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DEVELOPMENT AND VALIDATION OF A NOVEL METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL AND OLMESARTAN USING SECOND-ORDER UV DERIVATIVE SPECTROSCOPY

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ABSTRACT

A simple and economical UV 2nd derivative spectroscopic method has been developed for the simultaneous analysis of metoprolol succinate and olmesartan medoxamil. The chosen wavelengths for quantification were 224 nm for metoprolol succinate and 272 nm for olmesartan medoxamil, with water as diluent. The Beer-Lambert's range was found to be 1-80 µg/mL at 224 nm for metoprolol succinate and 1-60 µg/mL at 272nm for olmesartan medoxamil with a correlation coefficient of ≥ 0.990. The guidelines as set by the International Conference on Harmonization were followed for validation of the developed method, and the same were satisfied for all parameters. The method was applied for the analysis of fixed-dose combination, and the percentage assay results were within acceptable limits. The new method can thus be successfully employed for the regular analysis of metoprolol succinate and olmesartan medoxamil in bulk and combination.

Keywords: Second Derivative Method, Olmesartan, Metoprolol, ICH Guidelines.

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INTRODUCTION

Ultraviolet derivative spectrophotometry implies the transformation of a normal spectrum to a derivative spectrum using instrument software. Derivative methods offer advantages of enhanced resolution accompanied by discrimination in bandwidth, allowing the judicious determination of absorbing species in admixture, especially when simple spectrophotometric methods fail. In case of second derivative spectrophotometric methods, narrow spectral bands of benzenoid drugs are preferentially discriminated, while broad absorption bands of excipients are eliminated. Further, amplitudes of derivative spectrum correlate to the concentration of absorbing species (Beer's Law being followed by the fundamental absorption spectrum).[1,2] A fixed dose combination of metoprolol and olmesartan is used in the treatment of angina and hypertension.[3] Literature survey proved that UV spectroscopic methods,[4-6] like simultaneous equations method, absorption ratio method, dual-wavelength method, and more advanced and costly chromatographic methods,[7-10] like reverse phase HPLC and UHPLC, have been described for quantification of metoprolol succinate and olmesartan medoxamil either alone or in combination. However, to date, no second derivative method has been described for the estimation of metoprolol succinate and olmesartan medoxamil combination. As multi-component formulations have flooded the market today, instrumental methods are required for analyzing drugs in combination. Analytical methods like UV spectroscopy are rapid and offer cost benefits. Hence, it would be beneficial to develop an economical UV spectroscopic method for analyzing metoprolol succinate and olmesartan medoxamil in combination based on the second derivative method.

EXPERIMENTAL

Materials and Methods

Metoprolol succinate was procured from Indoco Remedies Limited, Verna Industrial Estate, and Olmesartan medoxamil was procured from Cipla, Pharm R&D, Vikhroli. The synthetic mixture was prepared according to the dose of the marketed pharmaceutical formulation 'OLMESAR-M 50' tablet,

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containing 0.050 g of metoprolol and 0.020 g of olmesartan, respectively. The instrument used for the analysis was a Shimadzu (1800 double beam UV spectrophotometer) with UV Probe software.

Preparation of Stock Solution (1000 µg/mL)
About 25 mg of metoprolol and olmesartan were dissolved with methanol (25 mL) to yield a concentration of 1000 µg/mL.

Preparation of Diluted Stock Solution (100 µg/mL)
Distilled water was used to dilute 10ml of the stock to 100 mL.

Detection Method
Choice of Analytical Wavelength
Standard solutions of metoprolol and olmesartan were subjected to scanning in the ultraviolet region of 200 nm to 400 nm against blank, and the wavelength maxima of both the drugs were recorded. Spectral conversion to the second derivative was employed using instrument software. The two analytical wavelengths selected for analysis were as follows:

- λ_1 –corresponding to λ_{max} of 1st drug
- λ_2 –corresponding to λ_{max} of 2nd drug

Method Validation
Linearity and Range
The linearity for metoprolol and olmesartan was determined for concentration levels ranging from 1-100 µg/ml for each drug. Solutions were subjected to scanning in the ultraviolet region of 200 nm to 400 nm against blank, and the obtained spectra were converted to second derivative using the instrument software. The amplitude for each spectrum obtained was recorded at selected predetermined wavelengths, and a graph of amplitude v/s concentration was plotted to determine the linear regression equation and correlation coefficient (r^2) for each drug.
Acceptance criteria: Correlation coefficient (r^2) $\geq$ 0.990

Precision
Repeatability
Repeatability for metoprolol and olmesartan was determined by performing an assay of a synthetic mixture in six replicates. The % RSD was calculated for each drug.
Acceptance criteria: % RSD $\leq$ 2

