Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X

RESEARCH ARTICLE

Received on: 27-11-2014
Accepted on: 15-12-2014
Published on: 27-12-2014

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Conflict of Interest: None Declared !

DOI: 10.15272/ajbps.v4i39.642

Spectrophotometric Determination of Some Angiotensin Converting Enzyme Inhibitors By Potassium Dichromate and Potassium Permanganate in Tablet Dosage Form.

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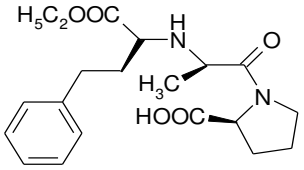
Abstract
Simple, cost effective and reproducible spectrophotometric methods are proposed for the determination of enalapril maleate, lisinopril dihydrate, moexipril hydrochloride and ramipril hydrochloride in pure and tablet doage forms. The methods are based on oxidation of these drugs by either potassium dichromate or potassium permanganate in sulphuric acid medium then measuring of the developed colored reaction products or the decrease in the intensity of color at λmax 610, 520 nm for the potassium dichromate (method A) and potassium permanganate (method B) respectively. Different variables affecting the reaction conditions were carefully studied and optimized. Under optimal experimental conditions the linear range is 20-900 $\mu g.ml^{-1}$ (method A) and 2-500 $\mu g.ml^{-1}$ (method B). The proposed methods were successfully applied to the analysis of the investigated drugs in pure and tablet dosage form. Results were comparable with those obtained by reported spectrophotometric methods.

Keywords: Spectrophotometric, Enalapril maleate, Lisinopril dihydrate, Moexipril hydrochloride, Ramipril hydrochloride, Potassium dichromate, Tablets.

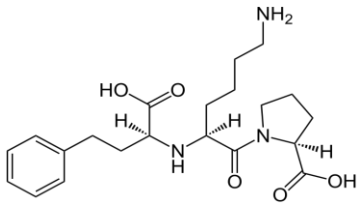
Cite this article as:
Horria A. Mohamed , Pakinaz Y. Khashaba , Reem Y. Shahin. Spectrophotometric Determination of Some Angiotensin Converting Enzyme Inhibitors By Potassium Dichromate and Potassium Permanganate in Tablet Dosage Form. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (39); 2014, 16-24.

INTRODUCTION

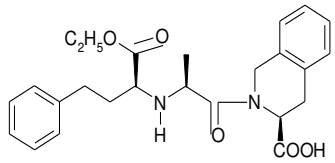
Enalapril (EN) (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-oxo-4-phenylbutan-2-yl]amino}propanoyl]pyrrolidine-2-carboxylic acid (I), lisinopril (LS) [N-[(1S)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline](II),moexipril (MOX) (3S)-2-[(2S)-2-{[(2S)-1-ethoxy- 1-oxo -4- phenylbutan -2-yl] amino} propanoyl]- 6, 7-dimethoxy-1 ,2, 3, 4-tetrahydroisoquinoline-3-carboxylic acid (III) and ramipril (RAM) (2S, 3aS, 6aS) – 1 - [(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta pyrrole-2-carboxylic acid (IV), belong to the class of dicarboxylate containing group of angiotensin convering enzyme inhibitors (ACEIs). They are widely used in the management of essential hypertension, stable chronic heart failure, myocardial infarction and diabetic nephropathy. They act mainly by suppressing the formation of angiotensin II by blocking its formation via rennin and angiotensin I. Thus, due to the vital importance of these drugs in pharmaceutical preparations and biological fluids, several spectroscopic [1-13] HPLC [14-17] and TLC methods [18-20] have been reported for the determination of the investigated drugs. The official methods include HPLC methods in the USP [21] and potentiometric methods in the BP [22]. Some of the reported methods lack adequate sensitivity, some are expensive or time consuming. The aim of this work is to develop simple, convenient, economical method that can be applied for routine analysis of these drugs in both pure and tablet dosage forms. Method A is based on the oxidation of the studied drugs by potassium dichromate in conc. sulphuric acid medium and measuring the green chromium (III) ions at λmax 610 nm. Method B is based on oxidation of the studied drugs by potassium permanganate in conc. sulphuric acid then measuring the decrease in the intensity of color at λmax 520 nm.



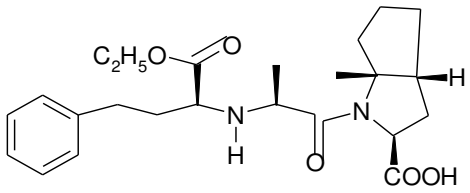
Enalapril (I)



Lisinopril (II)



Moexipril (III)



Ramipril (IV)

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical grade. EN and LS (Global-Nabi Pharmaceuticals, Cairo, Egypt), MOX (MinaPharm, Cairo, Egypt) and RAM (NODCAR, Cairo, Egypt). All drugs were complying with the requirements recommended by official methods and used as received without further purification. Purity was checked spectrophotometrically for EN [23], LS [24], MOX and RAM [25] and was found to be 99.67, 99.84, 100.94 and 101.02% respectively. Conc. Sulphuric acid (El-Nasr chemical Co., Cairo, Egypt) . Potassium dichromate (Rankem chemical Co., New Delhi, India) was 1.8 and 2 M aqueous solution. Dosage forms in this investigation were Ezapril tablets (Multi-Apex Pharma, Badr City, Egypt), Zestril tablets (AstraZeneca, Egypt), Primox tablets (Mina Pharm, Cairo, Egypt) and Tritace tablets (Aventis, Cairo, Egypt) labeled to contain 10, 20 , 15 and 5 mg of EN, LS, MOX and RAM respectively.

