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*Research Article*

# Ratio Spectra Derivative and Zero-Crossing Difference Spectrophotometric Determination of Olmesartan Medoxomil and Hydrochlorothiazide in Combined Pharmaceutical Dosage Form

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***Abstract.*** Two simple, economical, rapid, precise, and accurate methods for simultaneous determination of olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage form have been developed. The first method is based on ratio spectra derivative spectrophotometry, and the second method is zero-crossing difference spectrophotometry. The amplitudes in the first derivative of the corresponding ratio spectra at 231.0 and 271.0 nm were selected to determine olmesartan medoxomil and hydrochlorothiazide, respectively. Measurements of absorbance were carried out at zero-crossing wavelengths 257.8 and 240.2 nm for olmesartan medoxomil and hydrochlorothiazide by zero-crossing difference spectrophotometric method. Beer's law is obeyed in the concentration range of 08–24 μg/mL for olmesartan medoxomil (OLM) and 05–15 μg/mL for hydrochlorothiazide (HCT) by ratio spectra derivative and 05–30 μg/mL for OLM and HCT by zero-crossing difference spectrophotometric method. The results of the assay were found to be 100.46±0.95 for OLM and 100.4±0.27 for HCT by ratio spectra derivative and 99.06±1.14 for OLM and 100.05±0.90 for HCT by zero-crossing difference spectrophotometric method. These methods passes *F* test and *t* test. Both methods were validated statistically and by performing recovery study.

**KEY WORDS:** hydrochlorothiazide; olmesartan medoxomil; ratio spectra derivative spectrophotometry; zero-crossing difference spectrophotometry.

## INTRODUCTION

Olmesartan medoxomil (OLM) is chemically 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate, cyclic 2, 3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic "2, 3-carbonate", (1,2). It is a new selective angiotensin II receptor antagonist and is used as antihypertensive. It is unofficial in pharmacopoeia. Literature survey reveals that high-performance chromatography (HPLC) (3), high-performance thin-layer chromatography (HPTLC) (4), and estimations in biological samples were performed in highly sensitive methods like HPLC, liquid chromatography–mass spectrometry, etc. (5,6).

Hydrochlorothiazide (HCT) is chemically 6-chloro-3, 4 dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. It is the 3,4-dihydro derivative of chlorothiazide. It is a diuretic and antihypertensive (7). A literature survey reveals that UV (8,9), HPLC (10–15), and HPTLC (16,17) methods are reported for hydrochlorothiazide. No UV/Visible spectrophotometric method has been developed for this combination.

A simple, rapid, precise, and economic ratio spectra derivative (18) and zero-crossing difference spectrophotometric (19) ($\Delta A$) methods have been developed for the determination of OLM and HCT in combined pharmaceutical dosage forms.

The ratio spectra derivative method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is independent of the concentration of analyte used as a divisor. The ratio spectra of different OLM standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of HCT (12.5 μg/mL, scaling factor 4) and the first derivative of these spectra traced with the interval of $\Delta\lambda$=4 nm. Wavelength 231.0 nm was selected for the quantification of OLM in OLM and HCT mixture. The ratio and ratio derivative spectra of the solutions of HCT at different concentrations were obtained by dividing each with the stored standard spectrum of the OLM (20 μg/mL, scaling factor 4) and the first derivative of these spectra traced with the interval of $\Delta\lambda$=4 nm. Wavelength 271.0 nm was selected for the quantification of HCT in OLM and HCT mixture. Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. The amount of OLM and HCT in tablets was calculated using the following equations:

$$\text{At } 231.0 \text{ nm} : C_{OLM} = d/d\lambda[A_{OLM}/A_{HCT}] - \text{Intercept(C)}/\text{Slope(m)} \quad (1)$$

$$\text{At } 271.0 \text{ nm} : C_{HCT} = d/d\lambda[A_{HCT}/A_{OLM}] - \text{Intercept(C)}/\text{Slope(m)}. \quad (2)$$

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The technique of zero-crossing difference spectrophotometric method is based upon the measurement of absorbance difference ($\Delta A$) which can be induced by changing the pH of solvent medium of two equimolar solutions of olmesartan medoxomil and hydrochlorothiazide in phosphate buffer (pH 9) solution against their chloride buffer (pH 2) solution as blank (20). The choice of the optimum wavelength is based on the fact that the contribution of each component is zero at the wavelength at which other components exhibited maximum absorbance. Therefore, measurement of absorbance from difference spectra was carried out at zero-crossing wavelength at 257.8 nm for olmesartan medoxomil and 240.2 nm for hydrochlorothiazide.

