



SPEware uSPE (ultraSPE) Faster / Cleaner Sample Preparation

Benzoylecgonine from Urine

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

1. PREPARE SAMPLE

To 2 ml of urine sample add internal standard(s) and 0.5 ml of 100 mM phosphate buffer (pH = 6.0). Mix/Vortex. (Centrifuge sample for 2 minutes if cloudy.)

2. APPLY SAMPLE

Transfer the supernatant into the column and flow through the column at a flow rate of 10–20 ml/min.

3. WASH COLUMN (All wash steps flow at 1.0 ml/min)

1.0 ml DI H₂O;
1.0 ml 100 mM HCl
Dry column at 25 psi for 2 minutes
1 ml CH₃OH;
1ml Ethyl Acetate
Dry column at 25 psi for 2 minutes

4. ELUTE BENZOYLECGONINE

1 x 2.0ml CH₂Cl₂/IPA/NH₄OH (80:18:2); collect eluate at 1 ml/minute or allow to drip by gravity flow.
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):

TMS-BE: 240, 256, 361 TMS-d3-BE: 243, 259, 364