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Development and Validation of a Novel Isotope Dilution-Ultraperformance Liquid Chromatography-Tandem Mass Spectrometry Method for Serum C-Peptide

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Background: Mass spectrometry (MS) methods exhibit higher accuracy and comparability in measuring serum C-peptide concentrations than immunoassays. We developed and validated a novel isotope dilution-ultraperformance liquid chromatography-tandem MS (ID-UPLC-MS/MS) assay to measure serum C-peptide concentrations.

Methods: Sample pretreatment involved solid-phase extraction, ion-exchange solid-phase extraction, and derivatization with 6-aminoquinolyl-N-hydroxysuccinimidylcarbamate (Cayman Chemical, Ann Arbor, Michigan, USA). We used an ExionLC UPLC system (Sciex, Framingham, MA, USA) and a Sciex Triple Quad 6500$^+$ MS/MS system (Sciex) for electrospray ionization in positive-ion mode with multiple charge states of [M+3H]3+ and multiple reaction monitoring transitions. The total run time was 50 mins, and the flow rate was 0.20 mL/min. We evaluated the precision, trueness, linearity, lower limit of quantitation (LLOQ), carryover, and matrix effects. Method comparison with electrochemiluminescence immunoassay (ECLIA) was performed in 138 clinical specimens.

Results: The intra- and inter-run precision coefficients of variation were < 5% and the bias values for trueness were < 4%, which were all acceptable. The verified linear interval was 0.050–15 ng/mL, and the LLOQ was 0.050 ng/mL. No significant carryover or matrix effects were observed. The correlation between this ID-UPLC-MS/MS method and ECLIA was good (R = 0.995, slope = 1.564); however, the ECLIA showed a positive bias (51.8%).

Conclusions: The developed ID-UPLC-MS/MS assay shows acceptable performance in measuring serum C-peptide concentrations. This will be useful in situations requiring accurate measurement of serum C-peptide in clinical laboratories.

Key Words: Tandem mass spectrometry, C-peptide, Performance

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INTRODUCTION

C-peptide is a polypeptide of 31 amino acids derived from proinsulin, a prohormone synthesized in the beta cells of the pancreatic islets [1]. C-peptide and insulin are generated via proteolytic cleavage of proinsulin and released into the blood in equimolar amounts [2]. Although C-peptide and insulin are secreted together, plasma concentrations of C-peptide are higher than

those of insulin because of its longer half-life [1]. In patients treated with exogenous insulin, serum C-peptide is a good indicator of beta-cell activity because of the cross-reactivity between endogenous and exogenous insulin [3].

Various immunoassays are used in laboratories to measure serum C-peptide concentrations. An international comparison of different C-peptide measurement procedures conducted by the C-peptide Standardization Committee revealed significant variation among laboratories and measurement procedures and showed that C-peptide immunoassay results are often incomparable [4, 5]. The committee also reported considerable among-laboratory imprecision in C-peptide measurement and revealed that, although calibration with pure C-peptide standards (WHO IRR 84/510) does not improve comparability, patient samples can successfully be used to calibrate assays and reduce imprecision [5]. They reported that the comparability of clinical immunoassays among laboratories can be substantially improved by using a calibrator serum determined via liquid chromatography-tandem mass spectrometry (LC-MS/MS) [5, 6].

LC-MS/MS is used as a reference method for many clinical indicators owing to its high specificity, accuracy, and sensitivity, all of which are listed on the Joint Committee for Traceability in Laboratory Medicine (JCTLM) website (https://www/bipm.org/jctlm/home.do). This technology is highly effective for measuring small molecules; however, it is not extensively used for measuring biological macromolecules such as intact C-peptides for reasons such as the requirement for sample processing, sample complexity, and high cost [4]. However, LC-MS/MS is becoming increasingly popular for measuring such macromolecules owing to continuous complex method development. For example, Foreman, *et al*. [7] developed an LC-MS/MS-based detection method for circulating proinsulin-derived peptides that showed high specificity.

LC-MS/MS assays have ample potential to overcome the limitations of immunoassays [8, 9]. Previous MS assays for the quantification of serum C-peptide analyzed intact peptide, which has a low ionization efficiency, thus limiting its analytical sensitivity on many instruments [1]. This limited ionization efficiency has been overcome through the use of multiple dimensions of LC separation [1, 10-12] or immunoaffinity enrichment [1, 13, 14].

We developed and validated a novel isotope dilution-ultraperformance liquid chromatography (ID-UPLC-)MS/MS assay for measuring serum C-peptide. Additionally, we compared the results with those of the immunoassay.

MATERIALS AND METHODS

Experiments involving human specimens were performed according to the Declaration of Helsinki (2019) and approved by the Institutional Review Board (IRB) of GC Labs (IRB No. GCL-2023-1034-01).

