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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC ESTIMATION OF EBASTINE IN BULK AND TABLET DOSAGE FORM USING AREA UNDER CURVE METHOD

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ABSTRACT
The aim of this work is to develop a simple, accurate, reproducible and cost effective spectrophotometric method for determination of Ebastine in bulk and pharmaceutical dosage form. This method is basedon area under curve (AUC) in wavelength range of 247-257nm and method has followed linearity in the concentration range of 5-30µg/ml. Methanol was used as a solvent. The developed analytical method was validated as per International Conference on Harmonization (ICH) guidelines. The value of correlation coefficient (R^2) was 0.999. Limit of Detection and Limit of Quantitation were calculated as 0.78µg/ml and 2.37µg/ml, respectively. Results of the recovery studies showed good accuracy of the method. Validation results suggest that the developed method can be used for routine quality control studies for assay of Ebastine in bulk and tablet dosage form.
Keywords: Ebastine, UV Spectrophotometry, area under curve (AUC), Estimation, Validation.

INTRODUCTION

Ebastine is an antihistaminic agent, which is mainly used for treatment of allergic rhinitis and chronic idiopathic urticaria. It binds preferably to peripheral H_1 receptor and acts as a second generation H_1-receptor antagonist without giving central side effects i.e. effect on cardiovascular and psychomotor functions[1]. Ebastine is chemically known as 1-[4-(1, 1-dimethyl ethyl) phenyl]-4-[4-(diphenyl methoxy) piperidine-1-y] butan-1-one[2]. This drug is official in British Pharmacopoeia. Official method uses non-aqueous titrations for assay of this drug. Literature survey revealed that determination of Ebastine has been reported by RP-HPLC method[3], liquid chromatography ionspray tandem mass spectrometry[4,5], in single and combined dosage forms. Stability indicating spectrofluorimetric estimation of Ebastine has been also established[6,7]. There are only few papers available for estimation of Ebastine along with other drugs by UV spectrophotometry using simultaneous equation method, absorption correction method and Q-ratio method[8-10], but none of these techniques utilize integration technique. In this context, we wish to further explore UV spectrophotometry using area under curve for method development and validation for estimation of Ebastine in bulk and tablet dosage form in present study.

MATERIALS AND METHODS

Instrumentation and Apparatus

Shimadzu UV 1800 (Japan) double beam spectrophotometer with 1cm matched quartz cells, connected to computer loaded with UV Prob Software was employed for this work. Shimadzu AX200 (Japan) digital balance and Spectrolab UCB 40 (Germany) ultrasonicator, were also used. All the glasswares (Borosil®) were calibrated before use.

Chemicals and Reagents

Active pharmaceutical ingredient of Ebastine was received as a gift sample from BAL Pharma Pvt. Ltd., Bommasandra, Banglore, India. Commercially available tablets (Ebast® containing10mg of Ebastine) were procured from local market. The solvent (methanol) used was of analytical grade. It was purchased from Merck India Ltd.

Preparation of Standard Solution

The standard stock solution of Ebastine API was prepared by transferring, accurately weighed, 100mg of API to 100mL of volumetric flask. It was suitably dissolved and volume was made up to the mark by using methanol. This stock solution of the drug was further diluted with the same solvent to obtain 20µg/ml as working standard solutions. This solution was scanned in spectrum mode between 400 to 200nm in UV spectrophotometer against methanol as blank after baseline correction. The obtained spectrum was used to determine the absorption maxima (λ max). Here, working wavelength region (247-257nm) for area calculation study was selected around λ max. Series of working standard solutions were prepared from standard stock solution at six levels i.e. 5, 10, 15, 20, 25 and 30µg/ml using the same solvent.

Area under Curve (Area Calculation)

In this method area calculation involves calculation of integrated value of absorbance with respect to wavelength in indicated region of wavelenghts. Area calculation processing item calculates the area bounded by the curve and horizontal axis[11]. Here horizontal axis represents baseline.

$$\text{Area calculation: } (\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelenghts representing start and end point of curve region[12]. In this study area was integrated between wavelength ranges from 247 to257 nm.

