

# Kinetic Spectrophotometric Method for the Determination of Ramipril in Pharmaceutical Formulations

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## ABSTRACT

The objective of this research was to develop a kinetic spectrophotometric method for determination of ramipril in pure form and pharmaceutical formulations. The method was based on the reaction of carboxylic acid group of the drug with a mixture of potassium iodate (KIO<sub>3</sub>) and potassium iodide (KI) in aqueous medium at room temperature. The reaction is followed spectrophotometrically by measuring the increase in absorbance at 352 nm as a function of time. The initial-rate and fixed-time methods were adopted for constructing the calibration curves. Both the calibration curves were linear in the concentration range of 10.0–70.0 µg mL<sup>-1</sup>. The detection limits were 0.02 µg mL<sup>-1</sup> and 0.15-µg mL<sup>-1</sup> for initial rate and fixed time methods, respectively. The proposed methods are validated statistically and through recovery studies. The point and interval hypothesis tests have been performed confirming that there is no significant difference between the proposed methods and the reference method. The experimental true bias of all samples is less than ± 2%. The methods have been successfully applied to the determination of ramipril in tablets and capsules.

**KEYWORDS:** initial-rate method, fixed-time method, ramipril, pharmaceutical formulations, spectrophotometry, validation.

## INTRODUCTION

Ramipril, 2-[N- [(S)-1-ethoxy carbonyl-3-phenyl propyl]-L-alanyl]-(1S, 3S, 5S)-2-azabicyclo [3,3,0]-octane-3-carboxylic acid [CAS: 87333-19-5] is a prodrug<sup>1</sup> which is rapidly hydrolyzed with the cleavage of an ester group through hepatic metabolism forming an active metabolite ie, rami-prilat. This prodrug itself is a poor inhibitor of angiotensin converting enzyme (ACE) but its active metabolite has a higher affinity for ACE, thus blocking the conversion of the angiotensin I to the angiotensin II, a highly potent vaso-

constrictor and there by leading to a reduction in vasopressor activity and a decrease in peripheral vascular resistance.<sup>2,3</sup> The drug is officially listed in British Pharmacopoeia,<sup>4</sup> which describes a potentiometric titration procedure for its assay in bulk and dosage form.

The estimation of ramipril along with hydrochlorothiazide in binary mixture was performed by derivative compensation technique<sup>5</sup> as well as zero crossing derivative technique.<sup>6,7</sup> Two spectrophotometric methods have been reported for the assay of drug in commercial dosage forms, which are based on the formation of ternary complex of the drug with Cu (II) and eosin<sup>8</sup> and Fe (III) and ammonium thiocyanate.<sup>9</sup> The drug content in pharmaceutical formulations has been determined spectrophotometrically in visible region based on the charge transfer reaction of ramipril with  $\pi$ -acceptors such as 7,7,8,8,tetracyanoquinodimethane and p-chloranilic acid and subsequently measuring the absorbance at 840 and 520 nm, respectively.<sup>10</sup> Two extractive spectrophotometric methods<sup>11</sup> have been recommended based on extractive ion-pair complex of the drug with picric acid and bromocresol green. Potassium permanganate oxidizes ramipril in alkaline medium resulting in the formation of bluish green colored complex peaking at 610 nm.<sup>12</sup> The quantitation of ramipril<sup>13</sup> has been done by spectrophotometry and fluorimetry utilizing the reaction of the drug with 7-fluoro-4-nitrobenzo-2-oxo-1,3-diazole which exhibits maximum absorbance at 460 nm, and maximum fluorescence intensity at 530 nm after excitation at 465 nm. The literature is still poor in analytical procedures based on kinetic spectrophotometry for the determination of drug in pharmaceutical formulations. Therefore, there is a need for a simple kinetic spectrophotometric method for the determination of ramipril in commercial dosage forms.

This paper describes the development and validation of a kinetic spectrophotometric method for the determination of ramipril in pharmaceutical formulations. The method is based on the reaction of -COOH group of ramipril with a mixture of iodide-iodate in aqueous medium resulting in the formation of yellow color, which absorbs maximally at 352 nm. The absorbance increases with time and therefore, two calibration procedures ie, initial-rate and fixed time methods are adopted for the determination of ramipril in commercial dosage forms.

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## MATERIALS AND METHODS

### Apparatus

A Shimadzu UV-visible 1601 spectrophotometer (Model no1601, Kyoto, Tokyo, Japan) with matched quartz cells was used for all spectral and absorbance measurements.

A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature for the formation of degraded ramipril.

### Standard and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade.

Ramipril reference standard (Batch No. TR001FO2, purity 99.58%) was kindly provided by Dr. Reddy's Laboratories Limited (Andhra Pradesh, India). Standard solution of ramipril ( $0.5 \mu\text{g mL}^{-1}$ ) was prepared by dissolving 50 mg in 100 mL distilled water. This solution was used to prepare calibration curve and quality control samples. Quality control samples were prepared at three concentration levels of 20, 40 and  $70 \mu\text{g mL}^{-1}$ . The solution is stable for at least 1 week if kept stored in a cool and dark place.<sup>14</sup> Pharmaceutical formulations of ramipril such as Hopace-1.25 (Cardicare, Bangalore, India), Ramipres-1.25 (Cipla, Mumbai, India), Ramace-1.25 (AstraZeneca, Mumbai, India) and Variace-1.25 (Win Medicare, New Delhi, India) were obtained from commercial sources.

