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Trace determination of cobalt in heather leaf tea by matrix matching calibration assisted flame atomic absorption spectrometry following a dispersive liquid–liquid microextraction utilizing a Schiff base ligand

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ABSTRACT

In this study, an analytical method that involves dispersive liquid–liquid microextraction for extraction and enrichment of Co was developed for its determination in heather leaf tea infusions. N,N'-bis(2-hydroxyacetophenylidene)-1,3-propanediamine was utilized to form the complex extracted in chloroform with methanol as a dispersive solvent. The experimental parameters were optimized to allow for an enhancement of detection power by 35.6-folds compared to direct FAAS measurements while lowering the limit of detection to 0.0154 mg/L and the limit of quantification to 0.0513 mg/L and providing a linear range of 0.050–0.75 mg/L. Spiked recovery experiments were conducted on two brands of heather leaf tea where a matrix matching strategy was employed for further reduction of interferences. The recovery results obtained in the range of 78.7–128.4 % supported the accuracy of the proposed method that promises an economical and easy-to-apply process for the trace determination of cobalt in herbal tea matrices.

1. Introduction

The determination of trace metals in food products has been a globally recognized research topic [1,2,3]. While certain metals such as copper, zinc, iron, and cobalt are essential for maintaining a healthy human metabolism, elevated concentrations usually lead to toxic effects [4,5]. The vital importance of cobalt stems from the fact that it is integral to the structure of vitamin B12. On the other hand, because it is an element that is frequently utilized in various industrial applications, undesired amounts of Co are very likely to contaminate food and water resources [6]. While obtaining cobalt through vitamin B12 intake is crucial for health, mitigating harmful dietary exposure to this trace element is also necessary to avoid serious toxic effects [7]. For this reason, the significance of increasing the detection power for Co in commonly consumed food products comes forward.

Over the last decades, as consuming herbal teas for therapeutic and recreational purposes has become widespread without the prominent oversight that is common for drugs, determining their elemental content has become a crucial field of study to reveal the potential risks involved in using them [8,9]. In this manner, herbal teas can be considered as examples of food items that carry a heightened risk of containing harmful levels of heavy metals [10]. Heather, belonging to the *Erica* L. genus (Ericaceae), is commonly found in the Mediterranean and Western European regions [11]. It is known that the leaves and flowers of the plant are brewed as tea and used as a diuretic, laxative, and dietary supplement [11,12].

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Determination of trace metals in food samples is done by employing instrumental methods such as inductively coupled plasma optical emission spectrometry (ICP OES), inductively coupled plasma-mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS), and graphite furnace atomic absorption spectrometry (GFAAS) [13]. Arguably the most basic one of these, FAAS is a well-known quantitative elemental analysis method that is easy to use, low-cost, suitable for a wide variety of samples, and it provides rapid analysis. In addition, FAAS is considered to show less interference compared to many other methods. Despite the apparent advantages, using FAAS for heavy metal determinations at trace levels often makes it necessary to involve preconcentration procedures before analyses to lower the detection limits and increase the sensitivity and accuracy of the results [14].

Various extraction and enrichment techniques have been used in the determination of both organic and inorganic analytes in food samples. Analytical methods that involve preconcentration strategies such as solid phase extraction [15], liquid–liquid extraction, dispersive liquid–liquid microextraction (DLLME) [16,17], and cloud point extraction [18,19] are commonly studied for the determination of cobalt in food samples. Among these, DLLME stands out due to its quick, sensitive, and straightforward approach, typically offering high extraction efficiency. Because of the ease of application and high sensitivity, DLLME has become a preferred method, especially in analytical applications involving food and environmental samples with complex matrices [20,21].

In this study, a new analytical method was proposed, involving the application of DLLME utilizing the easily synthesized Schiff base N,N'-bis(2-hydroxyacetophenylidene)-1,3-propanediamine ($LACH_2$) as the complexing agent for enhancement of the limits of detection and sensitivity of determination of cobalt in heather leaf tea infusion samples. The experimental parameters were systematically optimized to obtain the highest extraction efficiency. As far as the authors are aware, the DLLME-FAAS combination has not been proposed before for the determination of heavy metals in heather leaf tea infusions and the use of $LACH_2$ ligand in any preconcentration application has not been reported. Heather leaf tea samples were collected from local stores and were brewed as in the conventional use to account for the applicability and accuracy of the analytical method in real herbal tea infusions.

2. Materials and methods

2.1. Instrumentation

An ATI Unicam SOLAAR 929 AA atomic absorption spectrometer was used for the detection and quantification of cobalt. A Varian (Australia) single-element hollow cathode lamp operating at a wavelength of 240.8 nm and a working current of 7 mA was utilized. Background corrections were done by using a deuterium (D_2) lamp. Atomization of analytes was achieved by a stoichiometric acetylene-air mixture with a moderate fuel flow rate. An Isolab vortex mixer (Germany) and an MRC Clean-01 ultrasonic bath (China) were used for mixing operations. A Biobased BKC-TL5II centrifuge apparatus (China) was employed for simple phase separation. Eppendorf Research plus pipettes (Germany) were utilized for precise and safe liquid transfer in the experiments. The pH values of the solutions were determined by employing a Mettler Toledo S220-SevenCompact pH meter (China).

