APPLICATION NOTE

No. 21578

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

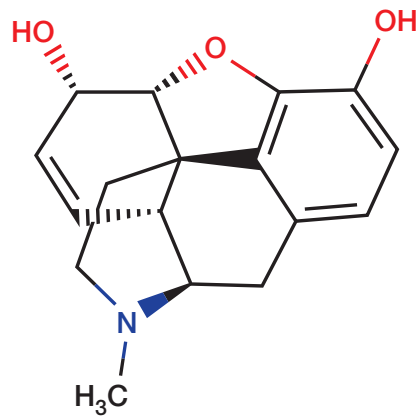


Figure 1. Structure of morphine.

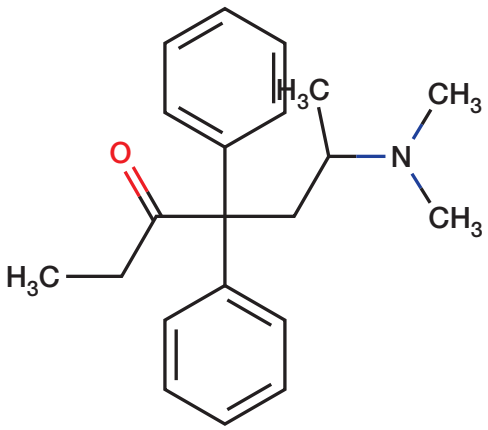


Figure 2. Structure of methadone.

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.

SPE method details

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

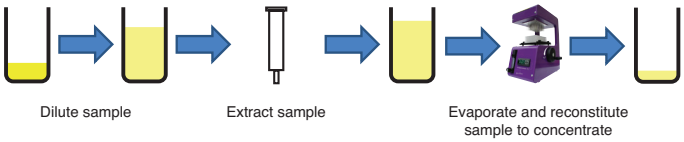
In Figure 3, an established method using SOLA SCX (10 mg SPE) is compared to a scaled-down method using SOLAμ SCX (2 mg SPE). The solvents used in each step are the same, as is the sample preparation, but the differences are in the volumes and time taken for each step.

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).

SOLA 10 mg			SOLAμ 2 mg	
Vol (µL)	Time (min)		Vol (µL)	Time (min)
500	5	Condition with methanol	200	5
500	5	Equilibrate with water	200	5
1000	5	Load pre-treated sample	1000	10
500	5	Wash with 0.1% formic acid (aq)	200	5
500	5	Wash with 0.1% formic acid (methanol)	200	5
Place a collection plate under the SPE device to capture the extract				
2 x 200	5	Elute with MeOH/ACN/TEA (45/45/10)	2 x 25	5
Post-extraction processing requirements				
-	-	Dilute with water	50	1
n/a	30	Evaporate under nitrogen	-	-
100	5	Reconstitute with mobile phase	-	-

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.

Traditional-scale SPE



Micro-scale SPE

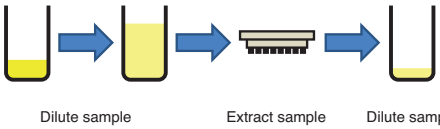


Figure 4. Workflow comparison of SOLA (top) and SOLAμ (bottom).

Separation conditions

Recommended LC system:

- Thermo Scientific™ Vanquish Horizon™

Recommended MS/MS system:

- Thermo Scientific™ TSQ Quantiva™

Recommended instrument software:

- Thermo Scientific™ Chromeleon™ Chromatography Data System v7.2 SR4

For full analytical conditions, including separation and detection, see Poster Note PN 20815.[1]

LC conditions

Column	Thermo Scientific™ Hypersil GOLD™ aQ, 3 µm, 100 x 4.6 mm
Flow rate	1 mL/min
Column temperature	30 °C
Injection details	20 µL
Injection wash solvent 1	Water
Injection wash solvent 2	45:45:10 (v/v/v) IPA / acetonitrile/acetone
Gradient	Table 1

Table 1. LC gradient conditions.

Time (min)	A %	B %
0	100	0
3	85	15
8	0	100
9	0	100
9.1	100	0
15	100	0

Results and discussion

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

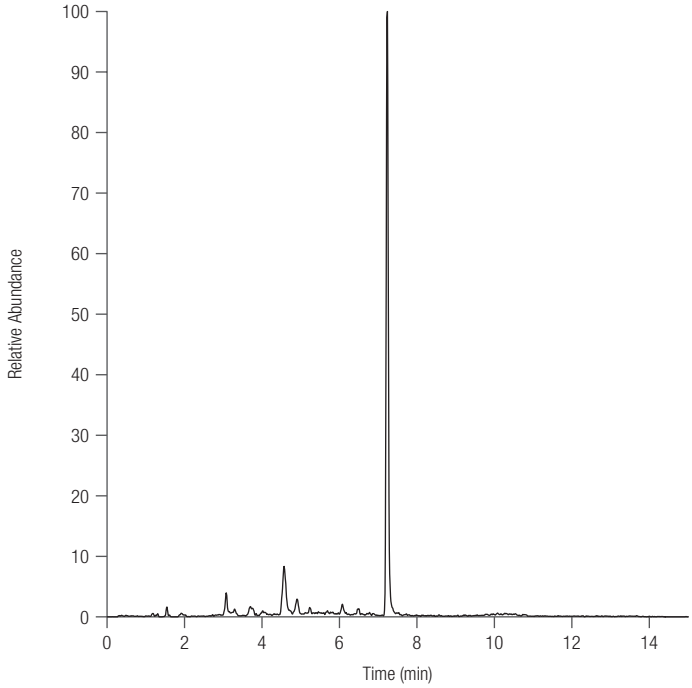


Figure 5. Example chromatogram of normeperidine obtained using the recommended LC conditions.

