thermo scientific

POSTER NOTE 65070

Development of an LC-MS/MS Method for Measurement of a Steroid Panel in Serum for Clinical Research

ABSTRACT

Purpose: To develop a simple and fast LC-MS/MS method for quantification of 11 steroids in serum

Methods: A solid phase extraction (SPE) method was developed on a Thermo Scientific™ SOLAμ™ HRP 96-well plate to simultaneously extract 11 steroids from serum (**11-deoxycortisol, 17-OH progesterone, aldosterone, androstenedione, corticosterone, cortisol, cortisone, estradiol, estrone, progesterone and testosterone**). Extracted compounds were separated on a reverse phase column chromatographically followed by analysis on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer.

Results: The entire SPE process takes less than 20 minutes, and no pre-conditioning, evaporation or reconstitution is required. Comparing to conventional SPE method which involves those steps, our method is simpler and faster. The recovery rate ranged from 42% (aldosterone) to 95% (testosterone). In neat solution, lower limit of quantitation (LOQ) of androstenedione is 1 pg/mL. LOQ of testosterone is 2 pg/mL. LOQ of 11-deoxycortisol, 17-OH progesterone, cortisone, estradiol, estrone, and progesterone is 5 pg/mL. LOQ of aldosterone, corticosterone, and cortisol is 10 pg/mL.

INTRODUCTION

Over the past few years there has been a growing interest to use LC-MS/MS method to measure steroids in serum. LC-MS/MS offers the potential for more reliable measurement compared to other detection systems, such as immunoassays. However, the challenges are time-consuming sample pre-treatment, matrix interference and isobars separation. We developed a simple and fast SPE sample preparation method for an 11-steroid panel and evaluated the analytical performance on an LC-triple quadrupole MS/MS system.

MATERIALS AND METHODS

Sample Preparation

SPE was performed on a SOLAμ HRP 96-well plate. Calibrators/QCs were spiked into neat solution or charcoal stripped serum and mixed with water and methanol. The mixture was loaded directly onto the SPE plate; no preconditioning was required. After washing the plate with 30% methanol, the elution was performed with two volumes of 25 μL of methanol each. Eluates were diluted with 50 μL water. 50 μL of the diluted eluate was injected for LC-MS/MS analysis.

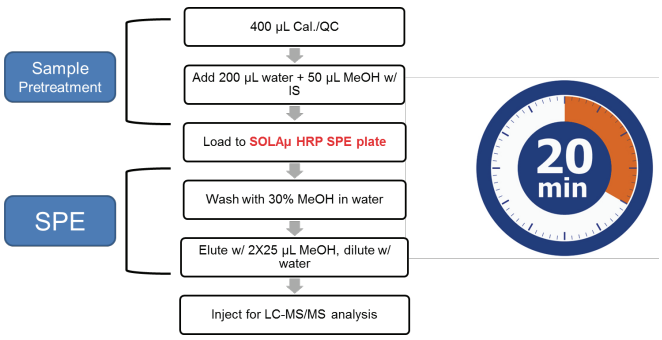
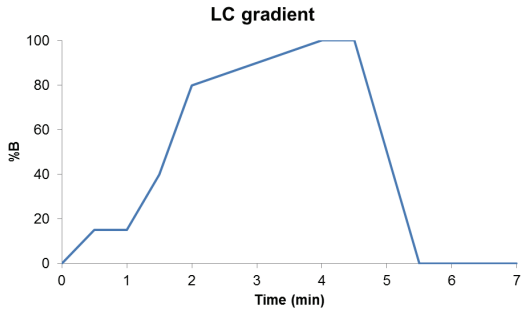


Figure 1. SPE process to extract 11 steroids from plasma.

