

## Solid Phase Extraction of Cd, Cu, and Ni from Leafy Vegetables and Plant Leaves Using Amberlite XAD-2 Functionalized with 2-Hydroxy-acetophenone-thiosemicarbazone (HAPTSC) and Determination by Inductively Coupled Plasma Atomic Emission Spectroscopy

G. PURNA CHANDRA RAO,<sup>†</sup> KALLURU SESHAIHAH,<sup>\*,†,‡</sup>  
YERRA KOTESWARA RAO,<sup>§</sup> AND M. C. WANG<sup>‡</sup>

Analytical Chemistry Division, Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, India, and Department of Environmental Engineering and Management and Institute of Biotechnology, Chaoyang University of Technology, Wufong 41349, Taichung County, Taiwan Republic of China

A method for solid phase extraction of trace metals, namely, Cd, Cu, and Ni, using Amberlite XAD-2 functionalized with a new chelating ligand, 2-hydroxy-acetophenone-3-thiosemicarbazone (Amberlite XAD-2-HAPTSC), has been developed. The optimum experimental conditions for the quantitative sorption of three metals, pH, effect of flow rate, concentration of eluent, sorption capacity, kinetics of sorption, and the effect of diverse ions on the sorption of analytes have been investigated. The chelating resin could be reused for more than 20 cycles of sorption–desorption without any significant change (<1.5%). The accuracy of the proposed procedure was evaluated by standard reference materials. The proposed method was applied for the determination of trace metal ions in leafy vegetable samples collected from different sources and medicinal plant leaves.

**KEYWORDS:** Solid phase extraction; Cd, Cu, and Ni; 2-hydroxy-acetophenone-3-thiosemicarbazone; Amberlite XAD-2; leafy vegetables; plant leaves

### INTRODUCTION

Copper and nickel metal ions play important roles in biological systems. Copper plays a key role during cell respiration in the blood of invertebrate animals and in the formation of hemocyanin and plays a definitive role in the intrinsic mechanisms regulating vital biological processes (1). Copper is involved in hemopiesis and in the maintenance of vascular and skeletal integrity in addition to the structure and function of the central nervous system. A variety of clinical disorders such as anemia, depression of growth, impaired reproductive performance, heart failure, and gastrointestinal disturbances have been associated with a dietary deficiency of copper (2). Overexposure to copper causes a metallic taste, nausea, vomiting, epigastric burning, diarrhea, hepatic neurosis, gastrointestinal bleeding, hemoglobinuria, hypertension, and convulsions. Nickel is an essential metal for plants and animals and has biological importance (3). Urease, which is a biologically important enzyme, is a nickel enzyme (4). As compared

with many transition metals, nickel is a moderately toxic metal. Inhalation of nickel and its compounds causes cancer of the respiratory system and a skin disorder called nickel eczema (5, 6). Cadmium is not an essential element but a toxic metal, which accumulates mostly in the liver and kidney and has a long biological half-life in humans. Chronic cadmium poisoning produces protein urea and causes the formation of kidney stones (7). There is a relationship between higher doses of cadmium and hypertension (8). A disease especially associated with cadmium poisoning is known as “itai-itai” (9).

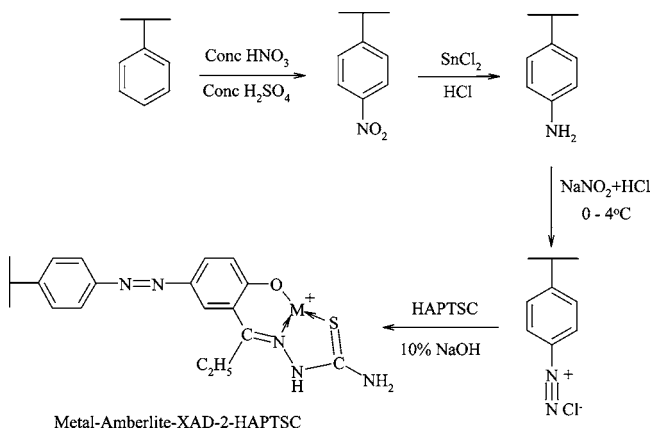
Hence, the determination of cadmium, copper, and nickel in food items plays an important role in the context of environmental protection and food and agriculture chemistry. Direct analysis of trace levels of metal ions in environmental and biological samples is not possible with conventional analytical techniques because of very low concentrations of these metal ions and high amounts of matrices in the analytical samples. The best way is to separate and preconcentrate the metal ions from the matrix constituent and then analyze them using a suitable analytical technique. Many methods such as extractive spectrophotometry (10–12), solid-phase spectrophotometry (13–16), and solid phase extraction (SPE) flame atomic absorption spectroscopy (AAS) are used for the separation and determination of Cd, Cu, and Ni from different matrices. Among