A 0.15 M KI (Fluka Chemie AG, Switzerland) solution was freshly prepared in distilled water. The solution was standardized by the recommended procedure.<sup>15</sup> A 0.1 M  $\text{KIO}_3$  (Fluka Chemie AG, Switzerland) was also freshly prepared in distilled water.

### Recommended Procedures for the Determination of Ramipril

#### Initial Rate Method

Aliquots of 0.2–1.4 mL reference standard solution of ramipril ( $0.5 \text{ mg mL}^{-1}$ ) were pipetted into a series of 10 mL standard volumetric flasks. In each flask, 2.2 mL of 0.1 M  $\text{KIO}_3$  followed by 3.3 mL of 0.15 M KI were added and then diluted to volume with distilled water at  $25 \pm 1^\circ\text{C}$ . The contents of each flask were mixed well and the increase in absorbance at 352 nm was recorded as a function of time against the reagent blank prepared similarly. The initial rate of the reaction ( $\nu$ ) 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 of reaction ( $\log \nu$ ) vs the logarithm of the molar concentration of the ramipril ( $\log C$ ). The amount of the drug was obtained either from the calibration graph or the regression equation.

#### Fixed-Time Method

In the fixed time method, the absorbance at 352 nm of each sample solution was measured at a preselected fixed time against a reagent blank prepared similarly. The calibration curve was constructed by plotting the absorbance against the final concentration of the drug. The amount of the drug in each sample was computed either from calibration curve or regression equation.

### Procedure for the Determination of Ramipril in Pharmaceutical Formulations

The sample solution containing ramipril at a concentration of  $0.5 \text{ mg mL}^{-1}$  was prepared. The powdered contents of 10 capsules of 1.25 mg (or 5.0 mg) strength were obtained by gentle tapping and the hard gelation shells were discarded. The ramipril was extracted into  $5 \times 10$  mL portions of methanol by shaking. Similarly, 10 tablets of 1.25 mg (or 5.0 mg) strength were taken in 10 mL methanol and left for 10-minute for complete dispersion and then extracted into  $5 \times 10$  mL portions of methanol by shaking and filtered through Whatmann No. 42 filter paper. The residue was washed well with methanol for complete recovery of the drug. The methanol was evaporated to dryness under vacuum and the remaining drug was dissolved in an appropriate volume of distilled water to give a concentration of  $0.5 \text{ mg mL}^{-1}$ . The assay was completed following the recommended procedures for the determination of ramipril.

### Procedure for Reference Method<sup>8</sup>

Aliquots of 0.2–1.0 mL of 0.1% ramipril were pipetted into a series of 50.0 mL separating funnels. Into each separating funnel, volume of solution was adjusted to 10.0 mL with distilled water and 3.0 mL of 0.2% Cu (II) sulfate solution followed by 1.0 mL of 0.1% eosin solution were added successively and mixed well. The funnels were shaken vigorously with  $3.0 \times 3.0$  mL portions of chloroform for 1.0-minute, and then allowed to pass the organic layer through anhydrous sodium sulfate into a 10.0 mL standard volumetric flask. The volume of chloroform layer was made up to 10.0 mL and the absorbance was measured at 535 nm against the reagent blank prepared similarly. The amount of the drug in a given sample was computed either from calibration graph or regression equation.

### Validation

The proposed method has been validated for specificity, linearity, precision, accuracy, and recovery.

### Specificity

Samples of composite of ramipril capsules (1.25 mg) were subjected to stress conditions of light, heat, acid, base and oxidants. All stressed samples were analyzed for ramipril content and compared with an unstressed time zero reference solution. The time zero solution provided a reference assay value for the unstressed product. The content of degradation in the stressed and control samples was calculated relative to this assay value.

### Linearity

For evaluation of linearity, determination of ramipril was done at seven concentration levels: 10.0, 20.0, 30.0, 40.0, 50.0, 60.0 and 70.0  $\mu\text{g mL}^{-1}$ . Each concentration was analyzed for five times.

### Precision and Accuracy

Three concentrations within the linearity range were selected: 20.0, 40.0, and 70.0  $\mu\text{g mL}^{-1}$ . Five sample solutions of each concentration were prepared and analyzed within one day. This assay was too repeated for five consecutive days. The intra and inter precision and accuracy were also determined by analyzing the quality control samples that were tested five times in one day and on five consecutive days.

### Recovery Studies

To study the accuracy of the proposed method and to check the interference from excipients used in the formulations, recovery experiments were performed by standard addition method. For this, 4.0 mL (or 6.0 mL) of sample solution (0.5  $\text{mg mL}^{-1}$ ) was transferred into a 100.0 mL volumetric flask followed by 4.0 mL (or 8.0 mL) of reference standard solution (0.5  $\text{mg mL}^{-1}$ ) and volume was completed to the mark with distilled water. The nominal value was determined by the proposed procedures.

