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Quantification of insulin-like growth factor 1 in human serum by Vanquish UHPLC with Q Exactive high-resolution accurate-mass mass spectrometry for clinical research

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Keywords
Insulin-like growth factor 1 (IGF-1), Q Exactive high-resolution accurate-mass mass spectrometry, Vanquish UHPLC, online solid phase extraction (SPE), clinical research

Goal

To develop an analytical method using Thermo Scientific™ Orbitrap™ powered HRAM mass spectrometry with a UHPLC system to quantify IGF-1 in human serum.

Introduction

Insulin-like growth factor 1 (IGF-1) is an important peptide hormone, usually used in clinical research to assess abnormal growth and development in adolescents caused by growth hormone disorders. At present, the main measurement method of IGF-1 is immunoassay, but the use of immunoassay has been proven to have the following deficiencies. First, the antibodies of IGF-1 produced by various manufacturers are not completely consistent with the affinity of the target protein, thus it is difficult to form a unified reference range.[1] Second, IGF-1 can bind to insulin-like growth factor binding protein 3 (IGFBP3) in the blood, and the interference from various manufacturers' immunoassays for IGFBP3 is also different.[2] Third, recent studies based on mass spectrometry have shown that about 0.6% of people in the sample population have protein single-point mutations,[3] in which case, immunoassays usually cannot distinguish the mutations causing the mismeasurement of protein level in serum.

At present, analytical methods based on mass spectrometry have been employed by increasing numbers of hospital laboratory departments and clinical laboratories. Mass spectrometry has excellent sensitivity and specificity, becoming the preferred detection method in clinical research fields, such as screening of inborn errors of metabolism and quantification of vitamin D in human serum. Currently, an LC-MS method mainly relies on a triple quadrupole mass spectrometer for the scanning method of selected reaction monitoring (SRM). This scanning method has strong quantitative

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power for many small-molecule compounds, peptides, and proteins. In previous studies, some laboratories piloted a triple quadrupole-based SRM method to measure IGF-1, but so far, few clinical laboratories have actually adopted triple quadrupole technology to routinely quanitfy IGF-1.[4,5] This is mainly because the three pairs of disulfide bonds in IGF-1 are very close to their N- end and C- end, so it is difficult to fragment and produce highly specific fragment ions to quantify the molecule. Since 2011, large third-party independent medical laboratories in the United States have been exploring the possibility of using high-resolution accurate-mass (HRAM) mass spectrometry to quantify IGF-1[3,6] and have proven its reliability for clinical research. In this note, an analytical method was developed by using Orbitrap-powered HRAM mass spectrometry with a UHPLC system to quantify IGF-1 in human serum.

Experimental

Sample pretreatment

To eliminate the interference of IGFBP3 on insulin-like growth factor in serum, the acidic ethanol precipitation method was used, and matrix interference was further reduced[7] by cyroprecipitate after neutralization. The experimental results show that the pretreatment method can significantly reduce matrix interference and achieve the recovery rate of IGF-1 at 85%–90%.

Liquid phase system online SPE

After two-step precipitation by acidic ethanol and neutralization pre-cooling, the sample solution contained a large amount of inorganic salts. To make the sample suitable for mass spectrometric analysis, further purification of the sample was required. When using offline solid phase extraction (offline SPE), both the target compound (human insulin-like growth factor 1) and the internal standard compound (rat insulin-like growth factor 1) were found to have non-negligible oxidation (Figure 1). This is probably because the dryness of the column bed catalyzed the oxidation of methionine in IGF-1 during offline SPE. Also, this oxidation was continuously intensified with the extension of the SPE time (data not shown), which adversely affected the accuracy of quantification.

