Supelco®
Analytical Products

Application Note

The use of DPX HLB tips and Hamilton® robot for extraction of opioid drugs from urine

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Introduction

DPX stands for Dispersive Pipette Extraction, a patented technology that introduces the benefits of solid phase extraction into a revolutionary, easy-to-use pipette tip. This device is unique from all other SPE devices because adsorbent is loosely contained within the tip. The Hydrophilic-Lipophilic Balanced (HLB) adsorbent used in this application was specifically developed to provide retention and cleanup of both polar and non-polar compounds from aqueous samples.

Dispersive Pipette Extraction provides an INTip solution for complete sample preparation and can be easily automated. In this work, an analytical sample preparation procedure for the determination of 13 opioids in urine was developed using a Hamilton® STARlet platform, DPX tips cleanup followed by LC-MS/MS analysis. The automated extraction method can process multiple samples simultaneously in under 10 minutes thereby minimizing within-run sample variability and maximizing throughput. DPX HLB tips are available with different amounts of HLB Adsorbent including 3 mg, 5 mg, 10 mg, and 20 mg to process different sample volumes or to clean the samples with various levels of matrix impurities. In this work the DPX HLB tips in a Hamilton® format with 5 mg of HLB adsorbent were used. This tips configuration was found acceptable to process urine samples.

Method and Materials

Spiked samples were prepared in an amber vial by combining urine (12 mL), 100 mM sodium phosphate buffer pH 6.0 (3 mL), and 10 ku β-glucuronidase solution (3 mL). 400 µL of this was then removed and disposed before being replaced with analyte stock solution at 1–10 µg/mL in methanol (200 µL) and internal standard stock solution at 1 µg/mL in methanol (200 µL). Final spiked concentration of analytes in urine is 10–100 ng/mL with internal standards spiked at 10 ng/mL. These samples are then heated to 60 °C for 2 hours for the β-glucuronidase enzymes to hydrolyze any glucuronide metabolites to the parent drug. The samples (300 µL) are then transferred to a 96 well plate to be processed on a Hamilton® Microlab® STARlet. Additional well plates are filled with wash solution (400 µL of 5% methanol in water), and elution solvent (300 µL of 5% formic acid in methanol). A 60 mL reservoir is filled with 5% methanol in water as a conditioning solution. DPX tips containing 5 mg of HLB adsorbent in a 1 mL Hamilton® format are then conditioned by aspirating 750 µL of 5% methanol solution from a solvent reservoir. Sample solutions are aspirated and dispensed four times to bind the analytes of interest to the sorbent. Wash solution is then aspirated and dispensed three times to remove salts and other common matrix interferences. Target compounds are eluted by aspirating and dispensing the elution solution twice. 150 µL of the elution solvent is then diluted with 350 µL of water. LC-MS/MS analysis was performed using a Shimadzu 8030 triple quad mass spectrometer. Chromatographic separation was performed using Ascentis® Express Phenyl-Hexyl HPLC column (2.7 µm; 5 cm x 2.1 mm) with a 5 µL injection. The details for this chromatographic separation are shown in **Figure 2**.

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Results and Discussion

Analytical results were achieved that are accurate and precise. Calibrations were linear with correlation coefficients (R^2) greater than 0.99 over the concentration range of 0.2–15 ng/mL for fentanyl and 2–150 ng/mL for all other analytes. **Table 1** and **Figure 3** present the results for recovery of pain drugs out of urine at 100 ng/mL spike levels for all analytes except fentanyl at 10 ng/mL. Good recovery values were achieved for all compounds between 78–111%. Relative Standard Deviations (%RSDs) were calculated using 8 replicate extractions and were under 11.2% for all compounds.

Conclusion

This DPX HLB method can process multiple samples in under 10 minutes allowing for a fast, automated, and high throughput workflow. The method is robust, linear, and provides the necessary sensitivity to meet most laboratories' needs.

