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Original Article

Development and validation of liquid chromatography- tandem mass pectrometry for determination of olanzapine in rabbit plasma

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Abstract

A sensitive, selective and novel LC-MS/MS method for the determination of Olanzapine in plasma was developed and validated for quantification of Olanzapine in rabbit plasma. Chromatographic separation was carried out on a Phenomenex (250*4.60mm) with an isocratic mobile phase consisting of 0.1% v/v formic acid in water and Methanol at a ratio of 08:92 v/v and a total run time of 2.5 min**.** The plasma Olanzapine concentrations were quantified using SCIEX API 3000 LC-MS/MS system equipped with electrospray ionization operated in the multiple reaction monitoring mode at *m/z* 313.4 → 256.3 for Olanzapine; and *m/z* 327.1 → 270.0 for Clozapine (internal standard) respectively. Calibration standards were prepared in the range 5 ng/mL to 1000 ng/mL for Olanzapine. The results were reproducible and precise with the samples prepared by Liquid-Liquid extraction technique using tert- butylmethyl ether extraction during method development trials. The novelty of this method involves the development and validation by using one step simple sample cleanup technique and the most sensitive method with shortest analysis time (2.50 min). Specificity, linearity, accuracy, precision, recovery, matrix effect and stability were evaluated during method validation.

Key words: Olanzapine; LCMS/MS; Methanol; 0.1% v/v formic acid in water; Plasma.

Introduction

OLANZAPINE

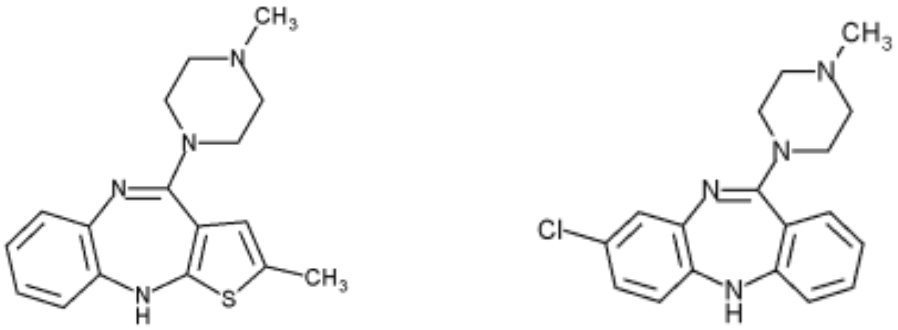
Olanzapine (fig1) is an atypical antipsychotic, approved by FDA for the treatment of schizophrenia, Olanzapine is a serotonin dopamine receptor antagonist indicated for the treatment of schizophrenia and other psychotic disorders. Drug is well absorbed from the gastro-intestinal tract. Food has no effect on its absorption[1].

There are several determination methods, HPLCUV[2], HPLC-ECD[3], GC-NPD[4], liquid chromatography/tandem mass spectroscopy (LC-MS/MS)[5, 6] published for the measurement of olanzapine in previous researches. However, a main drawback of LC-MS/MS is matrix effect from urine and blood sample[7]. The use of stable internal standards may effectively overcome the problem of matrix effect as they have almost identical chemical properties but are easily to be distinguished in the MS analysis providing very accurate and precise results and suitable as reference method[8].

Most of the previous researches have been reported solid phase extraction (SPE) as the method of sample preparation, it is effective for remove interferences but may be too cost for large amount bioanalysis[9–12].Liquid-liquid extraction was adopted in this study, which not only simplified the process but also saved the analytical time.

Based on previously published LC-MS/MS method [5, 6], a liquid chromatographic-tandem mass spectrometry (LC-MS/MS) was developed in this study and validated for the quantification of Olanzapine in rabbit plasma by spiking clozapine as an internal standard. A single step liquid-liquid extraction was adopted for sample preparation. Here we proposed an LCMS/MS method for simple, reliable and accurate determination for Olanzapine in rabbit plasma.

Figure 1 Chemical structures of olanzapine (1) and clozapine(2)



Materials and Methods

Reagents Olanzapine (99.9% purity) was provided at Dr. Reddy's Laboratories Ltd. (India), and compound 2, clozapine (internal standard, IS, 98% purity) was purchased from Bio organics & applied materials Pvt Ltd (Bangalore). Male, newzland rabbits; 1.8-2.5 kgs. Animal ethical committe registration no-1564/PO/a/11/CPCSEA.

