

High-performing Novel SPE Polymers Show Efficient Recoveries and Reduced Sample Preparation Times

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Introduction

The United States is currently under opiates epidemic, leading to an increase in demand for opiate drug testing in urine samples. At the same time, cuts in reimbursement rates from insurance companies are driving clinical labs to find ways to cut the cost and time required for sample processing. In this abstract, 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 Exciion LC system coupled to API 4500 mass spectrometer with a turbo ion spray ESI source operated in positive ion mode.

•**Materials:** Panthera Deluxe Polymeric SPE cartridges, 10 and 20 mg/cc (Orochem Technologies Inc) were used for all extractions. Twenty opiates drug standards and their isotope internal standards (IS) were purchased from Cerilliant. BG100 β -glucuronidase was provided by Biotec-La Piedra Biotechnologia SpA (Chile). 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.

Capacity and Recovery Tests

•**Sample preparation :** twenty analytes were spiked to blank urine at MOQ and ULOQ levels

•**Enzyme Hydrolysis:** Mix different volume of fortified urine with master mix (ammonium acetate buffer, glucuronidase, and IS) at 1:1 (v/v) ratio, incubate at 68 °C for 30 min.

Capacity Tests for 10 mg and 20 mg/cc cartridges

1. Condition Panthera Deluxe SPE cartridges with 1 mL of acetonitrile, followed by 1 mL of water.
2. Load 0.4 mL of hydrolyzed sample (ULOQ level), collect the flow thru. Label it as Load 1.
3. Load additional 0.2 mL of samples, collect the flow thru. This is Load 2.
4. Repeat step 3, and this is Load 3.
5. Inject Load 1-3 directly to UHPLC-MS/MS.

Recovery Tests of different volume of elution solution for 20 mg/cc cartridges:

1. Condition Panthera Deluxe SPE cartridges with 1 mL of acetonitrile, followed by 1 mL of water.
2. Load 0.8 mL of hydrolyzed sample (MLOQ level)
3. Wash with 0.5 mL of water followed by 0.5 mL of 5% methanol
4. Elute with 0.5 mL of 70% acetonitrile and collect it, added 0.75 mL of 0.1% FA/water to it, then dilute it 1:1 with 0.1% FA/water again. label it as Elute 1
5. Elute additional 0.5 mL of acetonitrile and collect it, added 0.75 mL of 0.1% FA/water to it, then dilute it 1:1 with 0.1% FA/water again. label it as Elute 2.
6. Inject Elute 1 and 2 directly to UHPLC-MS/MS

HPLC-MS/MS Conditions:

Column: Orochem Gazelle C18 UHPLC column 3x50 mm, 1.7 μ m.

Mobile phases: A: 5% methanol, 95% 20 mMNH₄AC/water, 0.1% FA; B: 0.1% FA/methanol. Gradient from 30% to 90% B in 3 min, and to 96% in 4 min

Flow rate: 0.3 mL/min

Injection volume: 10 μ L

Mass spectrometer: API4500, ESI+

Results



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

1) Capacity Test Results:

- **10 mg/cc cartridge:** most of the analytes show less than 1% loss with loading 0.4 mL of samples. After loading 0.6 mL of samples, the losses are more than 3% for most of the analytes, 3 analytes show over 5% losses. So, we recommend the loading capacity for 10 mg/cc cartridge is limited to 0.4 mL of sample (including buffer and enzyme)
- **20 mg/cc cartridge:** After loading 0.6 mL of sample, only 3 analytes show over 3% loss; two of them has 2-3% additional loss after loading 0.8 mL of sample. So, we recommend the maximum loading capacity for 20 mg/cc cartridge is about 0.8 mL of sample.

2) Recovery Test Results:

Except o-desmethyltramadol, all other analytes elute out by 0.5 mL of 70% ACN. Only o-desmethyltramadol shows additional 3% recovery at 2nd elution.

Compound	Recovery %	Matrix Effect %
6-MAM	84.3	96.5
Buprenorphine	73.1	125.7
Codeine	82.1	99.6
EDDP	82.1	104.5
Fentanyl	82.5	112.7
Hydrocodone	82.7	101.6
Hydromorphone	81.5	95.0
Meperidine	84.5	97.9
Methadone	75.5	107.5
Morphine	81.1	91.3
N-desmethyltapentadol	82.6	97.8
Norbuprenorphine	101.6	84.7
Norfentanyl	83.8	104.3
Norhydrocodone	82.6	98.9
Normeperidine	83.3	107.4
Noroxycodone	80.4	99.6
O-desmethyltramadol	83.3	92.9
Oxycodone	84.9	107.5
Oxymorphone	81.2	99.7
Tapentadol	83.9	92.6

Conclusions

For most of the opiates tested, 20 mg bed weight proved to be sufficient. The evaporation step was eliminated by eluting with 0.5 mL of ACN. Dilution with water is crucial for morphine and hydromorphone peak shapes.