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Simultaneous Estimation of Amtriptyline and Chlordiazepoxide in Bulk and Formulation by Reverse Phase High Performance Chromatography and Application of Stress Studies

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ABSTRACT

Amitriptyline and chlordiazepoxide in bulk and formulation were estimated by stability indicating reverse phase high performance liquid chromatographic method. For the proposed method the chromatographic conditions are C18 column (250mm×4.6mm i.d., 5µ particle size), mobile phase was mixture of acetonitrile and triethylamine buffer pH 2.5 at a ratio of 30:70. The injection volume was 20µl with a flow rate 1.0ml/min at a ambient temperature. The wave length selected was 220nm and retention times were found to be 4.55 and 5.893mins for amitriptyline and chlordiazepoxide respectively. Amitriptyline shows linear curve at a concentration range of 5-20µg/ml and for chlordiazepoxide the linearity was obtained for the concentration range of 1.8-6.4µg/ml and regression coefficient was found to be 0.997 and 0.999 for amitriptyline and chlordiazepoxide. The limit of detection and limit of quantification for amitriptyline was found to be 0.02µg/ml and 0.05µg/ml and for chlordiazepoxide was found to be 0.01µg/ml and 0.03µg/m. amitriptyline and chlordiazepoxide shows recoveries were ranged from 99.98% – 100.08 % and 99.96%- 100%respectively. The method was performed based on ICH guidelines. In the detection of these drugs degradation products were not interfered and the method was considered as new stability indicating method.

Keywords: Amitriptyline, Chlordiazepoxide, Method development and Validation, Stability indicating assay, forced degradation study.

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INTRODUCTION

Amitriptyline is chemically dimethyl({3-[2Z)-tricyclo[9.4.0.0^{3,8}]pentadeca-1(11),3(8),4,6,12,14-hexaen-2-ylidene]propyl})amine which is white crystalline powder in nature and soluble in methanol and water. It is an antidepressant agent used for that treatment of sleep disorders, agitation irritable bowel syndrome. Chlordiazepoxide HCl is 7-chloro-2- (methyl amino) -5-phenyl-3H-1, 4-benzodiazepine 4-oxide hydrochloride which is white or slightly yellow solid crystalline in nature and soluble in water, soluble or sparingly soluble in alcohol and practically insoluble in chloroform. It is an antianxiety drug used for irritable bowel syndrome along with Mebeverine. Literature survey explains that certain methods were established for the determination of Amitriptyline and Chlordiazepoxide includes UV spectrophotometric methods, HPLC methods. The previous methods were developed with more economical so the study was carried out to develop a simple, precise and less economy method for the estimation of Amitriptyline and Chlordiazepoxide which can be used in the routine analysis. The method was done according to I CH guidelines and validated and applied to stress conditions.

MATERIALS AND METHOD

Instrumentation include waters 2695-Empower software with Zodiac C18 column, UV detector and isocratic pump system. And P^H meter- Eutech, Weighing balance - Denver, Ultrasonicator-UCA 701 Unichrome were used. Chemicals and reagents were Tri ethyl amine HPLC grade, HPLC grade Distilled water and ortho phosphoric acid, Acetonitrile (Merck) was used.

Chromatographic conditions

Chromatographic conditions include Zodiac C18 column, Mobile phase consists of 30: 70 ratio of acetonitrile and Tri ethyl amine buffer P^H 2.5(v/v), flow rate was 1 mL/min at ambient temperature and UV detector for monitoring of the drugs at isobestic point 220nm. 20µL of injection was given.

Preparation of solutions:

Buffer preparation

Tri ethyl amine 1ml was added to 1lt of HPLC grade Distilled water and adjust the pH to 2.5 by ortho phosphoric acid and finally it was filtered through 0.45µ membrane filter.

Mobile phase preparation

It was prepared by Acetonitrile and Triethylamine buffer pH 2.5 with Orthophosphoric acid (30:70 v/v). It was also used as diluent.

