

SupelMIP™ SPE – Nitroimidazoles

Product Description:

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guide the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). **It is therefore critical for analysts to use the methodology described below when using this phase.** Conventional generic methodologies employed with conventional SPE chemistries (e.g., reversed-phase C18) will yield sub-optimal results when employed with this phase.

The following method(s) have been determined for Nitroimidazoles that can be optimized for a number of matrices. The nitroimidazoles that we have tested so far include: Dimetridazole (DMZ), Metronidazole (MNZ), Iprnidazole (IPZ), Ronidazole (RNZ); and their respective metabolites: DMZOH, MNZOH, and IPZOH.

The first procedure is a general procedure that can be followed if a matrix specific method is not included in this data/instruction sheet. This general procedure represents a recommended starting point for further optimization. The general procedure is followed by matrix specific procedures.

For the most recent matrix specific applications, please visit www.sigma-aldrich.com/supelmip and download the most recent version of the data/instruction sheet.

Important Note: The below procedure(s) may require further optimization. A special team of experts in SupelMIP SPE method develop has been formed to offer technical consultation. To reach a SupelMIP technical expert, please visit www.sigma-aldrich.com/supelmip-techsupport and fill out the questionnaire. A SupelMIP scientist will respond within 24 hours (barring holidays).

Protocol for Extraction of Nitroimidazoles – General Procedure:

Sample Pre-treatment

For solid/tissue samples:

1. Homogenize 2.5 g of sample with I.S.; and add 10 mL DI H₂O.
2. Remove particulate via centrifugation

For liquid samples:

1. Dilute samples with 1:1 to 1:5 with DI water or 10 mM ammonium acetate, pH 6

Note: Deuterated I.S. is recommended for each analyte for accurate quantitation.

Use polypropylene or silanized glassware only. Nitroimidazoles may adsorb onto standard glassware resulting in loss of recovery.

The sample should be completely aqueous prior to SPE processing. No organic modifiers should be present in the sample.

Additional sample pre-treatment may be required depending on the complexity of the sample. For example, a protein ppt step may be necessary for samples that contain high levels of protein.

Condition column with:
1 mL toluene
1 mL acetonitrile
1 mL 10 mM ammonium acetate, pH 6

Note: Do not allow the phase to go dry during conditioning. Recondition if the phase goes dry.

Load sample:
Apply a maximum of 2 mL sample

Wash #1:
A max volume of 1 mL ultra-pure water

Wash #2:
2 x 1 mL hexane

Wash #3:
1 mL heptane:toluene 3:1 (v/v)

Important: Apply a strong vacuum through cartridge for at least **5 min.** to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or -70 kPa).

Apply a gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Important: Apply a strong vacuum through cartridge for at least **5 min.** to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or -70 kPa).

Analyte elution:
Elute Nitroimidazoles with 2 x 1 mL acetonitrile: water (60:40, v/v) with 0.5 % acetic acid

Apply a gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each wash step unless noted otherwise.

Recommended flow rate during sample load is ≤ 0.5 mL/min. If possible use gravity flow during the sample load step.

A flow rate of 0.5-1 mL/min. is recommended for each wash step. The wash steps should be performed in the prescribed order.

Recommended flow rate during elution is ~ 0.2 mL/min

Evaporate the elution solvent to 20-50 μ L at 50 °C under N₂ and reconstitute in 500 μ L LC-MS mobile phase, filter prior to analysis if necessary.

Protocol for Extraction of Nitroimidazoles in Milk & Egg:

Sample Pre-treatment

For egg powder, combine 2.5 g of egg powder with 10 mL DI water in a centrifuge tube.

For raw egg, combine 10 g of raw egg with 10 mL DI water in a centrifuge tube.

For milk, transfer 10 mL milk to a centrifuge tube.

1. Add I.S. to 10 mL of egg powder, raw egg, or milk sample from above.
2. Shake vigorously for 2 min.; and add 10 mL MeCN. Shake for an additional 2 min.
3. Centrifuge for 15 min. at 4000 x g. Isolate supernatant and combine with 2 g NaCl.
4. Shake manually and centrifuge for 5 min. at 4000 x g. Isolate supernatant and evaporate to dryness at 50°C under slight nitrogen flow.
5. Reconstitute in 2 mL DI water or 10 mM ammonium acetate, pH 6. Sonicate for 3 min.

Note: Deuterated I.S. is recommended for each analyte for accurate quantitation.

Use polypropylene or silanized glassware only. Nitroimidazoles may adsorb onto standard glassware resulting in loss of recovery.



Condition column with:
1 mL toluene
1 mL acetonitrile
1 mL 10 mM ammonium acetate, pH 6

Note: Do not allow the phase to go dry during conditioning. Recondition if the phase goes dry.



Load sample:
Apply a maximum of 2 mL sample

Recommended flow rate during sample load is ≤ 0.5 mL/min. If possible use gravity flow during the sample load step.



