

Clinical research



Robust peptide quantitation using the TSQ Certis triple quadrupole mass spectrometer and OptiSpray ion source and cartridges

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Keywords

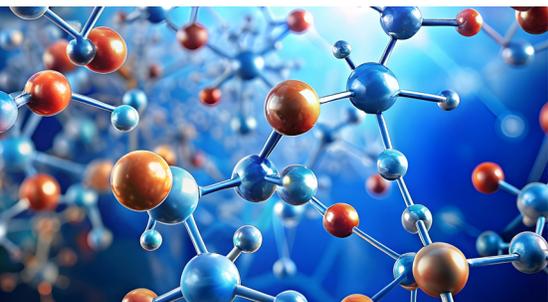
TSQ Certis mass spectrometer, Vanquish Neo UHPLC, OptiSpray ion source and cartridge, automation, peptides, biomarkers, human plasma, sensitivity, robustness

Application benefit

The newly introduced Thermo Scientific™ TSQ Certis™ Triple Quadrupole Mass Spectrometer delivers exceptional speed, reproducibility, and quantitative precision for targeted peptides and biomarkers. Its enhanced duty cycle and advanced ion optics enable fast acquisition speeds, as well as robust peptide quantitation across in a highly multiplexed assay. The Thermo Scientific™ OptiSpray™ Ion Source and Cartridge Columns improve precision and reproducibility for targeted workflows and make capillary-flow workflows easy to operate with an automated routine for optimized emitter positioning and integrated sheath gas. Additionally, OptiSpray technology used in combination with the TSQ Certis MS improves workflow operability, making it easier to acquire data for routine users and over large cohorts. Together, they are an ideal solution for translational research and peptide biomarker applications, which require confident and consistent results.

Goal

The goal of this work was to demonstrate the quantitative performance of targeted peptide analysis on the next-generation TSQ Certis triple quadrupole mass spectrometer with a focus on operability. The TSQ Certis MS is a high-speed, highly sensitive platform equipped with the OptiSpray ion source and cartridge columns, which provide an intelligent electrospray ionization (ESI) interface optimized for nano- and capillary flow applications (<5 $\mu\text{L}/\text{min}$). The study assessed reproducibility, retention time stability, and sensitivity for a multiplexed assay comprising 82 peptides representing 57 plasma proteins, including >20 recognized biomarkers, to illustrate increased functionality, efficiency, and manageability for users and operators, which is especially needed for large cohorts.



Introduction

Precise and accurate peptide quantitation is critical to advancing proteomics, biomarker discovery, and biotherapeutic development. Triple quadrupole mass spectrometers remain the gold standard for targeted peptide quantitation due to their superior sensitivity, selectivity, and quantitative precision. As peptide biomarker workflows increasingly demand higher throughput and greater robustness, innovations in acquisition speed and ion transmission efficiency have become essential. Thus, operability of the workflow becomes central where ease, efficiency, and manageability are key.

The TSQ Certis MS is the next evolution in Thermo Scientific™ triple quadrupole mass spectrometers, designed to deliver faster acquisition with uncompromised performance. The OptiSpray ion source offers simplified operation for both expert and routine users while delivering consistent ionization performance across instruments and analytical runs. This technical note presents a comprehensive evaluation of the TSQ Certis MS equipped with the OptiSpray ion source and column cartridges, highlighting its quantitative performance for targeted peptide analysis.

To evaluate the TSQ Certis MS with the OptiSpray ion source and column cartridges, we adapted a previously described Health Surveillance Panel (HSP) assay^{1,2} consisting of 82 peptides from 57 proteins, which was originally developed to quantify biomarkers associated with various diseases such as cardiovascular, inflammatory, and metabolic conditions and includes 24 well-recognized markers in the field. In this work, key performance characteristics—including reproducibility, retention time stability, and sensitivity—were determined using this multiplexed HSP assay.

Together, these results demonstrate the operability of the next-generation TSQ Certis MS with the OptiSpray ion source and cartridge columns to enable faster, more reliable, and higher-throughput peptide quantitation, supporting both research and applied targeted biomarkers laboratories in achieving consistent, high-quality results.

Experimental

Reagents

- Thermo Scientific™ Human plasma digest (Cat. No. NCI4852)
- Stable isotope–labeled (SIL) peptides, New England Peptide (Biosynth Gardner, MA, USA)
- Thermo Scientific™ Water, HPLC–MS grade (Cat. No. W8-1)
- Fisher Chemical™ Formic acid, Optima™ LC-MS grade, 99.0+% (Cat. No. A117-50)

- Thermo Scientific™ Acetonitrile, UHPLC–MS grade (Cat. No. A956-1)
- Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (PRTC) (Cat. No. 88321)

Calibration standards

Calibration and linearity experiments were conducted at a throughput of 100 samples per day (SPD) to evaluate instrument performance under high-throughput conditions. An 11-point SIL peptide calibration curve was generated, spanning load levels from 0 to 120 fmol on-column (0, 0.0061, 0.0183, 0.0548, 0.16, 0.49, 1.48, 4.4, 13.3, 40, 120 fmol). Each sample was prepared in a constant matrix containing 50 ng of digested control plasma and 50 fmol PRTC to maintain consistent background complexity. For each concentration point, six replicate injections were analyzed to assess reproducibility and quantitative precision.

