Original Article

Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form

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Abstract

Aim and Backrgound: A simple, rapid, precise and isocratic RP-HPLC (Reverse Phase High Performance Liquid Chromatography) method is aimed to develop for the simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in bulk drug and pharmaceutical dosage form. **Materials and Methods:** The quantification is carried out using YMC-Pack CN (250 × 4.6 mm, 5.0 μm) column and the mobile phase comprises of 0.05% Trifluoro acetic acid (TFA) and Acetonitrile (ACN) (70:30 v/v). The flow rate is 1.0 ml/min. The eluent is monitored at 220 nm. The retention times of Olmesartan Medoxomil and Metoprolol Succinate are 7.9 min and 4.1 min respectively. The method is validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation. **Results:** Linearity and percentage recoveries of both Olmesartan Medoxomil and Metoprolol Succinate are in the range of 5-35 μg/ml and 100 ± 2%, respectively. The stress testing of both the drugs individually and their mixture is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation (dry heat and wet heat) conditions and its degradation products are well resolved from the analyte peaks. **Conclusion:** This method was successfully validated for accuracy, precision, and linearity.

Key words: HPLC, Metoprolol Succinate, Olmesartan Medoxomil, stability indicating method

INTRODUCTION

Olmesartan Medoxomil (OLME) is chemically (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl) methyl 4-(2-hydroxy propan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-phenyl] phenyl}methyl) -1*H*-imidazole-5-carboxylate [Figure 1]. OLME belongs to a class of drugs known as angiotensin II (A2) receptor blockers (ARBs). These medicines are closely related to the common medications known as ACE inhibitors, which block an enzyme in the body that is responsible for causing the blood vessels to narrow.[1,2] Metoprolol succinate (METO) is chemically (*RS*)-1-(Isop ropylamino)-3-[4-(2- methoxyethyl)phenoxy] propan-2-ol succinate [Figure 2], is a cardio selective β-blocker, used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infraction and heart failure. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP and BP describe potentiometric method for its estimation.[3,4] Literature review revealed that few methods have been reported for the OLME which includes, LC-DAD method for its determination in tablets exposed to stress conditions,[5,6] RP-HPLC method for determination of the OLME in combination with hydrochlorotiazide[7] and amlodipine.[8] A few methods have also been reported for METO which includes RP-HPLC method for its determination in combination with ramipril[9] and amlodipine.[10] The stability of a drug substance or drug product is defined as its

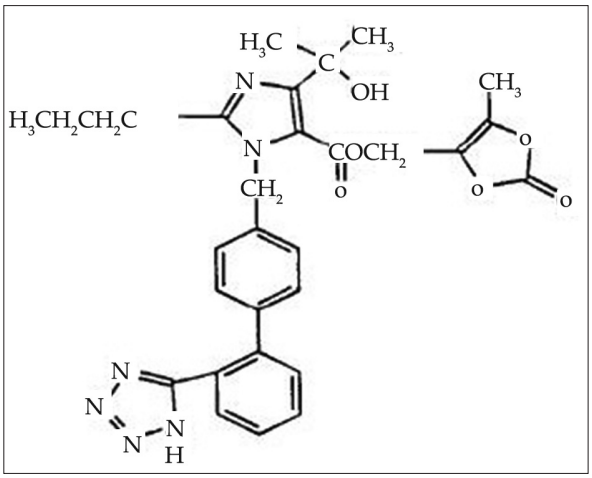

Figure 1: Structure of Olmesartan Medoxomil

capacity to remain within established specifications, i.e., to maintain its identity, strength, quality, and purity until the retest or expiry date. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life.[11,12] With the aim to develop accurate, precise and selective reverse phase isocratic HPLC assay procedure for the analysis of the titled analytes in individual bulk drug samples and in combined dosage formulation, the study was undertaken. Various trials on conventional C8 and C18 columns were conducted using different buffers including sodium phosphate, potassium phosphate in the wide pH range of 3 to 10. But these trials using amino column also did not meet up with the system suitability characteristics; hence the concept to use a novel CN column had to be selected. The proposed method was validated as per ICH guidelines.[13-15]

