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Simultaneous liquid chromatography-tandem mass spectrometry assay of amlodipine besylate and metoprolol succinate in human plasma: Development, validation, and application

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ABSTRACT

A rapid and easy liquid chromatography-tandem mass spectrometry (LC–MS/MS) method was developed, validated, and used to quantify amlodipine besylate and metoprolol succinate in human plasma. Hydrochlorothiazide was used as the internal standard. LC–MS/MS with electrospray ionization in the positive ionization mode was performed under multiple reaction monitoring to examine analytes, which were drawn from human plasma via liquid–liquid extraction using a mixture of diethyl ether and dichloromethane (6:4). The extracts were dried under a nitrogen stream at 40°C, after which the samples were dissolved in a mixture of methanol and water (80:20). The re-dissolved samples were injected onto a C18 column and eluted using a mixture of methanol and 0.4% formic acid (80:20) as the mobile phase. Linear standard calibration was performed, and lower limits of quantification were determined for each analyte. The developed method was fully validated with respect to selectivity and carryover, as well as intra- and inter-day accuracy and precision in accordance with guidelines of the United State Food and Drug Administration and the European Medicines Agency. Mean analyte recovery was between 71.72% and 97.55%, and matrix effects exerted only a minor influence on precision. The validated method was applied in a clinical bioequivalence study to evaluate the in vivo bioequivalence of two commercial products containing 5 mg amlodipine and 50 mg metoprolol. This randomized, single-dose, two-treatment, three-period, two-sequence, open-label, crossover study involved 14 healthy subjects, and a two-week washout period between the two phases of the research was implemented. Standard pharmacokinetic parameters were calculated to compare a test product with Selomax® as the reference.

Keywords: Amlodipine besylate; metoprolol succinate; LC–MS/MS, bioequivalence.

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Introduction

Hypertension is a common disease in communities, and its incidence increases with age. It is a fatal condition that leads to severe sequelae, such as hemiplegia and coma with a vegetable existence, and promotes coronary artery disease, heart failure, cerebrovascular disease, and chronic kidney disease, which affect quality of life. Hypertension is treated via monotherapy as the initial standard for blood pressure control in most patients, but many recent studies have shown that combination therapy is necessary for such regulation. Manica *et al.* [1], for example, indicated that the 2013 guidelines of the European Society of Cardiology/European Society of Hypertension on managing arterial hypertension recommend the use of least two drugs to achieve blood pressure targets for most patients. The guidelines also state that treatment may begin with monotherapy or a mixture of two drugs administered at low doses [1].

Combining drugs presents advantages such as increased effectiveness of blood pressure reduction, minimized side effects, improved tolerance by patients, and diminished cardiovascular events. The leading combination in the treatment of hypertension with coronary artery disease comprises amlodipine besylate (AML), which is a dihydropyridine calcium channel blocker, and metoprolol succinate (MET), which is a selective β1-adrenergic antagonist [2]. The chemical structures of AML and MET are shown in **Figure 1**. Notwithstanding the benefits of this combined regimen, however, few studies have used liquid chromatography–tandem mass spectrometry (LC–MS/MS) in analyzing AML [3] and MET [4], and only one investigation has been directed toward the simultaneous determination of these substances in human plasma [5]. To address this deficiency, the current work developed an easy and rapid LC–MS/MS method to determine AML and MET concentrations in human plasma and use the approach in evaluating the bioequivalence of a test product in comparison with Selomax® (Astra Zeneca) licensed at IPCA Laboratories Ltd. as the reference commodity.

MATERIALS AND METHODS

2.1. Chemicals and reagents

AML (100.43%), MET (99.80%), and hydrochlorothiazide (HCTZ) (99.55%) as the internal standard were supplied by the Institute of Drug Quality Control (Ho Chi Minh City, Vietnam). LCMS-grade methanol was obtained from JT Baker (USA), and LCMS-grade formic acid was derived from Prolabo. Diethyl ether and dichloromethane were obtained from Fisher Scientific in USA. Blank human plasma sources, which were supplied by the Blood Transfusion Hematology Hospital (Ho Chi Minh City, Vietnam), were stored at below –20°C prior to use.

