Evaluation of Streamlined SPE Processing using Novel Components prior to LC-MS/MS Analysis.

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Introduction

Solid phase extraction is generally regarded as the gold standard when comparing sample preparation approaches. However, SPE processing can be laborious and time consuming. In most cases you are required to condition the phase with an organic solvent, typically methanol, followed by equilibration with an aqueous based solvent prior to sample loading. Many modern polymer-based SPE sorbents are water-wettable. EVOLUTE® EXPRESS combines sorbent wettability with optimized SPE components, allowing better flow consistency and in many cases eliminating the need for SPE column conditioning; thus simplifying and reducing extraction processing. This poster compares the performance of various SPE column chemistries using 10-500 mg bed weights, in order to investigate whether sorbent conditioning and equilibration steps are truly required.

Experimental

Reagents

Standards were purchased from LGC Standards (Teddington, UK). Formic acid, ammonium acetate, and ammonium hydroxide were purchased from Sigma Aldrich (Poole, UK). Blank urine was obtained from healthy human volunteers. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

Various SPE column formats were compared: 10 and 30 mg/1 mL, 60 mg/3 mL and 150 and 500 mg/6 mL. Urine was spiked with various analyte suites depending on sorbent chemistry:

EVOLUTE® EXPRESS ABN:

Acidic, basic and neutral analytes: sulindac, ketoprofen, metoprolol, mianserin, prednisolone.

EVOLUTE® EXPRESS CX:

Weakly basic analytes: procainamide, salbutamol, atenolol, metoprolol, quinidine.

EVOLUTE® EXPRESS WCX:

Strong/weakly basic analytes: bretylium, metoprolol, quinidine, amitriptyline.

EVOLUTE® EXPRESS AX:

Weakly acidic analytes: ketoprofen, sulindac, mefenamic acid, diclofenac.

EVOLUTE® EXPRESS WAX:

Strongly acidic analytes. Dimethylthiophosphate, diethylthiophosphate, hexanesulfonic acid, benzenesulfonic acid.

Traditional SPE requires phase pre-conditioning. However, the comparison of eliminating these steps will be shown for all chemistries. Analytes were extracted using generic methodology for all sorbent chemistries. Full details are shown in ***Table 1***.

Table 1. *Generic methodology.*

Step	ABN	CX	WAX	AX	WCX
Condition	MeOH	MeOH	MeOH	MeOH	MeOH
Equilibrate	0.1% Formic acid	H_2O	H_2O	H_2O	H_2O
Sample Load	1:3 1% Formic acid	1:3 50mM NH_4OAc pH6	1:3 2% formic acid	1:3 50mM NH_4OAc pH7	1:3 50mM NH_4OAc pH7
Wash 1	95:5 (v:v) H_2O/MeOH	50mM NH_4OAc pH6	2% formic acid (aq)	50mM NH_4OAc pH7	50mM NH_4OAc pH7
Wash 2	NA	MeOH	MeOH	MeOH	MeOH
Elution	MeOH	5% NH_4OH/ MeOH	5% NH_4OH/ MeOH	2% formic acid/MeOH	2% formic acid/MeOH

UPLC Conditons

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).

ABN, CX, WCX and AX suites:

Column: ACE EXCEL 2 C18, 50 x 2.1 mm id 2 µm (ACT, UK).
Mobile Phase: A: 2 mM ammonium acetate with 0.1% FA (aq)
Mobile Phase B: 2 mM ammonium acetate with 0.1% FA in MeOH
Flow Rate: 0.4 mL/min
Gradient: Available on request
Injection Volume: 10 µL
Column Temperature: 40 ˚C

WAX suite:

Column: Kinetex HILIC, 100 x 2.1 mm id 2.6 µm (Phenomenex, Cheshire, UK).
Mobile Phase: A: 100 mM ammonium acetate (aq)
Mobile Phase B: MeCN
Flow Rate: 0.6 mL/min
Isocratic: 9/91 A/B
Injection Volume: 10 µL
Column Temperature: 40 ˚C

Mass Spectrometry

Instrument: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive and negative ions were acquired using the multiple reaction monitoring mode (MRM).
Desolvation Temperature: 450 °C
Ion Source Temperature: 150 °C
Collision Gas Pressure: 3.5 x 10^{-3} mbar

Results

Extensive flow testing using urine matrix for all sorbent chemistries was optimized at 1 psi positive pressure (PPM+ 96) or -0.1 bar vacuum. Flow rate comparisons between traditional SPE competitor product, and EVOLUTE® EXPRESS with and without conditioning steps were investigated. ***Figure 1***. demonstrates relative flow characteristics (n=12) between the three variants.

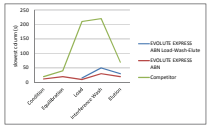

Figure 1. SPE procedure flow rates: SPE competitor product vs EVOLUTE® EXPRESS ABN, with and without conditioning and equilibration: 10 mg/1 mL column formats.