Apparatus

UV- visible spectrophotometer UVD 2950 (Labomed, U.S.A), with matching 1 cm quartz cell is used for all measurements and connected to PC using UV-WIN software.

Preparation of standard solution

Method A: Stock standard solution containing 2 mg ml-1 for each of EN, LS, MOX and RAM; respectively were prepared in distilled water. Working standard solution containing 25 – 900 µg ml^{-1}, 20-700 µg ml^{-1}, 30-750 µg ml^{-1} and 45- 650 µg ml^{-1} of EN, LS, MOX and RAM respectively was prepared by suitable dilution with the same solvent.

Method B: Stock standard solutions each containing 1 $mg.ml^{-1}$ of EN, LS, MOX and RAM was prepared in distilled water. Working standard solution containing 10-200 $\mu g.ml^{-1}$ for EN, 2-150 $\mu g.ml^{-1}$ for LS, 2-60 $\mu g.ml^{-1}$ for MOX and 1-500 $\mu g.ml^{-1}$ for RAM; was prepared by suitable dilution with the same solvent.

Preparation of sample solution

Twenty tablets were accurately weighed and finely powdered. An amount of powdered tablets equivalent to 100 mg (method A) or 50 mg(method B) of each of EN, LS, MOX and RAM; respectively was transferred into 50 ml volumetric flask and dissolved in water. The mixture was sonicated for 20 minutes and then completed to volume with the same solvent. The solution was filtered and the first portion of the filtrate was rejected. Working solution was prepared by further suitable dilution of the filtrate by the same solvent.

General assay procedure

Method A: One milliliter of standard or sample solution was accurately measured and transferred into 10 ml volumetric flask. A certain volume of potassium dichromate solution was added : 1.0 ml of 1.8 M solution (for EN), 1.0 ml of 2 M solution (for RAM), 2.0 ml of 2 M solution (for LS) and 2.0 ml of 1.8 M solution (for MOX), then 3.0 ml of conc. sulphuric acid (for EN, MOX and RAM) and 4.0 ml (for LS) were added. The mixture was allowed to stand for 10 minutes at room temperature. The solution was then completed to volume with distilled water and the absorbance (A) was measured at λmax 610 nm against a blank experiment treated similarly.

Method B: One milliliter of standard or sample solution was accurately measured and transferred into 10-ml volumetric flask. A certain volume of $KMnO_4$ solution was added: 1.5 ml of 0.018 M solution (for EN and MOX), 2.0 ml of 0.018 M solution (for RAM) and 2.0 ml of 0.024 M solution (for LS). Then a certain volume of H_2SO_4 was added: 1.5 ml of 5 M solution (for EN and MOX), 1.0 ml of 6 M solution (for RAM) and 1.0 ml of 17M solution (for LS). The mixture was allowed to stand at room temperature for 10 minutes (for MOX) and for 15 minutes for (EN, RAM and LS). The solution was then completed to volume with distilled water and the absorbance (ΔA) was measured at λmax 520 nm against a blank experiment treated similarly.

RESULTS AND DISCUSSION

Oxidation- reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds [26-29]. Method A depends on the oxidation of the studied drugs by potassium dichromate in acidic medium where it is converted into the green chromium (III) ion and measured at λmax 610 nm. The cited drugs were also found to be oxidized by potassium permanganate yielding Mn^{+2} ions resulting in decrease in color intensity at λmax 520 nm (method B). The liability of the studied drugs to oxidation may be due to the specific reactivity of the secondary amino group in EN, MOX and RAM as well as the primary and secondary amino groups in LS [3].

Determination of absorption maxima (λmax)

The absorption spectra of the reaction products of EN (as a representative example) with both potassium dichromate and potassium permanganate are given in Figures (1 and 2). EN has no absorbance at λmax 610 or 520 nm, respectively.

