

# Sensitive and rapid liquid chromatography/tandem mass spectrometry assay for the quantification of amlodipine in human plasma

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**ABSTRACT:** A simple, sensitive and rapid high-performance liquid chromatography/electrospray ionization tandem mass spectrometry method was developed and validated for the assay of amlodipine in human plasma. Following liquid–liquid extraction, the analytes were separated using an isocratic mobile phase on a reverse-phase C<sub>18</sub> column and analyzed by MS in the multiple reaction monitoring mode using the respective [M+H]<sup>+</sup> ions, *m/z* 409/238 for amlodipine and *m/z* 409/228 for the IS. The assay exhibited a linear dynamic range of 50–10,000 pg/mL for amlodipine in human plasma. The lower limit of quantification was 50 pg/mL with a relative standard deviation of less than 8%. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The average absolute recoveries of amlodipine and the IS from spiked plasma samples were 74.7 ± 4.6 and 72.1 ± 2.0%, respectively. A run time of 1.5 min for each sample made it possible to analyze more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies. The observed maximum plasma concentration (C<sub>max</sub>) of amlodipine (2.5 mg oral dose) was 1425 pg/mL, time to observed maximum plasma concentration (T<sub>max</sub>) was 8.1 h and elimination half-life (T<sub>1/2</sub>) was 50.1 h. Copyright © 2006 John Wiley & Sons, Ltd.

**KEYWORDS:** amlodipine; LC-MS/MS; pharmacokinetic study

## INTRODUCTION

Amlodipine, see Fig. 1, is a potent calcium antagonist, useful in the management of angina pectoris and hypertension (Meredith and Elliott, 1992), belonging to the generally termed '1,4-dihydropyridines' class. Like most other dihydropyridine calcium blockers, amlodipine is used as a racemic mixture. It has low plasma concentrations because after oral administration, amlodipine has a long elimination half-life in humans, ranging from 35 to 45 h due to the large volume of distribution (21 L/kg); moreover it is highly bound (>95%) to plasma proteins (Murdoch and Heel, 1991).

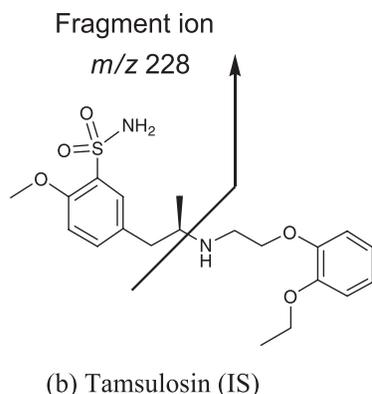
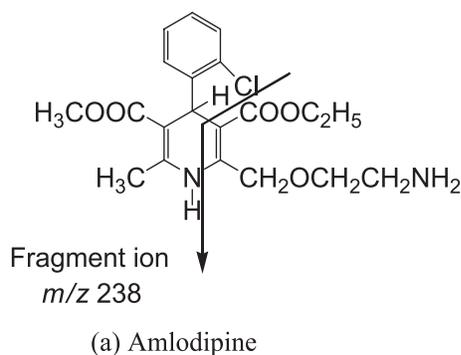
The bioanalytical component of pharmacokinetic study requires a drug assay with simplicity, selectivity, high sensitivity, small volume requirements and rapid turnaround time. Several analytical methods for the quantification of amlodipine in biological fluids have been reported, such as thin-layer chromatography (limit

of quantification, LOQ = 2.0 ng/mL) (Pandya *et al.*, 1995), gas chromatography (LOQ = 2.5 ng/mL; retention time, RT > 9.0 min) (Monkman *et al.*, 1996), liquid chromatography with amperometric detection (LOQ = 0.2 ng/mL; RT > 8 min; Josefsson *et al.*, 1995), liquid chromatography with ultraviolet detection (LOQ = 0.2 ng/mL; RT > 40 min, Luksa *et al.*, 1997; LOQ = 0.5 ng/mL, Zarghi *et al.*, 2005) and liquid chromatography with fluorescence detection (LOQ = 0.25 ng/mL, RT = 7 min, Tatar and Atmaca, 2001; LOQ = 0.25 ng/mL, RT = 3.7 min, Bahrami and Mirzaeei, 2004).

Quantification of drugs in biological matrices by liquid chromatography/tandem mass spectrometry (LC-MS/MS) is becoming more common, owing to the improved sensitivity and selectivity of this technique (Jemal, 2000; Ramakrishna *et al.*, 2004a–e, 2005a–f; Nirogi *et al.*, 2005a, b). LC-MS/MS has been used for the quantification of amlodipine concentrations in human plasma/serum. Yasuda *et al.* (1996) reported an LC-MS/MS method with an atmospheric pressure chemical ionization (APCI) interface using a deuterated internal standard, which is seldom commercially available. The LOQ was 0.014 ng/mL using 1 mL of human serum and the retention time was longer (4.5 min). Marzo *et al.* (2000) reported an LC-MS/MS method with an LOQ of 0.1 ng/mL using 1 mL of

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**Abbreviations used:** APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; LLE, liquid–liquid extraction; MRM, multiple-reaction monitoring.



