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Kinetic Spectrophotometric Determination of Ramipril in Commercial Dosage Forms

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***Abstract*—**This paper presents a simple and sensitive kinetic spectrophotometric method for the determination of ramipril in commercial dosage forms. The method is based on the reaction of the drug with 1-chloro-2,4-dinitrobenzene (CDNB) in dimethylsulfoxide (DMSO) at 100 ± 1°C. The reaction is followed spectrophotometrically by measuring the rate of change of the absorbance at 420 nm. Fixed-time (ΔA) and equilibrium methods are adopted for constructing the calibration curves. Both the calibration curves were found to be linear over the concentration ranges 20 - 220 μg/ml. The regression analysis of calibration data yielded the linear equations: $\Delta A = 6.30 \times 10^{-4} + 1.54 \times 10^{-3} C$ and $A = 3.62 \times 10^{-4} + 6.35 \times 10^{-3} C$ for fixed time (Δ A) and equilibrium methods, respectively. The limits of detection (LOD) for fixed time and equilibrium methods are 1.47 and 1.05 μg/ml, respectively. The method has been successfully applied to the determination of ramipril in commercial dosage forms. Statistical comparison of the results shows that there is no significant difference between the proposed methods and Abdellatef's spectrophotometric method.

***Keywords*—**Equilibrium method, Fixed-time (ΔA) method, Ramipril, Spectrophotometry.

I. 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 [M.W. 416.51] is an angiotensin-converting enzyme inhibitor (ACE). It is used in the treatment of hypertension, heart failure and following myocardial infarction. It is also used to reduce the risk of cardiovascular events in patients with certain risk factors. Ramipril acts as a prodrug of diacid ramiprilat. Ramipril owes its activity to ramiprilat to which it is converted after oral administration [1,2]. This drug is officially listed in British Pharmacopoeia [3] which describes a potentiometric titration procedure for its assay in tablets. In order to assure the quantity of ramipril in dosage forms, several methods have been reported which include LC-MS [4], HPLC [5-8], capillary electrophoresis [9], atomic absorption spectrophotometry [10,11], voltammetry [12], ion selective electrode potentiometry [13], and radioimmunoassay [14].

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The literature revealed that several spectrophotometric methods have been reported for the quantitative analysis of ramipril in pharmaceutical preparations and biological fluids. The estimation of ramipril in a binary mixture was carried out by derivative compensation technique [15] as well as zero crossing derivative technique [16,17]. The determination of ramipril was performed based on ion-pair complex of the drug with picric acid and bromocresol green [18], formation of ternary complex of the drug with Cu (II) and eosin [10], and Fe (III) and ammonium thiocyanate [11]. The charge transfer complexation reaction has also been utilized for its quantification using 7,7,8,8-tetracyanoquinodimethane, p-chloranilic acid and 2,3-dichloro-5,6-dicyano-p-benzoquinone [19]. Potassium permanganate oxidizes ramipril in alkaline medium and itself reduces to manganate ion, which was measured at 610 nm [20]. The determination of ramipril 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 [21]. The reaction of ramipril with a mixture of potassium iodide and potassium iodate has been utilized for its assay in commercial dosage forms [22].

This paper represents a simple and sensitive kinetic spectrophotmetric method for the determination of ramipril in bulk and dosage forms. The method is based on the reaction of ramipril with CDNB in DMSO at 100 ± 1°C. The absorbance (λ_{max} = 420 nm) of the colored product increases as a function of heating time. Therefore, two calibration procedures i.e. fixed time (ΔA) and equilibrium methods are adopted for the determination of ramipril in bulk and commercial dosage forms.

II. Materials

A. Apparatus

Spectral runs were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan). All other spectrophotometric measurements were made on Spectronic 20 D^+ spectrophotometer (Milton Roy Company, USA). A water bath shaker (NSW 133, New Delhi, India) was used for heating.

