Research Article

Determination of Olmesartan Medoxomil and Amlodipine in Tablet Formulations by Reversed-Phase HPLC

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ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Olmesartan medoxomil and Amlodipine in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column with mobile phase containing Acetonitrile:0.05 M KH2PO4 Buffer pH 3 (55:45). The flow rate was 1.5 ml/min and the eluent was monitored at 244 nm. The selected chromatographic conditions were found to effectively separate Olmesartan medoxomil (RT- 4.12 min) and Amlodipine (RT- 2.05 min). Linearity for Olmesartan medoxomil and Amlodipine were found in the range of 2-14 µg/ml. The values obtained of LODs were 0.184 and 0.154 µg/ml and LOQs were 0.558 and 0.467 µg/ml for Olmesartan medoxomil and Amlodipine, respectively. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous analysis of these drugs in tablet formulations.

Keywords: Chromatography, Olmesartan, Amlodipine, Validation.

INTRODUCTION

Olmesartan medoxomil (OLME) is a prodrug and hydrolyzed to Olmesartan during absorption from the gastrointestinal tract [1-4]. OMLE is a selective AT1 subtype angiotensin II receptor antagonist. OLME is described chemically as the (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl}-1*H*-imidazole-5-carboxylic acid [5,6].

Amlodipine(AMLO), chemically, 2-[(2-Aminomethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylic acid, 3-ethyl-5-methyl ester, is a calcium channel antagonist, used as an anti-hypertensive drug[7-8].

OLME has not yet been officially described in any pharmacopoeia. A literature survey revealed that several analytical methods were reported for the determination of OMLE including LC–MS–MS[9], capillary zone electrophoresis[10], HPLC[11-14] and HPTLC[15].

Literature survey revealed HPLC[16-17], HPTLC[18], LC-MS[19], LCMS/MS[20-22] and UV specrophotometric[23] methods for estimation of Amlodipine alone.

Spectrophotometric method for simultaneous estimation of OLME and AMLO[24]. The RP- HPLC method for simultaneous determination of OLME and AMLO in pharmaceutical formulation has not been reported so far. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.

MATERIALS

Instrumentation

- Shimadzu's HPLC (LC-10AT vp) with Photo diode array detector and Manual injector of 20-µl loop. Software used was Class-VP.
- Column used was Phenomenex C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size)
- Digital pH meter (Ecoscan)
- Corning volumetric flasks (10 ml, 50 ml,100 ml and 500 ml)
- Sartorius CP224S analytical balance
- Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai)
- Pipettes – 1 ml, 5 ml, 10 ml, beakers, measuring cylinders etc.
- Experiment was done in 2012-2013

Chemicals and materials

- Olmesartan medoxomil (OLME) - Torrent Pharmaceuticals Ltd.
- Amlodipine besylate (AMLO) - Cadila Pharmaceuticals Ltd.
- Acetonitrile (HPLC grade, S. D. Fine Chemicals Ltd., Mumbai)
- Water (HPLC grade, S. D. Fine Chemicals Ltd., Mumbai)
- Potassium Dihydrogen Phosphate (AR grade, S. D. Fine Chemicals Ltd., Mumbai)

- Ortho Phosphoric acid (AR grade, S. D. Fine Chemicals Ltd., Mumbai)

METHODS

Chromatographic conditions

The column used for chromatographic separations was Phenomenex C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size). The analytical wavelength was set at 244 nm and samples 20 µl were injected. The chromatographic separations were accomplished using mobile phase comprised of Acetonitrile: 0.05 M (pH 3) KH_2PO_4 Buffer (55:45, v/v) filtered through 0.45 µm filter (millipore) and de-aerated in ultrasonic bath. Mobile phase was pumped at a flow rate of 1.5 ml/min at ambient temperature.

Preparation of the mobile phase

Accurately weighed potassium dihydrogen phosphate (6.8 gm) was transferred to a 1000 ml volumetric flask, dissolved in and diluted up to the mark with water. pH 3 ± 0.02 was adjusted with ortho phosphoric acid. The mobile phase comprised of Acetonitrile: 0.05 M (pH 3) KH_2PO_4 Buffer (55:45, v/v). The mobile phase was filtered through nylon 0.45 µm membrane filter and was degassed before use.

