

Determination of Cadmium in Root Vegetables after Activated Carbon Enrichment by Flame Atomic Absorption Spectrometry

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Summary: Enrichment of trace levels of cadmium in vegetable materials by flame atomic absorption spectrometry has been carried out using anthranilic acid on activated carbon at pH 7. Up to hundred fold enrichment has been achieved by this reagent. Interferences due to matrix components are minimised by their addition at higher level to the standards and blank. Calibration curve is linear in 1-7 ppb range of cadmium. The relative standard deviation for 7 ppb of cadmium is found to be 3.5%. The observed cadmium level in root vegetables is 0.09-0.20 ppm.

Introduction

Soil-plant-man in a given eco-system is critical pathway for toxic elements such as cadmium and lead. Monitoring the concentration of trace elements in vegetables is very important in food and agriculture studies. Water, soil, sewage-sludge and fertilisers are amongst the sources of toxic metal introduction into the food chain [1]. Cadmium accumulates in liver and kidneys and it can cause irreversible damage to the later. Absorption of cadmium in kidney is a function of age and daily intake in food, drink and partly smoking.

Techniques such as spectrophotometry, electrothermal or flame atomic absorption

spectrometry (Et-AAS, FAAS), inductively coupled plasma - atomic emission spectrometry (ICP-AES) have been used for the determination of trace amounts of cadmium in environmental and biological matrixes. Et-AAS is a fast and sensitive technique for the determination of cadmium in biological and environmental samples [2-4].

The enrichment step in the analytical methodology enhances the sensitivity of FAAS for trace metal analyses. A number of pre-concentration methods such as precipitation and co-precipitation with 8-hydroxyquinoline and Mg^{2+} [5], co-precipitation with zirconium hydroxide [6], on-

line[7], flow injection [8], extraction with crown ethers [9], use of ion exchange resins [10,11], and activated carbon [12-18] have been applied for the trace metal enrichment, wherein the trace metals have been determined by atomic absorption spectrometry.

Use of metal complexing reagents such as cupferron, 8-hydroxyquinoline and dithizone for nanogram level determination of vanadium, lead and cadmium after activated carbon enrichment/pre-concentration has increased the enrichment factor by many fold [16-18]. We have recently reported the use of anthranilic acid as complexing reagent on the activated carbon for the determination of lead in root vegetables by FAAS [19]. Cadmium and lead are known to form a 1:2 complex with anthranilic acid [20].

The present study explores the application and usefulness of this reagent for complexation of cadmium. The complex is enriched by adsorption on activated carbon for the FAAS determination of metal in root vegetables i.e., beet, carrot, potato, radish, sweet potato and turnip.

Results and Discussion

After preparing vegetables samples from dried powders by ashing and dissolving in HNO_3 , they were subjected to cadmium analysis by FAAS using standard instrumental conditions. The resultant analytical signals were too weak to be measured. Thus the enrichment procedure was considered essential prior to FAAS analysis.

The effects of parameters such as pH, amount of activated carbon and complexing reagent, contact time and matrix components which are considered to be important in the determination of cadmium in root vegetables by activated carbon enrichment, were studied and optimised. The effect of each parameter was repeated three times on duplicate set of solutions to ensure the reliability.

Effect of pH

To investigate this effect on the enrichment of cadmium using anthranilic acid and activated carbon, a duplicate set of 500 ml of 7 ppb cadmium solution containing 2ml of matrix components and 4ml anthranilic acid was taken and enrichment procedure was applied. Each solution was maintained at a specific pH using 70 ml of appropriate buffer (slowly

added with mixing). After enrichment, resulting solutions were analysed for cadmium by FAAS using instrumental conditions given in experimental. Duplicate blanks were also treated as above. After blank correction, absorbance of cadmium was plotted as a function of pH (Fig.1). It is clear from the figure that the maximum absorbance is obtained in the pH range 6-8. Thus pH 7 was selected as an optimum for further studies.

Effect of the Amount of Activated Carbon

Different volumes (5-25 ml) of activated carbon suspension in water (25 mg/ml) were added to a duplicate set of cadmium solutions (500 ml, 7ppb) each containing 2 ml matrix components and 4ml anthranilic acid. pH 7 was maintained using 70 ml of phosphate buffer. After enrichment, measurement by FAAS and blank correction, absorbance of cadmium was plotted against the concentration of activated carbon. Figure 2 suggests that 375 mg of activated carbon is sufficient for complete adsorption of cadmium taken above as anthranilate complex. Further addition of activated carbon does not have significant effect on the absorbance of cadmium by FAAS.

