Article

Study on the Determination of Lead, Cadmium, Mercury, Nickel and Zinc by a Rapid Column High-Performance Liquid Chromatography

Guangyu Yang*,[a], Qiufen Hu[a,b], Zhangjie Huang[a,b] and Jiayuan Yin[a,b]

[a] *Department of Chemistry, Yunnan University, Kunming, 650091, P.R. China*

[b] *Department of Chemistry, Yuxi Teacher's College, Yuxi, 653100, P.R. China*

Um novo método para determinação simultânea de cinco íons de metais pesados em água, alimentos e cabelo, usando-se cromatografia líquida de alta eficiência foi desenvolvido. Os íons de chumbo, cádmio, mercúrio, níquel e zinco foram derivatizados (pré-coluna) com 2-(2-quinolinil-azo)-4-metil-1,3-dihidroxidobenzeno (QAMDHB) para formar quelatos coloridos. A seguir, os quelatos Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB e Zn-QAMDHB foram enriquecidos pela extração em fase sólida, com cartucho de C_{18}. O fator de enriquecimento de 100 foi obtido ao eluir os quelatos retidos no cartucho, com tetrahidrofurano (THF). Esses quelatos foram separados em uma coluna de análise rápida ZORBAX Stable Bound (4,6×50 mm, 1,8 μm) com 68% de metanol (contendo 0,05 mol L^{-1} da solução tampão de ácido acético-pirrolidina, pH 8,5, e 0,01 mol L^{-1} de CTMAB) como fase móvel, fluxo de 2,0 mL min^{-1}, em um detector por um detector de arranjo de fotodiodos a 450 ˜ 600 nm. Os quelatos Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB e Zn-QAMDHB foram separados completamente em 2,0 min. Os limites de detecção do chumbo, cádmio, mercúrio, níquel e zinco são 1,2 ng L^{-1}, 1,0 ng L^{-1}, 0,8 ng L^{-1}, 1,6 ng L^{-1} e 1,9 ng L^{-1}, respectivamente, nas amostras originais. Este método foi aplicado na determinação dos cinco íons desses metais pesados com bons resultados.

A new method for the simultaneous determination of five heavy metal ions in water, food and human hair by a rapid column high-performance liquid chromatography was developed. The lead, cadmium, mercury, nickel and zinc ions were pre-column derivatized with 2-(2-quinolinylazo)-4-methyl-1,3-dihydroxidebenzene (QAMDHB) to form colored chelates. Then the Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB and Zn-QAMDHB chelates were enriched by solid phase extraction with C_{18} cartridge. The enrichment factor of 100 was achieved by eluting the retained chelates from the cartridge with tetrahydrofuran (THF). These chelates were separated on a ZORBAX Stable Bound rapid analysis column (4.6×50 mm, 1.8 μm) with 68% methanol (containing 0.05 mol L^{-1} of pH 8.5 pyrrolidine-acetic acid buffer solution and 0.01 mol L^{-1} of CTMAB) as mobile phase at a flow rate of 2.0 mL min^{-1} using a photodiode array detector from 450-600 nm. The Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB and Zn-QAMDHB chelates were separated completely within 2.0 min. The detection limits of lead, cadmium, mercury, nickel and zinc are 1.2 ng L^{-1}, 1.0 ng L^{-1}, 0.8 ng L^{-1}, 1.6 ng L^{-1} and 1.9 ng L^{-1}, respectively in the original samples. This method was applied to the determination of these five heavy metal ions with good results.

Keywords: rapid column high-performance liquid chromatography, 2-(2-quinolinylazo)-4-methyl-1,3- dihydroxidebenzene, heavy metal ions

Introduction

The RP-HPLC technique with pre-column derivatization has been proved to be a favorable and reliable technique for the separation and determination of trace amount of metal ions. Many kinds of reagents have been examined as pre-column derivatization reagents, and several review articles have appeared on this approach.[1-8]

In previous work, Many azo dyes, such as pyridylazo reagents, thiazolylazo reagents, benzothiazolylazo reagents and the like have been used as chelating reagent for the determination of metal ions by RP-HPLC.[9-16] However, a long separation time (more then 10 min) is needed for the routine chromatographic methods. To shorten the separation time, in this paper, 2-(2-quinolinylazo)-4-methyl-1,3-dihydroxidebenzene

