ejpmr, 2024, 11(3), 139-146

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

METHOD DVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CLIDINIUM BROMIDE AND CHLORDIZEPOXIDE IN BULK AND TABLET DOSAGE FORM BY A RP-HPLC

Bairam Ravindar* and Yerra Shylaja

Department of Pharmaceutical Chemistry, Srikrupa Institute of Pharmaceutical Sciences, V. Velikatta, M. Kondapaka, Siddipet – 502277.

***Corresponding Author: Bairam Ravindar**

Department of Pharmaceutical Chemistry, Srikrupa Institute of Pharmaceutical Sciences, V. Velikatta, M. Kondapaka, Siddipet – 502277.

Article Received on 05/01/2024 **Article Revised on 25/01/2024** **Article Accepted on 15/02/2024**

ABSTRACT

New method was established for simultaneous estimation of Clidinium bromide and Chlordizepoxide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Clidinium bromide and Chlordizepoxide by using Inertsil C18 (4.6mm ×250mm, 5µm particle size), flow rate was 1.0 ml/min, mobile phase ratio was (55:45% v/v) Methanol: Phosphate buffer pH 4.8 (pH was adjusted with ortho phosphoricacid), detection wavelength was 282nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 1.688mins and 3.282mins. The % purity of Clidinium bromide and Chlordizepoxide was found to be 99.86%. The system suitability parameters for Clidinium bromide and Chlordizepoxide such as theoretical plates and tailing factor were found to be 7586, 1.69 and 6235 and 1.58, the resolution was found to be 10.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Clidinium bromide and Chlordizepoxide was found in concentration range of 100µg-500µg and 30µg-70µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.112% and 100.16%, %RSD for repeatability was 0.1702 and 0.043 respectively. The precision study was precise, robust, and repeatable. The LOD value was found to be 2.1µg/ml and 1.28µg/ml, and LOQ value was 6.3µg/ml and 3.84µg/ml for Clidinium bromide and Chlordizepoxide respectively. The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Clidinium bromide and Chlordizepoxide in pharmaceutical dosage form.

KEYWORDS: Clidinium bromide, Chlordizepoxide, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Clidinium is a synthetic anticholinergic agent which has been shown in experimental and clinical studies to have a pronounced antispasmodic and antisecretory effect on the gastrointestinal tract. It inhibits muscarinic actions of acetylcholine at postganglionic parasympathetic neuroeffector sites. It is used for the treatment of peptic ulcer disease and also to help relieve abdominal or stomach spasms or cramps due to colicky abdominal pain, diverticulitis, and irritable bowel syndrome.[1] IUPAC name 3-[(2-hydroxy-2,2-diphenylacetyl)oxy]-1-methyl-1-azabicyclo[2.2.2]octan-1-ium bromide. Molecular weight is 432.35 g/mole. Molecular formula is $C_{22}H_{26}BrNO_3$. Clidinium bromide was found to be Soluble in DMSO. Clidinium (bromide) is soluble in organic solvents such as DMSO and dimethyl formamide.

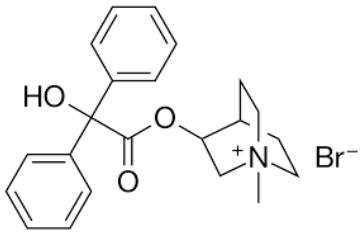

Figure 1: Structure of Clidinium bromide.

Chlordiazepoxide is a benzodiazepine used to treat the withdrawal symptoms of acute alcoholism, to treat preoperative anxiety, and to treat anxiety over a short term period. Chlordiazepoxide binds to stereospecific benzodiazepine (BZD) binding sites on GABA (A) receptor complexes at several sites within the central nervous system, including the limbic system and reticular formation. This results in an increased binding of the inhibitory neurotransmitter GABA to the GABA(A) receptor. BZDs, therefore, enhance GABA-

mediated chloride influx through GABA receptor channels, causing membrane hyperpolarization. The net neuro-inhibitory effects result in the observed sedative, hypnotic, anxiolytic, and muscle relaxant properties.[2,3] IUPAC name 7-chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepin-4-ium-4-olate. Molecular weight is 299.7 g/mole. Molecular formula is $C_{16}H_{14}ClN_3O$. Soluble in water; soluble or sparingly soluble in alcohol

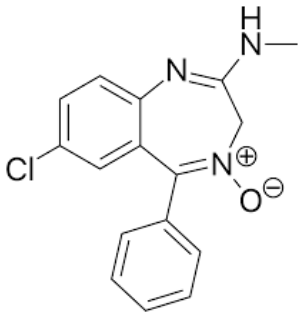

Figure 2: Structure of Chlordizepoxide.

