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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF EBASTINE AND PHENYLEPHRINE HYDROCHLORIDE IN TABLET FORMULATION BY RP- HPLC

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ABSTRACT

A simple, sensitive and reliable spectroscopic method for estimation of Ebastine and Phenylephrine hydrochloride in combined dosage form has been attempted. In RP-HPLC method, the analyte were resolved using MeOH: KH₂PO₄ Buffer (10 mM):(80:20 PH 5.5) at a flow rate of 1.5 ml/min, on Thermo Separation Product Quaternary Gradient HPLC pump Spectra System P4000 system containing of UV- 1000 UV- visible detector with Data Ace Software and ProntoSil C18 4.6 (id) x 250 mm. The method gave the good resolution and suitable retention time. HPLC with ProntoSil 4.6 (id) x 250 mm column and UV-1575 detector was used for the study. The standard and sample solution of EBS and PHE was prepared in methanol. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram. The detection was carried out at 275 nm..The selection of the wavelength was based on the λ_{max} obtained by scanning of standard laboratory mixture. This system gave good resolution and optimum retention time with appropriate tailing factor (less than 2).

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INTRODUCTION

Ebastine is chemically 1-[4-(1, 1-Dimethylethyl) phenyl]-4-[4- (diphenylmethoxy) piperidin-1- yl] butan-1-one. *It is used as Anti-Histaminic.* Ebastine is practically insoluble in water, sparingly soluble in methanol with pKa 10.3. Phenylephrine Hydrochloride is chemically (1R)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol hydrochloride is also used as Anti-Histaminic in cough and cold preparations. It is freely soluble in water and in ethanol (96%), as well as freely soluble in methanol with pKa 8.9.

The market survey revealed that Ebastine and Phenylephrine hydrochloride in combination is recently introduced in market as tablet dosage form. It is indicated as Anti-Histaminic. Literature survey revealed that Ebastine is official in BP and Phenylephrine hydrochloride is official in BP as well as IP. Although there are many methods reported for estimation of these drugs singly. However, no method is so far reported for simultaneous estimation of these drugs in combined dosage form.

The present work was undertaken with an objective to develop an accurate, simple, precise and reliable method for simultaneous estimation of these two drugs in their combined dosage form by HPLC.

EXPERIMENTAL WORK

Equipment and Chromatographic Conditions:

The chromatographic system consisted Thermo Separation Product Quaternary Gradient HPLC pump Spectra System P4000 Separation was carried out with a ProntoSIL C18 column (250×4.6mm ID), at 24°C temperature. Mobile phase selected was methanol: phosphate buffer 10mM at pH 5.5 (80:20v/v) at a flow rate of 1.5 ml/min. The UV detector was set at a wavelength of 275 nm. An injection volume of 20 µl was used.

Chemicals and Reagents:

Ebastine and Phenylephrine hydrochloride in combination EBAST-DC (claimed labeled amount 10mg EBS and 10 mg PHE per tablet) was procured from local pharmacies. HPLC-grade methanol was used and all other chemicals (analytical grade) were used. Ebastine and Phenylephrine hydrochloride in pure form were

donated as gift samples from Micro Labs Pvt. Ltd. Bangalore.

Stock and Working solutions:

Accurately weighed quantity of EBS 10 mg was transferred to 100 mL volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol and accurately weighed quantity of PHE 10 mg was transferred to 100 mL volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol.

The standard solution of EBS and PHE were mixed and diluted with methanol properly to obtain laboratory mixtures containing a concentration 10 µg/mL of EBS and 10 µg/mL of PHE. Separate standard calibration graphs were constructed for each component by plotting the peak area of the drug to the drug concentration.

Ruggedness, Accuracy and Precision:

The ruggedness (intra-day, inter-day, different analyst), specificity, precision and accuracy of the methods were estimated by assaying three replicate samples at three different concentrations, on the same day and on three different days. For checking the ruggedness and precision of the method, the relative standard deviations (RSD) were calculated and tabulated. The accuracy of the methods was expressed as percentage. Accuracy of the methods was also determined by recovery studies.

Analysis of marketed formulation:

Twenty tablets were weighed and contents were mixed. Accurately weighed tablet powder equivalent to 10 mg of EBS and 10mg of PHE was transferred in a 100 mL volumetric flask and methanol was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with methanol and was further diluted to get concentration 10 µg/mL of EBS and 10 µg/mL of PHE. The solution was filtered through Whatman filter paper No.42.

Equal volume (20µL) of standard and sample solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of EBS and PHE was calculated by comparing a sample peak with that of standard.

