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Original Article

VALIDATED KINETIC SPECTROPHOTOMETRIC DETERMINATION OF PITAVASTATIN CALCIUM USING ACIDIC PERMANGANATE OXIDATION

MARWA K. A. L. JAMAL

Beirut Arab University, Faculty of Pharmacy, Department of Pharmaceutical Technology
Email: marwa.jamal@bau.edu.lb

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ABSTRACT

Objective: Development and validation of a sensitive, indirect spectrophotometric kinetic method, based on oxidation-reduction reaction, using potassium permanganate, for the quantitative assay of pitavastatin calcium, a cardiovascular drug used for the treatment of hyperlipidemia.

Methods: The developed spectrophotometric kinetic method is based on the ability of potassium permanganate to oxidize Pitavastatin, where, the drug solution is treated with a fixed concentration of permanganate in acidic medium, and after a specified time, the unreacted permanganate is measured at 525 nm. All variables affecting the color development have been investigated and the conditions were optimized. Different kinetic methods, including initial rate, rate constant, fixed time and fixed concentration, were applied for the determination Pitavastatin.

Results: During the course of the reaction, the absorbance values, at 525 nm, related to $KMnO_4$, decreased linearly with increasing the concentration of the drug. The reaction rate obeyed was found to be pseudo-first-order and the kinetic method used was the fixed-time method. The assay of PITA in the concentration range of 16-80 µg/ml, using the fixed time method was successfully determined with a correlation coefficient value of 0.9999. The applicability of the developed method was also demonstrated by the determination of pitavastatin in its pure form and in its pharmaceutical formulation, where, the effect of excipients has also been studied and found to have no effect.

Conclusion: The developed indirect spectrophotometric kinetic method, using the fixed time method, was used for the determination of Pitavastatin in pharmaceutical tablets. This method was simple, accurate and easy to apply for routine assay and in quality control laboratories.

Keywords: Pitavastatin Calcium, Potassium Permanganate, Oxidation, Kinetic methods and Spectrophotometry

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INTRODUCTION

The kinetic spectrophotometric method is an analytical method in which the rate of a reaction is measured and utilized to determine the concentration of drugs [1]. The application of kinetic methods of chemical analysis has gained increasing importance because of their high selectivity and sensitivity. In order to determine the amount of substance being analyzed in solution, it is necessary to measure the rate of decrease of increase of substance concentration. By applying the isolation method, the concentration of one of the substances (indicator substance) changes in the course of the reaction, and the concentration of the remaining substances either do not change or its change is negligible [2]. Various kinetic methods have been applied for the determination of many pharmaceutical formulations [3–7]. Potassium permanganate, a strong oxidizing agent, has been used in an oxidimetric analytical method for the determination of many compounds [8–13].

Pitavastatin Calcium (PIT), chemically known as (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid (fig. 1), is a coenzyme A (HMG-CoA) reductase inhibitors (statins) that inhibits the synthesis of mevalonate, a rate-limiting step in cholesterol level. Competitive inhibition of HMG-CoA reductase by the statins decreases hepatocyte cholesterol synthesis, which results in increase extraction of LDL-C from the blood and decreases circulating LDL-C concentrations [14].

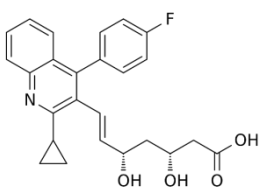

Fig. 1: Pitavastatin calcium

PIT has been determined in its pharmaceutical dosage form by simple spectrophotometric analysis [15–18], titrimetric analysis [19] and liquid chromatographic analysis [20–22]. Degradation pathway for PIT has been developed using LC/MS method [23], or UPLC method [24], also photostability of PIT has been studied [25], In many biological fluids, PIT has been assayed by LC/MS in human plasma [26], urine [27].