EXPERIMENTAL

Materials

OLME and METO were supplied as a gift sample by Cadila Healthcare Ltd., Ahmedabad, India. All chemicals and reagents used were of AR grade and purchased from Merck Chemicals, Mumbai. All the solvents used were of HPLC grade. ACN was purchased from S D Fine-chem Ltd., Mumbai and TFA from Sisco Research Lab Pvt. Ltd., Mumbai. Commercial formulation, Olmax-M tablet manufactured by Glenmark Pharmaceuticals Ltd.,

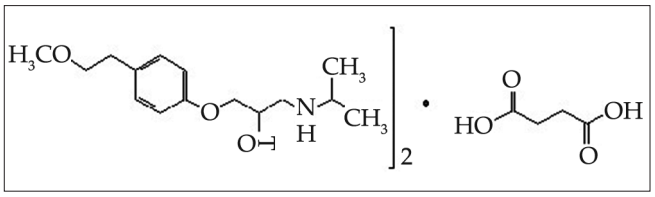

Figure 2: Structure of Metoprolol Succinate

Mumbai, containing 20 mg OLME and 25 mg METO was purchased from local market.

Instrumentation

The HPLC system (Model-Agilent 1200 series) consisted of a Binary pump. The detector consisted of UV/VIS PDA detector operated at a wavelength of 220 nm. Data was integrated using Chemstation software. The column used was YMC-Pack CN (250 × 4.6 mm, 5.0 µm) and the injection volume was 20 µL.

Preparation of standard stock solutions

The standard stock solutions were prepared individually. Accurately weighed OLME (20 mg) and METO (25 mg) were transferred to separate 100 ml volumetric flasks and then the volume was made up to the mark with ACN: Water (1:1). The stock solutions were further diluted with ACN: Water (1:1) to obtain a solution of OLME (20 µg/ml) and METO (25 µg/ml), respectively.

METHOD VALIDATION

Validation of the optimized HPLC method was carried out with respect to the following parameters:

Linearity and range

Linearity of the method was established by injecting six concentrations of the drugs prepared in the ACN: Water (1:1) in the range 5 to 35 µg/ml for both OLME and METO in six replicates into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (15, 20 and 25 µg/ml for OLME and METO) of the drugs three times on the same day (intra-day) and three consecutive days (inter-day).

Limit of detection and limit of quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × σ/*S* and LOQ = 10 × σ/*S*, where σ is the standard deviation of the peak areas of the drugs (*n* = 3), and *S* is the slope of the calibration plot in the lower part of linear range.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (OLME and METO combination tablet) to which known amount of OLME and METO standard solution corresponding to 50, 100 and 150% of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed in optimized chromatographic conditions. Base level amount of OLME and METO used for spiking were 6 µg/ml and 7.5 µg/ml respectively.

Analysis of a marketed formulation

To determine the content of OLME and METO in conventional tablet, twenty tablets were weighed; their mean weight was determined and was finely powdered. The weight of the tablet triturate equivalent to 20 mg of OLME (25 mg of METO) was transferred into a 100 ml volumetric flask and 80 ml ACN: Water (1:1) was added, sonicated for 30 min and diluted up to the mark with ACN: Water (1:1). The resulting solution was centrifuged at 1000 rpm for five min using G-force centrifuge machine. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 20 µg/ml OLME (25 µg/ml of METO). A 20 µL volume of sample solution was injected into HPLC system, under the conditions described above.

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently. The peak purity of OLME and METO was determined by comparing the retention time (t_R) of OLME and METO in standard and sample. The specificity was determined in absence of impurity under the various stress conditions like acidic, basic, peroxide, thermal, dry heat and wet heat.

FORCED DEGRADATION STUDIES

Acid degradation

The OLME + METO mixture was treated with 2 ml 0.1N HCl and heated at 100°C for 1hr. The mixture was cooled at RT and the volume was made up to 20 ml with ACN: Water (1:1).

