APPLICATION NOTE No. 20941

SOLAμ for pre-analysis sample concentration

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Goal

This application note demonstrates the use of Thermo Scientific™ SOLAμ™ Solid Phase Extraction (SPE) product to enhance sample pre-concentration prior to analysis. Additional benefits include reduced workflow and stability for analytes susceptible to loss or degradation during evaporation and reconstitution. 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

Despite advances in analytical detection technology, achieving required limits of sensitivity can still be an issue for many bioanalytical laboratories. In order to improve limits of detection analysts are looking to sample preparation in order to pre-concentrate their sample prior to analysis.

Traditional scale SPE helps to clean up the sample to minimize matrix effects, however in order to pre concentrate the sample a lengthy dry down and reconstitution step needs to be employed. This process is not only time consuming but can have a detrimental effect on the recovery of the analyte due to volatility or non specific binding.

Thermo Scientific SOLAμ products allow users to pre-concentrate the sample up to 20 times prior to injection, allowing greater limits of sensitivity to be achieved whilst maintaining a high level of analyte recovery, accuracy and precision.

SOLAμ products provide reproducibility, robustness and ease of use at low elution volumes by utilizing the revolutionary Thermo Scientific™ SOLA™ 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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Problem:
Improvement in assay sensitivity required

Option 1:
Introduce additional steps with associated issues

Option 2:
SOLAμ well plate provides twenty fold increase in sample concentration without changes to workflow

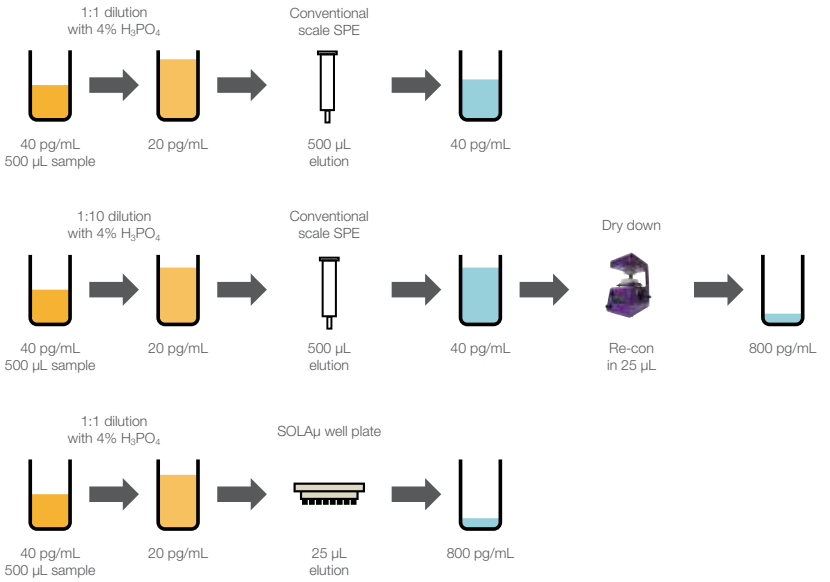

Figure 1: Summary of workflow required to achieve a twenty-fold increase in sample concentration prior to analysis without altering the workflow or compromising assay performance

Experimental details

Consumables		Cat. no.
Fisher Scientific™ LC-MS grade water		10777404
Fisher Scientific™ LC-MS grade methanol		10653963
Fisher Scientific™ analytical grade formic acid		10559570
Sample handling equipment		**Cat. no.**
Liquid handling hardware		-
SPE hardware	Thermo Scientific™ HyperSep™ 96 well plate vacuum manifold	60103-351
	Thermo Scientific™ HyperSep™ glass block vacuum manifold pump, European version	60104-241
Sample handling	Thermo Scientific™ Webseal™ 96-well square well microplate	60180-P212
	Thermo Scientific™ WebSeal™ mat	60180-M122
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, 2 mg/1 mL	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		

Separation conditions		
Instrumentation	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system	-
Column	Thermo Scientific™ Accucore™ RP-MS HPLC column 50 mm x 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	3 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**
0.0	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 500 µL of sample onto the SOLAµ plate and eluting in a total of 25 µL a twenty-fold concentration of the analyte was achieved. The results demonstrate that even with low elution volume, high levels of accuracy, precision, recovery and sample cleanliness were achieved.

The assay gave a linear dynamic range from 40 to 40000 pg/mL with an r2 coefficient of 0.998 (Figure 2, Table 1). The chromatography for the limit of quantitation sample at 40 pg/mL is significantly above the acceptable signal to noise limit (Figure 3).

