Analysis of estrogens in plasma with rapid chromatography and reduced sample volume

Using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

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Estrogens are a category of hormone responsible for, amongst other things, the development and regulation of the female reproductive system and secondary sex characteristics. There are 3 major endogenous estrogens that have estrogenic hormonal activity: estrone (E1), estradiol (E2) and estriol (E3). Estradiol is the most potent and prevalent. Estrogens are believed to be synthesized in all vertebrates and some insects.

Quantitatively, estrogens circulate at lower levels than androgens in both males and females, regardless of species. While estrogen levels are significantly lower in males compared to females, estrogens nevertheless have important physiological roles in males. This wide area of prevalence and biochemical importance leads the estrogen group of steroids to be a highly active space in terms of biochemical and metabolic research.

Steroids such as estrogens that are present in the bloodstream at such low concentrations are traditionally analyzed by radioimmunoassay (RIA). RIA approaches are known to suffer from issues such as cross-reactivity, however, leading to a lack of specificity and therefore inaccuracies at lower concentrations. LC-MS/MS analysis overcomes a number of these issues, but the measurement of a particular estradiol by LC-MS/MS poses some specific analytical challenges due to the low concentrations of this compound. While it is possible to achieve

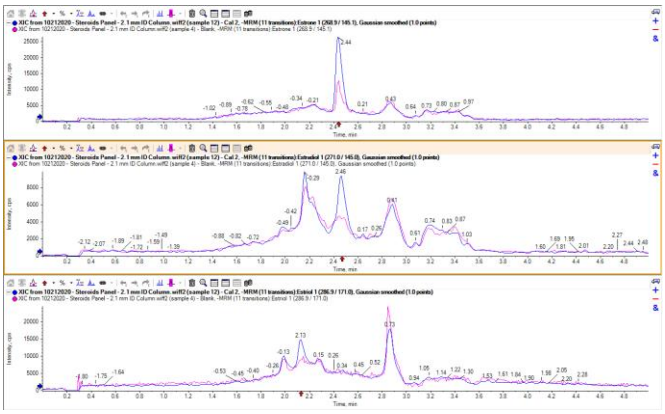

Figure 1. Chromatogram of 1 pg/mL plasma extract of estrone, estradiol and estriol from 200 µL of plasma. Here, 25 µL of extract was injected, equivalent to an amount on column for all 3 analytes of 0.05 pg. Signal-to-noise values (peak-to-peak) for all 3 compounds were 20:1, 12:1 and 6:1, respectively.

low limits of detection utilizing a standard quantitative LC-MS/MS workflow, it may potentially require the use of a large sample volume or employ a costly consumable-heavy sample preparation workflow to do so. To reduce sample volume to an acceptable level and still maintain a fast workflow with minimal consumable cost, the use of the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready to improve sensitivity was investigated. Figure 1 shows the analysis of a very low level (1 pg/mL, 0.05 pg on column) plasma extract of estradiol performed using the SCIEX 7500 System.

Key features of the SCIEX 7500 System for the analysis of estrogens

- Limits of quantification below 5 pg/mL (0.25 pg injected on column) were observed from a reduced (200 µL) volume of plasma
- Improved quantitative performance - including raw sensitivity, and reproducibility - was shown
- Sensitivity improvements allow for significantly simplified chromatography and faster run times as less interferences from larger matrix volumes or higher concentration steps in the extraction are needed

Methods

Sample preparation: Calibrators and quality controls were prepared by spiking analytes into blank matrix. Of these, 200 µL were spiked with internal standards at working concentrations, vortexed and then extracted by a liquid-liquid extraction (LLE) method using methyl-tertiary-butyl ether (MTBE). Following mixing and centrifugation, the organic layer was separated by snap-freezing and evaporated to dryness. It was then reconstituted in 100 µL mobile phase and 25 µL was injected on the LC-MS/MS system.

Chromatography: Chromatographic separation was achieved using an ExionLC™ AD System and a 50 mm Phenomenex Kinetex C8 column. A gradient of water and methanol (both containing ammonium fluoride) was used at a flow rate of 500 µL/min. The injection volume was set to 25 µL. The total run time for all compounds was 5 minutes.

Mass spectrometry: The SCIEX 7500 System was used to analyze the samples, operating in negative electrospray ionization mode. The OptiFlow® Pro Ion Source parameters were retuned as there are some key design differences compared to the earlier Turbo V™ Ion Source.

Data processing: All data were acquired and processed using SCIEX OS Software 2.0.

Results

Low-level quantification: Figures 2, 3 and 4 show an extracted matrix-spiked standard at a concentration of 1 pg/mL with a clear and distinct peak compared to the blank extract for each of the estrogens studied. Signal-to-noise is 20:1, 12:1 and 6:1 for estrone, estradiol and estriol, respectively. Signal-to-noise calculations are based on a peak-to-peak algorithm, and the number of data points across the peak are sufficient for reproducibility.

