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# Estimation of Olmesartan and Hydrochlorthiazide in Bulk and Tablet by Simultaneous Equation and Multi-component UV-Spectrophotometric Methods

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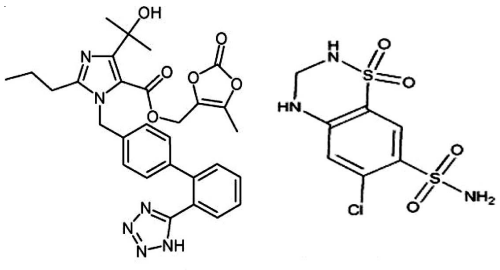
Olmesartan and hydrochlorthiazide are estimated simultaneously in bulk and tablets by two simple and rapid UV-spectrophotometric methods. The first method employs simultaneous equation for analysis, using methanol, as a solvent. The two wavelengths 255 nm ($\lambda_{max}$ of olmesartan) and 270 nm ($\lambda_{max}$ of hydrochlorthiazide) were selected for formation of simultaneous equations. Linearity was observed in the concentration range of 3 - 24 µg/mL for olmesartan and 2 - 16 µg/mL for hydrochlorthiazide. Statistical analysis and recovery studies validated the method. Multi-component method utilizes six mixed standards and two sampling wavelengths, as 255 and 270 nm. Six mixed standard solutions of both the drugs were prepared and concentrations were determined by the multi-component mode.

**Key Words: Simultaneous equation method, Multi-component mode.**

## INTRODUCTION

Chemically, olmesartan (OMS), (5-methyl-2-oxo-1,3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenylmethylimidazo-5 carboxylate), (Fig. 1a) is a selective angiotensin-II receptor blocker[1-3] and is advocated for the treatment of hypertension. Previously published work on the analysis of olmesartan in biological fluids such as human plasma and urine used LC-MS and LC-MS-MS[4-6]. Capillary in-tube solid phase micro-extraction technique has also been employed[7]. UV-spectrophotometric determination of olmesartan in tablets is reported by Celebier *et al.*[8]. A study identifying various degradation products in stressed tablets by use of HPLC is reported[9].




Olmesartan Medoxamil (OMS) Hydrochlorthiazide (HTZ)

Fig. 1. Chemical structures of olmesartan and hydrochlorthiazide

Hydrochlorthiazide (HTZ) (Fig. 1) chemically, is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide[10]. Literature survey reveals various methods for determination of hydrochlorthiazide individually or in its combination with other drugs, including the use of liquid chromatography[11-14], capillary zone electrophoresis[15], spectrophotometry[16-19].

The combination of olmesartan and hydrochlorthiazide was found to be more effective in comparison with individual administration of the drugs[20]. Literature survey highlights HPLC methods for estimation of olmesartan and hydrochlorthiazide in biological fluids and formulations[21]. HPTLC method has also been reported for simultaneous estimation of both drugs[22].

No official spectrophotometric method is reported so far for simultaneous estimation of both the drugs from pharmaceutical formulation. Therefore, it was thought worthwhile to develop simultaneous spectrophotometric method (applying cramer's rule) and multi-component mode method for estimation of hydrochlorthiazide and olmesartan from tablet formulations.

## EXPERIMENTAL

Simultaneous analysis of hydrochlorthiazide and olmesartan using simultaneous equation method and multi-component method has been developed in the present investigation. Instruments involved are UV/ VIS double beam spectrophotometer; model Shimadzu UV-2400PC series and UV/ VIS double beam spectrophotometer; model Shimadzu UV-1601 both having a spectral bandwidth of 2 nm and wavelength

accuracy of ± 0.1 nm with automatic wavelength correction and a pair of 10 mm matched quartz cells.

Drug samples were procured from Glenmark Pharmaceuticals, Nasik. The tablet formulations used were Brand-I (Olmax-H, Glenmark Pharmaceuticals, India) and Brand-II (Olmecip-H, Cipla Pharmaceuticals, India).

**Preparation of stock solution**

**Method - I: Simultaneous equation method**: Olmesartan (10 mg) and hydrochlorthiazide (10 mg) were accurately weighed and transferred to two separate 100 mL volumetric flasks; dissolved in methanol to obtain each stock solution of 100 µg/mL. From these stock solutions, working standard solutions of drugs were prepared by appropriate dilutions and were scanned in the entire UV range to determine the $\lambda_{max}$. Olmesartan has $\lambda_{max}$ at 255 nm while hydrochlorthiazide has $\lambda_{max}$ at 270 nm, respectively. Fig. 2, presents the overlain spectra of olmesartan and hydrochlorthiazide standards observed in UV range.

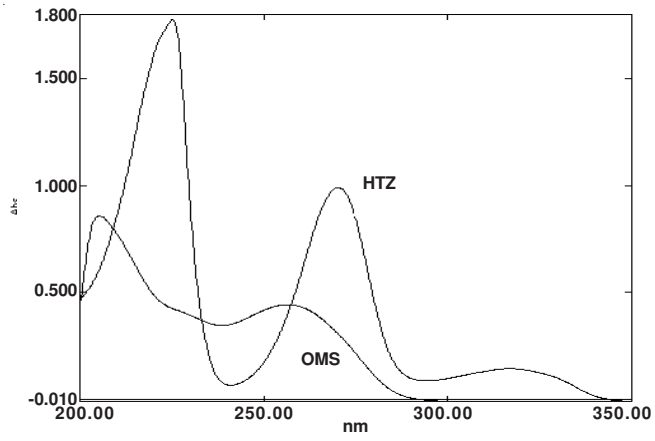



Fig. 2. Overlay spectra for OMS and HTZ

Standard solutions of concentration 3, 6, 9, 12, 15, 18, 21 and 24 µg/mL were prepared for olmesartan. For hydrochlorthiazide standard solutions having concentrations 2, 4, 6, 8, 10, 12, 14, 16 µg/mL were prepared. The absorbances of these standard solutions were measured at 255 and 270 nm. Calibration curves were plotted at these wavelengths and the optical characteristics of both the drugs are given in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND STATISTICAL DATA OF THE REGRESSION EQUATION FOR METHOD-I

| Parameters | Method – I | |
|---|---|---|
| | Olmesartan | Hydrochlorthiazide |
| Absorption maxima (nm) | 255 | 270 |
| Beer's law limit (µg/mL) | 3 – 24 | 2 – 16 |
| Absorptivity | 469.38 | 720 |
| Coefficient of correlation | 0.9992 | 0.9996 |
| Regression equation | Y = 0.048x – 0.009 | Y = 0.069x + 0.011 |
| Intercept (A) | – 0.009 | 0.011 |
| Slope (B) | 0.048 | 0.069 |

The absorptivity coefficients of two drugs were determined, using calibration curves; data is presented in Table-2. Two simultaneous equations[23] (in two variables $C_1$ and $C_2$) were formed using these absorptivity coefficient values.

$$A_1 = (469.38)\ C_1 + (228.14)\ C_2 \quad (I)$$

$$A_2 = (323.88)\ C_1 + (720)\ C_2 \quad (II)$$

where, $C_1$ and $C_2$ are the concentrations of olmesartan and hydrochlorthiazide measured in g/100 mL, in the sample solutions. $A_1$ and $A_2$ are the absorbance of mixture, at selected wavelengths of 255 and 270 nm respectively.

TABLE-2
ABSORPTIVITY VALUES FOR OLMESARTAN AND HYDROCHLORTHIAZIDE FOR METHOD–I

| S. No. | Olmesartan | | Hydrochlorthiazide | |
|---|---|---|---|---|
| | 255 nm | 270 nm | 255 nm | 270 nm |
| 01 | 452.19 | 307.10 | 206.50 | 754 |
| 02 | 456.48 | 311.40 | 213.71 | 751 |
| 03 | 473.79 | 326.24 | 230.71 | 715.52 |
| 04 | 472.83 | 306.83 | 236.23 | 709.79 |
| 05 | 474.78 | 340.12 | 234.31 | 702.49 |
| 06 | 473.58 | 327.61 | 231.85 | 715.43 |
| 07 | 475.37 | 342.31 | 230.10 | 696.69 |
| 08 | 475.99 | 329.42 | 241.69 | 715.10 |
| Mean | 469.38 | 323.88 | 228.14 | 720 |
| S.D. | ± 1.62 | ± 1.39 | ± 1.27 | ± 1.53 |
| % RSD | 0.35 | 0.43 | 0.56 | 0.21 |

By applying the Cramer's rule[24] to equations I and II, the concentration $C_{OMS}$ (for olmesartan) and $C_{HTZ}$ (for hydrochlorthiazide) can be obtained as follows:

$$C_{OMS} = \frac{A_2(228.14) - A_1(720)}{-264063.62} \quad (III)$$

and

$$C_{HtZ} = \frac{A_1(323.88) - A_2(469.38)}{-264063.62} \quad (IV)$$

**Method-II: Multi-component mode method:** Available marketed formulations contain olmesartan (20 mg) and hydrochlorthiazide (12.50 mg). Therefore mixed drug concentrations were selected, so as to maintain the constant ratio of 1.6: 1 *i.e.* for olmesartan and hydrochlorthiazide, respectively. Two sampling wavelengths, as 255 and 270 nm were selected for quantitation. Six mixed standards were selected to find out concentrations from multi-component mode of the instrument. Table-3, shows the concentrations of physical laboratory mixture. Fig. 3, presents the overlain spectra of mixed standards observed in the multi-component mode of instrument. Concentrations were estimated directly by the multi-component mode.

TABLE-3
CONCENTRATIONS OF MIXED STANDARDS APPLIED IN MULTI-COMPONENT MODE

| Drug (µg/mL) | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| Olmesartan | 00 | 8 | 12 | 16 | 20 | 20 |
| Hydrochlorthiazide | 12.5 | 5 | 7.5 | 10 | 12.5 | 00 |

**Preparation and analysis of tablet sample solution:** Brand-I (Olmax - H, Glenmark Pharmaceuticals, India) and Brand-II (Olmecip - H, Cipla Pharmaceuticals, India) each containing 20 mg of olmesartan and 12.50 mg of hydrochlorthiazide were weighed separately and crushed to

TABLE-4
RESULTS OF SIMULTANEOUS ESTIMATION OF TABLETS BY METHOD-I AND II

| Method | Tablet Sample | Label claim | Amount found* (mg/tab) | | % Label claimed | | Standard deviation | | % RSD | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (mg/ tab) | Brand-I | Brand-II | Brand-I | Brand-II | Brand-I | Brand-II | Brand-I | Brand-II |
| I | Olmesartan | 20.0 | 19.81 | 19.80 | 99.04 | 99.01 | ± 1.016 | ± 0.726 | 1.03 | 0.73 |
| | Hydrochlorthiazide | 12.5 | 12.47 | 12.51 | 99.74 | 100.11 | ± 0.031 | ± 1.213 | 0.31 | 1.21 |
| II | Olmesartan | 20.0 | 19.84 | 19.82 | 99.20 | 99.10 | ± 0.92 | ± 1.128 | 0.93 | 1.14 |
| | Hydrochlorthiazide | 12.5 | 12.49 | 12.52 | 99.92 | 100.16 | ± 1.045 | ± 1.015 | 1.05 | 1.01 |

Mean of six estimations.