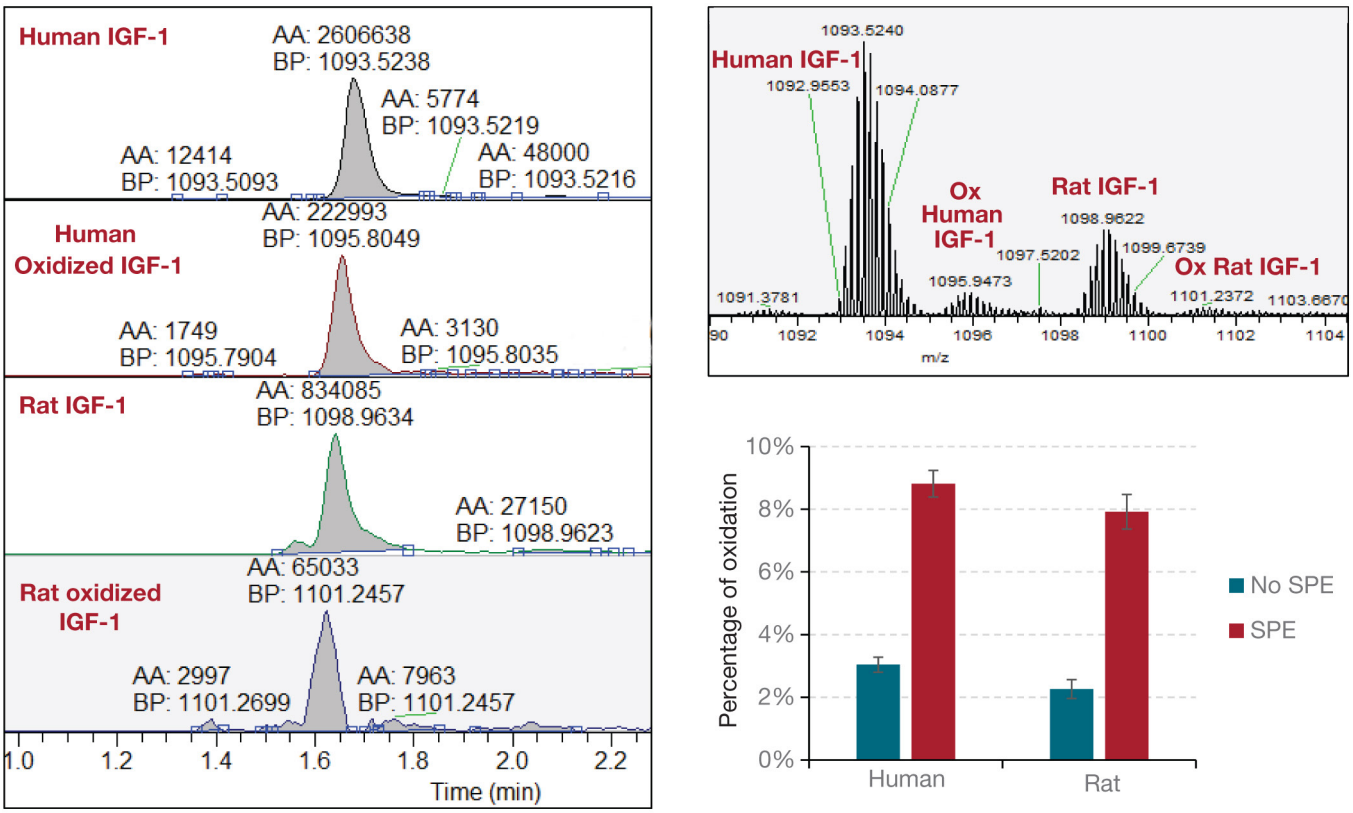


Figure 1. IGF-1 from both human and rat experienced some degree of oxidation after offline SPE

To overcome the problem of IGF-1 oxidation, an online SPE method was developed in which the target and internal standard compounds are always protected by the solution environment, effectively eliminating oxidation of methionine (data not shown). The liquid phase flow path of online SPE is shown in Figure 2. The whole analysis process is mainly divided into four parts:

1. Sample loading to the SPE column
2. Quickly filling the tubing before the extraction column with elution organic phase
3. Eluting the analyte from the extraction column, and loading to the analytical column after online dilution
4. Eluting gradient on the elution pump, and eluting the analyte from the analytical column into mass spectrometry for detection and quantification

