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BIOANALYTICAL STUDY OF A TYPICAL ANTIPSYCHOTIC DRUG OLANZAPINE USING RAT AS AN EXPERIMENTAL ANIMAL MODEL

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ABSTRACT

Present work is about the development of bioanalytical method of estimation for an atypical antipsychotic drug Olanzapine from endogenous matrix. Olanzapine is an atypical antipsychotic drug that chemically belongs to thieno benzodiazepine derivative. Bioanalytical estimation of antipsychotic drugs has clinical significance because of probable drug accumulation in body tissues because of long term treatment regime. Olanzapine has been recommended as the first-line drug for the treatment of schizophrenia. It is considered an atypical neuroleptic agent with fewer extrapyramidal side effects than classical neuroleptic agents. In the study initially an alternative bioanalytical estimation method, with the added advantages of decreased run time and minimization of endogenous matrix interferences was developed, which is indicated by high degree of specificity. Developed method was validated for quantization of drug Olanzapine. Developed method was finally cross validated for the estimation of Olanzapine after multiple dosing of drug in experimental animal. Result of study revealed linear detector response from 100 –500 ng/ml, with the correlation coefficient (r^2 =0.989). The limit of detection and limit of quantification of the assay were 27.76 ng/ml and 79.96 and 78.55% ng/ml respectively. The recoveries of Olanzapine from plasma and brain tissue were found to be 79.96 % w/v and 78.55% w/v respectively. The applicability of the assay was confirmed for multiple dose drug determination in blood plasma and brain tissue of Olanzapine in wistar rats after i.p. administration for 10 consecutive days. This method is suitable for studying Olanzapine pharmacokinetics and toxico-kinetics in single or multiple-dose studies.

Key words: Olanzapine, Bioanalytical, High Performance Liquid Chromatography.

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INTRODUCTION

Olanzapine {LY170053; 2-methy1-4-(4-methyl-1-piperazinyl) -10*H*- thieno[2,3-*b*] [1,5]benzodiazepine, OLA Figure 1} is an atypical neuroleptic drug for the treatment of schizophrenia. Pharmacological research has demonstrated that Olanzapine has nanomolar receptor affinity for dopamine D_1 –D_5, serotonin 5HT, $_{2A\ /\ 2B/\ 2C}$, $5HT_3$ and $5HT_6$ receptors. In addition Olanzapine is a potent antagonist of α_1 -adrenergic and histamine H_1 receptors [1, 2, 3]. It is important to note that the atypical antipsychotics offer many clinical benefits in the treatment of schizophrenia compared to traditional antipsychotics such as phenothiazines and butyrophenones, also called 'classical neuroleptic agents" and have emerged as first line therapy for schizophrenia[4].

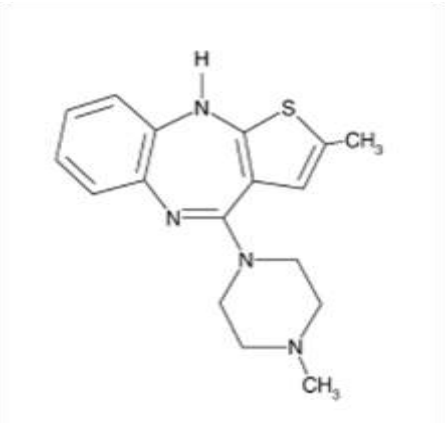


Figure 1: Chemical structure of Olanzapine

Differently from classical neuroleptic, OLA produces minimal extra pyramidal side effects or tardive dyskinesia. Animal studies indicated that Olanzapine has similar atypical pharmacological profiles to Olanzapine. It was effective in the treatment of both positive and negative symptoms of schizophrenia with low incidence of extra pyramidal motor symptoms. [5-6.] Bioanalytical methods for the quantitation of Olanzapine were developed to meet the requirements of drug disposition studies and therapeutic drug monitoring. The first published quantitation method for Olanzapine was developed by Goodwin et al. using gas chromatography-mass spectrometry (GC-MS) [7, 8]. Although this assay involved several extraction steps and derivatization. Several high performance liquid chromatography (HPLC) and liquid chromatography (LC)-MS methods have subsequently been reported for quantitative determination of Olanzapine in plasma and serum using solid phase extraction. Additionally, a few papers reported the determination of Olanzapine in human plasma and serum using liquid-liquid extraction for therapeutic drug monitoring[10, 11,12,13,14]. To the best of our knowledge, no

