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Original Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE HYDROCHLORIDE AND EBASTINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: A new simple, accurate, precise, robust, reproducible and economic RP-HPLC method was developed for Phenylephrine Hydrochloride and Ebastine in marketed tablet dosage form.

Methods: The Chromatographic separation was achieved on Thermo BDS Hypersil C_{18} column (250 mm × 4.6 mm, 5 µm) at ambient temperature. Mobile phase consist of Methanol: Phosphate buffer (30:70v/v), pH 4.0±0.05 was pumped at a flow rate was 1.0 ml/ min and Quantification was achieved with photodiode array (PDA) detection at 215 nm.

Results: The method was linear over the concentration range of 5-15 µg/mL (r^2 = 0.9994) for Phenylephrine Hydrochloride (PHE) and 5-15 µg/mL (r^2 = 0.9947) for Ebastine (EBS). The percentage content for PHE and EBS was found to be 101.08±0.74% and 99.11±0.52%, respectively in the marketed formulation. The LOD and LOQ values for PHE were 0.46 and 1.12 µg/ml, respectively and these values for EBS were 1.41 and 3.41 µg/ml, respectively. These values indicate the sensitivity of method. Percent recovery was 99.69% for PHE and 96.60% for EBS reflects the good accuracy of the method. The developed method was validated for linearity, precision, accuracy, and robustness as per ICH guideline.

Conclusion: A simple, precise, accurate, linear and rapid RP-HPLC method was developed and validated as per ICH guidelines. The results suggest that the developed can be applicable in routine analysis for tablets in the pharmaceutical industry.

Keywords: Phenylephrine Hydrochloride, Ebastine, Validation, RP- HPLC.

INTRODUCTION

The combination of Phenylephrine Hydrochloride and Ebastine has synergistic effect for the treatment of common cold and allergy [1]. Phenylephrine Hydrochloride is a selective α_1 agonist, it causes vasoconstriction by stimulating the post-synaptic α receptors. It is constituent of most of orally administered nasal decongestant preparations [2]. Phenylephrine Hydrochloride is chemically (*R*)-1-(3-hydroxyphenyl)-2- methylamino-ethanol hydrochloride [3] (Figure 1). It is official drug in Indian Pharmacopoeia [5], British Pharmacopoeia [6]. Ebastine is second generation H_1 receptor agonist and non sedating antihistamine drug. It is used for symptomatic relief of allergic conditions, including rhinitis and pruritic skin disorder [4,7]. Ebastine chemically known as 4-(4-benzhydryloxy-1-piperidyl)-1-(4- tert-butyl phenyl) butan-1- one [3] (Figure 2). Ebastine is official in British Pharmacopoeia [6] and European Pharmacopoeia [8].

Literature review revealed that several UV-Spectrophotometric methods [9], Electrochemical Determination [10], UPLC [11] and RP-HPLC [12, 13] methods have been developed for estimation of Ebastine. Similarly, UV-Spectrophotometric method [14], RP-HPLC [15], LC-MS-MS in plasma [16] methods have been developed for Phenylephrine Hydrochloride as single drug or combination of other drugs. The combination of Phenylephrine Hydrochloride and Ebastine is more effective for the treatment of allergy and decongestant without causing sedation as other antihistamine drugs. The objective of this work was to develop and validate a simple, accurate, precise, robust, reproducible and economic method for determination of Phenylephrine Hydrochloride and Ebastine in bulk and combined pharmaceutical dosage form as per ICH guidelines [17].

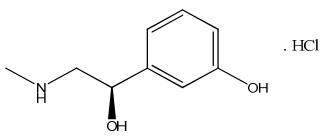

Fig. 1: Chemical structure of Phenylephrine Hydrochloride

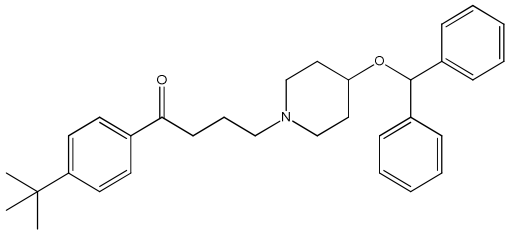

Fig. 2: Chemical structure of Ebastine

MATERIALS AND METHODS

Reagents and Materials

Phenylephrine Hydrochloride and Ebastine reference standard (RS) was obtained from Molecule Laboratory Pvt Ltd, Ahmedabad, India. The commercial fixed dose combination product containing 10 mg Ebastine and 10 mg Phenylephrine Hydrochloride (EBAST-DC®Micro Lab, India) was procured from the local pharmacy. Methanol (HPLC grade) and potassium dihydrogen phosphate of AR grade was obtained from Merck Ltd., Mumbai, India and S.D Fine Chemicals Ltd, Mumbai, India, respectively.

