ASSET™ EZ4-NCO Dry Sampler
Extraction Procedure.

Michael Halpenny
Jamie Brown
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Abstract:

This presentation introduces and details the procedure used for the extraction of isocyanates from the ASSET™ EZ4-NCO Dry Sampler.

The objective of the extraction is to quantitatively transfer the captured isocyanates from the sampler into the solvent medium for detection. This presentation is to be used as an instructional enhancer to demonstrate the proper techniques needed to perform the extraction.
Equipment, Supplies, Reagents

Equipment:
- **Shaker** (Example: IKA Vibrax VXR Basic Shaker available thru Sigma Aldrich)
- **Ultrasonic Bath**
- **Centrifuge** (Capable of 3000 rpm)
- **Evaporator/Dryer** (Examples: Zymark Turbo-Vap LV or Six Port Mini-Vap or Vacuum Centrifuge)
- **Mini Vortex**

Reagents:
- DBA-Isocyanate Standard Solution (Supelco 40141-U)
- Deuterated-DBA-diisocyanate Internal Standard Solution (Supelco 40142-U)
- Dibutylamine (Aldrich 471232)
- DI Water
- Acetic Acid (Sigma-Aldrich 320099)
- Sulfuric Acid (Sigma-Aldrich 435589)
- Acetonitrile (Sigma-Aldrich 34851)
- Methanol (Sigma-Aldrich 34860)
- Toluene (Sigma-Aldrich 34866)

Supplies:
- 2 mL Clear ABC Autosampler Vials, PTFE/Silicon Liner Cap (Supelco 27490-U)
- Borosilicate Glass Pipettes, Disposable 5 ¾”
- Pasteur pipette rubber bulb
- Nitrile Lab Gloves
- Polypropylene 15 mL Centrifuge Tubes (Ex: Cellstar Greiner Bio-one Graduated, Sterile, Blue Screw Cap (Sigma T1818-500ea))
- Glass Culture Tubes, 16 x 125 mm
- Various Pipettors: Range: 0.005 µL – 5.5 mL
- Various Pipette Tips
- Knife
- Tweezers
- Timer
- Culture tube Caps (Ex: Safe-T-Flex Cap, 16mm)
- Lab Tissues (Ex: Kimberly-Clark Kimwipes)
Preparation

Carefully cut the red heat shrink wrap between the filter cassette and the denuder with a knife to access the filter cassette.

Clean the tweezers between each ASSET™ sampler using a clean lab tissue and Methanol.
Filter Removal from Denuder

Carefully remove filter media from Denuder using the clean tweezers.

Place filter media in the properly labeled 15 mL polypropylene centrifuge tube.
Filter Removal from Cassette

Carefully remove the 13 mm filter and ring gasket from the cassette and place them in the properly labeled 15 mL centrifuge tube.
Denuder Rinse

Denuder is rinsed with 5.5 mL Toluene to extract any isocyanates that may have collected on the inlet rim.

ASSET™ Filters in the 15 mL centrifuge tube with 5.5 mL Toluene. Note the gasket with the 13 mm filter.
Addition of Reagents

The following reagents are added to the 15 mL centrifuge tube:

- 3.0 mL 1 mM H$_2$SO$_4$
- 3.0 mL Methanol
- 5.5 mL Toluene (Toluene has been added during the denuder rinse)
- 100 µL of 0.1 µg/mL of the deuterated Internal Standard Mix.
Prep of Recommended Extracted Calibration Curve

Calibration Standard Reagents:
- 3.0 mL 1mM H$_2$SO$_4$
- 1.5 mL Methanol
- 1.5 mL Matrix Solution
- 5.5 mL Toluene
- Internal Standard (See Table 1)
- External Calibration Standard (See Table 1)

- External Standard Mix 1 µg/mL (40141-U) volumes according to Table 1.
- Internal Standard Mix (40142-U, 10 times diluted in acetonitrile) volumes according to Table 1.

<table>
<thead>
<tr>
<th>Volume of 40141-U (µL)</th>
<th>Amount of analytes (µg) in 1 mL</th>
<th>Volume of ISTD 40142-U (µL) diluted to 0.1 µg/mL Final IS amount/sample: 0.01 µg</th>
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</table>
Extraction – Shaking, Sonication, Re-shaking

Shake samples 5 minutes on high setting. Vigorous shaking is recommended.

Sonicate samples for 10 minutes at ambient temperature.

Shake again

Re-shake samples for 20 minutes on high setting. At this point the filter media should start to fall apart.

The IKA Vibrax Orbital Shaker achieves the recommended vigorous shaking needed to pulverize the filter during the shaking procedure, providing the best conditions for the extraction of isocyanates.
Centrifuge

Centrifuge all samples for 10 minutes at 3000 rpm.

There should be a well defined separation of extraction solvents.

Filter media should be packed in the bottom of the centrifuge tube.
Transfer of Toluene Extract into Culture Tubes

Pipette Toluene layer into a new 16 x 125 mm culture tube*. Be careful not to pipette any of the other solvents with the Toluene extract. Cap and seal the culture tubes until the second extract layer is ready to be added.

*Dependent on drying equipment used, other size tubes can be used.
Addition of second 5.5 mL Toluene

Pipette 5.5 mL of Toluene into the centrifuge tube for the second extraction.
Loosen Packed Filter Media

For the second shake procedure, make sure that the compressed filter media is loosened from the bottom of the centrifuge tube. To do this, turn the centrifuge tube upside down and tap the tube on the counter to disperse the media throughout extraction solvents.
Second Extraction

Shake 5 minutes

Sonicate 10 minutes

Re-shake 20 minutes

Centrifuge at 3000 rpm for 10 minutes
Pipette 2\textsuperscript{nd} Toluene Extract into Culture Tubes

Pipette top toluene layer into the culture tubes with the previous organic extract.

Total volume of the toluene extract in the culture tube will be \(\sim 11\ \text{mL}\).
Sample Drying

Extracts are evaporated in a Zymark Turbo-Vap LV Evaporator set at 50°C with a 5 psi nitrogen purge.

Dry times under these conditions are between 100-145 minutes.

Samples are to be dried to complete dryness.
Re-dissolve the evaporated sample by adding 1 mL of Acetonitrile. Mix on a vortex for 10 seconds.
Transfer Reconstituted Sample to Autosampler Vials

Pipette reconstituted sample into clean, labeled 2 mL autosampler vial for analysis by LC-MS or LC-MS/MS.
Other Equipment that can be used for Sample Drying

Equipment Substitutions for Sample Drying

Six Port Mini-Vap
Evaporator/Concentrator
Supelco 22971

Vacuum Centrifuge with Cold Trap

Heating/Stirring Module with Nitrogen Purge
For additional information and instructions for analysis see:
Extraction and Analysis of ASSET™ EZ4-NCO Dry Sampler, Rev 1.5.