Development and Validation of SI/RS-UHPLC-PDA Method for Olmesartan Medoximil and Metoprolol Succinate Related Substance

Short Title in English: Stability Indicating UHPLC Method

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26.04.2022

ABSTRACT
Objectives
Olmesartan medoximil (OLM) and Metoprolol succinate (MPS) in fixed dose combination (FDC) tablet formulation prescribed extensively. Stability indicating (SI) method for impurities and related substance (RS) test quantitates the amount of these analytes in formulation; the manuscript presents stability indicating SI/RS-UHPLC-PDA method for OLM and MPS and their impurities.

Materials and Methods
Well-resolved separation of all analytes achieved with gradient elution on Shimadzu on Shimpack GIST-C18 (100mm X 2.1mm, 2 micron) column maintained at 25 °C. Mobile phase-A consist of 0.1% Orthophosphoric acid in water and mobile phase-B was acetonitrile at a flow rate of 0.4 ml/min, data integrated at 225 nm and 16 min. of short runtime for satisfactory elution of all the peaks.

Results
Proposed SI/RS-UHPLC-PDA method was developed and validated as per ICH guidelines. System suitability test complied by all eluted peaks of the interest with acceptable linearity, recovery and precision. Specificity, robustness and method sensitivity parameters determined; all the parameters found to be within the limits. All impurities and stress-degraded peaks were well resolved.

Conclusion
The proposed method found to be simple, fast, linear and accurate. Further, the method is precise, robust and specific; suitable for routine IPQC during API manufacturing, stability and impurity profiling studies of the titled bulk analytes. Furthermore, the method can be extended to assess the levels of impurities formed during life cycle of new FDCs of titled analytes.

Keywords: SI/RS-UHPLC-PDA, Related substances, Impurities, stability studies, Gradient elution.
INTRODUCTION
Olmesartan medoximil (OLM) is chemically (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl5- (2-hydroxypropan -2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl]phenyl] methyl] imidazole- 4- carboxylate (Figure 1a). Metoprolol succinate (MPS) is chemically butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol (Figure 1b). OLM is an angiotensin II type 1 (AT(1)) receptor antagonist. It inhibits actions of angiotensin II and administered once daily. OLM recommended in the dosage range of 10-40 mg to adult patients for treatment of hypertension. (1. Lesley J Scott 1, Paul L McCormack, 2007). MPS a β1-selective adrenoceptor blocking agent preferred in arrhythmia, hypertension, angina pectoris, and myocardial infarction. Extended-release tablet for controlled and predictable release of MPS achieved by once-daily oral administration. 2 These active pharmaceutical ingredients (APIs) are official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP) (IP 2010; BP 2010). For MPS Impurity A (Figure 1c) is reported in official books. Correspondingly, for OLM impurity B (Figure 1d), impurity C (Figure 1e), impurity D (Figure 1f) and dimer impurity (Figure 1g) reported in official books. ICH tripartite guidelines specify limits on impurity levels in APIs and their dosage. There is no need of testing impurity unless there is generation of impurity as part of drug degradation in dosages as per ICH guidelines (ICH Q1 2021, ICH Q3 2021) To support institutional research product development and stability studies and achieve faster quantitation and evaluations of combined formulation from stability and process samples, there was need of SI/RS-UHPLC-PDA method. Literature survey reveals that that there are various methods available for estimation of OLM. 3-20 Various methods are available for individual estimation of MPS. 21-25 Various UV spectrophotometric, TLC and HPLC methods available for estimation of MPS in combination with other drugs and OLM. 26-38 Literature survey also reveals that there is no impurity profiling UHPLC method reported for estimation of OLM and MPS. Therefore, SI/RS-UHPLC-PDA method development and validation for these analyte from formulation along with impurities and stress degradation product undertaken. Proposed method is simple, fast quantification and identification method for OLM and MPS along with their impurities RS. The proposed analytical methods is beneficial in achieving time and other resources efficiency. The method was developed and validated as per ICH guidelines. 39

