P . Koushik koteswara rao et al. / Journal of Pharmacy Research 2016,10(2),116-119

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Development and validation of RP-HPLC method for simultaneous estimation of atrovastatin and olmesartan in pharmaceutical formulations

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Received on:26-12-2015; Revised on: 17-01-2015; Accepted on: 28-02-2016

ABSTRACT

Combination therapy of Atorvastatin (AT) and Olmesartan (OLM) is used for the treatment of co existing essential hypertension and hyperlipidemia in adult persons. In the present study a simple, precise, rapid, efficient and reproducible reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of AT and OLM present in its tablet dosage forms. Chromatographic separations were carried out isocratically at 30°C ± 0.5°C on a hypersil bds C_{18}(100,4.6 mm,5µ) with a mobile phase composed of Sodium dihydrogen phosphate pH 4.5 with dilute phosphoric acid : Methanol: Acetonitrile in the ratio of 65:20:15 % v/v at a flow rate of 1.0 ml/min. Detection is carried out using a UV detector at 232 nm. The retention times for OLM and AT were 2.23 min and 3.46 min respectively. The linearity range for 20-100 µg/ml AT and OLM were found to be 40-200 µg/ml with correlation coefficient of 0.999 and 0.999 respectively. The % recovery of the proposed method was found in the range of 100.39 for AT and 98.53 for OLM. The relative standard deviations for three replicate measurements in three concentrations of standard solution were always less than 2%. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which may be useful for the routine estimation of AT and OM in bulk drug and in its pharmaceutical dosage form.

KEYWORDS: Atorvastatin, Olmesartan,RP-HPLC,Validation

INTRODUCTION:

Atorvastatin (AT),[(3*R*,5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid] is a white crystalline powder, slightly soluble in water and ethanol and freely soluble in methanol. AT is a HMG-CoA reductase inhibitor. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis[1, 2]. It is official in Indian pharmacopoeia[3]. The chemical structure AT was shown in fig.1. Olmesartan (OLM), [4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylic acid] is a white to yellow white crystalline powder, insoluble in water. OLM is an angiotensin II receptor blocker (ARB).The chemical structure OLM was shown in fig.2.

It blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle[2, 4, 5]. Several analytical methods that have been reported for the estimation of AT in biological fluids and/or pharmaceutical formulations include spectrophotometric [6, 7], high performance liquid chromatography [8-10], while OLM determinations have been reported by UV-VIS spectrophotometry [11, 12], HPLC[13] ,HPTLC [14] and LC [15,16].An attempt was made to develop a rapid, economic and sensitive method for the simultaneous determination of AT and OLM. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is mandatory, also[17].

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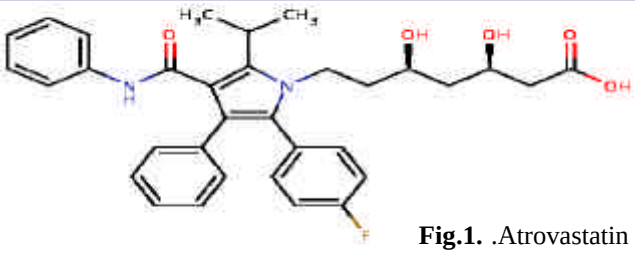

Fig.1. .Atrovastatin

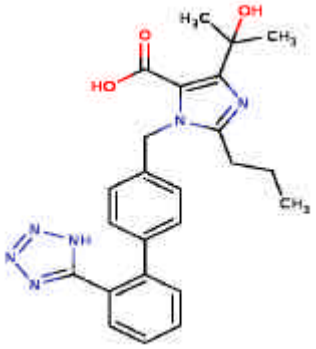

Fig.2. Olmesartan

MATERIAL AND METHODS:

AT and OLM of pharmaceutical grade were kindly supplied as gift samples by Aurobindo pharma limited,Hyderabad,India. Methanol, acetonitrile and water used were of HPLC grade.Phosphoric acid used as analytical grade from Merck. Olmesar av(Macleod Pharma, Mumbai) containing AT and OLM purchased from local market. The chromatographic analysis was performed using.

