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Research Article

Determination of Olanzapine in Human Plasma by LC/MS/MS and its Clinical Applications

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Abstract

Background: Olanzapine is a medication used in the treatment of schizophrenia and bipolar disorders which are typically appearing between the late teenage years and the mid-30s.

Aim: Development of a sensitive, robust, and cost effective bio-analytical method for rapid quantification of olanzapine in biological fluids and its application in pharmacokinetics, bioavailability studies, and clinical trials.

Methods: Olanzapine was extracted from plasma samples by liquid-liquid extraction method, and chromatographed with an eluting solvent consisting of Methanol: 0.5% Formic acid 60: 40 V/V at a flow rate of 0.5 ml/min, ESI positive mode, and m/z 313.2→256.1, 327→270 for olanzapine and clozapine as internal standard respectively. The method was applied for plasma sample analysis of comparative bioavailability study of Olanzapine 7.5mg generic versus reference tablets. The criteria used to assess the bioequivalence of the two products were the non significance of Cmax, AUC 0-72, and Tmax statistical analysis.

Results: The method showed to be more sensitive, specific, linear, accurate, precise, and cost-effective. The average recovery of olanzapine from human plasma was 91.118% with a limit of quantitation of 0.025ng/ml and the linearity (r^2) obtained was 0.9998. Moreover, statistical analysis (ANOVA) of the measured pharmacokinetic parameters showed that there was no significance between the two products.

Conclusion: The developed bioanalytical LC/MS/MS method is valid and reliable for olanzapine quantification in human plasma. Furthermore, the method is suitable for application in pharmacokinetic studies and therapeutic monitoring of olanzapine in the management of schizophrenia, bipolar disorders, and other diseases. On the other hand, the comparative bioavailability study results showed that both generic and reference tables are bioequivalent.

Keywords: Olanzapine; Schizophrenia; LC/MS/MS; Validation; Liquid-liquid Extraction

Introduction

Olanzapine is a second-generation (atypical) antipsychotic medication for treatment of schizophrenia if the patient is over the age of 13, and bipolar disorder, including mixed or manic episodes. Olanzapine is also approved for use with fluoxetine, a selective serotonin reuptake inhibitor, in patients with episodes of depression associated with bipolar disorder type 1 and treatment-resistant depression [1].

Nausea and vomiting, unrelated to chemotherapy, can be substantial symptoms in patients with advanced cancer. A study showed that olanzapine, at 5 mg/d, appeared to be effective in controlling nausea and emesis and in improving other symptoms and quality-of-life [2].

Olanzapine is commercially available in dose strengths ranging from 2 to 20 mg in the form of coated oral tablets named Zyprexa (Eli Lilly, Indianapolis, IN, USA) and an oral disintegrating tablet known as Zyprexa Zydis (Eli Lilly) [3].

Pharmacokinetics and pharmacology

Olanzapine is well absorbed after oral administration, reaching peak plasma concentrations within 5 to 8 hours. The absorption is not affected by food. The plasma protein binding of olanzapine was about 93% over the concentration range of about 7 to about 1000 ng/ml. Olanzapine is metabolized in the liver by conjugative and oxidative pathways. The major circulating metabolite is the 10-N-glucuronide, which does not pass the blood brain barrier. Cytochromes P450-CYP1A2 and P450-CYP2D6 contribute to the formation of the N-desmethyl and 2-hydroxymethyl metabolites, both exhibited significantly less in vivo pharmacological activity than olanzapine in animal studies [4].

The predominant pharmacologic activity is from the parent olanzapine. In healthy elderly (65 and over) versus non-elderly subjects, the mean elimination half-life was prolonged (51.8 versus 33.8 hr) and the clearance was reduced (17.5 versus 18.2 l/hr). The pharmacokinetic variability observed in the elderly is within the range for the non-elderly. In 44 patients with schizophrenia >65 years of age, dosing from 5 to 20 mg/day was not associated with any distinguishing profile of adverse events [4].

