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Quantification of γ-glutamyl-α-aminobutyric acid (γ-Glu-Abu) in beer by LC/MS/MS using stable isotope dilution

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Abstract

The quantification of the kokumi peptide, γ-glutamyl-α-aminobutyric acid (γ-Glu-Abu) in commercial beer was conducted. An analytical method using high-performance liquid chromatography-tandem mass spectrometry (LC/MS/MS) after a derivatization with 6-aminoquinoyl-N-hydroxysuccinimidyl-carbamate (AQC) was constructed. A stable isotope, γ-Glu (UL[uniformly labeled]-^{13}C, ^{15}N)-Abu was used as an internal standard for quantification. The detection of the AQC-derivative of γ-Glu-Abu was achieved by MS/MS using a multiple reaction monitoring (MRM) method. The coefficient of variation (CV) of analytical results of 1 μM γ-Glu-Abu solution (n = 6) was 1.65%. The recovery was assessed by analyzing the beer samples in which 0.1 μM γ-Glu-Abu was spiked, and the average recovery was 94%. These results suggest that this method enabled the precise and repeatable quantification of γ-Glu-Abu. All kinds of beer including Lager type and Ale type contained γ-Glu-Abu, and the contents varied from 12.5 μg / L to 24.0 μg / L (0.054 μM to 0.103 μM). These results suggest that γ-Glu-Abu is distributed in beer, and this study represents the first report documenting the content of γ-Glu-Abu in brewed alcoholic beverages.

Keyword: γ-glutamyl-α-aminobutyric acid, beer, kokumi, LC/MS/MS, stable isotope

Introduction

γ-Glutamyl-α-aminobutyric acid (γ-Glu-Abu) is reported to be a metabolic intermediate in the biosynthesis of ophthalmic acid (γ-glutamyl-α-aminobutyryl-glycine [γ-Glu-Abu-Gly]). Ophthalmic acid is known as a glutathione (GSH) analogue and is reported to be biosynthesized by GSH-synthesizing enzymes, including γ-glutamylcysteine synthetase (γGCS) and glutathione synthetase (GSHS). In addition, α-aminobutyric acid has been reported to be a natural substrate of γGCS (Griffith 1999). Therefore, γ-Glu-Abu and ophthalmic acid are thought to be related to GSH metabolism. Soga et al. (2016) conducted a metabolomics analysis and revealed that ophthalmic acid can serve as a oxidative stress biomarker indicating hepatic glutathione consumption in an animal model (Soga et al. 2016). They also showed that the hepatic level of γ-Glu-Abu increased after the administration of an excess dose of acetaminophen (150 mg/kg). Servillo et al. (2018) investigated the effects of various kinds of stress on the contents of ophthalmic acid and γ-Glu-Abu in oat leaves, and found that water stress, dark stress, paraquat stress, and cadmium stress significantly increased the contents of both ophthalmic acid and γ-Glu-Abu (Servillo et al. 2018). They also reported that γ-Glu-Abu is present in various kinds of flour, such as rye, oat, buckwheat, and chickpeas, at concentrations ranging from 0.1 to 0.5 mg/kg (Servillo et al. 2018).

Several γ-glutamyl peptides have been reported to be kokumi substances, which modify the basic taste and flavor characteristics such as complexity, mouthfulness, and continuity, although they are tasteless themselves at the concentrations at which they are used. A well-characterized kokumi substance is glutathione (GSH; γ-Glu-

Cys-Gly). Ueda et al. (1990) aimed to identify the kokumi substances in garlic and onion, and found that GSH is one of the kokumi substances present in garlic (Ueda et al. 1990) and onion (Ueda et al. 1994). Although GSH has only slight flavor in water, when added to an umami solution or various types of food, it substantially enhances the thickness, continuity, and mouthfulness of the food (Ueda et al. 1997). In addition, GSH has been reported to be distributed in a variety of foods, such as meats, vegetables, and brewed beverages including beer, wine, and sake (rice wine). From the 2000s to the 2010s, the research group led by Professor Thomas Hofmann at the Technical University of Munich investigated kokumi substances in various foodstuffs. They identified γ-Glu-Leu, γ-Glu-Val, and γ-Glu-Cys-β-Ala (homoglutathione) as kokumi substances in edible beans (*Phaseolus vulgaris L.*) (Dunkel and Hofmann 2007), and γ-Glu-Glu, γ-Glu-Gly, γ-Glu-Gln, γ-Glu-Met, γ-Glu-Leu, γ-Glu-Val, and γ-Glu-His as kokumi substances in cheese (Toelstede et al. 2009; Toelstede and Hofmann 2009).