2.2. Preparation of stock solutions, standards, and controls

Stock solutions of 150 µg/mL AML and 100 µg/mL MET were prepared by dissolving these respective amounts of substances in methanol, from which working solutions were prepared at the required concentrations in individual glass vials. Ten calibration standards were prepared in blank human plasma at the following concentrations: 0.045, 0.15, 0.75, 1.5, 3, 4.5, 7.5, 9, 12, and 15 ng/mL for AML and 0.3, 1, 5, 10, 20, 30, 50, 60, 80, and 100 ng/mL for MET.

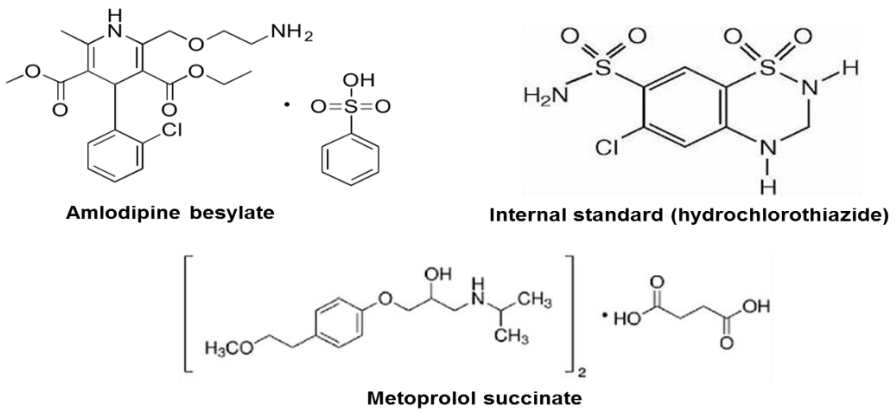


Figure 1. Chemical structures of AML, MET, and HCTZ

Quality controls (QCs), namely, lower limits of quantification (LLOQs) and low, medium, high, and upper limits of quantification (ULOQs) in blank human plasma were 0.45, 0.15, 0.75, 12, and 15 ng/mL for AML, respectively, and 0.3, 1, 50, 80, and 100 ng/mL for MET, respectively. The QCs were prepared separately from the calibration standards. Fresh calibration standards and QCs were prepared on each day of analysis during the validation and subject sample runs. An internal standard (IS) stock solution of 100 µg/mL was prepared by dissolving an appropriate amount of HCTZ in methanol. On each day of analysis, an aliquot of the IS stock solution was diluted in methanol to obtain a working IS solution (2000 ng/mL). The stock solutions of analytes and the IS were stored at 5 ± 3°C, and the calibration standards and QCs were kept at –20°C.

2.3. Sample treatment

Prior to analysis, all the frozen samples (i.e., subject samples, calibration standards, and QCs) were thawed and allowed to equilibrate at room temperature. Spiked plasma/subject samples (1 mL) were pipetted into a glass tube, to which 50 µL of the working IS solution was added. Diethyl ether and dichloromethane (2 × 3 mL, 3:1) was added for liquid–liquid extraction, after which the resultant mixture was vortexed for 1 min and shaken at 300 rpm for 5 min. The mixture was subjected to another 10 s round of vortexing. After the sample was centrifuged at 4000 rpm for 5 min, the organic layer was transferred to another tube and evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 500 µL of water and methanol (1:4), vortexed for 1 min, sonicated for 5 min, centrifuged for 5 min (4000 rpm, 0°C), and sieved through a 0.22 µm membrane filter. An aliquot of 2.5 µL was then injected into the LC–MS/MS system.

2.4. LC–MS/MS conditions

A Shimadzu UHPLC Nexera X2 coupled with the Triple Quadrupole Mass Spectrometer LCMS-8040 from Japan was used to establish reverse-phase liquid chromatographic conditions. The Shimadzu system consisted of an autosampler (SIL-30AC), two pumps (LC-30AD), a column oven (CTO-20A), and a controller (CBM 20A). The autosampler and oven temperatures were maintained at 5°C and 40°C, respectively. The analytes and IS were separated via high-performance liquid chromatography using a Gemini® C18 column (100 × 3 mm; 5 µm). An isocratic mobile phase composed of methanol and 0.4% formic acid (80:20, v/v) was delivered at a flow rate of 0.3 mL/min at an injection volume of 2.5 µL.

