

RATIO DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND ATORVASTATIN CALCIUM IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

Simple, accurate, precise and sensitive spectrophotometric method for simultaneous estimation of Olmesartan medoxomil and Atorvastatin calcium in their combined tablet dosage form has been developed and validated. The ratio derivative spectrophotometry method involves measurement of first derivative amplitude of ratio spectra at 223 nm for Olmesartan medoxomil and 313 nm for Atorvastatin calcium as two wavelengths for estimation. Linearity is observed in the concentration range of 8-32 µg/ml and 4-16 µg/ml for Olmesartan medoxomil and Atorvastatin calcium respectively. The accuracy and precision were determined and found to comply with ICH guidelines. The method was found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Olmesartan medoxomil and Atorvastatin calcium in their combined tablet dosage form.

Keywords: Olmesartan medoxomil, Atorvastatin calcium, Ratio derivative spectrophotometry.

INTRODUCTION

Olmesartan medoxomil (OLM) is a prodrug and hydrolysed to Olmesartan during absorption from the gastrointestinal tract¹. OLM is a selective AT1 subtype angiotensin II receptor antagonist. OLM is described chemically as the (5-methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[20-(1H-tetrazol-5-yl) [1, 10-biphenyl]-4-yl] methyl]-1H-imidazole-5-carboxylic acid². OLM has not yet been officially described in any pharmacopoeia. A literature survey revealed that several analytical methods were reported for the determination of OLM in biological fluids including liquid chromatography tandem mass spectrometry (LC-MS-MS)³⁻⁵, capillary electrophoresis (CE)^{6,7}, LC and high performance thin layer chromatography (HPTLC)⁸. OLM determination has been reported for single preparations or in combination with other antihypertensive drugs^{9,10}. Atorvastatin, (bR,R)-2-(4-fluorophenyl)-b-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt, is a second generation HMG-Coal reductive inhibitor

recently approved for clinic use as a cholesterol lowering agent¹¹. Atorvastatin calcium (AC) is a potent inhibitor of (HMG-CoA), the rate-limiting enzyme in cholesterol biosynthesis. This synthetic HMG-CoA reductase inhibitor induces a significant reduction in total cholesterol, low-density lipoprotein cholesterol, and plasma triglycerides in clinical studies¹²⁻¹³. Literature survey reveals that assay of Atorvastatin in bulk and tablet dosage form is official in Indian Pharmacopoeia 2007¹⁴⁻¹⁷.

The analytical methods reported for estimation of AC are Extractive spectrophotometry^{18, 19}, HPLC²⁰, HPTLC²¹ and LC-MS^{22, 23} methods. The proposed method is optimized and validated as per the ICH guidelines²⁴⁻²⁸. In the present work, a successful attempt has been made to estimate both these drugs simultaneously using ratio derivative UV spectrophotometric method²⁶. This study attempts to describe a simple, accurate and precise analytical spectrophotometric method, which can quantify these drugs simultaneously from a combined tablet dosage form. Structures of both the drugs (OLM and AC) are shown in figure 1.

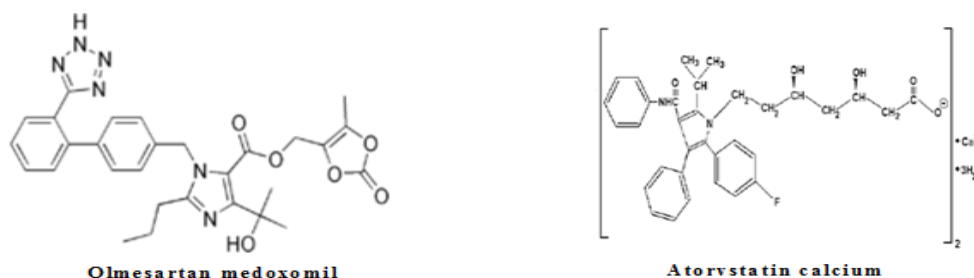


Fig. 1: Chemical structures of the analytes.

MATERIALS AND METHOD

Instruments

Instrument used was an UV-Visible double beam spectrophotometer, make: SHIMADZU (model UV-1800) with a pair of 1 cm matched quartz cells. All weighing was done on Shimadzu analytical balance (Model AU-220).

Reagents and chemicals

A pure drug OLM was obtained as gift sample from Alembic Pharmaceuticals, Vadodara and AC was procured as gift sample from

Torrent pharmaceuticals, Ahmedabad. Methanol AR was used as solvent. Calibrated glasswares were used throughout the work.

