

Study on the process of Determination of Cadmium and Lead in Some Vegetables in Lam Thao - Phu Tho by F-AAS Method

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Abstract

In this paper, we studied the process of analysis cadmium and lead in green vegetable samples by flame atomic absorption spectrometry (F-AAS). The conditions of analysis such as acid concentration, subtracted solution, releasing reagent have been investigated and optimized. Effect of foreign ions on spectral line intensity was also investigated. At the optimized conditions (HNO₃ concentration of 1%, CH₃COONH₄ concentration of 1%, LaCl₃ concentration of 1%), the analysis procedure showed the high accuracy and repeatability. The linear ranges of Cd and Pb were 0.01-4 ppm and 0.05-8 ppm, respectively. The limit of detection (LOD) and (LOQ) of Cd were 0.0092 and 0.0310 ppm, respectively and these values for Pb were 0.0090 and 0.0300 ppm. In addition, the procedure was applied to analyze the 10 green vegetable samples grown in Lam Thao district, Phu Tho province.

Keywords: F-AAS, Cadmium, Lead, Heavy metal, Analysis

1. Introduction

The process of industrialization and modernization as well as development of society have quickly created enormous consequences on the environment. At present, we are facing serious environmental problems such as land, water, air, and ecosystems [1]. Governments and scientists are very considered about addressing the source of environmental pollution. In particular, the analysis and evaluation of environmental indexes will play an important role in addressing the problem of pollution [2].

Recently, food contamination issue has received a great interest from society and government. Especially, green vegetables are food needs for daily life, the quality of them is very important to ensure human health. Normally, green vegetables accumulated heavy metals from fertilizers, soil and water source. It is therefore essential to analyze and evaluate the heavy metal contamination in green vegetables [3].

Lead and Cadmium are two unnecessary elements that accumulate the living organs for which these elements are toxic. Cadmium ion are easily absorbed by vegetables and animal food distributed in kidney and liver consequents to many health problems. vegetables grown in highly contaminated soils would

obviously contain a high cadmium level. Lead accumulates in the body and interferes in vitamin D and calcium metabolites, it is a neurotoxin which causes behavioral abnormalities. Vegetables, especially leaf vegetables may contain elevated lead level when grown near sources of lead [4]. Therefore, the determination of cadmium and lead in the vegetable samples at locations is becoming urgent.

In order to determine the heavy metal contents in food, water samples, and soil, there many methods have been developed such as catalytic kinetic method [5], micro-volume UV-Vis spectrophotometric [6], flame atomic absorption spectrometry (F-AAS) [7], and graphite furnace atomic absorption spectrometry (GF-AAS) [3]. X-ray fluorescence (XRF) [8]. And, atomic Absorption Spectroscopy (AAS) has been showed the high selectivity and accuracy. In each analytical method, the sample preparation and choosing the analytical conditions play the decisive roles in the accuracy and repeatability of the analytical method.

In the sample treatment technique, the heating on hot plate, decomposition by heating block, decomposition by high pressure, and microwave have been widely used. In which, microwave most suitable for AAS analysis due to the following characteristics: rapid decomposition rate, decomposition time for environmental samples from several minutes to less

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than 20 minutes, the sealed system that do not contaminate the environment and not lose the volatile metals Cd and Pb as compared to other techniques.

In this paper, we study and evaluate the analytical procedure of Cd and Pb in some green vegetable samples by F-AAS. The microwave oven was used to treat the samples. The effects acid concentration, substrate solution and releasing reagent on spectral line intensity were investigated to determine the optimized conditions. The effect of foreign ions also was studied. In addition, statistical evaluation methods were also used to evaluate the reliability and accuracy of the method.

2. Experimental

2.1. Apparatus and chemicals

The standard solutions, Cd²⁺ (Merck, 1000 ppm) and Pb²⁺ (Merck, 1000 ppm) were used to made the standard curves. The samples and standard solutions were prepared by diluting the stock solution in the respective proportions. Concentrated solutions, HNO₃ (Merck, 65%) and HCl (Merck, 37%) were used without further purification. The solutions, NH₄Cl 10%, CH₃COONH₄ 10%, CH₃COONa 10%, LaCl₃ 10% were used to studied the effect of operation parameters to the analytical methods. Double distilled water, volumetric flask, filter, filter paper, glass beaker, analytical balance (accuracy ± 0.0001g), technical weighing (accuracy ± 0.01 g), and heating plate were used to sample preparation. The flame atomic adsorption spectroscopy (F-AAS, NOVAA 350) was used to analyze the samples. The samples were prepared by a microwave system, Qlab pro. Canada, the microwave breaking capacity of 1200W, the frequency of 1250 MHz, power supply of 220V, 50Hz, 20A, and temperature range of 1-300 °C.

