Steroid deconjugation by helix pomatia – can we overcome snail speed?

Fozia Shaheen[1,2], Lorna C Gilligan[1,2], Camila Berner[3], Jose Luis Callejas[3], Cedric HL Shackleton[4], Wiebke Arlt[1] and Angela E Taylor[1,2]

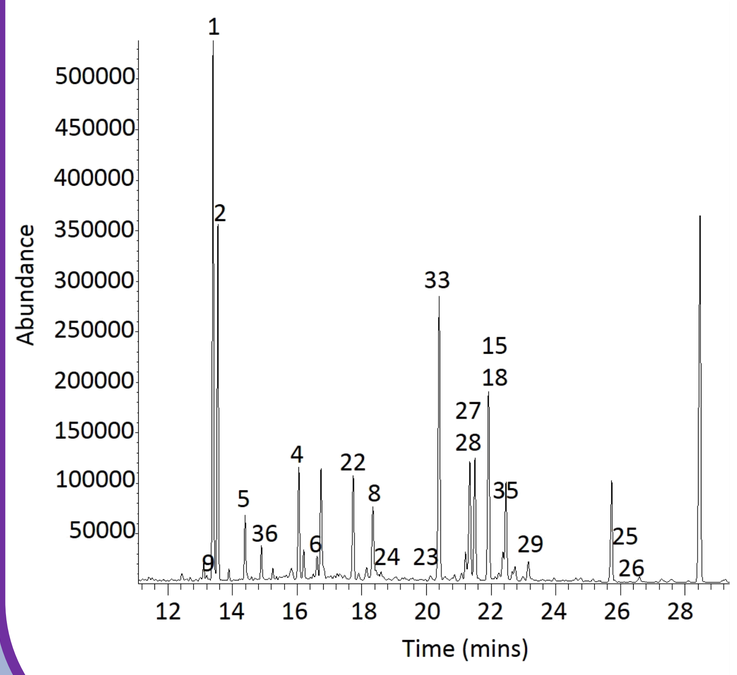
1. Steroid Metabolome Analysis Core, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK. 2. SMAC@contacts.bham.ac.uk. 3. KuraBiotech, Puerto Varas Chile. 4. Children's Hospital & Research Centre at Oakland, CA.

INTRODUCTION

Gas chromatography-mass spectrometry (GC-MS) has been used for urinary steroid analysis for over 50 years. The process of hydrolysis to release steroids from their conjugates is routinely performed using commercially available snail Helix-Pomatia sulfatase (HP). This enzyme contains both arylsulfatase and β-glucuronidase activity, making it effective for the study of the complete urinary steroid profile. However, as this is a naturally occurring enzyme its production process results in batch variation and so each batch requires experimental validation to adjust for the difference in efficiency of the enzymatic hydrolysis.

We aimed to find a more efficient and consistent enzyme. Here within we show a comparison in enzymatic hydrolysis of urinary steroid conjugates using either an optimised protocol for HP deconjugation (Sigma-Aldrich, UK) or a recombinant β-Glucuronidase/Sulfatase mix (BGS) enzyme (Kura Biotech Inc, Puerto Varas, Chile).