Marketed formulation

The marketed formulation studied was OLMESAR AV tablets manufactured by Macleods. Each tablet contains 10 mg Atorvastatin calcium and 20 mg Olmesartan medoxomil.

Preparation of standard stock solution

Accurately weighed quantity of OLM (40 mg) and AC (100 mg) were transferred to two separate 100 ml volumetric flasks, dissolved in

little amount of methanol and diluted to the mark with methanol (stock solutions: 400 µg/ml of OLM and 1000 µg/ml of AC).

Preparation of working standard solution

200 µg/ml of OLM solution was prepared by diluting 25 ml of stock solution to 50 ml with methanol. 200 µg/ml of AC solution was prepared by diluting 10 ml of stock solution to 50 ml with methanol.

Ratio derivative spectrophotometry

The method is based on dividing the spectrum for a mixture by the standard spectrum for each of the analyses and to obtain a spectrum that is independent of the analyte concentration used as a divisor. The use of standardized spectra as divisors minimizes experimental errors. An accurate choice of standard divisors and working wavelengths is fundamental for several reasons. Ratio spectra derivative permits the use of the wavelengths corresponding to maximum or minimum and also the use of the distance between consecutive maximum and minimum. Easy measurements on separate peaks, higher values of the analytical signals and no need to work only at zero crossing points (sometimes coexisting compounds have no maximum or minimum at these wavelengths) are

advantages for ratio spectra derivative spectrophotometry in comparison with the zero crossing derivative spectrophotometry. Also the presence of many maxima and minima in ratio spectra derivative data was another advantage, since these wavelengths gives an opportunity for the determination of these compounds in the presence of other active compounds and excipients that possibly interfered with the assay. The ratio spectra of OLM standard solutions at increasing concentrations were obtained by dividing each with the saved spectrum of the standard solution of AC (4 µg/ml) with the aid of UV Probe software as shown in figure 2.

The first derivative of these spectra traced at the interval of $\Delta\lambda = 4$ nm using Scaling factor 10 (the influence of $\Delta\lambda$ on the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval of $\Delta\lambda = 4$ nm) are illustrated in figure 3.

Wavelength 223 nm was selected for the quantification of OLM in combined tablet dosage form. The ratio spectra of the solutions of AC at different concentrations were obtained by dividing each with the saved standard spectrum of OLM (8 µg/mL). The first derivative of these spectra traced at the interval of $\Delta\lambda = 4$ nm using scaling factor 10 (figure 4 and 5 respectively).

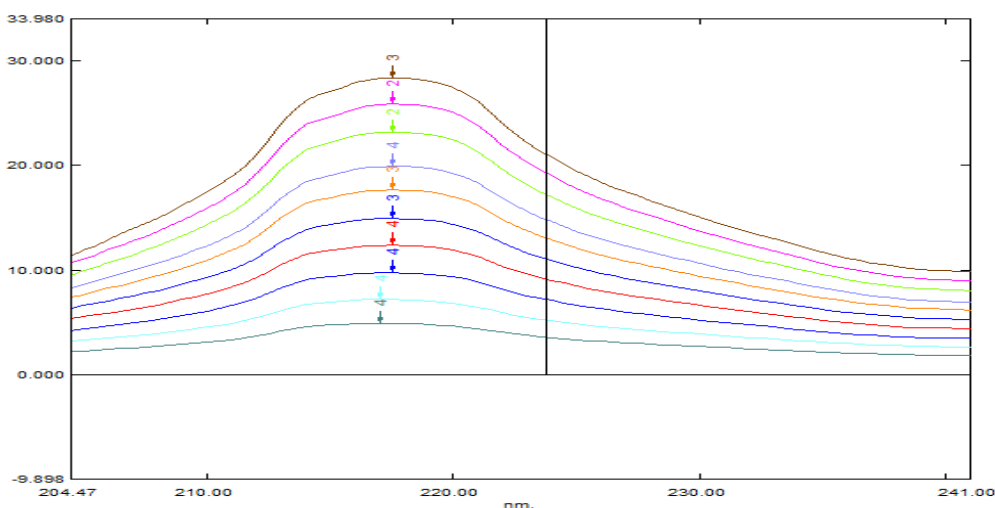


Fig. 2: Overlay zero order ratio spectra of standard Olmesartan medoxomil (Atorvastatin calcium 4 µg/ml used as divisor)

