



Method development and validation of HPLC tandem/mass spectrometry for quantification of perindopril arginine and amlodipine besylate combination in bulk and pharmaceutical formulations

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Abstract

A well-characterized and fully validated ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometric (UHPLC-ESI-MS/MS) method was developed to reliably analyze combination of perindopril arginine and amlodipine besylate in bulk and tablet formulations. The chromatographic separation was achieved on a Waters ACQUITY UPLC[®] BEH C18 column with 1.7 μ m particle packing which enabled the higher peak capacity, greater resolution, increased sensitivity, and higher speed of analysis using a volatile mobile phase ideally being at least 2 pH units below and above the perindopril arginine and amlodipine besylate pKa, respectively. Mass spectrometric detection was performed using electrospray ion source in positive ion polarity to profile the abundances of perindopril arginine and amlodipine besylate, using the transitions m/z 369 \rightarrow m/z 172, and m/z 409 \rightarrow m/z 238 for perindopril arginine and amlodipine besylate, respectively. Calibration curve was constructed over the range 0.25 – 500 ng/mL and 1.0 – 100 ng/mL for perindopril arginine and amlodipine besylate, respectively. The method was precise and accurate, and provided recovery rates > 80% for both compounds. Furthermore, the intra- and inter-assay precision in terms of % RSD was in between 0.1 – 3.7 for both perindopril arginine and amlodipine besylate. A specific, accurate, and precise UHPLC-MS/MS method for the determination of perindopril arginine and amlodipine besylate in bulk and tablet formulation.

Keywords: Tandem mass spectrometry; Perindopril; Amlodipine

INTRODUCTION

Perindopril arginine, an angiotensin converting enzyme (ACE) inhibitor, and amlodipine, a dihydropyridine calcium channel blocker, is indicated for the treatment of hypertension, to lower the blood pressure. This new combination medication is indicated for the treatment of arterial hypertension and/or stable coronary heart disease. Such fixed combination of two molecules that have been extensively evaluated according to evidence-based medicine offers the advantage of an excellent efficacy, associated with a good tolerance profile, and favors patient's compliance.

Patients with moderate-to-severe hypertension are at a relatively high risk of cardiovascular events (e.g., stroke, heart attack, and heart failure), kidney failure, and vision problems, so prompt treatment is clinically relevant.

Consider the patient's baseline blood pressure, target goal, and the incremental likelihood of achieving the goal with a combination product such as perindopril arginine and amlodipine besylate versus a monotherapy product when deciding upon initial therapy.

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A comprehensive literature survey revealed that, only a few analytical methods are available for determination of perindopril arginine and amlodipine besylate in bulk drugs and pharmaceuticals like *in vitro* dissolution study using high performance liquid chromatography (HPLC), thin-layer chromatography-densitometry, and stability-indicating reverse phase liquid chromatography (RP-LC) methods (1-6). Methods have also been developed for the estimation of perindopril arginine and amlodipine besylate in combination with other drugs simultaneously in bulk drugs and pharmaceutical formulations and in various biological matrices by using visible spectrophotometry, high performance thin layer chromatography (HPTLC), reverse phase high performance liquid chromatography (RP-HPLC), and capillary gas chromatography techniques (7-8). Further to the best of our knowledge, no sensitive method for determination of the perindopril arginine and amlodipine besylate as combination in bulk drugs or pharmaceuticals has been reported. Thus, there is a need for development of precise and sensitive analytical methods, which will be useful, for therapeutic drug monitoring (TDM) and optimization of laboratory resource utilization. In the present study, a standard analytical technique was developed for the quality detection capability and sensitivity, and also includes accurate measurement and reliable chemical fragmentation, which make the structure elucidations easier.

Most of the earlier methods are not ideal since they are time-consuming, have high limits of detections, use of surplus organic solvents, laborious sample preparation, involve expensive instrumentation and long chromatographic run times (9-12). In recent years, reverse phase ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (RP-UPLC-ESI-MS/MS) studies have emerged in the pharmaceutical field as a very crucial tool based on the reality that, studies has been used for the successful characterization of the *in vitro* behavior of drugs (13). As a result, the RP-UPLC-ESI-MS/MS are used not only to

evaluate batch-to-batch consistency of drug, but also in several crucial stages of formulation development, for screening and proper assessment of different formulations by characterizing the structural identification and information about behavior of drug alterations in the molecular mass of the molecules (14). The main purpose of the present work was to develop and validate a simple RP-UPLC-ESI-MS/MS method to be applied for the quantification perindopril arginine and amlodipine besylate. The developed and validated method is rapid, reproducible with simple mobile phase, trouble free sample preparation steps, improved sensitivity, short chromatographic run time, which therefore serves as a tool for the quality control of pharmaceutical dosage forms as per the ICH guidelines (15).

