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FULL GREEN ASSAY OF ROSUVASTATIN UTILIZING SULPHOPHTALEIN DYES: APPLICATION TO TABLET ANALYSIS

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The aim of the work *was to develop a «green» and extraction-free spectrophotometric procedure for the assay of rosuvastatin in tablets. The present work describes three new spectrophotometric procedures (A, B and C) that can be utilized for routine quality control of rosuvastatin in laboratories.*

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). Rosuvastatin calcium (purity≥98 % (HPLC)) was bought from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Rosuvastatin 10 mg tablets were acquired from a nearby drugstore. All solvents used in this study, including methanol, ethanol, chloroform, acetonitrile and ethyl acetate, were produced by Honeywell and had a purity of 99.9 %. BCG, BCP and BTB were acquired from Sigma-Aldrich Chemicals Co. (USA, St. Louis). All chemicals utilized in the experiment were of analytical purity.*

Results and discussion. *New simple «green» and extraction-free spectrophotometric procedures for assay of rosuvastatin in tablets involve the formation of ion-pair complexes with sulphophtalein dyes (BCG (Method A), BCP (Method B), BTB (Method C)) have been developed. The absorbances of the coloured reaction products were registered at 405 nm (Method A) and 400 nm (Methods B, C). The concentration was linearly proportional to absorbance values in the range of 2.51–20.08 μg/mL (method A), 2.50–24.90 μg/mL (method B) and 2.51–12.56 μg/mL (method C). Estimation of LOD and LOQ parameters were obtained as 0.67 μg/mL and 2.23 μg/mL (method A), 0.39 μg/mL and 1.32 μg/mL (method B), 0.30 μg/mL and 1.01 μg/mL (method C). The stoichiometric ratio of the reactive components of rosuvastatin – BCG, BCP, and BTB corresponded 1: 1. The %RSD values of intra-day and inter-day were obtained less than <1.5 %, which showed excellent repeatability and RE % data was ≤3 %. The effect on the environment of the proposed spectrophotometric procedures and their compliance with the GAC principles were endorsed by the output of AGREE, GAPI, and AES metrics tools. The values of these three «green» metrics show that the proposed spectrophotometric procedures had a low environmental impact compared with the reported ones.*

Conclusions. *The developed fast, simple and cost-effective methods A, B, and C can be used for routine analysis of rosuvastatin in tablets*

Keywords: *Rosuvastatin, Spectrophotometry, Sulphophtalein dyes, Bromocresol green, Bromocresol purple, Bromothymol blue, Tablet, Validation*

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1. Introduction

Rosuvastatin is a semisynthetic compound that belongs to the lipid-lowering class of statins. Such a class is commonly used to reduce low-density lipoprotein cholesterol levels. They are extremely effective in decreasing atherosclerosis. Atherosclerosis is defined as one of the leading causes of worldwide death. On the other hand, statins manage the risk of cardiovascular diseases. Prescribing statins is a general practice after cardiovascular events. In addition, statins are the first line of hypercholesterolemia therapy. Rosuvastatin is known as (E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]-pyrimidin-5-yl}-3,5-dihydroxyhept-6-enoic acid calcium (2:1) [1, 2] (Fig. 1).

In order to assure the quality of rosuvastatin in bulk and formulation, alone or in combination with other drugs, a variety of methods have been used [3, 4]. Multiple reported assays include spectrophotometry [5–18] and other analytical methods. Nevertheless, most of them have disadvantages, such as difficulty in performance, and are sophisticated and time-consuming. However, the spectrophotometric method is one of the environmentally focused analytical methods, widespread in various fields of application. According to the last literature data, few spectrophotometric procedures are presented and generally include extraction or dilution of the drug by subsequent registration of absorbance in the UV region, which leads to a lack of specificity and can be influenced by some UV-absorbing medicines, degradation compounds, flavours, dyes etc. [5–9]. According to the literature survey, it was summarized that reported spectrophotometric procedures associated with numerous drawbacks, such as exaction, using toxic and expensive solvents, the addition of buffer for pH control, being

harmful to nature, small concentration range and selectivity, as indicated in Table 1 [10–16].

The application of sulphophthalein dyes in pharmaceutical analysis is promising. In the last 30 years, a number of such spectrophotometric methods have been developed, but the principles of «green chemistry» were not taken into consideration during their development [13]. Our scientific group has developed a spectrophotometric procedure for the assay of rosuvastatin in tablets using a reaction with BPB [16]. Ramadan A. et al. have developed a spectrophotometric method for rosuvastatin determination in raw material and dosage form by reaction with BCG, but they used extraction with chloroform, which does not correspond to the principles of «green chemistry». However, the reported spectrophotometric procedures with dyes as reagents lack modern, sustainable environmental principles. Consequently, the development and validation of new environmentally sustainable methods and the moderation of previously developed procedures for the spectrophotometric determination of rosuvastatin in drugs is a prominent goal that will be performed during this research.

The aim of this study was to develop a «green» and extraction-free spectrophotometric procedure for the assay of rosuvastatin in tablets. The present work describes three new spectrophotometric procedures (A, B and C) that can be utilized for routine quality control in laboratories.