\* To whom correspondence should be addressed. Tel: +886-4-2332-3000 ext. 7486. Fax: +886-4-2333-9713. E-mail: seshaiyah@cyut.edu.tw.

<sup>†</sup> Sri Venkateswara University.

<sup>‡</sup> Department of Environmental Engineering and Management, Chaoyang University of Technology.

<sup>§</sup> Institute of Biotechnology, Chaoyang University of Technology.

**Scheme 1.** Functionalization of Amberlite XAD-2 with HAPTSC

these methods, SPE, based on a solid support modified with a complexing agent, has gained special attention due to the advantages in the use of these substances in metal ion enrichment. The advantages are a high degree of selectivity based on control of the pH, versatility, and durability and a good metal-loading capacity (17). Solid supports either loaded or functionalized with many ligands, such as piperidinedithiocarbamate (18),  $\alpha$ -nitroso- $\beta$ -naphthol (19), salicylic acid (20), 1-(2-pyridilazo)-2-naphthol (21), 1,3-dimethyl-3-aminopropan-1-ol (22), *O*-aminobenzoic acid (23), 2-(methylthio)aniline (24), 3,4-dihydroxybenzoic acid (25), and 2-aminothiophenol (26), are used for separation, preconcentration, and determination of Cd, Cu, and Ni, respectively, or along with other metal ions in different matrices. Thus, there is a continued interest in the development of functionalized resins that can provide good stability, high sorption capacity for metal ions, and good flexibility in working conditions. The sorption characteristics of the metal ions and the complexation properties of the reagent generally depend on the size of the chelate ring, the number of donor atoms/binding sites on the reagent, the type of donor atoms (hard or soft), and the metal atom.

The reagent 2-hydroxyacetophenone-3-thiosemicarbazone (HAPTSC) has three binding sites, nitrogen, oxygen, and sulfur, and is thus capable of forming chelate rings as shown in **Scheme 1**, which makes it a good reagent for metal ion enrichment. In addition, the reagent can be easily synthesized in the laboratory and the resin loaded with the reagent is stable in acid solutions. Hence, the reagent is more suitable for SPE methods, where elution of metals is carried out with acids for AAS or inductively coupled plasma atomic emission spectroscopy (ICPAES) analyses.

The aim of this work was to modify Amberlite XAD-2 by attaching HAPTSC ligand through an azo group spacer and to investigate the application of the modified resin for SPE of Cd, Cu, and Ni from plant leaves and determination by ICPAES. The proposed method was applied for the determination of metals in leafy vegetables and medicinally important plant leaves collected from different sources.

## MATERIALS AND METHODS

**Instrumentation.** An atomic emission spectrometer [Varian Liberty Series (II), Australia] with a wiproacer computer was used. The instrumental operating parameters were as follows: photomultiplier tube voltage, 700 V; incident power, 1.1 kW; plasma gas flow, 15.0 L min<sup>-1</sup>; auxiliary gas flow, 1.5 L min<sup>-1</sup>; observation height, 14.0 mm; pump rate, 15.0 rpm; and sample uptake, 25 times. The wavelengths used were 226.502 nm for Cd, 324.754 nm for Cu, and 221.647 nm for Ni.

