

Using SPE to Achieve a Twenty-fold Pre-concentration Without the Need for Evaporation and Reconstitution

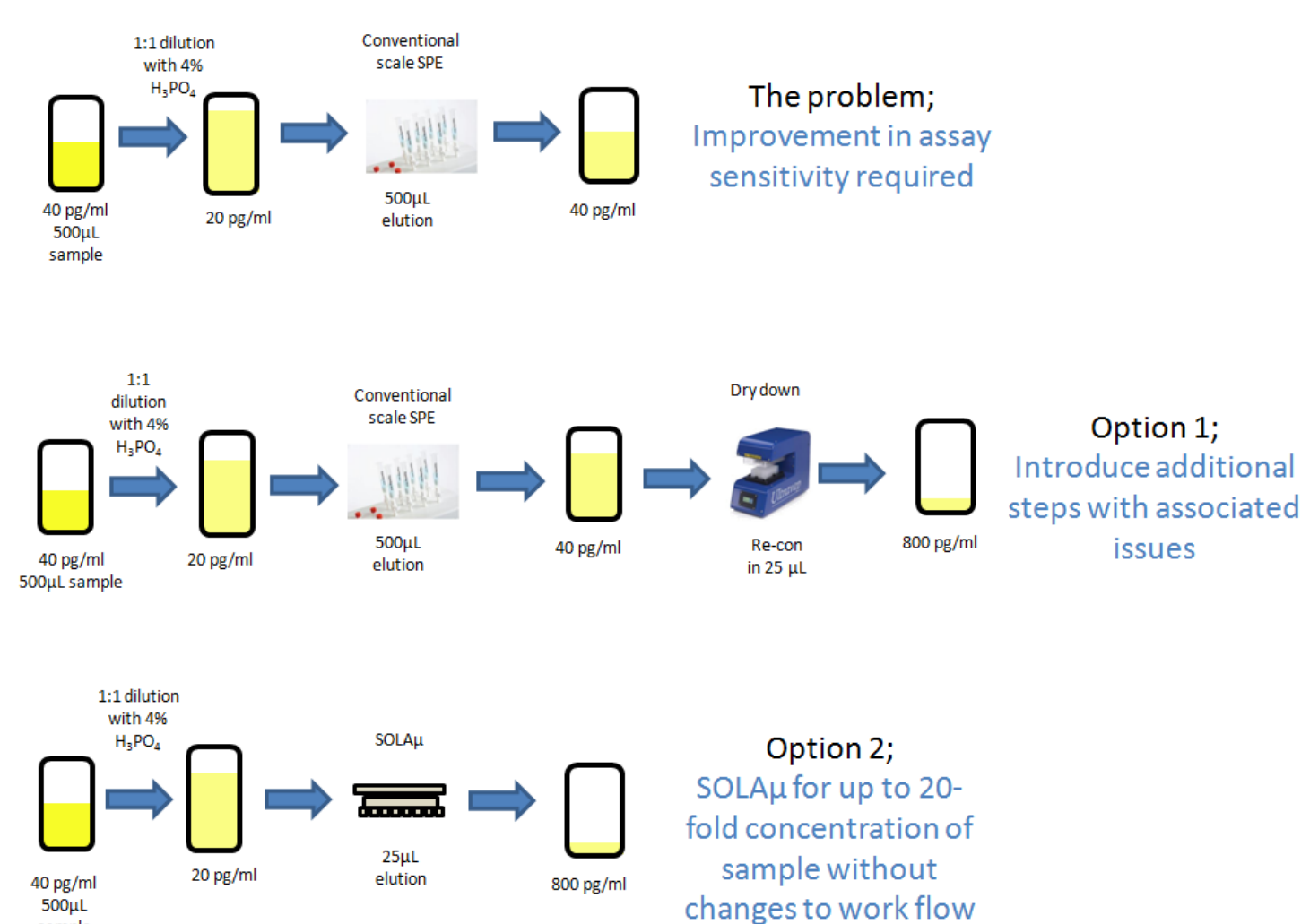
Jon Bardsley, Ken Meadows, Mike Oliver
Thermo Fisher Scientific, Runcorn, UK

Introduction

Despite advances in analytical detection technology achieving required limits of sensitivity, whilst maintaining reproducibility, can still be an issue for many bioanalytical laboratories.

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.

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



By using Thermo Scientific™ SOLAµ™ SPE micro elution plates a twenty-fold concentration of sample can be achieved without the need for a lengthy dry-down step, increasing sensitivity without compromising assay performance.



Did You Know?
SOLAµ plates have individually removable wells? This not only allows for efficient analysis but enables you to build your own bespoke method development plate, providing a fast option for screening different chemistries in one extraction.

