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BIOANALYTICAL METHOD VALIDATION FOR PERINDOPRIL AND AMLODIPINE IN HUMAN PLASMA USING UPLC/ESI-MS/MS

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ABSTRACT

Background: The main objective of this study is to develop and validate a simple mass compatible method for quantification of both perindopril and amlodipine in human plasma. **Methods:** A UPLC/ESI-MS/MS method for the determination of combined dosage form of perindopril and amlodipine in human plasma sample was developed. The gradient elution with flow rate at 0.3 mL per min of mobile phase was kept and 10 µL of sample was injected in each run. The total chromatographic run time was 5.5 min. Argon was used as the collision gas at the pressure of $3.5X10^{-5}$ Torr. **Results:** In this developed method, a high recovery of perindopril and amlodipine in plasma samples was proved with improved quality data in terms of increased detection limits and chromatographic resolution with greater sensitivity. As per ICH guidelines, the main characteristics of a bioanalytical method validation constraints that are essential to confirm the suitability and reliability of analytical results were evaluated. **Conclusion:** Quantification of perindopril and amlodipine dosage forms by this method is time saving, cost effective and it can be used in clinical studies from PK studies or clinical trials using LC-MS/MS to quantify the drug content in human plasma samples.

KEY WORDS: Amlodipine, Bioanalytical method, Perindopril, Plasma.

INTRODUCTION

A combination of perindopril, an angiotensin converting enzyme (ACE) inhibitor, and amlodipine, a dihydropyridine calcium channel blocker is specifically indicated for the treatment of hypertension.[1] Perindopril arginine is chemically described as L-arginine (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1H-indole-2-carboxylate.[2] Its empirical formula is $C_{19}H_{32}N_2O_5.C_6H_{14}N_4O_2$. Perindopril arginine is a white, crystalline powder with a molecular weight of 542.7.[3,4] The free acid has the molecular weight of 368.5. Amlodipine besylate is the benzene sulphonic acid salt of amlodipine, a long-acting dihydropyridine calcium channel blocker.[5] It is chemically described as 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate monobenzene sulphonate. Its empirical formula is $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$. Amlodipine besylate is a white crystalline powder with a molecular weight of 567.1.[6] It is slightly soluble in water and sparingly soluble in ethanol. The content of the tablets is expressed as Amlodipine (free base) which has a molecular weight of 409.1. A fixed-dose combination of these two drugs approved by FDA in Jan 2015, to lower blood pressure in patients not responding to monotherapy and as initial therapy in patients likely to need multiple drugs to achieve their blood pressure goals. It is supplied as a tablet for oral administration.

A complete literature search reveals that, a few analytical methods only are available for determination of Perindopril arginine (PER) and Amlodipine besylate (AMD) in combination form simultaneously in bulk drugs, pharmaceutical formulations and in various biological matrices by using visible spectrophotometry, High Performance Thin Layer Chromatography (HPTLC), Reversed phase- High Performance Liquid Chromatography (RP-HPLC), capillary gas chromatography techniques.[7-16] There is no method reported till date for determination of the Perindopril arginine and Amlodipine besylate as combination in biological matrix using LC-MS/MS technique. Among the several instrumental techniques available for the assay of drugs, usually visible spectrophotometric technique is the simple and less expensive method. The supremacy of traditional high-performance liquid chromatography with UV detection (HPLC-UV, DAD) can be readily extended by simply coupling a mass spectrometer (MS). In the early years of liquid chromatographic mass spectrometry (LC-MS), this coupling was considered exotic and complex. After more than 15 years of refinement, LC-MS/MS

systems are robust and easy to use, and provide specificity unattainable by any other detection scheme. With the increased analytical capability, challenges may be tackled from several different and complementary directions.

