ISSN: 2230-7346

Manorama. M et al. / JGTPS / 6(2)-(2015) 2534 – 2540

(Research Article)

Journal of Global Trends in Pharmaceutical Sciences

Journal home page: www.jgtps.com

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMITRIPTYLINE HYDROCHLORIDE AND CHLORDIAZEPOXIDE BY RP-HPLC

Manorama. M*,
Sathya Sowmya. P,
Abdul Ahad. H

Balaji College of Pharmacy, Ananthapuram, A.P, India

ABSTRACT

A simple, economic, selective, precise, and accurate Reverse Phase High Performance Liquid Chromatography method for analysis of Amitriptyline HCl and Chlordiazepoxide in tablet dosage form was developed and validated according to ICH guidelines. The quantification of the drug was carried out by using Agilent Zorbin C18 (250 X 4.6 mm, 5µ) column its equivalent in isocratic mode and maintain column at 30°C, using mobile phase composition of potassium dihydrogen phosphate buffer: Acetonitrile in the ratio of 50:50 v/v with a flow rate of 1.0 mL/min and the detection wavelength was carried at 274 nm. The retention time for Amitriptyline HCl and Chlordiazepoxide was found to be 1.975 and 2.773 min respectively. The % assay for Amitriptyline HCl and Chlordiazepoxide was found to be 100% & 99% respectively. The method was validated and the response was found to be linear in the drug concentration range of 50µg/ml to150 µg/mL for Amitriptyline HCl and 50µg/mLto150 µg/mL for Chlordiazepoxide. The LOD and LOQ for Amitriptyline HCl were found to be 2.53 and 8.44 µg/mL respectively. The LOD and LOQ for Chlordiazepoxide were found to be 1.961and 6.53 µg/mL respectively. 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.

Key words: Amitriptyline HCl, Chlordiazepoxide, Agilent Zorbin, potassium dihydrogen phosphate buffer, acetonitrile.

INTRODUCTION:

Benzodiazepines are being widely used for the treatment of anxiety and other related disorders. Sometimes these are also administered to patients along with tricyclic antidepressants (TCAs) as they both act on the central nervous system (CNS). Chloldiazepoxide, the first benzodiazepine developed in the 1950s is an anxiolytic benzodiazepine derivative, with anticonvulsant, sedative and amnesic properties[1].

Address for correspondence

Manorama. M*

Balaji College of Pharmacy,
Ananthapuram,
Andhra Pradesh, Mob: 9581884379
E-mail: shrey.shresta@gmail.com

Its IUPAC name is 7-Chloro-N-Methyl-5-Phenyl-3H-1, 4-benzodiazepin-2-amine4-oxide[2-4]. It has also been used in the symptomatic treatment of alcohol withdrawal and for the management of anxiety disorders. Depression, a disorder, is alarmingly on rise among the world population. For the treatment of depression, in general, tricyclic antidepressants (TCAs) are prescribed. These are one of the oldest classes of antidepressants that are still in extensive use. These antidepressants act on nerve cells in the CNS.

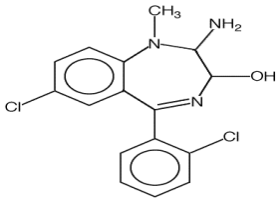

Figure 1: Chemical structure of Chloldiazepoxide

Amitriptyline hydrochloride (Figure 2) belongs to the class of tricyclic antidepressants and its IUPAC name is 3-(10, 11-Dihydro-5H-dibenzo [a, d] cycloheptен-5-ylidine)-N, N-dimethyl-1-propanamine[4]. Its function is to prevent serotonin and noradrenaline from being reabsorbed back into the nerve cells in the CNS. Thus it aids to relieve depression. It is sometimes prescribed in combination with benzodiazepines in treating depression in people who are also anxious and agitated, or who are suffering from disturbances in sleep.

