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Research Article

APPLICATION OF VISIBLE SPECTROPHOTOMETRIC FOR ESTIMATION OF PITVASTATIN IN BULK AND PHAMACEUTICAL FORMULATION

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Abstract

A sensitive and rapid extractive spectrophotometer method has been developed for the assay of pitvostatin in bulk and pharmaceutical formulations. The method is based on the formation of a chloroform soluble ion-pair complex between pitvostatin and bromophenol blue in an acidic medium. The complex shows absorption maximum at 420 nm. The system obeys Beer's law in the concentration range of 20-100 µg/ml. The results obtained by the proposed method were validated statistically and by recovery studies.

Keywords: Pitvostatin, Bromophenol Blue, Chloroform, Ion pair complex, Spectrophotometer.

Introduction

Pitvostatin is a member of the blood cholesterol lowering medication class of statins. Like other statins, it is an inhibitor of HMGCO-A reductase the enzyme that catalyses the first step of cholesterol synthesis can reduce risk of cardiovascular diseases[1,2]. Chemically it is (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid (Figure 1).

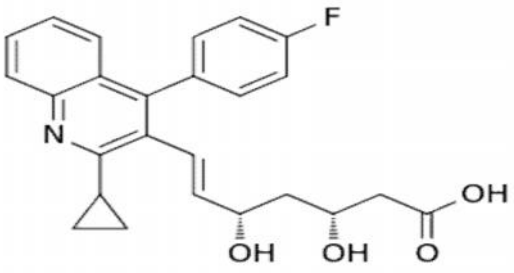

Figure 1.Chemical structure of pitvostatin

Literature search reveals following methods viz. spectrophotometric method by using acid potassium permanganate [3], spectrofluorometric method [4], liquid chromatography method [5,] HPLC [6,] UPLC [7] and HPTLC methods [8, 9], but there is no simple spectrophtometric method by using bromothymol blue (BTB). So the author made an attempt to develop a simple and specific spectrophotometric method for the estimation of pitvastatin by using BTB.

Materials and Methods

Instruments

All spectral measurements are made on Lab India 3000UV/visible spectrophotometer. Systronics pH meter was used for adjusting pH of the buffers.

Chemicals and reagents

All the chemicals used were of SD fine chemicals (analytical grade) and solutions were prepared with distilled water. Pitvostatin pure drug was gifted by **Mylan-Pharmaceuticals**-Private-Limited, Hyderabad and PIVASTA tablets – 4mg were procured from local market.

Preparation of solutions

Acetate buffer p^H 2.8: 4g of anhydrous sodium acetate was dissolved in about 840ml of water; sufficient glacial acetic acid was added to adjust the pH to 2.8 and diluted with water to 1000ml. **Bromo phenol blue:** 100mg of bromophenol blue was dissolved in small amount of distilled water and diluted to 100ml with distilled water.

Preparation of standard drug solution of pitavastatin

100mg of pitavastatin pure drug was accurately weighed and dissolved in 100ml of 0.1N HCL to get 1000µg/ml.

Selection of wavelength

The absorption spectrum of the colored species formed by mixing drug with dye and extracting into organic layer like chloroform shows absorbance maximum at 420nm for pitavastatin. Spectrum of the absorption curve is shown in the figure 2.