This technique provided a powerful method for increasing quantitative capability, providing peak identification, and elucidating the structure of analytes. This allows the analyst to monitor masses relevant only to the target analytes, and the resulting increased specificity provides multiple advantages like improves sensitivity, resolution, throughput, and productivity. The MS detection mode of multiple reaction monitoring (MRM) analysis are entirely different mechanisms, this facilitates problem-solving and to yield a single, powerful, orthogonal approach for quantification of analytes from the complex biological matrix. Chromatographic performance was maintained when switching the solvent system to MS-compatible solvents. In this present study, UPLC-MS/MS analytical technique was performed to quantitate for both perindopril and amlodipine in human plasma. The main objective of this study is to develop and validate a simple mass compatible method for quantification of both perindopril and amlodipine in human plasma.

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, formic acid (eluent additive for LCMS, ~ 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. The human plasma is collected from 6 volunteers.

METHODS

Liquid chromatographic and mass spectrometric conditions

UPLC analysis was performed on an AcQuity UPLC system (Waters, USA). The analytical column was an AcQuity UPLC™ BEH C_{18} with pressure-tolerant 1.7 µm bridged ethylsiloxane/silica hybrid particles with the dimension 2.1 × 100.0 mm (Waters). The mobile phase A consists of 0.1% formic acid in Milli Q water and mobile phase B consists of 0.1% formic acid in acetonitrile: Methanol (90:10,V/V) and pumped at a flow rate of 0.30 mL/min.

Chromatography was performed at 35 ± 2°C with a chromatographic gradient run time of 5.5 min. The auto sampler temperature was set at 5.0 ± 1.0°C. Mass spectrometry was performed using a Zevo TQD mass spectrometer (Waters) equipped with an ESI interface. The detection of PER and AMD was performed by ESI in positive ion mode with multiple reaction monitoring (MRM) using Lercanidipine (LID) as internal standard. The optimum operating conditions for the mass measurements are summarized in Table 1.

Table 1: Optimum operating mass spectrometric parameters for PER and AMD.

Parameter	Value
Source temperature, °C	150
Desolvation temperature, °C	500
Cone gas flow rate, L/h	20
Desolvation gas flow rate, L/h	300
Transition dwell time, s	0.02
Capillary voltage, kV	3.5
Cone voltage, V	35
Mode of analysis	Positive ion
Ion transition for PER, m/z	369.58 172
Ion transition for AMD, m/z	408.97 238
Ion transition for LID, m/z	612.79 280

Data acquisition and analysis were performed using the MassLynx™ NT 4.1 software with the TargetLynx™ program.

Stock and working standard solutions

Stock standard solutions of PER, AMD and LID, 1.0 mg/mL, were prepared by dissolving the appropriate amounts of the compounds in 50% methanol. A series of concentration ranges of 0.01-3.0 ng/mL for PER and 0.01-2.50 ng/mL for AMD working standard solutions was prepared by consequent dilution of the above mentioned stock standard solution in methanol/water (50:50, v/v).

Calibration curve and quality control standards in spiked plasma

Endogenous interference and drug free human plasma were screened prior to use and pooled the screened human plasma. The selectivity was ensured by free of endogenous interference at the retention times of the analytes and the IS (LID). From 5.0 mL of drug-free human plasma, spiked standards over the concentration range by appropriate dilutions of working standard solutions to 0.01, 0.70, 1.26, 1.80, 2.50 and 3.00 ng/mL for PER and 0.01, 0.58, 1.05, 1.50 2.09, and 2.50 ng/mL for AMD were prepared.

Stock solutions were prepared separately for calibration of standard solutions and quality control samples. Spiked at four concentration levels 0.03, 0.30, 1.70, 2.71 ng/mL for PER and 0.03, 0.25, 1.44, 2.31 ng/mL for AMD as limit of quantification (LOQC), low (LQC), medium (MQC) and high (HQC) quality controls respectively in human plasma. In a separate radio immune assay (RIA) vials, aliquot 250µL of all the calibration standard and QC samples. The samples were stored in -20°C until the assay.

