SIMULTANEOUS DETERMINATION OF ENALAPRIL AND STATIN'S IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC

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ABSTRACT

Simple, specific, economical and precise high performance liquid chromatographic method for the simultaneous determination of enalapril in presence of statins (rosuvastatin, atorvastatin and simvastatin) in API (active pharmaceutical ingredient) and formulation has been developed and validated. Chromatography was carried out at 25°C on a prepacked Purospher Star, C18 (5 mm, 250 x 4.6 mm) column with the isocratic mobile phase of acetonitrile: water (60:40 v/v) adjusting pH to 2.8. The UV detection was carried at 230 nm. The results obtained showed good agreement with the declared contents. Enalapril and statins separated in less than 10 mins with good resolution and minimal tailing and without interference of excepients. The method was linear in the range of 2.5–100 μgmL^{-1} for enalapril concentration with a correlation co-efficient 0.9995 and in the range 0.625–25 μgmL^{-1} for statins concentrations having correlation co-efficient 0.9990 (inter and intraday CV<2 .0%). The recovery was 99-102%. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The proposed method can be used for quantitative determination of enalapril and statins alone or in combination from API and formulations.

Keywords: Enalapril; statins; rosuvastatin; atorvastatin; simvastatin and RP-HPLC.

INTRODUCTION

In literature, it has been shown that antihypertensive drugs and statins are effective in preventing cardiovascular disease[1.] Chemically enalapril is designated as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenyl propyl]-L-alanyl]-L-proline,(Z)-2-butenedioate salt is a drug used in the treatment of hypertension and chronic congestive heart failure.[2]

Control of hypercholesterolemia is of prime importance for the primary and secondary prevention of coronary artery disease (CAD).[3,4]
3-hydroxy-3-methylglutarylcoenzymeA(HMG-Co A) reductase inhibitors (statins) are often prescribed in association with antihypertensiveagents5. Clinical trials have unequivocally demonstrated that treatment of dyslipidemias reduce cardiovascular (CV) evets[6]. Various HPLC methods have been reported for estimation of enalapril maleate. Tajerzadeh *et.al.,* [7] developed a method of enalapril maleate with UV detection having LOD of 0.125 and LOQ of 0.5 μgmL^{-1}. Kyung *et.al.*[8] and Pisarev *et.al*, [9] also developed a method for enalapril using mass spectrometrometry. Several methods are also reported for the determination of statins [10-12]. Our research group has worked on the simultaneous determination of a number of commonly co-adminstered drugs as olmesartan medoxamil and irbesartan and hydrochlorothiazide[13], atenolol**,** **r**osuvastatin**,** spironolactone, glibenclamide and naproxen sodium[14,] enalapril maleate and H_2-receptor antagonists[15], diltiazem and statins[16], lisinopril and statins[17], prazosine and statins[18], ceftriaoxone sodium and statins[19]and antidiabetic drugs with statins[20].

These methods can be applied for the quantitation of drugs as well as for clinical purposes. Other antihypertensive drugs such as amlodipine and statins are available in combined formulations. Simultaneous determinations of these combinations are also present in literature survey, but not a single method for the determination of enalapril and statins is available in literature although both of these drugs are given together. On this basis, it becomes apparent to develop and validate a simultaneous method for the determination of these drugs in bulk material and dosage formulation using RP-HPLC in a relatively short time with high linearity. Furthermore, this validated method was used to study the possible in vitro interactions of enalapril with statins (atorvastatin, rosuvastatin and simvastatin) at different simulating body environments, results of which are reported elsewhere.