TABLE-5
RESULTS FOR RECOVERY STUDIES

| Method | Drug added (µg/mL) | | Amount of drug recovered (µg/mL) | | | | % Recovery ± S.D. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brand-I | | Brand-II | | Brand-I | | Brand-II | |
| | OMS | HTZ | OMS | HTZ | OMS | HTZ | OMS | HTZ | OMS | HTZ |
| I | 08 | 5.00 | 7.95 | 4.92 | 7.94 | 4.95 | 99.31 ± 0.19 | 99.32 ± 0.14 | 99.21 ± 0.47 | 98.94 ± 1.22 |
| | 10 | 6.25 | 9.90 | 6.22 | 9.93 | 6.19 | 99.04 ± 0.03 | 99.49 ± 0.48 | 99.25 ± 0.29 | 99.05 ± 0.41 |
| | 12 | 7.50 | 11.88 | 7.44 | 11.93 | 7.43 | 99.00 ± 1.53 | 99.15 ± 0.74 | 99.40 ± 1.01 | 99.07 ± 0.42 |
| II | 08 | 5.00 | 7.96 | 4.99 | 7.95 | 4.97 | 99.52 ± 0.76 | 99.79 ± 0.51 | 99.37 ± 0.38 | 99.38 ± 0.78 |
| | 10 | 6.25 | 9.94 | 6.22 | 9.95 | 6.21 | 99.39 ± 0.36 | 99.52 ± 0.82 | 99.49 ± 0.53 | 99.36 ± 0.67 |
| | 12 | 7.50 | 11.96 | 7.46 | 11.89 | 7.42 | 99.67 ± 0.97 | 99.47 ± 0.37 | 99.08 ± 0.57 | 98.93 ± 0.81 |

Mean of six estimations; OMS = Olmesartan; HTZ = Hydrochlorthiazide.

TABLE-6
RESULTS FOR REPEATABILITY STUDIES

| Method | Drug (µg/mL) | | Inter-day amount of drug (µg/mL) | | | | Intra-day amount of drug (µg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OMS | HTZ | OMS | % RSD | HTZ | % RSD | OMS | % RSD | HTZ | % RSD |
| I | 8 | 5.00 | 7.94 | 0.53 | 4.97 | 0.88 | 7.98 | 0.30 | 4.98 | 0.49 |
| | 10 | 6.25 | 9.87 | 0.36 | 6.17 | 0.54 | 9.94 | 0.50 | 6.17 | 0.68 |
| | 12 | 7.50 | 11.84 | 0.34 | 7.48 | 0.45 | 11.77 | 0.29 | 7.55 | 0.44 |
| II | 8 | 5.00 | 7.95 | 0.24 | 4.94 | 0.33 | 7.91 | 0.51 | 4.92 | 0.26 |
| | 10 | 6.25 | 9.91 | 0.32 | 6.14 | 0.41 | 9.89 | 0.25 | 6.24 | 0.37 |
| | 12 | 7.50 | 11.88 | 0.27 | 7.42 | 0.32 | 11.81 | 0.39 | 7.48 | 0.18 |

Mean of six estimations; OMS = Olmesartan; HTZ = Hydrochlorthiazide.

fine powder. An accurately weighed powder sample equivalent to weight of one tablet was transferred to a 100 mL volumetric flask. It was dissolved in methanol with intermittent shaking and the resulting solution was filtered with Whatman paper # 41 and the volume was made up to the mark, using same solvent. Appropriate aliquots were subjected to above methods and the amounts of hydrochlorthiazide and olmesartan were determined. Per cent labeled claim and its standard deviation (S.D.) for both the brands by both the methods were calculated and results are given in Table- 4.

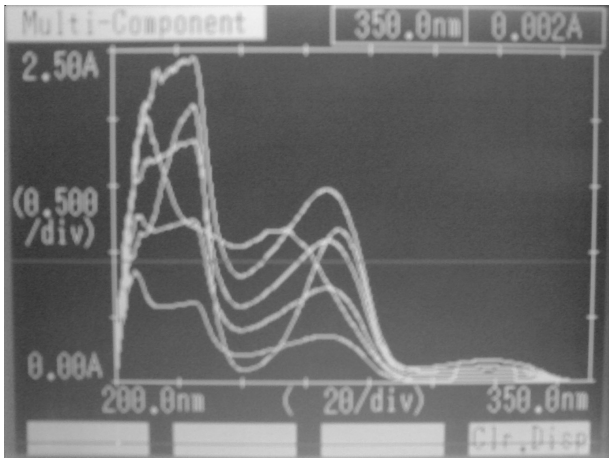



Fig. 3. UV multi-component mode overlay spectra for mixed standards of Olmesartan and Hydrochlorthiazide

