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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR CONTENT UNIFORMITY TEST OF CLIDINIUM BROMIDE CHLORDIAZEPOXIDE AND DICYCLOMINE HCl IN COMBINED DOSAGE FORM

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ABSTRACT
The aim of the present work was to develop simple, shorter and effective RP-HPLC method with UV detection (209nm) and subsequent validation for the content uniformity determination of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl in marketed tablet samples. The method uses isocratic mobile phase of phosphate buffer (pH 5) and methanol 60:40 compositions on reverse phase Shimadzu LC 2010 CHT C_{18} column. The linearity range of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was Linearity observed for 5-15 µg/ml, 10-30µg/ml and 20-60µg/ml. percentage of label claim established with 0.9999 correlation, Retention time of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was found to be 4.200, 3.420, 11.570 min. respectively and The percentage recoveries Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl obtained for were found to be in range of 99.81-100.47, 99.47-100.89, 100.23-100.71 respectively. different brands marketed samples were successfully analysed for content uniformity and compared the results with the USP and other guidelines for acceptance criteria. The developed method was found precise, linear, rugged and robust for validated parameters. The method can be used for assay and the content uniformity determination of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl in its tablet dosage form.

KEYWORDS: Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl; Content uniformity test; Tablet dosage form; Market sample analysis.

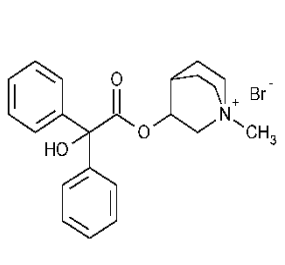
1.INTRODUCTION[1,2]
Content uniformity or the Uniformity of Dosage unit is defined as the degree of uniformity in the amount of active substance among dosage units. The risk assessment strategyunderlying content uniformity testing is the assumption that some pre-specified limits exist wheresafety and efficacy outcomes may change if content uniformity fails moreover drug content and content uniformity depends on a number of processes associated with its manufacture, hence it is obviously unrealistic to presume every unit to be contained exactly thesame amount of the active ingredient as of the label claim, and also due to increased awareness of physiological availability, pharmacopieal standards and specifications have been established toprovide limits for allowable variations for the active ingredients in single dosage units of all coated and uncoated tablets intended for oral administration where the range ofsize of the dosage form available include 25 mg or smaller sizes. the limite of this content uniformity is 85 to 115 %.

To study and compare the content uniformity test, a new class of substituted Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl. Clidinium bromide (3-hydroxy-methylquinuclidinium bromide benzilate) is a quaternary ammoniumcompound with anticholinergic andantispasmodic activity (3-hydroxy-1-methylquinuclidinium bromide) is one potential degradation product of clidinium bromide. impurity is monitored in clidinium bromide drug substance and in clidinium bromide pharmaceutical dosage forms.[4]

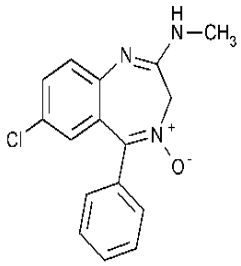
Chlordiazepoxide, 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide, is a benzodiazepine It is used for the short-term treatment of anxietydisorders and insomnia. CDO is also used in musclespasm and in alcohol withdrawal syndrome.[5]

2-(diethyamino) ethyl 1-cyclohexylcyclohexane-1-carboxylate It is anticholinergic drug used to treat a certain type of intestinal problem called irritable bowel

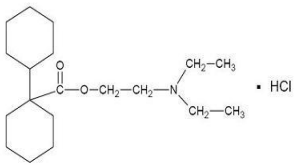
syndrome and it helps to reduce the symptoms of stomach.[6,7]



Clidinium bromide



Chlordiazepoxide



Dicyclomine HCl

This Combination of three drugs (Normaxin tablet) is highly effective and used in the treatment of peptic ulcer, nervous dyspepsia, gastritis, irritable spastic colon, mucous colitis, acute Enterocolitis.

Literature survey reveals that some analytical methods have been used for the estimation of Clidinium bromide, Chlordiazepoxide and Dicyclomine hydrochloride individually or combination with other drugs.

