

Determination of Five Heavy Metal Ions in Environmental Samples by a Rapid High Performance Liquid Chromatography

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A new method for the simultaneous determination of five heavy metal ions in water and food by a rapid high-performance liquid chromatography was developed. The lead, cadmium, mercury, nickel and zinc ions were pre-column derivatized with 2-(2-quinolinylazo)-resorcin (QAR) to form coloured chelates, then the Pb-QAR, Cd-QAR, Hg-QAR, Ni-QAR and Zn-QAR chelates were enriched by solid phase extraction with C₁₈ cartridge. The enrichment factor of 100 was achieved by eluting the retained chelates from the cartridge with tetrahydrofuran. These chelates were separated on a Zorbax stable bound rapid analysis column (4.6 × 50 mm, 1.8 μm) with 65% methanol (containing 0.05 mol/L of pH = 8.5 pyrrolidine-acetic acid buffer salt and 0.01 mol/L of CTMAB) as mobile phase at a flow rate of 2.0 mL/min and detected with a photodiode array detector from 450–600 nm. The Pb-QAR, Cd-QAR, Hg-QAR, Ni-QAR and Zn-QAR chelates were separated completely within 2.0 min. The detection limits of lead, cadmium, mercury, nickel and zinc are 1.5, 1.2, 1.0, 1.8 and 2.0 ng/L, respectively in the original samples. This method was applied to the determination of the five metal ions in water and food samples with good results.

Key Words: High-performance liquid chromatography, 2-(2-Quinolinylazo)-resorcin, Heavy metal ions.

INTRODUCTION

The RP-HPLC technique with pre-column derivatization has been proved to be a favourable 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 works, many azo dyes, such as pyridyl azo, thiazolyl azo, benzothiazolyl azo reagents and the like have been used as chelating reagents for the determination of metal ions by RP-HPLC^{9–16}. However, the routine chromatographic methods need a long separation time (more than 10 min is

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needed). To shorten the separation time, in this paper, a new synthesized reagent, 2-(2-quinolinylazo)-resorcin (QAR), was used as pre-column derivatization reagent for lead, cadmium, mercury, nickel and zinc and a Zorbax stable bound rapid analysis column (4.6×50 mm, $1.8 \mu\text{m}$) was used for the separation of metal-QAR 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 and food was developed.

EXPERIMENTAL

The HPLC system consisted of a Waters 2690 Alliance separation model and a 996 photodiode array detector (Waters Corporation, USA). The pH values were determined with a Beckman-200 pH-meter. The separation column used was a Zorbax stable bound microcolumn (4.6×50 mm, $1.8 \mu\text{m}$) (Agilent Corporation, USA). The cartridge used is Zorbax C₁₈ solid phase extraction cartridge (1 cc/50mg, $30 \mu\text{m}$) (Agilent Corporation, USA). The extraction was performed on Waters solid phase extraction (SPE) device (the device can prepare twenty samples simultaneously).

All the solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP reagent water system (Millipore Corporation, USA). QAR was dissolved with THF to make a 1.0×10^{-4} mol/L solution. Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) standard solutions (1.0 mg/mL) were obtained from Chinese Standards Center, and a working solution of 0.5 $\mu\text{g/mL}$ 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, pH = 8.5) and CTMAB solution (1%, dissolving with 10% ethanol) was used. The mobile phase used was 65% methanol (containing 0.05 mol/L of pH = 8.5 pyrrolidine-acetic acid buffer salt and 0.01 mol/L of CTMAB). All other reagents used were of analytical reagent-grade. The glass and teflon wares used were soaked in 5% of nitric acid for a long time and then thoroughly washed with pure water.

Synthesis of 2-(2-Quinolinylazo)-resorcin (b)

2-aminoquinoline (a) (7.2 g) was dissolved in 500 mL anhydrous ethanol, to which sodamide (2.0 g) was added and the mixture was refluxed in a boiling water bath for 5 h, followed by the addition of isoamyl nitrite (7.4 mL). The solution was refluxed for 30 min in a boiling water bath. The solution was cooled and placed overnight under 0°C. The diazo salt was obtained by filtering this solution with an isolation yield of 95%. Thereafter, the diazo salt was dissolved in 200 mL anhydrous ethanol, followed by the addition of resorcinol (5.5 g). Then carbon dioxide was ventilated into the solution with stirring until the pH reached about 8.0. The solution stood for 2 d, and then diluted with 400 mL water and extracted with chloroform. The solvent was evaporated and the residue was

recrystallized with 30% ethanol. QAR was obtained with 45% yield. The structure was verified by elemental analysis ($C_{15}H_{11}N_3O_2$, Found (Calcd.) (%): C 68.2 (67.9), N 16.2 (15.8), H 4.32 (4.18). IR (Fig. 1), 1H NMR (Fig. 2) and MS (Fig. 3).

