APPLICATION NOTE No. 20943

Achieve higher bioanalytical sensitivity with SOLAμ SPE for analytes susceptible to issues during pre-concentration dry down

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Keywords: SOLAμ, micro elution, reproducibility, matrix effects, SPE, no dry down

Goal

This application note demonstrates the use of Thermo Scientific™ SOLAμ™ Solid Phase Extraction (SPE) product for the extraction analytes which are 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

In order to achieve the required detection limits many bioanalytical methods utilize dry down and reconstitution steps to concentrate analytes prior to analysis. With conventional SPE formats the elution volume is often high and the final extract is diluted. This is a problem for assays requiring a challenging lower limit of detection and is especially prevalent for newer high efficacy compounds. Existing methodology will overcome this problem by evaporating the extract and reconstituting in a smaller volume (Figure 1).

In addition, for many analytes the process of drying and re-constituting extracts can prove to be problematic due to compound loss. Small analytes such as ibuprofen are volatile and evaporation stages result in losses of these analytes.[1] In many cases peptides and other biomolecules may undergo non specific binding with collection vessel surfaces which is often exacerbated by drying stages resulting in irreproducible data and poor sensitivity.[2]

SOLAμ products allow users to pre-concentrate the extract directly on the plate even with low sample volumes removing the need for evaporation steps and therefore the associated problems (Figure 1).

SOLAμ products provide provides reproducibility, robustness and ease of use at low elution volumes by utilizing the revolutionary 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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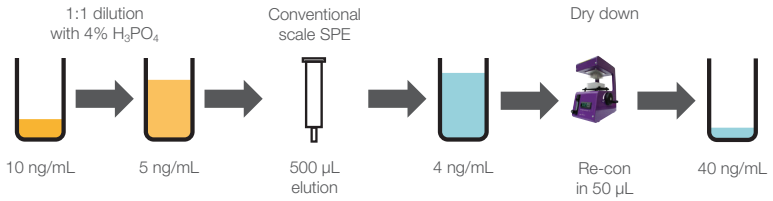

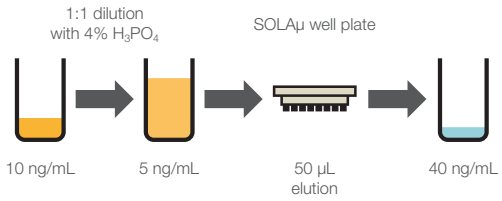

Figure 1: Summary of workflow required to ensure sufficient pre-concentration of analytes using dry down (top) and SOLAµ (bottom).

Experimental details

Consumables		Cat. no.
Fisher Scientific™ LC-MS grade water (ACN)		W/011217
Fisher Scientific™ LC-MS grade methanol (MeOH)		M/4062/17
Fisher Scientific™ analytical grade formic acid (HCOOH)		F/1900/PB08
Sample handling equipment		**Cat. no.**
Liquid handling hardware		-
SPE hardware	Thermo Scientific™ HyperSep™ 96 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 96 square well pre-slit	60180-M122
Sample pre-treatment		
	200µL of rat plasma diluted 1:1 with 4% H_3PO_4	
Sample preparation		
Compound(s)	Ibuprofen, ibuprofen d3 (IS), ketoprofen, ketoprofen d3 (IS)	-
Matrix	Rat plasma	-
	Thermo Scientific™ SOLAµ™ SAX 96 well plate, 2 mg/1 mL	60209-003
Condition	200 µL methanol	-
	200 µL H2O	-
Application	Load sample at 0.5mL/min	-
Wash	200 µL water with 1% ammonia	-
	200 µL methanol with 1% ammonia	-
Elute	2 × 25 µL 50/50 methanol/acetonitrile with 2% formic acid	-
Dilution	Add 50 µL water to each sample	-

