تطوير طريقة طيفية لتحديد بيتافاستاتين الكالسيوم في المستحضرات الصيدلانية باستخدام بروموكريزول الأرجواني

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الملخص

تم تطوير طريقة طيفية بسيطة وحساسة ومباشرة ولا تحتاج لخطوات تحضير سابقة لتحديد تراكيز بيتافاستاتين الكالسيوم بشكله النقي وفي المضغوطات بالاعتماد على تشكيل معقد أصفر اللون بين المادة الدوائية وكاشف البروموكريزول الأرجواني في وسط من الكلوروفورم، حيث كان للمعقد المتشكل امتصاصية عظمى عند طول الموجة 406 nm، وقد تم دراسة تأثير العوامل المختلفة المؤثرة في تشكل المعقد ومن ثم اختيار الشروط المثلى للتفاعل. وتم تحديد نسب الارتباط بين بيتافاستاتين الكالسيوم وكاشف البروموكريزول الأرجواني واقتراح آلية التفاعل الحاصل لتشكيل المعقد بالاعتماد على طريقة النسب الجزيئية وطريقة التغير المستمر، حيث تبين من خلال هاتين الطريقتين أن كل جزيئة من بيتافاستاتين كالسيوم ترتبط مع جزيئتين من الكاشف. تراوح المجال الخطي لعلاقة بيير لامبرت للمعقد المتشكل ضمن المجال (2-28)µg/mL وكان معامل الانحدار R^2= 0.9995 ومعامل الامتصاص الجزيئي5.66422×10^4 L/mol.cm وحساسية ساندل $0.016\ \mu g\ cm^{-2}$. وحد الكشف وحد التحديد الكمي 0.42 µg/mL و 1.27 µg/mL على الترتيب كما تم التأكد من صلاحية الطريقة وتطبيقها في تحديد بيتافاستاتين الكالسيوم في المضغوطات المتوفرة محلياً وكانت كمية المادة الدوائية المحددة ضمن الشروط الدستورية مقبولة.

الكلمات المفتاحية: بيتافاستاتين كالسيوم، بروموكريزول أرجواني، طريقة طيفية، زوج–شاردي

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Development of Spectrophotometric Method to Determine Pitavastatin Calcium in Pharmaceutical Preparation Using Bromocresol Purple

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Abstract

A simple, sensitive and direct spectrophotometric method without pretreatment has been developed and applied for the determination of Pitavastatin calcium (PTV) in raw material and tablets formulations.

This Spectrophotometric method is based on the formation of yellow colored ion-pair complex of drug and Bromocresol Purple (BCP) in chloroform. The complex has maximum absorptivity at 406 nm. The variable factors affecting the reaction such as solvents, time, temperature and reagent concentration were studied and optimized to achieve the best results. Molar ratio method and Job plot of continuous variation method indicated the ratio (1: 2) of PTV-BCP.

Beer's law is obeyed over concentration ranges (2 – 28) µg/mL. Regression analysis showed a good regression coefficient $R^2 = 0.9995$. The molar absorptivity was to be 5.66422 x 10^4 L/mol.cm with corresponding Sandell's sensitivity value of 0.016 μg cm^{-2}. The limit of detection (LOD) and limit of quantification (LOQ) were 0.42 µg/mL and 1.27 µg/mL respectively. The proposed method was successfully applied to commercial tablet products available in local markets, and the results showed good accuracy and precision without interference with common excipients.

Keywords: Pitavastatin calcium, Bromocresol purple, Spectrophotometric method, ion-pair.

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1 Introduction

Pitavastatin calcium (PTV) is Monocalcium bis{(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3,5-dihydroxyhept-6-enoate} with molar mass 880.98 g/mole [1]. The structure of PTV is shown in figure (1, a).

It is a novel statin compound. Like the other members in this class, it is an inhibitor of HMG-CoA reductase, the enzyme which catalyzes the first step of cholesterol synthesis. (PTV) is used for hypercholesterolemia treatment; it lowers total cholesterol and low density lipoprotein (LDL), and therefore, it is used for prevention of cardiovascular diseases (CVDs), [2,3].

A review of the literature revealed that several methods have been reported for the analysis of PTV in tablets like indirect visible spectrophotometric methods by using ferric chloride [4], acidic potassium permangana [5], 7,7,8,8-tetracyanoquinodimethane (TCNQ) [6], acid dyes [7,8] and bromate-bromide mixture [9], in spectrofluorometric method [10], UV spectrophotometric method [11], chromatographic methods [12-16], indirect titrimetric assay [17] and electrochemical methods [18,19]**.**

Most of the above visible spectrophotometric methods suffer from one or more of disadvantages such as use of expensive reagents, use of heating step, poor sensitivity, liquid-liquid extraction step, and close pH control.

