



# Simultaneous Quantitative Analysis of Olmesartan Medoxomil and Amlodipine Besylate in Plasma by High-performance Liquid Chromatography Technique

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## ABSTRACT

A rapid, simple and sensitive high-performance liquid chromatography (HPLC) method has been developed for quantification of olmesartan medoxomil (OLM) and amlodipine besylate (AM) in plasma. The assay enables the measurement of OLM and AM for therapeutic drug monitoring with a minimum detectable limit of 2 ng mL. The method involves a simple, one-step extraction procedure and analytical recovery was above 50%. The separation was performed on an analytical 250 × 4.6 mm Eurospher 100<sup>-5</sup> C18 column. The wavelength was set at 239 nm. The mobile phase was a mixture of acetonitrile:0.05 M ammonium acetate buffer: 0.1 mL triethylamine at pH 6.8 was selected at a flow rate of 1.0 mL/min. The calibration curve for the determination of OLM and AM in plasma was linear over the range 2–2500 and 8–10,000 ng/mL AM and OLM. The coefficients of variation for interday and intraday assay were found to be <15%. The method can be applied to a pharmacokinetic and pharmacodynamic study of OLM and AM in a combined dosage form.

**Key words:** Amlodipine besylate, high-performance liquid chromatography, olmesartan medoxomil, plasma

## INTRODUCTION

Amlodipine, R, S-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-ethoxycarbonyl-5-methoxy-carbonyl-6-methyl-1, 4-dihydropyridine [Figure 1], is a potent calcium

channel blocker used in treatment of hypertension and angina pectoris.<sup>[1]</sup> Amlodipine is well absorbed following oral administration with peak blood concentrations occurring after 6–12 h. The bioavailability is about 60–65%. It has a prolonged terminal elimination half-life of 35–50 h, and steady-state plasma concentrations are not achieved until after 7–8 days of administration. Amlodipine is extensively metabolized in the liver; metabolites are mostly excreted in urine together with <10% of a dose as unchanged drug.<sup>[2,3]</sup> Several analytical methods for quantifying of amlodipine in biological fluids have been reported; such as capillary gas chromatography (GC) with electron capture detection,<sup>[4,5]</sup> GC with electron-impact

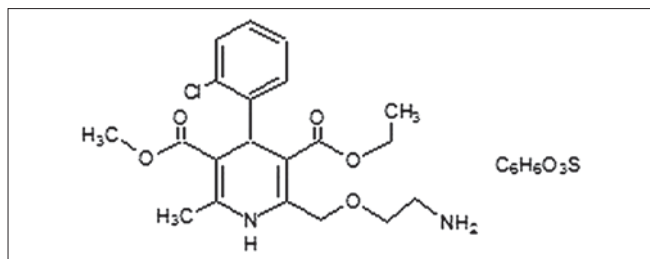
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**Figure 1:** Structure of amlodipine

mass spectrometry (EI-MS),<sup>[6,7]</sup> and high-performance liquid chromatography (HPLC).<sup>[8-12]</sup> Several HPLC procedures have been also reported for the analyses of amlodipine based on MS–MS detection by using tandem mass spectrometry.<sup>[13-16]</sup> Olmesartan medoxomil (OLM), (5-methyl-2-oxo-1,3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl} methyl imidazol-5-carboxylate) [Figure 2], is a potent and selective angiotensin AT<sub>1</sub> receptor blocker<sup>[17]</sup> which has been approved for the treatment of hypertension in the United States, Japan, and European countries. The drug contains a medoxomil ester moiety and is cleaved rapidly by an endogenous esterase to release the active metabolite olmesartan.<sup>[18]</sup> Up to date, olmesartan has been determined in plasma and other biological fluids using HPLC coupled to fluorescent detection.<sup>[19,20]</sup>

As per literature survey, no analytical methods have been reported for the simultaneous quantitative analysis of OLM and AM in biological fluid. The aim of our study was to develop a rapid, simple, accurate, precise, sensitive, and reproducible HPLC method, which can be utilized in pharmacokinetic research.

