

# Simple Titrimetric, Spectrophotometric and Gravimetric Methods for the Assay of Pitavastatin Calcium in a Green Manner

Niranjani S<sup>1</sup>, Venkatachalam K<sup>\*2</sup>

<sup>1</sup>Department of Chemistry, SDNB Vaishnav College for Women, Chromepet, Chennai-600044, Tamilnadu, India

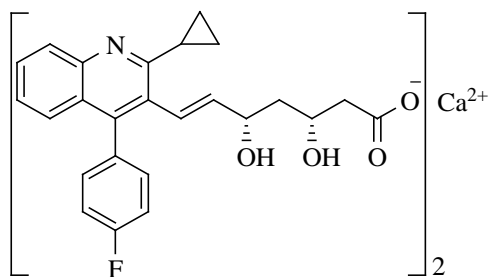
<sup>\*2</sup>Department of Analytical Chemistry, University of Madras, Guindy Campus, Chennai-600025, Tamilnadu, India

## Abstract

The conventional methods which consist of selective and accurate titrimetric, spectrophotometric and gravimetric methods are to develop and validate for the determination of pitavastatin calcium (PTC), an anti-cholesterol drug in pure and commercial dosage forms. Green reagents are used for developing the methods instead of toxic, direct brominating and iodinating reagents. The titration method which was carried out with bromometric mixture (potassium bromide and potassium bromate) is called bromometric titration (method A). Iodometric titration (method B) was done with iodometric mixture (potassium iodide and potassium iodate). Methods C and D involved the addition of a known excess of bromate-bromide mixture to PTC in acid medium followed by the estimation of unreacted bromine by its reaction with excess iodide and the liberated iodine ( $I_3^-$  ion) was either measured at 385 nm or liberated iodine which was reacted with starch followed by the measurement of the blue colored starch-iodide complex at 575 nm by spectrophotometry. In the gravimetric method (method E), 1% oxine was used as a precipitating reagent at the pH 10 and the precipitate was  $Ca(C_9H_6NO)_2 \cdot 2H_2O$ . The obtained results were calculated and validated as per ICH guidelines. The developed simple methods are used for the routine analysis of PTC in quality control laboratories in a green manner.

**Keywords:** bromometry, gravimetry, iodometry, pitavastatin calcium, spectrophotometry, titrimetry.

## INTRODUCTION



**Figure 1: Chemical structure of pitavastatin calcium (PTC)**

Pitavastatin calcium (PTC) is a drug which comes under the group of statin. Its chemical name is monocalcium(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolinyl]-3,5-dihydroxy-6-heptenoic acid with molar mass is 880.98 g/mol. The structure of PTC is shown in figure 1. It lowers cholesterol and low-density lipoprotein (LDL) in both animals and humans. PTC is used to control hypercholesterolemia and for the prevention of cardiovascular disease (CVD). PTC is an odorless and looks like a white powder. It is hygroscopic in nature and very slightly unstable in sunlight. It is freely soluble in pyridine, chloroform, dilute HCl, DMSO, and DMF.

Titrimetric analyses refer to the group of analytical techniques which takes advantage of titers or concentrations of solutions. In bromometry, bromine is employed as an oxidizing agent, because it is reduced quantitatively as the readily oxidized pharmaceutical organic substances in a reaction. In iodometry, an equivalent amount of iodine is liberated when the given sample of an oxidizing agent oxidizes potassium iodide in an acidic medium. The amount of iodine generated by these methods may be conveniently assayed by titration

against a standard sodium thiosulphate solution. In this situation, a point of caution must be observed while KI is being oxidized under a strongly acidic medium so as to avoid simultaneous oxidation of iodide by atmospheric oxygen that may result from high erroneous titer values leading to false estimations. Gravimetric methods are quantitative in nature that is based on determining the mass of a pure compound to which the analyte is chemically related. Literature survey revealed that titration based bromometric methods and spectrophotometric determination using bromate-bromide mixture was carried out for atenolol and timolol [1], simvastatin [2], sumatriptan succinate [3], tamsulosin hydrochloride [4], amlodipine besylate [5], terbinafine HCl, Telmisartan and Ramipril [6], spectrophotometric determination of enalapril maleate [7], Mesalamine using iodometry methods [8], vardenafil and sildenafil with a mixture of potassium iodide and potassium iodate [9], spectrophotometric method for determination of PTC through colour reactions [10-13], Stability indicating studies of PTC [15-17], Fluorimetric method [18], chromatographic methods like HPTLC [19-20], reverse-phase liquid chromatographic methods [21-22], HPLC [23], LC-MS/MS [24-25], electro analytical techniques [26-27]. No one has reported the titrimetric and gravimetric determination of PTC. The aim of the present work is to develop highly selective and rapid methods for the determination of PTC in pure and pharmaceutical dosage forms using titrimetric and gravimetric techniques. Simple titrimetric and spectrophotometric methods were developed and validated for the determination of PTC using Winkler's solution as bromometric and iodometric methods. Determination of calcium from PTC in pharmaceutical dosage forms using oxine (8-hydroxyquinoline) by standard gravimetric method was also carried out.

