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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CLIDINIUM BROMIDE, CHLORDIAZEPOXIDE, DICYCLOMINE HCl AND RABEPRAZOLE SODIUM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Clidinium Bromide, Chlordiazepoxide, Dicyclomine HCl and Rabeprazole Sodium has been developed. Method was studied by using Shimadzu 2010CHT, Chromatographic separation was achieved using ODS C_{18} RP column (250 mm × 4.6 mm i.d., 5µm) kept at ambient temperature, using a mobile phase consisting a mixture of Phosphate buffer (pH 4.5): Acetonitrile (80:20 v/v) and pH adjusted with 0.5% orthophosphoric acid at a flow rate of 1.0 ml/min. The detection was made at 215 nm. Retention time was 3.12 min, 4.28 min and 6.97 min and 13.29 for Clidinium Bromide, Chlordiazepoxide, Dicyclomine HCl and Rabeprazole Sodium respectively. Linear correlation was obtained between peak area and concentration in the range of 5-15 µg/ml for Clidinium Bromide, 10-30 µg/ml for Chlordiazepoxide, 20-60 µg/ml for Dicyclomine HCl and Rabeprazole Sodium. The percentage recoveries of four drugs Clidinium Bromide, Chlordiazepoxide, Dicyclomine HCl and Rabeprazole Sodium were found to be 100.48- 101.46 %, 98.87-101.75%,98.20-98.80% and 100.94-101.71 % respectively. The % RSD value was less than 2, for intraday and interday precision. It can be successfully used for routine analysis of Clidinium Bromide, Chlordiazepoxide, Dicyclomine HCl and Rabeprazole Sodium in combined solid dosage form without any interference from common excipients and impurity.

KEYWORDS: Clidinium Bromide, Chlordiazepoxide, Dicyclomine HCl, Rabeprazole Sodium, RP-HPLC method development.

INTRODUCTION
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.[1] Chlordiazepoxide is a sedative/hypnotic drug and benzodiazepine used to treat anxiety and acute alcohol withdrawl. It has GABA facilitator action. The drug has amnestic, anticonvulsant, anxiolytic, hypnotic and skeletal muscle relaxant properties. Chemically the drug is 7-chloro-2-methyl amino-5-phenyl -3H-1,4-benzodiazepine-4-oxide. Side effects with the drug includes clumsiness, confusion, dizziness, excessive daytime drowsiness, headache, lack of coordination, light headedness, unsteadiness, unusual weakness.[2]

Dicyclomine Hydrochloride is a gastrointestinal antispasmodic antacid. The chemical name of the drug is 2- (diethylamino) ethyl 1-cyclohexylcyclohexane-1-carboxylate HCl. Its action is achieved via a dual mechanism: (1) a specific anticholinergic effect (antimuscarinic) at the acetylcholine-receptor sites and (2) a direct effect upon smooth muscle (musculotropic) so it is used for the antispasmodic agent.[3]

Rabeprazole sodium is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in the treatment of Ulcerative Gastroesophageal RefluxDisease (GERD). It is chemically 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl] sulfinyl]-Hbenzimidazole sodium. Rabeprazole is officially used as PDR1.Rabeprazole belongs to a class of antisecretary compounds that suppress gastric acid secretion by inhibiting the gastric H+, K+ ATPase at the secretary surface of the gastric parietal cell. Because this enzyme is regarded as the acid (proton) pump within the parietal cell, rabeprazole has been characterized as a gastric proton pump inhibitor. Rabeprazole blocks the final step of gastric secretion. In gastric parietal cells, rabeprazole is protonated, accumulates and is transformed to an active sulphonamide2.[4] This Combination of four drugs (Coliwin- R Capsule) is highly effective and used in the treatment of peptic ulcer, nervous dyspepsia, gastritis,

irritable spastic colon.