Sample and standard solution preparations

Standard solution preparation

Accurately weighed amount of 12.5mg Amitriptyline and 5mg Chlordiazepoxide were transferred into two separate 100mL volumetric flasks and 70ml of diluent was added into each flask and allowed for sonication then made up the volume with diluent. Then 5mL each of Amitriptyline and Chlordiazepoxide solution into 50mL volumetric flask and made up the volume with diluent, from which different concentrations were prepared according to linearity range.

Sample solution preparation

20 tablets were accurately weighed and powdered. Equivalent to 12.5mg of Amixide-5 was weighed and transferred into 500mL volumetric flask. 400ml diluent was added and allowed for sonication and final volume was made up with the diluent. From the above a further dilution was prepared. 20 µL of Blank, standard and sample solutions were injected and chromatograms were recorded and showed in figure 1, 2, 3.

Specificity

Specificity is the ability to measure the analyte of interest without interference of any impurities. Due to this blank, standard and sample chromatograms were recorded in which blank chromatogram shows no response and the retention times of the drugs confirms the response was specific and showed in figure 1,2,3

Method validation

The HPLC method developed for the determination of Amitriptyline and Chlordiazepoxide as per the protocol. To validate the method, the following parameters were studied according to ICH guidelines.

System suitability

It was performed by using 12.5µg/mL Amitriptyline and 5µg/mL Chlordiazepoxide solution. Prior to the analysis the parameters include tailing factor, resolution, retention time and number of theoretical plates was checked. The results were given in Table 2 which was within the limits.

Linearity and range

Linearity of an analytical method will elicit the test results which are proportional to the concentration of the analyte sample within the range. Linearity range of Amitriptyline and Chlordiazepoxide were 5-22.5µg/mL and 1.8 – 6.6µg/mL and the results were shown in Table 3 and the linearity curves were validated by the high value of correlation co-efficient of regression equation (Figure 4, 5). The range is the interval between the upper and lower levels of analyte that have been demonstrated with different parameters include precision, accuracy and linearity.

Accuracy

Standard addition method was used for the accuracy study. The accuracy studies were represented in terms of percentage recovery. The accuracy was carried at 50%, 100% and 150% levels for Amitriptyline and 80%, 100% and 150% for Chlordiazepoxide and the results were shown in Table 4, 5.

Precision

The precision of the instrument was checked by repeated injection (n=6) solutions containing 12.5µg/mL of Amitriptyline and 5µg/mL of Chlordiazepoxide in combination. Intermediate precision (reproducibility) including interday and intraday precision were determined by the corresponding response three times on the same day and on three different days over a period of one week. The results were shown in Table 6.

Robustness and Ruggedness

Robustness was carried out by changing the flow rate ± 0.2mL/min and P^H ±0.5 and results were given in Table 8 which indicates that the method was robust. By changing the analyst, ruggedness was performed and the results were recorded in Table 7.

Limit of Detection and Limit of Quantification

The LOD and LOQ values for Amitriptyline and Chlordiazepoxide were calculated from the standard deviation and slope and the results were shown Table 9.

Stability study

Amitriptyline 12.5µg/mL and Chlordiazepoxide 5µg/mL were prepared and stability study was carried out at different time intervals (0, 6, 12, 24 hrs) and the results were recorded in Table 10.

Degradation study

Degradation study was carried out by performing different parameters which includes Acid degradation, Alkali degradation, Peroxide degradation, Thermal degradation and Photolytic degradation and the results were recorded in Table 11.

RESULTS AND DISCUSSION

A new stability indicating RP-HPLC method which was developed and validated for the simultaneous determination of Amitriptyline and Chlordiazepoxide in bulk and pharmaceutical dosage form. The chromatographic conditions include Zodiac C18 (250mm x 4.6mm, 5µm) column with UV detector with an injection volume of 20 µL was injected and eluted with the mobile phase containing Acetonitrile: Triethylamine buffer (30: 70v/v) pH adjusted to 2.5 with orthophosphoric acid. This was pumped at a flow rate of 1mL/min and detected by UV detector.

