

Quantitative Bioanalysis using the Q Exactive HRMS: Factors in choosing Resolution and Scan Type

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Overview

Purpose

The gold standard for quantitative bioanalysis is a triple stage quadrupole MS operating under SRM mode. This technique requires tuning of each analyte(s) and related internal standard(s) as well as the fragmentation of a molecule in order to achieve greater signal to noise and increased sensitivity. The Q Exactive MS coupled to a UHPLC system can provide quantitation under generic conditions without prior knowledge of the analyte(s) and without fragmentation. The combination of HRMS and SIM (no fragmentation) results in increased sensitivity and greater confidence in the results. The choice of resolution settings and scan types for the Q Exactive were explored and differences relative to sensitivity, linearity, specificity, precision and accuracy are presented.

Method

MK-5172 (m/z 767.34164) analysis completed under UHPLC conditions. GS-328083 was used as an internal standard and did not co-elute with the MK-5172. The study design included data acquisition with the following resolutions and scan mode combinations: 35K/SIM, 70K/SIM and 35K/MSMS.

Introduction

High resolution/accurate mass (HR/AM) mass spectrometry is rapidly becoming adopted for quantitative bioanalysis of both large and small molecules. The Q Exactive HR/AM MS can acquire data using SIM, Full Scan and MS/MS modes - each with various resolution settings. This flexibility provides scientists with a broad range of quantitative capability that cannot be achieved by even the most sensitive triple quadrupoles for routine bioanalysis.

Selected Reaction Monitoring (SRM) is the most common method of performing quantitation analysis by mass spectrometry. However, it does not provide the most intense signal. Selected Ion Monitoring (SIM) will have greater intensity compared to SRM because it does not lose signal from molecular fragmentation. Unfortunately, acquiring SIM data on a quadrupole instrument does not have the specificity of SRM in complex biological matrices like plasma. Quantitative SRM is typically accomplished using a triple quadrupole mass spectrometer capable of producing MS/MS fragmentation. Even though the overall signal will be lower than SIM, the combination of the specific precursor ion mass/charge and the product ion mass/charge improves signal-to-noise compared to SIM and is used to selectively monitor for the compound to be quantified.

HR/AM can overcome this limitation of SIM (Figure 1 showing isobaric compounds butyl-phthalate and ethinyl-estradiol with loss of water). This benefit becomes even more apparent when precursor ions fragment extensively or fragment poorly, high background still exists even with SRM, or because of less specificity for larger molecules (e.g., proteins and peptides) with typical triple quadrupole mass resolution.

Methods

Plasma Sample Processing

An aliquot of 50 μ L of plasma sample was treated with 200 μ L of cold acetonitrile (MeCN) containing ISTD. After the protein precipitation, about 200 μ L of the supernatant was transferred to a clean 96-well plate and mixed with 300 μ L of water.

Liquid Chromatography

Thermo Scientific HyPurity C₁₈ HPLC column (30 X 2.1 mm, 5 μ), (Part # 22105-032130).

Mobile phases

Mobile phase A contained 1 % acetonitrile in 10 mM ammonium formate aqueous solution with pH of 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate with pH of 4.6.

HPLC pumps

The HRMS analysis was performed using a Thermo Scientific Accela Open system with DLW (Dynamic Load and Wash) and with Accela™ 1250 UHPLC pumps.

Samples for HRMS & QQQ Analysis

MK-5172 calibration curves ranging from 1 nM to 5000 nM were prepared in human plasma. An aliquot of 50 μ L of plasma sample was treated with 200 μ L of cold acetonitrile containing ISTD. 200 μ L of the supernatant was transferred to a clean 96-well plate and mixed with 300 μ L of water.

| Time (min) | Flow Rate (mL/min) | Mobile Phase A (%) | Mobile Phase B (%) |
|------------|--------------------|--------------------|--------------------|
| 0.00 | 0.50 | 99 | 1 |
| 3.0 | 0.50 | 50 | 50 |
| 4.0–5.2 | 0.50 | 1 | 99 |
| 5.4–8.0 | 0.50 | 99 | 1 |

FIGURE 1. Low Resolution vs High Resolution SIM

- a) XIC of E Estradiol acquired at R=100,000. Interfering pththalate is resolved and doesn't interfere with the estradiol.
- b) XIC of same sample at lower resolution which is not capable of resolving matrix interference, resulting in a "masked" analyte (false negative).

