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Analytical Method Development and Validation of Anti-Psychotic drug Clozapine

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Abstract: Antipsychotic medications are used to treat psychiatric diseases and have a primary influence on psycho (mental processes). An accurate, precise, reproducible, easy, and speedy Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for quantifying the antipsychotic medication Clozapine was developed and validated in this study. The liquid chromatography was performed on an epic C12 analytical column with diameters of 250, 4.6 mm, and 5 mm, with a mobile phase of 70:30:0.1 v/v and a combination of methanol, water, and Trifluoro acetic acid. The sample was injected in a volume of 20 ml and measured at a flow rate of 1 ml/min at 245 nm (Methanol: water: TFA). The reference and sample medication had 3.43 minute retention times, respectively. Clozapine was shown to be linear in the calibration curve in the concentration range of 30-90g/mL, with a regression coefficient (R2) of 0.9998. The percentage recovery of clozapine was in the range of 98.10–103.65 percent, indicating that the current approach is extremely accurate. Reproducibility and recovery investigations revealed that the percent relative standard deviation with intra and inter-day precision was less than 2%, demonstrating that the devised approach was repeatable. The method was verified according to the (ICH) requirements, and it is the most reliable and quick approach for routine clozapine analysis. statistical validation of the data revealed that the suggested method can be used to estimate clozapine.

Keywords : antipsychotic, RP-HPLC, Clozapine, validation.

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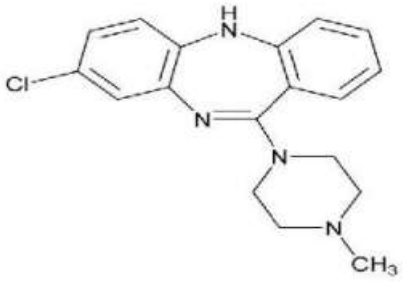
INTRODUCTION

Antipsychotic medications are used to treat psychiatric diseases and have a primary influence on psycho (mental processes). Antipsychotic drugs tend to block dopamine D2 receptors in dopaminergic pathways in the brain, reducing the action of dopamine produced in these pathways.[1] Clozapine has the chemical formula 3-chloro-6-(4-methylpiperazin-1-yl)-11H-benzo[b][1,4]. benzodiazepine [2] It acts by altering the chemical processes in the cerebrum. [3] It's used to treat severe schizophrenia, as well as to lower the risk of suicidal behaviour in patients with schizophrenia and other mental illnesses. It is also utilised in the treatment of Parkinson's disease. Clozapine has the following structure:



Chemical Structure of clozapine

Clozapine is included in the Indian Pharmacopeia6 as an official medication. The United States Pharmacopeia[7] and the British Pharmacopoeia[8] agree that a variety of analytical procedures are available for estimating Clozapine using the HPLC-UV method[9]. The goal of this work is to develop a simple, accurate, and time-saving RP-HPLC method for Clozapine measurement. This method is more efficient than the currently existing HPLC method. The ICH[10,12], and USP[11] guidelines were used to create and validate this approach.

EXPERIMENTAL WORK

Arochem pvt, Palghar sent a gift sample of clozapine standard. Clozapine pills with the label Clozapine (100mg) were acquired at the local market. All of the chemicals were HPLC quality and were purchased from Finar Limited in Ahmedabad, Gujarat, India. All HPLC solvents and solutions were filtered via a 0.2 pore Nylon membrane filter.

INSTRUMENTS AND CHROMATOGRAPHY CONDITION

Younglin SK Acme 9000 RP-HPLC system with Autochrome 3000 Software and Isocratic UV-Visible Detector was used. The ATX224 Shimadzu was used to weigh the standard and sample clozapine. The chromatographic conditions included utilising an epicC12 analytical column (250mm X 4.6mm, 5g internal diameter) with a mobile phase of Methanon:water:TFA (70:30:0.1 v/v). A total of 20 cc of material was injected. At a wavelength of 245 nm, the flow rate was changed to 1 ml/min.

