Mar 13, 2025

Sensitive targeted analysis of salivary steroids by liquid chromatography mass spectrometry for studies of infertility

DOI
https://dx.doi.org/10.17504/protocols.io.14egn9rzpl5d/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.14egn9rzpl5d/v1

Protocol Citation: Joanna Simpson, Scott Denham, Birgit Alsbjerg, Natalie Z M Homer 2025. Sensitive targeted analysis of salivary steroids by liquid chromatography mass spectrometry for studies of infertility . **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn9rzpl5d/v1

Protocol status: Working
We use this protocol and it's working

Created: February 10, 2025

Last Modified: March 13, 2025

Protocol Integer ID: 119903

Keywords: steroid profiling, saliva, LC-MS/MS, assisted reproductive technologies, progesterone, IVF, ART, measuring progesterone, assessment of progesterone level, analysis of progesterone, attractive for progesterone measurement, measurement of the salivary steroid profile, progesterone measurement, analysis of salivary steroid, salivary steroid hormone, profiles multiple salivary steroid hormone, progesterone level, low blood progesterone in the luteal phase result, salivary steroid profile, multiple salivary steroid hormone, low blood progesterone, including progesterone, progesterone, free progesterone in women, superior for steroid analysis, steroid analysis, estrone in human saliva sample, liquid chromatography mass spectrometry for study, free progesterone, liquid chromatography mass spectrometry, targeted liquid chromatography mass spectrometry, salivary steroid, liquid chromatography tandem mass spectrometry, targeted liquid chromatography tandem mass spectrometry, human saliva sample, saliva sample, mass spectr

Funders Acknowledgements:
Rosetrees Trust
Grant ID: Seedcorn2023\100344

Abstract

Progesterone plays a key role in implantation and early pregnancy (Mesen et al, 2015) and studies have shown that low blood progesterone in the luteal phase result in lower pregnancy rates and an increased pregnancy loss rate following fresh and frozen embryo transfer (Alpcetin et al, 2025). As such, infertility treatments require assessment of progesterone levels to determine success, typically measured in serum following a blood-draw.

It is important to develop treatment regimens with high reproductive outcome, that are safe and painless, and minimal time.To ease discomfort and clinic visit burden then non-invasive sampling using saliva is attractive for progesterone measurement. If progesterone levels can be measured and demonstrate a constant level during daytime this could improve patient care during infertility treatment.

Methods for measuring progesterone and sex steroids are commonly immunoassay based, which can be affected by cross-reactivity and imprecision at low levels. However, mass spectrometry based methods are superior for steroid analysis in saliva (Brouillard et al, 2025), enhanced by the ability to multiplex multi-steroid analysis without issues of sensitivity and cross-reactivity (Handelsman, 2013).

In order to assess the feasibility of using saliva to profile free progesterone in women undergoing infertility treatments then a sensitive bioanalytical method that reliably measures salivary steroid hormones is needed. We developed an automated extraction and targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) method that profiles multiple salivary steroid hormones, including progesterone. This method had suitable concentration ranges that allowed us to apply to saliva samples collected over a 12 hour cycle from women during the mid-luteal phase of IVF and FET cycles.

This protocol describes the extraction and targeted liquid chromatography mass spectrometry (LC-MS/MS) analysis of progesterone, 17alpha-hydroxyprogesterone, cortisol, cortisone, aldosterone, testosterone, androstenedione, dehydroepiandrosterone, 17β-estradiol and estrone in human saliva samples from women undergoing infertility treatment. It enables measurement of the salivary steroid profile. This targeted LC-MS/MS method was developed by adapting the method from Gregory et al, 2023, using updated instrumentation set up and improved isotopically labelled internal standards.

