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Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE QUANTIFICATION OF OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORMS

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ABSTRACT
Background: Olmesartan medoxomil is an angiotensin-II receptor antagonist prodrug whose medoxomil ester is hydrolytically labile, making a specific, stability-indicating assay essential for quality control. This study aimed to develop and validate a simple, precise, robust reverse-phase HPLC (RP-HPLC) method for olmesartan medoxomil in tablets using cost-effective, LC-MS-compatible reagents, and to apply it to marketed formulations. Methods: Separation used a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 um) with acetonitrile:10 mM ammonium acetate buffer pH 4.5 (65:35, v/v) at 1.0 mL/min, UV detection at 257 nm, 30 degC and a 20 uL injection. The method was validated per ICH Q2(R1), its specificity assessed by forced degradation, and applied to three commercial 20 mg brands. Results: Olmesartan eluted at 4.82 min with a tailing factor of 1.06 and 9,842 plates/m. The method was linear over 10-150 ug/mL (y = 12,486x + 246; r2 = 0.9998), accurate (99.24-101.12% recovery), precise (intra-day <= 0.48% RSD; inter-day <= 0.64% RSD), with LOD 0.84 ug/mL and LOQ 2.54 ug/mL. Acid and alkaline hydrolysis caused 42.6% and 68.4% degradation; all degradation products were resolved from the drug peak (Rs > 2.5) with confirmed peak purity. Assay of three brands gave 99.46-100.12% of label claim. Conclusion: The method is simple, rapid (8 min run), accurate, precise, robust and stability-indicating, and is suitable for routine assay, content uniformity, dissolution and stability studies of olmesartan medoxomil.

Keywords: *Olmesartan medoxomil; RP-HPLC; stability-indicating; forced degradation; method validation; ICH Q2(R1).*

INTRODUCTION

Pharmaceutical quality control comprises the testing, analysis and inspection that assure the identity, strength, quality and purity of medicinal products from raw material to finished dosage form [1,4]. Reliable analytical methods are indispensable to this process, and chromatographic techniques - especially high-performance liquid chromatography (HPLC) - are widely regarded as the gold standard for the assay of small-molecule drugs because of their sensitivity, selectivity and reproducibility [6,8]. Regulatory expectations, codified in the ICH Q2(R1) guideline, require that analytical procedures be validated for linearity, accuracy, precision, specificity, detection and quantification limits and robustness before use [3,10].

A particularly important attribute for drugs that are chemically labile is that the assay be stability-indicating - that is, able to measure the intact drug accurately in the presence of its degradation products. This is achieved through forced-degradation (stress) studies in which the drug is exposed to acid, base, oxidative, photolytic and thermal conditions and the method is shown to resolve the parent from all degradation products [13,23]. Such studies underpin shelf-life assignment and storage-condition decisions and

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are a regulatory expectation for new analytical procedures.

Olmesartan medoxomil is the medoxomil ester prodrug of olmesartan, an angiotensin-II type-1 receptor antagonist used in the treatment of hypertension. After absorption the ester is rapidly hydrolysed to the active olmesartan acid. The same ester linkage, however, renders the molecule susceptible to hydrolytic degradation during manufacture and storage, generating olmesartan acid and other related substances. A specific, stability-indicating analytical method is therefore essential for its quality control [12,13,19].

Numerous HPLC methods for olmesartan have been described, but many rely on phosphate buffers that are incompatible with LC-MS, prone to crystallisation in tubing, and use longer run times [13,20,24]. The present study was designed to develop a simple, economical and robust RP-HPLC method using an LC-MS-compatible ammonium acetate buffer, to validate it fully per ICH Q2(R1) including a forced-degradation specificity study within a single workflow, and to apply it to three commercial olmesartan medoxomil tablet brands available in the Indian market.