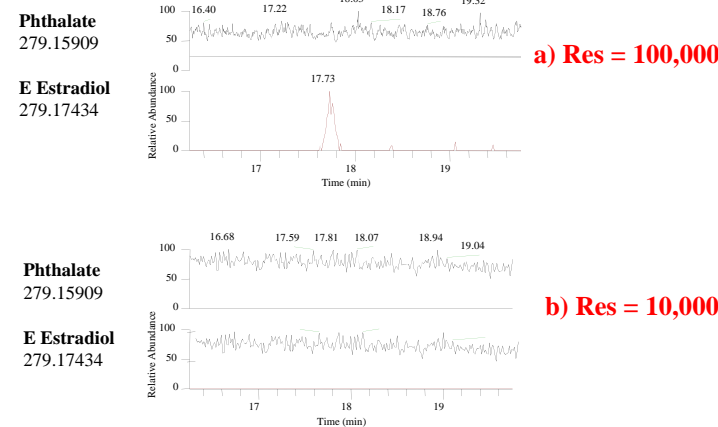
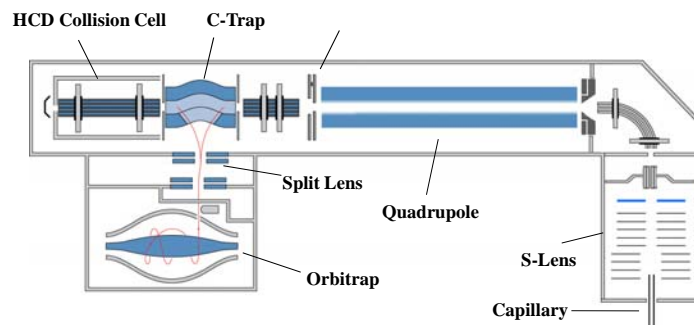


FIGURE 2. Q Exactive Instrument Schematic



Mass Spectrometry

UHPLC-HRMS analysis was performed using a Thermo Fisher Scientific Q Exactive MS (Figure 2) operating at different resolution settings and scan modes (i.e, SIM or MS/MS) to allow comparison. Analyte peak widths were approximately 3 seconds (baseline to baseline).

Generic ion source conditions were used for all sample collection including vaporizer temp. 475°C, capillary temp. 350°C, 4kV spray voltage, sheath gas 50, AUX gas 20, and sweep gas of 1 unit. The instrument was calibrated in positive ion mode before sample acquisition using Pierce LTQ Velos ESI Positive Ion Calibration Solution.

Data Analysis

Data was acquired using Thermo Scientific LCQuan 2.7 quantitation software.

Results

Figure 3: SIM with 35K Resolution

- The typical resolution setting for routine bioanalytical quantitation on the Q Exactive™.
- An isobar co-eluting with MK-5172 and only 0.1904 m/z difference (m/z 767.53518 vs. m/z 767.34476) resulted in imprecision and 4 standards greater than 20% theoretical.
- %RSD ranged from 6.67 to 19.2 for QC's.

Figure 4a and 4b: SIM with 35K and 70K Resolution Chromatograms

- Affect on resolution can be seen in highlighted spectra.
- Signal to noise is similar for both resolutions.

Figure 5: SIM with 70K Resolution

- Precision was greatly improved. Standards were <15% theoretical. %RSD ranged from 2.32 to 11.3 for QC's.