**Figure 1.** Chemical structures for amlodipine and IS (tamsulosin).

human plasma, and the retention time was 2.2 min. Carvalho *et al.* (2001) reported an LC-MS/MS method with positive ion electrospray ionization (ESI) using multiple reaction monitoring (MRM) mode with an LOQ of 0.1 ng/mL and the retention time was longer (3.4 min). Recently an LC-MS/MS method with an APCI interface was reported for a stereoselective analysis of amlodipine in plasma based on solid-phase extraction (Streel *et al.*, 2002). The LOQ was 0.1 ng/mL for each enantiomer using 1.8 mL of plasma and retention time was 15 and 20 min for the enantiomers. Recently, Massaroti *et al.* (2005) reported an LC-MS/MS method with an LOQ of 0.2 ng/mL and the retention time was 1.9 min.

The purpose of this investigation was to explore the high selectivity and sensitivity of a triple-quadrupole MS system with an ESI interface for the development and validation of a robust reversed phase LC-MS/MS method in MRM mode for the quantification of amlodipine in human plasma. It was essential to establish an assay capable of quantifying amlodipine at concentrations down to 50 pg/mL. At the same time, it was expected that this method would be efficient in analyzing large numbers of plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies after therapeutic doses of amlodipine.

## EXPERIMENTAL

**Chemicals.** Amlodipine was obtained from Torrent Pharmaceuticals Limited (Ahmedabad, India) and tamsulosin (internal standard, IS) was obtained from the R&D Institute (Hyderabad, India). Chemical structures are presented in Fig. 1. HPLC-grade LiChrosolv methanol and LiChrosolv acetonitrile were purchased from Merck (Darmstadt, Germany). Dichloromethane, diethyl ether, formic acid and sodium hydroxide pellets were purchased from Merck (Worli, Mumbai, India). HPLC-grade water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

**LC-MS/MS instrument and conditions.** The 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) was equipped with a G1312A binary pump, G1379A degasser, G1367A autosampler equipped with a G1330B thermostat, G1316A thermostatted column compartment and G1323B control module. The chromatography was on Waters symmetry C<sub>18</sub> column (5 μm, 150 × 4.6 mm i.d.) at 30°C temperature. The isocratic mobile phase composition was a mixture of water–acetonitrile–formic acid (30:70:0.03, v/v), which was pumped at a flow-rate of 1.0 mL/min with a split ratio of 50:50.

Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) using MRM. A turbo electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized in Table 1. Data processing was performed on Analyst 1.4.1 software package (SCIEX).

**Sample preparation.** A plasma sample (0.5 mL) was transferred to a 15 mL glass test tube, then 50 μL of IS working solution (20 ng/mL) and 50 μL of sodium hydroxide (0.1 M) were added. After vortex mixing for 10 s, 4 mL aliquot of the extraction mixture, diethyl ether–dichloromethane (7:3, v/v), was added using Dispensette Organic (Brand GmbH, Postfach, Germany). The sample was vortex-mixed for 3 min using vortexer. The organic layer (3 mL) was transferred to a 5 mL glass tube and evaporated to dryness using an evaporator at 40°C under a stream of nitrogen. Then the dried extract was reconstituted in 250 μL of water–methanol (50:50, v/v; diluent) and a 25 μL aliquot was injected into the chromatographic system.

**Bioanalytical method validation.** Standard stock solutions of amlodipine (1 mg/mL) and the IS (1 mg/mL) were separately prepared in methanol. Working solutions for calibration and controls were prepared by appropriate dilution in water–methanol (50:50, v/v; diluent). The IS working solution (20 ng/mL) was prepared by diluting its stock solution with diluent. Working solutions (0.5 mL) were added to drug-free human plasma (9.5 mL) as a bulk, to obtain amlodipine concentration levels of 50, 100, 200, 500 1000, 2000, 5000 and 10,000 pg/mL as a single batch at each concentration. Quality control (QC) samples were also prepared as a bulk on an independent weighing of standard drug, at concentrations of 50 (LLOQ), 150 (low), 4000 (medium) and 8000 pg/mL (high) as a single batch at each concentration. The calibration

**Table 1. Tandem mass-spectrometer main working parameters**

Parameter	Value
Source temperature, °C	250
Dwell time per transition, ms	200
Ion source gas 1, psi	20
Ion source gas 2, psi	20
Curtain gas, psi	25
Collision gas, psi	5
Ion spray voltage, V	5700
Entrance potential, V	10
Declustering potential, V	42 (Analyte) and 100 (IS)
Collision energy, V	15 (Analyte) and 45 (IS)
Collision cell exit potential, V	10 (Analyte) and 10 (IS)
Mode of analysis	Positive
Ion transition for amlodipine, <i>m/z</i>	409.3 ± 0.5/238.3 ± 0.5
Ion transition for tamsulosin, <i>m/z</i>	409.1 ± 0.5/228.1 ± 0.5

and control bulk samples were divided into aliquots in micro centrifuge tubes (Tarson, 2 mL) and stored in the freezer at below  $-50^{\circ}\text{C}$  until analysis.

