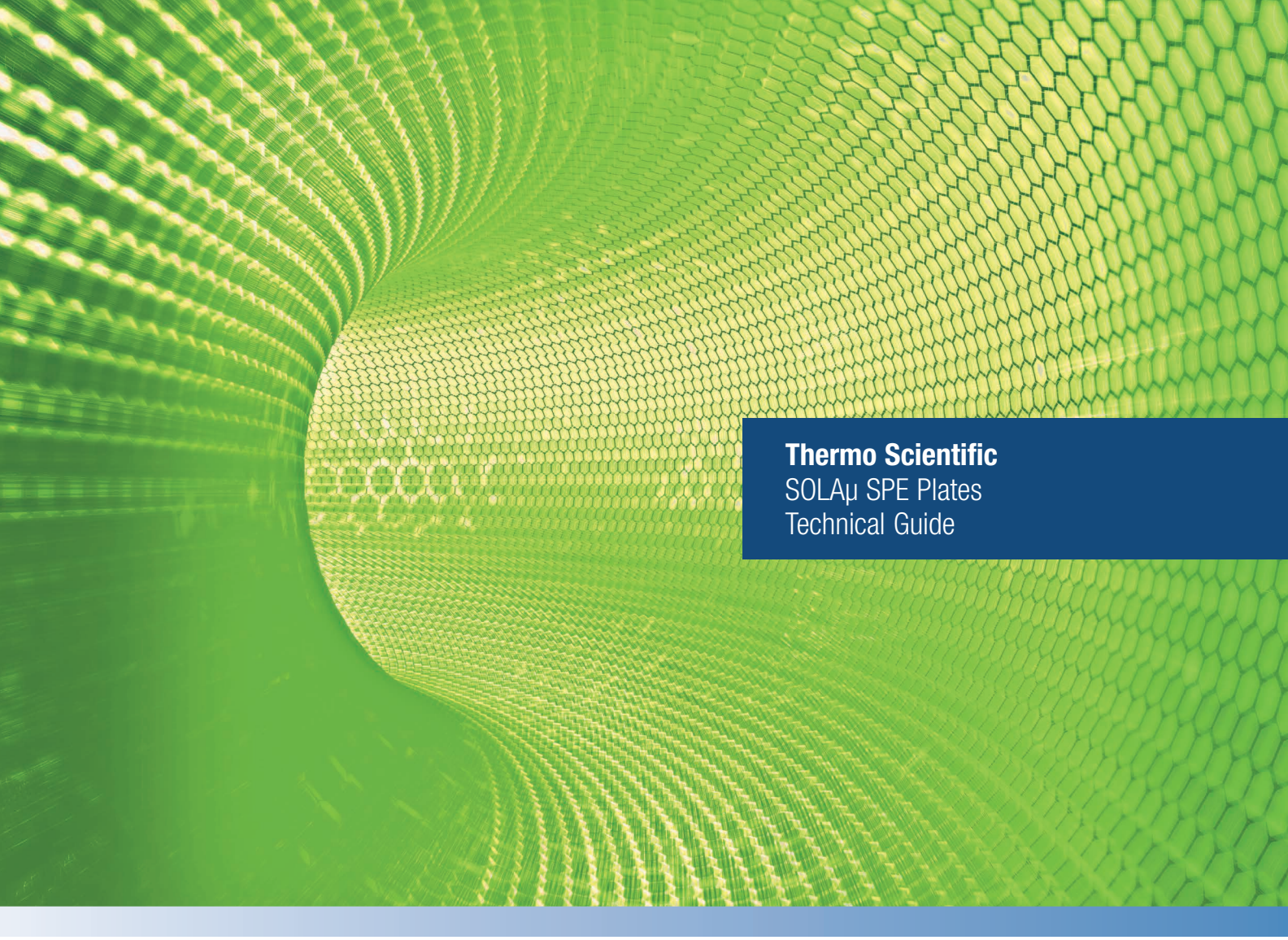


Thermo Scientific
SOLAμ SPE Plates
Technical Guide

Consistent excellence
for bioanalysis

Fisher Scientific

Thermo SCIENTIFIC

SOLAμ - delivering reproducible low volume extractions. Everytime!

Thermo Scientific™ SOLAμ™ plates are designed for bioanalytical and clinical research analyst's who require cleaner, highly reproducible and robust sample extraction at very low sample and solvent volumes in high throughput workflows. SOLAμ achieves this due to the unique and innovative frit-less SPE technology.

