ISSN : 0974-7419 Volume 10 Issue 1

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(1) 2011 [62-70]

Kinetic spectrophotometric method for determination of lisinopril in bulk material and tablets using alkaline potassium permanganate

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Received: 3rd July, 2010 ; Accepted: 13th July, 2010

ABSTRACT

A simple and sensitive kinetic spectrophotometric method has been developed and validated for the determination Lisinopril in bulk drug and in its pharmaceutical tablets. The method was based on the oxidation of lisinopril (LSP) with alkaline potassium permanganate resulting in a green colored reaction product. The reaction was monitored spectrophotometrically by measuring the absorbance of the reaction product at 610 nm. The factors affecting the reaction were studied and optimized. The stoichiometry of the reaction was determined and the reaction pathway was postulated. The activation energy of the reaction was calculated and found to be 29 k joule $mole^{-1}$. The initial rate and the fixed time methods were used for constructing the calibration graphs. The analytical performance of both methods was validated, and the results were satisfactory, however, the fixed time method was superior. The method was directly applied to the determination of LSP in bulk drug and by standard addition method in its commercial pharmaceutical tablets. The mean tablet label claim percentage was found to be 100.2 ± 0.25 (n=4). Statistical comparison of the results with those obtained by a reference method showed excellent agreement between the accuracy and precision of the two methods. The proposed method has a great value in its application to the analysis of LSP in quality control laboratories.

KEYWORDS

Lisinopril;
Kinetic spectrophotometry;
Initial rate method;
Fixed time method;
Standard addition;
Pharmaceutical analysis.

INTRODUCTION

Lisinopril dihydrate (LSP), (S)-1-(N_2-(1-Carboxy-3-phenylpropyl)-L-lysyl)-L-proline dihydrate, is a drug of the angiotensin converting enzyme (ACE) inhibitor class that is primarily used in treatment of hypertension, congestive heart failure, heart attacks and also in preventing renal and retinal complications of diabetes[1]. Historically, LSP was the third ACE inhibitor, after captopril and enalapril[2]. There are number of properties that distinguish LSP from other ACE inhibitors. It is hydrophilic, has long half-life and tissue penetration and is not metabolized by the liver[3].

Because of the therapeutic importance of LSP, there is much interest in its determination for the purpose of pharmaceutical quality control. The British Pharmacopoeia[4] recommends a potentiometric titration method for determination of LSP bulk material and an HPLC method for the tablets. The other analytical methods that have been reported for this purpose were the sub-

ject of many reports. The methods that have been reported before 1991 were reviewed by Ip. *et al*[5]. The methods that have been reported thereafter include gas chromatography[6-8], HPLC[9-17], capillary electrophoresis[18-23], electrochemical methods[24-25] immunoassays[26-28], spectrofluorimetry[11,30,31] and spectrophotometric methods[11,30-36]. Based on the number of reports cited in these articles, spectrophotometry is considered the most widely used technique. This is attributed to its inherent simplicity, low cost and wide availability in most quality control laboratories. Furthermore, kinetic spectrophotometric methods are becoming of great interest in the field of pharmaceutical analysis[37-40]. The application of these methods offers some specific advantages such as improved selectivity, avoiding the interference of the colored and/or turbid background of the samples, possibility of avoiding the interference of the other active ingredients present in the commercial products and reduction of the analysis time when the analytical reaction requires long time for completion.

LSP is weakly absorbing light in the UV region[5] and thus it is subjected to interference from excipients and/or impurities if direct spectrophotometric method is used. Therefore, a proper chromogenic reaction should be employed. In general, chemical reactions are characterized by two ways[41] the first one is the final equilibrium composition and the second is the speed with which the reactions reach to an end. The first type of these two approaches could successfully be applied if the system is thermodynamically favorable. The second type may be used if the system is thermodynamically unfavorable, and it is usually achieved by using the information obtained from the rate of the reaction[42]. The concentration of the substance under investigation may affect a certain reaction rate and this relationship could be analytically useful. No attempt has been made regarding the kinetic spectrophotometric determination of lisinopril. Therefore, the development of a kinetic spectrophotometric method for determination of LSP was very essential.

