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Continuous SPE Fractionation of a Raw Polar Sample into 7 Mixed Fractions

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We are still developing and optimizing this protocol

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Keywords: continuous spe fractionation, spe fractionation, raw polar sample, ethanol concentration, mixed fraction, mixed fractions this protocol, continuous change in solvent strength, solvent strength, ultrapure water, overlapping analyte distribution, analyte distribution, mixed component, adjacent fraction, fraction, subsequent analytical method, concentration, ethanol

Abstract

This protocol describes the SPE fractionation of a raw polar sample (70% ethanol in ultrapure water) using a continuous gradient. A gradient is generated by steadily increasing the ethanol concentration from 20% to 100% in water. The eluent is continuously collected into seven fractions, with the understanding that adjacent fractions may contain overlapping analyte distributions. This method can be useful when a continuous change in solvent strength is desired, but note that subsequent analytical methods may be required to resolve mixed components.

Materials

Materials

- **Sample:** Raw polar sample (70% ethanol in ultrapure water)
- **SPE Cartridges:** Appropriate reversed-phase cartridge (e.g., C18) based on analyte properties
- **Ultrapure Water (Water)**
- **Ethanol:** Analytical grade
- **Stock Solutions:**

20% ethanol in water (v/v)
40% ethanol in water (v/v)
60% ethanol in water (v/v)
80% ethanol in water (v/v)
100% ethanol (absolute)

- **Gradient Mixing Apparatus:** A dual-channel gradient pump or manual mixing system to continuously vary the ethanol concentration from 20% to 100%
- **Collection Tubes:** Clean, labeled glass or polypropylene tubes for seven fractions
- **Optional:** Internal standards for downstream analysis

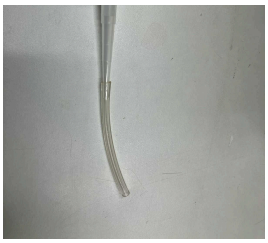
Equipment

- Vacuum or positive pressure manifold (if applicable)
- Gradient pump (or equivalent manual mixing device)
- Fraction collector or manual pipetting setup
- SPE cartridge holder
- Vortex mixer
- Timer
- Fume hood (for ethanol handling)
- Pipettes and tips

Safety warnings

Safety Considerations

- Work in a well-ventilated area or fume hood when handling ethanol and preparing solvent mixtures.
- Wear appropriate personal protective equipment (PPE), including gloves, a lab coat, and eye protection.
- Dispose of waste solvents according to institutional and regulatory guidelines.



Before start

Quality Control and Notes

- **Flow Rate and Gradient Control:** A steady and well-controlled flow rate is crucial for reproducible gradient formation. If available, use an automated gradient pump.
- **Fraction Volume:** Adjust the total elution volume and individual fraction volumes based on cartridge capacity and experimental goals.
- **Analyte Overlap:** Expect overlapping compound elution profiles between fractions; downstream analytical techniques (e.g., HPLC, MS) may be necessary to resolve the mixtures.
- **Documentation:** Record the actual volumes collected and any deviations from the intended gradient profile.
- **Optimization:** Depending on the complexity of your sample and the nature of your target analytes, fine-tune the gradient ramp (slope and volume) to improve separation.

Cartridge Conditioning

1

Cartridge to use: C18 1g (6mL head volume) - 1 g/6 mL 52606-U SUPELCO
https://www.sigmaaldrich.com/BR/pt/product/supelco/52607u
Stock Solutions (HPLC grade): 20% ethanol in water (v/v)
40% ethanol in water (v/v)
60% ethanol in water (v/v)
80% ethanol in water (v/v)
100% ethanol (absolute)
Sample: 500mg of dried sample diluted in 1 ml of solvent (70% ethanol in water)

Pre-rinse: Attach the SPE cartridge to the manifold. Flush the cartridge with 5–15 mL of 100% ethanol at a controlled flow rate (approximately 1–2 mL/min; under reduced pressure) to fully wet, wash and activate the stationary phase.

2 **Equilibration:** Follow with 15 mL of the solution 20% ethanol in ultrapure water to equilibrate the cartridge.

Note: Use the stopcock to stop the flow and prevent the column from going dry.

Sample Loading

3 **Mixing:** Vortex the raw polar sample (70% ethanol in uQ water) thoroughly to ensure homogeneity in the lowest volume possible.

4 **Application:** Slowly load the entire sample onto the cartridge.

Note: Unlike protocols that collect a flow-through fraction, this continuous gradient protocol retains the loaded sample on the cartridge and elutes it during the gradient run.

Continuous Gradient Elution

5 **Gradient Setup**:
- Separate every **Stock (gradient) Solutions:** 20%; 40%; 60%; 80% ethanol in uQ water (v/v); and 100% ethanol (absolute).

6 **Elution and Collection**:
- Begin the gradient elution at a controlled flow rate (under vacuum).
- Start the addition of the **Stock (gradient) Solutions** in the correct order. **10 mL** of each solution will be added for elution.
- Divide the collected eluent into 7 **sequential** fractions of **7 mL** continuously.
- Label the fractions as 1 through 7.

Note: Due to the continuous fraction collection, the boundaries between fractions are inherently mixed. You should observe **overlapping analyte profiles between adjacent fractions**.

Post-Elution Processing

7 **Cartridge Washing (Optional)**: If desired, wash the cartridge with an additional volume of 100% ethanol to ensure complete elution of strongly retained compounds. This wash can be analyzed separately or combined with the final fraction.

8 **Sample Concentration**: evaporate all the collected fractions under vacuum in new, pre-weighed vials to enable mass balance determination.

9 **Storage:** Store collected fractions at appropriate temperatures (−20 °C for longer durations) until further analysis.