Validated Method for the Simultaneous Determination of Lisinopril, Pravastatin, Atorvastatin and Rosuvastatin in API, Formulations and Human Serum by RP-HPLC

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Lisinopril is found to be useful in hypertension and statins as cholesterol lowering drug. Present work was designed for the simultaneous determination of lisinopril in presence of pravastatin, atorvastatin, and rosuvastatin using RP-HPLC method. A Purospher star C18 (5 μm, 25×0.46 cm) column was used with mobile phase consisting of acetonitrile : water (60 : 40 *V*/*V*, pH 3.0) with flow rate of 1.0 mL•min^{-1} and the quantitative evaluation was performed at 225 nm. The retention time of lisinopril was 2.0 min and for pravastatin, rosuvastatin and atorvastatin was found to be 3.1, 4.5 and 8.3 min respectively. Suitability of this method for the quantitative determination of the drugs was proved by validation in accordance with the requirements laid down by International Conference on Harmonization (ICH) guidelines. Application of the suggested procedures were successfully applied to the determination of these compounds in active pharmaceutical ingredient and in pharmaceutical preparations, with high percentage of recovery, good accuracy and precision.

Keywords Lisinopril, statins, method validation, HPLC determination

Introduction

Lisinopril (Figure 1), a synthetic peptide derivative, an oral long-acting, potent angiotensin converting enzyme (ACE) inhibitor, blocks the angiotensin converting enzyme that cleaves angiotensin I to form the potent vasoconstrictor, angiotensin II, as a result, the blood vessels enlarge or dilate and the blood pressure is reduced. It is indicated for the treatment of hypertension and congestive heart failure.[1]

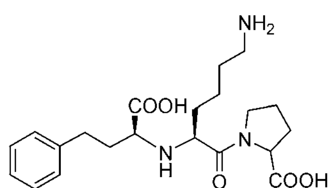
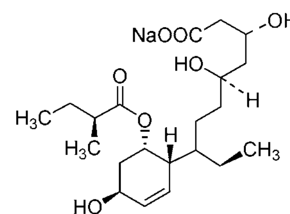
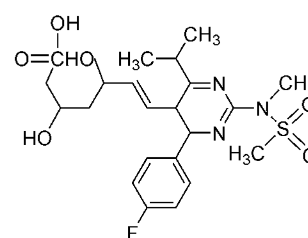


Figure 1 Structure of Lisinopril.

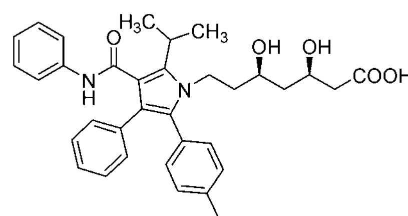
HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitors, called statins (Figure 2) was a breakthrough in the prevention of hypercholesterolemia and related diseases.[2-4] Cholesterol plays an important role in the everyday functioning of the body. Unfortunately, it can also have a negative effect, contributing to the development of atherosclerosis. These plaques can block the arteries, cutting off blood flow, or rupturing and causing a clot that increases blockage. The results



Pravastatin



Rosuvastatin



Atorvastatin

Figure 2 Structure of three kinds of statins.

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of such blockages are very serious and can include angina, claudication, heart attack and stroke. Hypertension and hyperlipidemia are correlated to each other and have additional effect on coronary heart disease and associated mortality rate, since cardiovascular disease is closely related to some factors such as hypertension, high cholesterol levels or diabetes. In literature there are many evidences which suggest additive beneficial effects of simvastatin combined with losartan in the treatment of hypercholesterolemia, hypertensive patients.[5]

Several methods have been reported for the determination of statins.[6-10] Several investigations have been performed for the determination of lisinopril by HPLC.[11-14] However, in literature no HPLC method for the separation and simultaneous determination of lisinopril and statins has been reported. In the present paper a universal HPLC method for the determination of these drugs in bulk material, pharmaceutical formulations and human serum has been described and validated. The aim of the present study was to establish an efficient, reliable, sensitive and accurate method for the separation and quantitative determination of lisinopril and statin drugs simultaneously. Simultaneous determination of these drugs would allow more efficient generation of clinical data and could be performed at more modest cost than separate assays. The applicability of the proposed method was demonstrated later for *in-vitro* interaction studies of lisinopril with statins.