Test Method(s)

Figure 2. Thermo Scientific™ UltiMate™ 3000 HPLC and TSQ Quantiva MS with heated ESI source



ThermoFisher SCIENTIFIC

Compound	RT (min)	RT Window (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
11-deoxycortisol	3.2	1	Positive	347.3	97	25	72
11-deoxycortisol	3.2	1	Positive	347.3	109	28	72
11-Deoxycortisol-d5	3.2	1	Positive	352.3	100	25	71
11-Deoxycortisol-d5	3.2	1	Positive	352.3	113	30	71
17-OH-P	3.4	1	Positive	331.2	97	24	69
17-OH-P	3.4	1	Positive	331.2	109.1	27	69
17OH-P-d8	3.4	1	Positive	339.3	100	26	70
17OH-P-d8	3.4	1	Positive	339.3	113	29	70
Aldosterone	3	1	Negative	359.3	189.1	17	69
Aldosterone	3	1	Negative	359.3	331.1	15	69
Androstenedione	3.3	1	Positive	287.2	97	23	64
Androstenedione	3.3	1	Positive	287.2	109.1	25	64
Androstenedione-^{13}C3	3.3	1	Positive	290.2	100.1	22	65
Androstenedione-^{13}C3	3.3	1	Positive	290.2	112	25	65
Corticosterone	3.2	1	Positive	347.3	121.1	25	68
Corticosterone	3.2	1	Positive	347.3	147.1	26	68
Cortisol	3	1	Positive	363.2	121.1	26	71
Cortisol	3	1	Positive	363.2	309.2	20	71
Cortisol-d4	3	1	Positive	367.3	121.1	26	72
Cortisol-d4	3	1	Positive	367.3	175.1	27	72
Cortisone	3	1	Positive	361.2	121.1	31	78
Cortisone	3	1	Positive	361.2	163	24	78
Estradiol	3.3	1	Negative	271.2	145	41	108
Estradiol	3.3	1	Negative	271.2	183	40	108
Estradiol-d5	3.3	1	Negative	276.1	147	40	115
Estradiol-d5	3.3	1	Negative	276.1	187	41	115
Estrone	3.4	1	Negative	269.2	145.1	40	90
Estrone	3.4	1	Negative	269.2	183.1	40	90
Estrone-^{13}C3	3.3	1	Negative	272.2	146	54	106
Estrone-^{13}C3	3.3	1	Negative	272.2	148.1	39	106
Progesterone	3.7	1	Positive	315.3	97.1	22	68
Progesterone	3.7	1	Positive	315.3	109.1	25	68
Progesterone-d9	3.7	1	Positive	324.5	100.1	22	67
Progesterone-d9	3.7	1	Positive	324.5	113	27	67
Testosterone	3.4	1	Positive	289.3	97	22	64
Testosterone	3.4	1	Positive	289.3	109.1	25	64
Testosterone-^{13}C3	3.4	1	Positive	292.3	100	23	66
Testosterone-^{13}C3	3.4	1	Positive	292.3	112	25	66

Figure 4. SRM transitions (two for each analyte/IS)

RESULTS

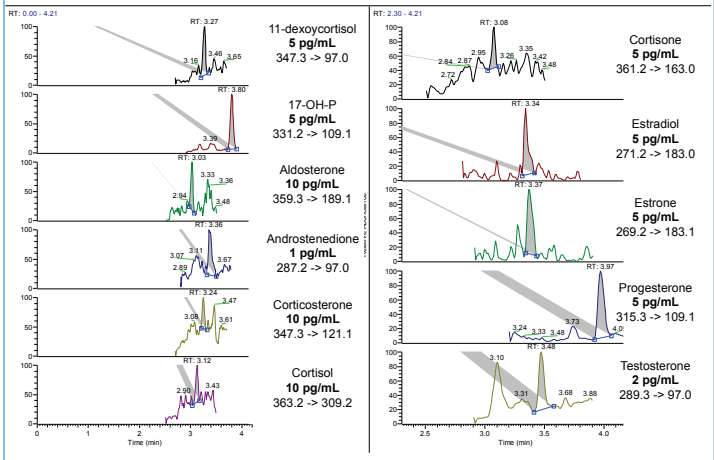
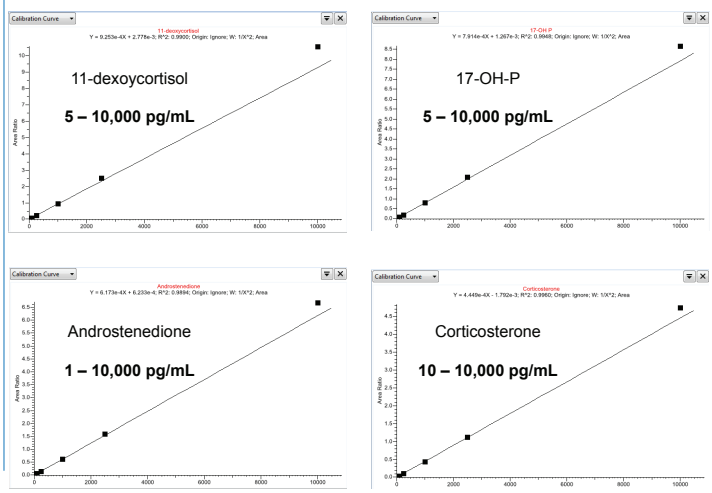


