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## DEVELOPMENT OF THE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ATORVASTATIN IN TABLETS BY USING BROMOTHYMOLO BLUE

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*The aim of the work* was to develop a simple, economic, fast, reliable, and eco-friendly spectrophotometric method for the determination of atorvastatin in tablets based on the reaction with bromothymol blue (BTB).

**Material and methods.** A double-beam Shimadzu UV-Visible spectrophotometer; with spectral bandwidth of 1 nm wavelength accuracy  $\pm 0.5$  nm, Model –UV 1800 (Japan), Software UV-Probe 2.62 was used to measure absorbance of the resulting solution. Pharmacopeial standard sample of atorvastatin calcium and BTB were provided by Sigma-Aldrich ( $\geq 98\%$ , HPLC). The used dosage forms of atorvastatin: tablets Atorvastatin 10 mg and 20 mg.

**Results and discussion.** The method of spectrophotometric determination of the quantitative content of atorvastatin calcium based on its reaction with BTB in ethyl acetate medium has been developed. The stoichiometric ratios of the reactive components as 1:1 were obtained by the methods of continuous changes and the saturation method. Linearity regression equation was  $y=0.0017x+0.0496$  and the obtained correlation coefficient was  $R^2=0.9993$ . The linear relationship was found between absorbance at  $\lambda_{max}$  and concentration of medicine in the range 15.48–154.80  $\mu\text{g/mL}$ . The LOD and LOQ values were calculated to be 4.85  $\mu\text{g/mL}$  and 14.71  $\mu\text{g/mL}$  respectively. Spectrophotometric method for the determination of atorvastatin in tablets using BTB was developed in accordance with GAC principles.

**Conclusions.** A simple, economic, fast, reliable and eco-friendly spectrophotometric method was developed for the determination of atorvastatin calcium in tablets based on the reaction with BTB and validated according to the standardized validation procedure by the standard method. It was proved that according to such validation characteristics as linearity, precision, accuracy, and robustness the proposed method met the requirements of SPhU

**Keywords:** atorvastatin, bromothymol blue, statins, spectrophotometry, validation, quantitative determination, pharmaceutical analysis, tablets

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## 1. Introduction

Statins are a class of cholesterol-lowering medicines that inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A-reductase and are commonly used to prevent or treat cardiovascular disease [1]. (3R,5R)-7-[2-(4-Fluorophenyl-5-isopropyl-3-phenyl-4-(phenyl-carbamoyl)-1H-pyrrol-1-yl)]-3,5-dihydroxyheptanoic acid calcium salt (atorvastatin) belongs to a group of medicines called 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, or “statins”. There is no monograph on the substance atorvastatin calcium in the State Pharmacopoeia of Ukraine (SPhU). European Pharmacopoeia (EP) [2] has a monograph on the atorvastatin calcium substance. EP regulates identification of atorvastatin calcium with the implementation of absorption spectrophotometry in the infrared region, determination of enantiomeric purity and reactions to the detection of the cation  $\text{Ca}^{2+}$ , quantitative determination – liquid chromatogra-

phy (LC). To quantify atorvastatin calcium, LC method is presented, which involves the use of octadecylsilyl column 0.25 m $\times$ 4.6 mm, gradient elution, and mobile phase A – tetrahydrofuran, acetonitrile, 3.9 g/l ammonium acetate solution pH 5.0 (12:21:67), mobile phase B – tetrahydrofuran, 3.9 g/l ammonium acetate solution pH 5.0, acetonitrile (12:27:61). The disadvantages of the proposed pharmacopoeial method include the long-time of chromatography (85–90 min) and the use of a significant volume of the mobile phase per chromatography. Several analytical methods, including spectrophotometric methods [3–12] and chromatographic methods [13–25] have already been reported for its determination, either alone or in combination with other medicines. Ukrainian scientists have developed a spectrophotometric method for the determination of atorvastatin calcium in tablets by reaction with bromocresol purple at a wavelength of 399 nm in acetone [3]. However, the methods of analysis described

in the scientific literature often have limited application due to not always sufficient sensitivity, specificity, pH dependence, the need for heating, complexity, durability, use of expensive instruments and toxic reagents (Table 1) [3–11]. There were two published analytical methods for determining of atorvastatin calcium either alone [12] and in combination with lisinopril [13], which were developed by our scientific group. Both are chromatographic methods, which require expensive equipment (liquid chromatograph, columns). For the needs of routine pharmaceutical analysis with less sophisticated equipment and budgets, spectrophotometric methods of analysis are undoubtedly preferred. There is, therefore, a need for a simple, fast, reliable and eco-friendly spectrophotometric methods for the determination of atorvastatin in tablets for routine pharmaceutical analysis with less sophisticated equipment and budgets.