## RESULTS AND DISCUSSION

### Spectral Studies

The spectrum of reference pure drug of ramipril in aqueous solution shows two absorption bands at 210 nm and 234 nm (Figure 1 A). The addition of aqueous solutions of KI and KIO<sub>3</sub> to the drug solution causes change in the absorption spectrum with new characteristic bands peaking at 298 and 352 nm (Figure 1 C). The reagent blank solution containing KI and KIO<sub>3</sub> shows one peak at 275 nm and a negligible absorbance (0.06) at 352 nm when measured against distilled water as a reference (Figure 1B). The absorbance obtained at 352 nm is higher than the absorbance at 298 nm,

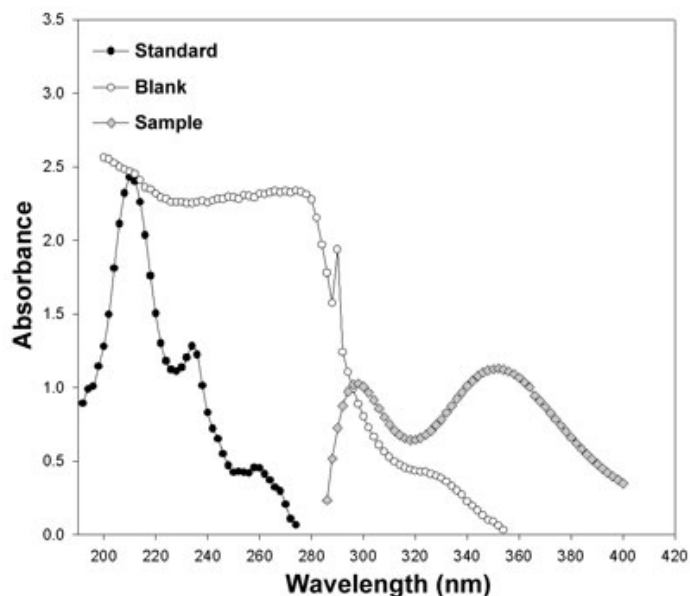
thus showing higher sensitivity at 352 nm. Therefore, the absorbance measurements for the determination of ramipril were made at 352 nm. The equilibrium is attained in ~50-minute. Therefore, a kinetically based spectrophotometric method was developed for the quantitative determination of ramipril by measuring the increase in absorbance at 352 nm as a function of time.

### Mechanism of the Color Reaction

It has been suggested that water-soluble acidic compounds liberate iodine from a solution containing both KIO<sub>3</sub> and KI according to the reaction<sup>16</sup>:



A yellowing of the solution reveals the occurrence of the reaction. The yellow color of the solution is due to the formation of I<sub>2</sub>, which immediately converted into triiodide ions in the presence of iodide ions ( $\text{I}_2 + \text{I}^- \rightarrow \text{I}_3^-$ ) exhibiting absorption maxima at 290 nm and 360 nm.<sup>17</sup> Ramipril, a water-soluble ACE inhibitor, possesses -COOH group in its moiety and hence undergoes a similar reaction with iodide-iodate mixture resulting in the evolution of iodine. The liberated iodine immediately reacts with potassium iodide to give triiodide ions showing absorption maxima at 298 nm and 352 nm. The reaction sequence is shown in



**Figure 1.** Absorption spectra of (A) 1.0 mL standard ramipril solution (0.05%) in distilled water, (B) blank solution: 3.3 mL of 0.15 M potassium iodide, 2.2 mL of 0.1 M potassium iodate, (C) sample solution: standard ramipril (50.0  $\mu\text{g mL}^{-1}$ ) + 3.3 mL of 0.15 M potassium iodide and 2.2 mL of 0.1 M potassium iodate. Each set is diluted to 10 mL with distilled water.

Figure 2. The confirmatory test for the presence of iodine in the final solution of the drug is established by the blue color, which appears on addition of starch solution.<sup>18</sup>

### Optimization of Variables

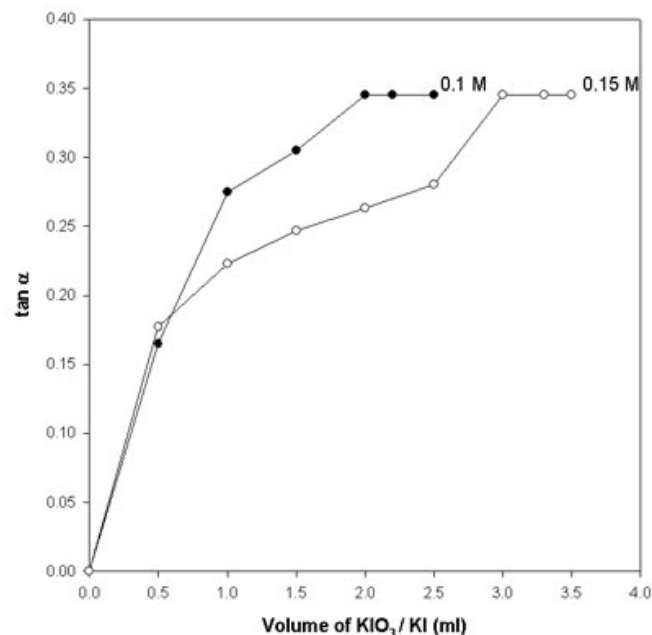
Preliminary experiments were performed to determine the optimum conditions of the variables used in the estimation of ramipril. The influence of the variables on the rate of reaction was studied and optimized. The optimum values of the variables were maintained throughout the experiment.

#### Effect of the Concentration of Potassium Iodate

The effect of the concentration of potassium iodate was studied by treating 50.0  $\mu\text{g mL}^{-1}$  ramipril with 3.3 mL of 0.15 M KI and varying volumes (0.5–2.5 mL) of 0.10 M  $\text{KIO}_3$ . The kinetic slope ( $\tan \alpha = dA/dt$ ) of the absorbance-time curves obtained at different volumes of  $\text{KIO}_3$  showed that the initial rate of reaction was increased with increasing volume of  $\text{KIO}_3$  and became constant at 2.0 mL; above this volume, the initial rate of reaction remained unchanged (Figure 3A). Therefore, 2.2 mL of  $\text{KIO}_3$  (0.10 M) was used in all measurements.

