Increased speed and sample throughput of opioid analysis from human urine using micro-elution solid phase extraction

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Key words
SPE, SOLA, SOLAμ, SCX, Hypersil GOLD aQ, opiates

Goal
To describe a reproducible optimization procedure for the isolation of opioids from human urine utilizing solid phase extraction (SPE). Subsequent analysis was carried out with liquid chromatography separation coupled to triple quadrupole mass spectrometry detection (LC-MS/MS).

Introduction
Solid phase extraction (SPE) has been beneficial in selective cleanup of complex biological matrices for the analysis of diverse species and is routinely used in laboratories ranging from drug discovery to forensics. The benefits of SPE are well known and include the following:

- Cleaner samples free from matrix interferences
- Concentration of sample with lower limits of detection
- Shorter chromatography due to a less complex sample being injected

Method optimization can help to streamline laboratory processes and increase productivity. Even small incremental changes can have a positive impact, particularly in a high-throughput laboratory environment. Established SPE methods can often be further optimized by changing to a more reproducible SPE format, implementing micro-elution SPE, or both.

Thermo Scientific™ SOLA™ solid phase extraction products are designed to selectively extract specific compounds classes from very complex matrices such as plasma or urine. The extract can then be analyzed using a variety of analytical techniques. SOLA SPE has a unique uniform packing design that provides higher
levels of reproducibility from extract to extract compared to traditional loose-packed SPE devices. SOLA media is packed into a variety of platforms that are scalable, from single sample analysis to high-throughput screening.

Thermo Scientific™ SOLAμ™ is a micro-elution version of the product that improves speed and solvent usage. Utilizing smaller bed weights can have a dramatic effect on minimizing the solvent volumes required for elution of the analytes from the SPE device. With micro-elution SPE, post-extraction processing such as evaporation and reconstitution can be removed and the laboratory workflow is optimized.

Here we focus on how a robust SPE workflow for the analysis of multiple opioids, including morphine and methadone (Figures 1 and 2), can be further optimized by introduction of micro-scale SPE to speed up the extraction process and increase sample throughput.

**Experimental**

**Consumables**
- SOLA SCX (10 mg, 96-well plates) (P/N 60309-002)
- SOLAμ SCX (2 mg, 96-well plates) (P/N 60209-002)
- Thermo Scientific™ 96-well square well plate (2 mL, V-shape) (P/N 60180-P212)
- Thermo Scientific™ WebSeal™ mat (Silicone/PTFE, blue, square, pre-slit) (P/N 60180-M122)
- Fisher Scientific™ LC-MS grade water (P/N 10728098)
- Fisher Scientific LC-MS grade methanol (MeOH) (P/N 10031094)
- Fisher Scientific LC-MS grade acetonitrile (ACN) (P/N 10489553)
- Fisher Chemical Analytical grade formic acid (FA) (P/N 10596814)
- Fisher Scientific Ammonia solution (35%) (P/N 10508610)
- Thermo Scientific™ Pierce™ HPLC grade triethylamine (TEA) (P/N 25108)

**Sample handling equipment**
- Thermo Scientific™ HyperSep™ 96-well plate vacuum manifold (P/N 60103-351)
- Thermo Scientific™ Ultra Vap (P/N 60180-P900)

**Sample pretreatment**

Compounds: cis-tramadol, codeine, EDDP, fentanyl, hydrocodone, hydromorphone, meperidine, methadone, morphine, norfentanyl, norhydrocodone, noroxycodone, O-desmethyltramadol, oxymorphone

Matrix: Human urine

All compounds and matrices were obtained from reputable sources.

Samples were prepared as follows:
1. A standard spiking solution of 14 opioids was prepared in MeOH.
2. Control urine was spiked with an appropriate volume of the solution to make a concentration of 150 ng/mL.
3. Six aliquots of 180 µL of the spiked urine were each diluted with 800 µL of 1% FA in water.
4. Blank samples were prepared in the same manner but with the absence of the compounds of interest.
5. All samples were vortexed for 30 seconds and then centrifuged for 5 minutes at 5000 rpm.

![Figure 1. Structure of morphine.](image1.png)

![Figure 2. Structure of methadone.](image2.png)
**SPE method details**

*NB: Practical considerations for SPE and manifold setup can be found in the appendix.*

In Figure 3, an established method using SOLA SCX (10 mg SPE) is compared to a scaled-down method using SOLAμ SCX (2 mg SPE). The solvents used in each step are the same, as is the sample preparation, but the differences are in the volumes and time taken for each step.

![Diagram of SPE method comparison](image)

The decreased sorbent size of the micro-elution SPE allows for a reduction in solvent usage. In this example, a solvent savings of 1100 μL per sample is made, and due to the removal of the post-extraction evaporation and reconstitution steps, a time savings of 29 minutes per plate (96 samples per plate) is achieved (Figures 3 and 4).

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**Figure 3.** Method details for SOLA SCX and SOLAμ SCX showing each step, volume of solvent required, and length of time in minutes for each step.
Results and discussion

An example chromatogram of normeperidine obtained using the recommended LC conditions is shown in Figure 5.

Recoveries for each compound were measured following extraction of all the compounds on both the SOLA SCX and SOLAµ SCX plates from urine. Recoveries were between 88% and 99% for the SOLA SCX method, and between 96% and 106% for the SOLAµ SCX method. Inter-method recoveries were comparable for each compound, with the exception of EDDP where a significantly better recovery was observed using the SOLAµ SCX plate (Figure 6).

Single digit percent relative standard deviations (%RSD) were observed (n=6) across all compounds from both SPE devices showing the exceptional reproducibility of SOLA and SOLAµ (Table 2). However, the reproducibility was approximately 2% better using SOLAµ with a range of 1.4% to 4.1% compared to a range of 3.2% to 6.2% for SOLA.

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**Table 1. LC gradient conditions.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A %</th>
<th>B %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
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</tr>
<tr>
<td>8</td>
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<td>100</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>100</td>
</tr>
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<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Conclusions

- SOLA SCX and SOLAµ SPE plates allow for a simple extraction method of opioids from urine.
- Excellent reproducibility is observed with both extraction methods.
- SOLAµ can be employed to decrease solvent use and increase throughput by eliminating post-SPE processing.
- Existing SPE methods can be further optimized with little to no method development by scaling directly down the solvent volumes when using the micro-scale SPE plates.

References

Appendix

Calculations

\[
\text{Recovery} = \frac{\text{average response of sample}}{\text{average response of overspike}} \times 100
\]

Practical considerations for SPE and manifold setup

Manifold setup and plate alignment

Points to consider
- No gap between SPE plate and collection plate.
- When under vacuum SPE plate will be held tightly in position.

Practical considerations for the use of SPE plates

1. Using an accurate pipette, aspirate the specified volume of either solvent/reagent, or sample.
2. Dispense solvent/reagent or sample into a unique ‘well’ of the SPE plate. Up to 96 wells can be used simultaneously.
3. Apply a gentle vacuum and increase the pressure until the liquid begins to flow through the SPE plate.
4. The collected eluent is then kept for analysis.

Note: The effluent from each load/wash step may also be collected and analyzed if method optimization is required.