Methods

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 x 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 x 2.1 mm 2.6 µm (17626-052130)

Guard column: Thermo Scientific™ Accucore™ RP-MS Defender™ Guard Cartridge (17626-012105)
Thermo Scientific™ Uniguard™ Direct-Connection Guard Cartridge Holder

Mass spec system: Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

Results

The assay gave a linear dynamic range from 40 to 40000 pg/mL with an r^2 coefficient of 0.998. The chromatography for the limit of quantitation sample at 40 pg/mL is significantly above the acceptable signal to noise limit.

Low, mid and high QC samples were prepared at concentrations of 400, 20000 and 30000 pg/mL respectively. Good levels of accuracy at all QC levels were observed. Assay is reproducible for replicate extractions (n=18) at both high and low QC levels.

FIGURE 2. Linear calibration line for Niflumic acid from 40 to 40000 pg/mL.

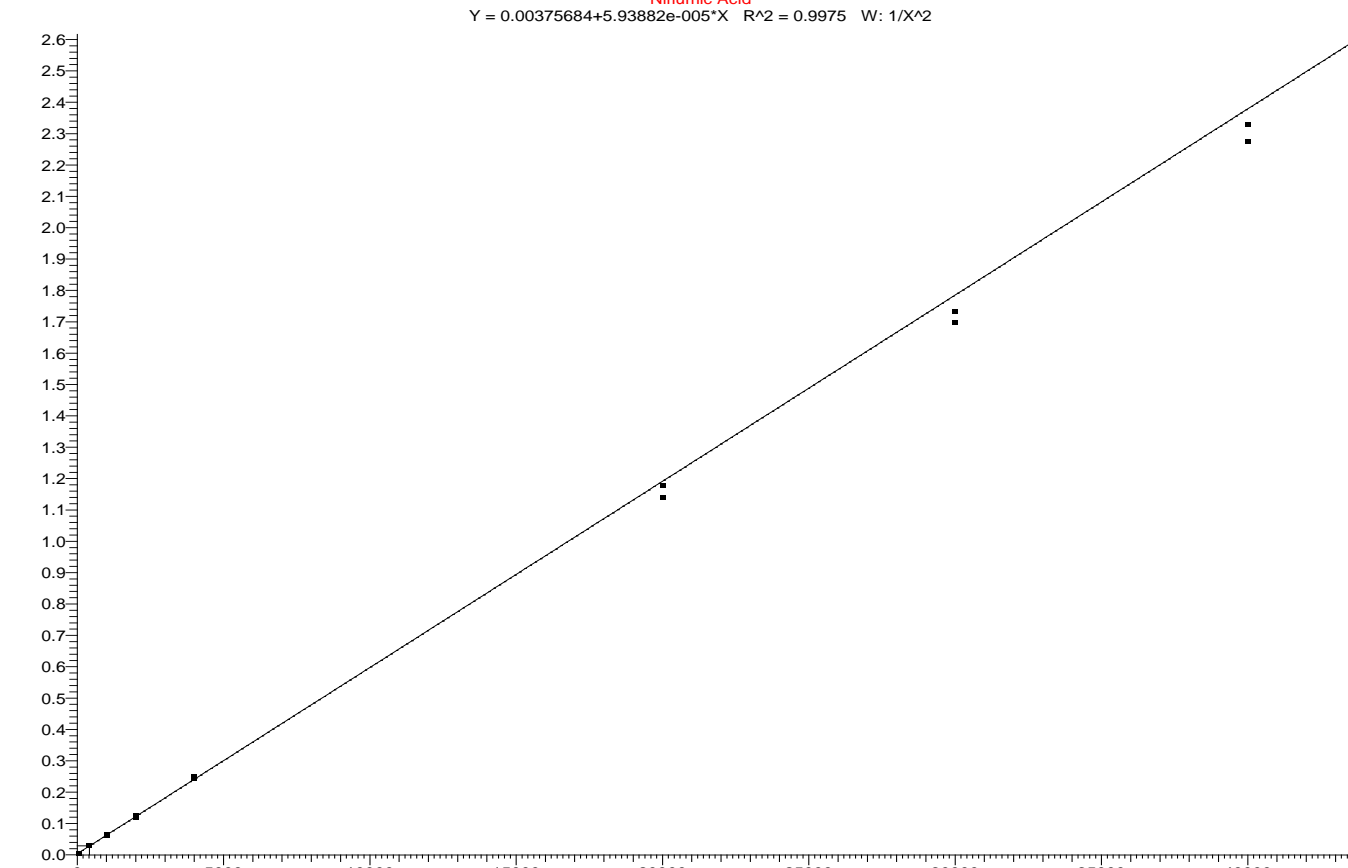
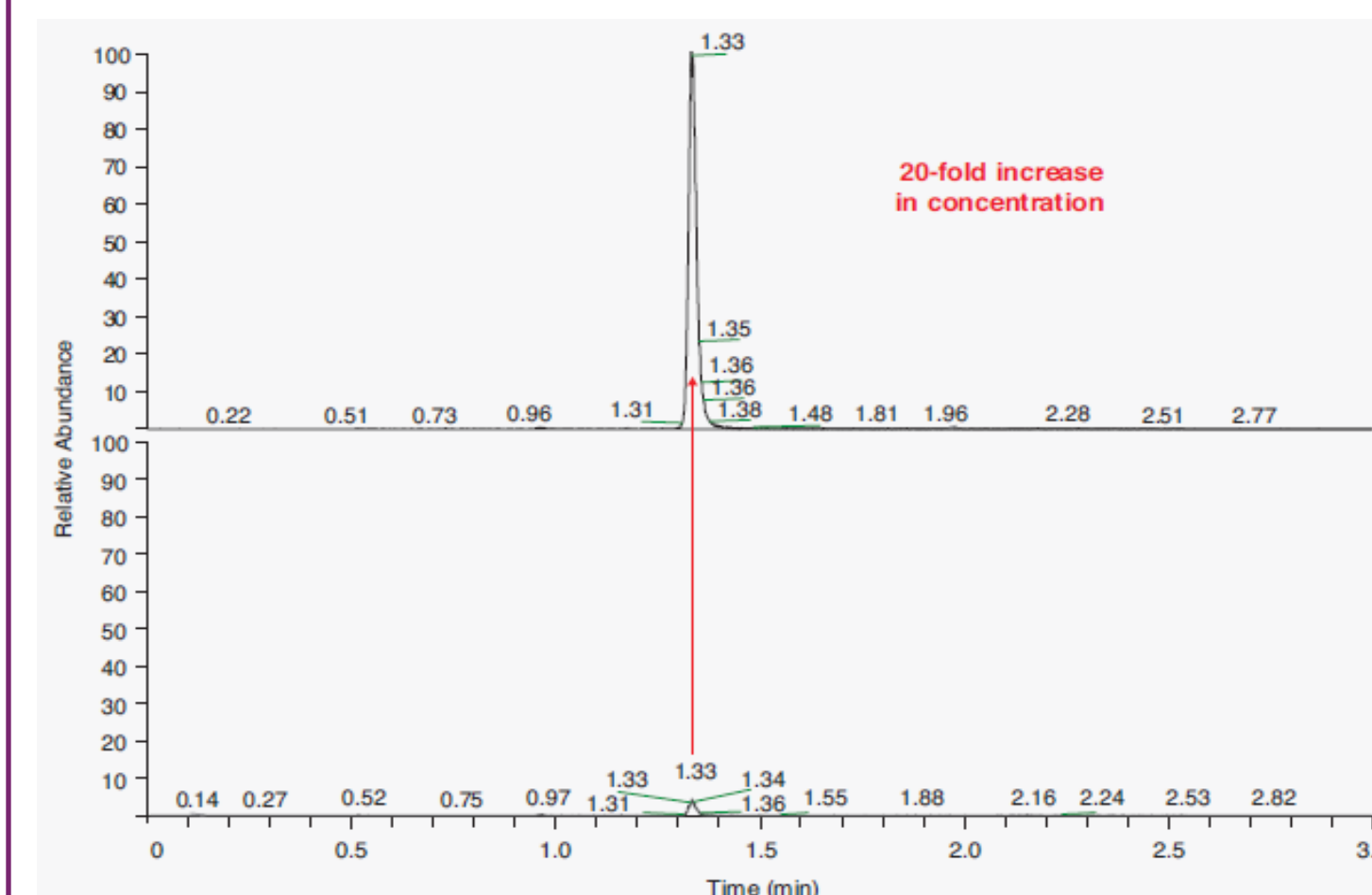


TABLE 1. Assay accuracy and precision data.

Standard	Specified Concentration (pg/mL)	Calculated Concentration (pg/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

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.

FIGURE 3. Twenty-fold increase in response achieved by using SOLAµ plate for sample concentration.



SOLAµ allows 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.

TABLE 2. Matrix effects results calculated by comparing fortified blank samples against pure reference standards.

	Matrix Effects (%)
Low QC	8.63
High QC	3.21



TABLE 3. Assay recovery results calculated by comparing extracted samples against fortified blank samples

	Recovery of Niflumic Acid (%)
Low QC	89.9
High QC	94.0

Analyte recovery was shown to be greater than 89.9% by comparison to post extraction fortified blank samples. 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.

Conclusion

This poster 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 sample integrity.

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

To download the full application note
SOLAµ for Pre-analysis Sample Concentration
visit: www.thermoscientific.com/sola-spe

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