Determination of Cadmium in Rice by Activated Aluminum Oxide Immobilized with Dithizone-Solid Phase Extraction-Hydride Generation Coupled Atomic Fluorescence Spectrometry

Xiufang Lu[1], Jianping Lu[2,*], Zhenglian Zhang[2] and Minhua Xue[2]

[1]China GuangXi Center for Disease Prevention and Control, Jinzhou Road 18, Nanning 530021, P.R. China
[2]College of Chemistry and Chemical Engineering, Guangxi Key Laboratory of Petrochemical Resource Processing and Process Intensification Technology, Guangxi University, Nanning 530004, P.R. China

*Corresponding author: E-mail: ljianpi@hotmail.com; look612_2006@163.com; 185316992@qq.com

Dithizone, as an absorbent was immobilized on activated aluminum oxide as a supporter, by impregnation to separate and enrich cadmium which was determined by hydride generation coupled atomic fluorescence spectrometry. The pH, eluting reagents of appropriate concentrations, eluting rate and interference were investigated. Under optimum experimental conditions, detection limit of 0.0060 μg/L, dynamic linearity of 0.005-10.0 μg/L and relative standard deviation of 2.23 % were obtained. The method validated by national standard certified rice materials showed a good agreement with the certified values. When applied to determination of the cadmium in rice from different origins, the method exhibited recoveries of 95.9-103 %. With environmental friendly, convenient operation, fast analysis, good accuracy and high sensitivity, the method demonstrated a great potential to be used in real samples.

Keywords: Activated aluminum oxide, Dithizone, Cadmium, Solid phase extraction, Atomic fluorescence spectrometry.

INTRODUCTION

Recently, great concerns are paid to the effects of heavy metals on human health and food safety. Cadmium is one of the toxic and poisonous elements. After accumulated a certain amount in human body, cadmium will damage kidney and make small protein molecules wash away through urea[1], leading to the developments of joint pain, rickets endocrine disorders, insomnia, loss of appetite, *etc*.[2]. In addition, cadmium, a strong carcinogen categorized by international cancer research institutes, may induce prostate cancer. Excess amount will deteriorate female physiology and fertility. Rice as human's main diet generates the great necessity to monitor its cadmium pollution or contamination.

Generally, the method of determining cadmium depends mainly on atomic spectrophotometry[3]. Atomic fluorescence spectrometry, demonstrating unique advantages of less inter-ference, wide linear range, high sensitivity, low cost, *etc*., increases applications in the determination of cadmium recently[4]. How-ever, the amount of cadmium in food sample is usually extremely low, which is very difficult to detect, so it requires some enrich-ment in sample preparation. Usually, enrichment applications are solid phase extraction, solid phase microextraction and liquid-liquid extraction[5-7]. Of which, the solid phase microex-traction is widely used in sample treatment in terms of its simple

setting up, convenient operation and no solvent involved[8,9]. Thus, a new solid phase microextraction was customized in this paper to investigate the separation and enrichment of cadmium, aimed at the realization of green chemistry and environmental safety.

Dithizone, a very common chelating reagent for heavy metal ions, possesses the ability of complexing approximately 20 heavy metal ions in different acidity and is widely used to determine these metals. Its application in cadmium determi-nation was reported[10,11]. In strong alkaline aqueous solution, cadmium reacts with dithizone to form a red coordinated compound as shown below. Unfortuately, the compound decomposes in an acidic solution.
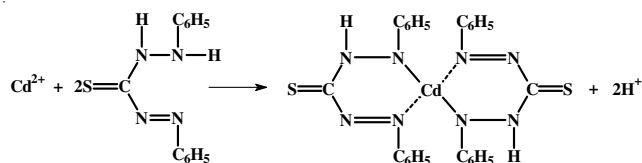
Absorbent is essential in solid phase extraction; active carbon, octadecaalkyl bonded silica, polyamide resin, tri sodium titanate whisker, polyurethane foam, activated aluminum oxide, *etc*. are usually used as absorbents[12,13]. Activated

aluminum, contributed to its large surface area, strong chemical absorption and large absorptive volume, is an ideal material in separation and enrichment of trace metals[14,15].

Loading type adsorbent with high absorptive capacity and good selectivity is preferably studied in chemistry[16]. However, using dithizone to modify activated aluminum oxide has not been reported. Thus, this adsorbent was investigated in this paper for its concentrating cadmium and the elution condition and the cadmium was determined by hydride generation-atomic fluorescence spectrometry. The method was attempted in the determination of cadmium in rice, showing simple operation, accuracy and no solvent extraction involved.