It was found that after single dose administration of olanzapine (Zyprexa 10 mg) film coated tablet, the mean values of C_{max}, AUC_{o-t}, AUC_{o-inf}, $T_{1/2}$, and median T_{max} was 12.99 ± 3.36 ng/ml, 524.24 ± 118.94 ng.h/ml, 559.86 ± 136.38 ng.h/ml, 34.51 ± 6.20 h, and 6 hours (range from 2-10 h) respectively [5].

Analytical methodologies and determination

There are several reports on the analysis of olanzapine in biological fluids available in the literature. Most of the analytical methods employed were based on the use of HPLC with ultraviolet, electrochemical, and amperometric detection. The shortcoming of these methods is their limited specificity [6-10].

The advent of the atmospheric pressure ionization (API) source was a breakthrough that allowed efficient coupling of liquid chromatography (LC) to mass spectrometry (MS), which enables the development of a more sensitive technique. The applicability of LC electrospray tandem MS has been demonstrated in a wide range of bioanalytical, environmental, and pharmaceutical applications such as olanzapine quantification [11,12].

Several LC/MS/MS analytical methods are using solid phase extraction [13,14] for sample cleanup which is costly and tedious. On the other hand, other methods are using non structurally related internal standards [15] that may not accurately compensate for sample cleanup, or detector response variation, or a non-familiar structurally related internal standard [16] that may be expensive or hardly to obtain in some countries.

The objective of this study was to introduce a simple, accurate, cost effective, and valid bioanalytical method for the determination of olanzapine in human plasma and its application in pharmacokinetic and bioavailability studies. Furthermore, all the used materials, reagents, and standards must be familiar, and easily obtainable.

When the method applied reliability in pharmacokinetic application, it can be applied in clinical trials and therapeutic monitoring of olanzapine in patients. This will provide a chance to ensure safety and efficacy of schizophrenia, and bipolar disorders management.

To examine the efficiency of the developed bioanalytical method for the quantitation of olanzapine in biological fluids, a comparative bioavailability study of olanzapine generic versus reference products was conducted according to FDA [17] international guidelines.

Methods

Bioanalytical method

Mass parameters and chromatography

The method was developed in-house as follow: eluting solvent composition is Methanol: 0.5% Formic acid 60: 40 V/V, the flow rate was set at 0.5 ml/min with injection volume set at 30 ul, the MS/MS 6410B detector was operated at ESI positive mode, m/z was 313.2 → 256.1, 327 → 270 for olanzapine and clozapine as internal standard respectively. The fragmentary energy was set at 104 for olanzapine and 130 for clozapine, and collision energy was set at 21 for olanzapine, 23 for clozapine.

Preparation of solutions

Olanzapine standard solution

An accurately weighed 10 mg of standard olanzapine was transferred into a 100 ml volumetric flask, 80 ml of methyl alcohol was added and sonicated for 10 minutes, then completed to volume with methyl alcohol, to obtain a solution with final concentration of 100 ug/ml olanzapine (Solution A) of which 0.2 ml was transferred to a 100 ml volumetric flask and completed to volume with methyl alcohol to obtain final concentration of 200 ng/ml olanzapine (Solution B).

Working solutions

Master Solution used	Milliliters taken	Final concentration obtained (ng/ml)	Final volume (ml)
"Solution B"	0.0125 ml	0.25	10
"Solution B"	0.025 ml	0.5	10
"Solution B"	0.05 ml	1	10
"Solution B"	0.25 ml	5	10
"Solution B"	0.5 ml	10	10
"Solution B"	1.25 ml	25	10
"Solution B"	2.5 ml	50	10
"Solution B"	5 ml	100	10
"Solution B"	7.5 ml	150	10
"Solution B"	10 ml	200	10

Table a

All dilutions are done with methanol.