## MATERIALS AND METHODS

Double-beam UV–visible spectrophotometer Shimadzu UV 2450 PC (Japan) with 10-mm matched quartz cell, bandwidth 1 nm was used for spectral measurement. Pure drug sample of olmesartan medoxomil (Ajanta Pharma Pvt. Ltd, Paithan) and hydrochlorothiazides (Glen Mark Pharmaceutical Ltd., Nashik) were used having 98.6% and 100.1% purity, respectively. Chloride buffer, pH 2 (6.57 g of potassium chloride, 119.0 mL of 0.1 M hydrochloric acid, and water up to 1,000 mL) and phosphate buffer, pH 9 (1.74 g of potassium dihydrogen orthophosphate in 80 mL water, adjusted pH with 1 M potassium hydroxide, and water up to 1,000 mL) were used as solvent for zero-crossing difference spectrophotometric analysis, and 0.1 N NaOH was used as solvent for ratio spectra first-derivative spectrophotometric method. All reagent used were of analytical grade. Tablet Olmesar H (olmesartan medoxomil 20 mg and hydrochlorothiazide 12.5 mg, Macleod Pharma. Pvt. Ltd.) were procured locally.

## RATIO SPECTRA DERIVATIVE METHOD

### Preparation of Standard Stock Solutions

Standard stock solutions of pure drug were prepared by dissolving 20 mg each of pure OLM and HCT in 0.1 N NaOH in a 100-mL volumetric flask separately. The working solutions of these drugs were obtained by dilution of the respective stock solution with 0.1 N NaOH.

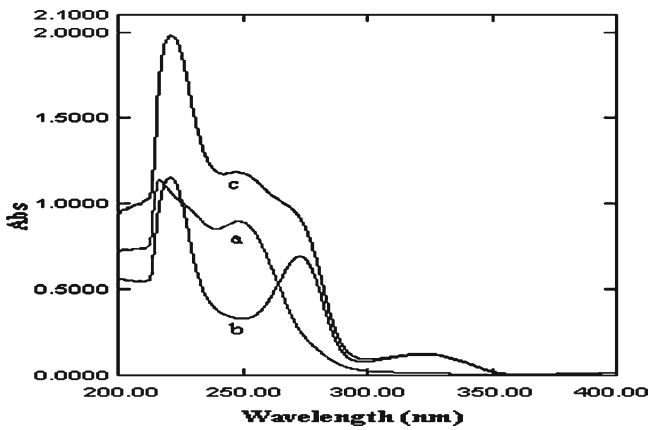


**Fig. 1.** Zero-order spectra of *a* OLM (20 μg/mL), *b* HCT (12.5 μg/mL), *c* their mix OLM (20 μg/mL) and HCT (12.5 μg/mL) in 0.1 N NaOH

**Table I.** Optical Characteristics of Linearity for OLM and HCT by Difference and Ratio Derivative Method

| | Ratio derivative method | | Difference method | |
|---|---|---|---|---|
| Method parameter | OLM | HCT | OLM | HCT |
| $\lambda_{max}$ (nm) | 248.6 | 272.8 | 257.8 | 240.2 |
| Beer's law limit(μg/mL) | 08–24 | 05–15 | 05–30 | 05–30 |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) | $2.483\times10^4$ | $1.654\times10^4$ | $7.541\times10^3$ | $4.426\times10^3$ |
| Correlation coefficient ($r^2$) | 0.998 | 0.9993 | 0.9952 | 0.9965 |
| Regression equation ($Y^*$) | – | – | – | – |
| Slope ($a$) | 0.0446 | 0.0538 | 0.0119 | 0.014 |
| Intercept ($b$) | 0.0116 | 0.0221 | 0.0181 | 0.0026 |

$Y^* = aX + b$ where $Y$ is absorbance and $X$ is concentration in micrograms per milliliter

### Linearity

The aliquots of the stock solution were transferred to a 10-mL volumetric flask in duplicate. Volumes were made up with 0.1 N NaOH to get final concentrations 08–24 μg/mL of OLM and 05–15 μg/mL of HCT. The statistical parameters (21) for linearity were calculated.