1. Reagents

Ramipril reference standard (Batch No. TR001FO2) was kindly provided by Dr. Reddy's Laboratories Limited (Andhra Pradesh, India). CDNB and DMSO were purchased from

Merck Limited, Mumbai, India. The pharmaceutical preparations of ramipril such as Ramipres-2.5 (Cipla, Mumbai, India), Ramace-2.5 (AstraZeneca, Mumbai, India) and Hopace-2.5 (Cardicare, Bangalore, India) were obtained from local market.

2. Standard Solutions

- 0.7% CDNB (3.46×10^{-2} M) was prepared in DMSO.
- 0.1% ramipril (2.40×10^{-3} M) was also prepared in DMSO.

II. Methods

A. Recommended Procedure for the Determination of Ramipril

Accurately measured aliquots of standard solution of ramipril corresponding to 0.1 - 1.1 mg were transferred into a series of 5 ml standard volumetric flasks. To each flask, 2.2 ml of 3.46×10^{-2} M CDNB was added and diluted to volume with DMSO. The content of the mixture was kept on water bath at 100 ± 1ºC for a preselected fixed time. The absorbance of the yellow colored product was measured at 420 nm against the reagent blank as a function of time. The following two procedures were adopted for constructing the calibration graphs:

- Fixed-time method (ΔA): The change in absorbance (ΔA) between the times, t_1 (3 min) and t_2 (6 min) was computed and plotted against the initial concentration of ramipril.
- Equilibrium method: The equilibrium was attained at 30 min of heating. The absorbance measured at 32 min of heating was plotted against the initial concentration of ramipril.

Alternatively, regression equations were also developed for estimation of the content of ramipril.

1. Analysis of Commercial Dosage Forms

To determine the content of ramipril in pharmaceutical preparations (label claim: 2.5 mg/tablet or capsule), the contents of 20 tablets or capsules were weighed and finely powdered. A portion of the powder equivalent to 50 mg ramipril was stirred with 20 ml DMSO and let stand for 10 min. The residue was filtered on Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) and washed with DMSO. The filtrate and washings were diluted to 30 ml with DMSO. This solution was further diluted as necessary to complete the analysis following the recommended procedures.

2. Procedure for Reference Method [10]

An aliquot of standard solution containing 0.1 - 1.0 mg of ramipril (0.1%) was transferred into a series of 50.0 ml separating funnels. The volume of each solution was adjusted to 10.0 ml with distilled water and 3.0 ml of 0.2% Cu (II) sulfate and 1.0 ml of 0.1% eosin were added with swirling. The funnels were shaken vigorously with 3.0 × 3.0 ml portions of chloroform for 1.0 min, 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 and a calibration graph was constructed.

B. Validation Parameters

1. 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 (ramipril diketopiperazine) and common excipients (sodium stearyl fumarate, magnesium stearate, starch, lactose and talc).

2. Recovery Studies

Recovery experiments were carried out by standard addition method. For this, 4.0 ml (or 8.0 ml) of reference ramipril solution (1.0 mg/ml) was transferred into a 100.0 ml volumetric flask followed by 6.0 ml of sample solution (1.0 mg/ml) and volume was completed up to the mark with DMSO. The total amount was determined by the recommended procedure.

3. Limits of Detection and Quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated using the following equations [23]:

$$LOD = 3.3 \times S_0 / b \text{ and } LOQ = 10 \times S_0 / b$$

where S_0 and b are the standard deviation and slope of the calibration line, respectively.

III. Results and Discussion

A yellow colored product with maximum absorption at 420 nm was formed when ramipril was allowed to react with CDNB in DMSO while the reagent, CDNB, exhibited absorption band at 350.5 nm. At room temperature, the reaction did not take place, hence the reaction was carried out at 100 ± 1°C to increase the rate of reaction and decrease the time for attaining equilibrium. At this temperature, the intensity of the colored product increased with time and attained equilibrium in 30 min. The increase in absorbance as a function of time was utilized to develop a kinetically based spectrophotometric method for the determination of ramipril.

A. Optimization of Reaction Conditions

The optimum conditions affecting the formation of Meisenheimer complex were studied and maintained throughout the experiment to determine the quantity of ramipril in bulk and drug formulations.

