Bulletin of Pharmaceutical Research 2016;6(2):36-44
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)
DOI: 10.21276/bpr.2016.6.2.1

RESEARCH PAPER

SIMULTANEOUS DETERMINATION OF AMLODIPINE AND OLMESARTAN IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY AND ITS APPLICATION IN PHARMACOKINETIC STUDY

Peeyush Jain[1], Yashumati R. Bhardwaj[2] and Dharma Kishore[2]*

[1]*Jubilant Clinsys Limited, Noida-201307, Uttar Pradesh, India*
[2]*Department of Pharmacy, Banasthali University, Banasthali-304 022, Rajasthan, India.*

**E-mails*: kishoredharma@yahoo.co.in, peeyush_jain108@yahoo.com
Tel.: +91 1438 228456.

Received: Feb 24, 2016 / Revised: May 22, 2016 / Accepted: May 23, 2016

The present study describes a sensitive, specific and rapid method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of amlodipine (AML) and olmesartan (OLM) in human plasma by using amlodipine D4 (IS1) and olmesartan D6 (IS2) as internal standards. Plasma samples were extracted by solid-phase extraction (SPE). The method was validated over parameters like selectivity, matrix effect, sensitivity, specificity, linearity, precision and accuracy, various stabilities in plasma, recovery and reinjection reproducibility. During the validation, inter and intra-batch precision were less than 15% and the accuracy was within 85-115%. Extraction recoveries were 75.30%, 81.41%, 79.19% and 81.72% for AML, OLM, IS1 and IS2 respectively. The method was applied to the pharmacokinetic study of OLM and AML in healthy subjects following a single oral dose of OLM and AML 40 mg/10 mg.

Key words: Tandem mass spectrometry, Amlodipine, Olmesartan, Solid-phase extraction.

INTRODUCTION

OLM Medoxomil is a pro-drug and is hydrolyzed to OLM during absorption from the gastrointestinal tract (Yanagisawa *et al* 1996; Mire *et al* 2005). OLM 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-{[20-(1*H*-tetrazol-5-yl)[1,10-biphenyl]-4-yl]methyl}-1*H*-imidazole-5-carboxylic acid. OLM is an angiotensin II receptor antagonist used for hypertension (Mizuno *et al* 1995). AML Beslylate chemically dihydropyridine is a potent calcium channel blocking agent, inhibits the calcium influx through slow channels in peripheral vascular and coronary smooth muscle cells, and thus is useful in hypertension and angina pectoris (Murdoch and Heel, 1991; Haria and Wagstaff, 1995). AML Besylate is described chemically as the 3-ethyl-*O*-5-methyl-2-(2-amino ethoxy methyl)-4-(2-chloro phenyl)-6-methyl-1, 4-dihydropyridine-3,5-dicarboxylate. New drugs and their combinations offers better inpatient compliance, than a single drug. Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. The US FDA has approved fixed dose combination of AML Besylate and OLM Medoxomil (5 mg /20 mg, 5 mg /40 mg, 10 mg/20 mg and 10 mg/40 mg) for patients with hypertension who do not respond efficiently to monotherapy of either drug (Punzi *et al* 2010; Bramlage *et al* 2010).

HPLC has been remained as a method of choice for determination of drugs alone or in

combination with other drugs in the pharmaceutical formulations (Bhimavarapu *et al* 2011; Basaveswara Rao *et al* 2012; Chhabra and Banerjee, 2013; Patwari *et al* 2014; Singh and Dahiya, 2014; Shrestha *et al* 2016). Till date, several methods have been reported for the determination of OLM and AML either individually or in combination with other drugs in plasma with LC–MS/ MS and HPLC-UV (Liu *et al* 2007; Chen *et al* 2008; Liu *et al* 2010; Sengupta *et al* 2010; Zhou *et al* 2013). Two methods reported for combination of these drugs for simulation determination in plasma are one each on HPLC-UV and LC-MS/MS (Shah *et al* 2012; Qi *et al* 2013). The method on HPLC-UV resulted in low sensitivity, long analytical run and complex sample preparation whereas the method reported on LC-MS/MS has higher sensitivity, small analytical run but at the same time has limitation of lower recovery of AML, narrow linearity range of OLM and OLM internal standard was not stable isotope of OLM. Recovery (non-ionization matrix effects) refers to loss of analyte during the sample preparation and separation steps, its matrix effects should not be ignored.

Here, we introduce a rapid LC-MS/MS method for simultaneous determination of OLM and AML in human plasma which is sensitive and specific, over a wide linearity range. The plasma samples extracted by SPE resulted in higher recovery and use of the stable isotope of the analytes as the internal standards to yield better performance results. This method has been successfully applied in a pharmacokinetic study of single dose of OLM and AML 40 mg/10 mg strength.