Saliva samples (200 µL) were enriched with isotopically labelled internal standards, diluted with water (0.1% formic acid v/v) and extracted alongside a (0.0025 - 400 ng) calibration curve, by automated 96-well supported liquid extraction (SLE), using dichloromethane and isopropanol as an organic solvent, on an Extrahera automated liquid handler by Biotage (Uppsala, Sweden). Extracted steroids were separated on an Acquity I-Class UPLC by Waters (UK) with gradient elution on a Kinetex C18 column (150 × 2.1 mm; 2.6 µm) by Phenomenex (UK) and a mobile phase of methanol and water (with 0.05 mM ammonium fluoride in water and methanol). The run time was 16 minutes, followed by analysis on a QTrap 6500+ mass spectrometer operated in multiple reaction mode in both positive and negative ionisation modes, scanning for 10 steroids and appropriate internal standards.

The amount of steroid in each sample was calculated using linear regression of the peak area ratio of the analytes to the isotopically labelled internal standard as determined by analysis of a calibration curve.

Image Attribution

Image generated by Created in BioRender. Homer, N. (2025) https://BioRender.com/t02r734Biorender

Guidelines

Ensure all training is up-to-date for operating the necessary laboratory instrumentation and equipment.

Materials

Consumables Table

	A	B	C	D
	Item	**Supplier**	**Part no.**	**Quantity**
	1.75 mL glass vials with lids	Scientific Laboratory Supplies Ltd	TUB1200	10
	7 mL glass vials with lids	Scientific Laboratory Supplies Ltd	TUB1220	5
	Isolute SLE+ 400 96 well plate	Biotage	820-0400-P01	1
	96-well plate sealing film	VWR	391-1250	1
	Adhesive Plate Seal	Waters	186006336	1
	Kinetex C18 (150 × 2.1 mm; 2.6 um)	Phenomenex	00F-4462-AN	1
	Kinetex KrudKatcher, 0.5 um	Phenomenex	AFO-8497	1
	Deep well 96 well collection plate	Biotage	121-5203	1
	Deep well (2 mL) 96 well collection plate	Waters	186002482	1

Table M1 - Consumables for extraction and liquid chromatography separation of steroids

	A	B	C
	Item	**Supplier**	**Article no.**

	A	B	C
	Water (HPLC grade)	Fisher Scientific	C-10449380-X
	Acetonitrile (LC-MS grade)	VWR	83640.320
	Methanol (LC-MS grade)	VWR	83638.320
	Water (LC-MS grade)	VWR	83645.320
	Isopropanol (HPLC grade)	VWR	20880.320
	Dichloromethane (HPLC grade)	Fisher Scientific	C-23373320-X
	Cortisol	Sigma-Aldrich/Cerilliant	(C-106) 1 mg/mL in methanol (certified)
	Cortisone	Sigma-Aldrich/Ceriliiant	(C-130) 100 µg/mL in methanol (Certified)
	Aldosterone	Sigma-Aldrich/Cerilliant	A-096) 100 µg/mL in acetonitrile (certified)
	Androstenedione	Sigma-Aldrich/Cerilliant	(A-075) 1 mg/mL in acetonitrile (certified)
	Testosterone	Sigma-Aldrich/Cerilliant	(T-037) 1 mg/mL in acetonitrile (certified)
	Dehydroepiandrosteron e	Sigma-Aldrich/Cerilliant	(D-063) 1 mg/mL in methanol (certified)
	17alpha-hydroxyprogesterone	Sigma-Aldrich/Cerilliant	(17OHP) H-085 1 mg/mL in methanol (certified)
	Progesterone	Sigma-Aldrich/Cerilliant	(P-069) 1 mg/mL in acetonitrile (certified)
	17beta-estradiol	Sigma-Aldrich/Cerilliant	(E-060) 1 mg/mL in acetonitrile (certified)
	Estrone	Sigma-Aldrich/Cerilliant	(E-075) 1 mg/mL in methanol (certified)
	13C3-Cortisol	Sigma-Aldrich/Cerilliant	(C-216) 100 µg/mL in methanol (certified)
	13C3-cortisone	Sigma-Aldrich/Cerilliant	(C-160) 100 µg/mL in methanol (certfied)
	13C3-aldosterone	Sigma-Aldrich/Cerilliant	(A-120) 10 µg/mL in acetonitrile (certified)

