APPLICATION NOTE No. 20942

SOLAμ SPE – achieve highly reproducible bioanalytical results with reduced sample volumes

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Keywords: Micro elution, reproducibility, matrix effects, SPE, no dry down, niflumic acid, reduced sample volume

Goal

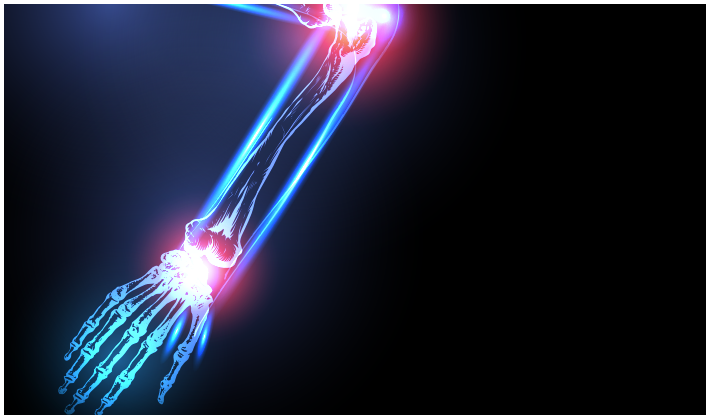
This application note demonstrates how the Thermo Scientific™ SOLAμ™ Solid Phase Extraction (SPE) product can be used to facilitate the scale down of an extraction method for use when sample volume is limited. The use of a Thermo Scientific™ Accucore™ HPLC column provided fast and efficient separation without the need for an ultra high pressure system. MS/MS detection was performed on a Thermo Scientific™ TSQ Vantage™ mass spectrometer.

Introduction

Ethical, analytical and sample availability considerations are a challenge faced by many bioanalytical laboratories and have resulted in a drive to limit sample volume.

In order to achieve the required detection limits many analytical methods utilize dry down and reconstitution steps to remove the dilution effects required by traditional scale SPE when operating with very low sample volumes.

In addition many analytes, such as small volatile molecules or larger biomolecules, suffer from loss of recovery attributed to the evaporation and reconstitution step.



SOLAμ products allow 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.

SOLAμ products provide reproducibility, robustness and ease of use at low elution volumes by utilizing the revolutionary Thermo Scientific™ SOLAμ™ Solid Phase Extraction (SPE) technology. This removes the need for frits delivering a robust, reproducible format which ensures highly consistent results at low elution volumes.

SOLAμ products deliver:

- lower sample failures due to high reproducibility at low elution volumes
- increased sensitivity due to lower elution volumes
- the ability to process samples which are limited in volume
- improved stability of bio-molecules by reduction of adsorption and solvation issues

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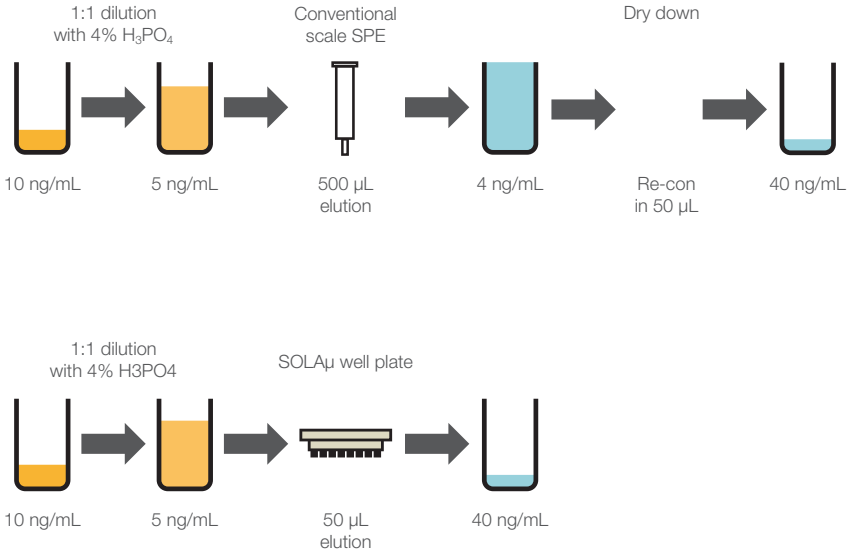

Figure 1: Summary of workflow required to achieve a ten-fold decrease in sample volume without altering the work-flow or compromising the final extracted analyte concentration.