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.

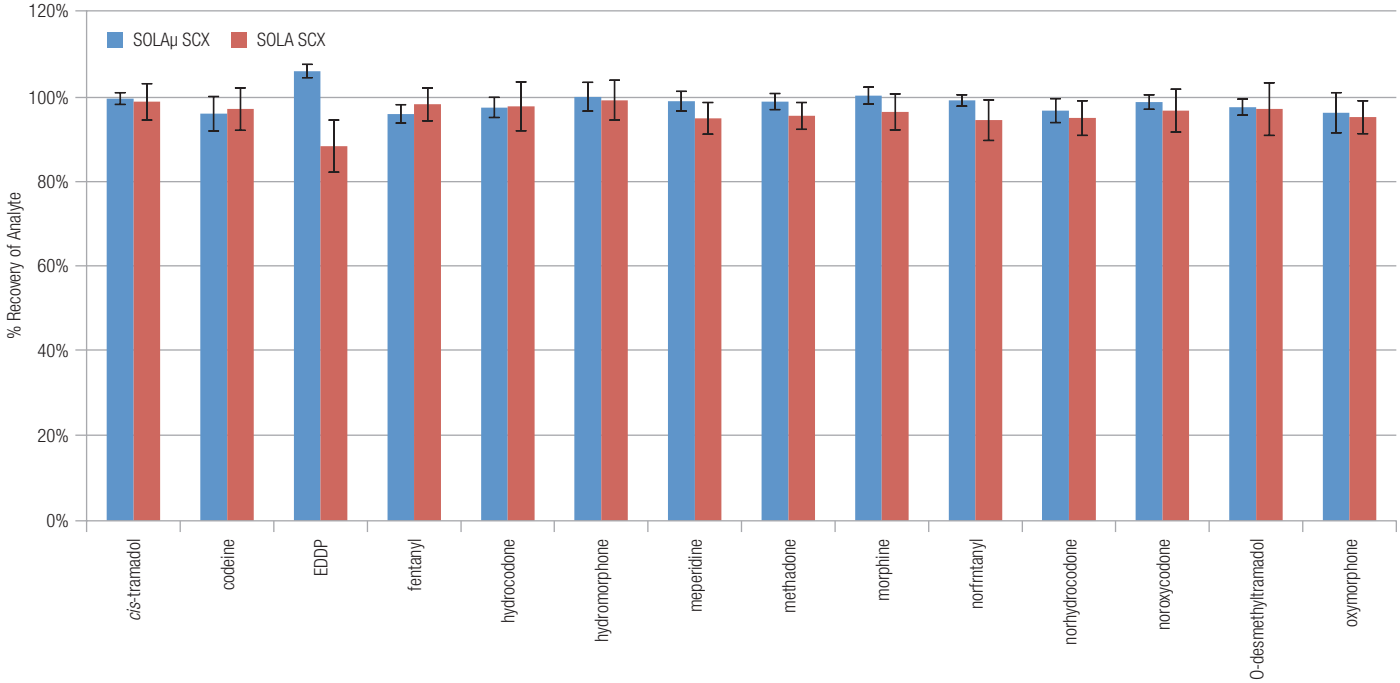


Figure 6. Comparison of SPE recovery between SOLA SCX and SOLAμ SCX.

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

1. Poster Note PN20815: LC-MS/MS Method for the Determination of 21 Opiates and Opiate Derivatives in Urine, J. Jones, S. Westwood, T. Liddicoat, L. Pereira, T. Edge, Thermo Fisher Scientific, Manor Park, Runcorn, UK, 2013.

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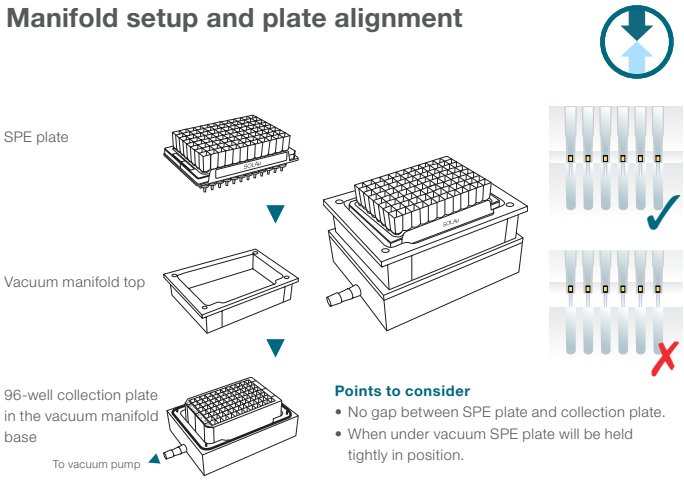
Appendix

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

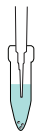


Points to consider

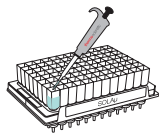
- No gap between SPE plate and collection plate.
- When under vacuum SPE plate will be held tightly in position.

Practical considerations for the use of SPE plates

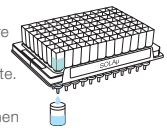
1. Using an accurate pipette, aspirate the specified volume of either solvent/reagent, or sample.



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.

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