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Development and Validation of Stability Indicating RP-UPLC Method for the Simultaneous Estimation of Chlordizepoxide and Amitriptyline Hydrochloride in Bulk and Tablet Dosage Forms

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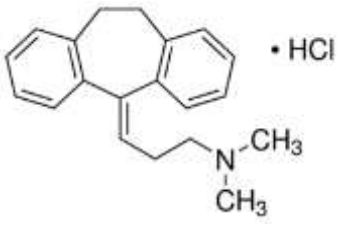
Abstract : The precise, accurate, sensitive and very rapid Isocratic Ultra Performance and Liquid Chromatography method has been developed for the estimation of chlordiazepoxide and amitriptyline hydrochloride. The method employs Waters Ecquity UPLC system with UV detector on X Bridge C18 column (4.6 X 50mm, 3.7). The optimum chromatographic separation was attained by usingacetonitrile:0.05M potassium dihydrogen phosphate buffer pH6.8 (70:30v/v) and pH is adjusted to 6.8 using sodium hydroxide(0.1N) as mobile phase at flow rate of 0.3mL /min^{-1}.The UV detection done at 240nm. Chlordiazepoxide and amitriptyline hydrochloride were eluted with the retention times of 0.84 and 1.16 min, respectively. Calibration plots of Chlordiazepoxide and amitriptyline hcl were linear over concentration ranges 10-50 and 25-125 µg/ml respectively. The percentage of relative standard deviation in accuracy and precision studies was found to be less than 1.5.Careful validation proved advantages of high sensitive, selectivity, accuracy, precision, and robust. Both drugs were subjected to stress conditions like acidic, alkaline, oxidative, thermal degradations and both are very sensitive to oxidative degradation. The developed and validated method was successfully used for the quantitative analysis commercially available dosage forms. The total analysis run time 3.0 minutes indicates speed and cost saving intiation of the method developed. No interfering peaks were found in chromatogram indicating that excipients used in the tablet dosage form does not interfered with the estimation of drugs by proposed RP-UPLC method.

Keywords: Chlordiazepoxide, Amitriptyline Hydrochloride, RP-UPLC, Stability Indicating Method, Validation.

Introduction:

Amitriptyline Hydrochloride is a tricyclic antidepressant and has at least equal efficacy against depression as the newer class of SSRIs according to a study from early. Chemically it is a3-(10,11-Dihydro-5H-dibenzo[a,d] cycloheptene-5-ylidene)-N,N-dimethylpropan-1-amine[1].Chemical structure of amitriptyline was shown in figure.1 .Chlordiazepoxide is indicated for the short term (2–4 weeks) treatment of anxiety which is severe and disabling or subjecting the person to unacceptable distress. It is also indicated as a treatment for the management of acute alcohol withdrawal syndrome[2].Chemically it is a7-choloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide4. Chemical structure of Chlordiazepoxide was shown in figure.2

Chlordiazepoxide and Amitriptyline Hydrochloride combination indicated in the treatment of severe depression, insomnia and alcohol withdrawl symptoms. This combination is available in market as a tablet dosage form.Several spectrophotometric[3-5], HPLC[6] and RP-HPLC[7-9] methods were reported for estimation of chlordiazepoxide and amitriptyline hclin single and combination. There is no UPLC method for estimation of these drugs and there is need to develop simple, sensitive, accurate and precise method for the estimation of chlodiazepoxide and amitriptyline hydrochloride in bulk and pharmaceutical dosage form. The method described is simple, sensitive, accurate and effectually used for routine quality control analysis of tablets. The developed method validated as per ICH guidelines[10].



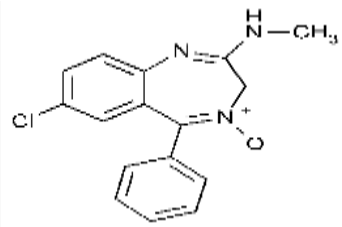


figure1. Structureof Amitriptyline HCL **figure 2.Structure of Chlordiazepoxide.**

2. Materials and Methods

Chemicals and reagents

Chlordiazepoxide and Amitriptyline Hydrochloride (working standards 99.67 and 99.97)were obtained as gift samples from Mylan labaratories, Hyderabad, India. Pharmaceuitical tablet formulation of Libotryp DS (10/25) was purchased from local market, Vijayawada. Acetonitrile, Hydrochloric Acid, Potassium Dihydrogen Phosphate, Methanol, Sodium hydroxide of analytical grade were obtained fromMerck India Ltd.

