

Original Research Article

Analytical method development and validation studies for estimation of anti-psychotic drug (Olanzapine)

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ABSTRACT

Background: Various sophisticated strategies have been raised in conformity with permit the fast separation and quantification about clue aspects concerning complex mixtures of biological matrix. These needs underscored the necessary of analytical instrumentation and the creation of new strategies.

Methods: A rapid, accurate, precise, and simple UV then LC-MS/MS analytical methods has been flourished for the determination of Olanzapine (OLZ) in tablet formulation.

Results: Chromatographic separation was conducted out on a Phenomenex 250×4.60 mm with an isocratic mobile phase consisting formic acid of 0.1% v/v in Methanol and water at a ratio regarding 92:08v/v and an aggregation run time of 2.5 min. The plasma Olanzapine concentrations were quantified the use of SCIEX API 3000 LC-MS/MS provision geared up along electro spray ionization cleft into the multiple reaction limit mode at m/z 313.4 to 256.3 for Olanzapine; and m/z 327.1 to 270.0 for clozapine respectively. Calibration requirements were organized into the range 5 ng/ml in conformity with 1000 ng/ml for Olanzapine. The effects had been unique and reproducible with the samples prepared by way of liquid-liquid extraction method using Tert-butyl methyl ether extraction in the course of approach improvement trials.

Conclusions: The novelty on its technique entails the improvement and validation by means of the usage of some quadrant easy pattern decontamination approach and the most sensitive technique with shortest analysis time.

Keywords: Bio-equivalence, LC-MS/MS, Matrix effects, Olanzapine

INTRODUCTION

Analytical chemists perform characteristic and quantitative analysis; usage the knowledge concerning sampling, defining, isolating, concentrating, then preserving samples; put in calamity limits; verify and confirm consequences via calibration then standardization; perform separations primarily based on differential chemical properties; propagate current methods in imitation of fulfil measurements; expound statistics within strong context; and communicate results.¹ They usage their skills of chemistry, instrumentation, computers, and data after resolve problems of almost entire areas concerning chemistry. For example, their

measurements are used in accordance with ensure assent with environmental and sordid regulations; in accordance with ensure the protection then virtue regarding food, pharmaceuticals, and water; after aid the legal process; after assist physicians diagnose disease; yet after provide chemical measurements indispensable in imitation of vocation and commerce. Analytical chemists are employed between all factors of chemical lookup among industry, academia, yet government. They operate basic laboratory research, increase tactics and products, design devices used among analytical analysis, teach, and assignment in marketing and law. Analytical chemistry is a difficult trade so much makes large contributions after much fields regarding science.² In unique evaluation of

tablets of biological matrix i.e., bioanalytical method, has main position both in the development of more selective than fantastic pills then in perception their therapeutic then toxic effects. It also presents a groundwork because bioavailability, toxicokinetic, tissue distribution, pharmacokinetics, clinical, bioequivalence, biopharmaceutics research and the influences of co-medications that hold in imitation of stay regarded because of new drug according to stay approved.^{3,4} Once an suitable made is elected out of medicine discovery or improvement that is required in imitation of enhance quantitative methods in imitation of determine awareness concerning prescript and postulate indispensable metabolites in biological matrix. These strategies are used in conformity with support a number of things to do in drug development including method research, GLP, toxicology, clinical pharmacology and clinical research studies.

Thus, the kindred among drug discovery, improvement and evaluation are a foremost trouble among the pharmaceutical industry. Clearly, regular techniques for evaluation are now not capable over assembly specialized desires made by improvements into remedy trace and development. Rapid, high throughput, sensitive and selective strategies are in modern times an essential for bioanalytical. Also, the capabilities in conformity with analyze trace mixtures, using a helpful configuration well suited along screening approaches, flourished as like an important feature. These demands underscored the necessary of analytical instrumentation and the introduction of fresh strategies.⁵ On literature survey, that was observed to that amount no longer a great deal assignment has been instituted on it precise category because its determination within bulk and pharmaceutical degree types using HPLC and UV techniques. OLZ have D2 blocking action. It has tremendous 5-HT₂ then α 1 blocking action, and some are notably selective for D4 receptors.

