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RP-HPLC analytical method development and validation for the determination of Olmesartan medoxomil in bulk drug and tablets

Vijaya Selvakumar
Selvakumar Duraipandi
Qing Wei Chan

School of Pharmacy, Taylor's University Lakeside Campus, Subang Jaya, 47500, Malaysia.

Corresponding author:
Vijaya Selvakumar (VS):
Tel: +60102503738;
Email: vijirx2008@gmail.com

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Abstract: *A simple, economic, accurate, sensitive and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of Olmesartan medoxomil in bulk drug and tablet dosage form was developed and validated as per the ICH Harmonized Tripartite Guideline. The chromatographic separation was carried out on Hypersil GOLD C_{18} column (150 mm × 4.6 mm, 5 µm) with acetonitrile and phosphate buffer pH 3 (35:65%) containing 1% of glacial acetic acid at a flow rate of 1.2 mL/min and the PDA detection set at 255 nm. A linear response was observed over the concentration of 5 µg/mL to 25 µg/mL (r^2 = 0.9999). The recoveries were found to be between 99.47-100.35% and the corresponding %RSDs between 0.52-0.68. The validation data proved that the assay method developed is sensitive, reproducible and economical and thus can be used for the routine estimation of Olmesartan medoxomil in bulk drug and tablets dosage form.*

Keywords: *Olmesartan medoxomil; RP-HPLC; method development; validation; ICH-guidelines*

1. Introduction

Olmesartan medoxomil (Fig 1), chemically known as (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylate with empirical formula of $C_{29}H_{30}N_6O_6$. It is a non-peptide, benzimidazole derivative acting as angiotensin II type 1 receptor antagonist with proven blood pressure lowering property. It is recently being approved by the US-FDA to treat patients with hypertension.(1,2)

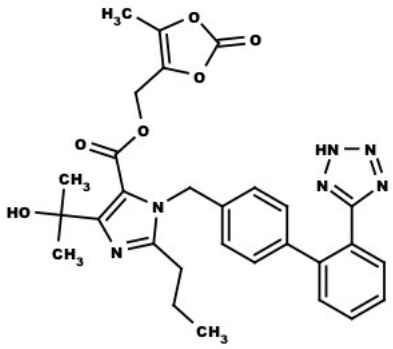

Figure 1. *Structure of Olmesartan Medoxomil*

Literature review revealed that several methods were reported for the estimation of Olmesartan medoxomil (OLM) in tablets, biological fluids using HPLC with UV and MS detector.(3-6) Various stability indicating RP-HPLC methods for the determination of OLM in bulk and pharmaceutical dosage forms was reported. (6-12) Simultaneous estimation of Olmesartan in combination with other drugs using LC and UV spectrophotometric methods were also been reported.(13-23) It is official in US pharmacopoeia, European pharmacopoeia and Japanese pharmacopoeia. The present research project was performed to develop a rapid, simple, accurate, sensitive, precise, reproducible and more importantly economic high performance liquid chromatographic (HPLC) method for the determination of OLM in bulk drug and tablet dosage form. The proposed method was validated according to ICH guidelines.(24) The proposed method aimed to utilize low percentage of acetonitrile, which is expensive, in the mobile phase in relation to the reported methods.

2. Results and Discussion

2.1. Method Development and Optimization

A number of eluting systems were studied for optimization of the mobile phase for separation of the drug. Gradient elution with the mobile phase composition of acetonitrile and 10 mM phosphate buffer (pH 3) was tried to get good peak shape and optimal retention time below 10 minutes. A mixture of acetonitrile and 10 mM phosphate buffer (pH 3) in the ratio of 35:65 containing 1% glacial acetic acid provided an efficient elution of the drug with good peak shape and retention time. A flow rate of 1.2 mL/min was found to be optimum and gave retention time 5.08 min with good baseline stability.

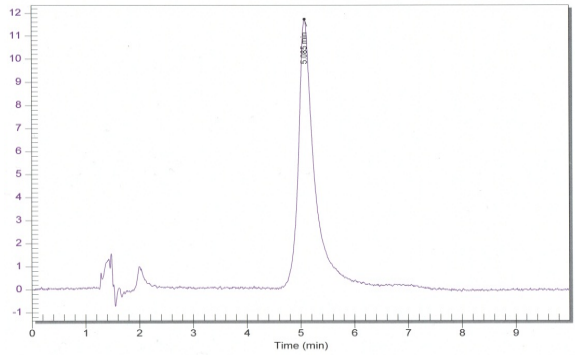

Figure 2. *Typical chromatogram of OLM*

Typical chromatogram of OLM was shown in Fig 2. The calibration curve was plotted utilizing the peak area of OLM against concentration of the drug. The calibration curve showed linearity, over a concentration range of 5-25 µg/mL for the drug as showed in Fig 3. Regression coefficient (R^2) was found to be 0.9999 for the Linear regression equation y = 12016x + 803.2. The number of theoretical plates obtained was 2092, which indicates the efficient performance of the column. The results of intra-day and inter-day precision are shown in Table 3. Assay of OLM tablets (Olmetec® 20 mg, Daiichi Sankyo Europe GmbH, Germany) using the developed method showed acceptable relative error values. The %RSD for assay of drug during intra-day and inter-day was 0.452 and 0.594. During injection of a standard solution, sample solution and bulk drug solution, the retention times were 5.085min, 5.093min and 5.095 min respectively.

