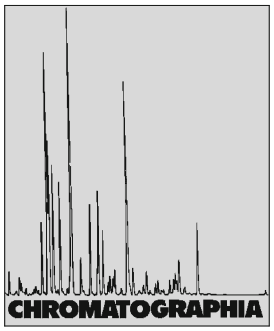
Stability-Indicating LC Determination of a New Antihypertensive, Olmesartan Medoxomil in Tablets



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Abstract

A stability-indicating liquid chromatographic method was developed and validated for quantitative determination of olmesartan medoxomil (OLM) in coated tablets in the presence of degradation products generated under stress conditions. An isocratic LC separation was performed using a Phenomenex RP-18 column using a mobile phase consisting of water:triethylamine:acetonitrile (60:0.3:40 *v/v/v*, pH adjusted to 6.3 with phosphoric acid). The flow rate was 1.2 mL min^{-1} and the detection was achieved with a photodiode array detector set at 257 nm. The response was linear over a range of 10.0 to 30.0 μg mL^{-1} ($r = 0.9999$). The specificity and stability-indicating capability of the method was verified subjecting the reference substance and drug product to hydrolytic, oxidative, photolytic, and thermal stress conditions. The method showed a good and consistent recovery (100.2%) with low intra- and inter-day relative standard deviation (RSD) ($\leq$1.0%). A considerable degradation occurred in all stress conditions and the degradation product was well resolved from the main peak. There was no interference of the excipients in the determination of the active pharmaceutical ingredient. Thus, the proposed method was found to be stability-indicating and can be used for routine analysis for quantitative determination of OLM in coated tablets without the interference of major degradation products.

Keywords

Column liquid chromatography
Stability-indicating method
Forced degradation
Validation
Olmesartan medoxomil

Introduction

Olmesartan medoxomil (OLM), (5-methyl-(2-oxo-1,3-dioxol-4-yl)methyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[2′(1*H*)-tetrazol-5yl)1,1′biphenyl(-4-yl)methyl]-1*H*-imidazole-5-carboxylate] (Fig. 1) is considered the latest angiotensin II receptor blocker approved in April 2002 by the Food and Drug Administration (FDA) for the treatment of hypertension. OLM is used, either alone or in combination with other drugs, to treat high blood pressure. This antihypertensive is a prodrug that is rapidly and completely de-esterified to the active metabolite olmesartan (OL; Fig. 1) by both arylesterase and albumin during gastrointestinal absorption [1, 2].

There is no reference for determination of this drug in both bulk and dosage forms in official compendia. OLM has been determined in biological fluids using LC coupled to fluorescence and tandem mass spectrometry [2–5]. There are also reports on spectrophotometry and capillary electrophoresis for the quantitative determination of this drug in coated tablets [6, 7]. To our knowledge, there is only one LC method applied for quality control and in vitro dissolution test for OLM combined with

hydrochlorothiazide in tablets [8]. Recently, an LC hyphenated technique was used to elucidate a degradation product obtained from stress condition of OLM tablets [9]. Even though, these articles are not related to stability-indicating technique.

The international conference on harmonization (ICH) guideline Q1A (R2) requires the stress testing to be carried out to elucidate the inherent stability characteristics of active substances [10]. In the present study, attempts were made to develop and validate a rapid stability-indicating liquid chromatographic (LC) method, in compliance with ICH guidelines, USP Pharmacopoeia and FDA [11–14].

Chemical and Reagents

OLM (purity 99.3%) was purchased from Sequoia Research Products (Oxford, UK). One batch of Benicar (Sankyo Pharma, Brazil) tablets containing 20 mg of OLM was obtained from commercial sources. The excipients contained in the dosage form (cellulose microcrystalline, low substitution hyprolose, lactose monohydrated, hyprolose, magnesium stearate, talc, titanium dioxide, hypromelose) were all pharmaceutical grades and acquired from different distributors. LC grade acetonitrile was obtained from Tedia (Fairfield, USA). Purified water was prepared using a Milli-Q Plus (Millipore, Bedford, USA). All other reagents and chemical were analytical grade.

