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Analytical Methods

Reductive amination derivatization for the quantification of garlic components by isotope dilution analysis

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ABSTRACT

In this work, we synthesized internal standards for four garlic organosulfur compounds (OSCs) by reductive amination with ^{13}C, D_2-formaldehyde, and developed an isotope dilution analysis method to quantitate these organosulfur components in garlic samples. Internal standards were synthesized for internal absolute quantification of *S*-allylcysteine (SAC), *S*-allylcysteine sulfoxide (alliin), *S*-methylcysteine (SMC), and *S*-ethylcysteine (SEC). We used a multiple reaction monitoring (MRM) to detect ^{13}C, D_2-formaldehyde-modified OSCs by ultrahigh-performance liquid phase chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) and obtained MS spectra showing different ratios of ^{13}C, D_2-formaldehyde-modified and H_2-formaldehyde-modified compounds. The resulting labeled and unlabeled OSCs were exhibited correlation coefficient (R^2) ranged from 0.9989 to 0.9994, respectively. The average recoveries for four OSCs at three concentration levels ranged from 89% to 105%. By ^{13}C, D_2-formaldehyde and sodium cyanoborohydride, the reductive amination-based method can be utilized to generate novel internal standard for isotope dilution and to extend the quantitative application.

1. Introduction

Garlic (Allium sativum) has been widely used as a food or condiment for millennia (Petrovska & Cekovska, 2010). Garlic also exhibits antioxidant, anti-inflammatory, insecticidal, antimicrobial, antibacterial, antiviral, antioxidant, and anticancer activities due to the highest percentage of sulfur components (Ariga & Seki, 2006; Hunter, Caira, & Stellenboom, 2005; Leelarungrayub, Rattanapanone, Chanarat, & Gebicki, 2006; Li et al., 2015; Tedeschi et al., 2011). Contents of garlic, such as Diallyl disulfide (DADS) and its analogs, which act as free-radical scavengers and have shown efficacy against Alzheimer's disease and breast cancer, are considered to be the major antioxidative and anticancer agents (Capasso, 2013; Huang et al., 2015; Manral, Saini, Meena, & Tiwari, 2015). Furthermore, garlic is rich in the trace element selenium (Se), which is also reported to have antioxidative and antitumor properties (Poldma, Tonutare, Viitak, Luik, & Moor, 2011).

In our previous study, we demonstrated the determination of four primary amine organosulfur compounds (OSCs) in garlic (i.e., *S*-methylcysteine (SMC), *S*-ethylcysteine (SEC), *S*-allylcysteine (SAC), and *S*-allylcysteine sulfoxide (alliin)) by derivatizing them with isotopic formaldehydes via reductive amination using sodium cyanoborohydride ($NaBH_3CN$). In that work, we prepared four OSC derivatives with H_2-formaldehyde as standards and four OSC analogs with D_2-formaldehyde as internal standards (ISs) (Tsai, Liu, Lin, Huang, & Liang, 2016). Similar techniques, such as isotopic dansylation (Guo & Li, 2009; Su et al., 2016; Wu & Li, 2014) and dimethyl labeling (Boersema, Raijmakers, Lemeer, Mohammed, & Heck, 2009; Hsu, Huang, Chow, & Chen, 2003) are utilized in metabolomics and proteomics research. For example, dimethyl labeling has been used to modify primary amines to produce standards and ISs for quantification of tamoxifen and its metabolites (Liang et al., 2016). In order to quantitate components in foods, which is a crucial activity in fields such as foodomics, it is important to accurately ascertain specific component levels and how they change under different conditions over time (Cifuentes, 2014; Gallo & Ferranti, 2016; Vallverdu-Queralt & Lamuela-Raventos, 2015). Unfortunately, it is often difficult to obtain

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accurate calibration curves with external standards for certain foodstuffs due to matrix effects. However, using the isotope dilution method which uses ISs, can reduce matrix effect and allow accurate determination of food components in otherwise problematic matrices (Yarita, Aoyagi, & Otake, 2015; Zhou, Wu, Li, Hu, & Tam, 2016).

In this study, we used the isotope dilution method to quantitate SMC, SEC, SAC, and alliin in garlic. We used ^{13}C, D_2-formaldehyde to generate isotope dilution ISs by reductive amination and developed a detection method using ultrahigh-performance liquid-phase chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC–MS/MS). Fragmented-ion searching and multiple reaction monitoring (MRM) was used to confirm the presence of the OSC components.

2. Materials and methods

2.1. Chemicals and materials

^{13}C, D_2-formaldehyde was obtained from Isotec Corp. (Miamisburg, OH, USA). SAC can be purchased from TCI (Tokyo Chemical Industry Co., Ltd. Tokyo, Japan). Garlic components including alliin, SEC and SMC were bought from Sigma (St. Louis, MO, USA), along with H_2-formaldehyde solution (36.5–38% in H_2O). Sodium cyanoborohydride ($NaBH_3CN$), formic acid (FA, 98–100%), trifluoroacetic acid (TFA) and sodium acetate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (MeCN, hypergrade for LCMS) and methanol (MeOH, hypergrade for LCMS) were bought from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Deionized water with a resistance of 18.3 MΩ cm was manufactured from a Milli-Q purification system (Millipore Co. Bedford, MA, USA). Polytetrafluoroethylene (PTFE) filters (pore size: 0.22 μm) were purchased from Millipore Co.

