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IJPBS |Volume 3| Issue 4 |OCT-DEC|2013|255-264

Research Article
Pharmaceutical Sciences

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DICYCLOMINE HYDROCHLORIDE AND DICLOFENAC POTASSIUM IN TABLET DOSAGE FORMS

Vijjigiri Chaitanya, Daravath Bhaskar*, Kamarapu SK

Department of Pharmaceutical Analysis, Sri Shivani College Of Pharmacy,
Mulugu Road, Warangal, A.P, India, 506007

***Corresponding Author Email:** daravathbaskar@gmail.com

ABSTRACT

The study describes method development and subsequent validation of RP-HPLC method for simultaneous estimation of Dicyclomine Hydrochloride, Diclofenac Potassium in combined tablet dosage forms. Chromatographic separation was achieved on a Kromasil C18 (250 mm × 4.6 mm id, 5µm) column using a mobile phase ratio consisting of (70:30) Methanol: Water at flow rate 1.0 ml/min. The detection wavelength is 263 nm. The retention times of Dicyclomine Hydrochloride and Diclofenac Potassium were found to be 2.951 min and 4.195 min respectively. The developed method was validated as per ICH guidelines using the parameters such as accuracy, precision, linearity, LOD, LOQ and robustness. The developed and validated method was successfully used for the quantitative analysis of Dicyclomine Hydrochloride and Diclofenac Potassium in tablet dosage forms.

KEY WORDS

Dicyclomine Hydrochloride, Diclofenac Potassium, Cataspa tablet dosage forms, HPLC, Method development, Method validation.

INTRODUCTION:

Diclofenac Potassium chemically, Potassium [2-[(2,6-dichlorophenyl)amino]phenyl]acetate is a non-steroidal anti-inflammatory agent (NSAID) that is widely used in pharmaceutical preparations for antipyretic and analgesic actions.

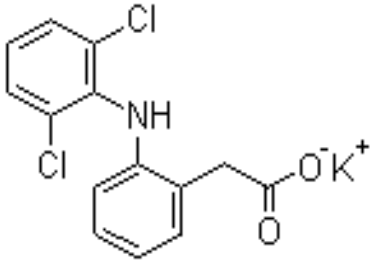


Figure-1: Chemical structure of Diclofenac Potassium

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).

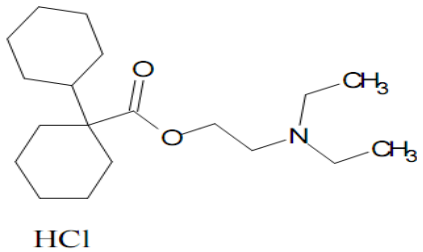


Figure-2: Chemical structure of Dicyclomine Hydrochloride

Many UV, HPLC and HPTLC based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. But no method had yet been reported for simultaneous estimation of these two drugs using HPLC in bulk drug and pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of Diclofenac Potassium and Dicyclomine Hydrochloride in pharmaceutical dosage forms.

EXPERIMENTAL: MATERIALS AND REAGENTS:

Dicyclomine Hydrochloride and Diclofenac Potassium were obtained from Active Pharma Labs, Hyderabad, India. A commercial preparation (Cataspa Tablet) used for analysis was procured from pharma market. Each tablet contains 20mg Dicyclomine Hydrochloride and 50mg Diclofenac Potassium. HPLC grade Methanol and water (Finar chemicals limited Ahmedabad).

Instrumentation: RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD plus, rheodyne sample injection port with 20 microlitre loop , SPD-M20A Photo diode array detector (PDA), LC solutions software , column used was Kromasil C18 (250 x 4.6mm, 5µm).

Chromatographic conditions:

A reverse phase column [Kromasil C18 (250 x 4.6mm, 5µm particle size)], equilibrated with mobile phase [Methanol: Water] (70:30) was used. Mobile phase flow rate was maintained at 1ml/min and effluents were monitored at 263nm. The sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 10 mins.

