A sensitive kinetic spectrophotometric method for the determination of captopril in bulk and dosage forms

NAFISUR RAHMAN[1]*
NISHAT ANWAR[1]
MOHAMMAD KASHIF[1]
NASRUL HODA[2]

[1] *Analytical Research Laboratory Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.) India*

[2] *Neurotoxicology Laboratory Department of Medical Elementology and Toxicology, Jamia Hamdard, Hamdard Nagar, New Delhi-62, India*

A simple and sensitive kinetic spectrophotometric method has been developed. The method is based on the reduction of Fe(III) with captopril. Fe(II) then reacts with potassium ferricyanide, resulting in the formation of a blue product. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 730 nm. Thus, 1.23×10^{-3} mol L^{-1} $FeCl_3$ and 3.04×10^{-4} mol L^{-1} potassium ferricyanide were used as optimum values for maximum concentration of captopril in the calibration graph. The initial rate is utilized for constructing the calibration graph, which was found to be linear in the range from 4.60×10^{-6} to 5.06×10^{-5} mol L^{-1}; detection limit is 1.99×10^{-7} mol L^{-1}. The proposed method has been validated; the mean recovery ranges from 99.8 to 101.4% with RSD < 2%. Common excipients do not interfere with the determination. The point and interval hypotheses tests have been performed and confirmed that there is no significant difference between the proposed method and the conventional spectrophotometric method. The experimental true bias of all samples is lower than ± 2%. The proposed method has been applied to the determination of captopril in bulk and dosage forms.

Captopril, 1-[(2S)-3-mercapto-2-methyl propionyl]-L-proline, is a sulphahydril containing angiotensin converting enzyme (ACE) inhibitor. It is used in the management of hypertension, heart failure and diabetic nephropathy (1). The drug is listed in *United States Pharmacopoeia* (2), which recommends a HPLC method for its assay in bulk and tablet formulations. In order to assure the quantity of captopril in dosage forms, several methods have been reported, including gas chromatography (3), high performance liquid chromatography (4), capillary electrophoresis (5), flow injection analysis (6), titrimetry (7), and atomic absorption spectrophotometry (8). The content of captopril in pharmaceutical preparations and biological fluids has also been determined using electrochemical methods such as potentiometry (9) and amperometry (10).

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* Correspondence, e-mail: cht17nr_amu@yahoo.com

Several spectrophotometric methods have been reported in the literature that are based on the reaction of the drug with Folin-Ciocalteau reagent (11), potassium iodate (12, 13), vanadate (14), bromate-bromide (15), chloramine-T (16), tetrazoline blue (17), palladium(II) chloride (18) and *p*–chloranilic acid (19). No kinetic spectrophotometric method has been reported in the literature for the assay of captopril. This communication describes a simple and sensitive kinetic spectrophotometric method for the determination of captopril in bulk and drug formulations.

<div align="center">EXPERIMENTAL</div>

Apparatus

Spectronic 20 D$^+$ spectrophotometer (Milton Roy, USA) was used for absorbance measurements with matched glass cuvettes.

Materials and reagents

Captopril was kindly provided by Wockhardt India Ltd. (India) and was used as received. Aceten tablets (25 mg each, Wockhardt India Ltd.), the only available commercial dosage forms, were purchased from the local market. All chemicals used were of AR grade.

Standard solution of captopril (2.30×10^{-3} mol L^{-1}) was prepared in doubly distilled water and diluted as necessary. Potassium ferricyanide solution (3.04×10^{-3} mol L^{-1}) was also prepared in water. Ferric chloride solution (6.16×10^{-3} mol L^{-1}) was prepared by dissolving in water, adding 70% HNO$_3$ and finally diluting to 100 mL water (final HNO$_3$ concentration was 0.14%).

