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ORIGINAL ARTICLE

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

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ABSTRACT

Objectives: Olmesartan medoxomil (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 SI/RS-ultra-high performance liquid chromatography-photodiode array (UHPLC-PDA) method for OLM and MPS and their impurities.

Materials and Methods: Well-resolved separation of all analytes was achieved with gradient elution on a Shimadzu on Shimpack GIST-C18 (100 mm x 2.1 mm, 2 µm) 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 peaks.

Results: The proposed SI/RS-UHPLC-PDA method was developed and validated as *per* International Conference on Harmonisation (ICH) of Technical Requirements guidelines. The system suitability test complied by all eluted peaks of the interest with acceptable linearity, recovery, and precision. Specificity, robustness, and method sensitivity parameters were determined; all the parameters were found to be within the limits. All the impurities and stress-degraded peaks were well resolved.

Conclusion: The proposed method was found to be simple, fast, linear, and accurate. Further, the method is precise, robust, and specific; suitable for routine IPQC during active pharmaceutical ingredient 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.

Key words: SI/RS-UHPLC-PDA, related substances, impurities, stability studies, and gradient elution

INTRODUCTION

Olmesartan medoxomil (OLM) is chemically (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole-4-carboxylate (Figure 1a). Metoprolol succinate (MPS) is a 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 was administered once daily. OLM recommended in the dosage range of 10–40 mg to adult patients for treatment of hypertension.[1] MPS a β1-selective adrenoceptor blocking agent preferred in arrhythmia, hypertension, angina pectoris, and myocardial infraction. Extended-release tablets 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. The International Council for Harmonisation (ICH) of Technical Requirements tripartite guidelines specify limits on impurity levels in APIs and their dosage. There is no need for

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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).[3,4] 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 a need of stability indicating/related substance-ultra-high performance liquid chromatography-photodiode array (SI/RS-UHPLC-PDA) method. Literature survey reveals that there are various methods available for estimation of OLM.[5-19] Various methods are available for individual estimation of MPS.[20-24] Various ultraviolet spectrophotometric, thin-layer chromatography (TLC), and HPLC methods are available for estimation of MPS along with other drugs and OLM.[25-37]

