ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AMITRIPTYLINE HYDROCHLORIDE AND CHLORDIAZEPOXIDE IN TABLET BY RP-HPLC

Neeli Sujatha* K Haritha Pavani

Department of Pharmaceutical Analysis and Quality Assurance, Nimra College of Pharmacy, Jupudi, Vijayawada, Andhra Pradesh, India

***Corresponding author: Email: neelisuji@gmail.com, Phone: 8142540237**

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 YMC Colimited C8 (250 X 4.6 mm,5µ) column its equivalent in isocratic mode and maintain column at 40^0C, using mobile phase comprising of Ortho phosphoric Acid : Methanol in the ratio of 50:50 v/v (Adjust pH -2 with Orthophosphoric Acid), with a flow rate of 1.0mL/min and the detection wavelength was carried at 253 nm. The retention time for Amitriptyline Hcl & Chlordiazepoxide was found to be 2.502&5.176. The percent assay was found to be 101%&99%. Proposed method was validated for precision, accuracy, linearity & range, specificity and robustness according to ICH guidelines. The method was successfully applied to Amitriptyline Hydrochloride and Chlordiazepoxide combination Tablet dosage form.

KEY WORDS: Amitriptyline Hydrochlorde, Chlordiazepoxide, RP-HPLC, YMC-Colimited Column, Validation.

INTRODUCTION

Amitriptyline Hydrochloride is a 3-**(**10,11-Dihydro-5H-dibenzo[a,d] cyclo hepten-5- ylidene)-N,N- dimethyl-1-propanamine hydrochloride.TricyclicAntidepressent drug. They contain a tricyclic ring system with an alkyl amine substituent on the central ring. In non-depressed individuals, amitriptyline does not affect mood or arousal, but may cause sedation.It is Decrease reuptake of nor epinephrine and serotonin. Amitriptyline appears to exert effect on both norepinephrine and serotonin (5-HT), although the selective acting desipramine is a more potent inhibitor of norepinephrine transport. The drug structure shows in figure no-1.

Chlordiazepoxide is a (7-chloro 2(methylamino)-5-phenyl-3-H-1,4 benzodiazepine 4-oxide).Tricyclic Antidepressent drug.It is bides to stereospecific benzodiazepine binding sites on GABA receptor complexes at several sites within the central nervous system including the limbic system and reticular formation.This result in an increased binding of the inhibitory neurotransmitter GABA receptor BZDs therefore enhance GABA-mediated chloride influx through GABA receptor channel causing membrane hyperpolarization.The drug structure shows in figure no-2.

Literature review reveals that several methods are reported for these drugs alone or in combination with other drugs. For combination of these drugs Spectroscopic method, HPTLC method is reported, there is no single work done for this combination by using RP-HPLC. Hence an attempt has been made for the development of RP-HPLC method for the combination of drugs.

The present study illustrate development and validation of simple, economical, selective, accurate, precise RP-HPLC method for the determination of Amitriptyline Hcl and Chlordiazepoxide in tablets dosage forms as per ICH guidelines.

MATERIALS AND METHODS

List of equipment: Quantitative HPLC was performed on a high performance liquid chromato graph -Waters e2695Alliance 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 YMC Colimited C8 (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.

List of chemicals: 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 was analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system. Amitriptyline Hcl & Chlordiazepoxide Tablets available in the market as 'Amixide' (Sun pharmaceutical Ind ltd ,Gujarat, India.) in composition of Amitriptyline Hcl (50mg), Chlordiazepoxide(20mg).

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 makeup 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 makeup 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.

System suitability: The purpose of system suitability is to ensure that the complete testing (including instrument,method,analyst) is suitable for the intended application. All system suitability parameters shows in the table no:2

Calibration curves for Amitriptyline Hcl & Chlordiazepoxide: Replicate analysis of solution containing 25-75µg/mL of Amitriptyline Hcl &10-30µg/mL of Chlordiazepoxide sample solutions respectively were injected into HPLC according to the procedure in a sequence and chromatograms were recorded. Calibration curves were constructed by plotting by taking concentrations on X-axis and ratio of peak areas of standards on Y-axis. Calibration graphs shows in the figure no:7, 8.