Intra-Day Precision
For determining intra-day precision, the assay of the synthetic mixture was performed in triplicate at three intervals on a day. % RSD was calculated for both drugs.
Acceptance criteria: % RSD $\leq$ 2

Inter-Day Precision
For determining inter-day precision, assay of a synthetic mixture in triplicate was performed on three consecutive days. % RSD was calculated for both drugs.
Acceptance criteria: % RSD $\leq$ 2

Accuracy
The accuracy in terms of % recovery was performed at three concentration levels, i.e., 80%, 100%, and 120%, in triplicate. About 25mg of metoprolol and olmesartan were weighed separately in 25ml volumetric flask, and methanol was added till it dissolved. Further, the volume was made up with methanol. Later, nine flasks of 25 mL were taken and divided into 3 groups marked 80%, 100%, and 120%. To all nine flasks, 25mg of placebo was added. Then, to 80% marked flasks, 0.4 mL of olmesartan solution and 1 mL of metoprolol solution were added. To 100% marked flasks, 0.5 ml of olmesartan solution and 1.25 ml of metoprolol solution were added. To 120% marked flasks, 0.6 mL of olmesartan solution and 1.5 mL of metoprolol solution were added. To all the above 9 flasks, sonication for 5 minutes was performed after adding 15 ml of methanol. Dilution to 25 mL was done using methanol, the solution was mixed, and filtered

using Whatman filter paper. Further, 2.5 mL from each flask was diluted to 10ml separately. Resultant solutions were subjected to scanning in the ultraviolet region against a blank. The spectra were converted to second derivatives using instrument software. The amplitude for each spectrum was recorded at predetermined wavelengths, and the percent recovery was calculated.
Acceptance criteria: % Recovery 95-105%

Limit of Detection and Limit of Quantification
These parameters were calculated using the standard deviation response and the slope of the calibration curves of drugs at their respective wavelengths.

Robustness
Robustness studies were performed by introducing minor changes in the methodology adopted in the assay of the synthetic mixture, i.e., change of UV spectrophotometer, analyst, and extraction technique.
Assay limits: Between 90-110 %.

Assay of a Synthetic Mixture of the Two Drugs
Powder mixture equivalent to 25mg of olmesartan was weighed into 25ml flask, and to this, 15 mL of methanol was added and the solution sonicated for 5 minutes. The solution was diluted to 25 mL with methanol. On filtration initial drops were discarded. From this, 0.5 mL of the solution was subjected to dilution (10ml with distilled water). The solutions in triplicate were scanned in the ultraviolet using a blank. The spectra were transformed to the second derivative using the software. The amplitude for each spectrum was recorded at selected wavelengths. The percent assay was calculated from the regression equation.

RESULTS AND DISCUSSION

Metoprolol succinate and olmesartan medoxamil are soluble in methanol. In the developed method, an attempt was made to minimize the use of methanol as a solvent to bring down the cost of analysis. Water was chosen as a diluent for the whole analysis. The overlain D^0 and D^2 spectra of metoprolol succinate and olmesartan medoxamil are displayed in Fig.-1 and Fig.-2, respectively.

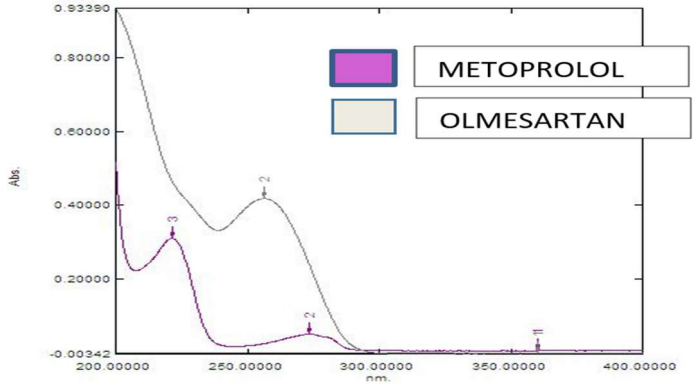

Fig.-1: Overlain Spectra of Metoprolol and Olmesartan

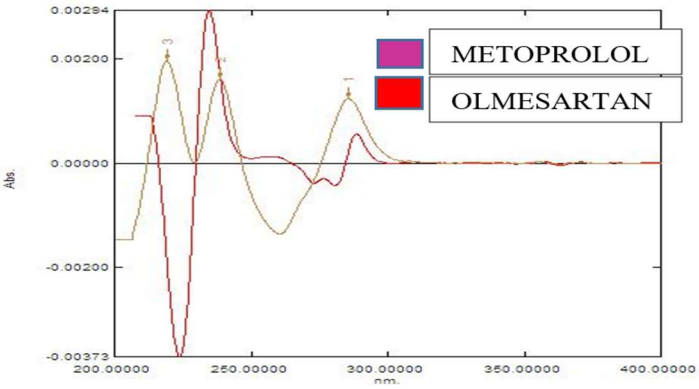

Fig.-2: Overlain Second Derivative Spectra of Metoprolol and Olmesartan

The two wavelengths selected for analysis of metoprolol and olmesartan by the second derivative method are:

- λ_1 – 224 nm (wavelength corresponding to λ_{max} of metoprolol)
- λ_2 – 272 nm (wavelength corresponding to λ_{max} of olmesartan)

The linearity data for metoprolol and olmesartan are given in Table-1.

