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Determination of Selenium in Black Tea Leaves Using the Air-assisted Cloud Point Extraction Method: Evaluation of the Method's Environmental Performance

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For the first time, air-assisted cloud point extraction (AA-CPE) procedure was applied to determine selenium in black tea samples at room temperature. The Air agitation is powered by a small fish tank generator to improve the dispersion phase and reduce the critical temperature for surfactants. The ligand and surfactant were 1-(2-hydroxy-5-ptolylazo-phenyl)-ethan-one (HPAPEO) and TritonX-100, respectively. Afterward, the extracted selenium complexes were injected into a hydride generation atomic absorption spectrometer (HG-AAS). At the optimal conditions of experimentation, linearity from 0.3 to 1000 μ g l⁻¹ was constructed with an enhancement factor, preconcentration factor, detection limit, and extraction recovery of 150, 100, 0.02 μ g l⁻¹, and 99.1-102%, respectively. The AA-CPE technique's accuracy has been proven using certified reference materials. Ultimately, the Red-Green-Blue (RGB12), Analytical Greenness Metric Approach (AGREE), and Complementary Green Analytical Procedure Index (ComplexGAPI) scales have been applied to assess the whiteness and greenness of this method. An accurate evaluation of the criteria has been performed to ensure this procedure is eco-friendly for the environment.

Keywords: Air-assisted cloud point extraction, Analytical greenness metric approach, Complementary green analytical procedure index, Whiteness scale, Selenium, Tea leaves

INTRODUCTION

Selenium is an essential trace element due to its use in a number of biological processes. About twenty-five human selenoproteins may be responsible for the redox mechanism of enzymes [1]. Selenium deficiency or higher concentrations in our bodies may cause many diseases. However, the suggested daily amount of selenium for adults, which ranges from 40 to 400 μ g, is still rather low [2]. The bioactivity and dangers of selenium in food depend on its concentration and

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chemical structure. Accurate and efficient separation and determination methods are essential because of the tiny distinction between the necessary and dangerous concentrations of selenium in food [3]. The most common beverage on earth is tea. Chemical pollutants contained in tea leaves caused by air pollution deposits represent possible hazards for users. Heavy metals and residues of pesticides are among the principal contaminants that have undergone substantial examination, and their identification and evaluation are critical [4,5].

Due to the low quantities of selenium naturally present in water, food, and beverages, determination is extremely difficult. Also, the determination approach must be utilized to address this issue. Therefore, the preconcentration process is an essential and required step before measurement. [6]. To deal with these challenges, many techniques were created to separate and determine the traces of selenium in food, such as solid-phase extraction (SPE) [7], liquid-liquid microextraction (LLME) [8,9], hollow fiber liquid-phase microextraction (HF-LPME) [10], and cloud point extraction (CPE) [11-13]. CPE is simpler, more inexpensive, quicker, and more effective than other commonly used organic solvents [14]. A surfactant solution must be applied in quantities greater than the critical micelle concentration (CMC) in order to form the cloud point layer (CPL) [15]. The solutes dissolve and partition into the micelles and aqueous solution after forming two insoluble, isotropic layers. The CPL layer contains the analytes. The CPL and the bulk of the aqueous solution are in equilibrium [16-19]. In order to create a cloudy solution and transfer the analyte to the CPL, the temperature is an essential factor in the CPE technique [20]. CPE uses a temperature well above cloud point temperature (CPT) to separate phases [21-23]. Above the CPT, the solution is separated into two distinct phases: an aqueous phase with surfactant quantities near the CMC and a CPL with higher surfactant quantities concentrated in a smaller region [24, 25]. Recently, the novel approach of air-assisted cloud point extraction (AA-CPE) was introduced as a green method. Combining CPE with air agitation as a dispersion system to improve extraction efficiency and enhance the rapid removal procedure. This procedure used air bubbles instead of higher temperatures to form a cloudy solution [26-28].

