The Application of QuEChERS in the Extraction of Anabolic Steroids in Whole Blood

UCT Part Numbers:
ECQUUS1015CT- Enviro-Clean® 15 mL centrifuge tube with 400 mg $MgSO_4$ and 100 mg NaCl
CUMPS2CT - Enviro-Clean® 2 mL dSPE tube with 150 mg $MgSO_4$ and 50 mg PSA
SLC-18100ID21-3UM - Selectra® C18 HPLC column, 100 x 2.1 mm, 3 µm
SLC-18GDC20-3UM - Selectra® C18 guard cartridge, 10 x 2.0 mm, 3 µm
SLDGRDHLDR - Guard cartridge holder

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Summary:

Anabolic steroids are drugs structurally related to the cyclic steroid ring system and behave similarly to testosterone in the body. Anabolic steroids are used therapeutically to stimulate muscle growth and appetite, induce male puberty and treat chronic wasting conditions, such as cancer and AIDS [1]. Ergogenic uses of anabolic steroids include bodybuilding, sport doping, and animal fattening. Developing a fast, simple and effective analytical method for anabolic steroids in complex biological samples is of great interest in clinical, anti-doping and food safety testing labs. This application utilizes the original non-buffered QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) technique to quantify anabolic steroids in human whole blood. Previous extraction techniques typically involved a protein precipitation step followed by liquid-liquid extraction (LLE) or solid phase extraction (SPE).

1 mL of human whole blood sample is extracted using 2 mL of acetonitrile (MeCN). 400 mg magnesium sulfate ($MgSO_4$) and 100 mg sodium chloride (NaCl) are used to enhance the phase separation and the partition of anabolic steroids into the organic phase (MeCN), no protein precipitation is needed when using QuEChERS for blood samples. After shaking and centrifugation, 1 mL of the

supernatant is purified using a 2-mL dispersive SPE tube containing 150 mg $MgSO_4$ and 50 mg PSA. $MgSO_4$ absorbs residual water in the extract, while PSA remove organic acids and other matrix co-extractives, resulting in a clean extract for LC-MS/MS analysis.

Matrix matched calibration curves were constructed for steroid quantification. The responses for the 12 representative compounds were linear with R^2 greater than 0.999 over the concentration range of 10 - 500 ng/mL. Excellent recoveries (81.4 - 101.6%) and relative standard deviations (RSD < 10%) were obtained. This method has been applied to 6 real whole blood samples, no steroids were detected above the quantitation limit of 10 ng/mL.

Procedure:

QuEChERS extraction

a) Add 2 mL of MeCN to 15-mL centrifuge tube containing 400 mg $MgSO_4$ and 100 mg NaCl (ECQUUS1015CT).
b) Add internal standards (IS), and appropriate amounts of steroids spiking solution to fortified samples.
c) Add 1 mL of the negative whole blood into the 15-mL tubes
d) Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
e) Centrifuge at 3000 g for 5 min.

dSPE cleanup

a) Transfer 1 mL of the supernatant to a 2-mL dSPE tube containing 150 mg $MgSO_4$ and 50 mg PSA (CUMPS2CT).
b) Shake 1 min at 1000 strokes/min using the Spex 2010 Geno-Grinder.
c) Centrifuge at 3000 g for 5 min.

d) Transfer 0.4 mL of the cleaned extract into a 2-mL auto-sampler vial; add 0.4 mL of reagent water, and vortex for 30 sec.

e) The samples are ready for LC-MS/MS analysis.

LC-MS/MS method:

System: Agilent 1200 Binary Pump SL with AB Sciex API 4000 QTrap MS/MS		
Column: UCT Selectra® C18 LC column, 100 x 2.1 mm, 3 µm		
Guard Column: UCT Selectra® C18 guard column, 10 x 2.1 mm, 3 µm		
Column Temperature: 50 °C		
Column Flow Rate: 0.30 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 methanol)
0	50	50
2	40	60
9	40	60
12	0	100
15	0	100
15.1	50	50
19	50	50

