



Selective Determination of Trace Co(II) and Cu(II) in Food and Environmental Matrices via Carrier Element-Free Coprecipitation Using a Novel Thiosemicarbazone-Derived Schiff Base

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Abstract

A carrier element-free coprecipitation (CEFC) method was developed for the preconcentration and determination of trace Co(II) and Cu(II) in environmental and food samples using a novel thiosemicarbazone-derived Schiff base ligand (MBITAH). Unlike conventional coprecipitation approaches, the proposed strategy eliminates the need for inorganic carriers, reducing potential interferences and improving selectivity toward the target analytes. Under optimized conditions (pH 7.0, 1.5 mg ligand), quantitative recoveries were achieved with limits of detection of 1.16 and 0.79 $\mu\text{g L}^{-1}$ for Co(II) and Cu(II), respectively. A preconcentration factor of 50 enabled sensitive determination by flame atomic absorption spectrometry (FAAS) with good precision ($\text{RSD} \leq 3.7\%$). The method exhibited good tolerance toward common matrix ions and was validated using certified-reference material (CRM-SA-C Sandy Soil C) and spike/recovery tests, showing agreement with certified values ($p > 0.05$). Its applicability was demonstrated in sea water, stream water, and food samples (red lentil and bulgur), confirming reliable performance in complex matrices. Compared with previously reported approaches, the proposed CEFC method offers a favorable balance between analytical performance, simplicity, and cost-effectiveness. In addition, greenness assessment using the AGREE tool yielded a score of 0.45, indicating a moderate-to-good level of environmental friendliness.

Keywords Carrier element-free coprecipitation (CEFC) · Thiosemicarbazone-based Schiff base · Co(II) and Cu(II) · Green analytical chemistry · Flame atomic absorption spectrometry (FAAS)

Highlights

- A novel thiosemicarbazone-derived Schiff base was synthesized for CEFC of Co(II) and Cu(II).
- The method eliminates the need for inorganic carriers, ensuring low blank signals.
- High preconcentration factor (50) and low detection limits (sub-ppb) were achieved.
- The procedure was successfully validated with CRM and applied to food and water samples.
- The method aligns with green analytical chemistry by minimizing reagent consumption.

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Introduction

The determination of trace metal ions in environmental samples remains a central issue in analytical chemistry due to their environmental persistence, toxicity, and bioaccumulation potential (Ali et al. 2019). In recent decades, highly advanced instrumental techniques such as inductively coupled plasma–mass spectrometry (ICP–MS), tandem ICP–MS (ICP–MS/MS), and high-resolution plasma-based

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systems have achieved extremely low detection limits and multi-element capability (Balcaen et al. 2015; Wilschefski and Baxter 2019).

Despite these technological advances, complex environmental matrices—including saline waters, wastewater, and organic-rich samples—continue to generate matrix-induced signal suppression/enhancement effects, spectral interferences, and analytical instability (Wilschefski and Baxter 2019; Yang et al. 2024). In addition, the high capital cost, continuous argon consumption, maintenance requirements, and need for trained personnel restrict the routine use of such sophisticated instrumentation in many environmental laboratories. Therefore, effective sample preparation remains indispensable, not only for enrichment but also for matrix elimination and improvement of analytical reliability (Williams et al. 2023).

In recent years, sample preparation strategies have evolved toward miniaturization, reduced solvent consumption, and improved environmental sustainability in accordance with the principles of green analytical chemistry (Płotka-Wasyłka 2018; Trujillo-Rodríguez et al. 2020). Microextraction-based techniques such as dispersive liquid–liquid microextraction (DLLME), solid-phase microextraction (SPME), magnetic solid-phase extraction (MSPE), liquid phase microextraction-based deep eutectic solvent, and ionic liquid-based systems have been extensively developed as efficient enrichment strategies for trace analytes (Sun et al. 2024; Jalili et al. 2021; Burato et al. 2020; Trujillo-Rodríguez et al. 2020). Although these approaches provide high enrichment factors and low detection limits, many require sophisticated sorbent synthesis, nanomaterials, ionic liquids, or multi-step extraction procedures, which may increase operational complexity, cost, and preparation time.

Coprecipitation has historically been employed as a simple and effective preconcentration technique and continues to attract attention as a simple and effective approach in modern analytical workflows. Beyond enrichment, it offers intrinsic matrix separation through phase isolation, which is particularly advantageous for complex environmental samples. Conventional coprecipitation methods rely on inorganic carrier elements such as Fe(III) or Al(III), which may introduce matrix contamination and elevated blank values. To overcome these drawbacks, an organic ligand-assisted approach without the addition of a carrier element was introduced (Bulut et al. 2008). The concept was later formalized under the term carrier element-free coprecipitation (CEFC) (Duran et al. 2009; Bulut et al. 2009), and subsequently applied to various trace metal determinations (Saracoglu and Soylak 2010; Gouda et al. 2022). Although CEFC is based on classical precipitation principles, its operational simplicity, minimal reagent consumption, and absence of nanomaterial synthesis or toxic organic solvents make it

consistent with contemporary green and minimalist analytical philosophies (Mabrouk et al. 2023).

From an instrumental standpoint, flame atomic absorption spectrometry (FAAS), while often considered conventional compared to plasma-based systems, remains widely available, robust, and economically accessible. When combined with an efficient preconcentration step, FAAS can provide satisfactory detection limits for numerous environmental monitoring applications without the need for highly sophisticated infrastructure. The relatively low operational cost, ease of maintenance, and straightforward method development continue to sustain its practical relevance, particularly in laboratories where access to ICP-based instrumentation is limited (Patriarca et al. 2023).

Despite its inherent advantages, CEFC has remained somewhat overshadowed by the recent surge in nanomaterial-based microextraction techniques. However, when re-evaluated through the lens of modern analytical chemistry—which demands sustainability, minimal sample handling, and superior matrix management—CEFC emerges not as a legacy method, but as a high-performance, rational alternative. This study seeks to bridge the gap between classical precipitation and modern selective recognition by exploring the synergistic potential of a novel organic scaffolding.

In this context, we report the development of a highly selective CEFC procedure utilizing a specifically engineered organic coprecipitating ligand. Unlike generic reagents, this novel organic compound exhibits a distinct molecular recognition behavior; while evaluated against a broad spectrum of transition metals, it achieved quantitative and selective recovery solely for Co(II) and Cu(II). This pronounced structure-dependent selectivity, coupled with the absence of any carrier element, minimizes blank signals and eliminates the need for complex sorbent synthesis. By integrating this targeted selectivity with a streamlined, mechanistically driven, and sustainable alternative for trace metal monitoring in complex environmental and food matrices.

Materials and Methods

Instrumentation

Trace metal determinations were performed using a Perkin Elmer AAnalyst 400 model flame atomic absorption spectrometer (FAAS) (Perkin Elmer, USA). An air-acetylene flame was employed as the oxidant/fuel mixture, and spectral background corrections were conducted using a deuterium (D_2) lamp. All quantitative analyses were carried out via the external calibration method. The measurements were recorded at the primary resonance wavelengths for each analyte: 324.75 nm for Cu(II) and 240.73 nm for Co(II).

Phase separation of the precipitates was achieved using a Sigma 3-16P model centrifuge (Sigma Laborzentrifugen GmbH, Germany). For pH measurements and adjustments, a Hanna pH-211 digital pH meter equipped with a combined glass electrode (Hanna Instruments, USA) was utilized. Solid samples were digested using a Milestone Ethos D closed-vessel microwave digestion system (Milestone Srl, Italy), capable of operating at a maximum pressure of 1450 psi and a maximum temperature of 300 °C. Throughout the experimental procedures, high-purity water (18.2 M Ω .cm) obtained from a Sartorius Milli-Q (arium® 611UV) ultra-pure water system (Sartorius AG, Germany) was used.