Table 1

Performance comparison of the previously reported and proposed spectrophotometric methods

No	Utilized reagents	Principle	Linearity	Comments	Reference
1	a) safranin O; b) methylene blue at pH 9.8 with further extraction in $CHCl_3$	The formation of ion association complexes was measured at 530 nm and 655 nm, respectively	a) 5.0–25.0 μg mL^{-1} (3.60×10^4 L $mole^{-1}$ cm^{-1}); b) 2.5–12.5 μg mL^{-1} (6.54×10^4 L $mole^{-1}$ cm^{-1})	Require close pH control and extraction	[10]
2	Iodine in acetonitrile (at a temperature of 50 °C 1 hour)	The formation of the triiodide (I_3^-) complex was measured at 291 and 360 nm	2.408–48.154 μg mL^{-1} (4.97×10^4 L $mole^{-1}$ cm^{-1})	It requires heating for 1 hour, and it is an expensive solvent	[11]
3	Quinalizarin in DMSO	The production of a charge transfer complex was observed at 579 nm	6.0-15.0 mg L^{-1}	Require using DMSO, wide linear range	[12]
4	Bromocresol green (BCG) in chloroform	The production of an ion-pair complex was observed at 416 nm	0.482–24.077 μg/mL (1.92×10^4 L $mole^{-1}$ cm^{-1})	Require extraction	[13]
5	safranin in chloroform and phosphate buffer pH 7.2	The production of an ion-pair complex was observed at 518 nm	5–25 μg/mL	Require close pH control and extraction	[14]
6	a) chloralinic acid in acetone; b) picric acid in chloroform; c) potassium permanganate (stand for 20 min)	a) oxidation (λmax 530 nm); b) salt formation (λmax 440 nm); c) oxidation (λmax 410 nm)	a)1–3 μg/mL (0.8345×10^4 L $mole^{-1}$ cm^{-1}); b) 0.25–1.25 μg/mL (1.91×10^5 L $mole^{-1}$ cm^{-1}); c) 0.25–1.25 μg/mL (2.3×10^5 L $mole^{-1}$ cm^{-1})	Require using organic solvents, narrow linear range	[15]
7	Bromophenol blue (BPB) in acetonitrile	The production of an ion-pair complex was observed at 595 nm.	7.99–23.97 μmol/L (1.55×10^4 L $mole^{-1}$ cm^{-1})	Require expensive solvent	[16]
8	a) bromocresol green (BCG) in ethyl acetate; b) bromocresol purpe (BCP) in acetonitrile; c) bromothymol blue (BTB) in ethyl acetate	a) the production of an ion-pair complex was observed at 405 nm; b) the formation of an ion-pair complex was observed at 400 nm; c) the formation of an ion-pair complex was observed at 400 nm	a) 2.51–20.08 μg/mL (3.54×10^4 L $mole^{-1}$ cm^{-1}); b) 2.50–24.90 μg/mL (3.41×10^4 L $mole^{-1}$ cm^{-1}); c) 2.51–12.56 μg/mL (3.47×10^4 L $mole^{-1}$ cm^{-1})	Extraction-free, highly sensitive with wide concentration ranges, without heating and pH-correcting, unitary step reaction and «green» solvents	Present methods

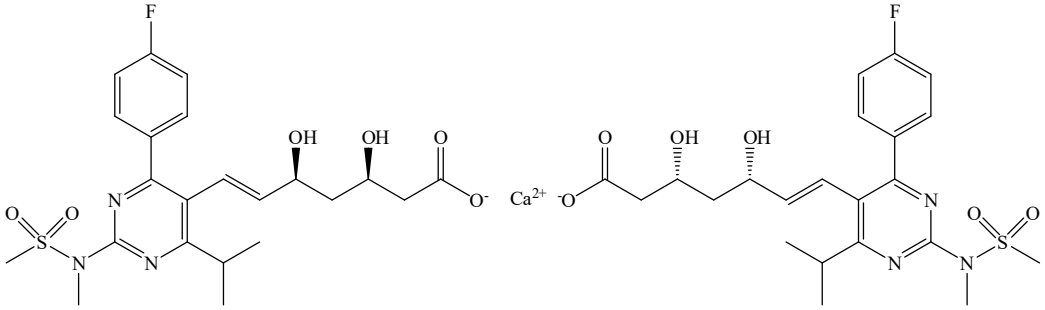


Fig. 1. Chemical structure of rosuvastatin calcium salt

2. Planning of the research

The methodology of investigation of development and validation of the spectrophotometric procedures for the determination of rosuvastatin in tablets includes:

1. Evaluation of the scientific sources.

2. Analysis of the SPhU and Ph. Eur. Monographs.

3. Selection of reaction conditions between rosuvastatin and BCG, BCP, BTB (choice optimal wavelength, solvent, concentration reagent, detection of stoichiometric indexes).

4. Development and validation of the spectrophotometric procedures for the determination of rosuvastatin in tablets.

5. Study of the greenness of the developed methods by analytical GREEnness, GAPI, and AES metric tools.

3. Materials and methods

Objects of study, 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). Rosuvastatin calcium (purity≥98 % (HPLC)) was bought from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Rosuvastatin 10 mg tablets were acquired from a nearby drugstore. All solvents used in this study, including methanol, ethanol, chloroform, acetonitrile and ethyl acetate, were produced by Honeywell and had a purity of 99.9 %. BCG, BCP and BTB were acquired from Sigma-Aldrich Chemicals Co. (USA, St. Louis). All chemicals utilized in the experiment were of analytical purity.

Proposed procedure for the determination of rosuvastatin calcium salt with BCG.

Twenty tablets of rosuvastatin calcium salt were thoroughly crushed and weighed. An accurate amount of the powdered tablets corresponding to 25.10 mg of rosuvastatin calcium salt was transferred into a 50.00 mL measuring flask containing 35 mL of ethyl acetate. The mixture underwent ultrasound treatment for 30 minutes to get it ready. The volume was filled to the mark with the needed solvent, stirred thoroughly and filtered using a Whatman No. 42 filtering paper. Aliquot 0.20 mL was transferred to a 10.00 mL measuring flask containing 0.8 mL of 5.1×10^{-4} M BCG, and the volume was made up to the mark by adding ethyl acetate. The absorbance of the resulting mixture was recorded at 405 nm against a reference solution prepared in the same way without adding the analyte.