A calibration curve was constructed from a blank sample (a plasma sample processed without the IS), a zero sample (a plasma processed with the IS) and eight non-zero samples covering the total range 50–10,000 pg/mL, including the LLOQ. The calibration curves were generated using the analyte to IS peak area ratios by weighted ( $1/x^2$ ) least-squares linear regression on five consecutive days. The acceptance criterion for a calibration curve was a correlation coefficient (*r*) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the nominal value except at the LLOQ, for which the maximum acceptable deviation was set at 20%. At least 67% of non-zero standards were required to meet the above criteria, including acceptable LLOQ and upper limit of quantification.

The within-batch precision and accuracy were determined by analyzing five sets of QC samples in a batch. The between-batch precision and accuracy were determined by analyzing five sets of QC samples on three different batches. The QC samples were randomized daily, processed and analyzed in a position (a) immediately following the standard curve, (b) in the middle of the batch or (c) at the end of the batch. The acceptance criteria for within- and between-batch precision were 20% or better for LLOQ and 15% or better for the other concentrations, and the accuracy was  $100 \pm 20\%$  or better for LLOQ and  $100 \pm 15\%$  or better for the other concentrations.

Recovery of amlodipine from the extraction procedure was determined by a comparison of the peak area of amlodipine in spiked plasma samples (five each of low, medium and high QCs) with the peak area of amlodipine in samples prepared by spiking extracted drug-free plasma samples with the same amounts of amlodipine at the step immediately prior to chromatography. Similarly, recovery of IS was determined by comparing the mean peak areas of extracted QC samples ( $n = 5$ ) to mean peak areas of IS in samples prepared by spiking extracted drug-free plasma samples with the same amounts of IS at the step immediately prior to chromatography.

**Application of the assay to pharmacokinetic study in healthy subjects.** To demonstrate the reliability of this assay

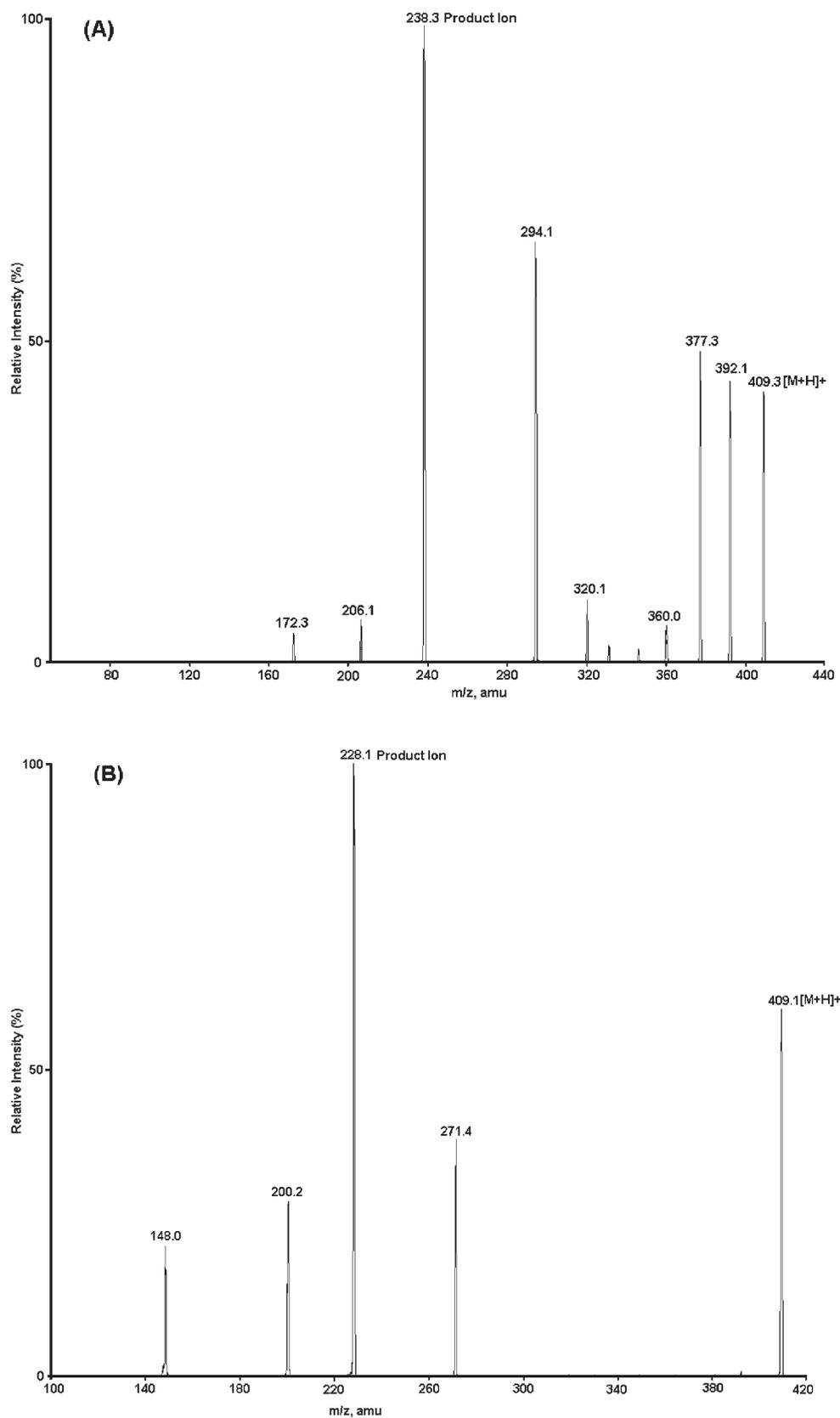
for the study of pharmacokinetics of amlodipine, it was used to determine amlodipine concentrations in plasma samples 0–192 h after administration of 2.5 mg amlodipine to 18 healthy subjects in a pharmacokinetic study approved by the Ethics Committee. The age of 18 male healthy subjects ranged from 18 to 27 years ( $23 \pm 5$  years), and the weight ranged from 57 to 74 kg ( $66 \pm 8$  kg). All subjects gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Serial blood samples (3 mL) were collected from the antecubital vein at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168 and 192 h post-dose. Plasma was separated by centrifugation at 2000 *g* for 10 min and stored frozen at below  $-50^{\circ}\text{C}$  until analysis.

