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

Nataliia Shulyak, Svitlana Protsyk, Tetyana Kucher, Liubomyr Kryskiw, Olha Poliak, Nadiya Zarivna, Liliya Logoyda

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 ±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 (≥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 λmax and concentration of medicine in the range 15.48–154.80 µg/mL. The LOD and LOQ values were calculated to be 4.85 µg/mL and 14.71 µg/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*

1. Introduction

Statins are a class of cholesterol-lowering medicines that inhibit the enzyme 3-hydroxy-3-methyl-glutaryl-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)-1*H*-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 Ca^{2+}, quantitative determination – liquid chromatography (LC). To quantify atorvastatin calcium, LC method is presented, which involves the use of octacedylsilyl column 0.25 m×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 Phar-macopoeia 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	λmax, nm	Concentration range	LOD/LOQ, µg/mL	Reference
1	Tablets	Bromcresol 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 µg/mL	LOD – 5.7, LOQ – 19	[4]
3	Pharmaceutical formulation	Pararosaniline Hydrochloride	Basic	547	1–8 µg/mL	LOD – 0.31, LOQ – 0.93	[5]
4	Pure form and tablets	Sulfo-Phospho-Vanillin	Acidic (sulphuric acid)	414	30–100 µg/ml	–	[6]
5	Pure and pharmaceutical formulations	Iodine	Acetonitrile	291 and 360	0.5586–11.173 µg/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 µg/ml	–	[9]
8	Combined tablet dosage form in the presents of amlodipine besylate	–	Methanol	256–238.5	5–50 µg/mL	LOD – 0.5, LOQ – 0.5	[10]
9	Tablet dosage form in the presence of telmisartan	–	Methanol	297	5–30 µg/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 ±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.

Pharmacopeial standard sample (CRS) of atorvastatin calcium and BTB were provided by Sigma-Aldrich (≥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 1.28×10^{-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 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 µm Nylon filter membrane. Aliquot 1.00 mL was added to 1.0 mL of 1.28×10^{-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 λ 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.
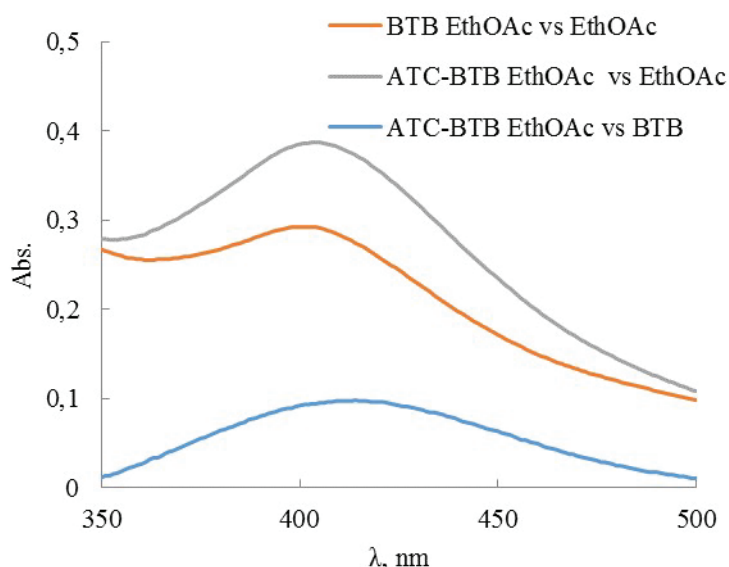

Fig. 1. Absorbance spectra of the reaction product of atorvastatin calcium from BTB against ethyl acetate (grey), against BTB (blue) and BTB against ethyl acetate (red)