MATERIALS AND METHODS

Chemicals and reagents

AML (> 99.59% *w/w* on as is basis), OLM (> 99.08% *w/w* on as is basis), IS1(> 99.44% *w/w* on as is basis) and IS2 (> 99.19% *w/w* on as is basis) were obtained from Clearsynth Labs imited, India. HPLC-grade acetonitrile, methanol and ammonium formate were obtained from SD Fine Chemical Ltd. (India). Ortho-phosphoric acid and formic acid were obtained from Merck (Fluka Chemie, GmbH, Germany). HPLC grade water 18.2 mΩ cm (milliohm centimeter) and TOC ≤ 50 ppb (parts per billion)] were obtained from Milli-Q system (Millipore SAS, Molsheim, France). All other reagents and chemicals used for the study were of HPLC grade unless specified. Genesis AQ C18, 5 μm (100 × 4.6 mm) HPLC column was obtained from Grace Jones Genesis, USA) and HLB cartridges (30 mg/1 cc) used for the extraction was obtained from Waters Corporation (Milford, MA, USA). Blank plasma lots were obtained from Laxmi Sai Clinical Labs, India.

HPLC-MS/MS condition

Liquid chromatography was performed on Shimadzu High Performance Liquid Chromatography (HPLC) unit (Shimadzu SIL HTC, USA) with Genesis AQ C18 100 × 4.6, 5 μm column. The analytes were chromatographically separated using isocratic program with mobile phase consisting [organic mixture (acetonitrile : methanol :: 60:40): buffer solution (5 mM ammonium formate in Milli-Q water containing 0.1% formic acid): 60:40 *v/v*)]. The flow rate was 0.8 ml/min and the injection volume was 10 μl. The column and sample temperature were maintained at 35°C and 5°C, respectively. An AB Sciex QTRAP 4000 triple quadruple mass spectrometer equipped with an Electro-Spray Ionization (ESI) source (Toronto, Canada) was used for mass spectrometric detection. The quantitative analysis of AML and OLM in human plasma was performed using Multiple Reactions Monitoring (MRM) method.

The dwell time was set to 200 ms for each MRM transition. The MRM transitions were *m/z* 409.2/ 238.0, *m/z* 447.3/207.1, *m/z* 413.2/238.0 and *m/z* 453.2/207.0 for AML, OLM, IS1 and IS2 respectively. The optimal MS parameters were as follows: Curtain gas (CUR) 35 psi, ion spray voltage (IS) 5500 V, source temperature (TEM) 500°C, collision gas (CAD)16 psi, GS1: 45 psi and GS2: 55 psi, while the declustering potential (DP), collision energy (CE) and cell exit potential (CXP) applied 48, 15 and 18 V for the AML and IS1 respectively. The declustering potential (DP), collision energy (CE) and cell exit potential (CXP) applied were 70, 16 and 12 V for the OLM and IS2 respectively.

Stock solutions, calibration standards and quality control (QC) sample preparation

Primary stock solution of AML and OLM that was used for preparation of calibration standard and quality control (QC) samples was prepared separately in methanol to obtain concentration of AML (1 mg/ml) and OLM (5 mg/ml). The primary stock solution of IS1 and IS2 were prepared in methanol to obtain concentration of 100 μg/ml.

The stock solution of internal standards were further diluted with diluent solution (Methanol :

Milli-Q water :: 50:50, *v/v*) to obtain the concentration of 55 ng/ml and 150 ng/ml for IS1 and IS2 respectively.

Aqueous dilutions for AML and OLM were prepared by serially diluting the primary stock solutions with diluents solution. Spiking of aqueous dilutions in human plasma was done to give eight-point calibration curve standards (0.209 to 15.197 ng/ml) for AML and (5.036 to 2081.502 ng/ml) for OLM. In a similar way spiking of aqueous quality control dilutions were done in human plasma to prepare the quality control samples consisting of AML and OLM (**Table 1**). Primary stock solutions were kept at 2-8°C and spiked calibration curve standards and QC samples were stored at -65°C.

Table 1. Recovery and matrix effect of OLM, AML and Internal standards (n = 6)

Analyte	Conc. added (ng/ml)	Recovery (%)		Mean % Recovery	% CV of Mean % Recovery	Matrix factor		Matrix effect
		Mean±SD	CV (%)			Mean±SD	CV (%)	
OLM	13.110	78.53±5.86	7.46	75.30	3.71	1.004±0.0315	3.14	-0.37
	874.029	74.20±2.63	3.55					
	1748.059	73.22±2.60	3.55			1.040±0.0122	1.17	-4.02
AML	0.578	82.45±3.66	4.44	81.41	1.43	0.993±0.0335	3.37	0.71
	7.647	81.68±3.08	3.77					
	12.166	80.18±3.34	4.16			1.019±0.0263	2.58	-1.91
IS1	55.000	79.19±2.75	3.48	79.19	3.48	0.997±0.0183	1.84	0.33
IS 2	150.000	81.72±1.14	1.39	81.72	1.39	1.011±0.0117	1.16	-1.06

Sample preparation

Plasma samples were extracted by SPE method using HLB cartridge (30 mg/1 cc). Plasma, 200 μl was mixed with 50 μl internal standard (55 ng/ml IS1 and 150 ng/ml IS2) and 200 μl of 1 % Ortho-phosphoric acid solution.