MATERIALS AND METHODS

Chemicals, reagents and Instrumentation: The drug sample of OLM (Assay - 99.81 %) and MPS (Assay - 99.77 %) were gifted by Cadila Healthcare Ltd., Ahmedabad, India. Impurities were gratis gift by Piramal Healthcare Limited. HPLC grade solvents and analytical grade reagents and chemical used in presented research work were purchased from Sisco Research Lab Pvt Ltd., Mumbai. Method development and validation work carried out on Shimadzu N-Series UHPLC instrument. Data was integrated using Shimadzu LabSolutions software version 6.89. The column used was Shimadzu Shimadzu Shimpack GIST-C18 (100mm X 2.1mm, 2 micron) and the injection volume was 5 µl using autosampler (LC40AD) mode. Mobile phase flow rate was 0.4 ml/min with online degasser. FDC combination tablets containing 20 mg of OLM and 25 mg of MPS, manufactured by Glenmark Pharmaceuticals Limited was used.

Standard solution preparation: Accurately about 100 mg OLM and 125 mg MPS transferred into separate 100 ml of VFs containing 50 ml of diluent (Water: Acetonitrile; 50:50 % v/v). Analytes dissolved by 5 min sonication and diluent used to makeup volume to get first standard stock solutions (SSS). 2 ml of these SSS transferred separately into 100 ml of VFs; volume made up to get second SSS with same solvent system. Combined OLM and MPS solution prepared by transferring 2 ml from each of first standard stock solutions of analytes. Further 5 ml of above second SSS transferred into 100 ml of volumetric flask; volume made up to the mark with same solvent.
system. 100 µg/ml standard stock solutions of impurity A of MPS as well as all impurities of OLM were prepared individually in diluent and used to spike solutions of actives.

**Sample preparation:**
Weight of 20 intact tablets recorded and tablets crushed to get powder, form this tablet powder equivalent to 100 mg OLM (125 mg MPS) transferred to 100 ml volumetric flask. To the flask 70 ml of diluent added and analytes dissolved by sonication for 20 minutes. Volume made up to the mark with diluent and mix well. The solution filtered through 0.45 µm PVDF syringe filter by discarding first 3 ml of filtrate.

**Preparation and treatment of mobile phase:**
Mobile phase A (MPA) contains 1 ml of Ortho-phosphoric acid (OPA) in 1000 ml of HPLC grade distilled water and sonicated for 15 min; filtered through 0.45 µ filter. Correspondingly, Acetonitrile used as mobile phase B.

**2.6 Method Validation:**
Validation of the optimized chromatographic method was carried out as per ICH guidelines for stability, impurity and analytical method validation. After multiple initial method development trials with different mobile phase compositions and different gradient programs, efficient separation and resolution of the degraded products and spiked impurities was achieved on Shimadzu Shim-pack GIST-C18 (100 mm X 2.1 mm, 2 µm) column maintained at 25 °C and data processed at isopiestic wavelength of 225 nm. Mobile phase-A consist of 0.1% Orthophosphoric acid (OPA) in water and mobile phase-B consist of acetonitrile with gradient elution at a flow rate of 0.4 ml/min. The instrument used was Shimadzu N-Series UHPLC. Method validation was performed for various parameters such as linearity, method sensitivity (LOD and LOQ), precision, accuracy, specificity (formulation specificity, stress degradation and impurity spiking) and robustness. To support validation data for Formulation studies, filter compatibility studies were also carried out using 0.45 µ PVDF and Nylon membrane filters. Solution stability studies were performed at room temperature and 5 °C. Standard mixture was injected in six replicates to perform System suitability test (SST) of analytes before start of each validation experiments and by determining % RSD of the peak areas; which was always < 5 % throughout the validation studies. Method validation parameters studied are as follows.

