LC/MS/MS Method for the Quantification of Endogenous Steroids in Oral Fluids using the QTRAP® 5500 LC/MS/MS System with SelexION™ Ion Mobility Technology

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INTRODUCTION

Measurement of hormone levels such as 17-Hydroxyprogesterone, Cortisol, Estradiol, Estriol, Estrone, Progesterone, and Testosterone can be used to help researchers identify potential markers of disease states in men, women, and children. Steroids are present in oral fluids at low concentrations so methods are needed that provide rapid and reliable analysis with high sensitivity. Traditional methods of analysis, such as immunoassays, can suffer from poor accuracy and reproducibility. A sensitive, selective and robust method was developed to enable the quantification of endogenous steroids in oral fluids.

MATERIALS AND METHODS

Sample Preparation:
1 mL of human oral fluid sample was spiked with deuterated internal standards corresponding to each of the 7 steroid analytes, and the sample was extracted with Biotage Evolute ABN SPE. The organic extracts were dried under nitrogen gas and derivatized with 100 µL of dansyl chloride solution. 2 µL of the derivatized sample was directly injected for LC-MS/MS analysis.

LC Conditions:
Separation was performed on the Eksigent ekspert™ microLC 200 system with a Halo C18 50x0.5mm, 2.7µm column. Mobile phase consisted of water and methanol with 0.1% formic acid. The fllow rate was 25µL/min with injection volume of 2µL. A 25µm ID PEEKSil capillary ion spray electrode with stainless steel tip was used in order to reduce band broadening due to the low flow rate of 30 µL/minute.

Figure 1. Eksigent ekspert™ microLC 200 system

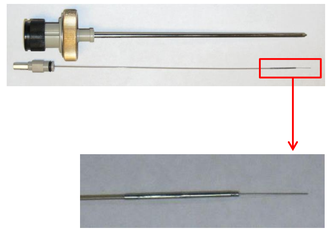


Figure 2. Turbo V™ probe next to 50µm ID PEEKSil® capillary ion spray electrode.

MS Conditions:
Mass spectrometric analyses were performed on an AB SCIEX QTRAP® 5500 system in positive ESI mode with SelexION™ ion mobility technology, also known as DMS (Differential Mobility Spectrometry) technology. The results were evaluated with and without DMS. The following MRM (Multiple Reaction Monitoring) transitions were monitored in this study for the steroid analytes: 17-Hydroxyprogesterone (331/97, 331/109), Cortisol (363/121, 363/115), Estradiol Derivative (506/171, 506/156), Estriol Derivative (522/171, 522/156), Estrone Derivative (504/171, 504/156), Progesterone (315/97, 315/109), and Testosterone (289/97, 289/109)

Table 1. Source/Gas Settings

Parameter	Value
Curtain Gas	20psi
Collision Gas	Medium
IonSpray Voltage	-5500V
Gas 1	25psi
Gas 2	50psi
Temperature	600C

Table 2. Optimized MRM conditions and compensation voltage (COV)

Analyte	Q1	Q3	DP	CE	CXP	COV
17OH-Progesterone 1	331	97	80	29	15	7.9
17OH-Progesterone 2	331	109	80	38	15	7.9
Cortisol 1	363	121	80	31	15	6.8
Cortisol 2	363	115	80	115	15	6.8
Estradiol Derivative 1	506	171	100	45	15	7.7
Estradiol Derivative 2	506	156	100	55	15	7.7
Estriol Derivative 1	522	171	100	45	15	7.2
Estriol Derivative 2	522	156	100	55	15	7.2
Estrone Derivative 1	504	171	100	45	15	6.5
Estrone Derivative 2	504	156	100	55	15	6.5
Progesterone 1	315	97	80	26	12	8.1
Progesterone 2	315	109	80	30	12	8.1
Testosterone 1	289	97	90	30	10	7.8
Testosterone 2	289	109	90	35	14	7.8

RESULTS

The method is sensitive for quantification of endogenous steroids in oral fluids. With and without DMS, lower limits of quantification (LLOQ) were established at 1 pg/mL for Testosterone, 5 pg/mL for 17-Hydroxyprogesterone, Cortisol, and Progesterone, and at 0.5 pg/mL for derivatives of Estradiol, Estrone, and Estriol.