Separation conditions		Cat. no.
Instrumentation	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system	-
Column	Thermo Scientific™ Accucore™ RP-MS HPLC column, 50 mm × 2.1mm 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	1200 µL/min	-
Run time	4 min	-
Column temperature	40 °C	-
Injection details	2 µL full loop injection	-
Injection wash solvent 1	Water	-
Injection wash solvent 2	45:45:10 (v/v/v) IPA / acetonitrile / acetone	-
Mobile phase A	Water with 0.005 % formic acid	-
Mobile phase B	Acetonitrile	-

Gradient conditions		
Time (min)	%A	%B
0.0	85	15
0.5	85	15
3.0	30	70
3.1	0	100
3.5	0	100
3.51	85	85
4.0	85	85

MS conditions		
Instrumentation	Thermo Scientific™ TSQ Vantage™ triple stage quadruple mass spec	
Compound	Ibuprofen	Ketoprofen
Ionization conditions	HESI	HESI
Polarity	-ive	+ive
Spray voltage (V)	3500	3500
Vaporiser temperature (°C)	300	300
Sheath gas pressure (Arb)	60	60
Aux gas pressure (Arb)	25	25
Capillary temp (°C)	300	300
Collision pressure (mTorr)	1.5	1.5
Scan time (s)	0.02	0.02
Q1 (FWHM)	0.7	0.7
Q3 (FWHM)	0.7	0.7

Compound	Parent *(m/z)*	S-Lens (V)	Product *(m/z)*	Collision Energy (V)
Ibuprofen	205.1	44	161.3	10
Ibuprofen d3 (IS)	208.4	51	164.2	10
Ketoprofen	255.1	63	209.0	13
Ketoprofen d3 (IS)	258.0	86	212.1	14

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

Results

By loading 200 µL of sample onto the SOLAµ plate and eluting in a total of 50 µL the required fourfold pre-concentration was achieved without the need for dry down. The results demonstrate that even with this low elution volume excellent data was achieved for both ibuprofen and ketoprofen.

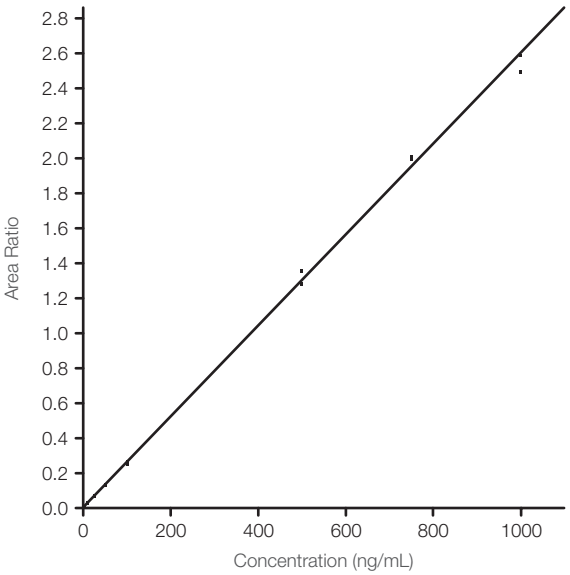

Figure 2: Ibuprofen linearity over the dynamic range 10–1000 ng/mL.

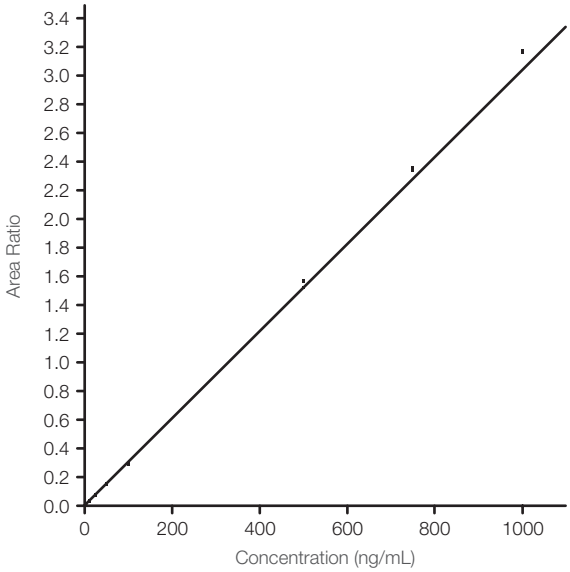

Figure 3: Ketoprofen linearity over the dynamic range 10–1000 ng/mL.