It has been reported that these kokumi γ-Glu-peptides, including GSH, are perceived by the calcium-sensing receptor (CaSR) expressed in taste cells. (Ohsu et al. 2010; Maruyama et al. 2012; Kitajima et al. 2026). Using human-CaSR-expressing HEK-293 cells, the CaSR activities of various kinds of γ-Glu-peptides were measured, and many kinds of kokumi γ-Glu-peptides were discovered (Ohsu et al. 2010; Amino et al. 2016). Among them, γ-Glu-Val-Gly was reported to be a potent kokumi substance (Ohsu et al. 2010), and it was indicated that the addition of this peptide to various foods enhanced the flavor characteristics such as complexity, thickness, mouthfulness, and continuity (Miyaki et al. 2015; Miyamura et al. 2015b; Kuroda 2024). The contents of γ-Glu-Val-Gly in various foods have been determined

by LC/MS/MS following derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate (AQC). γ-Glu-Val-Gly was quantified in scallop foods (Kuroda et al., 2012), and fermented foods (Kuroda et al. 2013a; Kuroda et al. 2013b; Miyamura et al. 2015a; Kuroda et al. 2020).

γ-Glu-Abu is one of the kokumi substances that were identified by screening using human-CaSR-expressing HEK-293 cells. The EC_{50} value of CaSR activation by γ-Glu-Abu (0.21 μM) has been reported to be lower than that of GSH (0.70 μM), suggesting that γ-Glu-Abu is a more potent CaSR agonist than GSH. In addition, γ-Glu-Abu has been approved as FEMA-GRAS (FEMA No. 4738), and its flavor has been described as kokumi, or kokumi-imparting (Marnett et al. 2013; Leffingwell and Leffingwell 2014). However, studies on the presence of γ-Glu-Abu in foods and beverages are limited. In the present study, an analytical method for the quantification of γ-Glu-Abu in foods was developed, and was applied to the quantification of this compound in beer.

Materials and methods

Chemicals

γ-Glu-Abu was purchased from Bachem AG (Bubendorf, Switzerland). The stable isotope of L-glutamic acid, UL-^{13}C, ^{15}N-L-glutamic acid was from Sigma-Aldrich (St Louis, MO). The AccQ Fluor reagent kit was supplied by Waters (Milford, MA). LC/MS grade methanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and HPLC grade formic acid (99%, Wako Pure Chemical Industries, Ltd.) were used as the mobile phase and in the derivatization procedure. Deionized water was prepared by a Milli-Q system (Millipore, Bellerica, MA).

Chemical synthesis of γ-Glu (UL-^{13}C, ^{15}N)-Abu

Chemicals for the synthesis of γ-Glu (UL-^{13}C, ^{15}N)-Abu were obtained from either Bachem AG, Watanabe Chemical industries Ltd. (Hiroshima, Japan), or Kokusan Chemical Co., Ltd. (Tokyo, Japan). Chemical synthesis of γ-Glu (UL-^{13}C, ^{15}N)-Abu was performed following the method of Amino et al. (2016). The scheme of chemical synthesis of γ-Glu (UL-^{13}C, ^{15}N)-Abu is indicated in Figure 1. Briefly, Cbz-glutamic acid (UL-^{13}C, ^{15}N) was prepared from L-glutamic acid (UL-^{13}C, ^{15}N) and N-carbobezoxyoxysuccinimide according to Nagasawa et al. (1973). N,N'-dicyclohexylcarbodiimide in ethyl acetate was added to the Cbz-Glu (UL-^{13}C, ^{15}N) solution and stirred overnight to yield the Cbz-Glu (UL-^{13}C, ^{15}N) anhydride. Then the Cbz-Glu (UL-^{13}C ,^{15}N) anhydride solution was added to Abu aqueous solution in the presence of sodium bicarbonate and N-hydroxysuccinimide. After the reaction, the reaction mixture was acidified with concentrated HCl. The product was extracted

into the organic phase, washed with aqueous NaCl solution, and the Cbz group was removed by hydrogenation over palladium catalyst. After filtration to remove the catalyst, the filtrate was concentrated, and methanol was added to afford crystals of γ-Glu (UL-^{13}C, ^{15}N)-Abu, which were collected by filtration and dried. The purity of obtained γ-Glu (UL-^{13}C, ^{15}N)-Abu was calculated as 86.9%.

Beer samples

Five brands of beer were purchased in Japanese markets. Table 1 indicates the country of origin, raw materials and alcohol contents of beer investigated in this study. Among them, four were Lager- type beer produced by bottom fermentation, while the remaining one was Ale-type beer produced by top fermentation. All samples were stored at 5℃ prior to the analysis.