Pharmacokinetic parameters were determined from the plasma concentration–time data. The elimination half-life ( $t_{1/2}$ ) was calculated with non-compartmental model of WinNonlin 4.0.1 software. The area under the plasma concentration–time curve from time zero to the last measurable plasma concentration point ( $t = 192$  h;  $\text{AUC}_{0-t}$ ) was calculated by the linear trapezoidal method. Extrapolation to time infinity ( $\text{AUC}_{0-\infty}$ ) was calculated as follows:  $\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + Ct/k_e$ , where *Ct* is the last measurable plasma concentration and  $k_e$  is the elimination rate constant.

## RESULTS AND DISCUSSION

### Mass spectrometry

In order to develop a method with the desired LLOQ (50 pg/mL), it was necessary to use MS-MS detection, as MS-MS methods provide improved limit of detection for trace-mixture analysis (Jemal, 2000). The inherent selectivity of MS-MS detection was also expected to be beneficial in developing a selective and sensitive method. The product ion mass spectra of amlodipine and the IS are shown in Fig. 2.  $[\text{M}+\text{H}]^+$  was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain product ion spectra. The most suitable collision energy was determined by observing the response obtained for the fragment ion peak *m/z*. When the collision energy was 15 V, the



**Figure 2.** Full scan positive ion turboionspray product ion mass spectra and the proposed patterns of fragmentation of (A) amlodipine and (B) tamsulosin (internal standard). The protonated molecules were used as precursor ions for MS/MS.

product ion mass spectra show the ions  $m/z$  238 and 294 from the protonated molecular ion at  $m/z$  409 (Fig. 2). It was demonstrated that, when the ion  $m/z$  294 was generated from the amlodipine molecule by applying an additional voltage in the API source, the product ion spectrum of  $m/z$  294 did not show the ion  $m/z$  238. This indicates that two different routes of fragmentation leading to different fragments exist (Yasuda *et al.*, 1996). Since the ratio of the amounts of the ions of  $m/z$  238–294 was about 1.5 under the LC-MS/MS conditions, the MS/MS transition 409–238 was selected. The most sensitive mass transition was from  $m/z$  409 to  $m/z$  238 for amlodipine and  $m/z$  409 to  $m/z$  228 for the IS.

LC-MRM is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. Thus, the MRM technique was chosen for the assay development. The MRM state file parameters were optimized to maximize the response for the analyte. The parameters presented in Table 1 are the result of this optimization.

### Method development

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and IS, as well as a short run time. It was found that a mixture of water–acetonitrile–formic acid (30:70:0.03, v/v/v) could achieve this purpose and was finally adopted as the mobile phase. The formic acid was found to be necessary in order to lower the pH to protonate the amlodipine and thus deliver good peak shape. The percentage of formic acid was optimized to maintain this peak shape while being consistent with good ionization and fragmentation in the mass spectrometer. The high proportion of organic solvent eluted both the analyte and the IS at retention time of 0.95 min. A flow rate of 0.8 mL/min produced good peak shapes and permitted a run time of 1.5 min.

Liquid–liquid extraction (LLE) was used for the sample preparation in this work. LLE can be helpful in producing a spectroscopically clean sample and avoiding the introduction of non-volatile materials onto the column and MS system. Clean samples are essential for minimizing ion suppression and matrix effect in LC-MS/MS analyses. Six organic solvents, diethyl ether, ethyl acetate, hexane, dichloromethane, chloroform and butyl-*tert*-methyl ether, and their mixtures in different combinations and ratios were evaluated. Finally, a mixture of diethyl ether and dichloromethane (7:3, v/v) was found to be optimal, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analyte from the plasma.

