Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 657–661
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Rapid quantitative analysis of oxiracetam in human plasma by liquid chromatography/electrospray tandem mass spectrometry

Junghyun Son, Jaeick Lee, Mijin Lee, Eunyoung Lee, Jung Hwa Lee, Sookie La, Dong-Hyun Kim*

Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, P.O. Box 131, Chungryang, Seoul, South Korea

Received 9 February 2004; accepted 25 July 2004
Available online 11 September 2004

Abstract

A rapid and accurate reversed-phase liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the quantitative determination of oxiracetam in human plasma. Oxiracetam, a highly polar compound, was successfully retained by Atlantis™ dC18 reversed-phase column and detected with triple-quadrupole tandem mass spectrometry. After addition of internal standard (piracetam) to human plasma, plasma was simply precipitated with two volume of acetonitrile, evaporated and dissolved in 0.1% acetic acid. This method for the determination of oxiracetam was accurate and reproducible, with a limit of quantitation of 0.2 μg/ml in human plasma. The standard calibration curve for oxiracetam was linear (r^2 = 0.999) over the concentration range 0.2–40.0 μg/ml in human plasma. The intra- and inter-day precision over the concentration range of oxiracetam was lower than 8.3% (relative standard deviation, %R.S.D.), and accuracy was between 92.5 and 106.4%.

Keywords: Oxiracetam; Pharmacokinetics; LC–MS/MS; Multiple reaction monitoring

1. Introduction

Oxiracetam (4-hydroxy-2-oxo-1-pyrrolidine acetamide), belongs to the class of highly polar achromophoric monohydroxy investigational nootropic drugs [1,2], is a compound effective in improving learning and memory [3,4]. The analytical tools developed for the determination of oxiracetam in biological matrices include normal-phase liquid chromatography with UV detection [5], reversed-phase liquid chromatography with fluorimetric detection combined with liquid phase extraction [6], and column-switching high-performance liquid chromatography [7]. These reported methods, however, are not adequate for pharmacokinetic studies due to relatively lower selectivity and sensitivity of short-wavelength UV detection system and complex sample pretreatment. Therefore, a simple and sensitive analytical tool for oxiracetam in human plasma is required to overcome such restrictions.

Recently, a tandem mass spectrometry has been widely used for the determination of various compounds because of its inherent accuracy and excellent sensitivity and selectivity [8]. Considering these points, an advanced reversed-phase LC–MS/MS technique combined with rapid sample preparation by simple precipitation seems to be the best candidate for the quantitation of oxiracetam. To our knowledge, no methods have been reported to determine oxiracetam in human plasma by reversed-phase LC–MS/MS with multiple reaction monitoring.

This paper describes a simple and sensitive LC–MS/MS technique for the quantitation of oxiracetam in human plasma. The method includes rapid sample preparation of oxiracetam from human plasma and reversed-phase HPLC chromatographic separation with detection by multiple reaction monitoring.

* Corresponding author. Tel.: +82 2 958 5055; fax: +82 2 958 5059.
E-mail address: dhkim@kist.re.kr (D.-H. Kim).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.07.031

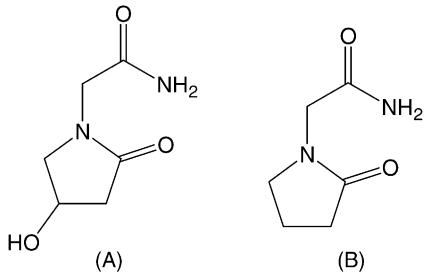

Fig. 1. Structures of (A) oxiracetam and (B) piracetam (internal standard).

2. Materials and methods

2.1. Materials

Oxiracetam reference standard and the internal standard piracetam (Fig. 1) were purchased from Sigma Chemicals (St. Louis, MO, USA). Human plasma was obtained from Korea Blood Bank Corp. (Seoul, Korea). Methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade and used as received. HPLC-grade water was prepared using a Milli-Q purification system (Millipore; Bedford, MA, USA). High-purity nitrogen (99.9999%) was purchased from Shin Yang Gas Co. (Seoul, Korea).