Optimized method: The quantification of the drug was carried out by using YMC Colimited C8 (250 X 4.6 mm,5 µ) column its equivalent in isocratic mode and maintain column at 40^0C, using mobile phase comprising of Ortho phosphoric Acid : Methanol in the ratio of 50:50v/v (Adjust pH 2 with Orthophosphoric Acid), flow rate of 1.0mL/min and the detection wavelength was carried at 253 nm. Mobile phase was used as diluent during the standard and test samples preparation. The optimized chromatographic conditions are mentioned in Table-1 and chromatogram for standard was shown in the figure no: 3.

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 (±0.2 mL/min), λmax (±5), column temperature (±5), mobile phase composition (±5%), and pH of the buffer solution.

RESULTS & DISCUSSION

Specificity: As no other extra peaks were found at retention time of 2.50 min & 5.17 min the proposed method was a specific for the detection of Amitriptyline Hcl & Chlordiazepoxide. The results are tabulated in the table no-3 and the chromatogram was shown in the figure no- 3,4,5,6.

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. The results are tabulated

in the table no-5 & Linearity graphs shows in the figure no-7,8.

Accuracy: The percentage recovery of Amitriptyline Hcl & 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 no-4.

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.

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.

Robustness: All the system suitability parameters are within limits for variation in flow rate (±0.2 mL). Hence the allowable flow rate should be within 0.8 mL to 1.2 mL. All the system suitability parameters are within limits for variation (±5^{0}C) in temperature. Hence the allowable variation in Temperature should be within 35^{0}C to 45^{0}C.The results shows Table no.7.

All validation parameters shows in the table no.6 All the results obtained were satisfactory and good agreement as per the ICH guidelines.