Fourier transform infrared spectrometry (FT-IR; Perkin-Elmer) was used to analyze the organic functional groups present in the chelating compound.

**Chemicals and Solutions.** All of the reagents used were of analytical grade. Deionized double-distilled water was used throughout the experimental study. The Amberlite XAD-2 resin (Aldrich, Milwaukee, WI) with a particle size of 20–60 mesh, a pore diameter of 90 Å, and a surface area of 330 m<sup>2</sup> g<sup>-1</sup> was used. Before use, the resin was washed with methanol and 4 mol L<sup>-1</sup> HCl to remove impurities. 2-Hydroxyacetophenone and thiosemicarbazide were obtained from Fluka. Nitric acid (Glaxo) was used without additional purification.

The following pH solutions were used for the SPE procedure: 1 mol L<sup>-1</sup> HCl for pH 1 and acetate buffer between pH 2 and pH 6, borate buffer at pH 7, and ammonium buffer between pH 8 and pH 10. A multielement standard solution (100  $\mu$ g mL<sup>-1</sup>) was prepared by dilution of ICP standards (Merck, Germany) of Cu(II), Ni(II), and Cd(II). Working standards were prepared by appropriate dilution of the multielement standard with double-distilled water.

**Synthesis of HAPTSC.** 2-Hydroxyacetophenone (0.1 mol dissolved in 15 mL of methanol), thiosemicarbazide (0.1 mol dissolved in 150 mL of water), and potassium hydroxide (1 g dissolved in 10 mL of water) were mixed in a round-bottomed flask and refluxed for 4 h and, after cooling, was poured in ice water. The yellow crystals obtained were washed repeatedly two or three times with ethanol and then purified by recrystallization in ethanol.

**Functionalization of Amberlite XAD-2 with HAPTSC.** For the functionalization of resin, the procedure described previously (20) was employed. Concentrated nitric acid (10 mL) and sulfuric acid (25 mL) were added to Amberlite XAD-2 (5 g), and the mixture was stirred at 60 °C for 1 h in a water bath. Thereafter, the reaction mixture was poured into an ice–water mixture. The nitrated mixture was filtered, washed repeatedly with water until free from acid, and then added to a reaction mixture of 40 g of SnCl<sub>2</sub>, 45 mL of concentrated HCl, and 50 mL of ethanol. The system was refluxed for 12 h at 90 °C. The solid precipitate was filtered and washed with water and 2 mol L<sup>-1</sup> NaOH, which released amino resin R-NH<sub>2</sub> from (R-NH<sub>3</sub>)<sub>2</sub>SnCl<sub>6</sub> (R resin matrix). The amino resin was first washed with 2 mol L<sup>-1</sup> HCl and finally with distilled water to remove the excess HCl. Then, the amino resin was suspended in an ice–water mixture (350 mL) and treated with 1 mol L<sup>-1</sup> NaNO<sub>2</sub> (added in small aliquots of 1 mL) until the reaction mixture showed a permanent dark blue color with starch–iodide paper. The diazotized resin was filtered, washed with ice-cold water, and reacted with HAPTSC (3.5 g dissolved in 100 mL of 10% NaOH solution) at 0–4 °C for 24 h. The resulting dark-brown resin was filtered, washed with water, and air-dried.

A schematic representation of functionalization of Amberlite XAD-2 with HAPTSC is shown in **Scheme 1**. The FT-IR spectrum of HAPTSC showed bands at 3398, 1622, 1234, 1370, and 1106 cm<sup>-1</sup> due to NH<sub>2</sub> stretching, C=N stretching, C=S stretching, O–H bending, and C–N stretching, respectively. In the FT-IR spectrum of XAD-2-HAPTSC, new bands appeared at 3436, 1659, 1545, 1387, 1199, and 1102 cm<sup>-1</sup>, which may be assigned to NH<sub>2</sub> stretching, C=N stretching, N=N stretching, O–H bending, C=S stretching, and C–N stretching, respectively. These similarities between the spectra of the ligand (HAPTSC) and the functionalized resin support the functionalization of HAPTSC onto Amberlite XAD-2.

