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Simultaneous Quantitation of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in Pharmaceutical dosage form by using HPLC

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Abstract: A simple, fast, and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in bulk drug and pharmaceutical dosage form. The quantification was carried out using thermo hypersil ODS–C_{18} (250 mm × 4.6 mm, 5.0 µ) column and mobile phase comprised of methanol: 0.05M potassium dihydrogen phosphate with triethylamine (80:20 v/v) pH 3 adjusted with orthophosphoric acid. The flow rate was 0.8 ml/min and the effluent was monitored at 230 nm. The retention time of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide were 5.25, 4.55 and 3.31 min respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide were in the range of 20 to 60 µg/ml, 5 to 25 µg/ml and 2.5 to 12.5 µg/ml respectively. The percentage recoveries of all the drugs were 99.46%, 99.92% and 100.01% for olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in pharmaceutical dosage form and bulk drug.
Keywords: Olmesartan Medoxomil, Amlodipine Besylate, Hydrochlorothiazide, HPLC,Validation.

INTRODUCTION

Olmesartan Medoxomil (OLME) is chemically (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl 4-(2-hydroxy propan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate (Fig.1a). OLME belongs to a class of drugs known as angiotensin II (A2) receptor blockers (ARBs). These medicines are closely related to the common medications known as ACE inhibitors, which block an enzyme in the body that is responsible for causing the blood vessels to narrow [1].

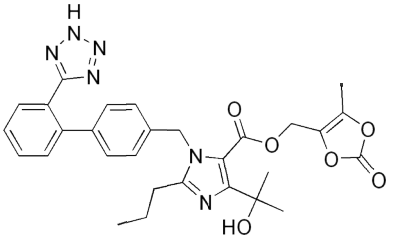

Figure 1(a) Structure of Olmesartan medoxomil

Amlodipine Besylate (AMLO) is chemically 3-ethyl O5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Fig.1b). Amlodipine is a calcium channel blocking agent. It 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 [2].

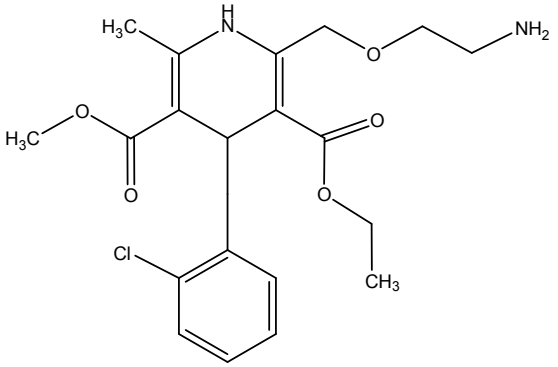

Figure 1(b) Structure of Amlodipine Besylate

Hydrochlorothiazide (HCTZ) is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-enzo[e][1,2,4] thiadiazine sulfonamide (Fig. 1c). As a diuretic, hydrochlorothiazide inhibits active chloride reabsorption at the early distal tubule via the Na-Cl cotransporter, resulting in an increase in the excretion of sodium, chloride, and water. Thiazides like hydrochlorothiazide also inhibit sodium ion transport across the renal tubular epithelium through binding to the thiazide sensitive sodium-chloride transporter. This results in an increase in potassium excretion via the sodium-potassium exchange mechanism[3].

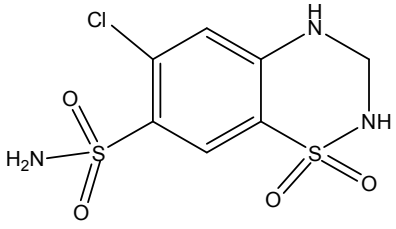

Figure 1(c) Structure of Hydrochlorothiazide

Literature review reveals that method have been reported for the olmesartan, Amlodipine and hydroclorothiazide, LC-DAD method for determination of Olmesartan Medoxomil in tablets exposed to stress conditions [4], RP-HPLC method for determination of the Amlodipine in combination with other drugs [5-10], Ion Pair-HPLC Method for the Simultaneous Estimation of Quinapril and Hydrochlorothiazide in tablets [11]. As no method is reported for Olmesartan Medoxomil, Amlodipine Besylate and hydrochlorothiazide in combination, the aim of the present study was to develop accurate, precise and selective reverse phase HPLC assay procedure for the analysis of the OLME. AMLO, HCTZ in bulk drug samples and in combined dosage formulation. The proposed method is validated as per ICH guidelines [12-14].

