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Chapter 11

Interaction Studies of ACE Inhibitors with Statins

Safila Naveed

Additional information is available at the end of the chapter

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1. Introduction

1.1. Angiotensin-Converting Enzyme Inhibitors

1.1.1. History

In the 1950s, it was discovered that angiotensin exists as both an inactive decapeptide angiotensin I and an active octapeptide angiotensin II. Human angiotensin-converting enzyme contains 277 amino-acid residues and has two homologous domains, each with a catalytic site and a region for binding Zn^{+2} [1, 2]. The degradation of bradykinin to inactive peptides occurs via action of ACE; ACE thus not only produces a potent vasoconstriction but also inactivates a potent vasodilator. In 1965, Ferreira [3] studied the physiological effects of snake poisoning and discovered a specific component from the venom of the pit viper, Bothrops jararaca, which inhibits degradation of the peptide bradykinin and potentiate hypotensive action of bradykinin potentiating factors (BPFs), basically amino-acid-containing peptides. Bakhle [4] reported that these same peptides had an inhibitory activity on ACE of dog lung homogenate and inhibited the enzymatic conversion of angiotensin I to angiotensin II. Brunner and Laragh [5] administered them to hypertensive patients and found them to be extremely effective in lowering blood pressure. The structural requirements for substrates of angiotensin-converting enzyme to cleave a substrate are found to be similar to those observed with carboxypeptidase A of bovine pancreas [6, 7].

The molecule ACE is a zinc metallopeptidase and has a similar mode of action to carboxypeptidase [8]. In 1970, the Bradykinin-potentiating pentapeptide BPP5a was isolated, which inhibited enzyme angiotensin and decreased blood pressure [9]. The significance of ACE in the pathogenesis of hypertension was not fully appreciated until 1977, when Ondetti [10] first isolated and then synthesized the naturally occurring non-peptide, teprotide. He proposed a hypothetical model of the active site of ACE and used it to predict and design compounds that would occupy the carboxy-terminal binding site of the enzyme captopril, a specific potent

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inhibitor of ACE. Clinical trials showed excellent anti-hypertensive properties and these results had a major impact on the treatment of cardiovascular disease [11]. The first demonstration of an orally active ACE inhibitor was made on 31 March 1975, when the succinyl group was replaced with a derivative of cysteine, increasing inhibitory potency about 2,000-fold because sulphhydryl of cysteine bound with zinc more tightly than the carboxyl of succinyl. This resulted in captopril, with a dramatic effect on renal function and on hypertension [12]. Enalapril is basically a first derivative of ACE inhibitor, which was developed to overcome the limitations of captopril. Lisinopril is a lysine analogue of enalaprilat (the active metabolite of enalapril). *In vitro* lisinopril is slightly more potent than enalaprilat. It is a non-sulphhydryl angiotensin-converting-enzyme (ACE) inhibitor active without metabolism and is absorbed in its active form.

1.1.2. Chemistry

Angiotensin enzyme inhibitors are basically ester-containing drugs that show 100-1000 times less activity than their active form; these inhibitors are synthetic in nature and can be classified on the basis of their chemical structure. They can be grouped as sulphhydral-containing (fentiapril, pivalopril, zofenopril, alacepril, etc.), dicarboxyl-containing (lisinopril, benazepril, quinapirl, perindopril, indopril, pentopril, indalapril, alazapril, moexipril, romipril, spirapril, etc.), phosphorous-containing (fosinopril) [13] and naturally occurring lactokinins and casokinins. [14]

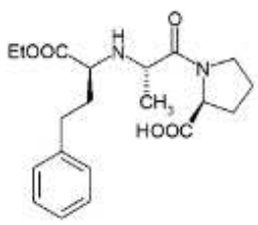
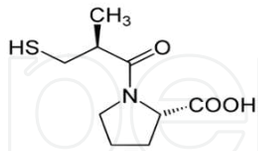
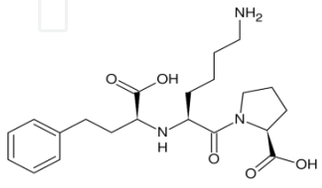
Drug	Nomenclature	Structure	Ref
Enalapril	(S)-1-[N-[1-(ethoxycarbonyl)-3-phenyl propyl]-L-alanyl]-L-proline,(Z)-2-butenedioate salt		[15]
Captopril	1-(3-mercapto-2-dmethyl-1-oxopropyl)-1-proline (S,S)		[16]
Lisinopril	((S)-1-[N2-(1-carboxy-3-phenylpropyl)-1-lysyl]-1-proline dehydrate		[16]

Table 1. ACE Inhibitors with structure and nomenclature

In general we can say that all ACE inhibitors differ by three properties: potency, conversion from pro-drug to active form, and pharmacokinetics (i.e., ADME). They also differ in terms of

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tissue distribution. All ACE inhibitors have a similar antihypertensive efficacy – they effectively block the conversion of angiotensin 1 to angiotensin II – and all have similar therapeutic indications, adverse effect profiles and contraindications.