Literature survey reveals that some analytical methods have been used for the estimation of Clidinium bromide, Chlordiazepoxide and Dicyclomine hydrochloride and Rabeprazole sodium individually or combination with other drugs. The United States Pharmacopoeia (USP) stated the non-aqueous titration method for the assay of Clidinium bromide and Chlordiazepoxide.[5] Very few methods for the determination of Clidinium bromide and Chlordiazepoxide in combined dosage forms including RP-HPLC[6-8], derivative spectroscopy[9] and spectrophotometry using multivariate calibration techniques [10] was reported. Chlordiazepoxide has been determined alone or with other compound in pharmaceutical formulations using first-derivative spectrophotometry[11], spectrophotometry[12], High-Performance Liquid Chromatography [13], HPTLC [14], RP-HPLC methods was reported for the estimation of Dicyclomine hydrochloride alone or in combination with other drugs. Rabeprazole sodium has been determined alone or with other compound in pharmaceutical formulations by RP-HPLC, derivative spectroscopy Since, there is no HPLC method has been reported till date for the simultaneous estimation of CDB, CDZ, DICY and RABE in bulk and combined pharmaceutical dosage forms.

Therefore the present research work, our aim is to develop a new analytical RP-HPLC method and validate according to the ICH guidelines[15] to estimate CDB, CDZ, DICY and RABE containing bulk drugs and combined pharmaceutical dosage forms in routine analysis.

MATERIALS AND METHODS

Instruments and Reagents

A double beam UV-visible Spectrophotometer (Lab India, UV-3000+), attached to a computer software UV Win, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells. The chromatography was performed by a HPLC instrument (Shimadzu LC 2010 CHT) equipped with a UV-Visible detector, injector with Isocratic pump, Sheisdo C_{18} column (250* 4.6mm, 5µm) and LC-solution software used. Analytical Balance and Ultra sonic cleaner were also used. Clidinium bromide was received as gift sample from MSN Laboratories Ltd., India. Chlordiazepoxide and Dicyclomine hydrochloride were received from Vapi Care Pharma Pvt. Ltd., Vapi. Rabeprazole Sodium were received from Indchemie Health Specialities Pvt. Ltd. The pharmaceutical preparations of combination of Clidinium bromide, Chlordiazepoxide, Dicyclomine hydrochloride and Rabeprazole Sodium that is Coliwin-R Capsule (Mankind Co.Group) contains 2.5mg of Clidinium bromide, 5mg of Chlordiazepoxide,10mg of Dicyclomine hydrochloride and 10mg of Rabeprazole Sodium was procured from local market. HPLC grade methanol (Merck Ltd., Mumbai, India), HPLC grade Acetonitrile (Merck Ltd., Mumbai, India), Analytical grade Orthophosphoric acid and water were used.

RP-HPLC Method: Chromatographic Condition

Method was developed using a Sheisdo C_{18} column (250 mm x 4.6 mm, 5µm). Mobile phase used was potassium dihydrogen phosphate buffer (0.05 M, pH 4.5 adjusted with 0.5% orthophosphoric acid): Acetonitrile (80:20 v/v) at flow rate is 1.0 mL/min. Samples were injected using Rheodyne injector with 20µl loop. Detection: At 215nm with UV detector.

Preparaton of Standard Stock Solution

An accurately weighed quantity of CDB 10mg, CDZ 20mg, DICY and RABE 40mg (Working standard drugs) were transferred to a 50ml volumetric flask and dissolved in mobile phase and finally the volume was adjusted up to the mark with mobile phase. From this stock solution working standard solution having concentration 10µg/ml, 20µg/ml and 40µg/ml were prepared by appropriate dilution with mobile phase for CDB, CDZ, DICY and RABE respectively.

Preparation of sample solution

Twenty capsules were weighed and powder was collected. The capsules powder equivalent to 10mg of CDB, 20mg of CDZ and 40 mg of DICY and RABE was transferred to a 50 ml volumetric flask and dissolved in mobile phase and the content was kept in ultrasonicator for 15 min. The flask was allowed to stand for 5 min at room tem perature and the volume was adjusted up to the mark with mobile phase. The solution was filtered through a nylon 0.45 µm membrane filter paper. The solution was suitably diluted with mobile phase to get a final concentration of 10µg/ml of CDB, 20µg/ml of CDZ and 40µg/ml of DICY and RABE respectively.

Preparation of Mobile Phase

The mobile phase used in this analysis consists of a mixture of Phosphate buffer (pH 4.5): Acetonitrile (80:20 v/v) and pH adjusted with 0.5% orthophosphoric acid at a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

A typical chromatogram obtained by using the optimal chromatographic conditions as shown below **Figure1**.

