EPH Fractionation SPE Cartridge cat.# 25859

Resprep



Red = methylene chloride Blue = hexane

Conditioning

- 1. Make sure the stopcock on the vacuum manifold is open.
- 2. Fit the cartridge tip securely in the vacuum manifold.
- 3. Add 6 mL of methylene chloride and allow gravity to pull it through the cartridge.
- 4. Close the stopcock when the methylene chloride meniscus touches the top frit of the cartridge. Then, let the cartridge soak in methylene chloride for 5 minutes.
- 5. Add ~ 1 mL of hexane and open the stopcock to draw the hexane down until the meniscus touches the top frit of the cartridge. Close the stopcock.
- 6. Add 15 mL of hexane to the cartridge and open the stopcock. Gravity will pull the hexane through the cartridge, but a slight vacuum can be applied to initiate and maintain the flow rate.
- 7. Close the stopcock when the hexane meniscus touches the top frit of the cartridge.
- 8. Discard waste solvent from the conditioning step appropriately.



Sample Loading

- 1. Confirm that the stopcock is still closed from the previous conditioning step.
- 2. Label "Fraction 1" on a clean collection vessel and place it under the tip of the cartridge.
- 3. Transfer 1 mL of sample from the sample container to the cartridge.
- 4. Rinsing the sample container with an additional 1 mL of hexane and transferring the rinse to the cartridge is recommended to ensure a more complete transfer.
- 5. Open the stopcock and let the sample go into the silica bed until the meniscus of the loaded sample touches the top frit of the cartridge.
- 6. Close the stopcock.

Blue = hexane

Fractionation and Elution of Aliphatic Compounds

- 1. Make sure that the stopcock is still closed from the sample loading step.
- 2. Add 18 mL of hexane to the cartridge.
- 3. Open the stopcock to initiate the flow and maintain a drop-wise flow rate of approximately 4 mL/min by controlling the stopcock appropriately.
- 4. Close the stopcock when the hexane meniscus reaches the top frit of the cartridge and remove the collection vessel labeled Fraction 1 from the manifold. This fraction contains the aliphatic compounds.

Elution of Aromatic Compounds

- 1. Make sure the stopcock is still closed from the previous step. Label "Fraction 2" on a new, clean collection vessel and place it under the tip of the same cartridge from the aliphatics elution step. Now you are ready to elute the aromatic compounds.
- 2. Add 20 mL of methylene chloride.
- 3. Open the stopcock to initiate the flow and maintain a drop-wise flow rate of approximately 4 mL/min by controlling the stopcock appropriately.
- 4. Close the stopcock when the methylene chloride meniscus reaches the top frit of the cartridge.
- 5. Remove the collection vessel labeled Fraction 2 from the manifold. This fraction contains the aromatic compounds.





Blue = Fraction 1 (aliphatics) in hexane

Red = Fraction 2 (aromatics) in methylene chloride



Volume Adjustment (Optional)

- 1. If the concentration volume is less than 1 mL after the concentration step, add the appropriate elution solvent to each fraction to bring up the final volume to 1 mL. Use hexane for Fraction 1 and methylene chloride for Fraction 2.
- 2. Transfer the concentrated samples from the collection vessels to 2 mL GC autosampler vials. The samples are now ready for analysis. (Pictures are for illustrative purposes only and do not represent actual volumes.)

Technical Tips

To ensure optimum fractionation:

- Do not open bags of cartridges until you are ready to begin the conditioning step. Cartridge conditioning should be started immediately once the bag has been opened.
- Flow rate may have an impact on the fractionation performance and a drop-wise flow rate of approximately 4 mL/min by gravity with appropriate stopcock positioning is strongly recommended for the sample loading and elution steps.
- Depending on the concentration apparatus, low molecular weight compounds can be lost during concentration when excessive temperature or vigorous drying is applied. Keep the concentration step conditions gentle to avoid the loss of low molecular weight compounds. When loss of recovery is observed, adjust the concentration conditions and refer to your lab's procedures for more specific concentration conditions.
- To avoid contamination, do not use any plastic-containing device for sample or solvent handling/transfer.

To ensure cartridge quality:

- Since all silica products are sensitive to moisture, exposure to atmospheric moisture should be avoided or minimized so that cartridge performance is
 not affected. Resprep EPH fractionation SPE cartridges are manufactured in a strictly controlled environment and housed in superior moisture-resistant
 packaging. However, once the packaging has been opened, the cartridges should be used immediately; if this is not possible, we strongly recommend
 that any unused cartridges be stored in a desiccated container until use.
- If a void is visible in the silica bed, orient the cartridges vertically with the cartridge tip at the bottom and tap the cartridge to resettle the silica bed. Press the top frit firmly down on top of the silica bed.

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Concentration

Concentrate each fraction separately by placing each collection vessel under a gentle stream of nitrogen and blowing the solvent down to a final volume of 1 mL or slightly less than 1 mL. Refer to your method or your lab's standard operating procedures for more specific concentration conditions and volumes. (Pictures are for illustrative purposes only and do not represent actual volumes.)