Optimization of *ex-situ* multielement preconcentration using µ-SPE cartridges based on C18 functionalized with dithizone for the determination of metals by capacitively coupled microplasma optical emission spectrometry with electrothermal vaporization from a Rh filament (SSETV-µCCP-OES) in food and dietary supplement samples

(Activity 1.2, CO-UBB)

The aim of this study was to evaluate and optimize an *ex-situ* (laboratory-based) mono- or multielement preconcentration procedure for the determination of Cu, Zn, Cd, and Pb by (µ-SPE)-SSETV-µCCP-OES. For this purpose, preconcentration was performed using micro solid phase extraction (µ-SPE) cartridges containing C18 sorbent functionalized with dithizone, followed by elution of the retained metal ions with a diluted HNO_3 solution in the presence of thiourea. HyperSep SPE cartridges (40–60 µm) with a 1 mL volume and 100 mg adsorbent material (Thermo Scientific, Massachusetts, USA) were used, along with dithizone (p.a., ACS, ≥98.0%, Sigma Aldrich, Massachusetts, USA), thiourea GR for analysis, glacial acetic acid 100% Suprapur, 25% ammonia solution Suprapur, and 65% HNO_3 Suprapur (Merck, Darmstadt, Germany). The procedure involved the following steps: (i) functionalization of the C18 cartridge with dithizone; (ii) preconcentration of metal ions on the column; and (iii) elution of the metal ions. Preconcentration optimization focused on evaluating the retention efficiency of metal ions as a function of the test solution pH, as well as the elution behavior with respect to the type, concentration, and volume of the eluent. Experimental determinations consisted of measuring the concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} ions in the eluate by SSETV-µCCP-OES under the conditions described in Activity 1.1.

Preparation of the µ-SPE columns involved preconditioning the C18 stationary phase with 2 mL of ethanol followed by 2 mL of ultrapure water, after which functionalization with dithizone was performed by passing 2 mL of a 0.5 mg mL^{-1} dithizone solution prepared in 1 mol L^{-1} ammonium acetate buffer (pH 8.6). The column was then rinsed with 2 mL of 1 mol L^{-1} HNO_3 and subsequently conditioned by passing a buffer solution with pH = 2–9. The conditioned column was used for metal preconcentration from the test solution adjusted to the same pH as that of the conditioned column. Ammonium acetate buffer solutions (50 mmol L^{-1}) were prepared from glacial acetic acid and 25% ammonia and adjusted to a pH between 3 and 9. The pH 2 solution was obtained by diluting glacial acetic acid with ultrapure water.

The preconcentration tests were performed on synthetic multielement solutions containing 0.25 mg L^{-1} Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+}, with pH values in the range 2–9, obtained by mixing appropriate ratios of acetic acid and ammonia. Elution of the metal ions retained on the dithizone-functionalized C18 column was tested using 1 mol L^{-1} HCl and 1 mol L^{-1} HNO_3 solutions, in the absence or presence of thiourea at concentrations of 0.1, 0.2, 0.5, and 1 mol L^{-1}. The results showed that elution in HCl was not suitable for Pb, while elution solely with 1 mol L^{-1} HNO_3 was not adequate for Cu (Figure 1). Therefore, optimization of the thiourea concentration in 1 mol L^{-1} HNO_3 was pursued. The influence of the test-solution pH and the thiourea concentration in the 1 mol L^{-1} HNO_3 eluent is presented in Figure 2. Retention and elution efficiencies were expressed as the ratio between the concentration of each element calculated in the extracted solution, based on the concentrations determined in the eluent by (µ-SPE)-SSETV-µCCP-OES, and the analytical concentration in the test solution (c_{spe} / c_{sol}).