Reagents and Solutions

All chemical reagents used in this study, including HNO_3 , NaOH, ethyl alcohol, dimethyl sulfoxide (DMSO), and various salts NaCl, KCl, CaCl_2 , $\text{Mg}(\text{NO}_3)_2$, NaNO_3 , Na_2CO_3 , Na_2SO_4 , Na_3PO_4 , KI, NaF, NaCH_3COO , NH_4NO_3 , V_2O_5 , $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Co}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3$, $\text{Cu}(\text{NO}_3)_2$, $\text{Mn}(\text{NO}_3)_2$, $\text{Al}(\text{NO}_3)_3$, glikol, $\text{KHC}_8\text{H}_4\text{O}_4$, KH_2PO_4 , and Na_2HPO_4 , were of analytical grade. These chemicals were purchased from Merck (Darmstadt, Germany) or Fluka (Buch, Switzerland).

A certified reference material (CRM), namely CRM-SA-C Sandy Soil C, was obtained from High Purity Standards Inc. (Charleston, SC, USA). Buffer solutions for various pH ranges were prepared according to the Sorensen buffer system.

For the preparation of 25 mL of the ligand solution (0.1% w/v), 0.025 g of 2-{3-(4-methylbenzyl)-4-[2-(1H-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-N'-(2-hydroxyphenylmethylene)acetohydrazide, as a coprecipitating agent (hereinafter referred to as MBITAH) (Bektaş et al. 2010), was accurately weighed and dissolved in a 25 mL mixture of DMSO and ethyl alcohol (1:4, *m/v*).

Stock solutions (1000 mg L^{-1}) of Co(II) and Cu(II) ions were prepared in 0.5% (*m/v*) HNO_3 . Working standard solutions were prepared daily by appropriate dilution of the stock solutions with ultra-pure water.

Synthesis of the MBITAH

The ligand used for the enrichment of Co(II) and Cu(II) ions, 2-{3-(4-methylbenzyl)-4-[2-(1H-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-N'-(2-hydroxyphenylmethylene)acetohydrazide (MBITAH), was synthesized at the Department of Chemistry, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey. The chemical reaction for the synthesis of this ligand is illustrated in Fig. 1.

For the synthesis, an equivalent amount of 2-hydroxybenzaldehyde (1.14 g, 0.01 mol) was added to a solution of compound (A) (4.04 g, 0.01 mol) in 50 mL of ethanol in a round-bottom flask. The mixture was heated under reflux for 2 h. After cooling to room temperature, the resulting precipitate was collected by filtration. The crude solid product was purified by repeated crystallization from a DMSO-water (1:1) mixture and dried over CaCl_2 under vacuum: yield, 4.31 g (85%); m.p., 273–274 °C (Bektaş et al. 2010).

The characterization data for the synthesized compound are as follows: yield 85%, m.p. 273–274 °C; *Anal.* Calcd. (%) for: $\text{C}_{29}\text{H}_{28}\text{N}_6\text{O}_3$ C, 68.49, H, 5.55, N, 16.52, Found; C, 68.51, H, 5.58, N, 16.50; IR (KBr, ν , cm^{-1}): 3396 (OH), 3198 (NH), 1710 (triazole C=O), 1684 (hydrazide-C=O), 1618 (C=N); ^1H NMR (DMSO- d_6) δ (ppm): 2.25 (s 3H, CH_3), 2.81 (t, 2H, trp- CH_2 , $j=6.6$ Hz), 3.40 (s, 2H, benzil- CH_2), 3.66 (t, 2H, trp- CH_2 , $j=6.6$ Hz), 4.87, 4.45 (s, 2H, CH_2 , *E/Z* amide conformer (%53*E*/%47*Z*)), 6.82–7.10 (m, 9H ar-H), 7.14–7.45 (m, 3H, ar-H), 7.58, 7.77 (s, 1H, ar-H, *E/Z* amide conformer), 8.34, 8.45 (s 1H, N=CH, *E/Z* amide conformer), 10.07 (s, 1H, OH), 10.97 (s, 1H, trp-NH), 11.62,

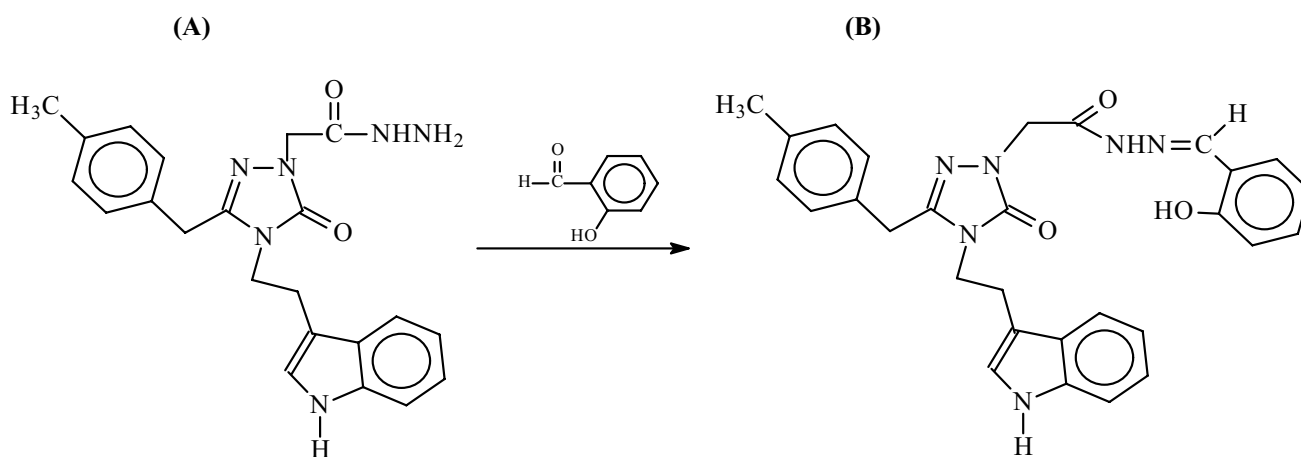


Fig. 1 Synthesis of the ligand MBITAH. (A) Acetohydrazide precursor used for ligand synthesis. (B) MBITAH, the Schiff base ligand used as the co-precipitating agent in this study

11.94 (s, 1H, NH, *E/Z* amide conformer); ^{13}C NMR (DMSO- d_6) δ (ppm): 20.49 (CH_3), 23.80 (trp- CH_2), 30.49 (benzil- CH_2), 41.90 (trp- CH_2), 46.43 (NCH_2), ar-C:[110.08 (C), 111.36 (C), 112.48 (C), 117.95 (C), 118.37 (C), 119.25 (C), 121.01 (C), 123.25 (C), 126.15 (C), 126.72 (C), 128.28 (2C), 129.00 (2C), 130.75 (C), 131.62 (C), 131.74 (C), 135.84 (C), 136.02 (C), 163.35 (C)], 145.28 ($\text{N}=\text{CH}$), 154.08 (triazole C-5), 157.14 (triazole C-3), 167.64 ($\text{C}=\text{O}$); MS (ESI): m/z (%) 509 ($\text{M}+1$, 48), 531 ($\text{M}+\text{Na}$, 100), 357 (28), 229 (100).

The obtained spectroscopic data (^1H NMR, ^{13}C NMR, and MS) are consistent with the proposed structure of the MBITAH ligand and confirm its successful synthesis. These techniques provide sufficient structural information for the identification of the synthesized compound.

Application of the CEFC Method

Before analyzing real environmental samples, the validity of the developed CEFC method was tested using synthetic working solutions to establish the general analytical protocol. The procedure was carried out as follows:

An aliquot of 50 mL of a synthetic working solution containing 5.0 μg of Cu(II) and 3.0 μg of Co(II) was adjusted to pH 7.0. Subsequently, 1.5 mL of 0.1% (w/v) MBITAH ligand solution (corresponding to 1.5 mg of ligand) was added to the mixture. The solution was allowed to stand for 10 min to ensure complete precipitate formation. The mixture was then centrifuged at 2500 rpm for 10 min.

Following the decantation of the supernatant, the obtained precipitate was dissolved in 0.5 mL of concentrated HNO_3 . The final volume was completed to 2.0 mL with ultra-pure water. Finally, the concentrations of Cu(II) and Co(II) in the resulting solution were determined by FAAS. The overall workflow of the proposed CEFC procedure is schematically illustrated in Fig. 2.