Reagents and chemicals

HPLC-grade water (Fisher, Pittsburgh, PA, USA), HPLC-grade acetonitrile (ACN) (Fisher), formic acid (Wako, Osaka, Japan), acetic acid (Wako), trifluoroacetic acid (TFA; Supelco, Bellefonte, PA, USA), protease-free bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), boric acid (Sigma-Aldrich), and Mass Spect Gold Human Serum (Golden West, Temecula, CA, USA) were used. D8-Val7,10-C-peptide (5 ng/mL; Bachem, Bubendorf, Switzerland) was used as an internal standard (IS).

C-peptide solution (100 μg/mL) in protease-free 1% phosphate-buffered saline (Cerilliant, Round Rock, TX, USA) and C-peptide (human) trifluoroacetate salt (Bachem, Bubendorf, Switzerland) were used as certified reference materials (CRMs). National Metrology Institute of Japan (NMIJ) CRM 6901-C (Wako) was used as a primary standard reference material.

Sample preparation materials

A Sep-Pak tC18 96-well plate with 100 mg solvent/well and 37–55-μm particle size (Waters, CT, USA) was used for solid-phase extraction (SPE). An Oasis MCX 96-well plate with 30 mg solvent/well and 30-μm particle size (Waters, Milford, MA, USA) was used for ion-exchange SPE. 6-Aminoquinolyl-N-hydroxysuccinimidylcarbamate (AQC; Cayman Chemical, Ann Arbor, MI, USA) was used for derivatization.

Calibrator, QC materials, and IS preparation

Calibrators were prepared by diluting the CRMs with 2% BSA and 5% acetic acid, and calibration curves were constructed using five calibrator concentrations (0.5, 1.0, 2.5, 5.0, and 10.0 ng/mL) (1 ng/mL = ~0.331 nmol/L).

Three QC samples for low, medium, and high concentrations (0.52, 1.04, and 10.4 ng/mL) were prepared by dissolving NMIJ CRM 6901-C in 2% BSA and 5% acetic acid.

Sample preparation

We used a two-step SPE method according to previous studies on ID-LC/MS for C-peptide measurement [15, 16].

For SPE, a mixture of serum (500 μL), IS (500 μL), and 500 mM acetic acid (1,000 μL) was loaded twice into a Sep-Pak

tC18 96-well plate. After a washing step, the samples were eluted with 50% ACN in 50 mM acetic acid.

For ion-exchange SPE, we diluted the eluted sample with 0.1% TFA at a 1:1 (v/v) ratio, loaded the diluted sample twice into an Oasis MCX 96-well plate, and eluted them with 50% ACN in 0.5% TFA.

For derivatization, we evaporated the samples with nitrogen gas for 3 hrs. Then, 0.2 M borate buffer (pH 8.8) was added, and the samples were vortexed for 10 mins. The samples were dried, and 1 mg/mL AQC in ACN was added. The samples were heated at 55°C for 40 mins for derivatization and then evaporated with nitrogen gas for 30 mins. We reconstituted the dried samples with 50 μL of 15% ACN in 1% TFA. The samples were filtered and loaded in the UPLC-MS/MS instrument.

UPLC–MS/MS settings

We used Exion UPLC (SCIEX, MA, USA) and Triple Quad 6500^{+} (SCIEX, MA, USA) instruments. We used a Capcell Pak C18 ACR (2.0×10 mm, 3 μm; OSAKA SODA, Osaka, Japan) guard cartridge column. A Capcell Pak C18 ACR (2.00×150 mm, 3 μL; OSAKA SODA, Osaka, Japan) column was also used. The LC conditions for mobile phases were 1.0% formic acid in water (A) and 100% ACN (B). The gradients were 85% A and 15% B at 0 min, followed by 70% of A and 30% of B at 35 mins. The total run time was 50 mins, with a flow rate of 0.20 mL/min. We used the integrated valve method, with a diverter valve in diverter mode (position A: 22–34 mins). We used the electrospray ionization (ESI) method in positive-ion mode with multiple reaction monitoring (MRM) transitions. Nitrogen gas was used for nebulation, desolvation, and collision.

The MRM transitions and other MS/MS settings are provided in Table 1.

Assay performance evaluation

Analytical performance characteristics, including precision, trueness, linearity, lower limit of quantitation (LLOQ), carryover, and matrix effects, were evaluated. Method comparisons were conducted in accordance with the US Food and Drug Administration Center for Drug Evaluation and Research Bioanalytical Method Validation Guidance for Industry [17], CLSI guidelines [18, 19], and review articles on LC-MS/MS laboratory development and operation [20-22].

Intra-run precision was assessed using five replicates in a single run in one day, and inter-run precision was assessed using 20 separate runs over 6 days, with a single run per day and three analyte concentrations (Table 2).

Spike recovery and trueness were assessed using NMIJ CRM 6901-C with a certified value of 104±5 ng/mL. The CRM was diluted with 2% BSA in 5% acetic acid and Mass Spect Gold Human Serum to values of 0.52, 1.04, and 10.4 ng/mL, respectively. They were analyzed three times in one day.

Acceptance limits were within 8.3% CV for precision and within ± 7.1% bias of nominal concentrations for trueness. Analytical performance goals for C-peptide measurement were set

Table 1. MRM transitions and other mass spectrometric settings

Analyte	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)	CUR (psi)	CAD (psi)	Temp (°C)	Dwell time (msec)
C-peptide1 (quantifier)	1,064.262 Da	171.2 Da	71	10	169	12	40	11	400	150
C-peptide2 (qualifier)	1,064.262 Da	955.2 Da	160	10	40	25	40	11	400	150
C-peptide IS (D_8-Val^{7,10} C-peptide)	1,069.915 Da	171.2 Da	76	10	159	16	40	11	400	300

Abbreviations: MRM, multiple reaction monitoring; IS, internal standard; DP, de-clustering potential; EP, exit potential; CE, collision energy; CXP, collision cell exit potential; CUR, curtain gas, CAD, collision gas; Temp, temperature.

Table 2. Intra- and inter-run precision and trueness

C-peptide	Target value (ng/mL)	Precision				Trueness	
		Intra-run (N=5)		Inter-run (N=20)			
		Mean (ng/mL)	% CV	Mean (ng/mL)	% CV	Mean (ng/mL)	% Bias
CRM 6901-C 104±5 ng/mL (1/200 dilution)	0.52	0.50	4.8	0.52	4.5	0.52	0.6
CRM 6901-C 104±5 ng/mL (1/100 dilution)	1.04	1.03	3.6	1.07	4.3	1.05	1.0
CRM 6901-C 104±5 ng/mL (1/10 dilution)	10.40	10.74	4.2	10.66	3.8	10.72	3.1

Abbreviation: CRM, certified reference material.

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according to the Westgard desirable biological variation database [23].

Response linearity was assessed using serial dilutions of CRM C-peptide (human) trifluoroacetate salt (Bachem) with 2% BSA in 5% acetic acid solution to yield seven concentrations of 0.05, 0.10, 0.25, 1, 5, 10, and 15ng/mL. The acceptance criteria of linearity were $R^2 > 0.99$, accuracy (% bias) < 15%, and CV (%) < 15%.

The LLOQ was evaluated using C-peptide (human) trifluoroacetate salt diluted with 2% BSA in 5% acetic acid, with < 20% bias for accuracy and < 20% CV for precision (seven concentrations × five times).

Carryover was evaluated according to the following equation, with four serial measurements of two concentrations of calibrators (calibrators 2 and 5);

$$\text{Carryover (\%)} = [\{L1-(L3+L4)/2\}/\{(H2+H3)/2-(L3+L4)/2\}] \times 100,$$

where L indicates the low concentration, and H is the high concentration. The acceptance criterion for carryover was ± 1.0%.

Ion suppression was evaluated using the post-column infusion method. In brief, a standard C-peptide solution with a concentration of 500 ng/mL was continuously infused directly into the MS detector at a flow rate of 5 μL/min, whereas Mass Spect Gold Human Serum, 2% BSA in 5% acetic acid, hemolysis sample, lipemic sample, icteric flag sample, and six extracted participant samples were injected into the UPLC column at a flow rate of 0.2 mL/min. A significant change in the detection level indicates that ion suppression has occurred at the point at which the change was observed.

Reference intervals (RIs)

We determined RIs for serum C-peptide according to CLSI EP28-A3c [24]. We measured C-peptide concentrations in residual samples from 150 healthy women (9–83 yrs of age) and 150 healthy men (12–84 yrs of age) with normal glucose values. For nonparametric confidence intervals of reference limits, we referred to Table 8 in CLSI EP28-A3c [24]. We compared the RIs with those in previous studies [13, 14].

Method comparison of LC–MS/MS and electrochemiluminescence immunoassay (ECLIA)

We measured serum C-peptide concentrations in another 138 random patients with unknown clinical histories, only having C-peptide concentrations of 0.55–12.54 ng/mL as determined by an ECLIA using the Roche Cobas 8000 instrument (Roche Diagnostics, Indianapolis, USA).

Statistical analysis

EP evaluator (Data Innovations, Burlington, USA) was used for correlation evaluation and RI validation, and Passing–Bablok regression analysis and percent bias plots were used for method comparisons. $P < 0.05$ was considered significant.