Mass spectrometry (MS) analysis was run at the positive ionization mode for the analytes and at the negative ionization mode for the IS through electrospray ionization (ESI). Interface voltage, heat block temperature, desolvation line temperature, nebulizer gas flow rate, and drying gas flow rate were set to 4,500 V, 400°C, 250°C, 3 L/min, and 15 mL/min, respectively. The multiple reaction monitoring (MRM) transitions for each analyte and the IS, as well as their respective optimum MS parameters, including voltage potential (Q1, Q3) and collision energy (CE), are summarized in **Table 1**. Quantification was carried out on the basis of peak area, and data were collected and processed using the LabSolutions software for the LCMS-8040 system.

2.5. Method validation

The proposed method was validated in accordance with the bioanalysis guidelines of the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [6,7].

2.5.1. Selectivity

Six individual blank plasma samples were analyzed to investigate interference peaks at the retention time of each analyte. Selectivity was accepted if the blank sample response was less than 20% at the LLOQ of each analyte and less than 5% of the IS response of HCTZ.

2.5.2. Carryover

Immediately following the injection of the ULOQ, carryover into injected blank plasma was assessed. Carryover was accepted if it was less than 20% at the LLOQ of an analyte and less than 5% of the IS response of HCTZ.

2.5.3. Calibration standard curve

The plasma samples with a series of known concentrations, prepared as described above, were examined in three separate runs, and linear regression analysis was performed on known concentrations of each analyte against the area ratios of the corresponding and IS peaks. Subsequently, the regression coefficient, slope, and y-intercept of the resultant linear calibration functions were determined.

2.5.4. Accuracy and precision

Four concentrations of each analyte (LLOQ and low, medium, and high QCs) were analyzed in six replicates per run. Three runs were carried out, each on a separate day. Intra-day (n = 6) and inter-day (n = 18) accuracy and precision were determined. Accuracy was accepted if the back-calculated concentration was within 15% of the nominal concentration of each QC and within 20% at the LLOQ. Precision was accepted at a coefficient of variation (CV) that did not exceed 15% of each QC and 20% at the LLOQ.

2.5.5. Matrix effects

Plasma samples from six individuals were tested at high and low QC concentrations. The matrix effects of each analyte and the IS were determined separately for each sample by ascertaining the ratio of the peak area in the post-extraction spiked plasma to the peak area in the spiked methanol.

2.5.6. Recovery

Three sets of six samples with concentrations at the high, middle, and low regions of the standard curve were separately prepared and analyzed. Then, the ratio of the recorded peak area to the peak area resulting from the direct injection of the solutions in methanol for the analytes with the same concentrations were determined as percentages in each case.

2.5.7. Stability

Freeze and thaw stability: Plasma samples with low and high levels of QC were kept at –20°C for 24 h and thawed unassisted at room temperature. The cycles were replicated, after which the samples were examined.

Short-term temperature stability: Plasma samples with low and high levels of QC were reserved at room temperature for 6 h before analysis.

Long-term stability: The stability of plasma samples with low and high levels of QC retained at –20°C was monitored for 80 and 110 d.

Post-preparative stability: The stability of the autosampler was evaluated by re-analyzing the extracted low- and high-QC samples retained under autosampling conditions (10°C) for 24 h.

2.6. Application to human samples

Table 1. Main parameters of tandem MS

Analytes	MRM transitions (m/z)	Q1 (V)	CE (V)	Q3 (V)	Acquisition time (min)
AML	409.10 → 238.00	–30	–10	–24	1.26
MET	268.15 → 116.00	–28	–11	–11	1.24
HCTZ	296.00 → 126.00	9	31	23	1.55

2.6.1. Bioequivalence study

The research was designed as an open-label, randomized, two-treatment, three-period, two-sequence, single-dose, crossover oral bioequivalence study involving a 50 mg/5 mg MET/AML test formulation and a reference formulation (Selomax™ 50/5 tablets). The study was conducted in accordance with the Declaration of Helsinki and the rules of good clinical trial practice following Circulars No. 29/2018/TT-BYT on "Regulations for Clinical Trials" and No. 03/2012/TT-BYT on "Guidelines for Clinical Trials" of the Vietnam Ministry of Health. All the subjects were informed of their right to terminate participation at any time, without restrictions. The study protocol was approved by the Ethics Committee of the Institute of Drug Quality Control, Ho Chi Minh City.

