

# Reverse phase SPE conditions optimized for peptide purification

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## Keywords

Solid Phase Extraction (SPE),  
small molecule purification

## Introduction

General reversed phase solid phase extraction (RP SPE) protocols are more suited for small molecule purification (where the molecule elutes from the RP stationary phase at 60–80% organic conditions). Published proteomics gradients show that all peptides have typically eluted by 30–40% organic solvent. Eluting peptides at a lower organic composition provides the additional advantage of leaving hydrophobic contaminants such as undigested proteins, detergents, and unreacted TMT free label bound to the stationary phase thereby yielding a sample with less hydrophilic (salts) and hydrophobic contaminants.

## Important notes

- Acidifying peptide sample prior to application to stationary phase increases peptide binding to stationary phase
- Collect flow through from loading step and reapply to stationary phase to minimize losses
- Collect flow through from loading and wash step and save for analysis while optimizing SPE method to ensure these fractions are free from sample
- Select RP SPE product that matches the capacity required by your sample
- Use solvent step volumes appropriate to your SPE bed volume

## Materials required

- Acetonitrile, Optima LC/MS Grade, Fisher Chemical (A955-500)
- Water, Optima LC/MS Grade, Fisher Chemical (W6500)
- Thermo Scientific™ Pierce™ Formic Acid (PI28905)
- Reversed Phase SPE of choice

## Protocol

1. Prepare SPE stationary phase for peptide binding adding 1 equivalent of Acetonitrile
2. Wash SPE stationary phase with 1 equivalent of 2% Acetonitrile 0.1% Formic Acid (Repeat 2 times)
3. Acidify sample containing peptides to 0.1% in Formic acid (sample should be in 100% aqueous conditions for optimal binding to stationary phase)
4. Load sample over SPE stationary phase (sample should be applied slowly)
5. Collect flow through from loading step and reapply
6. Wash SPE stationary phase with 1 equivalent of 2% Acetonitrile 0.1% Formic Acid (Repeat 2 times)
7. Elute peptides from stationary phase with 1 equivalent of 50% Acetonitrile 0.1% Formic Acid

## Related products

Description	Part Number
Thermo Scientific™ SOLA $\mu$ ™ HRP 2mg/1mL 96-well plate	60209-001
Thermo Scientific™ SOLA™ HRP 10mg/2mL 96-well plate	60309-001
SOLA HRP 10mg/1mL cartridge	60109-001
Thermo Scientific™ HyperSep™ Retain PEP Products	60107-201

Current versions of product instructions are available at [separatedbyexperience.com/chromexpert](https://separatedbyexperience.com/chromexpert)

Learn more about SOLA Solid Phase Extraction at [thermofisher.com/solaspe](https://thermofisher.com/solaspe)

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