

Mixed-mode (Reversed-phase/Cation eXchange) SPE in 3 or 4 steps

3-Step Protocol

Load*

Pre-treated sample diluted with a 1:1 solution of 200 mM ammonium formate with 4% H₃PO₄ (aq)



Wash 1

100% MeOH



Elute

5% Ammonium hydroxide in MeOH

* Note: The sample is diluted 1:1 to a final concentration of 100 mM ammonium formate and 2% H₃PO₄.

Preparation of sample diluent: 200 mM ammonium formate in 4% H₃PO₄ acid (aq)

Table of amounts required to prepare the final volume of solution

Total solution volume	20 mL	50 mL	100 mL	200 mL	500 mL	1000 mL
Water	19.2 mL	48 mL	96 mL	192 mL	480 mL	960 mL
Phosphoric acid 85% (ACS reagent grade)	0.8 mL	2.0 mL	4.0 mL	8.0 mL	20 mL	40 mL
Ammonium formate	252 mg	630 mg	1.26 g	2.52 g	6.31 g	12.6 g

For example, to prepare 100 mL of sample diluent solution:

1. Measure 96 mL of water into a 100 mL bottle.
2. Pipette 4 mL of phosphoric acid into the bottle.
3. Add 1.26 g of ammonium formate to the bottle.
4. Mix well.

Final solution is 200 mM ammonium formate with 4% phosphoric acid.

Please refer to the Oasis Sample Extraction Product Care and Use Manual for additional information.

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Mixed-mode (Reversed-phase/Cation eXchange) SPE in 3 or 4 steps

4-Step Protocol**

Load

Pre-treated sample diluted 1:1 with a solution of 4% H₃PO₄

Wash 1

100 mM Ammonium formate with 2% formic acid (aq)

Wash 2

100% MeOH

Elute

5% Ammonium hydroxide in MeOH

** Note: The extra wash step produces a cleaner sample by removing more polar matrix interferences if needed.

Preparation of wash 1 solution: 100 mM ammonium formate in 2% formic acid (aq)

Table of amounts required to prepare the final volume of solution

Total solution volume	20 mL	50 mL	100 mL	200 mL	500 mL	1000 mL
Water	19.6 mL	49 mL	98 mL	196 mL	490 mL	980 mL
Formic acid, 98%	0.4 mL	1.0 mL	2.0 mL	4.0 mL	10 mL	20 mL
Ammonium formate	126 mg	315 mg	630 mg	1.26 g	3.15 g	6.31 g

For example, to prepare 100 mL of wash 1 solution:

1. Measure 98 mL of water into a 100 mL bottle.
2. Pipette 2 mL of formic acid into the bottle.
3. Add 630 mg of ammonium formate to the bottle.
4. Mix well.

Final solution is 100 mM ammonium formate with 2% formic acid.

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