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DEVELOPMENT AND VALIDATION OF REVERSE PHASE – HIGH PERFORMACE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF CONTENT UNIFORMITY OF CETRIZINE HYDROCHLORIDE AND PHENYLEPHRINE HYDROCHLORIDE IN COMBINED DOSAGE FORM

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ABSTRACT

Combination of Phenylephrine hydrochloride (PHEN) (10mg) and Cetrizine hydrochloride (CET) (5mg) have additive role in cough and anti allergic preparation, since both acts by attenuating the sign and symptoms of common cold and allergy. Here PHEN and CET have low dose in their combined dosage form i.e. 10 mg and less than 10 mg. According to Indian Pharmacopoeia standards, Content Uniformity can be carried out for such formulations. It was carried out by using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method. The aim is to develop simple validated analytical method for simultaneous estimation of content uniformity of CET and PHEN. The RP-HPLC method was carried out by using mobile phase Methanol: Acetonitrile (94: 6, %v/v) with 1.0 min/ml flow rate and detection wavelength 220.0 nm. The regression analysis data for the calibration plot showed good linear relationship in the concentration range of 100-300 µg/ml (R2 = 0.999) for CET and 200-600 µg/ml (R2 = 0.999) for PHEN. The LODs were found to be 5.848 and 1.053 µg/ml for CET and PHEN respectively. The % relative standard deviation <2.0 was obtained for the proposed method. Statistical analysis proves that the method is reproducible and selective for the simultaneous determination of CET and PHEN.

Keywords: Cetrizine hydrochloride, Phenylephrine hydrochloride, Content Uniformity, RP-HPLC, Analytical method

INTRODUCTION:

Cetrizine is a potent second-generation histamine H_1 antagonist. It is effective in the treatment of allergic rhinitis, chronic urticaria and pollen-induced asthma. Unlike many traditional antihistamines, it does not cause drowsiness or anticholinergic side effects. Cetrizine, the active metabolite of the piperazine H_1-receptor antagonist hydroxyzine, is used to treat chronic idiopathic urticaria, perennial allergic rhinitis, seasonal allergic rhinitis, allergic asthma, physical urticaria, and atopic dermatitis **[1-2]**.

Phenylephrine is a selective α_1 adrenergic receptor agonist used primarily as a decongestant, as an agent to dilate the pupil and to increase blood pressure. Phenylephrine is a powerful vasoconstrictor. Phenylephrine is marketed as a substitute for the decongestant pseudoephedrine though clinical studies differ regarding phenylephrine's effectiveness in this role. Phenylephrine is a sympathomimetic amine that acts predominantly on α-adrenergic receptors **[3-4]**.

The chemical structures of Cetrizine and Phenylephrine are shown in **Figure 1 and 2** respectively.

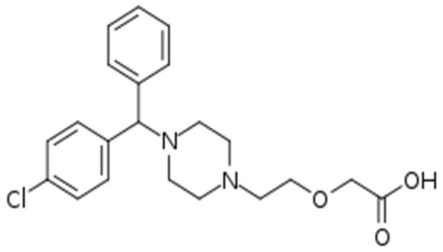

Figure 1: Chemical structure of Cetrizine

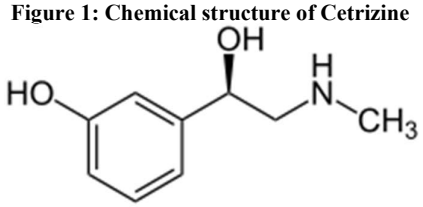

Figure 2: Chemical structure of Phenylephrine

This combination is launched by Unichem Laboratory Ltd. in market under the brand name **Zyncet D.** Recently it is manufactured by Torrent Pharmaceuticals Ltd. This formulation is widely used in the treatment of Allergic rhinitis.

