Challenges of Measuring an Acyl Glucuronide by LC-MS for Regulated Bioanalysis

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Best Poster Award

Introduction

Glucuronidation is a key elimination pathway for acidic drugs. Acyl glucuronide (AG) quantitation in vivo is vital to confirm if they cause toxicity through covalently binding with proteins and nucleic acids. Measurement of AGs can be challenging as AGs are inherently labile.

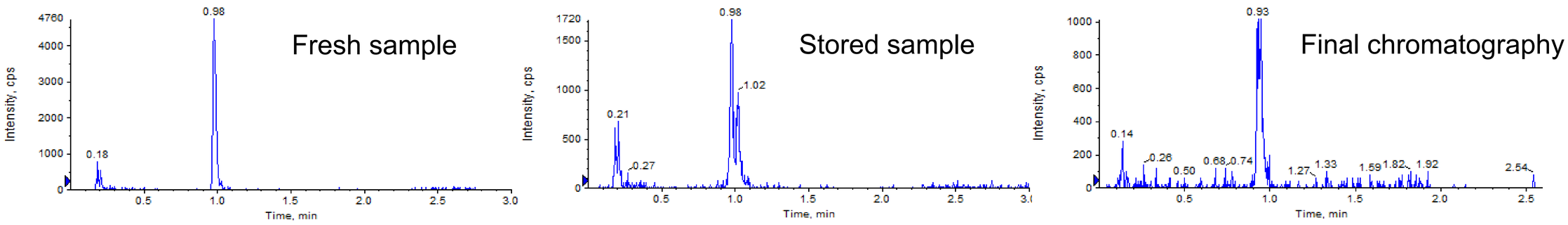
Background & Purpose

An LC-MS method was required in human plasma and urine for an AG metabolite. An internal standard (IS), a stable isotopically labelled parent was used for quantitation. Here, we present how we solved the challenges faced during assay development.

Challenge 1: Peak Shape Deterioration

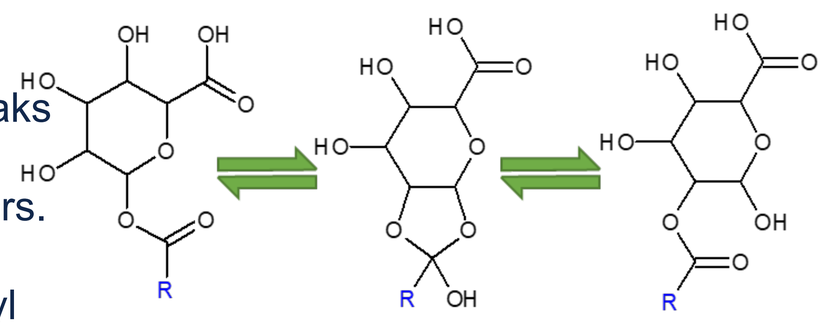
Chromatography was developed on a Waters Acquity CSH C18 using 10 mM Ammonium Formate pH 3 (aq) and Acetonitrile mobile phases, coupled to a Sciex API5000.

Chromatograms of peak shape deterioration after storage in biological matrix accompanied with overall loss of analyte response.



- Citric acid (aq) was added to lower the pH to stabilise the compound in biological matrix. This prevented the deterioration of peak shape in matrix for a sufficient time period for clinical sample analysis.
- However, clinical urine samples will reside within the bladder prior to excretion and upon collection will take time to cool to +4°C. Therefore, to provide a precise measurement we either needed to separate the peaks or promote coelution. Gradient steepness was increased to promote coelution of the peaks to improve peak shape from clinical samples.
- AGs undergo acyl migration, an intramolecular rearrangement to form isomers. These have the same product ion thus are indistinguishable by MS/MS detection. We hypothesise the peak shape deterioration could be due to acyl migration. By altering the chromatography we have a total measurement of the AG.

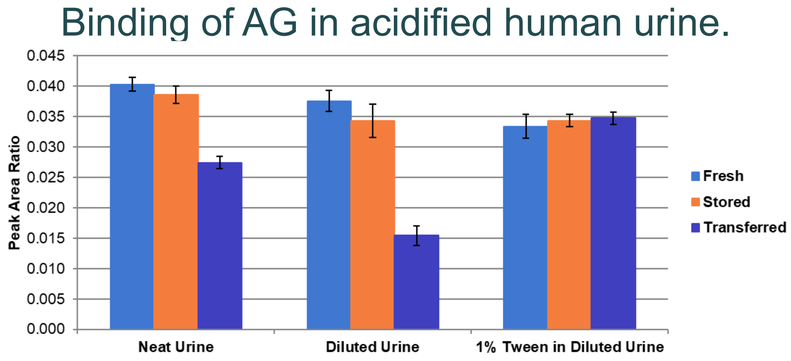
Acyl migration via orthoacid intermediate.



Challenge 2: Non-specific Binding

Non specific binding of AG observed in DMSO:MeOH (80:20) and urine.

Binding of AG in acidified human urine.



- Non specific binding in urine is common due to the lack of carrier proteins and lipids that can bind analytes. Tween surfactant was added to urine to prevent binding in polyethylene containers.
- Loss of AG at low concentrations in DMSO:MeOH (80:20) in glass and polypropylene containers over time mitigated by preparing calibration standards and quality control samples by serial dilution from stock solution in biological matrix.

Challenge 3: Interconversion

Interconversion of the AG into parent compound observed in biological matrix

- AGs can undergo hydrolysis to form their parent drug.
- Assessed by extracting AG only quality control samples after stress conditions and analysing them for the parent compound.
- Parent is chromatographically separated from the AG to prevent impact from any in source fragmentation.
- Sample extraction is kept at +4°C and pH controlled.
- A limit for 5% of AG converting into the parent compound agreed with client and samples potentially biased with be flagged.

Conclusion

We have developed a robust, reproducible method for the analysis of the AG compound in biological matrix that can now be used to analyse samples generated from a clinical study.