1.1.3. Mechanism of action

These inhibitors block the converting enzyme of angiotensin, which is responsible for cleavage from angiotensin I, which is decapeptide, to angiotensin II, which is octapeptide [17, 18], and lower the BP by reducing PVR (peripheral vascular resistance). They also decrease aldosterone secretion and the resulting sodium and water retention.

1.1.4. Pharmacokinetics

The oral bioavailability of ACE inhibitors ranges from 13% to 95% [19, 20]. Most ACE inhibitors are administered as pro-drugs that remain inactive until esterified in the liver [21]. Pharmakokinetic characteristics of different ACE inhibitors are given in Table 2

Drug	Oral resorption %	Protein binding %	Elimination half-life hr	Metabolism	Usual dose (mgd^{-1})
Enalapril	60	<08	11	Partly converted enalaprilate	5-20
Captopril	>25	30	1.7	Partly metabolized	25-50
Lisinopril	25	0	41	Non metabolized	5-20

Table 2. Pharmacokinetic of ACE Inhibitors

1.1.5. Therapeutic use

ACE inhibitors are effective in patients with mild to moderately severe hypertension, normal or low plasma renin activity, collagen vascular disease and cardiovascular disease [22, 23]. They are also used in the prevention and treatment of myocardial infarction [24, 25] and in the management of cardiac arrhythmias [26]. They can decrease the progression of atherosclerosis, microalbuminuria and diabetic retinopathy, and produce a beneficial effect in patients with Bartter's syndrome [27].

1.1.6. Adverse effects

ACE inhibitors have a relatively low incidence of side effects and are well tolerated; however, dry cough is common, appearing in 10-30% of patients. This appears to be related to the elevation in bradykinin [28-30]. Hypotension is seen especially in patients with heart failure [31], angiooedema (life-threatening airway swelling and obstruction; 0.1-0.2% of patients) and hyperkalaemia. ACE inhibitors are contraindicated in pregnancy, in the first trimester associated with a risk of major congenital malformations, particularly affecting the cardiovascular and central nervous systems [32]. The most common (≥1% of patients) adverse effects

include hypotension, fatigue, dizziness, headache, nausea and other gastrointestinal disturbances, dry cough, hyperkalaemia and renal impairment. Rash and taste disturbances are more prevalent with captopril and are attributed to its sulphhydryl moiety; eosinophilia has also been reported. Most of the adverse effects are reversible on withdrawing therapy [33]. Treatment with ACE inhibitor has been associated with the development of anaphylactoid reaction [34].

1.1.7. Drug interactions

Hypotensive effect of ACE inhibitors decreased when given in combination with non-steroidal anti-inflammatory drugs [35], but this effect was enhanced with calcium-channel blockers and beta-blockers [36]. Granulocytopaenia occurs after combined therapy of ACE inhibitors and interferones [37]. ACE inhibitors interact with different drugs, like NSAIDs [38]. Cytokines antagonize the hypotensive effect of ACE inhibitors [39]; severe hypokalaemia occurs with potassium-depleting diuretics [40] and potassium-sparing diuretics produce hyperkalaemia [41, 42]. ACE inhibitors were shown to increase potassium levels in the body [43]. Alpha-blockers enhance the hypotensive effect of ACE inhibitors [44]. Iron supplementation successfully decreases cough induced by ACE inhibitors [45] and can interfere with the absorption of ACE inhibitors [46]. Hypoglycaemic effect is enhanced with anti-diabetics and insulin [47, 48]. Combination of azathioprine and ACE inhibitors is associated with anaemia [49]. The risk of bone marrow depression is increased in patients taking concomitant therapy of ACE inhibitors and immunosuppressive agents.

1.2. HMG-CoA reductase inhibitors (statins)

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are the most effective among all hypolipidaemic agents [50]. These lipid-lowering drugs are increasingly used for primary and secondary hindrance of cardiovascular disease [51]; they have only been recognized for treatment of hyperlipidaemia. In clinical studies, statins are highly effective in enhancing HDL levels while reducing total cholesterol, LDL cholesterol, apolipoprotein B and triglyceride levels.

The normal treatment regimen for these drugs involves daily exposure over a period of many years [52, 53]. They have also been examined in combination with cures of multiple sclerosis, osteoporosis, Alzheimer's disease and dropping the superfluous increased occurrence in CHD in women on HRT treatment [54]. They have anti-thrombogenic, anti-inflammatory and anticoagulant properties [55, 56]. These therapeutic properties are independent of lipid lowering [57], and the benefits of statins appear to be independent of baseline cholesterol [58]. They can be classified into subclasses: the naturally or fungi-derived first generation, and the synthetic second generation. The first generation includes simvastatin, lovastatin and pravastatin, and the second atorvastatin and rosuvastatin. They can be further divided into the lipophilic group (simvastatin, lovastatin and atorvastatin) and the OH hydrophilic group (pravastatin and rosuvastatin) [59].