(QAMDHB) was used as pre-column derivatization reagents for lead, cadmium, mercury, nickel and zinc, and a ZORBAX Stable Bound rapid analysis column (4.6×50 mm, 1.8 μm) was used for the separation of metal-QAMDHB chelates. The five chelates were separated completely within 2.0 min in this method. The separation time was greatly shortened compared to the routine chromatographic methods. Based on this, a rapid, sensitive and selective method for the simultaneous determination of the five heavy metal ions in water, food and human hair was developed.

Experimental

Synthesis of QAMDHB
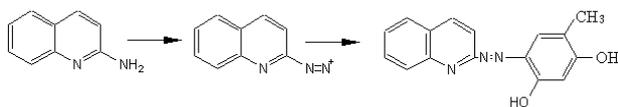
2-Aminoquinoline (7.2 g, 0.052 mol) was dissolved in a 500 mL anhydrous ethanol. To which, sodamide (2.0 g, 0.051 mol) was added and the mixture was refluxed in boiling water bath for 5 h, followed by the addition of isoamyl nitrite (7.4 mL, 0.052 mol). The solution was refluxed for 30 min with a boiling water bath, and then the solution was cooled and placed over night under 0 °C. The diazo solution was obtained by filtering this solution with an isolation yield of 95%. Thereafter, the diazo solution was dissolved in 200 mL anhydrous ethanol, followed by the addition of 4-methyl-1,3-dihydroxidebenzene (5.8 g, 0.047 mol). The carbon dioxide was ventilated into the solution with stirring until the pH reaches about 8.0. The solution was stood for two days, and then the solution was diluted with 400 mL water. The QAMDHB in diluted solution was extracted with chloroform. The chloroform was evaporated and the residue was re-crystallized with 30% ethanol. QAMDHB was obtained with 35% yield. The structure of QAMDHB was verified by elemental analysis [$C_{15}H_{11}N_3O_2$, found (calculated): C 68.8 (68.1), N 15.1 (14.8), H 5.06 (4.98)]. IR (Figure 1) (KBr) ν_{max}(cm^{-1}): 3580 (ν_{O-H}); 1615, 1565, 1530, 1429 ($\nu_{C=C, N=N}$); 2925, 2873 (ν_{C-H}); 1460, 1425, 1380 (δ_{C-H}); 3050, 3025 (σ_{Ar-H}); 1050 (ν_{C-O}); 1175, 1120, 865, 785, 750 (δ_{Ar-H}). ^{1}H NMR (Figure 2) (solvent: d_6-acetone) (δ ppm): 6.88-7.65 (m 8 H, Ar-H); 5.34, 5.46 (σ 1H, Ar-OH); 2.22 (σ3H, Ar-CH$_3$); MS (Figure 3): 279 (M$^+$).

Chemicals and apparatus

The HPLC system was consisted of a Waters 2690 Alliance separation model and a 996 photodiode array
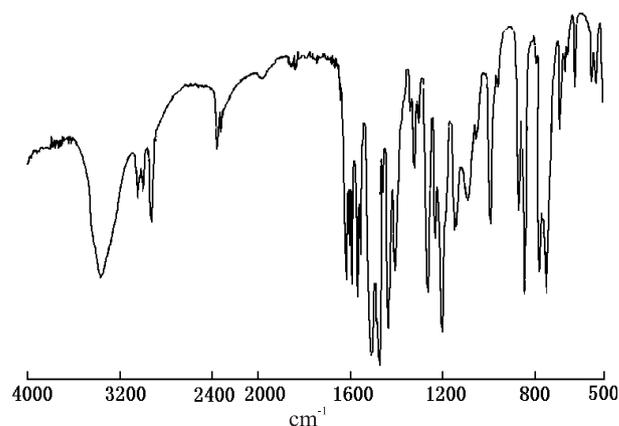
Figure 1. The infrared spectrum of QAMDHB.
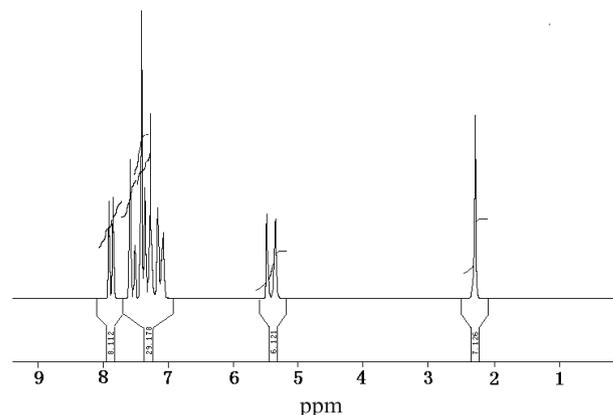
Figure 2. The ^{1}H nuclear magnetic resonance spectrum of QAMDHB.

