High-Throughput LC-MS/MS Quantification of 17-Hydroxyprogesterone (17-OHP) in Human Blood Serum for Clinical Research Purposes

Joe DiBussolo, Marta Kozak
Thermo Fisher Scientific, San Jose, CA

Application Note 651

Key Words
Steroid, 17-hydroxyprogesterone, 17-OHP, high-throughput, Transcend, TSQ Endura, multichannel, triple quadrupole MS

Goal
To develop a fast, reliable, and robust HPLC-MS/MS method for quantifying 17-hydroxyprogesterone in human blood serum for clinical research purposes.

Application Benefits
- Simple, economical sample extraction procedure
- Reliable quantitation from 10 to 1,000 ng/dL with ion-ratio confirmation
- Throughput capabilities of 14, 28, or 56 injections per hour with 1-, 2-, or 4-channel HPLC systems

Introduction
17-Hydroxyprogesterone (17-OHP) is a biosynthetic precursor to other steroids such as corticosteroids, androgens, and estrogens. It is converted to 11-deoxycortisol by 21-alpha-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) in blood serum. 17-OHP readily forms positive ions by atmospheric-pressure chemical ionization (APCI), which is less prone to matrix effects than electrospray ionization (ESI).

Methods
Sample Preparation
Aliquots of fresh blood serum specimens (200 µL), as well as calibrators prepared in 1% BSA and quality control specimens (QCs), were spiked with 17-OHP-D4 internal standard before being subjected to liquid-liquid extraction with 1 mL methyl *t*-butyl ether (MTBE). After drying the ether extracts with heated nitrogen, each residue was reconstituted with water and methanol (1:1) and subjected to reversed-phase liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) with an APCI probe.

Liquid Chromatography
Using one or more channels of a Thermo Scientific™ Transcend™ LX-4 multichannel HPLC, chromatographic separation of the steroid analytes from interfering sample components was achieved by gradient elution through a Thermo Scientific™ Accucore™ RP-MS column (2.6 µm, 50 x 2.1 mm, P/N 17626-05213) at room temperature. The chromatographic parameters are described in Figure 1. This method was multichanneled with other methods that utilized the same MS ion source at the same temperatures and gas flows.

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 1. Chromatographic parameters.

Mass Spectrometry and Data Analysis
A Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer was used with an APCI probe. 17-OHP and its deuterated internal standard (IS) were quantified by selected reaction monitoring (SRM), as described in Figure 2. All data were acquired and processed using Thermo Scientific™ TraceFinder™ software.

Thermo SCIENTIFIC

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

Figure 2. TSQ Endura parameters.

Method Evaluation

Method precision was assessed by calculating percent coefficient of variation (%CV) of peak areas from 20 replicate injections of two pools of test specimens. Carryover was measured in blanks immediately following injections of the highest calibrator. Matrix effects were evaluated by comparing IS peak areas in specimen samples to IS peak areas in calibrator and control samples. A method comparison experiment was performed by analyzing 40 donor samples (no informed consent was needed) following the procedures reported in this study and comparing results with those obtained by a reference lab.

Results and Discussion

As shown in Figure 3, the desired analytical range from 10 to 1,000 ng/dL (0.3 to 30 nmol/L) was achieved and was consistently linear ($r^2 \geq 0.999$ with 1/X weighting). Carryover never exceeded 0.1%. Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. The ion ratios for 17-OHP and 17-OHP-D_8 averaged 105% and 140%, respectively. Typical characteristics of chromatographic peaks from quantitation- and confirmation-ion transitions for 17-OHP and its deuterated internal standard from a donor specimen are shown in Figure 4.

The intra- and inter-batch precisions were better than 5% and 6%, respectively. IS peak areas among calibrators and QCs averaged 18,100 cps with an RSD of 20%. Specimen IS peak areas ranged from 11,000 to 21,300 cps with an average recovery of 87%, relative to the averaged IS peak areas in calibrators and QCs. In the method comparison experiment, 17-OHP values in analyzed samples ranged from 13 to 789 ng/dL and the percent difference between two analytical methods for 95% of analyzed samples was 20% or less (Table1).

