Method Development and Validation for Lead (Pb) Analysis in Natural Honey from East Kalimantan

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Submission date: 31-Dec-2021 09:15AM (UTC+0700)
Submission ID: 1736562350
File name: Article_Green_Chemistry_Proceeding_Update.pdf (608.99K)
Word count: 2629
Character count: 14490

ISBN 978-602-285-049-6

Method Development and Validation for Lead (Pb) Analysis in Natural Honey from East Kalimantan

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Abstract

Analysis method for determining Pb content in natural honey have been developed and validated. Analysis were carried out on one natural honey sample collected from Teririp (Balikpapan) with standard analysis method and three developed analysis methods and their analytical performance were compared. Pb content was determined with atomic absorption spectrometry after dry digestion (method A) or wet digestion (method B (HNO_3-H_2O_2;3:8), C (HNO_3-H_2O_2;2:1) and D (HNO_3)). Analytical performance parameter, such as linearity, working range, limit of detection, limit of quantification, precision and accuracy were tested. Content of Pb in natural honey sample which analysed with method A, B, C and D were found at 0.7923 ± 0.0311 μg/g, 0.7944 ± 0.0216 μg/g, 0.7998 ± 0.0218 μg/g and 0.7912 ± 0.0160 μg/g respectively. gAnalytical performance of four tested analytical method were still acceptable. Significance testing result showed that there were no significance differences in precision and accuracy between developed analysis method and standard analysis method, except for method D. Precision of method D was significantly better than standard analysis method.

Keywords : Honey; Lead; Method Validation

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Introduction

East Kalimantan Province, with land area covered 11% of Indonesia total land area, has vast forest region which covered 70% of its land. Honey is one of main commodity non-woods from forestry. East Kalimantan highest honey production dates back in 1996/1997 was 50.89 tons and keep declining until only 25.60 tons in 2008, either harvested from wild honey or beekeeping (BP2HP, 2010). Honey is natural liquid mostly has sweet flavour produced by honey bees from floral nectar or extra floral nectar or insects excretion (BSN, 2004).

Honey mostly used as food source, beside that honey bees and honey could also applied as indicator species of environmental quality. Honey bees are good biological indicators because they reveal the chemical impairment of the environment they live in through two signals: one is more evident, that is high mortality (in the case of pesticides), while the other is less so, consisting of residues present within their bodies or in beehive products (in the case of other contaminants like heavy metals and radionuclides) that may be detected by means of suitable laboratory analyses (Porrini et al., 2003). Have been reported that lead contamination to environment could be monitored with honey bees and its honey (Fakhimzadeh and Lodenius, 2000; Devillers and Pham-Delegue, 2002). Figure 1 shows interaction between honey bees and environment (Porrini et al., 2003).

Lead is fifth most used metal after iron, copper, aluminium and zinc (Enghag, 2004). Lead exposure could enter human body through breathing and swallowing (ATSDR, 2007). Inside the body, Lead has great impact, from hypertension and anemia until severely damage the nervous system, immunity system and reproduction, kidneys, and even resulting death (Radojevic and Bashkin, 1999; Enghag, 2004; ATSDR, 2007).

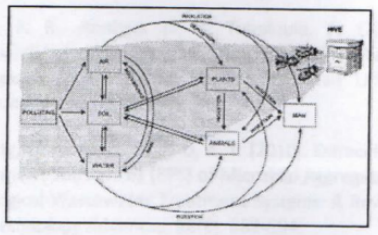

Figure 1. Honey bee interaction with its environment (Porrini et al., 2003)

Application of indicator species, such as honey, can be used to estimate how much lead species have direct impact to living things in certain ecosystem (Naidu, 2008). Analysis of lead content in honey was based on

SNI 01-2896-1998, for honey quality test, involving time consuming dry digestion procedure. Determination of toxicant content in environment for routine analysis must fulfill certain requirements such as highly specific, low detection limit, fast, but still economic (Funk et al., 2007). Dry digestion procedure in SNI 01-2896-1998 makes this method needs longer time and more costly because this method originally developed for food quality control not for environmental analysis.

This research was performed to developing lead analysis method in natural honey by using wet digestion method and validating the method to assure developed analysis method quality method not differ significantly with standard analysis method, therefore, this method could be used for environmental analysis purposes.