The HLB cartridge was conditioned with 1.0 ml methanol and 1.0 ml Milli-Qwater. The mixture, 450 μl was then loaded onto the cartridge. Cartridges were washed with 2.0 ml Milli-Q water. The cartridges were than dried for approximately 2 min. The elution of these compounds was carried out with 1.0 ml of methanol. The eluate evaporated to dryness at 50°C and at constant pressure in nitrogen evaporator. Finally, 200 μl of mobile phase added to the residues and well mixed before injection.

Bioanalytical method validation

The method was validated, before using it in pharmacokinetic study sample analysis in terms of selectivity, sensitivity, specificity, linearity, precision, accuracy, recovery, matrix effect, reinjection reproducibility and stability of analytes in plasma according to the United States Food and Drug Administration (FDA) guidelines for validation of a bioanalytical method (US FDA, Guidance for Industry, 2001).

Selectivity, specificity and sensitivity

Chromatogram comparisons of blank and spiked human plasma from six different lots were used to evaluate the selectivity of the method. Specificity performed to check the interference at the retention time of AML in presence of higher calibration curve standard concentration of OLM and vice- versa. The sensitivity was determined by quantifying the lower limit of quantification (LLOQ). The LLOQ was determined as the lowest concentration that could be quantified with an acceptable precision and accuracy within ±20%.

Matrix effect

To determine the matrix effect, six different normal blank plasma lots, three different haemolysed plasma lots and three different lipemic plasma lots samples were utilized to prepare QC samples and used for assessing the lot-to-lot matrix effect. Matrix effect estimated quantitatively through calculation of matrix factor, which is the ratio of peak response in the presence of matrix ions to the peak response in the absence of matrix ions. Matrix effect further evaluated from matrix factor as follows.

% Matrix effect = 1 – mean of the matrix factor for analyte / IS × 100

Goodness of fit, linearity, precision and accuracy
The data of three precision & accuracy batches used for the estimation for goodness of fit. The batches comprising of standard blank (blank without IS), standard zero (blank with IS), calibration standards and six replicates of quality control samples. The back-calculated concentrations of calibration curve standards using $1/x$ and $1/x^2$ weighing were considered for finding the best fit for regression. The precision of the assay measured by the calculation of percentage co-efficient of variation over the concentration range of quality control samples that were run within a day (intraday) or on different days (inter-day).Accuracy expressed in percentage and calculated asthe ratio of the calculated mean values of the quality control samples to their respective nominal values.

Stability
The stock solution stability was evaluated at room temperature and at 2-8°C for analytes (AML and OLM) and internal standards (IS1 and IS2).The stability experiments were also performed to evaluate analytes stability in human plasma under the following different conditions: short-term stability at room temperature for 15 h (Bench Top Stability); long-term stability at −22°C and −65°C for 90 days; five freeze (−65°C) thaw (room temperature) cycles on consecutive days. The extracted QC samples kept in the auto sampler at 5°C for 75 h, in refrigerator (2-8°C) for 73 h after reconstitute (Wet extract stability) and extracted QC samples kept in refrigerator (-17 to -27 °C) for 73 h before reconstitute (Dry extract Stability) were analyzed to evaluate post-preparation stability. All stability testing in plasma was determined by analyzing six replicates of QC samples at two concentration levels. The stability quality control samples and freshly spiked quality control samples, quantifying them against the freshly spiked calibration curve standards. The samples were concluded to be stable if the assay values were within the acceptable limits of ±15% deviation from the nominal concentration and percentage change from the assay values of freshly spiked quality control samples were within the acceptable limits of ±15%.

Reinjection reproducibility and recovery
Reinjection reproducibility performed by reinjection of a complete precision and accuracy batch after storage in the auto sampler for 68 hours at 5 °C. The recovery of analytes and internal standards was evaluated by comparing mean peak areas of analytes and internal standards in plasma spiked before and after extraction.

Data processing and regression
The MRM chromatographic peaks were integrated using Analyst software version 1.4.2 and regression was performed using Watson LIMS.

Application
The validated method successfully applied to evaluate the bioequivalence of two tablet formulations of AML/OLM in healthy volunteers: The study test product compared to reference product equal doses of (10 mg/40 mg tablet) of each product administered to healthy participants under fasting condition. Twenty eight volunteers were given written informed consent to participate in the study according to the principles of the Declaration of Helsinki. The study was carried using a open-label, balanced, randomized, two-treatment, two period, two-sequence, single-dose, two-way crossover design. The study conducted as per the ICH-GCP guidelines after getting approval of the study protocol from the independent ethics committee.

