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Validated Chromatographical methods for the Estimation of Antihypertensive Drugs in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

Two new, rapid, precise, accurate and specific chromatographic methods for the simultaneous determination of Olmesartan medoxomil and Hydrochlorothiazide in combined pharmaceutical dosage forms. The first method based on reverse phase liquid chromatography 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.The second method involved silica gel 60F254 high performance thin layer chromatography and densitometric detection at 270nm using chloroform : methanol(85:15) as the mobile phase

Keywords: Olmesartan medoxomil; Hydrochlorothiazide; high performance thin layer chromatography; reverse phase liquid chromatography.

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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[1]. 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[2].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[3].Validated Absorption Factor Spectrophotometric and Reversed-Phase High Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations[4].

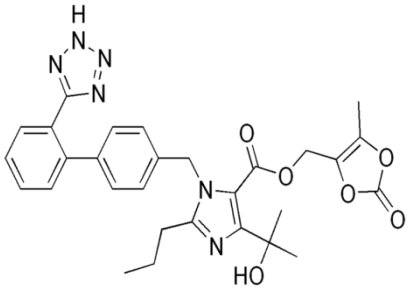

Figure 1: Structure of Olmesartan medoximil

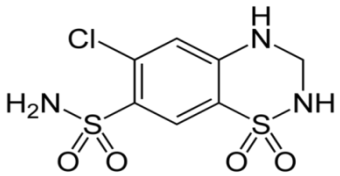

Figure 2: Structure of Hydrochlorothiazide

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[5]. Development and validation of Spectrophotometric method for simultaneous estimation of Metoprolol succinate and Olmesartan medoxomil in Tablets[6]. Simultaneous Quantitation of Olmesartan medoxomil and Amlodipine Besylate in Combined Tablets Using HPLC[7]. Spectrophotometric Method for Simultaneous determination of Olmesartan medoxomil and

Amlodipine Besylate from Tablet dosage forms[8]. UV spectrophotometric Determination of Hydrochlorothiazide and Olmesartan Medoxomil in pharmaceutical Formulation[9]. Spectrophotometric Estimation of Olmesartan Medoxomil and Hydrochlorthiazide in Tablet[10]. Spectrophotometric Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Form[11]. RP-HPLC Method for Simultaneous Estimation of Telmisartan & Hydrochlorothiazide in Tablet Dosage Forms[12]. A Validated Stability Indicating HPTLC Method for Simultaneous Estimation of Irbesartan and Hydrochlorothiazide[13]. Simultaneous Analysis of Eprosatan and Hydrochlorothiazide in Tablets by HPLC[14]. Development and Validation of a RP-HPLC for the Simultaneous Estimation of Atenolol and Hydrochlorothiazide in Pharmaceutical Dosage Forms[15].Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in combined tablet dosage form by Multicomponent Mode of analysis[16]. Spectrophotometric Simultaneous Determination Of Amlodipine Besylate And Hydrochlorothiazide In Combined Tablet Dosage Form By Simultaneous Equation, Absorption Ratio And First Order Derivative Spectroscopy Methods[17].

MATERIALS AND METHODS

The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. Samples were applied as 8 mm bands by means of a Camag Linomat V automatic samples applicator (Muttenz Switzerland) equipped with a 100 µL syringe. The distance between the bands was 11.4 mm. Silica gel 60 F_{254} HPTLC plates (20×10 cm, aluminium) were from Merck (Darmstadt, Germany). Densitometric scanning was performed at 270nm with a camag TLC scanner 3 equipped with camag Wincats software 1.42 using the deuterium light source and slit dimensions of 4.00 mm × 0.30 mm.

Chemicals

Olmesartan medoxomil & hydrochlorothiazide reference standards was supplied by M/s Microlabs limited, Bangalore**,** India. HPLC grade Acetonitrile, triethanolamine ,orthophosphoric acid,chlororm,methanol was purchased from Merck (Mumbai, India). All chemicals were of analytical grade. Commercially available tablets (Olmesar-H of Macleod, Gujarat, India), containing 20 mg OML and 12.5 mg HCT per tablet, were used for analysis. Stock solutions (1.0 mg mL-1) for RP-LC and HPTLC were prepared in methanol. 50 micro liter of the above stock solution was dissolved with 900 micro liter of methanol for HPTLC.

