



## SPEware uSPE (ultraSPE) Faster / Cleaner Sample Preparation

### Opiates from Urine (Including Synthetics)

For GC or GC-MS confirmations using:  
Trace-B 35mg.

#### 1. PREPARE SAMPLE

##### ACID/AUTOCLAVE HYDROLYSIS OF GLUCURONIDE:

To 1.0 ml of urine add internal standard(s)\* and 500 ul concentrated HCl followed by 0.5 ml 10% Methoxyamine. Mix/vortex.

Autoclave 20 minutes at 121 °C. Cool before proceeding.

Add 0.5 ml 45% KOH Mix/vortex

Add 0.5 ml saturated KHCO<sub>3</sub> solution slowly to avoid forming.

Mix/vortex Centrifuge for 5 minutes at 2000 rpm.

#### 2. APPLY SAMPLE

Decant the supernatant into the Trace-B column and draw through the column at a flow of 5-10 ml/minute.

#### 3. WASH COLUMN (All wash steps flow at 5-10 ml/min)

1 x 1ml pH 9.0 buffer\*\*\*

1 x 1 ml DI H<sub>2</sub>O;

1 x 1 ml 100 mM HCl

1 x 1 ml CH<sub>3</sub>OH

Dry for 2 minutes at 25 psi

#### 4. ELUTE OPIATES

1 x2.0 ml Ethyl Acetate containing 2% NH<sub>4</sub>OH; collect eluate at 1 to 2 ml/minute.

NOTE: Prepare elution solvent daily.

#### 5. DRY ELUATE

Evaporate to dryness at 40 °C.

#### 6. DERIVATIZE

Add 50 ul ethyl acetate vortex and transfer to a/s vial (**This transfer step is a very important clean up step. Do not omit.**)

Add 50 ul MSTFA. (Important—do not add MSTFA to dry down vial)

React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate MSTFA solution.

#### 7. QUANTITATE

Inject 1 to 3 ul sample onto chromatograph.

Monitor the following ions (GC/MS):

TMS-Codeine: 371\*\*, 372, 343 TMS- D3 -Morphine : 432\*\*, 417

TMS-D3 -Codeine: 374\*\*, 346 TMS-Morphine: 429\*\*, 414, 401

TMS-Hydrocodone: 328\*\*, 297, 271 TMS-Hydrocodone-D3: 331\*\*, 300

TMS-Hydromorphone: 386\*\*, 355, 371 TMS-Hydromorphone-D3: 389\*\*, 358

\* Suggested internal standards for GC/MS: D3 -Codeine, D3 -Morphine

\*\* Quantitation ion

\*\*\* (Prepared by dissolving 20 g of KHCO<sub>3</sub> and 10g of K<sub>2</sub>CO<sub>3</sub> in 500 ml of water, adjust the pH to 9.0 then bring the volume to 1,000 ml.)