Comparison of three digestion methods for determination of selenium in green tea samples using fluorescence spectrometry

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Abstract. Various digestions are available for the determination of selenium (Se) in environmental samples. This study aimed to investigate the most effective digestion method for the determination of Se in green tea samples that effective, eco-friendly, and low cost. Samples were digested with three different acid compositions, the aqua regia method (a mixture of nitric acid-hydrochloric acids HNO₃-HCl) in a ratio 1:3, only nitric acid HNO₃, and reverse aqua regia method (a mixture of nitric acid-hydrochloric acids HNO₃-HCl) in a ratio 3:1. Analysis of Se was conducted using fluorescence spectrometry after derivation by 2,3-diamino naphthalene (DAN). The results show that the aqua regia method was the highest fluorescence intensity. It had given a significant fluorescence intensity of 9.0510 a.u. for 2 mL sample, 26.3689 a.u. for 5 mL sample, 54.4308 a.u. for 10 mL sample, respectively. In addition, the aqua regia digestion resulted in the selenium form in the sample has been converted to tetravalent selenium, and there was a silicone compound as precipitation can be found after digestion. The aqua regia method provides a highly effective digestion method for selenium in green tea samples under fluorescence spectroscopic investigation.

1. Introduction
China is one of the largest countries in the world for planting and producing tea [1]. Chinese people believed that drinking tea has a lot of functions, such as medicine, tonic, beverage, food, intake energy, and improve well-being [2]. There are several kinds of tea, one of them is green tea. Green tea extracts have antioxidant activities due to its polyphenol compound [3]. Green tea contains the nutrient, such as polyphenols, amino acids, vitamins, and elements. Most researchers found the minerals in green tea, such as P, Ca, Na, F, Mn, Zn, Cu, and Se with the lowest concentration [4].

Selenium plays an important role in the human body. Generally, the determination of selenium is often difficult and requires techniques to obtain high sensitivity and selectivity. Due to the low concentration of selenium in the sample, preparation and digestion sample is important. Acid digestions are used and have a useful function to reduce the matrix effect. Combination of HNO₃:HCl (Reversed Aqua Regia, 3:1) is excellent in the Cold-Block digestion method for Au in four different Standard Reference Certified Materials [5]. A combination of HNO₃:HClO₃:HF as a reagent digestion method for selenium determination in the bovine sample was not recommended due to the potentially hazardous of perchloric acid [6]. Different heavy metal has a different species and its character. So the composition and types of reagent for the digestion method that eco-friendly and low cost is an important step in the experimental analysis.

Not only the preparation and digestion sample but also the method for determination of selenium
affected to obtain the precious amount of selenium. There are several methods for determination selenium, such as Capillary Microextraction Inductively Coupled Plasma-Mass Spectrometry (CME-ICP-MS) [7], Hydride Generation Atomic Absorption Spectrometry (HGAAS) [8], High-Performance Liquid Chromatography (HPLC) [9], Flame atomic absorption spectrometry (FAAS) [10]. The fluorescence spectroscopy method has the advantages: low cost, high detection sensitivity, easy operation. To conduct the fluorescence spectroscopy method need an appropriate fluorophore compound. One of the possible fluorophores for the detection of Se was 2,3-diaminonaphthalene (DAN) that it will react with tetravalent selenium to generate benzopiazselenol (BPS) complex [11]. Therefore, the aim of this research to investigate the combination of reagent digestion methods for the determination of trace level of selenium in green tea samples using fluorescence spectrometry.

2. Materials and Methods

2.1. Chemicals
Green tea samples were supplied from Wuhan Mingcha Tea Industry Co., Ltd., raw materials are collected from Enshi, Hubei. Green tea samples were weighed and brewed in boiling water. Centrifugation and filtration were conducted to obtain clear infused green tea. The determination of the fluorescence intensity was conducted using an FP-750 Spectrofluorometer. The oxidation-reduction potential value was performed using ORP, HOTEX, HTC-201U. Identification of precipitation after digestion was conducted using ATR-FTIR Perkin Elmer.

2.2. Method of digestions
Samples of Se-enriched tea (25 mL) were placed in a 370 x 25 mm Pyrex digestion tube. A mixture of nitric-hydrochloric acids HNO₃-HCl in a ratio 1:3 (20 mL), Nitric acid HNO₃ (20 mL), a mixture of nitric-hydrochloric acids HNO₃-HCl in a ratio 3:1 (20 mL) was added to the sample and digested at 90°C for 24 h, respectively. Then, the samples were quantified by deionized water (50 mL).

2.3. Method of derivatizations
Derivatization procedures were carried out in the darkroom using 2,3-Diaminonaphtalene (2 mL) at pH solution (1.5-2). To increase the reaction rate and to avoid the oxidation process, the samples were heated in a boiling water bath for 5 minutes. Therefore, the samples were extracted by solid-phase extraction technique.

3. Results and Discussions

3.1. Fluorescence Intensity of three digestion methods
Based on the experiment, the results showed that the aqua regia digestion method which represented the mixture of HNO₃-HCl (1: 3) gave the highest fluorescence intensity for selenium in green tea samples. In contrast, both reverse aqua regia which contained a mixture of HNO₃-HCl (3: 1) and nitric acid digestion method provided lower fluorescent intensity using various sample volumes. The fluorescence intensity for the three digestion methods is shown in Figure 1 and Table 1.

