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DEVELOPMENT OF TWO SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF BILASTINE IN TABLETS

Iryna Ivanusa, Alina-Mariia Horoshko, Anna Staranchuk, Mariya Mykhalkiv

The aim of the work *was to develop two simple, rapid, economically available spectrophotometric methods for the determination of bilastine in tablets based on the reaction with sulfonephthalein dyes (bromphenol blue (BPB) and thymol blue (TB)).*

Materials and methods. *Analytical instrumentation: Shimadzu UV-1800 double beam UV-VIS spectrophotometer (Japan) with attached UV-Probe ver. 2.62 software, RAD WAG AS 200/C precise analytical balance (Poland). Bilastine (purity ≥99% (LC)) was purchased from Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. Nixar tablets 20 mg were purchased from a local pharmacy.*

Results and discussion. *Two spectrophotometric methods for the determination of bilastine in tablets have been developed. Different sulfophthalein dyes (bromphenol blue, thymol blue, bromocresol green, bromthymol blue, bromocresol purple) have been tested in order to choose the optimal reagent for the method development. The experimental research results led to the selection of BPB and TB as the reagents. Methanol was used as the solvent in reaction of bilastine with BPB, while 20% methanol-ethyl acetate solution was used for TB.*

The optimal conditions for the quantitative determination of bilastine in tablets by using BPB were established: concentration – 1.08 × 10^{-3} mol/L, volume of BPB solution – 1.00 mL, wavelength – 596 nm, reaction time – 5 min, solution temperature – 25°C. The optimal conditions for the quantitative determination of bilastine in tablets by using TB were established: concentration – 4.34 × 10^{-4} mol/L, volume of TB solution – 1.00 mL, wavelength – 416 nm, reaction time – 5 min, solution temperature – 25°C.

The spectrophotometric method of the quantitative determination of bilastine in tablets by using BPB was linear in the concentration range of 0.5–7.5 μg/mL, LOD – 0.25 μg/mL, LOQ – 0.76 μg/mL; by using TB was linear in the concentration range of 2.00–18.00 μg/mL, LOD – 0.63 μg/mL, LOQ – 1.92 μg/mL. Both methods demonstrated acceptable robustness, accuracy, and precision, meeting all validation criteria. The «greenness» assessment results confirmed that both methods are excellent from a green analytical chemistry perspective.

Conclusions*. The developed methods can be used as an alternative method for the routine analysis of bilastine in tablets*

Keywords*: bilastine, tablets, spectrophotometry, sulfophthalein dyes, validation, quantitative determination*

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1. Introduction

Allergic diseases, such as allergic rhinitis, allergic asthma, atopic dermatitis, food allergy, and eczema, are systemic diseases caused by a disorder of the immune system. The constant increase in incidence is attracting more and more attention, as it is accompanied by a high recurrence rate. Allergic diseases are included by the World Health Organization as disorders that should be prevented and controlled [1].

Treatment of allergic rhinitis should combine allergen avoidance, pharmacotherapy, and allergen immunotherapy [2–4]. Bilastine (Fig. 1) (2-[4-(2-(4-[1-(2-Ethoxyethyl)-1H-benzimidazol-2-yl]-1-piperidinyl)ethyl) phenyl]-2-methylpropanoic acid) is one of effective antihistamine active ingredients, which was first approved in the European Union in 2010 for the symptomatic treatment of allergic rhinitis and hives (urticarial) [5–8].

Bilastine is rapidly absorbed in the gastrointestinal tract and is practically not metabolized. It is eliminated mainly in the feces (66.5%), some in the urine (28.3%).

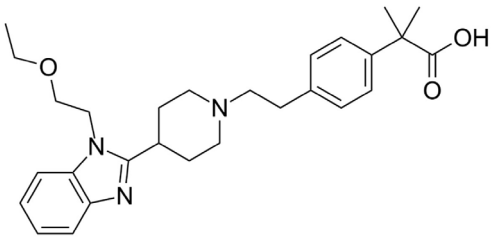


Fig. 1. The chemical structure of bilastine [3]

The pharmacopoeial monographs do not include spectrophotometric methods for the determination of bilastine. This means that official pharmacopoeias such as the European (Ph. Eur.), British (BP) or State Pharmacopoeia of Ukraine (USP) do not contain methods for the bilastine analysis which are based on the measurement of light absorption. Currently, the official pharmacopoeial monograph for bilastine is published in the Indian Pharmacopoeia (IP) [9]. This monograph presents methods for the bilastine assay (particularly in tablets), by using

high-performance liquid chromatography. Reversed-phase high-performance liquid chromatographic methods and methods of spectrofluorometric analysis for the bilastine determination are described in the scientific publications [10–15]. These quantitative analysis methods are difficult and expensive enough. Consequently, the aim of our research was to develop two simple, rapid, economic, alternative spectrophotometric methods for the bilastine determination in tablets, which based on the reactions with sulfophthalein dyes – bromophenol blue (BPB) and thymol blue (TB). These methods are a relevant and promising vector for the development of pharmaceutical science.

