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UV Spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness

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Bilastine is a novel nonsedative H1-receptor antagonist, which may be used for the symptomatic treatment of chronic idiopathic urticaria (CU). This study describes the validation of an UV spectrophotometric method for quantitative determination of bilastine in tablets using 0.1 mol L^{-1} HCl as solvent. The method was specific, linear, precise, exact and robust at 210 nm, confirming that the method is fast and useful to the routine quality control of bilastine in tablets. The validate method was compared to liquid chromatography (HPLC), which was previously developed and validated to the same drug, and no significative difference between the methods using Student´s t test was found to bilastine quantitation.

Keywords: *bilastine; experimental design, UV spectrophotometric method.*

Introduction

Bilastine (Figure 1) is a novel nonsedative H1-receptor antagonist, which may be used for the symptomatic treatment of chronic idiopathic urticaria and allergic rhinoconjunctivitis (1).

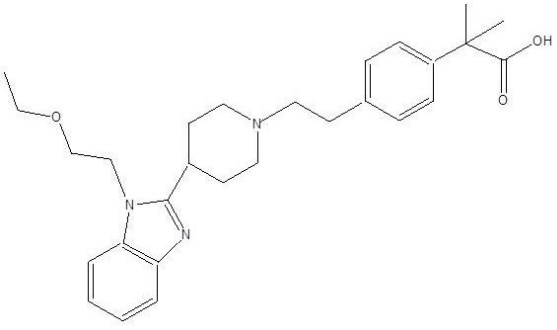

Figure 1 Chemical structure of bilastine.

Pharmacological studies have shown that the drug is highly selective for the H1 receptor in both *in vivo* and *in vitro* studies, and with no apparent affinity for other receptors. The absorption of bilastine is fast, linear and dose-proportional; it appears to be safe and well tolerated at all doses levels in healthy population (2).

Although there are some papers describing the determination of bilastine in pharmacokinetic studies by LC-fluorescence detection and LC-MS-MS (3, 4) and by hydrophilic interaction liquid chromatographic method (HILIC) (5), there are no studies describing quantification methods by UV spectrophotometry. Then the main objective of this study was to develop a simple, fast and low cost UV spectrophotometry method, without extraction process, derivatization process, and evaporation step, providing decrease in time and error in the quantitative determination of bilastine in tablets.

The method described in this study was validated through the evaluation of the following analytical parameters: specificity, linearity, precision (repeatability and intermediate precision), accuracy and robustness (6,7). Experimental design was used to evaluate method robustness (8). Besides, the

statistical comparison of the precision results with those obtained from the HPLC (previously validated by the same research group) was presented to show the suitability of the developed method.

Experimental

Reagents and Chemicals

Bilastine chemical reference standard (CRS) (99.7%) was acquired from TRC (Toronto, Ontario, Canada). Alektos® (Takeda, São Paulo, Brazil), composed of a 20 mg bilastine, was purchased in the market. The excipients contained in the dosage form (microcrystalline cellulose, sodium starch glycolate, magnesium stearate and colloidal silicon dioxide) were all pharmaceutical grades and were acquired from different suppliers. All chemicals and solvents used were of analytical grade. Methanol was purchased from Avantor® (J.T.BAKER, Center Valley, PA, USA) and Neon® (Suzano, SP, Brazil). Acetonitrile HPLC grade was purchased from Avantor® (J.T.BAKER, Center Valley, PA, USA). Purified water was prepared using Milli-Q Plus® (Millipore, Bedford, USA).

Instrumentation and Conditions

Spectral and absorbance measurements were performed with an UV-Vis Perkin-Elmer, model Lambda 35 using 10 mm quartz cells and detection at 210 nm.

A Shimadzu Prominence® liquid chromatograph (Kyoto, Japan) LC Solution V. 1.24 SP1 manager system software was used. The chromatographic separation was performed in a Shim-pack® RP-18 column (150 x 4.6 mm I.D., 5 µm, Shimadzu, Kyoto, Japan). The mobile phase comprising a mixture of 0.3% triethylamine (pH adjusted to 6.0 with 20% formic acid) and acetonitrile (55:45, *v/v*) at a flow-rate of 1.0 mL min^{-1} with isocratic elution at 25 °C. The injection volume was 20 µL for both reference substance and drug product solutions and the run time was 5 min. Bilastine was determined by UV detection at 207 nm using photodiode-array.