For an LC-MS/MS analysis, utilization of stable isotope-labeled drugs as internal standards proves to be helpful when a significant matrix effect is possible. Isotope labeled analyte was not obtainable to serve as IS, so, in the initial stages of this work, several compounds were investigated to find a suitable IS, and finally tamsulosin was found to be best for the present purpose. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant retention times were observed. However, in ESI, signal suppression or enhancement may occur due to co-eluting endogenous components of the sample matrix. These potential matrix effects were evaluated by spiking blank plasma extracts (after LLE treatment as described above) at the low and high QC levels. The resulting chromatograms were compared with those obtained for clean standard solutions at the same concentrations. Five independent plasma lots were used, with five samples from each lot. The results (data not shown) showed that there was no significant difference between peak responses for spiked plasma extracts and clean solutions. This result most likely reflects the efficacy of the sample clean-up with LLE. In any event, the use of matrix-matched calibration standards would have minimized any such effects on the quantification.

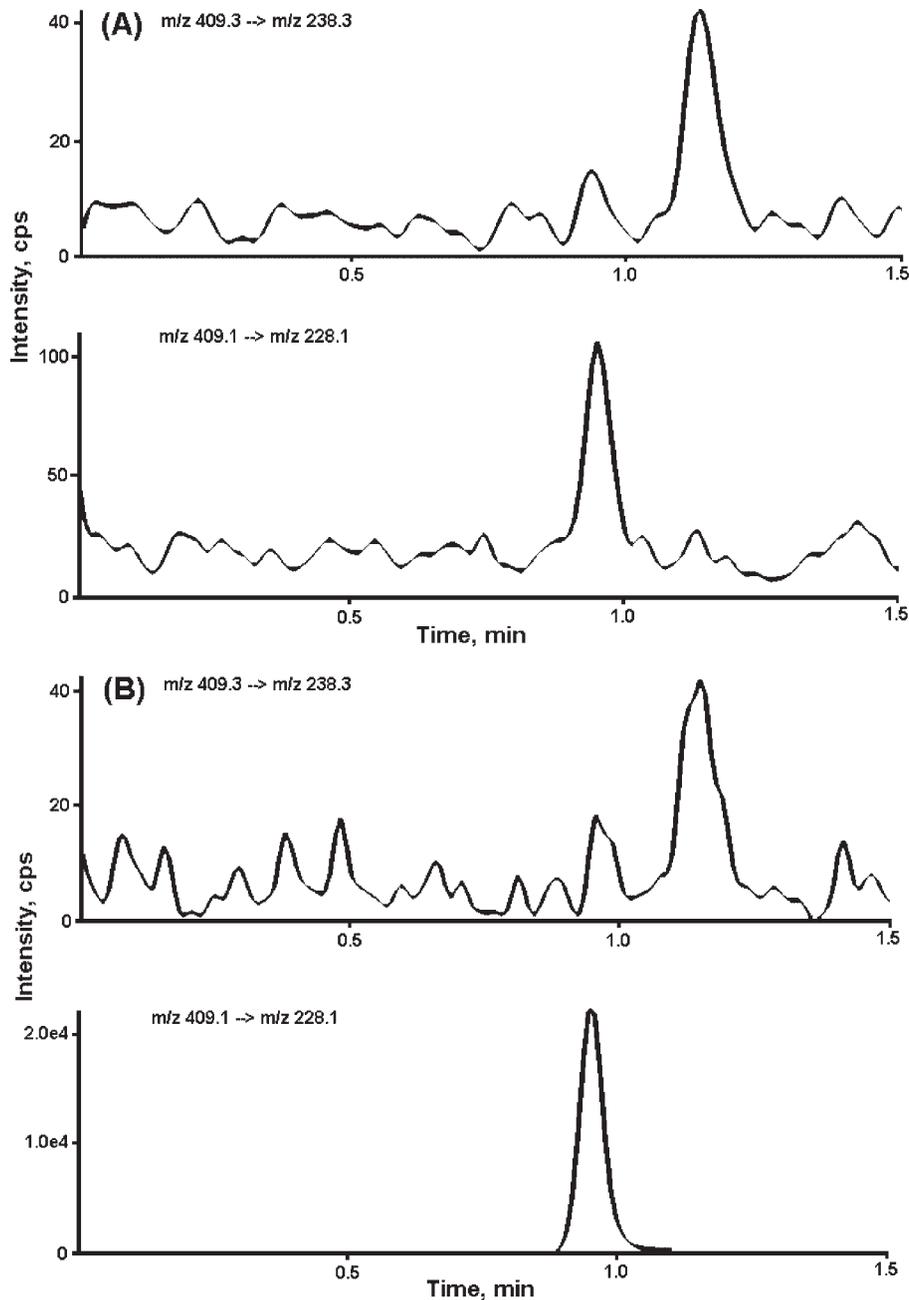
### Assay performance and validation

The eight-point calibration curve was linear over the concentration range 50–10,000 pg/mL. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ( $1/x$ ,  $1/x^2$  and  $1/\sqrt{x}$ ). The best linear fit and least-squares residuals for the calibration curve were achieved with a  $1/x^2$  weighing factor, giving a mean linear regression equation for the calibration curve of:  $y = 0.0006 (\pm 0.0007) x + 0.0022 (\pm 0.0015)$  where  $y$  is the peak area ratio of the analyte to the IS and  $x$  is the concentration of the analyte. The mean correlation coefficient of the weighted calibration curve generated during the validation was 0.999; Table 2 summarizes the calibration curve results.

