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Research Article

ABSORPTION CORRECTION METHOD AND SIMULTANEOUS EQUATION METHOD FOR THE SIMULTANEOUS ESTIMATION OF EBASTINE AND PHENYLEPHRINE HYDROCHLORIDE IN BULK AND INCOMBINED TABLET DOSAGE FORM

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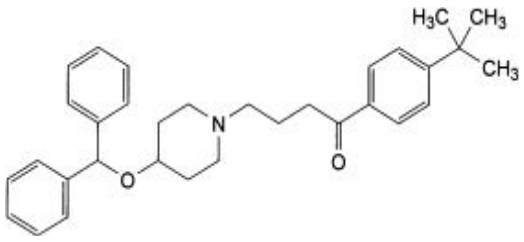
ABSTRACT

A new, simple, accurate and sensitive UV - spectrophotometric absorption correction method and simultaneous equation method has been developed for simultaneous determination of Ebastine and Phenylephrine HCL in bulk and in combined tablet dosage form. Methanol was used as solvent. The wavelengths selected for absorption correction method were 225 nm and 253 nm for Ebastine and Phenylephrine HCL respectively and in case of simultaneous equation method the wavelengths selected were 240 nm and 275 nm for Ebastine and Phenylephrine HCL respectively. Beer's law obeyed the concentration range of 4 – 40 µg/ ml, for both drugs. The percentage recovery was found in the range of 100.42 - 101.27% for Ebastine and 100.51 - 101.40% for Phenylephrine HCL. The developed methods were validated statistically and by recovery studies. The % RSD value was found to be less than 2. Thus the proposed methods are simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of Ebastine and Phenylephrine HCL in bulk and in combined tablet dosage form.

Keywords: Ebastine, Phenylephrine HCL, Absorption correction method, Simultaneous equation method.

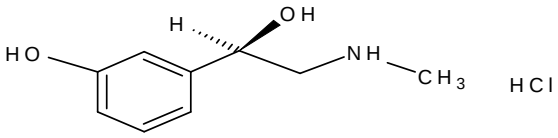
INTRODUCTION

1) Ebastine



Ebastine (EBS), 1-[4-(1,1-Dimethylethyl) phenyl]-4-[4- (diphenylmethoxy) piperidin-1-yl] butan-1-one is a potent H1 receptor antagonist, various analytical methods have been reported for the assay of EBS alone or in combination with other anti - Histaminic agents in pharmaceutical formulations. They include UV spectroscopy, high performance liquid chromatography, high performance thin layer chromatography, LC - MS and LC - MS/ MS.$C_{32}H_{39}NO_2$

2) Phenylephrine Hydrochloride



(1R)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol hydrochloridepotent H1 receptor antagonist, white or almost white, crystalline powder,Various Methods such as HPLC, LC - MS, Capillary electrophoresis and simultaneous UV spectrophotometric methods are reported for estimation of (PHE) alone or in combination with other drugs.Both the drugs are official in BP. PHE is also official in IP and Literature survey revealed that there are several methods were reported for the estimation of EBS and PHE individually as well as in combination with some other drugs. As no method is reported for EBS and PHE in combination, the aim of the present study was to develop accurate, precise and sensitive method for the simultaneous UV spectrophotometric estimation of EBS and PHE, in bulk and in combined tablet dosage form by absorption. For this purpose marketed tablets Ebast-DC containing 10 mg of EBS, 10 mg of PHE was used.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu - 1601 double beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Reagent and chemicals

Pharmaceutically pure sample of PHE, EBS were obtained as a gift samples from MICRO labs Pvt. Ltd. Bangalore. All solvents were of AR grade obtained from Loba chem India Pvt. Limited, Nagpur.

Experimental condition

According to the solubility characteristics, the common solvent for both the drugs was found to be methanol. Hence the stock solution was prepared in methanol and further dilutions were made up with methanol.

Preparation of standard stock solution

10 mg of EBS, 10 mg of PHE were accurately weighed and transferred in to 100 ml volumetric flasks separately. Dissolved in methanol and made up to the volume to 100 ml with the same. These solutions were observed to contain 100 µg/ ml, for both the drugs.

Study of spectral and linearity characteristics

The standard stock solutions of EBS and PHE were further diluted with methanol to get the concentration of 10 µg/ ml of each and the solutions were scanned between the range 200 - 400nm in 1cm cell against Methanol as blank and the overlain spectra was recorded. From the overlain spectrum of EBS and PHE in methanol, it was observed that PHE have zero absorbance at 253 nm, where as EBS has substantial absorbance. Thus EBS was estimated directly at 253 nm without interference of PHE. At 225 nm, EBS and PHE have substantial absorbance. The contribution of PHE was deducted from the total absorbance of sample mixture at 253 nm. The calculated absorbance was called as corrected

absorbance for EBS at 253 nm; these two drugs were showed the absorbance at 225 nm. In case of simultaneous equation method, maximum wavelength selected for EBS was 240 nm where absorbance of PHE is negligible and for PHE maximum wavelength selected was 275 nm where absorbance of EBS is negligible.

