Product No: JSM-CL-6500

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# Steroid Hormones in Serum LC-MS/MS Analysis Kit

Steroid hormones are defined as effective biological messengers which take part in crucial body processes even at low concentrations. Thus, reliable, and simultaneous analysis of a broad panel of steroid hormones plays vital role for the investigation of the hormone profile, which could be useful in detection endocrine disorders resulting from defects in steroid biosynthesis such as congenital adrenal hyperplasia (CAH). Due to the restrictions and shortcomings of immunoassay techniques for measurement of steroid hormones in body fluids, liquid chromatography tandem mass spectrometry (LC-MS/MS) is increasingly becoming the method for multi-class steroid hormone detection.

## Highlights of the Analysis Kit

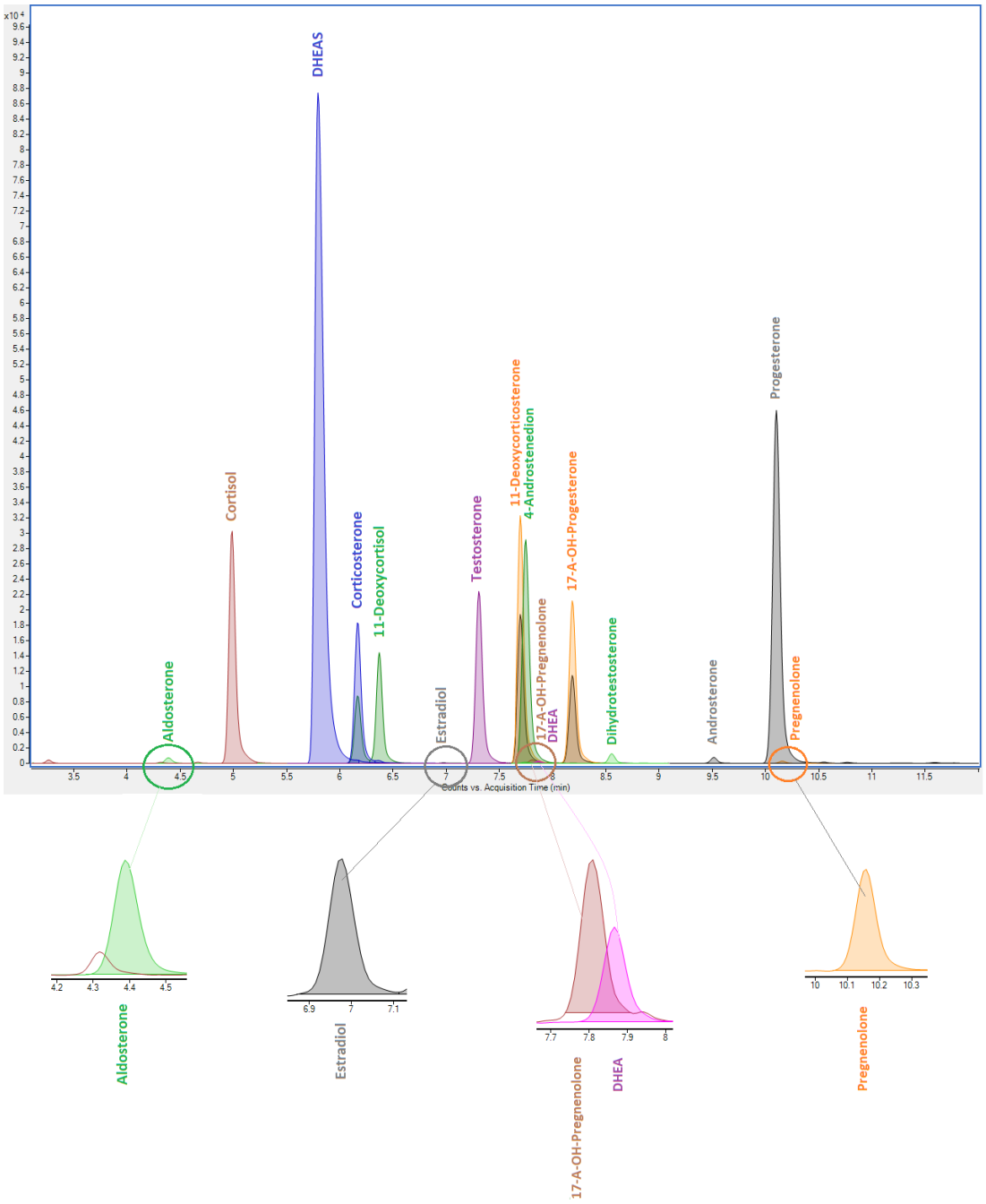
- Single-injection analysis of 16 clinically relevant steroid hormones
- Total run time is 16.0 min.
- Simple liquid–liquid extraction and protein precipitation, without SPE
- Key steroid hormones are covered by corresponding isotope-labeled internal standards
- Long life span of HPLC column

