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Strategies for Enhancing Productivity Using Biotage® Mikro Solid Phase Extraction Microelution Plates

Introduction

Solid phase extraction is a powerful extraction technique which delivers high analyte recoveries and clean extracts. Microelution SPE formats utilize very small amounts of extraction sorbents, suitable for processing low volumes of sample, and can deliver excellent analyte recovery in very low elution volumes.

But, solid phase extraction is sometimes viewed as a complex and time consuming sample preparation technique due to the number of processing steps.

In this technical note we examine some of the strategies that can be used to simplify the procedure, and at the same time improve throughput, and reduce the time you need to spend preparing samples before analysis.

Part 1 of this technical note will focus the use of the simplified Load-Wash-Elute solid phase extraction procedure for enhancing processing efficiency, using Biotage® Mikro SPE microelution plates.

In part 2, we look at how the use of microelution SPE can eliminate the need for the evaporation step, which is usually necessary when using traditional SPE formats. This can significantly reduce the overall processing time.

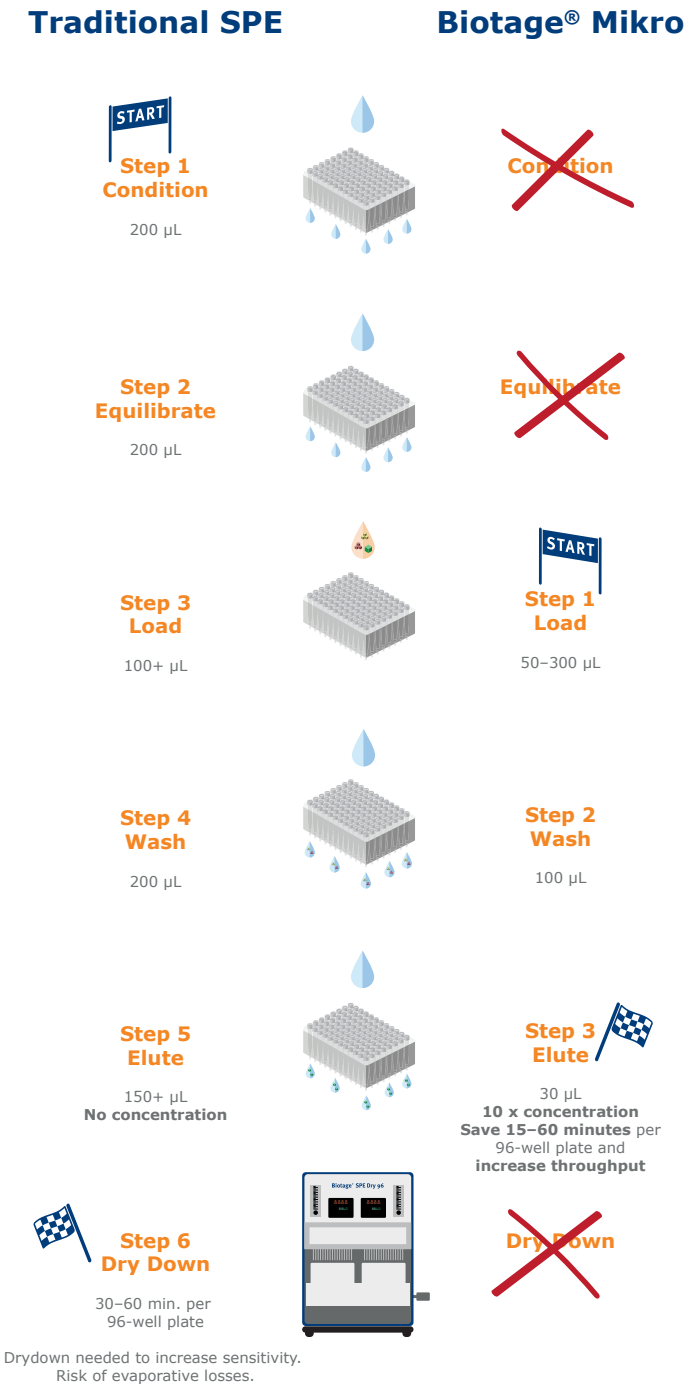

Figure 1. Eliminating sample preparation steps with Biotage® Mikro Plates.

