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technical brief

# Guidelines for the Efficient Extraction of Dried Blood from the Mitra® Microsampling Device

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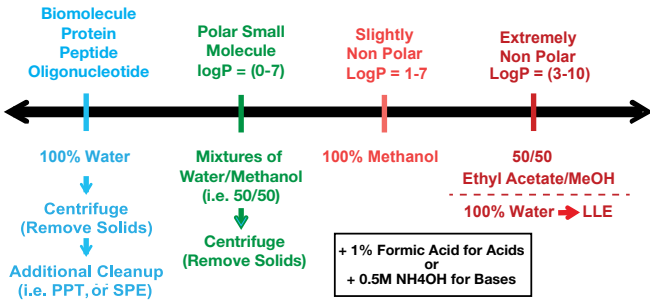
The patent pending Mitra microsampler collects 10 or 20 µL of blood ± 4 % RSD regardless of volumetric blood hematocrit level. This technical brief presents common extraction protocols for the collected dried blood samples based on the analytes LogP value. With minimal optimization for specific analytes, the recommended extraction protocols provide 80 % or greater extraction recovery.

## General Extraction Guidelines

An overview of simple extraction protocols are recommended 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 doped into 45 % hematocrit blood. Absolute recovery of > 85 % under these conditions will provide the best possible results

**Figure 1. Recommended extraction protocols based on analyte LogP**

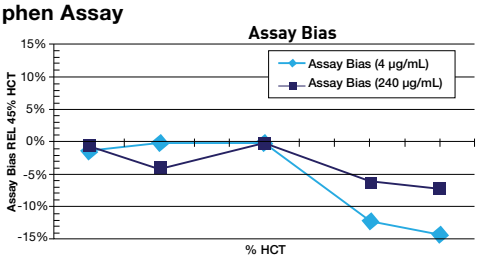



across a range of analyte concentrations and hematocrit levels. Suggested extraction materials include a 96-well deep well collection/extraction plate and a 20-200 µL pipettor or a liquid handler robot properly equipped to perform the protocol. Compatible extraction solvents include water, methanol, acetonitrile, ethyl acetate, and mixtures thereof. Extractions can be accelerated by masticating the tip, applying heat (heated solvents or heating block), and agitation (vortex or sonication).

## Solid Liquid Extraction with Methanol

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**




Denniff, P., Parry, S., Dopson, W., & Spooner, N. (2015). Quantitative bioanalysis of paracetamol in rats using volumetric absorptive microsampling (VAMS). Journal of Pharmaceutical and Biomedical Analysis, 108, 61–69.

**Suggested Protocol**

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96-well collection plate (part number 103) containing 200 µL Methanol (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
6. Blow down to dryness using $N_2$ or dilute to organic strength similar to initial mobile phase
7. 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)
8. Analyze extract

## Protein Precipitation Extraction

Protein precipitation is commonly used for fast sample clean-up and disrupting protein–drug binding. Most common way to perform protein precipitation is extracting the tip with aqueous and 1 % formic acid for acidic analytes and 1 % ammonium hydroxide for basic ones, followed by crashing the solids with methanol or acetonitrile. Another way of precipitating is by using salt organic combination, where zinc sulfate salt was found to be optimal at removing protein.

**Suggested Protocol A: Salt-Organic Extraction Using 1:4 $ZnSO_4$ : Methanol (One Step) for Small Molecules**

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96-well collection plate (part number 103) containing 200 µL of 4:1 Methanol: 2 % $ZnSO_4$ in water (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. See steps 6-8 in the solid liquid extraction with methanol protocol for remaining steps.