RESULTS AND DISCUSSION

Method development
The scanning and acquisition of the parent and the product ions for AML, OLM, IS1 and IS2 were carried out by continuous infusion of analytes and internal standards dilutions at appropriate concentration through a pump and sorting out appropriate polarity and ions. The highest intensity of all analytes was observed in ESI source in a positive ion mode. Cmax of OLM was higher than 546.2 ng/ml, hence linearity range was taken 5.036 to 2081.502 ng/ml to avoid the study samples further repeat in value above the concentration range (Yu *et al* 2006). OLM had wide range of linearity; therefore IS2 response was decreased with increased concentration of OLM linearity due to charge competition in ESI source. Chromatographic conditions were optimized to achieve best sensitivity, peak shape, separation of peaks and no charge competition in source. Mobile phase [Organic mixture (Acetonitrile: Methanol: 60:40): Buffer Solution (5 mM ammonium formate in Milli-Q water containing 0.1% formic acid) :: 60:40 *v/v*)] with column Genesis AQ C18 100*4.6, 5 μm served the desired purpose with utmost

effectiveness. AML and OLM retention times were different. Recovery of AML in protein precipitation and liquid-liquid extraction (LLE) methods using different solvents was low therefore we choose the SPE method in which recovery was higher than protein precipitation and LLE. Stable istope of analytes internal standards (IS1 and IS2) was used which had similar ionization condition, appropriate retention time and recovery compared to AML and OLM leading to better tracking of analytes during the quantitation.

Selectivity, specificity and sensitivity

No interference of endogenous matrix/ impurities found at the retention time of the analytes and internal standards. Representative chromatograms of extracted blank human plasma (**Figure 1a**) and blank human plasma fortified with IS (**Figure 1b**), demonstrated the selectivity of the method. The Specificity carried out in 6 plasma lots. There was no significant interference observed at the retention time of AML in presence of higher concentration of OLM and vice-versa. Sensitivity was determined by analyzing 6 replicates of blank human plasma spiked with the analytes at the lowest limit of the quantification (0.209 ng/ml for AML and 5.036 ng/ml for OLM). The precision and accuracy for AML at LLOQ was 4.63% and 103.35% and for OLM 3.54% and 94.22% respectively. The representative chromatogram for LLOQ showing sensitivity was depicted in **Figure 1c**.

Matrix effect assessment

The matrix effect in normal, haemolysed and lipemic human plasma were all between -4.02 to 0.71 for AML, OLM, IS1 and IS2 lower quality control (LQC) and higher quality control (HQC) levels (**Table 1**). The matrix effect on the ionization of the analytes and internal standards was not obvious under these conditions. These data indicated that the sample preparation method was satisfactory.

Goodness of fit, linearity, precision and accuracy

The goodness of fit results showed $1/x^2$ to be the best fit for regression. Calibration curves were linear over the concentration range 0.209 to15.197 ng/ml for AML and 5.036 to 2081.502 ng/ml for OLM. The correlation coefficient for AML and OLM was more than 0.99. The typical regression equation of these curves was calculated as follows: AML, y = (0.137104 x + 0.003715, r = 0.9992); OLM, y = (0.000486 x + 0.000339, r = 0.9996). As shown, all the standard calibration curves showed good linearity within the range using least squares regression analysis. The intra- and inter-day precisions and accuracies of low, medium and high QC levels of the analytes summarized in **Table 2**. The assay values for both intra- and inter-day were found to be within 15%. The results showed that the method was accurate and precise for simultaneous determination of two analytes in human plasma.

Stability study

Stability studies performed to evaluate the AML and OLM stability in across different parameters. Stock solution of AML, OLM, IS1 and IS2 were found stable at room temperature for 41 h with a percentage change of -2.29% to 0.66%, whereas the stock solutions were stable in refrigerator at 2-8°C for 10 days with a percentage change of -0.54% to 1.37%. OLM and AML were stable in human plasma under the follow condition: bench top storage (room temperature) for 15 h; at 5°C for 75 h post-extraction; after five freeze-thaw

Table 2. Inter and intra-day precision and accuracy of the method for OLM and AML

Analyte	QC Level	Conc. added (ng/ml)	Inter-day (n=6)			Intra-day (n=18)		
			Mean conc. (ng/ml)±SD	(%) Accuracy	CV (%)	Mean conc. (ng/ml)±SD	(%) Accuracy	CV (%)
OLM	LLOQQC	5.048	4.679±0.203	92.29	4.34	5.131±0.389	101.64	7.58
	LQC	13.110	13.137±0.242	100.21	1.84	13.638±0.534	104.03	3.92
	MQC	874.029	852.351±10.156	97.52	1.19	865.069±17.184	99.97	1.99
	HQC	1748.059	1691.574±27.816	96.77	1.64	1718.374±35.125	98.30	2.04
AML	LLOQQC	0.210	0.213±0.011	101.43	5.16	0.213±0.017	101.43	7.98
	LQC	0.578	0.567±0.018	98.10	3.17	0.560±0.025	97.89	4.46
	MQC	7.647	7.291±0.194	95.44	2.66	7.326±0.171	95.80	2.33
	HQC	12.166	11.576±0.360	95.15	3.11	11.631±12.166	95.60	2.14