Therefore, **the aim of research** was to develop a simple, economic, fast, reliable, and eco-friendly spectrophotometric method for the determination of atorvastatin in tablets based on the reaction with bromothymol blue (BTB).

## 2. Planning of the research

Methodology of the research of development of simple, economic, fast, reliable and eco-friendly spectrophotometric method for the determination of atorvastatin in tablets based on the reaction with BTB includes:

1. Study of the recommendations of the State Pharmacopoeia of Ukraine (SPHU) and EP, analysis of data from scientific literature.

2. The study of the conditions for reaction atorvastatin and BTB (choice of solvent, optimal wavelength and time, detection of stoichiometric coefficients) and its optimization for further use in the development of spectrophotometric methods.

3. The application of the proposed spectrophotometric method for the determination of atorvastatin by using BTB to the analysis of tablets.

4. Validation of the spectrophotometric method for determination of atorvastatin in tablets (specificity, linearity and range of application, accuracy, precision, robustness).

5. Study of the greenness profile assessment of the developed method (analytical GREENness, eco-scale).

Table 1

Comparison of the proposed and the existing spectrophotometric methods

No.	Drug	Reagent	Medium	$\lambda_{\max}$ , nm	Concentration range	LOD/LOQ, $\mu\text{g/mL}$	Reference
1	Tablets	Bromocresol Purple	Acetone	399	1.4–2.0 mg/100 mL	–	[3]
2	Bulk and dosage form	p-dimethylamino-benzaldehyde	Acidic (sulphuric acid)	540	20–160 $\mu\text{g/mL}$	LOD – 5.7, LOQ – 19	[4]
3	Pharmaceutical formulation	Pararosaniline Hydrochloride	Basic	547	1–8 $\mu\text{g/mL}$	LOD – 0.31, LOQ – 0.93	[5]
4	Pure form and tablets	Sulfo-Phospho-Vanillin	Acidic (sulphuric acid)	414	30–100 $\mu\text{g/mL}$	–	[6]
5	Pure and pharmaceutical formulations	Iodine	Acetonitrile	291 and 360	0.5586–11.173 $\mu\text{g/mL}$	LOD – 0.056, LOQ – 0.17	[7]
6	API Formulation	–	Methanol	244	–	–	[8]
7	Tablet dosage form in the presents of fenofibrate	–	Methanol	246	1–10 $\mu\text{g/mL}$	–	[9]
8	Combined tablet dosage form in the presents of amlodipine besylate	–	Methanol	256–238.5	5–50 $\mu\text{g/mL}$	LOD – 0.5, LOQ – 0.5	[10]
9	Tablet dosage form in the presence of telmisartan	–	Methanol	297	5–30 $\mu\text{g/mL}$	–	[11]
10	Tablets	Bromothymol blue	Ethyl acetate	420	15.48–154.80	LOD – 4.85, LOQ – 14.71	Proposed method. Simple, fast, ecofriendly and no heating step. Use of single reagent and no extraction step involved

### 3. Materials and methods

Objects of study, solvents and equipment.

A double-beam Shimadzu UV-Visible spectrophotometer, with spectral bandwidth of 1 nm wavelength accuracy  $\pm 0.5$  nm, Model – UV 1800 (Japan), Software UV-Probe 2.62, and a pair of 1 cm matched quartz cells, was used to measure absorbance of the resulting solution. Designed in accordance with the governing Japanese and European Pharmacopoeia, the new UV-1800 UV-VIS spectrophotometer achieves a resolution of 1 nm, the highest in its class, in a compact design.

Other used instruments: Analytical Balance RAD WAG AS 200/C, IKA orbital shaker KS4000i.

All the chemicals were used of analytical reagent grade.

Pharmacopoeial standard sample (CRS) of atorvastatin calcium and BTB were provided by Sigma-Aldrich ( $\geq 98\%$ , HPLC).

