Original Article

Development of the UV spectrophotometric method of Olmesartan medoxomil in bulk drug and pharmaceutical formulation and stress degradation studies

Abstract

A simple, sensitive, specific, spectrophotometric method was developed for the detection of Olmesartan medoxomil (OLM) in bulk and pharmaceutical formulations. The optimum conditions for the analysis of the drug were established. OLM was subjected to stress degradation under different conditions recommended by the International Conference on Harmonization (ICH). The samples so generated were used for degradation studies using the developed method. The λ_{max} of the OLM was found to be 257 nm. The method exhibited high sensitivity, with linearity, in the 2 to 20 µg/ml range. The lower limit of detection and the limit of quantification were found to be 1.012 µg/ml and 3.036 µg/ml, respectively. All the calibration curves demonstrated a linear relationship between the absorbance and concentration, with the correlation coefficient higher than 0.99. The regression equation of the curve was Y = 0.0579x + 0.0006. The precision of the method was found to be 40.043 ± 0.067 against the label claim of 40 mg. The percentage recovery was found to be 101.32 ± 0.452. The sample solution was stable for up to two hours. Hence, it could be concluded that the proposed method would be suitable for the analysis of OLM in bulk and pharmaceutical formulations.

Key words: Estimation, Olmesartan medoxomil, spectroscopy, stress degradation study

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INTRODUCTION

Chemically Olmesartan medoxomil is (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl] methyl]imidazole-4-carboxylate [Figure 1], and is a synthetic analog of the angiotensin II receptor blocker, which is widely utilized nowadays in the first line treatment of hypertension.[1,2]

Analysis is an important component in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drug(s) in bulk, in drug delivery systems, in dissolution studies (*in vitro*), and in biological samples (*in vivo*). If such a suitable method for a specific need is not available, then it becomes essential to develop a simple, sensitive, accurate, precise, and reproducible method for the estimation of drug samples. The literature survey reveals that OLM was analyzed by the Liquid Chromatography-Mass Spectrophotometry (LC-MS) and Reverse phase High Performance Liquid Chromatography (RP-HPLC) methods.[3-6] Moreover simultaneous Ultraviolet (UV) estimation of OLM and Amlodipine has been reported by Kumar M *et al.*, but a single estimation of this drug has not been reported in bulk or in pharmaceutical formulation. Thus, the present study was undertaken to develop and validate a simple, sensitive, accurate, precise, and reproducible UV method for OLM, and also to perform stress degradation studies on the drug as per ICH Guidelines, using the same method.[7-14]

MATERIALS AND METHODS

Instruments and materials

The instruments used were a SHIMADZU 1700 double beam UV / Visible Spectrophotometer and a SHIMADZU AX200 analytical balance. The OLM pure drug was obtained from the Torrent Research Center, Bhat, Gandhinagar, as a gift sample, with 99.9% w/w assay value, and was used without further purification. All chemicals and reagents used were of analytical grade. The OLM tablets (40 mg) were purchased from the local market with a trade name Benicar® (Daiichi Sankyo).

Preparation of the standard stock solution

A standard drug solution of OLM was prepared by dissolving 10 mg of OLM in 10 ml methanol, and this was transferred into a 100 ml volumetric flask. The volume was brought up to the mark with methanol to obtain a stock solution of OLM with 100 µg/ml final concentration. The solution was further sonicated for 15 minutes to obtain a clear solution.

Preparation of the working solution

From the above stock solution, a 2 ml sample was transferred into a 10 ml volumetric flask and the volume was made up to the mark with methanol to prepare a concentration of 20 µg/ml. The sample was further scanned by a UV-VIS Spectrophotometer in the range of 200 – 400 nm, using methanol as a blank. The wavelength corresponding to the maximum absorbance (λ_{max}) was found to be 257 nm [Figure 2]. This was further utilized to obtain a calibration curve.

Preparation of the calibration curve

Aliquots of 0.2 to 2 ml stock solutions were transferred to a series of 10 ml volumetric flasks, with subsequent volume adjustment by methanol up to 10 ml. The solutions were scanned in a double beam UV-VIS spectrophotometer. The samples were analyzed for their respective absorbance at 257 λ_{max}. The calibration curve was plotted and the optical characteristics summarized [Table 1].

