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METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF CHLORPHENIRAMINE MALEATE AND DIETHYLCARBAMAZINE CITRATE IN COMBINED TABLET DOSAGE FORMS

Gouru Santhosh Reddy, Daravath Bhaskar, Kamarapu SK*

Department of Pharmaceutical Analysis, Sri Shivani College Of Pharmacy,
Mulugu Road, Warangal, A.P, India, 506 007

***Corresponding Author Email:** kamarapu.sudheerkumar@gmail.com

ABSTRACT

The study describes method development and subsequent validation of UV Spectroscopic method for simultaneous estimation of Diethylcarbamazine citrate (DEC), Chlorpheniramine maleate(CPM) in combined tablet dosage forms.The solvent system used was 0.01N sodium hydroxide.Spectrum was scanned in the range of 200-400nm with 1cm pathlength.The Absorption maximum of Diethylcarbamazine citrate and Chlorpheniramine maleate were found to be 216nm and 261nm 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 Diethylcarbamazine citrate and Chlorpheniramine maleate in bulk and combined tablet dosage forms.

KEY WORDS

Diethylcarbamazine citrate,Chlorpheniramine maleate, Eofil Forte tablet dosage forms, UV, Method validation.

INTRODUCTION

Chlorpheniramine maleate (CPM) chemically, 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1-amine is an antihistamine drug that is widely used in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases.

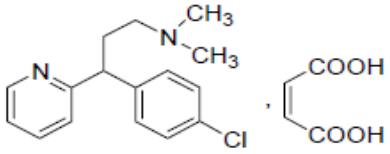


Figure-1: Chemical structure of Chlorpheniramine maleate

Diethylcarbamazine (DEC) is a piperazine anthelmintic agent indicated for the treatment of individual patients with lymphatic filariasis, tropical pulmonary eosinophilia and loiasis. The chemical name of the drug is N, N-diethyl-4-methylpiperazine-1-carboxamide citrate]. It acts by inhibiting arachidonic acid metabolism and it is a polar compound.

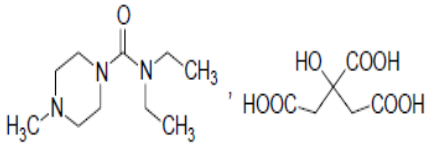


Figure-2: Chemical structure of Diethylcarbamazine citrate

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 UV in bulk drug and pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new UV Method for simultaneous estimation of CPM and DEC in pharmaceutical dosage forms.

EXPERIMENTAL

Materials and reagents:

Diethylcarbamazine citrate and Chlorpheniramine maleate were obtained from Green waves chemicals Pvt. Ltd., Bubeneshwar, India. A commercial preparation (EOFIL FORTE Tablet) used for analysis was procured from pharma market. Each tablet contains 250mg Diethylcarbamazine citrate and 4mg Chlorpheniramine maleate. HPLC grade acetonitrile and water (Finar chemicals limited Ahmedabad), Potassium dihydrogen ortho phosphate, Sodium hydroxide pellets,Ortho phosphoric acid (Qualikems Fine Chem Pvt. Ltd. Vadodhara).

Instrumentation: UV Spectroscopic method was performed using LABINDIA 3000+ system with UV win software **Solvent system:**

To prepare 0.01N sodium hydroxide solutions accurately weigh about 4gm of Sodium hydroxide pellets and transfer it to 1000ml volumetric flask. Dissolve and make up to volume with spectrophotometric grade distilled water.

Standard Solution preparation:

About 100 mg of pure samples of Diethylcarbamazine citrate and Chlorpheniramine maleate were accurately weighed and transferred to a 100 ml volumetric flask. Then they are 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 pharmaceutical dosage form:

Twenty tablets were weighed and crushed to fine powder. The tablet powder equivalent to 100 mg of Diethylcarbamazine citrate and Chlorpheniramine maleate was transferred to a 50 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 15min, then filtered the solution through 0.45µm filter paper .

Assay:

After the baseline run using blank, the sample solution of a concentration 30 µg/ml of each Diethylcarbamazine citrate and Chlorpheniramine maleate respectively. Each solution was scanned at their respective absorption maxima and the absorbances were found and amount of the drug and percentage of assay was calculated by regression equations which were tabulated in **Table-1** and chromatograms were recorded and presented in **Figure-5**.

Validation of UV Spectrophotometric method:

The proposed UV Spectrophotometric method was validated as per ICH guidelines.

