

A High Throughput and High Resolution LC-MS/MS Method to Measure IGF1 in Serum for Clinical Research

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ABSTRACT

Purpose: To develop a high throughput and high resolution LC-MS/MS method to quantify insulin-like growth factor 1 (IGF1) in serum for clinical research on a four-channel HPLC with online SPE and a high-resolution accurate-mass (HRAM) mass spectrometer (MS) powered by Orbitrap™ technology.

Methods: Serum samples were precipitated by adding acidified ethanol. After a second step precipitation by tris base, supernatant was diluted and injected onto an online sample clean-up column. LC-MS/MS was done on a four-channel HPLC system (Thermo Scientific™ Transcend™ II TLX-4) coupled with a high resolution mass spectrometer, Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ MS. Intact protein analysis under high resolution was performed for quantitation and isotope ion ratio was used for confirmation.

Results: High resolution LC-MS/MS can accurately quantify IGF1 in human serum with high specificity and selectivity. Our method was able to maintain the integrity of IGF1 protein by performing online sample clean-up. LLOQ was 7 ng/mL with ion ratio confirmation. The intra and inter-assay precision was good with CV less than 10%. Recovery was between 85% to 115%. By using a four-channel HPLC coupled with a HRAM mass spectrometer, we could analyze ~48 samples per hour.

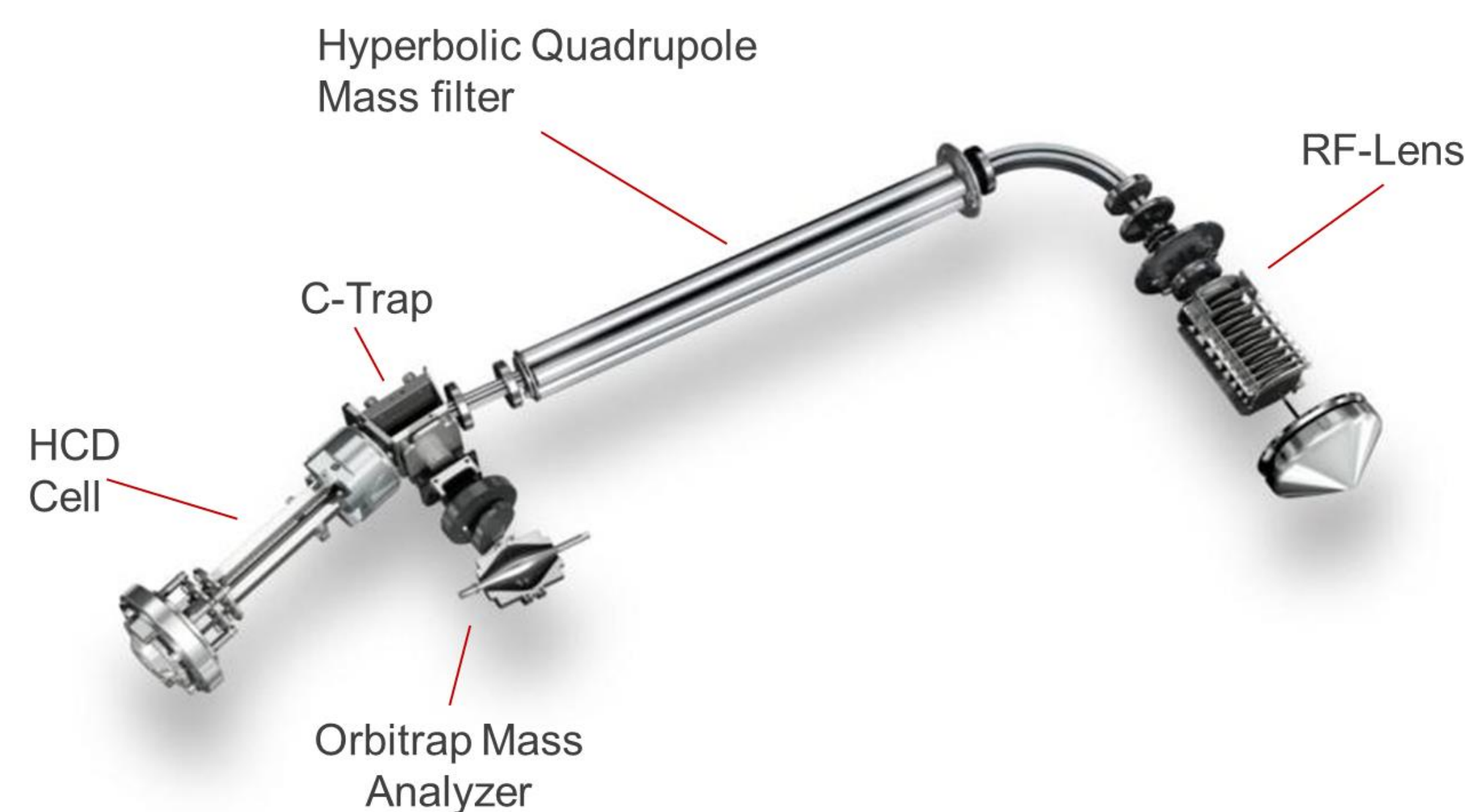
Figure 1. Transcend II TLX-4 and Q Exactive Focus LC-MS/MS system