MATERIALS AND METHODS

Clidinium Bromide Were Received As Gift Sample From MSN Laboratories Ltd., India Chlordiazepoxide And Dicyclomine HCl Was Gifted By The Vapi Care Pvt. Ltd. Distilled Water, Acetonitrile And Methanol Was RANKEM (HPLC Grade) UV Spectrophotometer was used for the wavelength selection. Model Lab India, UV 3000+ Software UV win software Range 200-800 nm Lamp Source: Deuterium and Tungsten lamp source.

HPLC method conditions

SCL-10A VP Shimadzu LC 2010 CHT Software: LC Solution Column: a Sheisdo C_{18} (250*4.6 mm,5µm)Pump: Isocratic Wavelength range Range as eluted with a mobile phase containing Phosphate buffer (pH 5) (60:40,v/v) at a flow rate of 1 mL min-Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was determined by UV detection at 209 nm. The injection volume of 20 µl and the run time was 10min.

PREPARATON OF STANDARD STOCK SOLUTION

Preparation of CLI, CHLO and DIC HCl stock solution: A 100 mg of Clidinium bromide was weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and sonicate the flask. The volume was made up to the mark with mobile phase to give 1000 µg/ml.

Working standard solution of Clidinium bromide: 10 ml of stock solution was diluted to 100 ml with mobile phase to prepare 100 µg/ml, Chlordiazepoxide 20 ml of stock solution was diluted to 100 ml with mobile phase to prepare 200 µg/ml. and Dicyclomine HCl 40 ml of stock solution was diluted to 100 ml with mobile phase to prepare 400 µg/ml.

Preparation of sample solution

Twenty Tablet were weighed and powder was collected. The tablet powder equivalent to 2.5 mg of Clidinium bromide, 10 mg of Chlordiazepoxide and 10 mg of Dicyclomine HCl was transferred to 100 ml volumetric flask. The content was mixed with mobile phase, sonicated for 15 min. dissolve the drug completely with mobile phase. The solution was filtered through whatman filter paper no. 42.

Method development

The developed RP-HPLC method was validated as per ICH guidelines.[11]

Selection of wavelength

Both the components show reasonably good response at 209 nm.

The standard solution of CLI, CHLO and DIC HCl were scaned between 200-400 nm using UV visible spectrophotometer. wavelength was selected from the overlay spectra of above solutions. Best response was achieved 209nm. So all three drugs were detected at this analytical wavelength.

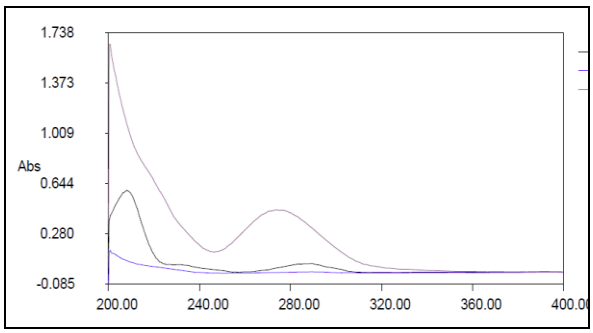


Figure no.:7.1.10 Selection of Wavelength.

Assay

Twenty tablet were weighed and triturate powder was collected. The powder equivalent to 2.5 mg of CLI, 5 mg of CHLO and 10 mg of DIC a was transferred to 100 ml volumetric flask. The content was mixed with mobile phase, sonicated for 15 min. Dissolve the drug completely with mobile phase. The solution was filtered through whatman filter paper no. 42 and first few drops of filtrate were discarded. The original stock solution

was further diluted to get sample solution of drug concentration of CLI (10µg/ml), CHLO (20µg/ml), DIC (40µg/ml). A 20 µl volume of sample solution was injected into HPLC. The peak area for the drugs was measured at 209 nm and amount of CLI, CHLO, DIC were determined using the related linear regression equations. The % assay of the drugs was calculated and the results are given in Table 1

SPECIFICITY

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (R*t*), resolution (*RS*), and asymmetry factor (*As*) and number of theoretical plates were calculated. Good correlation was found between the results of mixed standards and sample solutions.