Preparation of RS and sample solutions

Bilastine CRS was accurately weighed and dissolved in a 100 mL volumetric flask with 0.1 mol L^{-1} HCl to produce a concentration of 100.0 µg mL^{-1}. This solution was diluted appropriately in the same diluent to yield a final concentration of 10.0 µg mL^{-1}. To prepare a sample solution, twenty tablets of Alektos® were weighed and finely powdered. A quantity equivalent to 5.0 mg of the bilastine was transferred into a 50 mL volumetric flask with 30 mL of 0.1 mol L^{-1} HCl and kept in an ultrasonic bath for 30 min. The volume was completed with the same diluent, filtered and an aliquot of this solution was volumetrically diluted to yield a final concentration of 10.0 µg mL^{-1}. The stability of bilastine CRS and sample solutions in 0.1 mol L^{-1} HCl was evaluated at room temperature (23±1°C) during 24 h using the HPLC method. The stability of these solutions was verified by observing any change in the chromatographic pattern and in the decrease of the response of the peak (area), which can indicate the degradation of the solutions.

Method validation

Different solvents were investigated to develop a suitable UV spectrophotometric method for the analysis of bilastine in tablets. For selection of solvent the criteria employed was the easiness of sample preparation, solubility and stability of the drug, cost of solvent, toxicity and applicability of the method.

The evaluation of the method specificity was performed by preparing a placebo containing the same excipients of the commercial product. A quantity of excipients contained in the equivalent to 5.0 mg of the bilastine was transferred into a 50 mL volumetric flask with 30 mL of 0.1 mol L^{-1} HCl and kept in an ultrasonic bath for 30 min. The volume was completed with the same diluent, filtered and 1.0 mL of this solution was transferred into a 10 mL volumetric flask using 0.1 mol L^{-1} HCl.

Three calibration curves were prepared with six concentrations of bilastine CRS at 210 nm (3.0, 4.0, 5.0, 10.0, 15.0 and 20.0 μg mL^{-1}) to evaluate the linearity and for each concentration the solutions were prepared in triplicate. The obtained absorbances were plotted against the respective concentrations of drug to obtain the analytical curves. The calculation of the regression line was employed by the method of least squares and the curves were validated by means of the analysis of variance (ANOVA).

The determination of precision was done through six sample solutions at the same concentrations (10.0 μg mL^{-1}) under the same experimental conditions in the same day for intra-day precision (repeatability) and on three different days for inter-day precision (intermediate precision). The relative standard deviations (RSD) were determined.

Accuracy was calculated as percentage recovery by adding known amounts of bilastine CRS to synthetic mixtures of the formulation excipients. The analyses were done in three replicates in each concentration (3.0, 10.0, and 20.0 μg mL^{-1}).

The robustness tests using experimental design in the bilastine assay provided an effective approach as part of the method validation. Robustness testing was performed in order to evaluate the susceptibility of measurements due to deliberate variations in analytical conditions. The factors were examined in an experimental design of 8 experiments, which was selected as a function of the number of factors to investigate (8). This designs applied, so-called two-level screening designs, allows screening a relatively large number of factors in a relatively small number of experiments.

The factors and the levels investigated in robustness evaluation are summarized in Table 1. The construction of the experimental designs with 8 experiments was performed in accordance to Plackett–Burman described in Heyden and collaborators (8). After determination of the number of real factors to be examined, the remaining columns in the design were defined as dummy factors, which is an imaginary factor that has no physical meaning. A half-normal probability plot for the effects in combination with the dummy factors was used to estimate the error and identify significant effects.

Table 1 Factors and levels investigated in the robustness test.

