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Validated stability indicating LC-DAD method for determination of olmesartan medoxomil in tablets exposed to stress conditions

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Abstract

A sensitive and precise LC-DAD method was developed and validated for the determination of olmesartan medoxomil in the presence of its degradation products. Olmesartan medoxomil with its degradation products was resolved on a C_{18} column with mobile phase composed of methanol, acetonitrile and water (60:15:25 v/v/v; pH 3.5±0.5 by orthophosphoric acid) at 202 nm on DAD. Each peak resolved always with a resolution of >2.0 indicating the method to be specific and selective. Four degradation products (DP-I, II, III, IV) were formed during degradation study.

Keywords: Olmesartan medoxomil, stress degradation, liquid chromatography-DAD, stability indicating.

Introduction

Stability can be defined as the capacity of a drug substance or drug product to sustain its identity, strength, quality, and purity throughout the retest or expiration period (FDA 1998). Stability testing of an active substance or finished product provide evidence as to the quality of a drug substance or drug product that it remains acceptable up to the stated period under storage conditions as on label. The International Conference on Harmonization (ICH) guidelines Q1AR2 require the use of a validated stability-indicating assay method (SIAM) for stability testing of a drug substance or product (ICH 2003). It also emphasis on the conduct of a forced degradation study on the drug substance to generate information on degradation products that can form under the influence of hydrolytic, oxidative, thermal and photolytic degradation conditions.

Olmesartan medoxomil (OLM) (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4- (1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl methyl imidazo-5-carboxylate) (Fig.1a) is a prodrug and rapidly hydrolyzed in plasma by both arylesterase and albumin during Gastrointestinal absorption to form it's active metabolite olmesartan (Figure 1b) (Laeis, Puchler, Kirch 2001). It is a selective AT1 subtype angiotensin II receptor blocker (Chrysant et al. 2004, Nakamura 2005, Chrysant, Chrysant 2004) that was recently approved by the US-FDA to treat patients with hypertension. OLM is also reported to be effective in animal models of atherosclerosis, liver disorders and diabetic nephropathy (Puchler 1999).

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Method for analyses of olmesartan medoxomil in biological fluids such as human plasma and urine by florescence and LC–MS/LC–MS–MS has been reported previously (Lui et al. 2007, Vaidya et al. 2008, Dongyang et al. 2007). Use of capillary zone electrophoresis (CZE) for the determination of OLM in pharmaceutical dosage form has also been reported (Mustafa, Sacide 2007). As per our knowledge no stability indicating assay of OLM in bulk and solid dosage form could be traced in the literature which explain the degradation pattern of OLM in different conditions. However, the two methods first that identified the main degradation products obtained during long time storage condition using hyphenated techniques and second stability indicating LC-UV method unable to explain the degradation products under different condition have been reported (Murakami et al. 2008, Rane et al. 2009). So, in the present study we aimed to develop and validate a stability indicating LC-DAD assay method that allowed resolution, detection and quantitation of the olmesartan medoxomil in presence of degradation products of bulk substance and tablet dosage form.

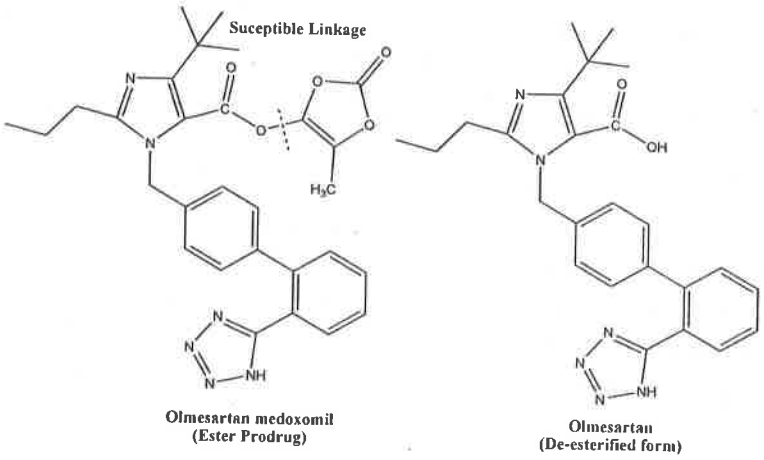

Figure 1. Chemical structure of olmesartan medoxomil and its de-esterified form olmesartan.

