various cognitive disorders (1–4). ORT is a chiral drug; at present, the form of ORT used in clinical treatment is a racemate (Fig. 1).

It is well known that chirality is a basic characteristic of biological systems. Major components of a biological system, such as carbohydrates, proteins, amino acids and lipids, all exhibit chirality. These components are closely related to intracorporeal characteristics of drugs, such as absorption, distribution, metabolism, and excretion (5–9). Two different enantiomers may exhibit different pharmacological effects as well as different pharmacokinetics and toxicology due to the chiral features of biological systems (10–12).

* Correspondence; e-mail: cpujihui@sina.com; dreamerltt@163.com

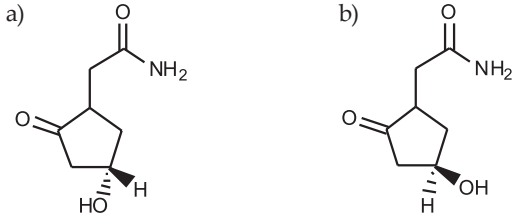

Fig. 1. Chemical structures of: a) *S*-oxiracetam and b) *R*-oxiracetam.

Two enantiomers of oxiracetam exhibit different pharmacological effects, as well. It was demonstrated that the *S* enantiomer of oxiracetam is more active than the *R* enantiomer in inducing long term potentiation (LTP) in the rat hippocampal slices *in vitro*, in potentiating glutamate-stimulated Ca^{2+} uptake in cultured cerebellar granule cells and in reverting the scopolamine induced amnesia in rats (13), suggesting that admistration of *S*-ORT would be therapeutically beneficial.

Differences in enantiomer effects may also occur from the pharmacokinetic aspect. Two enantiomers may show different pharmacokinetic features owing to their different spatial structure. Whether there were any pharmacokinetic differences between the two enantiomers of oxiracetam was not reported so far. In this paper, we carried out comparative pharmacokinetic studies of *S*-ORT and *R*-ORT in beagle dogs after studying the stereochemical stability of ORT enantiomers using a chiral separation column.

EXPERIMENTAL

Chemicals and reagents

ORT enantiomers (purity > 98 %) were provided by Chongqing Dongze Pharmaceutical Science and Technology Co. Ltd. (China), and piracetam (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). High-performance liquid chromatography (HPLC)-grade *n*-hexane was supplied by Tedia (USA). All other chemicals and solvents used were of analytical grade. The purity of all chemicals was above 99.9 %.

Animals

Six beagle dogs (3 males and 3 females), weighing 13.67 ± 2.04 kg and 6.3 ± 2.1 months of age, were purchased from the Shanghai Institute of Xingang Experimental Animal Center (China). All animal studies were approval by the Yijishan Hospital Ethics Committee (Wuhn PRC) and were carried out according to the Guide for Care and Use of Laboratory Animals of Yijishan Hospital.

Dogs were housed with unlimited access to food and water, but were fasted 12 h before the experiment. The animals were kept under a 12:12-h light-dark cycle (lights on from 8:00 to 20:00 h) at ambient temperature (22–24 °C) and 60 % relative humidity.

Study design

A random crossover experimental design was implemented to study conformational stability and pharmacokinetic differences between *S*-ORT and *R*-ORT in beagle dogs. Six dogs were divided into 2 groups. Dogs in the first group were given *S*-ORT (50 mg kg^{-1}, administered orally), while dogs in the other group were given *R*-ORT (50 mg kg^{-1}, administered orally). After a 1-week washout period, dogs in each group were switched and received the other drug.

After drug administration, 3 mL anticoagulant blood samples were collected from forelimb veins at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, and 24 h. Blood samples were centrifuged at 4000 rpm for 5 min immediately after collection to separate plasma and were stored at −70 °C until analysis.

Portions of the plasma samples of *S*-ORT and *R*-ORT collected at 0, 1, 1.5, and 2 h after oral administration were used for transformation studies. Plasma samples were used for pharmacokinetic analysis at all time points.

