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Development and Validation of RP–HPLC Method For Simultaneous Estimation of Cetrizine Hydrochloride and Phenylephrine Hydrochloride in Bulk and Tablet Dosage Form

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ABSTRACT

Cetrizine hydrochloride and Phenylephrine hydrochloride are used in combination in the treatment of allergy. The aim of this project work is to develop selective, accurate, specific and economic RP–HPLC method for simultaneous estimation of Cetrizine hydrochloride and Phenylephrine hydrochloride in Bulk and Tablet dosage form. The reverse phase C18 (250 mm × 4.6 mm i.d) column with 5 µm particle size was used. Acetonitrile: Water was taken as mobile phase was with a flow rate of 1 mL/min and detection was carried out at 222 nm. The retention time of Cetrizine hydrochloride and Phenylephrine hydrochloride was 1.956 min and 4.561 min respectively. The calibration curves were linear (>0.998) in the range of 5-25 µg/ml for Cetrizine hydrochloride and Phenylephrine hydrochloride. The Limit of Detection for Cetrizine hydrochloride and Phenylephrine hydrochloride was found to be 0.26 µg/mL and 0.51µg/mL. The Limit of Quantification was found to be 0.81µg/mL and 1.54 µg/mL respectively for Cetrizine hydrochloride and Phenylephrine hydrochloride. The developed method was simple, selective and precise and can be used for routine analysis of Cetrizine hydrochloride and Phenylephrine hydrochloride in tablet dosage form.

Keywords: Cetrizine hydrochloride (CET), Phenylephrine hydrochloride (PHE), RP-HPLC, Validation, Tablet.

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INTRODUCTION

Cetrizine Hydrochloride[1] (CET) is [2-[2-[4-[(4-chlorophenyl)(phenyl)methyl] piperazin-1-yl] ethoxy] acetic acid is a non-sedative second generation anti-histamine drug used in the treatment of seasonal allergic rhinitis, perennial allergic rhinitis, chronic urticaria also used as adjuvant in seasonal asthma. It is freely soluble in distilled water, soluble in alcohol, practically insoluble in acetone and in methylene chloride.

Phenylephrine Hydrochloride[2] (PHE) is [(R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride] (C9H13NO2, HCl) is potent H1 receptor antagonist useful as nasal and sinus decongestant. It is freely soluble in water and soluble in alcohol.

Many methods have been described in the literature[3-22] for the determination of Cetrizine hydrochloride and Phenylephrine hydrochloride individually with other drugs and in combination. However there is no RP-HPLC method reported for the simultaneous estimation of these drugs in combined dosage form. The present study involves development and validation of RP–HPLC method for estimation of CET and PHE in combined dosage form.

MATERIALS AND METHOD:

Chemicals and Reagents:

CET was obtained as a gift sample from Shine Pharmaceuticals Limited. PHE was obtained as a gift sample from Ethicare pharmaceuticals. HPLC grade Acetonitrile was obtained from Finar Chemicals Ltd. Tablet formulation (Allercet-DC, Micro Labs Limited) used for the study was purchased from pharmacy.

Instrumentation:

The chromatographic system consisted of a Shimadzu auto sampler with variable injection valve and UV-Visible detector. The chromatograms were recorded using LC solution software in a computer system for data collection and processing.

The Optimized Chromatographic Conditions:

Separation was performed by using reverse phase C_{18} (250 mm×4.6mm i.d.), 5 µm particle size analytical column. The mobile phase consisted of Acetonitrile: Water in the ratio of 45:55. Isocratic technique was used. It was prepared daily and degassed by sonicating it under vacuum through a 0.45 µm nylon filter. The experimental work was carried out by keeping flow rate of the mobile phase through the analytical column 1.0 mL/min. The detection wavelength was set at 222 nm.

Preparation of Standard Stock Solutions:

A Standard stock solution (1000 µg/mL) of CET and PHE were prepared by dissolving and diluting 100 mg of CET and PHE in different 100 mL volumetric flasks by using mobile phase. An aliquot of stock solution 1.0 mL was transferred in to 10 mL volumetric flask and adjusted up to mark with mobile phase having (100 µg/mL) concentration.

