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Original Article

Spectrophotometric Estimation of Olmesartan Medoxomil and Metoprolol Succinate in Tablet Dosage Form by Zero Order Derivative and Area Under the Curve Method

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ABSTRACT

Background: Olmesartan Medoxomil (OLM) and Metoprolol succinate (MET) formulations treat hypertension. The primary goal was to develop and evaluate a UV-spectrophotometric method for simultaneous estimation of OLM and MET in a combination tablet dosage form by ICH standards. **Aim:** The study aimed to develop a simple, swift, precise, accurate and cost-effective UV spectrophotometric method for the area under curve and zero-order method of OLM and MET in a combined tablet dosage form using distilled water as solvents. **Materials and Methods:** Using distilled water as solvents the approach is predicated on zero order equation. OLM has an absorbance maximum at 255 nm, while MET is at 215.5 nm in the zero-order equation. OLM had an absorbance maximum between 265-250 nm, while MET was 219-205 nm, as determined by the area under curve method. The suggested approach's reliability, precision, specificity, toughness and robustness were verified. Beer's rule was followed by OLM and MET between 2-10 µg/mL for both, respectively, with a correlation coefficient value is 0.999. **Results:** Recovery experiments determined the accuracy of all methods and revealed recoveries between 99-101%. Interday and intraday precision were evaluated and the mean %RSD for all approaches was less than 2%. **Conclusion:** This study demonstrates that all parameters, including precision, recovery, accuracy and robustness, were met by ICH guidelines. The analysis's findings have been scientifically and statistically validated. Therefore, it can be stated that the approach was novel, easy to implement, selective and specific in its ability to estimate both OlM and MET in bulk powder.

Keywords: Area under curve, Distilled water, Metoprolol succinate, Olmesartan medoxomil, Validation, Zero-order spectrophotometry.

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INTRODUCTION

There are multiple options for combining antihypertensive medications. These medication combinations improve hypertension treatment tolerance and reduce toxicity.[1]

Chemically Olmesartan Medoxomil (OLM) is known as 5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl] phenyl}methyl)-1H-imidazole-5-carboxylate.[2] The renin-angiotensin-aldosterone system is an important player in the development of hypertension and angiotensin II type 1 (AT1) receptor antagonists (ARBs) work by blocking the effects of angiotensin II on this system.[3]

Chemically Metoprolol succinate [MET] is known as (RS)-1-(Isopropyl amino)-3-[4-(2-methoxyethyl) phenoxy] propane -2-ol succinate.[4] It is used as an oral β-adrenoceptor antagonist (β-blocker) for the treatment of cardiovascular diseases, especially hypertension. However, MET also inhibits β-2 adrenoreceptors at greater doses, which are mostly found in the bronchial and vascular musculature.[5] Combined formulations of these two drugs are used for the treatment of hypertension. The chemical structure of the MET and OLM is presented in Figure 1.

Chromatographic separation techniques, including HPLC, HPTLC, GC and others, are typically used for the simultaneous evaluation of drug combinations. Although these approaches are accurate, exact and reproducible, they come with a hefty price tag due to the pricey equipment, reagents and expertise needed for analysis. So, it's beneficial to find easier and cheaper ways to estimate many medications at once for routine formulation analysis. Spectrophotometric analysis meets this need when it is possible to estimate the drug combination simultaneously using

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a method that is as effective as chromatographic approaches. An additional perk of the suggested procedures is that, instead of more expensive solvents like methanol and ethanol, the suggested methods make use of distilled water.

A Literature review[6-12] shows that various methods are reported such as spectrophotometric and HPLC for the estimation of MET and OLM individually and simultaneously.

The literature review reveals various methods for determining OLM in pharmaceutical dosage forms and biological fluids, including HPTLC,[13] RP-HPLC,[14] stability-indicating LC[15] and spectrophotometric and HPLC.[16] It also mentions HPTLC[17] and RP-HPLC.[18] The market produces MET and OLM in combination dosage forms for hypertension treatment.

