LC-MS/MS METHOD FOR SCREENING OF INTOXICATION AND DRUG ADHERENCE OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS IN PLASMA

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Background

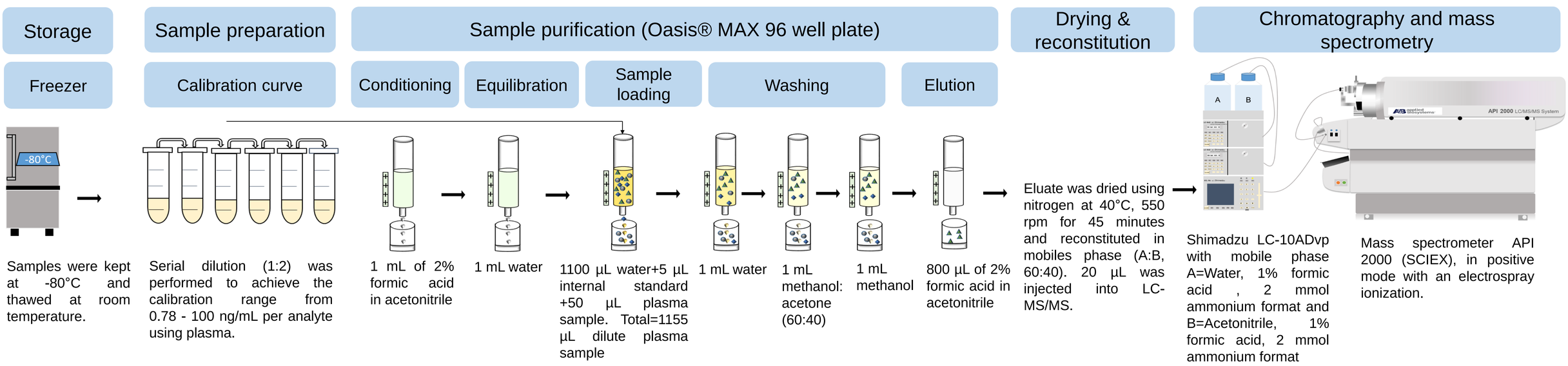
Cardiovascular diseases contribute largely to the portion of non communicable diseases and remain a high burden for the population causing more than 17 million deaths worldwide each year. Angiotensin converting enzyme inhibitors (ACEIs) are used for the treatment of cardiovascular diseases. The therapeutic effectiveness of these preventive agents is closely related to medication adherence by patients. Additionally, the increased availability of these drugs has led to the increased events of intoxication either intentionally or unintentionally (1,2). Qualitative screening of these agents using liquid chromatography-tandem mass spectrometry represents a reliable technique for monitoring medication adherence as well as intoxication.

Objective

The objective was to develop a qualitative screening method for commonly prescribed angiotensin converting enzyme inhibitors using residual blood volume 50 µL to avoid additional sampling stress both in paediatrics and adults.

Methods

Fit-for-purpose validation was performed for limit of detection (LOD), recovery and matrix effect. Further the accuracy and precision was also conducted for the semi-quantitation following EMA, FDA and ICH guidelines(3,4,5).



Samples were kept at -80°C and thawed at room temperature.

Serial dilution (1:2) was performed to achieve the calibration range from 0.78 - 100 ng/mL per analyte using plasma.

1 mL of 2% formic acid in acetonitrile

1 mL water

1100 µL water+5 µL internal standard +50 µL plasma sample. Total=1155 µL dilute plasma sample

1 mL water

1 mL methanol: acetone (60:40)

1 mL methanol

800 µL of 2% formic acid in acetonitrile

Eluate was dried using nitrogen at 40°C, 550 rpm for 45 minutes and reconstituted in mobiles phase (A:B, 60:40). 20 µL was injected into LC-MS/MS.