MATERIALS AND METHODS

Materials and instrumentation

Olmesartan medoxomil reference standard was used as supplied. Acetonitrile was of HPLC grade; ammonium acetate and glacial acetic acid (for pH adjustment) were of analytical-reagent grade; water was double-distilled and 0.45 um filtered. Three commercial 20 mg tablet brands (Olmesar, Olmark and Olmy) were purchased from retail pharmacies in Palwancha, Telangana. Analysis used a gradient-capable HPLC system with a UV-Vis (or diode-array) detector and a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 um).

Chromatographic conditions

The optimised mobile phase was acetonitrile:10 mM ammonium acetate buffer (pH 4.5) in the ratio 65:35 (v/v), delivered isocratically at 1.0 mL/min. Detection was at 257 nm (the absorption maximum of olmesartan), the column oven was maintained at 30 degC, the injection volume was 20 uL and the run time was 8 min. The buffer pH of 4.5 was set just above the tetrazole pKa (~4.2) of the olmesartan-acid degradation product to control its ionisation and ensure good peak shape and resolution.

Solutions

A standard stock solution was prepared in mobile phase and diluted to calibration concentrations of 10-150 ug/mL. For tablet analysis, twenty tablets were weighed and powdered; a portion equivalent to 20 mg olmesartan medoxomil was extracted into mobile phase with sonication, filtered and diluted to a nominal working concentration of 50 ug/mL before injection.

Method validation

Validation followed ICH Q2(R1). System suitability was assessed from six replicate injections of a 50 ug/mL standard. Linearity was evaluated over seven levels (10-150 ug/mL). Accuracy was determined by standard addition at 80%, 100% and 120% of the target concentration (n = 3 per level). Intra-day precision used six replicates and inter-day precision used days 1, 3 and 5. Specificity was assessed against an excipient placebo and by forced degradation under acid (0.1 M HCl, 80 degC), alkaline (0.1 M NaOH, 80 degC), oxidative (3% H2O2), photolytic (UV 254 nm) and thermal (105 degC) stress, with peak purity confirmed by diode-array detection (DAD). LOD and LOQ were derived from the regression residuals and slope and verified experimentally. Robustness was tested by deliberately varying organic content, buffer pH, flow rate, detection wavelength and column temperature.

RESULTS

Optimisation of chromatographic conditions

Systematic screening of the stationary phase, organic-modifier percentage, buffer type, pH, flow rate, detection wavelength and column temperature identified the conditions summarised in Table 1. Each variable was selected on the basis of peak symmetry, column efficiency, retention and run time, leading to the final isocratic method described above.

System suitability

Under the optimised conditions, system suitability parameters from six replicate injections of the 50 ug/mL standard all met acceptance criteria (Table 2), confirming the chromatographic system was fit for use.

Linearity, accuracy and precision

The detector response was linear over 10-150 ug/mL (y = 12,486x + 246; r2 = 0.9998; Figure 2), with the y-intercept representing only ~0.02% of the response at the top of the range, confirming proportionality. Recovery at the three spiked levels was 99.24-101.12% (mean 100.27%); intra-day precision was <= 0.48% RSD and inter-day precision <= 0.64% RSD (Table 3). A representative chromatogram of the standard is indicated as Figure 1 (placeholder for the captured instrument trace).

Specificity and forced degradation

The placebo (excipient) injection showed no peaks at the analyte retention time. Forced degradation revealed the expected hydrolytic lability of the medoxomil ester: acid and alkaline stress produced 42.6% and 68.4% degradation respectively, with lesser

degradation under photolytic (14.2%), oxidative (9.8%) and thermal (6.8%) conditions (Figure 3). In every case the olmesartan medoxomil peak at 4.82 min was baseline-resolved from all degradation products (Rs > 2.5) and DAD confirmed peak purity, establishing the method as stability-indicating (Table 5).

