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Determination of Endogenous Steroids in Human Blank Serum using a Triple Quadrupole Mass Spectrometer with Ion Funnel Technology in Positive/Negative Modes

Yanan Yang
MP 351
Agilent Technologies, Inc.,
Santa Clara, CA, USA
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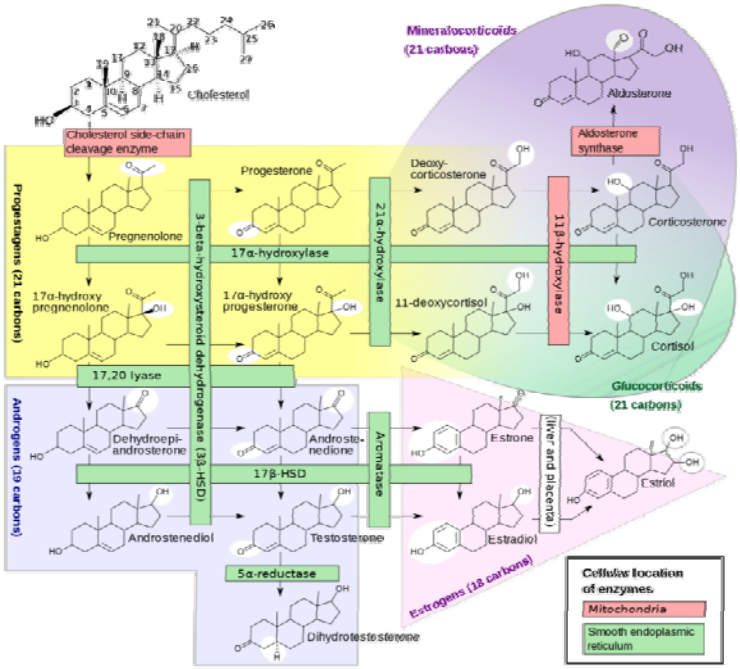
Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) coupled with ion funnel technology has become an essential clinical research tool for analysis of endogenous steroids because of its ability to simultaneously analyze multiple analytes with high sensitivity, and excellent specificity and reproducibility.

In this study, thirteen major steroids were quantified in human blank serum (see Figure 1 and Table 1). Some steroids exhibit a better response in negative ionization mode – for example, dehydroepiandrosterone sulfate (DHEAS) which forms anions in solution. Also, steroids with phenol groups, such as estradiol, estriol and estronem, lose a water molecule in positive ion mode and form more unique fragments in negative mode. The remaining steroids have better sensitivity in positive ion mode. A robust, sensitive, reliable and fast method is presented for the quantitation of the major endogenous steroids in human serum utilizing LC-MS/MS with positive/negative switching ionization in a single run. This quantitative method demonstrates a wide dynamic range, excellent linearity, accuracy and reproducibility in human serum.

Figure 1. Human Steroidogenesis



Copy from http://en.wikipedia.org/wiki/File:Steroidogenesis.svg

Experimental

Sample Preparation:

Sample information: Thirteen steroid standards and four isotopic labeled internal standards (obtained from Cerilliant) are listed in Table 1.

Serum sample preparation: 250 µL human blank serum (obtained from UTAK Laboratories, Inc.) was crashed with 500 µL acetonitrile, vortexed for 1 minute and centrifuged for 4 min at 10,000 rpm. 500 µL supernatant was transferred and diluted with 500 µL of water. This final extracted solution is used as serum matrix in the following dilution.