**Sample Preparation.** Sample preparation was carried out by following the procedure (27). Green leaves were air-dried followed by drying at 105 °C in a hot air oven for 2 h and then powdered. One gram aliquots of each sample were taken in Teflon flasks, and 5 mL of concentrated nitric acid and 5 mL of 30% (v/v) hydrofluoric acid were added to each flask. After they were left to stand for 8 h, the samples were digested in a microwave system for 10 min at 106 °C. The clear solutions obtained were transferred to a 100 mL beaker, the pH was adjusted to 5.0 using acetate buffer, and volumes were adjusted to 50 mL.

**Recommended Procedure for SPE and Determination of Metal Ions.** Amberlite XAD-2 functionalized with HAPTSC (0.5 g) was packed in a glass column (1.0 cm  $\times$  10.0 cm). This was treated with

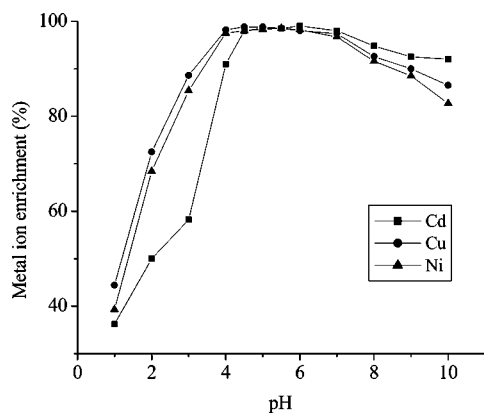


Figure 1. Effect of pH on metal ion enrichment on resin.

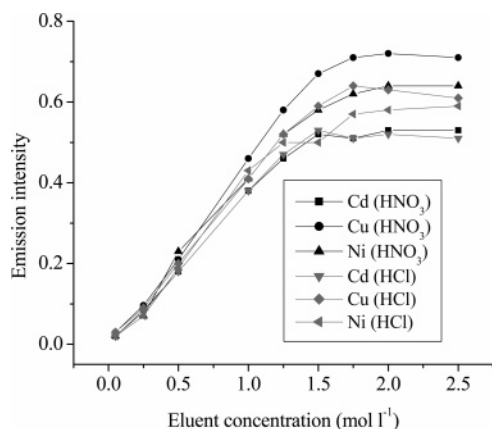


Figure 2. Effect of acid concentration on elution of metals.

4 mol L<sup>-1</sup> HCl and washed with double-distilled water until free from acid. Fifty milliliters of samples containing Cd, Cu, and Ni, with the pH adjusted to 5.0, was passed through the column, at a flow rate of 1.0–2.0 mL min<sup>-1</sup>, controlled by the peristaltic pump. The column was washed with double-distilled water. The bound metal ions were stripped from the column with 10 mL of 2.0 mol L<sup>-1</sup> HNO<sub>3</sub>. The concentrations of the metal ions in elutes were determined by ICPAES.

## RESULTS AND DISCUSSION

**Effect of pH on Metal Ion Preconcentration.** The effect of pH on the retention of metal ions on the HAPTSC functionalized Amberlite XAD-2- resin column was carried out over a wide range of pH values (2.0–9.0) by applying the proposed SPE-ICPAES analyses procedure. The variation in sorption of metal ions on the sorbent with pH is shown in **Figure 1**. These results revealed that at pH 5.0 the quantitative sorption of three metals was achieved. Hence, this pH was selected as the optimum pH for SPE of metal ions.

**Effect of Flow Rate and Eluent.** The degree of metal ion sorption on Amberlite XAD-2-HAPTSC was studied by varying the flow rate of the solution. It was found that the optimum flow rate for quantitative sorption of metal ions on the resin bed was between 1.0 and 2.5 mL min<sup>-1</sup>. However, at a flow rate more than 3.5, a decrease in percentage sorption was observed.