INTRODUCTION

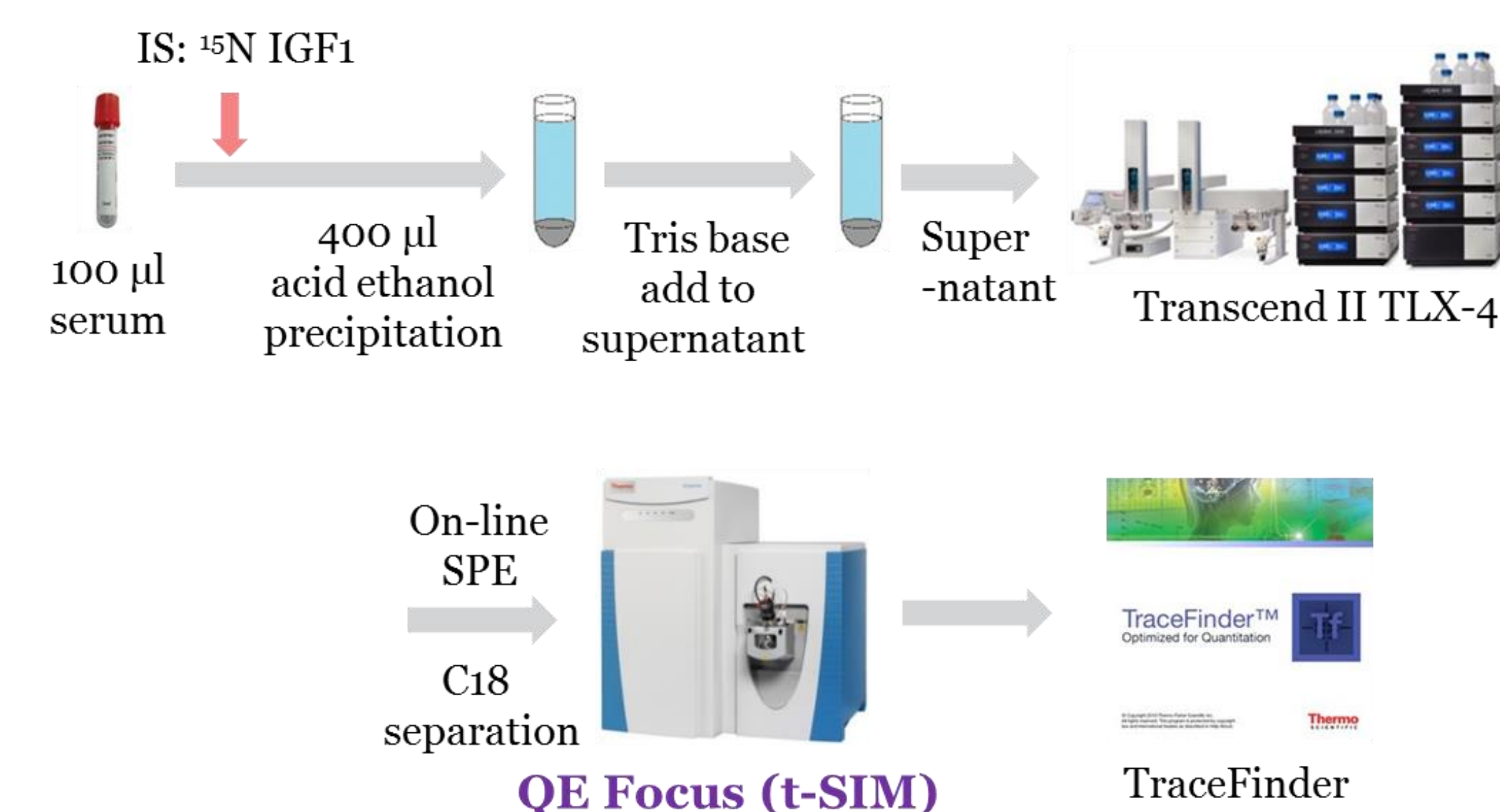
IGF1 is a 70-amino acid polypeptide (molecular weight 7.6kDa). IGF1 is the major mediator of the anabolic and growth-promoting effects of growth hormone (GH). Low IGF1 level is observed in GH deficiency or GH resistance. If acquired in childhood, these conditions result in short stature. Several immunoassays such as RIA, ICMA, or ECL have been developed to measure IGF1 level in serum. However, due to cross-reactivity, immunoassays result high values in some patients. LC-MS/MS removes cross-activity by direct measurement of IGF1 thus does not produce high results seen in immunoassays.