SOLAμ is the first micro elution product to truly meet the requirements of the bioanalyst.

Pharmaceutical and Biopharmaceutical analytical challenges

The modern bioanalytical and clinical research laboratory must provide high quality analytical results from complex biological samples in a high throughput environment while complying with strict legislation.

These demands are compounded by the continued drive to higher efficacy drugs and long acting formulations which continue to push the required quantification limits to lower levels. There is also the desire to take advantage of the replacement, refinement and reduction policy. The growth of biopharmaceuticals also brings into consideration additional analytical challenges such as solvation and non-specific binding.

What is required of the bioanalytical method to meet these demands?

- Robustness – low analytical failure rates
- Ability to process low sample volumes
- High sensitivity
- High reproducibility
- Ease of use
- High throughput processing
- Efficient and fast

The micro elution SPE format is uniquely positioned to deliver on these requirements.

The proprietary SOLA manufacturing process generates an SPE micro elution product which eliminates the issues with traditional loose-packed micro elution formats. By combining the support material and active media components into a solid uniform sorbent bed we remove the need for frits (Figure 1).

Stable and controllable flow through the SPE micro elution device is another key factor controlling reproducibility of the final analytical method. This is especially important in low bed weight devices where flow control is more difficult due to lower back pressure from the sorbent. The macro-porous structure found within SOLAμ is defined by a well controlled, reproducible manufacturing process which results in uniformity well to well, plate to plate and batch to batch. This provides an added advantage when dealing with viscous biological samples, preventing blocking and enabling high throughput processing of samples.