SELECTION OF LAMBDA MAX:

The UV spectrophotometer selection of lambda max is critical for the RP-HPLC method's sensitivity. An optimal wavelength can detect an exact absorption for any substance. A UV-1700 spectrophotometer was used to test quantities of pure clozapine ranging from 1 to 10 mg/ml utilising scanning in the 200–400 nm range (Shimadzu, Japan)

PREPARATION OF STANDARD STOCK SOLUTIONS:

A 61mg clozapine working standard was carefully weighed and put into a 100 ML volumetric flask. To dissolve the medication, 5.0 mL of HPLC-grade methanol was added to the volumetric flask and sonicated. The solution was cooled to room temperature before being topped up with HPLC grade methanol, yielding final concentrations of 1000 gm/ml (stock solution) clozapine.

PREPARATION OF SAMPLE SOLUTIONS:

Clozapine pills (sizipine100) were weighed and pulverised coarsely into a powder. An equal quantity of 20.91 mg clozapine was collected from this powdered tablet and diluted with methanol in a volumetric flask with a capacity of 100 ml, followed by 15 minutes of sonication. The basic RP-HPLC technique was used to examine a final concentration sample solution of 100 mg/ml.

METHOD OF ANALYSIS:

The chromatographic condition was maintained as stated and the baseline stabilization was performed for 20 min. After stabilization, the prepared concentration solution of the standard drug was recorded for reproducibility at the respective peak areas. The solution of the sample was injected for quantification. The response factor of standard peak ratio and sample peak ratio was calculated. The same procedure was repeated six times for conforming reproducibility of the developed method.[13]

RESULTS AND DISCUSSION:

DEVELOPMENT OF RP-HPLC METHOD:

Method development on clozapine done by RP-HPLC method. The mobile phase containing methanol as an organic phase and water as an aqueous phase and 0.1% Trifluoro acetic acid was used. To obtain a sharp and symmetric peak at acceptable retention time a chromatographic condition such as flow rate, column temperature and components ratio in the adopted mobile phase were investigated. The chromatographic conditions were kept constant, and the baseline stabilisation took 20 minutes. The produced concentration solution of the reference medication was tested for repeatability at the corresponding peak locations after stabilisation. For quantification, the sample solution was injected. The standard peak ratio and sample peak ratio response factors were determined. The identical approach was done six times to ensure that the new method was reproducible.[13]

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Column : Epic (C12), 250 x 4.6 mm i.d. 5µm particle size

Mobile Phase : Methanol: Water: Trifluoro acetic acid (70:30:0.1)

