SPE Method for Anabolic Steroids

Application Brief

Condition: Add 5 mL methanol to the cartridge. Apply vacuum and discard the eluant. Repeat with 5 mL water. Do not allow the sorbent to go dry at any point during this step.

Load: Attach reservoir to the cartridge using the coupling adaptor. Add 10 mL urine or 4 mL serum to the cartridge. Apply vacuum so that sample takes 30–60 seconds to pass through the cartridge. Serum may be diluted 1/1 with water to aid in handling.

Rinse: Add 3 mL 50% methanol to the cartridge. Apply vacuum and discard eluant. Leave vacuum on for 30 seconds after all of the 50% methanol has passed through the cartridge. Centrifuge the cartridge at 1000–1500 rpm for 5 minutes.[1]

Elution: Place a collection tube beneath the cartridge. Add 3 mL methanol to the cartridge. Apply vacuum and collect the eluant. Concentrate to dryness using gentle heat (<45°C) and under a stream of nitrogen (transfer to a small vial may be necessary). Dissolve the residue in 100 µL methanol. Inject 1–2 µL into GC.

[1] *Centrifuging removes additional water which aids in sample concentration.*

Equipment

- AccuBOND II ODS (C18) 6 mL/500 mg cartridge (P/N 188-1356)
- coupling fitting (P/N 5185-5794)
- 25 mL sample reservoir (P/N 700-4007)
- vacuum manifold (P/N 5185-5754, 10-port) (P/N 5185-5765, 20-port)

Reagents

- water (HPLC grade)
- methanol (HPLC grade)

Compound	k	$\bar{x}$	Std. Dev.
Dehydroisoandrosterone (prasterone)	9.55	76	7
5α-Androstan-17β-ol-3-one (stanolone)	9.85	22	6
19-Nortestosterone (nandrolone)	9.95	70	5
Mesterolone	10.24	90	8
Testosterone	10.37	70	4
1-Dehydrotestosterone (boldenone)	10.51	98	6
17α-Methyltestosterone	10.55	76	5
1-Dehydro-17α-methyltestosterone (methandrostenolone)	10.77	76	6
Norethandrolone	10.97	77	5
1-Dehydrotestosterone acetate	11.30	81	4
19-Nortestosterone-17-propionate	11.35	74	9
4-Chlortestosterone-17-acetate (clostebal)	12.47	60	8
Stanozolol	12.76*	46	10
1-Dehydrotestosterone benzoate	15.22	57	17
19-Nortestosterone-17-decanoate	15.48	59	9
1-Dehydrotestosterone undecylenate	16.60	55	12

**poor peak shape*

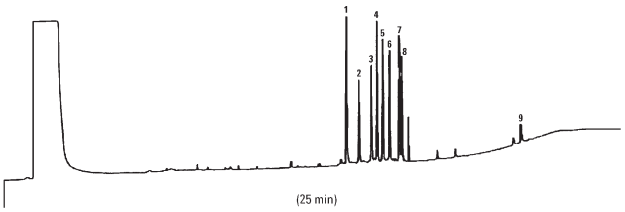
k = partition ratio (a measure of retention)

$\bar{x}$ = % recovery

Steroid Extract from Urine

Column:	DB-1 30 m x 0.25 mm I.D.
P/N:	122-1031
Film Thickness:	0.1 µm
Carrier:	Helium at 40 cm/sec (measured at 55°C)
Oven:	55°C for 1 min 55°C–175°C at 30°C/min 175°C–325°C at 10°C/min
Injector:	Splitless; 275°C 45 sec. purge activation time
Detector:	FID; 300°C

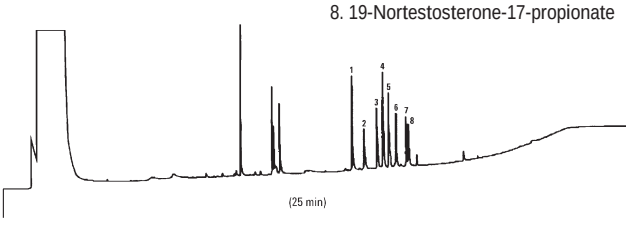
1. Dehydroisoandrosterone (prasterone)
2. 10-Nortestosterone (nandrolone)
3. Testosterone
4. 17α-Methyltestosterone
5. 1-Dehydro-17α-methyltestosterone (methandrostenalone)
6. Norethandrolone
7. 1-Dehydrotestosterone acetate
8. 19-Nortestosterone-17-propionate
9. 1-Dehydrotestosterone benzoate



Steriod Extract from Serum

Column:	DB-1 30 m x 0.25 mm I.D.
P/N:	122-1031
Film Thickness:	0.1 µm
Carrier:	Helium at 40 cm/sec (measured at 55°C)
Oven:	55°C for 1 min 55°C–175°C at 30°C/min 175°C–325°C at 10°C/min
Injector:	Splitless; 275°C 45 sec. purge activation time
Detector:	FID; 300°C

1. Dehydroisoandrosterone (prasterone)
2. 10-Nortestosterone (nandrolone)
3. Testosterone
4. 17α-Methyltestosterone
5. 1-Dehydro-17α-methyltestosterone (methandrostenolone)
6. Norethandrolone
7. 1-Dehydrotestosterone acetate
8. 19-Nortestosterone-17-propionate



To order or for technical assistance, contact your local authorized Agilent distributor, or visit our website at www.agilent.com/chem.

Printed in the U.S.A.
October 25, 2001
5988-4061EN

Agilent Technologies