Instrumentations

The analysis was performed on a Shimadzu LC system (Kyoto, Japan) which consisted of a LC-10AD pump, a SPD-M10ADVP photodiode array (PDA) detector, a SLA-10ADVP system controller, a DGU-14A degasser and a Rheodyne 7725i manual injector with a 20 µL loop. Data were acquired and processed using CLASS-VP software (version 6.1). The chromatographic separation was performed using a Phenomenex RP-18 column (250 mm × 4.6 mm i.d., particle size 5 µm) and the robustness was verified using an Ace RP-18 column (250 mm × 4.6 mm i.d., particle size 5 µm), both with a guard column (4 × 3 mm i.d.) packed with the same material. Photodegradation was carried out in a photostability UV chamber (1.0 × 0.17 × 0.17 m) with mirrors and equipped with an UV-A lamp (Orion, 352 nm, 30 W, 130 V) and UV cuvettes (Ultra Vette, São Paulo, Brazil) were used as a container for samples. For thermal stability studies, a dry air oven (Biomatic, Porto Alegre, Brazil) was used.

Chromatographic Conditions

The chromatographic analysis was performed isocratically at room temperature (23 ± 1 °C), using a mobile phase composed of water:triethylamine:acetonitrile (60:0.3:40 *v*/*v*/*v*, pH adjusted to 6.3 with phosphoric acid). This was filtered through a 0.45 µm membrane filter (Millipore, Bedford, USA) and degassed using an ultrasonic bath. The flow rate was 1.2 mL min^{-1} and the PDA detector was set at 257 nm. The injection volume was 20 µL for both reference substance and drug product solutions.

Preparation of Standard and Sample Solutions

OLM reference substance was accurately weighed (10.0 mg) and dissolved in a 20 mL volumetric flask with mobile phase to produce a concentration of 500.0 µg mL^{-1} of analyte. This solution was diluted appropriately in the same diluent to yield a final concentration of 20.0 µg mL^{-1}.

To prepare a sample solution, 20 units of Benicar tablets (20.0 mg of OLM) were accurately weighed and crushed to fine powder. An appropriate amount was transferred into a 20-mL volumetric flask with 10 mL of mobile phase. This flask was kept in an ultrasonic bath for 15 min. The volume was completed with the same diluent and an aliquot of 1.0 mL of this solution was diluted in a 25-mL volumetric flask to yield a final concentration of 20.0 µg mL^{-1}. The solutions were filtered through a 0.45 µm membrane filter before injection.

Validation of Method

The developed chromatographic method was validated for specificity, linearity, sensitivity, precision, accuracy, robustness and system suitability, following the guidelines [11–14]. Furthermore, stability-indicating capability was determined by forced degradation conditions in accordance with ICH guidelines [10–12].

Specificity/Forced Degradation Studies

The interference from inactive ingredients was investigated through recovery studies using the standard addition method. This procedure was carried out by adding known amounts of OLM reference substance into a mixture of excipients. Their concentrations were based on the literature [15].

Further, an intentional degradation was performed. A minimum of three samples were generated for each stress condition. Blank solutions were used during the analysis and both reference substance and drug product solutions were stored at room temperature (23 ± 1 °C) to compare the results.

Solutions containing 1.0 mg mL^{-1} of OLM were used. After the degradation period, these solutions were neutralized (if necessary) and diluted in mobile phase to achieve a concentration of 20.0 µg mL^{-1}. For peak purity test, a PDA detector was used in scan mode (200–350 nm) and the purity factor was observed. The stress conditions were the following:

Acid and basic hydrolysis. Each solution was prepared in a mixture of 0.01 M HCl—mobile phase (70:30, *v*/*v*) and 0.01 M NaOH—mobile phase (70:30, *v*/*v*), respectively. All were protected from the light and stored at room temperature (23 ± 1 °C) during 24 h.

Oxidative degradation. Solutions were prepared in a mixture of 3% H_2O_2 and mobile phase (70:30, *v*/*v*) and were protected from light and stored at room temperature (23 ± 1 °C) for 24 h.

Thermal degradation. Reference substance in powder (dry heat) and in solution (moist heat) were exposed at 80 °C in an oven for 24 and 1 h, respectively.

