

Reverse phase Liquid chromatographic method for the estimation of clozapine from tablet dosage forms

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Abstract : A simple and specific reverse phase high performance liquid chromatographic method was developed and validated for the estimation of clozapine in tablet dosage forms. A Lachrom C₁₈ column having 250 x 4 i.d in an isocratic mode with mobile phase containing acetonitrile: phosphate buffer (70:30% v/v) was used. The flow rate was 1.0 ml/min and the retention time of clozapine was found to be 3.06 min. The method was validated for specificity, linearity, accuracy, limit of quantification and limit of detection. The limit of quantification and limit of detection for the estimation of clozapine were found to be 0.1 µg and 0.5 µg respectively. Experimentally developed fast dissolving tablets of clozapine showed recovery levels in the range of 98.88-100.32 %, while assay of marketed samples showed a recovery of 97.85 to 101.45 % of their label claim. It was concluded that the proposed method could be an easy and economic method for the quantitative determination of clozapine in tablet dosage forms.

Key words: Clozapine, RP-HPLC, C₁₈, fast dissolving tablets

1. Introduction

Clozapine belongs to the chemical class of tricyclic dibenzodiazepine and is chemically 8-chloro-11- (4-methyl-1-piperazinyl)-5H-dibenzo [b,e][1,4] diazepine¹. An atypical antipsychotic drug, it is indicated for the management of severely ill schizophrenic patients who fail to respond adequately to standard drug treatment for schizophrenia. Physicochemical properties of clozapine are indicated in Table I.

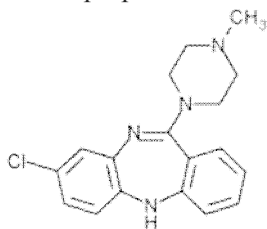


Figure 1: Structure of clozapine

Table I: Physicochemical properties of clozapine²

| | |
|---------------------------|--|
| Mol. wt | 326.83 |
| Melting point | 183-184°C |
| pKa | 3.7 & 7.6 |
| o/w Partition coefficient | 0.4 (pH 2) 600 (pH 7) 1000 (pH 7.4) 1500 (pH 8.0) |

The USP official method for the estimation of clozapine from tablets is by HPLC using C₈ column. Various methods have been reported for estimation of clozapine in biological matrices such as plasma. Clozapine having a strong chromophore shows UV absorption and hence most of these methods include the use of HPLC with UV detector^{3,4,5,6}. Spectrophotometric methods have also been developed for estimation of clozapine in samples of greater purity^{7,8}. Stability indicating methods have also been reported for its *in vitro* determination in gastric and intestinal fluids and pharmaceutical formulations⁹. Most of the reported HPLC methods use the C₈ column. However, in small-scale industries, educational institutions or moderate budget analytical sections, it may be difficult to have dedicated columns for analysis. In such cases a C₁₈ column is mostly preferred since most drugs have a considerable lipophilicity and can be analyzed using this column. The purpose of this work was to develop a reproducible, robust and sensitive method for the determination of clozapine from tablets using C₁₈ column.

2. Experimental

2.1. Chemicals

Pure clozapine was a gift sample from Torrent pharmaceuticals, Ahmedabad. All the solvents used were

of HPLC grade. Fast dissolving tablets of clozapine were prepared in the laboratory. Clozaril, a marketed sample was commercially procured for analysis.

2.2. Instrumentation

The estimation was carried out using a basic level isocratic system. The liquid chromatographic system consisted of the following components: Merck Hitachi Lachrom model containing L-7110 pump, variable wavelength programmable UV/VIS detector L-7400 and Rheodyne injector (7725i) with 20 μ L fixed loop. Chromatographic analysis was performed using winchrom software on a lichrospher-100 C18 column with 250 x 4 mm i.d. and 5 μ m particle size.