2. Planning of the research

Methodology of the research of simple, rapid, economic, alternative spectrophotometric methods for the determination of bilastine in tablets based on the reaction with sulfophthalein dye includes:

1. Analysis of the scientific literature, Ph. Eur., BP., USP and IP monographs.

2. Selection of reaction conditions between bilastine and sulfophthalein dyes (optimal solvent, choice of reagent (dye), its concentration and amount, wavelength for assay, detection of stoichiometric coefficients).

3. Development and validation of the spectrophotometric methods for determination of bilastine in tablets which based on the reaction with sulfophthalein dye (BPB and TB).

4. Greenness profile assessment of the proposed spectrophotometric methods (AGREE and MOGAPI).

3. Materials and methods

Objects of research, solvents and equipment.

Analytical instrumentation: Shimadzu UV-1800 double beam UV-VIS spectrophotometer (Japan) with attached UV-Probe ver. 2.62 software, RAD WAG AS 200/C precise analytical balance (Poland).

SPhU Reference Standard of Bilastine (purity ≥ 99% (LC)) was purchased from Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. All the chemicals were used of analytical reagent grade. Reagents (BPB and TB) were purchased from MERCK, Sigma-Aldrich (Switzerland) (≥98% (HPLC)). Nixar tablets 20 mg were purchased from a local pharmacy (BERLIN-CHEMIE, batch number 3565A).

Proposed procedure for the determination of bilastine with BPB.

The tablets (Nixar) were accurately weighed and crushed. The exact weight of powder equivalent 35.00 mg of bilastine was weighed on an analytical balance, transferred into a 100.00 mL volumetric flask, dissolved in 15.0 mL of methanol, adjusted with methanol to label, kept in an ultrasound bath for 2 min. and filtered, the first filtrate portions are discarded. Aliquot 5.00 mL was taken from the following portions of the filtrate and transferred into a 50.00 mL volumetric flask, dissolved in 20.0 mL of methanol, adjusted with methanol to label, and mixed. Aliquot 1.00 mL was transferred into a 10.00 mL volumetric flask and added 1.00 mL 1.08×10^{-3} M solution of BPB in methanol, adjusted with methanol to label, and mixed well. The absorbance of the resulting solution was measured against the background of the compensating solution at a wavelength of 596 nm.

Proposed procedure for the determination of bilastine with TB.

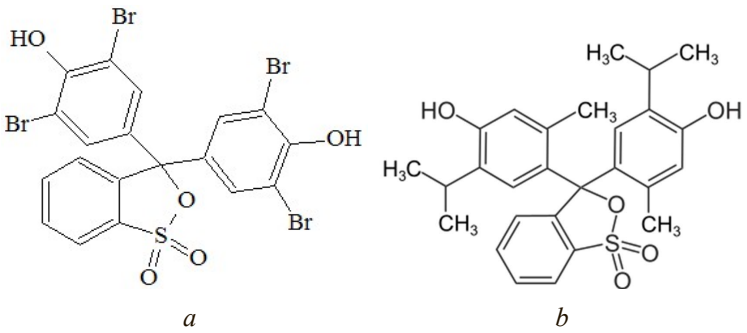
The tablets (Nixar) were accurately weighed and crushed. The exact weight of powder equivalent 20.00 mg of bilastine was weighed on an analytical balance, transferred into a 100.00 mL volumetric flask, dissolved in 15.0 mL of 20% methanol-ethyl acetate solution, adjusted with 20% methanol-ethyl acetate solution to label, kept in an ultrasound bath for 2 min. and filtered, the first filtrate portions are discarded. Aliquot 0.5 mL was taken from the following portions of the filtrate and transferred into a 10.00 mL volumetric flask, and added 1.00 mL 2.17×10^{-3} M solution of TB in 20% methanol-ethyl acetate solution, adjusted with solvent to label, and mixed well. The absorbance of the resulting solution was measured against the background of the compensating solution at a wavelength of 416 nm.

4. Results

4. 1. Selection of reaction conditions

Bilastine is a white crystalline powder, practically insoluble in acetonitrile, slightly soluble in water, acetone, glycerol, soluble in ethanol, methanol, ethyl acetate, chloroform, sparingly soluble in dimethylformamide. Melting point ~ 200–210°C. Lipophilicity (logP) is approximately 2.5–3.0 (high permeability through biomembranes). It is stable at room temperature, sensitive to light. Molecular weight: 463.62 g/mol. [16, 17].

No analytical method was found in which sulfophthalein dyes were used as reagents for spectrophotometric determination of bilastine in tablets, during the analysis of scientific articles. Different sulfophthalein dyes (bromphenol blue, thymol blue, bromocresol green, bromthymol blue, bromocresol purple) have been tested in order to choose the optimal reagent for the method development. The experimental research results led to the selection of BPB and TB as the reagents (Fig. 2).



a *b*

Fig. 2. The chemical structure of: *a* – BPB [18]; *b* – TB [19]

Optimizing the conditions for the reaction of bilastine with bromophenol blue (BPB) and thymol blue (TB) was necessary to ensure the formation of a stable colored product of sufficient intensity, thereby establishing the

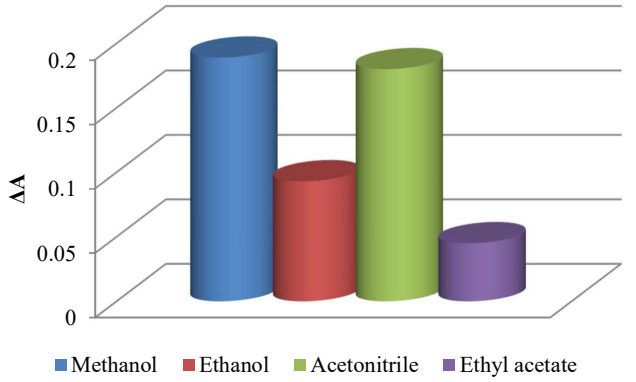
required sensitivity and reproducibility for the spectrophotometric determination.

