Original article

Development of LC Method for Estimation of Diethyl Carbamazine Citrate and Chlorpheniramine Maleate in Combined Dosage Form

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Liquid chromatographic method was developed for the quantitative determination of diethyl carbamazine citrate and chlorpheniramine maleate in combined dosage form. A Sunfire C_{18}, 5 µm (250 mm× 4.6 mm i.d.) particle size column with mobile phase containing water: methanol: 10% triethyl amine (10:90:0.1, v/v; pH 5.5) was used. The flow rate was 1.0 mL/min and effluents were monitored at 225 nm. The retention times of diethyl carbamazine citrate and chlorpheniramine maleate were 3.4 min and 5.2 min, respectively. The linearity for diethyl carbamazine citrate and chlorpheniramine maleate were in the range of 1-100 µg/mL and 0.1-20 µg/mL, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity and robustness. The method was successfully applied to the estimation of diethyl carbamazine citrate and chlorpheniramine maleate in combined dosage form.

Key words: Diethyl carbamazine citrate, Chlorpheniramine maleate, Liquid chromatography, Validation.

Dietilkarbamazin Sitrat ve Klorfeniramin Maleat'ın Kombine Dozaj Formlarında Miktar Tayinleri için LC Yöntemi Geliştirilmesi

Dietilkarbamazin sitrat ve klorfeniramin maleat'ın kombine dozaj formlarında miktar tayini için bir sıvı kromatografi yöntemi geliştirilmiştir. Yöntemde; 5 µM partikül çaplı bir Sunfire C_{18} kolonu (250 mm x 4.6 mm, iç çap) ve su:metanol: % 10 trietilamin (10:90:0.1, h/h, pH:5.5) içeren hareketli faz kullanılmıştır. Akış hızı 1 mL/dak'dır ve elüatlar 225 nm'de gözlenmiştir. Alıkonma zamanları dietilkarbamazin sitrat ve klorfeniramin maleat için sırasıyla 3.4 ve 5.2 dakikadır. Doğrusal çalışma aralığı sırasıyla dietilkarbamazin sitrat ve klorfeniramin maleat için 1-100 µg/mL ve 0.1-20 µg/mL'dir. Geliştirilen yöntem doğrusallık, doğruluk, kesinlik, seçicilik ve sağlamlılık açısından valide edilmiştir. Yöntem, kombine dozaj formları içindeki dietil karbamazin sitrat ve klorfeniramin maleat'ın tayini için başarıyla uygulanmıştır.

Anahtar kelimeler: Dietil karbamazin sitrat, Klorfeniramin maleat, Sıvı kromatografi, Validasyon.

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INTRODUCTION

Diethyl Carbamazine citrate (DEC) is chemically, N,N-diethyl-4-methylpiperazine-1-carboxamide (Figure 1). It is an inhibitor of arachidonic acid metabolism in filarial microfilaria. This makes the microfilaria more susceptible to immune attack. Diethylcarbamazine is an anthelmintic that is used in the treatment of lymphatic filariasis. It is active against the microfilariae and adult worms of *W. bancrofti, B. malayi* and *B. timori*.

Chlorpheniramine maleate (CPM) is chemically 3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine (Fig. 2). It is a histamine-1 receptor blocker. It is an antihistaminic drug used to relieve symptoms of allergy, hay fever, and the common cold. These symptoms include rash, watery eyes, itchy eyes/nose/throat/skin, cough, runny nose, and sneezing. Infections caused by worms are allergenic and hypersensitive reactions of the DEC to some patients can be cured by antihistaminic like CPM. So, combined dosage form of DEC and CPM is introduced in the market for the treatment of filariasis (1-3).
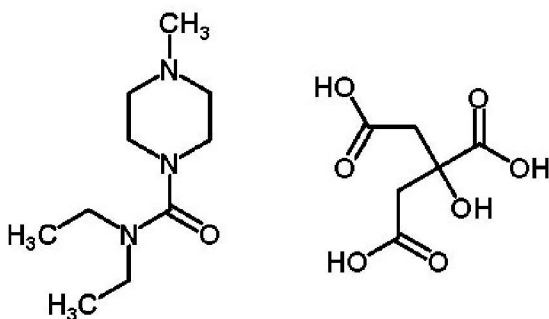