Cetrizine hydrochloride is official in Indian Pharmacopoeia and Phenylephrine hydrochloride is official in Indian Pharmacopoeia, British Pharmacopoeia and United State Pharmacopoeia. Hence official methods for their individual estimation are available.

Review of literature revealed that, there are some methods available for estimation of Cetrizine HCl and Phenylephrine HCl in individual and in combined (with other drugs) dosage forms. No reports were in literature on the determination of PHEN and CET simultaneously by RP-HPLC method **[5-15]**.

As well as this formulation contain low dose of the drug (CET – 5mg & PHEN – 10mg) so that according to Indian Pharmacopeia content uniformity test can be performed. According to Indian Pharmacopoeia, if any single dosage form contains 10 mg or less content of drug then content uniformity test should be performed rather than assay. So, it is thought of interest, to develop and validate methods for simultaneous estimation of content uniformity of Cetrizine HCl and Phenylephrine HCl in their combined dosage form. Here, RP-HPLC method was developed for simultaneous estimation of content uniformity of Cetrizine HCl and Phenylephrine HCl in their combined dosage form.

MATERIALS AND METHOD:

Materials:

Cetrizine hydrochloride and Phenylephrine hydrochloride working standard were received as a gift sample from Rhombus pharmaceuticals Pvt. Ltd. All the chemicals and reagents used were analytical grade and purchased from Chemdyes. Zyncet-D tablet formulation-Each tablet contains 10 mg Phenylephrine hydrochloride and 5 mg Cetrizine hydrochloride manufactured by Torrent Pharmaceuticals Ltd. was purchased from local market.

Method:

Instrumentation: RP-HPLC System

Make and Model: Young - Linn Clarity 9100 HPLC System

Degasser: Vacuum degasser YL - 9101

Pump: Quaternary pump YL - 9110

Detector: PDA detector YL - 9160

Column: Hibar® 250-4.6mm, Lichrospher® 100, RP-18 e (5µm), Merck ltd., India; 250 mm L × 4.6 mm Ø in size

Temperature: 20 ± 5 ˚C

Pressure: 1500 - 3000 psi

Ultra-Sonicator: Fast clean, Ultrasonic cleaner

Preparation of Standard stock Solutions

A 25 mg of both standard CET and PHEN were accurately weighed and transferred to a 25 ml volumetric flask and dissolved in 15 ml methanol. The flask was sonicated for 5 min. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml of CET and PHEN.

Selection of Analytical Wavelength

Solutions containing appropriate concentration of CET and PHEN in methanol were scanned using UV spectrophotometer in “Spectrum mode” in

the range of 400 - 200 nm and their spectra were overlaid. From overlaid spectra of both the drugs 220nm was selected as analytical wavelength for detection.