Analysis of Real Samples and Certified Reference Materials

The developed CEFC method was applied to various environmental water samples (Macka-Trabzon, Turkey) and solid food samples, including lentil and bulgur purchased from local markets in Trabzon. To evaluate the accuracy and validity of the method, it was also applied to a certified reference material, CRM-SA-C Sandy Soil C.

Real water samples were filtered through a 0.45- μm cellulose nitrate membrane filter, and 50 mL aliquots were directly subjected to the developed CEFC procedure. Solid samples, on the other hand, were first digested using a closed-vessel microwave system, and the resulting clear solutions were subsequently subjected to the CEFC method. For this purpose, accurately weighed 0.75 g of food samples and 0.1 g of CRM-SA-C were placed into PTFE (Teflon) vessels. The digestion reagent mixture for CRM-SA-C consisted of 4.5 mL HCl, 1.5 mL HNO_3 , 1.0 mL HF, and 2.0 mL H_2O_2 , whereas for food samples (lentil and bulgur), a mixture of 6.0 mL HNO_3 and 2.0 mL H_2O_2 was used.

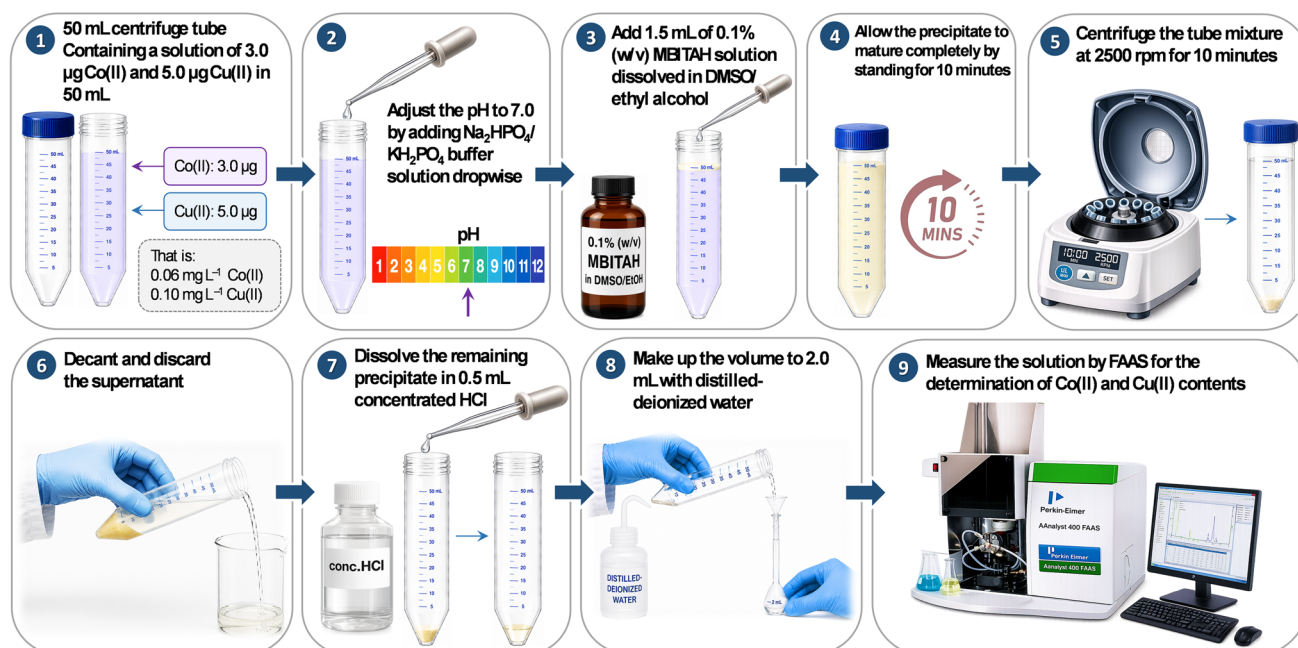


Fig. 2 Schematic illustration of the carrier-element-free coprecipitation (CEFC) procedure for the preconcentration and determination of Co(II) and Cu(II) ions prior to FAAS analysis

The digestion procedure was performed according to the manufacturer's recommended program. Specifically, the microwave digestion was carried out under controlled conditions with a maximum pressure of 45 bar. The program consisted of four sequential steps: (i) 250 W for 1 min at 180 °C; (ii) 0 W for 1 min at 180 °C (equilibration step); (iii) 650 W for 10 min at 200 °C; and (iv) 250 W for 5 min at 220 °C. After completion of the digestion process, a venting step of 3 min was applied to safely release the pressure. After the digestion process, the resulting clear solutions obtained from food samples (lentil and bulgur) were evaporated near to dryness to remove excess acids. Finally, the residues were diluted to 50 mL with ultra-pure water and subjected to the preconcentration procedure.

In the case of the certified reference material (CRM-SA-C Sandy Soil C), the digestion process resulted in a suspension due to the presence of insoluble residues. Therefore, the mixture was evaporated to near dryness and subsequently treated with 20 mL of 0.01 M HNO₃. The resulting suspension was filtered, and the filtrate was diluted to 50 mL with ultra-pure water prior to the preconcentration step (Senturk et al. 2007; Bulut et al. 2016).

Results and Discussion

In the development of a new analytical determination method, it is essential to optimize the method for specific analytical parameters and subsequently validate its performance. For this purpose, the recovery efficiencies of Co(II) and Cu(II) from aqueous solutions were systematically investigated as a function of several critical parameters, including solution pH, ligand amount, standing time, centrifugation speed and time, and sample volume.

Furthermore, the potential interference effects of co-existing foreign ions commonly found in environmental samples were investigated to ensure the analytical reliability of the proposed CEFC method. To rigorously evaluate the accuracy of the methodology, a certified reference material (CRM) were analyzed. Additionally, spike/recovery experiments were performed by adding known amounts of the analytes into the matrices of both solid and water samples to further validate the method's performance under complex conditions.

Influence of Solution pH on the Recovery of the Metal Ions

The solution pH is a critical parameter in CEFC as it influences both the surface charge of the precipitate and the degree of complexation between the analytes and the ligand (Mabrouk et al. 2023). The recovery of Co(II) and Cu(II) was investigated in the pH range of 2.0–10.0 (Fig. 3).

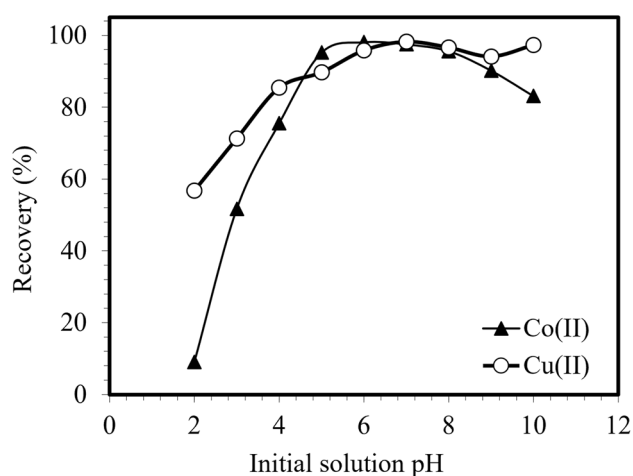


Fig. 3 Influence of solution pH on the recoveries of Cu(II) and Co(II) with MBITAH (sample volume=50 mL, $N=3$, ligand quantity=1.5 mg, standing time=10 min, centrifugation speed=2500 rpm, centrifugation time=10 min)

The coprecipitation mechanism in CEFC systems is inherently multifaceted and may involve metal-ligand complex formation, hydrophobic aggregation, surface adsorption, and partial entrapment processes (Hu et al. 2024). In the present system, the experimental results indicate that the dominant driving force is ligand-assisted complexation. The MBITAH ligand, possessing azomethine nitrogen, carbonyl oxygen, and phenolic donor groups, can act as a multidentate chelator, forming stable complexes particularly with Co(II) and Cu(II), as commonly reported for Schiff base and thio-semicarbazone derivatives (Abu-Dief and Mohamed 2015).