The present study describes, for the first time, the development of a simple and sensitive kinetic spectrophotometric method for the determination of LSP. The method involved the oxidation of LSP with alkaline potassium permanganate at room temperature and monitoring the reaction by measuring the absorbance at 610 nm. The initial rate constant and fixed time methods were tried and after their validation, the fixed time method was adopted for the direct determination of the drug in bulk material and by standard addition method in case of the commercial tablets.

EXPERIMENTAL

Apparatus

Double beam UV-Visible spectrophotometer (model UV-160IPC, Shimadzu., Japan) with matched 1-cm quartz cells was used throughout the present work.

Chemicals and dosage form

Lisinopril Dihydrate was obtained from Zeneca Ltd., UK. Zestril® tablets (20 mg LSP per tablet, lot LI 374) were obtained from local Saudi market. Potassium permanganate (Merck, Schuchardt, Munich, Germany) was prepared as an aqueous stock solution of 1×10^{-2} M. Sodium hydroxide (Aldrich Co Ltd., Gillingham-Dorst, Germany) was prepared as 4 M aqueous solution. Water used was distilled or demonized water. All other solvents and other chemicals used throughout this study were of analytical grade.

Preparation of standard and sample solutions

Preparation of stock standard solution

An accurately weighed amount (100 mg) of standard LSP was dissolved in about 70 ml of water in a 100-ml calibrated flask. The resulting solution was completed to volume with water. This stock solution (1 mg ml^{-1}) was diluted with water to obtain working standard solution containing 100 μg ml^{-1}.

Preparation of the tablet sample solution

Twenty tablets were weighed and finely powdered. A quantity of the mixed powder equivalent to 100 mg of anhydrous LSP was transferred into a 100-ml calibrated flask and about 70 ml of water were added. The flask was swirled and sonicated for 5 min, completed to volume with water and filtered rejecting the first portion of the filtrate. Portions of the filtrate were diluted with water to obtain solutions resembling the working standard concentration (100 μg ml^{-1}).

Effect of potassium permanganate concentration

The effect of $KMnO_4$ concentration was studied over

the range 3.5×10^{-4} – 3.5×10^{-3} M using constant concentrations of lisinopril and NaOH for a fixed time interval and plotting a graph of absorbance of the reaction product *versus* $KMnO_4$ concentration and the one corresponding to the maximum absorbance was chosen.

Effect of sodium hydroxide concentration

The concentration of sodium hydroxide was varied in the reaction mixture from 0.05–0.5 M keeping all the other parameters (including the concentration of the drug, concentration of $KMnO_4$, final volume of the reaction mixture and the time interval) constant. The absorbance values of the colored product was measured against a similarly treated reagent blank each time and plotted against NaOH concentration and the one resulting in the maximum absorbance value was chosen.

General recommended procedures and data treatment

Two identical sets of the reaction mixture were prepared by transferring varying volumes (1.0 – 6.0 ml) of LSP working standard solution (100 μg ml^{-1}) into 10-ml calibrated flasks followed by 1 ml of NaOH (4 M). Two milliliter of $KMnO_4$ solution (1×10^{-2} M) were added to each flask, immediately completed to volume with water and mixed. One of the two sets was monitored at 25±1°C and the other at 70±1°C by measuring the absorbance values at wavelength of 610 nm as a function of time against reagent blank treated similarly.

Regarding the tablet sample, a standard addition calibration curve was constructed. Different but gradually increasing volumes of the LSP working standard solution (100 μg ml^{-1}) were added to a fixed volume of the working sample solution in each of a series of 10-ml calibrated flasks except one. The procedure was completed as explained above.

The kinetic data that has been recorded were transformed to the Slide Write Plus software, version 5.011 (Advanced Graphics Software, Inc., CA, USA) for curve fitting, regression analysis, and statistical calculations. The initial rate (K) of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance-time curve. The calibration curve was constructed by plotting the logarithm of the initial rate (log K) of reaction versus logarithm of the concentration (log C) of the drug. Alternatively, the calibration curve was constructed by plotting the absorbance measured after a fixed time of 20 min *versus* the concentration of the drug.

