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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CHLORDIAZEPOXIDE AND CLIDINIUM BROMIDE IN COMBINED DOSAGE FORMS

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for the simultaneous estimation of Chlordiazepoxide and Clidinium bromide in combined dosage forms. Separation of Chlordiazepoxide and Clidinium bromide was successfully achieved on a ACE C_8 (150X4.6mm, 5µm Make: Agilent) in an isocratic mode utilizing H_3PO_4 buffer (pH 3.5): Methanol (50:50%v/v) at a flow rate of 1.0mL/min and with a retention time of 1.905 and 2.857 minutes for Clidinium bromide and Chlordiazepoxide. The method was validated and the response was found to be linear in the drug concentration range of 50µg/mL to 150µg/mL for Clidinium bromide and Chlordiazepoxide. This method was found to be good percentage recovery for Clidinium bromide and Chlordiazepoxide were found to be 100.52% and 100.44% respectively indicates that the proposed method is 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 extensively according validated to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

KEYWORDS: Clidinium bromide and Chlordiazepoxide.

1.0 INTRODUCTION

(a) (b)

Fig.1 (a,b): Structure of Chlordiazepoxide, Clidinium bromide

Chlordiazepoxide (7-chloro-N-methyl-5-phenyl-3H-1,4-benzodiazepine-2-amino-4-oxide) is use as an anxiolytic, sedative, hypnotic, anticonvulsant and skeletal muscle relaxant. The drug may inhibit monosynaptic and polysynaptic reflexes by acting as an inhibitory neuronal transmitter or by blocking excitatory synaptic transmission. The drug may also directly depress motor nerve and muscle function. Clidinium bromide (3-[(hydroxy-diphenylacetyl)-oxy]-1-methyl-1-azoniabicylo-[2.2.2] octane bromide is an anti cholinergic drug which may help symptoms of cramping and abdominal stomach pain by decreasing stomach acid and slowing the intestines. It is commonly prescribed in combination with Chlordiazepoxide by the name of Librax. Several methods have been published for the determination of Chlordiazepoxide in biological samples such as voltammetry, LC and spectrophotometry. So, in the present work, the aim is to develop a simple, rapid, accurate and sensitive method for simultaneous estimation of these two drugs for routine analysis.[1-7]

2.0 MATERIALS AND METHODS

2.1 Reagents and Chemicals

All solvents used were of HPLC grade. The reference standards of Chlordiazepoxide and Clidinium bromide

were obtained as gift samples from Lara drugs private limited (Hyderabad, India). The commercial fixed dose combination product Librax (Chlordiazepoxide 5mg and Clidinium bromide 2.5mg) was obtained from local pharmacy store. The solvents used were Methanol HPLC grade, Water HPLC grade (Fisher Scientific, Mumbai, India) and Acetonitrile HPLC grade (Merck, Mumbai, India).

2.2 Equipments

A high-performance liquid chromatographic system consisted of Waters 2695 series equipped with a quaternary pump (flow rate range of 50.000µL\ min−5.000mL\min) degasser and a column oven (temperature range of 1–85°C). All samples were injected (10µL) using an autosampler (injection volume range of 0.1–100 µL). Elutions of all analytes were monitored at 226 nm by using a PDA detector (190–900 nm). Each chromatogram was analyzed and integrated automatically using automation system software.

2.3 Chromatographic Conditions and Measurement Procedure

Chromatographic separation was performed on a reversed-phase ACE, C8 (150mm × 4.6mm, 5µ Make: Agilent) in an isocratic mode .The mobile phase was a mixture of H3PO4 buffer (pH 3.5): Methanol (50:50%v/v) respectively. The mobile phase was filtered through a 0.45µm nylon-membrane filter and degassed by ultra sonicator prior to use. A flow rate of 1.000mL/min was used in order to separate Clidinium bromide, Chlordiazepoxide. The injection volume was 10µL. Peak areas were measured and HPLC analysis was conducted at ambient temperature.