A Shimadzu IRAffinity-1 Fourier transform infrared spectrometer was utilized to acquire the IR spectrum of $LACH_2$. The spectrum was acquired at a resolution of 4 cm^{-1}. The ligand's mass spectrum was acquired by employing the direct inlet (DI) unit of a Shimadzu 2010 Plus Gas Chromatograph Mass Spectrometer equiped with an electron impact (EI) ionizer. The DI temperature range was established at 40 to 140 °C, and ionization was achieved using 70 eV electrons. The differential scanning calorimetry thermogram of the ligand was obtained in nitrogen atmosphere at a heating rate of 10 °C/min, utilizing platinum pans and a Shimadzu DSC 60 Differential Scanning Calorimeter. Metallic indium standard was utilized for temperature adjustment and heating calibration of the DSC 60, as outlined in the instruction manuals. Accuracy assessments were conducted using a metallic Pb standard, with a calculated relative error of less than 2 %.

2.2. Chemicals and reagents

All chemicals and reagents that were utilized throughout the experiments are of high purity and analytical grade. A standard solution of cobalt (1000 mg/L) in 0.50 M nitric acid (HNO_3), supplied from Merck Germany, was used for the preparation of Co working standards. An Elga Pure Flex 3 Ultrapure Water System was used to obtain ultrapure water for the preparation of the sample and working solutions. The buffer solutions in the pH range 4.0 – 6.0 were prepared from potassium hydrogen phthalate (Merck), pH 7.0 – 8.0 from tris (Merck), and pH 9.0 from sodium tetraborate decahydrate (Sigma Aldrich). The chloroform, dichloroethane, and sodium hydroxide (NaOH) were purchased from Merck Germany. Methanol, concentrated hydrochloric acid (37 %), and concentrated nitric acid (65 %) were purchased from Isolab Germany. HCl and NaOH were used for the adjustment of the pH of the solutions.

2.3. Preparation of the Schiff base ($LACH_2$)

The condensation reaction between 1,3-propanediamine and 2-hydroxyacetophenone was utilized for the preparation of N,N'-bis(2-hydroxyacetophenylidene)-1,3-propanediamine in hydrothermal conditions [22]. In the solution of 0.100 mol 2-hydroxyacetophenone in 100 mL warm ethanol at around 50 °C, 0.050 mol 1,3-propanediamine was dissolved. In a 250 mL round bottom flask, the solution was heated to boiling and kept at this temperature for about one minute. The flask was allowed to stay overnight, and the precipitate was filtered by using a black band filter paper. The product was rinsed with water and ethanol and was allowed to dry in a ventilated oven operating at 50 °C. The yield was calculated as approximately 90 %.

2.4. Dispersive liquid–liquid microextraction procedure

In a centrifuge tube, 0.75 mL of pH 9.0 buffer solution was added to 8.0 mL of standard/sample solution. Then, 1.0 mL of the solution of the ligand (0.1 % m/V) in methanol was added. The resulting solution was vortex-mixed for 15 s. Then, a mixture of 2.0 mL methanol and 400 μL chloroform was introduced and dispersed into the solution by using a disposable syringe with a needle. The obtained cloudy mixture was further mixed for another 15 s by using the vortex mixer. The mixture was centrifuged at 3000 rpm for 2.0 min. The aqueous phase was discarded, and the remaining chloroform phase was left at room conditions in a fume hood for 24 h to completely evaporate the extraction solvent. After this period, 100 μL of nitric acid (65 %, w/w) was added to dissolve the extract before directly sending it to the nebulizer of the FAAS system (Fig. 1).

2.5. Preparation of the heather leaf tea samples

Two heather leaf samples for tea preparation were purchased from different local herbalists in İstanbul. The leaves (1.0 g) were brewed in 200 mL water for 20 min. The resulting hot beverage was cooled down to room temperature. The leaves were filtered out and the liquid portion was diluted 10 times with water before determination.

3. Results and discussion

Details of the characterization study for the synthesized ligand can be found in Section 3.1. To improve the preconcentration efficiency acquired with the DLLME procedure, the univariate optimization strategy was embraced in the conducted studies. An isolated experimental parameter was put to test while the other parameters were kept

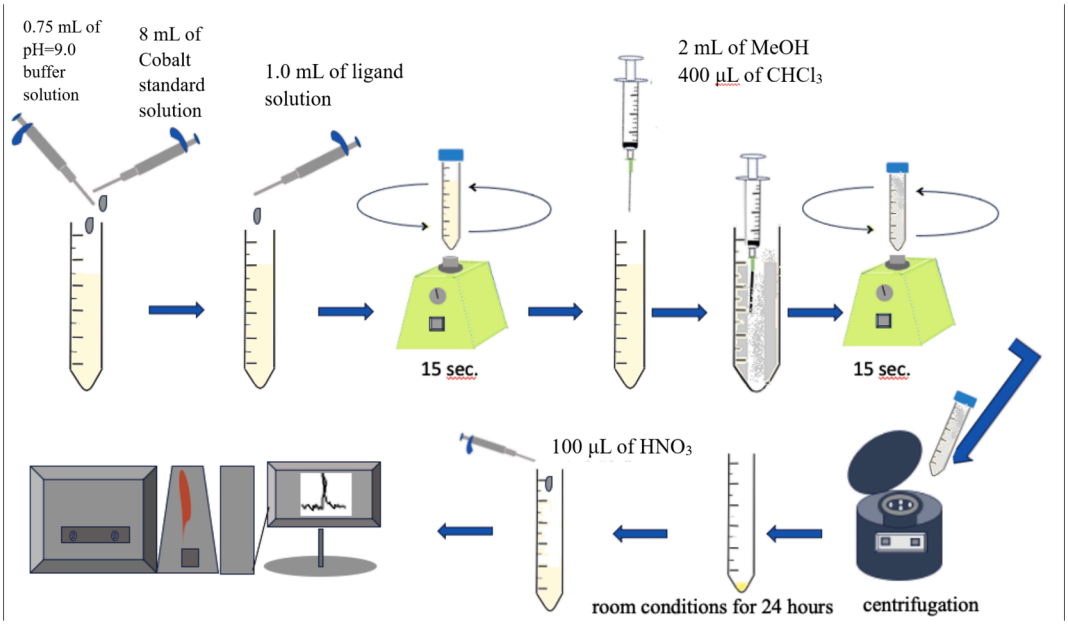

Fig. 1. Schematic representation of the preconcentration procedure.

constant. Three replicate measurements were made in each step of the study. The average absorbance values and standard deviations were calculated from these results.