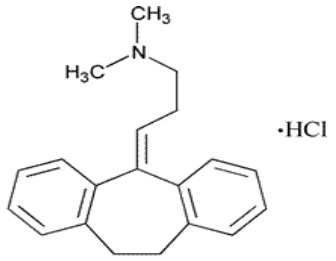

Figure 2: Chemical Structure of Amitriptyline hydrochloride

Literature survey reveals that various methods have been used for the determination chlordiazepoxide separately and in combination with other drugs. Chlordiazepoxide and its metabolites are determined by high performance liquid chromatography (HPLC) in human plasma[5-7], human serum and in urine and along with its impurities. Methods like non-aqueous titration and potentiometry[9] also have been demonstrated. Similarly, amitriptyline hydrochloride has been studied as a single drug and in combination with other drugs by different techniques. It is measured in human whole blood along with its metabolites[2-4, 9], plasma and bio-fluids.

To mention, non-aqueous titration, titrimetry and HPLC[11, 2-4, 12, 8, 10] voltammetry, conductometric[14], HPLC without organic solvent, spectrophotometry[19] and gas chromatography methods were demonstrated. Furthermore, spectrophotometry, first derivative spectrophotometry[16-20], gas chromatography, HPLC and high performance thin layer chromatography[19]are demonstrated for amitriptyline hydrochloride and chlordiazepoxide combination. So far, only two studies are reported for these drugs combination to the best of our knowledge[19, 20]. This necessitates more detailed investigations for quantitative estimation of these two drugs. In this paper, we report an improved simple and rapid method for simultaneous quantitative determination of chlordiazepoxide and amitriptyline hydrochloride in pharmaceutical formulations using RP-HPLC method. The developed method is validated following ICH[21].

Materials and Methods:

Quantitative HPLC was performed on Agilent 1220 infinity HPLC system connected with PDA Detector 2998 and Empower-2 Software. Analytical column was Agilent Zorbin C18 (250 X 4.6 mm, 5µ) Column. 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 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-Q-RO water purification system. Amitriptyline HCl & Chlordiazepoxide Tablets ('Amixide', Amitriptyline HCl (12.5mg), Chlordiazepoxide (5.0mg) Make: Sun pharmaceutical India ltd, Gujarat, India) was purchased from the local market.

Preparation of standard solutions: Transfer 62.5 mg of Amitriptyline HCl and 25 mg of Chlordiazepoxide in 50ml volumetric flask. Add 50ml of HPLC grade water. Pipette out 5ml from above solution transfer to 25ml volumetric flask. Make up the volume HPLC grade water.

Preparation of sample solution: Transfer the 727.5 mg of tablets powder into a 50 ml volumetric flask and add 10ml of diluent and sonicate for 20 min and makeup with diluent. Transfer 5 ml of above solution into 25ml volumetric flask dilutes the volume with the mobile phase.

Method validation

The proposed method was validated as per ICH guidelines[22].

Specificity (Forced decomposition studies)

Specificity is the ability of the method to measure the analyte response in the presence of its excipients. The specificity of the developed LC method for Amitriptyline HCl & Chlordiazepoxide was carried out in the presence of its degradants. Stress studies were performed for on tablets to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted with a stress condition of UV light (254 nm), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3.0% H_2O_2) to evaluate the ability of the proposed method to separate analytes from its degradation product. For light studies, study period was 10 days whereas for hydrolytic, acid, base and oxidation, it was 24 h. Peak purity test was carried out for the Amitriptyline HCl & Chlordiazepoxide peak by using PDA detector in stress samples.

Precision

The precision of the method verified by repeatability and by intermediate precision. Repeatability was checked by injecting six individual preparations of Amitriptyline HCl & Chlordiazepoxide real sample (tablets).

The intermediate precision of the method was also evaluated using different analyst and performing the analysis on different days. Precision of assay method was evaluated by carrying out six independent assays of real sample of Amitriptyline HCl & Chlordiazepoxide at 100 µg/ml level against qualified reference standard. The intermediate precision of the assay method was evaluated by different analysts by making use of different columns and different lot of the sample.