Instrumentation

Chromatographic separations of drugs were performed on Waters Acquity UPLC system, separation module integrated with auto sampler and equipped with Empower 2 software with waters 2996 photodiode Array Detector.

Chromatographic conditions

X Bridge C18 column (50 x 4.6. 3µm) was used for separation. The mobile phase containing acetonitrile:phosphate buffer pH6.8 (70:30 v/v) was delivered in isocratic mode at a flow rate of 0.3ml/min with the detection wavelength 240nm. The injection volume was 4µL and analysis was performed at ambient temperature.

Preparation of mobile phase or diluent

Accurately measured 300ml (30%) of 0.05M Potassium dihydrogen phosphate buffer (pH 6.8) and 700 ml (70%) of acetonitrlie were mixed and degassed in an ultrasonic water bath for 10minutes and the filtered through 0.45µm filter under vaccum filtration.

Preparation of stock solution

Accurately weigh and transfer the 25mg of chlordiazepoxide and 10mg of amitriptyline hydrochloride working standard into a 10ml clean dry volumetric flask add small amount of a diluents and sonicate to dissolve it completely finally make volume up to mark with same solvent. Further pipette 1ml of above solution into a clean and neat 10ml of volumetric flask and dilute upto the mark with diluent. Further pipette above solution into a 10ml volumetric flasks and make up to mark with diluents to achieve 10-50 and 25-125µg/ml concentrations of chlordiazepoxide and amitriptyline hydrochloride respectively. Mix well and filtered through the 0.45µm filter.

Preparation of sample solution

Accurately weigh 10tablets (LibotrypDS 10/25), crushed in a clean mortar and pestle and transfer equivalent to 10mg of chlordiazepoxide and 25mg of amitriptyline hydrochloride (marketed formulation) sample into a 10ml clean dry volumetric flask add little amount of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Mix well and filtered through the 0.45µm filter.

3.Results and Discussion

Method development and Optimization

To get optimized separation between the two components different buffer pH conditions and different proportions of solvents like water, ethanol and acetonitrile tested. However acetonitrile: 0.05M of potassium dihydrogen phosphate pH 6.8 (70:30) as mobile phase at flow rate of 0.3ml/min and detection at 240 nm was shown the both peaks well resolved, resolution 5.6,USP tailing 1.13 and 1.31 for chlordiazepoxide and amitriptyline hydrochloride respectively. Retention time of chlordiazepoxide was found to be 0.863min andfor amitriptyline hydrochloride was 1.191min.Chromatogram was shown in figure 3.