Determination of the molar ratio of the reaction

The Job's method of continuous variation was employed in this study. Master equimolar solutions (2.5×10^{-3} M) of each of LSP and $KMnO_4$ were prepared separately. Series of 10-ml total volumes containing mixtures of LSP and the analytical reagent ($KMnO_4$), were made up comprising different complementary ratios [0:10, 1:9, ….. 9:1, 10:0] of LSP:$KMnO_4$ in 10-ml calibrated flasks each containing 1.0 ml of 4 M NaOH. The solutions were further manipulated as described under the general recommended procedures and data treatment. The absorbance of the reaction mixture *versus* the molar ratio of LSP:KMnO4 was plotted from which the stoichiometry of the reaction was determined.

Evaluation of the kinetic methods

After conducting the experiments under the optimum reaction conditions, the data obtained from the experiments carried out at different time intervals were attempted to fit on one of the models corresponding to the initial rate or the fixed time kinetic methods. The most suitable method was selected taking into consideration linearity, the range of applicability, the sensitivity, the correlation coefficient (r) and the intercept.

Applications

After the method was established, added amounts of bulk lisinopril powder were analyzed to find the % recovery. Commercial tablets of lisinopril were also analyzed by the proposed standard addition method after preparation of the sample solution as described under preparation of the tablet sample solution.

RESULTS AND DISCUSSION

Involved reaction and the absorption spectrum

Potassium Permanganate ($KMnO_4$) is a strong oxidant, and its oxidation of the organic compounds is well known to be pH dependent. During the course of the reaction, the valance of manganese changes and the intermediate ions have been suggested as participating oxidants. The species that are considered as potential

oxidants depend on the nature of the substrate and the pH of the medium[40]. $KMnO_4$ has not been used previously for the spectrophotmetric determination of LSP. In the present study, the reaction of alkaline $KMnO_4$ with LSP was investigated, and it was found that LSP is vulnerable by $KMnO_4$. This was evident from the decrease in the violet color (λ_{max} at 525 nm) of $KMnO_4$ accompanied by the appearance of a green-colored reaction product (λ_{max} at 610 nm). The formation of this colored product was monitored spectrophotometrically. The absorption spectrum of the reaction product is shown in Figure 1.

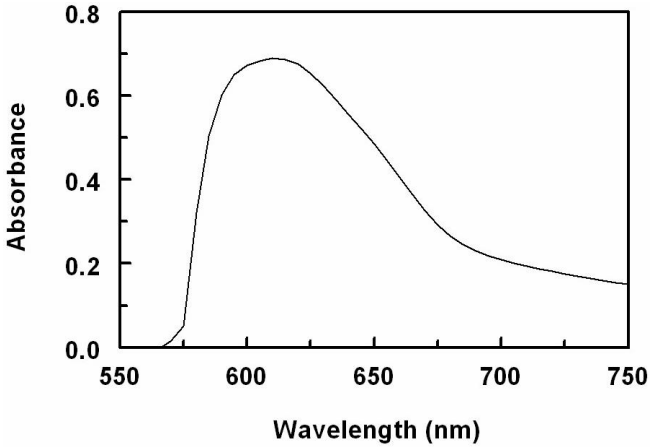

Figure 1 : Absorption spectrum of the reaction product between LSP (1.48×10^{-4} M) and $KMnO_4$ (2.0×10^{-3} M) in the presence of NaOH (0.4 M). The reactions were carried out at room temperature (25±2°C) for 20 min.

Optimization of the reaction conditions

The factors affecting the reaction conditions (concentration of $KMnO_4$, alkalinity, temperature, and the diluting solvent) were studied by altering each variable in turn while keeping the others constant. When the intensity of the developed color was recorded as a function of the concentration of $KMnO_4$, it was found that the absorbance values were proportional to the concentration of $KMnO_4$ up to a concentration of 1.5×10^{-3} M after which it was almost constant (Figure 2). Based on this finding, $KMnO_4$ at a final concentration of 2.0×10^{-3} M was added to the reaction mixture to ensure its presence in excess; and this concentration was kept constant in all the subsequent experiments.