RESULTS

Representative UPLC-MS/MS chromatograms of serum C-peptide are shown in Fig. 1. To demonstrate the specificity of our method, we used ion ratio confirmation using quantifier C-peptides (Q1: 1,064.3, Q3: 171.2) and qualifier C-peptides (Q1: 1,064.3, Q3: 955.2) showing adequate identification and assay specificity (Supplemental Data Fig. S1). The inter- and intra-run imprecision ranged from 3.6% to 4.8% CV, which is lower than the minimal requirement based on biological variation. The percentage bias for trueness ranged from 0.6% to 3.1% and was within the acceptable range (Table 2). Spike recovery test results (Table 3) reflect the trueness of the method and partly reflect the matrix effect.

The linearity range was 0.05–15 ng/mL, with $R^2 = 0.9995$. The LLOQ was 0.050 ng/mL. We observed no carryover effect. There was no matrix effect, as no significant ion suppression or enhancement was observed at the corresponding retention time (Fig. 2).

Using samples from 300 healthy individuals, including 150 men and 150 women, the RI was determined to be 0.21–3.92 ng/mL based on the values corresponding to the rank numbers of the lower three and upper 13 samples for the total sample size of 300 in the nonparametric distribution. This was slightly lower than that of the ECLIA, which was 1.1–4.4 ng/mL

The correlation between UPLC-MS/MS and Roche Cobas 8000 ECLIA results was good (R = 0.995, slope = 1.564) based on an analysis of 138 samples having C-peptide concentrations of 0.55–12.54 ng/mL (Fig. 3). However, the ECLIA showed positive percent bias (51.8 %) compared with UPLC-MS/MS for all 138 samples.

DISCUSSION

We developed and validated an ID-UPLC-MS/MS method for quantifying C-peptide in serum. This is a novel method with newly developed sample preparation procedures, enabling quantifying more lower C-peptide concentrations in serum com-

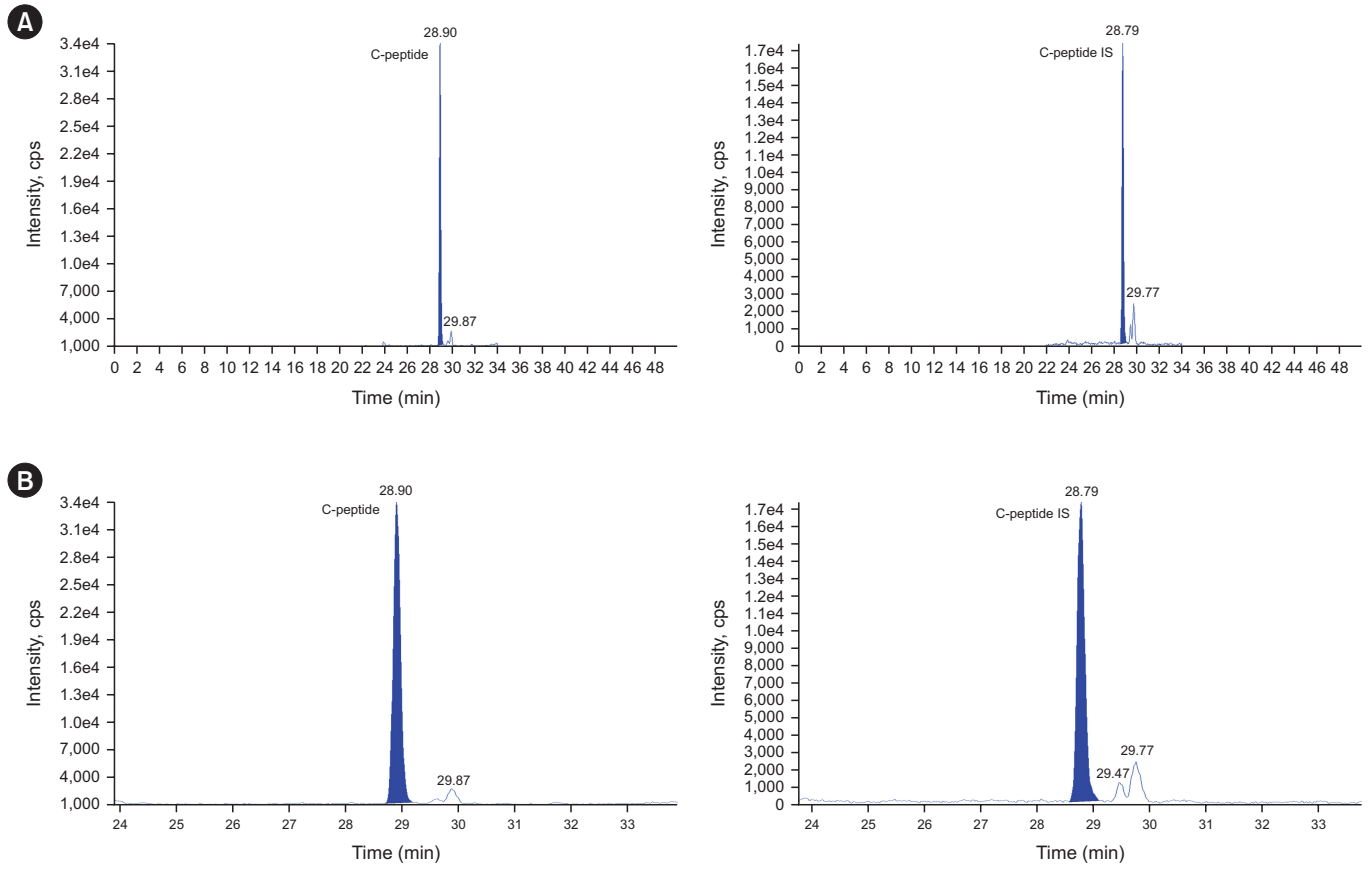

Fig. 1. Typical tandem mass spectrometry chromatograms of C-peptide in a patient serum sample (2.860 ng/mL). (A) Full chromatograms after 50 minutes of runtime. (B) Magnification of the major peaks in panel A.

pared with those of previous studies.

Existing sample preparation procedures for C-peptide commonly include protein precipitation, SPE, immune-affinity purification, ion-exchange chromatography, two-dimensional LC, and derivatization. Generally, to achieve sufficient enrichment and purification, combining multiple preparation procedures is essential [4]. The JCTLM list includes two reference procedures for C-peptide, both of which are based on LC-MS/MS. The first method, developed by Stoyanov, *et al*. [11] in 2011, uses protein precipitation and ion-exchange chromatography to effectively isolate C-peptide from the complex matrix. This method enables the measurement of low C-peptide concentrations because it involves an improved sample preparation procedure, including two-step purification or "double dilution" [12]. The second method, developed by Kinumi, *et al*. [13], involves immunoaffinity purification and precolumn derivatization with AQC to purify C-peptide before LC-MS/MS detection for improved sensitivity. AQC is also used to modify the N-terminal amino group of C-peptide, substantially increasing the fragmentation efficiency by adding a positive charge to the peptide. This currently represents the most sensitive published ID-MC-MS/MS measurement procedure for C-peptide [13].

The sample preparation procedure developed in the current study comprises 1) SPE, 2) ion-exchange SPE, and 3) derivatization using AQC, referring to the aforementioned procedures but using a novel combination. The total time required for sample pretreatment in a single 96-well plate is 5 hrs.

All published LC-MS/MS methods for C-peptide adopt the ESI mode, with most using positive-ion-mode ESI in MRM and selecting the highly abundant 3^+ charged ion as the precursor ion [4]. These settings are the same as those in our method.

Our laboratory-developed test (LDT) method has an inter- and intra-run imprecision of 3.6%–4.8% CV and a percentage bias for trueness of 0.6%–3.1%, which are substantially lower than the goals of <8.3% CV and <7.1% bias, respectively, in the Westgard desirable biological variation database [23], demonstrating the high performance of our method. The LLOQ of our method is 0.05 ng/mL, which is similar or even lower than the

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Table 3. Spike recovery test results

C-peptide	Targetvalue (ng/mL)	Dilution 1*			Dilution 2†		
		Spike recovery (N=3)			Spike recovery (N=3)		
		Mean (ng/mL)	% CV	% Bias	Mean (ng/mL)	% CV	% Bias
CRM 6901-C 104±5 ng/mL (1/200 dilution)	0.52	0.52	1.1	0.6	0.51	1.1	-1.3
CRM 6901-C 104±5 ng/mL (1/100 dilution)	1.04	1.12	1.4	7.4	1.07	1.4	3.2
CRM 6901-C 104±5 ng/mL (1/10 dilution)	10.40	10.20	2.5	-2.0	10.46	0.4	0.5

*Dilution with 2% BSA and 5% acetic acid. †Dilution with Mass Spect Gold Human Serum (Golden West).

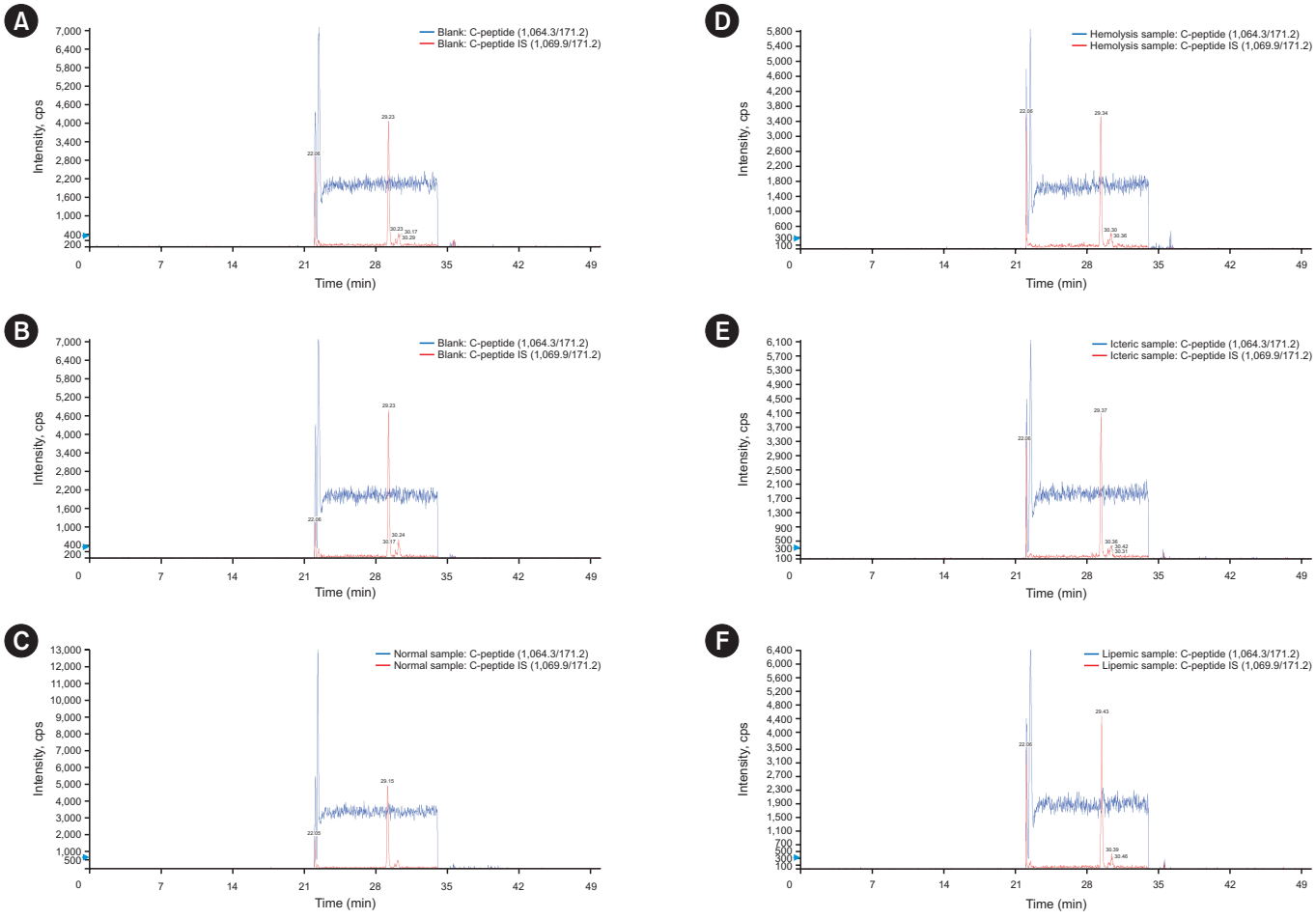

Fig. 2. Effect of ion suppression. No ion suppression was observed in blank samples (A, Mass Spect Gold Human Serum; B, 2% BSA in 5% acetic acid), (C) normal sample, (D) hemolytic sample, (E) icteric sample, or (F) lipemic sample.
Abbreviation: BSA, bovine serum albumin.

LLOQs of 0.1 ng/mL [3] and 0.06 ng/mL [1] reported previously.

In a previous study analyzing serum samples using an LC-MS/MS method and six routine immunoassays, all assays, except the Siemens and Beckman assays, displayed significant positive bias according to Bland–Altman analysis results (49.9%, 35.4%, 32.2%, and 81.7% for Roche, Abbott, Mindray, and Snibe, respectively) [3]. The Beckman and Siemens assays, both of which are magnetic particle chemiluminescence immunoassays, showed better agreement with the LC-MS/MS method than the other assays, indicating that the WHO material may have a lower matrix effect for this type of assay than for other assay methods [3]. The positive bias in immunological results is likely attributed

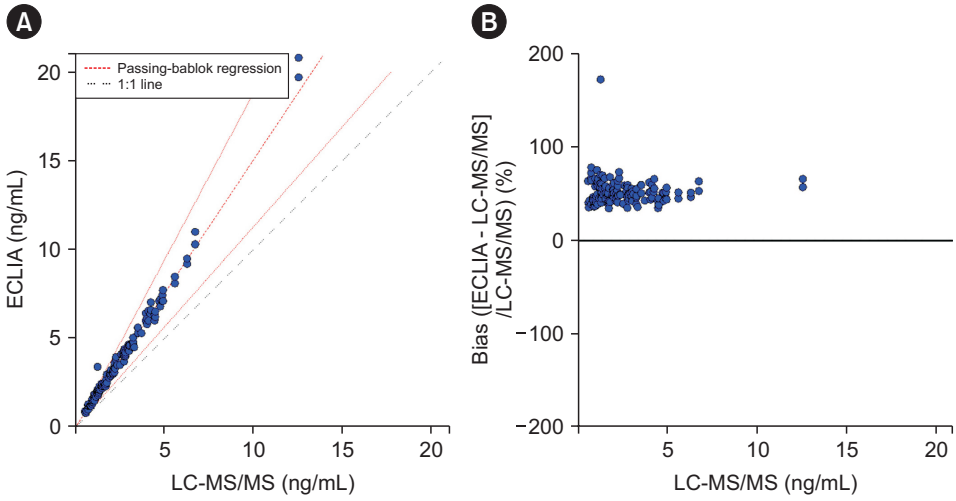

Fig. 3. Method comparison of ID-UPLC-MS/MS and the Cobas 8000 ECLIA. (A) Passing–Bablok regression scatter plot. (B) Percent bias plot. The correlation was good (R = 0.995, slope = 1.564) in 138 random individuals with C-peptide concentrations of 0.55–12.54 ng/mL, but positive percent bias was observed for all concentrations.
Abbreviations: ID-UPLC-MS/MS, isotope dilution-ultraperformance liquid chromatography-tandem mass spectrometry; ECLIA, electrochemiluminescence immunoassay.

to cross-reactions between C-peptide anti-serum and proinsulin or C-peptide metabolite fragments [4]. In line with the previous studies, we found that the ECLIA showed positive percent bias at all concentrations. In addition, the RI of our LDT method was lower than that of the ECLIA.

While LC-MS/MS for serum C-peptide measurement has numerous advantages, such as high throughput and high accuracy without cross-reactivity, which is observed with immunoassays, current obstacles to its application are the high initial cost and the requirement for professional operators [4]. In addition, the time-consuming pretreatment step increases the uncertainty of the entire procedure and hampers its application in routine testing in clinical laboratories.

For macromolecules with a molecular weight exceeding the detection limit of MS/MS, the detection of fragments rather than the intact form is often necessary, and in such case, the specificity of the measurement should be guaranteed. To guaranteeing the specificity of our method, we used ion ratio confirmation using quantifier and qualifier C-peptides.

As we developed this method for limited situations in which a high accuracy and wide linearity range are required, such as when establishing a reference standard for comparison with other immunoassays, a low LLOQ was an important consideration. To achieve this, we had to develop a lengthier pretreatment process than that used in a previous study [3].

As the reference measurement procedure (RMP) for C-peptide, LC-MS/MS plays a key role in C-peptide measurement standardization and CRM development. The international reference measurement system for C-peptide is still in development. The primary reference material (PRM) listed on the JCTLM website is NMIJ CRM6901. The JCTLM lists two primary RMPs (PRMPs) but no laboratories accredited to ISO 17025 and 15195 for providing C-peptide reference measurement services. There are no commutable (secondary) CRMs, either [4, 25]. The above-mentioned PRMP [13] adopted C-peptide CRM 6901-b as the calibrator to ensure that the results were traceable to the SI unit, and this PRM listed in the JCTLM was successfully produced by the NMIJ and can be used for RMP calibration [4].

In conclusion, while our method is unsuitable for routine clinical testing because of the long assay time and complex sample pretreatment, it is useful in limited situations requiring accurate measurement of serum C-peptide in clinical laboratories.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0072

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None.

AUTHOR CONTRIBUTIONS

Cho SE, Han J, Lee JH, Yi A, Lee SG, and Lee EH conceived and designed the study. You J and Han J investigated and validated the study. Cho SE wrote the manuscript. Lee SG and Lee EH provided feedback. Cho SE supervised all the study. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

None declared.

REFERENCES

1. Owusu BY, Pflaum H, Garner R, Foulon N, Laha TJ, Hoofnagle AN. Development and validation of a novel LC-MS/MS assay for C-peptide in human serum. J Mass Spectrom Adv Clin Lab 2021;19:1-6.
2. Oyer PE, Cho S, Peterson JD, Steiner DF. Studies on human proinsulin. Isolation and amino acid sequence of the human pancreatic C-peptide. J Biol Chem 1971;246:1375-86.
3. Deng Y, Zhang C, Wang J, Zeng J, Zhang J, Zhang T, et al. An accurate isotope dilution liquid chromatography-tandem mass spectrometry method for serum C-peptide and its use in harmonization in China. Ann Lab Med 2023;43:345-54.
4. Deng Y, Zhao H, Liu Q, Zhou W, Zhang C. The potential for isotope dilution-LC-MS/MS to improve laboratory measurement of C-peptide: reasons and critical determinants. J Mass Spectrom Adv Clin Lab 2021;21:1-9.
5. Wiedmeyer HM, Polonsky KS, Myers GL, Little RR, Greenbaum CJ, Goldstein DE, et al. International comparison of C-peptide measurements. Clin Chem 2007;53:784-7.
6. Little RR, Rohlfing CL, Tennill AL, Madsen RW, Polonsky KS, Myers GL, et al. Standardization of C-peptide measurements. Clin Chem 2008;54:1023-6.
7. Foreman RE, Meek CL, Roberts GP, George AL, Reimann F, Gribble FM, et al. LC-MS/MS based detection of circulating proinsulin derived peptides in patients with altered pancreatic beta cell function. J Chromatogr B Analyt Technol Biomed Life Sci 2022;1211:123482.
8. Hoofnagle AN and Wener MH. The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry. J Immunol Methods 2009;347:3-11.
9. Ghazal K, Brabant S, Prie D, Piketty ML. Hormone immunoassay interference: a 2021 update. Ann Lab Med 2022;42:3-23.
10. Kabytaev K, Durairaj A, Shin D, Rohlfing CL, Connolly S, Little RR, et al. Two-step ion-exchange chromatographic purification combined with reversed-phase chromatography to isolate C-peptide for mass spectrometric analysis. J Sep Sci 2016;39:676-81.
11. Stoyanov AV, Rohlfing CL, Connolly S, Roberts ML, Nauser CL, Little RR. Use of cation exchange chromatography for human C-peptide isotope dilution - mass spectrometric assay. J Chromatogr A 2011;1218:9244-9.
12. Stoyanov AV, Connolly S, Rohlfing CL, Rogatsky E, Stein D, Little RR. Human C-peptide quantitation by LC-MS isotope-dilution assay in serum or urine samples. J Chromatogr Sep Tech 2013;4:172.
13. Kinumi T, Mizuno R, Takatsu A. Quantification of serum C-peptide by isotope-dilution liquid chromatography-tandem mass spectrometry: Enhanced detection using chemical modification and immunoaffinity purification. Chromatogr B Analyt Technol Biomed Life Sci 2014;953-4:138-42.
14. Taylor SW, Clarke NJ, Chen Z, McPhaul MJ. A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide. Clin Chim Acta 2016;455:202-8.
15. Rodríguez-Cabaleiro D, Stöckl D, Thienpont LM. Improvement of sample pretreatment prior to analysis of C-peptide in serum by isotope dilution-liquid chromatography-tandem mass spectrometry. Rapid Commun Mass Spectrom 2005;19:3600-2.
16. Rodríguez-Cabaleiro D, Stöckl D, Kaufman JM, Fiers T, Thienpont LM. Feasibility of standardization of serum C-peptide immunoassays with isotope-dilution liquid chromatography-tandem mass spectrometry. Clin Chem 2006;52:1193-6.
17. U.S. Department of health and human services food and drug administration center for drug evaluation and research (CDER). Bioanalytical method validation guidance for industry. http://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry (Updated on May 2018).
18. CLSI. Liquid chromatography-mass spectrometry methods. 1st ed. C62-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2014.
19. CLSI. Evaluation of linearity of quantitative measurement procedures. 2nd ed. EP06-ED2. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.
20. Rappold BA. Review of the use of liquid chromatography-tandem mass spectrometry in clinical laboratories: part I-development. Ann Lab Med 2022;42:121-40.
21. Rappold BA. Review of the use of liquid chromatography-tandem mass spectrometry in clinical laboratories: part II-operations. Ann Lab Med 2022;42:531-57.
22. Cheng WL, Markus C, Lim CY, Tan RZ, Sethi SK, Loh TP; for the IFCC Working Group on Method Evaluation Protocols. Calibration practices in clinical mass spectrometry: review and recommendations. Ann Lab Med 2023;43:5-18.
23. Westgard desirable biological variation database https://www.westgard.com/biodatabase1.htm (Updated on June 2024).
24. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. 3rd ed. EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
25. Little RR, Wielgosz RI, Josephs R, Kinumi T, Takatsu A, Li H, et al. Implementing a reference measurement system for C-peptide: successes and lessons learned. Clin Chem 2017;63:1447-56.