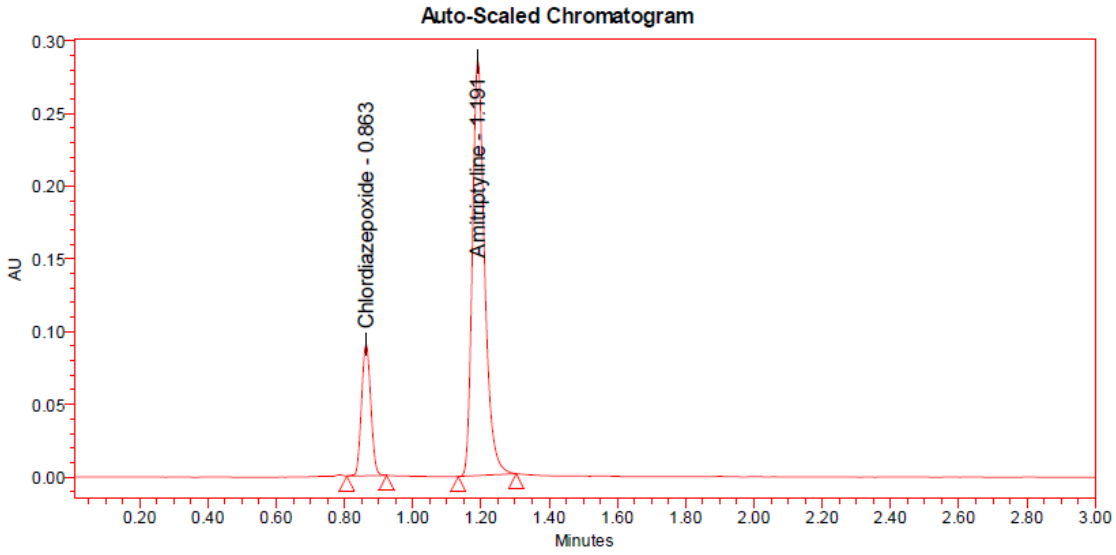


Fig.3. Representative Chromatogram of chlordiazepoxide and amitriptyline hydrochloride

Method validation

System suitability

System suitability is considered as importent portion in validation to evaluate the various parameters like USP plate count, USP tailing and USP resolution. System performance parameters for developed UPLC method were determined by injecting the standard solutions of chlordiazepoxide and amitriptyline hydrochloride. Various parameters such as retention time and area, USP plate count, USP tailing, USP resolution were shown in Table-1.

Table-1.Results of System suitabitlity parameters for chlordiazepoxide and amitriptyline hydrochloride

S.no	Name of the parameter	Chlordiazepoxide	Amitriptyline hcl
1	Retention time	0.863	1.191
2	Area	202531	841736
3	USP plate count	4631	5251
4	USP tailing	1.03	1.3
5	USP resolution	0	5.64

Specificity

The specificity studies were conducted to evaluate the possible interfence of placebo with analyte. Analyzing the blank, standard and sample solutions and proposed method showed the specificity by without interference. The chromatogram of blank, placebo, standard and sample solution were shown in figures 4a,4b,4c respectively.

