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Validated stability indicating gradient RP-HPLC method for the estimation of antihypertensive drugs in bulk and pharmaceutical dosage forms

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ABSTRACT

A rapid and precise RP-HPLC method for determination of Olmesartan medoxomil and Hydrochlorothiazide in bulk and pharmaceutical dosage forms. Olmesartan medoxomil & Hydrochlorothiazide are found to be degraded together under different set of conditions as followed according to ICH guidelines and the degradants so formed along with olmesartan & hydrochlorothiazide are separated by using INERTSIL ODS C18 3V (150 x 4.6, 5µ) using mobile phase 1ml triethanolamine in one litre water and the pH was adjusted to 2.5 with orthophosphoric acid and acetonitrile using a gradient program with a flow rate of 1ml/min, throughout the gradient program with a detection wavelength of 225nm for both the compounds with a injection volume of 10µl. The method was validated for selectivity, linearity, accuracy, robustness, precision and specificity. The results were indicating the method was selective in analysis of both olmesartan medoxomil and hydrochlorothiazide in the presence of degradation products formed under various stress conditions.

Key Words: Olmesartan medoxomil, Hydrochlorothiazide, INERTSIL ODS C_{18}, Orthophosphoric acid, Acetonitrile, ICH Guidelines.

INTRODUCTION

Olmesartan medoxomil (Figure 1) chemically it is 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester. It works by blocking a substance in the body that causes blood vessels to tighten. As a result, olmesartan relaxes blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart (The Merck Index). Hydrochlorothiazide (Figure 2) is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. It reduces the amount of water in the body by increasing the flow of urine, which helps lower the blood pressure (Black, 2009).Olmesartan medoxomil and Hydrochlorothiazide are introduced into the market in combined dosage form, which is widely used in the treatment of hypertension. Literature review reveals that the methods for olmesartan and hydrochlorothiazide alone or in combined dosage forms are Development and Validation of Spectrophotometric and RP-HPLC Method for Estimation of Olmesartan Medoxomil in Tablet dosage form (Jain *et al.*, 2010).Validated Absorption Factor Spectrophotometric and Reversed-Phase High Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations (Chintan V. Patela *et al* 2007). Development of UV Spectrophotometric method for the simultaneous estimation of olmesartan Medoxomil and atorvastatin calcium in tablet by simultaneous equation and first order derivative method (Nagavalli *et al.*, 2011). Development and

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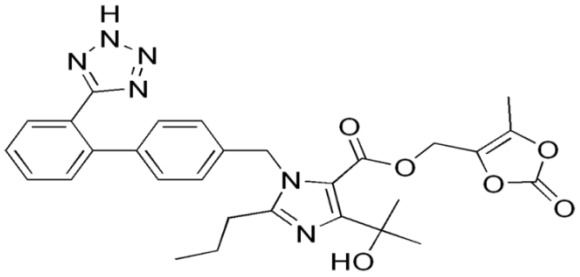

Figure 1: Structure of Olmesartan medoximil.

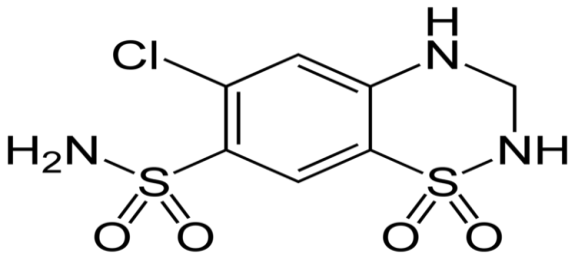

Figure 2: Structure of Hydrochlorothiazide.

