



Comparison of SPE vs. SLE for the Quantitative Analysis of Anabolic Steroids in Serum Using LC-MS/MS

UCT Part Numbers

CUQAX22Z

Clean-Up® C8 + QAX
200 mg, 10 mL cartridge

SLC-18100ID21-3UM

Selectra® C18 HPLC
100 X 2.1 mm, 3 µm

SLC-18GDC20-3UM

Selectra® C18 Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR

Guard Column Holder



Summary:

Analysis of anabolic steroids in serum typically requires a sample pre-treatment step such as solid-phase extraction (SPE) prior to instrumental analysis. Traditional SPE-based methods utilize C18 or alternative reversed phase sorbents to retain the hydrophobic steroids. Alternatively, the use of a supported liquid extraction, or SLE, is also gaining popularity for this panel of compounds.

UCT's approach for the analysis of anabolic steroids from serum utilizes a traditional reversed phase interaction, but also features the addition of strong-anion exchange functionality (QAX) within the sorbent. This added functionality aids in the removal of unwanted matrix components commonly found in serum such as amino acids and inorganic ions. To prove the effectiveness of this approach, a comparison study was conducted using UCT's recommended sorbent for steroid analysis (C8 + QAX) versus a traditional SLE, diatomaceous earth sorbent.



CLINICAL



FORENSICS

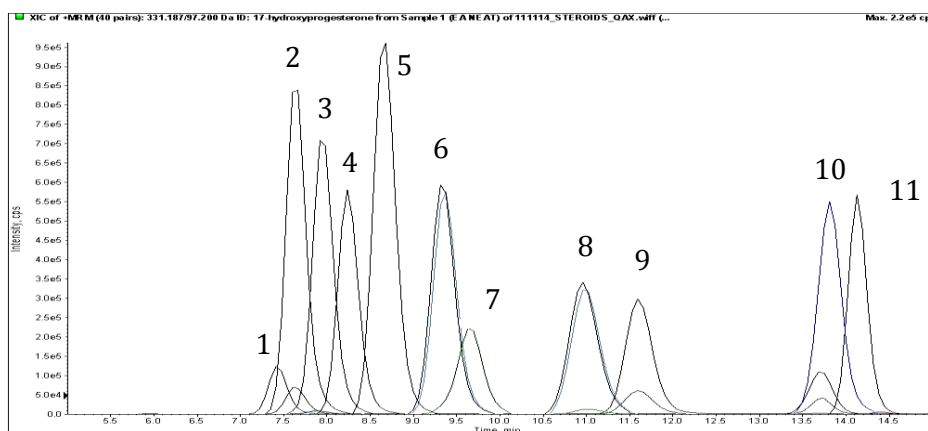
Sample Pretreatment:

To 200 µL serum sample add 100 µL 0.1 N HCl and appropriate amount of internal standard.

SPE Procedure (C8 + QAX):

1. Precondition SPE column with 3 mL of MeOH followed by 3 mL of D.I. H₂O.
2. Apply sample to SPE column.
3. Wash SPE column with 1 mL of 60:40 D.I. H₂O: MeOH.
4. Dry column (5 minutes at full vacuum or pressure).
5. Elute anabolic steroids with 3 mL of MeOH (collect eluate at 1- 2 mL/min).
6. Evaporate to dryness at < 50°C.
7. Reconstitute sample in 100 µL of mobile phase (50:50, A:B).

LC-MS/MS Parameters:



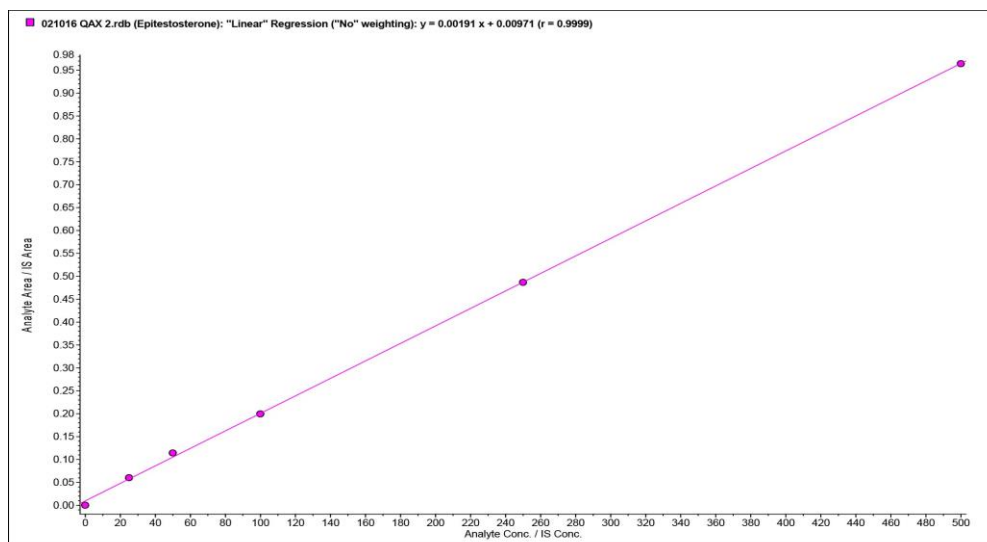
MRM transitions (ESI ⁺ , 50 ms dwell time)					
Compound		Rt (min)	Q1 ion	Q3 ion 1	Q3 ion 2
1	Trenbolone	7.42	271.1	115.1	-
2	Boldenone	7.63	287.1	120.9	-
3	Androstenedione	7.94	287.1	96.9	-
4	Nandrolone	8.24	275.1	109.2	78.9
5	Methandienone	8.65	301.1	120.9	91.1
-	Testosterone-D ₃	-	292.1	96.9	109.1
6	Testosterone	9.36	289.0	97.0	109.1
-	17-Alpha-Hydroxyprogesterone-D ₈	-	339.3	100.1	113.1
7	17-Alpha-Hydroxyprogesterone	9.65	337.1	97.2	109.2
8	Epitestosterone	10.95	289.0	97.0	109.1
9	Methenolone	11.60	303.2	83.1	90.9
-	Stanozolol-D ₃	-	332.3	81.1	95.1
10	Stanozolol	13.81	329.1	81.1	95.1
-	Progesterone-D ₉	-	324.1	100.1	113.1
11	Progesterone	14.13	315.1	97.1	109.2

System: AB Sciex API 4000 QTrap MS/MS with Agilent 1200 Binary Pump SL		
Column: UCT Selectra® C18, 100 x 2.1 mm, 3 µm		
Guard Column: UCT Selectra® C18, 10 x 2.0 mm, 3 µm		
Column Temperature: 50 °C		
Column Flow Rate: 0.3 mL/min		
Injection Volume: 10 µL		
Gradient Program:		
Time (min)	% Mobile Phase A (0.1% Formic Acid in Water)	% Mobile Phase B (0.1% Formic Acid in MeOH)
0	50	50
2	40	60
9.0	40	60
12.0	0	100
15.0	0	100
15.1	50	50
19.0	50	50

Results:

Recovery (%) from Serum Spiked at 125 ng/mL		
Steroid	Sorbent Chemistry	
	C8 + QAX	SLE
Trenbolone	97	90
Boldenone	98	92
Androstenedione	97	93
Nandrolone	93	81
Methandienone	95	88
Testosterone	95	90
17-Alpha-Hydroxyprogesterone	93	84
Epitestosterone	90	90
Methenolone	98	93
Stanozolol	96	91
Progesterone	94	90
Average Recovery:	95	89

Matrix-Matched Calibration Curve of Epitestosterone ($R^2=0.9999$)



Discussion:

For the analysis of anabolic steroids using SLE, the manufacturer recommended procedure was followed. This called for initial sample loading onto the respective column followed by a 10 minute adsorption period. Next, two elution steps were carried out using methyl tertiary-butyl ether (MTBE), a non-desirable solvent choice based on its EPA classification as a potential human carcinogen. A one minute adsorption time was allotted between each individual elution step. In regards to the ruggedness of this sample preparation technique, great care was taken to avoid any residual column breakthrough, commonly triggered by too much initial vacuum pressure during sample addition and elution.

For the UCT approach utilizing a copolymeric reversed phase and strong anion exchange sorbent, only a single wash step was needed to produce efficient sample cleanup. In addition, the required column conditioning prior to sample loading only contributed approximately another two minutes to the overall extraction procedure resulting in a very high-throughput methodology. Furthermore, economical, non-hazardous solvents, water and methanol, were utilized in all portions of the extraction including the conditioning, wash, and elution steps.

While excellent recoveries were achieved using both sorbent chemistries for the extraction of anabolic steroids in serum, distinguishable differences between sample preparation time and overall solvent usage between the two protocols were noted.

Conclusion:

When using the C8 + QAX sorbent, excellent recoveries were achieved for the 11 steroids included in the study, ranging from 90-98%. Matrix-matched calibration curves were used for quantification with R^2 values ranging from 0.9984 to 0.9999 over the entire concentration range (25 - 500 ng/mL). Compared to an SLE approach, the use of a mixed-mode CUQAX22Z column and corresponding method was found to be more effective at removing matrix interferences, which may otherwise cause ion suppression or enhancement.



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