To further clarify the origin of selectivity, a broad range of metal ions was investigated under varying pH conditions. The results showed that only Co(II) and Cu(II) exhibited quantitative recoveries (>95%) over the studied pH range, whereas other tested metal ions displayed significantly lower recoveries (typically 40–50%) and strong pH dependence.

This behavior indicates that the coprecipitation process is predominantly governed by selective coordination chemistry rather than nonspecific physical mechanisms. If simple hydroxide precipitation were dominant, comparable recoveries would be expected for multiple transition metal ions at higher pH values, which was not observed. Therefore, the phase separation is mainly driven by the formation of relatively hydrophobic metal-MBITAH complexes at near-neutral pH, followed by aggregation and sedimentation, while secondary contributions from surface interactions and entrapment cannot be completely excluded (Abu-Dief and Mohamed 2015).

The recovery was found to be low at $\text{pH} < 4.0$, which can be explained by the protonation of the coordination sites of the MBITAH ligand (Fig. 1B). In acidic media, the lone pairs on the azomethine nitrogen ($\text{N}=\text{C}$) and the

carbonyl oxygen are occupied by H^+ ions, preventing the formation of stable metal chelates. As the pH increased to 7.0, the deprotonation of the phenolic hydroxyl group and the availability of the nitrogen donor atoms facilitated the formation of neutral, hydrophobic complexes. The multi-dentate nature of MBITAH allows it to wrap around the metal ions, creating stable five- or six-membered chelate rings that lead to efficient coprecipitation (Babu et al. 2023; Kenawy et al. 2016; Dongare and Aswar 2022).

It should be noted that although Cu(II) and Co(II) may form hydroxide precipitates at higher pH values, the selected pH (7.0) minimizes this effect, and the coprecipitation process is mainly attributed to the formation of metal–MBITAH complexes.

Quantitative recoveries (> 95%) for both Co(II) and Cu(II) were achieved at pH 7.0. While Cu(II) maintained high recoveries even at pH 10.0, a decrease was observed for Co(II) at alkaline pH, likely due to the formation of soluble Co-hydroxyl species that compete with the ligand. Thus, pH 7.0 was selected for further studies.

Influence of Ligand Amount on the Recovery of the Metal Ions

The effect of the MBITAH amount on the recovery of Cu(II) and Co(II) ions was investigated in the range of 0–3.0 mg (corresponding to 0–3.0 mL of 0.1% w/v ligand solution). The relationship between the amount of ligand and the percentage recovery for the developed method is illustrated in Fig. 4.

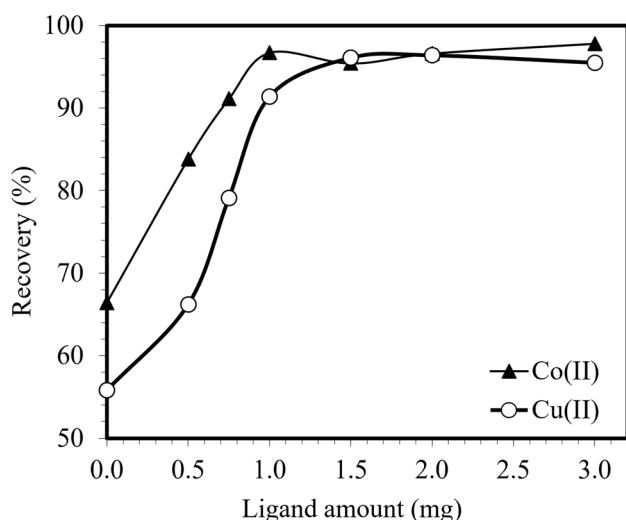


Fig. 4 Influence of MBITAH quantity on the recovery of Co(II) and Cu(II) ions (pH=7.0, sample volume=50 mL, $N=3$, standing time=10 min, centrifugation speed=2500 rpm, centrifugation time=10 min)

In the absence of the ligand (0.0 mg), the recoveries were significantly low (below 66%), indicating that although a minor contribution from hydroxide precipitation may occur, it is insufficient to achieve quantitative enrichment. This result confirms that the coprecipitation process is primarily governed by the formation of the metal–MBITAH complex. Upon increasing the ligand quantity, a sharp increase in recovery was observed as the stoichiometric requirements for complexation were met. Quantitative recoveries (> 95%) were achieved at 1.0 mg for Co(II) and 1.5 mg for Cu(II). The recoveries remained stable in the range of 1.5–3.0 mg, forming a plateau that indicates the robustness of the method against excess reagent.

Although increasing the ligand amount also introduces a small volume of DMSO/ethanol into the system, its proportion relative to the total sample volume is negligible and does not significantly affect the coprecipitation process.

Consequently, 1.5 mg of MBITAH was selected as the appropriate amount for all further experiments to ensure the quantitative and simultaneous enrichment of both analytes.

Effect of Standing Time, Centrifugation Time, and Rate

To achieve maximum separation efficiency in the carrier element-free coprecipitation (CEFC) procedure, physical parameters including standing time, centrifugation time, and centrifugation rate were systematically investigated. The results are illustrated in Fig. 5a, b, and c.

- **Effect of standing time:** The time required for the complete formation and maturation of the metal–MBITAH precipitate was investigated between 0 and 30 min (Fig. 5a). For both Co(II) and Cu(II), recoveries reached quantitative levels (> 95%) within just 10 min. A further increase in standing time did not significantly alter the recovery percentages, indicating a rapid complexation and precipitation kinetics. Thus, 10 min was selected as the appropriate standing time to maintain a high sample throughput.
- **Effect of centrifugation time:** The duration of centrifugation is crucial for the complete settling of the hydrophobic micro-precipitates. This effect was studied in the range of 0–30 min (Fig. 5b). It was observed that 10 min of centrifugation was sufficient to settle the precipitate quantitatively for both analytes. Prolonged centrifugation times had no additional benefit, and 10 min was adopted for subsequent experiments.
- **Effect of centrifugation rate:** The centrifugation rate was varied from 1000 to 3500 rpm to ensure the effective separation of the solid phase from the aqueous solution (Fig. 5c). Quantitative recoveries for Co(II) and Cu(II) were achieved at 2500 rpm. At lower speeds, the centrifugal force was insufficient to fully collect the precipitate,

