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Research Article

Development of Novel Method for Determination of Phenylephrine Hydrochloride and Cetirizine Hydrochloride in Tablet Dosage Form using RP-HPLC

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ABSTRACT

The literature survey shows that only single complicated method with multiple components of mobile phase is present for the simultaneous determination of phenylephrine hydrochloride and cetirizine hydrochloride in tablet dosage forms. An effort was made to develop a simple, efficient, less time consuming, economic and accurate reverse phase high performance liquid chromatographic method. An Xtera C-8 column (150mm x 4.6mm i.d., particle size 3.5µ) with mobile phase containing methanol: water 85:15 (v/v) with flow rate of 1.2mL/ min was used and effluents were monitored at 230nm. The retention times of phenylephrine hydrochloride and cetirizine hydrochloride were found to be 2.57 and 3.22 min, respectively as compared to 2.20 and 4.16 min in existing conventional method. The linearity was in the range of 5-35µg/mL for both drugs. The limit of detection and limit of quantification were found to be 0.5 and 1.5µg/mL for phenylephrine hydrochloride and 0.3 and 1.0µg/mL for cetirizine hydrochloride respectively. The % recoveries were found to be in the range of 99.82±0.85 and 100.86±1.54 for phenylephrine hydrochloride and cetirizine hydrochloride. The intermediate precision data was also subjected to statistical analysis (F-test and t-test at 95% confidence level). The proposed method was validated and successfully applied to the estimation of phenylephrine hydrochloride and cetirizine hydrochloride in combined tablet dosage forms.

Keywords: Phenylephrine hydrochloride, Cetirizine hydrochloride, RP-HPLC, Simultaneous determination, ICH

1. Introduction

Phenylepherine hydrochloride (PH) chemically described as (R)-1-(3-hydroxyphenyl)-2-methyl-amino ethanol hydrochloride is a sympatho-mimetic (α-adrenergic) agent that stimulates α -adrenergic receptors, producing pronounced vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations. Cetirizine hydrochloride (CH) or 2-[2-[4-[(4 chlorophenyl) phenylmethyl]- piperazin-1-yl]ethoxy]acetic acid dihydrochloride, is used for symptomatic relief of hypersensitivity reactions including rhinitis and chronic urticaria [1-5]. The structures of phenylephrine hydrochloride and cetirizine hydrochloride are shown in Figure 1. A number of UV and HPLC based methods are already in existence for evaluation of these drugs in various marketed dosage forms as well as in biological fluids [6-18]. UV spectrophotometer methods are less sensitive and accurate as compared to HPLC methods. Literature survey reveals that only one HPLC method has been published for the determination of PH and CH in tablet dosage form [6]. This method is complicated due to use of mobile phase systems along with buffer as 0.1 M Ammonium dihydrogen phosphate pH 5.2 ± 0.05. Keeping in view the present scenario, there was a need to develop simple, more economic method with shorter analysis time with use of simple mobile phase. So the main aim of present work was to develop ICH guidelines compliant, simple, economic and less time consuming RP-HPLC method for simultaneous estimation of PH and CH in pharmaceutical dosage forms [4-5].

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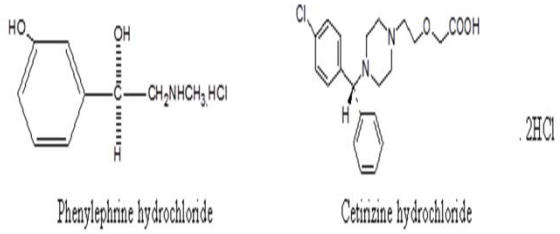

Figure 1: the structures of PH and CH

2. Experimental

2.1 Chemicals and Reagents

PH and CH reference standards were obtained from Sun Pharma, Mumbai, India. HPLC grade Methanol and water were obtained from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. The commercial tablet formulation, Ceticold (Marketed by Osho Pharma Pvt.Ltd., Delhi) containing 120mg PH and 5mg CH was procured from the local market [4-5,19].