Experimental details

Consumables		Cat. no.
Fisher Scientific™ LC-MS grade water (ACN)		10777404
Fisher Scientific™ LC-MS grade methanol (MeOH)		10653963
Fisher Scientific™ analytical grade formic acid (HCOOH)		10559570
Sample handling equipment		**Cat. no.**
Liquid handling hardware		-
SPE hardware	Thermo Scientific™ HyperSep™ 96 well vacuum manifold	60103-351
	Thermo Scientific™ HyperSep™ glass block vacuum manifold pump, European version	60104-241
Sample handling consumables	Thermo Scientific™ Webseal™ 96-well square well microplate	60180-P212
	Thermo Scientific™ WebSeal™ mat	60180-M122
Sample pre-treatment		
	25 µL of human plasma diluted 1:1 with 4% phosphoric acid	
Sample preparation		
Compound(s)	Niflumic acid, niflumic acid d5 (IS)	-
Matrix	Human plasma	-
	Thermo Scientific™ SOLAµ™ WAX 96 well plate , 2 mg/1 mL	60209-005
Condition	200 µL methanol	-
Equilibrate	200 µL water	-
Load	Apply 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		

Separation conditions		Cat. no.
Instrumentation	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
Flow rate	750 µL/min	-
Run time	4 min	-
Column temperature	30 °C	-
Injection details	2 µL full loop injection	-
Injection wash solvent 1	Water	-
Injection wash solvent 2	45:45:10 (v/v/v) propan-2-ol/acetonitrile/acetone (with 5% Ammonia)	-
Mobile phase A	Water with 0.1% formic acid	-
Mobile phase B	Acetonitrile with 0.1% formic acid	-

Gradient conditions		
Time (min)	**%A**	**%B**
0.02	70	30
2.0	10	90
2.01	70	30
3	70	30
MS conditions		
Instrumentation	Thermo Scientific™ TSQ Vantage™ triple stage quadruple mass spec	
Ionization conditions	HESI	
Polarity	+ive	
Spray voltage (V)	3000	
Vaporiser temperature (°C)	475	
Sheath gas pressure (Arb)	50	
Aux gas pressure (Arb)	60	
Capillary temp (°C)	300	
Collision pressure (mTorr)	1.5	
Scan time (s)	0.02	
Q1 (FWHM)	0.7	
Q3 (FWHM)	0.7	

Compound	Parent *(m/z)*	S-Lens (V)	Product *(m/z)*	Collision energy (V)
Niflumic acid	283.0	115	265.0	22
Niflumic acid d5 (IS)	288.8	115	271.1	22

Data processing	
Software	Thermo Scientific™ LCQUAN™ quantitative software, version 2.6

Results

By loading 25 µL of 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. The results demonstrate that even with this low elution volume equivalent method performance was achieved.