The study's overarching goal was to develop such a procedure and, following validation in conformity with ICH standards and the directions for conducting a safe experiment in a laboratory, how to implement the so that the medicinal composition of tablets can be examined. Distilled water as diluent spectrophotometric approaches have not been found in any literature surveys and neither the AUC method has been reported. By ICH standards, the current study seeks to create a zero-order and AUC spectrophotometric method of OLM and MET in combination tablet dosage form that is easy to use, quick, precise, accurate and economical.[19,20]

MATERIALS AND METHODS

Instruments

SHIMADJU UV-1800 double beam UV-visible spectrophotometer.

Digital weighing balance (AUX220 Shimadzu, Japan).

Ultrasonicator (model U311).

FTIR (FTIR-8400S, Shimadzu, Kyoto, Japan).

Chemical and reagents

The OLM and MET were utilised without additional purification; they were gift samples from Almon Healthcare Pvt. Ltd., Ahmedabad and Macleods Pharmaceutical Ltd., Baddi, respectively and had a 99.99% w/w assay value.

Marketed formulation

Olmezest Beta®25 (Sun Pharmaceutical Industries Ltd.,) contains METO IP 25 mg and OLME IP 20 mg was purchased from an open market. Analytical-grade chemicals and reagents were used in all experiments. In all of our tests, we used water that had been twice distilled.

Preparation of Stock Solution

Dissolving 100 mg of OLM and MET in 100 mL of distilled water yielded the standard stock solution. Make sure to sonicate the medication and adjust the volume to 1000 µg/mL with the same solvent.

Preparation of Working Standard Solution

To prepare 100 µg/mL MET and OLM working standard solutions, transfer 5 mL aliquots to a 50 mL volumetric flask and adjust volume with the same solvent.

Preparation of Calibration Curve of Standard OLM and MET

0.2, 0.4, 0.6, 0.8 and 1 mL volumes were taken from the MET and OLM working standard solution (100 µg/mL) and diluted to the correct concentrations in 10 mL volumetric flasks and the rest of the volume was filled up with diluent. The resulting MET and OLM concentrations are 2, 4, 6, 8 and 10 µg/mL. The samples were then examined with UV spectrophotometer and calibration graphs were created from the resulting data.[21]

FTIR

FTIR spectrum of standard OLM and MET and ranges are shown in Figure 2 and Table 1.

Method 1-Area Under Curve (AUC) method

When there is no strong peak or when a wide spectrum is achieved, the AUC (Area Under Curve) approach can be used.[7,22,23] OLM (10 mg/mL) and MET (10 µg/mL) solutions were made using a diluent (Distilled water) and the solutions were scanned in the ultraviolet spectrum from 200 to 400 nm. The wavelengths 265-250 nm and 219-205 nm were selected from the spectra to represent the OLM and MET, respectively. The AUC technique was used to determine the concentrations of OLM and MET in these two spectral regions; the results are illustrated in Figures 3 and 4. Both medications' concentrations can be calculated using a single calculation by plugging in the integrated absorbance value over the wavelength ranges of both drugs.

Method 2- Zero Order Spectrophotometry

OLM (10 mg/mL) and MET (10 µg/mL) solutions were made using a diluent (Distilled water) and the solutions were scanned in the ultraviolet spectrum from 200 to 400 nm. The wavelength of OLM is 255 nm and for MET is 215.50 nm. The zero-order method is employed to manipulate spectral data in analytical situations where a mixture exhibits interfering absorption and the results are illustrated in Figures 5 and 6. It is also utilised in spectral analysis to characterise any chemical conformation.[24]

Validation Parameters

Quantification, linearity, precision, specificity, accuracy, robustness, LOD and LOQ were all evaluated in the method's testing. The suggested procedure has been validated in accordance with the standards set out by the International Conference on

Harmonisation (ICH).[25-28] Descriptive statistics, including RSD and Mean±Standard deviation, were calculated.

Linearity and Range (Calibration Curve)

Calibration curves were generated using Area under curve and Zero order method for a concentration between 2-10 µg/mL of OLM and MET, respectively, as discussed in Figures 3 and 5. The calibration curves were made and the regression equations were found. As shown in Table 2, linear regression models with correlation coefficients (r^2) have been established.