### Calibration Graph

From appropriate volumes of the stock solution, 08–24 μg/mL of OLM and 05–15 μg/mL of HCT were prepared. The ratio and ratio derivative spectra of the solutions of OLM at different concentrations were obtained by dividing each with the stored standard spectrum of the standard solution of HCT (12.5 μg/mL, scaling factor 4), and the first derivative of these spectra were traced with the interval of $\Delta\lambda$=4 nm. Wavelength 231.0 nm was selected for the quantification of OLM in OLM and HCT mixture. The ratio and ratio derivative spectra of the solutions of HCT at different concentrations were obtained by dividing each with the stored standard spectrum of the standard solution of OLM (20 μg/mL, scaling factor 4), and the first derivative of these spectra were traced with the interval of $\Delta\lambda$=4 nm. Wavelength 271.0 nm was selected for the quantification of HCT in OLM and HCT mixture. The statistical parameters of the calibration graph were calculated.

### Preparation of Tablet Sample Solution

Twenty tablets were weighed and finely powdered. From tablet sample, an amount equivalent to 20 mg of olmesartan medoxomil was dissolved in minimum quantity of methanol and diluted up to the 100-mL mark with 0.1 N NaOH in a volumetric flask to get a working standard solution of 200 μg/mL. The solution was filtered through Whatman filter paper no. 41. Results of OLM and HCT were computed from calibration graph.

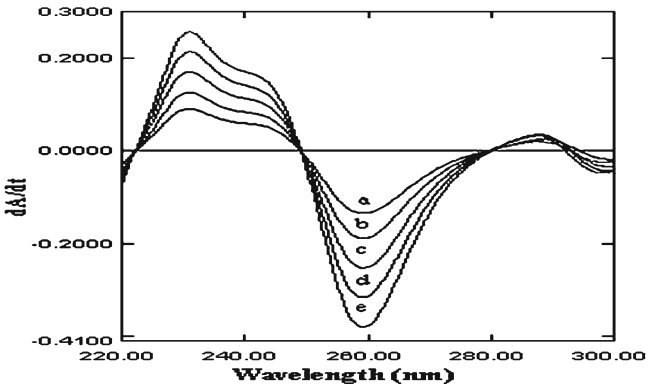


**Fig. 2.** First-derivative of the ratio spectra of solutions of OLM (*a–e* 08–24 μg/mL) when 12.5 μg/mL solution of HCT is used as divisor ($\Delta\lambda$=4 nm)

### Recovery Studies

The accuracy of the proposed method was checked by recovery studies by the addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (20%, 40%, and 60%) within the range of linearity for both the drugs.

## ZERO-CROSSING DIFFERENCE METHOD

### Preparation of Standard Stock Solution

Pure 20 mg each of olmesartan medoxomil and hydrochlorothiazide were weighed accurately and dissolved in minimum quantity of methanol, and final volumes were made with water to 100 mL in a volumetric flask. Then, further dilutions were made in respective buffer solution as (a) OLM 20 μg/mL in acidic pH (pH 2) with a maximum at 248.0 nm, (b) OLM 20 μg/mL in alkaline pH (pH 9), (c) HCT 15 μg/mL in acidic pH (pH 2) with a maximum at 271.3 nm, (d) HCT 15 μg/mL in alkaline pH (pH 9) with a maximum at 271.9 nm, and (e, f) mixture (20:15 μg/mL of OLM and HCT) in acidic pH (pH 2) and in alkaline pH (pH 9), and zero-order spectra were recorded.

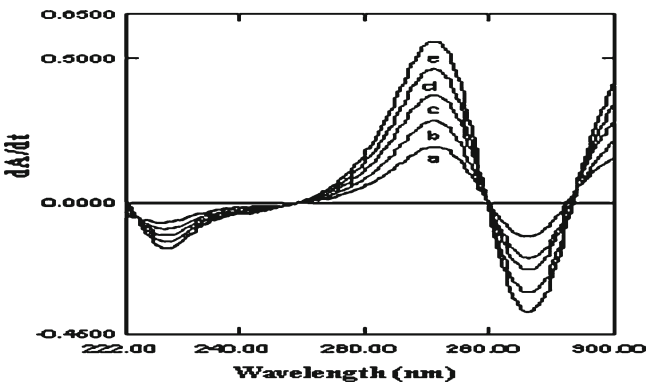


**Fig. 3.** First-derivative of the ratio spectra of solutions of HCT (*a–e* 05–15 μg/mL) when 20 μg/mL solution of OLM is used as divisor ($\Delta\lambda$=4 nm)

**Table II.** Optical Characteristics of Calibration Graph for OLM and HCT by Difference and Ratio Derivative Method

| Method | Ratio derivative method | | Difference method | |
|---|---|---|---|---|
| Parameter | OLM | HCT | OLM | HCT |
| $\lambda_{max}$ (nm) | 231.0 | 271.0 | 257.8 | 240.2 |
| Beer's law limit (μg/mL) | 08–24 | 05–15 | 08–24 | 05–15 |
| Regression equation ($Y^*$) | | | | |
| Slope ($m$) | 0.0113 | 0.0343 | 0.01207 | 0.0144 |
| Intercept (c) | 0.007 | 0.0127 | 0.00584 | 0.0272 |
| Correlation coefficient | 0.9989 | 0.9993 | 0.9998 | 0.998 |

$Y^* = aX + b$, where $Y$ is the absorbance and $X$ the concentration in micrograms per milliliter

### Linearity

The aliquots of the stock solution were transferred to a 10-mL volumetric flask in duplicate. Volumes were made up with chloride buffer (pH 2) and phosphate buffer (pH 9) to a series of equimolar solutions to get final concentrations of 5–30 μg/mL each of OLM and HCT. The statistical parameters for linearity were calculated.

### Calibration Graph

From appropriate volumes of the stock solution, two series of 10 mL equimolar solution of mixtures each of OLM and HCT in chloride buffer (pH 2) and phosphate buffer (pH 9) were prepared. The first series contained a constant concentration of OLM (20 μg/mL) and varying concentrations of HCT (5–30 μg/mL). The second series contained a constant concentration of HCT (12.5 μg/mL) and varying concentrations of OLM (08–24 μg/mL). Calibration graph of difference spectra was used for the calculation of results of OLM and HCT, and statistical parameters of the calibration graph were calculated.

### Tablet Sample Solution Preparation

Twenty tablets were weighed and finely powdered. From tablet sample, an amount equivalent to 20 mg of olmesartan

**Table III.** Results of Analysis of Commercial Formulation for OLM and HCT by Difference and Ratio Derivative Method

| | Ratio derivative method | | Difference method | |
|---|---|---|---|---|
| Method for estimation | OLM | HCT | OLM | HCT |
| Label claim | 20 | 12.5 | 20 | 12.5 |
| % of Label claim estimated[a] | 100.46 | 100.40 | 99.06 | 100.05 |
| Standard deviation | 0.948 | 0.266 | 1.135 | 0.899 |
| % RSD | 0.94 | 0.265 | 1.15 | 0.899 |
| $F$(0.05,5)=4.28 | | | 0.698 | 0.088 |
| $t$(0.05,5)=2.447 | 0.49 | 1.58 | 0.83 | 0.06 |

[a] Average of six determinations

**Table IV.** Recovery Studies of OLM and HCT by Difference and Ratio Derivative Method

| Level of % recovery | % Mean recovery[a] | | Standard deviation[a] | | % RSD[a] | |
|---|---|---|---|---|---|---|
| | OLM | HCT | OLM | HCT | OLM | HCT |
| Ratio derivative method | | | | | | |
| 20 | 97.75 | 99.65 | 0.27 | 0.39 | 0.270 | 0.39 |
| 40 | 98.06 | 99.40 | 0.31 | 0.21 | 0.32 | 0.21 |
| 60 | 98.80 | 100.00 | 0.11 | 0.19 | 0.112 | 0.19 |
| Difference method | | | | | | |
| 20 | 98.37 | 97.76 | 0.275 | 1.04 | 0.28 | 1.06 |
| 40 | 99.53 | 97.89 | 0.425 | 0.611 | 0.427 | 0.62 |
| 60 | 99.25 | 97.95 | 0.1 | 0.361 | 0.101 | 0.37 |