2.2. Sample preparation

2.2.1. Collection of samples

Vegetable samples were collected at locations and packed in clean plastic bags to bring to the laboratory. After cutting the yellow leaves, the vegetable samples were rinsed by tap water to remove the soil and sand, then rinsed with double distilled water several time. Fresh vegetable samples were keep at room temperature for 3h to for water drain, and then chopped in to small pieces and mixed well. The samples were weighed and then placed in an oven at 80 °C until dry. Dry samples were transferred into a desiccant to cool and weighed once again to determined the dry weight. The samples were stored in vials to avoid moisture then analyzed by a F-AAS.

2.2.2. Optimization of temperature and time for treatment of samples

The 8 vials of microwave-decomposition of sample were prepared. Dry vegetable sample (0.3 g) was put in each vials. Next, 10 mL of concentrated HNO₃ was added into vials and then placed in microwave oven. The temperature in range of 135-180 °C and the time in range of 4-12 min were used for treatment of sample to Fig.out the optimization temperature and optimization time.

2.2.3. Analysis conditions

Spectral measurement conditions: The selection of spectral measurement conditions based on the combination of theoretical and empirical aspects. The resonance line and slit width for lead were 288.8 nm and 0.5 nm, respectively, and the values for lead were 217.0 and 0.5 nm, respectively. The peak signal was sensitive enough, high stability, and 100 % of peak area was inside the slit. The currents of lamp for cadmium and lead (8 mA and 10 mA, respectively) were chosen to be had the high intensity and stability and of spectral line.

Atomization conditions: The height of lamp for atomization of Cd and Pb was chosen at 7 mm in order to have the high sensitivity and stability as well as the smallest error.

To evaluate the error and repeatability of the measurement, we used the formulas in the previous publication [9] as follows.

Relative error was calculated according to the formula:

$$\overline{Er} = \frac{|(x_1 - x_t)|}{x_t} \times 100 \quad (1)$$

Repetitivity of method was determined by formulas:

$$SD = \sqrt{\frac{\sum_1^i (x_i - \bar{x})^2}{(n-1)}} \quad (2)$$

$$RSD = 100 \times \frac{SD}{\bar{x}} \quad (3)$$

Where \bar{x} is mean value, \overline{Er} is average relative error (%), x_t is the true or acceptable value, SD is Standard deviation, and RSD is relative standard deviation.

Limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%):

$$LOD = \bar{x}_{\text{blank}} + 3SD \quad (4)$$

Limit of quantification (LOQ):

$$LOQ = \frac{10 LOD}{3} \quad (5)$$

The contents of Cd and Pb were determined by following equation:

$$X = \frac{C_x \times V}{m} \quad (6)$$

Where, X (mg/kg) is the content of heavy metal in fresh vegetable sample, C_x (mg/L) is the concentration of heavy metal from standard curves, V (L) is the volume of sample, m (kg) is mass of fresh vegetable sample.

3. Results and discussion

3.1. Study the effect of sample preparation on spectral line intensity

3.1.1. Effect of acid concentration on spectral line intensity

In this study, in order to investigate the effects of the type of acid and its concentration to spectral line intensity, we used HCl, H₂SO₄ and HNO₃ in concentration from 0 to 3% to analyze Cd and Pb at the concentrations of 1 and 2 ppm. The results in presented in Fig.1.

