thermo scientific

SOLA solid-phase extraction (SPE) method development guide

Method optimization for SPE bioanalysis

ThermoFisher SCIENTIFIC

Introduction

Solid-phase extraction (SPE) is said to be the most effective technique in modern bioanalysis with regard to isolation of analytes of interest from complex biological matrices. It improves accuracy, precision, allows for much lower levels of detection and reduces matrix effects (the most common problem in mass spectrometry).

Thermo Scientific™ SOLA™ products revolutionize solid-phase extraction (SPE). This fritless SPE product range provides greater reproducibility with cleaner, more consistent extracts.

SOLA products provide unparalleled performance characteristics compared to conventional SPE, phospholipid removal and protein precipitation products.

This includes:

- higher levels of reproducibility
- higher levels of extract cleanliness
- reduced solvent requirements
- increased sensitivity

The Embedded Particle Technology (EPT) and proprietary manufacturing process involved in the production of SOLA cartridges and plates, provides an SPE product which eliminates issues normally associated with conventional loose-packed SPE, by combining the polyethylene frit material and media components into a solid uniform sorbent bed, removing the need for frits (Figure 1). This provides an added advantage when dealing with viscous biological samples, preventing blocking and enabling high throughput processing of samples.

The manufacturing process has the additional benefit of removing extractables from component parts, resulting in cleaner sample extracts.

SOLA products provide reduced failure rates, higher analysis speeds and lower solvent requirements, which are critical in today's laboratory environment.

The increased performance delivered by SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Conventional SPE cartridges and well plates are packed with a loose powder of silica or polymeric material positioned between two frits. These packed beds are potentially prone to settling and voiding in production or transportation. This creates phase channeling and packing irreproducibility, resulting in reduced recovery and reproducibility in analytical results (Figure 2).

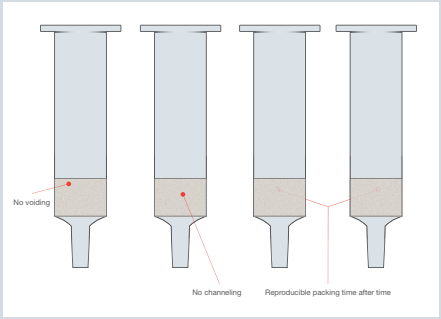


Figure 1: SOLA products eliminate common issues associated with conventional SPE

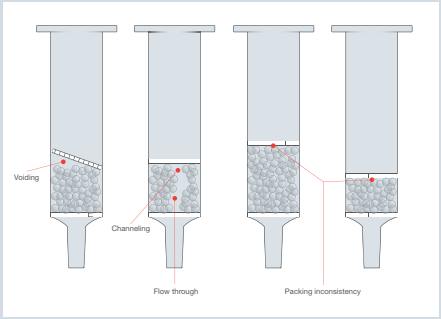
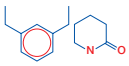
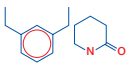
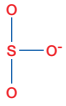
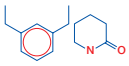
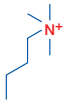
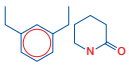
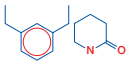


Figure 2: Examples of conventional SPE product issues

Selectivity options for SOLA cartridges and plates

Thermo Scientific SOLA and SOLAμ products are manufactured using high-quality polymeric material which provides a wide range of selectivity options (Table 1) to meet all your analytical requirements. The use of polymeric EPT sorbents in the design provides a robust high capacity bed which is stable over a wide range of pH (0-14) and does not lose sample capacity on drying.

Table 1: SOLA selectivity options and compound applicability

Chemistry	Base polymer	Functional groups	pK_a	Primary use	Secondary use	Description	Cat. no.
HRP Hydrophobic Reversed-Phase		-	-	Neutral compounds	Moderately polar compounds	Hydrophobic retention of compounds with complementary retention of moderately polar analytes. An all-purpose phase.	60109-001 60209-001 60309-001 60409-001 60509-001
SCX Strong Cation Exchange			<1	Weakly basic compounds (pK_a 8-10)	Neutral compounds	Strong ion-exhange retention of basic compounds. Complementary reversed-phase retention of neutral compounds.	60109-002 60209-002 60309-002 60409-002 60509-002
SAX Strong Anion Exchange			>18	Weakly acidic compounds (pK_a 2-4)	Neutral compounds	Strong ion-exhange retention of acidic compounds. Complementary reversed-phase retention of neutral compounds.	60109-003 60209-003 60309-003 60409-003 60509-003
WCX Weak Cation Exchange			~4.5	Strongly basic compounds (pK_a > 10)	Neutral compounds	Weak ion-exchange retention of basic compounds. Sorbent charge can be activated or deactivated. Complementary reversed-phase retention of neutral compounds.	60109-004 60209-004 60309-004 60409-004 60509-004
WAX Weak Anion Exchange			~8.5	Strongly acidic compounds (pK_a < 2)	Neutral compounds	Weak ion-exchange retention of acidic compounds. Sorbent charge can be activated or deactivated. Complementary reversed-phase retention of neutral compounds.	60109-005 60209-005 60309-005 60409-005 60509-005

Want to know more about how EPT and SOLA products can revolutionize your analysis?
thermofisher.com/solaspe

Bed size options for SOLA and SOLAμ SPE

The choice of bed weight is an important point to consider when developing an SPE protocol and is dictated by the volume and complexity of the sample matrix, along with the amount of analyte to be extracted in your application. When compared to traditional silica-based media, the SOLA packing sorbent has approximately 2-3 times more reversed-phase mass capacity. This allows SOLA and SOLAμ SPE to retain more analyte in your sample than the equivalently sized silica-based sorbent.

Smaller bed weights, such as the SOLAμ micro-elution plate, lend themselves to lower elution volumes, whereas larger bed weights, such as SOLA 30 mg, offer greater loading capacity for hard to retain analytes found in low concentrations. Both approaches can be used to increase extraction sensitivity. The deciding factor for bed size is often the sample concentration and volume of matrix used in your application.

Table 2: Bed size options for SOLA and SOLAμ SPE

SOLA bed size	Typical sample volumes pK_a	Elution volumes	Benefits of use
2 mg (SOLAμ)	Up to 500 µL	25 µL ≤	• Low volume samples • Reduced cost and increased throughput by removal of evaporation and reconstitution steps • Increased signal sensitivity by up to 20 fold
10 mg	Up to 1 mL	150 µL ≤	• Good option for most analyses • Lower elution volumes than silica based products thus reducing time for evaporation and increasing throughput
30 mg	Up to 2 mL	250 µL ≤	• When high loading volumes are required to reach lower limits-of-quantitation • For difficult to retain analytes • When experiencing analyte breakthrough on a smaller bedweight

Format options for SOLA and SOLAμ SPE

SPE devices come in various formats, and two common designs are the cartridge and the 96-well plate. SOLA is available in both formats, while SOLAμ is available in a 96-well plate format with individually removable wells, which are especially convenient for method development.

