

Development and Validation of First Order Derivative Spectroscopic Method for Content Uniformity for Simultaneous Estimation of Ebastine and Phenylephrine Hydrochloride in Combined Tablet Dosage Form

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Abstract: A novel, simple, accurate, sensitive and reproducible first order derivative spectroscopic method was developed and validated for estimation of Ebastine and Phenylephrine Hydrochloride in combined dosage form. The method obeys Beer's Law in concentration ranges of 5-40 µg/ml for Ebastine and Phenylephrine Hydrochloride both. The method was validated for linearity, accuracy and precision as per ICH guidelines. The zero crossing point for Ebastine and Phenylephrine hydrochloride was 231.61 nm and 242.21 nm respectively in methanol. The LOD and LOQ value were found to be 0.84 µg/ml and 2.54 µg/ml for Ebastine, 0.94 µg/ml and 2.85 µg/ml for Phenylephrine hydrochloride respectively. The accuracy of method was assessed by recovery studies was found to be 99.74 ± 0.1386 and 100.23 ± 0.0854 for Ebastine and Phenylephrine hydrochloride respectively. The developed and validated method was successfully used for quantitative analysis of commercially available dosage forms. The developed method further can be useful for dissolution study and bio analytical study for these drugs in combinations.

Key words: Ebastine, Phenylephrine hydrochloride, First order derivative spectroscopic method, Simultaneous estimation, Accuracy, Precision.

INTRODUCTION:

Ebastine (EBS) chemically is 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl) butan-1-one. It is a longacting non-sedating second-generation H1 receptor antagonist that is indicated mainly for allergic rhinitis and chronic idiopathic urticaria. The chemical structure of EBS is shown in Figure 1.

Phenylephrine hydrochloride (PHE) chemically is (1R)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol hydrochloride. It is a selective α₁-adrenergic receptor agonist used for treatment of stuffy nose, sinusitis, bronchitis,

vascular failure in shock, and drug-induced hypersensitivity. The chemical structure of PHE is shown in Figure 2.

Combined formulation of Ebastine and Phenylephrine hydrochloride have additive or synergistic role in cough and antiallergic preparation, since both acts by attenuating the sign and symptoms of common cold and allergy. So, quality of this formulation is most important. So, it is useful to develop method for testing of quality of this formulation.^[1]

Literature Survey reveals that no method for first order derivative spectroscopy for EBS and PHE in tablet dosage form has been reported. However simple UV methods^[2-5] and RP-HPLC method^[6] for EBS and PHE have been noted. Simple UV methods^[7-14] and RP-HPLC^[15-19] for EBS and simple UV methods^[20-24] and RP-HPLC^[25-42] for PHE have been noted. Hence attempt has been made to develop and validate in accordance with ICH guidelines, a simple, precise and accurate first order spectrophotometric method for simultaneous estimation of EBS and PHE in combined tablet dosage form.

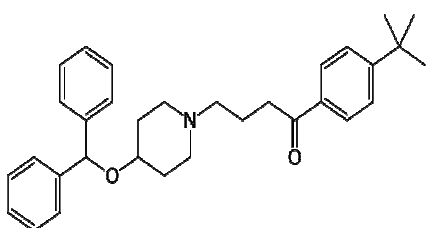


Figure 1: Structure of Ebastine

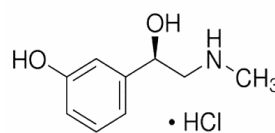


Figure 2: Structure of Phenylephrine hydrochloride

EXPERIMENTAL

Materials and equipment:

Reference standards of EBS and PHE were procured as gift samples from Bal Pharma Pvt. Ltd. (Bangalore) methanol AR grade was purchased from RFCL Ltd., India. Tablets of Ebast-DC were purchased from local market; each tablet was labeled to contain 10 mg EBS and 10 mg of PHE.

Instrumentation:

Double beam UV-Visible spectrophotometer (Shimadzu 1800 with UV Probe 2.42 software) and a pair of 1 cm matched quartz cells were used. Acculab AUX 220 electronic analytical balance.

Selection of solvent:

EBS and PHE are soluble in methanol. So, methanol is selected as solvent and used for preparation of stock solutions and working standard solutions.