The procedural parameters that were implemented in the steps of the optimization are summarized in Table 1 and the optimum parameters selected for the proposed method are summarized in Table 2. The details regarding the optimization studies are given in the following sections.

3.1. Characterization of the synthesized ligand

The synthesized ligand, $LACH_2$, was characterized by using IR Spectroscopy, Mass Spectrometry, and its melting behavior was recorded by using Differential Scanning Calorimetry.. The IR absorption bands corresponding to the major characteristic vibrations were observed as imine bond C=N stretching at 1613 cm^{-1}, phenol group C-O strechings between 1233 and 1159 cm^{-1}, aromatic C=C stretching at 1568 cm^{-1}, aromatic C–H stretchings between 3059 and 3017 cm^{-1}, aromatic C–H bending at 754 cm^{-1}, aliphatic C–H stretchings between 2945 and 2845 cm^{-1}. In the mass spectrum of $LACH_2$ (molecular mass: 310.4 u) obtained by EI, the molecular peak was observed at $m/z = 310$ and the base peak at $m/z = 148$. The m/z values corresponding to the major characteristic fragment ions are evaluated as $[O - C_6H_4 - CCH_3 = NH - CH_2 - CH_2]^+$ at $m/z = 174$, $[HO - C_6H_4 - CCH_3 = NH_2 - CH_2]^+$ at $m/z = 162$, $[HO - C_6H_4 - CCH_3 = NH_2]^+$ at $m/z = 148$, $[HO - C_6H_4 - CCH_3 = NH_2]^+$ at $m/z = 136$, $[HO - C_6H_4 - CH_2]^+$ at $m/z = 107$, $[C_6H_4 - CH_2]^+$ at $m/z = 91$. A sharp endotherm peaking at 123.7 °C clearly showing the melting of the product was observed in the DSC thermogram. Both the synthetic procedure and the results of the characterization study are in perfect agreement with the previously reported literature, directly supporting the successful synthesis and isolation of the ligand, $LACH_2$ [22,23].

Table 2
Optimum conditions for the proposed DLLME-FAAS system.

Parameter	Optimum condition
Sample volume	8.0 mL
pH buffer	0.75 mL, pH 9.0 buffer
Ligand solution	1.00 mL, 0.10 % (w/v) $LACH_2$ in MeOH
Dispersive solvent	2.0 mL methanol
Extraction solvent	400 µL chloroform
Mixing type and period	Vortex, 15 s
Elution solvent	100 µL, HNO_3

3.2. The effect of pH buffer type and volume on the analytical signal

It is widely known that the pH of the reaction medium dominantly affects the interaction of a ligand with metal ions. To determine the pH value providing the highest complexation efficiency, buffer solutions with pH values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were tried. In cases

Table 1
Respective experimental conditions in each step of the optimization study. The tested parameters are highlighted in bold.

		Optimized Parameters				
Experimental conditions	**pH buffer solution**	**Buffer sol. volume**	**Ligand sol. volume**	**$CHCl_3$ volume**	**Mixing period**	**HNO_3 volume**
Co^{2+} standard solution	1.0 mg/L	1.0 mg/L	1.0 mg/L	1.0 mg/L	0.5 mg/L	0.3 mg/L
	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL
pH buffer solution	**pH = 4.0–9.0**	pH = 9.0	pH = 9.0	pH = 9.0	pH = 9.0	pH = 9.0
	0.75 mL	**0.5–1.5 mL**	0.75 mL	0.75 mL	0.75 mL	0.75 mL
Ligand solution volume	1.00 mL	1.00 mL	**0.5–1.5 mL**	1.00 mL	1.00 mL	1.00 mL
Dispersive solvent	2.0 mL meOH	2.0 mL meOH	2.0 mL meOH	2.0 mL meOH	2.0 mL meOH	2.0 mL meOH
Extraction solvent	300 µL $CHCl_3$	300 µL $CHCl_3$	300 µL $CHCl_3$	**200–400 µL $CHCl_3$**	300 µL $CHCl_3$	300 µL $CHCl_3$
Mixing type and period	Vortex	Vortex	Vortex	Vortex	**Vortex**	Vortex
	15 s	15 s	15 s	15 s	**10–30 s**	15 s
Elution solvent	150 µL HNO_3	150 µL HNO_3	150 µL HNO_3	150 µL HNO_3	150 µL HNO_3	**75–250 µL HNO_3**