When to select cartridges or plates?

- 1 mL cartridges are typically used for routine or method development purposes
- 3 mL cartridges can be used in method development and in analyses where the larger cartridge volume is required e.g. analysis from urine
- 96-well plates are normally used in high throughput analyses where many samples are needed to be processed in parallel

Cartridges

SOLA SPE product	HRP	SCX	SAX	WCX	WAX
10 mg/2 mL	60109-001	60109-002	60109-003	60109-004	60109-005
30 mg/3 mL	60409-001	60409-002	60409-003	60409-004	60409-005

96-well plates

SOLA SPE product	HRP	SCX	SAX	WCX	WAX
2 mg/1 mL (SOLAμ)	60209-001	60209-002	60209-003	60209-004	60209-005
10 mg/2 mL	60309-001	60309-002	60309-003	60309-004	60309-005
30 mg/2 mL	60509-001	60509-002	60509-003	60509-004	60509-005

Using the 96-well plate format

The Thermo Scientific SOLA and SOLAμ plate design conforms to the standard footprint of 96-well formats (Figure 3) ensuring compatibility with standard positive and negative pressure manifolds and centrifugation devices. It is important, however to ensure that the height of the collection plate inside the manifold is correct in relation to the tips of the SOLA and SOLAμ plate. Ideally, the tips of the SPE plate should sit inside the top of the collection wells to ensure all liquid is transferred into the correct well (Figure 4).

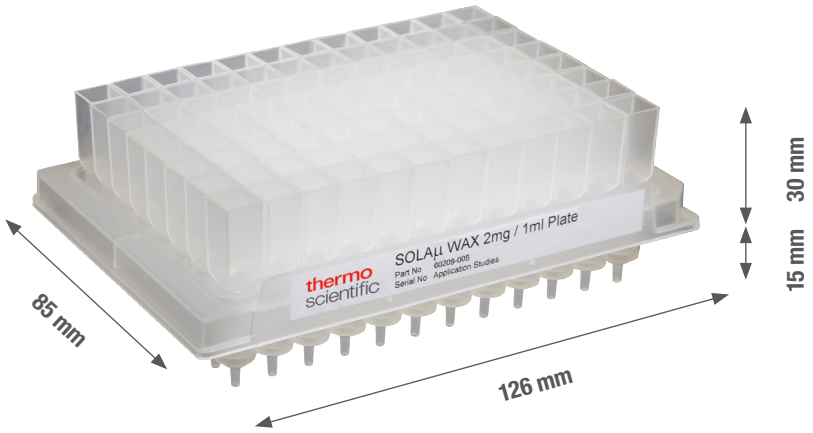


Figure 3: SOLAμ 96-well plate

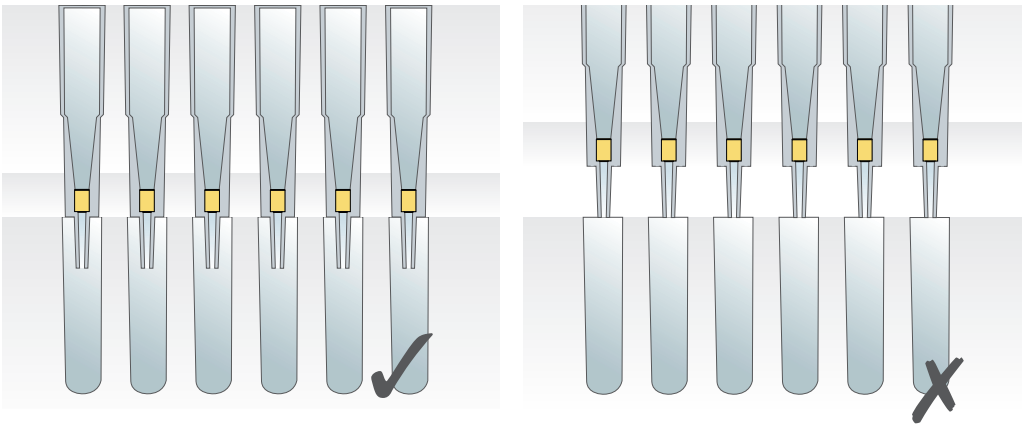


Figure 4: Correct plate height alignment

Keep your blanks blank

To ensure that the risk of cross-contamination from one well to another is minimized, the tip design of SOLA and SOLAμ plates has been carefully optimized to ensure droplet formation and extraction are precisely controlled. This minimizes the potential spray of liquids, even if high vacuum is applied to dry the sorbent bed.

Did you know SOLAμ plates have individually removable wells?
Save money and build your own bespoke method development plate – a fast option for screening different chemistries using one plate.

Method development and optimization

As with all SPE devices, the use of SOLA and SOLAμ products relies on fundamental chemistry principals for retention and elution of compounds. Generic methods can be used with SOLA and SOLAμ devices, but in order to achieve the best extraction, optimization and method development are advised. Optimized methods will always provide the best results, so where a method is already in use with a different product the starting approach should be to transfer this method to SOLA and SOLAμ for evaluation.

Find your starting point

For first time methods, or for method transfers, we recommend collecting each stage of the extraction. Analyzing these fractions provides you the information required to further optimize your method.