Proposed procedure for the determination of rosuvastatin calcium salt with BCP.

Twenty tablets of rosuvastatin calcium salt were thoroughly crushed and weighed. An accurate amount of the powdered tablets corresponding to 10.00 mg of rosuvastatin calcium salt was transferred into a 100.00 mL measuring flask containing 80 mL of acetonitrile. The mixture underwent ultrasound treatment for 30 minutes to get it ready. The volume was filled to the mark with the needed solvent, stirred thoroughly and filtered using a Whatman No. 42 filtering paper. Aliquot 1.00 mL was transferred to a 10.00 mL measuring flask containing 0.5 mL of 1.0×10^{-3} M BCP, and the volume was made up to the mark by adding acetonitrile. The absorbance of the resulting mixture was recorded at 400 nm against a reference solution prepared in the same way without adding the analyte.

Proposed procedure for the determination of rosuvastatin calcium salt with BTB.

Twenty tablets of rosuvastatin calcium salt were thoroughly crushed and weighed. An accurate amount of the powdered tablets corresponding to 16.00 mg of CRS rosuvastatin calcium salt (or equivalent in tablets) was transferred into a 50.00 mL measuring flask containing 35 mL of ethyl acetate. The mixture was shaken and then adjusted to the mark with the same solvent. The mixture underwent ultrasound treatment for 30 minutes to get it ready. The volume was filled to the mark with the needed solvent, stirred thoroughly and filtered using a Whatman No. 42 filtering paper. Aliquot 1.50 mL was transferred to a 10.00 mL measuring flask containing 0.5 mL of 1.0×10^{-3} M BTB, and the volume was made up to the mark by adding ethyl acetate. The absorbance of the resulting mixture was recorded at 400 nm against a reference solution prepared in the same way without adding the analyte.

Proposed procedure for preparing BCG solution.

A 5.1×10^{-4} M of BCG were prepared in a 50.00 mL measuring flask by dissolving 17.9 mg of BCG in 40 mL of ethyl acetate and adjusting to the mark with the same solvent.

Proposed procedure for preparing BCP solution.

A 1.0×10^{-3} M of BCP were prepared in a 25.00 mL measuring flask by dissolving 13.8 mg of BCP in 20 mL of acetonitrile and adjusting to the mark with the same solvent.

Proposed procedure for preparing BTB solution.

A 5.0×10^{-4} M of BTB were prepared in a 50.00 mL measuring flask by dissolving 16.0 mg of BTB in 20 mL of ethyl acetate and adjusting to the mark with the same solvent.

4. Results

4. 1. Selection of reaction conditions

Sulphophtalein dyes as reagents have attracted considerable attention from scientists in the analysis of pharmaceuticals due to their ability to react with substances of a basic nature. The advantage of such reactions is their simplicity, expressivity and greenness if advanced approaches are used. Taking into account our research team's expertise in developing and validating analytical methods for API routine pharmaceutical control, we previously developed a spectrophotometric method for determining rosuvastatin in tablets using a reaction with BPB in acetonitrile medium, which is a costly solvent. Rosuvastatin calcium in ethyl acetate nor acetonitrile doesn't absorb within the visible region, but when either of three dyes (BCG, BCP, or BTB) is added, colour is quickly developed, with absorbance maxima of about 405 nm (BCG) and 400 nm (BCP and BTB) (Fig. 2).

Sulphophtalein dyes, including BCG, BCP, and BTB are present in solution in two kinds of hydrogenated forms: monoanionic, in which the proton is broken off

from the sulfogroup, and dianionic, when another hydrogen breaks off from one of the phenolic hydroxylic groups. The monoanionic form of BCG, BCP and BTB absorbs in an aqueous solution at about 400–405 nm, and the dianionic form at about 600 nm. Rosuvastatin becomes protonated and produces an ion pair when combined with the dye [13]. Adding rosuvastatin calcium to the sulfophthalein dye solution increases the absorption band of the monoionized dye form (λmax=400–405 nm) (Fig. 2) while decreasing the absorbance band of the doubly ionized form. The solvatochromic effect of organic solvents causes these forms to shift in position. Therefore, 400 nm was chosen as the determination wavelength with BCG in ethyl acetate and 405 nm – with BCP in acetonitrile and BTB in ethyl acetate.

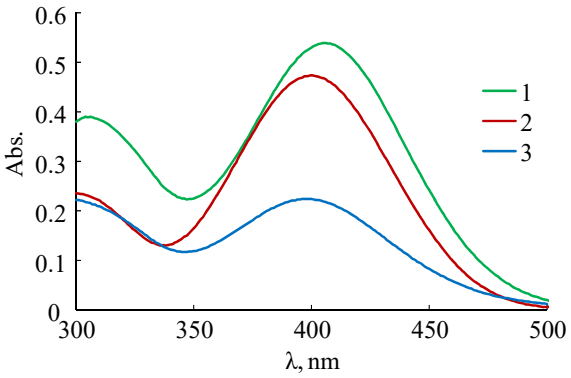


Fig. 2. Absorbance spectra of rosuvastatin (1.50×10^{-5} M) – BCG (4.10×10^{-5} M in ethyl acetate) (1) (method A), rosuvastatin (1.24×10^{-5} M) – BCP (5.00×10^{-5} M in acetonitrile) (2) (method B), rosuvastatin (7.53×10^{-6} M) – BTB (5.56×10^{-5} M in ethyl acetate) (3) (method C) complexes against the appropriate reagent blanks

Certain organic solvents, including methanol, ethanol, chloroform, methylene chloride, ethyl acetate and acetonitrile, were tested for their capability to produce ion pairs from drug and dye mixtures. Chloroform and methylene chloride are toxic solvents. However, we tested them for theoretical purposes. As already noted above, Syrian scientists used chloroform as a solvent in their work [13]. Effects of solvents on the formation of rosuvastatin-BCG, rosuvastatin-BCP, and rosuvastatin-BTB complexes are presented in Fig. 3–5.

