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Development and validation of new RP-HPLC method for determining impurity profiling in olmesartan medoxomil drug as well as in tablet dosage form

Nandini R Pai and Seema S. Sawant*

Department of Chemistry, D. G. Ruparel College of Arts, Science & Commerce, Mahim (West), Mumbai, Maharashtra, India

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ABSTRACT

A simple, rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic method was developed to estimate impurity profile for Olmesartan medoxomil in drug as well as in tablet dosage form. The HPLC analysis used a reversed phase Kromasil C18 (150 x 4.6mm, 5µm) column and a mobile phase constituted of buffer and acetonitrile (60:40 % v/v). The buffer was composed of 4.7 g of sodium dihydrogenorthophosphate and 1 mL of triethyl amine in 1000 mL of water and the pH of the solution was adjusted to 4.0± 0.05 with ort hophosphoric acid. The wave length of the detection was 225 nm. The validation data showed that the method is sensitive, specific and reproducible for the impurity determination of olmesartan in the dosage form. The method was found to be linear from 2 µg/mL to 7 µg/mL for Olmesartan medoxomil and from 0.25 µg/mL to 7 µg/mL for olmesartan Acid Impurity. The accuracy of the method was found to be 100.73% for olmesartan acid impurity. Inter and intraday assay relative standard deviation (RSD) was less than 0.71% in drug form and 1.10% in tablet dosage form for Olmesartan acid impurity. The proposed method provided an accurate and precise analysis of Olmesartan acid impurity in Olmesartan medoxomil Drug form as well as in pharmaceutical dosage form

Keywords: Olmesartan acid impurity, HPLC, validation

INTRODUCTION

Olmesartan medoxomil is chemically (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2' -(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-imidazole-5-carboxylate, As a selective and competitive, nonpeptide angiotensin II receptor antagonist, olmesartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II Olmesartan medoxomil (Benicar®, SankyoPharma) is currently being used as an alternative therapeutic antihypertensive agent for patients intolerant to angiotensin converting-enzyme inhibitors. This molecule was approved as drug by USFDA in the month of April 2002 for treatment of hypertension.

Methods of analysis of Olmesartan medoxomil in biological fluids such as human plasma and urine by LC-MS and LC-MS-MS were reported previously. Use of capillary zone electrophoresis (CZE) for the determination of OLM in pharmaceutical dosage form has also been reported. However, the method that identified the main degradation products obtained during short-time storage using different techniques has been reported. A thorough literature has revealed that several methods were reported for the determination of impurity of Olmesartan medoxomil. This method described the analysis and identification of Olmesartan acid impurity in Olmesartan medoxomil API and it's tablet dosage form and by complementary use of the HPLC techniques. In the present study, we aimed to develop and validate a RP-HPLC-DAD impurity study method that allowed resolution, detection and quantitation of Olmesartan medoxomil and olmesartan acid impurity in bulk substance and tablet dosage form

We report the development and validation of a simple HPLC impurity determination with UV detection for the quantitative determination of olmesartan acid impurity in bulk substance as well as in tablet dosage form

MATERIALS AND METHODS

Chemicals and reagents

All the reagents were of analytical-reagent grade. De-ionized water (Millipore), HPLC-grade acetonitrile, Sodium dihydrogen phosphate AR grade, Triethyl amine HPLC grade and Orthophosphoric acid AR grade were used

Instrumentation

The HPLC system was composed of LC 2010Shimadzu system fitted with Prominence PDA detector with LC Solution software. Analytical column used for this method was KromasilC18 (150 mm x 4.6 mm) 5µm

Buffer preparation

4.7 g of sodium dihydrogenorthophosphate and 1 mL of triethyl amine in 1000 mL of water and the pH of the solution was adjusted to 4.0± 0.05 with orthophosphoric acid

Standard Preparation

Olmesartan medoxomil reference substance was accurately weighed (25 mg) and dissolved in 15 mL quantity of acetonitrile: buffer (40:60) in a 50 mL volumetric flask and diluted up to the mark and it was further diluted to generate a concentration of 5µg/mL

Impurity Standard Preparation

Olmesartan acid impurity was accurately weighed (25 mg) and dissolved in 15 mL quantity of acetonitrile: buffer (40:60) in a 50 mL volumetric flask and diluted up to the mark and it was further diluted to generate a concentration of 5 µg/mL