Clozapine standard solution (Internal standard)

An accurately weighed 10mg of clozapine standard was transferred into a 100 ml volumetric flask and about 80 ml of methanol was added and was sonicated for 10 minutes, then volume was completed with methanol to obtain final concentration solution of 100ug/ml clozapine, 0.1 ml of which was transfer to 100ml volumetric flask and completed to volume with methanol to obtain 100 ng/ml clozapine.

Preparation of serial dilutions of standard olanzapine in human plasma

Serial dilutions of standard olanzapine in human plasma were prepared by transferring 50 ul aliquot of olanzapine of concentrations ranging from 0.25 to 200 ng/ml to a centrifuge tube containing 500 ul of blank plasma.

Preparation of plasma samples

The collected plasma samples of subjects, 500 ul, were transferred into centrifuge test tubes and 50 ul of clozapine working solution 100 ng/ml was added, then vortex-mix were for 30 seconds followed by addition 3 ml of diethylether: dichloromethane 70:30 v/v and vortex-mix for nearly 1 to 2 minutes. The samples were centrifuged at 4000 rpm for 5 minutes, the clear organic supernatant layer was transferred to a clean test tube and evaporate till dryness, 200 ul mobile phase were used to reconstitute dry residue and transferred to insert vial for analysis by LC/MS/MS.

Quantitation

The unknown plasma sample concentration was calculated as per formula: $Y = aX + b$. Where Y is the response ratio, X is the unknown concentration of drug in plasma samples, a is the calibration slope, b is the Y-Intercept

Clinical application

Ethics

This study was conducted in accordance with ICH and GCP guidelines adopted by (EMEA) and after Ethics Committee approval on Olanzapine 7.5 mg tablet bioequivalence study protocol (Study Code: BIO-BIOP-BES-1216/0242). All documents and records were archived according to Drug Research Center internal procedures.

The participant, clinical investigator, and other responsible persons signed a written informed consent. Before starting of screening step, all study aspects were discussed with participants. There were no any obligations on volunteers to continue the bioequivalence study if they didn't want to.

Principal investigator and clinical investigator were responsible for supervising all study procedures. Licensed physicians were responsible for physical examination and following-up of subjects for measurement of vital signs, including blood pressure, pulse rate, body temperature, respiratory rate, and monitoring appearance of any side or adverse events throughout the study. Blood sampling were performed by registered nurses.

Inclusion criteria

Subjects age within 18 to 55 years and calculated BMI within normal acceptable limits. Normal physiological examination and laboratory data were within normal limits. Subjects of no alcoholic or drug abusers and shouldn't have any known history for both, of no clinical study contribution history. Non-smoker subject was preferred over smoker subject and if smoker, this should be not more than 8 cigarettes per day.

Exclusion criteria

Any bisoprolol hypersensitivity, GIT problems, hematological abnormalities, kidney diseases, auto-immune diseases, CVS diseases, diabetics, hepatic disease, respiratory diseases, history of alcohol intake, and drug abuse, positive HIV, abnormal laboratory values, subject administered any medication less than two weeks of the study starting date, blood donation or participation in clinical studies that requires more than 500 ml of blood loss within month and half preceding starting date of the bioequivalence study.

Subjects

Twenty-four healthy adult subjects participated in the bioequivalence study and were subjected to general physical examination, neurological examination, clinical urine tests and blood analysis. The selected subjects had no history of drug or alcohol abuse and have no acute or chronic gastrointestinal, cardiac, vascular, hepatic, or renal disease. Concurrent medication was not allowed during the time course of the study, meals, beverages drink, coffee or tea are not allowed for four hours after study dose administration. At 10:30 a.m. they received a standard breakfast meal followed by a lunch meal at 2:30 p.m.

Study design

The design of this study was a single-center open-label randomized single-dose two-way crossover design to compare the bioavailability of generic versus reference olanzapine 7.5 mg tablet in 24 healthy male adults under fasting conditions with a washout period of 21 days.

Collection of sample

The number and disposition of the blood collections as well as the wash out period was designed with respect to pharmacokinetic parameters of olanzapine.