	A	B	C
	13C3-testosterone	Sigma-Aldrich/Cerilliant	(T-070) 100 ug/mL in acetonitrile (certified)
	13C3-androstenedione	Sigma-Aldrich/Cerilliant	(A-084) 100 ug/mL in acetonitrile (certified)
	d5-dehydroepiandrosterone	Sigma-Aldrich/Cerilliant	(D-064) 100 µg/mL in methanol
	d9-progesterone	Sigma-Aldrich/Cerilliant	P-070 100 ug/mL in acetonitrile
	d8-17hydroxyprogesterone	Sigma-Aldrich/Cerilliant	(H-096) 100 µg/mL in methanol
	13C3-estradiol	Sigma-Aldrich/Cerilliant	(E-073) 100 µg/mL in acetonitrile (certified)
	13C3-estrone	Sigma-Aldrich/Cerilliant	E-108) 100 µg/mL in methanol (certified)
	Ammonium Fluoride	Fisher Scientific	

Table M2 - Chemicals and Analytical Standards

Solutions Required

- 0.1% formic acid (aq) (200 mL) Make up to 200 mL with Water (HPLC grade). Mix thoroughly.
- 98:2 Dichloromethane:Isopropanol (1 L) - Add 20 mL Isopropanol (HPLC grade) to 980 mL Dichloromethane (HPLC grade). Mix thoroughly.
- Methanol (HPLC grade): for preparation of calibration standard/internal standard dilutions.
- Water (HPLC grade): for preparation of calibration standards.
- 70:30 Water:Methanol (100 mL) - Add 30 mL methanol (LC-MS grade) to 70 mL water (LC-MS grade). Mix thoroughly.

Equipment Table

	A	B	C
	Item	**Model**	**Supplier**
	Liquid Chromatography Pumps	I-Class UPLC	Waters

	A	B	C
	mass spectrometer	QTrap 6500+	AB Sciex
	Gilson Repetman	Gilson Repetman	Gilson
	Deepwell plate thermoshaker	TS-DW	Grant Scientific
	Liquid handling robot	Extrahera	Biotage, Sweden
	SPE Dry 96 dual evaporator	SPE Dry	Biotage, Sweden

Table M3 - Equipment required for automated extraction and LC-MS/MS steroid analysis

Safety warnings

Ensure risk assessments are up to date and that all local laboratory guidelines are followed for handling chemicals and biological samples.

Ethics statement

Ensure all human samples used in the analysis have been collected following ethical approval.

Before start

Prepare the automated robot for operation.
Prepare the liquid chromatography tandem mass spectrometer (LC-MS/MS) for operation and ensure sufficient mobile phase solutions and needle wash. Prime the solvents. Check that the chromatographic column is correctly installed and is not leaking when the mobile phase is pumping through.

Preparation of human saliva samples for extraction

1 Remove human saliva samples from the freezer and defrost on ice

Preparation of calibration standard stock solutions

2 Prepare a mixed stock of 10 steroids (**Table M2**) - progesterone, 17alpha-hydroxyprogesterone, cortisol, cortisone, aldosterone, testosterone, androstenedione, dehydroepiandrosterone, 17β-estradiol and estrone - by using 100 µg/mL stock solutions. Do this by adding 50 µL x 100 µg/mL **P4,** 50 µL x 100 µg/mL **17OHP4**, 50 µL x 100 µg/mL **F**, 50 µL x 100 µg/mL **E**, 50 µL x 100 µg/mL **Aldo**, 50 µL x 100 µg/mL **T**, 50 µL x 100 µg/mL **A4**, 50 µL x 100 µg/mL **DHEA,** 50 µL x 100 µg/mL **E2** and 50 µL x 100 µg/mL **E1** + 500 µL methanol to give a **5 µg/mL stock**.