Factors	Nominal	Level (-1)	Level (+1)
Solvent brand	J.T.Baker®	Neon®	J.T.Baker®
Time of Shaking (ultrasonic bath) (min.)	30	28	32
Wavelength (nm)	210	208	212

For each factor its resulting effect was calculated according to the Equation 1:

$$E_x = \frac{\sum Y(+)}{N/2} - \frac{\sum Y(-)}{N/2} \qquad \text{(Equation 1)}$$

Where: E_X is the effect of X on response Y (bilastine concentration); $\sum Y(+)$ and $\sum Y(-)$ are the sums of the responses where X is at the extreme levels (+) and (-), respectively, and N is the number of experiments of the design.

The effect of the estimate experimental error $(SE)_e$ allows concluding what is significant from dummy factors (Equation 2). This value was used to perform the statistical test.

$$(SE)_e = \sqrt{\frac{\sum E_{dummy}^2}{n_{error}}} \qquad \text{(Equation 2)}$$

Where: $\sum E^2$error is the sum of squares of the n_{error} dummy.

The statistical interpretation provides to the user a numerical limit value that allows defining what is significant and what is not. This limit value to identify statistically significant effects is usually derived from the *t*-test statistic, in accordance to the Equation 3:

$$t = \frac{|E_x|}{(SE)_e}$$ (Equation 3)

An effect is considered significant at a given α level if t calculated > *t* critical (8).

Methods comparison

In order to compare the developed UV spectrophotometry method at 210 nm with well characterized procedure (HPLC method previously validated), the precision results of these methods were statistically analyzed using Student´s t test, which indicates if there is significant difference between the methods at 5% significant level.

Results and Discussion

The UV-VIS method is very used in the quality control of pharmaceutical products due to the potential of the great majority of the drugs of absorbing energy in these wavelengths. The development of a simple, rapid, sensitive, and accurate UV spectrophotometric method for the routine quantitative determination of samples reduces unnecessary tedious sample preparations and the cost of analysis (9).

Considering the solubility and stability, the following solvents were used as diluent of the bilastine in the CRS and the sample solutions: methanol and 0.1 mol L^{-1} HCl. The stability of bilastine in acid solution was evaluated to verify if any spontaneous degradation occurs when the samples were prepared. The studies performed by HPLC method showed the stability of the drug in acid solution by 24 h. Besides that, the 0.1 mol L^{-1} HCl is considered less toxic than methanol. Then the utilization of this solvent was evaluated to perform the spectrophotometric method validation (Figure 2).

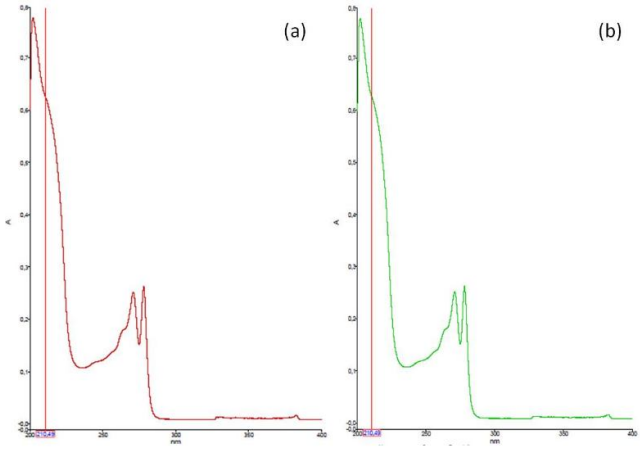

Figure 2 UV-absorption spectra of 10.0 μg mL^{-1} concentration of bilastine in tablets (a) and bilastine CRS solutions (b) in 0.1mol L^{-1} HCl.

The specificity test demonstrated that there was not interference of the excipients in the drug determination at 210 nm using 0.1 mol L^{-1} HCl as solvent.

Linearity was observed over the concentrations range of 3.0 to 30.0 μg mL^{-1} at 210 nm with significantly high value of correlation coefficient (r=0.9998). The validity of the assay was verified by means of ANOVA and according to it, there are linear regression and there are not deviation from linearity (α = 0.05, Table 2).

Table 2 Statistical data of the regression equations to analysis of bilastine.