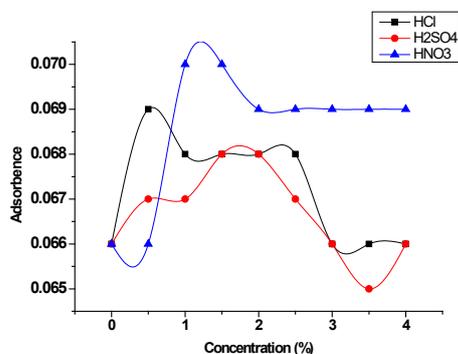


Fig. 1. Effect of acid concentration on spectral line intensity

It was seen that the type of acid and its concentration were significantly affected to spectral line intensity of Cd and Pb. The HNO₃ acid showed the high intensity compared to other acids. Therefore, we selected the HNO₃ at concentration of 1% to further study.

3.1.2. Effect of substrate solution on spectral line intensity

In some cases, the samples contain heat-resistant substances. These substances can inhibit the atomization, leading the reduction of the sensitivity of analytical method. We have added additional vaporized substances for elimination of the effect of heat-resistant substances. Therefore, in order to investigate the effect of substrate solution on spectral line intensity, the solutions CH₃COONa, CH₃COONH₄, and NH₄Cl in concentration range of 1-5% were used as substrate solutions to measure the absorbance of solution Cd 1ppm and Pb 2ppm in

HNO₃ 1%, the results are presented in Fig.2. The maximum absorbance for CH₃COONa, NH₄Cl, and CH₃COONH₄ were 0.064, 0.065, and 0.069, respectively. The absorbance for using CH₃COONH₄ solution approached maximum value at lower concentration than for using other solutions. Therefore, the solution CH₃COONH₄ 1 % was used to further investigation.

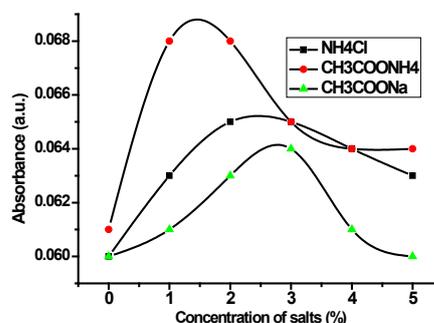


Fig. 2. Effect of substrate solution on absorbance of sample.

3.1.3. Effect of releasing reagent on spectral line intensity

In the previous publication [10], LiCl₃ can be used as releasing reagent to a voice possible interference by phosphate, sulfate and silicate ions presents in the matrix. However, LaCl₃ has the high vaporization temperature so that if the LaCl₃ content in the sample is large then the temperature of flame is not sufficient to vaporize the sample. Therefore, it is necessary to investigate the LaCl₃ content to Fig. out the suitable concentration. In this study, the LaCl₃ concentration was varied from 0 to 2.0% in sample solution, Cd 1 ppm and Pb 2 ppm in HNO₃ 1% and CH₃COONH₄ 1%. Fig.3 showed the absorbance of solution was depended on LiCl₃ concentration, it reached the maximum value at concentration of 1.0%.

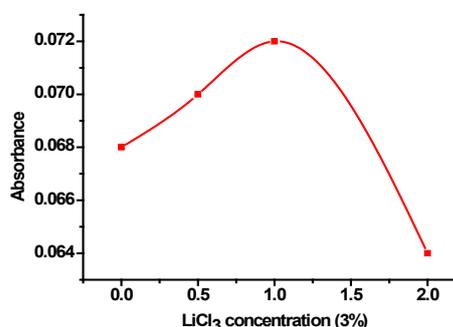


Fig. 3. The effect of releasing reagent on absorbance of sample.

3.1.4. Effect of interferences

In order to study the effect of various ions on determination of Cd and Pb, the solution of Cd 1ppm and Pb 2ppm in HNO₃ 1%, CH₃COONH₄ 1% and LaCl₃ 1% was used with the addition of different amount of foreign ions as mentioned in Table 1.