EXPERIMENTAL

Cadmium working solution: prepared by gradual dilution of a cadmium stock standard solution (1,000 µg/mL, GSB G 62040-90) acquired from National Center of Standard Materials, China. KBH$_4$ (15 g/L): 3.0 g KBH$_4$ dissolved in 200 mL of 5 g/L NaOH and mixed thoroughly; prepared when needed. Dithizone solution: a certain amount of dithizone weighed and dissolved in ammonia liquid to become 0.01 mmol/L. Activated alumina granule (The specific area is greater than or equal to 300 m^2/g) manufactured by Henan Gongyi Xinsheng Water Treatment Co. Ltd.: the activated alumina granule soaked in nitric acid of 5 mol/L for 24 h and stirred by a magnetic stirrer for 0.5 h, followed by rinsing with deionized water and drying in an oven. The granule was crushed and selected through a 1 mm diameter of sieve. Perchloric acid, nitric acid, anhydrous ethanol and hydrochloric acid were of analytic reagent grades. Water was deionized.

An atomic fluorescence spectrometer of AFS-2202E (Beijing Kechuang Haiguang Co. China) was used to obtain the fluorescence intensity emitted by the target element. A cadmium hollow vacuum cathode lamp (General Research Institute for non-ferrous Metals, China) provided an energy source to radiate the target element at wavelength of 228.8 nm. A thermostat magnetic stirrer with a timer (Shanghai Zhikuang Instrument, Co. Ltd. China) was applied to the preparation of immobilized activated aluminum oxide. An electronic balance of AL104 (Mettler Todelo Instruments (Shanghai) Co., Ltd, China) was conducted to achieve a desired weight. A sampling sieve (Shangyu Yarn and Sieve Plant, China) was utilized to obtain an appropriate size of sample.

Purification of dithizone: Dithizone of 0.50 g was dissolved in 100 mL chloroform and filtered. The filtrate was transferred into a separatory funnel and extracted three times using 30 mL ammonia each, respectively. Combining all aqueous extractants, which were acidified with hydrochloric acid afterwards, dithizone precipitated. The dithizone was extracted by 30 mL chloroform three times again, respectively. All chloroform extractants were combined and were further positioned on a 50 °C water bath to remove chloroform. The final product was kept in a desiccator for 24 h and stored in a brown vessel later on.

Preparation of absorbent: Dithizone immobilized on alumina: activated alumina of 50 g was added into a 200 mL ammonia solution containing 0.01 moL/L dithizone, stirred for 0.5 h and soaked for 12 h. Then, the granule was rinsed with water to neutral, filtered and dried at 40-50 °C. The final product was kept in a brown vial ready to use.

Preparation of column: The fine alumina granule (5 g) after modified by dithizone was filled in a 10 mL burette of acid type with bottom clogged by a little of quartz cotton.

Sample preparation: After the rice sample of 1 g was weighed in a 100 mL beaker, 12 mL mixture of nitric acid and perchloric acid (v/v, 5:1) was added and the beaker was covered with a watch glass for soaking overnight.

Analytical method: After the pH of Cd standard solution or sample solutions was adjusted to 8, 20 mL of this solution was transferred into the alumina column. With a flow rate of 3 mL/min, the sample solution gently and slowly flew through the column. Then, 100 mL of 1 % HCl were used as an eluent to strip the cadmium from the column, in which cadmium, finally, was determined by hydride generation-atomic fluorescence spectrometry.

RESULTS AND DISCUSSION

Principle of modification of activated alumina granule by dithizone: When the pH was high, the surface of activated alumina granule is covered with OH$^-$, resulting in a negatively charged surface. Thus, the NH in dithizone molecular structure reacting with the OH group on the granule surface forms a bonding configuration, which is stable and could be used repeatedly.

