Simultaneous LC-MS/MS Determination of Urine Sialic Acid, Pipecolic Acid and Creatinine and Serum Pipecolic Acid, for Diagnosis of Inherited Metabolic Disorders

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Introduction

- One Method
- Two Matrices
  - Urine
  - Serum
- Three Molecules
  - Sialic Acid
  - Pipecolic Acid
  - Creatinine
- Multiple Metabolic Disorders

Sialic acid is a diagnostic marker for several inherited metabolic disorders, notably Sialic Acid Storage Disease (SASD). SASD is an autosomal recessive neurodegenerative disorder caused by defective transport of sialic acid across the lysosomal membrane, leading to accumulation of sialic acid in tissues, fibroblasts, and urine. Quantitation of free sialic acid in urine provides a non-invasive first-line diagnostic test for this condition.

Pipecolic acid, a metabolite in the alternate lysine catabolism pathway, is relevant to other inborn errors of metabolism. The Zellweger spectrum disorders (ZSD) are a genetically-heterogeneous group of peroxisomal biogenesis disorders that result in impairment of peroxisomal function, leading to multi-organ disease. Pipecolic acid in urine and/or plasma is typically increased in ZSDs, but normal in clinically-similar disorders due to deficiencies in single peroxisomal enzymes. Plasma pipecolic acid is also increased in most patients with pyridoxine-dependent epilepsy, a disorder due to a specific defect of lysine catabolism. Quantitation of pipecolic acid has applications in diagnosis, differential diagnosis and monitoring of dietary treatment.

In order to increase working efficiency in our biochemical genetics diagnostic laboratory, we have developed a simple combined method permitting analysis of sialic acid, pipecolic acid and creatinine in urine and pipecolic acid in serum. The determination is performed by isotopic dilution with appropriate internal standards by LC-MS/MS in positive electrospray ionization mode with multiple reaction monitoring. Inclusion of appropriate controls provides capability to analyze different matrices.

Method

STANDARDS, CONTROL, SAMPLES PREPARATION

- 5 calibration levels in water
  - Sialic Acid: 10–200 µM
  - Pipecolic Acid: 0.1–500 µM
  - Creatinine: 0.05–30 mM
- Control SKML Red & Green from ERNDIM (QA Program)

LC METHOD

Agilent 1290 Infinity

HPLC CONDITIONS

- Column: Waters Acquity UPLC BEH Amide, 2.1x10mm, 1.7µm
- Column Temp: 30°C
- Injection volume: 10 µl
- Autosampler temp: 15°C
- Mobile phase A: H₂O/ACN 50:50, 10mM AmAc, 0.04% Formic acid, pH 9
- Mobile phase B: H₂O/ACN 95:5, 50mM AmAc, 0.04% Formic acid, pH 9
- Flow rate: 1 ml/min
- Run time: 4 min

LC Gradient

MRM Acquition Table

- Ion mode: Jet Stream Positive Mode
- Gas Temp (°C): 250
- Gas Flow (ℓ/min): 35
- Nebulizer (psl): 40
- SheathGasHeater (°C): 300
- SheathGasFlow (ℓ/min): 10
- Capillary (V): 3500
- Nozzle (V): 500
- Ion Funnel: High Pressure RF: 150
- Low Pressure RF: 60

MRM Acquisition Table

- Creatinine
  - RT (min): 0.78
  - MRM: 114.13 > 86.0
  - CE: 9
  - CAV: 5
- Pipecolic acid
  - RT (min): 1.36
  - MRM: 130.01 > 83.9
  - CE: 21
  - CAV: 5
- Sialic acid
  - RT (min): 1.73
  - MRM: 310.12 > 273.8
  - CE: 9
  - CAV: 5

Sample Preparation

- 25 µl urine or serum
- 400 µl of ISTD solution in ACN
- Vortex and centrifuge
- Inject supernatant

Discussion and Conclusion

- This is a simple method requiring a low volume of sample (25µl).
- Acceptable statistics have been obtained and demonstrate that the quantification method is robust, accurate, precise and specific.
- This method is now in service in our clinical laboratory.
- Positive diagnoses have already been obtained, using literature reference values.
- We are in the process of establishing in house age-related reference ranges.

References

- ACRONYM: SCPCIS Amino Columns Application Notebook, WAT0029
- ERNDIM, Quality Assurance in Laboratory Testing for Inborn Errors Metabolism, www.erndim.nl
- Reference values in urine and RIA in Serum, Techniques in Diagnostic Human Biochemical Genetics, A Laboratory Manual, 1984, Amsterdam, Editor, Wiley-Liss, 1991

Contact

LABORATORY TESTS LIST:

http://lab.chus.qc.ca ("Medicine Générale" in left pane)

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