One of the key factors that directly affect the efficiency of the reaction and the formation of an ion-association complex is the choice of solvent. The solvent performs several important functions: it ensures sufficient dissolution of both bilastine and the dye (BPB, TB), promotes their interaction at the molecular level, and also affects the stability of the formed complex.

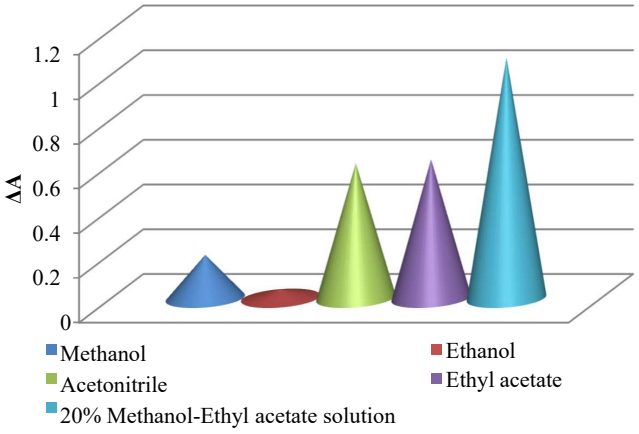
One of the main factors that directly influences on the reaction efficiency and formation of an ion-association complex is the solvent choice. The solvent performs several important functions: it ensures sufficient dissolution of both bilastine and the dye (BPB, TB), facilitates their reaction at the molecular level, and also affects the stability of the formed complex. An incorrectly selected medium can lead to a partial or complete absence of the reaction, low color intensity, signal instability, or by-product formation. Therefore, the influence of various solvents (such as ethyl acetate, ethanol, methanol, acetonitrile, a 20% methanol-ethyl acetate solution) on the formation of the bilastine–bromophenol blue and bilastine–thymol blue complexes was investigated during the experiment. Analysis of the obtained results showed that the highest absorbance was observed when methanol was used as the solvent and BPB was used as the dye (Fig. 3, *a*). Furthermore, it was established that the most intense and stable coloration of complex Bilastine-TB is observed at using a 20% methanol-ethyl acetate solution as the solvent, which indicates the best solubility of the components and favorable conditions for the complex formation (Fig. 3, *b*).

Based on the Hansen space green solvent selection tool, methanol and ethyl acetate had a *G* score of 5.8 and 6.7 accordingly [20], as shown in Fig. 4 (for methanol: waste = 4.0, health = 4.9, environment = 8.4, safety = 7.1; for ethyl acetate: waste = 4.9, health = 8.4, environment = 6.7, safety = 7.1).

The quantitative determination of bilastine, either as a pure substance or in pharmaceutical dosage forms, is possible through colour complex formation with sulfophthalein dyes such as bromophenol blue and thymol blue. Spectrophotometric data reveal that the formed complexes have characteristic absorption bands in the visible region, confirming the creation of a new chromophore. The most intense absorption is observed at wavelengths of 596 nm (complex with BPB, Fig. 5, *a*) and 416 nm (complex with TB, Fig. 5, *b*), which correspond to the absorption maxima (λ_{max}). These wavelengths were chosen for further analysis to ensure optimal method sensitivity and specificity.