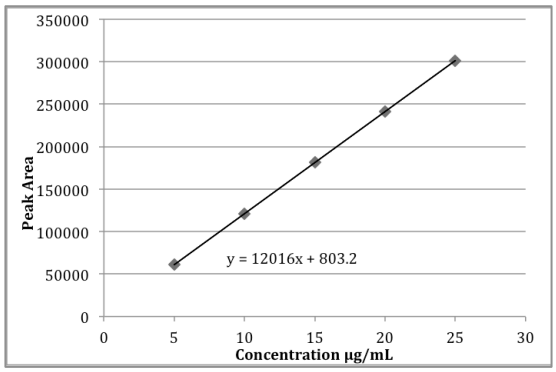

Figure 3. *Calibration curve of OLM*

2.1.1. Assay

With the optimized chromatographic conditions, 20 µL of the sample was injected in triplicates and the chromatograms were recorded. The retention time was found to be 5.08. The content of OLM in the tablets (Olmetec® 20 mg, Daiichi Sankyo Europe GmbH, Germany) was calculated from the peak area of the sample with reference to the peak area of the OLM reference standard injected.

2.2 Method Validation

2.2.1 Linearity

Linearity was demonstrated from the standard drug solution using five concentration levels for OLM. The peak areas were recorded and calibration plot was obtained by plotting peak area versus concentration of

OLM. The linear regression equation was found to be y = 12016x +803.2 (R^2 = 0.9999). The correlation coefficient results revealed that the developed analytical method having excellent linearity.

Table 1. *Estimation of OLM content in Tablets*

[a]Label Claim (mg)	[b]Amount estimated (mg)	[b]Amount estimated(%)
20	20.1±0.12	100.5±0.13

[a] Olmetec® 20 mg tablet; [b]n= 3

2.2.2. Accuracy

Recovery studies had been carried out to evaluate the degree of accuracy of the method. The study was conducted in triplicates by standard addition method at 50%, 100% and 150%. Known amounts of the standard solution were added to-pre-analyzed samples and subjected to the proposed RP-HPLC analytical method. The results were shown in Table 2. Average % recoveries obtained as 99.47% - 100.35%, which indicating that the method was accurate.

Table 2. *Recovery Studies*

Analyte	OLM content in pre-analysed sample (mg)	Amount added (%)	%Recovery estimated[a]	%RSD
OLM	20	50	99.72±0.67	0.68
OLM	20	100	100.35±0.56	0.56
OLM	20	150	99.47±0.52	0.52

[a]Mean ± SD of three samples; SD: Standard deviation; RSD – Relative standard deviation

2.2.3. Precision

Six repeated injections of standard and sample solutions were made and the response factor of drug peaks and %RSD were calculated. The result obtained was presented in Table 3.

Table 3. Precision

No.of Injections	Peak Area
1	220127
2	221914
3	220304
4	223268
5	221902
6	220251
Mean	221294.30
SD	1271.26
% RSD	0.57

SD: Standard deviation; RSD-Relative standard deviation

2.2.3.1 Intermediate Precision (Ruggedness)

The results of intra-day and inter-day precision studies were shown in Table 4. They revealed that the %RSD for assay of drug during intra-day and inter-day were within the permissible limits of 2.0%.

2.2.4. Limit of detection and limit of quantitation:

LOD is defined as the smallest level of analyte that gives a measurable response.(24) The LOQ is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision.(24) LOD and LOQ for OLM were calculated by using the linear regression equation obtained and was found to be 0.02 and 0.07 µg/mL respectively. This indicates the high sensitivity of the method.

Table 4. Results of intermediate Precision

Parameters	Intermediate precision			
	Intra-day precision	Inter-day precision	Analyst I	Analyst II
RT(min)	5.08	5.09	5.08	5.04
Mean	101.21	102.7	101.14	101.96
SD	0.44	0.46	1.05	1.01
%RSD	0.43	0.45	1.03	0.99

SD: Standard deviation; RSD – Relative standard deviation

2.2.5. Robustness

To evaluate robustness of the developed RP-HPLC analytical method, small deliberate variations in the optimized method parameters were applied. The effect of change in flow rate and wavelength of the detector on peak area was studied. The developed method was found to be unaffected by small changes such as ±0.2 mL/min change in flow rate and ±2 nm change in wavelength proposed. The results were shown in Table 5.