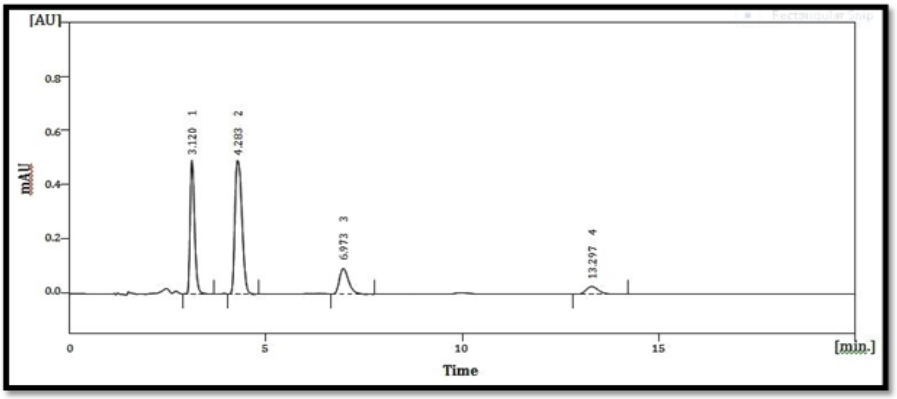

Figure 1.

METHOD VALIDATION

The developed RP-HPLC method was validated as per ICH guidelines.[15]

ASSAY: Twenty capsules were weighed and powder was collected. The capsule powder equivalent to 2.5 mg of CLD, 5 mg of CDZ and 10 mg of DICY and RABE 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 CLD (10µg/ml), CDZ (20µg/ml), DICY and RABE (40µg/ml). A 20 µl volume of sample solution was injected into HPLC. The peak area for the drugs was measured at 215 nm and amount of CLD, CDZ, DICY and RABE were determined using the related linear regression equations. The % assay of the drugs was calculated and the results are given in **Table-1**.

Table 1: Assay Parameters.

Drug	Label claim (mg)	Result (mg)	%Assay	Mean%	SD	% R.S.D
	2.5	2.491	99.643			
CLD	2.5	2.506	100.229	99.998	0.312	0.312
	2.5	2.503	100.121			
	5	4.981	99.626			
CDZ	5	4.988	99.764	99.819	0.225	0.226
	5	5.003	100.067			
	10	9.983	99.835			
DICY	10	9.755	97.546	99.160	1.404	1.416
	10	10.010	100.098			
	10	9.982	99.815			
RABE	10	10.020	100.199	100.036	0.198	0.198
	10	10.009	100.093			

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 (*R*S), and asymmetry factor (*As*) and number of theoretical plates were calculated. Good correlation was found between the results of mixed standards and sample solutions shown in figure 2 and figure 3.

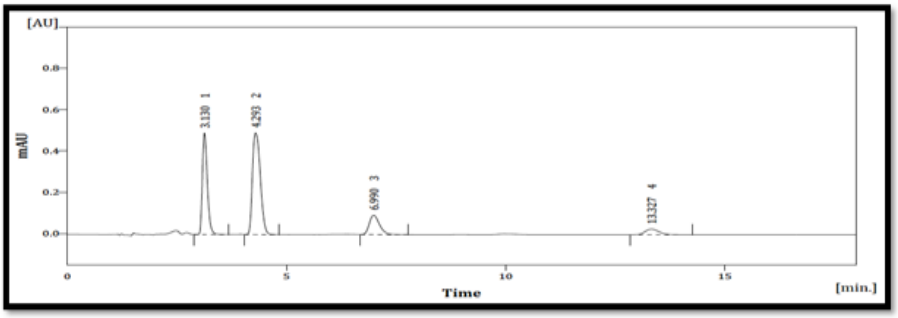

Figure 2: Chromatogram of CLD, CDZ, DICY and RABE of Std. Solution.