2.3. Determination of λ_{\max} of clozapine by UV spectrophotometric method

Clozapine was scanned using a UV spectrophotometer to determine its λ_{\max} . The drug was further scanned individually with the commonly used tablet excipients using different mobile phase to determine the effect of these excipients on λ_{\max} of the drug. Based on these studies, the λ_{\max} selected for the determination of clozapine was 290 nm.

2.4. Optimization of the mobile phase

Optimization of mobile phase was performed based on resolution, asymmetry factor and peak area obtained. Mobile phase was prepared using various combinations of polar and non-polar solvents. The pH of the mobile phase was varied from 5.8 to 8.3 using various ratios of 0.05 M phosphate buffer. The buffer was prepared by dissolving 2.04 g of potassium dihydrogen phosphate in 300 ml water and filtered using membrane filter. This solution was then mixed with required quantity of acetonitrile and the solution was sonicated for 20 mins before use.

2.5. Analytical blank

All the reagents used in analysis were mixed without the drug and the analytical blank was injected into the column and signal obtained was noted. This was repeated six times and the standard deviation was calculated.

2.6. Preparation of standard solution of clozapine

A stock solution of clozapine was prepared by accurately weighing 50 mg of drug, transferring to 100 ml volumetric flask, dissolving in 15 ml of methanol and diluting it upto mark with water. Appropriate aliquot of this solution was further diluted with 50 ml of water to obtain final standard solution of 5 μ g/ml of clozapine. Resultant solution was filtered through Whatman filter paper # 42 and then used. Further aliquots were prepared and diluted to 10 ml to obtain final concentration of 0.05, 1, 2, 3 and 4 μ g/ml of clozapine.

A reverse phase C-18 column was equilibrated with the prepared mobile phase. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 290 nm. The sample was injected using a 20 μ L fixed loop, and the total run time was 10 min. The solutions were injected into chromatographic system and chromatograms were developed and peak area ratio was determined for each concentration of drug solution. Calibration curve of clozapine was constructed by

plotting peak area ratio vs. applied concentration of clozapine and regression equation was computed.

2.7. Method validation¹⁰

The method was validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and solution stability

2.7.1. Limit of detection

This is the smallest amount of the drug, which can be detected by this method. This was determined by injecting much diluted solutions of clozapine with concentrations in decreasing order and appearance of the peaks was noted. Limit of detection was determined by the formula

$$x - x_B = 3 S_B$$

where x is the signal from the sample, x_B is the signal from the analytical blank and S_B is the SD of the reading for the analytical blank.

2.7.2. Limit of quantification

The limit of quantification is the smallest amount of the drug, which can be quantified reliably. It was calculated using the formula

$$x - x_B \geq 10 S_B$$

2.7.3. Robustness

Robustness was evaluated in terms of pH sensitivity and change in organic phase of the mobile phase. pH was changed by ± 0.5 and the organic phase composition was varied by $\pm 5\%$. In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 h at room temperature. Changes in terms of peak area and retention time were noted.

2.7.4. Selectivity

This parameter was determined by mixing drug solution with other excipients and noting the measure of separation.

2.7.5. Sensitivity

Sensitivity of the method was determined by deliberately changing the concentration of the analyte and noting the change in the slope of the response curve to a minute change in analyte concentration.

2.8. Analysis of clozapine from tablets

Sample solution containing tablet powder equivalent to clozapine concentration in standard solution was prepared. The triturated tablet powder equivalent to 25 mg of clozapine was sonicated with 15 ml methanol for 20 mins. The volume was made upto 50 ml with water and the solution was filtered using Whatman filter paper (# 42). The sample solution was chromatographed similar to the standard solution and concentrations of clozapine in tablet samples were found out using regression equation.