Table 5. Results of Robustness

Robustness condition	System suitability parameters		
	RT(min)	Theoretical plate	Tailing factor
Flow rate (1mL/min)	5.08	2088	1.32
Flow rate (1.4mL/min)	5.08	1996	1.43
Wavelength(253nm)	5.07	2021	1.39
Wavelength(257nm)	5.07	2014	1.44

2.2.6. System suitability

System suitability tests are used to verify that repeatability and resolution of critical parameter of system are adequate. The column efficiency, resolution and peak asymmetry were evaluated for the standard solutions. The values obtained, demonstrated the system suitability for the analysis. The variation in system suitability parameters fell within ± 3 % standard deviation range and was shown in Table 6.

2.3. Conclusion

A simple, sensitive, precise and accurate reverse phase high performance liquid chromatographic analytical method was developed for the estimation of OLM in bulk drug and tablet dosage form. The method was successfully validated and proved as linear, precise, accurate, and robust. Documented evidences of the present work suggesting that the developed method was an economical one in terms of lower acetonitrile concentration for the estimation of the drug and can be successfully employed for routine analysis in quality control laboratories.

Table 6. Regression analysis and system suitability parameters

Parameters	Results
Retention time (min)	5.08
Limit of detection (µg/mL)	0.02
Limit of quantitation (µg/mL)	0.07
Regression line	Y=12016x +803.2
Correlation coefficient (r)	0.9999
Theoretical plates	2092
Tailing factor	1.26

3. Experimental

3.1 Chemicals and reagents

OLM standard reference was certified with 99.3% w/w purity was purchased from Sigma Aldrich. OLM tablets (Olmetec® 20 mg) were purchased from a local retail pharmacy. HPLC grade methanol and acetonitrile were purchased from Merck. Analytical grade orthophosphoric acid 85% w/w, potassium dihydrogen orthophosphate and glacial acetic acid were purchased from Merck. Double distilled water for RP-HPLC was obtained from Arium® Pro (Sartorius Stedim Biotech). The analyses were performed on a PerkinElmer HPLC system containing Chromera Software and PDA detector set at 255 nm with an isocratic elution at a flow rate of 1.2 mL/min on a Hypersil GOLD C_{18} column (150×4.6 mm, 5 µ). Mobile phase composition of 35% v/v acetonitrile (HPLC grade) in 10mM phosphate buffer with 1% glacial acetic acid was used.

3.2. Method Development

3.2.1. Chromatographic condition

Column:	Hypersil GOLD C_{18} Column (150x4.6 mm; Particle Size: 5µ)
Mobile phase:	Acetonitrile: 10mM Phosphate buffer pH 3 (35:65) containing 1% Glacial acetic acid
Flow rate:	1.2 mL/min
Detector Wavelength:	255 nm
Column Temperature:	30 ºC
Injection Volume:	20 µL

3.2.2. Preparation of Buffer, pH 3

Phosphate buffer (10 mM) solution was prepared by dissolving 0.68 g of potassium dihydrogen orthophosphate in sufficient quantity of water and made up the volume to 1000 mL. The mixed solution is sonicated and then the pH was adjusted to 3.0 using orthophosphoric acid

3.2.3. Preparation of standard stock solution

Stock solution of Olmesartan, reference standard was prepared by accurately weighing 10 mg of the drug and dissolved in 50 mL of methanol and made up the volume to 100 mL (Final concentration 100 µg/mL). The stock solution was stored at 4 ºC in refrigerator. The calibration standards were prepared from stock solutions by diluting 0.5-2.5 mL with mobile phase to 10 mL to achieve the final concentration of 5-25 µg/mL in a series of 10 mL volumetric flasks respectively.

3.2.4. Preparation of the sample:

Twenty tablets of OLM (Olmetec® 20 mg) were weighed and grounded into fine powder using mortar and pestle. Amount equivalent to 20 mg of OLM was transferred into a 100 mL volumetric flask and dissolves in 50 mL quantity of HPLC grade methanol. The mixture is sonicated and filtered using Whatman No.41 filter paper. Finally the volume is made up to 100 mL in a volumetric flask. The mixture was sonicated and filtered using 0.45 µm membrane filter. It was further diluted to prepare a concentration of 20 µg/mL with mobile phase.

3.2.5. Preparation of the Bulk drug sample:

OLM bulk drug powder, 10 mg was accurately weighed and dissolved in 50 mL quantity of HPLC grade methanol. It was then diluted and the volume was made up to 100 mL in a volumetric flask. The solution was then sonicated and filtered using 0.45 µm membrane filter. It was further diluted to prepare a concentration of 20 µg/mL with mobile phase

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