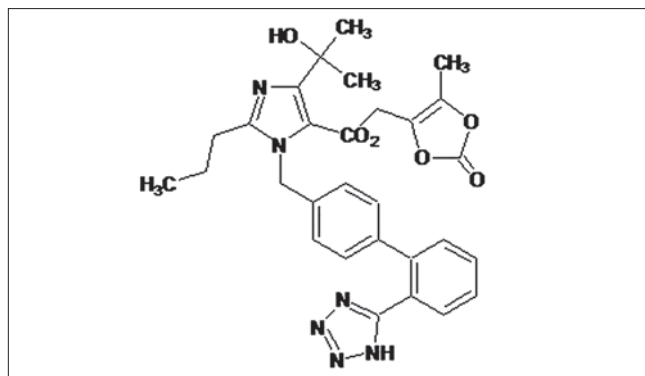
## EXPERIMENTAL

### Chemicals and reagents

Olmesartan medoxomil (OLM) and amlodipine besylate (AM) were kindly supplied by Ajanta Pharma Limited (Mumbai). All chemicals and reagents were HPLC grade, methanol and acetonitrile, ammonium acetate, ethylene diamine tetra acetic acid (EDTA) (Merck, India).

### Instruments and chromatographic conditions

A Shimadzu HPLC system (Japan) employed consisted of a model LC-20 AT pump, a model Rheodyne 7125 injector, and a model SPD-20A UV detector. The separation was performed on an analytical 250 × 4.6 mm Eurospher 100<sup>-5</sup> C18 (5 μm, particle size) column. The wavelength was set at 239 nm. The mobile phase was a mixture of



**Figure 2:** Structure of olmesartan

acetonitrile:0.05 M ammonium acetate buffer: 0.1 mL triethylamine at pH 6.8 was selected at a flow rate of 1.0 mL/min. The mobile phase was prepared daily and degassed by ultrasonification before use.

### Preparation of standard stock solution

The standard working concentration of OLM (10 μg/mL) and AM (10 μg/mL) were prepared in the mobile phase.

### Biological samples

The prepared suspension containing AM (0.4 mg/mL) and OLM (1.6 mg/mL) in 0.2% tween 80 were administered orally to Wistar rats (180–220 g) at a dose of 2 mg/kg (AM) and 8 mg/kg (OLM) body weight after overnight fasting. The blood samples were collected from the retrobulbar plexus into anticoagulant-treated polypropylene tubes from 0 to 12 h after drug administration. Blood samples collected were centrifuged immediately to separate the plasma. The plasma samples collected were stored at –20°C. The plasma samples then processed for drug recovery *via* solvent extraction.

### Samples preparation (extraction procedure)

To 1000 μL of plasma samples in a borosilicate glass tube were added 5 mL of HPLC grade acetonitrile. After vortex mixing for 10 min at room temperature, the samples were centrifuged at 10,500 rpm for 10 min. The upper organic layer was transferred to a glass container and evaporated inside a vacuum oven at 40°C. The dry residue was dissolved in 1 mL of the mobile phase. The mixture was sonicated well for 10 min, and 20 μL of this solution was injected into liquid chromatography.

### Stability

Stability assessments were defined as the resistance to change in concentration of an analyte in plasma under

specified conditions. The stability of OLM and AM in rat plasma was investigated by adding known amount of drug to blank plasma samples to give concentrations of 10, 200, and 500 ng/mL. These were stored at -20°C and aliquots taken for 1 and 4 weeks for analysis.

### Calibration curve

Linearity of instrument standard response was determined for each compound with different concentration calibration standards (final concentration ranging from 2 to 2500 ng/mL for AM and 8 to 10,000 ng/mL for OLM, with drug ratio maintaining constant 1:4). The calibration curves were constructed by plotting individual analyte peak area ratio as compared to the corresponding concentration and fitting these data in regression analysis. The calibration curves were constructed by the weighted regression method ( $1/x$ ) of the peak area of OLM or AM *vs.* actual concentrations.

## RESULTS AND DISCUSSION

### Method development

Considering the efficiency of HPLC, an attempt has been made to develop a simple, accurate, precise, rapid, and economic method for the simultaneous estimation of OLM and AM in the biological method. Thus, the method described enables to the quantification of OLM and AM. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. For the HPLC method development mobile phase consists of acetonitrile:0.05 M ammonium acetate buffer:0.1 mL triethylamine at pH 6.8 at a flow rate of 1.0 mL/min. The optimum wavelength selected was 239 nm. Under these chromatographic conditions described, OLM and AM

peaks were well resolved. Endogenous plasma components did not give any interfering peaks. The average retention times of OLM and AM were 3.1 and 5.0 min, respectively. Chromatogram obtained after drug administration of OLM and AM in Wistar rats for blank plasma and plasma samples collected after 2.5, 6 and 10 h are shown in Figure 3.

