Resprep Polymeric SPE Products

Pre-Extraction Steps:

- **Use of Acidified or Basified Diluents**

  The interactions between the solute, the various solvents used (including the sample diluent), and the sorbent can be drastically affected by the solvent pH.
  During method development, it may be advantageous to dilute your samples with acidified or basified water prior to extraction.

- **Solid Samples**

  - Ensure the sample is properly homogenized and initially extracted into either an aqueous or organic extract using conditions that have been optimized to provide the maximum initial extraction efficiency with minimal coextraction of unwanted matrix components. This step may benefit from the addition of buffers, dispersive salts, or the use of cosolvents to influence extraction efficiency.
  - Initial extracts of solid samples may require pH adjustment, solvent composition adjustment, or even evaporation and reconstitution using a different solvent in order to optimize polymeric SPE sorbent performance. Filtration or centrifugation may also be needed to remove particulates prior to extraction.

- **Aqueous Samples**

  Sample pH can play a significant role in solute retention on polymeric SPE sorbent. During method development, pH adjustments may be required, as well as the addition of buffering salts or dispersive agents. If the sample contains suspended matter, filtration or centrifugation may be needed prior to extraction.

- **Nonaqueous Liquid Samples**

  It may be possible to dilute a nonaqueous liquid sample using buffered water and organic cosolvents and then treat it as an aqueous sample in the following procedures.

- ***Note:*** *Do not use methods that were developed for silica-based SPE products because the solvent choices may not be appropriate for use with Resprep polymeric SPE products.*

Table I: Recommended Volumes for 1:1 Dilution

	Cartridges			96-Well Plates	
Cartridge size/sorbent mass	1 mL (30 mg)	3 mL (60 mg)	6 mL (150, 200, or 500 mg)	10 mg	30 mg
Condition/equilibration (mL)	1	2	3	0.5	0.5–1.0
Maximum load, matrix and dilution (mL)	1	2	5	1	1–2
Wash (mL)	1	2	4	0.5	0.5–1.0
Elute (mL)	1	2	4	0.15–0.3	0.4–1.0

Extraction of Acidic, Basic, and Neutral Compounds using HLB

1. **Setup**

   Mount HLB (hydrophilic-lipophilic balance) cartridges or plates to a vacuum manifold along with a waste collection vessel of sufficient size.

   a. Consult Table I for the appropriate volumes for each of the following steps based on the specific product you are using.

2. **Optional Conditioning and Equilibration**

   The following steps are not required for HLB extractions, but either or both of these steps can be performed if desired:

   a. Conditioning

      i. Add the appropriate volume of methanol to the cartridge/plate.

      ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel. Once eluted, turn vacuum off.

   b. Equilibration

      i. Add the appropriate volume of water to the cartridge/plate.

      ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel.

   c. Turn off the vacuum to the HLB cartridge/plate before proceeding to the next step.

Note: *When turning off the vacuum, be sure to reduce the vacuum to the lowest possible setting prior to turning it off. This will be important for Step 3 below.*

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3. **Sample Load**
    a. Load the diluted sample.
    b. Slowly apply vacuum, starting at the lowest possible setting, to load the entire sample volume onto the polymeric SPE sorbent bed. Gradually increase vacuum as necessary.
    c. Once the sample is fully loaded onto the sorbent bed, but before it has begun to elute, turn off the vacuum. Exceeding the volume in Table I is not recommended: when properly loaded, the sample liquid should not be above the top frit or drip from the tip.
4. **Wash**
    a. Add the appropriate volume of wash solvent (5% methanol in water).
    b. Apply vacuum to the cartridge/plate.
        i. Set vacuum initially to 5" Hg and adjust as necessary to fully elute.
        ii. To ensure sufficient interaction, establish a flow that results in the formation of discrete drops. For aqueous samples, you may need to increase the vacuum in order to obtain an appropriate flow.
    c. Continue to apply vacuum for an additional 30-60 seconds to ensure the elimination of any residual wash solvent.
5. **Discard Waste**
    a. Turn off the vacuum, making sure to first reduce it to its lowest setting so that it will be at the proper level for the elution step.
    b. Release the vacuum from the manifold and discard the waste liquid.
    c. Insert new, clean collection vessel(s) and replace the manifold cover.
6. **Elution**
    a. Add the appropriate volume of the elution solvent (100% methanol).
    b. Allow the elution solvent to flow by gravity before applying vacuum.
    c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.
    d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.
    e. Turn off the vacuum and release it from the manifold.
    f. Remove the collection vessel(s).
7. **Optional Evaporation and Reconstitution**

    Evaporation and reconstitution is an optional step that may be performed if needed (e.g., to concentrate the sample extract).
8. **Prepare Extract for Analysis**

    Transfer the extracts from the collection vessel to an appropriate analysis vessel, if necessary. If using plates, be sure to cover the well plate with a sealing mat.
9. **Evaluate Extraction Quality**

    The above procedure is designed for LC-MS/MS analyses, but if the initial analysis indicates cleaner extracts are needed to lower background noise and improve sensitivity and/or selectivity, the extraction process can be optimized by varying pH and the relative amounts of methanol in the wash and elution steps. This is similar to how an analytical LC method can be optimized for the relative retention and separation of analytes on a reversed-phase LC column. The same principles can be applied to optimizing the extraction procedure.

Extraction of:

- **Weak Basic Compounds (pKa 2–10) using MCX**
- **Strong Acidic Compounds (pKa <1) using WAX**

1. **Setup**

    Mount MCX (mixed-mode, strong cation exchange) or WAX (mixed-mode, weak anion exchange) cartridges or plates to a vacuum manifold along with a waste collection vessel of sufficient size. For compounds outside the pKa values given above, conduct a preliminary experiment to determine whether MCX or WAX products perform best.
    a. Consult Table I for the appropriate volumes for each of the following steps based on the specific product you are using.
2. **Conditioning and Equilibration**
    a. Conditioning
        i. Add the appropriate volume of methanol to the cartridge/plate.
        ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel. Once eluted, turn vacuum off.
    b. Equilibration
        i. Add the appropriate volume of water to the cartridge/plate.
        ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel.
    c. Turn off the vacuum to the MCX or WAX cartridge/plate before proceeding to the next step.

Note: *When turning off the vacuum, be sure to reduce the vacuum to the lowest possible setting prior to turning it off. This will be important for Step 3 below.*

3. **Sample Load**
    a. Load the diluted sample.
    b. Slowly apply vacuum, starting at the lowest possible setting, to load the entire sample volume onto the polymeric SPE sorbent bed. Gradually increase vacuum as necessary.
    c. Once the sample is fully loaded onto the sorbent bed, but before it has begun to elute, turn off the vacuum. Exceeding the volume in Table I is not recommended: when properly loaded, the sample liquid should not be above the top frit or drip from the tip.
4. **Wash**
    a. Add the appropriate amount of wash solvent (2% formic acid in water). (Other acids, e.g., 0.1 N HCl, may be used instead.)
    b. Apply vacuum to the cartridge/plate.
        i. Set vacuum initially to 5" Hg and adjust as necessary to fully elute.
        ii. To ensure sufficient interaction, establish a flow that results in the formation of discrete drops. For aqueous samples, you may need to increase the vacuum in order to obtain an appropriate flow.
    c. Continue to apply vacuum for an additional 30-60 seconds to ensure the elimination of any residual wash solvent.
    d. Turn off the vacuum, making sure to reduce it to its lowest setting first.
5. **First Elution (eluting neutral or uncharged ionic species, i.e., weak acids)**
    a. Add the appropriate volume of the first elution solvent (100% methanol).
    b. Allow the elution solvent to flow through by gravity before applying the vacuum.
    c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.
    d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.
6. **Discard Waste**
    a. Turn off the vacuum, making sure to first reduce it to its lowest setting so that it will be at the proper level for the elution step.
    b. Release the vacuum from the manifold and discard the waste liquid.
    c. Insert new, clean collection vessel(s) and replace the manifold cover.
7. **Second Elution (eluting basic or strongly acidic analytes of interest)**
    a. Add the appropriate volume of the second elution solvent (5% ammonium hydroxide in methanol).
    b. Allow the elution solvent to flow through by gravity before applying the vacuum.
    c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.
    d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.
    e. Remove the collection vessels.
8. **Optional Evaporation and Reconstitution**

    Evaporation and reconstitution is an optional step that may be performed if needed (e.g., to concentrate the sample extract).
9. **Prepare Extract for Analysis**

    Transfer the extracts from the collection vessel to an appropriate analysis vessel, if necessary. If using plates, be sure to cover the well plate with a sealing mat.
10. **Evaluate Extraction Quality**

    The above procedure is designed for LC-MS/MS analyses, but if the initial analysis indicates cleaner extracts are needed to lower background noise and improve sensitivity and/or selectivity, the extraction process can be optimized by varying pH and the relative amounts of methanol in the wash and elution steps. This is similar to how an analytical LC method can be optimized for the relative retention and separation of analytes on a reversed-phase LC column. The same principles can be applied to optimizing the extraction procedure.

Extraction of:
- **Weak Acidic Compounds (pKa 2–8) using MAX**
- **Strong Basic Compounds (pKa >10) using WCX**

1. **Setup**

    Mount MAX (mixed-mode, strong anion exchange) or WCX (mixed-mode, weak cation exchange) cartridges or plates to a vacuum manifold along with a waste collection vessel of sufficient size. For compounds outside the pKa values given above, conduct a preliminary experiment to determine whether MAX or WCX products perform best.
    a. Consult Table I for the appropriate volumes for each of the following steps based on the specific product you are using.
2. **Conditioning and Equilibration**
    a. Conditioning
        i. Add the appropriate volume of methanol to the cartridge/plate.

ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel. Once eluted, turn vacuum off.

b. Equilibration

i. Add the appropriate volume of water to the cartridge/plate.

ii. Set the vacuum manifold to 5" Hg and elute into a collection vessel.

c. Turn off the vacuum to the MAX or WCX cartridge/plate before proceeding to the next step.

Note: *When turning off the vacuum, be sure to reduce the vacuum to the lowest possible setting prior to turning it off. This will be important for Step 3 below.*

3. **Sample Load**

a. Load the diluted sample.

b. Slowly apply vacuum, starting at the lowest possible setting, to load the entire sample volume onto the polymeric SPE sorbent bed. Gradually increase vacuum as necessary.

c. Once the sample is fully loaded onto the sorbent bed, but before it has begun to elute, turn off the vacuum. Exceeding the volume in Table I is not recommended: when properly loaded, the sample liquid should not be above the top frit or drip from the tip.

4. **Wash**

a. Add the appropriate amount of wash solvent (5% ammonium hydroxide in water).

b. Apply vacuum to the cartridge/plate.

i. Set vacuum initially to 5" Hg and adjust as necessary to fully elute.

ii. To ensure sufficient interaction, establish a flow that results in the formation of discrete drops. For aqueous samples, you may need to increase the vacuum in order to obtain an appropriate flow.

c. Continue to apply vacuum for an additional 30-60 seconds to ensure the elimination of any residual wash solvent.

d. Turn off the vacuum, making sure to reduce it to its lowest setting first.

5. **First Elution (eluting neutral or uncharged ionic species, i.e., weak bases)**

a. Add the appropriate volume of the first elution solvent (100% methanol).

b. Allow the elution solvent to flow through by gravity before turning on the vacuum.

c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.

d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.

6. **Discard Waste**

a. Turn off the vacuum, making sure to first reduce it to its lowest setting so that it will be at the proper level for the elution step.

b. Release the vacuum from the manifold and discard the waste liquid.

c. Insert new, clean collection vessel(s) and replace the manifold cover.

7. **Second Elution (eluting basic or strongly acidic analytes of interest)**

a. Add the appropriate volume of the second elution solvent (2% formic acid in methanol).

b. Allow the elution solvent to flow through by gravity before applying the vacuum.

c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.

d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.

e. Remove the collection vessels.

8. **Optional Evaporation and Reconstitution**

Evaporation and reconstitution is an optional step that may be performed if needed (e.g., to concentrate the sample extract).

9. **Prepare Extract for Analysis**

Transfer the extracts from the collection vessel to an appropriate analysis vessel, if necessary. If using plates, be sure to cover the well plate with a sealing mat.

10. **Evaluate Extraction Quality**

The above procedure is designed for LC-MS/MS analyses, but if the initial analysis indicates cleaner extracts are needed to lower background noise and improve sensitivity and/or selectivity, the extraction process can be optimized by varying pH and the relative amounts of methanol in the wash and elution steps. This is similar to how an analytical LC method can be optimized for the relative retention and separation of analytes on a reversed-phase LC column. The same principles can be applied to optimizing the extraction procedure.

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