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Simultaneous Determination of Olanzapine and its Major Metabolite *N*-Desmethyl Olanzapine in Rat Plasma by HPLC-MS/MS; Application of PK in Rat

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Olanzapine is an atypical antipsychotic drug that is widely used for the treatment of schizophrenia[1] and acute bipolar disorder.[2] OLZ mainly metabolized to *N*-desmethyl olanzapine (N-DMO) by CYP1A2[3] that was clinically more important than CYP2D6.[4] Despite this importance, there have been no reports about the simultaneous determination of OLZ and N-DMO concentrations in rat plasma by HPLC-MS/MS. This study describes a simple and rapid method for the simultaneous determination of OLZ and N-DMO in rat plasma by HPLC-MS/MS with electrospray ionization, after sample pretreatment by deproteinization with methanol.

OLZ and N-DMO were purchased from Waterstone Technology (Carmel, IN, USA) and Toronto Research Chemicals, Inc. (North York, Ontario, Canada), respectively. Carbamazepine, used as an internal standard (IS), was purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario, Canada). HPLC-grade methanol was from Merck (Darmstadt, Germany). LC was performed on an Agilent 1100 HPLC system (Agilent technologies, Inc., Santa Clara, CA, USA). PE SCIEX API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) were used as a detector

Stock solutions (1 mg/mL) of OLZ and N-DMO were diluted serially with methanol. To optimize MS conditions, a solution of containing 100 ng/mL of OLZ or N-DMO was infused into the mass spectrometer at a flow rate of 10 μL/min. The precursor ions for the analytes were optimized as protonated molecular ions, $[M+H]^+$. OLZ and N-DMO were quantified using the multiple reaction monitoring mode and the peak-area ratio method with carbamazepine as the IS. The precursor-product ion transitions used for OLZ, N-DMO, and carbamazepine were *m/z* 313 → 256, 299 → 198, and 237 → 194, respectively. The turbo ion spray interface was operated in positive ion mode at 5500 V and 350 °C. For HPLC, a reversed-phase column (X-terra C18, 50 × 2.1 mm, 3.5 μm; Waters Ireland, Dublin, Ireland) was used with an isocratic mobile phase of 85% methanol and 15% ammonium formate (5 mM, pH 3.6) and a flow rate of 0.15 mL/min.

To prepare samples for calibration curve and Quality control (QC), 5 μL of the OLZ and N-DMO standard solutions were added to 40 μL of drug-free plasma at a known concentration. To construct a calibration curve, plasma samples containing OLZ or N-DMO at 0.01, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, and 3 μg/mL were analyzed. Low, medium, and high QC concentration was 0.01, 1, 3 μg/mL. For the IS, 150 μL of carbamazepine solution (500 ng/mL in methanol) were added to the plasma samples, and the samples were vortex-mixed for 5 min, followed by centrifugation at 12,000 rpm for 10 min. A 5-μL aliquot of the supernatant was injected into the column. The analytical data were processed using Analyst software (v. 1.4.1; Applied Biosystems, Foster City, CA, USA). The pharmacokinetic analysis was performed using noncompartmental methods with WinNonlin standard version 2.1 (Pharsight Corp., Palo Alto, CA, USA).

The specificity of the method was determined using blank plasma samples obtained from six rats. The intra-day and inter-day precision and accuracy were estimated by analyzing QC samples. The limits of precision and accuracy for acceptable data were within 15% or within 20% at the limit of quantitation. Short-term, long-term, freeze-thaw, and post-extraction stabilities were assessed at low (10 ng/mL) and high (3000 ng/mL) QC concentrations. The pharmacokinetics of OLZ were determined in five Sprague-Dawley rats administered a single oral dose of 30 mg/kg OLZ after an overnight fast. The plasma was separated from the collected blood and stored at −70 °C until analyzed. To determine the analyte concentrations based on the calibration curves, 50-μL aliquots of the rat plasma samples were analyzed.

The retention times of OLZ, N-DMO, and the IS were 1.29, 1.11, and 1.14 min, respectively. The chromatograms of OLZ, N-DMO and I.S in plasma sample are shown in Figure 1. The calibration curves for OLZ and N-DMO showed good linearity in the concentration range of 10 to 3000 ng/mL ($r^2 > 0.999$). The coefficients of variation (%CV) for intra-day and inter-day precision and accuracy are summarized in Table1. The mean recoveries of OLZ and N-DMO were 93.5 ± 6.37% and 89.4 ± 3.26%, respectively. The mean matrix effects for OLZ and N-DMO QC samples were 97.90 ± 3.86% and 99.87 ± 8.97%, respectively. Thus, no significant matrix effect or interference from endogenous compounds was present in the rat plasma.[5] Table 2 presents the stability test results for OLZ and N-DMO. The pharmacokinetics of OLZ and its metabolite N-DMO in rats administered a single oral dose of OLZ (30 mg/kg) are shown in Figure 2. The maximum plasma concentrations (C_{max}) of