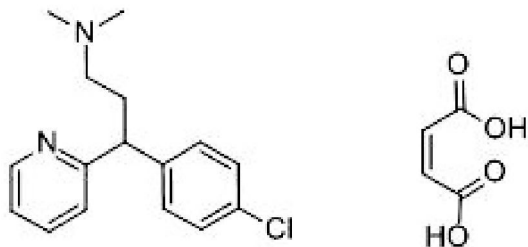**Figure 1.** Structure of diethyl carbamazine citrate

Figure 2. Structure of Chlorpheniramine maleate

Both of the drugs are official also in British Pharmacopoeia and United state Pharmacopoeia (4-5). A literature survey regarding quantitative analysis of these drugs revealed that attempts have been made to develop analytical methods for the determination of DEC alone and in combination with other drugs by liquid chromatographic (LC) (6-9), gas chromatography (10), paper chromatography (11) and spectrophotometric methods (12). Literature survey revealed that liquid chromatographic (LC) (13-18), HPTLC (19) and spectrophotometric methods (20) have been reported for the estimation chlorpheniramine maleate.

There is no method reported for the estimation of DEC and CPM in combined dosage form. Present study involves development and validation of liquid chromatographic method for the estimation of DEC and CPM in combined dosage form.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consist of Waters series 2998 (Shelton, USA) equipped with a PDA detector, series 515 quaternary isocratic pump and manual injector rheodyne valve with 20 µL fixed loop. The analytes were monitored at 225 nm. Chromatographic analysis was performed on Sunfire C_{18} column having 250 mm× 4.6 mm i.d. and 5 µm particle size. All drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

Reagents and materials

Analytically pure DEC and CPM were obtained as gift samples from Balaji Laboratory limited, Mumbai, India and Pramukhswami Pharma Limited, Ahmedabad, India, respectively. HPLC grade methanol and water were obtained from SRL Ltd., Mumbai, India. Tablet formulation (UNICARBAZAN FORTE, Unichem Laboratories, Mumbai, India) containing labeled amount of 250 mg of Diethyl carmbamazine citrate and 5 mg of Chlorpheniramine maleate was used for the study.

Chromatographic conditions

The Sunfire C_{18} column equilibrated with mobile phase water: methanol: 10% triethyl amine (10:90:0.1, v/v; pH 5.5) was used. The flow rate was maintained at 1 mL/min, eluent were monitored with UV detector at 225 nm, and the injection volume was 20 µL. Total run time was kept 10 min.

Preparation of standard stock solutions

DEC and CPM were weighed and transferred to two separate 10 mL volumetric flasks and dissolved in few mL of mobile phase. Volumes were made up to the mark with mobile phase to yield a solution containing 1000 µg/mL of DEC and CPM, respectively. Aliquot from the stock solutions of DEC and CPM were appropriately diluted with mobile phase to obtain working standard of 100 µg/mL of DEC and CPM, respectively.

Method validation

The method was validated for accuracy, precision, linearity, detection limit, quantitation limit and robustness as per ICH guideline (21).

Linearity

Appropriate aliquots of DEC and CPM working standard solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 1, 5, 20, 50, 100 µg/mL of DEC and 0.1, 0.5, 2, 10, 20 µg/mL of CPM, respectively. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both the drugs.

Precision

The repeatability studies were carried out by estimating response of DEC (20 µg/mL) and CPM (2 µg/mL) six times and results are reported in terms of relative standard deviation. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of DEC (1, 10, 50 µg/mL) and CPM (0.1, 2, 10 µg/mL), and

the results are reported in terms of relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of DEC and CPM by method of standard additions. Known amount of DEC (0, 12.5, 25, 37.5 µg/mL) and CPM (0, 0.25, 0.5, 0.75 µg/mL) were added to a pre quantified sample solution, and the amount of DEC and CPM were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Detection limit and quantitation limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

LOD = 3.3 $\times \sigma$ /S; LOQ = 10 $\times \sigma$ /S; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of organic phase.

Solution stability

Stability of sample solutions were studied at 25 ± 2°C for 24 h.

System suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of DEC and CPM to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

Analysis of marketed formulation

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 250 mg DEC and 5 mg of CPM was taken in 100 mL volumetric flask. Methanol (50 mL) was added to the above flask and the flask was sonicated for 15 minutes. The solution was filtered using whatman filter paper No.41 and volume was made up to the mark with the mobile phase.