1. Effect of Heating Time

To optimize the reaction time for color development, 2.2 ml of 3.46×10^{-2} M CDNB and 1.0 ml of 2.40×10^{-3} M ramipril were added and kept on water bath at 100±1°C for varied time. The maximum intensity of color was obtained at 30 min of heating and remained constant up to 34 min. Therefore, 32 min. of reaction time was used throughout the determination process in equilibrium method (Fig. 1).

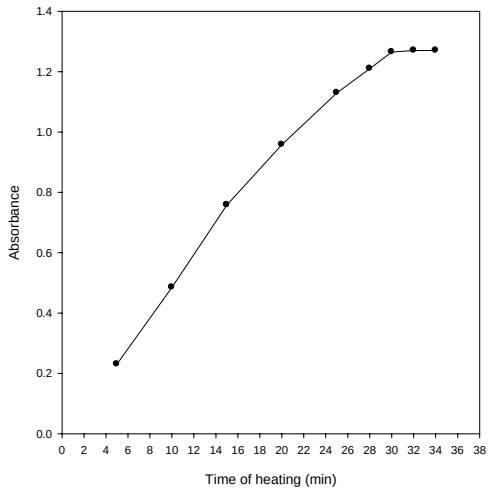


Fig. 1 Effect of heating time on the absorbance of the colored product (ramipril 200.0 □g/ml)

2. Effect of the Concentration of CDNB

The effect of the concentration of CDNB on the colored product was studied using the equilibrium procedure in the range 1.38×10^{-3} - 1.66×10^{-2} M. The absorbance increased with increase in concentration of CDNB. The maximum absorbance was obtained with 1.38×10^{-2} M; above this concentration absorbance remained constant (Fig. 2). Thus, 1.52×10^{-2} M was used for subsequent studies.

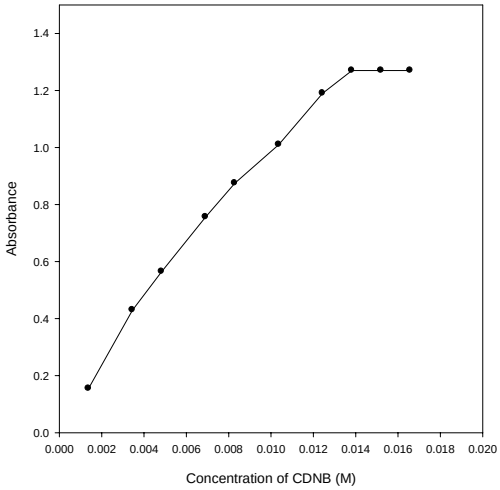


Fig. 2 Effect of the concentration of CDNB on the absorbance of the colored product (ramipril 200.0 □g/ml)

B. Stoichiometry

The stoichiometry of the reaction was established by Bent and French method [24]. The slopes of the plots of logarithm of absorbance versus logarithm of respective varied molar concentration were calculated and found to be 1:1 between ramipril and CDNB (Fig. 3). The mole ratio method was also employed to evaluate the combining ratio. The plot of absorbance versus mole ratio (ramipril/ CDNB) has also confirmed that one mole of ramipril reacted with 1 mole of CDNB (Fig. 4). The apparent formation constant and Gibbs free energy ($\Delta G^{\ddagger}$) were calculated and found to be 7.20×10^{7} and -56.12 KJ mol^{-1}, respectively.