The application of green analytical chemistry (GAC) and white analytical chemistry (WAC) is among the difficulties in analytical chemistry [29]. GAC aims to lessen the environmental and user effects of analytical procedures, whereas WAC tends to harmonize greenness with unbiased utility (analytical efficiency and applicability). The actual size of this difficulty appears to be very significant given the enormous number of various labs doing chemical analyses globally [30]. For evaluating the method's environmental performance, accurate scales were required. Recently, many scales have been introduced to measure greenness and whiteness, like AGREE, ComplexGAPI, the RGB12 model, and others [31]. The computational scales were used more than others because they are easy to evaluate and compare between different methods [32]. In this study, selenium in black tea samples was separated and determined using the AA-CPE technique at room temperature, followed by HG-AAS. Air agitation enhances the dispersion procedure. The whiteness of this method was determined by the RGB12 tool. The proposed method's green credentials were assessed using two scales (AGREE and ComplexGAPI). This method is white and green according to all the tools in the application.

EXPERIMENTAL

Chemicals and Reagents

The investigation was conducted with distilled water. The used chemicals (hydrochloric acid, sodium hydroxide, and salts) were provided by Merck, Darmstadt, Germany. The 1000 mg l⁻¹ selenium stock solution was provided by the National Standard Chemical Laboratory in Beijing, China. The standard operation solution was made every day by systematically diluting it in ultrapure water. The extracting medium was Triton X-100, a non-ionic surfactant from Sigma Aldrich. According to Tripathi et al. [33], the ligand 1-(2-hydroxy-5-ptolylazo-phenyl)-ethan-one (HPAPEO) was created. The Shanghai Botong Industrial Science Co., Ltd. of Shanghai, China, supplied the standard reference material (SRM) of the black tea leaves (GBW08513). All applied substances and instruments were kept at 20% HNO₃ for at least one day to avoid pollution before being washed multiple times in pure water.

Instruments

A Shimadzu AAS-6300 atomic absorption spectrometer from Kyoto, Japan, with a D₂-background correcting method, hydride generation framework, and selenium hollow cathode lamp. A wavelength of 196.3 nm, a lamp power of 10 mA, a slit width of 0.7 nm, and an argon flow rate of 150 ml min⁻¹ are used to run the device. The quartz T-cell was heated using an air-acetylene flame (900 °C) to induce atomization. In the process cell, the selenium in the sample reacted with 5.0% w/v sodium tetrahydridoborate and 0.05% w/v sodium hydroxide in an HCl solution to produce the hydrides. The air bubble generator (Big Bubble air motor, BB-8000, China) at a SunLab store in Najaf, Iraq, was the source of the airassisted source. Both the SRM and samples of tea leaves were digested using a microwave digestion machine (Speedwave® Four, Berghof Products, Germany). For mass evaluations, a four-digit EL-204 electrical balance from Mettle-Toledo in Shanghai, China, was used. A commercial centrifugal was utilized (Hinted Technologies Co., Ningbo, China). The pH scale (model pHS-3C, Shanghai Precision & Scientific Apparatus Co., Ltd., Shanghai, China) was used to calculate the pH. Deionized water, which is obtained from the Milli-Q purification system (Millipore, Bedford, MA, USA), was used to produce every solution.

Collection and Preparation of Samples

Ten black tea samples were obtained from local markets in Kufa, Iraq. The ordered samples investigated are 1- Lipton, 2- Barrie, and 3- Altair (both from Saudi Arabia); 4-Alokozay (from Germany); 5- Ahmad; 6- Mahmood; 7-Twinings; 8- Fila; 9- Al-Tore; and 10- Awaze (all from China). The samples were first dried to a fixed weight in a furnace at 70 °C and ground and sieved through a 100-mesh sieve [13]. Following that, 0.2 g of the crushed sample was transferred to the digestion tube, and mixed with 2.0 ml nitric acid (69.0%), 2.0 ml hydrogen peroxide (30% v/v), and 2.0 ml deionized water and the digestion procedure was proceed in the microwave system under the conditions specified in Table 1 [34]. Following the digestion procedure, the contents of the containers were diluted to 25.0 ml in volumetric flasks with deionized water. Ten ml of the digestion product was subjected to the extraction procedure as prescribed in the following section. The SRM was digested and processed by the same procedures.