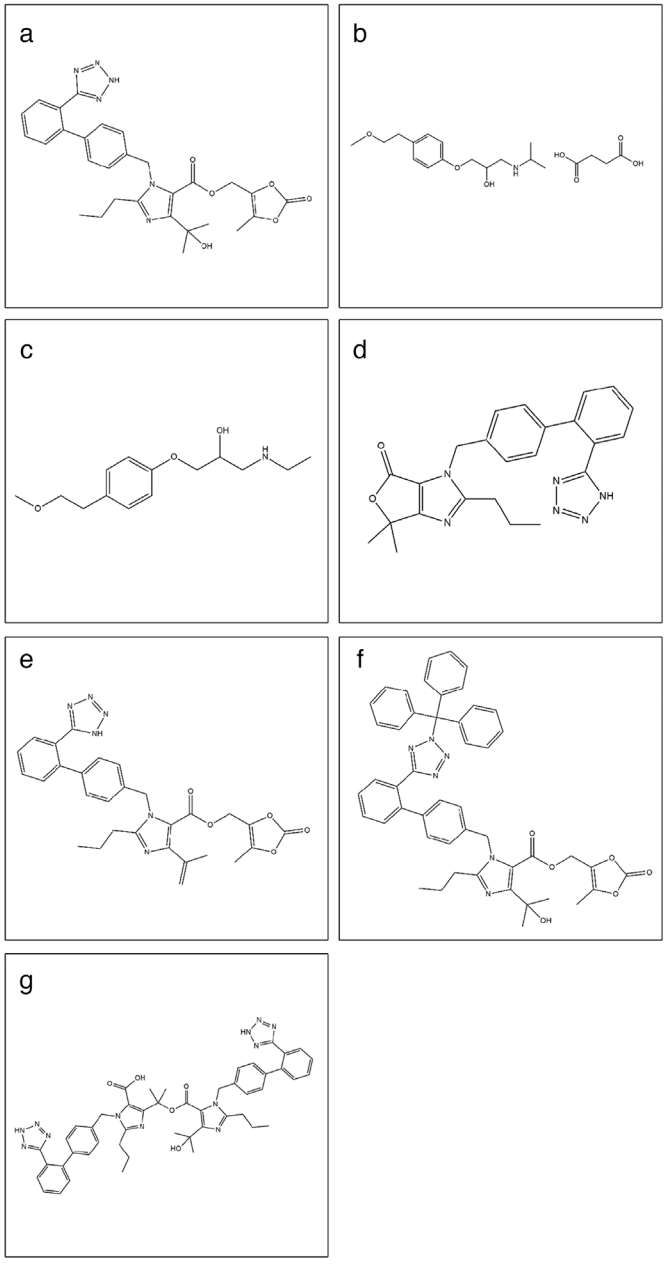

Figure 1. Structures of a) olmesartan, b) metoprolol succinate, c) MPS Imp-A, d) OLM Imp-B, e) OLM Imp-C, f) OLM Imp-D, g) OLM Imp dimer

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate, Imp: Impurity

The 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 analytes from formulations along with impurities and stress degradation products undertaken. Proposed method is a simple, fast quantification and identification method for OLM and MPS along with their impurities/RS. The proposed analytical methods are beneficial in achieving time and other resource efficiency. The method was developed and validated as *per* ICH guidelines.[38]

MATERIALS AND METHODS

Chemicals, reagents, and instrumentation

The drug samples of OLM (assay-99.81%) and MPS (assay-99.77%) were gifted by Cadila Healthcare Ltd., Ahmedabad, India. Impurities were gratis gifted 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 were integrated using Shimadzu LabSolutions software version 6.89. The column used was Shimadzu Shimpack GIST-C18 (100 mm x 2.1 mm, 2 µm) and the injection volume was 5 µL using an autosampler (LC40AD). Mobile phase flow rate was 0.4 mL/min with online degassed. Fixed-dose combination tablets containing 20 mg OLM and 25 mg MPS, manufactured by Glenmark Pharmaceuticals Limited was used.

Standard solution preparation

About 100 mg OLM and 125 mg MPS were transferred into separate 100 mL VFs containing 50 mL of diluent (water: acetonitrile; 50:50% v/v). Analytes dissolved by 5 min sonication and diluent were used to makeup volume to get first standard stock solutions (SSS). 2 mL of these SSS transferred separately into 100 mL VFs; volume made up to get second SSS with the same solvent system. Combined OLM and MPS solution was prepared by transferring 2 mL from each of first SSS of analytes. Further 5 mL of above second SSS was transferred into 100 mL of volumetric flask; volume made up to the mark with same solvent system. 100 µg/mL SSS 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 were 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 was added and analytes dissolved by sonication for 20 min. Volume made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 µm polyvinylidene fluoride (PVDF) syringe filter by discarding the first 3 mL filtrate.

Preparation and treatment of mobile phase

Mobile phase-A contains 1 mL of orthophosphoric 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.

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 were achieved on a 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% 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 a Shimadzu N-Series UHPLC. Method validation was performed for various parameters such as linearity, method sensitivity [limit of detection (LOD) and limit of quantitation (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 conducted 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 relative standard deviation (RSD) % of the peak area, which was always <5% throughout the validation studies. Method validation parameters studied are as follows:

Filter compatibility studies

Filter compatibility studies were performed using a standard solution for 0.45 µ nylon and 0.45 µ PVDF membrane filters. % Assay of filtered standard against the control centrifuged standard was calculated by discarding first 3 mL filtrate.