Base degradation

The OLME + METO mixture was treated with 2 ml 0.01N NaOH and heated at 100°C for 1 hr. The mixture was cooled at RT and the volume was made up to 20 ml with ACN: Water (1:1).

Peroxide oxidation

The OLME + METO mixture was treated with 2 ml 0.1% H_2O_2 and heated at 100°C for 1 hr. The mixture was cooled at RT and the volume was made up to 20 ml with ACN: Water (1:1).

Dry heat degradation

The OLME + METO mixture was heated at 105°C for 24 hrs in Hot Air Oven. The volume was made up to 20 ml with ACN: Water (1:1).

Humidity degradation

The OLME + METO mixture was treated at 75% RH for 24 hrs. The volume was made up to 20 ml with ACN: Water (1:1).

UV-stability

The OLME + METO mixture was treated at 254 nm in a UV chamber for 24 hrs. The volume was made up to 20 ml with ACN: Water (1:1).

Photo-stability

The OLME + METO mixture was treated in sunlight for 24 hrs. After that the volume was made up to 20 ml with ACN: Water (1:1).

RESULTS AND DISCUSSION

Development and optimization of HPLC method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for OLME and METO respectively. The mixed standard stock solution (20 µg/ml for OLME and 25 µg/ml for METO) was injected in HPLC. For HPLC method

optimization different ratios of different mobile phases were tried in combination with different columns including methanol, water, ACN, Phosphate buffers as the mobile phase and different C8, C18 and amino columns with various dimensions. But it was found that none of these combinations worked. Also Olmesartan showed stability problems in Methanol so ACN was used as solvent. In general in the commonly used C8 and C18 columns, it was found that in acidic conditions, there was a co-elution of both the peaks and on the contrary in neutral and basic conditions METO eluted early with retention time about five min and OLME was retained too long up to 35-40 min leading to a long method run time. After many trials it was found that YMC-Pack CN column gives good separation. Further using factorial design consisting of various buffers (sodium phosphate and potassium phosphate) at different pH ranges from 3 to 10 (3, 4, 5, 6, 7, 8, 9, 10), various trials were taken. It was found that acidic conditions (pH 3 to 5) gave good resolution but the peak shape was not good. Further a trial of 0.05% Trifluoro acetic acid (TFA) and ACN (70:30 v/v) with a flow rate of 1.0 ml/min in YMC-Pack CN column was taken which gave acceptable retention time (t_R), peak shape, plates counts and good resolution for OLME and METO [Figure 3]. Hence using these set of condition, system suitability parameters were studied by injecting six injections of the mixed standard of OLME and METO.

The proposed method for simultaneous estimation of OLME and METO in bulk drug and pharmaceutical dosage form was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines using 0.05% Trifluoro acetic acid (TFA): Acetonitrile (70:30 v/v) as mobile phase and YMC-Pack CN column. System suitability studies showed that the %RSD for six replicates of standard OLME and METO was less than 1.5%, resolution between both the drugs was 16.45, the plate count were 11833 and 9950 and the USP tailing factor for the drugs were 1.39 and 1.36 for OLME and METO, respectively.

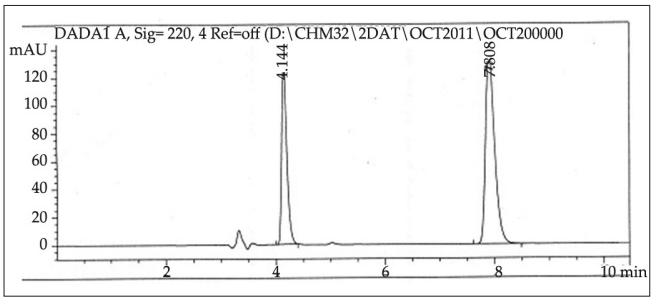

Figure 3: Chromatogram of standard olmesartan medoxomil and metoprolol succinate

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The Linearity range for both analytes was found to be 5 to 35 µg/ml. The regression equation for OLME and METO were found to be y = 73.6 × −14.32 and y = 33.64 × −10.03 with coefficient of determination, (r^2) 0.999 and 0.998 respectively.