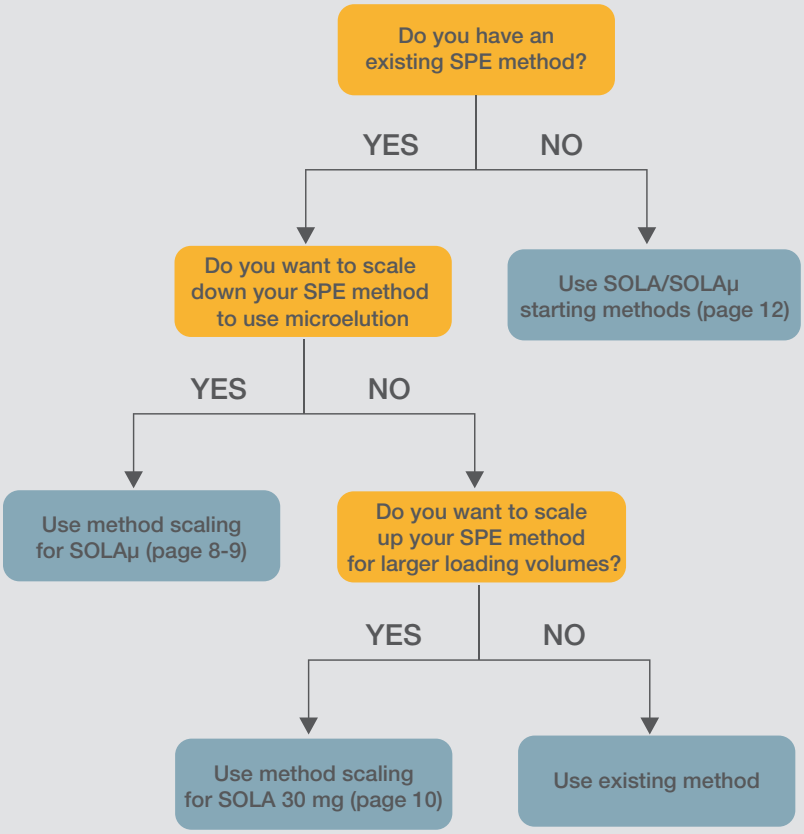


Figure 5: Decision tree for optimized method development

Method scaling for SOLAμ: processing restricted sample volumes

If a traditional SPE method is currently in use, it is often possible to scale-down the method to take advantage of SOLAμ well plate. The key to method scaling is to consider the following points:

To maintain final sample concentration, any reduction in sample load must be matched with a reduction in elution volume.

Reduction of conditioning, equilibration and wash volumes will reduce method cost and time. Direct scaling of the loading and elution volumes ensure that sample volume is reduced with no change to the concentration of the final extract.

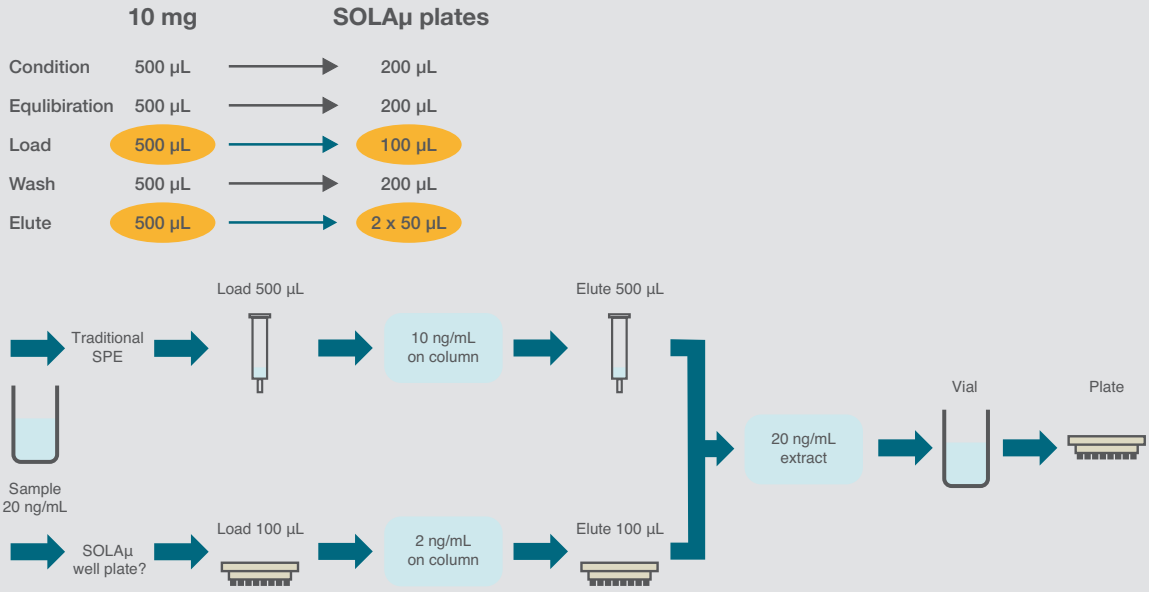


Figure 6: Method scale down to SOLAμ plate maintaining sample concentration

An example of this process can be seen below with equivalency of results obtained with niflumic acid (500 ng/mL) extracted with 10 mg SOLA WAX chemistry using 250 μL of sample and SOLAμ WAX chemistry using 25 μL of sample (Figure 7).

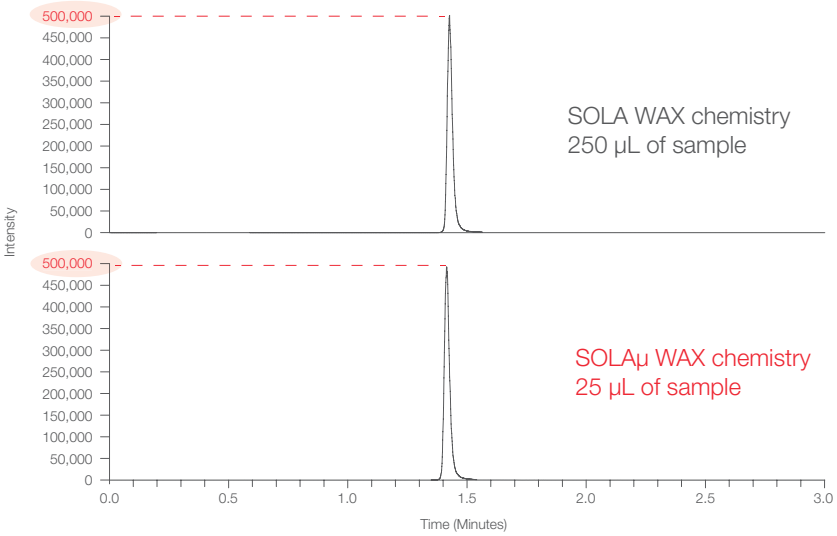


Figure 7: Equivalency of results obtained with niflumic acid (500 ng/mL) extracted with 10 mg SOLA WAX chemistry using 250 μL of sample and SOLAμ WAX chemistry using 25 μL of sample

Table 3: Precision data niflumic acid at Low QC 0.4 ng/mL and High QC 30 ng/mL (n=18)

	Analyte peak area (%RSD)	Peak area ratio (%RSD)
Low QC	7.32	0.356
High QC	5.33	0.195

Method scaling for SOLAμ: concentrating samples by using low elution volumes to reach quatitation limits

Reducing elution volume to be smaller than the sample load will provide an on-plate concentration of analyte in the final sample.

Reduction of conditioning, equilibration and wash volumes will reduce method cost and time. Maintaining sample load and reducing elution volume concentrates the analyte(s) in the final sample improving sensitivity or countering any dilution during sample pre-treatment without the need for additional drying and reconstitution.