2.2 Instrumentation

The analysis was carried out on a PerkinElmer series 200 HPLC using UV-Visible detector with rheodyne injector. Column used was An Xtera C-8 column with dimensions 150mm × 4.6mm i.d. and particle size 3.5µ. The software used for data integration was Total Chrom Navigator version®6.3.

2.3 Chromatographic conditions

The isocratic mobile phase consisting of methanol: water in a ratio of 85:15 (v/v) was used at a flow rate of 1.2mL/min. Analysis was performed with UV-visible detector at 230nm on ambient temperature. The mobile phase was used as diluent for preparation of standard and sample preparations [4-5].

2.4 Preparation of Mobile Phase

Mobile phase was prepared by mixing 850mL of methanol with 150mL of water in to 1000mL measuring cylinder and transferred in to reagent bottle. The mobile phase was sonicated for 15 min and filtered through 0.45µ membrane filter paper.

2.5 Preparation of Standard Stock Solution

Accurately weighed PH and CH (25mg each) and transferred in to 100mL volumetric flasks separately and dissolved in the mobile phase. The volume was adjusted with mobile phase to give composite solution of 100µg/mL each of PH and CH.

2.6 Preparation of Sample Solution

Twenty tablets (Ceticold tablet) were weighed and finely powdered. Tablet powder equivalent to 120 mg of PH and 5 mg of CH was transferred to a 100 mL volumetric flask and dissolved in 20 mL of mobile phase. The volume was made up to the mark with diluent and mixed well. The solution was sonicated for 15 min and filtered through 0.45µ membrane filter. The solutions were further diluted with mobile phase. The final concentration of solution was 120 g/mL of PH and 5 µg/mL of CH. The amount of drugs in samples was calculated from the peak area of PH and CH [4-5].

2.7 Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, accuracy, precision, robustness, limit of detection, limit of quantitation and system suitability parameters in accordance with the ICH guidelines [19-20].

2.7.1 Linearity and Range

Linearity study was performed by preparing standard solutions at seven different concentrations (5, 10, 15, 20, 25, 30, and 35µg/mL for both the drugs) and analysis was performed in duplicate. The response was measured as peak area and the calibration curves were obtained by plotting peak area against concentration.

2.7.2 Specificity

The specificity study was performed by injecting each drug individually and in drug mixture. The specificity studies proved the absence of any undesired interference.

2.7.3 Precision

Precision was considered at repeatability and intermediate precision in accordance with ICH recommendations. From the standard stock solutions, mixed standards containing PH and CH were prepared. Standard solutions (n=6) were injected using a universal rheodyne injector with injection volume of 20µL. The intraday and interday precisions were determined.

2.7.4 Accuracy

The accuracy of an analytical method was determined by the standard addition method. A known amount of standard PH and CH corresponding to 50%, 100%, and 150% of the label claim was added to pre-analysed sample of tablet dosage form separately. The recovery studies were carried out three times, at each level of recovery.

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

The LOD and LOQ were separately determined on the basis of residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve [4-5].

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions

For optimization of chromatographic conditions mainly focus was given in mobile phase, composition of mobile phase and stationary phase as well wavelength and flow rate. For mobile phase optimization two organic solvents (methanol and acetonitrile) was used while for selection of stationary phase three columns were used (Princeton sphere C-8 25 cm x 4.6mm, Kromasil C-18 250 × 4.6 mm and Xtera C-8 column 150mm x 4.6mm). Finally the optimum mobile phase containing methanol:water 85:15 (v/v) was selected because of ability to resolve the peaks of PH (RT = 2.57 ± 0.05) and CH (RT = 3.22 ± 0.09) with a resolution factor of 5.52. Due to basic nature of analytes**,** Xtera C-8 column 150mm x 4.6mm column was used as a stationary phase because properties of such columns are reversed-phase with hybrid-based for good peak shape performance for highly basic compounds. Quantification was achieved with UV detection at 230nm. HPLC chromatogram obtained during simultaneous determination of PH and CH is given in Figure 2 [4-5].