### Calibration curve

The calibration curve for the determination of OLM and AM in plasma was linear over the range 2–2500 and 8–10,000 ng/mL AM and OLM. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (*R*) for calibration curves were equal to or better than 0.997. The relative standard deviation (RSD) values of the slope were equal to or better than 5%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves.

### Recovery study

The relative analytical recovery for plasma at three different concentrations of OLM and AM was determined. Known amounts of drug were added to drug-free plasma in concentrations ranging from 0.002 to 10 µg/mL. The average recovery was in between 50% and 90% for a linear concentration range. The results are given in Tables 1 and 2 for the intraday and interday study.

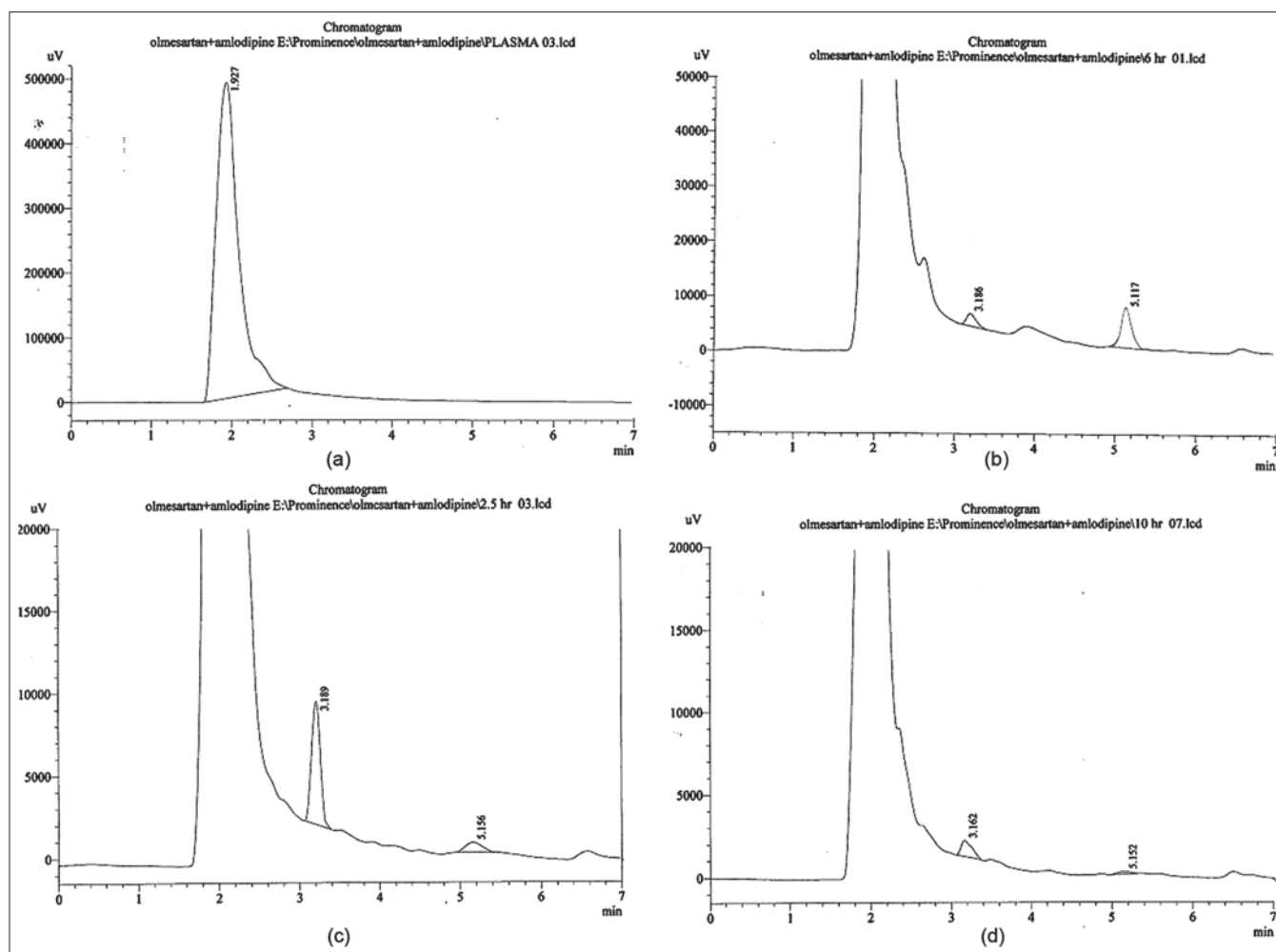
### Stability study

In the short-term stability studies, precision and accuracy for the analyte was <15% and <8% for OLM, <10% and 8% for AM, respectively. In the long-term stability studies, precision and accuracy for the analyte was <7% and <15%

**Table 1: Recovery studies for olmesartan medoxomil and amlodipine besylate (intraday) (RP-HPLC)**

Sample no.	Drug	Concentration added (µg mL)	Peak area of standard	Mean peak area sample <sup>a</sup>	% Recovery <sup>a</sup>	±SD	CV
1	AM	0.002	1888.81	1645.1	87.1	4.14	4.75
	OLM	0.008	1211.4	1069.5	88.29	4.43	5.02
2	AM	0.01	3739	3203.4	85.68	1.29	1.51
	OLM	0.04	2265.1	1966.2	86.8	2.51	2.89
3	AM	0.05	5695.2	4020	70.59	4.7	6.66
	OLM	0.2	11325.9	8689.2	76.72	2.82	3.68
4	AM	0.2	10781	8858.74	82.17	2.93	3.57
	OLM	80	44638.13	35049.84	78.52	6.49	8.27
5	AM	0.5	26952.5	17387.9	64.51	3.16	4.9
	OLM	2	113259.5	70583.26	62.32	5.66	9.08
6	AM	1.5	80857.5	38064.76	47.08	5.15	10.9
