

Extraction of Testosterone and Other Steroid Hormones From Human Plasma Using ISOLUTE® SLE+ 96-Well Plates

Introduction

This application note describes the extraction of testosterone and other steroid hormones from human plasma using ISOLUTE SLE+ supported liquid extraction plates (96-well) with LC-MS/MS analysis.

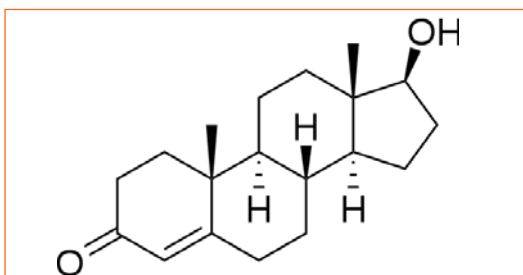


Figure 1. Structure of Testosterone

This method describes the use of ISOLUTE SLE+ supported liquid extraction plates (96-well) to extract a range of steroid hormones including testosterone and progesterone from human plasma. This simplified and efficient extraction method has significant analyte recoveries ranging from 90-107% with LOQs as low as 500 pg/mL.

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Testosterone, aldosterone, 21-deoxycortisol, 11-deoxycortisol, androstendione, 17- α -hydroxyprogesterone, dehydroepiandrosterone (DHEA), progesterone, androsterone.

ISOLUTE SLE+ procedure

ISOLUTE SLE+ 200 Supported Liquid Extraction plate, part number 820-0200-P01.

- | | |
|--------------------------------|--|
| Sample Pre-treatment: | Dilute human plasma (100 μ L) 1:1 with HPLC grade water (100 μ L). |
| Sample Load: | Load pre-treated sample (200 μ L) to plate followed by a pulse of vacuum to initiate flow and leave for five minutes. |
| Analyte Elution: | Elute with dichloromethane (1 mL) directly in to a deep well collection plate (121-5203). Leave to flow under gravity for 5 minutes then apply short pulse of vacuum. |
| Post extraction: | Evaporate to dryness at ambient temperature and reconstitute in 50% methanol (aq) (100 μ L). Vortex samples to ensure full reconstitution of all analytes. |
| Additional information: | Use of the reconstitution solvent combined with the vortex step addresses any issues that may arise from non-specific binding of testosterone and other analytes. All samples were processed using Biotage VacMaster-96 sample processing manifold and evaporated to dryness on a SPE Dry 96 Dual. |

UPLC Conditions

Instrument: Waters Acquity UPLC interfaced to a Quattro Premier XE triple quadrupole MS using electrospray ionization.

Column: Acquity BEH C18 100 x 2.1mm x 1.7u.

Mobile phase: **A:** 0.1% formic acid (aq) **B:** 0.1% formic acid in methanol.

Flow rate: 0.40 mL/min.

Injection: 15µL (partial loop, needle overfill).

Temperature: 40 °C.

Gradient:	Time (min)	% A	% B
	Initial	50	50
	0.8	50	50
	4.2	19	81
	4.3	0	100
	5.3	0	100
	5.8	50	50
	6.8	50	50

Mass Spectrometry Conditions

Source temp: 150 °C.

Desolvation temp: 450 °C.

Collision gas pressure: 3.58 e⁻³ mbar.

Table 1. MRM transitions for a range of steroids.

Scan function	Compound	MRM transition	Cone voltage	Collision energy (ev)
1	Aldosterone	361.4 > 315.4	30	22
2	11-Deoxycortisol	347.4 > 97	30	24
2	21-Deoxycortisol	347.4 > 311.4	30	20
3	Androstendione	287.3 > 97	30	22
3	Testosterone	289.4 > 97	35	25
3	DHEA	289.4 > 253.2	15	12
3	17a-Hydroxypregesterone	331.4 > 97	30	25
4	Androsterone	291.3 > 255.4	15	15
4	Pregesterone	315.4 > 97	30	21

Results

Figure 2 shows the mass chromatograms for all the extracted steroids spiked at 100 ng/mL for each analyte using this methodology. Figure 3 shows average analyte recoveries ranging from 90-107% (n=7) with table 2 showing the LOQ for each analyte ranging from 0.5 ng/mL for androstendione to 100 ng/mL for DHEA.