MATERIALS AND METHODS

Materials

Pure standards of perindopril arginine and amlodipine besylate were obtained from Tablets Pvt. Ltd, India. LC-MS grade organic solvents including acetonitrile, ammonium acetate, formic acid (eluent additive for LC-MS, ~ 98%) and methanol were purchased from Fluka, India. Lercanidipine standard was purchased from Sigma Aldrich, USA. Ultra purified water was obtained from Elix, India. All solvents and samples were filtered through MILLEX FG (Millipore, India), 13 mm, 0.2 mM, Fluoropore, non-sterile membrane sample filter paper before injecting into the system. All the chemicals used were of analytical reagent grade.

Mass spectrometry system set up

An advanced bench top tandem quadrupole mass detector with UHPLC-ESI-MS/MS method was used for the analysis of perindopril and amlodipine. The LC system consisted of the refurbished waters acquity UPLC binary solvent manager, sample manager, single column manager, and photodiode array detector (PDA) with the mass spectrometer (Waters ACQUITY Xevo TQD) equipped with MassLynx software version 4.1 workstation. The system

incorporates IntelliStart technology for automated system optimization and status monitoring, ensuring that the highest quality data is routinely available to all level of operators.

The chromatographic separation was achieved on a Waters ACQUITY UPLC® BEH C18 column (2.1 × 50 mm) with 1.7 µm particle packing which enabled the higher peak capacity, greater resolution, increased sensitivity, and higher speed of analysis using a volatile mobile phase ideally being at least 2 pH units below and above the perindopril arginine and amlodipine besylate pKa, respectively.

The mobile phase A consisting of 20 mM ammonium acetate with 0.1% formic acid and the mobile phase B consisting of acetonitrile: methanol (80:20 v/v) with 0.1% formic acid were used throughout the analysis. The flow rate of the mobile phase was 0.25 mL/min in gradient elution programme. The column oven temperature was kept at 40 °C and the sample injection volume was 5 µL. The Mass spectrometer was operated in the multiple reaction monitoring (MRM) mode. The sample introduction and ionization technique was electrosprayed with positive polarity. The tuning parameters and MRM condition of each individual analyte is summarized in Tables 1 and 2. The retention times of perindopril arginine and amlodipine besylate were observed at 3.0 ± 0.2 and 3.6 ± 0.2 min,

respectively. Lercanidipine was used as internal standard at the retention time of 4.0 ± 0.2 min.

Calibration curve

Appropriate dilutions were made to prepare 100 µg/mL test sample working solution of each dosage form and the pure compound stock solution. Working standard solutions were prepared for the linearity and construction of calibration curves. The concentrations of amlodipine besylate and perindopril arginine were calculated from the corresponding regression equations.

The content of 20 tablets of Coversyl® or Amlip® was separately powdered and mixed well. An amount of each powdered tablets equivalent to 100 mg of drug was accurately and separately weighed and transferred to 100 mL volumetric flask. 75 mL methanol was added and the prepared solutions were magnetically stirred for about 30 min. The solutions were allowed to cool and the volume was then adjusted with methanol to get 1 mg/mL of test sample stock solution.

Validation of the analytical method

The UPLC-MS/MS method was validated according to the International Conference on Harmonization (ICH) guidelines (15). The parameters evaluated were specificity, limits of detection and quantification, linearity, accuracy, precision, and robustness (16).

Table 1. Tuning parameters

Tuning parameters	Values
Source temperature	150 °C
Capillary voltage	3.5 KV
Cone voltage	35 V
Desolvation temperature	500 °C
Desolvation gas flow	800 L/Hr.
Cone gas flow	20 L/Hr.
Ionization mode	ES+
Data	continuum
Scan duration	0.6 sec

Table 2. MRM parameters

Compounds	Parent m/z	Product m/z	Cone (V)	Collision (V)
Perindopril arginine	369.58	172	35	28
Amlodipine besylate	408.97	238	35	18
Lercanidipine	612.79	280	35	16

Specificity

The specificity of the proposed method was proved by the analysis of each fixed dose matrix by comparing the chromatograms of blank and test sample. The response (presence of specific detectable peak) in spiked perindopril arginine and amlodipine besylate in matrix (mobile phase B) and the blank sample matrix (absence of specific peak) were used to assess the specificity of the method.

