Determination of Urinary Catecholamines and Metanephrines in a single run

Analysis by offline SPE and LC-MS/MS for Clinical Research

Linda Côté
Senior Clinical Application Specialist
Agilent Technologies
In this presentation, we will be discussing:

- A four minute method for quantifying catecholamines and their metabolites: epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine and 3-methoxytyramine
- The use of offline solid phase extraction (SPE) for simultaneous extraction of all six analytes from urine
- The chromatographic separation of all six analytes with conditions compatible with LC-MS/MS
- Typical method performance results
- Tips and tricks
Pathways of catecholamine synthesis and O-methylation

Tyrosine $\xrightarrow{TH}$ DOPA $\xrightarrow{AADC}$ Dopamine $\xrightarrow{COMT}$ Methoxytyramine

$\xrightarrow{DBH}$ Norepinephrine $\xrightarrow{COMT}$ Normetanephrine $\xrightarrow{PNMT}$ Epinephrine $\xrightarrow{COMT}$ Metanephrine
Molecular Genetics and Metabolism

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Mintreview

A new perspective on the treatment of aromatic L-amino acid decarboxylase deficiency


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* Neurometabolic Unit, The National Hospital for Neurology and Neurosurgery, London, UK

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**Diagram:**

- **Tyrosine** → BH4 + O₂ → TH → L-dopa → COMT → 3-O-Methyl dopa → MAO/COMT → HVA
- **Tryptophan** → BH4 + O₂ → TPH → 5-HTP → AADC → PLP → Serotonin → MAO → 5-HIAA
- **L-dopa** → TH → Dopamine → DBH → Noradrenaline → PNMT → Adrenaline
- **5-HTP** → PLP → 3-O-Methyl dopa → MAO/COMT → HVA
- **Serotonin** → N-acetyl-serotonin → HIOMT → Melatonin

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Which ones and in which matrix urine or plasma?

- Free catecholamines and metanephrines in urine
- Total catecholamines and metanephrines in urine
- Free catecholamines in plasma
- Free metanephrines in plasma
- VMA in urine
- HVA in urine
- 5-HIAA in urine
Which ones and in which matrix urine or plasma?

- Free catecholamines and metanephrines in urine
- Total catecholamines and metanephrines in urine
- Free catecholamines in plasma (in progress)
- Free metanephrines in plasma (in progress)
- VMA in urine (in progress)
- HVA in urine (in progress)
- 5-HIAA in urine (in progress)
Compound structures

Dopamine (D)
C8H11NO2
Neutral Mass: 153.08

Norepinephrine (NE)
C8H11NO3
Neutral Mass: 169.07

Epinephrine (E)
C9H13NO3
Neutral Mass: 183.09

3-Methoxytyramine (3-MT)
C9H13NO2
Neutral Mass: 167.09

Normetanephrine (NMN)
C9H13NO3
Neutral Mass: 183.09

Metanephrine (MN)
C10H15NO3
Neutral Mass: 197.11
Product Ion Scans (MS/MS)

D

NE

E

3-MT-H2O

NMN-H2O

MN-H2O

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Product Ion Scans (MS/MS)
Must separate E and NMN by chromatography

For Research Use Only. Not for use in diagnostic procedures.
Product Ion Scans (MS/MS)
Must separate 3-MT and MN by chromatography
Final Method Gradient to Include 3-MT

✓ Need to keep resolution between E and NMN
✓ Also between MN and 3-MT
## Internal Standards

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Internal Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>Dopamine-D4</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Norepinephrine-D6</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Epinephrine-D6</td>
</tr>
<tr>
<td>3-Methoxytyramine</td>
<td>3-Methoxytyramine-D4</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>Normetanephrine-D3</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>Metanephrine-D3</td>
</tr>
</tbody>
</table>

