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Simultaneous determination of amlodipine besylate, olmesartan medoxomil and chlorthalidone in pharmaceutical preparations using validated, LCMS compatible RP-HPLC method

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ABSTRACT

A simple, fast and precise LCMS compatible reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of Amlodipine Besylate, Olmesartan Medoxomil, Chlorthalidone in pharmaceutical preparations. Chromatographic separation of the three drugs was performed on a Merck Chromolith 4.6 X 50 mm, RP-18e as stationary phase with a mobile phase comprising of Buffer and Methanol in gradient system. Buffer was 0.1%v/v Formic acid (1ml Formic acid to 1000ml water.), filtered and degassed. The chromatographic system was run at a flow rate of 0.6 mLmin-1 and UV detection at 265nm. Method developed was LCMS compatible and the same was used to identify each drug. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ and can be conveniently adopted for routine quality control and pharmacokinetic analysis.

KEYWORDS

High performance liquid chromatography;
Mass spectrometry;
Validation;
Amlodipine besylate;
Olmesartan medoxomil;
Chlorthalidone.

INTRODUCTION

TRIOLMESAR CH which is a combination of Amlodipine Besylate (5mg), Olmesartan Medoxomil (20mg) and Chlorthalidone (12.5mg) manufactured by Macleods Pharmaceuticals Pvt Ltd, INDIA is very useful in the treatment of hypertension and congestive heart failure.

Amlodipine Besylate (3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]- 4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate) is a long-acting dihydropyridine-type (DHP) calcium channel blocker used to lower blood pressure and to treat anginal chest pain[1]. Like other calcium channel blockers, Amlodipine lowers blood pressure by relaxing arterial smooth muscles, which decreases total peripheral resistance and therefore reduces blood pressure. In angina, Amlodipine increases blood flow to the heart muscle (although DHP-class calcium channel blockers are more selective for arteries than the muscular tissue of the heart (myocardium), as the calcium ion channels of the heart are not of the dihydropyridine-type)[1].

Olmesartan Medoxomil (4- (1- hydroxy- 1-methylethyl)- 2- propyl- 1- [[2'- (2H- tetrazol- 5- yl)[1, 1'- biphenyl]- 4- yl]methyl]- (5- methyl- 2- oxo- 1, 3-dioxol- 4- (5- methyl- 2- oxo- 1, 3- dioxol- 4- (5-methyl- 2- oxo- 1, 3- dioxol- 4- (5- methyl- 2- oxo- 1, 3- dioxol- 4- y)methyl ester) is an antihypertensive agent, which belongs to the class of medications called angio-

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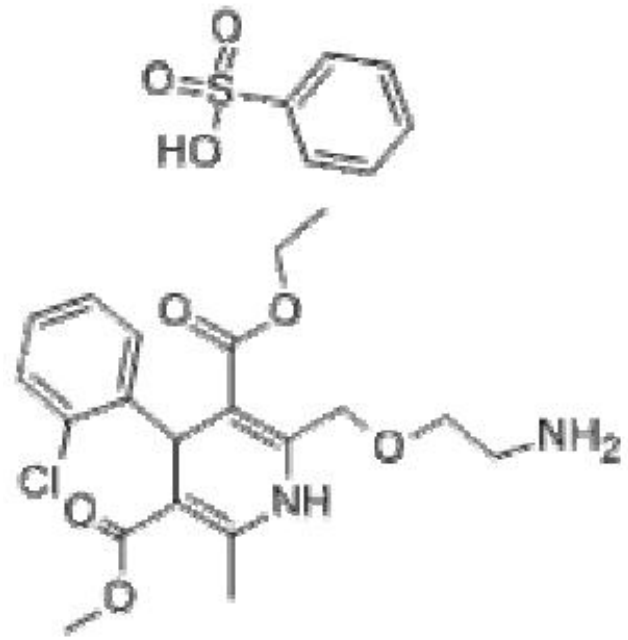

