A validated UPLC/ESI-MS/MS bioanalytical method for the quantification of Perindopril and Amlodipine in human plasma

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Scope of the Method Validation

Encompasses all possible well-characterized and fully validated bioanalytical method to yield reliable results that can be satisfactorily interpreted.

A primary concern in biopharmaceutics is the bioavailability of drugs.

Bioavailability

refers to the measurement of the rate and extent of active drug that reaches the systemic circulation.

means access to the bloodstream

METHODOLOGY

Selection of drugs combination and collection based on literature survey

Study of physicochemical properties of drug molecule. Find out solubility of combination in solvent

Tuning of the molecule of interest, source parameters, MS scanning and optimization.

Selection of chromatographic method (based on solubility study, retention of compound)

Concentration range of compounds in sample of interest and Method validation

Report the final results and discussion

OPTIMIZED UPLC-ESI-MS/MS ACQUISITION CONDITIONS FOR PERINDOPRIL & AMLODIPINE

UPLC Conditions

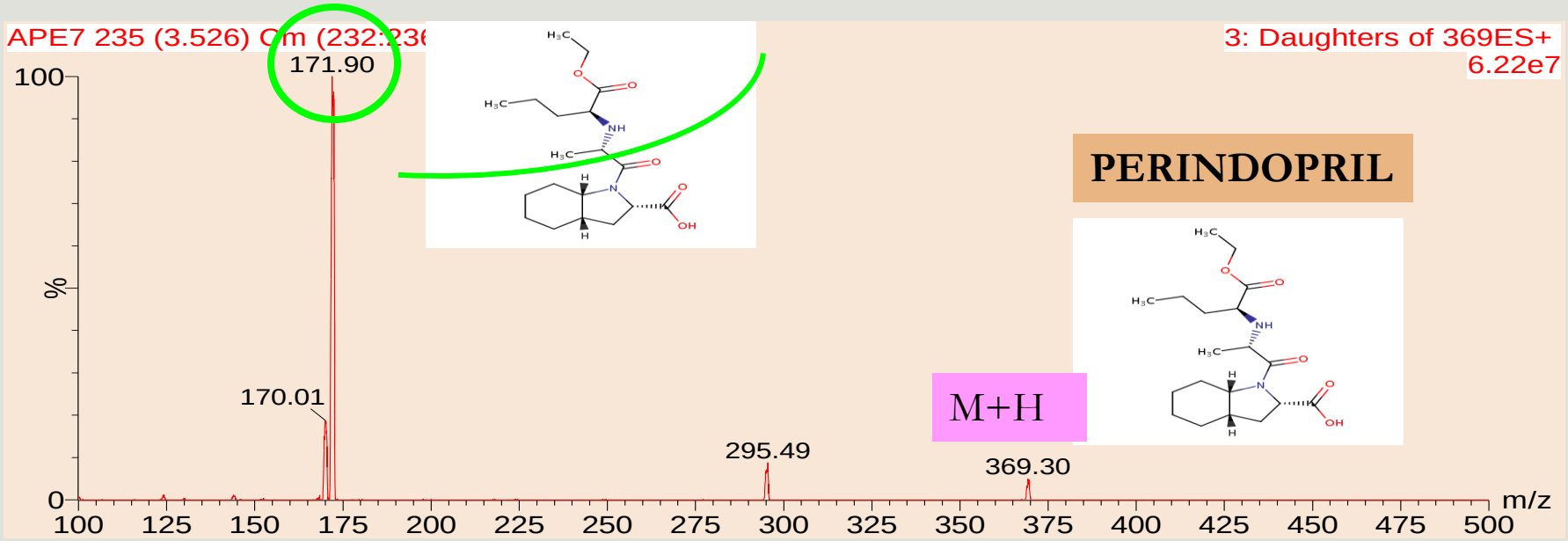
- Mobile phase:
  Solvent A: 0.1% Formic acid in MilliQ water (V/V)
  Solvent B: 0.1% Formic acid in Acetonitrile (V/V)
- Injection Volume: 10 µL

