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Analytical Methods

Quantification of selenomethionine in plasma using UPLC–MS/MS after the oral administration of selenium-enriched yeast to rats

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ABSTRACT

The *in vivo* determination of selenomethionine (SeMet) converted from selenium-enriched yeast (SeY) rather than the determination of *in vitro* hydrolyzed SeMet is a better parameter for the evaluation of SeY quality. A UPLC–MS/MS method was developed for the quantification of SeMet in rat plasma and the oral bioavailability of SeMet converted from SeY in rats. After a simple extraction with perchloric acid, SeMet and the internal standard methylselenocysteine (MSC) were separated on a C18 column with isocratic elution of water:acetonitrile:formic acid (99:1:0.1, *v/v/v*) and detected in the multiple reaction monitoring mode. The method was accurate (92.6–104.3%) and precise (1.8–11.0%), and the recovery was 79.4–95.4%. It was successfully applied to pharmacokinetic and bioavailability studies of SeY in rats following the intravenous administration of SeMet and intragastric administration of SeY. SeMet *in vivo*, converted from SeY, is reported for the first time, and the results suggested that the SeY bioavailability in rats is 87%.

1. Introduction

For approximately half a century selenium (Se) has been recognized as an essential trace element for mammals (Schwarz & Foltz, 1957). Selenium is involved in various biochemical and physiological functions in mammalian systems, such as immunity, antioxidant function, thyroid metabolism, and reproduction, which not only depend on the amount of Se but also on speciation of Se (Kubachka et al., 2017). The diet is the major source of Se, and the intake of Se is increased through dietary supplements or fortified foods because suboptimal dietary levels are relatively common. Selenium-containing supplements usually include inorganic Se (selenite and selenate), selenocysteine, selenomethionine (SeMet), methylselenocysteine (MSC) and selenium-enriched yeast (SeY) (Kubachka et al., 2017). Compared to inorganic Se and other organic Se, SeY is the most popular due to its rapid growth, ease of culture, high Se accumulation capacity, and high Se bioavailability (Anan, Mikami, Tsuji, & Ogra, 2011). During the bioconversion of Se in SeY, inorganic Se can enter the sulfur assimilation pathway and is, ultimately, transformed into SeMet, which is non-specifically incorporated into the protein in place of methionine at a rate of 30% (McSheehy, Yang, Sturgeon, & Mester, 2005). Most SeY products contain 97–99% of organic selenium in the total Se. In SeY, protein-bound SeMet, the most effective and active Se compound, is the predominant form of Se (Schrauzer, 2003) and a proper parameter for determining the effectiveness and bioavailability of Se in the yeast (Fagan et al., 2015). Numerous studies in humans and animals have indicated that the Se bioavailabilities of SeY and SeMet are approximately 1.4–2-fold higher than those of inorganic Se forms (Davis et al., 2017; Takahashi, Suzuki, & Ogra, 2017).

The identification and quantification of the total SeMet content in SeY have been used to evaluate SeY quality. The degradation of all proteins into their constituent amino acids via enzymolysis or hydrolysis is required for the quantification of SeMet. However, a disadvantage of this approach is that the effects of Se deposition on the subsequent oral bioavailability are not considered. SeMet may not be fully released from the proteins, peptides or other cellular components with which it is associated, and Se-containing proteins/peptides are labile and prone to degradation after being extracted, ultimately leading to an underestimation of the total SeMet content (Gammelgaard, Cornett, Olsen, Bendahl, & Hansen, 2003; Polatajko, Banas, Encinar, & Szpunar, 2005). Therefore, the *in vivo* level of SeMet after the oral administration of SeY is the optimal parameter for the evaluation of SeY quality.

Usually, the speciation analysis of Se compounds is achieved by

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hyphenated techniques interfacing a separation module (HPLC, GC or CE) with an element-specific detector (ICP-MS, AAS or AFS) (Anan, Nakajima, & Ogra, 2015). However, one obvious disadvantage of those systems is the loss of structural information during the element-specific detection process, resulting in the necessity of elemental species standards for the identification of species, which completely depends on a comparison of retention times. LC-ICP-MS is the primarily used analytical technique for Se speciation in biological samples due to its sensitivity and robustness, and several LC-ICP-MS methods have been reported for the detection of SeMet in plasma or serum (Encinar, Schaumloffel, Ogra, & Lobinski, 2004; Flouda et al., 2016; Kokarnig et al., 2015; Richie et al., 2014; Takahashi et al., 2017). However, the identification of Se compounds by LC-ICP-MS is only possible when the retention times of samples match those of some Se standard compounds since LC-ICP-MS provides only elemental information for Se compounds. In most cases, real samples often contain Se species that are not commercially available as standards or have not been identified. Therefore, it is imperative to develop a different approach including the use of a detector that provides structural information about the species for the identification of these species. Electrospray ionization (ESI)-mass spectrometry can provide structural information because ESI is a softer technique than ICP. For the identification and quantification of SeMet in biological matrices such as plasma, only one gas chromatography-mass spectrometry method was reported to measure the SeMet in plasma, which involved laborious and costly precolumn derivatization of the selenium species to render a volatile product for separation on the GC column (Matsukawa et al., 2011).

