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Improving Analytical Sensitivity and Sample Throughput 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.

Biotage® Mikro solid phase extraction microelution plates have a very low dead volume design, with relatively tall, narrow beds. These dimensions mean that the Mikro plates have high analyte capacity, but analyte recovery is achieved using much lower elution volumes than traditional SPE formats.

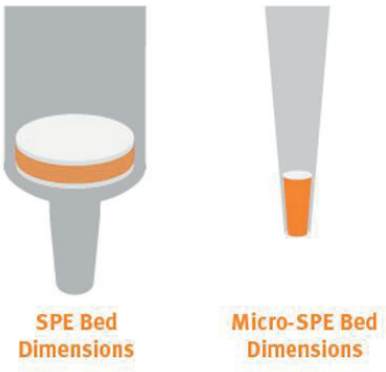

Figure 1. Comparison of SPE bed dimensions 10 mg SPE plate vs Biotage® Mikro Plate (not to scale).

The combination of high capacity and low elution volume leads to an increased concentration of analyte in the elution solvent.

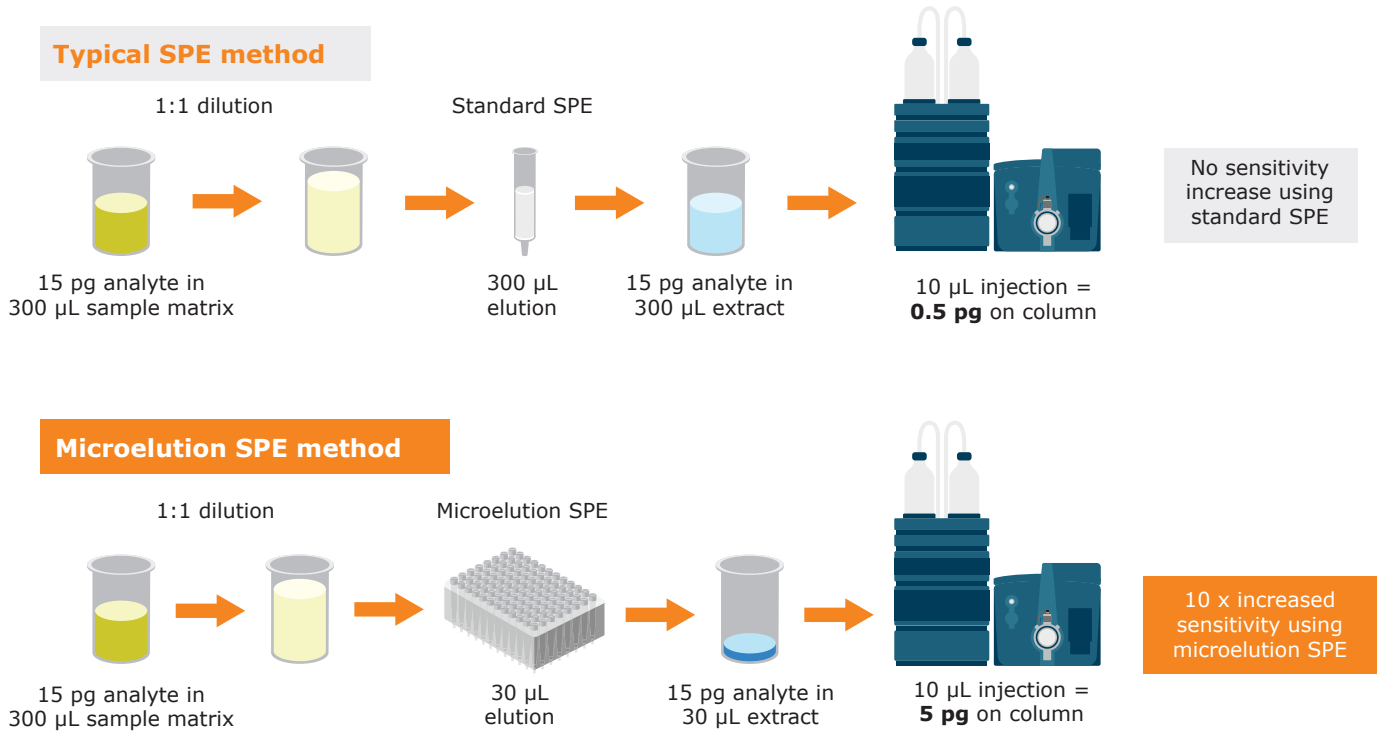

Figure 2. Schematic of workflow using 10 mg SPE plate vs Biotage® Mikro plate.

