**Pipette Tip Solid-Phase Extraction Combined with Fluorescence Spectroscopy for Determination of Selenium in Green Tea Samples**

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**Abstract.** In this study, the custom-built pipette-tip solid phase extraction (PT-SPE), was used for pre-concentrating the selenium (Se) in green tea samples. The PT-SPE was prepared using a pipette tip and C18 as the adsorbent that was placed between glass wool. This method afforded several advantages in terms of efficiency of extraction time (8 min), sample (2 mL), and solvent (1 mL) volumes with less wastage of solvent. Under optimal conditions of extraction, this method yielded good linearity ($r^2 = 0.998$) for the Se calibration curve and good limit of detection (0.2 $\mu$g/L) and quantification (0.6 $\mu$g/L). The standard addition method was used to remove the matrix effect. The recovery percentages of tea samples spiked with different Se concentrations ranged from 97.7 to 106.0%, indicating that the proposed method would be suitable for the determination of Se in green tea samples.

1. **Introduction**

Selenium (Se) is known to have a crucial function for the metabolism in the human body, processes involving active thyroid hormone production, the immune system’s functioning, and the inhibition of HIV-AIDS progression [1,2]. Unlike other trace elements, Se is very narrow between deficiency and excesses of the body's intake amount [3]. Due to its properties and low levels of Se in samples, the development of techniques with high sensitivity and selectivity for the determination of Se becomes crucial.

Spectrofluorometry offers high sensitivity, simplicity, low cost and versatile method for Se determination [4]. The important thing in determining Se using the spectrofluorometric method is the availability of fluorophore compounds that interact with Se. 2,3-diamino naphthalene (DAN) is a fluorophore compound that can react with Se(IV) to produce 4,5-benzopiazselenol (Se(IV)-DAN), which can be detected using fluorescence spectroscopy [5,7].

The pre-concentration method is crucial to concentrate Se for analytical assay. Some researchers have studied pre-concentration methods for determining Se in samples. Liquid-liquid extraction (LLE) [7], ultrasonic assisted dispersive $\mu$LLE [8], dispersive $\mu$LLE [9], solidified floating organic drop microextraction [10], pressurised liquid extraction (PLE) [11], and solid-phase extraction (SPE) [12]. In SPE, the sample's separation and purity depend on the adsorbent used and other influencing factors [13]. One of the non-polar adsorbents, C18, is suitable for Se analysis in water samples using SPE [14]. The current method, pipette tip solid-phase extraction (PT-SPE), has been applied as a miniature design filled with a relatively smaller amount of adsorbent than the SPE column [15]. In addition, PT-SPE overcomes the drawbacks of the SPE method in terms of large sample quantity [16]. It provides many advantages...
in terms of increased effectiveness for sample pretreatment, lower solvent consumption, saving extraction time, and ease of operation [17].

Therefore, this study aimed to investigate the feasibility of C_{18} as an adsorbent of 4,5-benzo selenium (BPS) based on PT-SPE method, and fluorescence spectroscopy was used to evaluate the performance of PT-SPE for the determination of trace-level Se in green tea samples. The amount of C_{18}, the composition of washing solution, and the sample volume were studied to determine their effect on the extraction performance.

2. Materials and Methods

2.1. Chemicals

High-purity chemicals and reagents were used for this study. Calibration and working solutions were diluted from the stock Se solution (1000 μg/mL). The reversed-phase silica gel C_{18} (mesh size 40-63 μm) was purchased from Silicycle (Canada), DAN (C_{10}H_{10}N_{2}) was purchased from Alfa aesar (England), hydroxylamine hydrochloride (H_{3}NO.HCl) was obtained from Alfa aesar (China), and ethylenediaminetetraacetic acid disodium salt (EDTA.2Na) was purchased from Choneye pure chemicals (China). The hydrochloric acid, nitric acid, ammonium chloride, methanol, cyclohexane were purchased from Fluka (Germany). The fluorescence measurements were performed using a spectrofluorometer (Jasco FP-750, Japan). The acidity of solutions were measured with a pH meter (Sartorius PB-10, Taiwan).

2.2. The preparation of sample, digestion, and derivatization procedures

Samples of green tea leaves (2.0 ± 0.1 g) were brewed in 100 mL boiling water for 5 min. Upon cooling, samples were filtered using filter paper (Advantec 70 mm) to remove the leaves and centrifuged (10,000 rpm) at 25 °C for 20 min. Then, samples were prepared for subsequent digestion and derivatization methods as described previously [18]. Upon digestion, the Se compound was oxidized to inorganic tetravalent selenium [19]. Under acidic conditions, the inorganic Se^{4+} would react with DAN to produce a BPS complex [20]. Meanwhile, the curve of linearity for Se was evaluated by spiking a series of standard solutions in the concentration ranges of 0.2, 0.4, 0.6, 0.8, and 1.0 μg/L, respectively.

2.3. The preparation of pipette tip-solid-phase extraction (PT-SPE) device and extraction of benzopiazselenol

The extraction was performed using a custom-built PT-SPE device (Figure 1a) with a 1000-μL pipette tip and a lidless 1.5-mL Eppendorf tube was placed in a glass connected to a vacuum pump and a rubber stopper. The pipette tip was filled with 100 mg of C_{18} which was placed between the glass wools, and each glass wool was about 5 mm in length (Figure 1b). Various amounts of C_{18} sorbent (10, 50, and 100 mg) was used to determine the effect of sorbent on the extraction efficiency. To assess the C_{18} solid-phase extraction column's ability to reduce matrix interferences, two variations of sample volumes (1 and 2 mL) were evaluated using three types of C_{18} solid-phase extraction columns.