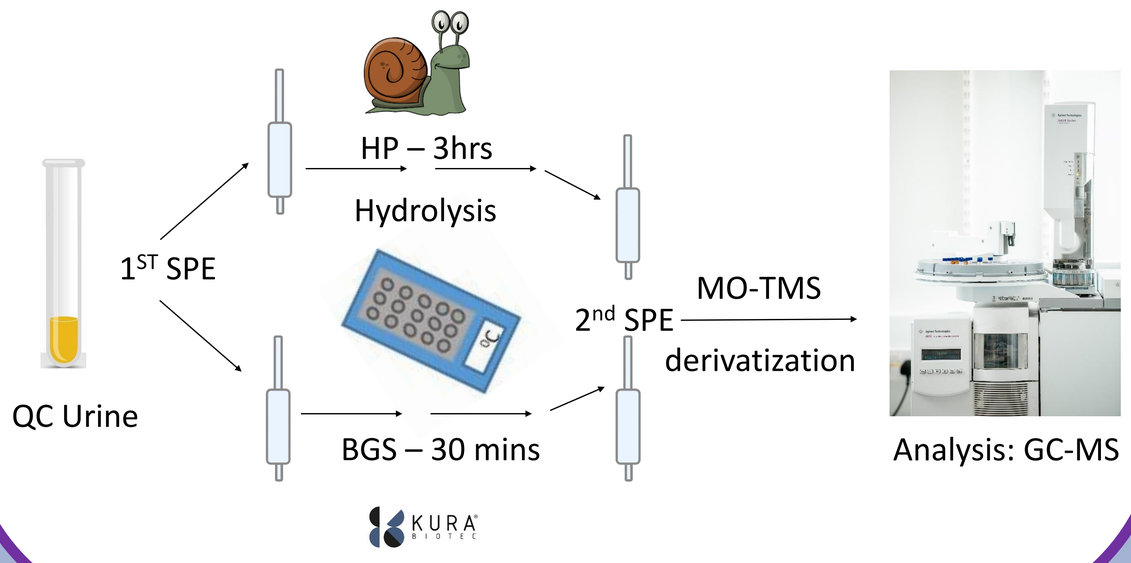
FIGURE 1. GC-MS Chromatogram



METHODS

Urine steroid conjugates were hydrolysed at both the glucuronide and sulfate moieties to release the corresponding free steroids. This was done using 1ml of pooled healthy male urine (QC). The urine underwent C18 solid-phase-extraction (SPE), followed by enzymatic hydrolysis using either an optimised protocol for HP deconjugation (Sigma-Aldrich, UK) or a recombinant β-Glucuronidase/Sulfatase mix (BGS) enzyme (Kura Biotech Inc, Puerto Varas, Chile). Then a second SPE was carried out followed by a two-step methyloxime-trimethylsilyl derivatization (MO-TMS) process. Samples were then analysed by GC-MS using an Agilent 5975 instrument (SIM mode) for steroid identification and quantification, followed by comparison of urinary steroid outputs from the two different enzyme hydrolysis methods.

FIGURE 2. Urinary Steroid Conjugate Hydrolysis Method



RESULTS

Comparison of urinary steroid outputs following hydrolysis of QC urine samples using HP versus the recombinant BGS enzyme mix:

FIGURE 3. HP vs BGS

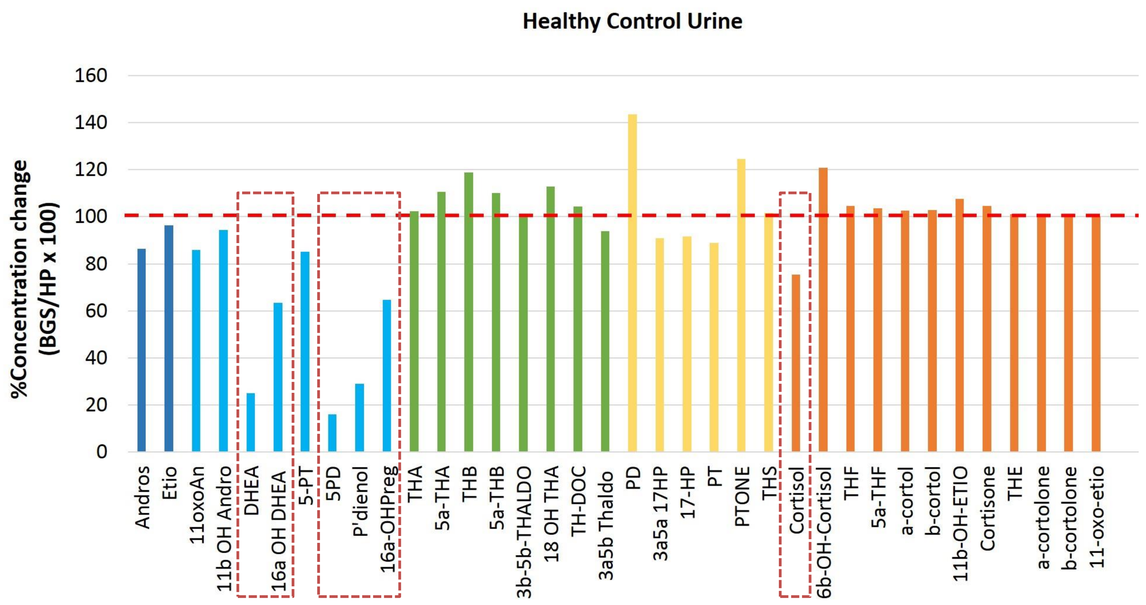
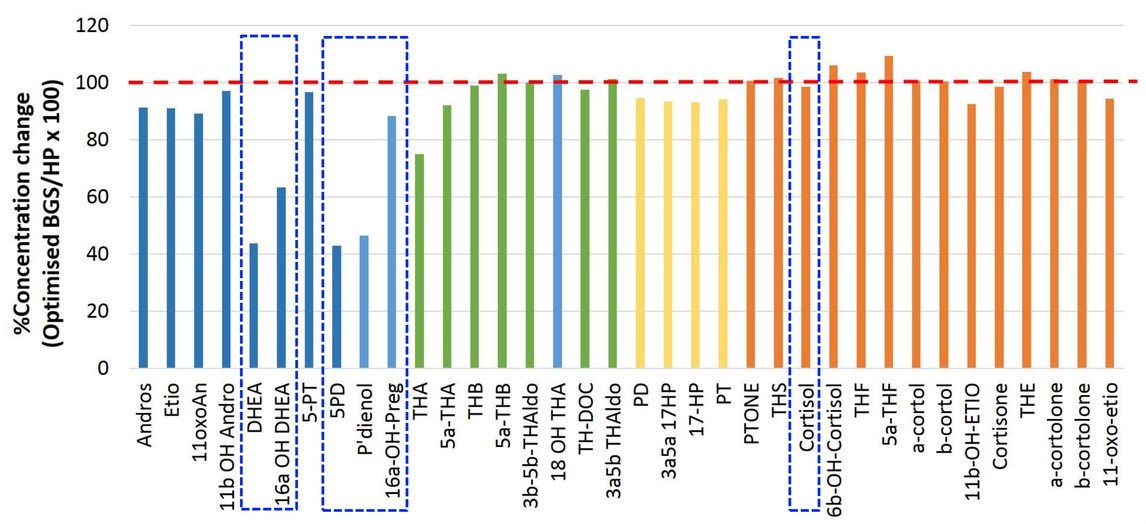


FIGURE 4. HP vs Optimised BGS



CONCLUSION

- The BGS hydrolysis method involved a shorter incubation (30-minutes) than HP (3-hours).
- HP and both versions of BGS performed equally for all examined glucuronides.

BGS:

- Showed a lower deconjugation ability for monosulfates ranging from 35-60% compared to the HP.
- Unlike HP, BGS did not efficiently hydrolyse 21-sulphated hydroxyls or 16β-and-17β sulfates.
- Under recommended hydrolysis conditions BGS had reduced ability to deconjugate delta-5-sulfated steroids; approximately a third of that observed with HP.

Optimised BGS:

The activity of sulfatase was doubled and the hydrolysis temperature further optimised.

- There was an improvement in the efficiency of optimised BGS to deconjugate delta-5-sulphated steroids by a further 12-20% compared to BGS.
- 21-sulfated hydroxyls e.g. cortisol hydrolysed equally well as HP.

With further optimisation of BGS enzymatic hydrolysis conditions we anticipate greater recovery of the delta-5 steroids.