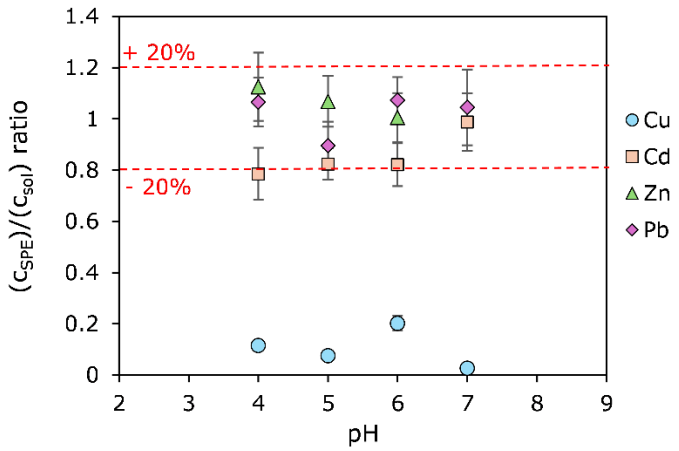

Figure 1. Influence of pH on the retention of Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+} on dithizone-functionalized C18 SPE cartridges and elution with 1 mol L^{-1} HNO_3. Error bars represent the expanded uncertainty (k = 2, n = 3 repeated measurements).

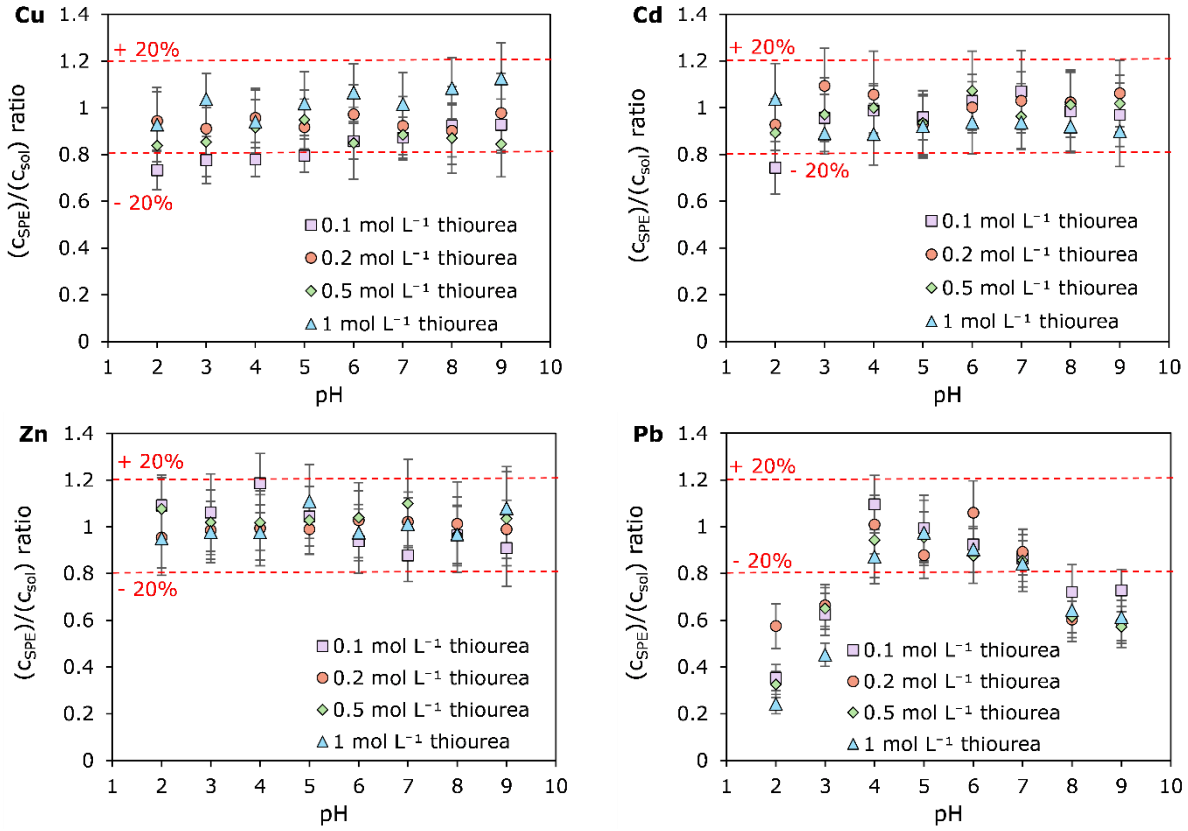

Figure 2. Influence of the test solution pH and thiourea concentration in 1 mol L^{-1} HNO_3 on the retention and elution of Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+} from dithizone-functionalized C18 SPE cartridges. Error bars represent the expanded uncertainty (k = 2, n = 3 repeated measurements)