Table:1 Optimized Chromatographic parameters

S.No	Parameters	Specifications
1	Column	ACE,C_8 (150× 4.6mm, 5µm)
2	Mobile phase	Ortho phosphoric acid: Methanol (50:50).
3	Flow rate	1.0mL/min
4	Detector Wavelength	226nm
5	Injection volume	10µL
6	Column temperature	30^0C
7	Autosampler temperature	25^0C

2.4 Preparation of standard stock solution

About 50mg of Chlordiazepoxide and Clidinium bromide were weighed and transferred into a 50mL clean volumetric flask and add about 10mL methanol and 10mL water dissolve it completely and make the volume up to the mark with the water (Stock solution concentration was 1000.000µg/mL). Further pipette 5mL of Chlordiazepoxide and Clidinium bromide from the above stock solution into a 25mL volumetric flask and dilute up to the mark with diluent final concentration of both analytes 200.000µg/mL respectively. The solutions were sonicated for 20min and filtered through whatmann filter paper.

2.5 Preparation of sample solution

Ten tablets were weighed accurately and their average weights were determined and powdered. About 2.3000g of Chlordiazepoxide and Clidinium bromide tablets powder was weighed and transferred into a 50mL clean dry volumetric flask and add about 10mL of methanol and 10mL of HPLC water and sonicated for 20 min to dissolve it completely and filter through vacuum filter to separate the excipients and make up to the mark with the HPLC water (stock solution) further pipette out 2.72mL of Chlordiazepoxide and Clidinium bromide of the above stock solution into a 25mL volumetric flask and dilute up to the mark with HPLC water and the aliquot portion of the filtrate was further diluted to get the final concentration of 50.000µg/mL of Chlordiazepoxide and Clidinium bromide respectively and 10µL of the above solution was injected into the HPLC under the set chromatographic conditions.

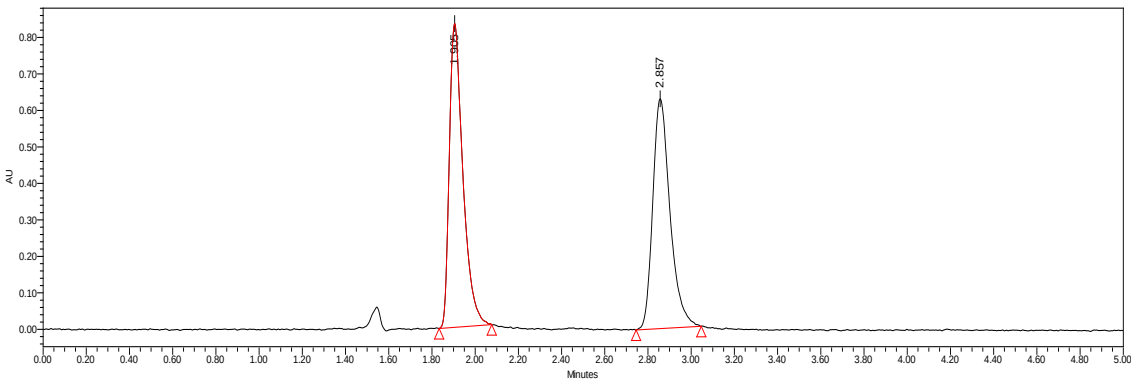

Fig.2: Chromatogram of mixture of Clidinium bromide and Chlordiazepoxide (50µg/mL)

3. METHOD VALIDATION

The developed method is validated for parameters such as system suitability, linearity, precision, accuracy, limit of detection, limit of quantification and robustness. Evaluation of analytical method validation report generated for the developed method as per ICH guidelines. Assay method precision was determined by using five independent test solutions. The intermediate precision of the assay method was also evaluated using on different days and the percentage RSD of area was calculated. The accuracy of the assay method was evaluated with the recovery. The linearity test solutions were prepared as described in section 4.2. linearity was studied by injecting concentrations of five of the standard Chlordiazepoxide and Clidinium bromide into the HPLC system. The peak area versus concentration data was performed by least squares linear regression analysis. The limits of detection (LOD) and quantification (LOQ) values were calculated. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±)0.2mL/min and the temperature of column modifier varied by (±)5°C.