Experimental

Instrumentation

Shimadzu HPLC system equipped with LC-10 AT VP pump and SPD-10 A VP UV-vIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 to P-IV computer loaded with Shimadzu CLASS-VP software (Version 5.03) for data acquisition and mathematical calculations. Rheodyne manual injector fitted with a 20 μL loop, Purospher® star C18 (5 μm, 25×0.46 cm) column a Hiber®, pre-packed column RT 250-4.6 and DGU-14 AM on-line degasser. In addition, Mettler Toledo electronic balance, microliter syringe and micropore filtration assembly were used in this study.

Material and reagents

Lisinopril, (Lisinopril® 5 mg) was a kind gift from Atco Loboratories Ltd, statins used include rosuvastatin, atorvastatin and pravastatin, these were obtained from Pharm Evo (Pvt.) Ltd., Atco Pharma (Pvt.) Ltd., and Bristol Meyers Pvt Ltd., respectively. All these drugs had an expiry of not less than one year at the time of study. All reagents used were of HPLC grade. Acetonitrile, methanol and phosphoric acid 85% (Merk, Germany) and HPLC grade deionized filtered water were used to prepare the mobile phase. Stock solutions of lisinopril and statins were prepared in the mobile phase. Fresh working solutions were prepared daily. All solutions were filtered (0.45 μm) and degassed using sonicator.

Preparation of solutions

Standard solutions of lisinopril and statins were prepared by dissolving appropriate amounts of each in mobile phase methanol : water : acetonitrile (80 : 17.5 : 2.5, *V/V*, pH 3.0) to obtain final drug concentrations of 100 μg•mL^{-1}. For the calibration standards, seven calibrators of each drug were prepared by making serial dilutions from stock solutions. For the assay preparation, the content of 20 tablets were powdered, weighed portion of the powder equivalent to the suitable amount of drug (according to the labeled claimed) was transferred into a 50 mL volumetric flask. The drug was fully dissolved in mobile phase and then diluted with this solvent up to the mark, seven dilutions of each drug were prepared, portion of this solution was filtered through a disposable 0.45 μm filter and then injected.

Serum drug analysis

Blood samples were collected from healthy volunteers and after cogulation centrifuged at 3000 r/min for 10 min. The supernatant (serum) obtained was stored at −20 ℃. After thawing, serum was deprotinated by acetonitrile and spiked daily with working solutions to produce desired concentrations of lisinopril and statins. 20 μL volume of each sample was injected and chromatographed under above conditions.

Chromatographic conditions

The chromatographic analysis was performed at ambient temperature with isocratic elution. The mobile phase consisted of methanol : water : acetonitrile (80 : 17.5 : 2.5, *V/V*) with pH adjusted to 3.0 with phosphoric acid (85%). The pump was set at a flow rate of 1.0 mL•min^{-1}, sample volume of 20 μL was injected in triplicate into the HPLC column and elute was monitored at 225 nm.

Method development

In order to select a proper mobile phase for the separation of lisinopril and statins isocratic elution was applied. The optimization of the analytical procedure has been carried out by varying the mobile phase composition, flow rate and pH of the mobile phase. Preliminary the mobile phases investigated were methanol and water, which were responsible in the broadening of peaks, therefore these could not be used as mobile phase. Optimal retention time for lisinopril was 2.0 min and for pravastatin, rosuvastatin and atorvastatin was found to be 3.1, 4.5 and 8.3 min respectively. The best resolution was achieved when mobile phase was methanol : water : acetonitrile (80 : 17.5 : 2.5, *V/V*) having pH adjusted to 3.0 with phosphoric acid. The criterion for the selection of mobile phase was based on its ease of formation, cost of mobile phase and its effect on peak

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parameters.