Apparatus

Quantification of γ-Glu-Abu was carried out using an LC/MS/MS system. Chromatographic separation of AQC-derivatized γ-Glu-Abu was achieved on a Shimadzu Nexera X2 UHPLC system (Shimadzu Corp., Kyoto, Japan), which was outfitted with a binary pump, a degasser, an auto-sampler, and a column oven. Mass spectrometric detection was performed using a SCIEX Triple Quad^{TM} 6500 LC/MS/MS system (SCIEX).

Procedure for the derivatization

All beer samples were filtered through a 0.45-μm syringe filter (25-mm GD/X disposable filter device, Whatman PLC, Maidstone, UK) to eliminate insoluble

particulates. The γ-Glu-Abu content was determined via pre-column derivatization using 6-aminoquinoyl-N-hydroxysuccinimidyl-carbamate (AQC) reagent (AccQ Fluor reagent kit) followed by LC/MS/MS analysis. The AQC solution was prepared in acetonitrile according to the manufacturer's instructions. The derivatization was performed as follows: 10 μL of each beer sample was combined with 60 μL of borate buffer, 5 μL of internal standard solution containing 2 μM γ-Glu (UL-^{13}C, ^{15}N)-Abu, 20 μL of AQC reagent solution, and either 5 μL of deionized water (for unspiked samples) or 5 μL of standard γ-Glu-Abu solution at the same concentration (for recovery assessment). The reaction mixture was heated at 55°C for 10 min on a block heater. Subsequently, 200 μL of a quench solution - composed of the Amino-Tag Wako eluent (Wako Chemicals., Co. Ltd., Osaka, Japan) mixed with 0.1% aqueous formic acid (3 : 1) - was added, and the mixture was vortexed and centrifuged using a CHIBITAN-R type CT6M centrifuge (Koki Holdings Co., Ltd., Tokyo, Japan). The resulting supernatant was then subjected to LC/MS/MS analysis.

LC/MS/MS analysis of AQC-γ-Glu-Abu

The chromatographic conditions for the AQC-derivative of γ-Glu-Abu were as follows. An InertSustainPhenyl column (2.1 mm ID, 100 mm, 3 μm; GL Science, Tokyo, Japan) equipped with a guard column, InertSustainPhenyl (1.5 mm ID, 10 mm, 3 μm; GL Science), were employed, with the column temperature maintained at 40 °C. Mobile phase A consisted of the Amino-Tag Wako eluent (Wako Chemical Co., Ltd.) adjusted to pH 6.5 with aqueous ammonia, while mobile phase B was water / methanol (20 / 80). Gradient elution was performed as follows: 0 min (10% B), 8 min (30% B), 8.1–9.0 min (95% B), and 9.1–15 min (10% B). The flow rate

was maintained at 0.25 mL/min throughout the analysis. The injection volume was 5 μL.

Mass spectrometric detection was conducted using a triple quadrupole mass spectrometer. The turbo ion spray interface was operated in positive ionization mode at 5500 V and 650 °C. Detection was performed by the MRM (Multiple Reaction Monitoring) method. The MS parameters, CUR, GS1, GS2, CAD, DP, EP and CXP, were set to 30, 50, 30, 8, 131, 10 and 48, respectively. Dwell times were set to 120 ms for the SCIEX Triple Quad^{TM} 6500 LC/MS/MS system. Operations were controlled using Analyst software (version 1.7.2). The AQC-γ-Glu-Abu was detected via MRM method. Three MRM transitions were monitored for AQC-γ-Glu-Abu, and one transition was monitored for the internal standard. The MRM transitions (Q1/Q3) and corresponding collision energies (CE) for γ-Glu-Abu were 403.2/171.1 (41V), 403.2/145.1 (25V), and 403.2/116.1 (95 V), respectively. For the internal standard, AQC-γ-Glu (UL-^{13}C, ^{15}N)-Abu, the transition 409.2/171.1 was monitored with a CE of 41 V.

Statistical analysis

The obtained data was indicated as average ± standard deviation. The statistical analysis was conducted using JMP 9.1 version (SAS Institute).

Results

Development of the analytical method for γ-Glu-Abu quantification in beer

Analysis was conducted using LC/MS/MS, after derivatization with the AQC reagent. The analytical run time was 8 minutes and the cycle time was 15 minutes, including washing and equilibration. The optimized separation conditions are detailed in the experimental section. Under these conditions, the AQC-γ-Glu-Abu peak was observed at a retention time of 6.7 minutes.

