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Detection of Amlodipine Levels in Human Plasma. Safety and Tolerability Issues

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Abstract

Background: Amlodipine is an antihypertensive drug used in the management of hypertension and other cardiovascular diseases. Compared to calcium antagonists, β-blockers or ACE inhibitors, amlodipine tolerability showed a significantly lower incidence of side effects. In addition, compared with enalapril, amlodipine significantly reduced non-fatal myocardial infarction and stroke, or transient ischaemic attack.

Aim: Development of a novel bio-analytical method for quantification of amlodipine in plasma and its clinical applications, including safety and tolerability evaluation and bioavailability studies.

Methods: After extraction of amlodipine from plasma, samples were chromatographed with a mobile phase consisting of 25mM Ammonium Formate: Acetonitrile 15:85 V/V with 0.1% Formic acid at flow rate 0.6ml/min, ESI positive mode, and m/z 409.4→238.1, 456.2→354.2 for amlodipine and lacidipine (internal Standard) respectively. The bioequivalence study involved 27 volunteers in a crossover pattern. Pharmacokinetic parameters AUC 0-72, Cmax, and Tmax used for assessment of bioequivalence of the generic and reference products.

Results: The developed bioanalytical method showed that the average recovery of amlodipine from human plasma was 80.166%. The limit of quantitation was 0.1ng/ml, and the correlation coefficient (r2) was equal to 0.999. Analysis of variance showed that there was no significant difference between generic and reference products.

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INTRODUCTION

Calcium channel blockers were first introduced for coronary heart disease, and they proved remarkable efficacy results in the management of hypertension (HTN). Calcium channel blockers are indicated besides HTN for angina, some arrhythmic conditions, and peripheral vascular disease [1].

Amlodipine is characterized by a lipophilic property and long duration of action. It exerts its action through inhibition of calcium influx into vascular smooth muscle cells and myocardial cells, which results in decreased peripheral vascular resistance [2].

The starting recommended dose of amlodipine is usually 5 mg with a 10mg maximum daily dose. In the elderly population and those with hepatic failure, the recommended starting dose is 2.5 mg [3].

Amlodipine is highly bioavailable, ranging from 60% to 80%. Amlodipine undergoes hepatic metabolism and has a slow rate of elimination half-life over 40–60 hours [3].

Clinical studies showed that amlodipine significantly reduced non-fatal myocardial infarction by 26% and stroke or transient ischaemic attack by 50%, compared to enalapril that showed no significant benefit compared with placebo. Moreover, amlodipine showed a statistically significant reduction in the hospitalization rate for angina versus enalapril [4].

Data from China during covid-19 patients screening showed that lower than one-third of hypertensive patients are receiving antihypertensive medication, and the most frequently prescribed drug is amlodipine and calcium channel blockers class as well (45.6%). On the other hand, prescriptions of angiotensin receptor blockers (ARBs) are about 21.7%, and those of ACEIs are 9.1% [5].

It was reported in the literature that after single-dose administration of amlodipine 10mg Tablet, mean plasma Cmax, AUCo-t, AUCo-inf, and T1/2 was 5.64± 0.91 ng/ml, 288 ± 78 ng.h/ml, 303 ± 88 ng.h/ml, and 46.2±10.9 h respectively. Moreover median Tmax was equal to 7 hours (range from 4 to 12 h) [6]. Another reported literature mentioned that Cmax, AUCo-t, AUCo-inf, and T1/2 was 4.3±0.82 ng/ml, 237±84 ng.h/ml, 263±87 ng.h/ml, and 45±10 h, respectively. Moreover median Tmax was equal to 8.5 hours (range from 5 to 13 h) [7].

It has been reported that low plasma amlodipine concentrations are achieved after oral administration [8], requiring a sensitive bioanalytical method for its determination in plasma.

Analytical methods for the quantification of amlodipine in plasma, such as gas chromatography with electron capture detection [9, 10], high-performance liquid chromatography with fluorimetric detection [11, 12] or with UV detection [13], or with electrochemical detection [14, 15], high-performance thin-layer chromatography-densitometry [16] and liquid chromatography-tandem mass spectrometry [17, 18, 19] have been reported.

Analysis of amlodipine in plasma was performed as per FDA requirements [20] through developing and validating an LC/MS/MS method in compliance with the international guidelines [21]. WinNonlin program was used to perform pharmacokinetic calculations, and SAS software was used to perform statistical analysis. The 90% C.I. for AUC0-t, AUC0-inf, and Cmax were calculated for the ratio between treatments, and results showed to be in the limit of 80% to 125% confidence limits [22].

MATERIALS

1.Chemicals and reagents

Purified water for LC/MS/MS grade, methanol HPLC-gradient (SIGMA Aldrich, Germany), acetonitrile HPLC-gradient (Scharlab, Spain), dichloromethane for HPLC (Fisher Scientific, UK), diethyl ether for HPLC (Scharlab, Spain), formic acid 98-100% essentQ (Scharlau, Spain), and ammonium formate anhydrous, reagent grade, 97% (SIGMA Aldrich, Germany).

2.Equipments

Adjustable pipettes (P200, and P1000), disposable plastic pipettes tips - labtip yellow (range 5 - 200 µL) and labtip blue (range 200 1000 µL), disposable glass test tubes 120 x 12 mm, vortex mixer (Boeco, Germany), vacuum pump (Boeco, Germany), PH-meters (Boeco, Germany), water purifier (Purelab option- R7ELGA, U. K.), sonicator (Crest, U.S.A.), analytical balance (Sartorius, U.S.A.), concentrator plus/vacufuge® plus (Eppendorf, Germany), LC-MS/MS Agilent 6410B triple quad, USA.

