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VALIDATED CHROMATOGRAPHICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF ANTIHYPERTENSIVE DRUGS IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Two new, rapid, precise, accurate and specific chromatographic methods for the simultaneous determination of Olmesartan medoxomil,Amlodipine besylate and Hydrochlorothiazide in combined pharmaceutical dosage forms. The first method based on reverse phase liquid chromatography by using Qualisil BDS C18 column (250 mm X 4.6 i.d., 5 µm). Mobile phase consists of 1.0 ml of triethylamine in one litre water and the pH was adjusted to 2.5 with orthophosphoric acid and Acetonitrile (60:40) with a flow rate of 1ml/min, with a detection wavelength of 231nm.The second method involved silica gel 60F254 high performance thin layer chromatography and densitometric detection at 231 nm using chloroform: methanol (85:15) as the mobile phase.

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

INTRODUCTION

Olmesartan medoxomil 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 chemically it 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]Amlodipine besylate is chemically 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5dicarboxylate benzene sulphonate salt of amlodipine, which is a dihydropyridine calcium channel blocker is a calcium antagonist inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load.[3] Olmesartan medoxomil, Amlodipine besylate 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 , Amlodipine besylate 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.[4]Validated Absorption Factor

Spectrophotometric and Reversed-Phase High Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations.[5] 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.[6]Development and validation of Spectrophotometric method for simultaneous estimation of Metoprolol succinate and Olmesartan medoxomil in Tablets.[7]Simultaneous Quantitation of Olmesartan medoxomil and Amlodipine Besylate in Combined Tablets Using HPLC.[8] Spectrophotometric Method for Simultaneous determination of Olmesartan medoxomil and Amlodipine Besylate from Tablet dosage forms.[9] UV spectrophotometric Determination of Hydrochlorothiazide and Olmesartan Medoxomil in pharmaceutical Formulations.[10] Spectrophotometric Estimation of Olmesartan Medoxomil and Hydrochlorthiazide in Tablets.[11] Spectrophotometric Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Forms.[12]RP-HPLC Method for Simultaneous Estimation of Telmisartan & Hydrochlorothiazide in Tablet Dosage Forms.[13] A Validated Stability Indicating HPTLC Method for Simultaneous Estimation of Irbesartan and Hydrochlorothiazide.[14]Simultaneous Analysis of Eprosatan and Hydrochlorothiazide in Tablets by HPLC.[15] Development and Validation of a RP-HPLC for the Simultaneous Estimation of Atenolol and Hydrochlorothiazide in Pharmaceutical Dosage Forms.[16]Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in combined tablet dosage form by Multicomponent Mode of analysis.[17] Spectrophotometric Simultaneous Determination Of Amlodipine Besylate And Hydrochlorothiazide In Combined Tablet Dosage Form By Simultaneous Equation, Absorption Ratio And First Order Derivative Spectroscopy Methods.[18]

EXPERIMENTAL

Chemicals: Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide reference standards was supplied by M/s Microlabs limited, Bangalore**,**India. HPLC grade Acetonitrile, triethylamine, orthophosphoric acid, chloroform, methanol was purchased from Merck (Mumbai, India). All chemicals were of analytical grade. Commercially available tablets (Olmat-AMH, India), containing 20mg Olmesartan medoxomil, 5mg Amlodipine besylate and 12.5mg Hydrochlorothiazide per tablet, were used for analysis. Stock solutions (1.0 mg mL-1) for RP-LC and HPTLC were prepared in methanol.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

***Apparatus and Chromatographic Conditions*:**
The determination was carried out on Agilent technologies 1220 series consisted of isocratic pump model G4286B liquid chromatographic system with 20µl loop manual injector was used. The analytes were separated on Agilent, Qualisil BDS C18 column (250 mm X 4.6 i.d.,5 µm particle diameters, made in USA). Mobile phase consists of 1.0 ml of triethylamine in one litre water and the pH was adjusted to 2.5 with orthophosphoric acid and Acetonitrile in the ratio of 60:40, filtered through 0.45µm Membrane filter and degassed through Agilent 1200 series vacuum degasser with flow rate 1 ml min.-1 with isocratic elution and the UV- Variable wave length detector Model G1314 was set at 231 nm using data handling system EZChrom Elite Compact 3.3.2 SP2 software. The column was conditioned for ≥ 30 min. All the determinations were performed at ambient temperature 25± 5 °C and the injection volume was 20 µl.

Calibration: For calibration purposes, a range of 2-30µg/ml for olmesartan, 2-25µg/ml for amlodipine & 2.5-17.5µg/ml for 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 100ml volumetric flask.100ml 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. 1ml of the above filtered solution was transferred into a 10ml 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

Apparatus and Chromatographic conditions:

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 231 nm 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. 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 60°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 for all the three drugs (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 $spot^{-1}$ 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 Whatman 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

HPLC method: A satisfactory separation was obtained (Olmesartan medoxomil Rt 5.78, Amlodipine besylate Rt 4.86 and Hydrochlorothiazide Rt 3.11) when using Qualisil BDS (250 x 4.6, 5µ) column using mobile phase 1.0 ml of triethylamine in one litre water and the pH was adjusted to 2.5 with orthophosphoric acid and acetonitrile (60:40) with a flow rate of 1ml/min with a detection wavelength of 231nm for both the compounds with a injection volume of 20µl. (Fig.1). A calibration curve was made and concentration examined within the detection range of 2-30µg/ml for olmesartan, 2-25µg/ml for amlodipine & 2.5-17.5µg/ml for hydrochlorothiazide and correlation coefficient was found to be 0.99 for all the drugs. 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 2. 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, Amlodipine besylate & hydrochlorothiazide in tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