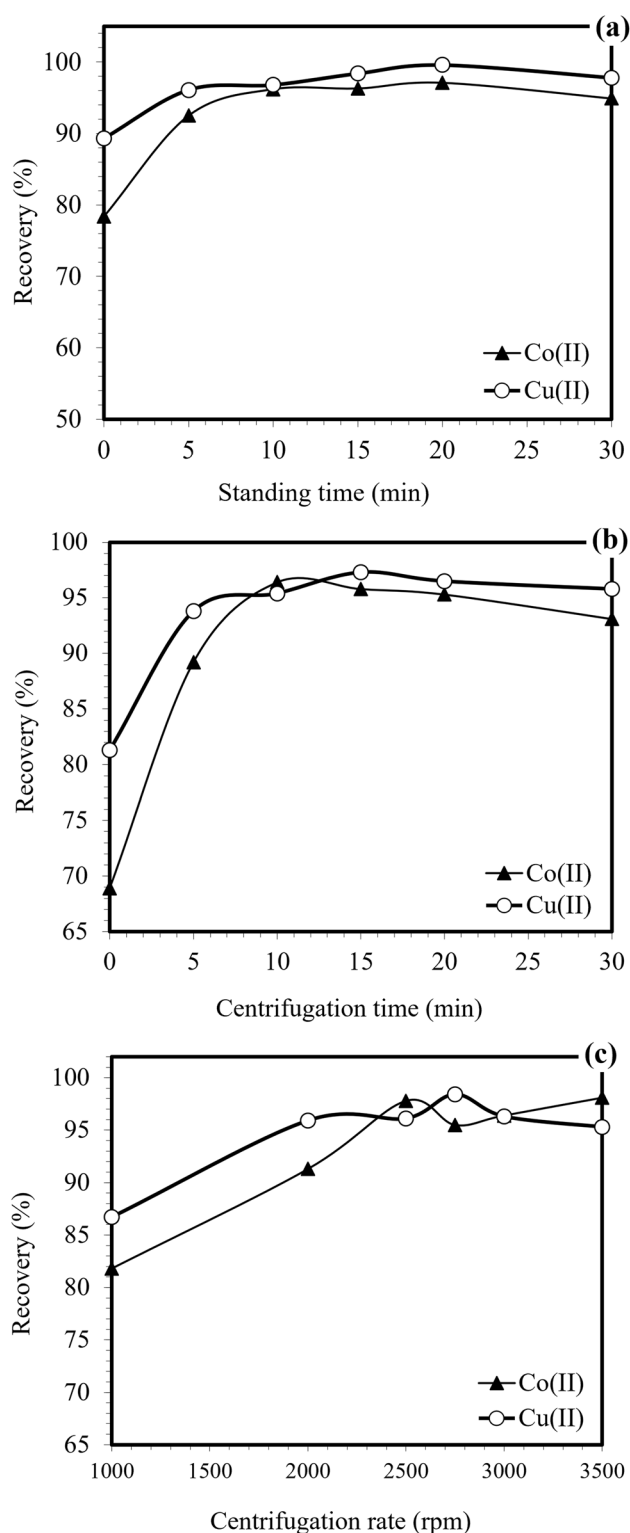


Fig. 5 Effects of (a) standing time, (b) centrifugation time, and (c) centrifugation rate on the recovery of Co(II) and Cu(II) (pH=7.0, ligand amount = 1.5 mg, sample volume = 50 mL, $N=3$)

leading to lower recoveries. Since no significant improvement was observed beyond 2500 rpm, this rate was chosen as the appropriate value.

Effect of Sample Volume and Preconcentration Factor

To enable the determination of trace Cu(II) and Co(II) in samples with very low concentrations, the effect of sample volume on the recovery was investigated. Various volumes of sample solutions (50, 100, 250, 500, 750, and 1000 mL) containing a fixed amount of analytes were processed under optimized conditions. The results are presented in Fig. 6.

The recovery of Cu(II) and Co(II) remained quantitative (> 95%) up to a sample volume of 100 mL. Beyond this volume, a gradual decrease in the recovery percentages was observed for both analytes, which is a common phenomenon in coprecipitation methods due to the solubility of the precipitate in large volumes of the aqueous phase. Under the standard operating conditions, a sample volume of 50 mL was selected, corresponding to a preconcentration factor of 25. However, the method was capable of handling sample volumes up to 100 mL without significant loss of recovery. Considering the final volume of 2.0 mL, this corresponds to a maximum preconcentration factor of 50. This high preconcentration factor allows for the sensitive detection of the target metal ions in real environmental and food samples.

Summary of Selected Experimental Conditions

The experimental conditions selected based on the systematic investigation of the main parameters are summarized in Table 1. All subsequent experiments, including the evaluation of analytical figures of merit and the application to real and spiked samples, were performed under these conditions.

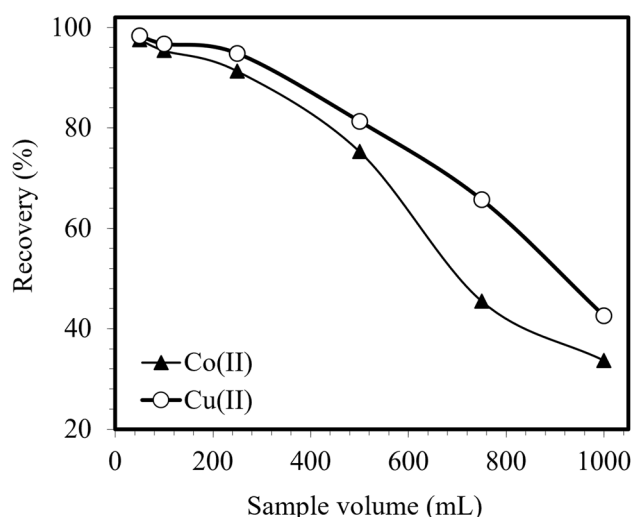


Fig. 6 Influence of sample volume on the recoveries of Co(II) and Cu(II) (pH=7.0, ligand amount = 1.5 mg, standing time = 10 min, centrifugation speed = 2500 rpm, centrifugation time = 10 min, $N=3$)

Table 1 Studied parameters and selected experimental conditions for the proposed CEFC method

Parameter	Range investigated	Selected condition
pH	2–10	7.0
Ligand amount (mg)	0–3.0	1.5
Standing time (min)	0–30	10
Centrifugation time (min)	1–30	10
Centrifugation rate (rpm)	1000–3500	2500
Sample volume (mL)	25–100	100

Analytical Figures of Merit

Analytical Performance of the CEFC Method

The sensitivity of the method was determined by calculating the limit of detection (LOD) and the limit of quantification (LOQ). These parameters were calculated using the standard deviation (s) of ten replicate measurements of the blank solution ($N=10$) and the slope (m) of the calibration curves ($y=mx+b$). The following internationally accepted approach was applied (Miller et al. 2018; Desimoni and Brunetti 2015; Fonollosa et al. 2014). In the signal domain, the detection limit is defined as:

$$y_{\text{LOD}} = y_{\text{blank}} + 3s_{\text{blank}} \quad (1)$$

where y_{blank} is the mean blank signal and s_{blank} is its standard deviation. Using the calibration equation $y=mx+b$, this signal can be converted into concentration as:

$$x_{\text{LOD}} = \frac{y_{\text{LOD}} - b}{m} \quad (2)$$

Assuming that $y_{\text{blank}} \approx b$, the LOD expression simplifies to:

$$\text{LOD} = \frac{3s}{m} \quad (3)$$

Similarly, the LOQ was calculated as:

$$\text{LOQ} = \frac{10s}{m} \quad (4)$$

Table 2 Analytical figures of merit of the proposed CEFC method for Co(II) and Cu(II)

Parameters	Co(II)	Cu(II)
LOD, limit of detection ($\mu\text{g L}^{-1}$)	1.16	0.79
LOQ, limit of quantification ($\mu\text{g L}^{-1}$)	3.87	2.63
RSD, relative standard deviation (%)	3.7	3.0
Linear dynamic ranges ($\mu\text{g L}^{-1}$)	4.0–120	3.0–120
Regression equations	$A=0.0015C+0.001$	$A=0.0026C-0.0006$

Furthermore, the precision and reproducibility of the proposed procedure were evaluated by calculating the relative standard deviation (RSD %). The RSD was determined from ten independent replicates of a standard solution using the following expression:

$$\text{RSD}(\%) = \frac{s}{\bar{x}} \times 100 \quad (5)$$

where s is the standard deviation and $\bar{x}$ is the arithmetic mean of the measured concentrations. The statistical treatment of replicate measurements is described in detail by Miller et al. (2018).

The analytical figures of merit of the proposed CEFC method are summarized in Table 2. Calibration curves were constructed by applying the CEFC procedure to standard solutions and plotting the measured absorbance values against the initial analyte concentrations. The method exhibited good linear dynamic ranges of 4.0–120 $\mu\text{g L}^{-1}$ for Co(II) and 3.0–120 $\mu\text{g L}^{-1}$ for Cu(II), with regression equations of $A=0.0015C+0.001$ ($R^2=0.9962$) and $A=0.0026C-0.0006$ ($R^2=0.9988$), respectively. The limits of detection (LOD) were found to be 1.16 $\mu\text{g L}^{-1}$ for Co(II) and 0.79 $\mu\text{g L}^{-1}$ for Cu(II), while the corresponding limits of quantification (LOQ) were 3.87 $\mu\text{g L}^{-1}$ and 2.63 $\mu\text{g L}^{-1}$. In addition, the relative standard deviation (RSD) values were calculated as 3.7% for Co(II) and 3.0% for Cu(II), indicating good precision of the proposed method. Since the preconcentration step was applied to both standards and samples, the measured absorbance values correspond to the enriched solutions, whereas the calibration curves are expressed in terms of the initial analyte concentrations. Therefore, the effect of the enrichment factor is inherently reflected in the slope of the calibration equations.

Effects of Foreign Ions

To evaluate the selectivity and the practical applicability of the proposed CEFC method, the influence of various co-existing ions commonly found in environmental and food samples was investigated. The interference effect is defined as a deviation in recovery exceeding $\pm 5\%$. The results, summarized in Table 3, demonstrate the robustness of the method against complex matrices.

