TECHNICAL NOTE 73770

Fast and accurate quantitation of intact IGF-1 from serum by HRAM LC-MS for clinical research

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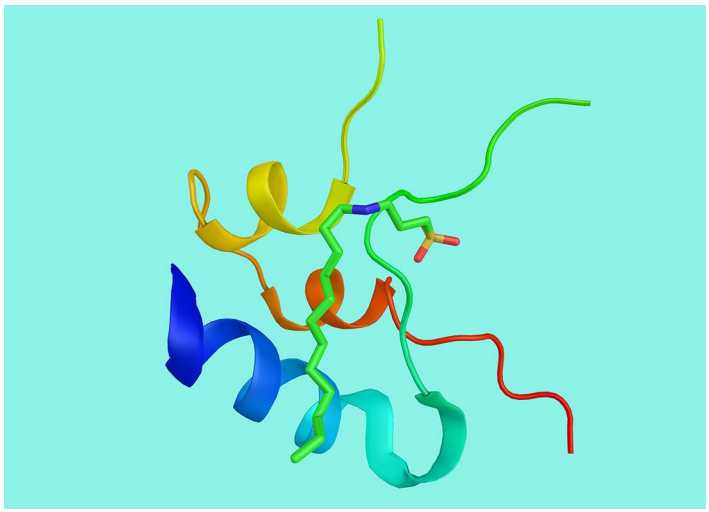
Keywords: Orbitrap Exploris 120 mass spectrometer, insulin-like growth factor 1, IGF-1, high-resolution accurate-mass (HRAM) mass spectrometry, Vanquish UHPLC, clinical research



Goal

To demonstrate the robustness and accuracy of the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer coupled with ultra-high-pressure liquid chromatography (UHPLC) for quantitation of IGF-1 in serum for clinical research.

Introduction

Insulin-like growth factor 1 (IGF-1) is an important polypeptide hormone whose quantification has many clinical research and anti-doping applications, such as the assessment of abnormal growth and development in adolescents caused by growth hormone disorders, as well as its use in athlete doping tests. High-resolution accurate-mass (HRAM) MS methods have unique advantages for reliably measuring and differentiating IGF-1 species, whose attributes might be missed by immunoassays and non-HRAM methods due to their comparative lack of selectivity and sensitivity. Quadrupole time-of-flight (Q-TOF)-based HRAM methods have been developed for IGF-1 quantification. However, these methods have practical challenges in a true routine context for differentiating IGF-1 and its variants due to limited mass resolving capability, compromised specificity, quantitative performance (using 20 ppm extraction windows), and mass deviation issues.

This technical note details a fast and robust HRAM data acquisition and data analysis workflow for serum IGF-1 on the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer. With fast scanning and enhanced mass accuracy enabled by the Thermo Scientific™ Easy-IC™ ion source system, the method shows good analytical sensitivity, linear dynamic range, and excellent reproducibility for reliable serum IGF-1 measurements. Intact IGF-1 was quantified down to an LLOQ of 10 ng/mL with a standard curve range of 15.6–2,000 ng/mL ($r^2 > 0.995$).

Experimental

Sample preparation

Internal standard (IGF-1-N^{15}) was added to the serum samples, which were first precipitated using acidic ethanol and then cryo-precipitated after neutralization with Tris base. Calibrators were prepared in 20% acetonitrile in 1× phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA). The internal standard, IGF-1-N^{15}, was purchased from Prospect International.

Chromatography and separation conditions

Automated online SPE cleanup and chromatographic separation was performed on a Thermo Scientific™ Vanquish™ Flex UHPLC system (Table 1 and Figure 1). A Thermo Scientific™ Hypersil GOLD™ C8, 50 × 2.1 mm, 5 µm column was used for separation. Total run time was 3 min. The high-quality full scan spectra were facilitated by the 3 min online SPE heart-cut technique. After the target fraction was eluted onto the analytical column using one pump, the autosampler and SPE trap column were cleaned and conditioned for the next run by a second pump. This reduced the sample-to-sample time needed if one pump alone performed all the work.

