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Quantitative determination of captopril, perindopril erbumine, moexipril hydrochloride, and ramipril in bulk and pharmaceutical preparations by high performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method was developed for simultaneous determination of four ACE-inhibitors: captopril (CAP), perindopril erbumine (PER), moexipril hydrochloride (MOEX), and ramipril (RAM) in pharmaceutical formulations. The chromatographic separation was performed on Shim-pack cyanopropyl column with a mobile phase consisting of methanol-10mM ammonium acetate buffer (pH 6.0) in a ratio of (40: 60, v/v) at flow rate 1 ml min^{-1}. the analysis was performed at ambient temperature using UV detector setting at 210 nm. All ACE-inhibitors were separated within seven min. The calibration curves were linear ($r \geq 0.9994$) over a concentration range from 5 to 50 µg ml^{-1}. the method was successfully applied to commercially available pharmaceutical preparations. The validity of the method was examined comparing the results obtained with official or published methods.

1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors are effective in the treatment of several medical conditions as hypertension (HT), congestive heart failure (CHF), post-myocardial infarction (MI), and diabetic nephropathy (Opie et al 1994., Wong et al 2004) that exhibit their antihypertensive effect by restraining the formation of angiotensin II (A II). These compounds competitively antagonize ACE, the rate-limiting enzyme in the formation of A II, and angiotensin receptor blockers, which block the binding of A II to its receptor in the renin-angiotensin-aldosterone neurohormonal system (RAAS). Besides this antihypertensive effect, the ACE inhibitors possess some additional properties (such as vasculoprotective and antithrombotic

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activities) that can play a favorable role in terms of cardiovascular morbidity (Okrucka et al 1998., Remkova et al 2000).

The first ACE inhibitor developed was captopril, a thiol-containing compound. The discovery of captopril was followed by enalapril, a compound that lacked the thiol group and the potentially related side effects. The tremendous therapeutic and commercial success of captopril and enalapril has fueled extensive research activity in this field of medicinal chemistry with the preference to develop nonthiol-containing ACE inhibitors (Opie et al 1994).

ACE inhibitors for medicinal use are currently classified into three classes. The first class represents thiol-containing ACE inhibitors (captopril, zofenopril, omapatrilat). To the second, and largest, class belong the dicarboxyl containing ACE inhibitors (perindopril, moexipril, ramipril, trandolapril, quinapril, benazepril, and cilazapril). The third class represents phosphorus containing inhibitors (fosinopril). It is well documented that the ACE inhibitors differ only moderately with respect to their pharmacodynamic efficacies (Gotti et al 2000).

Until now, capillary electrophoresis was the major technique used for simultaneous determination of some ACE-inhibitors (Gotti et al 2000., Hillaert 2002), other techniques as Gas chromatography (Ereda et al 1993), HPTLC (Kowalczuk et al 2004., Wyszomirska et al 2010), and flow injection assembly for rapid analysis of some ACE-inhibitors (Emara et al 2003) also applied. On another hand, several HPLC methods had published for the determination of studied drugs in pharmaceutical formulation (Khedr et al 1998., Ouyang et al 1999., Mirza et al 2001., Erk 2001., Gumieniczek et al 1998., Manna et al 2001., Rudzki et al 2007., Bonazzi et al 1997., Erturk et al 2003). Most of the reported methods involve troublesome preparation such as pre- or post-column derivatization, difficult detection method, application of ion-pair reagents that have some drawbacks, including slow column equilibrium, irreversible adsorption of ion pair reagents to the stationary phase resulting short column lifetime and poor reproducibility.

Structurally, some of ACE-inhibitors are L-proline containing drugs such as captopril, and other bear a proline analogous moiety such as moexipril, perindopril, and ramipril. Moreover, reversed phase high performance chromatography of these drugs, containing a proline or proline related residue, may show peak splitting owing to slow cis-trans isomerization, caused by hindered rotation around the N- substituted peptide bond (Gustafsson et al 1990). In spite of the separation was tried using the columns and mobile phase previously described in literature, but theses attempts were failed. The use of angiotensin converting enzyme (ACE) inhibitors are widely increased in the last few year, so the specific and sensitive methods are needed for the quantitative determinations of these drugs in pharmaceutical dosage forms.