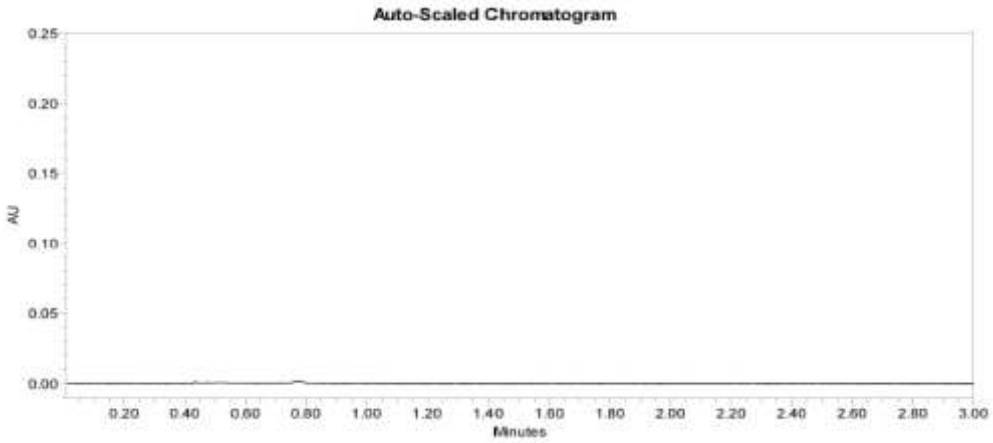


Figure 4a. Chromatogramof blank solution

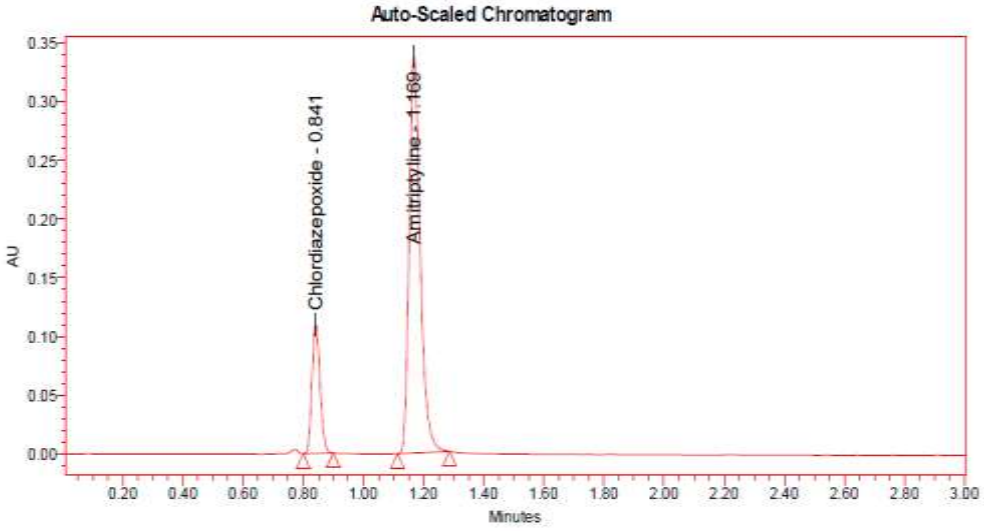


Figure 4b. Chromatogramof the standard solution

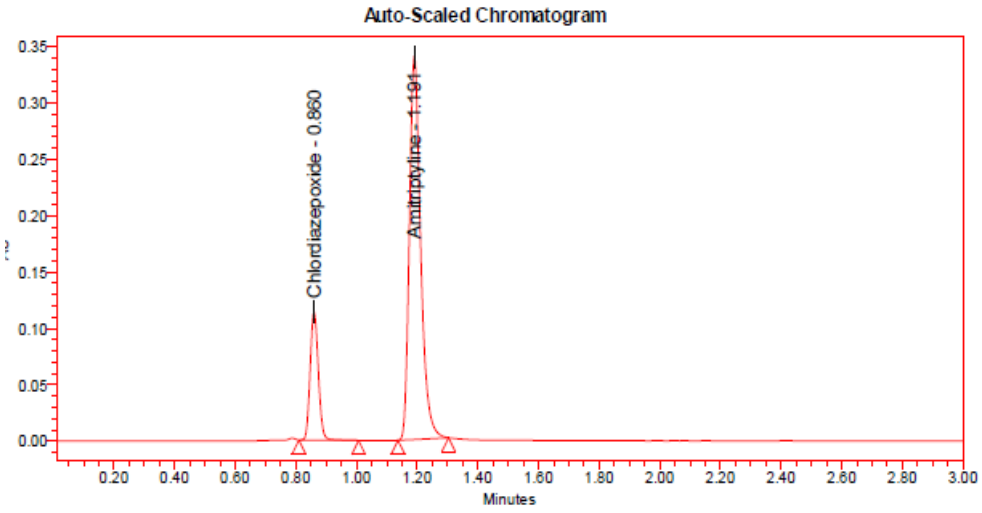


Figure 4c.Chromatogramof the sample solution

Linearity and Range

Linearity of the method was determined at five concentration level ranging from 10-50 and 25-125µg/ml of chlordiazepoxide and amitriptyline hydrochloride respectively. The calibration curves were constructed by plotting the response factor against concentrations of drugs. The regression equation, slope, intercept and correlation coefficient values are shown in Table-2 and calibration curve plots of chlordiazepoxide and amitriptyline hydrochloride shown in fig 5a and fig 5b respectively.

Table-2 linearity and range results

S.no	Parameters	Chlordiazepoxide	Amitriptyline hcl
1	Regression equation	Y=22854x+14008	Y=2359x+29157
2	Slope	22854	2359
3	Intercept	14008	29157
4	Correlation coefficient (R^2)	0.999	0.999