Standard	Specified concentration	Calculated concentration	% Diff
S1	10.00	10.23	2.30
S2	25.00	24.26	-2.94
S3	50.00	48.08	-3.83
S4	100.00	95.18	-4.82
S5	500.00	508.91	1.78
S6	750.00	773.27	3.10
S7	1000.00	1044.06	4.41
QC L	25.00	24.08	-3.68
QC M	500.00	513.14	2.63
QC H	750.00	782.83	4.38

Table 1: Ketoprofen accuracy data for the calibration range 10–1000 ng/mL

Standard	Specified concentration	Calculated concentration	% Diff
S1	10.00	10.15	1.50
S2	25.00	25.07	0.30
S3	50.00	49.57	-0.87
S4	100.00	97.44	-2.56
S5	500.00	506.99	1.40
S6	750.00	769.52	2.60
S7	1000.00	976.25	-2.37
QC L	25.00	24.89	-0.45
QC M	500.00	534.30	6.86
QC H	750.00	767.04	2.27

Table 2: Ibuprofen accuracy data for the calibration range 10–1000 ng/mL

Standards, extracted from rat plasma, gave a linear dynamic range from 10 to 1000 ng/mL with an R_2 coefficients of 0.999 and 0.997 respectively (Figures 2 and 3, Tables 1 and 2). The chromatography for the limit of quantitation sample at 10 ng/mL is shown in Figures 3 and 4 to be above the acceptable signal to noise limit.

Low, mid and high QC samples were prepared at concentrations of 25, 500 and 750 ng/mL. Tables 1 and 2 show high accuracy with variation less than 5% for all levels. Table 4 shows reproducibility data for replicate extractions of the two compounds (n= 18) at both high and low QC levels. RSD for ibuprofen is less that 4% and for ketoprofen less than 2%. Analyte recovery was shown to be greater than 90% for ibuprofen and ketoprofen by comparison to post extraction fortified blank samples (refer to Table 3). Matrix effects for ibuprofen and ketoprofen were calculated at less than 7% at both high and Low QC levels with the exception of ibuprofen at the Low QC which showed less than 16% (refer to Table 5).

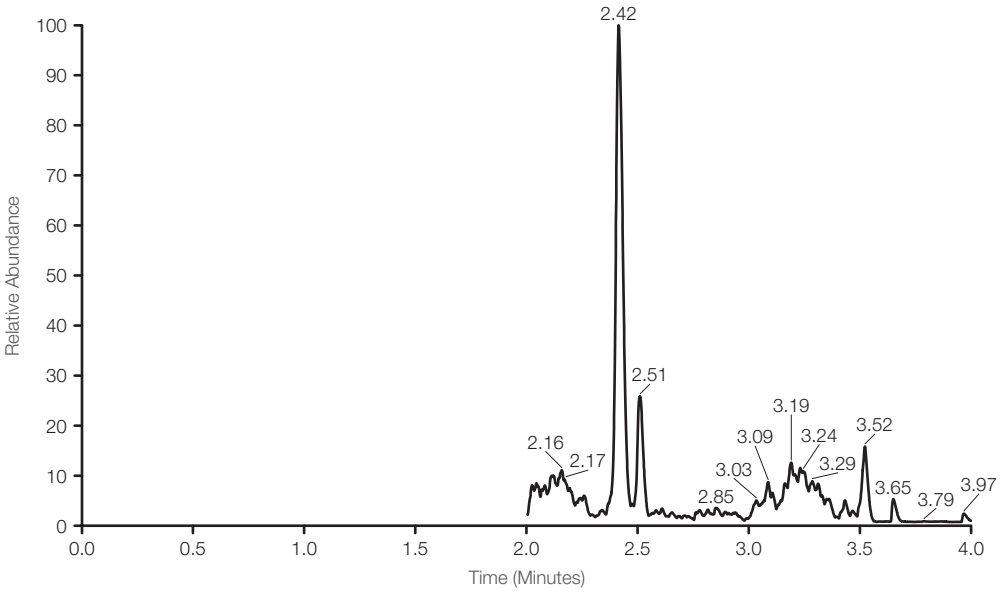

Figure 4: Example chromatogram 10ng/mL ibuprofen.

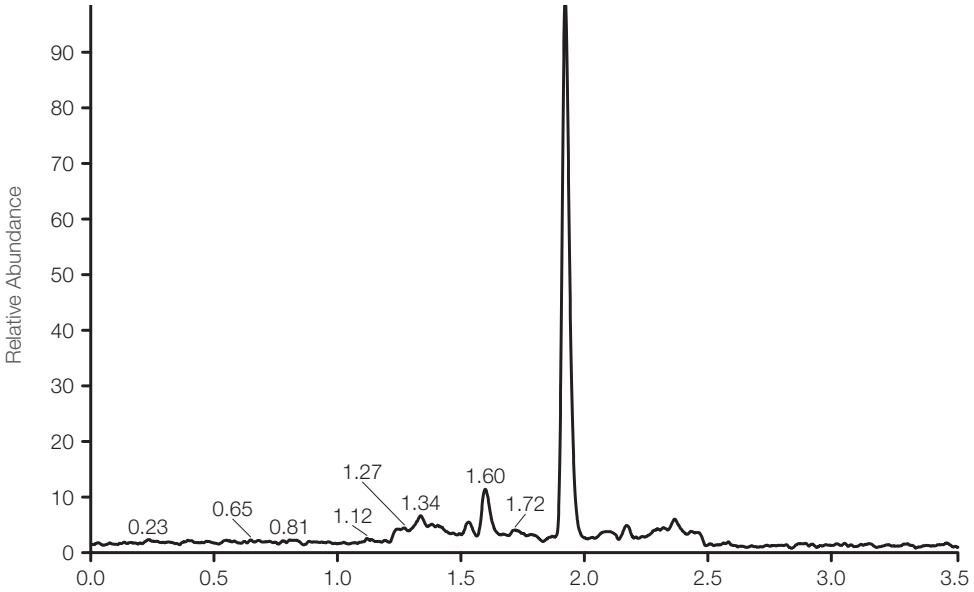

Figure 5: Example chromatogram 10 mg/mL ketoprofen.

	Ibuprofen (%)	Ketoprofen (%)
QC Low	95	91
QC High	90	92

Table 3: Percentage recovery for ibuprofen and ketoprofen at Low QC 25 ng/mL and High QC 750 ng/mL.

	Ibuprofen (%RSD)	Ketoprofen (%RSD)
QC High	4.00	1.57
QC Low	1.70	1.37

Table 4: Precision data for Ibuprofen and ketoprofen at low QC 25 ng/mL and high QC 750 ng/mL (n=18).

	Ibuprofen (%)	Ibuprofen d3 (%)	Ketoprofen (%)	Ketoprofen d3 (%)
QC High	1.61	-2.61	2.67	1.24
QC Low	15.34	2.60	6.91	-3.28

Table 5: Percentage matrix effects for ibuprofen and ketoprofen at Low QC 25 ng/mL and High QC 750 ng/mL.

Conclusion

The use of the SOLAμ SPE well plate in this case enables the removal of the evaporation and reconstitution steps typically required with larger format conventional SPE devices.

This results in:

- a fourfold pre-concentration step
- a faster more efficient process
- greater sample integrity because sample loss is minimized

This is achieved while realizing levels of accuracy and reproducibility required by the bioanalytical industry.

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