Instrumentation

The liquid chromatography system (Shimadzu, Germany) was equipped with two LC-20ADvp pumps, a DGU-20AD3 vacuum degasser, a CZ-0083 auto sampler and a controller module. Mass spectrometric detection was performed on an API 3000 triple quadruple instrument (ABI-SCIEX, Toronto, Canada). The data was acquired and processed with Analyst 1.4.2 software package.

Standard solutions

Stock solutions of olanzapine (1mg·mL−1) and IS (1mg·mL−1) were prepared with methanol. Working standard solution of olanzapine was diluted with 80% methanol solution. All standard solutions were stored at 4 °C. Calibration standards (5, 100, 400, 600, 800, and 1000, ng·mL−1) were prepared by spiking the working standard solutions of olanzapine into human plasma. Dilutions were used to prepare three levels of QCs, 50, 500, and 900 ng·mL−1 in human plasma. QCs were stored at -30^{0}C.

Sample preparation

10µL of Olanzapine is added to 10µL of ISTD and 190 µL of blank plasma. Vortex to mix. Add 2.000 µL of TBME. Vortex for 5mins. Centrifuge for 5mins at 4000 rpm. Supernant was evaporated to dryness under stream of N_2 at 40^{0}C. Reconstitute with methanol (0.2mL).Vortex and inject.

LC-MS/MS conditions

The chromatographic separation was performed on a Phenomenex (250 mm × 4.60 mm Luna 5 micron C_{18} 100A) an isocratic programming was used at a flow rate of 1.1 mL·min−1 with mobile phase consisting of 0.1% v/v formic acid in water and Methanol at a ratio of 08:92 v/v and a total run time of 2.5 min**.** 20 µL was injected. The nitrogen sheath gas and the auxiliary gas were set at 12 and 8 psi (1 psi ≈ 6.9 kPa), respectively. Tandem mass spectrometry analysis was carried out on an API 3000 triple quadruple instrument (Applied Biosystems, Bangalore) using multiple reaction monitoring (MRM) detection.

Method validation

The method was validated for specificity, linearity, lower limit of quantification (LLOQ), intra- and inter-assay precision and accuracy, matrix effect, recovery, and stability according to the recommendation for the bioanalytical method validation by FDA (2001).

The method specificity was evaluated by screening one batch of blank rabbit plasma prior to the main validation batch. This batch was spiked with known concentration of olanzapine at 0.01 ng·mL−1, extracted and analyzed along with a calibration curve.

The linearity for olanzapine was evaluated over the range of 5 − 1000 ng·mL−1. A linear regression model with 1/*x* weighted factor was constructed based on the measured peak area ratio of olanzapine to the IS versus the nominal concentration, where *x* is the concentration of olanzapine, was fitted to each standard curve.

QCs at three concentration levels (50, 500, and 900 ng·mL−1) were analyzed to evaluate intra- and interassay precision and accuracy of the method. Precision was expressed as RSD% for replicate measurements and accuracy (%) by the percentage of deviation between nominal and calculated concentrations.

The matrix effect was assessed by comparing the peak areas of analytes from the standards spiked after extraction and the pure QC standards at the same concentration levels. The recovery was assessed by comparing the standards spiked before and after extraction at three concentration levels (50, 500, and 900 ng·mL−1).

Clinical application

The developed method was applied to determining the plasma concentrations of olanzapine from 5 healthy rabbits.

Results and discussion

Optimization of chromatographic conditions

The precursor-to-product ion transitions 313→256 amu for olanzapine and 316→256 amu for IS were monitored with the collision energy set at 31 eV (Figure 2). The LC-MS/MS chromatograms of plasma samples are shown in Figure 3.

Optimization of sample preparation

In order to increase the extraction recovery, NaOH solution (0.1 mol·L−1) was added to adjust the pH value to 10 as olanzapine is an alkaline compound. The recovery was enhanced 20% by using the alkaline modifier. In addition, different extracting agents were tried. Finally *tert*-butyl methyl ether was selected considering the volatility and recovery rate. In the process of extraction, the toughest problem was the absorption of olanzapine. Different concentrations of solvent, different tubes and different sample preparation times were tested to find the causes of absorption.