Table 1. Effect of interference ions on absorbance of sample in optimum conditions

Ion added	Concentration, ppm				
	0	200	300	400	800
K ⁺ (ppm)	0	200	300	400	800
Na ⁺ (ppm)	0	100	200	400	500
Mg ²⁺ (ppm)	0	50	100	150	200
Ca ²⁺ (ppm)	0	50	100	150	200
Ba ²⁺ (ppm)	0	10	20	30	40
Sr ²⁺ (ppm)	0	10	20	30	40
Cu ²⁺ (ppm)	0	20	30	40	50
Ni ²⁺ (ppm)	0.	20	30	40	50
Zn ²⁺ (ppm)	0	20	30	40	50
Mn ²⁺ (ppm)	0	20	30	40	50
Al ³⁺ (ppm)	0	20	60	80	100
Fe ²⁺ (ppm)	0	20	60	80	100
Cr ³⁺ (ppm)	0	2	4	8	10
Abs-Cd	0.3052	0.3056	0.3049	0.3063	0.3060
Abs-Pb	0.0707	0.0700	0.0727	0.0728	0.0708

As seen in Table 1, the foreign ions in survey range concentration did not affect to determination of Cd and Pb. Moreover, in the practical samples, the concentration of these ion is lower than that of the survey. Therefore, it can be concluded that these ions did not affect to measurement. This is a great advantage of atomic absorption spectroscopy as compared to other methods.

3.2. Validation method

3.2.1. Determination of the linear ranges of Cd and Pb

To determine the linear ranges of Cd and Pb, we prepared the Cd and Pb standard solutions in HNO₃ 1%, CH₃COONH₄ 1%, 0.01-4 ppm and 0.01-8 ppm for Cd and Pb, respectively. The obtained absorbance values are presented in Tables 2 and 3.

The standard linear calibration curve of Cd obtained from the standard solutions analysis is presented in Fig.4. It showed a good linear relationship between the absorbance and concentrations of the standard solutions in the range of 0.01-4 ppm. The standard deviation was 0.099 and the linear regression coefficient was 0.995.

For the Pb, the calibration curve is presented in Fig.5. It also showed a good linear relationship between the absorbance and concentrations over the range of 0.05-8 ppm. The standard deviation was

0.047 and linear regression coefficient was 0.994. These results revealed that absorbance is directly proportional to concentration or in other work Lambert-Beer law is valid.

Table 2. Results for the determination of linear concentration range of Cd.

Cd concentration (ppm)	Absorbance			
	Abs1	Abs2	Abs3	Average value
0.01	0.0145	0.0145	0.0146	0.0145
0.025	0.0120	0.0120	0.0120	0.0120
0.1	0.0165	0.0165	0.0166	0.0165
0.25	0.0236	0.0237	0.0237	0.0237
0.5	0.0388	0.0390	0.0389	0.0389
1	0.0669	0.0670	0.0669	0.0669
2	0.1359	0.1359	0.1360	0.1359
3	0.2273	0.2272	0.2273	0.2273
4	0.3008	0.3008	0.309	0.3008

Abs1: first measurement, **Abs2:** second measurement,

Abs3: third measurement.

Table 3. Results for the determination of linear concentration range of Pb.

Cd concentration (ppm)	Absorbance			
	Abs1	Abs2	Abs3	Average value
0.01	0.0298	0.0297	0.0298	0.0298
0.05	0.0356	0.0357	0.0357	0.0357
0.1	0.0395	0.0394	0.0394	0.0394
0.25	0.0476	0.0475	0.0475	0.0475
1	0.0612	0.0613	0.0613	0.0613
2	0.0795	0.0795	0.0794	0.0795
3	0.0962	0.0962	0.0962	0.0962
4	0.1128	0.1127	0.1128	0.1128
6	0.1446	0.1445	0.1447	0.1446
7	0.1565	0.1565	0.1565	0.1565
8	0.1721	0.1722	0.1722	0.1722

Abs1: first measurement, **Abs2:** second measurement,

Abs3: third measurement.