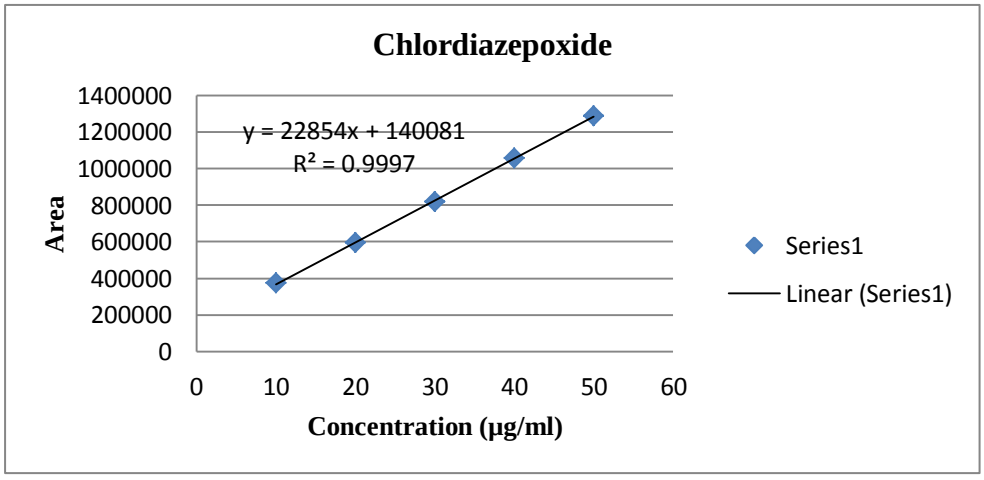


Figure 5a.Calibration curve plot of chlordiazepoxide

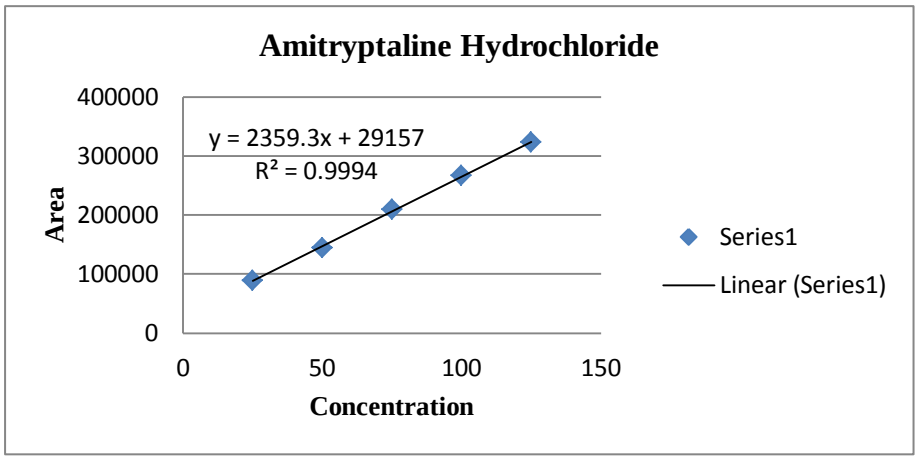


Figure 5b.Calibration curve plot of amitriptyline hydrochloride

Accuracy

Accuracy of the method can be determined by conducting the recovery studies. Recovery studies were conducted at three levels of 50, 100 and 150% of test concentrations in triplicate at each level of both drugs. Amount of drug recovered was quantified and % recovery was calculated and accuracy results were tabulated shown in Table-3

Table-3. Accuracy results

Sample	Level of recovery	Prenalyzed Conc. (µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)	ecovery (%)	%RSD
Chlordiazepoxide	50	20	10	29.5	98.33	0.9
	100	20	20	40.1	100.2	0.6
	150	20	30	50.2	100.4	0.2
Amitriptyline hydrochloride	50	50	25	74.8	99.7	0.5
	100	50	50	99.9	99.9	0.6
	150	50	75	125.2	100.1	0.5

Precision

Precision is usually expressed as standard deviation or relative standard deviation. In the present study, developed method was validated for method, system, repeatability, reproducibility and intraday precision. As values of % RSD of all the precision studies were within the acceptable limit (less than 2). Results of precision studies were tabulated shown in table-4.