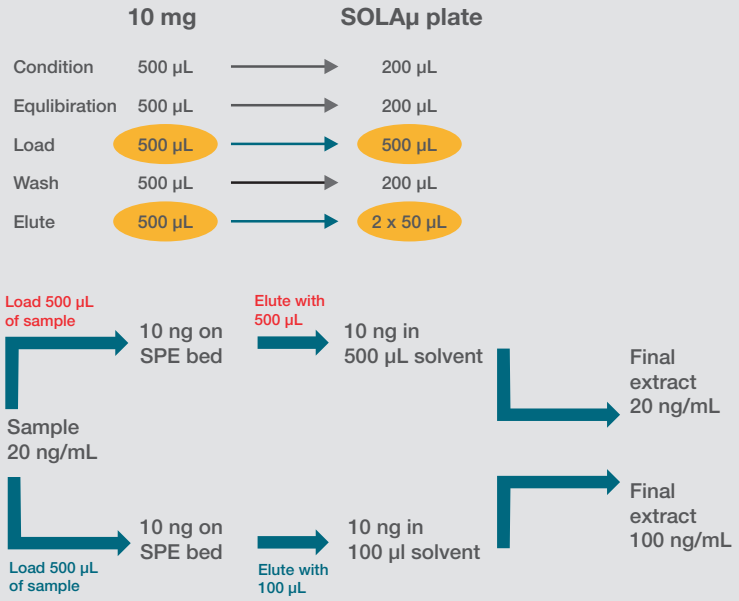


Figure 8: Method scale down to SOLAμ plate with a 5 fold pre-concentration step

In the following example 500 µL human plasma was loaded onto the SOLAμ plate for the analysis of niflumic acid. The compound was eluted in 25 µL providing a **20 times increase in concentration** whilst maintaining excellent precision (Figure 9).

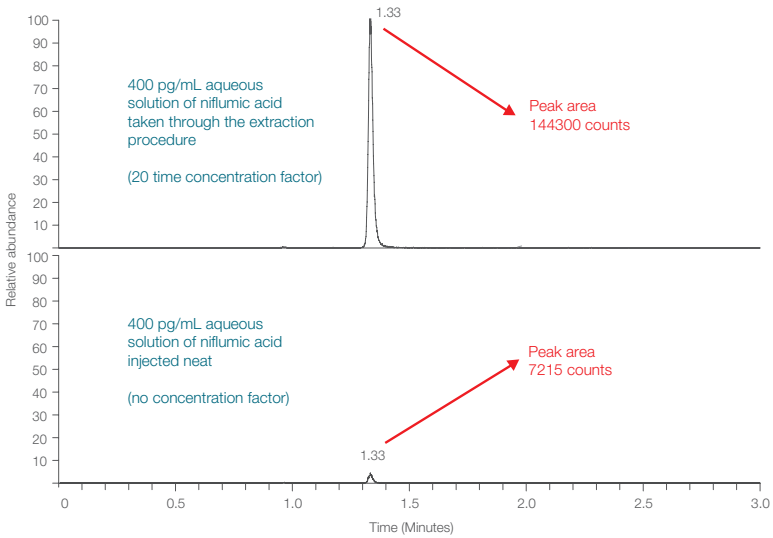


Figure 9: Sample enrichment (20 time pre-concentration)

Table 4: Precision, recovery and matrix effects data for niflumic acid at Low QC 0.4 ng/mL and High QC 30 ng/mL (n=18)

	Precision data for niflumic acid peak area ratio (%RSD) n = 18	Recovery of niflumic acid (%)	Matrix effects (%)
QC Low (0.4 ng/mL)	1.31	89.9	8.63
QC High (30 ng/mL)	1.06	94.0	3.21

Method scaling for SOLA 30 mg: concentrating large sample volumes to achieve low quantitation limits

Another simple way to increase the sensitivity of your assay from the very beginning of the workflow is to increase your sample loading volumes during SPE. Using larger sample volumes means more analyte is available to be retained by the SPE sorbent, and this in turn can boost the analyte signal response during later analysis. Boosts in signal response are particularly useful when trying to quantify low concentrations, and by combining large sample volumes with lower elution volumes, the final sample can be even more concentrated.

In order to increase loading volumes, an SPE bed weight with a high loading capacity should be used. SOLA 30 mg products are ideal for this purpose, as they have higher loading capacities than the equivalently sized silica-based products. They can be used for large sample volumes with low concentrations of analytes, as is often the case with many urine analyses. Additionally, high bed weight, polymeric SPE devices such as SOLA 30 mg are ideal compounds that are difficult to retain with smaller bed weights or when experiencing analyte breakthrough.

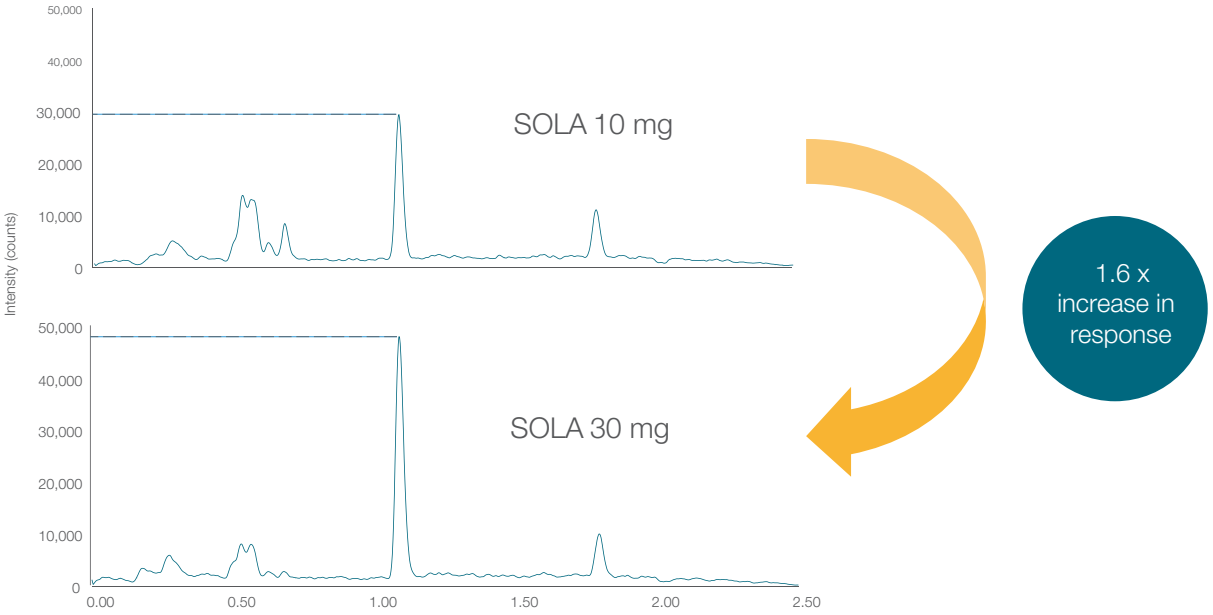


Figure 10. The high loading capacity of SOLA HRP 30 mg improves signal response when compared to smaller bed weights. The chromatograms above show an improved signal response of OH-Vit D_3 (150 ng/mL) when using SOLA HRP 30 mg to load 1 mL spiked human plasma compared to a 10 mg sorbent.