General AA-CPE Procedure

Samples of 10 ml of selenium solution (0.80-1000 μ g l⁻¹) at pH = 9 were transferred into centrifuge tubes with glass

Table 1. Microwave Decomposition Conditions of Operation

Condition	Stage 1	Stage 2	Stage 3
Power (w)	1000	1250	0
Temperature (°C)	160	200	60
Ramp time (min)	10	8	2
Hold time (min)	5	15	10
Pressure (bar)	25	30	20

stoppers that had a 25 ml capacity. In the beginning, 1 ml of 1.0×10^{-4} M ligand (HPAPEO) was injected into the test solutions. The tube then received 0.5 ml of 0.05% (v/v) Triton X-100. After that, the air pump is activated for 80 s to help disperse the analyte across the solution and enhance the separation procedure. The cloudy solution was made at room temperature at this stage. The phases were separated using centrifugation for eight minutes at a speed of 4000 rpm. The tubes were placed in an ice bath for five minutes to increase the viscosity of the surfactant-rich layer. The top aqueous layer was carefully withdrawn. Then, using a microsyringe to remove the CPL and dissolve it in 1 ml of ethanol, this layer contained the Se-complexes prior to their introduction to the HG-AAS system. The UV spectrophotometer was used to detect the formed Secomplexes in a diluted CPL via the AA-CPE method. SRM and blanks were prepared in a similar manner.

RESULTS AND DISCUSSIONS

The main variables affecting the extraction process were looked at, including complex formation, pH values, quantities (surfactant and chelating reagent), air-assisted periods, and centrifugal parameters. Extraction recovery (R%) was employed to gauge the method's effectiveness in accordance with equation (1) [24].

$$R(\%) = \frac{C_f V_f}{C_i V_i} \times 100$$
(1)

UV-Vis Spectra for Ligand and Complex

The spectroscopic characteristics of the chelating agent alone and its combination with selenium in ethanol are shown in Fig. 1. Due to intra-ligand transitions $\pi \rightarrow \pi^*$, the ligand (HPAPEO) shows an absorption band at $\lambda_{max} = 355$ nm, as illustrated [33]. After complexing with selenium, the absorption spectrum is moved to a longer wavelength (at $\lambda_{max} = 400$ nm) as a result of the creation of a brownish complex.

Impact of pH Value in Solutions

The pH of the sample solution has a significant impact on the combining of the metallic ions and ligands, in addition to the extraction effectiveness [35,36]. The impact of pH was





Fig. 1. The absorption spectra of the ligand and Secomplex at conditions (pH = 9, 80 μ g l⁻¹ of selenium, 0.05% TritonX-100, 1 × 10⁻⁴ M HPAPEO, 80 s air-assisted time, 8 min at 4,000 rpm centrifugal parameters).

examined between 2.0 and 12.0 to produce the greatest extraction efficiency. 0.1 M of HCl or 0.1 M of NaOH were added to the sample solution to change the starting pH. According to the findings in Fig. 2, the extraction recovery rises with pH and reaches a quantifiable value at pH 9.0. Since the selenium ions' hydrolysis occurs at higher pH levels, the extraction is poor. Therefore, pH 9.0 was selected for the next tests.



Fig. 2. The impact of pH value on formation of Se-complex at conditions (80 μ g l⁻¹ of selenium, 0.05% TritonX-100, 1 × 10⁻⁴ M HPAPEO, 80 s air-assisted time, 8 min at 4,000 rpm centrifugal parameters).

Impact of Ligand HPAPEO Quantities

The ligand used in the AA-CPE technique was HPAPEO. The efficiency of extraction is significantly influenced by the quantity of HPAPEO [37]. At quantities ranging from 1×10^{-6} to 1×10^{-3} M, the effect of HPAPEO quantity on selenium recovery was examined. According to Fig. 3, increasing the ligand quantity enhances extraction, which grows to a maximum at 1×10^{-4} M. A small concentration of HPAPEO results in poor complexation, which lowers the effectiveness of removal and affects the method's accuracy. Thus, the perfect ligand quantity was chosen to be 1×10^{-4} M.

Impact of Surfactant Quantities

TritonX-100 was selected as a surfactant because it is readily available in a pure state, has no dangerous side effects, and has a high density, making rotary evaporation the most straightforward way to isolate it. Because it is utilized to separate or extract a variety of metallic complexes, TritonX-100 also has a low CPT [38]. The impact of TritonX-100 on selenium recovery can be seen in Fig. 4.

The most acceptable amount for TritonX-100 to get a higher efficiency of extraction was 0.05%; however, ranges from 0.01% to 0.2% v/v were also examined. At quantities less than 0.05%, Se-complex extraction efficiency is poor, probably because the assemblies are unable to quantitatively



Fig. 3. The impact of ligand (HPAPEO) concertation on extraction recovery at conditions (pH = 9, 80 μ g l⁻¹ of selenium, 0.05% TritonX-100, 80 s air-assisted time, 8 min at 4,000 rpm centrifugal parameters).