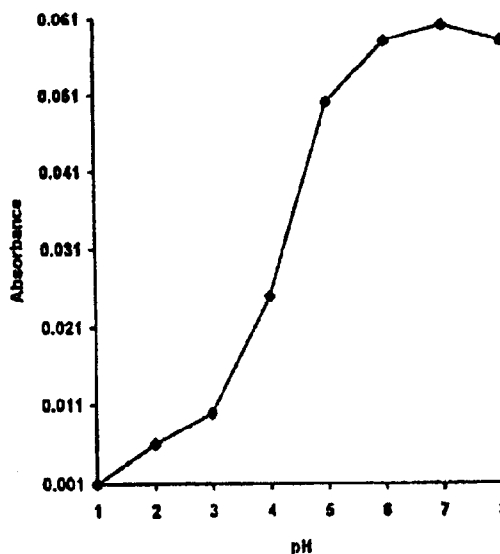


Fig. 1: Effect of pH on the absorbance of cadmium as anthranilate complex; concentration of Cd, 7 ppb (500 ml).

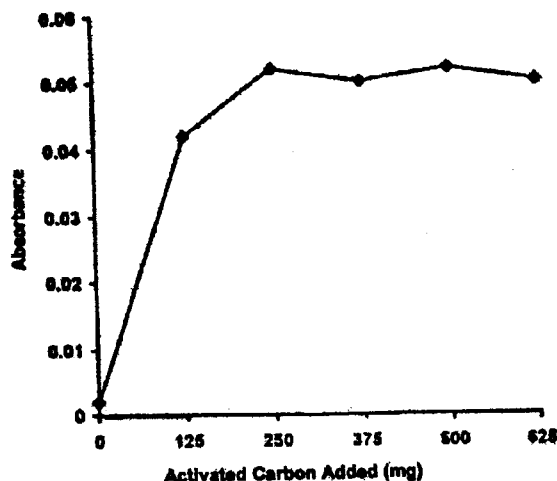


Fig. 2: Determination of optimum amount of activated carbon using 500 ml of 78 ppb cadmium solution plus matrix components.

Effect of Concentration of Anthranilic Acid

With Cd^{2+} ions anthranilic acid forms complex as $\text{Cd}(\text{C}_7\text{H}_6\text{O}_2\text{N})_2$ [20]. To study the amount of anthranilic acid to complex cadmium ions, different volumes (1-7.5 ml) of anthranilic acid solution (40 mg/ml) in methanol were used keeping other contents constant in the enrichment procedure. Absorbance of cadmium versus concentration of anthranilic acid plot (fig. 3) shows that 3 ml containing 120 mg of anthranilic acid is sufficient for complete complexation of cadmium.

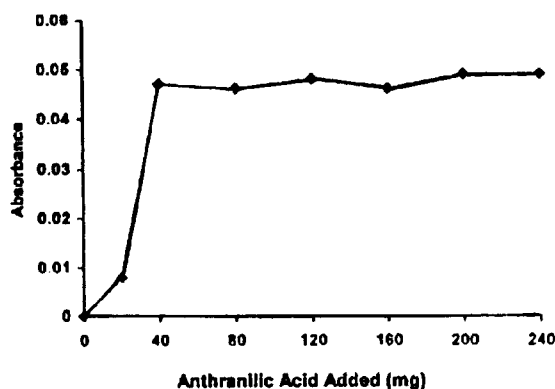


Fig. 3: Determination of optimum amount of anthranilic acid using 500 ml of 7 ppb cadmium solution plus matrix components.

Contact Time

The rate of adsorption of cadmium anthranilate on the activated carbon was investigated by taking 500 ml of cadmium solution (7 ppb) containing 2ml matrix components, 375 mg activated carbon, 120 mg anthranilic acid maintained at pH 7 and subjected to enrichment procedure. Variable contact timings (15, 30, 45, 60 & 90 minutes) were used in the stirring step. After blank correction absorbance of cadmium by FAAS was plotted against the contact time (Fig.4). It is clear from the shape of the curve that the absorbance approaches maximum value and becomes steady during 15-30 minutes stirring. However, a 30 minutes stirring time was chosen as optimum.