Screening the literature, no kinetic method has been reported for the determination of PITA in pharmaceutical preparations. In the present work, a simple and reliable kinetically based spectrophotometric method is proposed for the determination of PITA in its pharmaceutical dosage form Livazo®. The proposed method depends on the oxidation of the drug with potassium permanganate, where the A value of the excess unused permanganate is measured at 525 nm. The applied kinetic methods were validated according to ICH guidelines [28]. The results obtained were compared with those obtained by the RP-HPLC method [29], using the t-test and F-test [30].

MATERIALS AND METHODS

Apparatus

Spectrophotometer

The spectrophotometric measurements were carried out on a Jasco V-530 double beam UV-Vis Spectrophotometer connected to a computer loaded with Jasco UVPC software and an HP Deskjet 5652 printer. The absorption spectra were measured using 1 cm quartz cells. The absorption spectra were recorded on the same spectrophotometer, with 1 cm quartz cells and supported with Jasco Spectra Manager software for GULLIVER Ver. 1.53, and the same printer.

Chemicals

PIT supplied by Algorithm-Lebanon was used as a working standard. An acetic acid solution obtained from SIGMA-ALDRICH was used as solvents for the preparation of the standard solutions. $KMnO_4$ was

supplied by Fluka. The pharmaceutical preparation Livazo® was obtained from Algorithm-Lebanon.

Preparation of standard solutions

Standard stock solutions

PIT standard stock solution (0.16 mg/ml) was prepared by accurately transferring 16 mg into the 100-ml volumetric flask using 3:2, v/v acetic acid as solvent.

$KMnO_4$ standard solution (0.01 mole/l) was prepared by accurately transferring 158 mg into a 100-ml volumetric flask. The powdered was dissolved and diluted to the mark with distilled water.

Calibration graph

Accurate volumes of PIT standard stock solution were transferred into five separate 10-ml volumetric flasks. To each flask, 0.5 ml $KMnO_4$ were added, the flasks were diluted to the mark with water to obtain a calibration set in the range of 16-80 µg/ml. The prepared flasks were kept aside at room temperature, for 30 min. The absorbance values were measured at 525 nm using blank solutions prepared simultaneously. The corresponding regression equation, relating final concentration versus corresponding absorbance values was derived.

Tablet assay

Accurately ten tablets of Livazo 4 mg ® were separately weighed and powdered. Accurate weight equivalent to 16 mg PIT of the finely powdered tablets, were transferred into the 100-ml calibrated flask, 50 ml 3:2, v/v acetic acid was added and the flask was shaken for 15 min, filtered and completed to volume with 3:2, v/v acetic acid. 2-ml of the prepared solution were transferred into 10-ml calibrated flask, 0.5 ml, 0.01 mol/l $KMnO_4$ was added and the resulting solution was mixed and diluted to volume with water. The prepared tablet solution containing 32 µg/ml PIT was put aside for 30 min to allow enough time for the oxidation reaction to occur and the absorbance value was measured at 525 nm against a blank solution prepared similarly.

RESULTS AND DISCUSSION

The absorption spectrum of aqueous potassium permanganate solution in acidic medium exhibited an absorption band at 525 nm. The addition of the studied drug, PIT, to this solution produces a decrease in the intensity of this absorption band (fig. 2). This decrease in the absorption intensity is due to the oxidation of the drug by potassium permanganate in acidic medium, where potassium permanganate is consumed by the drug and is transformed into colorless manganese ions. Since the intensity of permanganate pink color decreases with time; therefore a kinetically based method was developed for the determination of PIT in its pharmaceutical formulation.

Conditions optimization

The various experimental factors affecting the development and stability of the reaction product were studied and optimized. Such factors that were changed individually, include the concentration of the reagents (KMnO4 and acetic acid), temperature and time.

Effect of $KMnO_4$ concentration

Potassium permanganate oxidizes PIT in the presence of acetic acid, where it is reduced into colorless manganese ions. Preliminary experiments were performed to determine the optimal $KMnO_4$ concentration, it was found that the use of $5x10^{-4}$ mol/l gave optimum absorbance values at 525 nm. Thus, when permanganate was reacted with increasing concentrations of PIT in acetic acid medium, there occurred a concomitant fall in the concentration of permanganate as is shown by the decreasing absorbance values (fig. 2). This decrease was proportional to the concentrations of PIT in the calibration graphs.