Chromatographic conditions :

1.19 gms sodium dihydrogen phosphate and 1 ml of triethylamine in 1ltr water adjust pH 4.5 with phosphoric acid.From this take 650ml of above solution ,the add 200 ml of hplc grade methanol and 150 ml of hplc grade acetonitrile.The flow through the column at a constant flow rate of 1.0 ml/min. A hypersil bds C_{18}, 100 X 4.6 mm, 5µ paricle size used as the stationary phase and 232 nm was selected as the detection wavelength for uv detector. Optimised Chromatographic conditions were mentioned in table-1.

Table-1. Optimized condition for simultaneous estimation of Olmesartan and Atrovastatin

Mode of separation	Isocratic elution
Mobile phase	Sodium dihydrogen phosphate P^H 4.5 H_3PO_4:Meoh: ACN-65:20:15
Column	HYPERSIL BDS C_{18}, 100 X 4.6 mm, 5µ,
Flow rate	1.0 ml/min
Detector wavelength	232 nm
Injection volume	20µl
Oven temperature	30°C

Standard stock solution preparation:

Standard stock solutions of 20mg AT and 40 mg OLM were prepared in 100ml of diluent.Working standard solutions were prepared in mobile phase by taking dilutions ranging 20-100 and 40-200 µg/ml for AT and OLM respectively.

Sample preparation:

Twenty tablets of Olmesar av (Macleod Pharma, Mumbai) containing AT and OLM in ratio of 10 mg: 20mg respectively was weighed and crushed to fine powder. Powder equivalent to 10 mg AT and 20 mg of OLM was weighed and dissolved in 100 ml of methanol, sonicated for 10 min and filtered through Whatmann filter paper No. 42. Appropriate volume of the aliquot was transferred to a 10 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain a solution containing 40 µg/ml of AT and 80 µg/ml of OLM. A 20 µl volume of above sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions. The chromatogram shown in the figure 3.

Calibration curves for AT and OLM:

Appropriate aliquots of AT and OLM stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 20-100 µg/ml of AT and 40-200 µg/ml of OLM. The solutions were injected and chromatograms were recorded. Calibration curves were constructed by plotting average peak areas vs. concentrations and regression equations were computed for both the drugs.

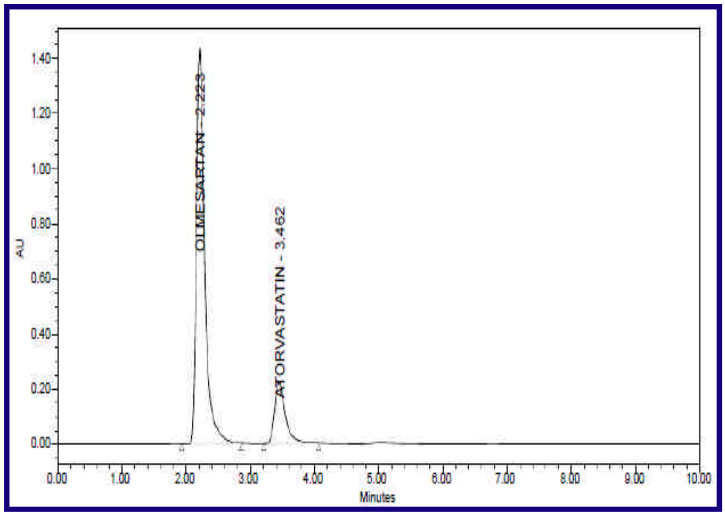

Fig-3 Chromatogram for simultaneous estimation of Olmesartan and Atrovastatin

VALIDATION OF METHOD

Linearity:

Inject the sample solutions containing AT and OLM concentrations in the range of 20-100 and 40-200 µg/ml.Every sample repetated six times.Plot the graph of peak area on y-axis vs concentration on x – axis.