Flow Rate : 1.0 ml/minute

Wave length : 245 nm

Injection volume : 20 µl

Run time : 08 minutes

Column temperature : Ambient

λ Max: clozapine 245

Injection Volume: 20µl

Retention Time: Clozapine 3.43

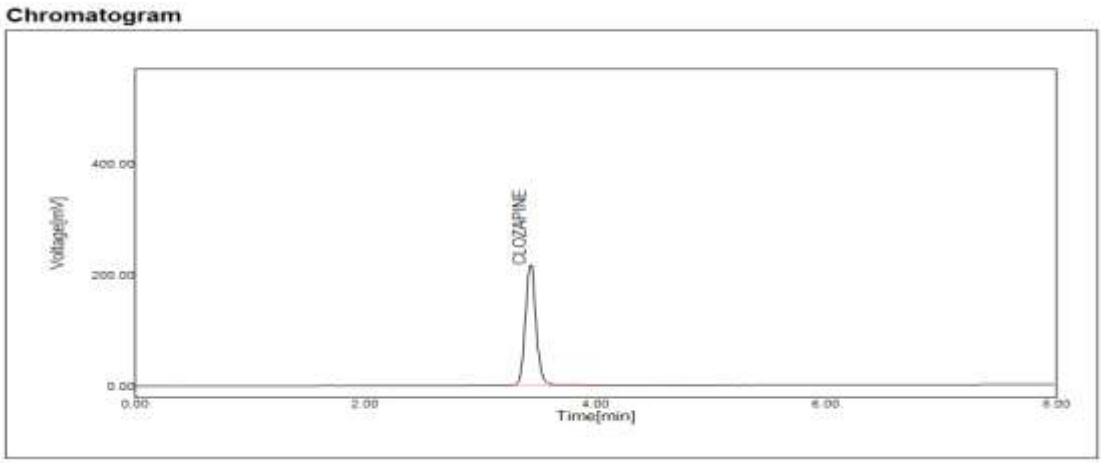


Fig 2: Optimized Chromatographic Condition

METHOD VALIDATION

SYSTEM SUITABILITY STUDIES:

The United States Pharmacopeia was used to determine system appropriateness (USP). For the five duplicate injections, the produced concentration solution of clozapine was utilised to determine metrics such as column efficiency, resolution, peak symmetry factor, percentage coefficient in peak area, or height14. The percent RSD, theoretical plate value, and tailing factor were all calculated using the observed value.

Table 1: System suitability parameters

Sr. No.	Parameter	Limit	Result
1.	Tailing factor	T ≤ 2	1.02
2.	Theorotical plates	> 2000	13666
3	%RSD	(NMT 2)	1.34

LINEARITY

To test the linearity of the analyte, a series of concentrations ranging from 30.20, 45.30, 60.40, 75.50, and 90.60g/ml were generated by diluting the stock (100g/ml) solution with mobile phase. 20l injections of each concentration were injected into the HPLC apparatus and chromatographed under optimum conditions using these solutions. By graphing the mean peak area (Y-axis) versus the concentration, a calibration curve[15] was created (X-axis). Table 2 shows that the findings were within acceptable bounds.

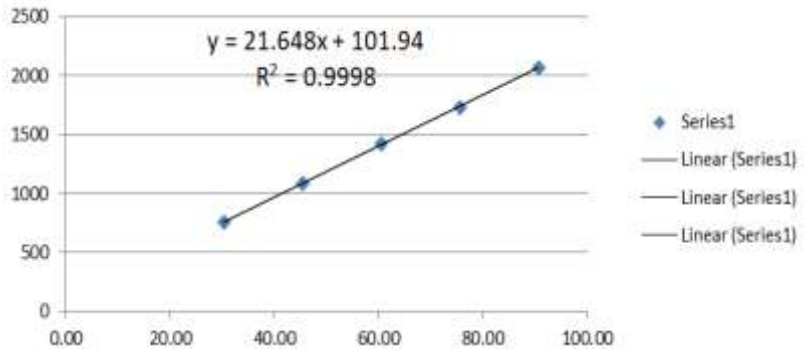


Fig 3: Standard Calibration curve of Clozapine

Table 2: Linearity results for Clozapine

Concentration of Clozapine in ppm or µg/ml	Peak area of Clozapine
0	0
30.20	754.2905
45.30	1080.4187
60.40	1419.7045
75.50	1728.2856
90.60	20648057
Intercept	**101.94**
Slope	**21.648**

The calibration curve for Clozapine (API) demonstrated high linearity in the range of 0-90.60g/mL g/ml, with a correlation value (r2) of 0.9999. (Fig-3). For Clozapine, a common calibration curve has the regression equation y = 21.648x +101.94 = 0.9998.

ACCURACY:

Recovery study:

The approach's accuracy was determined by estimating the clozapine recovery using the spiking method. This accuracy research was carried out by adding a known amount of clozapine to a pre-determined sample solution and determining the amount of clozapine by measuring peak area. The mean percent recovery values are within the acceptable range (limit is 98 percent and 103 percent).

Table 3: Recovery studies of CLOZAPINE.

