IMPLEMENTING AUTOMATED SAMPLE PREPARATION STRATEGIES FOR THE QUANTIFICATION OF DRUGS OF ABUSE AND PAIN MANAGEMENT DRUGS

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INTRODUCTION

Forensic toxicology panels typically analyze various compound classes like stimulants, opioids, benzodiazepines, and synthetic cathinones, otherwise considered to be drugs of abuse. Driven by sensitivity, selectivity, and robustness requirements for LC-MS/MS analysis, the choice of sample preparation techniques include: dilution, protein precipitation (PPT), liquid-liquid extraction (LLE), and solid phase extraction (SPE). Development and optimization of these assays, especially larger panels, can prove to be time consuming and difficult to transfer between scientists and laboratories. Implementing fully-automated devices frees up the analyst to do other tasks, streamlines the sample preparation process, and, by reducing human error, improving analytical method reproducibility and consistency.

This work aims to provide practical and broadly applicable, automated SPE strategy for the accurate and reproducible quantification of drugs of abuse and pain management drugs from urine samples in support of research. These automation strategies simplify and streamline the sample preparation workflow, maximize productivity, and reduce risk of human error, while ensuring peak, analytical performance.

METHODS

Standard solution preparation

All standards were obtained from Cerilliant (Round Rock, TX) and Cayman Chemical (Ann Arbor, MI). A mixed stock solution was prepared in methanol at concentrations of 2, 10, and 25 µg/mL, depending upon the analyte. Stable isotope labeled standards were used as internal standards (IS). The IS stock solution was prepared in methanol at a concentration of 1 µg/mL. Samples were prepared by diluting stock solutions into pooled, blank urine. External quality control material was obtained from UTAK Laboratories (Valencia, CA).

SPE Sample Extraction

This bioanalytical sample extraction and LC-MS workflow is highlighted in Figure 1. For this work, 100 µL of the prepared (containing pain management drugs and drugs of abuse) urine calibrators and quality control samples (QCs), were extracted manually and with the Hamilton Microlab® STAR™(STAR) using an Oasis MCX SPE 96-well plate in the µElution format according to the protocol highlighted in Figure 2. The STAR deck layout and accessories used for SPE sample extraction is shown in Figure 3.

LC-MS/MS Conditions

LC-MS/MS quantification of the various analytes was performed using a Waters Xevo® TQ-S micro MS (ESI+). Chromatographic separation was achieved using an ACQUITY I-Class PLUS UPLC® system ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 100 mm column. Mobile phases A and B consisted of 0.1% formic acid in water and acetonitrile, respectively. A linear gradient from 2-67% B over 3.33 min was used at a flow rate of 0.6 ml/min.

Full details of the analytes and LC-MS conditions can be found at www.waters.com (Application Note Ref. 720006187EN).

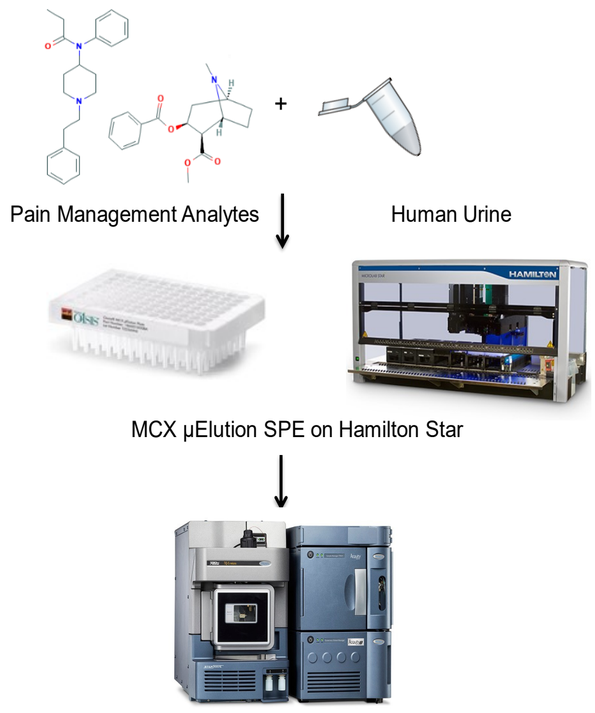


Figure 1. Standardized and automated sample preparation and LC-MS/MS bioanalytical workflow using Oasis MCX SPE 96-well plate in the µElution format with the STAR liquid handler to streamline the sample preparation process, maximize productivity, reduce errors, and improve analytical method performance.