Currently, to the best of our knowledge, none of the methods reported in the literature deal with the estimation of binary or ternary mixtures of ACE-inhibitors in a single chromatographic system. The aim of the present study is to develop and validate an efficient, reliable, accurate, sensitive, and generic liquid chromatographic method that allowed for the

determination of ACE-inhibitors in bulk and pharmaceutical formulation without the need for the development of separate and distinct m ethod for each drug having diversified structural features and difference in solubility profile. In order to have a universal acceptance and ensure ease of applicability, we have used a UV detector with no special assembly of detection systems for measurement of ACE-inhibitors. The major advantage of the proposed method is the four structurally related ACE-inhibitors (**Figure 1**) can be determined on a single system with reasonable resolution and better peak shape and symmetry, and applicability to clinical routine monitoring and pharmacokinetic study of the cited drugs because of its ability to determine the cited drugs in the presence of their main metabolite and their degradation products (Elshanawane et al 2008). Beside that the proposed method can applied for assay of the drugs in presence of co-administered drug like hydrochlorothiazide, indapamide, atenolol (Elshanawane et al 2008). For this purpose, the influence of column type, mobile phase composition, buffer type, buffer pH and flow rate was systematically investigated and the method validation studies were performed.

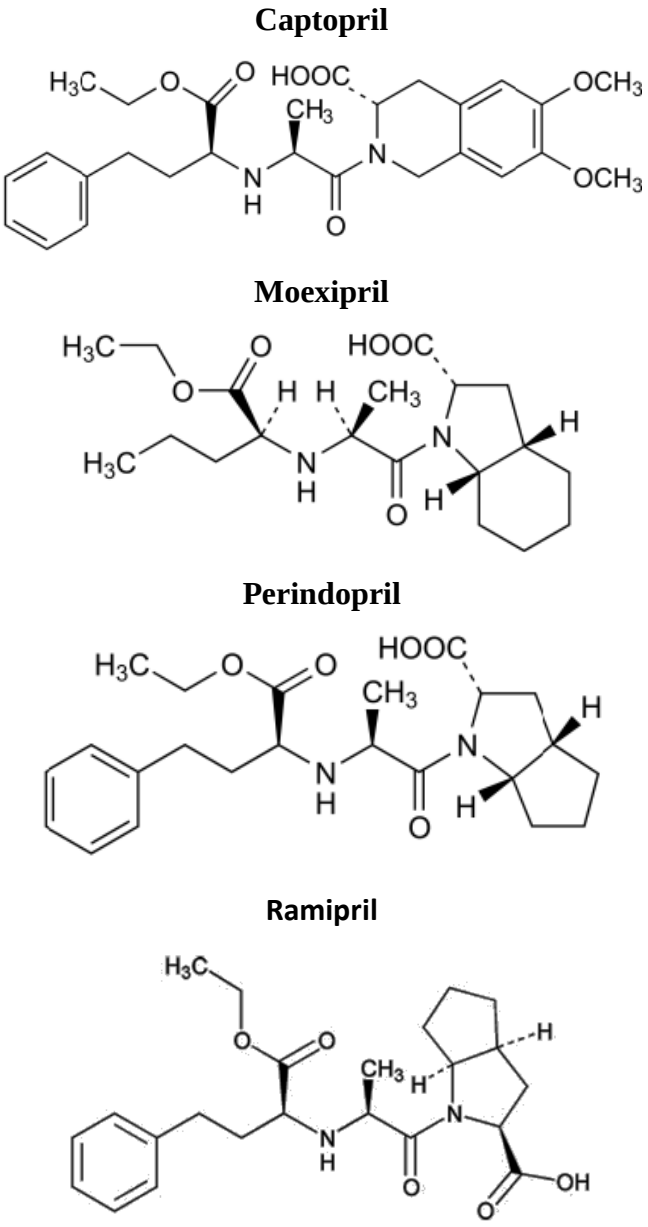

Figure 1: Chemical structures of captopril, moexipril, perindopril, and ramipril

2. Materials and methods

2.1. Instrumentation and chromatographic conditions

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 µl loop and a SPD-10AVP UV-VIS detector. The sample was injected with 25 µL Hamilton analytical syringe. Separation and quantitation were achieved on a 250 × 4.6 mm (i.d) Shim-pack 5 µm cyanopropyl column. The mobile phase was prepared by mixing methanol and 10 mM ammonium acetate; pH was adjusted to 6.0 using acet ic acid in a ratio (40: 60, *v/v*). The flow rate was set at 1 ml min^{-1}. The detector was set at 210 nm. All determinations were performed at ambient temperature and the injection volume was 20 µL. Operation, Data acquisition, and analysis were performed using Class-VP software.