System Suitability Solution Preparation

Olmesartan medoxomil reference substance and Olmesartan acid impurity were accurately weighed (each 25 mg) and dissolved in 15 mL quantity of acetonitrile: buffer (40:60) in a 50 mL volumetric flask and diluted up to the mark and it was further diluted to generate a concentration of each 5 µg/mL

Sample Preparation

Raw Material: Olmesartan medoxomil raw material was accurately weighed (25 mg) and dissolved in 15 mL quantity of acetonitrile: buffer (40:60) in 25 mL volumetric flask and diluted up to the mark and it was diluted to generate a concentration of 1000 µg/mL

Tablet: Twenty tablets of Olmesartan medoxomil (40 mg of Olmesartan medoxomil) were separately weighed and grounded to fine powder. An amount equivalent to 25mg of olmesartan was transferred into a25 mL volumetric flask and dissolved in 15 mL quantity of acetonitrile: buffer (40:60) and made up volume to 25mL to generate a concentration of 1000 µg/mL

Chromatographic conditions

Before the mobile phase was delivered into the system, buffer and acetonitrile were filtered through 0.2µm, PVDF membrane filter and degassed using vacuum. The chromatographic conditions which were used for the analysis are reproduced below
Column: KromasilC18 (150 mm x 4.6 mm) 5µm
Wavelength: 225 nm
Injection volume: 20 µl
Flow rate:1.0 mL/min
Column temperature:30ºC
Run time: 25 min

Method development

Detection wavelength for the HPLC study was selected as 225 nm after recording the UV spectrum from 190 to 800 nm of the drug and representative sample from standard, impurity standard solution and sample solution by using PDA detector HPLC. The suitable area and peak selectivity of Olmesartan medoxomil and olmesartan acid impurity was observed at this wavelength. The chromatographic conditions were optimized for resolution of the peak of the drug and it's impurity under each condition by varying the stationary phase, proportion of methanol/acetonitrile/water in the mobile phase and the flow rate using representative samples. Several trials using various proportions of methanol and water as mobile phase were carried out. However, to attain the selective resolution of Olmesartan medoxomil(OLM) and olmesartan acid impurity (Impurity A), acetonitrile and sodium

dihydrogen phosphate buffer was introduced as the third proportion; apparent pH 4.0 was adjusted by orthophosphoric acid. Subsequently, a mixture of different mobile phase composition was used to optimize the chromatographic conditions for resolving OLM and impurity A in a single run. An appropriate blank was injected before the analysis of all the samples. Such an optimized method was then used to study the impurity study of Olmesartan medoxomil drug form and it's tablet dosage form.

Method validation

Method validation was conducted according to published guidelines. Impurity profiling was evaluated by intraday and inter day (two different days) precision and determined from replicate analysis of samples (1000 µg/mL). Analysis of five different sample solutions was performed in the same day for intraday precision. The precision were expressed in terms of RSD from mean intra and inter day sample analysis

Accuracy of the method was tested by adding a known amount of Olmesartan acid impurity standard (4, 5 and 6µg/mL) in three sample solutions. Calculated the percent recovery from the peak areas obtained for diluted solutions Signal-to-noise ratios were employed to estimate limits of detection (3:1) and limits of quantitation (10:1) for olmesartan acid impurity

The specificity of a method is its suitability for analysis of a substance in the presence of impurities. Specificity of the method was established through the study of the resolution (Rs) of OLM samples. Overall selectivity was established through determination of drug purity and Rs peak area RSD each time

Various system suitability parameters were also evaluated on a mixture sample on different days using freshly prepared mobile phase each time

Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase composition and pH were evaluated. The chromatographic parameters monitored were peak retention time, tailing factor and theoretical plate number