In the case of the formation of rosuvastatin-BCG (Fig. 3) and rosuvastatin-BTB complexes (Fig. 5), the optimal solvent is ethyl acetate, while in the case of the formation of rosuvastatin-BCP complex (Fig. 4) is methylene chloride, however, we chose acetonitrile for the generation of the rosuvastatin-BCP complex due to its environmental safety.

The effect of reagent concentration on the intensity of colour formation was studied by tracking the absorbance of solutions containing a constant concentration of rosuvastatin and different amounts of BCG, BCP, and BTB. Maximum absorbance was achieved at concentrations of 5.1×10^{-4} M BCG, BTB and 1.0×10^{-3} M BCP.

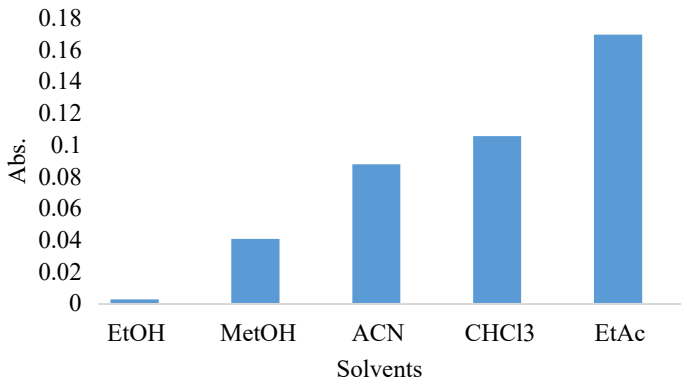


Fig. 3. Solvents impact on the generation of rosuvastatin-BCG complex ($C_{M\,BCG}=1.49\times10^{-5}$ M)

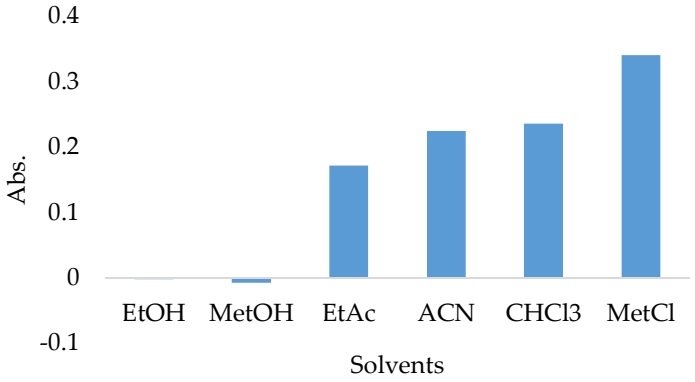


Fig. 4. Solvents impact on the generation of rosuvastatin-BCP complex ($C_{M\,BCP}=2.00\times10^{-5}$ M)

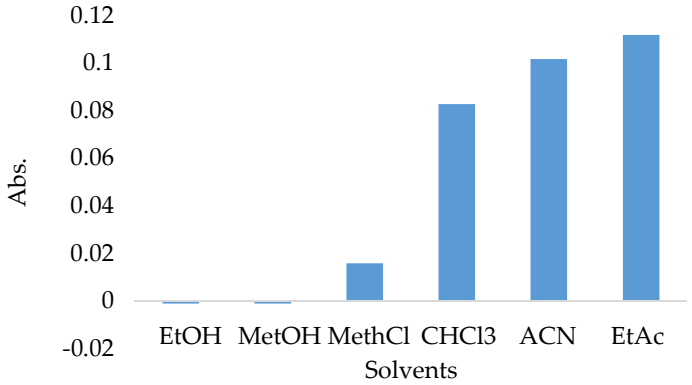


Fig. 5. Solvents impact on the generation of rosuvastatin-BTB complex ($C_{M\,BTB}=1.03\times10^{-5}$ M)

The effect of dye volume on the intensity of colour formation was studied by tracking the absorbance of solutions containing a constant concentration of rosuvastatin and different volumes of BCG, BCP, and BTB. It was determined that 0.8 mL of BCG and 0.5 mL of BCP and BTB were needed in order to achieve the highest absorbance.

At 25±2 °C, the interactions between rosuvastatin and the studied sulphophthalein dyes (BCG, BCP and BTB) were immediate. The colour solutions' absorbances were steady for 10 min before gradually decreasing. Although the reaction was rapid, readings in the following investigations were performed after 5 min to achieve greater measurement precision than measurements taken immediately after combining rosuvastatin with sulphophthalein dyes.

The stability of the ion-pair complexes generated by rosuvastatin with BCG, BCP or BTB was studied. Despite the fact that the ion pairs were formed immediately, steady absorbance values were measured after at least 5.0 min of remaining at a constant temperature (25±2) °C and remaining stable for twenty-four hours.

The molar ratio approach (saturation method) and Job's method of continuous variation were used to determine the composition of rosuvastatin:dye complexes.

Using varying volume ratios, in Job's method we combined 5.00×10^{-4} M solutions of dye and 5.00×10^{-4} M rosuvastatin calcium, so that the total volume of each mixture was the same as well. The analysis of the reacting components' stoichiometric ratios using Job's approach for continuous changes is shown in Fig. 6.

By using the saturation approach, one is able to determine how much absorbance depends on the concentration of a specific reaction mixture component while maintaining a constant amount of the other component and inversely. The component whose concentration has been varied has a stoichiometric coefficient equal to the inflexion point on the saturation curve (Fig. 7). The stoichiometric coefficients of the reaction mixture components between rosuvastatin calcium and dye (BCG, BCP and BTB) are equal to 1:1, as demonstrated in Fig. 7.