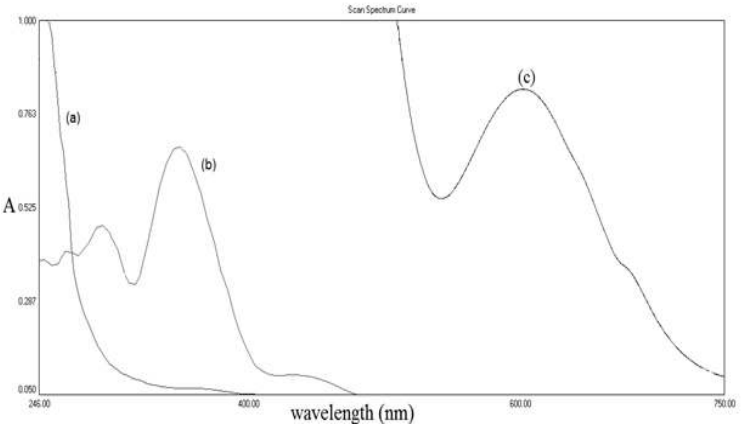


Figure 1: Absorbtion spectra of (a) EN (700 μgml^{-1}), (b) blank, and (c) the reaction product of EN (700 μgml^{-1}) with $K_2Cr_2O_7$ (1.8 **M).**

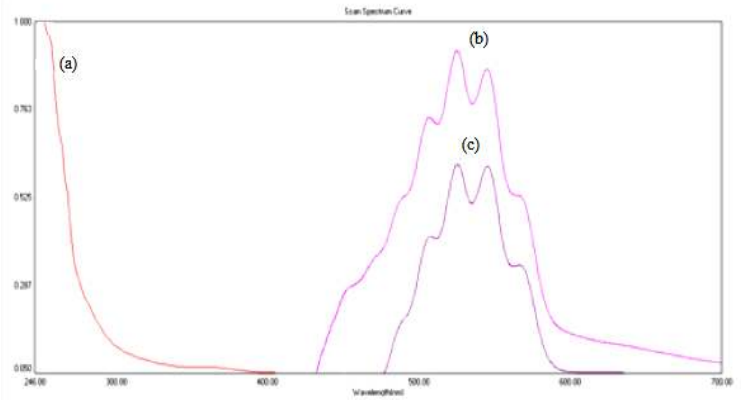


Figure 2: Absorption spectra of (a) En (120 μg.ml^{-1}), (b) blank and (c) Reaction product of En (120 μg.ml^{-1}) with $KMnO_4$ (0.018 M).

Optimization of the reaction conditions

The optimum conditions for the assay procedure have been established by studying reagent concentration and volume, type of acid and volume, variation in reaction time, diluting solvent and stability time. Such variables were changed individually while the others were kept constant.

Effect of reagent concentration

The effect of $K_2Cr_2O_7$ concentration on the reaction with the investigated ACEIs was studied by carrying out the reaction using 1.0 ml of each concentration in the range of 0.2 – 2.2 M $K_2Cr_2O_7$ (Fig. 3). It was observed that the absorbance of reaction product increases by increasing the concentration of $K_2Cr_2O_7$ until maximum absorbance was obtained. The optimum concentration of $K_2Cr_2O_7$ was 1.8 M for EN and MOX and 2.0 M for RAM and LS. The effect of $KMnO_4$ concentration on the reaction with ACEIs was

studied by carrying out the reaction using 1.0 ml of each concentration in the range of 0.006-0.03 M $KMnO_4$ (Fig. 4). It is observed that the absorbance difference (ΔA) increases by increasing the concentration of $KMnO_4$. The optimum concentration chosen of $KMnO_4$ was 0.018 M for EN, MOX and RAM and 0.024 M for LS.

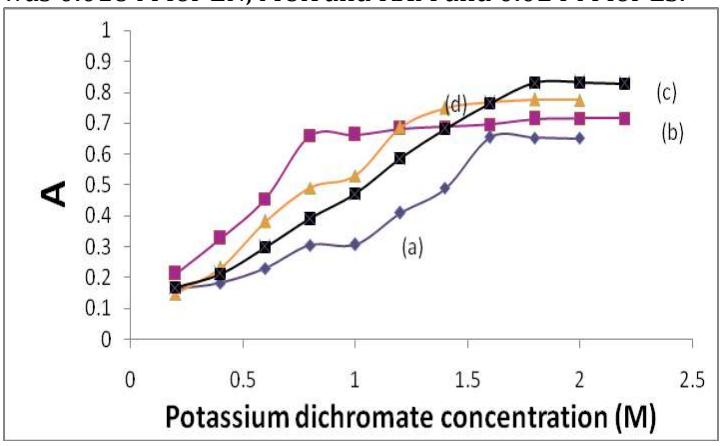


Figure 3: Effect of $K_2Cr_2O_7$ concentration on the absorption intensity of reaction product with (a) EN, (b)LS, (c) RAM and (d) MOX (500 µg.ml^{-1} of each).