Metal elution from the column was studied by using different concentrations (0.5–2.5 mol L<sup>-1</sup>) of nitric acid and hydrochloric acids, and results are presented in **Figure 2**. As seen from **Figure 2**, elution with nitric acid gave better results for three metals as compared to hydrochloric acid and total elution was attained at concentrations above 1.5 mol L<sup>-1</sup>. Hence, 2.0 mol L<sup>-1</sup> nitric acid was selected as the eluent for the analysis of the three metals.

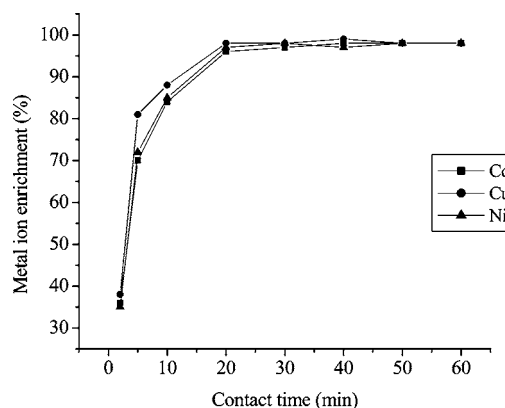


Figure 3. Kinetics of metal ion sorption on Amberlite XAD-2-HAPTSC resin.

Table 1. Tolerance Limits of Matrix Ions for Determination of Cd, Cu, and Ni by the Proposed Method

matrix ion	tolerance limit (mg L <sup>-1</sup> )	matrix ion	tolerance limit (mg L <sup>-1</sup> )
Mn <sup>2+</sup>	35	Ca <sup>2+</sup>	40000
Pb <sup>2+</sup>	50	Mg <sup>2+</sup>	35000
Pd <sup>2+</sup>	45	Cl <sup>-</sup>	50000
Zn <sup>2+</sup>	35	NO <sub>3</sub> <sup>-</sup>	35000
Co <sup>2+</sup>	40	PO <sub>4</sub> <sup>-</sup>	4000
Fe <sup>3+</sup>	45	HCO <sub>3</sub> <sup>-</sup>	25000
Na <sup>+</sup>	45000	SO <sub>4</sub> <sup>2-</sup>	35000
K <sup>+</sup>	40000		

Table 2. Determination of Trace Metals in Standard Reference Materials (NIST 1643e Water and NIST 1570a Spinach Leaves) by the Proposed Method (*n* = 4)

metal ion	NIST 1643e water (μg L <sup>-1</sup> )		NIST 1570a spinach leaves (μg g <sup>-1</sup> )	
	certified value	proposed method	certified value	proposed method
Cd	6.57	6.42 ± 0.21	2.89	2.92 ± 0.11
Cu	22.76	22.75 ± 0.85	12.20	11.84 ± 0.48
Ni	62.41	62.28 ± 1.81	2.14	2.17 ± 0.09

**Sorption Capacity and Detection Limits.** The sorption capacity of HAPTSC-functionalized Amberlite XAD-2 was determined by batch method. The resin (1 g) was saturated with Cd, Cu, and Ni ion solutions (concentration, 50 μg mL<sup>-1</sup>) by equilibrating the solutions on a mechanical shaker under optimum conditions. The solid matrix was filtered and washed with distilled water. The sorbed metal ions on the resin were desorbed with 10 mL of 2.0 mol L<sup>-1</sup> HNO<sub>3</sub> and determined by ICPAES. The sorption capacities in mg/g are 3.7 for Cd, 5.6 for Cu, and 4.8 for Ni, respectively. The detection limits defined as the concentration equivalent to three times the standard deviation of the reagent blank prepared as described in a section (sample preparation) of 10 measurements were found as 0.38 μg kg<sup>-1</sup> for Cd, 0.61 μg kg<sup>-1</sup> for Cu, and 0.54 μg kg<sup>-1</sup> for Ni.