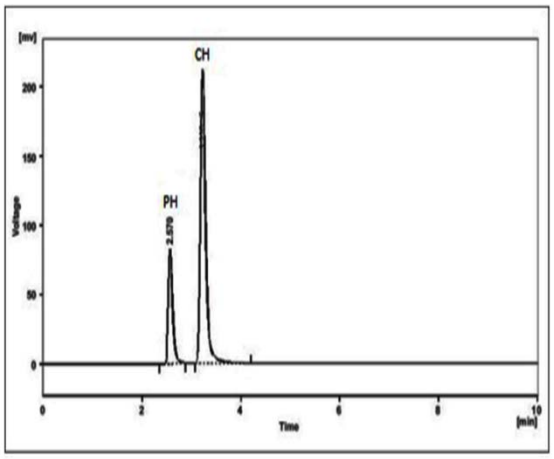

Figure 2: HPLC chromatogram obtained during simultaneous determination of PH and CH

3.2 Method Validation

3.2.1 Linearity and Calibration standards

Linear regression data showed a good relationship over a concentration range of 5-35 µg/mL for PH and CH. The linear regression equations for PH and CH were found to be y = 17,642.8571x - 8,206.4286, and y = 10,142.8571x + 1,793.5714, respectively. The regression coefficient values (r^2) were found to be 0.9978 and 0.9992 for PH and CH, respectively indicating a high degree of linearity. The linearity curves of PH and CH are given in Figure 3 and Figure 4, respectively [4-5]**.**

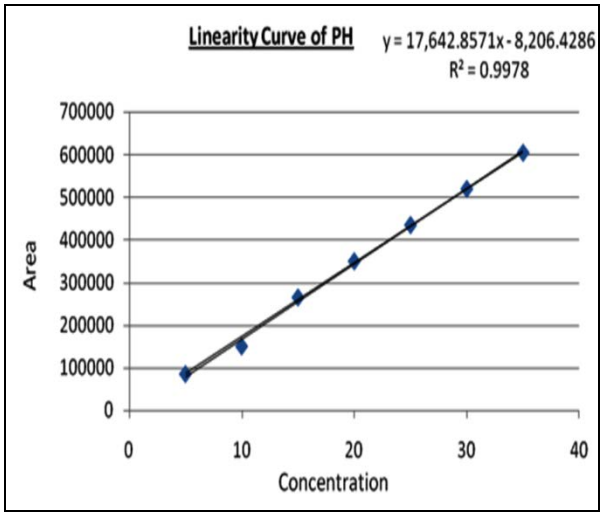

Figure 3: Linearity curve of PH

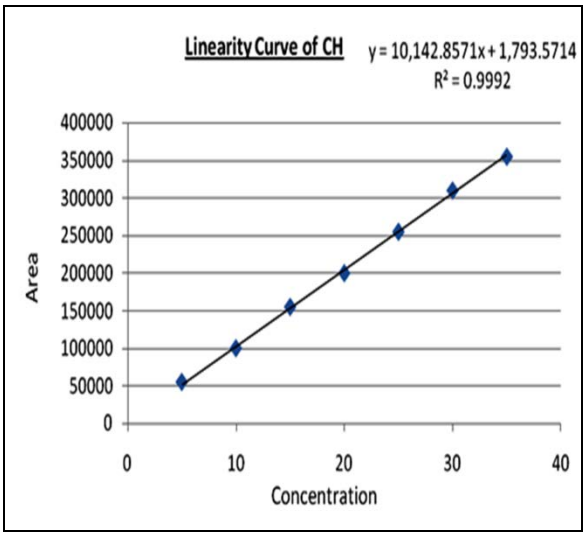

Figure 4: Linearity curve of CH

3.2.2 Specificity

The specificity studies revealed the absence of any interference by excipients since none of the peaks appeared at the same retention time of PH and CH. The interaction study in standard solution was also carried out by comparing peak of each drug individually and in drug mixture. Results indicated that the analytes did not interact with each other.