Wash #1:
A max volume of 1 mL ultra-pure water

A flow rate of 0.5-1 mL/min. is recommended for each wash step. The wash steps should be performed in the prescribed order.



Wash #2:
2 x 1 mL hexane



Wash #3:
1 mL heptane:toluene 3:1 (v/v)

Important: Apply a strong vacuum through cartridge for at least 5 min. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or -70 kPa).

Apply a gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each wash step unless noted otherwise.



Analyte elution:
Elute Nitroimidazoles with 2 x 1 mL acetonitrile: water (60:40, v/v) with 0.5 % acetic acid

Recommended flow rate during elution is ~0.2 mL/min

Important: Apply a strong vacuum through cartridge for at least 5 min. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or -70 kPa).

Apply a gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each wash step unless noted otherwise.



Evaporate the elution solvent to 20-50 μ L at 50 °C under N₂ and reconstitute in 500 μ L LC-MS mobile phase, filter prior to analysis if necessary.

Troubleshooting:

Improve Recovery:

- ♦ Do not exceed the recommended load and wash volumes.
- ♦ When evaporating the SPE eluent prior to reconstitution and analysis, do not evaporate to dryness. Analyte loss may occur.
- ♦ Use polypropylene or silanized glassware throughout the SPE procedure. Nitroimidazoles may adsorb onto standard glassware.
- ♦ Implement the SPE tube drying steps (e.g., between wash steps and elution steps) as recommended.
- ♦ Minimize flow rate during sample load and elution.
- ♦ Increase elution from 2 x 1 mL to 3 x 1 mL

Improve Sample Cleanup:

- ♦ Adjust the elution solvent from "2 x 1 mL acetonitrile:water (60:40, v/v) with 0.5 % acetic acid" to "2 x 1 mL acetonitrile:water (50:50, v/v) with 0.5 % acetic acid"

Recommended Analytical Method:

<p>Recommended Analytical Technique:</p> <p>LC-MS-MS or LC-MS</p>	column:	Ascentis® C18, 15 cm x 2.1 mm I.D., 3 µm particle size (581302-U)	
	instrument:	Sciex API 3200	
	mobile phase:	(A) 0.1% formic acid in LC-MS grade water (B) 0.1% formic acid in acetonitrile	
	gradient:	Min.	A% B%
		0.0	95 5
		1.0	95 5
		8.0	0 10 0
		12.0	0 10 0
		13.0	95 5
		18.0	95 5
	flow rate:	0.3 mL/min.	
	temp.:	ambient	
	det.:	MS/MS, MRM transitions	
		DMZ (142/96)	IPZOH (186/168)

	DMZ-d3 (145/99)	IPZOH-d3 (189/171)
	DMZOH (158/140)	MNZ (172/128)
	DMZOH-d3 (161/143)	MNZOH (188/126)
	IPZ (170/124)	RNZ (201/140)
	IPZ-d3 (189/171)	RNZ-d3 (204/143)
polarity:	Positive	
ion source:	Turbo spray	
ion spray voltage:	1200 V	
source temp:	350 °C	
collision gas:	4 psi	
curtain gas:	50 psi	
inj.:	30 µL	

Product Information:

Description	Pkg. Qty.	Cat. No.
SupelMIP SPE - Nitroimidazoles		
50 mg/3 mL	50	52734-U
SupelMIP SPE - Full Beta-receptors (beta-blockers & beta-agonists)		
25 mg/10 mL (LRC)	50	53223-U
25 mg/3 mL	50	53224-U
SupelMIP SPE - Beta-blocker (class selective)		
25 mg/10 mL (LRC)	50	53218-U
25 mg/3 mL	50	53213-U
SupelMIP SPE - Beta-agonists (class selective)		
25 mg/10 mL (LRC)	50	53202-U
25 mg/3 mL	50	53225-U
SupelMIP SPE - Clenbuterol		
25 mg/10 mL (LRC)	50	53201-U
SupelMIP SPE - TSNAs (NNK, NNN, NAB, NAT)		
50 mg/10 mL (LRC)	50	53221-U
50 mg/3 mL	50	53222-U
SupelMIP SPE – NNAL		
25 mg/10 mL (LRC)	50	53206-U
25 mg/3 mL	50	53203-U
SupelMIP SPE - Chloramphenicol		
25 mg/10 mL (LRC)	50	53210-U
25 mg/3 mL	50	53209-U
SupelMIP SPE – Fluoroquinolones		
25 mg/3 mL	50	53269-U
SupelMIP SPE – Amphetamines (class selective)		
25 mg/3 mL	50	53228-U
SupelMIP SPE - Riboflavin (Vitamin B2)		
25 mg/10 mL (LRC)	50	53207-U
SupelMIP SPE - Triazine 10		
25 mg/10 mL (LRC)	50	53208-U

SupelMIP SPE developed by MIP Technologies AB

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