Assay of Tablet Dosage Form

Twenty tablets were accurately weighed and average weight was calculated. These tablets were crushed and powdered in

glass mortar.Powder equivalent to 10mg of Ebastine was weighed accurately and transferred into a 100ml volumetric flask. It was dissolved with about 40ml methanol. The contents were sonicated for about 30minutes and diluted up to mark with methanol. The solution was filtered using Whatmann filter paper (No.41). The first 5ml of filtrate was discarded and suitable aliquot was diluted to obtain solution of 20µg/ml concentration. This solution was scanned in spectrum mode and area under curve was integrated at wavelength range of 247-257nm (Table 1).

Method Validation

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application[13]. The proposed method was validated for various parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines.

Linearity and Range

The working standard solutions were prepared by diluting stock standard solution with methanol to give a concentration range of 5 to 30µg/ml. The spectrums of these solutions were recorded (Figure 2) and area under curve was integrated in wavelength range 247-257nm. The relationship between area under curve (as a dependant variable) and concentration of standard working solution (as an independent variable) were established by simple linear regression method. The regression equation was obtained and this relationship is presented in the calibration curve (Figure 3). The range of solution has been decided according to correlation coefficient of regression equation.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions[13]. Intraday precision was studied by integrating area of a standard solution of 20µg/ml concentration at six independent series in the same day. Inter day precision studies were performed by integrating area of standard solution of 20µg/ml concentration on three subsequent days. The percentage relative standard deviation (%RSD) was calculated (Table 2).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness[13]. The method was applied to drug sample and recovery studies were performed where Ebastine corresponding to 80, 100, and 120% of label claim was present.Three determinations at each level were performed and results were expressed as % RSD (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy[13]. LOD and LOQ were determined by using the formulae as

$$LOD = 3.3\frac{SD}{S}$$

$$LOQ = 10\frac{SD}{S}$$

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 generated regression equation was

$$\int_{247}^{257} A\,d\lambda = 0.0175C + 0.0046$$ With R^2 value as 0.999

Where, $\int_{247}^{257} A\,d\lambda$ is area under curve between 247 to 257 nm, C is concentration and R is correlation coefficient.

Table 1: Assay of Tablet Dosage Form

Tablet solution containin Ebastine (µg/ml)	% Found	Mean % Found*	% RSD*
20	99.70	99.05	0.80
20	98.15		
20	99.31		

*n=3

Table 2: Precision Data of Ebastine

Parameters	Intra-day precision	Inter-day precision
Sample solution concentration (µg/ml)	20	20
Area Under Curve (Mean ± S.D)*	0.3457±0.0037	0.4113±0.0054
%RSD	1.08	1.30

*n=6

Table 3: Accuracy Data of Ebastine

Accuracy Level	Sample conc. (µg/ml)	Standard spiked (µg/ml)	Total Amount added (µg/ml)	% Recovery*	Mean % Recovery*	% RSD*
I 80 %	10	8	18	101.00±0.052	100.51%	0.88
II 100%	10	10	20	100.05±0.057		
III 120%	10	12	22	99.48±0.017		

*n=3

Table 4: Summary of Validation Parameters

Parameter	Results
λ max (nm)	252
Linearity Range (µg/ml)	5-30
Regression Equation (y=mx+c)	y=0.0175x+0.0046
Slope (m) ± SD*	0.0175±0.0010
Intercept (c) ± SD*	0.0046±0.0043
Correlation Coefficient ($R^{2)}$	0.999
Precision (% R.S.D*)	
Intraday	1.08
Interday	1.30
Accuracy (Mean % Recovery)	100.51
LOD	0.78 µg/ml
LOQ	2.37 µg/ml

*n=6

Abbreviations: % RSD: % Relative Standard Deviation, SD: Standard Deviation, n: No. of Determinants