	OLM	6	275224.5	135510.9	49.24	7.54	15.3
7	AM	2.5	134762.5	52579.76	39.02	2.55	6.54
	OLM	10	436810.5	181465.2	41.54	4.25	10.2

OLM: Olmesartan medoxomil; AM: Amlodipine besylate; <sup>a</sup>Results are mean of three samples; RP-HPLC: Reversed phase HPLC; SD: Standard deviation



**Figure 3:** Chromatogram of blank plasma, OLM, and AM after oral administration in rat plasma

for OLM, <12% and 18% for AM, respectively. The stability tests indicated that OLM and AM are stable in rat plasma over the period of 1 month. In addition, the stock solution remains stable for a duration of 1 month when stored at 4°C expressed by the % variation <2% for both OLM and AM. The results are given in Tables 3 and 4 for OLM and AM, respectively.

### Limit of detection

The limit of detection (LOD) was defined, as the amlodipine concentration that produced a signal-to-noise ratio greater than 3. The LOD in plasma was 2 ng/mL for AM and 8 ng/mL based upon this criterion. At this level, the RSD was lower than 15%.

### Accuracy and precision

Intraday and interday assay performed to evaluate precision (% RSD) and accuracy. The coefficient of variation for intraday studies was between 2–14% and 3–17% for

interday in rat plasma. The result suggests that within run and between run experiments, the precision and accuracy for the analyte met the acceptance criteria.

It does not require tedious derivatization or specialized detectors, making it readily amendable to routine laboratory use. However, this method is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of amlodipine and olmesartan. The coefficients of variation were less than 15%, which is acceptable for the routine measurement of OLM and AM. The results for intraday and interday precision and accuracy studies are given in Tables 5 and 6.

