# Validation Methods of Analysis Heavy Metals Level Lead (Pb) and Cadmium (Cd) with Oxidator Variations by Atomic Absorption Spectrofotometry In Herbal Medicine

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**Abstract**. The use of herbal medicines in Indonesia has been going on for thousands of years, before modern medicines were discovered and marketed. Traditional medicine is an ingredient or ingredients derived from plants, animals, minerals, sarian (galenic) preparations or mixtures of these materials, which for generations have been used for treatment based on experience. Indonesian traditional medicine or native Indonesian medicine, better known as herbal medicine, is generally a mixture of herbal medicines, which are medicines derived from plants. Plant parts that are used can be in the form of roots, stems, leaves, tubers or maybe all parts of the plant. In connection with the source and quality of raw materials for herbal medicines, effectiveness and safety are important roles in controlling the quality of herbal medicine formulations. Thick extracts or extracts derived from natural ingredients before being processed into herbal products must also meet the applicable quality requirements, such as lead (Pb) and cadmium (Cd) contamination. Lead (Pb) and cadmium (Cd) heavy metal contamination can occur in herbal medicinal products. The analytical method used to determine levels of lead and cadmium contamination must be validated. This study aims to validate the analysis method the levels of lead and cadmium heavy metal contamination in herbal medicine preparations in the City of Tasikmalaya. Sample preparation uses the wet destruction method using a variety of HNO3: H2O2 (3: 1) oxidizers, HNO3: H2SO4 (3: 1) and HNO3: HCl (3: 1). After obtaining the optimum destructive tools and processes, an analysis method was validated for the determination of levels of lead and cadmium contamination with Atomic Absorption Spectrophotometry (AAS) which included linearity, detection limits, quantification limits, precision and accuracy.

## 1. Introduction

In the last decade amidst the many types of modern medicine on the market and the emergence of new types of modern medicine, there is a global tendency to return to nature. Factors that encourage people to utilize natural medicines or herbal medicines include the high price of modern / synthetic drugs and the many side effects. In addition, promotion factors through the mass media also play a role in increasing the use of herbal medicines. Therefore herbal medicines are becoming increasingly popular and their use is increasing not only in developing countries like Indonesia, but also in developed countries such as Germany and the United States. Types of herbal medicines that are used can be homemade traditional medicine, herbal medicine as well as traditional industrial medicine factory. [1]

Regarding the source and quality of raw materials for herbal medicines, effectiveness and safety are important roles in controlling the quality of herbal medicine formulations. The World Healthy Organization (WHO) has made several resolutions using several analytical techniques to ensure quality control of plants. Metal contamination has been found in many herbal medicines due to unhygienic storage, packaging conditions or raw materials. High metal contamination can occur due to environmental effects that are damaged, namely from soil, water and air. For this reason, WHO is increasing efforts to eliminate heavy metal contamination in herbal medicines so that quality assurance is maintained [2]. Heavy metals can have effects on human health depending on the part of the body that is bound to metal. Heavy metals that are toxic in the body will endanger health and even cause death [3]. In accordance with the Regulation of the Drug and Food Control Agency No. 13 of 2014 the limit of heavy metal contamination in herbal medicines is 0.3 mg / Kg for cadmium (Cd) and 10 mg / Kg for lead (Pb) [4]. Previous research has been conducted by Zulharmita, et al., 2017 analysis of heavy metal contamination (Pb and Cd) in herbal medicine preparations at the Siti Rahmah Islamic Hospital in Padang by atomic absorption spectrophotometry. The sample consisted of 4 types of herbal medicines. The results of this research show that the four samples do not exceed the threshold limit of heavy metal contamination determined by the Indonesian Drug and Food Control Agency No. 13 of 2014. Based on this, this study aims to validate the method of analysis and test levels of lead heavy metal contamination (Pb ) and cadmium (Cd) with a variety of oxidizing spectrophotometry atoms in the preparation of herbal medicines that are sold in the City of Tasikmalaya.

## 2. Experimental and Method

### *2.1. Instruments*

The tools used Atomic Absorption Spectrophotometry, hollow cathode lamp for lead metals, destruction flask, heating mantle, 35 mL SPD cap vial, 1.0 mL, 2.0 mL, 5.0 mL, 10.0 mL volume pipette, 50.0 mL and 100.0 mL measured flask, and Mettler Toledo Dragon 204 analytical scale.