3.2.3 LOD and LOQ

The limit of detection and limit of quantification were found to be 0.5 and 1.5 µg/mL for PH and 0.3 and 1.0 µg/mL for CH respectively. The values indicate that the method is sensitive.

3.2.4 Stability studies

The stability of the analyte solutions was determined by comparing the analyte solutions at 3rd day and 7th day with that of the freshly prepared solution at 1st day. The differences determined on 3rd day were ±0.85, and ±1.14 for PH and CH respectively. The differences determined up to 7th day were ±1.75, and ±1.33 for PH and CH respectively.

3.2.5 Accuracy and precision

For evaluation of accuracy of the developed method, recovery studies were carried out using standard addition method at three different levels. The average % recoveries for PH and CH in marketed formulation were found to be between 99.82±0.85 and 100.86±1.54 respectively**.** The results revealed that there was no interference of excipients. The results of accuracy are given in Table 1. The intra-day and inter-day precisions were assessed by analyzing standard solutions. A low % RSD (Relative Standard Deviation) value of 1.02 and 0.69 for PH and CH respectively indicates that the method is precise [4-5]. The intra-day and inter-day results were calculated statistically by the F-test and student's t-test. The calculated values of F-test and student's t-test are given in Table 2. Data of intra-day and inter-day data did not differ significantly in terms of precision.

Table 1: Percent recovery data Phenylephrine hydrochloride (PH) and Cetirizine hydrochloride (CH)

Drug	% simulated dosage nominal	% Mean (n=3)	±SD	RSD (%)
PH	50	99.82	0.82	0.85
CH	50	99.10	1.78	1.79
PH	100	100.25	0.97	0.95
CH	100	99.23	0.53	0.55
PH	150	99.37	1.75	1.80
CH	150	100.86	1.52	1.54

Table 2: Statistical Treatment of the Precision Studies Data

Drug	F-test	t-test
PH	2.81 (4.85)	0.18 (1.89)
CH	2.56 (4.85)	0.35 (1.89)

[a]The values in parenthesis are the theoretical values of f-test and student's t test at 95% confidence level.

3.2.6 Analysis of Tablets

Analysis of marketed tablets (Ceticold) was performed on developed method. The % drug content found to be between 99.10% and 100.86%. Data indicated that the estimation of dosage forms were accurate. The results are given in the Table 3.

3.3 System Suitability Parameters

Six replicate injections of mixed standard solution were injected for checking system suitability. Resolution, capacity factor, tailing factor, theoretical plate, retention volume and asymmetry factor of the peaks were calculated. The results are summarized in Table 4.

Table 4: System suitability data

Parameters	PH	CH
Resolution	-	5.52
Capacity factor	0.15	0.69
Tailing factor	1.24	1.45
Theoretical plates	15020	10678
Asymmetry factor	1.1	1.19

4. CONCLUSION

A simple, economic and less time consuming RP-HPLC method for simultaneous estimation of PH and CH was developed and validated. The method is simple because there is no need to prepare buffer and maintain its pH throughout the experiment. The method is economic because it excludes the use of buffers and saves instrument working hours thus giving a long life to costly HPLC equipment and columns. The method gives good resolution for both the drugs with a short analysis time (<4.0 min).The time span reduction is very high because of removal of post column flushing session in each analysis. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when buffer are used in composition of mobile phase. This also ensures least carryover of buffer contaminants in next analysis. The method has been successfully tested for the analysis of marketed tablets and can be adopted for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the chromatographic conditions. The results obtained indicate that the proposed method is rapid, economic, accurate, selective, linear and reproducible as per ICH guidelines.

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