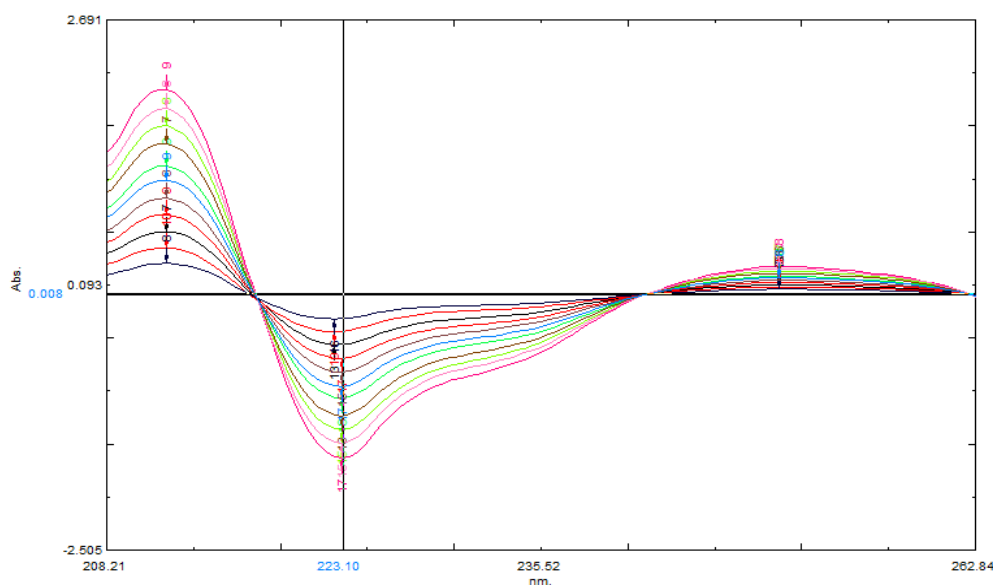


Fig. 3: Overlay first derivative ratio spectra of standard Olmesartan medoxomil (Atorvastatin calcium 4 µg/ml used as divisor)

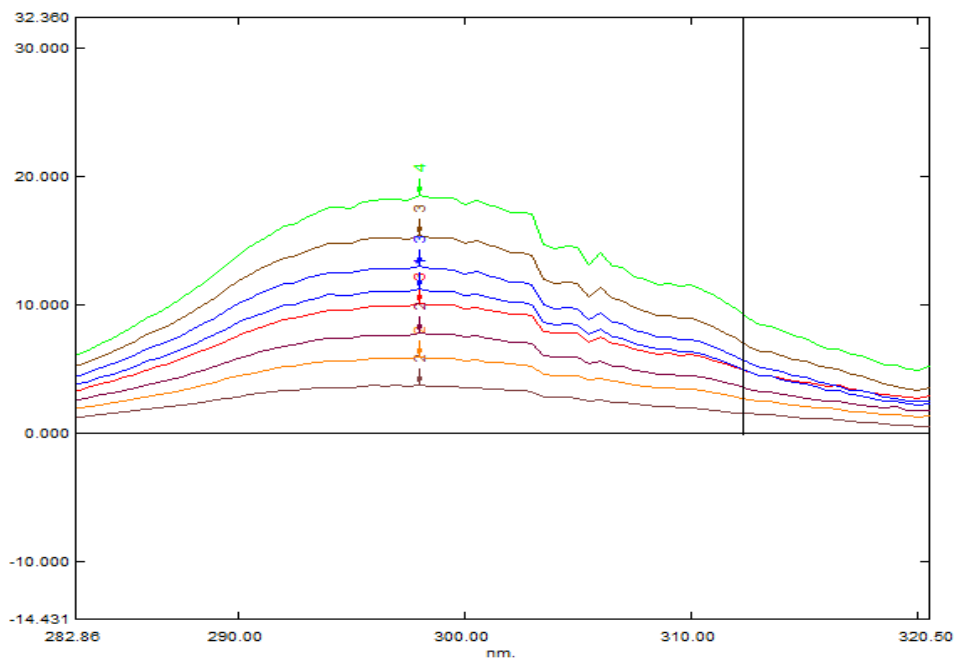


Fig. 4: Overlay zero order ratio spectra of standard Atorvastatin calcium (Olmesartan medoxomil 8 µg/ml used as divisor)