where buffer solutions with pH values ranging between 4.0 and 7.0 were used, no signal was detected. The absorbance values obtained with pH 8.0 buffer were dramatically lower than those obtained with pH 9.0. Therefore, pH = 9.0 was determined as the optimum buffer type because it gave the highest absorbances with a remarkably lower standard deviation. The complex formation was expected to be favored with the increasing pH, fundamentally due to the fact that the effective coordination of the metal ions by the $LACH_2$ ligand is strongly dependent on the Lewis basic nature of the imine group as is the case with other Schiff bases [24,25,26]. In other words, it is plausible to deduce that below the neutral pH, the hydronium ion concentration remains sufficient to protonate the C=N bond, leaving the lone pair of nitrogen unable to be involved in coordinative covalent bonds. Furthermore, the likelihood for protonation of the phenolic −O–H groups at lower pH levels would render the formation of the Co(II) $LACH_2$ complex unlikely. In contrast, at higher pH levels, the donor sites of the $LACH_2$ ligand can be easily deprotonated increasing the electron density around the donor atoms and facilitating the complex formation [27]. While definitive evidence of the imine bond's basicity and the phenolic hydrogens' acidity necessitates additional comprehensive research, the observed experimental variation in extraction effectiveness across different pH levels corroborates this proposed explanation. On the other hand, as the pH of the medium increases, there is a greater chance of complex formation starting to compete with the loss of cobalt ions from the solution due to precipitation in the form of hydroxides, which are insoluble at the higher pH levels [28]. To investigate the magnitude of such an effect, a control test without the addition of the ligand solution was performed to determine if the signals were resulting from the extracted complex and not from other sources such as the hydroxide precipitates at the selected pH. The readings of the control group show negligibly small signals compared to the complex formation case, proving that the recorded absorbances were, very dominantly, due to the preconcentration procedure. Buffer solutions of higher pH were not taken into consideration because of the rising favorability of precipitation of cobalt hydroxide species.

The volume of the added buffer solution is a significant factor in extraction studies due to the changes in buffer capacity and the overall dilution effects of the extraction procedure. The effect of buffer solution volume was evaluated by conducting experiments with 0.50, 0.75, 1.00, and 1.50 mL additions of pH 9.0 buffer. The optimum volume of 0.75 mL was selected with the highest absorbance value and the lowest standard deviation.

3.3. The effect of ligand solution volume on the analytical signal

The amount of the complexing agent needs to be evaluated to promote the quick and complete interaction between the ligand and analyte without causing any undesirable oversaturation that could become a problem in the form of interferences in detection. In this study, the concentration of the ligand was controlled by the volume of the solution of N,N'-bis(2-hydroxyacetophenylidene)-1,3-propanediamine in methanol added into the mixture. To determine the optimal volume, 0.50, 0.75, 1.00, and 1.50-mL additions of 0.10 % (m/V) $LACH_2$ (Fig. 2) solution were tested. The readings revealed that the difference between the average absorbance values was not very significant while the standard deviation within the sets showed remarkable differences (Fig. 3). Therefore, the volume of 1.00 mL, which yielded comparable repeatability without compromising the signal magnitude in the results, was selected.

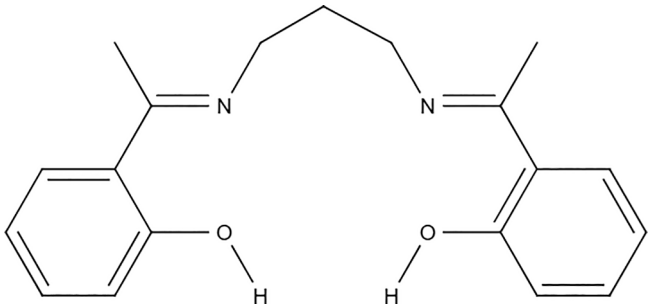

Fig. 2. Structural formula of the ligand $LACH_2$.

3.4. The effect of the type and volume of the extraction solvent on the analytical signal

The type and volume of the extraction solvent are among the most impactful parameters to obtain high extraction efficiencies and reduced dilution effects. Three solvents were put to the test, namely, chloroform, dichloroethane, and dichloromethane. A good phase separation could not be achieved with dichloromethane, apparently because of its tendency to partially dissolve in water. Chloroform was selected as the optimum extraction solvent due to the notably higher absorbance values observed than those obtained for dichloroethane. The average absorbances recorded were 0.275 ± 0.002 with chloroform and 0.150 ± 0.048 with dichloroethane.

To investigate the effect of the solvent volume, 200, 250, 300, 400 Âµl of chloroform were used. With 400 µL, increased absorbances along with noteworthily higher repeatability in the readings were obtained, hence it was selected as the optimum point (Fig. 4). Fig. 4 illustrates that augmenting the extraction solvent volume from 200 µL to 300 µL led to a nearly 50 % enhancement in the analytical signal. Conversely, the identical 100 µL increase in solvent volume yielded a diminished return, approximately increasing the signal magnitude by about 20 %. It is apparent that the decline in signal amplitude from sample dilution begins to outweigh the increased extraction efficiency from larger volumes, resulting in the analytical signals between 300 and 400 µL plateauing. Therefore, no larger extraction solvent volumes were tested as they would increase the solvent consumption and decrease the environmental friendliness of the method only for a marginal increase in the enrichment factor.

3.5. The effect of mixing time for the complexation reaction on the analytical signal

Vortex mixing was adopted to provide increased interaction of the ligand with the analyte particles in a short amount of time, which directly influenced the practicality and quickness of the method. The most efficient and shortest mixing time possible to achieve optimized results was investigated by experimenting with 10, 15, 20, and 30 s mixing times (Fig. 5). It was seen that 15 s yielded the highest average absorbance value and the lowest standard deviation.

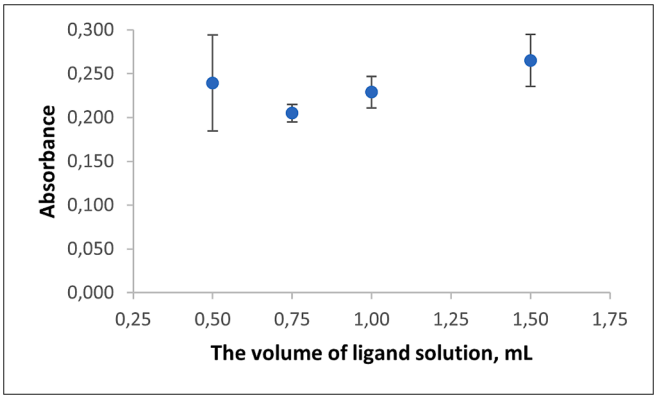

Fig. 3. The effect of ligand solution volume on absorbance.