Liquid chromatography-mass spectrometry

A Thermo Scientific™ Vanquish™ Neo UHPLC System was coupled to the next-generation TSQ Certis MS equipped with the OptiSpray ion source for data acquisition. Ionization was performed using the OptiSpray ion source fitted with a Thermo Scientific™ OptiSpray™ PepMap™ Neo 150 µm × 15 cm Cartridge with a tapered emitter (Cat. No. OS150150PN). Source parameters are summarized in Table 1. SIL-spiked plasma digests were analyzed in a trap-and-elute configuration with a total run time of 13 minutes (100 SPD throughput). The Thermo Scientific™ PepMap™ Neo Trap Cartridge (300 µm × 5 mm, Cat. No. 174500) was used for preconcentration and desalting. Mobile phases consisted of Solvent A: 0.1% formic acid in water; Solvent B: 0.1% formic acid in 80% acetonitrile. The LC gradient and flow rate conditions are detailed in Table 2. Thermo Scientific™ Xcalibur™ Software was used to acquire data on the TSQ Certis MS with SRM transitions of the target peptides listed in Table 3. Raw files and the LC-MS method are available for download from the [Thermo Scientific™ AppsLab Library](#).

Table 1. OptiSpray ion source parameters.

Ion source parameter	Set value
Positive ion voltage (V)	1,800
Sheath gas (arb)	10
Sweep gas (arb)	0
Ion transfer tube temperature (°C)	250
Cartridge temperature (°C)	45

Table 2. LC gradient conditions.

Time (min)	Flow rate ($\mu\text{L}/\text{min}$)	%A	%B
0.0	1.8	99	1
0.7	1.8	96	4
1.0	1.8	92	8
7.7	1.8	77.5	22.5
11.4	1.8	65	35
11.8	2.5	45	55
12.3	2.5	1	99
13.0	2.5	1	99
End	—	—	—

Data analysis

Following SRM data acquisition, the raw files were imported into Skyline-daily software (version 25.1.1.271, MacCoss Lab Software) for automated peak detection, integration, and visualization. The area under the curve (AUC) was determined for each peptide to quantify signal intensity. Limits of detection (LOD) and quantification (LOQ) were established individually for each peptide. In addition, the raw data files were processed using Thermo Scientific™ TraceFinder™ Software (TF, version 5.2 SP3) for automated peak detection, integration, and data review. The AUC was calculated for each peptide transition to quantify signal intensity. Skyline-daily software includes a built-in TF Export tool that enables seamless data export to TraceFinder software.

Results and discussion

The performance was evaluated using an 11-point stable isotope-labeled (SIL) peptide dilution curve spiked into a pooled human plasma digest. Quantitative performance was benchmarked by assessing points per peak, peak area, and relative standard deviation (RSD) for all 82 peptides across six replicate injections (Figure 1). For 1,080 transitions acquired, the number of data points acquired across chromatographic peaks ranged from 8 to 30 [$>96\%$ transitions (1,036 transitions) have data points >8 across the peak] with a median of 16, thereby exceeding standard practice for accurate quantification and precision. The average RSD of peak areas in six injections was 3.2%, with values ranging from 0.77% to 12.9%. Notably, $>98\%$ peptides (81 of 82) exhibited RSDs below 10%, demonstrating excellent reproducibility of signal intensity and overall method robustness.

Subsequently, the sensitivity, linearity, lower limit of detection (LLOD), and lower limit of quantification (LLOQ) for each of the 82 peptides were determined using a 11-point serial dilution curve with the 100 SPD method (Table 3). The median sensitivity observed across methods was in the sub-femtomole range (LLOD = 0.019 fmol; LLOQ = 0.164 fmol). Evaluation of the quantitative performance demonstrated excellent linearity for $>99\%$ of the peptides, exhibiting coefficients of determination (R^2) greater than 0.99 across the dilution series.

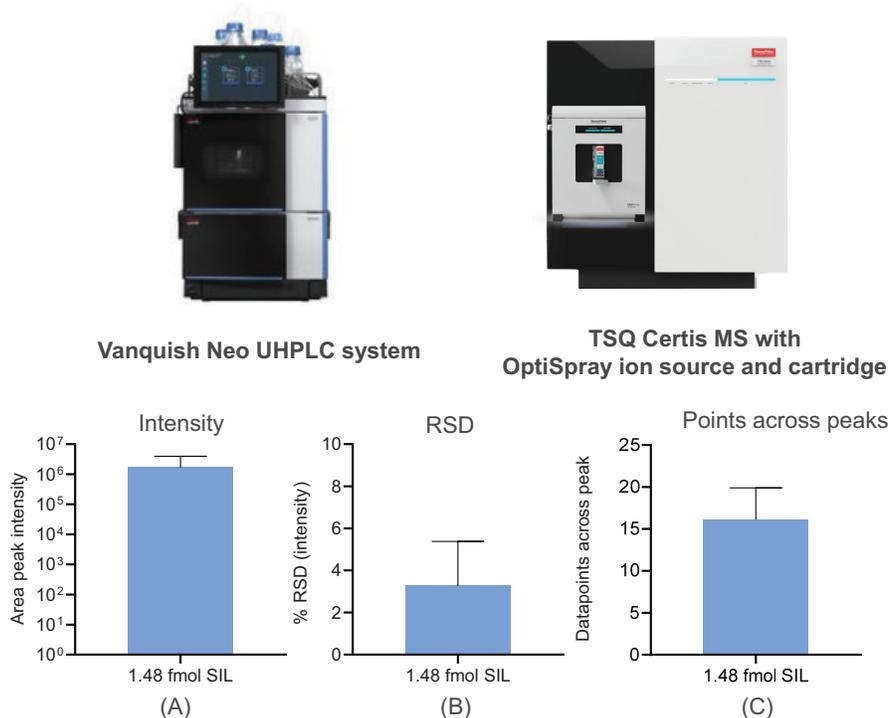


Figure 1. Technical evaluation with repeated injections. The performance of 100 sample per day (SPD) methods was evaluated using 1.48 fmol of the 82 SIL peptides spiked into 50 ng of digested human plasma used as the matrix ($n=6$ technical), and Skyline software was used to process the data. (A) The median peak intensities. (B) The median % RSD of the signal intensities. (C) The median points cross-peak points.

