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Stability-indicating RP-HPLC method for the simultaneous determination of clidinium bromide and chlordiazepoxidein pharmaceutical formulations

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ABSTRACT

A novel stability indicating RP-HPLC method was developed and validated for simultaneous determination of clidinium bromide and chlordiazepoxide in pharmaceutical dosage form. Kromasil RP- C18 (250 mm x 4.6 mm, 5 µm) column was used for separation of the components. A mobile phase comprising of a mixture of methanol: acetonitrile: water in the ratio 40:50:10 (v/v) was degassed under ultrasonication before use. The flow rate was maintained at 1.0 mL/min and the effluent was monitored at 228 nm. The retention times of clidinium bromide and chlordiazepoxide were found to be 5.56 min and 3.19 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection, limit of quantitation. Linearity was found to be in the range of 2.5-15 µg/mL for clidinium bromide and 5-30 µg/mL for chlordiazepoxide respectively. The method was successfully applied to separate and study the stability of the drugs. The results indicated that the drugs are stable at the sunlight, peroxide, UV and aqueous condition and sensitive in alkali and acidic conditions than others. The method was also successfully applied for the analysis of formulations in marketed samples.

KEYWORDS

Clidinium bromide;
Chlordiazepoxide;
RP-HPLC;
Forced degradation;
Assay.

INTRODUCTION

Clidinium bromide is an anticholinergic drug used to inhibit muscarinic acetylcholine receptors on smooth muscles, secretory glands, and in the central nervous system to relax smooth muscle and decrease biliary tract secretions[1]. Chemically the drug is 3-[(2-hydroxy-2,2-diphenylacetyl)oxy]-1-methyl-1-azabicyclo[2.2.2]octan-1-ium bromide. It may help symptoms of cramping and abdominal/stomach pain by decreasing stomach acid, and slowing the intestines[2,3]. The side effects with drug include drowsiness, dizziness, nausea, constipation, and dry mouth[4]. Chlordiazepoxide is a sedative/hypnotic drug and benzodiazepine used to treat anxiety and acute alcohol withdrawl[5-7].

The drug has amnestic, anticonvulsant, anxiolytic, hypnotic and skeletal muscle relaxant properties[8]. Chemically the drug is 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide. Side effects with the drug includes clumsiness, confusion, dizziness, excessive

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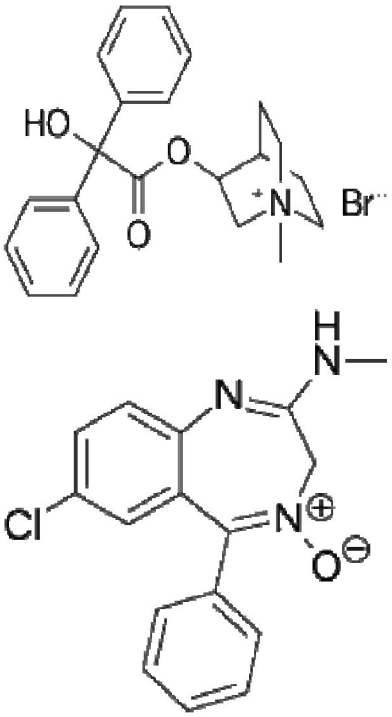

Figure 1 : Chemical structure of Clidinium bromide Chlordiazepoxide

daytime drowsiness, headache, lack of coordination, lightheadedness, unsteadiness, unusual weakness.

Chlordiazepoxide hydrochloride and clidinium bromide (Librax) combines the anti-anxiety action of chlordiazepoxide and the antispasmodic effects of clidinium. It also blocks the acid secretion of the gastrointestinal tract and inhibits the action of nerves that are very active in certain diseases. The combination used to treat peptic ulcers and irritable bowel syndrome. It also may be useful in the management of acutegastroenteritis. The combination of chlordiazepoxide and clidinium bromide comes as a capsule to be taken by mouth. Very few HPLC methods were reported[9-11] to analyse chlordiazepoxide hydrochloride and clidinium bromide in combined dosage forms. But, no stability indicating method is reported so far. Hence, the present work is aimed to develop a RP-HPLC method under forced degradation conditions.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump with variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK chromatographic software version 1.06. Rheodyne injector with 20µl fixed volume loop was used to inject the sample. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Ultrasonicator was used to sonicate the mobile phase and samples. Denver electronic analytical balance (SI-234) was used for weighing the sample.

