Spectrophotometric and Atomic Absorption Determination of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation with Molybdenum (V)-Thiocyanate (Mo(V)–SCN)

Mohamed Mohamed BARAKA,*[,a] Mohamed EL-SADEK,[a] Ewais Moussa Mohamed. MOUSSA,[b] and Nashat Mohamed Alanowar ABD-ALATY[b]

[a] *Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University; P.C.44519, Zagazig, Egypt: and* [b] *Nuclear Materials Authority; Cairo, P.O. Box 530 Almady, Egypt.*

Received December 29, 2007; accepted July 6, 2008; published online August 4, 2008

Three different sensitive and accurate spectroscopic procedures were developed for the determination of three angiotensin-converting enzyme inhibitors, namely, ramipril, enalapril maleate and fosinopril. The first two spectrophotometric (extractive and non-extractive) procedures were based on ternary complex formation with molybdenum(V) thiocyanate. The formed complex can be determined by extraction with chloroform measured at λ_{max} 517 nm Beer's law was obeyed in the concentration range from (10—90 μg ml^{-1}) for ramipril and fosinopril and (4—36 μg ml^{-1}) for enalapril maleate with molar absorptivity 1.2×10^4, 2×10^4 and 3.4×10^4 l mol^{-1} cm^{-1}, respectively, or by direct measurement after addition of benzalkonium chloride as surfactant and measuring the formed ternary complex at λ_{max} 545 nm with a linear relationship in the concentration range from (8—72 μg ml^{-1}), (3—27 μg ml^{-1}) and (8—72 μg ml^{-1}) for ramipril, enalapril maleate and fosinopril with molar absorptivity 1.5×10^4, 5×10^4 and 2.1×10^4 l mol^{-1} cm^{-1}, respectively. The third procedure is atomic absorption measurement through the quantitative determination of molybdenum content of the complex. These methods hold their accuracy and precision well when applied to the determination of ramipril, enalapril maleate and fosinopril in their dosage forms.

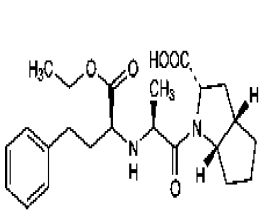
Key words ramipril; enalapril maleate; fosinopril; ternary complex; spectrophotometry; atomic absorption

The official drugs, ramipril: 4-[2-(1-ethoxycarbonyl-3-phenyl-propyl)aminopropionyl]-4-aza bicyclo[3.3.0]octane-3-carboxylic acid and enalapril maleate: 1-[2-(1-ethoxycarbonyl-3-phenyl-propyl)aminopropionyl]pyrrolidine-2-carboxylic acid and the non official fosinopril: 4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxy-propoxy)-(4-phenylbutyl)-phosphoryl]acetyl]-pyrrolidine-2-carboxylic acid are antihypertensive agents which their metabolites are active inhibitors of angiotensin-converting enzyme (ACE); inhibition of ACE results in decreased plasma angiotensin II which leads to decreased vasopressor activity.[1)] Ramipril and enalapril maleate are official in B.P.[2)] and enalapril maleate is official in USP 24,[3)] while fosinopril is unofficial in any pharmacopoeia.

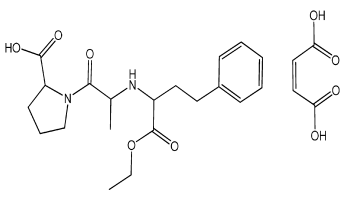
Various analytical methods have been reported for the assay of the cited drugs in their pure forms as well as in pharmaceutical formulations. They include for ramipril, a kinetic spectrophotometry,[4)] spectrophotometry and atomic absorption,[5)] fluorimetry,[6)] HPLC,[7)] bioavailability assessment of nanoemulsion,[8)] reversed-phase HPLC,[9)] and liquid chromatography-mass spectrometry.[10)] For enalapril, spectrophotometry using chelate formation with palladium(II) chloride,[11)] spectrophotometry in the presence of its photo degradation products,[12)] quantitative ^{1}H-NMR spectroscopy,[13)] second-derivative ultraviolet spectrophotometry,[14)] HPLC in the presence of the major active metabolite enalaprilate,[15)] linear regression analysis and its application to multivariate chromatographic calibration,[16)] HPTLC,[17)] radio-immunoassay,[18)] potentiometric titration[19)] and differential pulse polarography (DPP).[20)] For fosinopril with H_2-receptor antagonists by derivative spectroscopy,[21)] UV and third derivative spectrophotometry,[22)] microemulsion liquid chromatography,[23)] HPLC in the presence of its degradation product fosinoprilate,[24)] TLC procedures[25)] and reversed-phase ion-pair liquid chromatography.[26)]

Several colorimetric methods for determination of some drugs in pharmaceutical preparations using molybdenum (V) (Mo(V)) thiocyanate reagent forming ion-pair complex were reported such as; chloroquine and pyrimethamine,[27)] ampicillin, dicloxacillin, flucloxacillin and amoxacillin,[28)] some piperazine derivatives,[29)] trazodone, amineptine and amitriptyline hydrochloride,[30)] metoclopramide and oxybuprocaine,[31)] and some H_1-antihistaminics.[32)]

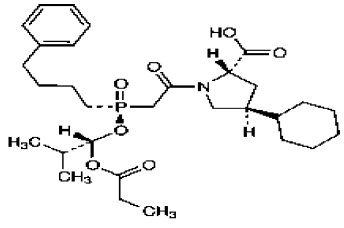
Although the chromatographic methods are sensitive enough, they are expensive and not easily manageable. On the other hand; spectrophotometry is still the technique of



Ramipril



Enalapril Maleate



Fosinopril

* To whom correspondence should be addressed. e-mail: m3baraka@yahoo.com

choice since it is sensitive, economical, rapid and more easily managed.