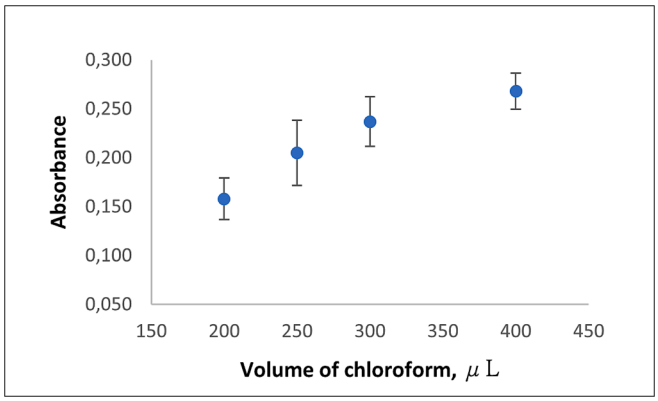

Fig. 4. The effect of the volume of the extraction solvent on absorbance.

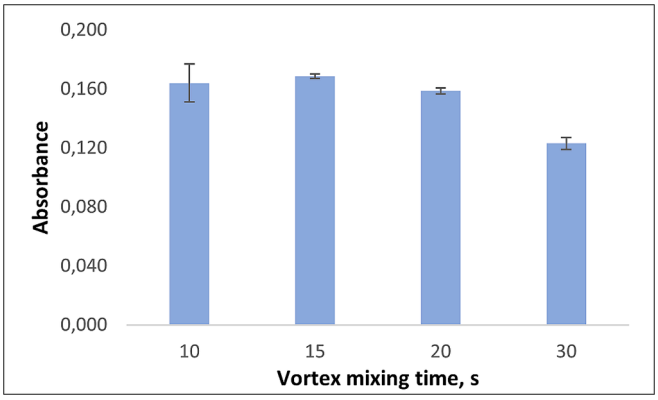

Fig. 5. The effect of mixing time on absorbance.

3.6. The effect of eluent volume on the analytical signal

After the extraction solvent was completely evaporated, nitric acid (65 %, w/w) was used to dissolve the non-volatile extract and make it suitable for nebulization. The final volume of the analytical phase introduced into the AAS system is a very important parameter in obtaining a high absorbance. An excess of the eluent would result in low absorbance values due to the decreased concentration of the analyte while too little eluent could render the volume of the final solution insufficient for the nebulizer or it could result in partially undissolved solid residue. To find out the optimum eluent volume for the proposed method, 75, 100, 125, 150, 200, 250 Âμl of HNO_3 were put to test (Fig. 6). The highest readings were observed with 75 μL nitric acid. However, 100 μL was determined as the optimum working volume due

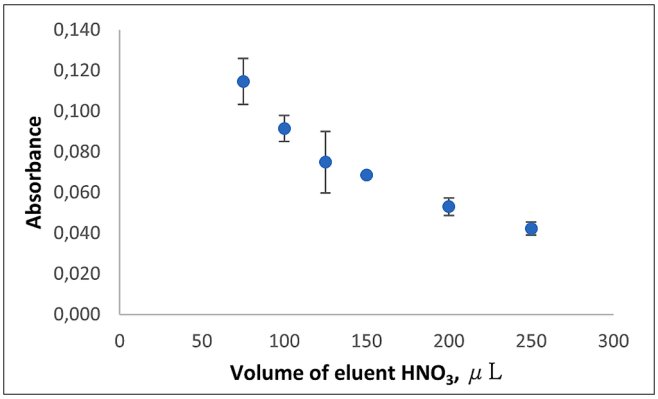

Fig. 6. The effect of HNO_3 volume on absorbance.

to the lower standard deviation and the remarkably easier operation with the nebulizer system.

3.7. Assessment of the analytical performance

The analytical performance parameters for the developed method were determined by constructing an analytical curve by utilizing the whole procedure under the optimum conditions for cobalt standard solutions of various concentrations between 0.050 and 5.0 mg/L. The linearity was provided by a linear working range between 0.050 – 0.75 mg/L with a regression coefficient (R^2 value) of 0.9926. The equation of the linear calibration curve was "y = 0.4134x + 0.0007" (Fig. 7). Limit of detection (LOD) and quantitation (LOQ) values were calculated using the formula 3.SD/m and 10.SD/m, respectively. Here, SD defines the standard deviation of the lowest concentration and m describes the slope of the calibration curve. LOD and LOQ values were found to be 0.0154 and 0.0513 mg/L, respectively and %RSD was calculated as 8.8 %.

An additional linear calibration run was conducted with the direct determination by FAAS without the preconcentration procedure for comparison of analytical performance. The linear calibration equation obtained for the FAAS system was "y = 0.0116x + 0.0211" within the cobalt concentration range of 0.50 – 20 mg/L. It was deduced that an enhancement of 35.6-fold in detection sensitivity was achieved. The preconcentration factor was obtained by calculating the ratio of the slope of the DLLME-FAAS calibration line to the slope of the FAAS calibration line.