For mass spectrometric detection, the MRM method was adopted for selective and sensitive detection. A precursor ion of m/z = 403.2 is equivalent to the univalent protonated ion of AQC-γ-Glu-Abu, and was set as the Q1 parameter for the MRM transition channels (Q1/Q3). Figure 2 indicates the product ion spectrum of AQC-γ-Glu-Abu using m / z 403.2 as the precursor ion. A product ion scan was performed in positive ionization mode with the precursor ion at m/z 403.2. The major product ions were observed at m/z 171.2, 145.2, and 116.2. The ions at m/z 171.2 and 145.2 were assigned to AQC-derived fragment ions, with theoretical m/z values of 171.1 and 145.1, respectively. These major product ions were selected as Q3 ions for the development of the MRM transitions. The top right diagram in Figure 2 shows the structures of fragment ions of m/z = 171.1 and 145.1. Fragment ion of m/z = 116.2 was assumed to be a cleavage fragment from the AQC moiety, however, the precise structure has not been clarified. Other MS parameters are outlined in experimental section. Figure 3A (i)-(iii) shows the peaks that correspond to the 1 μM standard γ-Glu-Abu, at each MRM transition, with Q3 values of 171.1 (i), 145.1 (ii), and 116.2 (iii). The relative peak intensities for standard solution at each channel were

calculated as 100 : 60 : 25.

Repeatability, linearity test and recovery assay

The repeatability of the present method was assessed by calculating the coefficient of variation (CV) for the analytical value of 1 μM γ-Glu-Abu standard solution (n = 6). In addition, to clarify the efficacy of approach using stable isotope (SI) as internal standard, the CV values obtained by analyses with or without SI as internal standard. The results were shown in Table 2. The CV value obtained by the analysis without SI standard was 5.80%, while that with SI standard was 1.65%. The linearity of the peak area ratio (γ-Glu-Abu / γ-Glu (UL-^{13}C, ^{15}N)-Abu) was confirmed in the range of 0.05 – 1 μM, and the correlation coefficient (*r*) was 0.9998 (Figure 4). The detection limit in the prepared sample was calculated to be 0.015 μM at the 403.2/171.1 (Q1/Q3) channel, based on a signal-to-noise ratio of 3. For the recovery assay, γ-Glu-Abu was spiked into the beer samples at a concentration of 0.1 μM (23.2 μg / L), and the samples were analyzed using the present method. The average recovery rates of three analyses for Beer A, Beer B, Beer C, Beer D, and Beer E were 90%, 100%, 98%, 93%, and 89%, respectively, with overall average recovery of 94% across all beer samples.

Identification of γ-Glu-Abu in the beer samples by LC/MS/MS analysis

The beer samples were analyzed by LC/MS/MS, as described in the experimental section. Figure 3B (i)-(iii) shows the mass chromatograms for the beer samples, monitored at three MRM transition channels at 171.1 (i), 145.1 (ii), and 116.2 (iii). Even using the selective MRM method, numerous other peaks were

observed at all channels, so it was difficult to conclusively identify any peaks as γ-Glu-Abu using only the retention times at one particular channel alone. The relative peak intensities of γ-Glu-Abu in beer A sample at MRM channel with Q3 values of 171.1, 145.1, and 116.1 were 100 : 58 : 26. The ratio of other beer samples (Beer B – E) ranged in 100 : 56 – 61 : 25 – 27 (Figure 5). These values from the beer samples agree well with those for standard solution (100 : 60 : 25).

Quantification of γ-Glu-Abu in the commercial beer by LC/MS/MS analysis

γ-Glu-Abu in beer was quantified by LC/MS/MS detection at the 403.2/171.1 (Q1/Q3) transition. The internal standard, γ-Glu (UL-^{13}C, ^{15}N)-Abu, was detected at the 409.2/171.1 transition. The γ-Glu-Abu contents were calculated from the ratio of the peak intensity of each sample to that of the internal standard. The results are presented in Table 3. All commercial beer samples tested in this study contained γ-Glu-Abu at concentrations ranging from 12.5 μg / L to 24.0 μg / L, corresponding to 0.054 μM to 0.103 μM.

Discussion

The analytical method for γ-Glu-Abu determination in beer was developed by modifying the analytical method for amino acids using a precolumn derivatization with AQC and an analysis with LC/MS/MS reported previously (Armenta et al. 2010). Shimbo et al. (2009) previously reported a simultaneous amino acid analysis method utilizing a similar strategy, based on precolumn derivatization with 3-aminopyridyl-N-hydroxysuccinimidyl carbamate (APDS) and *p*-(*N*,*N*,*N*-trimethylammonio)anilyl-*N*′-hydroxysuccinimidyl carbamate iodide (TAHS). These studies highlighted several advantages of this analytical approach: (1) the activated carbamate moiety reacts rapidly and selectively with amino groups under mild conditions; (2) the increased hydrophobicity of the derivatives extended their retention times in reversed-phase chromatography, thereby improving peak shapes and mitigating ion suppression or enhancement caused by matrix effects, which is beneficial for quantitative analysis; and (3) the backbone of the derivatization reagent tends to be ionized under positive detection mode, resulting in enhanced ionization efficiency.