detector (Waters Corporation, USA). The pH values were determined with a Beckman Φ-200 pH meter. The separation column used was a ZORBAX Stable Bound rapid column (4.6×50 mm, 1.8 μm) (Agilent Corporation, USA). The cartridge used is Zorbax C$_{18}$ solid phase extraction cartridge (1 cm^3 per 50 mg, 30 μm) (Agilent Corporation, USA). The extraction was performed on Waters Solid Phase Extraction (SPE) Device (The device can prepare twenty samples simultaneously).

All of the solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation, USA). QAMDHB was dissolved with THF to make a $1.0×10^{-4}$ mol L^{-1} of solution. Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) standard solution (1.0 mg mL^{-1}) was obtained from the Chinese Standards Center, and a working solution of 0.5 μg mL^{-1} was prepared by diluting this standard solution. The methanol and tetrahydrofuran (THF) used were HPLC grade (Fisher Corporation, USA). Pyrrolidine-acetic acid buffer solution (0.5 mol L^{-1}, pH 8.5) and CTMAB solution (1%, dissolving with 10% ethanol) was used. The mobile phase used was 68% methanol (containing 0.05 mol L^{-1} of pH 8.5 pyrrolidine-acetic acid buffer solution and 0.01 mol L^{-1} of CTMAB). All other reagents used were of analytical
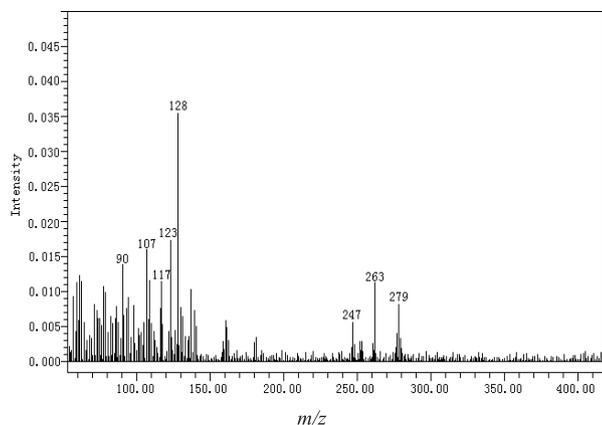
Figure 3. The mass spectrum of QAMDHB.

Figure 4. Chromatogram of standard sample and real sample: (1) Rice sample, (2) Standard sample. Injection volume 10 mL. The concentration of Pb, Cd, Hg, Ni, Zn is 20 mg L^{-1} in standard sample. Detection wavelength is 555 nm. Other conditions as in standard procedure.

reagent-grade. The glass and Teflon wares used were soaked in 5% of nitric acid for a long time, and then thoroughly wash with pure water.