MRM transitions (ESI positive, dwell time: 50 ms)				
Compound	Rt (min)	Q1 ion	Q3 ion	Linearity (R^2)
Trenbolone	4.56	271.1	115.0	0.9995
Boldenone	4.79	287.1	120.9	0.9999
Androstanedione	5.10	287.1	96.9	0.9992
Nandrolone	5.33	275.1	109.2	0.9999
Methandienone	5.69	301.1	120.9	0.9999
Testosterone	6.04	289.1	97.0	0.9999
17-hydroxyprogesterone	6.17	331.2	97.2	0.9998
Epitestosterone	6.85	289.1	97.0	0.9997
Methenolone	7.30	303.2	83.0	0.9994
Norethandrolone	8.49	303.2	79.0	0.9990
Stanozolol	8.78	329.2	81.1	0.9998

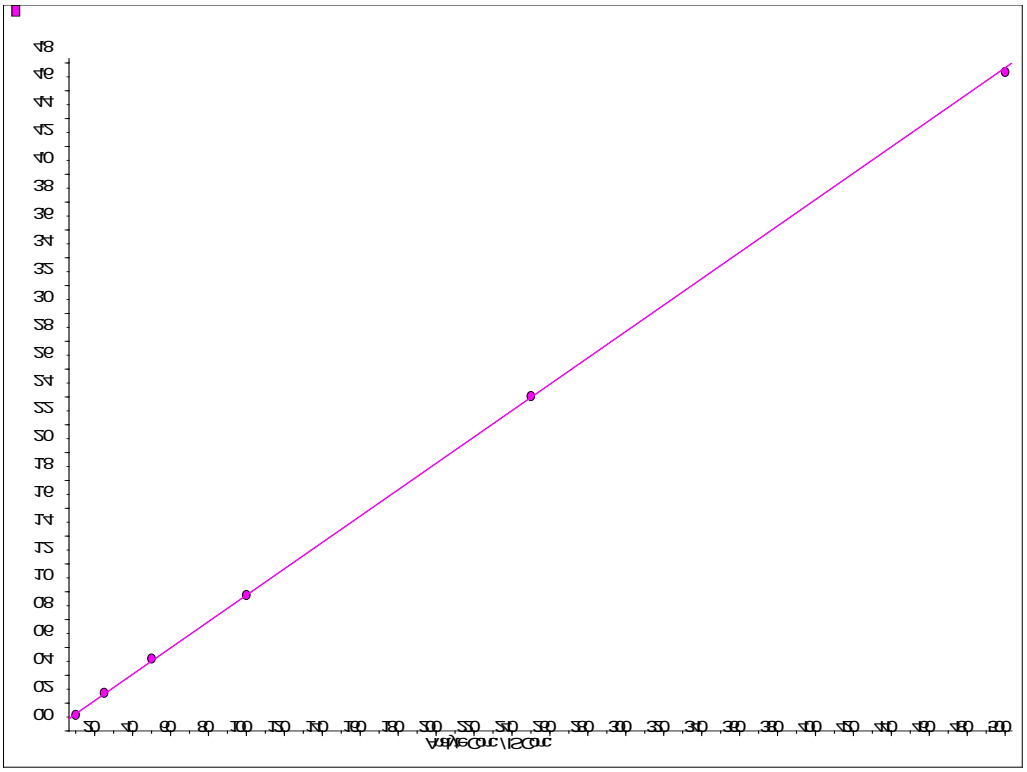
Progesterone	8.99	315.2	97.0	0.9995
Testosterone-D3	6.03	292.1	96.9	NA
17-hydroxyprogesterone-D8	6.05	339.3	100.0	NA
Stanozol-D3	8.69	332.3	81.0	NA
Progesterone-D9	8.84	324.2	100.1	NA

Results:

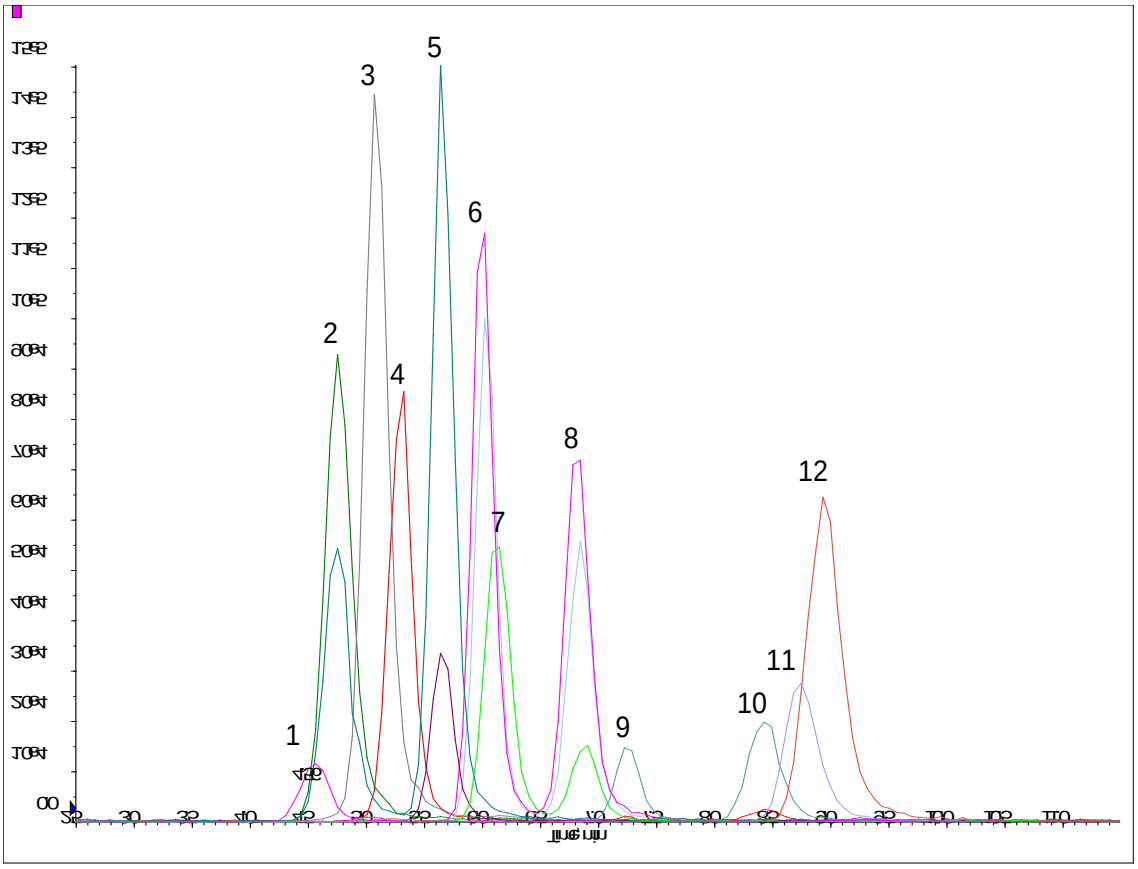
Recovery and RSD% from Human Whole Blood Spiked at 3 Levels

Compound	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	Recovery %	RSD% (n=6)	Recovery %	RSD% (n=6)	Recovery %	RSD% (n=6)
17-hydroxyprogesterone	89.6	6.6	99.2	5.7	99.3	3.2
Androstanedione	93.5	9.2	95.7	3.3	94.3	1.5
Boldenone	91.2	8.2	101.6	2.9	99.4	1.4
Methandienone	94.7	6.5	97.2	3.3	96.1	3.0
Methenolone	98.2	4.5	96.0	4.7	95.3	3.9
Norethandrolone	94.0	6.7	98.5	5.1	99.8	4.0
Nandrolone	96.4	9.6	92.3	1.1	89.8	1.6
Progesterone	101.6	5.0	95.5	1.3	94.8	4.0
Stanozolol	85.1	5.9	92.1	3.4	91.3	2.2
Testosterone	92.4	6.3	95.0	3.4	95.1	2.4
Trenbolone	81.4	9.0	93.2	6.9	95.0	3.0
Epitestosterone	89.8	5.4	97.6	4.4	99.3	2.8

Matrix Matched Calibration Curve of Testosterone (R^2=0.9999)



Chromatogram of Human Whole Blood Spiked with 200 ng/mL Steroids



Peak list: 1. Trenbolone; 2. Boldenone; 3. Androstanedione; 4. Nandrolone; 5. Methandienone; 6. Testosterone; 7. 17-hydroxyprogesterone; 8. Epitestosterone; 9. Methenolone; 10. Norethandrolone; 11. Stanozolol; 12. Progesterone

References:

[1] http://en.wikipedia.org/wiki/Anabolic_steroid

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