The scheduled method for the HSP panel includes 1,080 transitions (57 HSP proteins plus the Pierce Peptide Retention Time standard) with a 2-minute retention window (Table 3). Under these conditions, the system consistently maintains short dwell times of approximately 1.3–2 ms and cycle durations of ~0.4–0.6 s for the majority of transitions. This high duty cycle efficiency enables the acquisition of 10–20 data points

per chromatographic peak, ensuring quantitative robustness even in highly multiplexed targeted workflows. Additionally, the adaptive increase in dwell time as transition density decreases demonstrates the instrument's dynamic and intelligent scheduling capability, ensuring optimal sensitivity across the chromatographic run.

Table 3. Summary of LODs, LOQs, R² values, and retention times obtained for each peptide. Data was processed by Skyline software.

UniProt ID	Protein name	Peptide sequence	SIL-C-terminal residue modification	Retention time (min)	LOD (fmol on column)	LOQ (fmol on column)	R ²
O14791	APOL1_HUMAN	VAQELEEK	¹³ C ₆ , ¹⁵ N ₂	3.15	0.0438	0.0549	0.9972
P00734	THRB_HUMAN	ELLESYIDGR	¹³ C ₆ , ¹⁵ N ₄	9.35	0.0250	1.4815	0.9996
P00736	C1R_HUMAN	YTTEIHK	¹³ C ₆ , ¹⁵ N ₂	4.92	0.0175	0.0183	0.9971
P00738	HPT_HUMAN	VGYSVSWGR	¹³ C ₆ , ¹⁵ N ₄	6.62	0.0238	0.1646	0.9979
P00738	HPT_HUMAN	VTSIQDWVQK	¹³ C ₆ , ¹⁵ N ₂	7.99	0.0182	0.4938	0.9989
P00739	HPTR_HUMAN	TEGDGVYTLNDK	¹³ C ₆ , ¹⁵ N ₂	5.47	0.0680	0.4938	0.9984
P00742	FA10_HUMAN	MLEVPYVDR	¹³ C ₆ , ¹⁵ N ₄	8.84	0.0141	1.4815	0.9988
P00747	PLMN_HUMAN	LFLEPTR	¹³ C ₆ , ¹⁵ N ₄	7.16	0.0119	0.1646	0.9965
P00747	PLMN_HUMAN	LSSPAVITDK	¹³ C ₆ , ¹⁵ N ₂	5.39	0.0906	0.1646	0.9965
P00748	FA12_HUMAN	VVGGLVLR	¹³ C ₆ , ¹⁵ N ₄	7.5	0.0170	0.0549	0.9959
P00748	FA12_HUMAN	EQPPSLTR	¹³ C ₆ , ¹⁵ N ₄	3.87	0.0392	0.1646	0.9977
P00751	CFAB_HUMAN	EELLPADIK	¹³ C ₆ , ¹⁵ N ₂	7.31	0.0434	0.1646	0.9968
P00751	CFAB_HUMAN	ALFVSEEEK	¹³ C ₆ , ¹⁵ N ₂	5.91	0.0268	0.0549	0.9969
P01008	ANT3_HUMAN	DDLYVSDAFHK	¹³ C ₆ , ¹⁵ N ₂	7.27	0.0934	0.4938	0.9980
P01008	ANT3_HUMAN	FATTFYQHLADSK	¹³ C ₆ , ¹⁵ N ₂	7.54	0.0331	4.4444	0.9992
P01009	A1AT_HUMAN	SVLQQLGITK	¹³ C ₆ , ¹⁵ N ₂	8.84	0.0169	0.4938	0.9975
P01009	A1AT_HUMAN	QINDYVEK	¹³ C ₆ , ¹⁵ N ₂	4.27	0.0374	0.1646	0.9983
P01011	AACT_HUMAN	ADLSGITGAR	¹³ C ₆ , ¹⁵ N ₄	5.64	0.0065	0.0183	0.9967
P01011	AACT_HUMAN	EIGELYLPK	¹³ C ₆ , ¹⁵ N ₂	8.89	0.0227	0.4938	0.9980
P01023	A2MG_HUMAN	SDIAPVAR	¹³ C ₆ , ¹⁵ N ₄	3.77	0.0079	0.0183	0.9973
P01023	A2MG_HUMAN	TEHPFTVEEFVLPK	¹³ C ₆ , ¹⁵ N ₂	10.1	0.0406	13.3330	0.9984
P01024	CO3_HUMAN	TGLQEVEVK	¹³ C ₆ , ¹⁵ N ₂	5.1	0.0084	0.0183	0.9973
P01024	CO3_HUMAN	ISLPESLK	¹³ C ₆ , ¹⁵ N ₂	7.37	0.0266	0.1646	0.9975
P01031	CO5_HUMAN	LQGTLPVEAR	¹³ C ₆ , ¹⁵ N ₄	5.87	0.0048	0.0061	0.9961
P01031	CO5_HUMAN	TDAPDLPEENQAR	¹³ C ₆ , ¹⁵ N ₄	5.26	0.2558	0.4938	0.9989
P01871	IGHM_HUMAN	GFPSVLR	¹³ C ₆ , ¹⁵ N ₄	7.26	0.0164	0.1646	0.9969
P01876	IGHA1_HUMAN	TPLTATLSK	¹³ C ₆ , ¹⁵ N ₂	5.34	0.0126	0.0549	0.9961
P01876	IGHA1_HUMAN	DASGVFTWTPSSGK	¹³ C ₆ , ¹⁵ N ₂	9.8	0.0166	1.4815	0.9992
P02647	APOA1_HUMAN	ATEHLSTLSEK	¹³ C ₆ , ¹⁵ N ₂	3.19	0.0125	0.1646	0.9931
P02649	APOE_HUMAN	LGPLVEQGR	¹³ C ₆ , ¹⁵ N ₄	5.42	0.0069	0.0549	0.9961
P02649	APOE_HUMAN	AATVGSAGQPLQER	¹³ C ₆ , ¹⁵ N ₄	6.77	0.0057	0.1646	0.9991
P02652	APOA2_HUMAN	SPELQAEAK	¹³ C ₆ , ¹⁵ N ₂	3.24	0.0199	0.0183	0.9976
P02655	APOC2_HUMAN	TYLPVAVDEK	¹³ C ₆ , ¹⁵ N ₂	6.03	0.0092	0.0183	0.9968
P02655	APOC2_HUMAN	TAAQNLYEK	¹³ C ₆ , ¹⁵ N ₂	3.56	0.0412	0.1646	0.9977
P02656	APOC3_HUMAN	DALSSVQESQVAQQAR	¹³ C ₆ , ¹⁵ N ₄	6.89	2.3678	4.4444	0.9987
P02671	FIBA_HUMAN	NSLFEYQK	¹³ C ₆ , ¹⁵ N ₂	6.36	0.0057	0.1646	0.9944
P02741	CRP_HUMAN	ESDTSYVSLK	¹³ C ₆ , ¹⁵ N ₂	5.61	0.0186	0.1646	0.9966
P02741	CRP_HUMAN	GYSIFSATK	¹³ C ₆ , ¹⁵ N ₂	9.07	0.0329	1.4815	0.9993