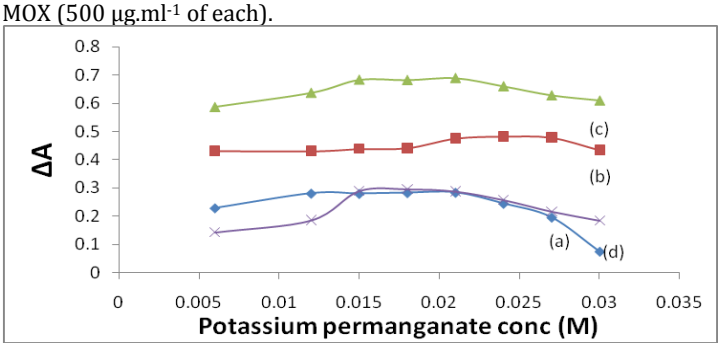


Figure 4: Effect of concentration of $KMnO_4$ on the absorption intensity of its reaction product with (a) EN, (b) MOX, (c) LS and (d) RAM (40 µg.ml^{-1} of each).

Effect of reagent volume

Different volumes of the selected $K_2Cr_2O_7$ molar solutions ranged from 0.5- 3.0 ml were tested, the optimum volume was found to be 1.0 ml for EN and RAM and 2 ml for LS and MOX. The effect of different volumes of the selected $KMnO_4$ concentration (0.5-2.5 ml) on the intensity of the reaction product with the investigated ACEIs was tested. The optimum volume of the selected concentration of $KMnO_4$ was found to be 1.5 ml for EN and MOX and 1.0 ml for LS and RAM.

Type of acid

In order to determine the most appropriate acid, different acids such as sulphuric, nitric, phosphoric, hydrochloric and acetic were tested. Sulphuric acid was selected as it gave the highest absorbance with all the investigated drugs for both methods A&B.

Effect of concentration of sulphuric acid

The preliminary experiments indicated that oxidation of the investigated drugs with $K_2Cr_2O_7$ needs high concentration of sulphuric acid, so in (method A) conc. sulphuric acid was used. While for (method B) Different concentrations of H_2SO_4 ranged from 1-7 M solutions (for EN, MOX and RAM) and 1-18 M solution (for LS) were tested (Table 1). It was found that the optimum conc. of sulphuric acid was 5 M for EN, MOX, and 6M for RAM. For LS, higher concentration of H_2SO_4 (17 M) is required to give maximum absorbance intensity with $KMnO_4$.

H_2SO_4 conc. (mol.ml^{-1})	Drug / absorbance**			
	EN*	MOX*	RAM*	LS*
1.0	0.187	0.269	0.261	0.092
2.0	0.190	0.348	0.286	0.201
4.0	0.195	0.569	0.291	0.238
4.5	0.278	0.678	0.300	–
4.8	0.279	0.679	0.327	–
5.0	**0.277**	**0.684**	0.349	–
5.2	0.274	0.682	0.384	–
5.5	0.261	0.619	0.423	–
5.8	0.246	0.608	0.426	–
6.0	0.230	0.578	**0.424**	0.241
6.2	0.214	0.539	0.427	–
7.0	0.208	0.478	0.397	–
8.0	–	–	–	0.251
10.0	–	–	–	0.255
15.0	–	–	–	0.258
16.0				0.265
16.8				0.284
17.0				**0.286**
17.2				0.279
18.0	–	–	–	0.253

Table 1. Effect of sulphuric acid concentration on the reaction product of the investigated ACEIs with $KMnO_4$.
**Absorbance values are the mean of three determinations.
* Drug concentration is 40 µg.ml^{-1}.

Effect of sulphuric acid volume

In order to determine the most suitable volume of acid used in method A, the reaction was performed using different volumes (0.5 – 6 ml) of sulphuric acid. Fig. 5 shows that the absorption intensity increased as the volume of the acid increased. Optimum volume of sulphuric acid was 3 ml for EN, MOX and RAM and 4 ml for LS. For method B, the reaction was performed using different volumes (0.5-4.0 ml) of H_2SO_4 (Table 2). The amount of H_2SO_4 at which maximum absorbance was obtained was 1.0 ml for EN, MOX and LS and 2.0 ml for RAM.

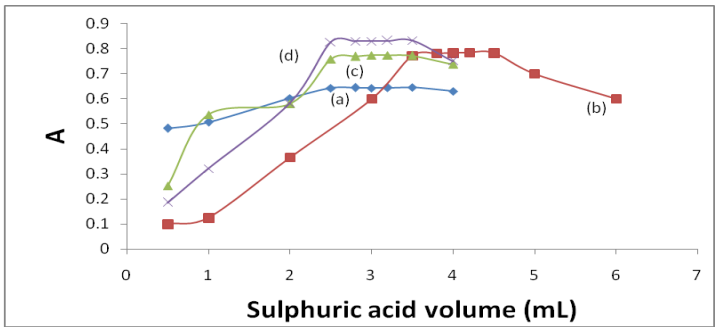


Figure 5: Effect of sulphuric acid volume on the absorption intensity of the $K_2Cr_2O_7$ reaction product with (a) EN, (b) LS, (c) MOX and (d) RAM (500 µg.ml^{-1} of each).