3. Results and Discussion

3.1. Method development

Clozapine is usually available as a single ingredient tablet of strength 25 mg and 100 mg. USP recommends HPLC for analysis of clozapine in dosage forms with C_8 column, which is less lipophilic than the traditional C_{18} column. The partition coefficient values of clozapine indicate that

the drug is strongly lipophilic and has two pK_a values 3.7 and 7.6. The structure also indicates that octadecylsilane group of the C_{18} column would be able to bind the drug. Hence an attempt was made to analyse the drug using C_{18} column.

The drug has a strong chromophore and the reported absorption maxima was 290 nm. Experimentally determined absorption maxima was found to match with the reported values. The molar extinction coefficient of the drug in acetate buffer (pH 4) was found to be 331211.8 which indicates a sensitive method can be developed on the basis of UV absorption.

Initial studies were carried out using a concentration of 1 $\mu\text{g}/\text{ml}$. The use of buffer was initially avoided and a strongly lipophilic mobile phase (75:25% v/v of acetonitrile: water) was used for the studies. When the drug solution was injected into the column, a peak was obtained with a retention time of 10 min. However this peak was bifurcated. A small peak just ahead of the main peak was noted. Since the drug has two pK_a values 3.7 and 7.6, at the pH of the mobile phase, the drug might have undergone partial ionization (9-10%), which could have resulted in the smaller peak. Decreasing the concentration of acetonitrile in the mobile phase to 30% increased the retention time and resulted in a broad peak, which was not bifurcated. The high values of retention time was also not acceptable since it increases the time of analysis. Since the drug has a strong lipophilicity due to the presence of aromatic groups, it was thought that even ionized moiety can be sufficiently lipophilic to effect proper separation. To obtain a single moiety the pH had to be kept either at 9.6 (where all the moieties would be totally unionized) or at 1.6 (where they would be completely ionized). However, this may not be practically possible because the pH limit for C_{18} columns is 2.5-7.5. Also at higher pH, silica column gets ionized, basic drug gets bonded to silica and results in tailing of the peaks.¹⁰

Hence finally a pH of 5.8, which is a value obtained by removing two units equally from both pK_a values was chosen. For this acetonitrile: 0.05 M phosphate buffer (70:30) was used. At this pH, a clear

sharp peak with remarkably lesser retention time(3.06 mins) was obtained.

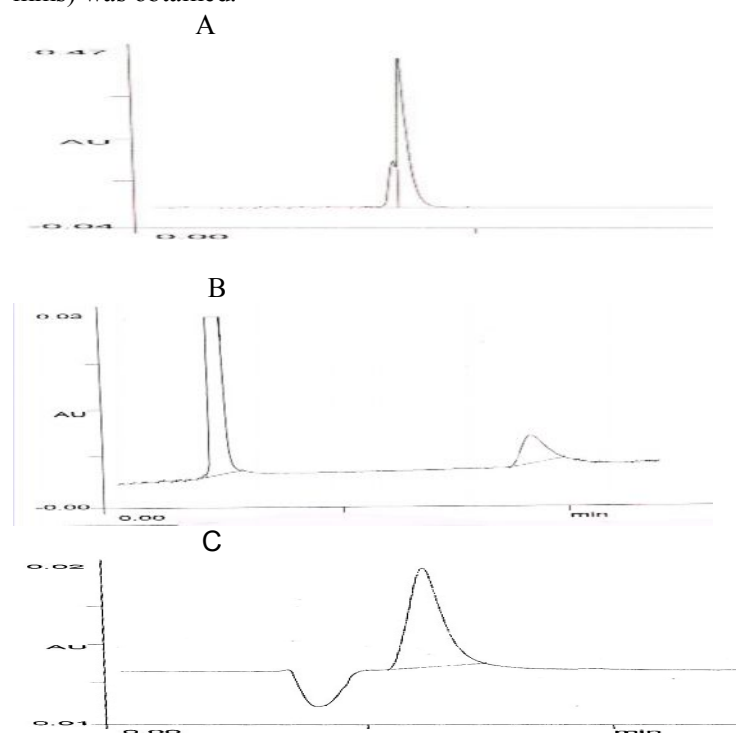


Figure 2: Chromatogram of clozapine obtained with various mobile phase A. acetonitrile: water 75:25) B. acetonitrile :water (30:70) C. acetonitrile: buffer (70:30)