High concentrations of alkali and alkaline earth metals, such as Na (10,000 mg L⁻¹), K, Ca, and Mg (1,000 mg L⁻¹ each), did not significantly interfere with the quantitative recovery of Co(II) and Cu(II). This is a significant advantage for the analysis of saline water and mineral-rich food samples. Furthermore, common anions like Cl⁻, NO₃⁻, SO₄²⁻, and PO₄³⁻ were well-tolerated at high levels.

Crucially, the simultaneous presence of transition metals and potential interferents (Fe, Al, Cr, Zn, Mn, and V) at a total concentration of 50 mg L⁻¹ did not hinder the efficiency of the MBITAH-based coprecipitation. The mixed-solution test also yielded recoveries within the acceptable range (92.9–94.2%). These findings indicate that the developed method possesses excellent selectivity, as the MBITAH

ligand forms highly stable and specific chelates with the target analytes even in the presence of a vast excess of foreign ions. The tolerance limits correspond to large excesses (up to ~10⁴–10⁵ fold) relative to the analyte concentrations.

Application to Real Samples and Recovery Studies

The applicability and accuracy of the proposed CEFC method were evaluated through recovery studies in water and food samples, as well as its application to real samples. The results obtained from these investigations are presented in Tables 4, 5, 6, and 7.

To validate the accuracy and applicability of the developed CEFC method for real-world environmental monitoring, spike/recovery tests were performed using various water samples. Known amounts of Co(II) and Cu(II) ions were added to the samples, and the recovery percentages were calculated. The results, summarized in Table 4, show that the recoveries for both analytes were in the range of 92.1–102.6%. These findings demonstrate that the proposed method is not affected by the matrix components of different water sources and can be reliably used for the determination of trace metals in environmental water samples.

To further evaluate the versatility and reliability of the proposed CEFC method, it was applied to the determination of Co(II) and Cu(II) in solid food samples, specifically red lentils and bulgur. These samples represent complex organic matrices that require a thorough digestion process. Known concentrations of analyte ions were spiked into the samples (0.75 g), and the results are presented in Table 5.

The recovery values for Co(II) and Cu(II) in food matrices ranged from 93.6 to 103.5%, with excellent precision as indicated by the low standard deviations (*N* = 3). In red lentils, the background concentration of Cu(II) was found to be 4.42 µg/0.75 g, while Co(II) was below the limit of detection (LOD). In wheat samples, both analytes were detected at trace levels. These results confirm that the digestion process with concentrated nitric acid effectively mineralized the organic content without any analyte loss. Furthermore, the

Table 3 Tolerance limits of various foreign ions on the recovery of Co(II) and Cu(II) (*N* = 3)

Ions	Added as	Concentration (mg L ⁻¹)	Recovery (%) ($\bar{x} \pm s$)	
			Co(II)	Cu(II)
Na ⁺	NaCl	10,000	94.2 ± 0.9	94.7 ± 3.1
K ⁺	KCl	1000	96.5 ± 2.4	99.1 ± 5.5
Ca ²⁺	CaCl ₂	1000	97.1 ± 3.4	92.2 ± 2.3
Mg ²⁺	Mg(NO ₃) ₂	1000	92.6 ± 3.1	93.7 ± 4.1
NH ₄ ⁺	NH ₄ NO ₃	250	101.3 ± 6.5	99.1 ± 4.4
NO ₃ ⁻	NaNO ₃	5000	95.6 ± 4.3	97.1 ± 4.3
Cl ⁻	NaCl	15,000	94.2 ± 0.9	94.7 ± 3.1
CO ₃ ²⁻	Na ₂ CO ₃	1000	98.4 ± 2.4	100.4 ± 5.1
SO ₄ ²⁻	Na ₂ SO ₄	1000	93.8 ± 2.9	98.7 ± 4.3
PO ₄ ³⁻	Na ₃ PO ₄	1000	99.4 ± 5.2	103.1 ± 4.3
F ⁻	NaF	250	95.3 ± 3.8	92.8 ± 2.5
Fe ³⁺ , Al ³⁺ , Cr ³⁺ , Zn ²⁺ , Mn ²⁺ , *V ⁵⁺		50	93.4 ± 4.9	92.1 ± 0.7
Mixed ^a			92.9 ± 5.1	94.2 ± 2.1

*V⁵⁺, added as V₂O₅, other ions added as their nitrate salts

^a10,000 mg L⁻¹ Na⁺, 15,000 mg L⁻¹ Cl⁻, 5,000 mg L⁻¹ NO₃⁻, 250 mg L⁻¹ K⁺, Ca²⁺, Mg²⁺, CO₃²⁻, SO₄²⁻, PO₄³⁻, 50 mg L⁻¹ F⁻, NH₄⁺, 10 mg L⁻¹ Fe³⁺, Al³⁺, Cr³⁺, Zn²⁺, Mn²⁺, V⁵⁺

Table 4 Spiked/recovery of analyte ions from water samples (*N* = 3, sample pH = 7.0, sample volume = 50 mL, quantity of MBITAH = 1.5 mg, final volume = 2.0 mL)

Metals	Added (µg)	Stream water		Sea water	
		Found (µg)	Recovery (%)	Found (µg)	Recovery (%)
Co(II)	0	0.93 ± 0.04	–	<LOD*	–
	3.75	4.78 ± 0.17	102.6	3.55 ± 0.12	94.6
	7.50	8.54 ± 0.33	101.5	6.91 ± 0.36	92.1
Cu(II)	0	2.73 ± 0.09	–	1.41 ± 0.07	–
	6.25	8.69 ± 0.34	95.4	7.17 ± 0.17	92.2
	12.50	14.98 ± 0.60	98.0	12.96 ± 0.33	92.4

*Below the limit of detection

The recovery values were calculated based on the net increase in analyte concentration after subtraction of the native analyte content in the unspiked sample

Table 5 Spiked recoveries of analyte ions from solid samples ($N=3$, sample pH = 7.0, quantity of MBITAH = 1.5 mg, sample quantities = 0.75 g, and final volume = 2.0 mL)

Metals	Added (μg)	Red lentils		Bulgur	
		Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
Co(II)	0	< LOD	–	0.38 ± 0.02	–
	3.75	3.85 ± 0.19	102.6	4.17 ± 0.18	101.1
	7.50	7.76 ± 0.34	103.5	8.03 ± 0.38	102.0
Cu(II)	0	4.42 ± 0.20	–	1.51 ± 0.09	–
	6.25	10.37 ± 0.37	95.2	7.36 ± 0.25	93.6
	12.50	16.67 ± 0.60	98.0	13.53 ± 0.43	96.2

The recovery values were calculated based on the net increase in analyte concentration after subtraction of the native analyte content in the unspiked sample

Table 6 Application of the present CEFC method to the certified reference material ($N=3$, quantity of CRM-SA-C sandy soil C = 0.1 g, and final volume = 2.0 mL)

Element	Certified value, x_{CRM} ($\mu\text{g g}^{-1}$)	Found value, x_{F} ($\mu\text{g g}^{-1}$)	Recovery (%)	$t_{\text{test}} = \frac{ x_{\text{CRM}} - x_{\text{F}} }{s/\sqrt{N}}$	t_{critical}	Comparison
Co(II)	12.4 ± 0.6	11.6 ± 0.4	93.5 ± 2.8	3.47	4.30	$3.47 (t_{\text{test}}) < 4.30 (t_{\text{critical}})$ (no significant difference)
Cu(II)	63.6 ± 4.0	59.9 ± 2.1	94.2 ± 3.3	3.05	4.30	$3.05 (t_{\text{test}}) < 4.30 (t_{\text{critical}})$ (no significant difference)

* $t_{\text{critical}} = 4.30$ (d.f. = 2, 95% confidence level)

Table 7 Determination of Co(II) and Cu(II) levels in real samples using the developed MBITAH-based CEFC method ($N=3$, sample volumes = 100 mL, sample quantities = 0.75 g, and final volume = 2.0 mL)

Sample	Co(II)	Cu(II)
Water samples ($\mu\text{g L}^{-1}$)		
Sea water	< LOD	28.2 ± 1.4
Stream water	18.6 ± 0.9	54.6 ± 2.8
Food samples ($\mu\text{g g}^{-1}$)		
Red lentil	< LOD	5.89 ± 0.20
Bulgur	0.51 ± 0.02	2.01 ± 0.10

MBITAH-based coprecipitation procedure successfully separated the target metal ions from the complex food matrix, proving its suitability for food safety and nutritional quality monitoring.

