Extraction of Low Level Testosterone and Androstenedione From Human Serum Samples Using ISOLUTE® SLE+

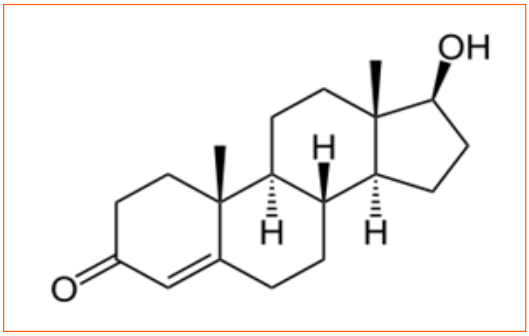

Figure 1. Structure of Testosterone

Introduction

This application note describes the extraction of testosterone and androstenedione from female patient serum samples using ISOLUTE SLE+ (96 well) plates.

This method has been optimized to extract testosterone and androstenedione from female clinical serum samples. The method has achieved very low limits of quantitation for testosterone and androstenedione 0.4 nmol/L and 0.9 nmol/L respectively. The recoveries for both analytes were greater than 90%.

Application Note

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid liquid extraction formation, and significantly reduced sample preparation time.

Analytes

Testosterone and androstenedione

ISOLUTE SLE+ procedure

ISOLUTE SLE+ 400 Supported Liquid Extraction plate, part number 820-0400-P01.

Sample pre treatment: dilute human serum (200 µL) with 0.5 mol/L ammonium hydroxide (200 µL) then mix.

Sample load: load pre treated sample (400 µL) to plate followed by a pulse of vacuum to imitate flow and leave for 5 minutes.

Analyte elution: apply 500 µL of diethyl ether, wait five minutes to allow the solvent to soak, apply a short pulse of vacuum if solvent not fully absorbed. Apply a second 500 µL of diethyl ether; allow to soak for five minutes, apply a short pulse of vacuum if not fully absorbed. Apply a third 500 µL of Diethyl ether, allow to soak for 5 minutes and then apply another short pulse of vacuum.

Post extraction: evaporate the eluate to dryness and reconstitute with 400 µL of methanol:water (1:1, vol:vol).

Additional information: testosterone has an affinity to bind to plastic so the extracts were collected in glass tubes held in a 96 well collection plate.

HPLC conditions

Instrument: Acquity UPLC

Column: Acquity UPLC HSS C18 2.1 x 50 mm column, 1.8 µm particle (Waters)

Mobile phase: A= Water with 2 mmol/L ammonium acetate and 0.1% formic acid, B= Methanol with 2mmol/L ammonium acetate and 0.1% formic acid at a flow rate of 0.6 mL/min.

Gradient

Time	%A	%B
0	50	50
0.20	50	50
1.50	30	70
2.25	2	98
2.75	30	70

Injection volume: 37.5 µL

Mass spectrometry conditions

Instrument: Waters Premier XE

Ion source temperature: 120 °C

Desolvation temperature: 450 °C

Table 1: MRM transitions for Testosterone, Androstenedione, D_2 testosterone and D_7 androstenedione

Analyte	MRM transitions	Cone voltage	Collision energy
Testosterone	289.2>97.0	35	25
D2 Testosterone	291.1>98.9	35	23
Androstenedione	287.2>96.9	36	22
D7 Androstenedione	294>99.8	35	23

Results:

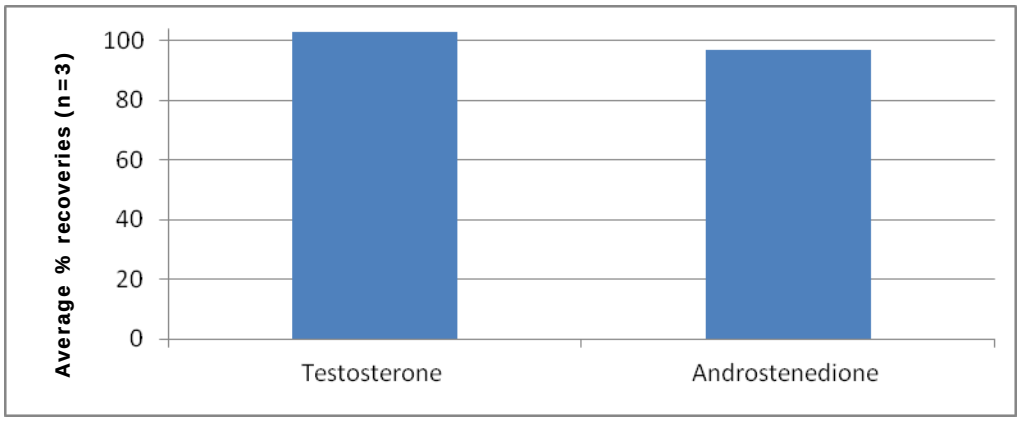

Figure 2. Average analyte recoveries up to 100 nmol/L

Table 2: LLOQ of Testosterone and Androstenedione

Analyte	LLOQ nmol/L
Testosterone	0.4
Androstenedione	0.9

References

'*Development and evaluation of a simple extraction method for the simultaneous measurement of female serum testosterone and androstenedione by UPLC-MSMS' M.Purcell, P.Reed. Department of Clinical Biochemistry, Salford Royal Hospital, M6 8HD, UK*. Poster presented at IBMS September 2011.

Ordering Information

Part number	Description	Quantity
820-0400-P01	ISOLUTE SLE+ 400 µL Supported Liquid Extraction Plate	1

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