### Pharmacokinetic study

The results of pharmacokinetics analysis of OLM and AM are mentioned in Table 7. The mean plasma concentration–time curve of OLM and AM shows:

**Table 2: Recovery studies for OLM and AM (interday) (RP-HPLC)**

Sample no.	Drug	Concentration added ( $\mu\text{g/mL}$ )	Peak area of standard	Mean peak area sample <sup>a</sup>	% Recovery <sup>a</sup>	$\pm\text{SD}$	CV
1	AM	0.002	1789.56	1726.7	96.48	3.18	3.3
	OLM	0.008	1045.65	1079.4	103.23	3.28	3.18
2	AM	0.5	25478.45	18597	72.98	5.14	7.05
	OLM	2	112457.48	72914	64.83	7.24	11.2
3	AM	2.5	132486.97	53425	40.32	1.45	3.62
	OLM	10	437849.98	259467	59.25	10.27	17.3

<sup>a</sup>Results are mean of three samples; AM: Amlodipine besylate; OLM: Olmesartan medoxomil; SD: Standard deviation; CV: Coefficient of variation, RP-HPLC: Reversed phase HPLC

**Table 3: Stability of OLM in rat plasma (RP-HPLC)**

Sample concentration (ng mL)	Concentration found (%)			±SD	Mean concentration (%) <sup>a</sup>	CV	% Deviation (inaccuracy)
Short-term stability for 24 h in plasma (−20°C)							
10	124.5	101.5	95.2	15.42	107.06	14.4	7.06
200	91.62	101.18	100.9	5.44	97.9	5.55	2.1
500	97.25	97.62	102.57	2.97	99.14	2.99	0.852
Long-term stability for 1 month in plasma (−20°C)							
10	96.5	88.4	101.12	6.43	95.34	6.75	4.64
200	87.82	80.12	90.67	5.45	86.20	6.33	13.79
500	90.57	95.07	88.91	3.18	91.51	3.48	8.41

<sup>a</sup>Results are mean of three samples, SD: Standard deviation; CV: Coefficient of variation; OLM: Olmesartan medoxomil; RP-HPLC: Reversed phase HPLC

**Table 4: Stability of AM in rat plasma (RP-HPLC)**

Sample concentration (ng mL)	Concentration found (%)			±SD	Mean concentration (%) <sup>a</sup>	CV	% Deviation (inaccuracy)
Short-term stability for 24 h in plasma (−20°C)							
10	111.45	134.5	122.4	11.53	122.78	9.3	2.28
200	87.27	107.77	92.12	10.714	95.72	11.1	4.3
500	97.89	88.51	94.24	4.72	93.54	5.05	7.58
Long-term stability for 1 month in plasma (−20°C)							
10	97.89	84.5	89.4	6.77	90.59	7.47	9.4
200	81.77	72.89	92.24	9.68	82.3	11.7	17.7
500	84.62	95.93	82.53	7.20	87.69	8.2	12.3

<sup>a</sup>Results are mean of three samples, AM: Amlodipine besylate; SD: Standard deviation; CV: Coefficient of variation; RP-HPLC: Reversed phase HPLC

**Table 5: Data for accuracy and precision studies (Intraday) (RP-HPLC)**

Sample no.	Drug	Concentration added ( $\mu\text{g mL}$ )	Peak area of standard	Mean concentration found <sup>a</sup>	$\pm\text{SD}$	CV	% deviation
1	AM	0.002	1888.81	0.0019	6.9	6.77	-0.05
	OLM	0.008	1211.4	0.0079	2.4	2.34	-0.012
2	AM	0.01	3739	0.0099	6.0	5.51	-0.01
	OLM	0.04	2265.1	0.0396	5.6	5.46	-0.01
3	AM	0.05	5695.2	0.051	3.3	3.25	0.028
	OLM	0.2	11325.9	0.198	2.8	2.75	-0.008
4	AM	0.2	10781	0.198	3.5	3.44	-0.012
	OLM	80	44638.1	0.78	7.0	7.03	-0.99
6	AM	0.5	26952.5	0.52	4.8	5.07	0.0304
	OLM	2	113260	1.95	7.5	6.95	-0.022
7	AM	1.5	80857.5	1.54	10.9	12.16	0.028
	OLM	6	275225	5.7	15.3	13.29	-0.0497
8	AM	2.5	134763	2.54	6.54	6.47	0.015
	OLM	10	436811	10.66	10.2	11.13	0.065

<sup>a</sup>Results are mean of three samples, RP-HPLC: Reversed phase HPLC; AM: Amlodipine besylate; OLM: Olmesartan medoxomil

Plasma concentration reached a maximum 2 h and 6 h for OLM and AM, after dosing with a level of 8 and 2 mg/kg body weight of OLM and AM, respectively. The value of pharmacokinetic parameters such as  $t_{1/2}$ ,