Part 1. Simplifying Sample Preparation Using Load-Wash-Elute SPE

The typical solid phase extraction method incudes 5 steps:

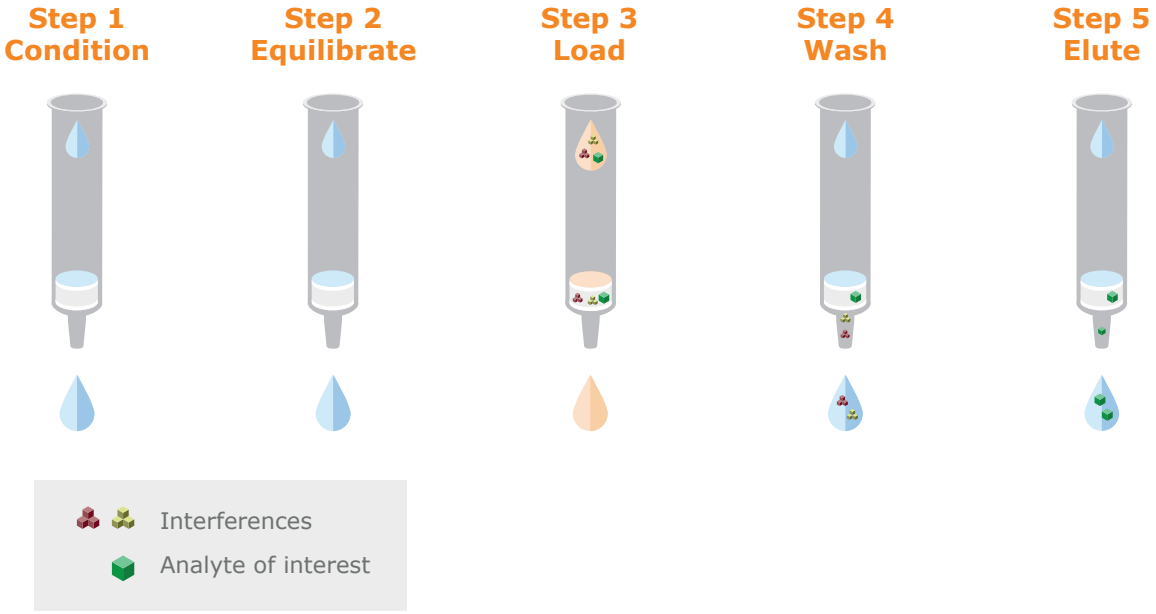

Figure 2. Traditional '5-step' SPE method.

Biotage® Mikro microelution plates contain EVOLUTE® SPE sorbents. These high-performance media are water wettable, so the traditional conditioning and equilibration step are not required to achieve the same high analyte recoveries, saving time and reducing solvent consumption.

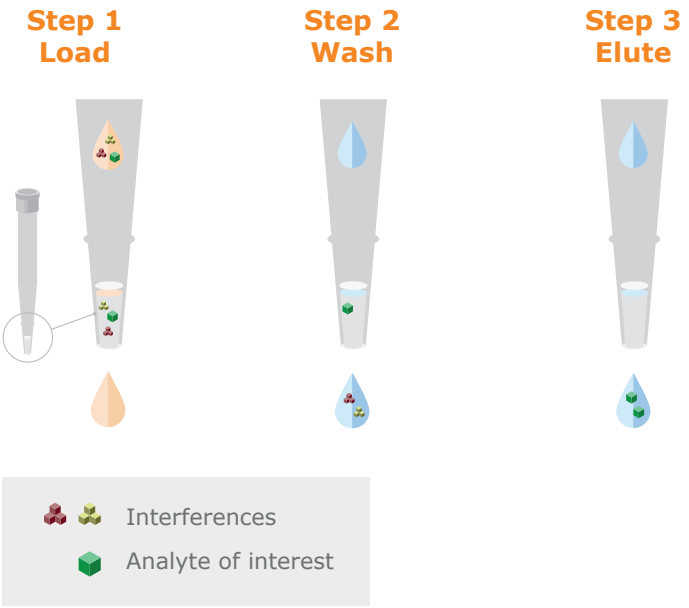

Figure 3. Load-Wash-Elute SPE method.