Sample pre-treatment options

It is normal protocol to perform some basic pre-treatment of your sample prior to loading onto the SPE plate. Below are some examples of cases where this is recommended.

Plasma and biological fluids

- Plasma and other biological samples may contain large clumps of material such as coagulated proteins and fatty tissue. Centrifugation of samples is recommended before loading onto the plate.
- Dilution 1:1 with an aqueous buffer is also recommended to further protect against blocked wells and to ensure the molecule and/or sorbent is in the correct charge state for interaction
- Most small molecules are protein bound to some extent. Dilution of sample 1:1 with a dilute acid buffer (0.1% formic acid or 4% phosphoric acid) releases the compound allowing interaction with the sorbent.

Urine

- Dilution 1:1 in an aqueous buffer with modified pH is recommended to ensure the molecule and/or sorbent is in the correct charge state for interaction

Tissue

- Analytes of interest need to be extracted from solid tissue prior to loading onto the SPE well. This is normally achieved through homogenising the sample, before performing a solvent extraction. If the solvent is highly organic it is recommended that the extract is diluted as much as possible with a water or an aqueous buffer prior to loading onto a reversed-phase mechanism.

Aqueous or high organic solvent samples

- Dilution 1:1 in an aqueous buffer with modified pH is recommended to ensure the molecule and/or sorbent is in the correct charge state for interaction
- Samples with a high organic solvent content should be diluted with water or an aqueous buffer prior to loading, if loading onto reverse phase mechanism

Even if you achieve 100% recovery from a generic starting point, or method transfer, it is recommended that the elution profile experiment (page 14) be performed for optimal clean-up from potential interferences

Starting points for method development

Below are a few recommended starting points and troubleshooting suggestions for SOLAμ plate extractions. They are intended as guidance for method development and should enable you to develop robust methodologies in logical steps.

Table 5: Recommended starting methods SOLAμ

	Sample pre-treatment	Dilute sample 1:1 with 0.1% formic acid (aq)	
	HRP chemistry	**SCX and WAX chemistry**	**SAX and WCX chemistry**
Equilibrate	200 µL methanol	200 µL methanol	200 µL methanol
Condition	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 2% formic acid in methanol

n = refer to elution profiles

Post extraction	Dilute extract with 50 µL of water

Table 6. Recommended starting methods for SOLA 10 mg

Stage	HRP	SCX and WAX	SAX and WCX
Equilibrate	500 µL methanol	500 µL methanol	500 µL methanol
Condition	500 µL water	500 µL water	500 µL water
Sample load*	Up to 1000 µL	Up to 1000 µL	Up to 1000 µL
Wash	500 µL n % methanol	500 µL 2% formic acid	500 µL 5% ammonia
Wash	-	500 µL methanol	500 µL methanol
Elute**	2 x 200 µL n % methanol	2 x 200 µL 5% ammonia in methanol	2 x 200 µL 2% formic acid in methanol

Table 7. Recommended starting methods for SOLA 30 mg

Stage	HRP	SCX and WAX	SAX and WCX
Equilibrate	500 µL methanol	500 µL methanol	500 µL methanol
Condition	500 µL water	500 µL water	500 µL water
Sample load*	Up to 2000 µL	Up to 2000 µL	Up to 2000 µL
Wash	1000 µL n % methanol	1000 µL 2% formic acid	1000 µL 5% ammonia
Wash	-	1000 µL methanol	1000 µL methanol
Elute**	2 x 500 µL n % methanol	2 x 500 µL 5% ammonia in methanol	2 x 500 µL 2% formic acid in methanol

* Maximum sample load will depend on dilution for pre-treatment. Total well volume allows for loading of up to 1 mL (SOLAμ) or 2 mL (SOLA) of liquid, e.g. with 1:1 dilution of sample a 1 mL total load will give a 500 µL sample load. For samples below 10 µL it is recommended to dilute the sample to make liquid handling easier.

** When you elute in a two-step process (e.g. 2 × 100 µL instead of 1 × 200 µL), a higher recovery is normally observed, particularly at lower elution volumes. This process allows more interaction time between the solvent and the sorbent.

Optimized Hydrophobic Reversed Phase (HRP)

Table 8: HRP summary information

Chemistry	Base polymer	Primary use	Secondary use	Cat. no.
HRP	Pyrrolidone graphed divinyl benzene	Neutral or hydrophobic compounds	Moderately polar compounds	60109-001 60209-001 60309-001 60409-001 60509-001

Hydrophobic Reversed Phase (HRP) has the ability to retain a mixture of hydrophobic and moderately polar compounds. For optimal retention of moderately polar compounds, it is recommended that the sample is pH adjusted to 2 pH units below the analytes pK_a for acids or 2 pH units above the analytes pK_a for bases.

For solvent and sample volumes see 'Recommended volumes' (Table 14).

It is recommended that the load, wash and elution stages all be collected during method development.
If expected recovery is not initially achieved then these can be analyzed and used to find the root cause of analyte loss, and determine which step of the method requires optimization.

