Evolution of Sample Preparation: Workflow Simplification Utilizing Sample Hold-up Technology in Forensic and Clinical Analyses

Lee Williams[1], Rhys Jones[1], Adam Senior[1], Geoff Davies[1], Helen Lodder[1], Katie-Jo Teehan[1], Alan Edgington[1], Steve Jordan[1], Claire Desbrow[1] & Paul Roberts[1]
[1]Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Cardiff, CF82 7TS, UK

Introduction

Sample preparation is generally regarded as a pain point for many labs as it can be laborious, time consuming and relatively expensive. In a high throughput setting performing offline chemistry also leads to increases in sample turnaround time and reporting. This poster evaluates simplified sample preparation workflows using optimized sample hold-up technology allowing chemistry in-situ. Reduced labour, extraction complexity, processing time, solvent usage and resultant instrument contamination and downtime can offset upfront costs associated with sample preparation.

Experimental

Reagents

Drug standards, ß-glucuronidase (HP-2) and additives were purchased from Sigma-Aldrich Company ltd. LC/MS grade solvents and water (18.2 MΩ.cm) were used throughout. Pooled human plasma was purchased from The Welsh Blood Service and urine kindly donated by healthy human volunteers.

Sample Preparation

Urine Hydrolysis: 1 mL of urine was mixed with 1 mL of 100 mM ammonium acetate buffer at pH 5 and 50 µL of ß-glucuronidase and incubated at 60 °C for 2 hours.

Extraction: Offline precipitation for plasma and dilute and shoot for urine were compared to 96-fixed well plate processing. Incubations and mixing were performed in-situ. ***Figure 1.*** illustrates workflow when using optimized sample hold-up frit technology.

Plasma: Protein precipitation, ISOLUTE® PPT+; ISOLUTE® PLD+; EVOLUTE® HYDRO SPE (ABN or CX).

Urine: Dilute and shoot; ISOLUTE® HYDRO DME+; EVOLUTE® HYDRO SPE (ABN or CX).

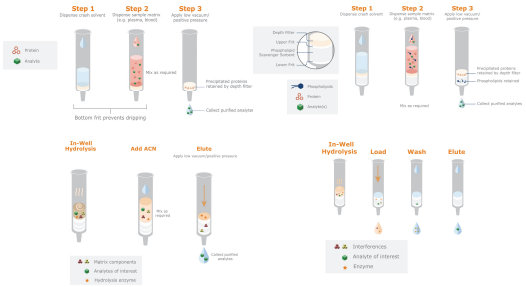

Figure 1. *Simplified processing using sample hold-up technology: protein precipitation, PPT+ (top left); phospholipid depletion, PLD+ (top right); dual mode extraction DME+ (bottom left); HYDRO SPE (bottom right).*

LC/MS Conditions

Details available on request.

Results

Plasma Extraction:

All plates held up solvent/matrix combinations overnight. Comparison of protein removal is demonstrated in ***Figure 2.*** Excellent removal is observed with all ACN protein precipitation techniques and SPE depending on pore size characteristics and retention mechanism.

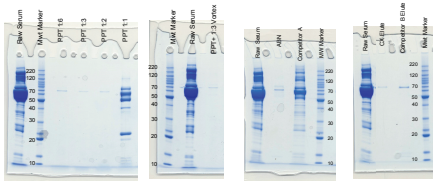

Figure 2. *Gel electrophoresis profiles.*

Phospholipid removal is demonstrated in ***Figure 3.*** Precipitation, offline or plate did not remove PLs from the samples. Whereas the use of a phospholipid scavenging technique resulted in excellent removal. SPE more complex and highly dependent on mechanism of interaction, wash and elution optimization.

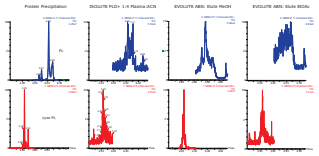

Figure 3. *TICs comparing PL extraction performance.*

Recovery profiles using generic methodologies for a multi-drug panel can be seen in ***Figure 4.*** PPT+ as a non-selective technique provides improved analyte recoveries compared to PLD+ and HYDRO CX SPE prep.

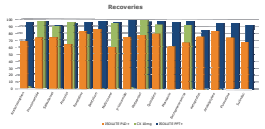

Figure 4. *Recovery profiles for multi-drug extraction from plasma.*

Figure 5. demonstrates matrix factors (evaporative/suppression effects) for the analyte panel. Suppression regions can be controlled by chromatography, so PL interferences do not always present issues immediately.