The used dosage forms of atorvastatin: tablets Atorvastatin 10 mg and 20 mg.

Proposed procedure for the determination of atorvastatin calcium with BTB.

7.74 mg of CRS atorvastatin calcium was transferred into a 50.00 mL volumetric flask with 35 mL ethyl acetate. The mixture was shaken and diluted to volume with ethyl acetate. Aliquot 1.00 mL was added to 1.0 mL of  $1.28 \times 10^{-4}$  M ethyl acetate solution of BTB. The volume 10.00 mL was made up to the mark by adding ethyl acetate. The absorbance of the resulting solution was measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 420 nm.

Procedure for tablets for the determination of atorvastatin calcium with BTB.

Twenty tablets were accurately weighed and powdered. A quantity of powder containing 7.74 mg of atorvastatin calcium was transferred into a 50.00 mL volumetric flask with 35 mL ethyl acetate. The mixture was

shaken for 15 min, diluted to volume with ethyl acetate and then filtered using  $0.2 \mu\text{m}$  Nylon filter membrane. Aliquot 1.00 mL was added to 1.0 mL of  $1.28 \times 10^{-4}$  M ethyl acetate solution of BTB. The volume 10.00 mL was made up to the mark by adding ethyl acetate. The absorbance of the resulting solution was measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 420 nm.

*Procedure of preparation of compensating solution.*

3.12 mg of BTB was transferred into a 50.00 mL volumetric flask with 35 mL ethyl acetate, diluted to volume with ethyl acetate and mixed.

### 4. Research results

#### 4. 1. Selection of reaction conditions

A number of works were devoted to the development of methods of analysis using sulfophthalein dyes, which we took for consideration in planning our research [14–19]. Various approaches were reported, including extraction-spectrophotometric methods [14], formation of ionic associative complexes with sulfonephthalein dyes in organic solvents [14–17], approaches in which organic solvents were not used [18, 19]. The main purpose of this study was to obtain non-extraction complexes, as they have several advantages in the development of analytical methods for determining API in medicine. We tested various sulfophthalein dyes to obtain a non-extractive binary complex between atorvastatin calcium and an ionic associate dye. Atorvastatin forms ion-pair complexes selectively with dyes, as evidenced by the formation of a coloured complex with BTB with  $\lambda_{\text{max}}$  at 420 nm. The formed ionic associates are sparingly soluble in water, but in optimized experimental conditions are freely soluble and do not require extraction with organic solvents, which was our main goal in developing the analytical method. The spectra of absorbance of the reaction product of atorvastatin calcium with BTB are presented in Fig. 1.

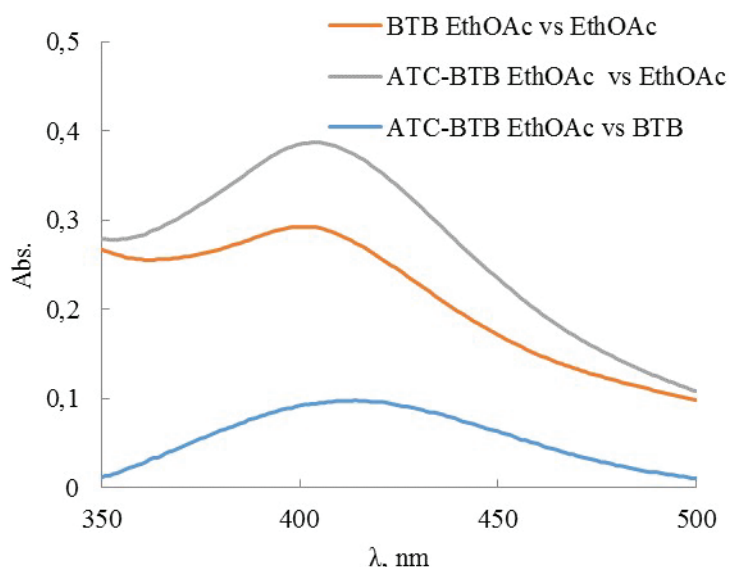


Fig. 1. Absorbance spectra of the reaction product of atorvastatin calcium from BTB against ethyl acetate (grey), against BTB (blue) and BTB against ethyl acetate (red)

Before starting development a spectrophotometric method for the determination of atorvastatin calcium by reaction with BTB, we optimized the conditions in order to form a color complex of ionic associates with maximum stability and sensitivity. Among the organic solvents studied (Fig. 2), the order of blank absorbance for all the dyes was: chloroform>ethyl acetate>acetonitrile>methanol>ethanol. Given the principles of «green» analytical chemistry (GAC), we did not take into account chloroform and ethyl acetate was chosen for further investigation.