Figure 2 Product ion mass spectrum of protonated ions obtained from olanzapine

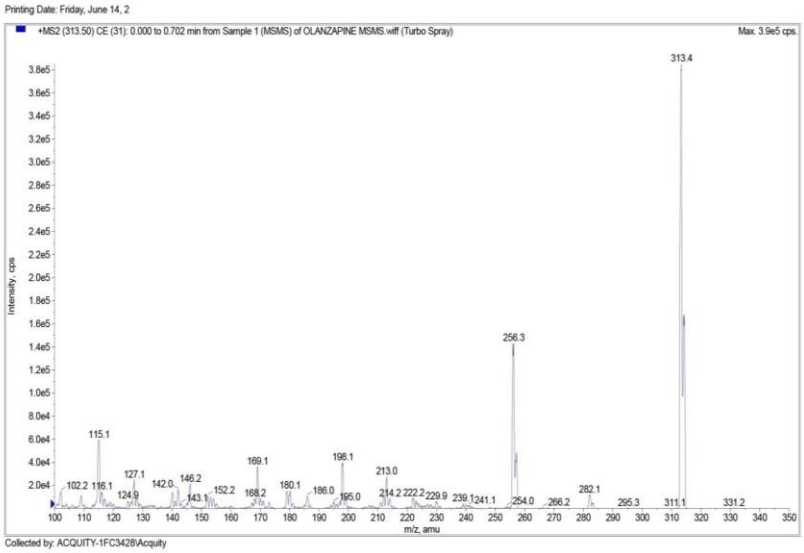
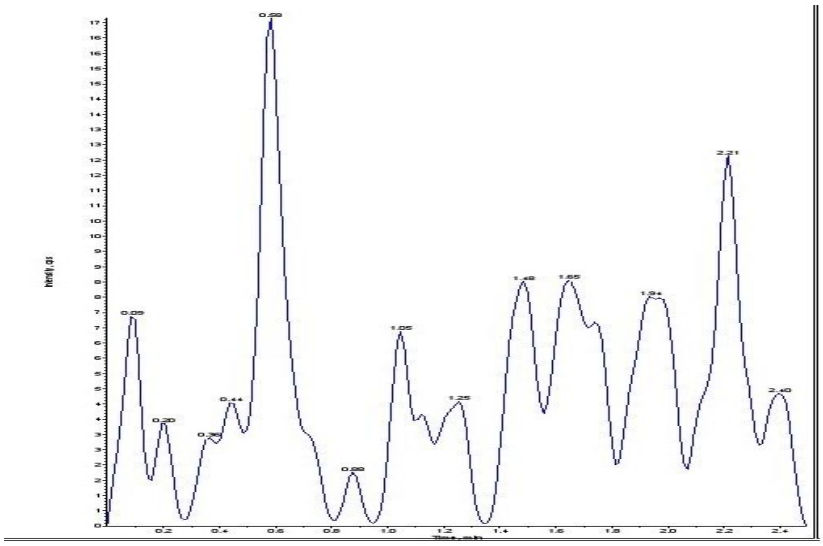
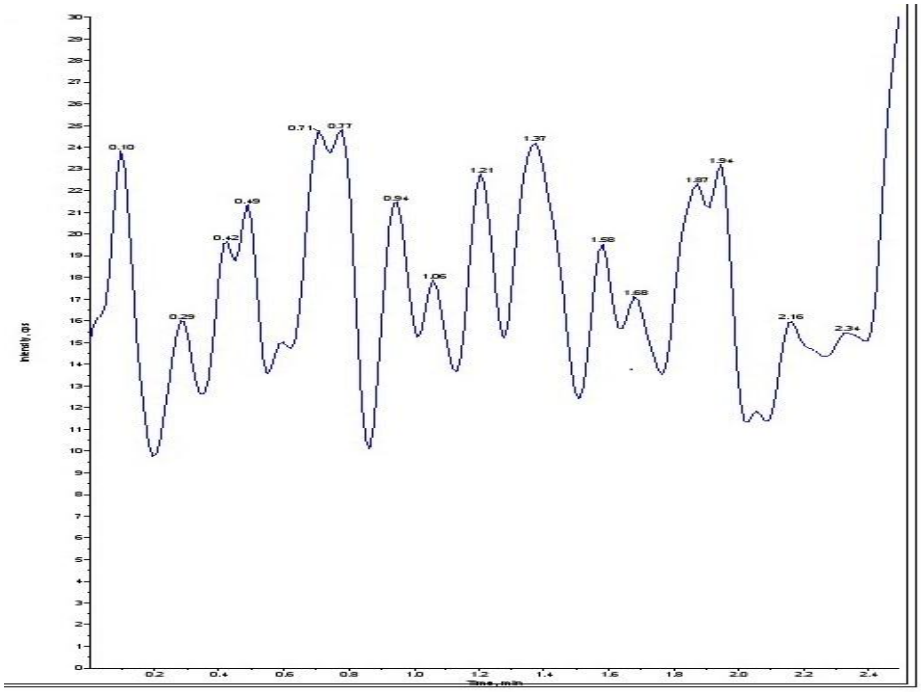


