Application Note 20943

Improving sensitivity with SOLAμ for analytes susceptible to issues during pre-concentration dry down

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Key Words
SOLAμ, micro elution, reproducibility, matrix effects, SPE, No dry down

Abstract
This application note demonstrates the use of Thermo Scientific™ SOLAμ™ 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 allows 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).

Thermo Scientific SOLAμ 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 delivers:

- 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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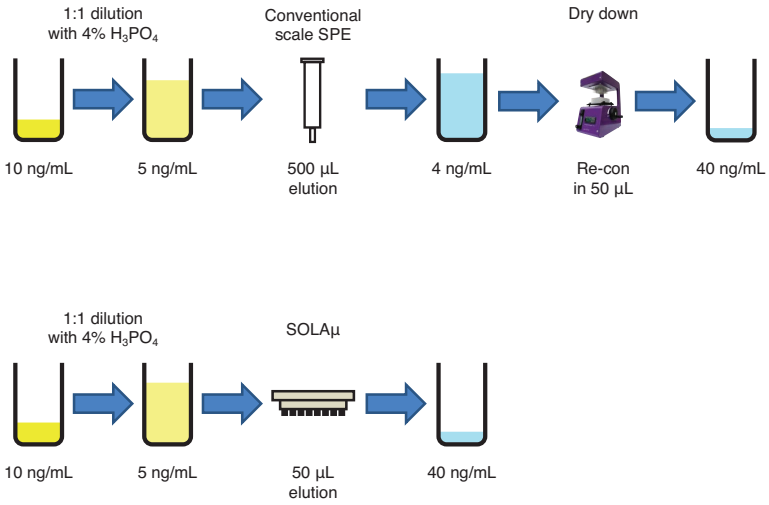

Figure 1: Summary of workflow required to ensure sufficient pre-concentration of analytes using dry down (top) and SOLAµ (bottom).

Experimental Details

Consumables	Part Number
Fisher Scientific™ LCMS grade water	W/011217
Fisher Scientific LCMS grade methanol	M/4062/17
Fisher Scientific™ analytical grade formic acid	F/1900/PB08

Sample Handling Equipment		Part Number
Liquid handling hardware:		
SPE hardware:	Thermo Scientific™ HyperSep™ 96 vacuum manifold	60103-351
	Vacuum pump, european version	60104-241
Sample handling:		
	Thermo Scientific™ Webseal™ 96 well square well microplate	60108-P212
	Thermo Scientific Webseal mat 96 square well pre-slit	60180-M122

Sample Pretreatment	
	200µL of rat plasma diluted 1:1 with 4% H_3PO_4

Sample Preparation		Part Number
Compound(s):	Ibuprofen, ibuprofen d3 (IS), ketoprofen, ketoprofen d3 (IS)	
Matrix:	Rat plasma	
	SOLAµ SAX 2 mg/1 mL 96 well plate	60209-003
Condition:	200 µL methanol	
	200 µL H_2O	
Application:	Load sample at 0.5mL/min	
Wash:	200 µL water with 1% ammonia	
	200 µL methanol with 1% ammonia	
Elution:	2 × 25 µL 50/50 methanol/acetonitrile with 2% formic acid	
Dilution:	Add 50 µL water to each sample	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system	
Column:	Accucore RP-MS 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	
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™ LC QUAN™ version 2.6 quantitative software

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.

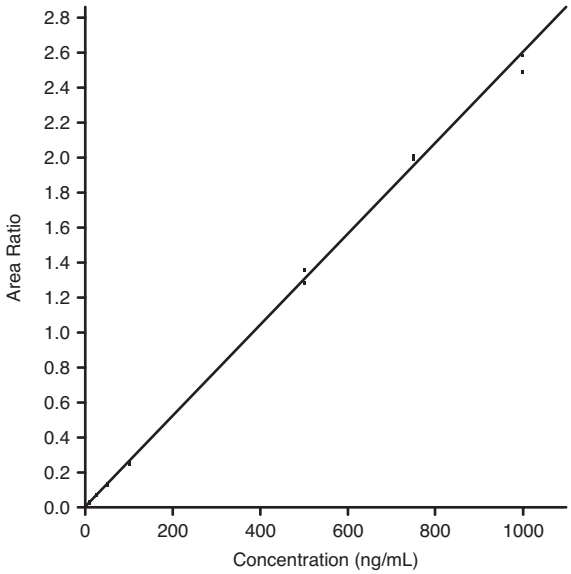

Figure 2: Ibuprofen linearity over the dynamic range 10–1000 ng/mL

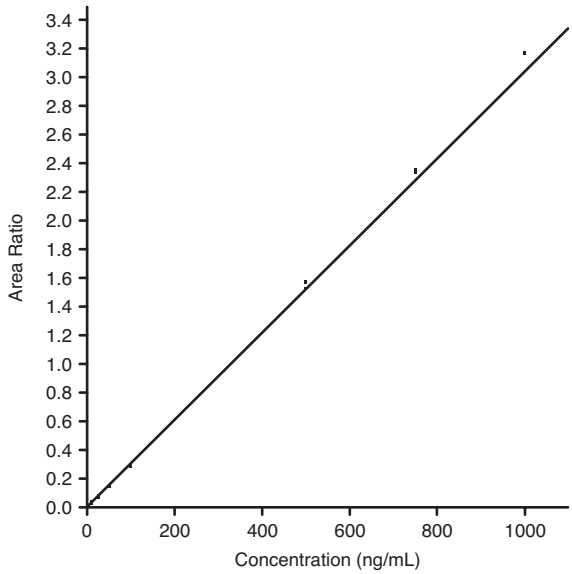

Figure 3: Ketoprofen linearity over the dynamic range 10–1000 ng/mL

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 (Figure 2 and 3, Table 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).

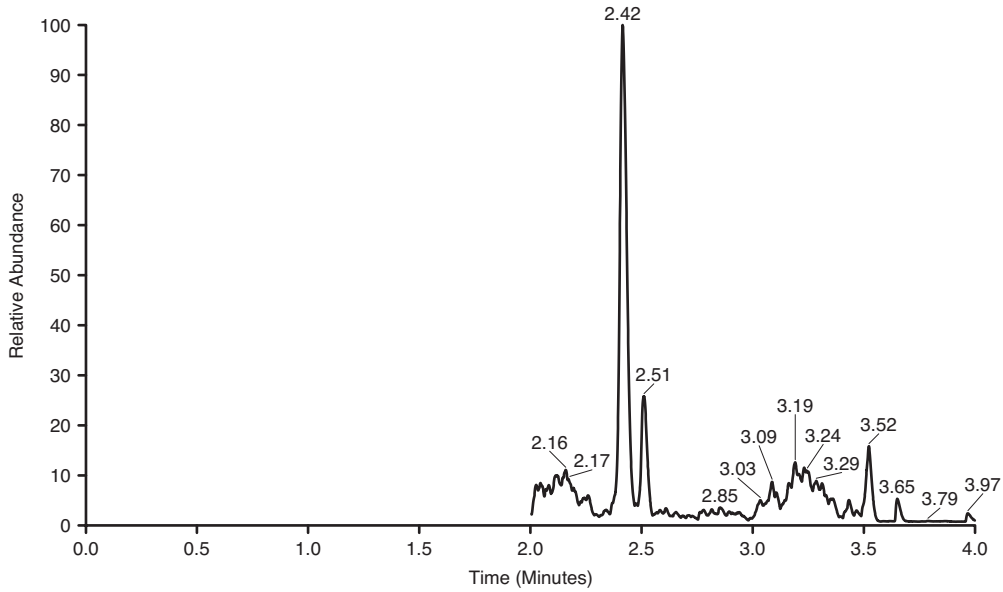

Figure 4: Example chromatogram 10ng/mL Ibuprofen

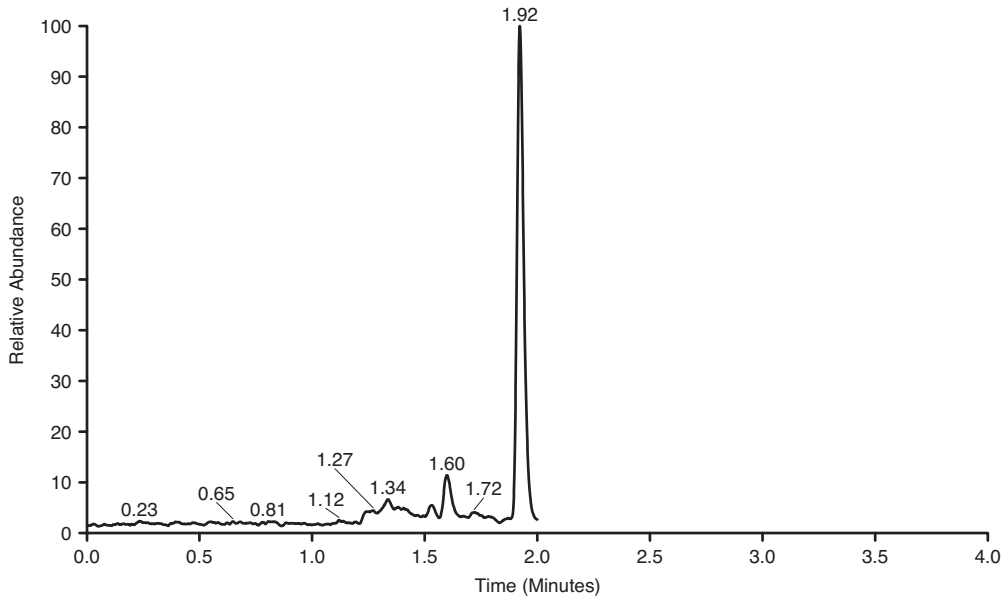

Figure 5: Example chromatogram 10 mg/mL ketoprofen

	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

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