Instrument and Apparatus

Chromatographic separation was performed using a HPLC instrument (LC-2010C_{HT}, Shimadzu, Japan) equipped with a photodiode array detector, manual injector with 20 µL loop system. Spinchrome software was employed for data collection and processing [21, 22]. Chromatographic separation was performed on BDS Hypersil C_{18} stainless steel column (250×4.6 mm, 5 µm). Digital pH meter (Metler Toledo) and Analytical balance (Metler Toledo) were employed for this study.

Chromatographic Condition

Stationary phase BDS Hypersil C_{18} was used. Mobile Phase comprised of Methanol: Phosphate buffer (30:70v/v), pH 4.0±0.05, Flow rate 1.0

mL/min, Injection volume 20 µL, HPLC analysis [18-20] was performed at ambient temperature with detection at 215 nm.

Preparation of mobile phase

Mobile phase used in a combination of 70:30 v/v of Phosphate buffer (0.05M): Methanol. Mobile phase was sonicate and filtered through 0.22µ nylon filter for 15 minutes in an ultrasonicator.

Preparation of mixed standard stock solutions

Mixed standard solution was prepared by transferring accurately weighed Phenylephrine Hydrochloride (10mg) and Ebastine (10mg) into a 100 ml volumetric flask. 50 ml of methanol was added to it and the solution was sonicated for 2 min. Then volume was made to 100 ml to obtain the final concentration 100 µg/ml.

Analysis of Marketed Formulation

20 tablets were accurately weighed and average weight was calculated. Then tablets were ground into a fine powder using a glass mortar and pestle. Powder equivalent to 10 mg of Phenylephrine Hydrochloride and Ebastine as well as accurately weighed and transferred to a 100 ml volumetric flask. Approximately 50 ml of mobile phase was added to the flask and the contents were sonicated for 15 min. Volume was adjusted upto the mark. The resulting solution was filtered using 0.22µ nylon filter. This sample stock solution was further diluted with the same mobile phase to obtain 10 µg/ml of Phenylephrine Hydrochloride and 10 µg/ml of Ebastine. The sample solutions were prepared in triplicate and 20 µl volume of each sample solution was injected into the sample injector of RP-HPLC under the optimized chromatographic conditions. The concentrations of the drugs in samples were calculated by measuring their peak areas and comparing with peak areas of standard drug solutions of respective concentrations.

Method validation

Validation of an analytical procedure is the process by which laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The developed chromatographic method was validated for system suitability, linearity & range, accuracy, precision, and robustness, as per ICH guidelines [18].

System suitability test

The system suitability test was performed by injecting five replicate of working standard solution. Results of retention time, theoretical plates and tailing factor (peak symmetry) were presented in Table 2.

Linearity and range

Working solutions of Phenylephrine Hydrochloride (5-15µg/ml) and Ebastine(5-15µg/ml) were injected under the operating chromatographic conditions and peak areas for each drug were calculated at 215 nm. The calibration curve was plotted between areas against corresponding concentrations of each drug. Linear regression data for calibration curves were shown in Table 3. The range of solution has been decided according to correlation coefficient of regression equation.

Accuracy (% recovery)

The accuracy of the method was determined by calculating % recovery of each drug by standard addition method. Percent recovery of Phenylephrine Hydrochloride and Ebastine was determined at three different level 80%, 100% and 120% of the target concentration in triplicate (Table 4).

Precision

Method Precision (Repeatability) was determined by injecting standard solution six times. The retention times and peak areas of six replicates are recorded. The intermediate (intra-day and inter-day) precision study of Phenylephrine Hydrochloride and Ebastine was carried out by estimating the corresponding responses three times on the same day and on three different days for the concentrations level at 50%, 100%, 150% of Phenylephrine Hydrochloride and 50%, 100%, 150% of Ebastine.

The precision is expressed as the % RSD of Peak areas and it should not be more than 2%. Precision study for Phenylephrine Hydrochloride and Ebastine were mentioned in Table 5 and 6.

Robustness

Robustness of the method was studied by changing flow rate (±0.2 ml/min), change in pH (±0.2), and change in mobile phase concentration (±2% v/v) during analysis. Sample solution of 100% concentration is prepared and injected in triplicate for every condition and %RSD was calculated for each condition (Table 7).

LOD and LOQ

The standard deviation of the Y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae [23] **(**Table 8).

$$\text{LOD} = \frac{3.3\ X\ SD}{S} \qquad \text{LOQ} = \frac{10\ X\ SD}{S}$$

LOD - Limit of detection,

LOQ – Limit of quantitation

Where, S is average value of slopes of calibration plots and SD is calculated using values of y intercepts of regression equations.