The peaks of Amitriptyline and Chlordiazepoxide were eluted at retention times of 4.455and 5.893 mins respectively. After method was developed, it was validated according to ICH guidelines for system suitability, specificity, linearity, sensitivity parameters, precision, accuracy and robustness studies. The linearity range for Amitriptyline and Chlordiazepoxide were 5-20µg/mL and 1.8-6.6 µg/mL and correlation coefficient was within the acceptable limit and showed in table 3. The recoveries obtained for 50%,100% and 150% levels were in-between 99.82 to 100.08% for Amitriptyline and 80%,100% and 150% for Chlordiazepoxide were in-between 99.96 to 100.00% and showed in table 4,5.The % RSD values of precision were less than 2 which explains that the method was precise. The low percentage RSD obtained in robustness due to change in pH and flow rate indicates that the method was robust. The LOD and LOQ values for Amitriptyline were found to be 0.02µg/mL and 0.05µg/mL. The LOD and LOQ values for Chlordiazepoxide were found to be 0.01µg/ mL and 0.03µg/mL and showed in table 9.The stability study for the sample solutions were carried up to 24hrs and shows that the solutions are stable. The method was forcibly degraded in the presence of Acid, Alkali, Peroxide, Thermal and Photolytic degradations and the results explains the method can be recovered in the presence of degradation products also.

Table 2: System suitability parameters

S.No	Drug	Retention time(min)	Plate count	Tailing factor	Resolution
1	Amitriptyline	4.455	3286	1.44	3.65
2	Chlordiazepoxide	5.893	3630	1.491	

Table 3: Results of linearity for Amitriptyline and Chlordiazepoxide

S.No	Amitriptyline		Chlordiazepoxide	
	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area
1	5	695453	1.8	601792
2	7.5	1237064	2.6	1081034
3	10	1782606	3.4	1515311
4	12.5	2454732	4.2	2096345
5	15	3101105	5	2629345
6	17.5	3841522	5.8	3256750
7	20	4389636	6.6	3740288
Regression equation		y = 244946x – 578083	y = 695401x – 828796	
Slope		244946	695401	
Intercept		578083	828796	
R^2		0.997	0.999	

Table 4: Accuracy results of Amitriptyline by RP-HPLC method

S.no	% Level of Std	Conc. of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	%R.S.D
1	50	2.5+5	1237064 1237859 1237568	7.502	100.03		
2	100	5+5	1782606 1782586 1782025	9.998	99.98	100.03	0.045
3	150	7.5+5	2454732 2456895 2458956	12.51	100.08		

Table 5: Accuracy results of Chlordiazepoxide by RP-HPLC method

S.no	% Level of Std	Conc. of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	%R.S.D
1	80	2 +2.5	2246629 2245866 2246592	4.49	99.98		
2	100	2.5+2.5	2629345 2629856 2625896	4.998	99.96	99.98	0.037
3	120	3 +2.5	3088297 3088958 3088456	5.5004	100.0		

Table 6: Precision studies by RP-HPLC method

S.no	Type	Amitriptyline			Chlordiazepoxide		
		Mean area(n=6)	Std. deviation	% RSD	Mean area(n=6)	Std. deviation	% RSD
1	System precision	2881858	23302.32	0.808	2461439	40611.12	1.64
2	Method precision	3022518	35770.93	1.18	2556901	30800.86	1.2
3	Intermediate precision	2975484	411026.65	1.38	2514617	27141.84	1.07

Table 7: Results of Ruggedness study by RP-HPLC

S.No	Parameter	Amitriptyline	Chlordiazepoxide	Limit
1	% RSD	0.808	1.2	NMR 2.0%

Table 8: Results of Robustness study by RP-HPLC

Variations	Amitriptyline				Chlordiazepoxide			
	Retention time	Peak area	Plate count	% RSD	Retention time	Peak area	Plate count	% RSD
P^H=2	5.097	3644925	6383	0.0185	7.107	2972688	7450	0.099
P^H=3	4.110	2949173	6149	0.057	5.428	2465826	8173	0.0715
Flow rate 1.1mL/min	3.391	2974887	5114	0.0571	4.740	1923753	6729	0.0825
Flow rate 0.9mL/min	4.648	2313834	6289	0.058	6.424	2692874	7415	0.034