Experimental

Chemical and reagents

Olmesartan medoxomil was kindly provided by Torrent Pharmaceutical Ltd, (India) with 99.94% purity as gift sample. Tablet dosage form of olmesartan medoxomil (20 mg) was purchased from local pharmacy. HPLC grade methanol and acetonitrile were purchased from S.D Fine Chemical, India. Hydrochloric acid, sodium hydroxide pellets, hydrogen peroxide solution, and orthophosphoric acid also were purchased from S.D Fine Chemical (India) and were of analytical grade. The water for RP-HPLC was prepared by triple distillation in glass still and filtered through a nylon 0.45 µm–47 mm membrane filter (Gelman Laboratory, India).

Instruments

The Shimadzu (Kyoto, Japan) LC system (LC-2010c HT) equipped with a diode array detector (SPD-M20A), auto sampler (SIL-10ADvp) and column oven CTO-10A(C) vp. Chromatographic separation were performed using Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 µm particle

size) at 35 °C column oven temperature and analyzed by LC solution software. Sartorius CP224S analytical balance (Gottingen, Germany), and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the study. For photolytic degradation, UV cabinet producing short wave length (254 nm) from (India) was used. High precision water bath and hot air oven (Narang Scientific Works, India) capable of controlling the temperature with in ±1 and ±2 °C were used for the hydrolytic and thermal degradation studies, respectively.

Preparation of standard solutions

A stock solution of olmesartan medoxomil was prepared by dissolving a 10 mg of drug in 10 mL of methanol to get concentration of 1.0 mg mL^{-1}. During experiment every time fresh stock solution was prepared. Working solution containing 100 µg mL^{-1} was prepared from this stock solution and seven calibration standards 1–18 µg mL^{-1} and quality control samples of 4, 10 and 14 µg mL^{-1} concentrations were prepared by diluting the appropriate aliquots of standard solutions.

Preparation of sample solution

Twenty tablets were weighed, transferred to a clean, dry mortar and well grind. Powder equivalent to 50 mg drug was then transferred to a 100 mL volumetric flask containing 50 mL methanol. The flask was attached to a rotary shaker for 10 min to disperse the material completely. The mixture was then sonicated for 20 min and centrifuged at 3,000 rpm for 5 min. Aliquot of supernatant solution was diluted appropriately to give a solution of 500 µg mL^{-1} and 10mL of this solution was used for forced degradation study. Further, the 10 µg mL^{-1} of sample from different conditions were analyzed by the LC-DAD method to quantify OLM and to study the effect of forced conditions on OLM in tablets.

Forced degradation studies

Forced degradation of drug substance and the drug product was carried out under acid/neutral/base hydrolytic, oxidative, thermolytic, and photolytic stress conditions. For hydrolytic and oxidative degradation solution were prepared in 10 mL volumetric flask with concentration of 500 µg mL^{-1} drug samples. After degradation, aliquots from these solutions were diluted with methanol to achieve a concentration of 10 µg mL^{-1}. Methanol (60% v/v) was used for solubilization of the drug in acidic, neutral and oxidative media.

Hydrolytic degradation studies were carried out in acid (0.1 N HCl), neutral (water), and basic (0.01 N NaOH) conditions at 60 °C as well as at room temperature over 12 h to 48 h. Oxidative degradation was carried out in 3% H_2O_2 solution at room temperature over 48 h. During the optimization of hydrolytic degradation process, drug sample were initially placed in 0.1N HCl, 0.1N NaOH and in water at 60 °C. However after 24 and 48 h, OLM sample in HCl and water shown 31 and 12% degradation respectively, but OLM in 0.1 N NaOH was completely degraded. So, we decided to carry out degradation at room temperature for 0.1 N NaOH sample. Still compound was completely degraded to DP-I instantly at room temperature, than strength of NaOH was lowered down to 0.01 N. The sample containing OLM was then 90% degraded in 0.01 N NaOH at room temperature after 4 h.

Thermal and photo degradation of drug substances and drug product was carried out in the solid state. For thermal degradation, the drug was spread in borosilicate glass petri-dish and placed in the hot air oven maintained at 60 °C for 10 days. Also the photolytic studies were carried out by exposing a thin layer of the solid drug in a petri-dish in the UV chamber for 10 days during which the total light exposure equaled to 1.2 million lux h. After degradation stock solution were prepared by dissolving the samples in methanol to achieve a concentration of 1 mg mL^{-1}. From these solutions, aliquots were diluted with methanol to get final concentration of 10 µg mL^{-1} of OLM. Samples were withdrawn initially, subsequently at prefixed time intervals and stored at 2-8 °C till analysis for all stress conditions.