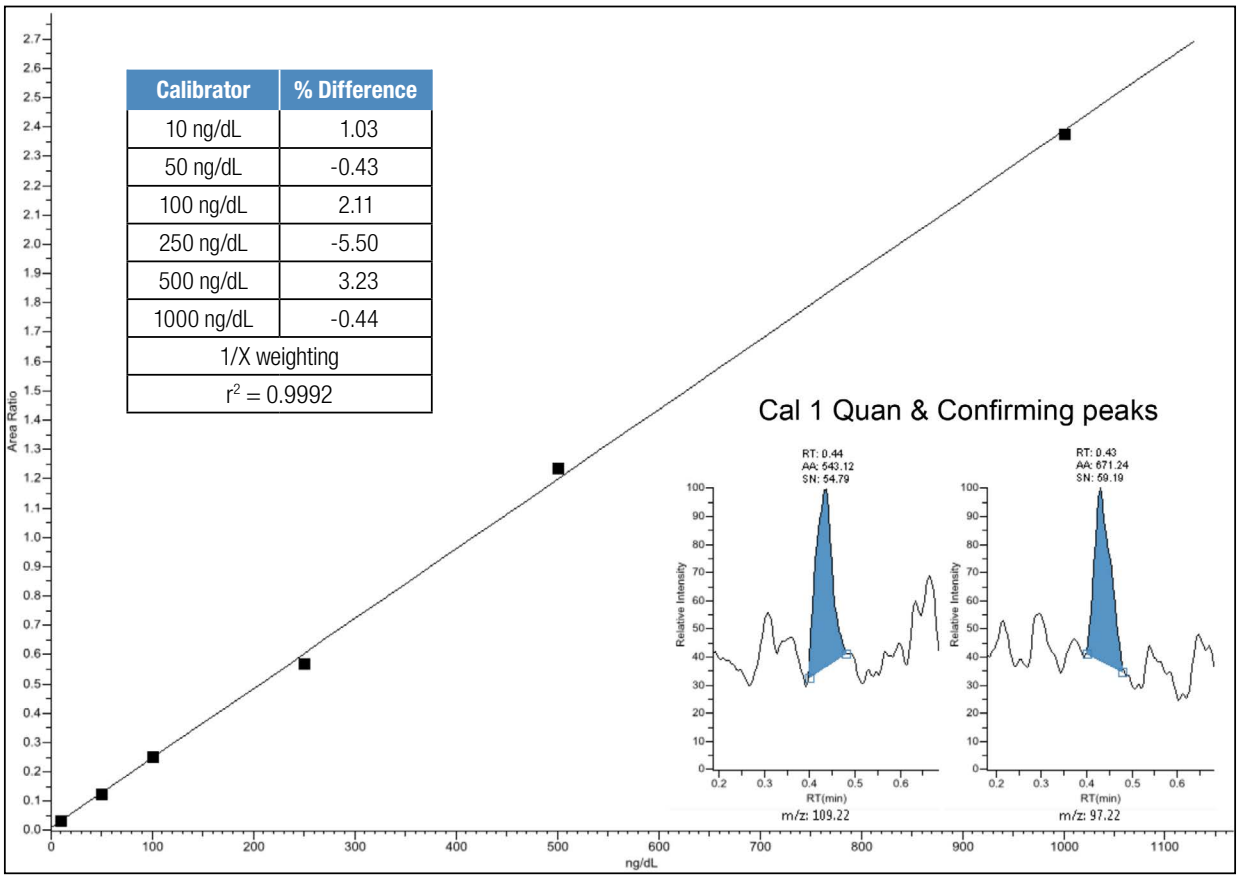

Figure 3. Typical 17-OHP quantitation results.