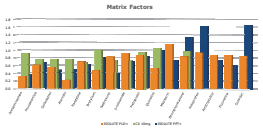

Figure 5. *Matrix factor profiles for multi-drug extraction from plasma.*

Assay issues can present in other ways. ***Figure 6.*** demonstrates UPLC column stability. Simple precipitation approaches can result in increased column backpressure and limited lifetime. Using more advanced sample preparation in the form of ISOLUTE PLD+ technology extends UPLC column lifetime reducing assay costs.

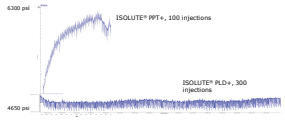

Figure 6. *UPLC column backpressure plot comparing PPT+ and PLD+ techniques.*

Urine Extraction:

Interferences such as pigment, ionic strength, creatinine, urea and protein can make for challenging urine assays. Dilute and shoot is dominant due to speed, cost and simplicity. However, cheap upfront can lead to more expensive downstream issues. ***Figure 7.*** illustrates protein interference when using ß-glucuronidase and associated removal with D/S and ISOLUTE HYDRO DME+. ***Figure 8.*** shows hydrolyzed urine and post extracted matrix using ISOLUTE HYDRO DME+.

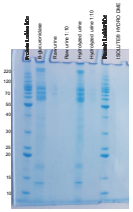

Figure 7. *Gel electrophoresis profile demonstrating protein content in various matrices and extracts.*

Figure 8. *Hydrolyzed urine (left) and post-extraction eluent (right).*

Figure 9. compares creatinine and urea levels for D/S and ISOLUTE HYDRO DME+ extractions. SPE pigment, creatinine and urea removal will be highly dependent on chemistry, wash and elution protocols (data not shown).

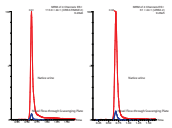

Figure 9. *MRM chromatograms illustrating creatinine and urea content in hydrolyzed urine: (red) following ACN crash, (blue) post processing through HYDRO DME+.*

Drugs of abuse recoveries and matrix factors (evaporative and suppression effects) for HYDRO CX and HYDRO DME+ are demonstrated in ***Figures 10 and 11.*** respectively. Slightly lower recoveries were returned for the flow-through technique, but excellent matrix factors returned.

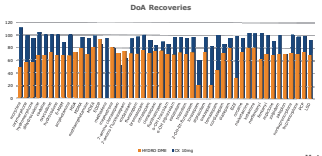

Figure 10. *Recovery profiles for DoA extraction from urine.*

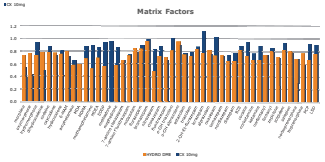

Figure 11. *Matrix factor profiles for DoA extraction from urine.*

Figure 12. transfers data into chromatogram form for signal:noise comparison. Lower S:N for dilute and shoot due to suppression effects also manifests into signal drop off over a number of sequential injections as demonstrated in ***Figure 13.*** ultimately resulting in instrument cleaning and downtime.

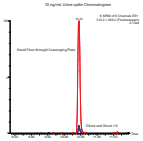

Figure 12. *Flunitrazepam MRMs overlaid at fixed signal intensity: (blue) 1:9 dilute and shoot; (red) HYDRO DME+.*

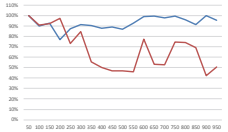

Figure 13. *Peak area response comparison over 900 injections: (blue) 1:9 dilute and shoot; (red) HYDRO DME+.*

Figures 14. demonstrate plasma and urine sample preparation costs, respectively. When including better sample preparation as expected assay costs increase. However, simplified workflows reduce labour time which can partially offset this increase.

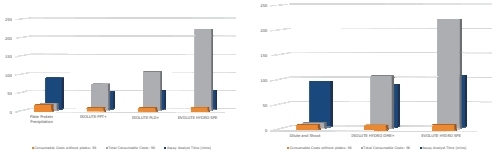

Figure 14. *Sample preparation costs: plasma (left); urine (right).*

As demonstrated in ***Figures 6. and 13.*** instrument issues will need to be costed into the final figure: For example, source cleaning requires instrument downtime which can impact throughput needs; while UPLC column backpressure increases, result in more frequent replacement. These instrument related issues can offset increased sample preparation costs.

Conclusions

- This poster illustrates the use of a novel sample hold up technology for workflow improvements when dealing with biological matrices.
- The removal of endogenous matrix components, sample cleanliness and the associated benefits to assay performance are highlighted.
- Improved sample preparation, although increase assay costs, the hidden benefit to instrument performance, increased throughput due to lower downtime and issues more than outweigh the advantages of cheaper precipitation or dilute and shoot approaches.

ASMS 2019, Atlanta, GA
Part Number: P200