2.2. Instrumentation and software

The Xcalibur software package (version 2.2, Thermo Finnigan Inc., San Jose, CA, USA) was utilized to control the instruments including UHPLC–MS/MS apparatus and an autosampler along with acquiring and processing the raw LC–MS/MS data. Xcalibur LCquan software package (version 2.7, Thermo Finnigan Inc., San Jose, CA, USA) was utilized for statistical analysis. Injection and separation were achieved with an Acella 1250 autosampler (Thermo Fisher Scientific Inc., Waltham, MA, USA) linked to a 10 μL sample loop, and a Shiseido Capcell Pak MG II column (C18, i.d. 1.5 mm × 150 mm, 3 μm, Tokyo, Japan), respectively. The UHPLC mobile phases: 0.1% FA in water (A) and 0.1% FA in 100% MeCN (B) under the following linear gradient: 2% (B) for 1 min; 2–30% (B) over 7 min; 30–98% (B) over 1 min; and 98% (B) for 1 min, was set at the flow rate with 150 μL/min. The UHPLC separated system was performed with a Thermo Finnigan Acella 1250 UHPLC pump (Thermo Fisher Scientific Inc., Waltham, MA, USA), and was equipped with a micro electrospray ionization (ESI) ion source in positive ion mode to connect to a triple quadrupole MS system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The ESI applied voltage for MS was set at 2200 V with the vaporizing and capillary temperatures set at 250 and 300 °C, respectively. The auxiliary gas and sheath gas pressures were maintained at 10 and 35 absolute units, respectively, and the collision pressure was set at 1.0 unit with the collision energy adjusted to 22 V. The MS survey scan mode utilized MRM to select the precursor and product ion m/z values and the MRM fragmentation transitions are listed in Table 1.

2.3. Preparation of isotope diluted standards

Four OSCs were dissolved in 20 μL of ethanol, and the individual OSCs solutions (500 μg/mL) were prepared in volumetric flasks with deionized water. Individual OSC solutions (20 μL) were transferred into Eppendorf tubes, and the pH of the buffer solutions was adjusted to 5.6 using a sodium acetate buffer (160 μL). The OSC samples were reacted for 5 min with 10 μL of 4% ^{13}C, D_2-formaldehyde and then reduced for 1 h using 0.6 M $NaBH_3CN$. A schematic of the generation of isotope diluted standards is represented in Fig. 1.

2.4. MRM transitions of the four OSCs

The four ^{13}C, D_2-formaldehyde-modified garlic standards were diluted to 2.0 μg/mL in 50% MeCN solution and infused into the MS in order to determine their MRM transitions based on the values of the precursor ion and the product ions, as detected by MS/MS fragmentation. The MS/MS fragmentations of the four ^{13}C, D_2-formaldehyde-modified OSCs are given in Fig. 2. The modified OSC MRMs were set as follows: m/z 170 → 73 and 170 → 119 for SMC; 184 → 87 and 184 → 133 for SEC; 196 → 73 and 196 → 145 for SAC; and 212 → 76 and 212 → 122 for alliin. For the H_2-formaldehyde-modified OSCs compounds, the MRM transitions were set as follows: m/z 164 → 73 and 164 → 119 for SMC; 178 → 87 and 178 → 133 for SEC; 190 → 73 and 190 → 145 for SAC; and 206 → 70 and 206 → 116 for alliin. The MRM transitions are listed in Table 1.

2.5. Method validation

SMC, SEC, SAC, and alliin were modified using H_2-formaldehyde or ^{13}C, D_2-formaldehyde, and the labeled and non-labeled products for each OSC were mixed at ratios of 20:1, 10:1, 5:1, 1:1, 1:5, 1:10, and 1:20, to investigate the linear range of the quantitative analysis by our reductive amination method (see Fig. 4). The y axis in Fig. 4 shows the ratios 1:5 (0.2), 1:10 (0.1), and 1:20 (0.05), at −5, −10 and −20, respectively.

Table 1
Characteristics of OSCs including molecular formula, molecular weight, and MRM transitions with m/z values of the precursor ion and product ions.

Name	Molecular formula	Molecular weight (Da)	MRM transitions	
			Precursor ion	Product ions
H_4-SMC	$C_6H_{13}NO_2S$	163.23	164	73, 119
$^{13}C_2$, D_4-SMC	$C_4^{13}C_2H_9D_4NO_2S$	169.25	170	73, 119
H_4-SEC	$C_7H_{15}NO_2S$	177.26	178	87, 133
$^{13}C_2$, D_4-SEC	$C_5^{13}C_2H_{11}D_4NO_2S$	183.27	184	87, 133
H_4-SAC	$C_8H_{15}NO_2S$	189.27	190	73, 145
$^{13}C_2$, D_4-SAC	$C_6^{13}C_2H_{11}D_4NO_2S$	195.29	196	73, 145
H_4-Alliin	$C_8H_{15}NO_3S$	205.27	206	70, 116
$^{13}C_2$, D_4-Alliin	$C_6^{13}C_2H_{11}D_4NO_3S$	211.28	212	76, 122