Chemicals and solvents

The drug samples, clidinium bromide and chlordiazepoxide working standard was obtained as gift sample from Nicholas Piramal India Limited, Hyderabad. The pharmaceutical formulation was procured from local market. Methanol, acetonitrile, perchloric acid used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of mobile phase and stock solutions

The mobile phase consisting of methanol, acetonitrile and water were mixed in 40:50:10 ratio and pH of mixture was adjusted to 5.3. This mixture was sonicated for 10 min and filtered through 0.22 µm membrane filter and used as mobile phase. Stock solutions were prepared by weighing 10 mg each of clidinium bromide and chlordiazepoxide. The weighed drugs were transferred to two separate 100 ml volumetric flasks. Volumes were made up to the mark with methanol to obtain a solution containing 1000 µg/ml of clidinium bromide and chlordiazepoxide. The solutions were further diluted with the same solvent to obtain final concentrations of 100 µg/ml of each drug. The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of methanol: acetonitrile: water in 40:50:10 (v/v) ratio with pH 5.3, using Kromasil RP- C18 (250×4.6 mm, 5 µm particle size) with 1 ml/min flow rate at 228 nm using UV detector.

Preparation of formulation solution

Twenty tablets of the formulation (LIBRAX-clidinium bromide 2.5mg and chlordiazepoxide 5mg)

were accurately weighed and crushed into a fine and uniform size powder. An amount of the tablet powder equivalent to 10mg of clidinium bromide was accurately weighted and quantitatively transferred into a 10ml volumetric flask. Approximately 5ml methanol were added and the solution was sonicated for 15 min and made up the volume with same solvent and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 10µg/ml. The combined solution was used for the simultaneous estimation of clidinium bromide and chlordiazepoxide in combined fixed dosage form.

Method validation

The method of analysis was validated as per the recommendations of ICH and USP for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness and stability. The accuracy of the method was determined by calculating percentage recovery of clidinium bromide and chlordiazepoxide. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of clidinium bromide and chlordiazepoxide corresponding to 50, 100 and 150% of label claim had been added (standard addition method). At each level of the amount, six determinations were performed and the results obtained were compared. Intraday and interday precision study of clidinium bromide and chlordiazepoxide was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 50 µg/ml and 10 µg/ml of clidinium bromide and chlordiazepoxide, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the following formulae: LOD= 3.3(SD)/ S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve. System suitability tests are an integral part of chromatographic method which are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg/ml and 20 µg/ml for clidinium bromide and chlordiazepoxide, respectively to check the reproducibility of the system and the results are shown in TABLE 2. For robustness evaluation of HPLC method, a few parameters like flow rate, percentage of methanol in the mobile phase and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 10 µg/ml and 20 µg/ml for clidinium bromide and chlordiazepoxide respectively.

Forced degradation studies

To perform the forced degradation study, 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. For acidic degradation,the drug was exposed to 0.1 M HCl for 48 h and the mixture was neutralized. For alkaline degradation the drug was treated with 0.1 M NaOH for 48 h and the mixture was neutralized. For degradation under oxidizing conditions, the drug was exposed for H_2O_2 for 48 h. For thermal degradation, the powdered drug was exposed at 70º for 48 h. For photolytic degradation, the powdered drug was exposed to sunlight for 48 h. After completion of the treatments the solutions were neutralized and diluted with solvent mixture to furnish 30 µg/ml solutions. The purity of the drug peak obtained from the stressed sample was measured by UV detector and compared the chromatogram of untreated drugs in tablet solution. The results indicated that the drugs are mostly stable at the sunlight, peroxide, UV and aqueous

TABLE 2 : Results of intraday and interday precision

Clidinium bromide (10µg/ml)		Chlordiazepoxide (20µg/ml)	
Intra day	**Interday**	**Intra day**	**Interday**
215231	213823	422557	417336
210964	214424	421510	414749
214247	219405	421878	414124
213726	215092	427660	425121
219717	216576	427745	408717
211178	213156	417915	417943
1.49	1.05	0.90	1.29

condition and sensitive in alkali and acidic conditions than others.

