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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AMLODIPINE IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY/MASS SPECTROMETRY

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ABSTRACT

Objective: The objective of the present investigation was to develop a novel, simple, and economic method for the estimation of amlodipine in positive ion mode in human plasma using amlodipine maleate d4 as an internal standard.

Methods: The chromatographic separation was performed on Zorbax SB, C_{18}, 50 mm*4.6 mm, and 3.5 mm. The mobile phase was prepared with a mixture of 5 mm ammonium acetate in 0.1% formic acid: High performance liquid chromatographic (HPLC) grade methanol:HPLC grade acetonitrile (40:30:30) that run isocratically at the flow rate of 0.700 ml/min and run time at 2.50 min.

Results: The analytical method is valid for the estimation of amlodipine, in human plasma over a range of 0.100 ng/ml–9.990 ng/ml with the detection of amlodipine m/z - 409.10 (parent) and 238.00 (product), and internal standard Amlodipine Maleate d4 m/z - 413.20 (parent), and 238.00 (product) in positive ion mode. The results of carryover test, matrix effect, linearity, precision and accuracy, stabilities, dilution integrity, and run size evaluation test presented in this report are within the acceptance range.

Conclusion: A sensitive method for the separation and determination of amlodipine in plasma has been developed based on solid-phase extraction with disposable extraction cartridges in combination with LC and mass spectrophotometers (MS/MS).

Keywords: Amlodipine, Amlodipine maleate d4, Liquid chromatography-mass spectrophotometers/mass spectrophotometers, Human plasma, Validation, Stability studies.

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INTRODUCTION

Amlodipine is a three-ethly-5-methly-2-[(2-aminoethoxy) methyl]-four-(2-chlorophenyl)6-methyl,1,4-dihydro pyridine-3,five- dicarboxylate [1]. It is a calcium channel blocker that dilates (widens) blood vessels and improves blood waft. It's so far used to treat chest pain (angina) and different situations due to coronary artery illnesses. It is also used to treat excessive blood pressure. It is also used alone or together with other drug treatments to treat chest pain and high blood pressure. It affects the movement of calcium into the cells of the heart and blood vessels as amlodipine is a calcium antagonists it reduces coronary and peripheral vascular resistance, decreases blood pressure and myocardial oxygen intake. Amlodipine is norm frequent (with everyday heart rate) and has 24 h long impact. Chemical formulation of amlodipine is $C_{20}H_{25}ClN_2O_5$, molecular weight is 567.1, and it is barely soluble in water and sparingly soluble in ethanol (Fig. 1) [5].

Amlodipine is a dihydropyridine derivative calcium antagonist (Calcium ion antagonist or slow channel blocker) that inhibits the transmembrane inflow of calcium ions into vascular smooth muscle and cardiac muscle. After oral management of therapeutic doses of amlodipine, absorption produces height plasma concentrations between 6 and 12 h. Amlodipine is considerably (approximately 90%) transformed to inactive metabolites through hepatic metabolism with 10% of the determined compound and 60% of the metabolites excreted in the urine. Amlodipine is slowly and nearly absolutely absorbed from the gastrointestinal tract [6]. Peak plasma attention is reached 6–12 h following oral management. Its predicted bio-availability is 64–90%. Absorption is not stricken by meals.

Inner standard decided on for the estimation of amlodipine is amlodipine maleate d4 (Fig. 2).

METHODS

Amlodipine and Amlodipine maleate d4 have been procured from Vivan Life Technology Pvt. Ltd., acetonitrile (high performance liquid chromatographic [HPLC] grade), ammonium acetate and formic acid were obtained from Merck and methanol (HPLC grade) is from JT Baker. The water was purified using Milli-Q device (Rankem).

Calibration curve requirements and quality control samples

Calibration curve trendy together with a set of 9 non-zero concentrations ranging from 0.100 ng/ml to 9.990 ng/ml of amlodipine were organized. Organized high-quality manage samples consisted of concentrations of 0.101 ng/ml (lloq quality control), 0.287 ng/ml (lqc), 1.198 ng/ml (mqc1), 4.991 ng/ml (mqc2), and 7.799 ng/ml (hqc) for amlodipine. Those samples have been saved at −70°C until use.