Level of % Recovery	Ref. Std. (mg)	Amount of Std. Drug Added (µg/ml)	Amount Found (µg/ml)	Total Amount Recovered (µg/ml)	%Recovery (97 to 103%)	Peak Area
80 %	60	48.00	108.6761	48.6761	101.41	2668.0022
	60	48.00	107.7536	47.7536	99.49	2645.3548
	60	48.00	107.9170	47.9170	99.83	2649.3664
100%	60	60.00	118.9188	58.9188	98.20	2919.4607
	60	60.00	119.8293	59.8293	99.72	2941.8146
	60	60.00	119.5458	59.5458	99.24	2934.8557
120%	60	72.00	131.9358	71.9358	99.91	3239.0288
	60	72.00	132.7287	72.7287	101.01	3258.4964
		72.00	131.9677	71.9677	99.96	3239.8124

Table 4: Statistical Validation of Recovery Studies

Level of % Recovery	Clozapine		
	Mean*	±SD	%RSD
80%	100.24	1.0255	1.02
100%	99.05	0.7766	0.78
120%	100.29	0.6235	0.62

PRECISION

INTERMEDIATE PRECISION:

Intra-assay & inter-assay: Precision is defined as the measuring of real value between different quantities' outcomes. Analyzing concentration solutions ranging from 61 to 100 mg/ml on the same day and two separate days revealed intra-day and inter-day variability. The following equation was used to calculate the percent relative standard deviation (RSD). The normal acceptability limit for percent RSD is less than 2% .[16]

Table 5: Result and statistical data for intraday precision study

Set	Preparation	Wt of sample mg	Area	Assay
Morning	Test Prep 1	61	1387.9708	99.31
	Test Prep2			99.80
Evening	Test prep1	61	1386.8976	98.25
	Test Prep2			98.35
Mean				98.93
SD				0.7528
%RSD				0.76

Table 6: Result and statistical data for interday precision study

Days	Preparation	Wt of sample mg	Area	Assay
Day 1	Test Prep 1	59.9	1443.9918	99.31
	Test Prep2			99.80
Day 2	Test prep1	59.7	1428.7612	99.83
	Test Prep2			99.11
Mean				99.51
SD				0.3589
%RSD				0.36

ROBUSTNESS

The impact of small changes in chromatographic conditions such as flow rate (0.1ml/min), wavelength of detection (2nm), and mobile phase concentration (2%), which were studied to determine the method's robustness[16], are also in favour of the developed RP-HPLC method for the analysis of clozapine (Table-8, percent RSD 2%). (API).

Table 7: Robustness of clozapine

Variables	Clozapine			
	RT (min)	Area	TF	TP
Flow rate (+02 ml/min)	3.12	1293.0657	1.09	15326
Flow rate (-0.2 ml/min)	3.78	1607.9254	1.17	16585
Mobile phase (+2%)	2.98	1229.4663	1.03	14058
Mobile phase (-2%)	4.10	1712.1003	1.16	8850
Change in Wavelength (+2)	3.40	1400.9681	1.09	11932
Change in Wavelength (-2)	3.42	1462.4390	1.11	11518

Table 8: Result and statistical data of Robustness for clozapine

Variables	Clozapine		
	Mean	**SD**	**%RSD**
Flow rate (+0.2 mL.min-1)	99.79	0.7826	0.78
Flow rate (-0.2 mL.min-1)	99.63	0.6385	0.64
Mobile phase (+2%)	99.13	0.7424	0.75
Mobile phase (-2%)	99.45	0.4784	0.48
Change in avelength(+2)	99.47	1.1008	1.11
Change in mWavelength(-2)	100.17	0.8335	0.83

LOD & LOQ

The LOD stands for the lowest detection limit. The LOQ is the smallest concentration that can be determined quantitatively. Taking into account the steyx and the slope The detection limit (LOD&LOQ) can be written as

$$\mathbf{LOD} = \frac{3.33XSTEYX}{SLOP}$$

$$\mathbf{LOQ} = \frac{10XSTEYX}{SLOP}$$

where,

S = Slope of Calibration Curve

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) [were found to be µg/ml & 1.07 and 3.57µg/ml respectively.