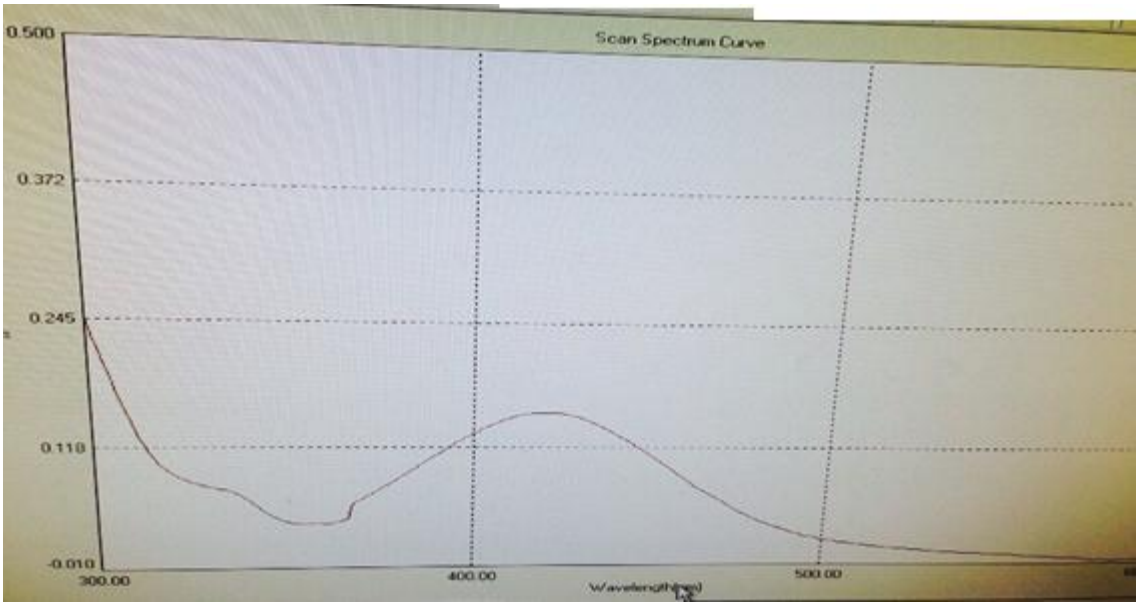

Figure 2.spectrum of pitvostatin showing absorption maxima

Procedure for calibration curve:

Different aliquots of the standard drug ranging from 0.1 to 0.5 ml was taken from standard stock solution of pitavastatin were into a series of 60ml separating funnels. To each separating funnel 1.5ml of pH 2.8 buffer and 1.5 ml of dye solution was added. Then 10ml of chloroform was added to each separating funnel to extract the drug –dye complex. Then contents were shaken for 1 min for thorough mixing of the two layers and were allowed to stand 10 min for separation both the layers. The separated chloroform layer was collected and absorbance was measured against the reagent blank at 420nm. The results obtained are presented in the Table 1.

Table 1. Calibration curve data for the developed method

S.NO	Concentration µg/ml	Volume of 1% reagent	Volume of buffer	Volume of chloroform	Shaking time	Absorbance at 420nm
1.	10	1.5 ml	1.5ml	10ml	1min	0.157
2.	20	1.5ml	1.5ml	10ml	1min	0.255
3.	30	1.5ml	1.5ml	10ml	1min	0.373
4.	40	1.5ml	1.5ml	10ml	1min	0.465
5.	50	1.5ml	1.5ml	10ml	1min	0.550

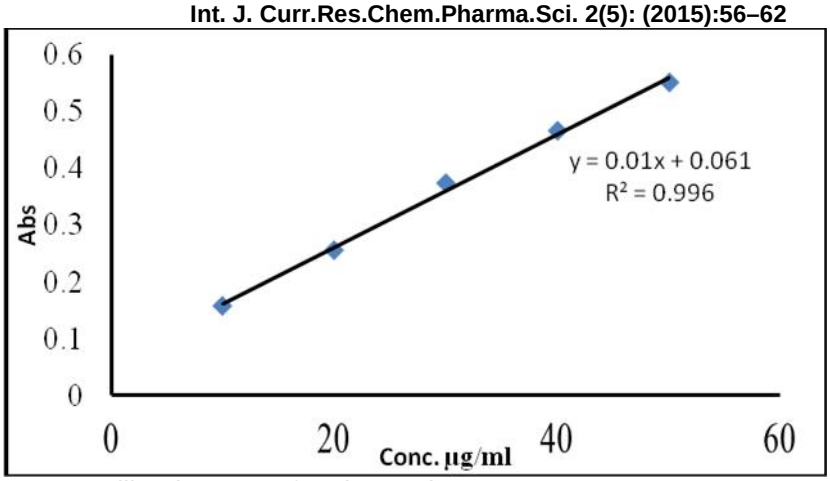

Figure 3. Calibration curve for pitvostatin

Validation of analytical method

The developed method was validated as per ICH guidelines for parameters like linearity, precision, accuracy, LOD, LOQ, robustness and ruggedness.

Linearity:

Different aliquots of the standard drug 0.2-1ml of solution were taken into series of 60ml separating funnels. To each separating funnel 1.5ml of pH 2.8 buffer and 1.5 ml of dye solution was added. To each separating funnel 10ml of chloroform was added. Then contents were shaken for thorough mixing of the two layers and were allowed to stand for separation of the chloroform layer. The separated chloroform layer absorbance was measured against the reagent blank at 420nm and the results obtained are presented in the table 2. Calibration curve was plotted by taking absorbance on the y axis and conc. on the x-axis and shown in the Figure 4.