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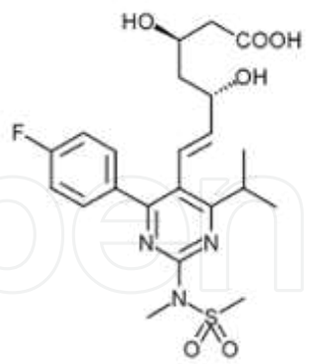
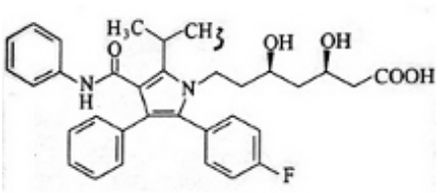
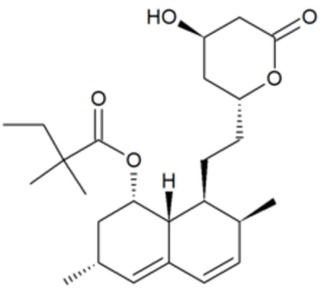
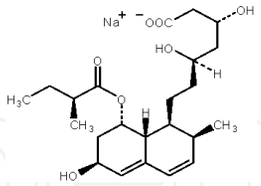
Statin	Nomenclature	Structure
Rosuvastatin	(3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N* methyl-methanesulphonamido)-6-(propan-2-yl) pyrimidin -5-yl]-3,5-dihydroxyhept-6-enoic acid	
Atorvastatin	[R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino) carbonyl]-1 H -pyrrole-1-heptanoic acid	
Simvastatin	[1S-[1α,3α,7β,8β(2S*,4S*), 8aβ]]-2,2-dimethyl-butanoic acid1,2,3,7,8, 8a-hexahydro-3,7-dimethyl -8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl ethyl]-1-naphthalenyl ester	
Pravastatin	[1 S- [1alpha(beta S*, delta S*), 2, 6alpha ,8beta(R*):8]]-1,2,6,7,8,8a-hexahydro-beta, delta,[6-trihydroxy-2-methyl-8-[2-methyl-1-oxobutoxy]-1-phthaleneheptanoic acid salt	

Table 3. Statins with structure and chemical name

2. Experiment

2.1. Materials

Raw materials used were of pharmaceutical purity and were obtained from different pharmaceutical companies (Table 4). Tablets were purchased from a local pharmacy; each product was labelled with an expiry date not earlier than two years from the time of these studies.

Class	Drugs	Brands	Potency (mg)	Pharmaceutical industry
ACE inhibitors	Enalapril	Renitec	10	MSD
	Captopril	Capoten	25	Bristol Meyers (Pvt.) Ltd.
	Lisinopril	Lisinopril	5	Atco Laboratories Ltd.
Statins	Rosuvastatin	X-plended	20	Pharm Evo (Pvt.) Ltd.
	Atorvastatin	Atopitar	10	Atco Pharma (Pvt.) Ltd.
	Pravastatin	Pravachol	20	Bristol Meyers (Pvt.) Ltd.
	Simvastatin	Atcol	10	Geofman Pharma (Pvt.) Ltd.

Table 4. Drugs, brands and manufacturers

2.1.1. Reagents

Analytical-grade solutions were used for the performance of the experiment. Methanol and acetonitrile were of HPLC grade and other reagents included HCl, sodium hydroxide (NaOH), sodium chloride (NaCl), disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, ammonium chloride, NH_3 solution (10%), phosphoric acid (8%) (Merck Germany). Organic solvents used were methanol, ethanol, ethyl acetate, chloroform, acetronitrile, triethylamine and DMSO (Merck HPLC Grade Germany).

2.1.2. Equipment

A UV-visible spectrophotometer (Shimadzu Model 1601, Japan) with 10-mm path length was connected to a computer with UVPC version 3.9 software. A Stedec CSW-300 was used for deionization of water. Dissolution was accomplished using BP 2009 standards. Chromatographic studies were carried out by using two Shimadzu HPLC systems, one equipped with an LC-10 AT VP pump (SPD-10 A VP), and the second with an LC-20AT UV/VIS detector utilizing Hypersil, ODS, C18 (150×4.6 mm, 5 micron) and a Purospher® STAR RP-18 column. Chromatographic data peaks were analysed using Shimadzu Japan CBM-102, class GC 10 software.

Infrared studies were performed using Shimadzu FTIR Prestige-21. Spectral analysis was performed using Shimadzu software. The proton H^1-NMR spectra were calculated on a Bruker (AMX 500 MHz) spectrometer using TMS as an internal standard. Melting points were recorded using Gallen kamp melting-point apparatus Minnesota Mining And Manufacturing Company.