RESULT AND DISCUSSION

This method provides a simple procedure to determine simultaneously the concentration of EBS and PHE in bulk drugs and pharmaceutical dosage forms. To develop a rugged and suitable LC method, various mobile phase compositions, flow rate and different temperatures were tested.

Different trials of chromatographic conditions:

- 1) MeOH: KH₂PO₄ Buffer (10 mM) (95:05 pH 3.0)
- 2) MeOH: KH₂PO₄ Buffer (10 mM) (90:10 pH 2.8)
- 3) MeOH: KH₂PO₄ Buffer (10 mM) (85:15 pH 4.0)
- 4) MeOH: KH₂PO₄ Buffer (10 mM) (80:20 pH 7.2)
- 5) MeOH: KH₂PO₄ Buffer (10 mM) (80:20 pH 6.5)
- 6) MeOH: KH₂PO₄ Buffer (10 mM) (80:20 pH 5.5)

Chromatographic conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout method.

Column	: Prontosil 4.6 (id) x 250 mm
Particle size packing	: 5 μm
Stationary phases	: C18
Mobile phase	: Methanol: KH ₂ PO ₄ Buffer (10mM) (80:20) pH 5.5
Detection wavelength	: 275 nm
Flow rate	: 1.5 ml/min.
Temperature	: Ambient
Sample size	: 20 μL

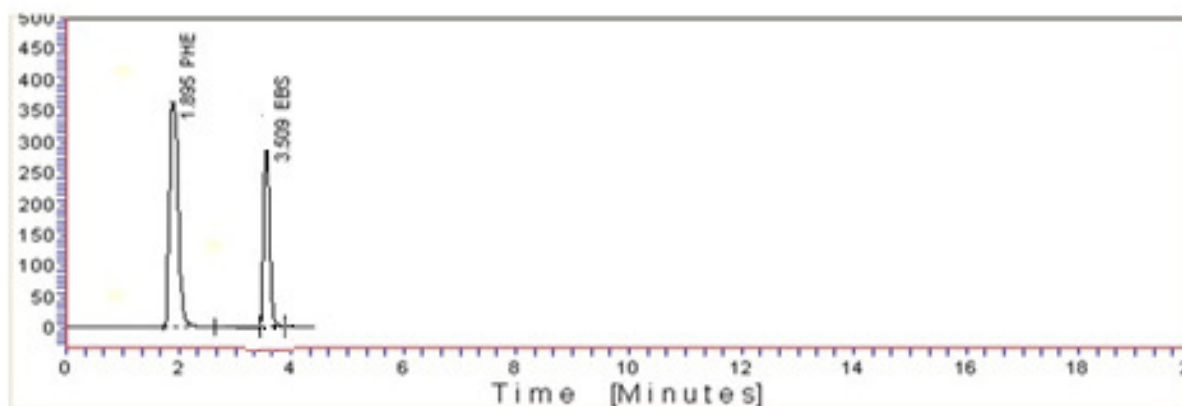


Fig. No. 1: chromatogram obtained by using MeOH: KH₂PO₄ Buffer (10 mM) (80:20 pH 5.5) as mobile phase.

The USP suggests that system suitability tests to be performed prior to analysis. The parameters include tailing factor, capacity factor, theoretical plate number, retention time, asymmetric factor, and selectivity and RSD % of peak height or area for repetitive injections. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. Some of tests were carried out on freshly prepared standard solutions. Tailing factors of 1.6 and 1.9 were obtained for EBS and PHE respectively, with asymmetry factors of 1.77 and 1.51 for EBS and PHE respectively. The chromatographic conditions described ensured adequate retention and resolution for both of the analytes. The retention times of EBS and PHE were 3.5 and 1.8 min. respectively the variation in retention

time for five replicate injections of two compounds reference solutions gave RSDs of 0.12% for EBS and 0.13% for PHE. The results obtained from the system suitability tests satisfy the USP requirements. The calibration curve and equations for EBS and PHE in methanol was calculated by plotting the peak area and was found to be linear.

