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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PHENYLEPHERINE HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT: A simple, economical, specific, accurate, precise and validated Reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous estimation of Phenylepherine hydrochloride (PPEH) and Cetirizine hydrochloride (CETH) in combine dosage form. The chromatographic separation was achieved on Princeton SPHER C18 column (250 mm x 4.6 mm id, 5 µ particle size) at ambient temperature using mobile phase buffer (0.1 M Ammonium dihydrogen phosphate pH 5.2 ± 0.05) : Acetonitrile (50:50% v/v) at flow rate 1.0 ml/min. Quantification was achieved with UV detector at 225 nm. Retention time of Phenylepherine hydrochloride and Cetirizine hydrochloride were found to be 2.19 ± 0.05 minute and 4.16 ± 0.05 minute respectively. Linearity was studied in the concentration range 5-30 µg/ml and 10-60 µg/ml for Cetirizine hydrochloride and Phenylepherine hydrochloride respectively with a correlation coefficient of 0.9998 and 0.9998 respectively. The proposed method was validated according to ICH guidelines with respect to linearity, accuracy, precision, robustness, LOD, and LOQ. The developed method with good separation, successfully applied for determination of PPEH and CETH in its pharmaceutical dosage form.

INTRODUCTION: Phenylepherine hydrochloride (PPEH) chemically described as (R)-1-(3-hydroxyphenyl)-2-methylamino ethanol hydrochloride is a sympathomimetic (alpha-adrenergic) agent [1] who stimulates alpha-adrenergic receptors, producing pronounced vasoconstriction [2]. It is also a frequent constituent of orally administered nasal decongestant preparations [3]. It is officially in IP [1], BP [4] and USP[5].

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Cetirizine hydrochloride (CETH) chemically described as [2-[4-[(4-chlorophenyl) phenyl methyl]-1piperazinyl]ethoxy] acetic acid, dihydrochloride is an antihistaminic [1], histamine H1 receptor antagonist [4] and an anti-allergic agents used in the treatment of seasonal rhinitis, hay fever, running nose, control sneezing of allergic origin [3].

The levorotatory enantiomer of Cetirizine, known as Levocetirizine is more active form [7]. Cetirizine hydrochloride is official in IP [1], BP [4] and USP [5]. Literature survey reveals that several analytical methods have been reported for the determination of PPEH and CETH as individual and combined dosage form with each other and with other combination of other drugs such as RP-HPLC, Spectrophotometric, HPTLC, Fluorimetry and ion-

pair Chromatographic method [8-20]. Our objectives in the present investigation are to develop and validate new RP-HPLC method for simultaneous determination of PPEH and CETH. The proposed RP-HPLC method utilise economical solvent system having advantages like better retention time, very sharp and symmetric peak shapes. The proposed method was validated according to ICH guidelines [6].

Chemical structures of PPEH and CETH:

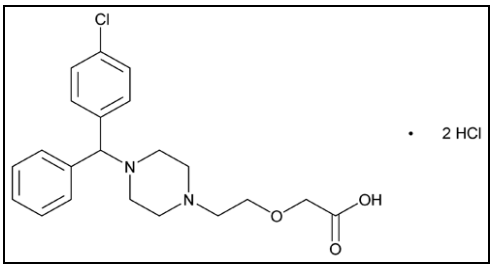

FIG. 1: CHEMICAL STRUCTURE OF CETH

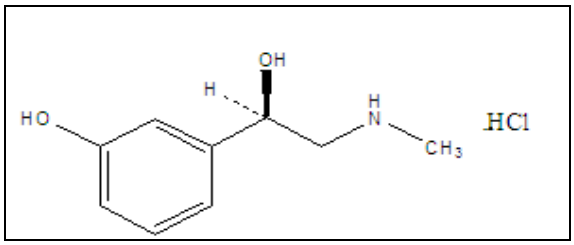

FIG. 2: CHEMICAL STRUCTURE OF PPEH

MATERIALS AND METHOD:

Instrument and apparatus:

A HPLC Instrument (LC-2010 CHT, Shimadzu, Japan) equipped with UV detector, auto injector and LC-Solution Software was used. The chromatographic analysis was performed on Princeton SPHER C18 Column (250mm x 4.6mm id, 5 µ particle size). Analytical balance (Mettler Toledo), digital pH meter (Eutech instruments pH tutor) was used during analysis.