This paper reports simple, sensitive, economical and accurate spectrophotometric methods for the analysis of ramipril, enalapril maleate and fosinopril in their pure and dosage forms. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used as excipients in the pharmaceutical dosage forms do not interfere in the determination of the studied drugs.

Experimental

Instruments Metertech Inc. SP-8001 UV–VIS spectrophotometer (Taiwan, R.O.C.) with 1 cm quartz cells connected to an IBM computer loaded with software application, GBC 932 AA atomic absorption spectrophotometer.

Materials and Reagents Chemicals used were of the highest purity available from their sources in the form of pure analytical grade: Ramipril pure drug (purity 99.42): Manufactured by Dr. Reddy's laboratories, Batch No. ED004A0L, LTD. Ameerpet, Hyperabad (India).

Tritace tablets containing 2.5 mg ramipril per tablet: Manufactured in Egypt under license from Aventis Pharma-Germany, Batch No. 13E13.

Enalapril maleate pure drug (purity 99.64): Manufactured by Dr. Reddy's laboratories, Batch No. EMFPO18, LTD. Norsapur TQ. Medak DT, A.P, (India).

Ezapril tablets containing 10 mg enalapril per tablet: Offered by Kahira Pharm. and Chem. Ind. Co. for Multipharma. Co., Batch No. 0410067.

Fosinopril pure drug (purity 99.33): Offered by Bristol-Myers Squib, Egypt, Batch No. L4132.

Monopril tablet containing 10 mg fosinopril per tablet: Offered by Bristol-Myers Squib Egypt under license from Bristol-Myers Squib, New York, Batch No. L43541.

Hydrochloric acid: Ubchem, U.N. 1789, code H014C.

Ammonium molybdate: Adwic, Batch No. 8110027.

Ammonium thiocyanate: Fluka, Chemika, Batch No. AG CH-9470.

Ascorbic acid solution: AR laboratory and fine chemicals L (+) ascorbic acid, Batch No. 100127.

Chloroform: Adwic, Batch No. C0068111.

Working Solutions. Pure Drugs Spectrophotometric method: 1mg ml^{-1} of ramipril, fosinopril and 0.5 mg ml^{-1} enalapril maleate solution in distilled water.

Atomic absorption method (AAS): 0.18 mg ml^{-1} ramipril, 0.19 mg ml^{-1} enalapril maleate and 0.16 mg ml^{-1} fosinopril solution in distilled water.

Ammonium molybdate: prepared as 1×10^{-3} M in distilled water solution.

Ammonium thiocyanate: prepared as 10% (w/v) solution in distilled water.

Ascorbic acid solution: prepared as 10% (w/v) solution in distilled water.

Benzalkonium chloride: prepared as 5% (w/v) solution in distilled water.

General Procedures. Spectrophotometric Method Extractive Method: Into 50 ml separating funnels, 5 ml of 1×10^{-3} M ammonium molybdate solution, 2 ml of 3 M HCl, 1 ml 10% ascorbic acid solution and 4 ml of 10% ammonium thiocyanate solution were transferred. Left for 15 min, till formation of Mo(V)– thiocyanate (SCN) complex, varying aliquots of the standard ramipril, enalapril maleate and fosinopril solutions containing (0.25—2.25 mg), (0.1—0.9 mg) and (0.25—2.25 mg) respectively were added. The reaction was left for 10 min. The aqueous solution was extracted with 2×10 ml portions of chloroform, shacked for 1 min and the extract was transferred into 25 ml volumetric flasks after drying over anhydrous sodium sulphate, the volume was completed to 25 ml with chloroform and the absorbance measured at the convenient λ_{max} 517 nm against a blank solution omitting the drugs.

Non-extractive Procedure: Appropriate volumes of the standard solution containing (0.2—1.8 mg), (0.075—0.675 mg) and (0.2—1.8 mg) of ramipril, enalapril maleate and fosinopril, respectively, 5 ml of 1×10^{-3} M ammonium molybdate solution, 2 ml 3 M HCl, 1 ml 10% ascorbic acid solution and 4 ml of 10% ammonium thiocyanate were added in heating tubes. Left for 15 min until complete formation of Mo(V)–SCN complex, 3 ml of 5% benzalkonium chloride was added, the mixture was homogenized by shaking, immersed in a water bath at 50 °C for 30 min, then cooled to room temperature. The mixture was transferred into a 25 ml volumetric flask then diluted to volume with distilled water and the absorbance was measured at λ_{max} 545 nm against a blank.