**Filter Compatibility studies**
Filter compatibility studies were performed using standard solution for 0.45 µ Nylon and 0.45 µ PVDF membrane filters. The % assay of filtered standard against control centrifuged standard was calculated by discarding first 3 ml of filtrate.

**Linearity method sensitivity and Specificity**
Linearity assessed visually as well as by means of a lack-of-fit test; interval between the upper and the lower levels of the analyte considered as the method range. Furthermore, method linearity evaluated from LOQ level to 150 % of specification level. Slope, intercept, correlation coefficient and % Y intercept bias was calculated. For method specificity chromatographic peak interference from blank, placebo and impurities at the retention time of both analyte peaks as well as stress-degraded products in stressed samples were observed for accepted resolution. Purity of the analyte peaks for each of these conditions was assessed by peak purity test. To evaluate peak purity criteria peak purity index and peak purity threshold values generated by the software system were noted and interpreted. Peak purity index value less than peak purity threshold values of relevant peak indicate that the peak is pure.

**Method sensitivity study**
Solution were prepared at 0.03 ppm to 100 ppm for all impurities and injected for determination of LOD and LOQ, respectively as method sensitivity parameters. These parameters were estimated based on S/N ratio (LOD: S/N >3; LOQ: S/N >10). As part of this study LOQ precision was performed by injecting 6 replicates and % RSD was determined.

**Method Precision**
Six different sets were prepared by spiking all the impurities at 100% Level (5 ppm) in the API at sample concentration level (1000 ppm OLM + 1250 ppm MPS) and % RSD was determined.

**Accuracy (Recovery)**
Method recovery was evaluated by standard spiking technique. Known amount of standard impurities were spiked in API and placebo mixture preparation at 50%, 100% and 150% level. Accuracy studies were performed in triplicate.

**Forced degradation and specificity**
Forced degradation study on formulation was carried out in sample solution state. For acid stress, 5 ml of tablet stock solutions were transferred into 25 ml of volumetric flask and 2.5 ml of 0.01N HCl was added, the solutions was subjected to stress at 60°C for 10 min. Stressed sample was neutralized with 2.5 ml of 0.01N NaOH. Similarly, solution for base stress was prepared. For oxidation stress, 3% hydrogen peroxide was used and sample was subject to stress at room temperature for 2 hours. For thermal stress, tablet formulation was kept at 60°C for 1 week and for humidity stress tablets were exposed to 75% RH at 60°C for 1 week. Heat and humidity stress samples were appropriately extracted, filtered and diluted as per sample preparation procedure and used for the study. Peak purity of all stressed samples was checked for specificity.

**Robustness**
Robustness was performed for method parameters like flow rate, column oven temperature and concentration of OPA in mobile phase buffer. System suitability parameters was reported for all the conditions.

**Solution Stability Studies**
Solution stability studies were performed at room temperature and at 5°C temperature in mix standard solution for 4 hrs. Further study extended to 24 hours at 5°C; results of the study were analysed against fresh standard.

**RESULT AND DISCUSSION**

**Method Development:**
The stepwise process was adopted for logical and scientific analytical method development. Development efforts undertaken are presented along with the reasoning. Method development was started considering HPLC method as base method. Initial trial condition comprised of 0.1 Ortho Phosphoric Acid (OPA) as Mobile phase A and Acetonitrile as Mobile Phase B with a gradient elution. Various trials with C18 column with length 50mm, 75mm and 100mm were taken with various gradient conditions. With shorter columns of 50 mm and 75 mm known impurities were getting merged in to the tailing of main peak (Figure 2). A reasonable and acceptable separation was achieved with 100 mm column. Hence various gradient trials were taken and the final optimized method parameters are given in Table 1 and system suitability test (SST) parameters are presented in Table 2, optimised chromatographs of OLM, MPS and their impurities is presented in Figure 3.