Table-1: Linearity Data of Metoprolol and Olmesartan

Parameters	Metoprolol	Olmesartan
	At 224 nm	At 272 nm
Linearity range	1- 60 µg/ml	
Regression equation	y = 38.598x + 1.8801	y = 11.109x + 0.5253
Slope	38.598	11.109
Intercept	1.8801	0.5253
Correlation coefficient (r^2)	0.9998	0.9998

The overlain second derivative linearity spectra of metoprolol and olmesartan are depicted in Fig.-3 and Fig.-4, respectively.

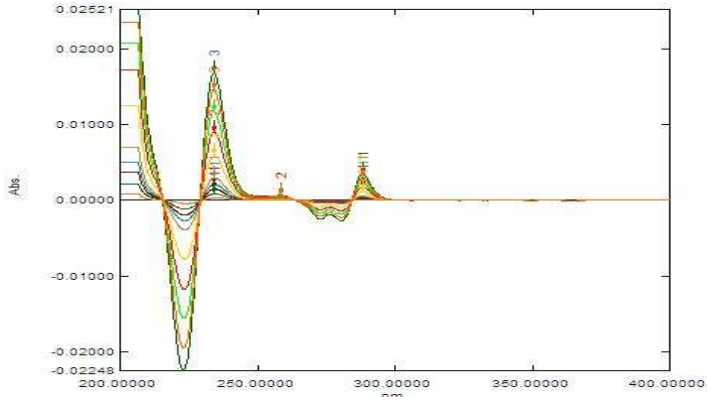

Fig.-3: Overlain Second Derivative Linearity Spectra of Metoprolol at 224 nm

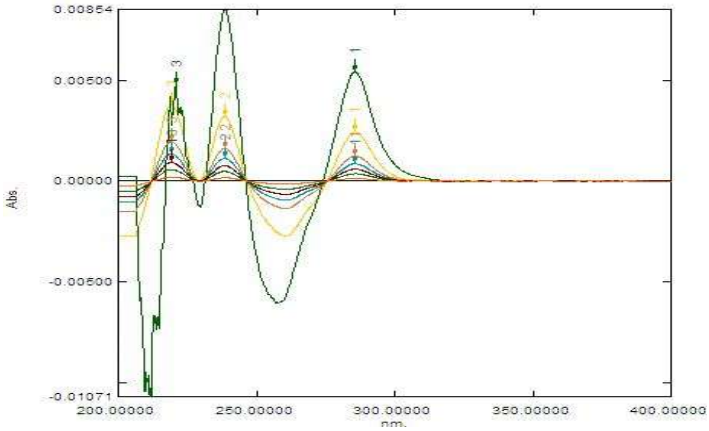

Fig.-4: Overlain Second Derivative Linearity Spectra of Olmesartan at 272 nm

Beer Lambert's plots for the drugs are depicted in Fig.-5 and Fig.-6. Beer Lambert's range was established from 1-60 µg/mL for metoprolol and olmesartan at 224 nm and 272 nm, respectively. The calibration curves constructed for metoprolol and olmesartan were found to be linear with $r^2 > 0.999$ for both drugs. The average absorptivity for metoprolol was 39.09 L/g cm^{-1} at 224 nm and for olmesartan was 11.26 L/g cm^{-1} at 272 nm. The results for precision studies are depicted in Table-2. The % RSD was within acceptable limits of less than 2 %. Hence, the developed method was found to be precise.