**Suggested Protocol B: Salt-Organic Extraction Using 2:1 $ZnSO_4$ : Methanol for Small Molecules**

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8mm 96 well collection plate (part number 103) containing 100 µL of water (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. Add 100 µL of 8% $ZnSO_4$ to collection plate and mix for 5 minutes
6. Add 100 µL of methanol with internal standard (optional) to collection plate and mix for 5 minutes
7. Centrifuge at 1800 g for 10 minutes at room temperature (can transfer extract to centrifuge tubes if desired)
8. Online solid phase extraction (SPE) is recommended after centrifugation for further clean up
9. Analyze the extract

**Suggested Protocol C: Acidic Water-Organic Extraction Using Water-Acid and Acetonitrile for Small Molecules**

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96-well collection plate (part number 103) containing 200 µL of water and 1 % formic acid (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. Transfer 100 µL of the supernatant to a Neoteryx Protein Precipitation Plate (part number 104)
6. Add 300 µL of acetonitrile into the protein precipitation plate well containing supernatant and mix for 10 minutes
7. Vacuum at 5” Hg and collect in collection plate )
8. See steps 6-8 in the solid liquid extraction with methanol protocol for remaining steps

## Liquid-Liquid Extraction (LLE) Protocol for Small Molecules

For non-polar analytes with LogP >3, liquid-liquid extraction will be quite effective. The analytes are extracted using water (addition of acid or base) and then partitioned to another immiscible liquid (non-polar).

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96-well collection plate (part number 103) containing 200 µL of water (internal standard is optional). Add 1 % formic acid (for neutrals and acids) or 0.25M ammonium hydroxide (for neutrals or bases) to neutralize the analyte
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. If particulate is present, centrifuge the extraction plate at 1500g for 5 mins
6. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
7. Add 1 mL organic solvent to each well (e.g. EtOAc, DCM, MTBE)
8. Mix 10 minutes using platform mixer
9. Centrifuge the extraction plate at 1800 g for 10 minutes at room temperature
10. Freeze / transfer supernatant
11. See steps 6-8 in the solid liquid extraction with methanol protocol for remaining steps

## Aqueous Extraction

When working with biomolecules an aqueous extraction is needed. The Mitra microsampler will turn white with an aqueous extraction due to removal of the majority of the solids from the tip. Centrifugation is required to remove solids from sample. An appropriate immunoassay or DNA, RNA isolation should follow.

### Suggested Protocol

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96 well collection plate (part number 103) containing 200 µL of water (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. If particulate is present, centrifuge the extraction plate at 1500 g for 5 minutes
6. Remove supernatant from extraction plate and transfer to a secondary plate (optional) followed by an appropriate immunoassay or DNA, RNA isolation.
7. Analyze the extract followed by an appropriate immunoassay or DNA, RNA isolation

## 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**

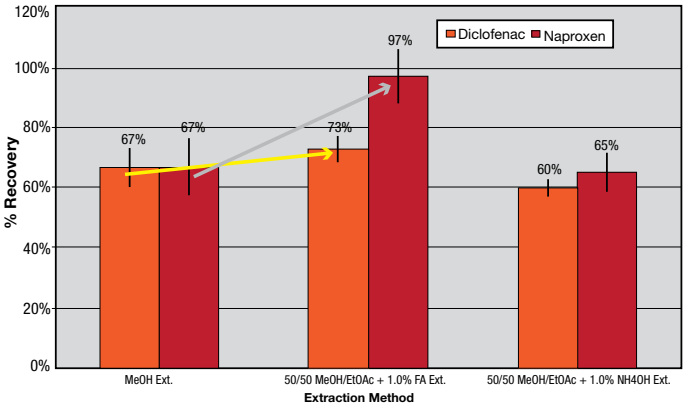
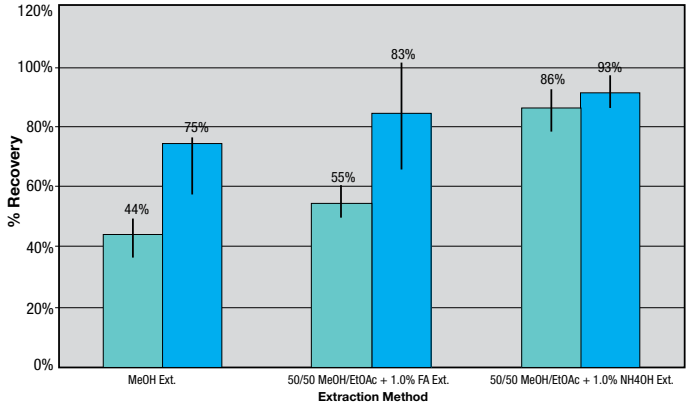