Figure 2. Schematic design view of Q Exactive Focus MS, which allows for small and large molecule analysis in high resolution (up to 70,000 FWHM)



MATERIALS AND METHODS

Figure 3. From sample to knowledge IGF1 method



Why HRAM MS

- IGF1 has 3 disulfide bonds, close to N- and C- terminal, not easy to generate fragmental ion using CID on QqQ
- Be able to monitor IGF1 SNP which generate loss-of-function IGF1

Why online SPE

- Offline SPE is prone to Met oxidation

Figure 4. Transcend II HPLC online SPE method

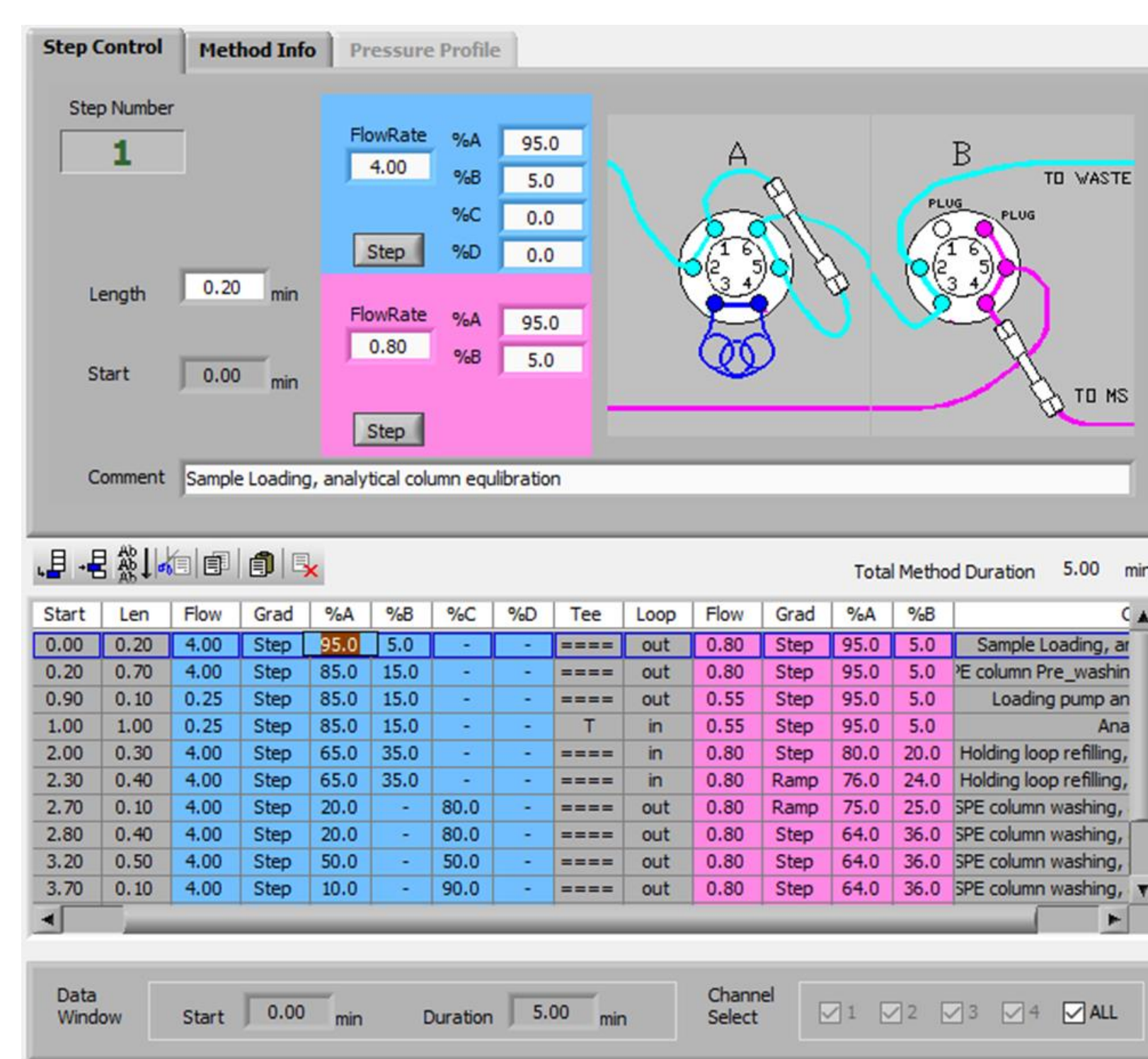
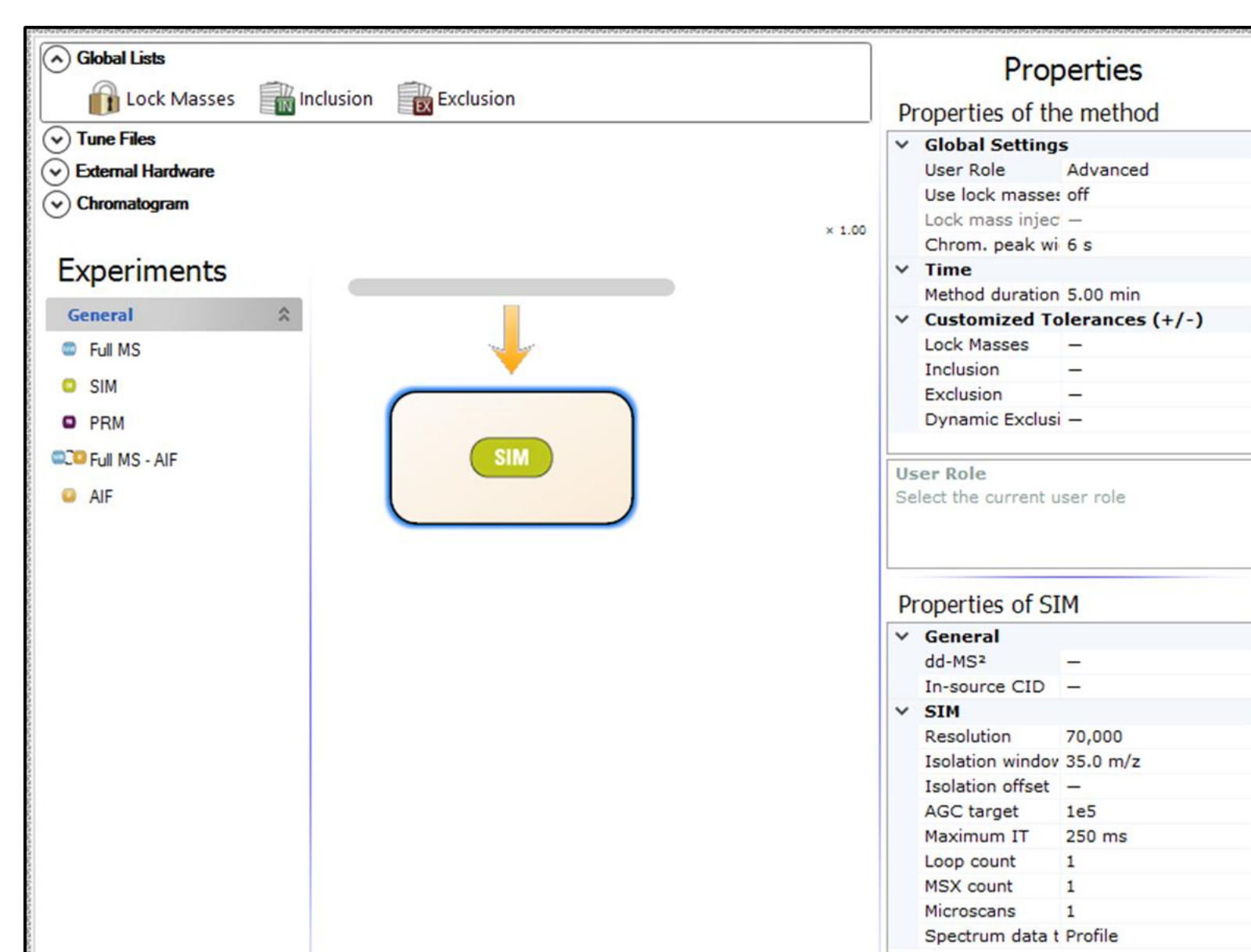