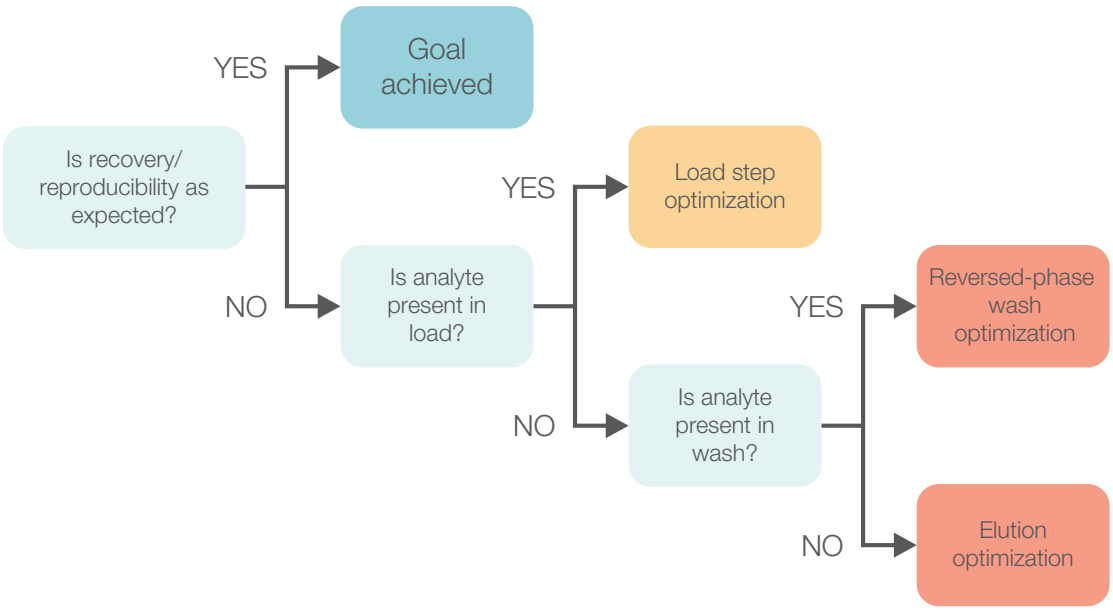


Figure 11: Decision tree for optimized HRP method

Elution profile

Elution profiling is a simple yet powerful tool for optimization of reverse phase SPE methods. By loading your sample and performing subsequent washes with incremental increases in organic strength; you can optimize the wash step to be as aggressive as possible without disrupting your analytes interaction with the stationary phase. Furthermore, you can also optimize the strength of your elution solution to fully disrupt the retention of your analyte whilst leaving other interferences retained on the sorbent. This provides optimum extract cleanliness.

Example experiment using SOLAμ
Load 100 µL of analyte in an aqueous solvent
Wash with 100 µL of 10 % methanol (aq) and collect
Wash with 100 µL of 20 % methanol (aq) and collect
Increase organic composition by increments of 10% and collect each aliquot
Wash with 100 µL of 100 % methanol (aq) and collect

Analyze each wash step for your analyte of interest. Plot analyte response against strength of organic component. Determine the optimal wash strength one increment prior to observation of analyte breakthrough. Determine the optimal elution strength one increment after all the analyte has eluted (Figure 11).

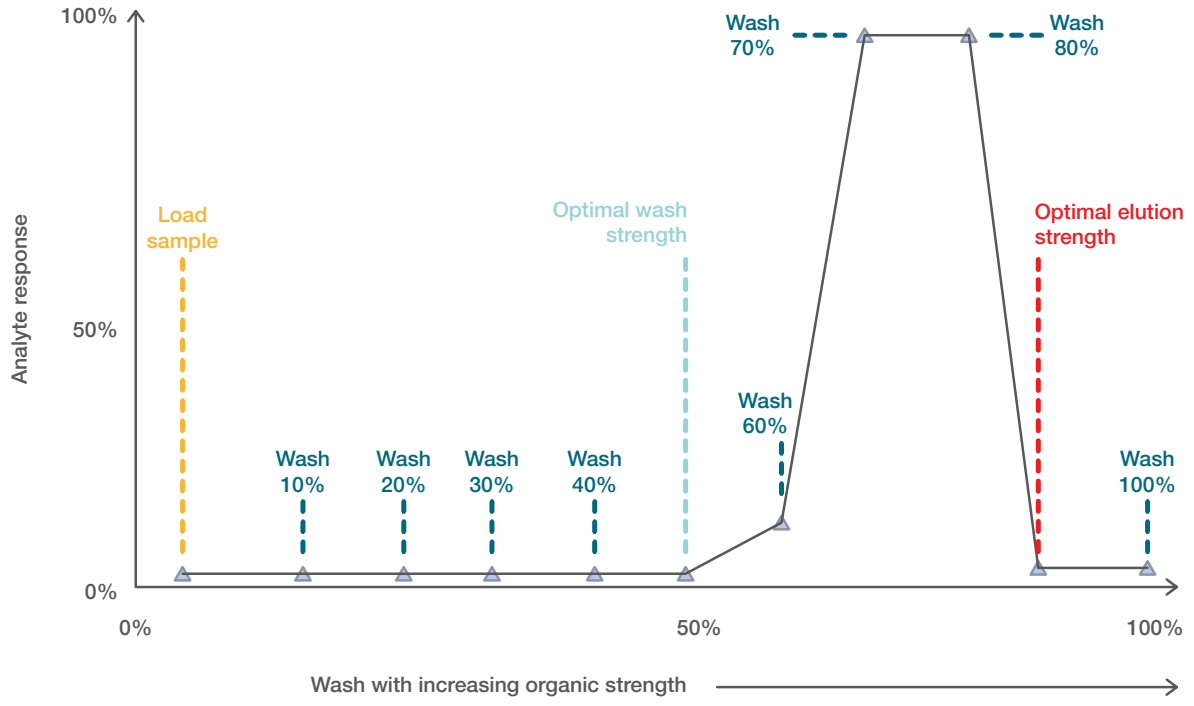


Figure 12: Example of an elution profile data set

Following this procedure, allow for the optimum wash and elution steps to be applied in subsequent analysis. This ensures maximum recovery and sample extract cleanliness.

Optimized mixed-mode phases

Mixed-mode phases offer a more targeted approach to SPE extractions by combining ion exchange and reversed-phase interactions on one sorbent bed. By taking advantage of the two retention mechanisms it is possible to utilize more aggressive washing procedures to improve matrix clean up.

Table 9: Mixed-mode summary information

Chemistry	Base polymer	Primary use	Secondary use	Cat. no.
SCX Strong Cation Exchange	Pyrrolidone graphed Divinylbenzene	Weakly basic compounds	Neutral compounds	60109-002 60209-002 60309-002 60409-002 60509-002
SAX Strong Anion Exchange	Pyrrolidone graphed Divinylbenzene	Weakly acidic compounds	Neutral compounds	60109-003 60209-003 60309-003 60409-003 60509-003
WCX Weak Cation Exchange	Pyrrolidone graphed Divinylbenzene	Strongly basic compounds	Neutral compounds	60109-004 60209-004 60309-004 60409-004 60509-004
WAX Weak Anion Exchange	Pyrrolidone graphed Divinylbenzene	Strongly acidic compounds	Neutral compounds	60109-005 60209-005 60309-005 60409-005 60509-005

It is recommended that the load, wash and elution stages all be collected during method development.
If expected recovery is not initially achieved then these can be analyzed and used to find the root cause of analyte loss, and determine which step of the method requires optimization (Table 12). Ion-exchange retention has additional factors to optimize such as pH, organic strength and counter-ion strength.