#### Effect of the Concentration of Potassium Iodide

The influence of the volume of 0.15 M KI on the rate of reaction was investigated in the range of 0.5–3.5 mL. The initial rate of reaction (Figure 3B) was increased with increasing volume of KI and became constant at 3.0 mL;



**Figure 3.** Effect of the volume of (A) 0.10 M potassium iodate, (B) 0.15 M potassium iodide on the initial rate of reaction.

beyond this volume, the initial rate remained constant. Therefore 3.3 mL of 0.15 M KI was recommended for determination procedures.

### Analytical Data

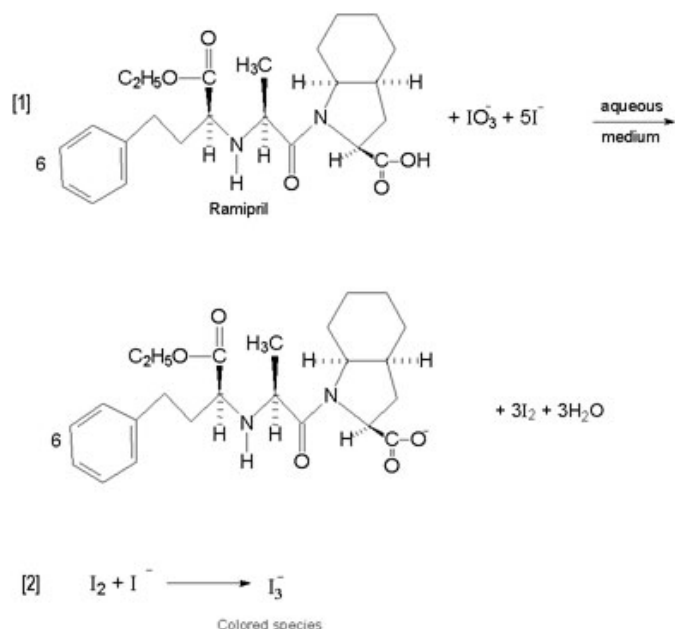
Under the optimized experimental conditions, the assay of ramipril was performed in presence of excess concentration of  $\text{KIO}_3$  and KI in aqueous solutions with respect to ramipril concentration. Therefore, a pseudo zero order reaction condition was worked out with respect to the concentration of the reagents. The kinetic plots (Figure 4) are all sigmoid in nature and therefore, the initial rate of reaction was obtained by measuring the slopes ( $\tan \alpha = dA/dt$ ) of the initial tangent to the absorbance-time curves at different concentrations of the drug. The order with respect to ramipril was evaluated by plotting the logarithm of the initial rate of reaction vs logarithm of the molar concentration of ramipril and was found to be one.

The initial rate of reaction would follow a pseudo first order and obeyed the following rate equation:

$$\text{Rate} = \frac{\Delta A}{\Delta t} = kC^n \quad (1)$$

where  $k$  is the rate constant,  $C$  is the concentration of ramipril,  $n$  is the order of reaction. The logarithmic form of the equation is written as:

$$\log_{10}(\text{rate}) = \log_{10} k + n \log_{10} C \quad (2)$$



**Figure 2.** Reaction sequence of the proposed method.

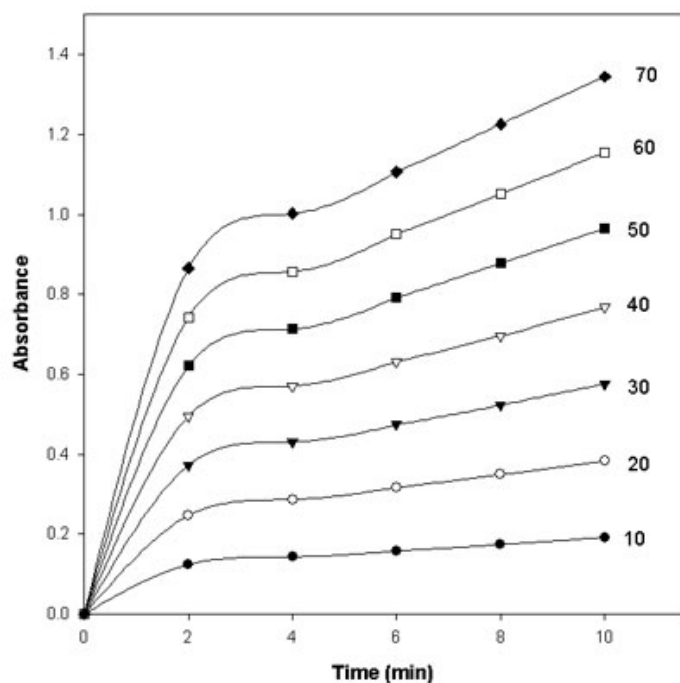
A calibration curve was constructed by plotting the logarithm of the initial rate of reaction ( $\log v$ ) vs logarithm of initial concentration of ramipril ( $\log C$ ), which showed a linear relationship over ramipril concentration of 10.0–70.0  $\mu\text{g mL}^{-1}$ . The linear regression analysis using the method of least square treatment of calibration data ( $n = 7$ ) was made to evaluate slope, intercept and correlation coefficient. The regression of  $\log$  rate vs  $\log C$  gave the following linear regression equation:

$$\log \hat{v}(rate) = 3.5665 + 1.0277 \log \hat{v} C \quad (3)$$

with a correlation coefficient ( $r$ ) of 0.9999. The value of ‘ $n$ ’ representing order of reaction in the regression equation is one, confirming the first order reaction with respect to the ramipril concentration. The limit of detection (LOD) for initial rate method was calculated<sup>19</sup> by statistical treatment of calibration data ( $n = 7$ ) by considering seven calibration points using the following equation:

$$LOD = \frac{t}{b} \left[ S_0^2 \tilde{n} \frac{n-2}{n-1} \right]^{1/2} \quad (4)$$

where  $n$  is the number of standard samples ( $n = 7$ ),  $t$  is the value of student’s  $t$  for  $n-2$  degrees of freedom at 95% confidence level,  $b$  is the slope of the regression line and  $S_0^2$  is the variance characterizing the scatter of the experi-



