High-Throughput LC-MS/MS Measurement of 17-Hydroxyprogesterone in Human Blood Serum for Research Purposes

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OVERVIEW

Purpose: To develop an LC-MS/MS method for researchers to accurately measure 17-hydroxy-progesterone (17-OHP) in blood serum from 10 to 1,000 ng/dL with a throughput of at least 12 injections per hour and can be multi-channeled with other LC-MS methods utilizing the same ion source.

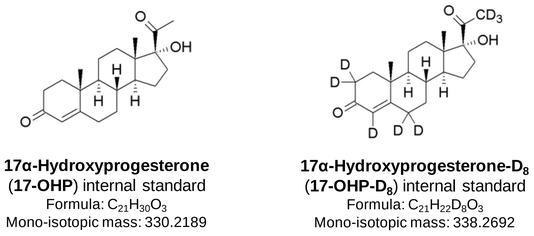
Methods: Liquid-liquid extraction (LLE) of blood serum followed by quantitative analysis using a 4-channel ultra high-performance liquid chromatography (UHPLC) system coupled to a tandem mass spectrometer (MS/MS). 17-OHP and its internal standard were separated from sample matrix components by water-to-methanol gradient elution through a UHPLC column packed with solid-core silica particles having an alkyl-bonded phase on its surfaces. Analytes were eluted to an atmospheric-pressure chemical ionization (APCI) probe of a triple-quadrupole mass spectrometer where selected-reaction monitoring (SRM) detected the analytes.

Results: An analytical range of 10 to 1,000 ng/dL was achieved with inter- and intra-batch reproducibility less than 6% CV, carryover less than 0.1% and acceptable correlation of specimen results with those from a reference laboratory. Throughputs of 14 to 56 injections/hour were achieved. Method accuracy and reproducibility were maintained while other channels ran other methods, such as testosterone.

INTRODUCTION

17-OHP is a biosynthetic precursor to other steroids, such as cortisol, androgens and estrogens. It is converted to 11-deoxycortisol by 21α-hydroxylase or to androstenedione by 17, 20 lyase. Researchers investigating how these enzymes function need to measure 17-OHP within an analytical range of 10 to 1,000 ng/dL (0.3 to 30 nmol/L) of blood serum. The chemical structures of 17-OHP and its deuterated internal standard (IS) are shown in Figure 1.

Figure 1. Chemical structures of analyte and IS



17α-Hydroxyprogesterone (17-OHP) internal standard
Formula: $C_{21}H_{30}O_3$
Mono-isotopic mass: 330.2189

17α-Hydroxyprogesterone-D_8 (17-OHP-D_8) internal standard
Formula: $C_{21}H_{22}D_8O_3$
Mono-isotopic mass: 338.2692

MATERIALS AND METHODS

Consumables

Fisher Scientific™ Optima™ solvents were used for LC mobile phases and wash solutions, as well as for preparations of calibrators, quality controls (QCs) and donor blood serum samples. 17α-Hydroxy-progesterone and 17α-Hydroxyprogesterone-D8 were purchased from Cerilliant Corporation (Round Rock, TX). Calibrators were made by mixing stock standard with a diluent of 1% bovine serum albumin in phosphate-buffered saline. 17-OHP controls were purchased from UTAK Laboratories Inc. (Valencia, CA).

Sample Preparation

200 µL aliquots of fresh blood serum specimens, as well as calibrators and quality control specimens (QCs), were spiked with 17-OHP-D8 internal standard (IS) before being subjected to liquid-liquid extraction with 1 mL methyl t-butyl ether (MTBE). After drying the ether extracts by heated nitrogen flow, the residue of each tube was reconstituted with water and methanol (1:1) to a total volume of 150 µL, and 50 µL injections were made into the LC-MS/MS system.

Test Methods

Using one or more channels of a Thermo Scientific™ Transcend™ LX-4 system, chromatographic segregation of the steroids from unwanted sample components was accomplished by gradient elution through a Thermo Scientific™ Accucore™ RP-MS column (2.6 µm, 50 x 2.1 mm), which was heated to 50ºC. Chromatographic conditions are described in Figure 2. The Thermo Scientific™ TSQ Endura™ triple-quadrupole mass spectrometer was used with an APCI probe. Ion source and MS/MS conditions are described in Figure 3.

Instrument Control & Data Analysis

Thermo Scientific™ TraceFinder™ software with Aria™ MX software was used to control the Transcend LX-4 and Endura MS/MS systems, submit batches to desired channels, as well as for analyzing data and reporting results.

Figure 2. Liquid chromatography parameters

Column:	Accucore RP-MS, 2.6 µ, 50 x 2.1 mm
Solvent A:	Water + 0.1% Formic Acid
Solvent B:	Methanol

Step	Start	Sec	Flow	Gradient	%A	%B
1	0.00	10	0.5	Step	90	10
2	0.17	20	0.5	Step	50	50
3	0.50	60	0.5	Ramp	30	70
4	1.50	30	0.5	Ramp	-	100
5	2.00	30	0.5	Step	-	100
6	2.50	30	0.5	Step	90	10
7	3.00	60	0.7	Step	90	10

Start data: 2.0 min Duration: 1.0 min

Total run time: 4.0 min

Figure 3. Mass spectrometer APCI & SRM parameters

Ion Source:	APCI, + Ion Current 3 µA, vaporizer temp: 400°C
SRM Transitions:	Q1 & Q3 resolutions: 0.7

Analyte	Q1	Q2	CE	RF
17-OHP (Confirm)	331.20	97.20	22	150
17-OHP (Quan)	331.20	109.20	26	150
17-OHP-D_8 (Confirm)	339.25	100.22	24	160
17-OHP-D_8 (Quan)	339.25	113.22	28	160

RESULTS

Quantitative Analysis Reliability

Typical results from calibrators, QCs and specimens are shown in Figure 4. Ion ratios (Confirming/Quan transitions peak areas) averaged 110% for 17-OHP and 140% for 17-OHP-D8.