The United States Pharmacopeia (USP) stated the nonaqueous titration method for the assay of clidinium bromide and chlordiazepoxide.[4] Few methods for the determination of clidinium bromide and chlordiazepoxide in combined dosage forms including HPLC,[5–7] derivative spectrophotometry,[8,9] spectrophotometry using multivariate calibration techniques,[10] and capillary SFC,[11] have been reported. Literature survey revealed that some analytical methods have been used for the individual estimation of clidinium bromide and chlordiazepoxide. Capillary electrophoresis,[12] and kinetic spectrophotometric,[13] methods for clidinium bromide have been described. Chlordiazepoxide has been determined either alone or with other compounds in pharmaceutical formulations using high-performance liquid chromatography,[14–23] first-derivative spectrophotometry,[17] spectrophotometry,[23,24] HPTLC,[23,25] voltammetry,[26] and flow-injection potentiometry.[27] Several methods have been published for the determination of chlordiazepoxide in biological samples such as voltammetry,[26] LC,[28] and spectrophotometry.[29] In this work, a new reversed-phase high-performance liquid chromatographic method is proposed for the simultaneous determination of clidinium bromide and chlordiazepoxide in combined dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents: Clidinium bromide and Chlordizepoxide were obtained as a gift sample from sura training lab, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Phosphate Buffer (pH-4.8): Methanol (55:45% v/v), dimensions at 35^0C temperature. The optimized mobile phase consists of. Flow rate was maintained at 1 ml/min and run time for 6 min.

Preparation of solutions

Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation

Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of the Clidinium bromide and Chlordizepoxide standard solution

Preparation of standard solution: (Clidinium bromide)

Accurately weigh and transfer 10 mg of Clidinium bromide, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Chlordizepoxide)

Accurately weigh and transfer 10 mg of Chlordizepoxide working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 3ml of Clidinium bromide, 0.5ml of Chlordizepoxide from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Clidinium bromide, Chlordizepoxide sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure

Further pipette 1.2ml of Clidinium bromide, Chlordizepoxide from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C 18 column (4.6 x 250mm, 5µm), the mobile phase of composition Phosphate Buffer (pH-4.8): Methanol (55:45% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Clidinium bromide and Chlordizepoxide in their pharmaceutical dosage form. The result obtained for Clidinium bromide and Chlordizepoxide was comparable with the corresponding labeled amounts and they were shown in Table-2.

RESULTS AND DISCUSSION

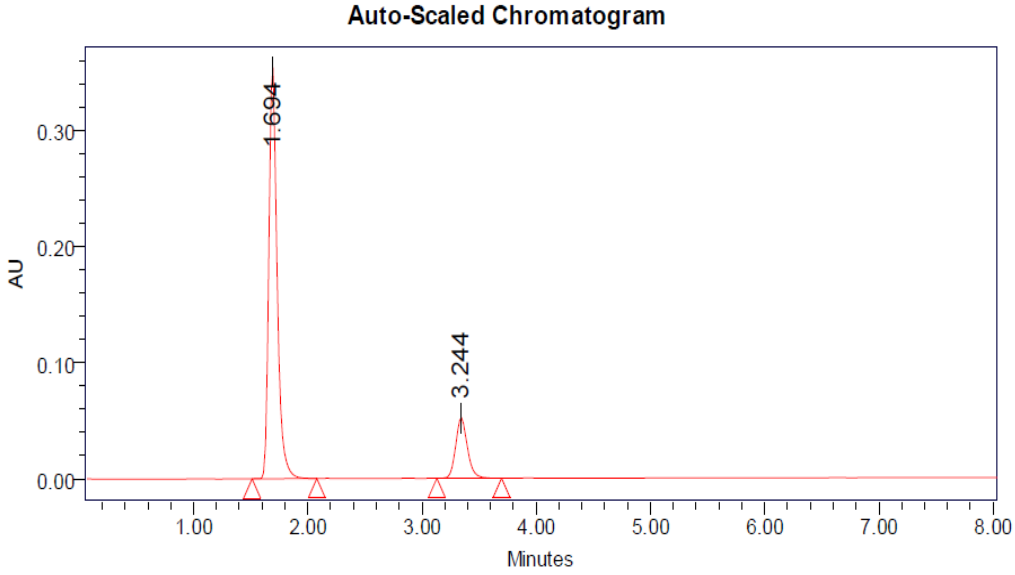

Figure 3: Standard chromatogram.