Atomic Absorption Method (AAS) Variable volumes ranging from 0.25—2.25 ml (pure drugs AAS method) of ramipril, enalapril maleate and fosinopril were treated as mentioned under extractive procedure until the volume was completed to 25 ml with chloroform. The collected chloroformic extract was evaporated to dryness, the residue was dissolved in 1 ml conc. HCl and the volume was completed with distilled water to 25 ml in a volumetric flask. A blank (omitting addition of the drugs) was performed and the absorbance was measured by atomic absorption using the following conditions: analysis wavelength, 313.3 nm; lamp current, 7 mA; slit width, 0.2 nm; work head height, 15 mm; burner slot/flame, 5 cm (air–C_2H_2); supporting gas flow, air acetylene; fuel gas flow, 2 l/min; absorption sensitivity, 0.11 ppm; air pressure, 10 l min^{-1}; atomizing temperature, 1700 °C.

The concentration of the consumed molybdenum was calculated from a calibration graph of standard ammonium molybdate solution.

For Pharmaceutical Preparation Twenty tablets were weighed and finely powdered, the quantity of the powdered tablets of each drug equivalent to the concentration taken in the pure drug was transferred and dissolved in distilled water. The assays for ramipril, enalapril maleate and fosinopril were done as described under general procedures applying the standard addition technique. The amount of drug was calculated either from the calibration graph or the regression equation.

Results and Discussion

Ternary complexes of the general formula ($L_NM_XS_Y$) have been widely used in spectrophotometric analysis.[33—37] The complex is extractable with organic solvents, such as hydrocarbons and their halogenated derivatives with higher values of molar extinction coefficient than the binary complexes; in respect to this paper the main ligand L is the investigated drug , the second ligand S is SCN^- and M is Mo.

The main object of this work focused on the fact that an ion-pairs is formed between the secondary amine group of both ramipril and enalapril and the tertiary amine group of fosinopril and molybdenum(V)–thiocyanate binary complex through the protonated nitrogen atom of these drugs.[31] The reduction probability of Mo(VI) may occur by ascorbic acid or SCN^- in acidic media[31] and react with thiocyanate to form a red binary Mo(V)–SCN^- complex, non extractable with chloroform.

$$\mathrm{Mo(V)} \xrightarrow[3\ \mathrm{M\ HCl}]{\text{ascorbic acid}} \mathrm{Mo(VI)} \xrightarrow{6SCN^-} \mathrm{Mo(SCN)_6^-}$$

It was also found that the sensitivity and stability of the molybdenum(V)–thiocyanate ion-pair binary complex is enhanced by using ascorbic acid. Ascorbic acid gives reproducible values and masks many interfering ions.[38]

On addition of the cited drugs, an orange red complex was formed, which is extractable with chloroform. The cited drugs were determined by three different techniques (extractable, non extractable and atomic absorption). The optimal conditions for each of the above procedures were carefully studied as follows.

Optimization of Reaction Conditions The formation of ternary complex was confirmed by studying the absorption curves for the three drugs (lower than 200 nm in ethanol) and the formed ternary complex (drug–Mo(V)–SCN) with λ_{max} 517 nm in chloroform, while the binary complex of Mo(V)–SCN is not extractable in chloroform and so it dos not interfere with the formed complex (Fig. 1).

In the non-extractive method the formed binary complex of Mo(V)–SCN in distilled water was measured at λ_{max} 465 nm, while the ternary complex of the three drugs was measured at λ_{max} 545 nm (Fig. 2).

The effect of different variables of the reagent concentration, reaction time, solvent of extraction, and stability has

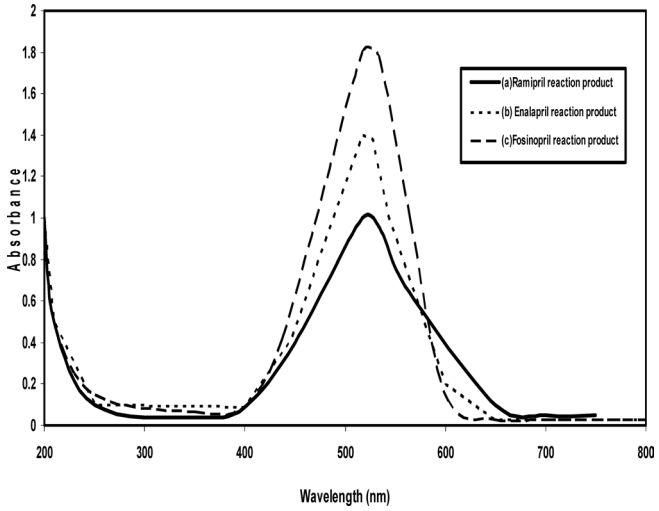


Fig. 1. Absorption Spectra of the Ternary Complex of (a) Ramipril 30 μg ml^{-1}, (b) Enalapril Maleate 20 μg ml^{-1}, (c) Fosinopril 50 μg ml^{-1} and Mo(V)–SCN 1×10^{-3} M in Chloroform at λ_{max} 517 nm