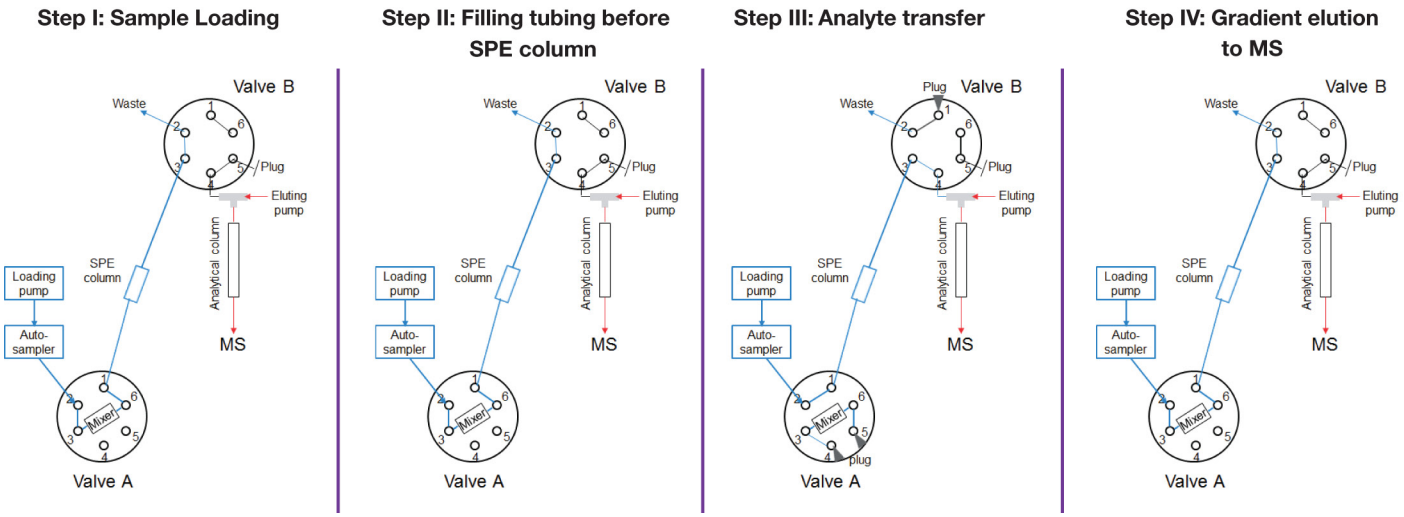
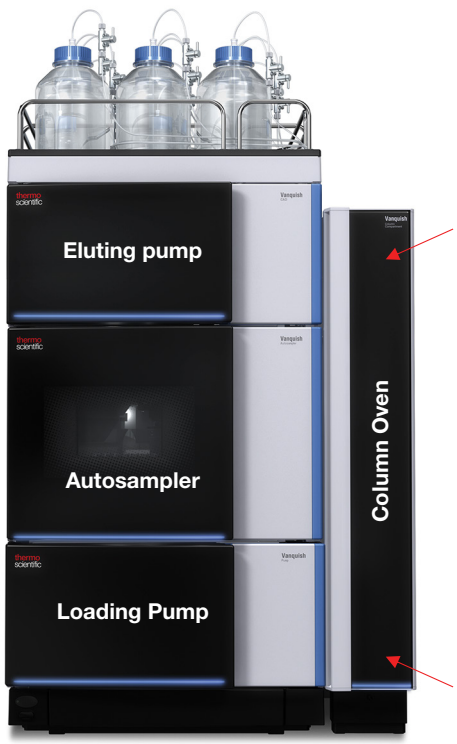


Figure 2. Online SPE flow path of liquid phase system (Focus mode)



Valve B

- **Thermo Scientific™ Vanquish™ Binary Flex UHPLC system w/o Detector (P/N 5400.0225)**
- **Quaternary Pump F (P/N VF-P20-A)**
- **Valve 2-p 6-p 150 MPa bio VH-C * 2 (P/N 6036.1560)**
- **Mixing system 100 µL, for Vanquish * 2 (P/N 6044.5100)**
- **Sample loop volume 100 µL, for Vanquish (P/N 6850.1913)**
- **nanoViper tubing**
- **Inline mixer**
- **Tee for mixing (PEEK or SST)**