Linearity method sensitivity and specificity

Linearity assessed visually and by using a lack-of-fit test; interval between the upper and the lower levels of the analyte considered as the method range. Furthermore, method linearity was evaluated from the LOQ level to 150% of specification level. Slope, intercept, correlation coefficient, and Y% intercept bias were calculated. For method specificity, chromatographic peak interference from blank, placebo, and impurities at the retention time of both analyte peaks and stress-degraded products in stressed samples were observed for accepted resolution. Purity of the analyte peaks for each of these conditions was assessed by the peak purity test. To evaluate the 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 was 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 a standard spiking technique. Known amounts of standard impurities were spiked in API and placebo mixture preparation at 50%, 100%, and 150% levels. Accuracy studies were performed in triplicate.

Forced degradation and specificity

Forced degradation study on formulation was conducted in a 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 0.01 N HCl was added and the solutions were subjected to stress at 60°C for 10 min. Stressed sample was neutralized with 2.5 mL of 0.01 N NaOH. Similarly, a solution for base stress was prepared. For oxidation stress, hydrogen peroxide (3%) was used and sample was subjected to stress at room temperature for 2 h. 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 such as flow rate, column oven temperature, and concentration of OPA in mobile phase buffer. System suitability parameters were reported for 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 h 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 the HPLC method as a base method. Initial trial condition comprised 0.1 OPA as mobile phase-A and acetonitrile as mobile phase-B with a gradient elution. Various trials with C18 columns with length 50 mm, 75 mm, and 100 mm were taken under 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. SST parameters are

presented in Table 2, while optimized chromatographs of OLM, MPS, and their impurities are 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 toward the 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 compared to nylon-filtered solution. Therefore, based on the study data PVDF filter was selected for all further validation studies.

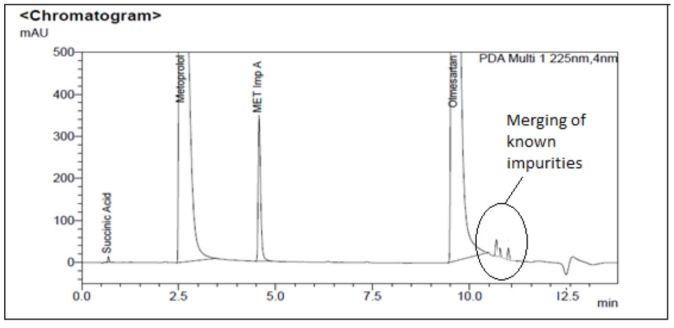

Figure 2. Trial 1 chromatogram of impurity spiked standard

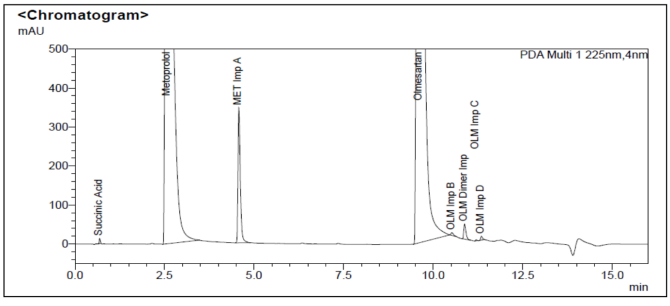

Figure 3. Optimised chromatograph of impurity spiked standards

Linearity of the proposed method was studied as described in procedure section by spiking impurities in analyte solutions. Chromatographs were acquired using optimized chromatographic conditions and the data integrated using system software to generate linearity equation values (slope and intercept). One of the indicators of the linearity, coefficient of correlation (r) generated by system software was noted; the values for the analytes of the current interest were always more than 0.999. Linearity data were 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 indicate that the recovery values and RSD% were always within the limit at the three levels of accuracy for 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 (Figures 4-8). Peak purity data for the stress conditions for both analytes shows that peak purity index values were always less than peak purity threshold. Results of stress degradation display that the OLM is very sensitive to acid and base stress (Table 4).

Robustness

Robustness was performed for changes in optimized chromatographic method parameters such as MP flow rate, column oven temperature, and concentration of OPA in mobile phase buffer. Resolution was the most important parameter considered for the study. Robustness study data are 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.