Limits of detection and quantification

The signal-to-noise ratio method was used to find out the limits of detection (LOD) and the limits of quantification (LOQ). The LOD was estimated at a signal-to-noise of 3:1 and the LOQ was estimated as the peak at a signal-to-noise ratio of at least 10:1. The stock solutions were diluted in mobile phase until the smallest detectable peaks were observed.

Linearity

To validate linearity relationship between concentration and analyte peak area, six concentrations of each analyte were analyzed in triplicate and calibration curve were produced. This study was carried out using serial dilution of stock solution at different concentration ranging from 0.5 - 500 ng/mL (0.5, 100, 200, 300, 400, and 500 ng/mL) and 1.0 - 100 ng/mL (1.0, 10, 25, 50, 75, and 100 ng/mL) for perindopril arginine and amlodipine besylate, respectively which were diluted in mobile phase. The slope, intercept and regression coefficient (r^2) was calculated by weighted $1/x^2$ linear regression for perindopril and amlodipine, respectively using the theoretical concentration vs observed mean concentration ($n = 3$).

Accuracy

Precision and accuracy of the method was checked by determination of pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations. Precision and accuracy for this method was controlled by calculating the intra- and inter-day variations at different concentrations of samples with various replicates.

For intra-day variations, three concentrations of 0.5, 150, and 450 ng/mL for

perindopril and 1.0, 40, and 80 ng/mL for amlodipine for this method were analyzed three times intraday using the proposed methods. For inter-day variations, the previous procedures were repeated interdaily on three different days.

Precision and accuracy for the back calculated concentrations of the calibration points, should be within $\leq 15\%$ and $\leq 5\%$ of their nominal values. However, for lower limit of quantification (LLOQ), the precision and accuracy should be within $\leq 20\%$ and $\pm 20\%$.

Robustness

In this proposed method, during method development phase robustness was evaluated by making small changes in the composition of mobile phase and column temperature in LC compartment, desolvation gas flow and desolvation temperature in source compartment.

RESULTS

Settings

MRM transitions of m/z 409 \rightarrow 238 for amlodipine besylate, m/z 369 \rightarrow 172 for perindopril arginine, and m/z 612 \rightarrow 280 for internal standard with a scan time of 0.6 s per transition (Fig. 1).

Specificity

There were no interfering peaks at the retention time of analyte, which indicate the selectivity of the method (Figs. 2, 3, and 4).

Limits of detection and quantification

The LOD obtained from the signal-to-noise ratio study was 0.15 ng/mL and 0.65 ng/mL for perindopril and amlodipine, respectively. In the signal-to-noise ratio study, the concentration of LOQ was 0.35 ng/mL and 0.85 ng/mL for perindopril and amlodipine, respectively, but considering 0.5 ng/mL as LOQ for perindopril and 1.0 ng/mL for amlodipine, the precision and accuracy of the assay at this concentrations was evaluated.

Linearity

Good linearity was obtained for analytes with the correlation coefficients (r^2) above 0.99 (Table 3).

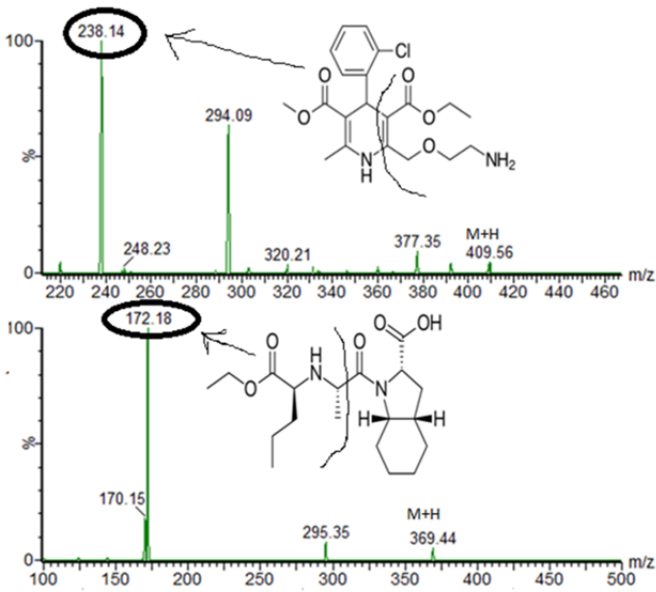


Fig. 1. MRM transitions of amlodipine besylate and perindopril arginine.
MRM transition m/z 409 → 238 of amlodipine besylate and m/z 369 → 172 of perindopril arginine.

