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Research Article

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## Simultaneous Estimation Amitriptyline Hydrochloride and Chlordiazepoxide by HPLC Method

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### ABSTRACT

A simple, economic, selective, precise, and accurate Reverse Phase High Performance Liquid Chromatography method for analysis of Amitriptyline HCl & Chlordiazepoxide in tablet dosage form was developed and validated according to ICH guidelines. The quantification of the drug was carried out by using Agilent Zorbix C18 (250 X 4.6 mm, 5 $\mu$ ) column its equivalent in isocratic mode and maintain column at 30°C, using mobile phase comprising of potassium dihydrogen phosphate buffer: Acetonitrile in the ratio of 50:50s v/v with a flow rate of 1.0ml/min and the detection wavelength was carried at 274 nm. The retention time for Amitriptyline HCl & Chlordiazepoxide was found to be 1.975min & 2.773min. The percent assay was found to be 100% & 99%. The method was validated and the response was found to be linear in the drug concentration range of 50 $\mu$ g/ml to 150 $\mu$ g/ml for Amitriptyline HCl and 50 $\mu$ g/ml to 150 $\mu$ g/ml for Chlordiazepoxide. The LOD and LOQ for Amitriptyline HCl were found to be 2.53  $\mu$ g/ml, 8.44  $\mu$ g/ml respectively. The LOD and LOQ for Chlordiazepoxide were found to be 1.961  $\mu$ g/ml, 6.53  $\mu$ g/ml respectively. This method was found to be good percentage recovery for Amitriptyline HCl and Chlordiazepoxide were found to be 100.00 and 100.00 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was successfully applied to Amitriptyline Hydrochloride and Chlordiazepoxide combination Tablet dosage form.

**Keywords:** Amitriptyline HCl, Chlordiazepoxide, Zorbix C18, Acetonitrile, Tablet.

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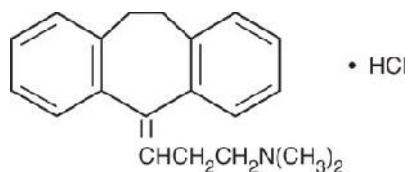
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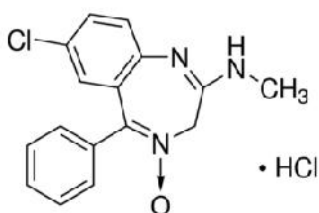
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## 1. Introduction

Amitriptyline hydrochloride (AMI) is chemically 3-(10,11-dihydro-5h-dibenzo[a,d] cyclo heptane-5-ylidene)-N,N-dimethyl-1-propanamine hydrochloride<sup>1</sup> (Figure 1). Its molecular formula is C<sub>20</sub>H<sub>23</sub>N·HCl having molecular weight of 313.86 g/mol<sup>2</sup>. It is a tricyclic anti-depressant in case of anxiety and also exerts anticholinergic activity. Amitriptyline acts primarily as a serotonin-norepinephrine reuptake inhibitor, with strong actions on the serotonin transporter and moderate effects on the norepinephrine transporter. AMI is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). Official methods have been used non-aqueous titrations<sup>2&3</sup> and acid-base titration<sup>4</sup> for assay of AMI. Chlordiazepoxide (CLR) is chemically (7-chloro-2-(methylamino) -5- phenyl -3-H-1,4-benzodiazepine-4-oxide) [2] (Figure 2). Its molecular formula is C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O having molecular weight of 299.755 g/mol<sup>2</sup>. Chlordiazepoxide acts on benzodiazepine sub receptors of the main GABAA receptor and this result in an increased binding of the inhibitory neurotransmitter GABA to the GABAA receptor thereby producing inhibitory effects on the central nervous system and body similar to the effects of other benzodiazepines. Chlordiazepoxide is an anxiolytic agent and also a anticonvulsant. Chlordiazepoxide is official in Indian Pharmacopoeia (IP). The IP<sup>2</sup>, BP<sup>3</sup> and USP<sup>4</sup> describe non-aqueous titration, potentiometry titration and HPLC methods, respectively for estimation of chlordiazepoxide. Literature survey reveals that for this combination derivative spectroscopic method<sup>5</sup>, HPTLC<sup>5</sup> and RP-HPLC<sup>6</sup> methods are reported and spectrophotometric, HPTLC, HPLC methods for AMI in combination with nortriptyline<sup>8</sup> and CLR with imipramine<sup>9,10</sup>, mebavarine<sup>11</sup> and clidinium bromide<sup>12</sup> are reported. However there is no work reported on combination of these drugs by Rp-HPLC. Hence in the present communication we propose fast, simple, and accurate Rp-HPLC method, was developed for the simultaneous estimation of both the drugs in tablet dosage form.