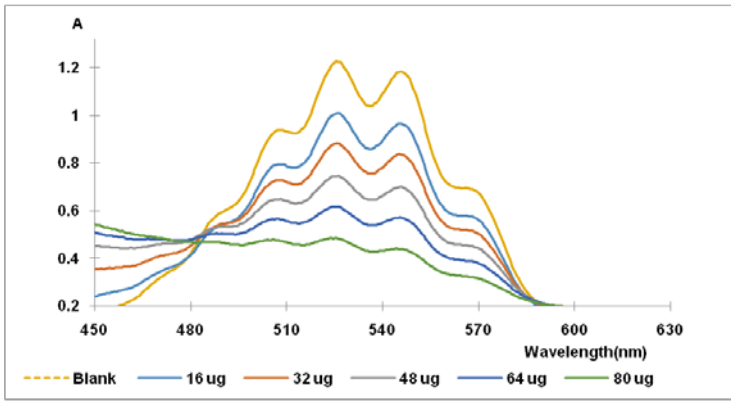

Fig. 2: Absorption spectrum of $5x10^{-4}$ mol/l $KMnO_4$, 16-80 µg/mlof PIT with $5x10^{-4}$ mol/l$KMnO_4$, at room T, for 30 min

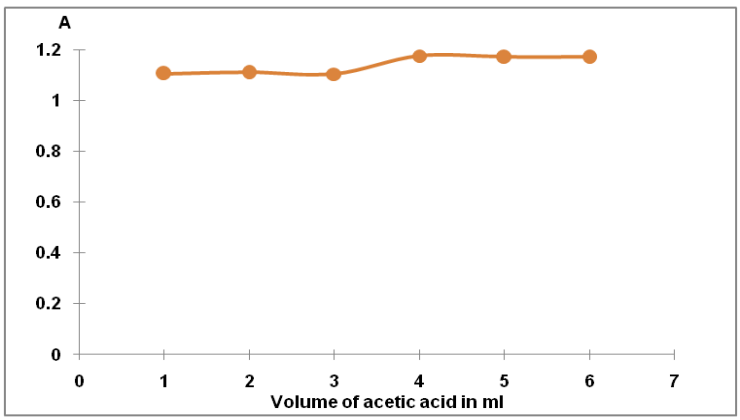

Fig. 3: Effect of volume of 3:2, v/v acetic acid on the absorbance of the reaction product of 32 µg/ml of PIT with 0.5 ml, 0.01 mol/l $KMnO_4$ at 525 nm

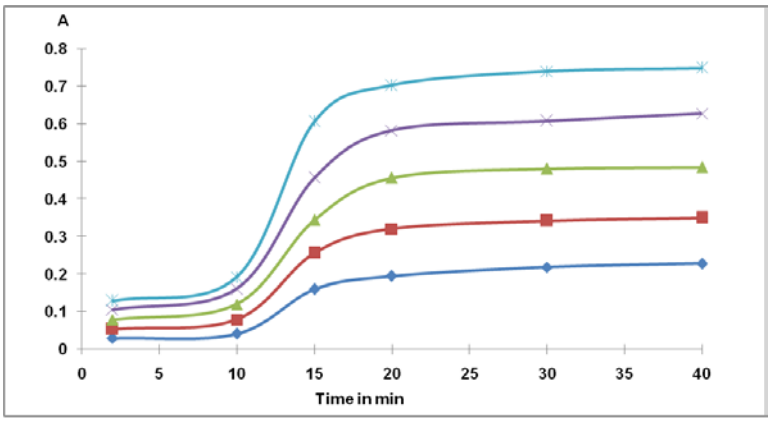

Fig. 3: Absorbance-time curve for the reaction of PIT (16-80 µg/ml) with $KMnO_4$ in acidic medium