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Sample preparation

• Calibrators are prepared with clean urine matrix from Golden West Biologicals
• Isotopically labelled Internal standards
• 24 hours collection of urine
• Native urine for free catecholamines and metabolites (typically for catecholamines)
• Acid-hydrolysed urine for total (typically for metanephrines)
• Solid phase extraction (SPE) is used to cleanup urine
Solid Phase Extraction (SPE)
“The diphenyl boronate forms a stable negatively charged complex (Fig. 1) with cis-hydroxyl groups of catecholamines, which is strongly retained on a C18 extraction sorbent when operating in alkali media. This allows for column washing with methanol-buffer solutions to remove interfering compounds without the loss of the catecholamines which are eluted by disrupting the complex under acid conditions”.

Fig. 1. Diphenyl boronate–catecholamine complex structure. (For noradrenaline: R1=OH, R2=H; for adrenaline: R1=OH, R2=CH3; for dopamine: R1=H, R2=H). Adapted from Ref. [6].

Catecholamines and metanephrines in a single run

- One SPE cartridge is used to recover both catecholamines and metanephrines
- SPE Bond Elut Plexa was chosen for best recoveries
- Simple acid elution for direct injection into LC-MS/MS
- pH control for stabilization of catecholamines
- Metanephrines are also retained under the same conditions, even though they are methylated and do not contain the cis-diol moiety for the covalent linkage binding mechanism
- Studies have shown that metanephrines do have some affinity for this sorbent

Bond Elut Plexa
A Unique “Polar Enhanced” Polymeric Sorbent

- Unique chemistry with Polarity gradient:
  - Hydrophilic OH ligands on the exterior
  - Frequency of OH is reduced as getting inside
  - Hydrophobic deep inside the pore

Going thru the Plexa cartridge

Plexa particle
Bond Elut Plexa – How does it works

**Apply Sample**
Water Rich Hydrophilic component allows excellent phase transfer of analytes into the polymer core

**Washing**
Analytes remain tightly bound in the hydrophobic core.

**Elution**
Specially engineered pore structure allows excellent mass transfer out of polymer.

Large endogenous proteins do not bind to the surface of the polymer and cannot access pore structure.

Interferences (lipids and proteins) washed away without into the analytes of interest.

Clean extract with high recovery.
Sample Preparation
Solid Phase Extraction (SPE)

Prepare complexed samples:
- 0.5 mL urine*, calibrators, QCs*
- Add 40 µL of internal standards mix
- Add 0.8 mL of complexing agent
- Verify pH, must be between 7.5-9.5. If necessary adjust to pH 8.5 with NH4OH

Step 1: Condition SPE cartridge (Bond Elut Plexa, 30 mg, 3 mL) with:
- 1 mL of MeOH
- 1 mL of wash buffer 0.2 M NH4Cl-NH4OH

Step 2: Add complexed samples

Step 3: Wash with 1 mL of 5% MeOH wash buffer 0.2 M NH4Cl-NH4OH
- Dry at full vacuum for 5 minutes

Step 4: Elute with 1 mL of 5% formic acid in water. Apply vacuum 5” Hg for 30 seconds
- Transfer to autosampler vial

* Native for free catecholamines, hydrolyzed for total metanephrines
  (add 25 µL HCl 6N, incubate at 90 deg. C for 25 min., cool at RT)

**Four Minute Method LC Conditions**

**Agilent 1290 LC**

- **Analytical Column:** Pursuit 3 PFP, 2 x 150 mm, 3 µm
- **Guard Column:** Pursuit 3 PFP MetaGuard 2 mm
- **Columns Temp:** 40 °C
- **Injection volume:** 20 µL
- **Needle Wash:** 1:1:1:1 MeOH:ACN:IPA:H₂O + 0.1% formic acid (20 sec)
- **Injector Temp:** 4 °C
- **Mobile Phase:**
  - A: 0.2% Formic Acid in Water
  - B: Methanol
- **Flow rate:** 0.3 mL/min.