Figure 3 Representative LC-MS/MS chromatograms of olanzapine (OZP) and D3-olanzapine (IS) in rabbit plasma. A: Blank rabbit plasma; B: LLOQ plasma sample with 0.1 ng·mL−1 olanzapine.

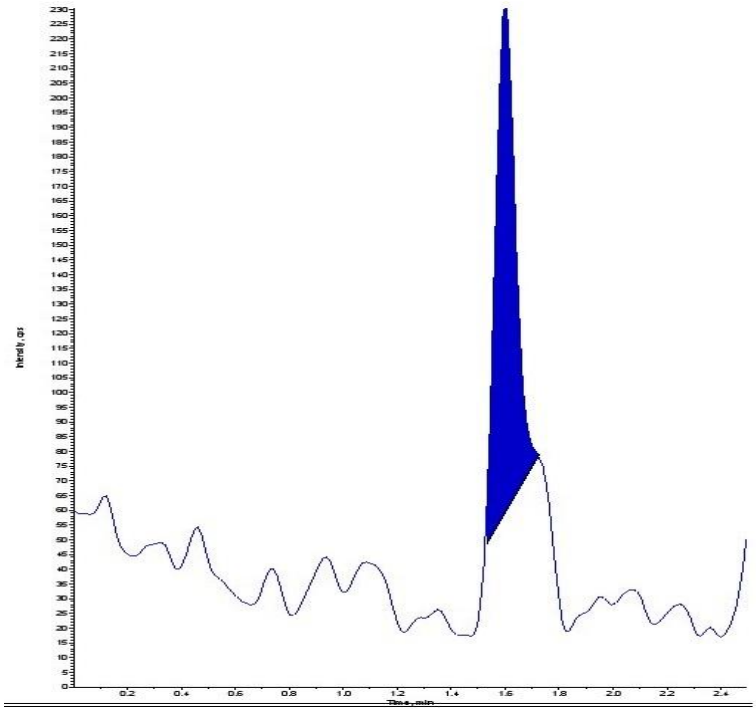


Olanzapine

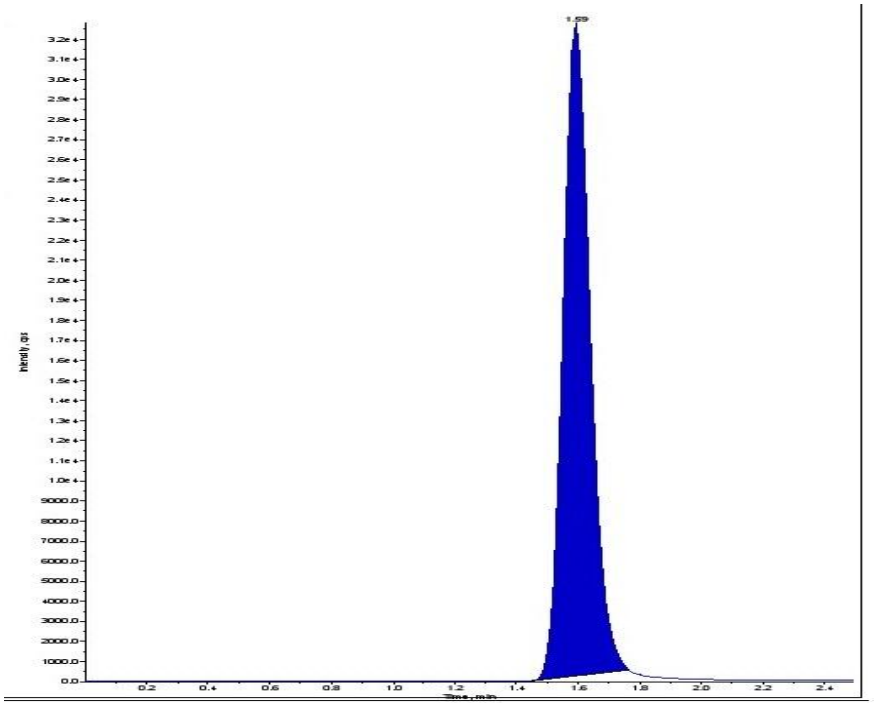


Internal standard

Representative chromatograms of Plasma blank



Olanzapine



Clozapine

Method validation

Specificity

Blank plasma was tested for endogenous interferences. The olanzapine retention time region was free from interferences. The back-calculated values spiked at concentrations of 5, 100, and 400 ng·mL−1 for olanzapine, the RSD was less than 15% and the accuracy was 80%−120%, respectively, indicating no significant batch-to-batch variation.

Linearity and LLOQ

Linear regression analysis for olanzapine was performed by using internal standard method. Olanzapine fixed good linearity over a relatively wide concentration range, and the mean correlation coefficient (*r*) was 0.9993. Based on the standard data presented here, it was concluded that the calibration curves used in this method were accurate for the determination of olanzapine. The LLOQ was 5 ng·mL−1 for olanzapine. Though LLOQ is adequately low for olanzapine quantification, the sensitivity is achieved using a limited sample volume of 200µL plasma. The extracts were diluted two folds in the reconstitution step and only 0.2 mL was injected onto LC-MS/MS for analysis. This indicates that a more sensitive linear range could be achieved if it is necessary for future studies.

Accuracy and precision

A summary of the individual QC data is obtained in the three runs for the validation. The inter-batch precision RSD of the assay was no more than 3% at four concentration levels of the QC samples, the RSD