**Kinetics of Sorption.** The rate of uptake of metal ions on Amberlite XAD-2-HAPTSC was studied by batch method. Fifty micrograms of individual metal ion solutions was added to 1.0 g of resin and stirred for 2, 5, 10, 20, 30, 40, 50, 60, 90, and 120 min at room temperature. The metal ions on the resin surface were eluted with 2 M HNO<sub>3</sub> and analyzed by ICPAES as described in the recommended procedure. The saturation of resin with time is graphically presented in **Figure 3**. The loading half-time, *t*<sub>1/2</sub>, needed to reach 50% sorption of the total loading

**Table 3.** Determination of Trace Metals from Leafy Vegetables by the Proposed Method ( $n = 4$  and Concentration of Metal Ions in  $\mu\text{g g}^{-1}$ )

leafy sample	Mallamgunta area			Korlagunta area		
	Cd	Cu	Ni	Cd	Cu	Ni
Chukkaku ( <i>R. vesicarius</i> )	0.35 ± 0.01	18.6 ± 0.83	3.46 ± 0.16	1.64 ± 0.06	30.85 ± 1.21	8.63 ± 0.40
Palakura ( <i>I. frutescens</i> )	0.25 ± 0.01	16.14 ± 0.61	1.68 ± 0.08	0.86 ± 0.04	24.64 ± 0.95	7.82 ± 0.38
Thotakura ( <i>A. tricolor</i> )	0.28 ± 0.01	25.39 ± 1.04	3.76 ± 0.18	2.24 ± 0.08	40.64 ± 1.66	6.0 ± 0.27
cabbage ( <i>B. oleracea</i> var. <i>capitata</i> )	0.21 ± 0.01	17.4 ± 0.75	4.28 ± 0.21	1.86 ± 0.07	31.26 ± 1.35	9.14 ± 0.42

**Table 4.** Determination Metals from Medicinal Plant Leaves ( $n = 4$ )

plant name	metal ion ( $\mu\text{g g}^{-1}$ )		
	Cd	Cu	Ni
Vepa ( <i>A. indica</i> )	0.18 ± 0.01	13.76 ± 1.44	8.12 ± 0.36
Uttareni ( <i>A. aspera</i> )	0.22 ± 0.01	40.81 ± 1.68	10.02 ± 0.43
Neredu ( <i>S. cumini</i> )	0.28 ± 0.01	16.02 ± 0.72	11.62 ± 0.55
Caanuga ( <i>P. pinnata</i> )	0.27 ± 0.01	24.14 ± 0.96	7.46 ± 0.32

capacity (estimated from **Figure 3**) has been found to be less than 5 min for each metal ion (Cd, 3.4; Cu, 4.2; and Ni, 4.0 min, respectively). The kinetics of the resin–metal interaction is sufficiently rapid for all of the metal ions at optimum pH. The faster uptake of these metal ions on Amberlite XAD-2-HAPTSC reflects a good accessibility of the chelating sites of the resin to metal ions.

**Resin Stability and Reusability.** The stability of HAPTSC-loaded Amberlite XAD-2 was studied in acid (1.0–6.0 mol L<sup>-1</sup> HNO<sub>3</sub>). It was shaken with acid solutions of varying concentrations for 4 h and filtered. The solid was washed with distilled water until free from acid and air-dried, and its sorption capacity was determined using a batch method. The sorption capacity of the acid-treated resin was found to be similar (variation, <3%) to that of the untreated one. This shows that the present resin can resist an acid concentration up to 6 mol L<sup>-1</sup>. It can also be reused for more than 20 cycles of sorption–desorption without any significant change in the sorption capacity (<1.5%).

**Effect of Foreign Ions.** The efficiency of the ligand functionalized on the matrix to sorb the metal ions has been assessed in the presence of anions such as chloride, nitrate, sulfate, and phosphate and cations such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> by studying the sorption efficiency of Cd, Cu, and Ni on Amberlite XAD-2-HAPTSC using the recommended method. The tolerance limits of various foreign species on the sorption of the metal ions are given in **Table 1**. The tolerance limit of foreign ions was taken as that value which caused an error of not more than ±5% in the emission reading. Metal ions such as Mn, Co, Fe, Pb, Pd, and Zn also form complexes with the ligand, but

there was no significant effect on the emission signal at the selected wavelengths of the analytes by their presence in the elutes.