Enantiomer transformation

HPLC. – ORT enantiomers were separated using a Waters (USA) HPLC system equipped with a binary pump (1525), CTO-10Asvp thermostated column oven, 717 plus auto-sampler, and 2487 UV detector. Chromatographic separation was performed on a Chiralel OC Daicel (4.6 × 250 mm) column. The isocratic mobile phase, a mixture of hexane-ethanol (75:25, *V*/*V*), was delivered at 1.0 mL min^{-1}. The temperature of the column was maintained at 30 °C, the detection wavelength was 210 nm and the injected volume was 50 μL. The chromatographic run time for each sample was 35 min.

Samples and standards. – Five hundred microliters of methanol and 500 μL plasma were vortexed for 3 min. Then, 10 mL ethyl acetate was added into the mixture and vortexed for 5 min. The tubes were centrifuged at 15,000 rpm at 4 °C for 10 min. Supernatants (9 mL) were enclosed in a thermostatic water bath at 38 °C and dried with nitrogen. After drying, 100 μL of mobile phase solution was added. The tubes were then centrifuged at 15,000 rpm at 4 °C for 10 min again. Supernatants (80 μL) were pipetted into autosampler vials, 50 μL of which was injected into the column for analysis.

S-ORT and *R*-ORT, at three concentrations of 10, 50, and 100 μg mL^{-1}, were used as quality control (QC) samples. Intra-day precision was tested by analysis of QC samples (n = 6 each) at 5 different times in the same day. Interday precision (n = 6 each) was determined by repeated analyses of the same samples over 5 consecutive days. Precision was determined as relative standard deviation of peak areas.

Pharmacokinetics

HPLC. – *S*-ORT and *R*-ORT concentrations were measured using a sensitive and specific ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) method. A Shimadzu (Japan) LC system equipped with a binary pump (LC-20AD), vacuum degasser (DGu-20A5), CTO-10Asvp thermostated column oven and an SIL-HTc autosampler were used. Chromatographic separation was performed on an HP Amide LC-MS/MS column (100 mm × 3.00 mm, 5 μm). The isocratic mobile phase, a

mixture of methanol-water (85:15, *V*/*V*), was delivered at 0.2 mL min^{-1}. Piracetam was used as an internal standard (IS). The temperatures of the column and autosampler were maintained at 40 and 4 °C, respectively. The chromatographic run time for each sample was 5 min. Detection was located in the positive ion mode with multiple reaction monitoring (mass transition [*m*/*z*] 159.0→114.1 and 143.0→126.1 for ORT and piracetam).

Samples and standards. – Plasma samples were added to an Eppendorf tube spiked with IS working solution (piracetam). Samples were extracted with methanol and then centrifuged, and supernatants were pipetted into an autosampler vial for analysis. Duplicate QC samples at 3 concentration levels spanning the assay range (low, medium, and high) were included in each analytical run to measure assay performance. Analyses were performed within a concentration range of 0.05–50 μg mL^{-1} for both enantiomers (14). Inter-assay precision and accuracy were determined using QC samples from all study sample runs.

Pharmacokinetic analysis and statistics

Transformations of the enantiomers were calculated by determining the peak area ratio of *S*-ORT to *R*-ORT.

The relationship of time *versus* plasma concentration for *S*-ORT and *R*-ORT was analyzed using a Drug and Statistics computer program (version 1.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China), using a weighted least squares regression analysis. Compartment model selection was based on the Akaike Criterion. The $AUC_{0-\infty}$ was determined using the linear trapezoidal rule. The pharmacokinetic parameters and mean plasma concentrations were reported as mean ± SD and compared by Student's *t*-test using the Drug and Statistics computer program (version 1.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China). The differences were considered to be significant when *p*-values were less than 0.05.

RESULTS AND DISCUSSION

The pharmacokinetics of ORT in humans and rats has been reported in previous studies (15–18). As mentioned above, the form of ORT used in clinical treatment is a racemate of *S*-ORT and *R*-ORT. Previous pharmacodynamic studies have shown that *S*-ORT was the superior isomer (13). But until now no reports have described the pharmacokinetic differences between *S*-ORT and *R*-ORT. Because in some chiral drugs the enantiomers may be transformed into each other, the premise of our pharmacokinetic comparison was to determine whether *S*-ORT could be transformed into *R*-ORT *in vivo.* Thus, enantiomer transformation studies were necessary and critical. As the UPLC-ESI-MS/MS method could not separate enantiomers, we developed a normal phase high performance liquid chromatographic (NP-HPLC) method for chiral separation of ORT enantiomers in beagle dog plasma to study conformational transformation.

Separation of ORT enantiomers

The NP-HPLC method was developed for chiral separation of ORT enantiomers in beagle dogs' plasma to study conformational stability. Under the current chromatographic

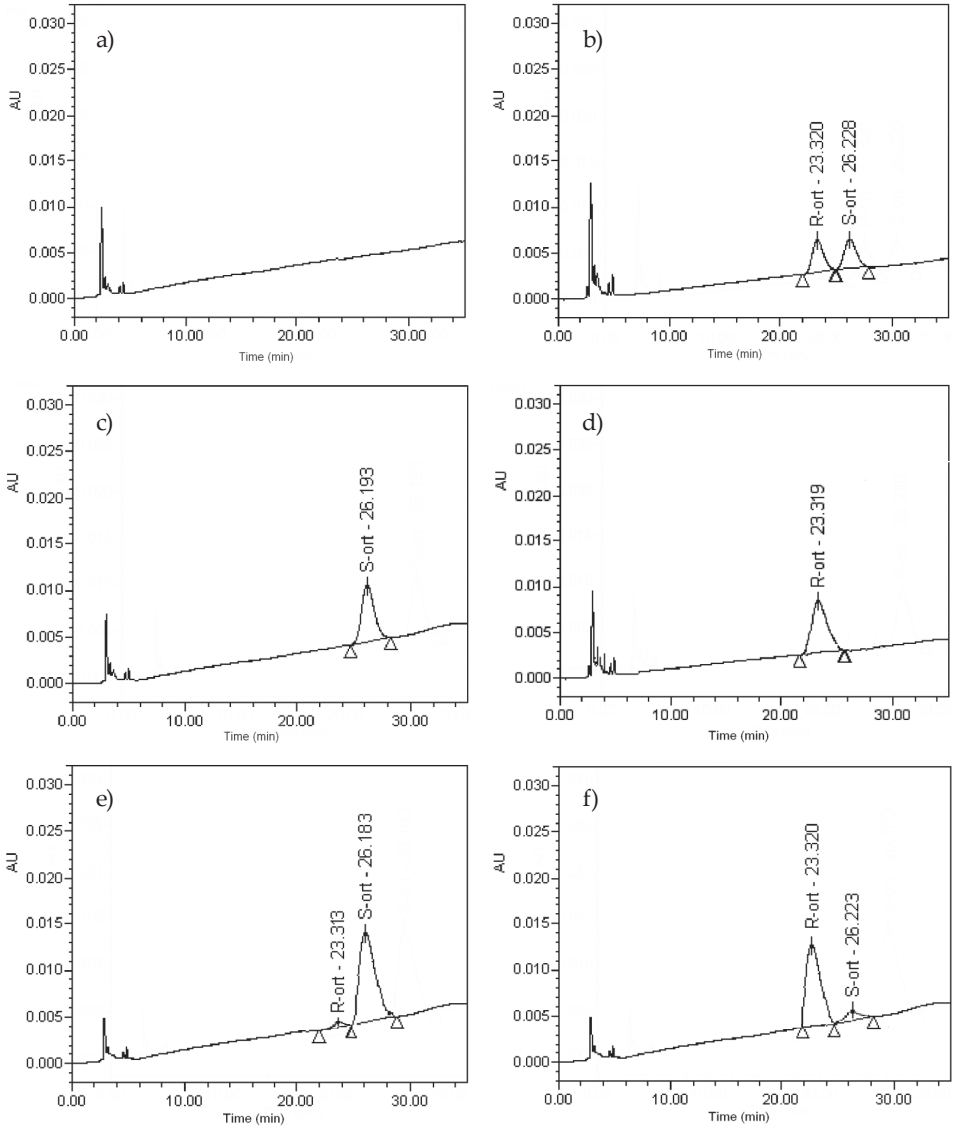

Fig. 2. NP-HPLC chromatograms of: a) blank dog plasma, b) blank dog plasma spiked with ORT racemate (10 μg mL^{-1}), c) blank dog plasma spiked with *S*-ORT (10 μg mL^{-1}), d) blank dog plasma spiked with *R*-ORT (10 μg mL^{-1}), e) plasma sample 1.5 h after oral administration of *S*-ORT (50 mg kg^{-1}), and f) plasma sample 1.5 h after oral administration of *R*-ORT (50 mg kg^{-1}).