EXPERIMENTAL

Instrumentation and chromatographic conditions

Shimadzu HPLC system equipped with LC-10 AT VP pump, DGU-14 AM on-line degasser, from Shimadzu Corporation (Chromatographic and Spectrophotometric Division, Kyoto, Japan) consisted of a ternary gradient system, column and UV detector. Isocratic mobile phase consisted of acetonitrile: water (60:40 v/v) filtered and degassed through membrane filter of 0.45 micron porosity. Purospher Star, C18 (5 mm, 250 x 4.6 mm) column was used as stationary phase. The flow rate was l.8 ml min^{-1} and the detector was set at 230 nm. All analyses were made at 25°C and the volume of solution injected was 20 µL .Chromatograms were recorded and integrated on PC installed with Shimadzu CLASS-GC software (Version 5.03).

Reference substances, reagents and chemicals

Reference standards of enalapril from BMS and statins (rosuvastatin, atorvastatin and simvastatin) were obtained from Pharm Evo (Pvt.) Ltd., Atco Pharma (Pvt.) Ltd., and Geofman Pharma (Pvt.) Ltd., respectively. Enalapril (renitec 10) was a gift from BMS Pvt Ltd. Statins used were rosuvastatin (X-plended 20mg), atorvastatin (Atopitar 10mg) and simvastatin (Atcol 10mg) which were purchased from the local pharmacy. Acetonitrile (HPLC grade) was obtained from Merck, Germany Deionizer; Stedec CSW-300 was used for deionization of water. All the chemicals and reagents were of analytical or reagent grade.

Solution preparation

Enalapril and statins standard stock solutions

Stock standard solutions of 100 μgmL^{-1} of enalapril, and 25 μgmL^{-1} simvastatin, atorvastatin, and rosuvastatin were prepared by dissolving 10 mg of enalapril and 2.5 mg of statins in 100 mL volumetric flask, 10 milliliters of diluent was added initially and sonicated for a few minutes to solubilize these drugs, then 30 ml of mobile phase was added, sonicated to dissolve drugs. The solution was diluted to volume with the mobile phase and mixed.

Standard solution

Solutions were also prepared by diluting these to obtain concentration between 2.5-100 μgmL^{-1} for enalpril and 0.25-25 μgmL^{-1} for statins. These solutions were stored at 20ºC, they were prepared once and analyzed daily for inter-day and inter-operator variations of the method and analyzed each time before drug analysis in biological samples. 20 µL of these solutions were injected into LC system and chromatographed.

Determination from formulations

For testing the suitability of the proposed method for the estimation of the drugs in dosage forms, 20 tablets of each drug were powdered and equivalent

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to 2.5 g of statins (simvastatin, atorvastatin, rosuvastatin and pravastatin) and 10 mg of enalapril were transferred individually to 100 mL volumetric flask, dissolved and diluted with mobile phase. The resulting solutions were filtered through Whatman filter paper no. 41 and diluted to the desired concentration and analyzed for the drug content.

RESULTS AND DISCUSSION

Chromatographic condition

Initially, a Purospher Star, C18 (5 mm, 250 x 4.6 mm) column in isocratic mode, with mobile phase acetonitrile and water in proportion of 60:40 (v/v) at a flow rate of 1.8 ml/min at a detection wavelength of 230 nm was used. To optimize the operating conditions for isocratic RP-LC detection of all analytes, a number of parameters such as the mobile phase composition, pH and the flow rate were varied. Various ratios (80:20, 70:30, 60:40 v/v) of methanol: water was tested as starting solvent for system suitability study then acetonitrile and water having the above ratios was tried. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry, capacity factor and retention time. The pH effect showed that optimized conditions are reached when the pH value is 2.8, producing well resolved and sharp peaks for all drugs assayed. Henceforth, in the present method pH was adjusted to 2.8 using wavelength 230 nm (isobestic point). However, the peak shape and resolution were found to be good when the mobile phase comprising of the ACN: water having pH adjusted to 2.8 with phosphoric acid was used in the ratio of (60:40 v/v) at a flow rate of l.8 $mLmin^{-1}$ (filtered through a 0.45 micron filter). For simultaneous determination of enalapril with simvastatin, rosuvastatin, and atorvastatin individual drug solutions were injected into the column at the concentration of 100 μgmL^{-1} and both elution pattern and resolution parameters were studied. System suitability data is given in table 1. The retention time for enalapril was found to be 2.4 minute, rosuvastatin 3.3 minute, atorvastatin 4.0 minute and for simvastatin was 8.0 minute meeting the resolution criteria specified in USP 2008. A typical chromatogram of test solution is shown in figure 1**.**