As far as the alkalinity is concerned, best results were obtained when NaOH was used. The optimum concentration of NaOH was obtained in the same manner as with the $KMnO_4$ and it was found to be a final concentration of 0.4 M (Figure 3). The reaction was carried out at room temperature (25±2 °C) and at an elevated temperature of 70±2 °C, using a thermostatically controlled water bath (Grant Type GD100, England). The rate of color development was higher at the elevated temperature (Figure 4), with obviously ultimate better sensitivity. Nevertheless, the subsequent experiments were carried out at room temperature to simplify the analytical procedure, by avoiding the use of extra equipment of controlled-temperature water bath, on the expense of lower limit of detection. This decision was based on the ICH guidelines for validation of analytical procedures[43], which do not include the limit of detection (LOD) as a part of the validation.

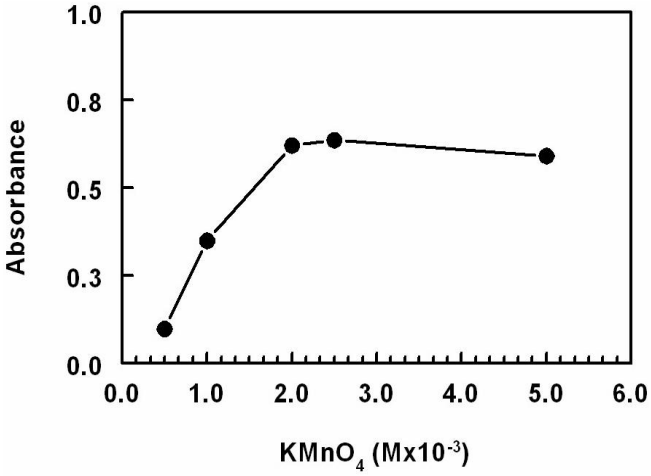

Figure 2 : Effect of $KMnO_4$ concentration on the reaction of $KMnO_4$ with LSP (1.23×10^{-4} M) in presence of NaOH (0.4 M). The reactions were carried out at room temperature (25 ±2°C) for 20 min.

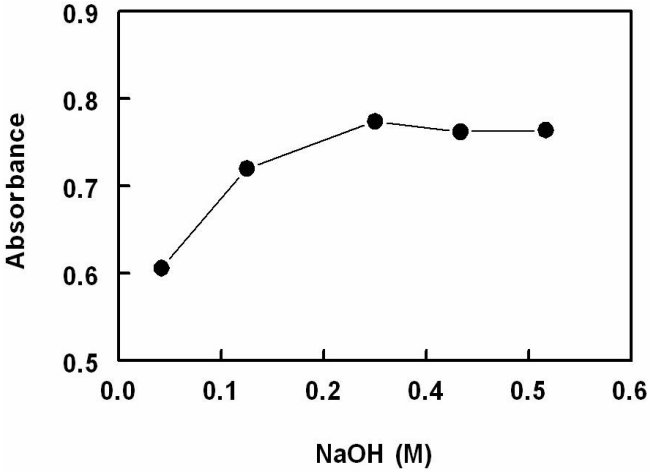

Figure 3 : Effect of NaOH concentration on the reaction of $KMnO_4$ (2.0×10^{-3} M) with LSP (1.23×10^{-4} M). The reactions were carried out at room temperature (25±2°C) for 20 min.

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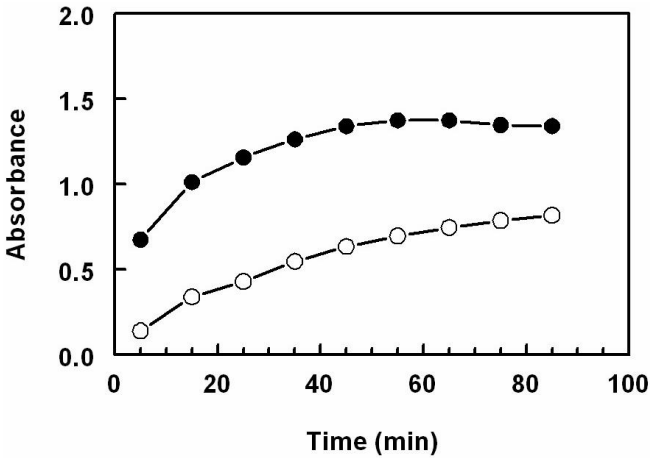

Figure 4 : Effect of temperature on the reaction between alkaline $KMnO_4$ (2×10^{-3} M) and lisinopril (9.86×10^{-5} M) with time at room temperature, 25±2°C (O) and at 70±2°C (●).