Optimized bioanalytical conditions (Table 1)

Sample preparation

The samples were thawed at room temperature and vortexed to make certain entire blending of the contents. 300 µl of the plasma sample was pipetted 5 ml polypropylene ria vial tubes, 30 µl of internal standard dilution (49.985 ng/ml of amlodipine maleate d4) became delivered to it and vortexed, besides in blank plasma samples where 30µl diluent was introduced and vortexed. Then, 500 µl of HPLC grade water turned into introduced and vortex. The sample aggregate was loaded onto strata ×33 µm polymeric sorbent (30 mg/1 cc) cartridges that have been pre-conditioned with 1.0 ml of HPLC grade methanol followed through 1.0 ml Milli-Q/HPLC grade water (new cartridge for each pattern). After making use of the most pressure, the extraction cartridge becomes washed with 1 ml of 50 mm ammonium acetate and 2 ml of Milli-Q/HPLC grade water (1 ml every time). Then, the samples

were eluted with 2 ml of methanol and evaporated to dryness at 45°C beneath gentle movement of nitrogen and the dried extract changed into reconstituted with 300 µl of the cellular segment. The samples had been transferred to sampler loading vials (ambered coloration) and loaded.

Validation

Method validation consists of all of the techniques required to demonstrate that a method to quantify the attention of amlodipine in plasma is dependable for the intended software.

Carry overcheck

Certainly organized extracted matric clean (cot clean), excessive (cot uloq), and low (lloq) attention of analyte while injected, the effects display that there is no deliver over the impact of the analyte and internal preferred. Fig. 3 is the representative chromatogram of extracted blank plasma sample analyzed for carryover test.

Accuracy and precision (Tables 2-4)

Inside-batch, intraday, and between-run/interday precision and accuracy have been evaluated at three nice control samples concentrations (lqc, mqc1, mqc2, and hqc). Inside-batch, intraday, and between-run/interday assay precision was determined as coefficient of variation %, and within-batch, intraday, and between-run/interdayassay accuracies have been expressed as percent nominal.

Linearity

Use a regression equation with the perfect weighting thing for determining the detector reaction/concentration courting. Encompass trendy and widespread in training of calibration curve. Fig. 4 suggests representative calibration curve for regression evaluation of amlodipine.

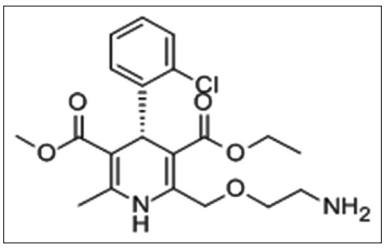

Fig. 1: Structure of amlodipine [1]

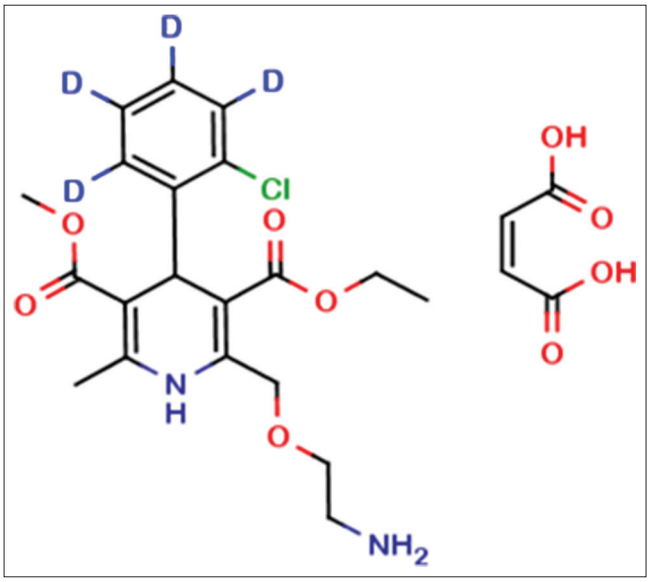

Fig. 2: Structure of amlodipine maleate d4 [10]

Sensitivity (Table 5)

Decide the sensitivity in phrases of lloq in which the reaction of the lloq need to be at least 5 times greater than the reaction of interference in a clean matrix on the retention time or mass transitions of the analyte(s).