UniProt ID	Protein name	Peptide sequence	SIL-C-terminal residue modification	Retention time (min)	LOD (fmol on column)	LOQ (fmol on column)	R ²
P02748	CO9_HUMAN	TSNFNAAISLK	¹³ C ₆ , ¹⁵ N ₂	7.24	0.0514	0.1646	0.9986
P02748	CO9_HUMAN	LSPIYNLVPVK	¹³ C ₆ , ¹⁵ N ₂	10.48	0.0089	4.4444	0.9990
P02750	A2GL_HUMAN	ALGHLDLSGNR	¹³ C ₆ , ¹⁵ N ₄	5.18	0.0392	0.1646	0.9947
P02750	A2GL_HUMAN	DLLLQPDLR	¹³ C ₆ , ¹⁵ N ₄	10.77	0.0250	1.4815	0.9992
P02760	AMBP_HUMAN	ETLLQDFR	¹³ C ₆ , ¹⁵ N ₄	8.82	0.0281	0.4938	0.9976
P02763	A1AG1_HUMAN	SDVYTDWK	¹³ C ₆ , ¹⁵ N ₂	7.16	0.0510	0.0549	0.9976
P02765	FETUA_HUMAN	FSVYAK	¹³ C ₆ , ¹⁵ N ₂	5.8	0.0111	0.0183	0.9957
P02768	ALBU_HUMAN	LVNEVTEFAK	¹³ C ₆ , ¹⁵ N ₂	7.29	0.0164	0.1646	0.9969
P02768	ALBU_HUMAN	DLGEENFK	¹³ C ₆ , ¹⁵ N ₂	5.58	0.1156	0.0549	0.9979
P02774	VTDB_HUMAN	VLEPTLK	¹³ C ₆ , ¹⁵ N ₂	5.18	0.0100	0.0549	0.9925
P02787	TRFE_HUMAN	DGAGDVAFVK	¹³ C ₆ , ¹⁵ N ₂	6.51	0.0204	0.0183	0.9970
P02787	TRFE_HUMAN	SASDLTWDNLK	¹³ C ₆ , ¹⁵ N ₂	8.41	0.0289	0.0549	0.9993
P02790	HEMO_HUMAN	NFPSPVDAEFR	¹³ C ₆ , ¹⁵ N ₄	9.44	0.0183	1.4815	0.9993
P03952	KLKB1_HUMAN	YSPGGTPTAIK	¹³ C ₆ , ¹⁵ N ₂	4.42	0.0072	0.0549	0.9968
P04003	C4BPA_HUMAN	EDVYVGTVLR	¹³ C ₆ , ¹⁵ N ₄	9.6	0.0281	1.4815	0.9992
P04004	VTNC_HUMAN	FEDGVLDPDYPR	¹³ C ₆ , ¹⁵ N ₄	9.01	0.0263	1.4815	0.9996
P04114	APOB_HUMAN	TEVIPLIENR	¹³ C ₆ , ¹⁵ N ₄	9.23	0.0109	1.4815	0.9990
P04217	A1BG_HUMAN	NGVAQEPVHLDSPAIK	¹³ C ₆ , ¹⁵ N ₂	6.35	0.0209	0.1646	0.9932
P04217	A1BG_HUMAN	LLELTGPK	¹³ C ₆ , ¹⁵ N ₂	6.83	0.0122	0.1646	0.9969
P04275	VWF_HUMAN	ILAGPAGDSNVVK	¹³ C ₆ , ¹⁵ N ₂	5.76	0.0071	0.0549	0.9961
P04278	SHBG_HUMAN	VVLSQGSK	¹³ C ₆ , ¹⁵ N ₂	3.14	0.0154	0.0549	0.9960