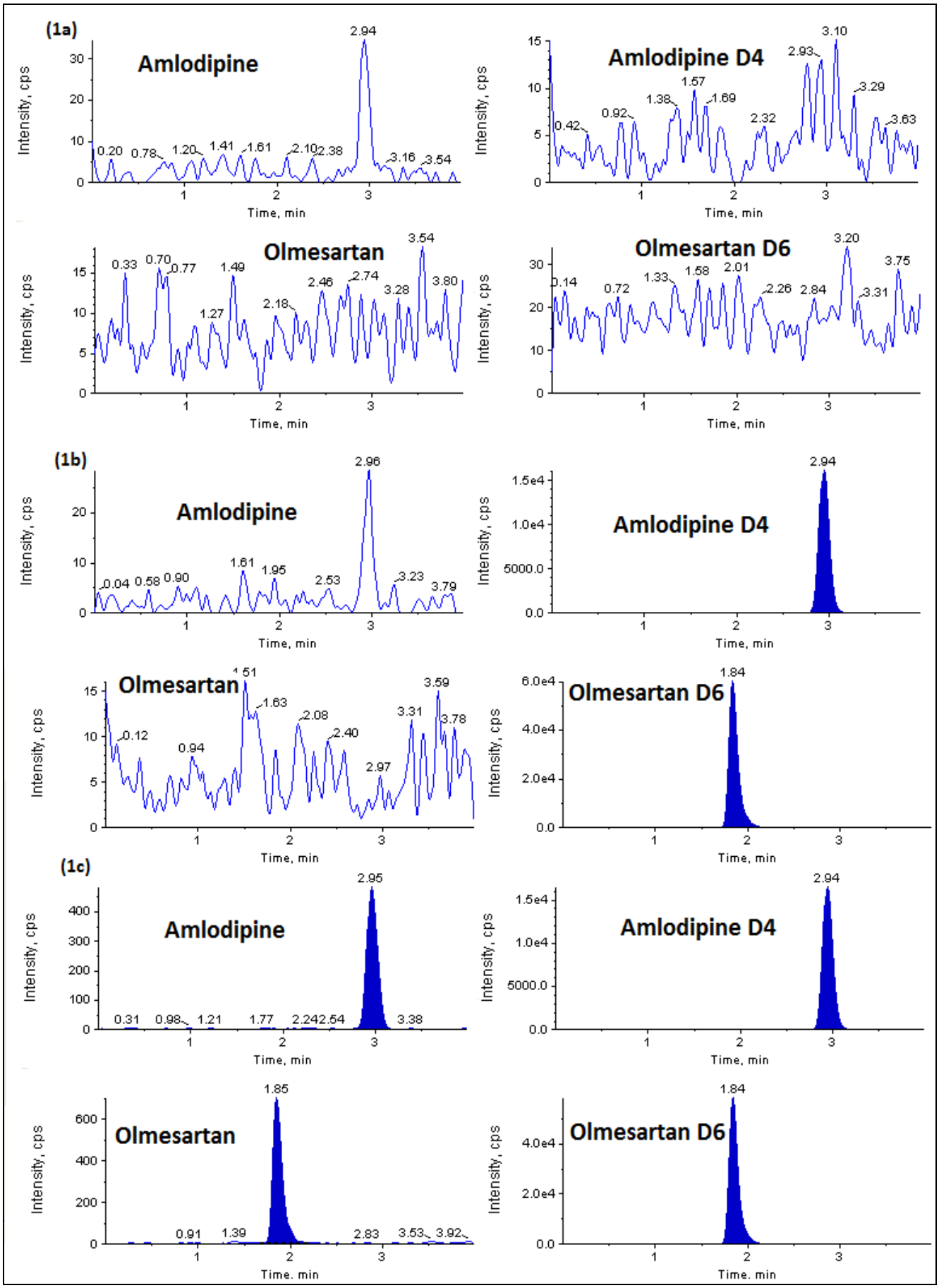

Fig. 1. Representative Chromatograms of (a) extracted blank human plasma (b) extracted blank human plasma fortified with Internal Standard (c) extracted LLOQ

cycles; long-term storage (−22°C and −65°C) for 90 days; wet extract stability (2 to 8°C) for 73 h and dry extract stability (-17 to -27°C) for 73 h. The results were found to be within the assay variability limits. All stability results are shown in the **Table 3**.

