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# Quantitative analysis of estradiol and testosterone in plasma for clinical research using the TSQ Altis triple quadrupole mass spectrometer

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## Goal

To develop a sensitive LC-MS/MS method for quantitative analysis of estradiol and testosterone in plasma for clinical research using liquid chromatographic separation coupled to a triple quadrupole mass spectrometer.

## Introduction

Analysis of estradiol and testosterone in plasma samples for clinical research requires a sensitive analytical method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been widely adopted as an analytically sensitive and selective technique for estradiol and testosterone analysis in complex matrices such as human serum or plasma.

## Experimental

### Sample preparation

To prepare the samples, 10 µL of spiking solution (final concentration range: 0.5–10 ng/mL) and 20 µL of internal standard (2 ng/mL testosterone-$^{13}$C3 and 20 ng/mL estradiol-d5) were added to 400 µL of plasma. Then 2 mL of MTBE were added and the sample was vortexed. After liquid-liquid extraction (LLE), the MTBE layer was evaporated under nitrogen, and 200 µL of 50:50 methanol/water were added to reconstitute. From this, 10 µL were injected in triplicate for LC-MS/MS analysis.

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## Liquid chromatography

Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Flex Binary HPLC system equipped with a Thermo Scientific™ Accucore™ aQ C18 Polar Endcapped LC column (100 × 2.1 mm, 2.6 µm particle size, P/N 17326-102130). Mobile phases A and B were 0.5 mM ammonium fluoride in Fisher Chemical™ Optima™ grade water and pure methanol, respectively. The column temperature was 40 °C. The total run time was 9 minutes (Table 1).

**Table 1. LC gradient.**

| No | Time (min) | Flow (mL/min) | %B | Curve |
|---|---|---|---|---|
| 1 | 0 | 0.25 | 30 | 5 |
| 2 | 1 | 0.25 | 30 | 5 |
| 3 | 1.5 | 0.25 | 55 | 5 |
| 4 | 5 | 0.25 | 85 | 5 |
| 5 | 6 | 0.25 | 100 | 5 |
| 6 | 7 | 0.25 | 100 | 5 |
| 7 | 7.01 | 0.25 | 30 | 5 |
| 8 | 9 | 0.25 | 30 | 5 |

## Mass spectrometry

MS analysis was carried out on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer equipped with heated electrospray ionization. Table 2 shows the mass spectrometer source parameters.

Two selected reaction monitoring (SRM) transitions were monitored for estradiol and testosterone and their isotope-labeled internal standards to provide ion ratio confirmations (IRC). The scans were run in timed selected reaction monitoring (t-SRM) mode with a cycle time of 0.4 seconds. Table 3 shows SRM properties used in this analysis.

**Table 2. Source parameters for the TSQ Altis mass spectrometer.**

| Ion Source Parameter | Value |
|---|---|
| Positive Ion | 3500 V |
| Sheath Gas | 40 Arb |
| Aux Gas | 12 Arb |
| Sweep Gas | 1 Arb |
| Ion Transfer Tube Temp | 350 °C |
| Vaporizer Temp | 350 °C |

## Data analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software.

## Results and discussion

Lower limits of quantitation (LLOQ) for estradiol and testosterone were defined as the lowest concentration at which the back-calculated calibrator concentration on the linear calibration curve was within 20% of theoretical, the ion ratio was within 20% of the target, and replicate injections had a %RSD of less than 20%. The LLOQ of testosterone in plasma was 2 pg/mL (linearity range: 2–1000 pg/mL - Figure 1). For estradiol in plasma, the LLOQ was 20 pg/mL (linearity range: 20–10,000 pg/mL - Figure 2). The precisions were less than 8% and 7% for testosterone and estradiol, respectively, for all replicates at all concentrations.

**Table 3. SRM properties for analysis of estradiol and testosterone.**

| Compound | Retention Time (min) | RT Window (min) | Polarity | Precursor (*m/z*) | Product (*m/z*) | Collision Energy (V) | RF Lens (V) | Quantifying or Confirming Ion |
|---|---|---|---|---|---|---|---|---|
| Estradiol | 4.8 | 1 | Negative | 271.1 | 183.1 | 40 | 100 | Quantifying |
| Estradiol | 4.8 | 1 | Negative | 271.1 | 145.1 | 40 | 100 | Confirming |
| Estradiol-d5 | 4.8 | 1 | Negative | 276.1 | 187.1 | 41 | 100 | Quantifying |
| Estradiol-d5 | 4.8 | 1 | Negative | 276.1 | 147.1 | 38 | 100 | Confirming |
| Testosterone | 4.9 | 1 | Positive | 289.1 | 109.1 | 25 | 51 | Quantifying |
| Testosterone | 4.9 | 1 | Positive | 289.1 | 97.1 | 22 | 51 | Confirming |
| Testosterone-$^{13}$C3 | 4.9 | 1 | Positive | 292.3 | 112.1 | 25 | 51 | Quantifying |
| Testosterone-$^{13}$C3 | 4.9 | 1 | Positive | 292.3 | 100.1 | 21 | 51 | Confirming |