**Method Validation**
Proposed RP-HPLC method for RS of both the titled drugs was validated for various parameters as described in procedure section. Efforts were also directed towards separation of the stress degraded products of both the analytes. Results for various validation parameters are described as follows.

**Filter Compatibility, Linearity, method sensitivity and precision**
Filter compatibility studies for PVDF Filter and Nylon Filter were studied as described in procedure section. Study data results of PVDF filtered solutions were close to standard assay results as compared to nylon-filtered solution. Therefore, based on the study data PVDF filter was selected for all further validation studies.

Linearity of the proposed method was studied as described in procedure section by spiking impurities in analyte solutions. Chromatographs were acquired using optimised
chromatographic conditions and the data integrated using system software to generate linearity equation values (slope and intercept). One of the indicator of the linearity, coefficient of correlation (r) generated by system software was noted; the values for all the analytes of the current interest were always more than 0.999. Linearity data was used to determine method sensitivity values (LOD and LOQ); relevant precision values; intermediate precision, precision at LOQ and repeatability were determined. All the parameter values are depicted in Table 3.

Accuracy (Recovery)
Accuracy was estimated by recovery studies as described. As presented in Table 3, amount of impurities was spiked at the given recovery level with respect to test the concentration of OLM (1.0 mg/ml) and MPS (1.25 mg/ml). Data indicates that the recovery values and % RSD were always within the limit at all the three levels of accuracy for all the impurities in Table 3.

Forced degradation and Specificity
Results of acid, base, oxidation, heat and humidity stress degradation study on formulation solutions are shown in Table 4. Specificity was performed by checking interference from blank and placebo at the retention of main peak and impurities (Figure 3); no interference was observed during the forced degradation studies (Figure 4 – Figure 8). Peak purity data for all the stress conditions for both the analytes shows that peak purity index values were always less than peak purity threshold. Results of stress degradation shows that the OLM is very sensitive to acid and base stress (Table 4).

Robustness
Robustness was performed for changes in optimised chromatographic method parameters like MP flow rate, column oven temperature and concentration of OPA in mobile phase buffer. Resolution was most important parameters considered for the study. Robustness study data is presented in Table 5 and indicate that the proposed SI/RS-RP-HPLC method is robust and small variation within the experimental limits does not affect the results in Table 3.

Solution Stability Studies
Solution stability studies were performed at room temperature and 5 °C temperature in mix standard solutions. Percent of impurities in solution were determined at each of the time point; results of the study are shown in Table 6.

CONCLUSION
SI/RS-UHPLC-PDA method for estimation of impurities of OLM and MPS in tablet formulation was developed and validated. All system suitability and peak purity parameters of analyte peaks during stressed studies and stability studies were in acceptable range. Linearity of the developed method was near to 1.0 within the specified range. % RSD was found to be less than 2 % for repeatability. % Recovery of all impurities was found to be within 95-105% across all levels with % RSD values always less than 2. The said method can go an LOD level as low as 0.03ppm, which is otherwise 2ppm for the reported methods. This ultimately results in lower linear range of as low as 0.05-7.5 ppm. These results indicate that the developed method is fast, accurate, precise and specific. It can be used in the routine quality control of API manufacturing and formulations. Resolution between actives and impurities was more than 2.5. USP S/N achieved more than 3 for LOD and more than 10 in LOQ preparation. Total run time per sample analysis was 16 minutes, which ultimately reduces the overall analysis time and cost of analysis.

ACKNOWLEDGEMENTS:
The authors are thankful to Spinco Biotech Private Limited, Chennai (India), for providing facility and necessary guidance for the research work.

CONFLICTS OF INTEREST:
The authors declare that there is no conflict of interest.