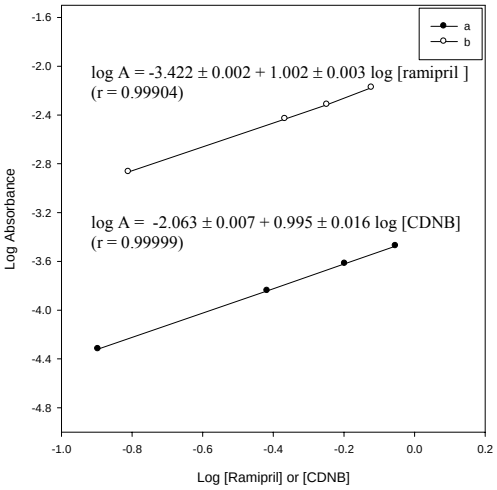


Fig. 3 Limiting logarithmic plots for molar combining ratio between ramipril and CDNB: (a) log Absorbance versus log [Ramipril]; (b) log Absorbance versus log [CDNB]

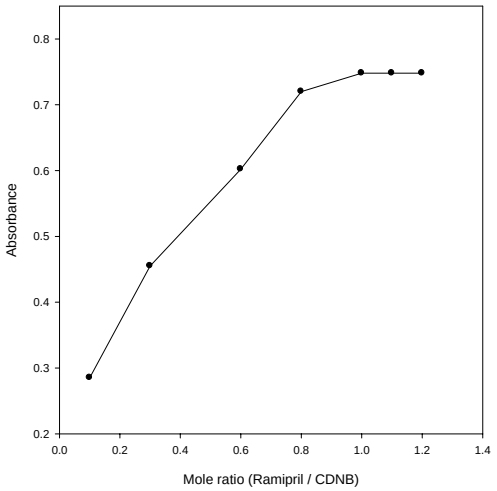
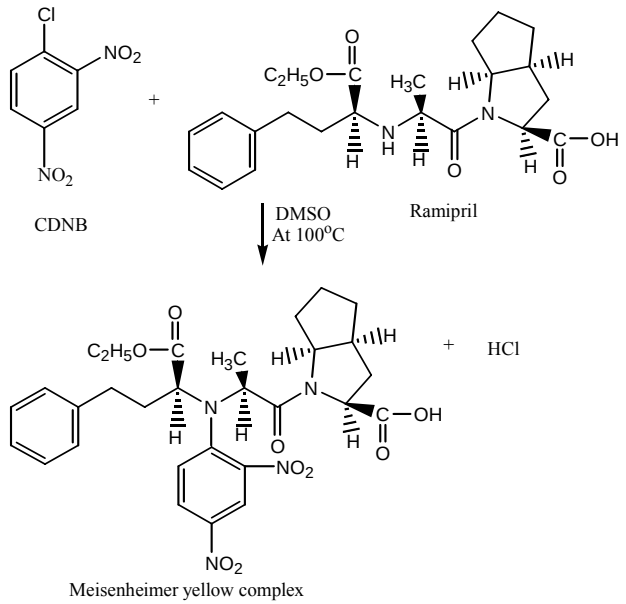


Fig. 4 Mole ratio plot for the reaction between ramipril and CDNB in DMSO ([Ramipril]= [CDNB] = 3.46×10^{-3} M)

C. Mechanism of the Reaction

A literature survey revealed that brightly colored products are formed when polynitroaromatic and halopolynitroaromatic compounds react with bases. The formation of colored products is due to various interactions depending upon the degree to which the base participates through its unshared electron pair with the nitro compounds [25]. Ramipril possesses –NH group in its structure thus behaves as a base. The addition of CDNB to ramipril in DMSO yielded the 1-

substituted Meisenheimer complex, which absorbs maximally at 420 nm. On the basis of our experimental findings and literature background [26], the reaction mechanism is proposed and given in Scheme 1.



Scheme 1

D. Solution Stability

The solution stability was ascertained from UV spectra of reference standard and quality control samples. The reference standard of the drug showed an absorption peak at 260 nm. The standard and quality control sample solutions were kept at room temperature for five days and it was observed that there was no change in the absorption spectra of these solutions. The solution stability was also ascertained by applying the standard and quality control samples on TLC plates coated with silica gel G (Merck Limited, Mumbai, India) and developed in methanol-chloroform (2:8 v/v) solvent system. The plates were air-dried and spots were detected in the iodine chamber. In both the cases, a single spot was observed with R_f = 0.80 corresponding to ramipril.