## MATERIALS AND METHODS

### Instrument

A Systronics model 105 digital spectrophotometer (Systronics, India) which was supplied with 1 cm matched quartz cells was used for all absorbance measurements.

### Reagents and materials

All reagents and chemicals were used of analytical or pharmaceutical grade and all the solutions were prepared in the distilled water.

### Preparation of bromate-bromide mixture

A stock standard bromate-bromide mixture equivalent to 5mM  $\text{KBrO}_3$  and  $\text{KBr}$  (10-fold molar excess) was prepared by dissolving 0.21 g of potassium bromate (S.D. Fine-Chem. Ltd., India) and 1.49 g of potassium bromide (Merck, India) in a 100 ml volumetric flask containing distilled water and directly used for titrimetric methods. Another stock standard  $\text{KBrO}_3$ - $\text{KBr}$  solution equivalent to 100  $\mu\text{g/ml}$   $\text{KBrO}_3$  was prepared by dissolving 10 mg of  $\text{KBrO}_3$  and 100 mg  $\text{KBr}$  in a 100 ml volumetric flask. This solution was diluted appropriately with distilled water to get 30 and 15  $\mu\text{g/ml}$   $\text{KBrO}_3$  as working concentrations used for spectrophotometric method B and method C respectively.

### Preparation of iodate-iodide mixture

In the same way, standard stock solution of iodate-iodide mixture equivalent to 5mM  $\text{KIO}_3$  and 10 fold molar excess of  $\text{KI}$  were prepared by dissolving accurately weighed 0.107g of potassium iodate and 0.830g of potassium iodide in 100ml of calibrated flask containing distilled water which was used for titrimetric method B.

### 10% and 2% potassium iodide solution

10g of potassium iodide (Merck, India) was weighed accurately in 100 ml distilled water and used for titrimetric works. This solution was diluted as 2%  $\text{KI}$  for spectrophotometric methods. Both the solutions were prepared afresh daily.

### 1% starch solution

1g of starch (LOBA Chemie, India) was made into a paste with little amount of water and the paste was slowly poured into 100 ml boiling water, boiled for 2 minutes and cooled. This solution was freshly prepared and used.

### Preparation of 2M HCl

Concentrated hydrochloric acid (Merck, India, Sp. gr. 1.18) was diluted in 250 ml distilled water to get 2M  $\text{HCl}$  and used for all the developed methods.

### Sodium acetate

3 M aqueous solution of sodium acetate was prepared by dissolving sodium acetate trihydrate (Merck, India) in distilled water.

### Preparation of 0.03 M sodium thiosulphate

An accurately weighed 0.75g of sodium thiosulphate pentahydrate in 100ml of deionized water was standardized iodometrically using potassium dichromate and used for the titrimetric methods.

### 1% oxine

1g of 8-hydroxyquinoline was prepared by dissolving in 100 ml of 2N acetic acid.

### $\text{NH}_3$ - $\text{NH}_4\text{Cl}$ buffer (pH =10)

142 ml of ammonia was taken in 400 ml beaker and 17.5 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) salt was weighed accurately

and transferred into beaker and made into 250 ml with water. This solution was adjusted with the value of pH 10.

### Standard solution of pitavastatin calcium

Pharmaceutical grade pitavastatin calcium (purity 99.5%) was gifted from Sun Pharmaceuticals, Cuddalore, India and used as received. A stock standard solution equivalent to 1.0 mg/ml of PTC was prepared by dissolving initially 100mg of PTC in 5ml of DMF and then made up to the mark using distilled water in a 100ml standard flask. This was directly used for titrimetric methods A and B and also for method E. 10ml of this solution was taken in a 100ml volumetric flask and diluted to the mark with water. This solution (100  $\mu\text{g/ml}$  PTC) was used for spectrophotometric method C and diluted to 50  $\mu\text{g/ml}$  for method D. The pharmaceutical formulations Pivasta 1, 2 and 4 (Zydus Cardiva, India) were purchased from a local pharmacy in Chennai and subjected to the analysis.

### The green friendly developed methods

#### Method A – Bromometric Titration

Different volumes of 1.0-10.0 ml standard PTC (1 mg/ml) solution were taken into 100 ml iodine flask and the total volume was made up to 10 ml with distilled water. It was acidified by pouring 5 ml of 2M  $\text{HCl}$  followed by the addition of 10 ml of 5mM bromate-bromide mixture using a graduated pipette. The content was shaken well and kept aside for 10 min with periodic shaking. 5 ml of 10%  $\text{KI}$  was added to that flask followed by the addition of 1ml of 1% starch as an indicator. The liberated iodine was titrated with 0.03M sodium thiosulphate. A blank solution was prepared without adding PTC and followed the same procedure. The amount of PTC in the aliquot was calculated from the amount of bromine consumed.