Photodegradation. Reference substance in powder and in solution were placed in a glass dish and quartz cuvettes, respectively, were placed in a light chamber and exposed to UV-A radiation (352 nm) for 48 h, at room temperature (23 ± 1 °C). Control samples were protected from light with aluminum foils, placed in a light chamber and exposed concurrently.

Following the degradation period, all samples were prepared for analysis as previously described.

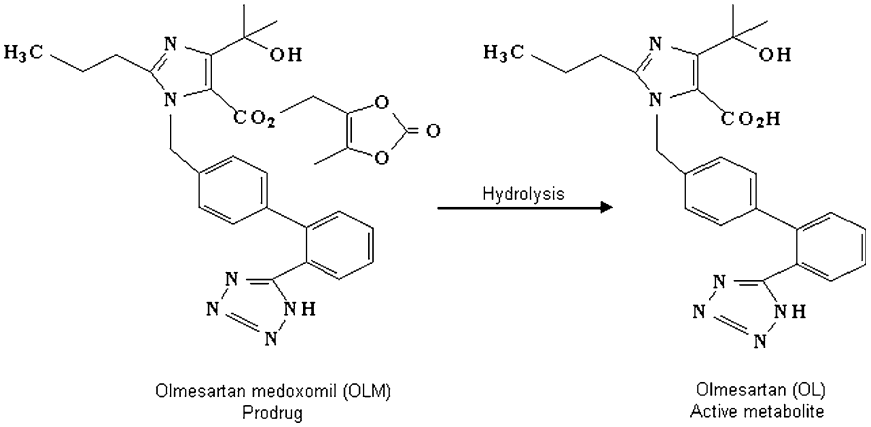

Fig. 1. Hydrolysis of olmesartan medoxomil

Linearity and Limits of Detection and Quantitation

Three calibration curves were prepared in mobile phase and analyzed in triplicate at five different OLM concentrations (10.0, 15.0, 20.0, 25.0 and 30.0 μg mL^{-1}). The results were tested by ANOVA and linear regression analysis was used to obtain the equation and correlation coefficient. The limits of detection (LOD) and quantitation (LOQ) were calculated using the calibration line directly. The aforementioned factors (3.3 and 10) were multiplied by the ratio from the residual standard deviation and the slope (corresponding to the standard error of slope), according to the guidelines [11–14].

Precision

The precision was determined using the parameters of repeatability (intra-day) and intermediate precision (inter-day), analyzing six sample solutions of OLM prepared at 20.0 μg mL^{-1} in triplicate on three different days. They were expressed as relative standard deviation (RSD) of the measurements.

Accuracy

Accuracy was calculated as the percentage of recovery by adding known amounts of OLM reference substance to sample solutions (12.0 μg mL^{-1}) to yield final concentrations of 18.0, 20.0 and 22.0 μg mL^{-1}, corresponding to 80, 100 and 120% of the nominal analytical concentration.

Robustness

In this study, the chromatographic parameters (peak retention time, theoretical plates, tailing, capacity factor and repeatability) were evaluated using both sample and reference substance solutions (20.0 μg mL^{-1}) changing flow rate (1.0 and 1.4), acetonitrile concentration (38 and 42%) and pH (6.1 and 6.5) of mobile phase, column (Ace RP-18 column) and equipment (Shimadzu liquid chromatograph equipped with a SCL-10AVP system controller, LC 10AVP pump).

The stability of reference and sample solutions was also evaluated at room temperature (23 ± 1 °C) and in the refrigerator (8 ± 2 °C), during 24 and 48 h. The stability of these solutions was verified by performing the experiment and observing any change in the chromatographic pattern, comparing with freshly prepared solutions. The RSD of the assay was calculated for the study period during solution stability experiments.

Results and Discussion

Selection of Chromatographic Conditions

The chromatographic conditions were optimized to develop a stability-indicating method to separate the degradation products from the drug. During the procedure three columns (Hypersil RP-18 250 mm × 4.6 mm i.d., particle size 5 μm; Merck® RP-18 250 mm × 4.6 mm i.d., particle size 5 μm; Phenomenex RP-18 250 mm × 4.6 mm i.d., particle size 5 μm) and different mobile phases with distinct proportions of organic solvent (acetonitrile or methanol) and water, with and without triethylamine, in two distinct pH (3.3 and 6.3) were evaluated.