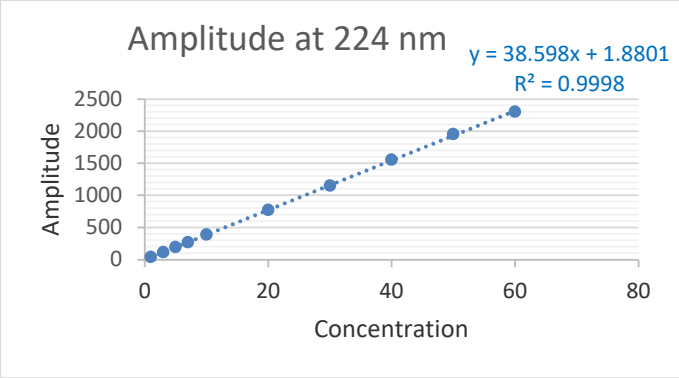

Fig.-5: Calibration Curve of Metoprolol at 224 nm

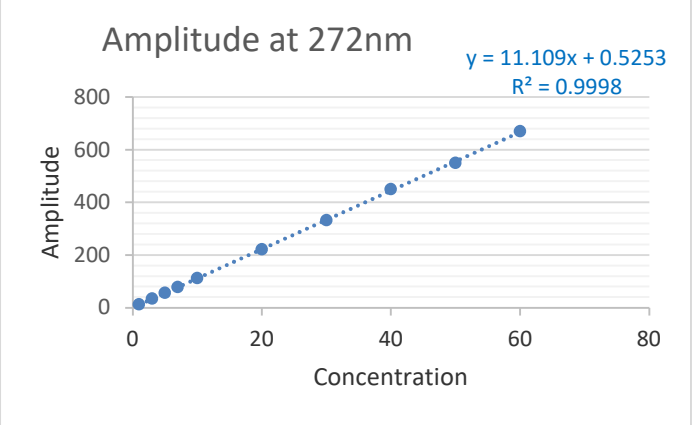

Fig.-6: Calibration Curve of Olmesartan at 272 nm

Table-2: Precision Data of Metoprolol and Olmesartan

Drug	Conc. (µg/ml)	Mean % purity	% RSD
	Repeatability (n=6)		
Metoprolol	12.5	99.933	0.439
Olmesartan	5	101.6	1.58
	Intra-day (n=3)		
Metoprolol	12.5	99.697	0.24
Olmesartan	5	99.933	0.84
	Inter-day (n=3)		
Metoprolol	12.5	99.662	0.213
Olmesartan	5	100.91	1.33

Results of accuracy are depicted in Table-3. As can be seen, the % recovery is within acceptance limits, indicating the method is accurate.

Table-3: Accuracy Data of Metoprolol and Olmesartan

Concentration level (%)	Quantity of drug added (µg/mL)		Quantity of drug recovered ((µg/mL)		% Recovery		Mean % recovery	
	MET	OLM	MET	OLM	MET	OLM	MET	OLM
80	10.0	4	10.00	3.91	100	97.75	100.00	97.00
	10.0	4	10.00	3.82	100	95.50		
	10.0	4	10.00	3.91	100	97.75		
100	12.5	5	12.48	4.99	99.84	99.80	100.07	99.80
	12.5	5	12.51	4.99	100.08	99.80		

	12.5	5	12.53	4.99	100.31	99.80		
120	15.0	6	14.50	6.16	96.67	102.76	97.02	102.76
	15.0	6	14.58	6.16	97.2	102.76		
	15.0	6	14.58	6.16	97.2	102.76		

Results for LOD and LOQ, as displayed in Table 4, proved the method was sensitive.

Table-4: LOD and LOQ Data

Parameters	Metoprolol	Olmesartan
	At 224 nm	At 272 nm
LOD (g/L)	0.129	0.39
LOQ (µg/mL)	0.041	0.135

Results of robustness studies of a developed method performed by introducing deliberate changes in the methodology are outlined in Table-5. As observed, minor changes in the methodology during the analysis did not significantly affect the analysis results. Hence, the developed second derivative method was robust.

Table-5: Robustness Data

Parameters	Drugs	Mean % assay	% RSD
Instrument 1	Metoprolol	99.65	0.20
	Olmesartan	99.53	0.46
Instrument 2	Metoprolol	100.38	0.12
	Olmesartan	96.31	0.0010
Analyst 1	Metoprolol	100.42	0.32
	Olmesartan	100.73	1.60
Analyst 2	Metoprolol	100.52	0.20
	Olmesartan	96.31	0.001
Sonication time 1	Metoprolol	99.01	0.20
	Olmesartan	99.53	0.46
Sonication time 2	Metoprolol	100.72	0.20
	Olmesartan	100.00	0.0057

Percent assay values are depicted in Table-6. The results of the % assay of the synthetic blend for metoprolol were 99.933, and for olmesartan, it was 101.6, which met the acceptance limits (90-110 %).

Table-6: % Assay of Drugs

Drug	Mean % purity (n=6)	% RSD
Metoprolol	99.933	0.439
Olmesartan	101.6	1.58

CONCLUSION

A novel and economical UV method has been developed for the simultaneous analysis of metoprolol succinate and olmesartan medoxamil based on second derivative UV spectroscopy. The developed method minimized the use of methanol as a solvent. The wavelengths chosen for the estimation of drugs were 224 nm and 272 nm. Method validation was done as per ICH guidelines. The Beer Lambert's range for both drugs was found to be 1-60 µg/mL. The method was accurate, precise, sensitive, and robust. Hence, the developed method is a cost-effective alternative for the simultaneous analysis of the drugs using UV spectroscopy.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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