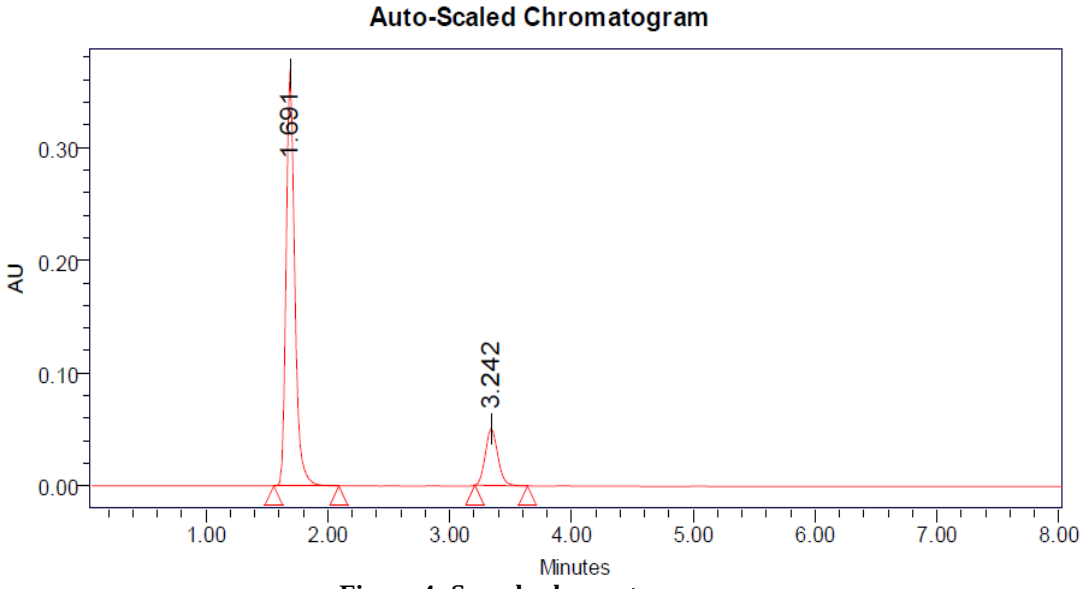

Figure 4: Sample chromatogram.

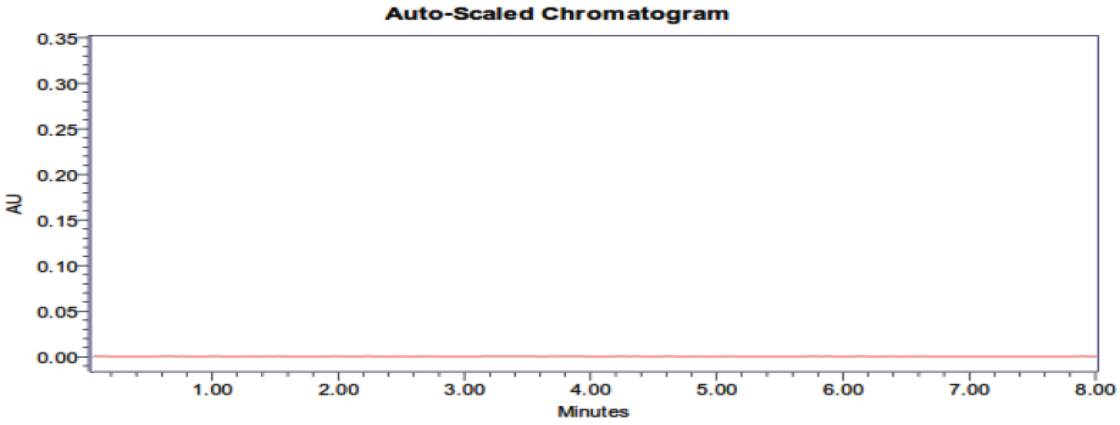

Figure 5: Blank chromatogram.

Table 1: System suitability parameters.

Parameters	Clidinium bromide	Chlordizepoxide
Retention time	1.688	3.282
USP Plate count	7586	6235
USP Tailing	1.69	1.58

Table 2: Assay results for Clidinium bromide and Chlordizepoxide.

	Label Claim (mg)	% Assay
Clidinium bromide	80	99.86
Chlordizepoxide	20	99.86

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and30 ppm to 70 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Table 3: Linearity results for Clidinium bromide and Chlordizepoxide.

Clidinium bromide		Chlordizepoxide	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Area**
100	585985	30	268764
200	1182468	40	356958
300	1768785	50	445631
400	2326852	60	535186
500	2856874	70	624698
Correlation coefficient	0.999	Correlation coefficient	0.999