Effect of acetic acid concentration

To investigate the effect of acetic acid concentration on the reaction, 1.0-6.0 ml of 3:2, v/v acetic acid were added to a fixed concentration of PIT (32 µg/ml) and $KMnO_4$ ($5x10^{-4}$ mol/l). After 30 min, it was observed that constant absorbance readings were obtained when different volumes of 3:2, v/v acetic acid was used (fig. 3). Hence, acetic acid concentration does not have any effect on the reaction pathway and its volume was maintained constant in all the flasks of the calibration graphs.

Effect of time

The effect of time was also studied, where the reaction was found to be complete and quantitative when the mixture was allowed to stand for 30 min, beyond this standing time and up to 40 min, the absorbance values remained constant (fig. 3).

Effect of temperature

The effect of temperature was also studied and it was found that providing heat to the mixture resulted in the loss of the pink color of permanganate where manganese dioxide brown precipitate appeared. Thus, room T has been chosen as the optimal temperature for the assay of PIT.

Evaluation of the kinetic methods

The quantitative determination of PIT under the optimized experimental conditions outlined above would result in a pseudo-first-order reaction with respect to PIT concentration where the rate will be directly proportional to drug concentration in a pseudo-first-order rate equation as follows:

$$\text{Rate} = K' [C]^n \ldots\ldots\ldots \text{(eq. 1)}$$

Where K′ is the rate constant and n is the order of reaction

Eq. (1) was the basis for several experiments, which were carried out to obtain drug concentration. The rate constant, fixed-concentration, and fixed time methods [2], were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r), and the intercept.

Taking logarithms of rates and concentrations, the above equation becomes:

$$\text{Log (rate)} = \log \frac{\Delta A}{\Delta T} = \log k' + n \log [C] \ldots\ldots\ldots \text{(eq. 2)}$$

Where A is the absorbance, t is the time in seconds and K is the pseudo-first-order rate constant.

From the absorbance-time plot, for different PIT concentration (fig. 3), the reaction rate may be estimated by the variable-time method, measured as $\frac{\Delta A}{\Delta T}$, where A is the absorbance and t is the time in seconds. Regression of log (rate) versus log [C] gave the regression equations:

Log (rate) =-2.46+1.10 log C (fig. 4)

With a correlation coefficient (r) = 0.990 and with, $k' = 3.46 \times 10^{-3}\ s^{-1}$ and the order of reaction is first order (n ~1).

It can be concluded that the quantitative determination of PIT under the optimized experimental conditions outlined above would result in a pseudo-first-order reaction with respect to PIT concentration where the rate will be directly proportional to drug concentration in a pseudo-first-order rate equation (fig. 4).

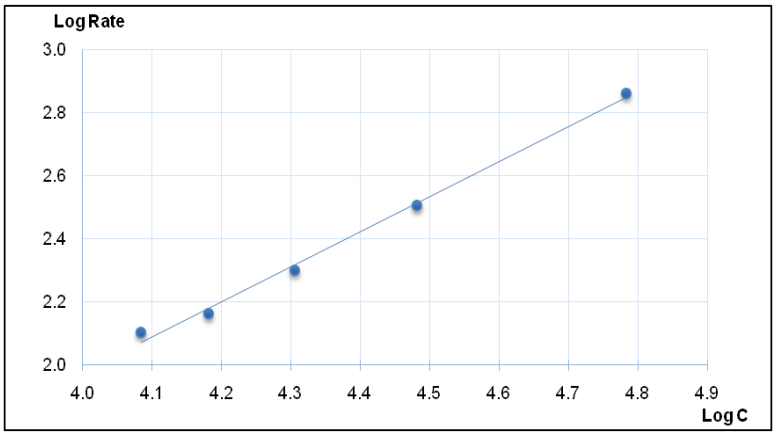

Fig. 4: Log rate versus log C of PIT

Rate-constant method

Graphs of Log (Absorbance) versus time, over the concentration range of (1.65 x10^{-5}-8.24 x10^{-5} mole/l/) (fig. 5), were plotted and all appeared to be rectilinear. The obtained graphs were used to calculate the first-order rate constants corresponding to different PIT concentrations. These constants were calculated from the slopes multiplied by (-2.303) and are presented in table 1.