Validation procedure

All validation steps were carried out according to the ICH guidelines.[15]

Method validation establishes that the method performance characteristics are suitable for the intended use. Various parameters as system suitability, selectivity, specificity, linearity (concentration-detector response relationship), accuracy, precision, sensitivity, detection and quantification limit recovery from the matrix were studied.[16]

The system suitability was assessed by five replicate analyses of the drug at a concentration of 250 ng•mL^{-1}. System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks of statins and lisinopril, mass distribution ratio (capacity factor) and relative retention.

Specificity is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample.[17]

The specificity of the method was evaluated to ensure separation of lisinopril and statins. For demonstrating the specificity of the method for drug formulation, the drug was spiked and the excipients used in formulation products. The linearity of the method was evaluated at seven different concentrations that ranged from 2.5—100 μg•mL^{-1} for lisinopril and 0.625—25 for statins. Here the peak area using absorbance detection was studied for each drug. The accuracy of the method was evaluated from recovery assay which was made on the formulation samples. Thus, known amounts of each drug were prepared in triplicate at three levels (80%, 100% and 120%) and spiked into their corresponding formulation and the average recovery was calculated as the mean value obtained. To test the precision of the method analysis were carried out in two different non-consecutive days. LOD and LOQ were calculated by the equation given in ICH guidelines. Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy University of Karachi, while Lab 2 was in the Department of Chemistry, Faculty of Science, and University of Karachi.

Results and discussion

Development of HPLC method for the determination of drugs has received considerable attention in recent years because of their importance in routine quality control analysis and comparison of different reported methods.[18] HPLC methods generally require complex and expensive equipment, provision for use and disposal of solvents, labor-intensive sample preparation procedure and personal skilled in chromatographic techniques. The goal of this study was to develop a rapid, more accurate, precise, reliable and least time consuming HPLC method for the simultaneous determination of lisinopril and statins in the form of bulk drug samples, its formulations and human serum using the most commonly employed C-18 column with UV detector.

Method development

In the present investigation the best separation of lisinopril and statins was achieved using a Purospher® star C18 (5 μm, 25×0.46 cm) column which provides efficient and reproducible separation of the components. Using other type of column under similar experimental condition, the separation lasted about 15 min. A mobile phase of methanol : water : acetonitrile (80 : 17.5 : 2.5, *V/V*) having pH adjusted with phosphoric acid to 3.0 provided a reproducible, baseline resolved peak. Small changes in pH of the mobile phase had a great influence on the chromatographic behavior of these substances. It is obvious from the chromatogram (Figures 3 and 4) that statins and lisinopril were eluted out forming symmetrical peaks and were well separated from each other. The method was found to be rapid as lisinopril seperated in 2.0 min and pravastatin, rosuvastatin and atorvastatin were seperated in 3.1, 4.5 and 8.3 min respectively, which is important for routine analysis. In comparison with other published methods for determination of lisinopril and statins the advantages of this method are ease of operation, short analysis time (total run time $<$10 min), utilization of readily available cost-effective solvents, no matrix interferences, and satisfactory limit of quantification to enable pharmacokinetic studies of lisinopril and statins. Rapidness, sensitivity, simplicity, economical nature, acceptable resolution, good recovery and precision of this method give it an advantage over the other reported HPLC methods for the determination of lisinopril and statins.

Method validation

The newly developed method has been validated and holds well for the determination of drug in raw materials, dosage formulations and serum. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use have recommended the accomplishment of selectivity, specificity, linearity, accuracy test, precision, sensitivity, limit of detection and quantification of the method.

Selectivity and specificity

The selectivity and specificity of the method were established through the study of resolution factor of the peak of lisinopril from that of statins. The method demonstrated good resolutions and was found to be free of interference from the excipients (Figure 3) used in formulation products and thus, the method is specific for lisinopril and statins.