Fourteen healthy adult subjects who had given informed consent were selected for participation on the basis of their medical histories, physical examinations, vital signs (blood pressure, temperature, heart frequency), electrocardiogram records, and standard laboratory test results (blood cell count, biochemical profile, hepatitis B and C, HIV, urinalysis). The subjects were 20 to 26 years old (mean 23.1 ± 1.8 age) and had a height of 165 to 172 cm (mean 168.2 ± 2.40 cm), a weight of 53 to 73 kg (mean 63.6 ± 7.20 kg), and a body mass index of 18.7 to 25.9 kg/m2 (mean 22.5 ± 2.7 kg/m2). All the 14 subjects (100%) were male. They had no history of drug or alcohol abuse or hypersensitivity to the drugs used in the research and did not take any medication within 2 weeks before dosing in this study. Alcohol, cigarettes, tobacco, and beverages containing caffeine were forbidden for 48 h before the study and during the course of the investigation. Standard meals were served to the subjects at four and 11 h after drug administration.

Samples taken from the subjects were centrifuged at 4000 rpm for 10 min to obtain plasma, which was kept at –20°C until the analysis was finished. In the first and second periods of observation, healthy volunteers were administered a single oral dose of the test and reference drugs under fasting conditions to assess the bioavailability of AML and MET. In the third period, the healthy volunteers were given a single dose of the test drugs under fed conditions to evaluate the effects of food on the bioavailability of MET. Each period lasted 5 d.

The developed method was applied efficaciously to quantify AML and MET concentrations in the human plasma samples. The test and reference products, comprising 5 mg AML and 50 mg MET, were administered, after which blood samples were collected prior to dosing (time 0) and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 48, 72, 96, and 120 h after dosing.

2.6.2. Pharmacokinetic analysis

The pharmacokinetic parameters for AML and MET were determined using WinNonlin 7.0. C_{max} and T_{max} were read directly from the experimental data. The elimination rate constant (K_e) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The total area under the plasma concentration curve from time 0 to the last measurable concentration (AUC_{0-t}) was calculated using the trapezoidal rule-extrapolation method. The extrapolated parameter AUC_{0-inf} (AUC from time 0 to infinity) was determined by adding the value of C_t/K_e to the calculated AUC_{0-t}, where C_t is the last observed concentration higher than the LLOQ.

RESULTS AND DISCUSSION

3.1. Optimization of LC–MS/MS conditions

During the development of the proposed method, ESI was operated in the positive ionization mode. The full scan Q1 MS

spectra of AML, MET, and HCTZ (IS) predominantly contained a protonated precursor $[M+H]^+$ ion at *m/z* 409.10, 268.15, and 296.00, respectively. The most abundant product ion reflected in the Q3 MS spectra of AML, MET, and the IS was observed at *m/z* 238.00, 116.00, and 126.00, respectively. The MS/MS parameters were systematically optimized for each analyte and the IS to obtain a consistent and adequate response. A dwell time of 100 ms was sufficient.

Chromatographic conditions were investigated by considering column type, mobile phase component, pH of buffer, ionic strength, and flow rate. The examination was initiated by modifying the aforementioned parameters to derive a symmetric peak shape, a short run time, minimum matrix interference, and solvent consumption. On the basis of the outcomes of various trials, the Gemini® C18 (100 × 3 mm; 5 µm) column was selected. The mobile phase consisting of methanol–0.4% formic acid (80:20, v/v) , injected at a flow rate of 0.3 mL/min, ensured the separation of AML, MET, and the IS at retention times of 1.27, 1.24, and 1.55 min, respectively, at a total run time of 3.0 min. **Figure 2** shows the chromatograms of the blank plasma and analytes at medium-level QC (MQC) levels with the IS. The blank plasma chromatogram was clean and showed no endogenous interfering peak at the retention times of the analytes and IS.