#### Method B – Iodometric Titration

Different volumes of 1.0-10.0 ml standard PTC (1 mg/ml) solution were taken into 100 ml iodine flask and the total volume was completed to 10 ml with distilled water. It was acidified by pouring 5 ml of 2M  $\text{HCl}$  followed by the addition of 10 ml of 5mM iodate-iodide mixture using a graduated pipette. The content was shaken well and kept aside for 10 min with periodic shaking. 5 ml of 10%  $\text{KI}$  was added to that flask followed by the addition of 1ml of 1% starch as an indicator. The liberated iodine was titrated with 0.03M sodium thiosulphate. A blank solution was prepared without adding PTC and followed the same procedure. The amount of PTC in the aliquot was calculated from the amount of iodine consumed.

#### Spectrophotometric method C (based on the measurement of triiodide ion)

Different volumes (0.25 - 4.0 ml) of standard PTC solution (100  $\mu\text{g/ml}$ ) were transferred into various 10 ml volumetric flask and 4.0 ml as the total volume was filled with distilled water. In each flask, 1 ml of 2M  $\text{HCl}$  followed by 1 ml of 30  $\mu\text{g/ml}$  bromate-bromide mixture (w.r.t  $\text{KBrO}_3$ ) was added. The contents were shaken well and allow for 15 min with occasional shaking. Then, 1.0 ml of 3 M sodium acetate followed by 1 ml of 2% potassium iodide was added to each flask. The total volume was adjusted with water and the absorbance of the resulting triiodide ion was measured at 385 nm after 5 min against water as the blank.

**Spectrophotometric method D (based on the measurement of starch-iodine complex)**

Into a different 10 ml volumetric flask, various aliquots (0.25-4.0 ml) of standard PTC (50 µg/ml) solution were transferred using the micropipette. The total volume in each flask was adjusted to 4 ml by adding distilled water. The content was acidified by adding 1 ml of 2M HCl followed by the addition of 1 ml of bromate-bromide (15 µg/ml in KBrO<sub>3</sub>) solution. The flasks were kept aside for 15 min with periodic shaking. To the content, 1 ml of 2% potassium iodide was added and mixed well. After 5 min, 1 ml of 1% starch was added to each flask as an indicator. The volume was made up to the mark with water and shaken well. The absorbance of the resulting blue colored chromogen was measured at 575 nm against water as the blank after 5 min.

A standard linear graph was prepared by plotting absorbance against the concentration of known PTC and the unknown concentration was examined and computed from the regression equation which was derived using Beer's law.

**Method E – Gravimetric Method**

40 ml of standard PTC solution containing 0.016 g of calcium was taken in a 250 ml beaker and diluted to 50 ml using distilled water. To the content, added 50 ml of ammonium chloride followed by the addition of 20 ml of 1% oxine solution with continuous stirring. The pH of the resulting clear solution was adjusted to 10-11 by dropwise addition of 5ml of 1N ammonia with stirring. The bulky yellow precipitate was digested on a hot water bath for 60 minutes, followed by filtration in a sintered-glass crucible (porosity No. 4), and washed with hot water till the washings were colorless, then kept in an oven dried at 90-110°C for 2 h and cooled in a desiccator containing anhydrous calcium chloride. The resulting dried precipitate (Ca(C<sub>9</sub>H<sub>6</sub>NO)<sub>2</sub>·2H<sub>2</sub>O) was accurately weighed in a digital weighing balance.

**Procedure for tablet analysis**

Fifty tablets each containing 2 mg or 4 mg of PTC were weighed and finely powdered. An amount of the powder equivalent to 100 mg of PTC was accurately weighed and transferred to a 100 ml volumetric flask. This was initially dissolved in 15 ml of DMF (3×5ml) and the content was put in sonication for about 30 min. It was filtered using Whatman No. 42 filter paper in another 100 ml volumetric flask where the first 10 ml portions of the filtrate were discarded. The volume was diluted to the mark with the distilled water and mixed well. This solution was directly assayed by titrimetric methods A and B and also for method E. 10ml of this solution was taken in a 100ml volumetric flask and diluted to the mark with water. This solution (100 µg/ml PTC) was used for spectrophotometric method C and diluted to 50 µg/ml for method D.

**Procedure for placebo blank and synthetic mixture analyses**

A placebo blank containing lactose (10 mg), hydroxylpropylcellulose (20 mg), hypromellose (10 mg), magnesium stearate (25 mg), titanium dioxide (15 mg), triethyl citrate (10 mg) and anhydrous silica (20 mg) was

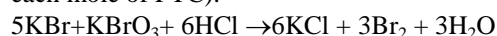
prepared, and 250 mg of the placebo blank was prepared in a 100ml standard flask as described under the procedure for tablet analysis. A synthetic mixture was prepared by adding 100 mg of PTC to the placebo blank (250 mg) prepared above, homogenized and the solution was prepared by following the same procedure. The filtrate was collected in a 100 ml standard flask. The synthetic mixture solution was analyzed by titrimetric methods A and B and then appropriately diluted with distilled water to get 100 µg/ml PTC solution which was used for method C and diluted to 50 µg/ml for method D, respectively.