The analytical figures of merit of this study along with previously reported analytical performance parameters from studies regarding the preconcentration of cobalt are given in Table 3. Ojeda et al. reported an analytical method for the preconcentration of cobalt in food, environmental, and water samples. They obtained an 8-fold sensitivity enhancement with the DLLME-FAAS method using 1,5-bis(di-2-pyridyl) methylene thiocarbohydrazide (DPTH) as the chelating agent [17]. Sivrikaya et al. presented preconcentration of cobalt 7.3 times in water and food samples after a modified silica gel-based flow injection solid phase extraction method [15]. In 2021, de Almeida et al. reported a preconcentration factor of 38 with a method for the determination of cobalt at trace levels in water, black tea, and chocolate powder samples utilizing a synthetic polymer as the solid phase extraction agent and a hyphenated flow system for sample introduction. In their study involving a method for the separation of Co from water samples and serum, Shokrollahi and Ebrahimi observed a 22.4-fold enhancement in sensitivity [29]. In light of these outputs, the DLLME-FAAS method that is proposed by this study was considered a good alternative for the extraction and preconcentration of cobalt from complex matrices.

3.8. Recovery studies

The applicability and accuracy of the developed method to real samples were evaluated by performing recovery studies on heather-leaf tea using the optimum conditions. Recovery studies were carried out by calculating the ratio of cobalt that was determined by utilizing the procedure to the known concentration of cobalt in two different heather leaf tea samples following a matrix-matching calibration strategy. Firstly, the brewed tea samples were filtered, diluted, and studied under the optimum conditions to determine whether there was an amount of cobalt in them detectable by the developed DLLME-FAAS method. These readings showed no detectable absorbance. Then, proper volumes of concentrated Co^{2+} standard solutions were spiked into the samples producing different concentrations of cobalt in the range of 0.075–0.50 mg/L followed by the application of the proposed method for determination. 4 repetitions were performed for each data point. Due to the matrix effects, using the external calibration line, obtained from the standard solutions, resulted in very poor recovery values. To help overcome these by improving the accuracy and suitability of the developed method matrix matching calibration was embraced.

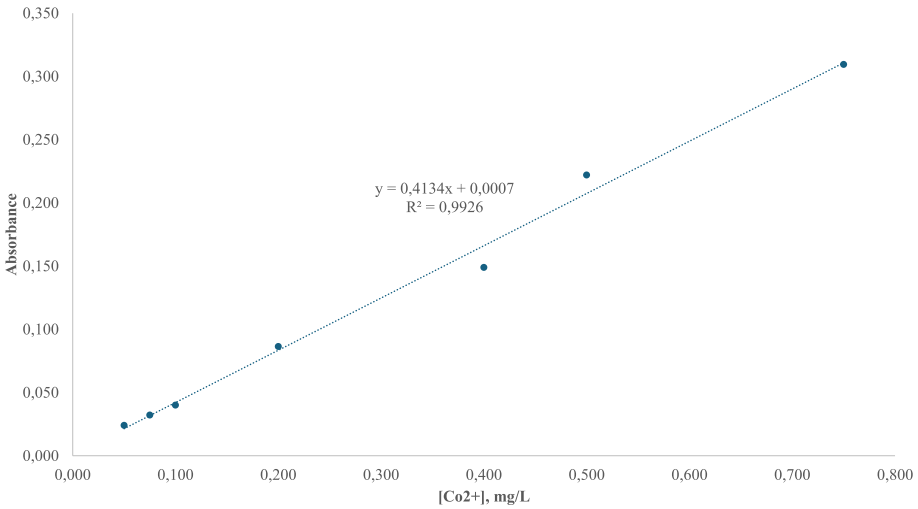

Fig. 7. The calibration line acquired with the proposed DLLME-FAAS method.

Table 3
System analytical performance parameters for the proposed method and comparable methods previously reported in the literature.

Method	LOD, mg/L	R^2	Linear Range, mg/L	PF[1]	Related study
DLLME-FAAS[2]	0.0142	0.9990	0.050 – 0.50	8	[17]
FI-SPE-FAAS[3]	0.0014	–	0.050 – 0.50	7.3	[15]
SPE by Poly (vinylpyridine-co-protoporphyrin)-FAAS[4]	0.00138	0.9991	0.0046 – 0.20	38	[30]
UA-DSLLME-FAAS[5]	0.0035	0.9981	0.005 – 0.5	22.4	[29]
DLLME-FAAS[2]	0.0154	0.9926	0.050 – 0.75	35.6	Present study

[1] Preconcentration factor.
[2] Dispersive liquid–liquid microextraction-flame atomic absorption spectrometry.
[3] Flow injection-solid phase extraction-flame atomic absorption spectrometry.
[4] Solid phase extraction by Poly(vinylpyridine-co-protoporphyrin)-flame atomic absorption spectrometry.
[5] Ultrasonic-assisted dispersion solidification liquid–liquid microextraction-flame atomic absorption spectrometry.

For this purpose, 2 calibration curves were drawn using the absorbance values obtained with the 2 sets of spiked samples, one set in the Brand A matrix and the other in the Brand B matrix. The equations of the calibration lines obtained from the 1st (Brand A) and the 2nd (Brand B) set of samples were found as "y = 0.2075x + 0.0070" and "y = 0.1604x + 0.0088", respectively. Then, the concentrations and percent recoveries for the spiked samples in the Brand A matrix were calculated by using the calibration equation acquired with the spiked sample set prepared in the Brand B matrix. Likewise, the percent recoveries of the spiked samples in the Brand B matrix were calculated by using the calibration equation obtained with the Brand A matrix. The results from the recovery studies are given in Table 4 along with the standard deviation values. It is apparent in the results that the recovery values obtained for Brand A tea samples are within the range of 105.7–128.4 %, while those in Brand B tea samples are considerably lower, lying within the range of 78.7–86.7 %. This discrepancy between the brands is due to the slight difference in matrix components of tea extracts. Meanwhile, the overall range of 78.7–128.4 % is indicative of the accuracy and applicability of the developed method.