Precision:

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of DIethylcarbamazine citrate and Chlorpheniramine maleate was carried out by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of Diethylcarbamazine citrate and Chlorpheniramine maleate and the results are reported in terms of % relative standard deviation (%RSD) however, all results fall within acceptance limits (RSD < 2.0), as shown in **Table 3.**

Accuracy:

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 50%, 100% and 150%. The recovery studies were carried out by adding known amounts of standard Diethylcarbamazine citrate and Chlorpheniramine maleate were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries results of Diethylcarbamazine citrate and Chlorpheniramine maleate in pharmaceutical preparation are shown in the **Table 2.**

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

The LOD and LOQ were separately determined based on the calibration curves. The limit of detection (LOD) and limit of quantification (LOQ) of developed method were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The limit of detection

(LOD) and limit of quantification (LOQ) were calculated as 3.3 σ/S and 10 σ/S, were respectively as per ICH guidelines σ is standard deviation of response(y – intercept) and S is the slope f calibration plot. Results are shown in the **Table 4.**

Linearity:

Linearity was determined for Diethylcarbamazine citrate and Chlorpheniramine maleate separately by plotting a Calibration curve of peak area against their respective concentration. From the calibration curve it was found that linearity range between 10-50µg/ml and 10-50µg/ml for Diethylcarbamazine citrate and Chlorpheniramine maleate respectively. The slope and y- intercept value for calibration curve was y=10972x+14199(R^2=0.998) for Diethylcarbamazine citrate, y = 1099x+1143 (R^2=0.999) for Chlorpheniramine maleate. Results are shown in the **Table-6** and **Table-7.**

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable UV spectrophotometric method for simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in tablet dosage form different solvents were used and finally 0.01N sodium hydroxide was chosen which give good absorption maximum for Diethylcarbamazine citrate and Chlorpheniramine maleate**.**The linear relationship was carried out between the absorption and concentration from a range of 10-50µg/ml for Diethylcarbamazine citrate and 10-50µg/ml for Chlorpheniramine maleate. The linearity can be expressed as correlation coefficient 0.998 and 0.998 for Diethylcarbamazine citrate and Chlorpheniramine maleate respectively. Correlation coefficient, y-intercept, slope of regression line is shown in **Table 5**. 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 3**. System suitability parameters for proposed method are shown in **Table 5**. Assay of tablets Diethylcarbamazine citrate and Chlorpheniramine maleate was evaluated. Three replicate determinations were carried out on tablets. Percentage purity was found to be 99.72% and 99.75%. Results of tablet analysis were shown in **Table 1**. Percentage of recovery shows that method is free from interference of the excipients used in the formulation shown in **Table 2.**