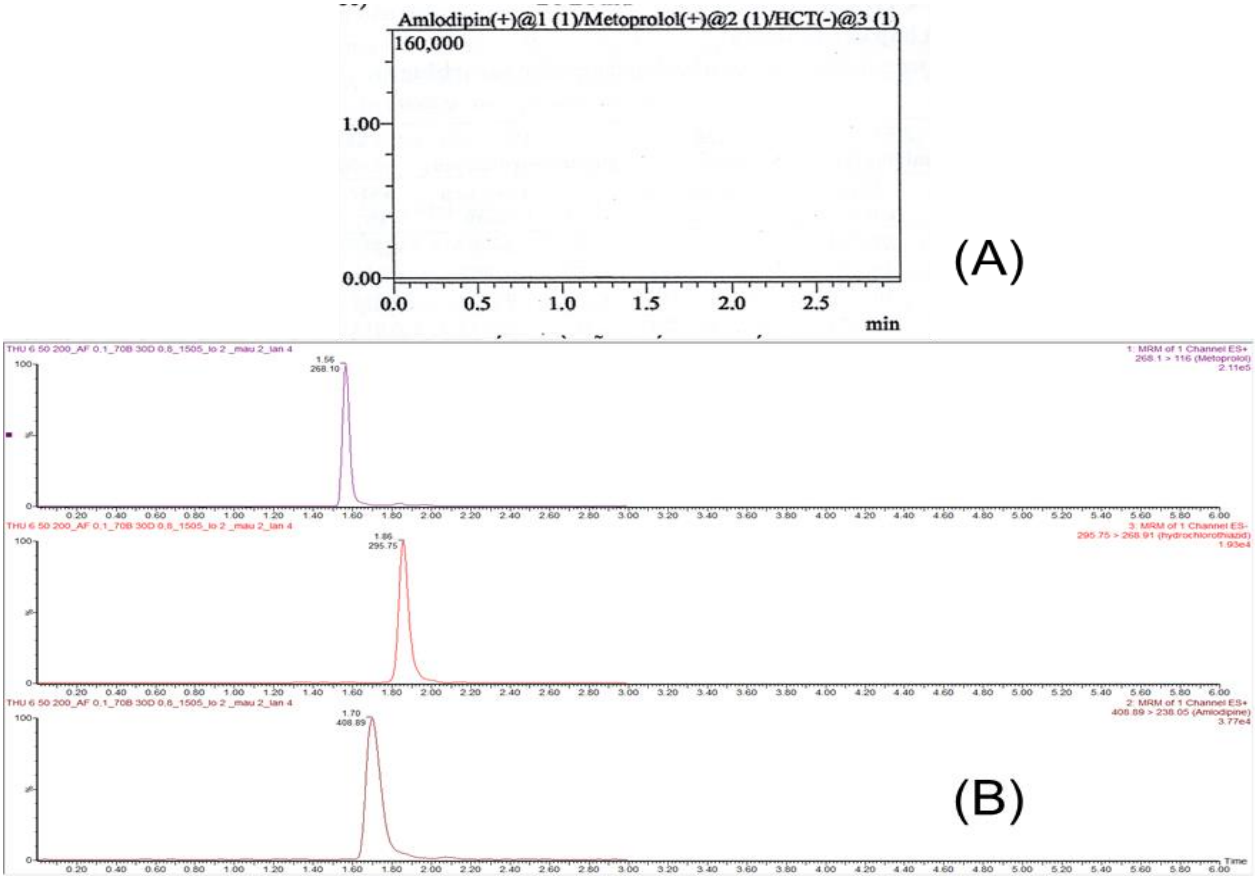


Figure 2. Chromatograms of (A) blank plasma and (B) analytes at MQC levels with IS

3.2. Extraction procedure

Liquid–liquid extraction was chosen because this technique only purifies a sample but also concentrates it. The extraction was initiated with diethyl ether, dichloromethane, and a mixture of these two substances (3:1). The investigation revealed that the combined mixture achieved a satisfactory recovery of all the analytes and caused low interference. This finding was an additional driver of the selection of the procedure.

3.3. Method validation

The bioanalytical method described here fully satisfied the validation criteria for calibration curve, selectivity, carryover, accuracy, precision, matrix effect, and stability in accordance with the US FDA and EMA guidelines. The results are provided in **Table 2**.

Carryover evaluation was conducted to ensure that it does not affect the accuracy and precision of the proposed method. No carryover (0.0%) was observed during the experiment. No response at the retention times of the analytes and IS occurred after subsequent injection at the ULOQ. The calibration curve was validated for all the analytes over the following ranges: 0.045 to 15 mg/mL for AML and 0.3 to 100 ng/mL for MET. Calibration lines were drawn to derive the following linear regression equations: $y = 1.1742x + 0.0019$ (AML) and $y = 0.2553x + 0.1140$ (MET). In these equations, y is the peak area ratio of an analyte and the IS, and x is the concentration of an analyte. The squared correlation coefficient (R^2) was greater than 0.99, while the accuracy levels of the calibration curve standard ranged from 95.86% to 109.98% and 86.21% to 107.85% for AML and MET, respectively. These values satisfied predetermined requirements. The LLOQs in the standard curve that can be measured with acceptable accuracy and precision were 0.045 ng/mL (for AML) and 0.3 ng/mL (for MET) at a signal-to-noise ratio of more than 5.