Sample preparation procedure

Liquid-liquid extraction (LLE) procedure was carried out for cleanup of human plasma and extraction of analytes samples. The frozen plasma samples were retrieved from -20°C for each assay and thawed at room temperature. In a RIA tubes, aliquot 200µL of each calibration and QC samples separately and spiked with IS, 50 µL of 50 ng/mL LID working standard solution prior to the extraction. Added 2.5mL of ethyl acetate, vortex-mixed at 2500rpm for 15 min and then centrifuged at 4000rpm at 10°C for 10 min. All these centrifuged calibration and QC samples were frozen using dry ice for 10 min. The organic layer was transferred into another set of labeled RIA tube. All the samples were evaporated under gentle stream of nitrogen at 30°C for 40 minutes. The dried residual in RIA tubes were reconstituted in 200µL of mobile phase B as diluent and vortex-mixed for 30 s. The samples were transferred in the 96-well plate and allowed to stand for 20 min at 5°C before starting the sequence. All the samples were injected at 10µL into the chromatographic system for analysis.

Validation

Calibration standards at six different concentration levels ranging from 0.01, 0.70, 1.26, 1.80, 2.50, 3.00 ng/mL for PER and 0.01, 0.58, 1.05, 1.50 2.09, 2.50 ng/mL for AMD were spiked in human plasma (each standards triplicate injections, n=5) were analyzed. The standard curve of calibration standards were plotted for each run based on the peak area ratio of the analyte to that of the IS versus the theoretical concentration. Least-squared linear regressions, weighted ($1/y^2$) were used to achieve the equation of the calibration curves.

In order to assess the intra, inter-assay accuracy and precision, QC samples were processed in six replicates at each concentration (0.30, 1.70, 2.71 ng/mL for PER and 0.25, 1.44, 2.31 ng/mL for AMD) for five different analytical runs. To evaluate the recovery of the LLE procedure, at two concentration levels (0.30, 2.71 ng/mL for PER and 0.25, 2.31 ng/mL for AMD) and at 50 ng/mL for the IS were used by comparing the peak areas obtained from the

QC samples. In spiked human plasma the analytes stability were also investigated at two concentration level (0.30, 2.71 for PER and 0.25, 2.31 for AMD) under various storage conditions like at bench-top stability (ambient temperature for 6 hours), Long term stability at -20°C for 180 days, Freeze/thaw cycles stability (-20°C for 3 cycles), autosampler stability at 4°C for 24 hrs and dry residue stability at 4°C for 24 hrs, which were compared with the absolute peak area measurements obtained from the analysis of freshly prepared spiked samples.

RESULTS

Mass spectrometric conditions optimization

To achieve the maximum abundance of the parent and daughter ions of PER, AMD and LID (IS), acquisition parameters were optimized by LC combine mode using Intelistart automatic software. Acquisition of tune parameters for MRM of each compound separately into the tandem mass spectrometer of a 300 ng/mL olution (50% methanol) at a flow rate of 0.3mL/min.

Full scan and product ion mass spectra of PER, AMD and LID (IS) were obtained in positive ESI modes are presented in Figure: 1 to 3 respectively. PER, AMD and LID (IS) revealed a protonated molecule $[M+H]^+$ at *m/z* 369.58, 408.97 and 612.79 respectively, which were chosen as the precursor ion. The $[M+H]^+$ ion fragmented under collision-induced decomposition to produce product ions at *m/z* 172, 238 and 280 for PER, AMD and LID respectively. The optimized mass spectrometric conditions along with mass transitions in MRM are presented in Table 1. The MRM transitions of *m/z* 369.30 → 171.90, 409.35 → 238.14and *m/z* 613.02 → 280.35 were selected for the quantitation of PER, AMD and LID (IS) respectively.

