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Determination of Cd, Cr, Cu, Ni, Zn in River Fish by Ultrasound Assisted Extraction-Atomic Absorption Spectrometry

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The conditions for determining heavy metals in river fish through ultrasound assisted extraction were optimized and a method of ultrasound assisted extraction-atomic absorption spectrometry was established for the determination of Cd, Cr, Cu, Ni and Zn present in fish. By ultrasound extraction, quantitative recoveries were reached for all of these five heavy metals investigated, recoveries were found to be 91-110 %. Good linearity of the calibration curves was obtained for all the five elements (r = 0.9936-0.9998). The detection limits of the method were in the range of 0.003-0.010 mg/L and the relative standard deviations were in the range of 1.3-5.7 %. The GBW08573 standard reference material was determined under the optimum experimental conditions by both ultrasound extraction and wet digestion. Results obtained by these two methods are close to each other and in good agreement with the reference values.

Key Words: Ultrasound assisted extraction, River fish, Heavy metals.

INTRODUCTION

Fish is widely consumed by humans for it contains high protein content, low saturated fat, essential vitamins, trace elements and omega fatty acids known as good health supporting nutrition[1]. However, fish will be contaminated with toxic metals (cadmium, chromium, lead, *etc*.) by water pollution from industrial and domestic waste wate. Jinzhou is an important industrial city in Liaoning province,industrial waste water discharged by petrochemical and ferroalloy factory caused Xiaoling river and Nver river were polluted by heavy metals. While water pollution leads to fish contaminated with toxic metals. After accumulation of these toxic metals from aquatic environments, fish will become a carrier of concentrated toxicant and subsequently transfer to human through food chain[2]. The ingested toxic elements are very harmful for human health even at low content over a long time period[3]. The essential metals (copper, zinc, *etc*.) can also produce toxic effects when the metal intake is excessively elevated[4]. For this reason determination of contents of heavy metals in river fish is extremely important for human health in Jinzhou[5].

As the most common used methods for the digestion of heavy metals in biological samples, microwave and conventional wet acid digestion have been widely applied for the dissolution of elements[6,7]. However, these techniques generally require the use of concentrated acids, high temperatures and often high pressures to achieve the total dissolution of elements from solid samples[8]. These steps are time-limiting, requiring more than 60 % of the total time to perform the complete procedure[9]. Hence, the aim of this study is to establish a method with shorter time, lesser contamination, lower reagent consumption and lesser residue or waste generation[10].

It has been demonstrated that ultrasound assisted extraction could speed up and simplify sample treatment[11]. In solution, ultrasonic energy causes acoustic cavitation, which means bubble formation and their subsequent implosion[12]. The collapse of bubbles created by the sonication of solutions generates extremely high local temperatures and pressure gradients leading to enhanced chemical reactivity[13]. The combined effect of extremely high temperatures and pressures at the interface of the sonicated solution and the solid matrix, along with the oxidative power of strong acids, results in high extractive power. This phenomenon makes ultrasonic extraction compatible with the traditional methods without the utilization of expensive and complicated equipment[4,14]. Here, in this work, parameters influencing ultrasonic-assisted extraction such as presonication time, sonication time, temperature, solvents and particle size were optimized. Thereafter, a rapid and suitable method for determination of heavy metal elements in fish, namely ultrasound assisted extraction-atomic absorption spectrometry, was established.

EXPERIMENTAL

H_2O_2, HNO_3 and $HClO_4$ were of suprapur quality. The standard solutions of Cd, Cr, Cu, Ni and Zn for calibration

procedure were produced by diluting a stock solution of 1000 mg/L of the investigated element supplied by Institute for Environmental Reference Materials of Ministry of Environmental Protection. GBW08573 (yellow croaker) were chosen as certified reference materials. Double distilled deionized water was used in all experiments.

AA320N atomic absorption spectrometer equipped with GA 3202 graphite furnace system was used for determination of trace elements. Teflon reaction vessels were used in all digestion procedures. Ultrasonic extractions were carried out in an ultrasound cleaner (KQ-100DB), programmable for temperatures ranging from 30-80 ºC with an intensification frequency of 40 kHz. A total volume of 4 L was used to induce the acid leaching process. All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1:9, v/v) and were rinsed with distilled water prior to use. Cu, Ni and Zn were determined using flame atomic absorption spectrometry, Cd and Cr were determined using graphite furnace atomic absorption spectrometry. The analytical conditions are summarized in Tables 1 and 2.

