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Absorbance correction method for simultaneous determination of Olmesartan Medoxomil and Hydrochlorothiazide in combined tablet dosage form

Dharmesh T. Patel* and Dipti B. Patel

S. K. Patel College of Pharmaceutical Education & Research, Department of Pharmaceutical Chemistry, Ganpat University, Kherva, India

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ABSTRACT

A new, simple, accurate and sensitive UV-Spectrophotometric absorbance correction method has been developed and validated for simultaneous estimation of Olmesartan Medoxomil (OLME) and Hydrochlorothiazide (HCTZ) in a combined tablet dosage form. Methanol was used as solvent. The wavelengths selected for the absorbance correction method were 256 nm & 318 nm for Olmesartan Medoxomil and Hydrochlorothiazide respectively. The method was found to be linear between the range of 8-28 µg/ml for Olmesartan Medoxomil and 5-30 µg/ml for Hydrochlorothiazide. The mean percentage recovery was found in the range of 100.87% and 100.65% for Olmesartan Medoxomil and Hydrochlorothiazide respectively at three different levels of standard additions. The precision (intra-day, inter-day) of method were found within limits (RSD <2%). Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of Olmesartan Medoxomil and Hydrochlorothiazide in combined tablet dosage form.

Keywords: Olmesartan Medoxomil, Hydrochlorothiazide, Absorbance correction method, UV spectrophotometric, Validation.

INTRODUCTION

Olmesartan Medoxomil (Figure.1) is (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl 1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)]phenyl}methyl)-1 H-imidazole-5 carboxylate and it is a prodrug used as antihypertensive, which blocks the vasoconstrictor effect of angiotensin-II by selectively blocking the binding of angiotensin-II to the AT1 receptor in vascular smooth muscle[1,2]. This drug is official in United States Pharmacopoeia [3]. Literature survey reveals that HPLC [4-6], HPTLC [7], Capillary zone electrophoresis [8] and spectroscopic [9-11] methods have been reported for its determination alone and in combination with other drugs.

Hydrochlorothiazide (Figure.2) is chemically 6-chloro-3, 4- dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide. It is a diuretic and antihypertensive drug, which inhibits the reabsorption of sodium and calcium at the beginning of distal convoluted tubules. This drug is official in British Pharmacopoeia [12], Indian Pharmacopoeia [13] and United States Pharmacopoeia [14]. Literature survey reveals that HPLC [15-17], HPTLC [18], Capillary zone electrophoresis [19], GS-MS/MS [20, 21] and spectroscopic [22-24] methods have been reported for the estimation of Hydrochlorothiazide alone and with other drugs from pharmaceutical formulations and in biological fluids.

Figure 1: Structure of Olmesartan Medoxomil

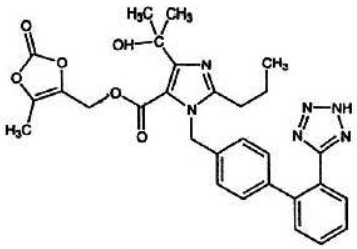
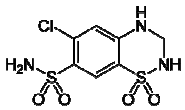


Figure 2: Structure of Hydrochlorothiazide



Literature survey reveals that several HPLC [25, 26], HPTLC [27] and spectroscopic [28- 30] methods have been reported for the estimation of Olmesartan Medoxomil and Hydrochlorothiazide in combined dosage form. So far no UV-visible spectroscopic method was reported by absorbance correction method of quantitative estimation of Olmesartan Medoxomil and Hydrochlorothiazide in combined dosage form. So, it was thought of interest to develop a new, simple, precise, accurate and cost effective method of analysis for the simultaneous estimation of Olmesartan Medoxomil and Hydrochlorothiazide in combined tablet dosage form by absorbance correction method.

MATERIALS AND METHODS

Olmesartan Medoxomil and Hydrochlorothiazide pure powder was obtained as a gift sample from Intas Pharmaceutical Limited (Ahmedabad, Gujarat, India). All the reagents used were of AR grade and procured from S. D. Fine chemicals Ltd., Mumbai, India. A double beam UV-visible spectrophotometer (UV–1800, Shimadzu, Japan) attached to computer software UV Prob 2.0 with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells. A Sartorius (CP224S) analytical balance and ultrasonic cleaner (Frontline FS-4) sonicator were used during the study. Tablet of Olmesartan Medoxomil and Hydrochlorothiazide were purchased from local pharmacy.

