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A NOVEL RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DICYCLOMINE AND ETHYL MORPHINE IN BULK AND PHARMACEUTICAL FORMULATION

Aruna Gundala [*1], Divya Sree Kammuri [1] and Prasanthi Chengalva [2]

Department of Pharmaceutical Analysis and Quality Assurance [1], Department of Pharmaceutical Analysis [2], Krishna Teja Pharmacy College, Tirupati - 517506, Andhra Pradesh, India.

Keywords:

Dicyclomine, Ethyl morphine, RP-HPLC, Method development and Validation

Correspondence to Author:
Aruna Gundala

Associate Professor, Department of Pharmaceutical Analysis and Quality Assurance, Krishna Teja Pharmacy College, Tirupati - 517506, Andhra Pradesh, India.

E-mail: garuna.mpharm@gmail.com

ABSTRACT: The present study was designed to develop and validate a simple, sensitive, precise and accurate RP-HPLC method for simultaneous estimation of dicyclomine and ethylmorphine in bulk and tablet dosage form. The chromatographic separation was achieved on Discovery C_{18} column (250 × 4.6 mm, 5 µm) as stationary phase with a mobile phase of water (pH 5.4 adjusted with orthophosphoric acid): acetonitrile (40:60 v/v) at a flow rate of 1 ml/min and PDA detection at 215 nm. The proposed method was validated for system suitability, specificity, linearity, accuracy, precision, LOD, LOQ, and robustness as per ICH guidelines. The retention times of dicyclomine and ethylmorphine were found to be 3.166 ± 0.02 and 4.204 ± 0.19 min respectively. The calibration curves were linear in the concentration range of 50% to 150% of the working concentration (r^2=0.999) for both the drugs in a binary mixture. The accuracy was found to be 98.61 % and 99.24 % for dicyclomine and ethylmorphine respectively. The LOD was found to be 0.05 µg/ml, and 0.20 µg/ml and LOQ were found to be 0.17 µg/ml and 0.62 µg/ml for dicyclomine and ethylmorphine respectively. The percentage recoveries for both drugs were in the range of 98-101%. Hence the proposed RP-HPLC method can be used in routine analysis of tablets containing dicyclomine and ethylmorphine.

INTRODUCTION: Dicyclomine is chemically known as (1, 1-Bicyclohexyl)-1-carboxylic acid, 2-(diethylamino) ethyl ester [1] **Fig. 1**. It is an anti cholinergic agent, synthetic tertiary amine and antispasmodic. It works by relaxing the muscles in the stomach and gut. It inhibits sudden muscle contractions and relieves cramps, pain and bloating. Ethylmorphine is chemically known as (5*α*, 6*α*)-3-ethoxy- 17- methyl- 7, 8- didehydro- 4, 5-epoxymorphinan-6-ol **Fig. 2**.

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It is an opioid analgesic. It works by decreasing the perception of pain by blocking the transmission of pain signals to the brain. Dicyclomine and ethylmorphine are used to relieve muscle spasms, muscle cramps, pain and bloating of stomach or intestine.

Extensive literature survey revealed that there were few analytical methods for the estimation of specified drugs with other combinations [1-10]. There was no reverse phase high-performance liquid chromatography (RP-HPLC) method reported for the estimation of dicyclomine and ethylmorphine. Hence we planned to develop a simple analytical method for simultaneous estimation of dicyclomine and ethylmorphine in bulk and pharmaceutical preparations.

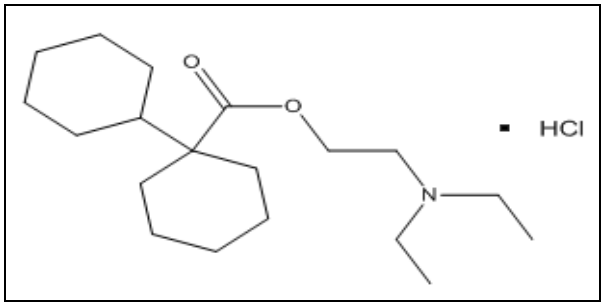

FIG. 1: STRUCTURE OF DICYCLOMINE

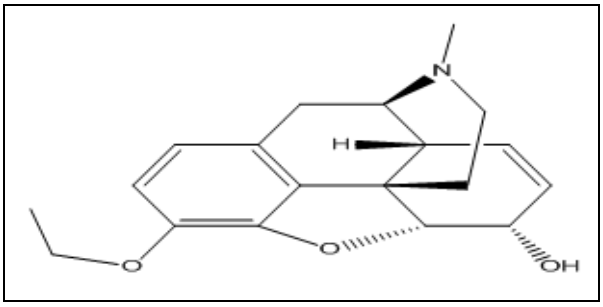

FIG. 2: STRUCTURE OF ETHYL MORPHINE

MATERIALS AND METHODS:

Materials: Dicyclomine and ethylmorphine were obtained from spectrum pharma research laboratory, Hyderabad as a gift sample. Spasmindon T tablets were purchased from the local market which contains 20 mg dicyclomine and 11 mg ethylmorphine. Acetonitrile, ortho-phosphoric acid (OPA) and HPLC grade water were procured from Merck, Mumbai. Analytical column used for the separation of analytes was Discovery C_{18} column (250 × 4.6 mm, 5 µm).

Methods:

Diluent: Water: acetonitrile has taken in the ratio 50:50 % v/v.

Preparation of Standard Stock Solution: 20 mg of dicyclomine and 11 mg of ethylmorphine standards were accurately weighed and transferred into a 10 ml clean dry volumetric flask, 5 ml of diluent was added, sonicated for 10 min and made up to the final volume with diluent. Further, 1 ml from the above solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Preparation of Sample Solution: 20 tablets were weighed, the average weight of each tablet was calculated and crushed then the weight equivalent to one tablet was transferred into a 10 ml clean dry volumetric flask, 5 ml of diluent was added, sonicated for 10 min and made up to the final volume with diluent. Further, 1 ml from the above solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Chromatographic Conditions: The chromatographic condition was performed on Discovery C_{18} column (250 × 4.6 mm, 5 µm particle size) at 30 ºC. The samples were eluted using water whose pH adjusted to 5.4 with orthophosphoric acid and acetonitrile (40:60 v/v) as the mobile phase. The measurements were carried out with an injection volume of 10 µl; the flow rate was set to 1 ml/min at a detection wavelength 215 nm by using a PDA detector.

RESULTS:

Method Development: A series of trails were conducted with different columns with different mobile phase ratios to develop a suitable RP-HPLC method for estimation of dicyclomine and ethylmorphine in bulk and tablet dosage form. Discovery C_{18} column was found to be satisfactory for better separation and good resolution, analytes were checked with PDA detector at 215 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of dicyclomine and ethylmorphine from standard preparation and pharmaceutical formulation was shown in **Fig. 3** and **4**.

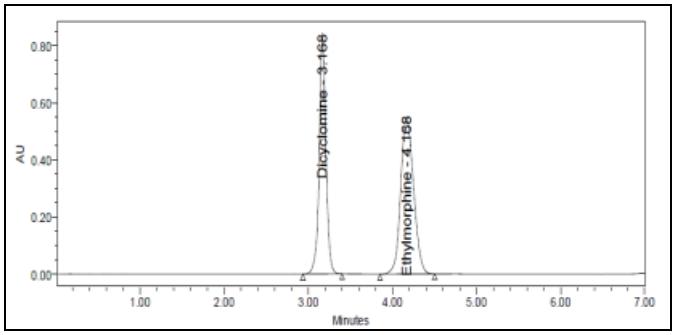

FIG. 3: CHROMATOGRAM OF DICYCLOMINE AND ETHYL MORPHINE IN STANDARD PREPARATION

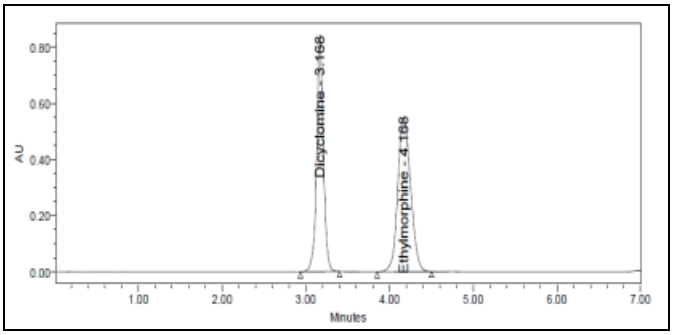

FIG. 4: CHROMATOGRAM OF DICYCLOMINE AND ETHYL MORPHINE IN SAMPLE PREPARATION

Method Validation: The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines [11].

The developed RP-HPLC method was validated for parameters like system suitability, specificity, linearity, accuracy, precision, LOD, LOQ, and robustness.

System Suitability: To check the system suitability, standard solutions were prepared as per the test method and injected into the chromatographic system. The parameters such as theoretical plates, resolution and asymmetric factor were evaluated. The system suitability parameters were tabulated in **Table 1**. All the parameters were found to be within limits.