Table 2 displays the optimal parameters for the spectrophotometric measurement of rosuvastatin calcium via the ion-pair formation of complexes utilizing BCG, BCP and BTB. We have determined the parameters for using spectrophotometric procedures to determine the content of rosuvastatin in tablets and estimated the reaction's sensitivity factors. The molar absorptivity of the previously developed method was 1.55×10^4 L mole^{-1} cm^{-1}, while the proposed methods have 3.54×10^4 L mole^{-1} cm^{-1} (method A), 3.41×10^4 L mole^{-1} cm^{-1} (method B) and 3.47×10^4 L mole^{-1} cm^{-1} (method C).

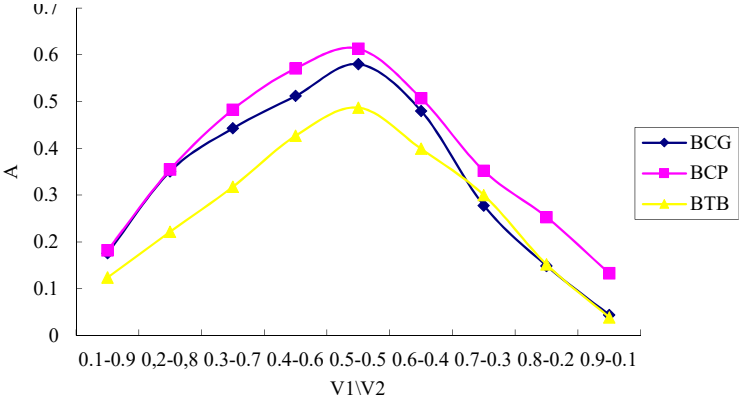


Fig. 6. Rosuvastatin:BCG complex continuous variation method at λ=405 nm, rosuvastatin:BCP complex at λ=400 nm, rosuvastatin:BTB complex at λ=400 nm

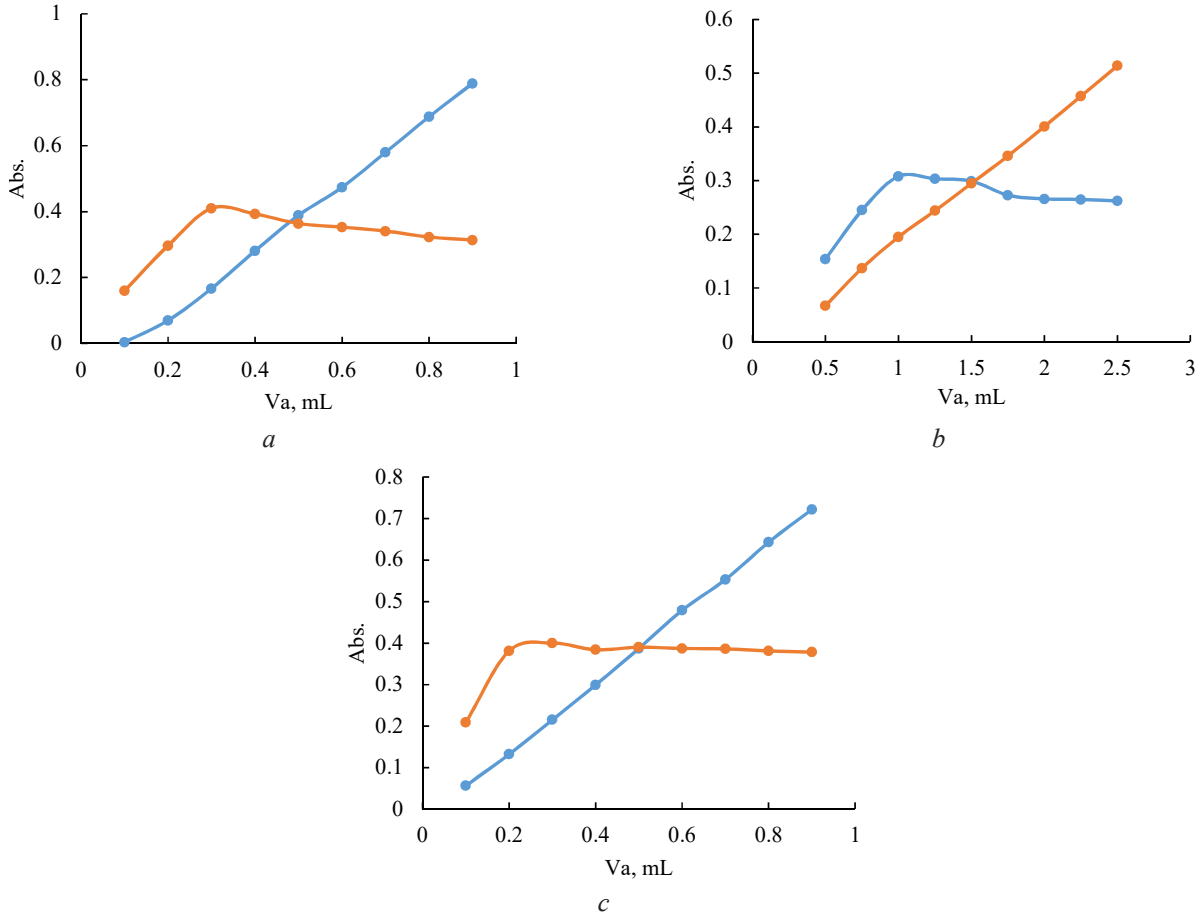


Fig. 7. Molar ratio method of rosuvastatin:BCG complex at λ=405 nm (*a*), rosuvastatin:BCP complex at λ=400 nm (*b*), rosuvastatin:BTB complex at λ=400 nm (*c*) ($C_{M\,BCG}=5.13\times10^{-4}$ M, $C_{M\,BCP}=1.02\times10^{-4}$ M, $C_{M\,BTB}=5.13\times10^{-4}$ M)