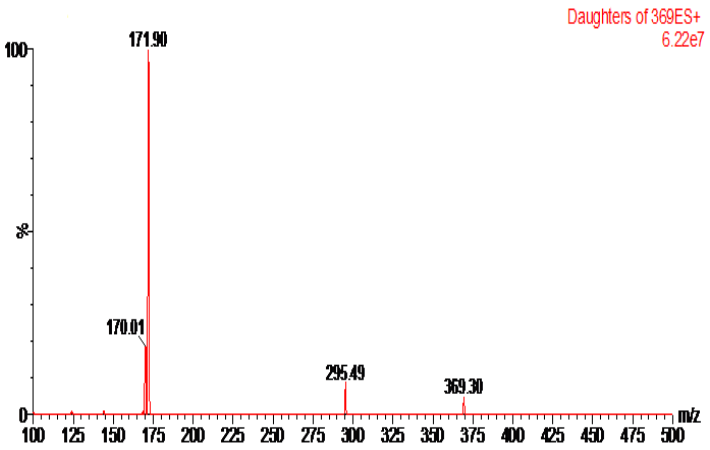

Figure 1: MS scan of a 300 ng/mL PER, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 369.58 → 172.

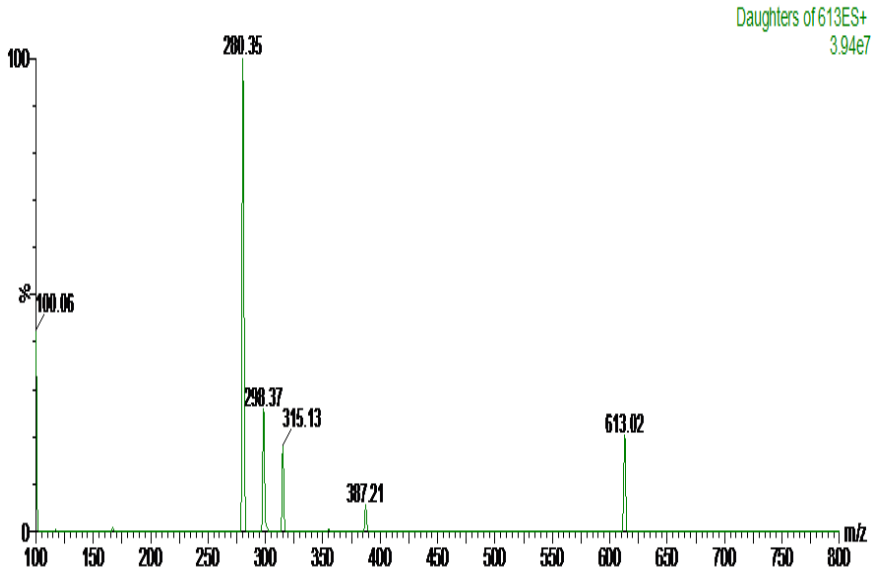

Figure 2: MS scan of a 300 ng/mL AMD, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 408.97 → 238.

