Chemometric Optimization of UPLC-MS/MS Assay for Evaluation of Rare Causes of Kidney Stones and Kidney Failure

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Outline

• Adenine phosphoribosyltransferase (APRT) deficiency
  ✓ Diagnosis
  ✓ Therapeutic drug monitoring

Icelandic Patients
Adenine phosphoribosyltransferase (APRT) deficiency

- Rare autosomal recessive disorder of adenine metabolism
- Excessive production and urinary excretion of 2,8-dihydroxyadenine (2,8-DHA) which is poorly soluble
- Crystal aggregates accumulate in the renal tubules, tubular epithelial cells and interstitium
- Results in chronic kidney disease, kidney failure, recurrent kidney stones and kidney transplantation
Epidemiology of APRT deficiency

• The majority of reported cases of APRT deficiency have come from France, Iceland and Japan

• The prevalence has been estimated to be 0.5 to 1 per 100,000 in the Caucasian population

• Accordingly, one would expect 500-1000 cases in the UK and 3000-6000 cases in the US

• In Iceland we have identify 34 patients and the prevalence is around 1:10,000 population
  
  – All Icelandic patients have the same mutation and show wide variation in disease severity and/or age of onset
Variable Clinical Symptoms!

- Recurrent radiolucent urinary tract stones
- Unexplained chronic kidney disease
- Asymptomatic disease
2,8-Dihydroxyadenine Stone
"Jóhonnukristallar"
"It takes a redheaded woman to get a dirty job done." ...  
(Redheaded Woman, Bruce Springsteen)
Diagnosis

• Urine microscopy
• Analysis of APRT activity in red cell lysates
• Stone analysis using infrared spectrophotometry or x-ray crystallography
• Kidney biopsy
• Molecular genetic testing
• Urinary DHA screening assay?
Urine Sediment from Patient with APRT Deficiency

Light microscopy

Polarized light microscopy
Treatment

- Allopurinol 5-10 mg/kg/day
- Febuxostat 40-80 mg/day
- High fluid intake and low-purine diet
- Stone removal by ureteroscopy
- Surgery

- Monitoring of pharmacotherapy
  - Detection of crystal burden by urine microscopy
  - LC-MS/MS purine assay
Early diagnosis is the Key!
Aim

• To develop a specific high-throughput method for simultaneous quantification of urinary 2,8-DHA and other purine metabolites

• To implement the assay for diagnosis of rare cases of kidney stones and kidney failure

• To implement the assay for therapeutic monitoring of allopurinol treatment of APRT deficient patients
Analytical Strategy

• Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) with electrospray ionization was used for the analysis

• Chemometrics was utilized for optimization of the UPLC-MS/MS quantification method
Experimental Design

Screening
Identifications of important Parameters

Optimize chromatography and MS parameters
Ensure chromatographic separation

Maximize precursor ion signal
Optimisation of source

Maximize product ion signal
Optimize CID settings

Experiment with optimal parameter settings
Experimental set-up

- Waters Quattro Premier™ XE coupled to ACQUITY UPLC
- Ionization in a positive mode
  - Column: Acquity HSS T3 and Acquity BEH C18 (1.7µm, 100 x 2.1mm)
  - Mobile phase: H₂O and methanol containing ammonium acetate at pH 6.7
No Sample preparations needed!

• Matrixes
  – Human urine

➢ No Sample preparations needed
  • Samples vortexed for few seconds
  • Diluted with 20mM NH$_4$OH down to creatine concentration of 0.5 mmol/L
Optimization of the UPLC-MS/MS

Design
• D-optimal Design, Interaction model
• Multi-level qualitative factors
  ➢ Type of slope
  ➢ Column type
• Four quantitative factors

Responses
➢ Peak Area
➢ Retention time

Modeling
• Partial least square regression (PLS)

Software
• Modde 8.0, Umetrics AB
## D-Optimal Design

<table>
<thead>
<tr>
<th>Variable parameters</th>
<th>Experimental domain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
</tr>
<tr>
<td><strong>Capillary voltage (kV)</strong></td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Gradient steepness (min)</strong></td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Flow rate (ml/min)</strong></td>
<td>0.45</td>
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<tr>
<td><strong>Ionic strength (mM)</strong></td>
<td>2</td>
</tr>
<tr>
<td><strong>Column type</strong></td>
<td>Acquity HSS T3</td>
</tr>
<tr>
<td><strong>Type of slope</strong></td>
<td>4</td>
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</tbody>
</table>
Results
Effect of pH on Solubility