4.0 RESULTS AND DISCUSSION

4.1 System suitability

In the system suitability tests, five replicate injections of freshly prepared working standard solutions of Chlordiazepoxide and Clidinium bromide (150.0µg/mL) were injected into the HPLC and the retention time, theoretical plates, resolution factor, tailing factor and % relative standard deviation (% RSD) of peak areas were determined. The results (Table:2) obtained from system suitability tests were in agreement with the USP requirements. The variation in peak area among five replicate injections of Chlordiazepoxide and Clidinium bromide standard solutions was very low.

Table:2 Results of System suitability parameters

S.No	Parameter	Chlordiazepoxide	Clidinium bromide
1	Retention time(Rt)	2.876	1.924
2	Theoretical plates(N)	5868.600	4185.600
3	Resolution(Rs)*	6.830	-
4	Tailing factor(T)	1.340	1.542
5	%RSD for five injections	0.400	1.000

*The resolution factor is calculated between each peak and its nearest preceding neighbor

4.2 Linearity

Several aliquots of standard solutions of Chlordiazepoxide and Clidinium bromide were taken in different 10mL volumetric flasks and the volume was made upto the mark with mobile phase such that final concentration of Clidinium bromide and Chlordiazepoxide is 50-150µg/mL. Evaluation was performed using the UV-V detector at 226nm, peak area recorded for all the peaks, results are displayed in Table:2 and calibration curve was plotted as time against peak area as shown in Figure:3 and 4. The slope and intercept values for calibration curve were y = 35674.3x+ 3111.6 (r^2 = 1.000) for Clidinium bromide and y = 16928.63x+3876.6 (r^2 = 1.000) for Chlordiazepoxide.

Table:3 Results of Linearity study

S.No	Concentration (µg/mL)	Peak area of Chlordiazepoxide	Concentration (µg/mL)	Peak area of Clidinium bromide
1	50	1699930	50	1789226
2	75	2542925	75	2671219
3	100	3385098	100	3577243
4	125	4233026	125	4461818
5	150	5087038	150	5353218
Correlation coefficient(r^2)		1.000	1.000	

Table:4 Rawdata summary of Linearity study

Peak Name: CLIDINIUM BROMIDE

	SampleName	Peak Name	RT	Area
1	LINEARITY-50%	CLIDINIUM BROMIDE	1.935	1789226
2	LINEARITY-75%	CLIDINIUM BROMIDE	1.922	2671219
3	LINEARITY-100%	CLIDINIUM BROMIDE	1.925	3577243
4	LINEARITY-125%	CLIDINIUM BROMIDE	1.902	4461818
5	LINEARITY-150%	CLIDINIUM BROMIDE	1.902	5353218

Peak Name: CHLORDIAZE POXIDE

	SampleName	Peak Name	RT	Area
1	LINEARITY-50%	CHLORDIAZE POXIDE	3.000	1699930
2	LINEARITY-75%	CHLORDIAZE POXIDE	2.999	2542925
3	LINEARITY-100%	CHLORDIAZE POXIDE	3.024	3385098
4	LINEARITY-125%	CHLORDIAZE POXIDE	3.016	4233026
5	LINEARITY-150%	CHLORDIAZE POXIDE	3.037	5087038