2.2. Methods

2.2.1. Preparation of simulated gastric juice and buffers

0.1 N HCl was prepared by using 9 mL HCl (11 N) in a volumetric flask; the volume was made up with de-ionized water. Chloride buffer at pH 4 was prepared by dissolving 3.725 g of KCl (potassium chloride) in deionized water and 0.1N HCl was used for pH adjustment. For

preparation of PO_4 (phosphate buffer pH 7.4) 0.6 gm of potassium dihydrogen orthophosphate was used, plus 6.4 g of disodium hydrogen orthophosphate and 5.85 g of NaCl (sodium chloride), and the pH was adjusted. Preparation of NH_3 ammonia buffer at pH 9 was done using 4.98 g of NH4Cl ammonium chloride and pH-adjusted with 10% ammonia.

2.2.2. Construction of the calibration curve of drugs

The above standard solutions of all drugs were scanned in the region 200-700 nm against the reagent blank, and absorbance maxima were recorded as shown in Table 5. Calibration curves were constructed between concentration and absorbance. Epsilon values and linear coefficients were calculated in each case at all the above-described pH values. Beer Lambert's law was obeyed at all concentrations and pHs.

Class of drugs	Analytes	Wavelength (nm)	Conc. range (m Mole)
ACE inhibitors	Enalapril	203, 206, 207, 208	$1\text{-}9 \times 10^{-5}$
	Captopril	203, 204, 206	$5\text{ -}14 \times 10^{-7}$
	Lisinopril	206	$1\text{-}10 \times 10^{-5}$
Statins	Atorvastatin	241	$0.5\text{-}4.5 \times 10^{-2}$
	Rosuvastatin	240	$1\text{-}5 \times 10^{-5}$
	Simvastatin	231, 238, 246	$1\text{-}9 \times 10^{-5}$
	Pravastatin	235	$1\text{-}9 \times 10^{-5}$

Table 5. Please Add Caption

2.2.3. Monitoring of drug interactions of enalapril, captopril and lisinopril by high-performance liquid chromatography

HPLC methods for simultaneous determination of enalapril, captopril and lisinopril with statins in raw materials, pharmaceutical dosage forms or in human serum were developed and validated according to ICH guidelines. These methods were then applied to drug-drug, drug-metals and drug-antacid interaction studies.

2.2.4. Chromatographic conditions

Isocratic elution was performed at ambient temperature with two different types of column. Hypersil, ODS, C18 (150×4.6 mm, 5 micron) and Purospher® STAR RP-18, for assay of enalapril, captopril and lisinopril and simultaneous determination of these drugs with interacting drugs, respectively. The mobile phase, flow rate, wavelength and UV detection were varied as shown in Table 6. A sample volume of 20 μL was injected in triplicate onto the HPLC column and the elute was monitored at different wavelengths.

2.2.5. Preparation of standard solutions

Stock reference standard solutions of all drugs were prepared daily by dissolving appropriate amounts of each drug in mobile phase to yield final concentration of 300 μgmL^{-1}. For the calibration standards, calibrators of each drug were prepared by making serial dilutions from stock solutions. All solutions were filtered through 0.45 μm filter and degassed using sonicator.

2.2.6. Preparation of pharmaceutical dosage from samples

Pharmaceutical formulations of the respective brands commercially available in Pakistan were evaluated. In each case, groups of 20 tablets were individually weighed and finely ground in a mortar. The portion of the powder equivalent to the amount of drug was transferred into a volumetric flask and completely dissolved in mobile phase, and then diluted with this solvent up to the mark. After filtration using a 0.45 micrometre μm filter this was then injected.

2.2.7. Preparation of standard plasma solutions

Samples of blood used were collected then centrifuged at 3000 rpm for at least ten minutes, Supernatant solution was stored at –20°C. The solution serum was deprotinated by using (ACN) acetonitrile, and this solution was spiked daily with working solutions for required concentrations of ACE inhibitors and interacting drugs (statins). 10 μL of sample was injected and chromatographed under the above conditions.

Drug	Mobile phase			pH	Flow rate	Detection
	MeOH	ACN	H_2O		$mLmin^{-1}$	nm
Enalapril assay	70	-	30	3.5	1	215
Enalapril + statins		60	40	3	1.8	230
Captopril	50	-	50	2.9	1	220
Captopril + statins	-	60	40	2.9	1.5	230
Lisinopril	80	2.5	17.5	3	1	225
Lisinopril + statins	-	60	40	3	1	225

Table 6. Chromatographic conditions of HPLC methods

2.2.8. Method development and optimization

HPLC methods were developed and optimized for certain parameters before method validation. The optimization of the analytical procedure was carried out by varying the mobile-phase composition, flow rate, pH of the mobile phase, diluent of solutions and wavelength of analytes in order to achieve symmetrical peaks with good resolution at reasonable retention time.

2.2.9. Method validation

All validation parameters were established according to the guidelines given by ICH: system suitability, linearity, selectivity of drugs, specificity, (concentration-detector response rela-

tionship), accuracy or precision and sensitivity with systems, i.e., D and Q (detection and quantification) limit.

