APPLICATION NOTE

1599-9837

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Steroid Panel Analysis with the PerkinElmer QSight® 220 Triple Quadrupole Mass Spectrometer

Introduction

Achieving adequate sensitivity across the whole panel is quite difficult because optimum ionization conditions vary depending on the steroid. For example, the optimum source conditions for delta-5 steroids (e.g. pregnenolone) are much different than for cortisol.

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2. Method

The purpose of this study was to assess the performance of the HPLC-MS/MS system in the presence of a matrix but independent of extraction effects. Therefore, samples were prepared in an extracted matrix instead of in serum or plasma.

A blank matrix was created by mixing one volume of steroid free human serum (MP Biomedical) with two volumes of cold acetonitrile. The mixture was vortexed and centrifuged. The supernatant was removed and used as the matrix.

Standard solutions were prepared in this matrix over a variety of concentration ranges (shown in Table 3). Each sample was analyzed 5 times. Accuracy is the average of the difference between the prepared and measured concentrations over the entire calibration range. CV is determined over 5 injections at each concentration.

2.1. Mass Spectrometry Conditions

This method utilized the QSight® 220 mass spectrometer in positive ESI mode.

Table 1: Mass Spectrometer Conditions

ESI Voltage (V)	5500
HSID Temp (°C)	350
Nebulizer Gas Setting	350
Drying Gas Setting	120
Heating Gas	350
Source Temp. (°C)	350
Dwell Time (ms)	100
Pause Time (ms)	5

Quick Facts:

- Method for a single run quantitative steroid panel using the QSight® 220 triple quadrupole mass spectrometer.
- MRM Analysis of: Testosterone, Progesterone, 17-OH-Progesterone, Deoxycorticosterone, Corticosterone, 11-deoxycortisol, DHEA, 17-OH-pregnenolone, and allopregnanolone
- LLOQs in low pg/mL for all compounds
- Accuracy ranging from 91-106%

Table 2: MRM Transitions

Testosterone	289.2/97.1	289.2/109.1
Progesterone	315.1/97.1	315.1/109.1
17-OH-progesterone	331.0/97.1	331.0/109.1
Deoxycorticosterone	331.0/97.1	331.0/109.1
Corticosterone	347.2/121.1	-
11-deoxycortisol	347.2/97.1	347.2/109.1
DHEA	253.0/197.0	-
17OH-pregnenolone	315.2/279.2	-
Allopregnanolone	301.1/135.1	-

2.2 LC Conditions

Sample injections of 10 µL were loaded onto a Phenomenex Kinetex® C18 (2.1x50 mm, 2.7 µm) column at a flow rate of 0.5 mL/min. Solvent A: MeOH/H2O (5/95) with 5mM ammonium acetate and 0.1% formic acid. Solvent B: MeOH/H2O (95/5) with 5mM ammonium acetate and 0.1% formic acid.

3. Results

Table 3: Results Obtained During the Steroid Panel

Compound	LLOQ (ng/mL)	Accuracy (%)	CV (%)	Calibration Range (ng/mL)
Testosterone	0.0024	101	13	0.0024-10
Progesterone	0.0024	96	7	0.0024-5
17OH-Progesterone	0.0012	96	17	0.0012-10
Deoxycorticosterone	0.0024	97	5	0.0024-20
Corticosterone	0.025	104	15	0.025-25
11-deoxycortisol	0.0048	106	12	0.0048-10
DHEA	0.098	98	9	0.050-25
17OH-Pregnenolone	0.025	91	18	0.025-50
Allopregnanalone	0.025	100	17	0.025-100

3.1 Extracted Ion Chromatograms (EICs)

Below, Figures 1-4 illustrate the EICs of Testosterone, Progesterone, DHEA, and 11- Deoxycortisol respectively. All of the compounds are shown at a concentration of 0.01 ng/mL in matrix. These chromatograms are representative of the panel as a whole.