Experimental

Study 1: Drugs of Abuse Panel in Hydrolysed Urine

In this experiment, we spiked a panel of 49 drugs of abuse including opioids, cocaine and metabolites, benzodiazepines and amphetamines, into hydrolyzed urine samples at a concentration of 1 ng/mL and extracted them using Biotage® Mikro ABN microelution plates.

We compared the recovery of the drugs using the full ('5 step') SPE method and the simplified Load-Wash-Elute method.

Table 1. Solid phase extraction methodology: Drugs of abuse in urine.

Step	Biotage® Mikro ABN Full SPE Method	Biotage® Mikro ABN L-W-E SPE Method
Condition	Methanol	N/A
Equilibrate	0.1% Ammonium Hydroxide (aq) (100 µL)	N/A
Sample load	200 µL of pre-treated sample	200 µL of pre-treated sample
Wash	a. 0.1% Ammonium Hydroxide (100 µL) b. H_2O:MeOH (90:10, v/v, 100 µL)	a. 0.1% Ammonium Hydroxide (100 µL) b. H_2O:MeOH (90:10, v/v, 100 µL)
Elute	DCM:MeOH (90:10, v/v, 30 µL)	DCM:MeOH (90:10, v/v, 30 µL)

After elution, samples were evaporated to dryness and reconstituted in H_2O:MeOH (90/10, v/v). containing 0.1% formic acid (30 µL) before LC-MS/MS analysis.

Figure 4 shows the recovery obtained from each approach.

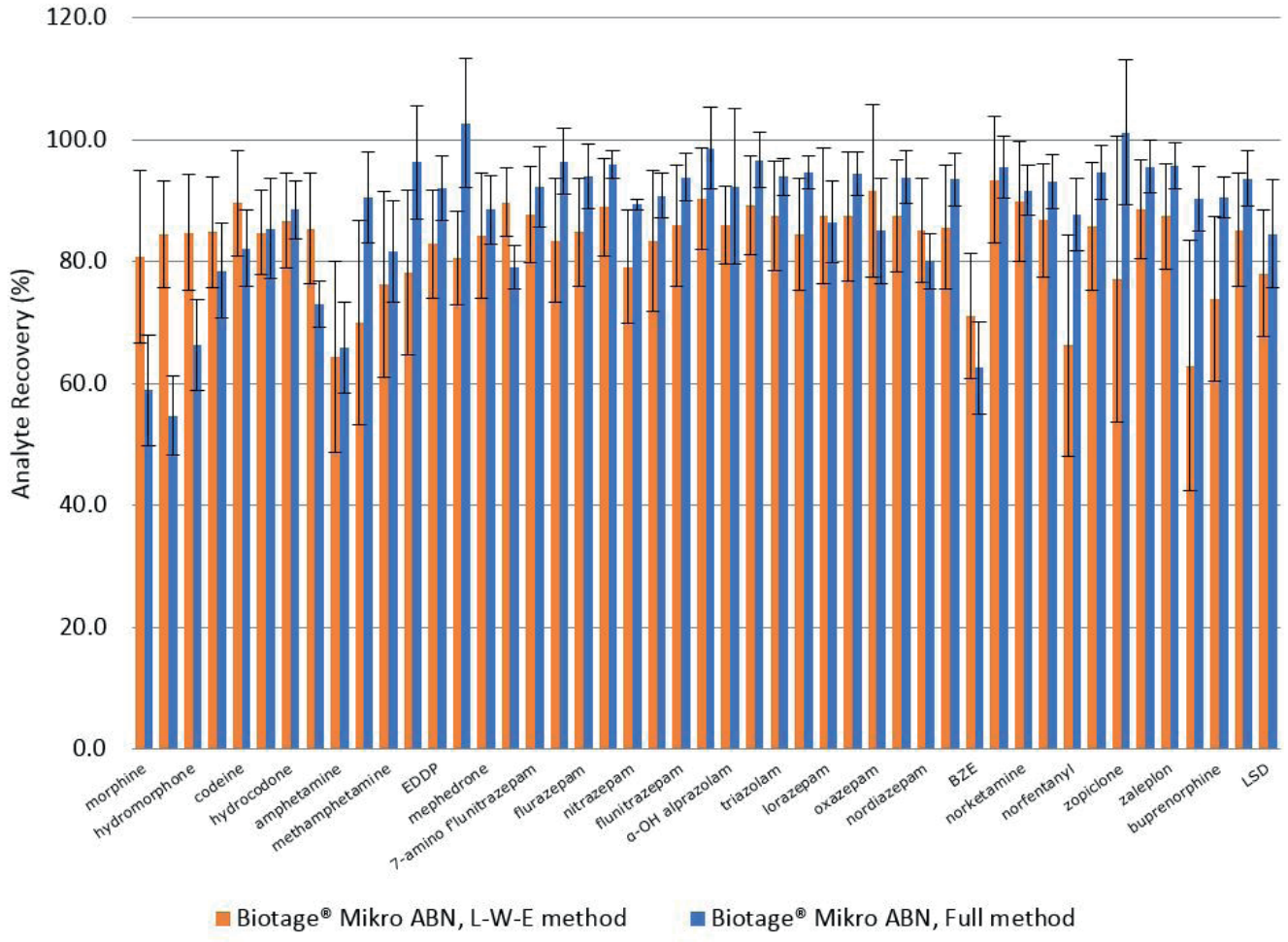

Figure 4. Analyte Recovery (n=7) with RSD for 49 Drugs of Abuse in hydrolyzed urine.

Study 2: NSAIDS in Urine

In this experiment, we spiked a panel of non-steroidal anti- inflammatory drugs (NSAIDs) into urine samples at a concentration of 5 ng/mL, and extracted them using Biotage® Mikro AX microelution plates, containing EVOLUTE® AX media.

EVOLUTE® AX is a mixed-mode, strong anion exchange media, retaining acidic analytes using a dual retention mechanism. This allows for enhanced interference removal, reducing matrix effects without analyte loss.

Again, we compared the recovery of the drugs using the full ('5 step') SPE method and the simplified Load-Wash-Elute method.

Table 2. Solid Phase Extraction Methodology: NSAIDs in Urine.

Step	Biotage® Mikro AX Full SPE method	Biotage® Mikro AX L-W-E SPE Method
Condition	Methanol (100 µL)	N/A
Equilibrate	pH 7 50mM Ammonium Acetate (100 µL)	N/A
Sample load	100 µL of pre-treated sample	100 µL of pre-treated sample
Wash	a. pH 7 50mM Ammonium Acetate (200 µL) b. Methanol (200 µL)	a. pH 7 50mM Ammonium Acetate (200 µL) b. Methanol (200 µL)
Elute	Methanol/Formic Acid (98:2, v/v, 50 µL)	Methanol/Formic Acid (98:2, v/v, 50 µL)

After elution, samples were evaporated to dryness and reconstituted in 200 µL of mobile phase before LC-MS/MS analysis.

Figure 5 shows the recovery obtained from each approach.

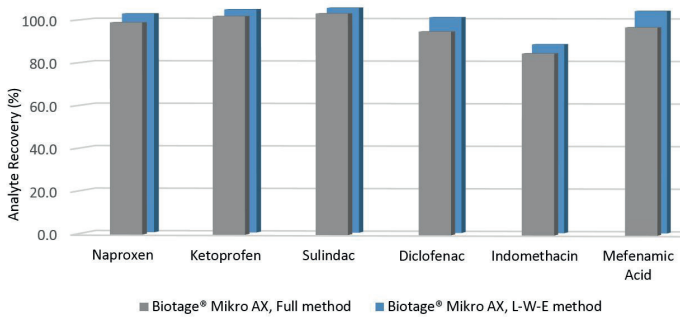

Figure 5. Analyte Recovery, NSAIDs in urine.

Results and Discussion

For both analyte suites, comparable recovery and reproducibility was achieved using each approach, demonstrating that elimination of the conditioning and equilibration steps does not adversely impact on assay performance when using Biotage® Mikro solid phase extraction microelution plates. Elimination of 2 steps from the sample preparation process reduced the overall sample preparation time by ~20%.

Part 2: Eliminating the Evaporation Step using Biotage® Mikro Plates

Evaporation and reconstitution of an extract following SPE is an approach which is often used to achieve the sensitivity required to reach low detection limits. However, this can be a time-consuming step in the overall assay.

Microleution SPE using Biotage® Mikro plates can be used as an alternative approach to achieve improved analytical sensitivity, without the need for evaporation.

Experimental

Using a typical SPE procedure (including all possible steps) we compared the time taken to dispense solvent (96 wells per plate using an 8 position pipette) and process each step using a Biotage® PRESSURE+ 96 Positive Pressure Manifold. Recommended processing conditions were used for each format. The high capacity Biotage® Mikro plate allowed loading of an equal sample volume (in this case 400 µL of diluted matrix), despite the reduced bed mass, without analyte breakthrough. Solvent volumes used for all other steps were optimized for the format.

Table 3 shows the solvent volume and processing time taken for each step.

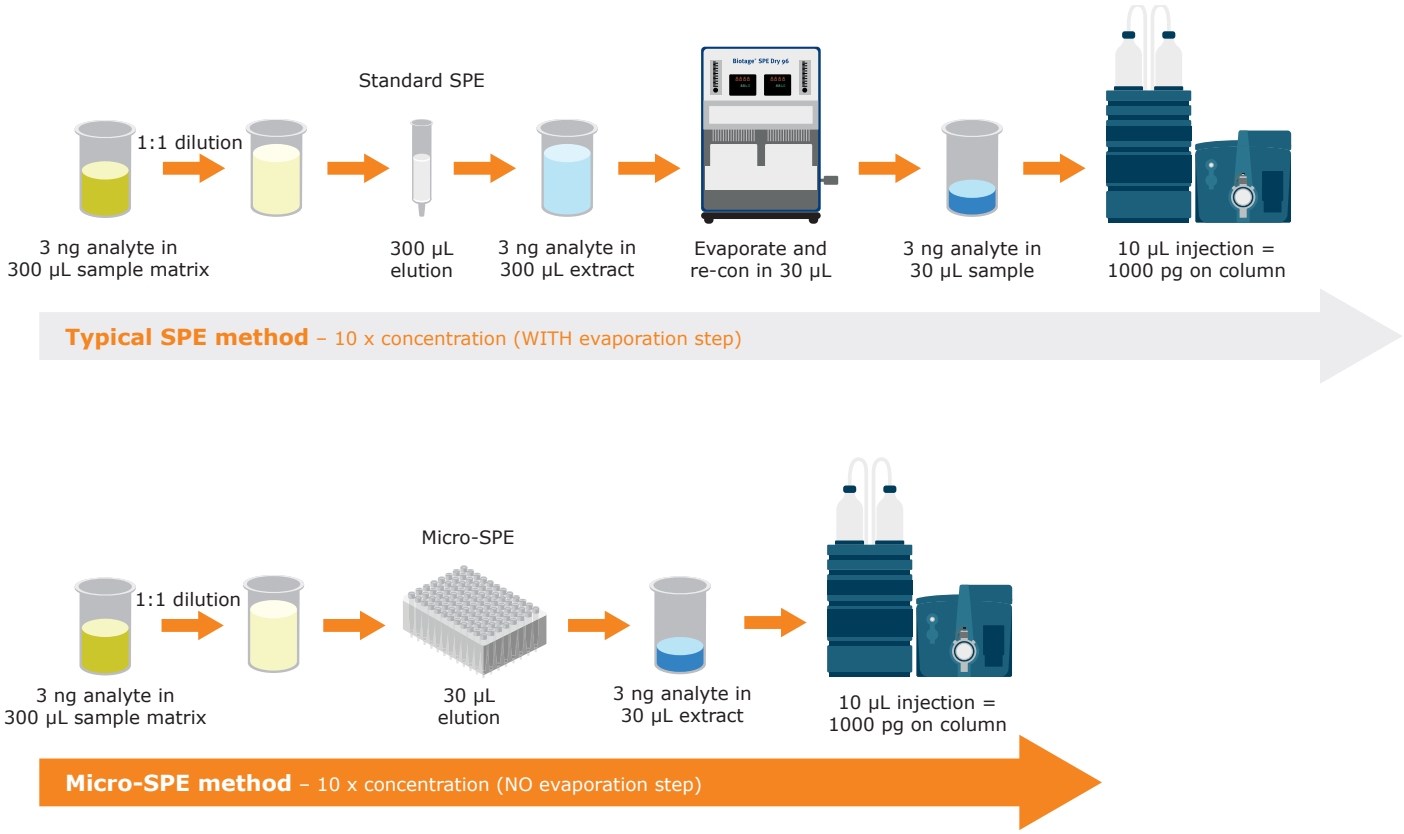

Figure 6. Schematic illustrating how Biotage® Mikro plates can deliver concentrated extracts without evaporation.

Table 3. Processing time comparison.

	2 mg, Direct Injection		10 mg, with Evaporation Step	
	Volume	Time	Volume	Time
Condition	100 µL	1 min 50 s	500 µL	1 min 50 s
Equilibrate	100 µL	2 min	500 µL	2 min
Load	400 µL	3 min	400 µL	2 min