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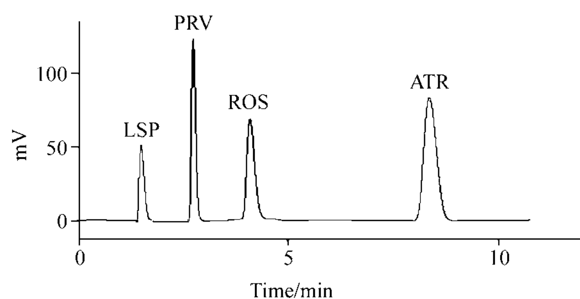

Figure 3 Lisinopril (LSP), pravastatin (PRV), rosuvastatin (ROS) and atorvastatin (ATR) in dosage form.

Range and linearity

Table 1 shows the regression statistics of concentration analytical response, the standard deviation of the regression line and the optimum linear range (2.5—100 μg•mL^{-1}) for lisinopril and 0.625—25μg•mL^{-1} for statins and were found to be linear within the quantification ranges for all the assayed drugs using a linear regression. Excellent linearity was obtained in all cases with correlation coefficients$<$0.999.

Table 1 Regression equations

Drug	Regression equation	r^2
Lisinopril	$y=808.24x+89.01$	0.9994
Pravastatin	$y=315.41x+97.37$	0.9995
Rosuvastatin	$y=315.08x+98.83$	0.9995
Atorvastatin	$y=276.37x+66.89$	0.9994

r^2: correlation coefficient.

Accuracy and recovery

Data corresponding to these recovery assays for the studied analytes are presented in Table 2. The accuracy ranged from 98.9%—101%, at low, medium and high levels for all investigated analytes. The data given in Table 2 show that there is no significant difference between the amount of drug spiked in serum (Figure 4) and the amount recovered. Thus, serum did not interfere with the estimation.

Table 2 Accuracy of lisinopril and statins

Drug	Conc./(μg•mL^{-1})	RSD/%	Recovery/%
Lisinopril	8	0.01	101.3
	10	0.32	98.9
	12	0.63	100.36
Pravastatin	8	0.01	99.9
	10	0.63	100.36
	12	0.63	99.9
Rosuvastatin	8	0.22	99.8
	10	0.96	100.3
	12	0.36	99.7
Atorvastatin	80	0.08	101.3
	100	0.04	100.3
	120	0.36	101

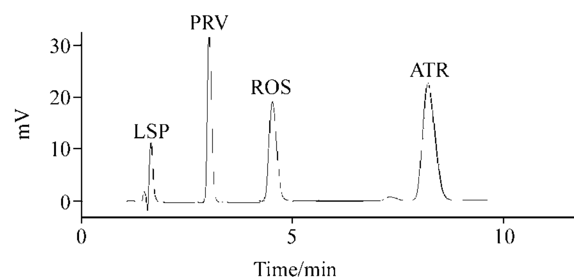

Figure 4 Lisinopril (LSP), pravastatin (PRV), rosuvastatin (ROS) and atorvastatin (ATR) in serum.

Limit of detection and quantification

The analytical sensitivity of the method was anticipated from the signal to noise ratio 3∶1 and 10∶1. The minimum limits at which the analytes can be readily detected (LOD) and quantified (LOQ) for lisinopril 0.4 and pravastatin, rosuvastatin and atorvastatin were 0.6, 1.20, 1.3 ng•mL^{-1} and 1.4, 1.8, 3.6, 4.1 ng•mL^{-1} respectively.

Precision

The intra-and inter-day precisions were evaluated by assaying the samples (Table 3). In this assay, the intraday precision and the inter-day precision were $\leqslant$1.2. The results demonstrated that the values were within the acceptable range and the method was sufficiently accurate and precise.

Ruggedness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy University of Karachi, while Lab 2 was in the Department of Chemistry, Faculty of Science University of Karachi. The method did not show any notable deviations in results from acceptable limits.

Conclusion

The new HPLC method described in this paper provides a simple, universal, convenient and reproducible approach for the simultaneous identification and quantification that can be used to determine lisinopril and any of the four statins. In summary, the proposed method can be used for the drug analysis in routine quality control. In addition, this method has the potential application to clinical research of drug combination, multi-drug pharmacokinetics and interactions.