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.
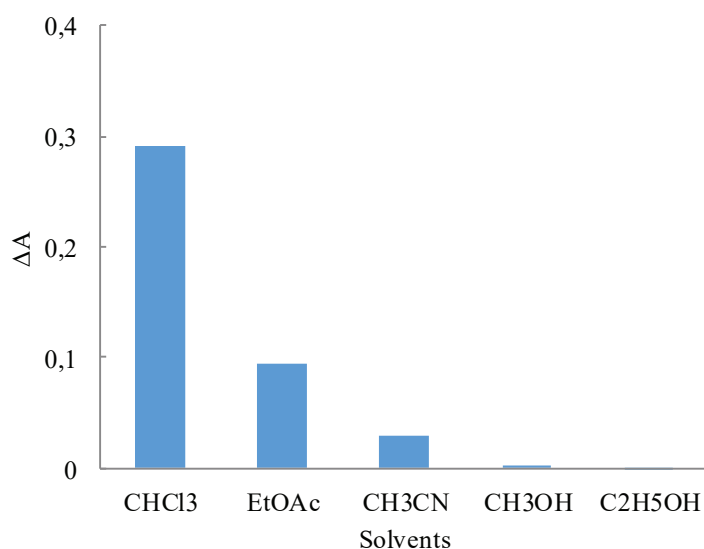

Fig. 2. Effect of solvents on the formation of Atorvastatin-BTB complex

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 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 1.28×10^{-4} M, the dependence levels off. The concentration of BTB was 1.28×10^{-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 (1.28×10^{-4} M) and mixed them in the antibate 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
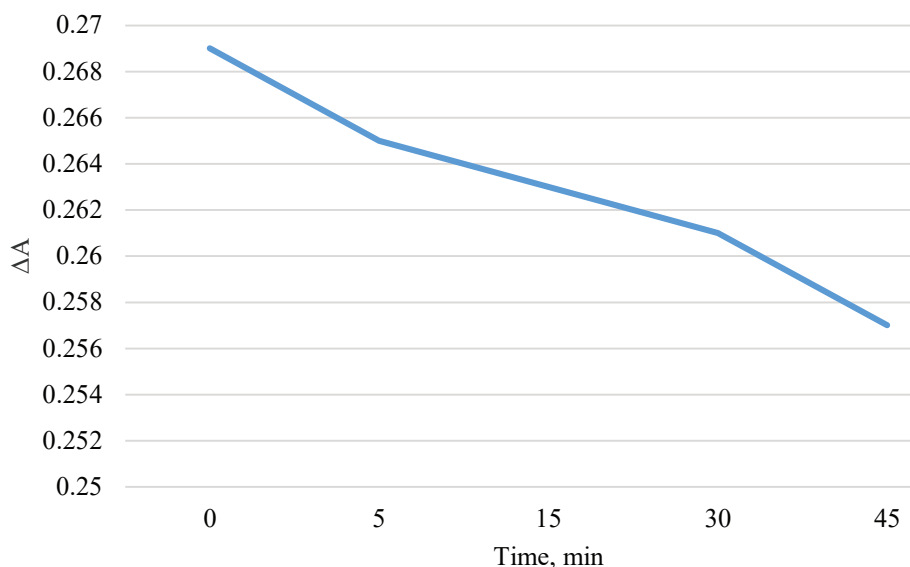

Fig. 3. Graph of dependence of absorbance of the product of the reaction of atorvastatin calcium with BTB on time

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).
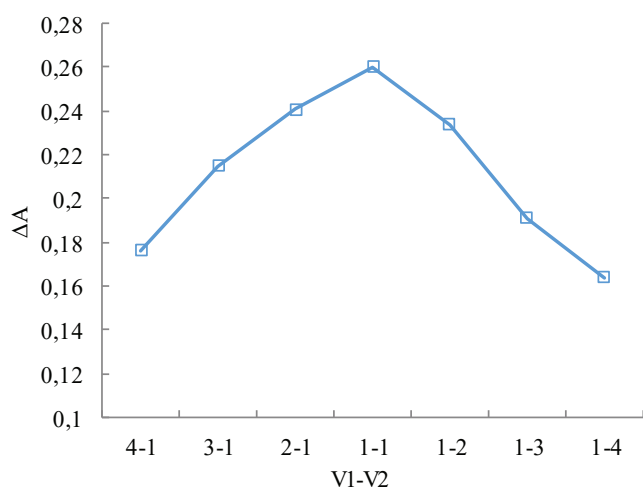
sorption (ε) was 2.03×10^3, the specific absorption (a) was 1.68×10^{-3}, the Sendel coefficient (Ws) was 0.09 respectively.