Regarding the most appropriate solvent for dilution, water was selected being the most convenient available solvent besides its excellent role in keeping the oxidizing reagent in ionic form.

Stoichiometry and mechanism of the reaction

The stoichiometry of the reaction between $KMnO_4$ and LSP was investigated by Job's method (continuous variation method). The results indicated that the ratio of LSP: $KMnO_4$ was 1:3 (Figure 5A). Based on this ratio, the reaction pathway was postulated to proceed as shown in Figure 5B. These findings were in agreement with the reported results for an oxidation procedure involving alkaline $KMnO_4$ [44].

Evaluation of the kinetic methods of the reaction

The rate-constant method

Under the optimum reaction conditions, the absorbance-time curves for the reaction of varying concentrations of LSP: 2.47×10^{-5} M – 1.48×10^{-4} M (10 – 60 μg ml^{-1}) with a fixed concentration of $KMnO_4$ (2.0×10^{-3} M) in presence of NaOH (0.4 M) were generated (Figure 6). From the graphs, the rate of the reaction was found to be dependant on LSP concentration [LSP]. It increased as the [LSP] increased indicating that the reaction rate obeys the following equation:

$$K\ (rate) = k'\ [LSP]^n \qquad (1)$$

Where k' is the pseudo-order rate constant of the reaction. The initial reaction rates were determined from the slopes of the variable-time method measurements (as $\Delta A/\Delta t$), where A is the absorbance and t is the time

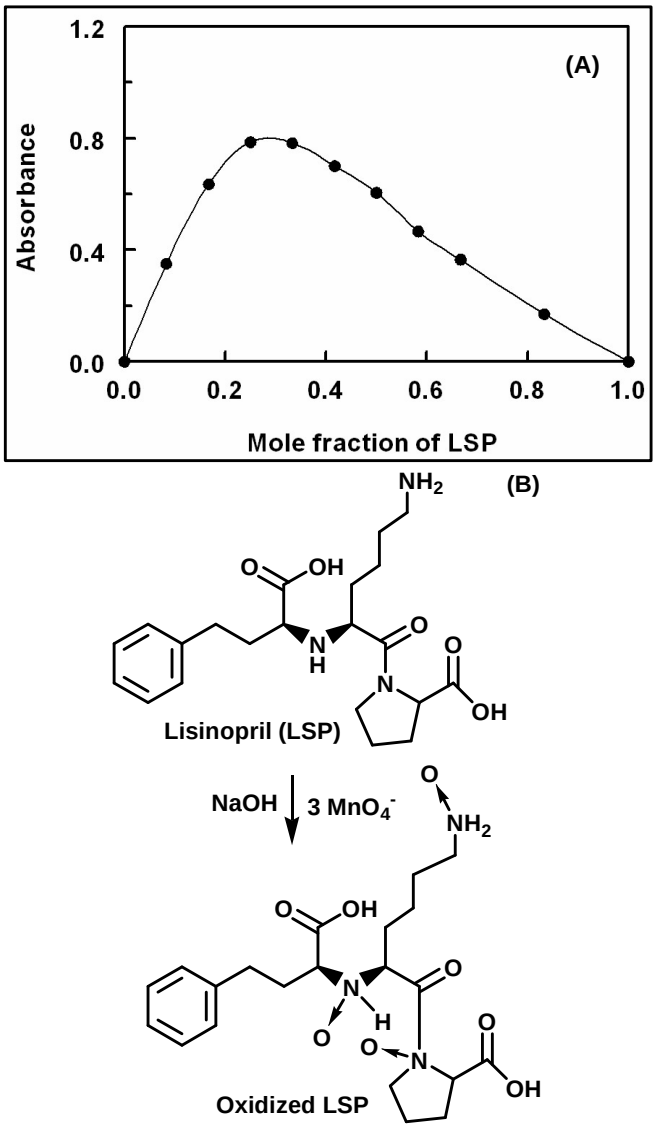

Figure 5 : Job's plot for absorbance *versus* molar ratio of LSP:$KMnO_4$ (A) and the scheme for the reaction pathway postulated for the reaction of $KMnO_4$ with LSP in presence of NaOH (B).