Method precision (Repeatability)

Precision is the degree to which, when subjected to identical analytical conditions, many measurements provide results that are statistically similar to one another. The accuracy of the instrument was evaluated by scanning the solution repeatedly (n=3) and measuring its absorbance (n=3) for OLM (5 µg/mL) and MET (5 µg/mL), without changing any of the zero-order spectrophotometry parameters as shown in Table 2.

Intermediate precision (Reproducibility)

To find the intraday and interday variations, we compared three separate OLM and MET solutions on the same day and three separate days during the week and fluctuations were determined by a precision investigation elaborated in Table 2.

Accuracy (Recovery study)

OLM and MET recovery were calculated using the standard addition method to test process precision. Sample solutions of OLM (5 µg/mL) and MET (5 µg/mL) were supplemented with standard solutions at 50%, 100% and 150% concentrations (Table 3).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The results of the linearity tests were used to determine the LOD and LOQ illustrated in Table 2. Using the supplied formulae.[22]

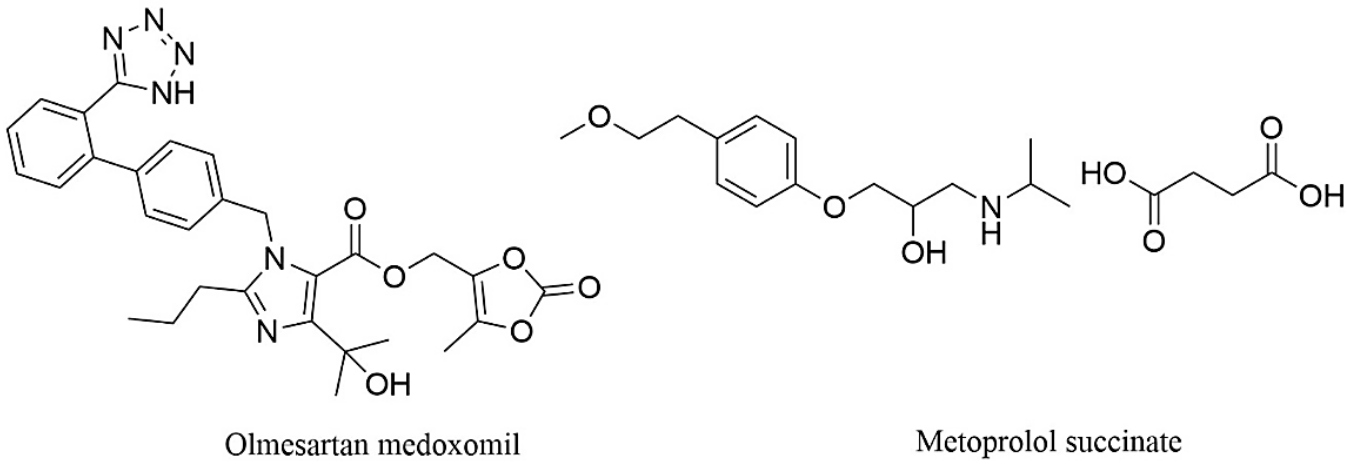

Figure 1: Structure of OLM and MET.