In the present investigation, we established a sensitive and reliable ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method employing a simple one-step extraction for the quantification of SeMet in rat plasma. Additionally, this method was successively applied to pharmacokinetic and bioavailability studies of SeMet in rats following the intravenous administration of SeMet and intragastric administration of SeY. *In vivo* SeMet converted from SeY was reported and quantified for the first time.

2. Materials and methods

2.1. Materials

SeY with a Se content of 2166.8 μg g^{-1} was provided by the Angel Yeast Co. Ltd. (Yichang, China). L-SeMet (purity ≥98%) and methylselenocysteine (purity ≥95%) as an internal standard (IS) were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade acetonitrile, formic acid and water were obtained from Fisher Scientific Inc. (Geel, Belgium). All other reagents were commercially available and of analytical grade.

2.2. Chromatographic conditions

The Waters Acquity XEVO TQ UPLC system consisted of a binary pump solvent management system, an online degasser and an autosampler. The chromatographic analysis was performed on a Waters Acquity UPLC HSS T3 C18 column (2.1 mm × 100 mm, 1.8 μm particle size) using a mobile phase of water: acetonitrile: formic acid (99:1:0.1, *v/v/v*) delivered at a flow rate of 0.3 mL min^{-1}. The total run time was 3 min, the auto-sampler was conditioned at 15 °C and the injection volume was 5 μL.

2.3. Mass spectrometric conditions

The Acquity UPLC system was coupled to a TQ mass spectrometer operated in the positive ESI mode. Quantification was performed using the multiple reaction monitoring (MRM) mode to monitor the precursor-product ion transitions of *m/z* 198.0 → 181.1 for SeMet and 184.0 → 167.0 for MSC. The optimal instrument operating conditions were as follows: capillary voltage, 3.5 kV; source temperature, 120 °C; and desolvation temperature, 350 °C. The nitrogen gas flows were 700 L/h and 50 L/h for the desolvation and cone gases, respectively. Argon was employed as the collision gas with a flow rate of 0.15 mL min^{-1}. The cone voltage was set at 14 and 12 V for SeMet and MSC, respectively. The collision voltage values of SeMet and MSC were 12 and 14 eV, respectively. The scan time was 0.2 s per transition.

2.4. Preparation of stock and standard working solutions

Stock solutions of SeMet (1 mg mL^{-1}) and MSC (1 mg mL^{-1}) were prepared in water and stored at 4 °C. Working standard solutions of SeMet were prepared in water at concentrations from 20 to 2000 ng mL^{-1}. The working IS solution was 4 μg mL^{-1}, which was made by diluting the stock solution of MSC with water.

2.5. Sample preparation

The calibration standard samples for SeMet were prepared by spiking 90 μL of blank rat plasma with 10 μL of SeMet working solution. The ultimate concentrations of SeMet in rat plasma were 2–200 ng mL^{-1} including the lower limit of quantification (LLOQ) concentration. The LLOQ in rat plasma was defined as the lowest concentration detectable with a signal-to-noise ratio of at least 10, an accuracy of 80–120% and a precision no greater than 20%. Quality control (QC) samples in rat plasma were prepared in a similar way with three SeMet concentrations of 5, 80 and 150 ng mL^{-1}.

A 10 μL aliquot of MSC solution was added to 100 μL of plasma. Following the addition of 100 μL of 6.2% (*w/v*) perchloric acid aqueous solution, the sample was vortex-mixed for 2 min and centrifuged at 12,000*g* for 5 min. The supernatant was transferred to an autosampler vial, and a 5-μL aliquot of the sample was injected into the UPLC–MS/MS.

2.6. Method validation

The method was validated for its selectivity, matrix effect, recovery, accuracy, precision, linearity and stability according to our previous methods (Zhang, 2015, 2016).

2.7. Pharmacokinetic and bioavailability studies of SeMet and SeY

All procedures involving animals were executed according to the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, eighth edition in 2011) and were approved by our Institutional Animal Care and Use Committee. Blank rat plasma was obtained from normal rats and stored at −70 °C. Twelve adult Sprague-Dawley rats weighing 295 ± 11 g were obtained from the Academy of Military Medical Sciences Animals Center (Beijing, China), and they were housed in temperature-controlled (22 ± 2 °C) and relative humidity-controlled (50 ± 10%) rooms with a 12 h light/dark photoperiod regime for at least 7 days prior to the study. The animals had free access to demineralized water and Se-deficient diets (the content of Se was < 0.02 mg/kg) with all other nutrients at the standard levels according to the AIN-93M formula. Before the experiment, the rats were fasted overnight for 12 h and were allowed free access to food 4 h after the administration. Both before and during the experiment, water was provided *ad libitum*. Those rats were equally assigned into two groups, in which each group included three males and three females. SeMet was dissolved in 0.9% (w/v) physiological saline to produce a concentration of 2 μg Se mL^{-1} (Se equivalent), and the solution was intravenously (*i.v.*) injected into the tail veins of rats under mild isoflurane anesthesia at a single dose of 8 μg Se kg^{-1}. One gram of carboxymethylcellulose sodium (CMC-Na) was dispersed in 99 mL of water and stirred until dissolved to obtain a 1% (w/v) CMC-Na aqueous solution. SeY was suspended in the 1% (w/v) CMC-Na aqueous solution to obtain a level