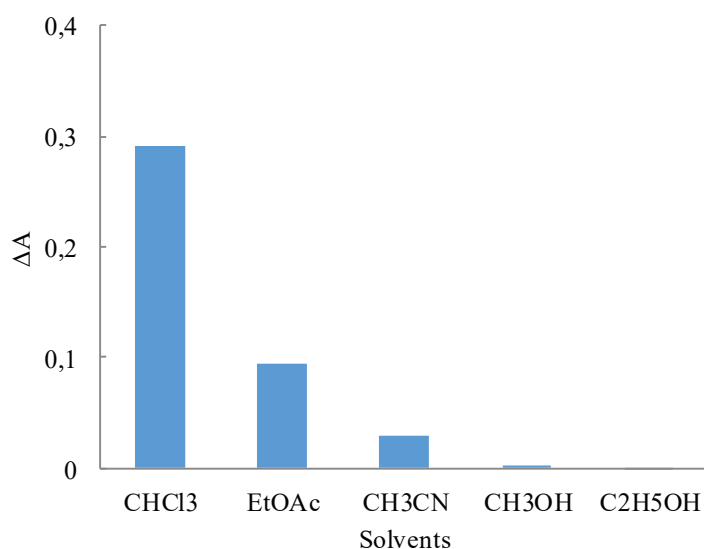


Fig. 2. Effect of solvents on the formation of Atorvastatin-BTB complex

The next and important step in the development of spectrophotometric method was the study of the stability of the analyzed solutions over time. If the solutions are unstable, it is necessary to use certain analytical techniques to stabilize them (eg, pH adjustment). To study the stability, the absorbance of the

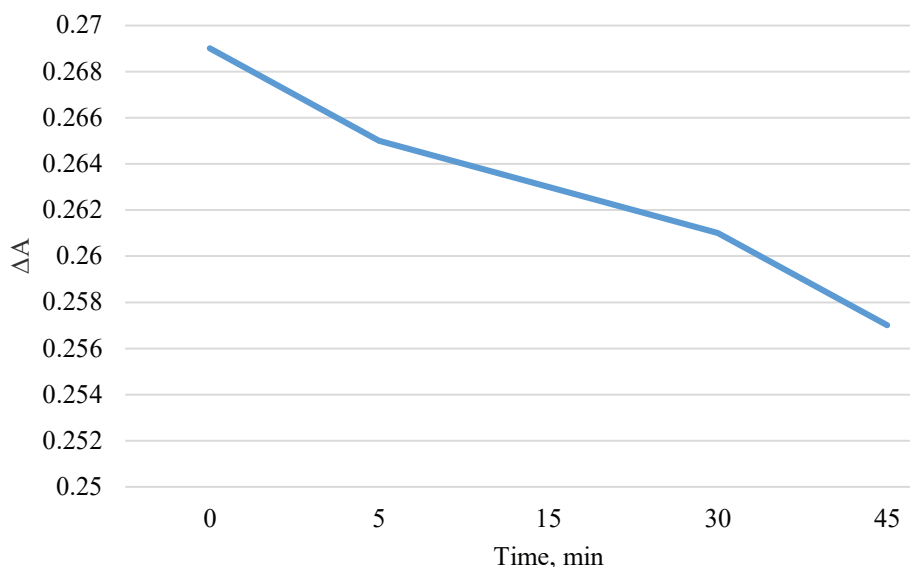


Fig. 3. Graph of dependence of absorbance of the product of the reaction of atorvastatin calcium with BTB on time

obtained solution was measured under optimal conditions for 45 min. It was found that the tested solutions are stable for 45 minutes (Fig. 3). This fact is one of the advantages of the future developed method, as it does not require pH adjustment and the addition of buffer solutions, which would complicate sample preparation.

The study of the influence of the BTB concentration on the absorbance at 420 nm at a constant concentration of atorvastatin showed that at the concentrations of BTB above  $1.28 \times 10^{-4}$  M, the dependence levels off. The concentration of BTB was  $1.28 \times 10^{-4}$  M was chosen as optimal.