Accuracy:

The accuracy of the method was determined by calculating percentage recovery of AT and OLM. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of AT and OLM corresponding to 50, 100 and 150% of label claim had been added (standard addition method). At each level of the amount three determinations were performed and the results obtained were compared.

Precision:

Precision was measured by analysis of working standard solution at three different concentrations level. The precision of the method, as intraday precision (%RSD) was determined by analysis of AT and OLM standard solutions in the range 10-60 µg/ml and 20-120 µg/ml three times on the same day. Inter-day precision (%RSD) was assessed by analysis of the same solution on three different days over a period of one week.

Limit of detection and limit of Quantification:

The limit of detection (LOD) and limit of quantitation (LOQ) were

Table2. LOD and LOQ values for Olmesartan and Atrovastatin

	LOD (µg/ml)	LOQ (µg/ml)
Olmesartan	0.016	0.048
Atorvastatin	0.013	0.41

Robustness:

Robustness was performed by flow rate was varied to 0.8ml/min and 1.2ml/min (± 0.2ml/min). Robustness values of AT and OLM mentioned in table 3.

Table. 3. Robustness values

Inj. Sample	Flow Rate (ml/min)	USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
Olmesartan	0.8	2477	1.37	2650	1.35
	1.2	2839	1.37	2627	1.42
Atorvastatin	0.8	2959	1.30	3023	1.37
	1.2	3275	1.31	3340	1.36

Ruggedness:

A wide range of the method was studied by carrying out the experiments by changing the conditions such as **.** Different operators in the same laboratory (using waters HPLC system & spectra physics HPLC system). Rugged values of AT and OLM mentioned in table 4.

Table.4. Ruggedness values

	Compound	Tailing factor	Number Theoretical Plates
Analyst-1	Olmesartan	1.37	2665
	Atorvastatin	1.32	3276
Analyst-2	Olmesartan	1.39	2678
	Atorvastatin	1.35	3272

System suitability parameters:

System suitability parameters were showed in table 5.

Table 5. Ststem Suitabvility Parameters:

Parameters	Olmesartan	Atorvastatin
Tailing factor (T)	1.37	1.31
Number of theoretical plate(n)	2676	3265
Retention time (R	2.223	3.462
%RSD	0.23	0.12

ASSAY:

Assay results for combined dosage form using proposed method showed in table 6.

Table 6: Analysis of marketed formulation

Tablet	mg/tablet		Amount found (mg)		Assay (% of label claim)*	
Olmesar Av	AT	OLM	AT	OLM	AT	OLM
	10	20	10.03	19.825	100.39	99.12

Average of three estimation

RESULTS AND DISCUSSIONS:

The present method was a sensitive, precise, and accurate HPLC method for the analysis of Atorvastatin (AT) and Olmesartan (OLM) .To optimize the mobile phase, various combinations of buffer, acetonitrile and methanol were studied on an hypersil C18 column. Then the mobile phase containing a mixture of Sodium di hydrogen phosphate pH 4.5 with dilute phosphoric acid : Methanol: Acetonitrile in the ratio of 65:20:15 (v/v/v)was carried out and found that the resulted peaks with good shape and resolution. A flow rate of 1.0 mL/min was maintained.The retention times of OLM and AT were found to be 2.21 min and 3.45 min respectively. Quantitative linearity was obeyed in the concentration range of 20-100 and 100-200 µg/mL of AT and OLM respectively. The number of theoretical plates obtained was 3265 (AT) and 2676 (OLM) respectively which indicates the efficient performance of the column. The limit of detection and limit of quantitation were found to be 0.13µg/mL and 0.41µg/mL (AT); 0.016 µg/mL and 0.048 µg/mL (OLM) respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

CONCLUSION

In the proposed study, RP-HPLC method was developed for the simultaneous determination of AT and OLM and validated as per ICH guidelines. The developed method was found to be simple, sensitive and selective for analysis of AT and OLM in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation.

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Source of support: Nil, Conflict of interest: None Declared