*RSD* relative standard deviation
[a] Average of three determinations

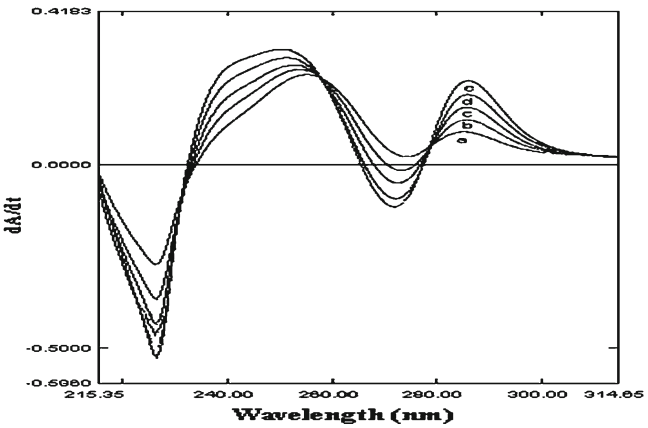


**Fig. 5.** Difference spectra of OLM in mixture *a–e* (12.5 μg/mL HCT and 08, 12, 16, 20, 24 μg/mL OLM) in acidic (pH 2) *vs* alkaline (pH 9) pH solution

medoxomil was dissolved in 20 mL of methanol and diluted with buffers acidic pH (pH 2) and alkaline pH (pH 9) up to the 100-mL mark in a volumetric flask to get a working standard solution of 200 μg/mL. The solution was filtered through Whatman filter paper no. 41. Results were computed from the calibration graph.

### Recovery Studies

The accuracy of the proposed method was checked by recovery studies with the addition of standard drug solution to pre-analyzed tablet sample solution at three different concentration levels (20%, 40%, and 60%) within the range of linearity for both the drugs.

## RESULTS AND DISCUSSION

### For Ratio Spectra Derivative Method

Zero-order spectra of OLM (20 μg/mL), HCT (12.5 μg/mL), and their mix OLM (20 μg/mL) and HCT (12.5 μg/mL) in 0.1 N NaOH were recorded, which is shown in Fig. 1.

The linearity for pure drug was found to be 08–24 μg/mL for OLM and 05–15 μg/mL for HCT. The correlation coefficients 0.998 for OLM and 0.9993 for HCT show good precision for linearity (Table I).

The ratio and ratio derivative spectra of the solutions of OLM at different concentrations were obtained by dividing each with the stored standard spectrum of the standard solution of HCT (12.5 μg/mL, scaling factor 4), and the first derivative of these spectra were traced with the interval of $\Delta\lambda$=4 nm.. Wavelength 231.0 nm was selected for the quantification of OLM in OLM and HCT mixture. The ratio and ratio derivative spectra of the solutions of HCT at different concentrations were obtained by dividing each with the stored standard spectrum of the standard solution of OLM (20 μg/mL, scaling factor 4), and the first derivative of these spectra were traced with the interval of $\Delta\lambda$=4 nm. Wavelength 271.0 nm was selected for the quantification of HCT in OLM and HCT mixture. From the ratio and ratio derivative spectra of OLM and HCT (Figs. 2 and 3), calibration graph was plotted and used for the quantization of concentration of OLM and HCT in formulation. The correlation coefficients 0.9989 for OLM and 0.9993 for HCT show good precision for calibration (Table II). The proposed

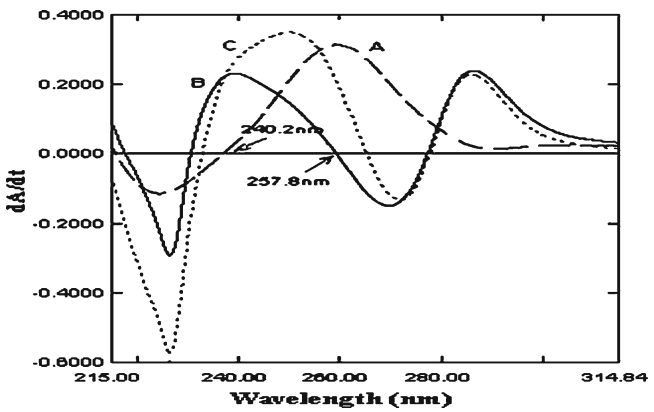


**Fig. 4.** Difference spectra of pure *A* (OLM 20 μg/mL), *B* HCT (15 μg/mL) and mixture *C* (20:15 μg/mL of OLM and HCT) in acidic (pH 2) *vs* alkaline (pH 9) pH solution