Table 3 Intra-day and inter-day precision of the method

Drug	Conc. injected/(μg•mL^{-1})	Inter-day		Intra-day	
		RSD/%	Recovery/%	RSD/%	Recovery/%
Lisinopril	2.5	0.36	100	0.08	99.9
	1.2	0.35	99.98	0.23	100
	0.23	0.003	99.6	0.36	100.1
	0.52	0.003	99.87	0.63	100.2
	0.26	0.059	100	0.36	100
	0.23	0.089	100.32	0.36	100.23
Pravastatin	0.625	0.23	100.32	0.26	99.9
	1.25	0.36	99.94	0.64	99.9
	2.5	0.26	100.23	0.098	100.23
	6.25	0.29	99.99	0.12	100.02
	12.5	0.26	100.23	0.56	100.02
	25	0.69	100	0.032	100.03
Rosuvastatin	0.625	0.65	101.12	0.48	101.1
	1.25	0.36	100.03	0.098	100
	2.5	0.56	100.01	0.089	100.23
	6.25	0.36	99.91	0.5	99.9
	12.5	0.36	100.32	0.045	100
	25	0.59	100	0.23	100
Atorvastatin	0.625	0.89	99.87	0.09	99.9
	1.25	0.32	99.86	0.01	99.9
	2.5	0.02	99.97	0.089	100
	6.25	0.12	100.23	0.089	100.2
	12.5	0.04	99.97	0.58	100
	25	0.023	100	0.99	100.3

References

1 Goa, K.; Balfour, J.; Zuanetti, G. *Drugs* **1996**, *52*, 564.
2 Tobert, J. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 517.
3 Jones, P. H.; Davidson, M. H.; Stein, E. A.; Bays, H. E.; McKenney, J. M.; Miller, E. *Am. J. Cardiol.* **2003**, *92*, 152.
4 Caslake, M. J.; Stewart, G.; Day, S. P.; Daly, E. *Atherosclerosis* **2003**, *171*, 245.
5 Koh, K. K.; Quon, M. J.; Han, S. H. *J. Am. Heart Assoc.* **2004**, *110*, 3687.
6 Khalid, M. P.; Muzeeb, M.; Jafar, S. S.; Basha, S. D.; Ramesh, M.; Srinivas, N. R. *Biomed. Chromatogr.* **2005**, *20*(3), 282.
7 Erturk, S.; Sevinc, A. E.; Ersoy, L. *J. Pharm. Biomed. Anal.* **2003**, *33*, 1017.
8 Zarghi, A.; Shafaati, A.; Foroutan, S. M.; Khoddam, A.; *Arzneimittelforschung* **2005**, *55*, 451.
9 Ravi, K. T.; Raja, R. K.; Ramesh, M.; Srinivas, N. R. *J. Pharm. Biomed. Anal.* **2005**, *39*, 661.
10 Bořek-Dohalský, V.; Huclová, J.; Barrett, B. *J. Anal. Bioanal. Chem.* **2006**, *386*, 275.
11 Sane, R. T.; Valiyare, G. R.; Deshmukh, U. M.; Singh, S. R.; Sodhi, R. *Indian Drugs* **1992**, *29*(12), 558.
12 Aytan, F.; Alpdogan, G.; Sungur, S. *Anal. Chem.* **2007**, *26*, 55.
13 El-Emam, A. A.; Hansen, S. H.; Moustafa, M.; El-Ashry, S. M.; El-Sherbiny, D. T. *J. Pharm. Biomed. Anal.* **2004**, *34*, 35.
14 Olcay, S.; Lale, E. *J. Chromatogr. B* **2004**, *809*, 159.
15 ICH Guideline Q2B, *Validation of Analytical Procedures, Methodology*, **2003**.
16 Mehta, A. C. *Analyst* **1997**, *122*, 83.
17 Krull, I. S.; Swartz, M. E. *LC-GC* **1997**, *15*, 534.
18 Xu, Y. J.; Geng, J. P.; Zhang, X. Z.; Zhang, S. J.; Tian, X. H.; Liu, H. H. *Chin. J. Chem.* **2010**, *68*, 86.

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