IJPSR (2010), Vol. 1, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 July, 2010; received in revised form 07 October, 2010; accepted 01 November, 2010

DEVELOPMENT AND VALIDATION OF A REVERSED PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM

Moynul Hasan*[1], Abdullah Al Masud [2] and Jamiuddin Ahmed [3]

Department of Pharmacy, Dhaka International University [1], Banani, Dhaka, Bangladesh
Product Development Officer, ACI Pharmaceuticals Ltd., [2] Dhaka, Bangladesh
Department of Pharmacy, Primeasia University [3], Banani, Dhaka, Bangladesh

Keywords:

Reversed Phase HPLC,

Olmesartan medoxomil,

Hydrochlorothiazide,

Validation

Correspondence to Author:

Moynul Hasan

Department of Pharmacy, Dhaka International University, Banani, Dhaka, Bangladesh

ABSTRACT

A simple reversed phase HPLC method has been developed for the simultaneous determination of olmesartan medoxomil in combination with hydrochlorothiazide. The method was based on reversed phase liquid chromatography using a Grace Smart RP C_{18} column (250 × 4.6 mm, 5µ) with UV detection at 256 nm. The mobile phase consisting of acetonitrile and triethylamine buffer adjusted to pH 2.7 in a ratio of (30:70, v/v) and at a flow rate of 2 ml**/**min. The method was linear over the concentration range for olmesartan medoxomil 4.0-40.0 µg/mL and for hydrochlorothiazide 5.0-50.0 µg/mL. The recoveries of olmesartan medoxomil and hydrochlorothiazide were found to be in the range of 99.25-100.46% and 99.31-102.13% respectively. The method was validated and was successfully employed for the analysis of pharmaceutical formulations containing olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage form.

INTRODUCTION: Chemically Hydrochlorothiazide (HCT) is 6- chloro- 3, 4-dihydro- 2H- 1, 2, 4- benzothiadiazine- 7-sulphonamide 1, 1- dioxide (**Fig. 1**), one of the oldest and widely used thiazide diuretics [1]. On the other hand Olmesartan medoxomil (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- {[2′- (1H- tetrazol- 5-yl) [1, 1′- biphenyl]- 4- yl] methyl}- 1H- imidazole-5- carboxylic acid (**Fig. 2**) and is a selective AT_1 subtype angiotensin II receptor blocker [2, 3].

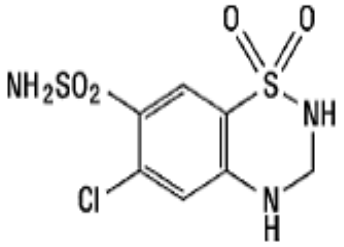

FIG. 1: HYDROCHLOROTHIAZIDE

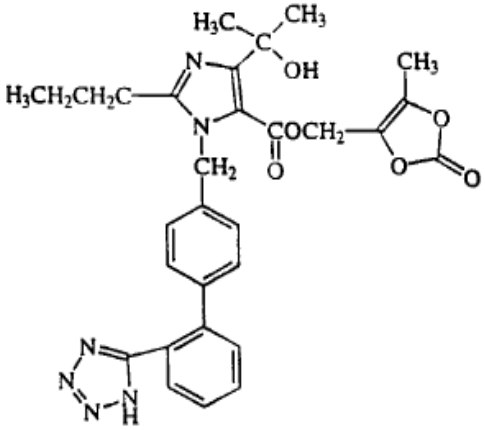

FIG. 2: OLMESARTAN MEDOXOMIL

The USP describes an RP-HPLC method for the determination of HCT in tablets. Several analytical methods have been reported for the determination of HCT in pharmaceutical formulations including polarography, LC, HPTLC and spectrofluorometry [4-10]. OLM has not yet been officially described in any pharmacopoeia and several analytical methods were reported for its determination in biological sample such as plasma [11-13]. OLM determination has been reported for single preparations or in combination with other antihypertensive drugs [10, 14, 15]. A literature survey revealed that very few analytical methods have been reported for the determination of HCT and OLM in a combined tablet formulation. So in this present investigation, an attempt has been made to develop accurate, precise and economically viable reversed phase HPLC method for the simultaneous estimation of hydrochlorothiazide and olmesartan medoxomil in combined tablet dosage form.

MATERIALS AND METHODS:

Apparatus and chromatographic condition: The chromatographic separation was performed on a Shimadzu HPLC Promoinence series, integrated with PDA (Photo Diode Array) detector. The analytical Grace Smart C18 column (25cm x 4.6mm i.e., 5 µm) was used for the separation. The mobile phase consisted of acetonitrile and triethylamine buffer (consisting of 5ml triethylamine in 1000 ml distilled water adjusted to pH 2.7) in a ratio of 70:30 (v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 2 ml/min and the detector wavelength was set at 256 nm. The injection volume was 20 µl.

Chemicals and Reagents: The pharmaceutical grade pure samples of Olmesartan medoxomil (99.28%) supplied by Zhejiang Tianu Pharma co. Ltd. China, Hydrochlorothiazide (99.55%) from INFA, Italy. HPLC grade Acetonitrile (Scharlau Chemie S. A., Spain) and Analytical grade Triethylamine and Orthophosphoric acid (Scharlau Chemie S. A., Spain) were used.