Table 1. Chromatographic conditions

Property	Setting
Column	Hypersil GOLD C8, 50 × 2.1, 5 µm (P/N 25205-052130)
Trap column	MilliporeSigma™ Chromolith™ RP-18e 5-2 mm Guard Cartridge Kits
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Gradient	Time (min) %A %B 0 80 20 0.51 80 20 2.5 5 95 2.6 80 20 3 80 20
Flow rate	0.6 mL/min
Column temperature	35 °C
Autosampler temperature	10 °C
Injection volume	50 µL

Mass spectrometry

IGF-1 was detected on an Orbitrap Exploris 120 mass spectrometer operating in SIM mode, monitoring IGF-1 in the 7+ charge state (*m/z* 1093.5225). Mass spectrometer method conditions are shown in Table 2.

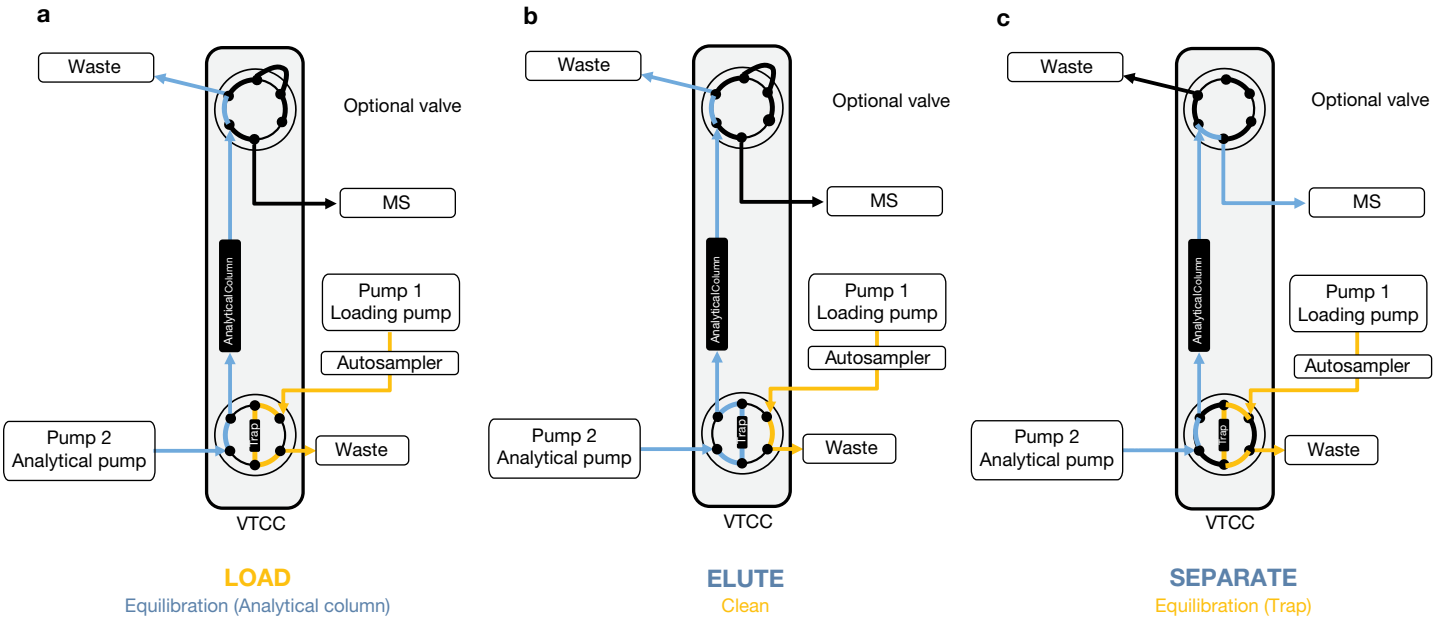


Figure 1. Configureation of online SPE cleanup and chromatographic separation. a) Sample loading and washing; b) sample transferring from trap to analytical column; c) sample separation.

Table 2. Mass spectrometry conditions

Property	Setting
Instrumentation	Orbitrap Exploris 120 mass spectrometer
Spray voltage	3.5 kV Positive
Sheath gas	50 Arb
Aux gas	15 Arb
Sweep gas	5 Arb
Capillary temperature	300 °C
Vaporizer temperature	100 °C
Ion polarity	Positive
SIM scan data type	Profile
Isolation window (*m/z*)	2
Multiplex ions	2
Orbitrap resolution	60,000
Maximum injection time	Auto
Microscans	1
AGC target	Standard
EASY-IC	On

Mass accuracy and mass stability

To demonstrate robustness and to explore the quantitative performance of the LC-MS system, more than 1,500 injections, including calibrators, spiked serum samples, blanks, and spiked high concentration samples (out of reference range for carryover monitoring) organized into 54 batches were analyzed over a period of 130 hours. Tables 3 and 4 show the precision and recovery across the 54 batches of eight calibrators and seven spiked serum samples at different concentration levels.