The number of blood collections for drug analysis was 17 samples in each period, 5ml per sample collected at the following intervals: 0 (directly prior to dosing), 25, 50, 75min, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, and 72 hr after dose administration in tubes containing anticoagulant EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes and plasma samples were separated in a 5 ml-plastic wassermann tube. The collected samples were stored at a -80°C until analysis. The study code, subject number, study period, time interval was recorded on the tubes. Total blood amount withdrawn during the whole study did not exceed 170 ml.

Analysis of samples

Determination of bisoprolol in plasma samples of the participants was performed by LC-MS/MS using the developed bioanalytical method was validated according to the international guidelines.

Calculation of the pharmacokinetics parameters

Pharmacokinetic calculations were done using WinNonLin program. The following pharmacokinetic parameters of olanzapine were assessed; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), half-life of drug elimination during the terminal phase ($t_{1/2e}$), terminal rate of elimination (K_e), area under plasma concentration-time curve from zero to time (72) (AUC_{0-72}).

Statistical analysis

Statistical analysis of the calculated pharmacokinetic data was performed using statistical computerized program SAS software for determination of analysis of variance (ANOVA). Bioequivalence could be demonstrated for olanzapine within the prescribed 90% confidence limit of 80.00 to 125.00% for AUC_{0-72}, and C_{max} with respect to the parametric method on Ln-transformed data.

Tolerability and safety

Subject medical histories, physical examination and laboratory reports, and all incidents of possible adverse reactions to the study formulations were reported.

Measurement of blood pressure and heart rate

Blood pressure cyctolic/diastolic and pulse rate measurements before dosing and at regular intervals (at 2, 4, 6, and 10 hours) after

drug administration were included in tolerability assessments. A 120/80 mmHg blood pressure reading and 50 to 100 beats per minute resting heart rate are considered normal.

Results

The developed validated LC/MS/MS method was in compliance with the international guidelines for the bioanalysis of plasma samples [18].

Validation results of bionalytical procedure

Chromatograms of olanzapine

It is apparent from figures (1), (2), and (3) that olanzapine and clozapine were well separated and their retention time was 0.9, and 1 minute respectively. The chromatograms showed sharp and symmetrical peaks with a good baseline and minimum tailing thus facilitating the accurate measurement of the peak response. The in house developed chromatographic conditions was in accordance with published literature [11-16] with modifications in extraction procedure, internal standard used, and chromatographic conditions.

Figure 1: Chromatogram - an MRM Data of Blank Plasma Spiked with Internal Standard Clozapine.

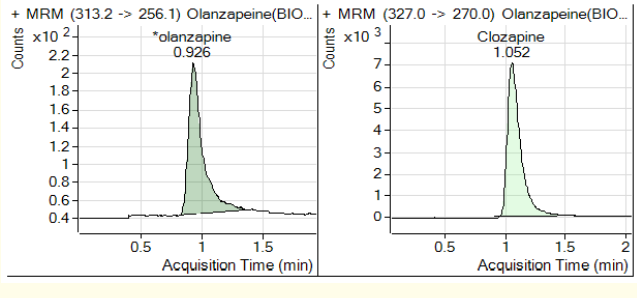

Figure 2: Chromatogram - an MRM Data of Blank Plasma Spiked with 0.025 ng/ml Olanzapine and Internal Standard Clozapine.

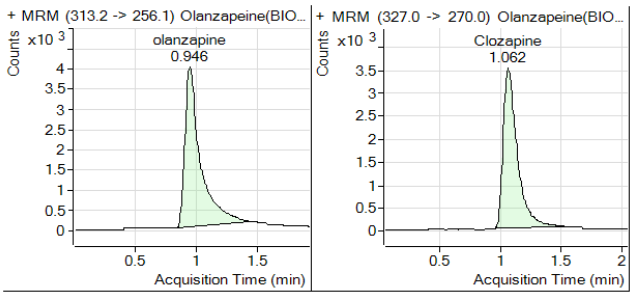

Figure 3: Chromatogram - an MRM Data of Blank Plasma Spiked with 10 ng/ml Olanzapine and Internal Standard Clozapine.

