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Evaluation of selenium in dietary supplements using elemental speciation

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ABSTRACT

Selenium-enriched dietary supplements containing various selenium compounds are readily available to consumers. To ensure proper selenium intake and consumer confidence, these dietary supplements must be safe and have accurate label claims. Varying properties among selenium species requires information beyond total selenium concentration to fully evaluate health risk/benefits

A LC-ICP-MS method was developed and multiple extraction methods were implemented for targeted analysis of common "seleno-amino acids" and related oxidation products, selenate, selenite, and other species relatable to the quality and/or accuracy of the labeled selenium ingredients. Ultimately, a heated water extraction was applied to recover selenium species from non-selenized yeast supplements in capsule, tablet, and liquid forms. For selenized yeast supplements, inorganic selenium was monitored as a means of assessing selenium yeast quality. A variety of commercially available selenium supplements were evaluated and discrepancies between labeled ingredients and detected species were noted.

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1. Introduction

Within the scientific community, the essential element selenium (Pinsent, 1954) continues to be a topic of interest and debate. The health impact of selenium is multifaceted and complicated by its narrow range between toxic and beneficial effects, as well as its uneven distribution among the earth's crust (Rayman, 2004, 2008). Currently in the United States, the Institute of Medicine and other entities recommend 55 μg/day for adults (Dietary Reference Intake (DRI), 2000; Hurst et al., 2013). Selenium plays a vital role as an antioxidant, in proper organ function and development, and although recently questioned, (Vinceti et al., 2014) is a possible chemopreventor (Clark et al., 1996). The Tolerable Upper Intake Level for adults is 400 μg/day with some evidence that selenium intake above this can increase occurrence of alopecia, dermatitis and type-2 diabetes (Dietary Reference Intake (DRI), 2000; Rayman, 2012).

Regardless of definitive evidence for selenium's health benefits/risks, the importance of controlling selenium intake, coupled with its possible chemopreventive nature, has no doubt contributed to an increase in marketing selenium-enriched dietary supplements. These supplements are commercially available to the public from a plethora of sources, in numerous dosage forms, and containing a variety of selenium species. The various forms of selenium dictate the role and efficacy of the functions it carries out (Fairweather-Tait, Collings, & Hurst, 2010).

Supplements commonly include selenium in the forms selenite (Se(IV)) or selenate (Se(VI)) (referred to as inorganic selenium (iSe)), selenomethionine (SeMet), Se-methylselenocysteine (MeSeCys), and selenized yeast (Se-yeast). Specifics vary among studies, but in general, inorganic forms of selenium (iSe) are absorbed less effectively than the organic forms, with the latter being slightly less toxic (Dietary Reference Intake (DRI), 2000; Tiwary, Stegelmeier, Panter, James, & Hall, 2006). Selenocysteine (SeCys) and SeMet have been shown to be incorporated into proteins in humans and plants (Cheajesadagul, Bianga, Arnaudguilhem, Lobinski, & Szpunar, 2014). To our knowledge, SeCys is not commercially available as a standard or dietary

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supplement. Recent debates have suggested reported findings of selenocystine ($SeCys_2$, a commercially available standard, not typically listed as a dietary supplement ingredient) may be incorrect, including possible confusion with SeCys (Dernovics & Lobinski, 2008). There are other reports of common selenium species in supplements such as phenylselenocysteine, methaneseleninic acid (methylseleninic acid, MSeA), and selenocyanate (Gosetti et al., 2007), but obtaining supplements labeled to contain these ingredients proved to be difficult.

Se-yeast has been reported to contain over 60 unique selenium species (Arnaudguilhem et al., 2012) and for virtually all yeast types tested, SeMet was the most abundant. The lack of a formal definition of Se-yeast and the extensive variation of types complicates any verification. Bierla, Szpunar, Yiannikouris, and Lobinski (2012) noted that the typical criteria for Se-yeast of industrial use is >60% SeMet and <2% iSe of the total selenium. Currently, FDA regulates Se-yeast related to animal feed additives for which iSe must be <2% of total selenium (Selenium, 2015). Inorganic selenium content >2% could indicate a lower quality Se-yeast (Bierla et al., 2012).

Recent publications have noted that many selenium supplements available to the public either do not contain the labeled amount of selenium or lack the labeled form (Bakirdere, Volkan, & Ataman, 2015; Gosetti et al., 2007; Niedzielski et al., 2016). Total selenium content can routinely be determined using inductively coupled plasma mass spectrometric (ICP-MS) analysis. A greater challenge lies in quantifying the individual species, which is often complicated by the conversion that may occur during storage or extraction of the supplement. Many publications have examined selenium speciation in various matrices and this topic has been well reviewed (Wuilloud & Berton, 2014).