Preparation of Standard solutions:

A. Standard OLME stock solution (100 µg/ml):

Standard OLME powder (5 mg) was weighed accurately and transferred into 50 ml volumetric flask, dissolved and diluted to 50 ml with mobile phase to obtain a standard solution having concentration of 100 µg/ml (OLME).

B. Standard AMLO stock solution (100 µg/ml):

Standard AMLO powder (5 mg) was weighed accurately and transferred into 50 ml volumetric flask, dissolved and diluted to 50 ml with mobile phase to obtain a standard solution having concentration of 100 µg/ml (AMLO).

Determination of wavelength of maximum absorbance:

The standard solution of OLME and AMLO were scanned in the range of 200-400 nm against mobile phase blank. OLME and AMLO showed maximum absorbance at 244 nm. So, the wavelength selected for the determination of OLME and AMLO was 244 nm.

Calibration curve of standard OLME and AMLO

A calibration curves were plotted over a concentration range of 2 - 14 µg/ml for OLME and AMLO. Accurately measured standard stock solutions of OLME and AMLO (0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solution (20µl) was injected into the column and the peak area obtained at retention time 2.059 min and 4.129 min and flow rate 1.5 ml/min were measured at 244 nm for OLME and AMLO respectively. Calibration curves were constructed for OLME and AMLO by plotting peak area versus concentration at 244 nm. Each reading was average of three determinations.

Sample solution

Twenty tablets were weighed accurately and powdered. Powder equivalent to 20 mg of OLME and 5 mg of AMLO was weighed and transferred in a 100 ml volumetric flask and mobile phase (50 ml) was added. This solution was sonicated for 15 minutes, and final volume was made to the mark with mobile phase. The solution was filtered through Whatman filter paper No. 41. The filtrate (5ml) was transferred in a 100 ml volumetric flask and diluted to the mark with mobile phase to obtain sample solution of OLME (10 µg/ml) & AMLO (2. µg/ml).

METHOD VALIDATION

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determined with precision and accuracy using the method. The linear response of OLME and AMLO were determined by analyzing five independent levels of the calibration curve in the range of 2 - 14 µg/ml. Result should be expressed in terms of Correlation co-efficient.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

A. Repeatability (Precision on replication):

It is precision under the same condition (same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of OLME (10 µg/ml) and AMLO (10 µg/ml) for six times and analyzed as per the proposed method. Percentage relative standard deviation (% RSD) or coefficient of variation (CV) was not more than 2%.

B. Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Variation of results within same day is called Intra-day precision and variation of results amongst days called Inter-day precision. The Intra-day precision (CV) was determined for standard solution of OLME and AMLO (2-14 µg/ml) for four times at the same day. The Inter-day precision (CV) was determined for standard solution of OLME and AMLO (2 - 14 µg/ml) for four times at different four days.

Accuracy (% Recovery)

Accuracy of an analysis is determined by systemic error involved. It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (OLME 4 µg/ml and AMLO 1 µg/ml) with three different concentrations of standards (OLME 2,4,6 µg/ml and AMLO 2,4,6 µg/ml).

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 × N/S

Where N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × N/S

Where N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

System suitability: System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

RESULTS AND DISCUSSION

Selection of mobile phase

Various compositions of mobile phase were used. The best results were obtained with Acetonitrile: 0.05 M (pH 3) KH_2PO_4 Buffer (55:45 v/v) at 1.5 ml/min flow rate which gave the retention times were 2.059 min for AMLO and 4.129 min for OLME as shown in Fig 1. The calibration graphs for OLME and AMLO were constructed by plotting the peak area versus their corresponding concentrations, respectively; good linearity for both was found over the range of 2 - 14 µg/ml.