Method development

Detection wavelength for HPLC study was selected as 202 nm after recording UV spectrum from 190 to 400 nm of the drug and representative sample from each forced condition. The maximum area, peak selectivity and less hindrance were observed at this wave length. The chromatographic conditions were optimized for resolution of peak of drug and degradation products in each stressed condition by varying stationary phase, proportion of methanol-acetonitrile-water in mobile phase and flow rate using representative samples from each forced condition. Several trials using various proportions of methanol and water as mobile phase were carried out. However, to attain the selective resolution of OLM and its degradation products, acetonitrile as third solvent was introduced; apparent pH 3.5 was adjusted by orthophosphoric acid. Subsequently a mixture of different stress conditions was used to optimize the chromatographic conditions for resolving OLM and all the degradation products in a single run. Appropriate blank was injected before analysis of the all forced samples. This optimized method was then used to study the forced degradation behavior of OLM and also applied in stability indicating assay of OLM tablets.

Validation of the method

The optimized method was validated in accordance with the ICH guidelines (ICH. 1996, ICH 2005). Linearity was determined by analyzing, in triplicate, standard drug solutions of concentrations 1, 2, 4, 6, 10, 14 and 18 μg mL^{-1} using 20 μL of injection volume. For intra-day precision three quality control drug concentrations 4, 10 and 14 μg mL^{-1} were analyzed seven times on the same day whereas for inter-day precision the same drug concentrations were analyzed on three different days. Accuracy/recovery was evaluated by spiking the mixture of degradation samples with three known drug concentrations and calculating the percent recovery from differences between the peak areas obtained for strengthen and weaken solutions. The specificity of a method is its suitability for analysis of a substance in the presence of potential impurities. Specificity of the method was established through the study of the resolution (*Rs*) of OLM samples. Overall selectivity was established through determination of purity and *Rs* of each peak using a DAD detector at 202 nm wavelength. Ruggedness of the method was established through separation studies on the mixture of degradation samples by different persons on the same chromatographic system as well as on a different chromatographic system in a different laboratory on a different day by another analyst. Various system suitability parameters were also evaluated on the mixture sample on six different days by using freshly prepared mobile phase each time.

Results and Discussion

Method development and optimization

Peak of pure OLM obtained using 50% methanol and 50% acetonitrile (v/v) suggested us to employ 70% methanol and 30% water (v/v) pH 3.5 adjusted with orthophosphoric acid as mobile phase. Now forced degradation samples were analyzed by using a same mobile phase flowing at a rate of 1.0 mL min^{-1} on a C18 column employing DAD detection. The method resolved the drug and degradation products in neutral, basic and oxidative condition, but it could not resolved the cluster of peaks observed in acidic condition. To separate the degradation products formed under acidic condition acetonitrile was introduced in mobile phase and all other variables were kept the same. However, degradation products in acidic condition still remained unresolved. The alteration in stationary phase from C18 to C8 and flow rate of the mobile phase also did not furnish promising results. Many composition of different strength of mobile phase were tried and, finally mobile phase composition of methanol, acetonitrile and water (60:15:25, v/v/v) apparent pH 3.5 adjusted with orthophosphoric acid at a flow rate of 1.0

mL min^{-1} on a C18 column was used. This mobile phase could optimally resolve the OLM and all the degradation products formed under different conditions in the mixture sample in a single run (Figure 2a). The relative retention time (*RRT*) of each peak of degradation product with respect to Olmesartan medoxomil is given in Table1.

Table 1. Relative retention time, peak purity data and system suitability parameters of olmesartan medoxomil and its degradation products.

Parameter	Peaks				
	DP-I	DP-II	DP-III	DP-IV	OLM
RR_T	0.740	0.809	0.932	1.154	1.000
Peak purity index	0.789	1.000	0.999	0.999	1.000
Purity threshold	0.842	0.998	0.883	0.974	0.999
Asymmetry (As)	1.11	1.28	1.23	1.32	1.06
Tailing Factor (T)	1.044	-	-	-	1.058
Resolution (R_s)	1.91	1.24	1.99	2.05	1.16
Capacity factor (k')	4.93	5.48	6.47	7.02	8.26
Selectivity (α)[a]	1.25	1.34	1.67	1.33	
Theoretical plate (N)	2725.85	3164.63	3195.41	4215.38	2688.93

[a] with respect to succeeding peak

Validation of the method

Linearity- Peak area and concentrations were subjected to least square linear regression analysis to calculate the calibration equations and correlation coefficients. The calibration plot for assay of OLM was linear over the calibration range 1–18 μg mL^{-1}, and the regression coefficient, slope and intercept were 0.999, 5412.14 and 340.9, respectively. These results demonstrate an excellent correlation between the peak area and analyte concentration.