P05155	IC1_HUMAN	FQPTLLTLPR	¹³ C ₆ , ¹⁵ N ₄	10.43	0.0115	4.4444	0.9995
P05546	HEP2_HUMAN	TLEAQLTPR	¹³ C ₆ , ¹⁵ N ₄	5.82	0.0185	0.0183	0.9971
P06396	GELS_HUMAN	TGAQELLR	¹³ C ₆ , ¹⁵ N ₄	4.9	0.0125	0.0549	0.9979
P06396	GELS_HUMAN	AGALNSNDAFVLK	¹³ C ₆ , ¹⁵ N ₂	8.17	0.0146	0.1646	0.9982
P06727	APOA4_HUMAN	ISASAEELR	¹³ C ₆ , ¹⁵ N ₄	4.4	0.0086	0.0549	0.9961
P08603	CFAH_HUMAN	IDVHLVPDR	¹³ C ₆ , ¹⁵ N ₄	6.39	0.0305	0.1646	0.9946
P08603	CFAH_HUMAN	SPDVINGSPISQK	¹³ C ₆ , ¹⁵ N ₂	6.02	0.0155	0.1646	0.9978
P08697	A2AP_HUMAN	LGNQEPGGQTALK	¹³ C ₆ , ¹⁵ N ₂	3.92	0.0199	0.0549	0.9977
P09871	C1S_HUMAN	TNFDANDIALVR	¹³ C ₆ , ¹⁵ N ₄	8.7	0.0378	1.4815	0.9997
P0C0L5	CO4B_HUMAN	DHAVDLIQK	¹³ C ₆ , ¹⁵ N ₂	4.53	0.0309	0.0183	0.9975
P0C0L5	CO4B_HUMAN	VGDTLNLNLR	¹³ C ₆ , ¹⁵ N ₄	8.11	0.0148	0.4938	0.9984
P10909	CLUS_HUMAN	EIQNAVNGVK	¹³ C ₆ , ¹⁵ N ₂	3.93	0.0133	0.1646	0.9974
P10909	CLUS_HUMAN	TLLSNLEEAK	¹³ C ₆ , ¹⁵ N ₂	8.27	0.0078	1.4815	0.9982
P19823	ITIH2_HUMAN	SLAPATAAK	¹³ C ₆ , ¹⁵ N ₂	3.19	0.0323	0.1646	0.9956
P19823	ITIH2_HUMAN	IYLPGR	¹³ C ₆ , ¹⁵ N ₄	4.8	0.0106	0.0183	0.9972
P19827	ITIH1_HUMAN	AAISGENAGLVR	¹³ C ₆ , ¹⁵ N ₄	5.18	0.0062	0.1646	0.9955
P25311	ZA2G_HUMAN	AGEVQPELRL	¹³ C ₆ , ¹⁵ N ₄	4.83	0.0239	0.0183	0.9974
P32119	PRDX2_HUMAN	GLFIDGK	¹³ C ₆ , ¹⁵ N ₂	9.55	0.0201	0.0549	0.9975
P43652	AFAM_HUMAN	FLVNLVK	¹³ C ₆ , ¹⁵ N ₂	8.99	0.0243	0.4938	0.9977
P51884	LUM_HUMAN	SLEYLDLSFNQIAR	¹³ C ₆ , ¹⁵ N ₄	11.81	6.8849	13.3330	0.9656
P69905	HBA_HUMAN	VGAHAGEYGAEALER	¹³ C ₆ , ¹⁵ N ₄	4.85	0.0466	0.1646	0.9982
Q14624	ITIH4_HUMAN	LALDNGGLAR	¹³ C ₆ , ¹⁵ N ₄	6.27	0.0599	0.1646	0.9970
Q14624	ITIH4_HUMAN	LGVEYELLLK	¹³ C ₆ , ¹⁵ N ₂	11.29	0.0213	4.4444	0.9990