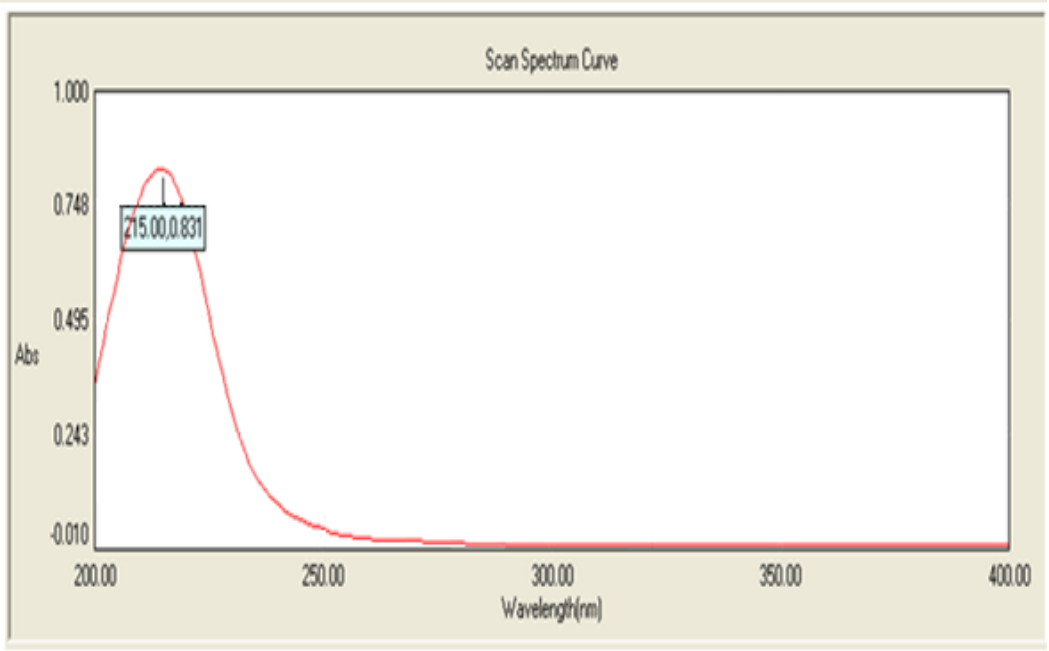


Figure-3: UV Spectrum of Diethylcabamazine citrate

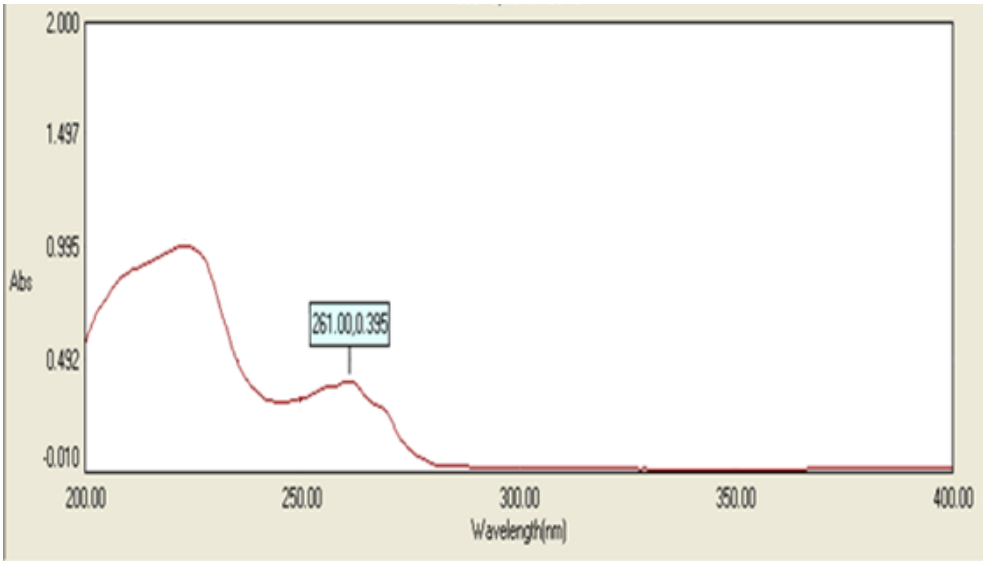


Figure-4:UVSpectrum of Chlorpheniramine maleate

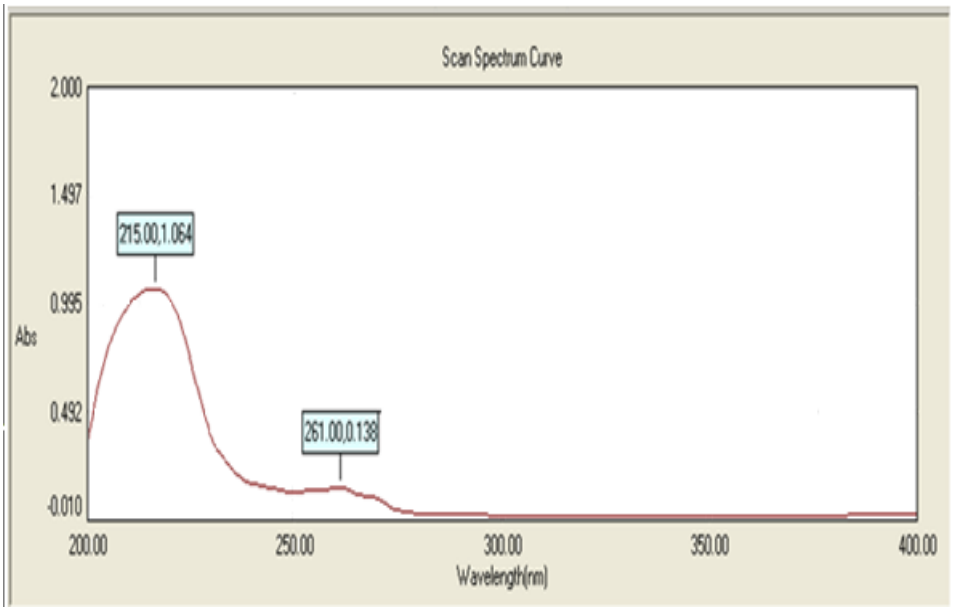


Figure-5:UV Spectrum of combined Tablet Dosage form

Drug	Label claim (mg)	Amount Added (mg)	Amount Recovered (mg)	% Recovery
		125 (50%)	122.93	98.35
DEC	250	250 (100%)	245.25	98.1
		375 (150%)	370.76	98.87
		2 (50%)	1.97	98.9
CPM	4	4 (100%)	3.95	98.9
		6 (150%)	5.91	98.52

Table-2: Results of Recovery studies.