Preparation of standard solutions:

Preparation of EBS and PHE stock – working standard solution:

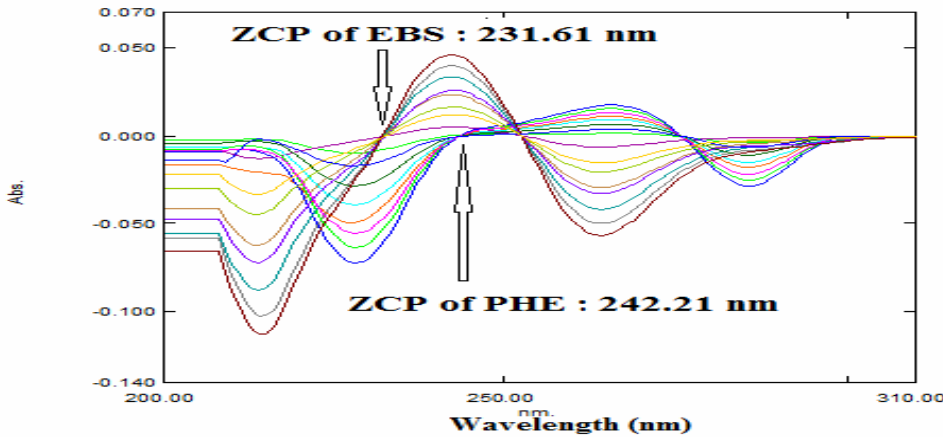
Accurately weighed 25 mg of EBS and 25 mg PHE were transferred to 25 ml volumetric flask individually, dissolved and diluted up to mark with methanol to obtain final concentration of 1000 µg/ml EBS and 1000 µg/ml PHE. Solution was further diluted with methanol to obtain working standard solutions of 100 µg/ml of EBS and 100 µg/ml PHE respectively.

Determination of zero crossing point:

Solutions having concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for EBS and concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for PHE were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The zero order spectra of both the drugs were derivatised to first order. First order derivative spectra were selected for analysis of both the drugs. From the

overlay spectra of both drugs (Figure 3), wavelength selected for quantitation were 242.21 nm for EBS (zero cross for PHE) and 231.61 nm for PHE (zero cross for EBS).

Figure 3: Overlay first order spectra of EBS and PHE in methanol



Preparation of Calibration curve for EBS and PHE:

Solutions having concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for EBS and concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for PHE in mixture were prepared from working standard solution and spectra were recorded in spectroscopic condition. Spectra were converted to first order derivative spectra using UV probe software (ver. 2.42). Amplitude (dA/dλ) of both the drugs was measured at 242.21 nm for EBS (zero cross for PHE) and 231.61 nm for PHE (zero cross for EBS). Standard calibration curves of dA/dλ vs. concentration were plotted (Figure 4 and 5).

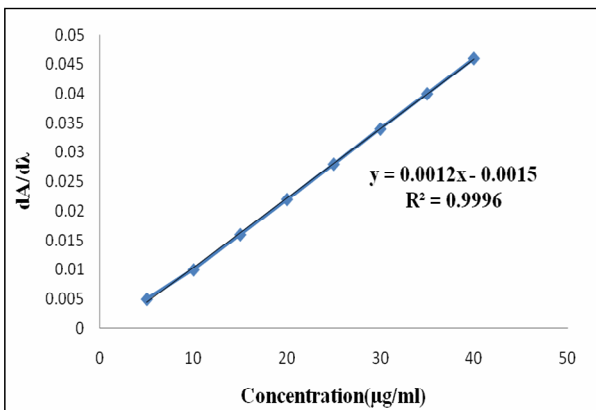


Figure 4: Calibration curve of EBS (at ZCP of PHE)

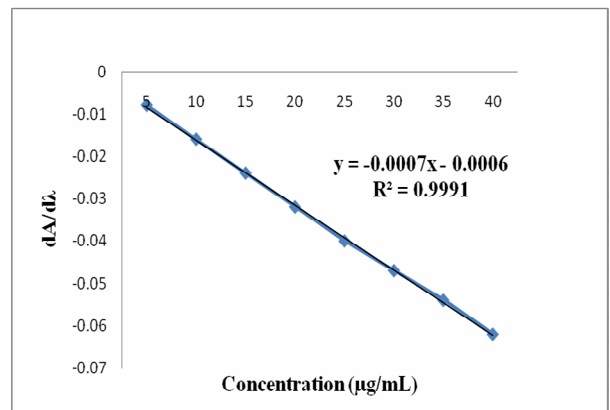


Figure 5: Calibration curve of PHE (at ZCP of EBS)

Method validation:^[43]

The method was validated by validation parameters linearity, precision, accuracy, robustness, LOD and LOQ.