Standard procedure

An appropriate volume of sample solution or standard solution was transferred into a 100 mL of volumetric flask. To which, 8.0 mL 1.0×10^{-4} mol L^{-1} of QAMDHB THF solution, 10 mL pH 8.5 pyrrolidine-acetic acid buffer solution and 2.0 mL of 1% of CTMAB solution were added. The solution was diluted to the volume with water and mixing well. After 10 min, the solution was passed through the C_{18} cartridge at a flow rate of 10 mL min^{-1}. When the enrichment had finished, the retained chelates were eluted from the cartridge with 1.0 mL of THF at a flow rate of 5 mL min^{-1} in an opposite direction. The solution was filtered with 0.45 mm of filters and adjusted to the volume of 1.0 mL. 10 μL of sample was injected for HPLC analysis. A tridimensional (X axis: retention time, Y axis: wavelength, Z axis: absorbance) chromatogram was recorded from 450~650 nm with photodiode array detector and the chromatogram of 555 nm was shown in Figure 4.
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Results and Discussion

Precolumn derivation

The optimal pH for the QAMDHB reacts with metal ions is 7.5 ~ 11.2 for lead, 7.8 ~ 12.5 for cadmium, 8.0 ~ 11.8 for mercury, 4.8 ~ 9.2 for nickel and 7.2 ~ 9.4 for zinc. Therefore, a 0.5 mol L^{-1} of pyrrolidine-acetic acid buffer solution (pH 8.5) was recommended to control pH.

It was found that 1.0 mL of 1.0×10^{-4} mol L^{-1} QAMDHB THF solution was sufficient to chelate 10.0 μg of Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) ions. But in the real samples, the foreign ions can react with QAMDHB to consume

reagents. So the amount of QAMDHB must in excess. In this experiment, 8.0 mL of 1.0×10^{-4} mol L^{-1} QAMDHB was sufficient to chelate the ions in real samples, and 8.0 mL QAMDHB solution was recommended.

The experiments show that in the nonionic surfactants and cationic surfactants medium, the sensitivity of the metal-QAMDHB chelates was increased markedly. Various nonionic surfactants and cationic surfactants enhance the absorbance in the following sequence: CTMAB > Tween-80 > CPB > Tween-20 > TritonX-100. Therefore, CTMAB was selected as additive in this experiment. The use of 1 ~ 4 mL of CTMAB solution give a constant and maximum absorbance in this experiment. Accordingly, 2.0 mL CTMAB solution was recommended.

Solid phase extraction

Both the enrichment and the elution were carried out on a Waters SPE device, which can prepare twenty samples simultaneously, the flow rate was set to 10 mL min^{-1} when enrichment and 5 mL min^{-1} when elution.

Some experiments were carried out in order to investigate the retention of metal-QAMDHB chelate on the cartridge. It was found that the Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB and Zn-QAMDHB chelates could be retained on cartridge quantitatively when they pass the cartridge as aqueous solution. The capacity of the cartridge for QAMDHB was 30 mg and for its metal-QAMDHB chelate was 25 mg in a 100 mL of solution. In this experiment, the cartridge has adequate capacity to enrichment the metal-QAMDHB chelate and the excessive QAMDHB.

Various organic solvents were studied as eluant for eluting the retained QAMDHB and its metal-chelate from the cartridge. It was found that the THF, acetone, acetonitrile, ethanol and methanol could elute the QAMDHB and its metal-chelate from cartridge quantitatively. For completely eluting the metal-QAMDHB chelates from cartridge, the volume of the solvent needed is THF 0.9 mL, isopentyl alcohol 1.4 mL, acetone 1.7 mL, acetonitrile 1.9 mL, ethanol 2.2 mL, methanol 2.4 mL. The maximal enrichment factor was achieved when THF was selected as eluant. Therefore, THF was selected as eluant in this experience. The metal-QAMDHB chelate has a good stability in weak alkaline medium. The eluant containing a 0.05 mol L^{-1} pyrrolidine-acetic acid buffer (pH 8.5) can increase the stability of the metal-QAMDHB chelate in the course elution. Therefore, the THF (containing 0.05 mol L^{-1} pyrrolidine-acetic acid buffer solution (pH 8.5)) was selected as eluant in this experience. The experiment show that it was easier to elute the retained QAMDHB and its metal-chelate in reverse direction than in forward direction (only 0.9 mL of eluant is needed when eluted in reverse direction, however, 2.2 mL of eluant is needed when eluted in forward direction), so it is necessary to upturned the cartridge when elution. 1.0 mL of eluant was sufficient for elute the QAMDHB and its metal-chelate from cartridge quantitatively at a flow rate of 5 mL min^{-1}. Therefore the volume of 1.0 mL eluant was selected in this experiment.