Chromatographic Conditions

The mobile phase consists of 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. 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 1.2ml/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 30ºc.

Table 1: Gradient Program

Time (minutes)	**Flow Rate ml/min**	**%Mobile Phase A** (pH 2.5 Triethanolamine)	**%Mobile Phase B** (Acetonitrile)
0	1.2	75	25
5	1.2	30	70
7	1.2	30	70
8	1.2	25	75
12	1.2	25	75

Calibration

For calibration purposes, a range of 25-150µg/ml & 15-90µg/ml for olmesartan & hydrochlorothiazide solutions were prepared and 20 µL injections were carried out in triplicate.

Analysis of tablet formulations

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 & dilute to the volume with diluent.

Recovery study

The accuracy of the proposed method was evaluated by the addition of a standard drug solution to a pre-analysed tablet sample solution at three different concentrations levels at 50,100 and 150% of linearity for both drugs.

High Performance Thin Layer Chromatography

Chromatographic conditions

Chromatography was performed on 20 × 10 cm aluminum HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck). Before use plates were washed with methanol and dried in an oven at 120°C for 20 min. ascending development of the plate with a migration distance of 50

mm was performed at 23± 2°C using chloroform: methanol (85:15 v/v) as the mobile phase and a Camag twin-trough chamber previously saturated with mobile phase for 20 min. the average development time was 5 minutes.

Calibration

Mixed working standard solutions equivalent to 4, 6, 8, 10, 12, 14, 16 µL were separately stopped on the TLC plate in order to obtain final concentrations at 200, 300, 400, 500, 600,700,800 ng spot^{-1} for both drugs respectively. The plates were developed in a 20 × 10 cm twin through chamber using 20 mL freshly prepared mobile phase.

Analysis of Tablet Formulation

The tablets were weighed, triturated and the average weight was calculated. A 1.0 mg/mL solution was prepared in methanol and filtered through Whatmann filter paper no. 41. The above stock was diluted in the ratio of 1:1 with methanol which was used as the working standard solution. The 4µL solution was spotted on the HPTLC plate and the concentrations were calculated from the calibration graph.

Recovery study

The accuracy of the proposed method was evaluated by the addition of a standard drug solution at three different concentration levels at 50, 100, and 150% of linearity for both drugs.

RESULTS AND DISCUSSION

High Performance Liquid Chromatography

A satisfactory separation was obtained when 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 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. (Figure.3).

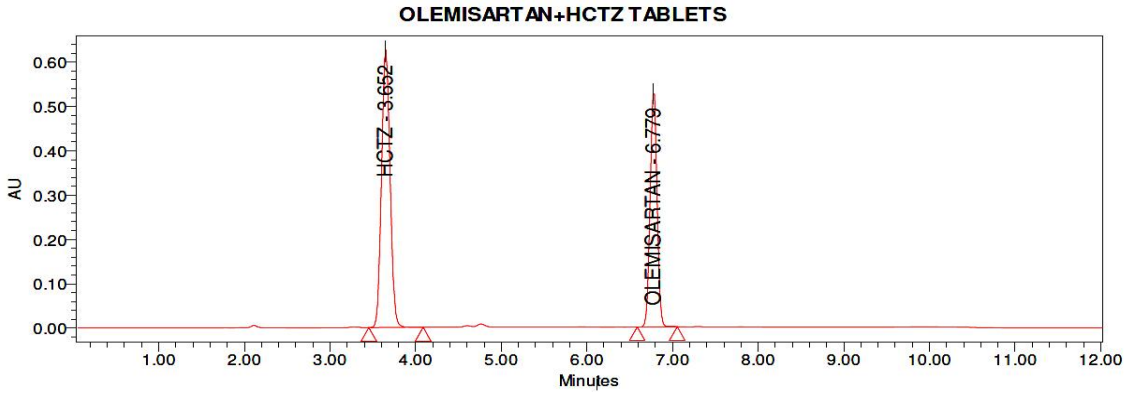

Figure 3: HPLC Sample chromatogram for Hydrochlorothiazide and Olmesartan

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 and the results was tabulated in Table 2 . 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.