| Parameters | | | |
|---|---|---|---|
| 11-Deoxycorticosterone<br>11-Deoxycortisol<br>17-α-Hydroxypregnenolone<br>17-α-Hydroxyprogesterone | Aldosterone<br>Androstene-3,17-dione<br>Androsterone<br>Corticosterone | Cortisol<br>DHEA<br>DHEAS<br>Dihydrotestosterone | Estradiol<br>Pregnenolone<br>Progesterone<br>Testosterone |
| **Sample Type** | | | |
| Serum/Plasma | | | |

## Sample Preparation

| | |
|---|---|
| 1 | Pipette 425 µl of serum/plasma sample into a glass centrifuge tube and add 25 µl of internal standard mixture, vortex for 5 sec. |
| 2 | Add 450 µl of Reagent-1 into tube-1 and vortex for 15 sec. Then, centrifuge at 4000 rpm for 5 min. |
| 3 | Decant the supernatant into a new glass centrifuge tube (tube-2) |
| 4 | Wash the precipitate in tube-1 with 1.5 mL Reagent-2 (two times!) and transfer the washing fluids into tube-2 |
| 5 | Agitate tube-2 for 3 min. and centrifuge at 4000 rpm for 5 min. |
| 6 | Transfer 2.4 ml of the upper phase to a new glass tube and evaporate under nitrogen stream |
| 7 | Reconstitute tube-3 with 100 µl of Reagent-3 and transfer to a HPLC vial prior to injection |

## Example Chromatogram




Total and extracted ion chromatogram of steroid hormones

## Method Performance

All validation results were obtained using Agilent 6465 TQ (Ultivo) system

| Analytes | LOQ (µg/L) | Linearity (µg/L) | Recovery (%) | Repeatability (%CV) |
|---|---|---|---|---|
| 11-Deoxycorticosterone | 0.12 | 0.12–12.5 | 97 | 4.56 |
| 11-Deoxycortisol | 0.05 | 0.05–10.625 | 97 | 3.84 |
| 17-α-Hydroxypregnenolone | 1.25 | 1.25–50 | 89 | 2.44 |
| 17-α-Hydroxyprogesterone | 0.08 | 0.08–12.5 | 106 | 3.81 |
| 4-Androstene 3,17-dione | 0.19 | 0.19–25 | 112 | 3.98 |
| Aldosterone | 0.10 | 0.10–3.125 | 95 | 1.71 |
| Androsterone | 0.24 | 0.24–12.5 | 103 | 4.53 |
| Corticosterone | 0.42 | 0.42–62.5 | 113 | 3.90 |
| Cortisol | 0.69 | 0.69–312.5 | 117 | 0.97 |
| DHEA | 0.33 | 0.33–37.5 | 91 | 3.47 |
| DHEAS | 38.01 | 38.01–6250 | 98 | 2.46 |
| Dihydrotestosterone | 0.26 | 0.26–7.5 | 88 | 2.21 |
| Estradiol | 0.23 | 0.23–5.0 | 105 | 3.25 |
| Pregnenolone | 0.37 | 0.37–12.5 | 94 | 3.08 |
| Progesterone | 0.08 | 0.08–20.0 | 111 | 2.27 |
| Testosterone | 0.027 | 0.027–6.25 | 107 | 1.04 |

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