In addition to the spike/recovery studies in real matrices, the accuracy of the proposed CEFC method was further validated by analyzing a certified reference material (CRM-SA-C Sandy Soil C). The determination of Co(II) and Cu(II) in the CRM was performed under optimized conditions, and the results were statistically compared with the certified values using the Student's t -test. The findings are summarized in Table 6 (Miller et al. 2018).

As shown in the table, the found values for Co(II) and Cu(II) were $11.6 \pm 0.4 \mu\text{g g}^{-1}$ and $59.9 \pm 2.1 \mu\text{g g}^{-1}$, respectively, which are in excellent agreement with the certified values ($12.4 \pm 0.6 \mu\text{g g}^{-1}$ and $63.6 \pm 4.0 \mu\text{g g}^{-1}$).

The recovery percentages were calculated as 93.5% and 94.2%, confirming the high efficiency of the MBITAH-based coprecipitation.

To evaluate the significance of the difference between the experimental results (x_{F}) and the certified values (x_{CRM}), the t -values were calculated. The calculated t_{test} values for both analytes (3.47 for Co and 3.05 for Cu) were found to be significantly lower than the critical t -value ($t_{\text{critical}} = 4.30$) at a 95% confidence level for two degrees of freedom ($N=3$). These results indicate that there is no significant difference between the results obtained by the developed method and the certified reference values, demonstrating the high accuracy and reliability of the procedure for trace metal analysis in complex soil matrices.

After validating the accuracy and precision of the developed CEFC method through interference studies, spike/recovery tests, and CRM analysis, the procedure was finally applied to the determination of Co(II) and Cu(II) in various environmental water and food samples. The results of these applications are summarized in Table 7.

The results for liquid samples (sea water from Trabzon and stream water from Trabzon/Maçka) were reported in $\mu\text{g L}^{-1}$. In the stream water sample, Co(II) and Cu(II) concentrations were found to be $18.6 \pm 0.9 \mu\text{g L}^{-1}$ and $54.6 \pm 2.8 \mu\text{g L}^{-1}$, respectively. For the solid food samples (red lentil and bulgur), the results were calculated as $\mu\text{g g}^{-1}$ (ppm). The Cu(II) levels in red lentil and bulgur were determined as $5.89 \pm 0.20 \mu\text{g g}^{-1}$ and $2.01 \pm 0.10 \mu\text{g g}^{-1}$, respectively, which are consistent with typical values reported in the literature for such matrices.

Table 8 Analytical performance comparison of the proposed CEFC method and various microextraction/preconcentration techniques for trace metal determination

Method	Analyte	Sample	Detection technique	LOD	LOQ	RSD (%)	EF	Ref
Surfactant-induced aqueous two-phase system (ATPS)	Co(II)	Food (peanut, hazelnut, shrimp, etc.)	FAAS	0.330 $\mu\text{g kg}^{-1}$	1.10 $\mu\text{g kg}^{-1}$	6.32	20.3	Neves et al. 2026
	Ni(II)			0.0370 $\mu\text{g kg}^{-1}$	0.890 $\mu\text{g kg}^{-1}$	7.37	16.7	
Cloud point extraction (CPE)	Co(II)	Food (spinach, tea, tomato sauce vs.)	UV-Vis	0.4 $\mu\text{g mL}^{-1}$	1.56 $\mu\text{g mL}^{-1}$	3.3	66.66	Mussa et al. 2020
	Cu(II)			0.1 $\mu\text{g mL}^{-1}$	0.65 $\mu\text{g mL}^{-1}$	2.8	50	
Coprecipitation ($\text{Mg}(\text{OH})_2$ carrier-based)	Co(II)	Fish and shell-fish	MIP-OES	0.038 $\mu\text{g g}^{-1}$	0.127 $\mu\text{g g}^{-1}$	–	25	Moreira et al. 2020
	Cu(II)			0.035 $\mu\text{g g}^{-1}$	0.035 $\mu\text{g g}^{-1}$	–	24	
Carrier element-free coprecipitation (CEFC)	Co(II)	Water and food	FAAS	0.30 $\mu\text{g/L}$	1.0 $\mu\text{g/L}$	3.50	100	Mabrouk et al. 2023
	Cu(II)			0.50 $\mu\text{g L}^{-1}$	1.50 $\mu\text{g L}^{-1}$	2.40	100	
Carrier element-free coprecipitation (CEFC)	Cu(II)	Water and vegetable	FAAS	0.51 $\mu\text{g L}^{-1}$	1.71 $\mu\text{g L}^{-1}$	3.2	50	Duran et al. 2024
	Cd(II)			2.28 $\mu\text{g L}^{-1}$	7.59 $\mu\text{g L}^{-1}$	4.7	50	
Carrier element-free coprecipitation (CEFC)	Co(II)	Water, brewed tea, tobacco	FAAS	1.3 $\mu\text{g L}^{-1}$	–	2.4	50	Mohammadi et al. 2018
	Cu(II)			1.5 $\mu\text{g L}^{-1}$	–	2.5	50	
Carrier element-free coprecipitation (CEFC)	Cu(II)	Water and food	FAAS	0.5 $\mu\text{g L}^{-1}$	–	≤ 5.0	100	Gouda 2016
	Pb(II)			1.2 $\mu\text{g L}^{-1}$	–	≤ 5.0	100	
Carrier element-free coprecipitation (CEFC)	Cu(II)	Water and food	FAAS	0.80 $\mu\text{g L}^{-1}$	–	8.0	25	Mendil et al. 2015
	Pb(II)			3.08 $\mu\text{g L}^{-1}$	–	8.0	25	
DLLAPE (dispersive liquid–liquid aerosol phase extraction)	Cu(II)	Seawater	ICP-OES	–	0.2 $\mu\text{g L}^{-1}$	3–9	~ 1.4	Guijarro-Ramírez et al. 2024
	Pb(II)			–	0.2 $\mu\text{g L}^{-1}$	3–9	~ 1.4	
DLLME (dispersive liquid–liquid microextraction)	Cu(II)	Water and mint leaves	FAAS	1.23 $\mu\text{g L}^{-1}$	6.14 $\mu\text{g L}^{-1}$	0.9–8.4	~ 20	Divrikli et al. 2021
	Co(II)			2.48 $\mu\text{g L}^{-1}$	9.01 $\mu\text{g L}^{-1}$	0.9–8.4	~ 20	
UA-DES-DLLME-SFO (ultrasound-assisted, deep eutectic solvent)	Co(II)	Water, food (broccoli, spinach)	FAAS	0.4 $\mu\text{g L}^{-1}$	1.3 $\mu\text{g L}^{-1}$	2.5	50	Tavakoli et al. 2021
	Ni(II)			0.3 $\mu\text{g L}^{-1}$	1.1 $\mu\text{g L}^{-1}$	2.3	50	
IL-CPE (ionic liquid–cloud point extraction)	Cu(II)	Water/food (oranges, bananas, onions, spinach)	UV-Vis	0.1 $\mu\text{g L}^{-1}$	0.3 $\mu\text{g L}^{-1}$	0.9–2.9	90.5	Wannas et al. 2024
IS-VA-DLLME (in-syringe vortex-assisted DLLME)	Cu(II)	Food (vegetables, fruits, juices, rice, fish, chicken)	ICP-OES	0.09 $\mu\text{g L}^{-1}$	0.40 $\mu\text{g L}^{-1}$	1.6–2.2	20.3	Mortada et al. 2023
	V(V)			0.07 $\mu\text{g L}^{-1}$	0.30 $\mu\text{g L}^{-1}$	1.8–2.1	19.8	
MSPE (magnetic solid phase extraction, Fe_3O_4 @coPANI-PTH)	Co(II)	Beverages, spices, vegetables, water	MIS-FAAS	1.6 $\mu\text{g L}^{-1}$	5.3 $\mu\text{g L}^{-1}$	2.5	60	Elci (2021)

