



ANABOLIC STEROIDS IN URINE FOR GC OR GC/MS CONFIRMATIONS USING:

200 mg CLEAN SCREEN[®] EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE - β -GLUCURONIDASE HYDROLYSIS

To 5 mL of urine add internal standard(s)* and 2 mL of β -Glucuronidase.

β -Glucuronidase: 5,000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0).

Mix/vortex.

Hydrolyze for 3 hours at 65°C. Cool before proceeding.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Adjust sample pH to 6.0 \pm 0.5 with approximately 700 μ L of 1.0 N NaOH.

2. PREPARE CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 1 mL 100 mM phosphate buffer (pH= 6.0).

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 to 2 mL/minute.

4. WASH COLUMN

1 x 3 mL 10% (v/v) CH₃OH in D.I. H₂O.

Dry column (5 minutes at > 10 inches Hg).

1 x 1 mL hexane or hexane/ethyl acetate (50:50).

** Part # SMSTFA-0-1,10, 25, 100

5. ELUTE ANABOLIC STEROIDS (Choose a, b, c or d)

a. 1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2); Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily. Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

b. 1 x 3 mL CH₂Cl₂/IPA (80:20).

c. 1 x 3 mL ethyl acetate.

d. 1 x 3 mL CH₃OH.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 μ L ethyl acetate and 50 μ L MSTFA** (with 3% trimethylsilyliodide).

Over layer with N₂ and cap.

Mix/vortex.

React 20 minutes at 70°C.

Remove from heat source to cool.

NOTE: Do not evaporate MSTFA solution.

8. QUANTITATE

Inject 1 to 2 μ L onto chromatograph.

Monitor the following ions (GC/MS):

<u>Compound</u>	<u>Primary*</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>OTHER</u>
Testosterone-TMS	432	301	209	
19-Nortetiocholanone-TMS	405	315	225	
Oxymethalone	640	52	462	370,143
Dehydroepiandrosterone-2TMS	432	327	297	
10-Nortestosterone-2TMS	418	287	194	
Oxymethalone Metabolite #1	640	52	462	143
Oxymethalone Metabolite #2	625	462	370	143
11- β -Hydroxyandosterone	522	417	158	
Methandienone	409	313	281	
19-Norandosterone-2TMS	405	315	225	
Alpha-Hydroxyetiocholanone	504	417		
17- α -Epiandrosterone-TMS	432	341	327	209
Stanozolol	472	381	342	149

*Quantitation Ion