Fig. 4. The impact of Triton-X100 concentrations on extraction recovery at conditions (pH = 9, 80 μ g l⁻¹ of selenium, 1 × 10⁻⁴ M HPAPEO, 80 s air-assisted time, 8 min at 4,000 rpm centrifugal parameters).

entrap hydrophobic compounds. On the other hand, if the quantity and viscosity of the surfactant have increased in greater amounts, the signals could become weaker in recoveries.

Impact of Air-assisted Time

The application of a tiny fish-tank engine in AA-CPE was thoroughly investigated. The air-generating device was a plastic pipette with an internal diameter of 3 mm and a length of 5 ml. A polyethylene tube resistant to the chemicals and fluids was employed for this extraction method. A Big Bubbles air pump (BB-8000) running at its maximum output (50 Hz) was selected as the bubble producer, and its effectiveness was examined over time periods ranging from 5 to 100 s. As demonstrated in Fig. 5, the recovery with growing air-assisted time lasted up to 80 s before the signal started to fade. This indicates that greater extraction efficiency results from breaking more air bubbles; micelles encapsulate the surface area of each bubble. Continuing bubbling expansion can help increase the opportunity for contact between the Se-complex and the surfactants before it separates, leading to quick preconcentration and effective CPL aggregation. The rate of recovery decreases when air assistance times increase by more than 80 s [39].



Fig. 5. The impact of air-assisted time on extraction recovery at conditions (pH = 9, 80 μ g l⁻¹ of selenium, 0.05% TritonX-100, 1 × 10⁻⁴ M HPAPEO, 8 min. at 4,000 rpm centrifugal parameters).

Centrifugal Parameters

The impact of the centrifuge period (5 to 15 min) and range (2000 to 4500 rpm) was examined to enable the completion of the quantified separation quickly. The AA-CPE technique's ideal spin settings of eight minutes at 4000 rpm were approved. These specifications had high removal and recovery rates. The effects of centrifugation conditions (rate and time) on the AA-CPE method are shown in Table 2.

The Impacts of Interference

To effectively separate and recover selenium utilizing the proposed AA-CPE, the matrix ions influence was studied. Table 3 summarizes the allowed limit for interfering ions. The approved level is the quantity of each of the cations and anions that generated a $\pm 5\%$ difference in recoveries. It has been proven that the presence of additional anions and cations in a specified ratio has no influence on selenium separation by AA-CPE at optimal conditions. As a result, given the specified parameters, the procedure is suitable for selenium analysis in a real sample. The AA-CPE method offers outstanding selenium sensitivity [37,38].

Evaluation Figures

After preparing the samples utilizing the AA-CPE process, the calibration curve linearity of selenium at a

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Rate of centrifugation	Time of centrifugation	Recovery % ± SD
(rpm)	(min)	
2000	5	66.70 ± 1.4
	8	82.00 ± 2.0
	15	59.00 ± 1.5
2500	5	59.70 ± 1.5
	8	90.00 ± 1.7
	15	79.90 ± 1.8
3000	5	77.00 ± 2.5
	8	78.00 ± 0.5
	15	69.00 ± 1.4
4000	5	87.90 ± 1.7
	8	99.50 ± 1.2
	15	85.00 ± 1.5
4500	5	49.80 ± 2.1
	8	62.00 ± 2.5
	15	73.00 ± 1.9

Table 2. Effect of Centrifugation Parameters on the AA-CPE Procedure

Table 3. The permissible Ranges for Several Ions Used in Selenium Detection

Ions	The interfering ions/selenium	Recovery %
	(w/w)	
Cd ²⁺ , Pb ²⁺ , Hg ²⁺ , Ca ²⁺ , Na ⁺ , K ⁺ , Li ⁺	2000	99.00 ± 1.2
La ³⁺ , Fe ³⁺ , Th ⁴⁺ , As ⁴⁺ , Nd ³⁺ , Al ³⁺	1500	97.00 ± 1.8
$CO_3^{=}$, $SO_4^{=}$, PO_4^{3-} , $C_2O_4^{=}$	1000	99.00 ± 1.1
Cl ⁻ , Br ⁻ , NO ₃ ⁻ , CH ₃ COO ⁻	800	98.00 ± 2.1

concentration range of 0.3-1000 μ g l⁻¹ was produced with correlation scores of 0.998. The proposed approach's limit of detection (LOD) and limit of quantification (LOQ) have been estimated to be 0.06 and 0.20 μ g l⁻¹, respectively. The relative standard deviation (n = 6) was 1.1%. The ratio of the slopes of the calibration graphs generated with and without preconcentration of selenium showed an enhancement factor (EF) of 150 for the AA-CPE approaches [39,40]. For the AA-CPE methodology, the ratio of sample volume prior to and after the pre-concentration step operation was utilized to obtain the preconcentration factor (PF) of 100. Table 4 involves further analytical properties like linearity, LOD, LOQ, EF, and PF.

By assessing a standardized SRM (GBW08513 tea leaves) test with acknowledged measurements of selenium, the precision of the novel AA-CPE and traditional CPE procedures was demonstrated. The AA-CPE technique is appropriate for the identification of trace selenium in black tea tests due to its excellent sensitivity and low detection limits. Table 5 shows the quantities of selenium in RSM using two methods: CPE and AA-CPE. Also, the recoveries and t-values were determined based on Eqs. (1) and (2), respectively [41].

$$t - test = \frac{Found - Certified}{SD \sqrt{n}}$$
(2)

where SD is the standard deviation and n denotes the number of observations. The results reveal that the tested and certified values agree more with the AA-CPE method than CPE. Furthermore, the t-value is less than the threshold t-value with 95% assurance (2.850) for three degrees of freedom, showing that the process for determining selenium is accurate.

Table 4. Evaluation Figures of Quantification Selenium Using AA-CPE Procedure

Evaluation figures	Values
LOD (3S/m)	0.02 (µg l ⁻¹)
LOQ (10S/m)	0.06 (µg l ⁻¹)
Linearity	0.3-1000 (µg l ⁻¹)
EF (Slope with AA-CPE/slope without)	150
PF (C _o /C _{aq})	100

Table 5. The Amount of Selenium in the RSM (GBW08513 Tea Leaves)

	$(\mu g g^{-1})$	$(\mu g g^{-1})$	5		
AA-CPE CPE	40.0 ± 0.3	39.8 ± 0.2 35.9 ± 0.4	99.5 89.7	0.854 0.541	

Table 6. Compassion with Several CPE Studies for the Determination of Selenium in Water and Food Samples

		Ligands	Samples	Detectors	Optimal parameters			Evaluation figures			Published		
Methods	Surfactants				pН	T (°C)	LOD (µg l ⁻¹)	PF	RSD (%)	Linearity (µg l ⁻¹)	Recovery (%)	year	Ref. year
AA-CPE	Triton X-100	HPAPEO	Black tea	HG-AAS	9	25	0.02	100	0.1-2.9	0.3-1000	99.1-102	2023	This work
CPE	Triton X-114	Dithizone	Rice	GFAAS	1	50	0.08	82	2.1	1-100	90.3-106	2013	[11]
CPE	Ponpe 7.5	Pyronine B, FeCl ₃	Food	FAAS	4	40	3.8	-	2.45	20-1700	97.8- 102.8	2013	[12]
CPE	Triton X-100	DDTC	Tea	ICP-MS	5	85	0.10	20	3.2	5-100	97-103	2013	[13]
CPE	Triton X-114	DAN	walnut	UV-Vis	3	80	0.09	-	1.6	0.05-4	92-99.1	2015	[20]
d-CPE	Triton X-114	APDC	Food	HG-AFS	4	51	0.023	11.8	4.04	0.5-6.0	95.6-105.2	2017	[22]
UA-CPE	Triton X-114				1	50	0.3	103	3.2				
RS-CPE	Dithizone Water Triton X-114, octanol	Water	UV-Vis	1.4	25	0.2	124	4.3	1-100	93-105	2014	[19]	
UA-CPE	CTAB, PONPE 7.5	NRH^+	Water, food	HG-AAS	6	35	0.0024	120	5.3	8-300	≥96	2016	[21]

DDTC: Diethyl-dithiocarbamate, DAN: 2,3-diaminonaphthalene, NRH⁺: 3-amino-7-dimethylamino-2-methylphenazinehydrochloride, APDC: ammonium pyrrolidine dithiocarbamate.