Limits of Detection and Quantification- Limits of detection (LOD) and quantitation (LOQ) were estimated from the signal-to-noise ratio. The LOD was 0.03 μg mL^{-1} for OLM at a signal-to-noise ratio of 3:1. The limit of quantification was determined as 0.1 μg mL^{-1} for OLM at a signal-to-noise ratio of 10:1.

Precision-Relative standard deviation (RSD) in study of precision for the assay of OLM was less then 1.0 % and confirmed the method is highly precise. The results of precision study for OLM by proposed LC-DAD method are given Table 2.

Table 2. Precision data of the proposed LC-DAD method.

Actual conc. (μg mL^{-1})	Measured concentration (μg mL^{-1}) Mean± SD; %RSD	
	Inter day (n=7)	Intra day (n=7)
4	4.04±0.12; 0.47	3.94±0.42; 0.73
10	9.84±0.31; 0.39	10.24±0.52; 0.67
14	14.13±0.22; 0.68	13.83±0.82; 0.85

Recovery-Standard addition method was used to examine the recovery of the LC-DAD method (Ewing 1995). Recovery of OLM from bulk drug samples ranged from 99.30 to 100.73% and that from tablet dosage forms ranged from 99.46 to 100.80% (Table 3).

Specificity-There was no interference due to placebo and sample diluents and degradation products. Resolutions between closely eluting degradation products, i.e. between DP-I, DP-II, and between DP-III, OLM were greater than 2.0 illustrated the specificity of the method.

Table 3. Results of recovery studies for bulk drug substance and drug product .

Recovery studies for bulk drug substance				
Amount of drug taken (µg mL^{-1})	**Amount of standard added (µg mL^{-1})**	**Recovered conc. (µg mL^{-1}); Mean± SD**	**%RSD (n=3)**	**Recovery (%)**
10	5	14.98±0.21	0.45	99.60
10	10	19.93±0.41	0.82	99.30
10	15	25.11±0.32	0.73	100.73
Recovery studies for drug product				
10	5	15.04±0.52	0.90	100.80
10	10	20.03±0.39	0.87	100.30
10	15	24.92±0.28	0.98	99.46

Stability in stock solution and in mobile phase

During solution stability and mobile phase stability experiments, (%) RSD for assay of OLM was within 1% for three replicates. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for up to 48 h during assay of OLM.

Forced degradation profile

Degradation pattern in all stress condition was similar with respect to DP-I, it is the major degradation product of OLM formed during every degradation study. As illustrated in Figure 2b for acid hydrolysis all the degradation products (DP-I, II, III, IV) were obtained in good amount. The OLM was fairly degraded after 24 h at 60 °C in 0.1N HCl. DP-II, was comparatively more in acid hydrolysis then other stress condition samples. For hydrolysis in basic medium 0.1N NaOH, almost all the drug had degraded at room temperature however 0.01N NaOH at room temperature was appropriate for significant degradation. Three degradation products (DP-I, DP-II and DP-III) were present in this stress sample, DP-I was the major (Figure 1c). Degradation in neutral hydrolytic medium at 60 °C (Figure 1d) occurred at slower rate then other hydrolytic conditions. Results proved that OLM was more degraded in basic hydrolytic condition then acidic and neutral.

Degradation under the oxidative condition 3% H_2O_2 at room temperature for 48 h produced DP-I, II and III but, DP-I and II formed in minor amount (Figure 1e). No degradation was seen in solid drug kept at 60 °C for 10 days and 8-9% degradation was observed upon exposure to light intensity of 1.2 million lux h but no major degradation product was observed. Summary of all the forced degradation results is given in Table 4.

Peak purity test results suggested that the OLM peak as well as the peaks of OLM degradation products were harmonized and pure for all the stress samples analyzed (Table 1). No peak was observed after 5 min in chromatograms obtained for the extended runtime of 25 min in every study sample.

The UV spectrum of pure drug OLM was compared with the spectrum of drug subjected to the different stress conditions, all the spectra showed slight changes in the absorption pattern.