Mobile phase was filtered through a 0.45µm nylon membrane filter (Millipore, Milford, MA) under vacuum and degassed by ultrasonication (Cole Palmer, Vernon Hillis, USA).

2.2. Materials and reagents

All solvents were of HPLC grade and all reagents were of analytical grade. Methanol was obtained from (Riedel- de Haen laboratory chemicals, Germany). Deionized water was prepared using a Barnstead Nanopure Diamond analytical (Ihlas, USA) ultrapure water system and applied throughout the experiment. All solvents and solution were filtered through 0.45 um membrane filters (Millipore, Milford, MA) and degassed before used. Raw material CAP (Pharaonia Company for pharmaceutical industry, Alexandria, Egypt, 99.78%), MOEX (Minapharm pharmaceutical co, Egypt, 99.85%) PER (Servier Company for pharmaceutical industry, Egypt, 99.9%), and RAM (Aventis pharma, Egypt, 99.9 %) were kindly supplied by local pharmaceutical industries were used.

2.3. Standard solution

Stock solution of the cited drugs (1 mg ml^{-1}) prepared by dissolving 25 mg each drug separately in 25 ml of methanol. The solutions were stable for one month (one week for captopril) when stored in refrigerator at 4 ^{0}C

2.4. Preparation of calibration curve

The working solution was prepared separately by further dilution of the stock standard solution with the mobile phase to reach the concentration range of 5-50 µg $ml^{-1}$for the four drugs. Triplicate 20 µL injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area of each drug was plotted against corresponding concentrations, linear relationship was obtained.

2.5. Sample preparation

Ten tablets of the four drugs (**Table1**) were weighed and finely powdered. A portion of the powder equivalent to 25 mg of each drug was accurately weighed, transferred separately to 25 ml volumetric flask, and dissolved in 25 ml methanol using ultrasonic bath (30 min) and then filtered through 0.45 um membrane filters (Millipore, Milford, MA). Further dilution was carried out with mobile phase to reach calibration range of each compound.

Table 1: Commercial pharmaceutical dosage form of ACE-inhibitors used as sample in the research

Drug	Trade name	Pharmaceutical dosage form
Captopril	Capoten®	Tablets (50 mg)
Perindopril Erbumine	Coversyl®	Tablets (4 mg)
Moexipril-HCl	Primox®	Tablets (15 mg)
Ramipril	Tritace® protect	Tablets (10mg)

3. Results and discussion

In order to validate an efficient method for analysis of drug in pharmaceutical formulations, preliminary test was performed with the objective to select adequate and optimum conditions. Parameters, such as type of column, ideal mobile phase and their proportions, optimum pH, flow rate, detection wave length, ionic strength the buffer were exhaustively studied.

A reversed phase high performance chromatography of these drugs, containing a proline or proline related residue, may show peak splitting owing to slow cis-

trans isomerization, caused by hindered rotation around the N- substituted peptide bond (Gustafsson et al 1990). Therefore, with a view to providing practical method suitable for reliable quality control of ACE-inhibitors, this study was concerned with the development of HPLC method able to avoid peak splitting and band broadening.

Three columns were used for simultaneous determination of the studied drugs namely; C18, C8, CN columns. Both C18 and C8 gave better peak shape and inadequate separation at low pH, and better resolution with peak splitting and band broadening at high pH. The better resolution and peaks shape without excessive tailing was obtained when applied the cynopropyl column.

Variation of pH of the ammonium acetate buffer result in maximum capacity factor ($k^{/}$) value at PH 6.0, with better shape and reasonable retention time. At pH from 2.5-5.5 the drugs more retained and the peaks eluted very broadening, splitting, and with excessive tailing.