Table-4. Precision results

Repeatability		
Parameter	Chlordiazepoxide	Amitriptyline hydrochloride
Mean	207281	864945.1
Standard deviation	2981.6	3649.1
%RSD	1.4	0.4
Intradayprecision		
Parameter	Chlordiazepoxide	Amitriptyline hydrochloride
Mean	208200.2	864989.4
Standard deviation	1694.7	6085.2
%RSD	0.8	0.7

Reproducibility		
Parameter	Chlordiazepoxide	Amitriptyline hydrochloride
Mean	210166.2	868399.8
Standard deviation	2459.3	8058.3
%RSD	1.2	0.9

Limit of detection and limit of quantification

The limit of detection and limit of quantification was calculated on the basis of signal to noise ratio of 3:1 and 10:1 respectively. The lowest limit detection of chlordiazepoxide and amitriptyline hydrochloride was found to be 0.015 and 0.113µg/ml respectively. The lowest limit of quantification for chlordiazepoxide and amitriptyline hydrochloride was found to be 0.045 and 0.386 µg/ml. The chromatograms of LOD and LOQ were shown in 6a and 6b respectively.

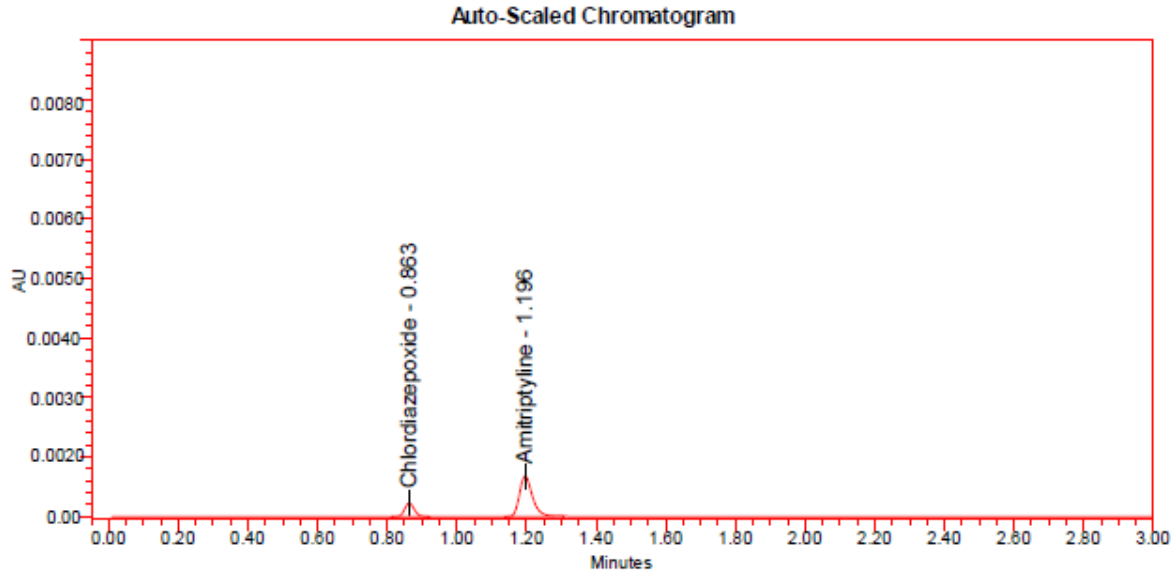