Subsequently, 1 mL of each derivatized sample was loaded to PT-SPE, followed by 1 mL washing solution and 1 mL cyclohexane as an elution. A combination of methanol and deionized water was used as a washing solution under the same condition. The eluent sample containing the BPS complex was collected using an Eppendorf tube. Finally, the fluorescence absorbance of eluent samples was determined at the excitation wavelength of 375 nm and an emission wavelength of 519 nm [21].
3. Results and Discussions

3.1. Optimization of the amount of C\textsubscript{18} sorbent, sample volume, and washing solution.
Various amounts of C\textsubscript{18} sorbent were filled into the end of the pipette tip to determine the effect of the sorbent amount on the extraction efficiency. The best extraction efficiency of the C\textsubscript{18} sorbent amount was obtained using 100 mg. Figure shows the fluorescence intensity of BPS, in which increasing the quantity of C\textsubscript{18} would yield more effective adsorption while reducing the effect of matrix interference in the sample. In this respect, 50 and 100 mg of C\textsubscript{18} could eliminate the matrix disturbance in the derived sample, but 10 mg C\textsubscript{18} could not do so. Besides, the fluorescence intensity of BPS for sample volumes of 1 mL and 2 mL at the emission wavelength of 519 nm was 9.94 and 9.41, respectively. Given that the difference in fluorescence intensity between these sample volumes was not too large. Thus, 100 mg C\textsubscript{18} and a sample volume of 2 mL were used as the optimal C\textsubscript{18} solid-phase extraction column for the subsequent analysis for actual samples because it had the highest ability to reduce matrix interference.
Figure 2. Effect of the amount of C<sub>18</sub> adsorbent on the fluorescence intensity on the variation sample volume (a) 1 mL, (b) 2 mL.

One of the important steps of PT-SPE is the washing process. In this study, Combinations of methanol and deionized water were used as a washing solution. Various volume comparisons of methanol and water were investigated under the same conditions. Figure 3 shows that methanol: deionized water (50:50) produced the lowest fluorescent intensity, and the highest fluorescent intensity was achieved with methanol below 20%. Besides that, the highest fluorescent intensity was produced by deionized water. Thus, deionized water was used as a washing solution in the next analysis due to the lowest cost.

Figure 3. Effect of volume ratio of methanol and water on fluorescence intensity.

3.2. Linearity, limit of detection, and accuracy.

The curve's linearity was studied by spiking a series of standard solutions in the concentration ranges of 0.2, 0.4, 0.6, 0.8, 1.0 μg/L, respectively. Each standard concentration was prepared and analyzed in triplicate. Figure 4 shows a clear emission peak signal at the wavelength of 519 nm with no matrix signal in the wavelength range of 460-500 nm.
Indeed, the Se standard's calibration curve showed good linearity with a correlation coefficient (r²) of 0.998. The limit of detection (LOD) and the limit of quantification (LOQ) were obtained 0.2 μg/L and 0.6 μg/L, respectively.

3.3. Real samples analysis.

The standard addition method was used to evaluate the feasibility of PT-SPE for Se determination in green tea samples. To further confirm the practical application of the proposed method in real samples, two different teas (tea leaves and tea powder) were spiked with three different concentration levels of Se at 0.4, 0.8, and 1.2 μg/L which the data are presented in Table 1.

Table 1. Determination of Se in tea samples by PT-SPE combined with fluorescence spectrometry.

| Samples           | Se (μg/L) Found | Added | Sum   | Recovery (%) |
|-------------------|----------------|-------|-------|--------------|
|                   |                |       |       |              |
| Green tea leaves  | 0.58±0.004     | 0.00  | 0.58  | 99.20        |
|                   | 0.00           | 0.40  | 0.98  | 105.97       |
|                   | 0.00           | 0.80  | 1.43  | 98.17        |
|                   | 0.00           | 1.20  | 1.76  |              |
| Green tea powder  | 0.71±0.010     | 0.00  | 0.71  | 97.68        |
|                   | 0.00           | 0.40  | 1.10  | 104.86       |
|                   | 0.00           | 0.80  | 1.55  |              |
|                   | 0.00           | 1.20  | 1.94  | 102.33       |

Table 1 gives the result of the feasibility of PT-SPE for the determination of Se in green tea samples in which a satisfactory recovery for the trace-level Se was attained. Moreover, this method (PT-SPE) was time efficient with a total extraction time of 8 min, 1 min for loading sample, 1 min to elute the washing solution (deionized water), and 6 min to elute the BPS derivative with cyclohexane.
substantially lower LOD value and higher recovery in this study suggested that this PT-SPE method would be feasible for the determination of Se in green tea samples.

4. Conclusions
The sensitive, economical, and miniature custom-built PT-SPE device combined with the spectroscopy was developed to determine Se in green tea samples. The C18 was used as an adsorbent in PT-SPE. The advantages of this method included a reduced extraction time (8 min), solvent-savings (1 mL), a lower sample volume (2 mL), and ease of operation.

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