SOLAµ for pre-analysis sample concentration

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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 nonspecific binding.

Thermo Scientific™ 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.

SOLAµ provides 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.

Key Words
SOLAµ, micro elution, reproducibility, matrix effects, SPE, no dry down, niflumic acid, sensitivity, sample concentration

Abstract

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

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
Experimental Details

Consumables                                      Part Number
Fisher Scientific™ LCMS grade water             10777404
Fisher Scientific™ LCMS grade methanol          10653963
Fisher Scientific™ Analytical grade formic acid 10559570

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

Sample Pretreatment                            
500 µL of human plasma diluted 1:1 with 4% phosphoric acid.

Sample Preparation                            Part Number
Compound(s): Niflumic acid, niflumic acid d5 (IS) 
Matrix: Human plasma                          
SOLAµ WAX 2 mg/1 mL 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 × 12.5 µL 50/50 methanol/acetonitrile with 2% ammonia

Direct injection of eluent
## Separation Conditions

<table>
<thead>
<tr>
<th>Instrumentation:</th>
<th>Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>Thermo Scientific™ Accucore™ RP-MS HPLC column 50 mm x 2.1 mm 2.6 µm</td>
</tr>
<tr>
<td></td>
<td>17626-052130</td>
</tr>
<tr>
<td>Guard column:</td>
<td>Thermo Scientific™ Accucore™ RP-MS Defender™ guard cartridge</td>
</tr>
<tr>
<td></td>
<td>17626-012105</td>
</tr>
<tr>
<td></td>
<td>Thermo Scientific™ Uniguard™ drop-in guard holder 852-00</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>750 µL/min</td>
</tr>
<tr>
<td>Run time:</td>
<td>3 min</td>
</tr>
<tr>
<td>Column temperature:</td>
<td>30 °C</td>
</tr>
<tr>
<td>Injection details:</td>
<td>2 µL full loop injection</td>
</tr>
<tr>
<td>Injection wash solvent 1:</td>
<td>Water</td>
</tr>
<tr>
<td>Injection wash solvent 2:</td>
<td>45:45:10 (v/v/v) propan-2-ol / acetonitrile / acetone (with 5% Ammonia)</td>
</tr>
<tr>
<td>Mobile phase A:</td>
<td>Water with 0.1% formic acid</td>
</tr>
<tr>
<td>Mobile phase B:</td>
<td>Acetonitrile with 0.1% formic acid</td>
</tr>
</tbody>
</table>

### Gradient Conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>2.0</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>2.01</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

### MS Conditions

<table>
<thead>
<tr>
<th>Instrumentation:</th>
<th>Thermo Scientific™ TSQ Vantage™ triple stage quadruple mass spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization conditions</td>
<td>HESI</td>
</tr>
<tr>
<td>Polarity</td>
<td>+ive</td>
</tr>
<tr>
<td>Spray voltage (V)</td>
<td>3000</td>
</tr>
<tr>
<td>Vaporiser temperature (°C)</td>
<td>475</td>
</tr>
<tr>
<td>Sheath gas pressure (Arb)</td>
<td>50</td>
</tr>
<tr>
<td>Aux gas pressure (Arb)</td>
<td>60</td>
</tr>
<tr>
<td>Capillary temp (°C)</td>
<td>300</td>
</tr>
<tr>
<td>Collision pressure (mTorr)</td>
<td>1.5</td>
</tr>
<tr>
<td>Scan time (s)</td>
<td>0.02</td>
</tr>
<tr>
<td>Q1 (FWHM)</td>
<td>0.7</td>
</tr>
<tr>
<td>Q3 (FWHM)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent (m/z)</th>
<th>S-Lens (V)</th>
<th>Product (m/z)</th>
<th>Collision Energy (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niflumic Acid</td>
<td>283.0</td>
<td>115</td>
<td>265.0</td>
<td>22</td>
</tr>
<tr>
<td>Niflumic Acid d5 (IS)</td>
<td>288.8</td>
<td>115</td>
<td>271.1</td>
<td>22</td>
</tr>
</tbody>
</table>

### Data processing

| Software: | Thermo Scientific™ LC QUANT™ version 2.6 quantitative software |
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 $r^2$ 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).

![Graph showing niflumic acid linearity](image)

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

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

**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.

<table>
<thead>
<tr>
<th></th>
<th>Precision Data for Niflumic Acid Peak Area Ratio (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC 1.31</td>
</tr>
<tr>
<td></td>
<td>High QC 1.06</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Recovery of Niflumic Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC 89.9</td>
</tr>
<tr>
<td></td>
<td>High QC 94.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Matrix Effects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC 8.63</td>
</tr>
<tr>
<td></td>
<td>High QC 3.21</td>
</tr>
</tbody>
</table>

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 provides 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

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