3 Dilute the **5 µg/mL stock** Mixed STOCK by 1:10 dilution (100 µL x 5 µg/mL + 900 µL methanol) to give **500 ng/mL stock**

4 Dilute the **500 ng/mL** mixed STOCK by 1:10 dilution (100 µL x 500 ng/mL + 900 µL methanol) to give **50 ng/mL stock**

5 Dilute the **50 ng/mL** mixed STOCK by 1:10 dilution (100 µL x 5 µg/mL + 900 µL methanol) to give **5 ng/mL stock**

6 Dilute the **5 ng/mL** Mixed STOCK by 1:10 dilution (100 µL x 5 µg/mL + 900 µL methanol) to give **500 pg/mL stock**

7 Dilute the **500 pg/mL** Mixed STOCK by 1:10 dilution (100 µL x 5 µg/mL + 900 µL methanol) to give **50 pg/mL stock**

Preparation of internal standard solution

8 Prepare **100 µg/mL solutions** of each isotopically labelled internal standard (**Table M2**) (d9-progesterone, d8-17a-hydroxyprogesterone, $^{13}C_3$-cortisol, $^{13}C_3$-cortisone, $^{13}C_3$-aldosterone, $^{13}C_3$-testosterone, $^{13}C_3$-androstenedione, d5-dehydroepiandrosterone, $^{13}C_3$-estrone and $^{3}C_3$-estradiol) in methanol.

9 Prepare a **mixed 5 µg/mL** Internal Standard mix stock solution of the isotopically labelled steroids by adding 25 µL x 100 µg/mL d9-progesterone, 25 µL x 100 µg/mL d8-

17a-hydroxyprogesterone, 25 µL x 100 µg/mL $^{13}C_3$-cortisol, 25 µL x 100 µg/mL $^{13}C_3$-cortisone, and 250 µL x 100 µg/mL$^{13}C_3$-aldosterone, 25 µL x 100 µg/mL $^{13}C_3$-testosterone 25 µL x 100 µg/mL $^{13}C_3$-androstenedione, 25 µL x 100 µg/mL d5-dehydroepiandrosterone, 25 µL x 100 µg/mL $^{13}C_3$-estrone, and 25 µL x 100 µg/mL $^{3}C_3$-estradiol to 25 µL methanol.

10 Prepare a **5 ng/mL Working Internal Standard** solution by taking 10 µL x 5 µg/mL Int Std Mix + 1990 µL methanol.

Set up of supported liquid extraction of steroids from calibration standards and samples

11 Label a 2 mL deep well 96-well collection plate (Table M1). Label a Supported Liquid Extraction SLE400 plate with batch details. Label a 2 mL deep well 96-well collection plate (Waters).
Design and prepare batch of standards and saliva samples in Microsoft Excel template, following a column-wise plate map design as below (Table S1).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1 Double Blank	A2 0.250 STD	A3 100.0 STD	A4 Plasma 4	A5 Plasma 12	A6 Plasma 20	A7 Plasma 28	A8 Plasma 36	A9 Plasma 44	A10 Plasma 52	A11 Plasma 60	A12 Plasma 68
B	B1 0 STD	B2 0.500 STD	B3 200.0 STD	B4 Plasma 5	B5 Plasma 13	B6 Plasma 21	B7 Plasma 29	B8 Plasma 37	B9 Plasma 45	B10 Plasma 53	B11 Plasma 61	B12 Plasma 69
C	C1 0.0025 STD	C2 1.0 STD	C3 400.0 STD	C4 Plasma 6	C5 Plasma 14	C6 Plasma 22	C7 Plasma 30	C8 Plasma 38	C9 Plasma 46	C10 Plasma 54	C11 Plasma 62	C12 Plasma 70
D	D1 0.0050 STD	D2 2.5 STD	D3 Double Blank	D4 Plasma 7	D5 Plasma 15	D6 Plasma 23	D7 Plasma 31	D8 Plasma 39	D9 Plasma 47	D10 Plasma 55	D11 Plasma 63	D12 Plasma 71
E	E1 0.010 STD	E2 5.0 STD	E3 QC	E4 Plasma 8	E5 Plasma 16	E6 Plasma 24	E7 Plasma 32	E8 Plasma 40	E9 Plasma 48	E10 Plasma 56	E11 Plasma 64	E12 Plasma 72
F	F1 0.025 STD	F2 10.0 STD	F3 Plasma 1	F4 Plasma 9	F5 Plasma 17	F6 Plasma 25	F7 Plasma 33	F8 Plasma 41	F9 Plasma 49	F10 Plasma 57	F11 Plasma 65	F12 Plasma 73
G	G1 0.050 STD	G2 25.0 STD	G3 Plasma 2	G4 Plasma 10	G5 Plasma 18	G6 Plasma 26	G7 Plasma 34	G8 Plasma 42	G9 Plasma 50	G10 Plasma 58	G11 Plasma 66	G12 QC
H	H1 0.100 STD	H2 50.0 STD	H3 Plasma 3	H4 Plasma 11	H5 Plasma 19	H6 Plasma 27	H7 Plasma 35	H8 Plasma 43	H9 Plasma 51	H10 Plasma 59	H11 Plasma 67	H12 Solvent blank