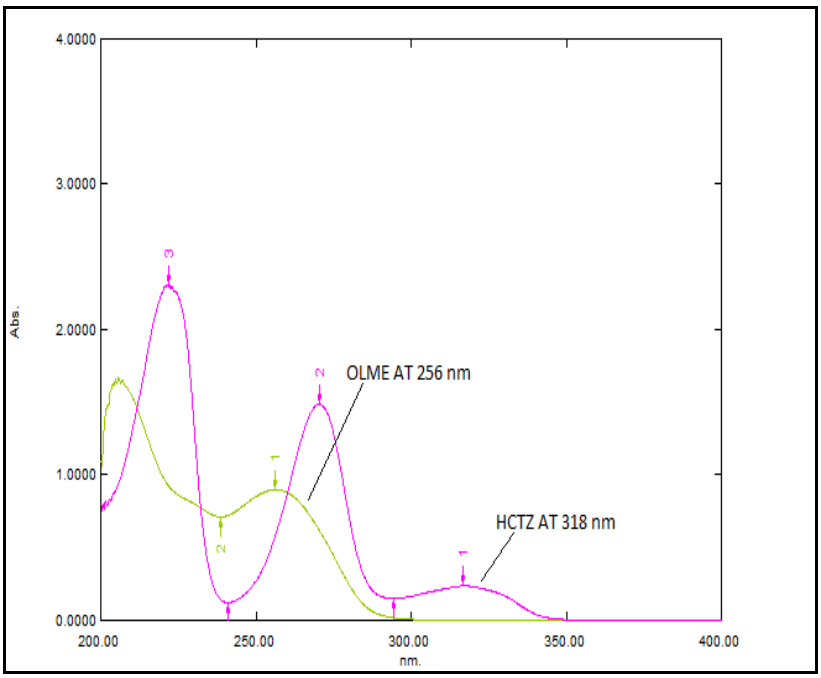


Figure 3. Overlay UV absorption spectra of Olmesartan Medoxomil (20 µg/ml) and Hydrochlorothiazide (20 µg/ml) in methanol

Method:
Standard solution of Olmesartan Medoxomil (20 µg/ml) and Hydrochlorothiazide (20 µg/ml) were scanned in UV range of 200 to 400 nm for determination of wavelength for estimation of Olmesartan Medoxomil and Hydrochlorothiazide. From the overlain spectrum of Olmesartan Medoxomil and Hydrochlorothiazide (Figure.3), the wavelength selected for estimation of Hydrochlorothiazide was 318 nm (λ1), where Olmesartan Medoxomil has no significant absorbance and Hydrochlorothiazide has some absorbance. For estimation of Olmesartan Medoxomil it was 256 nm (λ2), where absorbance of Olmesartan Medoxomil is corrected.

A standard stock solution of Olmesartan Medoxomil and Hydrochlorothiazide (100 µg/ml) was prepared by dissolving 10 mg of pure drug powder to 100 ml volumetric flask separately with methanol. Aliquots of standard stock solution of Olmesartan Medoxomil and Hydrochlorothiazide were suitably diluted with methanol to obtain the final concentration in the range of 8-28 µg/ml and 5-30 µg/ml respectively. The solution was scanned in the range of 200 nm to 400 nm against methanol as a blank, to obtain the absorbance. The measured absorbance at 256 nm for Olmesartan Medoxomil and at 318 nm Hydrochlorothiazide. The calibration curve was prepared by plotting concentration of Olmesartan Medoxomil or Hydrochlorothiazide vs. Absorbance of solution. Absorptivity of Hydrochlorothiazide was calculated at 318 nm (a_1) and 256 nm (a_2) and absorptivity of Olmesartan Medoxomil was calculated at 256 nm (a_3).

Method Validation
The method was validated according to the ICH guidelines [31].

Calibration curve (linearity)
Calibration curves were plotted over a concentration range of 8-28 µg/ml for OLME and 5-30 µg/ml HCTZ. Accurately measured standard working solutions of OLME (0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 ml) and HCTZ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were transferred to a series of 10 ml of volumetric flasks separately and diluted to the mark with methanol. Absorbance of all the solutions was measured at 256 nm and 318 nm against methanol solution as blank. Calibration curves were constructed by plotting absorbance vs. concentration of OLME and HCTZ, and the regression equations were calculated.

Accuracy (% Recovery)
The accuracy of the proposed method was determined by calculating recoveries of OLME and HCTZ by standard addition method. Known amounts of standard solutions of OLME and HCTZ were added at 50%, 100% and 150% levels to prequantified solutions of OLME (10 µg/ml) and HCTZ (6.25 µg/ml).