A Comparative Study with Alternative CPE Procedures

As noted in Table 6, various investigations measured selenium in water and food samples by CPE or assisted CPE [11-13,19-22]. The analytical properties of the AA-CPE to

detect selenium in tea leaves using the ligand HPAPEO have been compared with those of earlier published techniques. Many CPE modes were used to separate selenium, such as CPE [11-13], rapid synergistic cloud point extraction (RS-CPE) [19], ultrasound-assisted cloud point extraction (UA- CPE) [19,21], dual-cloud point extraction (d-CPE) [22], and the proposed AA-CPE. Table 6 provides several factors and analysis features, such as LOD, RSD, and PF. The reported LOD in the AA-CPE method was sufficiently low to be appropriate for identifying selenium in several tea tests. Also, this technique covers a broad range of concentrations.

Assessing Selenium Levels in Black Tea Leaves

The ten samples of black tea from the market were

collected and handled following the recommended technique of analysis. Tea leaf samples used for evaluating the results of the recently devised technique showed great agreement with those obtained from the AA-CPE. The levels of selenium in the chosen samples were within acceptable ranges. The recovered amounts from the spiked samples were within the allowed range of 96.1 to 105%. Table 7 contains an analysis of the outcomes. According to the study, selenium is present in trace amounts in the tea leaves bought from

Sample number	Added	$Found \pm SD$	RSD%	Recovery %	Er %
	0	-	-	-	-
Sample No. 1	50	50.1 ± 1.1	2.1	100.2	0.11
	90	89.2 ± 0.1	0.1	99.1	-0.89
	0	-	-	-	-
Sample No. 2	50	49.9 ± 1.2	2.4	99.8	-0.20
	90	90.1 ± 1.3	1.4	100.1	0.11
	0	-	-	-	-
Sample No. 3	50	49.9 ± 1.2	2.4	99.8	-0.20
	90	90.1 ± 1.3	1.4	100.1	0.11
	0	-	-	-	-
Sample No. 4	50	49.9 ± 1.2	2.4	99.8	-0.20
-	90	90.1 ± 1.3	1.4	100.1	0.11
	0	-	-	-	-
Sample No. 5	50	49.8 ± 1.1	2.2	99.6	-0.40
-	90	90.1 ± 1.5	2.9	100.1	0.11
	0				
	50	-	-	-	-
Sample No. 6	90	49.8 ± 1.1	2.2	99.6	-0.40
		90.1 ± 1.3	2.9	100.1	0.11
	0	-	-	-	-
Sample No. 7	50	50.1 ± 1.1	2.1	100.2	0.11
-	90	89.2 ± 0.1	0.1	99.1	-0.89
	0	-	-	-	-
Sample No. 8	50	50.1 ± 1.1	2.1	100.2	0.11
-	90	89.2 ± 0.1	0.1	99.1	-0.89
	0	-	-	-	-
Sample No. 9	50	50.1 ± 1.1	2.1	100.2	0.11
-	90	89.2 ± 0.1	0.1	99.1	-0.89
	0	-	-	-	-
Sample No. 10	50	50.1 ± 1.1	2.1	100.2	0.11
-	90	89.2 ± 0.1	0.1	99.1	-0.89

Table 7. Assessing Selenium Levels in Ten Tea Leaves Using AA-CPE Procedure

neighborhood shops. Also, the relative errors (Er%) were calculated for all samples based on Eq. (3). Further demonstrating the great precision and accuracy of the AA-CPE techniques for quantification of the analytes in their actual state were the RSD% and Er% findings, both of which were smaller than 2.5% [36].

$$Er\% = \frac{Measured \ value - True \ value}{Measured \ value} \times 100$$
 (3)

Evaluation of Whiteness for AA-CPE-HG-AAS Method

A new Red, Green, and Blue (RGB12) model was recognized in 2021 based on WAC principles. RGB12 separates into three categories. Each category symbolizes a different color and contains certain criteria that assess crucial facets of the analytical method. The red category assesses the analytical effectiveness in terms of validating factors, including the scope of usage, precision, accuracy, and minimum LOD and LOQ. The famous GAC concepts are assigned to the green category, and productivity variables including cost and time effectiveness, minimal practicalities, and simplicity of operation are represented in the blue region. The RGB 12 simulation is displayed as an Excel spreadsheet that adheres to WAC's guidelines [41]. The amount of environmental quality as determined by the whiteness assessment is estimated. A well-balanced analytical procedure that is appropriate for the situation is referred to as "white" in the WAC methodology. Figure 6 looked at the suggested approach's whiteness using the RGB12 model.