assay have been published for the determination of Olanzapine in both plasma and brain tissue using the same HPLC method. In order to determine the concentration of Olanzapine in plasma and brain tissue and study its pharmacokinetic properties in plasma, a sensitive analytical assay was developed utilizing a one step liquid-liquid extraction. Further, Preliminary assay were carried out using mobile phase with a mixture of ammonium buffer and methanol (70:30, v/v). This mobile phase resulted unsuitable for the analysis of OLA since interference from brain tissue homogenate was detected near to the Olanzapine chromatographic peak. Decreasing the methanol percentage from 30 to 20%, the interference from matrix was separated from OLA peak. This is the second advantage of this method.

MATERIALS AND METHODS

Olanzapine were purchased from Vega Biotech. Ltd., Baroda, and other chemicals of analytical grade purity were purchased from commercial sources. All solvents including water used for extraction and in the mobile phase were of HPLC grade purity.

Preparation of Calibration curve solutions

- Preparation of Solvent mixture:
  It was prepared by 60 volume of Acetonitrile and 40 volume of water.
- Preparation of standard curve solution and validation solutions:
  Stock solutions of Olanzapine were prepared by dissolving the 2.5mg of pure substances in 25 ml solvent mixture by sonication. This stock solution was further diluted with the same solvent mixture to obtain the working solution of 100-500 ng/ml.
- Preparation of ammonium buffer pH-2.5:
  3 g of ammonium dihydrogen orthophosphate was dissolved in 900 ml of water and add 2 ml of triethylamine was added. Finally made up the volume up to 1000 ml and adjust pH to 2.5 with ortho-phosphoric acid
- Preparation of Mobile Phase:
  80 vol. of ammonium buffer of pH-2.5 + 20 Vol. of Methanol

Chromatographic specifications

- Column : Phenomenex Luna C_{18} column (250 mm × 4.6mm, 5 µm)
- Flow rate : 1ml/min
- Injection volume : 10µl
- Detector : UV-Visible detector at 220 nm
- Runtime : 12 min.

- Temperature : 37ºC

Extraction procedure

In a 10 ml Borosilicate test tube, 1ml of Plasma/Brain tissue homogenate was taken. To this 1ml of known concentration of drug was added. The mixture was adjusted to pH 10 with 1ml of 1M carbonate buffer. Further, 5 ml of organic solvent (15% dichloromethane in n-hexane) was then added. The mixture was vortex mixed for 1 min and then centrifuged at 2000g for 5 min. After that, the organic layer, which contains the analyte of interest, was aspirated into a clean test tube and the solvent was evaporated. The residue was reconstituted in 250 µl of mobile phase and 10 µl was injected in HPLC.

Assay Validation parameters

- Specificity:

  Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix.

- System Suitability:

  These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns.

- Linearity:

  The linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation.

- Limit of Detection & Limit of Quantitation:

  The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitate. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

- % Recovery (accuracy):

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value. Accuracy is measured as the percentage of analyte recovered by assay, by spiking samples in a blind study.

Rat pharmacokinetic study

Female wistar rats received from Shree Dhanvantary Pharmaceutical Analysis and Research Centre were housed in groups of four under controlled conditions of temperature (22±2ºC) and humidity (65%) and were allowed free access to standard laboratory diet and tap water for three days. Olanzapine was administered intra-peritoneal at a dose of 20 mg/kg for 7 consecutive days. After administration of final dose of Olanzapine, blood and brain tissue homogenate(prepared by homogenizing brain tissue in 3ml of water) of animals were collected at respective time intervals (0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, and 24 hr). Then, extraction procedure was followed according to the previously discussed method and extracted samples stored at 4ºC before analysis.