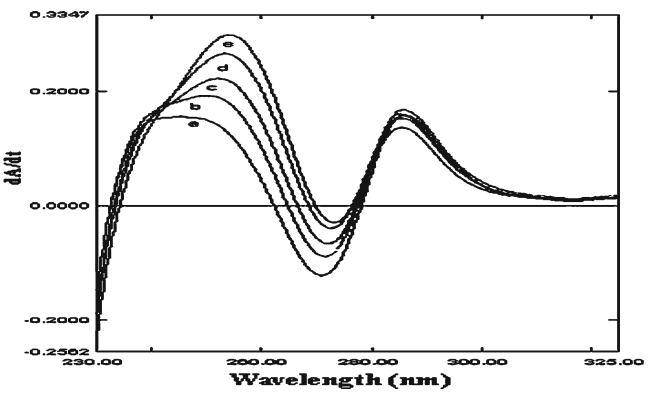


**Fig. 6.** Difference spectra of HCT in mixture *a–e* (20 μg/mL OLM and 05, 7.5, 10, 12.5, 15 μg/mL HCT) in acidic (pH 2) *vs* alkaline (pH 9) pH solution

method was also evaluated by performing the assay of commercially available tablet formulation Olmasar H containing olmesartan medoxomil 20 mg and hydrochlorothiazide 12.5 mg. The results of the assay are shown in Table III. The percent relative standard deviation (RSD) values for recovery study ranging from 0.112 to 0.32 for OLM and from 0.19 to 0.39 for HCT show good precision and reproducibility. The results of recovery for OLM and HCT are shown in Table IV.

### For Zero-Crossing Difference Method

In Fig. 4, we show the absorption (zero-order) spectra of the following: (a) OLM 20 µg/mL in acidic pH (pH 2) with a maximum at 248.0 nm, (b) OLM 20 µg/mL in alkaline pH (pH 9) with a maximum at 248.4 nm, (c) HCT 15 µg/mL in acidic pH (pH 2) with a maximum at 271.3 nm, (d) HCT 15 µg/mL in alkaline pH (pH 9) with a maximum at 271.9 nm, and (e, f) mixture (20:15 µg/mL of OLM and HCT) in acidic (pH 2) and in alkaline (pH 9) pH. Difference spectra of equimolar OLM 20 µg/mL, HCT 15 µg/mL, and mixture (20:15 µg/mL of OLM and HCT) in acidic pH (pH 2) and alkaline pH (pH 9) solutions were recorded. The linearity was found to be in the concentration range of 05–30 µg/mL for OLM and HCT, respectively. The correlation coefficients 0.9952 for OLM and 0.9965 for HCT show good precision for linearity (Table I). The difference spectra of calibration graph for OLM and HCT are depicted in Figs. 5 and 6. The correlation coefficients 0.9998 for OLM and 0.998 for HCT show good precision for calibration (Table II).

The proposed method was also evaluated by performing the assay of commercially available tablets. The results of the assay are shown in Table III. The percent RSD values for recovery study ranging from 0.28 to 0.427 for OLM and from 0.37 to 1.06 for HCT show good precision and reproducibility. The results of recovery for OLM and HCT are shown in Table IV.

Both methods were compared by performing *F* test and *t* test. Calculated *F* values 0.698 for OLM and 0.088 for HCT are less than theoretical $F(0.05,5)=4.28$. We conclude that there is no significant difference in the precision of the two methods, and the calculated *t* values 0.49, 0.83 for OLM and 0.06, 1.06 HCT are less than the theoretical $t(0.05,5)=2.447$ value, showing no significant difference in the precision of the two proposed methods.

## CONCLUSION

The validated ratio spectra derivative and zero-crossing difference spectrophotometric methods employed proved to be simple, economical, rapid, precise, and accurate. Thus, it can be used for routine simultaneous determination of OLM and HCT in combined pharmaceutical tablet dosage form. The ratio spectra derivative method has greater sensitivity and accuracy than the difference spectrophotometric method.

## ACKNOWLEDGMENT

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