Precision

The results of the repeatability and precision experiments are shown in Table 1. The developed method was found to be precise as the % RSD values for repeatability and precision studies were <2% as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.085 and 0.259 µg/ml for OLME, and 1.05 and 3.19 µg/ml for METO respectively.

Accuracy (recovery studies) and analysis of the commercial formulation

Results of recovery studies were in the range of 98 to 102% [Table 2]. The result of analysis of tablet formulation is in the range of 100 ± 0.5% and is reported in Table 3.

Table 1: Precision studies

Drug	Concentration (µg/ml)	Intraday precision (% RSD)	Inter day precision (% RSD)
OLME	15	0.12	0.56
	20	0.26	0.45
	25	0.45	0.61
METO	15	0.51	0.68
	20	0.79	0.86
	25	0.85	0.94

Table 2: Recovery studies

Concentration (mg/tablet)	Amount added (%)	Total amount present (mg)	Amount recovered (mg)	% Recovery
OLME	50	30	29.50	98.03
	100	40	39.72	98.63
	150	50	48.91	98.28
METO	50	37.5	37.41	99.55
	100	50	50.17	100.71
	150	62.5	63.02	101.41

Table 3: Assay of commercial tablet

Drug	Label claim (mg)	Drug content (%)± S.D	% R. S. D
OLME	20	100.504±0.6855	0.682
METO	25	100.308±0.9533	0.95