Table S1 - Plate Map - Column-wise plate layout for automated Supported Liquid Extraction on an Extrahera liquid handling robot (Biotage, Sweden)

Preparation of calibration standard curve and samples

12 Prepare calibration standards directly into the **96-well deep well plate** using the following table for volumes of each stock concentration, into a final volume of

200 µL water.

	A	B	C	D
	Standard name	**Amount (ng)**	STD Mix Vol (uL)	Vol water (uL)
	0 STD	0	0	200
	0.00250 STD	0.00250	5 uL x 500 pg/mL	195
	0.00500 STD	0.00500	10 uL x 500 pg/mL	190
	0.01000 STD	0.0100	20 uL x 500 pg/mL	180
	0.0250 STD	0.0250	5 uL x 5 ng/mL	195
	0.0500 STD	0.0500	10 uL x 5 ng/mL	190
	0.100 STD	0.100	20 uL x 5 ng/mL	180
	0.250 STD	0.250	5 uL x 50 ng/mL	195
	0.500 STD	0.500	10 uL x 50 ng/mL	190
	1.00 STD	1.00	20 uL x 50 ng/mL	180
	2.50 STD	2.50	5 uL x 500 ng/mL	195
	5.00 STD	5.00	10 uL x 500 ng/mL	190
	10.0 STD	10.0	20 uL x 500 ng/mL	180
	25.0 STD	25.0	5 uL x 5 ug/mL	195
	50.0 STD	50.0	10 uL x 5 ug/mL	190
	100 STD	100.0	20 uL x 5 ug/mL	180
	200 STD	250	5 uL x 50 ug/mL	195
	400 STD	400.0	8 uL x 50 ug/mL	192

Table 2 - Calibration standard preparation table

13 Aliquot 200 µL saliva sample into the correct well according to the plate map design.

Supported liquid extraction of steroids from calibration standards and saliva samples

14 Using a multi-step pipette enrich the plate containing calibration standards with WIS by adding 20 µL **x 5 ng/mL Working Internal Standard** into each calibration standard, including 0 std and each sample (human saliva), except for the double blank and solvent blank.

15 Using the Extrahera liquid handling robot, set up with the batch labelled SLE400 extraction plate and the deep well extraction plate, containing the calibration standards and samples. Programme Extrahera to aliquot 200 µL 0.1% formic acid in water (v/v) into each well of the 96-well deep well plate containing the samples and standards.

16 Programme the Extrahera to transfer 400 µL of liquid from each well (containing sample and the diluent, into a 400 µL volume Supported Liquid Extraction plate (SLE400), pre-placed into the deck on the Extrahera, with a deep well Waters 2 mL deep well collection plate below, pre-labelled with the batch details and date of extraction.

17 Allow the diluted sample to adsorb onto the SLE extraction bed for 00:05:00 before eluting with 600 µL x 98:2 (v/v) dichloromethane/isopropanol and repeating twice more, each time collecting the eluent into the collection plate

18 Dry down the eluent collected into the 2 mL collection plate using the SPE Dry down for 96-well plates under nitrogen.

19 Resuspend in 100 µL x 70:30 water/methanol, seal the plate with a zone-free plate seal and shake on ThermoShaker for 00:05:00 at 300 rpm 5m

20 Place the plate in the autosampler for LC-MS/MS or store at -20 °C until ready for analysis.

Steroid measurement by LC-MS/MS

21 Set up an acquisition batch in Analyst software using the electronic excel file of the calibration standards and sample list. Set to inject 20 µL per sample and use a

method of chromatographic separation as described in steps 22 and 23 and mass spectrometer settings as outlined in steps 24 and 25.