Table 1. Optimized chromatographic conditions

Sr. no.	Optimized chromatographic conditions		Gradient program (time and mobile phase composition)		
	Parameters	Details	Time (min)	Mobile phase-A	Mobile phase-B
1.	Column	Shimadzu Shimpack GIST-C18 (100 mm x 2.1 mm, 2 µm)	0.01	80	20
2.	Mobile phase-A	0.1% orthophosphoric acid in water	5.0	65	35
3.	Mobile phase-B	Acetonitrile	7.5	65	35
	Mobile phase program	Gradient	10.0	45	55
4.	Column temperature	25°C	12.5	45	55
5.	Injection volume	5 µL	13.5	80	20
6.	Flow rate	0.4 mL/minute	16.0	80	20

Table 2. System suitability parameters of OLM, MPS and impurities

Peak	Name	Retention time	Area	Area%	Resolution (Rs >1.5)	Tailing (T <2.0)	TP (TP >2000)	K' (k' >2)
1	Succinic acid	0.688	26825	0.058	-	1.33	2959	2.44
2	Metoprolol	2.576	18008242	38.812	8.258	1.83	2680	11.88
3	ME Imp-A	4.572	1560580	3.363	7.763	1.57	23552	21.86
4	Olmesartan	9.604	26568380	57.261	23.408	1.79	15281	47.02
5	OLM Imp-B	10.520	32047	0.069	4.181	1.27	109125	51.60
6	OLM dimer Imp	10.872	162154	0.349	2.920	1.55	146291	53.36
7	OLM Imp-C	11.196	7016	0.015	3.438	1.20	358621	54.98
8	OLM Imp-D	11.344	33524	0.072	1.720	1.34	217633	55.72
Total			46398767	100.000	-	-	-	-

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate, Imp: Impurity

Table 3. Linearity, LOD, LOQ, precision, and accuracy data of drugs and impurities

Analytes → Parameter ↓	MPS impurity Imp-A	OLM impurities			
		Imp-B	Dimer impurity	Imp-C	Imp-D
LOQ (ppm)	0.05	0.05	0.05	0.65	0.15
LOD (ppm)	0.03	0.03	0.03	0.25	0.05
Range (ppm)	0.05-7.5	0.05-7.5	0.05-7.5	0.65-7.5	0.15-7.5
Slope (b)	313459.7	6274.8	32228.2	1447.3	6869.4
Intercept (a)	-3182.4	877.8	1083.2	-222.6	-1158.8
Correlation coefficient (r)	0.99993	0.99976	0.99983	0.99977	0.99957
Y% intercept @ 100% level	-0.20	2.74	0.67	-3.17	-3.46
Precision of repeatability (% RSD)[#]	0.65	0.93	1.11	1.98	0.87
Intermediate precision[#] precision (RSD%)[#]	0.99	0.87	1.34	1.45	1.32
Precision at LOQ (RSD%)[#]	1.45	1.83	1.67	2.54	2.11
Recovery at level[€] and recovery limit	**% Accuracy data for impurities (± RSD%)**				
50% (**RSD% ≤5.00%**)[$]	98.6 ± 1.81	98.7 ± 1.57	99.1 ± 1.32	98.1 ± 1.36	98.2 ± 1.22
100% (**RSD% ≤5.00%**)[$]	99.0 ± 0.92	98.5 ± 1.07	98.7 ± 1.01	99.1 ± 1.12	98.7 ± 1.38
150% (**RSD% ≤5.00%**)[$]	98.7 ± 1.23	99.2 ± 0.88	99.3 ± 1.26	98.6 ± 0.82	99.4 ± 0.91

[#]Average RSD% for six determinations, [€]Amount spiked with respect to test concentration of OLM (1.0 mg/mL) and MPS (1.25 mg/mL), [$]Mean ± RSD% for three determinations. OLM: Olmesartan medoxomil, MPS: Metoprolol succinate, LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation

Solution stability studies

Solution stability studies were performed at room temperature and 5°C temperature in mix standard solutions. Percent of impurities in solution was determined at each of the time points; 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 and stability studies were in an acceptable range. Linearity of the developed method was near 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 to an LOD level as low as 0.03 ppm, which is otherwise 2.2 ppm for the reported methods. This ultimately results in a lower linear range of as low as

