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Original Article

DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL, AMLODIPINE BESYLATE, CILNIDIPINE IN COMBINATION TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate a simple, sensitive and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous determination of olmesartan medoxomil (OLM), amlodipine besylate (AML) and cilnidipine (CIL) in pharmaceutical tablet formulation.

Methods: In this work we have taken 2 combinations, combination 1-OLM & AML and combination 2-OLM & CIL. HPLC analysis was carried out by using reverse phase isocratic elution with a C 18 column and a mobile phase of 0.05 M ammonium acetate, acetonitrile and methanol in the ratio of 30:50:20, v/v, pH was adjusted to 7.3. Detection of the analyte was achieved by using UV detector at 240 nm.

Results: The retention time of olmesartan medoxomil, amlodipine besylate and cilnidipine were 2.2, 3 and 4.5 minutes respectively. Linearity of the method was found to be in the concentration range of 10-100µg/ml for olmesartan medoxomil, 5-50µg/ml for amlodipine and 10-100µg/ml for cilnidipine. The correlation coefficient value was greater than 0.999 for all the analytes.

Conclusion: The method was validated as per ICH guidelines and is applied for the estimation of these components simultaneously in pharmaceutical tablet formulation.

Keywords: HPLC, Acetonitrile, Olmesartan, Amlodipine, Cilnidipine.

INTRODUCTION

Olmesartan medoxomil (OLM) is a pro drug that hydrolysed to olmesartan during absorption [1-3]. Olmesartan is a competitive and selective Angiotensin II receptor antagonist. The hydrolysis of olmesartan medoxomil occurs readily by the action of esterase which is present abundantly in gastro intestinal tract, liver and plasma. It is used alone or with other antihypertensive agents to treat hypertension [4, 5]. It is chemically designated as 5-methyl-2-oxo-1, (3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-proply-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl} methylimidazol-5-carboxylate [6]. Olmesartan is also beneficial in animals and is a strong agent to show activity against atherosclerosis, liver disorders and diabetic nephropathy [7, 8]. Amlodipine besylate (AML) is chemically known as 3-ethyl-5-methyl (±)-2-[(2aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylate, monobenzene sulphonate is long acting calcium channel blocker [9, 10] used to treat hypertension and angina. Amlodipine blocks the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [11-13]. It is metabolized in the liver and metabolites are mostly excreted in urine together with less than 10% of the dose as unchanged drug [14, 15]. Cilnidipine (CIL) is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function [16]. It is chemically, 1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine di carboxylic acid 2-methoxy ethyl (2E)-3-phenyl-2-propenyl ester [17]. It reduces the incidence of pedal edema unlike amlodipine. It does not cause reflex tachycardia. It is superior to amlodipine in preventing the progression of proteinuria in patients with hypertension and chronic renal disease [18]. Most hypertensive patients require more than one agent in order to achieve adequate blood pressure control. Fixed dose combination antihypertensive treatments such as OLM/AML or OLM/CIL have advantage over monotherapy including increased efficacy, reduced side effect and lower cost. Several analytical methods have already been developed for the determination of olmesartan, amlodipine and cilnidipine either individually or in combination with other drugs [19-21]. According to the information extracted from literature, till date there is no method reported for the simultaneous determination of olmesartan medoxomil, amlodipine besylate and cilnidipine in pharmaceutical formulation. Aim of present work is to develop simple, precise and accurate RP HPLC method for simultaneous determination of olmesartan medoxomil, amlodipine besylate and cilnidipine in pharmaceutical formulation. The developed method can be applied successfully to quality control and other analytical purposes.

MATERIALS AND METHODS

Chemicals and reagents

The working standard Olmesartan medoxomil was gifted by Madras pharmaceuticals, Chennai. Amlodipine besylate was gifted by Strides Arco lab Ltd, Bangalore and cilnidipine working standard was gifted by J. B. Chemicals & pharmaceuticals Ltd, Mumbai. Combination products Amlovas-OL and Nexovas-O manufactured by Macleods pharmaceuticals Pvt Ltd, Mumbai with label claim olmesartan medoxomil 20 mg, amlodipine besylate 5 mg and olmesartan medoxomil 20 mg, cilnidipine 10 mg was purchased from local market. Acetonitrile and Methanol (HPLC grade) purchased from Himedia Laboratories Pvt Ltd, Mumbai. Ammonium acetate (Extra pure) was purchased from Finar chemicals Ltd, Ahmadabad. The 0.45 µm nylon filters were purchased from Millipore India pvt Ltd. Whatmann filter paper No. 41 purchased from the local market. Purified water for chromatography was obtained from Mille-Q system was used throughout an experiment.