The stability of sample was checked by forced degradation in different conditions and the studies indicate that any other impurity is not merging with the main peak The analyte solution was stable up to 24hrs.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.

Table 2: Calibration graphs of Olmesartan & Hydrochlorothiazide

Parameter	**HPLC**		**HPTLC**	
	OLM	**HCTZ**	**OLM**	**HCTZ**
Linearity range	25-150µg/ml	15-90µg/ml	200-800 ng/ml	200-800 ng/ml
Regression equation				
Slope	1.844	1.644	7.315	5.876
Intercept	1761	1126	1023	696.7
Coefficient of correlation	0.9998	0.9998	0.9982	0.9990
Limit of detection (LOD)				
Limit of quantitation (LOQ)			50ng	50 ng
Tailing Factor	1.41	1.12	150 ng	150 ng

Table 3: Assay and Recovery studies of Olmesartan & Hydrochlorothiazide

Brand name	**Compound**	**% Assay**	**% recovery**
HPLC	Olmesartan	100.2	99.80
	Hydrochlorothiazide	101.7	99.60
HPTLC	Olmesartan	95.8	100.5
	Hydrochlorothiazide	99.9	101.6

High Performance Thin Layer Chromatography

A number of experimental parameters, such as mobile phase composition, scan modes and detection wavelengths, were optimized during method development in order to provide accurate, precise and reproducible results for the simultaneous determination of OML and HCT. Maximum separation (OML Rf 0.31, HCT Rf 0.44) and minimum tailing were obtained when using a mobile phase composition of chloroform: methanol (85:15 v/v) respectively (Figure. 4). A calibration curve was made and concentration examined within the detection range of 200-800ng/ml & 15-90µg/ml for olmesartan & hydrochlorothiazide and correlation coefficient was found to be 0.998 & 0.999 for both the compounds respectively and the results was tabulated in

Table 2. The LOD values were 50 ng spot -1, while LOQ values were 150 ng spot -1 for both drugs respectively. The proposed method was used for the determination of both drugs in tablets and results are also shown in Table 3. Good recoveries and standard deviations were observed.

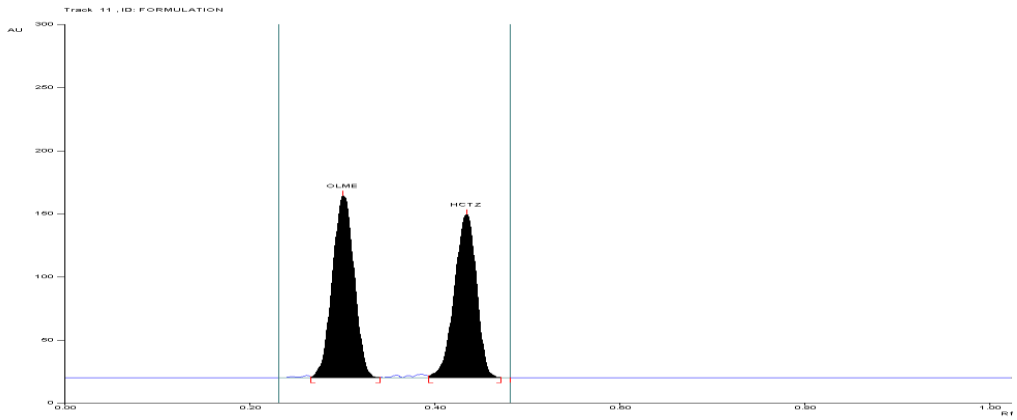

Figure 4: HPTLC Sample chromatogram for Hydrochlorothiazide and Olmesartan

CONCLUSION

A method was developed for the determination of tablets which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

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