MW: 167.13

2,8-DHA

MW: 135.13

Adenine
Response Contour Plot for Peak Area of 2,8-DHA

Optimum conditions:
Flow rate: 0.55 ml/min
Gradient steepness: 3.4 min
Capillary voltage: 0.5 kV
Slope type: Sl4
Ionic strength: 2mM
Desolvation temp: 130
MRM Chromatogram of Adenine and other Selected Purines at Optimal UPLC-MS/MS Conditions
Validation Results for Determination of 8 Urinary Purines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linearity (mmol/mol Cr*)</th>
<th>R²</th>
<th>Intra-Day Variation (n=6)</th>
<th>Accuracy (%)</th>
<th>Precision (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,8-Dihydroxyadenine (2,8-DHA)</td>
<td>0.24 - 59.8</td>
<td>0.9989</td>
<td>-3.9 - 5.5</td>
<td>-3.9 - 5.5</td>
<td>2.2 - 7.7</td>
</tr>
<tr>
<td>2-Deoxyadenosine</td>
<td>0.16 - 7.96</td>
<td>0.9970</td>
<td>1.1 - 13.8</td>
<td>1.1 - 13.8</td>
<td>2.9 - 4.1</td>
</tr>
<tr>
<td>2-Deoxyguanosine</td>
<td>0.07 - 37.4</td>
<td>0.9997</td>
<td>-2.8 - 0.9</td>
<td>-2.8 - 0.9</td>
<td>1.6 - 2.4</td>
</tr>
<tr>
<td>2-Deoxynosine</td>
<td>0.08 - 39.6</td>
<td>0.9953</td>
<td>-3.4 - 0.2</td>
<td>-3.4 - 0.2</td>
<td>2.3 - 10.8</td>
</tr>
<tr>
<td>Adenine</td>
<td>2.96 - 74.0</td>
<td>0.9978</td>
<td>-2.3 - 0.2</td>
<td>-2.3 - 0.2</td>
<td>3.4 - 11.3</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.75 - 26.2</td>
<td>0.9980</td>
<td>-6.7 - 4.7</td>
<td>-6.7 - 4.7</td>
<td>1.6 - 3.6</td>
</tr>
<tr>
<td>Guanosine</td>
<td>0.14 - 24.7</td>
<td>0.9967</td>
<td>-12.8 - 9.4</td>
<td>-12.8 - 9.4</td>
<td>1.2 - 4.5</td>
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<tr>
<td>Inosine</td>
<td>0.30 - 37.3</td>
<td>0.9989</td>
<td>-2.9 - 6.7</td>
<td>-2.9 - 6.7</td>
<td>0.8 - 3.9</td>
</tr>
</tbody>
</table>

*Cr - creatine
Healthy Control Subject versus Patient with APRT deficiency

2,8-DHA

A - Healthy control subject
B – Patient with APRT deficiency

62.2 µmol

BLOQ

5.0 µmol

BLOQ

Adenine
Analyzes of 2,8 DHA and Adenine in Urine from Patient

2,8 - DHA

Adenine
Key Objectives of the APRT Deficiency Program

• Collect longitudinal clinical data into an APRT Deficiency Registry
  – Study the epidemiology and natural history of the disorder
  – Develop strategies to increase the awareness and detection of APRT deficiency and thus improve clinical outcomes

• Establish a Biobank (DNA, urine, kidney tissue)

• Identify factors affecting clinical expression
  – Stones, CKD/crystalline nephropathy, asymptomatic state
    • Risk factors associated with stone formation and recurrence.
    • Potential genotype-phenotype correlations
    • Role of modifying genes

• Study the efficacy of pharmacologic treatment

• Interface with patient organizations, health care professionals and researchers through our APRT Deficiency Websites to further enhance the educational mission of this project and disseminate knowledge to the community
Conclusions

• The DoE was useful for evaluation of the factors and optimization of the method

• The UPLC-MS/MS assay method provides significant improvements in sensitivity and analysis run time for determination of urinary excretion of 2,8-DHA and other key purines

• A rapid and robust method for quantitative determination of 2,8-DHA and other key purines was successfully developed and validated

• This method facilitates the diagnosis of APRT deficiency and greatly enhances monitoring of pharmacotherapy in affected patients
Acknowledgements

University of Iceland, Faculty of Pharmacy
– Finnur Freyr Eiríksson

ArcticMass;
– Baldur Bragi Sigurðsson

Landspítalí University Hospital;
– Vidar O Edvardsson
– Runolfur Palsson

Financial support:
The Icelandic Research Fund
University of Iceland Research Fund

NIDDK Support, Other NIH Support-Office of Rare Diseases Research (ORDR)
Thanks for your attention!