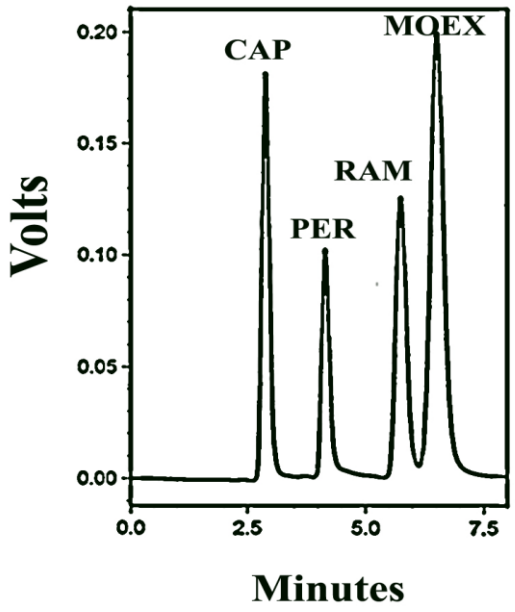

Figure 2: HPLC chromatogram of 20 µl injection of sample containing 25 µg ml-1 of captopril (CAP), perindopril (PER), moexipril (MOEX), and ramipril (RAM)

The effect of type and concentration of organic modifier was studied and observed the following, increasing acetonitril concentration than 40% the drugs peaks eluted with better shape and symmetry but with inadequate resolution, and at concentration less than 30% the peaks splitting was observed. The combination of acetonitril with methanol gave inadequate separation of the four dugs. While at 40 % methanol a better resolution with reasonable retention time was obtained.

In order to avoid multiple peaks of peptides on reversed-phase columns, the pH must be controlled with buffers, such as ammonium acetate. A major reason for using a concentration of 0.01M was to allow for maximal sensitivity of the UV detection at low wavelengths. In fact, the structural features of this class exhibit weak benzene chromophores and are characterized by low molar absorptivity values, so the detector was set at 210 nm to increase the sensitivity of the method. The chromatogram of sample containing CAP, PER, RAM, and MOEX can be observed in **Figure 2**. The proposed HPLC method was described for the first time the chromatographic technique to simultaneous determination of structurally related compounds. The method has advantage that is simple, fast, and do not involve laboratories time-consuming sample preparation when compared with published method.

3.1. System suitability

The system suitability parameters including capacity factor ($k^{/}$), selectivity (α), resolution (Rs), tailing factor (T), and theoretical plate (N) listed in **Table 2**.

Table 2: Chromatographic characteristic of captopril, perindopril, ramipril, and moexipril

Compound	Retention time	Capacity factor	Selectivity	Resolution	Tailing factors	Theoretical plates
	(min)	(k')	α	R_s	(T)	(N)
Captopril	2.875	2.14	1.64	1.98	1.15	1571
Perindopril	4.142	3.52	1.49	4.22	1.25	3054
Ramipril	5.742	5.26	1.16	4.47	1.13	3080
Moexipril	6.49	6.1		1.67	1.16	2696

All parameters were satisfactory with good specificity for the assessment of captopril, perindopril, ramipril, and moexipril.

3.2. Analysis of pharmaceutical formulations

The proposed method was successfully applied to determine Captopril, perindopril, ramipril and moexipril in their dosage forms (Capoten® Batch No. A70168 was manufactured by Bristol- Myers Squibb Egypt Company under license of Bristol- Myers Squibb Company New York, labeled to contain 50 mg of captopril per tablet), (Coversyl® Batch No.5699 was manufactured by servier Egypt, under license of Les laboratories Servier-France, labeled to contain 4mg of Perindopril Erbumine per tablet), (Tritace®protect Batch No. 15E04 was manufactured by Aventis pharma, Egypt, under license of Aventis pharma-Germany, labeled to contain 10 mg of ramipril per tablet), and (Primox® Batch No. 031131 was manufactured by Minapharm, Egypt, under license of Schwarz pharma, Germany, labeled to contain 15mg of moexipril-HCL per tablet) tablets, respectively. Six sample (n = 3) determination were made. Satisfactory results were obtained for each