METHODS

(a) Chromatographic conditions

The developed chromatographic conditions were used. The mobile phase composition was 25mM ammonium formate: acetonitrile 15:85 V/V with 0.1% formic acid. The flow rate was set at 0.6ml/min. Injection volume was set at 5ul. MS/MS 6410B detector was operated at ESI positive mode, m/z was 409.4→238.1, 456.2→354.2 for amlodipine and lacidipine (internal standard), respectively.

Fragmentor energy was set at 100, and 135 for amlodipine and (internal standard) lacidipine. The collision energy was set at 2, and 0 for amlodipine and (internal standard) lacidipine.

(b) Preparation of Solutions

1.Master standard solution

Accurately weighed 13.92mg of amlodipine besylate standard equivalent to 10mg amlodipine were transferred to a 100 ml volumetric flask, about 80 ml methanol was added, and sonication was done for 10 minutes. The volume was completed with methanol to obtain a solution containing 100ug/ml amlodipine “Solution A”.

From “Solution A” 0.1 ml was transferred to a 100 ml volumetric flask and volume completed with methanol to obtain a solution of 100ng/ml “Solution B”.

2. Working Solutions:

Solution used	Volume taken	Conc. obtained	Final volume (ml)
"Solution B"	0.1ml	1 ng/ml	10
"Solution B"	0.25ml	2.5 ng/ml	10
"Solution B"	0.5ml	5 ng/ml	10
"Solution B"	1ml	10 ng/ml	10
"Solution B"	2.5ml	25 ng/ml	10
"Solution B"	5ml	50 ng/ml	10
"Solution B"	7.5ml	75 ng/ml	10
"Solution B"	10ml	100 ng/ml	10

Lacidipine solution

An accurately weighed 10mg of lacidipine standard was transferred to a 100 ml volumetric flask followed by addition of 80 ml of methanol and sonication for 10 minutes, the volume was then completed with methanol to obtain a solution of concentration of 100ug/ml lacidipine solution (A). From solution (A) 300ul was transferred to a 100ml volumetric flask, and the volume was completed with methanol to obtain 300 ng/ml lacidipine solution (B).

(c) Preparation of Amlodipine Standard Concentrations in Human Plasma:

The standard samples in plasma were prepared by transferring a 30 ul aliquot of prepared working solutions of amlodipine at concentrations ranging from 1 to 100 ng/ml to centrifuge tubes containing 300 ul of blank plasma.

(d) Sample Preparation

Volunteers human plasma samples and standard samples (300 ul) were transferred into appropriate centrifuge test tubes 30 ul of the internal standard (Lacidipine working solution 300ng/ml), were added, then samples were vortex-mixed for approximately 30 seconds. 0.3ml of 25mM borate buffer pH 9 was added, then vortex-mix was done for approximately 1 minute. 3ml of (diethyl ether: dichloromethane 70:30 V/V) were added and vortex-mix was done for approximately 1 to 2 minutes. Centrifugation of samples was done at 3500rpm for 5 minutes; clear supernatant layer evaporated at 45oC till dryness. Reconstitution of dry residue was performed with 200ul mobile phase and injected on LC/MS/MS.

(e) Quantitation:

Unkown drug concentrations in plasma samples withdrawn calculated using the following equation: y = ax + b, where; Y: response ratio, X: unknown concentration of drug in plasma samples, a: calibration curve slope, b: Y-Intercept

(III) Bioequivalence Study:

(a) Study ethics:

This study was conducted as per ICH and GCP guidelines adopted by the European agency for the evaluation of medicinal products (EMEA), and after Ethics Committee approval on the bioequivalence study protocol of amlodipine 10mg tablet (Study Code: VAS-ARAD-BES-1017/0255). Essential documents and records were all archived according to drug research center (DRC) internal procedures for authorized direct access.

Written informed consent was signed by the participant and clinical investigator, and all study aspects were discussed with participants before starting screening. There were no obligations on volunteers to continue the study if they didn't want to.

Clinical investigator, study director (principal investigator), licensed physicians responsible for physical examination and following-up of the subjects for the appearance of any side or adverse effects, measurement of vital signs throughout the study including blood pressure, pulse rate, body temperature, respiratory rate before and all over the study and registered nurses were responsible for blood sampling.

(b) Inclusion criteria:

Volunteers age should be within 18 to 55 years, and calculated body mass index should lie within normal acceptable limits, no history of contribution in any pharmacokinetics study, and normal physiological examination, laboratory data within normal, limits. Subjects should not be alcoholics or drug abusers and shouldnot have any known history for both. It is preferred to select non-smoker subjects, and if subjects are smokers, so they should not smoke more than 8 cigarettes per day.

(c) Exclusion criteria:

A known drug hypersensitivity, GIT problems, auto-immune diseases, kidney diseases or kidney dysfunction, CVS diseases, diabetics, hepatic disease, hematological abnormalities, respiratory diseases, alcohol intake or drug abuse history, positive HIV-I, (smoking and if including they should be identified), abnormal laboratory values, subject administered any medication less than two weeks of the study starting date, subjects who have donated blood or who participated in clinical studies that require more than 500 ml of blood to be withdrawn within a month and a half preceding study starting date.