Table 4
Results of the recovery studies in the spiked heather leaf tea samples.

Sample set	Spiked Concentration, mg/L	External Calibration		Matrix Matching Calibration	
		% Recovery	SD	% Recovery	SD
Brand A	0.075	80.4	8.2	124.3	19.6
	0.1	65.5	4.5	105.7	10.6
	0.2	64.4	5.9	128.4	14.2
	0.4	57.2	7.0	124.0	16.7
	0.5	57.9	3.5	128.3	8.3
Brand B	0.075	66.4	5.9	81.9	11.0
	0.1	63.5	5.6	86.7	10.4
	0.2	54.3	2.7	84.9	5.0
	0.4	46.8	1.4	78.7	2.5
	0.5	46.1	5.0	79.0	9.3

4. Conclusion

In this study, a simple and environmentally friendly extraction method was developed for the trace level determination of cobalt in heather leaf tea samples. The extraction and preconcentration of cobalt were achieved by an optimized DLLME procedure before FAAS measurements. In the procedure, N,N'-bis(2-hydroxyacetophenylidene)-1,3-propanediamine was utilized as the complexing agent, and chloroform and methanol were employed as the extraction and dispersive solvents, respectively. Spike and recovery experiments were performed to determine the accuracy and applicability of the proposed method. A conventional, external calibration approach led to unpractically low recovery results due to negative interferences by the matrices. Meanwhile, acceptable percent recovery values spanning in the range of 78.7–128.4 % were obtained via matrix matching calibration strategy. The analytical outputs along with the results from the recovery studies revealed that the developed method can be used to determine cobalt with comparable accuracy and precision in heather leaf tea samples using a matrix matrix-matching calibration strategy.

5. Code availability

Not applicable.

6. Conformed consent

Not applicable.