Table 3: Statistical comparison between the proposed method and the published method for the determination of Captopril in (Capoten®), moexipril in Primox®, perindopril in (Coversyl®), and ramipril in Tritace® Protect tablets

Commercial product	Recovery[a] ± S.D.	Reference method
Captopril	100.56 ± 0.88	101.41 ± 0.97
t	1.58	2.57[b]
F	1.22	5.05[b]
Moexipril	99.87 ± 0.85	99.43 ± 0.72
t	0.96	2.57[b]
F	1.39	5.05[b]
Perindopril	99.22 ± 0.5	98.86 ± 1.04
t	0.76	2.57[b]
F	4.33	5.05[b]
Ramipril	99.92 ± 0.63	100.6 ± 0.905
t	1.51	2.57[b]
F	2.06	5.05[b]

[a] **mean of six determination**

[b] **the theoretical values of t and F at P = 0.05**

Table 4: Assay parameters and regression characteristic of captopril, moexipril, perindopril, and ramipril determined by the proposed HPLC method

parameters	captopril	Moexipril	Perindopril	Ramipril
Linearity range(μgml^{-1})	5-50	5-50	5-50	5-50
Detection limit (μgml^{-1})	0.022	0.02	0.029	0.09
Quantitation limit (μgml^{-1})	0.076	0.052	0.099	0.32
Regression equation (y*)				
n	6	6	6	6
Slope (b)	3.7×10^4	7.2×10^4	2.3×10^4	3.8×10^4
Standard deviation of slope	0.8×10^3	1.2×10^3	0.6×10^3	1.5×10^3
Relative standard deviation of slope	2.16	1.71	2.61	3.8
Intercept (a)	136×10^3	199×10^3	23×10^3	54×10^3
Standard deviation of intercept	26×10^3	35×10^3	18×10^3	44×10^3
Correlation coefficient (r)	0.9998	0.9999	0.9997	0.9995
Standard error of regression	0.033	0.034	0.011	0.04

*Y = a + bC, where Y= peak area ratio of drug/ internal standard, and C is drug concentration

compound in good agreement with label claims. The results obtained were compared statistically by Student's t-test (for accuracy), and variance ratio F-test (for precision) with the published method (Erturk et al 2003, The United States Pharmacopoeia 2005, Ayad et al 2003). The results in **Table 3** showed that the t and F values were smaller than the critical values indicating that there was no significant difference between the proposed and published methods.

3.3. Validation of the method

3.3.1. Linearity

The linearity of the proposed method for determination of captopril, perindopril moexipril, ramipril and was evaluated by analyzing different concentration of the drugs. According to the international conference on harmonization (The United States Pharmacopoei 2005), at least five concentrations must be used. In this study six concentrations were chosen, ranging between 5-50 μg ml^{-1} for the studied drug. Each concentration was repeated three times. The high value of the correlation coefficient and the intercept value that was not statistically ($p<0.05$) different from zero (**Table 4**) validate the linearity of the calibration graphs. Typically, the regression equations were: $\mathbf{y = 3.7\times10^4 x + 136\times10^3}$ (*r* = 0.9998) for captopril and $\boldsymbol{y = 7.2\times10^4 x + 199\times10^3}$ (*r* = 0.9999) for perindopril, $\mathbf{y = 2.3\times10^4 x + 23\times10^3}$ (*r* = 0.9997) for moexipril, $\mathbf{y = 3.8\times10^4 x + 54\times10^3}$ (*r* = 0.9995) for ramipril respectively.

3.3.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels (5, 30, 50 μg ml^{-1}) for each drug. The precision expressed as relative standard deviation (RSD %) of the mean measured concentration. Within the examined range, the intra-day reproducibility of the assay was

excellent, with R.S.D. % being in the range of 0.16–0.52. The inter-day R.S.D. % was 0.13– 0.96.

assessed practically as 0.022, 0.02, 0.029, 0.09 µg ml^{-1} and 0.076, 0.052, 0.099, 0.32 µg ml^{-1} for captopril,

Table 5: The application of standard addition technique to the analysis of the studied drugs