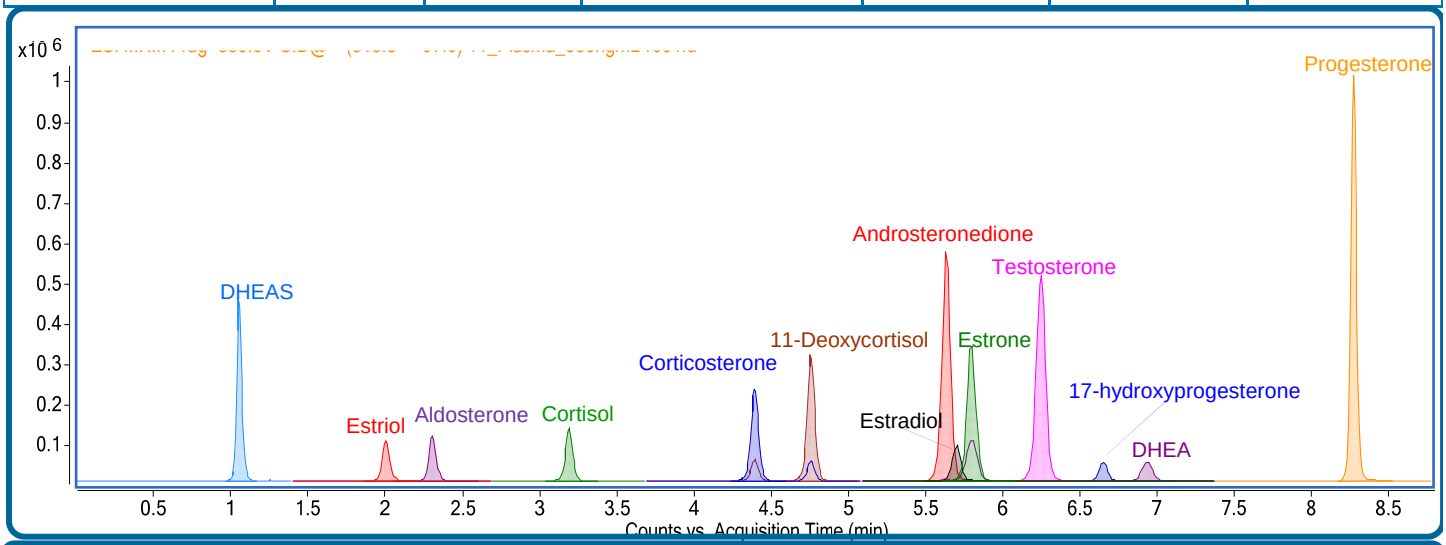
Calibration curve in both clean solvent and serum matrix: The calibration range of DHEA, estrone, estradiol, and estriol is from 0.05 to 500 ng/mL. The calibration range of the other nine steroids is from 0.0025 to 50 ng/mL. Serum matrix curves were only used for analytes with undetectable concentrations in blank serum (table 2).

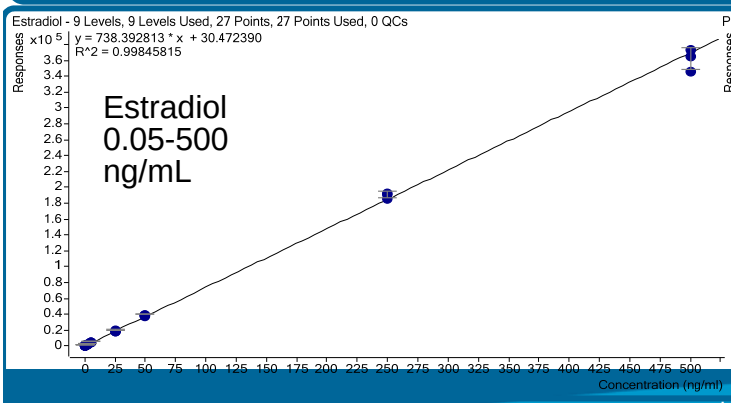
LC Method:
Agilent 1290 Infinity UHPLC series binary pump, well plate sampler, thermostatted column compartment
Column: Extend C18, 2.1x50mm 1.8 um, 600 bar
Column temperature: 50 ºC
Injection volume: 2 µL
Autosampler temp: 4 ºC
Needle wash: flushport (MeOH:water 75:25), 10 sec
Mobile phase: A = 0.02 % ammonium hydroxide in water
B = methanol:isopropanol 75:25
Flow rate: 0.4 mL/min
Gradient: 20% B to 47% B in 7 minutes and up to 95% B in 1 min, hold at 95% B for 0.5 min, post run is 1.5 min. The total time for each run is 10 min.