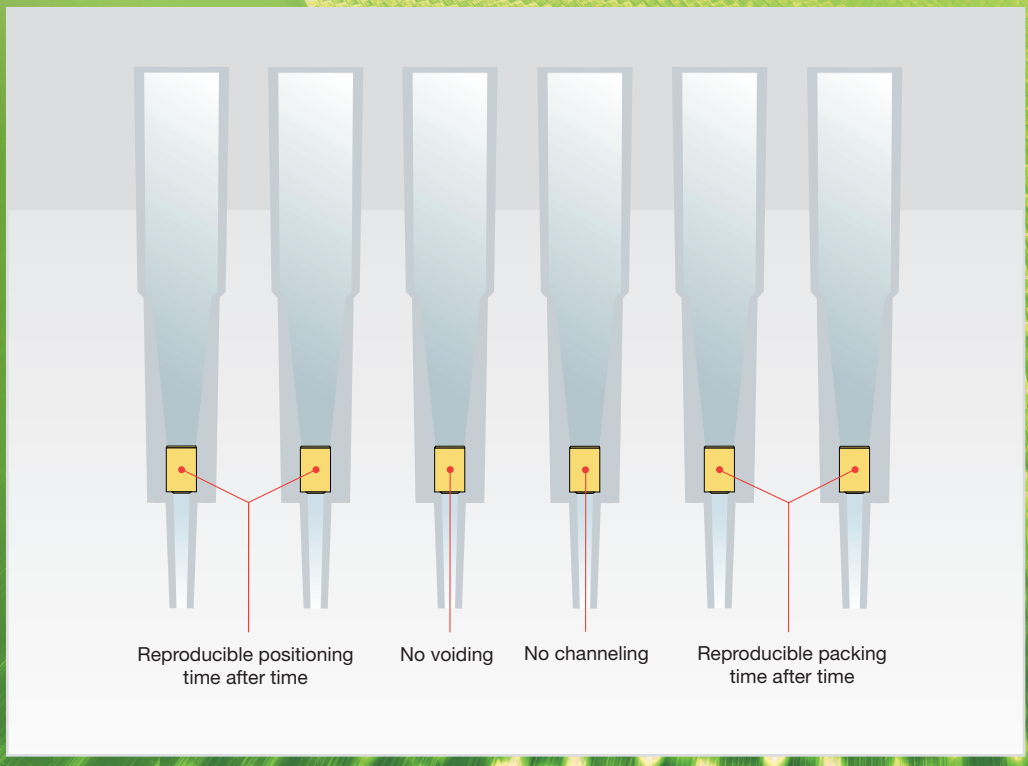


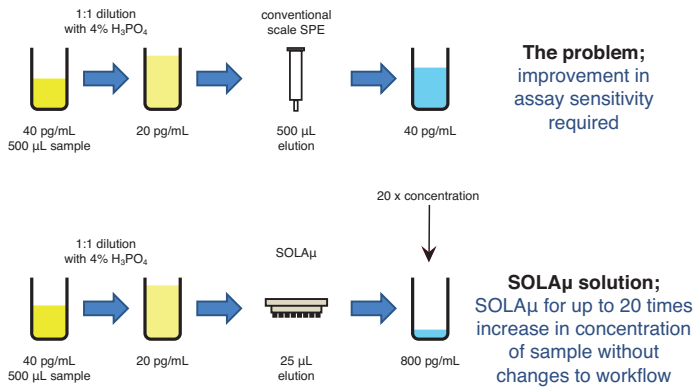
Figure 1: SOLAμ SPE design – limiting issues associated with conventional SPE formats

SOLAμ provides you with reproducible sensitivity

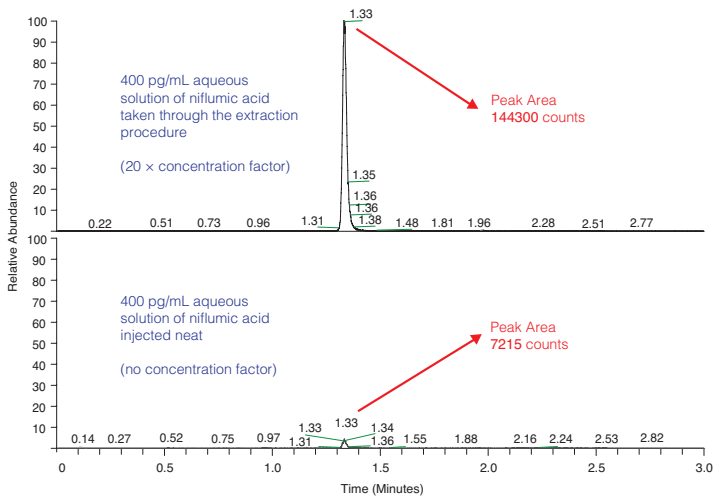
Concentration of a large sample volume to achieve quantitation limits

Up to 20 times increase in sensitivity can be achieved by loading a large volume of sample and eluting in a low volume.

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.



Sample enrichment (20 x pre-concentration)



Sample preparation protocol

Sample pre-treatment

500 µL of human plasma diluted 1:1 with 4% phosphoric acid

Sample preparation

Compound(s):	niflumic acid, niflumic acid d5 (IS)
Matrix:	human plasma
	SOLAμ WAX 96 well plate (60209-005)
Condition:	200 µL methanol
Equilibrate:	200 µL 4% phosphoric acid
Load:	apply sample at 0.5 mL/min
Wash:	200 µL 25 mM ammonium acetate (pH4)
	200 µL 70% methanol (pH4)
Elute:	2 × 12.5 µL 50/50 methanol/acetonitrile with 2% ammonia

Direct injection of eluent

HPLC system:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column:	Thermo Scientific™ Accucore™ RP-MS HPLC column 50 mm × 2.1 mm 2.6 µm (17626-052130)
Guard column:	Thermo Scientific™ Accucore™ RP-MS Defender™ guard cartridge (17626-012105) Thermo Scientific™ Uniguard™ drop-in guard holder (852-00)
Mass spec system:	Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

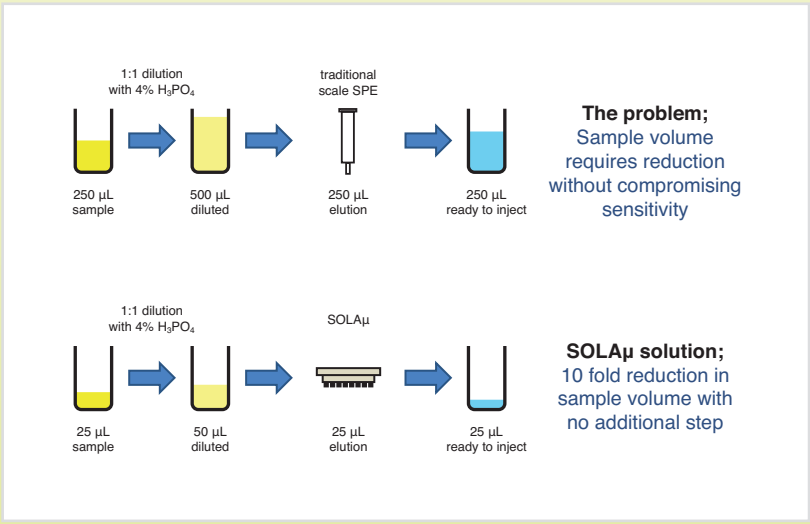
	Precision Data for Niflumic Acid Peak Area Ratio (%RSD) n = 18	Recovery of Niflumic Acid (%)	Matrix Effects (%)
QC Low (0.4ng/mL)	1.31	89.9	8.63
QC High (30ng/mL)	1.06	94.0	3.21