Valve A

Figure 3. Preparation of online SPE liquid phase system instruments

Mass spectrometry conditions

Table 1. Mass spectrometry analysis conditions for IGF-1

Parameter	Value
Source parameters	
Sheath gas	50 Arb
Aux gas	15 Arb
Sweep gas	1 Arb
Spray voltage	3.8 kV
Capillary temp.	320 °C
Probe heater temp.	300 °C
S-lens RF	60
Probe height	B
Ion channels	
Human IGF-1 quantitative ion	1093.520
Human IGF-1 qualitative ion 1	1093.378
Human IGF-1 qualitative ion 2	1093.664
Internal standard ion (oxidized rat IGF-1)	1101.248
Mass spectrometry parameters	
SIM scan resolution	70 K
Isolation window	36 Da
Central mass	1091.5
AGC target	1e5
MaxIT	200 ms
MicroScan	1
Data type	Profile
Software	
Data acquisition	Thermo Scientific™ Xcalibur™ software
Quantitative analysis	Thermo Scientific™ TraceFinder™ software, version 4.1
XIC mass window	15 ppm

Results and discussion

Perform quantification and qualification by high-resolution MS1

The ultra-high resolution of Orbitrap mas spectrometry allows a very accurate parent-ion *m/z* of IGF-1 to be obtained (Figure 4). In the end, the 7-valent monoisotopic peak of 1093.520 was chosen as the quantitative ion, and two adjacent monoisotopic peaks (1093.378, 1093.664) as the qualitative ions. A resolution of 70,000 FWHM was used (Table 1), while setting the allowable mass deviation window to 15 ppm. In the detection of donor serum samples, no interference from the matrix was found (data not shown).

Linear range, accuracy, and precision

Previous data show that the reference range for insulin-like growth factors in the human population is 14–800 ng/mL (quantified by high-resolution mass spectrometry), and this value is related to age and gender. Therefore, the range of standard curve was set as 15.6–2,000 ng/mL, and lower limit of quantification (LLOQ) as 10 ng/mL. It can be seen from Table 2 and Table 3 that the standard curve shows excellent linearity (Figure 5A), and deviations between all the points on the standard curve and the actual values are less than 10%. In the samples of the matrix spiked standard, the coefficient of variation for the internal standard is less than 5% (Table 2). Even if the donor samples are considered, the coefficient of variation for the internal standard intensity is also within 10% (data not shown), which indicates that: 1) the instrument system is highly robust, and 2) the method does not have significant matrix effect.