RESULTS AND DISCUSSION

The mobile phase consisting of methanol: acetonitrile: water in 40:50:10 (v/v) with pH 5.3, at 1ml/ min flow rate was optimised which gave two sharp, well-resolved peaks with minimum tailing factor for clidinium bromide and chlordiazepoxide (Figure 1). The retention times for clidinium bromide and chlordiazepoxide were 5.56 min and 3.19 min. respectively. UV spectra of both clidinium bromide and chlordiazepoxide showed that both drugs absorbed appreciably at 228 nm, so this wavelength was choosen as detection wavelength. The calibration curve for clidinium bromide and chlordiazepoxidewas were found to be linear over the range of 2.5-15µg/ml and 5-30µg/ ml, respectively. The data of regression analysis of the calibration curves is shown in TABLE 1. The proposed method was successfully applied to the determination of clidinium bromide and chlordiazepoxidein in their combined dosage form. The results for the combination were comparable with the corresponding labelled amounts. The developed method was also found to be specific, since it was able to separate other excipients present in formulation from the two drugs.

High intra- and inter-day precisions are shown in TABLE 2. Intra- day precision was assessed by injection of the standard solution of the drug at three concentrations levels six times during a day. The same was done for inter- day precision test except that the analysis of the samples was done every day for six days.

The robustness of the present method was evaluated in terms of pH of the mobile phase and wave length. The results are given in TABLE 3. The slight variations

TABLE 3 : Results of Robustness

Condition	Clidinium bromide		Chlordiazepoxide	
	Area	% Change	Area	% Change
MP 1	213828	0.88	419191	0.22
MP 2	218239	1.15	424091	0.94
pH 1	217709	0.90	427286	1.70
pH 2	216667	0.42	422344	0.52
WL 1	218449	1.25	423552	0.81
WL 2	214241	0.69	428035	1.87

TABLE 4 : Results of Recovery

	Clidinium bromide					Chlordiazepoxide				
S. No	Target µg/ml	Spiked µg/ml	Total µg/ml	Amount found	% Recovery	Target µg/ml	Spiked µg/ml	Total µg/ml	Amount found µg/ml	% Recover
1	5	2.5	7.5	7.47	99.62	10	5	15	15.17	101.13
2	5	2.5	7.5	7.63	101.83	10	5	15	15.28	101.93
3	5	2.5	7.5	7.31	97.48	10	5	15	15.08	100.53
4	5	5.0	10.0	9.86	98.65	10	10	20	20.33	101.69
5	5	5.0	10.0	9.85	98.58	10	10	20	20.09	100.48
6	5	5.0	10.0	9.87	98.70	10	10	20	20.16	100.84
7	5	7.5	12.5	12.81	102.52	10	15	25	25.06	100.25
8	5	7.5	12.5	12.60	100.86	10	15	25	25.23	100.94
9	5	7.5	12.5	12.86	102.89	10	15	25	24.77	99.10