In the present study, AQC was employed to capitalize on these advantages. However, numerous extraneous peaks were observed across all MRM transition channels in the chromatograms. This was presumably attributable to the detection of other peptides or amino-group-containing compounds sharing the same molecular weight as γ-Glu-Abu. Consequently, identification of γ-Glu-Abu based solely on retention times proved challenging. To address this issue, the relative peak intensities in each MRM channels, and the identification of AQC-γ-Glu-Abu peak was

conducted. As shown above, the relative peak intensities of standard γ-Glu-Abu at each MRM transition channel, with Q3 values of 171.1, 145.1, and 116.2 were calculated as 100 : 60 : 25, while those of γ-Glu-Abu in representative beer sample (Beer A) were 100 : 58 : 26. The other beer samples revealed similar ratio. These values obtained for the tested samples were in close agreement with those of the γ-Glu-Abu standard, suggesting the identification of γ-Glu-Abu in beer samples was achieved by the MRM approach.

Quantification was carried out via LC/MS/MS analysis, utilizing the most sensitive MRM transition at m/z = 403.2/171.1 (Q1/Q3). In our previous studies, γ-Glu-Val-Gly, a potent kokumi peptide, was identified and quantified in various food samples by a same methodology using ^{15}N-uniformly labeled Arg (^{15}N-UL-Arg) as an internal standard. Since preliminary tests indicated that the γ-Glu-Abu content in beer was below the ppm (mg/kg) level, we developed an analytical method based on stable isotope dilution. A stable isotope of γ-Glu-Abu, γ-Glu (UL-^{13}C, ^{15}N)-Abu, was chemically synthesized and used as an internal standard. The coefficient of variation (CV) for the analytical value of γ-Glu-Abu standard solution was 1.65%, indicating that the present method provides highly repeatable quantification. It has been reported that the stable isotope dilution is effective in overcoming matrix effects in quantification of biomolecules by HPLC-MS/MS method (Matuszewski et al. 2003). In addition, the average recovery rate of γ-Glu-Abu, calculated from peak areas, was 94 % for beer samples, demonstrating that this method enables accurate quantification.

This analytical method was applied for the quantification of γ-Glu-Abu in commercial beer. As presented in Table 3, all beer samples examined—

encompassing both Lager (bottom-fermentation) and Ale (top-fermentation) types—contained γ-Glu-Abu at concentrations ranging from 12.5 μg/L to 24.0 μg/L, corresponding to 0.054 μM and 0.103 μM in molar terms. These findings suggest that γ-Glu-Abu is widely distributed across beer beverages. Regarding kokumi peptides in beer, previous reports have documented the presence of glutathione (γ-Glu-Cys-Gly) and γ-Glu-Val-Gly in commercial beer. In beer, glutathione and γ-Glu-Val-Gly was reported to be contained at 1.6 mg / kg (Ueda et al.,1997), and 0.08 – 0.18 mg / kg (Miyamura et al. 2015), respectively. However, the occurrence of γ-Glu-Abu in beer has not been previously reported. A prior metabolomics study on rice wine (sake) indicated the existence of γ-Glu-Abu in Ginjo sake (Takahashi & Kohno, 2016), although its concentration was not reported therein. Therefore, to the best of our knowledge, this study represents the first report documenting the content of γ-Glu-Abu in brewed alcoholic beverages. The contribution of γ-Glu-Abu on the taste and flavor of foods including beer is now in progress in our laboratory.

In conclusion, the kokumi peptide γ-Glu-Abu was successfully quantified in commercial beer samples using LC/MS/MS following derivatization with AQC, with a stable isotope-labeled internal standard employed for accurate quantification. Further investigations aimed at the identification and quantification of γ-Glu-Abu in other food matrices are currently underway in our laboratory.

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Author contribution Junko Yamazaki: Writing – review & editing, Visualization, Methodology, Investigation, Data curation, Formal analysis. Motonaka Kuroda: Writing – original draft, Writing – review & editing, Visualization, Investigation, Data curation, Conceptualization. Kazuhiro Hayashi: Writing – review & editing, Conceptualization, Supervision. Masakazu Nakazawa: Writing – review & editing, Investigation. Seiji Kitajima: Writing – review & editing, Project administration, Supervision. Conceptualization.

Data availability

Data will be made available on request.

Declaration

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends.

Figure 1. Scheme for the chemical synthesis of γ-L-Glu (UL-^{13}C, ^{15}N)-L-Abu. The asterisks indicate stable isotope.