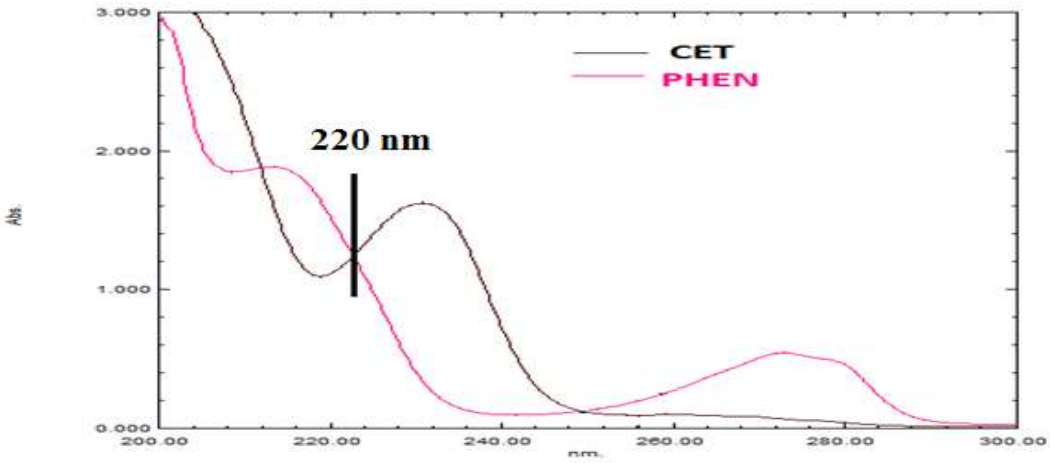

Figure 3: Overlain spectra of CET and PHEN in methanol

Selection of Mobile Phase

The pure drug mixture of CET and PHEN were injected into HPLC system and run in different solvent systems. All the solvents were filtered through 0.45 µm membrane filter paper and sonicated for 20 min. Different mobile phases like, Methanol: Water, Acetonitrile: Water and Methanol: Acetonitrile were tried in order to find best condition for separation of CET and PHEN. It was found that methanol: Acetonitrile gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and different flow rates to optimize mobile phase for best possible separation of both the drugs.

Preparation of mobile phase:

The HPLC grade methanol and acetonitrile were filtered through 0.45 µm membrane filter paper separately. Filtered solutions were ultrasonicated for 20 min. Solutions were allowed to come at room temperature if they were warmed due to sonication.

Chromatographic conditions:

Stationary Phase: Hibar® 250-4.6mm, Lichrospher® 100, RP-18 e (5µm), Merck ltd., India; 250 mm L × 4.6 mm Ø in size

Mobile Phase: Methanol: Acetonitrile 94:6 (% v/v)

Flow Rate: 1.0 ml/min

Detection: 220 nm

Injection Volume: 20 µl

Temperature: 20 ± 5 ˚C

Pressure: 1000 - 2000 psi

Preparation of binary mixture of CET and PHEN

By appropriate dilution using metanol, from the standard stock solutions of CET

and PHEN, binary mixture containing 100+200, 150 + 300, 200 + 400, 250 + 500, 300 +600 µg/ml CET + PHEN were prepared and sonicated for 5 min. Six replicate series of binary mixture were prepared.

Preparation of Calibration Curves

Prepared standard solutions were injected to system with stated chromatographic conditions as described in section above. The run was stopped after separation achieved completely. Peak areas were recorded for all the peaks using YL -Clarity software (Ver.3.0.04.444). Standard calibration curve of AUC against concentration were plotted.

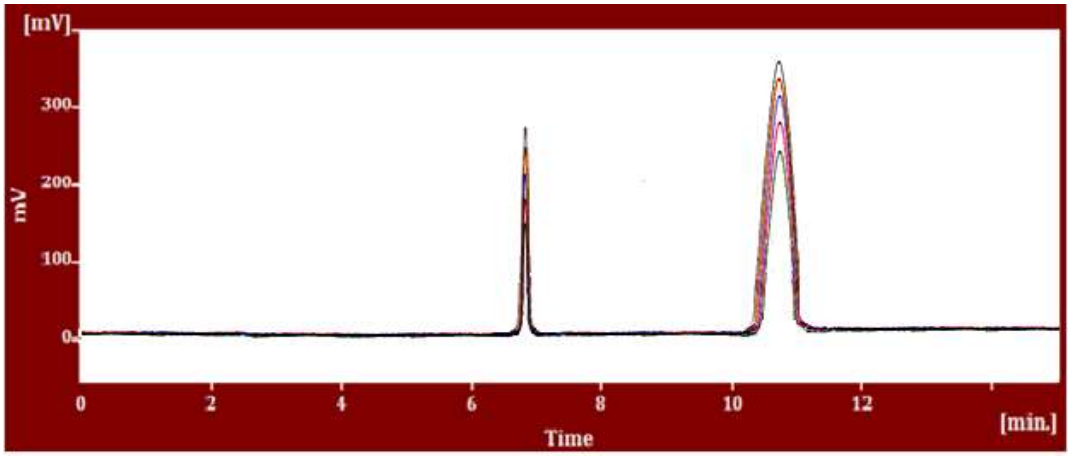

Figure 4: Overlaid chromatogram of standard mixture solutions in 2D view (100-300µg/ml CET) and (300-600µg/ml PHEN)