The intra- and inter-day accuracy and precision of the LLOQ and QCs of all the analytes were within the acceptable range. The

intra-day precision (%CV) varied from 5.71% to 11.73%, and the accuracy fell within 90.02% to 105.15% for all the analytes. Similarly, the inter-day precision (%CV) varied from 5.92% to 8.05%, and the accuracy was within 95.12% to 103.15%. The stability of the analytes and IS in the human plasma and stock solutions was investigated under various conditions. The analytes were stable for up to 6 h at room temperature and for three freeze and thaw cycles at –20°C. The spiked plasma samples were also stable for up to 110 d after a long-term stability experiment. Autosampler stability was determined, and the results indicated that the spiked samples were stable up to 24 h without a significant loss of analytes at 10°C.

Analyte responses were stable in plasma on the benchtop (room temperature for 6 h) after three freeze–thaw cycles, after 110 d of storage (at –20°C), and in the autosampler (24 h, set at 10°C). Moreover, analyte responses in the stored stock were within 15% of the fresh solution responses, which was considered acceptable.

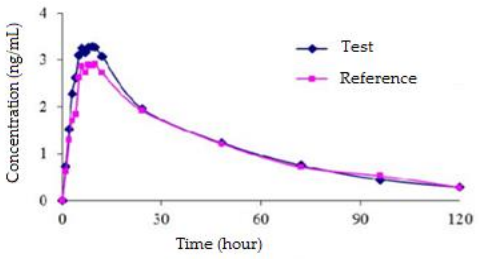
3.4. Application

As previously stated, the method put forward in this work was used to determine the concentrations of AML and MET in a bioequivalence study involving a single fixed dose of the test and reference formulations containing 50 mg/5 mg MET/AML administered to the 14 healthy Vietnamese subjects. **Figure 3** illustrates the mean concentration time curve of the reference and the test results on AML and MET in human plasma after the oral administration of the test and reference formulations under fasting and fed conditions. **Table 3** summarizes the mean pharmacokinetic parameters of AML and MET.

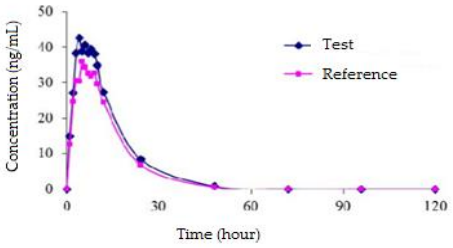
Table 2. Summary of validation results

Property		QC	AML	MET	IS
Selectivity	Analyte (%LLOQ)	-	0.0	0.0	
	Internal standard (%IS)	-	0.0	0.0	
Carryover	Analyte (%LLOQ)	-	0.0	0.0	
	Internal standard (%IS)	-	0.0	0.0	0.0
Calibration standard curve	R square (R^2)	-	0.9977	0.9939	
Intra-day accuracy and precision	Accuracy (%)	LLOQ	105.15	90.02	
		LQC	98.01	90.70	
		MQC	93.36	93.63	
		HQC	100.43	95.14	
	Precision, CV (%)	LLOQ	6.96	11.73	
		LQC	6.81	5.85	
		MQC	5.71	6.01	
		HQC	8.92	7.95	
Inter-day accuracy and precision	Accuracy (%)	LLOQ	101.86	101.28	
		LQC	99.60	95.12	
		MQC	99.47	103.15	
		HQC	98.78	100.03	
	Precision, CV (%)	LLOQ	6.41	8.05	
		LQC	6.68	5.92	
		MQC	6.32	5.18	
		HQC	6.15	7.22	
Matrix effect	Precision, CV (%)	LQC	1.09	5.73	
		HQC	3.28	2.95	
Recovery	Mean	LQC	93.47	85.36	95.21
		MQC	92.20	97.55	96.74
		HQC	71.72	84.52	84.23
	Precision, CV (%)	LQC	2.11	5.72	2.00
		MQC	9.32	2.20	3.91
		HQC	9.57	1.47	4.29
Free-thaw stability	% of nominal concentration	LQC	98.14	118.27	
		HQC	97.40	89.72	
At room temperature for 6 hours	% of nominal concentration	LQC	104.70	99.47	
		HQC	102.28	108.52	
In autosampler for 24 hours	% of nominal concentration	LQC	109.44	101.85	
		HQC	107.86	108.00	
Long-term stability for 80 days	% of nominal concentration	LQC	99.60	86.91	
		HQC	110.35	104.29	
Long-term stability for 110 days	% of nominal concentration	LQC	104.78	103.10	
		HQC	94.51	96.72	