**Figure 4.** Absorbance-time curves for the reaction of varying concentration of ramipril with potassium iodate and potassium iodide. Concentration of ramipril ( $\mu\text{g mL}^{-1}$ ): (A) 10, (B) 20, (C) 30, (D) 40, (E) 50, (F) 60, (G) 70.

**Table 1.** Spectral and Statistical Data for the Determination of Ramipril by Initial Rate Method

| Parameters                                     | Initial Rate Method                          |
|--|--|
| $\lambda_{\max}(\text{nm})$                    | 352  |
| Linear dynamic range ( $\mu\text{g mL}^{-1}$ ) | 10.0–70.0                                    |
| Regression equation                            | $\log(\text{rate}) = 3.5665 + 1.0277 \log C$ |
| $S_0^*$  | $6.388 \times 10^{-3}$                       |
| Intercept (a)                                  | 3.5665                                       |
| $S_a$  | $3.595 \times 10^{-2}$                       |
| $\pm t S_a^\dagger$                            | $9.243 \times 10^{-2}$                       |
| Slope (b)                                      | 1.0277                                       |
| $S_b$  | $8.768 \times 10^{-3}$                       |
| $\pm t S_b^\ddagger$                           | $2.254 \times 10^{-2}$                       |
| Correlation coefficient (r)                    | 0.9998                                       |
| Variance ( $S_0^2$ )                           | $4.080 \times 10^{-5}$                       |
| Detection limit ( $\mu\text{g mL}^{-1}$ )      | 0.020  |

\*Standard deviation of the calibration line.

<sup>†</sup>Confidence interval of the intercept at 95% confidence level.

<sup>‡</sup>Confidence interval of the slope at 95% confidence level.

mental data points with respect to the line of regression. The variance was calculated using the equation<sup>20</sup>:

$$S_0^2 = \frac{\sum (\log \hat{v}_{exp} - \log \hat{v}_{reg})^2}{n-2} \quad (5)$$

Linear dynamic range, correlation coefficient, variance, detection limit, standard deviations and confidence limits for slope and intercept of the calibration line are summarized in Table 1. The low values of variance confirmed negligible scattering of the experimental data points around the line of regression and good sensitivity of the proposed method.

### Fixed Time Method

In this method, the absorbance of a yellow colored solution ( $\lambda_{\max} = 352 \text{ nm}$ ) was recorded at a preselected fixed time. Calibration graphs of absorbance vs initial concentration of ramipril at seven concentration levels were plotted at a fixed time of 2, 4, 6, 8, and 10-minute. Beer’s law limit, molar absorptivity, linear regression equation, coefficient of correlation, detection limit, variance, standard deviation and confidence limits for slope and intercept are summarized in Table 2. Test of significance of the intercepts,  $a$ , of regression lines of the fixed time method at different intervals of time showed that these values of ‘ $a$ ’ did not differ significantly from the theoretical value, zero. For this, a simplified method was used to calculate the quantities ‘ $t$ ’ from the relation  $t = a/S_a$ <sup>21,22</sup> and their comparison with the tabulated data from the student’s  $t$ -distribution.<sup>23</sup> The  $t$ -values for fixed time method at 2, 4, 6, 8 and 10-minute do not exceed the 95% criterion ( $t = 2.571$ ). It confirmed