The selectivity of the method was examined by analyzing ( $n = 5$ ) blank human plasma extract [Fig. 3(A)] and an extract spiked only with the IS [Fig. 3(B)]. As shown in Fig. 3(A), no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free human plasma at the retention time of the analyte. Similarly, Fig. 3(B) shows the absence of direct interference from the IS to the MRM channel of the analyte. Figure 3(C) depicts a representative ion-chromatogram for the LLOQ (50 pg/mL). Excellent sensitivity was observed for a 25- $\mu$ L injection volume; the LLOQ corresponds to ca. 1.25 pg on-column.

**Table 2. Precision and accuracy data of back-calculated concentrations of calibration samples for amlodipine in human plasma**

Concentration added (pg/mL)	Concentration found (mean $\pm$ SD, $n = 5$ ; pg/mL)	Precision (%)	Accuracy (%)
50	52.3 $\pm$ 2.2	4.3	103.4
100	100.7 $\pm$ 4.4	4.4	99.5
200	205.1 $\pm$ 8.0	3.9	101.4
500	500.6 $\pm$ 16.1	3.2	99.0
1000	1008.9 $\pm$ 26.2	2.5	99.7
2000	1928.6 $\pm$ 112.9	5.8	95.3
5000	5101.8 $\pm$ 175.3	3.4	100.8
10,000	10,165.7 $\pm$ 159.7	1.5	100.5

**Figure 3.** MRM chromatograms for amlodipine and the IS resulting from analysis of: (A) blank (drug and IS free) human plasma; (B) blank (drug-free spiked with IS) human plasma; (C) 50 pg/mL (LLOQ) of amlodipine spiked with the IS.

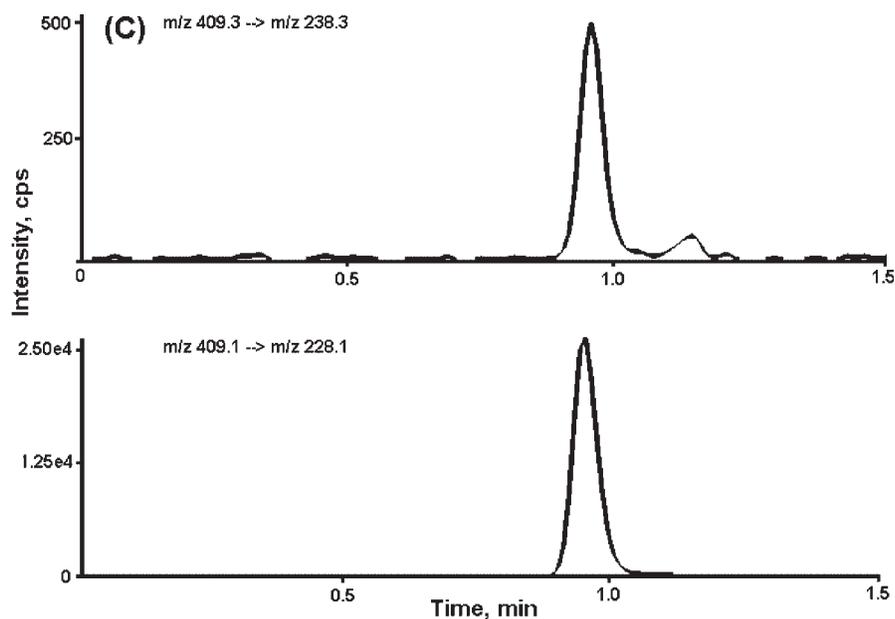


Figure 3. (Continued)

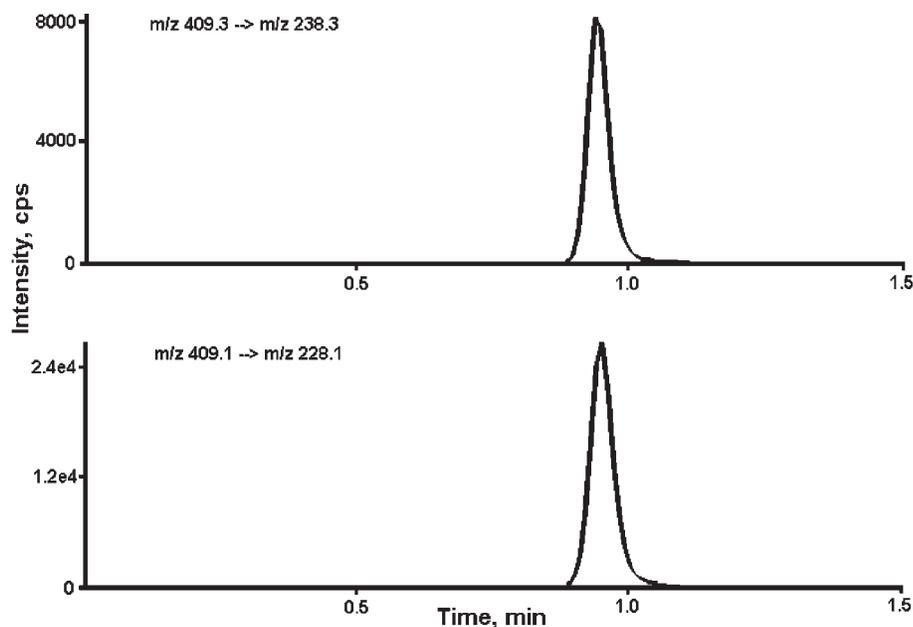


Figure 4. MRM chromatograms resulting from the analysis of a subject plasma sample after the administration of a 2.5 mg oral single dose of amlodipine. The sample concentration was determined to be 1009 pg/mL.