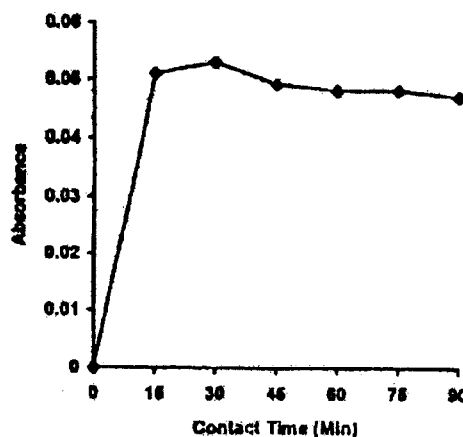


Fig. 4: Determination of optimum contact time for 500 ml of 7 ppb cadmium solution plus matrix components.

Calibration, Precision and Accuracy

Calibration graph was obtained by applying enrichment procedure and measurement by FAAS on a duplicate set of 500 ml of blank, 1-7 ppb of cadmium solutions, each containing 2ml of matrix components at maximum level using optimised experimental conditions of pH, concentration of reagents and contact time.

Calibration curve was linear in 1-7 ppb range with a correlation coefficient of 0.9996. Standard deviation (S.D.) for the measurement of cadmium concentration of 7 ppb was calculated using optimised experimental conditions and RSDs for 7 ppb cadmium ($n = 8$) was found to be 3.5%. The pre-concentration step gives a 100-fold enrichment factor

Table: 1 Cadmium contents in fresh root vegetables (dried mass basis) by the proposed method.

Sample	Proposed Method			Literature Method[17]
	Cd added ppm	Cd Found ppm	Recovery %	Cd Found ppm
Beet	0	Mean ± S.D. 0.14±0.02	-	Mean ± S.D. 0.15±0.02
	0.10	0.25±0.02	110	
Carrot	0	0.13±0.02	-	0.12±0.02
	0.10	0.23±0.02	100	
Potato	0	0.09±0.02	-	0.10±0.02
	0.10	0.20±0.02	110	
Radish	0	0.10±0.02	-	0.09±0.02
	0.10	0.19±0.02	90	
Sweet Potato	0	0.09±0.02	-	0.11±0.02
	0.10	0.19±0.02	100	
Turnip	0	0.20±0.02	-	0.19±0.02
	0.10	0.30±0.02	100	

for the determination of cadmium in the root vegetables under study. As the standard solution used contained matrix components, therefore, the method is said to be accurate. For validation of sample preparation, enrichment procedure and extraction, dried vegetable samples in duplicate were fortified with a known amount of cadmium, put through all steps and the resultant clear solutions were analysed by FAAS. The data is given in table 1. The recoveries of cadmium in vegetable samples are found to be 90-110%.

The measured level of cadmium in 500ml of reagent blank was 0.7 ppb with a standard deviation of 0.2 ppb. The detection limit, defined as three times the S.D. of blank value, 0.6 ppb is achieved. Thus the proposed method can be applied to nanogram level of cadmium in biological matrixes per gram of dried vegetable.

Application

The developed method was applied to root vegetables for the determination of cadmium. The vegetables include beet, carrot, potato, radish, sweet potato and turnip. The samples were also analysed by reported method [17] for vegetables. The analytical results are listed in table.1. A good agreement can be seen within permitted limits. Their lead contents have already been reported by us [19].

The results of cadmium analysis are calculated on dry mass basis (ppb) after blank subtraction. Each value is an average of at least six independent determinations of the same sample at different times and/or different samples. The observed level of cadmium in root vegetables (table 1) in the present study is 0.09-0.20 ppb. It is also observed that potato and sweet potato contain the lowest and turnip the highest level of cadmium amongst the vegetables analysed. Reported concentration of cadmium in vegetable samples ranges from 0.15-1.02 µg/g [17]. British prescribed concentration of value for cadmium is 5µg/l in drinking water [21]. The permitted cadmium levels in European and WHO food standards are 23±3 ppm [22] and 25±5 ppm [23] respectively for normal body weights. Thus the cadmium contents in vegetable studied in this work are well below these reported values.

