

1599-9830

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## Metanephrine and Normetanephrine Quantification by SPE-LC-MS/MS

### Introduction

Pheochromocytomas are catecholamine producing tumors found mainly in the adrenal medulla. These tumors can present as unexplained hypertension and, though rare, can be fatal if not diagnosed and treated appropriately.<sup>[1]</sup> Metanephrine (ME) and Normetanephrine (NME), are routinely screened in both urine and plasma serving as the best biochemical markers for Pheochromocytomas. However, measurement of metanephrines and normetanephrine in plasma is challenging due to the low physiological concentrations, their hydrophilic nature<sup>1</sup>, and time consuming traditional sample preparation. With use of the PerkinElmer QSight® 220 triple quadrupole mass spectrometer, LC-MS/MS analysis was performed using the recently advanced solid phase extraction (SPE) sorbent technology (“load, wash, elute”) method protocol. As a result low levels of ME and NOR are detectable in plasma with short sample preparation and LC run time. This LC-MS/MS method provides a fast, sensitive, accurate, and reproducible solution for the analysis of ME and NOR in plasma.

## 2. Method

### 2.1. Chemicals and Solvents

ME and NME were purchased from Cerilliant Corp (Round Rock, TX). All the HPLC grade water (H<sub>2</sub>O), methanol (MeOH), acetonitrile (MeCN), and formic acid (FA) were purchased from Caledon Lab (Georgetown, ON). The pooled human plasma with EDTA K<sub>2</sub> was obtained from BioChemed Services (Winchester, VA). All the chemicals were stored in the freezer. The stock solutions of ME and NME (10µg/mL) were prepared and used to be diluted sequentially to lower concentration for calibration curve preparation. No IS was used.

### 2.2. Solid Phase Extraction

SPE was carried out with the Biotage EVOLUTE EXPRESS WCX (30mg) sorbent selection 96-well plate. The washing step was performed with a Vacmaster-96 manifold, and the evaporation step was carried out with the SPE Dry 96 Evaporator, all of which were supplied by Biotage (Charlotte, NC). Refer to Table 1 the steps used.

Table 1: SPE Steps & Procedure

Step	Procedure
Sample	300µL 50mM NH <sub>4</sub> Ac + 100µL Sample
Sample load	0.4mL
Wash 1	1mL H <sub>2</sub> O
Wash 2	1mL 50/50% (v/v) MeCN/MeOH
Elute	2 x 0.9mL 95/5% (v/v), Wash 2/FA
Evaporate	Evaporate 5min after well appeared dry
Reconstitution	0.5mL mobile phase

### 2.3 Mass Spectrometry Conditions

The SPE-LC-MS/MS analysis was performed using the PerkinElmer QSight® 220 triple quadrupole mass spectrometer. Table 2 outlines the instrumental parameter settings used during this method. Multiple MRM experiments were used to accommodate the fast UHPLC system. The optimized MRM parameters for Metanephrine and Normetanephrine are shown in Table 3.

### Quick Facts:

- High sensitivity SPE LC-MS/MS method for quantitation of Metanephrine and Normetanephrine using the QSight® 220 triple quadrupole mass spec
- Calibration curve generated for Metanephrine and Normetanephrine with R<sub>2</sub> > 0.996 over 0.005 to 1ng/mL concentration range
- LOQ's for Metanephrine and Normetanephrine were 0.005 and 0.01pg/µL respectively.

Table 2: MS conditions used on the QSight® 220 instrument during the method.

ESI Voltage (V)	5850
HSID Temp (°C)	300
Nebulizer Gas Setting	450
Drying Gas Setting	120
Heating Gas	350
Source Temp. (oC)	300
Dwell Time (ms)	20
Pause Time (ms)	5

Table 3: Optimized MRM Parameters

Compound	Precursor (m/z)	Fragment (m/z)	CCL 2	CE
Metanephrine	180.0	148.0	-50	23
	180.0	165.0	-40	23
Normetanephrine	166.0	134.0	-30	22
	166.0	122.0	-30	24

### 2.4. LC Conditions

A Restek PFPP (2.1mm X 100mm) 3.0 µm particle size column was used. The LC was run with a gradient flow with a run time of 2.5min and the following conditions:

**Mobile Phase:** A (0.1% Formic Acid in H<sub>2</sub>O)  
 B (0.1% formic acid in ACN)

**Flow rate:** 0.5 mL/min

**Injection volume:** 50 µL

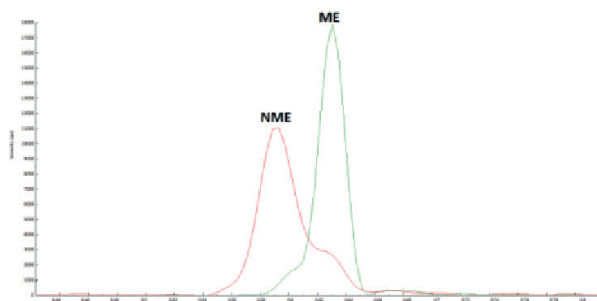
**Column temperature:** 22 °C

### 3. Results

#### 3.1. Extracted Ion Chromatograms (EICs)

The retention times for Normetanephrine and Metanephrine are about 0.58 and 0.63 minutes, respectively. A representative overlaying chromatogram of NME and ME is shown in Figure 1.