Specificity and linearity

The drugs were spiked with pharmaceutical formulations containing different excepients. The linearity of the proposed method was checked at different levels of concentration with different groups. Correlation coefficient was linear; intercept and slope values were also calculated.

Suitability of system

The system suitability of the method was evaluated by analysing five replicate analyses of the drug at a specific concentration for repeatability, (peaks) symmetry factor, theoretical plates for columns, resolution of peaks between interacting drugs, and relative retention of drugs.

Accuracy and precision

Accuracy was calculated at three different levels of concentration (80±20%) by spiking a known amount of the drug. Three or four injections of each drug were injected into the system and the percentage recovery was calculated.

For precision, six replicates of each level were injected into the system on two different non-consecutive days in each case, and the %RSD was calculated.

Limit of detection and quantification

The detection limit (LOD) of the method was calculated by the formula LOD = 3.3 SD/slope. The quantitation limit (LOQ) – the lowest level of analyte that is accurately measured – was set at ten times the noise level (LOQ = 10σ/S, where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve).

Robustness

Robustness was established by changing the concentration of mobile phase (water, methanol and acetonitrile), wave length, flow rate and pH. At least five repeated solutions were used with small variations of different parameters. Parameters that were changed mainly had a small deviation: ± 0.2% flow rate/pH, and ±5% for wave length.

Ruggedness

Ruggedness was determined in different labs. Lab 1 was the (RIPS) Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Karachi University, and the other at the same university in the Department of Chemistry. Two different instruments (LC 10/LC 20) and two different columns (Purospher STAR C_{18}/Hypersil ODS) were used.

2.2.10. Interaction studies by HPLC

Enalapril solution was mixed with a solution of the interacting drug (statins), which gave a final concentration of 100 μgmL^{-1} for each constituent). These solutions were kept in a water bath at 37 °C for three hours. An aliquot of 5 mL was withdrawn at 30-minute intervals; after making appropriate dilutions it was filtered through 0.45 μ filter paper and three replicates were injected into the HPLC system. The concentration of each drug was determined and the

percentage recovery was calculated; the same procedure was applied for captopril and lisinopril.

2.3. Synthesis of ACE inhibitors and interacting-drugs complexes

Complexes of enalapril, captopril and lisinopril with all interacting drugs were synthesized. Equimolar solutions of enalapril and interacting drugs were prepared in methanol. An equivolume solution of enalapril was mixed with each drug individually and the respective pH was adjusted either by 1-2 drops of ammonia or 0.1 N HCl. These mixtures were refluxed for three hours then filtered and left for crystallization at room temperature. Melting points and physical characteristics of these complexes were noted. Solubility of all these complexes was checked in different solvents: water, methanol, ethanol, chloroform and DMSO. A similar procedure was adopted for captopril and lisinoril.

2.3.1. Spectroscopic studies of complexes

2.3.1.1. Infrared studies

ACE inhibitors and their complexes were characterized by using a FT-IR spectrophotometer in the region 400-4000 cm^{-1}. The infrared spectra were recorded using a potassium bromide disc. ATR (attenuated total reflection) or smart performer accessory was used for the sample (minimum amount).

2.3.1.2. Proton NMR analysis

Proton ^{1}H NMR analysis was performed using a Bruker instrument in deuterated H_2O, chloroform and methanol using (TMS) tetramethyl silane as an IS (internal standard).

3. Results and discussion

3.1. Method development/validation by HPLC

Simple, cheap and very precise, HPLC was used for the determination of ACE inhibitors (captopril, enalapril and lisinopril) in the presence of different statins: ROS (rosuvastatin), ATR (atorvastatin) and SMV(simvastatin) in active ingredients as well as in formulations. It was developed according to guidelines ICH. All inhibitors with statins separated out in less than 10 mins without interference from any ingredients. The recovery of drugs was within the desired range (99-102%). These methods were validated according to ICH and the criteria for acceptance (accuracy/linearity/precision/specificity) and for system suitability were met. The methods can easily be used for quantitative analysis of ACE inhibitors and statins as single drugs or in formulations.

3.2. Interaction of ACE inhibitors with statins

Hyperlipidaemia and hypertension correlate with each other. They can effect coronary heart disease (CHD), because cardiovascular disease (CVD) is closely related to different factors,