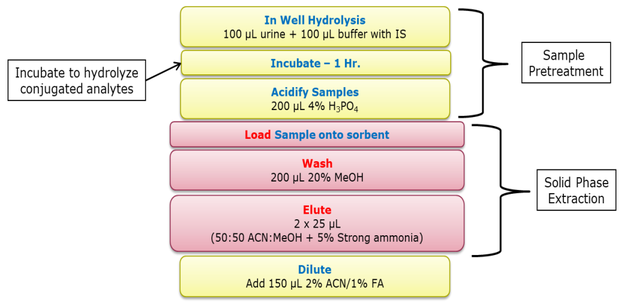
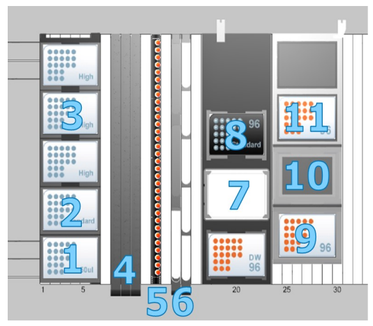


Figure 2. OASIS MCX SPE protocol for the extraction of various drugs of abuse and pain management drugs from urine, performed by the STAR liquid handler with SPE extraction time < 30 minutes.



1. 50 µL Tip Carrier
2. 300 µL Tip Carrier
3. 1000 µL Tip Carrier
4. Sample Tube Rack
5. IS Buffer Tube Rack
6. MCX Reagent Reservoir Holder
7. Hamilton Heater Shaker (HHS)
8. Tip Storage Location
9. Vacuum Manifold
10. MCX µElution SPE Plate Place Holder
11. 700 µL Round-Well Collection Plate

Figure 3. STAR deck layout for SPE sample extraction and purification using the OASIS MCX 96-well µElution SPE plate.

RESULTS

I. COMPARABLE AUTOMATED VS. MANUAL SPE EXTRACTION PERFORMANCE

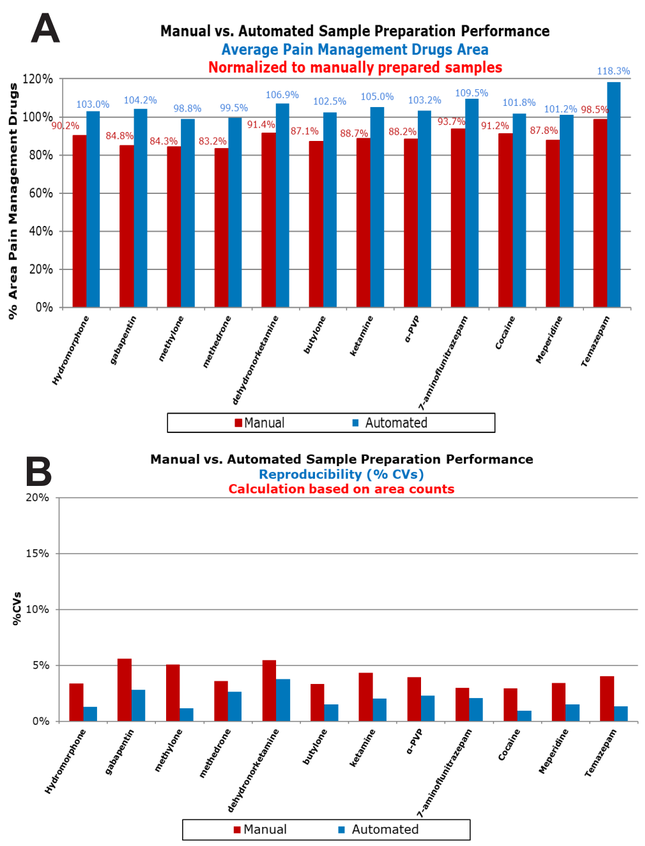


Figure 4. Comparable automated (STAR) vs. manual sample SPE performance using the OASIS MCX 96-well µElution SPE plate and LC-MS/MS analysis of pain management drugs. Panel A: Comparison of % drug area and Panel B: Reproducibility (%CV) comparison. These results demonstrate improved analyte recovery and improved CVs as compared to manual SPE extraction.

II. ACCURATE AND REPRODUCIBLE QUANTIFICATION USING SPE SAMPLE EXTRACTION PERFORMED WITH AN AUTOMATED LIQUID HANDLER

Standard Curve Performance

Drug/Analyte	Standard Curve Range (ng/mL)	Weighting	Linear Fit (r^2)	Mean % Accuracy Range
Hydromorphone	25-2,500	1/x	0.998	91.4-104.7
Gabapentin	10-1,000		0.997	89.7-104.9
Methylone	10-1,000		0.998	85.8-105.7
Methedrone	10-1,000		0.997	100.1-108.5
Butylone	10-1,000		0.996	97.5-108.2
Dehydronorketamine	10-1,000		0.996	97.4-111.5
Ketamine	10-1,000		0.996	96.2-109.3
Alpha-PVP	10-1,000		0.996	96.2-108.3
7-Aminoflunitrazepam	10-1,000		0.994	80.0-112.4
Cocaine	10-1,000		0.994	95.6-108.9
Meperidine	10-1,000		0.997	85.9-105.5
Temazepam	10-1,000		0.996	82.0-105.0

Table 2. Representative standard curves for select drugs of abuse and pain management drugs extracted from urine using the OASIS MCX 96-well µElution plate performed on the STAR liquid handler. Quantification performance was excellent with dynamic ranges from 10-1,000 ng/mL, linear fit, with R^2 values ≥ 0.99, and accuracy ranges within 15% meeting standard bioanalytical performance criteria for method validation.