Figure 1: Overlaying Chromatogram of ME and NME



Figures 2-4 shows the EIC for ME MRM transition 180.0/148.0. The plasma was spiked with neat ME standard at the given concentrations before running through the SPE procedure.

Figure 2: EIC plasma blank post SPE cleanup for ME showing the LC-MRM Transition (180.0/148.0)

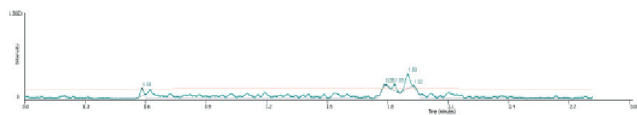


Figure 3: EIC for 0.005ng/mL ME pre spiked plasma following SPE cleanup showing the LC-MRM Transition (180.0/148.0)

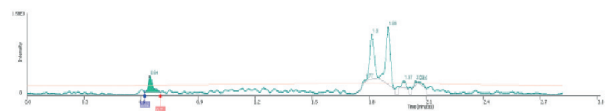
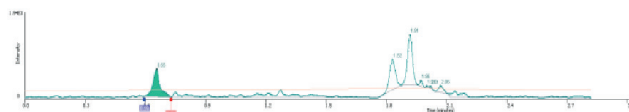


Figure 4: EIC for 0.01ng/mL ME pre spiked plasma following SPE cleanup showing the LC-MRM Transition (180.0/148.0)



#### 3.2. Linearity

A calibration curve for doublet injections for ME and NME standard was created for a concentration range of 0.005ng/mL to 1.0ng/mL shown below (Figures 5 & 6 respectively). The R<sup>2</sup> value is >0.996 for linear regression fit with 1/x weighting. All the calculated CV and accuracy for ME and NME were within the variation of 15 % .

Figure 5: Calibration curve for ME (180.0/148.0) with doublet injections for each concentration

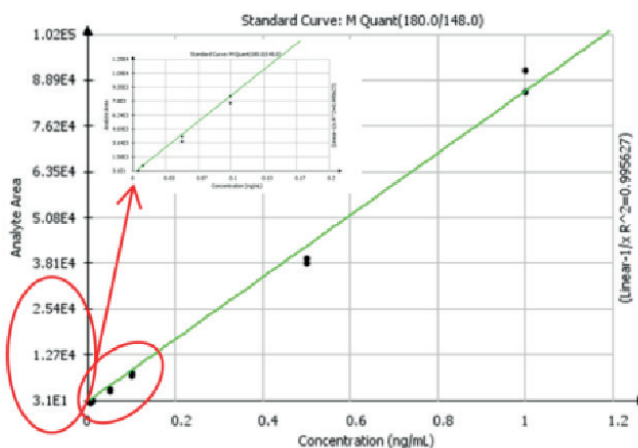
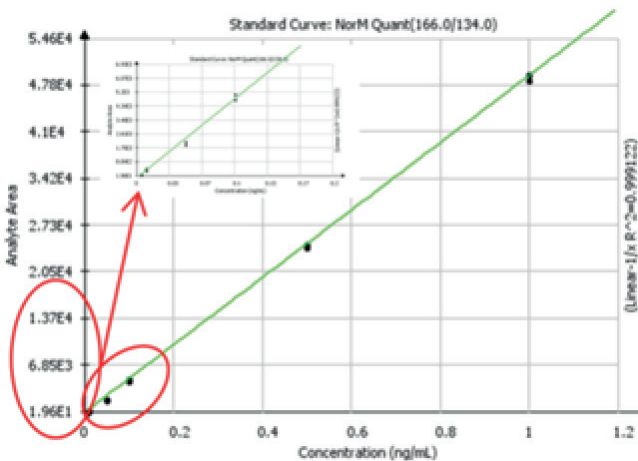


Figure 6: Calibration curve for NME (166.0/134.0) with doublet injections for each concentration



### 3.3. Solid Phase Extraction

The extraction performance results using Biotage EVOLUTE EXPRESS WCX 96-well plate is shown in Table 4, for ME at 0.005ng/ mL.

Table 4: Extraction Performance Results for

Recovery Rate	82.4%
Matrix Effect	103.0%
Process Efficiency	86.8%

### 4. Conclusion

The PerkinElmer QSight® 220 triple quadrupole mass spectrometer proved to be a successful analyzer with high sensitivity for SPE-LC-MS/MS analysis of Metanephrine and Normetanephrine in plasma. The SPE-LC-MS/MS process showed no signs of interferences, and allowed for a good recovery rate of 84% at the LOQ for ME. The fast and efficient method allowed for LOQ's of 0.005 and 0.01ng/ mL for ME and NME, respectively.

### 5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

### Reference

1. Gabler, J., Chao Y., and Sihe W. Chromatography Separation Techniques S2 (2012).

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