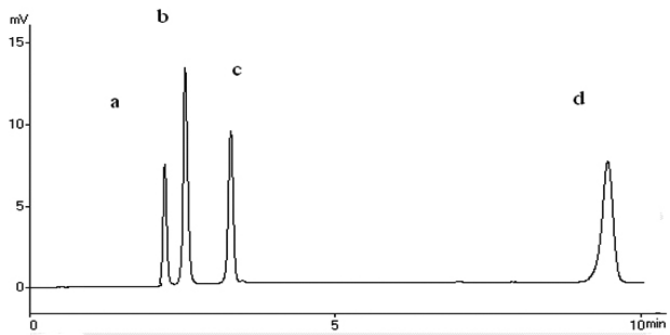

Figure.1: A representative chromatogram of (a)Enalapril (b) rosuvastatin (c)atorvastatin and(d) simvastatin in raw material.

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 system suitability, selectivity, specificity, linearity, accuracy test, precision, sensitivity, limit of detection and quantification of the method.

System suitability testing

Typical system suitability results are summarized in table 1, all the values for the system suitability parameters are within limits. The method was validated according to the ICH guidelines [21].

Table 1: System suitability parameters.

	(% RSD)					
Parameters →	Retention time(Rt)	Capacity factors(K')	Theoretical plates(N)	Tailing factor(T)	Resolution (R)	Separation factor
Enalpril	0.421	0	0.71	0.607	0.415	0.68
Rosuvastatin	0.439	0	0.722	0.786	0.419	0.74
Atorvastatin	0.50	0	0.733	0.911	0.421	0.62
Simvastatin	0.51	0	0.725	0.885	0.413	0.72

Selectivity and Specificity

The selectivity and specificity of the method was established through the study of resolution factor of the peak of enalapril from that of statins (table 1). The method demonstrated good resolutions and was found to be free of interference from the excipients (Fig. 2) used in formulation products and thus, the method is specific for enalapril and statins

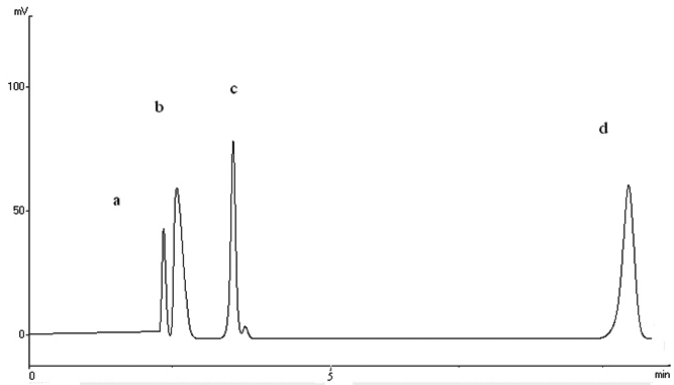

Figure.2: A representative chromatogram of (a)Enalapril (b) rosuvastatin (c)atorvastatin and(d) simvastatin in formulation.

Linearity

Enalapril and statins showed linear calibration curves in the range of 2.5, 5, 10, 25, 50 and100 µgmL- for enalapril and 0.625, 1.25, 2.5, 6.25, 12.5 and 25 µgmL for statins respectively ($r2 > 0.999$)**.** Table 2 shows the regression statistics of enalapril and statins.

Table 2: Regression equations with LOD, LOQ.