Standard Solution preparation:

About 100 mg of pure samples of Dicyclomine Hydrochloride and Diclofenac Potassium were accurately weighed and transferred to a 100 ml volumetric flask. Then they were dissolved in mobile phase and the solution was made up to volume with the same. Each ml of stock solution contained 1000 µg/ml. 10 ml of this stock solution was diluted to 100 ml with mobile phase to give 100 µg/ml solution (Working Stock).

Preparation of sample solution from dosage form:

Twenty tablets were weighed and crushed to fine powder. The tablet powder equivalent to 100 mg of Dicyclomine Hydrochloride and Diclofenac Potassium was transferred to a 100 ml volumetric flask and dissolved in mobile phase and the content was made up to mark with mobile phase. Then the sample solution kept in sonicater for 15 min and the solution was filtered through 0.45µm filter paper.

Assay:

With the optimized chromatographic conditions mentioned early, a steady base line was recorded. After the stabilization of baseline, inject the sample solution of a concentration 30 µg/ml of each Dicyclomine Hydrochloride and Diclofenac Potassium respectively. Each solution was run an interval of 10 minutes and the peak areas were found and amount of the drug and percentage of assay was calculated by regression equations which were tabulated in **Table-6** and chromatograms were recorded and presented in **Figure-3**.

Validation of HPLC method:

The proposed RP-HPLC method was validated as per ICH guidelines.

Linearity:

Linearity was determined for Dicyclomine Hydrochloride and Diclofenac Potassium separately by plotting a Calibration curve of peak area against their respective concentration. From the calibration curve it was found that the curve was linear in the range of 20-80 µg/ml for Dicyclomine Hydrochloride and Diclofenac Potassium. The regression equations for calibration curve was y=303.7x+0.428 (R^2=1) for Dicyclomine Hydrochloride, y=1961.x+1.5 (R^2=1) for Diclofenac Potassium. Results were shown in the **Table-1.**

Precision:

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of Dicyclomine Hydrochloride and Diclofenac Potassium was carried out by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of Dicyclomine Hydrochloride and Diclofenac Potassium and the results were reported in terms of % relative standard deviation (%RSD). However, all results fall within acceptance limits (RSD < 2), as shown in **Table-4**.

Accuracy:

The accuracy of the method was determined by calculating the recovery studies at three levels (50%, 100% and 150%) by standard addition method. Known amounts of standard Dicyclomine Hydrochloride and Diclofenac Potassium were added to the pre quantified samples and they were subjected to proposed HPLC method. The recoveries results of Dicyclomine Hydrochloride and Diclofenac Potassium in pharmaceutical preparation are shown in the **Table-3.**

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ for Dicyclomine Hydrochloride and Diclofenac Potassium were separately determined by based on calculating the signal-to-noise ratio (s/n is 3.3 for LOD and 10 for LOQ) and from the calibration curves the standard deviation of the y-intercepts and slope of the regression lines were used. σ is standard deviation of response (y – intercept) and S is the slope of calibration plot. Results are shown in the **Table 5.**

Robustness:

The robustness study was done by making small changes in the optimized method parameters like ±1% change in pH and ±1% change in flow rate. There was no significant impact on the retention time and tailing factor.

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable HPLC method for simultaneous estimation of Dicyclomine Hydrochloride and Diclofenac Potassium in tablet dosage form different mobile phases such as Methanol and Water in different proportions were used and finally Methanol: Water (70:30) was selected as an appropriate mobile phase, which give good retention time and acceptable peak parameters for Dicyclomine Hydrochloride and Diclofenac Potassium. The linear relationship was carried out between the peak area and concentration from a range of 20-80µg/ml for Dicyclomine Hydrochloride and 20-80µg/ml for Diclofenac Potassium. The linearity can be expressed as correlation coefficient 0.998 and 0.999 for Dicyclomine Hydrochloride and Diclofenac Potassium respectively. Correlation coefficient, y- intercept, slope of regression line is shown in **Table-1**. Precision was determined as intermediate precision as per ICH guidelines. It was assessed at 3 concentration levels %RSD obtained was less than 2% for both drugs. The results of precision are shown in **Table-4**. System suitability parameters for proposed method are shown in **Table-8**. Assay of tablets Dicyclomine Hydrochloride and Diclofenac Potassium was evaluated. Three replicate determinations were carried out on tablets. Percentage purity was found to be 98.6% and 99.26%. Results of tablet analysis were shown in **Table-2**. Robustness studies were carried out after deliberate alterations of flow rate and mobile phase compositions. It was observed that did not lead to changes of retention times of peak of interest. Percentage of recovery shows that method is free from interference of the excipients used in the formulation shown in **Table-3.**