Table 3. Stability data for AML and OLM in plasma

Stability	Analyte	Conc. (ng/ml)	Mean conc. found in stability samples (ng/ml)	Nominal (%)	CV (%)	Mean conc. found in comparison samples (ng/ml)	Nominal (%)	CV (%)	Change (%)
Bench Top Stability (15 h)	AML	0.578	0.532	92.04	4.89	0.557	96.70	2.15	-4.82
		12.166	11.295	92.85	2.55	11.919	98.07	2.58	-5.33
	OLM	13.110	14.101	107.56	2.85	13.636	104.20	3.70	3.22
		1748.059	1724.581	98.66	2.38	1713.873	98.23	1.69	0.44
Auto sampler Stability (75 h)	AML	0.578	0.571	98.79	4.03	0.563	97.57	5.15	1.25
		12.166	11.725	96.38	3.22	11.754	96.55	3.25	-0.18
	OLM	13.110	14.328	109.29	7.87	13.311	101.60	3.87	7.57
		1748.059	1811.233	103.61	6.81	1763.454	100.94	2.25	2.64
Freeze-Thaw Stability (5-cycles)	AML	0.578	0.515	89.10	4.27	0.563	97.57	5.15	-8.68
		12.166	10.995	90.37	1.96	11.754	96.55	3.25	-6.40
	OLM	13.110	13.578	103.57	0.84	13.311	101.60	3.87	1.94
		1748.059	1786.570	102.20	2.17	1763.454	100.94	2.25	1.25
Long term stability at-65°C±10°C (90 days)	AML	0.578	0.541	93.60	4.44	0.557	96.70	2.15	-3.21
		12.166	11.935	98.10	1.47	11.919	98.07	2.58	0.04
	OLM	13.110	13.612	103.83	2.18	13.636	104.20	3.70	-0.36
		1748.059	1710.313	97.84	1.86	1713.873	98.23	1.69	-0.40
Long term stability at -22°C±5°C (90 Days)	AML	0.578	0.541	93.60	5.36	0.557	96.70	2.15	-3.21
		12.166	11.459	94.19	2.34	11.919	98.07	2.58	-3.95
	OLM	13.110	13.435	102.48	5.83	13.636	104.20	3.70	-1.65
		1748.059	1724.486	98.65	2.40	1713.873	98.23	1.69	0.43
Dry Extract Stability (73 h)	AML	0.578	0.534	92.39	4.49	0.563	97.57	5.15	-5.32
		12.166	11.599	95.34	2.51	11.754	96.55	3.25	-1.25
	OLM	13.110	13.585	103.62	2.69	13.311	101.60	3.87	2.00
		1748.059	1726.690	98.78	2.64	1763.454	100.94	2.25	-2.15
Wet Extract Stability (73 h)	AML	0.578	0.571	98.79	4.23	0.563	97.57	5.15	-7.80
		12.166	11.090	95.58	4.13	11.754	96.55	3.25	-5.59
	OLM	13.110	13.646	104.09	5.39	13.311	101.60	3.87	2.45
		1748.059	1786.658	102.21	1.61	1763.454	100.94	2.25	1.25

Reinjection reproducibility and recovery

The reinjection reproducibility percentage change ranged from -3.79% to 2.01% for AML and -4.06% to 3.05% for OLM at 5°C for 47 h. The mean percentage recovery of AML, OLM, IS1 and IS2 were 75.30%, 81.41%, 79.19% and 81.72% with a precision of 3.71%, 1.43%, 3.48% and 1.39% respectively (**Table 1**).

Pharmacokinetic evaluation

The developed and validated bioanalytical method was successfully applied for the determination of plasma concentrations of both AML and OLM in the plasma samples harvested during a bioequivalence study on 28 healthy male volunteers.

The mean pharmacokinetic profiles are illustrated in **Figure 2a-b**, whereas the pharmacokinetic parameters of Cmax, Tmax, and AUC0-∞ were evaluated. The 90% confidence interval of the individual ratio geometric mean for test/reference was within 80–125% forAUC0-∞ and Cmax. These findings suggest that the drugs are bioequivalent.