Shimadzu LC-10ADvp with mobile phase A=Water, 1% formic acid , 2 mmol ammonium format and B=Acetonitrile, 1% formic acid, 2 mmol ammonium format

Mass spectrometer API 2000 (SCIEX), in positive mode with an electrospray ionization.

Results

Method validation

Following results were obtained from the semi-quantitative validation for linearity, accuracy and precision along with recovery and matrix effect.

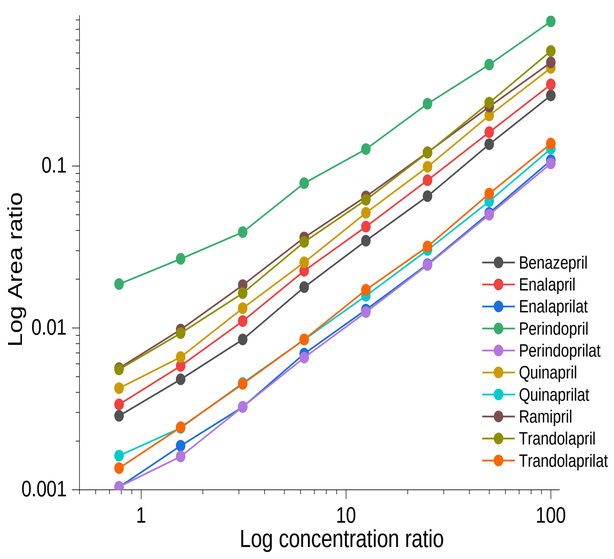


Figure 1: *Mean (n=3) calibration curve for all analyte with linearity range of 0.78-100 ng/mL. Area ratio=Analyte peak area/internal standard peak area. Concentration ratio= Analyte concentration/internal standard concentration. Least square weighted regression was applied. Weighting=1/x^2*

Analyte name	r-value	LOD ng/mL	LOQ ng/mL	Recovery %	Absolute ME %
Benazepril	0.997	0.56	1.72	90.69	2.70
Enalapril	0.999	0.56	1.70	95.38	4.23
Enalaprilat	0.998	0.60	1.82	91.07	4.26
Perindopril	0.993	0.41	1.26	93.03	0.00
Perindoprilat	0.998	0.54	1.65	87.74	1.88
Quinapril	0.998	0.58	1.56	94.07	2.50
Quinaprilat	0.998	0.60	1.83	88.89	13.41
Ramipril	0.996	0.65	1.99	95.81	7.28
Trandolapril	0.999	0.59	1.79	93.04	3.60
Trandolaprilat	0.999	0.50	1.76	95.08	1.93

Table 1: *Obtained results of validation parameters including co-efficient of correlation values (r-value) for linearity (0.78-100 ng/mL, mean (n=3)) for all analytes, LOD, LOQ, recovery and absolute ME (n=2). LOD=Limit of detection, LOQ=Limit of quantification. ME=Matrix effect*

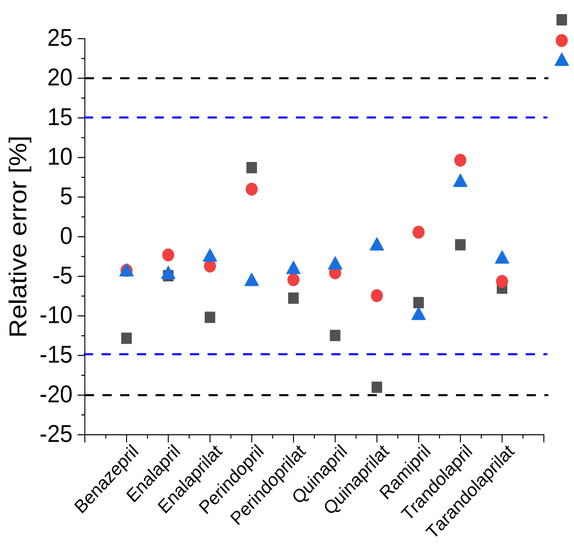


Figure 2: *Inter-day accuracy in terms of relative error [%] at three quality control levels. n=3, LQC=Lower quality control (3.13 ng/mL), MQC=Middle quality control (25 ng/mL), HQC=High quality control (100 ng/mL). Black dotted line represents acceptance limit for LQC (±20%) and blue represents for MQC and HQC (±15%) for relative error [%]*