Table 9: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Parameter	Amitriptyline		Chlordiazepoxide	
	µg/mL	Area	µg/mL	Area
LOD	0.02	29818	0.01	3343
LOQ	0.05	6954	0.03	10029

Table 10: Results of stability study

Time period (hours)	Amitriptyline				Chlordiazepoxide				Resolution
	Retention time	Peak area	Tailing factor	Plate count	Retention time	Peak area	Tailing factor	Plate count	
0	4.568	2991521	1.413	3246	5.868	2497648	1.406	3778	3.63
6	4.568	3020364	1.468	3286	5.867	2567774	1.480	3700	3.61
12	4.567	3000442	1.414	3265	5.863	2513692	1.396	3788	3.63
24	4.568	2961581	1.415	3306	5.862	2513365	1.424	3750	3.62

Table 11: Results of degradation study

Degradation	Sample	Amitriptyline			Chlordiazepoxide		
		Mean area(n=6)	% label claim	% degradation	Mean area(n=6)	% label claim	% degradation
Control	1180.5	2377581	100.2	-0.2	1979990	100.9	-0.9
Acid	1120.5	1735294	77.1	23.9	1448498	77.8	23.1
Alkali	1125.6	1727512	76.4	23.8	1413421	75.5	25.4
Peroxide	1132.3	1702372	74.8	25.4	1415642	75.2	25.7
Thermal	1134.7	1696836	74.4	25.8	1386141	73.5	27.4
Photo	1135.8	1716663	75.2	25	1438169	76.2	24.7