<table>
<thead>
<tr>
<th>1290 Pump Gradient:</th>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
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<td>0.5</td>
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<td>1.5</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

- **Stop time:** 4 min.
- **Re-equilibration time:** 3 min. (use automated column regeneration for increased throughput)
Automated Column Regeneration (ACR)
For increased throughput

Position 1 (Port 1 > 2)
- Pump 1
- ALS
- Column A
- Waste
- Pump 2
- Column B
- Mass Spec

Position 2 (Port 1 > 10)
- Pump 1
- ALS
- Column A
- Waste
- Pump 2
- Column B
- Mass Spec

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**Agilent 6460 QQQ MS/MS**

**Ion Source:** Agilent Jet Stream (AJS)

**Ion Mode:** Positive Ionization

**Ion source conditions**

- Drying gas temperature: 325°C
- Drying gas flow: 5 L/min
- Nebulizer pressure: 35 psi
- Sheath gas temperature: 375°C
- Sheath gas flow: 12 L/min
- Capillary voltage: 3000 V
- Nozzle voltage: 0 V
- Q1/Q3 resolution: 0.7 unit
- $\Delta$ EMV: 200 V
## MRM Transitions Table

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prec Ion</th>
<th>Prod Ion</th>
<th>Dwell</th>
<th>Frag (V)</th>
<th>CE (V)</th>
<th>CAV (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine*</td>
<td>154.1</td>
<td>137.1</td>
<td>20</td>
<td>75</td>
<td>8</td>
<td>3</td>
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<tr>
<td>Dopamine</td>
<td>154.1</td>
<td>91.1</td>
<td>20</td>
<td>75</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Dopamine-D4</td>
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<td>141.1</td>
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<td>75</td>
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<tr>
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<td>152.1</td>
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<td>65</td>
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<tr>
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<td>107</td>
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<td>Norepinephrine-D6</td>
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<td>Epinephrine*</td>
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<td>166.1</td>
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<td>70</td>
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<td>5</td>
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<tr>
<td>Epinephrine</td>
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<td>107.1</td>
<td>20</td>
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<td>Epinephrine-D6</td>
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<tr>
<td>3-Methoxytyramine*</td>
<td>151.1</td>
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<td>135</td>
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<td>3-Methoxytyramine-D4</td>
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<td>95.1</td>
<td>20</td>
<td>135</td>
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<td>Normetanephrine*</td>
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<td>134</td>
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<td>Normetanephrine</td>
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<td>106.1</td>
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<td>Normetanephrine-D3</td>
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<td>137.1</td>
<td>20</td>
<td>105</td>
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<td>3</td>
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<tr>
<td>Metanephrine*</td>
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<td>165.1</td>
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<td>120</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>180.1</td>
<td>148.1</td>
<td>20</td>
<td>120</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Metanephrine-D3</td>
<td>183.1</td>
<td>168.1</td>
<td>20</td>
<td>120</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

* = Quantification transition
Example chromatogram

1. Norepinephrine
2. Epinephrine
3. Normetanephrine
4. Dopamine
5. Metanephrine
6. 3-Methoxytyramine
Example chromatogram
E/NMN and MN/3-MT Resolution is Critical

1. Norepinephrine
2. Epinephrine
3. Normetanephrine
4. Dopamine
5. Metanephrine
6. 3-Methoxytyramine

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Calibration curves

- **Dopamine**
  \[ y = 0.003 \times x - 7.61 \times 10^{-4} \]

- **Norepinephrine**
  \[ y = 0.002 \times x - 9.603 \times 10^{-5} \]

- **Epinephrine**
  \[ y = 0.003 \times x - 3.078 \times 10^{-4} \]

- **3-Methoxytyramine**
  \[ y = 0.005 \times x + 0.004 \]

- **Normetanephrine**
  \[ y = 0.004 \times x - 4.174 \times 10^{-4} \]