Preparation of Stock Solutions of Standard OLM and HCT: Stock solutions of 1.0 mg/mL OLM and 1.25 mg/mL HCT were prepared in mobile phase. The standard working concentrations of mixed OLM (20 µg/mL) and HCT (25 µg/mL) were prepared in the mobile phase. This solution was subjected to liquid chromatographic analysis.

Analysis of Tablet Formulation: Ten tablets were weighed accurately and powdered. Powder equivalent to 100 mg OLM and 125 mg HCT was weighed and transferred to a 100 mL volumetric flask. It was dissolved in 60 mL mobile phase by shaking the flask for 15 min and sonicated for 3 minutes. Then the volume was adjusted up to the mark with the same solvent and mixed well. Then it was first filtered through a 0.45µm whatman filter paper and then with 0.2µ disk filter. A final concentration of 20 µg/mL of OLM and µg/mL of HCT were prepared and concentrations of the OLM and HCT were calculated from the calibration graph.

RESULT AND DISCUSSION: All of the analytical validation parameters for this proposed method were determined according to ICH guideline [16]. Obtained validation parameters are presented in **Table 1**.

TABLE 1: ANALYTICAL VALIDATION PARAMETERS

Parameter	OLM	HCT
Linearity range	4.0-40.0 µg/mL	5.0-50.0µg/mL
Correlation Coefficient	1.00	0.999
Limit of detection (LOD)	103.68 ng/ml	38.592 ng/ml
Limit of quantitation (LOQ)	345.6 ng/ml	120.06 ng/ml
Accuracy (%)	99.25-100.46	99.31-102.13
Retention time (min)	7.91	1.93
Resolution	18.737	0.00
No. of theoretical plates	4434	2288
Tailing factor	1.56	1.65

Linearity: The linearity for HPLC method was determined at ten concentration levels ranging from 5-50 µg/mL for HCT and 4-40 µg/mL for OLM**.** The calibration curve was constructed by plotting response factor against respective concentration of OLM and HCT. The plots of peak area Vs respective concentration of OLM and HCT were found to be linear in the range of 4.0-40.0 µg/mL and 5.0-50.0 µg/mL with coefficient of correlation (r^2) 1.00 and 0.999 for OLM and HCT respectively (Table 1).

Recovery: Five different samples of known concentration ranging from 4-40 µg/mL for OLM and 5-50 µg/mL for HCT were prepared and these are analyzed against standard solution**.** The result of recovery analysis of olmesartan medoxomil and hydrochlorothiazide were found to be in the range of 99.25-100.46% and 99.31-102.13% respectively reported in Table 1**.**

Sensitivity: The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 103.68 ng/ml and 345.6 ng/ml for OML and 38.592 ng/ml and 120.064 ng/ml for HCT (Table 1). The LOD and LOQ showed that the method is sensitive for OLM and HCT.

System suitability test: The specificity of this method was determined by complete separation of OLM and HCT as shown in **Fig. 3** with parameters like retention time, resolution and tailing factor (Table 1). Here tailing factor for peaks of OLM and HCT was less than 2% and resolution was satisfactory. The average retention time ± standard deviation for OLM and

HCT were found to be 7.911±0.004 and 1.939±0.005 respectively, for five replicates. The peaks obtained for OLM and HCT were sharp and have clear baseline separation. Analysis were also performed for active OLM & HCT, placebo (All the ingredients except active OLM & HCT) and sample both stressed (at 105°C for 24hr) and unstressed condition. After analysis it was found that there is no interference of peak in the Olmesartan medoxomil & Hydrochlorothiazide region for the stressed sample, Placebo & Active. Hence the developed method was specific for the analysis of this product**.**