Regression analysis	λ 210 nm
Linearity range (μg mL^{-1})	3 – 20
Slope	0.0525
Intercept	-0.0042
Correlation coefficient (r)	0.9998
Lack of fitting (critical F-value)[a]	0.21 (3.26)
Linear regression F-value (critical F-value)[a]	6460.6 (3.11)

a Theoretical value of *F* is based on one-way ANOVA test at α = 0.05 level of significance.

The Student's *t*-test was performed to verify the significance of the experimental intercept in the regression equation. According to the results, it is not significantly different from the theoretical zero value with a significance level of 5% (p > 0.05).

The experimental values obtained for the determination of the analytical method precision (repeatability and intermediary precision) are presented in Table 3. The low relative standard deviations (RSD) obtained for the repeatability and intermediary precision showed the good precision of the analytical method.

Table 3 Repeatability values obtained for bilastine in tablets by UV spectrophotometry at 210 nm.

Sample (n)	Repeatability		
	Day 1	Day 2	Day 3[a]
1	99.55	101.93	99.74
2	101.26	100.32	99.11
3	103.30	99.05	97.39
4	103.48	101.95	99.27
5	102.45	102.98	99.86
6	102.12	103.78	100.25
Mean (%)	102.03	101.67	99.27
RSD (%)	1.43	1.70	1.01
Inter-day - Mean values % (n=18)			
Mean (%)		100.99	
RSD (%)		1.82	

[a]AnalystB

The accuracy was calculated in relation of the percentage of recovery of the known added amount of bilastine CRS to the excipients solution. The accuracy of the method ranged from 99.53 to 100.54%. These values showed the good accuracy of the purposed method.

The results of the robustness experiments are presented in Table 4. They are expressed in percentage of bilastine in the sample solution calculated using standard solution in the same experimental condition. The effects of the factors in analysis, the error estimated starting from the factors dummy and the value of *t* - calculated are also showed in Table 4. The analysis of the results of the robustness study demonstrated that the factors in analysis did not present significant effect on the quantitation of the drug, indicating the robustness of the UV spectrophotometric method in the analytical conditions.

Table 4 The selected Plackett-Burman design, results of the experiments and effects (Ex) of the factors.

Exp	Sonic	λ (nm)	Dummy	Dummy	Dummy	Solvent	Dummy	% bilastine
1	+	+	+	-	+	-	-	100.00
2	-	+	+	+	-	+	-	100.00
3	-	-	+	+	+	-	+	97.94
4	+	-	-	+	+	+	-	98.68
5	-	+	-	-	+	+	+	101.03
6	+	-	+	-	-	+	+	99.56
7	+	+	-	+	-	-	+	97.94
8	-	-	-	-	-	-	-	101.47
E_x	-1.06	0.33	-0.40	-1.87	-0.33	0.48	-0.92	
$(SE)_x$	1.075							
t_{calc}	-0.99	0.31				0.44		
t_{crit}	2.983							

Sonic = shaken in ultrasonic bath; λ = Wavelength; Ex = effects; (SE)$_x$ = estimate experimental error; t_{calc} = calculated; t_{crit} = critical

The statistical comparison between the UV spectrophotometry at 210 nm and HPLC method was performed through Student´s t test using the mean experimental values, obtained in the precision of the methods (Table 5). The test did not show statistical difference between the techniques ($t_{calc} = 0.0011 < t_{crit} = 1.691$, $p > 0.05$), showing also the capacity of this developed method to quantify the drug bilastine in tablets with accuracy and precision.

Table 5 Statistical analysis between HPLC and UV methods.

	HPLC assay (%)	UV assay (%)
Mean (%) (n=18)	99.21	100.99
SD	1.90	1.84
RSD	1.91	1.82
Student´s t test	0.0011 (p > 0.05)	
T critical	1.69	

Conclusions

An HPLC-UV method was developed for determining the encapsulation efficiency of zidovudine in nanoparticles. This chromatographic method was considered

simple and rapid, since the preparation of the samples did not involve complex and prolonged processes. Furthermore, the HPLC-UV method was validated in terms of selectivity, stability, linearity, limits of quantitation and detection, precision, accuracy and robustness. Finally, it provided unequivocal determination of the amount of zidovudine in nanoparticule formulation which may be applied to stability or quality control.

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Conflits of Interest

Nothing to declare.

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