Development of the Spectrophotometric Method for the Determination of Atorvastatin Calcium in Tablets by using Bromophenol Blue

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A simple, rapid, and inexpensive spectrophotometric method for the determination of atorvastatin calcium in tablets has been developed. It requires only one reagent (BPB) and suitable for routine pharmaceutical analysis with less sophisticated equipment. The procedure for atorvastatin determination is based on its interaction with bromophenol blue (BPB) in medium of organic solvent. As a result of the reaction, the intensity of the band of the doubly ionized form of the BPB with a maximum at 595 nm increases. The maximum analytical signal is observed in a solution of methanol, chloroform, or acetonitrile, while ethanol and ethyl acetate turned out to be unsuitable. The optimal concentration of BPB is $4.12 \cdot 10^{-4}$ M. To study the stability, the absorbance of the obtained solution was measured under optimal conditions for 45 min. The stoichiometric ratio of components in the resulting associate, determined by the method of continuous variations, is 1:1. The calibration graph is linear in the range from 4.1 to 33 μmol/l. The calculated LOD and LOQ values were 1.36 μmol/L and 4.13 μmol/L, respectively. The systematic error of the method is statistically insignificant in the whole range of calibration graph. The developed method was successfully applied for the determination of atorvastatin in tablets.

Keywords: atorvastatin, bromophenol blue, spectrophotometry, validation, quantitative determination, pharmaceutical analysis, tablets

Statins are a class of cholesterol-lowering drugs that work by inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and are usually used to prevent or treat heart disease. Atorvastatin is one of the first anti-atherosclerotic drugs that found intensive use about 40 years ago [1]. Chemically, atorvastatin calcium is calcium salt of (3R,5R)-7-[2-(4-fluorophenyl-5-isopropyl-3-phenyl-4-(phenyl-carbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid [2]. Doses from 10 mg to 80 mg of atorvastatin calcium are recommended. There is no monograph on the substance atorvastatin calcium in the State Pharmacopoeia of Ukraine (SPhU). European Pharmacopoeia (EP) has a monograph on the substance atorvastatin calcium. Quantitative determination of atorvastatin calcium EP regulates the implementation of liquid chromatography (LC).

The scientific literature describes a significant number of methods for the quantitative determination of atorvastatin calcium in drugs [3–26]. However, the described analytical methods require the use of expensive equipment (in the case of LC) or toxic solvents and long-term sample preparation (in the case of spectrophotometry). Spectrophotometric methods of analysis are of interest because they do not require the use of expensive equipment and have a number of advantages. An overview of spectrophotometric methods for determining atorvastatin calcium in medicines is given in Table 1.

The formation of ionic associative complexes between the API of pharmaceutical preparations having nitrogen atoms capable of protonation and forming cations and anionic forms of intensely colored dyes is a popular trend in pharmaceutical analysis. Various approaches are being used, including extraction-spectrophotometric methods [27, 28], formation of ionic associative complexes with sulfonephthalein dyes in organic solvents [27–32], approaches in which organic solvents are not used [33, 34], formation of ternary complexes between xantene dyes, palladium(II) and nitrogen containing drug [35].Only one spectrophotometric method for determining atorvastatin by reaction with sulfonephthalein dye, namely bromocresol purple, is 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 is not suitable, as it evaporates very easily, is toxic and therefore the method cannot correspond to «green» analytical chemistry. Thus, there is a need to develop simple and rapid spectrophotometric method for the quantitative determination of atorvastatin calcium tablets for routine pharmaceutical analysis. The aim of this work was to develop a simple, rapid and inexpensive spectrophotometric method for the determination of atorvastatin in tablets based on the reaction with bromophenol blue (BPB).

Table 1. Overview of spectrophotometric methods for determining atorvastatin calcium in drug.

№	Drug	Reagent	Medium	λ_{max}, nm	Concentration range, μg/mL	LOD/LOQ, μg/mL	Ref.
1	Tablets	Bromcresol Purple	Acetone	399	14–20	-	[3]
2	Bulk and dosage form	p-dimethyl-amino-benzaldehyde	Acidic (sulphuric acid)	540	20-160	LOD - 5.7, LOQ - 19	[4]
3	Pharmaceutical formulation	Pararosaniline Hydrochloride	Basic	547	1-8	LOD - 0.31, LOQ -0.93	[5]
4	Pure form and tablets	Sulfo-Phospho-Vanillin	Acidic (sulphuric acid)	414	30-100	-	[6]
5	Pure and pharmaceutical formulations	Iodine	Acetonitrile	291 and 360	0.5586-11.173	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	-	[9]
8	Combined tablet dosage form in the presents of amlodipine besylate	-	Methanol	256-238.5	5-50	LOD - 0.5, LOQ - 0.5	[10]
9	Tablet dosage form in the presence of telmisartan	-	Methanol	297	5-30	-	[11]

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. Other used instruments: Analytical Balance RAD WAG AS 200/C, pH–meter I-160MI and IKA orbital shaker KS4000i.