Figure 1 : Chemical structure of amlodipine besylate

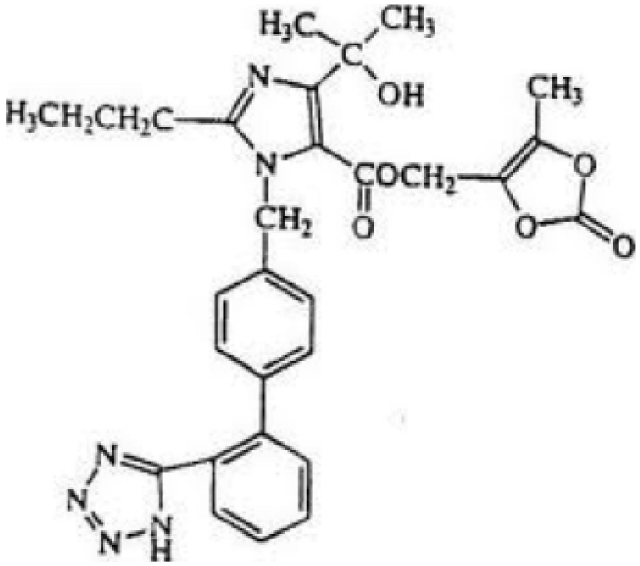

Figure 2 : Chemical structure of olmesartan medoxomil

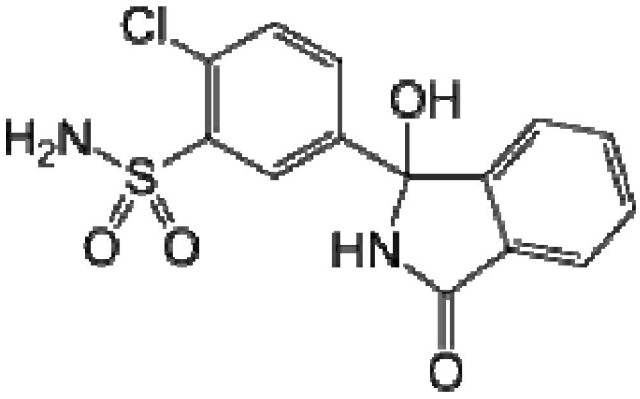

Figure 3 : Chemical structure of chlorthalidone

tensin II receptor blockers{ARB}[1]. It is indicated for the treatment of high blood pressure. Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstriction and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure[1].

Chlorthalidone ((*RS*)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzene-1-sulfonamide) is a diuretic drug used to treat hypertension[1]. Chlorthalidone prevents reabsorption of sodium and chloride by inhibiting the Na^+/Cl^- symporter in the distal convoluted tubule. Thiazides and related compounds also decrease the glomerular filtration rate, which further reduces the drug's efficacy in patients with renal impairment (e.g. renal insufficiency). By increasing the delivery of sodium to the distal renal tubule, Chlortalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism (i.e. apical ROMK/Na channels coupled with basolateral NKATPases)[1].

While many methods are available for single or dual combinations of the drugs under study[2-4], literature search revealed no method was available for simultaneous determination of these three drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed for determination of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone from their combined dosage form. Also the method was developed as an LCMS compatible method so that future studies could be easily carried out on LCMS. The method described is simple, fast, precise and accurate for simultaneous determination of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone from pharmaceutical preparation.

EXPERIMENTAL

Chemicals and reagents

Standards were supplied from National Chemical Co., Mumbai, India. TRIOLMESAR CH (Amlodipine Besylate - 5mg, Olmesartan Medoxomil - 20mg and Chlorthalidone - 12.5mg) manufactured by Eris Life Science Pvt Ltd, India was procured from the market.

TABLE 1: Chromatographic conditions

HPLC Column	Merck Chromolith 4.6 X 50 mm, RP-18e		
Mobile Phase	A) 0.1%v/v Formic acid (1000ml filtered and degassed) B) Methanol (HPLC Grade)		
Flow Rate	0.6mLmin^{-1}		
Gradient Program	**Time**	**%A**	**%B**
	0	60	40
	10	30	70
	15	15	85
	17	60	40
	20	60	40
UV Wavelength	265nm		
Temperature	25°C		
Injection Volume	10µL		
Diluent	Methanol		

Methanol and Formic acid were from procured from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Also harsh buffers like phosphate buffers were not used to develop a LCMS compatible method[5].