Results obtained by applying the RP-HPLC method showed that the concentrations of OLME and AMLO can be simultaneously determined in tablets. The proposed method has been applied to the assay of OLME and AMLO in tablet dosage forms. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives present do not interfere with analysis of the tablets. The regression characteristics and validation parameters are reported in Table VII. Calibration curve of OLME and AMLO are shown in Fig 2 and 3 respectively.

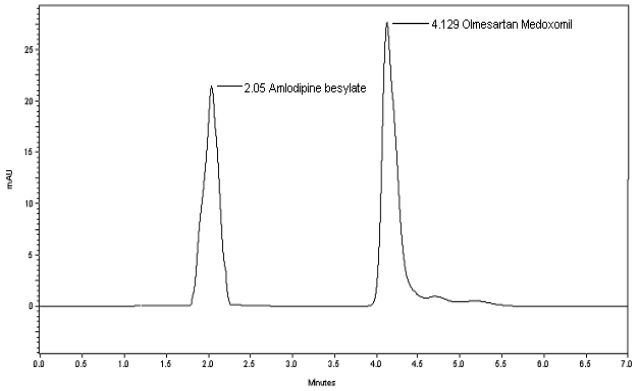

Figure 1: HPLC Chromatogram OLME (10 µg/ml) and AMLO (10 µg/ml)

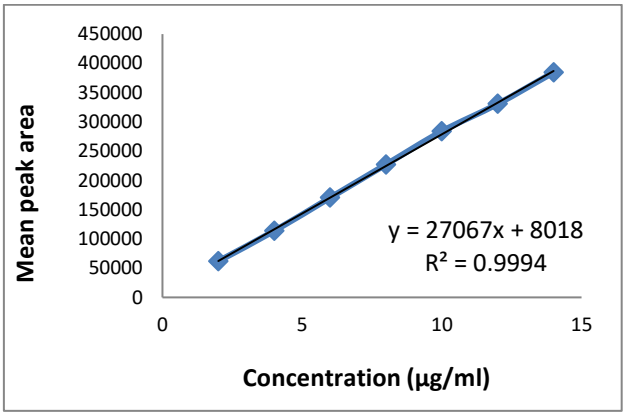

Figure 2: Calibration curve of OLME

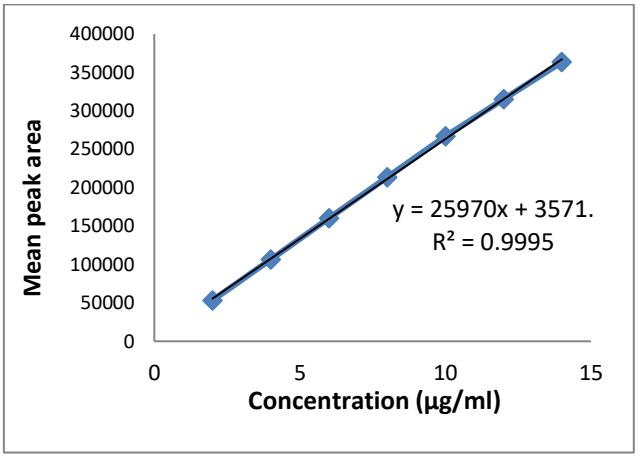

Figure 3: Calibration curve of AMLO

Validation:

Linearity and range: The seven-point calibration curves that were constructed were linear over the selected concentration range for both OLME and AMLO ranging between 2-14 µg/ml. Each concentration was repeated 3

times. The assay was performed according to the experimental conditions previously described. The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the correlation coefficient and the intercept value.

Accuracy: Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The good recoveries with the standard addition method (Table I) prove the good accuracy of the proposed methods.

Precision: For evaluation of precision, repeatability of the results for a concentration of 10 µg/ml was evaluated by 6 replicate determinations. For evaluation of intermediate precision, the results over the concentration range 2-14 µg/ml were evaluated by 4 replicate determinations to estimate intraday variation and another replicate determination on different 4 days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

Limit of Detection: LOD for OLME and AMLO were found to be 0.184 µg/ml and 0.154 µg/ml respectively.

Limit of Quantification: LOQ for OLME and AMLO were found to be 0.558 µg/ml and 0.467 µg/ml respectively.