(d) Subjects:

Twenty-seven healthy adult volunteers participated in the comparative bioavailability study after being subjected to complete medical and laboratory assessment and ensuring that they are in compliance with the required inclusion/exclusion criteria. Concurrent medications were not allowed during the study time course. No food intake was allowed for four hours after study dose administration. At 11:00 they received a standard meal, and at 15:00 a second standardized meal was introduced.

(e) Study design:

The design of this study was a randomized two-way crossover design comparing the bioavailability of generic versus reference amlodipine 10mg tablets in 27 healthy adult volunteers under fasting state with a washout period of three weeks. The number of required blood samples, and their disposition after collection, besides the required washout period, was designed according to amlodipine pharmacokinetics.

(f) Sample collection:

The number of blood collections for drug analysis was 17 samples each

5 ml for each study period at the following time intervals; 0 (directly prior to dosing), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 24, 48, and 72 hours after drug administration.

Blood sample collection was performed into a tube containing anticoagulant EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes in order to obtain plasma samples which were kept at -80 oC until analysis.

(g) Analysis of Plasma Samples:

The withdrawn volunteers' plasma samples were analyzed using LC-MS/MS technique for the quantitation of amlodipine in human plasma.

(h) Pharmacokinetic Calculations:

The following pharmacokinetic parameters (variables) of amlodipine were assessed; Cmax, tmax, t1/2e, Ke, and AUC0-72.

(i) Measurement of Blood Pressure and Heart Rate

Blood pressure cyctolic / diastolic and pulse rate measurements before dosing and at regular intervals (at 2, 4, 6, and 10 hours) after drug administration were included in tolerability assessments. A 120/80 mmHg blood pressure reading and 50 to 100 beats per minute resting heart rate are considered normal.

(j) Safety and Tolerability:

Subject's medical history and medication history, physical examination, laboratory reports, and all incidents of possible side and/or adverse effects to the study formulations were reported.

(k) Statistical Analysis of Data:

Analysis of variance (ANOVA) was performed by using SAS software. Bioequivalence could be demonstrated for amlodipine within the prescribed 90% confidence interval of 80.00% to 125.00% for AUC0-72, and Cmax with respect to the parametric method on Ln-transformed data.

RESULTS

Analytical Method Validation:

(a) Chromatograms of Amlodipine:

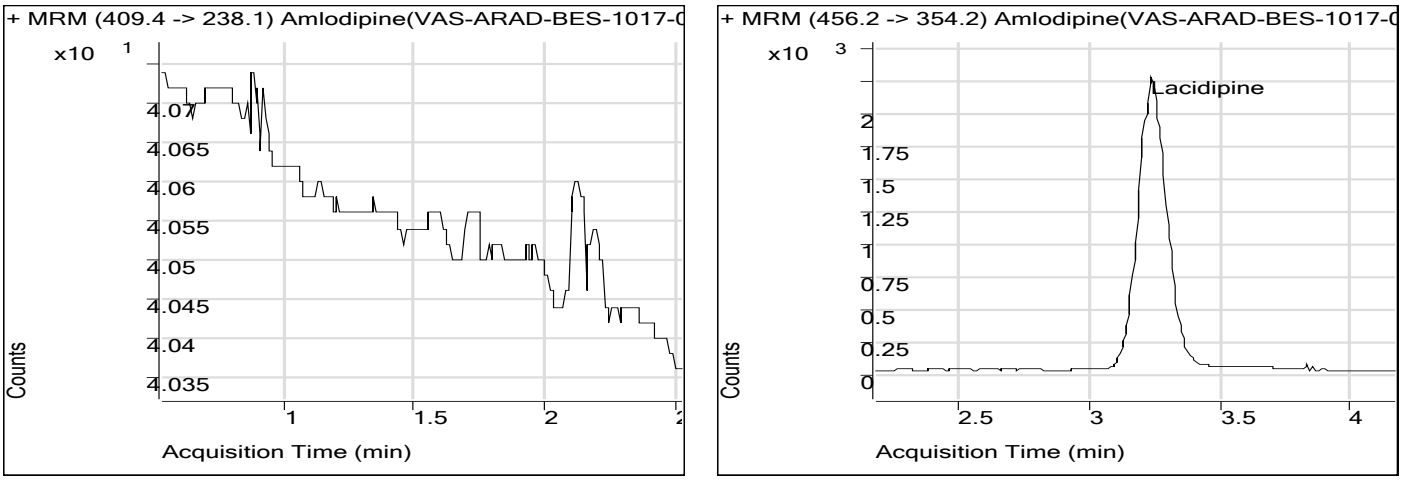

Figure 1: Chromatogram representing an MRM data of blank plasma sample spiked with internal standard lacidipine.