RESULTS AND DISCUSSION

The composition, flow rate of mobile phase and column as well as column temperature was suitably optimized for better separation of Phenylephrine Hydrochloride and Ebastine combined dosage form. Finally, potassium dihydrogen phosphate (0.05M KH_2PO_4) Buffer: Methanol (70:30v/v) at pH 4.0±0.5, 1 ml/min. flow rate and Hypersil BDS C_{18} column at ambient temperature was selected.

These optimized conditions had following system suitability parameters. Number of theoretical plates for Phenylephrine Hydrochloride and Ebastine were 6724 and 7099, respectively.

Tailing Factors for Phenylephrine Hydrochloride and Ebastine were 1.39 and 1.40, respectively. LOD and LOQ for Phenylephrine Hydrochloride was 0.46 and 1.12 that for Ebastine was 1.41 and 3.41 respectively (Table 8). Low value of LOD and LOQ shows that method is sensitive and can be apply for detection of lowest amount of analyt. The retention time for Phenylephrine Hydrochloride and Ebastine were 3.60 and 5.84 min., respectively.

The values of correlation coefficient for Phenylephrine Hydrochloride and Ebastine (Table 2) demonstrated the good relationship between peak area and concentration. Therefore, the developed method was linear in concentration range of 5-15 µg/mL for Phenylephrine Hydrochloride and 5-15µg/mL for Ebastine. The percentage assay of Phenylephrine Hydrochloride and Ebastine in tablets was 101.08% and 99.11%, respectively (Table 1).

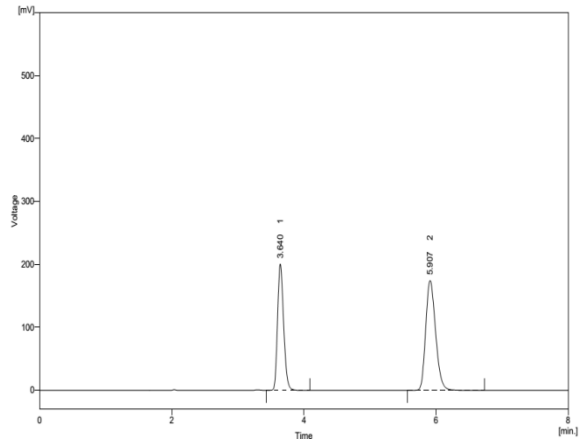

Fig. 3: Standard Solution of Phenylephrine HCl and Ebastine

Peak 1.Phenylephrine HCl 2.Ebastine

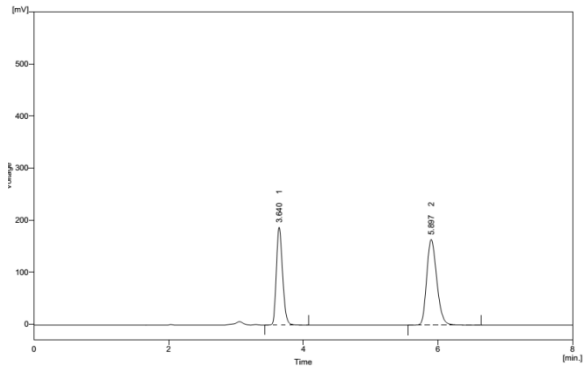

Fig. 4: Sample solution of Phenylephrine HCl and Ebastine

Peak 1.Phenylephrine HCl 2.Ebastine

Percent recovery was 99.69% for Phenylephrine Hydrochloride and 96.60% for Ebastine demonstrated accuracy. The low value of % RSD in intra-day and inter-day precision (Table 5 and 6) indicated reproducibility of this method. Finally, deliberate variations were made to check the significant variations in experimental conditions (Table 7) suggested robustness of developed method.

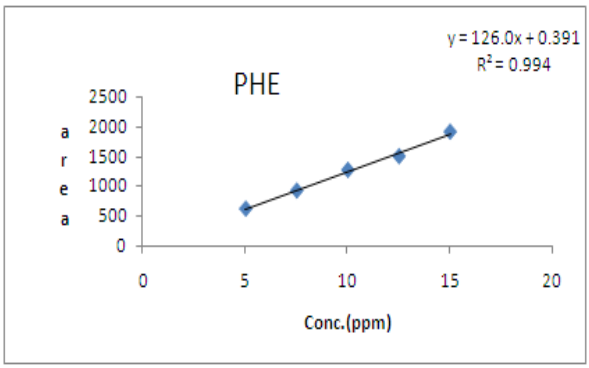

Fig. 5: Calibration curve of Phenylephrine HCl

Table 1: Results of Assay of Marketed formulation

Sample	Label Claim (mg/tab)	Drug contain% ± SD*	% RSD
Ebastine	10	99.11± 0.5186	0.986
Phenylephrine HCl	10	101.08± 0.738	0.730