**RESULTS AND DISCUSSION**

In pharmaceutical compounds, bromate mixture is generally used as an oxidizing or brominating reagent [13-17]. It is also a specific reagent for the bromination of organic compounds, particularly in double bond positions [17-20]. As bromate mixture is a strong oxidizing agent, the only possible reaction between PTC and bromine molecule would be bromination at carbon-carbon double bond present in PTC. Hence, the present study was involved two titrimetric, two spectrophotometric and one gravimetric method for the determination of PTC using in-situ generated bromine /iodine as the green brominating/iodinating agent. The reaction between PTC and excess bromate-bromide mixture or iodate-iodide mixture in acidic medium involved through electrophilic substitution reaction. This was supported by a stoichiometry reaction between PTC and bromine, which was found at 1:2 using titrimetry. The possible reaction mechanism was presented in figure 2. All the developed methods were indirect and involved the determination of the unreacted bromine after allowing the reaction between PTC and a measured amount of bromine. The amount of iodine liberated, by the reaction of the unreacted bromine with potassium iodide was calculated using back titration whereas in spectrophotometry it was measured directly at 385nm in which yellow colored triiodide ion was formed or indirectly at 575 nm by reacting with starch in which blue colored starch-iodine complex chromogen was formed. PTC content in the measured aliquot was calculated from the following equation in titrimetric methods:

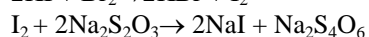
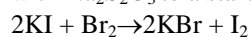
$$\text{PTC amount in mg} = \frac{(B - S) \times M_w \times R}{n}$$

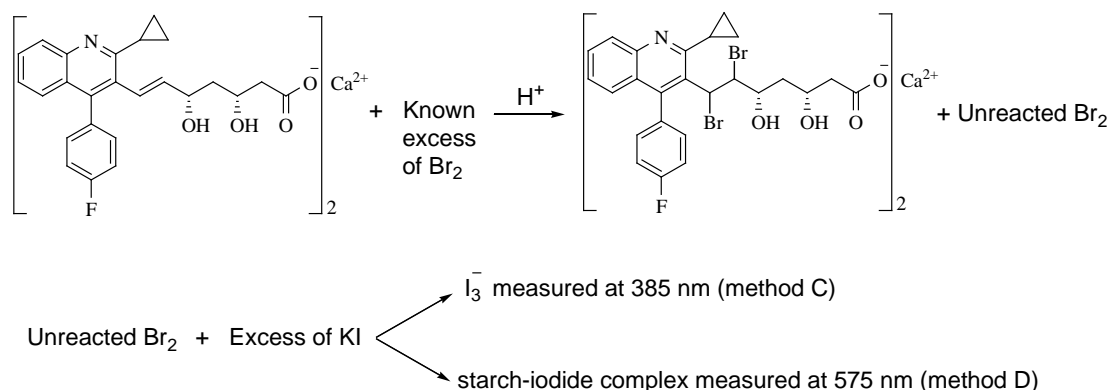
where B is volume of the titrant in the absence of PTC, S is volume of the titrant in the presence of PTC, M<sub>w</sub> is relative molecular mass of PTC, R is molarity of bromate in the bromate-bromide mixture and n is the reaction stoichiometry (number of moles of bromate reacting with each mole of PTC).



PTC + known excess of Br<sub>2</sub> → brominated product of PTC + unreacted Br<sub>2</sub>

unreacted Br<sub>2</sub> + excess of KI → liberated iodine (titrated with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to a starch endpoint)

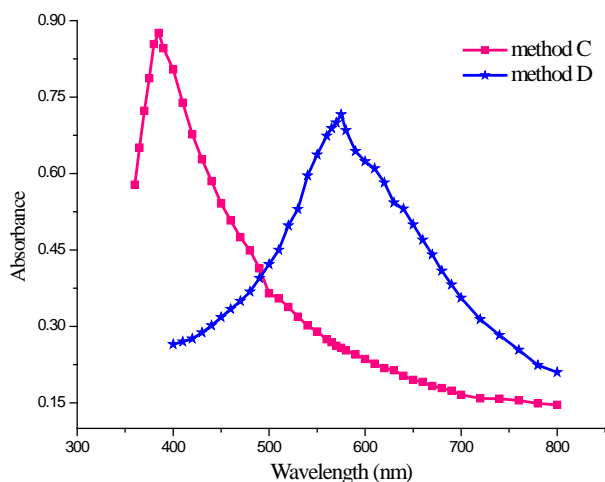




**Figure 2: The proposed mechanism for the bromination of PTC**

Since, 1 mole of PTC reacted with 2 mols, 4 equivalent of bromine were involved under the conditions of the assay.

Therefore, the equivalent weight of PTC is  $220.25 \text{ g} \cdot \frac{1}{4}$  gram molecular weight (i.e.,  $\frac{881}{4} = 220.25 \text{ g}$ ). Therefore, each ml of 5mM bromine consumed in the reaction with PTC is equivalent to  $0.005 \times 220.25 = 1.1010 \text{ g}$ .