TABLE-1
WORKING CONDITIONS OF THE FAAS

Element	Wavelength (nm)	Lamp current (mA)	Slit (nm)	Acetylene (L/min)	Air (L/min)
Cu	324.8	10	0.4	0.6	8.0
Ni	232.0	10	0.1	0.8	9.0
Zn	213.9	8	0.7	0.7	8.0

Sample collection and pretreatment: The common economic representative and intact river fish samples were randomly purchased from retail outlets located in Liaoning province from August to October in 2011. Five kinds of river fish including carp, grass carp, crucian, silver carp and catfish with similar size were selected. After cleaning surface sludge, specimens collected were frozen in prewashed polyethylene bags and then brought to the laboratory in ice chests[15].

After sequent washing twice with distilled water and deionized water, the samples were dried. After dissected by a stainless steel knife, the same fish species were homogenized and stored in polyethylene bottles at -20 ºC. The homogenized samples were dried in refrigeration for over 24 h until constant weight was obtained. The dried samples were further homogenized by grinding in an agate ball mixer mill and sieving through a nylon sieve (< 74 μm mesh size) and then separately stored in prewashed and dried polyethylene vessels. separately at room temperature in desiccators until further treatment[3].

Ultrasonic acid extraction procedure: About 0.15 g of certified sample or fish muscle samples were directly weighted into centrifuge tubes, then 5 mL of HNO_3-H_2O_2 (2:1, v/v) was added and allowed to stand for 10 min without ultrasonic stirring at room temperature (marked as presonication time). The tubes were then placed inside the ultrasonic water bath for ultrasound treatment at 40 kHz for 10 min. The temperature of the water bath was controlled in the range of 70 ºC. The resulting suspension in the tubes was then centrifuged at 3000 rpm for 10 min to separate the acid liquid phase from solid sample. The liquid phase was collected as acid leachate. In order to collect as much elements as possible, the same procedure was repeated twice by re-adding 2 mL volume of ultrapure water to the solid residue, centrifuging and collecting liquid phase again. All the collected solutions were put together and then diluted to 10 mL with ultrapure water and stored in polyethylene bottles at 4 ºC. The blank digestions were carried out in the same way[11].

Wet acid digestion method: About 0.5 g of certified sample or fish muscle samples were weighed in digesting flasks and 10 mL of HNO_3-H_2O_2 (2:1, v/v)was added. The flasks were covered with watch glasses stay overnight and then heated on a hot plate until the colour of the digestion solution became transparent. After cooling, the solutions were diluted with ultrapure water to a final volume of 50 mL in volumetric flasks and stored in polyethylene bottles at 4 ºC. The blank digestions were carried out in the same way.

RESULTS AND DISCUSSION

Influence of pre-sonication time: The effect of the pre-sonication time on the recoveries of heavy metals was investigated by varying the time from 2-12 min and the results were shown in Fig. 1. Each value in line graph represents the mean of generally six replicates. For Cd and Zn the results were multiplied by a factor (indicated on the bar) in order to be plotted in the same graph. Without pre-sonication, the recovery concentrations are relatively low. When presonication is applied, the increases on the recovery concentrations for all the five elements are observed. The maximum recoveries of all the elements are obtained at 10 min of pre-sonication time. Longer pre-sonication time has no effect on the recoveries[16].

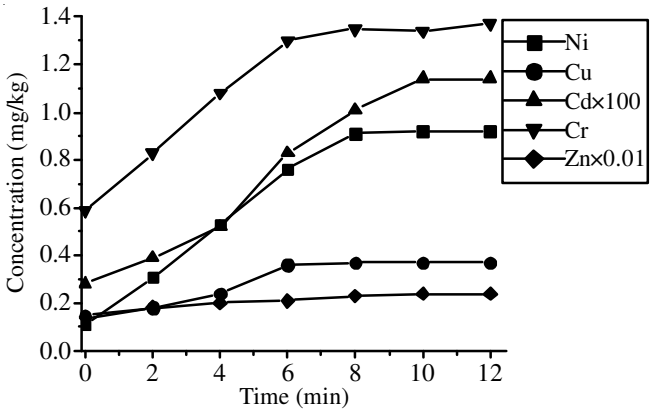

Fig. 1. Effects of pre-sonication time

TABLE-2
WORKING CONDITIONS OF THE GFAAS

Element	Wavelength (nm)	Slit (nm)	Lamp current (mA)	Temperature (ºC), ramp time(s), hold time(s)							
				Drying		Ashing		Atomization		Cleaning	
Cd	228.8	0.7	8.0	120	10/10	350	5/10	2300	2/3	2400	3/3
Cr	357.9	0.7	10.0	110	20/20	1000	10/20	2800	3/3	2850	3/3