**Figure 4. Basic modifier improves absolute recovery for bases**




### Suggested Protocol

1. Place Mitra Drying AutoRack (part number 108) containing dried microsamplers onto a 2 mL, round bottom 8 mm 96-well collection plate (part number 103) containing 200 µL of methanol/ethyl acetate (50:50) (internal standard is optional)
2. Sonicate collection plate and AutoRack assembly for 15 minutes
3. Shake collection plate and AutoRack assembly on Vibramax 100 (Heidolph) for 1 hour @ 1200 rpm
4. Remove AutoRack containing microsamplers from the collection plate and discard
5. Remove supernatant from extraction plate and transfer to a secondary plate (optional)
6. Blow down to dryness using $N_2$
7. 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)
8. Analyze extract

## 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 device instead of a plate. If there is a tube that you want to use and the Mitra sampler does not fit, the sampling tip can be removed from the end of the sampler body and put directly into the tube.
- For extremely hydrophobic analytes, use glass tubes and glass vials because they have lower non-specific binding.
- 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.

20 µL 96-autorack (PN 20101) & 20 µL Mitra 2-sampler Cartridge (PN 20103)

20 µL 96-rack (PN 20006) & 20 µL Mitra 4-sampler Clamshell (PN 20004)

## Ordering Information

| Part No. | Description | Unit |
|---|---|---|
| **96-rack / 96-autorack format** | | |
| 10006 | 10 µL Mitra Microsampler 96-rack | 6/pk |
| 20006 | 20 µL Mitra Microsampler 96-rack | 6/pk |
| 10101 | 10 µL Mitra Microsampler 96-autorack | 1 ea |
| 20101 | 20 µL Mitra Microsampler 96-autorack | 1 ea |
| **clamshell format** | | |
| 10004 | 10 µL Mitra Microsampler 4-sampler Clamshell | 52/pk |
| 20004 | 20 µL Mitra Microsampler 4-sampler Clamshell | 52/pk |
| 10002 | 10 µL Mitra Microsampler 3-sampler Clamshell | 52/pk |
| 20002 | 20 µL Mitra Microsampler 3-sampler Clamshell | 52/pk |
| 10001 | 10 µL Mitra Microsampler 2-sampler Clamshell | 52/pk |
| 20001 | 20 µL Mitra Microsampler 2-sampler Clamshell | 52/pk |
| **cartridge format** | | |
| 10103 | 10 µL Mitra Microsampler 2-sampler Cartridge / Desiccant Pack | 52/pk |
| 20103 | 20 µL Mitra Microsampler 2-sampler Cartrdige / Desiccant Pack | 52/pk |
| **reformatting and extraction accessories** | | |
| 100 | Mitra Drying Rack | 1 ea |
| 108 | Mitra Drying AutoRack | 1 ea |
| 104 | Neoteryx Protein Precipitation Plate | 2/pk |
| 103 | 96-Well Collection Plate, 2 mL Round Well, Round Bottom, 8 mm | 50/pk |
| 104 | Protein Precipitation Plates | 2 pk |
| 106 | Pre-Slit Sealing Mat, 96-Round Well, 8 mm, Silicone | 50/pk |
| 107 | Sealing Tape Pad | 10/pk |

CE IVD

The Mitra Microsampling Device is a FDA listed Class 1 device (D254956). Neoteryx complies with FDA good manufacturing practices, CFR 820 regulations, and ISO 13485

Trademarks
VAMS is a trademark and Mitra is a registered tradmark of Neoteryx LLC. TurboVap is a registered trademark of Biotage.

Disclaimer
Mitra is patent pending. The Mitra Microsampler class I medical device is for direct specimen collection of blood and other biological fluids. It is not specific to any clinical test, and is not for use in diagnostic procedures. Use of the Mitra Microsampler in Laboratory Developed Tests (LDTs) requires further processing including the establishment of performance characteristics and successful validation by the laboratory in a manner consistent with CLIA requirements.

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