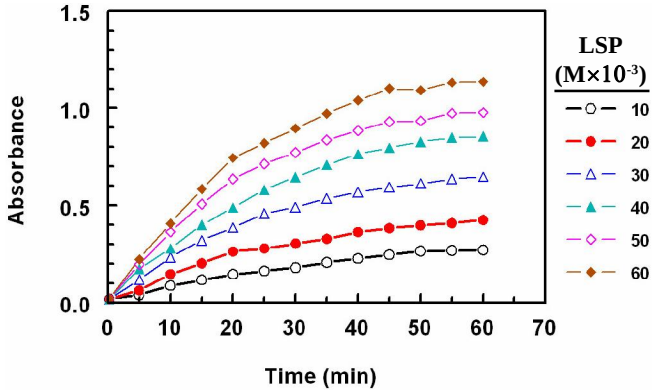

Figure 6 : Absorbance versus time curves for the reaction products between alkaline $KMnO_4$ (2.0×10^{-3} M) and different concentrations of LSP.

in seconds. By determining the logarithms of the rates and the corresponding lisinopril concentration, equation (1) is transformed into:

Log K= Log (ΔA/Δt) = Log *k′* + n Log [LSP] (2)

A plot of log K (log ΔA/t) *versus* log [LSP] resulted in a straight line (Figure 7).

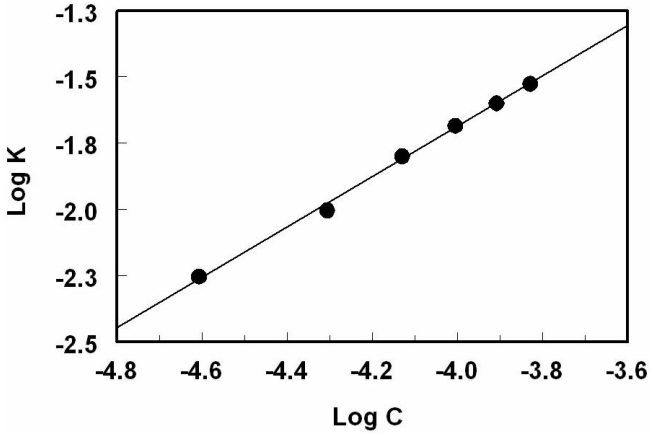

Figure 7 : A plot of log K (rate) *versus* log [LSP] concentration obtained from the reaction of $KMnO_4$ (2.0×10^{-3} M) with varying concentrations of LSP in presence of NaOH (0.4 M). The reactions were carried out at room temperature (25±2°C) for 20 min.

Alternatively, the regression analysis of the data of Log (K) *versus* Log [LSP] by the least squares method yielded the following equation:

Log K (rate) =1.9321 + 0.9127 Log [LSP] (r = 0.9987)

From which the rate constant (*k′*) was found to be: 85.5 sec^{-1} and the reaction is first order (n = 0.9127 i.e. approximately 1) with respect to LSP. However under the optimized reaction conditions, the concentrations of $KMnO_4$ and NaOH were in large excess compared to that of LSP in the reaction solution, and therefore, the reaction was regarded as a pseudo-first order reaction. The LOD and LOQ values were calculated according to ICH guidelines[43] and they were found to be 2.22 μg ml^{-1} (5.47×10–^{6} M) and 11.2 μg ml^{-1} (2.76×10^{-5} M) respectively.

The fixed-time method

In this method, the absorbance values of the reaction mixtures containing varying amounts of LSP were measured at pre-selected fixed time intervals. Calibration plots of the absorbance *versus* the concentration of LSP were established after allowing the reactions to proceed for the pre-selected fixed time intervals. The regression equations, correlation coefficients (r), limits of detection (LOD) and limits of quantification (LOQ) are given in TABLE 1. The widest linear ranges were obtained at 5, 10, 15 and 20 min reaction times. However the best linearity (r = 0.9993), the lowest standard deviation of intercept, the lowest standard deviation of slope, the lowest LOD and the lowest LOQ were found when the reaction time was fixed at 20 min.

TABLE 1 : Analytical parameters for the proposed fixed time spectrophotometric method for determination of LSP.