EXPERIMENTAL

Materials

Olmesartan Medoxomil and Hydrochlorothiazide as a gift sample supplied by Taj Pharmaceuticals Ltd., Mumbai, India and Amlodipine Besylate supplied by Sandoz Pharmaceuticals, Mumbai, India. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Instrumentation

The HPLC system consisted of a Pump (model Jasco PU 2080); Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 230 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil ODS–C_{18} (250 mm × 4.6 mm, 5.0 µm).

Preparation of Standard Stock Solutions

Accurately weighed OLME (20 mg), AMLO (5 mg) and HCTZ (12.5 mg) were transferred to 100 ml volumetric flask and dissolved in, and then diluted to the mark with methanol. The stock solution was further diluted with methanol to obtain a solution of OLME (20µg/ml), AMLO (5 µg/ml) and HCTZ (12.5 µg/ml), respectively.

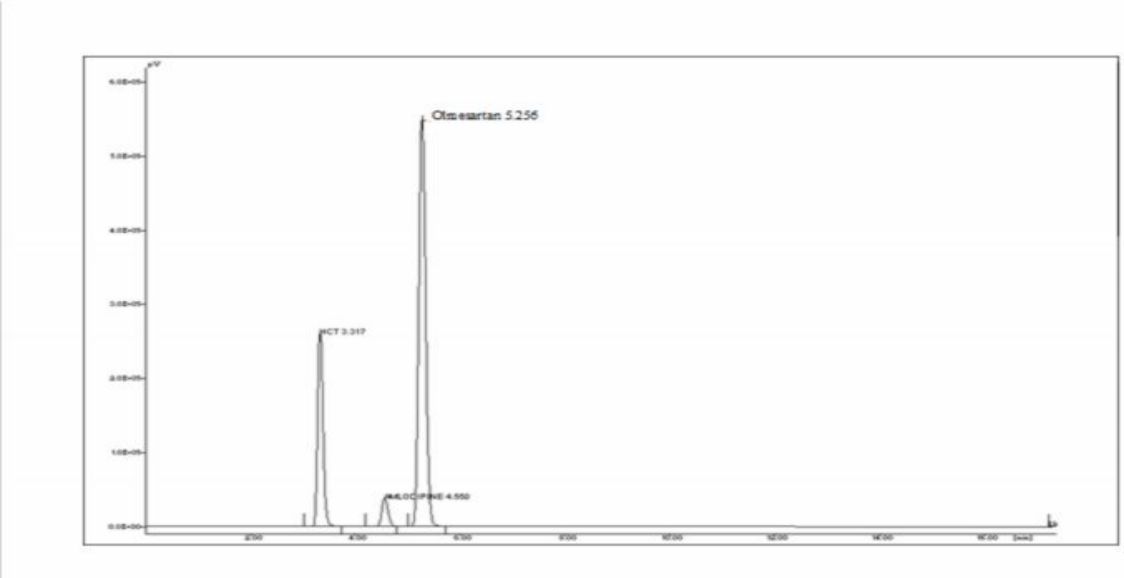

Figure 2:Chromatogram of standard Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorothiazide (20 µg/mL, 5µg/mL and 12.5 µg/mL)

Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for OLME, AMLO and HCTZ respectively. The mixed standard stock solution (20 µg/ml for OLME and 5 µg/ml for AMLO and 12.5 µg/ml for HCTZ) was injected in HPLC. For HPLC method optimization different ratios of methanol and water were tried but it was found that methanol: 0.05M potassium dihydrogen phosphate with triethylamine (80: 20 v/v) pH 3 adjusted with orthophosphoric acid gives acceptable retention time (t_R), plates and good resolution for OLME, AMLO and HCTZ (Fig. 2).