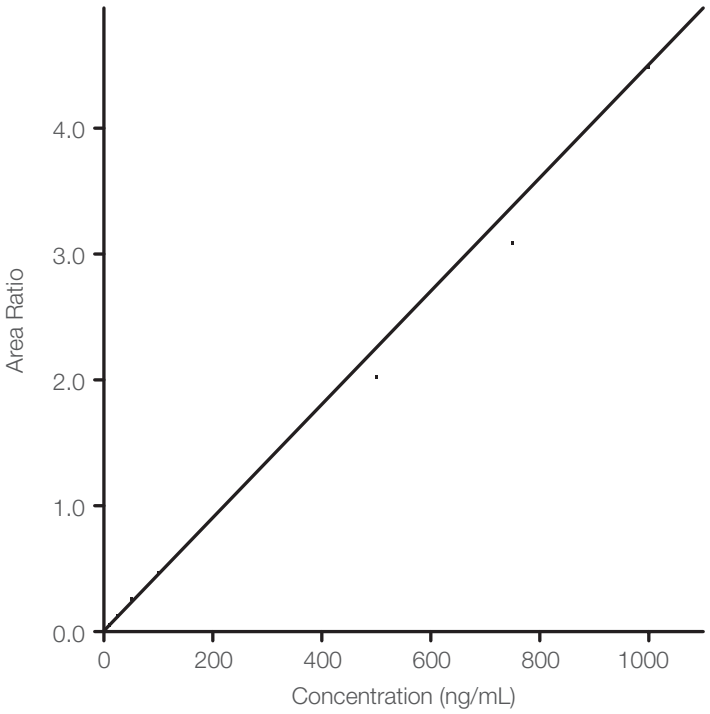

Figure 2: Niflumic acid linearity over the dynamic range 1-1000 ng/mL.

Standard	Specified Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (% difference)	Precision (%RSD n=18)
S1	1.00	0.995	-0.450	-
S2	10.0	10.0	-0.0200	-
S3	25.0	26.4	5.69	-
S4	50.0	55.9	11.9	-
S5	100	102	2.22	-
S6	500	448	-10.4	-
S7	750	685	-8.63	-
S8	1000	997	-0.330	-
QC L	10.0	9.73	2.66	0.355
QC M	500	440	11.9	0.142
QC H	750	671	10.5	0.195

Table 1: Niflumic acid accuracy data for the calibration range 1 to 1000 ng/mL.

The assay gave a linear dynamic range from 1 to 1000 ng/mL with an R^2 coefficient of 0.995 (Figure 2, Table 1). The chromatography for the limit of quantitation sample at 1 ng/mL is significantly above the acceptable signal to noise limit (Figure 3).

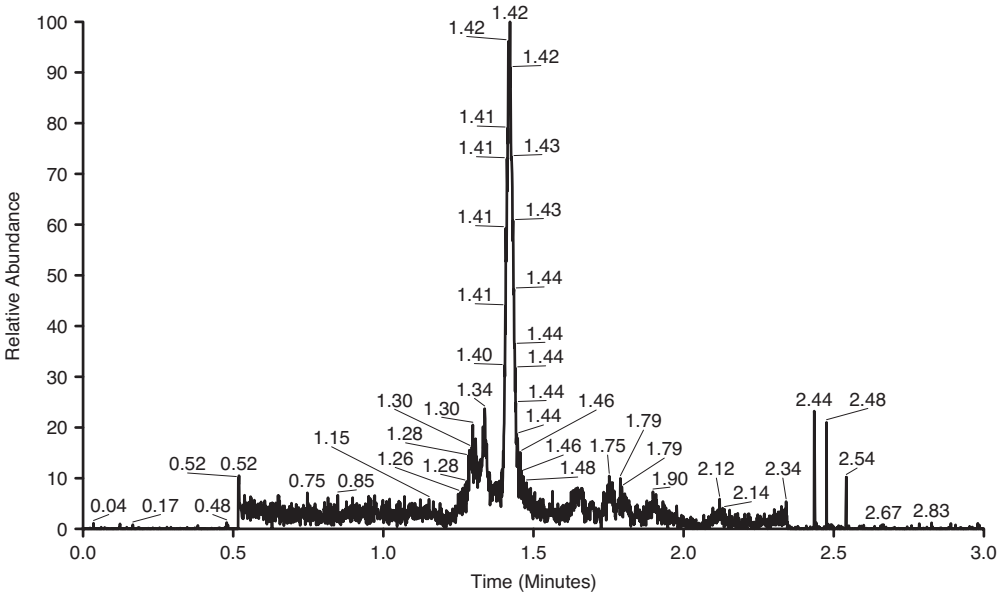

Figure 3: Example chromatogram 1 ng/mL niflumic acid.

Low, mid and high QC samples were prepared at concentrations of 10, 500 and 750 ng/mL respectively. Table 1 shows good level of accuracy at all QC levels. Table 2 shows reproducibility data for replicate extractions (n=18) at both high and low QC levels. Relative standard deviation for niflumic acid was less than 8%.