Chromatographic separation

The experiments showed that the Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB and Zn-QAMDHB chelates have a good stability in the presence of alkaline acid buffer solution and CTMAB medium. The pH of mobile phase within 7.8-9.4 and containing a 0.005-0.3 mol L^{-1} of CTMAB in the mobile phase can avoid the metal-chelate decomposing in the course of separation and get a good peak shape. So methanol/water (68/32, v/v) (containing 0.05 mol L^{-1} of pH 8.5 pyrrolidine-acetic acid buffer solution and 0.01 mol L^{-1} of CTMAB) was selected as mobile phase. To shorten the chromatographic separation time, A ZORBAX Stable Bound rapid analysis column (4.6×50 mm, 1.8 μm) was selected in this experiment. With rapid analysis column, the five chelates were separated completely within 2 min. Compared to the routine chromatographic method, more then 80% of separation time was shortened.

Spectrophotometric properties

The absorption spectrum of metal-QAMDHB chelates was obtained by measured with a Shimidzu UV-2401 spectrophotometer. The absorption data were given in Table 1. In this experiment, to get maximum sensitivity, each metal-QAMDHB chelates was monitored at its maximum absorption wavelength (Pb-QAMDHB 562 nm, Cd-QAMDHB 556 nm, Hg-QAMDHB 570 nm, Ni-QAMDHB 568 nm, Zn-QAMDHB 548 nm).

Calibration graphs

Under the optimum conditions, the regression equations of metal-QAMDHB chelates were established based on the standard sample injected and its peak area. The limits of detection are calculated by the ratio of signal to noise (S/N=3). The results were shown in Table 2. The reproducibility of this method was also examined for 20 mg L^{-1} of Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II). The relative standard deviations (n=9) were shown in Table 2 too.

Table 1. Spectrophotometric properties of metal-QAMDHB chelates

Metal chelates	Pb-	Cd-	Hg-	Ni-	Zn-
λ_{max} (nm)	562	556	570	568	548
ε (×10^4 L mol^{-1} cm^{-1})	8.23	8.68	8.94	7.76	6.67

Interference

Under the pre-column derivatization conditions, the foreign ions of Fe(III), Co(II), Mn(II), Ag(I), Cu(II), V(V) which can reacts with QAMDHB to form color chelates. To examine the selectivity of this method, the

Table 2. Regression equation, coefficient and detect limit

Components	Regression Equation[a]	Linearity Range (μg L^{-1})	Coefficient	Detect limits (ng L^{-1}) [b]	RSD % (n=9)
Pb-QAMDHB	A=5.48×10^4 C+158	0.7-800	r=0.9995	1.2	1.9
Cd-QAMDHB	A=5.99×10^4 C-229	0.5-650	r=0.9992	1.0	2.1
Hg-QAMDHB	A=6.68×10^4 C+246	0.6-600	r=0.9994	0.8	2.2
Ni-QAMDHB	A=5.28×10^4 C+287	0.8-900	r=0.9992	1.6	2.4
Zn-QAMDHB	A=5.15×10^4 C-229	0.6-950	r=0.9993	1.9	1.8

[a] In the measured solution; [b] in the original digested sample.

interference of these foreign ions was investigated. When 5.0 mL of 1.0×10^{-4} mol L^{-1} QAMDHB was used, with 20 μg L^{-1} of Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) respectively, the tolerance amount with an error of ±5% was 3000 μg L^{-1} for Fe(III), Cu(II), Co(II), Mn(II) and 500 μg L^{-1} for Ag(I), V(V). This method is high selectivity.

Applied to the rice and human hair samples

A sample of 0.2-0.50 g (rice and human hair) was weighted accurately into the teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China). To which, 5.0 mL of concentrated nitric acid and 6.0 mL of 30% hydrogen peroxide were added. The bombs were sealed tightly and then positioned in the carousel of the microwave oven (Model WL 5001, 1000 W, Fei Yue Analytical Instrument Factory, Shanghai, China). The system was operated at full power for 6.0 min. The digest was evaporated to near dryness. The residue was dissolved with 5 mL of 5% of nitric acid and transferred into a 100 mL of calibrated flask quantitatively, then diluted the solution to volume with water. The Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) contents were analyzed by using a proper volume of this solution according to general

procedure. The results (deducted the reagents blank) were shown in Table 3.