RESULTS AND DISSCUSION

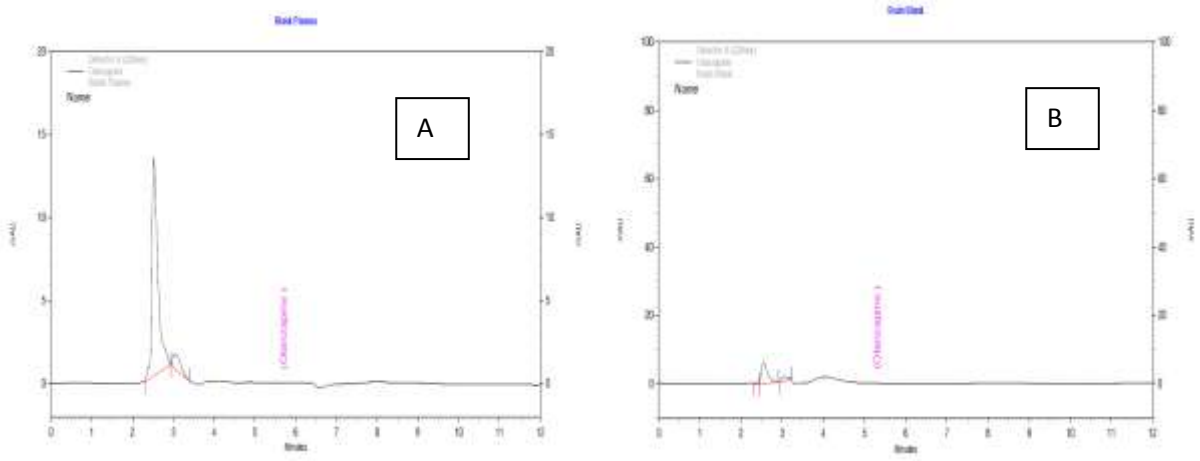
- The objective of the present work was development and validation of bioanalytical estimation of Olanzapine using HPLC with UV detector.
- Substantiation of developed bioanalytical method of estimation of drug Olanzapine in an endogeneous matrix of blood and brain.
- Use of developed method for the determination of pharmacokinetic profile of drug Olanzapine as conformational check through, about the developed method of estimation.
- In the present work the reverse phase HPLC method was developed using Phenomenex Luna C_{18} column (250 mm × 4.6 mm id, 5 µm particle sizes) with ammonium acetate Buffer and methanol in the ratio (80:20) as a mobile phase at the flow rate 1 ml/min. and detection wave length at 220 nm.
- Results of the study indicated clear advantage of developed bioanalytical method over the reported method of estimation of drug Olanzapine in that it is an isocratic estimation method which reduced the time of retention of drug in the column to a very great extent (i.e. time of eluting solvent run from 30 min to 12 min), which is advantageous in increasing column life span and there by number of runs injected to the column. As main problematic issues in the estimation of drugs from the biological matrix are interference from endogenous substances which interfere in the estimation as well as clog the column because of the high molecular weight of these substances. If developed method would reduce the runtime of estimation then

it helps in increasing life of column and number of runs given to the column, interfering substances lag time inside the column, decreased utilization of mobile phase, all these in other term increases the economy of the method.

- Another specific advantage of the developed method is use of n-hexane and dichloromethane in the ratio of 85:15 as an extraction solvent which was easy to evaporate and no residue being left after the evaporation. Acetonitrile and water at the ratio of 60:40, which was used as a diluents. When drug after extraction was mixed with the diluents no precipitation of extraction solvent was found, which was a major problem incurred during our study with reported drug extraction method.
- Developed bioanalytical method of estimation of the atypical antipsychotic drug Olanzapine showed validated system suitability parameters like theoretical plates per meter, asymmetry, %RSD for retention time and area were well within the limit of acceptance.

Specificity

Representative HPLC chromatograms of Olanzapine in rat plasma and brain tissue were shown in Figure 2. Olanzapine was detected around at 6.67 min. No interference peaks were observed at the retention time of Olanzapine for blank plasma and brain tissue samples.