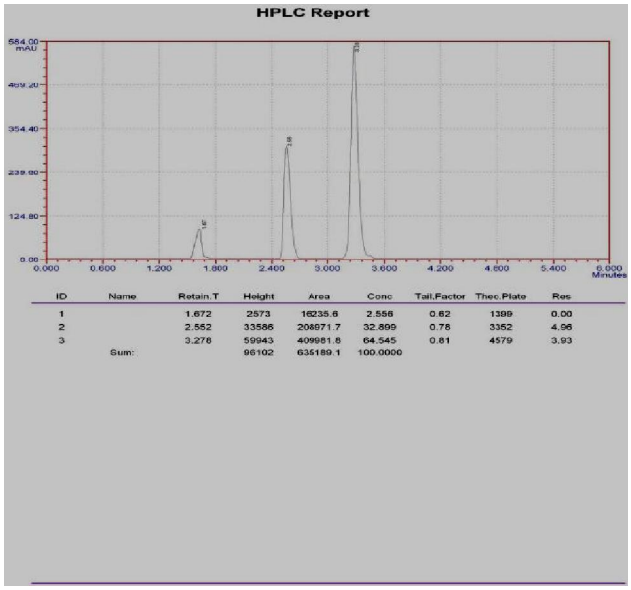

Figure 2 : Hydrogen peroxide

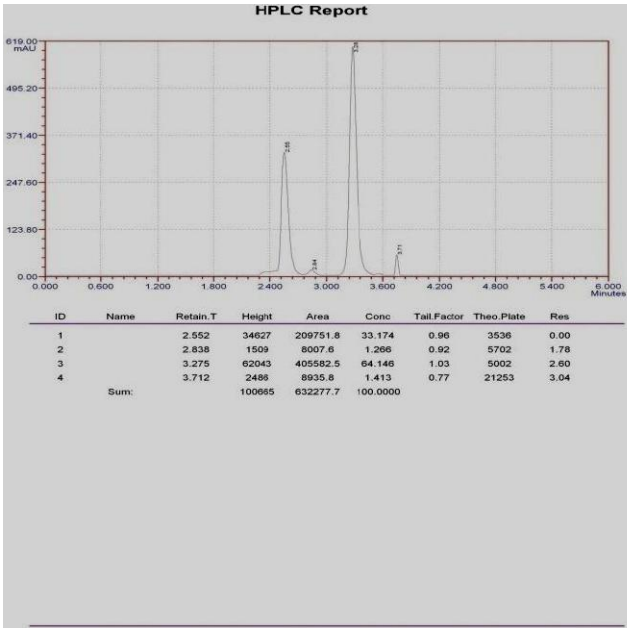

Figure 3 : Thermal

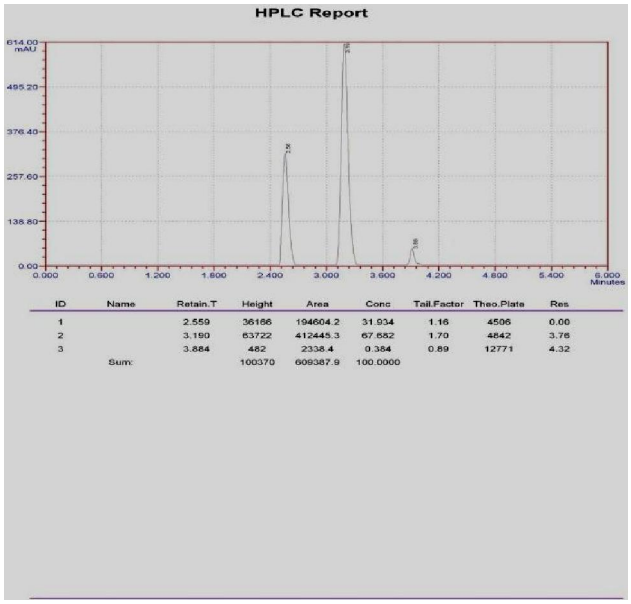

Figure 4 : UV light

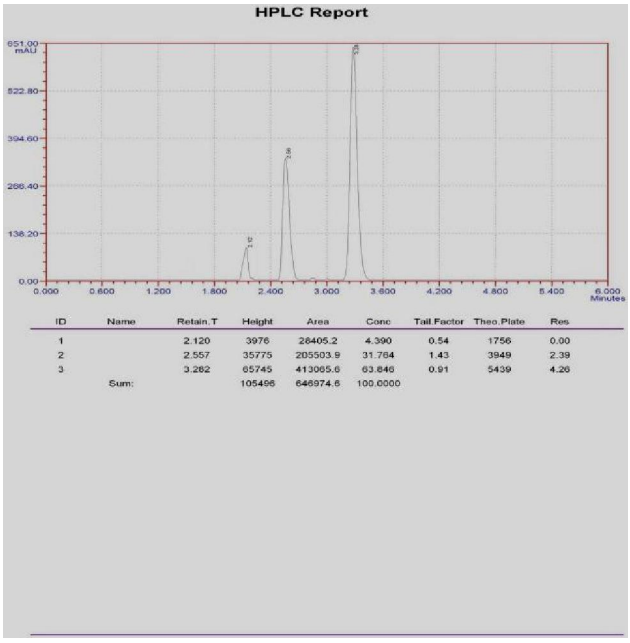

Figure 5 : Sunlight

in the examined factors had no significant effect on the shape of the peak. The results indicated that the method is more sensitive to changes in the pH, wave length and the change in contentsof mobile phase than to changes in the other factors. The standard parameters of the method are: mixture of methanol: acetonitrile: water in 40:50:10 (v/v) and pH 5.3 as a mobile phase, injection volume 20 µl, column temperature 40°C, detection wavelength 228 nm and flow rate 1.0 ml/min. Recovery