Analysis of Laboratory mixture of EBS and PHE

Different mixtures of the two drugs were prepared by transferring different volumes of EBS and PHE from standard stock solutions into 100 ml volumetric flasks and diluting to volume with Methanol. The concentrations of both drugs EBS and PHE were determined by measuring the absorbance of the prepared mixtures at 225 nm, 253 nm for absorption correction method and 240 nm, 275 nm for simultaneous equation method .From these absorbance values, the concentrations of EBS and PHE were determined using absorbance correction method and simultaneous equation method. The results are shown in Table no.1a and Table no.1b for both the methods respectively.

Analysis of Tablet formulation

Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder was transferred into100mlvolumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. The absorbance of sample solution was measured at all selected wavelengths for both the methods. The content of EBS and PHE in sample solution of tablet was calculated. This procedure was repeated for six times. The results are shown in Table no.2a and Table no.2b for both the methods respectively.

Validation of methods

The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision, accuracy and ruggedness.

Linearity

Linearity was checked by diluting standard stock solution at six different concentrations. EBS was linear with the concentration range of 4 – 40 µg/ ml at 225 nm, 253 nm and PHE showed the linearity in the range of 4 – 40 µg/ ml at 225nm for absorption correction method. For simultaneous equation method, EBS was linear with the concentration range of 4 – 40 µg/ ml at 240 nm and PHE at 275 nm.

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations; LOD =3.3σ/ s and LOQ = 10σ/ s, where σ is standard deviation of y intercept of calibration curve (n = 6) and s is slope of regression equation.

Precision

The precision of the methods was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The

Intermediate precision of the methods was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated. The results are shown in Table no.3a and Table no.3b for both the methods respectively.

Accuracy

To check the accuracy of the developed methods and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated. The results are shown in Table no.4a and Table no.4b for both the methods respectively.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of

reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of EBS and PHE were carried out by using different instruments and different analysts. The results are shown in Table no.5a and Table no.5b for both the methods respectively.

RESULTS AND DISCUSSION

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical methods for simultaneous estimation of EBS and PHE in pure and in combined tablet dosage form. The proposed methods are based on spectrophotometric absorption for the simultaneous estimation of EBS and PHE in UV region using methanol as solvent.The overlain spectra of EBS and PHE are shown in Fig. 1.

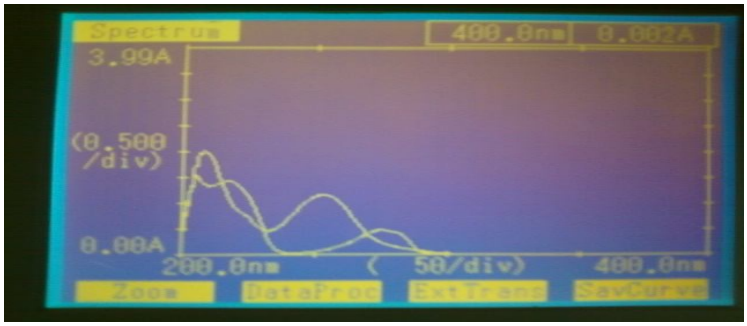


Fig. 1: Overlain spectra of EBS and PHE

Beer's law obeyed in the concentration range of 4- 40 µg/ ml at 225nm, 253 nm, 240 nm for EBS, 4 - 40 µg/ ml for PHE at 253 nm, 275 nm considering wavelengths selected for both the methods. The correlation coefficient values were found above 0.999, which shows that absorbance of all the drugs was linear with concentration.