Reagents and Materials:

Working standards of PPEH (potency = 99.45%) and CETH (potency = 99.12%) were obtained as a gift samples from Cipla Ltd. Mumbai. HPLC grade Acetonitrile, AR grades Orthophosphoric acid and Ammonium dihydrogen phosphate were procured from Merck Ltd. Mumbai India. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a 0.45µ membrane filter paper. The commercial fixed dose combination product Alerid-D tablet (Marketed by Cipla Ltd. Mumbai) containing 10 mg PPEH and 5 mg CETH was procured from the local market.

Chromatographic Condition:

HPLC system	LC-2010 CHT , Shimadzu
Software	LC Solution
Detector	UV Detector
Wavelength	225 nm
Pump	Isocratic Pump
Stationary phase	Princeton SPHER, C18(250 mm x 4.6 mm id, 5µ particle size)
Mobile phase	0.1 M Ammonium dihydrogen phosphate buffer (pH 5.2 ± 0.05) : ACN (50:50 v/v)
Flow rate	1.0 ml/min
Injection volume	20 µl

Preparation of stock and standard solutions:

A Stock solution of PPEH (1 mg/mL) was prepared by dissolving 100 mg PPEH in 100 ml volumetric flask and volume make up by mobile phase. CETH (0.5mg/ml) was prepared by dissolving 50 mg CETH in 100 ml volumetric flask and volume make by mobile phase. Appropriate volumes of the stock solution were transferred to appropriate volumetric flask and solution was diluted with mobile phase to furnish final concentration of PPEH and CETH in the range 10-60µg/ml and 5-30µg/ml respectively.

Preparation of Tablet dosage form:

20 tablets (Alerid-D Tablet) each contained 10 mg PPEH and 5mg CETH were accurately weighed. Their average weight determines and finally powdered. Quantity of the powder containing weight equivalent to 100 mg PPEH and 50 mg CETH were transferred to 100 ml volumetric flask and 50 ml mobile phase was added followed by ultrasonication for 10 minute and make up the volume up to 100 ml with mobile phase. The resulting solution stirred for 1 hour. After that centrifuged at 2000 RPM for 5 minute further dilution was performed with mobile phase to reach the calibration range for each compound.

Method Validation:

The proposed method was validating according to ICH (Q2) B Guidelines for validation of analytical procedures. As per the ICH guidelines the methodvalidation parameters checked were

linearity, accuracy, precision, assay, robustness, LOD, LOQ.

Linearity (Calibration Curve):
For constructing calibration curve, series of six dilutions in the concentration range 10-60 (10, 20, 30, 40, 50, and 60) µg/ml for PPEH and 5-30 (5, 10, 15, 20, 25, and 30) µg/ml for CETH was taken. Calibration curve were constructed by plotting peak area vs. concentration of PPEH and CETH and regression equation calculated from calibration curve. Linearity curves have shown in **Fig. 5** and **6** respectively.

Accuracy (% Recovery):
The accuracy of the method was determined by calculating recovery of PPEH and CETH by the standard addition method. Known amounts of standards solutions of PPEH and CETH added at 80,100 and 120% level to prequantified sample solution of PPEH and CETH. Three samples were prepared for each recovery level solutions were then analysed and the percentage recovery were calculated by using formula.

Precision:
The precision of analytical procedure express degree of the agreement among individual test when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision are considered at two levels: Repeatability and Reproducibility.

Method Precision (Repeatability):
The precision of the instrument was checked by repeatedly injecting (n =6) standard solutions of the PPEH and CETH. Under the same Chromatographic condition and measurements of % RSD of peak area, retention time, and tailing factor should not be more than 2%.

Intermediate Precision (Reproducibility):
The intraday and interday precision of the proposed method was determine by analysing the corresponding responses 3 times of the same day and on three different days over a period of 1 week for three different concentration of standard solutions of PPEH (10, 20 and 30 µg/ml) and CETH (5, 10 and 15 µg/ml) the results were reported in the terms of % RSD.

Robustness:
The robustness of the method was established by introducing small changes in various parameters like, pH of mobile phase, flow rate, wavelength, column temperature and mobile phase composition. The method was evaluated by calculating % RSD.

Limit of Detection & Limit of Quantification:
Limit of detection (LOD) was lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of quantification (LOQ) the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3 (SD/M) and LOQ = 10(SD/M). The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1.

RESULTS AND DISCUSSION:
To optimize the HPLC parameters, several mobile phase composition were tried.