Repeatability
The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n=6) of OLME (20 µg/ml) and HCTZ (20 µg/ml) without changing the parameters of the proposed method. The results are reported in terms of percentage relative standard deviation (%RSD).

Intermediate precision
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of OLME (12, 16 and 20 µg/ml) and HCTZ (10, 15 and 20 µg/ml). The results are reported in terms of percentage relative standard deviation (%RSD).

Limit of detection (LOD) and limit of quantification (LOQ)
The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were calculated by using the following equations as per ICH guideline.

$$\mathbf{LOD = 3.3 \times \sigma / S}$$

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where, σ = the standard deviation of the response, S = slope of the calibration curve

Estimation of drug in combined dosage form:
For analysis of Olmesartan Medoxomil and Hydrochlorothiazide in tablet dosage form, twenty tablets were accurately weighed and powdered. A quantity of accurately weighed the tablet powder equivalent to 20 mg of Olmesartan Medoxomil and equivalent to 12.5 mg Hydrochlorothiazide was transferred to 10 ml volumetric flask containing 10 ml methanol, sonicated for 30 min. Finally volume was made up to the mark with methanol and further shaken for 15 min for complete extraction of from its matrix. Above solution filtered through whatman filter paper No.42 and diluted up to mark with methanol. Aliquot of above prepared sample solution was suitably diluted with methanol to obtain solution of Olmesartan Medoxomil (20 µg/ml) and Hydrochlorothiazide (12.5 µg/ml) and analyzed by absorbance correction method calculated as following,

$A = a * b * c$

$C_X = A_1 / a * b$

$C_X = A_1 / a_1 * b$..... 1

$A_2 = A (HCTZ) + A (OLME)$

$A_2 = (a_2 * C_X * b) + (a_3 * C_Y * b)$

$A_2 = (a_2 * C_X) + (a_3 * C_Y)$

$C_Y = [A_2 - (a_2 * C_X)] / a_3$..... 2

Where,
A_1 = Absorbance of sample solution at 318 nm
A_2 = Absorbance of sample solution at 256 nm
a_1 = Absorptivity of Hydrochlorothiazide at 318 nm
a_2 = Absorptivity of Hydrochlorothiazide at 256 nm
a_3 = Absorptivity of Olmesartan Medoxomil at 256 nm

Test solution containing Olmesartan Medoxomil (20 µg/ml) and Hydrochlorothiazide (12.5 µg/ml) was analyzed by proposed absorbance correction method. The absorptivity values calculated for Hydrochlorothiazide at 318 nm and 256 nm were 119.4 and 290.9, respectively and for Olmesartan Medoxomil at 256 nm were 449.5. Quantitative estimation of Olmesartan Medoxomil and Hydrochlorothiazide tablet was carried out by using above formula 1 & 2.

1. Concentration of Hydrochlorothiazide at 318 nm :
$A_1 = a_1 * b * C_X$

$C_X = A_1 / a_1 * b$

Where,
A_1 = absorbance of sample solution at 318
a_1 = A (1%, 1cm) of Hydrochlorothiazide at 318 nm
b = path length (b = 1)
C_X = concentration of Hydrochlorothiazide at 318 nm
C_X = 0.1488/119.4485 gm/100ml
C_X = 0.001245 * 1000 (Dilution factor)
C_X = 12.45 mg

2. Absorbance of Hydrochlorothiazide at 256 nm :

$A = a_2 * b * C_X$

Where,
A = absorbance of Hydrochlorothiazide at 256 nm

a_2 = A (1%, 1cm) of Hydrochlorothiazide at 256 nm
b = path length (b = 1)
C_X = concentration of Hydrochlorothiazide at 318 nm
A = 0.001245 * 290.9815
A = 0.3622

3. Calculation of concentration of Olmesartan Medoxomil from the corrected absorbance at 256 nm :

Corrected absorbance = A_{256} (Sample Solution) – A_{256} (Hydrochlorothiazide)

= 1.2688 - 0.3622

= 0.9066

Concentration of Olmesartan Medoxomil at 256 nm from corrected absorbance

$A_2 = a_3 * b * C_Y$

$C_Y = A_2 / a_3 * b$

Where,
A_2 = Corrected absorbance of Olmesartan Medoxomil at 256 nm,
a_3 = A (1%, 1cm) of Hydrochlorothiazide at 256nm
b = path length (b = 1)
C_Y = concentration of Olmesartan Medoxomil at 256 nm
C_Y = 0.9066 / 449.5718 gm/100ml
C_Y = 0.002016 * 1000 (Dilution factor)
C_Y = 20.16 mg

RESULTS AND DISCUSSION

The developed method was validated as per ICH guideline and validation parameters are summarized in Table 1.