The objective behind the current study was antipsychotics action may additionally depend regarding a specific line concerning work of the drugs of several neurotransmitter receptors. In discriminate regarding the want for a suitable approach for analysis, attempts are wight taken in accordance with develop simple, particular or perfect analytical techniques for the addition regarding antipsychotics. Analytical validation is the corner cobble concerning the procedure validation. Without an established pardon regulation that is impossible to ensure whether the manufactured technique has performed what that purports to do. Hence at that place is a need according to try the recent techniques developed.

METHODS

This research is Interventional study and has been conducted at Vijaya College, Bangalore, Karnataka during the period of Aug 2020 to Dec 2020.

Apparatus and software in spectrophotometric estimation of olanzapine

Shimadzu UV-1800 spectrophotometer linked in conformity with a computer loaded together with Shimadzu UV Probe 2.10 software program used to be used for all the Spectrophotometric measurements. The spectral bandwidth was 1nm and the wavelength scanning pace used to be 2800nm/min. The absorbance spectra of the reference or check options were led out in 1cm quartz cells above the extent of 200-400 nm.

Reagents and substances used into spectrophotometric estimation

Analytically pure sample of Olanzapine procured as like a sample by way of Dr. Reddy's Laboratories Ltd. (India). The drug was once used except similarly purification and it was 99.9%. A.R standard Methanol (Merck), Pharmaceutical system Olanzapine drugs (label declares 10mg) batch no. BSL1782, Mfg. Lic. No. M/5B12/09, Manufactured via Dr. Reddy's Laboratories Ltd. (India), Clozapine as an internal standard and the purity was 99.9% procured from Bio organics & applied materials Pvt. used to be used in UV analysis.

Selection of analytical wavelength

Appropriate dilutions had been prepared for drug beyond the standard stock solution and the options were scanned in the wave extent of 200-400nm.

Table 1: Optimized method parameters.

Method parameters	Optimized values in zero order derivative
Solvent	Methanol
Scanning range	200 to 400nm
Slit width	2nm
Scan speed	Fast
Analytical wavelength for OLZ	273 nm

Preparation of stock solutions

100 mg on standard Olanzapine was weighed and transferred in conformity with a 100 ml volumetric flask yet dissolved in methanol. The decanter was once shaken and aggregation used to be taken on according to the mark with methanol in accordance with assign a solution containing 1000 μ g/ml (stock solution 'A').

From this stock solution, 10 ml concerning the solution used to be pipette out then placed between after 100ml volumetric flask and volume used to be taken above according to remark along methanol according to commend a solution containing 100 μ g/ml (stock solution 'B').

Selection of analytical concentration ranges

From the standard stock solution over Olanzapine, splendid aliquots have been pipetting out into in imitation of 10ml volumetric flask and dilutions had been taken including water in conformity with attain work standard solutions on concentrations from 1-12 µg/ml. Absorbance for it options have been reasonable at 273 nm. For the value solution analytical concentration measure has been found to remain 1-12 µg/ml.

Determination of molar absorptivities of olanzapine at selected analytical wavelengths

The absorbance of the drug Olanzapine in the concentration range 1-12 µg/ml was determined at the selected analytical wavelength 273 nm.

The molar absorptivity of the drugs was calculated using the following equation.

$$A = \epsilon bc$$

Where, A = Absorbance of the sample solution.

ϵ = Molar Extinction Coefficient

b = Path length of the sample cell (b = 1cm).

c = Concentration of the sample.

Thus the molar absorptivity can be determined by the following equation.

$$\epsilon = A/c$$

Calibration curve for Olanzapine (1-12 µg/ml)

Appropriate volume of aliquots from standard Olanzapine stock solutions were delivered to different 10ml volumetric flasks. The volume was managed to the mark with water to get concentrations of 1, 2, 4, 6, 8, 10 and 12 µg/ml. Absorbance spectra of each solution against concentrations were plotted (Figure 1).