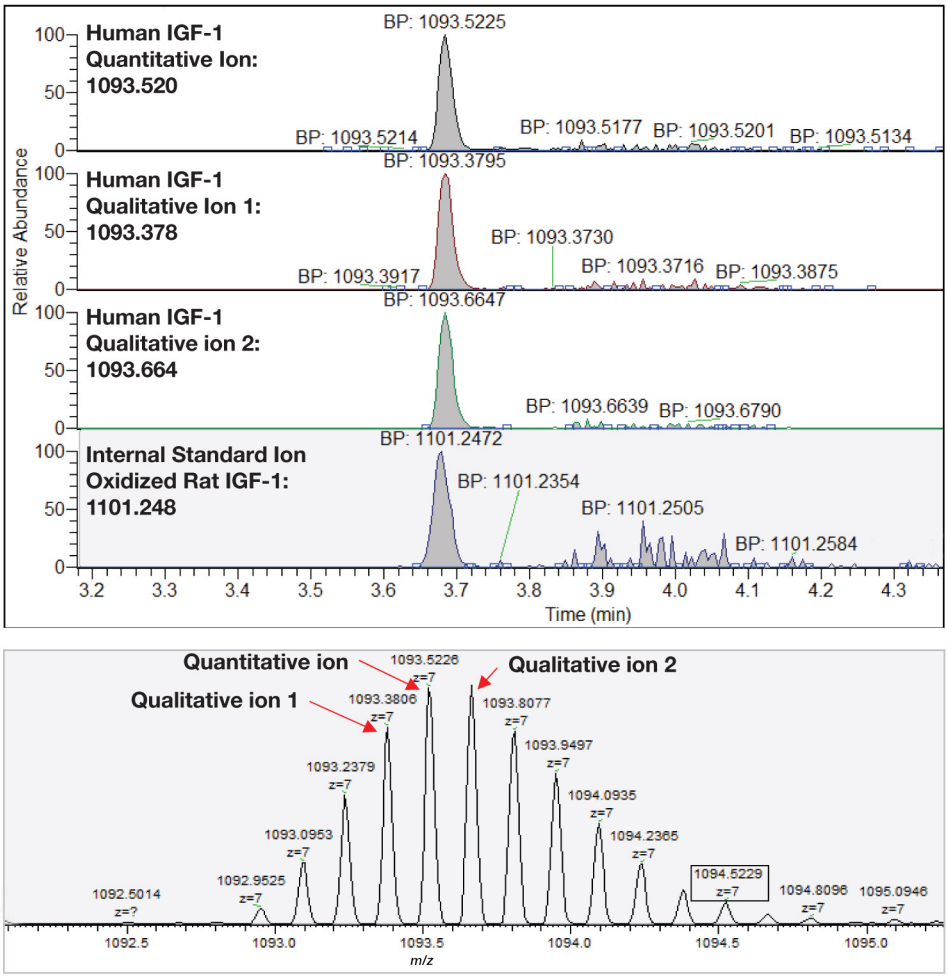


Figure 4. Chromatography mass spectra for insulin-like growth factor quantitative, qualitative, and internal standard ions

Table 2. Standard curve range and r^2

Analyte	Linearity range	r^2 linear fit, 1/x weight	LLOQ	Internal standard CV%
Human IGF-1	15.6–2000 ng/mL	0.9996	10 ng/mL	3.96

Table 3. Recovery rate at each point of the standard curve

	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Concentration (ng/mL)	15.625	31.25	62.5	125	250	500	1000	2000
Recovery rate (%)	93.64	99.14	105.69	102.27	97.97	99.87	102.76	98.65