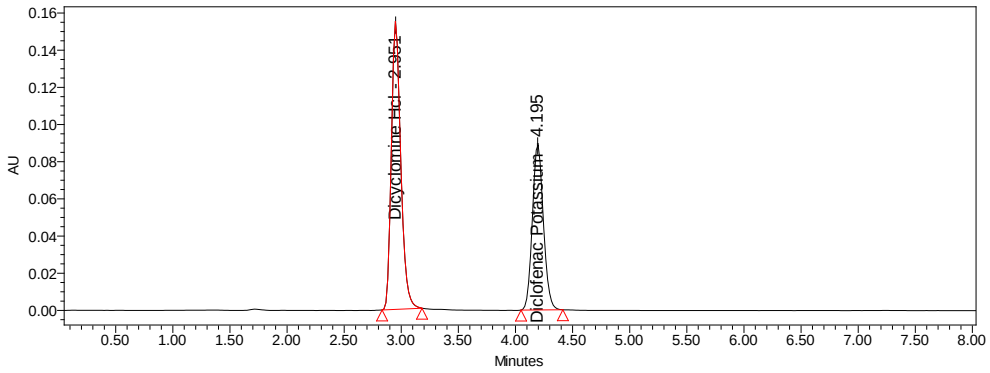


Figure-3: Chromatogram of combined Tablet dosage form

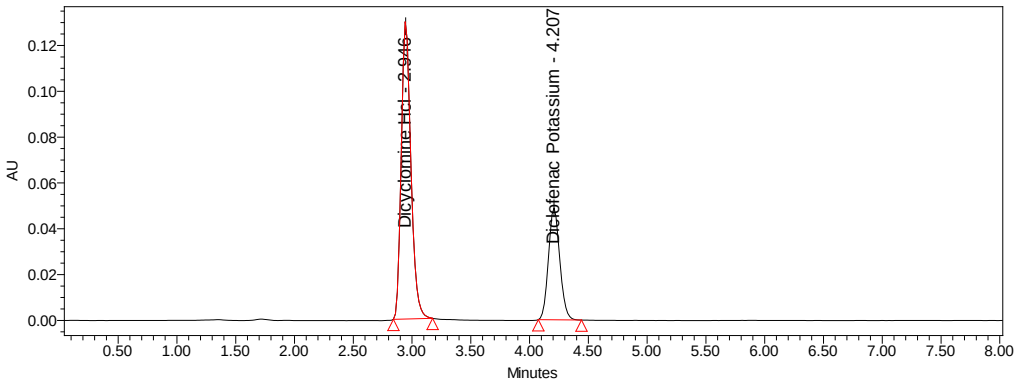


Figure-4: Chromatogram of Dicyclomine Hydrochloride and Diclofenac Potassium for Recovery studies by HPLC method (50%).