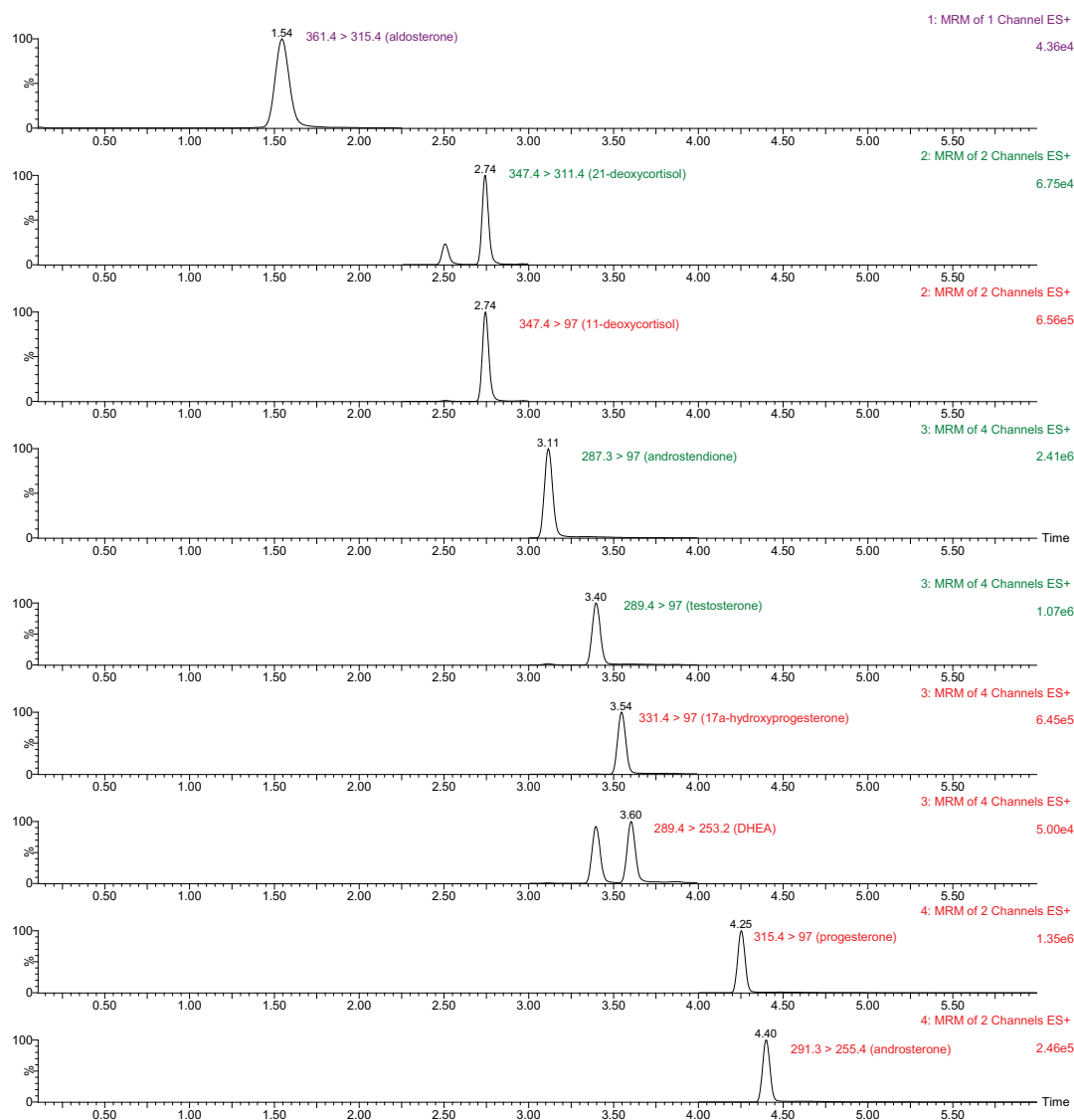


Figure 2. Extracted ion chromatograms for all hormone analytes at DHEA LOD 100 ng/mL

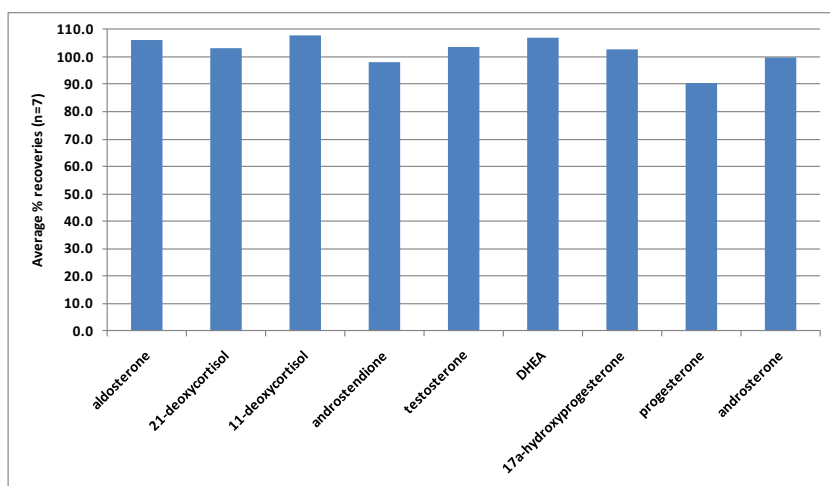


Figure 3. Average analyte recoveries of steroid analytes at 100 ng/mL (n=7)

Table 2. Steroid analytes LOQ (ng/mL)

Analyte	LOQ (ng / mL)
Aldosterone	2
21-deoxycortisol	5
11-deoxycortisol	1
Androstendione	0.5
Testosterone	1
17α-hydroxyprogesterone	0.5
DHEA	100
Progesterone	0.5
Andosterone	2

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 µL supported liquid extraction plate	1
121-5203	Deep well collection plate 2 mL	50
121-9600	VacMaster-96 Sample processing manifold complete (without vacuum control)	1
SD2-9600-DHS-UK	SPE Dry 96 Dual, 240 V UK	1

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