**Figure 1:** Chemical structure of Amitriptyline hydrochloride



**Figure 2:** Chemical structure of Chlordiazepoxide

## 2. Materials and Methods

Quantitative HPLC was performed on a high performance liquid chromatograph – Agilent technology 1220 infinity HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC and Agilent Zorbix C18 (250 X 4.6 mm, 5μ) Column. In addition an analytical balance (DENVER 0.1mg sensitivity), digital pH meter (Eutech pH. 510), a Sonicator (Unichrome associates UCA 701) were used in this study. Pharmaceutical grade Amitriptyline HCl & Chlordiazepoxide were kindly supplied as a gift sample by Dr. Reddy's Laboratory, Hyderabad, and Andhra Pradesh, India. Methanol was of HPLC grade and Purchased from E. Merck, Darmstadt, Germany. Ortho Phosphoric Acid and Potassium Dihydrogen Phosphate was analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.

### Preparation of mobile phase:

The mobile phase was prepared by mixing ortho phosphoric acid Buffer and Methanol in the ratio 50:50 v/v. Then it was sonicated for 15min and filtered through 0.45μ membrane filter.

### Preparation of standard solution:

Accurately 50mg of Amitriptyline HCl and 20mg of Chlordiazepoxide was weighed and transferred into 50mL volumetric flask and diluted with 30mL diluent and sonicated for 15minutes. Then the volume was make up to 50mL with diluent and filtered through 0.45μ nylon filter. Further 10mL of above solution was diluted to 50 mL and mixed to get a concentration of 100 μg/mL. From this stock solution further dilutions were made by taking the two drugs for the validation of the method developed.

### Preparation of sample solutions:

20 tablets were powdered and weigh and transfer tablet powder equivalent to 50 mg(291.6mg) of Amitriptyline HCl&20mg of chlordiazepoxide into 100 mL volumetric flask, diluted to 60 mL diluent and sonicated for 15 mins and make up to final volume with diluent and filtered through 0.45μ membrane filter. Further dilute 5 mL of this solution to 25 mL diluent and mixed to get a concentration of 100 μg/mL. From this stock solution further dilutions were made for the validation of the method developed.

### Validation parameters:

#### Specificity:

Specificity is the ability of analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample. The specificity of method was determined by spiking possible impurities at specific level to standard drug solution (100ppm). The diluent and placebo solutions were also injected to observe any interference with the drug peak.

**Linearity:**

Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with given range. Linearity in the concentration range of 25-75µg/mL for Amitriptyline Hcl, 10-30µg/mL for Chlordiazepoxide. From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient was found to be 0.999 and shows good linearity for both the drugs.

**Precision:**

Precision is the degree of closeness of agreement among individual test results when the method is applied to multiple sampling of a homogeneous sample. Study was carried out by injecting six replicates of the same sample preparations at a concentration of 100ppm.

**Accuracy:**

Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Accuracy of the method was evaluated by standard addition method. Recovery of the method was determined by spiking an amount of the pure drug (50%,100% ,150%) at three different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug.

**LOD & LOQ:**

Limit of detection and limit of quantification were calculated using following formula  $LOD=3.3(SD)/S$  and  $LOQ=10(SD)/S$ , where SD= standard deviation of response (peak area) and S= average of the slope of the calibration curve.

**Robustness:**

The robustness is evaluated by the analysis of Amitriptyline HCl& Chlordiazepoxide under different experimental conditions such as making small changes in flow rate ( $\pm 0.2$  mL/min), max ( $\pm 5$ ), column temperature ( $\pm 5$ ), mobile phase composition ( $\pm 5\%$ ), and pH of the buffer solution.

**3. Results and Discussion**

**Analytical Method Validation**

**Specificity:** As no other extra peaks were found at retention time of 1.96 min & 2.74 min the proposed method was a specific for the detection of Amitriptyline HCl & Chlordiazepoxide. The results of chromatograms were shown in the figure 3-5.