Table 3. Robustness statistics for 54 replicates of calibrators across 130 hours of non-stop run time for IGF-1

Level (n=54)	Concentration	%Bias (average)	Ion ratio	% CV
Cal1	15.6 ng/mL	-2.0%	Pass	7.0%
Cal2	31.3 ng/mL	2.3%	Pass	4.0%
Cal3	62.5 ng/mL	1.6%	Pass	4.2%
Cal4	125 ng/mL	2.1%	Pass	3.0%
Cal5	250 ng/mL	1.8%	Pass	3.7%
Cal6	500 ng/mL	0.6%	Pass	3.0%
Cal7	1000 ng/mL	-1.0%	Pass	2.8%
Cal8	2000 ng/mL	-6.0%	Pass	2.6%

Table 4. Robustness statistics for 54 replicates of seven spiked serum samples across 130 hours of non-stop run time for IGF-1

Spiked sample # (n=54)	Concentration	% Recovery (average)	% Ion ratio	% CV
Sample 1	15.6 ng/mL	-3.7%	Pass	12.7%
Sample 2	31.3 ng/mL	-5.4%	Pass	6.2%
Sample 3	62.5 ng/mL	-7.3%	Pass	4.0%
Sample 4	125 ng/mL	-7.3%	Pass	3.5%
Sample 5	250 ng/mL	-16.3%	Pass	3.0%
Sample 6	500 ng/mL	-12.4%	Pass	2.9%
Sample 7	1000 ng/mL	-19.6%	Pass	3.0%

Data analysis

Automated quantification of SIM data was performed by Thermo Scientific™ TraceFinder™ 5.1 software. Mass extraction (5 ppm mass extraction width) of a single isotope of IGF-1 in the 7+ charge state (*m/z* 1093.5225) was used to generate extracted ion chromatograms, and the 7+ charge state (*m/z* 1106.7671) from N^{15}-labeled IGF-1 was used as the internal standard (Figure 2).

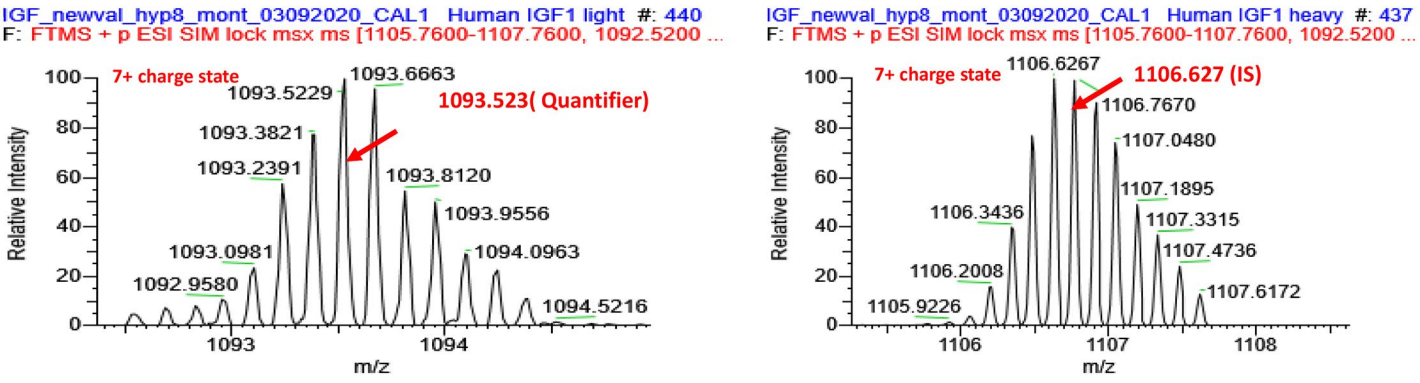


Figure 2. Example mass spectra for IGF-1 in a) low level calibrators (15.6 ng/mL), and b) internal standard (500 ng/mL). The 7+ charge state (*m/z* 1093.523) of a single isotope of IGF-1 was used for quantitation.