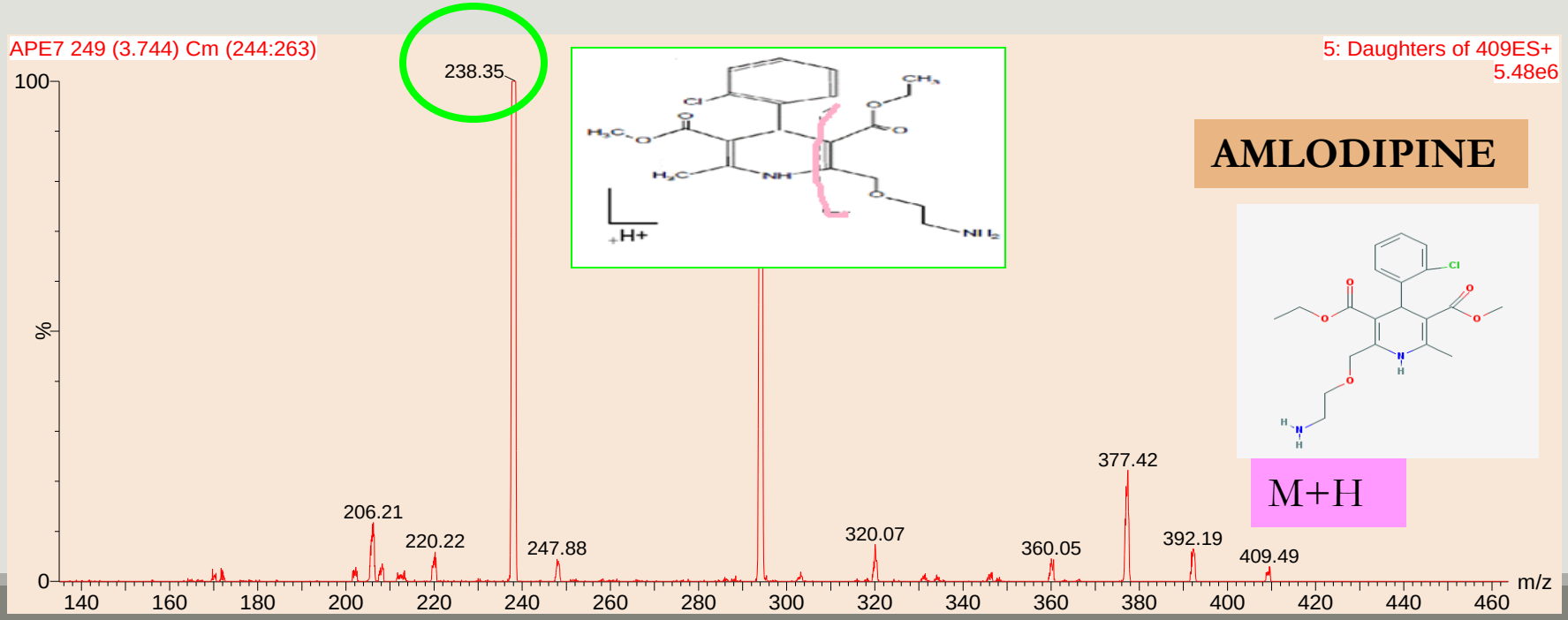
Parameters	Conditions
Flow rate	0.2mL/min
Run time	5 .5 min
TCC	25°C ± 2°C
Column	AQUITY UPLC BEHC18 , 2.1 x 100 mm, 1.7 µm
Gradient Program	
Time (min)	**%B**
0	2
2	40
3	90
3.5	90
4	2
5.5	2

OPTIMIZED UPLC-ESI-MS/MS ACQUISITION CONDITIONS FOR PERINDOPRIL & AMLODIPINE (Continued..)

MS/MS Conditions

Ion polarity			**Positive**	
Data storage			Continnum	
Source				
Source temperature (°C)			150	
Gas flow (L/H)			300	
Desolvation temperature (°C)			500	
Capillary Voltage (KV)			3.5	
Cone			35	
MRM				
Compound	Parent m/z	Product m/z	Cone (V)	Collision (V)
PER	369.58	172	35	28
AMD	408.97	238	35	18
LID	612.79	280	35	16
Mass		Mass range		
		Min range		Max range
		100 m/z		700 m/z


APE7 235 (3.526) Cm (232:23
3: Daughters of 369ES+
6.22e7
171.90
170.01
295.49
369.30
100
%
0
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
m/z
PERINDOPRIL
M+H


APE7 249 (3.744) Cm (244:263)
5: Daughters of 409ES+
5.48e6
238.35
206.21
220.22
247.88
320.07
360.05
377.42
392.19
409.49
100
%
0
140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460
m/z
AMLODIPINE
M+H

Extraction Method-LLE

Fifty microlitres of internal standard (50ng/ml of Licarnidipine in 50% methanol) was added to each 200 µl of human plasma samples

vortexed for 10 s

Ethyl acetate (2.5 ml) was added, followed by vortexing at 2500rpm for 15 min

centrifuged at 4000rpm, 10°C for 10 min

Organic layer was transferred to another set of labeled test tubes

Organic layer was evaporated under nitrogen

Residue was dissolved in 200µL of Reconstitution solution (1:1, Mobile phase A:B)

CALIBRATION STANDARDS AND QC SAMPLE CONCENTRATION

Final Conc. of perindopril (ng/mL)	Final Conc. of amlodipine (ng/mL)	Final Conc. of lercanidipine (IS)(ng/mL)	Standard ID
0.951	0.761	50.686	AQ.STD1
19.011	15.224	50.686	AQ.STD2
63.370	50.745	50.686	AQ.STD3
90.529	72.493	50.686	AQ.STD4
125.734	100.685	50.686	AQ.STD5
150.580	120.580	50.686	AQ.STD6

QC Samples ID	Final Conc. of perindopril (ng/mL)	Final Conc. of amlodipine (ng/mL)	Final Conc. of lercanidipine (IS)(ng/mL)
LLOQ.QC	0.943	0.707	50.686
LQC	26.195	19.636	50.686
MQC	65.486	49.089	50.686
HQC	145.525	109.087	50.686