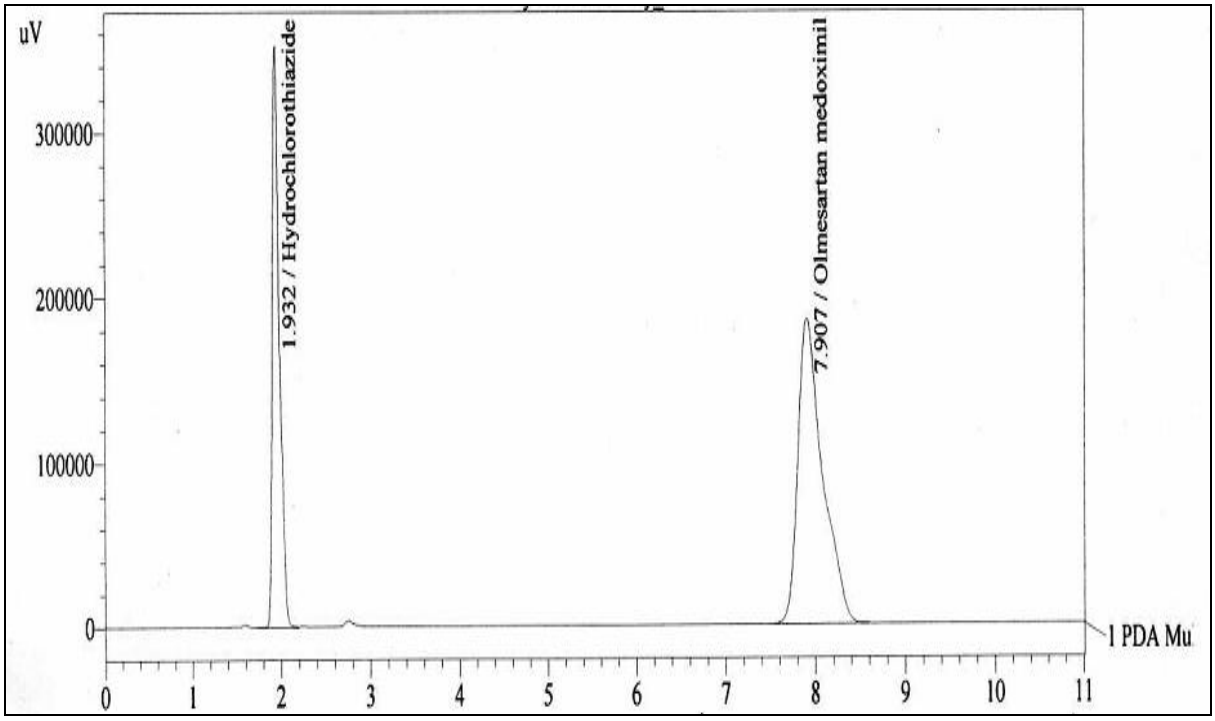

FIG. 3: TYPICAL HPLC CHROMATOGRAM OF OLMESARTAN MEDOXOMIL OLM (20 µg/mL) AND HYDROCHLOROTHIAZIDE HCT (25 µg/mL)

Ruggedness and Robustness: Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results show that for both solutions, the retention time and peak area of HCT and OLM remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process.

CONCLUSION: The developed HPLC method is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of

olmesartan medoxomil and hydrochlorothiazide in a combined tablet dosage form.

ACKNOWLEDGEMENT: The authors express their gratitude to ACI Pharmaceuticals Ltd. for providing necessary facilities.

REFERENCES:

1. British pharmacopoeia: The Stationery Office, London, Vol I, 2007: 1036-1037.
2. Martindale: The complete drug reference. Lambeta high street, London, 35th Edition, 2006.
3. Julie AB and John MB: Olmesartan Medoxomil: New Signs in Hypertension, New drug, Olmesartan medoxomil, Anangiotensin II-receptor blocker. Clinical Therapeutics 2004; 26 (1): A28-A32.
4. Fei L, Yu X, Shu G, Jundong Z and Qingxiang G: Determination of hydrochlorothiazide in human plasma by liquid chromatography/tandem mass spectrometry. J Pharm Biomed Analysis 2007; 44:1187–1191.
5. Hillaert S and Van den Bossche W: Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis. J Pharm Biomed Analysis 2003; 31:329-339.
6. Taomin H, Zhong He, Bei Y, Luping S, Xiaowei Z and Gengli D: Simultaneous determination of captopril and hydrochlorothiazide in human plasma by reverse-phase HPLC from linear gradient elution. J Pharm Biomed Analysis 2006; 41:644–648.
7. Sathe SR and Bari SB: Simultaneous analysis of losartan potassium, atenolol and hydrochlorothiazide in bulk and in tablets by high-performance thin-layer chromatography with uv absorption densitometry. Acta chromatographica 2007; 19:270-278.
8. Al-momani F: Determination of Hydrochlorothiazide and Enalapril Maleate in Tablet Formulations by Reversed-Phase HPLC. Turk J Chem 2001; 25:49-54.
9. Hao L, Yingwu W, Yao J, Yunbiao T, Jiang W, Limei Z and Jingkai G: A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma. Euro J Pharmacology 2003; 460 (1):19-26.
10. Shah NJ, Suhagia BN, Shah RR and Petal NM: Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007; 69(6):834-836.
11. Klaus OS: Olmesartan compared with other angiotensin II receptor antagonists: Head-to-head trials. Clin Ther 2004; 26:A33-37.
12. Gonzalez L, Lopez JA, Alonso RM, Jimenez RM: A fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr 2002; 949:49-60.
13. Venkata C, Ram S, Silfani T: A comparative analysis of the efficacy of olmesartan medoxomil (O)/hydrochlorothiazide (HCTZ), valsartan (V)/HCTZ, irbesartan (I)/HCTZ and telmisartan (T)/HCTZ combinations. Am J Hypertens 2004; 17:S121.
14. Sagirli O, Onal A, Toker SE and Ensoy DS: Simultaneous HPLC Analysis of Olmesartan and Hydrochlorothiazide in Combined Tablets and *in vitro* Dissolution Studies. Chromatographia 2007; 66:213-218.
15. Chen M, Zhou B and Wei D: HPLC determination of olmesartan medoxomil- hydrochlorothiazide tablets. Chinese J New Drugs 2006; 15(23):2050-2052.
16. ICH guidelines, analytical method validation (Q3). Geneva: July 2000.

****