The MRM chromatograms obtained for an extracted plasma sample of a healthy subject who participated in a bioequivalence study conducted on 18 subjects are depicted in Fig. 4. Amlodipine was identified and was quantified as 1009 pg/mL.

The LLOQ was defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision, and was found to be

50 pg/mL in human plasma. The mean response for the analyte peak at the assay sensitivity limit (50 pg/mL) was ~12-fold greater than the mean response for the peak in five blank human plasma samples at the retention time of the analyte. The between-batch precision at the LLOQ was 3.8%, and the between-batch accuracy was 99.8% (Table 3). The within-batch precision was 7.2% and the accuracy was 102.2% for amlodipine.

**Table 3. Precision and accuracy of the method for determining amlodipine concentrations in plasma samples**

Concentration added (pg/mL)	Within-batch precision ( $n = 5$ )			Between-batch precision ( $n = 3$ )		
	Concentration found (mean $\pm$ SD; pg/mL)	Precision (%)	Accuracy (%)	Concentration found (mean $\pm$ SD; pg/mL)	Precision (%)	Accuracy (%)
50	51.6 $\pm$ 3.7	7.2	102.2	50.4 $\pm$ 1.9	3.8	99.8
150	149.6 $\pm$ 5.8	3.9	98.6	150.9 $\pm$ 10.5	6.9	99.5
4000	4102.8 $\pm$ 68.5	1.6	101.4	4084.3 $\pm$ 205.3	5.0	100.9
8000	8216.6 $\pm$ 81.8	0.9	101.5	8234.7 $\pm$ 339.5	4.1	101.7

**Table 4. Absolute recoveries of amlodipine from human plasma**

Sample concentration (pg/mL)	Absolute recovery (%; mean $\pm$ SD, $n = 5$ )
150	74.5 $\pm$ 2.1
4000	70.3 $\pm$ 3.8
8000	79.4 $\pm$ 2.5

The middle and upper quantification levels of amlodipine ranged from 150 to 8000 pg/mL in human plasma. For the between-batch experiments the precision ranged from 4.1 to 6.9% and the accuracy from 99.5 to 101.9% (Table 3). For the within-batch experiments the precision and accuracy for the analyte met the acceptance criteria ( $<\pm 15\%$ ).

The average absolute recoveries for amlodipine at three different concentrations (low, medium and high QC samples) are shown in Table 4. The recovery of the analyte was high (74.7  $\pm$  4.6%). The recovery of the IS was 72.1  $\pm$  2.0% at the concentration used in the assay (20 ng/mL). The recovery of the analyte and the IS was high and also the extent of recovery of the analyte and IS was consistent, precise and reproducible. Therefore the assay has proved to be robust in high throughput bioanalysis.

### Stability studies

The stability of the analyte and IS in human plasma under different temperature and timing conditions, as well as their stability in the stock solutions, was evaluated as follows. QC samples were subjected to short-term room temperature conditions, to long-term storage conditions ( $-50^\circ\text{C}$ ), and to freeze–thaw stability studies. All the stability studies were conducted at two concentration levels (150 and 8000 pg/mL as low and high values) with five determinations for each.

For short-term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of time exceeding that expected to be encountered during routine sample preparation (around 24 h). Samples were extracted and analyzed as described above, and the results are given in Table 5. These results indicate reliable stability behavior under the experimental conditions of the regular analytical procedure. The stability of QC samples kept in the autosampler for 24 h was also assessed. The results indicate that solutions of amlodipine and the IS can remain in the autosampler for at least 24 h without showing significant loss in the quantified values, indicating that samples should be processed within this period of time (Table 5).

The data representing the stability of amlodipine in plasma at two QC levels over three freeze–thaw

**Table 5. Stability of amlodipine in human plasma**

Sample concentration (pg/mL; $n = 5$ )	Concentration found (pg/mL)	Precision (%)	Accuracy (%)
<i>Short-term stability for 24 h in plasma</i>			
150	154	4.2	102.6
8000	7893	3.4	98.6
<i>Three freeze–thaw cycles</i>			
150	146	3.5	97.3
8000	8149	3.9	101.8
<i>Autosampler stability for 24 h</i>			
150	149	5.1	99.3
8000	7968	2.8	99.6
<i>Stability for 21 days at <math>&lt;-50^\circ\text{C}</math></i>			
150	144	4.8	96.0
8000	7928	3.4	99.1

cycles are given in Table 5. These tests indicate that the analyte is stable in human plasma for three freeze-thaw cycles, when stored at below  $-50^{\circ}\text{C}$  and thawed to room temperature.