conditions, the retention times of *R*-ORT and *S*-ORT were 23.3 and 26.2 min, respectively (Fig. 2). No endogenous interference was detected at the retention time of the peaks. The degree of separation was 1.2, the theoretical plate number was 3310 m^{-1}, which was

Table I. chromatographic parameters for the NP-HPLC method

Analyte	Capacity factor (k')	Symmetry factor	Retention time, t_R (min)	Selectivity factor	Resolution
S-ORT	9.93	1.01	26.2	1.12	1.20
R-ORT	8.76	0.98	23.3		

Table II. Precision and accuracy for determination of R-*ORT and* S-*ORT in dog plasma by the NP-HPLC method*

Analyte	Theoretical concentration (μg mL^{-1})	Concentration found (μg mL^{-1})[a]	Intraday RSD (%)[b]	Interday RSD (%)[b]	e_r (%)[b]
S-ORT	10.00	10.97 ± 0.81	4.3	8.4	9.7
	50.00	51.23 ± 3.51	2.2	6.8	2.5
	100.00	106.37 ± 9.75	4.0	6.1	6.4
R-ORT	10.00	10.85 ± 0.84	5.1	9.0	8.5
	50.00	51.62 ± 3.67	2.6	6.9	3.2
	100.00	109.10 ± 9.99	2.6	8.1	9.1

[a] Mean ± SD, $n = 5$.
[b] $n = 5$.

calculated for *S*-ORT (Table I). Intra- and inter-day precision and accuracy are presented in Table II. The results showed acceptable precision and accuracy of the analyses in dog plasma samples. At all the 3 concentrations (10, 50 and 100 μg mL^{-1}), the peak area was linear in relation to its concentration in the concentration range 5–150 μg mL^{-1}.

Stereochemical stability of ORT enantiomers

After intragastric administration of *S*-ORT and *R*-ORT at the chosen time points, the mean peak area ratio of *R*-ORT to *S*-ORT was 0.025 and that of *S*-ORT to *R*-ORT was 0.019. This indicated that only ca 2 % of ORT was converted in beagle dogs, which could be ignored. This result shaped our pharmacokinetic study of *S*-ORT.

Pharmacokinetic differences between S-*ORT and* R-*ORT in beagle dogs*

Because the sensitivity of NP-HPLC could not satisfy the requirements of pharmacokinetic research, we chose the UPLC-ESI-MS/MS method, which was already established in our laboratory (14). Due to the facts that the UPLC-ESI-MS/MS method could not separate the enantiomers and that the enantiomers did not transform into each other, this method seems to be appropriate for pharmacokinetic studies.

The time-concentration curves of *S*-ORT and *R*-ORT are depicted in Fig. 3. Both *S*-ORT and *R*-ORT concentration-time data after oral administration were best fitted as one-com-

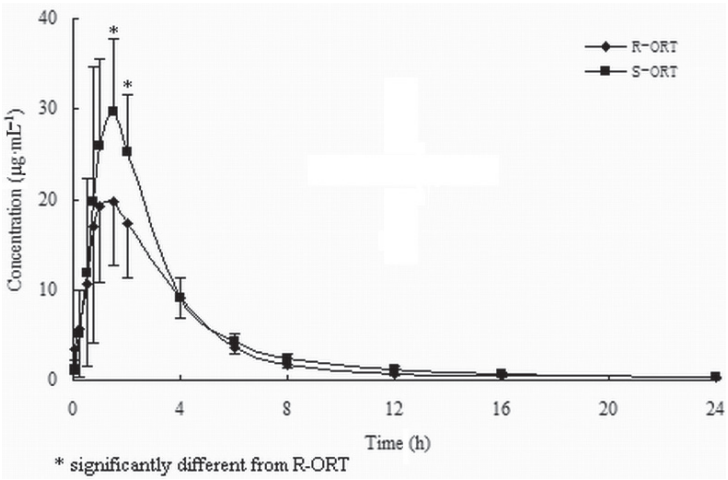

Fig. 3. Mean plasma concentration *vs*. time profiles in 6 dogs after intragastric administration of a single 50 mg kg^{-1} dose of *S*-ORT and *R*-ORT. Each point and bar represent mean ±SD, $n = 6$.