Effect of pH on the absorption: As known, the acidity of a solution will alter the stability of Cd-dithizone complex, similarly, so it influences the absorption of Cd on the activated alumina surface. This pH influence was examined in the pH range of 5-11 (Fig. 1). As the pH increases, the fluorescence intensity increases, indicating more cadmium absorbed and the intensity reaches a maximum when pH is 8. Afterwards, the increment of pH causes the decrement of the intensity, which suggests more cadmium precipitate formed due to the higher pH.
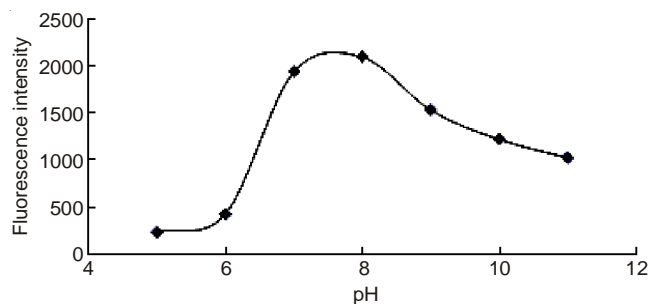

Fig. 1. Effect of pH on Cd absorption

Effect of different eluents on the absorption: As observed in Fig. 1, Cd absorption prefers an alkaline environment, which indicates that an acidic condition benefits its depriving from the column. The effect of different acids, such as hydrochloric acid, sulfuric acid and nitric acid on the stripping of Cd, was investigated. It is found that hydrochloric acid yields the best result, nitric acid in between and sulfuric acid the worst.

The effect of hydrochloric acid on the Cd stripping was further studied in the concentrations of 0-5 % (mass conc.) depicted in Fig. 2, revealing the best Cd deprivation at HCl of 1 %.
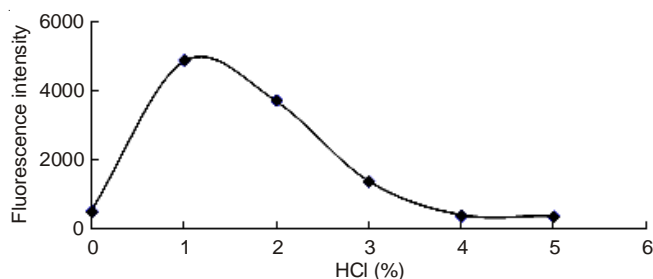
Fig. 2. Effect of hydrochloric acid concentration on Cd stripping

Fig. 3. Effect of eluent flow rate on the Cd desorption

Effect of eluent flow rate on Cd desorption: The degree of Cd stripping from the column was also governed by the eluent flow rate. It is evident that the flow rate of eluent, 1 % hydrochloric acid, varying from 1 to 4 mL/min, generates almost the same Cd amount (Fig. 3). However, the stripping efficiency deteriorates after 4 mL/min, showing that fast flow rate does not favour the Cd desorption.

Interferences: The co-existing ions, which could potentially disturb Cd determination, were examined. When 5 µg/L Cd existed, following ions with their amounts did not generate any interference listed in Table-1.

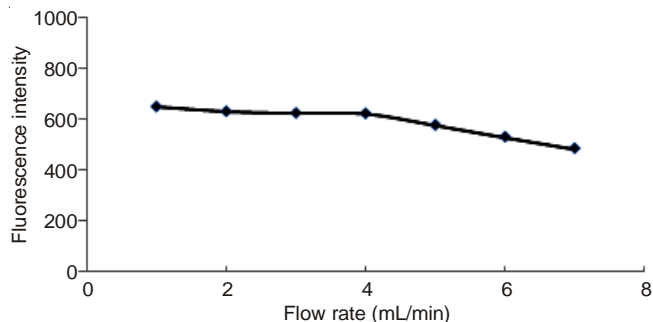**Detection limit, precision and standard calibration curve:** Under optimum experimental conditions, Cd shows a good linear relationship between its fluorescence intensities and concentrations of 0.005-10.0 µg/L with an equation of $y = 76.675x + 7.3365$ and a relative regression coefficient of 0.9955; where x is the Cd concentration and y the fluorescence intensity. Based on the definition from IUPAC, the limit of detection 0.0060 µg/L is calculated and the relative standard error is 2.23 %.