RESULTS AND DISCUSSION

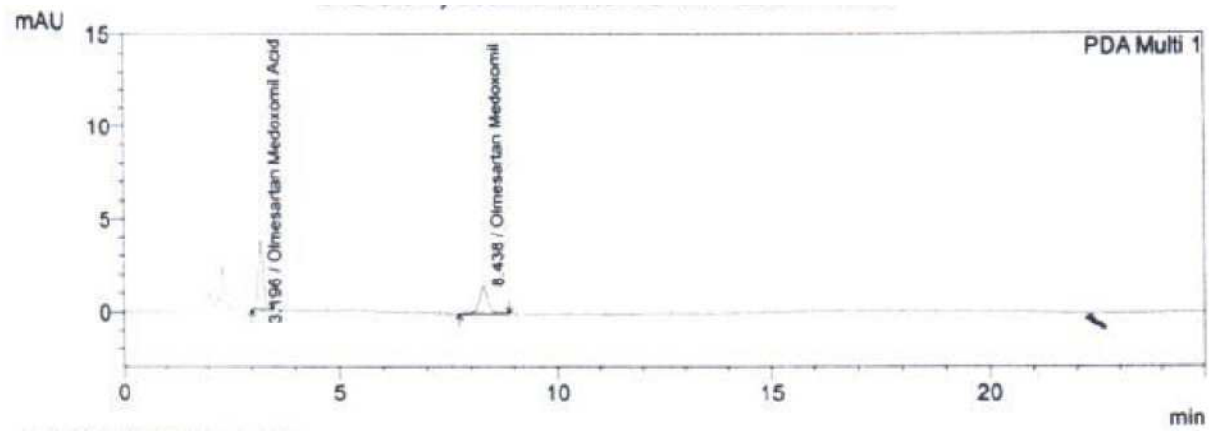

Figure 1: Chromatogram of the standard solution (System Suitability Solution)

Method development and optimization

Using a mobile phase consisting of different buffers with methanol and acetonitrile at different concentrations, methanol and acetonitrile ratios and at different mobile phase pH values were attempted. Changes in the analytical procedure were tested. Different mobile phases with different proportions of organic modifier (acetonitrile) were tried. The pH value of the mobile phase was checked over a wide range (3.8-4.2). The pH of the aqueous phase was adjusted with orthophosphoric acid. It was observed that the peak shape and retention time of olmesartan was found to be broad compared to the buffer-acetonitrile composition as mobile phase. After various trials of different buffer and acetonitrile ratios as mobile phase, sodium dihydrogen phosphate with trietylamine was selected as buffer, pH was adjusted to 4.0 with orthophosphoric acid and buffer-acetonitrile ratio was chosen to be 60:40. Chromatographic run was evaluated using Kromasil C18 column. After selecting the best conditions based on peak performance, the run time of the proposed method was 25 min with isocratic elution. During injection of a standard and sample solution, the retention times found were about 8.300 minute for Olmesartan medoxomil and about 3.200 minute for Olmesartan acid impurity respectively. It shows good resolution of chromatogram with symmetrical peak. The proposed chromatographic conditions were found to be appropriate for the quantitative determination. System

suitability tests were carried out as per ICH guidelines and the parameters are summarized in Table 2 referred to in Specificity validation parameter. Refer Figure 1 and figure 2 for standard and sample solution graph.