Figure 6: MSMS with 35K Resolution

- Precision was not assessed. However, a discovery style single replicate curve was acquired. Standards were <15% theoretical and with the exception of one point, all were single digit differences.

Discussion

- The same plasma extracts were used for the entire study. The method development method consisted of three acquisition modes: SIM w/35K Resolution, SIM w/70K Resolution, MSMS w/35K Resolution. For bioanalytical method development, a set of samples could be injected unattended at various resolution settings using SIM, Full Scan and/or MSMS mode to determine what settings are preferred.
- HR/AM SIM acquisition at 35K Resolution is an ideal starting point for quantitation based on points across the peak and specificity.
- With the generic discovery based UHPLC method, an isobaric interference was present with significant intensity resulting in imprecision.
- Increased specificity was possible by either increasing the resolution to 70K or by using MSMS at 35K resolution. Both of these options greatly improved the quantitative results.

FIGURE 3: SIM with 35K Resolution Standard Calibrator Summary

| File Name | Comment | Sample | Level | Specified Conc | Vol Pk | RT | Integration | Response | ISTD Response | Response | % Diff | % RSD | % CV | Calculated | Units |
|-----------|---------------|-------------|---------|----------------|--------|------|-------------|----------|---------------|----------|---------|-------|------|------------|-------|
| 1 | GS-328083-004 | Std 1 - 1nm | Stand 1 | 1.000 | 1000 | 4.87 | Method 5 | 25376 | 510762 | 510540 | -2.40 | 0.00 | 0.00 | 5.976 | 1 |
| 12 | GS-328083-004 | Std 1 - 1nm | Stand 1 | 1.000 | 1000 | 4.87 | Method 5 | 26888 | 509549 | 509549 | 0.0000 | NA | NA | 5.976 | 1 |
| 13 | GS-328083-004 | Std 2 - 2nm | Stand 2 | 2.000 | 2000 | 4.87 | Method 5 | 51380 | 1018542 | 1018542 | 0.0000 | NA | NA | 11.434 | 1 |
| 14 | GS-328083-004 | Std 2 - 2nm | Stand 2 | 2.000 | 2000 | 4.87 | Method 5 | 88180 | 1736270 | 1736270 | 0.0000 | NA | NA | 2.108 | 1 |
| 15 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 126820 | 2555130 | 2555130 | -0.0000 | NA | NA | 4.933 | 1 |
| 16 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 204820 | 4090260 | 4090260 | 0.0000 | NA | NA | 5.161 | 1 |
| 17 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 264820 | 5296520 | 5296520 | 0.0000 | NA | NA | 6.667 | 1 |
| 18 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 324820 | 6493040 | 6493040 | 0.0000 | NA | NA | 7.520 | 1 |
| 19 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 384820 | 7690060 | 7690060 | 0.0000 | NA | NA | 8.877 | 1 |
| 20 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 444820 | 8887080 | 8887080 | 0.0000 | NA | NA | 10.232 | 1 |
| 21 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 504820 | 10084100 | 10084100 | 0.0000 | NA | NA | 11.587 | 1 |
| 22 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 564820 | 11280200 | 11280200 | 0.0000 | NA | NA | 12.942 | 1 |
| 23 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 624820 | 12476300 | 12476300 | 0.0000 | NA | NA | 14.297 | 1 |
| 24 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 684820 | 13672400 | 13672400 | 0.0000 | NA | NA | 15.652 | 1 |
| 25 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 744820 | 14868500 | 14868500 | 0.0000 | NA | NA | 17.007 | 1 |
| 26 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 804820 | 16064600 | 16064600 | 0.0000 | NA | NA | 18.362 | 1 |
| 27 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 864820 | 17260700 | 17260700 | 0.0000 | NA | NA | 19.717 | 1 |
| 28 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 924820 | 18456800 | 18456800 | 0.0000 | NA | NA | 21.072 | 1 |
| 29 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 984820 | 19652900 | 19652900 | 0.0000 | NA | NA | 22.427 | 1 |
| 30 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1044820 | 20849000 | 20849000 | 0.0000 | NA | NA | 23.782 | 1 |
| 31 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1104820 | 22045100 | 22045100 | 0.0000 | NA | NA | 25.137 | 1 |
| 32 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1164820 | 23241200 | 23241200 | 0.0000 | NA | NA | 26.492 | 1 |
| 33 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1224820 | 24437300 | 24437300 | 0.0000 | NA | NA | 27.847 | 1 |
| 34 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1284820 | 25633400 | 25633400 | 0.0000 | NA | NA | 29.202 | 1 |
| 35 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1344820 | 26829500 | 26829500 | 0.0000 | NA | NA | 30.557 | 1 |
| 36 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1404820 | 28025600 | 28025600 | 0.0000 | NA | NA | 31.912 | 1 |
| 37 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1464820 | 29221700 | 29221700 | 0.0000 | NA | NA | 33.267 | 1 |
| 38 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1524820 | 30417800 | 30417800 | 0.0000 | NA | NA | 34.622 | 1 |
| 39 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1584820 | 31613900 | 31613900 | 0.0000 | NA | NA | 35.977 | 1 |
| 40 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1644820 | 32810000 | 32810000 | 0.0000 | NA | NA | 37.332 | 1 |
| 41 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1704820 | 34006100 | 34006100 | 0.0000 | NA | NA | 38.687 | 1 |
| 42 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1764820 | 35202200 | 35202200 | 0.0000 | NA | NA | 40.042 | 1 |
| 43 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1824820 | 36398300 | 36398300 | 0.0000 | NA | NA | 41.397 | 1 |
| 44 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1884820 | 37594400 | 37594400 | 0.0000 | NA | NA | 42.752 | 1 |
| 45 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 1944820 | 38790500 | 38790500 | 0.0000 | NA | NA | 44.107 | 1 |
| 46 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 2004820 | 39986600 | 39986600 | 0.0000 | NA | NA | 45.462 | 1 |
| 47 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 2064820 | 41182700 | 41182700 | 0.0000 | NA | NA | 46.817 | 1 |
| 48 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 2124820 | 42378800 | 42378800 | 0.0000 | NA | NA | 48.172 | 1 |
| 49 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 2184820 | 43574900 | 43574900 | 0.0000 | NA | NA | 49.527 | 1 |
| 50 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 2244820 | 44771000 | 44771000 | 0.0000 | NA | NA | 50.882 | 1 |