Linearity

Linearity test solutions for the assay method were prepared from Amitriptyline HCl & Chlordiazepoxide stock solution at six concentration levels from 50 to 150% of assay analyte concentration (50, 75, 100, 125 and 150 µg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the method were prepared by diluting stock solution to the required concentrations.

Accuracy

Accuracy of the assay method was evaluated in triplicate using three concentration levels 50, 100 and 150µg/ml on real sample (tablets). Standard addition and recovery experiments were conducted on real sample to determine accuracy the method. Study was carried out in triplicate using three (50, 100 and 150%) concentration levels. The percentages of recoveries for Amitriptyline HCl & Chlordiazepoxide were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the system suitability parameters were evaluated. Tailing factor for Amitriptyline HCl & Chlordiazepoxide was recorded. The flow rate of the mobile phase was 0.8 ml/min, to study the effect of flow rate on the retention time; flow was changed by ± 0.2 units from 0.6 to 1.0 ml/min. The effect of the column temperature on retention time was studied at 25 and 35 ºC.

RESULTS AND DISCUSSION:

The absorption wavelength for chlordiazepoxide and amitriptyline hydrochloride is determined after several trials. The absorbance spectra of the diluted standard and working solutions of chlordiazepoxide and amitriptyline hydrochloride in methanol are recorded on a UV spectrophotometer. They are scanned in the wavelength 200 nm - 400 nm range using quartz cuvettes with 10 mm path length. The maximum absorption wavelength was observed at 274 nm for the two drugs. This is in good agreement with the reported wavelengths for these drugs combination.

Specificity (Forced decomposition studies)

Chlordiazepoxide and Amitriptyline hydrochloride was found to degrade significantly in acid hydrolysis and in base hydrolysis and mild degradation was observed in UV and peroxide stress conditions. Figure 3a, 3b shows the representative chromatogram of standard and sample Chlordiazepoxide and Amitriptyline hydrochloride respectively and figure 4 shows the chromatograms of degradation studies. Photodiode array detector was employed to check and ensure the homogeneity and purity of Chlordiazepoxide and Amitriptyline hydrochloride peak in all the stressed sample solutions. Assay studies were carried out for stress samples against Chlordiazepoxide and Amitriptyline hydrochloride qualified working standard. The results are presented in Table 1. The purity and assay of Chlordiazepoxide and Amitriptyline hydrochloride was unaffected by the presence of its degradation products and thus confirms the stability-indicating power of the developed method.

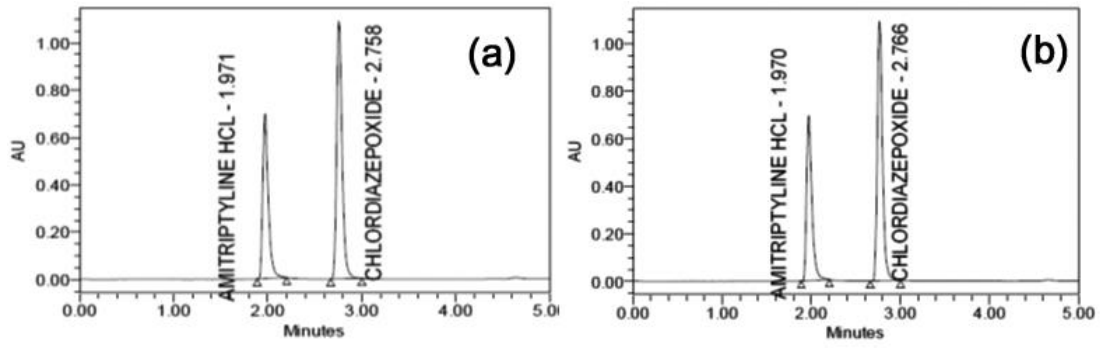

Figure 3. Representative chromatograms of a) Standards of Chlordiazepoxide and Amitriptyline hydrochloride b) Tablet sample of Chlordiazepoxide and Amitriptyline hydrochloride