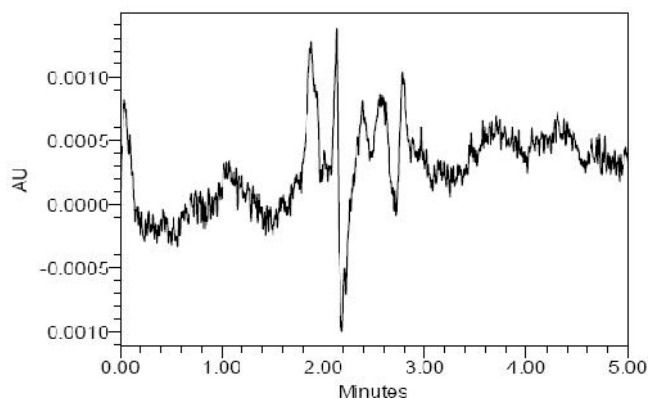


Figure 3: Typical chromatogram of the blank

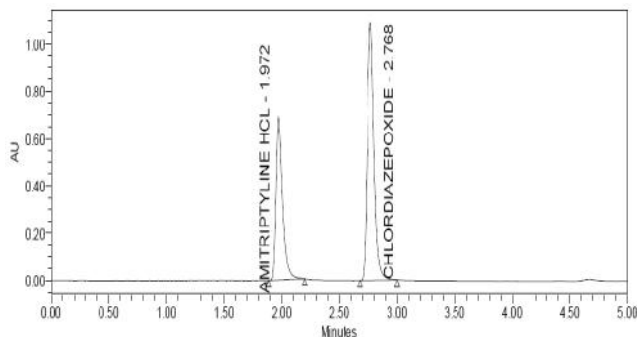


Figure 4: Chromatogram representing specificity of standard

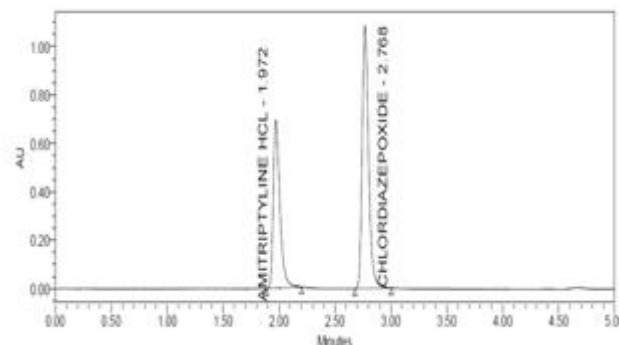


Figure 5: Chromatogram representing specificity of sample

**Linearity:** From the Linearity data it was observed that the method was showing linearity in the concentration range of 25-75µg/mL for Amitriptyline HCl 10-30µg/mL for Chlordiazepoxide. Correlation coefficient was found to be 0.999 for both the compounds. Linearity curves were shown in figure 6& 7 for Amitriptyline HCl and Chlordiazepoxide respectively.

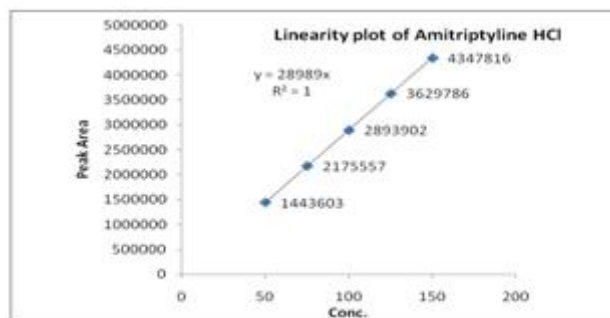


Figure 6: Linearity plot of Amitriptyline HCl

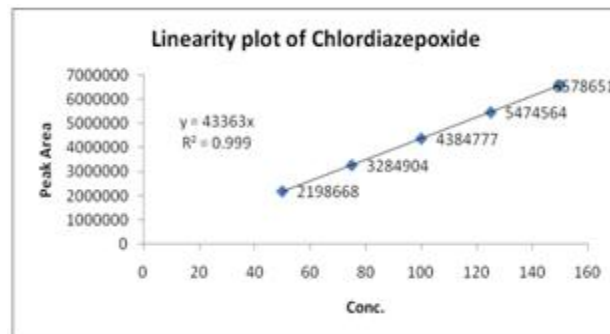


Figure 7: Linearity plot of Chlordiazepoxide

**Accuracy:** The percentage recovery of Amitriptyline HCl and Chlordiazepoxide was found 101% and 99% respectively. The percentage RSD of the samples was found less than 2. The results are tabulated in the table 3.

**Precision:** The percentage relative standard deviation value for precision of six replicate samples of Amitriptyline HCl & Chlordiazepoxide was found to be 0.43&0.22, which was well within the acceptance criteria limit. The results of precision were shown in table 1 and 2.

**LOD & LOQ:** The limit of detection was obtained as 0.154mg/mL for Amitriptyline HCl and 0.130mg/mL for Chlordiazepoxide. The limit of quantitation was obtained as 0.466mg/mL for Amitriptyline HCl and 0.395mg/mL for Chlordiazepoxide.

**Table 1:** Precision data for Chlordiazepoxide

S. No	RT	Area	% Assay
injection1	2.759	4383792	99
injection2	2.751	4386122	99
injection3	2.749	4388966	99
injection4	2.754	4388131	99
injection5	2.751	4386162	99
injection6	2.755	4389845	99
Mean			99
Std. Dev.			0.05
% RSD			0.05

**Table 3:** Accuracy data

Accuracy Level	Peak Area		Amount added	Amount found	% Recovery
	Amitriptyline HCl	Chlordiazepoxide			
50 %	1448235	2199613	124.625	124.43	100
100 %	2894954	4388815	249.250	249.08	100
150 %	4341729	6571981	373.892	373.56	100

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**Table 2:** Precision data for Amitriptyline HCl

S. No	RT	Area	%Assay
injection1	1.970	2890564	99
injection 2	1.966	2890300	99
injection 3	1.963	2891207	100
injection 4	1.967	2898491	100
injection 5	1.966	2893307	100
injection 6	1.971	2892802	100
Mean			100
Std. Dev.			0.10
%RSD			0.11

## 4. Conclusion

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust, rugged and economical. Hence the method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Amitriptyline HCl & Chlordiazepoxide in Tablets dosage forms.

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