General procedure

Aliquots (0.2–2.2 mL) of 2.30×10^{-4} mol L^{-1} captopril test solution were pipetted into a series of 10-mL volumetric flasks. To each flask, 2.0 mL of 6.16×10^{-3} mol L^{-1} FeCl$_3$ solution was added, followed by 1.0 mL of 3.04×10^{-3} mol L^{-1} potassium ferricyanide and then diluted with water. Flask contents were shaken and immediately transferred to the spectrophotometric cell. Absorbance was recorded as a function of time at λ_{max} 730 nm against the reagent blank prepared simultaneously. The initial rate of the reaction (*v*) at different concentrations was evaluated from the slope of the tangent to the absorbance-time curves. The calibration graph was constructed by plotting the logarithm of the initial rate (log *v*) against the logarithm of the molar concentration of the drug (log *c*).

Analysis of captopril in pharmaceutical preparations

Ten tablets of Aceten were accurately weighed and powdered. A portion equivalent to 50 mg of captopril was extracted with methanol by shaking and filtered on Whatman No. 42 filter paper. The filtrate and washings were evaporated to dryness. The residue was dissolved in doubly distilled water and analyzed by the recommended procedure.

Analysis of the synthetic mixture for solid dosage forms

Synthetic mixture containing captopril was prepared with excipients commonly used in solid dosage forms and analyzed to check the applicability of the proposed method. The following excipients were used to prepare a synthetic mixture for solid dosage forms: captopril (100 mg), lactose (280 mg), silicon dioxide (40 mg), starch (360 mg), microcrystalline cellulose (100 mg) and magnesium stearate (25 mg).

In the synthetic mixture, the mass ratio of captopril to lactose, silicon dioxide, starch, microcrystalline cellulose and magnesium stearate was 1:3:0.4:4:1:0.3, respectively. A portion of the synthetic mixture equivalent to 25 mg of captopril was transferred to a 50 mL conical flask and treated as described in *Analysis of captopril in pharmaceutical formulations*.

Stoichiometry

The stoichiometry of the reaction was studied by the Bent and French method (20). For this, three sets of experiments were performed. In the first set, the concentration of captopril was varied while keeping excess concentrations of $FeCl_3$ (2.07×10^{-3} mol L^{-1}) and potassium ferricyanide (3.04×10^{-3} mol L^{-1}). In the second set, excess concentrations of captopril (2.30×10^{-3} mol L^{-1}) and potassium ferricyanide (3.04×10^{-3} mol L^{-1}) were employed and a varying concentration of $FeCl_3$. In the last set, concentration of potassium ferricyanide was varied while keeping excess concentrations of captopril (2.30×10^{-3} mol L^{-1}) and $FeCl_3$ (2.07×10^{-3} mol L^{-1}).

Validation

For linearity evaluation, captopril was determined at eleven concentration levels: 4.60×10^{-6}, 9.20×10^{-6}, 1.38×10^{-5}, 1.84×10^{-5}, 2.30×10^{-5}, 2.76×10^{-5}, 3.22×10^{-5}, 3.68×10^{-5}, 4.14×10^{-5}, 4.60×10^{-5} and 5.06×10^{-5} mol L^{-1}. Each concentration was analyzed five times.

The detection limit (21) was evaluated as:

$$LOD = 3.3 \frac{s_0}{b}$$

where b is the slope and s_0 is the standard deviation of the regression line.

Three concentrations of reference captopril solution within the linearity range were selected: 9.20×10^{-6}, 1.84×10^{-5} and 3.22×10^{-5} mol L^{-1}. Six solutions of each concentration were prepared and analyzed within the same day. The assay was repeated for five consecutive days. Recovery experiments were performed using the standard addition method. The total amount was determined by the proposed procedure.

Interval hypothesis

For practical purposes, the acceptable bias can be calculated statistically (22). For example, a test method, *i.e.,* the initial rate method (method 2) is considered acceptable if

its true mean is within ± 2% of that of the literature spectrophotometric method (11) (method 1):

$$-0.02\ \mu_1 < (\mu_2 - \mu_1) < 0.02\ \mu_1$$

This can be written as:

$$0.98 < \mu_2/\mu_1 < 1.02$$

and generalized to:

$$\theta_L < \mu_2/\mu_1 < \theta_u$$

where θ_L and θ_u represent the lower and the upper acceptance limits, respectively, when μ_2 is expressed as a proportion of the reference mean μ_1. Statistically, θ_L and θ_u are calculated from the relation:

$$\theta^2\left(\bar{x}_1^2 - \frac{S_p^2 t_{tab}^2}{n_1}\right) - 2\theta\bar{x}_2\bar{x}_1 + \left(\bar{x}_2^2 - \frac{S_p^2 t_{tab}^2}{n_2}\right) = 0$$

where $\bar{x}_1$ and $\bar{x}_2$ are estimates of μ_1 and μ_2 based on n_1 and n_2 measurements, respectively. S_p^2 is the variance of pooled measurements, t_{tab} is the tabulated one-sided *t*-value with n_1+n_2-2 degrees of freedom at the specified level of significance.

RESULTS AND DISCUSSION

Captopril is a reducing agent owing to the presence of thiol group (–SH) in its structure. In aqueous solution, captopril reduces Fe(III) to Fe(II), but it is oxidized to disulphide. Fe(II) immediately reacts with ferricyanide, resulting in the formation of a blue product that absorbs maximally at 730 nm. The absorbance of the coloured solution increases with time and hence, a kinetically based method was elaborated to assay the captopril in pharmaceutical formulations. The following specific advantages of the kinetic method can be expected: (*i*) some experimental steps such as filtration and extraction are eliminated before the absorbance measurement; (*ii*) selectivity is improved owing to measuring the evolution of absorbance with time instead of measuring the concrete absorbance value; (*iii*) usually, no interference is observed due to the coloured background of samples.
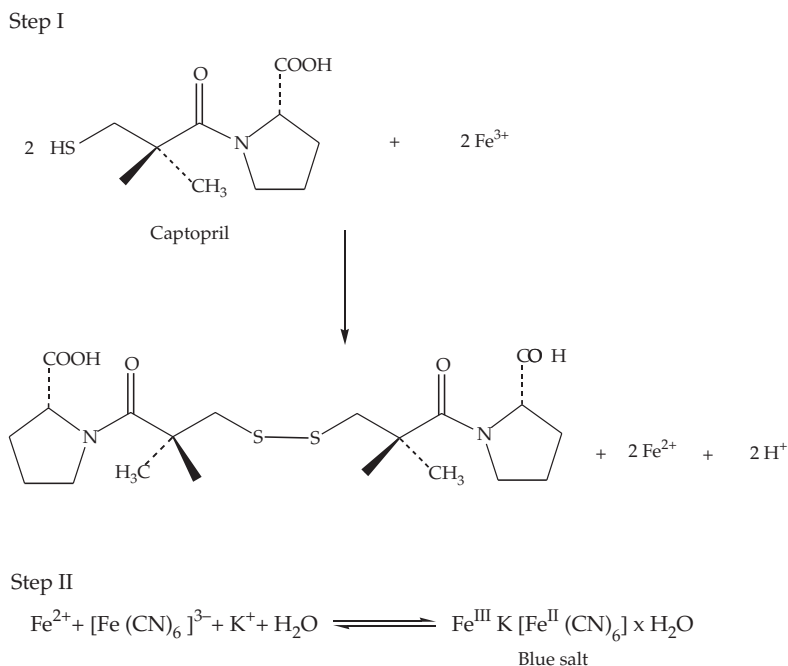
Reaction conditions optimization

During the optimization of the proposed procedure, it was found that the rate of reaction or absorbance of the solution remained unchanged in the temperature range of 20–28 °C. Therefore, all the studies were performed at 25 ± 1 °C. It was found that the

absorbance remained constant in the pH range of 2.5–3.0. Therefore, all the studies were performed at pH 2.8. To study the effect of $FeCl_3$ concentration, aliquots equivalent to 3.68×10^{-5} mol L^{-1} of drug were pippeted into a series of 10.0 mL volumetric flasks, followed by varying the concentration of $FeCl_3$ solution ($1.23 \times 10^{-4} - 1.23 \times 10^{-3}$ mol L^{-1}) and 3.04×10^{-4} mol L^{-1} potassium ferricyanide solution. The absorbance was measured at a fixed time of 24 minutes because the equilibrium was attained at this time point. The absorbance increases with increasing concentration of the $FeCl_3$ solution and becomes constant at 8.63×10^{-4} mol L^{-1}.