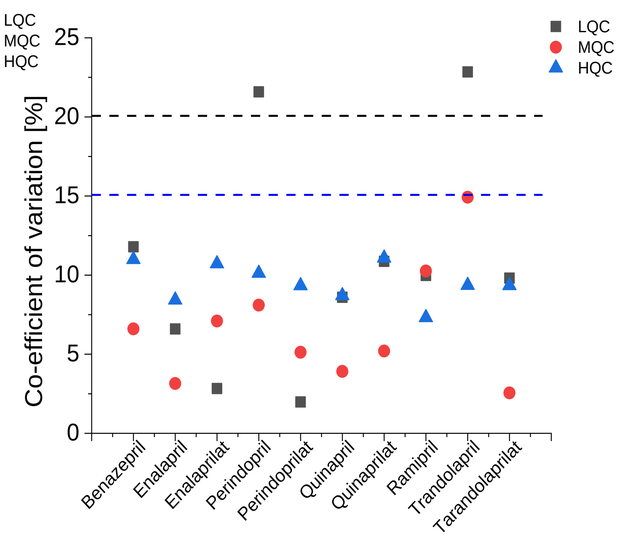


Figure 3: *Inter-day precision in terms of co-efficient of variation [%] at three quality control levels. n=3, LQC= Lower quality control (3.13 ng/mL), MQC=Middle quality control (25 ng/mL), HQC=High quality control (100 ng/mL). Black dotted line represents acceptance limit for LQC (20%) and blue dotted line represents acceptance limit for MQC and HQC (15%) for variation*

Optimized sample purification

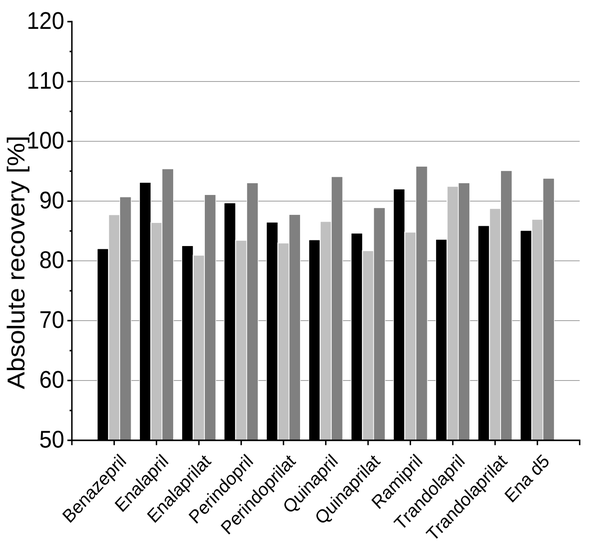


Figure 4: *Effect of different conditioning, washing and elution solution on absolute recovery of all analytes and internal standard enalapril D5 (Ena D5), **Black**=Water and methanol: acetone (60:40) for washing and 2% formic acid in methanol for conditioning and elution, **Light grey**=Water, methanol: acetone (60:40) and methanol for washing and 2% formic acid in methanol for conditioning and elution, **Dark grey**=Water, methanol: acetone (60:40) and methanol for washing and 2% formic acid in acetonitrile for conditioning and elution. n=2*