Bio Analytical Method validation Results

Methods used for quantitative measurement of analytes in any given biological matrix must be

reliable and reproducible for the intended use…

- ❖Selectivity
- ❖ Carry-over
- ❖ Calibration curve
- ❖ Accuracy & Precession
- ❖ Recovery
- ❖Matrix effect
- ❖Dilution integrity
- ❖Suitability for the assay

C_{max} (ULOQ)

$AUC_t/AUC_\infty \geq 80\%$ (LLOQ)

Carry-over (LLOQ ≤5% *Cmax)*

15–20% Bias / Precision

Bio Analytical Method validation Results (contd..,)

1. **Screening and Selectivity**

Specificity : for an analyte

Selectivity: for a matrix

Experimental Design:

- Matrix blanks: 8 lots, n=1 for each lot
- Matrix blank fortified with IS: 8 lots, n=1 for each lot
- LLOQ Selectivity Sample: 6 lots, fortified with analyte at LLOQ level and IS. n=3 for each lot

Result: 7 out of the 8 lots meet the following criteria:

✓Response for the analyte in matrix blanks and matrix blank fortified with IS were ≤20% of the mean analyte response in the acceptable LLOQ.

✓ Selectivity LLOQ replicates for each lot meets accuracy acceptance limit, and the mean accuracy was within ±20.0% of the nominal concentration

Bio Analytical Method validation Results (contd..,)

2. ASCOT (AUTO SAMPLER CARRIES OVER TEST)

Sequence:

Aqueous blank (without spiked drug)-1
Highest aqueous concentration
Aqueous blank (without spiked drug)-2
Lowest aqueous concentration.
Blank matrix without drug-1
Extracted Highest concentration
Blank matrix without drug -2
Extracted Lowest concentration

%Carry Over for Aqueous samples={Area of Aq.Blank-2 - Aq.Blank-1/Area of Aq.LLOQ} * 100

%CarryOver for Extracted samples={Area of Ex.Blank-2 - Ex.Blank-1/Area of Ext STD8} * 100

Result: Calculated the % carryover at the RT of analyte/ISTD in both unextracted and Extracted samples. The %Carry over for RT of analyte & ISTD not more than 5% .

Bio Analytical Method validation Results (contd..,)

3. Linearity

Experimental Design: A calibration curve consist of

- ❖A blank sample (matrix sample processed without analyte or internal standard),
- ❖A zero sample (matrix sample processed without analyte but with internal standard), and
- ❖Six non-zero samples (matrix samples processed with analyte and internal standard)

  covering the expected range, including LLOQ.
- ❖Four concentrations (including LLOQ, low, medium, and high), each concentration n=3

Results:

- ✓Standards were not more than 15% of nominal concentrations, except at LLOQ where the standard was not more than 20%.
- ✓The analyte response at the LLOQ was five times the response compared to blank response.
- ✓Correlation coefficient: r2 was 0.9889 to 0.9985 for both drugs .

Bio Analytical Method validation Results (contd..,)

4. Accuracy and precision

Experimental Design: For both the inter-run and intra-run experiments, as followed and used the linearity data to calculate the accuracy and precision

Result:

✓For Accuracy, the mean values for both PER and AMD were within 15% of the nominal value, except at LLOQ, where it was not more than 20%.

✓The precision determined at each concentration level and it were not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it showas not exceed 20% of the CV.

Bio Analytical Method validation Results (cond..,)

5. Recovery

Experimental Design: Analyte at LQC and HQC levels, and IS at the level of use: pre extraction spiked samples (n=6) are compared with mean response of post extraction spiked matrix samples (n=6)

$$\% \text{ Recovery} = \frac{\text{Extracted sample Response}}{\text{Un-extracted sample response}} \times 100$$

Result: Recovery of the analyte were not be 100%, but the extent of recovery of an analyte and of the internal standard was observed consistent, precise, and reproducible.

Bio Analytical Method validation Results (cond..,)

6. Dilution (Parallelism)

➢Dilution of samples should not affect the accuracy and precision. If applicable, dilution integrity should be demonstrated by spiking the matrix with an analyte concentration above the ULOQ and diluting this sample with blank matrix. Accuracy and precision should be within the set criteria, i.e. within ±15%.

Experimental Design: Two level at ULOQ concentration (2 fold and 4fold dilution); each dilution, n=6.

Result:

Mean accuracy was within ±15.0% RE of nominal; precision was ≤15.0% RSD.

Bio Analytical Method validation Results (cond..,)

7. Matrix Effect

Experimental Design: It was evaluated by processing post extracted spiked samples at six replicates of LQC and HQC concentration and analyzed with aqueous LQC and HQC concentration and difference of response is calculated.

Calculation:

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.

Result:

Both QC samples MF was within 0.85 to 1.15 and %CV for each set of LQC and HQC were not more than 15%.

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Thank you for your attention