Stoichiometric coefficients between atorvastatin and BTB were determined by the method of continuous changes (or Job's method) and by the method of saturation (by the method of molar ratios) [3, 15]. Job's method is based on determining the ratios of isomolar concentrations of reactants, which corresponds to the maximum yield of compounds formed because of the reaction. To do this, prepare solutions of BTB and atorvastatin calcium of the same molar concentration ( $1.28 \times 10^{-4}$  M) and mixed them in the antipate ratio (1/4 to 4/1), while the total volume of the solution remains unchanged. The reaction was carried out according to the proposed method. The graph of the dependence of the amount of absorbance on the ratio of the volumes of the components of the isomolar series is presented on Fig. 4. The method of molar ratios determines the dependence of the absorbance on the concentration of one of the components of the reaction mixture at a constant concentration of the other component and vice versa. The inflection point on the saturation curve is equal to the stoichiometric coefficient of the component whose concentration varied (Fig. 5). As could be seen from Fig. 4, 5, the obtained stoichiometric coefficients of the reacting components

of the reaction of the interaction of atorvastatin calcium with BTB agree with each other and are 1:1. The results obtained from both method were in agreement with the probable mechanism for the formation of ion-pair complex of atorvastatin with BTB (Fig. 6).

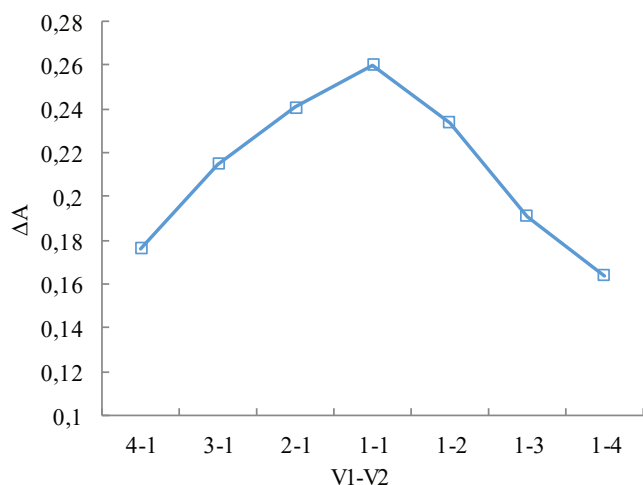


Fig. 4. Graph of the dependence of the amount of absorbance on the composition of the isomolar solution (Job's method): V1 –  $1.28 \times 10^{-4}$  M atorvastatin calcium solution; V2 –  $1.28 \times 10^{-4}$  M solution BTB) at 420 nm

In order to establish the analytical sensitivity of the reaction of atorvastatin calcium with BTB, the sensitivity of the reaction was calculated. The molar ab-

sorption ( $\epsilon$ ) was  $2.03 \times 10^3$ , the specific absorption ( $a$ ) was  $1.68 \times 10^{-3}$ , the Sendel coefficient ( $Ws$ ) was 0.09 respectively.

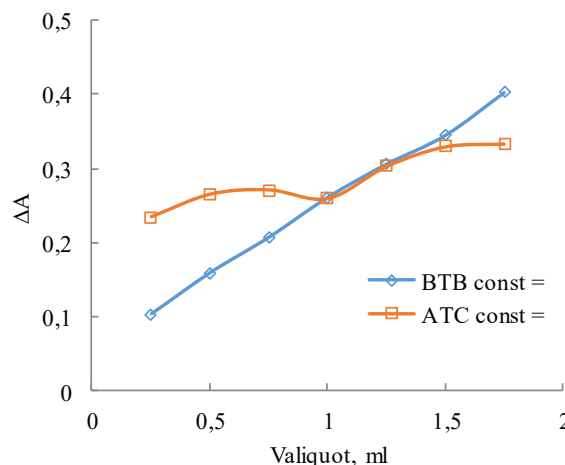


Fig. 5. Saturation curves: atorvastatin calcium solution at a constant concentration of reagent (1.00 mL of  $1.28 \times 10^{-4}$  M solution), BTB solution at a constant concentration of atorvastatin calcium (1.00 mL of  $1.28 \times 10^{-4}$  M solution)

#### 4. 2. Determination of validation characteristics

The developed analytical method was validated in accordance with the requirements of SPbU [20] for the following indicators: specificity, linearity, range of application, accuracy, precision and robustness.

