

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CHLORPHENIRAMINE MALEATE AND DIETHYLCARBAMAZINE CITRATE IN PHARMACEUTICAL DOSAGE FORMS

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Received: 24 April 2014, Revised and Accepted: 6 May 2014

ABSTRACT

Objective: The present study describes method development and subsequent validation of RP-HPLC method for the simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in tablet dosage forms.

Methods: Reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in combined dosage forms. RP-HPLC separation was achieved by using Kromasil C18 column (250mm 4.6mm, 5mm) with mobile phase consisting of (80:20) Acetonitrile: Potassium di hydrogen phosphate solution (0.01M, pH 3.0 adjusting with Ortho phosphoric acid) with a flow rate 1.0 ml/min (UV detection 238nm).

Results: The retention time of Diethylcarbamazine citrate and Chlorpheniramine maleate were found to be 2.808 min and 4.042 min respectively. The developed method was validated as per ICH guidelines using the parameters such as accuracy, precision, linearity, LOD, LOQ and robustness. Linearity was observed over the concentration range 10–50 µg/ml with regression equation $y=1099x+1143$, $R^2=0.999$ for CPM and $y=10972x+14199$, $R^2=0.998$ for DEC.

Conclusion: The developed and validated RP-HPLC method was successfully used for the quantitative analysis of Diethylcarbamazine citrate and Chlorpheniramine maleate in combined tablet dosage forms.

Keywords: Diethylcarbamazine citrate, Chlorpheniramine maleate, Eofil Forte tablet dosage forms, HPLC, Method validation.

INTRODUCTION

Chlorpheniramine maleate (CPM) chemically, (RS)-3-(4-chlorophenyl)-3-(pyrid-2-yl) propyldimethylamine hydrogen maleate (Fig 1)[1]. It is an antihistamine drug that is widely used in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases. It inhibits the effects of histamine on capillary permeability and bronchial smooth muscles [2]. It is a first generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria.

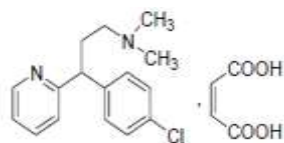


Fig.1: Chemical structure of Chlorpheniramine maleate

Diethylcarbamazine citrate (DEC) chemically, N,N-diethyl-4-methylpiperazine-1-carboxamide dihydrogen citrate (Fig 2)[1]. It is a piperazine anthelmintic agent indicated for the treatment of individual patients with lymphatic filariasis, tropical pulmonary eosinophilia and loiasis. It acts by inhibiting arachidonic acid metabolism [2].

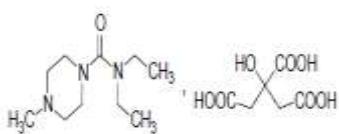


Fig.2: Chemical structure of Diethylcarbamazine citrate

There are many UV reported methods to determine either CPM or DEC alone or in combination with other drugs [3-8], NMR spectroscopy [9], polarographic method [10], electrokinetic chromatography [11], HPLC method [12-25] and UV method [26] is reported for simultaneous estimation of both drugs. But to the best of our knowledge, none has been reported the simultaneous determination of CPM and DEC by HPLC method in bulk and pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of CPM and DEC in pharmaceutical dosage forms.

MATERIALS AND METHODS

Materials and reagents

Diethylcarbamazine citrate and Chlorpheniramine maleate were procured from Green waves chemicals Pvt. Ltd., Bubeneshwar, India. A commercial tablet (EOFIL FORTE Tablet) used for analysis was procured from pharmacy. HPLC grade acetonitrile and water were procured from Finar chemicals limited, Ahmedabad, Potassium dihydrogen ortho phosphate and Ortho phosphoric acid were procured from Qualikems Fine Chem Pvt. Ltd., Vadodhara.

Instrument RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD, 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 [Acetonitrile: 0.01M Phosphate solution of pH 3.0 adjusted with Ortho phosphoric acid]

(80:20) was used. Mobile phase flow rate was maintained at 1ml/min and effluents were monitored at 238nm. The sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 10 minutes.