APPLICATION OF PROPOSED METHOD TO MARKETED FORMULATION

The test Clozapine chromatogram is shown in its simplest form (Fig No4) Extrapolating the area value from the calibration curve, the quantities of Clozapine per tablet were estimated. The analysis was conducted five times using tablet formulation tablet Assay for percent Label claim for percent RSD Calculated, and the result was shown in **(Table No.9,10).**

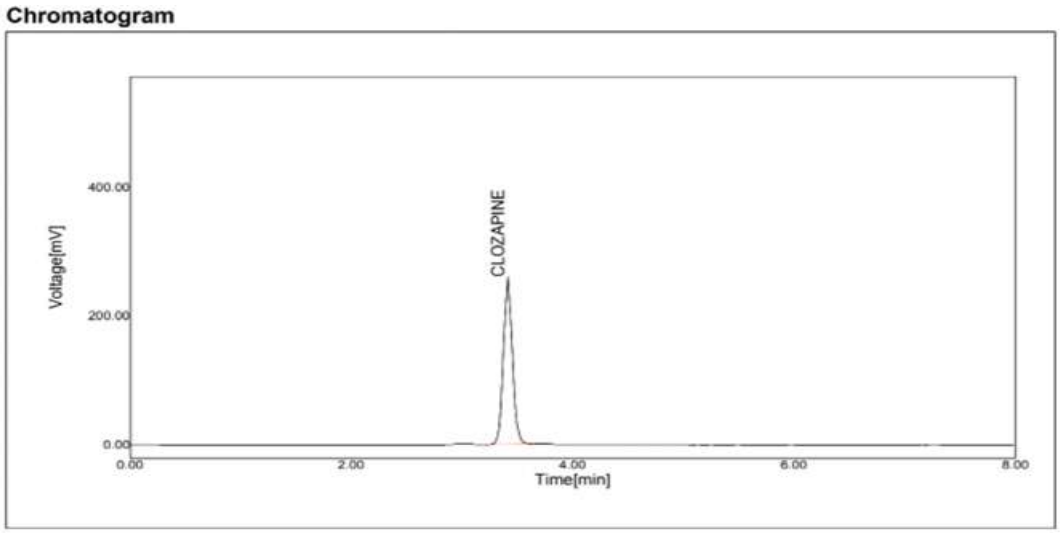


Fig 4 Sample Chromatogram of Clozapine of Marketed formulation

Table.9 Results for estimation of Clozapine in marketed formulation

Sr no	Conc.(.(µg/ml)	Area	Label claim
1	116.8	1448.4298	100
2	117.9	1473.5581	100
	Mean	1473.5581`	
	SD	12.56	
	%RSD	0.8	

Table. 10. Statistical data for estimation of Clozapine in marketed Formulation

Sr. No.	Clozapine
	Assay (%)
1	97.68
2	98.44
Mean	98.06
SD	0.38
% RSD	0.38

The proposed method was applied to the determination of Clozapine in marketed formulation. The mean % amount found was 98.06 (Table No20.) with % RSD values was NMT 2.0% indicates the developed method was successfully applied for analysis of marketed formulation. All the results found were in good agreement with the label content of marketed formulation.

CONCLUSION

Attempts to develop Clozapine estimating methods have been successful in the current project effort. In the current study, the RP-HPLC technique was used. Clozapine was determined using a new simple, rapid, precise, and accurate RP-HPLC technique that was developed without interference from other active components or excipients. According to the ICH Q2 (R1) Guidelines, the created approach was validated (Validation of Analytical Procedures: Text and Methodology). The analytical method for determining Clozapine test conforms with the analytical parameters' approval requirements. As a result, the method is considered valid. The procedure can be used for quality control on a regular basis.

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