All the chemicals used were of analytical reagent grade. Pharmacopeial standard sample (CRS) of atorvastatin calcium and BPB were provided by Sigma-Aldrich (≥ 98 %, HPLC). The used dosage forms of atorvastatin were10 mg and 20 mg Atorvastatin tablets.

Procedure for the determination of atorvastatin calcium with BPB

20.0 mg of CRS atorvastatin calcium was transferred into a 50.00 mL volumetric flask with 35 mL methanol. The mixture was shaken and diluted to volume with methanol. Aliquot 0.60 mL was added to 0.6 mL of $4.12 \cdot 10^{-4}$ M methanol solution of BPB. The volume 10.00 mL was made up to the mark by adding methanol. The absorbance of the resulting solution was measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 594 nm.

Procedure for tablets for the determination of atorvastatin calcium with BPB

Twenty tablets were accurately weighed and grounded. A quantity of powder containing 20.0 mg of atorvastatin calcium was transferred into a 50.00 mL volumetric flask with 35 mL methanol. The mixture was shaken for 15 min, diluted to volume with methanol and then filtered using 0.2 μm Nylon filter membrane. Aliquot 0.60 mL was added to 0.6 mL of $4.12 \cdot 10^{-4}$ M methanol solution of BPB. The volume 10.00 mL was made up to the mark by adding methanol. The absorbance of the resulting solution was measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 594 nm.

Results and Discussion

Selection of reaction conditions

A number of scientific works are devoted to the development and validation of spectrophotometric methods for determining API (active pharmaceutical ingredient) in bulk and drugs using different dyes [3, 27–34]. However, in the literature, only one spectrophotometric method for the determination of atorvastatin calcium has been described based on the reaction with bromocresol purple in acetone [3]. 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 BPB, these forms absorb in aqueous solution at 437 nm (monoanionic form) and at 592 nm (dianionic form). In organic solvents, the position of these bands is slightly shifted due to the solvatochromic effect. The reactions described in the article and in the mentioned works are associated with the formation of ionic associative complexes, where the nature of the chemical bond lies in the electrostatic attraction of oppositely charged ions – the formation of so-called ion pairs or ionic associative complexes. Therefore, in principle, only two forms of ionic associative can be formed: with a singly ionized or a doubly ionized form of the dye. Sometimes, especially in aqueous solutions, other interactions are added to the ion association, such as hydrophobic interaction or the formation of aggregates. In certain cases, the formation of charge-transfer complexes cannot be excluded.

In a methanolic solution, the band of the monoanionic form of BPB predominates, while in the presence of atorvastatin, the acid-base balance of the dye shifts towards the doubly deionized form, since atorvastatin forms more stable ionic associate with this form of the dye. The presence of such an equilibrium explains why the calibration curves in such methods are often non-linear. If the stability of the ionic associate is low, then the addition of the drug to the dye does not completely shift the equilibrium and not all of the added drug reacts.

The absorbance spectra of the reaction product of atorvastatin calcium with BPB are presented in Fig. 1. In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagent was prepared separately in different solvents such as methanol, chloroform, acetonitrile, ethanol and ethyl acetate. Maximum absorbance was observed in a solution of methanol, chloroform and acetonitrile with BPB, while ethanol and ethyl acetate were unsuitable (Fig. 2). Methanol was chosen for further studies, since in this case the maximum analytical signal was observed. The wavelength of 595 nm was chosen as the analytical wavelength, since at this wavelength the maximum difference with respect to the blank solution was observed.