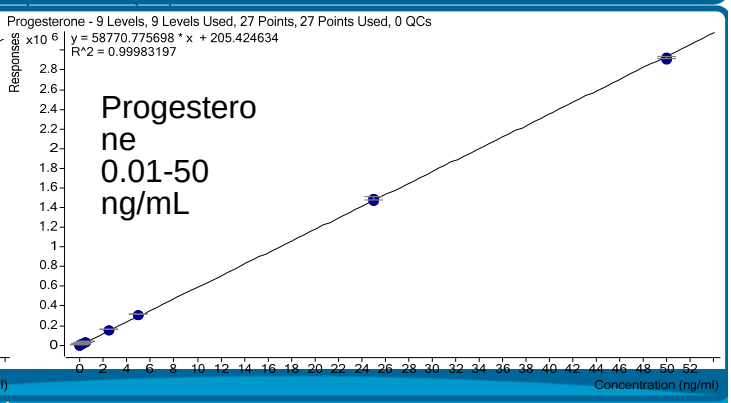
MS Method:
Agilent 6490 triple quadrupole mass spectrometer with ion funnel technology
Ion mode: Agilent Jet Stream pos/neg
Gas temperature: 200 ºC
Drying gas (nitrogen): 15 L/min
Nebulizer gas (nitrogen): 35 psi
Sheath gas (nitrogen): 250 ºC
Sheath flow: 11 L/min
Capillary voltage: +4000V/-3000V
Nozzle voltage: +0V/-2000V
Q1/Q2 Resolution: 1.2/0.7 unit
Switching dwell time: 40 msec
Delta EMV: +100V/-100V

Results and Discussion

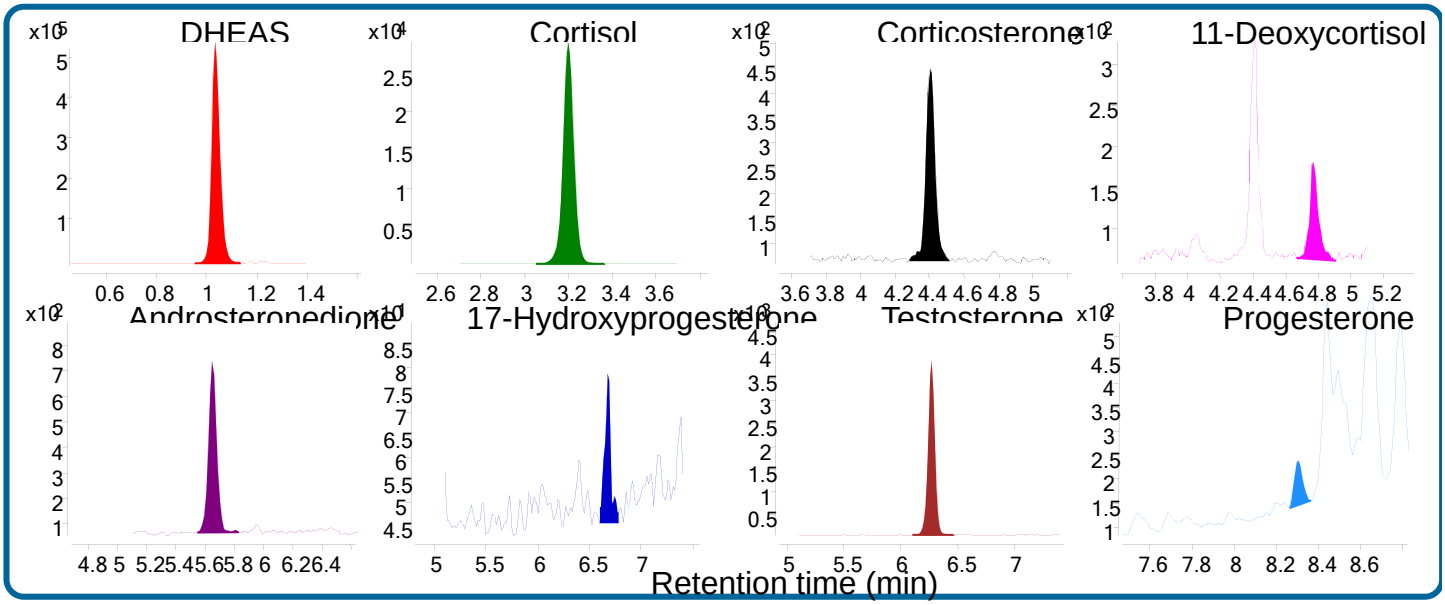
Compound	Ion Mode	RT (min)	MRM	Dwell (msec)	Fragmentor (V)	CE (V)
DHEAS	ESI-	1.05	367.2>97.0	400	380	35
Estriol	ESI-	2.00	287.2>171.0, 145.0	100	380	43,43
Aldosterone	ESI+	2.32	361.3>343.3, 315.2	100	380	18,18
Cortisol	ESI+	3.21	363.2>327.2, 121.1	200	380	15, 23
Corticosterone	ESI+	4.41	347.3>329.3, 311.2	100	380	15, 18
11-Deoxycortisol	ESI+	4.78	347.3>109.1, 97.0	100	380	33, 36
Androsteronedione	ESI+	5.65	287.2>109.1, 97.0	50	380	28, 23
Estradiol-d5	ESI-	5.70	276.2>147.1	50	380	43
Estradiol	ESI-	5.72	271.2>183.1, 145.1	50	380	36, 43
Estrone	ESI-	5.82	269.2>183.1, 145.1	50	380	36, 43
Testosterone-d3	ESI+	6.24	292.2>97.0	50	380	28
Testosterone	ESI+	6.27	289.2>109.1, 97.0	50	380	28, 28
17-Hydroxyprogetserone	ESI+	6.68	331.3>109.1, 97.0	50	380	33, 33
DHEA-d5	ESI+	6.93	276.2>258.2	50	380	8
DHEA	ESI+	6.96	271.2>253.2, 197.2	50	380	8, 18
Progesterone-d9	ESI+	8.30	324.3>100.2	100	380	20
Progesterone	ESI+	8.30	315.3>109.2, 97.0	200	380	25, 20







