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Technical Brief

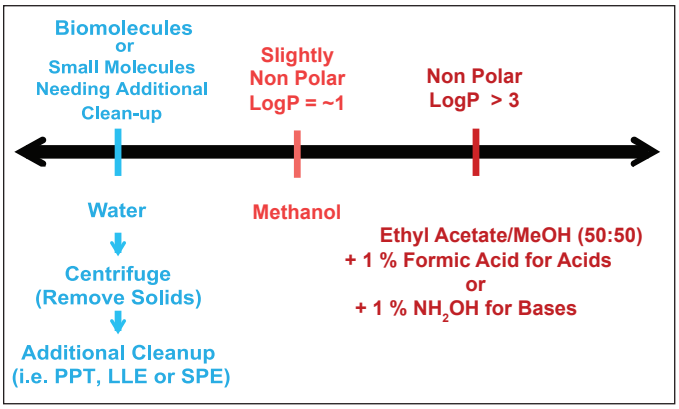
Extraction Guidelines for the Mitra™ (RUO) Microsampling Device

The patent pending Mitra microsampler collects a fixed volume of blood regardless of volumetric blood hematocrit level. This technical brief presents common extraction protocols for the collected blood samples based on the analytes LogP value. With minimal optimization for specific analytes, the recommended extraction protocols typically provide 80 % or greater extraction recovery.

General Extraction Guidelines

An overview of simple extraction protocols is given in **Figure 1.** The basic protocols recommended produce results in an acceptable recovery range. Method optimization is always suggested for higher absolute recovery. As a general guideline, extraction of the dried blood from the Mitra microsampler should be optimized using your target analyte at a concentration of 5x your LLOQ spiked into the highest percentage hematocrit blood available. Absolute recoveries of > 85 % under these conditions will provide the best possible results across a range of analyte concentrations and hematocrit levels.

Figure 1.
Recommended extraction protocols based on analyte LogP

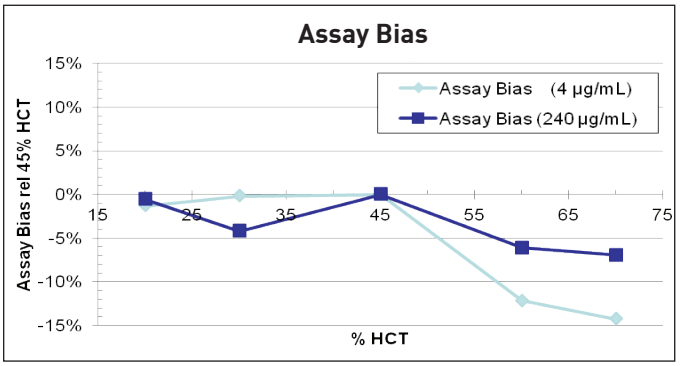


Suggested extraction materials include a 96-well deep collection/extraction plate (P/N: AH0-8636), the Mitra Sampling Tool (P/N: AK-9270-A), a 20-200 µL pipettor or a liquid handler robot properly equipped to perform the protocol. Compatible extraction solvents include water, methanol, ethyl acetate, acetonitrile, DCM, MTBE, chloroform, hexanes, IPA, and mixtures thereof. Extractions can be accelerated by applying heat (heated solvents or heating block) and agitation (vortex or sonication).

Methanol Extraction

When extracting low LogP, slightly non-polar analytes, polar solvents like methanol are suggested. Without the use of modifiers, methanol extractions commonly result in biases within the ± 15 % accuracy accepted for analytical assays **(Figure 2)**.

Figure 2.
Results from basic methanol extraction of acetaminophen assay



Data Courtesy of Neil Spooner and Phil Denniff, GSK (Analytical Chemistry, in press)

Suggested Methanol Extraction Protocol

1. Add methanol to extraction plate (200-500 µL).
2. Add internal standard (optional)
3. Insert Mitra microsamplers into wells of extraction plate
4. Vortex extraction plate for 15-60 minutes at 800-1200 RPM using a platform mixer/rotator.
5. Remove Mitra microsamplers from extraction plate and discard
6. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
7. Blow down to dryness using N2
8. Reconstitute sample with a solvent suitable for dissolution of analytes. (Consider keeping the organic percentage similar to initial mobile phase conditions for LC analysis methods)
9. Analyze the extract.

Aqueous Extraction

When working with biomolecules or small molecules that require additional clean-up, an aqueous extraction is needed. Data has shown > 80 % recovery with these types of extractions across a variety of hematocrit. The Mitra microsampler will turn white with an aqueous extraction due to removal of the majoriiy of the solids from the tip and centrifugation is required to remove solids from sample

Suggested Aqueous Extraction Protocol for Biomolecules

1. Add water to extraction plate (200-500 µL).
2. Add internal standard (optional)
3. Insert Mitra microsamplers into wells of extraction plate
4. Vortex extraction plate for 15-60 minutes at 800-1200 RPM using a platform mixer/rotator
5. Remove Mitra microsamplers from extraction plate and discard
6. If particulate is present, centrifuge the extraction plate at 1500 g for 5 minutes
7. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
8. Analyze the extract.