**Figure 3: Absorption spectra for method C (40 µg/ml) and method D (15 µg/ml)**

#### Method development in titrimetry

Direct titration of PTC with bromine was not successful due to the slow reaction. So, the indirect titrimetric method, a known excess of bromine or iodine generated *in situ* from bromate-bromide mixture or iodate-iodide mixture was allowed to react with PTC in acidic medium and the unreacted bromine or iodine was subsequently back-titrated by iodometrically after ensuring the completion of the reaction. Among different acids, hydrochloric acid was found to be the best one. At optimum acid concentration, 0.4M HCl in a total volume of 25ml was used and the reaction was completed within 10 min in which there was no stoichiometry effect in the contact time upto 25 min. The reaction stoichiometry was slightly less at lower acid concentration ( $\leq 0.24\text{M}$  HCl) and at a higher acid concentration ( $\geq 0.64\text{M}$  HCl) the regular

stoichiometry was observed. 10ml of 5 mM KBrO<sub>3</sub>-50 mM KBr was used for the investigation. Under the optimized reaction conditions, there was found to be a definite reaction stoichiometry of 1:2 between PTC and bromine within the range of 1-10 mg of PTC.

#### Method development in spectrophotometry

There were two methods in spectrophotometer in which unreacted bromine reacted with potassium iodide liberating iodine. The amount of liberated iodine was found directly at 385 nm in method C. In method D, blue colored chromogen of starch-iodide complex which was obtained by the reaction of iodine with starch was measured at 575 nm (figure 3).

#### Optimization of Reaction Conditions

##### Selection of the solvent

PTC is insoluble in water, ethanol, methanol, acetonitrile, diethyl ether whereas it shows maximum solubility in dimethyl sulfoxide (25 mg/ml) and dimethyl formamide (30 mg/ml). The drug PTC was first dissolved in DMF and then with water to get the maximum solubility.

##### Effect of HCl concentration

Various acids such as hydrochloric acid, sulphuric acid, nitric acid were used in the reaction between PTC and bromate-bromide mixture. By fixing the concentration of PTC and bromate-bromide mixture, different acids were tested in which hydrochloric acid showed better results when compared with other acids. After fixing HCl, the strength of HCl was chosen by varying the concentration from 0.13 M to 0.75 M. The constant absorbance was observed in 0.25M HCl with great sensitivity and constancy.

##### Reaction time and color stability

By fixing every reaction conditions, the effect of reaction time on the reaction between PTC and bromate-bromide mixture in hydrochloric acid medium was tested from 5 to 30 min. The reaction involved in method C in which yellow colored tri-iodide ion was formed was completed in 15 min and stable up to 1 h. The reaction involved in method D in which blue colored starch-iodine complex was formed was completed in 10 min and stable up to 1 h.

##### Role of sodium acetate

The liberation of iodine was stopped by adding sodium acetate in method C because iodine liberation occurred even after 30 min in acidic condition. 1ml of 3M sodium

acetate was fixed in method C whereas 1ml of 1% starch was optimized in method D by studying the effect of sodium acetate and the effect of starch in which other variables were fixed.

#### Assay of calcium ion ( $\text{Ca}^{2+}$ ) using 8-hydroxyquinoline (oxine)

8-hydroxyquinoline, which is also termed as oxine is an effective precipitating reagent used for the precipitation of PTC due to its chelating behavior. It is highly specific,

critical and quantitative method used for the determination of PTC.

#### Results from Gravimetric Data

In gravimetric method, the results generally to calculate were the amount from two experimental measurements which were the mass of sample and the mass of a product of known composition.

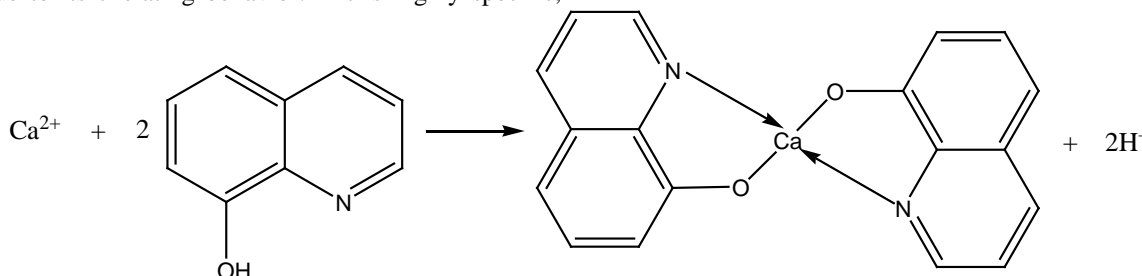


Figure 4: The proposed mechanism for the gravimetric method of PTC

$$\text{Percentage of desired constituent} = \frac{\text{Weight of precipitate} \times \text{gravimetric factor}}{\text{Weight of sample}} \times 100$$

The term gravimetric factor is generally represented as the number of grams of the desired constituent in 1 g of the substance weighed [28].