HPLC analysis was carried out using a Waters HPLC system consisting of two pumps, (Waters 515 pumps with Waters pump control module-II), an automatic sampling unit (Waters 717 plus auto sampler), a column oven, a photodiode array detector (Waters 2996) and temperature control module-II. Waters empower software was used for data analysis and data processing. The UV spectrum of all the three drugs was scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs was checked using photo diode array detector.

The chromatographic conditions used are noted in TABLE 1:

A triple quadrupole mass spectrometer, Agilent 6410 (Agilent Technologies, USA), equipped with an electrospray ionization (ESI) source was used for the mass analysis. Full scan data were acquired by scanning from m/z 100 to 1000 in profile mode, using a cycle time of 4.94 s, with a step size of 0.1 µs. Both the

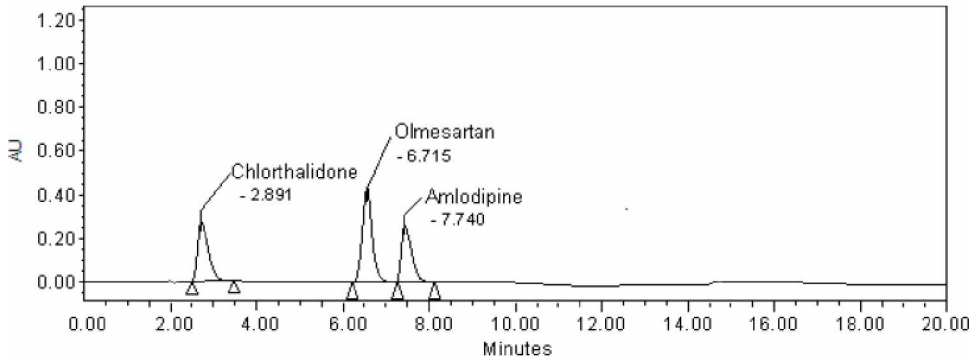

Figure 4 : Chromatogram of amlodipine besylate, olmesartan medoxomil and chlorthalidone in standard preparation

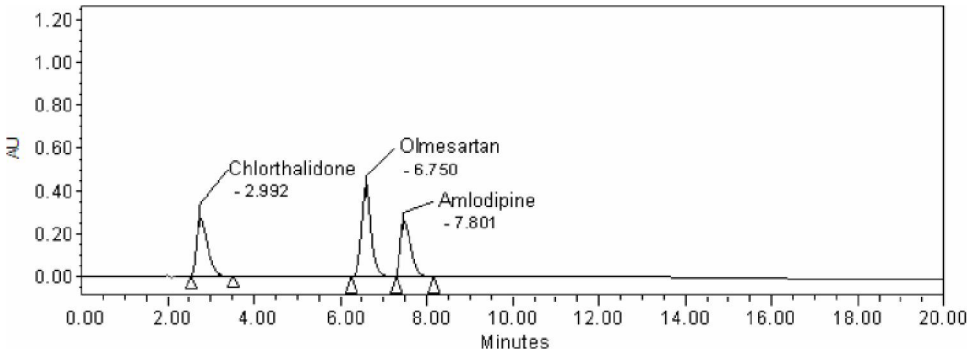

Figure 5 : Chromatogram of amlodipine besylate, olmesartan medoxomil and chlorthalidone in sample preparation