Apparatus and chromatographic conditions

HPLC apparatus consisting of Waters 515 systems with binary pump, Rheodyne manual injector with a 20 µl loop and coupled with UV detector (Waters 2489). Data was integrated by Waters Empower 2 software and used for development and evaluation of this method. A chromatographic separation was achieved on Waters Symmetry C 18 column-3.5 µm (4.6 ×75 mm). Mobile phase consist of 0.05M ammonium acetate, acetonitrile and methanol in ratio of (30:50:20) v/v. The mobile phase pumped through the column with flow rate of 0.3 ml/min in isocratic mode. The mobile phase filtered through 0.45 µm nylon filter and degassed in ultrasonicator bath for 30 minutes prior to use. Injection volume was 20 µl throughout the

study. Based on the response of all the analyte the optimum wavelength 240 nm was selected. HPLC system was operated at room temperature (25±2 °C)

Preparation of standard solution

1 mg/ml of standard stock solutions of drug substance were prepared in methanol. Prior to measurement, stock solutions of olmesartan medoxomil, amlodipine besylate and cilnidipine were diluted with mobile phase so as to prepare working standard solution of 100µg/ml. various dilutions were made to prepare working solutions. HPLC analysis was carried out with 20 µl aliquots of various concentration of working solutions.

Assay procedure for pharmaceutical preparation

Ten tablets of each formulation Amlovas OL (composition 1) and Nexovas O (composition 2) were accurately weighed and finely powdered. A quantity of the powdered tablets equivalent to 20 mg OLM and 5 mg AML for combination 1 and 20 mg OLM and 10 mg CIL for combination 2 was accurately weighed and transferred to 50 ml volumetric flask separately. 50 ml of methanol was added each volumetric flask and extraction were performed mechanically for 20 minutes and sonicated for 30 minutes. The dilution was made with methanol to give the solution containing 40 µg/ml of OLM and 10 µg/ml of AML for combination 1, 40 µg/ml of OLM and 20 µg/ml of CIL for combination 2. From each of these solutions, 1 ml of extract was transferred to 10 ml volumetric flasks and diluted to the mark with the mobile phase. The working tablet solution containing 4 µg/ml OLM, 1 µg/ml AML for combination 1 and 4 µg/ml OLM, 2 µg/ml CIL of combination 2.20 µl of sample from each working tablet solution was directly injected in to HPLC column. All measurements were repeated six times for each concentration. Nominal contents of pharmaceutical preparation were calculated using regression equation of calibration graph.

Method validation

The optimized RP-HPLC method was validated according to the ICH guidelines [22] with respect to specificity, accuracy, precision, linearity, limit of detection, limit of quantification and robustness.

Specificity

The specificity of the method was performed by analysis of drug standard and samples. The mobile phase resolved both drugs very efficiently. The peak purity of OLM, AML and CIL was determined by comparing the retention time

Precision

Precision of an analytical method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (30.2, 60.4 and 90.7 µg/ml for OLM and CIL, 15.2, 30.1 and 45.2 µg/ml for AML) of the drugs for six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Accuracy

Accuracy of the method was evaluated by recovery studies. It was employed by the standard addition method. Three levels of solution were made at nominal analytical concentration such as 40%, 50%, and 60% respectively. Each level was made in triplicate.

Linearity

Linearity of the method was studied by injecting six concentration of the drug prepared in mobile phase in the range of 10-100µg/ml of OLM and CIL(10,20,40,60,80,100µg/ml), 5-50µg/ml for AML (5,10,20,30,40,50 µg/ml) respectively in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentration to obtain calibration graphs.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection LOD and quantification LOQ was determined based on the standard deviation of response and slope. Detection limit and quantification limit were calculated by [(3.3 ×σ)/S and (10×σ)/S] respectively. Where 'σ' is the standard deviation of the response and 'S' is slope of the calibration curve.

Robustness

The robustness is the capacity of the method to remain unaffected by small but deliberate change in chromatographic conditions. Robustness of the developed method was tested by studying the influence of small change in flow rate (±0.1 ml/min) and change in pH of the buffer (±0.2units).