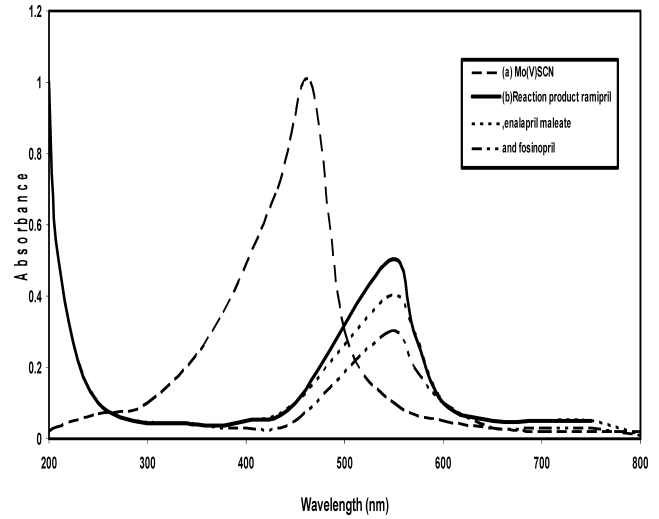


Fig. 2. Absorption Spectra of (a) Mo(V)–SCN in Distilled Water at λ_{max} 465 nm and (b) Ternary Complex of 8 μg ml^{-1} Ramipril, 3 μg ml^{-1} Enalapril Maleate, and Mo(V)–SCN 1×10^{-3} M in Distilled Water at λ_{max} 545 nm by Non-extractive Method

been studied.

Extractive Method Five milliliters of ammonium molybdate and 4 ml of ammonium thiocyanate maximize the color intensity. The maximum absorption of the ternary complex was observed after 10 min and it was found to be stable more than 24 h.

Chloroform, methylene chloride, toluene and benzene were tried, chloroform gave the highest color intensity.

Two milliliters of 3 M HCl and 1 ml of 10% ascorbic acid were sufficient to give maximum color absorpitivity.

Non-extractive Method To avoid the extraction problem and to increase sensitivity of the method, many surfactants were examined; sodium lauryl sulphate, methylcellulose, benzalkonium chloride, tween 40, myrj and brij, the results obtained rivaled the maximum color observed in the presence of 3 ml of benzalkonium chloride.

Other factors including the reagent volumes used in the extractive method are the same, the only difference is heating the reaction mixture at 50 °C for 20 min to enhance the formation of the ternary complex.

Atomic Absorption Method (AAS) The effect of reagents, time, order of additions and solvents which give maximum sensitivity were found the same as the extractive procedure.

The organic solvent of the ternary complex in the atomic absorption spectrometer and the high chlorine/carbon ratio would lead to the formation of a large quantity of HCl in the flame which would damage the instrument.[39,40] To avoid this, the formed complex was extracted with organic solvent, the solvent was evaporated to dryness then the residue was dissolved in HCl and diluted with distilled water.

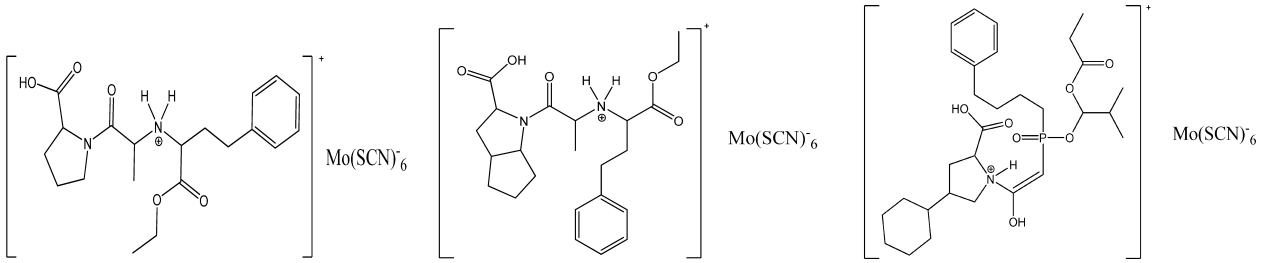
Stoichiometry of the Reaction To study the stoichiometry of the reaction, the molar ratio between Mo(V) and each of the investigated drug in equimolar solutions and in the presence of an excess amount of ammonium thiocyanate was determined using job's method.[41] It was found that the ratio was 1 : 1 for the three drugs when using an enalapril base as shown in the proposed Chart 1, but when enalapril used as maleate the ratio was 1 : 2 (Figs. 3, 4).

Linearity and Quantification. Extractive Method A linear relationship was obtained for the absorbance of Mo(V)–SCN with the cited drugs in the concentration ranges of (10—90 μg ml^{-1}), (4—36 μg ml^{-1}) and (10—90 μg ml^{-1}) for ramipril, enalapril maleate and fosinopril, respectively.

Non-extractive Method A linear relationship was obtained over concentration ranges of (8—72 μg ml^{-1}), (3—27 μg ml^{-1}) and (8—72 μg ml^{-1}) for ramipril, enalapril maleate and fosinopril, respectively.

Under the optimized condition, the optical and statistical parameters for the proposed methods are summarized in Table 1. The molar absorptivity, Sandell's sensitivity, correlation coefficients, slopes and intercepts were listed.