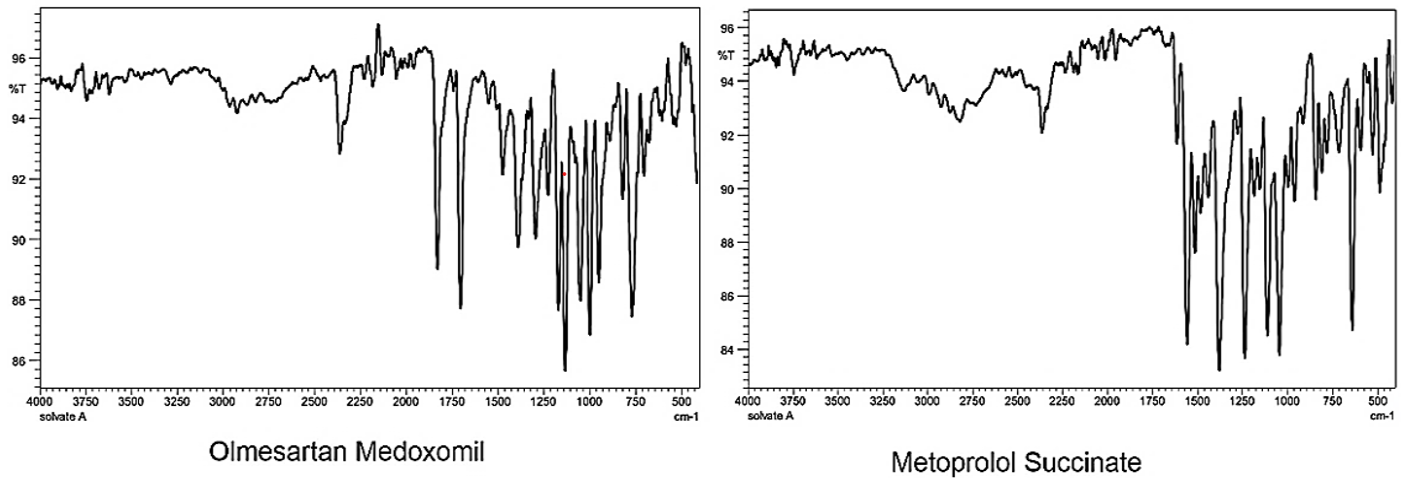

Figure 2: FTIR spectra of Olmesartan Medoxomil and Metoprolol Succinate.

Table 1: **FTIR spectrum values of OLM and MET.**

Sl. No.	Stretching (str)/ Bending (bend) vibration	Obtained value (cm^{-1})	Obtained value (cm^{-1})
		OLM	MET
1.	OH str	3501.11	3267.42
2.	C-H str	2851.33	3083.60
3.	C=O str	1724.20	1724.20
4.	C=C str	1590.25	1593.10
5.	N-H str N-H bend	3499.69 1590.25	3469.76
6.	C-O str	1192.69	1132.84
7.	C-N str	-	1020.26
8.	C-H bend	-	1410.71

Table 2: **Summary of validation parameters.**

Parameters	Zero Order Derivative (Method-1)		Area Under Curve (AUC) (Method-11)	
	OLM	MET	OLM	MET
Linearity range (µg/mL)	2-10	2-10	2-10	2-10
Regression equation	Y=0.0235x+0.0021	Y=0.023x+0.018	Y=0.0484x+0.0108	Y=0.0177x+0.0029
Regression coefficient (r^2)	0.99	0.99	0.99	0.99
Precision (%RSD) Repeatability	0.47	0.44	0.71	1.36
Intra-day (*n*=3) (%RSD)	1.13	1.13	1.33	0.48
Inter-day (days=3) (%RSD)	1.12	1.13	1.33	0.50
Robustness Analyst to Analyst (%RSD)	0.371-0.742	0.136-0.525	0.184-0.392	0.259-0.479
Robustness Lab to Lab (%RSD)	0.255-0.508	0.397-0.523	0.252-0.548	0.162-0.542
LOD (µg/mL)	1.27	1.89	0.67	0.71
LOQ (µg/mL)	3.86	5.74	2.03	2.16

µ: micro, RSD: Relative Standard Deviation.

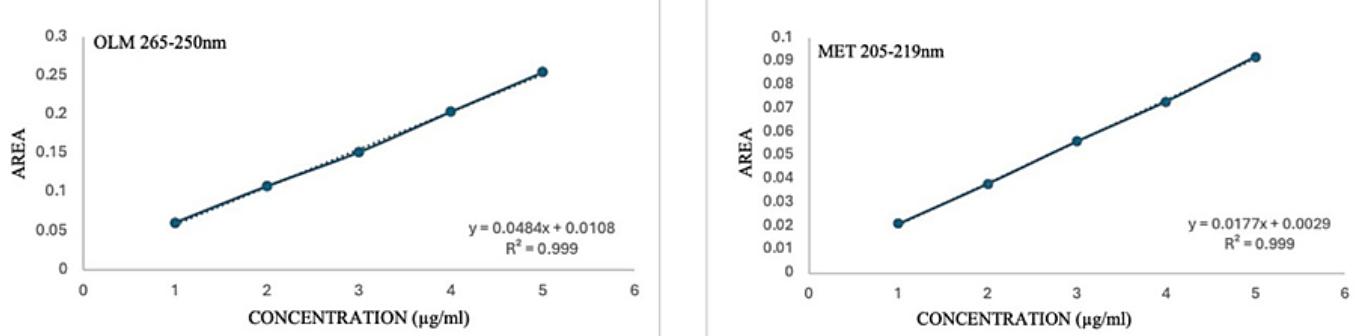

Figure 3: Calibration graph of OLM and MET by Area Under Curve.