Many studies have been done using Bromocresol purple (figure (1, b)) for estimation of several drugs in their bulk and pharmaceutical dosage forms. [20-22]

In this research, a simple direct extractive-free spectrophotometric method was developed by using Bromocresol purple for the estimation of PTV in raw material drug and in tablets of pharmaceutical preparations.

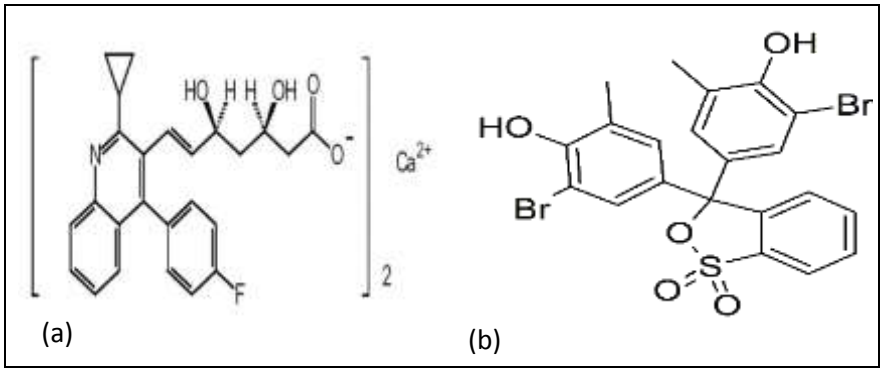

Figure 1: the chemical structure of: a- (PTV) and b- (BCP).

2 Aim and Goal of Study

An attempt was made to develop a direct, simple, sensitive, rapid and extraction-free validated visible- method for the determination of Pitavastatin calcium in the tablets formulations and raw materials.

3 Experimental

3.1 Apparatus

All spectral measurements were carried out using a T80+ UV/V spectrophotometer instrument Ltd (UK) connected to computer, with quartz cells 1 cm., ultrasonic bath (Powersonic, model 405, Korea), adjustable micro pipette covering a volume range from 100 to 1000 µL (LABGILLS, Germany), and analytical balance (Sartorius, model 2474, Germany).

3.2 Chemical reagents:

Chloroform Emsure (Merck, Germany), Dichloromethane (Riedel-de Haën, Germany), Methanol (Merck, Germany), Ethanol (Eurolab, UK), Bromocresol Purple from (Merck, Germany), Pitavastatin calcium raw material (Ultra Medica Pharmaceutical Industeries), and Ezitemibe raw material (Barakat pharmaceutical industries).

3.3 Tablets pharmaceutical preparations:

The listed tablets' commercial products were subjected to analytical procedure:

- Londalop tablets, Ultramedica Pharmaceutical company, Damascus- Syria, (2018/2021), labeled 2, 4 mg/tab of Pitavastatin as (calcium) with batch No. H1458 and H2221 respectively.
- Lipid Free tablets, Ibn-Roshed pharmaceutical company, Aleppo- Syria, (2018/2022), labeled 2, 4 mg/tab of Pitavastatin as

(calcium) with Bbtch No. 3 for both products.

3.4 Pitavastatin calcium stock solution:

Stock solution of (1mg /mL) of Pitavastatin calcium 99.5 % was prepared by dissolving (25.1) mg raw material equivalent to 25 mg (after taking the purity in consideration) of Pitavastatin calcium in 25 mL of chloroform in volumetric flask. Then 1 mL of the solution was taken to volumetric flask 10 mL and diluted with Chloroform to give concentration (0.1 mg /mL).

Pitavastatin calcium stock solution is stable for a period of 3 days when refrigerated (4 - 8°C). It was concluded after absorbance measurement of the solution over 3 days, and RSD % which was less than 2% was calculated.

3.5 Bromocresol purpule stock solution:

Stock solution of reagent BCP was prepared with a concentration of (1×10^{-3} M) by dissolving suitable weight of the reagent (0.0135) g and diluting to mark with chloroform in 25 ml volumetric flasks.

3.6 Preparation of solutions of optimizing reaction conditions:

First, we compare the absorptivity of the complex in different medium of organic solvents in which the drug and the dye were dissolved and the chloroform selected. To study the effect of reagent concentration on the colored complex solution, we made a series of 5 mL of separated volumetric flasks, by adding 1 mL of Pitavastatin calcium 1×10^{-4} M, added between (0.2 - 2 mL) of (BCP) 1×10^{-3} M, equivalent to (40 - 400 µM), and completed to 5 mL by chloroform to have drug concentration equivalent to 20 µM. After we determined the optimal concentration, we studied the effect of the time and the temperature.

3.7 Preparation of working standard solutions:

The working standard solutions of the raw material were prepared by taking appropriate volumes of the stock solution among (100 - 1400) μL and diluting them with chloroform in 5 mL volumetric flasks that contain optimum volume of BCP which is equivalent to 1.13×10^{-3} M ($C_{BCP} = 10\ C_{PTV}$). Working standard solutions contained (2 - 28) µg/ml of Pitavastatin calcium.

