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UV-Spectrophotometric estimation of Ebastine and Phenylephrine Hydrochloride in tablet dosage form using absorption ratio method

Love Kumar Soni*, Tamanna Narsinghani and Charu Saxena

School of Pharmacy, Devi Ahilya University, Takshashila Campus, Ring Road, Indore, India

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ABSTRACT

The proposed method for simultaneous analysis of EBS and PHE uses the ratio of observed absorbance at two selected wavelengths. The two selected wavelengths for this method were 252 (λ_{max} of Ebastine) and 272 nm (isoabsorptive point). These were used to determine the unknown concentration of pharmaceutical formulation. The method was validated according to ICH guidelines. The proposed method is accurate and precise, require no separation steps and can be used for the routine analysis of both drugs in the pharmaceutical formulations.

Key words: absorption ratio method, Ebastine, Phenylephrine hydrochloride.

INTRODUCTION

Ebastine (EBS) is second generation H_1 – receptor antagonist and antihistaminic agent which binds preferentially to peripheral H_1 receptor without central side effects. It is indicated for the sympatomatic treatment of seasonal and perennial allergic and chronic urticaria [1-7]. Phenylephrine hydrochloride (PHE) is α_1-adrenoceptor agonist which stimulates postsynaptic alpha receptor cause vasoconstriction ,systolic and diastolic pressure.It is indicated for nasal congestion, minor eye irritations and open angle glaucoma [8,9]. EBS has been determined by RP-HPLC method [10,11], liquid chromatography ionspray tandem mass spectrometry [12,13], high performance thin layer chromatography [14] in single and combination with other drugs . The estimation of PHE using UV-spectroscopy has been reported in combination with other drugs [15-19]. The UV spectrophotometric determination is a well established analytical technique to determine the active pharmaceutical ingredients in their pharmaceutical formulations. [20-22] The objective of the work is to develop new absorption ratio method for estimation of EBS and PHE in tablet dosage form with good accuracy, simplicity, precision and economy.

MATERIALS AND METHODS

The instrument used for the analysis was UV double beam spectrophotometer (Shimadzu Model 1700).Gift sample of ebastine was procured from Tonira Pharma Ltd. Gujarat and phenylephrine hydrochloride was procured from Torrent Pharma Ltd. Gujarat. Combined ebastine and phenylephrine hydrochloride tablets (Ebast-Dc containing ebastine-10 mg and phenylephrine hydrochloride -10 mg and manufactured by micro labs ltd.Pondicherry) were purchased from local market. Analytical grade methanol was used as the common solvent.

In absorption ratio, absorbances of both the drugs were calculated at two selected wavelengths; among which λ_1 is the wavelength of iso-bestic point (where both shows same absorbance) and λ_2 is the λmax of either drug among the drugs to be analyse. From the overlain spectra wavelength 272 (λ_1 –isobestic point) and 252 (λ2- λmax of EBS) were selected for study. The concentration of the individual drug components were calculated by using the following equations.

$$C_x = \frac{Q_m - Q_v}{Q_x - Q_y} \times \frac{A_1}{ax_1} \quad \text{……Equation. 1} \qquad Cy = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{ax_1} \quad \text{….Equation. 2}$$

$$\frac{A_1}{Ax_1}$$

Where Q_m = A1 is absorbance of sample at λ_1 (isobestic point), A_2 is absorbance of sample at (λmax of EBS).

$$Qx = ax_2 / ax_1 \qquad Qy = ay_2 / ay_1$$

where, ax_1 and ax_2 represents absorptivities of EBS at λ_1 and λ_2 having value of 0.0153 and 0.0162 and ay_1 and ay_2 denote absorptivities of PHE at λ_1 and λ_2 having value 0.0154 and 0.0473 respectively: C_x and C_y be the concentration of EBS and PHE respectively. Q_x having value 1.058 and Q_y having value of 3.071.

2.1. Preparation of Standard stock solutions

10mg each of EBS and PHE were accurately weighted and dissolved in 100 ml of methanol to get solution of 100 µg/ml.