- **Metanephrine**
  \[ y = 0.003 \times x - 0.002 \]
Calibration curves in log scales

Dopamine

\[ y = 0.003 \times x \times 7.61\times10^{-4} \]

Norepinephrine

\[ y = 0.002 \times x \times 9.603\times10^{-5} \]

Epinephrine

\[ y = 0.003 \times x \times 3.078\times10^{-4} \]

3-Methoxytyramine-H2O

\[ y = 0.005 \times x + 0.004 \]

Normetanephrine-H2O

\[ y = 0.004 \times x - 4.174\times10^{-4} \]

Metanephrine-H2O

\[ y = 0.003 \times x - 0.002 \]
## Results

### Summary of Analyte Performance

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^2$</th>
<th>Concentration (ng/mL)</th>
<th>Concentration (nmol/L)</th>
<th>Accuracy (%) n = 3</th>
<th>Intraday CV (%) n = 3</th>
<th>Interday CV (%) n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>0.9997</td>
<td>1.56</td>
<td>10.2</td>
<td>107.5</td>
<td>1.0</td>
<td>2.7</td>
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<td></td>
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<td>62.5</td>
<td>408.0</td>
<td>99.1</td>
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<td>1000</td>
<td>6528.3</td>
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<td>0.3</td>
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<td>Norepinephrine</td>
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<td>1.56</td>
<td>9.2</td>
<td>102.9</td>
<td>0.9</td>
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<td></td>
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<td>62.5</td>
<td>369.4</td>
<td>101.1</td>
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<td>Epinephrine</td>
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<td>1.56</td>
<td>8.5</td>
<td>101.6</td>
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<td>2.7</td>
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<tr>
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<td>62.5</td>
<td>341.2</td>
<td>100.9</td>
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<td>1000</td>
<td>5458.4</td>
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<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Note: Signal to noise ratios and CVs indicate that LLOQs are lower than measured here for all analytes.
### Results

#### Summary of Analyte Performance

<table>
<thead>
<tr>
<th>Compound</th>
<th>R²</th>
<th>Concentration (ng/mL)</th>
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<th>Interday CV (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>n = 3</strong></td>
<td><strong>n = 3</strong></td>
<td>n = 5</td>
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<tr>
<td>3-Methoxytyramine</td>
<td>0.9999</td>
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<td>28</td>
<td>95.7</td>
<td>1.1</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
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<td>187.5</td>
<td>1121.4</td>
<td>102.9</td>
<td>0.9</td>
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</tr>
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<td></td>
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<td>100.0</td>
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<td>0.3</td>
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<td>25.6</td>
<td>100.1</td>
<td>1.5</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
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<td>187.5</td>
<td>1023.45</td>
<td>102.0</td>
<td>1.1</td>
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<td>3000</td>
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<tr>
<td>Metanephrine</td>
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<td>23.8</td>
<td>100.5</td>
<td>0.3</td>
<td>2.8</td>
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<tr>
<td></td>
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<td>187.5</td>
<td>950.7</td>
<td>102.0</td>
<td>0.5</td>
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<td>3000</td>
<td>15210.6</td>
<td>100.8</td>
<td>0.1</td>
<td>0.2</td>
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</table>

Note: Signal to noise ratios and CVs indicate that LLOQs are lower than measured here for all analytes.
### Results

Inter-run Over 3 Days for Commercial QC (BioRad Lyphocheck)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Free/Total</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range (HPLC)</td>
<td>Measured</td>
</tr>
<tr>
<td>Dopamine Free</td>
<td></td>
<td>44.4 – 75.0</td>
<td>61.4</td>
</tr>
<tr>
<td>Norepinephrine Free</td>
<td></td>
<td>31.3 – 51.6</td>
<td>38.4</td>
</tr>
<tr>
<td>Epinephrine Free</td>
<td></td>
<td>9.62 – 19.1</td>
<td>14.3</td>
</tr>
<tr>
<td>3-Methoxytyramine Total</td>
<td></td>
<td>28.6 – 48.7</td>
<td>44.7</td>
</tr>
<tr>
<td>Normetanephrine Total</td>
<td></td>
<td>220 - 366</td>
<td>300.7</td>
</tr>
<tr>
<td>Metanephrine Total</td>
<td></td>
<td>69.0 - 116</td>
<td>91.2</td>
</tr>
</tbody>
</table>