Accuracy

The % recovery experiment was performed by the Standard Addition Method. Fixed amounts of sample mixture of Clidinium bromide (5 µg/ml), Chlordiazepoxide (10 µg/ml) and Dicyclomine HCl (20 µg/ml) and increasing amount of its working standard solutions were added at 80, 100 and 120 % level of Clidinium bromide (4, 5 and 6 µg/ml), Chlordiazepoxide (8,10 and 12 µg/ml) and Dicyclomine HCl (16, 20, and 24 µg/ml). Area of peak obtained with each solution was measured for Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl. The mean % recovery from the peak areas was calculated. Table 3, 4, 5.

Precision

A. Repeatability

1 ml of combined working standard solutions (100 µg/ml of CLI, 200 µg/ml of CHLO and 400µg/ml of DIC HCl) were transferred into separate 10 ml volumetric flasks and diluted up to mark with mobile phase to get 10 µg/ml of CLI, 20 µg/m CHLO and 40 µg/ml of DIC HCl. Each concentration was prepared and analyzed. The peak area obtained with each solution was measured and % R.S.D was calculated.

B. Intra-day precision

Standard solution of Clidinium bromide (5-15 µg/ml),Chlordiazepoxide (10-30µg/ml) and Dicyclomine HCl(20-60 µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed on the same day and % R.S.D was calculate 0.337-0.464 % of CLI, 0.269-0.297 % of CHLO and 0.638-0.836% of DIC.

C. Inter-day precision

Standard solution of Clidinium bromide (5-15 µg/ml),Chlordiazepoxide (10-30µg/ml) and Dicyclomine HCl(20-60 µg/ml) were prepared from working standard solutions and injected in to system with stated chromatographic conditions and analyzed on different days and % R.S.D was calculated. 0.900-0.946 % of CLI 0.712-0.942 % of CHLO 0.962-1.900% of DIC.

LOD and LOQ

The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

LOD = 3.3 × (SD / Slope)

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves. Table

The linearity is expressed in term of correlation co-efficient of linear regression analysis. Aliquots of standard solutions of CLI in range of 5-15 µg/ml CHLO in range of 10-30 µg/ml and DIC HCl in range of 20-60 µg/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analysed. Linear correlation was obtained between peak areas versus concentration of CLI in range of 5-15 µg/ml CHLO in range of 10-30 µg/ml and DIC HCl in range of 20-60 µg/ml The calibration curves of CLI, CHLO and DIC HCL at 209 nm.

LINEARITY

The linearity is expressed in term of correlation co-efficient of linear regression analysis. Aliquots of standard solutions of CLI in range of 5-15 µg/ml CHLO in range of 10-30 µg/ml and DIC HCl in range of 20-60 µg/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analysed. Linear correlation was obtained between peak areas versus concentration of CLI in range of 5-15 µg/ml CHLO in range of 10-30 µg/ml and DIC HCl in range of 20-60 µg/ml The calibration curves of CLI, CHLO and DIC HCL at 209 nm. Show table.

ROBUSTNESS

The robustness study was done by making small changes in the optimized method parameters like changing in pH of the mobile phase by ± 2%, mobile phase ratio by ±2%, and flow rate by ± 2 ml/min and the chromatographic characteristics were evaluated. No significance change was observed.

Estimation of Content Uniformity of CLI, CHLO and DIC HCl.

- 10 tablets are taken in 10 separate 100ml volumetric flask.
- Add 60ml of methanol in each volumetric flask.
- Sonicated the solution for 15 min.
- Dilute up to the mark by using same solvent.

- Mix well & filtered using whatman filter paper no.42.
- 2.5mg drug sample diluted up to 100ml with methanol to obtain 25µg/ml.
- 4ml solution dilute up to 10ml with same solvent obtain CLI, CHLO and DIC HCl 10µg/ml, 20 µg/ml and 40 µg/ml.
- Absorbance of resulting solution was measured at 209nm.