The good linearity of the calibration graph and the negligible scatter of the experimental points were clearly evident from the value of the correlation coefficient and variance.



Mo (SCN)$^-_6$ – Ramipril ion –pair complex Mo (SCN)$^-_6$- Enalapril ion –pair complex Mo (SCN)$^-_6$ –Fosinopril ion –pair complex

Chart 1

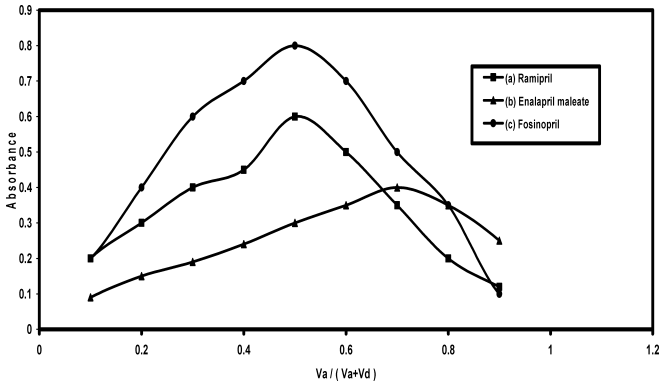


Fig. 3. Continuous Variation Plot for (1×10^{-3} M) Ammonium Molybdate and (1×10^{-3} M) of (a) Ramipril, (b) Enalapril Maleate and (c) Fosinopril by Extractive Method Va=Drug and Vd=Mo(V)–SCN

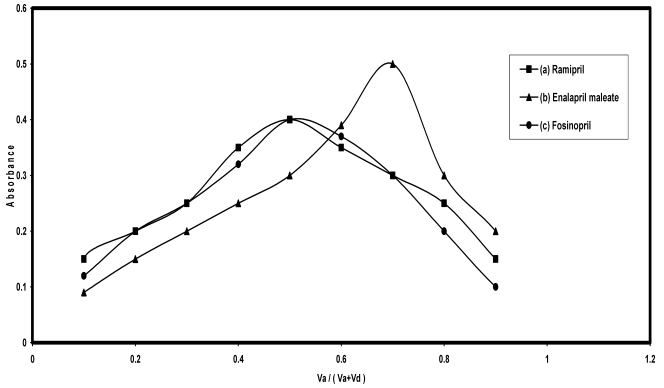


Fig. 4. Continuous Variation Plot for (1×10^{-3} M) Ammonium Molybdate and (1×10^{-3} M) of (a) Ramipril, (b) Enalapril Maleate and (c) Non-extractive Method Using Surfactant Va=Drug and Vd=Mo(V)–SCN

Table 1. Assay Parameter and Spectral Data for Spectrophotometric Determination of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation with Mo(V)–SCN by Extractive and Non-extractive Method

Parameter	Ramipril		Enalapril maleate		Fosinopril	
	Extractive method	Non-extractive method	Extractive method	Non-extractive method	Extractive method	Non-extractive method
Reaction time (min)	10	30	10	30	10	30
Temperature (°C)	Room temperature	50	Room temperature	50	Room temperature	50
Ammonium molybdate concentration	5 ml (1×10^{-3})	5 ml (1×10^{-3})	5 ml (1×10^{-3})	5 ml (1×10^{-3})	5 ml (1×10^{-3})	5 ml (1×10^{-3})
Ammonium thiocyanate concentration 10%	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml
λ_{max} for pure drug in ethanol (nm)	Lower than 200	Lower than 200	Lower than 200	Lower than 200	Lower than 200	Lower than 200
λ_{max} for pure drug in methanol (nm)	210	210	208	208	210	210
λ_{max} of Mo(V)–thiocyanate (nm) in distilled water	—	465	—	465	—	465
λ_{max} for the ternary complex (nm)	517	545	517	545	517	545
Linearity range $\mu g\,ml^{-1}$	10—90	8—72	4—36	3—27	10—90	8—72
Slope (b)	0.02	0.03125	0.025	0.083	0.0625	0.0375
Intercept (a)	0.4	0.25	0.55	0.15	0.15	0.001
Variance	0.977	0.693	0.763	0.893	0.367	0.571
Correlation coefficient	0.9997	0.9999	0.9994	0.9999	0.9998	0.9999
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	1.2×10^4	1.5×10^4	3.4×10^4	5×10^4	2×10^4	2.1×10^4
Sandell's sensitivity ($\mu g\,cm^{-2}$)	3×10^{-2}	2.4×10^{-2}	1.8×10^{-2}	1×10^{-2}	1.4×10^{-2}	2.6×10^{-2}
Limit of detection LOD ($\mu g\,ml^{-1}$)	1.05	0.096	0.84	0.036	0.336	0.08
Limit of quantification LOQ ($\mu g\,ml^{-1}$)	3.5	0.32	2.8	0.12	1.12	0.26

The proposed methods were successfully applied for the determination of pure drugs. Performance of the proposed was assessed by comparing the calculated *t* and *F* values with the reference method (A_{max}. UV spectra in methanol). The results showed that the *t* and *F* values were less than the tabulated ones indicating that there was no significant difference between the proposed and reference method, Table 2.