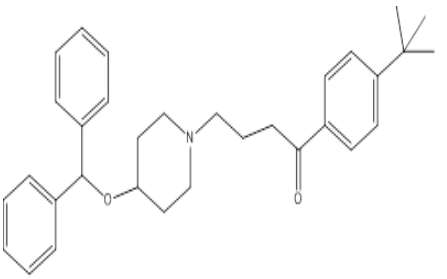

Figure 1: structure of Ebastine

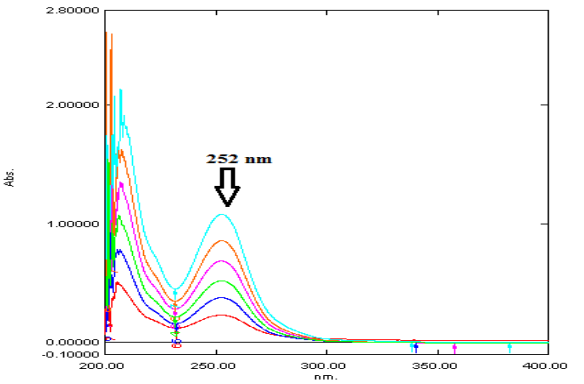

Figure 2: UV spectrum of Ebastine (5-30µg/ml)

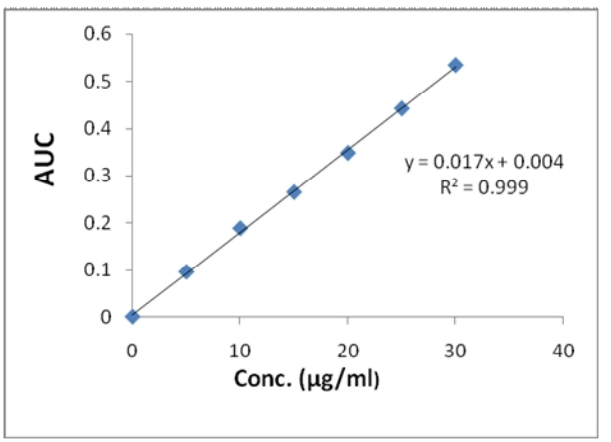

Figure 3: calibration curve of Ebastine (5-30µg/ml)

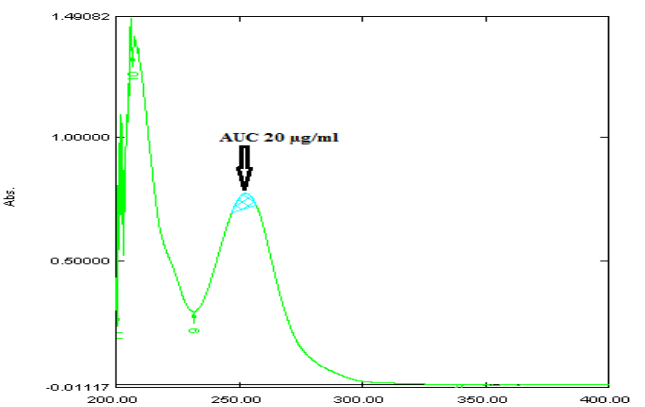

Figure 4: AUC of Ebastine (20µg/ml)

Value of correlation coefficient suggests that the developed method is following linearity in the concentration range 5-30µg/ml of drug. Results obtained by assay of Ebastine tablet dosage form indicate that applicability of developed methods to the tablets, asan average amount found was 99.05% with low% RSD (0.80). Percent relative standard deviation (%RSD) values for the intra-day and inter-day precision were 1.08 and 1.30, respectively, which is under acceptable range. This show the developed method is precise. LOD and LOQ values suggests that lowest amount of drug that can be detected using this analytical procedure is 0.78µg/ml and lowest amount of drug in a sample that can be quantitatively determined with suitable precision and accuracy is 2.37µg/ml. Percent (%) recovery was calculated as amount of drug found/drug added X 100. The range of % recovery was 99.48 to 101.00% (with mean 100.51%). Results of the recovery studies indicated good accuracy of the method. There was no interference from the excipients of tablet formulation. The validation parameters are summarised in Table 4.

CONCLUSION

A simple, economical, precise and accurate UV Spectrophotometric method for the estimation of Ebastine has been developed.This method was validated as per ICH guidelines. Results suggest that the developed method can be used for routine quality control studies for assay of Ebastine in bulk and tablet dosage form.

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