REFERENCES


List of Tables

Table 1: Optimized chromatographic conditions

8
<table>
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<tr>
<th>Sr. No.</th>
<th>Optimized Chromatographic conditions</th>
<th>Gradient Program (Time and Mobile phase composition)</th>
</tr>
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<td>Parameters</td>
<td>Details</td>
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<tr>
<td>1.</td>
<td>Column</td>
<td>Shimadzu Shimpack GIST-C18 (100mm X 2.1mm, 2 micron)</td>
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<td>2.</td>
<td>Mobile phase A</td>
<td>0.1% Orthophosphoric acid (OPA) in water</td>
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<td>3.</td>
<td>Mobile phase B</td>
<td>Acetonitrile</td>
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<td>Mobile Phase program</td>
<td>Gradient</td>
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<td>Column temperature</td>
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<td>5.</td>
<td>Injection volume</td>
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<td>6.</td>
<td>Flow rate</td>
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**Table 2:** System suitability parameters of OLM, MPS and impurities.

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Name</th>
<th>Ret Time</th>
<th>Area</th>
<th>Area %</th>
<th>Resolution (Rs&gt;1.5)</th>
<th>Tailing (T&lt;2.0)</th>
<th>TP (TP&gt;2000)</th>
<th>K' (k'&gt;2)</th>
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<td>Succinic Acid</td>
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<td>2</td>
<td>Metoprolol</td>
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<td>18008242</td>
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<td>46398767</td>
<td>100.000</td>
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**Table 3:** Linearity, LOD, LOQ, precision and accuracy data of drugs and impurities

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<tr>
<th>Analytes Parameter →</th>
<th>MPS impurity Imp-A</th>
<th>OLM impurities</th>
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<td></td>
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<td>Imp-B</td>
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9
<table>
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<th>Parameter</th>
<th>LOQ (ppm)</th>
<th>LOD (ppm)</th>
<th>Range (ppm)</th>
<th>Slope (b)</th>
<th>Intercept(a)</th>
<th>Correlation Coefficient (r)</th>
<th>% Y Intercept @ 100%</th>
<th>Precision of Repeatability (% RSD)#</th>
<th>Intermediate Precision #</th>
<th>Precision at LOQ (%) RSD#</th>
<th>Recovery at level and recovery limit</th>
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<td>0.05</td>
<td>0.03</td>
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<td>0.65</td>
<td>0.99995</td>
<td>1.45</td>
<td>98.6 ± 1.81, 98.7 ± 1.57, 99.1 ± 1.32, 98.1 ± 1.36, 98.2 ± 1.22, 99.0 ± 0.92, 98.5 ± 1.07, 98.7 ± 1.01, 99.1 ± 1.12, 98.7 ± 1.38, 98.7 ± 1.23, 99.2 ± 0.88, 99.3 ± 1.26, 98.6 ± 0.82, 99.4 ± 0.91</td>
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<tr>
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<td>0.05-7.5</td>
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<td>877.8</td>
<td>0.99976</td>
<td>2.74</td>
<td>0.65</td>
<td>0.99983</td>
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<td>98.6 ± 1.81, 98.7 ± 1.57, 99.1 ± 1.32, 98.1 ± 1.36, 98.2 ± 1.22, 99.0 ± 0.92, 98.5 ± 1.07, 98.7 ± 1.01, 99.1 ± 1.12, 98.7 ± 1.38, 98.7 ± 1.23, 99.2 ± 0.88, 99.3 ± 1.26, 98.6 ± 0.82, 99.4 ± 0.91</td>
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<td>98.6 ± 1.81, 98.7 ± 1.57, 99.1 ± 1.32, 98.1 ± 1.36, 98.2 ± 1.22, 99.0 ± 0.92, 98.5 ± 1.07, 98.7 ± 1.01, 99.1 ± 1.12, 98.7 ± 1.38, 98.7 ± 1.23, 99.2 ± 0.88, 99.3 ± 1.26, 98.6 ± 0.82, 99.4 ± 0.91</td>
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<td>98.6 ± 1.81, 98.7 ± 1.57, 99.1 ± 1.32, 98.1 ± 1.36, 98.2 ± 1.22, 99.0 ± 0.92, 98.5 ± 1.07, 98.7 ± 1.01, 99.1 ± 1.12, 98.7 ± 1.38, 98.7 ± 1.23, 99.2 ± 0.88, 99.3 ± 1.26, 98.6 ± 0.82, 99.4 ± 0.91</td>
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</table>