Table 8 (continued)

Method	Analyte	Sample	Detection technique	LOD	LOQ	RSD (%)	EF	Ref
PV-IS-DLLME (pressure variation in-syringe DLLME)	Co(II)	Food (banana flour, eggplant, spice, herbal samples)	Digital image colorimetry	1.14 $\mu\text{g L}^{-1}$	3.29 $\mu\text{g L}^{-1}$	4.6	65	Barreto et al. 2020
TI-DSPE (temperature-induced dispersive solid phase extraction)	Co(II)	Food (broccoli, spinach) and water samples	FAAS	0.55 $\mu\text{g L}^{-1}$	1.83 $\mu\text{g L}^{-1}$	2.6	50	Jamali et al. 2024
UA-SUPRAS-DLLME (ultrasound-assisted supramolecular solvent DLLME)	Cr(VI)/total Cr	Tap water, wastewater, beverages, vegetables	FAAS	0.03 $\mu\text{g L}^{-1}$	0.1 $\mu\text{g L}^{-1}$	~2.1	134	Tuzen et al. 2021
US-VA-DLLME (NADES-based ultrasound + vortex-assisted DLLME)	Cd(II)	Water, rice wheat, water-melon	GFAAS	$0.37 \times 10^{-4} \mu\text{g L}^{-1}$	$1.24 \times 10^{-4} \mu\text{g L}^{-1}$	2.65	125	Shamsipur et al. 2022
Carrier element-free coprecipitation (CEFC)	Co(II)	Water, red lentil, bulgur	FAAS	1.16 $\mu\text{g L}^{-1}$	3.87 $\mu\text{g L}^{-1}$	3.7	50	This study
	Cu(II)			0.79 $\mu\text{g L}^{-1}$	2.63 $\mu\text{g L}^{-1}$	3.0	50	

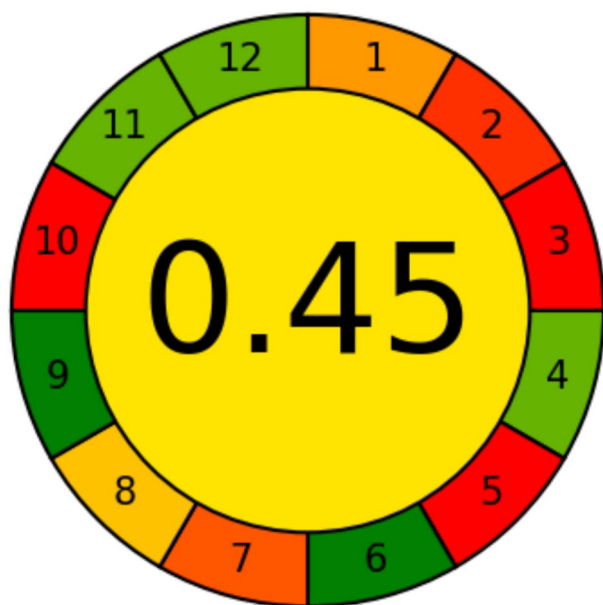


Fig. 7 AGREE (Analytical GREENness Calculator) pictogram illustrating the greenness profile of the proposed CEFC method, with an overall score of 0.45 (*The obtained score provides a realistic assessment of the method, highlighting both its environmental advantages and the limitations associated with conventional instrumentation.*)

These applications demonstrate that the developed CEFC method is a robust and reliable analytical tool for the routine monitoring of trace metal ions in both environmental water systems and complex organic food samples. The ability to detect analytes at these levels confirms the practical utility of the preconcentration step.

Comparative Assessment of the Proposed CEFC Method with Recently Reported Preconcentration Approaches

As presented in Table 8, a wide variety of preconcentration and microextraction techniques have been reported for the determination of trace metal ions in environmental and food samples, including ATPS, CPE, DLLME-based approaches, MSPE, and CEFC methods. These techniques differ significantly in terms of sensitivity, enrichment capability, operational complexity, and instrumental requirements.

Among the compared methods, advanced microextraction techniques such as UA-SUPRAS-DLLME and NADES-based DLLME exhibit very low detection limits and high enrichment factors (EF up to 134 and 125, respectively). However, these methods typically require additional

operational steps such as ultrasonic assistance, vortex mixing, or the preparation of complex solvent systems, which may limit their routine applicability.

Similarly, IL-CPE and ATPS-based methods provide relatively high enrichment factors, but they rely on specific solvent systems or surfactants, which may introduce additional complexity and potential environmental concerns. MSPE-based approaches offer good adsorption capacity and satisfactory precision, yet they often involve the synthesis or preparation of specialized sorbent materials.

In contrast, CEFC-based methods stand out due to their simplicity, low cost, and minimal use of reagents. As shown in Table 8, previously reported CEFC methods generally provide moderate to high enrichment factors (25–100) with acceptable precision. The proposed CEFC method (this study) demonstrates comparable analytical performance, with LOD values of 1.16 and 0.79 $\mu\text{g L}^{-1}$ for Co(II) and Cu(II), respectively, and satisfactory precision ($\text{RSD} \leq 3.7\%$).

Although some advanced techniques achieve lower detection limits, they often require sophisticated instrumentation such as ICP-OES or GFAAS, or involve multi-step extraction procedures. In contrast, the proposed CEFC method enables efficient preconcentration using a simple and rapid procedure combined with widely accessible FAAS instrumentation.

Furthermore, unlike many reported methods that are limited to single-element analysis, the proposed approach allows the simultaneous determination of multiple metal ions and can be readily applied to both environmental and food matrices.

Overall, the proposed CEFC method offers a favorable balance between analytical performance, operational simplicity, cost-effectiveness, and applicability, making it a strong alternative for routine trace metal analysis.

In addition to analytical performance, the environmental impact of the method was also considered in order to provide a more comprehensive evaluation. Therefore, the greenness of the proposed CEFC method was evaluated using the AGREE (Analytical GREENness Calculator) tool (Pena-Pereira et al. 2020). The obtained score (0.45) corresponds to a moderate-to-good level of greenness. This value reflects the use of FAAS instrumentation, moderate sample volume, and manual handling steps, while the method still benefits from low reagent consumption, limited use of organic solvents, and a relatively simple and rapid procedure. The corresponding AGREE pictogram is presented in Fig. 7.

Conclusions

A carrier element-free coprecipitation strategy based on the MBITAH ligand was successfully developed for the determination of trace Co(II) and Cu(II). The method combines satisfactory analytical performance with operational simplicity

and does not require complex extraction steps or specialized instrumentation.

The results demonstrate that the proposed approach provides a practical alternative to more elaborate microextraction techniques, particularly for routine analysis of complex environmental and food samples. In addition, the AGREE evaluation (score = 0.45) indicates a moderate-to-good level of greenness, reflecting a reasonable balance between analytical performance and environmental considerations.

Overall, the developed method offers a straightforward, cost-effective, and reliable option for trace metal analysis, with potential applicability in routine monitoring laboratories.

Author Contribution V.N.B. and C.D. conceived and designed the study. H.B. synthesized and characterized the novel Schiff base ligand. H.D. carried out the experimental work, including optimization of the carrier-element-free coprecipitation procedure, with contributions from V.N.B. and D.O. A.G., V.N.B., and D.O. participated in the FAAS analyses and evaluation of Co and Cu determinations. H.D., V.N.B., and D.O. conducted the application and validation studies on food and environmental samples. A.G. performed the literature review, prepared the figures and tables, and drafted the manuscript. V.N.B., C.D., and M.S. contributed to the interpretation of the results, critically revised the manuscript, and provided scientific supervision. All authors reviewed, revised, and approved the final version of the manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval This study did not involve human participants or animals; therefore, ethical approval was not required.

Declaration of Generative AI Use During the preparation of this work, the corresponding author used ChatGPT (OpenAI) to improve clarity and language expression in certain sections. After using this tool, the corresponding author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

Conflict of interest The authors declare no competing interests.

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