TABLE 1: RESULTS OF SYSTEM SUITABILITY

Analytes	Retention times	Resolution	Theoretical Plates	Tailing Factor
Dicyclomine	3.166 ± 0.02 min	-	7363	0.98
Ethyl morphine	4.204 ± 0.19 min	9.4	3462	1.06

min: minutes

Specificity: To ensure the specificity of the developed analytical method blank and placebo injections were prepared as per the test method and injected into the chromatographic system. The chromatograms of blank and placebo were shown in **Fig. 5** and **6**. From the results, it was found that there were no interfering peaks at retention times of analytes.

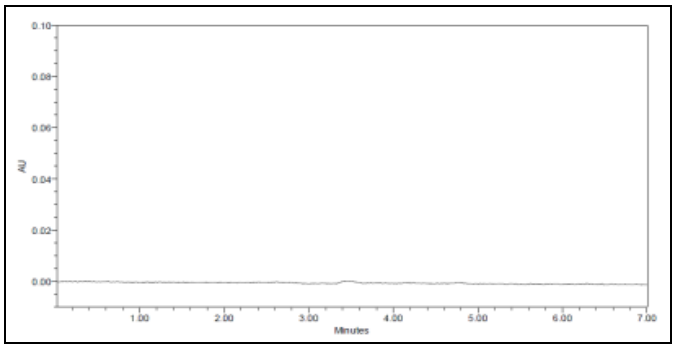

FIG. 5: CHROMATOGRAM OF BLANK

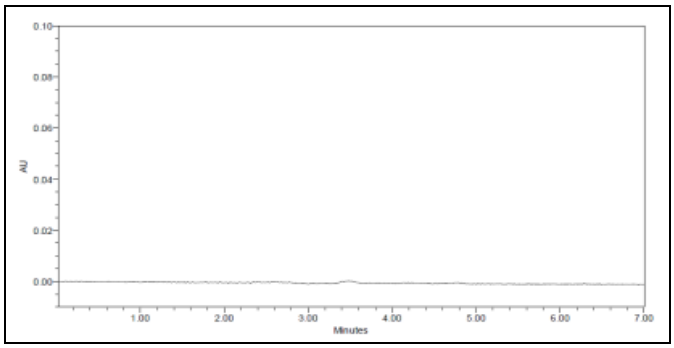

FIG. 6: CHROMATOGRAM OF PLACEBO

Precision: Method precision was determined by performing the analysis of the sample under the test of repeatability at working concentration. The sample solutions of dicyclomine and ethylmorphine were prepared as per the test method and injected six times into the column. The results of precision were tabulated in **Table 2**. RSD values were calculated and reported. The values are found within limits, indicating the developed method was precise.

TABLE 2: RESULTS OF PRECISION

N*	Dicyclomine		Ethylmorphine	
	Rt (min)	Peak area	Rt (min)	Peak area
1	3.160	2371551	4.186	3036710
2	3.162	2388646	4.186	3062269
3	3.162	2354505	4.186	3037302
4	3.163	2386204	4.187	3067680
5	3.163	2374109	4.189	3045354
6	3.163	2387617	4.191	3003649
Mean		2377105		3042161
SD		13244.4		22813.3
RSD (%)		0.6		0.7

* Number of replicates = 6, SD = standard deviation and RSD = relative standard deviation.

Linearity: To check the linearity of the test solutions for the assay method was prepared from dicyclomine and ethylmorphine standard stock solutions at five concentration levels from 50% to 150% of assay concentrations. The peak area versus concentration data was treated by least-

square linear regression analysis was shown in **Fig. 7** and **8**. The results were tabulated in **Table 3** have shown an excellent correlation between peak areas and concentration range of 100-300 µg/ml for dicyclomine and 55-165 µg/ml for ethylmorphine. The correlation coefficients were found to be 0.999 for both the drugs, which meet the method validation acceptance criteria and hence the method was said to be linear at the specified concentration range for the mentioned drugs.