Tablets containing ramipril, enalapril maleate and fosinopril were analyzed by the proposed methods applying the standard addition technique. The obtained results compared with the reference method and statistical analysis of the results showed that there is no interference from the common additives and excipients, indicating a high selectivity for determining the studied drugs in their dosage forms, Table 3.

Atomic Absorption Method (AAS) The sensitivity of the method is increased by using atomic absorption measurements; a linear relationship was obtained in the concentration ranges of (1.8—16.2 $\mu g\,ml^{-1}$), (1.9—17.1 $\mu g\,ml^{-1}$) and (1.6—14.4 $\mu g\,ml^{-1}$) for ramipril, enalapril maleate and fosinopril respectively, according to the molar ratio of their respective complexes, it was found that, 0.91 $\mu g\,ml^{-1}$ Mo= 9 $\mu g\,ml^{-1}$ ramipril, 0.81 $\mu g\,ml^{-1}$ Mo=9.5 $\mu g\,ml^{-1}$ enalapril maleate and 0.59 $\mu g\,ml^{-1}$ Mo=8 $\mu g\,ml^{-1}$ fosinopril.

The method was successfully applied for the determination of both pure form and the pharmaceutical preparation containing the selected drugs. Statistical analyses of the data revealed that there was no significant difference between the AAS method and the reference method, and moreover the AAS method is more highly sensitive than the spectrophotometric methods, Tables 4, 5.

Sensitivity The detection limit (LOD) for the two spectrophotometric methods (extractive and non-extractive) method was calculated using the following equation.[42)]

$$LOD=3S/K$$

where *S* is the standard deviation of the replicate determina-

Table 2. Statistical Data for Determination of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation Using Mo(V)–Thiocyanate by Extractive and Non-extractive Method Compared with the Reference Method

Statistic	Ramipril			Enalapril maleate			Fosinopril		
	Reference method	Extractive method	Non-extractive method	Reference method	Extractive method	Non-extractive method	Reference method	Extractive method	Non-extractive method
Mean recovery[a] ±S.D.	99.62±1.0137	99.08±0.988	99.7±0.833	99.8±0.9028	99.94±0.874	99.66±0.945	100.14±1.176	99.62±0.606	99.358 ±0.756
n	5	5	5	5	5	5	5	5	5
Variance	1.0276	0.977	0.693	0.815	0.763	0.893	1.383	0.367	0.571
S.E.	0.453	0.442	0.373	0.4037	0.391	0.423	0.526	0.271	0.338
t-test[b]		0.852	0.237		0.552	0.341		0.879	1.251
F-test[b]		1.05	1.483		1.07	1.096		3.77	2.422

a) Average of three experiments. *b*) Theoretical *t* and *F* value are 2.77 and 6.39, respectively for 4 degree of freedom at 95% confidence level.

Table 3. Statistical Data for Determination of Pharmaceutical Preparation of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation with Mo(V)–Thiocyanate by Extractive and Non-extractive Method Compared with the Reference Method

Statistic	Ramipril (Tritace 2.5 mg)			Enalapril maleate (Ezapril 10 mg)			Fosinopril (Minopril 10 mg)		
	Reference method	Extractive method	Non-extractive method	Reference method	Extractive method	Non-extractive method	Reference method	Extractive method	Non-extractive method
Mean recovery[a] ±S.D.	99.62±1.0137	98.92±0.557	99.92±0.887	99.8±0.9028	99.74±0.623	99.7±0.623	100.14±1.176	99.68±0.859	99.34±0.918
n	5	5	5	5	5	5	5	5	5
Variance	1.0276	0.31	0.787	0.815	0.388	0.955	1.383	0.737	0.843
S.E.	0.453	0.249	0.397	0.4037	0.279	0.437	0.526	0.384	0.384
t-test[b]		1.35	0.498		0.122	0.168		0.198	1.19
F-test[b]		3.314	1.305		2.10	1.172		1.876	1.641

a) Average of three experiments. *b*) Theoretical *t* and *F* value are 2.77 and 6.39, respectively for 4 degree of freedom at 95% confidence level.

Table 4. Statistical Data for Determination of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation with Mo(V)–Thiocyanate by AAS Method Compared with the Reference Method

Statistic	Ramipril		Enalapril maleate		Fosinopril	
	Reference method	AAS method	Reference method	AAS method	Reference method	AAS method
Mean recovery[a] ±S.D.	99.62±1.0137	100.5±0.748	99.8±0.9028	99.96±0.844	100.14±1.176	100.784±0.111
n	5	5	5	5	5	5
Variance	1.0276	0.56	0.815	0.713	1.383	1.232
S.E	0.32077	0.335	0.4037	0.377	0.526	0.496
t-test[b]		1.562		0.29		0.891
F-test[b]		1.835		1.143		1.23

a) Average of three experiments. *b*) Theoretical *t* and *F* value are 2.77 and 6.39, respectively for 4 degree of freedom at 95% confidence level.