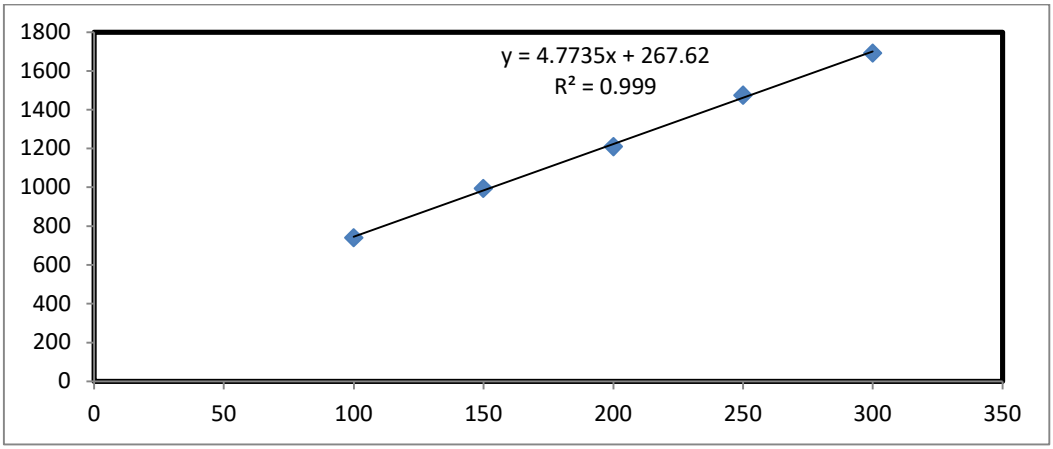

Figure 5: Calibration curve of CET for RP-HPLC Method

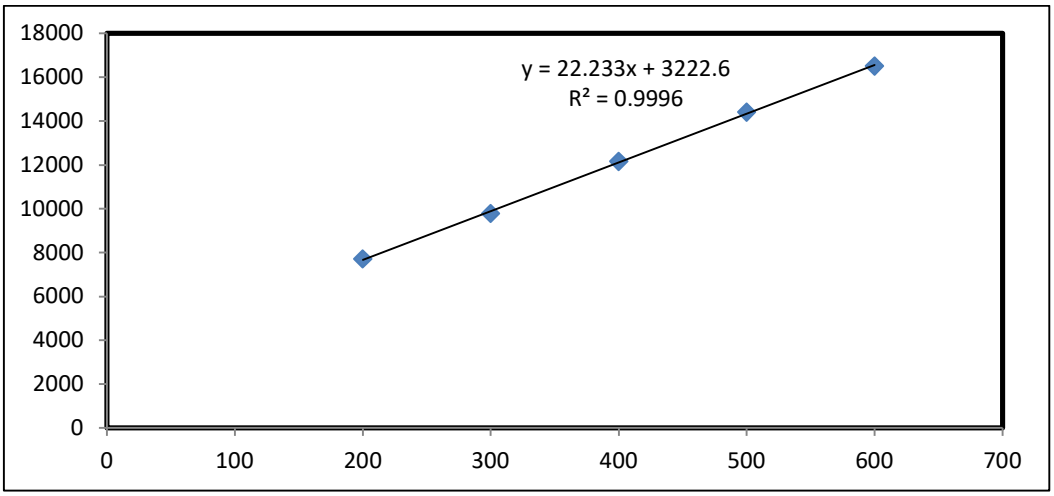

Figure 6: Calibration curve of PHEN by RP-HPLC Method

Analysis of Pharmaceutical Formulation by Content Uniformity

Randomly ten tablets of brand ZYNCET D were selected. Each individual tablet was weighed and finely crushed. Then there were transferred in 10 different 10ml volumetric flask. It was dissolved in methanol by ultra sonication. Then volume was made up to mark with methanol. It was filtered through Whatman filter paper resulting solution was sonicated. It was further diluted to get final concentration 50 µg/ml of CET and 100 µg/ml of PHEN.