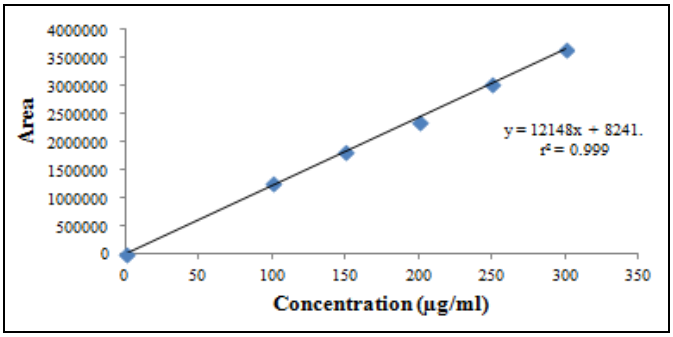

FIG. 7: LINEARITY CHART OF DICYCLOMINE

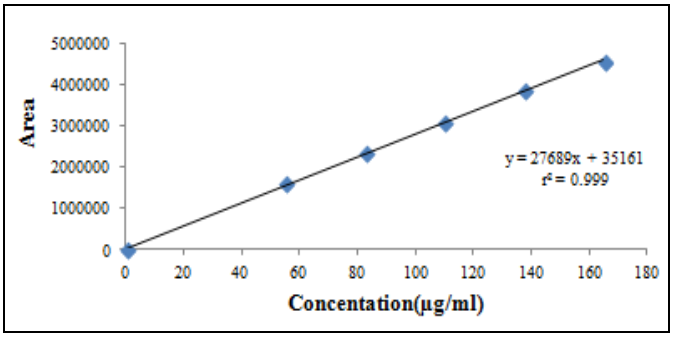

FIG. 8: LINEARITY CHART OF ETHYL MORPHINE

TABLE 3: RESULTS OF LINEARITY

Analytes	Correlation Coefficients (r^2)
Dicyclomine	0.999
Ethylmorphine	0.999

Accuracy: To check the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to the analysed sample, and the contents were reanalyzed by the proposed method, and the percentage recovery was reported. The results were given in **Table 4 and 5**. The recovery was found to be within limits; hence the method was accurate for the determination of dicyclomine and ethylmorphine.

TABLE 4: RESULTS OF ACCURACY OF DICYCLOMINE

% Level	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	% Recovery	% Mean Recovery
50	100	98.77	98.77	
100	200	197.23	98.61	98.61
150	300	295.39	98.46	

TABLE 5: RESULTS OF ACCURACY OF ETHYL MORPHINE

% Level	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	% Recovery	% Mean Recovery
50	55	94.75	99.52	
100	110	109.57	99.61	99.24
150	165	162.71	98.61	

Limit of Detection and Limit of Quantitation: To determine the lowest amount of analyte in the sample, limit of detection (LOD) and limit of quantitation (LOQ) were established at a signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of dicyclomine and ethylmorphine were experimentally determined by injecting six injections of each drug and results were given in **Table 6**.

TABLE 6: RESULTS OF LOD AND LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Dicyclomine	0.05	0.17
Ethyl morphine	0.20	0.62

Robustness: To check the reliability of the method by altering the chromatographic conditions like mobile phase composition, temperature, flow rate, *etc*. can be reported. Small changes in the operational conditions were allowed, and the extent to which the method was robust was determined. A deviation of ± 2 ºC in the column temperature and ± 0.2 ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in duplicate. The results were reported in **Table 7**.

From the results it was found that there was no significant difference was observed in system suitability parameters. Hence the method was found to be robust.

TABLE 7: RESULTS OF ROBUSTNESS

	Dicyclomine		Ethylmorphine	
Parameter	**Tailing***	**Place count***	**Tailing***	**Plate count***
Less flow rate (1.1 ml/min)	1.00	8188	1.07	4132
More flow rate (1.3 ml/min)	1.02	7588	1.40	4203
Less mobile phase (35:65)	1.03	8226	1.05	4335
More mobile phase (45:55)	1.00	8099	1.07	3464
Less temperature (±25 ºC)	0.99	7544	1.12	3056
More temperature (±35 ºC)	1.00	7494	1.11	3110

*: Average of two determinations.

DISCUSSION: The developed method can be used for routine analysis because the linearity found to be 0.999 for both drugs which show better regression for linearity. The percentage of recoveries obtained for both drugs were in the range of 98-101%. Therefore the method can be used for routine analysis and one more important reason is that the developed method does not involve any buffer; so that the life of the column was increased. There were various RP-HPLC methods have reported for the determination of dicyclomine and ethylmorphine in individual and in combination with other drugs [1-10]. However, to date, there was no RP-HPLC method had been reported for simultaneous estimation of dicyclomine and ethylmorphine in the combined dosage form. So this method was first of its kind.

CONCLUSION: The proposed RP-HPLC method was found to be simple, accurate, precise, robust, rapid and economical. This method gives good resolution between two compounds with a short analysis time and can be used for routine quality control analysis in quality control departments for the determination of dicyclomine and ethylmorphine in the tablet dosage form.

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AUTHORS CONTRIBUTIONS: All the authors have contributed equally.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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