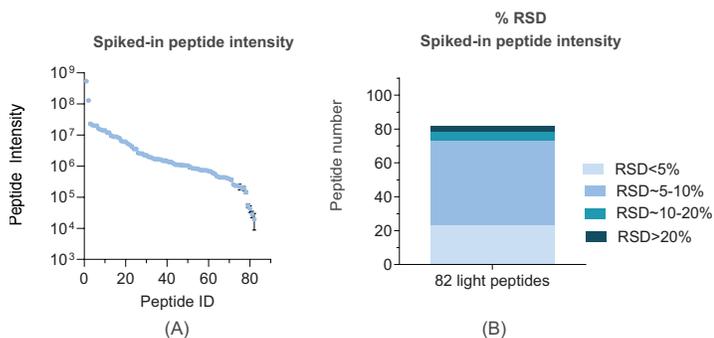


Figure 2. Reproducibility of peptide quantitation using the TSQ Certis mass spectrometer with the OptiSpray ion source and PepMap Neo 150 μ m x 15 cm cartridge column across 71 injections of 50 ng plasma digest. Skyline software was used for data processing. (A) Average light peptide intensity with standard deviations. (B) Relative standard deviation (RSD) for all 82 native peptides.

The reproducibility and robustness of the TSQ Certis MS equipped with the OptiSpray ion source and PepMap Neo 150 μ m x 15 cm cartridge column were evaluated using a panel of 82 peptides representing 57 HSP proteins. A total of 71 injections of 50 ng plasma digest spiked with internal standards was analyzed. The average and standard deviation of the light peptide signals are shown in Figure 2A, and % RSD values

across the 71 injections are summarized in Figure 2B. The results demonstrated excellent reproducibility, with approximately 89% (73) of native peptide signals exhibiting RSDs below 10%, and 95% (78) showing RSDs below 20%. The remaining 5% (4 peptides) with RSDs greater than 20% were attributed to low endogenous peptide concentrations in plasma.

A critical aspect in the development of a SRM targeted peptide assay is the maintenance of consistent retention times, which allows for accurate and efficient scheduling of multiple precursor ions. To evaluate chromatographic reproducibility, retention time variations were analyzed for the SIL peptides. Specifically, the assay includes 24 well-recognized protein markers represented by 35 peptides.

As shown in Figure 3A, the retention times of these 35 peptides ranged from 3.09 to 10.42 minutes, with %RSD between 0.084% and 0.56% across 71 injections, indicating exceptional retention time stability (Figure 3B).

Furthermore, Figure 3C illustrates the reproducibility of peptide peak areas across 71 injections. The % RSD values for the 35 peptides (representing 24 recognized protein markers) ranged from 4.3% to 55.1%, with a median RSD of 3.2%. Notably, 88% of the peptides (31 out of 35) exhibited peak area RSDs below 10% (Figure 3D).

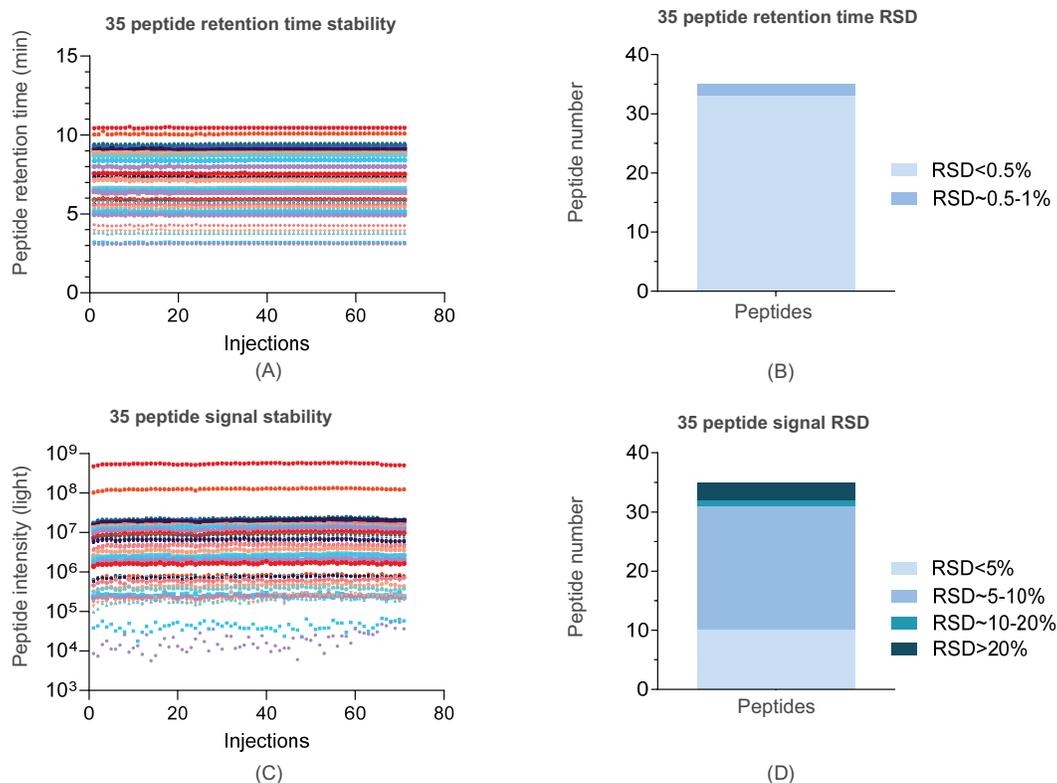


Figure 3. Retention time and peak area reproducibility. Over 71 injections, 35 peptides representing 24 well-recognized protein markers were investigated. Skyline software was used for data processing. (A) Retention times ranged from 3.09 to 10.42 min with RSDs between 0.084% and 0.56%. (B) 33 peptides showed RT RSD < 0.5%, and 2 peptides had RT RSDs between 0.5% and 0.56% across 71 injections, indicating excellent chromatographic stability. (C) Peak area reproducibility and (D) variation across 71 injections, with 31 peptides showing RSD < 10% and 33 peptides showing RSD < 20%.

The remaining 47 peptides also exhibited excellent signal reproducibility (average RSD = 6.9%) and stable retention times (average RSD = 0.3%). The consistent performance highlights their potential analytical robustness and suitability for research or pre-clinical biomarker studies. Overall, the peptide quantitation data acquired on the TSQ Certis MS demonstrates outstanding quantitative performance.