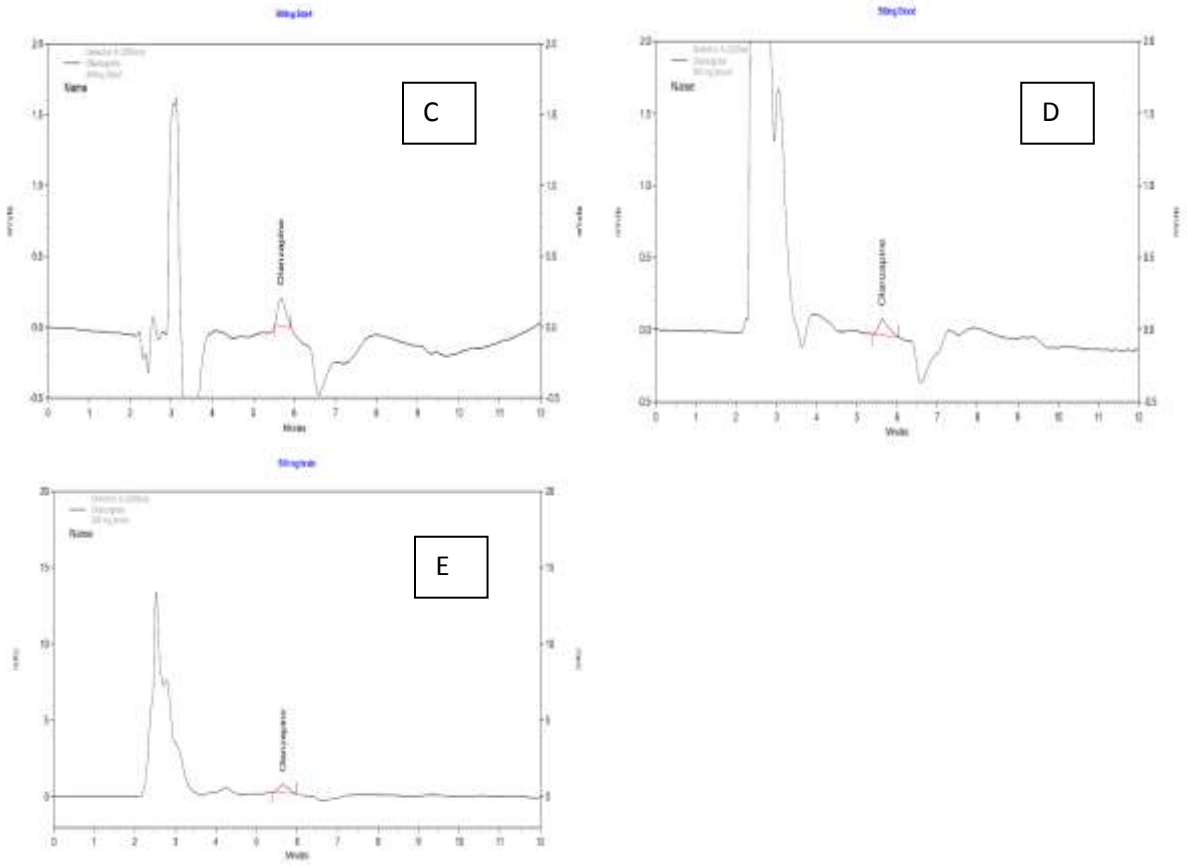


Figure 2. Representative HPLC chromatograms of (A) blank plasma sample (B) blank brain tissue homogenate (C) Olanzapine 500 ng/ml standard solution (D) blank plasma spiked with 500 ng/ml of Olanzapine (E) blank brain tissue homogenate spiked with 500 ng/ml of Olanzapine.

- Specificity of developed method indicated there was no visible peak in chromatogram with diluents and Blank plasma and Blank Brain homogenate, indicating a high degree of specificity for the proposed method.

Selectivity

Result: % RSD of retention time, area, theoretical plates and asymmetry (10%) were within the limit of acceptance criteria of system suitability shown in Table 1.

Table 1: System Suitability data for Olanzapine.

Name	No. of injection	Retention time (min)	Area	Theoretical plates	Asymmetry (10%)
Blank	-	-	-	-	-
	1	5.708	1057	1832.18	1.241
	2	5.684	1046	1831.26	1.239
	3	5.719	1079	1841.47	1.242
Olanzapine working	Average	5.704	1060.66	1834.97	1.240

standard.(100ng/ml)					
	SD	0.0194	16.802	5.647929	0.0015
	%RSD	0.34	1.58	0.003078	0.1231
	1	5.679	3196	2947.45	1.209
	2	5.637	3127	2932.59	1.203
	3	5.651	3205	2927.24	1.207
Olanzapine working	Average	5.655	3176	2935.767	1.206
standard.(300ng/ml)					
	SD	0.0213	42.673	10.48244	0.0031
	%RSD	0.3781	1.34	0.003571	0.2533
	1	5.677	4917	3938.47	1.227
	2	5.625	5014	3949.41	1.223
	3	5.65	4853	3954.72	1.221
Olanzapine working	Average	5.637	4928	3947.533	1.223
standard.(500ng/ml)					
	SD	0.0176	81.061	8.285954	0.0031
	%RSD	0.3136	1.64	0.002099	0.2497

Linearity

Calibration curves for Olanzapine (100-500 ng/ml) were obtained using quadratic least-squares regression of the peak height ration versus concentrations. A weighing factor of $1/x^2$ was applied to each standard curve. Linearity shown in Figure 3.