Dependence of potassium ferricyanide concentration on the formation of the blue product was examined by taking a fixed amount of captopril (3.68×10^{-5} mol L^{-1}), 1.23×10^{-3} mol L^{-1} of $FeCl_3$ solution and varying the concentration of potassium ferricyanide (6.07×10^{-5} mol $L^{-1} - 4.86 \times 10^{-4}$ mol L^{-1}). It was observed that maximum absorbance was obtained with 2.43×10^{-4} mol L^{-1}.

Stoichiometry

The stoichiometry of the reaction product was established from the Bent and French plots (27). The plot of log absorbance *vs.* log [Fe(III)], [$K_3Fe(CN)_6$] and [captopril] gave values of the slopes of 0.98 ($R = 0.9999$), 1.0 ($R = 0.9998$), and 0.96 ($R = 0.9990$), respectively. Hence, it is concluded that the reaction proceeds at the molar ratio of 1:1:1. The probable reaction sequence is shown in Scheme 1.

Step I

Step II

$$Fe^{2+} + [Fe(CN)_6]^{3-} + K^+ + H_2O \rightleftharpoons Fe^{III} K[Fe^{II}(CN)_6] \times H_2O$$

Blue salt

Scheme 1

Initial-rate method

The initial absorbance *vs.* time curves are shown in Fig. 1. The order with respect to the Fe(III) was determined by studying the reaction at different initial concentrations of Fe(III) with fixed molar concentration of captopril. The plot of initial rate ($\Delta A / \Delta t$) against initial absorbance was linear, passing through the origin. This suggested that the order of the reaction with respect to Fe(III) at the start is one. The order with respect to captopril was determined from the measurement of initial rates at several captopril concentrations, keeping a constant concentration of Fe(III), which was also found to be one. The simple rate expression can be written as:

$$\text{rate} = k \, [\text{Fe(III)}]^m \, [\text{captopril}]^n$$

where *m* and *n* are the respective orders of the reaction, being 1 for both [Fe(III)] and [captopril].

Under optimized experimental parameters, the pseudo-first order condition was worked out by taking excess concentration of $FeCl_3$ and potassium ferricyanide with respect to the initial concentration of captopril. Therefore, the above equation is reduced to:

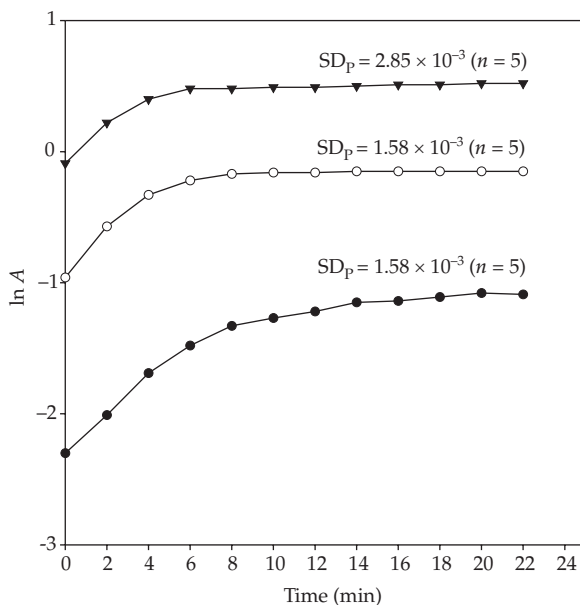$$\text{rate} = K_\psi \, [\text{captopril}]^n$$

Fig. 1. Initial absorbance *vs.* time graph showing the dependence of the reaction on captopril concentration: (●) 9.204×10^{-6} mol L^{-1}; (○) 2.761×10^{-5} mol L^{-1}; (▼) 4.602×10^{-5} mol L^{-1}; SD_p – pooled standard deviation.