22 Set up the liquid chromatography system and fit with a Phenomenex Krud Katcher and a Phenomenex 150 × 2.1 mm; 2.6 µm Kinetex C18 liquid chromatography column, using mobile phase A - water with 0.05 mM ammonium fluoride and mobile phase B - methanol with 0.05 mM ammonium fluoride at 0.3 mL/min and 50 °C diverting to the mass spectrometer at 0.2 mins and returning to waste at 15.9 mins

23 Set up chromatographic gradient as below (**Table S3**) with a run time of 00:16:00 per sample

	A	B	C	D
	Time (min)	Flow (mL/min)	A (%)	B (%)
	Initial	0.3	50	50
	4	0.3	50	50
	9	0.3	25	75
	10	0.3	0	100
	12	0.3	0	100
	12.1	0.3	50	50
	16.00	0.3	50	50

Table S3 - Chromatographic gradient details. A - water w/ 0.05 mM ammonium fluoride; B - methanol w/ 0.05 mM ammonium fluoride. 50^{O}C. Kinetex C18 (150 x 2.1 mm; 2.6 µm)

24 Set up the mass spectrometer for Multiple Reaction Monitoring (MRM) method in positive mode, with electrospray ionisation as below, with divert of LC flow into the mass spectrometer set at 1 minute and 15.9 minutes.

	A	B
	Instrument	Sciex QTrap 6500+

	A	B
	Source, Ionisation Mode	IonDrive Turbo V Source, ESI
	Scan Mode, Polarity	MRM, Positive and Negative
	Resolution (Q1/Q3)	unit/unit
	Mass range	Low mass
	Pause Time	5.007 ms
	Acquisition time	16.0 min
	Delay time	0 sec
	Curtain Gas (CUR) (N2)	30 units
	Collision Gas (CAD) (N2)	Medium
	IonSpray Voltage (IS) (Positive)	5500 V / -4500 V
	Temperature (TEM)	600 °C
	Ion Source Gas 1 (GS1) (Air)	40 units
	Ion Source Gas 2 (GS2) (Air)	60 units
	Entrance Potential (EP) (Positive)	10 V
	Probe position (x – axis)	5
	Probe position (y – axis)	2

Table S4 - Mass Spectrometry source settings for positive and negative ion electrospray ionsiation on QTrap 6500+ mass spectrometer

25 Set up the mass spectrometer to monitor for the following multiple reaction monitoring (MRM) transitions for each steroid and each isotopically labelled steroid in positive and negative mode (**Table S5**).

	A	B	C	D	D	E	F	G
	Q1 Mass (Da)	**Q3 Mass (Da)**	**Scan time (msec)**	**Polarity**	**Steroid Name**	**DP (V)**	**CE (V)**	**CXP (V)**
	363.1	121.2	10	+	Cortisol 1	66	31	12
	363.1	91.0	10	+	Cortisol 2	76	83	10
	361.1	163.1	10	+	Cortisone 1	81	31	26
	361.1	77.1	10	+	Cortisone 2	81	107	10
	289.1	97.0	10	+	Testosterone 1	101	29	12
	289.1	109.2	10	+	Testosterone 2	101	31	6
	287.1	97.0	10	+	Androstenedione 1	61	27	14
	287.1	78.9	10	+	Androstenedione 2	61	67	10
	271.1	235.1	10	+	Dehydroepiandrosterone 1	106	17	12
	271.1	188.1	10	+	Dehydroepiandrosterone 2	106	17	12
	315.0	97.1	10	+	Progesterone 1	96	23	10
	315.0	109.1	10	+	Progesterone 2	96	27	10
	333.1	109.1	10	+	17a-hydroxyprogesterone 1	66	31	12
	333.1	96.9	10	+	17a-hydroxyprogesterone 2	66	29	12
	359.1	188.9	10	-	Aldosterone 1	-70	-24	-21