Table 2. demonstrates flow characteristics for mixed-mode strong ion exchange sorbents in the 60 mg/3 mL formats using 1 psi positive pressure processing.

Table 2. *Flow rates comparing EVOLUTE® EXPRESS mixed-mode strong ion exchange sorbents with standard SPE.*

	CX Flow (sec)			AX Flow (sec)		
Step	STD	EXPRESS	L-W-E	STD	EXPRESS	L-W-E
Condition	17-20	23-24	/	25-27	20-22	/
Equilibration	27-30	27-31	/	33-34	26-30	/
Load	**81-107**	**32-34**	**36-47**	**65-107**	**27-31**	**26-29**
Wash 1	**38-64**	**29-33**	**36-45**	**41-47**	**32-36**	**34-39**
Wash 2	25-26	29-30	27-33	29-30	27-29	26-28
Elute	26-28	25-27	22-26	25-27	21-25	19-21

As demonstrated, flow characteristics were much faster and more consistent, column to column using EVOLUTE® EXPRESS with both the full processing and the load-wash-elute regimens. The problematic steps with traditional SPE are during analyte loading (particularly when using viscous matrices) and the first aq wash step. These issues were eliminated when using EVOLUTE® EXPRESS.

By eliminating the need for column conditioning and equilibration steps, time saving for processing 96 samples using EVOLUTE® EXPRESS columns with the load-wash-elute methodology is up to 30%. ***Table 3.*** demonstrates the time taken to process 96 1 mL EVOLUTE EXPRESS columns using traditional processing and with the modified load-wash-elute protocol.

Table 3*.Processing time for EVOLUTE® EXPRESS ABN using traditional processing and load-wash-elute methodology: Processed using the Biotage Extrahera Automated SPE Platform.*

Processing Step	Traditional SPE	EVOLUTE® EXPRESS
Condition	✓	Not required
Equilibrate	✓	Not required
Pre-treat sample and Load	✓	✓
Wash	✓	✓
Elute	✓	✓
Total time for 96 samples	33 minutes	24 minutes

Multiple analyte suites were investigated according to the extraction mechanisms involved for the appropriate polymeric SPE sorbent. ***Figures 2-6.*** demonstrate the recovery profiles using EVOLUTE® EXPRESS chemistries with both traditional and load-wash-elute processing regimens. The extraction protocols were not optimized for the specific suites, and generic methodology used throughout.

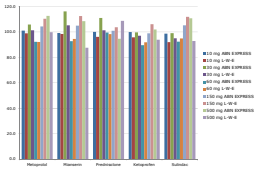

Figure 2. *Acidic, basic and neutral recoveries from urine using EVOLUTE® EXPRESS ABN, with and without conditioning and equilibration steps.*

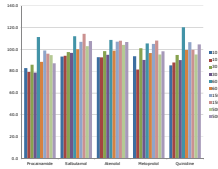

Figure 3. *Basic analyte recoveries from urine using strong cation exchange: EVOLUTE® EXPRESS CX, with and without conditioning and equilibration steps.*

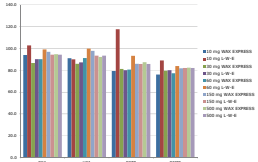

Figure 4. *Phosphate and sulfonate recoveries from urine using weak anion exchange: EVOLUTE® EXPRESS WAX, with and without conditioning and equilibration steps.*

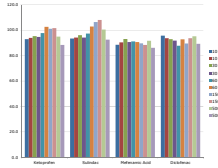

Figure 5. *Acidic analyte recoveries from urine using strong anion exchange: EVOLUTE® EXPRESS AX, with and without conditioning and equilibration steps.*

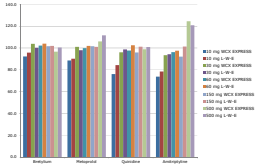

Figure 6. *Basic analyte recoveries from urine using weak cation exchange: EVOLUTE® EXPRESS WCX, with and without conditioning and equilibration steps.*

As can be seen in the data, the elimination of the pre-conditioning steps make negligible difference on final recoveries. RSDs across all analyte suites were comparable and below 10% in all cases.

Conclusion

- Improved flow rates over conventional SPE formats was observed using the EVOLUTE EXPRESS using standard processing and the load-wash-elute protocol.
- Excellent recoveries and RSDs were observed using the load-wash-elute methodology and demonstrated minimal difference compared to standard processing.
- The results demonstrated that it is possible to perform SPE without conditioning and equilibration steps when using the optimized components and water wettable sorbent chemistries in EVOLUTE® EXPRESS.
- Less clogging of aqueous samples was seen in these experiments. This is especially important when automation is involved.
- As such faster work flow can be achieved for higher throughput and increased lab efficiency.

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