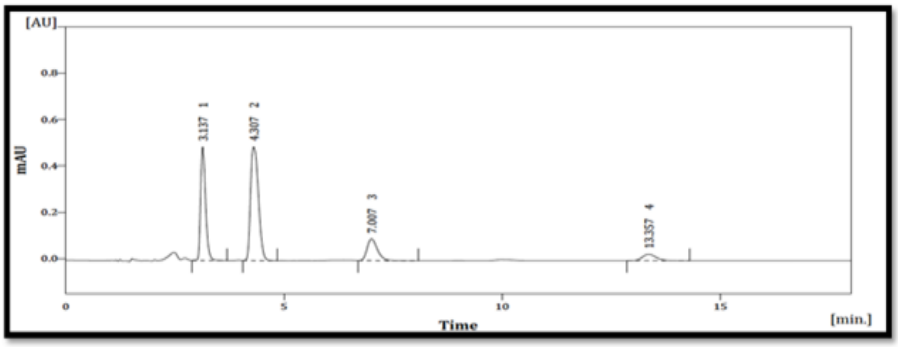

Figure 3: Chromatogram of CLD, CDZ, DICY and RABE of Sample Solution

ACCURACY

The accuracy of the method was determined by calculating the recovery studies at three levels (80%, 100% and 120%) by standard addition method. Known amounts of standard CDB, CDZ, DICY and RABE were added to the pre quantified samples and they were subjected to proposed HPLC method. The results of the recovery studies are given in **Table - 2**.

Table 2: Accuracy.

Drug	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	% Recovery
	5	4	9	4.046	101.161
CLD	5	5	10	5.059	101.185
	5	6	11	6.025	100.430
	10	8	18	8.147	101.842
CDZ	10	10	20	9.87	98.733
	10	12	22	12.23	101.95
	20	16	36	15.816	98.848
DICY	20	20	40	19.804	98.816
	20	24	44	23.61	98.209
	20	16	36	16.12	100.80
RABE	20	20	40	20.13	100.279
	20	24	44	24.32	101.361

PRECISION

Precision study was performed to find out intra-day and inter-day variations. In this process the combined solution (10µg/ml, 20µg/ml, 40µg/ml and 40µg/ml of CDB, CDZ, DICY andRABE respectively) analyzed by same day (Intra-day precision) and on three different days (Inter-day precision). The % relative standard deviation (RSD) for intra-day precision was 0.1406-0.8869 % of CDB, 0.3189-1.1936 % of CDZ, 0.3970-0.7175 % of DICY and 0.3147-0.6920 % of RABE and for inter-day precision was 0.6415-1.7525 % of CDB, 0.3588-1.0207 % of CDZ, 0.8709-1.6885 % of DICY and 0.4994-1.0164 % of RABE respectively, which is less than 2% indicating high degree of precision.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The LOD and LOQ for CDB, CDZ, DICY and RABE were separately determined by based on calculating the signal-to-noise ratio and from the calibration curves the standard deviation of the y-intercepts and slope of the regression lines were used. Results of LOD and LOQ are given in Table 3.

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations designated by International Conference on Harmonization (ICH) guidelines (15).

$$\mathbf{LOD = 3.3 \times \sigma/S \quad LOQ = 10 \times \sigma/S}$$

Where, σ = the standard deviation of the response S = slope of the calibration curve.

Table 3: Results from validation and system suitability studies.

Validation parameters	CLD	CDZ	DICY	RABE
Theoretical plates	4238	2485	8384	3583
Resolution	6.95	4.21	12.52	-
Asymmetry factor	1.554	1.615	1.325	1.593
Intra-day precision (%RSD)	0.141-0.887	0.319-1.194	0.397-0.718	0.318-0.692
Inter-day precision (%RSD)	0.6415-1.7525	0.3588-1.0207	0.8709-1.6885	0.4994-1.0164
LOD (µg/ml)	0.4963	0.8603	3.5786	1.9727
LOQ (µg/ml)	1.5041	2.6070	10.8443	5.9779

LINEARITY

An accurately weighed quantity of CDB 10mg, CDZ 20mg, DICY 40mg and RABE 40mg (Working standard drugs) were transferred to into a separate 50mL clean and dry volumetric flasks and dissolved in mobile phase and finally each volumetric flask volume was adjusted up to the mark with mobile phase respectively. From this stock solution prepare 5, 10, 15, 20 and 25µg/ml of CDB, 10, 20,30, 40 and 50 µg/ml of CDZ, 20, 30, 40, 50 and 60µg/ml of DICY and 20, 30, 40, 50 and 60µg/ml of RABE concentrations respectively. 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 results were shown in **Table-4**.