	A	B	C	D	D	E	F	G
	359.1	331	10	-	Aldosterone 2	-70	-22	-35
	269.1	144.9	10	-	Estrone 1	-150	-48	-15
	269.1	142.9	10	-	Estrone 2	-150	-70	-15
	271.0	144.9	10	-	Estradiol 1	-110	-52	-21
	271.0	182.9	10	-	Estradiol 2	-110	-52	-19
	292.1	100.0	10	+	13C3-Testosterone	96	29	12
	290.2	100.1	10	+	13C3-Androstenedione	31	27	12
	294.1	258.2	10	+	d5-dehydroepiandrosterone	21	13	28
	324.1	100.0	10	+	d9-progesterone	151	31	15
	339.2	96.9	10	+	d8-17hydroxyprogesterone	66	29	12
	367.2	121.1	10	+	13C3-cortisol	80	29	16
	364.2	166.0	10	+	13C3-cortisone	81	31	26
	362.0	192.0	10	-	13C3-aldosterone	-70	-24	-12
	272.1	147.8	10	-	13C3-estrone	-110	-52	-21
	274.0	147.9	10	-	13C3-estradiol	-110	-48	-29

Table S5 - Multiple reaction monitoring (MRM) settings for each steroid, including quantitative (1) and qualitative (2) ions for each steroid. DP - declustering potential, CE - collision energy, CXP - collision exit potential

26 Check the retention times of the steroids are as expected, as shown in the chromatogram in **Table S6**:

Expected result

Retention times; aldosterone at **2.6 mins**, cortisol at **3.5 mins**, cortisone at **2.9 mins**, estrone at **7.2 mins**, 17beta-estradiol at **7.0 mins**, androstenedione at **6.9 mins,** testosterone at **7.6 mins,** dehydroepiandrosterone at **7.9 mins**, 17alpha-hydroxyprogesterone at **8.0 mins** and progesterone at **9 mins**

27 Inject a mid-level standard. Check the chromatography and each steroid retention time is consistent with expected times and peak area response is as expected. Once satisfied then set the batch of samples to analyse, injecting 20 µL per sample.

Method specific data evaluation of LC-MS/MS data

28 Use the data analysis parameters to assess the peak area of the chromatograms for each steroid in the Steroid analytes and their assigned internal standards (Table S6)

	A	B	C	D
	Steroid Name	**Abbreviation**	**Retention Time (min)**	**Internal Standard**
	Progesterone	P4	9.0	d9-P4
	Cortisol	F	3.5	13C3F
	Cortisone	E	2.9	13C3E
	Androstenedione	A4	6.9	13C3A4
	Testosterone	T	7.6	13C3T
	Dehydroepiandorsterone	DHEA	7.9	d5-DHEA
	Aldosterone	Aldo	2.6	13C3-Aldo
	Estrone	E1	7.2	13C3-E1
	17beta-estradiol	E2	7.0	13C3-E2
	17alpha-hydroxyprogesterone	17OHP4	8.0	d817OHP4
	Internal Standards			
	13C3-cortisol	13C3F	3.5	Int Std

	A	B	C	D
	13C3-cortisone	13C3E	2.8	Int Std
	13C3-Androstenedione	13C3A4	6.9	Int Std
	13C3-Testosterone	13C3T	7.6	Int Std
	d9-progesterone	d9P4	8.9	Int Std
	d8-17hydroxyprogesterone	d817OHP4	7.9	Int Std

Table S6 - **Method specific summary of retention time and specific internal standard of the steroids**

Data Evaluation of steroid profiling LC-MS/MS data

29 Use MultiQuant software and Microsoft Excel to evaluate the LC-MS/MS steroid profiling data, by defining calibration standard levels, ensuring accuracy of the calibration standards and linear regression > 0.99. Use the Table above, to calculate the concentration of steroids in each sample, as detailed in the 'MultiQuant and Excel' protocol below. Remember to account for the volume of sample extracted and express as ng/mL.

Protocol

NAME

Using MultiQuant and Excel software to evaluate and report multi-analyte targeted LC-MS/MS data

CREATED BY

Natalie Z M Homer

Preview

Protocol references

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