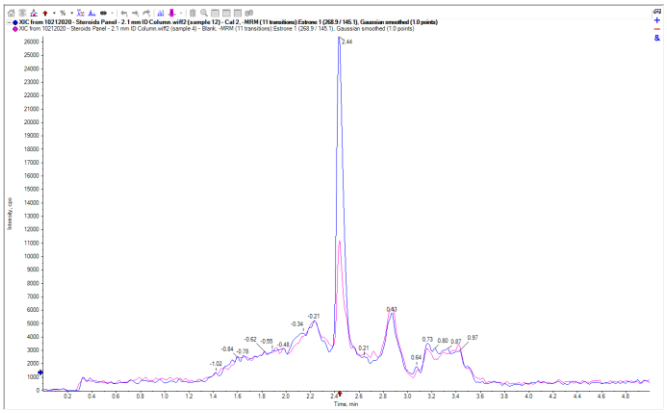

Figure 2. Estrone results. Plasma extract of estrone at 1 pg/mL (approximately 3 pmol/L) analyzed on the SCIEX 7500 System by the proposed method from 200 µL of plasma (0.05 pg injected on column).

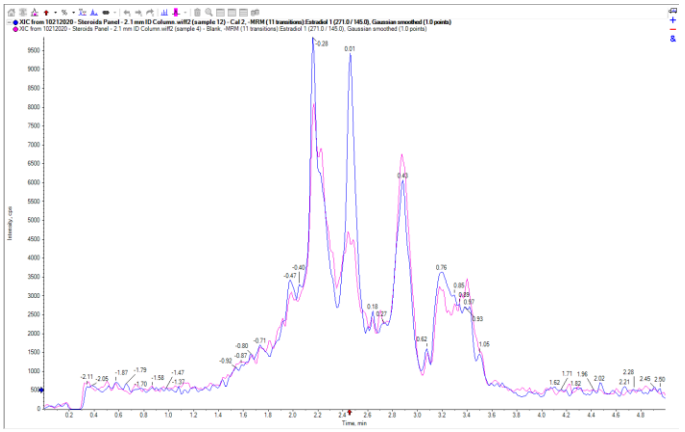

Figure 3. Estradiol results. Plasma extract of estradiol at 1 pg/mL (approximately 3 pmol/L) analyzed on the SCIEX 7500 System by the proposed method from 200 µL of plasma (0.05 pg injected on column).

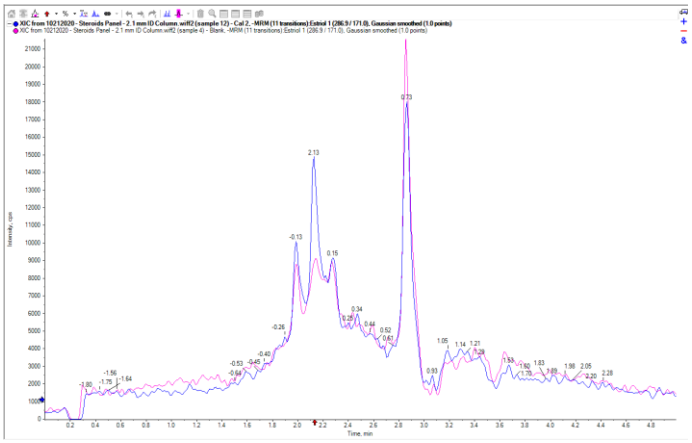

Figure 4. Estriol results. Plasma extract of estriol at 1 pg/mL (approximately 3 pmol/L) analyzed on the SCIEX 7500 System by the proposed method from 200 µL of plasma (0.05 pg injected on column).

Linearity: Linearity was assessed over a concentration range of 5–10,000 pg/mL. Linear regression was used based on peak areas and a 1/x weighting was used. R^2 values were greater than 0.9996 for all compounds analyzed. Calibration curves are shown in Figure 5.

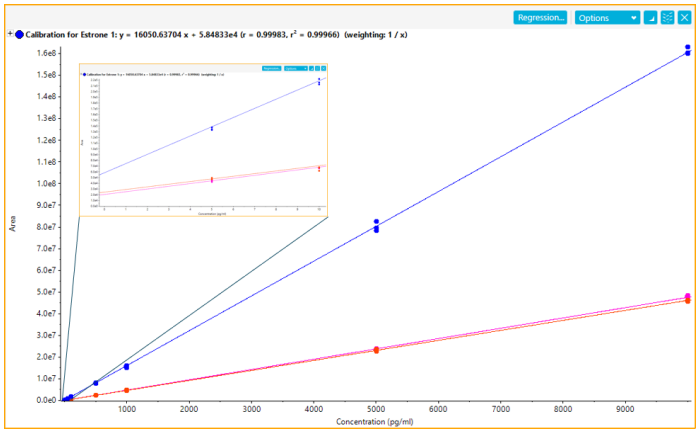

Figure 5. Calibration curves. Linearity of the response of calibration standards in matrix from 5–10,000 pg/mL for all compounds.

Reproducibility and robustness: To assess intra-assay performance, multiple replicates (n=3) of calibration standards in matrix were analyzed. A summary of the results obtained are shown in Table 1.

Table 1. Summary of assay performance.

Analyte	Conc. (pg/mL)	n	Mean	CV	Accuracy
Estrone	*5*	*3*	*4.77*	*2.95*	*95.5*
Estradiol	*5*	*3*	*4.93*	*6.24*	*98.6*
Estriol	*5*	*3*	*5.01*	*5.7*	*100.2*

Conclusions

The SCIEX 7500 System was used to analyze estrone, estradiol and estriol spiked into human plasma. Sensitivity was shown to be significantly less than 3 pg/mL from only 200 µL of sample, linearity was over 4 orders of magnitude and reproducibility in terms of %CV was demonstrated at low concentrations (less than 6.5%).

References

1. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.

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