Table 2

The optimal parameters for rosuvastatin calcium spectrophotometric analysis via complex formation using BCG, BCP and BTB

Conditions	Operating modes		
	Method A BCG	Method B BCP	Method C BTB
Temperature of solution, °C	25±5		
Solvent	Ethyl acetate	Acetonitrile	Ethyl acetate
Reagent concentration, mol/l	5.1×10^{-4}	1.0×10^{-3}	5.1×10^{-4}
Reaction time, min	5		
Stability, h	24		
λmax of complex, nm	405	400	400
Molar absorptivity, L $mole^{-1}$. cm^{-1}	3.54×10^{4}	3.41×10^{4}	3.47×10^{4}
Sandell sensitivity, μg cm^{-2}	0.03	0.03	0.03
Working C, mol/l	5.0×10^{-4}	1.0×10^{-4}	5.0×10^{-4}

4. 2. Determination of validation characteristics

Proposed methods have been validated to meet the requirements specified by the International Conference on Harmonization (ICH) [19].

4. 2. 1. Linear Range and Sensitivity

Model mixtures were used to evaluate the linearity within the method's applicability range. These results were statistically analyzed using the least squares approach in compliance with ICH guidelines. The absorbance and concentration of rosuvastatin were shown to be linearly correlated in the following ranges: 2.51–20.08 μg/mL (method A), 2.50–24.90 μg/mL (method B), and 2.51–12.56 μg/mL (method C). The regression parameters are displayed in Table 3.

Out of the three suggested approaches, the BCP and BTB methods are the most sensitive, as seen by the low LOD and LOQ values in methods B and C. Within the analytical methods' range of application, the linearity parameters satisfied the criteria established by ICH.

Table 3

Analytical parameters for the spectrophotometric determination of rosuvastatin calcium via complex formation with BCG, BCP and BTB

Analytical parameters	Value for method A BCG	Value for method B BCP	Value for method C BTB
Linearity range, μg/mL	2.51–20.08	2.50–24.90	2.51–12.56
Intercept (*a*)	0.0075	–0.0212	0.0071
Slope (*b*)	0.0345	0.0398	0.0341
Correlation coefficient	0.9990	0.9994	0.9989
S.D. of slope (S*b*)	0.0017	0.0008	0.00097
S.D. of intercept (S*a*)	0.0214	0.0118	0.0079
LOD, μg/mL	0.6695	0.3947	0.3033
LOQ, μg/mL	2.2315	1.3156	1.0109

4. 2. 2. Accuracy and Precision

The intra-day and inter-day precision and accuracy outcomes resulting from the recommended methods are summarized in Table 4. The RE % data was less than 3 %, and the intra-day and inter-day RSD values were determined to be less than 1.5 %, indicating the methods' applicability to routine control. The above high recovery, along with low error values, showed high precision for all three described rosuvastatin quantification procedures. The results of the research proved the precision and accuracy of the recommended procedures.

Table 4

Assessment of the methods' intra-day and inter-day accuracy and precision

Method	Concentration, μg/mL	Intra-day accuracy and precision (*n*=7)			Inter-day accuracy and precision (*n*=7)		
		Recovery, %	%RSD	%RE	Recovery, %	%RSD	%RE
Method A	5.00	100.11	0.92	1.89	99.92	1.12	1.59
	12.54	99.95	0.87	1.22	99.46	0.95	1.71
	20.08	101.43	1.02	1.31	100.67	1.34	1.94
Method B	4.00	100.86	1.28	1.93	101.62	0.69	1.25
	14.45	101.38	1.47	1.78	99.64	1.19	1.93
	24.90	99.05	0.95	1.36	100.06	1.34	1.40
Method C	4.00	100.07	0.68	1.10	99.95	1.27	0.98
	8.28	100.94	0.92	1.27	100.12	1.28	1.39
	12.56	99.38	0.79	1.07	100.84	1.08	0.92

Note: RSD – Relative standard deviation; RE – Relative error.

4. 2. 3. Specificity

The proposed spectrophotometric procedures for the determination of rosuvastatin in tablets demonstrate specificity for two main reasons. Firstly, excipients do not interfere with sulfophthalein dyes, as confirmed by conducting measurements within the visible spectrum range. Secondly, the use of organic solvents in dissolving and preparing tablet samples ensures that only rosuvastatin dissolves, while the excipients remain undissolved due to their solubility in water but not in acetonitrile or ethyl acetate.

4. 2. 4. Robustness and Ruggedness

The robustness of the proposed spectrophotometric methods, which refers to how minor variations in the methods' variables impact their analytical performance, was thoroughly examined. These variables included the concentrations of BCG, BCP, and BTB, as well as reaction times, each adjusted by 10 % from their optimal values. It was observed that these adjustments had no significant effect on the method's efficacy. The recovery values fell within the range of 98.9 % to 101.5 %, with relative standard deviation (RSD) values ranging from 0.5 % to 1.4 %. These findings validate the suitability of the three proposed spectrophotometric methods for the routine analysis of rosuvastatin. Furthermore, the ruggedness of the methods was evaluated in terms of day-to-day reproducibility. The RSD values obtained did not surpass 1.5 %, indicating that the proposed spectrophotometric methods exhibit ruggedness, ensuring consistent and reliable performance over time.

4. 2. 5. Application to tablet analysis

The suitability of the proposed spectrophotometric procedures for quantifying rosuvastatin in tablet formulations was assessed based on the satisfactory validation outcomes outlined earlier. These methods were employed to evaluate rosuvastatin concentrations in tablet samples across various predetermined levels (as detailed in Table 5).