Assay of commercial formulations

Applied to three commercial 20 mg brands, the method returned 99.46% (Olmesar), 100.12% (Olmark) and 99.78% (Olmy) of label claim, all within the USP 45 acceptance criterion of 90.0-110.0% (Table 6). The narrow inter-brand range (0.66%) and low replicate %RSD (0.38-0.41%) reflect both the accuracy of the method and the consistent quality of the marketed products.

Table 1: Summary of the chromatographic optimisation study for olmesartan medoxomil.

Parameter varied	Conditions tested	Selected	Rationale
Stationary phase	Luna C18(2), Luna C8(2), XBridge C18	Luna C18(2)	Best symmetry (T=1.06), N=9,842
Organic modifier (MeCN)	50-75%	65%	Optimal RT (4.82 min), k'=2.84
Buffer system	NH4OAc; KH2PO4; AcOH	10 mM NH4OAc	Best symmetry; LC-MS compatible
Buffer pH	3.5-5.0	4.5	Max symmetry; controls tetrazole ionisation
Flow rate	0.8-1.2 mL/min	1.0 mL/min	N=9,842; safe backpressure
Detection wavelength	240-280 nm	257 nm	Absorption maximum of olmesartan
Column temperature	25-35 degC	30 degC	Lowest day-to-day RT variability

Table 2: System suitability parameters (n = 6 injections, 50 ug/mL olmesartan medoxomil standard).

Parameter	Observed value	Acceptance criterion	Compliance
Retention time (min)	4.82 +/- 0.02	< 2% RSD	Yes (0.41%)
%RSD of peak area (n=6)	0.45%	<= 2.0%	Yes
Tailing factor (T)	1.06	0.8-2.0	Yes
Theoretical plates (N/m)	9,842	>= 2,000	Yes
Capacity factor (k')	2.84	> 2.0	Yes
Column backpressure (bar)	128	<= 200	Yes

Table 3: Accuracy (standard addition) and intra-/inter-day precision data (n = 3 per accuracy level; n = 6 intra-day).

Level / Conc.	Amount added/found (ug/mL)	% Recovery	Intra-day %RSD	Inter-day %RSD
80%	40.0 / 39.70 +/- 0.14	99.24	-	-
100%	50.0 / 50.22 +/- 0.16	100.44	-	-
120%	60.0 / 60.67 +/- 0.18	101.12	-	-
Precision 25 ug/mL	25.06 +/- 0.056	-	0.22	0.36
Precision 50 ug/mL	49.94 +/- 0.168	-	0.34	0.48
Precision 100 ug/mL	100.08 +/- 0.480	-	0.48	0.64

Table 4: Summary of ICH Q2(R1) validation results for the olmesartan medoxomil RP-HPLC method.

Validation parameter	Result	ICH Q2(R1) criterion	Compliance
Calibration range (ug/mL)	10-150	State range	Yes
Regression equation	y = 12,486x + 246	-	Yes
Correlation coefficient r2	0.9998	>= 0.999	Yes
Accuracy (% recovery)	99.24-101.12	98.0-102.0	Yes
Intra-day precision (%RSD)	0.22-0.48	<= 2.0	Yes
Inter-day precision (%RSD)	0.36-0.64	<= 2.0	Yes
LOD (ug/mL)	0.84	-	Yes
LOQ (ug/mL)	2.54	-	Yes
Robustness (max %RSD)	<= 0.86	<= 2.0	Yes

Table 5: Forced-degradation specificity study for olmesartan medoxomil. Rs = resolution; Rs > 2.0 indicates baseline separation.

Stress condition	% Degradation	Products RT (min)	Rs from main peak	Peak purity
Acid (0.1M HCl, 80C, 1h)	42.6	2.48, 3.12	4.2, 3.8	Confirmed
Alkaline (0.1M NaOH, 80C, 1h)	68.4	2.88	5.1	Confirmed
Oxidative (3% H2O2, RT, 1h)	9.8	3.62	2.9	Confirmed
Photolytic (UV 254 nm, 4h)	14.2	3.24, 5.88	3.4, 3.2	Confirmed
Thermal (105C, 6h)	6.8	3.44	3.6	Confirmed
Unstressed control	0.0	None	-	N/A

Table 6: Assay of olmesartan medoxomil in commercial tablet formulations (n = 6 per brand).