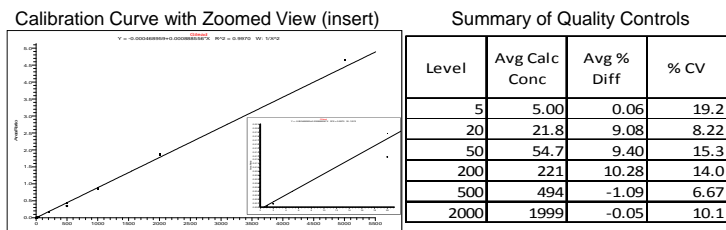


FIGURE 4a: Representative Chromatogram at 35K Resolution
Results from 35K resolution (7 Hz). Chromatogram displaying the ion of interest and the large interference ion.

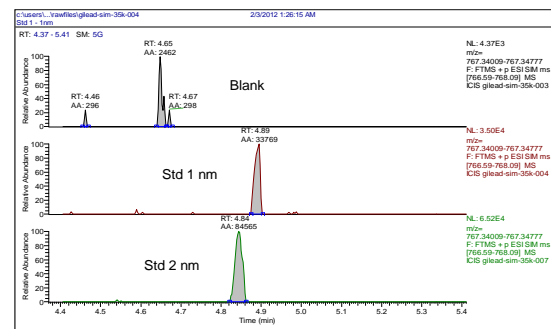


FIGURE 4b: Representative Chromatogram at 70K Resolution
Results from 70K resolution (7 Hz). Chromatogram displaying the ion of interest and the large interference ion.