Robustness

According to ICH, robustness is a gauge of a method's capacity to hold up against minor but deliberate changes in its input parameters and results are discussed in Table 2. Instrument, laboratory and observer swaps were used to test the proposed method's stability.[21]

Analysis of OLM and MET in combined marketed formulation

Twenty Olmezest Beta®25 tablets containing 25 mg MET and 20 mg OLM were carefully weighed to determine the average. The 10 mg OLM and 10 mg MET tablet powder was weighed and placed in a 100 mL volumetric flask. Add a minimum of 100 µg/mL diluent to dissolve the substance and fill the volume (OLM and MET). The mixture was centrifuged at 100 rpm and filtered with Whatman filter paper after 15 min of sonication. To obtain 4 µg/mL of OLM and MET, 1 mL of the clear solution was transferred to a 25 mL volumetric flask and filled with d/w. Six formulation absorbance measurements were taken at 219-205 and 265-250 nm. The amount of MET and OLM was calculated by using an area under the curve method, results are discussed in Table 4.

RESULTS

Using the area under the curve technique and zero-order method, we were able to produce calibration curves and calculate robustness, LOD, LOQ and regression equations (Figure 2). Tables 2 and 3 summarize the results of the accuracy, precision and inter and intra-day data. As shown in Table 4, the marketed product was evaluated for its labelled claim using the proposed assay methods.

DISCUSSION

While most active chemicals absorb in the UV region, spectrophotometric techniques are the simplest, fastest and most widely applicable to all laboratories. Two approaches, the area

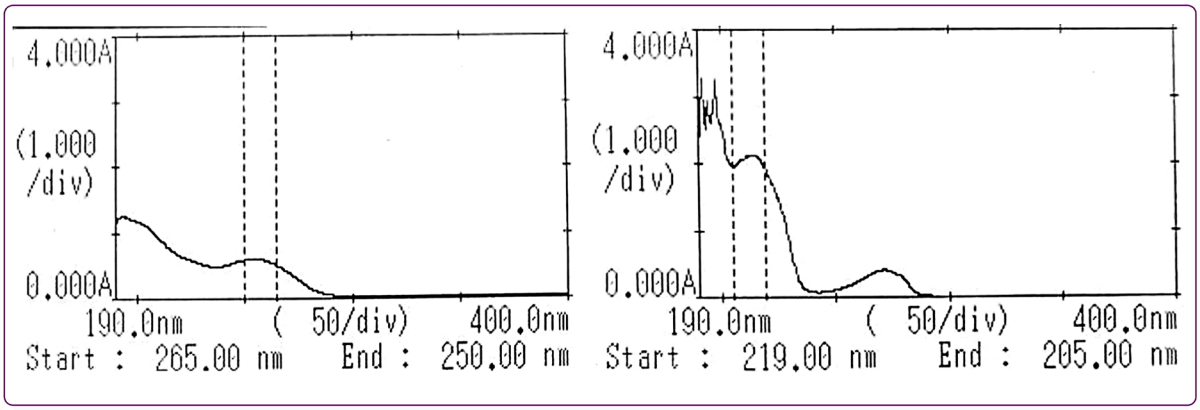

Figure 4: UV Area under curve spectrum of OLM and MET.

Table 3: Accuracy data of determination of Olmesartan and Metoprolol using zero-order Spectroscopy and area under curve method.