	Claimed µg ml^{-1}	Added µg ml^{-1}	Found µg ml^{-1}	Recovery %
Captopril	5	5	5.05	101.02
	5	10	10.05	100.54
	5	20	20.42	102.10
	5	25	25.31	101.22
	5	35	35.51	101.45
	5	45	44.76	99.47
Mean				100.97
S.D.				0.90
	Claimed µg ml^{-1}	**Added µg ml^{-1}**	**Found µg ml^{-1}**	**Recovery %**
Moexipril	5	5	4.96	99.25
	5	10	9.98	99.84
	5	20	19.71	98.53
	5	25	24.90	99.60
	5	35	35.03	100.08
	5	45	44.62	99.15
Mean				99.41
S.D.				0.55
	Claimed µg ml^{-1}	**Added µg ml^{-1}**	**Found µg ml^{-1}**	**Recovery %**
Perindopril	5	5	5.03	100.69
	5	10	9.90	99.01
	5	20	19.91	99.56
	5	25	25.26	101.03
	5	35	34.63	98.94
	5	45	45.13	100.30
Mean				99.92
S.D.				0.88
	Claimed µg ml^{-1}	**Added µg ml^{-1}**	**Found µg ml^{-1}**	**Recovery %**
Ramipril	5	5	4.97	99.39
	5	10	10.10	101.00
	5	20	20.02	100.09
	5	25	25.26	101.05
	5	35	35.39	101.12
	5	45	45.76	101.69
Mean				100.72
S.D.				0.83

3.3.3. Detection and Quantitation Limits

According to ICH recommendations (The European Agency for The Evaluation of Medical Products 1996) the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were perindopril, moexipril, and ramipril, respectively

3.3.4. Selectivity

The selectivity of the proposed method was evaluated through the possible interference due to excipients presented in pharmaceutical formulation. For that, placebo of each tablet sample was prepared by

mixing respective excipients and solutions were prepared following the procedure described in section. The influence of the commonly used tablet excipients (lactose, starch, magnesium stearate, talc, povidon, aerosil, sodium laryl sulfate and microcrystalline cellulose, hydroxypropyl cellulose) was investigated and no interference was observed with the proposed methods.

3.3.5. Accuracy

This study was performed by adding known amounts of the studied drug compound to known concentration of the commercial pharmaceutical tablet (standard addition method). The resulting mixtures were analyzed and the results obtained were compared with the expected results (**Table 5**) suggested the good accuracy of the proposed methods. The mean results ± SD from six determinations were 100.97 ± 0.90, 99.41 ± 0.55, 99.92 ± 0.88, and 100.72 ± 0.83 for captopril, moexipril, perindopril, and ramipril, respectively, indicative of the high accuracy of the method.

3.3.6. Robustness

Robustness is the measure of capacity of analytical methods to remain unaffected by small but deliberating variations of the operation parameters. Variation of the pH of 10 mM ammonium acetate of the mobile phase by ± 0.2, organic solvent strength of the mobile phase by ± 2 %, and detector wavelength by ± 2 nm did not have significant effect on chromatographic resolution of in HPLC method. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. The determination of 25 µg ml^{-1} for CAP, PER, RAM, and MOEX under the various conditions was performed. Each mean value was compared with the mean value obtained by optimum conditions. The statistically comparison was done with t test (Miller 1993) and no difference was found between results (p = 0.05). Therefore, the method is robust to the small changes in experimental conditions.

3.3.7. Solutions stability

In order to demonstrate the stability of both standard working solutions and tablets sample solutions during analysis, both solutions were analyzed over a period of 12 h at room temperature. The results showed that, the retention time and peak area of the cited drugs remained almost unchanged and no significant degradation was observed within the indicated period, suggesting that both solutions were stable for at least 12 h, which was sufficient for the whole analytical process.

4. Conclusion

An isocratic RP-LC method has been developed and validated for evaluation of ACE- inhibitors and their determination in bulk drugs and pharmaceuticals dosage forms simultaneously. The developed method has been found to be selective, sensitive, and precise. The shorter duration of analysis for cited drugs makes these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The method is capable of detecting 0.02 – 0.09 µg ml-1 of the studied drugs making it successfully applied for clinical routine monitoring and pharmacokinetic studies of ACE- inhibitors.

5. Conflict of interest

The authors report no declaration of conflict of

interest.

6. Acknowledgements

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