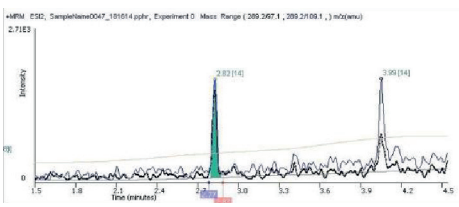


Figure 1: EIC of Testosterone in Matrix.

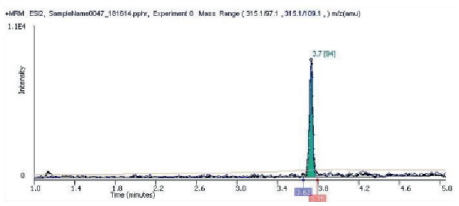


Figure 2: EIC of Progesterone in Matrix.

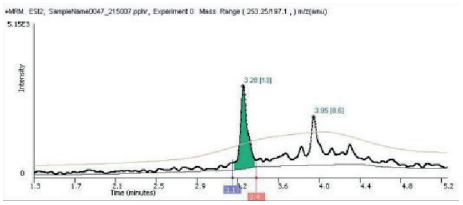


Figure 3: EIC of DHEA in Matrix.

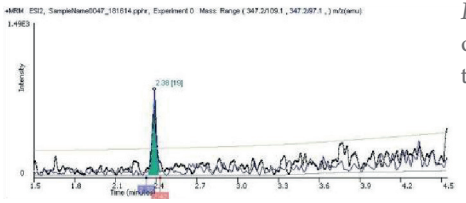


Figure 4: EIC of 11-Deoxycortisol in Matrix.

3.2 Linearity

All analytes produced a linear response over the measured concentration range. The accuracy and precision was acceptable for bioanalysis. Incorporating heavy atom labeled internal standards into the analysis will further improve the accuracy and precision of the assay. Figures 5 and 6 illustrate the linearity of Testosterone and DHEA and are representative of results achieved for all of the analytes.

Figure 5: Calibration Curve for Testosterone (289.2/97.1)

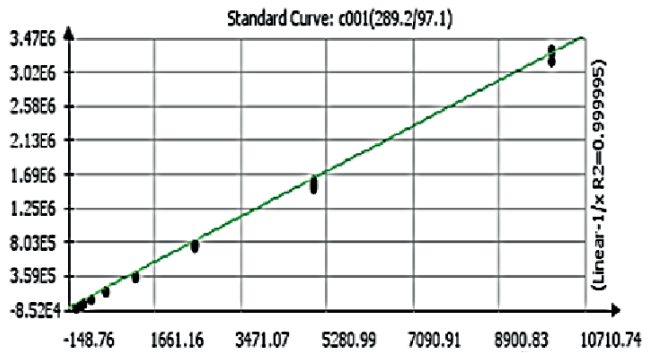
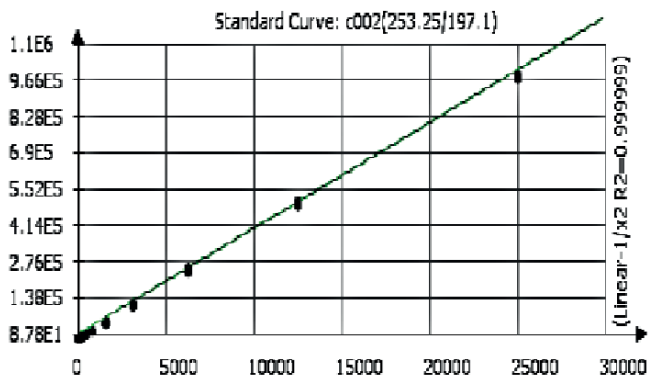


Figure 6: Calibration Curve for DHEA (253.1/197.1)



4. Conclusion

The PerkinElmer QSight® 220 triple quadrupole mass spectrometer has the sensitivity to measure low pg/mL levels of steroids from biological samples. This sensitivity can be achieved for androgens, glucocorticoids, and progestagens together in one analytical run; thereby enabling the analysis of a panel of steroids by one method.

5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

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