Preparation of Working Standard Solutions:

Accurately measured Standard stock solutions of CET and PHE (0.5, 1.0, 1.5, 2.0, 2.5 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase.

Preparation of Sample Solution (Marketed Preparation):

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 10 mg of CET and 10 mg of PHE was transferred to a 100 mL volumetric flask. The content was mixed with mobile phase (50 mL), sonicated for 20 min to dissolve the drug as completely as possible. Then it was filtered through a Whatman filter paper no. 41.The volume was adjusted up to mark with mobile phase. From this solution, 1.5 mL was transferred in to a 10 mL volumetric flask and the volume was adjusted up to mark with mobile phase.

Method Development:

Different combinations of mobile phase were tried in different concentration to measure CET and PHE in combined form like Methanol:Water(50:50), Acetonitrile:Water(70:30), Acetonitrile: Water(60:40), Acetonitrile: Water(50:50). Finally Acetonitrile: Water (45:55) was selected for measurement of CET and PHE in combined dosage form.

System Suitability:

A system suitability test is an integral part of the method development to verify that the system is adequate for the analysis of CET and PHE to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of standard working solutions and one injection of a sample solution of 10 µg/mL were injected. Area, retention time (RT), tailing factor and theoretical plates were determined. System suitability test was carried out, and the results are summarized in Table 1.

Method Validation:

The RP-HPLC assay validation was done as per ICH Q2A and Q2B guidelines. To check validation of method different validation parameters were performed like accuracy, precision, robustness, linearity, detection limit and quantitation limit.

Linearity:

Calibration curves for CET and PHE were constructed by plotting peak areas vs. concentration of drug and the regression equations were calculated. The calibration curves were plotted over a

wide concentration range in which a linear response was observed. Accurately measured std. working solutions of CET and PHE (0.5, 1.0, 1.5, 2.0, 2.5 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. The injection volume was 20µL.

Precision:

The repeatability studies were carried out by estimating response of CET (15 µg/mL) and PHE (15 µg/mL) six times to determine the relative standard deviation. The intraday and interday 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 CET and PHE (10, 15 and 20 µg/mL) and relative standard deviation was calculated.

Accuracy:

The accuracy of the method was determined by calculating % recovery of CET and PHE by the standard addition method. Known amounts of standard solutions of CET (6, 7.5 and 9 µg/mL) and PHE (6, 7.5 and 9 µg/mL) were added to prequantified separate sample solutions of CET (7.5 µg/mL) and PHE (7.5 µg/mL). The amounts of CET and PHE were estimated by applying obtained values to the regression equation of the calibration curve.

Detection Limit and Quantitation Limit:

LOD i.e. Limit of Detection was estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as

$$\mathbf{LOD = 3.3 \times (SD/Slope)} \quad \text{(1)}$$

LOQ i.e. Limit of quantification was estimated from the set of 5 calibration curves used to determine method linearity. The LOQ may be calculated as

$$\mathbf{LOQ = 10 \times (SD/Slope)} \quad \text{(2)}$$

Where, SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Robustness:

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and wavelength. The Robustness change was measuring by deliberate change in flow rate (± 0.2 mL/min) and detection wavelength (± 2 nm). %RSD for area was calculated which should be less than 2%.

Analysis of Marketed Formulation:

The response of the sample solution was measured at 222 nm under the chromatographic condition mentioned earlier for the quantitation of CET and PHE. The amounts of CET and PHE

present in sample solution were determined by applying values of the peak area to the regression equations of the calibration graph.

RESULTS AND DISCUSSION:

The retention time for CET and PHE was found to be 1.956 and 4.561(Figure 1). The calibration curve of both CET and PHE were found to be linear in the range of 5–25 µg/mL with a correlation co-efficient of 0.9992 and 0.9991, respectively. The regression analysis of calibration curves is reported in Table 2.

Table 1: System suitability parameter for the proposed method

Parameters	CET ± RSD (n=6)	PHE ± RSD (n=6)
Retention time (min)	1.956 ± 0.31	4.561 ± 0.53
Tailing Factor	1.11 ± 0.33	1.05 ± 0.64
Theoretical plates	2775 ± 0.67	2714 ± 0.59
Resolution	7.23 ± 1.20	

Table 2: Linearity Data

Parameter	CET	PHE
Range	5-25 µg/mL	5-25 µg/mL
Correlation co-efficient	0.9992	0.9991
Slope of regression	20314	16595
Intercept of regression	1642	2559

Instrument precision was determined by performing injection repeatability test and the % RSD values for CET and PHE were found to be 0.95% and 0.39%, respectively. The intraday and interday precision studies were carried out. The % RSD values (<2%) indicate that the method is precise.

