Evaluation of a Novel Low-Volume 96-well SPE Format for Forensic and Clinical Toxicology Prior to UHPLC-MS/MS Analysis

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Introduction

Miniaturisation within various fields of analytical chemistry is not a new phenomenon. Early phase small animal drug trials have largely been the driver due to limited sample sizes available. However, this trend is gaining popularity in forensic/clinical toxicology with respect to alleviating patient discomfort, particularly in paediatrics. Increased LC-MS/MS sensitivity has reignited this focus area. This poster will evaluate a novel low-volume 96-well solid phase extraction (SPE) format and potential application to forensic and clinical toxicology. Emphasis on matrix load volumes, wash and elution volume minimisation and processing will be investigated for: target analyte recoveries, RSDs, matrix effects and overall signal. Comparison of the novel low-volume plate and traditional SPE plate formats will be presented.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Steroids, catecholamine standards, ammonium fluoride, ammonium formate, ammonium acetate and formic acid were purchased from Sigma-Aldrich Company ltd. (Gillingham, UK). HPLC and 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 samples were kindly donated by healthy human volunteers. Plasma was purchased from Welsh Blood Service (Pontyclun, UK). Serum was purchased from Golden West Biologicals, Inc. (Temecula CA).

Sample Preparation

SPE Information

DoA: Biotage® Mikro CX Plate, 2 mg (601-0002-LVP)
Plasma cats/mets: Biotage® Mikro WCX Plate, 2 mg (602-0002-LVP)
Steroids: Biotage® Mikro ABN Plate, 2 mg (600-0002-LVP)

All methods follow a typical SPE procedure incorporating additional wash steps (***Figure 1***).

Figure 1. *Schematic of a Typical SPE Procedure.*

Matrix Preparation:

Drugs of Abuse Panel:

Urine was spiked with standards at 100 pg/mL. Urine hydrolysis was performed 1:1 with buffered enzyme 95/5 100 mM ammonium acetate pH 5 and ß-glucuronidase at 60 °C for 2 hrs.

Plasma catecholamine Panel:

Plasma was spiked with standards at 100 pg/mL and pre-treated 1:1 with 0.05% formic acid (aq) prior to extraction.

Steroid Panel:

Serum was spiked with standards at 100 pg/mL and pre-treated with 1% formic acid (aq) prior to extraction.

Final extraction protocols for each are shown in ***Table 1***.

Table 1. Optimized Extraction Protocols.

Conditions	Steroids	DoA	Cats/Mets
Condition		MeOH	
Equilibrate	0.1% formic acid aq	4% phosphoric acid aq	10 mM Ammonium acetate pH 6.0 aq
Load	Pre-treated Serum	Pre-treated Urine	Pre-treated Plasma
Wash 1	0.1% formic acid aq	4% phosphoric acid aq	10 mM Ammonium acetate pH 6.0 aq
Wash 2	60:40 water:MeOH	50:50 water:MeOH	IPA
Wash 3	/	/	DCM
Elution	MeOH	DCM:MeOH:NH_4OH 78:20:2	2% formic acid 80:20 MeOH:H_2O
Recon	50:50 water:MeOH	90:10 Water:MeOH 0.1% formic acid	-

LC/MS Conditions

Steroids & DoA

Instrument: Shimadzu Nexera X2 UHPLC and 8060 (Shimadzu Europa GmbH, Duisburg, Germany)
Steroid Column: ACE C18 1.7 µm 100 x 2.1 mm + guard (ACT, UK)
Mobile phase: A, 0.2 mM NH_4F (aq); B, MeOH **Flow rate:** 0.4 mL/min
Column temp: 40 °C **Injection volume:** 10 µL

DoA Column: Restek Raptor™ Biphenyl 2.7 µm (100 x 2.1 mm)
Mobile phase: 2 mM ammonium formate; 0.1% formic acid (aq), MeOH
Column temp: 30 °C **Flow rate:** 0.4 mL/min **Injection volume:** 10 µL

Catecholamines

Instrument: Shimadzu Nexera UHPLC (Shimadzu Europa GmbH, Duisburg, Germany): 5500 AB Sciex (Framingham, USA)
Column: ACE Excel C18-PFP 1.7 um (3.0 x 100 m)
Mobile phase: A, 0.05 mM NH_4F (aq); B, MeOH **Flow rate:** 0.4 mL/min
Column temp: 40 °C **Injection volume:** 10 µL

For details on MS conditions and MRM transitions for each analyte panel visit Biotage.com

Results

Drugs of Abuse Analysis

Due to the presence of an EVOLUTE® EXPRESS CX SPE method initial work simply involved scaling the 10 mg method to the 2 mg format. Method scalability is demonstrated in ***Figure 2***.