Results and discussion

The ultra-high resolution and fast scanning ability of Orbitrap Exploris 120 MS allow accurate IGF-1 quantification in SIM mode. We used the 7-valent monoisotopic peak of *m/z* 1093.520 as the quantitative ion, two adjacent monoisotopic peaks (*m/z* 1093.378, 1093.664) as the qualitative ions, and *m/z* 1106.767 from N^{15}-labeled internal standard ion (Figure 2). A resolution of 60,000 (FWHM) at *m/z* 200 was used for data collection.

Chromatograms were generated with an extraction window of 5 ppm and used to calculate calibration curves. Linearity of analyte measurements in the matrix (1XPBS with 20% acetonitrile and 1% BSA) was measured using weighted $1/X^2$ linear regression analysis. An excellent R^2 value of 0.9978 was obtained (Figure 3). No significant interference was found from the serum matrix (data not shown) using a 5 ppm mass extraction window.

Figure 4 shows an overlay of 54 calibration curves, acquired across 130 hours of non-stop run time. After analyzing 54 batches over five days of consecutive runs, the calibration curves did not show degradation, which suggests that the whole system, including samples, columns, UHPLC and MS, has great stability. The linearity of the calibration curve was 0.9964. Across all 54 calibration curves, the median %RSD of IS was 6.97%. Variation within the individual batch was even smaller (around 3.58%). The mass accuracy was stable within 1.5 ppm throughout for calibrators (Figure 5) and within 2 ppm for spiked serum samples (Figure 6).

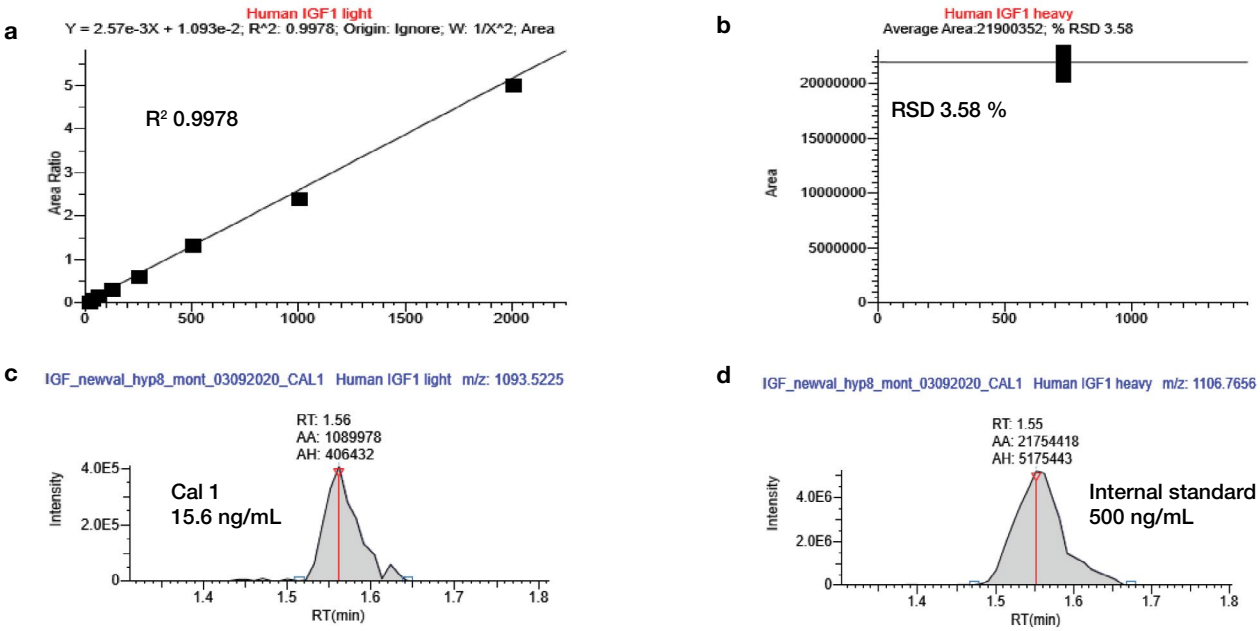


Figure 3. a) Linearity of analyte measurements in the matrix (1XPBS with 20% acetonitrile and 1% BSA); b) The internal standard also shows excellent precision with a %RSD of 3.5% over eight injections; c) An example chromatograph of calibrator 1; d) An example chromatograph of IS.