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Experimental

In this example, we compared the use of Biotage® Mikro ABN plates with traditional format EVOLUTE® EXPRESS ABN 10 mg 96-well plates for the extraction of steroids from human serum. Serum was spiked at a concentration of 1 ng/mL. The same extraction sorbent (EVOLUTE ABN) was packed into both formats, and the same methodology was used in each case. Solvent volumes were reduced for the Mikro format (see table 1 for the SPE methods used). Both plates were processed using the Biotage® PRESSURE+ 96 Positive Pressure Manifold.

After extraction, the plate was sealed with a sealing mat until analysis. 5 µL of diluted extract was analysed by LC-MS/MS (see Biotage application note AN939 for full analytical details), and analyte sensitivity was compared.

Table 1. SPE Methods.

Step	EVOLUTE® EXPRESS ABN 10 mg Plate	Biotage® Mikro ABN 2 mg Plate
Condition	Methanol (500 µL)	Methanol (100 µL)
Equilibrate	0.1% formic acid (500 µL)	0.1% formic acid (100 µL)
Load	400 µL diluted serum 200 µL serum diluted with 200 µL 1% formic acid	400 µL diluted serum 200 µL serum diluted with 200 µL 1% formic acid
Wash 1	Water (500 µL)	Water (100 µL)
Wash 2	H_2O:MeOH (60:40, v/v, 500 µL)	H_2O:MeOH (60:40, v/v, 100 µL)
Elute	Methanol (150 µL)	Methanol (30 µL)
Dilute*	Water (150 µL)	Water (30 µL)
Inject	5 µL	5 µL

*Note after elution, the eluent was diluted (1:1, v/v) with water for compatibility with the analytical HPLC conditions.

Results and Discussion

Using the methods described, very clean extracts were produced and excellent recovery with low RSDs was achieved using both SPE formats. Extracts produced using the Mikro format showed a significant gain in sensitivity compared to the traditional 10 mg format (see figure 3).

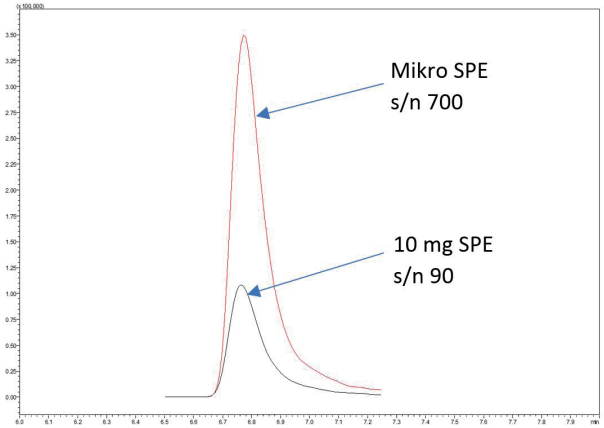

Figure 3. Extracted ion chromatogram for estradiol extracted from human serum using the methods outlined in table 1. Microelution SPE using Biotage® Mikro ABN plates resulted in a ~8 x increase in signal/noise.

Conclusion

Biotage® Mikro ABN solid phase extraction microelution plates provide high analyte recoveries with excellent reproducibility. Compared to the traditional 10 mg SPE plate format, improved sensitivity and reduced LLOQs have been demonstrated. In order to achieve equivalent sensitivity using the 10 mg plate format, an evaporation and reconstitution step would be required, adding approximately 15 minutes to the overall sample preparation time and introducing the possibility of processing errors.

Low volume microelution can lead to immediate improvements in analyte sensitivity, without the need for an evaporation step.

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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Literature Number: PPS619.V.1

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