The use of SelexION™ ion mobility technology, in combination with MRM analysis, increased data quality by removing endogenous background, and improving peak shapes, which is especially important for low concentrations. When the SelexION device was employed, linearity was also improved: 0.9993 (17-Hydroxyprogesterone), 0.9995 (Cortisol), 0.9994 (Estradiol), 0.9998 (Estriol), 0.9993 (Estrone), 0.9962 (Progesterone), and 0.9975 (Testosterone).

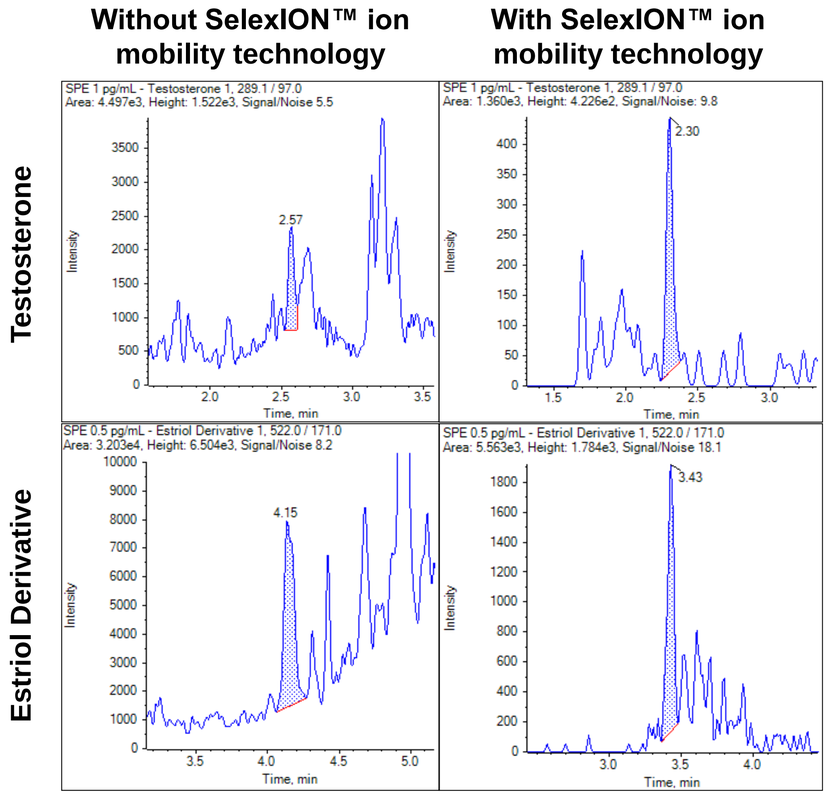
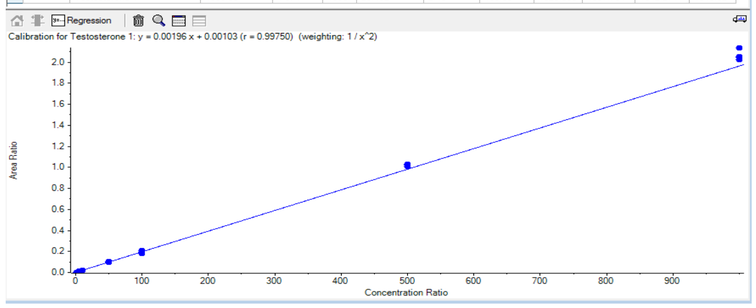
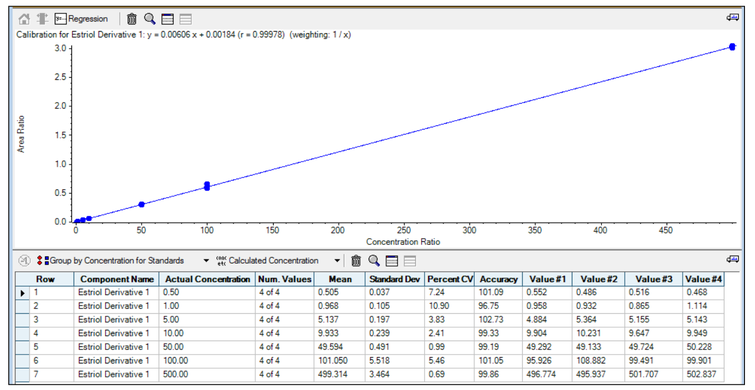


Figure 3. Representative chromatogram for testosterone at 1pg/mL (top) and estriol derivative at 5pg/mL (bottom) without DMS (left) and with DMS (right).

