Guidance: ISOLUTE® ENV+ Troubleshooting

How to Use This Document

The purpose of this technical note is to provide guidance on optimization of methodology if issues are experienced while transitioning from original ISOLUTE® ENV+ to new formulation ISOLUTE ENV+.

To identify any issues, we recommend that you test your existing method using the same methodology and processing conditions with original and new formulation product (ideally side by side, to minimize variables) and compare results. Then evaluate the suggested method modifications to improve performance, if required.

For additional information on method development using ISOLUTE ENV+, refer to TN109.

Problem: Reduced Analyte Recovery

Possible causes:

- Analyte breakthrough
- Enhanced retention
- Drying conditions

Analyte Breakthrough

New formulation ISOLUTE® ENV+ has a slightly reduced capacity for caffeine and slightly reduced surface area.

ISOLUTE ENV+ is rarely used at full capacity, so it is unlikely that this will affect analyte recovery, except for the most water soluble of analytes, at high loading concentrations.

How to test for breakthrough?

1. The simplest way is to stack 2 columns (using column adapters (p/n 120-1101 for 1, 3 or 6 mL columns)).
2. Using the existing method load sample through both columns.
3. Dry, and disconnect the columns.
4. Elute the lower column.
5. If analyte is present on the lower column, breakthrough has occurred.

Suggested Method Modifications

- For acidic analytes, consider adjusting pH to 2 pH units lower than lowest analyte pK. This will protonate acidic groups on the analytes, increasing their hydrophobic character, and improve retention.
- For basic analytes, consider adjusting pH to 2 pH units higher than lowest analyte pK. This will deprotonate basic groups on the analytes, increasing their hydrophobic character, and improve retention.
- Decrease loading flow rate. This will increase contact time between analyte and sorbent, and may improve retention for weakly retained analytes.
- Reduce strength of wash (interference elution) solvents. For example, if a 50% (v/v) methanol/water mix is used for interference elution, evaluate the use of 40% or 30% methanol.
- Consider a larger bed mass: for weakly retained analytes, a larger bed mass may be required. Evaluate the next largest bed mass available.

Enhanced Retention

Some analytes may be retained more strongly by new formulation ISOLUTE ENV+ via enhanced hydrophobic retention mechanisms. This is most likely for very hydrophobic analytes.

There is some indication that new formulation ISOLUTE ENV+ may exhibit increased secondary ionic interactions. This could lead to reduced recovery of basic or acidic analytes.

How to test for enhanced retention?

1. The simplest way is to elute with multiple aliquots of elution solvent.
2. Collect each aliquot separately.
3. Analyse each aliquot.
4. If analyte is present in the second or subsequent aliquots, analyte is retained more strongly.
5. For acidic or basic analytes only: If NO analyte is present in any aliquot, secondary interactions are likely.

Suggested Method Modifications

- Increase elution volume. Note that 2 aliquots with a soak step may be more effective than a single larger aliquot.
- Consider use of a stronger elution solvent. This is highly analyte dependent. Choose solvents or solvent mixtures in which the analytes are highly soluble.
- Consider a smaller bed mass. For strongly retained analytes, a smaller bed mass may be adequate, and may allow elution in a slower solvent volume. Note: this may have additional benefits of reduced solvent cost, or reduced evaporation times
- Consider addition of a modifier to the elution solvent
  - For acidic or basic analytes, add a low % of formic or acetic acid to the elution solvent.
  - For acidic or basic analytes, add a low % of ammonium hydroxide to the elution solvent.

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Drying Conditions

» Consider extended drying time. For water immiscible elution solvents, an extended column drying time may be required achieve full recovery. To ensure complete removal of traces of aqueous sample from the column, extend the drying time or increase vacuum/pressure. This will allow more efficient contact of the immiscible solvent with the analyte.

Problem: Dirtier Extracts or Increased Matrix Effects

Any factors that enhance analyte retention or elution may also increase the retention or elution of unwanted matrix components, leading to an increase of interference concentrations in the final extract.

Care should be taken that any modifications used to decrease interference levels the final extract do not adversely impact analyte recovery.

There are two primary ways to improve extract cleanliness:

» Reduce interference retention.

» Reduce interference elution.

Reducing Interference Retention

Suggested method modifications

» For strongly retained analytes, consider addition of a low % of solvent (such as 10–20% methanol) to the sample before loading. This will reduce interferences that were weakly retained via hydrophobic retention mechanisms.

» For neutral analytes, consider adjusting the pH of the sample by adding a low % acid (formic or acetic) or base (ammonium hydroxide). This may reduce the retention of polar basic or acidic interferences via secondary interactions.

Reducing Interference Elution

Suggested method modifications

» Increase wash solvent strength. For strongly retained analytes, an increase in wash solvent strength (for example from 30% methanol to 50% methanol) may remove weakly retained interferences without impacting analyte recovery.

» Decrease elution solvent strength. For strongly retained interferences, decreasing the elution solvent strength may prevent interferences from eluting and decrease their concentration in the final extract, without impacting analyte recovery.

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Literature Number: UI483

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