Figure 2. Product ion spectrum of AQC-derivatized γ-Glu-Abu obtained in positive ionization mode using m/z 403.2 as the precursor ion. Top right diagram indicates the structures of fragment ions of m/z = 171.1 and 145.1. Fragment ion of m/z = 116.2 was assumed to be a cleavage fragment from the AQC moiety, however, the precise structure has not been clarified.

Figure 3. Mass chromatograms obtained by MRM analysis are shown. X and Y axes indicate retention time (minutes) and intensity (cps), respectively. (A) 1.0 μM γ-Glu-Abu standard, (B) Beer A sample, a representative beer sample. MRM transition channels were 403.2/171.1 (i), 403.2/145.1 (ii), and 403.2/116.2 (iii). Triangles highlight the γ-Glu-Abu peaks.

Figure 4. Linearity of the analytical method. γ-Glu-Abu standard solution (0 – 1.0 μM) was analyzed.

Figure 5. Relative peak intensities normalized for that monitored at 403.2/171.1 (Q1/Q3) MRM channel. A, Beer A: B, Beer B; C, Beer C; D, Beer D; E, Beer E. Error bars indicate standard deviation over a triplicate analysis.

Table 1, Characteristics of commercial beer tested in this study

Sample	Origin of Country	Type	Raw materials	Alcohol content (v /v)
Beer A	Netherland	Lager (Bottom fermentation)	Malt, Hop	5.0%
Beer B	United States	Lager (Bottom fermentation)	Malt, Hop, Rice, Corn starch	5.0%
Beer C	Ireland	Ale (Top fermentation)	Malt, Hop, Barley	4.5%
Beer D	Czech Republic	Lager (Bottom fermentation)	Malt, Hop	4.4%
Beer E	Japan	Lager (Bottom fermentation)	Malt, Hop, Rice, Corn starch	5.0%

Table 2. Analytical results of γ-Glu-Abu standard solution (1μM) with or without stable isotope (SI) as an internal standard.

Sample	γ-Glu-Abu Peak area	Peak area ratio (γ-Glu-Abu / SI-γ-Glu-Abu)	SI-γ-Glu-Abu-Abu Peak area (reference)
	cps (x 10^6)		cps (x 10^6)
	without SI	with SI	
No.1	1.85	1.78	1.04
No.2	1.60	1.80	0.89
No.3	1.85	1.83	1.01
No.4	1.86	1.81	1.03
No.5	1.88	1.83	1.03
No.6	1.84	1.75	1.05
Average	1.81	1.80	1.01
STD	0.105	0.031	0.059
CV(%)	5.81	1.65	5.86

SI, stable isotope; STD, standard deviation; CV, coefficient of variation.

Table 3. Contents of γ-Glu-Abu in commercial beer

Sample	Origin of Country	Contents*	
		γ-Glu-Abu (μM)	γ-Glu-Abu (μg / L)
Beer A	Netherland	0.103 ± 0.0100	24.0 ± 2.4
Beer B	United States	0.0560 ± 0.0025	13.0 ± 0.6
Beer C	Ireland	0.0704 ± 0.0051	16.3 ± 1.2
Beer D	Czech Republic	0.0757 ± 0.0055	17.6 ± 1.3
Beer E	Japan	0.0538 ± 0.0032	12.5 ± 0.7