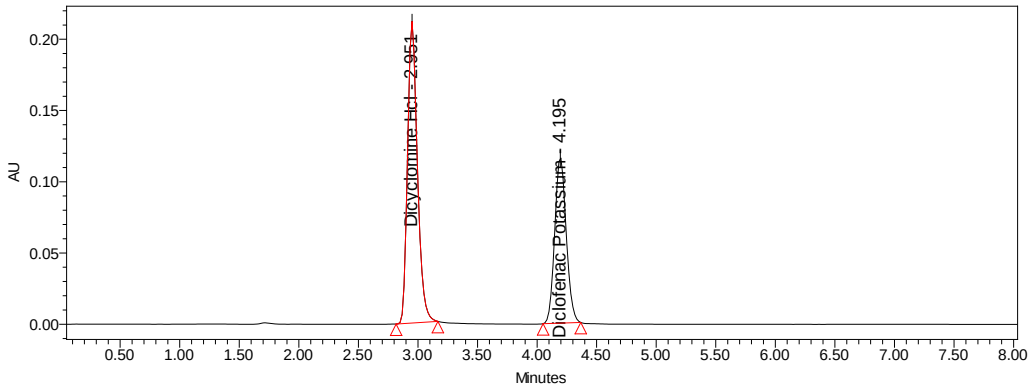


Figure-5: Chromatogram of Dicyclomine Hydrochloride and Diclofenac Potassium for Recovery studies by HPLC method (100%).

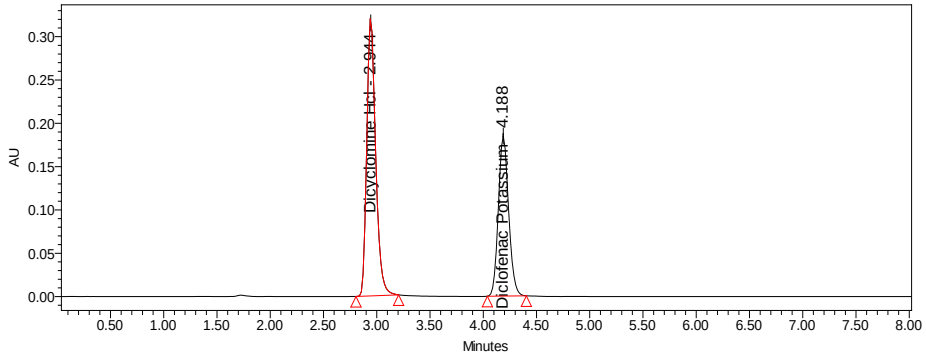


Figure-6: Chromatogram of Dicyclomine Hydrochloride and Diclofenac Potassium for Recovery studies by HPLC method (150%).

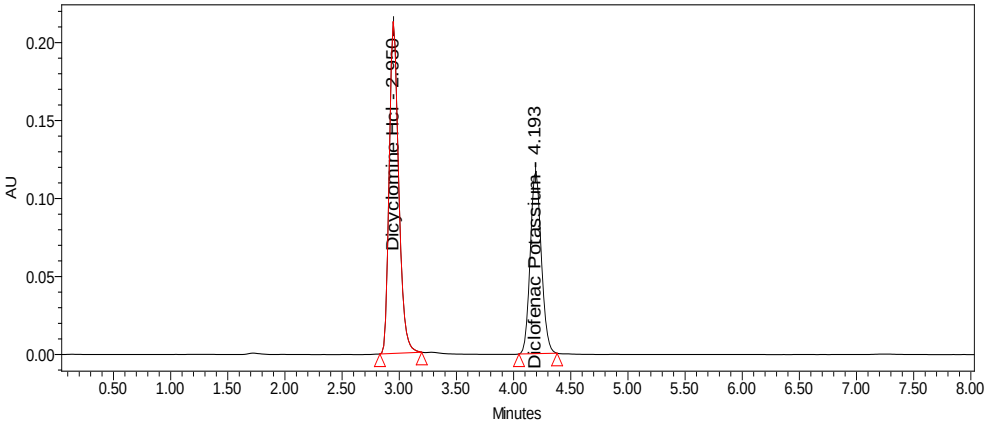


Figure-7: Chromatogram of Dicyclomine Hydrochloride and Diclofenac Potassium for Robustness by HPLC method.

