



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

### Cocaine and Benzoyllecgonine from Whole Blood

For GC or GC-MS confirmations using:  
Trace-B 50mg.

#### 1. PREPARE SAMPLE

To 1 ml of blood sample add internal standards(s) and 3 ml of 100 mM phosphate buffer (pH = 6.0)  
Mix/Vortex for 30 seconds. Sonicate sample in an ultrasonic bath for 5 minutes. Centrifuge sample at 3000 rpm for 5 minutes

#### 2. APPLY SAMPLE

Transfer the supernatant into the Trace-B column and flow through the column at 2-5 ml/min

#### 3 WASH COLUMN (All wash steps flow at 2-5 ml/minute)

2 ml DI H<sub>2</sub>O;  
2ml pH 9.0 buffer (Prepared by dissolving 20 g of KHCO<sub>3</sub> and 10 g 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 1000 ml.)  
2 ml 100 mM HCl  
2 ml CH<sub>3</sub>OH;  
2ml Ethyl Acetate  
Dry column (3 min at 20 psi N<sub>2</sub>)

#### 4 ELUTE COCAINE AND BENZOYLECGONINE

2.0ml (78/20/2//Dichloromethane/IPA/NH<sub>4</sub>OH); collect eluate at 1-2 psi  
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  
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):

Cocaine: 182, 198, 303  
TMS-BE: 240, 256, 361

D3-Cocaine: 185, 201, 306  
TMS-D3-BE: 243, 259, 364