Appropriate volume of the aliquot was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 25 μg/mL of DEC and 0.5 μg/mL of CPM. The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak areas were recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

Optimization of mobile phase

The objective of the method development was to resolve chromatographic peaks for active drug ingredients with less asymmetric factor. Various mixtures containing methanol and water were tried as mobile phases in the initial stage of method development. Mixture of methanol: water (80:20, v/v) and methanol: water (75:25 v/v) was tried as mobile phase but peaks were broad and tailed. The mixture of methanol: water (90:10, v/v) gave sharp resolved peaks but tailing was observed for both DEC and CPM so, triethyl amine was used to adjust the pH. The pH was adjusted to 3, 4 and 5.5 using tri ethyl amine but satisfactory peak shape with less asymmetry factor was obtained with pH 5.5.

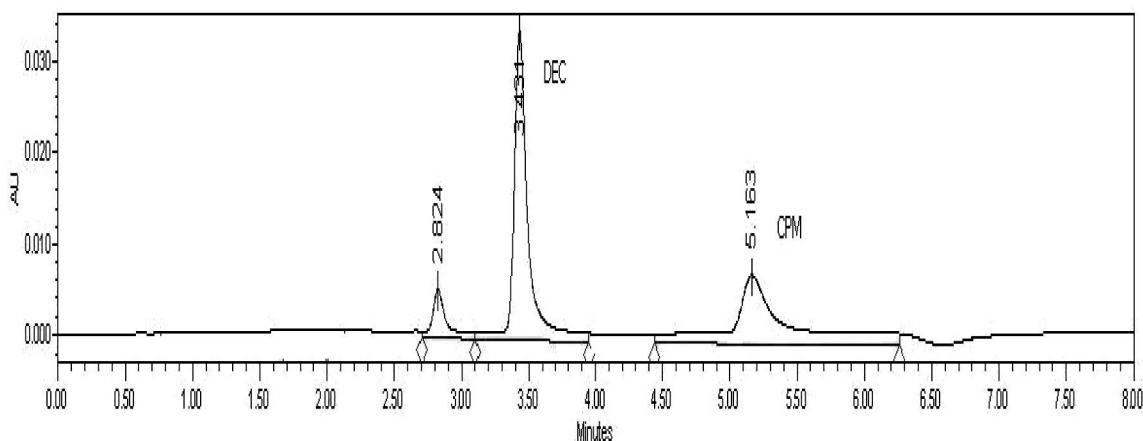The mobile phase water: methanol (10:90 v/v) adjusted to pH 5.5 with 0.01% tri ethyl amine

Figure3. Liquid chromatogram of DEC (20 μg/mL) and CPM (2 μg/mL)

was found to be satisfactory which gave two symmetric and well-resolved peaks for DEC and CPM. The retention time for DEC and CPM were 3.4 min and 5.2 min, respectively (Fig. 3). The resolution between DEC and CPM was found to be 5.5, which indicates good separation of both the compounds. The asymmetric factors for DEC and CPM were 0.9 and 0.8, respectively. The mobile phase flow rate was maintained at 1 mL/min. Overlaid UV spectra of both the drugs showed that DEC and CPM absorbed appreciably at 225 nm, so detection was carried out at 225 nm.

Method validation

The calibration curve for DEC was found to be linear in the range of 1 - 100 μg/mL with a correlation coefficient of 0.9958. The calibration curve for CPM was found to be linear in the range of 0.1-20 μg/mL with a correlation coefficient of 0.9987. The regression

analysis of calibration curves are reported in Table 1. Instrument precision was determined by performing injection repeatability test and the RSD values for DEC and CPM were found to be 0.96 % and 0.58 %, respectively.

The accuracy of the method was determined by calculating recoveries of DEC and CPM by

Table 1. Regression analysis of calibration curve.

Parameter	DEC	CPM
Linearity (μg/mL)	1-100	0.1-20
Correlation coefficient (r)	0.9958	0.9987
Slope of Regression	11726.6	23356.6
Standard deviation of slope	122.08	167.59
Intercept of Regression	-22352.4	3441.3
Standard deviation of intercept	892.53	117.28

The accuracy of the method was determined by calculating recoveries of DEC and CPM by method of standard addition. The recoveries were found to be 98.81 %–101.44 % and 98.17

%-101.68 % for DEC and CPM, respectively (Table 2). The high values indicate that the method is accurate.