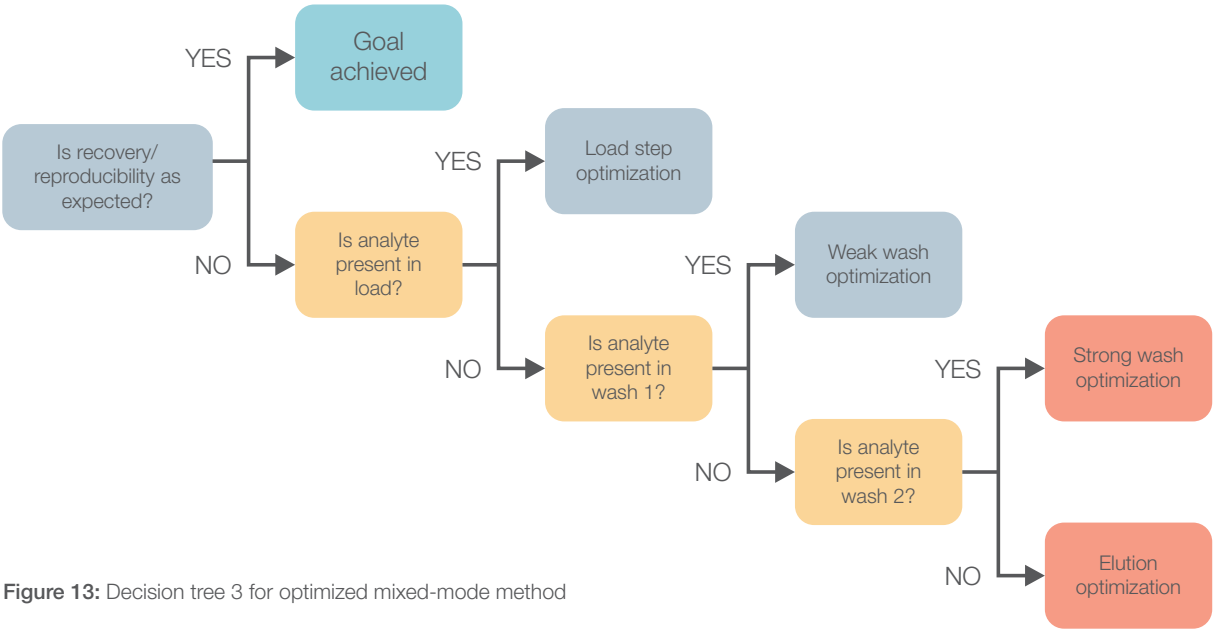


Figure 13: Decision tree 3 for optimized mixed-mode method

Optimized mixed-mode phases (continued)

Table 10: Considerations for mixed-mode SPE methods

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 effectively. Note that sample dilution with acidified water may be required to remove any protein binding to the matrix. Confirm correct phase chemistry is being used.
Analyte present in the Wash 1 step	Confirm the wash pH is adjusted to ensure the molecule or and/or sorbent is fully ionized. Confirm correct phase chemistry is being used.
Analyte present in the Wash 2 step	Confirm the wash pH **in Wash 1 step** is adjusted to ensure the molecule or 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	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.

Table 11: Troubleshooting

General troubleshooting	Solution
Slow sample flow	Due to the uniform nature of the EPT design, flow is more controlled and slower than conventional loose packed SPE products. This provides more consistent extracts. Dilute sample 1:1 with aqueous buffer. 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. For products with removable wells, ensure a full plate is used, or empty spaces are capped (p/n 300 022)

Mixed-mode chemistries can also be used in simple reversed-phase mode for the retention of your analyte, with the added benefit of having additional retention mechanisms to trap unwanted interferences.

Optimized ion-exchange methods

Table 12: Further considerations for ion-exchange methods

Conditioning and equilibration	Polymeric phases such as SOLA do not need to be 'activated' in the same way as silica-based sorbents and so conditioning with methanol is not always necessary. However, the inclusion of the step ensures that the sorbent is effectively wetted, allowing the sample to enter the other-wise hydrophobic environment for partitioning to occur. An aqueous buffer wash step is then applied to equilibrate the sorbent for sample compatibility. Higher recoveries are seen if these steps are included.
Sample load	Ideally your analyte should be in its ionized form to allow optimum interaction to the ion-exchange mechanism. Depending on the matrix to be analyzed it may not always be possible to use the optimum pH to ionize your analyte (see 'sample pre-treatment options'). If this is the case, then it is possible to initially load your analyte using, the reversed-phase mechanism. In order to do this, the organic strength of the sample must be low enough as to allow interaction with the stationary phase. If loading via ion-exchange only care should be taken to reduce the loading speed as far as possible to ensure maximum recovery.
Wash 1	This aqueous wash step is designed to ensure that the analyte retained onto the sorbent is converted into its ionized form and retain via the ion-exchange mechanism. It also ensures the weak ion-exchange sorbent is charged. The pH of this wash can be adjusted to optimize retention based on analyte pK_a (all ion-exchange modes), or pK_a of sorbent (WCX and WAX modes only).
Wash 2	Your analyte is now retained by an ion-exchange mechanism and so it is possible to wash with an aggressive, highly organic solvent that will remove many more interferences than if using a simple reverse phase method. The solvent used is normally 100% methanol or acetontitrile, although this may be pH adjusted. Loss of recovery on this step may suggest that the analyte is not retained fully by ion-exchange. This can be optimized in wash 1, or by reducing the organic content of Wash 2.
Elution	By altering the pH of your elution solvent you can switch off the ion exchange mechanism by neutralizing your analyte (or sorbent in the case of weak exchangers). High organic composition is usually required to counter-act the reversed-phase retention of the sorbent, although this can be optimized by use of elution profiles. If loss of recovery is observed at this stage then optimization of either solvent volume or switching to a solvent of higher eluotropic strength) may be required (Table 11).
Counter ions	Throughout all steps, the use of any counter-ions should be considered. Any buffer or pH modifying agent used can be optimized if recovery is low. This occurs if the counter-ion used to achieve an appropriate pH is more strongly affiliated with the sorbent than your compound of interest, and so will retain preferentially over your analyte. If this is the case, then change to a weaker counter-ion.

Table of solvents, ordered by polarity strength

If low recovery is observed optimization of the elution solvent may be required. Modifying the solvent composition in relation to the analyte properties can help to optimize recovery. For example, the more polar the compound of interest is, then more polar solvent should be used to elute for solubility purposes.