The 11-point serial dilution peptide dataset was also analyzed with TraceFinder software. In Figure 4, extracted data are presented for two well-recognized protein biomarkers: the FSVVYAK peptide from Alpha-2-HS-glycoprotein and the SDIAPVAR peptide from Alpha-2-macroglobulin. Serial dilutions of the heavy peptides were analyzed over a dynamic range of 0–120 fmol on-column (Figure 4A). Both peptides exhibited

excellent linearity, with correlation coefficients ($R^2 = 0.9981$ and $R^2 = 0.9974$, respectively), confirming accurate quantitative response across the tested range. Insets highlight expanded views of the low-concentration regions (0–2 fmol), demonstrating consistent sensitivity ($R^2 > 0.996$).

Signal intensity reproducibility for the corresponding light (unlabeled) peptides was evaluated across 71 consecutive injections, yielding RSD of 3.6% for SDIAPVAR and 2.8% for FSVVYAK, indicating high analytical precision. Retention time reproducibility showed minimal variation, with mean retention times of 3.775 min (RSD = 0.195%) for SDIAPVAR and 5.80 min (RSD = 0.0975%) for FSVVYAK, demonstrating excellent chromatographic stability across repeated injections.

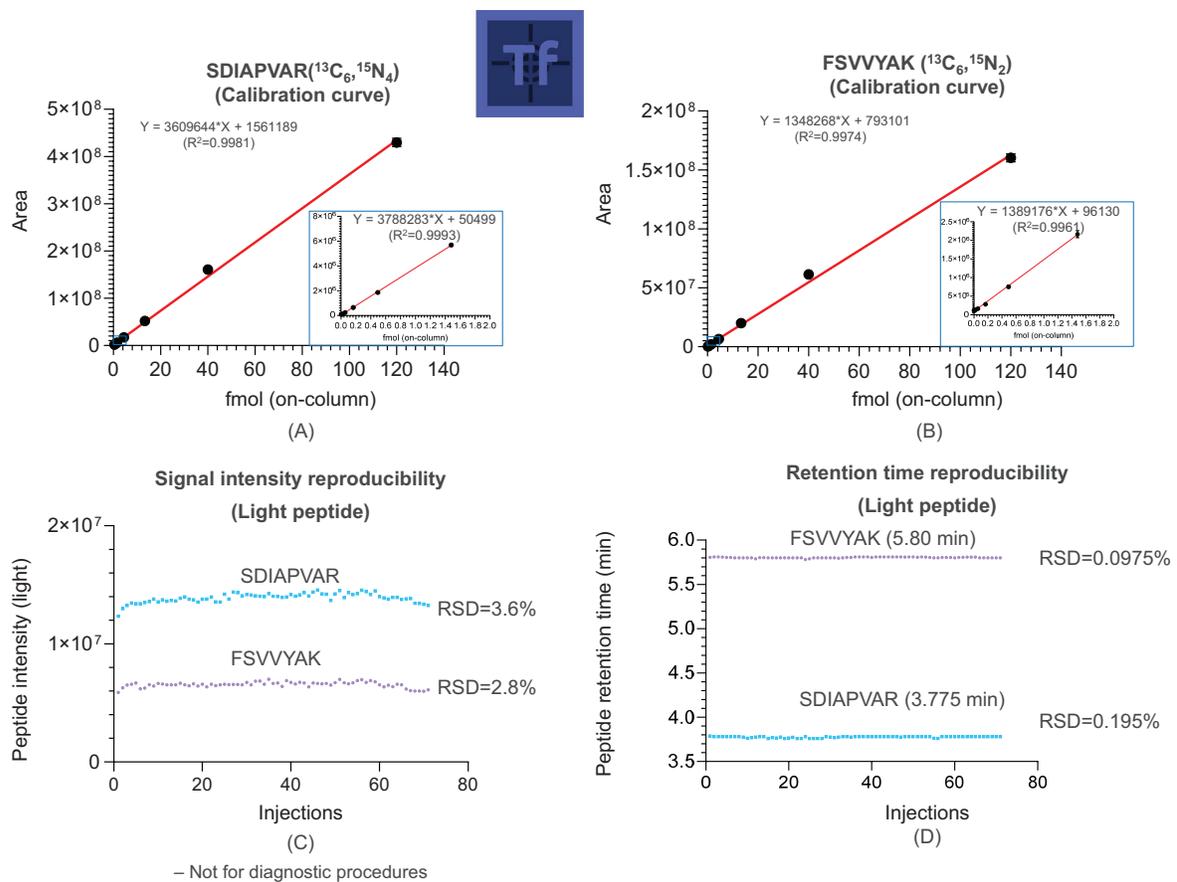


Figure 4. Calibration curves and reproducibility assessment of targeted peptide quantification using TraceFinder software.

(A) and (B) Calibration curves for the stable isotope–labeled internal standard peptides SDIAPVAR ($^{13}\text{C}_6, ^{15}\text{N}_4$) and FSVVYAK ($^{13}\text{C}_6, ^{15}\text{N}_2$), respectively. (C) Signal intensity reproducibility of the corresponding light (unlabeled) peptides was assessed across 71 consecutive injections. (D) Retention time reproducibility for the same light peptides. TraceFinder software was utilized for data generation.

