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# Quantification of steroid hormones in serum or plasma by liquid chromatography-tandem mass spectrometry for research use

**Authors**
Zuzana Škrabáková,
Thermo Fisher Scientific,
Hemel Hempstead, UK

Claudio De Nardi,
Thermo Fisher Scientific,
Dreieich, Germany

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## Application benefits

- Simple and economical sample preparation method involving liquid-liquid extraction (LLE)
- Sensitive LC-MS/MS workflow with wide quantitative range

## Goal

Development of an analytical workflow for the quantification of a panel of steroid hormones in human plasma or serum using a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer aided by a simple sample preparation approach

## Introduction

Quantitative analysis of steroids in clinical research laboratories is usually performed by liquid chromatography coupled to mass spectrometry. An analytical method for clinical research for quantitative analysis of a panel of steroids in human plasma or serum utilizing liquid-liquid extraction (LLE) and the TSQ Quantiva mass spectrometer is reported. The analytical performance of the method was assessed using calibrators, controls, and internal standards from Biocrates®. Samples were extracted by LLE and analyzed using a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to a TSQ Quantiva triple quadrupole mass spectrometer with heated electrospray

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ionization. The Vanquish Flex UHPLC system was fitted with normal phase piston seals, as these are more resistant to the mobile phases used in this application. Selective reaction monitoring (SRM) was used for detection and quantitation. Linear quantitative range, limit of quantitation, accuracy, intra- and inter-assay precision, and matrix effects were evaluated for each analyte.

## Experimental

### Target analytes

- Aldosterone
- Androstenedione
- Corticosterone
- Cortisol
- Cortisone
- 11-Deoxycorticosterone
- 11-Deoxycortisol
- Estradiol-17β (E2)
- Estrone (E1)
- 17α-Hydroxyprogesterone
- Progesterone
- Testosterone
- DHEAS

### Sample preparation

Lyophilized calibrators and quality controls were reconstituted using 1300 µL of calibrator matrix and 650 µL of water, respectively. Lyophilized internal standard mix was reconstituted using 3000 µL of methanol. A sample volume of 200 µL was combined with 10 µL of reconstituted internal standard mix and thoroughly mixed with 1 mL of methyl tert-butyl ether for liquid-liquid extraction. Extraction vials were kept at -80 °C for 15 minutes to freeze the aqueous layer and allow for quantitative recovery of the organic layer. The latter was evaporated to dryness and reconstituted with 50 µL of mobile phase. 25 µL of sample were injected onto the LC-MS/MS system.

### Liquid chromatography

A 9-minute chromatographic elution through a Thermo Scientific™ Hypersil GOLD™ column (50 × 2.1 mm, 1.9 µm) at 40 °C was performed using a Vanquish Flex UHPLC system. Mobile phases consisted of 0.02% ammonium hydroxide in water (Optima™, Fisher Chemical™) and methanol (Optima, Fisher Chemical) for phases A and B, respectively.

Injection volume: 25 µL
Flow rate: 400 µL/min
Gradient:

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 70 | 30 |
| 0.50 | 70 | 30 |
| 1.00 | 55 | 45 |
| 6.00 | 20 | 80 |
| 6.50 | 0 | 100 |
| 7.00 | 0 | 100 |
| 7.01 | 70 | 30 |

### Mass spectrometry

Compounds were detected by acquisition of SRM data on a TSQ Quantiva triple quadrupole mass spectrometer with heated electrospray ionization in positive and negative mode. Two SRM transitions were acquired for each compound for quantitation and confirmation, respectively. SRM transitions and electrospray polarity for each analyte are reported in Table 1.

### Method evaluation

The analytical method performance was evaluated by obtaining limit of quantitation, linearity range, accuracy, intra- and inter-assay precision, and matrix effects for each analyte. Intra-assay precision was evaluated in terms of percentage RSD (%RSD) using controls at three levels in replicates of five (n=5) prepared and analyzed in one day. Inter-assay precision was evaluated on the same control replicates as for intra-assay precision but prepared on three different days (n=15). Analytical accuracy was evaluated using control samples at three levels by comparing experimental concentrations with acceptance concentration range for each analyte. Matrix effect was evaluated by comparing a peak area in spiked pooled donor plasma samples with peak area in neat solution at the same concentration for each analyte.

### Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

**Table 1. SRM transitions and parameters.**

| Compound | Polarity | PrecursorIon (*m/z*) | Quantifier Ion (*m/z*) | Qualifier Ion (*m/z*) |
|---|---|---|---|---|
| 11-Deoxycorticosterone | + | 331.2 | 97.0 | 109.0 |
| d8-11-Deoxycorticosterone | + | 339.3 | 100.1 | |
| 11-Deoxycortisol | + | 347.2 | 109.0 | 97.0 |
| d5-11-Deoxycortisol | + | 352.3 | 100.0 | |
| 17α-Hydroxyprogestrone | + | 331.2 | 109.0 | 97.0 |
| d8-17α-Hydroxyprogestrone | + | 339.4 | 100.1 | |
| Aldosterone | - | 359.2 | 189.0 | 297.2 |
| d7-Aldosterone | - | 366.1 | 193.0 | |
| Androstenedione | + | 287.2 | 97.0 | 109.1 |
| d3-Androstenedione | + | 290.3 | 100.1 | |
| Corticosterone | + | 347.2 | 121.0 | 147.1 |
| d8-Corticosterone | + | 355.2 | 125.1 | |
| Cortisol | + | 363.3 | 121.1 | 309.2 |
| d4-Cortisol | + | 367.3 | 121.1 | |
| Cortisone | + | 361.2 | 163.1 | 121.1 |
| d7-Cortisone | + | 368.3 | 100.1 | |
| Estradiol | - | 271.2 | 183.0 | 145.0 |
| d3-Estradiol | - | 274.4 | 147.1 | |
| Estrone | - | 269.2 | 145.0 | 183.1 |
| d4-Estrone | - | 273.1 | 147.1 | |
| Progesterone | + | 315.3 | 97.0 | 109.0 |
| d9-Progesterone | + | 324.3 | 100.1 | |
| Testosterone | + | 289.3 | 97.0 | 109.0 |
| d5-Testosterone | + | 294.3 | 100.1 | |
| DHEAS | - | 367.2 | 96.9 | 79.9 |
| d5-DHEAS | - | 372.2 | 98 | |

## Results and discussion

The reported analytical method was found to be linear in the concentration range covered by the calibrators utilized, with correlation coefficient ($R^2$) above 0.995 for all the analytes. Limits of quantitation (LOQs) are reported in Table 2, and representative chromatograms for the lowest calibrator are shown in Figure 1.

Method accuracy and intra- and inter-assay precision are reported in Table 3 and Table 4, respectively. The analytical method proved to be very accurate, with all results within the acceptance range. Intra-assay precision was always below 14.0% while inter-assay precision was always below 15%, with the exception of cortisone, which had slightly higher values of %RSD.

Matrix effect is expressed as analyte peak area percentage recovery in plasma compared to neat solution. The effects were determined at three different concentrations, and results are reported in Table 5. Observed matrix effects are compensated by the use of deuterated internal standards.

## Conclusions

An analytical method for the quantification of a panel of steroids in human serum or plasma by liquid chromatography-tandem mass spectrometry using a Thermo Scientific TSQ Quantiva MS is reported. The analytical method employs a simple and cost-effective sample preparation and meets research laboratory requirements of sensitivity, linearity of response, accuracy, precision, and matrix effect.

Table 2. Lower (LOQ) and upper (ULOQ) limits of quantification.

| Compound | LOQ (ng/mL) | ULOQ (ng/mL) |
|---|---|---|
| Aldosterone | 0.05 | 5.0 |
| Androstenedione | 0.03 | 7.0 |
| Corticosterone | 0.03 | 28 |
| Cortisol | 1.00 | 923 |
| Cortisone | 0.10 | 92 |
| 11-Deoxycorticosterone | 0.03 | 14 |
| 11-Deoxycortisol | 0.01 | 9.0 |
| Estradiol 17 beta (E2) | 0.02 | 18 |
| Estrone (E1) | 0.03 | 14 |
| 17α-Hydroxyprogesterone | 0.05 | 46 |
| Progesterone | 0.06 | 14 |
| Testosterone | 0.01 | 9.0 |
| DHEAS | 30 | 7385 |