Dilution integrity (Table 6)

Put together 12 units of qcs spiked with approximately 1.5–1. Eight instances the concentration of the highest preferred (uloq) method six units of above qcs with the aid of diluting them 2 times and another six sets using diluting four instances before extraction by addition of screened blank matrix. Inject these quality control samples along with the calibration curve requirements processed without dilution and calculate the quality control concentrations using multiplication issue as 2 (for 2 times diluted samples) and four (for 4 times diluted samples).

Stabilities

Freeze and thaw stability (Table 7)

The samples have been saved at −20°C and thawed at room temperature (≤27°C). The stability of quality controls concentrations was envisioned after three cycles of freezing and thawing of samples. The samples have been analyzed underneath a fresh calibration curve and the concentrations obtained have been as compared with the nominal attention of exceptional manage samples.

Re-injection stability (Table 8)

In having access to the reinjection balance, six sets of quality control samples (lqc and hqc) had been processed and analyzed with calibration curve general. The qc samples have been retained within the vehicle sampler and re-injected after a period of 46 h 30 min and quantified in opposition to the preliminary calibration curve statistics. The mean concentration of re-injected qcs becomes in comparison against the imply of the qcs while injected for 1st time.

Wet extract stability (Table 9)

Moist extract balance is finished to evaluate the integrity of analyze samples which had been kept at room temperature over a time period after processing. Good enough wide variety of low and excessive quality control samples (6 replicates) to facilitate injection at proposed balance durations. Maintain the processed samples in vehicle sampler at room temperature/special temperature. At the day of balance, put together sparkling trendy inventory solution of analyte(s). Inject quality controls samples in replicates (6 samples each) in conjunction with the freshly spiked calibration standards of awareness variety equal to that used for the calculation of precision and accuracy. Calculate the autosampler stability intervals at the time of injection of first quality control; much less the sample training completed time.

Autosampler stability (Table 10)

Autosampler stability of the analyte and inner widespread is done at a selected temperature for the time frame relying at the anticipated run time for the whole evaluation of a batch length. Number of low and excessive quality controls samples (6 replicates) is facilitated to inject at proposed stability durations, hold the processed samples at room temperature. On the day of stability, inject qc samples in replicates (6 samples every) along with the freshly spiked calibration requirements of concentration range equal to that used for the calculation of precision and accuracy.

RESULTS

Carryover test
Precision and accuracy
Linearity
Sensitivity
Dilution integrity
Stabilities