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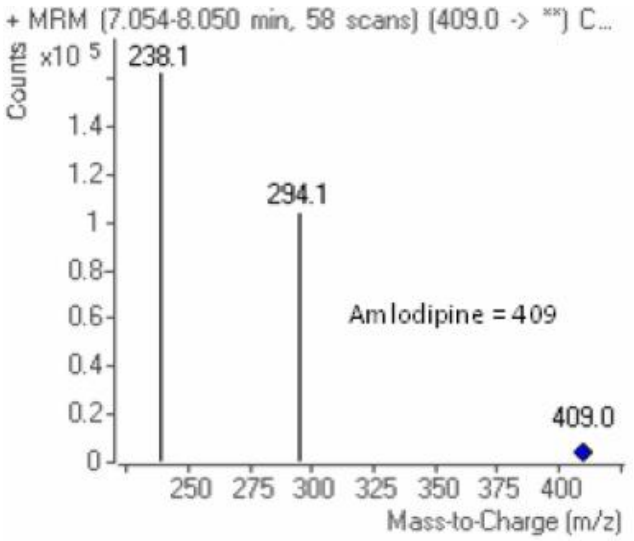

Figure 6 : MS spectrum of amlodipine besylate

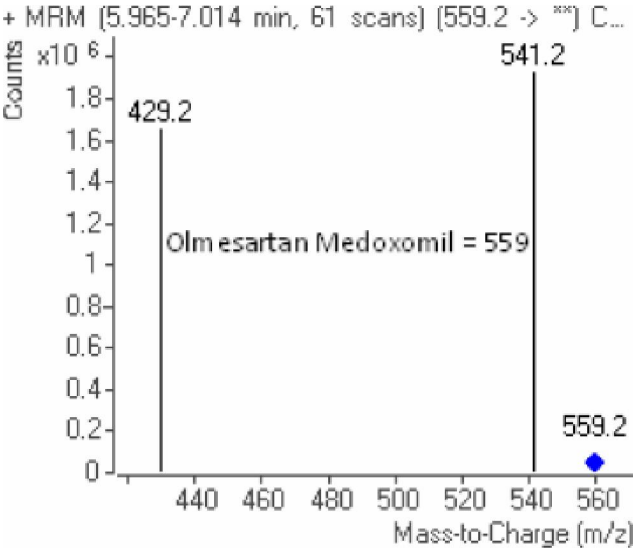

Figure 7 : MS spectrum of olmesartan medoxomil

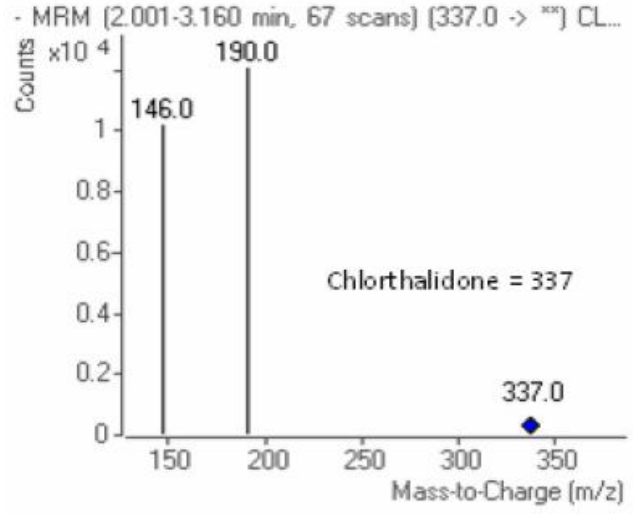

Figure 8 : MS spectrum of chlorthalidone

TABLE 2 : Results of system suitability

Parameters	Chlorthalidone	Olmesartan Medoxomil	Amlodipine Besylate
Resolution	--	8.5	2.2
Tailing Factor	1.4	1.1	1.3

TABLE 3 : Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation Coefficient(r^2)n=7
Amlodipine Besylate	38182.1	-3103.0	0.9996
Olmesartan Medoxomil	19609.9	27210.3	0.9989
Chlorthalidone	18618.9	61203.2	0.9984

quadrupoles (Q1 and Q3) were operated at unit resolution. ESI source in positive mode was chosen for the detection of investigated compounds with following operating conditions: Source and Mode; ESI, Polarity; positive (+), Collision gas; nitrogen, Capillary voltage; ±4000 V, Ion source temperature; 3000C, Gas flow; 10L/min, Dwell time; 10ms, Resolution (Q1 and Q3); unit.

Typical HPLC chromatograms for simultaneous determination of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone from pharmaceutical formulation is shown in Figures 4 and 5.