The accuracy of the method was determined by calculating recoveries of CET and PHE from regression equation. The recoveries were found to be 98.75-101.33% and 99.08–100.75% for CET and PHE, respectively (Table 3).

Table 3: Precision Data

Precision	CET(% RSD)	PHE(% RSD)
Repeatability	0.95	0.39
Intraday Precision	0.38 - 0.76	0.41 – 0.95
Interday Precision	0.58 – 0.73	0.39 – 0.71

% RSD = % Relative Standard Deviation

The detection limits (LOD) for CET and PHE were found to be 0.26 µg/mL and 0.51 µg/mL, respectively, while quantitation limits (LOQ) were found to be 0.81 µg/mL and 1.54 µg/mL, respectively for CET and PHE (Table 2).

%RSD for Robustness parameters (flow rate change, wavelength change) was found to be less

than 2%.

The proposed method was successfully applied to the determination of CET and PHE in their tablet dosage form using the same chromatographic parameters (Figure 1). The assay was found to be 98.5% and 101% for Cetrizine hydrochloride and Phenylephrine hydrochloride respectively in marketed dosage form (Allercet-DC) which was described in table-4.

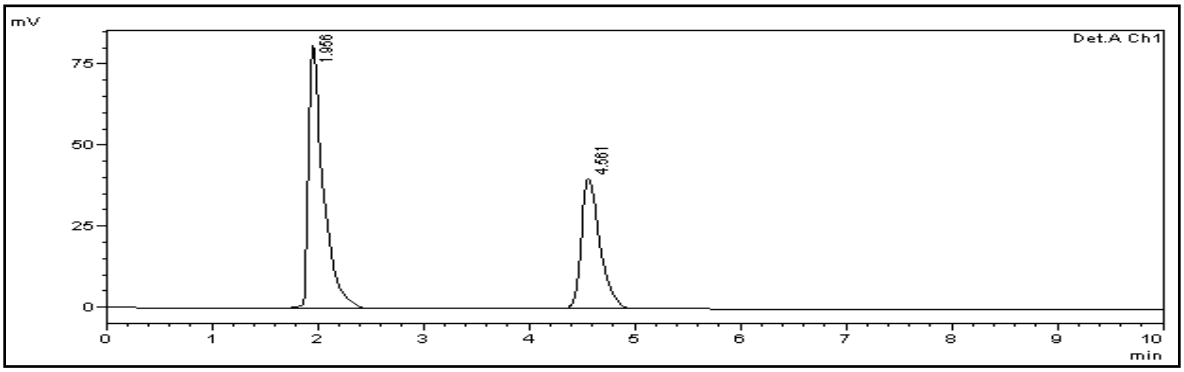

Figure I: Chromatogram of Product (CET and PHE)

Table 4: Summary of validation parameters

Parameters	CET	PHE
Retention time (min)	1.956	4.561
Detection limit (µg/mL)	0.26	0.51
Quantification Limit (µg/mL)	0.81	1.54
Accuracy (%)	98.75–101.323	99.08-100.75

Table 5: Analysis of marketed formulation

Formulation	Label Claim (mg/tablet)		% Assay	
	CET	PHE	CET	PHE
1	10	10	98.5 ± 1.31	101 ± 2.04

CONCLUSION:

RP-HPLC method was developed and validated for simultaneous estimation of Cetrizine hydrochloride and Phenylephrine hydrochloride in bulk and tablet dosage form. Acetonitrile: Water was selected for estimation of CET and PHE. Linearity of the developed method was near to 1 and range was found to be 5-25 µg/mL for CET and PHE both. The % RSD was found to be less than 2% for repeatability and intraday precision. The % recoveries were found to be 98.75–101.33% and 99.08-100.75% for CET and PHE, respectively. These results indicate that the developed method is accurate, precise and simple. It can be used in the routine quality control of dosage form.

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