Weight of the empty crucible = 30.1953g

Weight of the crucible + precipitate = 30.0650g

Weight of the precipitate = 0.1303g

328.318 g of calcium oxinate contains 40.078 g of calcium

One mole of calcium oxinate (328.318 g) contains one mole of calcium atoms (40.078g). Hence,

$$\begin{aligned} \text{Gravimetric factor} &= \frac{\text{calcium}}{\text{calcium oxinate}} \\ &= \frac{40.078}{328.318} \text{ g} \\ &= 0.1221 \text{ g} \end{aligned}$$

$$0.1303 \text{ g of calcium oxinate contains} = \frac{40.078}{328.318} \times 0.1303 \text{ g}$$

$$0.1303 \text{ g of calcium oxinate contains} = 0.01591 \text{ g of } \text{Ca}^{2+} = 15.91 \text{ mg of } \text{Ca}^{2+}$$

Table 1: Gravimetric results of PTC

S.No	Amount of calcium oxinate in mg	Amount of calcium present in calcium oxinate in mg	Taken PTC in mg	Theoretical value in mg	Assay in %
1	130.3	15.91	352	16.01	99.37
2	130.5	15.94	352	16.01	99.56
3	130.8	15.97	352	16.01	99.75
4	131.0	16.00	352	16.01	99.93
5	129.4	15.79	352	16.01	98.62
Average	130.4	15.92	352	16.01	99.44
%SD	0.5549	0.07252			
%RSD	0.4260	0.4560			

$$\text{Percentage of calcium by calculation} = \frac{0.01591 \times 0.12207 \times 100}{0.352} = 0.552\%$$

$$\text{Percentage of calcium theoretically} = \frac{0.01601 \times 0.12207 \times 100}{0.352} = 0.555\%$$

$$\text{Percentage of error} = \frac{0.003 \times 100}{0.352} = 0.85\%$$

**Table 2: Regression and analytical parameters**

Parameter	Method C	Method D
$\lambda_{\max}$ , nm	385	575
Linear range, $\mu\text{g ml}^{-1}$	2.5-40	1.25-20
Molar absorptivity ( $\epsilon$ ), $\text{L mol}^{-1} \text{cm}^{-1}$	$1.95 \times 10^4$	$3.87 \times 10^4$
Sandell sensitivity*, $\mu\text{g cm}^{-2}$	$4.53 \times 10^{-8}$	$2.27 \times 10^{-8}$
Limit of detection (LOD), $\mu\text{g ml}^{-1}$	4.07	2.27
Limit of quantification (LOQ), $\mu\text{g ml}^{-1}$	12.32	6.89
Regression equation, Y**		
Intercept (a)	0.0167	0.1721
Slope (b)	0.2151	0.0363
Standard deviation of a ( $S_a$ )	0.0042	0.0004
Standard deviation of b ( $S_b$ )	0.0002	0.0046
Variance ( $S_a^2$ )	$1.76 \times 10^{-5}$	$1.6 \times 10^{-7}$
Regression coefficient (r)	0.9996	0.9995

\*Limit of determination as the weight in  $\mu\text{g/ml}$  of solution, which corresponds to an absorbance of  $A = 0.001$  measured in a cuvette of cross-sectional area  $1 \text{ cm}^2$  and  $l = 1 \text{ cm}$ . \*\* $Y = a + (bX)$ , Where Y is the absorbance, X is concentration in  $\mu\text{g ml}^{-1}$ , a is an intercept, b is a slope

**Table 3: Intra-day and inter-day precision and accuracy studies**

Developed methods	PTC taken	Intra day (n=7)			Inter day (n=5)		
		PTC found <sup>a</sup>	%RSD <sup>b</sup>	%RE <sup>c</sup>	PTC found <sup>a</sup>	%RSD <sup>b</sup>	%RE <sup>c</sup>
Method A (Titrimetry)	3.0	3.07	0.96	2.33	3.05	1.03	1.67
	5.0	4.92	1.39	1.60	4.89	1.27	2.20
	7.0	7.15	1.04	2.14	7.06	0.94	0.86
Method B (Titrimetry)	3.5	3.54	2.01	1.14	3.58	1.83	2.29
	5.5	5.41	0.82	1.64	5.46	1.07	0.73
	7.5	7.56	1.68	0.80	7.62	1.53	1.60
Method C (Spectrophotometry)	10.0	9.84	2.25	1.60	9.91	2.09	0.90
	20.0	20.16	1.43	0.80	20.20	1.36	1.00
	30.0	29.84	1.07	0.53	29.91	0.99	0.30
Method D (Spectrophotometry)	5.0	5.12	0.83	2.40	5.09	1.11	1.80
	10.0	9.84	1.57	1.60	9.93	1.39	0.70
	15.0	15.21	1.94	1.40	15.17	1.83	1.13

\*In methods A and B, PTC taken/found are in mg and they are  $\mu\text{g/ml}$  in method C and D. <sup>a</sup>Mean value of five determinations, <sup>b</sup>Relative standard deviation (%), <sup>c</sup>Relative error (%)

### Method Validation

All the developed methods have been validated as per ICH guidelines with respect to linearity, sensitivity, precision, accuracy, selectivity and recovery [29].

