High Throughput SPE and LC-MS/MS Methods for Drugs of Abuse in Urine

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Introduction

The United States is currently under opiates epidemic, leading to an increase in demand for drug testing. At the same time, cuts in reimbursements from insurance companies are driving clinical labs to find ways to cut the cost and time required for sample processing. Here, the sample preparation process was examined in an effort to address this goal. The dilute and shoot method is fast and cheap, but it causes more downtime due to increased mass spectrometer cleaning, and it reduces the column lifetimes. Orochem has chosen to optimize the solid phase extraction plate's sorbent weight and the elution solvent with HPLC mobile phases.

Instruments and Materials

- **Instruments:** All automated extractions were carried out using Orochem's Oroflex Personal Pipettor Robot.

All LC-MS/MS methods used AB Sciex Exion LC system coupled to API 4500 mass spectrometer with a turbo ion spray ESI source.

- **Materials:** Panthera Deluxe Polymeric SPE plates (Orochem Technologies Inc) were used for all extractions.

Drugs' standards and their isotope internal standards (IS) were purchased from Cerilliant. *E. Coli* β-glucuronidase (BG) was purchased from Campbell. Mass spec grade methanol, acetonitrile, water and formic acid (FA) were purchased from Pharmco- Aaper. Ammonium acetate buffer was purchased from Sigma-aldrich. Orochem's EZYPRESS HT 96-well plate positive pressure manifold unit was used for conditioning, washing and processing of SPE plates. Evaporation was carried out using Quikvap 96-well plate evaporator (Orochem Technologies Inc). Human urine was fortified with standards.

Procedures

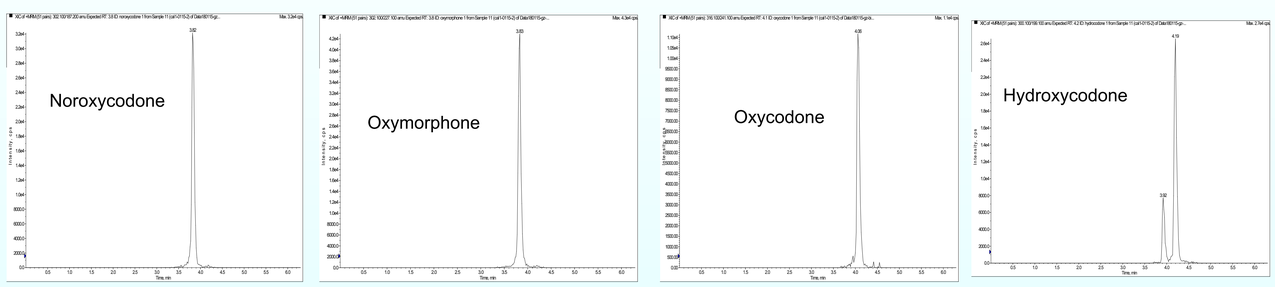
- **Barbiturates and THC carboxylic acid (THCA) panel in urine (negative panel):** Each well was conditioned with 1 mL of methanol and 1 mL of water, loaded with 0.1 mL of fortified urine, mixed with 0.05 mL of IS spiking solution, vortexed with 0.1 mL of a mix of 200 mM ammonium acetate pH 6.8 buffer and a BG solution, incubated (55 deg C, 30 min), loaded on SPE, washed with water and mixture of methanol/water, and eluted with methanol solvent. Elute was evaporated and reconstituted in mobile phase prior to analysis.

- **Main pain management drug panel in urine (positive panel):** Each well was conditioned with 1 mL of acetonitrile and 1 mL of water, loaded with 0.6 mL of enzyme hydrolyzed fortified urine (see above method) mixed with 0.05 mL of IS spiking solution. After loading, SPE was washed with water and mixture of methanol/water, and eluted with 0.5 mL of 70% acetonitrile. Elute was diluted with 1/1 0.1% formic acid/water, and analyzed directly.

- **HPLC-MS/MS conditions for negative panel:** Orochem Gazelle C18 column (2.1 x 50 mm, 1.7 µm) was used with a 30-95% methanol (0.1% FA) gradient mobile phase and Orochem Gazelle C18 guard column. Analysis was performed in ESI negative ion mode. Total run time was 7 minutes.