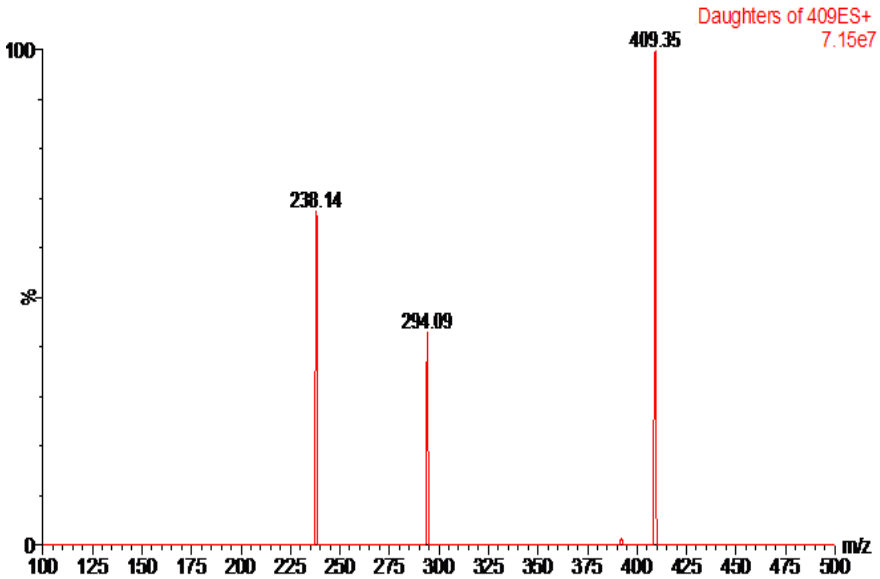

Figure 3: MS scan of a 300 ng/mL LID, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 612.79 → 280.

Ultra-performance liquid chromatography

A representative UPLC-MS/MS- MRM chromatogram obtained from the analysis of a sample spiked with 3.0 ng/mL of the PER, 2.5 ng/mL of the AMD and 50 ng/mL of the IS is presented in Figure: 4. Under the current chromatographic conditions PER, AMD and LID were eluted at 3.06, 3.49 and 4.17 min, respectively.

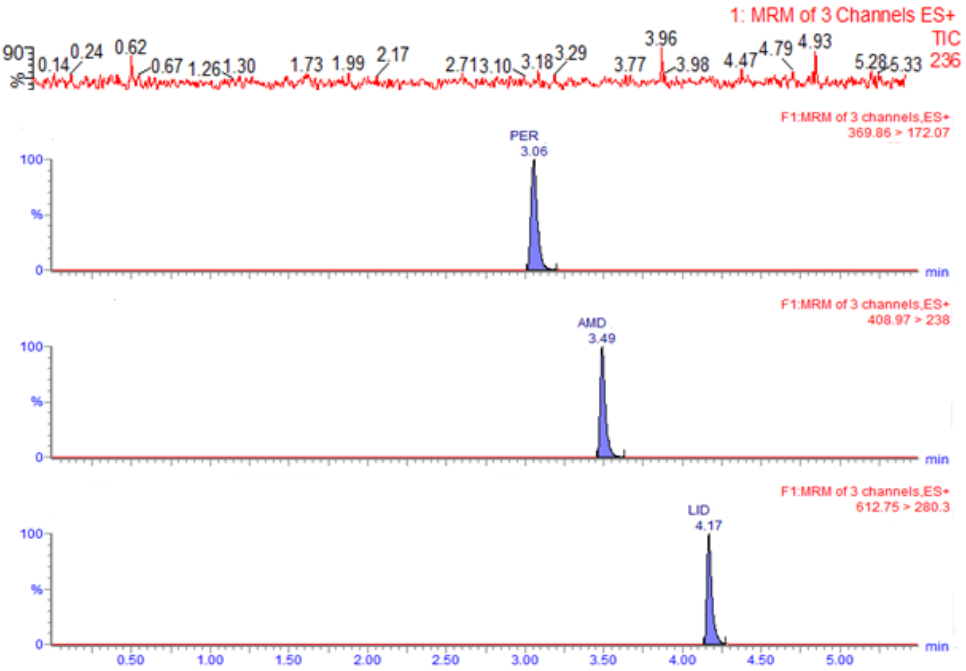

Figure 4: A representative UPLC/MS/MS chromatogram of a blank plasma extract along calibration plasma sample spiked with 3.0 ng/mL of the PER 2.5ng/mL of the AMD and 50ng/mL of the IS (top to bottom).

Statistical analysis

Linearity

Calibration curves for the samples spiked with plasma were linear over the range of 0.01-3.0 ng/mL for PER and 0.01-2.50 ng/mL for AMD. All the samples were analyzed in triplicate in five analytical runs. Correlation coefficients (r^2) for both analytes were >0.9981 with a weighted factor $1/x^2$, which were determined by relationships between the ratios of the peak area signals of PER and AMD to that of the IS and the corresponding concentrations were observed, as result was presented in Table 2. The back calculated concentrations in all case of the calibration curves were within 15% of the nominal values except LLOQ concentration which was less than 20% CV. The analyte response at the LLOQ was more than five times as compared to blank response. The linear model reasonably expresses the relationship between concentration and response of the both analytes and which are in agreement with international guidelines.