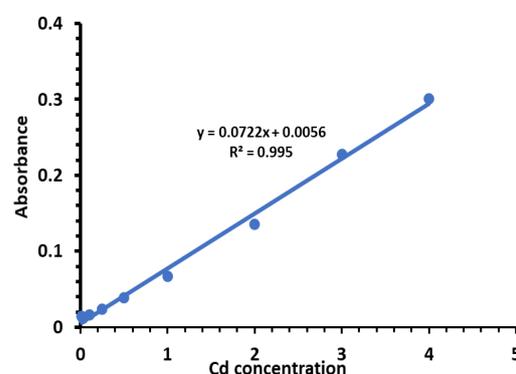


Fig. 4. Calibration curve for standard solution

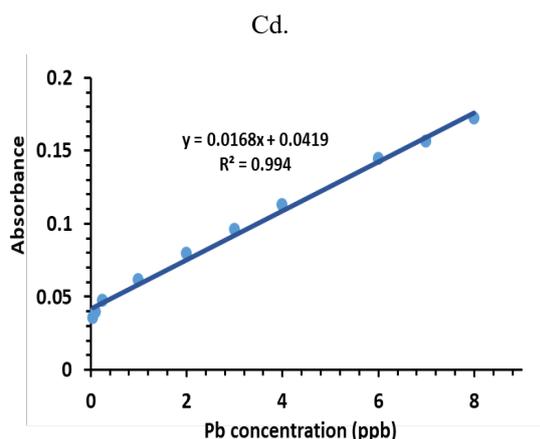


Fig. 5. Calibration curve for standard solution Pb

3.2.2. Determination of LOD and LOQ of Cd and Pb

To determine the LOD and LOQ, we prepared 20 bank samples and then analyzed at optimized condition. The obtained results of absorbance were used to calculate the SD, b (intercept of standard curve), LOD and LOQ by equations 1-5. The results are recorded in Table 4.

Table 4. Statistical values of Cd and Pb analysis method.

Statistical values	Pb	Cd
SD	0.0014	0.0051
b	0.017	0.062
LOD (ppm)	0.0090	0.0092
LOQ (ppm)	0.0300	0.0310

Table 5. Accuracy and repeatability for Cd analysis

Sample	1		2		3	
CCd	0, 025 ppm		1 ppm		2 ppm	
x_t (Abs)	0,0120		0,0669		0,1359	
Test	x_i	\bar{E}_r	x_i	\bar{E}_r	x_i	\bar{E}_r
1	0.0122	1.67	0.0673	0.60	0.1343	1.18
2	0.0118	1.67	0.0658	1.64	0.1315	3.24
3	0.0116	3.33	0.0655	2.09	0.1342	1.25
4	0.0119	0.83	0.0662	1.04	0.1312	3.45
5	0.0117	2.50	0.0665	0.60	0.1345	1.03
6	0.0121	0.83	0.0675	0.90	0.1336	1.69
7	0.0123	2.50	0.0670	0.15	0.1328	2.28
8	0.0115	4.20	0.0662	1.05	0.1332	1.99
9	0.0118	1.67	0.0665	0.60	0.1325	2.50
10	0.0122	1.67	0.0656	1.86	0.1320	2.87
\bar{x} (Abs)	0.0119	0.83	0.0664	0.75	0.1330	2.13
SD	0.00026		0.00069		0.00109	
RSD	2.185		1.0392		0.8595	

Table 6. Accuracy and repeatability for Pb analysis

Sample	1		2		3	
CPb	1 ppm		3 ppm		6 ppm	
x_t (Abs)	0,0613		0,0962		0,1446	
Test	x_i	\bar{E}_r	x_i	\bar{E}_r	x_i	\bar{E}_r
1	0.0602	1.79	0.0958	0.42	0.1422	1.66
2	0.0595	2.94	0.0955	0.73	0.1410	2.48
3	0.0589	3.92	0.0945	1.78	0.1404	2.90
4	0.0592	3.43	0.0948	1.46	0.1427	1.31
5	0.0580	5.38	0.0942	2.01	0.1420	1.80
6	0.0585	4.57	0.0966	0.42	0.1419	1.87
7	0.0597	2.61	0.0939	2.39	0.1425	1.45
8	0.0582	5.06	0.0935	2.81	0.1416	2.07
9	0.0590	3.75	0.0951	1.14	0.1405	2.83
10	0.0596	2.77	0.0954	0.83	0.1412	2.35
\bar{x} (Abs)	0.0590	3.75	0.0949	1.35	0.1416	2.07
SD	0.0007		0.0011		0.0008	
RSD	1.197		1.148		0.567	

Table 7. The recoveries of Cd and Pb

Element	Added standard (ppm)	Analysis value (ppm)	Recovery (%)	
Cd	0.5	0.461	98.16	
		0.482		
		0.513		
		0.476		
		0.522		
Cd	1.0	1.037	98.46	
		0.982		
		0.985		
		0.967		
		0.951		
Pb	1.0	0.984	97.98	
		0.976		
		0.968		
		1.016		
	Pb	2.0	0.955	98.2
			1.947	
			1.895	
Pb	2.0	1.925	98.2	
		1.942		
2.108				