2.2. Preparation of standards solutions for linearity study

From the standard stock solutions of 100µg/ml of both EBS and PHE, different dilutions were prepared for each drug having concentration as shown in Table 1 with methanol. Then these solutions were scanned over the range of 400-200nm and absorbances were measured in the spectrum mode at the respective analytical wavelengths 272nm ($\lambda_{1)}$ and 252 nm ($\lambda_{2)}$ respectively. The calibration curves were plotted between the mean value of the observed absorbance and respective concentration. From the calibration curve it was found that both the drugs follows Beer's – Lamberts law within the range of 5-40µg/ml for EBS and 5-40 µg/ml for PHE respectively.

2.3. Analysis of Mixed Samples

The method was checked by analyzing a solution containing known concentration of both the drugs. The mixed solutions were prepared from the standard stock solutions. Absorption was measured at the analytical wavelengths and put these values in the respective Eqn.1 and 2 to

calculate the results which shows the accuracy of the method. The results are given in the Table 2.

2.4. Analysis of Ebast-DC

The dilutions of the marketed formulation were prepared as per section 6.3.4, then these dilutions were scanned over the range of 400-200nm in the spectrum mode and measured the absorbance at 272 nm and 252 nm, and these values were put in the respective Eqn. 1 and 2 to calculate the % of drug content. The results are present in Table 3 and its statistical validation result are reported in Table 4.

2.5. Recovery Study

The dilutions for the recovery study were prepared as per scheme 6.3.5 and these dilutions were scanned over the range of 400-200nm and measured its absorbances at the selected analytical wavelengths to measured the % of drug content by applying these values in Eqn. 1 and 2. The results and its statistical validation results are reported in Table 5 and 6.

2.6. LOD and LOQ study

From the standard stock solutions of both the drugs 1µg/ml solutions of both the drugs were prepared by further dilution with methanol. From these 1µg/ml solutions further dilutions of concentration 0.2, 0.3, 0.4, 0.5, 0.6 µg/ml were prepared for both the drugs by diluting with methanol. Then these solutions were scanned over the range of 400-200nm and absorbances were measured at the analytical wavelengths 272 and 252 nm. This is repeated five times and the standard deviation of the analyte was calculated to determine the value of LOD and LOQ. The absorbances of EBS are reported in Table 7.

2.7. Intermediate precision (inter-day and intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. To determine the intraday precision absorption of the prepared solutions of the tablet were taken on the very day at an interval of 1hr, 2hr and 3rdhr at the selected analytical wavelengths respectively. Then by applying these values in the respective Equations 1 and 2, calculate the percentage of drug content. For determination of interday precision the same solutions were used to determine the absorbance at the particular analytical wavelengths on 1st, 2nd and 3rd day calculate the percentage of drug content. The results of intra and inter day precision study for both drugs EBS and PHE are reported in Table 8 and 9.

RESULTS AND DISCUSSION

The % recovery of EBS and PHE was found to be close to 100 %. The low value of % Coefficient of variance (% COV) and standard deviation (S.D) indicating that developed methods were accurate and precise for quantitative estimation of both EBS and PHE in tablet dosage form.

The sample recovery was in good agreement with respective label claim, which suggested non interference of formulation additives in estimation. The developed method is simple, rapid, inexpensive, accurate, precise and convenient for the routine analysis.

Table 1. Linearity of EBS at 272 nm

Replicate no.	Absorbance of EBS at concentration (µg/ml)							
	5	10	15	20	25	30	35	40
Replicate 1	0.075	0.148	0.228	0.302	0.378	0.448	0.525	0.585
Replicate 2	0.078	0.152	0.225	0.308	0.375	0.445	0.528	0.580
Replicate 3	0.076	0.154	0.229	0.305	0.376	0.442	0.527	0.582
Replicate 4	0.075	0.146	0.226	0.301	0.382	0.452	0.522	0.583
Replicate 5	0.072	0.151	0.232	0.302	0.381	0.456	0.523	0.586
Mean	0.075	0.150	0.228	0.303	0.378	0.448	0.525	0.583
Standard Deviation (S.D.)	0.0021	0.0028	0.0027	0.0028	0.0030	0.0055	0.025	0.031
Standard Error (S.E.)	0.0009	0.0012	0.0012	0.0012	0.0013	0.0024	0.011	0.013

Table 2. Analysis of mixed standard (Absorption ratio method)

