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Research Article

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DICYCLOMINE HYDROCHLORIDE, ACETAMINOPHEN AND CLIDINIUM BROMIDE IN SOLID DOSAGE FORM

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ABSTRACT

Dicyclomine hydrochloride in combination with Clidinium bromide and Acetaminophen is used in management of Irritable bowel syndrome and other gastrointestinal related disorders.

Objective: To develop and validate a high performance liquid chromatographic method for simultaneous estimation of Dicyclomine hydrochloride, Clidinium bromide and Acetaminophen in solid dosage form.

Method: The drugs were separated using a Kromasil 100 C18 column by isocratic elution with a flow rate of 1.2 ml/min. The mobile phase composition was pH 7 Phosphate Buffer:Methanol: Acetonitrile (30:40:30 v/v/v) and spectrophotometric detection was carried out at 218 nm. The method was validated as per ICH Q2 Guidelines for Linearity, Accuracy, Precision, Sensitivity and Robustness.

Results: The retention time were 2.24, 3.41 and 9.39 min for Acetaminophen, Clidinium bromide and Dicyclomine hydrochloride respectively. The linear range of determination for Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide were 10-60 µg/ml, 26-58.5 µg/ml and 2.5-15 µg/ml respectively. Recovery of Dicylomine hydrochlroride, Acetaminophen and Clidinium bromide were found to be 98.98%, 99.21% and 98.83% respectively.

Conclusion: The method was found to be reliable for simultaneous estimation of Dicyclomine hydrochloride, Clidinium bromide and Acetaminophen in solid dosage form.

Keywords: Dicyclomine hydrochloride (DLC), Acetaminophen (ACP), Clidinium bromide (CLB), Chromatography, Validation.

INTRODUCTION

Dicyclomine [1] (bicyclohexyl]-1-carboxylic acid is an antispasmodic and anticholinergic agent. Its action is achieved via a dual mechanism: a specific anticholinergic effect at the acetylcholine-receptor sites, a direct effect upon smooth muscle. It is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping.

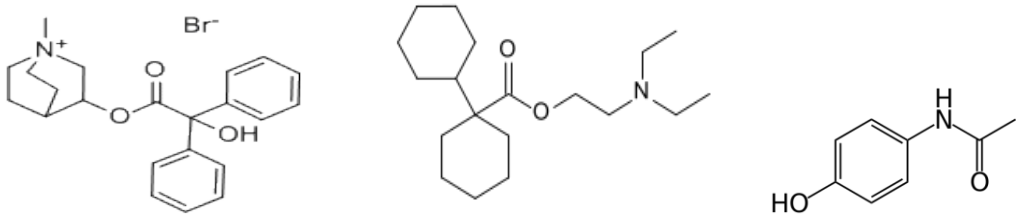
Clidinium bromide (3-[(hydroxy-diphenylacetyl)-oxy]-1-methyl-1-azoniabicyclo-[2.2.2]) octane bromide [2] is an anticholinergic drug which is reported to be effective for anxiety-related conditions including spastic colon.

Acetaminophen (Paracetamol) [3] has non-narcotic analgesic antipyretic but rather weaker anti-inflammatory effects than other NSAIDS. It may act through inhibition of central nervous system – specific cyclo oxygenase (COX) isoform such as COX-3.

Combination of Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide is used in various gastrointestinal tract related disorders like Irritable bowel syndrome,

Hyper peristalsis, Peptic ulcer, Functional diarrhoea, Morning sickness and Motion sickness and Dysmenorrhoea [4].

Detailed literature survey for Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide revealed that many methods of estimation are available based on techniques [5-21] viz. Spectrophotometry, HPLC, HPTLC, and GC. However no analytical method is reported for simultaneous estimation of Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide in combination. So, in the present research work, the aim is to develop a simple, rapid, accurate and sensitive method for simultaneous estimation of these three drugs for routine analysis.