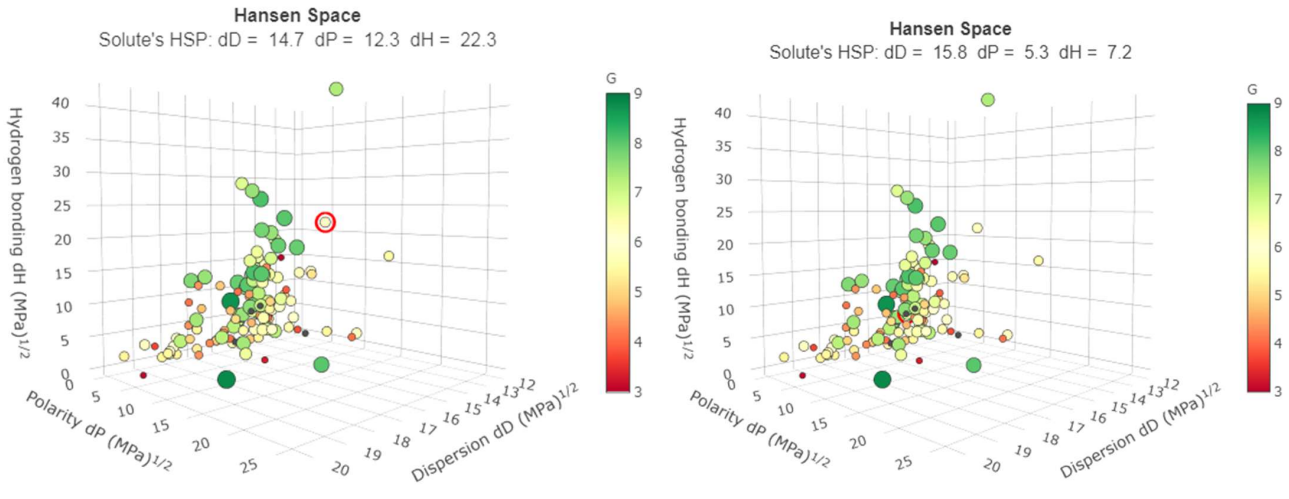


a



a

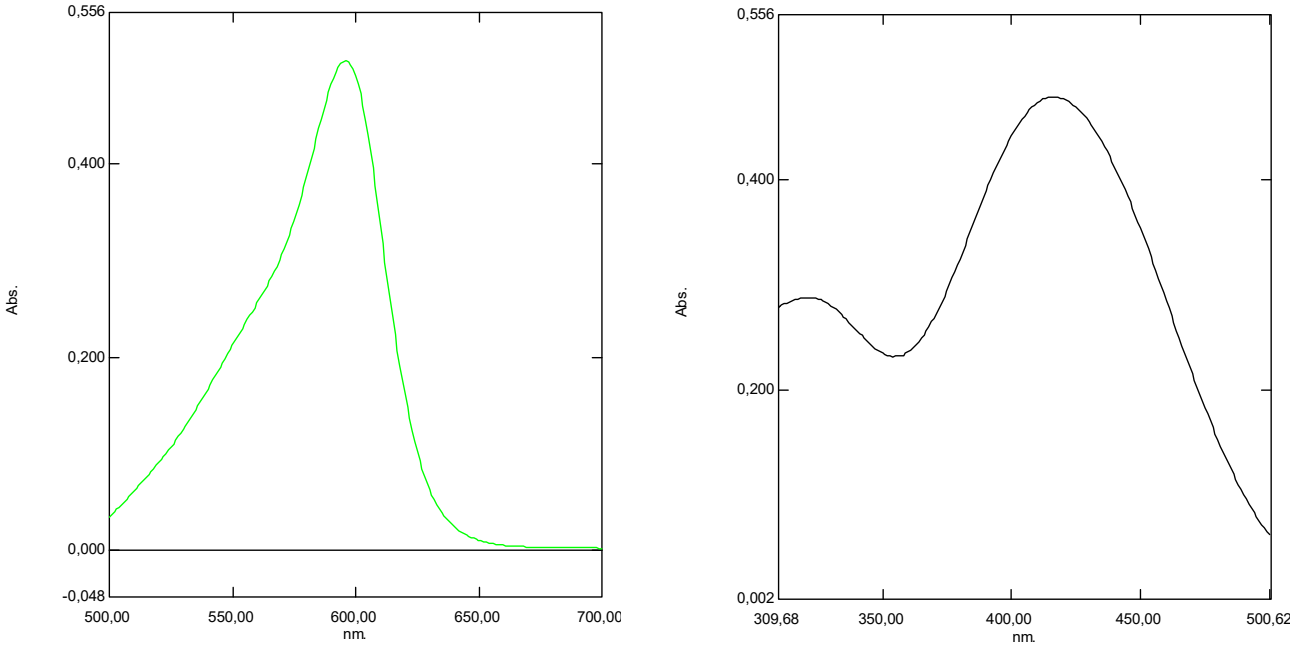
Fig. 3. Solvents impact on the complex formation of bilastine with: *a* – BPB (1.08×10^{-4} M); *b* – TB (2.17×10^{-4} M)



a *b*

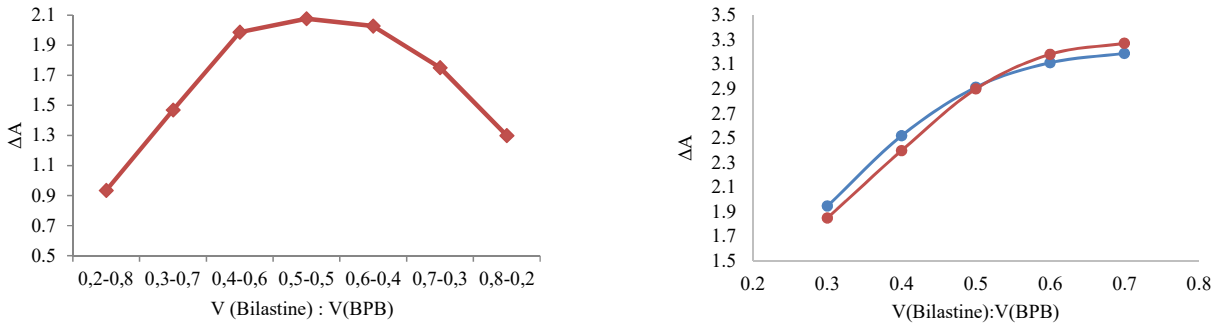
Fig. 4. Solvent sustainability level generated by the Hansen space green solvent selection tool: *a* – methanol; *b* – ethyl acetate