Table 1: Regression analysis data and summary of validation parameters by proposed absorption correction method

Parameters	OLME	HCTZ
Wavelength (nm)	256	318
Beer's law limit	8-28	5-30
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.085	0.403
Molar extinction coefficient (l . mol^{-1} . cm^{-1})	3.56×10^4	25.09×10^4
Regression equation (y=mx+ C) **Slope (m)** **Intercept (C)**	 Y=0.0437X+0.0186 0.0437 0.0186	 Y=0.0118X+0.002 0.0118 0.002
Correlation coefficient (R^2)	0.9997	0.9999
LOD[a] (µg/ml)	0.62	0.34
LOQ[b] (µg/ml)	1.89	1.04
Repeatability (n=6) %RSD[d]	0.30	0.67
Precision (%RSD) **Intraday(n=3)** **Interday(n=3)**	 0.56-0.92 0.69-1.64	 0.81-0.99 0.97-1.82
%Recovery± S.D.[c] (n=3)	100.87±1.12	100.65±1.16
%Assay ± S.D. (n=6)	100.56±0.74	100.06±0.96

[a]Limit of detection, [b]Limit of quantification, [c]Standard deviation, [d]Relative standard deviation

The drugs obeys beer's law in the concentration range of 8-28 µg/ml and 5-30 µg/ml for Olmesartan Medoxomil and Hydrochlorothiazide respectively. The high value of correlation coefficient suggested that the proposed method is linear in the stated range.

The %RSD value for repeatability study was Olmesartan Medoxomil and Hydrochlorothiazide was found to be <2%, which indicates that the proposed method was repeatable. The low values of % RSD for intraday and interday precision indicate that the method was precise and reproducible. Low value of LOD and LOQ describe the method was sensitive.

Recovery studies were done by standard addition method by adding known quantity of standard solution (50 %, 100 % and 150 % levels) to preanalyzed sample solution and the mixtures were reanalyzed by proposed method. The results are shown in Table 1.

The assay results obtained was 100.56±0.74 for Olmesartan Medoxomil and 100.06 ± 0.96 for Hydrochlorothiazide, indicate that the excipients do not interfere during the analysis normally present in the tablet. The results are shown in Table 2.

Table 2. Analysis of Olmesartan Medoxomil and Hydrochlorothiazide in tablets by proposed absorbance correction method

Sample No.	Label Claim		Amount Found		% Label Claim	
	OLME (mg/tab)	HCTZ (mg/tab)	OLME (mg/tab)	HCTZ (mg/tab)	OLME	HCTZ
1	20	12.5	20.19	12.32	100.95	98.56
2	20	12.5	20.12	12.57	100.6	100.56
3	20	12.5	20.16	12.49	100.8	99.92
4	20	12.5	20.23	12.54	101.15	100.32
5	20	12.5	19.82	12.68	99.1	101.44
6	20	12.5	20.16	12.45	100.8	99.6
Mean					**100.56**	**100.06**
±S.D.[a]					**0.74**	**0.96**

[a]*Standard deviation.*

CONCLUSION

The proposed absorbance correction method was found to be linear between the range of 8-28 µg/ml for Olmesartan Medoxomil and 5-30 µg/ml for Hydrochlorothiazide. The mean percentage recovery was found 100.87% and 100.65 for Olmesartan Medoxomil and Hydrochlorothiazide, respectively at three different levels of standard additions. The precision (repeatability, intra-day and inter-day) of methods were found within limits (RSD <2%).

It could be concluded from the results obtained in the present investigation that the proposed method for the simultaneous estimation of Olmesartan Medoxomil and Hydrochlorothiazide in tablet dosage form is simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

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REFERENCES

[1] Maryadele, J. O'Neil., Eds., In; The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edn., Merck & Co., Inc., Whitehouse Station. NJ, **2006**, 827, pp. 1178.
[2] Goodman & gillman, Pharmacology basis of therapeutics: 9th ed.; Mcgraw-hill medical publishing division New Delhi, **1996**,pp. 780-781.
[3] The United States Pharmacopoeia: 32, NF, 27 Vol.3, The U.S. Pharm. Convention, INC. Rockville, MD, **2009**: 4057-4058.
[4] Purnima. D. Hamrapurkar, Kamalesh. K. Gadapayale, *International J. Sci. & Engineering.*, **2013**, 11(2), 137-147.
[5] Jayvadan K. Patel, Nilam K. Patel, *sci. pharm.*, **2014**, 82, 541-554.
[6] Kevin H. Vachhani, Satish A. Patel, Himanshu H. Vachhani, *International J. Intitutional pharm. Life Sci.*, **2012**, 2(2), 390-399.