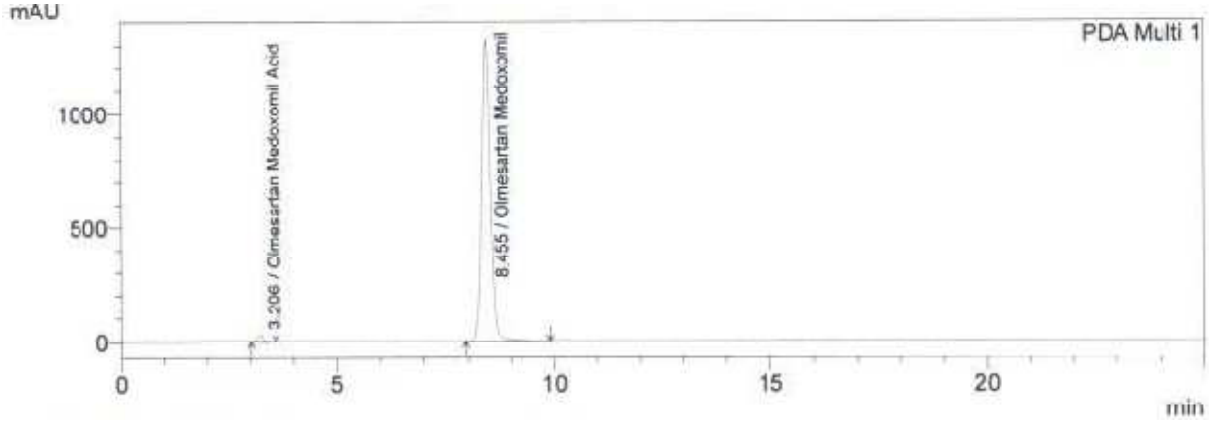

Figure 2: Chromatogram of the sample solution

Method Validation

Linearity: Linearity was studied by preparing standard solutions at different concentration levels for Olmesartan medoxomil and it's acid impurity. The linearity range for OLME was found to be 2-7µg/ml for Olmesartan medoxomil and 0.25-7 µg/ml for olmesartan acid impurity. Refer Table1 for linearity values observed for Olmesartan medoxomil and olmesartan acid impurity. Refer Figure 3 and figure 4 for linearity graph of olmesartan acid impurity and Olmesartan medoxomil respectively.

Table 1: Linearity values observed for Olmesartan medoxomil and olmesartan acid impurity

Linearity Parameter	Olmesartan medoxomil	Olmesartan acid impurity
Concentration range	2-7 µg/ml	0.25- 7 µg/ml
Correlation coefficient	0.99990	0.999998
Slope	1886.9257	2004.45
Y - Intercept	4361.3524	-11.36
R-square	0.99980	0.999996

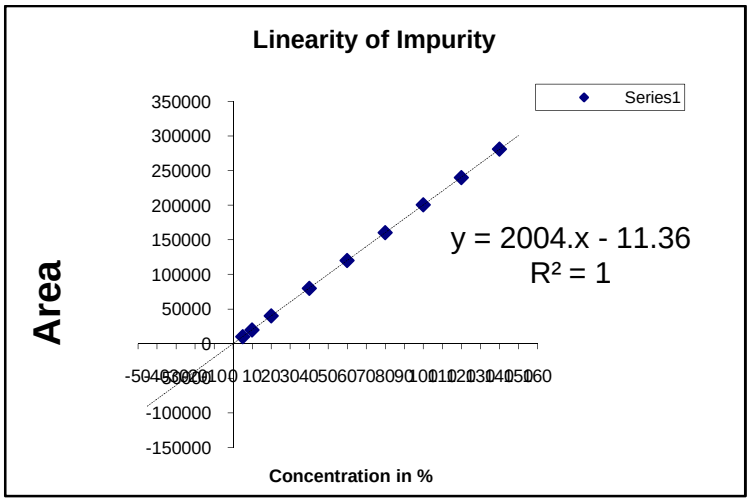

Figure 3: Linearity of Olmesartan acid impurity

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that maybe expected to be present. Typically, these might include impurities, degradants, matrix, etc. Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from the blank and placebo. Specificity of the peak purity of OLME and Olmesartan acid impurity were assessed by comparing the retention time of standard OLME and the sample and good correlation was obtained. Injected the individual identification solutions of Olmesartan medoxomil and Olmesartan acid impurity each; for the identification purpose. Both the peaks found pure in presence of each other. Also there were no peaks when the placebo and blank were

injected and no interferences, hence the method is specific. System suitability solution was injected to determine the resolution, tailing factor and theoretical plates for both the peaks.

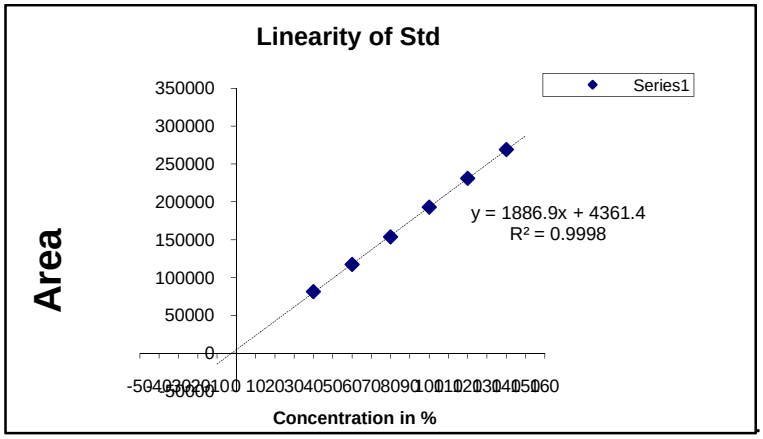

Figure 4: Linearity of Olmesartan medoxomil standard

Refer Table 2 for specificity study values observed for Olmesartan medoxomil and olmesartan acid impurity. Refer figure 5 and figure 6 for Olmesartan medoxomil and olmesartan acid impurity peak purity graph respectively