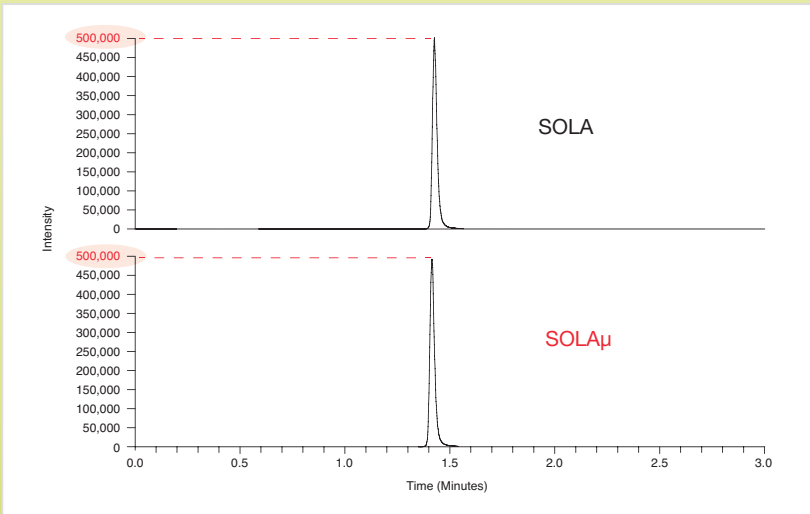
Precision, recovery and matrix effects data for niflumic acid at Low QC 0.4ng/mL and High QC 30ng/mL (n=18)

Sample limited assays or scaling down a conventional SPE method and obtaining equivalent sensitivity

SOLAμ allows users to directly scale down the volumes used in their analytical methods, allowing for a reduction in sample usage and eliminating issues caused by evaporation without compromising the sensitivity of their assay. This is also an important consideration when sample volumes are limited.

The following example shows that by loading 25 µL of niflumic acid sample onto the SOLAμ plate and eluting in a total of 25 µL a ten-fold decrease in sample volume was achieved when compared to a traditional scale higher bed weight product. Equivalent method performance and high levels of reproducibility provided by SOLA technology were still maintained.





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

Sample preparation protocol

Sample pre-treatment

Human plasma diluted 1:1 with 4% phosphoric acid

Sample preparation

Compound(s):	niflumic acid, niflumic acid d5 (IS)
Matrix:	human plasma
	SOLAμ WAX 96 well plate (60209-005)
Condition:	200 µL methanol
Equilibrate:	200 µL water
Load:	apply 25 µL sample at 0.5 mL/min
Wash:	200 µL 25 mM ammonium acetate (pH4)
	200 µL methanol
Elute:	2 × 12.5 µL methanol with 2% ammonia

Direct injection of eluent

HPLC system:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column:	Thermo Scientific™ Accucore™ RP-MS HPLC column 50 mm × 2.1 mm 2.6 µm (17626-052130)
Guard column:	Thermo Scientific™ Accucore™ RP-MS Defender™ guard cartridge (17626-012105) Thermo Scientific™ Uniguard™ drop-in guard holder (852-00)
Mass spec system:	Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