**Table 2.** Spectral and Statistical Data for the Determination of Ramipril by Fixed Time Method

| Parameters                                    | Fixed-time method                                   |   |  |  |   |
|---|---|---|--|--|---|
|   | 2-minute  | 4-minute  | 6-minute   | 8-minute   | 10-minute   |
| Beer's law limit ( $\mu\text{g mL}^{-1}$ )    | 10.0–70.0   | 10.0–70.0   | 10.0–70.0  | 10.0–70.0  | 10.0–70.0   |
| Molar absorptivity ( $\text{L mol cm}^{-1}$ ) | $5.161 \times 10^3$                                 | $5.954 \times 10^3$                                 | $6.591 \times 10^3$                                  | $7.288 \times 10^3$                                  | $7.989 \times 10^3$                                 |
| Linear Regression equation                    | $A = 5.714 \times 10^{-4} + 1.238 \times 10^{-2} C$ | $A = 7.143 \times 10^{-4} + 1.428 \times 10^{-2} C$ | $A = -2.857 \times 10^{-4} + 1.583 \times 10^{-2} C$ | $A = -2.571 \times 10^{-3} + 1.755 \times 10^{-2} C$ | $A = 5.714 \times 10^{-4} + 1.916 \times 10^{-2} C$ |
| Intercept                                     | $5.714 \times 10^{-4}$                              | $7.143 \times 10^{-4}$                              | $-2.857 \times 10^{-4}$                              | $-2.571 \times 10^{-3}$                              | $5.714 \times 10^{-4}$                              |
| $S_a$   | $9.389 \times 10^{-4}$                              | $1.264 \times 10^{-3}$                              | $8.601 \times 10^{-4}$                               | $1.846 \times 10^{-3}$                               | $1.284 \times 10^{-3}$                              |
| $\pm t S_a$                                   | $2.414 \times 10^{-3}$                              | $3.249 \times 10^{-3}$                              | $2.211 \times 10^{-3}$                               | $4.746 \times 10^{-3}$                               | $3.302 \times 10^{-3}$                              |
| Slope   | $1.238 \times 10^{-2}$                              | $1.428 \times 10^{-2}$                              | $1.583 \times 10^{-2}$                               | $1.755 \times 10^{-2}$                               | $1.916 \times 10^{-2}$                              |
| $S_b$   | $2.099 \times 10^{-5}$                              | $2.826 \times 10^{-5}$                              | $2.082 \times 10^{-5}$                               | $4.128 \times 10^{-5}$                               | $2.871 \times 10^{-5}$                              |
| $\pm t S_b$                                   | $5.398 \times 10^{-5}$                              | $7.264 \times 10^{-5}$                              | $5.353 \times 10^{-5}$                               | $1.061 \times 10^{-4}$                               | $7.382 \times 10^{-5}$                              |
| Correlation coefficient (r)                   | 0.9999  | 0.9999  | 0.9999   | 0.9999   | 0.9999  |
| Variance ( $S_o^2$ )                          | $1.234 \times 10^{-6}$                              | $2.235 \times 10^{-6}$                              | $1.036 \times 10^{-6}$                               | $4.771 \times 10^{-7}$                               | $2.309 \times 10^{-6}$                              |
| $S_o^*$                                       | $1.111 \times 10^{-3}$                              | $1.495 \times 10^{-3}$                              | $1.018 \times 10^{-3}$                               | $2.184 \times 10^{-3}$                               | $1.520 \times 10^{-3}$                              |
| Detection limit ( $\mu\text{g mL}^{-1}$ )     | 0.211   | 0.246   | 0.151  | 0.292  | 0.186   |
| $^{\dagger}t = a / S_a$                       | 0.609   | 0.565   | 0.332  | 1.393  | 0.445   |

\*Standard deviation of the calibration line.

$^{\dagger}$ Calculated t-value, which is less than the theoretical value of t (2.365) at 95% confidence level.

that the calculated intercepts for the fixed time method are not significantly different from zero. Thus, the fixed time methods are free from constant errors independent of the concentration of ramipril. It is apparent from Table 2 that the values of intercept, standard deviation of the slope and intercept and detection limit were found to be lowest at a fixed time of 6-minute. Therefore, on the basis of lowest values of these parameters, the fixed time of 6.0-minute was recommended for the assay of ramipril in pharmaceutical formulations.

### Specificity

The specificity of the proposed method was evaluated by determining the ramipril concentration in the presence of varying amounts of degraded product of ramipril such as

ramipril diketopiperazine (ester), which is a principal degradant of the ramipril. It was found that the specified degradant did not react with either of the reagents.

The various excipients commonly used in dosage forms such as sodium stearyl fumarate, magnesium stearate, starch, lactose, and talc did not interfere with the assay procedure. The solubility of stearic acid in distilled water is negligible, it is only soluble in chloroform, acetone and ether,<sup>24</sup> and therefore stearic acid is not interfering with the determination process as the developed method is performed only in distilled water.

The samples were stressed by light, acid and oxidants such as potassium persulfate, ammonium molybdate, sodium metavanadate,  $\text{KBrO}_3$ , N-bromosuccinimide, chloramine T, and sodium nitrate for 2, 5 and 5-day-time points, respectively.

**Table 3.** Intra Day Assay: Test of Precision of the Proposed Methods for the Determination of Ramipril

| Proposed Methods    | Concentration ( $\mu\text{g mL}^{-1}$ ) |                       | Recovery $\pm$ RSD* (%) | SAE $^{\dagger}$ | CL $^{\ddagger}$ |
|---------------------|---|-----------------------|-------------------------|------------------|------------------|
|                     | Theoretical                             | Nominal $\pm$ SD $^a$ |                         |                  |                  |
| Initial rate method | 20.0                                    | $20.012 \pm 0.113$    | $100.058 \pm 0.563$     | 0.050            | 0.140            |
|                     | 40.0                                    | $39.994 \pm 0.105$    | $99.985 \pm 0.264$      | 0.047            | 0.131            |
|                     | 70.0                                    | $69.988 \pm 0.117$    | $99.983 \pm 0.167$      | 0.052            | 0.145            |
| Fixed time method   | 20.0                                    | $20.031 \pm 0.053$    | $100.153 \pm 0.264$     | 0.024            | 0.066            |
|                     | 40.0                                    | $39.993 \pm 0.053$    | $99.980 \pm 0.133$      | 0.024            | 0.066            |
|                     | 70.0                                    | $70.037 \pm 0.072$    | $100.053 \pm 0.103$     | 0.032            | 0.089            |

$^a$ Mean for five determinations.

$^{\dagger}$ SAE, standard analytical error.

$^{\ddagger}$ CL, confidence limit at 95% confidence level and four degrees of freedom ( $t = 2.776$ ).