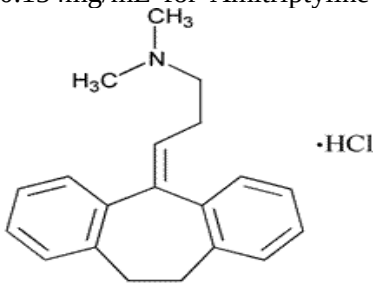

Fig:1 Structure of Amitriptyline Hcl

Fig:2 Structure of Chlordiazepoxide

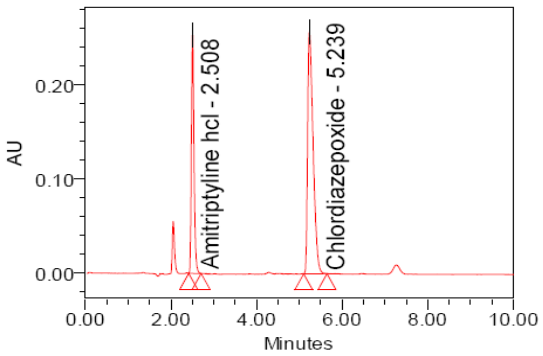

Fig:3 Chromatogram for Standard

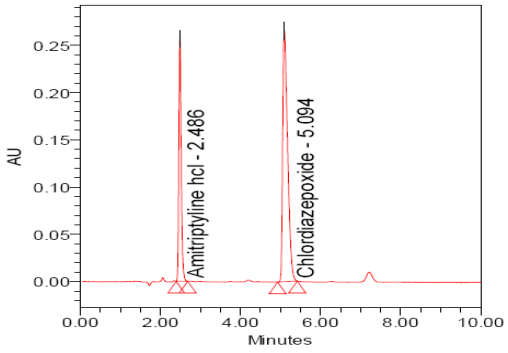

Fig:4 Chromatogram for Sample

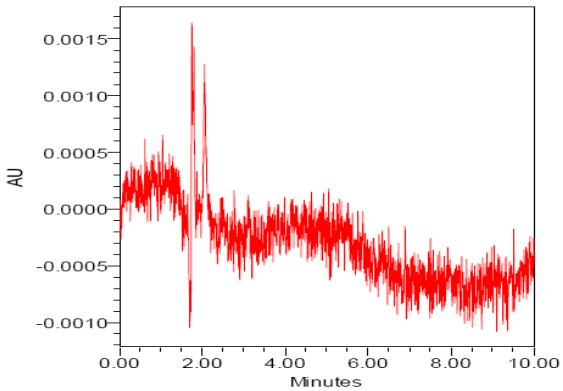

Fig:5 Chromatogram for Blank

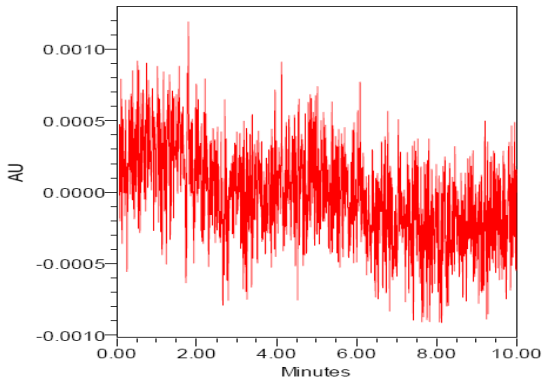

Fig:6 Chromatogram for Placebo

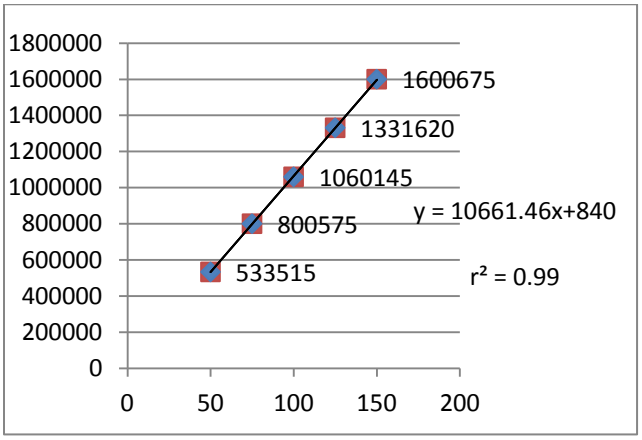

Fig:7 Linearity plot for Amitriptyline Hcl

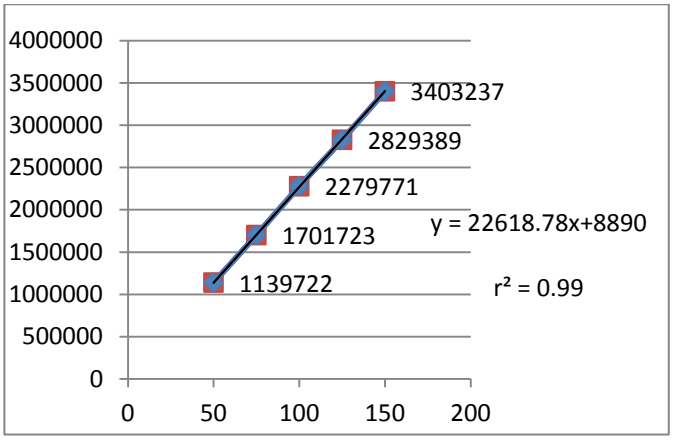

Fig:8 Linearity plot for Chlordiazepoxide

Table.1. Optimised Conditions for Amitriptyline Hcl & Chlordiazepoxide

Parameter	**Chromatographic Condition**
Column	YMC-Co-limited column, C_8 (150×4.6)mm, 5µ
Mobile phase	OPA Buffer : Methanol(50:50) v/v
Flow rate	1.0 mL/ min
Wavelength	253 nm
Injection volume	5 µl
Column temperature	40^0C
Run time	10 min

Table.2. System Suitability Parameters

Parameter	**Amitriptyline Hcl**	**Chlordiazepoxide**	**Acceptance criteria**
Theoretical plates	8604	7548	>2000
Tailing factor	1.263	1.810	<2
Asymmetric factor	0.4	0.38	0.9-1.2
Retention time	2.502	5.176	±10% of Actual Rt
%RSD	0.43	0.22	<2