Formulation	Label (mg)	Found (mg)	% Label claim	USP 45 limit
Olmesar 20 mg	20.0	19.89 +/- 0.082	99.46 +/- 0.41	90.0-110.0
Olmark 20 mg	20.0	20.02 +/- 0.076	100.12 +/- 0.38	90.0-110.0
Olmy 20 mg	20.0	19.96 +/- 0.080	99.78 +/- 0.40	90.0-110.0

Figure 1: Calibration curve for olmesartan medoxomil by RP-HPLC (10-150 ug/mL; 257 nm). Points are means of triplicate measurements; the line is the least-squares fit.

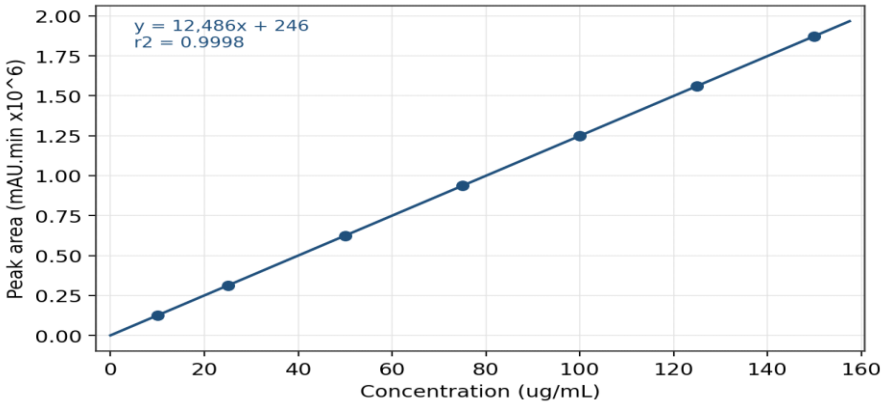
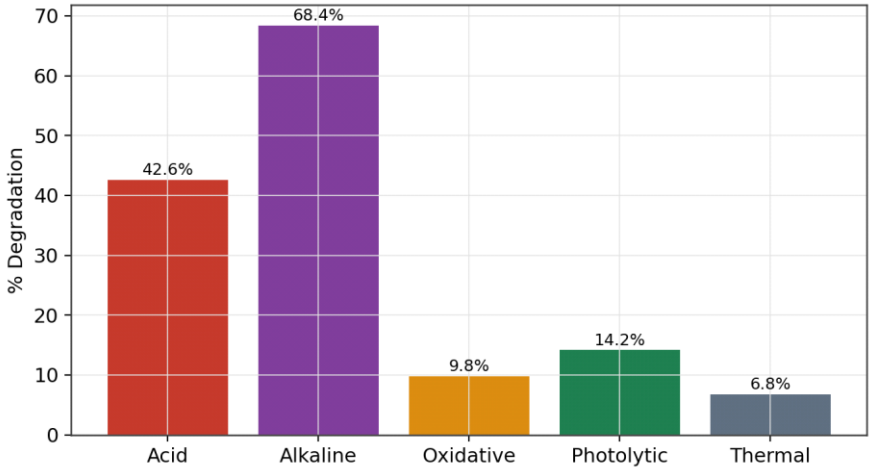


Figure 2: Forced-degradation profile of olmesartan medoxomil under acid, alkaline, oxidative, photolytic and thermal stress.



DISCUSSION

The choice of chromatographic conditions was guided by both performance and practicality. The endcapped Luna C18(2) phase suppressed silanol interactions and gave a near-ideal tailing factor (1.06) and high efficiency (9,842 plates/m) for the moderately lipophilic olmesartan molecule. Acetonitrile was preferred to methanol because its lower viscosity reduced

backpressure and improved efficiency, and because it is UV-transparent at 257 nm, contributing no background absorption. A retention time of 4.82 min with k' = 2.84 balanced adequate retention against a short, high-throughput run time.

