High-Throughput High-Sensitivity Oasis® µElution Technology

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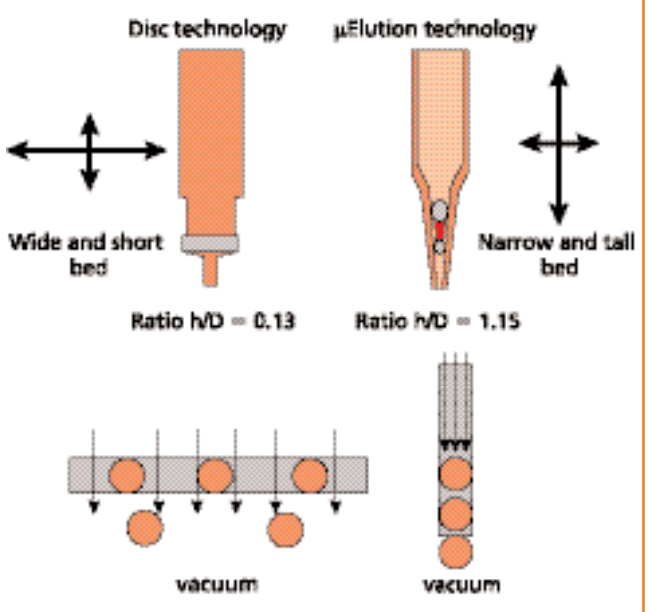
Novel Oasis® µElution Design Enables Elution Volumes as Low as 25 µL

The purpose of sample preparation is to selectively isolate analytes of interest from a complex sample matrix in a highly concentrated form prior to identification and quantification.

Protein precipitation (PPT) provides the advantage of simplicity and low cost. However, PPT results in a higher background and increased interference, which causes ion-suppression, reduced column lifetime and instrument downtime. The current trend in sample cleanup is to use the solid-phase extraction (SPE) technique. The SPE methods contain the steps of conditioning, loading, washing off of interferences, eluting analytes of interest, evaporating and reconstituting the eluate before the analysis (e.g., LC–MS/MS).

The evaporation and reconstitution 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 the amount of time required.

Figure 1: Comparison of disc and µElution Technology. Disc technology provides good flow properties with a lower capacity and a larger hold-up volume than the µElution Technology. Larger hold-up volumes require a larger elution volume because more liquid droplets remain in the hold-up volume.



Currently, there are two technologies (Disc vs µElution) to minimize the elution volume for SPE (depicted in Figure 1). In the disc design (e.g., membrane/glass fibre disc), a small mass of sorbent is embedded into a thin structure with an aspect ratio (defined as the ratio of bed height, h, to bed diameter, D) of 0.13. The disc technology functions more like a filter and is designed to provide good flow properties and a low elution volume (in the range of 75–500 µL). The hold-up volume is measured to be approximately 35–65 µL.

In the µElution design, a much smaller sorbent mass (e.g., 2 mg) is packed into an internally tapered well with an aspect ratio of 1.15. The µElution technology functions more like a chromatography column and enhances the capture of target analytes. It also helps to prevent breakthrough during the load and wash steps. The hold-up volume for the µElution technology was determined to be as little as 15 µL.

A small hold-up volume is important for achieving a low elution volume. Larger hold-up volumes result in larger elution volumes. This is illustrated schematically in Figure 1. After applying a vacuum, more solvent droplets are still held-up in the disc plate due to its large hold-up volume. However, this is not the case with the µElution plate since it has much smaller hold-up volume. Typical elution volumes are determined to be 75–300 µL for the disc technology and only 25–100 µL for the µElution plate.

Comparison of Protein Precipitation (PPT), Disc 96-Well SPE Plate, and Oasis® µElution 96-Well SPE Plate

In order to compare the different techniques, terfenadine-alcohol was added to raw uncentrifuged rat plasma at a concentration of 0.5 ng/mL. For the PPT method, sufficient acetonitrile (1 mL) was added to 250 µL of sample solution to induce protein precipitation. This solution was centrifuged, and the supernatant was then evaporated to dryness. The sample was reconstituted with 25 µL acetonitrile/isopropanol (40:60) with 2% ammonium hydroxide and finally further diluted with 50 µL water. For SPE, 96-well

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extraction plates were conditioned with 200 µL MeOH, equilibrated with 200 µL water. Then 250 µL of sample solution was loaded into the wells, which were then washed with 5% methanol in water, and eluted with acetonitrile/isopropanol 40:60 with 2% formic acid (25 µL for the Oasis® HLB µElution plate, 100 µL for the disc plates). Finally, the eluate was diluted with water (50 µL for µElution plate, 200 µL for disc plates) through the plates, and 25 µL was injected into the LC–MS/MS system.

The results are shown in Figure 2. Good peak shape and signal-to-noise (S/N) were observed from protein precipitation. However, the disc technology provided an inferior S/N to the protein precipitation. Clearly, the best results were obtained using the Oasis® µElution technology.

Summary

The Oasis® µElution technology is designed to make an elution volume as low as 25 µL possible, eliminating time-consuming and tedious evaporation steps involved in the SPE technique. Much better peak shape and signal-to-noise ratio could be easily achieved with the µElution plate compared with the disc technology or protein precipitation.

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Figure 2: Comparison of signal-to-noise ratio results from protein precipitation, Brand S and E disc 96-well extraction plates and Oasis® HLB µElution plate. Much better results were achieved with the Oasis® µElution technology than with protein precipitation or disc technology.