Table 2: Analytical parameters of the calibration equations for the determination of PER and AMD (5 runs).

PER			AMD		
Conc. range (ng/mL)	**Regression equations[a]**	**r[b]**	**Conc. range (ng/mL)**	**Regression equations[a]**	**r[b]**
0.01–30.00	$R_{per} = 40.2866 \times C_{per} + 28.7116$	0.9958	0.01-2.50	$R_{amd} = 15.3826 \times C_{amd} + 9.26622$	0.9966
0.01–30.00	$R_{per} = 28.4699 \times C_{per} + 15.338$	0.9965	0.01-2.50	$R_{amd} = 10.3551 \times C_{amd} + 5.12636$	0.9991
0.01–30.00	$R_{per} = 37.0594 \times C_{per} + 22.1733$	0.9926	0.01-2.50	$R_{Amd} = 16.0174 \times C_{Amd} + 9.0441$	0.9902
0.01–30.00	$R_{per} = 52.1587 \times C_{per} + 41.1145$	0.9992	0.01-2.50	$R_{Amd} = 15.2584 \times C_{Amd} + 10.0045$	0.9994
0.01–30.00	$R_{per} = 53.5409 \times C_{per} + 39.7141$	0.9988	0.01-2.50	$R_{Amd} = 20.9887 \times C_{Amd} + 12.0538$	0.9974

a. Ratio of the peak area amplitude of PER and AMD to that of the IS, Rper and Ramd, vs. the corresponding concentration, Cper and Camd.

b. Correlation coefficient.

Accuracy and Precision

The closeness of individual measures of an analytes were calculated for the determinations of precision of inter day and intraday precision. The procedure is applied on multiple aliquots of single homogeneous volume of 3 different concentration spiked in plasma and 6 determinations per concentration. Intra-day precision and Inter-day precision was evaluated as coefficient of variation (CV) at each concentration level, as mentioned in Table 3. The observed results explains for accuracy and precision of this method. The intraday precision were between 1.8 %, 3.0 % for PER and 3.5 %, 8.5 % for AMD. The inter assay % CVs were lower than 6.3 % for PER and 6.7% for AMD. The overall accuracy was assessed by the relative percentage error (absolute % E_r), which ranged from 1.8 to 12.2% for PER and -3.2 to 4.0 for AMD.

Table 3: Accuracy and precision evaluation of QC samples for PER & AMD (in 3v validation days, 6 replicates per day).

Parameters		**Concentration of PER (ng/mL)**			**Concentration of AMD (ng/mL)**		
		0.3	**1.7**	**2.71**	**0.25**	**1.44**	**2.31**
Run 1	Mean ± SD	0.34±0.010	1.72±0.047	2.75±0.050	0.27±0.023	1.35±0.047	2.37±0.090
	% Er[a]	12.5	1.3	1.3	9.8	-6.4	2.73
Run 2	Mean ± SD	0.33±0.018	1.71±0.035	3.05±0.108	0.24±0.027	1.49±0.052	2.35±0.053
	% Er[a]	10.9	0.3	12.4	-3.9	3.5	1.8
Run 3	Mean ± SD	0.34±0.034	1.76±0.022	2.73±0.020	0.27±0.023	1.34±0.113	2.39±0.105
	% Er[a]	11.8	3.2	0.8	7.3	-7.2	3.3
Overall mean		0.34	1.73	2.84	0.26	1.39	2.37

Overall accuracy						
% Er[a]	12.2	1.8	4.9	4.0	-3.2	2.6
Intra-assay % CV[b]	3.0	2.8	1.8	8.5	3.5	3.8
Inter-assay % CV[b]	1.7	1.5	6.3	6.7	6.0	0.8

a - % Er: Relative percentage error.

b- % CV: coefficient of variation; intra- and inter-assay CV.