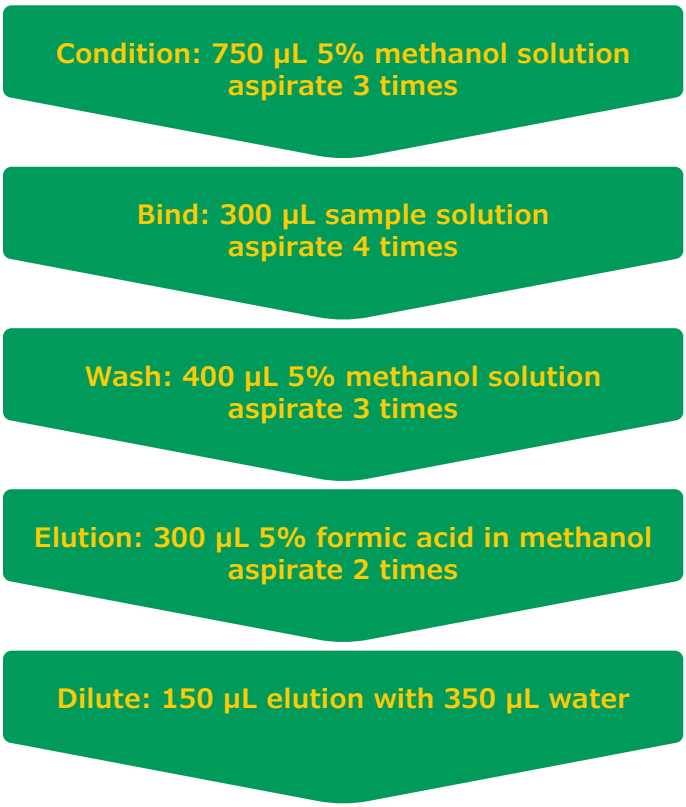

Figure 1. Flow chart for DPX sample preparation method

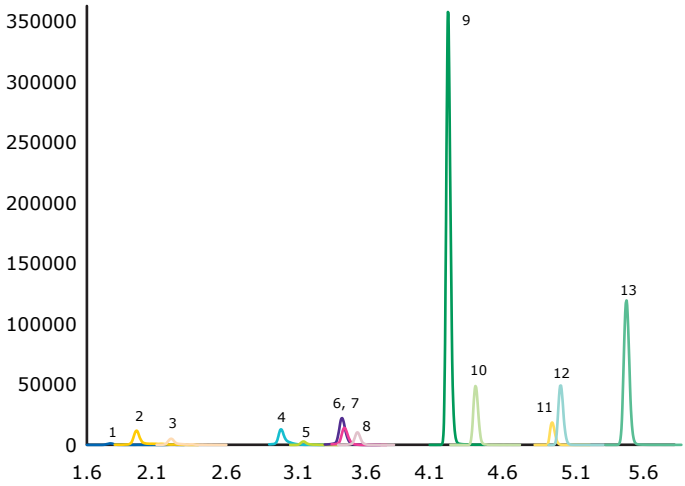

Figure 2.

Column	Ascentis® Express Phenyl-Hexyl column 10 cm x 2.1 mm, 2.7 µm (53336-U)
Mobile phase A	water with 0.1% formic acid
Mobile phase B	methanol with 0.1% formic acid
Column Temp	30 °C
Inj. Vol	5 µL
Flow Rate	0.4 mL/min
Gradient	5 to 20% B in 2.25 minutes; to 60% B in 2.25 minutes; held for 1.5 mins; to 95% B in 0.1 minutes; held for 1.4 mins; reset to 5% for 3.4 mins to re-equilibrate.

Peak	Analyte	Concentration (ng/mL)
1	morphine	50
2	oxymorphone	50
2-IS	oxymorphone-D3	10
3	hydromorphone	50
4	naloxone	50
5	codeine	50
6	naltrexone	50
7	oxycodone	50
7-IS	oxycodone-D3	10
8	hydrocodone	50
8-IS	hydrocodone-D3	10
9	tramadol	50
9-IS	tramadol-D3	10
10	meperidine	50
10-IS	meperidine-D4	10
11	fentanyl	5
12	buprenorphine	50
13	methadone	50
13-IS	methadone-D9	10