E. Analytical Performance

Under the optimized experimental conditions mentioned above, the absorbance of each solution was recorded as a function of time at a regular interval of 3 min. The change in absorbance (ΔA) between the times t_1 (3 min) and t_2 (6 or 9, 12, 15 min) was computed and plotted against the initial concentration of ramipril. The corresponding linear regression equations with coefficient of correlations are summarized in Table I. As can be seen from the table that the most acceptable linearity was obtained when the change in absorbance between 3 and 6 min (i.e. $\Delta A = A_6 - A_3$) was plotted against the initial concentration of ramipril. This fixed time was adopted for the assay procedure.

TABLE I
CALIBRATION EQUATIONS AT DIFFERENT FIXED TIMES

ΔA	Calibration equation	r	S_a	S_b
$A_6 - A_3$	$\Delta A = 6.30 \times 10^{-4} + 1.54 \times 10^{-3} C$	0.9999	4.14×10^{-4}	3.14×10^{-4}
$A_9 - A_3$	$\Delta A = -1.43 \times 10^{-3} + 2.04 \times 10^{-3} C$	0.9998	1.81×10^{-3}	1.37×10^{-5}
$A_{12} - A_3$	$\Delta A = -3.36 \times 10^{-3} + 2.62 \times 10^{-3} C$	0.9998	2.17×10^{-3}	1.64×10^{-5}
$A_{15} - A_3$	$\Delta A = -6.90 \times 10^{-3} + 3.16 \times 10^{-3} C$	0.9997	4.18×10^{-3}	3.16×10^{-5}

The reaction between ramipril and CDNB required 30 min to complete. Therefore, in equilibrium procedure, the calibration curve was constructed by plotting absorbance measured after attaining the equilibrium (32 min) against the initial concentration of ramipril. The linear dynamic range, molar absorptivity, regression equation, correlation coefficient, confidence limits for slope ($\pm tS_b$) and intercept ($\pm tS_a$), limits of detection and determination, and variance of calibration line for both the procedures are summarized in Table II. The high values of correlation coefficient indicated the excellent linearity of the calibration curves. The small values of confidence limit of slope and intercept pointed towards good reproducibility of the proposed procedures.

TABLE II
OPTICAL AND REGRESSION CHARACTERISTICS OF FIXED TIME AND EQUILIBRIUM METHODS

Parameters	Fixed time method (ΔA)	Equilibrium Method
Linear dynamic range (μg/ml)	20 - 220	20 - 220
Molar absorptivity (l/mol/cm)	-	2.654×10^3
Regression equation	$\Delta A = 6.30 \times 10^{-4} + 1.54 \times 10^{-3} C$	$A = 3.62 \times 10^{-4} + 6.35 \times 10^{-3} C$
$\pm t S_a$	9.79×10^{-6}	3.88×10^{-3}
$\pm t S_b$	7.42×10^{-4}	2.61×10^{-5}
Correlation coefficient (r)	0.9999	0.9999
LOD (μg/ml)	1.47	1.05
LOQ (μg/ml)	4.46	3.18
Variance (S_o^2) of calibration line	4.72×0^{-7}	4.08×0^{-6}

$\pm t S_a$ & $\pm t S_b$ are C.L. for intercept and slope, respectively

The accuracy and precision of the fixed time and equilibrium methods were evaluated by performing five successive measurements within one day at three different concentration levels (40, 120 and 220 μg/ml). The inter day precision was measured by assaying the bulk sample on five consecutive days. The results are summarized in Table III. The results showed that the RSD (< 0.22%) and standard analytical error (< 0.04) found in intra and inter day assays can be considered to be very satisfactory.