BRAND (Cataspa)		% Amount found ± SD
Dicyclomine Hydrochloride 250mg + Diclofenac Potassium 4mg	Dicyclomine Hydrochloride Diclofenac Potassium	98.6 ± 1.4 99.26 ± 0.74

Table -1: Analysis of tablet formulation

Drug	Label claim (mg)	Amount added (mg)	Amount Recovered	% Recovery
Dicyclomine Hydrochloride	20	10(50%)	9.7	97.0%
		20(100%)	19.62	98.1%
		30(150%)	29.81	99.36%
Diclofenac potassium	50	25 (50%)	24.76	99.04%
		50(100%)	49.51	99.02%
		75(150%)	74.11	98.81%

Table 2: Results of Recovery studies

Drug	Theoretical concentration (µg/ml)	Intra-day concentration (µg/ml)		Inter-day concentration (µg/ml)	
		Mean	% RSD	Mean	% RSD
Dicyclomine Hydrochloride	20	607512	0.001	607501	0.022
	40	1214937	0.004	1214897	0.008
	60	1822432	0.001	1822411	0.026
Diclofenac Potassium	20	389916	0.007	389891	0.103
	40	784655	0.022	784615	0.024
	60	1176982	0.006	1176927	0.148

Table-3: Intra – day and Inter – day precision of Dicyclomine Hydrochloride and Diclofenac Potassium Standard solutions

Parameters	Dicyclomine Hydrochloride	Diclofenac Potassium
Limit of detection	0.028	12.53
Limit of quantitation	0.084	37.99

Table -4: Results of precision and LOD & LOQ

Flow rate	Drug	Mean (Peak area)	Rt value	% RSD	Overall %RSD
0.8	Dicyclomine Hydrochloride	1539568	2.949	0.020	Dicyclomine Hydrochloride (0.0163)
	Diclofenac Potassium	987674	4.193	0.108	
1.0	Dicyclomine Hydrochloride	1219784	2.945	0.016	
	Diclofenac Potassium	780921	4.188	0.112	Diclofenac Potassium (0.106)
1.2	Dicyclomine Hydrochloride	1000535	2.938	0.013	
	Diclofenac Potassium	649221	4.176	0.099	

Table-5: Robustness-Effect of Flow rate (HPLC)

Parameters	Dicyclomine Hydrochloride	Diclofenac Potassium	Acceptance limits
Theoretical plates	3562	2591	>2000
Tailing Factor	1.230	1.117	<2.0
Asymmetry Factor	1.026	1.041	<2.0

Table-6: System suitability parameters

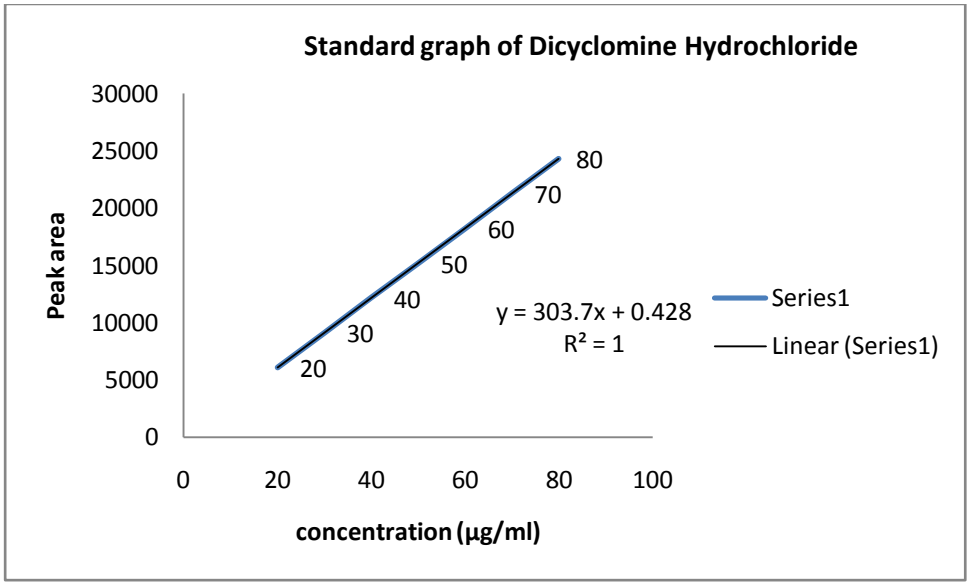
Concentration (µg/ml)	Peak Area
20	6075
30	9112
40	12149
50	15188
60	18223
70	21264
80	24297