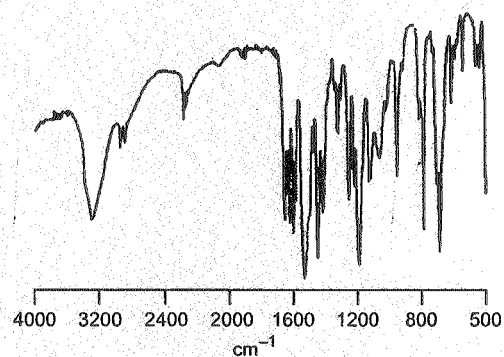
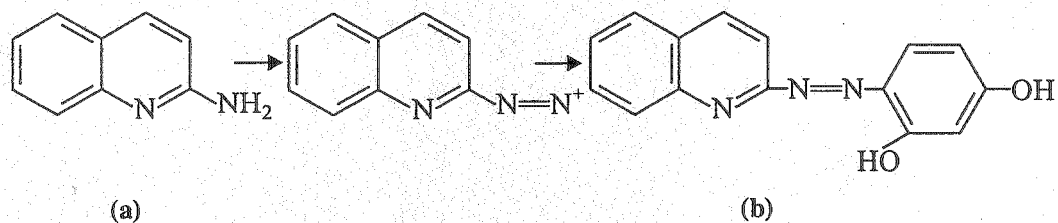


Fig. 1. Infrared spectrum of QAR

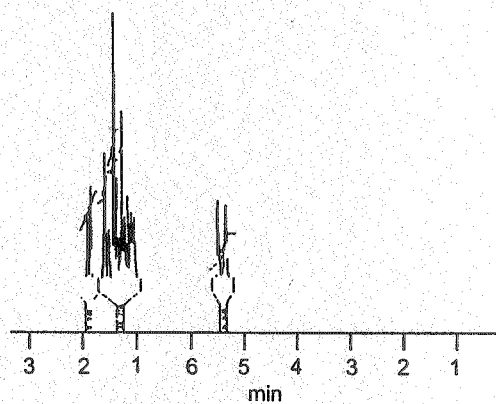


Fig. 2. 1H nuclear magnetic resonance spectrum of QAR

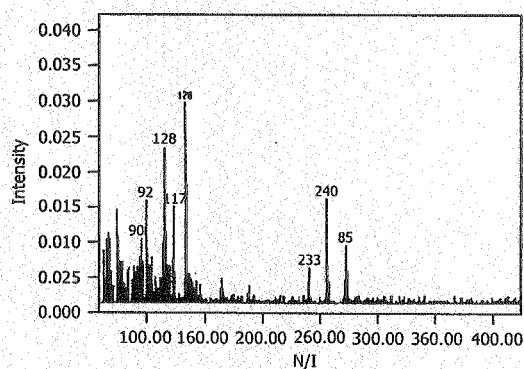


Fig. 3. The mass spectrum of QAR

Standard procedure

An appropriate volume of sample solution or standard solution was transferred into a 100 mL volumetric flask, to which 8.0 mL of QAR THF solution (1.0×10^{-4} mol/L), 10 mL pyrrolidine-acetic acid buffer solution (pH = 8.5) and 2.0 mL of CTMAB solution (1%) were added. The solution was diluted to 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. 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 in an opposite direction. The solution was filtered with $0.45 \mu\text{m}$ filters and adjusted to a volume of 1.0 mL. 10 μL of the 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 550 nm as shown in Fig. 4.

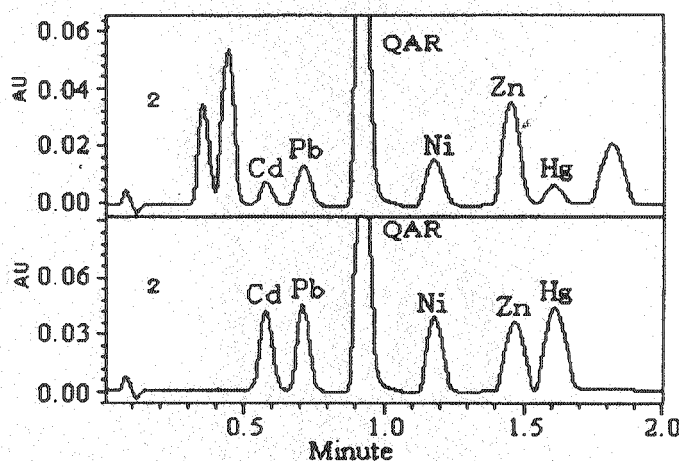


Fig. 4. Chromatogram of standard sample and real sample: (1) Real sample, (2) Standard sample. Injection volume 10 μL . The concentration of Pb, Cd, Hg, Ni, Zn is 20 $\mu\text{g/L}$ in standard sample. Detection wavelength is 550 nm. Other conditions as in standard procedure.