Figure 4: Plasma Concentration (Mean ± S.D.) of Olanzapine following Single Dose Administration of olanzapine 7.5 mg Tablets of Generic and Reference Products.

Linearity, precision and accuracy

The peak area ratios of serial dilutions of olanzapine in human plasma of concentrations ranging from 0.025 to 20 ng/ml was highly linear with r^2 of 0.9998. The C.V.% of the average results of inter-day variation was 1.096% in accordance with FDA Guidelines [18] which strengthen the possibility of its application in pharmacokinetics and bioavailability studies of olanzapine.

Accuracy and precision were assessed on within-day and between-day basis at three levels of drug concentrations at the expected range. Moreover, the results of intra-day inter-day accuracy showed an average recovery percentage of 100.212% and 99.941% respectively. The results of freeze-thaw, short term and long-term stability in human plasma showed that the average

recovery of olanzapine was greater than 95% providing that both targeting drug and internal standard were stable in the studied conditions.

Comparative bioavailability study results

Clinical observation (Safety and Tolerability)

The drug was to some extent tolerated by most of the participants. No treatment related adverse events or laboratory abnormalities were observed. Blood sampling during the whole study was performed at the proper time. No subjects withdrew from the study for any reason attributable to drug side effects.

Pharmacokinetic data and assessment of bioequivelance

Results of pharmacokinetic parameter's presented in Tables (1) and (2) showed that the mean values for C_{max} was 12.136 ± 1.421 ng/ml and 11.874 ± 1.418 ng/ml, t_{max} was 3.708 ± 0.464h and 3.750 ± 0.442h, $t_{1/2e}$ 34.475 ± 5.313 and 32.061 ± 4.905h, AUC_{0-72} 219.617 ± 32.170 ng.h/ml and 228.583 ± 44.554 ng.h/ml, for generic and reference products respectively which were in accordance with those reported in the literature [4,5].

Statistical analysis

Two-way ANOVA was performed for C_{max}, AUC_{0-72} of the two products, also, 90% confidence limit of 80 to 125% for AUC_{0-72}, and C_{max} with respect to the parametric method on Ln-transformed data should be fulfilled. In this bioequivalence study the point estimate (%) results for C_{max}, AUC_{0-72}, were 102.247, 96.797% respectively and the 90% confidence intervals of parametric means of C_{max}, AUC_{0-72}, were 98.529 to 106.107%, 88.853 to 105.453% respectively, (table 3) thus, providing a 90% confidence intervals limits within FDA acceptance limits [17].

Blood pressure and pulse rate

The reported measurements of blood pressure and pulse rate were all approaching normal levels and within the safe limits (Figures 5 and 6).

It is clear from the blood pressure results represented in figure (7), for the generic product, that all approaches normal levels, as the reported mean values of systolic blood pressure were 115, 116, 114, 116, 113 mmHg and 75, 74, 74, 76, 75 mmHg for diastolic blood pressure at Zero (predose), 2, 4, 6, and 10 hours of drug administration respectively.

Figure 5: Blood pressure systolic/diastolic (Mean ± SD) after single oral administration of Olanzapine generic and reference products.

Figure 6: Pulse rate (Mean ± SD) after single oral administration of Olanzapine generic and reference products.

Figure 7: Blood pressure systolic/diastolic and Olanzapine plasma Conc (Mean ± SD) after single oral administration of reference product.

On the other hand, concerning the reference product, mean values of systolic blood pressure were 117, 114, 115, 115, 115 mmHg and 74, 74, 73, 74, 76 mmHg for diastolic blood pressure at Zero (predose), 2, 4, 6, and 10 hours of drug administration respectively (Figure 8).

Figure 8: Blood pressure systolic/diastolic and Olanzapine plasma Conc (Mean ± SD) after single oral administration of generic product.