Results and Discussion



Eight steroids were detected in pooled human serum blank after being crashed with acetonitrile and diluted with water (Figure 4). The detected human blank serum levels are listed in Table 2. The simple sample preparation procedure results in a 6 factor dilution from the original serum. In the future study, a lower LOQ for Estradiol, estrone, estriol, aldosterone and DHEA could be achieved by using alterations to the sample preparation method, such as enrichment, evaporation, and etc.

Compound	LOQ (ng/mL)	Range (ng/mL)	R^2	Accuracy (%)	Reproducibility (%)	Blank Serum (ng/mL)
DHEAS	0.0025	0.0025-25	0.9995	85.3-116.7	0.04-12.2	1360
Estriol	0.05	0.05-250	0.9998	86.3-103.6	0.01-5.41	n.d.
Aldosterone	0.005	0.005-50	0.9996	94.5-104.6	0.30-5.05	n.d.
Cortisol	0.0025	0.0025-25	0.9998	87.8-113.1	0.26-5.75	149
Corticosterone	0.01	0.01-25	0.9998	93.4-109.0	0.35-6.28	2.94
11-Deoxycortisol	0.0025	0.0025-25	0.9999	95.8-111.0	0.39-5.21	0.22
Androsteronedione	0.0025	0.0025-25	0.9996	92.1-117.1	1.05-9.65	0.77
Estradiol	0.05	0.05-500	0.9984	92.3-106.4	0.68-7.24	n.d.
Estrone	0.05	0.05-500	0.9985	82.3-114.6	1.25-10.9	n.d.
Testosterone	0.0025	0.0025-25	0.9993	94.1-113.2	0.97-10.4	3.94
17-Hydroxyprogesterone	0.0025	0.0025-25	0.9995	86.4-102.3	1.22-3.63	0.24
DHEA	0.025	0.025-250	0.9951	81.9-111.7	1.33-5.22	n.d.
Progesterone	0.0025	0.0025-25	0.9997	95.5-102.5	0.27-1.06	0.05

Conclusion

- Baseline separation of thirteen steroids with the exception of estradiol is achieved under 8.5 minutes. However, estradiol is not isobaric to androsteronedione or estrone, so the quantitation calculation is not effected.
- The calibration curves show excellent linearity (> 0.995) with greater than three orders of dynamic range.
- Great accuracy, precision, reproducibility, and signal stability of LC-MS/MS (QQQ) analyses were observed for the 13 steroids.
- This fast and simple LC-MS/MS method is suitable for analyzing several endogenous steroids in biological matrices in a single run.