Influence of sonication time: Under optimum pre-sonication time, the effect of the sonication time on the recoveries of heavy metals was investigated by varying the time from 2 to 14 min and the results were shown in Fig. 2. The recovery concentrations for all the five elements show an upwards increase tendency as the time prolonged until a maximum value reached. The optimum time for Cr and Cu is 8 min, for Ni and Zn is 10 min and for Cd is 12 min.

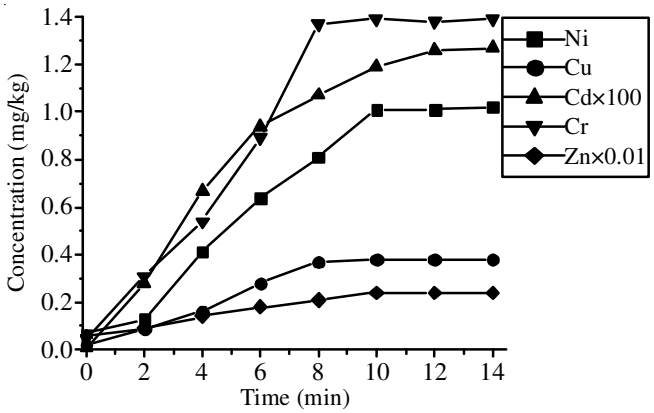

Fig. 2. Effects of sonication time

Influence of temperature: The influence of temperature, ranged from 30 to 80 ºC, was investigated by fixing other variables at their optimal values. The results obtained in this study are shown in Fig. 3. It is found that high temperature is necessary for enhancing recoveries of all the elements tested. The maximum recoveries of all the elements are obtained at 70 ºC.

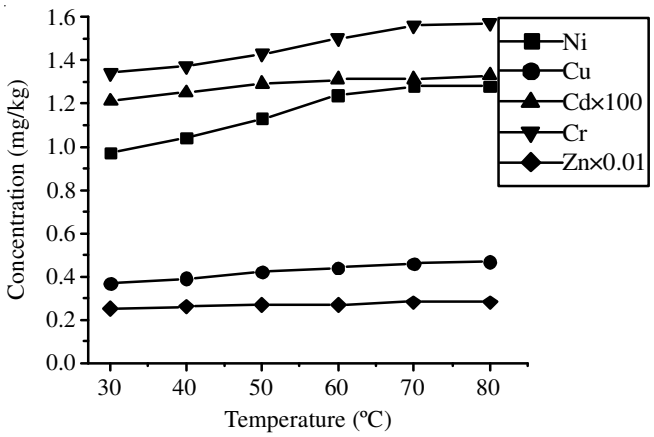

Fig. 3. Effects of temperature

Influence of solvents: The influence of solvents, such as HNO_3, HNO_3-H_2O_2 (2:1,v/v) and HNO_3-$HClO_4$ (2:1,v/v) was investigated by fixing other variables at their optimal values. As can be seen in Fig. 4, higher recoveries of all heavy metals are obtained in acid-oxidant solvents (HNO_3-H_2O_2 and HNO_3-$HClO_4$). This result indicated that samples with high content of organic matter usually can not be completely oxidized by HNO_3 only. The recoveries of all the five heavy metals obtained from HNO_3-H_2O_2 and HNO_3-$HClO_4$ are almost same. However, considering the danger of $HClO_4$, HNO_3-H_2O_2 (2:1,v/v) is selected as the solvent for all of the samples.

