thermo scientific

SOLAμ well plate method development

Manifold set-up considerations for micro elution SPE plates

1

Setting up a method

SOLAμ well plate

Thermo Scientific™ SOLAμ™ well plate is a micro elution device built on Embedded Particle Technology (EPT), which removes the need for frits, thus eliminating issues associated with traditional loose-packed solid phase extraction (SPE). This results in highly reproducible, accurate data.

SOLAμ well plate enables:

- an increase in your sensitivity by up to 20 fold
- removal of evaporation and reconstitution steps
- removal of analyte volatility, adsorption and solubility issues

Did you know SOLAμ well plates have individually removable wells?

This saves you money and allows you to build your own bespoke method development plate. This provides a fast option for screening different chemistries in one plate.

2

Phase selection

Choose the most appropriate SOLAμ product by using the chemical properties of your analyte to find the most selective stationary phase.

Chemistry	pKa	Description	Cat. no.
HRP Hydrophobic Reversed Phase	-	Hydrophobic retention of neutral compounds with complementary retention of polar analytes. An all-purpose phase.	60209-01
SCX Strong Cation Exchange	<1	Strong ion-exhange retention of basic compounds. Complementary reversed-phase retention of neutral compounds.	60209-02
SAX Strong Anion Exchange	>18	Strong ion-exhange retention of acidic compounds. Complementary reversed-phase retention of neutral compounds.	60209-03
WCX Weak Cation Exchange	~4.5	Weak ion-exchange retention by adjusting the pH of basic compounds. Sorbent charge can be activated or deactivated. Complementary reversed-phase retention of neutral compounds.	60209-04
WAX Weak Anion Exchange	~8.5	Weak ion-exchange retention by adjusting the pH of acidic compounds. Sorbent charge can be activated or deactivated. Complementary reversed-phase retention of neutral compounds.	60209-05

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Starting method

Select a starting method from the options below:

	Sample pre-treatment	Dilute sample 1:1 with 0.1% formic acid (aq)	
	HRP	**SCX and WAX**	**SAX and WCX**
Condition	200 µL methanol	200 µL methanol	200 µL methanol
Equilibrate	200 µL water	200 µL water	200 µL water
Sample load	Up to 1000 µL	Up to 1000 µL	Up to 1000 µL
Wash	200 µL n % methanol	200 µL 2% formic acid	200 µL 5% ammonia
Wash	-	200 µL methanol	200 µL methanol
Elute	2 × 25 µL n % methanol	2 × 25 µL 5% ammonia in methanol	2 × 25 µL 5% formic acid in methanol
	Post extraction	**Dilute extract with 50 µL of water**	

n = refer to elution profiles

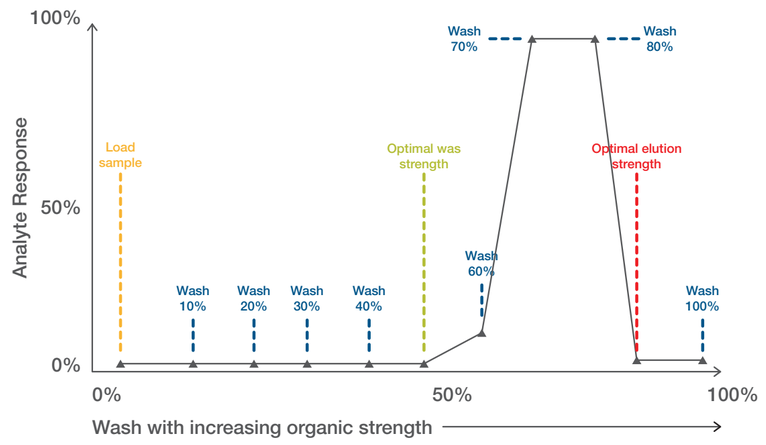
Collect and analyze each step from the chosen procedure. This can help with the optimization of your assay.

4

Method optimization

Optimization of HRP method

Load sample and wash with increasing organic composition. Collect all fractions separately and analyze to give an elution profile. Use this to determine optimal wash and elution conditions.

