### Abstract

The determination of creatinine and purine metabolites can be an important marker for renal function, and the concentrations of various metabolites are an indicator for certain disease states. However, this method creates a separation problem because of the dramatic differences in chromatographic behavior of the analytes. A novel HPLC method was developed that separates and quantifies these compounds of interest on an aminoepoxy phase without the use of ion pairing additives. Important variables that were optimized for this method include stationary phase selection, sample solvent composition, mobile phase additives, and pH.

A single column, multi-wavelength method will be shown for five following analytes: creatinine, xanthine, alanthoin, and uric acid. This method is currently being used for routine urine analysis.

### Structures for Creatinine and Purine Metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar Extinction Coefficient</th>
<th>LogP</th>
<th>MW</th>
<th>TIC</th>
<th>MWD</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>4,000</td>
<td>4.9</td>
<td>115</td>
<td>1.0</td>
<td>4.6</td>
<td>315</td>
</tr>
<tr>
<td>Xanthine</td>
<td>3,000</td>
<td>3.9</td>
<td>155</td>
<td>1.0</td>
<td>5.0</td>
<td>113</td>
</tr>
<tr>
<td>Allantoin</td>
<td>2,500</td>
<td>3.8</td>
<td>165</td>
<td>1.0</td>
<td>5.5</td>
<td>118</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>2,000</td>
<td>3.7</td>
<td>145</td>
<td>1.0</td>
<td>5.0</td>
<td>119</td>
</tr>
</tbody>
</table>

Analytical Problems

- The four analytes have significantly different chromatographic characteristics.
- Range of polarity makes column selection difficult.
- Absorbance spectra are almost identical.
- Single-wavelength analysis not practical.

### Optimized Chromatographic Conditions

**Gradient Program**

- *Amino Acids and Purine Standards*:
  - Column: Amino (3um), 150 x 4.6mm (3um), autosampler
  - Flow: 1.5 mL/min
  - Injection: 5 µL
  - Optimized Chromatographic Conditions
- *Uranium 235-238*:
  - Column: Amino (3um), 150 x 4.6mm (3um), autosampler
  - Flow: 1.5 mL/min
  - Injection: 5 µL
  - Optimized Chromatographic Conditions

### Sample Preparation

- **Collect urine sample**
- **Dilute 1:10 with 50 mM ammonium formate**
- **Filter through 0.45 µm filter**
- **Autosampler vial**

### Acknowledgments

- **Amino Acids and Purine Standards**
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- **Sara Sheppard**
- **Dr. Craig Johnson**
- **Dr. Mike Licklider**

### References

- **A Novel Aminoepoxy Column with Revolutionary Aqueous Dioxane**
- **YAZAWA Itaru, TACHIKAWA Hiroshi, OKAMURA Itaru**
- Intact Corporation, Kyoto, Japan
- **HPLC 2008 – Baltimore, MD, USA**

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**Merlin K. Bicking, Ph.D.**
ACCTA, Inc.
PO Box 25602
St. Paul, MN 55125
Phone: 651-731-3670
Email: mbicking@accta.com

**Bryan Evans**
Intakt USA
1511 Walnut St., Ste 310
Philadelphia, PA 19102
Phone: 888-456-HPLC
Email: bevans@intaktusa.com