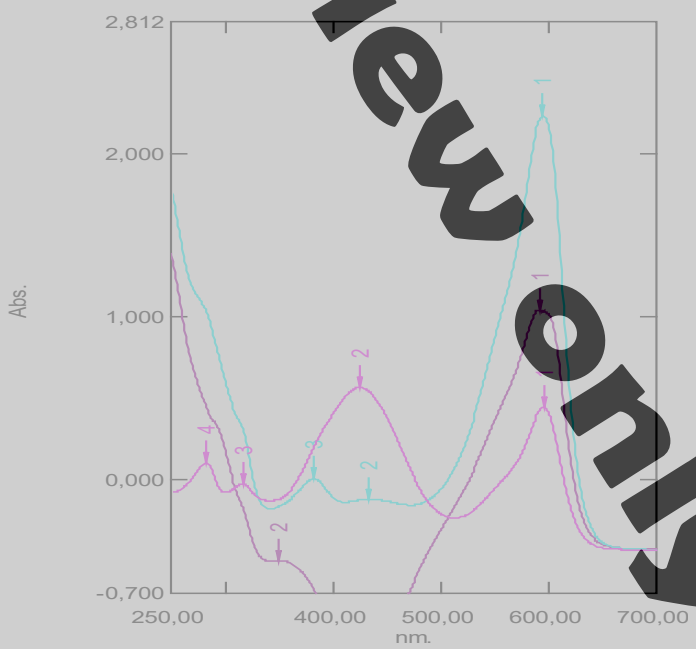

Fig. 1. Absorbance spectra of the reaction product of atorvastatin calcium from BPB against methanol (green), against BPB (purple) and BPB against methanol (pink).

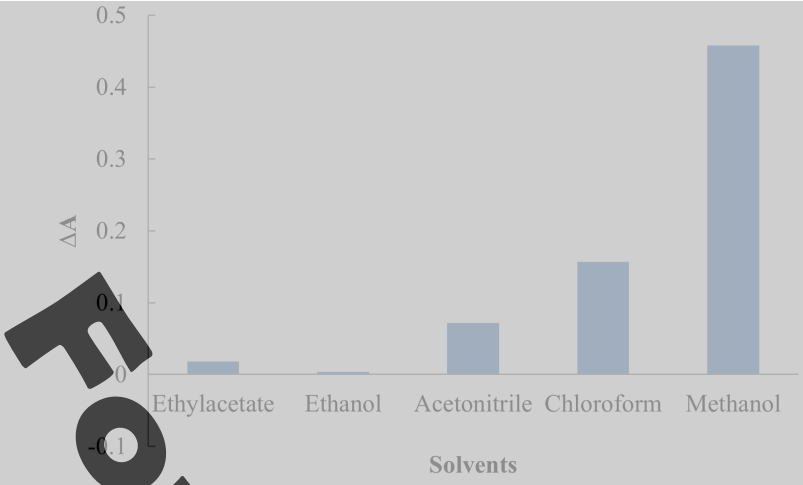

Fig. 2. Effect of solvents on the formation of atorvastatin-BPB complex ($4.12 \cdot 10^{-4}$ M solutions at 594 nm).

The study of the influence of the BPB concentration on the absorbance at 594 nm at a constant concentration of atorvastatin showed that at the concentrations of BPB above $4 \cdot 10^{-4}$ M, the dependence levells off. The concentration of BPB was $4.12 \cdot 10^{-4}$ M was chosen as optimal. It was found that the solutions of ion associate were stable for 45 min. This is an advantage of the developed method, as it does not require long-term sample preparation of solutions due to the stability of solutions without pH adjustment. We recommend measuring the absorbance immediately after preparing the solution.

Stoichiometric coefficients of atorvastatin and BPB in the compostion of ion pair were determined by the continuous variations (or Job`s method). The graph of the dependence of the absorbance on the ratio of the volumes of the components of the isomolar series is presented on Fig. 3.

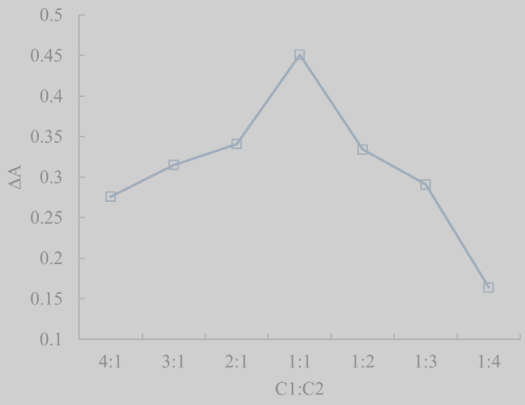

Fig. 3. Graph of the dependence of the amount of absorbance on the composition of the isomolar solution: C1 – $4.12 \cdot 10^{-4}$ M atorvastatin calcium solution; C2 – $4.12 \cdot 10^{-4}$ M solution BPB) at 594 nm.

According to the data obtained in Fig. 3, the stoichiometric ratios of the reactive components of «atorvastatin calcium - BPB» is 1:1.