Purity of all degradation products were confirmed by comparing the spectra of the same with the spectrum of OLM (Figure 3a-d). The comparative spectra of two major degradation products DP-I and II with spectrum of OLM Fig.3a and b respectively, suggest the absence of ester moiety in both the DP-I and II as the required UV absorption maxima at 260 nm characteristic of the 5-methyl-2-oxo-1,3-dioxolen-4-yl-methyl group (ester moiety) in OLM, was not visible. Olmesartan medoxomil is a prodrug and easily de-esterifies to its active metabolite olmesartan in hydrolytic condition (Laeis et al. 2001).

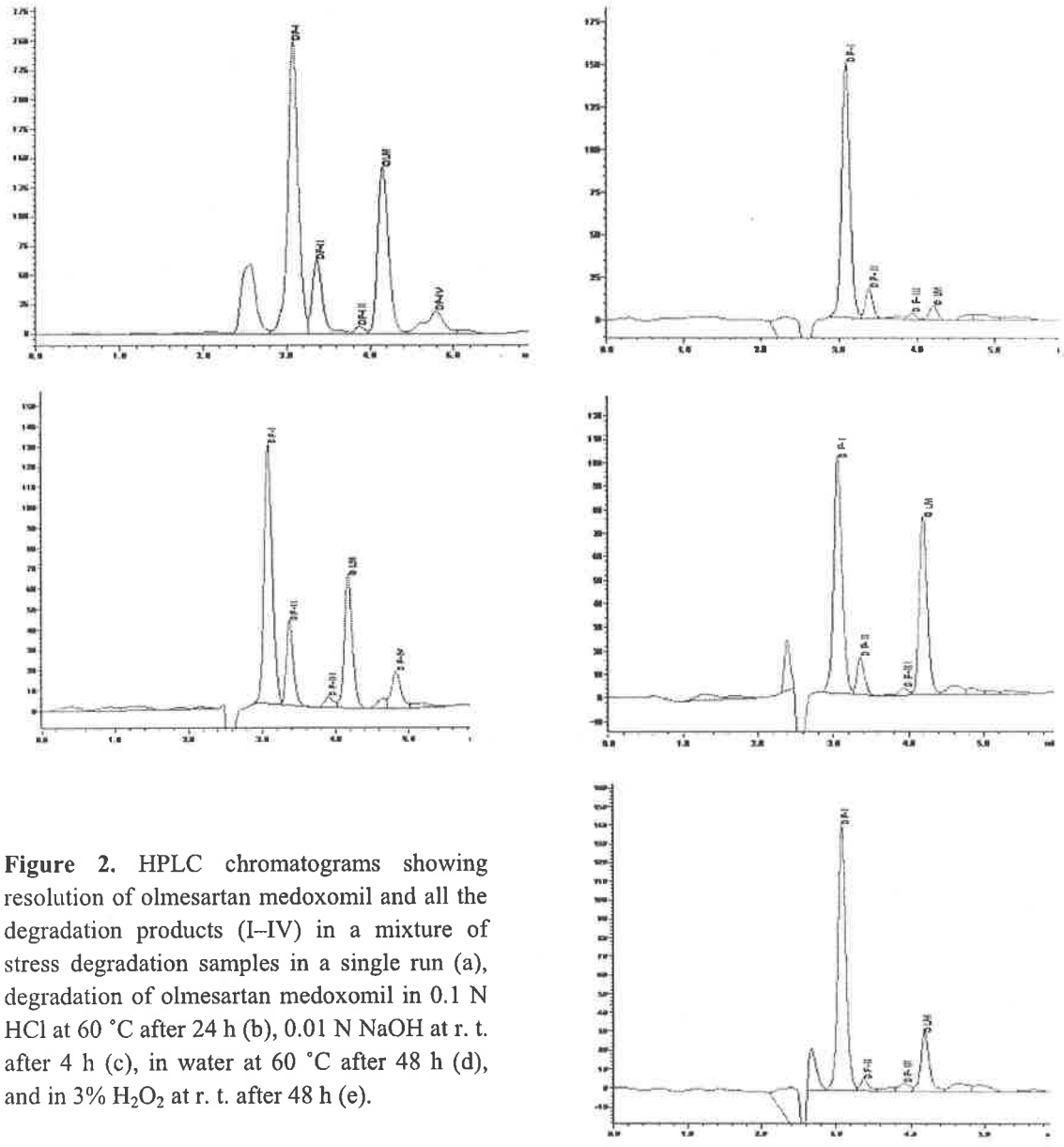

Figure 2. HPLC chromatograms showing resolution of olmesartan medoxomil and all the degradation products (I–IV) in a mixture of stress degradation samples in a single run (a), degradation of olmesartan medoxomil in 0.1 N HCl at 60 °C after 24 h (b), 0.01 N NaOH at r. t. after 4 h (c), in water at 60 °C after 48 h (d), and in 3% H_2O_2 at r. t. after 48 h (e).