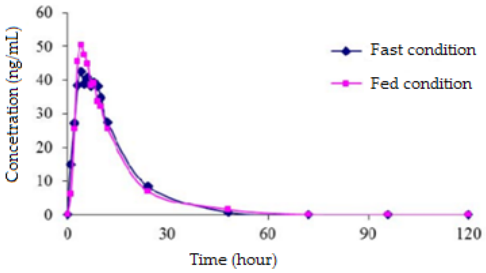
Abbreviations: LLOQ = lower limit of quantification, LQC = low-level quality control, MQC = medium-level quality control, HQC = high-level quality control, IS = internal standard, CV = coefficient of variation



A. Amlodipine



B. Metoprolol in fasting conditions



C. Metoprolol in fed conditions

Figure 3. Mean plasma concentration–time curves of AML (A) and MET in fasting conditions (B) and MET in fed conditions (C) after the oral administration of 5 mg AML and 50 mg MET (fixed-dose tablet formulation) to 14 healthy Vietnamese subjects

Table 3. Mean pharmacokinetic parameters (±SD) of 14 healthy Vietnamese subjects after the oral administration of 5 mg AML and 50 mg MET (fixed-dose tablet formulation)

	Parameters	Drug	Mean ± SD	Range (Min - Max)
Amlodipine besylate in fasting conditions	AUC_{0-t} (ng.hour/ml)	Test	146.910 ± 40.46	65.855 – 197.304
		Reference	139.832 ± 42.38	60.750 – 196.081
	AUC_{0-inf} (ng.hour/ml)	Test	162.001 ± 46.54	67.816 – 221.824
		Reference	155.251 ± 48.61	64.198 – 213.235
	C_{max} (ng/ml)	Test	3.758 ± 0.80	2.160 – 4.978
		Reference	3.450 ± 0.97	1.609 – 5.211
	T_{max} (hour)	Test	7.69	5.00 – 12.00
		Reference	7.92	5.00 – 12.00
	$T_{1/2}$ (hour)	Test	35.18	24.20 – 52.37
		Reference	37.04	29.06 – 56.17
	K_e ($hour^{-1}$)	Test	0.0209	0.0132 – 0.0286
		Reference	0.0194	0.0123 – 0.0239
Metoprolol succinate in fasting conditions	AUC_{0-t} (ng.hour /ml)	Test	718.336 ± 479.26	103.697 – 1705.877
		Reference	604.097 ± 342.43	144.891 – 1223.920
	AUC_{0-inf} (ng.hour /ml)	Test	730.569 ± 483.00	106.198 – 1727.012
		Reference	615.081 ± 340.35	150.952 – 1232.768
	C_{max} (ng/ml)	Test	48.280 ± 25.59	11.321 – 99.064
		Reference	40.945 ± 19.42	8.743 – 75.673
	T_{max} (hour)	Test	5.31	3.00 – 8.00
		Reference	6.15	2.00 – 12.00
	$T_{1/2}$ (hour)	Test	6.72	3.85 – 8.37
		Reference	7.07	5.85 – 9.60
	K_e($hour^{-1}$)	Test	0.1087	0.0828 – 0.1802
		Reference	0.1000	0.0722 – 0.1185

Metoprolol succinate in fed conditions	AUC_{0-t} (ng.hour/ml)	Test	702.808 ± 466.68	101.834 – 1523.373
	AUC_{0-inf} (ng.hour/ml)	Test	725.127 ± 484.84	106.982 – 1534.304
	C_{max} (ng/ml)	Test	57.121 ± 29.07	11.491 - 98.423
	T_{max} (hour)	Test	4.85	3.00 – 8.00
	$T_{1/2}$ (hour)	Test	6.96	3.20 – 15.69
	K_e(hour^{-1})	Test	0.1180	0.0442 – 0.2169