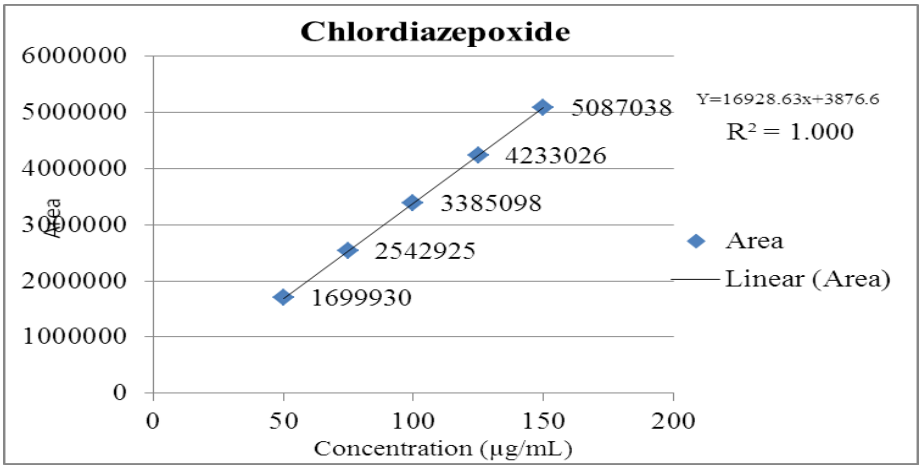

Fig.3: Calibration curve of Chlordiazepoxide

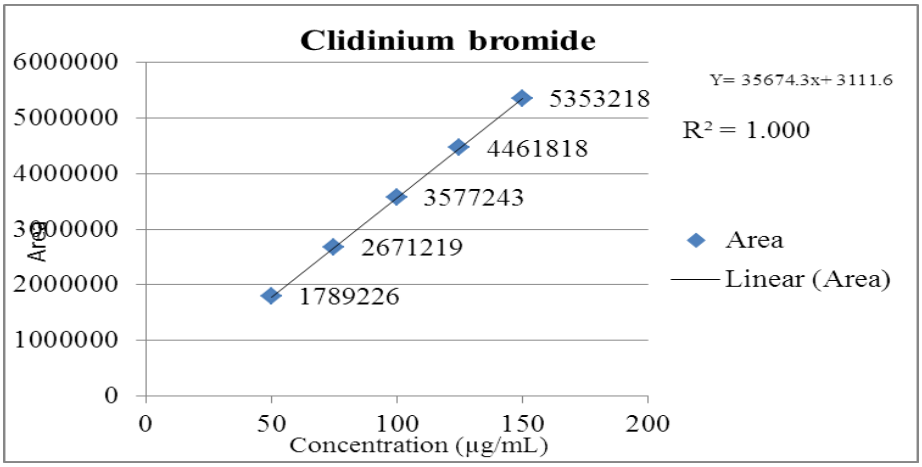

Fig.4: Calibration curve of Clidinium bromide

4.3 Precision

To determine the precision of method, six replicates of the sample prepared from the commercial tablets were injected and assay was calculated to measure the repeatability of retention times and peak area of standard and sample. Precision of the method was verified by using tablet stock solution. Intraday and inter day precision were determined by repeating assay six times in same day for intraday precision and on different days for inter day precision studies. The results of these analyses are shown in Table:5.

Table:5 Results of Intra day and inter day Precision

Analyte	Intra day precision		Inter day precision	
	Assay	% RSD	Assay	%RSD
Chlordiazepoxide	100.0	0.080	99.8	0.152
Clidinium bromide	100.0	0.090	99.7	0.237

4.4 Accuracy

Accuracy of the method was calculated by recovery studies at three levels (50%, 100% and 150%) by standard addition method. The accuracy was expressed as the percentage of the analyte recovered. Accuracy of proposed method was checked as per ICH guidelines. Tablet powder equivalent to 5.0mg of Chlordiazepoxide and 2.5mg of Clidinium bromide was taken individually into three different 100mL volumetric flasks and then 1.15g (50%), 2.29g (100%) and 3.44g (150%) of standard Chlordiazepoxide and Clidinium bromide were added to each of the volumetric flasks. After that 50mL of the HPLC water was added to each of the volumetric flask and sonicated for 20 min. The solutions were then filtered through vacuum filter. Transfer 2.5mL, 5 mL and 7.5mL of the filtrate from each was taken into 25mL volumetric flasks individually and diluted upto the mark with diluent. The solutions were injected in triplicates into the chromatographic system and the peak areas were observed and evaluated to give percent Recovery and Standard deviation.