The findings of this final investigation on whiteness are depicted in Fig. 6. The combined results demonstrated that the suggested strategy is superior, with a score of 95.3%. The suggested approach was found to have a wide range of potential applications for the validation criterion (red region). As long as the procedure adheres to the ICH criteria, the method is accurate [40,41]. The HG-AAS detector received an additional 20 merit points for assessing unique samples that had been identified in the initial RGB12 algorithm design. Usually, the assigned AA-CPE receives a high value in the red category. Studying the environmental influences (green region) revealed that this approach was the most environmentally friendly because it used the fewest chemicals, produced the least waste, didn't use any harmful



Fig. 6. The whiteness of the AA-CPE-HG-AAS method by applying the RGB12 model.

substances, and consumed the least energy [42]. Additionally, neither animal products nor genetically modified organisms (GMOs) were used in this technique [43,44]. The AA-CPE-HG-AAS was cheap, quick, and simple to adjust in terms of efficiency and environmental sustainability (blue region).

Evaluation of Greenness for AA-CPE-HG-AAS Method

The greenness of the proposed method was assessed using two simple, free, and accurate programs as follows:

Analytical Greenness Metric Approach (AGREE) software. The most commonly used greenness evaluation tool is AGREE. It operates using a math program that may be downloaded (https://mostwiedzy.pl/AGREE), which applies all 12 GAC presumptions. The center of the AGREE symbol represents the final rating, which is a fraction of one from 0 to 1. The color scheme displaying the results of the twelve AGREE symbol sections is the result-associated color in the center. The best approach yields a score of 1, using the color dark green [43,44]. As a result, the AGREE criteria are regarded as being user-friendly, thorough, simple to use, and extremely quick [29-32]. The graphics in Fig. 7 demonstrate the new technique's excellent greenness. The AA-CPE-HG-AAS approach received a 0.87 value with excellent green efficiency because it did not have waste or hazards (sections 7, 10, and 11). This method was safe and rapid (sections 4, 8, and 12).



Fig. 7. The greenness of the AA-CPE-HG-AAS method using the AGREE software.

Complementary Green Analytical Procedure Index (ComplexGAPI) software. The most recent tool created to gauge a technique's greenness is ComplexGAPI software. It is a modification to the widely used GAPI [41] indicator. The software offers a widely accepted criterion to assess the analytical method's greenness. Five pentagrams are used to depict this examination, and they offer a thorough analysis of the complete approach, starting with sample collection and preparation and moving on to the liquids and chemicals used, the instruments, the waste generated, and the energy consumption. The pre-analysis stages are reflected in the additional hexagonal shape at the bottom of the assessment. This assessment employs a color-coded system; red indicates a significant risk to the environment, while green and yellow indicate a medium and low risk, respectively [42-44]. The only red spots in the middle pentagram that apply the extraction approach are for our recommended approach (Fig. 8). The graph's central ring designates this process as qualifying. The reagent quantities and sample preparation pentagrams were green. With 19 green sections, the approach demonstrated excellent greenness.

CONCLUSIONS

At room temperature, a novel extraction method (AA-CPE) paired with HG-AAS was introduced for the preconcentration of selenium in tea leaves. The dispersion process was improved by air agitation. The process is



Fig. 8. The greenness of the AA-CPE-HG-AAS method using ComplexGAPI software.

accurate, sensitive, rapid, and economical. The extraction time is brief compared to other preconcentration techniques like SPE and conventional CPE. The AA-CPE-HG-AAS method has good analytical figures for determining selenium, including minimal LOD, a broad dynamic quantitative range, and an excellent enhancement factor. Three computational scales were used to assess the whiteness and greenness of this method. The RGB12 scale was applied to measure whiteness. The whiteness was 95.3%. The AGREE software was used to measure greenness, which equals 0.87. The result of the ComplexGAPI software involved 19 green parts, 5 yellow parts, and 1 red part. This method is white and green according to these scales.

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