Validation of analytical method

The developed spectrophotometric method has been validated according to the following parameters: linearity, calibration range, accuracy, precision and robustness.

Linearity and calibration range

Determination of linearity was performed over the calibration range of the method using model solutions. The results were statistically processed by the method of least squares in accordance with the requirements of the SPhU (State Pharmacopoeia of Ukraine). It was found that in the ranges indicated in Table 2, there is a linear relationship between absorbance and concentration of atorvastatin calcium.

Table 2. Analytical parameters.

Parameter	Value
Beer's law limits (μmol/l)	4.12–33
Molar absorptivity	$1.71 \cdot 10^4$
Sandell sensitivity (μg cm^{-2})	0.07
Limit of detection (μmol/l)	1.4
Limit of quantification (μmol/l)	4.1
Intercept (a)	0.14
Slope (b)	0.0487
Correlation coefficient (r)	0.9990
Standard deviation of intercept (Sa)	0.02
Standard deviation of slope (Sb)	0.0034

Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and quantification calculated are compiled in Table 2 and are indicative of the high sensitivity of the method.

Accuracy and precision

The precision of the method was determined at the level of convergence. For this purpose, nine parallel determinations were carried out (three measurements/three repetitions) and metrological characteristics were calculated (Table 3). At the same time, the optical density of the comparison solution was measured. The calculated one-sided confidence interval did not exceed the maximum allowable uncertainty of the analysis, therefore the method is precise at the level of convergence.

In order to determine possible systematic errors due to the influence of excipients included in the dosage form, the correctness of the methodology was determined by the method of additives. To do this, different amounts of the solution of CRS atorvastatin calcium were added to three identical aliquots of the dosage form and analyzed three times. The practical insignificance of the systematic error did not exceed the maximum permissible uncertainty of the analysis (0.32·ΔAs).

Table 3. The results of the study of the precision of the method of quantitative determination of atorvastatin calcium in drug.

Drug	$\bar{X}$, g	S	RSD	Δx
Atorvastatin 0.02 g	0.0202	$2.48 \cdot 10^{-4}$	1.23	$2.61 \cdot 10^{-4}$
Atorvastatin 0.01 g	0.0104	$6.06 \cdot 10^{-4}$	5.85	$6.36 \cdot 10^{-4}$

Robustness

During the study of robustness it was found that the analyzed solutions were stable for 45 min, and fluctuations in the amount of added reagent (BPB solution) within ± 16.67 % did not significantly affect the absorbance (Table 4).

Table 4. Determination of the robustness of the analytical method.

Amount of BPB, mL	% BPB	ΔA
0.50	83.33	0.435
0.55	91.67	0.436
0.60	100.00	0.438
0.65	108.33	0.441
0.70	116.67	0.442

Assessment of the impact of analytical methods on the environment

Assessment of the «greenness» of the analytical method was performed using AGREE tool (Analytical GREEnness) and analytical eco-scale. Pictogram of analytical method using AGREE tool is presented in Fig. 4. The score of the analytical eco-scale was 90 (Table 5).

Results show that developed spectrophotometric method for the quantitative determination of atorvastatin calcium in tablets based on the reaction with BPB was ecofriendly.

Fig. 4. Pictogram of analytical method using AGREE tool.

Table 5. Analytical eco-scale for assessing the «greenness» of the developed method.

Parameters	Penalty points
Reagent BPB	1
Methanol	3
Energy	1
Waste	5
Total number of penalty points	10
Ball of analytical eco-scale	90
Conclusion	Exellent «green» analysis

Conclusions

A simple, inexpensive and rapid spectrophotometric method was developed and validated for the determination of atorvastatin calcium in tablets based on the reaction with BPB. The maximum absorbance was observed in a solution of methanol, chloroform, and acetonitrile, while in solutions of ethanol and ethyl acetate, the analytical signal was insignificant. It was established that the optimal concentration of BPB is $4.12 \cdot 10^{-4}$ M. The absorbance of the analytical form is stable at least for 45 min. The stoichiometric ratios of the reactive components as 1:1 were obtained. The linear relationship was established between absorbance at 594 nm and concentration of drug in the range 4.12–33.00 μmol/l. The LOD and LOQ values were calculated to be 1.36 μg/ml and 4.13 μg/ml, respectively. Proposed spectrophotometric method is simple, requires only one reagent (BPB), and suitable for routine pharmaceutical analysis with common spectrophotometric instruments.

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Conflicts of Interest

The authors declare no conflict of interest.

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