Experimental

Apparatus

A Hitachi Model A-1800 atomic absorption spectrometer equipped with standard atomiser burner and air-acetylene flame was used for all FAAS measurements. The operated instrumental conditions used were : Wavelength 228.8nm, slit width 1.3 nm, lamp current 7.5 mA, air flow rate 9.5 l/min, acetylene flow rate 2.3 l/min and burner height 7.5 mm.

pH measurements were made on Corning Model-125 pH meter. Magnetic stirrer Model KHS-2 Kayagki (Tokyo) and centrifuge Model YJ03-043-4000 (China) were used for enrichment of cadmium. For drying purpose oven Model Memmert D-911226 (Germany) was employed. Vegetables were ashed using muffle furnace Model RJM-1.8-10A (China).

Reagents

Deionized water (obtained from Pak-Arab Fertiliser, Multan, Pakistan) was used throughout. All the filtrations were carried out using Toyo filter paper No.1. Chemicals used were Analar grade from Merck unless otherwise stated. Stock standard solution of cadmium (1000 ppm) was prepared by dissolving 2.74 g of Cd(NO₃)₂·4H₂O per litre in 0.1 M HNO₃. Working standard solutions were always freshly prepared by appropriate dilution of stock solution with 0.1 M HNO₃.

4% anthranilic acid solution was prepared by dissolving 4 g of the reagent per 100 ml in methanol.

Buffers

Buffer solutions of pH 1-3 were prepared from 0.1 M glycine and 0.1 M HCl solution, pH 4-5 from 0.1 M sodium acetate and 0.1 M acetic acid and pH 6-8 from 0.066 M disodium hydrogen phosphate and 0.066 M potassium dihydrogen phosphate.

Purification of Activated Carbon

Purification of activated carbon was achieved by soaking it in concentrated HCl (37%, sp.g.1.19) for three hours, washing with water and drying at 105°C. It was then treated with 3+1/HCl + HNO₃ (65%, sp.g.1.4) over night, filtered (Toyo filter paper No.1), washed with water, dried at 110°C and stored in an air tight glass container. It was used as 25 mg/ml suspension in water.

Vegetables Samples

Collection and Storage

Six types of fresh root vegetables: Beet, carrot, potato, radish, sweet potato and turnip were purchased from local city market (more than one places). They were thoroughly washed, cut into small pieces, weighed, spread on stainless steel dishes and placed in a preheated oven at 80°C. It took 2-3 days to get complete drying of vegetables. They were ground to fine powder in agate pestle-mortar, weighed and stored in tightly stoppered glass bottles. Percent loss in weight was calculated from wet and dry mass weights of samples. Loss in weight was as follows: beet (85%), carrot (91%), potato (79%), radish (93%), sweet potato (73%) and turnip (92%). These dried powders were used in preparing samples for analysis by FAAS.

Preparation of Sample for Analysis

Accurately weighed amount (about 20-30g) of dried vegetable was taken in a china dish and heated on flame burner till smoke from the sample stopped. Transferred carefully into a porcelain crucible and ashed in furnace at 550°C for four hours. A whitish residue was obtained.

The residue was carefully dissolved in 25-35 ml Conc. HNO₃ (heating is required) and resulted solution was diluted with water and filtered. The

filtrate and washings were collected and volume was made up to one litre with water.

Matrix Components

Aluminium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium and zinc are reported as matrix components in vegetables [17]. These elements were analysed by FAAS. The observed upper limits of the matrix elements in ppms (on dried mass basis) are: Cr, Co, Ni = 4; Cu = 8; Mn = 16; Zn = 32; Al = 200; Fe = 400; Mg = 2000; Ca = 5000; Na = 10,000; K = 20,000. Interferences due to these components were minimized by their addition at the highest levels in blank and standard solutions and during standardisation of cadmium. It gave good matrix matching.

Enrichment Procedure

Working standard solutions in the range of 1-10 ppb cadmium were prepared after appropriate dilution of stock standard solution. 500ml of each working standard cadmium solution was taken and 2 ml of matrix components solution was added. Addition of matrix components to standards was necessary for the matrix matching with vegetable samples. pH of the solution was adjusted to 7 by adding 70 ml of buffer solution (0.066M KH₂PO₄/0.066M Na₂HPO₄) and anthranilic acid 3 ml and 15 ml of activated carbon suspension was added and mixed. pH of the solution was readjusted, if required. Mixture was then stirred for 30 minutes and filtered. The filtrate was discarded and the residue was dried at 105°C for one hour. It was then transferred to a glass beaker, treated with 10 ml conc. HNO₃ and evaporated to dryness. To the resultant residue 5ml of 2M HNO₃ was added, mixed thoroughly, centrifuged twice (at 2000 rpm) and the clear solution was separated for FAAS measurement. Final volume of the clear solution was made up to mark using 2 M HNO₃ in 5ml volumetric measuring flask. The cadmium contents of the solution were determined by FAAS. Blanks were also enriched in the same way.