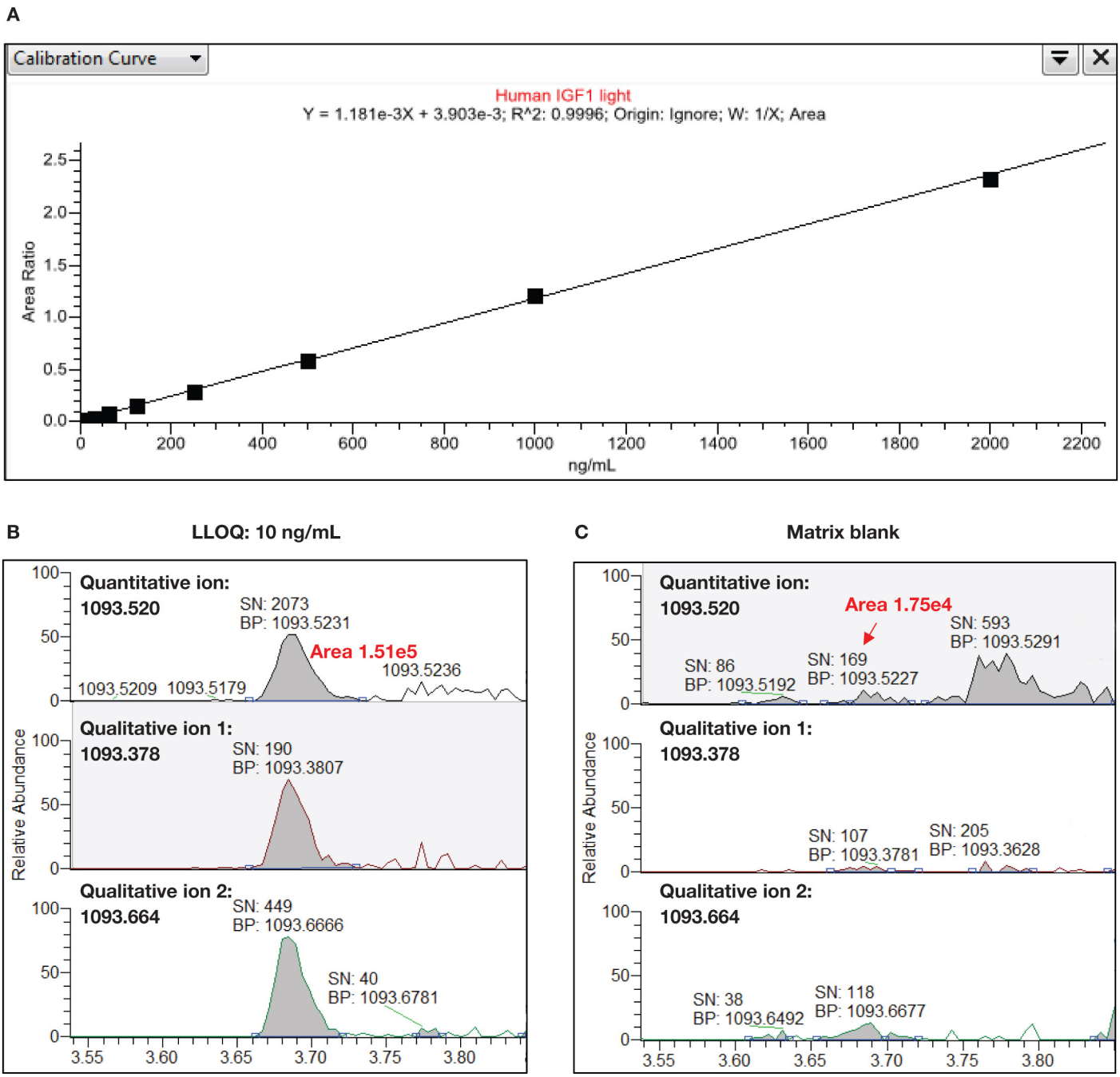


Figure 5. Standard curve and LLOQ and matrix blank chromatograms

It can be seen from Figure 5B that the quantitative and qualitative ions at the LLOQ point (10 ng/mL) still present a good chromatographic peak shape and an excellent signal-to-noise ratio. In fact, considering that the lower limit of the reference range is 14 ng/mL, the quantitative performance was only examined with LLOQ of 10 ng/mL, and there is no pressure to continue to do so at a lower concentration. Immediately after running the sample of upper limit of quantification (ULOQ), the matrix blank samples were loaded and found to have a residue of about 10% of LLOQ, which satisfactorily met requirements of clinical research laboratories (Figure 5C). Because there is interference from a certain amount of target compound quantitative ion in blank serum and the internal standard, if the stable isotope-labeled insulin-like growth factor 1 is used as an internal standard, interference from the target compound in matrix blank will be further reduced.

Biocompatibility of liquid chromatography tubing systems

For peptide or protein analytes, biocompatibility of the liquid chromatography system is a problem that must be investigated; otherwise it may affect chromatographic peak shape and carryover effects. It can be seen from the results of Figure 6 that Thermo Scientific™ nanoViper™ tubing provides outstanding biocompatibility, not only obtaining good chromatographic peak shape, but also minimizing residue. In addition, compared to the most frequently used PEEK material tubing, the fingertight nanoViper tubing withstands up to 1200 bar of pressure and is seamlessly compatible with ultra-high-performance liquid chromatography, truly enabling uncompromising analysis of biomacromolecules.