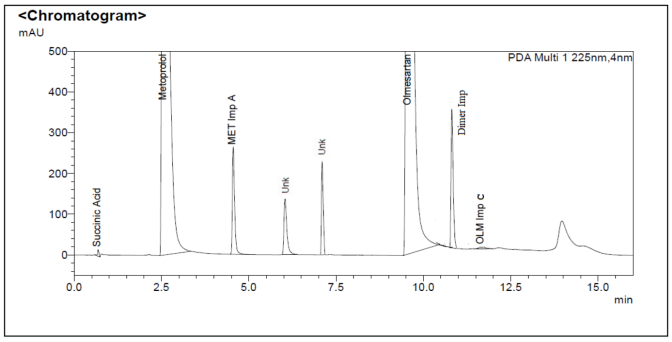

Figure 4. Stress degradation chromatographs of OLM and MPS tablet solution - acid stress

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

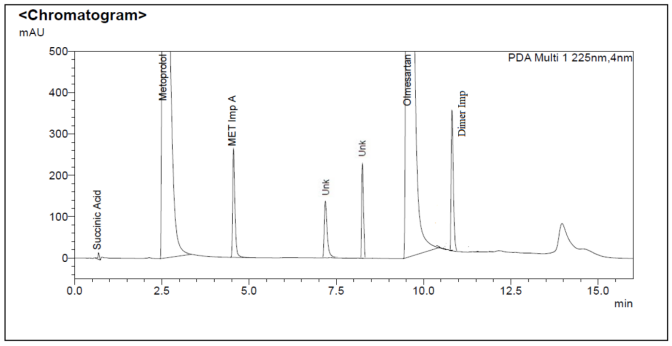

Figure 5. Stress degradation chromatographs of OLM and MPS tablet solution - base stress

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

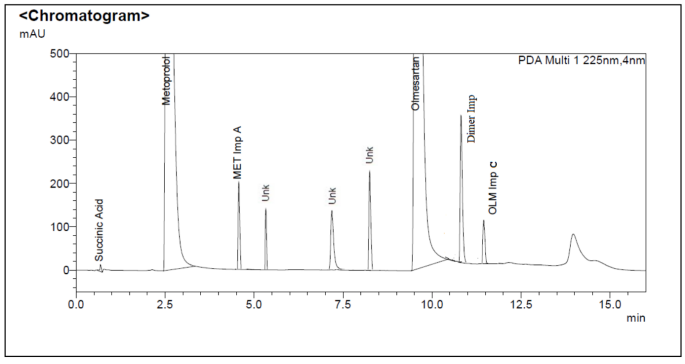

Figure 6. Stress degradation chromatographs of OLM and MPS tablet - oxidative stress