where K_ψ is the pseudo-first order rate constant, $[c]$ is the molar concentration of captopril. The above equation is written in logarithmic form as:

$$\log \text{rate} = \log K_\psi + n \log c = 4.248 + 1.173 \log c$$

The calibration graph was obtained by plotting the log initial rate *vs.* log *c*, which showed a linear relationship over the concentration range $4.60 \times 10^{-6} - 5.06 \times 10^{-5}$ mol L^{-1}. The value of *n* also confirmed that the reaction order with respect to captopril concentration at the initial stage is one. The regression equation, confidence interval of slope and intercept at 95% confidence level, correlation coefficient, variance and detection limit were calculated as well and are summarized in Table I. The low value of the calibration line variance (2.38×10^{-4}) confirmed the negligible scattering of the calibration data points around the regression line. The low value of the detection limit (1.99×10^{-7} mol L^{-1}) pointed to good sensitivity of the proposed procedure.

The intraday and interday precisions were found to be in the range 0.7–1.6% and 0.6–1.0%, respectively (Table II). The percent error was found to vary from 0.1–1.0%. The results of recovery studies are summarized in Table III. The mean percentage recovery ranged from 99.8–101.4%, with relative standard deviation values < 2%. The selectivity of the proposed method was preliminarily checked by monitoring the standard solution of captopril in the presence of other compounds of the tablet (excipients). The results are summarized in Table IV. It is evident from Table IV that the excipients had no effect on the captopril estimation. Hence, the determination of the drug is considered to be free from interference due to excipients.

The proposed procedure has been successfully applied to the determination of captopril in pharmaceutical preparations. The results are summarized in Table V. The results obtained by the proposed procedure were compared with the spectrophotometric method (11) using point and interval hypotheses. The results from Table V show that there is no significant difference between the performances of the methods compared. The Canadian Health Protection Branch (23) has recommended that a bias, based on recovery experiments, of ± 2% is acceptable for pharmaceutical analysis. The data obtained

Table I. Regression data for captopril using the initial-rate method

Parameter	Initial-rate method
Linear range (mol L^{-1})	$4.60 \times 10^{-6} - 5.06 \times 10^{-5}$
Regression equation	$\log v = 4.248 + 1.173 \log c$
Coefficient of correlation (*R*)	0.9992
$\pm t \, s_a$ [a]	2.768×10^{-2}
$\pm t \, s_b$ [b]	2.705×10^{-2}
Detection limit (mol L^{-1})	1.99×10^{-7}
Variance of regression line (s_0^2)	2.38×10^{-4}

[a] $t s_a$ – confidence interval of the intercept at 95% confidence level.
[b] $t s_b$ – confidence interval of the slope at 95% confidence level.

Table II. Intraday and interday assays

Concentration (mol L^{-1})		Error (%)	RSD (%)[a]	SE[b]
Taken	Found			
Intraday assay				
9.20×10^{-6}	9.25×10^{-6}	0.5	1.1	0.01
1.84×10^{-5}	1.84×10^{-5}	0.3	0.7	0.01
3.22×10^{-5}	3.19×10^{-5}	1.0	1.6	0.04
Interday assay				
9.20×10^{-6}	9.25×10^{-6}	0.5	1.0	0.01
1.84×10^{-5}	1.84×10^{-5}	0.3	0.6	0.01
3.22×10^{-5}	3.21×10^{-5}	0.3	0.7	0.02

[a] Six independent analyses of reference captopril solution.
[b] Standard error.

Table III. Determination of captopril in pharmaceutical formulations using the standard addition technique

Formulation	Concentration (mol L^{-1})			Recovery (%)	RSD (%)[a]	SE[b]
	Taken	Added	Found			
Aceten	9.20×10^{-6}	9.20×10^{-6}	1.86×10^{-5}	101.4	1.8	0.03
	9.20×10^{-6}	2.76×10^{-5}	3.67×10^{-5}	99.8	1.1	0.03
	3.22×10^{-5}	1.38×10^{-5}	4.63×10^{-5}	100.6	0.6	0.02

[a] Six independent analyses.
[b] Standard error.