studies to check the degree of accuracy of the method were performed in triplicate by standard addition method at 50%, 100% and 150% (standard addition method). Known amounts of standard clidinium bromide and chlordiazepoxide was added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in TABLE 4.

It was found that there is no degradation and changes in the unstressed sample and comparatively very less changes in the stressed samples except acidic and alkali conditions. There different degrading peaks were observed in acidic and alkali conditions compared with other forced degradation studies. The method is

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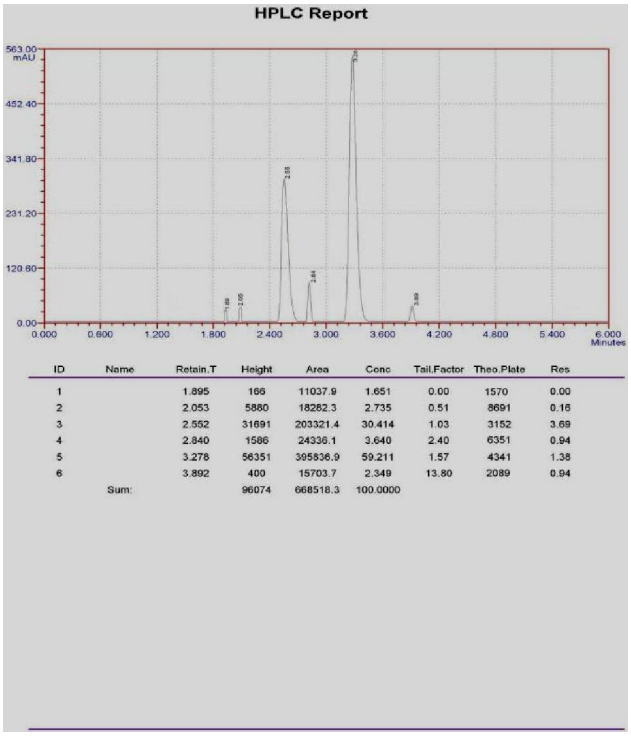
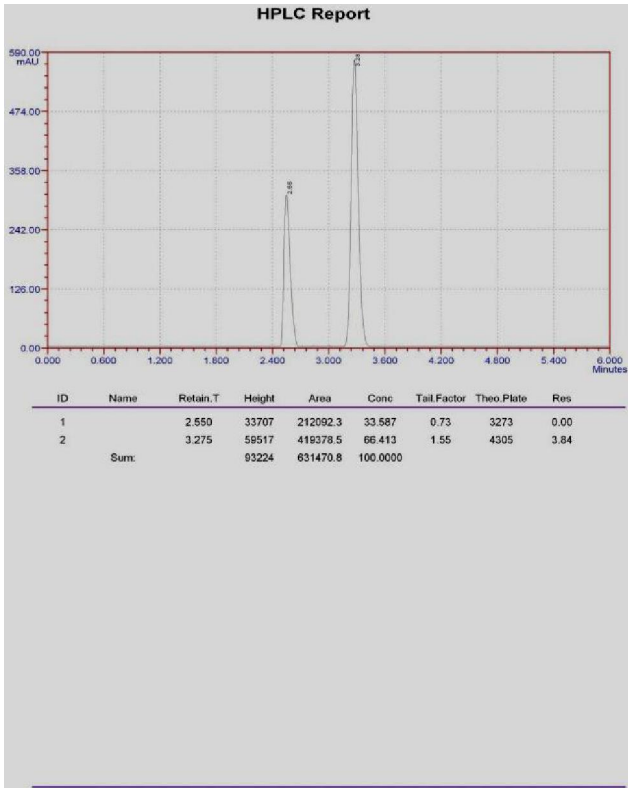