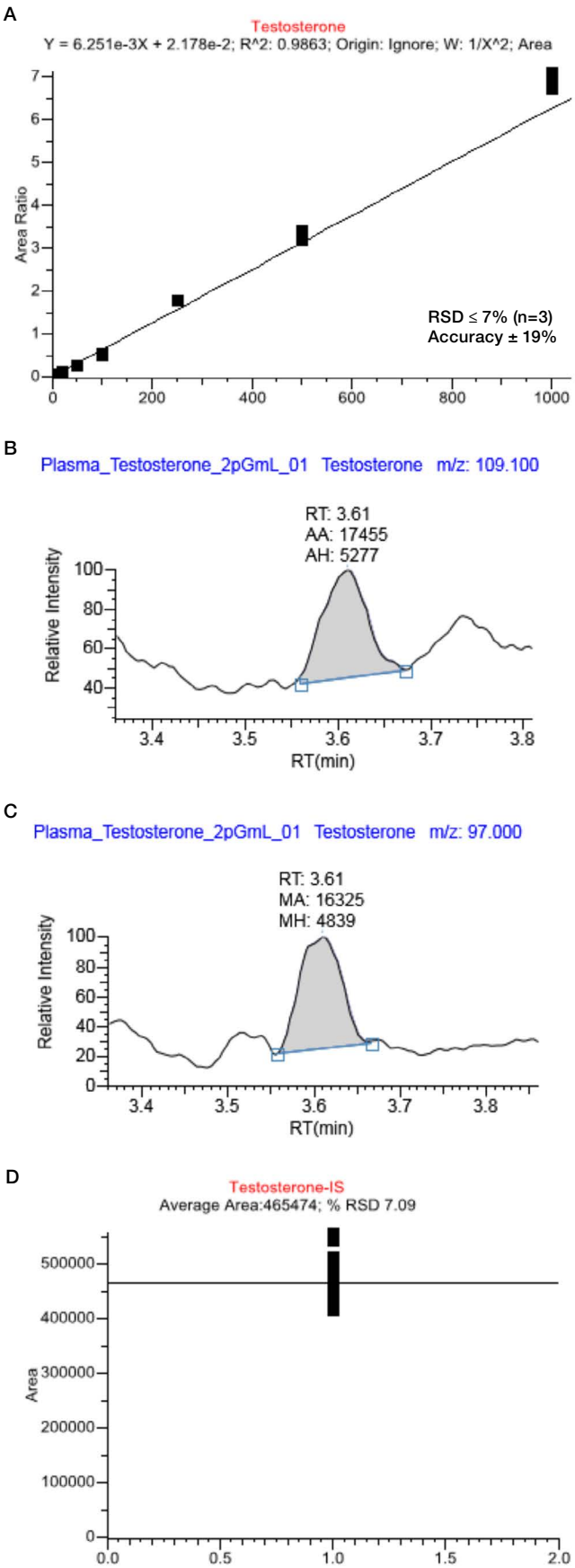


Figure 1. Testosterone data: (A) calibration curve, (B) LLOQ chromatogram for quantifying ion, (C) LLOQ chromatogram for confirming ion, and (D) internal standard.

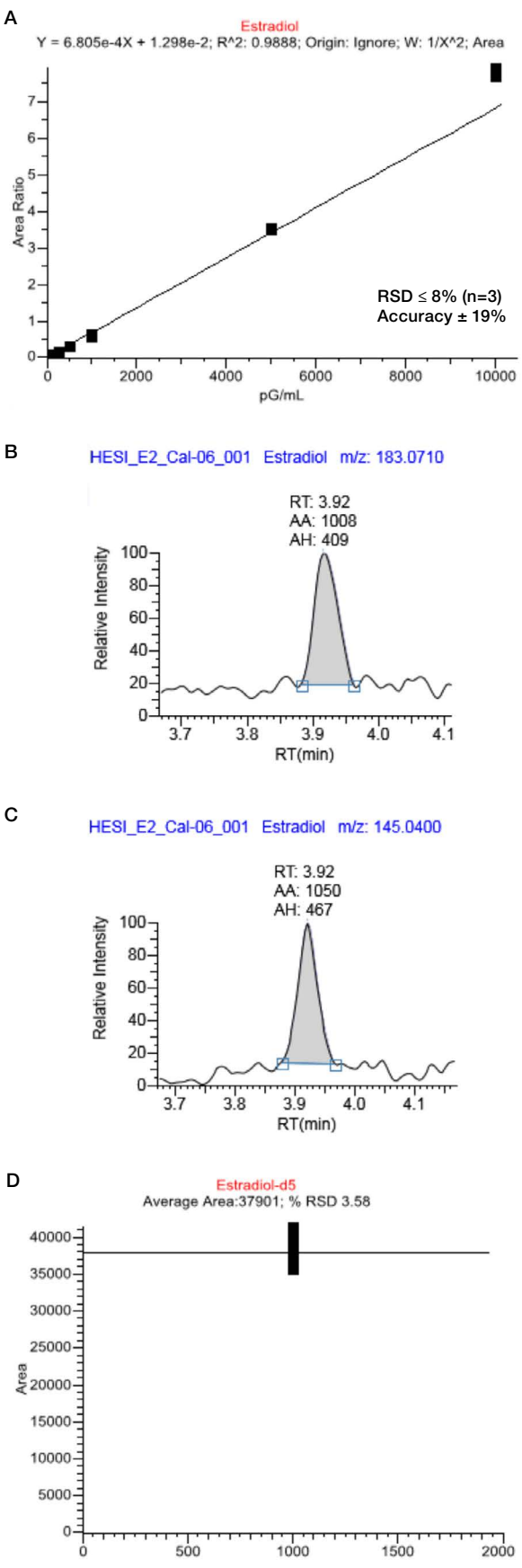


Figure 2. Estradiol data: (A) calibration curve, (B) LLOQ chromatogram for quantifying ion, (C) LLOQ chromatogram for confirming ion, and (D) internal standard.

## Conclusion

- The TSQ Altis triple quadrupole mass spectrometer provides the superior sensitivity required for the analysis of estradiol and testosterone in plasma for clinical research.
- Limits of quantitation in plasma of 2 pg/mL for testosterone and 20 pg/mL for estradiol were obtained with the methodology described in this technical note.

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