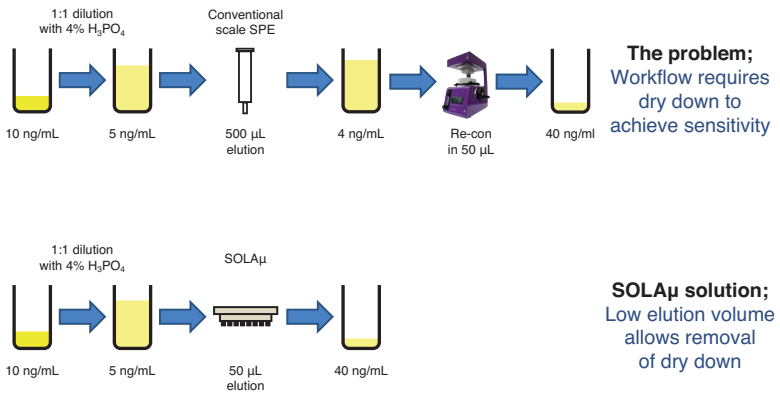
Precision Data for Niflumic Acid		
	Analyte Peak Area (%RSD)	Peak Area Ratio (%RSD)
Low QC	7.32	0.356
High QC	5.33	0.195

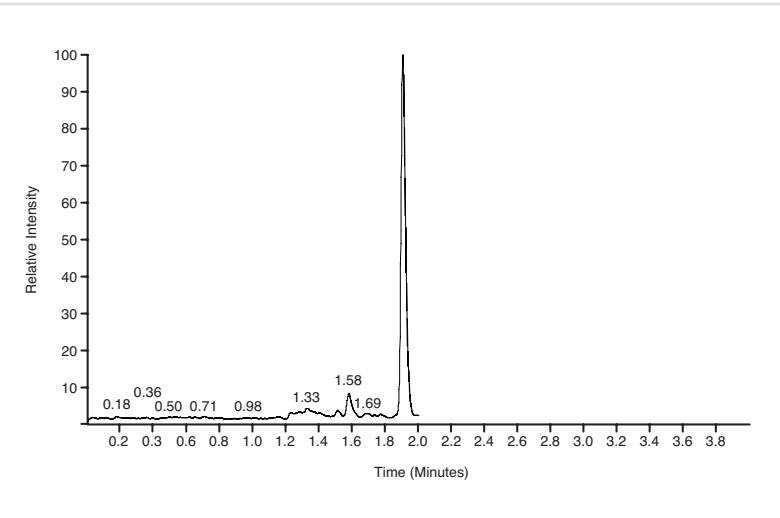
Precision data niflumic acid at Low QC 0.4ng/mL and High QC 30ng/mL (n=18)

Extracting samples which are susceptible to solvation and non-specific binding issues

With traditional SPE the eluted sample is typically blown down to increase the concentration of the sample and thus improve the sensitivity. This causes an issue for certain compound types which can be lost during this step resulting in reduced sensitivity. SOLAμ allows the sample to be extracted without the need for dry down and reconstitution. Not only does this maximize recovery of the analytes it also improves workflow efficiency and increases productivity.

In the case of extraction of ibuprofen a four-fold pre-concentration was achieved without the need for dry down by loading 200 µL of sample onto the SOLAμ plate and eluting in a total of 50 µL. The results demonstrate that even with this low elution volume, excellent reproducibility was achieved.





Example chromatogram at quantitation limit of 10 ng/mL for ibuprofen

Sample preparation protocol

Sample pre-treatment	
200 µL of rat plasma diluted 1:1 with 4% phosphoric acid	
Sample preparation	
Compound(s):	ibuprofen, ibuprofen d3 (IS),
Matrix:	rat plasma
	SOLAμ SAX 1 mL 96 well plate (60109-002)
Conditioning:	200 µL methanol
	200 µL water
Application:	load sample at 0.5 mL/min
Washing:	200 µL water with 1% NH4
	200 µL methanol with 1% NH4
Elution:	2 × 25 µL 50/50 methanol/acetonitrile with 2% formic acid
Dilution:	add 50 µL water to each sample
Direct injection of eluent	
HPLC system:	Thermo Scientific™ Dionex™ Ultimate™ 3000 RS system
Column:	Accucore RP-MS 50 mm × 2.1mm 2.6 µm (17626-052130)
Guard column:	Accucore RP-MS defender (17626-012105) Uniguard drop-in guard holder (852-00)
Mass spec system:	Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