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such as hypertension (HT) or high cholesterol levels. Factors include family history, age, sex, and diabetes [60-66]. Co-administration of antihypertensive, lipid-lowering and antidiabetic drugs is used in the treatment [67-72]. The most commonly used combinations of diuretic (chlorthalidone, hydrochloroth-iazide, etc.) and an angiotensin II receptor antagonist to control hypertension, as well as with a statin (fluvastatin, simvastatin, etc.) to reduce the cholesterol [73]. Co-administration of an antihypertensive agent with statin is an effective therapeutic option for treatment of multiple cardiovascular risk factors, and especially for high blood pressure (BP) and LDL-C [74-78]. In addition, statins may improve the vasodilatation capacity of large arteries and may thus contribute to BP-lowering in patients treated with both an anti-hypertensive and a statin [79]. Hypercholesterolaemia is often accompanied by hypertension, an associated risk factor for coronary-artery disease (CAD) [80-82]. ACE inhibitors are effective for the management of hypertension, supraventricular arrhythmias and angina pectoris. Other antihypertensive drugs such as propranolol [83] and atenolol [84] also interact with HMG-CoA reductase inhibitor. In the light of the above results, ACE inhibitors may interact and effect a change in each other's availabilities. Methods were developed by HPLC for both ACE inhibitors and statins before starting interaction studies [85-88]. *In vitro"* interactions of ACE inhibitors with statins (atorvastatin, rosuvastatin, pravastatin and simvastatin) were studied in stimulated body environments utilizing the HPLC technique.

3.2.1. Interaction of enalapril with statins using HPLC

In vitro interactions of enalapril in the presence of statins drugs (rosuvastatin, atorvastatin and simvastatin) were observed in 1:1 ratio buffers of pH 4 and 7.4 at 37°C. Simultaneous determination of both interacting drugs was also developed, as described above. The results are summarized in Table 7 and Figures 1-3. There was no significant increase or decrease in the concentration of enalapril and interacting drugs at pH 4 and pH 7.4. When enalapril interacted with rosuvastatin, atorvastatin and simvastatin, concentration remained at nearly 99-103% at pH 4 and 99-107% at pH 7.4. Collectively, *in vitro* interaction of enalapril with rosuvastatin, atorvastatin and simvastatin using HPLC at pH 4 and pH 7.4 did not show any significant interactions.

Time	% Availability at pH 4						% Availability at pH 7.4					
min	**ENP**	**ROS**	**ENP**	**ATR**	**ENP**	**SIM**	**ENP**	**ROS**	**ENP**	**ATR**	**ENP**	**SIM**
0	100	100	98	98.3	99	99.8	100	99	99	99.99	99.02	99.97
30	100	100	99	99.3	100	101	99.78	99	98	100	99.9	99.9
60	99	100	104	98.3	99	101	98.99	95	98	102	99.99	100.3
90	100	101	100	99.4	100	102	102.6	106	106	101.8	100.3	100
120	100	100	104	99.4	101	102	99.79	106	104	101.9	101	101.3
150	100	100	101	99.4	100	103	98.93	106	105	102.6	101.3	102.1
180	100	100	99.7	102	101	102	102	106	107	106.5	102	103

Table 7. Percentage availability of enalapril and statins at pH 4 and 7.4 using HPLC