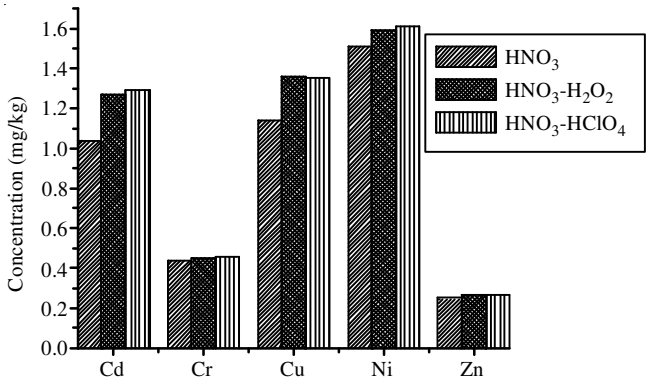

Fig. 4. Effects of solvent systems

Influence of sample amount: The influence of sample amount, ranged from 0.05 to 0.30 g, for 5 mL volume of solvent was investigated by fixing other variables at their optimal values. As can be seen in Fig. 5, decrease in all heavy metals recovery is observed when the sample amount is larger than 0.15 g. Therefore, the optimum sample amount/solvent volume ratio is fixed at 0.15 g/5 mL.

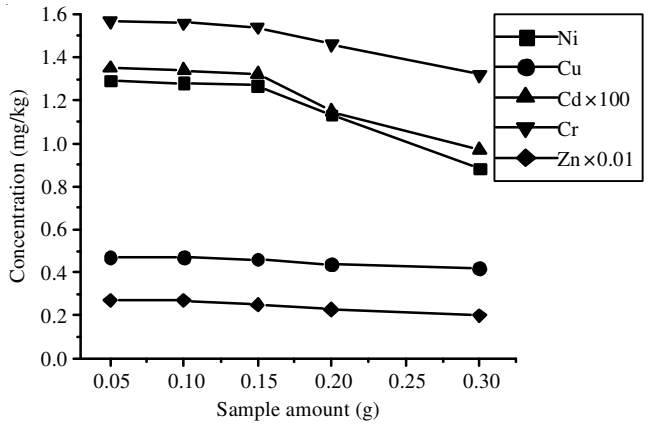

Fig. 5. Effects of sample amount

Influence of particle size: It was reported that reactions could be enhanced by increasing the contact surface[17]. The influence of particle size, ranged from less than 50 μm to more than 150 μm, was investigated by fixing other variables at their optimal values. As can be seen in Fig. 6, recoveries decrease when the particle size is larger than 74 μm for all the metals. Therefore, the optimum particle size is fixed at less than 74 μm in this work.

Comparison of wet digestion and ultrasound extraction: Performances of wet digestion and ultrasound extraction (the optimal condition are shown in Table-3) for certified reference materials (GBW08573 yellow croaker) were compared in this work. As can be seen in Table-4, quantitative recoveries for all the heavy metals are obtained by both of these two digestion methods. However, wet digestion generally requires long time and the blank value is high. Moreover the acid mist formed by wet digestion procedures is harmful for human health. Compared with wet digestion, ultrasound extraction is simpler, faster, safer and lesser acid is consumed.

Calibration curves and detection limits: The calibration curves were obtained by the standard solution for the

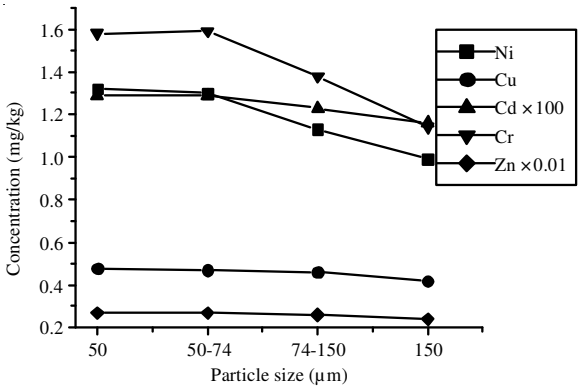

Fig. 6. Effects of particle size

TABLE-3
OPTIMUM LEACHING CONDITIONS

Variable	Interval	Best condition
Presonication time (min)	0~12	8
Sonication time (min)	0~14	10
Temperature (ºC)	30~80	70
Solvent systems	HNO_3, HNO_3-H_2O_2, HNO_3-$HClO_4$	HNO_3-H_2O_2
Sample amount (g)	0.05~0.30	0.15
Particle size (μm)	< 50, 50~74, 74~150, > 150	50~74