Method	Drug	Percentage (%)	Drug taken (Formulation) (µg/mL)	Drug added (Pure Drug) (µg/mL)	Total amount found* (µg/mL)	%Mean recovery ±S.D (*n*=3)
1	OLM	50	5	2.5	7.47	99.66±0.80
		100	5	5	10.04	100.38±0.56
		150	5	7.5	12.47	99.79±0.36
	MET	50	5	2.5	7.45	99.29±0.60
		100	5	5	9.95	99.47±0.20
		150	5	7.5	12.44	99.58±0.15
2	OLM	50	5	2.5	7.47	99.62±0.43
		100	5	5	10.04	100.42±0.57
		150	5	7.5	12.47	99.77±0.57
	MET	50	5	2.5	7.44	99.22±0.57
		100	5	5	10.08	100.88±0.80
		150	5	7.5	12.44	99.53±0.57

*Mean of three observations.

under the curve and zero-order method, have been devised to evaluate OLM and MET in combination tablet dose form.

AUC and zero-order methods

AUC technique was used to measure standard solution absorbance to determine linearity. OLM and MET were dissolved in distilled water for this sample solution. Calibration curves were created by plotting concentration vs. absorbance for both drugs at 265-250 nm for OLM and 219-205 nm for MET and for zero-order method, at 255 nm (OLM) and 215.50 nm (MET), as shown in Figures 2-4.

Table 2 summarises the analytical findings for synthetic mixtures. Beer's law conformity is shown by the linearity of the calibration curves and a correlation coefficient of 0.99 for OLM and 0.99 for MET. Validation of the suggested procedures was performed by ICH recommendations. Results shown in Table 2, LOD was found to be 0.67 µg/mL and 0.7 µg/mL for OLM and MET respectively and LOQ was found to be 2.03 µg/mL and 2.16 µg/mL for OLM and MET, respectively for AUC. For zero order, LOD was found to be 1.27 and 1.89 for OLM and MET respectively and LOQ was found to be 3.86 and 5.74 for OLM and MET respectively. The accuracy and precision of the data were tabulated in Table 2. Both intra-day and inter-day %RSD were found to be 1.13, 1.33, 1.13, 48 and 1.12, 1.33, 1.13 and 0.50 for OLM and MET. Recovery experiments at 50%, 100% and 150% were used to determine the method's accuracy; Table 4 shows that a low %RSD result indicates the method's accuracy. For quantification of OLM and MET, the % purity of tablets was 99.33±0.21 to 99.93±0.57, respectively. OLM and MET concentrations in tablet dosage forms were successfully measured using this method. The results agreed well with their labelled counterparts, as illustrated in Table 4.

Table 4: Analysis of marketed formulation.

Method	Drug	Labelled amount (mg)	Amount found* (mg)	%Labelled claim* (±S.D.) (*n*=6)	%RSD
1	Olmesartan	25	24.92	99.66±0.43	0.44
	Metoprolol	20	19.92	99.86±0.36	1.40
2	Olmesartan	25	24.96	99.93±0.57	1.27
	Metoprolol	20	19.73	99.33±0.21	1.34

*Mean value of six determinations.

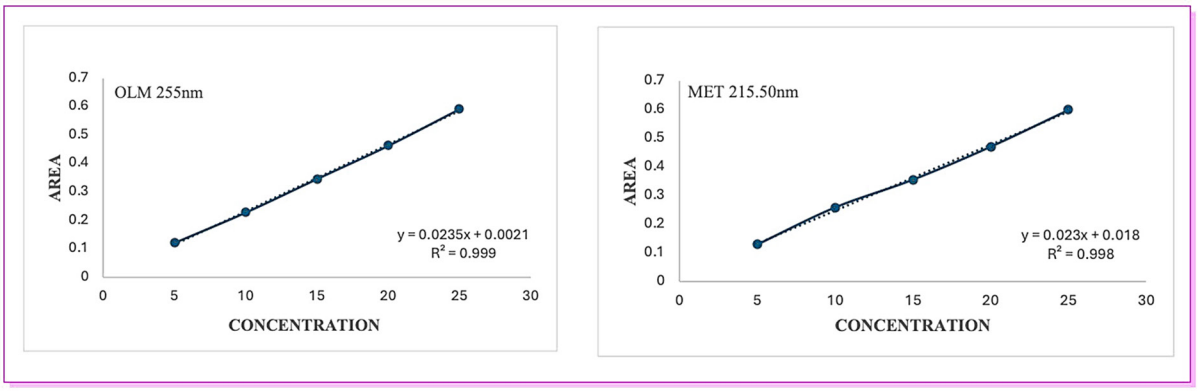

Figure 5: Calibration graph of OLM and MET by Zero order.