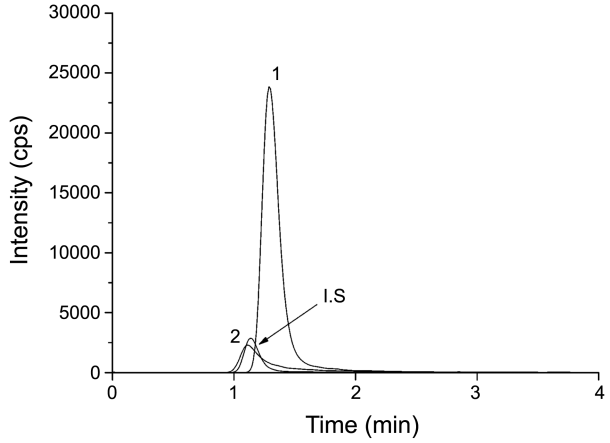

Figure 1. The chromatograms of olanzapine, *N*-desmethyl olanzapine and I.S in plasma sample. 1, Olanzapine; 2, *N*-desmethyl olanzapine.

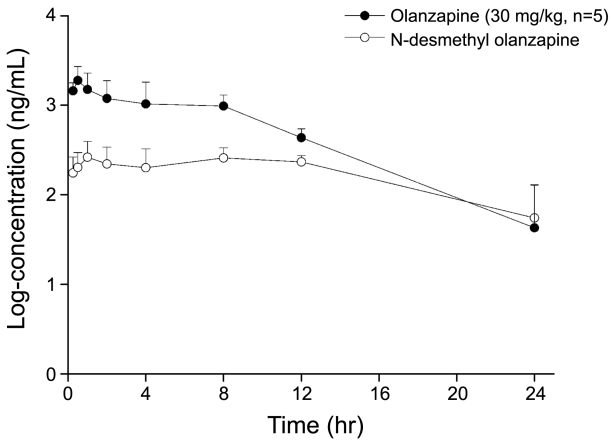

Figure 2. Time course of olanzapine and *N*-desmethyl olanzapine log plasma concentration in rats (n=5)

OLZ and N-DMO were 2108.84 ± 762.24 ng/mL and 309.98 ± 88.06 ng/mL, respectively. The $AUC_{0-\infty}$ was 16180.84 ± 4871.33 ng/mLh for OLZ and 5579.20 ± 1216.20 ng/mLh for N-DMO. The half-life estimated at the terminal phase was 3.86 ± 1.32 h for OLZ and 8.13 ± 3.04 h for N-DMO.

In conclusion, the HPLC-MS/MS method described here is a simple, rapid, and accurate method for simultaneously determining the concentrations of OLZ and N-DMO in rat plasma. This method was successfully applied to an in vivo pharmacokinetic study in rats. HPLC-MS/MS methods for OLZ and N-DMO determinations in human plasma[6] and cerebrospinal fluid[7] have been reported, but these methods require complicated sample preparation procedures, need large amount of sample, expensive column, and a long run time. In contrast, our method is simple, has an easy and rapid sample preparation procedure, uses a low-cost column with isocratic elution, and has a short run time. And there had been reported about HPLC-ECD method for the quantification of OLZ and N-DMO in rat plasma,[8] but this previous study needed complicate pretreatment of sample and long run time compared our study that need simple and rapid sample preparation and short run time. Also there are no previous reports describing the simultaneous determination of OLZ and N-DMO in rat plasma by HPLC-MS/MS methods. Finally, this simple, rapid, and accurate method can be successfully applied for both in vitro and in vivo studies.

Table 1. The intra-, inter-day precision and accuracy (n=5)

OLZ / N-DMO	Nominal concentration (ng/mL)	Mean calculated concentration (ng/mL)	CV%	RE (%)
Intra-day	10	9.71/9.87	10.41/15.41	-3.03/-1.30
	1000	998.99/949.37	9.46/12.99	-0.10/-5.33
	3000	3059.39/2857.00	3.24/13.81	1.94/-5.01
Inter-day	10	9.30/9.79	10.94/15.20	-7.02/-2.07
	1000	1096.87/1133.40	7.83/3.07	9.69/13.33
	3000	3208.20/3196.58	13.91/13.77	6.94/-5.64

Table 2. Stabilities of Olanzapine and N-desmethyl olanzapine in rat plasma (n=3)

Stability Test	Storage condition	10 ng/mL Stability (%)	3000 ng/mL Stability (%)
OLZ / N-DMO			
Short term in plasma	Room temperature for 6h	101.20/ 98.44	101.56/ 96.86
Long term in plasma	-70 °C for 14 days	111.29/ 103.50	99.18/ 110.31
Freeze-thaw cycle in plasma	-70 °C after the third cycle	98.09/ 90.80	97.44/ 98.18
Process (extracted sample)	4 °C, for 24 h	97.57/ 99.09	113.36/ 111.89
Stock solution	-20 °C, for 14 days	98.83/ 109.30	95.02/ 105.39

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