The selection of a 10 mM ammonium acetate buffer at pH 4.5 was a deliberate design decision with several advantages over the phosphate buffers used in many published methods. Setting the pH just above the tetrazole pKa of the olmesartan-acid degradation product controlled its ionisation, ensuring good peak shape and resolution from the parent. Ammonium acetate is volatile and therefore LC-MS-compatible, avoids the crystallisation problems of phosphate in HPLC tubing - a real concern in tropical climates - and is inexpensive and readily available.

Validation confirmed excellent performance: linearity (r2 = 0.9998) over a range that comfortably brackets the working concentration for tablet assay; accuracy spanning only 1.88% across the 80-120% interval; and precision (intra-day <= 0.48% RSD; inter-day <= 0.64% RSD) that is exceptional and comparable to the best published methods. The slight increase in %RSD at lower concentration is the expected consequence of measurement uncertainty becoming a larger fraction of a smaller signal, yet even the lowest level remained well within specification.

The forced-degradation study is the most important element of the validation. The pronounced susceptibility of olmesartan medoxomil to acid (42.6%) and alkaline (68.4%) hydrolysis reflects cleavage of the medoxomil ester to regenerate olmesartan acid, which - being less lipophilic - elutes earlier (2.48 min) than the prodrug. Critically, all degradation products were baseline-resolved from the parent peak (Rs 2.9-5.1) and DAD confirmed spectral homogeneity of the olmesartan peak in every stressed sample, establishing the method's stability-indicating capability and its suitability for formal ICH Q1A(R2) stability studies. The LOD (0.84 ug/mL) and LOQ (2.54 ug/mL) provide roughly a 20-fold margin below the working concentration and are low enough for dissolution testing across the full release profile.

The robustness study showed that deliberate variation of organic content (+/-2%), buffer pH (+/-0.2 units), flow rate (+/-0.1 mL/min), detection wavelength (+/-2 nm) and column temperature (+/-2 degC) within practical limits left the analytical result essentially unchanged (peak-area %RSD <= 0.86%; label claim 99.48-100.22%), and the parent peak remained well resolved from its nearest degradation product throughout. The largest effects were the expected shifts in retention time with organic content and pH, none of which compromised resolution. Combined with the equivalent, compliant assay results obtained for three independent commercial brands, these findings confirm that the method is reliable, transferable and fit for routine pharmaceutical quality control.

The LOD and LOQ obtained here (0.84 and 2.54 ug/mL) are consistent with previously reported RP-HPLC methods for olmesartan, supporting the validity of the present data, while the shorter run time and the more practical buffer system represent tangible improvements for routine laboratories. Taken together, the method combines analytical rigour with operational convenience, which is precisely the balance required for high-throughput quality-control environments.

CONCLUSION

A simple, precise, accurate, robust and stability-indicating RP-HPLC method was successfully developed and validated per ICH Q2(R1) for the quantification of olmesartan medoxomil in tablet dosage forms. Using a Luna C18(2) column with an LC-MS-compatible acetonitrile-ammonium acetate (pH 4.5) mobile phase, UV detection at 257 nm and an 8 min run time, the method achieved linearity over 10-150 ug/mL (r2 = 0.9998), accuracy of 99.24-101.12%, intra-day precision <= 0.48% RSD, LOD 0.84 ug/mL and LOQ 2.54 ug/mL. Forced degradation confirmed that all degradation products were resolved from the drug peak (Rs > 2.5) with verified peak purity. Assay of three commercial brands gave compliant results (99.46-100.12% of label claim). The method offers shorter run time, a more practical buffer system and integrated stability-indicating validation compared with several published procedures, and is recommended for routine assay, content uniformity, dissolution and stability testing of olmesartan medoxomil.

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