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate	Res
1		1.895	166	11037.9	1.651	0.00	1570	0.00
2		2.053	5880	18282.3	2.735	0.51	8691	0.16
3		2.552	31691	203321.4	30.414	1.03	3152	3.69
4		2.840	1586	24336.1	3.640	2.40	6351	0.94
5		3.278	56351	395836.9	59.211	1.57	4341	1.38
6		3.892	400	15703.7	2.349	13.80	2089	0.94
	Sum:		96074	668518.3	100.0000			

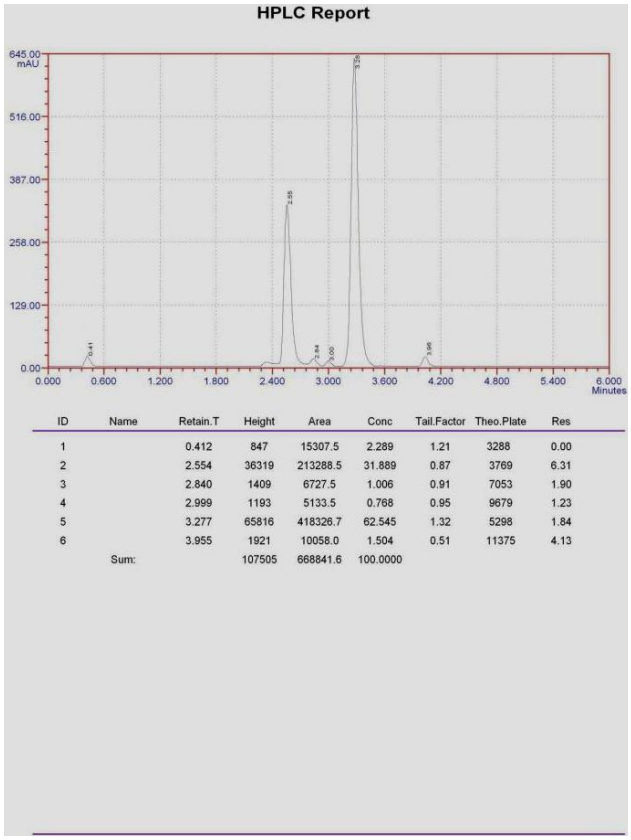
Figure 6 : Acidic



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate	Res
1		2.550	33707	212092.3	33.587	0.73	3273	0.00
2		3.275	59517	419378.5	66.413	1.55	4305	3.84
	Sum:		93224	631470.8	100.0000			

Figure 7 : Aqeous

capable to separate degraded compounds and useful to study the stability characters of both drugs. The chromatograms of the degradation studies were presented in the Figures 2-8.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate	Res
1		0.412	847	15307.5	2.289	1.21	3288	0.00
2		2.554	36319	213288.5	31.889	0.87	3769	6.31
3		2.840	1409	6727.5	1.006	0.91	7053	1.90
4		2.999	1193	5133.5	0.768	0.95	9679	1.23
5		3.277	65816	418326.7	62.545	1.32	5298	1.84
6		3.955	1921	10058.0	1.504	0.51	11375	4.13
	Sum:		107505	668841.6	100.0000			

Figure 8 : Basic

CONCLUSION

In the proposed study, stability-indicating HPLC method was developed for the simultaneous determination of clidinium bromide and chlordiazepoxide and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise, and repeatable. The developed method was found to be simple, sensitive and selective for analysis of clidinium bromide and chlordiazepoxide in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.78% of clidinium bromide and 98.6 % of chlordiazepoxide. The results indicated the suitability of the method to study stability of clidinium bromide and chlordiazepoxide under various forced degradation conditions viz. acid, base, dry heat, photolytic,neutral and UV degradation. It can be concluded that the method separates the drugs from their degradation products and it can be employed for analysis of stability samples of clidinium bromide

and chlordiazepoxide. However characterization of degradation products was not carried out.

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