[7] Ambadas R. Rote, Sadhana K. Kande, *J. Anal Bioanal. Techniques*, **2011**, 2, 128.
[8] Mustafa Celebier, Sacide Altinoz, *Chromatographia*, **2007**, 66, 929-933.
[9] Tripti Sharma, Sudam Chandra Si, D. Gowrishankar, *International J. Drug Delivery*, **2012**, 4, 134-138.
[10] Nikita N. Patel, Parag R. Patel, Falguni A. Tandel, Charmy S. Kothari, Shailesh A. Shah, *International J. Pharmacy & Pharm. Sci.*, **2012**, 4(5) , 222-226.
[11] Isha J. Soni, Hiral J. Panchal, *Indian J. Pharm. Biol. Res.*, **2014**, 2(1), 76-81.
[12] British Pharmacopeia, 4th Ed., Vol.I, Her Majesty's Stationary Office, London, UK, **2010**, 1051.
[13] Indian Pharmacopoeia, Vol. I., The Controller of Publication, New Delhi, **2010**, 2, 1451-1452.
[14] The United States Pharmacopoeia: 32, NF, 27 Vol.2, the U.S. Pharm. Convention, INC. Rockville, MD, **2009**: 2566-2567.
[15] Sachin Bhagwate, N. J. Gaikwad, *J. Applied Pharm. Sci.*, **2013**, 3(2), 88-92.
[16] Aruna G., Chanukya M., Reddy Mohan V., *International J. Medi. Chemi. Anal.*, **2012**, 2(1), 57-61.
[17] Mahesh. M, Kumanan. R, Jayaveera. K.N, *International J. Pharm. Res.*, **2011**, 3(2), 119-122.
[18] Pradip N. Bhoya, Emanual M Patelia, Gautambhai, *J. Chromat. Separation Techniq.* **2013**, 4(1), 1-4.
[19] Manoela R. Balesteros, Adriana F. Faria, Marcone A. L. de Oliveira, *J. Brazil Chem. Soc.*,**2007**, 18(3), 554-558.
[20] Lindstrom B., Molander M., *J. Chromat.*, **1975**, 114, 459-462.
[21] Olga Zaporozhets, Iuna Tsyrulneva, Mykola Ischenko, *American J. Anal. Chemi.* **2012**, 3, 320-327.
[22] S. A. Hapse, V. S. Wagh., P. T. Kadaskar, M. D. Dokhe., A. S. Shirsath., *Der Pharma Chemica*, **2012**, 4(1), 10-14.
[23] Khan SA, Kulkarni SS., Biyani KR, Khan BA, *International J. Res. Pharma. Biomedical Sci.*, **2013**, 4(3), 795-801.
[24] Sridharan D., Thenmozhi A., Rajamanickam V., Sundaranandavalli S., Palanikumar B., *International J. Chem Tech Res.*, **2010**, 2(2), 876-879.
[25] Raja B., A. Lakshmana Rao, *International J. Res. Pharm. Chem.*, **2011**, 1(3), 714-717.
[26] Maitreyi Zaveri , Bhavita Dhru, Jemish Parmar, Viral Brahmbhatt, Khandhar Amit., *Indian J. Pharm. Res. Bio-Sci.*, **2012,** 1(1), 1-13.
[27] N. J. Shah, B. N. Suhagia, R. R. shah, N. M. Patel, *Indian J. Pharma. Sci.*, **2007**, 69(6), 834-836.
[28] K. P. Bhusari, P. B. Khedekar, S. Dhole, V. S. Banode, *Indian J. Pharm. Sci.*, **2009**, 71(5), 505-508.
[29] Jyoti V. Jadhav, K. B. Burade, *Der Pharma Chemica*, **2013**, 5(4), 252-261.
[30] A. R. Rote, P. D. Bari, *Indian J. Pharm. Sci.*, **2010**, 72, 111-113.
[31] ICH, Q2A Text on validation of analytical procedures. International Conference on Harmonization: Oct. **1994**.