TABLE III
EVALUATION OF ACCURACY AND PRECISION OF THE PROPOSED METHODS

Proposed methods	Amount (μg/ml)		RSD (%)	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]			
Fixed time (Δ A)					
Intra day assay	40.0	40.007 ± 0.058	0.15	0.026	0.072
	120.0	119.985 ± 0.057	0.05	0.026	0.071
	220.0	219.994 ± 0.071	0.03	0.032	0.088
Inter day assay	40.0	39.967 ± 0.080	0.20	0.036	0.099
	120.0	119.987 ± 0.064	0.05	0.029	0.080
	220.0	219.942 ± 0.074	0.03	0.033	0.092
Equilibrium method					
Intra day assay	40.0	39.990 ± 0.080	0.20	0.036	0.100
	120.0	119.981 ± 0.068	0.06	0.031	0.085
	220.0	219.981 ± 0.072	0.03	0.032	0.089
Inter day assay	40.0	40.006 ± 0.086	0.22	0.039	0.107
	120.0	120.012 ± 0.078	0.07	0.035	0.097
	220.0	220.031 ± 0.082	0.04	0.036	0.101

[a]Mean for 5 independent analyses.
[b]SAE, standard analytical error.
[c]C.L., confidence limit at 95 % confidence level and 4 degrees of freedom (t = 2.776).

To test the applicability of the proposed methods, recovery experiments were performed using the standard addition method. For this purpose, a known amount of pure ramipril was spiked to its formulated preparations and the total amount of the drug was estimated. The results are summarized in Table IV. As can be seen from the table that the mean recovery ranged from 99.98 -100.03% with low RSD values. Moreover, it was also found that the degraded product of ramipril and common excipients such as sodium stearyl fumarate, magnesium stearate, starch, lactose and talc did not interfere with determination process.

The proposed methods have been applied for the analysis of ramipril in commercial dosage forms. The analysis of the same batch of samples was also completed by Abdellatef's spectrophotometric method [10]. The results of the proposed method (fixed time or equilibrium method) were compared with those obtained by the Abdellatef's spectrophotometric method statistically. The student's t- and F-values at 95% confidence level did not exceed the tabulated t- and F- values. The interval hypothesis test [27] has also been performed to compare the results at 95% confidence level. The results presented in Table V showed that the true bias of all samples is smaller than ±2% indicating the compliance of the regulatory authorities [28]. These tests have confirmed that there is no significant difference between the performances of the methods compared.

IV. CONCLUSION

The proposed method does not require any pretreatment of the drug or any laborious clean up procedure before measurement. In addition, the method has wider linear dynamic range with good accuracy and precision. Point and interval hypothesis tests proved that the proposed methods have acceptable bias of ± 2% and hence the data presented, demonstrate that the method is accurate and precise for the determination of ramipril in commercial dosage forms. Therefore, the proposed method can be used in the laboratories of research, hospitals and pharmaceutical industries.

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REFERENCES

[1] D. N. Franz, "Cardiovascular Drugs (Ed: A. R. Gennaro)," in *Remington: The Science and Practice of Pharmacy*, 19th ed., Vol. II, Mack Publishing Company, Pennsylvania, 1995, p. 951.
[2] T.G. Warner and M. C. Perry, "Ramipril: a review of its use in the prevention of cardiovascular outcomes," *Drugs,* vol. 62, pp.1381-1405, 2002.
[3] Royal Pharmaceutical Society, "*British Pharmacopoeia,"* vol. II, H. M. Stationery Office, Royal Pharmaceutical Society, London, UK, 2000, pp.1331-1333.
[4] Z. Zhimeng, V. Andre and N. Len, "Liquid chromatography-mass spectrometry method for determination of ramipril and its active metabolite ramiprilat in human plasma," *J. Chromatography B.,* vol. 779, pp. 297-306, 2002.
[5] B. Francesco, M. Patrizia and Q. Fabiasa, "Analysis of ACE inhibtor drugs by high performance liquid chromatography," *Il Farmaco*, vol. 49, pp. 457-460, 1994.
[6] U. J. Dhorda and N. B. Shetkar, "RP-HPLC determination of ramipril and amlodipine in tablets," *Indian Drugs,* vol. 36, pp. 638-641, 1999.
[7] S. S. Zarapakar and S. H. Rane, "RP-HPLC determination of ramipril and hydrochlorothiazide in tablets," *Indian Drugs,* vol. 37, pp. 589-593, 2000.
[8] J. N. Harlikar and A. M. Amlani, "Simultaneous determination of perindopril, indapamide, ramipril, trandapril in pharmaceutical formulations using RP- HPLC," *Res. J. Chem. Environ.,* vol. 7, pp. 59-62, 2003.
[9] S. Hillaer, K. De Grauwe and W. Van den Bossche, "Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis," *J.Chromatogr. A*, vol. 924**,** pp**.** 439- 449, 2001.
[10] H. E. Abdellatef, M. M. Ayad and E. A. Taha, "Spectrophotometric and atomic absorption spectrophotometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu (II)," *J. Pharm. Biomed. Anal.,* Vol. 18, pp. 1021-1027, 1999.
[11] M. M. Ayad, A. A. Shalaby, H. E. Abdellatef and M. M. Hosny, "Spectrophotometric and AAS determination of ramipril and enalapril