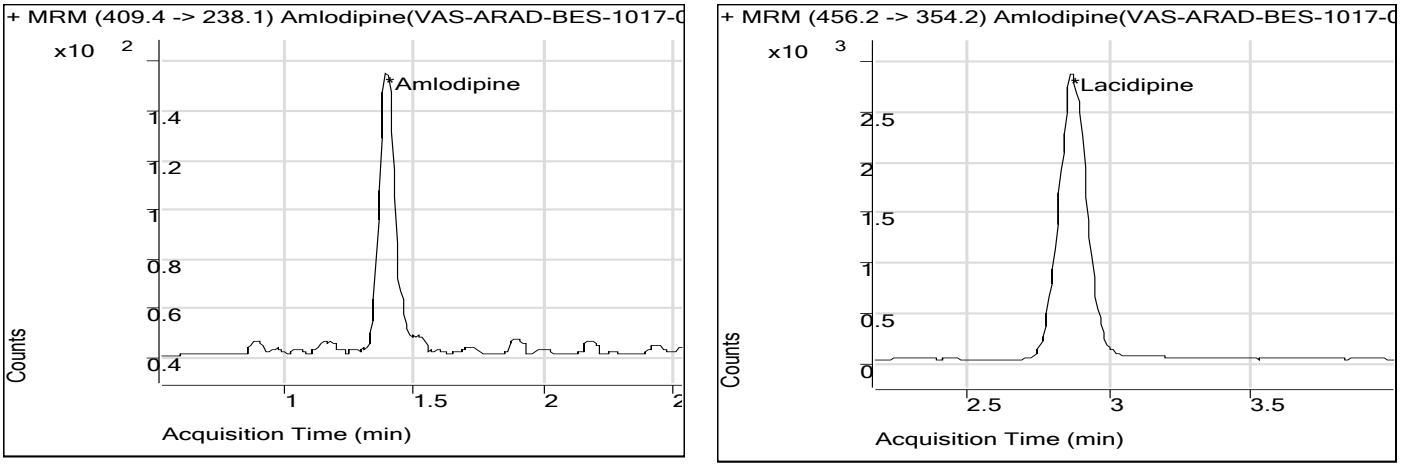

Figure 2: Chromatogram representing an MRM data of blank plasma sample spiked with 0.1ng/ml amlodipine and internal standard lacidipine.

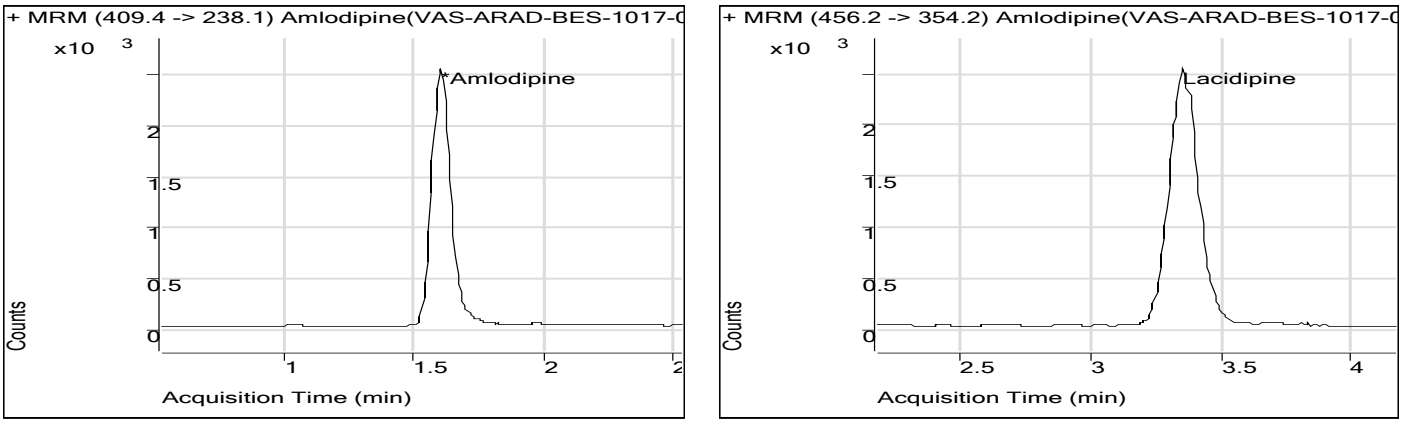

Figure 3: Chromatogram representing an MRM data of blank plasma sample spiked with 5ng/ml amlodipine and internal standard lacidipine.

It is apparent from Figures (1), (2), and (3) that amlodipine was well separated and its retention time was 1.4 min. The obtained peaks were sharp, symmetrical with good baseline resolution and minimum tailing, thus facilitating accurate measurement of the peak responses

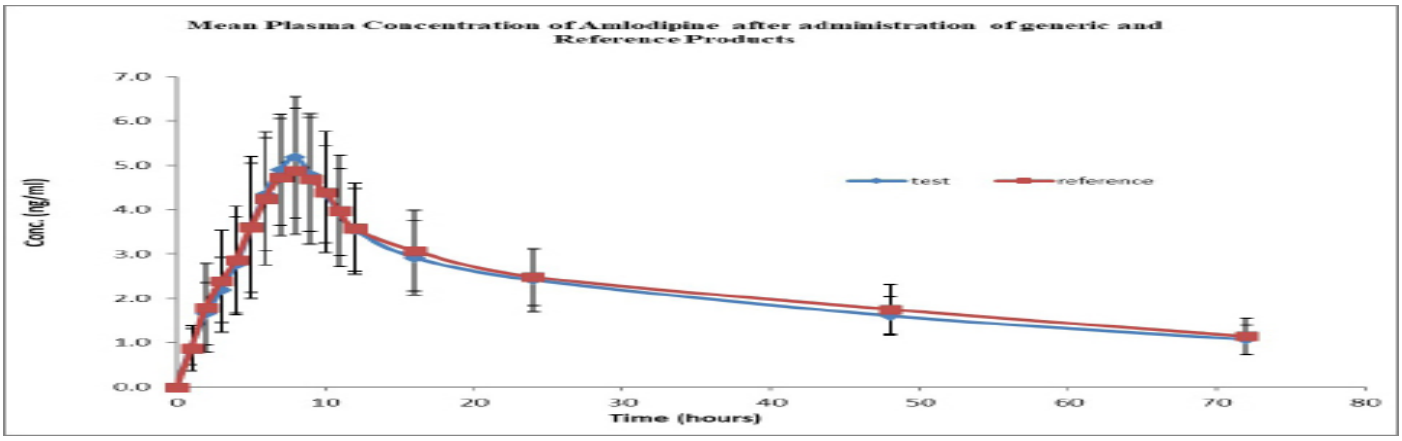

Figure 4: Plasma concentration (Mean±S.D.) of amlodipine following single dose administration of amlodipine 10mg tablets generic and reference products.

