# Urinary steroid profiling by ultra-high-performance liquid-chromatography tandem mass spectrometry: Method Validation and comparison to GC-MS

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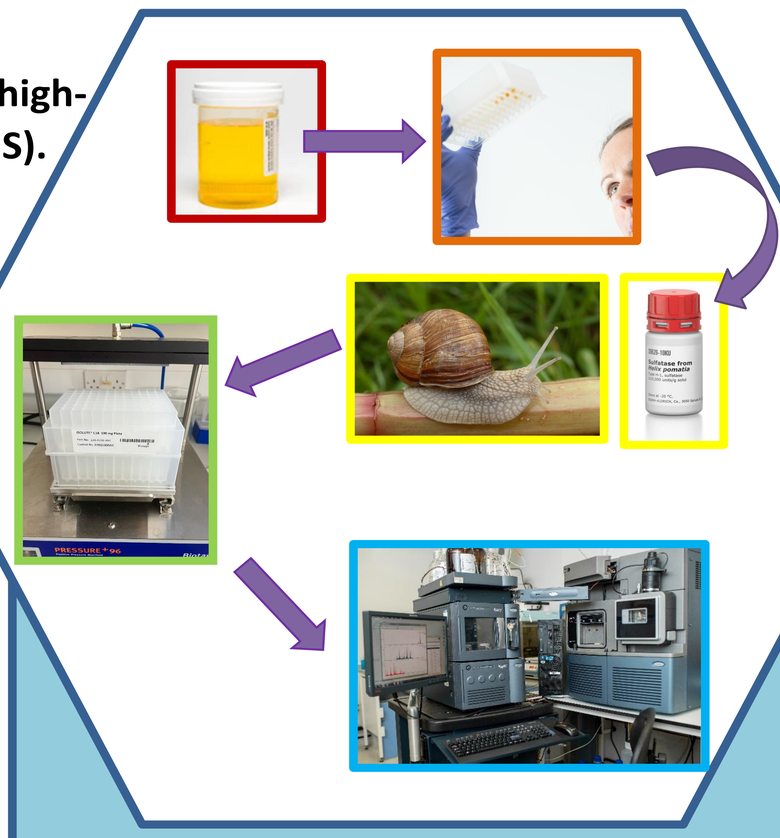
## Introduction

Gas chromatography-mass spectrometry (GC-MS) is the gold standard method for urinary steroid profiling. A valuable discovery and diagnostic tool, our established GC-MS method quantifies steroids from mineralocorticoid, glucocorticoid, and androgen classes. However, GC-MS assays are laborious since samples require two-step chemical derivatisation and long run times, rendering this approach unsuitable for high-throughput analysis.

**The aim was to develop and validate a urinary steroid profiling method using ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-LC-MS/MS).**

**UPLC-MS/MS method for 29 urinary steroids**




## Methods

Chromatography and mass spectrometry parameters were optimised for 29 urinary steroids.

Corresponding isotopically labelled internal standards were added to 200 µl of sample, followed by hydrolysis to remove sulfate and glucuronide conjugates. The subsequent unconjugated steroids were extracted using a $C_{18}$ 96-well plate solid-phase extraction cartridge.

Separation of steroids was achieved using a Waters HSS T3 column (1.8 µm 1.2 x 50 mm) with a water and methanol (both 0.1 % formic acid) gradient maintained at a flow rate of 0.6 mL/ min, on a Waters Acquity UPLC system coupled to a Waters TQ-XS mass spectrometer.

## Results

- Steroids separated in a total run time of 22 minutes.
- The method quantifies steroids across the large concentration ranges commonly observed in the urine metabolome (0.5-3000 ng/ mL).
- Lower limits of quantification (LLOQ) ranged from 0.5 to 10 ng/ ml.
- Mean recovery was 89% (range 61-131%) with acceptable matrix effects.
- A comparison of GC-MS and UPLC-MS/MS revealed similar quantitation for all steroids.