3.8 Preparation of pharmaceutical sample solutions:

20 tablets were powdered and mixed accurately. An amount of average weight of 20 tablets was taken which is theoretically equivalent to labeled content (2.09 mg or 4.18 mg) of Pitavastatin calcium, dissolved in 25 ml of chloroform, and sonicated for 20

minutes. Then a sample was filtered by using Büchner funnel. After that, further dilutions were made to obtain an appropriate concentration. Accordingly, 1 ml was taken and diluted to 10 mL. We theoretically obtained concentrations of 8.36 μg/mL and 16.72 μg/mL respectively.

3.9 Procedure for stoichiometric relationship:

To study the Stoichiometric Relationship between Pitavastatin calcium and the dye, the stock solutions of the drug and dye were prepared under the same concentration (1×10^{-3}M) in 25 mL volumetric flasks. For molar ratio method, a series of solutions was prepared in 10 mL volumetric flask in which the ratio of the drug to dye changed. Whereas for Jobs method for continuous variation, a series of solutions was prepared in which the total volume of PTV and dye was kept at the same value. The drug and dye solutions were mixed in various complementary proportions and completed as directed under the recommended procedures to have a resultant concentration of ([PTV] + [BCP] = 40 μM). The absorbance of the resultant ion-pair complex was measured at 406nm**.**

4 Results:

4.1 Spectrophotometric optimal conditions:

The different experimental parameters affecting the spectrophotometric determination of Pitavastatin calcium through ion-pair complex formation with (BCP) were studied in order to determine the optimal conditions for the drug determination.

4.1.1 Effect of organic solvents:

According to (PTV) and (BCP) solubility, few solvents (Acetone, Chloroform, Dichloromethane, Ethanol and Methanol) were studied to select the best one which exhibits both high molar absorptivity coefficient and negligible blank absorption. As a result, chloroform was the preferred solvent to be used. Figure 2 shows the spectra of the PTV-BCP complex and BCP blank in medium of chloroform.

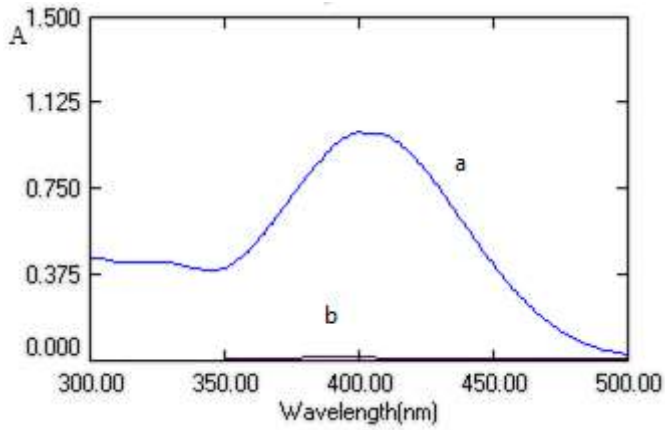

Figure 2: Spectra of: a-PTV-BCP complex in chloroform, b-BCP blank in chloroform

4.1.2 Effect of dye concentration:

It was found that the completed colored complex formation was when adding 1 mL of (BCP) solution as it is shown in figure 3. The best concentration addition of (BCP) was 200 µM with ten times of Pitavastatin calcium concentration.

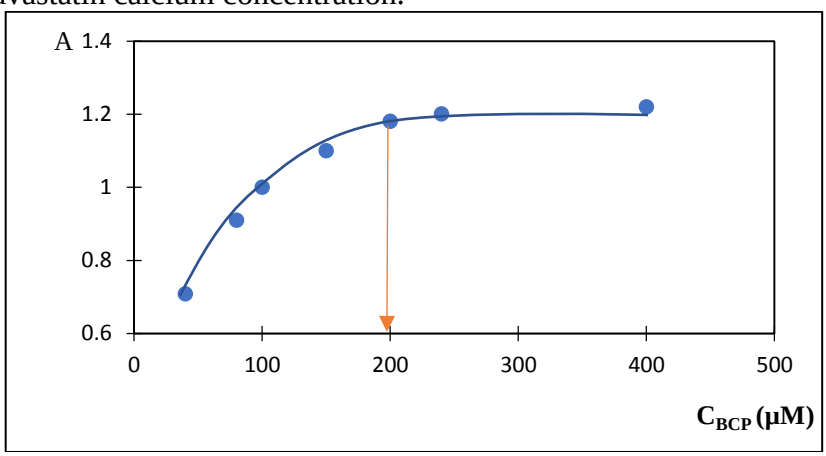

Figure 3: Effect of BCP concentration

4.1.3 Effect of temperature:

Studies showed that the temperature has a negligible effect on the formation of PTV: BCP complex in the range of (20 - 30^{o}C) as shown in figure 4.