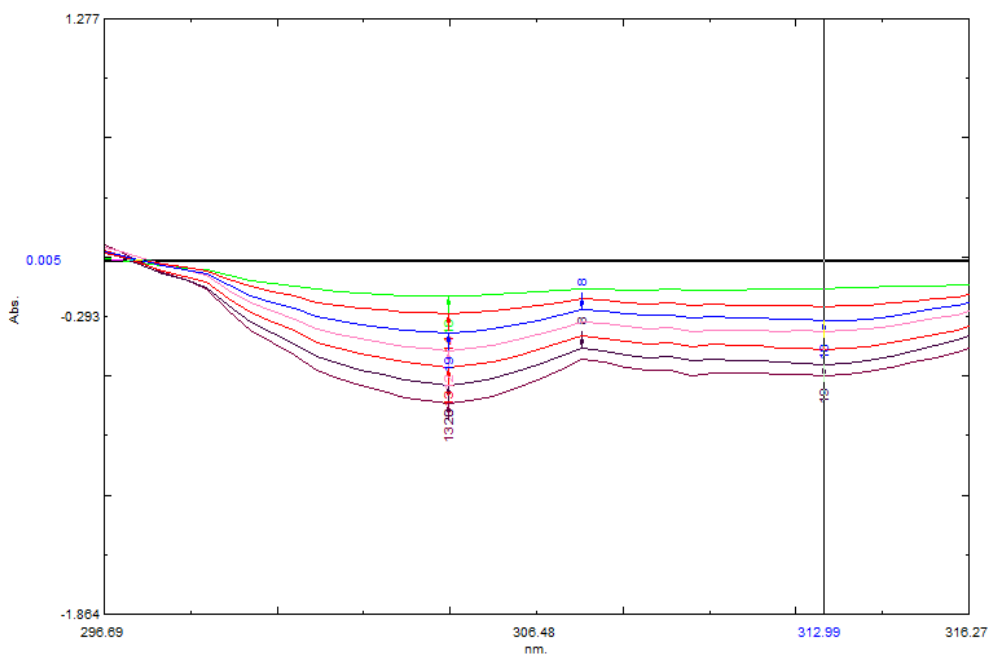


Fig. 5: Overlay first derivative of ratio spectra of standard Atorvastatin calcium (Olmesartan medoxomil 8 µg/ml used as divisor)

Wavelength 313nm was selected for the quantification of AC in combined tablet dosage form. Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. The amount of OLM and AC in tablets was calculated by using following equations:

$$\text{At 223 nm: } C_{\text{OLM}} = d/d\lambda [A_{\text{OLM}} / A_{\text{AC}}] - \text{Intercept (C) / Slope (m)} \dots\dots (1)$$

$$\text{At 313 nm: } C_{\text{AC}} = d/d\lambda [A_{\text{AC}} / A_{\text{OLM}}] - \text{Intercept (C) / Slope (m)} \dots (2)$$

Preparation of calibration curve

Aliquots of working standard solutions were further diluted in methanol to get the solutions in the range of 8-32 µg/ml for OLM

and 4-16 µg/ml for AC and were scanned in the wavelength range of 200–400 nm. Derivative amplitude of ratio spectra was obtained and was used for construction of calibration curve.

Assay of tablet formulation by ratio spectra derivative spectrophotometry

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to about 10 mg of AC and 20 mg of OLM was transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol. The volume was made up to the mark using methanol as solvent. The resulting solution was filtered through Whatmann filter paper and 10 ml of this filtrate was appropriately diluted to get concentration of 20 µg/ml of OLM and

10 µg/ml of AC. The sample solution was scanned in the wavelength range of 200–400 nm. The ratio spectra of the final tablet solutions were obtained by dividing the spectrum with standard spectrum of the OLM (8 µg/mL) and AC (4 µg/mL). Derivative amplitude of ratio spectra was obtained and concentrations of the drugs were calculated by using calibration curve.

Method validation

Linearity and range

Aliquots of working standard solutions of OLM and AC were diluted with methanol to get final concentrations in range of 8-32 µg/ml for OLM and 4-16 µg/ml for AC. This calibration range was prepared five times and derivative amplitude of ratio spectra was obtained for each drug separately.

Precision

Precision of the method was determined by performing interday variation, intraday variation and method repeatability studies. In interday variation, the absorbance of standard solutions of OLM (8-32 µg/ml) and AC (4-16 µg/ml) were measured on three consecutive days. In intraday variation the absorbances were measured three times in a day. In repeatability study, three concentrations of both the drugs were analyzed in triplicate.

Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different concentration levels. A known amount of drug pre-analyzed tablet powder and percentage recoveries were calculated.

Ruggedness

The data for ruggedness were obtained from two different analysts.

RESULTS AND DISCUSSION

Method development and Validation

The overlain derivative amplitude of ratio spectra of the drugs suggested that ratio derivative spectrophotometric method was a suitable method for simultaneous determination of Olmesartan medoxomil and Atorvastatin calcium. Methanol was taken as solvent system as both the drugs were soluble in this solvent. In this method 223 nm and 313 nm were selected for determination of Olmesartan medoxomil and Atorvastatin calcium respectively. Optimized method parameters for ratio derivative spectrophotometry are shown in table 1.