Quality Control Performance

A

	Inter-Day Precision and Accuracy of QC Samples (3 Days)							
Expected QC Concentration	LOQC		LMQC		MQC		HQC	
(ng/mL)	15		75		250		750	
Analyte/Drug	%CV	% Accuracy	%CV	% Accuracy	%CV	% Accuracy	%CV	% Accuracy
Hydromorphone	0.58	103.2	0.31	107.2	0.47	105.9	0.80	103.0
Gabapentin	0.67	106.4	2.12	107.5	0.64	105.6	1.03	102.7
Methylone	1.07	105.7	0.40	107.3	0.75	106.2	0.67	104.1
Methedrone	0.53	107.2	1.06	109.0	0.78	106.5	0.59	104.7
Butylone	1.49	105.1	0.44	109.6	0.33	109.4	0.25	105.3
Dehydronorketamine	1.05	104.8	1.59	109.8	0.24	109.3	0.87	103.9
Ketamine	0.81	107.5	1.03	113.6	0.42	111.2	0.25	103.5
Alpha-PVP	1.57	105.9	0.69	111.3	0.36	108.9	0.15	103.1
7-Aminoflunitrazepam	1.33	110.2	1.94	109.3	0.59	102.9	0.65	101.7
Cocaine	1.28	107.4	0.41	114.2	0.34	111.1	0.41	103.7
Meperidine	0.32	105.4	1.09	107.4	0.20	106.9	0.58	103.1
Temazepam	0.80	109.1	1.00	110.9	1.19	109.8	0.55	106.1

B

	Intra-Day Precision and Accuracy of QC Samples, Day 3 (N=4)							
Expected QC Concentration	LOQC		LMQC		MQC		HQC	
(ng/mL)	15		75		250		750	
Analyte/Drug	%CV	% Accuracy	%CV	% Accuracy	%CV	% Accuracy	%CV	% Accuracy
Hydromorphone	2.47	97.6	1.66	106.4	1.19	104.6	2.17	100.2
Gabapentin	1.71	100.1	1.40	107.5	2.44	104.9	2.31	99.9
Methylone	1.17	97.5	1.63	109.9	1.58	108.6	2.19	104.6
Methedrone	2.15	100.7	1.82	109.7	1.31	108.9	1.54	105.4
Butylone	3.67	95.2	1.50	111.7	1.95	107.5	1.24	103.0
Dehydronorketamine	1.19	96.2	2.24	105.6	0.92	107.3	1.92	100.1
Ketamine	2.83	99.7	0.87	112.6	1.28	110.2	1.33	102.2
Alpha-PVP	3.51	96.6	1.31	110.6	1.97	107.2	1.68	99.6
7-Aminoflunitrazepam	2.20	103.6	1.11	106.2	0.50	95.2	2.51	93.7
Cocaine	4.04	99.6	2.35	112.9	1.29	110.5	1.79	100.7
Meperidine	1.77	99.6	2.05	102.5	1.72	105.0	1.50	98.7
Temazepam	2.64	98.6	1.21	110.2	3.22	111.9	2.22	102.7

Table 3. Inter (Panel A) and intra-day (Panel B) QC sample statistics for select drugs of abuse and pain management drugs extracted from urine using the OASIS MCX 96-well µElution plate performed on the STAR liquid handler. Inter and intra-day mean QC sample % accuracies ranged from 98.65-106.1 %, with CVs < 4.0 %, demonstrating a highly accurate and reproducible sample preparation and LC-MS analysis method.

STUDY HIGHLIGHTS

- A total sample preparation workflow (Figure 1), which is a robust, flexible, and scalable solution, addresses the increasing demand for accurate and reproducible quantification
- Rapid, simplified sample preparation of a comprehensive drug panel using a liquid handler and standardized SPE protocol, which streamlines the process, maximizing productivity, reducing errors, ensuring analytical method performance (Figure 2) and frees up the analysts time to perform other tasks.
- This standardized and automated approach yields excellent quantitative performance (Tables 2 and 3) with standard curve and QC accuracies between 98.65-106.8 % and mean CVs 2.5 % for the select drugs of abuse and pain management drugs extracted from urine.

CONCLUSION

Robust and accurate quantification of pain management drugs and drugs of abuse in urine was achieved, using a fully automated sample preparation workflow for SPE sample preparation with a standardized protocol.

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