System Suitability:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Table No. 1: Summary of system suitability test results

Sr.No.	Parameter	EBS	PHE
1.	Peak area	583.124	561.700
2.	Retention time (min)	3.518	1.889
3.	Asymmetry	1.778	1.511
4.	Efficiency	23772.85	16641.75

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analyzed by following procedure described under experimental and results. It gave accurate, reliable results and was

extended for estimation of drugs in marketed tablet formulation. The summary of results of laboratory mixture and marketed formulation are given in the Table. No2

Table No.2: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method:

Sr. no.	Sample	Statistical data	% Estimation		% Recovery	
			EBS	PHE	EBS	PHE
1.	Standard Laboratory mixture	Mean	99.65	99.47	-	-
		S.D.	1.05	1.016	-	-
		C.V.	1.05	1.021	-	-
2.	Ebast-DC	Mean	99.74	99.72	99.97	99.98
		S.D.	0.45	0.29	0.0378	0.02
		C.V.	0.451	0.29	0.0378	0.02

Recovery studies were also conducted with the tablets using the standard addition method to determine the accuracy and precision. The recovery was measured by spiking the already analyzed samples of tablets with known concentrations of standard solutions of the studied compounds. From the results, it is concluded that the method is sufficiently accurate and precise in order to be applied to the tablet dosage forms.

Validation:

Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guidelines for the following criteria.

1) Accuracy - Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Result are shown in the Table No. 3

Table No.3: Results and statistical data of accuracy Study:

Sr. no.	Weight of tablet powder (mg)	Amount of Drug Added in($\mu\text{g/ml}$).		Peak Area of stand.		Peak Area of sample		% Recovery	
		EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE
1	10	2	2	150.317	145.575	180.356	174.68	99.93	99.96
2		4	4			210.4435	203.806	99.99	100
3		6	6			240.5073	229.91	100	99.98
Mean								99.97	99.98
\pmS.D.								0.0378	0.02
C.V								0.0378	0.02

2) Precision:

Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

3) Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for EBS and PHE without any interference from component of matrix.

Mean retention time for –

EBS -3.5 min

PHE- 1.8 min.

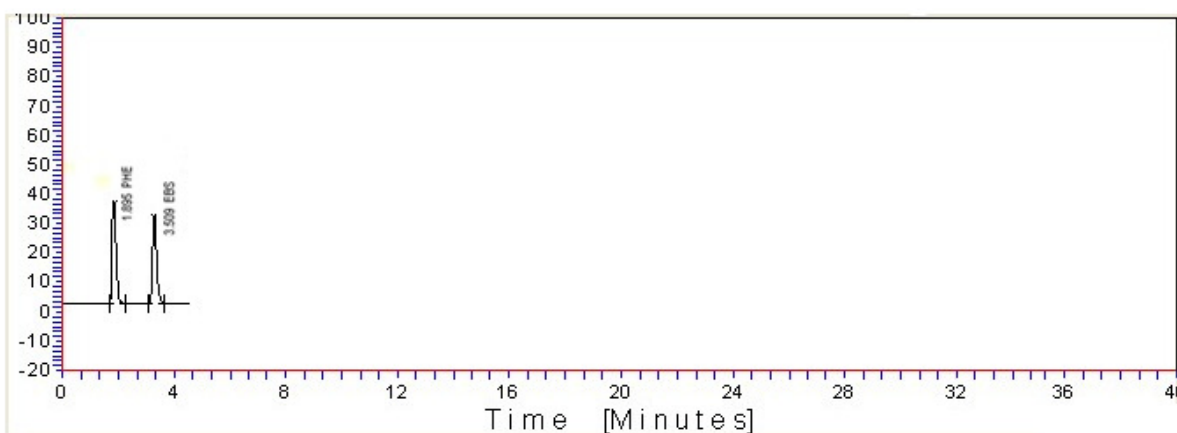


Fig. No.2: Chromatogram obtained by marketed formulation of EBS and PHE showing retention time for EBS– 3.5 min. and PHE – 1.8 min.

4) Ruggedness - Studies were carried out only for the two different parameters like different time, different

day and different analyst. Results of estimation by proposed method are very much similar under variety of

conditions. This study signifies the ruggedness of the method under varying condition of its performance.

Table No.4: Summary of results of Ruggedness by RP-HPLC method

Parameter	Statistical data	% Estimation by RP-HPLC method	
		EBS	PHE
Interday	Mean	99.51	99.45
	± S.D.	0.82	0.40
	C.V.	0.824	0.402
Intraday	Mean	99.20	99.62
	± S.D.	0.579	0.456
	C.V.	0.583	0.459
Different analyst	Mean	99.86	99.77
	± S.D.	0.365759	0.298378
	C.V.	0.36625	0.299049

CONCLUSION

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Ebastine and Phenylephrine hydrochloride in their combined dosage formulations.

The method shows good reproducibility Compared to UV-spectrophotometric methods, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of combined dose formulation of Ebastine and Phenylephrine hydrochloride can also be successfully performed by the UV-spectrophotometric methods.

The UV spectrophotometric method is also simple, accurate, precise, reproducible, economical and rapid too. It may be adopted for routine control analysis of these two drugs in combined dosage form.

No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

Suitability of these methods on biological samples also needs study.

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