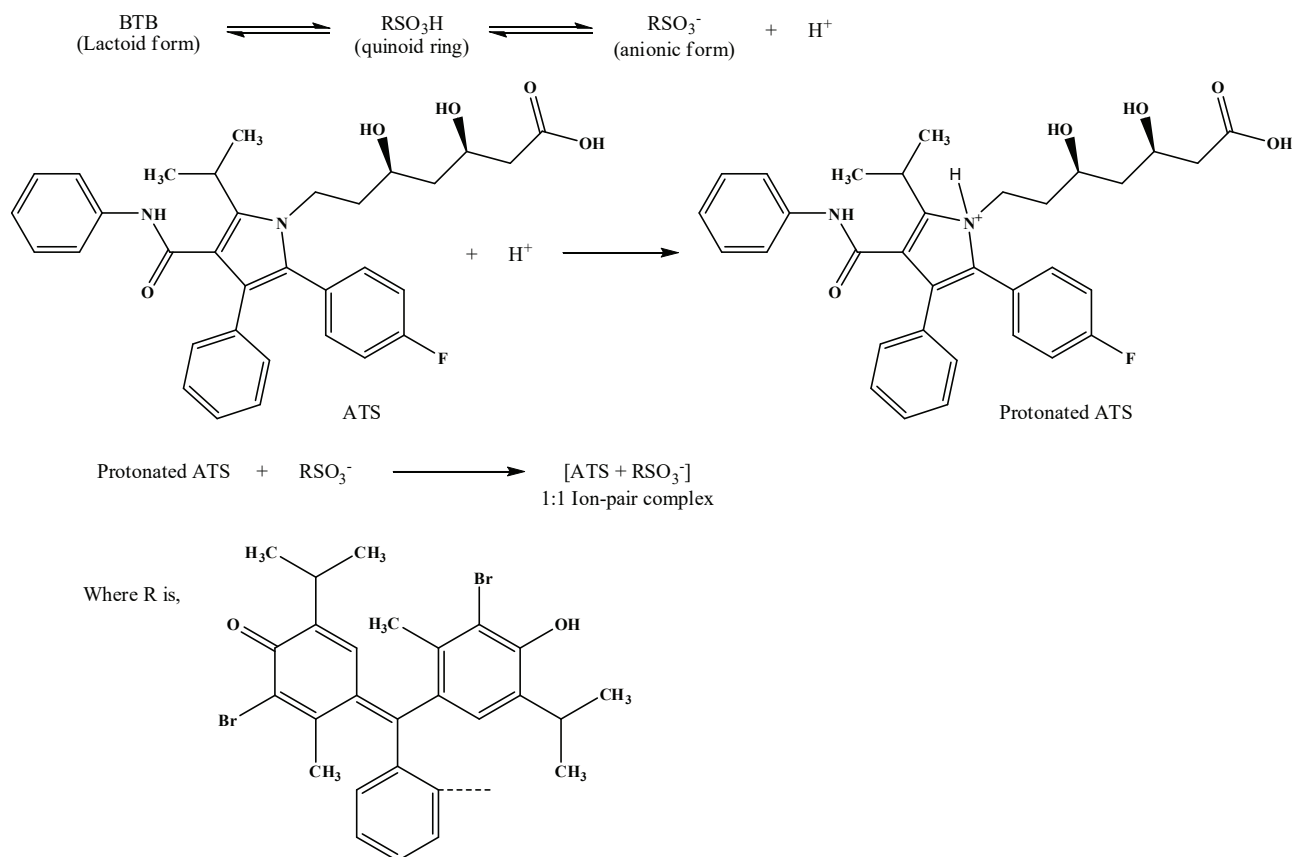


Fig. 6. The probable mechanism for the formation of ion-pair complex of atorvastatin with BTB

#### 4.2.1. Specificity

To confirm the specificity of the spectrophotometric method of determining atorvastatin in tablets by reaction with BTB, a solution of excipients ("placebo") was prepared. The influence of impurities on the results of the quantitative determination of atorvastatin calcium was not studied, since during the performance of this work, industrially produced medicines were used that did not contain an unacceptable amount of impurities, as evidenced by the quality certificates of the manufacturers. The results of studying the specificity of the analytical method are presented in the Table 2.