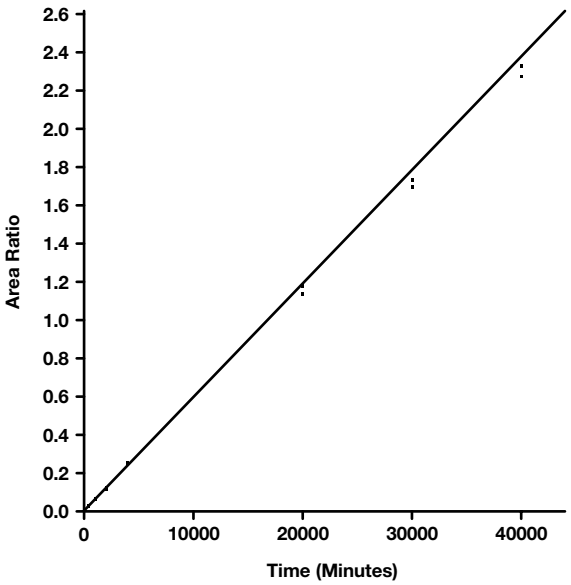

Figure 2: Niflumic acid linearity over the dynamic range 40-40000 pg/mL

Standard	Specified Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (% difference)	Precision (%RSD n=18)
S1	40.0	39.7	-0.775	-
S2	400	429	7.37	-
S3	1000	1006	0.592	-
S4	2000	2000	0.00	-
S5	4000	4112	2.81	-
S6	20000	19445	-2.78	-
S7	30000	28809	-3.97	-
S8	40000	38702	-3.25	-
QC L	400	420	5.00	1.31
QC M	20000	19200	4.00	0.77
QC H	30000	28800	4.00	1.06

Table 1: niflumic acid accuracy data for the calibration range 40 to 40000 pg/mL

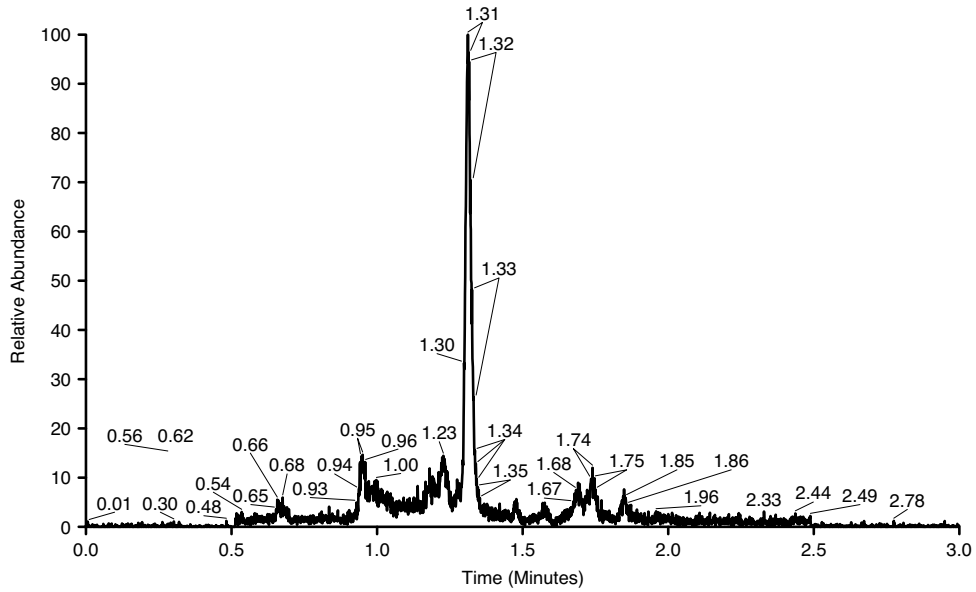

Figure 3: Example chromatogram 40 pg/mL niflumic acid

Low, mid and high QC samples were prepared at concentrations of 400, 20000 and 30000 pg/mL respectively. Table 1 shows a 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.

	Precision data for niflumic acid peak area ratio (%RSD)
Low QC	1.31
High QC	1.06

Table 2: Precision data niflumic acid at Low QC 40 pg/mL and High QC 30000 pg/mL (n=18)

	Recovery of niflumic acid (%)
Low QC	89.9
High QC	94.0

Table 3: Percentage recovery for niflumic acid at Low QC 40 pg/mL and High QC 30000 pg/mL

	Matrix effects (%)
Low QC	8.63
High QC	3.21

Table 4: Percentage matrix effects for niflumic acid at Low QC 40 pg/mL and High QC 30000 pg/mL

Analyte recovery was shown to be greater than 89.9% 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 9% at both high and Low QC levels (refer to Table 4).

Conclusion

This application note demonstrates the advantages of SOLAμ for sample concentration prior to analysis while maintaining high levels of precision, accuracy, recovery and sample cleanliness.

By loading a sample volume of 500 µL and eluting in a volume of 25 µL it is possible to decrease the lower limit of quantitation by a factor of twenty without the need for lengthy evaporation procedures that may compromise analytical results.

SOLAμ products provide users with the ability to:

- achieve a high level of confidence in analytical results at low elution volumes due to high reproducibility at low elution volumes
- increase sensitivity by increasing sample loading and reducing elution volumes
- improve productivity by removing requirement for lengthy evaporation and reconstitution