Table 1a: Results and statistical data for estimation of EBS or PHE in Laboratory mixture for absorption correction method

S. No.	Conc. Of Pure Drug in mg/100ml		Absorbance		% Estimation	
	EBS	PHE	A_1	A_2	EBS	PHE
1	10	10	0.220	0.399	100	103.31
2	9.97	10.1	0.219	0.397	99.54	102.84
3	10.2	9.99	0.215	0.398	97.72	104.83
				Mean	99.08	103.66
				± S.D.	1.20	1.04
				C.V.	1.21	1.003

Table 1b: Results and statistical data for estimation of EBS or PHE in Laboratory mixture for simultaneous equation method

S. No	Conc. Of Pure Drug in mg/100ml		Absorbance		% Estimation	
	EBS	PHE	A_1	A_2	EBS	PHE
1	10.2	9.83	1.718	1.569	102	98.3
2	10	10.2	1.717	1.568	100	102
3	10.1	9.9	1.712	1.564	101	99
				Mean	101	99.76
				± S.D.	1	1.96
				C.V.	0.99	1.96

Table 2a: Results and statistical data for estimation of EBS and PHE in Marketed formulation for absorption correction method

S. No.	Weight of tablet powder (mg)	Absorbance		% Label claim	
		A_1	A_2	EBS	PHE
1	310	0.211	0.394	95.90	104.50
2	309.5	0.215	0.398	97.72	104.83
3	310.3	0.212	0.394	96.36	104.07
			Mean	96.66	104.46
			± S.D.	0.94	0.38
			C.V.	0.97	0.36

Brand name EBAST-DC
Average weight =310 mg

Table 2b: Results and statistical data for estimation of EBS and PHE in Marketed formulation for simultaneous equation method

S. No	Weight of tablet powder (mg)	Absorbance		% Label claim	
		A_1	A_2	EBS	PHE
1	312	1.713	1.565	102	103
2	310	1.712	1.564	101	99
3	310.5	1.717	1.568	100	102
			Mean	101	101.33
			± S.D.	1	1.98
			C.V.	0.99	1.95

Brand name EBAST-DC
Average weight =310 mg

Table 3a: Results and statistical data for Precision study of EBS and PHE for absorption correction method

S. No.	Weight of tablet powder (mg)	Absorbance		% Label claim	
		A_1	A_2	EBS	PHE
1	309	0.219	0.397	99.54	102.84
2	310	0.221	0.395	100.45	101.04
3	310.5	0.220	0.399	100	103.31
			Mean	99.99	102.39
			± S.D.	0.45	1.19
			C.V.	0.45	1.16

Table 3b: Results and statistical data for Precision study of EBS and PHE for simultaneous equation method

S. No.	Weight of tablet powder (mg)	Absorbance		% Label claim	
		A_1	A_2	EBS	PHE
1	310	1.712	1.564	101	99
2	311	1.713	1.565	102	103
3	310.5	1.717	1.568	100	102
			Mean	101	101.33
			± S.D.	1	1.98
			C.V.	0.99	1.95

Table 4a: Results and statistical data for Accuracy study of EBS and PHE in absorption correction method

S.No	Weight of tablet powder (mg)	Amount Added in µg/ml		Absorbance		Amount Recovered in µg/ml		% Recovery	
		EBS	PHE	A_1	A_2	EBS	PHE	EBS	PHE
1	310	2	2	3.201	3.169	1.984	2.002	99.2	100.1
2		4	4	3.324	3.260	3.964	3.956	99.1	98.9
3		6	6	3.441	3.351	5.854	5.924	97.5	98.73
						Mean		98.6	99.2
						± S.D.		0.95	0.74
						C.V.		0.96	0.74

Table 4b: Results and statistical data for Accuracy study of EBS and PHE in simultaneous equation method

S. No	Weight of tablet powder (mg)	Amount Added in µg/ml		Absorbance		Amount Recovered in µg/ml		% Recovery	
		EBS	PHE	A_1	A_2	EBS	PHE	EBS	PHE
1	310	2	2	2.795	1.161	1.99	1.96	99.88	98
2		4	4	2.880	2.256	3.97	4.00	99.41	100.09
3		6	6	2.966	2.308	5.99	5.96	99.96	99.36
						Mean		99.75	99.15
						± S.D.		0.29	1.06
						C.V.		0.29	1.06

Table 5a: Results and statistical data of Different analyst study in absorption correction method

S. No.	% Label claim			
	ANALYST I		ANALYST II	
	EBS	PHE	EBS	PHE
1	97.72	104.83	99.54	102.84
2	96.36	104.07	100.45	101.04
3	95.90	104.50	100	103.31
Mean	96.66	104.46	99.99	102.39
± S.D.	0.94	0.38	0.45	1.19
C.V.	0.97	0.36	0.47	1.16

Table 5b: Results and statistical data of Different analyst study in simultaneous equation method

S. No.	% Label claim			
	ANALYST I		ANALYST II	
	EBS	PHE	EBS	PHE
1	99.6	99.6	100.3	99.3
2	99.35	100.3	99.2	98.8
3	98.7	99.2	99.8	99.8
Mean	99.21	99.7	99.76	99.3
± S.D.	0.464	0.556	0.550	0.500
C.V.	0.467	0.557	0.551	0.503

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