Table 5. Statistical Data for Determination of Pharmaceutical Preparation of Ramipril, Enalapril Maleate and Fosinopril through Ternary Complex Formation with Mo(V)–Thiocyanate by AAS Method Compared with the Reference Method

Statistic	Ramipril (Tritace 2.5 mg)		Enalapril maleate (Ezapril 10 mg)		Fosinopril (Minopril 10 mg)	
	Reference method	AAS method	Reference method	AAS method	Reference method	AAS method
Mean recovery[a] ±S.D.	99.62±1.014	100.136±0.867	99.8±0.903	100.278±0.594	100.14±1.176	99.986±0.468
n	5	5	5	5	5	5
Variance	1.0276	0.571	0.815	0.352	1.383	0.219
S.E.	0.453	0.388	0.4037	0.266	0.526	0.209
t-test[b]		1.45		0.989		0.272
F-test[b]		1.799		2.315		6.315

a) Average of three experiments. *b*) Theoretical *t* and *F* value are 2.77 and 6.39, respectively for 4 degree of freedom at 95% confidence level.

Table 6. Evaluation of the Accuracy and Precision of the Proposed Methods

Compared method	Statistical parameter				
	Taken (μg ml^{-1})	Found±S.D.[a)]	RSD (%)	SAE[b)]	Confidence limit[c)]
Ramipril					
Extractive method	30	29.400±0.0130	0.01	0.006	0.011
	50	49.608±0.0096	0.02	0.0043	0.008
	70	69.832±0.0130	0.027	0.0085	0.017
Non-extractive method	24	23.436±0.0290	0.12	0.013	0.025
	40	40.040±0.1140	0.28	0.051	0.09
	56	55.830±0.0354	0.063	0.016	0.031
AAS method	2	2.0164±0.020	0.99	0.008	0.0.18
	2.2	2.2300±0.016	0.72	0.007	0.017
	2.4	2.3970±0.006	0.25	0.003	0.006
Enalapril maleate					
Extractive method	12	12.10±0.0580	0.48	0.025	0.051
	20	19.826±0.031	0.062	0.014	0.027
	28	27.68±0.0187	0.065	0.008	0.016
Non-extractive method	9	8.94±0.0350	0.39	0.016	0.031
	15	14.822±0.019	0.013	0.0085	0.017
	21	21.08±0.1240	0.59	0.055	0.11
AAS method	2.3	2.328±0.023	0.99	0.01	0.02
	2.5	2.530±0.016	0.63	0.006	0.014
	2.8	2.812±0.013	0.46	0.005	0.011
Fosinopril					
Extractive method	30	29.660±0.060	0.2	0.027	0.053
	50	50.068±0.110	0.22	0.049	0.096
	70	69.344±0.038	0.055	0.017	0.031
Non-extractive method	24	23.95±0.035	0.15	0.016	0.031
	40	39.56±0.048	0.12	0.021	0.017
	56	54.96±0.038	0.07	0.017	0.11
AAS method	2	2.030±0.016	0.79	0.007	0.014
	2.4	2.412±0.013	0.54	0.006	0.011
	2.8	2.812±0.012	0.46	0.005	0.01

a) Mean±standard deviation for five determinations. *b*) Standard analytical error. *c*) Confidence limits at $p=0.95$ and 4 degree of freedom.

tion values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were calculated and listed in Table 1.

The limit of quantification, LOQ is defined as[42)]

$$\mathrm{LOQ}=10S/K$$

According to this equation, the limits of quantification were calculated and are listed in Table 1.

Precision and Accuracy Precision and accuracy studies of the proposed methods were done by carrying out five independent determinations at three concentration levels. The small RSD% and SAE indicate excellent precision and accuracy, Table 6.

As an additional confirmation of accuracy and precision, recovery experiments were performed by adding known amount of the pure drugs to the preanalyzed dosage forms. No interference from the common excipients was observed in the results.

Conclusion

The described methods are based on the formation of an ion-pair ternary complex between the cited drugs and molybdenum(V)–thiocyanate. The methods can use both spectrophotometric and AAS techniques for the final measurement step. Moreover, the simplicity and convenience at low cost as well as the reproducibility, accuracy and sensitivity of the quantitation procedure are superior to those obtained from the official titrimetric method[2)] and other spectrophotometric methods.[11,43)]

The commonly used additives such as starch, silicon dioxide, magnesium stearate, glucose and talc do not interfere with the assay procedure. Therefore, the methods are useful for routine analytical and quality control assay of the investigated drugs.