The next stage of our work involved determining the optimal stoichiometric ratio for the complex formation of bilastine and sulfophthalein dyes. Two methods were used: the Job's method (the method of continuous variation) and the saturation method (the method of molar ratios). Model solutions of bilastine and BPB with a molarity 1.08×10^{-3} M were prepared for determination of the optimal stoichiometric ratio by both methods (Fig. 6), as well as solutions of bilastine and TB with a molarity 4.34×10^{-4} M (Fig. 7). As seen from Fig. 6, 7, the stoichiometric coefficients of the reacting components between bilastine and dyes equals 1 : 1.



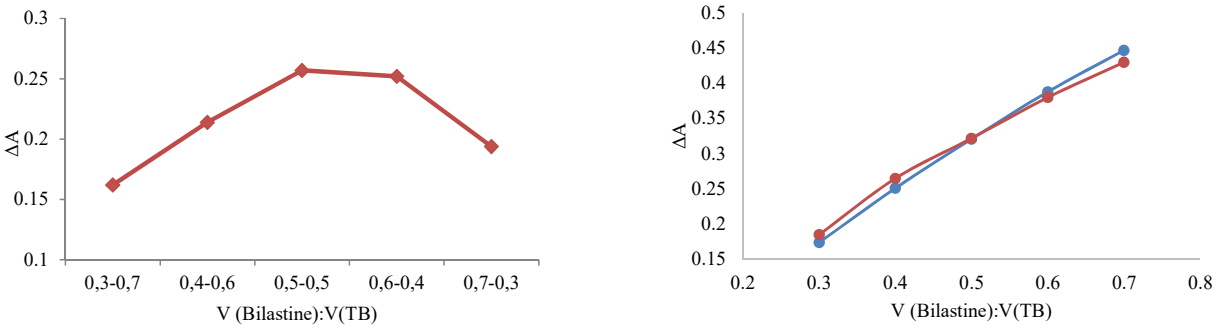
a *b*

Fig. 5. Absorption spectra of: *a* – bilastine (1.08×10^{-4} M) – BPB (1.08×10^{-3} M in methanol) complex; *b* – bilastine (4.34×10^{-4} M) – TB (2.17×10^{-3} M in 20% methanol-ethyl acetate solution) complex



a *b*

Fig. 6. Study of stoichiometric coefficients by the reaction bilastine with BPB ($C_M = 1.08 \times 10^{-4}$ M) by: *a* – continuous variation method at λ = 596 nm; *b* – Molar ratio method at λ = 596 nm (blue – constant molarity of BPB, brown –constant molarity of bilastine)



a *b*

Fig. 7. Study of stoichiometric coefficients by the reaction bilastine with TB ($C_M = 4.34 \times 10^{-4}$ M) by: *a* – continuous variation method at λ = 416 nm; *b* – Molar ratio method at λ = 416 nm (blue – constant molarity of TB, brown – constant molarity of bilastine)

4. 2. Determination of validation characteristics

Two spectrophotometric methods for the determination of bilastine in tablets have been validated in accordance with the requirements of the SPhU [21] for the parameters: robustness, accuracy and precision, linearity, range of application.

4. 2. 1. Specificity study

The absorptivity of a solution containing only excipients was measured to confirm the "specificity" as validation parameter for the spectrophotometric method of bilastine assay which is based on its reaction with methanol solution of BPB and 20% methanol-ethyl acetate solution of TB (Table 1). This investigation allowed for the assessment of the potential influence of extraneous components of the dosage form on the measurement results.

Table 1

Specificity study of spectrophotometric methods for bilastine assay in tablets

Placebo absorbance ($A_{placebo}$)		Solution absorbance (A_{st})		Found value δ_{noise},%		Acceptance criterion	
BPB	TB	BPB	TB	BPB	TB	BPB	TB
0.001	0.001	0.506	0.460	0.20	0.22	Not more 0.5%	

The specificity study results demonstrated that the excipients do not significantly interfere with the quantitative determination of the active pharmaceutical ingredient (bilastine). This is confirmed by the placebo absorbance value falling within the acceptable range defined by the acceptance (δ_{noise} = 0.20 % and δ_{noise} = 0.22 %, at the maximum allowable value 0.5 %).

4. 2. 2. Linearity and range of application.

The linearity of the analytical method was studied over its entire concentration range (0.5–7.5 μg/mL for the BPB-bilastine complex; 2.00–18.00 μg/mL for the TB-bilastine complex) in accordance with the SPhU requirements. The main metrological characteristics of the linear relationship are presented in Table 2. All parameters for linearity satisfied the SPhU specifications over the entire concentration range of the method.

Linearity of the analytical procedure for both methods is sufficient because the correlation coefficient is more than 0.998. The Limit of Detection (LOD) was 0.25 μg/mL and the Limit of Quantification (LOQ) was 0.76 μg/mL for the bilastine complex with BPB. For the bilactine complex with TB, the LOD and LOQ were 0.63 μg/mL and 1.92 μg/mL, respectively. These values confirm the high sensitivity of the method.