Table 2

The results of the study of the specificity of the spectrophotometric method of determining atorvastatin calcium in tablets by reaction with BTB

Absorbance of placebo (A <sub>placebo</sub> )	Absorbance of a solution of impurities (A <sub>impurities</sub> )	Absorbance of the compensating solution (A <sub>st</sub> )	Value $\delta_{noise}$ , %	Criteria
0.001	–	0.267	0.37	Not more than 0.5 %

From the data presented in the Table 2, it follows that the absorbance of excipients was insignificant (value of  $\delta_{noise}$  was 0.37 %) and did not exceed the acceptance criterion.

#### 4.2.2. Linearity and range of application

Determination of linearity was performed over the entire range of application of the method using model solutions. The obtained results were statistically processed by the method of least squares in accordance with the requirements of the SPhU. The linearity of the analytical method was studied using the procedure described in sample preparation. Aliquots of 0.2–1 mL were taken. The results of calculations of the linear regression equation and analytical parameters are given in Table 3.

Table 3

Numerical indicators of linear dependence and analytical parameters ( $p=95\%$ ;  $n=5$ )

Indicator	Value	Criteria	Conclusion
$b \pm (S_b)$	$0.0017 \pm \pm(0.036)$	–	
$a \pm (S_a)$	$0.0496 \pm \pm(0.0025)$	$ a  \leq \Delta a = t(2.77) \cdot S_a = 0.0069$	Corresponds
$R^2$	0.9993	$> 0.9984$	Corresponds
LOD ( $\mu\text{g/mL}$ )	4.85	–	
LOQ ( $\mu\text{g/mL}$ )	14.71	–	
Beer's law limits ( $\mu\text{g/mL}$ )	15.48–154.80	–	

The linear relationship was found between absorbance at  $\lambda_{max}$  and concentration of medicine in the range 15.48–154.80  $\mu\text{g/mL}$ .

#### 4.2.3. Accuracy and precision

To verify the accuracy and precision of the analytical method, mixtures with a well-known content of API were prepared, which covered the range of application of the method (with concentrations of 70–130 % of nominal). The results of the calculations are given in Tables 4, 5.

Table 4

The results of the study of the precision of the method of quantitative determination of atorvastatin calcium in tablets

Dosage form	$\bar{Z}\%$ ( $n=9$ )	$S_{z\%}$	$\max \Delta_{AS}$	$\Delta_{\%}$	Conclusion
Atorvastatin 20 mg	100.07	0.15	1.60	0.35	Corresponds
Atorvastatin 10 mg	100.04	0.13	1.60	0.30	Corresponds

Table 5

The results of the study of the correctness of the method of quantitative determination of atorvastatin calcium in tablets

Dosage form	$\bar{Z}\%$ ( $n=9$ )	$S_{z\%}$	$\Delta_{\%}$	$\delta$	$0.32 \cdot \Delta_{AS}$	Conclusion
Atorvastatin 20 mg	99.68	0.72	1.34	0.32	0.51	Corresponds
Atorvastatin 10 mg	100.23	0.26	0.48	0.23	0.51	Corresponds

According to the obtained results in Table 4, the one-sided confidence interval  $\Delta_{\%}$  did not exceed the maximum allowable uncertainty of the analysis  $\max \Delta_{AS}$ , so the method was accurate at the level of convergence. According to the obtained results in Table 5, calculated criteria of practical insignificance for dosage forms did not exceed the maximum allowable uncertainty of the analysis.

#### 4.2.4. Robustness

The study of robustness was carried out at the stage of development of spectrophotometric method for determining atorvastatin calcium by reaction with BTC during the establishment of optimal conditions of reaction (stability of solutions over time, the amount of reagent added). It was found that the analyzed solutions are stable for 45 min (provided the cell was tightly closed during absorption measurement) (Fig. 3), and fluctuations in the amount of added reagent (BTC solution) (Table 6) within  $\pm 10\%$  does not significantly affect the absorbance.