Regression of [C] versus k′ gave the equation:

$$k' = -0.056 + 118.86\ C \quad r = 0.835$$

The value of the correlation coefficient (r) indicates poor linearity, which is probably due to changes in the rate constant (k ')

Initial rate method

The initial rate of the reaction was determined from the absorbance-time plots (fig. 3), by measuring the slopes of the initial tangents to the absorbance-time curves at different concentrations of the investigated drugs. The values of the calculated slopes are summarized in table 2.

Regression of the initial rate between two and ten minutes versus [C] gave the equations:

$$\upsilon = \Delta A/\Delta t = -0.00017 + 101.7\ C,\ r = 0.995 \text{ for PIT, } k' = 101.7\ min^{-1}$$

The values of the correlation coefficients (r) indicate poor linearity, the high wale of the rate constant Kindicates that the first step is too fast and not rate determining.

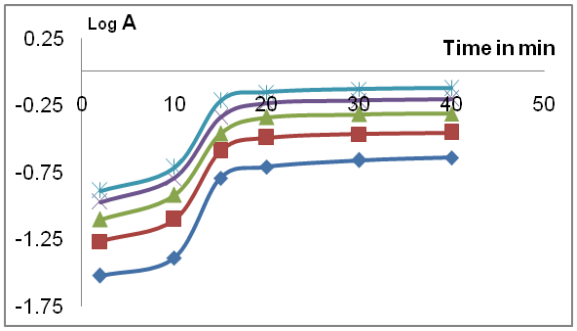

Fig. 5: Values of k′, calculated from the slopes of log A versus time graphs multiplied by (-2.303), four different concentrations of PIT

Table 1: These constants were calculated from the slopes multiplied by (-2.303) and are presented

Drug	K′(s^{-1})	C, [mol/l]
PIT	-0.0562	1.647 x10^{-5}
	-0.0488	3.294 x10^{-5}
	-0.0491	4.941 x10^{-5}
	-0.0477	6.588 x10^{-5}
	-0.0470	8.235x10^{-5}

Table 2: Values of slopes calculated for different concentrations of PIT at room T with 0.5 ml 0.01 mol/l potassium permanganate

Drug	C, mol/l	Slope, s^{-1}
PIT	1.647 x10^{-5}	0.00579
	3.294 x10^{-5}	0.00853
	4.941 x10^{-5}	0.01177
	6.588 x10^{-5}	0.01487
	8.235x10^{-5}	0.01760

Fixed concentration method

The kinetic study of PIT was followed up at different concentration levels by recording the time in seconds required for the absorbance to reach a preselected value. This preselected value was chosen as it gave the widest calibration range. The reciprocals of time (1/Δt) were plotted versus the initial concentration of the PIT and the equations of calibration graphs are given in table 3. The values of the correlation coefficients indicate poor linearity, which is considered a disadvantage.

Table 3: Value of (1/Δt) taken at fixed absorbance* for different concentrations of PIT at room T with 0.5 ml, 0.01 mol/l potassium permanganate

Drug	Δt (min)	1/Δt(s^{-1})	C, mol/l	Regression equation	Regression coefficient (r)
PIT	15.00	0.00111	1.647 x10^{-5}		
	13.30	0.00125	3.294 x10^{-5}	1/Δt = 0.000751+11.79 C	r = 0.960
	12.00	0.00139	4.941 x10^{-5}		
	10.90	0.0153	6.588 x10^{-5}		
	10.00	0.0167	8.235x10^{-5}		

