Evaluation of Matrix Component Removal Using a Novel Flow-through Scavenging Plate for Drugs of Abuse Testing in Urine

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Introduction

Dilute and shoot (D/S) is the most common form of sample preparation for the analysis of drugs of abuse in urine. High analyte cutoffs combined with sensitive mass spectrometers allow substantial sample dilution while still reaching desired limits of quantitation. However, this technique presents various issues resulting in increased MS downtime. This poster evaluates the extraction of a range of drugs of abuse from hydrolyzed and non-hydrolyzed urine using a novel flow-through matrix scavenging plate. Specific investigation of matrix component removal in terms of creatinine and urea, salt residue, pigmentation associated with urobillin content and protein removal will be demonstrated.

Experimental

Reagents

Standards, ammonium acetate, ammonium formate and formic acid were purchased from Sigma-Aldrich Company ltd. (Gillingham, UK). LC/MS grade solvents were from Honeywell Research Chemicals (Bucharest, Romania). Water (18.2 MΩ.cm) was drawn fresh daily from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK). Urine was kindly donated by healthy human volunteers.

Sample Preparation

Extractions were performed using a novel flow-through matrix scavenging plate in 96-well format. Non-hydrolyzed or hydrolyzed urine (100 µL) was pipetted into the wells and allowed to incubate if necessary. Acetonitrile (600 µL) was added and mixed using 5x aspirate and dispense steps. Elution was performed using 5 psi positive pressure for 2 minutes.

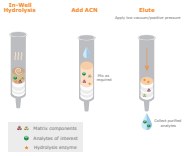


Figure 1. *Schematic of the novel flow-through scavenging plate.*

Post extraction: Extracts were either injected directly or evaporated at 40 °C and reconstituted in respective mobile phase for analysis.

LC/MS Conditions

Instrument: Waters Acquity UPLC interfaced via electrospray ionization to a Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK). Positive ions were acquired in the multiple reaction monitoring (MRM) mode.
Desolvation Temp: 450 °C **Ion Source Temp:** 150 °C
Gradient and MRM transitions: Details on Biotage.com

Creatinine & Urea Analysis

Column: Thermo Scientific BetaMax Acid 5 µm (100 x 2.1 mm) with C8 guard cartridge.
Mobile Phase: A: 10 mM Ammonium Acetate pH4 (aq)
Mobile Phase B: Acetonitrile.

DoA Analysis

Column: Restek Raptor™ Biphenyl 2.7 µm (100 x 2.1 mm) with EXP guard cartridge (Thames Restek UK Ltd., Saunderton, UK.)
Mobile Phase: A: 2 mM Ammonium Formate (aq) 0.1% formic acid
Mobile Phase B: 2 mM Ammonium Formate (MeOH), 0.1% formic acid

Gel Electrophoresis Conditions

Protein removal from hydrolyzed urine was investigated using a NuPAGE Novex 12% bis-tris mini gel with MOPS SDS running buffer. Gels were run for approximately 65 minutes and compared to a protein benchmark molecular weight ladder.

Results

Matrix Component Removal

While dilute and shoot is a method designed to save time, the interfering components from the original urine sample are not removed. Specifically, creatinine, urea, pigments and salts and in the case of enzyme-hydrolyzed urine; protein, remain in the sample and are injected into the LC/MS instrument. Samples with poor cleanliness will reduce the lifetime of the subsequently used liquid chromatography systems and increase the requirement for column and mass spectrometer maintenance. ***Figure 2.*** Illustrates pigment (urobilin) present when using D/S in a 1:10 ratio.

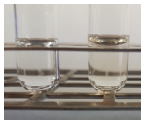
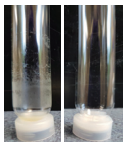


Figure 2. *Comparison of HPLC-grade water (left) with a 1:10 urine/aq dilution (right).*

Hydrolyzed urine, as a worst case due to presence of matrix pigment and ß-glucuronidase is shown in ***Figure 3.*** The image on the left shows the unprocessed matrix while post extracted solution is documented on the right.

Figure 3. *Visual appearance of hydrolyzed urine (left) and post-extraction eluent (right).*

Extracted samples were evaporated in glassware to highlight any salt residue and pigmentation remaining after processing. ***Figure 4.*** demonstrates non-hydrolyzed (left) and hydrolyzed (right) urine extracts comparing ACN precipitated urine and the novel flow-through matrix scavenging plate.