3.2.3 Accuracy and repeatability of method

To evaluate the accuracy of the measurement, we prepare the standard samples with concentration in linear range of Cd and Pb. Each sample was measured and repeated in ten times, the E_r , SD, and RSD were calculated by equation 1, 2 and 3. The results are presented in Table 5 and Table 6. It was showed that the standard deviation (SD) and relative standard deviation (RSD) of the Cd and Pb measurements are small within the permissible limitations (7.3-15%)

according to AOAC. These mean that the F-AAS method is a stable, good repeatability, high precision, and fully consistent to the determination of trace metal in the vegetable samples.

The repeatability of method was also evaluate by recovery. It was determined from added standard sample at the addition of 0.5 and 1.0 ppm for Cd and 1.0 and 2.0 ppm for Pb. The recovery was presented in Table 7. It can be seen in the Table 7, the recoveries were 98.16 and 98.46% for Cd at concentrations of 0.5 and 1.0 ppm, respectively, these values were 97.98 and 98.2% for Pb at concentrations of 1.0 and 2.0 ppm, respectively. These values were compatible with AOAC.

3.2.4. Analysis of the real samples

Table 8. The results of analysis of Cd in vegetable samples.

No.	Vegetable sample	Concentration from calibration curve (ppm)	Concentration in vegetable samples (mg/kg)	Limit of QCVN 8-3:2012/BYT
1	Moringa oleifera	0.0347	0.1735	0.2 ppm
2	Cabbage	0.0300	0.1503	
3	Vegetables shrinkage	0.0338	0.1688	
4	Ceylon spinach	0.0359	0.1797	
5	vegetable chrysanthemum	0.0348	0.1740	
6	Watercress	0.0365	0.1827	
7	Tomato	0.0384	0.1922	
8	Choy sum	0.0340	0.1698	
9	Spinach	0.0370	0.1850	
10	Water spinach	0.0373	0.1863	

The 10 vegetable samples collected from Lam Thao, Phu Tho province were treated by procedure in section 2.2 and then analyzed by F-AAS (NOVAA350) at optimized conditions. The results are presented in Tables 8 and 9. The results showed that the Cd and Pb content in the samples was low. The Cd content in 10 vegetable sample was in range 0.1503-0.1922 ppm and it was 0.2433-0.2841 ppm for Pb content. And, both of metals were in allowed range as followed the national technical regulation on the limits of heavy metals contamination in food (QCVN 8-3:2012/BYT).

Table 9. The results of analysis of Pb in vegetable samples.

No.	Vegetable sample	Concentration from calibration curve (ppm)	Concentration in vegetable samples (mg/kg)	Limit of QCVN 8-3:2012/BYT
1	Moringa oleifera	0.0518	0.2588	0.3 ppm
2	Cabbage	0.0487	0.2433	
3	Vegetables shrinkage	0.0545	0.2723	
4	Ceylon spinach	0.0533	0.2663	
5	vegetable chrysanthemum	0.0529	0.2645	
6	Watercress	0.0536	0.2680	
7	Tomato	0.0568	0.2841	
8	Choy sum	0.0496	0.2480	
9	Spinach	0.0495	0.2473	
10	Water spinach	0.0536	0.2680	

4. Conclusion

The optimum conditions for determination of Cd and Pb contents in some green vegetable samples in Lam Thao district, Phu Tho Province, Vietnam by F-AAS were investigated and it was indicated following: The acid HNO₃ concentration was 1%, substrate solution was CH₃COONH₄ with the concentration of 1%, releasing reagent was LaCl₃ with the concentration of 1%. At this condition, the analysis method had high accuracy and repeatability with the a tolerance was less than $\pm 5\%$. The foreign ions such as Na⁺, Ca²⁺, Cu²⁺, Al³⁺, etc. did not affect to measurement. In addition, 10 vegetable samples were analyzed, Cd and Pb were detected in all samples. However, the Cd and Pb contents in these samples were in allowed range as followed the national technical regulation on the limits of heavy metals contamination in food (QCVN 8-3:2012/BYT).

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