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 were dissolved in mobile phase and the solution was made up to the required volume. 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

Twenty tablets were weighed and pulverized. The tablet powder equivalent to 100 mg of Diethylcarbamazine citrate and Chlorpheniramine maleate 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 sonicator for 15 min and the solution was filtered through 0.45µm filter paper.

Validation of HPLC method

The proposed method was validated with the aspect of system suitability test, specificity, linearity and range, accuracy, precision, LOD, LOQ, stability and robustness according to the ICH guidelines[27-30].

RESULTS AND DISCUSSION

Specificity

No peaks were obtained while running the solution containing placebo ingredients which proves that the method is specific (Fig 3).

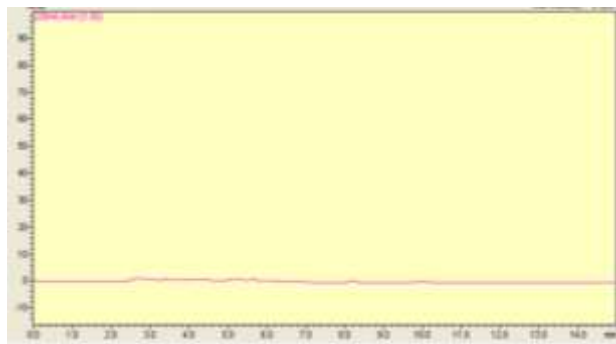


Fig.3: Blank Chromatogram

System suitability test

System suitability was determined by six replicate injections of the system suitability solution. A typical chromatogram for the system suitability test is shown in Table-1(Fig 4).

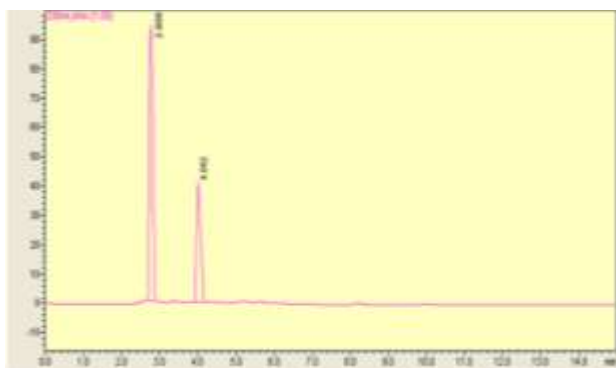


Fig.4: Chromatogram of System suitability parameters for DEC and CPM

Table 1: System suitability parameters for DEC and CPM by RP-HPLC method

System suitability parameters	DEC	CPM
Retention time (min)	2.808	4.042
Theoretical plates	2809	2124
Tailing Factor	1.024	1.035
Asymmetric factor	1.633	1.529

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 the curve was linear in the range of 10-50 µg/ml for Diethylcarbamazine citrate and Chlorpheniramine maleate. The regression equations 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 were shown in the Table-2(Fig-5 & Fig-6).

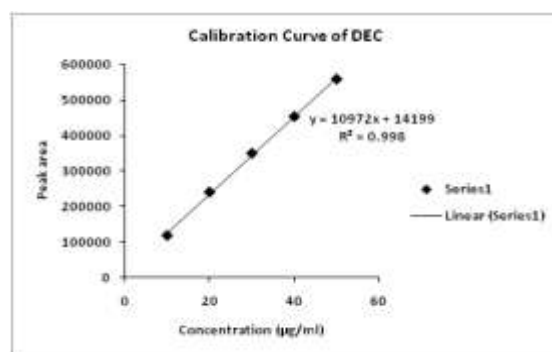


Fig.5: Calibration Curve of DEC

Table 2: Linearity data for DEC & CPM by RP-HPLC method

Concentration* (µg/ml)	Peak Area	
	DEC	CPM
10	116719	11908
20	239439	23418
30	349158	34196
40	452878	45132
50	558598	56046