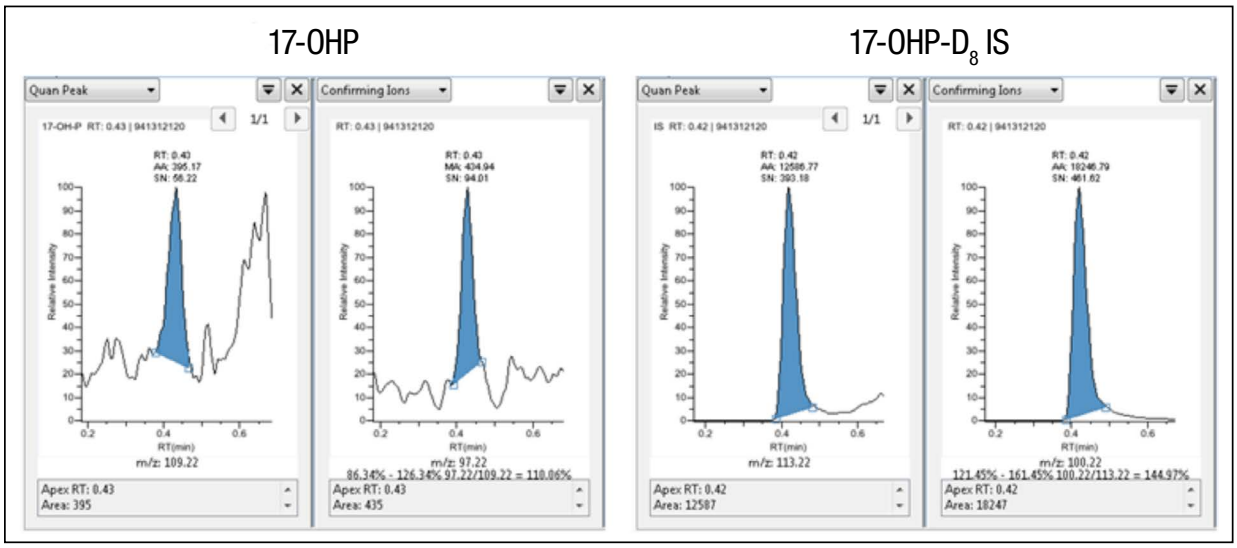

Figure 4. Analyte & IS chromatograms from donor specimen containing 10 ng/dL 17-OHP.

Table 1. 17-OHP results comparison with reference lab.

Test Sample	Current Method	Reference Lab	Difference %
1	35	27	29.63
2	37	32	15.63
3	28	27	3.70
4	34	27	25.93
5	48	48	0.00
6	23	23	0.00
7	22	26	-15.38
8	18	18	0.00
9	31	32	-3.13
10	71	72	-1.39
11	67	77	-12.99
12	82	85	-3.53
13	53	55	-3.64
14	93	98	-5.10
15	41	41	0.00
16	44	43	2.33
17	46	48	-4.17
18	121	114	6.14
19	42	39	7.69
20	25	24	4.17

Test Sample	Current Method	Reference Lab	Difference %
21	81	71	14.08
22	54	58	-6.90
23	108	97	11.34
24	112	127	-11.81
25	177	161	9.94
26	34	35	-2.86
27	34	31	9.68
28	164	168	-2.38
29	37	35	5.71
30	91	82	10.98
31	94	88	6.82
32	207	181	14.36
33	53	49	8.16
34	63	61	3.28
35	84	70	20.00
36	13	11	18.18
37	80	85	-5.88
38	246	213	15.49
39	185	199	-7.04
40	798	828	-3.62

Conclusion

A sensitive, robust, high-throughput quantitation assay for 17-hydroxyprogesterone was developed in this application note. Some of the key features of this research method are:

- Sensitive quantitation from 10 to 1,000 ng/dL (0.3 to 30 nmol/L)
- Excellent reliability with inter- and intra-batch precisions less than 6% and carryover less than 0.1%
- High-throughput capabilities and multichanneling with other APCI methods

Acknowledgements

We thank Dr. Hashim Othman and his staff at Bio-Reference Laboratories (Elmwood Park, NJ) for providing donor specimens and resources to evaluate this research method.

For research use only. Not for use in diagnostic procedures.

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN64658-EN 0616S