Typical LCMS spectrum of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone from pharmaceutical formulation is shown in Figures 6, 7 and 8.

Sample preparation

Standard stock solutions

The standard stock solution of Amlodipine Besylate (50 μg mL^{-1}), Olmesartan Medoxomil (200 μg mL^{-1}) and Chlorthalidone (125 μg mL^{-1}) was prepared in methanol in a standard 100mL volumetric flask. The standard stock solution was used to prepare working standard solutions and other concentrations for validation tests.

Working standard solution

Working Standard solution of Amlodipine Besylate (5 μg mL^{-1}), Olmesartan Medoxomil (20 μg mL^{-1}) and Chlorthalidone (12.5 μg mL^{-1}) was prepared by pipetting 10mL of Standard Stock Solution in a standard 100mL volumetric flask and diluting up to mark with methanol.

Sample preparation

To prepare sample, 5 TRIOLMESAR tablets were

TABLE 4: Results of precision

	Amlodipine Besylate	Olmesartan Medoxomil	Chlorthalidone
Drug found in mg/mL (mean)	4.98	19.89	12.46
Mean%	99.60	99.45	99.68
RSD	0.20	0.41	0.35

weighed and transferred to 500mL standard volumetric flask. About 300mL of methanol was added to the volumetric flask and the mixture was sonicated till the tablets were dissolved. After equilibration to achieve room temperature, the solution was diluted up to mark and mixed well. This solution was filtered through filter paper (25mm) and the first 3 mL was discarded. Further 10 ml of this solution was pipetted into a 100mL standard volumetric flask and diluted up to mark with methanol.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were performed as per the USP to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10µl of working standard solution of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone in five replicates. The RSD values of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone were 0.20, 0.25 and 0.21 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP (RSD less than 2.0%). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 4.

Linearity

Linearity was evaluated by analysis of working standard solutions Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone of seven different concentrations. The range of linearity was from 2.5 – 7.5 µg mL-1 for Amlodipine Besylate, 10 to 30 µg mL-1 for Olmesartan Medoxomil and 6.25 to 18.75 µg mL-1 for Chlorthalidone. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals are represented in TABLE 5. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

Limits of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone were experimentally determined by six injections of each drug. The LOD of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone were 0.1 µg mL^{-1}, 0.8 µg mL^{-1} and 0.3 µg mL^{-1}. The LOQ of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone were 0.3 µg mL^{-1}, 2 µg mL^{-1} and 0.9µg mL^{-1}.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3]. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone in the product. The RSD were 0.20, 0.41 and 0.35 respectively. Refer TABLE 4.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of each drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of each drug was added, to determine if there are positive or negative interferences from excipients present in the formulation. Each set of addition was repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drugs from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of the three drugs.

CONCLUSION

Several mobile phases such as buffer-methanol,

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TABLE 5 : Result of accuracy

Analyte	Initial Conc (mg)	Conc Added (mg)	Total Conc (mg)	Conc Found (mg)	RSD% (n=3)	Recovery%
Amlodipine Besylate	5	0	5	4.98	0.19	99.60
	5	0.5	5.5	5.48	0.20	99.64
	5	1.0	6	5.96	0.21	99.33
	5	1.5	6.5	6.47	0.22	99.54
Olmesartan Medoxomil	20	0	20	19.96	0.19	99.80
	20	2	22	21.84	0.24	99.27
	20	4	24	23.90	0.38	99.58
	20	6	26	25.86	0.22	99.46
Chlorthalidone	12.5	0	12.50	12.41	0.15	99.28
	12.5	1.25	13.75	13.65	0.24	99.27
	12.5	2.50	15.00	14.95	0.31	99.67
	12.5	3.75	16.25	16.12	0.37	99.20

buffer-acetonitrile in different ratios were tried but good peak shape and good resolution between Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and because of the use of LCMS compatible buffers it can be used for simultaneous determination of Amlodipine Besylate, Olmesartan Medoxomil and Chlorthalidone from pharmaceutical preparations in both HPLC-PDA and HPLC-MS systems.

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