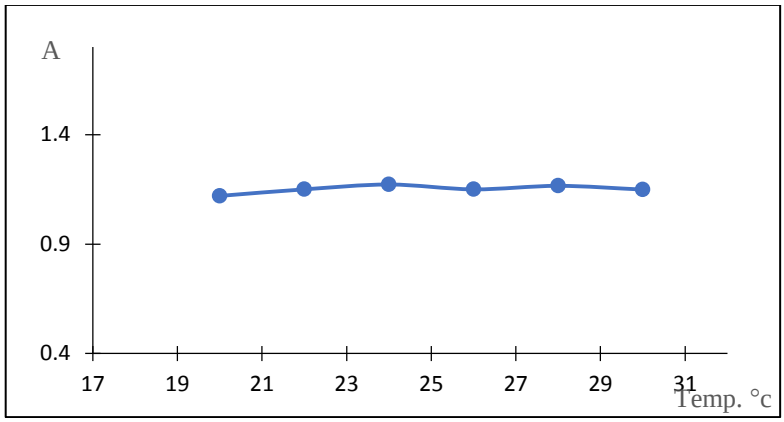

Figure 4: Effect of temperature on PTV-BCP complex formation

4.1.4 Effect of reaction time and stability:

It was found that the formation of PTV: BCP complex was direct after the mixing of drug and reagent. The absorbance of the complex was stable for 24 hours.

4.2 Stoichiometric ratio:

The stoichiometric ratio of PTV-BCP complex formation was determined by applying two methods; molar ratio and Jobs' continuous variation methods (figures 5 and 6).

4.2.1 Molar ratio method:

In this method, the concentration of the reagent was constant 20 µM, and the drug concentrations were variable between the range (5 - 35) µM.

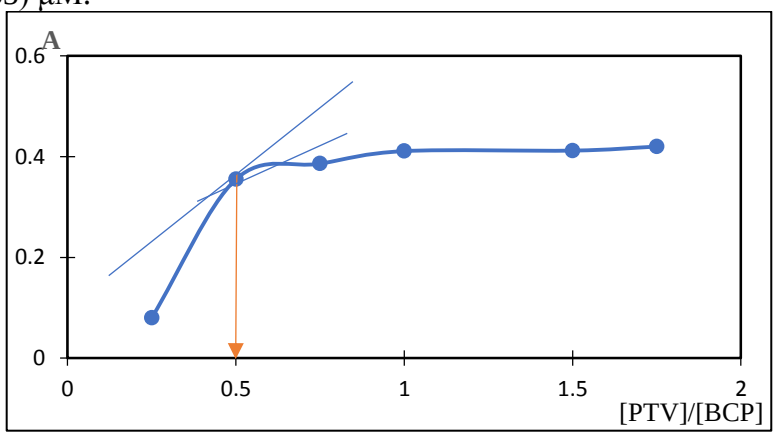

Figure 5: Molar ratio plot for PTV-BCP

4.2.2 Job's continuous variation method:

In order to study the stoichiometric ratio of complex formation, continuous variation volumes were taken from drug and reagent to

have a resultant concentration of ([PTV] + [BCP] = 40 µM) in 10 mL volumetric flasks using chloroform for diluting.

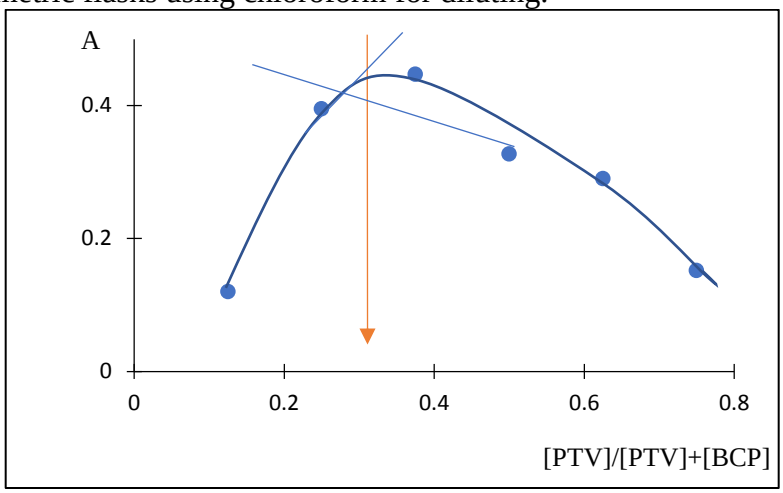

Figure 6: Job's method plots for PTV-BCP complex

4.2.3 Mechanism of reaction:

Anionic reagents like sulfonephthaleins dyes form ion-pair complexes via electrostatic reaction between positively charged nitrogen of PTV and negatively charged sulfonic group of the BCP. Accordingly, we summarized that 2 Pitavastatin moles or 1 mole of Pitavastatin calcium react with 2 moles of the dye. So we can say that the molar stoichiometric ratio is (2:2) for Pitavastatin or (1:2) for Pitavastatin calcium.