Analogous to degradation products reported to be formed in tablets of losartan stored at 40 °C and 75% relative humidity (Zhao et al. 1999), the major degradation product DP-I and II probably may be the olmesartan (de-esterified form, Figure 1b) and dimer of olmesartan respectively. The goal of determining OLM in the presence of degradation product by this proposed LC-DAD method was successfully achieved and method also can be used for routine quality control of tablets.

Assay of OLM from its tablet dosage forms

The assay results of OLM tablet dosage form was comparable with the value claimed on the label. The %assay±%CV of OLM for formulation I and II was 98.4±1.32 and 99.7±1.25 respectively, indicate the suitability of method for routine analysis of OLM from their dosage form.

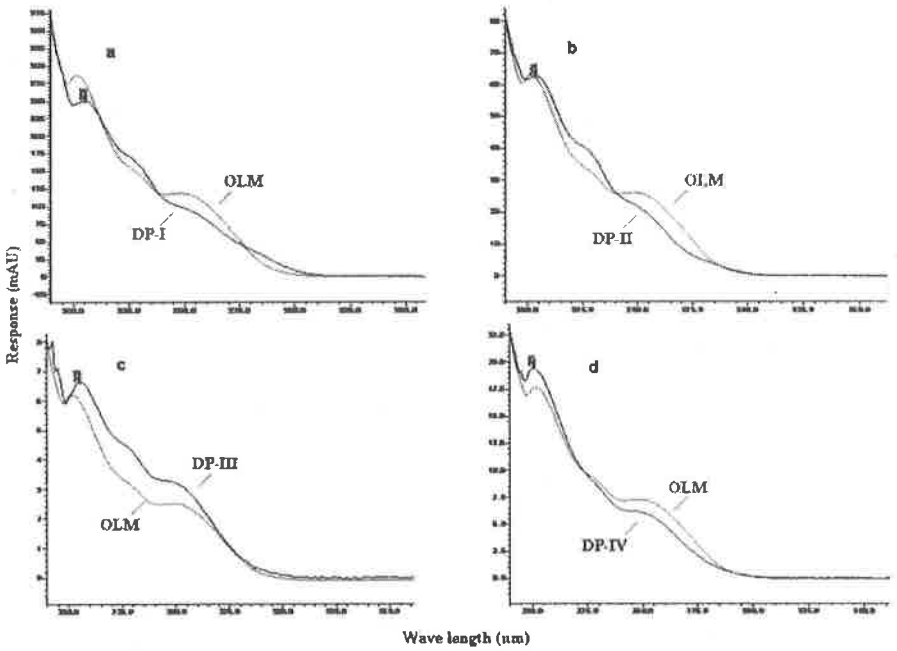

Figure 3. Comparative UV spectra between olmesartan medoxomil (OLM) and degradation product (DP-I) (a), DP-II (b), DP-III (c), DP-IV (d). Lack of UV absorption maximum at 260 nm characteristic of ester moiety in both degradation product DP-I and II.

Table 4. Summary of forced degradation study results.

Stress condition	Time	Assay of active substance (%)	Remarks (major degradation product)
Acidic hydrolysis (0.1 N HCl at 60°C)	24 h	68.7	Degraded to DP-I and II
Basic hydrolysis (0.01 N NaOH at r.t.)	4 h	10.3	Degraded to DP-I
Aqueous hydrolysis (at 60°C)	48 h	88.1	Degraded to DP-I and II
Oxidation (3% H_2O_2 at r.t.)	48 h	54.3	Degraded to DP-I
Thermal (60°C)	10 days	99.4	No degradation product formed
Photo (UV 254nm)	10 days	90.8	No major degradation product observed

r.t., Room temperature

Conclusions

It can be concluded that the method is specific, sensitive for the estimation of olmesartan medoxomil in the presence of degradation products obtained in different stress conditions. The method is linear with in the mentioned range and is found to be accurate and precise. The drug has been found susceptible to hydrolytic and oxidative degradation.

Although no attempt was made to quantify the degradation products but the quantitation is possible after isolation of degradation product in pure form. The proposed method can be used as stability indicating method for assay of olmesartan medoxomil in drug substance and drug product.

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