(b) Linearity, Accuracy and Precision:

Peak area ratios of varying amounts of amlodipine in human plasma from 0.1 to 10 ng/ml were highly linear (r2 value of 0.999). The results of intraday precision CV% was 5.588% which is following the latest FDA Guidelines. Accuracy and precision were assessed at three different concentrations in the range of predicted drug concentrations on a within, and between-day basis. Intra-day accuracy results showed an average recovery percentage of 98.878%. Inter-day accuracy results showed an average recovery percentage of 98.700%, with an average CV% of 3.510%. Stability study results in plasma showed that the average stability percentage was greater than 95% ensuring stability in the studied conditions.

Bioequivalence Study:

1. Clinical observation (safety and tolerability):

The drug was well tolerated by all participating subjects as there was no side effects or adverse effects reported throughout the whole study in both periods. Moreover, both blood pressure and pulse rate reported were approaching normal ranges.

2. Pharmacokinetic data and assessment of bioequivance:

Results of pharmacokinetic parmeters presented in Tables (1) and (2) showed that the mean maximum plasma concentration (Cmax) was 5.633±1.357ng/ml and 5.328±1.440ng/ml, time point of maximum plasma concentration (tmax) 7.407±1.217h and 8.000±1.301h, half-life of drug elimination during the terminal phase (t1/2e) 44.439±14.553h and 47.750±19.888h, area under the curve from zero to 72h (AUC0-72) 155.033±37.523ng.h/ml and 161.306±38.847ng.h/ml for generic and reference products respectively.

Subject	**T_{max} (h)**	**C_{max} (ng/ml)**	**AUC_{0-72} (ng.h/ml)**	**K_{el} (h^{-1})**	**$T_{1/2}$ (h)**	**MRT_{inf} (h)**
Mean± S.D.	8.000±1.301	5.328±1.440	161.306±38.847	0.017±0.007	47.750±19.888	68.024±28.190
CV%	16.261	27.019	24.083	42.484	41.650	41.441
Range (Median)	6.00-10.00 (8.000)	3.144-7.937 (5.205)	93.830-229.508 (160.359)	0.007-0.032 (0.017)	21.707-95.897 (41.952)	32.715-135.581 (59.340)

Table 1: Pharmacokinetics of amlodipine following administration of single oral dose of (Reference product) to 27 Volunteers.

Subject	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-72} (ng.h/ml)	K_{el} (h^{-1})	$T_{1/2}$ (h)	MRT_{inf} (h)
Mean± S.D.	7.407±1.217	5.633±1.357	155.033±37.523	0.017±0.005	44.439±14.553	62.538±19.766
CV%	16.432	24.098	24.203	29.455	32.748	31.606
Range (Median)	5.00- 10.00 (8.000)	2.680-7.575 (5.860)	92.602-232.005 (158.495)	0.009-0.029 (0.018)	23.909-78.853 (39.607)	35.647-110.194 (56.002)

Table 2: Pharmacokinetics of amlodipine following administration of single oral dose of (Generic product) to 27 Volunteers.

3. Pharmacodynamic results :
The reported measurements of blood pressure and pulse rate were all approaching normal levels and within the safe limits (Figures 5 and 6).