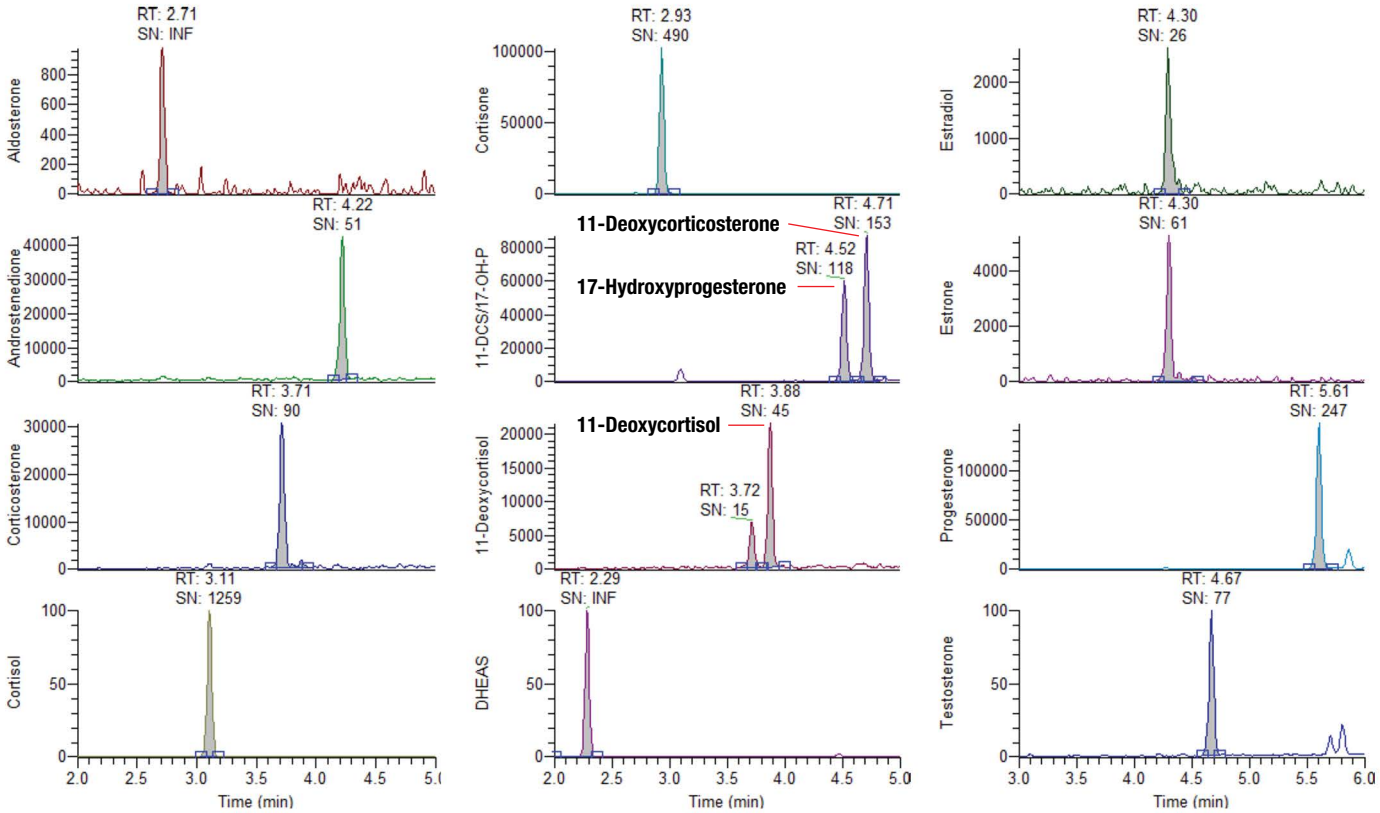


Figure 1. Representative chromatograms using the lowest calibrator.

Table 3. Analytical accuracy (n=15).

| Analyte | QC1 | | QC2 | | QC3 | |
|---|---|---|---|---|---|---|
| | Acceptance Range (ng/mL) | Measured Concentration (ng/mL) | Acceptance Range (ng/mL) | Measured Concentration (ng/mL) | Acceptance Range (ng/mL) | Measured Concentration (ng/mL) |
| Aldosterone | 0.066–0.175 | 0.118 | 0.273–0.507 | 0.414 | 1.86–3.45 | 2.73 |
| Androstenedione | 0.392–1.034 | 0.745 | 0.725–1.35 | 1.10 | 3.34–6.21 | 5.38 |
| Corticosterone | 0.82–2.18 | 1.56 | 2.24–4.17 | 3.47 | 12.6–23.4 | 19.2 |
| Cortisol | 45.1–119 | 77.6 | 101–187 | 145 | 420–780 | 616 |
| Cortisone | 7.87–20.8 | 13.8 | 16.1–29.7 | 25.9 | 48.1–89.3 | 70.8 |
| 11-Deoxycorticosterone | 0.073–0.191 | 0.135 | 0.849–1.57 | 1.36 | 5.90–11.0 | 9.81 |
| 11-Deoxycortisol | 0.108–0.284 | 0.210 | 0.581–1.08 | 0.910 | 3.95–7.34 | 5.89 |
| Estradiol 17 beta (E2) | 0.097–0.255 | 0.191 | 1.08–2.00 | 1.73 | 7.32–13.6 | 13.3 |
| Estrone (E1) | 0.083–0.220 | 0.143 | 0.793–1.48 | 1.10 | 5.49–10.2 | 8.14 |
| 17α Hydroxyprogesterone | 0.358–0.942 | 0.637 | 2.83–5.27 | 4.11 | 19.4–36.1 | 27.4 |
| Progesterone | 0.582–1.54 | 0.844 | 1.56–2.90 | 1.75 | 6.69–12.5 | 7.68 |
| Testosterone | 0.152–0.402 | 0.239 | 0.671–1.25 | 0.986 | 3.88–7.20 | 5.42 |
| DHEAS | 639–1684 | 1089 | 1276–2369 | 1877 | 3710–6889 | 5592 |