The best chromatographic condition was achieved using aqueous 0.3% triethylamine (pH adjusted to 6.3 with phosphoric acid) and acetonitrile (60:40, *v*/*v*). The flow rate used was 1.2 mL min^{-1}. PDA detection was used to select the best wavelength for analysis as well as to verify peak purity during analysis. With this mobile phase, adequate results were obtained in terms of peak parameters (shape peak, selectivity, theoretical plates, symmetry, capacity factor, repeatability). A typical chromatogram obtained by the proposed method for OLM is shown in Fig. 2a.

Specificity/Degradation Behavior

The high percentage of recovery observed with sample solutions indicates that the proposed method was not affected by interferences from excipients used in the dosage form (Fig. 2a). Photodiode array detection also supported

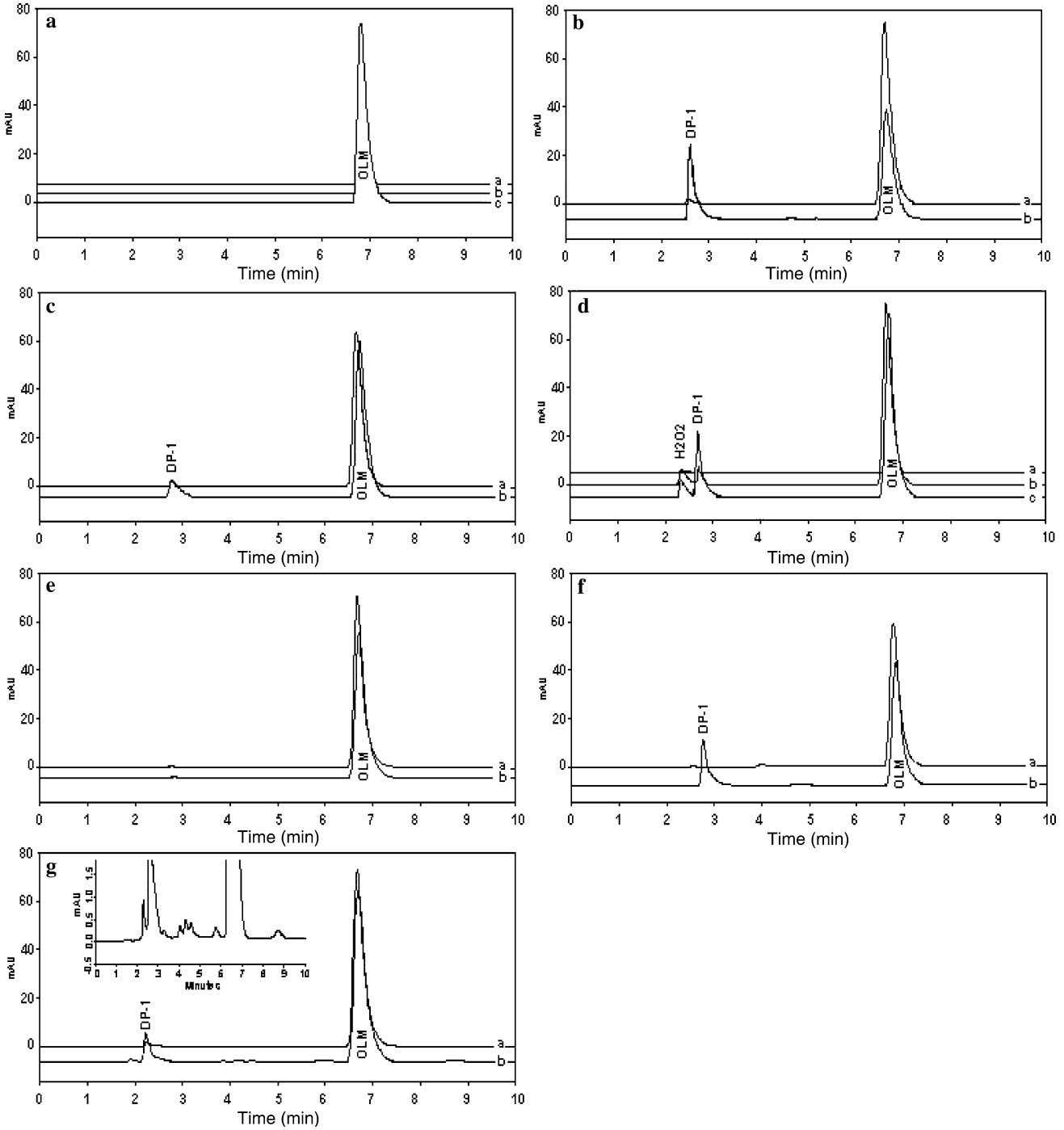

Fig. 2. **a**–**g** Chromatograms obtained from olmesartan medoxomil (20.0 μg mL^{-1}): **a** blank (*a*), placebo (*b*), reference substance (*c*); **b** acid hydrolysis 0 h (*a*) and 24 h (*b*); **c** basic hydrolysis 0 h (*a*) and 24 h (*b*); **d** oxidative degradation: 3% H_2O_2 blank (*a*), 0 h (*b*) and 24 h (*c*); **e** dry heat 0 h (*a*) and 24 h (*b*); **f** moist heat 0 h (*a*) and 1 h (*b*); **g** photodegradation 0 h (*a*) and 48 h (*b*). Chromatographic conditions: Phenomenex RP-18 column (250 × 4.6 mm i.d., particle size 5 μm); 1.2 mL^{-1} flow rate; mobile phase water:triethylamine:acetonitrile (60:0.3:40 *v*/*v*/*v*, pH adjusted to 6.3 with phosphoric acid); UV detection at 257 nm

the specificity of the method and provided evidence of peak purity for OLM.

Forced degradation studies showed that the OLM drug product was considered more stable and reveal the same profile degradation as the OLM reference substance. The results presented in Table 1 concern the OLM reference substance under various stress conditions.

A complete degradation occurred when 0.1 M HCl and 0.1 M NaOH