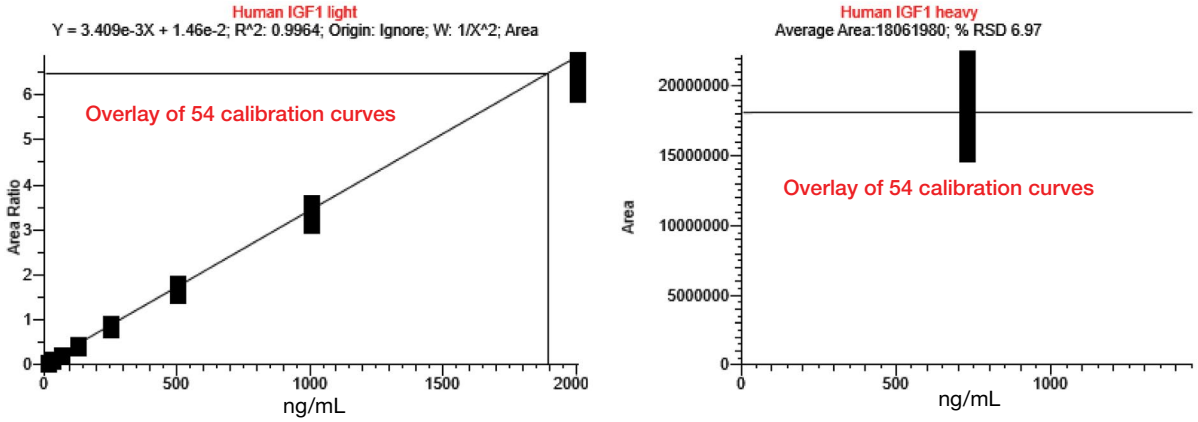
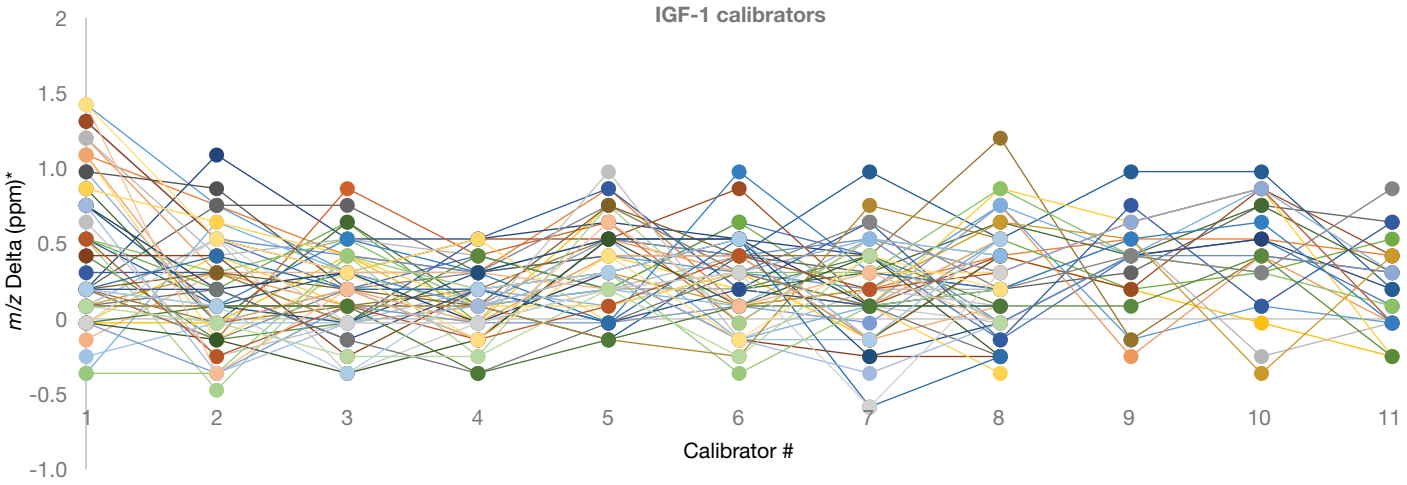