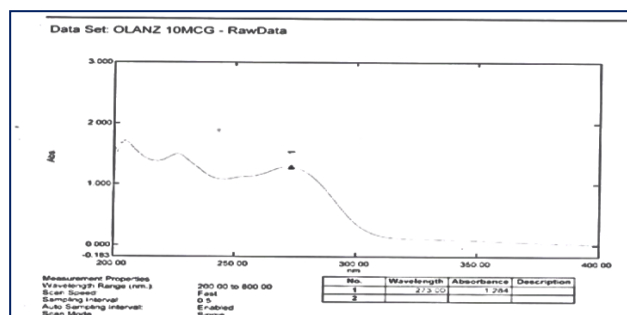


Figure 1: Zero order derivative spectra of Olanzapine.

Sample preparation for determination of Olanzapine from dosage form

Twenty tablets were weighed and finely powdered. The dust equivalent after 10 mg of Olanzapine was once precisely conversant and transferred in imitation of volumetric decanter over 10 ml capacities containing 5ml about the methanol and sonicated for 5 min. The vial was once shaken and volume was instituted above according to the take notice together with methanol to commend a solution of 1000 µg/ml. The over solution was once filtered through what man filter paper (No. 41). From it solution 10ml was dilute in conformity with 100 ml with methanol after consign a solution of 100 µg/ml then used for the addition concerning OLZ.

Validation of spectrophotometric method⁶⁻¹⁴

Linearity and range

The linearity over analytical method is its ability to bring out test consequences that are directly proportional in imitation of the attention on analyzed between samples. The range regarding analytical method is the end between the higher and lower stages of analyze so much have been verified in imitation of stay determined with within a suitable level about precision, precision then linearity.

Precision

The precision on an analytical method is the degree over settlement amongst unaccompanied take a look at results, then the approach is applied often in conformity with a couple of samplings over homogenous samples. It offers an indication regarding random oblivion outcomes and was once expressed as coefficient of variation (CV)/ Relative standard deviation (RSD).

Intra and inter-day precision

Validation on effects inside the same day is acknowledged as Intra-day. Validation regarding results of days is acknowledged as like Inter-day. Intra-day precision was once decided through analyzing Olanzapine for pair instances into the same day at 273 nm. Inter-day obviousness used to be decided via analyzing daily once for two days at 273 nm and % RSD was calculated.

Repeatability

Standard solutions on Olanzapine were prepared and absorbance was once adequate at 273 nm taking the water as the blank. The absorbance about the same attention solution was moderate three instances and standard deviation was deliberated and presented.

Accuracy

Accuracy is the closeness on the take a look at consequences present with the aid of the method according to the authentic value. To study the accuracy, 20 drugs had been expert and powdered then analysis on the same was received out. Recovery studies have been received out through including recognized aggregation about standard drug solution to the sample solution. The percent quotation used to be deliberated and reported.

LOD and LOQ

Calibration curve was once repeated for 5 instances and the standard deviation (SD) regarding the intercepts was once calculated.

LC-MS/MS method development¹⁵⁻²⁰

The mass spectrometer used to be run between positive mode and multiple reaction monitoring (MRM) passion to screen the ions together with m/z over 313.4 (parent ion) and 256.3 (product ion) for Olanzapine; 327.1 (parent ion) and 270 (product ion) for Clozapine (IS) along dwell time of 100ms.

In method according to bear and most excellent selectivity and sensitivity special types of column and mobile Phase have been used. Length regarding the columns different beyond 50 cm in conformity with 250 cm, and the particle size different from 3.5 μ to 5 μ . Columns over different types concerning stationary phase as C8, C18 have been used who showed big matrix impact of peak shape and intensity. Finally, Phenomenex C18, 250 \times 4.6 mm ID, particle size 5 μ , used to be chosen for analysis based on strong peak shape and no form effects.

The effect on ignoramus molarity, pH, yet types over natural modifier about the sign intensities was once also strong at the optimized declustering potential.

Based over Peak shape and intensity of Olanzapine and IS formic acid among water and methanol at 08:92 v/v; at a go with the flow dimensions over 1.1ml/min had been elected namely optimum.