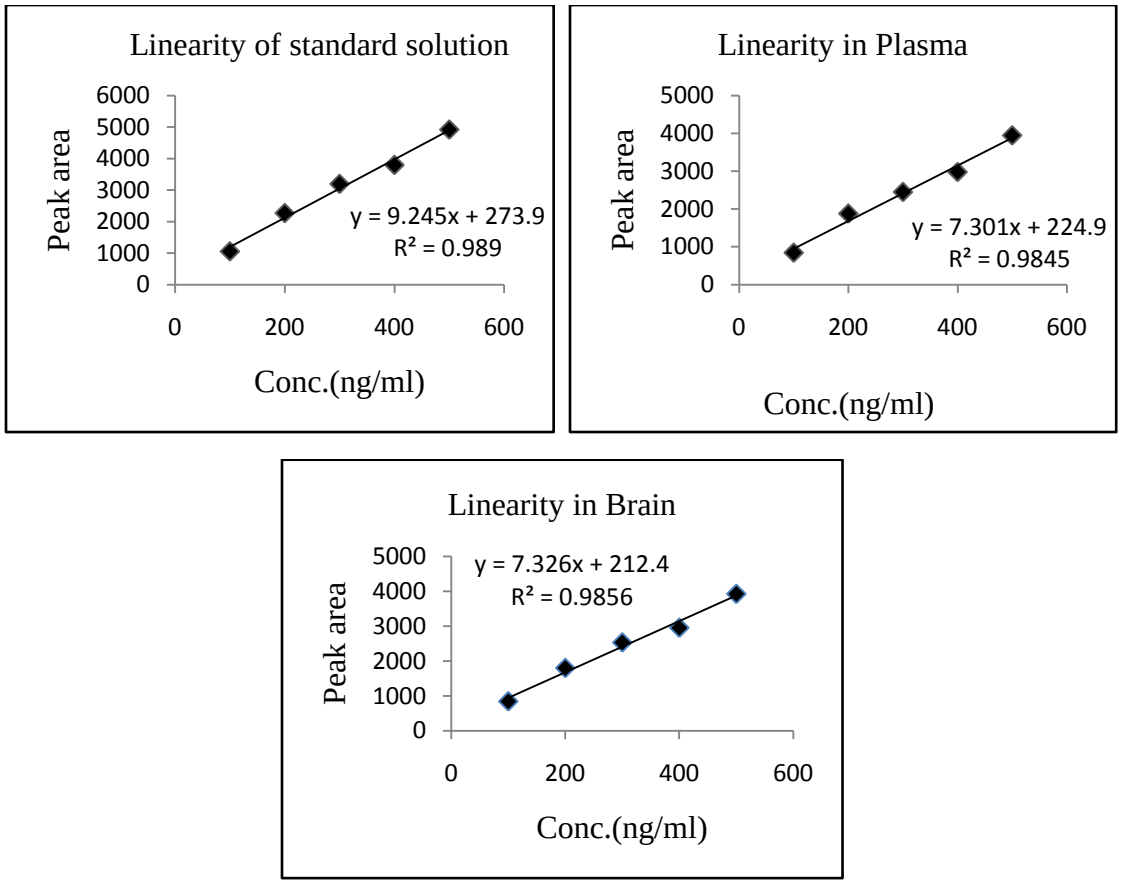


Figure 3: linearity of standers drug, drug in plasma, drug in brain

- Linearity in the estimation for the developed method for the drug Olanzapine was estimated in the range of 100-500 ng/ml of Olanzapine, which was found to be linear with the regression coefficient value of 0.988 in diluents matrix, 0.984 for blood plasma and 0.985 in brain tissue.

Limit of Detection and Limit of Quantitation.