Figure 6a.Limit of detection chromatogram

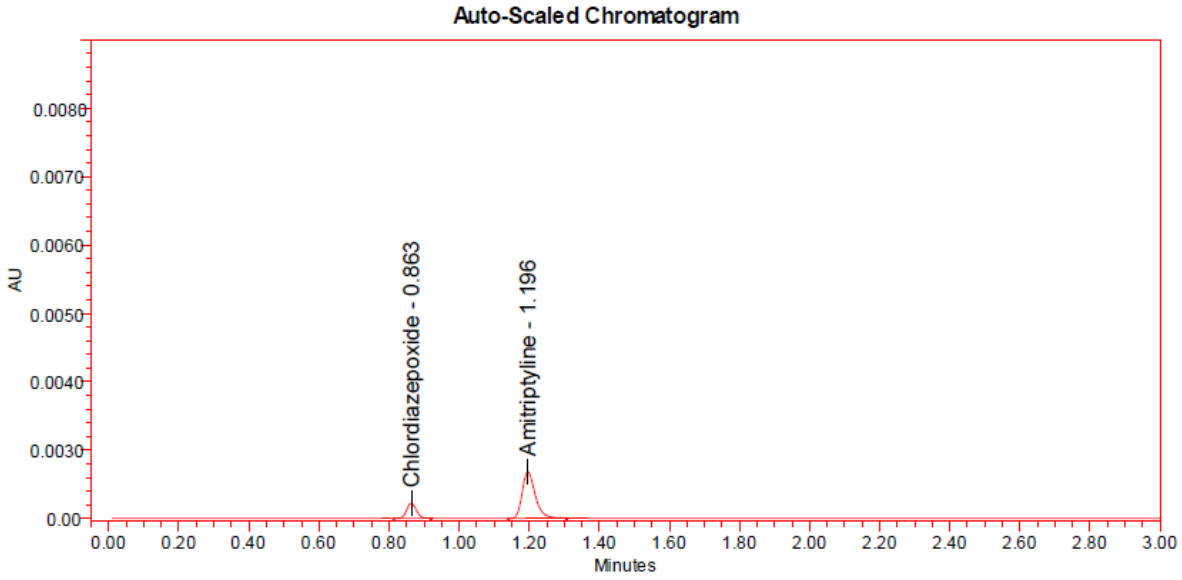


Figure 6b.Limitof quantification chromatogram

Forced Degradation Studies

Stress testing was carried using different stress conditions like such as acidic, basic, oxidative and thermal stresses. The acidic(0.1N HCl, at 60 ^{0}c for 6 hours), basic(0.1N Naoh, at 60 ^{0}c for 6 hours), peroxide(3% hydrogen peroxide, at 60^{0}c for 6 hours) and thermal degradation(at 105^{0}c for 6 hours)of chlordiazepoxide and amitriptyline hydrochloride results were shown in Table 6.

Table 6. Stability studies data

	Chlordiazepoxide		**Amitriptyline hydrochloride**	
	Area	**% Degraded**	**Area**	**% Degraded**
Standard	144632	-	589256	-
Acid	103254	6.40	338060	3.88
Base	924687	4.80	439607	5.13
Peroxide	116345	8.42	338066	7.64
Thermal	139642	5.86	383077	3.79

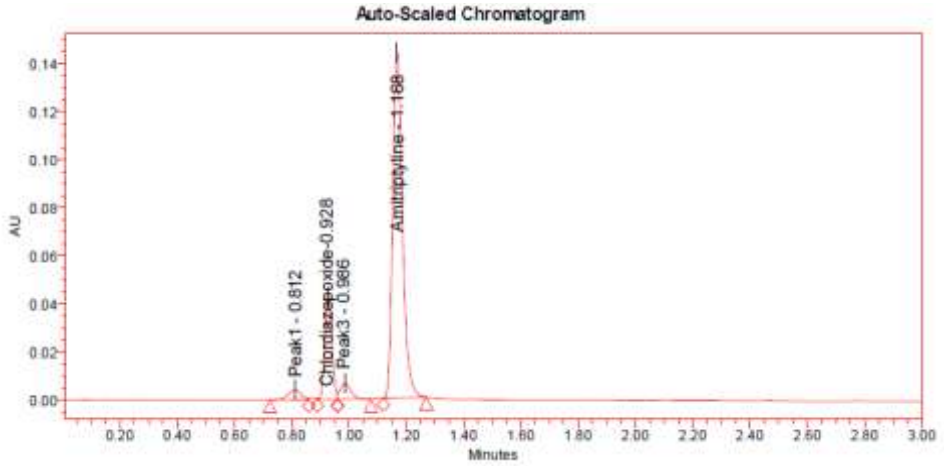


Figure 7a.Chromatogram of chromatogram of chlordiazepoxide and amitriptyline hcl in acid degradation