Figure 5. QE Focus MS method



RESULTS

Figure 6. Four-channel HPLC maximizes the productivity of MS

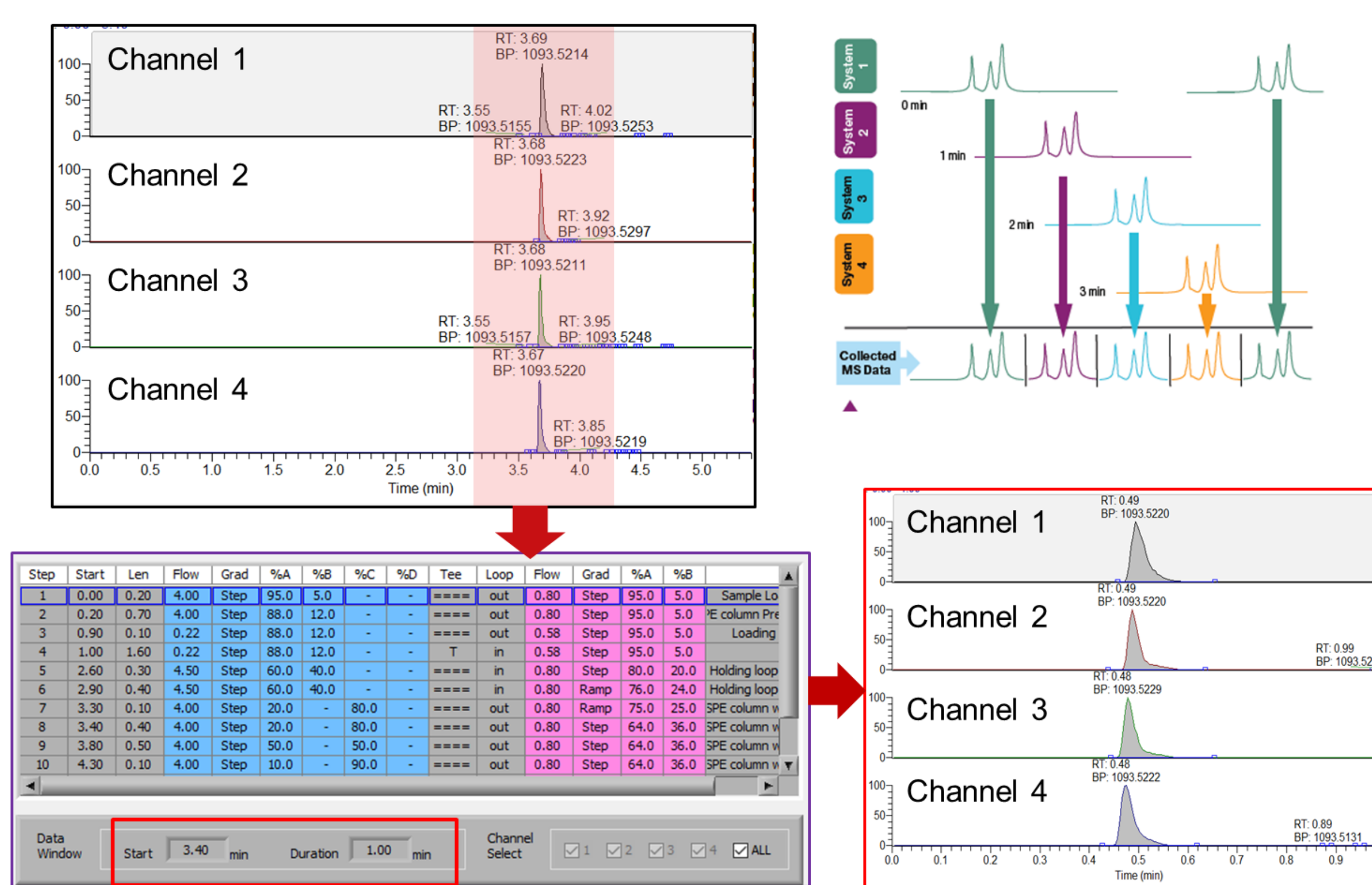


Figure 7. Calibration curve with good linearity (R²>0.999)