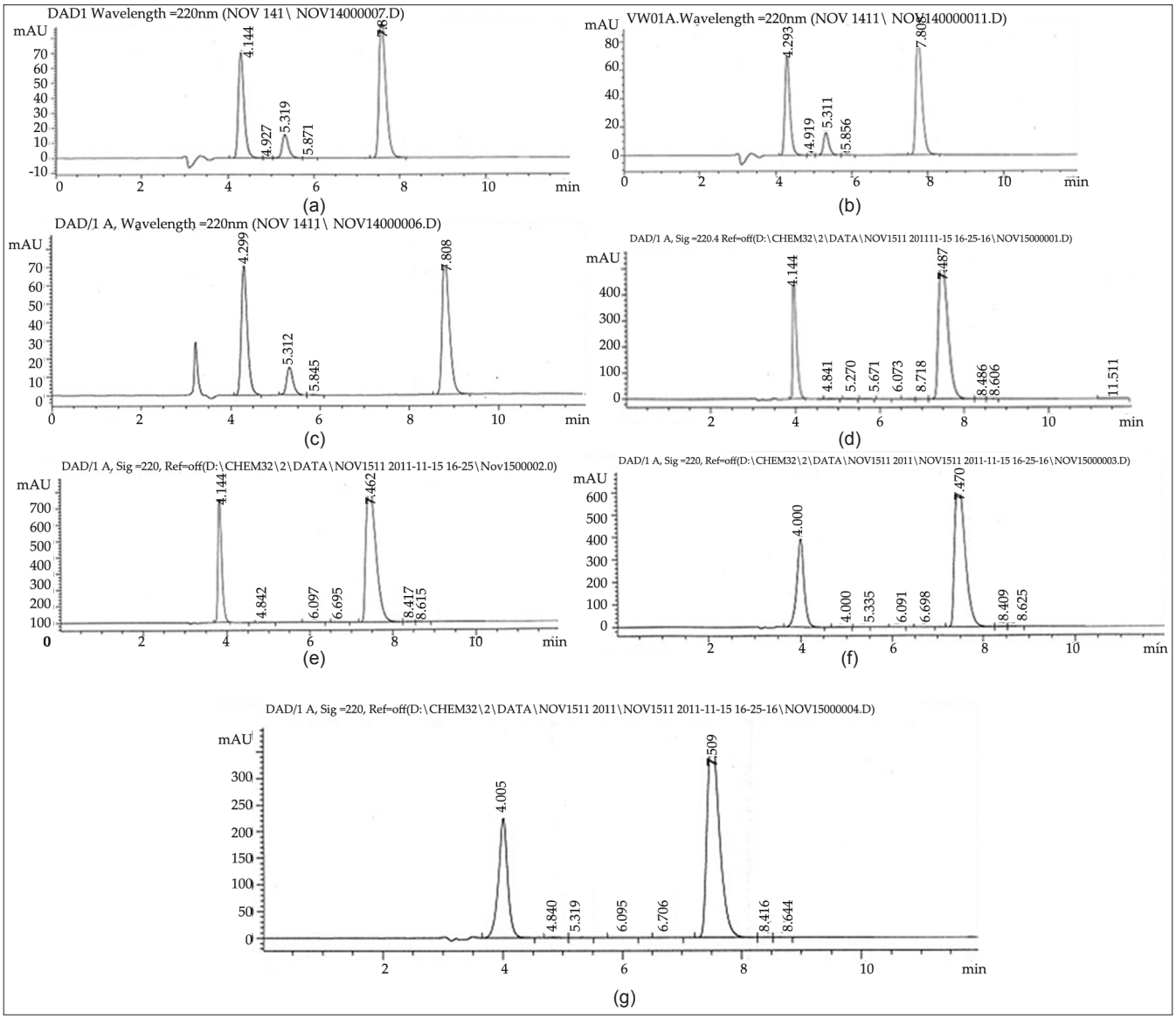

Figure 4: (a) Acid treated OLME+METO, (b) Base treated OLME+METO, (c) Peroxide treated OLME+METO, (d) Thermal treated OLME +METO, (e) Humidity treated OLME+METO, (f) UV treated OLME+METO, (g) Sunlight treated OLME+METO

Table 4: Forced degradation studies

Agent	Exposure	Condition	Degradation peaks at (t_R in min)		Total % degradation	
			OLME	METO	OLME	METO
0.1N HCl	1 hr	Heat at 100°C	5.8	4.9, 5.3	1.3	6.67
0.01N NaOH	1 hr	Heat at 100°C	5.9	4.9, 5.3	1.3	6.72
0.1% H_2O_2	1 hr	Heat at 100°C	5.8	5.3	2.5	4.49
Dry heat	24 hrs	Heat at 105°C	8.4, 8.6	4.8, 6.1, 6.7, 6.8	0.24	0.58
Humidity	24 hrs	75% RH	8.4, 8.6	4.8, 6.1, 6.7	0.48	0.43
UV-Light	24 hrs	254 nm	8.4, 8.6	4.8, 5.3, 6.1, 6.7	0.41	0.31
Sunlight	24 hrs	Normal sunlight	8.4, 8.6	4.8, 5.3, 6.1, 6.7	0.21	0.36

Specificity and forced degradation

The peak purity of OLME and METO were assessed by comparing the retention time (t_R) of standard OLME and METO. Good correlation was obtained between the retention time of standard and sample of OLME and METO. The chromatograms of the mixture after stress treatment are shown in Figures 4a-g. The percentage degradation of OLME and METO were calculated by comparing the peak area of OLME and METO before and after treatment. The amounts of OLME and METO

after treatment were calculated and the results are reported in Table 4. The degradation peaks were well resolved from that of drugs in the absence of impurity.

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. As all the stress degraded products are well resolved from the analyte peaks and there is no co-elution it can be concluded that the method is specific for simultaneous estimation of OLME and METO in pharmaceutical dosage form. The method has linear response in stated range and is accurate and precise. Statistical analysis proves that the method is suitable for the analysis of OLME and METO as bulk drugs and in pharmaceutical formulations without any interference from the excipients and degradation products. As the mobile phase is Mass Spectrometry compatible, it may be extended for the estimation of OLME and METO in plasma and other biological fluids.

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