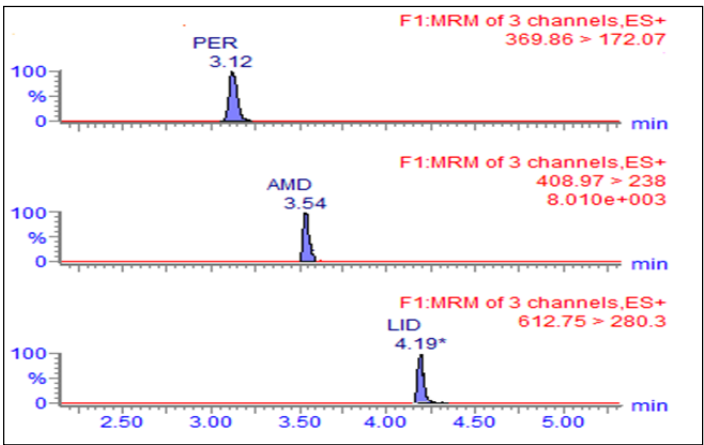


Fig. 2. EIC at LLOQ level of amlodipine besylate (1.0 ng/mL) and perindopril arginine (0.5 ng/mL) with lercanidipine (50.0 ng/mL) as internal standard.

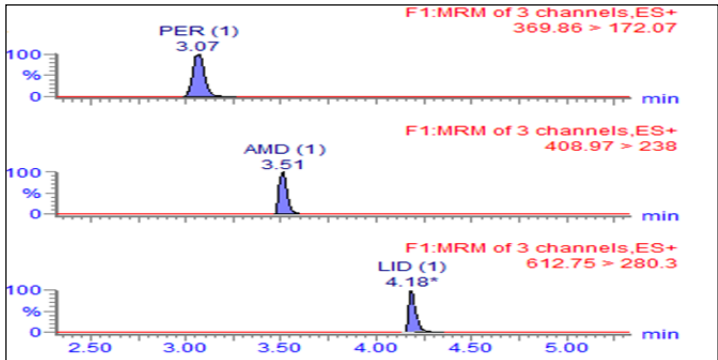


Fig. 3. EIC at ULOQ level of amlodipine besylate (100 ng/mL) and perindopril arginine (500 ng/mL) with lercanidipine (50.0 ng/mL) as internal standard.

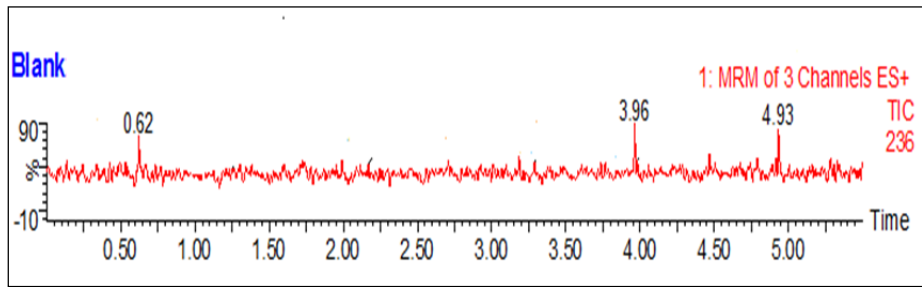


Fig. 4. TIC of blank chromatogram (mobile phase).

Table 3. The results of calibration curves of perindopril arginine and amlodipine besylate from one batch of validation

Concentration (ng/mL)		Mean concentration (ng/mL) ± SD, (n = 3)		RSD ^a (%)		Regression equations	
PER	AMD	PER	AMD	PER	AMD	PER	AMD
0.5	1	0.40 ± 0.03	0.90 ± 0.05	6.5	5.7		
100	10	98.50 ± 1.25	10.5 ± 0.89	1.3	8.5		
200	30	202.2 ± 0.58	30.0 ± 0.29	0.3	1.0	y = 0.9994x + 0.0090 R ² = 0.9999	y = 0.9999x + (-0.1673) R ² = 0.9998
300	50	300.4 ± 1.96	49.7 ± 0.89	0.7	1.8		
400	75	397.9 ± 1.22	75.9 ± 0.58	0.3	0.8		
500	100	500.2 ± 1.97	99.4 ± 1.03	0.4	1.0		

(RSD^a) standard deviation / mean concentration measured) × 100.

(Y) Observed concentration (of PER and AMD), (X) concentration in ng/mL, and (R) Correlation coefficient.

(PER) Perindopril arginine, (AMD) amlodipine besylate.