Applied to the water samples

For the fresh water (tap water, river water and lake water), the water sample was analyzed according to the general procedure. The results (deducted the reagents blank) were shown in Table 4, together with the results of a recovery test by added 0.2 μg of Pb, Hg, Cd, Ni and Zn in water sample and diluted to 100 mL of final solution. For planting effluents, the sample was digested as literature[17] and analyzed according to the general procedure. The results (deducted the reagents blank) were shown in Table 4 too, together with results of a recovery test by added 0.2 μg of Pb, Hg, Cd, Ni and Zn in water sample and diluted to 100 mL of final solution. A standard method using atomic absorption spectrometry reported in literature [17] had also been used as a reference method. The results are shown in Table 5.

Conclusions

In this paper, 2-(2-quinolinylazo)-4-methyl-1,3-dihydroxidebenzene was used as pre-column

Table 3. Determination results of certified standard food samples

Samples	Standard value (μg g^{-1})	By this method (μg g^{-1})	RSD % (n=5)
Rice (GBW08458)	As(0.285), Ba(21.2), Bi(0.342), Ca(2900), Cd(0.318),Ce(1.25), Co(4.71), Cr(3.76), Cu(10.2), Fe(54), Hg(0.186), Mg(360), Mn(22.5), Mo(0. 735), Ni(3.83), Pb(0.852), V(2.86), Zn(8.76)	Cd(0.301), Hg(0.197), Ni(3.64), Pb(0.884), Zn(8.49)	2.9
Human Hair (GBW08126)	As(0.121), Ba(11.7), Bi(0.825), Ce(0.643), Cd(0.528), Co(6.25), Cr(0.8), Cu(11.2), Fe(123), Hg(0.242), Mn(68.2), Ni(5.61), Pb(1.06), Sn(1.24), Se(0.0412), Zn(12.7), V(3.86)	Cd(0.504), Hg(0.214), Ni(5.82), Pb(1.15), Zn(13.4)	2.7

Table 4. Determination results (μg L^{-1}) of the water sample with this method

Components	Samples (μg L^{-1})				RSD % (n=5)	Recovery % (n=5)
	River water	Lake water	Plant effluent	Tap water		
Pb	19.6	26.5	75.8	10.5	2.8	96
Cd	7.26	11.5	23.2	-	3.2	106
Hg	5.76	7.43	38.6	-	3.0	97
Ni	24.9	32.5	58.7	6.84	3.2	93
Zn	76.2	63.5	121	36.4	3.4	102

Table 5. Determination results (μg L^{-1}) of the water sample with reference method

Components	Samples (μg L^{-1})				RSD % (n=5)	Recovery % (n=5)
	River water	Lake water	Plant effluent	Tap water		
Pb	20.6	28.4	79.6	11.3	3.4	105
Cd	6.94	10.2	21.8	-	3.6	97
Hg	5.18	6.89	39.5	-	3.8	108
Ni	26.1	33.4	57.1	7.16	3.8	92
Zn	77.9	64.3	114	37.1	3.5	96

derivatization reagent for Pb, Hg, Cd, Ni and Zn ions, and the ZORBAX rapid analysis column was used for the separation of Pb-QAMDHB, Cd-QAMDHB, Hg-QAMDHB, Ni-QAMDHB and Zn-QAMDHB chelates. The QAMDHB can reacts with Pb, Hg, Cd, Ni and Zn rapidly at room temperature. The five chelates were separated completely within 2 min. Compared to the routine chromatographic method, more then 80% of separation time was shortened. The metal-chelates were preconcentrated by C_{18} cartridge and the enrichment factor of 100 was achieved. The detection limits of this method reach the ng L^{-1} level. Most foreign ions do not interfered with the determination. This is one of the sensitive, selective and rapid methods for the simultaneous determination of Pb, Hg, Cd, Ni and Zn.

Acknowledgments

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