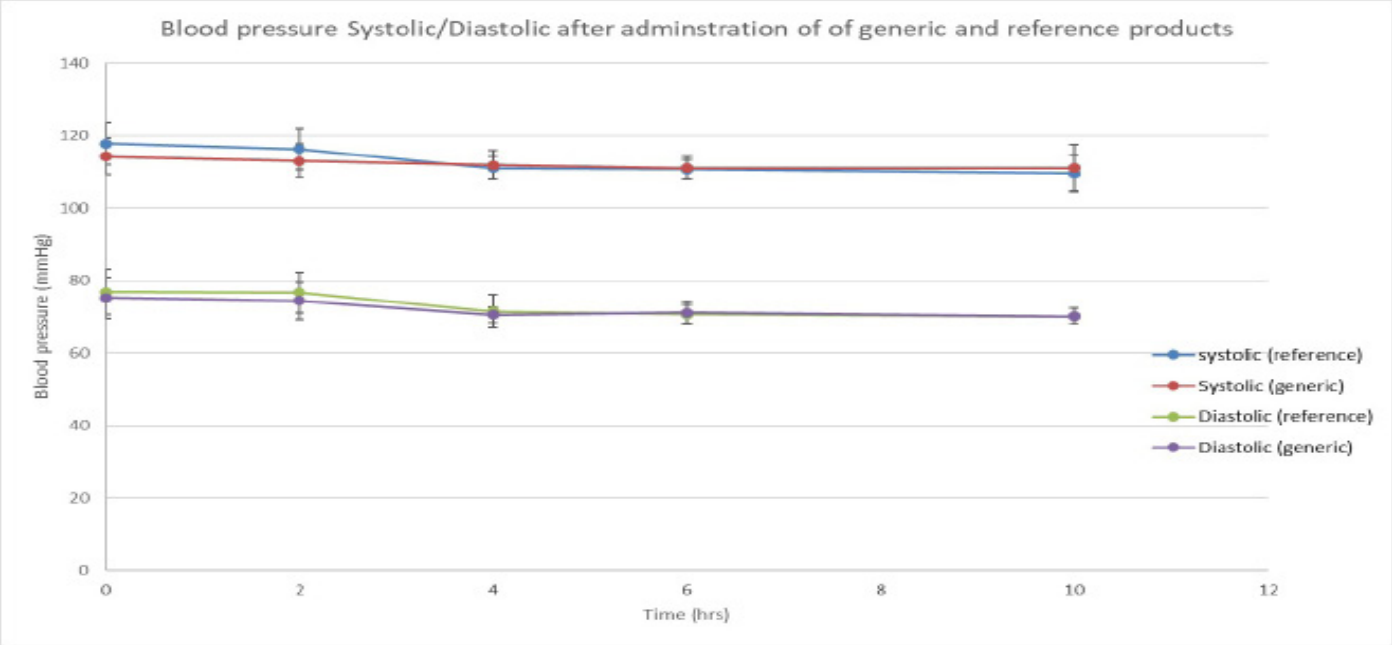

Figure 5: Blood pressure (Mean±S.D.) following single dose administration of amlodipine 10mg tablets generic and reference products.

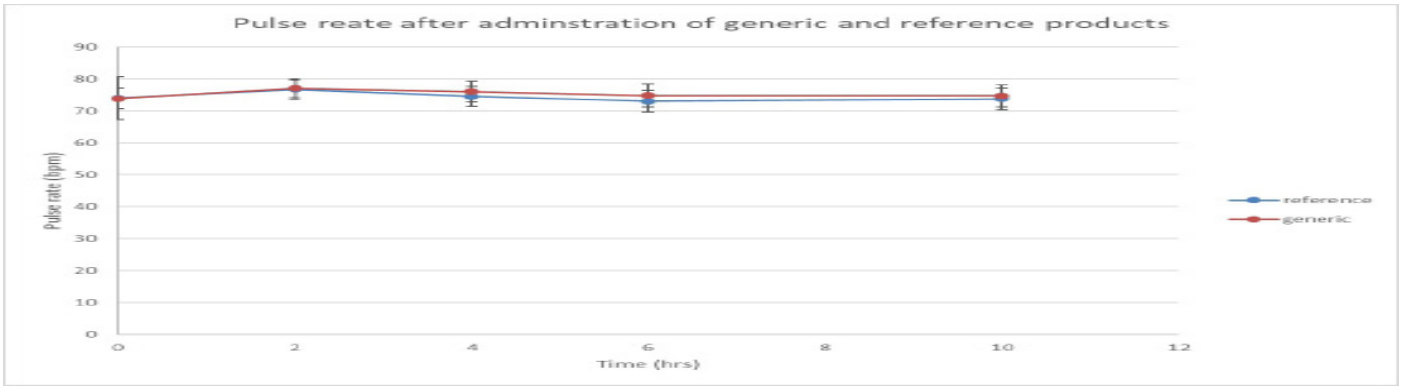

Figure 6: Pulse rate (Mean±S.D.) following single dose administration of amlodipine 10mg tablets generic and reference products.