Selectivity

The selectivity towards endogenous plasma compounds were tested in six different batches of drug-free human plasma by analyzing blanks (non-spiked plasma samples) and plasma samples (spiked with 0.03 ng/mL of PER, 0.03 ng/mL of AMD and 50 ng/mL of LER). Mass chromatograms of six batches of drug-free plasma contained no co-eluting peaks not greater than 20% of the area of PER, AMD at the LLOQ level, and no co-eluting peaks not greater than 5% of the area of LER (IS). The concentration of PER and AMD obtained after the analysis of the six different lots of human plasma was 0.032 ± 0.002 ng/mL and 0.029 ± 0.001 ng/mL with a relative percentage error (% E_r) of 6.7 and -3.3% respectively. Selectivity LLOQ replicates for each lot meets accuracy acceptance limit, and the mean accuracy was within ±20.0% of the nominal concentration (Table 4).

Table 4: Selectivity result of PER and AMD.

Lot Number	Mean conc. ng/mL (n=3)		Average Conc. (Mean ± SD)		%Er	
	PER	AMD	PER	AMD	PER	AMD
LLOQ-Lot1	0.032	0.029	0.032 ±002	0.029 ±001	6.7	-3.3
LLOQ-Lot2	0.029	0.028				
LLOQ-Lot3	0.033	0.031				
LLOQ-Lot4	0.032	0.031				
LLOQ-Lot5	0.033	0.028				
LLOQ-Lot6	0.031	0.029				

Recover and Matrix Effect

The matrix effect susceptibility of ion suppression or enhancement was evaluated as the co-elution of matrix ions on the ionization of the target analysis. Six samples of drug-free human plasma were processed according to the sample preparation procedure and then spiked with at 0.30 ng/mL, 2.71 ng/ml for PER and 0.25ng/mL, 2.31ng/mL for AMD concentration. The corresponding peak areas of PER and AMD were then compared with those of aqueous standard solutions at equivalent concentrations. Calculation for matrix effect percentage were calculated as,

Matrix factor = B/A

% Matrix effect = [(B-A)/A] * 100

Where, A, is the response of the aqueous sample and B is response for the post extracted spiked samples.

Both QC samples Matrix factor was within 0.85 to 1.15 and % CV for each set of LQC and HQC were not more than 15%. The results indicate that the matrix effect does not appreciably affect the assay. The proposed LLE procedure and instruments efficiency was evolved as the percent recovery by calculating the ratio of the absolute peak areas of extracted spiked plasma samples to the absolute peak areas of aqueous standard solutions containing equivalent concentrations of PER and AMD (unextracted standards) that represent 100% recovery. The data presented in Table 5 indicate average recovery of LQC and HQC was 97.3, 100.4 for PER and 96.1, 100.1% for AMD respectively.

% Recovery = (Extracted sample response/ Un-extracted aqueous sample response) X 100.

Table 5: Extraction recovery and matrix effect of PER and AMD.

Nominal concentration of PER (ng/mL)	Extraction recovery of PER (Mean ± SD)	Aqueous recovery of PER (Mean ± SD)	Post spiked-matrix recovery of PER (Mean ± SD)	% Extraction recovery	% Matrix effect
0.3	97.3 ± 5.97	100.6±1.91	101.7±8.10	97.3	1.09
2.71	101.4 ± 2.83	101.0±1.61	100.2±3.79	100.4	-0.79
Nominal concentration of AMD (ng/mL)	**Extraction recovery of AMD (Mean ± SD)**	**Aqueous recovery of AMD (Mean ± SD)**	**Post spiked-matrix recovery of AMD (Mean ± SD)**	**% Extraction Recovery**	**% Matrix effect**
0.25	94.5±6.04	98.3±9.06	99.3±4.68	96.1	1.02
2.31	100.2±3.69	101.7±3.77	100.6±1.70	98.5	-1.08