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

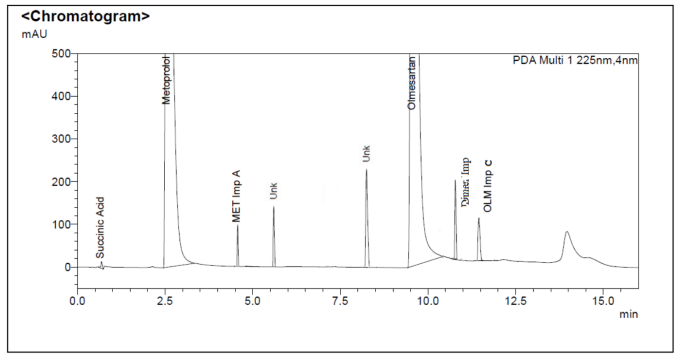

Figure 7. Stress degradation chromatographs of OLM and MPS tablet - heat stress

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

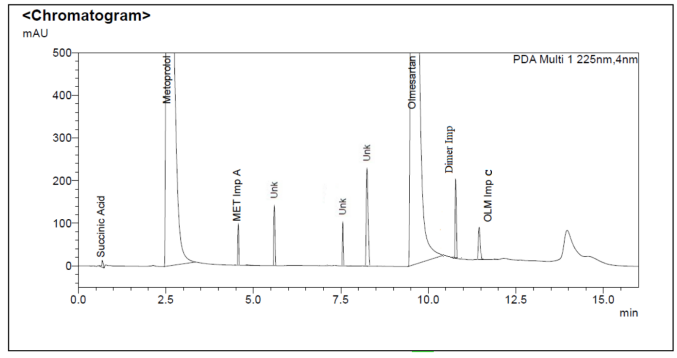

Figure 8. Humidity stress degradation chromatographs of OLM and MPS tablet

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

Table 4. Forced degradation study results

Stress conditions	Total degradation (%)	Peak purity index for OLM	Peak purity threshold for OLM	Peak purity index for MPS	Peak purity threshold for MPS	Peak purity results
As such condition	3.06	0.999991	0.999978	1.000000	0.999987	Pass
Acid stress	13.42	0.999984	0.999910	1.000000	0.999956	Pass
Base stress	17.91	0.999990	0.999938	0.999993	0.999978	Pass
Peroxide stress	10.22	0.999978	0.999937	0.999995	0.999983	Pass
Heat stress	5.11	0.999999	0.999983	0.999998	0.999972	Pass
Humidity stress	6.02	0.999996	0.999902	1.000000	0.999967	Pass

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate

Table 5. Robustness data

Parameter Variations→ *Analytes↓	Adjacent peaks resolution (R_s) values						
	Optimised chromatographic conditions	Flow rate (mL/min)		Temperature (ºC)		OPA composition of MP (% v/v)	
		0.38	0.42	23	27	0.09	0.11
MPS	NA	NA	NA	NA	NA	NA	NA
MPS Imp-A	7.8	7.9	7.6	7.9	7.8	7.8	7.8
OLM	23.4	23.2	23.5	23.3	23.5	23.3	23.5
OLM Imp-B	4.2	4.0	4.1	4.2	4.1	4.2	4.3
OLM dimer Imp	2.9	2.9	2.7	2.8	3.0	2.9	2.9
OLM Imp-C	3.4	3.5	3.2	3.4	3.3	3.1	3.3
OLM Imp-D	1.7	1.7	1.6	1.7	1.7	1.7	1.7

*Imp: Impurity, OLM: Olmesartan medoxomil, MPS: Metoprolol succinate, NA: Not applicable

Table 6. Solution stability data, n: 3

Time point and storage conditions	Imp contents (% w/w)					Other unknown total
	MPS	OLM				
	Imp-A	Imp-B	Dimer	Imp-C	Imp-D	Total (Unk)
Initial	3.37	0.07	0.35	0.02	0.07	0.12
1 hour (R_t)	3.65	0.08	0.46	0.02	0.07	0.19
2 hour (R_t)	4.27	0.09	0.55	0.03	0.08	0.31
4 hour (R_t)	5.12	0.09	0.86	0.02	0.07	0.45
1 hour (5˚C)	3.38	0.07	0.37	0.02	0.08	0.13
2 hour (5˚C)	3.35	0.08	0.36	0.03	0.07	0.14
4 hour (5˚C)	3.38	0.08	0.38	0.02	0.08	0.13
8 hour (5˚C)	3.39	0.07	0.35	0.03	0.08	0.13
16 hour (5˚C)	3.37	0.08	0.36	0.02	0.07	0.14
24 hour (5˚C)	3.38	0.08	0.35	0.03	0.08	0.14
24 hour (5˚C)	3.38	0.08	0.35	0.03	0.08	0.14

OLM: Olmesartan medoxomil, MPS: Metoprolol succinate, R_t: Retention time, Imp: Impurity

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 min, which ultimately reduced the overall analysis time and cost of analysis.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.T., A.D., Design: N.T., G.S., Data Collection or Processing: N.T., Analysis or Interpretation: N.T., V.C., Literature Search: N.T., G.S., V.C., Writing: N.T., V.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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