Table.3. Specificity data for Amitriptyline Hcl & Chlordiazepoxide

Sample name	**Amitriptyline Hcl Area**	**Rt**	**Chlordiazepoxide Area**	**Rt**
Standard	1077129	2.508	2293861	5.239
Sample	1064380	2.486	2274042	5.094
Blank	--	--	--	--
Placebo	--	--	--	--

Table.4. Accuracy data for Amitriptyline Hcl & Chlordiazepoxide

Amitriptyline HCI						
Spiked Level	**Sample Weight(mg)**	**Sample Area**	**µg/mL added**	**µg/mL found**	**% Recovery**	**% Mean**
50%	145.80	535849	49.500	49.83	101	101
100%	291.60	1068848	99.000	99.40	100	
150%	437.40	1600269	148.500	148.82	100	
Chlordiazepoxide						
50%	145.80	1137087	20.000	19.84	99	99
100%	291.60	2279745	40.000	39.77	99	
150%	437.40	3401849	60.000	59.34	99	

Table.5. Linearity data for Amitriptyline Hcl & Chlordiazepoxide

sample	Linearity level (µg/mL)	Peak area	Slope	Y-intercept	r^2
Amitriptyline Hcl	25	533515	10661.4	840	0.999
	37.5	800575			
	50	1060145			
	62.5	1331620			
	75	1600675			
Chlordiazepoxide	10	1139722	22618.78	8890	0.999
	15	1701723			
	20	2279771			
	25	2829389			
	30	3403237			

Table.6. Summary of validation parameters

Parameter	Amitriptyline Hcl	Chlordiazepoxide
Specificity(Rt)	2.50min	5.17min
Range,Linearity(r^2)	25-75µg/mL,0.999	10-30µg/mL,0.999
Precision(% RSD)	0.43	0.22
Accuracy	101%	99%
LOD & LOQ	0.154,0.466	0.130,0.395

Table.7. Robustness data for Amitriptyline Hcl & Chlordiazepoxide

sample	Parameters	Optimized	Used	Rt	Peak area	Plate count
Amitriptyline Hcl	Flow rate (±0.2)	1mL/min	0.8	2.487	1063950	7324
			1	2.500	1065899	8291
			1.2	2.492	1072653	7432
	Temperature (±5°C)	40°C	35	2.482	1084297	7423
			40	2.501	1062198	8684
			45	2.481	1076311	7245
Chlordiazepoxide	Flow rate (±0.2)	1mL/min	0.8	5.146	2240382	7942
			1	5.173	2256757	7613
			1.2	5.156	2306841	7413
	Temperature (±5°C)	40°C	35	5.040	2330109	7701
			40	5.158	2262283	7674
			45	5.044	2127480	7563

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.

ACKNOWLEDGEMENT

The authors would like to thank beloved parents and all my well wishers, one and all who have helped me directly and indirectly in completing this project work.

REFERENCES

Barbai C, Hotopf M, The British Journal of Psychiatry, The Journal of Mental Science, 178(2), 129-144.

Bhatt, Mitesh, Shah, Sanjay, Shiv Prakash, Method development and validation of amitriptyline and its metabolites in human plasma by ultra performance liquid chromatography; Tandam mass spectrometry and its application to a bioequivalence study, Bio med chromotogr, 24(11), 2010, 1247-54.

Dhara patel, Vivek patel, Simultaneous estimation of amitriptyline hcl and perphenazine by absorption ratio (Q-Analysis) UV spectrophotometric method in combined tablet dosage form, International journal of pharmaceutical sciences and research, 1(12), 2010, 133-137.

Sejal patel, N.T Patel, Spectrophotometric and chromatographic simultaneous estimation of amitriptyline and chlordiazepoxide in tablet dosage form, Indian Journal of Pharmaceutical Sciences, 7 (4), 2009, 472-476.