solutions were used at room temperature (23 ± 1 °C). In 0.1 M NaOH the solution became yellow instantaneously. Considering this, 0.01 M HCl and 0.01 M NaOH solutions were used and an additional peak was detected at ~2.70 min, in both conditions (Fig. 2b, c). The same was observed when 3% H_2O_2 was used. An third peak observed at 2.20 min could be attributed to the H_2O_2 stabilizer (phenacetin), because it was also detected in the blank solution (Fig. 2d).

No degradation of OLM reference substance was observed under dry heat condition, while a peak appeared at 2.72 min when a solution was heated (Fig. 2e, f).

Under photolytic condition a peak also emerged at 2.72 min, however other small peaks also appeared (Fig. 2g). Control samples demonstrated that the heat generated inside the light chamber did not interfere in the degradation of the drug.

Observing all the stress conditions the OLM reference substance was less stable in peroxide medium but stable when the powder was exposed to dry heat.

The peak purity indexes (Table 1) obtained for both OLM and major degradation product (2.7 min) peaks in all samples analyzed indicate that they were pure. The resolution between these two peaks was higher than 2.0. The same retention time was achieved for the main degradation product (about 2.70 min) in all stress conditions tested. Since OLM is the ester prodrug of OL, it is reasonable to think that this degradation product is OL. However, OL reference substance should be used to confirm this assumption.

Table 1. Results of analysis of forced degradation study using proposed method

Stress condition	Degradation (%)	Peak purity[a]
Control sample	No degradation	0.9999
Acid hydrolysis (0.01 M HCl, 24 h)	50.7	0.9999
Basic hydrolysis (0.01 M NaOH, 24 h)	36.3	0.9999
Oxidation (3% H_2O_2, 24 h)	55.6	0.9999
Dry heat (80 °C, 24 h)	No degradation	0.9999
Moist heat (80 °C, 1 h)	15.0	0.9999
Photolytic degradation (48 h)	8.7	0.9999

[a] Peak purity values in the range of 0.9990–1.0000 indicate a homogeneous peak

Table 2. Evaluation of robustness for the olmesartan medoxomil LC assay ($n = 3$)