Table 2

Metrological parameters of the linear regression

Parameters	Value	
	BPB	TB
Slope (b) ± SD*	0.156 ± 0.0075	0.0466 ± 0.0022
Intercept (a) ± SD	–0.0476 ± 0.0304	0.0087 ± 0.0248
Correlation coefficient	0.9982	0.9992
**LOD μg/mL)	0.25	0.63
***LOQ (μg/mL)	0.76	1.92
Linear range (μg/mL)	0.5–7.5	2.00–18.00

*Note: SD – Standard Deviation; ** – LOD: Limit of detection; *** – LOQ: Limit of quantitation.*

4. 2. 3. Accuracy and precision

During the study of the validation parameters "accuracy" and "precision" of the developed methods, model solutions with an accurately defined content of the active pharmaceutical ingredient were prepared in the concentration range from 80% to 120% of the nominal value. The accuracy of the methods was evaluated by calculating the systematic error (δ,%), which confirmed the method's compliance with this criterion. The precision was evaluated using the relative confidence interval (Δz), which reflects the degree of result reproducibility. The experimental results obtained, along with the acceptance criteria for the specified validation parameters, are presented in Table 3.

A systematic error of 0.16% was obtained, which is statistically and practically negligible, demonstrating the method's accuracy for the analysis of all tested concentrations.

Table 3

The results of the accuracy and precision study

Model solution	Content of bilastine,%				The ratio of found to added, $Z_i = (Y_i / X_i)\cdot 100\%$	
	Added, $X_i = (C_i / C_{rs})$ 100%		Found, $Y_i = (A_i / A_{rs})$ 100%			
	BPB	TB	BPB	TB	BPB	TB
M_1	83.43	82.00	83.71	82.99	100.34	101.20
M_2	88.57	86.40	88.04	85.57	99.40	99.04
M_3	93.14	93.10	93.99	92.61	100,91	99.47
M_4	98.57	99.80	99.95	99.12	101.40	99.32
M_5	100.57	106.50	99.98	105.77	99.41	99.31
M_6	102.60	113.90	103.00	114.42	100.39	100.45
M_7	110.00	115.60	109.97	114.22	99.97	98.88
M_8	112.57	117.20	113.25	117.50	100.60	100.25
M_9	117.14	119.40	118.02	119.69	100.75	100.24
The average value, Z, %					100.35	99.80
Standard deviation, S_z, %					0.67	0.77
Relative confidence interval $\Delta z = t(95\%, 8)\cdot S_z = 2.3060\cdot S_{z\%}$					1.54	1.78
The critical value for the convergence of results $\Delta z \le \max\Delta_{4s} = 2.4\%$					Corresponds (< 2.4)	
Systematic error $\delta = \lvert Z - 100\rvert$, %					0.07	0.09
The criterion of uncertainty of systematic error $\delta \le \max \delta\%$: $\delta\% \le \max\delta\% = \frac{\Delta z}{\sqrt{n}} = \frac{1.54}{\sqrt{9}} 0.51$					Corresponds (0.35 < 0.51)	Corresponds (0.2 < 0.59)
General conclusion					Correct	

Six samples of the same series of tablets, which contains bilastine were analyzed to evaluate the validation parameter "intra-laboratory precision". The study was designed so that different analysts, on different days, performed the experimental procedure using different volumetric glassware. The results presented in Table 4 were evaluated using the relative confidence interval, which was required to be within permissible uncertainty of the analysis results (Δz)

$\Delta z \leq 2.4$ (at $B = 7.5\%$).

Thus, based on the analysis of intermediate precision (Table 4), it was established that the value of the relative confidence interval for six independent determinations of the same series tablets does not exceed the permissible limit specified by the acceptance criterion.

Table 4

Results of intra-laboratory precision study

No. solution	Value Z_i, %					
	BPB			TB		
	Experiment			Experiment		
	1	2	3	1	2	3
1	99.84	100.11	99.85	99.79	100.20	99.86
2	99.80	99.95	99.98	99.81	99.99	100.11
3	100.12	99.79	100.09	100.25	100.54	100.10
4	100.20	100.15	100.11	100.44	100.09	100.20
5	100.10	100.25	100.20	100.17	99.79	99.85
6	99.90	100.07	100.02	99.84	99.87	100.13
Average Z (%)	99.99	100.05	100.04	100.05	100.11	100.04
RSD_x, %	0.17	0.16	0.12	0.27	0.25	0.15
Relative standard deviation, RSD_z (%)	0.15			0.22		
Relative confidence interval, Δ_z	$0.10 \leq 2.4$			$0.10 \leq 2.4$		
The critical value of the convergence of results Δ_{As}, %	2.4			2.4		

4. 2. 4. Robustness study

The stability of the sample solutions was evaluated over a defined time period to determine the robustness of the analytical method. The results of the absorbance measurements are presented in Table 5. The test solutions remained stable for 45 minutes as the obtained results indicate.