The results of the pH optimization showed an optimal range of 4–7 for the simultaneous retention of the metals, with recoveries between 92–102%. At pH values below 4 and above 7, a significant decrease in the retention efficiency of Pb^{2+} was observed, a phenomenon associated with the instability of the Pb–dithizone complex and the formation of partially hydroxylated species. In contrast, for Cu^{2+}, Cd^{2+}, and Zn^{2+} the measured values remained within the ± 20% accuracy limits, considered satisfactory for an accurate preconcentration. This behaviour can be explained by the protonation of the thiol and azomethine groups in the dithizone molecule, which reduces its complexation ability and leads to poor metal retention by μ-SPE, particularly for Pb^{2+}, and to a lesser extent for Cd^{2+}. Efficient ion retention within the pH range of 4–7 is an important practical finding, as the optimized method can be applied over a broad pH interval, including the natural pH of surface waters, which represents a target matrix for future applications. In this pH domain, dithizone is partially deprotonated, forming anions capable of establishing strong coordination bonds with metal ions, resulting in high complex stability and thus efficient μ-SPE retention on the C18 cartridge. Above pH 7–8, the accuracy of the preconcentration method based on μ-SPE followed by elution with 0.2 mol L^{-1} thiourea in HNO_3 decreases significantly. This behaviour can be attributed to the formation of hydroxylated species ($M(OH)_2$) or even metal precipitates, which compete with the complexation reaction with dithizone.

The thiourea concentration in the eluent, in the range of 0.1–1 mol L^{-1} in 1 mol L^{-1} HNO_3, did not significantly influence the elution efficiency at concentrations higher than 0.2 mol L^{-1} thiourea. Thus, the average recoveries within the pH range of 4–7 were 95 ± 12% for 0.1 mol L^{-1} thiourea, 98 ± 5% for 0.2 mol L^{-1}, 96 ± 8% for 0.5 mol L^{-1}, and 96 ± 7% for 1 mol L^{-1} thiourea. Consequently, simultaneous elution of the elements was performed using 0.2 mol L^{-1} thiourea in 1 mol L^{-1} HNO_3. The influence of the eluent volume in the range of 0.25–5 mL on the efficiency of releasing the metal ions from the functionalized cartridges for 0.25 mg L^{-1} test solutions at pH 6.5 is shown in Figure 3.

The results in Figure 3 indicate a rapid increase in elution efficiency up to 2 mL of eluent, followed by stabilization at volumes greater than 2 mL, where the c_{spe}/c_{sol} ratio remained within the required ± 20% accuracy range. For volumes below 1 mL, elution was incomplete, particularly for Cu^{2+} and Pb^{2+}, which can be explained by the formation of metal–dithizone complexes that are less soluble in the eluent. Therefore, it was concluded that the optimal eluent volume of 0.2 mol L^{-1} thiourea in 1 mol L^{-1} HNO_3 is 1–2 mL, ensuring recoveries of 94 ± 11% for 1 mL and 94 ± 13% for 2 mL for all studied elements. The optimized method can also be applied for single element preconcentration, depending on the nature and concentration of the analyte in the samples.

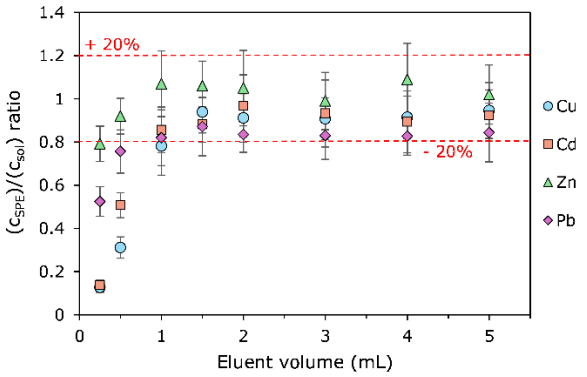

Figure 3. Influence of the eluent volume of 0.2 mol L^{-1} thiourea in 1 mol L^{-1} HNO_3 on the c_{spe}/c_{sol} ratio obtained by μ-SPE preconcentration of a 0.25 mg L^{-1} synthetic multielement solution containing Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+}. Error bars represent the expanded uncertainty (k = 2, n = 3 repeated measurements).

Results: Optimized *ex-situ* (laboratory) method for multielement preconcentration of Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+} by µ-SPE using dithizone-functionalized C18 cartridges, applicable for determination by (µ-SPE)-SSETV-µCCP-OES.