Analyte	Internal Standard	N	Recovery (%)	RSD (%)
morphine	oxymorphone-D3	8	106.8	7.8
oxymorphone	oxymorphone-D3	8	93.8	9.3
hydromorphone	oxymorphone-D3	8	111.3	6.1
naloxone	oxycodone-D3	8	87.4	7.9
codeine	oxycodone-D3	8	78.2	7.3
oxycodone	oxycodone-D3	8	102.5	6.8
naltrexone	oxycodone-D3	8	91.2	7.0
hydrocodone	hydrocodone-D3	8	93.3	11.2
tramadol	tramadol-D3	8	101.4	2.4
meperidine	meperidine-D4	8	97.9	4.7
fentanyl	meperidine-D4	8	104.2	4.2
buprenorphine	meperidine-D4	8	84.7	3.0
methadone	methadone-D9	8	98.9	4.4

Table 1. Analyte recovery

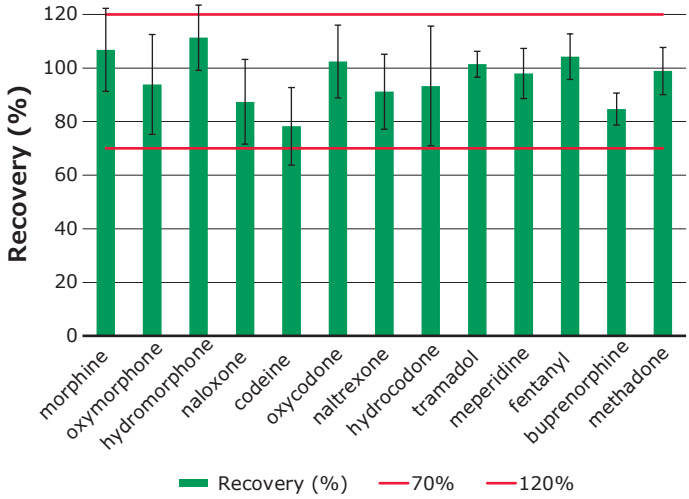

Figure 3. Chart

Reagents and consumables

Catalogue Number	Description
52984-U	SUPEL™ SWIFT HLB - DPX TIP 5 mg HAMILTON® 1 mL PK96

Catalogue Number	Description	
O-003	Oxymorphone-D3 solution	100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®
O-005	Oxycodone-D3 solution	100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®
M-038	Meperidine-D4 solution	1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®
M-088	(±)-Methadone-D9 solution	100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®
H-005	Hydrocodone-D3 solution	100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®
T-029	cis-Tramadol-13C, D3 hydrochloride solution	100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®
P-071	Pain Management Multi-component Opiate Mixture-13 solution	100 µg/mL each component (10 µg/mL Fentanyl), ampule of 1.0 mL, certified reference material, Cerilliant®
1.06035	Methanol	hypergrade for LC-MS LiChrosolv®
33015	Formic acid	puriss. p.a., ACS reagent, reag. Ph. Eur., ≥98%
SRE0093	β-Glucuronidase from limpets (Patella vulgata)	aqueous solution
S9638	Sodium phosphate monobasic monohydrate	ACS reagent, ≥98%
S9390	Sodium phosphate dibasic heptahydrate	ACS reagent, 98.0–102.0%
5.33003	Ammonia solution 25%	for LC-MS LiChropur™
P5368	Phosphate buffered saline	BioPerformance Certified, pH 7.4
52989-U	SUPEL™ SWIFT HLB - DPX TIP 5 mg UNIVERSAL 1 mL PK96	
52992-U	SUPEL™ SWIFT HLB - DPX TIP 10 mg HAMILTON® 1 mL PK96	
52995-U	SUPEL™ SWIFT HLB - DPX TIP 10 mg UNIVERSAL 1 mL PK96	
52997-U	SUPEL™ SWIFT HLB - DPX TIP 20 mg HAMILTON® 1 mL PK96	
52999-U	SUPEL™ SWIFT HLB - DPX TIP 20 mg UNIVERSAL 1 mL PK96	
53001-U	SUPEL™ SWIFT HLB - DPX TIP 3 mg MICROELUTION HAMILTON® 300 µL PK96	

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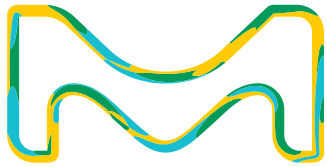

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