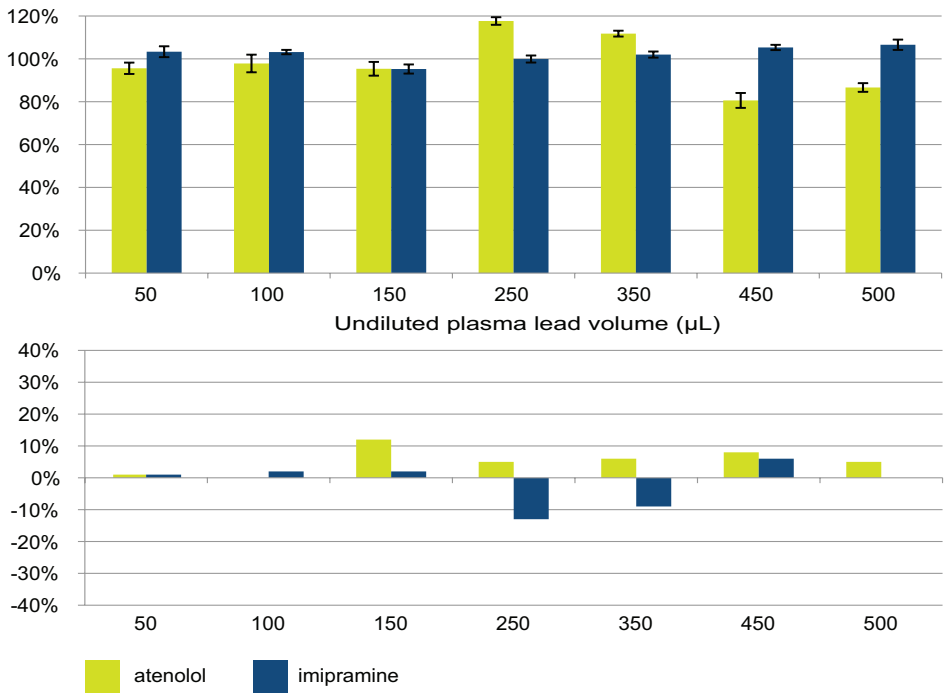
	Ibuprofen (%RSD) n=18	Ibuprofen recovery (%)
Low QC (25 ng/mL)	4.00	90
High QC (750 ng/mL)	1.70	95

Precision data for ibuprofen at Low QC 25 ng/mL and High QC 750 ng/mL (n=18)

SOLAμ loading capacity

The utilization of our advanced polymeric technologies in SOLAμ provides an SPE phase with excellent loading capacity. This ensures that good retention of analyte and removal of matrix interferences is achieved when a larger range of sample volumes are applied.

In the following example incremental volumes of human plasma spiked at 200 ng/mL with a polar (atenolol) and non polar (imipramine) analyte were extracted. Recovery and matrix effects were monitored across the loading range to demonstrate acceptable assay performance.



Loading capacity: recovery and reproducibility

Matrix effects

Conclusion

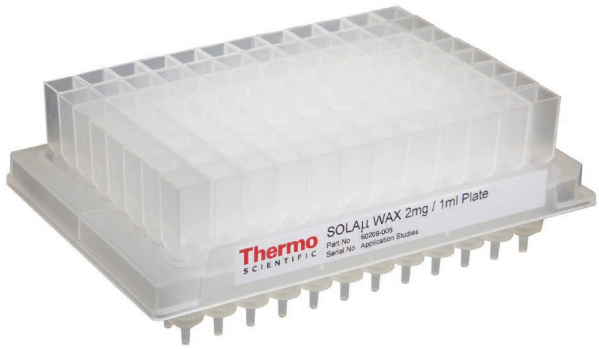
SOLAμ meets bioanalytical needs by providing:

- A robust low sample volume preparation platform
- Reproducibility at low sample and solvent levels
- Processing of low volume samples
- Sample enrichment (20 times)
- Mitigates against solvation and non-specific binding issues

*NB to view the complete application note please visit www.thermoscientific.com/sola-spe

Ordering information

Description	Part Number
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**



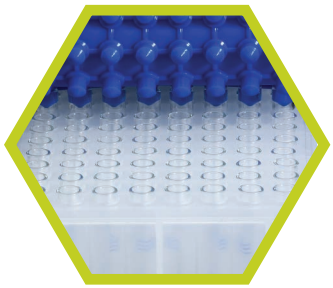
Complimentary products

Description	Part Number
HyperSep-96 Well Plate Manifold	**60103-351**
Vacuum Pump, European Version	**60104-241**
Vacuum Pump, North American Version	**60104-243**

A comprehensive product offering for your complete bioanalytical workflow

Sample Preparation

Sample Handling



LC Columns and Accessories

Separation

Analysis

Data Processing

Fisher Scientific

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