Reaction time (min)	Linear range (μg ml^{-1})	Intercept	Standard deviation of intercept	Slope	Standard deviation of slope	Correlation coefficient	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
5	25-260	0.00033	0.01203	0.0039	0.00031	0.9877	10.2	30.8
10	10-150	0.02493	0.01044	0.00654	0.00027	0.9967	5.3	16.0
15	8-100	0.02340	0.00956	0.00590	0.00025	0.9987	5.3	16.2
20	6-80	0.02367	0.00871	0.01203	0.00022	0.9993	2.4	7.2
25	5-75	0.03220	0.01847	0.01347	0.00047	0.9975	4.5	13.7
30	4-65	0.03547	0.01984	0.01466	0.00051	0.9976	4.5	13.5
35	3-60	0.04647	0.02447	0.01578	0.00063	0.9968	5.1	15.5
40	2-55	0.05973	0.02452	0.01663	0.00063	0.9971	4.9	14.7
45	2-50	0.06467	0.02207	0.01746	0.00057	0.9979	4.2	12.6
50	2-50	0.09247	0.03225	0.01703	0.00083	0.9953	6.2	18.9
55	2-50	0.09000	0.03114	0.01777	0.00080	0.9960	5.8	17.5
60	2-50	0.10093	0.02980	0.01767	0.00077	0.9962	5.6	16.9

Comparing the *rate-constant method* and the *fixed-time method* (at 20 min), the later was found better with respect to the correlation coefficient (r) and the LOQ while the LOD values for both methods were comparable. In addition, the fixed-time method is convenient and easier to perform. Therefore, the fixed time method at 20 min as a pre-selected fixed time was preferably used in the present study.

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The apparent rate constant and the energy of activation

The absorbance-time curves were generated at two temperatures (25±2°C and 70±2 °C) using fixed concentration of LSP (7.4×10^{-5} M) and $KMnO_4$ (2.0×10^{-3} M) (Figure 4). From these curves the apparent rate constant (k) for each of the two temperatures was calculated. The activation energy, defined as the minimum kinetic energy that a molecule possess in order to undergo a reaction, was determined based on Arrhenius equation:

$$\text{Log } k = \log A - Ea / 2.303\ RT$$

Where k is the apparent rate constant, A is the frequency factor, Ea is the activation energy, T is the absolute temperature (°C + 273), and R is the gas constant (1.987 calories degree^{-1} mole^{-1}). Since only two temperatures (T_1 and T_2) were used in the present study, then the above equation could be written as:

$$\text{Log } k_1 = \log A - Ea / 2.303\ RT_1 \quad (1)$$

$$\text{Log } k_2 = \log A - Ea / 2.303\ RT_2 \quad (2)$$

Substracting equation (1) from equation (2) will yield the following equation:

$$\text{Log } (k_2 / k_1) = Ea\ (T_2 - T_1) / 2.303R\ T_2T_1 \quad (3)$$

The values of (k) obtained at each of the two temperatures were substituted in equation (3) together with the values of T_1 (298 °K), T_2 (343 °K) and the gas constant R (1.987). From these data, the activation energy was calculated and found to be 29 k joule mole^{-1}.

Validation of the proposed methods

Selectivity, linearity, and sensitivity

Under the optimized conditions the reagent blank treated similarly showed absorption characteristics similar to those of $KMnO_4$ with λ_{max} at 525 nm while the λ_{max} of the reaction mixture was found at 610 nm indicating a selective oxidation reaction between lisinopril and KMnO4. It is also worth mentioning that the proposed kinetic method was performed in the visible region away from the UV region where tablet excipients, which might be co-extracted from the dosage forms, may interfere.

Fixed time method was adopted for measuring the absorbance values of the reaction mixtures. Linear relationship with good correlation coefficient (r = 0.9993, n = 5) was found between the absorbance of the reaction mixture at $\lambda_{610\,nm}$ *versus* LSP concentration in the range of 10 μg ml^{-1} (2.47×10^{-5} M) – 60 μg ml^{-1} (1.48×10^{-4} M); the data is presented in TABLE 1. The regression equation of the calibration curve obtained was: Y = 0.02367 (± 0.00871) + 0.01203 (± 0.00022) X; where X and Y are the LSP concentration and the absorbance value at λ_{610nm} respectively. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the ICH guidelines for validation of analytical procedure[43]. The calculation was based on the standard deviation of the intercept and the slope of the calibration curve using the formula: LOD or LOQ = κσ/S, where κ = 3.3 for LOD and 10 for LOQ σ is the standard deviation of the intercept, and S is the slope of the calibration curve. LOD and LOQ were found to be 2.4 μg mL^{-1} (5.92×10^{-6} M) and 7.2 μg mL^{-1} (1.77×10^{-5} M), respectively. These values of LOD and LOQ indicate good sensitivity of the proposed kinetic specrophotometric method.