Table:6 Results of recovery

Analyte	Accuracy Level	Amount added (µg/mL)	Mean of Amount founded (µg/mL)	Mean of recovered (%) ±SD	%RSD
Chlordiazepoxide	50%	99.267	99.730	100.44± 0.238	0.237
	100%	198.533	199.303	100.39 ± 0.105	0.104
	150%	297.800	299.260	100.49 ± 0.089	0.088
Clidinium bromide	50%	49.335	49.603	100.54 ± 0.135	0.134
	100%	98.669	99.217	100.56 ± 0.108	0.108
	150%	148.004	148.670	100.45 ± 0.047	0.047

4.5 LOD and LOQ

The minimum level at which the compounds can be reliably detected (Limit of Detection, LOD) and quantified (Limit of Quantification, LOQ) was determined experimentally (Table:7). The LOD was expressed as the concentration of drug that generated a response to three times of the signal to noise (S/N) ratio, and the LOQ was 10 times of the S/N ratio.

Table:7 LOD and LOQ of Chlordiazepoxide and Clidinium bromide

Parameter	Chlordiazepoxide (µg/mL)	Clidinium bromide(µg/mL)
Limit of Detection (LOD)	2.970	2.941
Limit of Quantification (LOQ)	9.901	9.804

4.6 Robustness

As part of the Robustness, deliberately changed flow rate and temperature to evaluate the impact on the method.

a) The flow rate variation: To demonstrate flow rate robustness, flow rate was altered with ±0.2mL/min of method flow (i.e. 0.800mL/min and 1.200mL/min).

Standard solution

About 200ppm of Chlordiazepoxide and 100ppm of Clidinium bromide were prepared and analyzed using the varied flow rates along with method flow rate.

b) The column temperature variation: To demonstrate temperature robustness, temperature of column oven was altered with ±5ºC of method temperature (i.e, 25ºC &35ºC).

Standard solution

About 200ppm of Chlordiazepoxide and 100ppm of Clidinium bromide was prepared and analysed using the varied column oven temperature along with the actual column temperature used in the method. The results (Table:8) of Robustness of the present method had shown that changes made in the flow and temperature did not produce significant impact on analytical results which were presented in the above table. Based on the results obtained and insignificant effect of the robustness experiment it concluded that the developed method is a Robust.

Table:8 Results of Robustness study

Parameter		Retention time (RT)		Tailing factor (T)		Resolution (RS)		Theoretical plates (N)	
	Analyte →	CBD	CZD	CBD	CZD	CBD	CZD	CBD	CZD
Flow rate (mL/min)	0.800	2.322	3.828	1.68	1.37	NA	9.22	4762	7234
	1.000	1.905	2.851	1.57	1.33	NA	7.23	4484	6473
	1.200	1.562	2.584	1.61	1.28	NA	6.74	4209	5861
Column (Temparature ºC)	25	1.902	3.08	1.61	1.31	NA	8.49	4222	6368
	30	1.916	2.875	1.59	1.32	NA	7.47	4085	5780
	35	1.882	3.081	1.64	1.33	NA	8.73	4592	6673

4.7 Specificity

The Specificity study of Chlordiazepoxide and Clidinium bromide was found to be free from interference (Table:9) and good separation between all peaks. Commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. This indicates that the method is Specific.

Table:9 Results of specificity study

S.No	Sample name	Peak area of CBD	Retention time (RT)	Peak area of CZ D	Retention time (RT)
1	Standard	3558963	1.916	3404123	2.875
2	Sample	3636260	1.905	3495808	2.857

3	Blank	-	-	-	-
4	Placebo	-	-	-	-

5.0 CONCLUSION

Literature review [8-17] revealed that only few instrumental methods have been reported to determine Chlordiazepoxide and Clidinium bromide in combined dosage form**.** The developed RP-HPLC method for simultaneous assay of Chlordiazepoxide and Clidinium bromide in combined tablets dosage forms is simple, precise, accurate, specific and robust. So, it can be employed for the routine analysis for simultaneous estimation. Hence, this RP-HPLC method is suitable for quality control of raw materials and formulations and also for dissolution studies.

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