A Reduced Workflow SPE- LC-ESI-MS/MS Method To Distinguish Healthy From Elevated Concentrations Of Metanephrine And Normetanephrine In Patient Plasma Samples

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Introduction

Adrenal neuroendocrine tumors known as pheochromocytomas induce excessive production of catecholamines in mammalian blood and urine. The salient metabolites, metanephrine and normetanephrine, are routinely screened as biomarkers for this condition in both matrices. The bottleneck of these analytical methods has traditionally been laborious sample preparation methods that mitigate the variability in matrix inherent with patient samples. Additional issues include the complexity of the measurement that challenges detectors that lack sensitivity and robustness. This report details a "load, wash, elute" weak cation exchange solid phase extraction procedure amenable to both plasma and urine samples. The extracts are subsequently injected into an LC-MS/MS system. The preliminary sample preparation method was developed at the Biotage US Applications lab (Charlotte, NC).The method was then transferred to Ionics (Bolton, ON, Canada) to facilitate the nmole/L measurements of the selected biomarkers by laminar flow tandem mass spectrometry. The SPE-LC-ESI-MS/MS method parameters were first optimized using pooled mixed gender plasma. A set of patient samples (n=32) was later supplied by the Mayo Clinic (Rochester, MN) that had previously been analyzed by a validated referee method. The population represented measured values across a range of clinical relevance.

Experimental Procedure

Patient samples (n=32) – proof-of-concept study

A split sample study was performed to determine the performance of the candidate method versus the gold standard method from the Mayo clinic. A brute force comparison of the data is given in Figures 1 and 2. The nominal values of the obtained measurements tracked in close agreement with the historical data obtained from the Mayo clinic for these samples. Correlation plots are provided. Since the Mayo Clinic does not report values <0.2 nmole/L , samples measuring at or below these values were excluded from these graphs. In addition, an outlier (patient 16) was excluded from the metanephrine correlation plot. An example of a real patient chromatogram from this study has previously been reported at MSACL 2014 (Ye et al.).

Mass Spectrometry

Detection of the target analytes was optimized using an Ionics 3Q 220 triple quadrupole mass spectrometer operated in MRM mode The details of the instrument method were described previously[1].

Chromatography

Chromatographic separation was accomplished using a Shimadzu UFLCxr Liquid Handling System. Optimized gradient chromatographic conditions were identified using the Restek PFP column (2.1 mm x 100 mmx 3.0 µm)[1] . The injection volume was 50 µL. The method was isocratic (80/20, A/B) with a 1 mL flow rate. Positve ion mode. The mobile phase A and B were 0.1% formic acid (aq) and 0.1% formic acid in acetonitrile.

Solid Phase Extraction (SPE)

Table 1: solid phase extraction procedure

Step	EVOLUTE Express WCX 96 well plate (30mg)
Sample	100µL plasma 300µL 50mM $NH_4Ac_{(aq)}$
Sample load	0.4 mL
Wash 1	1 mL H2O
Wash 2	1 mL 50//50% MeCN/MeOH
Elute	2 x 0.9 mL 47.5/47.5/5% MeCN/MeOH/Formic acid
Evaporation / recon	0.1 mL mobile phase

Results

A summary of the optimized method performance (with pooled plasma) is given in table 2. Correllation plots are provided in Figures 1a and 1b. A split sample comparison is provided in Figure 2.

Conclusions

It is anticipated that this time saving and sensitive SPE-LC-ESI-MS/MS method will have significant impactin population screening strategies for these metabolites.

Reference

1. IONICS Application note 125A (ionics.com)
2. Clinical Chemistry 50:3, 603–611 (2004)

Acknowledgement

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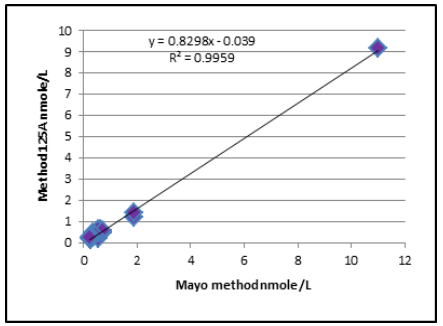
Table 2 Development summary – pooled plasma

	metanephrine	normetanephrine
Structures		
[2]clinical reference range (nmole/L)	**0.05- 0.477**	**0.12-1.1**
Candidate Method LOQ (nmole/L)	**0.05**	**0.1**
Linearity 0.1 – 20 (nmole/L)	**r^2=0.999**	r^2=**0.999**
%Recovery Pooled plasma	**82.4**	**89.6**
%Matrix suppression Pooled plasma	**<10**	**<10**
Method repeatability (%RSD)	**<15**	**<15**

a)



b)

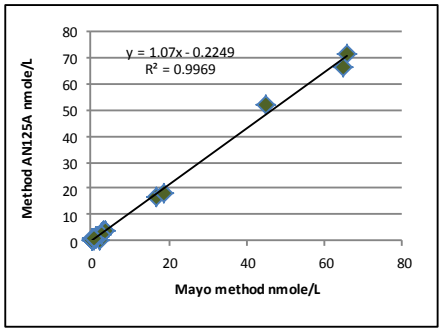


Figure 1: *Correlation plots: a) metanephrine b) normetanephrine*

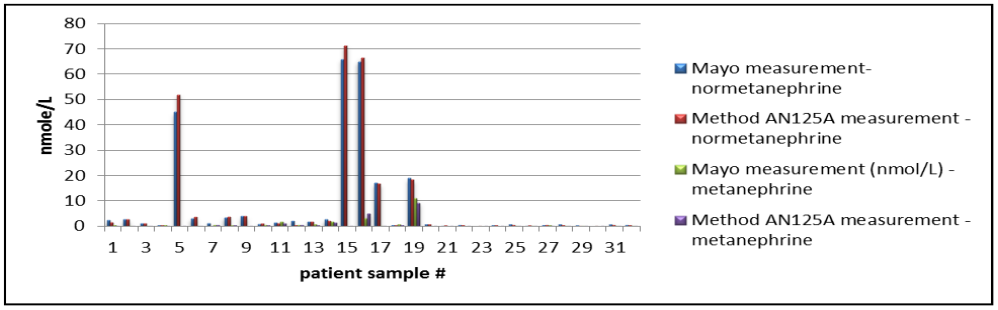


Figure 2: *Split patient sample comparison – full data set (n=32)*

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