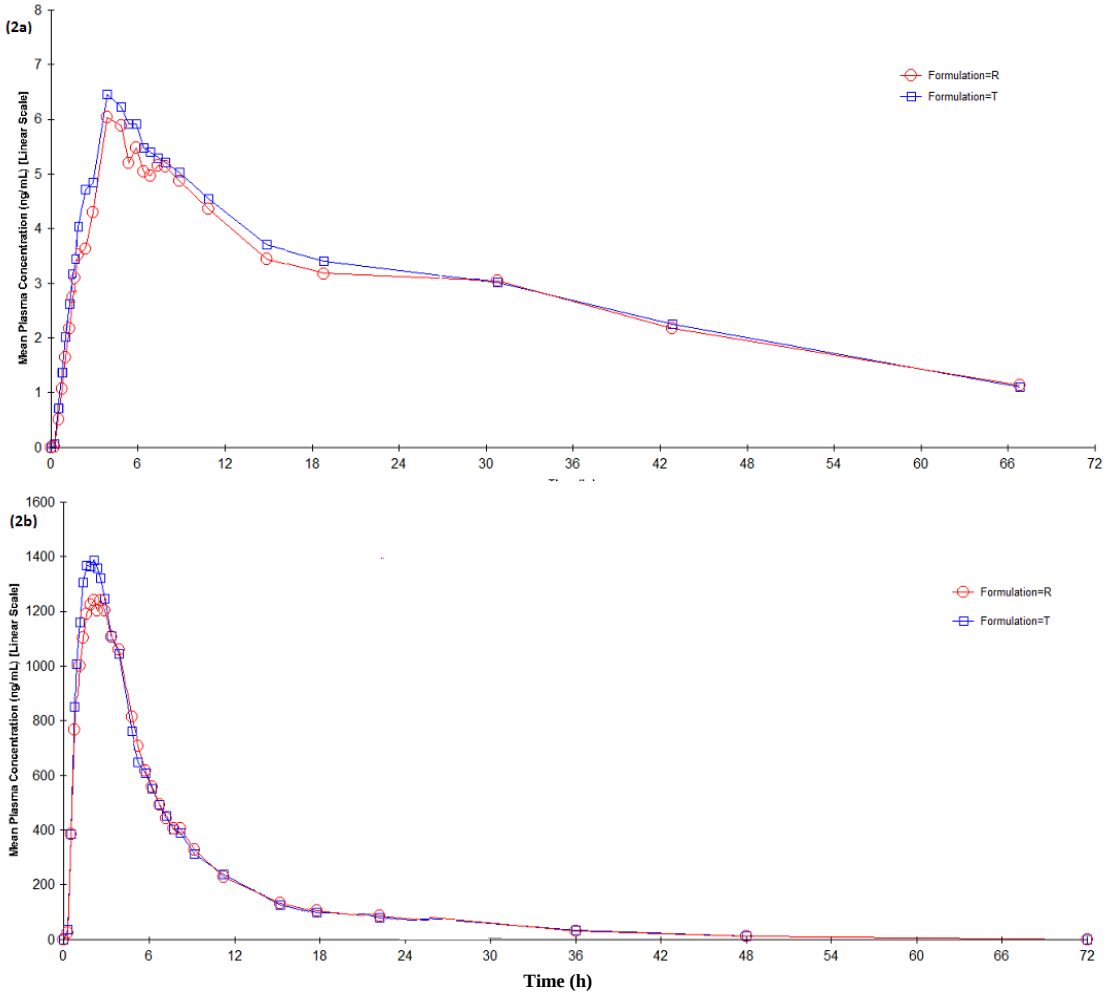

Fig. 2. Mean plasma concentration-time curves for (a) AML (b) OLM following single dose administration of test and reference tablets

CONCLUSION

A highly specific, selective and sensitive LC-MS/MS method based on SPE extraction has been developed and validated for simultaneous determination of AML and OLM concentration in human plasma. The validated method provides better linearity range of OLM and higher recovery of ALM and OLM. The method was successfully applied for performing the pharmacokinetic evaluation to adjudge the bioequivalence of two formulations of AML and OLM with 10 mg/40 mg dose respectively in 28 healthy volunteers.

Conflict of interest

All the authors declare no conflict of interest.

REFERENECS

Basaveswara Rao MV, Nagendrakumar AVD, Sivanadh M, Venkata Rao G. Validated RP-HPLC method for the estimation of telmisartan in tablet formulation. *Bull. Pharm. Res.* 2012;2(2):50-5.

Bhimavarapu R, Chitra KP, Meda H, Kanikanti D, Anne M, Gowthami N. Forced degradation study of paracetamol in tablet formulation using RP-HPLC. *Bull. Pharm. Res.* 2011; 1(3):13-7.

Bramlage P, Wolf WP, Stuhr T, Fronk EM, Erdlenbruch W, Ketelhut R, Schmieder RE. Effectiveness and tolerability of a fixed-dose combination of olmesartan and amlodipine in clinical practice. *Vasc. Health Risk Manag.* 2010;6:803-11. [DOI: 10.2147/VHRM.S13441]

Chen SH, Wu CF, Chen BM, Pei Q, Tan HY, Yang L, Yang GP. High-performance liquid chromatography-mass spectrometry for determining olmesartan in human plasma. *Nan Fang Yi Ke Da Xue Xue Bao* 2008;28(6):1104-5.

Chhabra GS, Banerjee SK. Stability indicating assay method development and validation of dronedarone hydrochloride in its bulk form by RP-HPLC. *Bull. Pharm. Res.* 2013;3(2): 58-65.