Drugs	Regression equations	LOD ng/mL	LOQ ng/mL	r2
Enalapril	y = 1883.8x - 1856.6	3.9	12	0.9995
Rosuvastatin	y = 21249x + 2854.7	0.03	0.09	0.9990
Atorvastatin	y = 27570x - 1123	0.04	0.1	0.9995
Simvastatin	y = 17239x - 1479.9	0.02	0.07	0.9995
LOD=Limit of detection, LOQ=limit of quantification				
Correlation coefficient (r2)				

Accuracy

The accuracy of the method was evaluated from the recovery results of spiked placebo samples. Appropriate portions of stock solution of enalapril and statins were spiked into blank placebo matrix to produce concentrations of 80, 100 and 120% of the theoretical concentration. Mean recovery of samples was 100.1% for enalapril and 99.40% for statins data given in table 3.

Table 3: Accuracy of Enalapril and Statins.

Drugs	Conc%	%RSD	% Recovery
	80%	0.019	100
Enalapril	100%	0.0095	99.99
	120%	0.0058	100
	80%	0.00076	100.01
Rosuvastatin	100%	0.0004	100.01
	120%	0.0002	100
	80%	0.0008	100.01
Atorvastatin	100%	0.0004	100.01
	120%	0.0002	101
	80%	0.0044	100.01
Simvastatin	100%	0.0002	100
	120%	0.0011	100.02

Precision

Precision was determined by six replicate determinations of standard solution and the relative standard deviations were <2% for enalapri and statins. Method precision or intra-assay precision was performed by preparing six different samples involving different weightings. Each solution was injected in triplicate under the same conditions and the mean values of peak area responses for each solution were taken. Intermediate precision was performed by analyzing the samples on two different days (table 4) employing different instruments.

Table 4: *Inter day and intraday precision of Enalapril and Statins.*

Drugs	Conc. Injected µg mL^{-1}	Inter-day		Intra-day	
		%RSD	%Recov-ery	%RSD	%Recov-ery
Enalapril	2.5	0.006	97.44	1.077	100.9
	5	0.014	100.5	1.074	101.05
	10	0.001	99.87	1.292	99.49
	25	0.0009	99.2	0.602	101.18
	50	0.001	100.8	1.055	98.94
	100	0.0015	99.92	1.094	101.13
Rosuvastatin	0.625	0.01	97.44	0.817	100.9
	1.25	0.003	101.9	0.337	101.08
	2.5	0.001	99.52	0.546	100.92
	6.25	0.0005	100.6	0.577	100.52
	12.5	0.0001	100.7	0.497	99.45
	25	0.0009	100.4	1.033	100.64
Atorvastatin	0.625	0.005	97.6	1.286	99.99
	1.25	0.003	100.8	0.573	101.26
	2.5	0.001	100	0.711	100.59
	6.25	0.001	102	0.534	100.55
	12.5	0.001	100.2	0.423	99.09
	25	0.0006	101.8	0.649	99.57
Simvastatin	0.625	0.0173	99.2	0.695	98.85
	1.25	0.0034	103.6	0.52	99.5
	2.5	0.0017	100	0.416	100.5
	6.25	0.0005	102	0.522	101.1
	12.5	0.0002	100.12	0.148	98.14
	25	0.0002	101.6	0.742	99.58

Robustness

Robustness of the proposed method was estimated by changing:(i) mobile phase composition from methanol:water to acetonitrile:water (60:40 v/v); (ii)the pH (iii) the flow rate from 1.0 ml to 1.8ml min^{-1}. System suitability parameters in table 1 were found to be within acceptable limits.

CONCLUSIONS

In short, our method is specific, sensitive, rapid and easy to perform for simultaneous determination of enalapril and statins (rosuvastatin, atorvastatin and simvastatin). The limit of quantification, small sample volume and short chromatographic time of this method makes it advantageous for adaptation to routine assay requirements and enables simultaneous determination of enalapril and statins because of good separation of the chromatographic peaks. The obtained results are in good agreement with the declared contents of dosage formulations. Results are accurate and precise and are confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this method give it advantage over the other reported methods.

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