Aqua regia is a mixture of 3:1 concentration of hydrochloric acid to concentrated nitric acid. It can be yellow to orange color when initially made but will fade as the solution degrades. It can be used to dissolve precious metals. Aqua regia has been proven as the most efficient digestion method for recovery As, Cd, Pb, Ni, Zn, Fe in herbal medicine samples [12]. By using the aqua regia digestion method, selenium was more easily extractable. It was mainly obtained in the form of selenium tetravalent (oxidation state, +4) and selenium hexavalent (oxidation state, +6). For determining selenium total, sample reduction is important. The general reaction of the aqua regia yields nitrosyl chloride (NOCl), Chlorine (Cl₂), and water [13].
Conversely, reduction of selenium was not effective using only nitric acid due to its oxidizing agent properties. Another research found that a low detection limit (0.03 μg g⁻¹) of selenium was found in dry sediment samples based on a combination of aqua regia-HCl digestion method and determination by ICP-MS using $^{82}$Se [14].

![Fluorescence intensity graphs](image)

**Figure 1.** Fluorescence intensity of (a) 2 mL sample volume, (b) 5 mL sample volume, (c) 10 mL sample volume.
Table 1. Comparison of fluorescence intensity for three digestion methods at various sample volumes.

| Reagent Digestions          | Volume of loading samples (mL) |
|-----------------------------|---------------------------------|
|                             | 2                              | 5                   | 10                 |
| HNO₃                        | 3.1377                         | 8.1280              | 18.0541            |
| HNO₃:HCl (1:3)              | 9.0510                         | 26.3689             | 54.4308            |
| HNO₃:HCl (3:1)              | 3.7396                         | 10.6782             | 22.6311            |

The sample volume is proportional to the fluorescence intensity. The more increases of sample volume, the more increases of the derivative benzopiazselenol signal and matrix signal. There is a significant emission peak signal at a wavelength of 519 nm for the benzopiazselenol complex and at a wavelength of 465-500 nm for the matrix interferences. Benzopiazselenol complex was formed from DAN reacted with selenite. In this case, selenate must be reduced prior to DAN derivatization by the addition of hydrochloric acid to the sample followed by heating [15].

3.2. The oxidation-reduction potential
In order to confirm that the selenium form in the sample has been converted to tetravalent selenium (selenite), it was conducted testing the oxidation-reduction potential of the digested sample spike 1 ppm selenium standard (Table 2). The oxidation-reduction potential value was greater than 500 mV at pH less than 2 shows the existence of tetravalent selenium.

Table 2. The oxidation-reduction potential of the digested sample spike 1 ppm selenium standard.

| Sample                     | ORP (mV) |
|-----------------------------|----------|
| Se 1 ppm                    | 526      |
| Sample + Se 1 ppm (10x)     | 645      |
| Sample + Se 1 ppm (100x)    | 720      |

Dissolved selenium can exist in the hexavalent selenium Se(VI) and tetravalent selenium Se(IV). Under oxidizing conditions will be dominant in the Se(VI) species HSeO₄⁻ at pH value less than 2, whereas, selenium under reduction conditions will exist in the Se(IV) species H₂SeO₃ at pH value less than 2.5 [16,17,18]. It means that the digestion method in this experiment converted selenium types into tetravalent selenium (H₂SeO₃) to facilitate subsequent derivatization experiments of tetravalent selenium and DAN.

3.3. Analysis of precipitation after digestion
Detection of precipitation in the solution was conducted by ATR-FTIR analysis. This analysis shows that the peaks were characteristic peaks of silicone (Figure 3). 2920, 2850 cm⁻¹ are the characteristic of sp² and sp³ CH₃, and 1709 cm⁻¹ is the C=O functional group. 1641 cm⁻¹ is the characteristic of C=C. 1262 is the characteristic of Si-CH₃ cm⁻¹. 1102, 1018 cm⁻¹ are the characteristic of Si-O-Si functional groups. This peak is appropriate with the silicone spectrum. There are four peaks unique of Silicone oil, 1412 cm⁻¹ is Si-CH₃ asymmetric, 1260 cm⁻¹ is Si-CH₃ symmetric, 1091 cm⁻¹ and 1020 cm⁻¹ are Si-O-Si stretch, 798 cm⁻¹ is Si-C stretch [19].
Figure 2. ATR-FTIR spectrum of precipitation after aqua regia digestion method.

Silicone in plants comes from the soil which is adsorbed by plant roots in the form of silicic acid [20]. Silicone can react with hydrofluoric acid or strong alkaline solutions [21]. Crystalline silicon is a nonmetallic solid with a covalent bond and it has melting point 1414°C [22].

4. Conclusion
Aqua regia had proven a highly effective acid combination as reagent digestion for the determination of selenium in green tea samples. Selenium was extracted by aqua regia and hexavalent selenium had been converted into tetravalent selenium to facilitate the generating of benzopiazselenol. On another hand, there was a silicone compound obtained as precipitation after the digestion process.

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