Table 4. Precision and accuracy data

Batch	Concentration (ng/mL)		Mean concentration (ng/mL) ± SD, (n = 3)		RSD ^a (%)		Accuracy (%)	
	PER	AMD	PER	AMD	PER	AMD	PER	AMD
Intra-day	0.5	1	0.48 ± 0.07	0.98 ± 0.09	13.6	9.06	95.73	97.62
	150	40	149.2 ± 2.00	40.3 ± 0.56	1.34	1.39	99.46	100.8
	450	80	449.3 ± 1.21	80.1 ± 0.36	0.27	0.45	99.84	100.1
Inter-day	0.5	1	0.51 ± 0.04	1.07 ± 0.14	7.78	12.93	102.5	106.9
	150	40	149.9 ± 1.41	39.7 ± 0.83	0.94	2.08	99.90	99.30
	450	80	450.6 ± 1.41	79.8 ± 1.43	0.31	1.80	100.1	99.78

(RSD^a) Standard deviation / mean concentration measured × 100.

(PER) Perindopril arginine, (AMD) amlodipine besylate.

Table 5: Robustness data

Concentration (ng/mL)		Mean concentration (ng/mL) ± SD, (n = 3)		RSD ^a (%)		Accuracy (%)	
PER	AMD	PER	AMD	PER	AMD	PER	AMD
0.5	1	0.49 ± 0.06	1.18 ± 0.06	13.0	5.16	98.53	117.5
150	40	149.1 ± 2.17	40.1 ± 2.05	1.45	5.10	99.41	100.4
450	80	449.1 ± 2.32	79.1 ± 3.05	0.52	3.86	99.81	98.89

(RSD^a) standard deviation / mean concentration measured × 100.

(PER) perindopril arginine, (AMD) amlodipine besylate.

Accuracy and precision

The results are shown in Table 4. Accuracy was further assessed by applying the standard addition technique on Coversac[®] and Amlip[®]

tablets where good recoveries were obtained revealing no interference from excipients and good accuracy of the proposed methods (Table 4).

As shown in Table 4, the intra-day % RSD was less than 13.59% and the accuracy ranged from 97.6% to 100.07%. Inter-day % RSD was less than 12.93% and the accuracy ranged from 99.3% to 106.9%. These results indicate the adequate reliability and reproducibility of this method within the analytical curve range.

Robustness

All that deliberate small changes in the studied factors did not lead to a significant change in terms of retention values, area and/or symmetry of the peaks, where the % RSD (Table 5) was less than 13.04%.

DISCUSSION

Since no sensitive method for quantification of the perindopril arginine and amlodipine besylate as combination in bulk drugs or pharmaceuticals using LC-MS technique has been reported, the validated RP-UPLC/ESI-MS/MS assay method was developed which yields highly reproducible chromatographic results when quantifying perindopril and amlodipine and provides an accurate and precise assay for analyzing combined dosage form of bulk drugs and tablet formulations. Moreover, currently available methods (9-12) are developed either for each individual drug or their combination with other drug molecules and also they are time consuming with laborious sample preparation, so there is a need for developing a fully automated online method for the determination of perindopril and amlodipine.

In this method high-performance liquid chromatography on MS/MS detection for the quantification of combined dosage form of perindopril and amlodipine using simple sample preparation and acquisition parameter for analysis was used. UPLC was carried out using a C18 column with 20 mM ammonium acetate with 0.1% formic acid and acetonitrile:methanol with 0.1% formic acid (80:20, v/v) as mobile phase A and B, respectively. There were no interfering peaks, which indicate the selectivity of the method. Recovery values within $100 \pm 20\%$ were obtained for three different concentrations by the proposed method indicating the method

was precise. The LOD values of 0.15 ng/mL and 0.65 ng/mL and LOQ of 0.35 ng/mL and 0.85 ng/mL respectively for perindopril and amlodipine obtained by the proposed method indicates sufficient sensitivity of the method. Analysis using UPLC/ESI-MS/MS is so fast and it is possible to analyze almost all pharmaceutical compounds due to the presence of wide variety of detection system. Although the instrument is costly and not affordable by each laboratory, simple, fast, and cost-effectiveness procedures with adequate accuracy and precision, makes this technique still popular and attractive. As a powerful complementary new technique to HPLC, RP-UPLC/ESI-MS/MS has rapidly spread into a wide array of analytical areas due to its excellent separation efficiency, short analysis time, minimal need of samples and solvents and high versatility in terms of separation modes (13).

CONCLUSION

In conclusion the ion spray MS/MS method has advantages in providing shorter analytical run time, higher selectivity and much lower limit of quantification compared with previous analytical methods. This proposed method can be suitably used for determination of these combined dosage form in plasma for therapeutic drug monitoring analysis as clinical study sample analysis.

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