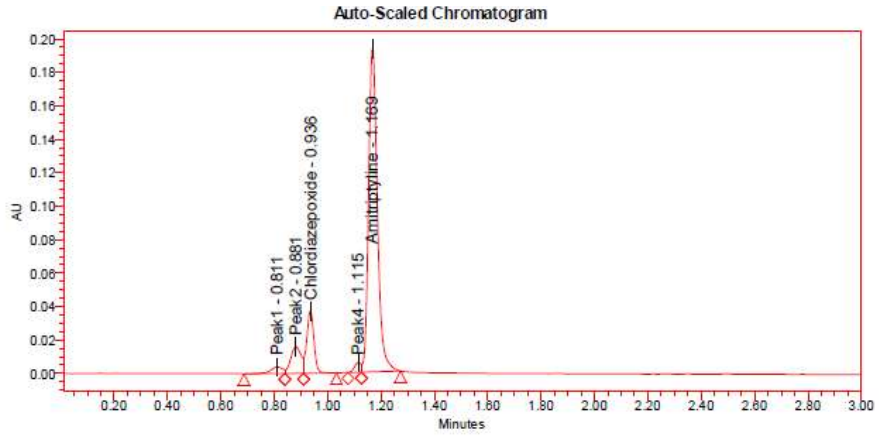


Figure 7b. Chromatogram of chlordiazepoxide and amitriptyline hcl in base degradation

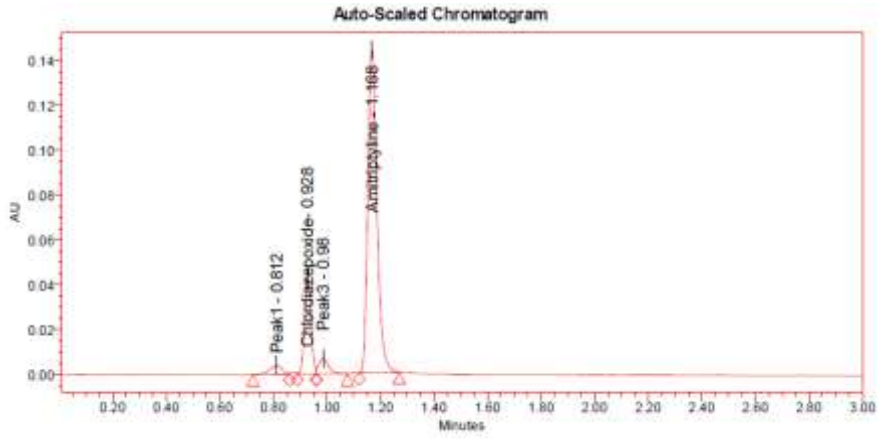


Figure 7c. Chromatogram of chlordiazepoxide and amitriptyline hcl in oxidative degradation

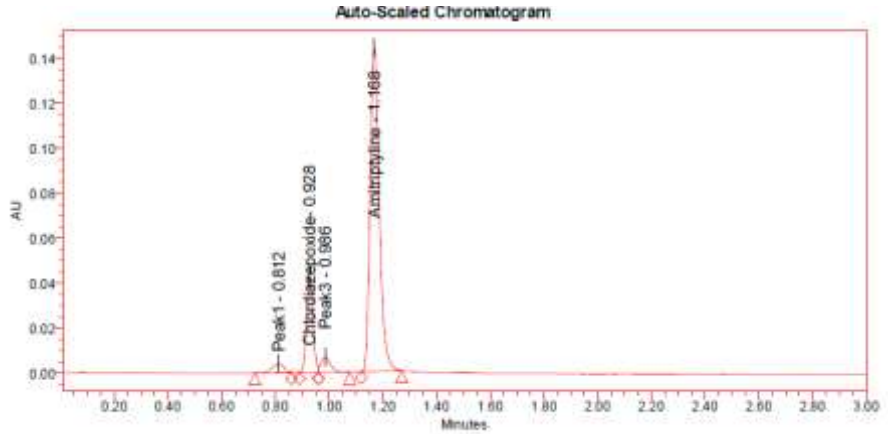


Figure 7d. Chromatogram of chlordiazepoxide and amitriptyline hcl in thermal degradation

Conclusion

The proposed method was ultra fast, sensitive, simple and reliable and found to be more accurate, precise, specific, stability indicating, rugged and robust hence it can be applied for estimation of chlordiazepoxide and amitriptyline hydrochloride. Conventional spectrophotometric and HPLC methods can be replaced by proposed UPLC method because of its superiority in cost effectiveness, time savings of analysis of time per sample and better detection. The developed method was validated as per ICH regulatory guidelines. The percent RSD values for all validation parameters were found less than 2, indicating the proposed is trusty worthy for simultaneous analysis of these drugs.

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