H_2SO_4 vol. (mL)	Drug / absorbance[a]			
	EN[b]	LS[b]	MOX[b]	RAM[b]
0.5	0.251	0.437	0.669	0.288
1	**0.253**	**0.435**	**0.669**	0.289
1.5	0.248	0.438	0.665	0.292
2	0.250	0.434	0.671	**0.296**
2.5	0.254	0.438	0.666	0.288
3.5	0.251	0.436	0.663	0.285
4	0.250	0.433	0.665	0.284

Table 2. Effect of sulphuric acid volume on the absorption intensity of the reaction product of $KMnO_4$ with the investigated ACEIs.
[a] Absorbance values are the mean of three determinations.
[b] Drug concentration is 40µgml^{-1}.

Effect of variation in reaction time

The reaction was carried out for different periods of time (5 – 30 min), and was found to be time dependant. Maximum absorption intensity was obtained after 10 and 15 min. for method A&B respectively for all the investigated drugs (Fig. 6,7).

Effect of diluting solvent

The effect of diluting solvent was also studied by using different solvents of different polarities [30] such as : water, methanol, ethanol, isopropanol and acetone for both methods. Results shown in table 3 revealed that water was the optimum diluting solvent as it gave the maximum absorbance with all the investigated drugs. Dilution with water is advantageous as it is the most cheap and environmentally safe solvent.

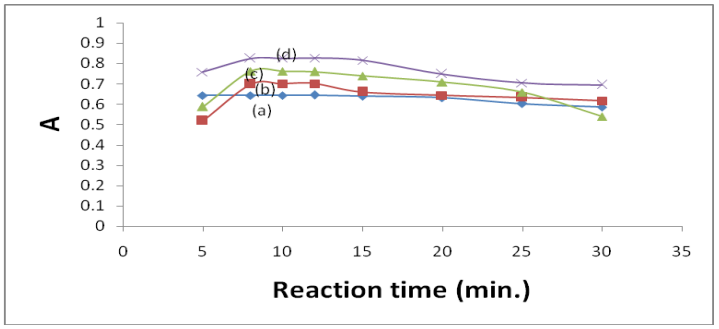


Figure 6: Effect of time on the absorption intensity of the reaction product of $K_2Cr_2O_7$ with (a) EN, (b) LS, (c) MOX and (d) RAM (500 µg.ml^{-1} of each).

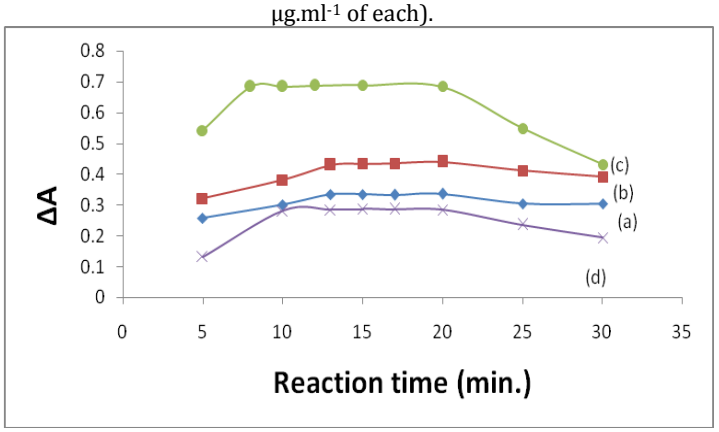


Figure 7: Effect of time on the absorption intensity of reaction product of each of (a) EN (b) LS (c) MOX and (d) RAM with $KMnO_4$ (40 µg.ml^{-1} each).

Solvent	Drug / Absorbance*							
	Method A**				Method B***			
	EN	LS	MOX	RAM	EN	LS	MOX	RAM
Water	**0.651**	**0.701**	**0.774**	**0.828**	**0.334**	**0.434**	**0.694**	**0.281**
Methanol	0.514	0.575	0.637	0.691	0.293	0.398	0.533	0.245
Ethanol	0.506	0.567	0.629	0.683	0.283	0.310	0.507	0.241
Isopropanol	0.511	0.572	0.634	0.688	0.267	0.287	0.521	0.246
Acetone	0.540	0.601	0.663	0.717	0.270	0.296	0.562	0.266

Table 3. Effect of diluting solvent on the absorption intensity of the oxidation product of the investigated ACEIs with $K_2Cr_2O_7$.
* Absorbance values are mean of three determinations.
** Drug concentration (500 µg.ml^{-1}). ***Drug concentration (40 µg.ml^{-1}).

Stability time

The stability time of the reaction product (method A&B) was studied by carrying out the reaction and leaving for different time intervals after dilution with water (figure 8 and 9). It was found that the absorption intensity was stable for at least 1 hour after diluting the reaction mixture.