TABLE-4
RESULTS OF TWO METHOD AGAINST CERTIFIED REFERENCE MATERIAL (mg/kg)

Elements	Cd	Cr	Cu	Ni	Zn
Reference value	0.015	0.43	1.36	1.50	28.8
Wet digest	0.014	0.40	1.41	1.43	26.4
Ultrasound extraction	0.013	0.47	1.32	1.59	27.2

determination of each element. As shown in Table-5, good linearity of the calibration curves is obtained for all these five elements (r = 0.9936-0.9998). The linear range of the calibration curve covers from the detection limit to 10 mg/L for all heavy metals. The detection limit was defined as three times the relative standard deviation of 11 absorbance readings of the reagent blank values for each element.

Precision and accuracy: The precision of a method is expressed as the relative standard deviation of eight independent analyses of the same sample. As shown in Table 6, the RSD values for ultrasound extraction are in the range from 1.3 to 5.7 %. In order to investigate the accuracy of the method, the standard addition experiments for these five elements were made. After adding a certain amount of standard solution to the samples, the samples were digested using ultrasound extraction, the concentrations of heavy metals were determined according to the methods metioned in experimental part. The recoveries are 90-110 %.

TABLE-5
LINEAR CORRELATION COEFFICIENTS, LINEAR RANGES AND DETECTION LIMITS (3S/N)

Elements	Standard curve	Correlation coefficient	Linear range (mg/L)	Detection limit (mg/L)
Cd	y=0.0692x-0.0129	0.9936	0-10	0.010
Cr	y=0.0344x+0.0025	0.9998	0-10	0.009
Cu	y=0.2065x-0.0114	0.9973	0-10	0.008
Ni	y=0.5960x+0.0057	0.9991	0-10	0.003
Zn	y=0.3057x+0.0131	0.9981	0-10	0.007

TABLE-6
AVERAGE RECOVERIES AND RSD

Elements	Background (μg/mL)	Add (μg/mL)	Measured (μg/mL)	Recoveries (%)	RSD (%)
Cd	0.14	0.20	0.36	110.0	5.7
Cr	0.25	0.30	0.57	106.7	4.0
Cu	0.73	1.00	1.64	91.0	2.9
Ni	0.49	0.50	1.03	108.0	2.5
Zn	8.42	10.0	17.83	94.1	1.3

Analytical application: The concentrations of heavy metal elements in fish muscle were determined by atomic absorption spectrometry under the optimal ultrasound extraction conditions. The results obtained in this study are shown in Table-7.

The concentration levels of Cr, Cu, Ni and Zn in fish muscles are lower than the maximum legal limits, but Cd levels in grass carp are 1.30 times of the maximum legal limits. Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies[21].

Conclusion

Ultrasound assisted extraction offers a fast, easy, safe, inexpensive and efficient sample preparation for direct determination of Cd, Cr, Cu, Ni and Zn in fish muscle samples by AAS. When conditions such as presonication time, sonication time, temperature, solvent systems, sample amount and sample particle size are fixed at optimum value, the method has high precision and accuracy. The results obtained by ultrasound assisted extraction are similar to those obtained by the wet digestion. However, ultrasound assisted extraction seems more promising for the treatment time is shorter, the costs and pollution are lower and the operation is simpler.

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TABLE-7
CONCENTRATIONS OF HEAVY METALS OF FISH MUSCLES AND VEGETABLES (mg/kg)

Elements	Cd	Cr	Cu	Ni	Zn
Carp	0.05 ± 0.01	0.13 ± 0.04	1.67 ± 0.39	0.49 ± 0.17	10.6 ± 4.20
Grass carp	0.13 ± 0.02[a]	0.27 ± 0.11	0.53 ± 0.18	0.51 ± 0.34	8.37 ± 3.52
Crucian	0.01 ± 0.00	0.09 ± 0.04	1.50 ± 0.34	1.24 ± 0.53	21.2 ± 6.79
Silver carp	0.04 ± 0.02	0.64 ± 0.31	4.22 ± 1.76	0.70 ± 0.12	4.94 ± 1.90
Catfish	ND	0.48 ± 0.12	6.75 ± 2.01	0.85 ± 0.24	16.7 ± 3.17
Maximum levels	0.1 [Ref. 18]	2.0 [Ref. 18]	50 [Ref. 19]	–	[Ref. 20]

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