**Table 4.** Inter Day Assay: Test of Precision of the Proposed Methods for the Determination of Ramipril

| Proposed methods    | Concentration ( $\mu\text{g mL}^{-1}$ ) |                    | Recovery $\pm$ RSD* (%) | SAE <sup>†</sup> | CL <sup>‡</sup> |
|---------------------|---|--------------------|-------------------------|------------------|-----------------|
|                     | Theoretical                             | Nominal $\pm$ SD*  |                         |                  |                 |
| Initial rate method | 20.0                                    | 19.992 $\pm$ 0.124 | 99.957 $\pm$ 0.618      | 0.055            | 0.153           |
|                     | 40.0                                    | 40.105 $\pm$ 0.253 | 100.262 $\pm$ 0.631     | 0.113            | 0.314           |
|                     | 70.0                                    | 70.044 $\pm$ 0.182 | 100.062 $\pm$ 0.260     | 0.082            | 0.260           |
| Fixed time method   | 20.0                                    | 20.005 $\pm$ 0.056 | 100.026 $\pm$ 0.282     | 0.025            | 0.070           |
|                     | 40.0                                    | 40.031 $\pm$ 0.085 | 100.077 $\pm$ 0.213     | 0.038            | 0.106           |
|                     | 70.0                                    | 69.987 $\pm$ 0.072 | 99.981 $\pm$ 0.103      | 0.032            | 0.089           |

\*Mean for five determinations.

<sup>†</sup>SAE, standard analytical error.

<sup>‡</sup>CL, confidence limit at 95% confidence level and four degrees of freedom ( $t=2.776$ ).

It was observed that stress by such conditions did not cause significant degradation. There was no change in the absorption spectra of the reference drug and stressed sample solutions. However, the samples degraded significantly when stressed by base and heat for 1 and 2 hours, respectively. All stressed samples (light, acid and oxidants) and unstressed reference solution were analyzed for ramipril content, which gave acceptable recoveries of the drug. Thus the proposed method is stability-indicating assay for the determination of intact ramipril in the presence of its degradation products.

**Accuracy and Precision of the Proposed Methods**

The accuracy and precision of the proposed methods was established by measuring the content of ramipril in pure form at three different concentration levels (20.0, 40.0 and 70.0  $\mu\text{g mL}^{-1}$ ). The intra day precision of the proposed methods was performed by carrying out five independent analyses at each concentration level within one day (Table 3). In the same manner, the inter day precision was also evaluated by measuring the ramipril content at each concentration level on five consecutive days by initial rate and fixed time

**Table 5.** Determination of Ramipril in Drug Formulations by Standard Addition Method

| Formulations   | Initial rate method                     |        |                    |                         |       |       |
|----------------|---|--------|--------------------|-------------------------|-------|-------|
|                | Concentration ( $\mu\text{g mL}^{-1}$ ) |        |                    | Recovery $\pm$ RSD* (%) | SAE   | CL    |
|                | Theoretical                             | Spiked | Nominal $\pm$ SD*  |                         |       |       |
| Capsule        |   |        |                    |                         |       |       |
| Hopace-1.25    | 20.0                                    | 20.0   | 39.993 $\pm$ 0.078 | 99.985 $\pm$ 0.195      | 0.034 | 0.097 |
| (Cardicare)    | 30.0                                    | 40.0   | 70.044 $\pm$ 0.117 | 100.063 $\pm$ 0.167     | 0.052 | 0.146 |
| Ramipres-1.25  | 20.0                                    | 20.0   | 40.008 $\pm$ 0.101 | 100.021 $\pm$ 0.251     | 0.045 | 0.125 |
| (Cipla)        | 30.0                                    | 40.0   | 69.988 $\pm$ 0.117 | 99.983 $\pm$ 0.167      | 0.052 | 0.145 |
| Tablet         |   |        |                    |                         |       |       |
| Ramace-1.25    | 20.0                                    | 20.0   | 40.022 $\pm$ 0.105 | 100.056 $\pm$ 0.263     | 0.047 | 0.131 |
| (AstraZeneca)  | 30.0                                    | 40.0   | 70.016 $\pm$ 0.131 | 100.023 $\pm$ 0.187     | 0.059 | 0.163 |
| Variace-1.25   | 20.0                                    | 20.0   | 40.008 $\pm$ 0.101 | 100.020 $\pm$ 0.251     | 0.045 | 0.125 |
| (Win Medicare) | 30.0                                    | 40.0   | 70.002 $\pm$ 0.125 | 100.003 $\pm$ 0.179     | 0.056 | 0.155 |

| Formulations   | Fixed time method                       |        |                    |                         |       |       |
|----------------|---|--------|--------------------|-------------------------|-------|-------|
|                | Concentration ( $\mu\text{g mL}^{-1}$ ) |        |                    | Recovery $\pm$ RSD* (%) | SAE   | CL    |
|                | Theoretical                             | Spiked | Nominal $\pm$ SD*  |                         |       |       |
| Capsule        |   |        |                    |                         |       |       |
| Hopace-1.25    | 20.0                                    | 20.0   | 39.993 $\pm$ 0.053 | 99.980 $\pm$ 0.133      | 0.024 | 0.066 |
| (Cardicare)    | 30.0                                    | 40.0   | 70.012 $\pm$ 0.063 | 100.017 $\pm$ 0.089     | 0.028 | 0.078 |
| Ramipres-1.25  | 20.0                                    | 20.0   | 40.005 $\pm$ 0.050 | 100.013 $\pm$ 0.112     | 0.020 | 0.056 |
| (Cipla)        | 30.0                                    | 40.0   | 69.999 $\pm$ 0.053 | 99.999 $\pm$ 0.075      | 0.024 | 0.065 |
| Tablet         |   |        |                    |                         |       |       |
| Ramace-1.25    | 20.0                                    | 20.0   | 39.993 $\pm$ 0.053 | 99.980 $\pm$ 0.133      | 0.024 | 0.066 |
| (AstraZeneca)  | 30.0                                    | 40.0   | 70.037 $\pm$ 0.072 | 100.053 $\pm$ 0.103     | 0.032 | 0.089 |
| Variace-1.25   | 20.0                                    | 20.0   | 40.005 $\pm$ 0.078 | 100.013 $\pm$ 0.194     | 0.035 | 0.096 |
| (Win Medicare) | 30.0                                    | 40.0   | 69.999 $\pm$ 0.083 | 99.998 $\pm$ 0.118      | 0.037 | 0.102 |

\*Mean for five determinations.