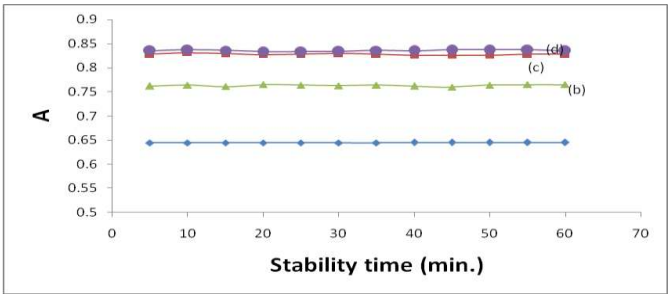


Figure 8: Effect of time on the stability of reaction product of $K_2Cr_2O_7$ with (a) EN, (b) LS, (c) MOX and (d) RAM (500 µg.ml^{-1} of each).

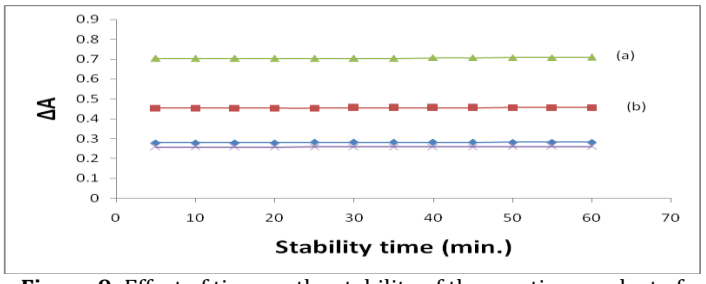


Figure 9: Effect of time on the stability of the reaction product of $KMnO_4$ with (a) EN (b) LS (c) MOX and (d) RAM (40 µg.ml^{-1} each).

Method validation

The proposed methods was validated according to ICH guidelines [31] and USP 31- NF 26 [21] validation guidelines for the following parameters:

Quantification

Regression analysis for the results was carried out using least-square method [32]. in both proposed

methods Beer's plots were linear with small intercepts and good correlation coefficients in the concentration ranges cited in tables (4). The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated using the formula: LOD or LOQ = K.SDa/b where K=3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

Validation parameter	Method A				Method B			
	EN	LS	MOX	RAM	EN	LS	MOX	RAM
Linearity range (µg.ml^{-1})	25-900	20-700	30- 750	45-650	10-200	2-150	2-60	1-500
Correlation coefficient (r) ± SD[a]	0.9998 ± 4.8×10^{-3}	0.9998 ± 4.6×10^{-3}	0.9997 ± 5.9×10^{-3}	0.9994 ± 1.01×10^{-3}	0.9998 ± 4.5×10^{-3}	0.9998 ± 3.6×10^{-3}	0.9997 ± 4.9×10^{-3}	0.9999 ± 4.8×10^{-3}
Intercept (a) ± SD[a]	0.12± 2.3×10^{-3}	0.14 ± 2.3×10^{-3}	0.14± 3.8×10^{-3}	0.13± 5.9×10^{-3}	0.13 ± 2.8×10^{-3}	0.23 ± 2.2×10^{-3}	0.17 ± 2.4×10^{-3}	0.22 ± 2.2×10^{-3}
Slope (b) ± SD[a]	0.001 ± 5.2×10^{-3}	0.001 ± 6.4×10^{-3}	0.001 ± 1.3×10^{-3}	0.001 ± 1.76×10^{-3}	0.004 ± 2.4×10^{-5}	0.05 ± 2.86×10^{-5}	0.01 ± 7.71×10^{-5}	0.001 ± 1.08×10^{-5}
LOD (µg.mL^{-1})	7.59	5.97	9.78	13.92	2.4	0.14	0.59	0.46
LOQ (µg mL^{-1})	22.99	18.1	29.63	42.19	7.12	0.43	1.79	1.4

Table4. Quantitative parameters of the proposed $K_2Cr_2O_7$ spectrophotometric method.
[a] Average of three results.

Precision

Precision (Interday and intraday) of the proposed methods was excellent as indicated from the relative standard deviation (RSD ≤ 1.73 for $K_2Cr_2O_7$ and 1.88 for $KMnO_4$ oxidation methods respectively) calculated from replicate analysis of six separate solutions of the working standard of each of the studied ACEIs at three concentration levels (table 5 & 6).

Method A												
Interday precision												
Drug	EN			LS			RAM			MOX		
Concentration (µg.ml^{-1})	75	500	800	50	300	700	75	400	700	100	300	600
%Recovery* ± SD	101.00 ± 1.75	100.6 ± 1.21	100.8 ± 1.05	99.5 ± 1.68	99.6 ± 0.80	101.1 ± 0.65	100.3 ± 1.09	100.4 ± 0.78	99.9 ± 1.06	100.0 ± 1.24	100.4 ± 1.34	99.8 ± 1.51
%RSD	1.73	1.20	1.04	1.69	0.80	0.64	1.09	0.78	1.06	1.24	1.32	1.51
Intraday precision												
Drug	EN			LS			RAM			MOX		
Concentration (µg.ml^{-1})	75	500	800	50	300	700	75	400	700	100	300	600
%Recovery* ± SD	99.1 ± 1.23	99.8 ± 1.04	99.9 ± 1.03	97.9 ± 0.32	100.5 ± 1.02	101.1 ± 0.91	100.1 ± 1.14	100.4 ± 1.23	99.8 ± 1.34	98.1 ± 1.26	102.2 ± 1.08	99.3 ± 0.27
%RSD	1.24	1.04	1.03	0.33	1.02	0.90	1.14	1.23	1.34	1.29	1.06	0.27

Table 5. Interday and intraday precision of the proposed $K_2Cr_2O_7$ spectrophotometric method for the analysis of the studied ACEIs at three concentration levels.