validation of Spectrophotometric method for simultaneous estimation of Metoprolol succinate and Olmesartan medoxomil in Tablets (Vachhani *et al.,* 2011). Simultaneous Quantitation of Olmesartan medoxomil and Amlodipine Besylate in Combined Tablets Using HPLC (Syed *et al.,* 2009). Spectrophotometric Method for Simultaneous determination of Olmesartan medoxomil and Amlodipine Besylate from Tablet dosage forms (Pournima Patil *et al.,* 2011). UV spectrophotometric Determination of Hydrochlorothiazide and Olmesartan Medoxomil in pharmaceutical Formulation (Hemke *et al.,* 2010). Spectrophotometric Estimation of Olmesartan Medoxomil and Hydrochlorthiazide in Tablet (Rote *et al.,* 2010). Spectrophotometric Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Form (Rekha *et al.,* 2010). RP-HPLC Method for Simultaneous Estimation of Telmisartan & Hydrochlorothiazide in Tablet Dosage Forms (Wankhede *et al.,* 2008). A Validated Stability Indicating HPTLC Method for Simultaneous Estimation of Irbesartan and Hydrochlorothiazide (Amol *et al.,* 2010). Simultaneous Analysis of Eprosatan and Hydrochlorothiazide in Tablets by HPLC (Harsha U. Patel *et al.,* 2011). Development and Validation of a RP-HPLC for the Simultaneous Estimation of Atenolol and Hydrochlorothiazide in Pharmaceutical Dosage Forms (Zaveri *et al.,* 2010).Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in combined tablet dosage form by Multicomponent Mode of analysis (Tarte *et al.,* 2008). Spectrophotometric Simultaneous Determination of Amlodipine Besylate And Hydrochlorothiazide In Combined Tablet Dosage Form By Simultaneous Equation, Absorption Ratio And First Order Derivative Spectroscopy Methods(Vijaya *et al.,* 2011).

EXPERIMENTAL

Chromatographic Conditions: Olmesartan medoxomil & hydrochlorothiazide reference standards was supplied by M/s Microlabs limited, Bangalore, India. HPLC grade Acetonitrile was purchased from Merck (Mumbai, India) and triethanolamine and orthophosphoric acid from Merck (Mumbai, India). All chemicals were of analytical grade. The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. The column used in the development for the determination is INERTSIL ODS C18 (150 x 4.6, 5µ). The detector wavelength was set at 225nm for both the components. A flow rate of 1ml/min was used for the determination of olmesartan and hydrochlorothiazide. The samples and standards were dissolved in diluent (water: ACN, 30:70) and 10µL sample were injected into HPLC system at the column and sample temperature of 30°C.

Mobile phase

1.0 ml of Triethanolamine was mixed with 1000mL of milli Q water and pH was adjusted to 2.5±0.05 with Orthophospharic acid, filtered through 0.45µm Membrane filter. pH 2.5 Triethanolamine: Acetonitrile mixture used as a mobile phase injected into the system through a gradient flow indicated in Table 1.

Table 1: Gradient Program.

Time (min)	Flow Rate ml/min	%Mobile Phase A (pH 2.5 Triethanolamine)	%Mobile Phase B (Acetonitrile)
0	1	75	25
5	1	50	50
7	1	50	50
8	1	75	25
12	1	75	25

Table: 2 Method Development conditions.

Trial	Type of column	Mobile phase composition	Injection volume	Flow	Defect
1	C_{18}(150 x 4.6),5µm	Isocratic Water : Methanol (40:60)	20µl	1ml/min	More Rt & less no. of theoretical plates
2	C_{18} (150 x 4.6),5µm	Isocratic Water : Acetonitrile (30: 70)	20µl	1ml/min	Split peeks, less number of theoretical plates
3	C_{18}(150 x 4.6),5µm	Isocratic Methanol:acetonitrile (40: 60)	20µl	1ml/min	More Rt
4	C_{18}(150 x 4.6),5µm	Isocratic Buffer: Acetonitrile (30: 70)	20µl	1ml/min	Resolution was not so good
5	C_{18}(150 x 4.6),5µm	Gradient Buffer (pH 3.5): Acetonitrile	20µl	1ml/min	Resolution was not so good
6	C_{18}(150 x 4.6),5µm	Gradient Buffer (pH 2.5):Acetonitrile	20µl	1ml/min	Less Rt, More theoretical plates, less tailing, symmetrical peek shape, Good resolution

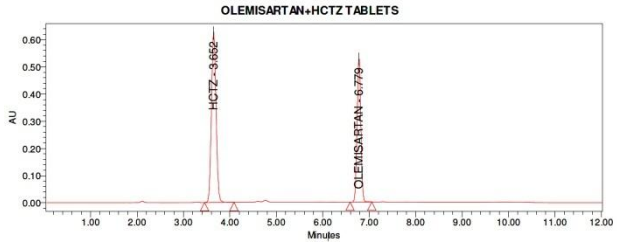

Figure 3: Sample chromatogram for Hydrochlorothiazide and Olmesartan.