Table 1: Data of Forced decomposition studies

S. No	Sample name	Assay of Chlordiazepoxide (%)	% of Net Degradation	Assay of Amitriptyline HCl (%)	% of Net Degradation
1	Acid	79.24	19.76	67.59	32.41
2	Base	79.92	19.08	67.33	32.67
3	Peroxide	78.14	20.86	71.44	28.56
4	Heat	78.44	20.56	73.97	26.03
5	UV light	90.95	08.05	78.02	21.98

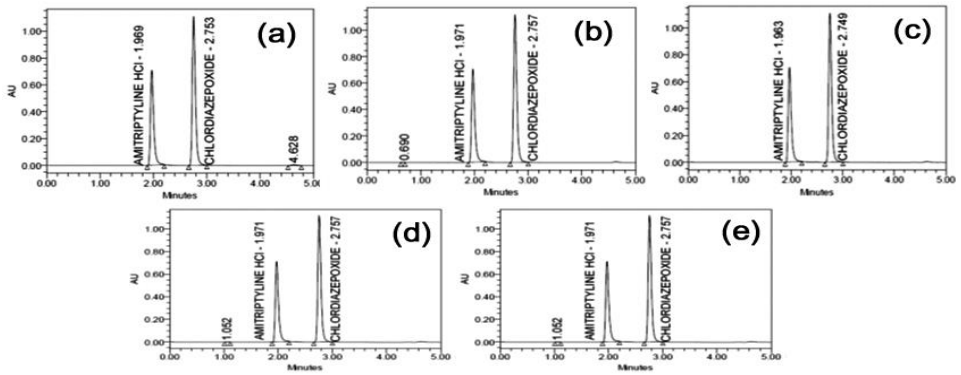

Figure 4: Specificity Chromatograms of a) Acid Degradation b) Base Degradation c) Peroxide Degradation d) Heat Degradation e) UV light Degradation

Precision

The % RSD during the method precision study of Amitriptyline hydrochloride was 0.15 and 0.11% for retention time and Peak area respectively and 0.13 % and 0.05 % for retention time and Peak area of Chlordiazepoxide respectively. The % RSD for the area of Chlordiazepoxide and Amitriptyline hydrochloride were well within 2 %, conforming good precision of the method. The % RSD values are presented in Table 2.

Table 2: Precision data

S.no	Amitriptyline hydrochloride		Chlordiazepoxide	
	RT	Area	RT	Area
Injection-1	1.970	2890564	2.759	4383792
Injection-2	1.966	2890300	2.751	4386122
Injection-3	1.963	2891207	2.749	4388966
Injection-4	1.967	2898491	2.754	4388131
Injection-5	1.966	2893307	2.751	4386162
Injection-6	1.971	2892802	2.755	4389845
Mean	1.97	2892778.50	2.75	4387169.67
Std. Dev.	0.003	3047.26	0.00	2228.45
% RSD	0.15	0.11	0.13	0.05

Linearity

Linear calibration plot for above method was obtained over the calibration range 50 µg/ml to 150 µg/ml (20–150% of Chlordiazepoxide and Amitriptyline hydrochloride, nominal concentration 100 µg/ml). The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Table 3: Linearity data

S.no	Conc.(µg/ml)	Amitriptyline hydrochloride	Chlordiazepoxide
		Peak Area	Peak Area
1.	50	1443603	2198668
2.	75	2175557	3284904
3.	100	2893902	4384777
4.	125	3629786	5474564
5.	150	4347816	6578651
Slope		29051	43799
Intercept		6929.2	4462.4
Correlation coefficient (r^2)		1	0.999

Accuracy

Accuracy was determined by analyzing a sample of known concentration (reference standard solutions) and comparing the measured value with the true value, and using the method of standard additions. Tables 3 summarize the accuracy results, expressed as percent recovery. The method showed good recovery.