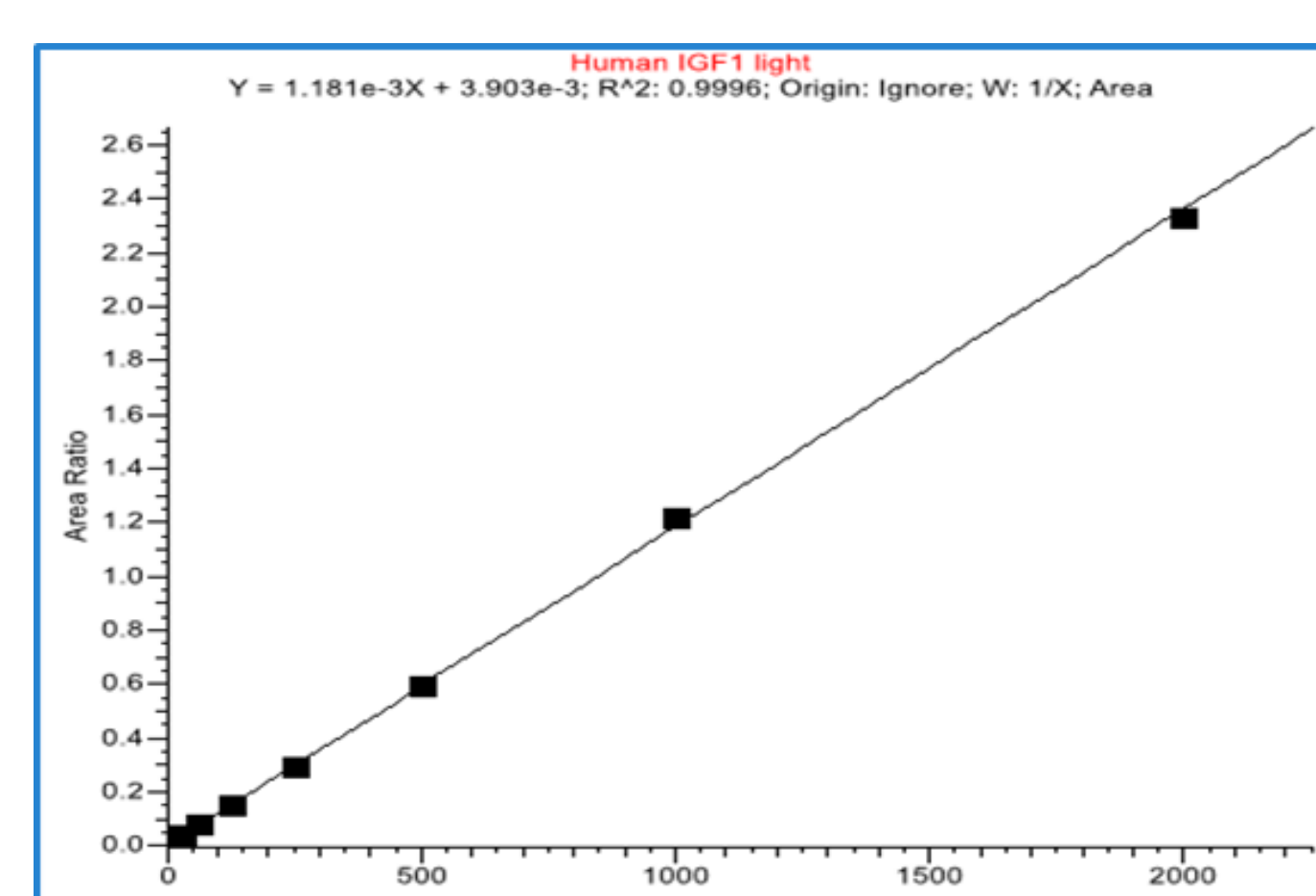


Figure 8. LLOQ: 7 ng/mL with ion ratio confirmation. Quantitation range: 7-2000 ng/mL