Linearity:

Series of standard solutions were prepared by dilution of the working standard solutions with methanol which having concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for EBS and concentration 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml for PHE. The absorbances of the derivatised spectra were measured at 242.21 nm and 231.61 nm for EBS and PHE, respectively. Six replicate analyses were carried out.

Precision:

Repeatability: Solution containing mixture of 10 µg/ml of EBS and 10 µg/ml of PHE (100 % test concentration) were prepared from working standard solution and analyzed as per the proposed method for system precision, method precision, reproducibility, intra-day and inter-day precision. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for the drugs.

Intraday Precision: Solution containing the mixture of 15 and 15 µg/ml, 20 and 20 µg/ml and 25 and 25 µg/ml of EBS and PHE respectively were prepared and analyzed. Analysis was replicated for 3 different times within same day and %RSD was calculated.

Interday Precision: Solution containing the mixture of 15 and 15 µg/ml, 20 and 20 µg/ml, 25 and 25 µg/ml EBS and PHE respectively were prepared and analyzed. Analysis was replicated for 3 different days and %RSD was calculated.

Accuracy:

Tablet powder equivalent to 10 mg was transferred to three individual 100 ml volumetric flask, 15 ml methanol was added to dissolve the drugs and add 8 mg (Flask 1), 10 mg (Flask 2), and 12 mg (Flask 3) of standard powder of both EBS and PHE, and sonicated for 10 minutes then made up to the mark with methanol to made them 80%, 100% and 120% spiking. The solution was then filtered through a Whatmann filter paper. Pipette out 1.0 ml filtered solution from each flask is diluted 10 times. At each level of the amount three determinations were performed and the result obtained was compared with expected results.

Robustness:

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. The parameters were change of scanning speed and change in manufacturer of methanol. The result is expressed in percentage RSD. Three replicate analyses were carried out.

Ruggedness:

Solution containing mixture of 10 µg/ml EBS and 10 µg/ml PHE was prepared from their respective stock-working standard solutions prepared. Prepared solution was analyzed as per proposed method by 2 different analyst. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for each analysis.

Limit of detection (LOD) and limit of quantitation (LOQ):

Limit of Detection (LOD) and Limit of Quantitation were determined on the basis of standard deviation and slop of the regression equation.

$$\text{LOD} = (3.3 \times \text{SD}) / \text{Slope}$$

$$\text{LOQ} = (10 \times \text{SD}) / \text{Slope}$$

Content uniformity:

Randomly select 10 units and perform testing on each individual. Each individual tablet was weighed and finely powdered. For a single tablet a portion of powder equivalent to the weigh of 10 mg was accurately weighed and transferred into 10 ml volumetric flask and 5 ml methanol was added. The volumetric flask was sonicated for 20 min to effect complete dissolution of the EBS and PHE, the solution was then made up to volume with methanol. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 20 µg/ml of EBS and 20 µg/ml of PHE. The % stated value of the drugs was calculated.

Table 1 - Result of linearity, range, LOD and LOQ for First order derivative spectroscopy

Parameter		EBS	PHE
Linearity	Range	5-40 µg/ml	5-40 µg/ml
	Equation	$y = 0.0012x - 0.0015$	$y = -0.0007x - 0.0006$
	R ²	0.9996	0.9991
LOD		0.84 µg/ml	0.94 µg/ml
LOQ		2.54 µg/ml	2.85 µg/ml

Table 2 - Result of accuracy study for First order derivative spectroscopy

Level	Total Amount Taken (mg)		Amount Obtained (mg)		% Recovery		SD		%RSD		
	EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE	
L-1	1	18	18	17.83	18.17	99.07	100.94	0.1450	0.1021	0.8066	0.5646
	2			17.98	18.14	99.88	100.78				
	3			18.12	17.98	100.67	99.89				
	Mean % Recovery			17.98	18.09	99.87	100.54				
L-2	1	20	20	19.91	20.08	99.58	100.04	0.1386	0.0854	0.6955	0.4257
	2			20.10	20.15	100.5	100.75				
	3			19.83	19.98	99.15	99.90				
	Mean % Recovery			19.94	20.07	99.74	100.23				
L-3	1	22	22	21.83	21.86	99.24	99.35	0.1500	0.1429	0.6824	0.6524
	2			22.13	22.14	100.59	100.63				
	3			21.98	22.05	99.90	100.22				
	Mean % Recovery			21.98	21.91	99.91	100.07				

