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Innovative SPE Technology: Ultra Low Elution Volumes and No Evaporation Steps

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The μElution technology combines a patented plate design, proven Oasis® chemistries, and detailed extraction protocols enabling SPE elution volumes as low as 25 μL for the first time.

Introduction

The importance of sample preparation stems from three major concerns — removing interferences from the biological sample matrices, concentrating analyte(s) of interest, and improving analytical system performance. Protein precipitation (PPT) provides the advantage of simplicity and low cost, but results in a relatively dirty sample. SPE provides a cleaner sample, especially if more selective (e.g., ion exchange) resins are used. However, SPE methods often involve evaporating and reconstituting the eluent before LC–MS analysis. These steps not only take time and effort, but can also lead to loss of valuable sample. Therefore, the ability to elute in very small volumes of solvent is desirable to minimize sample preparation time and reduce sample loss.

Experimental Conditions

1. Spike rat plasma with 0.1 ng/mL of amitriptyline and acidify with H_3PO_4 (2% of total sample volume).
2. For protein precipitation, add 1 mL of acetonitrile to 250 μL of the spiked rat plasma. Centrifuge, evaporate supernatant, and reconstitute with 25 μL ACN/IPA (40:60) containing 2% NH_4OH.
3. For Oasis® HLB μElution plate, condition and equilibrate with 200 μL each MeOH and water. Load 250 μL of spiked plasma, wash with 5% MeOH in water, elute with 25 μL ACN/IPA (40:60) with 2% formic acid.
4. For Oasis® MCX μElution plate, condition and equilibrate with 200 μL each MeOH and water. Load 250 μL of spiked plasma, first wash with 0.1 N HCl, second wash with 100% MeOH and elute with 25 μL ACN/IPA (40:60) with 5% NH_4OH.
5. Dilute all samples with 50 μL water (SPE samples are diluted through the plate.)

Column:	XTerra® MS C_{18} 2.1 × 30 mm, 3.5 μm
Mobile phase A:	10 mM NH_4HCO_3, pH 10
Mobile phase B:	MeOH with 10 mM NH_4HCO_3, pH 10
Gradient:	0 to 95% B in 5 min
Flow rate:	0.4 mL/min
Injection volume:	25 μL
Instruments:	Waters Alliance 2795 HPLC, Micromass® Quattro Ultima™ (ESI^+ in MRM)

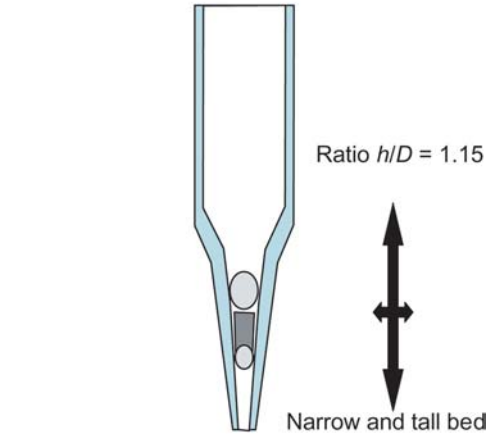

Figure 1: Schematic of the μElution plate design. Tip design of the 96-well plate provides a smaller surface area and larger depth, more like an HPLC column, and allows for good flow through the plate and low hold up volume (~15 μL). This unique design facilitates the elution volume as little as 25 μL.

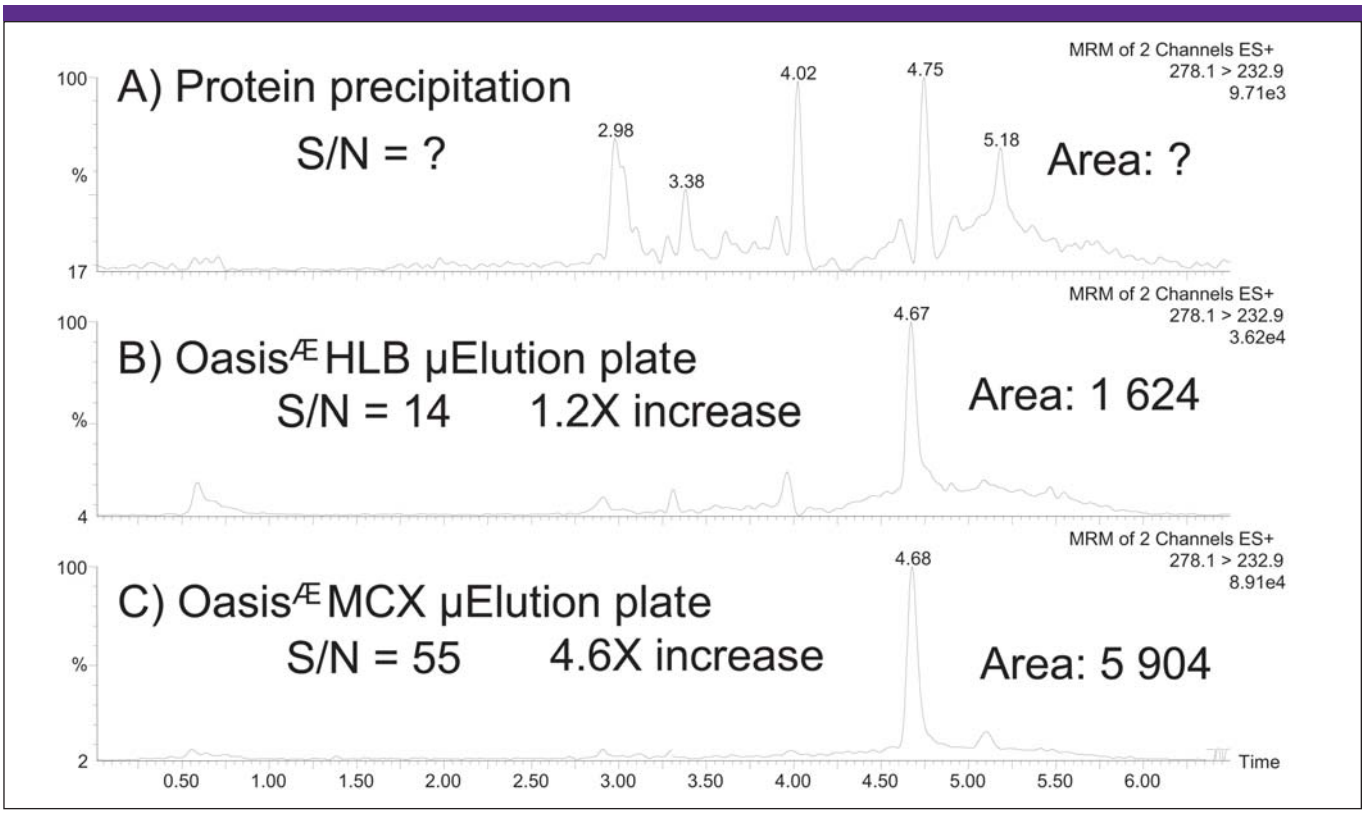

Figure 2: MRM data for amitriptyline spiked into rat plasma at 0.1 ng/mL, followed by (a) protein precipitation, (b) SPE with Oasis® HLB µElution plate and (c) SPE with Oasis® MCX µElution plate. S/N is the signal-to-noise ratio. Area is the peak area.

Results

As seen in Figure 1, the novel µElution design applies an internally tapered well, packed with high capacity sorbent having an aspect ratio of 1.15. The µElution plate functions more like a chromatography column enhancing the efficiency of capturing target analytes and preventing breakthrough during the load and wash steps, as well as facilitating elution volumes as low as 25 µL.

Figure 2 is a comparison of the LC–MS–MS data for amitripyline extracted from rat plasma using A) protein precipitation (PPT), B) Oasis® HLB (reversed-phase) sorbent, and C) Oasis® MCX (mixed-mode cation exchange) sorbent. With PPT, the peak of interest is very difficult to identify. The Oasis® HLB results in a better clean-up than PPT. Because Oasis® MCX has a higher selectivity for basic analytes than the HLB sorbent, this method provides the cleanest extracts and highest sensitivity.

Conclusions

The design of the µElution plate allows each well to function as an HPLC column, preventing breakthrough and wash off of analytes. This design also allows for elution volumes as low as 25 µL, eliminating the time-consuming evaporation and reconstitution steps, as well as providing enhanced sensitivity for samples of low concentration. By combining the µElution plate with Oasis® SPE sorbents, sensitive and selective sample preparation methods can be developed.

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