Table 6

Determination of the robustness of the analytical method

Amount of BTB, mL	% BTB	$\Delta A$
0.90	90.00	0.265
0.95	95.00	0.267
1.00	100.00	0.268
1.05	105.00	0.268
1.10	110.00	0.270

### 4.3. Assessment of the impact of analytical methods on the environment

As mentioned above, the aim of our study was to develop a non-extraction spectrophotometric method for the determination of atorvastatin calcium in tablets, considering GAC principles [21, 22]. Assessment of the «greenness» of the analytical methodology was performed using the analytical eco-scale and the method AGREE (Analytical GREENess). The score of the analytical eco-scale was 90 (Table 7). The results of the study of the «greenness» of the analytical methodology using the AGREE method are shown in Fig. 7.

Table 7  
Analytical eco-scale for assessing the «greenness» of the developed method

Parameters	Penalty points
Reagents	
BTB	1
Ethyl acetate	3
Energy	1
Waste	5
Total number of penalty points	10
Ball of analytical eco-scale	90
Conclusion	Excellent «green» analysis

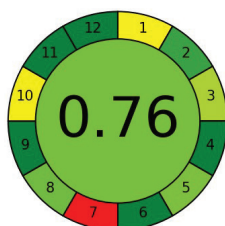


Fig. 7. Pictogram of analytical methodology using AGREE method

### 5. Discussion of research results

Only one spectrophotometric method for determining atorvastatin by reaction with sulfonephthalein dye, namely bromocresol purple, was described in the scientific literature [3]. The cited article also used a sulfonephthalein dye and an organic solvent. However, the molar absorptivity was lower, the calibration range was unexpectedly narrow from 14 to 20 mg/L, and the acetone used as a solvent was not suitable, as it evaporates very easily, was toxic and therefore the method cannot correspond to GAC principles.

Sulfonephthalein dyes exist in solution mainly in two protonated forms – monoprotinated, where the proton is split off from the sulfogroup, and in the dianionic form, where the second proton is split off from one of the phenolic hydroxyls. For BTB, these forms absorb in aqueous solution at 420 nm (monoanionic form) and at 590 nm (dianionic form). In organic solvents, the position of these bands is slightly shifted due to the solvatochromic effect. We considered the possibility of applying the method of differential spectrophotometry by reaction with BTB using the maximum absorption of the reaction product at a wavelength of 420 nm. Optimal spectrophotometric conditions were established. The stoichiometric ratios of the reactive components as 1:1 were obtained by the methods of contin-

uous changes and the saturation method. Linearity regression equation was  $y=0.0017x+0.0496$  and the obtained correlation coefficient was  $R^2=0.9993$ . The linear relationship was established between absorbance at  $\lambda_{max}$  and concentration of medicine in the range 15.48–154.80  $\mu\text{g/mL}$ . The LOD and LOQ values were calculated to be 4.85  $\mu\text{g/mL}$  and 14.71  $\mu\text{g/mL}$  respectively. The proposed method of quantitative determination of atorvastatin calcium is characterized by specificity, linearity, accuracy, precision, robustness. The results, presented in Table 7 and Fig. 7, indicate that the spectrophotometric method for the determination of atorvastatin in tablets using BTB was developed in accordance with GAC principles.

**Study limitations.** The developed method cannot be used to determine atorvastatin calcium in the presence of other statins and antihypertensive medicines.

**Prospects for further research.** The article describes the main stages of spectrophotometric method development of atorvastatin calcium in tablets based on the reaction with BTB. The next stage of research is planned to develop the spectrophotometric method for determination of atorvastatin calcium in tablets based on the reaction with BPB (bromophenol blue).

### 6. Conclusions

A simple, economic, fast, reliable, and eco-friendly spectrophotometric method was developed for the determination of atorvastatin calcium in tablets based on the reaction with BTB and validated according to the standardized validation procedure by the standard method. Maximum absorbance was observed in a solution of chloroform and ethyl acetate, while ethanol, methanol and acetonitrile were unsuitable. Given the principles of GAC, we did not consider chloroform and chose ethyl acetate as optimal solvent. It was established that the optimal concentration of BTB was  $1.28 \times 10^{-4}$  M. The stoichiometric ratios of the reactive components as 1:1 were obtained. The linear relationship was established between absorbance at  $\lambda_{max}$  and concentration of medicine in the range 15.48–154.80  $\mu\text{g/mL}$ . The LOD and LOQ values were calculated to be 4.85  $\mu\text{g/mL}$  and 14.71  $\mu\text{g/mL}$  respectively. Proposed method does not require various elaboration treatment and tedious extraction procedure required in other traditional extractive spectrophotometric methods.

### 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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