Suggested LLE Protocol for Small Molecules

1. Add water to extraction plate (200-500 µL). Add 1% formic acid (for neutrals and acids) or 0.25M ammonium hydroxide (for neutrals or bases) to neutralize.
2. Add internal standard (optional)
3. Insert Mitra microsamplers into wells of extraction plate
4. Vortex extraction plate for 15-60 minutes at 800-1200 RPM using a platform mixer/rotator
5. Remove Mitra microsamplers from extraction plate and discard
6. If particulate is present, centrifuge the extraction plate at 1500 g for 5 minutes
7. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
8. Add 1 mL organic solvent to each well (e.g. EtOAc, DCM, MTBE)
9. Mix 10 minutes using platform mixer
10. Centrifuge the extraction plate at 2700 g for 10 minutes at room temperature
11. Freeze / Pour off supernatant
12. Evaporate at 40 °C with N_2 for 25 minutes
13. Reconstitute sample with solvent suitable for dissolution of analytes. (Consider keeping the organic percentage similar to initial mobile phase conditions for LC analysis methods)
14. Analyte the extract

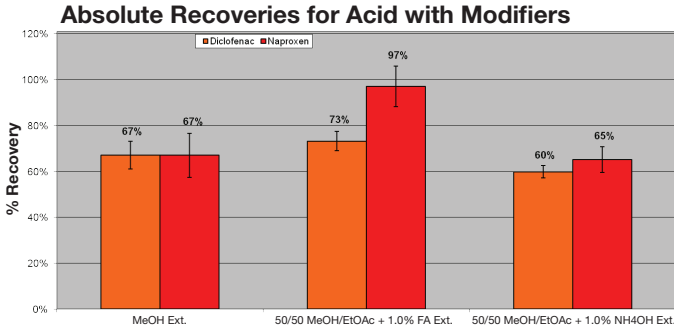
Suggested Protein Precipitation Protocol for Small Molecules

1. Add 100 µL water to extraction plate
2. Insert Mitra microsamplers into wells of extraction plate
3. Vortex extraction plate for 15-60 minutes at 800-1200 RPM using a platform mixer/rotator
4. Remove Mitra microsamplers from extraction plate and discard
5. Add 100 µL of 2.8 % ZnSO4 to extraction plate and mix for 5 minutes
6. Add 100 µL of methanol with internal standard (optional) to extraction plate and mix for 5 minutes
7. Centrifuge at 2700 g for 10 minutes at room temperature (can transfer extract to centrifuge tubes is desired)
8. Analyte the extract

Organic Extraction with Modifiers

For extractions of high LogP, non-polar analytes, use lower polarity solvents such as ethyl acetate. Extraction efficiency is increased by neutralizing the analyte. 1 % formic acid can be used for acidic analytes **(Figure 3)** and 1 % ammonium hydroxide for basic ones **(Figure 4)**.

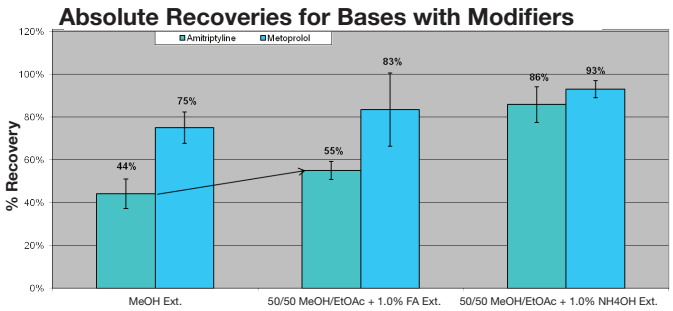
Figure 3.
Acidic modifier improves absolute recovery for acids



Suggested Organic Extraction with Modifiers Protocol

1. Add methanol/ethyl acetate (50:50) to extraction plate (200-500 µL). The additon of 1 % formic acid or 1 % ammonium hydroxide may improve extraction efficiencies.
2. Add internal standard (optional)
3. Insert Mitra microsamplers into wells of extraction plate
4. Vortex extraction plate for 15-60 minutes at 800-1200 RPM using a platform mixer/rotator
5. Remove Mitra microsamplers from extraction plate and discard
6. Remove supernatant from extraction plate and transfer to secondary extraction plate. (optional)
7. Blow down to dryness using N_2
8. Reconstitute sample with a solvent suitable for dissolution of analytes. (Consider keeping the organic percentage similar to initial mobile phase conditions for LC analysis methods)
9. Analyze the extract.

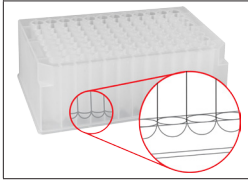
Figure 4.
Basic modifier improves absolute recovery bases



Helpful Tips

- When working with aggressive solvents (e.g. DCM) there is a higher likelihood of extracting potential interferences, such as leachates, from extraction plates. Try to minimize contact time with of these solvents with the plates to avoid/minimize this.
- A tube or vial can be used for extraction of the Mitra tip instead of a plate. If there is a tube that you want to use and the Mitra sampler does not fit, the tip can be removed from the end of the sampler body and put directly into the tube.
- To use smaller extraction volumes, the tip can be removed from the sampler body and dropped into the well of the extraction plate or a microcentrifuge tube to reduce volumes required.
- The recommend 2 mL/well 8 mm round well & bottom collection plate (P/N AH0-8636) fits the Mitra microsampler perfectly for extractions. You can also use P/N: AH0-7193, which is a 1 mL /well square well conical V-bottom collection plate. However, the Mitra sampler tip will touch the bottom of the well.

Ordering Information

Product	Part No.	Description	Unit
	9R-K002-CA	Mitra (RUO) 10 µL Microsampling Device 96-Well Plate Assembly	1 ea
	9R-K002-CD	Mitra (RUO) 10 µL Microsampling Device 96-Well Plate Assembly	12/pk
	9R-K002-BF	Mitra (RUO) 10 µL Microsampling Device 4-Pack Clamshell	60/pk
	AK-9268-A	Mitra Drying Rack 96-Well Plate	1 ea
	AK-9269-A	Mitra SBS Deck Adapter 96-Well Plate	1 ea
	AK-9270-A	Mitra Sampling Tool	1 ea
	AH0-8636	96-Well Collection Plate 2 mL round well with round bottom	50/pk