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

Table 2: Precision data niflumic acid at low QC 10 ng/mL and high QC 750 ng/mL (n=18).

	Recovery of niflumic acid (%)
Low QC	101
High QC	98.5

Table 3: Percentage recovery for niflumic acid at low QC 10 ng/mL and high QC 750 ng/mL.

	Matrix effects (%)
Low QC	1.70
High QC	4.78

Table 4: Percentage matrix effects for niflumic acid at low QC 10 ng/mL and high QC 750 ng/mL.

Analyte recovery was shown to be greater than 98% by comparison to post extraction fortified blank samples (refer to Table 3). Post extraction fortified blank samples were also compared against pure reference standards to demonstrate matrix effects which were calculated at less than 5% at both high and Low QC levels (refer to Table 4).

A mid QC sample (500 ng/mL) was extracted using SOLA WAX well plate and SOLAμ WAX well plate. Sample volume was reduced ten fold from 250 µL to 25 µL respectively as outlined in Figure 1. A high level of comparison is shown in Figure 4 between the extracts, demonstrating the ability to directly scale volumes with no adverse effect on assay performance.

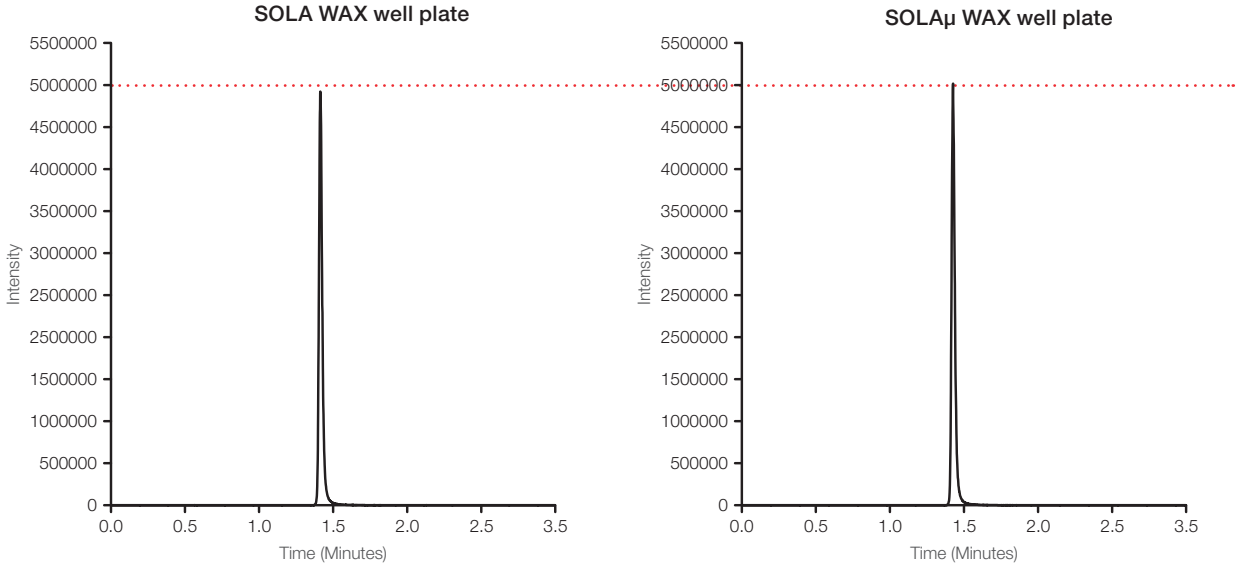

Figure 4: Comparison of niflumic acid extracted with 10 mg SOLA WAX well plate using 250 µL of sample and from SOLAμ WAX using 25 µL of sample. Both methods show equivalent assay performance.

Conclusion

This application note demonstrates the advantages of SOLAμ products for the reduction of sample volume compared to traditional scale SPE while maintaining high levels of precision, accuracy, recovery and sample cleanliness.

By eluting in less solvent it is possible to achieve equivalent limits of detection requiring only a fraction of the sample volume and without relying on lengthy evaporation procedures that may compromise sample integrity. This is particularly important for sample limited applications.

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