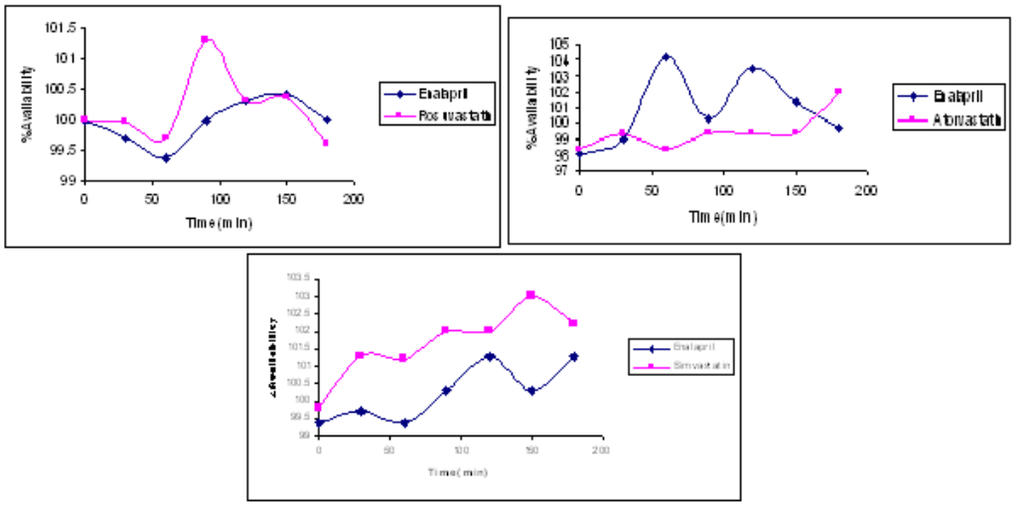


Figure 1. Percentage availability of enalapril and statins at pH 4

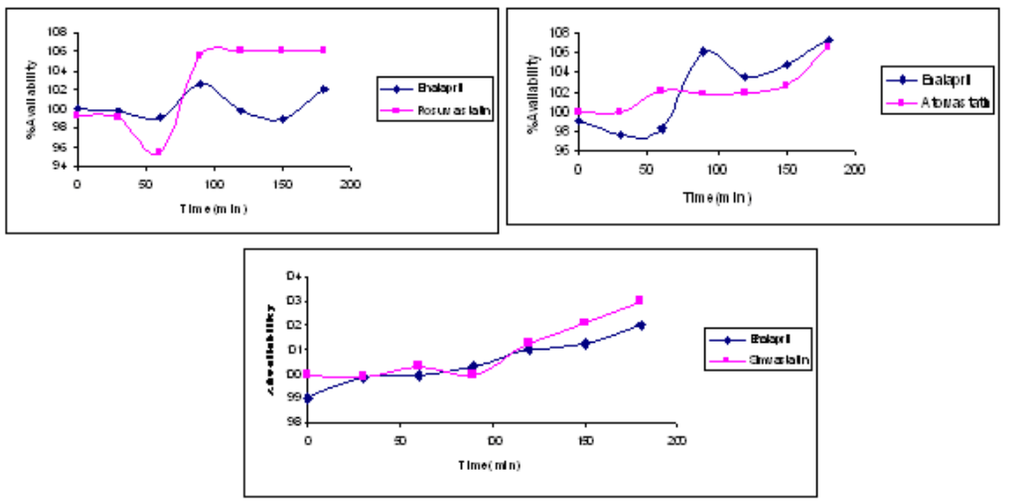


Figure 2. Percentage availability of enalapril and statins at pH 7.4

3.2.2. Interaction of captopril with statins using HPLC

In vitro interactions of captopril in the presence of statins drugs (rosuvastatin, atorvastatin and simvastatin) were observed in 1:1 ratio solutions at 37°C. Simultaneous determination of both interacting drugs was also developed, as described above. Interaction results (Table 8 and Figure 3) show that availability of all drugs was 100% at zero minutes; after that, availability of atorvastatin and simvastatin increased in ascending order, but the percentage availability of captopril decreased in the presence of atorvastatin and simvastatin and remained the same in the presence of rosuvastatin. The availability of atorvastatin and simvastatin was 173% and 115% after 180 min, respectively. Retardation effect was observed at availability of captopril of 97.8%, and 58.2% was available at the end of experiment. Rosuvastatin showed no effect on

captopril and availability of rosuvastatin and captopril at the end of experiment was 100% was 99.12%, respectively.

Time min	CAP	ROS	CAP	ATOR	CAP	SIM
0	100	100.4	100.3	100	100.7	100.3
30	100.2	100.4	100.3	101.5	100.36	100.23
60	100.3	100.3	101.3	100.3	62.1	105.3
90	100.2	99.98	100.2	102.2	67.3	108.2
120	99.9	99.69	100.2	160.8	59.3	110.3
150	100.3	99.87	98.9	162.1	59.3	113.6
180	100.2	99.12	97.8	173.1	58.2	115.6