Table 2.Linearity data of the proposed method

S.No	Volume of drug solution	Conc µg/ml	Absorbance at 420nm
1.	0.2ml	20	0.209
2.	0.4ml	40	0.438
3.	0.6ml	60	0.648
4.	0.8ml	80	0.856
5.	1ml	100	1.07

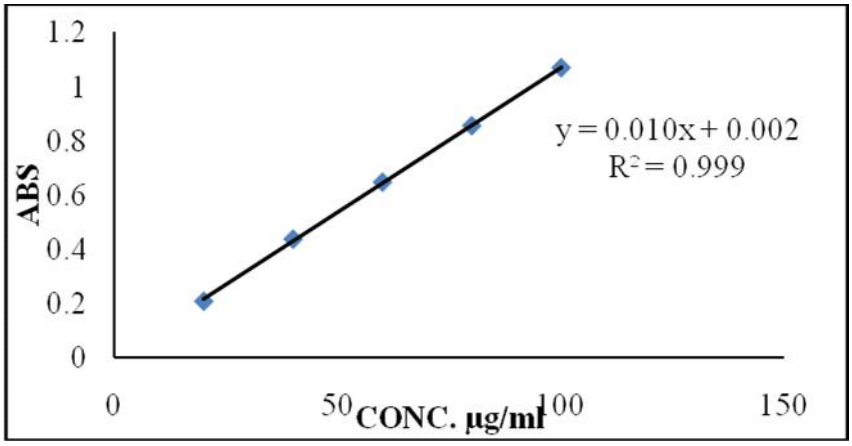

Figure 4.Standard linear curve for pitvostatin

Precision

Repeatability was performed by applying six replicates of sample analysis. For intermediate precision and intraday and inter day precision were performed by determiningon the same day and on three different days. The results are reported in terms of % RSD in the following Table 3 and 4.

Table 3. System precision of the proposed method

S NO	Drug conc. µg/ml	Absorbance at 420 nm
1.	60	0.934
2.	60	0.955
3.	60	0.937
4.	60	0.934
5.	60	0.944
6.	60	0.947
MEAN	…..	0.9418
SD	…..	0.00837
%RSD		0.8887

Table 4. Results of Intra and inter day precision

Drug	Concentration µg/ml	Intra day (n=3)	%RSD	Inter day (n=3)	%RSD
pitvostatin	40	39.99µg/ml	0.452	39.89	0.365
pitvostatin	60	60.02 µg/ml	0.235	60.01	0.587
pitvostatin	80	79.98 µg/ml	0.186	79.97	0.269

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed by preparing of concentration 60 µg/ml of pitvastatin standard solution. Recovery studies were carried out by standard addition method at three different levels 50%, 100% and 150% respectively. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The % recovery of the added pure drug was calculated as % recovery = [(Ct–Cs)/Ca] x 100, where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; Ca, drug concentration added to formulation. The result of recoveries obtained by proposed method was validated by statistical evaluation and presented in Table 5.

Table 5. Results of accuracy of the proposed method

Level	Amount added (µg/ml) (n=3)	Amount recovered (µg/ml)±SD⋆	%Recovered
50%	30	29.98±0.021	99.93
100%	60	60.23±0.102	100.38
150%	90	90.14±0.121	100.1

Average of three determinations⋆

Stability:

The stability of color produced for both standard and sample solutions were determined by keeping the solutions over a period of 8 hrs at room temperature and then the absorbance of both sample and standard solutions were measured. There is no appreciable change in the absorbance after 8hrs. The results are shown in the Table 6.

Table 6. Results showing the stability of the color formed

Time	Initial	After 2hrs	After4 hrs	After 6hrs	After 8hrs
Absorbance Standard (60µg/ml)	0.648	0.645	0.644	0.642	0.640
Sample 60 µg/ml	0.642	0.641	0.638	0.635	0.633

Limit of Detection and Limit of Quantification

The LOD and LOQ of pitvastatin were determined by using standard deviation of response and slope approach as defined by ICH guidelines. The LOD and LOQ were found to be 0.23 and 0.42 respectively**.** The results are given in the Table 7

.