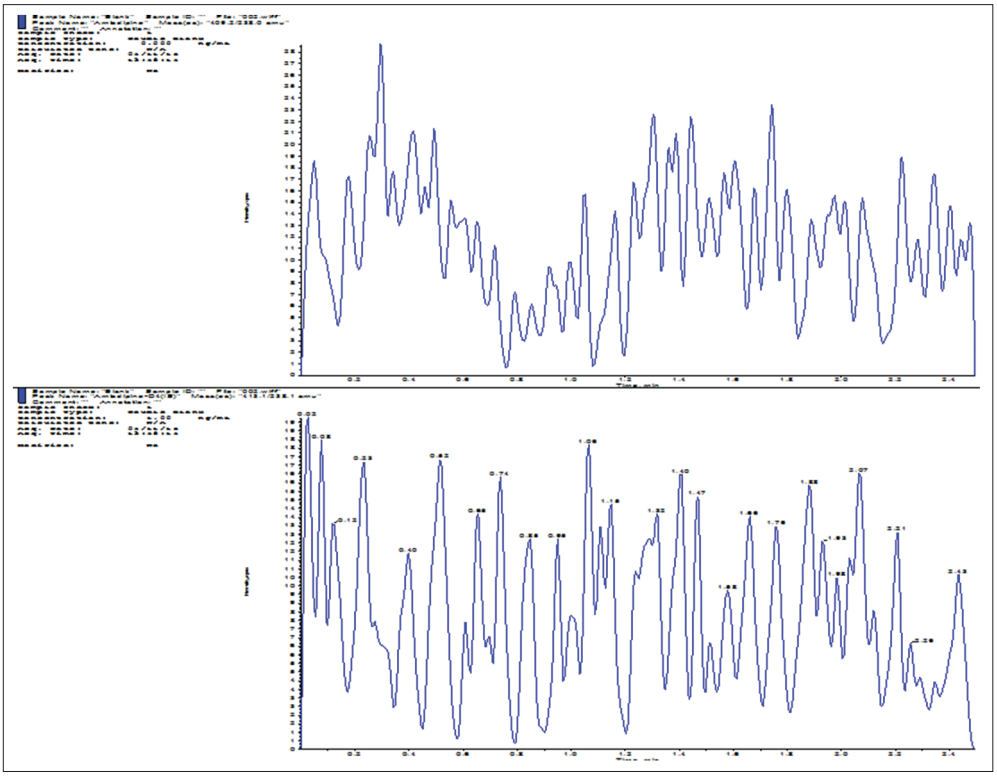

Fig. 3: Representative chromatogram of blank plasma sample of amlodipine

Table 1: Optimized chromatographic conditions

Chromatographic conditions	
Column	Zorbax SB, C18, 50 mm*4.6 mm, and 3.5 µm
Mobile phase	5 mm ammonium acetate in 0.1% formic acid: methanol: acetonitrile (30:30:40)
Rinsing solution	Acetonitrile: Milli-Q water (60:40)
Flow rate	0.7 mL/min
Sampler cooler temperature	10°C
Injection volume	20 µL
Split ratio	50:50
Needle rinsing volume	1000 µL
Retention times	1.35±0.5 (amlodipine) 1.35±0.5 (amlodipine maleate d4)
Run time	2.50 min

Table 2: Within, batch precision and accuracy for analysis of amlodipine in human plasma

Nominal concentration	Within batch		
ng/ml	Mean±SD	Accuracy (%)	CV (%)
LQC (0.28 ng/ml)	0.26±0.01	91.35	7.61
MQC1 (1.1 ng/ml)	1.11±0.007	93.36	0.69
MQC2 (4.9 ng/ml)	4.97±0.05	99.65	1.01
HQC (7.7 ng/ml)	7.37±0.11	94.62	1.55

SD: Standard deviation, CV: Coefficient of variation

DISCUSSIONS

The chromatographic method was optimized by changing various parameters, such as PH of the mobile phase, organic modifier, and buffer used in the mobile phase. Under the presently prescribed conditions, it was found that there was no carryover test in the proposed method. Representative chromatogram is shown in Fig. 3. Hence, this method is very useful for determination of amlodipine in the pharmaceutical dosage form. It was also found that the differences of <5.0% for within, intra- and inter-day data reflect the precision of the method. The observation of percentage CV <10 for within, intra- and inter-day measurements also indicates a high degree of precision. The results of accuracy and precision are shown in Tables 2-4. In this study, the developed chromatographic method the linearity ranges from 0.100 ηg/ml to 9.990 ηg/ml. The regression curve of amlodipine is shown

Table 3: Intraday precision and accuracy for analysis of amlodipine in human plasma

Nominal concentration	Intra batch		
ng/ml	Mean±SD	Accuracy (%)	CV (%)
LQC (0.28 ng/ml)	0.28±0.02	100.03	10.35
MQC1 (1.1 ng/ml)	1.11±0.01	93.12	1.00
MQC2 (4.9 ng/ml)	4.96±0.04	99.39	0.86
HQC (7.7 ng/ml)	7.36±0.08	94.44	1.21