Table 5 also summarizes the long-term stability data for amlodipine in plasma samples stored for a period of 21 days at below  $-50^{\circ}\text{C}$ . The stability study of amlodipine in human plasma showed reliable stability behavior, as the mean of the results of the tested samples were within the acceptance criteria of  $\pm 15\%$  of the initial values of the controls. These findings indicate that storage of amlodipine in plasma samples at below  $-50^{\circ}\text{C}$  is adequate, and no stability-related problems would be expected during routine analyses for pharmacokinetic, bioavailability or bioequivalence studies.

The stability of the stock solutions was tested and established at room temperature for 2 h, 24 h, and under refrigeration ( $\sim 4^{\circ}\text{C}$ ) for 6 months. The recoveries for amlodipine and tamsulosin were 101.5 [coefficient of variance (CV) 1.6%], 103.1 (CV 1.6%), 104.4 (CV 0.8%) and 100.4 (CV 1.9%), 104.1 (CV 0.5%) and 101.1 (CV 2.3%) respectively. The results revealed optimum stability for the prepared stock solutions throughout the period intended for their daily use.

### Pharmacokinetic study

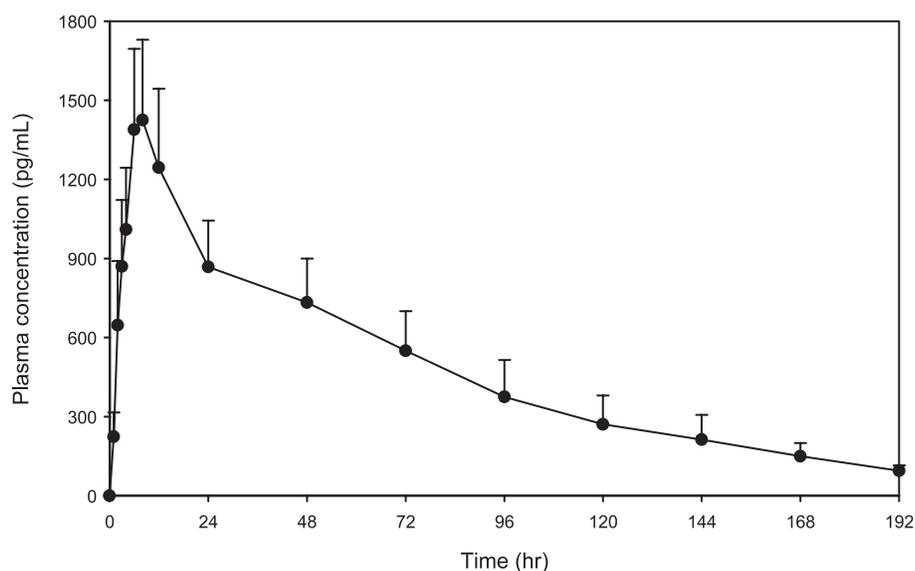
The method was applied to determine the plasma concentration of amlodipine following a single oral administration (2.5 mg) to 18 healthy subjects. Mean plasma concentration-time profiles of amlodipine are presented in Fig. 5. The main pharmacokinetic parameters of amlodipine in 18 subjects were calculated.

After oral administration of 2.5 mg amlodipine,  $T_{\max}$  and  $C_{\max}$  values were found to be  $8.1 \pm 2.1$  h and  $1425 \pm 244.1$  pg/mL, respectively. Plasma concentrations declined with  $t_{1/2}$  of  $50.12 \pm 4.9$  h. The elimination rate constant ( $k_e$ ) was calculated as  $0.014 \pm 0.002$  h $^{-1}$ . The  $\text{AUC}_{0-t}$  and  $\text{AUC}_{0-\infty}$  values obtained were  $91,423.3 \pm 20,206.4$  and  $98,292.7 \pm 22,269.5$  pg/mL, respectively.

### CONCLUSIONS

In summary, the method is described for the quantification of amlodipine in human plasma by LC-MS/MS in positive electrospray ionization mode using multiple reaction monitoring and fully validated according to commonly accepted criteria (Shah *et al.*, 1991). This method offers significant advantages over those previously reported, in terms of improved sensitivity and selectivity, faster run time (1.5 min) and rapid extraction. The current method has shown acceptable precision and adequate sensitivity for the quantification of amlodipine in human plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies. The desired sensitivity of amlodipine was achieved with an LLOQ of 50 pg/mL, which has a within- and between-batch CV of 7.2 and 3.8%, respectively. Many variables related to the electrospray reproducibility were optimized for both precision and sensitivity to obtain these results.

Amlodipine was shown to be stable in routine analysis conditions and in human plasma for up to 21 days when stored at below  $-50^{\circ}\text{C}$ . The cost-effectiveness, simplicity of the assay, using rapid liquid-liquid extraction and sample turnover rate of 1.5 min per sample,



**Figure 5.** Representative data showing plasma concentration-time profiles of six healthy subjects after the administration of an oral single dose of 2.5 mg of amlodipine.



make it an attractive procedure in high-throughput bioanalysis of amlodipine. The validated method allows quantification of amlodipine in the 50–10,000 pg/mL range.

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