Resulting solutions were injected separately into column and 10 different runs were taken. The concentration of each drug was calculated using calibration curve equation.

Validation of method

Validation of developed method was carried out according to ICH guideline for Validation of Analytical Procedures Q2 (R1).

Linearity:

Binary mixture containing 100 + 200, 150 + 300, 200 + 400, 250 + 500, 300 + 600 µg/ml CET + PHEN were prepared from working standard. Prepared solutions were analyzed as per the proposed method. Three replicates analysis were carried out. The mean area with its standard deviation and % relative standard deviation of peak areas were calculated. Mean AUC against concentration were plotted to obtain the calibration curve. Regression equations, co-relation coefficients were computed from calibration curves.

Precision:

Repeatability

Sample solution containing 200 µg/ml of CET and 400 µg/ml of PHEN were analyzed six times in same day. The absorbance of the each solution was measured at selected wavelengths and % RSD was calculated.

Intraday precision: Replication within same day at different time

Sample solutions containing 100, 200, 300 µg/ml CET and 200, 400, 600 µg/ml of PHEN were analyzed three times on the same day and % RSD was calculated.

Interday Precision: Replication in different days:

Sample solutions containing 100, 200, 300 µg/ml CET and 200, 400, 600 µg/ml of PHEN were analyzed on three different days. Each concentration was prepared in triplicate and % RSD was calculated.

Accuracy (Recovery Studies):

In case of the assay of a drug in a formulated product, Accuracy is determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i .e. to spike).

Here, accuracy of the method was carried out at three levels (80%, 100%, and 120%). Known amount of standard CET (80, 100, 120 mg) and standard PHEN (160, 200, 240mg) were added to a pre quantified sample powder which contains 100mg CET and 200mg PHEN, and the amount of CET and PHEN were estimated from regression equation.

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

LOD and LOQ were calculated from the data obtained from the linearity studies. For each of the three replicate determinations, slope and y-intercept of the linearity plot was determined. Average of slope (S) and standard deviation of the y intercept (σ) were computed. From these values, the parameters LOD and LOQ were determined using following equations (On the basis of response and slope of the regression equation):

$$LOD = 3.3\ \sigma \backslash S$$

$$LOQ = 10\ \sigma \backslash S$$

Where; σ = Standard deviation of Response,

S = Slope of calibration curve

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of blank in methanol was prepared and spectrum was taken. The spectrum of blank was compared with those acquired from CET (200µg/ml) and PHEN (400 µg/ml) standards. Peak purities of main peaks were computed using PDA detector YL - 9160 and YL - Clarity software (Ver.3.0.04.444).

Robustness:

Solution containing mixture of 200µg/ml CET and 400µg/ml PHEN were prepared

from their respective standard stock solutions. Prepared solution was analyzed six times as per proposed method with small but deliberate change in chromatographic conditions as listed below:

1) Change in flow rate: 0.8 ml/min; 1.0 ml/min; 1.1 ml/min

The mean peak area with its standard deviation and % relative standard deviation was computed at each level.

System suitability parameters:

Solution containing mixture of 200µg/ml CET and 400µg/ml PHEN was prepared from their respective standard stock solution. Data related to peak like area, height, width, retention time, resolution, tailing (symmetry) factor, column efficiency (theoretical plates) etc. was recorded using YL - Clarity software (Ver.3.0.04.444). All system suitability parameters were computed using these recorded data.

RESULTS AND DISCUSSION:

Analysis of Marketed Formulations

The proposed method was successfully applied to determine CET and PHEN in pharmaceutical formulations **(Table 1)**. The results obtained for CET and PHEN were comparable with the corresponding label claims.