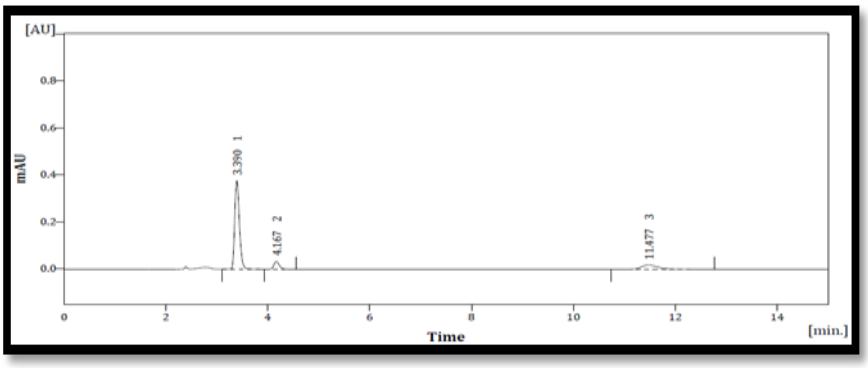


Figure 1: Chromatogram of CHLO, CLI & DIC HCL of std. solution.

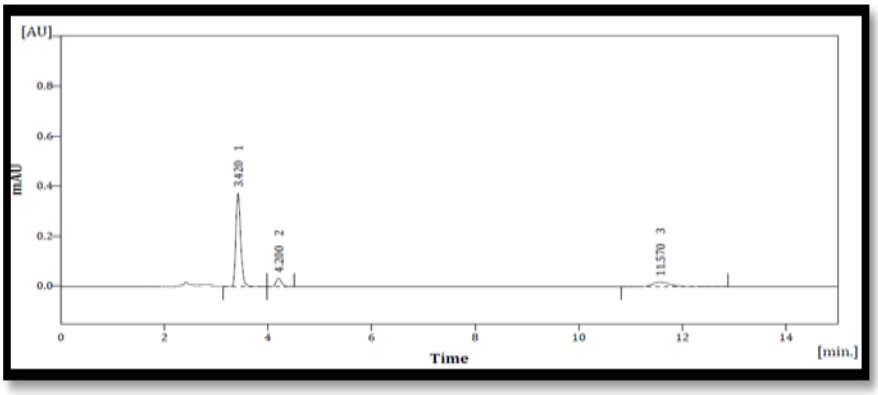


Figure 2: Chromatogram of CHLO, CLI & DIC HCl of sample solution,

Table 1: Assay Parameters.

Formulation (tablet)	Tablet amount (mg)			Amount found (mg)			% Assay (n=3)		
	CLI	CLO	DIC HCl	CLI	CLO	DIC HCl	CLI± S.D	CLO± S.D	DIC ± S.D
1	2.5	5	10	2.46	4.95	9.85	98.515	98.677	98.668
2	2.5	5	10	2.46	4.94	9.88			
3	2.5	5	10	2.45	4.91	9.82			

Table 2: Results from validation and system suitability studies

Validation parameters	CLI	CHLO	DIC
Theoretical plates	7021.167 ± 208.693	1513.73 ± 2.470	6088.533 ± 0.690
Resolution	-	4.155	18.643
Asymmetry factor	1.352 ± 0.036	1.389 ± 0.037	1.489 ± 0.116
Intra-day precision (%RSD)	0.337-0.464	0.269-0.588	0.638-0.836
Inter-day precision (%RSD)	0.900-0.946	0.712-0.769	0.962-1.900
LOD (µg/ml)	0.713	0.631	0.713
LOQ (µg/ml)	2.161	1.913	2.169

Table 3: Accuracy CLI.

Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	5	4	9	4.020	100.518	99.870 ± 1.345
	5	4	9	3.932	98.324	
	5	4	9	4.030	100.769	
100%	5	5	10	5.076	101.533	100.471 ± 0.945
	5	5	10	5.007	100.159	
	5	5	10	4.986	99.722	
120%	5	6	11	6.084	101.405	99.817 ± 1.480
	5	6	11	5.974	99.572	
	5	6	11	5.908	98.474	

Table 4: Accuracy CHLO.