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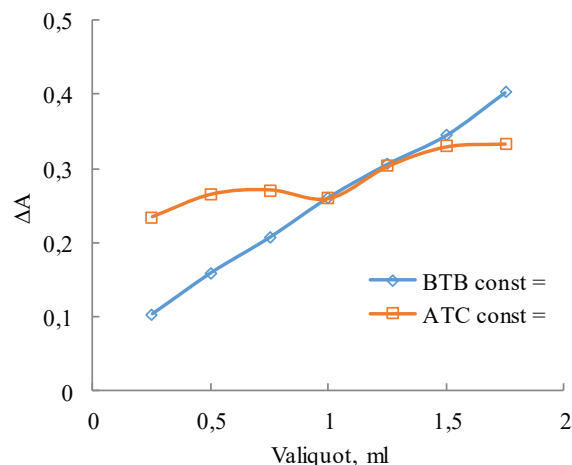

Fig. 5. Saturation curves: atorvastatin calcium solution at a constant concentration of reagent (1.00 mL of 1.28×10^{-4} M solution), BTB solution at a constant concentration of atorvastatin calcium (1.00 mL of 1.28×10^{-4} M solution)

4. 2. Determination of validation characteristics

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

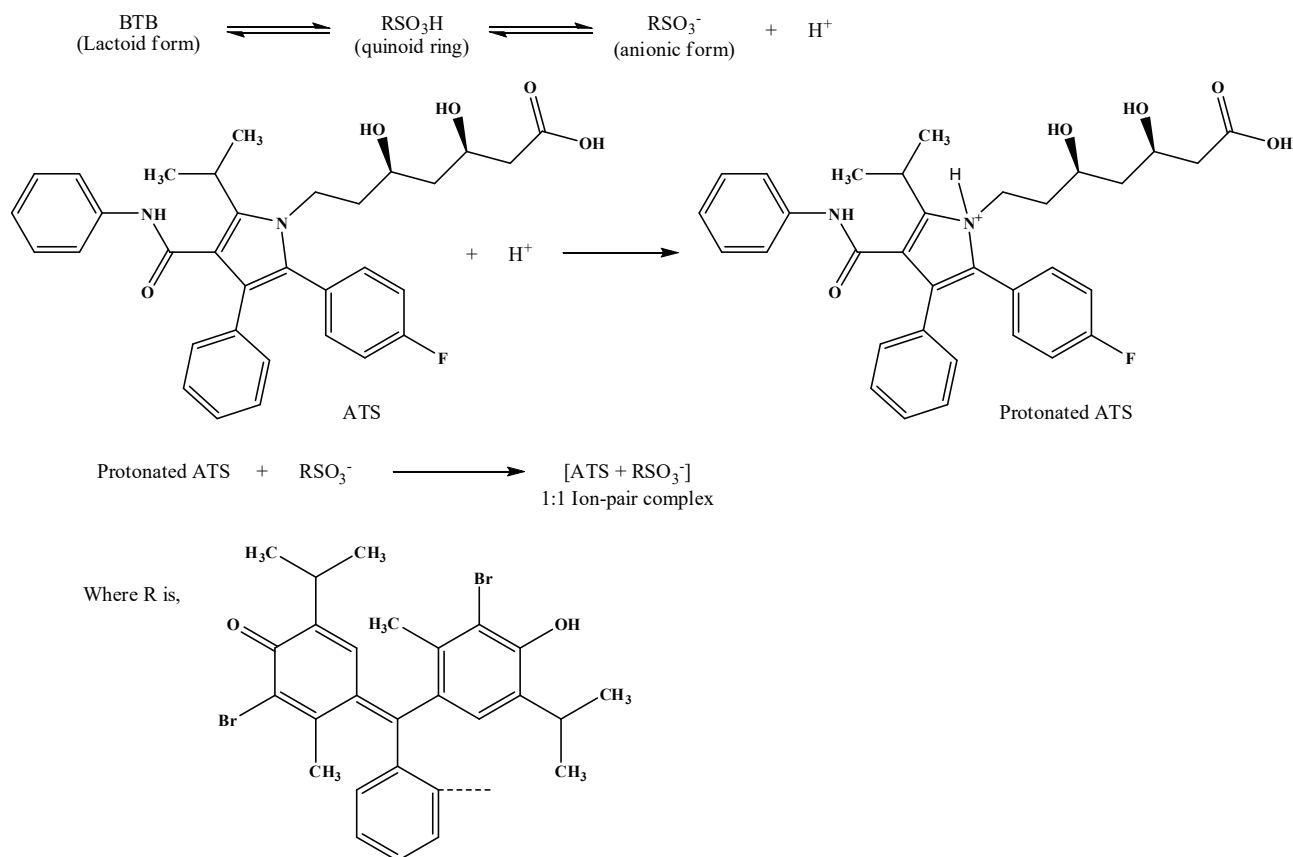In order to establish the analytical sensitivity of the reaction of atorvastatin calcium with BTB, the sensitivity of the reaction was calculated. The molar ab-