LOD: The Limit of Detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value determined with statistical method by using Statistical formula. The limit of Detection (L.O.D.) was calculated as per below equation:

$$LOD = \frac{3.3\ X\ SD}{Slope}$$

LOQ: The Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with statistical method by using statistical formula. The limit of Quantification (L.O.Q.) was calculated as per below equation: LOD and LOQ value for Olanzapine shown in Table 2.

$$LOQ = \frac{10\ X\ SD}{Slope}$$

Table 2. Limit of Quantification (LOQ) & Limit of Detection (LOD) value for Olanzapine.

Parameters	Drug used (Olanzapine)
Slope	9.369
S.D.	86.69
L.O.D. (ng/ml)	27.78
L.O.Q. (ng/ml)	93.6

- Limit of detection for the drug Olanzapine in developed HPLC method of estimation was found to be 27.78 ng/ml and Limit of quantification for the drug Olanzapine in developed HPLC method of estimation was found to be 93.6 ng/ml which indicated drug Olanzapine even in the 20ng/ml can also be detected using developed method. The result of limit of quantification indicated that any concentration of the drug Olanzapine above 94 ng is an quantifiable using this developed method.

Recovery/Accuracy

The average recovery of analyte from plasma at different drug concentrations in both blood & brain were found to be 79.96% w/v and 78.55% w/v respectively shown in Table 3 & 4.

Table 3: Recovery data of Olanzapine from spiked plasma sample.

	Conc. Level (ng/ml)	Std. area	Spiked area	% Recovery	% mean Recovery	% RSD
BLOOD	100	1057	841	79.56	79.96	2.77
	200	2271	1874	82.56		
	300	3196	2446	76.54		
	400	3796	2973	80.97		
	500	4917	3942	80.18		

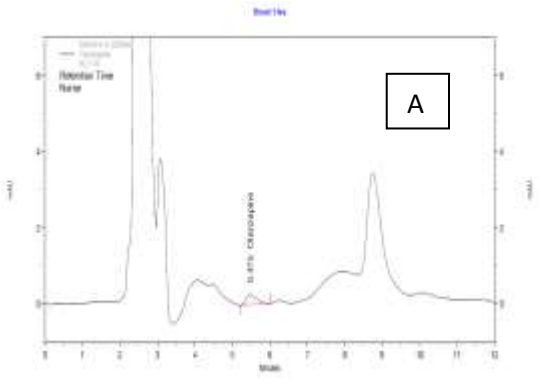
Table 4: Recovery data of Olanzapine from brain spiked tissue sample.

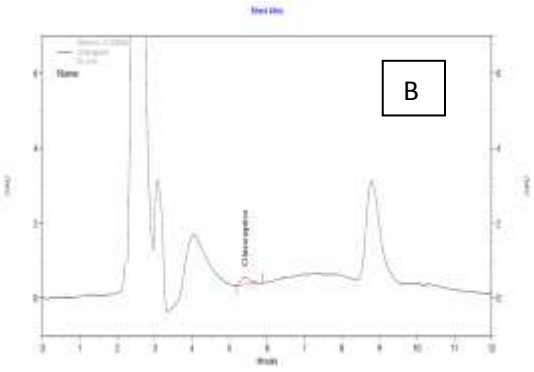
	Conc. Level (ng/ml)	Std. area	Spiked area	% Recovery	% mean Recovery	% RSD
BRAIN	100	1057	807	76.35	78.55	1.85
	200	2271	1788	78.76		
	300	3196	2502	78.30		
	400	3796	3034	79.91		
	500	4917	3926	79.85		

- Accuracy was determined through recovery studies of the Olanzapine. Olanzapine showed 79.96% w/v recovery of drug from blood plasma and 78.55% w/v recovery of drug from brain tissue. Percentage recovery from different biological matrix were found to be well within the acceptance limit, indicating practically no interference of the drug with the plasma content and brain homogenate of experimental animal.

Pharmacokinetic study

The analytical method has been successfully applied to a pharmacokinetic study on rat plasma and brain tissue homogenate following i.p. administration of Olanzapine for 7 consecutive days.