### *2.2. Material*

The ingredients are Pb Standard solution 1000 mg / L (Merck), nitric acid 65% (Merck), hydrogen peroxide 30% (Merck), hydrocloride acid (Merck), sulfuric acid (Merck), aquabidest, herbal medicine sample obtained in Tasikmalaya area.

### *2.3. Experiments*

Development of analytical methods for determining lead and cadmium in herbal preparations for preparation of tools, materials and sample preparation. The first step is optimizing the Atomic Absorption Spectrophotometer (SAA). After the optimum tool condition is obtained, the next step is to optimize the wet digestion process using a destructive device which includes the use of various reactants as destructors. After obtaining optimum digestion tools and processes with oxidizing variations, a calibration curve is made, followed by validation of the analysis method of lead and cadmium contamination with SAA which includes linearity, detection limits, quantification limits, precision and accuracy. The stages in the linearity test include the creation of an intermediate and working standard solution for making a calibration curve. The detection limit and the quantization limit of the tool are determined statistically from the linear regression equation obtained from the calibration curve. Precision and accuracy are carried out by adding standard Pb and Cd to the sample. At this stage begins with the preparation of samples of herbal preparations in wet destruction with Pb and Cd levels to be known. Sample preparation of herbal medicines using the wet destruction method using a mixture of nitric acid 65% and oxidizing variation (3: 1). Destruction is done with 65% nitric acid as much as 15 ml added to the destruction flask and while heated at a temperature of approximately 100oC. This process is carried out until the loss of brown smoke. After that the solution was added with variations of oxidizers (sulfuric acid, 30% hydrogen peroxide, and hydrochloric acid) as much as 5 ml little by little while heating at a temperature of approximately 100oC. The destruction process is stopped until the solution is clear which indicates that the destruction process has been completed. After the digestion

process is complete, the solution is allowed to stand until it cools down, then the solution is put into a 50 ml volumetric flask and add the aquabidest to the mark of the flask, then the solution is homogenized. Then filtered using filter paper and put in a vial. Determination of the most optimal oxidizer seen from the results of the best validation calculation. Destruction of samples was carried out twice. The results of the destruction were measured by Atomic Absorption Spectrophotometry at wavelength 217.0 nm for Pb and 228.8 nm for Cd.

## 3. Result and Discussion

Before the sample is analyzed with AAS, the sample must be destructed. Destruction is a way of treating samples to break down organic substances into simpler forms and dissolve metal analytes into cations so that measurements can be made. Destruction method in sample preparation before being measured with AAS can be done by the method of dry destruction (dry ashing) and wet digestion method (wet digesiton). In the wet destruction method, the decomposition of the sample is carried out using strong acids both single and mixed. Strong acids that can be used to destroy samples are nitric acid (HNO3), sulfuric acid (H2SO4), percholic acid (HClO4), and hydrochloric acid (HCl) which can be used singly or in mixture. The perfection of destruction is characterized by obtaining a clear solution after the destruction is complete, which shows that all the existing constituents have completely dissolved or the decomposition of organic compounds has gone well. After the AAS conditions and destruction have been optimum, it can be continued to verify methods including linearity, detection limits, quantity limits, accuracy and precision. Determination of the linearity is done by plotting the instrument's response expressed by the measured absorbance / absorbance value, with the concentration of a standard metal solution Pb and Cd consisting of 5 levels of concentration.

**Table 1**. Linear Regression Parameters of Lead and Cadmium Calibration Curves

| Parameter | Pb | Cd |
|---|---|---|
| Regression equation | y = 0,0125x + 0,0014 | y = 0,1237x + 0,0247 |
| Slope (b) | 0,0125 | 0,1237 |
| Intersect with the y-axis line (a) | 0,0014 | 0,0247 |
| X average (ppm) | 3,67 | 3,67 |
| Sy/x (residual standard) | 0,0010075 | 0,0260404 |
| (Sy/x)/b | 0,0805978 | 0,2105122 |
| $V_{x0}$ /coefficient of variance regression (%) | 2,20 | 5,74 |
| r (correlation coefficient) | 0,9998 | 0,9982 |
| LOD/Limit of Detection (ppm) | 0,24 | 0,63 |
| LOQ/Limit of Quantity (ppm) | 0,81 | 2,11 |

From the above table it can be seen that the linear Pb metal regression parameters in the concentration range of 1.0 - 8.0 ppm all show good results with the regression line equation y = 0.0125x + 0.0014 and the correlation coefficient r = 0.9998. The detection limit (LOD) and the quantization limit (LOQ) are calculated statistically from the calibration curve of 0.24 ppm and 0.80 ppm. While from the table above it can be seen that the linear regression parameters of Cd metals in the concentration range of 1.0 - 8.0 ppm all show good results with the regression line equation y = 0.1237x + 0.0247 and the correlation coefficient r = 0.9982. The detection limit (LOD) and the quantization limit (LOQ) are calculated statistically from a calibration curve of 0.63 ppm and 2.11 ppm.