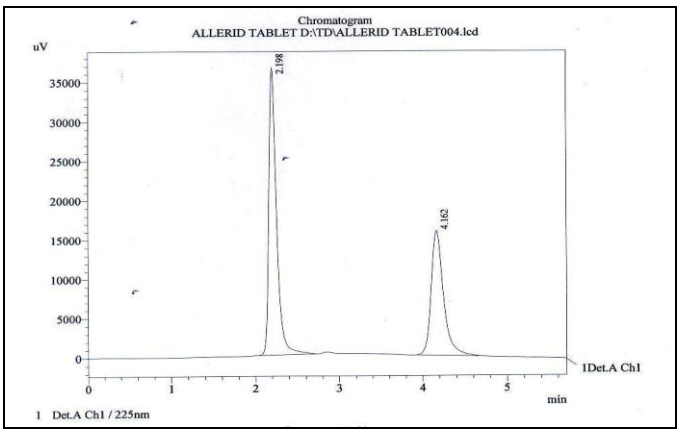

FIG. 3: CHROMATOGRAM OF PPEH AND CETH IN STANDARD SOLUTION.

A good peak symmetry, a satisfactory resolution for PPEH and CETH was obtained with mobile phase buffer (pH 5.2 ± 0.05): ACN (50:50 v/v) at a flow rate 1 ml/min to get better reproducibility and

repeatedly optimization of method was done by changing mobile phase composition, pH of mobile phase, column packing, flow rate, temperature, detection wavelength and the effect on retention and peak shape were monitored for PPEH and CETH. Typical chromatogram of PPEH and CETH.

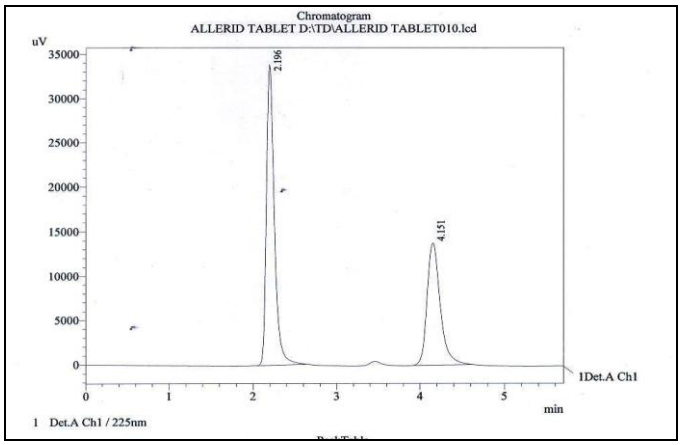

FIG.4: CHROMATOGRAM OF PPEH AND CETH IN SAMPLE SOLUTION.

1] System suitability test parameters for PPEH and CETH for the developed method are reported in **Table 1.**

TABLE 1: SYSTEM SUITABILITY TEST PARAMETERS FOR PPEH & CETH

Parameters	PPEH ± RSD (n=6)	CETH ± RSD (n=6)
Retention Time (min)	2.19 ± 0.187	4.16 ± 0.104
Tailing Factor	1.42 ± 0.365	1.38 ± 0.373
Theoretical plates	2919 ± 0.316	5004 ± 0.367
Resolution		9.094 ± 0.652

RSD: - Relative standard deviation

2] The method showed good linear response in the concentration range 10-60 µg/ml for PPEH (r^2 = 0.9998) and 5-30 µg/ml for CETH (r^2 = 0.9998)