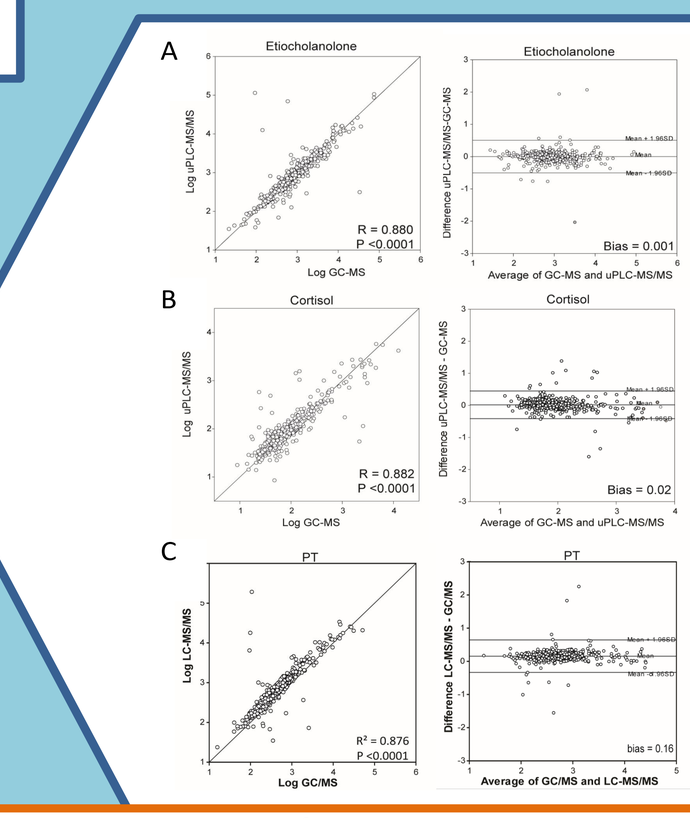



**Fig 1. GC-MS and LC-MS/MS comparison.**
Bland-Altman and correlation plots for comparison of steroid quantitation by uPLC-MS/MS and GC-MS. A. shows androgen Etiocholanolone, B. shows glucocorticoid Cortisol, and C. shows glucocorticoid precursor Pregnanetriol (PT).

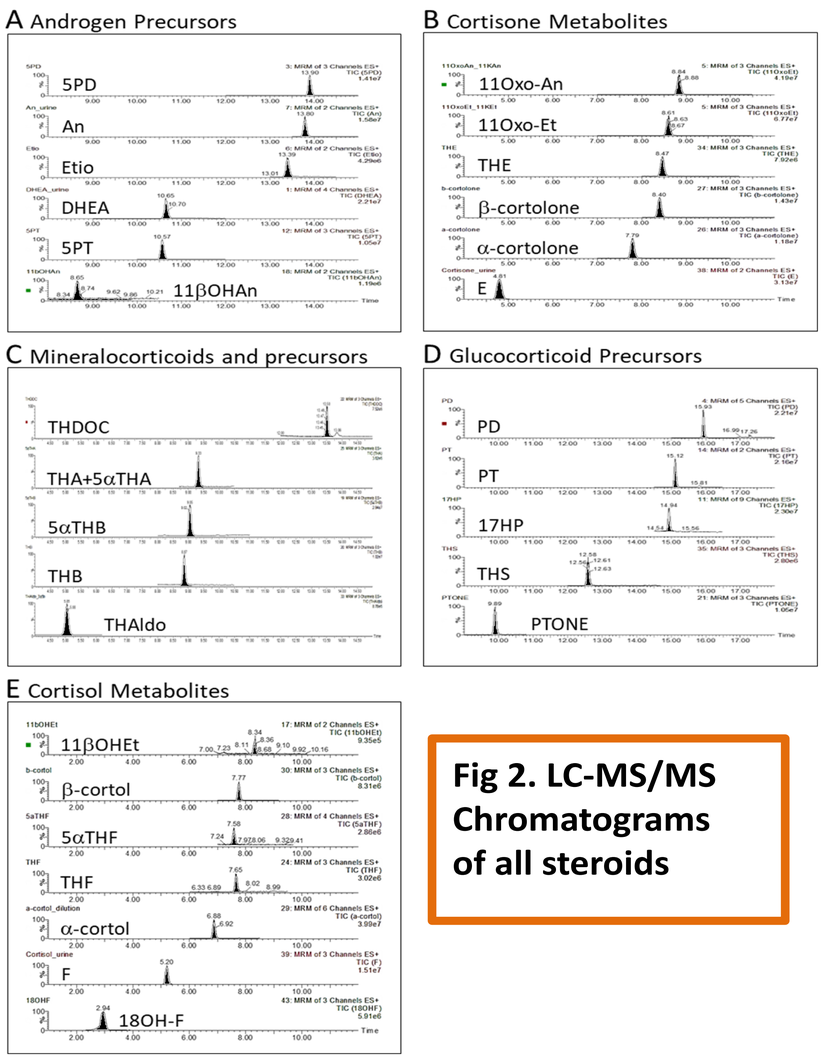



**Fig 2. LC-MS/MS Chromatograms of all steroids**

## Conclusions

- We have developed a powerful tool for the comprehensive profiling of urinary steroids using UPLC-MS/MS.
- 29 steroids from three steroid classes; mineralocorticoids, glucocorticoids, and androgens.
- Compared to an established GC-MS method, whilst maintaining the resolution of a large steroid panel, this assay has the advantages of
  - reduced sample preparation
  - reduced run time
  - greater sample throughput