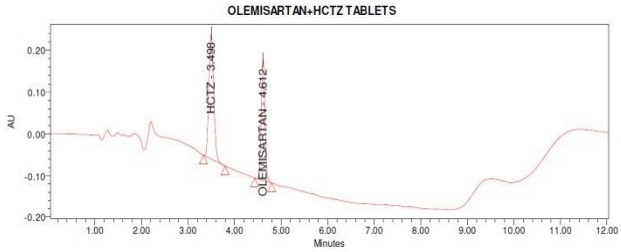

Figure 4: Acid degadation chromatogram of olmesartan & hydrochlorothiazide.

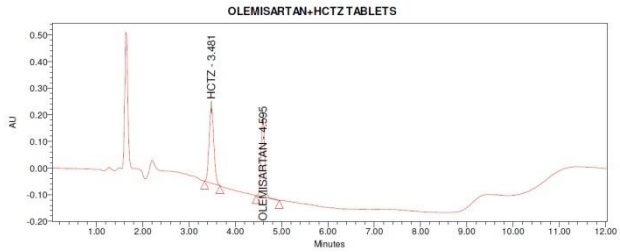

Figure 5: Basic degradation chromatogram of olmesartan & hydrochlorothiazide.

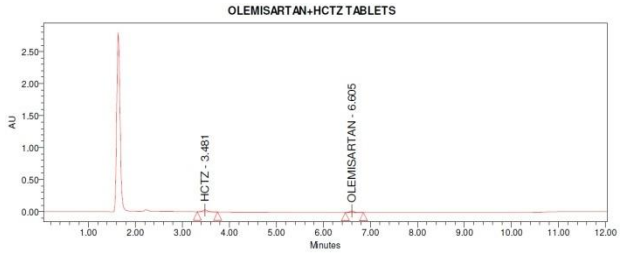

Figure 6: Peroxide degradation chromatogram of olmesartan & hydrochlorothiazide.

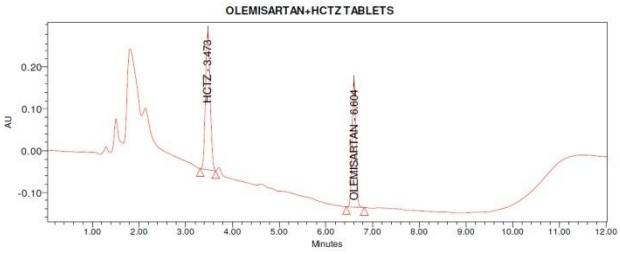

Figure 7: Reduction degradation chromatogram of olmesartan & hydrochlorothiazide.

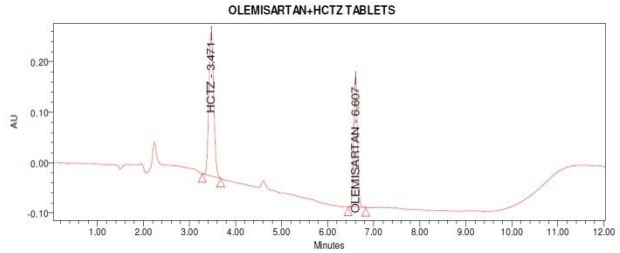

Figure 8: Thermal degradation chromatogram of olmesartan & hydrochlorothiazide.

Table 3: Assay of Olmesartan & Hydrochlorothiazide.

Brand name	Compound	Amount found (mg)	% Assay
OLSAR-H	Olmesartan	20.04	100.2
	Hydrochlorothiazide	12.7	101.7
OLMEZEST-H-20	Olmesartan	20.1	100.5
	Hydrochlorothiazide	12.7	101.6

Table 4: Precision studies for Olmesartan & Hydrochlorothiazide.

Compound	% Assay (Day-1, Analyst-1, Instrument-1)	% RSD of Assay (N=6)	% Assay (Day-2, Analyst-2, Instrument-2)	% RSD of Assay (N=6)
Olmesartan medoximil	99.45	0.26	99.40	0.08
Hydrochlorothiazide	100.3	0.31	100.6	0.22

Table 5: Recovery studies for Olmesartan & Hydrochlorothiazide.