**Table 2**. Lead (Pb) and Cadmium (Cd) Precision In Herbal preparation

| Day- | Concentration Pb (µg/g) | Concentration Cd (µg/g) |
|---|---|---|
| 1 | 54,4 | 47,00 |
| 2 | 54,3 | 46,60 |
| 3 | 54,4 | 46,20 |

| | | |
|---|---|---|
| Average | 54,4 | 46,60 |
| SD | 0,11 | 0,69 |
| RSD(%) | 0,20 | 0,22 |
| CV Horwitz | 24,64 | 19,01 |
| 0,67 CV Horwitz | 16,43 | 12,67 |
| HORRAT | 0,01 | 0,02 |

From the table above obtained RSD (Relative Standard Deviation) 0.2% for Pb and 0.22% for Cd. Thus the results for precision parameters meet the requirements between day and day which is less more tha 2% HORRAT.

Accuracy tests are carried out by the standard addition method, by adding standards with known concentrations into the herbal preparation and the following accuracy test results are obtained.

**Table 3**. Accuracy of Lead (Pb) in herbal preparation

| No | Analyte Concentration without spike (µg/g) | Analyte Concentration with spike (µg/g) | Obtained Analyte Concentration (µg/g) | Added Analyte Concentration (µg/g) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 134,4 | 186,4 | 52 | 50 | 104 |
| 2 | 134,4 | 186,4 | 52 | 50 | 104 |
| 3 | 134,4 | 182,4 | 48 | 50 | 96 |
| 4 | 134,4 | 230,4 | 96 | 100 | 96 |
| 5 | 134,4 | 234,4 | 100 | 100 | 100 |
| 6 | 134,4 | 238,4 | 104 | 100 | 104 |
| 7 | 134,4 | 410,4 | 276 | 250 | 110 |
| 8 | 134,4 | 394,4 | 260 | 250 | 104 |
| 9 | 134,4 | 402,4 | 268 | 250 | 107 |
| Average of Recovery (%) | | | | | 102,78 |

**Table 4**. Accuracy of Cadmium (Cd) in herbal preparation

| No | Analyte Concentration without spike (µg/g) | Analyte Concentration with spike (µg/g) | Obtained Analyte Concentration (µg/g) | Added Analyte Concentration (µg/g) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 9,42 | 59,94 | 50,52 | 50 | 101,04 |
| 2 | 9,42 | 59,94 | 50,52 | 50 | 101,04 |
| 3 | 9,42 | 60,35 | 50,92 | 50 | 101,84 |
| 4 | 9,42 | 114,11 | 104,68 | 100 | 104,68 |
| 5 | 9,42 | 114,91 | 105,49 | 100 | 105,49 |
| 6 | 9,42 | 114,11 | 104,68 | 100 | 104,68 |
| 7 | 9,42 | 264,40 | 255,05 | 250 | 102,02 |
| 8 | 9,42 | 265,68 | 256,26 | 250 | 102,51 |
| 9 | 9,42 | 266,09 | 256,66 | 250 | 102,67 |
| Average of Recovery (%) | | | | | 102,89 |

Accuracy of Pb and Cd metals is expressed as percent of recovery calculated by recovery calculation. From the table above, Pb and Cd recovered in herbal preparation are between 96-110% and 101.04 - 105.49%, respectively. This fulfills the acceptance requirements for accuracy in the range 80-120%

## 4. Conclusion

The results of the validation of the analysis method show the following results with variation oxidator:

- Pb metal linear calibration curves in the concentration range of 1-8 ppm with linear regression equation is y = 0.0125x + 0.0014 with a correlation coefficient r = 0.9998. Cd metals in the concentration range of 1-8 ppm obtained linear regression equations y = 0.1237x + 0.0247, the value of the correlation coefficient = 0.9982
- The limit of detection (LOD) and limit of quantitation (LOQ) were 0.242 ppm and 0.806 ppm for Pb and 0.631 ppm and 2.105 ppm for Cd
- The precision is relative standard deviation 0.20% for Pb and 0.22% for Cd.
- The recovery and accuracy for Pb is 96-110% and for Cd is 101.64 - 105.49%.

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