System suitability testing for HPLC: (Table V).

Application to the tablets

The proposed validated method was successfully applied to determine OLME and AMLO in tablet dosage forms. Results are given in Table VI. No interference of the excipients with the peaks of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of OLME and AMLO in pharmaceutical dosage forms.

Table I: Data of recovery study

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
OLME	4	2	5.89	98.16 ± 0.52
	4	4	8.14	101.75 ± 0.35
	4	6	10.19	101.90 ± 0.47
AMLO	1	2	3.07	102.33 ± 0.58
	1	4	5.09	101.80 ± 0.34
	1	6	6.94	99.14 ± 0.26

Table II: Method precision data

Parameters	Area of OLME	% Assay	Area of AMLO	% Assay
1st	279748	100.39	266634	101.29
2nd	284201	102.03	265427	100.83
3rd	283305	101.70	262739	99.79
4th	283029	101.61	266452	101.22
5th	276385	99.14	265438	100.83
6th	283982	101.95	264325	100.40
Mean		101.13		100.72
SD		1.1441		0.5596
%RSD		1.1313		0.5556

Table III: Intra-day precision data

Concentration (µg /ml)	Intra-day precision			
	OLME		AMLO	
	Mean ± S.D (n=4)	% RSD	Mean ± S.D (n=4)	% RSD
2	61177 ± 864	1.41	53330 ± 504	0.94
4	114132 ± 1038	0.91	106352 ± 495	0.47
6	170545 ± 1597	0.94	160348 ± 1823	1.14
8	227188 ± 1269	0.56	213197 ± 1081	0.51
10	283408 ± 1820	0.64	267525 ± 1369	0.51
12	330207 ± 1407	0.43	315633 ± 1451	0.45
14	382957 ± 2578	0.67	363079 ± 1773	0.49

Table IV: Inter-day precision data

Concentration (µg /ml)	Inter-day precision			
	OLME		AMLO	
	Mean ± S.D (n=4)	% RSD	Mean ± S.D (n=4)	% RSD
2	61743 ± 749	1.21	53793 ± 612	1.13
4	115648 ± 1205	1.04	107347 ± 927	0.86
6	171239 ± 1745	1.01	161038 ± 1105	0.69
8	227836 ± 963	0.42	212873 ± 1842	0.86
10	289547 ± 1683	0.58	267093 ± 2136	0.80
12	331286 ± 2446	0.73	314821 ± 1532	0.48
14	382356 ± 2090	0.54	363828 ± 1986	0.54

Table V: Parameters of chromatogram

System Suitability Parameters	OLME	AMLO
Retention Time	4.12	2.05
Tailing factor	1.49	0.96
Theoretical plate	2452	5372

Table VI: Estimation of OLME and AMLO tablets

Formulation	OLME			AMLO		
	Amount labeled (mg)	Amount found (mg)	% Amount Found ± S.D. (n=3)	Amount labeled (mg)	Amount found (mg)	% Amount Found ± S.D. (n=3)
Brand I	20	20.41	100.76 ± 0.83	5	4.93	100.60 ± 0.62
Brand II	20	20.32	100.15 ± 0.74	5	4.97	99.46 ± 0.93

Table VII: Regression characteristics and validation parameters

Parameters	OLME	AMLO
Calibration range	2-14 µg/ml	2-14 µg/ml
Detection limit	0.184 µg/ml	0.154 µg/ml
Quantitation limit	0.558 µg/ml	0.467 µg/ml
Slope	27067	25970
Intercept	8018	3571
Correlation co- efficient(r)	0.9996	0.9997
Intraday (% RSD)	0.43 -1.41	0.45 - 1.14
Interday (% RSD)	0.42 -1.21	0.48 - 1.13

Intraday and interday relative standard deviation (RSD) values of the whole concentration range

CONCLUSION

Results are in good agreement with claim which indicates there is no interference of routinely used excipients. The proposed method was accurate, precise, simple, sensitive and rapid. The proposed method can be used for routine analysis of OLME and AMLO in tablets.

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