Wash 1	100 µL	2 min	500 µL	2 min
Wash 2	100 µL	2 min 40 s	500 µL	2 min 20 s
Dry	-	2 min	-	5 min
Elute	30 µL	1 min 40 s	150 µL	1 min 35 s
Evaporation	-	-	-	15 min
Reconstitution	-	-	30 µL	1 min 30 s
Total		14 mins 50 s		33 mins 15 s

Results and Discussion

The time taken for processing 96-samples using the Biotage® Mikro plate was ~15 minutes, compared to ~ 33 minutes for the traditional plate. Similar analyte recovery, RSD and sensitivity was achieved in both situations. The microelution approach, eliminating extract evaporation, reduced overall sample preparation time by more than half, or 18 minutes per 96 samples.

Conclusions

Load-Wash-Elute SPE, eliminating conditioning and equilibration steps can provide excellent extraction performance while reducing complexity and processing time. Elimination of the evaporation step using microelution SPE can also bring significant time savings.

Note: The specific saving will be solvent and volume dependant. It is not always possible to employ direct injection in microelution SPE experiments, as some elution solvents may not be compatible with the analytical technique. In that situation strategies such as extract dilution or evaporation/reconstitution prior to analysis may be required. However, due to the reduced volume of elution solvent, evaporation times are likely to be significantly reduced compared to the traditional formats.

Ordering Information

Part Number	Description	Quantity
600-0002-LVP	Biotage® Mikro ABN Plate, 2 mg	1
601-0002-LVP	Biotage® Mikro CX Plate, 2 mg	1
602-0002-LVP	Biotage® Mikro WCX Plate, 2 mg	1
603-0002-LVP	Biotage® Mikro AX Plate, 2 mg	1
604-0002-LVP	Biotage® Mikro WAX Plate, 2 mg	1
Collection Plate and Accessories		
121-5202	Collection Plate, 1 mL Square	50
121-5204	Pierceable Sealing Mat	50
Positive Pressure Manifold		
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1

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