The sample clean-up technique used to be also optimized within order after reach minimal interference about endogenous compounds or matrix effects and excellent analyte recovery.

Different techniques, such namely protein precipitation, liquid-liquid extraction and Solid phase extraction have been used for sample extraction, based about non-interference and finest recovery, Liquid-liquid extraction used to be determined in imitation of stay the good appropriate for sample preparation.

Table 2: Optimized method.

Biological matrix	Rabbit Plasma
Anti Coagulant	K2EDTA
Volume of biological matrix required	200 μ l
Analyte	Olanzapine
MW of Fluoxetine	313.4
Internal Standard	Clozapine
MW of Clozapine	327.1
Calibration curve range for Olanzapine	5 ng/ml to 1000 ng/ml
Analytical technique	Liquid Chromatography
Detection mode	Mass Spectrometer
Sample extraction method	Liquid-liquid Extraction
Quantitation method	Peak area ratio
Regression	Analyte to internal standard peak area ratio versus analyte to IS concentration ratio
Calibration function fit	Linear
Weighing method	1/X ²

Table 3: Chromatographic and MS conditions for the analysis.

Chromatographic conditions as follows;	
Mobile Phase	0.1% v/v Formic acid in water: Methanol (08:92, v/v)
Column/Needle wash Solution	1% v/v Formic acid in water: Methanol (08:92, v/v)
Pump Mode	Binary
Flow rate	1.1ml/min
Injection volume	5 μ l
Auto Sampler temperature	5 \pm 2 $^{\circ}$ C
Column Oven temperature	NA
Acquisition time	2.5min
Column	Phenomenex C18250 \times 4.6 mm ID, particle size 5 μ
MS conditions	Source parameters
Collision gas	12 psi
Curtain gas	30 psi
Gas1	35 psi
Gas2	45 psi
Ion spray voltage	5000 V
Temperature	500 $^{\circ}$ C

Table 4: Compound parameter.

Analyte	Parent Mass Q1 (amu)	Daughter Mass Q3 (amu)	Dwell Time (m sec)	DP	CE	CXP	EP
				Volts			
Olanzapine	313.5	256	100	45	31	5	10
Clozapine	327.1	270	100	45	31	5	10

Preparation of reagents and solutions

Preparation of 0.1% v/v formic acid in water:

Add 0.5ml of formic acid to 495.5ml of milli Q water in a glass bottle and sonicated.

Diluents (Methanol: Water (50:50 v/v)):

Mixed 50 volumes of Methanol and 50 volumes of water and degassed.

Preparation of standard stock solutions

Olanzapine stock solution (1mg/ml): 10mg equivalent of Olanzapine working standard was weighed and transferred into a 10mL volumetric flask and dissolved and diluted to the mark with methanol.

Mix Intermediate solution (60 µg/ml): Take 0.6 ml from stock solution and dilute to 10ml with diluents.

Internal standard working stock solution (1 µg/ml): 5.000 mg equivalent of Clozapine working standard was weighed and transferred into a 5 ml volumetric flask. Dissolved and diluted to the mark with diluents.

Internal standard working solution (3 µg/ml): 0.03 ml Clozapine main stock is transferred to 10 ml volumetric flask. Volume made up to the mark with diluents.

Table 5: Preparation of spiking solutions for calibration standards and quality control samples.

Spiking solution taken	Volume taken(µl)	Volume of plasma taken(µl)	Concentration of OLZ in plasma(ng/ml)
STD-01	10	190	5.000
STD-02	10	190	100.000
STD-03	10	190	400.000
STD-04	10	190	600.000
STD-05	10	190	800.000
STD-06	10	190	1000.000
Preparation of quality control samples			
LQC	10	190	50.000
MQC	10	190	500.000
HQC	10	190	900.000

Preparation of samples

Preparation of plasma blank 20µl of diluents is added to 190µL of screened rabbit plasma (Table 5).

Preparation of zero standard 190µl of screened plasma was transferred to a pre-labelled centrifuge tube. 10µl of Internal Standard dilution working solution (0.300 µg/ml) and 10µl diluents was added and vortexed for 15 seconds.