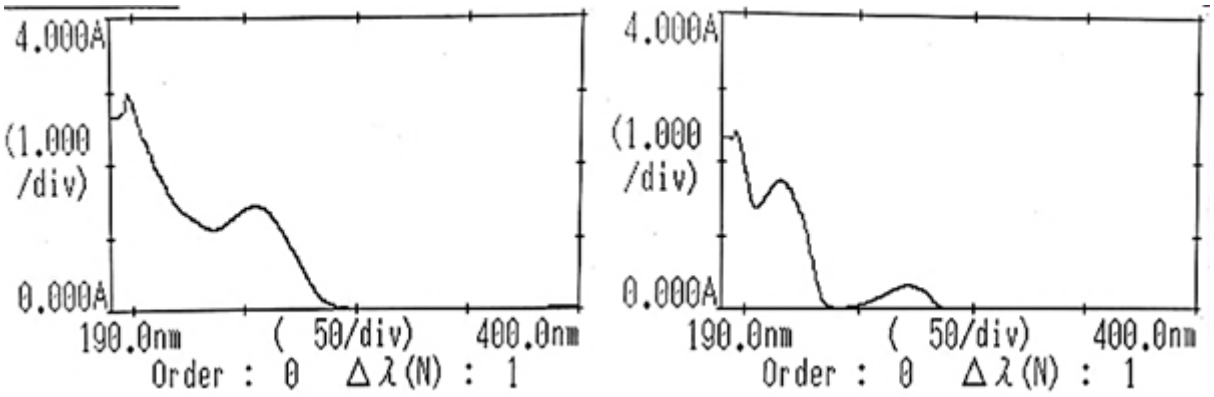

Figure 6: Zero Order spectra of OLM and MET.

CONCLUSION

The results of the experiments indicate that the suggested spectrophotometric methods such as zero-order estimation and area under the curve for OLM and MET were simple, robust, accurate and precise by ICH guidelines and, can be used for the bulk drug and pharmaceutical dosage formulation assay. This formulation quantification approach is validated. In pharmaceutical tablet dosage forms, the suggested method can be used for routine simultaneous assessment of MET and OLM because the excipients did not appear to interact with the absorbance of interest. The sample recoveries in all formulations matched their label claims, facilitating routine evaluation of pure drug or pharmaceutical formulations. The technique for analyzing MET and OLM makes use of inexpensive and readily available solvents, such as distilled water. For future routine quality control of OLM and MET combination dose formulations, the proposed methods were thus found to be both cost-effective and appropriate.

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ABBREVIATIONS

ICH: International Council for Harmonization**; UV-vis:** Ultraviolet-visible; **RP-HPLC:** Reverse Phase High-Performance Liquid Chromatography; **FTIR:** Fourier Transform Infrared Spectroscopy; **OLM:** Olmesartan Medoxomil; **MET:** Metoprolol Succinate; **IP:** Indian Pharmacopoeia; **LOD:** Limit od Detection; **LOQ:** Limit of Quantification; **AUC:** Area Under Curve.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This work developed and validated a UV-vis spectrophotometer by estimating OLM and MET using AUC and the Zero Order Method. The study's findings informed the choice of distilled water as the solvent and the analysis wavelengths: 255 nm for OLM and 215.5 nm for MET in the AUC technique and 256-250 for OLM and 219-205 for MET in the Zero order approach. Aspects of validity include linearity, precision, accuracy, LOQ, LOD and robustness as per ICH Q2 (R^1) standards. At concentrations ranging from 2 to 10 µg/mL, the Area Under the Curve (AUC) was linear for both OLM and MET (R^2=0.999) and zero-order was similarly linear for OLM (R^2=0.999) and MET (R^2=0.998). During the accuracy testing, the drug's recovery rate was determined to be between 99.22% and 100.88%. As the percentage of RSD was less than 2%, it was determined that the precision and robustness parameter values fell within the necessary range.

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