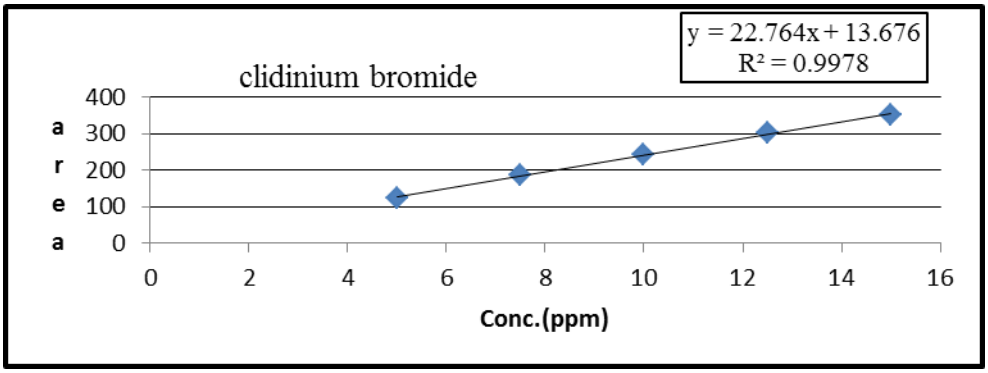
Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	10	8	18	8.090	101.126	100.897 ± 0.818
	10	8	18	8.126	101.576	
	10	8	18	7.999	99.988	
100%	10	10	20	10.088	100.882	99.932 ± 1.313
	10	10	20	10.048	100.480	
	10	10	20	9.843	98.433	
120%	10	12	22	12.098	100.819	99.477 ± 1.180
	10	12	22	11.881	99.015	
	10	12	22	11.831	98.599	

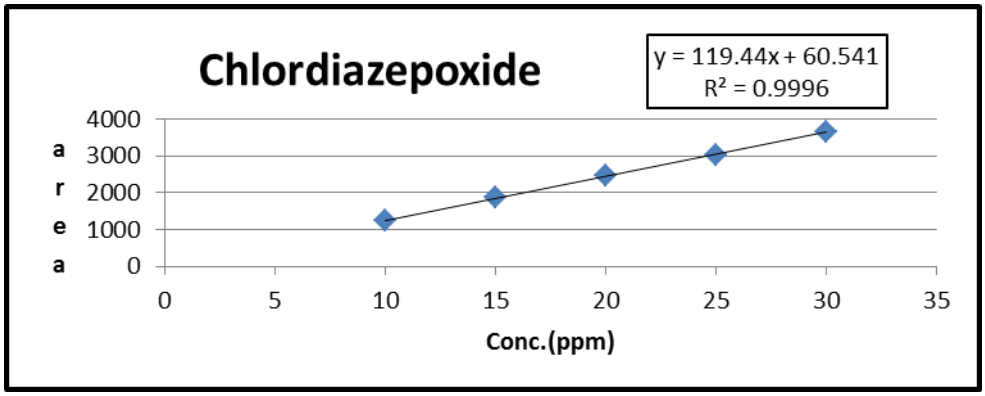
Table 5: Accuracy DIC HCl.

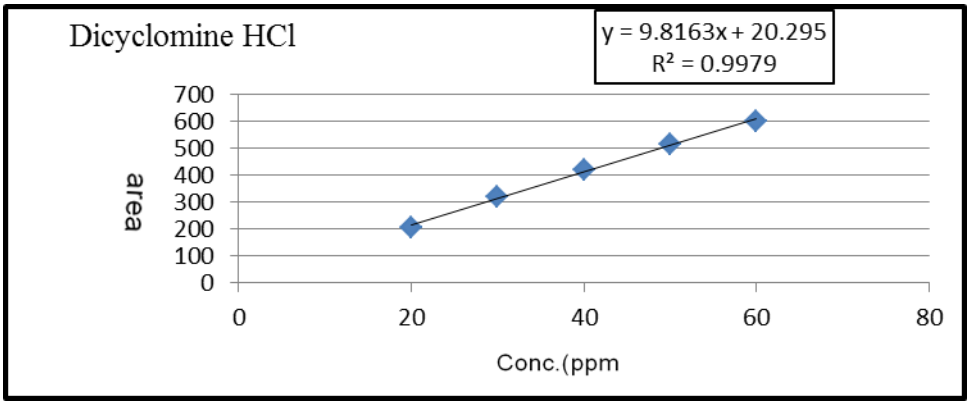
Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery ± S.D (n=3)
80%	20	16	36	16.281	101.761	100.711 ± 1.021
	20	16	36	15.955	99.719	
	20	16	36	16.104	100.652	
100%	20	20	40	20.226	101.131	100.445 ± 0.640
	20	20	40	19.972	99.862	
	20	20	40	20.068	100.341	
120%	20	24	44	24.309	101.290	100.235 ± 1.066
	20	24	44	24.062	100.258	
	20	24	44	23.797	99.157	