RESULTS AND DISCUSSION

Method optimization

In this work, a simple, sensitive and validated HPLC method has been developed for simultaneous estimation of olmesartan OLM, amlodipine AML and cilnidipine CIL using liquid chromatography with ultraviolet visible detection. A number of mobile phase were initially attempted to elute three components simultaneously.

The main focus was to achieve sharp and Gaussian shaped peak with tailing less than 1.5 and resolution greater than 1.5. In order to achieve this goal acetonitrile, methanol, water in different proportion were used but no sharp peaks were observed. Ammonium acetate was then added to increase the polarity of mobile phase. A 0.05M concentrations of ammonium acetate at pH 7.3 with acetonitrile and methanol in the ratio of 30:50:20 v/v was strong enough to elute the three components with resolution greater than 5, theoretical plate greater than 3500 and tailing less than 1.5 for the three components. The best mobile phase composition was then found to be 0.05M ammonium acetate pH 7.3, acetonitrile and methanol in the ratio of 30:50:20 (v/v) Under mentioned chromatographic condition sharp peaks belonging to olmesartan medoxomil, amlodipine besylate and cilnidipine were obtained at retention time of 2.2, 3 and 4.5 minutes respectively (fig. 1 and 2).

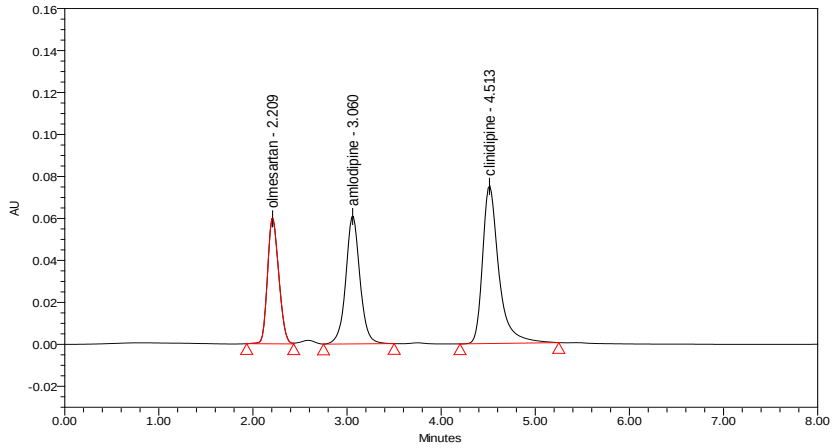

Fig. 1: Chromatogram for standard OLM, AML and CIL

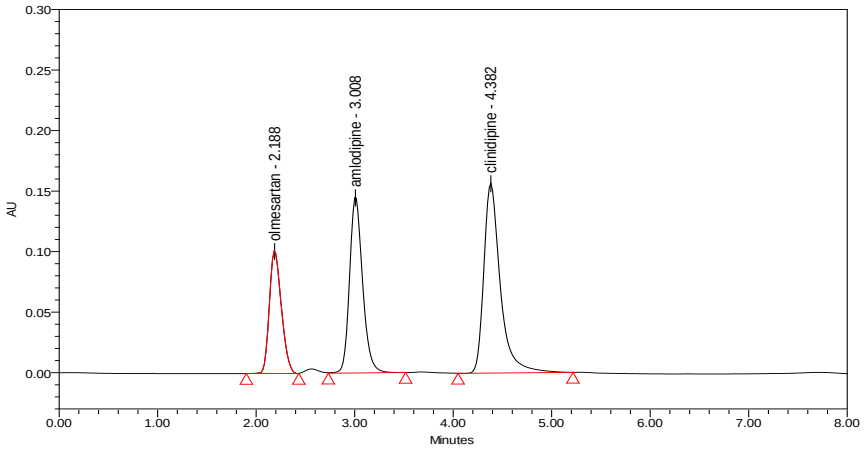

Fig. 2: Chromatogram for sample on simultaneous estimation of OLM, AML and CIL

Method validation

Linearity

Linearity was studied by preparing the standard solution at different concentration levels. The linearity range for OLM, AML, and CIL was found to be 10-100 µg/ml, 5-50 µg/ml and 10-100µg/ml respectively. The regression equation for OLM, AML and CIL were found to be Y=5.24e+004X-7.13e+004, Y=6.98e+004X-2.91e+004 and Y=1.12e+005X-2.47e+005 with correlation coefficient (R2) 0.998, 0.999 and 0.998 respectively for all the compounds, the coefficients of determination prove that the method was linear in specific range.