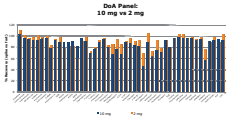


Figure 2. DoA *recovery profiles comparing 10 mg and 2 mg CX plates.*

Subsequent evaluation involved determining the maximum load for the low-volume plate. ***Figure 3.*** demonstrates that up to 600 µL total pre-treated urine can be loaded without deterioration.

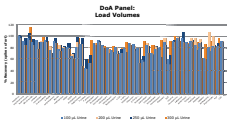


Figure 3. DoA *recovery profiles loading 200-600 µL of pre-treaded urine.*

Investigation of processing volumes for conditioning/equilibration and washing are demonstrated in ***Figure 4.*** Wash steps work well at 100 µL but can be tailored for individual assay.

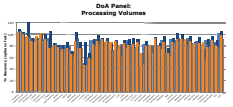


Figure 4. DoA *recovery profiles processing with 50-200 µL steps.*

Minimum elution volumes, 20-50 µL were investigated for recoveries and RSDs as shown in ***Figures 5 and 6.*** respectively. As observed elution volumes of 30 µL in a single aliquot can be used without recovery or RSDs deterioration.

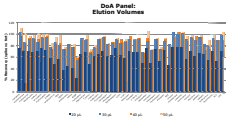


Figure 5. *Recovery profiles evaluating minimum elution volumes of 20-50 µL.*

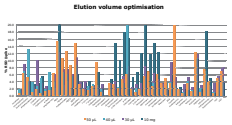


Figure 6. *RSD profiles evaluating minimum elution volumes of 20-50 µL.*

Catecholamine Analysis

Previous catecholamine methods were developed on 10 or 30 mg WCX plate formats. Using a scaled down process, the 2 mg plate was compared to larger plate sizes. ***Figure 7.*** demonstrates plate comparison from 2-60 mg.

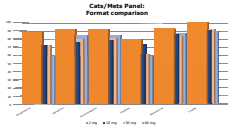


Figure 7. *Recovery profiles of cats/mets using various WCX plate formats.*

Further investigation of the low-volume WCX plate involved evaluating minimum elution volumes as demonstrated in ***Figure 8.***

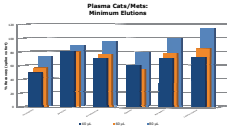


Figure 8. *Recovery profiles of cats/mets evaluating minimum elution volumes.*

Steroid Analysis

An existing ABN method was used to evaluate the potential of a novel low-volume SPE plate for steroid analysis. 10 mg plates were compared to the 2 mg plate, as seen in ***Figure 9.***

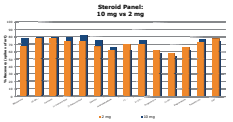


Figure 9. *Recovery profiles comparing 2 mg and 10 mg plate performance for steroids.*

Maximum matrix load volumes were investigated as shown in ***Figure 10.*** Load volumes of 600 µL (1:1) can be used with this format without deterioration of performance.

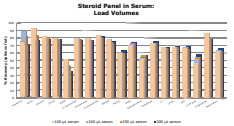
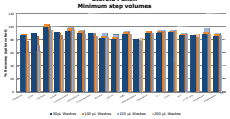


Figure 10. *Recovery profiles for maximum load volumes.*

Figures 11. compares processing steps for the steroid assay. Volumes between 50-200 µL can be utilised with 100 µL being the optimum for a 2 mg bed.

Figure 11. *Recoveries comparing step volumes of 50-200 µL.*



Minimum elution volumes, 20-50 µL were investigated for recoveries and RSDs as shown in ***Figures 12 and 13.*** respectively. As observed elution volumes of 30 µL in a single aliquot can be used without recovery or RSDs deterioration.

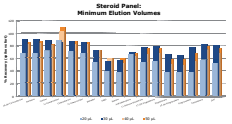


Figure 12. *Recovery profiles of minimum elution volumes for an ABN steroid method.*

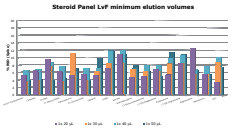


Figure 13. *RSD profiles of minimum elution volumes for an ABN steroid method.*

Conclusion

- This poster demonstrates the use of a new low-volume SPE offering for clinical and forensic toxicology.
- We have demonstrated scalability of existing methodologies for drugs of abuse extraction from urine, catecholamine metabolites from plasma and steroid hormones from serum.
- Polymer-based SPE combined with low dead volume components allows extraction of up to 600 µL loading volumes while allowing minimum elution volumes of 30 µL or below in a single aliquot.

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