Drug	Theoretical concentration (µg /ml)	Intra-day concentration measured* (µg/ml)		Inter-day concentration measured* (µg /ml)	
		Mean	RSD %	Mean	RSD %
DEC	10	0.142667	0.809	0.139333	1.096
	30	0.395667	0.145	0.388	0.257
	50	0.632	0.158	0.632333	0.182
CPM	10	0.195333	0.591	0.191	0.907
	30	0.543	0.184	0.538333	0.107
	50	0.842333	0.181	0.836667	0.138

Table-3: Intra – day and Inter – day precision of Diethylcarbamazine citrate and Chlorpheniramine maleate Standard solutions

Parameters	Diethylcarbamazine citrate	Chlorpheniramine Maleate
Limit of detection	2.28	2.30
Limit of quantitation	6.91	6.97

Table-4: Results of LOD & LOQ

UV Method - Parameters	Diethylcarbamazine citrate	Chlorpheniramine maleate
Linearity range(µg/ml)	10-50	10-50
Regression Equation	Y=0.012x+0.018	Y=0.016x+0.044
Slope	0.012	0.016
Correlation Coefficient(R^2)	0.998	0.998
λ_{max}	215nm	261nm
Recovery %	99.728	99.8

Table-5: System suitability parameters

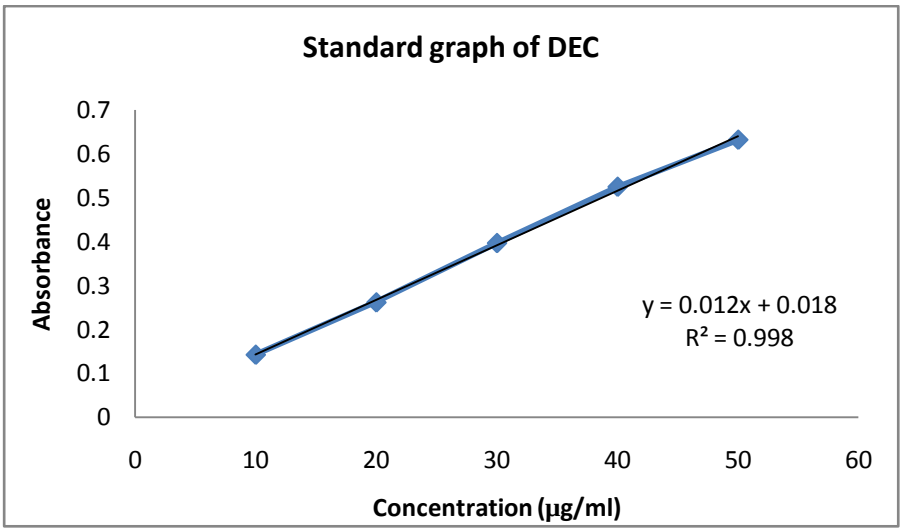
Linearity studies:

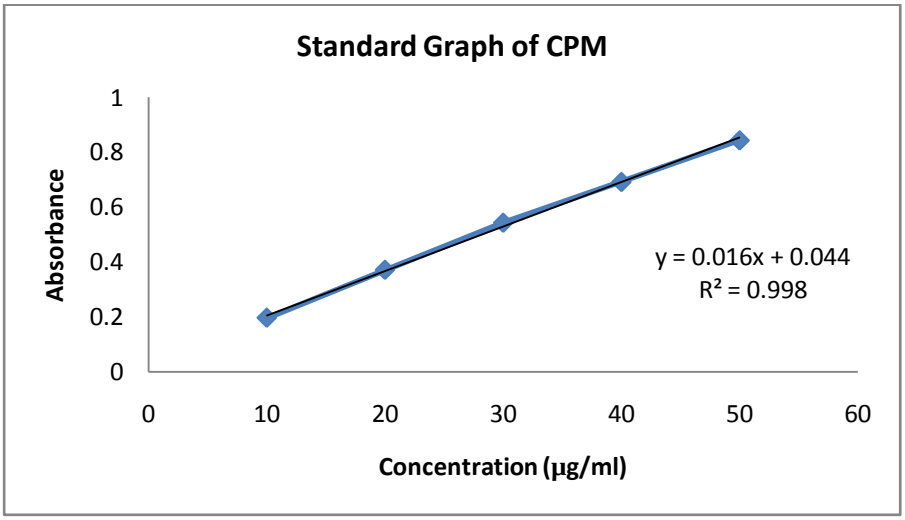
Concentration (µg/ml)	Absorbance
10	0.141
20	0.260
30	0.395
40	0.524
50	0.632

Table-6: Linearity data of UV method for Diethylcarbamazine citrate

Concentration (µg /ml)	Absorbance
10	0.195
20	0.371
30	0.542
40	0.691
50	0.843

Table-7: Linearity data of UV method for Chlorpheniramine maleate





CONCLUSION

The present paper describes proposed UV Spectroscopic method for the simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in tablet dosage form is accurate, precise, linear, 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 results of this developed UV Spectroscopic method can be could be conveniently adopted for quality control analysis of Diethylcarbamazine citrate and Chlorpheniramine maleate simultaneously from tablet dosage form.

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***Corresponding Author:**
SudheerKumar Kamarapu *
Department of Pharmaceutical Analysis,
Sri Shivani College Of Pharmacy,
Mulugu Road, Warangal,
A.P, India, 506 007.