HPTLC method: 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 the three drugs.Maximum separation (Olmesartan medoxomil Rf 0.50, Amlodipine besylate Rf 0.22 and Hydrochlorothiazide Rf 0.33) and minimum tailing were obtained when using a mobile phase composition of chloroform: methanol (85:15 v/v) respectively (Fig.2). Table 1 shows that correlation coefficients were 0.998 for all the drugs. The LOD values were 50 ng spot -1, while LOQ values were 150 ng spot -1 for both Olmesartan medoxomil and Hydrochlorothiazide respectively and for Amlodipine besylate LOD values were 100 ng spot -1, while LOQ values were 300 ng spot -1. The proposed method was used for the determination of both drugs in tablets and results are also shown in Table 2. Good recoveries and standard deviations were observed.

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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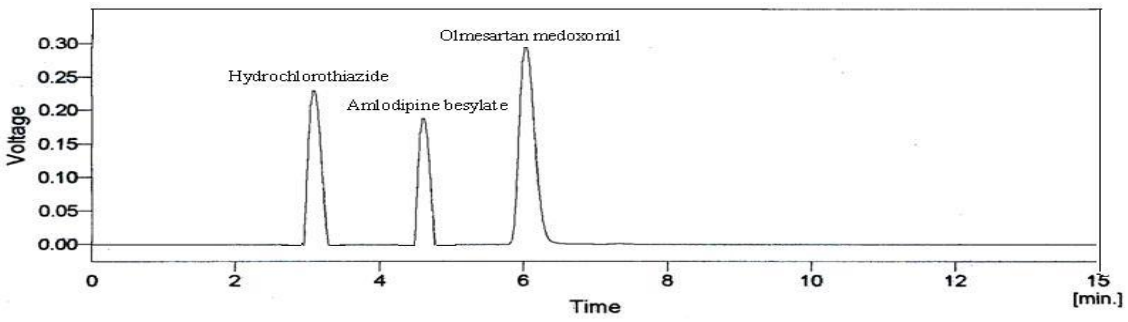

Figure 1: HPLC chromatogram for Olmesartan medoxomil,Amlodipine besylate and hydrochlorothiazide

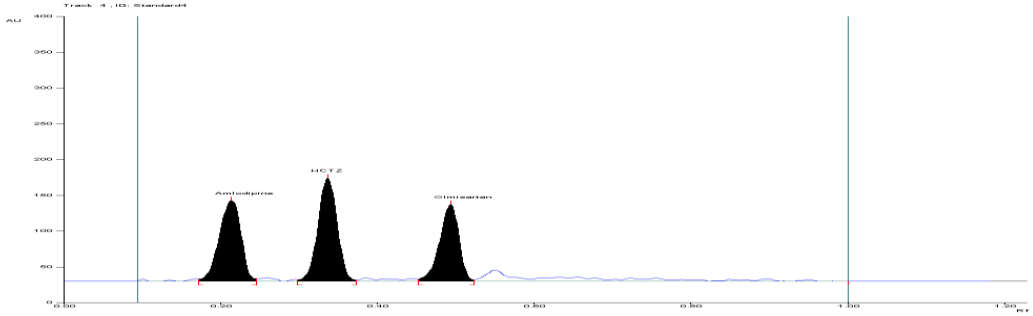

Figure 2: HPTLC chromatogram for Olmesartan medoxomil,Amlodipine besylate and hydrochlorothiazide

Table 1: Calibration graphs of Olmesartan medoxomil,Amlodipine besylate and hydrochlorothiazide

Parameter	**HPLC**			**HPTLC**		
	Olm	**Amlo**	**Hctz**	**Olm**	**Amlo**	**Hctz**
Linearity range	2-30µg/ml	2-25µg/ml	2.5-17.5µg/ml	200-800 ng/spot	400-1600 ng/spot	300-800 ng/spot
Regression equation						
Slope	23720	13539	40716	3.813	2.395	4.101
Intercept	22921	14784	12686	432.5	432.5	713.9
Coefficient of correlation	0.9954	0.9983	0.9932	0.9989	0.9986	0.9987
Limit of detection (LOD)	1µg/ml	1µg/ml	0.1µg/ml	50 ng/spot	100 ng/spot	50 ng/spot
Limit of quantitation (LOQ)	3µg/ml	3µg/ml	0.4µg/ml	150ng/spot	300 ng/spot	150 ng/spot

Table 2: Assay and Recovery studies of Olmesartan medoxomil,Amlodipine besylate and hydrochlorothiazide

Brand name	Method	Compound	% Assay	% recovery
OLMAT AMH	HPLC	Olmesartan	100.24	99.97
		Amlodipine	101.70	100.90
		Hydrochlorothiazide	100.46	100.74
	HPTLC	Olmesartan	95.8	100.35
		Amlodipine	99.9	103.14
		Hydrochlorothiazide	100.07	99.84

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