through ternary complex formation," *J. Pharm. Biomed. Anal.,* vol. 28, pp. 311-321, 2002.
[12] J. A. Prieto, R. M. Jimenez and R. M. Alonso, "Square wave voltammetric determination of angiotensin- converting enzyme inhibitors cilazapril, quinapril and ramipril in pharmaceutical formulation," *Il Farmaco,* vol. 58, pp. 343-350, 2003.
[13] H. Y. Aboul-Enein, R. I. Stefen and J. F. Van Staden, "Analysis of several angiotensin-converting enzyme inhibitors using potentiometric, enantioselective membrane electrodes," *Anal. Lett.,* vol. 32, pp. 623-632, 1999.
[14] H. G. Eckert, G. Muenscher, R. Oekonomopulos, H. Strecker, H. Urbach and H. A. Wissmann, "Radioimmunoassay for the II angiotensin-converting enzyme inhibitor ramipril and its active metabolite," *Arzneim.-Forsch,* vol. 35, pp.1251-1256, 1985.
[15] H. H. Abdine, F. A. El-Yazbi, R. A. Shaalan and S. M.Blaih, "Direct differential solubility and compensatory- derivative spectrophotometric methods for resolving and subsequently determining binary mixtures of some anti hypertensive drugs," *S. T. P. Pharm Sci.,* vol. 9, pp.587-591, 1999.
[16] H. Salem, "Derivative spectrophotometric determination of two component mixtures," *Chin. Pharm. J.,* vol. 51, pp. 123-142, 1999.
[17] N. Erk, "Ratio-spectra-zero-crossing derivative spectrophotometric determination of certain drugs in two component mixtures," *Anal. Lett.*, vol. 32, pp. 1371-1388, 1999.
[18] S.M. Blaih, H. H. Abdine, F. A. El-Yazbi and R. A. Shaalan, "Spectrophotometric determination of enalapril maleate and ramipril in dosage forms," *Spectrosc. Lett.,* vol. 33, pp. 91-102, 2000.
[19] F. M. Salama, O. I. A. El-Sattar, N. M. El-Aba Sawy and M.M. Fuad, "Spectrophotometric determination of some ACE inhibitors through charge transfer complexes," *Al Azhar J. Pharm. Sci.,* vol. 27, pp. 121-132, 2001.
[20] A. A. Al-Majed, F. Belal and A. A. Al-Warthan, "Spectrophotometric determination of ramipril (a novel ACE inhibitor) in dosage forms," *Spectrosc. Lett*., vol. 34, pp. 211-220, 2001.
[21] A. A. Al-Majed and J. Al-Zehouri, "Use of 7- fluoro-4-nitrobenzo-2-oxo-1,3-diazole for the determination of ramipril in tablet and spiked human plasma," *Il Farmaco,* vol. 56, pp. 291-296, 2001.
[22] N. Rahman, Y. Ahmad and S. N. H. Azmi, "Kinetic spectrophotometric method for the determination of ramipril in pharmaceutical formulations," *A. A. P. S. PharmSciTech.,* vol.6, pp. 543-551, 2005.
[23] J. Ermer, "Validation in pharmaceutical analysis. Part I: An integrated approach.," *J. Pharm. Biomed. Anal.,* vol. 24, pp.755-767, 2001.
[24] H. E. Bent and C. L. French, "The structure of ferric thiocyanate and its dissociation in aqueous solution," *J. Am. Chem. Soc.,* vol. 63, pp. 568-572, 1941.
[25] E. Buncell, A. R. Norris and K. E. Russell, "The interaction of aromatic nitro compounds with bases," *Q. Rev. Chem. Soc.*, vol. 22, pp.123-125, 1968.
[26] E. Buncell, A. R. Norris and K. E. Russell, "The interaction of aromatic nitro compounds with bases," *Q. Rev. Chem. Soc.*, vol. 22, pp.123-125, 1968.
[27] M. J. Strauss, "Anionic sigma complexes," *Chem. Rev.,* vol. 70, pp. 667-712, 1970.
[28] C. Hartmann, J. Smeyers-Verbeke, W. Pinninckx, Y.V. Heyden, P. Vankeerberghen and D. L. Massart, "Reappraisal of hypothesis testing for method validation: detection systematic error by comparing the means of two methods or of two laboratories," *Anal. Chem*., vol. 67, pp.4491-4499, 1995.
[29] Canada Health Protection Branch, "*Drugs Directorate Guidelines, Acceptable methods",* Ministry of National Health and Welfare, Draft, Ottawa, Canada , 1992.