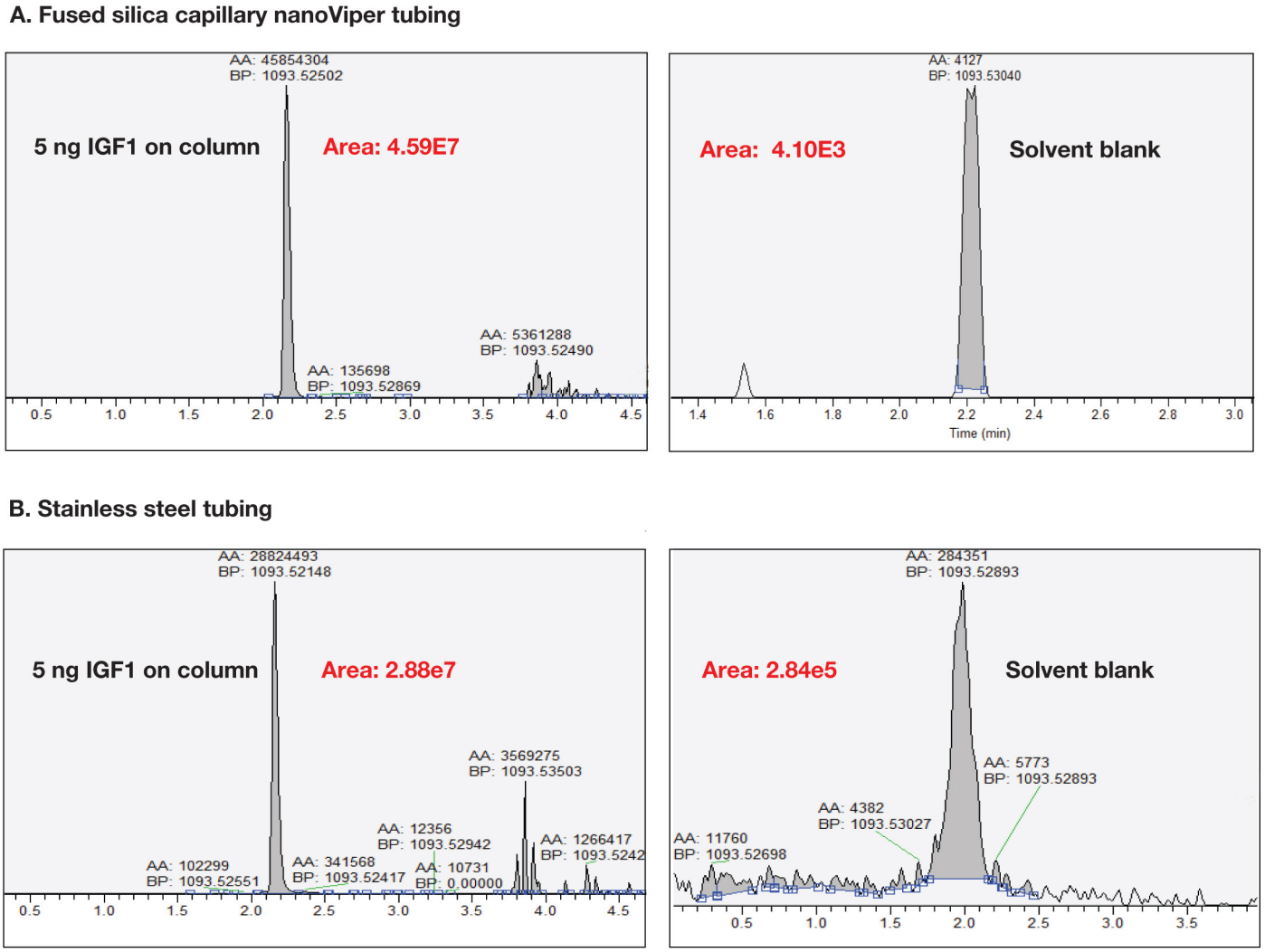


Figure 6. Residues of insulin-like growth factor 1 in different LC tubing systems

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Conclusion

In this note, an analytical method based on Thermo Scientific™ Vanquish™ UHPLC and Thermo Scientific™ Q Exactive™ Focus HRAM mass spectrometry was developed to quantify IGF-1 in human serum. The method uses two-step protein precipitation and online SPE to extract the sample, and finally obtains the results that satisfy the needs of clinical research laboratories. It takes 5 minutes to analyze each sample with this method, but the time window of the peak for the target and the internal standard compounds is actually less than 20 seconds. In subsequent work, a method was developed on the Thermo Scientific™ Transcend™ II TLX-4 four-channel online pre-treatment liquid chromatography system to increase the laboratory throughput by a factor of 4; that is, one minute and a half for testing one sample.

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