Preparation of the sample solution

The proposed method was applied to analyze the commercially available OLM tablet (Benicar®–40 mg). Ten tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of OLM was weighed accurately and transferred to a 100 ml volumetric flask containing 10 ml of methanol, which was further sonicated for 15 minutes with frequent shaking. The volume was brought up to 100 ml by methanol. The solution was subjected to filtration through Whattman filter paper #44. The filtrate was diluted suitably with methanol to get a final solution of 5 µg/ml concentration. This was subsequently analyzed for OLM using a double beam UV-VIS spectrophotometer and methanol as the blank. The drug content of the sample was calculated using the standard calibration curve.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result, or a product meeting its predetermined

Table 1: Validation parameters

Parameter	Result
Absorption maxima (nm)	257 nm
Linearity range (mg/ml)	2 – 20 µg/ml
Standard regression equation	y = 0.0579x + 0.0006
Correlation coefficient (R^2)	0.9972
Accuracy (% recovery ± SD)	100.57 ± 0.565
Precision (% recovery ± SD)	0.3306 ± 0.006
Specificity (% recovery ± SD)	99.98 ± 0.325
Sandell's sensitivity (mg/cm^2/0.001 absorbance unit)	0.03222
LOD (µg/ml)	0.2 µg/ml
LOQ (µg/ml)	0.665 µg/ml

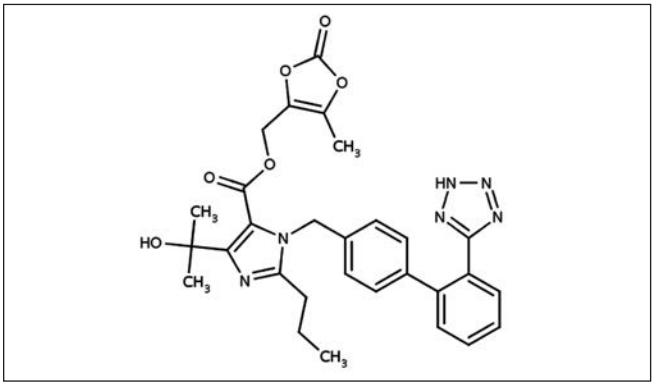

Figure 1: Structure of Olmesartan medoxomil

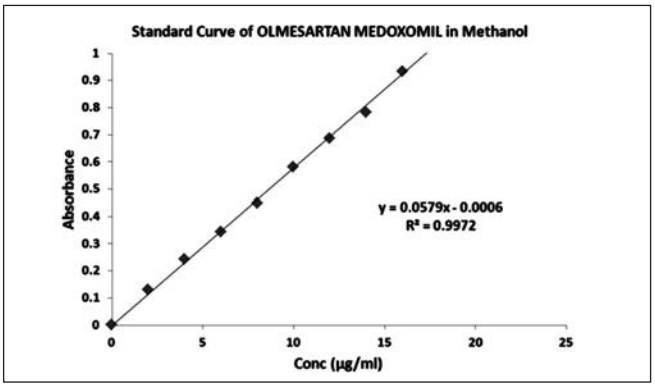

Figure 2: UV spectrum of Olmesartan medoxomil in methanol

Table 2: Linearity in working standards

Concentration (µg/ml)	Absorbance (Average)	Standard deviation
0	0	0
2	0.0865	± 0.005859
4	0.1625	± 0.014856
6	0.2340	± 0.010201
8	0.3185	± 0.006341
10	0.4100	± 0.007279
12	0.4800	± 0.006723
14	0.5610	± 0.004376
16	0.6465	± 0.002359
18	0.7165	± 0.003467
20	0.8032	± 0.004467

Table 3: Determination of accuracy by the percentage recovery method

Ingredient	Tablet amount (µg/ml)	Level of addition (%)	Amount added (mg)	Drug found (µg/ml)	% Recovery	Average recovery ± SD (%)
Olmesartan medoxomil	5	80	4	9.2341	102.55	101.43 ± 1.25
	5	100	5	10.1355	101.35	
	5	120	6	11.0444	100.40	