* Average of five replicates

Method B												
Interday precision												
Drug	EN			LS			RAM			MOX		
Conc. (μg.ml^{-1})	40	120	160	30	50	120	50	200	450	10	30	50
%Recovery± SD*	97.2 ± 1.53	100.4 ± 1.08	99.2 ± 0.54	98.4 ± 1.04	99.1 ± 0.88	99.5 ± 0.60	98.9 ± 1.48	99.7 ± 1.15	98.0 ± 1.49	99.4 ± 1.20	99.4 ± 0.72	99.8 ±0.43
%RSD	1.57	1.08	0.54	1.02	1.03	0.60	1.50	1.15	1.52	1.21	0.73	0.43
Intraday precision												
Drug	EN			LS			RAM			MOX		
Conc. (μg.ml^{-1})	40	120	160	30	50	120	50	200	450	10	30	50
%Recovery± SD*	98.9 ± 1.31	100.8 ± 0.40	99.1 ± 0.46	99.3 ± 1.37	99.7 ± 0.68	100.0 ± 1.22	100.2 ± 1.61	99.8 ± 1.88	97.9 ± 1.14	98.1 ± 1.26	102.2 ± 1.08	99.3 ±0.27
%RSD	1.32	0.4	0.46	1.38	0.68	1.22	1.61	1.88	1.16	1.29	1.06	0.27

Table 6. Interday and intraday precision of the proposed $KMnO_4$ spectrophotometric method for the analysis of the studied ACEIs at three concentration levels. * Average of five replicates

Accuracy

Applying the suggested spectrophotometric procedure for the analysis of commercially available tablets (Ezapril®, Zestril®, Primox® and Tritace® tablets) validated the accuracy of the proposed methods. Table (7) shows mean percentage recoveries of 98.84-99.82 (± 1.09-1.35) and 98.58-99.54 (± 0.93-1.62) of the labeled amount for method A and method B respectively. This indicates an excellent concordance between experimental and nominal values. The performance of the current methods was judged by comparing with other visible spectrophotometric methods [1, 12, 13, 2] for EN, LS, MOX and RAM respectively. According to the variance ratio test (F-test), and t-test, the calculated values of F and t indicate the absence of significant difference between the proposed and reported method with respect to precision and accuracy.

	Method A				Method B			
Dosage Form (tablet)	**EN** Ezapril	**LS** Zestril	**MOX** Primox	**RAM** Tritace	**EN** Ezapril	**LS** Zestril	**MOX** Primox	**RAM** Tritace
Proposed (%recovery [a]± SD)	99.77± 1.21	98.84± 1.11	99.82 ± 1.09	99.22 ± 1.35	98.58 ± 1.62	99.54 ± 0.93	99.52 ± 0.95	99.09 ± 1.31
Reported (%recovery [*]± SD)	99.19± 0.99	98.85± 1.30	99.35 ± 1.57	99.42 ± 0.97	99.19 ± 0.99	98.85 ± 1.30	99.36 ± 1.57	99.42 ± 0.97
t-value	1.23	0.99	0.65	0.68	0.54	0.70	0.86	0.71
F-value	1.49	1.73	1.48	1.93	2.65	1.96	1.37	1.80

Table 7. Accuracy of the proposed spectrophotometric methods to tablet dosage form.

[a] Average of three determinations.

[b] Theoretical values for t and F at 95% confidence limit (t= 2.447, F= 9.28) (method A) and (t=2.228, F=5.053) (method B).

Interference study

The effect of common excepients that often accompany the studied drugs in pharmaceutical dosage form was tested for possible interference in the assay. An attractive feature of the procedure is its relative freedom from interference by the usual tablet diluents and excepients such as sucrose, lactose, starch, citric acid and gum acacia. This was performed by analyzing sample solution containing a fixed amount of each of the studied ACEIs mixed with 5 folds of common additives, an amount far in excess of their normal occurrence in the dosage form. No effect due to these excepients was found, indicating the suitability of the proposed methods for the analysis of dosage forms without interference from common reducing excipients.

Robustness

Robustness was examined by evaluating the influence of small variations of the method variables on the performance of the proposed methods [33].It was found that none of these variables significantly affect the proposed method. The percentage recoveries ranged from 98.95-103.9 and 97.5-101.02 for method A and B respectively (Table 8,9) provide an indication for the reliability of the proposed methods during their routine application for the analysis of the investigated drugs.