Preparation of calibration standards and quality control samples 190 µl of plasma was transferred to a pre-labelled Centrifuge tube. 10µl of Calibration standard / quality control spiking solution was added for the preparation (Table 5).

LC-MS/MS method validation**Spiking solution check**

RS vials at each level of concentration of calibration standards and quality control samples as per Standard testing procedure were prepared. Vials for quality control samples were prepared in duplicate and calibration standards in singlet. Quality control samples along with calibration standards were injected and analyzed against calibration standards to calculate its concentrations.

Acceptance criteria

Acceptance criteria were % Accuracy (% nominal), 80.00 to 120.00 for lowest calibration standard, 85.00 to 115.00 for other calibration standards and quality control samples. All calibration standards should meet the above-mentioned criteria for accuracy. Regression coefficient (r) >0.9900, and minimum one quality control sample should pass from two samples at each level.

Specificity/selectivity evaluation

Samples of two normal matrices were obtained. The Lowest calibration par sample and blank sample among each matrix was once organized so per Standard testing procedure. The Interference between every blank sample used to be checked with the aid of comparing the area response obtained into lowest calibration pattern over up to expectation matrix the use of established system for analysis.

% Interference = Response obtained in blank sample * 100/Response obtained in lowest calibration sample of that matrix.

Acceptance criteria

The entire matrix tested should not have more than 20% response of interference at Analyte retention time when compared with the LLOQ response. The entire matrix tested should not have more than 5% response of interference at internal standard retention time when compared with the internal standard response in corresponding LLOQ samples.

Limit of quantification

Three LLOQ and three ULOQ samples along with calibration standards were processed & injected. The concentrations of the LLOQ and ULOQ samples against calibration curve were back-calculated. As per Standard testing procedure the samples were processed and analyzed.

% CV = SD * 100/Mean

Acceptance criteria

For Accuracy: the % accuracy around the Nominal concentration should be within 80.00 to 120.00 for LLOQ and 85.00 to 115.00 for ULOQ. At least 67% of total samples should meet the above-mentioned criteria for accuracy at each level.

For Precision: the % CV around the nominal concentration should be <20.00 for LLOQ and <15.00 for ULOQ.

Calibration curve

Plasma blank, zero standard and six calibration standards of different concentration were Prepared and injected. The regressed line b/w the area ratio and concentration ratio of Analyte to internal standard was plotted. The correlation coefficient of regressed line was determined and calculated the % accuracy of calibration standards and quality control samples from calibration curve.

Acceptance criteria

Plasma blank should not have more than 20% response at Analyte retention time when compared with the LLOQ response and should not have more than 5% response at internal standard retention time when compared with the internal standard response in corresponding LLOQ sample.

Zero standards should not have more than 20% response at Analyte retention time when compared with the corresponding LLOQ sample.

% Accuracy (% nominal) 80.00-120.00 for lowest calibration standard

85.00-115.00 for other levels. 75% of total standards should meet the above criteria for accuracy.

Two consecutive calibration standards should not fail.

First and last calibration standard should not fail.

Regression coefficient (r) ≥ 0.9900

Intraday and inter day precision and accuracy

The following samples were injected in sequence for a Precision and Accuracy batch

Reconstitution solution, reference solution, reconstitution solution, plasma blank, zero standards, calibration standards (Three LQC, Three MQC and Three HQC).

As per standard trying out procedure samples were processed then deliberated the concentrations about attribute control samples by way of quantifying such against calibration standards. One precision and accuracy batch concerning single day was once generated for Intraday precision and accuracy. Three precision then propriety batches had been generated regarding different days for Inter day precision and accuracy.

Acceptance criteria

For precision: the % CV for the QCs must be <15.00

For accuracy: the % accuracy around the nominal concentration should be within 85.00 to 115.00. At least 67% of total QCs should meet the above-mentioned criteria for accuracy at each level.