**Table 6.** Point Hypothesis Test: Comparison of the Proposed Methods with the Reference Method at 95% Confidence Level

| Formulations                | Initial rate method |        |                      |                      | Fixed time method |        |                      |                      | Reference method |        |
|-----------------------------|---------------------|--------|----------------------|----------------------|-------------------|--------|----------------------|----------------------|------------------|--------|
|                             | Recovery %          | RSD* % | t-value <sup>†</sup> | F-value <sup>†</sup> | Recovery %        | RSD* % | t-value <sup>†</sup> | F-value <sup>†</sup> | Recovery %       | RSD* % |
| Capsule                     |                     |        |                      |                      |                   |        |                      |                      |                  |        |
| Hopace-1.25 (Cardicare)     | 99.988              | 0.399  | 0.123                | 1.853                | 100.045           | 0.188  | 0.053                | 2.426                | 100.070          | 0.293  |
| Ramipres-1.25 (Cipla)       | 100.083             | 0.337  | 0.092                | 1.687                | 99.961            | 0.258  | 0.151                | 1.018                | 100.035          | 0.260  |
| Tablet                      |                     |        |                      |                      |                   |        |                      |                      |                  |        |
| Ramace-1.25 (AstraZeneca)   | 99.988              | 0.400  | 0.074                | 2.359                | 100.045           | 0.188  | 0.023                | 1.906                | 100.035          | 0.260  |
| Variace-1.25 (Win Medicare) | 99.988              | 0.400  | 0.074                | 2.359                | 99.961            | 0.258  | 0.151                | 1.018                | 100.035          | 0.260  |

\*Mean for five determinations.

<sup>†</sup>Theoretical t-value ( $\nu = 8$ ) and F-value ( $\nu = 4, 4$ ) at 95% confidence level are 2.306 and 6.39, respectively.

methods (Table 4). The results of standard deviation (SD), relative standard deviation (RSD) and recoveries by initial rate and fixed time methods in Table 3 and 4 can be considered to be very satisfactory. Thus the proposed methods are very effective for the assay of ramipril in drug formulations.

The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and capsules at two different concentration levels and the nominal value of drug was estimated by the proposed method. Each level was repeated five times. The results (Table 5) were reproducible with low SD and RSD. No interference from the common excipients was observed. The applicability of the proposed methods for the determination of ramipril has been tested on commercially available pharmaceutical formulations. The results of the

proposed method (initial rate or fixed time) were compared with those obtained by the reference method<sup>8</sup> using point hypothesis test. The student's t and F-values (Table 6) at 95% confidence level did not exceed the tabulated t- and F-values, confirming no significant differences between the performance of the proposed methods and the reference method. Once this is done, the evaluation of the bias is made using the interval hypothesis test as an alternate. Therefore, the interval hypothesis test<sup>25</sup> has been performed to compare the results of the proposed methods with those of the reference method at 95% confidence level (Table 7). The Canadian Health Protection Branch has recommended that a bias, based on recovery experiments, of  $\pm 2\%$  is acceptable.<sup>26</sup> It is evident from Table 7 that the true bias of all samples is smaller than  $\pm 2\%$ . The interval hypothesis test has also confirmed that accuracy and precision are within the acceptable limits and indicated no significant differences between the performance of the methods compared at 95% confidence level.

**Table 7.** Interval Hypothesis Test: Comparison of the Proposed Methods with the Reference

| Formulations                | Initial rate method         |                             | Fixed time method           |                             |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Lower limit* ( $\theta_L$ ) | Upper limit* ( $\theta_U$ ) | Lower limit* ( $\theta_L$ ) | Upper limit* ( $\theta_U$ ) |
| Capsule                     |                             |                             |                             |                             |
| Hopace-1.25 (Cardicare)     | 0.981                       | 1.017                       | 0.987                       | 1.013                       |
| Ramipres-1.25 (Cipla)       | 0.986                       | 1.015                       | 0.986                       | 1.013                       |
| Tablet                      |                             |                             |                             |                             |
| Ramace-1.25 (AstraZeneca)   | 0.982                       | 1.018                       | 0.988                       | 1.012                       |
| Variace-1.25 (Win Medicare) | 0.982                       | 1.017                       | 0.986                       | 1.013                       |

\*In pharmaceutical analysis, a bias, based on recovery experiments, of  $\pm 2\%$  ( $\theta_L = 0.98$  and  $\theta_U = 1.02$ ) is acceptable.

## CONCLUSION

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear dynamic range with good accuracy and precision. Point and interval hypothesis tests proved that the proposed methods have acceptable bias of  $\pm 2\%$ . Hence the data presented in the manuscript by kinetic spectrophotometric method for the determination of ramipril in pharmaceutical formulations demonstrate that the proposed method is accurate, precise, linear, specific and robust for the determination of ramipril in tablets and capsules and thus can be extended for routine analysis of ramipril in pharmaceutical industries, hospitals and research laboratories.



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