Stability

The stability of the method was determined at two concentration level spiked in drug free plasma (for PER and for AMD) and stability under various temperatures were investigated for each concentration. The results of stability were compared with the zero day sample results and were evaluated the recovery as well as % coefficient of variation. The different stability of spiked samples were evaluated in long term plasma stability at -20°C for 180 days, three successive freeze/thaw cycles, auto sampler stability at 4°C for 4 hrs, dry residue stability at -4°C for 48 hrs and bench top stability at ambient temperature for 6 hrs. There was no degradation products were observed in any of the above mentioned stability samples,

which indicate that the both analytes were can be considered stable under the various temperature conditions (it was presented in Table 6). The recovery was 80-120% of the initial concentration and the % CV was not more than 10.7% for both analytes.

Table 6: Stability data for PER and AMD in human plasma under various storage conditions (n = 6).

Storage conditions/time	Nominal Concentration levels (ng/mL)		Calculated concentration (ng/mL) (mean ± SD)				%CV		% Recovery	
			Freshly prepared zero day samples		Stability samples					
	PER	AMD	PER	AMD	PER	AMD	PER	AMD	PER	AMD
Bench-top stability (at ambient temperature/6 h)	0.30	0.25	0.31 ± 0.027	0.25 ± 0.032	0.35 ± 0.026	0.27 ± 0.024	7.3	8.7	112.9	108.0
	2.71	2.31			2.86 ± 0.074	2.43 ± 0.104	2.6	4.3	103.6	104.7
Long-term stability (−20°C/180 days)	0.30	0.25			0.31 ± 0.021	0.27 ± 0.029	6.9	10.7	103.3	108.0
	2.71	2.31			3.01 ± 0.185	2.40 ± 0.050	6.2	2.1	111.1	100.4
Freeze-thaw stability (−20°C/ 3 freeze/thaw cycle)	0.30	0.25			0.31 ± 0.028	0.25 ± 0.026	9.1	10.2	104.0	100.7
	2.71	2.31	2.76 ± 0.038	2.32 ± 0.067	2.76± 0.119	2.36 ± 0.043	4.3	1.8	102.0	102.2
Auto-sampler stability (4°C/24hrs)	0.30	0.25			0.31 ± 0.022	0.27 ± 0.017	7.2	6.4	102.8	100.7
	2.71	2.31			2.68± 0.127	2.50 ± 0.223	4.7	8.9	98.8	108.2
Dry residue stability (4°C/24hrs)	0.30	0.25			0.32 ± 0.027	0.25± 0.008	8.3	3.2	108.2	100.4
	2.71	2.31			2.76± 0.113	2.40 ± 0.084	4.1	3.5	102.0	104.1

DISCUSSION

A innovative method to quantitative PER and AMD in human plasma by using UHPLC with Waters Xevo^{TM} Triple Quadrupole MS was successfully developed and validated. AQUITY BEH C18, 1.7µ column provided excellent peak shape, sensitivity and selectivity for the quantitation of both analytes with a total LC cycle time of 5.5 minutes. A simple straightforward LLE strategy method of extraction procedure was successfully applied for extraction of analyte from the biological matrix. Detection and quantification limits 0.01-3.0ng/mL for PER and 0.01-2.5ng/mL were achieved from only 200 µL of human plasma extracted with appropriate accuracy and precision at each concentration level. All the QC samples at all levels were passed regulatory guidelines, with mean accuracies ranging from 90% to 113%. Average CV of all points on three independent standard curves was 4%. Recovery of the analytes were not be 100%, but the extent of recovery of an analyte and of the internal standard was observed consistent, precise, and reproducible.

CONCLUSION

The method shows promise for high sensitivity quantification of patient samples from PK studies or clinical trials using LC/MS/MS. The validated method data was showed satisfactory for all the parameters tested. UPLC with MS/MS has the advantage over comes the problems of poor chromatography, wearisome extraction steps, uncertain characterized peak and high injection load.

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