S. No.	Amount Present (µg/ml)		Amount Found			
			µg/ml		%	
	EBS	PHE	EBS	PHE	EBS	PHE
1	5	40	4.99	39.97	99.91	99.90
2	10	35	9.94	34.75	99.40	99.30
3	15	30	14.99	29.66	99.97	98.90
4	20	25	19.80	24.51	99.02	98.04
5	25	20	24.97	19.62	99.89	98.10
6	30	15	29.66	14.85	98.86	99.00
7	35	10	34.97	9.82	99.9	98.20
8	40	5	39.66	4.96	99.15	99.20

Table 3. Result of Analysis of Ebast-DC

Replicate no.	Lable claim (mg/Tab)		Conc. Found (mg/Tab)		Percentage found	
	EBS	PHE	EBS	PHE	EBS	PHE
Replicate - 1	10	10	9.99	9.96	99.9	99.6
Replicate - 2	10	10	9.98	9.96	99.8	99.6
Replicate - 3	10	10	9.95	10.02	99.5	100.2
Replicate - 4	10	10	9.92	9.95	99.2	99.5
Replicate - 5	10	10	9.88	10	98.8	100

Table 4. Result of statistical validation of Ebast-DC

S. No	Drug	Mean %	S.D.	% COV	S.E.
1	EBS	99.44	0.502	0.506	0.259
2	PHE	99.78	0.380	0.39	0.171

Table 5. Result of recovery study of Ebast-DC

Replicate no.	Amount taken (µg/ml)		Amount added (µg/ml)			% Recovery	
	EBS	PHE	%	EBS	PHE	EBS	PHE
Replicate-1	10	10		7	6.5	98.45	100.01
Replicate-2	10	10	80	7	6.5	98.17	100.53
Replicate-3	10	10		7	6.5	98.29	100.11
Replicate-1	10	10		10	10	99.34	101.5
Replicate-2	10	10	100	10	10	99.23	101.7
Replicate-3	10	10		10	10	99.21	101.5
Replicate-1	10	10		13	11.5	98.53	101.45
Replicate-2	10	10	120	13	11.5	98.53	101.47
Replicate-3	10	10		13	11.5	99.9	101.83

Table 6. Result of statistical validation of recovery study

S. No.	Drug	Mean (%)	S.D.	% COV
80%	EBS	98.30	0.175	0.179
	PHE	100.21	0.336	0.337
100%	EBS	99.26	0.011	0.011
	PHE	101.56	0.114	0.112
120%	EBS	98.98	0.545	0.556
	PHE	101.58	0.263	0.261

Table 7. LOD and LOQ of EBS

Replicate No.	Absorbance of EBS at concentration (µg/ml)				
	0.2	0.3	0.4	0.5	0.6
Replicate - 1	0.012	0.028	0.035	0.047	0.056
Replicate -2	0.011	0.026	0.036	0.048	0.055
Replicate -3	0.013	0.025	0.033	0.046	0.052
Replicate -4	0.014	0.028	0.032	0.047	0.051
Replicate -5	0.012	0.027	0.035	0.045	0.053
Mean	0.012	0.026	0.034	0.046	0.053
S.D.	0.0011	0.0013	0.0016	0.0011	0.002

Table 8. Intra and Inter day precision study of EBS

Replicate No.	% Obtained					
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate - 1	99.97	99.95	99.89	99.95	98.55	98.15
Replicate - 2	100.08	100.08	100.02	100.15	98.62	98.22
Replicate - 3	99.85	100.12	99.78	99.93	98.52	98.12
Replicate - 4	100.15	99.83	100.10	100.02	98.60	98.18
Replicate - 5	99.91	99.88	99.82	99.87	98.48	98.08
Mean	99.99	99.97	99.90	99.98	98.55	98.15
S.D.	0.122	0.125	0.108	0.107	0.057	0.053
% COV	0.122	0.125	0.108	0.107	0.058	0.054

Table 9. Intra and Inter day precision study of PHE

Replicate No.	% Obtained					
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate - 1	100.28	100.25	99.98	100.12	99.65	99.15
Replicate - 2	100.32	100.25	99.92	100.08	99.48	98.93
Replicate - 3	100.25	100.18	99.88	99.82	99.25	98.72
Replicate - 4	100.85	100.65	100.36	99.78	99.12	98.58
Replicate - 5	100.76	100.63	100.28	99.95	99.35	98.76
Mean	100.49	100.37	100.08	99.95	99.35	98.76
S.D.	0.288	0.247	0.220	0.151	0.208	0.287
% COV	0.287	0.246	0.220	0.151	0.210	0.291

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