TABLE IV
STANDARD ADDITION METHOD FOR THE DETERMINATION OF RAMIPRIL IN TABLETS AND CAPSULE

Formulations	Fixed time (ΔA) method					Equlibrium method				
	Amount (μg/ml)			Recovery (%)	RSD (%)	Amount (μg/ml)			Recovery (%)	RSD (%)
	Taken	Added	Found ± SD[a]			Taken	Added	Found ± SD[a]		
Tablets										
Ramipres (Cipla)	60.0	40.0	99.98 ± 0.06	99.98	0.06	60.0	40.0	99.98 ± 0.07	99.98	0.07
	60.0	80.0	139.98 ± 0.06	99.99	0.05	60.0	80.0	140.01 ± 0.06	100.00	0.04
Ramace (AstraZeneca)	60.0	40.0	99.99 ± 0.08	99.99	0.08	60.0	40.0	99.99 ± 0.07	99.99	0.07
	60.0	80.0	139.98 ± 0.05	99.99	0.04	60.0	80.0	139.99 ± 0.06	99.99	0.04
Capsule										
Hopace (Cardicare)	60.0	40.0	100.01 ± 0.06	100.01	0.06	60.0	40.0	100.02 ± 0.09	100.02	0.09
	60.0	80.0	140.04 ± 0.06	100.03	0.05	60.0	80.0	139.99 ± 0.07	99.99	0.05

[a] Mean for 5 independent analysis

TABLE V
COMPARISON OF THE PROPOSED METHODS WITH THE REFERENCE METHOD

Formulations	Fixed time method		Equilibrium method		Reference method	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Tablets						
Ramipres (Cipla)	100.03 $\theta_L = 0.986$ t = 0.115	0.05 $\theta_U = 1.012$ F = 2.628	99.99 $\theta_L = 0.986$ t = 0.016	0.07 $\theta_U = 1.013$ F = 1.818	99.98	0.09
Ramace (AstraZeneca)	100.01 $\theta_L = 0.987$ t = 0.063	0.06 $\theta_U = 1.012$ F = 1.889	100.00 $\theta_L = 0.987$ t = 0.038	0.07 $\theta_U = 1.013$ F = 1.443	99.98	0.08
Capsule						
Hopace (Cardicare)	99.95 $\theta_L = 0.986$ t = 0.081	0.06 $\theta_U = 1.014$ F = 2.819	99.98 $\theta_L = 0.983$ t = 0.029	0.09 $\theta_U = 1.016$ F = 1.217	100.00	0.10

[a]Theoretical t- (ν = 8) and F-values (ν = 4, 4) at 95 % confidence level are 2.306 and 6.39, respectively
θ_L and θ_U are within the acceptable limits of ±2%

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