Table 5

Results of the stability study for test solution of bilastine-dye complex (1) and reference solution of bilastine-dye complex (2)

No.	t, min						Mean absorbance	RSD_t, %
	0	15	30	45	90	120		
Bromophenol blue								
1	0.508	0.512	0.493	0.492	0.490	0.490	0.497	1.98
2	0.502	0.500	0.504	0.498	0.495	0.492	0.498	0.89
Thymol blue								
1	0.483	0.478	0.487	0.479	0.481	0.480	0.481	0.68
2	0.492	0.495	0.490	0.489	0.487	0.485	0.490	0.73
Thymol blue								

4. 3. Application to tablet analysis

The validated analytical method was applied to the assay of bilastine in its tablet. The results of this analysis and metrological characteristics are summarized in Table 6.

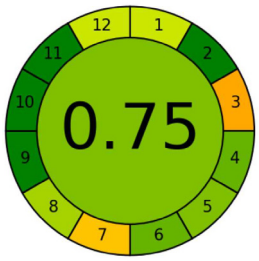
Table 6

The results of the quantitative determinations of bilastine in tablets ($n = 6$, $p = 0.95$)

Dosage form	Found, g		metrological characteristics	
	BPB	TB	BPB	TB
Bilastine tablets (20 mg)	0.0208	0.0210	$\overline{m} = 0.0201$ g; $S = 1.52 \times 10^{-4}$; $t_\alpha = 2.57$ $\Delta x = 3.91 \times 10^{-4}$; RSD = 1.85% ε = 1.94%	$\overline{m} = 0.0204$ g; $S = 2.17 \times 10^{-4}$; $t_\alpha = 2.57$ $\Delta x = 5.57 \times 10^{-4}$; RSD = 2.60% ε = 2.73%
	0.0199	0.0204		
	0.0201	0.0198		
	0.0197	0.0206		
	0.0202	0.0208		
	0.0201	0.0197		

4. 4. Environmental impact assessment

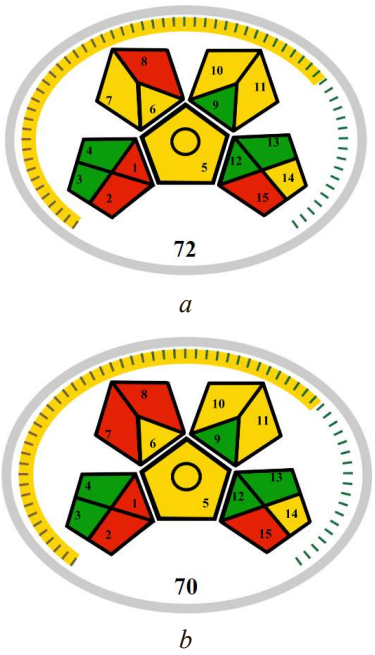
The next stage of the experiment involved calculating the "greenness" of the quantitative method for assay of bilastine with BPB and TB in tablets that we developed. The greenness of the analytical method was assessed using the AGREE (Analytical GREEnness) tool [22], proposed by researchers from the Gdańsk University of Technology (Poland) and MOGAPI tool [23]. The results are presented in Fig. 8, 9. The AGREE tool scores are 0.75 and 0.7, the MOGAPI tool scores are 0.72 and 0.70. These indicate an excellent green analysis.



a

b

Fig. 8. Pictograms of analytical methods using AGREE tool: *a* – BPB; *b* – TB



a

b

Fig. 9. Pictograms of analytical methods using MOGAPI tool: *a* – BPB; *b* – TB

5. Discussion of research results

Other scientists of pharmaceutical chemistry department I. Horbachevsky Ternopil National Medical University have developed methods for API assay by using dyes, for example, amlodipine, perindopril, rosuvastatin with sulfophthalein dye; however, we have not found any scientific publications about bilastine assay with sulfophthalein dye [24–26].

A review of the scientific literature revealed no methods for the quantitative determination of bilastine using sulfophthalein dyes. Several dyes were tested. Based on the results of preliminary screening, BPB and TB were selected as the most suitable for the formation of colored ion-association complexes with bilastine. This choice is justified by both the high reactivity of the dyes and the stability of the formed complexes, which ensures the reliability of the analytical signal.

The solvent is critical important for the successful reaction course between bilastine and the dye. Several solvents were investigated: methanol, ethanol, ethyl acetate, acetonitrile, acetone, as well as their mixtures. The best results were obtained for:

– BPB in methanol. It provides maximum color intensity and signal stability (Fig. 3, *a*). Methanol facilitated the complete dissolution of the reagents and promoted the rapid formation of a complex with a specific color effect;

– TB in a 20% methanol-ethyl acetate solution. It gives stable color (Fig. 3, *b*). This medium provided the necessary balance between polarity and solubility for both components.

Methanol and ethyl acetate had a G score of 5.8 and 6.7 accordingly, which are based on the Hansen space green solvent selection tool (Fig. 4).

Based on spectrophotometric studies, it was found that:

– λ_{max} for bilastine-BPB complex is 596 nm (Fig. 5, *a*);

– λ_{max} for bilastine-TB complex is 416 nm (Fig. 5, *b*).

These values indicate the formation of new chromophore fragments and they are optimal for further measurements. The difference in wavelengths is due to the distinct nature of the dyes, which affects the energy state of the electronic transitions in the molecular complex.