Table III. Pharmacokinetic parameters of S*-ORT and* R*-ORT given orally to beagle dogs*[a]

Parameter	*S*-ORT	*R*-ORT
$t_{1/2}$ (h)	3.33 ± 0.63	3.11 ± 0.63
$t_{1/2ka}$ (h)[b]	0.64 ± 0.33	0.51 ± 0.42
V_d (L kg^{-1})	4.09 ± 2.34	5.06 ± 2.44
Cl/F (L h^{-1} kg^{-1})	0.89 ± 0.39	1.07 ± 0.61
$AUC_{0-\infty}$ (mg L^{-1} h^{-1})	110.77 ± 19.69[c]	86.43 ± 14.97
$MRT_{0-\infty}$ (h)	4.62 ± 0.66	4.65 ± 0.87
t_{max} (h)	1.33 ± 0.26	1.79 ± 1.17
c_{max} (mg L^{-1})	31.15 ± 10.82	23.10 ± 12.31

[a] Dose: 50 mg kg^{-1}, mean ± SD, $n = 6$.
[b] Absorption half time.
[c] Significantly different from *R*-ORT ($p < 0.05$).

partment open models. In Fig. 3, at 1.5 and 2 h after administration, the *S*-ORT concentration was significantly higher than that of *R*-ORT ($p < 0.05$).

The pharmacokinetic parameters are given in Table III. The elimination half-life ($t_{1/2}$), mean resident time (*MRT*), absorption half-life ($t_{1/2ka}$), peak time (t_{max}), volume of distribution (V_d), and systemic clearance (*Cl*) of *S*-ORT were all similar to those of *R*-ORT after oral administration. In contrast, the $AUC_{0-\infty}$ of *S*-ORT after oral administration tended to be higher than that of *R*-ORT ($p < 0.05$). Having in mind the equation $AUC_{0-\infty} = FD/Cl$, one can

speculate that in the case of *AUC* changes, *Cl* and *F* are parameters that can interfere assuming the same dose. As there are no significant differences in *Cl* and $t_{1/2}$, we can conclude that parameter *F*, which depicts the extent of absorption, is significantly higher in the case of *S*-form. Moreover, this conclusion is supported by the fact that c_{max} of *S*-ORT tends to be significantly higher than c_{max} of *R*-ORT. Many reasons can lead to different absorption, such as the different destruction degree of the two enantiomers in the gastrointestinal tract, the different binding capacity to transporters, and so on. The exact reasons for different absorption of the two enantiomers of ORT need to be further studied.

As mentioned above, concentration of *S*-ORT was significantly higher than that of *R*-ORT 1.5 and 2 h after administration, and the $AUC_{0\text{-}\infty}$ of *S*-ORT after oral administration tended to be higher than that of *R*-ORT, indicating that the absorption rate of *S*-ORT was higher than that of *R*-ORT. According to the classical theory of pharmacology, higher absorption and higher concentration result in stronger effects. This result showed that the different effects of *S*-ORT and *R*-ORT (13) may be partly associated with their distinctive absorption at least. Other pharmacokinetic parameters of *S*-ORT after oral administration were very similar to those of *R*-ORT, which indicates that there were no significant differences in intracorporeal processes such as distribution, metabolism and excretion.

CONCLUSIONS

In this study, only ca 2 % of ORT enantiomers were converted to each other (*S*-ORT and *R*-ORT), which indicated that ORT enantiomers were well maintained in their original conformations in beagle dogs. There were significant differences in absorption between the two enantiomers of oxiracetam, which may resulted in different pharmacological effects. The reasons for different absorption of the two enantiomers need to be further studied.

Acknowledgements. – This research received support from the Key Clinical Medicine Application Technology Item of the Anhui Provincial Health Department (No. 2010A013).