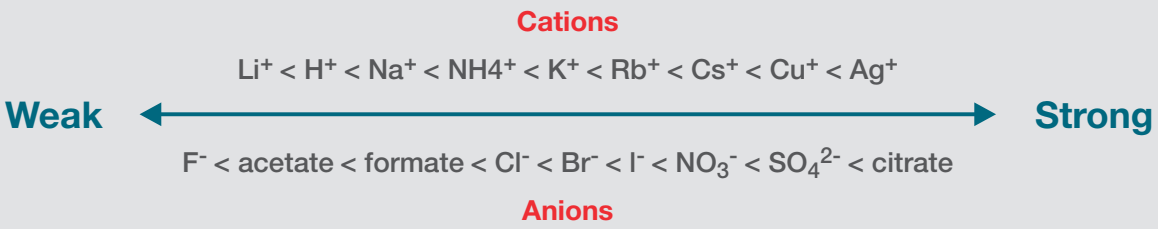
Table 13: Table of solvents, ordered by polarity

Solvent	Polarity (p')
Water	10.2
Methanol	6.6
Acetonitrile	6.2
Acetic acid, glacial	6.2
Acetone	5.4
Pyridine	5.3
Chloroform	4.4
Ethyl acetate	4.3
2-Propanol	4.3
Methylene chloride	3.4
Toluene	2.4
n-Hexane	0.06
Cyclohexane	0.0

Solvent mixtures can be used to optimize elution e.g. 50:50 MeOH:MeCN

List of counter-ion strengths

The use of counter-ions must be considered for all ion-exchange SPE. The choice and strength of counter-ion during the load step must be such as to charge the molecule and/or sorbent whilst not so strong as to preferentially bind at the expense of the compound of interest. The inverse is true for the elution step.



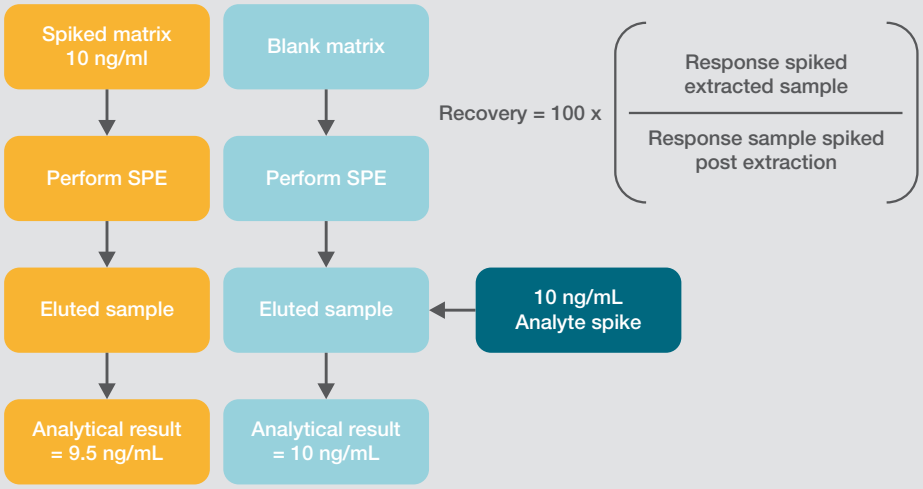
Calculation of SPE recovery and matrix effects

In order to assess extraction robustness a set of simple experiments can be used to determine SPE recovery and effects of matrix on the analysis. You will require:

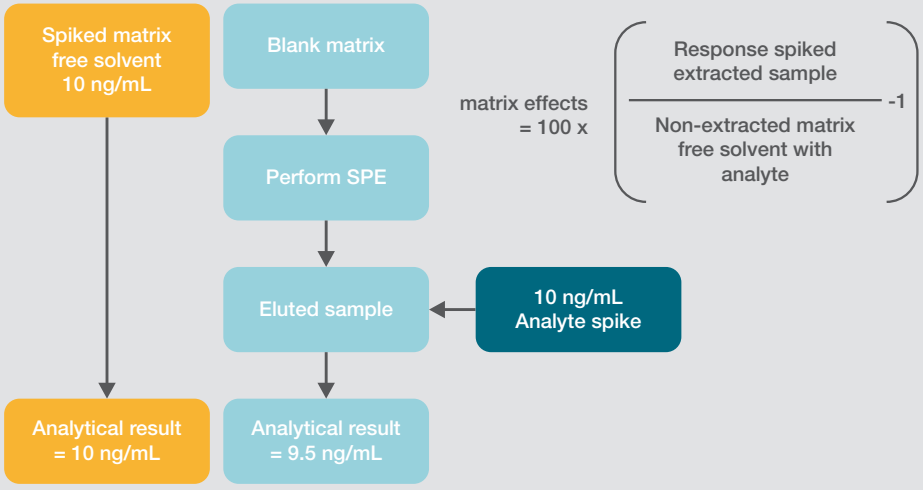
- matrix sample
- matrix blank
- aqueous sample

Extract both the matrix sample and matrix blank. Once extracted, spike the aqueous sample on-top of the matrix blank to give a concentration equal to that of the extracted sample assuming all the anlayte is recovered. This is sometimes referred to as a post-spiked sample, or an over-spiked sample.

1. By comparison of the extracted sample and the over-spiked sample you can determine the SPE recovery. The example below gives a recovery of 95%.



2. By comparison of the over-spiked sample and the aqueous sample you can determine the matrix effects of the assay. The example below gives a matrix effect of -5%.



Matrix effects can hinder assay robustness and accuracy of the final result. By ensuring extract cleanliness, this provides greater confidence in your analytical results.

thermo scientific

Cartridges ordering information

SOLA SPE product	HRP	SCX	SAX	WCX	WAX
10 mg/1 mL (100/pk)	60109-001	60109-002	60109-003	60109-004	60109-005
30 mg/3 mL (50/pk)	60409-001	60409-002	60409-003	60409-004	60409-005

96-well plates ordering information

SOLA SPE product	HRP	SCX	SAX	WCX	WAX
2 mg/1 mL (SOLAμ) (1/pk)	60209-001	60209-002	60209-003	60209-004	60209-005
10 mg/2 mL (1/pk)	60309-001	60309-002	60309-003	60309-004	60309-005
30 mg/2 mL (1/pk)	60509-001	60509-002	60509-003	60509-004	60509-005

Complementary products

Description	Cat. no.
Thermo Scientific™ HyperSep™ Vacuum Manifold	60103-351
Thermo Scientific™ HyperSep™ Glass Block Vacuum Manifold Pump, European Version	60104-241
Thermo Scientific™ HyperSep™ Glass Block Vacuum Manifold Pump, North American Version	60104-243

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