VALIDATION OF THE METHOD

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and Range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range 20 to 60 µg/ml, 5 to 25 µg/ml, and 2.5 to 12.5 µg/ml for OLME, AMLO and HCTZ, respectively in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (20, 40 and 60 µg/ml for OLME and 5, 15 and 25 µg/ml for AMLO and 2.5, 7.5 and 12.5 µg/ml for HCTZ) of the drugs six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration plot.

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently. The peak purity of OLME, AMLO and HCTZ was determined by comparing the retention time (T_R).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (OLME, AMLO and HCTZ combination tablet) to which know amount of OLME, AMLO and HCTZ standard powder corresponding to 50, 100 and 150 % of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of OLME, AMLO and HCTZ in conventional tablet (Brand name: OLMET-AMH, Label claim: 20mg OLME, 5mg AMLO and 12.5mg HCTZ per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 20mg of OLME, 5mg of AMLO and 12.5mg of HCTZ was transferred into a 100 ml volumetric flask containing 80 ml methanol, sonicated for 30 min and diluted up to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (200, 50 and 125 µg/ml for OLME, AMLO and HCTZ respectively). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 20, 5 and 12.5 µg/ml for OLME, AMLO and HCTZ, respectively. A 20-µL volume of sample solution was injected into HPLC system, under the conditions described above.

Table 1 Precision study

Drug	Concentration (µg/ml)	Intra day precision % RSD	Inter day precision % RSD
AMLO	5	0.78	1.10
	15	0.87	1.25
	25	0.98	1.20
OLME	20	1.45	1.23
	40	0.59	1.01
	60	0.78	1.20
HCTZ	2.5	1.20	0.87
	7.5	0.67	0.34
	12.5	0.88	0.92

Table 2 Recovery studies

Concentration (mg/tablet)	Amount added (%)	Total Amount present mg	Amount Recovered (µg/ml) ± SD	% Recovery
AMLO 5	50	7.5	7.46 ± 0.11	99.46
	100	10	9.9 ± 0.15	99
	150	12.5	12.54 ±0.21	100.32
OLME 20	50	30	29.2 ± 1.48	98.93
	100	40	39.2 ±1.92	100.2
	150	50	48.9 ±2.23	99.92
HCTZ 12.5	50	18.75	18.83 ±0.19	99.10
	100	25	24.75 ±0.43	99
	150	31.25	31.34 ±0.34	101

Table 3 Assay of commercial tablet.

Drug	Label claim (mg)	Drug content (%) ± S.D	% R.S.D.
AMLO	5	96.6 ± 0.25	0.26
OLME	20	98.96 ± 0.41	0.42
HCTZ	12.5	96.83 ± 0.25	0.26

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of for OLME, AMLO and HCTZ dosage form was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines using methanol: 0.05M potassium dihydrogen phosphate with triethylamine (80: 20 v/v) pH 3 adjusted with orthophosphoric acid as mobile phase.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The Linearity range for OLME, AMLO and HCTZ were found to be 20 to 60 µg/ml, 5 to 25 µg/ml, and 2.5 to 12.5 µg/ml, respectively. The regression equation for OLME, AMLO and HCTZ were found to be y = 22921x-23720, y = 14784x-13539 and y=12686x+ 40716with coefficient of correlation, (r^2) 0.995, 0.998 and 0.993, respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 1 µg/ml and 3 µg/ml for OLME, 1 µg/ml and 3 µg/ml for AMLO and 0.1 µg/ml and 0.4 µg/ml for HCTZ.

Specificity

The peak purity of OLME, AMLO and HCTZ were assessed by comparing the retention time (T_R) of standard OLME, AMLO and HCTZ. Good correlation was obtained between the retention time of standard and sample of OLME, AMLO and HCTZ. Thus the method was specific for OLME, AMLO and HCTZ.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard OLME, AMLO and HCTZ were added to pre-analysed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 2.

Analysis of the commercial formulation

The amounts OLME, AMLO and HCTZ per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation is reported in Table 3.

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. It can be concluded that the method is specific for estimation of OLME, AMLO and HCTZ in pharmaceutical dosage form. The method has linear response in stated range and is accurate and precise. Statistical analysis proves that the method is suitable for the analysis of OLME, AMLO, and HCTZ as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of OLME, AMLO, HCTZ and also for its estimation in plasma and other biological fluids.

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