Sample analysis and method validation: This method was attempted to determine a variety of rice samples with different origins; simultaneously, blanks and recovery experiments were accompanied, giving recoveries of 95.9-103 %. When national certified standard rice material GBW10010 (87 ± 5

TABLE-1
INTERFERENCES ON Cd DETERMINATION

Ion	K^+	Na^+	Zn^{2+}	Mg^{2+}	Fe^{3+}	Ba^{2+}	Ca^{2+}	Hg^{2+}	Cu^{2+}	Ag^+
Mole ratio	2000	1000	500	500	500	200	200	100	100	100
Recovery (%)	100	100	97	101	102	98	100	96	102	99

TABLE-2
ANALYTICAL RESULTS OF SAMPLES AND RECOVERIES (n = 6)

Sample No.	Origins	Determined values (µg/kg)	RSD (%)	Added (µg/kg)	Total (µg/kg)	Recoveries (%)
1	Fragrant rice (Ubon Ratchathani, Thailand)	84	2.4	80 90 100	164 170 187	100 102 98.4
2	Fragrant rice (Cambodia)	89	1.3	70 90 100	156 178 187	101 100.5 101
3	Rice (San Francisco, USA)	29	2.1	20 30 40	47 60 67	95.9 101 97.1
4	Hydride rice (California, USA)	24	2.5	15 20 40	38 45 64	97.4 102 100
5	White glutinous rice (Laos)	121	1.8	120 130 140	244 260 260	101 103 99.6
6	White rice (Pampa, brazil)	35	2.7	20 30 40	53 63 73	96.3 96.9 97.3
7	White rice (Lafite, France)	67	2.3	50 60 70	114 128 133	97.4 100.7 97.1
8	Pearl rice (Liaoning, China)	50	0.6	40 50 60	90 102 108	100 102 98.2
9	Precious rice (Liuzhou, China)	148	2.5	140 150 160	295 297 310	102 99.6 100.6
10	GBW10010	84	0.9	/	/	/

µg/kg) was used to validate the method, the result was in a good agreement with the reported value (Table-2).

Conclusion

Based on the activated alumina granule modified by dithizone through impregnation to separate and enrich cadmium, a novel method to determine the cadmium in rice was established. In optimized conditions, the method showed a Cd detection limit of 0.0060 µg/L and a linear calibration curve in the range of 0.005-10.0 µg/L. The relative standard deviation was 2.23 %. When the method was applied to determination of a number of rice samples from different origins, recoveries of 95.9-103 % were obtained. The method, with advantages of simple operation, fast analysis, non-solvent involved, high sensitivity and good accuracy, demonstrates a great potential to be used in the determination of trace amount cadmium.

REFERENCES

1. A.M.H. Shabani, S. Dadfarnia and Z. Dehghani, *Talanta*, **79**, 1066 (2009).
2. K. Li, *J. Health Lab. Technol.*, **17**, 1016 (2007).
3. S.K. Xu, M. Sperling and B. Welz, *Fresenius J. Anal. Chem.*, **344**, 535 (1992).
4. H. Wu, C. Fang, B.X. Du and C.C. Zhao, *Mikrochim. Acta*, **160**, 173 (2008).
5. M. Chamsaz, A. Atarodi, M. Eftekhari, S. Asadpour and M. Adibi, *J. Adv. Res.*, **4**, 35 (2013).
6. E.L. Silva, P.S. Roldan and M.F. Giné, *J. Hazard. Mater.*, **171**, 1133 (2009).
7. A.S. Amin and A.A. Gouda, *Food Chem.*, **132**, 518 (2012).
8. N. Pourreza and K. Ghanemi, *J. Hazard. Mater.*, **178**, 566 (2010).
9. V.A. Lemos, E.M. Gama and A. da Silva Lima, *Mikrochim. Acta*, **153**, 179 (2006).
10. S.H. Ba, *Chem. Ind.*, **28**, 50 (1999).
11. Q.X. Zhou, N. Zhao and G.H. Xie, *J. Hazard. Mater.*, **189**, 48 (2011).
12. H.L. Liu, T.S. Zhao, Y.H. Wang and C.J. Hu, *J. Instrumental Anal.*, **23**, 25 (2004).
13. Y.L. Mao, Y.J. Shi, R.Q. Liu, K. Zhang, X.C. Xiao and Y.S. Yan, *Metallurgical Anal.*, **29**, 25 (2009).
14. T.T. Gong, S.C. Shen, Y.C. Bai and L. Guo, *J. Sci. Teachers' Coll. Univ.*, **31**, 73 (2011).
15. X.L. Pu, H.H. Wang, Q.M. Dong, H.Z. Pan, B. Hu and H. Zhang, *Guangdong Weiliang Yuansu Kexue*, **15**, 50 (2008).
16. B. Puzio, B. Mikula and B. Feist, *Mikrochim. Acta*, **160**, 197 (2008).