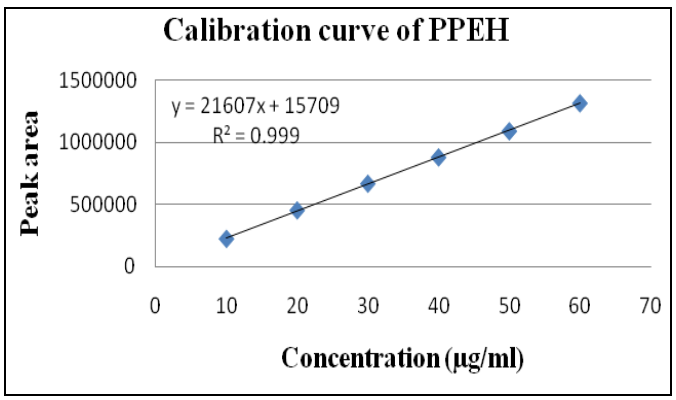

FIG.5: LINEARITY CURVE OF PPEH

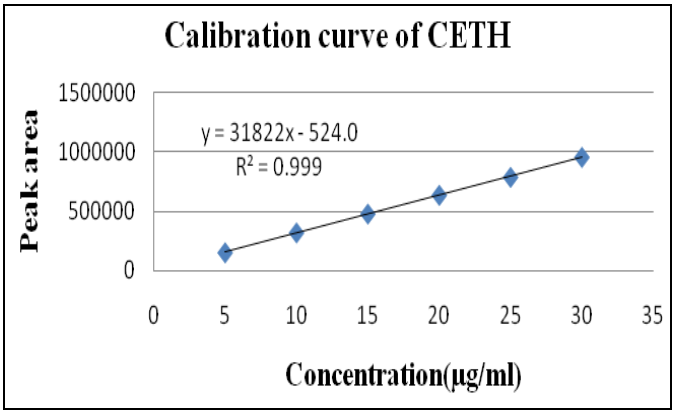

FIG. 6: LINEARITY CURVE OF CETH

TABLE 2: REGRESSION ANALYSIS OF THE CALIBRATION CURVES FOR PPEH & CETH (N=6).

Parameters	PPEH	CETH
Linear range (µg/ml)	10-60	5-30
Slope	21607	31822
Intercept	15079	524.07
Correlation Coefficient (r^2)	0.9998	0.9998

3] The method was found to be precise and RSD was found to be less than 2% are given in **Table 3.**

TABLE 3: PRECISION OF RP-HPLC METHOD

Drugs	Level (n=3)	Intraday amount found (%) ±RSD	Interday amount found (%) ±RSD
PPEH	1	101.63±0.541	100.68±0.682
	2	100.02±1.201	99.25±0.861
	3	98.92±0.925	98.75±1.652
CETH	1	98.65±0.621	99.56±1.203
	2	98.95±0.792	100.62±1.652
	3	99.45±0.994	98.65±0.256

4] The results of recovery of PPEH and CETH with the RSD less than 2% are given in **Table 4**.

TABLE 4: RECOVERY STUDIES OF PPEH AND CETH

Drugs	Level (n=3)	% Recovery ± % RSD	Mean recovery
PPEH	80 %	100.96±1.18	100.47%
	100 %	100.95±1.88	
	120 %	99.50±0.95	
CETH	80 %	98.43±0.80	99.34%
	100 %	98.88±1.13	
	120 %	100.72±0.18	

5] Assay of PPEH and CETH shown in **Table 5**.

TABLE 5: RESULTS OF TABLET ASSAY OF PPEH AND CETH

Drugs	Label claim (mg/tab)	Amount of drug estimated (mg/tab)	% Amount found
PPEH	10.0	10.095	100.95
CETH	5.0	4.944	98.88

6] LOD and LOQ value of PPEH and CETH was determined by residual standard deviation method. The results are given in **Table 6**.

TABLE 6: LOD AND LOQ OF PPEH AND CETH

Drugs	LOD (µg/ml)	LOQ (µg/ml)
PPEH	0.176	0.533
CETH	0.248	0.750

7] Robustness was evaluated by varying different parameters. The results of these variations are given in **Table 7**.

TABLE 7: RESULTS OF ROBUSTNESS OF PPEH AND CETH

Parameters	Variation	PPEH		CETH	
		Retention time(min)	Assay (%)	Retention time(min)	Assay (%)
Flow rate(ml/min)	0.9	2.44	99.52	4.63	98.65
	1.0	2.19	100.21	4.16	98.11
	1.1	1.99	98.56	3.78	99.83
pH	5.0	2.19	99.56	4.16	100.62
	5.2	2.19	99.23	4.16	98.22
	5.4	2.19	99.98	4.16	98.78
Column temperature (^{0}C)	24	2.19	100.95	4.16	98.36
	25	2.19	99.56	4.16	100.34
	26	2.19	101.65	4.16	101.53
Wavelength(nm)	224	2.19	101.85	4.16	99.52
	225	2.19	100.90	4.16	98.88
	226	2.19	99.37	4.16	99.63
Mobile Phase Composition	(60:40)	2.34	101.88	6.34	100.11
	(50:50)	2.19	99.51	4.16	98.63
	(40:60)	1.55	98.66	3.88	98.99

CONCLUSION: A validated RP-HPLC method has been developed for the determination of Phenylephrine Hydrochloride and Cetirizine Hydrochloride in tablet dosage form. The proposed method is simple, rapid, linear, accurate, precise and specific. Results from the validation experiments showed that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Phenylepherine Hydrochloride and Cetirizine Hydrochloride in tablet dosage form.

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