for intra-batch precision was less than 3%. The inter- and intra-batch accuracy of the assay ranged from 85% to 115%. The assay for olanzapine was accurate and precise between runs and within individual run for each level.

Recovery and matrix effect

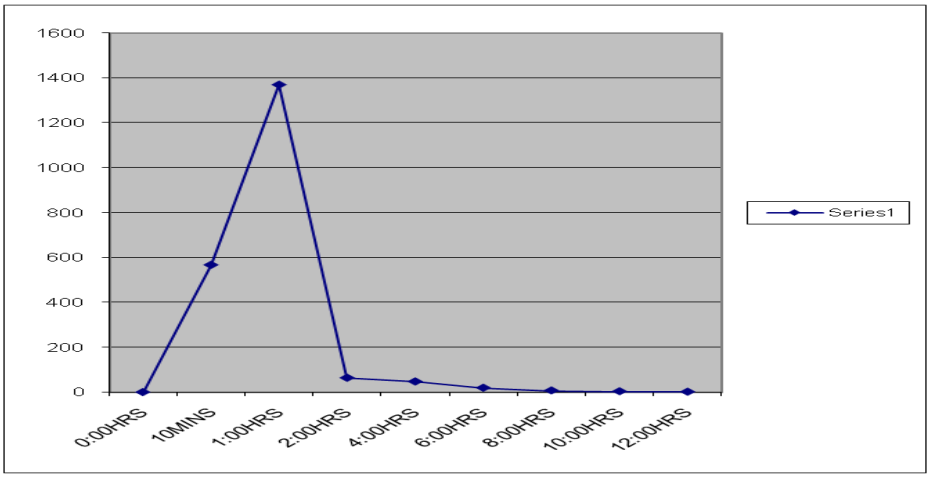
The mean recoveries of olanzapine at 50, 500, and 900 ng·mL−1 were 85.36%, 96.84% and 88.12%, respectively. The mean recoveries of IS were 90.90%. Both these replicates presented with a RSD of less than 15%.

No interferences from other compounds present in plasma were observed to the analytes assay. To date, no significant interferences from endogenous substances were observed during the process of method validation and sample assay.

Application study

The described LC-MS/MS method was successfully applied to determining the rabbit plasma samples of olanzapine up to 12 h after a single oral dose administration of a 10 mg olanzapine tablet to 5 healthy animals. Figure 4 showed the concentration-time curve of olanzapine after an oral administration of 10 mg reference and test olanzapine tablet.

Figure 4 Concentration-time curve of olanzapine after an oral administration of 10 mg reference and test olanzapine tablets



Conclusion

A liquid chromatography-tandem mass spectrometry was developed for the quantification of plasma olanzapine. No interference with the LCMS/MS method from endogenous substances has been

observed. The validated method has been successfully applied to the determination of olanzapine in the plasma samples.

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