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 placebo)	Absorbance of a solution of impurities (A impurities)	Absorbance of the compensating solution (Ast)	Value δnoise, %	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 δnoise 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± ±(0.036)	–	
$a\pm(S_a)$	0.0496± ±(0.0025)	$\|a\|\leq\Delta a=$ $=t(2.77)\cdot S_a=$ $=0.0069$	Corresponds
R^2	0.9993	> 0.9984	Corresponds
LOD (µg/mL)	4.85	–	
LOQ (µg/mL)	14.71	–	
Beer's law limits (µg/mL)	15.48–154.80	–	

The linear relationship was found between absorbance at λmax and concentration of medicine in the range 15.48–154.80 µg/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	$\overline{Z}\%$ (n=9)	$S_{z\%}$	max Δ_{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	$\overline{Z}\%$ (n=9)	$S_{z\%}$	$\Delta_{\%}$	δ	$0.32\cdot\Delta As$	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 Δ_{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 ±10 % does not significantly affect the absorbance.

Table 6

Determination of the robustness of the analytical method

Amount of BTB, mL	% BTB	ΔA
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 GREEnness). 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

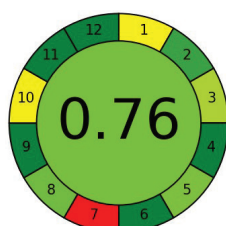| Parameters	Penalty points
Reagents	
BTB	1
Ethyl acetate	3
Energy	1
Waste	5
Total number of penalty points	10
Ball of analytical eco-scale	90
Conclusion	Exellent «green» analysis

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 – monoprotonated, 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 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 established between absorbance at λmax and concentration of medicine in the range 15.48–154.80 μg/mL. The LOD and LOQ values were calculated to be 4.85 μg/mL and 14.71 μg/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 1.28×10^{-4} M. The stoichiometric ratios of the reactive components as 1:1 were obtained. The linear relationship was established between absorbance at λmax and concentration of medicine in the range 15.48–154.80 μg/mL. The LOD and LOQ values were calculated to be 4.85 μg/mL and 14.71 μg/mL respectively. Proposed method does not require various elaboration treatment and tedious extraction procedure required in other traditional extractive spectrophotometric methods.