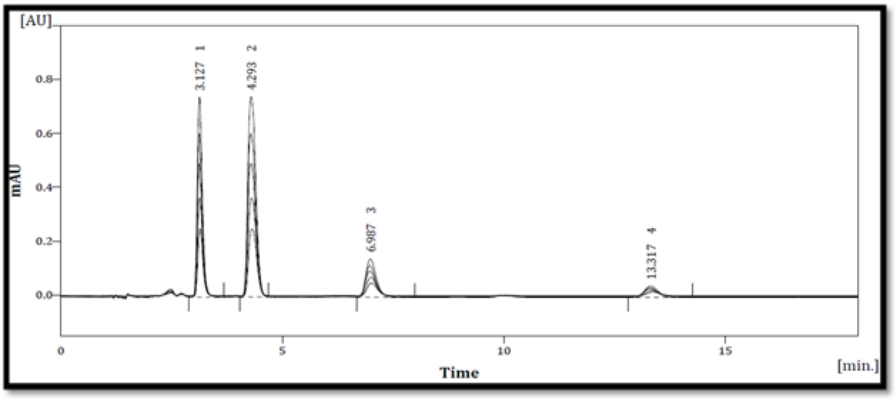

Figure 3.

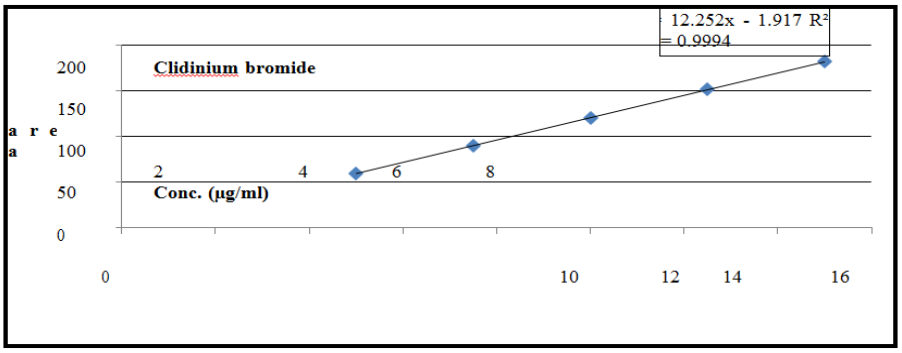

Figure 4.

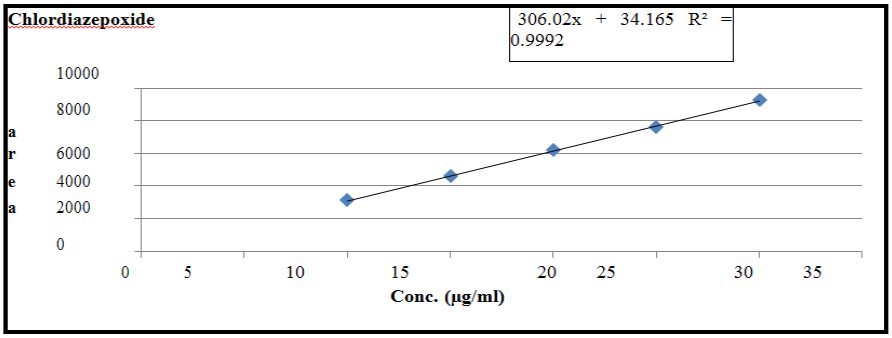

Figure 5.

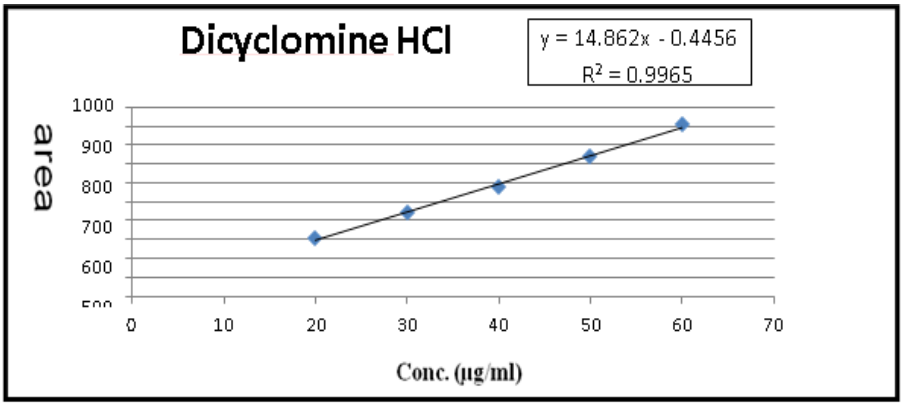

Figure 6.