Matrix factor

Three samples each of LQC and HQC were prepared and each blank plasma as per Standard testing procedure. These QCs along with calibration standards prepared from one blank matrix were injected and these QCs were quantified against calibration curve to calculate concentration.

Acceptance criteria

For precision were % CV for all the QCs should be <15.00 at each level

For Accuracy were % accuracy for all QCs around the nominal concentration should be within 85.00 to 115.00 at each level. At least 67% of total QCs should meet the above-mentioned criteria for accuracy at each level in each matrix.

Ruggedness

Ruggedness run was constituted with calibration standards and three samples each of LQC, MQC and HQC samples. Processing was carried out by different analyst and by using different column as per standard testing procedure. The concentrations of quality control samples were calculated by quantifying it against calibration standards.

Acceptance criteria

For Precision were % CV for the QCs must be <15.00.

For Accuracy were % accuracy around the nominal concentration should be within 85.00 to 115.00. At least 67% of total QCs should meet the above-mentioned criteria for accuracy at each level.

Recovery

The recovery about Olanzapine and inner grade through evaluating the Bio analytical results for extracted QC samples including aqueous solutions equal to a hundred percent recovery over LQC, MQC and HQC was evaluated. As by the extraction manner devoted into ternary samples each of LQC, MQC and HQC had been processed. The Comparison samples of aqueous moderate at Low, middling then excessive Quality monitoring stage were prepared as represent spiking of same concentration degrees among a similar aggregation as that of extracted samples. These assessment samples have been analyzed alongside with extracted QC samples then% excerpt was once calculated by using evaluating area arrived of extracted samples along so much concerning aqueous samples.

% Recovery =Area response observed in individual extracted sample × 100/Mean area response observed in aqueous samples

The mean recovery and %CV of the recovery observed in six samples at each level for Analyte was calculated. The mean recovery of internal standard was calculated at all level.

Acceptance criteria

The %CV of Analyte recovery must be < 15.00 at low, medium and high quality control level. The %CV of internal standard recovery must be < 15.00.%CV of mean recovery at low, medium and high quality control level should be <20.00 for Analyte.

Carry over check

Blank reconstitution solution is added to aqueous ULOQ, and then adds blank RS to blank sample that gives extracted ULOQ.

Acceptance criteria

Analyte area should be < 20% of area of LLOQ.

Study sample analysis

The study sample analysis was performed by taking healthy rabbit by giving tablet Olanzapine 10mg orally and collected time points at regular time intervals.

Statistical analysis

The data's been collected from Shimadzu UV-1800 spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software and was subjected to analyze by performing statistics tools using the Microsoft Excel software.

RESULTS

Results of calibration curve at 273 nm for Olanzapine by Zero order derivative spectroscopy

The proposed method showed good linearity in the concentration range of 1- 12 µg/ml. The correlation coefficient was found to be 0.997 (Figure 2).

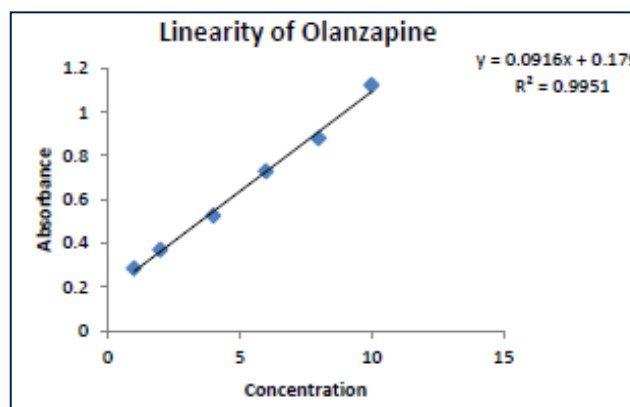


Figure 2: Linearity curve for olanzapine at 273 nm by zero order derivative spectroscopy.

Table 6: Optimum conditions, optical characteristics and statistical data of the regression equation in UV method.