Variation	%Recovery*± SD*			
	EN**	LS**	MOX**	RAM**
No variation	97.59 ± 0.78	101.03 ± 0.73	99.41 ± 1.24	99.84 ± 1.51
$K_2Cr_2O_7$ conc.		**2.0** ± 0.1		**2.0** ± 0.1
1.7 M	**1.8** ± 0.1		**1.8** ± 0.1	
1.9 M	99.2 ± 1.04	99.6 ± 0.64	100.1 ± 1.16	99.6 ± 0.32
2.1 M	98.9 ± 0.87	101.0 ± 0.91	99.9 ± 1.82	100.0 ± 0.37
Volume of $K_2Cr_2O_7$		**2.0** ± 0.2	**2.0** ± 0.2	
0.8 ml	**1.0** ± 0.2			**1.0** ± 0.2
1.2 ml	100.5 ± 1.94			99.6 ± 0.30
1.8 ml	100.2 ± 1.35	101.0 ± 0.91	100.4 ± 1.38	100.5 ± 0.36
2.2 ml		100.7 ± 0.61	102.1 ± 1.17	
H_2SO_4 volume (ml)		**4.0** ± 0.2		
2.8 ml	**3.0** ± 0.2		**3.0** ± 0.2	**3.0** ± 0.2
3.2 ml	101.2 ± 1.62		98.3 ± 0.89	99.9 ± 0.50
3.8 ml	103.9 ± 0.98	100.1 ± 0.52	100.1 ± 1.15	100.4 ± 0.18
4.2 ml		100.2 ± 1.92		
Reaction time	**10.0** ± 2.0	**10.0** ± 2.0	**10.0** ± 2.0	**10.0** ± 2.0
8 min.	102.3 ± 0.65	99.9 ± 0.57	99.4 ± 1.22	99.3 ± 0.36
12 min.	100.7 ± 0.98	101.3 ± 0.66	99.6 ± 1.01	99.7 ± 0.15

Table 8: Robustness of the proposed K2Cr2O7 spectrophotometric method.
* Average of three determinations. ** Conc. of the drug is 500 µg.ml^{-1}.

Variation	%Recovery± SD*			
	EN**	LS**	MOX**	RAM**
No variation	99.15 ± 1.10	99.05 ± 1.47	99.38 ± 0.72	98.83 ± 1.12
$KMnO_4$ conc.	**0.018 ± 0.01**	**0.024 ± 0.01**	**0.018 ± 0.01**	**0.018 ± 0.01**
0.016 M	98.5 ± 1.25		98.5 ± 1.23	98.6 ± 1.70
0.020 M	98.3 ± 1.14		99.4 ± 0.82	101.0 ± 0.96
0.022 M		99.5 ± 1.99		
0.026 M		100.4 ± 1.27		
$KMnO_4$ vol.	**1.5 ± 0.2**	**1.0 ± 0.2**	**1.5 ± 0.2**	**1.0 ± 0.2**
0.8 ml		98.0 ± 0.58		98.0 ± 1.48
1.0 ml		99.9 ± 1.31		97.6 ± 1.06
1.3 ml	98.6 ± 0.35		98.6 ± 0.47	
1.7 ml	97.9 ± 0.94		99.8 ± 0.46	
H_2SO_4 vol.	**1.0 ± 0.2**	**1.0 ± 0.2**	**1.0 ± 0.2**	**2.0 ± 0.2**
0.8 ml	99.2 ± 2.01	99.9 ± 1.09	98.5 ± 0.65	
1.2 ml	98.4 ± 0.89	100.0 ± 1.66	99.1 ± 0.94	
1.8 ml				98.1 ± 0.52
2.2 ml				99.3 ± 0.96
Reaction time	**15.0 ± 2.0**	**15.0 ± 2.0**	**10.0 ± 2.0**	**15.0 ± 2.0**
8 min.			97.5 ± 1.06	
12 min.			99.3 ± 0.80	
13 min.	100.4 ± 1.27	100.2 ± 1.04		99.1 ± 1.99
17 min.	99.7 ± 0.61	99.5 ± 0.83		98.1 ± 1.74

Table 9: Robustness of the proposed $KMnO_4$ spectrophotometric method.
* Average of three determinations. ** Conc. of the drug is 40 µg.ml^{-1}.

Proposed methods versus other spectrophotometrically methods:

When the propposed method was compared to other spectrophotometric methods, it was found that most of these methods require extraction [5-7], heating [8-12] or derivatization [14], while the proposed method requires none of these time consuming steps.

CONCLUSIONS

The present work represents validated spectrophotometric method for the determination of the investigated ACEIs in pure and tablet dosage forms after oxidation with potassium dichromate and potassium permanganate in sulphuric acid medium. The proposed methods were found to be simple, rapid, precise, economic, robust and stable. It has the advantages of avoiding expensive instrumentation, heating, extraction or derivatization. The proposed methods can be routinely applied in quality control laboratories.

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