Table 2: Specificity study values observed for Olmesartan medoxomil and olmesartan acid impurity

Specificity Study	Olmesartan medoxomil	Olmesartan acid impurity
Retention Time in minute	8.455	3.206
Relative retention time	1.0	0.38
Resolution	15.70	-
Tailing Factor (NMT 2.0)	1.062	1.304
Theoretical plates (More than 2000)	8782.56	3707.34
Peak Purity	Peak Purity Index : 1.00	Peak Purity Index : 1.00
Blank/Placebo Interference	Not detected	Not detected
% RSD peak area (NMT 2.0 %)	0.06 %	0.60 %

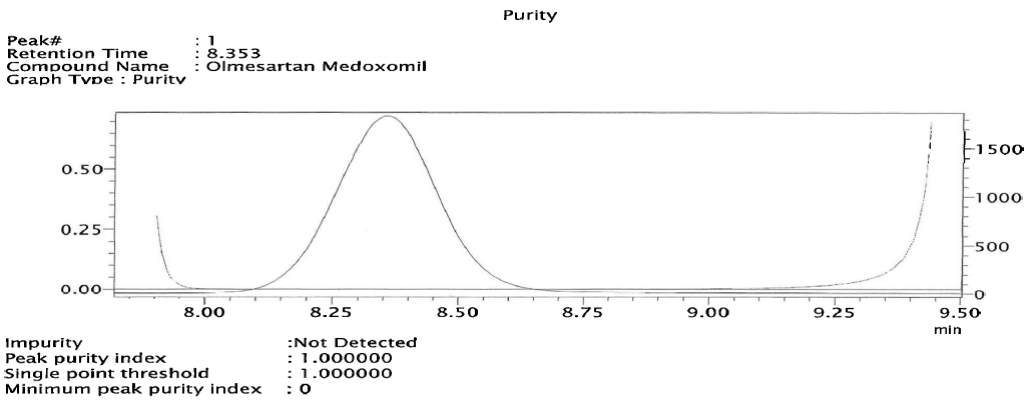

Figure 5: Olmesartan medoxomil standard peak purity graph

Precision& Ruggedness

Precision was carried out for Inter and Intraday analysis for both drug forms as well as for tablet dosage form. Precision was evaluated by carrying out five independent sample preparations of a single lot of bulk drug and formulation. The sample preparation for bulk product was carried out in same manner as described in sample preparation for raw material. The sample preparation for tablet dosage form was carried out in same manner as described in sample preparation for tablet and spiking of Impurity solution to the concentration of 5 µg/mL

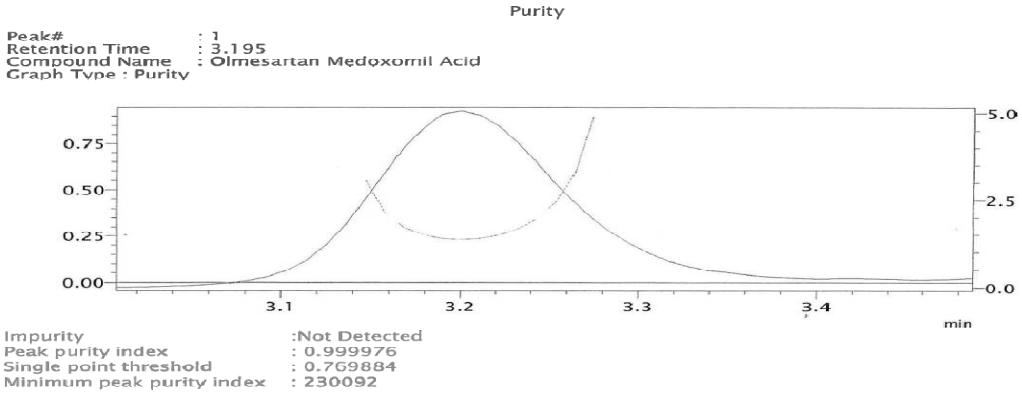

Figure 6: Olmesartan acid impurity peak purity graph

Relative standard deviation (% RSD) was found to be less than 2%, which proved that the method is precise. Refer Table 3 for Method precision and intermediate precision study