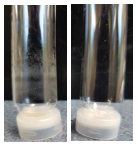


Figure 4. *Visual appearance of evaporated non-hydrolyzed urine (left), and hydrolyzed urine (right) following ACN crash compared to extracts following the novel flow-through scavenging plate.*

The extracts were analyzed for creatinine and urea, which are found in significant concentrations in urine and can have a detrimental effect on the quantitation of desired analytes. To demonstrate the removal of these matrix components, MRM acquisition was performed with and without sample extraction. ***Figure 5.*** charts the extent of removal of these matrix compenents while ***Figure 6.*** compares the relative MRM signal of the components on a fixed axis.

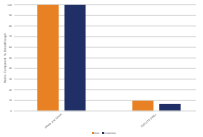


Figure 5. *Creatinine and Urea content chart for 1:9 dilute and shoot and novel flow-through scavenging plate.*

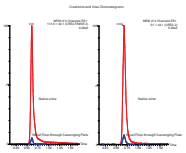


Figure 6. *MRM chromatograms illustrating relative Creatinine and Urea content in hydrolyzed urine: (red) following ACN crash, (blue) post processing through the novel flow-through scavenging plate.*

Continuing the investigation into component removal, a gel electrophoresis experiment was established to determine protein content following extraction. ***Figure 7.*** demonstrates protein content from enzyme constituents in various stages of urine processing. Full protein removal was achieved that are otherwise not removed using dilute and shoot approaches.

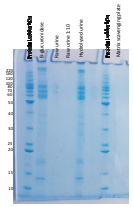


Figure 7. *Gel electrophoresis profile demonstrating protein content in various matrices and extracts.*

Finally the extracts were acquired in full scan mode (50-800 m/z) to compare baseline cleanliness. ***Figure 8.*** shows the offset, overlaid TICs comparing dilute and shoot and novel flow-through scavenging plate extracts with a blank mobile phase injection.

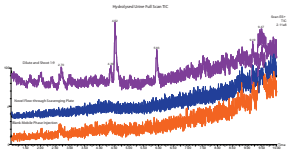


Figure 8. *Full scan TIC: blank mobile phase (red), novel flow-through scavenging plate (blue), dilute and shoot 1:9 (purple).*

Analyte Recovery

Typical analyte recoveries are demonstrated in ***Figure 9***. This data was acquired by direct injection of the flow-through eluent. However the use of evaporation and concentration is an option if greater signal is required.

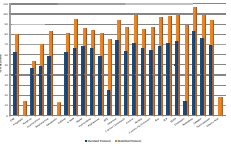


Figure 9. *Typical recovery profile using the novel flow-through matrix scavenging plate.*

Even though recoveries are lower than dilute and shoot approaches, the majority of analytes exhibit similar or improved signal response and hence sensitivity. ***Figure 10***. demonstrates the peak area comparison with a dilute and shoot approach.

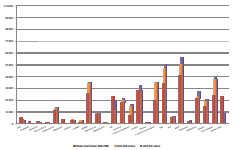


Figure 10. *Peak area comparison with dilute and shoot.*

Figure 11. demonstrates overlaid Flunitrazepam chromatograms, and the improvement seen in the signal versus dilute and shoot.

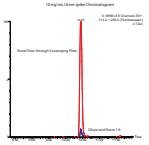


Figure 11. *Flunitrazepam MRMs overlaid at fixed signal intensity: (blue) 1:9 dilute and shoot; (red) novel flow-through scavenging plate.*

Figure 12. illustrates LOQ improvements when this product is used with hydrolyzed urine, compared to dilute and shoot.

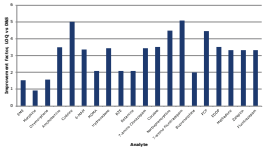


Figure 12. *Chart showing LOQ improvement factor of the novel flow-through scavenging plate over dilute and shoot.*

Calibration curves were constructed from 10-400 ng/mL. Good LOQs, linearity and coefficients of determination (r^2) were returned for all analytes with an example shown in ***Figure 13.***

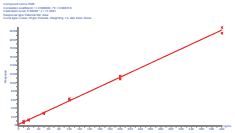


Figure 13. *Calibration curve for EME using the novel flow-through scavenging plate with direct injection.*

Conclusion

- This poster describes the use of a novel flow-through matrix scavenging plate for drugs of abuse extraction from urine.
- The removal of described endogenous matrix components and the associated benefits to quantitation are highlighted.
- The advantages to the workflow are: small number of steps, minimal volume of solvent, no evaporation required.

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