- All measurements are in **ng/mL**
- Bio-Rad QC material was used. Ranges provided were for free catecholamines and total metanephrines
## Results

**Inter-run Over 3 Days for Commercial QC (BioRad Lyphocheck)**

<table>
<thead>
<tr>
<th>Compound</th>
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<th>Level 1</th>
<th></th>
<th>Level 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range (HPLC)</td>
<td>Measured</td>
<td>CV (%)</td>
<td>Range (HPLC)</td>
</tr>
<tr>
<td>Dopamine Free</td>
<td>290-490</td>
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<td>Norepinephrine Free</td>
<td>185-305</td>
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<td>920-1410</td>
<td>1135</td>
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<td>Epinephrine Free</td>
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<td>3335</td>
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<tr>
<td>Normetanephrine Total</td>
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<td>Metanephrine Total</td>
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<td>462</td>
<td>2</td>
<td>2200-3320</td>
<td>3103</td>
</tr>
</tbody>
</table>

- All measurements are in **nmol/L**
- Bio-Rad QC material was used. Ranges provided were for free catecholamines and total metanephrines
Example chromatogram
Dopamine and Dopamine-D4

Calibration 62.5 ng/mL
QC Level 1
QC Level 2

Target
Quant

ISTD

For Research Use Only. Not for use in diagnostic procedures.
Example chromatogram
Epinephrine and Epinephrine-D6

Separation from Normetanephrine and other interference is critical

Calibration 62.5 ng/mL
QC Level 1
QC Level 2

Target
Quant

ISTD

Counts vs. Acquisition Time (min)

For Research Use Only. Not for use in diagnostic procedures.
Example chromatogram
Norepinephrine and Norepinephrine-D6

Separation from interferences is critical

Calibration 62.5 ng/mL
QC Level 1
QC Level 2

Target
Quant

ISTD

For Research Use Only. Not for use in diagnostic procedures.
Example chromatogram
3-Methoxytyramine and 3-Methoxytyramine-D4

Separation from Metanephrine is critical

Calibration 187.5 ng/mL

QC Level 1

QC Level 2

For Research Use Only. Not for use in diagnostic procedures.
Example chromatogram
Normetanephrine and Normetanephrine-D3

Counts vs. Acquisition Time (min)

Target
Quant

ISTD

Calibration 187.5 ng/mL

QC Level 1

QC Level 2

For Research Use Only. Not for use in diagnostic procedures.
Example chromatogram
Metanephrine and Metanephrine-D3

Separation from interference is critical

Calibration 187.5 ng/mL
QC Level 1
QC Level 2

Target
Quant
ISTD

Counts vs. Acquisition Time (min)

For Research Use Only. Not for use in diagnostic procedures.
## Results

Recoveries Observed Using SPE Procedure

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absolute Recoveries %* (n = 9)</th>
<th>Relative recoveries % With ISTDs corrections** (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
</tr>
<tr>
<td>Dopamine</td>
<td>73.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>112.5</td>
<td>4.9</td>
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<tr>
<td>Epinephrine</td>
<td>90.3</td>
<td>3.6</td>
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<tr>
<td>3-Methoxytyramine</td>
<td>53.2</td>
<td>3.6</td>
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<tr>
<td>Normetanephrine</td>
<td>88.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>93.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* ISTDs peak areas spiked in formic acid subjected to SPE compared with spiked formic acid without SPE