Row	Component Name	Actual Concen	Num. Val	Mean	Standard Dev	Percent CV	Accuracy	Value #1	Value #2	Value #3	Value #4
1	Testosterone 1	1.00	4 of 4	1.011	0.091	8.98	101.11	1.105	1.073	0.934	0.933
2	Testosterone 1	5.00	4 of 4	4.798	0.353	7.35	95.96	4.940	4.413	4.624	5.216
3	Testosterone 1	10.00	4 of 4	9.712	0.670	6.90	97.12	9.780	9.459	10.599	9.012
4	Testosterone 1	50.00	4 of 4	50.16	2.077	4.14	100.32	50.657	52.896	48.598	48.495
5	Testosterone 1	100.00	4 of 4	96.65	7.096	7.34	96.66	100.180	90.565	104.953	90.937
6	Testosterone 1	500.00	4 of 4	518.7	5.055	0.97	103.75	514.104	515.196	524.990	520.624
7	Testosterone 1	1000.00	4 of 4	1050.	25.492	2.43	105.08	1029.358	1087.754	1042.271	1043.62





Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Dev	Percent CV	Accuracy	Value #1	Value #2	Value #3	Value #4
1	Estriol Derivative 1	0.50	4 of 4	0.505	0.037	7.24	101.09	0.552	0.486	0.516	0.468
2	Estriol Derivative 1	1.00	4 of 4	0.968	0.105	10.90	96.75	0.958	0.932	0.865	1.114
3	Estriol Derivative 1	5.00	4 of 4	5.137	0.197	3.83	102.73	4.884	5.364	5.155	5.143
4	Estriol Derivative 1	10.00	4 of 4	9.933	0.239	2.41	99.33	9.904	10.231	9.647	9.949
5	Estriol Derivative 1	50.00	4 of 4	49.594	0.491	0.99	99.19	49.292	49.133	49.724	50.228
6	Estriol Derivative 1	100.00	4 of 4	101.050	5.518	5.46	101.05	95.926	108.882	99.491	99.901
7	Estriol Derivative 1	500.00	4 of 4	499.314	3.464	0.69	99.86	496.774	495.937	501.707	502.837

Figure 4. Standard curve and statistics for testosterone (top) and estriol (botttom) in matrix, analyzed on the QTRAP® 5500 system equipped with SelexION™ ion mobility technology (n=4).

Analyte	Concentration (pg/mL)	S/N	CV (%)	Accuracy (%)	Linearity
17OH-Progesterone	5	8.0	2.0	100	0.996
Cortisol	5	4.9	8.9	100	0.985
Estradiol Derivative	0.5	5.9	10	98	0.996
Estriol Derivative	1	8.2	9.0	99	0.997
Estrone Derivative	1	22	6.0	102	0.997
Progesterone	5	9.9	15	106	0.992
Testosterone	1	5.5	5.0	99	0.997

Table 3. Statistical data for steroids at lowest calibration curve concentration without DMS (n=4)

Analyte	Concentration (pg/mL)	S/N	Precision (%)	Accuracy (%)	Linearity
17OH-Progesterone	5	15	4.2	94	0.999
Cortisol	5	15	7.0	102	0.999
Estradiol Derivative	0.5	9.0	6.0	93	0.999
Estriol Derivative	1	18	7.0	101	0.999
Estrone Derivative	1	17	4.6	110	0.999
Progesterone	5	13	9.1	103	0.996
Testosterone	1	10	8.0	101	0.997

Table 4. Statistical data for steroids at lowest calibration curve concentration with DMS (n=4)

CONCLUSIONS

The method proved to be accurate, precise and easy to perform for the routine measurement of endogenous steroids in oral fluids. Use of the SelexION™ ion mobility technology provided a significant improvement to data quality, by removing endogenous interferences.

TRADEMARKS/LICENSING