Table 7. Results of LOD and LOQ

LOD	LOQ
0.23 µg/ml	0.42 µg/ml

Ruggedness

Ruggedness of the proposed method was carried out by 2 different analysts. The result did not show any considerable statistical difference suggesting that the method developed was rugged. Results obtained are summarized in the Table 8.

Table 8. Results of ruggedness

S.No.	Amount used (µg/ml)	Analyst 1 Amount ±SD⋆	Analyst 2 Amount±SD⋆	Instrument 1 Amount±SD⋆	Instrument 2 Amount±SD
1.	30	30.01±0.215	29.99±0.237	29.97±0.203	30.03±0.231
2.	60	59.99±0.341	60.01±0.301	60.03±0.106	60.02±0.206
3.	80	80.03±0.365	80.01±0.314	80.03±0.305	79.96±0.404

⋆ n=6

SD –Standard deviation

Robustness

Robustness of the proposed method was determined by estimating a drug at slightly different wave length from the selected wavelength. No significant difference was found in the absorbance of samples. Therefore, the proposed method was considered as robust.

Assay

Preparation of sample solution

The proposed method was successfully applied for the determination of pitvastatin in tablet dosage form.
Twenty tablets of PIVASTA were weighed and powdered. The amounts of tablet powder equivalent to 10 mg of pitvostatin was weighed accurately and transferred to 10 ml of volumetric flask containing 5 ml 0.1M HCL and volume was made up to mark with 0.1M HCL in 10 ml Volumetric flask. The solution was then filtered through Whatmann filter paper. From the above solution 0.3ml was taken to that 1.5ml of pH 2.8 acetate buffer of pH 2.8 and 1.5ml of 1% BPB dye solution was added. This solution was transferred into separating funnel and 10ml of chloroform was added. Then contents were shaken for 1 min for thorough mixing of the two layers and allowed to stand for 10 min for separation of the chloroform layer. The color complex formed is extracted into the chloroform layer and absorbance was measured against the reagent blank at the wavelength of 420nm. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in Table 9.

Table 9.Results obtained for marketed product

Formulation	Labeled amount	Amount found	% recovery
Pivasta	4 mg	4.01	100.25

Results and Discussion

The development of spectrophotometric methods for the determination of drugs has increased considerable in recent years because of their importance in pharmaceutical analysis.

By OVAT(one variable at a time) all the parameters like buffer, buffer strength, shaking time, dyes, organic solvents were fixed and optimized the method and the color complex formed was extracted into chloroform layer. To determiner λ_{max} the chloroform layer was scanned from 300 to 800nm.and absorption maxima was found to be 420nm. Linear regression of absorbance on concentration gave equation y = 0.010x−0.002 with a correlation coefficient 0.999. The accuracy and reproducibility is evident from the data as results are close to 100 % and the value of % RSD were found to be < 2 %; shows the high precision of the method. Percent Relative standard deviation observed for analysis was < 2 indicating developed method is precise. The low value of LOD and LOQ values shows that the method is sensitive. The high recovery values show that the method is accurate. The absorbance of the drug solution was observed by changing the wavelength by±2nm and no significant difference was observed in the absorbance of the sample, so the method is robust. The low % RSD values in the study of ruggedness show that the method is rugged. All the regression and validation parameters are presented in the Table 10. The results obtained in the assay are in good agreement with the labeled claim as there is no interference from other excipients.

Table 10.Regression and validation parameters of the proposed method for pitavostatin

S.No.	Parameter	Results
Regression Parameters		
1.	Slope	0.010
2.	Intercept	0.002
3.	Standard regression equation Y=bx+c	0.010x+0.002
4.	Correlation coefficient (R^2)	R^2=0.999
Validation Parameters		
1.	Absorption Maxima	420nm
2.	Linearity Range	20-100µg/ml
3.	Accuracy	100.13%
4.	LOD	0.23µg/ml
5.	LOQ	0.42µg/ml

y = bx + c, where x is the concentration of drug in µg/ml; Average of six determinations

Conclusion

Validation parameters complies the developed spectrophotometric method of analysis was found to be simple, sensitive, accurate and satisfactory capable for determination of pitvostatin in tablet formulation with reproducible specific results. The linear concentration range of pitvostatin elaborated method was observed wider. Thus, proposed visible spectrophotometric method is applicable for the quality control and routine analysis of drug in bulk and marketed formulations.

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