Table 3 - Result of Precision for First order derivative spectroscopy

Parameter	Drug	SD	% RSD
Repeatability (n=6)	EBS	0.3040	0.3061
	PHE	0.3889	0.3913
Intra-day Precision (n=3)	EBS	0.7082	0.7041
	PHE	0.9192	0.9203
Inter-day Precision (n=3)	EBS	0.4198	0.4206
	PHE	0.4681	0.4689

Table 4 - Result of Robustness study for First order derivative spectroscopy

Variation and Level		Concentration ($\mu\text{g/ml}$)		% Labelled Claim		Mean		SD		%RSD	
		EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE
Change in Scanning speed	Slow	10	10	99.11	99.34	99.61	99.77	0.5156	0.4457	0.5176	0.4467
	Medium	10	10	99.58	99.74						
	Fast	10	10	100.14	100.23						
Change in methanol manufacture	Merck	10	10	99.14	98.76	99.62	99.34	0.6858	0.8273	0.6884	0.8327
	Rankem	10	10	100.11	99.93						

Table 5 - Result of Ruggedness Study for First order derivative spectroscopy

Variation and Level		Concentration ($\mu\text{g/ml}$)		% Labelled Claim		Mean		SD		%RSD	
		EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE
Different Analyst	Analyst-1	10	10	99.34	98.76	99.93	99.26	0.8414	0.7071	0.8420	0.7123
	Analyst-2	10	10	100.53	99.76						

Table 6 - Content uniformity result by First order derivative spectroscopy

Sr. no.	Labelled claim (mg)		$dA/d\lambda$		Amount obtained (mg)		%Labelled Claim	
	EBS	PHE	EBS	PHE	EBS	PHE	EBS	PHE
1	10	10	0.0227	-0.0145	10.08	9.92	100.83	99.28
2	10	10	0.0223	-0.0147	9.91	10.07	99.16	100.71
3	10	10	0.0228	-0.0144	10.12	9.85	101.25	98.57
4	10	10	0.0224	-0.0146	9.95	10.00	99.58	100.00
5	10	10	0.0226	-0.0145	10.04	9.92	100.41	99.28
6	10	10	0.0222	-0.0148	9.87	10.14	98.75	101.42
7	10	10	0.0229	-0.0144	10.16	9.85	101.66	98.57
8	10	10	0.0225	-0.0147	10.00	10.07	100.00	100.71
9	10	10	0.0221	-0.0148	9.83	10.14	98.33	101.42
10	10	10	0.0223	-0.0147	9.91	10.07	99.16	100.71

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of EBS and PHE. In first order derivative spectroscopy, wavelengths selected for quantitation were 242.21 nm for EBS (ZCP for PHE) and 231.61 nm for PHE (ZCP for EBS). Both the drugs obey the Beer's law with the concentration range (EBS: 5 – 40 $\mu\text{g/ml}$, PHE: 5 – 40 $\mu\text{g/ml}$) with R^2 value of 0.9996 and 0.9991 for EBS and PHE respectively ($n = 6$) (Figure 4 and 5, Table 1). The mean % recovery was found to be 99.74% and 100.23%

for EBS and PHE respectively (Table 2).The Limit of Detection (LOD) and Limit of Quantitation (LOQ) value was found to be 0.84 μ g/ml and 2.54 μ g/ml for EBS and 0.94 μ g/ml and 2.85 μ g/ml for PHE respectively (Table 1).A result of precision, robustness and ruggedness for this method was also discussed in tables (Table 3 ,4 and 5).The Content uniformity results are discussed in table (Table 6).

The proposed method was found to be simple, accurate and rapid for the routine estimation of Ebastine and Phenylephrine hydrochloride in tablet formulation. To study the validity and reproducibility of proposed method, recovery studies were carried out. The method was validated in terms of linearity, accuracy, precision and robustness. So, method can be successfully used for simultaneous estimation of Ebastine and Phenylephrine hydrochloride in combined dosage form. The developed method further can be useful for dissolution study and bio analytical study for these drugs in combinations.

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