



ANABOLIC STEROIDS IN URINE BY LC-MS/MS OR GC-MS CLEAN SCREEN[®] DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN[®] DAU, 200 mg, 10mL Tube

BETA-GLUC-10 - Selectrazyme[®] Beta-glucuronidase

SMSTFA-1-1 – SELECTRA-SIL[®] MSTFA w/ 1% TMCS

SLDA50ID21-5UM - Selectra[®] DA HPLC Column, 50 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:

To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme[®] β -glucuronidase.

Optionally, add 1 mL of acetate buffer and 25-50 μ L of concentrated β -glucuronidase.

Vortex and heat for 3 hours at 65°C.

Allow sample to cool

Adjust sample pH to 7.0 \pm 0.5 with approximately 3-4mL of D.I. H₂O

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

NOTE: Aspirate at full vacuum or pressure

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 (10 minutes at > 10 inches Hg).

5. ELUTE ANABOLIC STEROIDS (Choose a or b):

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).

6. DRY ELUATE:

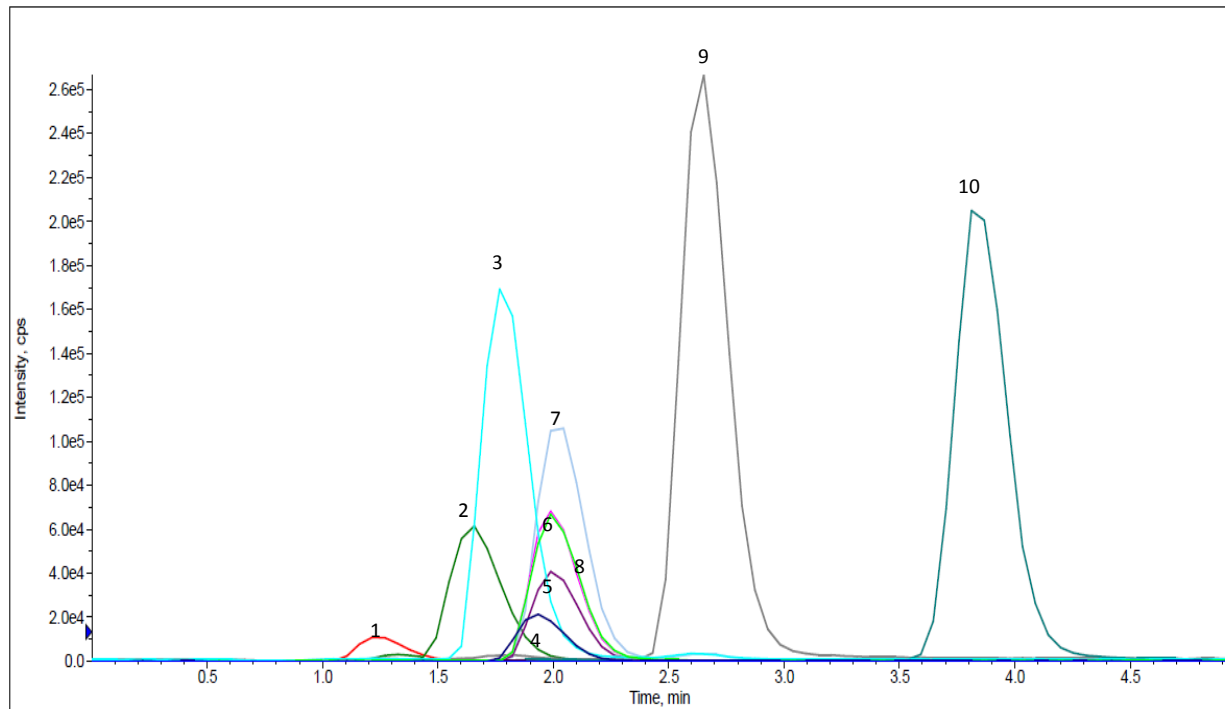
Evaporate to dryness at < 40°C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and
50 μ L MSTFA (with 1%TMCS)
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 -2 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Cortisone	363.2	121.1	1.24
2. 11-Deoxycortisone	347.1	97.1	1.65
3. Boldenone	287.0	121.0	1.78
4. 17-OH Progesterone D ₈	339.5	100.1	1.93
5. 17-OH Progesterone	331.3	97.1	1.99
6. Testosterone-D ₃	292.0	97.1	2.00
7. Testosterone	289.3	97.1	2.02
8. Nandralone	275.0	109.0	2.00
9. Androstendione	287.3	97.1	2.64
10. Progesterone	315.3	97.1	3.84

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.35mL/minute

Polarity: Positive

Reconstitute: 100µl

Injection Volume: 10µl

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

LC Column: Selectra® DA HPLC Column 50 x 2.1mm 3µm

Isocratic:

Time	%A	%B
0.00	20	80
5.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
Testosterone-TMS	432	301	209
19-Noretiocholanone-TMS	405	315	225
Oxymethalone	640	52	462
Dehydroepiandrosterone-2TMS	432	327	297
10-Nortestosterone-2TMS	418	287	194
Oxymethalone Metabolite #1	640	52	462
Oxymethalone Metabolite #2	625	462	370
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
Stanozolol	472	381	342