SD: Standard deviation, CV: Coefficient of variation

Table 4: Interday precision and accuracy for analysis of amlodipine in human plasma

Nominal concentration	Inter-batch		
ng/ml	Mean±SD	Accuracy (%)	CV (%)
LQC (0.28 ng/ml)	0.29±0.02	103.79	6.95
MQC1 (1.1 ng/ml)	1.11±0.01	93.03	1.16
MQC2 (4.9 ng/ml)	4.95±0.04	99.19	0.86
HQC (7.7 ng/ml)	7.35±0.08	94.26	1.12

SD: Standard deviation, CV: Coefficient of variation

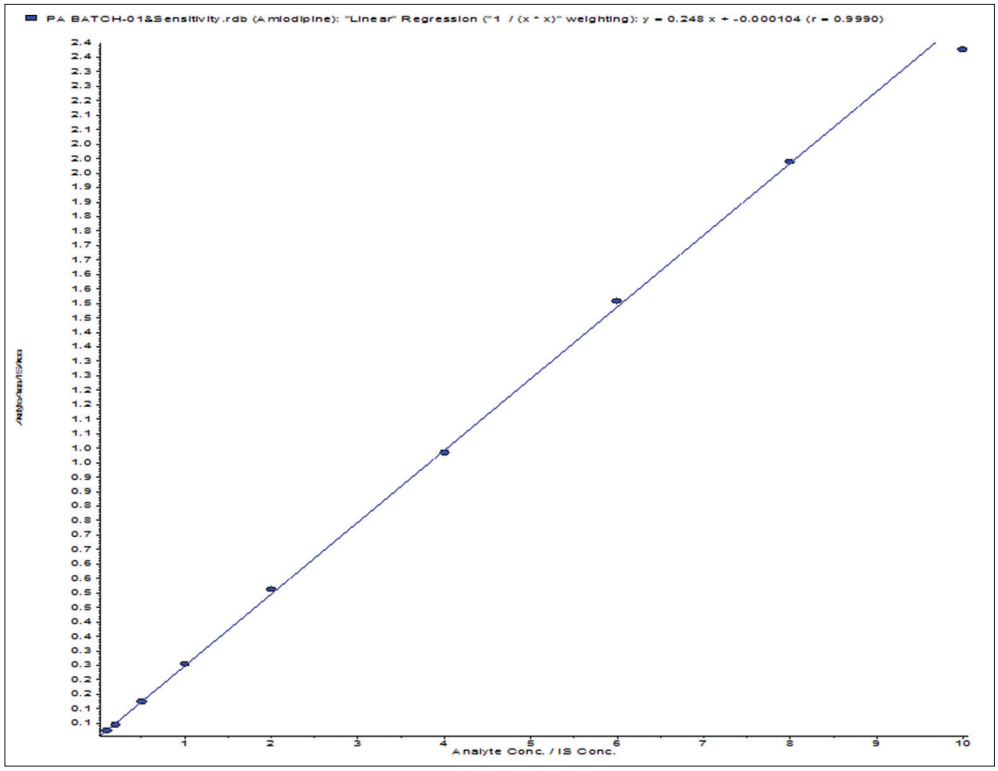

Fig. 4: A representative calibration curve for regression analysis of amlodipine

Table 5: Within batch precision and accuracy for the sensitivity of amlodipine

QC level	Mean±SD	% CV	% Accuracy
LLOQ	0.099±0.004	4.13	99.83

SD: Standard deviation, CV: Coefficient of variation

Table 6: Dilution integrity data for amlodipine (20 times and 4 times)

Dilution factor	Mean±SD	% CV	% Accuracy
½	17.0±0.06	0.04	105.67
¼	34.1±0.19	0.57	105.81

SD: Standard deviation, CV: Coefficient of variation

Table 7: Stability data for free-thaw stability

Analyte (amlodipine)	LQC (%)		HQC (%)	
	Nominal	CV	Nominal	CV
Freeze-thaw stability	107.74	1.48	96.14	0.86