Abbreviations: C_{max} = Maximum plasma concentration, T_{max} = time point of maximum plasma concentration, $t_{1/2}$ = half-life of drug, AUC_{0-t} = area under the plasma concentration–time curve from 0 to 120 h, AUC_{0-inf} = area under the concentration–time curve from 0 h to infinity, SD = standard deviation

DISCUSSION

Sarkar et al. [5] proposed an LC/ion trap MS method for the quantification of AML and MET in human plasma, with HCTZ as the internal standard. The authors used liquid–liquid extraction with chloroform. They found mean recovery levels of 77.68% for AML, 99.06% for MET, and 71.51% for the IS. The analytes and IS were separated on a Peerless Basic C18 column (33 mm × 4.6 mm; 5 µm). The mobile phase consisted of methanol and water containing 0.5% formic acid (8:2, v/v) and was isocratically delivered at a flow rate of 1 mL/min and a total run time of approximately 3 min. The method linearity range was 1.25 to 12.5 ng/mL for AML and 1.5 to 100 ng/mL for MET. The method was also used in a bioequivalence study involving healthy volunteers, who were administered separately commercial products containing 5 mg AML and 50 mg MET.

In the current work, another novel, simple, economical, and rapid approach to the simultaneous quantification of AML and MET via LC–MS/MS was established. The process involved liquid–liquid extraction with diethyl ether–dichloromethane (6:4) as the agent. AML, MET, and the IS were recovered at 93.47%, 97.55%, and 96.74%, respectively. A single IS (HCTZ) enabled the monitoring the chromatographic separation of all the analytes, which was performed on a Gemini® C18 column (100 × 3 mm; 5 µm). The mobile phase, composed of 0.4% formic acid and methanol (20:80, v/v), was delivered at a flow rate of 0.3 mL/min. The total chromatographic run time was 3.0 min, which was short enough to be used for routine analysis wherein a large number of samples need to be quantified. Our method was established as linear in nature in the range of 0.045 to 15 ng/mL (for AML) and 0.3 to 100 ng/mL (for MET). The accuracy and precision at the LLOQ were within the acceptable range, indicating the considerable sensitivity of the developed method.

This low-level LLOQ was considered adequate for application in an inquiry into the bioequivalence of formulations containing AML and MET (5 and 50 mg, respectively).

CONCLUSION

We developed and validated a novel, sensitive assay for the quantification of AML and MET in human plasma. The approach offers several advantages, such as a simple extraction procedure and a short chromatographic run time, which renders the method suitable for the analysis of large sample batches of products containing AML and MET.

CONFLICTS OF INTERESTS

The authors have no conflicts of interests to declare.

REFERENCES

1. Darvishi, M., Forootan, M., Noori, M., Nazer, M. R., Eghbali, M., & Hormozi, S. F. (2019). An investigation into the effect of needle stick injuries on the healthcare workers in besat hospital a brief report. International Journal of Pharmaceutical Research, 11(2), 337–338. https://doi.org/10.31838/ijpr/2019.11.02.056
2. Vietnam Ministry of Health. Vietnam national pharmacopoeia. Hanoi: Medicine Publishing House;2009.
3. Chan-Mei Lv., Chun-Min Wei, Fan-Long Bu, Rui Chen, Xiao-Lu Wang, Rong Li, et al. Determination of Amlodipine in Human Plasma by LC-MS/MS and its Bioequivalence study in Healthy Chinese Subjects. Pharmacol Pharm 2013;4:191-200.
4. Narmada P, Nalini K, Bhujanga Rao AKS, Jogi KV. Int J Pharm Phytopharmacol 2013;3(2):117-20.
5. Sarkar AK, Ghosh, Das A, Senthamil P, Veeran GK, Uttam M, et al. J Chromatogr B: Biomed Sci Appl 2008;873(1):77-85.
6. FDA. Guidance for Industry – Bioanalytical Method Validation, Rockville, MD, USA, 2013, 1-27.
7. Sibi G. "Factors Influencing Heavy Metal Removal By Microalgae-A Review." Journal of Critical Reviews 6.6 (2019), http://dx.doi.org/10.22159/jcr.2019v6i6.35600