Ethical approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Arda Atakol: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Damla Zeydanli:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Tuğçe Göver:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Melike Atakol:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Hakan Serbest:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Kübra Karakebap:** Formal analysis, Validation, Visualization, Writing – original draft. **Muhammed Ali Büyük:** Formal analysis, Validation, Visualization. **Orhan Atakol:** Conceptualization, Investigation, Methodology, Writing – original draft. **Sezgin Bakirdere:** Conceptualization, Investigation, Methodology, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] Z. Guo, et al., Detection of heavy metals in food and agricultural products by surface-enhanced Raman spectroscopy, *Food Rev. Int.* 39 (3) (Apr. 2023) 1440–1461, https://doi.org/10.1080/87559129.2021.1934005.
[2] N.A. Kasa, Ç. Büyükpinar, A.F. Erulaş, S. Bakirdere, A sensitive and accurate analytical method for the determination of cadmium in food samples: molybdenum coated T-shape slotted quartz tube flame atomic absorption spectrophotometry, *Food Chem.* 319 (Jul. 2020) 126572, https://doi.org/10.1016/j.foodchem.2020.126572.
[3] M. Soylak, A. Aydin, Determination of some heavy metals in food and environmental samples by flame atomic absorption spectrometry after coprecipitation, *Food Chem. Toxicol.* 49 (6) (Jun. 2011) 1242–1248, https://doi.org/10.1016/j.fct.2011.03.002.
[4] M. Jaishankar, T. Tseten, N. Anbalagan, B.B. Mathew, K.N. Beeregowda, Toxicity, mechanism and health effects of some heavy metals, *Interdiscip. Toxicol.* 7 (2) (Jun. 2014) 60–72, https://doi.org/10.2478/intox-2014-0009.
[5] M.A. Zoroddu, J. Aaseth, G. Crisponi, S. Medici, M. Peana, V.M. Nurchi, The essential metals for humans: a brief overview, *J. Inorg. Biochem.* 195 (Jun. 2019) 120–129, https://doi.org/10.1016/j.jinorgbio.2019.03.013.
[6] R. Lauwerys, D. Lison, Health risks associated with cobalt exposure — an overview, *Sci. Total Environ.* 150 (1) (Jun. 1994) 1–6, https://doi.org/10.1016/0048-9697(94)90125-2.
[7] P. Banerjee, P. Bhattacharya, Investigating cobalt in soil-plant-animal-human system: dynamics, impact and management, *J. Soil Sci. Plant Nutr.* 21 (3) (Sep. 2021) 2339–2354, https://doi.org/10.1007/s42729-021-00525-w.
[8] M. Ekor, The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, *Front. Pharmacol.* 4 (Jan. 2014), https://doi.org/10.3389/fphar.2013.00177.
[9] E. Marguí, et al., Determination of essential elements (Mn, Fe, Cu and Zn) in herbal teas by TXRF , FAAS and ICP-OES, *X-Ray Spectrom.* 51 (3) (May 2022) 204–213, https://doi.org/10.1002/xrs.3241.
[10] N.E. Karlıdağ, R. Demirel, H. Serbest, F. Turak, S. Bakırdere, Determination of cobalt in chamomile tea samples at trace levels by flame atomic absorption spectrophotometry after poly(vinyl alcohol)-magnetic hydrogel based dispersive solid phase extraction, *Anal. Methods* 15 (1) (2023) 56–62, https://doi.org/10.1039/D2AY01493A.
[11] A. Güvenç, G. Kend, A. Eken, and A. Aydin, "Evaluation of Inorganic Compounds of Erica L. Species (Ericaceae) Native to Turkey," 2007.
[12] H. Nazemiyeh, et al., Antioxidant phenolic compounds from the leaves of Erica Arborea (Ericaceae), *Nat. Prod. Res.* 22 (16) (2008) 1385–1392, https://doi.org/10.1080/14786410701824007.
[13] J. Żukowska, M. Biziuk, Methodological evaluation of method for dietary heavy metal intake, *J. Food Sci.* 73 (2) (2008) R21–R29, https://doi.org/10.1111/j.1750-3841.2007.00648.x.
[14] X. Song, X. Huang, Recent developments in microextraction techniques for detection and speciation of heavy metals, *Adv. Sample Prep.* 2 (May 2022) 100019, https://doi.org/10.1016/j.sampre.2022.100019.
[15] S. Sivrikaya, M. Imamoglu, S.Z. Yıldız, D. Kara, Novel functionalized silica gel for on-line preconcentration of cadmium(II), copper(II), and cobalt(II) with determination by flame atomic absorption spectrometry, *Anal. Lett.* 49 (7) (May 2016) 943–957, https://doi.org/10.1080/00032719.2015.1067812.
[16] R. Khani, F. Shemirani, Simultaneous determination of trace amounts of cobalt and nickel in water and food samples using a combination of partial least squares method and dispersive liquid–liquid microextraction based on ionic liquid, *Food Anal. Methods* 6 (2) (Apr. 2013) 386–394, https://doi.org/10.1007/s12161-012-9449-8.
[17] C.B. Ojeda, F.S. Rojas, J.M.C. Pavón, Determination of cobalt in food, environmental and water samples with preconcentration by dispersive liquid-liquid microextraction, *Am. J. Anal. Chem.* 03 (02) (2012) 125–130, https://doi.org/10.4236/ajac.2012.32018.
[18] D. Citak, M. Tuzen, A novel preconcentration procedure using cloud point extraction for determination of lead, cobalt and copper in water and food samples using flame atomic absorption spectrometry, *Food Chem. Toxicol.* 48 (5) (May 2010) 1399–1404, https://doi.org/10.1016/j.fct.2010.03.008.
[19] A.A. Gouda, A.M. Summan, A.H. Amin, Development of cloud-point extraction method for preconcentration of trace quantities of cobalt and nickel in water and food samples, *RSC Adv.* 6 (96) (2016) 94048–94057, https://doi.org/10.1039/C6RA20900A.
[20] E.G. Primel, S.S. Caldas, L.C. Marube, A.L.V. Escarrone, An overview of advances in dispersive liquid–liquid microextraction for the extraction of pesticides and emerging contaminants from environmental samples, *Trends Environ. Anal. Chem.* 14 (Apr. 2017) 1–18, https://doi.org/10.1016/j.teac.2017.03.001.
[21] P. Viñas, N. Campillo, I. López-García, M. Hernández-Córdoba, Dispersive liquid–liquid microextraction in food analysis. a critical review, *Anal. Bioanal. Chem.* 406 (8) (Mar. 2014) 2067–2099, https://doi.org/10.1007/s00216-013-7344-9.
[22] M. Aksu, et al., Investigation on the thermal decomposition some heterodinuclear NiII-MII complexes prepared from ONNO type reduced Schiff base compounds (MII=ZnII, CdII), *J. Therm. Anal. Calorim.* 90 (2) (Nov. 2007) 541–547, https://doi.org/10.1007/s10973-006-7729-5.
[23] M. Sönmez, H. Nazir, E. Emir, I. Svoboda, L. Aksu, O. Atakol, Two dinuclear NiII–CdII complexes of reduced ONNO-type Schiff bases, *J. Therm. Anal. Calorim.* 131 (3) (Mar. 2018) 3077–3091, https://doi.org/10.1007/s10973-017-6720-7.
[24] P. D. Bernardo, P. L. Zanonato, S. Tamburini, P. Tomasin, and P. A. Vigato, "Complexation behaviour and stability of Schiff bases in aqueous solution. The case of an acyclic diimino(amino) diphenol and its reduced triamine derivative," *Dalton Trans.*, no. 39, pp. 4711–4721, Oct. 2006, doi: 10.1039/B604211B.
[25] A. Rauf, et al., Synthesis, spectroscopic characterization and pH dependent photometric and electrochemical fate of Schiff bases, *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* 138 (Mar. 2015) 58–66, https://doi.org/10.1016/j.saa.2014.10.111.
[26] A. Rauf, et al., Synthesis, pH dependent photometric and electrochemical investigation, redox mechanism and biological applications of novel Schiff base and its metallic derivatives, *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* 176 (Apr. 2017) 155–167, https://doi.org/10.1016/j.saa.2017.01.018.
[27] L.-S. Long, pH effect on the assembly of metal–organic architectures, *CrstEngComm* 12 (5) (Apr. 2010) 1354–1365, https://doi.org/10.1039/B921146B.
[28] E. Garcia, J. Santos, E. Pereira, M.B.J.G. Freitas, Electrodeposition of cobalt from spent li-ion battery cathodes by the electrochemistry quartz crystal microbalance technique, *J. Power Sources* 185 (Oct. 2008) 549–553, https://doi.org/10.1016/j.jpowsour.2008.07.011.
[29] A. Shokrollahi, F. Ebrahimi, Supramolecular-based ultrasonic-assisted dispersion solidification liquid–liquid microextraction of copper and cobalt prior to their flame atomic absorption spectrometry determination, *J. AOAC Int.* 100 (6) (Nov. 2017) 1861–1868, https://doi.org/10.5740/jaoacint.17-0042.
[30] F.G. de Almeida, et al., Synthesis and performance of cross-linked poly (vinylpyridine-*co*-protoporphyrin) for effective cobalt determination using a micro-packed column hyphenated system coupled to FAAS, *React. Funct. Polym.* 164 (Jul. 2021) 104934, https://doi.org/10.1016/j.reactfunctpolym.2021.104934.