For dietary supplements, a variety of extraction/dissolution procedures have been employed, including water, acid, and enzymatic mixtures. Enzymatic extracts are commonly used for Se-yeast samples, but batch variability of proteases can lead to inconsistent recoveries and require rigorous control of conditions for optimal operation (Bierla et al., 2012). Bakirdere et al. (2015) reported that water, dilute hydrochloric acid, and enzymatic extractions all achieved comparable extraction efficiencies for non-Se-yeast labeled tablets. Methane sulfonic acid (MSA) has previously been demonstrated to extract SeMet from the selenized yeast reference material SELM-1 (Mester et al., 2006) and other Se-yeast sources (Barrientos, Wrobel, Guzman, Escobosa, & Wrobel, 2016). A basic extraction using sodium hydroxide (NaOH) was applied to selenium-enriched animal feeds with some success (Stadlover, Sager, & Irgolic, 2001). One primary goal of this project was to use a one-step extraction to quantitatively extract the compounds of interest while preserving their *in situ* forms; therefore multiple extractions were explored each with various limitations as subsequently explained.

Following extraction, multiple separations are typically needed to resolve the selenium species of interest using ICP-MS for detection. The most commonly used separation methods are reversed phase ion pairing (RP-IP), anion exchange, and cation exchange. The work by Niedzielski et al. (2016) examined 86 selenium fortified supplements for iSe versus the organic forms, but they did not distinguish among organic species. The report by Bakirdere et al. (2015) separated Se(IV), Se(VI), SeMet and $SeCys_2$, while Hsieh and Jiang (2013) included MeSeCys; neither report accounted for the oxidized form of SeMet (SeOMet) or other related degradation products. Gosetti et al. (2007) examined a few supplements using LC-MS/MS, but multiple extractions, chromatographic separations, and relatively long analysis time made this method less attractive as a routine method. In general, anion exchange methods have difficulty resolving organic selenium compounds while RP-IP methods are limited by the resolution of iSe and $SeCys_2$. While many research groups have utilized similar methodology to examine yeast based supplements, often with the objective of identifying the gamut of interesting selenium species (Goenaga Infante et al., 2004; Larsen, Hansen, Fan, & Vahl, 2001; Larsen et al., 2004); this approach is not desirable for routine analysis. With a few exceptions, separations published in the selenium supplement field have not considered common oxidation products or adequate support for $SeCys_2$ identification. The other primary goal of this work is to develop a simple, robust, and more efficient method for routine detection of targeted selenium species commonly present or claimed present in selenium-enriched dietary supplements.

2. Material and methods

2.1. Reagents and standards

Water used throughout the experiments was ultrapure deionized water with resistance >18 MΩ·cm obtained from a Milli-Q system (Bedford, MA, USA). All chemicals were reagent grade or higher. For total selenium analysis, samples were digested using Optima grade concentrated nitric acid and 30% (w/w) hydrogen peroxide both from Fisher Scientific (Pittsburgh, PA, USA). Individual standards of selenium species, including Se(IV) and Se(VI), were obtained from Inorganic Ventures (Christiansburg, VA, USA) at concentrations ca. 10 μg/mL. SeMet and $SeCys_2$ were purchased from Acros organics (Fisher Scientific) and stock solutions were prepared in degassed water and 0.1% hydrochloric acid (Optima grade, Fisher Scientific), respectively. Se-methylselenocysteine hydrochloride (MeSeCys) was obtained from Sigma (St Louis, MO, USA) then diluted in degassed water. Methylselenomethionine (MeSeMet) was prepared similar to Wrobel, Wrobel, Kannamkumarath, and Caruso (2003) and SeOMet was synthesized based on Larsen et al. (2004). The oxidized form of Se-methylselenocysteine (MeSeOCys) and MSeA were prepared by adding 50 μL of H_2O_2 to 5 mL of 10 μg mL^{-1} MeSeCys; after 20 min, the primary product was MeSeOCys and after 16 h MSeA was the primary product. The concentrations of the selenium species are described on a total selenium concentration basis rather than the molecule as a whole. Total selenium concentrations of each stock solution were verified versus NIST traceable selenium standards (MES-2A, Spex Certiprep, Metuchen, NJ, USA) and NIST 1643e (NIST, Gaithersburg, MD, USA). For speciation analysis, mobile phase components included ammonium acetate (Fisher Scientific), tetrabutylammonium hydroxide (TBAH, Aldrich, Milwaukee, WI, USA), acetic acid, methanol (Fisher Scientific), pyridine (Fisher Scientific), and formic acid (Fisher Scientific). Sodium hydroxide (NaOH), hydrochloric acid (HCl), hexane and MSA were used for extraction and obtained from Fisher Scientific. For the bioaccessibility extraction, pepsin, pancreatin, bile salt, and ammonium carbonate were obtained from Sigma.

2.2. Samples

A total of 13 dietary supplements labeled to be selenium-enriched were purchased through various reputable online retailers; peer to peer transactions were avoided. The labeled selenium ingredients included SeMet, MeSeCys, selenium amino acid complex or chelates, selenium yeast, selenate, or selenite, and are summarized in Table 1. The form of the supplement included liquids, a tablet, capsules, and softgels. Additionally, SELM-1 (NRC, Ottawa, Ontario, Canada) was used as a reference, primarily for total selenium analysis. SELM-1 is certified for SeMet, but the SeMet is incorporated into the yeast proteins. To our knowledge, no applicable non-Se-yeast SRMs were available for selenium speciation. Unless otherwise noted, intact individual dosage forms