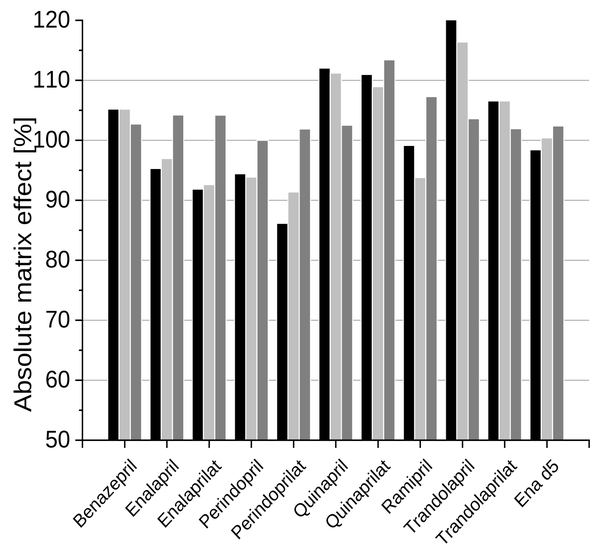


Figure 5: *Effect of different conditioning, washing and elution solution on absolute matrix effect of all analytes and internal standard enalapril D5 (Ena D5), **Black**= Water and methanol: acetone (60:40) for washing and 2% formic acid in methanol for conditioning and elution, **Light grey**=Water, methanol: acetone (60:40) and methanol for washing and 2% formic acid in methanol for conditioning and elution, **Dark grey**=Water, methanol: acetone (60:40) and methanol for washing and 2% formic acid in acetonitrile for conditioning and elution. n=2*

Optimized chromatographic separation

The chromatographic gradient was finalised for achieving the maximum intensity and proper resolution specifically among the pro-drug and active metabolite.

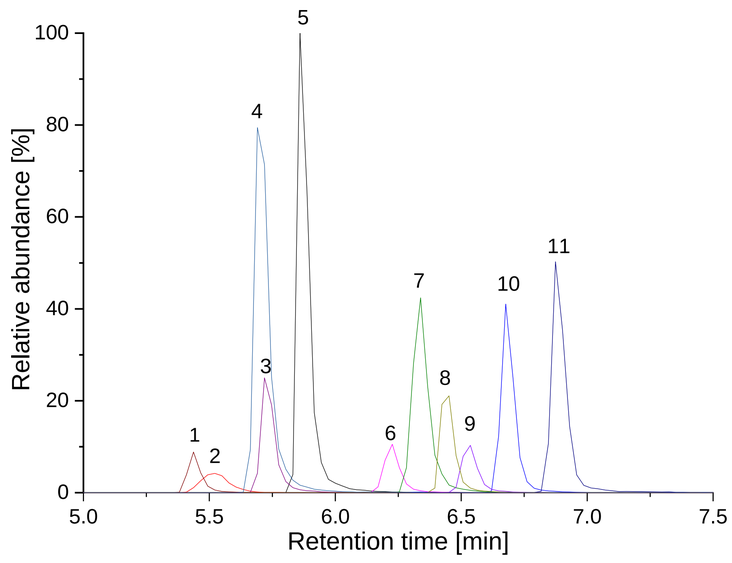


Figure 6: *Chromatogram for all analytes. 1. Enalaprilat (RT=5.40 min), 2. Perindoprilat (RT=5.48 min), 3. Enalapril D5 (RT=5.56 min), (IS) 4. Enalapril (RT=5.69 min), 5. Perindopril (RT=5.83 min), 6. Quinaprilat (RT=6.17 min), 7. Ramipril (RT=6.29 min), 8. Benazepril (RT= 6.40 min), 9. Trandolaprilat (RT=6.47 min), 10. Quinapril (RT=6.62 min), 11. Trandolapril (RT=6.82 min). RT = Retention time*

Conclusion:

The screening method was successfully developed and partially validated qualitatively for monitoring of medication adherence and intoxication of 10 ACEIs in 50 µL residual blood samples.

References

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Disclosure

The results presented here have already been published in BIOANALYSIS VOL. 10, NO. 23 and permission was duly obtained from the journal editor to present the contents as a poster on congress.

More information

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