IS peak areas among calibrators & QC averaged 8,050 with RSD < 6%. IS peak areas in specimens ranged from 6,670 to 10,290 and showed an average recovery of 102%. Carryover was less than 0.1%. Inter- and intra-batch precisions were less than 5 and 6% coefficient of variation (CV), respectively (Tables 1a & 1b).

Throughput

Single-channel throughput was 14 injections per hour. When multi-channeled across 2, 3 or 4 channels, the throughput increased to 28, 42 and 56 injections per hour, respectively. 17-OHP batches were also multi-channeled with MMA (1) batches, which utilized the same MS source conditions.

Figure 4. Typical 17-OHP quantitative results

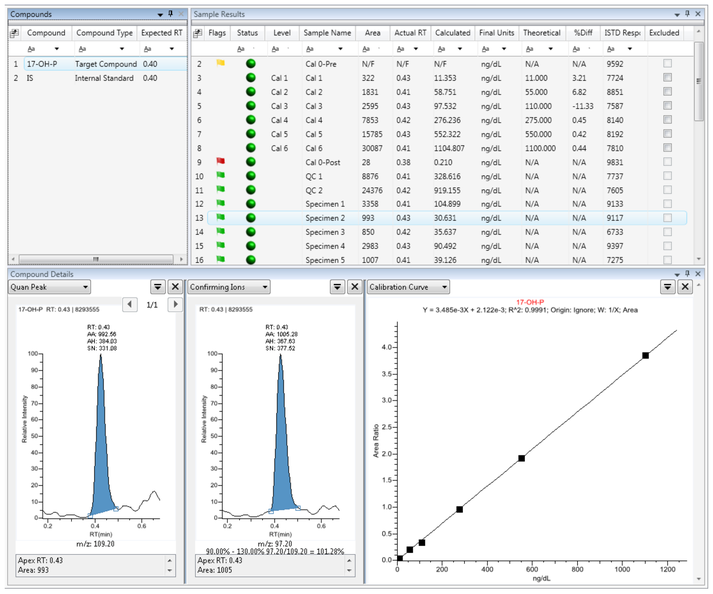


Table 1. Inter- and intra-batch precision results

a. Inter-batch

Injection	17-OHP (ng/dL)
1	86
2	74
3	80
4	85
5	84
6	84
7	84
8	79
9	83
10	76
11	75
12	86
13	81
14	78
15	83
16	83
17	87
18	83
19	84
20	86
Mean	**82.05**
STD	**3.85**
CV %	**4.7**

a. Inter-batch

b. Intra-batch

Date	Injection	17-OHP (ng/dL)
Day 1, 10/7/14	1	86
	2	74
	3	80
	4	85
	5	84
Day 2, 10/8/14	1	80
	2	77
	3	78
	4	82
	5	89
Day 3, 10/9/14	1	85
	2	85
	3	76
	4	77
	5	87
Day 4, 10/10/14	1	77
	2	82
	3	74
	4	86
	5	77
	Mean	**81.05**
	SD	**4.63**
	CV %	**5.71**

b. Intra-batch

Accuracy Assessment

Comparison of 40 specimen results ranging from 11 to 828 ng/dL with reference-lab results showed differences that averaged 3.0% and ranged from -29.6 to 15.4 (Table 2). Only 3 of 40 test samples (7.5%), deviated 20% or more. Deming regression analysis showed $R^2 = 0.9961$, Slope = 0.979, Intercept = 2.8, Standard Error of Estimate = 10.6%.

Table 2. Reference-lab results comparison

Test Sample	Current Method	Reference Lab	Difference %	Test Sample	Current Method	Reference Lab	Difference %
1	35	27	-29.6	21	81	71	-14.1
2	37	32	-15.6	22	54	58	6.9
3	28	27	-3.7	23	108	97	-11.3
4	34	27	-25.9	24	112	127	11.8
5	48	48	0.0	25	177	161	-9.9
6	23	23	0.0	26	34	35	2.9
7	22	26	15.4	27	34	31	-9.7
8	18	18	0.0	28	164	168	2.4
9	31	32	3.1	29	37	35	-5.7
10	71	72	1.4	30	91	82	-11.0
11	67	77	13.0	31	94	88	-6.8
12	82	85	3.5	32	207	181	-14.4
13	53	55	3.6	33	53	49	-8.2
14	93	98	5.1	34	63	61	-3.3
15	41	41	0.0	35	84	70	-20.0
16	44	43	-2.3	36	13	11	-18.2
17	46	48	4.2	37	80	85	5.9
18	121	114	-6.1	38	246	213	-15.5
19	42	39	-7.7	39	185	199	7.0
20	25	24	-4.2	40	12	11	-9.1

CONCLUSIONS

Robust, reliable and sensitive quantification of 17-OHP in donor blood serum samples was achieved using this research method with a four-channel LC-MS/MS system. We demonstrated:

- Analytical range from 10 to 1,000 ng/dL
- Throughputs of 14, 28 or 56 injections per hour from a 1-, 2- or 4-channel system
- Inter- & intra-batch precisions less than 6% and carryover less than 0.1%
- Multi-channeling with other methods utilizing the same APCI source

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