(A) **(B)** **(C)**

Fig. 1: (A) Dicyclomine hydrochloride, (B) Acetaminophen, (C) Clidinium bromide

MATERIALS AND METHODS

Reagents and Chemicals

All solvents used were of HPLC grade. The reference standards of Dicylomine hydrochloride and Clidinium bromide were obtained as gift samples from Mercury Laboratories (Vadodara, India) and Cadila Pharmaceuiticals (Ahmedabad, India) respectively. The reference standard of Acetaminophen was obtained from K.B. Institute of Pharmaceutical Education and Research (Gandhinagar, India). The commercial fixed dose combination product Cypa (Dicyclomine

hydrochloride 10 mg, Acetaminophen 325 mg and Clidinium bromide 2.5 mg) was obtained from local pharmacy store. The solvents used were Methanol HPLC grade (Finar chemicals, Ahmedabad, India) and Acetonitrile HPLC grade (RFCL chemicals,New Delhi, India). The distilled water was produced by double distillation and filtered through Nylon 0.45 µm membrane filter.

Instrument and Chromatographic conditions

Chromatographic seperation was carried out using Analytical Technologies Ltd HPLC system with UV-2230 UV-Vis detector and P-2230 HPLC pump(Analytical technologies Ltd, Vadodara, India). The elution was carried out isocratically.

Table 1: Optimized Chromatographic conditions

S. No.	Parameter/Condition	Specification
1	Column	Kromasil 100 C18, 5µ, 250x4.6mm
2	Mobile phase	pH 7 Phosphate buffer: Methanol: Acetonitrile (30:40:30)
3	Flow rate	1.2 ml/min
4	Wavelength of detection	218 nm
5	Sample load	10 µl
6	Column temperature	40 ˚C

Preparation of Standard Stock solution

The standard stock solutions of DCL (100µg/ml), ACP (100µg/ml) and CLB (50µg/ml) were prepared by transferring 10 mg, 10 mg and 5 mg respectively in 100 ml Volumetric flasks. The volume was made upto the mark using mobile phase. The solutions were sonicated for 15 min and filtered through Whatmann filter paper.

Preparation of Sample solution

Twenty tablets were weighed accurately, their average weight was determined and powdered.

The powder of the tablets equivalent to 2.5 mg of CLB, 10 mg of DCL and 325 mg of ACP was transfererred into 100 ml volumetric flask. 25 ml of methanol was added into the volumetric flask and sonicated for 15 min to effect complete dissolution of the drugs. Then the volume was made upto the mark with mobile phase. The solution was filtered through the Whatmann filter paper and the aliquot portion of the filtrate was further diluted to get the final concentration of 2.5 µg/ml of CLB, 10 µg/ml of DCL and 325µg/ml of ACP. 10 µl of the above solution was injected into the HPLC under the set chromatographic conditions.

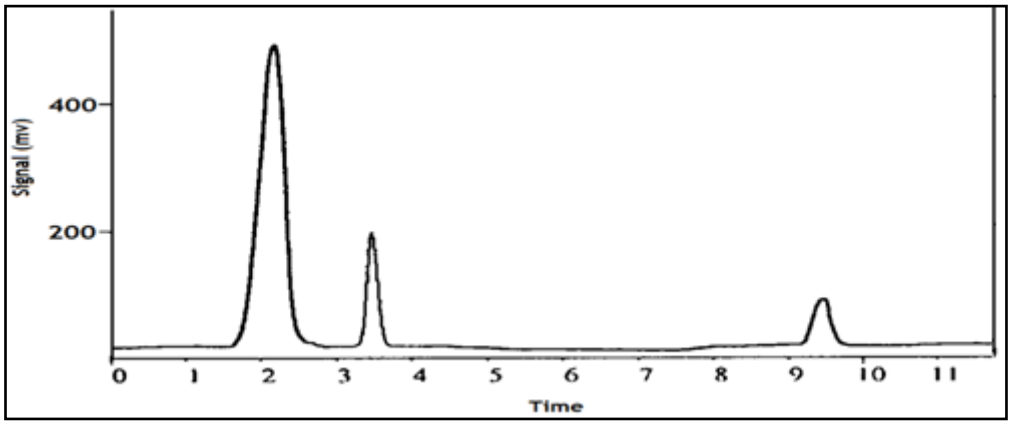


Fig. 2: Chromatogram of the mixture of ACP (100 µg/ml), CLB (50 µg/ml) and DCL (100 µg/ml)

Method Validation

Validation of any analytical method shall be done to establish by laboratory studies, that the performance of the method meet the requirement for the intended analytical application.

Linearity

Several aliquots of standard solutions of DCL, ACP and CLB were taken in different 10 ml volumetric flasks and and the volume was made upto the mark with mobile phase such that final concentration of DCL, ACP and CLB were 10-60 µg/ml, 26-58.5 µg/ml and 2.5-15 µg/ml respectively. Evaluation was performed using the UV-Vis detector at 218 nm, peak area recorded for all the peaks, results are displayed in Table 2. Calibration curve was plotted as time against peak area as shown in Figure 3, 4 and 5. The slope and intercept value for calibration curve were y = 3282.x + 11254 (R^2 = 0.993) for DCL, y = 15148x + 42258 (R^2 = 0.993) for ACP and y = 12263x + 12096 (R^2 = 0.990) for CLB.

Table 2: Linearity study

S. No.	Concentration of DCL (µg/ml)	Peak Area	Concentration of ACP (µg/ml)	Peak area	Concentration of CLB (µg/ml)	Peak area
1	10	141232	26	423667	2.5	159228
2	20	176265	32.5	528795	5	174269
3	30	219331	39	657993	7.5	208384
4	40	244658	45.5	733974	10	245685
5	50	278454	52	835362	12.5	277459
6	60	304587	58.5	913761	15	304510

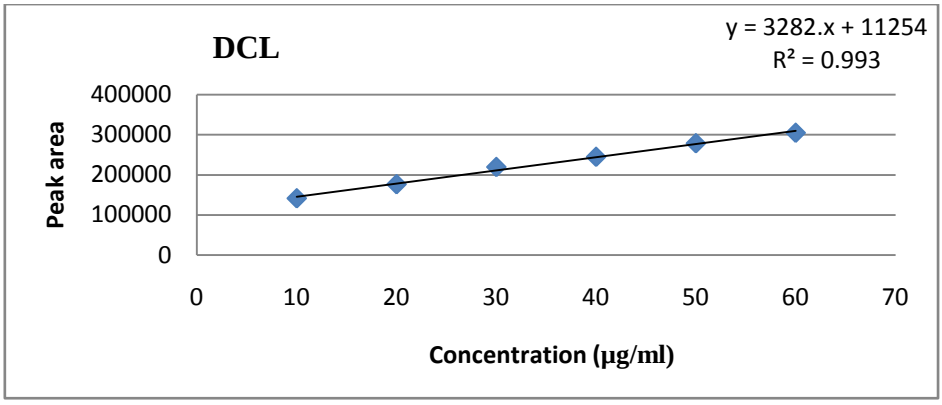


Fig. 3: Calibration curve of DCL

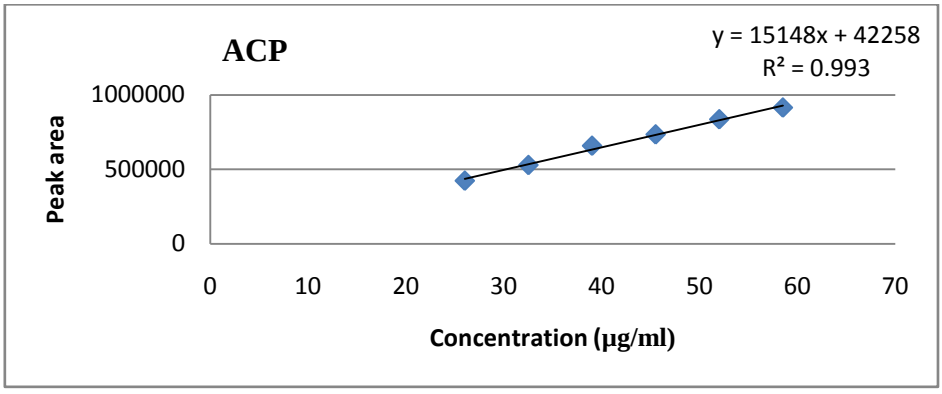


Fig. 4: Calibration curve of ACP

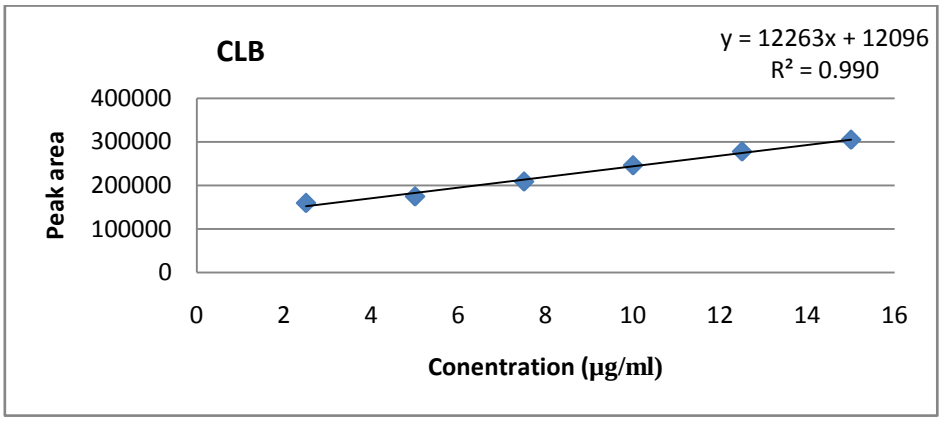


Fig. 5: Calibration curve of CLB

Percent Recovery

Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) 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. For DCL, tablet powder equivalent to 10 mg DCL was taken individually into three different 100 ml volumetric flasks and then 8 mg (80%), 10 mg (100%) and 12 mg (120%) of standard DCL were added to each of the volumetric flasks. After that 25 ml of the mobile phase was added to each of the volumetric flask and sonicated for 5 min. The solutions were then filtered and 1 ml of the filtrate from each was taken in 10 ml volumetric flasks individually and diluted upto the mark with mobile phase. The solutions were injected in triplicates into the chromatographic system and the peak area were evaluated to give Percent Recovery and Standard deviation. Similar procedure was repeated for other two drugs.

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 interday precision were determined by repeating assay six times in same day for intraday precision and on different days for interday precision studies. The results of these analyses are shown in Table 3.

Table 3: Percent Recovery

Drug	Label claim	Amount of Standard spiked		%Recovered± SD	%RSD
Dicyclomine hydrochloride	10 mg	80%	8 mg	99.05±0.096	0.53
		100%	10 mg	98.88±0.079	0.39
		120%	12 mg	99.01±0.098	0.61
Acetaminophen	325 mg	80%	260 mg	98.84±0.581	0.10
		100%	325 mg	99.60±0.770	0.118
		120%	390 mg	99.20±0.641	0.089
Clidinium bromide	2.5 mg	80%	2 mg	98.64±0.031	0.68
		100%	2.5 mg	99.15±0.034	0.66
		120%	3 mg	98.86±0.3329	0.90

Table 4: Precision

Drug	Intraday		Interday	
	% Obtained	%RSD	%Obtained	%RSD
Dicyclomine Hydrochloride	99.41	0.45	98.49	0.564
Acetaminophen	100.85	0.185	99.67	0.196
Clidinium bromide	98.62	0.785	98.28	0.885

Limit of detection and Limit of quantification: (LOD, LOQ)

The LOD and LOQ of the proposed method were determined by progressively injecting lower concentrations of the standard solutions under the set chromatographic conditions.

The results obtained are displayed in Table 5.

Robustness

The robustness of the proposed method was verified by varying the buffer pH and solvent ratio in the mobile phase and column temperature. Sample solutions were injected as 10 µl injection into the chromatographic system. The parameters studied were Retention time, tailing factor and % Content found.

Table 5: LOD and LOQ

Parameter	Dicyclomine hydrochloride	Acetaminophen	Clidinium bromide
LOD	0.1281	0.014	0.069
LOQ	0.5750	0.0189	0.295

Table 6: Change in pH of buffer

pH	Retention time			Tailing factor			% Content found		
	DCL	ACP	CLB	DCL	ACP	CLB	DCL	ACP	CLB
7.1	9.34	2.28	3.45	1.41	1.28	1.47	98.54	99.26	98.31
7.3	9.15	2.34	3.52	1.34	1.22	1.31	99.15	99.54	98.39
7.5	9.12	2.41	3.42	1.14	1.12	1.24	98.17	99.24	98.44
Mean							98.62	99.34	98.38
SD							0.351	0.215	0.651

Table 7: Change in Column temperature

Column Temp	Retention time			Tailing factor			% Content found		
	DCL	ACP	CLB	DCL	ACP	CLB	DCL	ACP	CLB
38°C	9.22	2.14	3.18	1.25	1.55	1.21	99.24	99.17	98.36
40°C	9.26	2.25	3.42	1.34	1.58	1.31	98.64	99.36	98.10
42°C	9.45	2.47	3.51	1.34	1.69	1.28	99.01	100.4	98.33
Mean							98.96	99.59	98.26
SD							0.0812	0.121	0.098

Table 8: Solvent ratio in mobile phase

Solvent Ratio	Retention time			Tailing factor			% Content found		
	DCL	ACP	CLB	DCL	ACP	CLB	DCL	ACP	CLB
32:38:30	9.45	2.87	3.35	1.14	1.27	1.37	98.11	100.2	98.04
28:40:32	9.31	2.85	3.65	1.25	1.68	1.24	98.54	99.85	98.02
30:42:28	9.14	2.15	3.30	1.34	1.52	1.29	98.63	99.10	99.14
Mean							98.42	99.71	98.40
SD							0.0887	0.099	0.108

RESULTS AND DISCUSSION

The aim of the study was to develop an appropriate HPLC method for estimation of Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide simultaneously in a commercial tablet formulation using commonly employed C18 column and UV detector. In order to make the method effective preliminary adequate and optimum chromatographic conditions were selected. Parameters such as detection wavelength, ratio of the solvents in the mobile phase, pH of the buffer and concentrations of the standard solutions were studied.

The method was validated according to the ICH guidelines and no interfering peaks were found in the chromatogram indicating that the tablet excipients did not interefered with the analysis of the drugs.

Table 9: System Suitability parameters

Parameters	Observation		
	ACP	**CLB**	**DCL**
Linearity	26-58.5 µg/ml	2.5-15 µg/ml	10-60 µg/ml
Regression equation	y = 15148x + 42258	y = 12263x + 12096	y = 3282.x + 11254
Correlation coefficient	R^2=0.993	R^2=0.990	R^2=0.993
Retention time	2.24	3.41	9.39
Resolution	13.03	16.54	
Theoretical plates	54908.64	6258.11	82173.57
%Recovery	99.21	98.83	98.98
Precision	%RSD		
Intraday	0.185	0.785	0.45
Interday	0.196	0.885	0.564
Robustness	Robust	Robust	Robust
LOD	0.014	0.069	0.1281
LOQ	0.0189	0.295	0.5750

CONCLUSION

The developed method gives good resolution between Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide with short analysis time. The method is simple, accurate, rapid, precise and can be easily used for routine analysis of the three drugs Dicyclomine hydrochloride, Acetaminophen and Clidinium bromide without involving any complicated sample preparation.

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