**Accuracy of the Method.** The accuracy of the developed procedure was evaluated by determining the metal ions in the standard reference material NIST 1643e natural water and NIST 1570a spinach leaves. According to results in **Table 2**, concentrations of the three metal ions determined by the proposed method are in good agreement with the certified values. Results indicate the applicability of the developed procedure in metal ions determination free of interference.

**Application of the Method to the Real Samples.** The developed method was applied for the determination of three metals in leafy vegetables, namely, Chukkaku (*Rumex vesicarius*), Palakukra (*Ichnocarpus frutescens*), Thotakura (*Amaranthus tricolor*), and cabbage (*Brassica oleracea* var. *capitata*), collected from two different sources (Mallamgunta area and Korlagunta area) from the nearby agricultural fields of Tirupati town, and medicinal plant leaves such as Vepa (*Azadirachta indica*), Uttareni (*Achyranthes aspera*), Caanuga (*Pongamia pinnata*), and Neredu (*Syzygium cumini*), collected from Tirumala hill ranges. Metal concentrations in these samples are presented in **Tables 3** and **4**. The concentrations of the three metals in leafy vegetables collected from the agricultural fields of the Mallamgunta area irrigated by Kalyani river water were found in the range of 0.21–0.35 for Cd, 16.14–25.39 for Cu, and 1.68–4.28  $\mu\text{g g}^{-1}$  for Ni. The high concentrations for Cd, Cu, and Ni found from the samples collected from the Korlagunta area could possibly reflect the influence of Tirupati urban drainage water entering the irrigated water in that region. This indicates the great impact of irrigation water on the elemental concentrations in leafy vegetables. The concentrations of the three metals in medicinal plant leaves were found in the range of 0.18–0.28 for Cd, 13.76–40.81 for Cu, and 7.46–11.62  $\mu\text{g g}^{-1}$  for Ni.

In conclusion, the newly developed method was successfully applied to the SPE of Cd, Cu, and Ni in real samples of leafy vegetables and plant leaves with determination by ICPAES.

**Table 5.** Comparison of the Proposed Method with Other Methods Reported in the Literature

method	reagent	detection limit ( $\mu\text{g L}^{-1}$ )	remarks	ref
extractive spectrophotometry	benzylidithiosemicarbazone	Cu, 68.0	less sensitive; applied to determination of Cu only; Ag, Ni, Co, Pd, and Zn interfere	10
extractive spectrophotometry	pyridoxal-4-phenyl-3-thiosemicarbazone	Cu, 68.0	less sensitive; applied to determination of Cu only; Cd, Ni, Co, Pd, and Zn interfere; needs phosphate masking	12
SPE spectrophotometry	o-carboxyphenyldiazo-aminoazobenzene loaded XAD-4	Ni, 0.22	sensitive; applied to determination of Ni only; Cd, Co, and Hg interfere; needs acetyl acetone masking	16
SPE flame AAS	2-aminothiophenol	Cd, 0.81	sensitive; determination of Cd and Cu;	26
SPE	functionalized Amberlite XAD-2	Cu, 1.31	flame AAS	
SPE	2-hydroxy-acetophenone-3-	Cd, 0.38	more sensitive; determination of three	present
ICPAES	thiosemicarbazone functionalized Amberlite XAD-2	Cu, 0.61	metals: Cd, Cu, and Ni; no interferences	method
		Ni, 0.54		



Amberlite XAD-2-HAPTSC has a good resin-loading capacity, the half loading time ( $t_{1/2}$ ) of this chelating resin is also very short, and the kinetic studies ensure faster exchange kinetics in the process of metal ion uptake. The reusability of the resin is more than 20 times without affecting its sorption capacity. The method has high tolerance limits from the matrix ions. A comparison of the proposed method with other methods used for the determination of these metals is given in **Table 5**. Low detection limit, reusability of the resin, and high tolerance to the interferences from the matrix ions are the advantages of the proposed method over the methods reported in the literature.

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