** Calculated concentrations with ISTDs peak area ratios corrections (with SPE) versus theoretical concentrations
## Results

Matrix Effects Observed Using SPE Procedure

<table>
<thead>
<tr>
<th>Compound</th>
<th>Matrix effects %* (n = 9)</th>
<th>Accuracies % With ISTDs corrections** (n = 9)</th>
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<tbody>
<tr>
<td></td>
<td>Average</td>
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<tr>
<td>Dopamine</td>
<td>102.1</td>
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<tr>
<td>Norepinephrine</td>
<td>30.1</td>
<td>5.2</td>
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<tr>
<td>Epinephrine</td>
<td>107.1</td>
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<tr>
<td>3-Methoxytyramine</td>
<td>88.2</td>
<td>8.5</td>
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<td>Normetanephrine</td>
<td>93.8</td>
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<tr>
<td>Metanephrine</td>
<td>103.0</td>
<td>1.3</td>
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</tbody>
</table>

* ISTDs peak areas spiked in urine subjected to SPE compared with spiked formic acid subjected to SPE

** Calculated concentrations with ISTD corrections (urine with SPE) versus theoretical concentrations
### Stability of extracted spiked urine

18 hours in Autosampler at 6ºC

<table>
<thead>
<tr>
<th>Calibration Levels</th>
<th>Stability in Autosampler 18 hours at 6 ºC</th>
<th>% Difference</th>
</tr>
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<tr>
<td></td>
<td>D</td>
<td>NE</td>
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<tr>
<td>1</td>
<td>0.7</td>
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<td>2</td>
<td>0.8</td>
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<tr>
<td>3</td>
<td>0.0</td>
<td>-1.8</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>-0.7</td>
<td>-0.9</td>
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<tr>
<td>6</td>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>9</td>
<td>-0.1</td>
<td>-0.3</td>
</tr>
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</table>
Stability of Extracted Spiked Urine

Urine Extracts Instability

Urine extracts kept at 4°C

Norepinephrine-D6 extracted on Oc. 11th and injected on Oc. 25th

Norepinephrine-D6 extracted on Oc. 11th and injected on Oc. 30th
Tips and Tricks

• Mobile phase quality; grade and freshness
• Inline filter between needle seat and injection valve
• If sensitivity decreases, check cleanliness of spray chamber and run Scan method to monitor background ions levels
• Use needle wash to reduce carryover
• Perform a wash method with 100% methanol at the end of a batch
• Use guard column and change when necessary
• Minimize extra column volume
• Delay volume adjustment
Conclusions

- A four minute method has been developed for quantifying catecholamines and their metabolites: epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine, and 3-methoxytyramine for research.
- Offline solid phase extraction (SPE) for simultaneous extraction of all six analytes from urine is shown with excellent recoveries.
- Chromatographic separation of all six analytes with conditions compatible with LC-MS/MS have been developed.
- Typical method performance results are well within acceptable criteria.


[1] Simultaneous measurement of urinary metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection
M J Whiting, Clinical Biochemistry and Pharmacology Laboratory, SA Pathology, Flinders Medical Centre, Bedford Park 5042, South Australia

[2] Extraction and separation of urinary catecholamines as their diphenyl boronate complexes using C solid-phase extraction 18 sorbent and high-performance liquid chromatography
Dinesh Talwar*, Cathie Williamson, Allison McLaughlin, Alan Gill, Denis St.J. O'Reilly
Department of Clinical Biochemistry, Macewen Building, Royal Infirmary, Glasgow G4 OSF, UK

[3] Extraction of Catecholamines from Urine
AN 1071A, Argonaut, Dr Wéber Consulting KFT,
www.weber.hu/PDFs/SPE/AN1071S_CatecholaminesUrine.pdf
## Solutions preparations