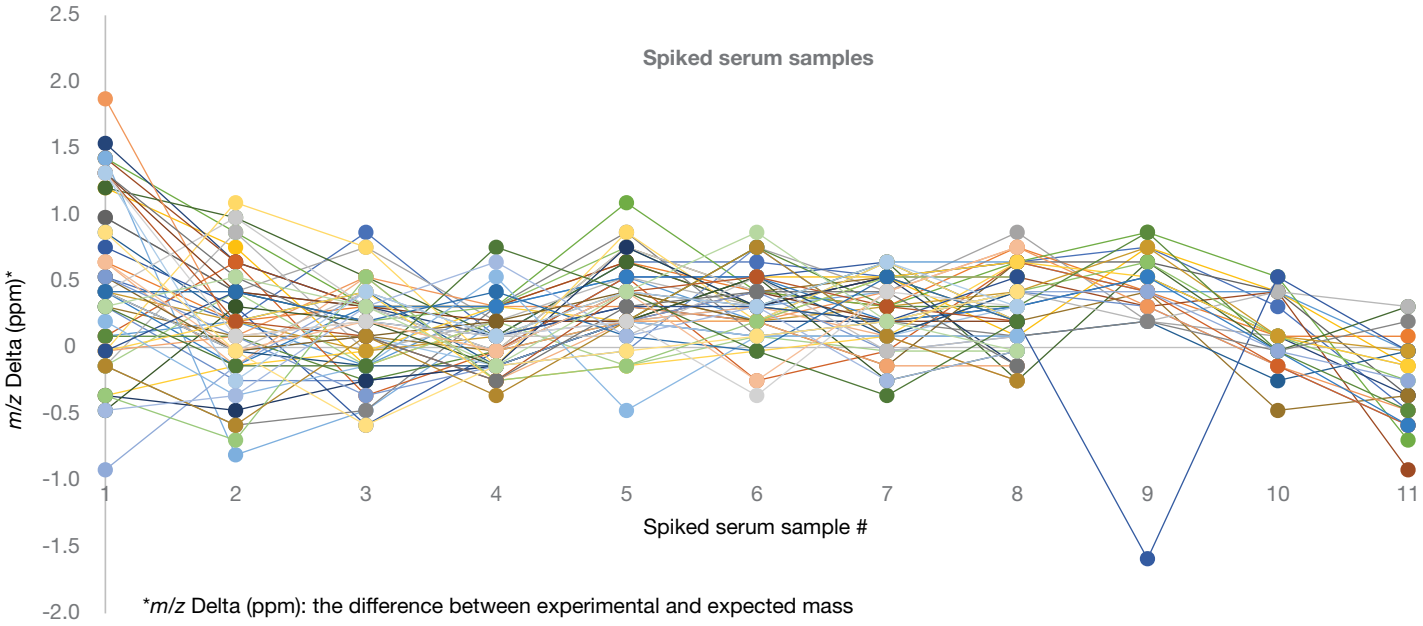
Figure 4. Accumulation of 54 calibration curves acquired across 130 hours non-stop run time for the IGF-1 quantification



*m/z Delta (ppm): the difference between experimental and expected mass

Calibrator #	1	2	3	4	5	6	7	8	9	10	11
Concentration (ng/mL)	15.6	31.3	62.5	125	250	500	1000	2000	4000	8000	16,000

Figure 5. The Orbitrap Exploris 120 mass spectrometer with EASY-IC ion source enables IGF-1 mass accuracy within 1.5 ppm in calibrators across 130 hours non-stop run time.



*m/z Delta (ppm): the difference between experimental and expected mass

Spiked sample #	1	2	3	4	5	6	7	8	9	10	11
Concentration (ng/mL)	17	31	59	69	117	206	443	771	807	1315	1575

Figure 6. The Orbitrap Exploris 120 mass spectrometer with EASY-IC ion source enables IGF-1 mass accuracy within 2 ppm in serum samples across 130 hours non-stop run time. The deviation of the dark blue point in sample # 9 can be attributed to autosampler blockage by small particles from donor samples.

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Conclusion

The Vanquish UHPLC platform combined with Orbitrap Exploris 120 mass spectrometer provides a fast and robust HRAM based quantitation workstream for IGF-1 in serum.

The Vanquish UHPLC platform provided both sample cleanup and analytical separation in a 3-minute method, enhancing the ability of the mass spectrometer to detect compounds.

The Orbitrap Exploris 120 mass spectrometer provides excellent robustness of data over long run times without maintenance.

Find out more at **thermofisher.com/clinicalresearch**

TN73770-EN 0920S

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