Table 1: Optimized method parameters for ratio derivative spectrophotometry

Method parameters	Optimized parameters
Solvent	Methanol
Scanning range	200 nm to 400 nm
Scan speed	Medium
Δλ for tracing	4 nm
Divisor conc. for determination of AC	8 µg/ml of OLM
Divisor conc. for determination of OLM	4 µg/ml of AC
Analytical wavelength for determination of AC	313 nm
Analytical wavelength for determination of OLM	223 nm

Linearity

The calibration curves of Olmesartan medoxomil and Atorvastatin calcium were linear in the range of 8-32 µg/ml and 4-16 µg/ml respectively. The regression equations of calibration curves were

$$Y = -0.08405x + 0.1032, R^2 = 0.9987 \text{ for Olmesartan medoxomil,}$$

$$Y = -1.5233x + 0.576821, R^2 = 0.9991 \text{ for Atorvastatin calcium.}$$

Precision

Relative standard deviation (% R.S.D.) for repeatability was found to be 1.586-1.731% and 0.814-2.590 % for Olmesartan medoxomil and Atorvastatin calcium respectively. The intraday precision showed % R.S.D. 1.176-2.384 % for Olmesartan medoxomil and 0.607-2.072% for Atorvastatin calcium. The inter day precision showed % R.S.D. ranging from 1.015-3.252 % and 1.058-3.492 % for Olmesartan medoxomil and Atorvastatin calcium respectively. Results of repeatability, intraday and inter day precision of method is illustrated in table 2.

Table 2: Validation Parameters for ratio derivative spectrophotometry

Parameters	OLM	AC
Linearity range(µg/ml)	8-32	4-16
Correlation Coefficient	0.9987	0.9991
Precision	% RSD	
Repeatability	1.59-1.73	0.81-2.59
Intraday	1.18-2.39	0.61-2.07
Interday	1.02-3.25	1.06-3.49
Ruggedness(%RSD)	1.18-2.39	0.20-2.79
% Recovery	98.67-100.79	98.10-100.83

*OLM-Olmesartan medoxomil; AC-Atorvastatin calcium; RSD-Relative Standard Deviation.

Accuracy

The percentage recovery of drugs from marketed formulation was determined by standard addition of pure drugs at three known concentrations and excellent recovery was obtained at each concentration level. The percentage recovery values for Olmesartan medoxomil at three levels were found 100.79±0.0235, 98.67±0.0771 and 99.78±0.0489. The percentage recovery values for Atorvastatin calcium at three levels were found 98.10±0.0545, 98.22±0.0376 and 99.20±0.1613. The results of accuracy studies are shown in table 3.

Table 3: Recovery studies

Name of Drug	Amount of Drug Added (µg/ml)	Ratio Derivative Spectrophotometry	
		%Recovery*	SD
OLM	8	100.79	0.0235
AC	4	98.10	0.0545
OLM	12	98.67	0.0771
AC	6	98.22	0.0376
OLM	16	99.78	0.0489
AC	8	99.20	0.1613

*Mean of three estimations.

OLM- Olmesartan medoxomil; AC-Atorvastatin calcium; SD-Standard Deviation.

Ruggedness

Relative standard deviation (% R.S.D.) for ruggedness was found to be 1.176-2.384 % and 0.204-2.785 % for Olmesartan medoxomil and Atorvastatin calcium respectively.

Application of the method in tablets

The proposed UV method was applied for the determination of Olmesartan medoxomil and Atorvastatin calcium in their combined pharmaceutical formulation and the results are shown in table 4. The percentage recovery values (98.10-100.79 %) confirm the suitability of the proposed method for the routine determination of these components in combined formulation.

Table 4: Results of simultaneous estimation of OLM and AC in marketed formulation by ratio derivative spectrophotometry method

mg/tablet		% of label claim* ± S.D.	
OLM	AC	OLM	AC
20	10	99.14±0.025	98.7±0.074

*Average of three determinations;

OLM- Olmesartan medoxomil; AC-Atorvastatin calcium; SD-Standard Deviation.

CONCLUSION

The proposed ratio derivative spectrophotometry method gives accurate and precise results for determination of Olmesartan medoxomil and Atorvastatin calcium in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of the ratio derivative spectrophotometry method is its simplicity and accuracy. Method validation has been demonstrated by various tests for linearity, accuracy, precision and ruggedness.

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