Table IV. Preliminary selectivity data[a]

Captopril concentrations (mol L^{-1})		Recovery (%)[b]
Taken	Found	
6.40×10^{-6}	6.38×10^{-6}	99.68
1.25×10^{-5}	1.26×10^{-5}	100.80
4.80×10^{-5}	4.78×10^{-5}	99.58

[a] Synthetic mixture containing excipients (lactose, silicon dioxide, starch, microcrystalline cellulose and magnesium stearate, 1.4, 0.2, 1.8, 0.5 and 0.13 mg mL^{-1}, respectively) in water.
[b] Mean of five independent analyses.

for the analysis of commercial dosage forms (Table V) show that the true bias is less than ± 2%. Thus, the interval hypothesis test has also indicated the acceptable performance of the proposed method.

Table V. Comparison of the proposed kinetic spectrophotometric method with conventional spectrophotometry

	Proposed method						Literature method (18)	
Formulation	Mean recovery (%)[a]	RSD (%)[a]	*t*-value[b]	*F*-value[b]	θ^c_L	θ^c_U	Mean recovery (%)[a]	RSD (%)[a]
Aceten	99.3	1.5	0.46	2.87	0.98	1.01	99.6	0.9

[a] Six independent analyses.
[b] Theoretical *t*- and *F*- values at 95% confidence level are 1.812 and 5.05, respectively.
[c] In pharmaceutical analysis, a bias in recovery experiments of ± 2% ($\theta_L = 0.98$ and $\theta_U = 1.02$) is acceptable.

The performance of the proposed method has been compared with other existing methods. It has been found that the proposed method has the following advantages: (*i*) more rapid and easy to perform analysis compared to other methods (11, 14, 17); (*ii*) low cost compared to GC (3), HPLC (4) and CE (5).

CONCLUSIONS

The initial-rate method can be easily applied to the determination of captopril in bulk and in dosage forms. The proposed method is sensitive enough to enable the determination of a lower amount of drug. These advantages encouraged the application of the proposed method in routine quality control of captopril in industrial laboratories.

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S A Ž E T A K

Osjetljiva kinetička spektrofotometrijska metoda za određivanje kaptoprila

NAFISUR RAHMAN, NISHAT ANWAR, MOHAMMAD KASHIF i NASRUL HODA

Razvijena je jednostavna osjetljiva kinetička spektrofotometrijska metoda za određivanje kaptoprila. Metoda se temelji na redukciji Fe(III) u Fe(II) koji zatim s kalijevim fericijanidom daje plavo obojeni produkt. Nastajanje produkta praćeno je spektrofotometrijski na valnoj duljini 730 nm. Optimalne koncentracije $FeCl_3$ i kalijeva fericijanida bile su $1{,}23 \times 10^{-3}$ mol L^{-1}, odnosno $3{,}04 \times 10^{-4}$ mol L^{-1}. Početna brzina upotrebljena je za izradu baždarnog pravca. Linearnost je postignuta u koncentracijskom području od $4{,}60 \times 10^{-6}$ do $5{,}06 \times 10^{-5}$ mol L^{-1}; granica detekcije bila je $1{,}99 \times 10^{-7}$ mol L^{-1}. Predložena metoda je validirana. Srednja vrijednost analitičkog povrata iznosila je 99,8–101,4% uz RSD < 2%. Uobičajeni ekscipiensi nisu smetali određivanju. Ispitivanja hipoteze točke i intervala potvrdila su da nema značajne razlike između predložene metode i opisane spektrofotometrijske metode. Stvarna eksperimentalna pogreška za sve uzorke bila je manja od ± 2%. Opisana metoda primijenjena je za određivanje kaptoprila kao čiste supstancije i u ljekovitom pripravku.

Ključne riječi: kaptopril, određivanje, kinetička spektrofotometrijska metoda

Analytical Research Laboratory, Department of Chemistry, Aligarh Muslim University, Aligarh-202002 (U.P.), India

Neurotoxicology Laboratory, Deptt. of Medical Elementology and Toxicology, Jamia Hamdard, Hamdard Nagar, New Delhi-62, India