Table 4: Precision results showing repeatability of Olmesartan medoxomil

Concentration (µg/ml)	Absorbance	Mean ± SD
12	0.4800	0.4809 ± 0.002005
12	0.4851	
12	0.4786	
12	0.4792	
12	0.4823	
12	0.4812	
12	0.4791	
12	0.4799	
12	0.4811	
12	0.4827	

Table 5: Intra-assay study

Concentration (µg/ml)	% RSD			Average % RSD
	Absorbance 1	Absorbance 2	Absorbance 3	
12	0.22	0.23	0.19	0.21

Table 6: Inter-assay study

Concentration (µg/ml)	% RSD			Average % RSD
	Day 1	Day 2	Day 3	
12	0.15	0.19	0.22	0.19

specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD), and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared from the stock solution (100 µg/ml) ranging from 2 – 20 µg/ml. The samples were analyzed with the help of a UV-VIS Spectrophotometer, using methanol as the blank. The linearity of the above-mentioned sample can be observed in Table 2.

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations, that is, 80, 100, and 120%, in which the amount of marketed formulation (Benicar®) was kept constant (5 mg) and the amount of pure drug was varied, that is, 4 mg, 5 mg, and 6 mg for 80, 100, and 120%, respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery [Table 3].

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of same concentration (12 µg/ml) were prepared and analyzed thrice, for three consecutive days, and the absorbances were recorded [Tables 4 and 5]. In the intra-day variation study, nine different solutions of the same concentration (12 µg/ml) were prepared and analyzed thrice a day (morning, afternoon, and evening). The result was indicated by % RSD [Table 6].

Specificity

Olmesartan medoxomil of 5 mg was spiked with 50% (5 mg), 100% (10 mg), and 150% (15 mg) of excipient

Table 7: Test for specificity

Excipient concentration added (%)	Amount of pure drug added (mg)	Total drug recovered (mg)	Total drug recovered (%)	Mena recovery	% RSD
50	5	7.5255	107.42	103.87	3.91
100	5	10.4532	104.53		
150	5	12.4653	99.68		

Table 8: Ruggedness study

Concentration (%)	Analyst 1		Analyst 2	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis
12	0.4778	Mean: 0.4799	0.4732	Mean: 0.4787
12	0.4801	% RSD: 0.21	0.4839	% RSD: 0.53
12	0.4820	Standard error: 0.0014	0.4791	Standard error: 0.0030

mix (Magnesium Stearate) and the sample was analyzed for % recovery of OLM [Table 7].

Ruggedness

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbances were noted. The results are indicated in Table 8.

Limit of detection

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1 – 0.5 μg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

Limit of quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

Degradation studies

The ICH guidelines entitled stability testing of new drug substances and products that required stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this study was to perform the stress degradation studies on OLM using the method developed.

Stress degradation by hydrolysis under acidic condition

A stock solution of OLM was prepared by dissolving 10 mg of the drug in 10 ml of methanol to produce 1000 μg/ml of the solution. To 1 ml of the stock solution (1000 μg/ml), 1 ml of 1 N HCl was added in a 10 ml volumetric flask and the volume was brought up to the mark with methanol. The volumetric flask was kept under normal conditions for 90 minutes. After a 60-minute time interval, 1 ml of solution was sampled out from the flask, which was further neutralized and diluted with methanol in order to bring the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (10 μg/ml). For the blank sample, 0.5 ml solution of 1N HCl and 0.5 ml solution of 1N NaOH were diluted with methanol in a 10 ml volumetric flask. After 90 minutes, again 1 ml of the solution was pipetted out from the flask and the above procedure was repeated.

Stress degradation by hydrolysis under alkaline condition

To 1 ml of OLM stock solution, 1 ml of 0.1 N NaOH was added in a 10 ml of volumetric flask, and the volume was brought up to the mark with methanol. The volumetric flask was kept under normal conditions for 90 minutes. After a 60-minute time interval, 1 ml of the solution was pipetted out from this flask, neutralized, and diluted with methanol, to bring the volume up to 10 ml, and the dilutions were carried out to achieve the appropriate concentration (10 μg/ml). For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH were diluted with methanol in a 10 ml of volumetric flask. After, 90 minutes 1 ml of the solution was again pipetted out from the flask and the above-mentioned procedure was repeated.