Using the Job's method (the method of continuous variation) and saturation method (the method of molar ratios), it was established that bilastine reacts with both dyes in the 1:1 ratio (Fig. 6, 7). This indicates the formation of a stable ion-association complex, in which one molecular ion of the dye associates with one molecule of bilastine. This finding is crucial for the standardization of assay, as it allows for predicting and controlling the chemical interaction in calculations.

It was confirmed that the excipients of the tablet formulation do not affect the accuracy of the analysis. The absorbance of the placebo solution was significantly lower than the bilastine solution and it did not exceed the permissible limits ($\delta\_noise \leq 0.5\%$). This indicates the high selectivity of the method and the absence of cross-interference with other components of the dosage form.

The method meets the requirements of the SPhU for the parameter of linearity:

– for BPB: the concentration range is 0.5–7.5 μg/mL; correlation coefficient is 0.9982;

– for TB: the concentration range is 2.0–18.0 μg/mL; correlation coefficient is 0.9992 (Table 2).

The obtained correlation coefficients confirm the linearity of both methods within the specified concentration range, which is crucial for accurate dosing.

The limits of detection (LOD) and quantification (LOQ) were as follows:

– for BPB: LOD = 0.25 μg/mL; LOQ = 0.76 μg/mL;

– for TB: LOD = 0.63 μg/mL; LOQ = 1.92 μg/mL.

These values demonstrate the high sensitivity of the methods and theirs ability to detect even low concentrations of bilastine in a complex matrix.

It was demonstrated that the methods provide reliable results within the range of 80–120% of the nominal content (Table 3):

– mean systematic error: 0.35% (BPB), 0.20% (TB);

– relative confidence interval (Δz): 1.54% (BPB), 1.78% (TB).

These values indicate the high accuracy of the method and its ability to produce reproducible results regardless of repeated analyses. Compliance with the SPhU criteria confirms the analytical reliability.

The obtained values of the relative confidence interval for six parallel determinations of one series of tablets confirm the acceptance criterion (≤2.4%) for the intra-laboratory precision (Table 4).

The solutions of bilastin in the presence of the selected dyes maintain stable absorption characteristics for at least 45 minutes that confirm the robustness of the method (Table 5). This stability allows the analysis to be performed without the urgent need for immediate measurement, which is practically convenient under laboratory conditions.

Based on the results from the AGREE and MOGAPI tools (Fig. 8, 9), the method is confirmed to be green and en-

vironmentally friendly. The obtained AGREE score was 0.75 for BPB and 0.70 for TB. The obtained MOGAPI score was 0.72 for BPB and 0.70 for TB. These values indicate environmental safety, minimal use of toxic reagents, and a low volume of organic solvents. The method meets modern requirements for sustainable development in analytical chemistry.

Thus, the developed spectrophotometric methods for the quantitative determination of bilastine with BPB and TB are validated. They are sensitive, specific, accurate, and environmentally friendly. They can be implemented into pharmaceutical practice for the quality control of bilastine in dosage forms.

Practical relevance. The proposed analytical methods can be used to determine bilasine in tablets.

Study limitations. The developed spectrophotometric method cannot be used to determine bilastine in the presence of other APIs in medicines.

Prospects for further research. The next stage of research is planned to investigate possibility of using bromocresol green and bromothymol blue for determination of bilastine in tablets.

6. Conclusions

1. The optimal conditions for the quantitative determination of bilastine in tablets by using BPB were established: solvent – methanol, concentration – 1.08×10^{-3} mol/L, volume of BPB solution – 1.00 mL, wavelength – 596 nm, reaction time – 5 min, solution temperature – 25°C.

2. The optimal conditions for the quantitative determination of bilastine in tablets by using TB were established: solvent – 20% methanol-ethyl acetate solution, concentration – 4.34×10^{-4} mol/L, volume of TB solution – 1.00 mL, wavelength – 416 nm, reaction time – 5 min, solution temperature – 25°C.

3. The stoichiometric coefficients of the reacting components between bilastine and dyes equal 1:1, which were determined by the Job's method and the saturation method.

4. The spectrophotometric method of the quantitative determination of bilastine in tablets by using BPB was linear in the concentration range of 0.5–7.5 μg/mL, LOD – 0.25 μg/mL, LOQ – 0.76 μg/mL; by using TB was linear in the concentration range of 2.00–18.00 μg/mL, LOD – 0.63 μg/mL, LOQ – 1.92 μg/mL. Both methods demonstrated acceptable robustness, accuracy, and precision, meeting all validation criteria.

5. The *«greenness»* assessment results confirmed that both methods are excellent from a green analytical chemistry perspective.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

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Authors' contributions

Iryna Ivanusa: Conceptualization, Methodology, Analysis, Investigation, Visualization, Validation, Writing – original draft; **Alina-Mariia Horoshko:** Software, Visualization, Analysis, Investigation, Funding acquisition; **Anna Staranchuk:** Software, Visualization, Analysis, Investigation, Funding acquisition; **Mariya Mykhalkiv:** Investigation, Analysis, Visualization, Validation, Writing – original draft.

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