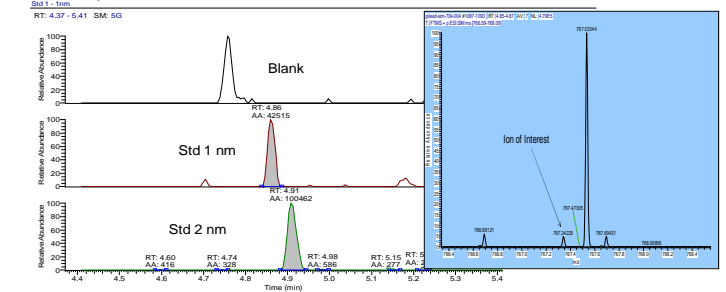


FIGURE 3: SIM with 70K Resolution Standard Calibrator Summary

| File Name | Comment | Sample | Level | Specified Conc | Vol Pk | RT | Integration | Response | ISTD Response | Response | % Diff | % RSD | % CV | Calculated | Units |
|-----------|---------------|-------------|---------|----------------|--------|------|-------------|----------|---------------|----------|---------|-------|------|------------|-------|
| 1 | GS-328083-004 | Std 1 - 1nm | Stand 1 | 1.000 | 1000 | 4.87 | Method 5 | 42115 | 6654780 | 6654780 | 0.0004 | 2.40 | 0.00 | 5.976 | 1 |
| 2 | GS-328083-004 | Std 1 - 1nm | Stand 1 | 1.000 | 1000 | 4.87 | Method 5 | 37323 | 6654780 | 6654780 | -4.92 | 0.00 | 0.00 | 5.976 | 1 |
| 3 | GS-328083-004 | Std 2 - 2nm | Stand 2 | 2.000 | 2000 | 4.87 | Method 5 | 83143 | 13310300 | 13310300 | 0.0000 | NA | NA | 11.434 | 1 |
| 4 | GS-328083-004 | Std 2 - 2nm | Stand 2 | 2.000 | 2000 | 4.87 | Method 5 | 159462 | 26829500 | 26829500 | 0.0000 | NA | NA | 2.108 | 1 |
| 5 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 267169 | 47122200 | 47122200 | 0.0000 | NA | NA | 4.933 | 1 |
| 6 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 466428 | 83642800 | 83642800 | 0.0000 | NA | NA | 5.161 | 1 |
| 7 | GS-328083-004 | Std 3 - 3nm | Stand 3 | 3.000 | 3000 | 4.87 | Method 5 | 665687 | 12018400 | 12018400 | 0.0000 | NA | NA | 6.667 | 1 |
| 8 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 864946 | 15674600 | 15674600 | 0.0000 | NA | NA | 7.520 | 1 |
| 9 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 107622 | 66225400 | 66225400 | 0.0000 | NA | NA | 12.603 | 1 |
| 10 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 107622 | 66225400 | 66225400 | 0.0000 | NA | NA | 12.603 | 1 |
| 11 | GS-328083-004 | Std 4 - 4nm | Stand 4 | 4.000 | 4000 | 4.87 | Method 5 | 107622 | 66225400 | 66225400 | 0.0000 | NA | NA | 12.603 | 1 |
| 12 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 13 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 14 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 15 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 16 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 17 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 18 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 19 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 20 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 21 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 22 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 23 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 24 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 25 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 26 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 27 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 28 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 29 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 30 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
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| 32 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 33 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
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| 35 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
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| 45 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 46 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
| 47 | GS-328083-004 | Std 5 - 5nm | Stand 5 | 5.000 | 5000 | 4.87 | Method 5 | 209482 | 66219940 | 66219940 | -0.0001 | NA | NA | 2.405 | 247 |
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