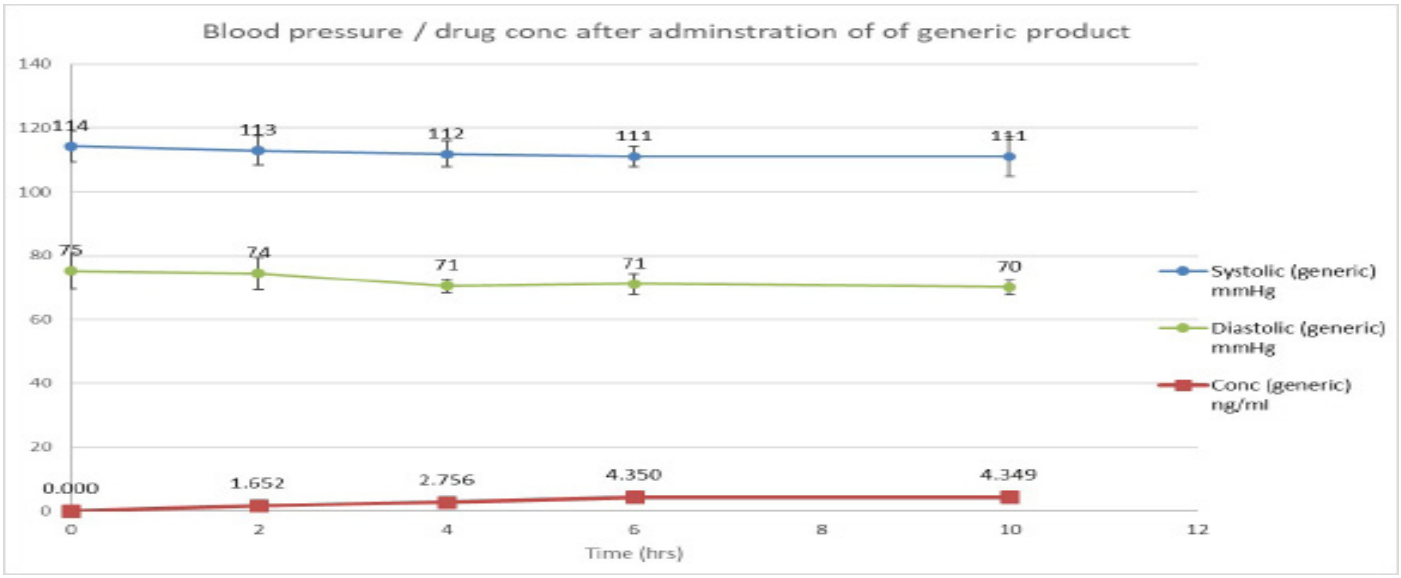

Figure 7: Blood pressure / drug conc (Mean±S.D.) following single dose administration of amlodipine 10mg tablets generic product.

It is clear from the results of blood pressure represented in Figure (7), for the generic product, that all approaches normal levels, as the reported mean values of systolic blood pressure were 114, 113, 112, 111, 111 mmHg and 75, 74, 71, 71, 70 mmHg for diastolic blood pressure at zero (predose), 2, 4, 6, and 10 hours of drug administration respectively.

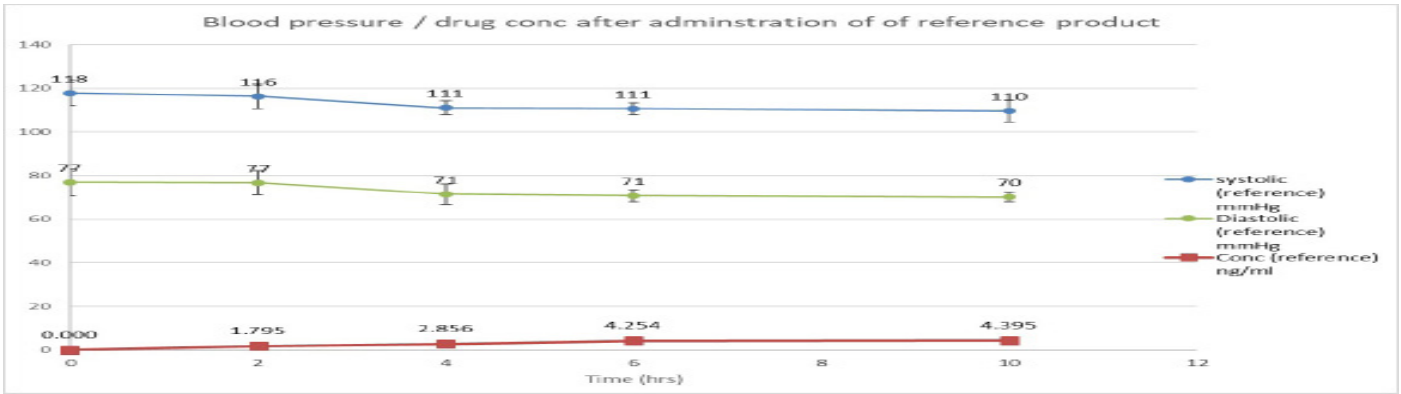

Figure 8: Blood pressure / drug conc (Mean±S.D.) following single dose administration of amlodipine 10mg tablets reference product.

On the other hand, concerning the reference product, mean values of systolic blood pressure were 118, 116, 111, 111, 110 mmHg and 77, 77, 71, 71, 70mmHg for diastolic blood pressure at Zero (predose), 2, 4, 6, and 10 hours of drug administration respectively. (Figure 8)

Significant amlodipine side effects like peripheral edema, heart failure, pulmonary edema, flushing, dizziness, headache, sleepiness, skin rash, nausea, and abdominal pain were not reported during both periods of the study [24].

5. Statistical Analysis:

The results of 2-way ANOVA on Cmax and AUC0-72 for amlodipine were presented in Table (3) showed that there was no significant difference between generic and reference products. The point estimate (%) results for Cmax, AUC0-72 was 98.507%, and 93.998% respectively. The 90% C.I. of parametric means of Cmax, AUC0-72, were 95.082% to 102.055%, and 88.458% to 99.885% respectively.

Table 3: 90 % C.I for amlodipine generic and reference Products.

Pharmacokinetic Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}(ng/ml)	105.929	96.917	115.780
AUC_{0-72}(ng.h/ml)	96.155	92.509	99.943

DISCUSSION

The LC/MS/MS method used in this study was simple and of excellent sensitivity, specificity, precision, and accuracy. The calibration curve was linear over the concentration range of 0.1 to 10 ng/ml, and r2 was 0.999, which is following the latest FDA Guidelines [21], moreover, the developed bioanalytical method could be applied in different clinical applications, including; pharmacokinetics and bioavailability studies, clinical trials, and therapeutic monitoring of amlodipine to insure achievement of therapeutic goals.

One of the amlodipine advantages is what was proved in clinical studies that amlodipine was associated with lower blood pressure variability than other calcium channel blockers for both hypertensive patients and hypertensive patients with comorbidities. Study results indicated that hypertensive patients prescribed amlodipine showed lower blood pressure variability than patients prescribed other calcium channel blockers. Also, the hypertensive patients with comorbidity prescribed amlodipine had lower blood pressure variability than patients prescribed other calcium channel blockers [23].