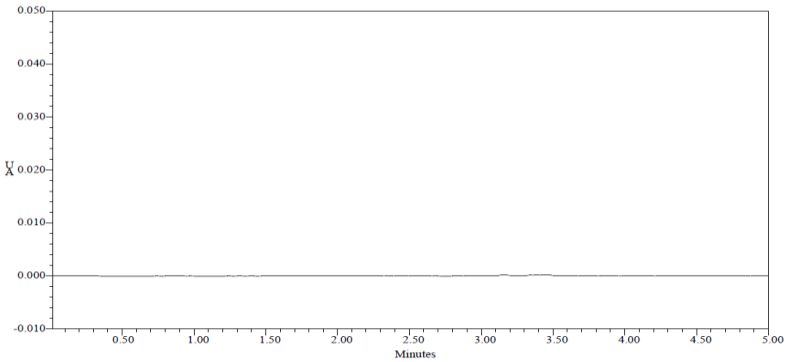

Figure 1: Chromatogram of Blank at 220nm

Standard and Sample Chromatograms

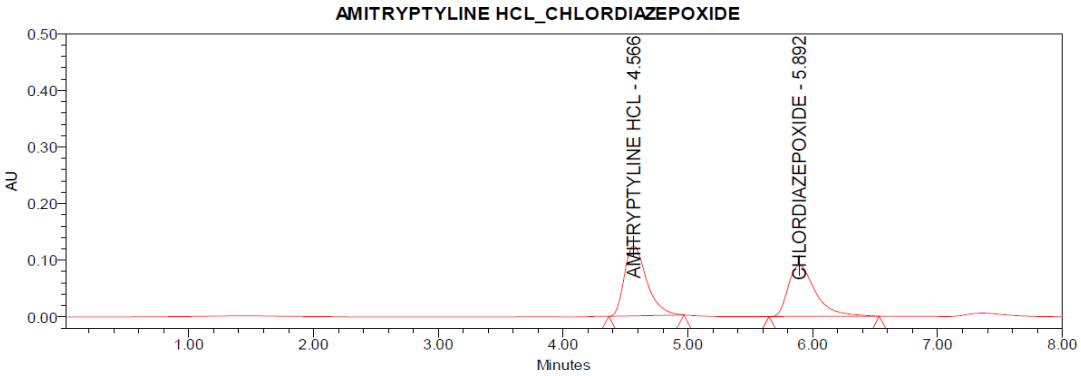

Figure 2: Chromatogram of Amitriptyline and Chlordiazepoxide standard at 220nm

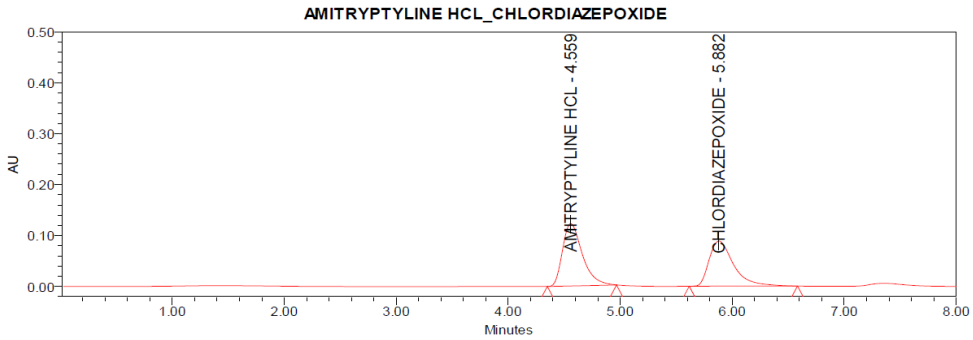

Figure 3: Chromatogram of Amitriptyline and Chlordiazepoxide formulation at 220nm

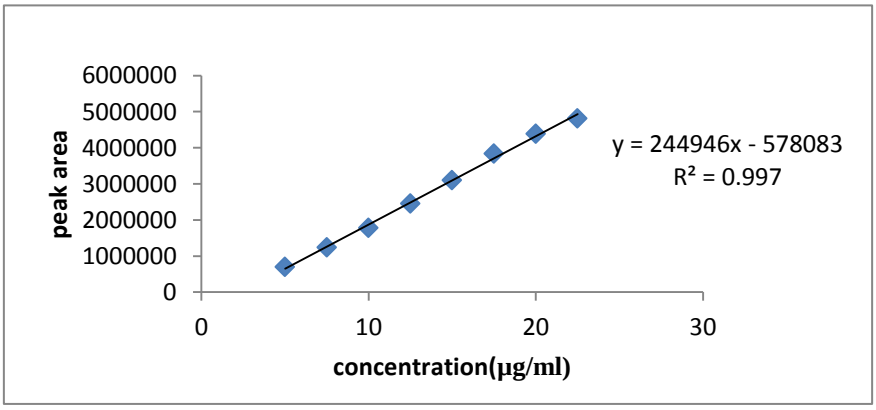

Figure 4: Calibration curve for Amitriptyline at 220 nm

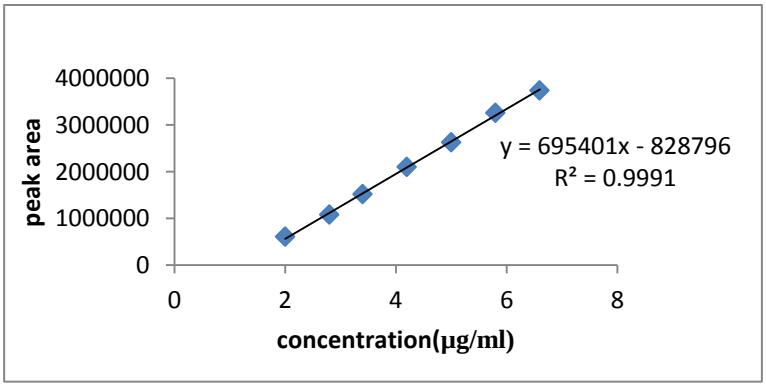

Figure 5: Calibration curve for Chlordiazepoxide at 220 nm

CONCLUSION

It was an attempt made to develop a new stability indicating RP-HPLC method which was validated for the simultaneous determination of Amitriptyline and Chlordiazepoxide in bulk and pharmaceutical dosage form. The method is significant as it does not use methanol in the mobile phase whereas previous methods uses methanol as mobile phase in different ratios. The retention times were different from other methods and isobestic point was 220nm which is not occurred in any other method. The limits of detection values are very low when compared to other methods. Hence the proposed method is concluded as simple, rapid, less economical, accurate and specific, sensitive and also stable from the stability and degradation studies.

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