3.2. Validation of the method

Calibration curve for clozapine was developed using clozapine over the concentration range of 1-5 $\mu\text{g}/\text{ml}$ (Figure 3) The slope and intercept value for calibration curve was $y = 247165x + 16797$, and it was found to be linear over entire calibration range studied with R^2 value of 0.998. Detection limit for clozapine was found to be 0.1 $\mu\text{g}/\text{ml}$ and quantification limit was 0.5 $\mu\text{g}/\text{ml}$, which suggest that the method was suitable to analyse the microgram levels of the drug. The validation parameters are summarized in Table II.

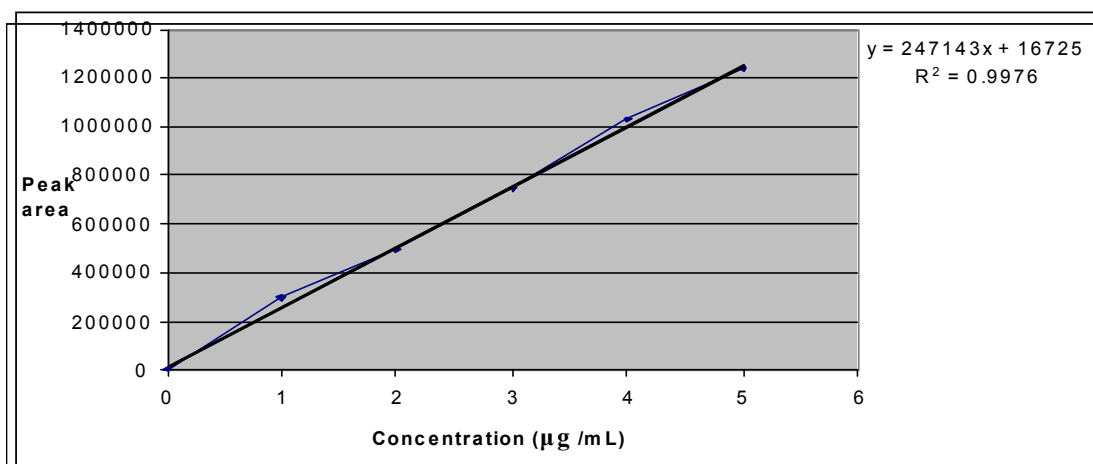


Figure 3: Calibration curve of clozapine

Table II. System suitability parameters

| Sl.No. | Parameters | Observed values |
|--------|-------------------------|-----------------|
| 2 | Tailing factor | 0.3461 |
| 3 | Retention time | 3.06 mins |
| 4 | Calibration range | 1-5 µg /ml |
| 5 | Limit of detection | 0.1 µg /ml |
| 6 | Limit of quantification | 0.5 µg /ml |

3.3. Analysis of clozapine from tablets

Recovery of clozapine was found to be in the range of 98.88-100.32 % from in house developed fast dissolving tablets and 97.85 to 101.45 % from marketed samples as indicated in Table III. The result were comparable with the corresponding labeled amount The test for robustness showed that retention time and peak area of clozapine remained almost unchanged when the pH and

composition of organic phase of the mobile phase were changed by ± 0.5 and $\pm 5\%$ respectively.

Table III : Assay results of clozapine from tablets using the proposed method

| Formulations | Labelled amount (mg) | % recovery* |
|--------------|----------------------|-------------------|
| A | 25.00 | 99.31 \pm 0.871 |
| B | 25.00 | 100.70 \pm 2.55 |

* average of 3 readings.

4. Conclusion

In conclusion, the developed HPLC method was found to be suitable for the determination of clozapine from dosage forms using C₁₈ column.

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