$C_{\text{max}}$  and  $k_{\text{el}}$  obtained reveals that there was less difference in the profile of a individual drug when administered simultaneously in a single oral dosage form. These results are agreement with the previous reports.<sup>[19,21,22]</sup> Plasma



**Table 6: Data for accuracy and precision studies (interday) (RP-HPLC)**

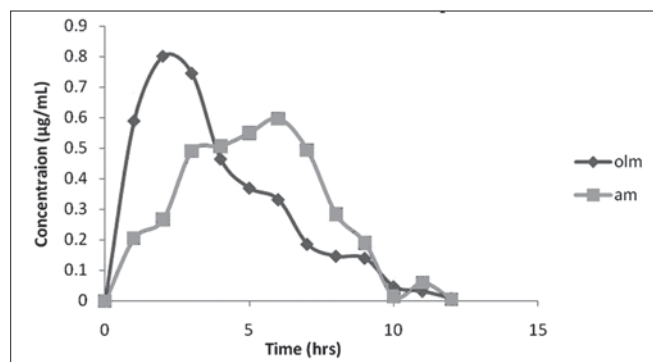
Sample no.	Drug	Concentration added ( $\mu\text{g/mL}$ )	Peak area of standard	Mean concentration found <sup>a</sup>	$\pm\text{SD}$	CV	% Deviation
1	AM	0.002	1789.56	0.0021	3.18	5.2	0.05
	OLM	0.008	1045.65	0.0079	3.28	3.7	-0.0125
2	AM	0.5	25478.45	0.554	5.14	2.15	0.108
	OLM	2	112457.48	2.338	7.24	2.9	0.169
3	AM	2.5	132486.97	2.7531	1.45	5.64	0.101
	OLM	10	437849.98	12.859	10.27	2.96	0.28

<sup>a</sup>Results are mean of three samples, AM: Amlodipine besylate; OLM: Olmesartan medoxomil; RP-HPLC: Reversed phase HPL

**Table 7: Pharmacokinetic parameters of a single oral administration of OLM and AM in combined dosage form in Wistar rats**

Parameters	Drug administered	
	OLM	AM
$t_{\text{max}}$ (hrs)	6	2
$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	0.807	0.597
$\text{AUC}_{0-4}$ ( $\mu\text{g/mL/hr}$ )	3.8165	3.5973
$\text{AUC}_{\text{INF}}$ ( $\mu\text{g/mL/hr}$ )	14.6869	18.3447
$t_{1/2}$ (hrs)	0.7473	0.9076
CL ( $\mu\text{L/hr}$ )	2091.88	554.915
$\text{AUMC}_{\text{LAST}}$ ( $\mu\text{g/mL}\cdot\text{hr}^2$ )	14.5854	18.3447
$\text{AUMC}_{\text{INF}}$ ( $\mu\text{g/mL}\cdot\text{hr}^2$ )	14.7066	18.4353
$\text{MRT}_{\text{LAST}}$ (hrs)	3.8216	5.0995
$\text{MRT}_{\text{INF}}$ (hrs)	3.8404	5.115

<sup>a</sup>Results are mean of three samples, OLM: Olmesartan medoxomil; AM: Amlodipine besylate; AUC: Area under curve; CL: Drug clearance; AUMC: Area under the moment curve

**Figure 4: Plasma time profile curve**

concentration–time profile curve following a single oral administration of OLM and AM in a combined dosage form in Wistar rats is shown in Figure 4.

## CONCLUSION

The HPLC method presented is direct, simple, selective, reproducible, sensitive, and linear. The procedure was successfully applied to the simultaneous determination of the studied compounds in biological fluid without any interference from the additives and endogenous substances. The procedure was fully validated to meet the requirements of the food and drug administration center for drug

evaluation and research guidelines for bioanalytical method validation.<sup>[23]</sup> These methods are well-suited for routine application in the quality control laboratories and clinical laboratories because of the simplicity, economic, accuracy, sensitivity, and reproducibility. The HPLC method is applicable to pharmacokinetics studies of OLM and AM in rats. This method can also be used to study the mechanism of metabolism of OLM and AM.<sup>[24]</sup>

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