* Mean of 6 determinations

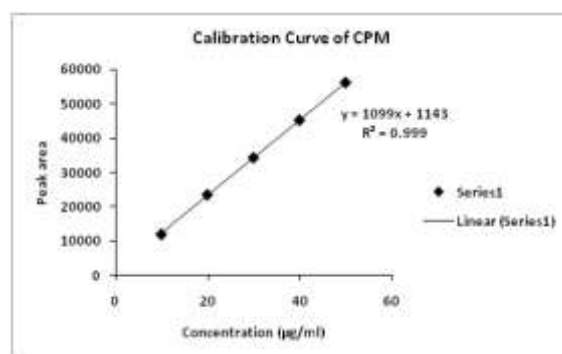


Fig.6: Calibration Curve of CPM

Accuracy

The accuracy of the method was determined by calculating the recovery studies at three levels (80%, 100% and 120%) by standard addition method. Known amounts of standard DEC and CPM were added to the pre quantified 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-3(Fig.7, Fig. 8 and Fig. 9).

Table 3: Accuracy results (%Recovery) for the determination of DEC and CPM by RP-HPLC

Drug	Amount Added (mg)	Amount Recovered (mg)	% Recovery	SD	%RSD
DEC	200 (80%)	198.72	99.36	0.14	0.14
	250 (100%)	248.57	99.43		
	300 (120%)	297.45	99.15		
CPM	3.2 (80%)	3.17	99.06	0.15	0.15
	4.0 (100%)	3.97	99.25		
	4.8 (120%)	4.77	99.37		

Table-4: Intra - day and Inter - day precision of DEC and CPM Standard solutions by RP-HPLC

Drug	Concentration* ($\mu\text{g/ml}$)	Peak Area	% RSD	Peak Area	% RSD
		Mean		Mean	
DEC	10	117720.60	0.004	117703.00	0.008
	30	349159.30	0.001	349076.30	0.022
	50	558595.00	0.001	558424.00	0.026
CPM	10	11909.00	0.022	11895.00	0.148
	30	34198.30	0.007	34157.30	0.103
	50	56045.00	0.006	56001.60	0.024

* Mean of each 3 determinations

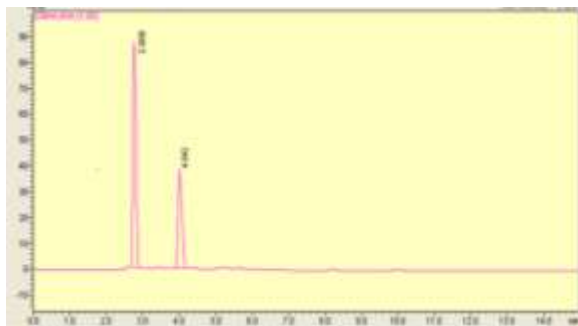


Fig.7: Recovery studies of DEC and CPM by RP-HPLC method (80%).

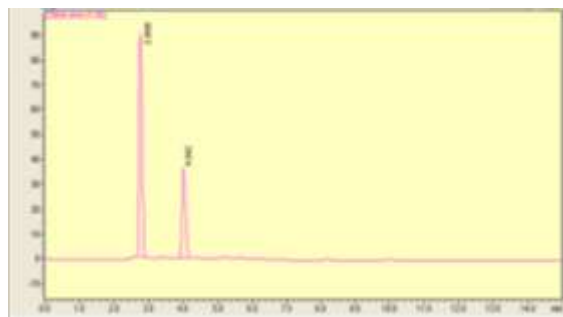


Fig.8: Recovery studies of DEC and CPM by RP-HPLC method (100%).