**n=3*

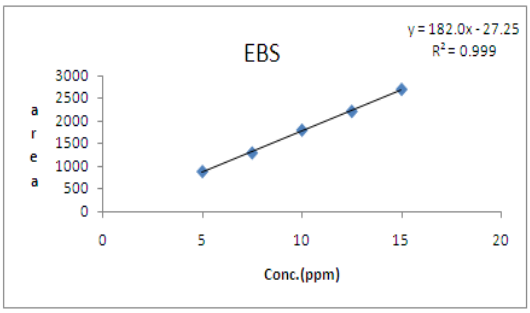

Fig. 6: Calibration curve of Ebastine

Table 2: Results of system suitability test

Parameters	Ebastine	Phenylephrine HCl
Retention time (min)	3.605	5.847
Tailing factor	1.391	1.405
Theoretical plates	6724	7099
Resolution	9.915	

Table 3: Linear regression data for calibration curves of Phenylephrine HCl and Ebastine

Parameters	Ebastine	Phenylephrine HCl
Linearity range (µg/ml)	5-15	5-15
Coefficient of correlation	0.9994	0.9947
Slope± SD*	643.848 ± 182	446.75± 126
Intercept	27.25	0.391

**n=5*

Table 4: Accuracy data of Phenylephrine HCl and Ebastine

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (µg/mL)	Mean % Recovery ± SD*	%RSD*
Phenylephrine HCl	80%	5	4	99.82±1.12	1.254
	100%	5	5	99.66±0.75	0.749
	120%	5	6	99.59±0.32	0.324
Ebastine	80%	5	4	99.94±1.12	1.124
	100%	5	5	99.25±0.23	0.234
	120%	5	6	99.60±0.59	0.594

**n=3*

Table 5: Results for method precision (Repeatability)

Drug	Concentration of drug (µg/ml)	Area (Mean ± SD *)	% RSD*
Phenylephrine HCl	10	1277.75±4.8363	0.3785
Ebastine	10	1802.384±10.153	0.5633

**n=6*

Table 6(a): Results for intermediate precision (Inter-day)

	Interday	
Conc.	**Phenylephrine HCl %RSD***	**Ebastine %RSD***
50 %	0.7113	0.6626
100 %	0.7762	0.7448
150 %	0.4650	0.8864

****n=3***

Table 6(b): Results for intermediate precision (intra-day)

Intraday		
Conc.	**Phenylephrine HCl %RSD***	**Ebastine %RSD***
50 %	0.9282	0.5530
100 %	0.9077	1.0642
150 %	0.6068	0.7164

n=3

Table 7: Robustness studies of Phenylephrine HCl and Ebastine

Change in flow rate (1 ml/min ± 0.2 ml/min)			
	Flow rate (ml/min)	**Area (Mean±SD*)**	**%RSD***
Phenylephrine HCl	1.2 ml/min	1250.113±17.62	1.41
	0.8 ml/min	1326.512±19.11	1.44
Ebastine	1.2 ml/min	1754.164±32.76	1.86
	0.8 ml/min	1872.860±17.77	0.94
Change in mobile phase composition (± 2% v/v)			
	Mobile phase (70:30)v/v	**Area (Mean±SD*)**	**%RSD***
Phenylephrine HCl	(72:28) v/v	1250.164±32.76	1.11
	(68:32) v/v	1313.800±14.04	1.06
Ebastine	(72:28) v/v	1762.058±17.22	0.97
	(68:32) v/v	1853.114±15.28	0.82
Change in pH (4.0±0.2)			
	Change in pH	**Area (Mean±SD*)**	**%RSD***
Phenylephrine HCl	4.2	1226.226±14.47	1.18
	3.8	1315.910±14.13	1.07
Ebastine	4.2	1723390±17.64	1.02
	3.8	1852.540±15.69	0.84

n=3

Table 8: LOD and LOQ

Parameters	**Phenylephrine HCl**	**Ebastine**
LOD (µg/ml)	1.12	0.46
LOQ (µg/ml)	3.41	1.41

CONCLUSION

It can be concluded from the results that the proposed RP-HPLC method was found to be simple, accurate, robust, precise, reproducible and economic for the analysis Phenylephrine Hydrochloride and Ebastine in bulk and tablet dosage forms. This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of Phenylephrine Hydrochloride and Ebastine.

CONFLICT OF INTERESTS

Declared None

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