References

1) Adithan C., Swaminathan M. D., *Drug Alert Regional Pharmacovigilance Centr.*, **2**, 1177—1181 (2006).
2) "The British Pharmacopoeia," Vol. 1, 2, HM Stationery Office, London, 2007, pp. 763—765, 1786—1788.
3) "The United States Pharmacopoeia," 24 Revision, The National Formulary, United States Pharamacopeial Convention, Inc., 2000, p. 638.
4) Nafisur R., Yasmin A., Syed N. A., *Uttar Pradesh, India*, **6**, 543—551 (2005).
5) Abdellatef H. E., Ayad M. M., Taha E. A., *J. Pharm. Biomed. Anal.*, **18**, 1021—1027 (1999).
6) Al-Majed A. A., Al-Zehouri J., *Il Farmaco*, **56**, 291—296 (2001).
7) Hanysova L., Vaclavcova M., Dohnal J., Klimes J., *J. Pharm. Biomed. Anal.*, **37**, 1179—1183 (2005).
8) Sheikh S., Faiyaz S., Sushma T., Farhan J., Roop K., Mushir A., *Eur. J. Pharm. Biopharm.*, **66**, 227—243 (2007).
9) Baing M. M., Vaidya V., Sane R. T., Menon S. N., Dalvi K., *J. Chromatogr.*, **64**, 293—296 (2006).
10) Zhu Z., Vachareau A., Neirinck L., *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.*, **779**, 297—306 (2002).
11) Ayad M. M., Shalaby A. A., Abdellatef H. E., Hosny M. M., *J. Anal. Bioanal. Chem.*, **375**, 556—560 (2003).
12) de los A. Oliva M., Sombra L. L., Olsina R. A., Masi A. N., *J. Fluo-*

resc., **2005**, 723—728 (2005).
13) Zoppi M., Marcela L., *J. Pharm. Biomed. Anal.*, **37**, 627—630 (2004).
14) Carlucci G., Digiusipi E., Mazzo P., *Int. J. Pharm.*, **93**, 245—248 (1993).
15) Phensri T., Chotima P., *J. Pharm. Biomed. Anal.*, **37**, 763—769 (2004).
16) Erdal D., Abdil D., *Il Farmaco*, **60**, 591—597 (2005).
17) Bhushan R., Shravankumarsingh J. B., *Biomed. Chromatogr.*, **20**, 217—224 (2006).
18) Arafat T., Awad R., Hamad M., Azzam R., Al-Nasan A., Jehanli A., Matalka, K., *J. Clin. Pharm. Ther.*, **30**, 319—328 (2005).
19) Avdeef A., Berger C. M., *Eur. J. Pharm. Sci.*, **14**, 281—291 (2001).
20) Fikriye E., Guzin A., Sule A., Sidika S., *Turk. J. Chem.*, **27**, 65—69 (2003).
21) Sultana N., Arayne M., Sana A., *Pak. J. Pharm. Sci.*, **20**, 19—25 (2007).
22) Mashru R., Sutariya V. B., Thakker A. J., *Ind. J. Pharm. Sci.*, **68**, 643—645 (2006).
23) Biljana J., Mirjana M., Darko I., Andelija M., Slavko, M., *J. Anal. Bioanal. Chem.*, **383**, 687—694 (2005).
24) Vanovic D., Medenica M., Jancic B., Malenovic A., Markovic S., *J. Chromatogr.*, **60**, S87—S92 (2004).
25) Jadranka O., Biljana S., Mirjana A., *J. Serb. Chem. Soc.*, **71**, 621—628 (2006).
26) Manna L., Valvo L., Alimonti S., *J. Chromatogr.*, **53**, S271—S275 (2001).
27) Khalil S. M., Mohamed G. G., Zayed M. A., Elqudaby H. M., *J. Microchem.*, **64**, 181—186 (2000).
28) Mohamed G. G., *J. Pharm. Biomed. Anal.*, **24**, 561—567 (2001).
29) Abou-ELkheir A., Saleh H. M., El-Mammli M. Y., Emam O. A., Alex J., *Pharm. Sci.*, **16**, 115 (2002).
30) Mohamed G. G., Nour El-dien F. A., Farag E. U., *Spectrochim. Acta A*, **65**, 11—19 (2006).
31) Abdel-Gawad F. M., El-Ginudi N. M., *Anal. Lett.*, **28**, 1437—1447 (1995).
32) Hassan W. S., El-Henawee M. M., Gouda A. A., *Spectrochim. Acta A*, **69**, 245—255 (2008).
33) Ayad M. M., Abdellatef H. E., Taha E. A., Soliman S. M., *Zagazig J. Pharm. Sci.* (Egypt), **8**, 7—12 (1999).
34) El-Walily A. M., Belal S. F., Bakry R. S., *J. Pharm. Biomed. Anal.*, **14**, 561—569 (1996).
35) Issopouls P. B., Economou P. T., Fresenius J., *Anal. Chem.*, **343**, 518—522 (1992).
36) Issopouls P. B., Economou P. T., Fresenius J., *Anal. Chem.*, **345**, 595—599 (1993).
37) Fujita Y., Mori I., Fujita K., Tanaka T., Koshiyama Y., Kawabe H., *Chem. Pharm. Bull.*, **34**, 2236—2238 (1986).
38) Biazek J., Mares V., *Chem. Abstr.*, **1967**, 22266f (1967).
39) Cristina N., Agustin G., *Anal. Chem.*, **57**, 34—38 (1985).
40) Nerin C., Cacho J., Garania A., *Anal. Lett.*, **18**, 1887—1891 (1985).
41) Rose J., "Advanced Physico-Chemical Experiments," 1964, p. 54.
42) Miller J. C., Miller J. N., "Statistics for Analytical Chemistry," 3rd ed., Ellis Horwood, Chichester, 1993.
43) Ayad M. M., Shalaby A. A., Abdellatef H. E., Hosny M. M., *J. Pharm. Biomed. Anal.*, **28**, 311—321 (2002).