Validation Parameters	Olanzapine
Mobile phase	Methanol
Detection wavelength	273 nm
Beers limit	1-10 µg /ml
Linearity	1-10 µg/ml
R2	0.995
LOD	1.88 µg/ml
LOQ	5.7 µg/ml
Precision	% RSD <2
Recovery	98-102%

Calibration curve was repeated for 5 times and the standard deviation (SD) regarding the intercepts used to be calculated. The values over LOD and LOQ on Olanzapine are 1.88 and 5.7 µg/ml respectively (Table 6).

Calibration curves via weighted then un-weighted linear regression; the functional dependency on the norm dislodgement on the Analyte/Internal Standard area ratio about pattern attention was once evaluated. It used to be located that auspicious in shape and weighing is linear with offset 1/X2.

Calibration curve was determined according to keep consistently correct and particular over the 5 ng/ml to 1000 ng/ml for Olanzapine. The regression coefficient (r) used to be 0.995 for Olanzapine respectively (Table 6).

Table 7: Summary of the present study (LC-MS/MS) Olanzapine.

Calibration curve range	5.000 ng/ml to 1000.000 ng/ml	
Lower limit of quantification	5.000 ng/ml	
Inter day precision and Accuracy	Accuracy (% Nominal)	88.1 to 101.5
	Precision (% CV)	2.15 to 4.911
Intraday precision and Accuracy	Accuracy (% Nominal)	87.8 to 102.8
	Precision (% CV)	2.46 to 7.22
Limit of quantification	Accuracy (% Nominal)	For LLOQ is 100.2 For ULOQ is 104.66
	Precision (% CV)	For LLOQ is 0.11 For ULOQ is 7.78
Ruggedness	Accuracy (% Nominal)	88.4 to 100.2
	Precision (% CV)	0.609 to 2.8
Recovery	% Recovery	For LQC is 85.36 For MQC is 96.84 For HQC is 88.12
	Precision (% CV)	For LQC is 142.27 For MQC is 15.30 For HQC is 48.78
Matrix factor	Accuracy (% Nominal)	For LQC is 100 For HQC is 104.72
	Precision (% CV)	For LQC is 6.18 For HQC is 4.104

Three LLOQ and three ULOQ samples have been processed and injected along with the calibration standards of identical length used in directness and

accuracy batch as by standard checking out procedure. Back thought concentrations of LLOQ and ULOQ samples were determined against calibration curves (Table 7).

Calibration curve parameters and returned thought concentrations of calibration requirements and regression coefficient (r) are presented in the Table 7.

Ruggedness was once defined along special analyst. The conduct consisted of a calibration curve, three LQC, three MQC or three HQC samples.

One precision then accuracy batch was constituted a calibration curve yet ternary samples each regarding LQC, MQC and HQC. The samples had been processed and analyzed as per standard checking out technique. Three samples concerning each LQC or HQC out of each three plasma plenty have been prepared as like Standard testing system then injected alongside including calibration requirements prepared besides blank plasma. The under time directness and accuracy evaluation had been assessed by way of evaluation of three precision and accuracy batches on Olanzapine over different days (Table 7).

Recovery regarding Olanzapine yet internal norm have been evaluated or the percent excerpt and Precision% CV concerning Clozapine (internal standard) have been 90.90 or 96.55% respectively.

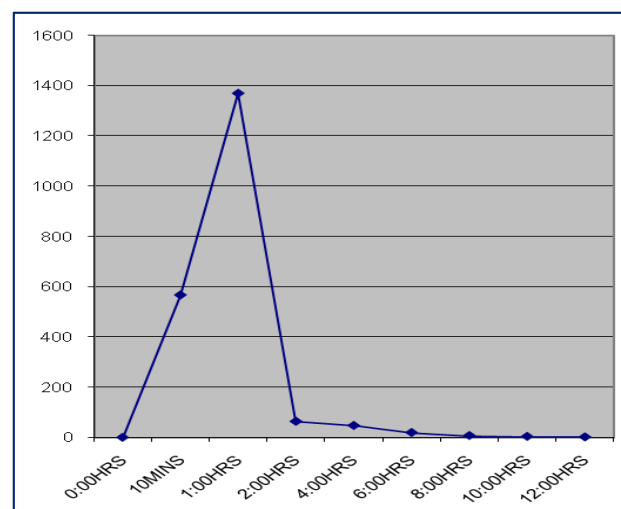


Figure 3: Study sample analysis.