### Linearity

The stoichiometry of 1:2 (PTC:KBrO<sub>3</sub>) was resulted from method A and method B. Based on these results, spectrophotometric calculations were made. A linear graph was attained between absorbance and concentration of PTC in the range of 2.5-40.0  $\mu\text{g/ml}$  (method C) and 1.25-20.0  $\mu\text{g/ml}$  (method D). The equation  $Y = a + b X$  (where Y = absorbance, a = intercept, b = slope and X = concentration of PTC in  $\mu\text{g/ml}$ ) was used to calculate calibration data such as intercept, slope and correlation coefficient which was drawn by the method of least squares. Sensitivity parameters such as molar absorptivity and Sandell's sensitivity, limit of detection and quantification were calculated as per current ICH guidelines [29] were given in table 2. The obtained results confirmed the sensitivity of the developed methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$\text{LOD} = 3.3\sigma/s \text{ and } \text{LOQ} = 10\sigma/s$$

Where  $\sigma$  is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

### Precision and accuracy

Table 3 explained the intra-day and inter-day precision results as a percentage relative standard deviation (%RSD) and intra-day and inter-day accuracy results as percentage relative error (%RE) for the assay of PTC in pure drug by the developed methods in which three different concentrations of PTC were used. To evaluate repeatability (intra-day precision), the assay procedures were repeated seven times, and percentage relative standard deviation (%RSD) values were obtained within the same day. To evaluate the intermediate precision (inter-day precision), the procedures were repeated five different days. In the same way, intra-day and inter-day accuracy studies were carried out and the results were summarized in table 3. The percentage RSD values were found to be  $< 2.25\%$ , reflecting the usefulness of the methods in routine use, and %RE data was  $\leq 2.4\%$ .

### Robustness and ruggedness

The robustness of the developed methods were evaluated by slightly changing the volume of hydrochloric acid in the

optimal value of titrimetric methods. However, in spectrophotometric methods, the reaction time and volume of HCl were slightly changed. Three different burettes in methods A and B were taken to verify the ruggedness whereas for methods C and D, three different intra and inter analysts were chosen. Both robustness and ruggedness were checked at three different PTC concentrations. The values of %RSD reflected the intermediate precision which was within the satisfactory limits as exposed in table 4.

### Selectivity

The selectivity of the developed methods was analyzed by considering placebo blank and synthetic mixture. The liquid portions within the concentration limits were taken and tested in both titrimetry and spectrophotometry. It was found there was no interference by the inactive ingredients present in the placebo mixture.

All the developed methods were again executed with the synthetic mixture analysis. The obtained results were tabulated in table 5. The inactive ingredients which were present in the synthetic mixture did not interfere with the assay. All these results further confirm that the accuracy, as well as the precision, of the developed methods is good.

### Application studies in pharmaceutical dosage forms

To evaluate the applicability of the developed methods to the assay of PTC in commercially drugs containing PTC, the obtained results were compared with the reference method [10]. The results of the assay revealed good agreement with the declared content and percent found. The results were also compared with those obtained by the official method by applying Student's t-test and F-test at the 95% confidence level given in table 6. The calculated Student's t-test and F-test value did not exceed the tabulated values for five degrees of freedom, suggesting that there was no significant difference between the developed methods and the reference method in respect of accuracy and precision.

### Recovery studies

By using recovery studies, accuracy of the developed methods was validated by the standard addition method. Pre-analyzed tablet's powder, pivasta-2 and pivasta-4 were spiked with API PTC at three concentration levels. The total amount obtained was then determined by the developed methods. From table 7 results, it was found that the results obtained by the developed methods indicated good recovery and there was an absence of interference from the co-formulated substance in the determination.

**Table 4: The results obtained for verifying robustness and ruggedness**

Method A			Method B			Method C				Method D					
PTC taken (mg)	Robustness %RSD Volume of HCl <sup>a</sup> (n=3)	Ruggedness %RSD Inter-burettes(n=3)	PTC taken (mg)	Robustness %RSD Volume of HCl <sup>a</sup> (n=3)	Ruggedness %RSD Inter-Burettes(n=3)	PTC taken (µg/ml)	Robustness %RSD		Ruggedness %RSD		PTC taken (µg/ml)	Robustness %RSD		Ruggedness %RSD	
							Volume of HCl <sup>a</sup> (n=3)	Reaction time <sup>b</sup> (n=3)	Intra-analyst (n=3)	Inter-analyst (n=3)		Volume of HCl <sup>a</sup> (n=3)	Reaction time <sup>b</sup> (n=3)	Intra-analyst (n=3)	Inter-analyst (n=3)
3	0.94	1.24	3.5	1.21	0.89	10	0.56	0.62	1.19	0.62	5	0.55	0.64	1.06	0.61
5	0.82	0.69	5.5	0.94	1.05	20	0.74	1.11	0.97	0.71	10	1.07	0.82	0.91	0.89
7	1.06	0.75	7.5	1.09	0.64	30	1.08	0.93	0.75	0.96	15	0.87	1.20	0.75	1.09

<sup>a</sup>In methods A and B, volumes of 2M HCl varied were 5 ± 1 ml, in methods C and D, 2M HCl (1 ml ± 0.2 ml); <sup>b</sup>In method C, the reaction time was 10 ± 1 min and in method D, 15 ± 1min