Table 5

Applying the recommended spectrophotometric procedures for estimating the content of rosuvastatin in tablets using reactions with BCG, BCP, and BTB (method A, method B and method C)

Method	Concentration level, μg/mL	Labeled Claim (%±RSD)[a] Dosage form (tablets) 1	Label Claim (%±RSD)[a] Dosage form (tablets) 2
Method A	5.00	99.84±1.09	101.78±1.88
	12.54	100.37±1.39	101.63±1.12
	20.08	99.69±0.95	100.70±0.78
	–	Mean 99.97±1.14	101.37±1.26
Method B	4.00	99.28±1.67	99.28±1.11
	14.45	100.50±0.92	100.24±0.94
	24.90	101.12±0.86	100.85±1.07
	–	Mean 100.30±1.15	100.12±1.04
Method C	4.00	101.27±1.02	99.08±0.94
	8.28	99.49±1.85	101.43±1.07
	12.56	99.10±1.19	100.39±1.53
	–	Mean 99.95±1.35	100.30±1.18

Note: [a]n – average of 3 determinations.

The findings revealed label claim percentages with mean values of 99.97±1.14 % (Method A), 100.30±1.15 % (Method B), and 99.95±1.35 % (Method C) for dosage form 1, and 101.37±1.26 % (Method A), 100.12±1.04 % (Method B), and 100.30± ±1.18 % (Method C) for dosage form 2. These results demonstrate notably high label claim percentages, underscoring the effective applicability of the proposed spectrophotometric methods for the precise and accurate determination of rosuvastatin content in tablets.

4. 3. Greenness levels of the proposed spectrophotometric methods

According to the current trends in environmental protection, the approaches of «green» chemistry are widely used and demanded. Nowadays, scientists have applied a number of techniques and approaches for calculating the «greenness» of existing, developed and newly created procedures. These techniques entailed miniaturizing analytical procedures in accordance with the «green analytical chemistry» (GAC) tenets, which include elimination of extraction, utilizing lower amounts of sample and reagent, and minimization of waste. For the calculation of the greenness of the proposed methods, three effective metric tools were applied: Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI) and Analytical Greenness Metric Approach (AGREE). These resources offer a thorough review of environmental-friendly analytical techniques, and their evaluation criteria and outcomes are available in modern scientific papers [20–22].

The outcomes of the first metric tool analysis, AES, are shown in Table 6 and contain all three analyzed reagents annexed to the same reagent category, operating conditions, and procedures.

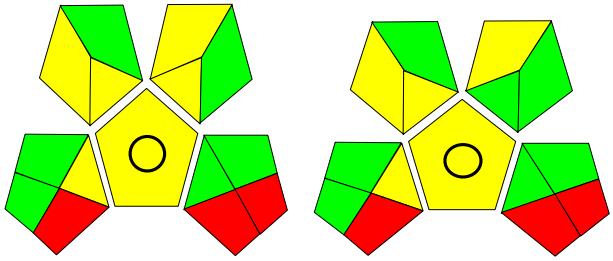
As can be mentioned, penalty points (PP) associated with the amount of reagents and solvent were 3. Considering the potentially harmful effects of the reagents and solvents utilized in each procedure, the intermediate summary of PP was 6. Instrument energy consumption parameters and occupational exposures satisfied GAC requirements and did not give any PP. The intermediate summaries of 5 and 3 PP, respectively, indicate the parameters of waste generation and treatment. The whole PP for each method was 17, and the analyses' corresponding environmental scale scores were 83 (100–17). This high score demonstrated the suggested approaches' excellent result of greenness in compliance with metric requirements.

Table 6

Analytical Eco-Scale for determining the greenness of the recommended spectrophotometric procedures for the assay of rosuvastatin through its interaction with BCG (Method A), BCP (Method B) and BTB (Method C)

Eco-scale score parameters	Penalty Points (PPs)		
	Method A	Method B	Method C
Amount of solvent/reagent	–	–	–
Solvent:	10–100 mL (mL (g) per sample) (2)	10–100 mL (mL (g) per sample) (2)	10–100 mL (mL (g) per sample) (2)
Reagent:	Reagent: <1 mL (mL (g) per sample) (1)	Reagent: <1 mL (mL (g) per sample) (1)	Reagent: <10 mL (mL (g) per sample) (1)
Hazard of solvent/reagent	–	–	–
Solvent:	3	3	3
Reagent:	3	3	3
Instrument: energy used (0.1 kWh per sample)	0	0	0
Occupational hazards	–	–	–
The analytical process is hermetic	0	0	0
Emission of vapors and gases to the air	0	0	0
Waste	–	–	–
Production	(>10 mL (g) per sample) (5)	(>10 mL (g) per sample) (5)	(>10 mL (g) per sample) (5)
Treatment (no treatment involved)	3	3	3
Total PPs	17	17	17
Eco-Scale score	83	83	83

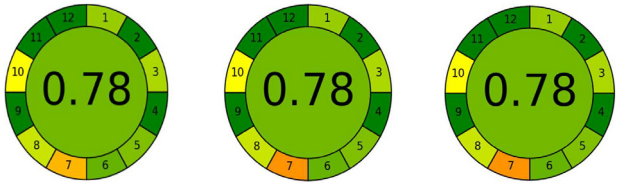
The outcomes of the GAPI tool for 15 criteria are illustrated in Fig. 8. Three of the fifteen parameters – parameters 2, 14, and 15 – were colored red in the pictograms. Due to the incorrect handling of the waste tests, these parameters acquired the color red. The difference between the methods was in parameter 9 (amount of reagents and solvents). Other parameters that complied with guidelines and the green processes' requirements were coloured green and yellow.



a *b*

Fig. 8. GAPI pictograms for determination of the greenness of the analyzed procedures: *a* – A; *b* – B, C

The obtained pictograms using the AGREE tool are shown in Fig. 9. Parameter 7 is more or less marked in red in all methods, which shows significant waste. Parameter 10 (type of reagents) is marked in yellow colour. All other parameters are green. The overall score is 0.78, which confirms the green analysis. A comparison of the three environmentally friendly tools' outputs attested to the greenness of the suggested spectrophotometric methods and their adherence to the GAC principles.