RESULTS AND DISCUSSION

The optimal pH for the QAR reacting with metal ions is 7.2–11.5 for lead, 7.8–12.3 for cadmium, 7.6–11.8 for mercury, 4.6–9.4 for nickel and 6.8–9.8 for zinc. Therefore, a 0.5 mol/L pyrrolidine-acetic acid buffer solution (pH = 8.5) was recommended to control pH. It was found that 1.0 mL of QAR THF solution (1.0×10^{-4} mol/L) 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 QAR to consume reagents. So the amount of QAR must be in excess. In this experiment, 8.0 mL of QAR (1.0×10^{-4} mol/L) was sufficient to chelate the ions in real samples. The experiments show that in the non-ionic surfactants and cationic surfactants medium, the sensitivity of the metal-QAR chelates increased markedly. Various nonionic surfactants and cationic surfactants enhance the absorbance in the following sequence: CTMAB > Tween-80 > CPB > Tween-20 > Triton-X-100. Therefore, CTMAB was selected as additive in this experiment. The use of 1–4 mL of CTMAB solution gives 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 during enrichment and 5 mL/min during elution.

Some experiments were carried out in order to investigate the retention of metal-QAR chelate on the cartridge. It was found that the Pb-QAR, Cd-QAR, Hg-QAR, Ni-QAR and Zn-QAR chelates can be retained on cartridge quantitatively when they pass the cartridge as aqueous solution. The capacity of the cartridge for QAR was 28 mg and for its metal-QAR chelate was 22 mg in 100 mL of solution. In this experiment, the cartridge has adequate capacity to enrich the metal-QAR chelate and the excessive QAR.

In order to choose a proper eluant for the retained QAR and its metal-chelate, various organic solvents were studied. It was found that the THF, acetone, acetonitrile, ethanol and methanol could elute the QAR and its metal-chelate from cartridge quantitatively. For eluting the metal-QAR 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 and methanol 2.4 mL. The maximal enrichment factor was achieved when THF was selected as eluant. Therefore, THF was selected. Metal-QAR chelate has a good stability in weak alkaline medium. Containing a 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH = 8.5) in THF could increase the stability of the metal-QAR chelate in the course of elution. So THF (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH = 8.5)) was selected as eluant. Experiments show that it was easier to elute the retained QAR and its metal-chelate in reverse direction than in forward direction; so it is necessary to upturn the cartridge during elution. 1.0 mL of eluant was sufficient for eluting the QAR and its metal-chelate from cartridge quantitatively at a flow rate of 5 mL/min. The volume of 1.0 mL was selected.

Chromatographic separation

The experiments showed that the Pb-QAR, Cd-QAR, Hg-QAR, Ni-QAR and Zn-QAR 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 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 (65/35) (containing 0.05 mol/L of pH = 8.5 pyrrolidine-acetic acid buffer salt and 0.01 mol/L 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 than 80% of separation time was shortened.

Spectrophotometric properties

The absorption spectrum of metal-QAR chelates was obtained by measuring with a Shimadzu UV-2401 spectrophotometer. The absorption data are given in Table-1. In this experiment, to get maximum sensitivity, each metal-QAR chelate was monitored at its maximum absorption wavelength.

TABLE-1
THE SPECTROPHOTOMETRIC PROPERTIES OF METAL-QAR CHELATES

Chelates	Pb-QAR	Cd-QAR	Hg-QAR	Ni-QAR	Zn-QAR
λ_{\max} (nm)	560	555	568	565	545
ϵ ($\times 10^{-4}$ L mol ⁻¹ cm ⁻¹)	7.86	8.12	8.65	7.22	6.48

Calibration graphs

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

TABLE-2
REGRESSION EQUATION, COEFFICIENT AND DETECT LIMIT

Components	Regression equation ^a	Linearity range ($\mu\text{g/L}$)	Coefficient	Detect limits (ng/L) ^b	RSD (%) (n = 9)
Pb-QAR	$A = 5.22 \times 10^4 C - 212$	0.6–850	$r = 0.9994$	1.5	1.8
Cd-QAR	$A = 5.74 \times 10^4 C - 186$	0.4–680	$r = 0.9998$	1.2	2.2
Hg-QAR	$A = 6.25 \times 10^4 C + 186$	0.6–650	$r = 0.9995$	1.0	2.4
Ni-QAR	$A = 5.08 \times 10^4 C + 208$	0.8–890	$r = 0.9997$	1.8	1.7
Zn-QAR	$A = 4.87 \times 10^4 C - 159$	0.8–956	$r = 0.9996$	2.0	2.2

^aIn the measured solution; ^bIn the original digested sample.