REFERENCES

1. Z. Hlinak, J. Vinsova and E. Kasafirek, Effect of alaptide, its analogues and oxiracetam on memory for an elevated plus-maze in mice, *Eur. J. Pharmacol.* **314** (1996) 1–7.
2. Z. Hlinak and I. Krejci, Oxiracetam pre- but not post-treatment prevented social recognition deficits produced with trimethyltin in rats, *Behav. Brain Res.* **161** (2005) 213–219; DOI: 10.1016/j.bbr.2005.02.030.
3. C. Mondadori, B. Hengerer, T. Ducret and J. Borkowski, Delayed emergence of effects of memory-enhancing drugs: implications for the dynamics of long-term memory, *Proc. Natl. Acad. Sci. USA* **91** (1994) 2041–2045.
4. C. Mondadori, H. J. Möbius and J. Borkowski, The GABAB receptor antagonist CGP 36,742 and the nootropic oxiracetam facilitate the formation of long-term memory, *Behav. Brain Res*. **77** (1996) 223–225.
5. W. H. Brooks, W. C. Guida and K. G. Daniel, The significance of chirality in drug design and development, *Curr. Top. Med. Chem*. **11** (2011) 760–770; DOI: 10.2174/156802611795165098.

6. A. J. Hutt, Chirality and pharmacokinetics: an area of neglected dimensionality, *Drug Metabol. Drug Interact.* **22** (2007) 79–112; DOI: 10.1515/DMDI.2007.22.2-3.79.
7. T. Kolev, M. Spiteller and B. Koleva, Spectroscopic and structural elucidation of amino acid derivatives and small peptides: experimental and theoretical tools, *Amino Acids* **38** (2010) 45–50; DOI: 10.1007/s00726-008-0220-9.
8. H. Lu, Stereoselectivity in drug metabolism, *Expert Opin. Drug Metab. Toxicol.* **3** (2007) 149–158; DOI: 10.1517/17425255.3.2.149.
9. Q. Shen, L. Wang, H. Zhou, L. S. Yu and S. Zeng, Stereoselective binding of chiral drugs to plasma proteins, *Acta Pharmacol. Sinica* **34** (2013) 998–1006; DOI: 10.1038/aps.2013.78.
10. N. Inotsume and M. Nakano, Stereoselective determination and pharmaco-kinetics of dihydropyridines: an updated review, *J. Biochem. Biophys. Methods* **54** (2002) 255–274; DOI: 10.1016/S0165-022X(02)00120-3.
11. R. Mehvar and D. R. Brocks, Stereospecific pharmacokinetics and pharmacodynamics of beta-adrenergic blockers in humans, *J. Pharm. Pharm. Sci.* **4** (2001) 185–200.
12. L.A. Nguyen, H. He and C. Pham-Huy, Chiral drugs: an overview, *Int. J. Biomed. Sci.* **2** (2006) 85–100.
13. L. Chiodini, G. Pepeu, *Composition Comprising S-oxiracetame for Use as Nootropic*, EP. WO 1,993,006,826 A1, 15 April 1993; ref. *Chem. Abstr.* 119 (1993) 139083.
14. W. S. Wang, H. Ji, H. T. Xie, M. Dai, Y. W. Jia, D. H. Liang, L. Ye and Z. Y. Rong, A sensitive and specific UPLC–MS/MS analysis and preliminary pharmacokinetic characterization of S-oxiracetam in beagle dogs, *Chin. J. Clin. Pharmacol Ther.* **17** (2012) 988–994.
15. J. B. Lecaillon, J. P. Dubois, H. Coppens, T. Darragon, W. Theobald, G. Reumond and H. Beck, Pharmacokinetics of oxiracetam in elderly patients after 800 mg oral doses, comparison with non-geriatric healthy subjects, *Eur. J. Drug Metab. Pharmacokinet.* **15** (1990) 223–237.
16. E. Perucca, A. Albrici, G. Gatti, R. Spalluto, M. Visconti and A. Crema, Pharmacokinetics of oxiracetam following intravenous and oral administration in healthy volunteers, *Eur. J. Drug Metab. Pharmacokinet.* **9** (1984) 267–274.
17. E. Perucca, J. Parini, A. Albrici, M. Visconti and E. Ferrero, Oxiracetam pharmacokinetics following single and multiple dose administration in the elderly, *Eur. J. Drug Metab. Pharmacokinet.* **12** (1987) 145148.
18. X. Wan, H. Wang, P. Ma, L. Xi, J. Sun, Z. He, X. Zhang and X. Liu, Simultaneous determination of oxiracetam and its degraded substance in rat plasma by HPLC–MS/MS and its application to pharmacokinetic study after a single high-dose intravenous administration, *J. Chromatogr. B.* **969** (2014) 95–100; DOI: 10.1016/j.jchromb.2014.07.041.