# Average % RSD for six determinations. € Amount spiked with respect to test concentration of OLM (1.0 mg/ml) and MPS (1.25 mg/ml). OLM= olmesartan medoxomil, MPS= Metoprolol succinate $ Mean ± % RSD for three determinations.

Table 4: Forced degradation study results

<table>
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<tr>
<th>Stress Conditions</th>
<th>Total Degradation (%)</th>
<th>Peak Purity index for OLM</th>
<th>Peak Purity threshold for OLM</th>
<th>Peak Purity index for MET</th>
<th>Peak Purity Results</th>
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<td>As such</td>
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<td>0.999978</td>
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<tr>
<td>Acid Stress</td>
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<td>Base Stress</td>
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Table 5: Robustness data

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<tr>
<th>Parameter Variations → *Analytes ↓</th>
<th>Optimised Chromatographic conditions</th>
<th>Adjacent peaks resolution (Rs) values</th>
<th>Flow rate (ml/min)</th>
<th>Temperature (ºC)</th>
<th>OPA composition</th>
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<tbody>
<tr>
<td>Adjacent peaks resolution (Rs) values</td>
<td>Optimised Chromatographic conditions</td>
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<td>NA</td>
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</tr>
<tr>
<td>MPS Imp A</td>
<td>7.8</td>
<td>7.9</td>
<td>7.6</td>
<td>7.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Time Point and storage conditions</td>
<td>Impurity (Imp) contents (% w/w)</td>
<td>Other unknown total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------------------</td>
<td>---------------------</td>
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</tr>
<tr>
<td></td>
<td>MPS Imp-A</td>
<td>MPS Imp-B</td>
<td>OLM Dimer</td>
<td>OLM Imp-C</td>
<td>OLM Imp-D</td>
</tr>
<tr>
<td>Initial</td>
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<td>0.07</td>
<td>0.35</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>1 hour (RT)</td>
<td>3.65</td>
<td>0.08</td>
<td>0.46</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>2 hour (RT)</td>
<td>4.27</td>
<td>0.09</td>
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<td>0.08</td>
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<td>4 hour (RT)</td>
<td>5.12</td>
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</tr>
<tr>
<td>1 hour (5°C)</td>
<td>3.38</td>
<td>0.07</td>
<td>0.37</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>2 hour (5°C)</td>
<td>3.35</td>
<td>0.08</td>
<td>0.36</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>4 hour (5°C)</td>
<td>3.38</td>
<td>0.08</td>
<td>0.38</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>8 hour (5°C)</td>
<td>3.39</td>
<td>0.07</td>
<td>0.35</td>
<td>0.03</td>
<td>0.08</td>
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<tr>
<td>16 hour (5°C)</td>
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<td>0.36</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>24 hour (5°C)</td>
<td>3.38</td>
<td>0.08</td>
<td>0.35</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>24 hour (5°C)</td>
<td>3.38</td>
<td>0.08</td>
<td>0.35</td>
<td>0.03</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Imp= Impurity, OLM= olmesartan medoxomil, MPS= Metoprolol succinate

**Table 6:** Solution stability data, n=3

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**Figure 1:** Structures of a) Olmesartan, b) Metoprolol succinate, c) MPS Imp A, d) OLM Imp B, e) OLM Imp C, f) OLM Imp D, g) OLM Imp Dimer

**Figure 2:** Trial 1 Chromatogram of impurity spiked standard

**Figure 3:** Optimised chromatogram of impurity spiked standard

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**Figure 5:** Stress degradation chromatographs of OLM and MPS tablet solution - base stress

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