The importance of therapeutic monitoring of amlodipine was emerged from being a used therapeutic agent in the management of hypertension HTN, angina, some arrhythmic conditions, and peripheral vascular disease [1]. Additionally, therapeutic monitoring ensures that the patients' drug levels are within the required therapeutic range and absence of subtherapeutic levels or toxic levels, which increases the incidence of adverse events.

Compared to other medications like nifedipine and others in the dihydropyridine class, amlodipine has the longest half-life of 30 to 50 hours, providing an advantage of the ability to have once-daily dosing. Caution should be taken into consideration to avoid possible severe hypotension, and thus, the recommendation is to gradually titrate the dose with an initial low dose, besides the importance of long-term patient monitoring to determine its effectiveness [24].

An LC-MS/MS bioanalytical method for quantification of amlodipine in human plasma was reported in the literature. The method used positive ESI mode and tizanidine as internal standard (I.S). Extraction was performed by simple one-step liquid-liquid extraction with (diethyl ether: dichloromethane 70:30 V/V). The chromatography performed on a C18 column using a mobile phase of 10mM ammonium formate methanol : acetonitrile (30:50:20 V/V/V) at a flow rate of 1.0 mL/min. Quantitation was performed using multiple reaction monitoring (MRM) mode with ion transitions of m/z 409.4 → 238.1 for amlodipine and m/z 254.2 → 44.1 for I.S., respectively. Validation results were within the acceptance criteria. Linearity was obtained over the concentration range 0.3-15.0 ng/mL, with a coefficient of determination (r2) of 0.9993. The lower limit of quantification (LLOQ) was 0.3 ng/mL [25].

Another method was reported using LC-MS/MS for the quantification of amlodipine in human plasma. The internal standard (I.S) used as gliclazide. The extraction procedure was performed with ethyl acetate, and chromatography was done using Diamond C18 (150 mm × 4.6 mm, 5 µm) column. The used mobile phase was methanol: 10 mM ammonium acetate with gradient flow rates and gradient conditions at positive ionization mode with multi-reaction monitoring (MRM) mode detection and using ion transitions of m/z 409.2→238.1, 294.1, and m/z 324.2→127.3, for drug and internal standard respectively. The linearity concentration range was 0.05 - 12 ng/ml, and the method was fully validated [26].

The developed bioanalytical method, in the current study, provides a linear dynamic range more than those reported in the literature. Besides, it is nearly in accordance with them regarding sensitivity [25, 26]. The extraction method was performed using liquid-liquid extraction technique as mentioned in literature methods [25, 26] with some changes in extraction procedures. Also, some changes were performed on chromatographic conditions.

A meta-analysis of 16 consecutive studies addressed the tolerability and safety of amlodipine in a large population of patients (n = 12831). An open questionnaire was used for the detection of adverse events reported by patients after administration of amlodipine. The overall percentage of patients experiencing adverse effects was 15%, and only 3% of patients were withdrawn from amlodipine therapy because of drug intolerance. This percent was not influenced by drug dosage or disease status [27].

Amlodipine's tolerability compared to alternative calcium antagonists, β-blockers, or ACE inhibitors showed a significantly lower incidence of amlodipine side effects, 17.3 versus 39.7% of patients, indicating safety and tolerability of amlodipine use [27].

It was reported that amlodipine/valsartan combination single-pill was effective in reducing blood pressure and well tolerated in hypertensive

patients who were not adequately monotherapy controlled in a daily clinical setting [28].

More protection against stroke and myocardial infarction was provided by amlodipine than by angiotensin II receptor blockers. In addition, amlodipine prevented more strokes than angiotensin-converting enzyme (ACEis) inhibitors and older drug classes (diuretics and β-blockers), in accordance with prior meta-analyses [29].

Amlodipine was given to 35 hypertensive patients with renal dysfunction at 2.5-5.0 mg/day for 8 weeks, and its efficacy and safety were assessed. The goal of reducing blood pressure was achieved in 28 of the 35 patients (80%), while blood pressure decreased in 4 patients (11.4%) and 3 patients remained unchanged (8.6 percent). In 27 of the 35 patients (77.1 percent), the drug was rated as clinically beneficial (77.1 percent) and slightly beneficial in another 5 patients (14.3 percent). Thus, amlodipine significantly reduced blood pressure in hypertensive patients with renal impairment while causing little or no worsening of renal dysfunction [30].

The results of amlodipine pharmacokinetic parameters obtained were nearly in accordance with reported literature regarding Tmax, Cmax, AUC, and T1/2 [6, 7]. Besides, the pharmacodynamic results of amlodipine 10mg tablet generic and reference products showed that both treatments nearly equal in the reduction of systolic and diastolic blood pressure.

In a bioequivalence study, a 90% confidence interval of 80% to 125% for AUC0-72 and Cmax on Ln-transformed data should be fulfilled. In this study, the point estimate (%), and 90% confidence intervals of parametric means of Cmax, AUC0-72 were lying within FDA acceptance limits (80 % to 125%) [22].

CONCLUSION

It could be concluded that the bioanalytical method developed for the determination of amlodipine in plasma is valid, sensitive, specific, precise, and accurate, and could be used for the determination of drug pharmacokinetic parameters. Besides, the results of the bioequivalence study of amlodipine 10mg tablets generic compared to the reference product showed that both products are bioequivalent. The developed method could be used in amlodipine bioavailability and bioequivalence studies, clinical trials, and therapeutic drug monitoring, efficacy, and safety studies. The developed method provides accurate monitoring of amlodipine blood levels for long term follow-up and evaluation of treatment effectiveness.

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