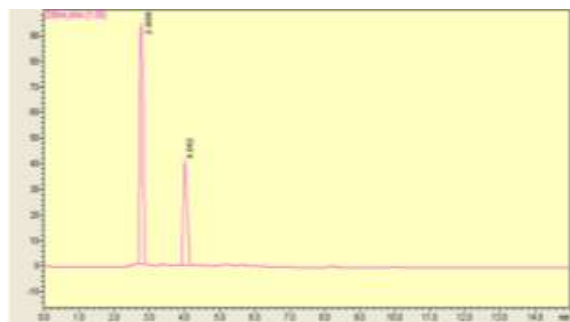


Fig.9: Recovery studies of DEC and CPM by RP-HPLC method (120%).

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 were reported in terms of % relative standard deviation

(%RSD). All results fall within acceptance limits ($\text{RSD} < 2$), as shown in Table-4.

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

The LOD and LOQ for DEC and CPM were separately determined by based on calculating the signal-to-noise ratio. Detection limit= $3.3 \sigma/s$; quantification limit= $10 \sigma/s$; where σ is the standard deviation of y-intercept of regression line and 's' is the slope of the calibration curve. Results were shown in the Table-5.

Table 5: LOD & LOQ for DEC and CPM by RP-HPLC method

Parameters	DEC	CPM
Limit of detection	2.03	0.65
Limit of quantitation	6.15	1.98

Robustness

The robustness study was done by making small changes in the optimized method parameters like $\pm 1\%$ change in pH and $\pm 1\%$ change in flow rate. There was no significant impact on the retention time and tailing factor. Results were shown in Table-6 & Table-7.

Assay

Three batches of compound tablets were analyzed using the developed method. Satisfactory results were obtained that the mean percentage found for DEC and CPM were in good agreement with the label claimed. The mean percentage found and the %RSD values (Table-8) indicated that the proposed method could be adopted for the determination of DEC and CPM in compound tablets.

CONCLUSION

The RP-HPLC method has been developed and validated for the simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in tablet dosage form. The results show that the method is accurate, precise, linear, robust, simple and rapid. Acceptable regression values, %RSD and standard deviations which

make it is versatile and valuable for simultaneous estimation of two drugs in bulk and pharmaceutical dosage forms. The run time is relatively short. The results of this developed RP-HPLC method could be conveniently adopted for quality control analysis of Diethylcarbamazine citrate and Chlorpheniramine maleate simultaneously from tablet dosage form.

ACKNOWLEDGEMENT

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 heartfelt 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.

Declaration of interest: The authors report no conflicts of interest.

Table 6: Robustness Data of the RP-HPLC Method at Different Flow Rate for DEC and CPM

Flow Rate (ml)	Drug	Concentration ($\mu\text{g/ml}$)	Mean (Peak area)	Rt	% RSD	Over all %RSD
1.0	DEC	20	239182.30	2.808	0.099	(DEC)
	CPM	20	23418.14	4.042	0.013	0.106
0.9	DEC	20	239239.30	2.811	0.112	(CPM)
	CPM	20	23314.24	4.053	0.016	0.016
1.1	DEC	20	239288.00	2.798	0.108	
	CPM	20	23412.37	4.037	0.020	

Table 7: Robustness Data of the RP-HPLC Method at Different pH for DEC and CPM

pH	Drug	Concentration ($\mu\text{g/ml}$)	Mean (Peak area)	Rt	% RSD	Over all %RSD
3.0	DEC	20	239172.23	2.808	0.038	(DEC)
	CPM	20	23415.67	4.042	0.013	0.059
2.5	DEC	20	239282.32	2.788	0.136	(CPM)
	CPM	20	22637.00	4.016	0.105	0.057
3.5	DEC	20	239186.24	2.918	0.004	
	CPM	20	23361.33	4.121	0.054	

Table 8: Assay of combined Tablet dosage form

Tablet (Eofil Forte)	Label Claim (mg)	Amount Estimated* (mg)	% Amount Estimated	Acceptance Range
DEC	250	248.3	99.32	98%-102%
CPM	4	3.96	99.00	

* Mean of 3 determinations

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