References

1. Lippi, G., Mattiuzzi, C., & Cervellin, G. (2019). Statins popularity: A global picture. British Journal of Clinical Pharmacology, 85 (7), 1614–1615. doi: http://doi.org/10.1111/bcp.13944

2. European Pharmacopoeia (2020). Available at: https:www.edqm.eu/en/european-pharmacopoeia-ph-eur-10th-edition

3. Myronova, O. Yu., Vasjuk, S. O. (2015). Spectrophotometric determination of atorvastatin calcium. Current issues in pharmacy and medicine: science and practice, 1 (17), 32–35. doi: http://doi.org/10.14739/2409-2932.2015.1.41370

4. Al Adl, S. M., Abdel Aziz, L. M., Mohamed, M. A. (2017). Spectrophotometric Determination of Atorvastatin Calcium and Rosuvastatin Calcium in Bulk and Dosage Form Using P-Dimethylaminobenzaldehyde. Journal of Applied Pharmacy, 9 (1). doi: http://doi.org/10.21065/1920-4159.1000233

5. Alshabrawy, A., Mostafa, A., Abotaleb, N. (2017). Sensitive Spectrophotometric Determination of Atorvastatin in Pharmaceutical Formulation by Ion Pair Complexation with Pararosaniline Hydrochloride. Journal of Advanced Pharmacy Research, 1 (4), 193–200. doi: http://doi.org/10.21608/aprh.2017.4040

6. Bernard, S., Rani, S., Babitha, M. C. (2018). New spectrophotometric method for the estimation of atorvastatin calcium and aspirin using urea as hydrotropic solubilizing agent, Hygeia. Journal for Drugs and Medicines, 9 (2), 11–19. doi: http://doi.org/10.15254/h.j.d.med.9.2018.169

7. Ramadan, A. A., Mandil, H., Sabouni, J. (2015). Determination of atorvastatin calcium in pure and its pharmaceutical formulations using iodine in acetonitrile by UV-Visible spectrophotometric method. International Journal of Pharmacy and Pharmaceutical Sciences, 7 (9), 427–433. Available at: https://innovareacademics.in/journals/index.php/ijpps/article/view/7535/pdf_1137

8. Naveed, S. (2014). Simple UV spectrophotometric assay of atorvastatin API formulation and their comparative study. D Global Journal ofMedical research: BPharma, Drug Discovery, Toxicology and Medicine, 14 (2), 211–216. Available at: https://globaljournals.org/GJMR_Volume14/4-Simple-UV-Spectrophotometric-Assay.pdf

9. Hirave, R., Bendgude, R. D., Maniyar, M. G., Kondawar, M. S., Patil, S. B. (2013). Spectrophotometric method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet Dosage Form. International Journal of Drug Development and Research, 5 (1), 38–42. Available at: https://www.itmedicalteam.pl/articles/spectrophotometric-method-for-simultaneous-estimation-ofatorvastatin-calcium-fenofibrate-in-tablet-dosage-form-100789.html

10. Jani, D. J., Ahmed, M., Shetty, S. K. A, Sridhar, B. K., Shah, J. S. (2010). Simultaneous spectrophotometric estimation of atorvastatin calcium and amlodipine besylate in combined tablet dosage form by area under curve method. International Journal Chemical Science, 8 (1), 701–710. Available at: https://www.tsijournals.com/articles/simultaneous-spectrophotometric-estimation-of-atorvastatin-calcium-and-amlodipine-besylate-in-combined-tablet-dosage-for.pdf

11. Tomlesh, B. D., Sujata, S. D., Farhin, S. I. (2018). Simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage form by spectrophotometry. International journal of pharmacy and pharmaceutical research, 11 (2), 46–58. Available at: https://ijppr.humanjournals.com/wp-content/uploads/2018/02/5.Tomlesh-B.-Deshmukh-Sujata-S.-Deo-Farhin-S.-Inam.pdf

12. Shulyak, N., Piponski, M., Kovalenko, S., Bakovska Stoimenova, T., Balkanov, T., El-Subbagh, H. I. et. al. (2021). Development of a Novel, Fast, Simple HPLC Method for Determination of Atorvastatin and its Impurities in Tablets. Scientia Pharmaceutica, 89 (2), 16. doi: http://doi.org/10.3390/scipharm89020016

13. Shulyak, N., Piponski, M., Kovalenko, S., Stoimenova, T. B., Drapak, I., Piponska, M. et. al. (2021). Chaotropic salts impact in HPLC approaches for simultaneous analysis of hydrophilic and lipophilic drugs. Journal of Separation Science, 44 (15), 2908–2916. doi: http://doi.org/10.1002/jssc.202100168