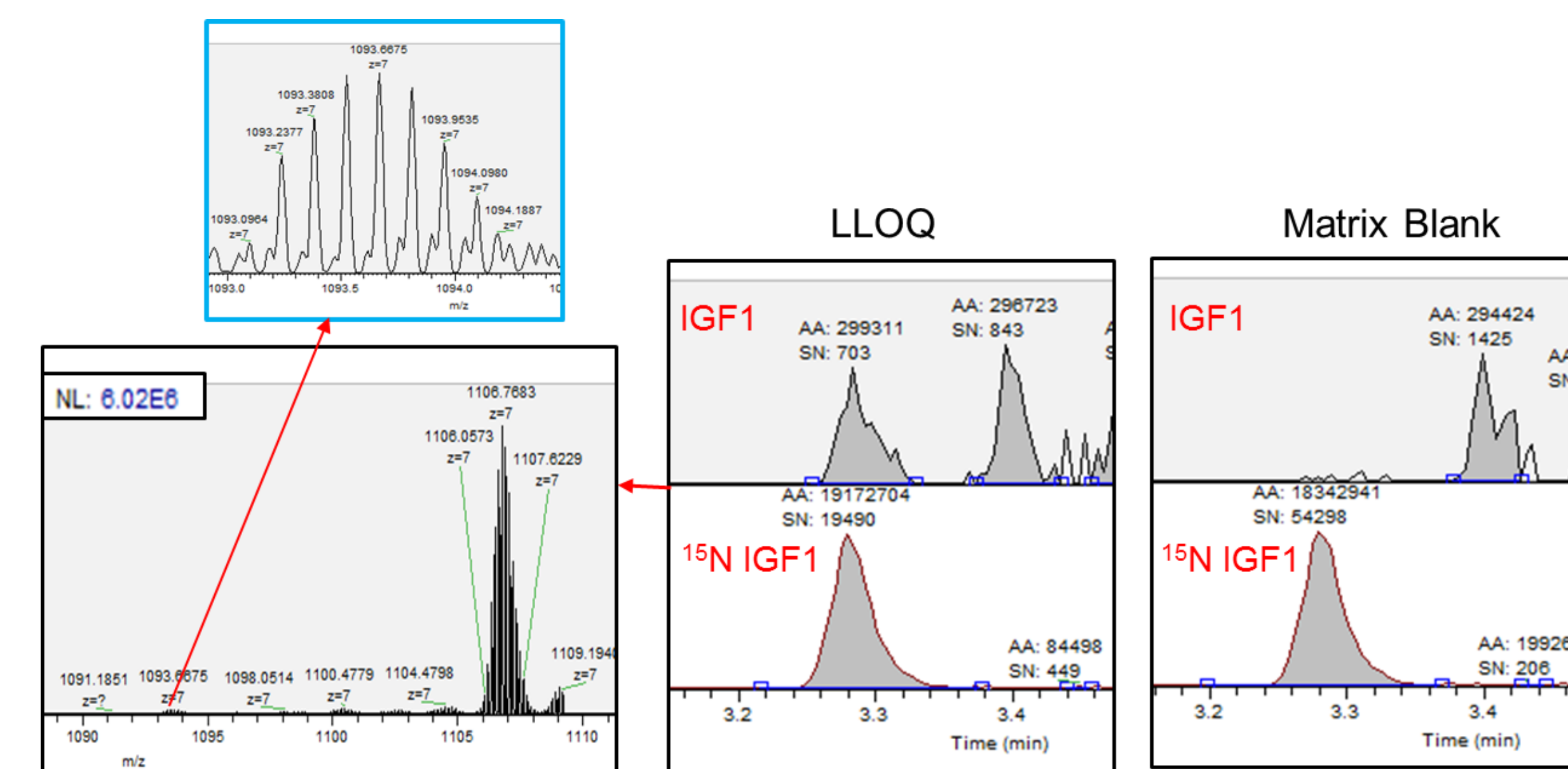
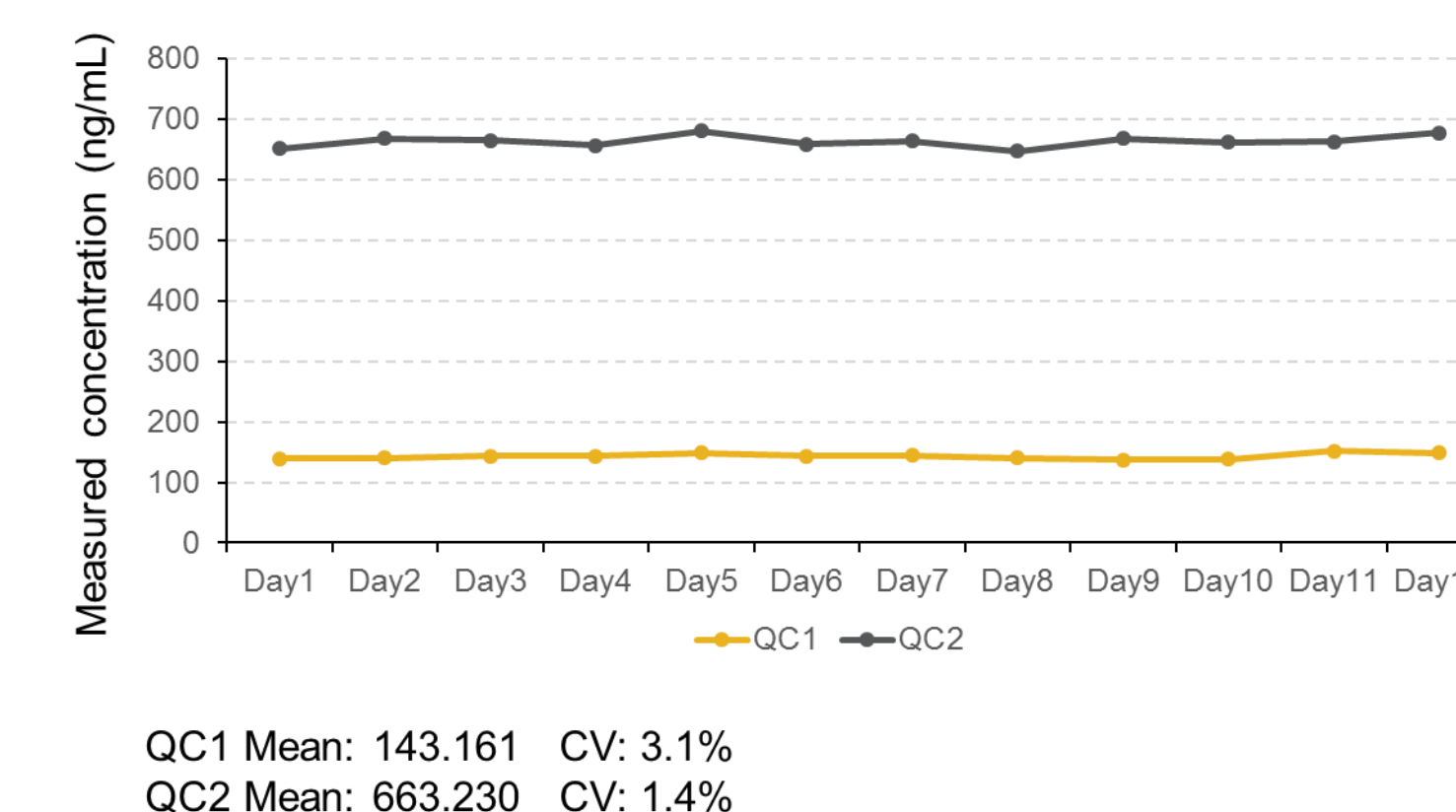