**Table 5: Results from synthetic mixture analysis**

Methods	PTC in synthetic mixture taken	% recovered ± SD <sup>a</sup>
Method A (mg)	3.0	101.15 ± 0.75
	5.0	99.62 ± 1.33
	7.0	99.97 ± 0.88
Method B (mg)	3.5	98.69 ± 1.28
	5.5	100.01 ± 0.63
	7.5	101.06 ± 0.79
Method C (µg/ml)	10.0	101.21 ± 0.72
	20.0	99.55 ± 1.06
	30.0	98.97 ± 1.26
Method D (µg/ml)	5.0	100.08 ± 0.34
	10.0	99.74 ± 0.65
	15.0	100.09 ± 0.77

<sup>a</sup>Mean value of five determinations

**Table 6: Results of tablet analysis by the developed methods**

Trade brand	Labelled amount (mg/tablet)	Found (% of label amount $\pm$ SD) <sup>a</sup>				
		Reference Methods [13,14]	Developed methods			
			Method A	Method B	Method C	Method D
Pivasta	2	99.39 $\pm$ 0.033	100.10 $\pm$ 0.63 t=2.04 F=3.68	99.69 $\pm$ 0.76 t=1.49 F=2.95	100.32 $\pm$ 0.41 t=1.91 F=3.05	99.74 $\pm$ 0.62 t=2.57 F=1.98
Pivasta	4	100.25	99.83 $\pm$ 0.62 t=1.87 F=2.12	100.23 $\pm$ 0.54 t=1.56 F=2.08	101.2 $\pm$ 0.69 t=2.09 F=4.1	100.08 $\pm$ 0.48 t=2.58 F=3.05

<sup>a</sup>Mean value of five determinations a Manufactured by Zydus cadila, India; <sup>b</sup>Tabulated t-value at the 95% confidence level for five degrees of freedom is 2.78; Tabulated F-value at the 95% confidence level for five degrees of freedom is 6.39

**Table 7: Results of recovery study by the standard addition method**

Tablets studied	Method A				Method B				Method C				Method D			
	PTC in tablet (mg/ml)	Pure PTC added (mg/ml)	Total PTC found (mg/ml)	Pure PTC recovered <sup>a</sup> , % $\pm$ SD	PTC in tablet (mg/ml)	Pure PTC added (mg/ml)	Total PTC found (mg/ml)	Pure PTC recovered <sup>a</sup> , % $\pm$ SD	PTC in tablet ( $\mu$ g/ml)	Pure PTC added ( $\mu$ g/ml)	Total PTC found ( $\mu$ g/ml)	Pure PTC recovered <sup>a</sup> , % $\pm$ SD	PTC in tablet ( $\mu$ g/ml)	Pure PTC added ( $\mu$ g/ml)	Total PTC found ( $\mu$ g/ml)	Pure PTC recovered <sup>a</sup> , % $\pm$ SD
Pivasta-2	2.05	1	3.04	99.00 $\pm$ 1.25	2.10	1.5	3.58	98.67 $\pm$ 2.13	8	2	9.97	98.5 $\pm$ 2.56	3	2	5.01	100.5 $\pm$ 1.64
	2.05	3	5.03	99.33 $\pm$ 1.04	2.10	3.5	5.65	101.43 $\pm$ 0.52	8	12	20.15	101.25 $\pm$ 0.76	3	7	9.95	99.29 $\pm$ 1.28
	2.05	5	7.06	100.20 $\pm$ 0.92	2.10	5.5	7.54	98.91 $\pm$ 2.37	8	22	29.93	99.68 $\pm$ 1.43	3	12	15.08	100.67 $\pm$ 0.83
Pivasta-4	2.08	1	3.09	101.00 $\pm$ 0.84	1.95	1.5	3.48	102.00 $\pm$ 0.21	6	4	10.03	100.75 $\pm$ 0.94	4	1	4.99	99.0 $\pm$ 2.13
	2.08	3	5.09	100.33 $\pm$ 1.32	1.95	3.5	5.43	99.43 $\pm$ 1.36	6	14	19.96	99.71 $\pm$ 2.38	4	6	10.10	101.67 $\pm$ 0.54
	2.08	5	7.00	99.40 $\pm$ 2.11	1.95	5.5	7.49	100.72 $\pm$ 0.84	6	24	30.11	100.46 $\pm$ 1.08	4	11	15.06	100.54 $\pm$ 1.2

<sup>a</sup>Mean value of four determinations

## CONCLUSION

The presented study developed a simple, inexpensive, precise and accurate method for the determination of pitavastatin calcium in pure and pharmaceutical dosage forms as a green way. The developed methods can be rapidly carried out, simple to perform, and do not require any specific sample treatments. The reagents utilized in the developed methods are cheaper and readily available besides being less time-consuming. Bromate-bromide mixture or iodate-iodide mixture allows the reactions in a green way because hazardous and highly toxic liquid bromine or costly solid iodine is avoided. The developed methods can be used as an alternative method to the reported methods for the routine quality control determination of pitavastatin tablets in laboratories. Since gravimetric analysis is potentially one of the primary methods of measurement, it is sometimes useful for purity evaluation of substances. In gravimetry, comparison of different weighing forms, solubility error, and mechanical loss are important in order to attain an accurate purity evaluation of PTC with a low uncertainty. The developed methods have been demonstrated to be superior to the reported methods with respect to simplicity, selectivity, and cost-effectiveness.

**Conflict Of Interests** - Declared none

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