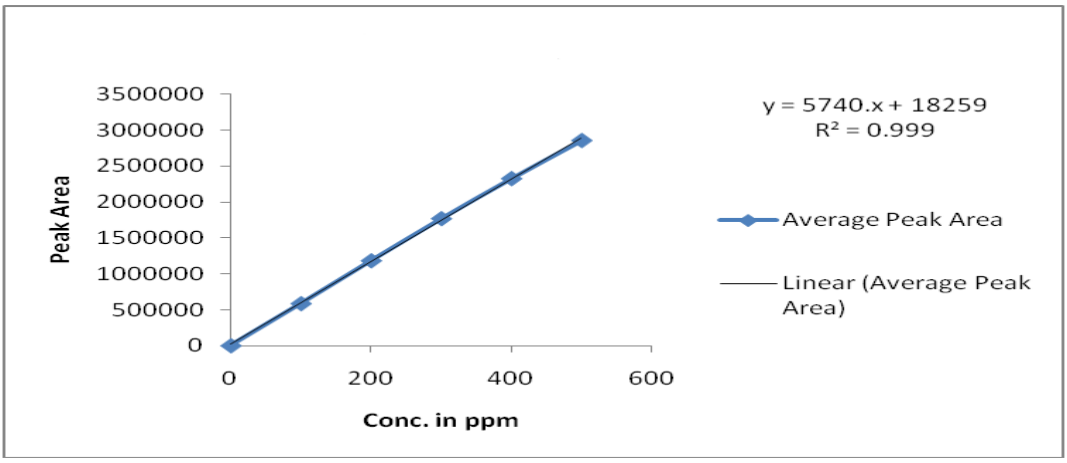

Figure 4: Linearity graph for Clidinium bromide.

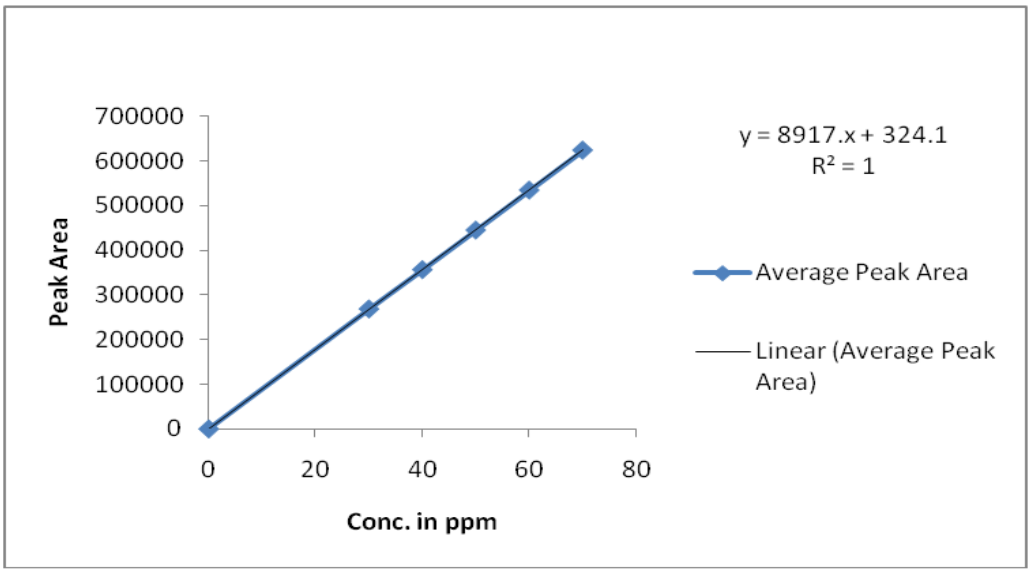

Figure 5: Linearity graph for Chlordizepoxide.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Table 4: Showing accuracy results for Clidinium bromide.

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table 5: Showing accuracy results for Chlordizepoxide.

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Table 6: Precision results for Clidinium bromide and Chlordizepoxide.

S. No	Clidinium bromide	Chlordizepoxide
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7 and 8.

Table 7: Intermediate precision resultes for Clidinium bromide and Chlordizepoxide on day 1.

S. No	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Table 8: Intermediate precision resultes for Clidinium bromide and Chlordizepoxide on day 2.

Injection	Area for Clidinium bromide	Area for Chlordizepoxide
Injection-1	1648598	415985
Injection-2	1642587	415267
Injection-3	1649852	415986
Injection-4	1648754	415265
Injection-5	1645289	415874
Injection-6	1647581	415632
Average	**1647110**	**415668.2**
STD Deviation	**2699.291**	**337.2106**
%RSD	**0.16388**	**0.081125**

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The Wavelength varied from 243nm to 247nm. The resulte are shown in table 9,10,11,12.

Robustness results for Clidinium bromide

Table 9: Organic Composition results for Clidinium bromide.

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Table 10: Wavelength variation results for Clidinium bromide.

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Robustness results for Chlordizepoxide

Table 11: Flow variation results for Chlordizepoxide.

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Table 12: Organic Composition results for Chlordizepoxide.

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 13.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 13: LOD, LOQ of Clidinium bromide and Chlordizepoxide.

Drug	LOD	LOQ
Clidinium bromide	2.10	6.30
Chlordizepoxide	1.28	3.84

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Clidinium bromide and Chlordizepoxide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Clidinium bromide and Chlordizepoxide in pure and its pharmaceutical dosage forms.

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