14. Issa, Y. M., Abdel-Gawad, F. M., Abou Table, M. A., Hussein, H. M. (1997). Spectrophotometric Determination of Ofloxacin and Lomefloxacin Hydrochloride with Some Sulphonphthalein Dyes. Analytical Letters, 30 (11), 2071–2084. doi: http://doi.org/10.1080/00032719708001722

15. Prashanth, K., Basavaiah, K., Raghu, M. (2012). Rapid and sensitive spectrophotometric measurement of non-specific beta blocker propranolol hydrochloride using three sulphonphthalein dyes in pure form, pharmaceuticals and human urine. Chemical Sciences Journal, 2012, 2 14. Available at: https://www.hilarispublisher.com/open-access/rapid-and-sensitive-spectrophotometric-measurement-of-non-specific-beta-blocker-propranolol-hydrochloride-.2150-3494.1000056.pdf

16. Abdine, H., Belal, F., Zoman, N. (2002). Simple spectrophotometric determination of cinnarizine in its dosage forms. Il Farmaco, 57 (4), 267–271. doi: http://doi.org/10.1016/s0014-827x(02)01204-1

17. Derayea, S. M. S. (2014). An application of eosin Y for the selective spectrophotometric and spectrofluorimetric determination of mebeverine hydrochloride. Anal. Methods, 6 (7), 2270–2275. doi: http://doi.org/10.1039/c3ay41371c

18. Hedjazi, M., Vishnikin, A. B., Balanenko, A. D. (2021). A green spectrophotometric method for determination of drotaverine hydrochloride in pharmaceutical preparations using formation of ion association complex with erythrosine. Journal of Chemistry and Technologies, 29 (3), 467–475. doi: http://doi.org/10.15421/jchemtech.v29i3.242348

19. El-Enany, N. (2004). Spectrophotometric determination of gliclazide in pharmaceuticals and biological fluids through ternary complex formation with eosin and palladium (II). Il Farmaco, 59 (1), 63–69. doi: http://doi.org/10.1016/j.farmac.2003.08.007

20. Derzhavna farmakopeia Ukrainy. Vol. 1 (2015). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovo-ekspertnyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 1128.

21. Gałuszka, A., Migaszewski, Z. M., Konieczka, P., Namieśnik, J. (2012). Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC Trends in Analytical Chemistry, 37, 61–72. doi: http://doi.org/10.1016/j.trac.2012.03.013

22. Pena-Pereira, F., Wojnowski, W., Tobiszewski, M. (2020). AGREE – Analytical GREEnness Metric Approach and Software. Analytical Chemistry, 92 (14), 10076–10082. doi: http://doi.org/10.1021/acs.analchem.0c01887

Nataliia Shulyak, Postgraduate Student, Department of Pharmaceutical Chemistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Voli ave., 1, Ternopil, Ukraine, 46001, Lecturer, Municipal Institution of Higher Education «Volyn Medical Institute» of Volyn Oblast Council, Lesi Ukrainky str., 2, Lutsk, Ukraine, 43016

Svitlana Protsyk, Department of Pharmaceutical Chemistry**,** Ivan Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine**,** Voli ave., 1, Ternopil, Ukraine, 46001

Tetyana Kucher. PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** Ivan Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine**,** Voli ave., 1, Ternopil, Ukraine, 46001

Liubomyr Kryskiw, PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** Ivan Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine**,** Voli ave., 1, Ternopil, Ukraine, 46001

Olha Poliak. PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** Ivan Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine, Voli ave., 1, Ternopil, Ukraine, 46001

Nadiya Zarivna, PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** Ivan Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine**,** Voli ave., 1, Ternopil, Ukraine, 46001

Liliya Logoyda*, Doctor of Pharmaceutical Sciences, Professor, Head of Department, Department of Pharmaceutical Chemistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Voli ave., 1, Ternopil, Ukraine, 46001

***Corresponding author:** Liliya Logoyda, e-mail: logojda@tdmu.edu.ua*