Benefits of Using the Agilent EMR—Lipid Polish Pouch Protocol

Technical Overview

Abstract
Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is the next generation of sample preparation product. It is implemented in a convenient dispersive solid phase extraction (dSPE) format for highly selective lipid removal from sample matrices. The introduction of the Agilent EMR—Lipid MgSO₄ Final Polish Pouch as a post dispersive SPE step improves the removal of water and nonmatrix solid residue from the final sample extract. The new pouch format ensures content integrity by preventing the absorption of water and cross-contamination that can occur in bulk containers. This improved methodology includes a highly recommended extra drying step to remove any water or salt residue remaining in the samples, especially samples requiring a concentration step.

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**Introduction**

Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is a novel sorbent material that selectively removes major lipid classes from sample matrices. Techniques such as QuEChERS and protein precipitation (PPT) alone have not been a good match for the preparation of high fat samples. This incompatibility is due to the tendency of these techniques to co-extract target analytes along with large amounts of matrix. Previously published applications describe the EMR—Lipid workflow, and demonstrate that EMR—Lipid dispersive solid phase extraction (dSPE) provides superior sample cleanup in complex matrices as well as excellent quantitation results for multiclass, multiresidue analysis [1-4].

Multiresidue analyses can require either sample concentration (LC or GC) or solvent exchange (GC). The original polish tube introduced with the EMR—Lipid workflow as a post-dSPE sample step contains NaCl in addition to MgSO₄. NaCl can potentially hinder water removal, and leave behind sodium salt residue when samples are highly concentrated. The use of anhydrous MgSO₄ alone in the polish step, along with the addition of an extra dry step, will address both potential outcomes.

The anhydrous MgSO₄ is preweighed and sealed into pouches. Due to the hygroscopic nature of MgSO₄, this pouch format is advantageous to protect against repeated exposure to atmospheric moisture, which occurs when bulk sorbents are used. This also gives the added benefit of protecting against cross-contamination that can occur when multiple users are handling bulk sorbent containers.

The benefits of the EMR—Lipid workflow using the Agilent EMR—Lipid MgSO₄ Final Polish Pouch include:

- The ability to decant the entire sample instead of a 5 mL transfer
- Convenience of a preweighed pouch
- Pouch format assures content integrity
  - NO atmospheric water introduction
  - NO cross contamination
- Elimination of NaCl salt residue in the sample
- Removal of unwanted residues, including water

For the MgSO₄ Polish Pouch procedure, the entire extract post EMR—Lipid cleanup should be transferred to an empty 15 or 50-mL centrifuge tube. Add the entire contents of the 3.5 g MgSO₄ Polish Pouch (p/n 5982–0102) to the extract. The slim-pack pouch design is easier to pour. This allows the MgSO₄ to be added gradually into the extract. After salt addition, cap the sample tube tightly, and immediately vortex or shake the sample tube vigorously. Vortexing immediately prevents clumping of the MgSO₄ to provide optimal conditions for water and residue removal.
The extra-dry step is optional, but highly recommended. This is especially important for GC analysis or when the sample is evaporated to dryness either for concentration or solvent exchange. This extra-dry step insures complete water and residue removal.

### Extra-dry step

**Sample after polish protocol**

- Prepare a drying tube with an appropriate amount of anhydrous MgSO$_4$ from a Polish Pouch (~300 mg/1 mL ACN extract)
- Transfer the desired amount of the ACN layer to the final drying tube
- Vortex immediately and centrifuge

**Supernatant**

- Evaporate to dryness and reconstitute, or directly dilute with mobile phase in a 2-mL autosampler vial
- Samples are ready for LC/MS/MS analysis
- Transfer to a 2-mL autosampler vial or another tube for solvent exchange, if necessary
- Samples are ready for GC/MS/MS analysis

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**Polish step**

**Sample after EMR—Lipid dSPE**

- Transfer 5 mL of supernatant to an EMR—polish tube
- Vortex immediately and centrifuge
- Transfer the upper layer for analysis or to dry step

**Agilent EMR—Polish Tube (p/n 5982-0101)**

- Decant entire supernatant into empty centrifuge tube
- Vortex immediately and centrifuge
- Transfer the upper layer for analysis or to dry step

**Agilent EMR—Lipid MgSO$_4$ Final Polish Pouch (p/n 5982-0102)**

- Pour ENTIRE contents of polish pouch into tube and cap tube tightly
- Vortex immediately and centrifuge
- Transfer the upper layer for analysis or to dry step

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Benefits of MgSO₄ Post Sample Protocol

Both LC and GC applications will benefit from the EMR—Lipid post-sample protocol using the MgSO₄ Polish Pouch. The removal of water for GC applications has a significant impact, including higher analyte responses, better peak shape, and more consistent reproducibility over multiple injections. The reduction of nonmatrix salt residue benefits both LC and GC applications.

Conclusions

The Agilent Bond Elut Enhanced Matrix Removal—Lipid workflow, including a post sample polishing step with the Agilent EMR—Lipid MgSO₄ Final Polish Pouch, followed by the extra-dry step with anhydrous MgSO₄ reduces the water residue and nonmatrix salt residue in the final sample extract. This alternate post sample protocol maintains the superior matrix removal efficiency and analyte recovery associated with EMR—Lipid dSPE while improving long-term analysis consistency and reliability, especially for GC applications.

References