The study sample analysis was performed by taking healthy rabbit by giving tablet Olanzapine 10mg orally and collected time points at regular time intervals (Figure 3).

DISCUSSION

UV spectrophotometric technique used to be applied without the use of some prior chemical pre-treatment in

the emergence about the high overlapping spectra. Accurate consequences had been learnt by means of using the proposed method because the quantitation concerning Olanzapine yet a good agreement including the consequences obtained via the pointed out method was once found. The above study compared with the reference of Basavaiaha et al has reported on Spectrophotometric Determination of Antipsychotic Drug Olanzapine in Pharmaceuticals the calculated molar absorptivity, Sandell sensitivity and LOQ for the methods are reported.²¹ %RSD from 0.25 to 2.89% and %RE ranged from 0.9 to 3.0%, and the inter-day RSD 3.5% and RE values were 4.0% within the limit range. For UV Spectrophotometric method, linearity used to be obtained among concentration spread of 1-12 µg/ml for Olanzapine at 273 nm. High percent removal higher than 98% confirmed up to expectation the method is broad besides the thrusting of excipients back into the formulation. The worth of standard dislodgment and % R.S.D. were found to be <2, confirmed the high obviousness regarding the method. High % removal and low % RSD suggests the approach perform be relevant for the activity's analysis on commercial formulations.

The reported data which has given by Raggi et al, on a sensitive high-performance liquid chromatographic method using electrochemical detection for the analysis of Olanzapine and dimethyl olanzapine in plasma of schizophrenic patients using a new solid-phase extraction procedure the analysis was carried out on a reversed phase column, linear responses were obtained from 5 and 150 ng/ml with repeatability <3.3% and followed by 97% recovery, the compared with the current reports, chromatograms concerning Mobile phase, Reference solution, extracted blank plasma sample, Zero standard, LLOQ, ULOQ, LQC, MQC and HQC, calibration curve, discipline pattern analysis and Mass fragmentation are illustrated.²² The assumption times of Olanzapine and Internal Standard i.e. clozapine had been approximately 1.59 and 1.6 respectively.

The ordinary chromatography run time used to be 2.5 minutes. Two normal blank plasma, had been led through the extraction technique and chromatographed after decide the volume according to who endogenous factors may also make contributions to chromatographic interference with Internal standard response. No enormous interference used to be observed among every six one of a kind plenty over blank plasma samples. Calibration corner was once determined in imitation of keep consistently mathematic or particular over the length on 5.000 ng/ml according to 1000.000 ng/ml for Olanzapine. The regression coefficient (r) is larger than 0.9986 for Olanzapine. Back-calculations have been instituted beyond the calibration curves in accordance with determine Olanzapine concentrations over every calibration standard.

Recovery over Olanzapine and Internal value were evaluated by way of evaluating individual area of 3

extracted samples about low, average or high characteristic control samples mean area over ternary replicates injections of aqueous low, middling then high-quality monitoring samples. The percent excerpt and Precision % CV concerning Clozapine have been thought and presented (Table 7).

CONCLUSION

The proposed UV- spectrophotometric techniques are suitable techniques for dedication concerning Olanzapine. All the parameters for analyzing Olanzapine met the standards over ICH guidelines for approach validation. The UV-Spectrophotometric approach is rapid, simple and cost effective. The developed approach might also remain advocated because pursuits QC analysis over the investigated drugs after grant simple yet unerring quantitative evaluation for the determination about Olanzapine. A simple, sensitive, selective, precise, accurate, and quick LCMS\MS approach for determination about Olanzapine in rabbit plasma, over a range about 5 in accordance with 1000 ng/ml for Olanzapine, was advanced then validated. This method requires shortest run time i.e. 2.50 min; such permits high pattern throughput with almost 800 samples per day. The method does be effectively utilized to bio equivalence study of Olanzapine then according to discipline bioavailability of two specific formulations.

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