Table 4. Analytical intra- and inter-assay precision.

| Analyte | QC1 | | | QC2 | | | QC3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nominal Conc. (ng/mL) | Intra-day % RSD (%, n=5) | Inter-day % RSD (%, n=15) | Nominal Conc. (ng/mL) | Intra-day % RSD (%, n=5) | Inter-day % RSD (%, n=15) | Nominal Conc. (ng/mL) | Intra-day % RSD (%, n=5) | Inter-day % RSD (%, n=15) |
| Aldosterone | 0.121 | 14 | 15 | 0.390 | 7.6 | 9.6 | 2.66 | 6.6 | 8.1 |
| Androstenedione | 0.714 | 7.1 | 8.8 | 1.03 | 6.5 | 6.8 | 4.78 | 3.9 | 4.9 |
| Corticosterone | 1.51 | 6.5 | 8.9 | 3.21 | 4.1 | 6.7 | 18.1 | 5.4 | 5.8 |
| Cortisol | 82.1 | 7.8 | 7.9 | 144 | 4.2 | 4.6 | 600 | 5.0 | 5.2 |
| Cortisone | 14.3 | 3.0 | 17 | 22.9 | 6.1 | 23 | 68.8 | 4.2 | 9.4 |
| 11-Deoxycorticosterone | 0.132 | 8.5 | 12 | 1.21 | 4.8 | 9.5 | 8.4 | 3.6 | 6.1 |
| 11-Deoxycortisol | 0.197 | 6.6 | 18 | 0.829 | 3.9 | 6.0 | 5.65 | 4.0 | 4.6 |
| Estradiol 17 β (E2) | 0.175 | 9.7 | 9.5 | 1.54 | 11 | 15 | 10.4 | 7.8 | 9.3 |
| Estrone (E1) | 0.151 | 9.7 | 9.7 | 1.13 | 3.5 | 3.4 | 7.84 | 5.1 | 4.8 |
| 17α-Hydroxyprogesterone | 0.655 | 9.0 | 14 | 4.05 | 4.1 | 4.1 | 27.8 | 4.7 | 4.7 |
| Progesterone | 1.06 | 6.6 | 9.0 | 2.22 | 4.6 | 9.6 | 9.60 | 3.4 | 6.3 |
| Testosterone | 0.277 | 8.0 | 9.7 | 0.959 | 4.1 | 7.2 | 5.54 | 3.9 | 4.5 |
| DHEAS | 1161 | 11 | 8.4 | 1822 | 5.1 | 5.4 | 5299 | 6.5 | 6.7 |

**Table 5. Matrix effect expressed as percentage recovery from plasma at three different concentrations.**

| Analyte | Recovery 50 pg/mL (%) | Recovery 100 pg/mL (%) | Recovery 10 ng/mL (%) |
|---|---|---|---|
| Aldosterone | – | 93 | 120 |
| Androstenedione | 107 | 80 | 85 |
| Corticosterone | 102 | 55 | 64 |
| Cortisol | – | 59 | 76 |
| Cortisone | 13 | 63 | 87 |
| 11-Deoxycorticosterone | 104 | 96 | 92 |
| 11-Deoxycortisol | 68 | 54 | 65 |
| Estradiol 17 β (E2) | – | 71 | 74 |
| Estrone (E1) | 80 | 58 | 58 |
| 17α-Hydroxyprogesterone | 98 | 97 | 98 |
| Progesterone | 31 | 29 | 38 |
| Testosterone | 16 | 72 | 69 |
| DHEAS | – | – | 85 |

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