



# SPEware uSPE (ultraSPE) Faster / Cleaner Sample Preparation

## Opiates from Saliva (Including Synthetics)

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

### 1. PREPARE SAMPLE

To 0.3ml saliva sample add internal standard then add the following in sequence:

1.0ml 0.1M Acetate Buffer pH4.5

0.5ml 2.4N HCL

0.25ml 10% methoxyamine and vortex for 20 secs

Incubate at room temperature for 15 minutes( **Note: Longer incubation will lower recovery values**)

### 2. APPLY SAMPLE

1x 3ml Methanol

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

Decant the sample into the column and draw through the column at a flow of 1-2ml/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 x 2.0 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 )