Linearity

The calibration curves were found to be linear over the ranges 10 – 30 µg/ml and 20 – 60 µg/ml for CET and PHEN respectively. Parameters for the regression equation and correlation coefficients are given in **Table 3**. The linearity of the calibration curves was validated by the high value of correlation coefficients of the regression.

Accuracy

The recovery experiments were carried out by the standard addition method. The percent recoveries obtained were 99.787 and 99.887 for CET and PHEN respectively **(Table 2)**.

Precision

The results of the repeatability, intraday and interday precision are shown in Table 3. The low values of relative standard deviation (RSD) of the repeatability, intraday and interday determinations show that the proposed method is precise.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of the drugs are given in **Table 3**. These data shows that this method is sensitive for the determination of CET and PHEN.

Robustness

A result of robustness study is depicted in **Table 3**.

System suitability parameters

Result for all system suitability parameters is depicted in **Table 4**.

Table 1: Results of analysis of marketed formulation by content uniformity of CET and PHEN by RP-HPLC method

Sr. No.	Concentration (µg/ml)		Concentration obtained (µg/ml)		% Labelled Claim		Standard value according to IP 2010
	CET	PHEN	CET	PHEN	CET	PHEN	
1.	5	10	4.82	10.13	96.40	101.3	85-115%
2.	5	10	4.96	9.65	99.20	96.50	
3.	5	10	4.74	9.99	94.80	99.93	
4.	5	10	5.01	9.93	100.2	99.30	
5.	5	10	5.18	9.87	103.6	98.70	
6.	5	10	4.89	10.11	97.80	101.1	
7.	5	10	4.84	10.06	96.8	100.6	
8.	5	10	4.97	9.79	99.40	97.90	
9.	5	10	4.91	10.32	98.20	103.2	
10.	5	10	5.21	9.93	104.2	99.30	
					94.80-104.2%	96.50-103.2%	

Table 2: Summary of Recovery studies by RP-HPLC method

Level	Tablet powder (mg)		Standard added (mg)		Final amount (mg)		% Recovery	
	CET	PHEN	CET	PHEN	CET	PHEN	CET	PHEN
80%	100	200	80	160	180	360	99.58	99.96
100%	100	200	100	200	200	400	99.94	99.91
120%	100	200	120	240	220	440	99.87	99.89

Table 3: Summary of Validation Parameters of RP-HPLC method

Sr. No.	Validation Parameters	Results		Standard Values
		CET	PHEN	
1	Linearity Range	100-300µg/ml	200-600µg/ml	-
2	Straight line equation	y=4.7734x-267.6	y=22.23x+3222	-
3	Correlation Coefficient	0.999	0.999	> 0.998
4	Precision (% RSD) Repeatability Intraday Interday	 0.633 1.204 1.336	 0.103 0.134 0.136	< 2.0 %RSD
5	Mean % Recovery	99.787	99.887	98 – 102
6	Specificity	Specific		
7	LOD (µg/ml)	5.848	1.053	-
8	LOQ (µg/ml)	17.722	3.191	-
9	Robustness	1.005-1.211	0.108-0.120	< 2.0 %RSD

Table 4: System suitability parameter

Parameter	Value obtained	
	CET	PHEN
Retention time (t_R, min)	6.97	10.98
Theoretical plates (N)	2596	25973
Resolution (R_S)	9.687	
Tailing factor (A_S)	1.21	0.63

CONCLUSION:

The proposed RP-HPLC method provide simple, specific, precise, accurate, economic and reproducible quantitative analysis for simultaneous determination of CET and PHEN in combined tablet dosage

form. The method was validated according to ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and robustness. The proposed method can be used for routine analysis and quality control assay of CET and PHEN in combined dosage form. Furthermore, developed method can be useful for Content Uniformity test for blend mixture in process validation.

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