Table 2. Accuracy study of DEC and CPM by the proposed HPLC method.

Amount of sample taken (μg/mL)		Amount of standard drug added (μg/mL)		Amount of drug recovered (μg/mL)		% recovery ± % RSD (n = 3)	
DEC	CPM	DEC	CPM	DEC	CPM	DEC	CPM
25	0.5	0.0	0.0	24.91	0.491	99.90±0.21	98.17±0.45
25	0.5	12.5	0.25	38.04	0.762	101.44±1.39	101.68±1.45
25	0.5	25	0.5	49.40	1.004	98.81±1.67	100.06±1.74
25	0.5	37.5	0.75	62.65	1.249	100.40±0.97	99.99±1.51

method of standard addition. The recoveries were found to be 98.81 %–101.44 % and 98.17 %-101.68 % for DEC and CPM, respectively (Table 2). The high values indicate that the method is accurate.

The intra-day and inter-day precision studies were carried out and the results are reported in Table 3. The low RSD values indicate that the

method is precise. The detection limits for DEC and CPM were found to be 0.26 μg/mL and 0.02 μg/mL respectively, while quantitation limits were found to be 1 μg/mL and 0.1 μg/mL, respectively. The above data shows that a nano gram quantity of both the drugs can be accurately and precisely determined. Robustness study was performed by deliberately

Table 3. Summary of validation parameters.

Parameters	DEC	CPM
Retention time (min)	3.4	5.1
Detection limit (μg/mL)	0.26	0.02
Quantitation limit (μg/mL)	1	0.1
Accuracy (%)	98.81-101.44%	98.17-101.68%
Precision (RSD[a] %)		
Intra-day precision (n=3)	0.68-1.28%	0.74-1.28%
Inter-day precision (n=3)	1.16-1.78%	1.43-1.81%
Instrument precision (RSD[a] %) (n=6)	0.96%	0.58%

[a]RSD is relative standard deviation and 'n' is number of determinations

Table 4. System suitability parameters for the proposed method.

Parameter	DEC	CPM
Retention time (min)	3.4	5.2
Relative standard deviation of retention time (%)	0.42	0.67
Theoretical plates	5012	6323
Assymetric factor	0.9	0.8
Resolution	-	5.5
Capacity factor	0.26	0.92

changing the experimental conditions like flow rate from 1 mL/min to 0.8 mL/min and 1.2 mL/min. The composition of mobile phase was changed varying the proportion of methanol by 5 %. In both the conditions the recoveries of both the drugs were determined and the RSD was found to be less than 2%. The validation parameters are summarized in table 3. System suitability test was carried out and the results are summarized in Table 4.

Stability of standard and sample solution of DEC and CPM were evaluated at room temperature for 24 hr. Both the drugs were found to be stable with a recovery of more than 98%.

Analysis of marketed formulations

The proposed method was successfully applied to the determination of DEC and CPM in their combined dosage form. The % recovery was found to be 100.04 ± 1.34 and 100.33 ± 1.57 respectively, for DEC and CPM (Table 5)

Which were comparable with the corresponding labeled amount.

CONCLUSION

Proposed study describes LC method for the estimation of DEC and CPM combination in mixture. The method was validated and found to be simple, sensitive, accurate and precise. Study proved that method was repeatable and selective for the analysis of DEC and CPM in combination with out any interference from the excipients. The method was successfully used for determination of drugs in their pharmaceutical formulations.

ACKNOWLEDGEMENTS

Table 5. Analysis of marketed formulation.

Formulation	Labeled Amount (mg)		Amount found (mg)		% Recovery [b]	
	DEC	CPM	DEC	CPM	DEC	CPM
A	250	5	250.1	5.01	100.04 ±1.34	100.33 ± 1.57

[b] mean value ± standard deviation of three determinations; tablet formulation A is UNICARBAZAN FORTE (Unichem, India) containing labeled amount of 250 mg of diethyl carbamazine citrate and 5 mg of chlorpheniramine maleate.

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