Figure 9. Intra-assay precision (CV<11%). Recovery between 85% to 115%

No.	Patient sample (ng/mL)	No.	pool-L (ng/mL)	pool-H (ng/mL)
Rep1	39.04	Rep1	54.88	124.67
Rep2	29.27	Rep2	79.81	122.43
Rep3	31.94	Rep3	61.68	117.96
Rep4	34.73	Rep4	58.32	116.79
Rep5	37.70	Rep5	58.31	117.55
Rep6	33.96	Rep6	56.44	119.06
Rep7	34.98	Rep7	60.17	126.31
Rep8	32.37	Rep8	57.70	122.79
Rep9	34.69	Rep9	60.51	124.74
Rep10	31.44	Rep10	58.34	125.81
Rep11	33.53	Rep11	59.50	122.40
Rep12	35.33	Rep12	62.91	131.09
Mean	34.08	Mean	60.71	122.63
SD	2.67	SD	6.40	4.25
CV	7.85	CV	10.54	3.46

Figure 10. Inter-assay precision (CV<4%)



CONCLUSIONS

- Sample preparation
 - Simple and reliable, just two steps of protein precipitation, no speedvac needed
- Online SPE
 - The focus mode on Transcend II TLX system enables easy and reliable online SPE method
- Throughput
 - Transcend II TLX-4 enables four-channel online sample prep, analyzing one sample in 1.25min
- Mass Spec
 - QE Focus MS offers high selectivity and sensitivity
- Economical efficiency
 - Low cost for daily run, the consumables are majorly internal standard, SPE column and analytical column

TRADEMARKS/LICENSING

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