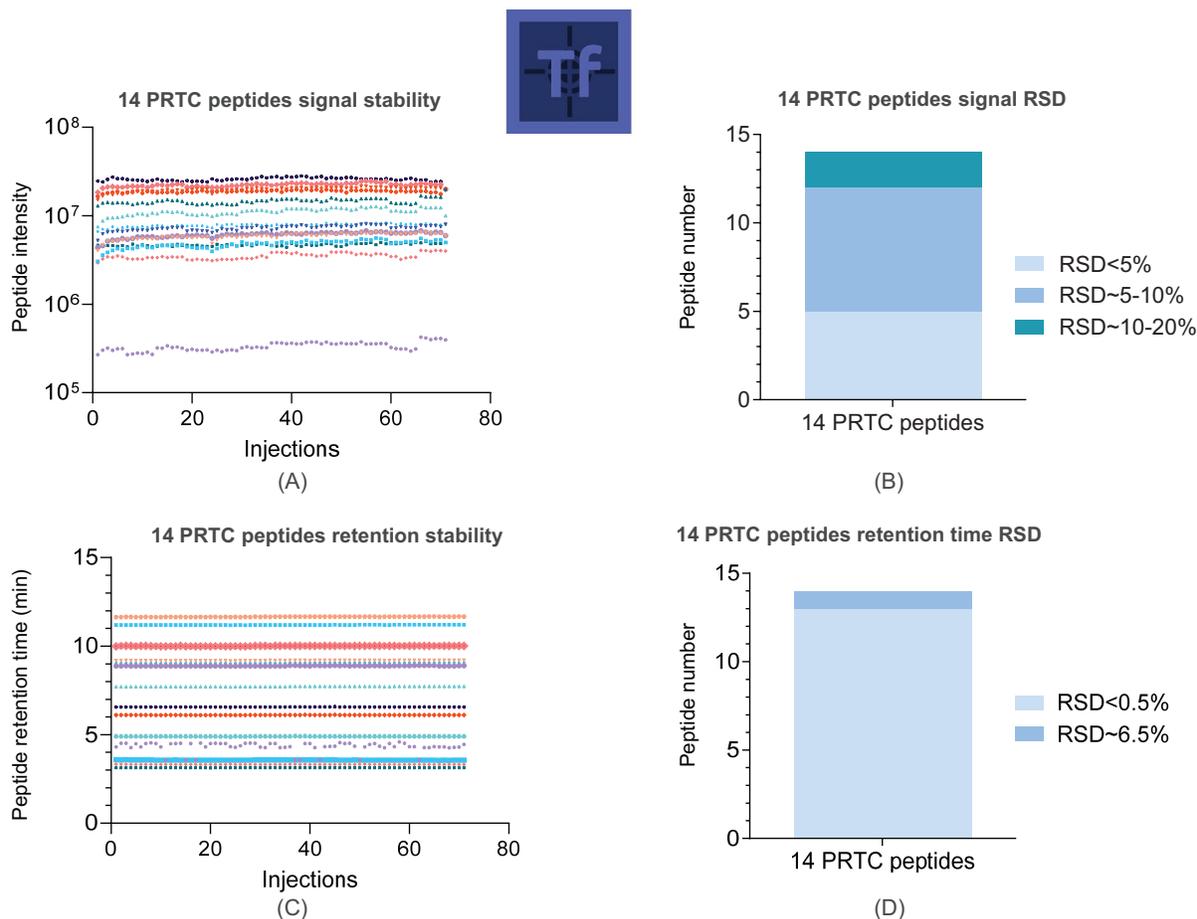


Figure 5. Robust performance of Pierce Peptide Retention Time Calibration Mixture (PRTC) intensity and retention time. (A) Signal stability of 14 PRTC peptides across 71 injections. (B) Signal RSD distribution for the 14 PRTC peptides. (C) Retention time stability of 14 PRTC peptides across 71 injections. (D) Retention-time RSD distribution for the 14 PRTC peptides. TraceFinder software was utilized for data generation.

All samples were spiked with Pierce Peptide Retention Time Calibration Mixture and 14 PRTC peptides were targeted. As expected, PRTC peptides demonstrated excellent LC–MS performance across 71 consecutive injections. As shown in Figure 5A, peptide signal intensities remained highly stable throughout the run. Correspondingly, the signal RSD analysis in Figure 5B revealed that most peptides exhibited RSD values below 10%, and 4 out of 14 were below 5%, indicating strong quantitative reproducibility. Retention time stability (Figure 5C) was also consistently maintained, with all peptides displaying tightly clustered retention times across injections. The retention time RSD distribution (Figure 5D) further confirmed this stability, as nearly all peptides (13 out of 14) showed RSD values below 0.5%, with the remainder still under 10%. Together, these results demonstrate robust instrument stability and reproducible chromatographic performance suitable for high-confidence LC–MS analyses.

Conclusion

The TSQ Certis triple quadrupole mass spectrometer equipped with the OptiSpray source and cartridge columns provides exceptional reproducibility, sensitivity, and stability for multiplexed peptide quantitation. The key outcomes include sub-femtomole sensitivity; >99% linearity for >98% of the peptides, <0.6% retention time variation; and high reproducibility at 100 SPD (13 minutes per run) throughput. These results demonstrate the instrument's suitability for peptide quantitation, pre-clinical biomarker verification, and translational research applications.

The TSQ Certis MS is a next-generation, high-performance triple-quadrupole platform designed for fast, precise quantitative analyses. Its rapid-scanning (900 SRM/second) architecture supports large transition loads without compromising data quality.

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