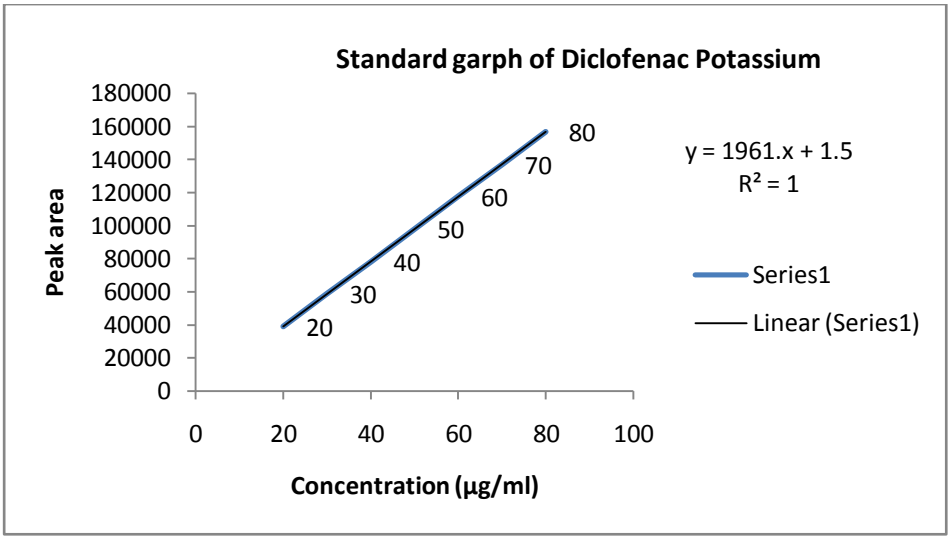
Table-7: Linearity graph data of HPLC method for Dicyclomine Hydrochloride

Concentration (µg /ml)	Peak Area
20	39233
30	58849
40	78465
50	98080
60	117696
70	137312
80	156928

Table-8: Linearity graph data of HPLC method for Diclofenac Potassium

Figure-8: Linearity studies





CONCLUSION

The present paper describes proposed RP-HPLC method for the simultaneous estimation of Dicyclomine Hydrochloride and Diclofenac Potassium in tablet dosage form is accurate, precise, linear, robust, simple and rapid. Acceptable regression values, RSD % and standard deviations which make it versatile and valuable for simultaneous estimation of two drugs in tablet formulation. Acceptable values of precision and accuracy have been obtained as per guidelines for assay validation. The run time is relatively short i.e. within 10 mins. So, A large number of samples can be analysed in short period of time. The results of this developed RP-HPLC method can be could be conveniently adopted for quality control analysis of Dicyclomine Hydrochloride and Diclofenac Potassium simultaneously from tablet dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Active Pharma Labs, Hyderabad, India for providing sample of Dicyclomine Hydrochloride and Diclofenac Potassium for research. The authors are grateful to Department of Pharmaceutical Analysis, Sri Shivani College of Pharmacy, Affiliated to Kakatiya University, Warangal, Andhra pradesh, India, for providing all the facilities to carry out the work. The authors express heartful thanks to Principal and Management of Sri Shivani college of Pharmacy for their best wishes and constant support and encouragement during course of project work.

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***Corresponding Author:**
Daravath Bhaskar *
Department of Pharmaceutical Analysis,
Sri Shivani College Of Pharmacy,
Mulugu Road, Warangal,
Andhra Pradesh, India, 506001.