Discussion

The developed bioanalytical method proved to be sensitive, specific, precise and accurate, showing linearity in the range of 0.025 to 20 ng/ml with r^2 of 0.9998, and fulfilling FDA Guideline [18] requirements. The method could be applied in bioavailability and clinical studies, clinical trials, therapeutic monitoring of olanzapine to assure safety and efficacy in schizophrenia management.

A quantitative ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for determination of olanzapine in human plasma was developed. The results showed that separation was achieved on Waters XBrige C18 column. ESI+ was involved and mass-to-charge ratio transitions were at m/z 313.2→256.1 for olanzapine and m/z 316.2→256.1 IS (d3-olanzapine). The linear range was 0.1-20 ng/ml with LLOQ of 0.1 ng/ml [19]. Although this analytical method showed adequate linearity, sensitivity, and validity, but the use of labelled isotope internal standard (d3-olanzapine) is considered a drawback, since labelled internal standards are often expensive, and in some instances not commercially obtainable.

Another bioanalytical method based on LC/MS/MS detection was developed and validated for quantification of olanzapine in human plasma. Venlafaxine was used as the internal standard (IS), and the samples were extracted using liquid-liquid extraction from 400-ul human plasma with methyl tert-butyl ether. Chromatography was performed using an ACE C18, 125 X 4.6-mm i.d., 5-um column. The mobile phase consisted of water with 0.1% formic acid and acetonitrile with 0.1% formic acid (50: 50 v/v) in isocratic mode. The flow rate was 1.2 mL/min. Mass detection operated in positive ESI mode was used to detect olanzapine and the IS (m/z: 313.1 > 256.1 and 278.1 > 260.2, respectively). The mean recovery of olanzapine was 90.08%. The assay was linear in the concentration range of 1 to 20 ng/mL (r^2 = 0.9976). The intra- and interday precision were < 11.60% and the accuracy was < 1.66% [15]. The method lower limit of 1 ng/ml may not adequate to characterize olanzapine during elimination phase in some clinical settings. On the other hand, the use of a non-structurally related internal standard (venlafaxine) may not be the best choice for compensating sample cleanup and detector response variation.

Further LC/MS method was validated for determination of olanzapine in human plasma. Liquid-liquid extraction was used for sample cleanup. Chromatographic separation used eluting solvent of acetonitrile: 10 mM ammonium acetate solution (pH 4) (56: 44 v/v) on a column XDB-CN (2.1 mm x 150 mm, 5 µm). Mass parameters was SIM mode and m/z 313.15 for olanzapine and m/z 383.00 for loratadine (Internal standard). Run time was less than 6.0 min. The method showed linearity in the range of 0.5-50 ng/ml with good accuracy and precision [20]. The use of a non-structurally related internal standard (loratadine) may not be the best choice for compensating sample cleanup and detector response variation. Furthermore, achievement of chromatographic analysis in less than 6 minutes on LC/MS/MS may be time, and solvent consuming.

In our in-house developed bioanalytical method, we overcome the poor sensitivity in previously published methods [6-10] and performed some modifications to avoid the drawbacks of other methods [11-16,19]. Extraction procedure was based on liquid-liquid extraction and the use of clozapine as a structurally related internal standard, since clozapine is commercially available with appropriate price. Furthermore, the chromatographic analysis was achieved in less that 2.5 minutes. It is worthy to mention that, the

development of an accurate and precise bioanalytical assay was important for ensuring accurate and precise therapeutic monitoring and testing the validity of generic products for commercial use with targeted clinical outcomes [21].

The importance of olanzapine therapeutic drug monitoring is that it proved to be effective against schizophrenia and bipolar disorders [1,2]. Furthermore, clinical studies showed that metabolic dysfunction caused by olanzapine is more severe and dose-dependent in drug-naive individuals, but not in chronic schizophrenia patients. That's why various recommendations advocate therapeutic drug monitoring to balance effectiveness and negative effects [22].

