



Analysis of Stability Over 5000 Injections of Testosterone and 17-Hydroxyprogesterone on IONICS 3Q 220 Triple Quad Mass Spec

Quick Facts:

- >5000 injections were performed.
- Liquid Chromatography: Shimadzu Prominence UFLC
- Mass Spectrometry: IONICS 3Q 220 triple quadrupole mass spectrometer
- ESI Source
- CV <3.2% in final 1200 injections

1. Introduction

Robustness and stability are important considerations when choosing a mass spectrometry platform. Instruments with reduced cleaning and maintenance requirements are able to be run for longer periods of time enabling greater laboratory efficiency. LC-MS/MS continues to be the platform of choice when high performance, high throughput and high sensitivity are required. Already accepted in big pharma, environmental and food testing labs for small molecule analysis, new applications in clinical chemistry labs are increasing the demand for simplified, easy to use systems.

IONICS 3Q triple quadrupole mass spectrometers are designed to maximize uptime by utilizing a variety of innovative features. This experiment, conducted on an IONICS 3Q 220 illustrates the day-after-day robustness of the instrument. Over the course of this experiment more than 5000 injections of testosterone and 17-hydroxyprogesterone in human serum were analyzed with remarkably consistent, high quality results.

2. Method

Testosterone and 17-hydroxyprogesterone were purchased from Sigma (Milwaukee, WI) and used when received. The two compounds were spiked in protein precipitated human serum. The injection concentration used was 1 ng/mL. All solvents used were HPLC grade.

2.1 Mass Spectrometry Conditions

An IONICS 3Q 220 equipped with the Torrent Multi-Option Dual Source™ interface with both probes set in ESI mode was used to perform the analysis of both testosterone and 17-hydroxyprogesterone.

Below, **Table 1** outlines the instrument settings used during the method.

Table 1: Instrument Settings

| | |
|-----------------------|------|
| ESI Voltage (Volts) | 5000 |
| HSID Temp. (°C) | 225 |
| Nebulizer Gas Setting | 200 |
| Drying Gas Setting | 120 |
| Heating Gas Setting | 250 |
| Source Temp. (°C) | 250 |
| Dwell Time (ms) | 100 |
| Pause Time (ms) | 5 |

The Q1 and Q2 mass filters were set to unit resolution. For each compound, two MRM transitions were monitored: m/z at 289.2/97.1 and 289.2/109.1 for testosterone as well as m/z at 331.2/109.1 and 331.2/97.1 for 17-hydroxyprogesterone.

2.2 LC Conditions

The separation was performed on a Shimadzu UFLC system (including two pumps, an autosampler, a degasser, and a column oven). 1 μ L of the sample was loaded on a Imtakt Cadenza CD-C18 HT column (50x2mm, 3 μ m) at 40 °C. The total LC cycle time was 3 min. Solvent A was composed of 5% methanol in water with 0.1% formic acid and 5 mM ammonium acetate. Solvent B was composed of 5% water in methanol with 0.1% formic acid and 5 mM ammonium acetate. The flow rate was 450 μ L/min.

3. Results

3.1 Extracted Ion Chromatogram (EIC)

LC-MS/MS EICs of testosterone and 17-hydroxyprogesterone in protein precipitated human serum are shown in **Figures 1a and 1b**. More than 5000 injections were completed within a week. The performance and calibration of the 3Q 220 instrument remained stable and maintained very low CVs.

Figure 1(A): LC-MS/MS EIC of testosterone in protein precipitated human serum.

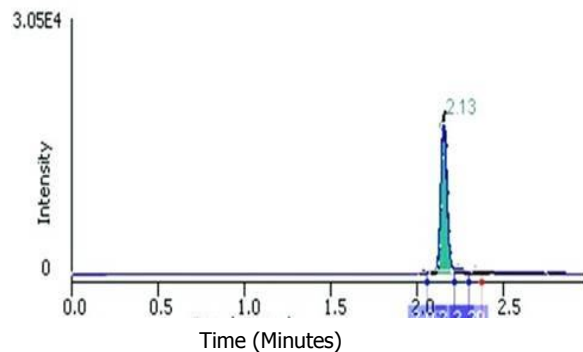
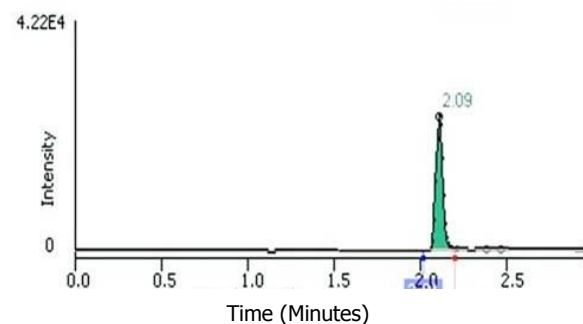


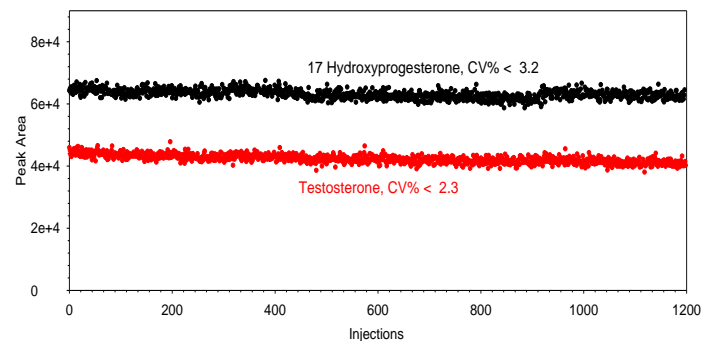
Figure 1 (B): LC-MS/MS EIC of 17-hydroxyprogesterone in protein precipitated human serum.



3.2 Reproducibility

For testosterone and 17-hydroxyprogesterone, the changes of peak area with injections are shown in **Figure 2**. The data in **Figure 2** represents the final 1200 injections (5000 total). The repeated injections have excellent percent CV's; 3.2% for 17-hydroxyprogesterone and 2.3% for testosterone.

Figure 2: Over the final 1200 injections of testosterone and 17-hydroxyprogesterone, the instrument maintained CVs of 2.3 and 3.2% respectively an indication that despite the high volume of samples the instrument had run, results remained remarkably consistent.



4. Conclusion

After more than 5000 injections of testosterone and 17-hydroxyprogesterone in human serum, the 3Q 220 maintains excellent performance. The CVs for testosterone and 17-hydroxyprogesterone were 2.3% and 3.2% respectively demonstrating exceptional reproducibility of results over a long period. With ease of maintenance, unmatched stability and the ability to run for an extended time without the need for cleaning, IONICS 3Q 220 is perfectly suited to meet the needs of high volume laboratories.

5. Contact Information

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