2.2. Standard solutions

Standard stock solutions of oxiracetam and piracetam were made up at 1.0 mg/ml in methanol as their free forms. They were further diluted with methanol to obtain working standard solutions at several concentration levels. The calibration curves were obtained using eight calibration standards, i.e. spiked plasma samples prepared by addition of the stock solution to blank human plasma giving final concentrations of oxiracetam of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 μg/ml.

2.3. Sample extraction

Piracetam (20 μl of 10 μg/ml) was added as an internal standard to 200 μl of plasma sample and 400 μl acetonitrile was added to each human plasma and blank samples. The samples were vortexed for 30 s and centrifuged for 5 min at 13,000 rpm. The supernatant was taken and evaporated to dryness under nitrogen gas in a TurboVap evaporator (Zymark, Hopkinton, MA, USA). The dried residue was dissolved in 100 μl of 0.1% acetic acid and 4.0 μl was injected into the LC–MS/MS system.

2.4. Reversed-phased liquid chromatography–mass spectrometry

The HPLC was performed using LC-10ADvp binary pump system, SIL-10ADvp autosampler and CTO-10ASvp oven (Shimadzu, Kyoto, Japan). The analytical column was an Atlantis^{TM} dC18 (50 mm × 3.0 mm i.d., 3 μm, Waters, Mass, USA). The HPLC mobile phases consisted of 0.1% acetic acid (A) and 100% methanol (B). The flow rate was 0.3 ml/min. Separations were conducted using a gradient: 1% (B) for 1 min, followed by a linear increase to 80% B over 1 min, then maintained for 1 min. The column was re-equilibrated to initial condition for 1.5 min.

The HPLC was coupled to an API2000 triple-quadrupole mass spectrometry (Applied Biosystems SCIEX, Concord, Canada) equipped with a Turbo Ion Spray source. Electrospray ionization (ESI) was performed in the positive mode with nitrogen as the nebulizing, turbo spray and curtain gas with the optimum value set of 40, 80, and 40 (arbitrary values). The heated nebulizer temperature was set at 425 °C. The mass spectrometer operated with low and unit resolution for Q1 and Q3, respectively. Multiple reaction monitoring (MRM) detection was employed using nitrogen as the collision gas (4 arbitrary value) with a dwell time of 150 ms for each transition, monitoring the transition of the protonated molecular ion *m/z* 159 and 143 to their corresponding product ion *m/z* 114 and 126 for oxiracetam and piracetam (IS), respectively. Collision energy was set to be 19 and 13 eV for oxiracetam and piracetam, respectively. The data acquisition was ascertained by Analyst 1.3.1 software.

2.5. Calibration and validation

The calibration curves for oxiracetam in human plasma were generated by plotting the peak area versus the concentrations in the standard spiked plasma samples by least-square linear regression. The calibration curves consisted of eight calibration standards and each standard was prepared in triplicate. Intra-day coefficient of variation (CV) and accuracy of the method were evaluated by the analysis of five plasma samples spiked with 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 μg/ml. The CV and accuracy for inter-day assay were assessed at the same concentration, and repeated for five different days.

2.6. Pharmacokinetic parameters

The pharmacokinetic parameters were determined using standard non-compartmental methods. Plasma AUC was calculated using WinNonlin (version 3.1, Scientific Consulting, KY, USA) with a log-linear trapezoidal method.

3. Results and discussion

3.1. Sample preparation and analysis

Rapid sample analysis is especially of importance for the studies that require a large number of sample analyses such as pharmacokinetic investigation. The developed analytical tools for oxiracetam require a complex sample preparation

due to its polarity and low UV responsiveness. Recovery of oxiracetam after solvent extraction or solid-phase extraction was relatively low and separation of this compound from endogenous compounds was difficult in ultraviolet absorption detection. Due to high selectivity and sensitivity of MS/MS system, a simple acetonitrile precipitation can be used with the highest recoveries than any other preparation (data not shown). In addition, the present extraction method provided excellent closeness of agreement between intra- and inter-batch data.

Compounds of high polarity show almost no retention on reversed-phase columns, and normal-phase chromatography should be used. Moreover, aqueous solutions cannot be injected into polar bonded-phase columns as they lead to excessive band broadening. This problem can be solved by using Atlantis^{TM} dC18 column. Atlantis^{TM} dC18 column is a silica-based line of difunctionally bonded C18 columns that provide the optimal balance of retention for polar and non-polar compounds in reversed-phase chromatography. High-polarity oxiracetam was successfully retained in our systems at 1.3 min without ion suppression. Therefore, a gradient mobile phase condition (from 20 to 80%, for 4.5 min) resulting in a better chromatographic properties and shorter run time for the simultaneous determination of oxiracetam.

The product ion mass spectra and its postulated fragmentation patterns of oxiracetam, and piracetam are illustrated in Fig. 2. Loss of amine group of the protonated molecule of oxiracetam (*m/z* 159) and piracetam (*m/z* 143) yielded a fragment at *m/z* 142 and 126, respectively. The loss of carbonyl moiety from the fragment ion at *m/z* 142 and 126 resulted in fragment ions at *m/z* 114 and 98, respectively. Among these product ions, the most abundant ions (*m/z* 114 for oxiracetam and *m/z* 126 for piracetam) were selected for MRM analysis.

The specificity and selectivity of the method were investigated by preparing and analyzing human plasma blanks from four different batches of pooled human plasma. No interference was observed in drug-free plasma samples (Fig. 3A) at the retention times of oxiracetam and piracetam. In addition, the carry-over effect was not observed in our system. A significant carry-over effect was noted when acetonitrile was used as organic modifier. Fig. 3B shows the MRM chromatograms

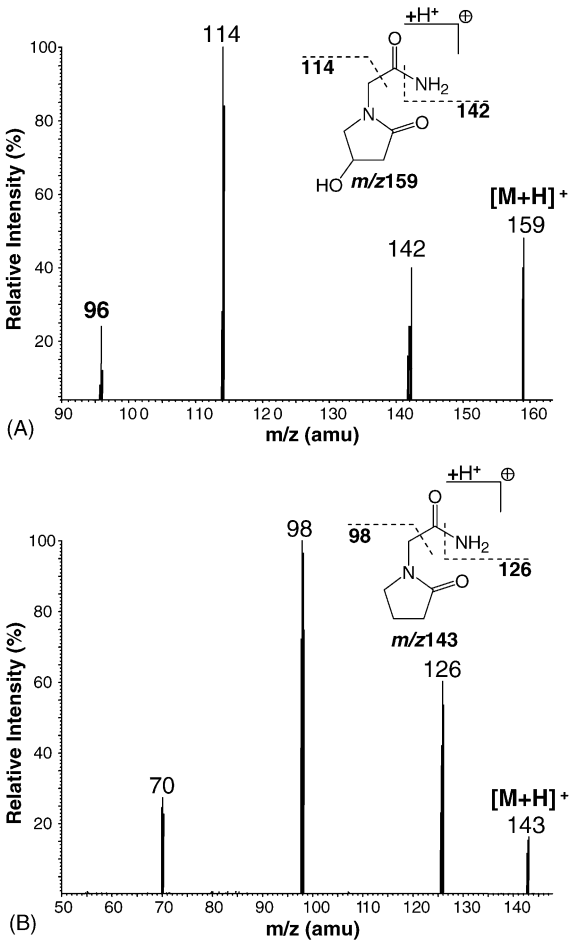

Fig. 2. Product ion mass spectra of $[M + H]^+$ ions of (A) oxiracetam and (B) piracetam (IS).

obtained by the analysis of the plasma spiked with 10 μg/ml oxiracetam and 1.0 μg/ml internal standard.

3.2. Method validation

The calibration curve was constructed using ten different concentrations and processed by weighted least-square linear regression analysis. The calibration for oxiracetam was linear

Table 1
Intra- and inter-day coefficient of variation and accuracy for determination of oxiracetam in human plasma

Theoretical concentration (μg/ml)	Intra-day			Inter-day		
	Concentration found (μg/ml) (mean ± S.D.)	CV (%)	Accuracy (%)	Concentration found (μg/ml) (mean ± S.D.)	CV (%)	Accuracy (%)
0.2	0.19 ± 0.02	8.3	92.5	0.20 ± 0.01	3.8	98.2
0.5	0.50 ± 0.03	5.3	99.9	0.47 ± 0.02	4.4	93.6
1.0	1.06 ± 0.06	6.1	106.3	0.99 ± 0.06	5.6	99.0
2.0	2.13 ± 0.08	3.8	106.4	2.01 ± 0.11	5.5	100.3
5.0	5.07 ± 0.11	2.2	101.3	4.82 ± 0.13	2.8	96.3
10.0	11.1 ± 0.3	2.3	110.5	10.4 ± 0.4	3.6	103.8
20.0	20.7 ± 0.6	2.9	103.7	19.4 ± 0.6	3.3	96.9
40.0	39.7 ± 1.0	2.5	99.4	38.1 ± 1.6	4.2	95.4

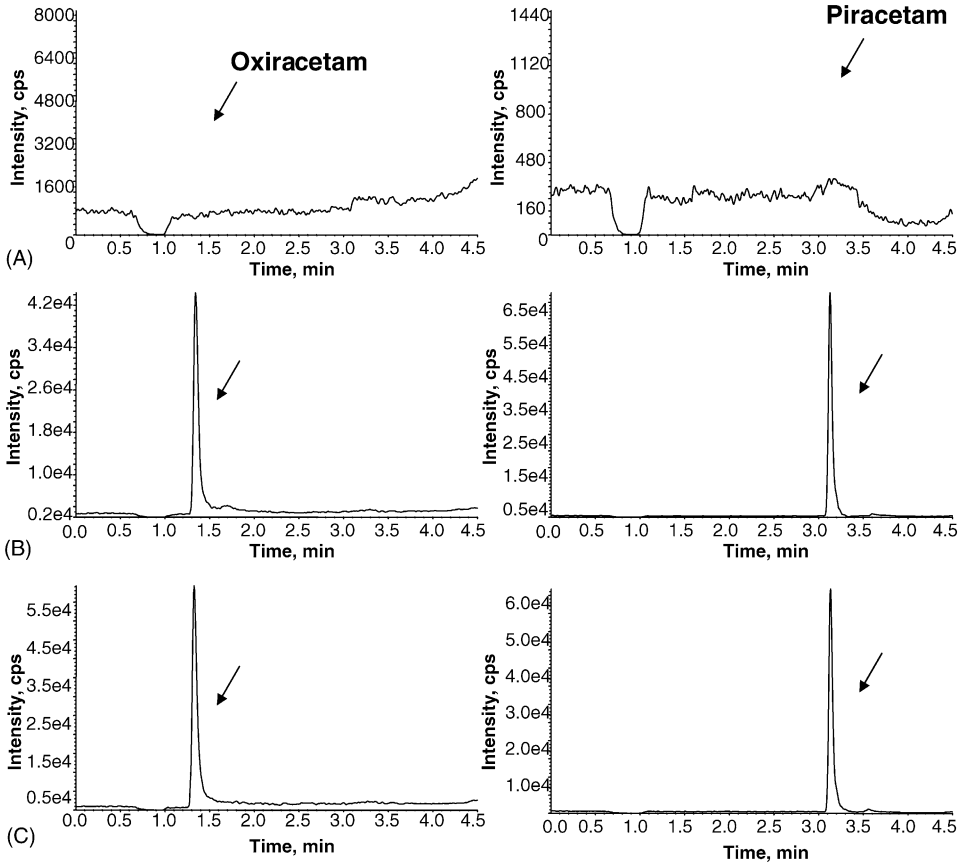

Fig. 3. LC–MS/MS chromatograms of (A) blank human plasma, (B) plasma spiked with oxiracetam (10 μg/ml) and internal standard (1.0 μg/ml), and (C) plasma collected 2.0 h after single oral administration of oxiracetam tablet (800 mg).

in the range 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 40.0 μg/ml. A typical equation of each calibration curve for oxiracetam was: $y = 0.0625 \pm 0.00033x + 0.002 \pm 0.0050$, $r^2 = 0.9996$.

The intra- and inter-day variations of oxiracetam determination in human plasma are summarized in Table 1. The intra-day coefficients of variation were between 2.2 and 8.3% and the accuracies ranged from 92.5 to 106.4%. The inter-day coefficients of variation were between 2.8 and 5.6% and the accuracies were between 93.6 and 103.8%. Under these conditions a lower limit of quantitation (LLOQ) of 0.2 μg/ml was achieved for oxiracetam using a 0.2 ml plasma sample volume. This was the lowest concentration of the analyte that can be measured with a coefficient of variation and accuracy both less than 15%. These LLOQ was sufficient for pharmacokinetic studies, although lower LLOQ for oxiracetam could be achieved when considering reconstitution (100 μl) and injection (4 μl) volume.

3.3. Pharmacokinetic investigation in healthy volunteers

The validated method was applied to determine oxiracetam concentration in human plasma after single oral administration of 800 mg oxiracetam in tablets to 24 healthy volunteers. Blood samples were collected at 0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h post dose. Fig. 4 shows the mean plasma concentration-time curve of oxiracetam. The AUC of oxiracetam was 110 ± 28 μg h/ml. The average C_{max} of oxiracetam was 21.6 μg/ml.

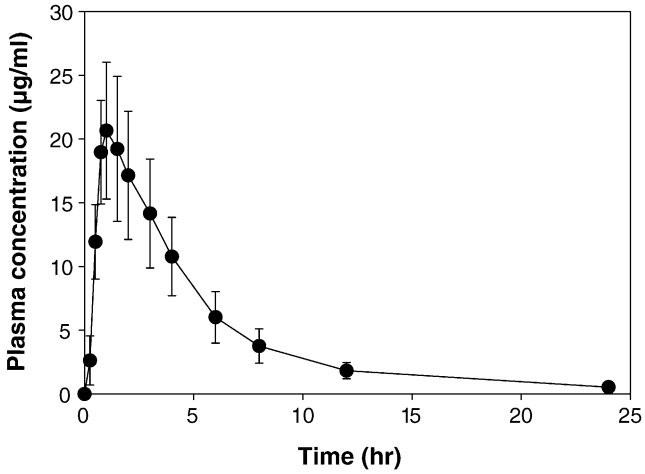

Fig. 4. Mean plasma concentrations of oxiracetam after oral administration of oxiracetam tablet (800 mg) to 24 healthy volunteers. Each point represents the mean ± S.D.

4. Conclusions

The LC–MS/MS method combined with protein precipitation has been developed for the quantitative determination of oxiracetam in human plasma. The present method affords the sensitivity, accuracy and precision necessary for quantitative measurements in pharmacokinetic studies and therapeutic monitoring of oxiracetam.

Acknowledgement

The work presented was supported by National Research Laboratory grants, M1-0204-00-0167, funded by Korean Ministry of Science and Technology.

References

[1] G. Pifferi, M. Pinza, Farmacol. Ed. Sci. 32 (1977) 602–613.
[2] B. Saletu, L. Linzmayer, J. Grunberger, H. Pietschmann, Neuropsychobiology 13 (1985) 44–52.
[3] S. Banfi, L. Dorigotti, Clin. Neuropharmacol. 9 (1986) S19–S26.
[4] G. Spignoli, G. Pepeu, Eur. J. Pharm. 126 (1986) 253–257.
[5] M. Visconti, R. Spalluto, T. Crolla, G. Pifferi, M. Pinza, J. Chromatogr. 416 (1987) 433–438.
[6] R.C. Simpson, V.K. Boppana, B.Y. Hwang, G.R. Rhodes, J. Chromatogr. 631 (1993) 227–232.
[7] J.B. Lecaillon, C. Souppart, F. Le Duigou, J.P. Dubois, J. Chromatogr. 497 (1989) 223–230.
[8] J. Lee, J. Son, M. Lee, K.T. Lee, D-H. Kim, Rapid Commun. Mass Spectrom. 17 (2003) 1157–1162.