Figure 5. Chromatograms on the lower limit of quantitation level in neat solution



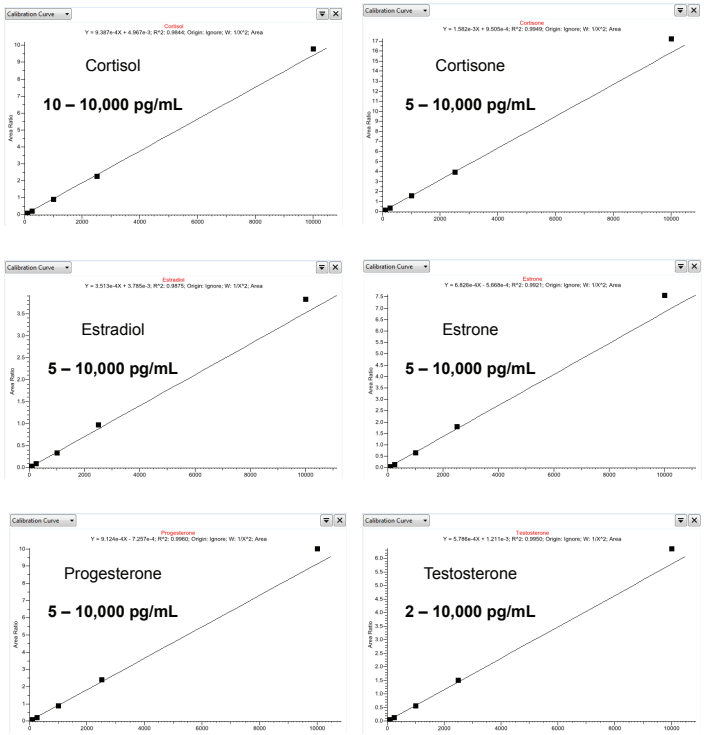


Figure 6. Linearity range

Compound	SPE Recovery Rate (%)	Lower Limit of Quantitation (pg/mL)
11-deoxycortisol	63	5
11-Deoxycortisol-d5	64	
17-OH-P	84	5
17OH-P-d8	83	
Aldosterone	42	10
Androstenedione	93	1
Androstenedione-^{13}C3	95	
Corticosterone	67	10
Cortisol	68	10
Cortisol-d4	70	
Cortisone	71	5
Estradiol	87	5
Estradiol-d5	88	
Estrone	89	5
Estrone-^{13}C3	89	
Progesterone	84	5
Progesterone-d9	86	
Testosterone	95	2
Testosterone-^{13}C3	97	

Figure 7. Recovery rate and lower limit of quantitation

CONCLUSIONS

- A simple and fast SPE method (~20 min) was develop to simultaneously extract 11 steroids from serum with recovery rate ranged from 42% to 95%.
- A sensitive LC-MS/MS analytical method was demonstrated to analyze 11 steroids with lower limit of quantitation ranged from 1 pg/mL to 10 pg/mL.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC