A compact flow-based system for the determination of copper and zinc in water

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Abstract

Metals such as copper and zinc are commonly found in natural and drinking water sources. However, at high concentrations, they are considered toxic and pose significant risks to human health, highlighting the importance of routine water monitoring. In this context, the main goal of this work was to develop a sequential injection system with in-line solid phase extraction for the bi-parametric determination of copper(II) and zinc(II) in water samples. The selectivity of the quantification was controlled by adjusting the pH conditions of the coulourimetric reaction and by implementing an in-line solid-phase extraction strategy. Copper(II) was determined at pH 5, whereas zinc(II) was determined at pH 9 after retention of copper(II) on the resin column. The developed method enabled the quantification of copper(II) and zinc(II) in the concentration ranges of 11.3–500 μg/L and 19.9–500 μg/L, with detection limits of 4.10 and 6.61 μg/L, respectively. The method was validated through the analysis of nine natural water samples, and the results were compared with those obtained with the reference procedure (ICP-OES). Additionally, recovery studies were performed, yielding 97 ± 3% and 98 ± 7% for copper and zinc, respectively. The greenness of the developed method and the reference procedure was assessed using the AGREE tool. The reference method (ICP-OES) achieved a score of 0.59, whereas the developed method obtained a higher score of 0.71. The improvement in the score was associated with the more miniaturised, energy-efficient, direct, and potentially on-site analysis nature, which makes it a promising tool for routine environmental monitoring.

Keywords: spectrophotometry; sequential injection analysis; solid phase extraction; Zincon

1. Introduction

Metals such as copper and zinc are essential micronutrients for the proper functioning of living organisms, contributing to several vital biological processes (1–3). Because of their high density and long half-life, they are commonly known as heavy metals (2,4). Metal ions are commonly found in trace amounts in natural and drinking water sources, originating from both natural processes and anthropogenic activities, such as industrial waste, mining, and agricultural runoff (5). However, at high concentrations, they are known to become toxic, posing relevant risks to the environment and to human health, either through direct ingestion or through bioaccumulation in the food chain(3,6). Consequently, regular monitoring of copper and zinc levels in water sources is crucial to ensure safety and compliance with environmental standards.

Currently, the most common methods for the quantification of metal ions are atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP), owing to their high sensitivity, selectivity and accuracy (7). Nevertheless, these methods require expensive and bulky equipment, as well as high maintenance costs (8–10). Additionally, since these metal ions are usually present in trace levels in natural waters, accurate quantification often requires sample preparation prior to analysis. Techniques such as solid phase extraction (SPE) and liquid-liquid extraction (LLE) have been widely used for this purpose (1,6). While LLE separates the analytes between two immiscible liquids (1,11,12). SPE involves the use of sorbent materials to retain metal ions from solutions, which is a particularly attractive feature as it enables the selective retention and pre-concentration of analytes. In particular, cation-exchange resins can selectively retain positively charged metal ions, such as copper and zinc, thus facilitating their separation and improving analytical performance (11-14).

Flow-based methods, such as sequential injection analysis (SIA), represent an appealing analytical strategy for metal ions monitoring, as they provide automatic and miniaturized tools based on relatively low-cost equipment (8,13). A SIA system, allowing a fully automatic flow-based determination, is typically composed of a propulsion system, a holding coil, a multi-position valve, a reaction coil and the detector (10,14). The programmable operation and precise control of timing and flow conditions inherent to this technique highlight its strong potential for methodological enhancement and improved analytical performance (9). Moreover, these systems reduce reagent consumption, waste generation, and analyst exposure, thereby contributing to safer and more sustainable analytical procedures (9,10). The versatility of SIA also enables its coupling with SPE, which is particularly advantageous for the selective separation and quantification of metal ions with improved sensitivity and accuracy. Moreover, this feature potentially enables the implementation of multiple determinations on a single manifold. This capability has already been demonstrated in previous studies (1,3,15–17). However, four of those methods (3,15–17) rely on subtraction-based algorithm approaches, which may affect precision. Nevertheless, the most recent method (1) enables direct determination of both analytes but requires two extraction procedures. In this context, this work addresses some of these constraints by modulating the selectivity of the colorimetric reagent through pH control and the use of a single in-line extraction procedure, avoiding indirect subtraction-based quantification, multiple extraction steps or dual-line manifolds.

Accordingly, a flow-based system incorporating an in-line cation-exchange resin column (Toyopearl AF-Chelate-650M) was developed for the determination of copper(II) and zinc(II) ions in water. To improve the overall apparatus robustness and portability, the method was implemented using a GlobalFIA, Inc. flow platform (13) that integrates all essential components, including the propulsion unit, multiport valve, flow cell, light source, and detector within a single compact unit. This configuration enhances operational simplicity and enables straightforward application to in-situ analysis. By exploiting the affinity of the resin for metal cations, particularly Cu(II), and by modulating the selectivity of the colorimetric reagent (Zincon) through pH control with appropriate buffer solutions, the proposed system enables the effective separation and accurate bi-parametric quantification of the target metal ions. More specifically, copper(II) is determined under acidic conditions, whereas zinc(II) is quantified after copper retention in the resin column under alkaline conditions. This approach provides a simpler and compact alternative for the routine monitoring of these metal ions in water samples.

2. Methods and materials

2.1. Reagents and solutions

The solutions were prepared using analytical-grade chemicals and Milli-Q water (resistivity > 18 MΩ cm, Millipore).

A 4 mM stock solution of Zincon (Merck) was prepared by dissolving 10 mg of the solid in 5.0 mL of a NaOH 0.02 M solution. A 100 µM working solution was then prepared by dilution of the stock solution with a 0.02 M NaOH solution. The 0.02 M NaOH solution was prepared by dissolving 80 mg of the solid (Panreac) in 100 mL of water.

A 1.00 mg/L stock solution of each metal ion, copper (II) and zinc (II), was prepared by proper dilution from the respective 1000 mg/L atomic absorption standard solutions (Fluka and Spectrosol). Calibration standards were prepared for each metal, ranging from 10 to 500 µg/L, in 0.01 M HNO_3.

A 0.6 M sodium hydrogen carbonate buffer solution was prepared by dissolving 5 g of the solid (Merck) in 100 mL of water, and the pH was adjusted to 10.3 with NaOH pellets (Panreac).

A 0.2 M sodium acetate buffer solution was prepared by dissolving 0.34 g of sodium acetate (Merck) in 20 mL of water. The pH was then adjusted to 5.0 with acetic acid (Merck).

A 0.25 M boric acid buffer solution was prepared by dissolving 0.31 g of the solid (Sigma-Aldrich) in 20 mL of a solution containing 0.05 M KCl (Merck) and 0.5 M NaOH (Panreac). The final pH was adjusted to 9.0 with HCl.

2.2. Apparatus

The flow system was assembled using a FloPro Researcher platform, controlled by FloZF data acquisition, and device control software (GlobalFIA) was used. This equipment included a bi-directional milliGAT™ pump, connected to the central channel of a 10-port multi-position selection valve (VICI Cheminert® C25Z-3480-M17) and to an absorbance flow cell with an optical path of 5 cm. To perform the detection, an Ocean Optics USB 4000 charge-coupled device (CCD) spectrophotometer detector was connected to the flow cell and to a polychromatic light source using optic cables. Absorbance measurement was carried out at

600 nm. All components of the flow system were connected using PTFE tubing from Omnifit® (0.8 mm i.d., Merck).

2.3. Sequential injection system manifold and procedure

The developed flow manifold used for the spectrophotometric determination of Cu(II) and Zn(II) in water is shown in Figure 1.

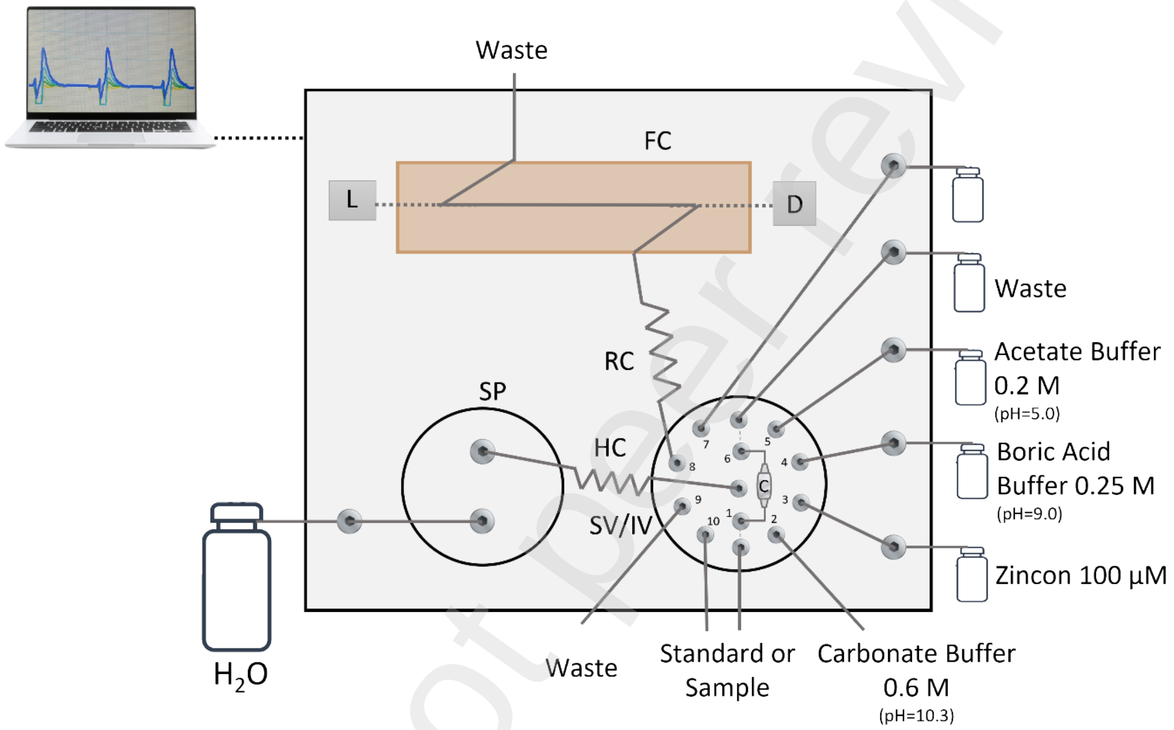

Figure 1. Sequential injection manifold for the spectrophotometric determination of metals in water; SP, syringe pump; SV/IV, selection valve; HC, holding coil; RC; reaction coil (150 cm); C, column packed with Toyopearl® AF-Chelate-650M cationic resin; FC, flow-cell (50 mm path length); L, light source; D, CCD - charge-coupled device detector (λ= 600 nm).

The sequence of steps, including the operational flow-rate and volume, for the determinations performed, is shown in Table 1. For the determination of Cu (II), Zincon solution, acetate buffer solution and the sample/standard solution were sequentially aspirated to the holding coil (steps A, B and C), after which these plugs were propelled towards the detector (step D). The analytical signal was taken as the absorbance registered 48 seconds after initiating the propelling step.

Table 1. Sequential injection protocol for the determination of Cu(II) and Zn(II) in water.

	Step	*SV position*	*Flow Rate (µL/s)*	*Volume (µL)*	*Description*
Cu(II)	A	3	80	200	Aspirate Zincon solution
	B	5	5	20	Aspirate acetate buffer solution
	C	10	25	500	Aspirate sample/standard solution
	D	8	30	3250	Send to detector and absorbance measurement (λ= 600 nm)
Zn(II)	E	2	50	500	Aspirate carbonate buffer solution
	F	1	25	1000	Send to waste through the column (column conditioning)
	G	3	80	200	Aspirate Zincon solution
	H	4	5	30	Aspirate borate buffer solution
	I	6	25	700	Aspirate sample/standard solution through the column
	J	8	30	3250	Send to detector and absorbance measurement (λ= 600 nm)
	K	1	25	500	Send water through the column for cleaning

For the determination of Zn(II), the column was first conditioned by aspirating carbonate buffer solution to the holding coil and propelling it through the column to waste (steps E and F). Then, the Zincon solution was aspirated, followed by the borate buffer solution to the holding coil and, afterwards, the sample/standard solution was aspirated through the column towards the holding coil, enabling copper(II) retention (steps G - I). The plugs were then propelled to the detector (step J). The analytical signal for the zinc(II) quantification was calculated as the difference between the absorbance measured at 40 seconds and the value measured at 32 seconds, after initiating the propelling step. After measurement, water was propelled through the column to clean the system (step K).

2.4. Solid-phase extraction column assembly

A laboratory-made column was assembled using 2.5 cm of Tygon tubing (Gilson, Villiers-le-Bel, France) with an inner diameter of 3.18 mm. Toyopearl AF-Chelate-650M resin (mesh size 650, Tosoh Bioscience) was packed into the column and secured between two pieces of dishwashing sponge, resulting in a final resin column length of 1 cm. (SI-Fig. 1)

2.5. Sample collection and preparation

The water samples were collected from different locations in the Porto district, acidified to pH 2 with nitric acid according to the reference procedure (7), and stored at 4 °C until analysis.

2.6. Validation

The validation of the developed method was performed by comparing the results obtained with the herein described flow-based method, for the quantification of copper(II) and zinc(II), with those obtained using the reference procedure (7), the inductively coupled plasma-optical emission spectrometry, ICP-OES (Perkin Elmer Avio 220 Max). Additionally, recovery studies were performed by spiking water samples with two known concentrations of each analyte (addition of 1 and 3 mL of a 1 mg/L standard of each metal ion to 20 mL of the water samples), and the corresponding recovery percentages were calculated according to the IUPAC recommendations (18).

3. Results and discussion

In this work, the goal was to develop a bi-parametric flow-based analytical method for the quantification of copper(II) and zinc(II) in natural waters. The individual quantification of these metal ions relied on two complementary strategies: (i) modulation of the metal-Zincon reaction by pH control using appropriate buffer solutions, and (ii) implementation of an in-line solid-phase extraction procedure using a cation-exchange resin column. Under the selected conditions, using an acetate buffer solution (pH 5 buffer) enabled the selective quantification of copper(II) since zinc(II) did not react with the chromogenic reagent, whereas a borate buffer solution (pH 9) allowed the determination of both metal ions. The incorporation of the cation-exchange resin column coupled with alkaline conditions enabled the retention of copper(II) in the column, allowing the direct quantification of zinc(II) without the need for subtraction-based calculations.

Given the described individual quantification strategy (sub-section 2.3) and the manifold portrayed in Figure 1, all optimisation studies were carried out by establishing individual calibration curves. The condition was chosen based on the highest sensitivity (slope of the calibration curve) while preferentially combining it with a lower intercept.

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3.1. Study of the colorimetric reaction

3.1.1. Copper(II)

The reaction between copper(II) and Zincon occurs over a wide range of pH values, and in the herein described method, pH values of 5 and 9 were employed. In this context, the optimization of the Cu(II) determination was carried out at pH 5. After evaluation of the absorbance peak profile obtained for each standard injection, it was observed that using the absorbance measured at 48 seconds (after initiating the propelling step) provided a substantially higher calibration curve sensitivity (15%) than the commonly used approach of registering the maximum peak absorbance (corresponding in this case to 51 seconds after initiating the propelling step). Therefore, all subsequent studies for Cu(II) were based on the absorbance signal measured at 48 seconds after initiating the propelling step.

The performance of the flow-based method was evaluated over a range of physical and chemical parameters. First, sample volumes of 400, 500 and 600 µL were tested using 200 µL of Zincon and 30 µL of acetate buffer solution (pH=5). Results in Figure 3(A) showed that the differences in sensitivity between the tested conditions were lower than 10%, and 500 µL of sample volume was chosen for the remaining optimization studies.

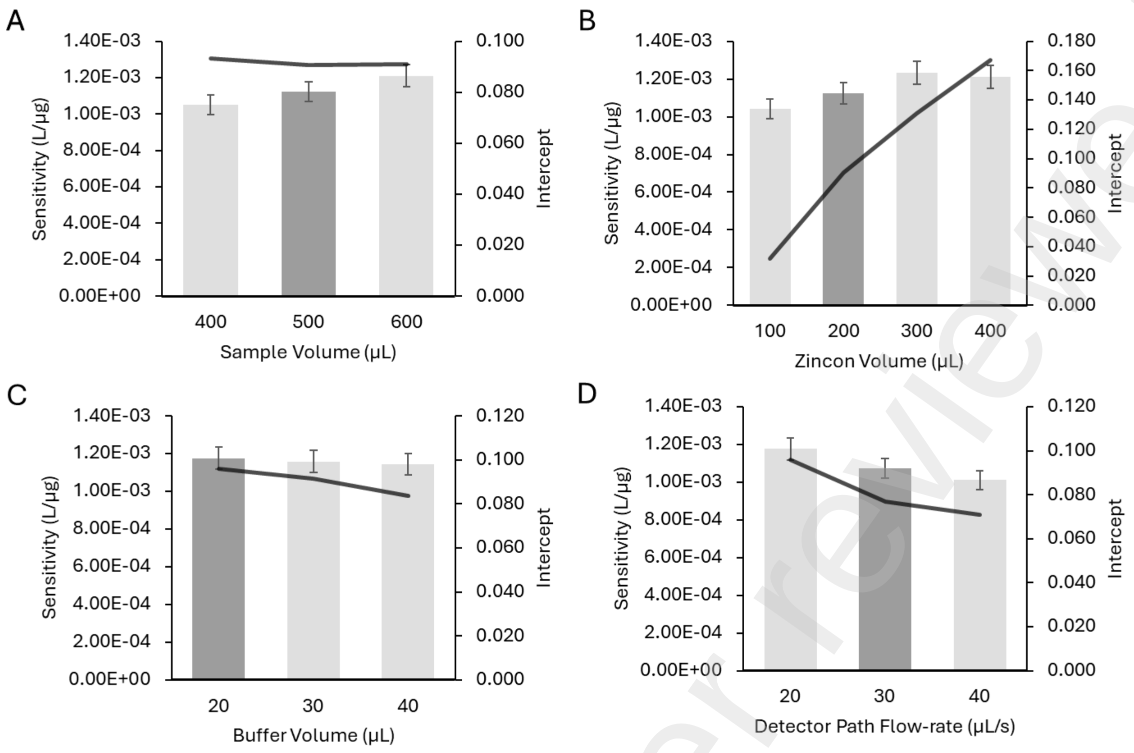

Figure 3. Influence of the (A) sample volume, (B) Zincon reagent volume, (C) acetate buffer (pH=5) volume, and (D) detector path flow-rate, on the calibration curve slope (sensitivity); the black line represents the intercept; the dark grey bar corresponds to the chosen parameter; the error bars represent 5% deviation of the average sensitivity.

The influence of the volume of Zincon was also evaluated. Figure 3(B) shows the resulting sensitivities when using 100, 200, 300 and 400 µL of reagent. Since decreasing or increasing the volume of Zincon relative to 200 µL did not lead to differences in sensitivity greater than 10%, 200 µL was the volume selected for the Cu(II) determination.

Next, the effect of the acetate buffer volume was studied by testing 20, 30 and 40 µL buffer volume (Figure 3(C)). Since there were no relevant differences (<10%) between the sensitivities of the calibration curves obtained under the three tested conditions, 20 µL of buffer was the volume chosen for the Cu(II) determination.

The last parameter optimized for the Cu(II) determination was the flow rate used to propel the solutions to the detector. Flow rates of 20, 30, and 40 µL/s were tested (Figure 3(D)), and no differences in sensitivity greater than 10% were observed. Even though 20 µL/s provided a slightly higher sensitivity, it resulted in a longer analysis time. Therefore, 30 µL/s was chosen as the most appropriate compromise between sensitivity and sample throughput.

3.1.2. Zinc(II)

For the zinc determination, the studies were performed using borate buffer at pH 9, since zinc(II) only reacts with Zincon under alkaline conditions. To minimize variations in the baseline and ensure positive absorbance values, the analytical signal was defined as the difference between the absorbance measured at 40 seconds and the baseline absorbance measured at 32 seconds after initiating the propelling step.

Sample volumes of 400, 500, 600 and 700 µL were studied, as shown in Figure 4(A). For this study, 500 µL of Zincon solution and 20 µL of borate buffer solution (pH=9) were used. A 500 µL sample volume was chosen since it was the one that provided higher sensitivity (corresponding to an increase of approximately 20% if compared to using a 400 µL volume), while also yielding a lower intercept.

Next, the influence of the volume of Zincon reagent was evaluated, using volumes ranging from 100 µL to 500 µL (Figure 4(B)). Results showed no relevant differences (< 10%) in the sensitivity when using 200, 300, 400 or 500 µL of Zincon. In contrast, 100 µL resulted in a lower sensitivity (16%), so 200 µL was the volume of Zincon chosen for the Zn(II) determination.

The influence of the borate buffer solution volume was also studied by testing 20, 30 and 40 µL. The results presented in Figure 4(C) showed that the sensitivities obtained with 20 µL and 30 µL did not differ by more than 10%, but 30 µL of buffer solution also produced a lower intercept (41%), so this was the set volume.

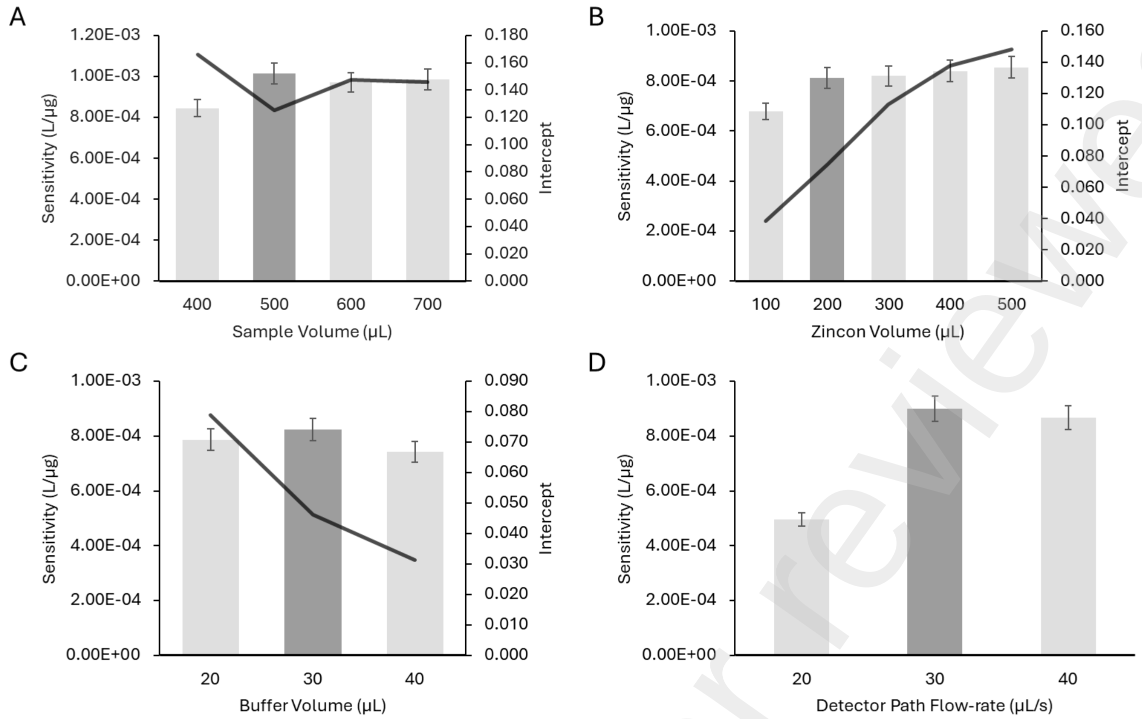

Figure 4. Influence of the (A) sample volume, (B) Zincon reagent volume, (C) boric acid buffer (pH=9) volume, and (D) detector path flow rate, on the calibration curve slope (sensitivity); the black lines represent the intercept; the dark grey bars correspond to the option chosen; the error bars represent 5% deviation of the average sensitivity.

The flow rate used when sending the solutions to the detector was also studied, as shown in Figure 4(D). Of the three flow rates tested, 20, 30 and 40 µL/s, the one that provided a higher sensitivity was 30 µL/s (RD≈81% when comparing the sensitivities obtained with the 20 µL/s flow-rate). When comparing with a 40 µL/s flow-rate, the latter showed a slight decrease. Therefore, as a compromise, 30 µL/s was selected as the flow rate for the Zn(II) determination.

3.2. Resin column

To separate the metal ions and perform their individual quantification, a laboratory-made column packed with Toyopearl AF-Chelate-650M resin was incorporated into the manifold. As the resin has affinity for several metal ions, particularly copper(II) and zinc(II), its retention capacity was evaluated by comparing calibration curves obtained by aspirating standards of the different metal ions (Cu(II) and Zn(II)) with and without passage through the column. The results shown in Figure 5 indicate that the column fully retained the copper ions under the selected conditions. Zinc, on the other hand, was only partially retained in the column, as demonstrated by the lower sensitivity obtained when the standards were aspirated through the column.

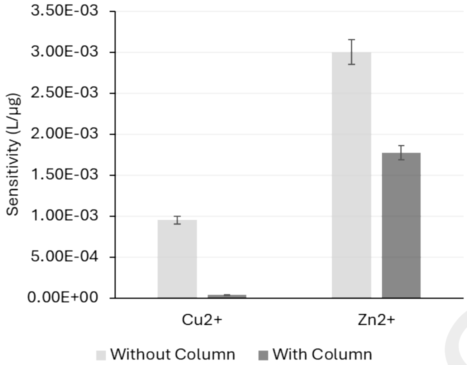

Figure 5. Calibration curves sensitivity when aspirating Cu(II) and Zn(II) individually without the column (light grey) and through the column (dark grey); the error bars represent 5% deviation of the average sensitivity.

3.2.1. Zinc quantification

Zincon at pH 9 is known to react with both Cu(II) and Zn(II) (17). Therefore, to achieve the selective determination of Zn(II), the sample was aspirated through the Toyopearls column to retain Cu(II) before detection. Because the column also partially retained zinc(II), the sensitivity of the Zn(II) under these conditions was lower. For this reason, the study of the sample volume was revisited after the incorporation of the in-line extraction strategy to increase the sensitivity of the final zinc(II) quantification method. Sample volumes of 500, 600, 700 and 800 µL were tested (Figure 6), and the highest sensitivity was obtained with 700 µL, corresponding to an increase of approximately 30% relative to 500 µL. This volume was therefore selected for the Zn(II) determination.

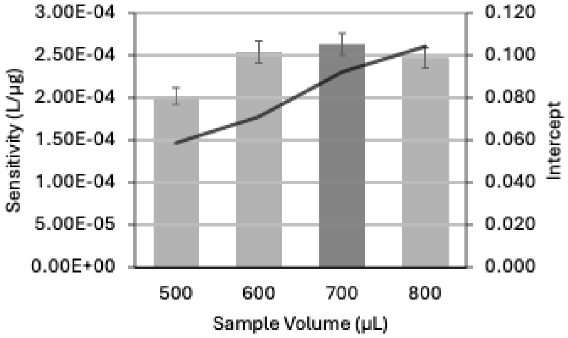

Figure 6. Influence of the sample volume after the incorporation of the in-line extraction strategy on the calibration curve slope (sensitivity); the black line represents the intercept; the dark grey bar corresponds to the option chosen; the error bars represent 5% deviation of the average sensitivity.

3.3. Interference studies

Zincon is a non-specific chromogenic reagent that can react with a variety of different ions. Consequently, a study was performed to assess the potential interference caused by other metal ions commonly present in natural waters on the method´s performance. To evaluate the potential interference, standards of copper(II) and zinc(II) were prepared in the absence and presence of the potential interfering species. These solutions were analysed and the interference percentage calculated (Table 2). The tested concentrations of interferents were selected based on concentrations reported for streams, and in some cases, higher concentrations were also evaluated to provide a conservative assessment of interference (7).

Regarding the copper(II) determination, the only interferences exceeding 10% was observed for iron(III). This ion produced a marked positive interference, indicating that the proposed procedure may be more suitable for waters with low iron content unless an additional masking or separation step is introduced. However, although the allowed iron concentration in natural waters can reach 0.7 mg L^{-1}, the concentration expected does not exceed 0.1 mg L^{-1} (7). In the zinc(II) determination, on the other hand, no significant interferences were found for the ions tested under the selected conditions.

Table 2. Assessment of potential interference in the copper(II) and zinc(II) determinations from metal ions, commonly present in natural waters. The absorbance signal obtained for a 50 µg/L Cu(II) or Zn(II) standard was compared in the absence and presence of the potential interfering species.

Tested Metal Ion	*$[M]_{max}$ in streams (ug/L)*	*Tested [M] (ug/L)*	*Interference %*	
			Cu(II) Determination	Zn(II) Determination
A(III)	400	440	-4.7%	0.0%
Ca(II)	15000	5000	-	0.0%
		10000	-	1.9%

		15000	-3.2%	-1.9%
Co(II)	0.2	5	8.6%	-
		10	23%	-6.0%
Fe(III)	700	250	44%	6.3%
		500	91%	6.3%
		750	116%	0.0%
Mg(II)	4000	2500	-2.8%	-4.2%
		5000	-7.3%	3.8%
Ni(II)	1	5	-	-7.7%
		10	-4.1%	6.3%

3.4. Features

The main analytical features of the developed multiparametric SIA method for copper (II) and zinc (II) are summarized in Table 3.

The limits of detection (LOD) and quantification (LOQ) were calculated according to IUPAC recommendations as the concentration corresponding to the sum of three and ten times the standard deviation to the mean value of ten consecutive blank solution measurements, respectively (19,20). The relative standard deviation (RSD) for Cu(II) and Zn(II) determination was below 5% and was calculated with nine replicate analyses (three consecutive cycles) of a standard with 50 µg/L of each metal ion. Additionally, the corresponding reagents consumption for a complete analytical cycle (three replicas) for the analysis of both metal ions were: 58 µg of Zincon, 1.3 mg of sodium hydroxide, 1.0 mg of sodium acetate, 75 mg of sodium hydrogencarbonate, 0.2 mg of potassium chloride and 0.9 mg of boric acid. It should be emphasized that the cation exchange column can be reused for about 350 analysis (in triplicate).

Table 3. Features of the developed SIA system for the copper(II) and zinc(II) determinations: LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Metal Ion (M^{2+})	*Dynamic Range (µg/L)*	*Calibration Curve ^{a} A = Slope x [M^{2+}] + Intercept*	*LOD (µg/L)*	*LOQ (µg/L)*	*RSD (%)*

Copper (II)	11.3 – 500	A = $9.13\text{x}10^{-4}$ ($\pm1.24\text{x}10^{-6}$) x [Cu^{2+}] + $5.64\text{x}10^{-2}$ ($\pm4.63\text{x}10^{-4}$)	4.10	11.3	2.0
Zinc (II)	19.9 – 500	A = $4.95\text{x}10^{-4}$ ($\pm1.21\text{x}10^{-5}$) x [Zn^{2+}] + 1.01 $\text{x}10^{-2}$ ($\pm6.78\text{x}10^{-3}$)	6.61	19.9	4.7

^{a} n=3

To evaluate the greenness of the developed method, the AGREE tool proposed by Pena-Pereira et al. (21) was employed by assessing the compliance with the 12 principles of Green Analytical Chemistry. Greenness scores were determined for both the developed SIA method and the reference technique (ICP-OES), with detailed results presented in SI-Fig.2. The reference method achieved a score of 0.59, whereas the SIA method obtained a higher score of 0.71. This improvement is primarily associated with the miniaturised nature of the proposed flow system, lower reagent consumption, and reduce energy requirements (criteria 1, 3, 5, and 9). The developed SIA system not only enables more direct and potential in-site analysis but is also more miniaturized and energy efficient. These findings demonstrate that the proposed method aligns more closely with the principles of Green Analytical Chemistry, representing a more sustainable approach for the quantification of copper(II) and zinc(II), with potential for field-oriented application.

3.5. Validation

The accuracy of the developed multiparametric method was assessed by comparing the results obtained for nine natural water samples with those obtained with a reference procedure (ICP-OES) (Table 4). Whenever necessary, water samples were diluted in a multistep approach, in order to fit the linear range of the analytical method calibration. A linear relationship was established between the concentrations obtained with the developed SIA system (C_{SIA} (µg/L)) and the ICP-OES (C_{ICP} (µg/L)). For the copper (II) determination, the linear regression equation was $C_{SIA} = 1.08 (\pm 0.09) \times C_{ICP} - 3.01 (\pm 10.2)$, whereas for the zinc determination was $C_{SIA} = 1.04 (\pm 0.05) \times C_{ICP} - 4.53 (\pm 20.2)$. The values in the brackets represent the 95% confidence intervals. Since the confidence intervals for the slope and intercept include 1 and zero, respectively, the results indicate acceptable agreement between the two methods (22).

Table 4. Results obtained with the developed SIA system and the results obtained with ICP-OES. SD, standard deviations, RD, Relative Deviation

Sample ID	Cu(II) (µg/L)					Zn(II) (µg/L)				
	SIA	*SD*	*ICP*	*SD*	*RD (%)*	*SIA*	*SD*	*ICP*	*SD*	*RD (%)*
1	31.9	±0.6	34.2	±1.2	-6.1%	119	±11.8	108	±1.7	10%
2	20.4	±2.4	22.1	±0.5	-7.4%	34.5	±1.5	31.9	±0.9	7.7%
3	202	±11.2	195	±1.7	3.7%	845	±14.2	795	±8.2	6.3%
4	57.4	±5.0	54.6	±0.9	4.4%	181	±17.7	189	±3.0	-4.1%
5	116	±1.0	122	±0.8	-5.0%	734	±75.1	730	±15.8	0.5%
6	10.0	±0.0	8.6	±0.5	16%	18.0	±2.9	21.2	±0.7	-15%
7	18.5	±4.1	16.7	±0.9	11%	38.6	±10.2	41.8	1.3	-7.6%
8	<LOD	-	<LOD	-	-	32.2	±5.7	28.5	±1.7	13%
9	<LOD	-	<LOD	-	-	112	±1.4	126	±1.4	-11%

Table 5. Recovery studies performed with spiked water samples assessed with the developed SIA system for copper(II) and zinc(II) determination.

	Cu(II)				*Zn(II)*			
Sample ID	Initial (µg/L)	Added (µg/L)	Found (µg/L)	Recovery %	Initial (µg/L)	Added (µg/L)	Found (µg/L)	Recovery %

11	20.4	50.0	70.4	100%	20.8	50.0	73.0	104%
		150	169	99.1%	34.5	150	184	100%
12	24.6	50.0	72.4	95.6%	95.9	50.0	148	104%
		150	175	100%		150	229	88.8%
13	-	-	-	-	<LOD	50.0	48.9	97.7%
		-	-	-		150	161	107%
14	33.3	50.0	79.9	93.2%	53.4	50.0	99.9	93.0
		150	178	96.1%		150	186	88.3

To further assess the accuracy of the developed method, recovery studies were performed by spiking 4 samples with two known concentrations of the target metal ions. The calculation of the recovery percentage was made according to IUPAC recommendations (18) (Table 5). The average recoveries obtained for Cu(II) and Zn(II) were 97 ± 3% and 98 ± 7%, respectively. A two-tailed one-sample statistical test (Student t-test) was applied to evaluate whether the mean recovery differed significantly from 100%. For a 95% significance level, the calculated t-values for Cu(II) and Zn(II) were 2.36 and 0.82, respectively, while the corresponding critical t-values of 2.57 and 2.36. Since the calculated values were lower than the critical values in both cases, there was no significant difference between the mean recovery and 100%.

4. Conclusions

In this work, an automatic, biparametric sequential injection system was developed for the quantification of copper(II) and zinc(II) in freshwater samples. The selective quantification of the target ions was achieved by combining pH-controlled modulation of the Zincon-metal reaction with an in-line solid phase extraction strategy based on a cation-exchange resin (Toyopearl AF-Chelate-650M).

The developed method enabled the determination of copper(II) and zinc(II) in the concentration ranges of 11.3–500 µg/L and 19.9–500 µg/L, with detection limits of 4.10 and 6.61 µg/L, respectively. Since the concentration of copper(II) and zinc(II) in water is recommended to be below 100 µg/L (7), the herein described method is suitable for the monitoring of natural waters.

Since one of the objectives of this work was to develop a more sustainable alternative to commonly used techniques, the greenness of the proposed method was also evaluated using the AGREE tool reported by Pena-Pereira et al. (21), and compared with that of the reference ICP-OES method. The sequential injection method achieved a higher score (0.71) than the reference ICP-OES procedure (0.59), mainly due to the compact and fully integrated nature of the GlobalFIA flow system, together with its lower reagent consumption and reduced energy requirements. Overall, the proposed system represents a compact and more sustainable alternative for the determination of copper(II) and zinc(II) in water samples.

CRediT authorship contribution statement

Francisca T.S.M. Ferreira: Investigation, Data curation, Writing – Original draft; Tânia C. F. Ribas: Conceptualization, Methodology, Validation, Writing – Review and Editing; Ana Machado: Investigation, Resources, Project administration, Writing – Review and Editing; Adriano Bordalo: Resources, Project administration, Writing – Review and Editing; Raquel B. R. Mesquita: Conceptualization, Methodology, Writing – Review and Editing António O. S. S. Rangel: Resources, Funding acquisition, Supervision, Conceptualization, Supervision – Review and Editing.

Acknowledgements

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project 2022.08713.PTDC (https://doi.org/10.54499/2022.08713.PTDC). R.B.R. Mesquita thanks FCT for the 2022.00962.CEECIND (https://doi.org/10.54499/2022.00962.CEECIND/CP1745/CT0006). We would also like to thank the scientific collaboration under the FCT project UID/50016/2025, LA/P/0076/2020 (https://doi.org/10.54499/LA/P/0076/2020), UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020).

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