<table>
<thead>
<tr>
<th>Solutions preparations:</th>
<th></th>
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<tbody>
<tr>
<td><strong>0.2 % Formic acid in water:</strong></td>
<td>2 mL of formic acid in 1 L water</td>
</tr>
<tr>
<td><strong>5 % Formic acid in water:</strong></td>
<td>50 mL of formic acid in 1 L water</td>
</tr>
<tr>
<td><strong>HCl 0.1N in water:</strong></td>
<td>830 uL of HCl 6N in 50 mL water</td>
</tr>
<tr>
<td><strong>2 M NH4Cl-NH4OH buffer:</strong></td>
<td>Dissolve 107g of NH4Cl in 1 L water and adjust pH to 8.5 with NH4OH (about 8 ml of NH4OH 50%). Store at 4 deg.C</td>
</tr>
<tr>
<td><strong>Diphenyl-boronate complexing agent:</strong></td>
<td>To 200 mL of 2 M NH4Cl-NH4OH buffer, add 400 mg of diphenylboronic acid ethanolamine ester and 1 g of disodium EDTA. Diphenylboronic acid does not dissolve easily and may require to mixing slowly overnight to dissolve completely. Adjust pH to 8.5 with NH4OH. Store at 4 deg.C and check pH before use</td>
</tr>
<tr>
<td><strong>Wash buffer 0.2 M NH4Cl-NH4OH:</strong></td>
<td>Add 50 mL of 2 M NH4Cl-NH4OH buffer to 450 mL of water. Add 250 mg EDTA. Adjust pH to 8.5 with NH4OH (about 3 ml of NH4OH 50%). Store at 4 deg.C and check pH before use</td>
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<tr>
<td><strong>5% Methanol in wash buffer 0.2 M NH4Cl-NH4OH:</strong></td>
<td>Add 25 mL of methanol to 475 mL of wash buffer 0.2 M NH4Cl-NH4OH. Adjust pH to 8.5 with NH4OH. Store at 4 deg.C and check pH before use</td>
</tr>
<tr>
<td>Supplier:</td>
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## Standards

### Cerilliant ([http://cerilliant.com](http://cerilliant.com))

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<thead>
<tr>
<th>Item</th>
<th>Description</th>
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<tbody>
<tr>
<td>C-109</td>
<td>Catecholamine Mix 1 (Epinephrines), 1.0 mg/mL (as free base) of each component</td>
</tr>
<tr>
<td>C-110</td>
<td>Catecholamine Mix 2 (Metanephrines), 1.0 mg/mL (as free base) of each component</td>
</tr>
<tr>
<td>C-111</td>
<td>Catecholamine Metabolites Mix, 1.0 mg/mL of each component</td>
</tr>
<tr>
<td>D-072</td>
<td>Dopamine-D, HCl, 100 µg/mL (as free base)</td>
</tr>
<tr>
<td>D-081</td>
<td>Dopamine HCl, 1.0 mg/mL (as free base)</td>
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<tr>
<td>E-077</td>
<td>(±)-Epinephrine-D₉, 100 µg/mL</td>
</tr>
<tr>
<td>M-148</td>
<td>(±)-Metanephrine-D, HCl, 100 µg/mL (as free base)</td>
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<tr>
<td>N-068</td>
<td>(±)-Normetanephrine-D, HCl, 100 µg/mL (as free base)</td>
</tr>
<tr>
<td>N-069</td>
<td>(±)-Norepinephrine-D, HCl, 100 µg/mL (as free base)</td>
</tr>
</tbody>
</table>

**Cambridge Isotopes:** PN: DLM2739-0.05g, Description: 3-methoxytyramine-d₄ HCl

**Medical Isotopes:** PN: 5376, Description: 3-methoxytyramine HCl
Thank You!

Acknowledgements

Kevin McCann  
Agilent Technologies  
Clinical Applications Specialist

Rory Doyle  
Agilent Technologies  
Clinical Applications Specialist

Christophe Deckers  
Agilent Technologies  
Sample preparation Applications Scientist