Table 6: Linearity.

SR.NO	Concentration(µg/ml)			Area		
	CLI	CHLO	DIC	CLI	CHLO	DIC
1	5	10**1**0	20	123.673 ± 2.299	1235.126 ± 3.416	208.376± 1.926
2	7.5	15	30	184.752 ± 3.436	1873.982 ± 1.925	323.523 ± 3.702
3	10	20	40	244.269 ± 3.476	2455.174 ± 2.898	426.624 ± 5.435
4	12.5	25	50	294.017 ± 4.841	3019.103 ± 2.703	526.354 ± 6.764
5	15	30	60	346.174 ± 4.183	3649.751 ± 3.163	613.482 ± 9.175







Linearity of CLI, CHLO, DIC

Table 7: Content Uniformity.

Sr.No	A			B			C		
	CLI	CHLO	DIC	CLI	CHLO	DIC	CLI	CHLO	DIC
1	95.75	95.80	96.76	94.75	95.40	95.70	92.28	93.81	95.40
2	98.40	98.46	99.44	95.58	97.41	96.76	95.40	95.58	95.40
3	96.74	96.78	94.15	96.20	96.55	94.52	95.40	99.25	94.55
4	99.20	99.25	100.24	96.20	99.40	100.85	96.20	92.63	92.40
5	99.58	99.63	98.73	92.40	99.45	92.74	94.75	95.40	99.15
6	101.48	100.59	102.55	101.45	100.54	99.55	93.48	99.60	99.21
7	99.88	99.75	99.36	99.80	93.72	98.30	101.05	97.55	96.56
8	100.09	99.96	97.38	98.27	94.68	97.61	96.63	94.68	97.45
9	96.63	96.68	97.65	95.63	95.63	97.63	98.80	94.68	92.00
10	96.74	96.49	95.87	99.48	96.81	99.44	95.65	100.52	99.15
Avg	98.45	98.34	98.21	96.98	96.96	97.31	82.70	96.37	96.13
S.D.	1.89	1.74	2.38	2.72	2.13	2.468	2.50	2.69	2.670
%C.V.	1.92	1.77	2.43	2.81	2.19	2.536	3.03	2.79	2.778

RESULTS AND DISCUSSION

In RP-HPLC method, estimation of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was carried out at 209 nm for simple quantitative determination.

In RP- HPLC estimation of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was carried out by using Shiseido C_{18} (250mm x 4.6mm, 5 µm) was used at ambient temperature. Mobile Phase: Potassium Dihydrogen Phosphate buffer(pH-5)**:** methanol (60:40 v/v) at a flow rate of 1.0 ml/min was used. Detection was carried out at 209 nm. Retention time of Clidinium bromide,Chlordiazepoxide and Dicyclomine HCl was found to be 4.200, 3.420, 11.570 min.

The RP-HPLC method for Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl was found to be linear over the range of 5-15 µg/ml, 10-30µg/ml and 20-60µg/ml.

The method has been validated for linearity, accuracy and precision, LOD, LOQ and system suitability according to ICH guideline. The % recovery found to be 99.81-100.47, 99.47-100.89, 100. 23-100.71.

CONCLUSION

All the method was found to be simple, accurate, economical, robust and reproducible. The proposed method was successfully applied for the RP-HPLC of Clidinium bromide, Chlordiazepoxide and Dicyclomine HCl.

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