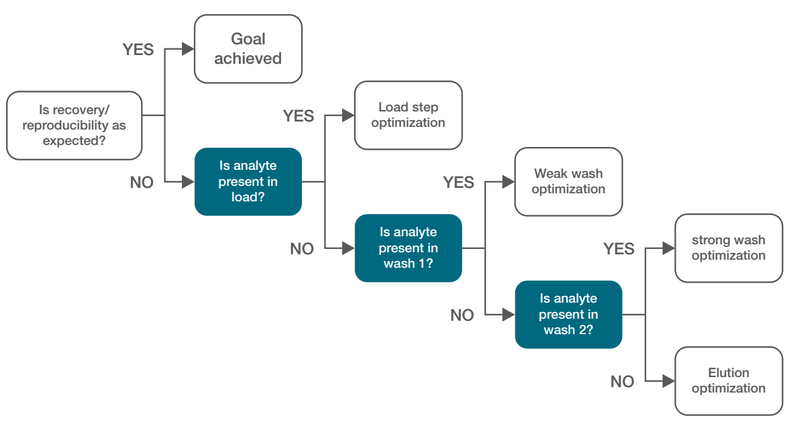


*Even if you achieve **100% recovery** from a generic starting point with HRP, it is recommended that the elution profile experiment is performed for optimal removal of potential interferences.*

5

Optimization of mixed-mode ion-exchange method

Collect each fraction of your method and analyze. This information can be used to identify which step of the process requires optimization if low recovery is observed.



Mixed-mode phases can be used with reversed-phase methods to provide cleaner extracts by removing more unwanted interferences.

Step optimization	Solution
Analyte present in the load step	Compound can be retained via reversed-phase mechanism or ion-exchange. Confirm the sample pH is adjusted to ensure the molecule is either fully neutralized or fully ionized in order to retain efficiently. Note that sample dilution with acidified water may be required to remove any protein binding to the matrix. Ion-exchange chemistry is sensitive to flow speed, ensure a low flow speed is used. Confirm correct phase chemistry is being used.
Analyte present in wash step 1	Confirm the wash pH is adjusted to ensure the molecule and/or sorbent is fully ionized. Confirm correct phase chemistry is being used.
Analyte present in wash step 2	Confirm the wash pH in wash step is adjusted to ensure the molecule and/or sorbent is fully ionized. Organic strength may be reduced but not normally required. Confirm correct phase chemistry is being used.
Analyte recovery low in elution step	Confirm the elution pH is adjusted to ensure the molecule and/or sorbent is fully neutralized and sufficient organic composition is used to break reverse phase retention. Solvents and mixtures of differing polarity and eluotropic strength may be used. Solvent volume may be increased. Confirm sufficient sample pre-treatment is used to break any protein binding prior to load.

FAQs

General	Solution
Slow sample flow	SOLAμ well plate requires a higher vacuum to initiate flow compared to traditional SPE due to uniformity of the packing material. Dilute sample 1:1 with aqueous buffer to aid flow though the plate. Centrifuge sample prior to load to remove particulates. Clean sample with partial solvent crash prior to load (dilution of supernatant may be required).
Contamination of blank samples by SPE process	Ensure the collection plate is sufficiently close to the underside of the SPE plate within the vacuum manifold.
No vacuum pressure	Ensure the SPE is fitted correctly in the vacuum manifold and the collection plate is not lifting the SPE plate causing a breach of seal.

Ordering information

Description	Cat. no.
Thermo Scientific™ SOLAμ™ products	
SOLAμ HRP 96 well plate	60209-001
SOLAμ SCX 96 well plate	60209-002
SOLAμ SAX 96 well plate	60209-003
SOLAμ WCX 96 well plate	60209-004
SOLAμ WAX 96 well plate	60209-005

Description	Cat. no.
Complementary products	
Thermo Scientific™ HyperSep-96™ Well Plate Manifold	60103-351
Vacuum Pump, European Version	60104-241
Vacuum Pump, North American Version	60104-243

Find out more at **thermofisher.com/solaspe**

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