*The preselected absorbance values for PIT is 0.2

Fixed time method

Reaction rates were determined for different concentrations of PIT at a preselected fixed-time. Calibration graphs of absorbance versus concentration of PIT were established at fixed times of 15, 20, 30 and 40 min in the concentration range of 1.647 x10^{-5}-8.235x10^{-5} mol/l (16-80 µg/ml). The calculated regression equations are assembled at table 4. It is clear at 15 min the value of the correlation coefficient is poor and that indicates that the kinetic reaction did not reach stability. However, at 20 min the slopes increase with time and the most suitable values for the correlation coefficient (r) and the intercept (a) were obtained for a fixed-times of 30 min (table 4). This was therefore chosen as the most suitable time interval for measurement.

Table 4: Regression equations at different fixed times for PIT at 15, 20, 30 and 40 min in the concentration range of 1.647 x10^{-5}-8.235x10^{-5} mol/l (16-80 µg/ml)

Drug	Time (min)	Regression equation*	Correlation coefficient (r)
PIT	15	A= 0.0372+0.00681 C	0.9940
	20	A= 0.0682+0.00798 C	0.9997
	30	A= 0.00818+0.00818 C	0.9999
	40	A= 0.09225+0.00824 C	0.9997

*Regression equation calculated using concentrations in µg/ml.

Method validation

Statistical evaluation of the regression line (table 5) gave small values for the standard deviation of residuals ($S_{y/x}$), the standard deviation of the slope S_b. These small values reflect the high reproducibility of the proposed method. The limit of detection LOD and quantitation LOQ were calculated using the statistical treatment of calibration data. These statistical data challenged for the robustness of the fixed-time method under the optimum reaction condition for carrying it in the assay of PIT.

Table 5: Analytical parameters for the determination of PIT using the fixed-time method

Parameters	PIT
λ_{nm}	525
Linearity range (µg/ml)	16-80*
a (intercept)	0.08495
b (slope)	0.008175
r (correlation coefficient)	0.9999
S_a	0.00346
a/S_a	24.54
S_b^2	4.25 x10^{-9}
S_b	6.52 x10^{-5}
F	15714
Sig F	1.119 x10^{-6}
$S_{y/x}$	3.26 x10^{-5}
LOD (µg/ml)	1.26
LOQ (µg/ml)	4.23

*5 points, at 16-µg/ml intervals

Pharmaceutical application

Assay of PIT in its pharmaceutical formulation Liv`azo® 4 mg using the developed method was successfully applied without interference from the excipients. Excellent percent recovery and RSD demonstrated the applicability of the method. t-test and F-test values were also calculated using a standard reference method [29]. The student t-test and variance ratio-F-test values at 95% confidence level did not exceed the theoretical values [30], indicating no significant difference in accuracy and precision of the proposed kinetic spectrophotometric method and the RP-HPLC-method [29].

Table 6: Determination of PIT in pharmaceutical preparations using the fixed-time method and RP-HPLC method

Drug	Pharmaceutical preparation	Mean recovery±SD[a] RSD %[b] Er %[c]	
		Fixed-time method	RP-HPLC method
PIT	Livazo®4 mg	100.32±0.552 0.744 1.59 t** = 1.49 F** = 3.25	101.08±0.996 0.985 1.08

[a]mean±SD for the five determinations, [b]% Relative standard deviation, [c]% Relative error, **Theoretical values of t-and F-at P = 0.05 are 2.13 and 6.93, respectively.

CONCLUSION

The low cost of the kinetic spectrophotometric technique makes it highly desirable for the determination of Pitavastatin in pharmaceutical formulations. Although the poor selectivity of the proposed methods, yet it is more simple, time-saving and more economic compared with HPLC and other sophisticated chemometric methods. These facts encourage to apply such methods in drug quality control laboratories.

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Nil

AUTHORS CONTRIBUTIONS

Marwa k. Al Jamal designed, planned, and performed the experiments and the measurements. She processed the experimental data along with the calculations, drafted the manuscript, designed the fig. and interpreted the results.

CONFLICT OF INTERESTS

Declared none

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