CV: Coefficient of variation

Table 8: Stability data for re-injection stability

Analyte (amlodipine)	LQC (%)		HQC (%)	
	Nominal	CV	Nominal	CV
Re-injection stability (46 h–30 min)	107.61	2.29	94.46	0.46

CV: Coefficient of variation

Table 9: Stability data for wet extract stability

Analyte (amlodipine)	LQC (%)		HQC	
	Nominal	CV	Nominal	CV
Wet extract stability (69 h–50 min)	105.92	2.12	98.87%	1.02

CV: Coefficient of variation

Table 10: Stability data for autosampler stability

Analyte (amlodipine)	LQC (%)		HQC (%)	
	Nominal	CV	Nominal	CV
Autosampler stability (74 h–25 min)	105.05	1.25	94.44	2.70

in Fig. 4. The sensitivity results gave good recovery and results are shown in Table 5 due to high sensitivity, the method was used to detect the drug in different stabilities studies. The different stabilities were performed at different suitable conditions, and their results were within acceptance criteria, and their results are represented in Tables 7-10.

CONCLUSION

In summary, a enormously, unique, reproducible, and high-throughput LC-mass spectrophotometers (MS)/MS approach become advanced and tested based on the procedure of solid-phase extraction for quantification of Amlodipine pharmacokinetic and bioequivalence research. The extraction manner and LC-MS/MS conditions have been optimized to be able to enhance the sensitivity and robustness of the technique. In the end, the technique turned into completely tested to fulfill the necessities for sensitivity, accuracy, and precision defined through kingdom food and drug administration and GLP guidelines.

From the optical characteristics, of the proposed method, it changed into determined that the results of sensitivity, carryover test, linearity, precision accuracy, and stabilities provided in this article are in the recognition range, and the analytical approach which is defined above

is legitimate for the estimation of amlodipine, in human plasma over various 0.100 ŋg/ml–9.990 ŋg/ml with the detection of amlodipine m/z-409.10 (parent) and 238.00 (product) and internal amlodipine maleate d4 m/z-413.20 (parent) and 238.00 (product) in positive ion mode. A summary of the validation parameters and the effects are provided above.

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AUTHORS' CONTRIBUTION

All the authors contributed equally.

CONFLICTS OF INTEREST

Declared none.

REFERENCES

1. Available from: http://www.wikipedia.org/wiki/Amlodipine.
2. Gohil K, Trivedi P, Molvi KI. Spectrophotometric analysis of amlodipine besylate in bulk and in tablet dosage forms. Indian J Pharm Sci 2005;67:376.
3. Bhatt J, Singh S, Subbaiah G, Shah B, Kambli S, Ameta S. A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the estimation of amlodipine in human plasma. Biomed Chromatogr 2007;21:169-75.
4. Macek J, Klíma J, Ptácek P. A rapid determination of valsorton and amlodipine in human plasma by protein precipitation and high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2006;832:169-72.
5. Rahman N, Azmi SN. 'Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. Farmaco 2001;56:731-5.
6. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies. Farmaco 2005;60:789-92.
7. Rao JR, Kadam SS, Mahadik KR. Methods of estimation of multi-component formulations: A review. Indian Drugs 2002;39:378-81.
8. González L, López JA, Alonso RM, Jiménez RM. Fast screening method for the determination of angiotensinII receptor antagonists in human plasma by high performance liquid chromatography with flourimetric detection'. J Chromatogr A 2002;9499:49-60.
9. Philipp T, Smith TR, Glazer R, Wernsing M, Yen J, Jin J, *et al*. Two multicenter, 8-week, randomized, double-blind, placebo-controlled, parellel-group studies evaluating the efficacy and tolerability of amlodipine and valsarton in combination and as monotherapy in adult patients with mild to moderate essential hypertension. Clin Ther 2007;29:563-80.
10. Available from: https://www.pubchem.ncbi.nlm.nih.gov.