Precision

The result of the repeatability and intermediate precision experiment 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 (ICH Q2 (R1), 2005).

Accuracy

The accuracy of the method was performed by the standard addition technique. Three levels of solution were made which corresponds to 40, 50, and 60 % of the nominal analytical concentration. Each level was made in triplicate. The recovery and relative standard deviation for each of the analytes are given table 2. From the recovery studies it is evidence that the method is highly accurate and can give excellent results.

Limit of quantitation and limit of detection

Limit of quantification (LOQ) and Limit of detection (LOD) can be determined based on visual evaluation, signal-to noise approach and standard deviation of the response and slope. The LOD of OLM was 0.26 µg/ml, AML is 0.05µg/ml and CIL is 0.22µg/ml was determined. The LOQ of OLM is 0.79µg/ml, AML is 0.14µg/ml and CIL is 0.67µ/ml was determined.

Specificity

The peak purity of OLM, AML and CIL was assessed by comparing the retention time of standard OLM, AML and CIL. Good correlation was obtained between the retention time of the standard and sample of OLM, AML and CIL. Thus, the method was specific for OLM, AML and CIL.

Robustness

Robustness of the method was performed by small deliberate changes in the optimized chromatographic condition such as flow rate (±0.1 ml/min) and PH of ammonium acetate buffer (±0.2units). The result shown that have the negligible effect on retention time, recoveries and peak area of three drugs indicating the developed method is robust.

Table 1: Precision of the proposed HPLC method

Drug	Concentration (µg/ml)	N	Within day precision		Between dayprecision	
			Mean	RSD %	Mean	RSD%
Olm	30.2	6	30.59	1.16	29.98	1.34
	60.4	6	60.54	0.59	61.43	0.52
	90.7	6	90.70	0.52	90.56	0.51
Aml	15.2	6	15.68	1.16	15.86	1.34
	30.1	6	30.51	1.12	30.53	1.15
	45.2	6	45.58	0.82	44.85	0.60
CIL	30.2	6	30.32	1.29	29.86	0.89
	60.4	6	60.68	0.67	61.04	0.83
	90.7	6	90.63	0.52	90.27	0.51

Table 2: Accuracy of the proposed HPLC method

Drug	Level %	Concentration (µg/ml)	Amount recovered (µg/ml)	% Recovery	%RSD
OLM	40	40	40.15	100.38	1.16
	50	50	49.66	99.32	0.97
	60	60	59.78	99.64	1.06
AML	40	20	19.96	99.83	1.55
	50	25	24.64	98.58	0.79
	60	30	30.36	101.27	1.66
CIL	40	40	40.24	100.6	0.92
	50	50	49.77	99.54	0.99
	60	60	60.3	100.5	1.55

Table 3: Assay of commercial tablets

Drug	Label claim (mg)	Drug content(%)±SD	% R. S. D
Combination-I			
OLM	20	99.33±0.97	0.98
AML	5	98.59±0.78	0.79
Combination-II			
OLM	20	99.33±0.97	0.98
CIL	10	99.54±0.99	0.99

Analysis of commercial formulation

The amount of OLM, AML and CIL per tablet was calculated by extrapolating the value of an area from the calibration curve. Analysis procedure was repeated six times with the tablet formulation. The result of analysis of the tablet formulation is reported in table 3.

DISCUSSION

In previous method Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide combination, cilnidipine and olmesartan medoxomil combination was estimated by RP-HPLC in tablet dosage form. Here two combinations such as OLM and AML, OLM andCIL combinations were estimated simultaneously by HPLC. So this is new method in such combination. The retention time was less than 5 minutes. The method was validated as per ICH guidelines (ICH Q2 (R1), 2005). The method was simple, selective, cost effective and reproducible and can be reliably used by almost every drug laboratory.

CONCLUSION

HPLC method was developed and validated as per ICH guidelines (ICH Q2 (R1), 2005). It is simple, selective, cost effective and reproducible and can be reliably used by almost every drug laboratory. The method enables simultaneous determination of OLM, AML and CIL in pharmaceutical preparation. Due to the fact that these substances are mainly used as combination for hypertension therapy, this new procedure is very important. In the process of developing the method and validation studies were carried out. Finally the method was applied to analysis for two drug formulation including combination I and II, the run time was less than 5 minutes.

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CONFLICT OF INTERESTS

We wish to confirm that there are no known conflicts of interest associated with this publication.

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