Interference

Under the pre-column derivatization conditions, the foreign ions of Fe(III), Co(II), Mn(II), Ag(I), Cu(II), V(V) can react with QAR to form colour chelates. To examine the selectivity of this method, the interference of these foreign ions was investigated. When 5.0 mL of QAR (1.0×10^{-4} mol/L) was used, with 20 $\mu\text{g/L}$ of Pb(II), Cd(II), Hg(II), Ni(II) and Zn(II) respectively, the tolerance amount with an error of $\pm 5\%$ was 3000 $\mu\text{g/L}$ for Fe(III), Cu(II), Co(II), Mn(II) and 500 $\mu\text{g/L}$ for Ag(I), V(V). This method is highly selective.

Applied to the food samples

0.2–0.50 g of sample was weighed 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% nitric acid and transferred into a 100 mL 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 reagent blanks) are shown in Table-3.

TABLE-3
DETERMINATION RESULTS OF CERTIFIED STANDARD FOOD SAMPLES

Samples	Standard value ($\mu\text{g/g}$)	By this method ($\mu\text{g/g}$)	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(0.347), Hg (0.166), Ni (3.52), Pb (0.782), Zn (8.54)	3.1
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 (0.556), Hg (0.231), Ni (5.52), Pb (1.18), Zn (11.8)	3.3

Applied to the water samples

For fresh water (tap water, river water and lake water), the water sample was analyzed according to the general procedure. The results (deducted the reagent blanks) are shown in Table-4, together with the results of a recovery test by adding 0.2 μg of Pb, Hg, Cd, Ni and Zn in water sample and diluting to 100 mL of final solution. For planting effluents, the sample was digested as in literature¹⁷ and analyzed according to the general procedure. The results (deducted the reagent blanks) are also shown in Table-4, together with results of a recovery test by adding 0.2 μg of Pb, Hg, Cd, Ni and Zn in water sample and diluting to 100 mL of final solution. A standard method using atomic absorption spectrometry reported in literature¹⁷ has also been used as a reference method. The results are shown in Table-5.

TABLE-4
DETERMINATION RESULTS ($\mu\text{g/L}$) OF THE WATER SAMPLE WITH THIS METHOD

Metals	Samples ($\mu\text{g/L}$)				RSD (%) (n = 5)	Recovery (%) (n = 5)
	River water	Lake water	Plant effluent	Tap water		
Pb	22.80	31.80	82.5	12.40	3.2	103
Cd	7.26	11.50	23.2	—	3.1	105
Hg	6.27	8.57	42.3	—	3.4	98
Ni	28.60	36.40	56.5	8.29	3.5	104
Zn	78.50	65.50	108.0	38.40	3.2	102

TABLE-5
DETERMINATION RESULTS ($\mu\text{g/L}$) OF THE WATER SAMPLE WITH REFERENCE METHOD

Metals	Samples ($\mu\text{g/L}$)				RSD (%) (n = 5)	Recovery (%) (n = 5)
	River water	Lake water	Plant effluent	Tap water		
Pb	20.60	28.40	79.6	11.30	3.4	105
Cd	7.85	12.90	20.5	—	3.6	97
Hg	5.18	6.89	39.5	—	3.8	108
Ni	26.10	33.40	57.1	7.16	3.8	92
Zn	77.90	64.30	114.0	37.10	3.5	96

Conclusion

In this paper, 2-(2-quinolinylazo)-resorcin (QAR) was first used as pre-column derivatization reagent for Pb, Hg, Cd, Ni and Zn ions and the Zorbax rapid analysis column was used for the separation of Pb-QAR, Cd-QAR, Hg-QAR, Ni-QAR and Zn-QAR chelates. The QAR can react 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 than 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 level. Most of the foreign ions do not interfere with the determination. This is one of the sensitive, selective and rapid methods for the simultaneous determination of Pb, Hg, Cd, Ni and Zn. In a word, for the simultaneous determination of Pb, Hg, Cd, Ni and Zn in water and food, this method possesses high sensitivity, high selectivity and high rapidity.

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