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Evaluation of mutual interference between bovine α-lactalbumin peptide and its isotope-labeled peptide in whey protein analysis using liquid chromatography-tandem mass spectrometry

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ABSTRACT

Internal standard (IS) method is commonly used to correct the matrix effect of samples in the liquid chromatography-tandem mass spectrometric (LC–MS/MS) analysis of whey proteins. However, the presence of mutual interference between some peptides and their isotope-labeled peptides distorts the MS signals, requiring a fundamental evaluation to understand the phenomenon of signal variations. In this study, a simple strategy is proposed to evaluate the effects of sample pretreatment, materials and dilution of solutions on the MS signals of α-lactalbumin (VGINYWLAHK) and β-lactoglobulin peptides using two typical LC–MS/MS systems, Q-Trap and Q-Orbitrap. The strategy adapts the experimental MS data to optimize methods, thus providing meaningful solutions to suppress the mutual interference presented in the analysis of peptides. As a result, the strategy through the combination of 100-fold dilution and plastic injection vial improves the quantitation results of α-lactalbumin peptide significantly. While the β-lactoglobulin peptide presents different phenomenon of signal variations when compared with that of α-lactalbumin peptide, revealing that each peptide needs to be optimized individually. The calibration effect of different IS was also studied in fifteen infant milk powders to confirm the mutual interference impact to quantification result. These results indicated that a simple strategy through the combination of sample dilution and plastic injection vial could be well extended to quantitative analysis of any other peptide in the complex systems.

1. Introduction

Alpha-lactalbumin (α-lactalbumin) reperesents the dominant protein in human milk that comprises 25–35% of the total protein content [1]. It primarily contributes to the neurodevelopment, regulating sleep and mood of infants [2–4]. However, the routine infant formula is typically produced from cow's milk, where the α-lactalbumin only accounts for 2%–5%. α-Lactalbumin is thus often added as a strengthening agent in the infant formula to match the nutrition profile in breast milk [5,6]. This creates a need for detecting α-lactalbumin in infant formula efficiently.

Liquid chromatography–mass spectrometry (LC–MS) has become the main method for the determination of α-lactalbumin content, due to its advantages of high sensitivity and high selectivity. For example, Ren and Zhang applied high-performance liquid chromatography (HPLC)-tandem MS with α-lactalbumin signature peptide VGINYWLAHK, isotope-labelled signature peptide (ILSP) VGI*NYWL*AHK, whose molar conversion quantitates the bovine α-lactalbumin accurately. Through the analysis of three representative tryptic peptides by comparing the theoretical and endogenous peptides, VGINYWLAHK was selected because of its highest abundance, intensity and sensitivity in all the LC–MS/MS analyses [7,8]. However, the matrix effect may distort the LC–MS signals, and thus spoiling the accuracy and precision of the measurement [9,10]. In order to validate the quantification results of LC–MS, the US Food and Drug Administration (FDA) has suggested methods to reduce the matrix effect in its guidelines in developing bioanalytical methods [11]. Among the current methods to correct matrix effect, the internal standard method is the most effective [12–14]. It not only offsets the matrix effect of ionization [15,16], but also eliminates the variations in the sample pretreatment process [17,18]. However, the usage of internal standads (IS) could

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also reciprocally interfere the MS signal of the analyte, which is seldom reported so far.

Even with the IS technique, the LC–MS requires extensive optimization to suppress the matrix interference presented in the internal standard analysis. The International Union of Pure and Applied Chemistry (IUPAC) defines the interfering substances as specific substances that may produce matrix interference effect [9]. We had found an abnormal phenomenon in the internal standard analysis of α-lactalbumin content in infant formula. The concentration of α-lactalbumin peptide (α-peptide) significantly affects the MS response of its corresponding ILSP under positive electrospray source (ESI), while the specific peptide of β-lactoglobulin, another major whey protein show little influence on the MS intensity of its ILSP. This observation in α-peptides is inconsistent with the characteristics of internal standards, which assumes that they have consistent response to the pretreatment condition and the instrument, and does not interfere with the each other [19,20]. Such abnormal phenomenon rarely occurs, which is only reported in few literatures. For example, Liang et al. reported the matrix interference effect of methadone and eight other drugs with their corresponding internal standards [21].

In this study, we proposed a simple strategy for adapting the experimental mass spectrometry data to optimize methods, which provides meaningful solutions to suppress the mutual interference between bovine α-lactalbumin peptide and its ILSP in the internal standard analysis. This study reveals how different mass spectrometers, analyte concentrations, and other factors affect the mutual interference of α-peptide and the ILSP. This could provide an alternative thought that is helpful to study the mechanism of matrix effect, because there are only two peptides in a solvent under simple control conditions, especially for the analysis of the relationship between the structure of peptides and interference. Other materials used for handling the samples, such as filtration membrane and vial material that might affect the quantification of peptides were also evaluated. The results provide the reference and basis for further study of methods to compensate/suppress the matrix effect, thus enhancing the accuracy and reproducibility of the quantitation of the whey protein in infant formula using LC-tandem MS.

2. Material and methods

2.1. Instruments and reagents

There are two LC–MS/MS system used in the study. One was the acquity ultra-high performance liquid chromatography system (Waters, Milford, MA, USA) combined with an AB Sciex Q-Trap 5500 MS/MS (AB Sciex, Framingham, MA, USA), which is named Q-Trap for short. Another was the UHPLC/ESI Q-Exactive system consisted of a Dionex 300UHPLC system (Dionex Corporation, Sunnyvale, CA, USA) with an autosampler, a rapid separation quaternary pump, a column compartment coupled by a heated electro-spray ionization (HESI) electrospray source to a Q-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen,Germany), which is named Q-Orbitrap. An ultrasonicator KQ-500 (ShuM, Kunshan, China) and a vortex mixer Qilinbeier-5 (Jinghong, Shanghai, China) were used to support the extraction. In sequence, the temperature water bath HH-US-B (Spring Instrument. Co., Ltd, iangSu, China) supported the enzymatic process. 10 ml volumetric flasks 100 (Beijing Glass Instrument Factory, Beijing, China) and 2 ml polypropylene vials (CNW, Beijing, China) were used for milk powder dissolution and process. The 0.22 rane polyethersulfone membranes (Jinteng, Tianjin, China) and 1ml syringe (KDL, Shanghai, China) were used for removal of macromolecular impurity in milk powder solution. The 2 ml pipette (Kangwei, Jiangsu, China) was used for transfering milk powder solution. The 1 ml plastic vial and glass vial (Agilent Technologies, Shanghai, China) were used as the container of sample for LC analysis.

Formic acid (FA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was of HPLC grade (Merck, Darmstadt, Germany). Ultrapure water was obtained through a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA) during all the experiments. What should be stressed that bovine functional protein assay kit was the key materials purchased from Realgoal Technology Co., Ltd. (Hangzhou,China), which contains α-peptide (VGINYWLAHK, 1199.7 Da, 2.0 μmol/L), β-peptide (IDALNENK,915.5 Da, 4.0 μmol/L), ILSP-α-peptide (VGI*NYWL*AHK, 1214.4 Da, 2.0 μmol/L), ILSP-β-peptide (I*DAL*NENK, 929.5 Da, 4.0 μmol/L), ILSP-α-lactalbumin internal standard (KILDKVGI*NYWL*AHKAL CSEK, 2443.9 Da, 2.0 μmol/L), ILSP-β-lactoglobulin internal standard (KIPAVFKI*DAL*NENKVLVLDTDYK, 4.0 μmol/L), bovine trypsin solution (500 μg/mL), ammonium bicarbonate solution (NH_4HCO_3, 500 mmol/L), iodoacetamide solution (IAA, 500 mmol/L), dithiothreitol solution (DTT, 500 mmol/L), and calcium chloride solution ($CaCl_2$, 100 mmol/L).

Fifteen types of infant milk powders were purchased at the local market.

2.2. Instrument operating conditions

Chromatographic separation was achieved on a ACQUITY UPLC peptide BEH C18 column (pore 300 Å, 100 mm × 2.1 mm, 1.7 μm) with a mobile phase flow rate of 0.30 mL min^{-1}. The mobile phase consisted of (A) water containing 0.1% FA and (B) acetonitrile containing 0.1% FA. A gradient elution program was applied as follows (all values are expressed as % (v/v)): initial 5% B and kept for 0.8 min, linearly from 5% to 10% B in 0.4 min; linearly from 10% to 17% B in 1.3 min; linearly from 17% to 23% B in 0.1 min; isocratic elution at 23% B in 1.2 min; linearly from 23% to 100% B in 0.2 min; isocratic elution at 100% B in 0.8 min; column reconditioning to initial mobile phase composition in 0.2 min; The total run time was 5 min. The injection volume was 5 μl while column temperature was 40 °C.

For Q-Trap, the MS system was operated using electrospray ionization (ESI) in positive MRM mode. Nitrogen was used as the nebulizer, heater, and curtain gas, as well as the collision gas. The optimum conditions were set as following: 40 psi curtain gas, 5500 V ion spray voltage, 450 °C heated nebulizer temperature (TEM), 40 psi nebulizing gas (GS1), and 50 psi heater gas (GS2). The ion pairs of α-peptide, β-peptide and the corresponding ILSPs for quantitative analyses and the corresponding collision voltage (CE) were optimized as shown in Table 1. The aquired data were processed using Analyst 1.6 software.

For Q-Orbitrap, the MS system was operated using HESI in positive MRM mode, The optimum conditions were set as fol-

Table 1
Mass parameters of peptides.

Compound	Precursor ion (m/z)	Product ion (m/z)	CE (eV)	MS
α-peptide	600.8	284.3	28	Q-Trap
		355.3[a]	24	
ILSP-α-peptide	607.8	284.3	26	Q-
		355.3[a]	24	Trap
β-peptide	458.8	616.6	22	Q-
		503.8[a]	22	Trap
ILSP-β-peptide	465.8	623.6	22	Q-
		503.8[a]	22	Trap
α-peptide	600.83	284.1713	25	Q-Orbitrap
		355.2074[a]	25	
ILSP-α-peptide	607.82	284.1709	25	Q-Orbitrap
		355.2079[a]	25	

[a] Ion for quantitative analysis.

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