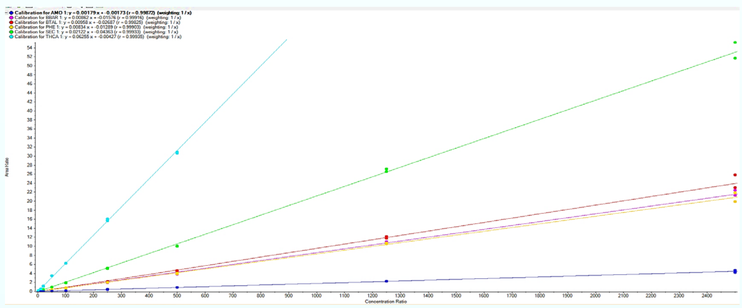
- **HPLC-MS/MS Conditions for positive panel:** Orochem Gazelle biphenyl column (3.0 x 50 mm, 1.7 µm) was used with a 30-90% methanol (0.1% FA, 20 mM ammonium acetate) gradient mobile phase and Orochem Gazelle biphenyl guard column. Analysis was performed in ESI positive ion mode. Total run time was 7 minutes.

Results



Mass chromatograms of SPE extracts of some opiate drugs and metabolites from fortified urine

Results



Linear regression for barbiturates and THCA

	LOD (ng/mL)	LLOQ (ng/mL)
AMO	0.36	1.08
BBAR	0.83	2.51
BTAL	0.41	1.25
PHE	0.37	1.14
SEC	0.46	1.39
THCA	0.14	0.42

	TARGET VALUES			ACCURACY & PRECISION: n= 15 (5 per level x 3 days)								
	LQC	MQC	HQC	AVE (L)	STDEV	CV %	AVE (M)	STDEV	CV %	AVE (H)	STDEV	CV %
AMO	40	80	1000	44.3	1.6	3.6%	81.6	3.4	4.2%	1015.8	38.7	3.8%
BBAR	40	80	1000	43.0	0.8	2.0%	80.1	2.6	3.2%	1002.3	21.9	2.2%
BTAL	40	80	1000	43.9	1.3	3.0%	80.9	3.6	4.4%	1017.7	33.0	3.2%
PHE	40	80	1000	43.7	1.1	2.6%	81.1	2.6	3.2%	1010.8	31.5	3.1%
SEC	40	80	1000	44.4	1.1	2.4%	81.8	3.4	4.1%	1022.6	25.8	2.5%
THCA	8	16	200	8.3	0.2	2.6%	16.0	0.5	2.9%	204.4	5.6	2.7%

MATRIX EFFECT	
CUT-OFF SPIKE (50 and 10^{THCA} ng/mL)	Mean absolute bias (n=10 blank urines)
AMO	7.8%
BBAR	3.8%
BTAL	4.9%
PHE	5.7%
SEC	4.9%
THCA	10.5%

MATRIX INDUCED ION SUPRESSION AND ENHANCMENT (n=3)		
Analyte	Ratio in water	Ratio in urine
AMO	0.0752	0.0692
BBAR	0.3137	0.3244
BTAL	0.3539	0.3550
PHE	0.3463	0.3262
SEC	0.7882	0.8073
THCA	0.6629	0.5714

Recovery test for pain drug panel (positive ion mode):

Name	Recovery%	Name	Recovery%	Name	Recovery%
6-MAM	84.3	lorazepam	102.2	normeperidine	83.3
amphetamine	81.1	MDMA	100.5	noroxycodone	90.9
benzoylecgonine	96.1	meperidine	84.5	o-desmethyltramadol	115.6
buprenorphine	73.1	meprobamate	91.2	oxazepam	104.2
carisoprodol	91	methadone	75.5	oxycodone	84.9
codeine	82.1	methamphetamine	92.5	oxymorphone	81.2
cotinine	91.3	morphine	81.1	PCP	115
EDDP	82.1	N-desmethyltapentadol	82.6	tapentadol	83.9
fentanyl	82.5	norbuprenorphine	101.6	temazepine	100.2
hydrocodone	82.7	nordiazepam	83.8	zolpidem	100.2
hydromorphone	81.5	norfentanyl	116.9	α-hydroxyalprazolam	102.4
ketamine	89.3	norhydrocodone	82.6	α-hydroxymidazolam	101.1
		norketamine	74.2		

Conclusions

We developed fast and reliable SPE methods for analysis main drug panels including THCA and barbiturates in urine toxicology setting. The improved methods demonstrated reduced matrix effect and expended a lower end of AMR for almost an order of magnitude. The sample processing time per plate is about 15 minutes, while maintaining the total cost of sample preparation at a very competitive level.

Acknowledgement

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