Table 3: Method precision and intermediate precision study

	Sr. No.	Impurity in RM (%)		Impurity in Tablet (%)	
		Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
Precision Study	**1**	0.201	0.202	0.552	0.554
	2	0.202	0.200	0.552	0.566
	3	0.202	0.203	0.555	0.565
	4	0.202	0.204	0.557	0.553
	5	0.203	0.205	0.568	0.558
	Mean	**0.202**	**0.203**	**0.557**	**0.559**
	SD	**0.0007**	**0.0019**	**0.0066**	**0.0061**
	RSD	**0.35**	**0.95**	**1.19**	**1.08**
Precision - Intermediate Precision :	**Mean**	**0.202**		**0.558**	
	SD	**0.0014**		**0.0061**	
	RSD	**0.71**		**1.10**	

Accuracy (recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by impurity standard addition method at 80, 100 and 120% concentration levels of Impurity standard(5 µg/mL). Known amounts of standard solution of impurity were added to the pre-analyzed raw material samples and were subjected to the proposed HPLC method. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 100.73% for olmesartan acid impurity.

Refer Table 4 for results of recovery studies

Table 4: Results of recovery studies

Recovery	Olmesartan acid impurity
Level	% Recovery
80 %	100.48
100 %	101.34
120 %	100.37
Mean	**100.73**
% RSD	**0.48**

Limit of quantification and limit of detection

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach, standard deviation of the response and slope (calibration curve method). LOQ and LOD were calculated as LOD = 3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas of the drugs(n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve. Limit of detection of OLME was found to be 0.10µg/ml and the limit of quantification of OLME was determined to be 0.30µg/ml. Limit of detection of Olmesartan acid impurity was found to be 0.02 µg/ml and the limit of quantification of Olmesartan acid impurity was determined to be 0.05 µg/ml

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in mobile phase composition, change in pH of mobile phase and filter

paper change was studied. Tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes like ±5% in mobile phase composition,±0.2 change in pH and filter paper from 0.45 µto whatmann 41 no.

Refer table 5 for the results of different robustness parameter

Table 5: Results of different robustness parameter

Robustness Study	pH 3.8	pH 4.2	Mobile phase composition : 62 : 38	Mobile phase composition : 58 : 42	Filter paper 41 no.
Tailing Factor OLM peak	1.070	1.057	1.076	1.082	1.052
Theoretical plates OLM peak	9027.94	8828.71	8352.32	9133.72	8671.73
Resolution OLM & Impurity	15.66	15.65	14.16	17.45	15.62
% RSD OLM peak	0.067	0.079	0.183	0.281	0.454
RT of OLM	8.387	8.394	7.303	9.717	8.398
RT of OLM acid impurity	3.190	3.206	2.999	3.400	3.198
% RSD for Impurity content in RM	0.202	0.204	0.204	0.203	0.204
% RSD for Impurity content in Tablet	0.553	0.558	0.551	0.551	0.553

Stability of stock solution

During solution stability experiments, RSD for the Olmesartan acid impurity content was found 0.38% for bulk product and 0.70% for tablet dosage form which was within 2% RSD. Results of the solution stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for up to 12 hour during the analysis

CONCLUSION

As described in ICH guidelines, the identification and isolation of impurities is a very important task during drug synthesis and storage. It can provide crucial toxicology and safety data of the final drug and dosage forms. We have identified one impurity in samples of Olmesartan medoxomil drug substance and drug product, characterized by HPLC analytical data

The HPLC method developed and validated allows a simple and fast quantitative determination of olmesartan acid impurity from bulk drug and its formulation. A mobile phase composed of solvent A and acetonitrile with a short run time (25 min) and isocratic elution used were advantageous and made the routine analysis easy. Among the significant advantages of this method are simplicity, selectivity, accuracy and precision ensuring that it is suitable for determining the impurity content of olmesartan in tablet dosage form

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