Drug	Amount recovered (mg)	% recovery	Mean % recovery	% RSD
Olmesartan medoximil	100.80	99.8	99.8	0.170
	200.03	99.5		0.080
	300.50	99.5		0.030
Hydrochlorothiazide	63.20	99.5	99.60	0.160
	125.70	99.7		0.170
	187.60	99.5		0.170

Table 6: Stability studies for Olmesartan & Hydrochlorothiazide.

Experiment		% Degradation	Purity Angle	Purity Threshold
Acid	Olmesartan	25.9	0.024	1.085
(1N HCL,1ml)	Hydrochlorothiazide	26.8	0.022	1.058
Alkali	Olmesartan	22.6	0.095	1.041
(1N NaOH, 1ml)	Hydrochlorothiazide	27.1	0.023	1.022
Oxidation	Olmesartan	26.7	0.051	1.005
(30% H_2O_2, 2ml)	Hydrochlorothiazide	27.1	0.099	1.072
Reduction	Olmesartan	17.0	0.059	1.051
(10% Sodium bisulphate, 2 ml)	Hydrochlorothiazide	27.2	0.039	1.095
Thermal	Olmesartan	29.7	0.026	1.014
(Heating at 70°C for 1hr)	Hydrochlorothiazide	27	0.044	1.066

Table 7: Robustness studies for Olmesartan & Hydrochlorothiazide.

Sl. No.	Condition	% RSD	
		Olmesartan	Hydrochlorothiazide
1	Flow (+20%)	0.55	0.2
2	Flow (-20%)	0.11	0.15
3	Temperature (35°C)	0.29	0.06
4	Temperature (40°C)	0.3	0.21
5	Wavelength (+5nm)	0.29	0.2
6	Wavelength (-5nm)	0.13	0.38
7	pH of buffer 2.6	0.55	0.68
8	pH of buffer 2.4	0.25	0.3
9	Organic (+2%)	0.4	0.32
10	Organic (-2%)	0.25	0.1

Preparation of standard and sample solutions

Accurately weighed and transferred about 40 mg of Olmesartan Medoxomil, 30 mg of Hydrochlorothiazide working standards into two separate 100ml volumetric flasks, add about 70 ml of diluent and sonicated to dissolve, cool the solution to room temperature & dilute to the volume with diluent. 5ml of Olmesartan medoxomil standard stock solution, 4ml of Hydrochlorothiazide Standard Stock solution was transferred into 20ml volumetric flask and dilute to the volume with diluent.

Ten tablets were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 20mg of Olmesartan Medoxomil was transferred into a 250ml volumetric flask. 200ml of diluent was added, sonicated for 30minutes with occasional stirring. Cool the solution to room temperature and dilute to the volume with diluent, filtered the solution through 0.45µm Teflon filter syringe. 3ml of the above filtered solution was transferred into a 25ml volumetric flask and diluted to the volume with diluent.

RESULTS AND DISCUSSION

The conditions tested for method development (Table 2) indicates that all the system suitability parameters according to ICH guidelines was achieved by using INERTSIL ODS C18 (150 x 4.6, 5µ) column using mobile phase Triethanolamine in water and the pH was adjusted to 2.5 with Ortho-phosphoric acid and acetonitrile using a gradient program with a flow rate of 1ml/min throughout the gradient program with a detection wavelength of 225nm for both the compounds with a injection volume of 10µl (Figure 3).

To validate the RP-HPLC method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range of 25-150µg/ml & 15-90µg/ml for olmesartan & hydrochlorothiazide and correlation coefficient was found to be 0.99988 & 0.99984 for both the compounds respectively. The precision (expressed as the relative standard deviation (RSD) was determined for olmesartan & hydrochlorothiazide for repeated analysis and the values are presented in Table 4. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding a fixed amount of drug to preanalysed formulation summarized in Table 3 and Table 5.

The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The following values in Table 6 indicate that any other impurity is not merging with the main peak (Figure 4-8). The analyte solution was stable up to 24hrs.The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values (Table 7) obtained, an indication of its reliability on normal usage. A method was developed for the determination of olmesartan & hydrochlorothiazide in tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

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