The results of the pharmacokinetics application of olanzapine, in the current study, by using the developed validated bioanalytical method were nearly complying with those reported in the literature [4,5] which might be evidence for the successful application of this method for clinical studies used for evaluation of olanzapine outcomes in patients.

Importance of therapeutic drug monitoring

Compared with conventional antipsychotics, olanzapine has greater affinity for serotonin 5-HT2A than for dopamine D2 receptors. In large, well controlled trials in patients with schizophrenia or related psychoses, olanzapine 5 to 20 mg/day was significantly superior to haloperidol 5 to 20 mg/day in overall improvements in psychopathology rating scales and in the treatment of depressive and negative symptoms, and was comparable in effects on positive psychotic symptoms. The 1-year risk of relapse (rehospitalisation) was significantly lower with olanzapine than with haloperidol treatment [23].

Different diseases and therapeutic agents may be associated with undesired complications, and side effects that need therapeutic intervention. At these cases therapeutic drug monitoring is beneficial to avoid any drug-drug or drug-disease interactions. Delirium has been reported often in patients with COVID-19, and it is associated with a poor prognosis. A recent meta-analysis of randomised controlled trials found that olanzapine and risperidone were beneficial in preventing delirium [24]. Furthermore, olanzapine is considered as the most commonly prescribed antipsychotic agent for COVID-19 patients [25].

Diabetic individuals are more vulnerable to the severity and frequency of COVID-19; thus, antidiabetic medications may interact with antiviral drugs and other therapeutic agents used in COVID-19 treatment [26]. Moreover, according to clinical studies, it was suggested that olanzapine may induce hyperglycemia [27] so caution should be taken when given to diabetic patients. On the other hand, another clinical study showed that on day 19 of olanzapine administration, it caused hyperinsulinemia and decreased insulin sensitivity [28].

Furthermore, olanzapine may not be the best prescription for someone who has a history of gestational diabetes mellitus; nonetheless, if olanzapine is prescribed, frequent blood glucose monitoring is advised to control and take proper action in case of olanzapine-induced hyperglycemia [29]. That is why we need a reliable bioanalytical method for therapeutic drug monitoring. Furthermore, we need to monitor adverse events (AEs).

Subject	T_{max}(h)	C_{max}(ng/ml)	AUC_{0-72}(ng.h/ml)	K_{el}(h^{-1})	$T_{1/2}$(h)
Mean	3.750	11.874	228.583	0.022	32.061
CV%	11.795	11.942	19.491	16.220	15.298
Range (Median)	3.00-4.00 (4.00)	8.957-14.485 (12.009)	167.148-326.063 (215.794)	0.017-0.028 (0.021)	24.556-40.186 (32.991)

Table 1: Pharmacokinetics Parameters of Olanzapine following Administration of Reference Product.

Subject	T_{max}(h)	C_{max}(ng/ml)	AUC_{0-72}(ng.h/ml)	K_{el}(h^{-1})	$T_{1/2}$(h)
Mean	3.708	12.136	219.617	0.021	34.475
CV%	12.521	11.709	14.648	13.953	15.413
Range (Median)	3.00-4.00 (4.000)	9.224-14.941 (11.850)	164.785-303.116 (216.160)	0.013-0.028 (0.020)	24.933-51.854 (34.096)

Table 2: Pharmacokinetics Parameters of Olanzapine following Administration of Generic Product.

Pharmacokinetic Parameter	90% C.I. of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	102.247	98.529	106.107
AUC_{0-72}	96.797	88.853	105.453

Table 3: 90 % C.I. for Generic and Reference Products.

Conclusion

The developed bioanalytical method for the quantitation of olanzapine in plasma is fully validated. It showed to be more sensitive and cost effective compared to previously reported literature methods. The method can be applied for bioavailability studies, clinical trials, therapeutic drug monitoring. Moreover, the results of the comparative bioavailability study showed that both the generic and the reference products were as bioequivalent. Furthermore, therapeutic drug monitoring is beneficial in certain clinical settings to avoid undesired adverse events of drug-drug, drug-disease interactions.

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