Precision and accuracy

Intra-day and inter-day precisions were studied treating concentration levels ranging from 20 μg ml^{-1} to 60 μg ml^{-1} of LSP in replicates according to the general procedure and analyzed by the proposed method during the same day and on four different days. The mean % recovery values found ranged from 97.80 – 103.82 (with RSD ranging from 0.38 – 1.61) for the intra-day and from 99.17 – 102.22 (with RSD ranging from 1.27 – 3.61) for the inter-day recovery study (TABLE 2).

TABLE 2 : Precision for the fixed-time spectrophotometric method for determination of LSP

Concentration added (μg ml^{-1})	Recovery (± RSD)[a]			
	Intra-assay		Inter-assay	
	Concentration found (μg ml^{-1})	Recovery (±RSD)[a]	Concentration found (μg ml^{-1})	Recovery (±RSD)[a]
20	19.56	97.8±1.09	20.41	102.06±2.97
30	31.15	103.82±1.64	30.66	102.22±3.61
40	39.44	98.59±0.57	39.80	99.51±2.48
50	50.28	100.57±0.38	49.58	99.17±2.90
60	59.41	99.02±1.54	59.78	99.63±1.27
Mean		99.96±2.38		100.52±1.49

[a] Values are mean of three determinations.

Obviously, the small values of RSD especially for the intra-day recovery studies confirmed the high precision of the proposed method.

Similarly, the accuracy of the proposed method was determined by the recovery studies of different concentrations of lisinopril covering the linear range of the method within a single day (intra-day) and on four different days (inter-day). The % recovery values were calculated using the formula: (found experimental concentration/nominal concentration×100). The mean recovery values were found to be 99.96% ± 2.38% and 100.52%±1.49% from intra-day and inter-day analysis respectively, indicating the accuracy of the proposed method (TABLE 2).

Robustness and ruggedness

In order to measure the extent of the method robustness, some of the critical reaction conditions such as the $KMnO_4$ concentration, NaOH concentration, the room temperature and the selected fixed time of the reaction were changed while keeping the other parameters unchanged. The changes made were within the range of 1-10% of the optimum recommended conditions. The results revealed that the method was robust for these small changes and the results were not significantly different.

The ruggedness of the method was evaluated by carrying out the analysis of LSP using the proposed method independently on different days on the analysis of a series of LSP samples. The results obtained were comparable indicating the ruggedness of the proposed method.

Application of the proposed methods

Bulk material of LSP powder was analyzed to find the % recovery using a simple fixed time method of 20 min. reaction time. A calibration curve was constructed, as described under *general recommended procedures and data treatment*. Absorbance values obtained were plotted versus the concentration of LSP in $\mu g\ ml^{-1}$. Commercial tablets of LSP (Zestril® 20 mg tablets, lot LI 374) were also analyzed employing a standard addition method. This modification was necessary and successful for overcoming the interference occurred from the tablet excipients. The results obtained for the pure sample and the commercial tablets were compared with those obtained by a reference method[30] by calculating the t- and F-values. The mean % recovery values obtained by the proposed method and the reference method were 100.2±0.25 and 101.1±0.70 respectively. t = 1.76 (tabulated t-value = 2.78), F = 7.84 (tabulated F-value = 19.25). These calculated t-value and F-value indicate that the proposed method is accurate and precise.

CONCLUSION

A simple kinetic standard addition spectrophotometric method was developed, for the first time, for determination LSP in bulk material and in tablet formulations. Both initial rate and fixed time kinetic methods can easily be applied although the fixed time method was found to be superior in the present study. The method does not require pre-treatment of the samples nor tedious procedures for the analysis. Furthermore, the proposed method does not require expensive instruments and/or critical analytical reagents. The analytical results demonstrated that the proposed method is suitable for the accurate quantification of LSP in quality control laboratories.

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