Quantitative Analysis of Orotic Acid by LC-ESI-MS/MS

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Overview

- Increased orotate excretion a biomarker for several IEMs
- Quantitation of orotate useful since:
  - Can help with differential diagnosis
    - QAA not conclusive
    - Qualitative organic acid analysis misleading
- Stable isotope dilution LC-ESI-MS/MS method\(^1\)
  - Sample preparation rapid and simple
  - Good accuracy
  - Good precision
  - Capable of detecting elevated concentrations of clinical significance

Why Quantitate?

[Orotate] uM / mmol creatinine

0 250 500 750 1000

CPS Deficiency & Unaffected

OCT

Citrullinaemia

ASA

Argininaemia

Hereditary orotic aciduria

LPI

HHH syndrome

Choice of Instrumentation

**HPLC**
- Long procedure
  - Clean up
- Drug interference

**GC-MS**
- Long procedure
  - Clean-up/extraction
  - Derivatisation

**LC-ESI-MS/MS**
- Simple, rapid sample preparation:
  - 20ul sample + 150ul $^{15}$N$_2$-orotic acid (IS) (200µM)
    - Calibration standards 0-200µM
    - EQA samples used as IQC
- C18 reversed phase LC separation
  - 5µ x 4.6mm x 150mm Phenomenex Gemini
  - 60% acetonitrile with 2.5mM ammonium acetate
Multiple Reaction Monitoring (MRM)

LC-ESI(-ve)-MS/MS in MRM mode

Orotic acid [M-H]⁻  m/z 155.1 → 111.1  [M-H – CO₂]⁻

¹⁵N₂-Orotic acid [M-H]⁻  m/z 157.1 → 113.1  [M-H – CO₂]⁻
Linearity

- Linear to 200uM
- $r^2 = 0.9977$
Accuracy

- 21 EQA samples analysed
- Direct comparison of results and target values
- $r^2 = 0.9898$
Precision

**Inter-Assay**
- EQA samples
  - $C_1 = 2.6 \, \mu M$
  - $C_2 = 39.9 \, \mu M$
  - $C_3 = 89.5 \, \mu M$
- $CV_1 = 29\% \ (n=7)\$
- $CV_2 = 8 \% \ (n=11)$
- $CV_3 = 7 \% \ (n=11)$

**Intra-Assay**
- 22 replicates of EQA material analysed
- $C = 36.5 \, \mu M$
- $CV = 6\%$
Limit of Detection

- LOD = 3.125 µM
- S/N = 42
- Fit for purpose – clinically significant increase detectable