Dry heat-induced degradation

The OLM sample was taken in a petri plate and exposed to a temperature of 70°C for 48 hours in a hot air oven. After 48 hours, 10 mg of the sample was diluted with methanol in order to bring the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20 μg/ml) and the solution was analyzed using the previously validated UV method.

Oxidative degradation

To 1.5 ml of the stock solution of OLM (1000 μg/ml), 1 ml of 30% w/v of hydrogen peroxide was added in a 10 ml volumetric flask and the volume was brought up to the mark with methanol. The volumetric flask was then kept at room temperature for 15 minutes. For the blank, 1 ml of 30% w/v hydrogen peroxide was kept under normal conditions, overnight, in a 10 ml volumetric flask. Both solutions were heated in a boiling water bath to remove the excess hydrogen peroxide. Finally, after 15 minutes, dilutions were made from the stock solution to achieve the required concentration (12 μg/ml). The solution was further analyzed with the help of a UV-VIS spectrophotometer.

Photolytic degradation

A sample of OLM was exposed to a near ultraviolet lamp in a photostablity chamber, providing

illumination of not less than 1.2 million lux hours. Ten milligrams of the sample was dissolved in methanol and the volume was brought up to 10 ml. From this solution, an appropriate dilution (12 μg/ml) was made using methanol and taken in a cuvette, for UV analysis.

RESULT AND DISCUSSION

The calibration curve of Olmesartan medoxomil in methanol was found to be linier in the concentration range 2-20 μg/ml [Figure 3]. The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. The method was also found to be specific, indicated by the % recoveries ranging from 100.4 to 102.55%. The LOD and LOQ were found to be in the sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values, which were less than 2%. The results of the assay showed that the amount of drug was in good agreement with the label claim of the formulation as indicated by the % recovery. A summary of the validation parameters of the proposed spectrophotometric method is shown in Table 2. The stress degradation studies showed that OLM underwent degradation in acidic and alkaline conditions, whereas, it was relatively stable when exposed to dry heat, oxidation, and in photolytic conditions. A summary of the results of the stress degradation studies of OLM are shown in the Table 9.

Table 9: Stress degradation study

Condition	Time	Degradation (%)
0.1 N NaOH (1ml)	60 minutes	42.45
	90 minutes	88.90
3 N HCl (1 ml)	60 minutes	85.92
	90 minutes	99.90
30% Hydrogen peroxide (1 ml)	15 minutes	11.67
Dry heat (70°C)	48 hours	1.32
Photolytic	3 hours	45.78
	6 hours	56.67

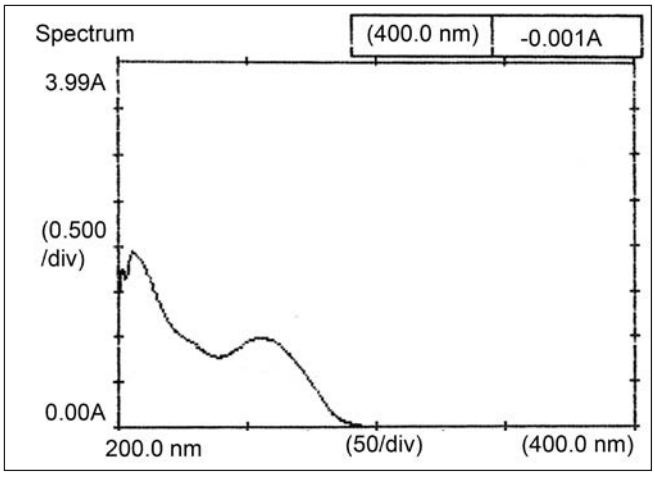

Figure 3: Standard curve of Olmesartan medoxomil in methanol

CONCLUSIONS

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust, and cost-effective, and can be applied successfully for the estimation of OLM in bulk and pharmaceutical formulations. The proposed method is also useful for the determination of OLM stability in samples of pharmaceutical dosage forms.

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