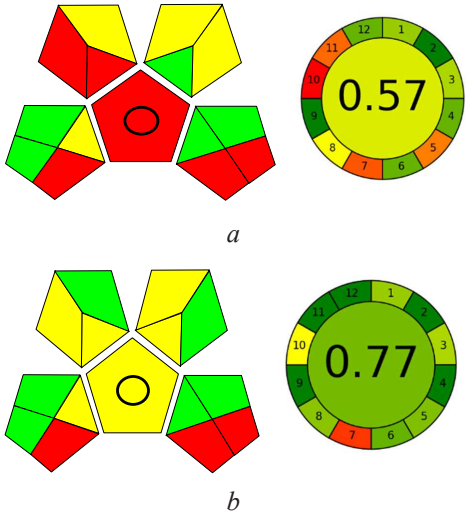


a *b* *c*

Fig. 9. AGREE pictograms for determination of the greenness of the analyzed procedures: *a* – A; *b* – B; *c* – C

4. 4. Matching the analyzed methods' greenness levels with the reported methods

The levels of greenness of the analyzed spectrophotometric methods were compared with the levels of greenness of existing spectrophotometric methods [13, 16]. The GAPI and AGREE tools were utilized for conducting comparisons, and Fig. 10 shows the pictograms prepared for the described methods using these approaches. The greenness levels for the reported procedures specified by the GAPI tool were determined to be lower than the analyzed ones [13]. There were red parameters 1, 5, 6, 7, 14, and 15 (corresponding to the amount of reagent and solvent, the use of chloroform and extraction, and the amount of waste, respectively), and the result of the method [16] was similar (method was also developed by our scientific group). The AGREE tool gave a score of 0.57 for the method [13], which is unsatisfactory due to the use of chloroform and extraction, and 0.77 for the method [16], which is a good score due to the lack of extraction and the use of acetonitrile as a solvent.



a

b

Fig. 10. Comparison of the reported methods' greenness according to the AGREE (right) and GAPI (left) metric tools: *a* – [13]; *b* – [16]

Therefore, it can be concluded that the conducted research significantly expands the bank of simple and «green» spectrophotometric methods, which are developed in accordance with the modern methodology of pharmaceutical analysis and can be an alternative to chromatographic methods.

5. Discussion of research results

We focused on selecting sulphophtalein dyes for the development of «green» spectrophotometric procedures for determining rosuvastatin in tablets, aiming for suitability in routine quality control laboratories during the method development process. The three suggested spectrophotometric procedures required the application of sulphophtalein dyes (BCG, BCP, and BTB) in various «green» solvents. The absorbances of the coloured reaction products were registered at 405 nm (method A) and 400 nm (methods B, C). All three spectrophotometric methods have been validated in compliance with ICH guidelines. The concentration is linearly proportional to absorbance values in the range of 2.51–20.08 μg/mL (method A), 2.50–24.90 μg/mL (method B) and 2.51–12.56 μg/mL (method C). Estimation of LOD and LOQ parameters were obtained as 0.67 μg/mL and 2.23 μg/mL (Method A), 0.39 μg/mL and 1.32 μg/mL (Method B) 0.30 μg/mL and 1.01 μg/mL (Method C). The stoichiometric ratio of the reactive components of rosuvastatin – BCG, BCP, and BTB corresponds 1:1. The %RSD values of intra-day and inter-day were obtained less than <1.5 %, which showed excellent repeatability and RE % data was ≤3 %.

Chemists find the spectrophotometric procedures easy to use, making them suitable for a wide range of applications, including regular pharmaceutical analysis and labs lacking sophisticated equipment. Furthermore, the suggested procedures are rapid, cost-effective, and «green». The suggested spectrophotometric methods' potential environmental impact using greenness

tools (AGREE, GAPI, and AES) was evaluated. In comparison to the reported procedure, the analyzed spectrophotometric procedure has a lower ecological impact based on the greenness assessment criteria. Compared to the methods described in the literature [13, 16], the proposed methods are simpler, do not require extraction, are more environmentally friendly and more cost-effective.

Practical Relevance. The content of rosuvastatin in its tablets can be determined using the proposed spectrophotometric procedures.

Study limitations. The proposed analytical method can not be used to determine rosuvastatin in the presence of other antihypertensive and metabolic APIs in medicines.

Prospects for further research. The next stage of research is planned to develop and validate the spectrophotometric method for the determination of rosuvastatin in tablets based on the reaction with other dyes.

6. Conclusions

Three spectrophotometric methods (A, B, C) for the determination of rosuvastatin in tablets have been developed. Stoichiometric ratios of reactive components (rosuvastatin – BCG, BCP, BTB) correspond 1:1. All three spectrophotometric methods have been validated in compliance with ICH guidelines. The concentration is linearly proportional to absorbance values in the range of 2.51–20.08 μg/mL (method A), 2.50–24.90 μg/mL (method B) and 2.51–12.56 μg/mL (method C). Estimation of LOD and LOQ parameters were obtained as 0.67 μg/mL and 2.23 μg/mL (method A), 0.39 μg/mL and 1.32 μg/mL (method B) 0.30 μg/mL and 1.01 μg/mL (Method C). In summary, the developed fast, simple and cost-effective methods can be used for routine analysis of rosuvastatin in tablets.

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 at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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