*: Average ± standard deviation

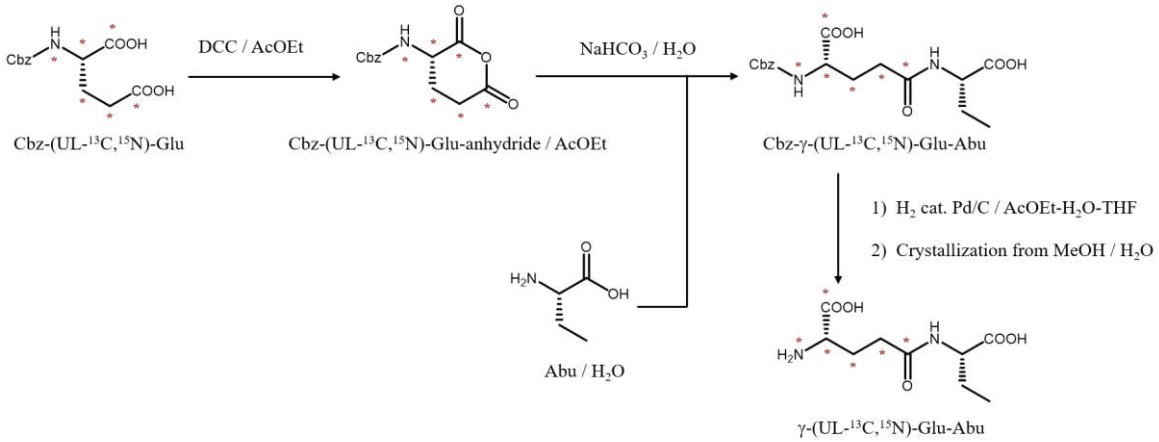

DCC / AcOEt
NaHCO3 / H2O
Cbz-(UL-13C,15N)-Glu
Cbz-(UL-13C,15N)-Glu-anhydride / AcOEt
Cbz-γ-(UL-13C,15N)-Glu-Abu
1) H2 cat. Pd/C / AcOEt-H2O-THF
2) Crystallization from MeOH / H2O
Abu / H2O
γ-(UL-13C,15N)-Glu-Abu

Fig. 1

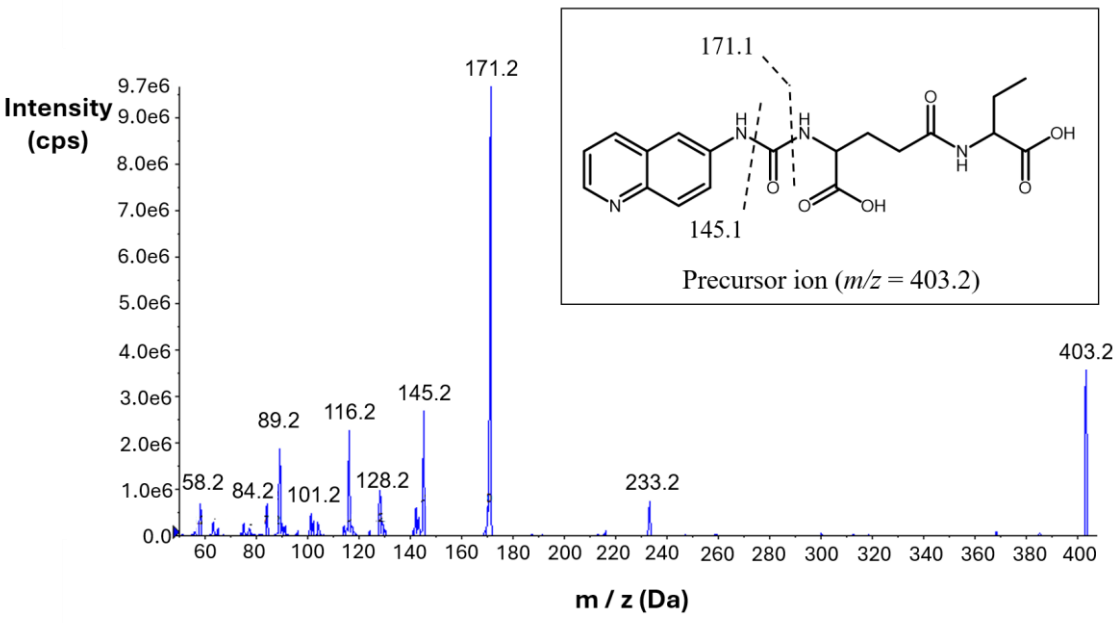

Intensity (cps)
m / z (Da)
171.2
403.2
145.2
116.2
89.2
233.2
128.2
58.2
84.2
101.2
171.1
145.1
Precursor ion (m/z = 403.2)