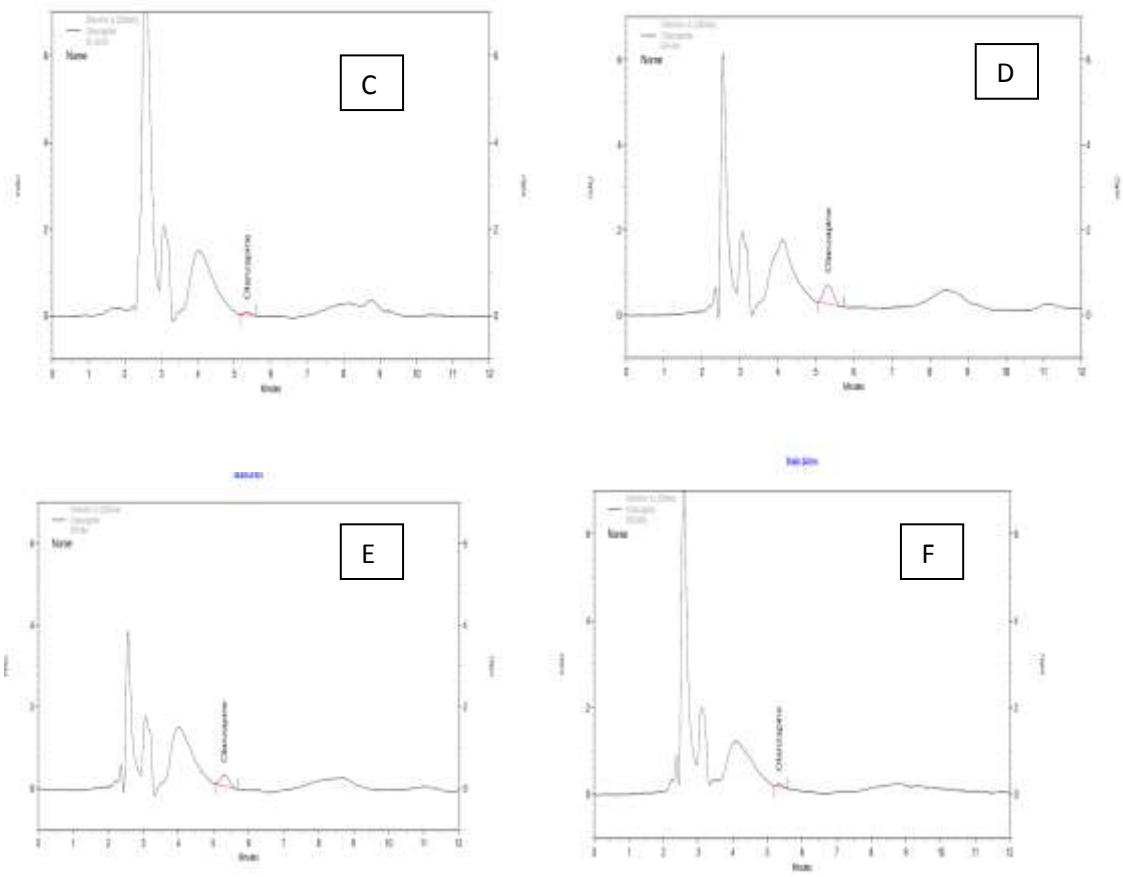


Figure. 4. Representative HPLC chromatograms of (A),(B) (C) represent an extracted blood plasma sample collected at 1,4, 24 hr respectively following a 20 mg dose of Olanzapine to a rat and (D),(E),(F) represent an extracted brain tissue sample collected at 1,4, 24 hr respectively following a 20 mg dose of Olanzapine to a rat.

Estimation of Olanzapine in plasma

C_{max} and T_{max} of Olanzapine in blood Plasma was found to be 452.53 ng/ml and1 hr, respectively shown in figure 5&6.