Parameter	R_t[b] (min)	T[c] (≤2.0)	N[d] (>2,000)	k[e] (>2.0)	(%)
Proposed method[a]	6.98	1.76	3,019	2.48	100.18
Flow rate (1.0 mL^{-1})	8.36	1.82	3,107	3.18	101.04
Flow rate (1.4 mL^{-1})	6.27	1.72	3,081	2.10	101.38
pH (6.1)	6.89	1.76	3,033	2.45	97.37
pH (6.5)	6.74	1.78	2,972	2.37	96.84
Acetonitrile (−5%)	7.86	1.65	3,744	2.93	97.49
Acetonitrile (+5%)	5.80	1.89	2,289	4.80	97.93
Stability at 8 ± 2 °C	7.10	1.73	3,095	2.55	101.03
Stability at 23 ± 1 °C	7.05	1.73	3,063	2.53	101.49
Different column	5.92	1.91	2,644	2.95	100.24
Different equipment	7.37	1.97	6,174	2.75	99.86
%RSD					1.78

[a] Mobile phase with water:triethylamine:acetonitrile (60:0.3:40 *v/v/v*, pH adjusted to 6.3 with phosphoric acid), Phenomenex RP-18 (250 × 4.6 mm i.d., particle size 5 µm)
[b] R_t retention time
[c] T tailing factor
[d] N theoretical plate number
[e] k retention factor

Linearity and Limits of Detection (LOD) and Quantitation (LOQ)

Linearity was evaluated in the 10.0–30.0 µg mL^{-1} range. The slope and intercept (±SD, $n = 3$) were 11,444 ± 11,282 and 50,716 ± 376, respectively, and the correlation coefficient was 0.9999. ANOVA results showed that the regression equation was linear ($F_{calculated} = 5.8.10^4 > F_{critical} = 4.96$; $P = 0.05$) with no deviation from linearity ($F_{calculated} = 1.89 < F_{critical} = 3.71$; $P = 0.05$). The LOD and LOQ were 0.73 and 2.22 µg mL^{-1}, respectively.

Precision and Accuracy

The intra-day ($n = 6$) precision, performed by assaying the samples in three different days by the same analyst, showed good results: 99.63 ± 0.83; 99.41 ± 1.06 and 99.02 ± 0.96% (mean ± RSD). The RSD for inter-day precision was 0.95% ($n = 3$). The average results were equivalent ($F_{calculated} = 6.3.10^{-1} < F_{critical} = 3.68$; $P = 0.05$).

In the accuracy test three concentrations were evaluated (18, 20 and 22 µg ml^{-1}) and mean recoveries were 101.28 ± 0.18, 100.05 ± 0.56 and 99.38 ± 0.15% (mean ± RSD), respectively.

Robustness

The results and experimental range of the selected variables evaluated in the robustness assessment are shown in Table 2. Flow rate (from 1.0 to 1.4) and acetonitrile proportion (±5%) changes yielded the expected results: earlier elution with faster flow rates and higher acetonitrile concentration. Different columns and equipments resulted in changes in the retention time, tailing factor and theoretical plate number of the major peak in comparison with the proposed analytical conditions. However, no significant changes (RSD < 2.0%) were observed regarding quantitation of OLM. The solutions can be considered stable up to 24 h in both temperatures tested. After 48 h an extra peak was observed. The results were: theoretical plates (from 2,289 to 6,174), retention time (from 5.80 to 8.36) and tailing factor (from 1.65 to 1.91). The area peak

RSD of five injections was 0.85%, demonstrating the injection repeatability. The values for these parameters were satisfactory in accordance with the literature [10–14]. None of these alterations caused a significant effect on the determination of this drug in coated tablets, indicating robustness or stability of the method.

Conclusion

The LC method developed and validated for quantitative determination of OLM in coated tablets was considered simple, sensitive, specific, precise, accurate and reproducible. The method uses simple reagents and minimum sample preparation procedures, encouraging its application in routine analysis.

Based on results of specificity procedure and forced degradation studies, undertaken according to the ICH guidelines, the method can be considered specific and stability-indicating, for the reason that there was no interference of the excipients and of the degradation product formed in the determination of OLM in bulk samples. Thus, the method can be applied in stability testing of the commercially available OLM coated tablets.

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