Table 8. Percentage availability of captopril and statins using HPLC

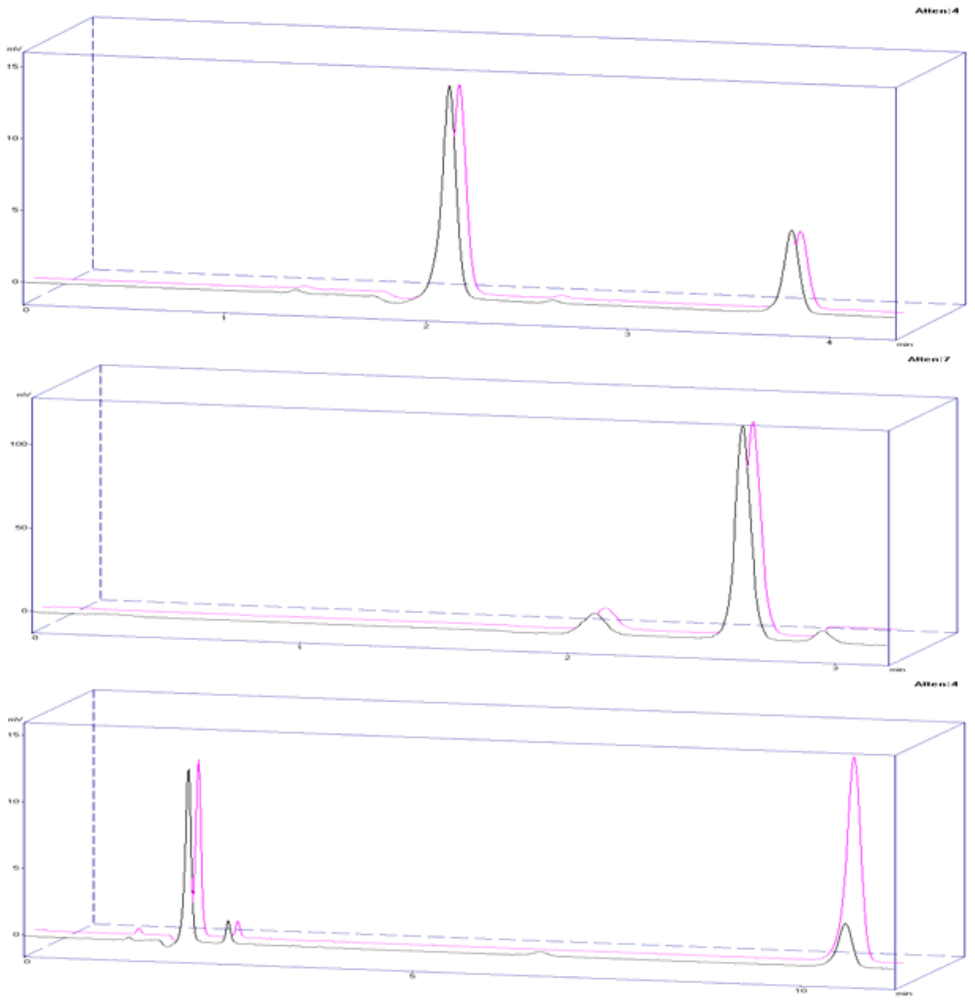


Figure 3. Chromatogram showing change in AUC of drugs. CAP+ATR, CAP+ROS and CAP+SIM (pink before and black after interaction).

3.2.3. Interaction of lisinopril with statins using HPLC

In vitro interactions of lisinopril in the presence of statin drugs (pravastatin, rosuvastatin and atorvastatin) were carried out in solution of 1:1 ratio at 37°C. Simultaneous determination of these interacting drugs was also developed as described above. Results of these interactions (Table 9 and Figure 4) show that when lisinopril interacted with pravastatin, rosuvastatin and atorvastatin its concentration remained in the range 100.3- 102%, 99.9-101.4% and 99.36-102% at 37°C. Pravastatin, rosuvastatin and atorvastatin amplified the availability of lisinopril, unaffected at 37 °C using HPLC. Availability of pravastatin and rosuvastatin after interaction almost stayed unchanged at 37 °C. Interactions of lisinopril in the presence of atorvastatin showed that availability of atorvastatin was enhanced, while that of lisinopril after interaction was unchanged. Collectively, *in vitro* interaction of lisinopril with rosuvastatin, atorvastatin and simvastatin using HPLC at 37 °C did not show any significant results.

Time min	LIS	PRA	LIS	ROS	LIS	ATOR
0	100.3	99.98	99.98	100.3	99.36	97.34
30	100.6	99.63	99.36	100.2	101.5	100.2
60	100.3	99.36	101	100.6	101.3	105.2
90	100.6	100.3	101	100.2	102.5	106.3
120	100.9	100.3	102.3	100.9	101.1	106.20
150	102.0	100.3	102.3	100.4	102.4	106.2
180	102.3	101.3	101.4	100.5	102.4	106.02

Table 9. Percentage availability of lisinopril and statins using HPLC

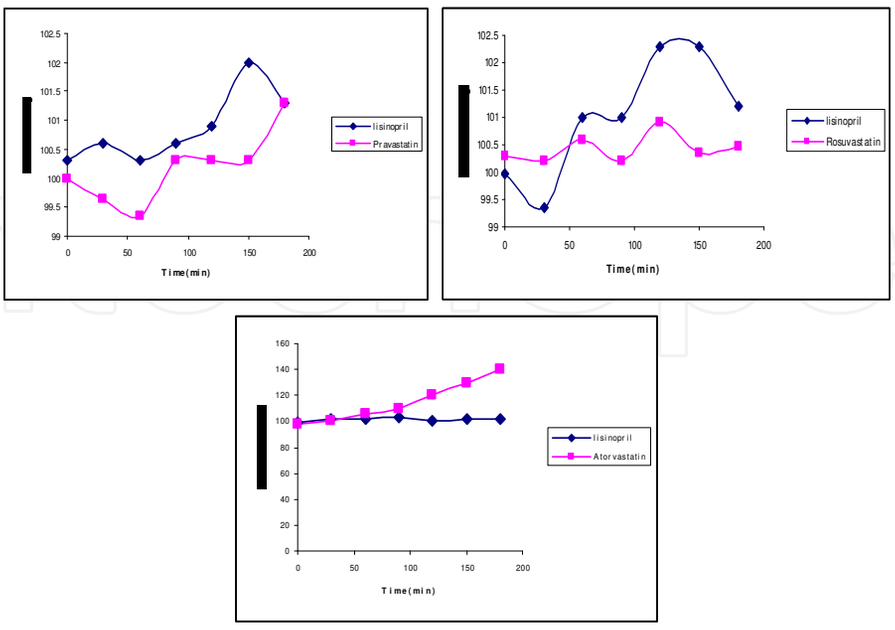


Figure 4. Percentage availability of lisinopril and statins

4. Conclusion

Interaction studies suggest that enalapril and lisinopril are not affected by statins but captopril changes the availability of drugs. *In vivo* studies are required to prove this relationship.

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