Fig. 2

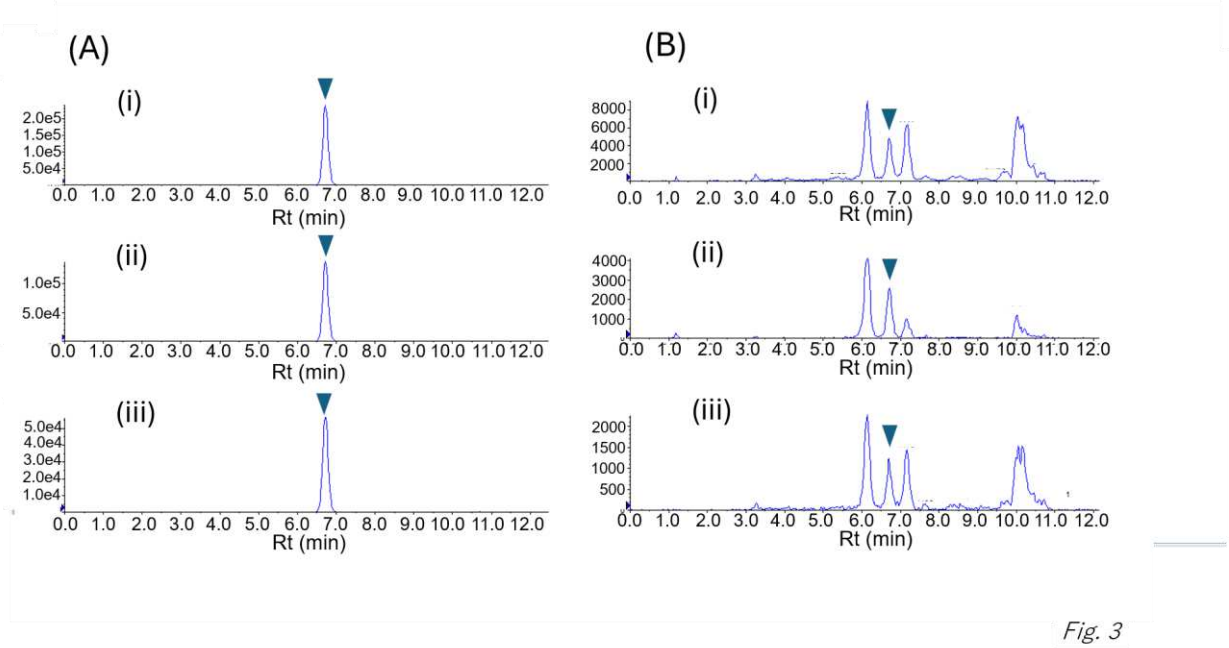

(A)
(i)
2.0e5
1.5e5
1.0e5
5.0e4
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)
(ii)
1.0e5
5.0e4
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)
(iii)
5.0e4
4.0e4
3.0e4
2.0e4
1.0e4
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)
(B)
(i)
8000
6000
4000
2000
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)
(ii)
4000
3000
2000
1000
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)
(iii)
2000
1500
1000
500
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
Rt (min)

Fig. 3

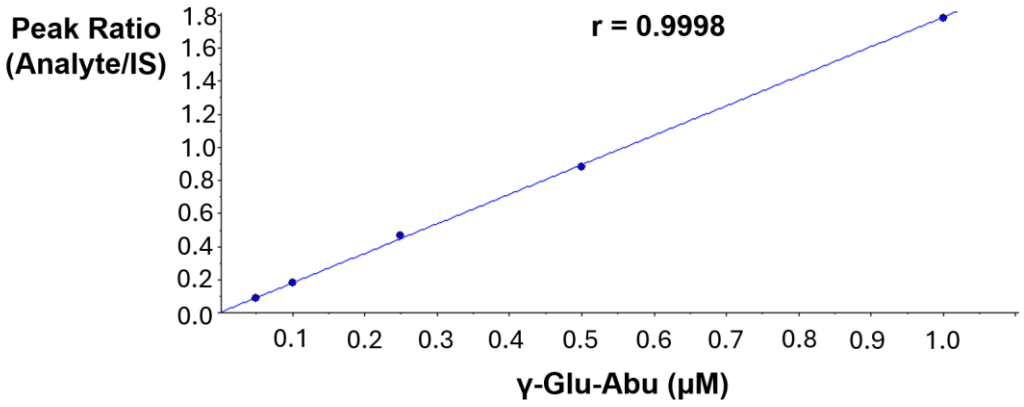

Peak Ratio
(Analyte/IS)
r = 0.9998
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
γ-Glu-Abu (μM)

Fig. 4

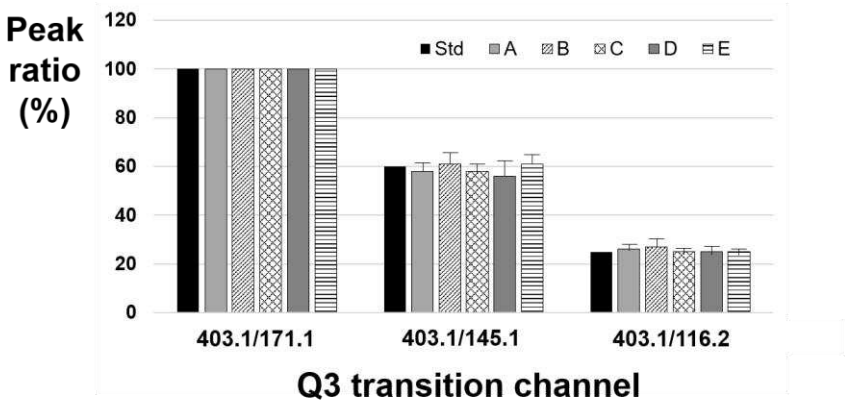

Peak ratio (%)
120
100
80
60
40
20
0
Std
A
B
C
D
E
403.1/171.1
403.1/145.1
403.1/116.2
Q3 transition channel

Fig. 5

1