Haria M, Wagstaff AJ. Amlodipine: A Reappraisal of its pharmacological properties and therapeutic use in cardiovascular disease. *Drugs* 1995;50(3):560-86.

Liu D, Hu P, Matsushima N, Li X, Li L, Jiang J. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* 2007;856(1-2):190-7. [DOI: 10.1016/j.jchromb.2007.05.049]

Liu D, Jiang J, Wang P, Feng S, Hu P. Simultaneous quantitative determination of olmesartan and hydrochlorothiazide in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2010;878(9-10):743-8. [DOI: 10.1016/j.jchromb.2010.01.009]

Mire DE, Silfani TN, Pugsley MK. A review of the structural and functional features of olmesartan medoxomil, an angiotensin receptor blocker. *J. Cardiovasc. Pharmacol.* 2005;46(5): 585-93.

Mizuno M, Sada T, Ikeda M, Fukuda N, Miyamoto M, Yanagisawa H, Koike H. Pharmacology of CS-866, a novel nonpeptide angiotensin II receptor antagonist. *Eur. J. Pharmacol.* 1995;285(2):181-8.

Murdoch D, Heel RC. Amlodipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cardiovascular disease. *Drugs* 1991; 41(3): 478-505.

Patwari A, Dabhi M, Rathod I, Desai U, Suhagia BN, Sharma M. Simultaneous determination of ofloxacin and cefixime in combined tablet dosage form by HPLC and absorbance correction method. *Bull. Pharm. Res.* 2014;4(3):112-7.

Punzi H, Neutel JM, Kereiakes DJ, Shojaee A, Waverczak WF, Dubiel R, Maa JF. Efficacy of amlodipine and olmesartan medoxomil in patients with hypertension: the AZOR trial evaluating blood pressure reductions and control (AZTEC) study. *Ther. Adv. Cardiovasc. Dis.* 2010;4(4): 209-21. [DOI: 10.1177/1753944710374745]

Qi W, Zhao Q, Jiang J, Hu P. Simultaneous determination of olmesartan and amlodipine in human plasma and urine by ultra performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* 2013;938:27-34. [DOI: 10.1016/j.jchromb.2013.08.026]

Sengupta P, Sarkar AK, Bhaumik U, Chatterjee B, Roy B, Chakraborty US, Pal TK. Development and validation of an LC-ESI-MS/MS method for simultaneous quantitation of olmesartan and pioglitazone in rat plasma and its pharmacokinetic application. *Biomed. Chromatogr.* 2010;24(12):1342-9. [DOI: 10.1002/bmc.1447]

Shah S, Asnani A, Kawade D, Dangre S, Arora S, Yende S. Quantitative analysis of olmesartan medoxomil and amlodipine besylate in plasma by high-performance liquid chromatography technique. *J. Young Pharm.* 2012;4(2):88-94. [DOI: 10.4103/0975-1483.96622]

Shrestha DM, Srinivasa Rao T, Sreedhar C, Akkamma HG, Bharadwaja Reddy G, Yadav PK. Development and validation of new analytical method for the simultaneous estimation of naproxen and esomeprazole in bulk and pharmaceutical formulation. *Bull. Pharm. Res.* 2016; 6(1): 28-35.

Singh S, Dahiya R. Stability-indicating RP-HPLC method for estimation of atorvastatin calcium in solid dosage form. *Bull. Pharm. Res.* 2014;4(1):9-13.

Yanagisawa H, Amemiya Y, Knazaki T, Shimoji Y, Fujimoto K, Kitahara Y, Sada T, Mizuno M, Ikeda M, Miyamoto S, Furukawa Y, Koike H. Nonpeptide angiotensin II receptor antagonists: synthesis, biological activities, and structure-activity relationships of imidazole-5-carboxylic acids bearing alkyl, alkenyl, and hydroxyalkyl substituents at the 4-position and their related compounds. *J. Med. Chem.*1996;39(1):323-38. [DOI: 10.1021/jm950450f]

Zhou Y, Li J, He X, Jia M, Liu M, Li H, Xiong Z, Fan Y, Li W. Development and validation of a liquid chromatography-tandem mass spectrometry method for simultaneous determination of amlodipine, atorvastatin and its metabolites ortho-hydroxy atorvastatin and para-hydroxy atorvastatin in human plasma and its application in a bioequivalence study. *J. Pharm. Biomed. Anal.* 2013;83: 101-7. [DOI: 10.1016/j.jpba.2013.04.021]

US FDA, Guidance for industry: Bioanalytical Method Validation. US Food and Drug Administration, Rockville, MD, 2001.

Yu Y, Hou YN, HU YQ, Wang J, Liu JF, Zhao W. Pharmacokinetics of olmesartan, a new angiotensin II receptor blocker, in Chinese healthy subjects. *Asian J. Pharmacodyn. Pharmacokinet.* 2006;6(2):219-23.

*****