Table 3: Results of Accuracy

S. No	Analyte	Accuracy Level	Sample weight	Amount added (µg/ml)	Amount Found (µg/ml)	% Recovery	% Mean
1	**Chlordiazepoxide**	50%	363.75	49.550	49.52	100	100
2		100 %	727.50	99.100	99.24	100	
3		150%	1091.30	148.657	148.61	100	
4	**Amitriptyline Hydrochloride**	50%	363.75	124.625	124.08	100	100
5		100 %	727.50	249.250	249.47	100	
6		150%	1091.30	373.892	373.92	100	

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In order to perform the robustness study of the proposed method deliberate modifications in flow rate and column temperature were made. The results are shown in Table 4. It can be seen that every employed condition, the chromatographic parameters are in accordance with established value. A change of ± 0.2 unit of flow rate and column temperature had no impact on chromatographic performance (Table 4). According to the data of robustness test study proposed criteria for system suitability test (tailing factors, theoretical plates number and repeatability (R.S.D.)). It is used to verify that the repeatability of the system is adequate for the analysis intended.

Table 4: Results of Robustness

Parameter	Amitriptyline Hydrochloride			Chlordiazepoxide		
	RT	Theoretical plates	Asymmetry	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	2.466	2974823	1.2	3.442	12761	1.1
Increased flow rate(1.2ml/min)	1.701	2987456	0.9	2.367	11569	1.0
Decreased temperature(25^0c)	1.963	2958340	0.8	2.749	11509	1.0
Increased temperature(35^0c)	1.964	2939314	0.9	2.744	11521	0.9

Detection and Quantification Limit:

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10 were determined experimentally for the proposed methods and results are given in Table 5.

Table 5: LOD and LOQ

s.no	Sample name	LOD(µg/ml)	LOQ (µg/ml)
1	Amitriptyline HCl	2.533	8.445
2	Chlordiazepoxide	1.961	6.536

Assay:

The proposed validated method was successfully applied to determine Amitriptyline HCl Chlordiazepoxide in tablet dosage form. The result obtained was comparable with corresponding labeled amounts. The results were shown in table 6.

Table 6: Results of Assay

Sample no.	Amitriptyline HCl		Chlordiazepoxide	
	Area	**%Assay**	**Area**	**%Assay**
1	2890564	99	4383792	99
2	2890300	99	4386122	99
3	2891207	100	4388966	99
4	2898491	100	4388131	99
5	2893307	100	4386162	99
6	2892802	100	4389845	99
Mean	2892778.50	100	4387169.67	99
Std. Dev.	3047.26	0.10	2228.45	0.05
% RSD	0.11	0.11	0.05	0.05

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Chlordiazepoxide and Amitriptyline Hydrochloride in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Chlordiazepoxide and Amitriptyline Hydrochloride. The method was stable at even in stressed conditions.

REFERENCES:

1. Sternbach LH. The story of benzodiazepines. J Med Chem 1979; 22(1): 1-7.
2. Indian Pharmacopoeia. Indian pharmacopoeia commission: Vol. 2. Ghaziabad, p 1054 and p 804.
3. British Pharmacopoeia. Her Majesty's stationary office, Vol.1. London, 2005, p 124 and p 438.
4. The United States Pharmacopoeia. United States Pharmacopoeial convention, 28, Revision: Rockville, MD, 2005, p 135 and p 435.
5. Ascalone V. Determination of chlordiazepoxide and its metabolites in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr. B 1980; 181 (1): 141-146.
6. Roberts SE, Determination of chlordiazepoxide, its hydrochloride and related impurities in pharmaceutical formulations by reversed-phase high performance liquid chromatography. J Chromatogr. A 1984; 238: 265-272.
7. Soentjens-Werts, High performance liquid chromatographic determination of chlordiazepoxide, its metabolites and oxaziridines generated after UV irradiation. J Chromatogr. A 1994; 662 (2): 255-262.
8. Vree, TB, Baars, AM, Hekster, YA, van der Kleijn E. Simultaneous determination of chlordiazepoxide and its metabolites in human plasma and urine by means of reversed phase high-performance liquid chromatography. J Chromatogr. B 1981; 224 (3): 519-525.
9. French WN, Matsui FF, Smith SJ. Determination of major impurity in chlordiazepoxide formulations and drug substance. J Pharm Sci 2006; 64: 1545-7.
10. Gabrielle AS, Pierre S, Kathleen MG, Terrence FB. High pressure liquid chromatographic determination of amitriptyline and its major metabolites in human whole blood. J Pharm Sci 1981; 71: 581-3.
11. Queiroz RHC, Lanchote VL, Bonato PS, de Carvalho D. Simultaneous HPLC analysis of tricyclic antidepressants and metabolites in plasma samples. Pharm. Acta Helvet 1995; 70 (2): 181-186.
12. Samanidou VF, Nika MK, Papadoyannis IN. Development of an HPLC method for the monitoring of tricyclic antidepressants in biofluids. J Separ Sci 2007; 30(15): 23912400.
13. Biryol I, Uslu B, Kucukyavuz Z. Voltametric determination of imipramine hydrochloride and amitriptyline hydrochloride using a polymer modified carbon paste electrode. J Pharm Biomed Anal 1996; 15: 371-381.
14. Ghani NTA, El-Nashar RM, Biome AA. Conductometric determination of the antidepressants amitriptyline and dothiepin hydrochlorides and tranylcypromine hemisulphate in their pharmaceutical formulations. FABAD J Pharm Sci 2004; 29: 195-2001.
15. Fernández-Navarro JJ, Ruiz-Ángel MJ, Reversed-phase liquid chromatography without organic solvent for determination of tricyclic antidepressants. J Separat Sci 2012; 35: 10-11.

16. Deshmane GV, Kadam SS. Simultaneous spectrophotometric estimation of amitriptyline HCL and chlordiazepoxide. Indian drugs 1997; 34: 443.
17. Karpinska J, Suszynska J, Trace J. The spectrophotometric simultaneous determination of amitriptyline and chlorpromazine hydrochlorides in their binary mixtures. Microprobe Tech 2001; 19: 355-364.
18. Bakal RL, Manwar JV, Sahare AY, Bhajipale NS, Manikrao AM. Spectrophotometric estimation of amitriptyline HCL and chlordiazepoxide in pharmaceutical dosage form. Ind J Pharm Educ Res 2008; 42(1): 23-26.
19. Patel S, Patel NJ. Spectrophotometric and chromatographic simultaneous estimation of amitriptyline hydrochloride and chlordiazepoxide in tablet dosage forms. Indian J Pharm Sci 2009; 71(4): 472-476.
20. Mustafa AA, Mohamed E, Abdel-Hamid. Simultaneous high performance liquid chromatographic and first derivative spectrophotometric determination of amitriptyline hydrochloride and chlordiazepoxide in capsules. Anal Lett 1989; 22: 951-962.
21. Sane RT, Surve SR, Gangrade MG, Bapat VV, Chankar NL. Simultaneous gas chromatographic estimation of combined dosages. I-Amitriptyline hydrochloride with chlordiazepoxide, II-Methocarbamol with ibuprofen, III-Paracetamol with diclofenac sodium, IV-Paracetamol with promethazine hydrochloride. Indian Drugs 1993; 30: 66-72.
22. ICH guidlines. ICH Harmonized Tripartite Guideline Q2 (R1), Validation of analytical procedures: text and methodology, Current Step 4 Version, 2005.

How to cite this article:
M.Manorama*, P. Sathya Sowmya, H. Abdul Ahad, Stability indicating method development and validation for simultaneous estimation of amitriptyline hydrochloride and chlordiazepoxide by RP-HPLC, 6(2): 2534 – 2540 (2015)