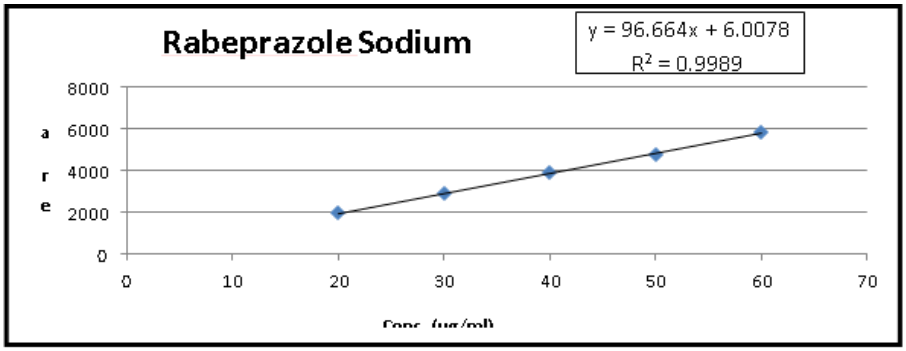

Figure 7.

Table 5: Results for construct the calibration curve.

SR.NO	Concentration(µg/ml)				Area (n=5)			
	CDB	CDZ	DICY	RABE	CDB	CDZ	DICY	RABE
1	5	10	20	20	778.01 ± 5.6	3143.70±7.9	309.58±2.2	1968.16±8.4
2	7.5	15	30	30	1137.70 ±11.4	4571.58±9.5	445.77±2.1	2870.92±12.1
3	10	20	40	40	1534.79 ±5.9	6184.02±5.7	576.17±2.1	3882.64±8.48
4	12.5	25	50	50	1879.69 ±5.3	7569.23±11.0	736.39±2.3	4772.56±11.6
5	15	30	60	60	2312.2± 6.2	9286.73±43.4	907.97±2.6	5865.17±15.2

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.

SUMMARY AND CONCLUSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CDB, CDZ and DICY was obtained using ODS C_{18} column (250 mm ×4.6 mm, 5µm) with a mobile phase consisting of a mixture of Phosphate buffer (pH 4.5): Acetonitrile (80:20 v/v) and pH adjusted with 0.5% orthophosphoric acid at a flow rate of 1.0 ml/min, detection was performed at 215 nm. The retention times of Clidinium bromide, Chlordiazepoxide,, Dicyclomine hydrochloride and Rabeprazole Sodium were found to be 3.12 min, 4.28 min, 6.97 min and 13.29 min respectively (**Figure 1**). The amount of CDB, CDZ, DICY and RABE present in the sample solutions were determined respectively and the results obtained were comparable with the corresponding labeled claim (**Table 1**). The % recovery was found to be 98-102% within the limits for CDB, CDZ and DICY (**Table 2**) which indicates high degree of accuracy of developed method. The results of system suitability testing are given in Table 3. The %RSD of CDB, CDZ and DICY for intra-day precision and inter-day precision was less than 2% it reveal that the proposed method is precise (**Table 3**). The sensitivity of method LOD and LOQ is shown in (**Table 3)**. Linear correlation was obtained between concentration versus peak area of CDB, CDZ and DICY in the concentration ranges of 5-15 µg/ml for CLD, 10-30 µg/ml for CDZ, 20-60 µg/ml for DICY and RABE (**Table 4**). The correlation co-efficient (' *r2* ' value) for CDB, CDZ and DICY was 0.998, 0.996 and 0.995 respectively. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions.

CONCLUSION

The proposed study describes RP-HPLC method for the estimation of CDB, CDZ and DICY in bulk drugs as well

as in tablet formulation. The method was validated according to the ICH guidelines. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, and selective and it can be employed successfully for the estimation of CDB, CDZ and DICY in their bulk drugs and tablet formulation in routine analysis.

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