**Validation of methods[25,26]:** Method-I was validated in terms of linearity, accuracy, precision, specificity and reproducibility of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of olmesartan and hydrochlorthiazide and measured the absorbance values at 270 and 296 nm. Calibration curves were constructed by plotting the absorbances against the amount of drug in µg/mL. Since, six mixed standards were used in method - II, it was validated for accuracy, precision, specificity and reproducibility.

**Recovery studies:** A known amount of each drug was added to preanalyzed tablet samples, at three levels of 80, 100 and 120 % of assay concentrations and the percentage recoveries were calculated. The results of recovery studies for both the methods are presented in Table-5.

**Repeatability:** Repeatability is given by inter and intraday precision. Intraday precision was determined by analyzing, the three different concentration of drug for three times in the same day. Interday precision was determined by analyzing the three different concentration of the drug for three days in a week; results are presented in Table-6. The precision of the assay was determined and % RSD was found to be lower than 0.88 % in method I and II.

**Ruggedness:** Ruggedness of the proposed methods was determined by analysis of aliquots from homogenous slot by different analyst, using similar operational and environmental onditions; the data for method I and II is presented in Table-7.

TABLE-7
RUGGEDNESS DATA

| Method | Drug (μg/mL) | | Analyst-I, Amount of drug (μg/mL) | | | | Analyst-II, Amount of drug (μg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OMS | HTZ | OMS | % RSD | HTZ | % RSD | OMS | % RSD | HTZ | % RSD |
| I | 10 | 6.25 | 9.89 | 1.08 | 6.25 | 0.79 | 9.88 | 1.12 | 6.22 | 1.04 |
| II | 10 | 6.25 | 9.89 | 1.61 | 6.23 | 0.82 | 9.84 | 1.22 | 6.27 | 0.69 |

Mean of six estimations; OMS = Olmesartan; HTZ = Hydrochlorthiazide.

## RESULTS AND DISCUSSION

Two wavelengths 255 nm ($\lambda_{max}$ for olmesartan) and 270 nm ($\lambda_{max}$ for hydrochlorthiazide) were selected for the simultaneous analysis of the drugs. Olmesartan and hydrochlorthiazide follow linearity in the concentration range of 3-24 and 2-16 μg/mL, respectively. Two brands of tablets were analyzed and amount of drug determined by proposed methods; it was in good agreement with the label claim. The proposed methods were validated as per the ICH guidelines. The recovery of drugs was determined at 80, 100 and 120 % level. The recovery ranges from 99.00 to 99.40 for olmesartan and 98.94 to 99.49 for hydrochlorthiazide respectively for method-I, which shows the accuracy of method. Method-II exhibits the recovery in a range of 99.08 to 99.67 for olmesartan and 98.93 to 99.79 for hydrochlorthiazide, respectively. Inter-day and intra-day precision of the assay were determined by analyzing the drug sample at three different concentrations. The inter-day and intra-day % RSD values for both the methods were calculated and were observed in the range of 0.25-0.53 for olmesartan and 0.18-0.88 for hydrochlorthiazide, respectively.

Ruggedness of proposed methods was determined with the help of two different analyst and results were evaluated by calculating the % RSD values found lying within the range of 1.08-1.61 for olmesartan and 0.69-1.04 for hydrochlorthiazide, respectively.

### Conclusion

The proposed methods for simultaneous estimation of olmesartan and hydrochlorthiazide in combined dosage form were simple, rapid, accurate, reproducible and useful for the routine determination of olmesartan and hydrochlorthiazide in tablet formulations.

## ACKNOWLEDGEMENTS

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