The above enrichment procedure was applied for the optimisation of analytical conditions stated in results and discussion by taking 500ml of 7 ppb cadmium solution to which 2ml of matrix components solution was added. Vegetable samples

were similarly enriched without the addition of matrix components solution.

Conclusion:

An FAAS method has been developed for the determination of trace levels of cadmium in fresh root vegetable using anthranilic acid as a complexing reagent after pre-concentration on activated carbon. The reagent has been previously reported by us for the determination of lead in root vegetable materials using activated carbon enrichment [19] and gives reliable results for cadmium as that of cupferron and/or oxine reagents [17]. The addition of matrix components at upper level to all standard solutions and blanks minimises the interference from these components. The observed cadmium concentration in studied vegetables (dried mass basis) is very low, i.e. 0.09-0.20 ppm. The analytical results obtained can be used to correlate the concentration of cadmium to the sources of toxic metal introduction i.e. water, soil [8,14], etc.

The observed working pH range for lead determination using anthranilic acid and activated carbon is 4.5-8 [19] and that of cadmium 6-8 [this work]. Thus simultaneous determination of these metals can be accomplished using pH 7 in the enrichment step.

References

1. T. P. Coutate, "Food, the Chemistry of its Components", 2nd edn; Royal Soc. of Chemistry, Cambridge, pp 265 (1992)
2. C. D. Stalikas, G. A. Pilidis and M. I. Karayannis, *J. Anal. At. Spectrom.*, **11**, 595 (1996).
3. F. Dolinsek, J. Stupar, V. Vrscaj, *J. Anal. At. Spectrom.*, **6**, 653 (1991).
4. K. Ohta, W. Aoki and J. Mizuno, *Microchim. Acta*, **1**, 81 (1990).
5. Z. S. Chen, M. Hiraide and H. Kawaguchi, *Mikrochim. Acta*, **124**, 27 (1996).
6. S. S. Brown and Y. Kodama, Eds., "Toxicology of Metals, Clinical & Experimental Research", Ellis Horwood, pp 185 (1987)
7. A. Karakaya and A. Taylor, *J. Anal. At. Spectrom.*, **4**, 261 (1989).
8. S. R. Bysouth, J. E. Tyson and P. B. Stockwell, *Anal. Chim. Acta*, **214**, 329 (1988).
9. N. Mateeva, S. Arpadjan, T. Deligeorgiev and M. Mitewa, *Analyst*, **117**, 1599 (1992).
10. V. K. Jain, S. S. Sait, P. Shrivastav and Y. K. Agrawal, *Talanta*, **45**, 397 (1997).
11. M. Soylak, L. Elcl and M. Dogan, *Anal. Letters*, **26**, 1997 (1993).
12. H. Berndt, E. Jackwert and U. Kimura, *Anal. Chim. Acta*, **93**, 45 (1977).
13. S. Gucer and M. Demir, *Anal. Chim. Acta*, **196**, 277 (1987).
14. P. Ramadevi and G. R. K. Naidu, *Analyst*, **115**, 1469 (1990).
15. M. Demir, S. Gucer and T. Essen, *J. Agric. Food Chem.*, **38**, 726 (1990).
16. M. Yaman and S. Gucer, *Fresenius J. Anal. Chem.*, **350**, 504 (1994).
17. M. Yaman and S. Gucer, *Analyst*, **120**, 101 (1995).
18. Y. P. Depena, M. Gallego and M. Valcarcel, *J. Anal. At. Spectrom.*, **9**, 691 (1994).
19. S. B. Niazi, A. A. Gill & M. Y. Khokhar, *J. Chem. Soc. Pak.* **21**, 386 (1999).
20. J. Bassett, R. C. Denny, G. H. Jeffery and J. Mendham, "Vogel's Textbook of Quantitative Inorganic Analysis", 4th ed., ELBS & Longman. (1978)
21. *British Water Quality Act, Sec. 20(5)(b)*, Department of Environment (UK) (1989).
22. European Food Standards (1998).
23. WHO Permitted Levels of Metals in Foods, Standards Bulletin (1998).