4.3 Method validation:

The proposed method was validated for linearity, sensitivity, precision (reported as RSD %), accuracy (reported as recovery percentage), robustness and specificity according to International Conference on Harmonization guidelines (ICH) [23]

4.3.1 Linearity:

We studied the linearity of Pitavastatin calcium concentrations at the optimal conditions where we made a series of 5 mL volumetric flasks; each flask contained equivalent volumes of (BCP) 1.13×10^{-3} M and (PTV) 1.13×10^{-4} M, then the volume was made up to the mark with chloroform. The absorbance of yellow colored complex was measured at 406 nm against the blank of BCP in chloroform. Figure 7 presents PTV-BCP complex spectra, and the linearity range of Beer law was obeyed to be (2-28) μg/mL as shown in figure 8.

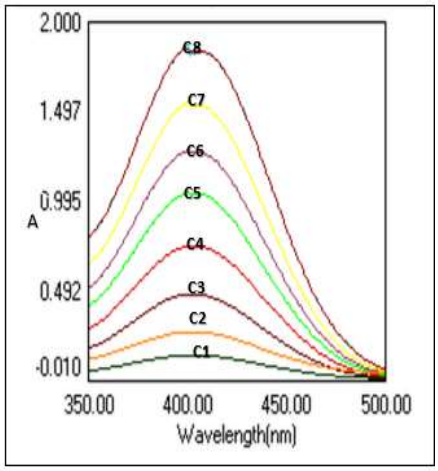

Figure 7: spectra of (PTV-BCP): C_1: 2 µg/mL, C_2: 4 µg/mL, C_3: 8 µg/mL, C_4 : 12 µg/mL, C_5: 16 µg/mL, C_6: 20 µg/ mL, C_7: 24 µg/mL, C_8: 28 µg/ mL.

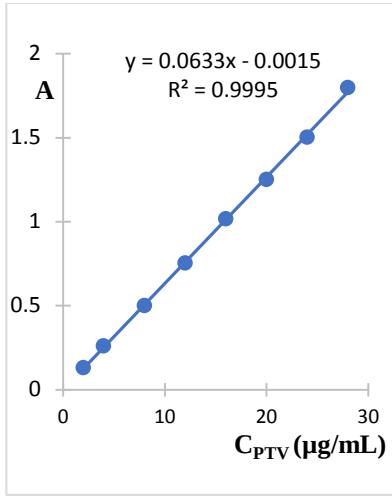

Figure 8: Calibration curve for (PTV-BCP), n = 5 for each concentration.

4.3.2 Detection limit, quantitation limit and sensitivity Sandell's:

The mean molar absorptivity ε, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) were calculated to estimate the sensitivity of the developed method. The values are presented in table 1:

Table 1: Statistical data for calibration graphs of Pitavastatin calcium

Parameter	Value
λ_{max} (nm)	406
Beer's law range (µg/mL)	2 - 28
Stability of the complex	24
Temperature of solution, °c	25 ±5
Solvent	Chloroform
C_{BCP}: C_{PTV}, M	10
Molar absorptivity, ε (L/mol.cm)	5.66422×10^4
Sandell's sensitivity SS (µg/cm^2)	0.016
Slope (b)	0.0633
Intercept (C)	0.0015
R^2	0.9995
Correlation coefficient (r)	0.9997
LOD* (µg/mL)	0.42
LOQ* (µg/mL)	1.27

*LOQ = 10 × SD/m LOD = 3.3 × SD/m

Where SD is the standard deviation of y-intercepts (a) of regression lines and (b) is the slope of the equitation of calibration curve, y = a + b x.

4.3.3 Precision and accuracy:

To determine the precision and accuracy of the proposed method, three replicates per day were carried out on three different concentrations of standards (PTV) over three different days. The results of this study are summarized in table 2. The relative standard deviation (RSD%) values were ≤1.89 % (intra-day) and ≤2.33% (inter-day), indicating the good precision of the proposed method. The accuracy was assessed as the percentage of mean concentration, and it demonstrates the high accuracy as the results shown in tables 2 and 3.

Table 2: Intraday accuracy and precision for PTV determination

PTV taken (μg/mL)	PTV found ± CL (μg/mL)	Percent*(%)	SD (μg/mL)	RSD (%)
8	7.95 ± 0.35	99.37	0.14	1.76
16	15.85 ± 0.74	99.06	0.30	1.89
20	20.17 ± 0.82	100.85	0.33	1.64

Table 3: Inter-day accuracy and precision for PTV determination

PTV taken (μg/mL)	PTV found ± CL (μg/mL)	Percent* (%)	SD (μg/mL)	RSD (%)
8	7.98 ± 0.42	99.75	0.17	2.13
16	15.87 ± 0.92	99.19	0.37	2.33
20	19.65 ± 0.45	98.25	0.18	0.92

*Percent (%) = (found concentration / taken concentration) ×100.

4.3.4 Recovery:

The recovery was studied by three addition standards at levels (80%-100%-120%) for Londalop 2 and Lipid Free 2 products. Table 4 presents the recovery study results for the two Syrian trademark drugs.

Table 4: Recoveries study for accuracy estimation

Product	Sample (µg/mL)	Added (µg/mL)	Total found (µg/mL)	Recovery %	SD	RSD %	Recovery average %
Londalop 2	6.29	5.00	11.37	101.60	2.02	1.99	100.76
		6.30	12.55	99.37	1.02	1.01	
		7.55	13.94	101.32	0.93	0.92	
Lipid Free 2	6.20	5.00	11.19	99.80	0.70	0.70	99.73
		6.20	12.43	100.48	1.46	1.45	
		7.44	13.56	98.92	1.88	1.90	

Five separate determinations were performed and the mean was calculated.

Recovery% = (total found concentration – sample

concentration)/added concentration ×100

4.3.5 Robustness:

In order to demonstrate the robustness of the developed method, three replicates were carried out on two different concentrations (8, 20 µg/mL) changing variable parameters of measurement such as wavelength, scanning interval and scanning speed. The changes had a negligible impact on the results, as revealed by the small intermediate precision values expressed as %RSD (≤ 1.95%). The obtained results are presented in table 5:

Table 5: Robustness study results

Parameter	Change	PTV (µg/mL)	RSD %	Per %
λ max (406 nm)	+2nm	8.03	0.90	100.38
		19.85	0.86	99.25
	-2 nm	8.03	0.98	100.38
		19.66	0.33	98.30
Scanning Interval	1 nm	8.17	1.03	102.13
		20.05	0.74	100.25
	2 nm	8.21	0.38	102.63
		19.70	0.46	98.50
Scanning Speed	Fast	8.09	1.89	101.13
		20.05	0.74	100.25
	Slow	8.08	1.95	101.00
		19.63	0.29	98.15

4.3.6 Specificity:

To assure that the developed method was specific for (PTV) determination in pharmaceutical products without interfering of the excipients in these formulations, the method was applied to Syrian tablets products and the obtained results were compared with a validated reference method [11]. The results showed that no interference was exhibited by excipients as shown in tables 6 and 7.

Ezitemibe (EZT) has synergistic effect with statins in treating dyslipidemia and it is suggested to develop fixed dose combination in future. Accordingly, we had studied Ezitemibe interaction with the reagent BCP and in combination with pitavastatin. As it is shown in (figure 9), Ezitemibe did not react with the reagent, did not demonstrate the yellow color of the complex formation, and did not interfere in Pitavastatin determination when it is in ratio (2:5) for Pitavastatin and Ezitemibe respectively [24].

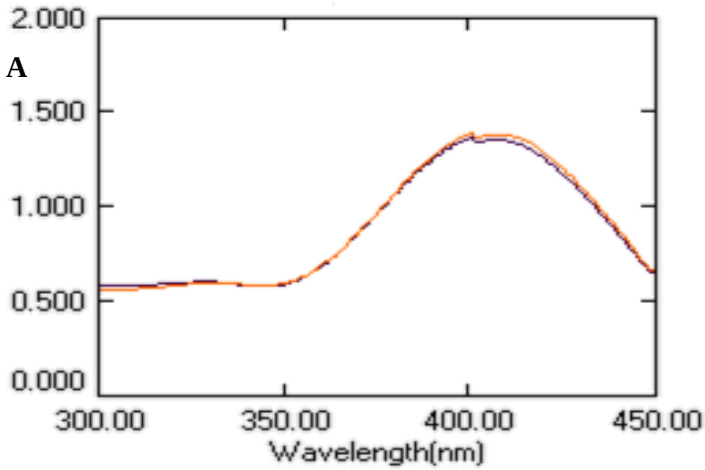

Figure 9: Overlay of PTV-BCP complex (c= 20µg/mL) and mix of PTV-BCP (C= 20µg/mL) and EZT 50 µg/mL

4.4 Application of the proposed method for (PTV) estimation in two Syrian brands:

The developed method was applied for the quantitative determination of (PTV) in different Syrian tablets formulation drugs. The samples were prepared as described in the section of samples preparation and then they were analyzed. Quantitative analysis was done by using calibration curve. The results are shown in tables 6 and 7:

Table 6: Estimation of (PTV) in Londalop (2 and 4)

Londalop 2				
Claim Amount (µg/mL)	Average recovery ± SD (%(		t- test	F-test
	Reference method[11]	Developed method		
8.36	104.67 ± 0.72	104.09 ± 1.141	0.96	2.50
Londalop 4				
Claim Amount (µg/mL)	Average recovery ± SD (%)		t- test	F-test
	Reference method[11]	Developed method		
16.72	101.94 ± 0.36	101.56 ± 0.62	1.19	2.92

*n=5

The value of t (critical) at 95% confidence level and for four degrees of freedom is 2.78.

The value of F (critical) at 95% confidence level and for four degrees of freedom is 6.39.

Table 7: Estimation of (PTV) in Lipid Free (2 and 4).

Lipid Free 2				
Claim Amount (µg/mL)	Average recovery ± SD(%)		t- test	F-test
	Reference method[11]	Developed method		
8.36	97.75 ± 1.85	97.67 ± 2.36	0.04	1.63
Lipid Free 4				
Claim Amount (µg/mL)	Average recovery ± SD(%)		t- test	F-test
	Reference method[11]	Developed method		
16.72	102.70 ±1.85	102.76 ± 1.74	0.05	1.10

*n = 5

The value of t (tabulated critical) at 95% confidence level and for four degrees of freedom is 2.78.

The value of F (tabulated critical) at 95% confidence level and for four degrees of freedom is 6.39.

The results obtained by the proposed method were compared to those of the reference method [11] by applying Student's t-test for accuracy and F- test for precision. The results (Tables 6 and 7) showed that the Student's t- and F-values at 95% confidence level did not exceed the tabulated critical values, which confirmed that there is a good agreement between the results obtained by the proposed method and the reference method with respect to accuracy and precision.

The dosages of (PTV) were conformed to JP legislation. [1] (The tablets must contain no less than 95.00% and no more than 105.00 % of labelled amount).

5 Conclusion:

In this research, simple extractive free method was developed and validated to be applied directly in room temperature without previous treatments. A comparison study between the developed method and previous visible spectrophotometric methods was done. It has been concluded that the proposed method is simpler than the reported methods because it does not require adjusting pH or heating; the used medium of reaction was a cheap solvent available in quality control laboratories. The method was direct and not time-consuming. The linearity range of Beer law was (2 – 28) µg/mL which was better than the previous methods, and the molar absorptivity coefficient (Ɛ) of the formed yellow complex in chloroform was 5.66422 x 10^4 L/mol.cm higher than the other methods, and that means it is a more sensitive method. We summarized some of these comparison points in table 8:

Table 8: Comparison between the developed method and some visible spectrophotometric methods.

Method Reagents / Reference	Methodology Remarks	Solvent	Linearity range (µg/ml)	λmax (nm)	Molar absorptivity (L/mole/cm)
Permanganate [5]	Indirect, less sensitive method, required pH control, narrow linearity range	Water-acetic Acid	8.0 – 18.0	550	NA
BTB [7]	Required pH control and involved extraction step	Chloroform	20-100	420	NA
BCP [8]	More expensive solvents, less sensitive method	DMF: acetonitrile (1:1)	20-90	601	9820
BTB [8]	More expensive solvent, less sensitive method	DMSO	20-80	637	12000
BCP (Developed Method)	Direct	Chloroform	2-28	406	566422

6 References

[1] Japanese Pharmacopoeia 17th Edition,2016, 1424-1425.

[2] KAJINAMI K., TAKEKOSHI N., SAITO Y., 2003 - **Pitavastatin: Efficacy and Safety Profiles of a Novel Synthetic HMG CoA Reductase Inhibitor. Cardiovascular**. *Drug Reviews*. 21(3): 2003; 199-215.

[3] FAZIO S., 2011 - **The Role of Statin Therapy in Primary Hyperlipidemia and Mixed Dyslipidemia**. *US Endocrinology*, **7(1)**, 23-32.

[4] VAMSIKRISHNA M., SANKAR D. G., 2007 - **Adaptation of Color Reactions for Spectrophotometric Determination of Pitavastatin Calcium in Bulk Drugs and in Pharmaceutical Formulations**. *E-Journal of Chemistry*, **4(2)**, 272-278.

[5] VIRUPAXAPPA B. S., SHIVAPRASAD K. H., LATHA M. S., 2011 - **Novel Spectrophotometric Method for The Assay of Pitavastatin Calcium in Pharmaceutical Formulations.** *Der Chemica Sinica,* **2(4)**, 1-5.

[6] ERGIN G., AGLAR S., ONAL A., TOKER E., 2013 - **Spectrophotometric Determination of 3-Hydroxy-3-Methylglutaryl Coenzyme-A Reductase Inhibitors in Pharmaceutical Preparations.** *Turkish Journal of Chemistry*, **37**, 171-181.

[7] TULJARANI G., CHETHANA G., RASHEEDA, ARCHANA CH., 2015 - **Application of Visible Spectrophotometric for Estimation of Pitvastatin in Bulk and Phamaceutical Formulation.** *International Journal of Current Research in Chemistry and Pharmaceutical Sciences,* **2(5)**, 56-62.

[8] NIRANJANI S., VENKATACHALAM K., 2019 - **Spectrophotometric Determination of Atorvastatin Calcium and Pitavastatin Calcium Through Ion-Pair Complex Formation Using Acid Dyes in Pharmaceutical Formulations and Human Urine Samples.** *International Journal of Applied Pharmaceutics*, **11(6)**, 171-180.

[9] NIRANJANI S., VENKATACHALAM K., 2019 - **Simple Titrimetric, Spectrophotometric and Gravimetric Methods for the Assay of Pitavastatin Calcium in a Green Manner.** *Journal of Pharmaceutical Sciences and Research,* **11(5)**, 1766-1774.

[10] EL-BAGARY R., ELKADY E. F., KADRY A. M., 2012 - **Spectrofluorometric Determination of Certain Antihyperlipidemic Agents in Bulk and Pharmaceutical Preparations**. *Spectroscopy: An International Journal*, **27(2)**, 83-92.

[11] AGLAWE K. V., KHARAT U. P., DONGAONKAR C. C., CHAVAN V. A., 2016 - **Development and Validation of Stability Indicating UV Spectrophotometric Method for The Determination of Pitavastatin Calcium.** *World Journal of Pharmacy and Pharmaceutical Sciences*, **5(7)**, 1773-1787.

[12] SATHEESH N.K., BAGHYALAKSHMI J., 2007 -

Determination and Quantification of Pitavastatin Calcium in Tablet Dosage Formulation by HPTLC Method. *Analytical Letters*, **40**, 2625-2632.

[13] HIRAL J.P., BHANUBHAI N.S., NATVARLAL J.P., BHAVESH H.P., 2008 - **A Simple and Sensitive HPTLC method for estimation of Pitavastatin Calcium in Tablet Dosage Form**. *Journal of Planar Chromatography*, **21**, 267-270.

[14] PANCHAL H. J., SUHAGIA B. N., PATEL M.M., PATEL B.H., 2009 - **Estimation of Pitavastatin Calcium in Tablet Dosage Forms by Liquid Chromatography and Ultraviolet Spectrophotometry**. *AOAC Int*, **92(1)**, 158-164.

[15] GOMAS A. R, RAM P.R, SRINIVAS N., SRIRAMULU J., 2010 - **Degradation Pathway for Pitavastatin Calcium by Validated Stability Indicating UPLC Method**. *American Journal of Analytical Chemistry*, **2**, 83-90.

[16] SATHEESH N.K., NARAYANAN N., JAYABALAN N., NARAYANAN S., BAGYALAKSHMI J., 2011 - **HPLC Determination of Pitavastatin Calcium in Pharmaceutical Dosage Forms**. *Pharmaceutica Analytica Acta*, **2(2)**, 1-4.

[17] JANAGIRAMAN S., RAJU T., GIRIBABU K., 2014 - **Simple Titrimetric Analysis for Determination of Pitavastatin Calcium in Bulk and Formulation Dosage**. *International Journal of Modern Chemistry*, **6(1)**, 18-27.

[18] JANAGIRAMAN S., RAJU T., GIRIBABU K., NARAYANAN V., 2014 - **Electrochemical Determination of Pitavastatin Calcium as Bulk Drug by Voltammetry Techniques**. *International Journal of Innovative Research in Science & Engineering*.

[19] PANDIT U.J., NAIKOO G.A, SHEIKH M.U., KHAN G.A., RAJ K.K., 2017 - **Electrochemical Determination of an Anti-Hyperlipidimic Drug Pitavastatin at Electrochemical Sensor Based on Electrochemically Pre-Treated Polymer Film Modified GCE**. *Journal of Pharmaceutical Analysis*, **7(4)**, 258-264.

[20] SAKUR A. A., OBAYDO R., 2015 - **Sensitive Spectrophotometric Methods for Determination of Zolmitriptan in Bulk Form and in Tablets Via Complex Formation with Two Sulphonphtalein Acid Dyes**. *International Journal of academic and Scientific Research*, **3(4)**, 106- 114.

[21] SAKUR A. A., NASHED N., 2018 - **Validated Direct**

Spectrophotometric Methods for Determination of Cefpodoxime proxetil using Sulphonphtalein Acid Dyes. *Research Journal of Pharmacy and Technology*, **11(6)**, 1-6.

[22] ANTAKLI S., NEJEM L., AHMAD A.W., 2019 - **Determination of Amoxicillin Trihydrate by Analytical Spectrophotometry.** *Research Journal of Pharmacy and Technology*. **12(6)**, 2716-2720.

[23] ICH Q2(R1). 2005- **Validation of analytical procedures: text and methodology**. International Conference on Harmonization of Technical Requirements for registration of Pharmaceuticals for Human Use.

[24] PANCHAL H., SUHAGIA B.N., 2011 - **Simultaneous Determination and Validation of Pitavastatin Calcium and Ezetimibe in Binary Mixture by Liquid Chromatography.** *International Journal of PharmTech Research*, **3(4)**, 2155-2161.