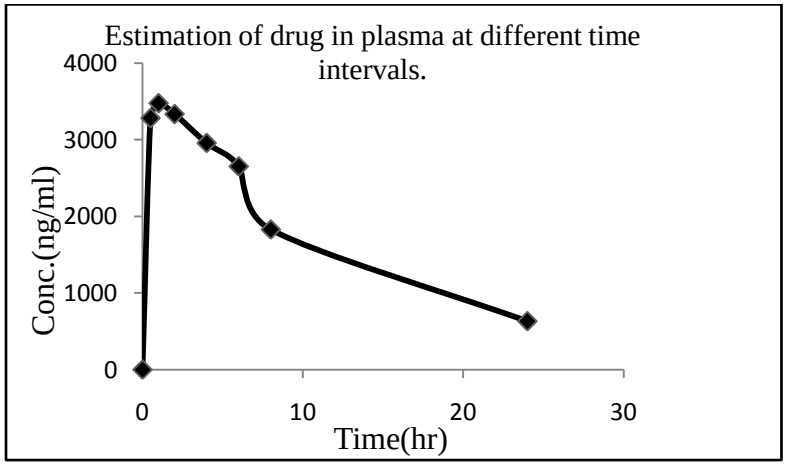


Figure 5. Graph showing plasma peak area with respect to time

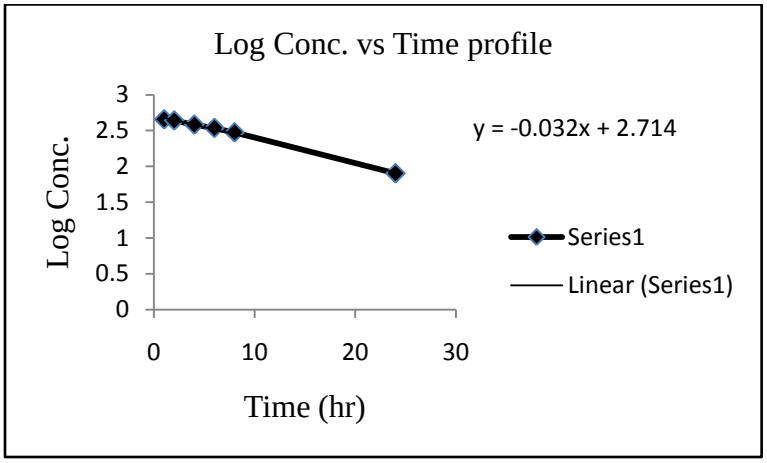


Figure 6. Log Conc Vs. time profile in plasma

Estimation of Olanzapine in brain tissue homogenate

Result: C_{max} & T_{max} of Olanzapine in brain was found to be 407.51 ng/ml and 1hr respectively shown in Figure 7.

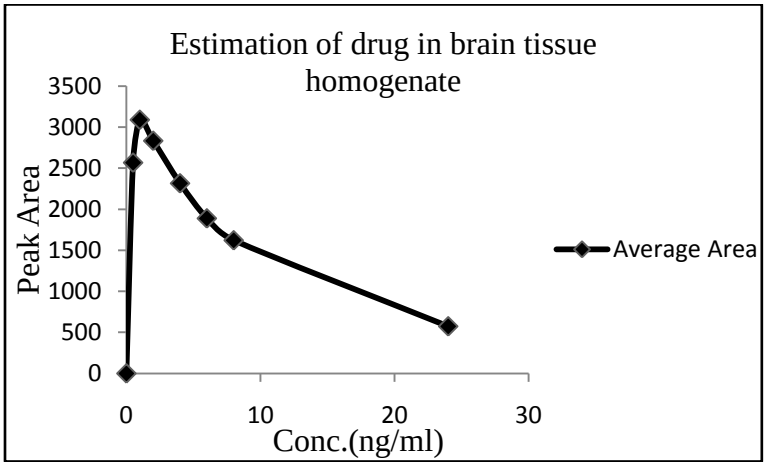


Figure. 7: Graph showing brain tissue drug concentration with respect to time.

Pharmacokinetic parameter estimation:was shown in table 5.

Table 5: Results of Pharmacokinetic Parameters:-

PK PARAMETERS	OLA	Units
Dose	20	mg/day
C_{max}	452.53	ng/ml
T_{max}	1	Hr
K_E	0032	hr^{-1}
$t_{1/2}$	9.42	Hr
V_d	3579.85	ml/kg
AUC_{0-t}	74.78	ng.hr/ml
CL_T	4.38	ml/min/kg

CONCLUSION

- The bioanalytical method developed for the determination of Olanzapine in endogenous matrix is rapid, sensitive and accurate. The method is quite faster with a run time of 12 minutes and achieves to address the advantageous features stated in the discussion over the reported method of quantization. The developed method exhibits a good range of quantization.
- Substantiation of developed bioanalytical method for estimation of drug Olanzapine in an endogenous matrix was good at both its specificity and accuracy.
- Developed method was found to be suitable in establishing pharmacokinetic profile of drug Olanzapine as conformational check through, about the developed method of estimation.
- Estimated pharmacokinetic parameters of drug indicated developed method is fit to carry out estimation of drug residues after BABE studies and clinical studies in experimental animals.

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