Methodology

Materials

The samples for this research were natural honey collected from beekeepers at Teritip, Balikpapan, East Kalimantan. The samples were stored in glass tube and cool place until determination process. 1000μg.mL^{-1} lead stock solution was made from $PbNO_3$ salt from Merck. All other reagents, such as HNO_3 65%, H_2O_2 30%, Glucose, $Mg(NO_3)_2$ and ethanol were reagent grade from Merck. Determination process was carried out by using Flame-AAS Shimadzu AA-6200 spectrometer.

Methods

Establishing External Calibration Curve

In this research, the effect of matrices correction addition for external calibration qualities was investigated. Calibration curve was made by dilution method 5.0; 10.0; 15.0; 20.0; 25.0; 30.0; 35.0; 40.0; 45.0 and 50.0μL of 1000μg.mL^{-1} lead stock solution were pipetted into 10mL volumetric flask, then glucose solution equivalent to 1.2g glucose were added into it and diluted with HNO_3 5% until volume. Therefore standard series with monosaccharide matrices correction achieved. Standard series absorbance were measured with Atomic Absorption Spectrophotometer at λ=283.3nm, then calibration curve established between standard concentration (μg.mL^{-1}) at x axis and standard absorbance at y axis. For calibration without matrices correction, no glucose were added.

Honey Samples Preparation

Preparation with dry digestion (Method A) (SNI 01-2896-1998)

1.5g honey samples were mixed with 1.5 mL $Mg(NO_3)_2$ 10% (ethanol solution). Ethanol in mixture were evaporated, then mixture were charred. Char were transferred into furnace and heated at 450°C for 16h. The produced ashes were mixed with 5mL HNO_3 5% and heated until dissolved. Solutin transferred to 10mL volumetric flask, added with 10μL of 1000μL.mL^{-1} lead stock solution and diluted with HNO_3 5% until volume.

Preparation with wet digestion using HNO_3-H_2O_2 (Method B) (AOAC Official Method 997.15)

1.5g honey samples were mixed with 0.75mL conc. HNO_3 then slowly heated from 30 until 95 °C for 30 minutes. Heating continued until yellowish smoke disappear. Let it cool and added with 1.0mL conc. H_2O_2 then heated again at 95 oC for 5 minutes. Let it cool again and added with 1.0mL conc. H_2O_2 then heated again at 95 °C for 5-10 minutes until solution became clear. Solution transferred to 10mL volumetric flask, added with 10mL of 1000μL.mL^{-1} lead stock solution and diluted with HNO_3 5% until volume.

Preparation with wet digestion using HNO_3-H_2O_2 (Method C) (Ouyang et al., 2010)

1.5g honey samples were mixed with 1.5mL conc. HNO3 and 0.75mL conc. H_2O_2 then stored for 12h. Solution were heated until yellowish smoke formed then let it cool. Solution transferred to 10mL volumetric flask, added with 10μL of 1000μL.mL^{-1} lead stock solution and diluted with HNO_3 5% until volume.

Preparation with wet digestion using HNO_3 (Method D) (Birge and Price, 2001; Bogdanov et al., 2007)

1.5g honey samples were mixed with 1.0mL conc. HNO_3 then heated until yellowish smoke disappear and solution became clear. Solution transferred to 10mL volumetric flask, added with 10μL of 1000μL.mL^{-1} lead stock solution and diluted with HNO_3 5% until volume.

Determination of Lead

Absorbance of samples solution were measured by Atomic Absorption Spectrophotometer at λ= 283,3nm. Lead concentration in sample solution calculated from calibration curve. The actual lead content in honey calculated after dilution factor correction.

Analytical Performance Determination of Test Methods

Linearity and working range

Linearity and working range of analytical methods were done by establishing external calibration standard with (n=10) and without (n=9) matrices correction. Linearity and working range of calibration

determined by residual analysis, correlation coefficient calculation and homogeneity test.

Limit of detection and limit of quantification

Limit of detection and limit of quantitation analysis method determined by measuring absorbance of blank samples (n=7) with 4 analysis method. Limit of detection defined as mean of blank content ± 3sd and limit of quantitation defined as mean of blank content ± 10sd.

Precision test

Precision of four analysis methods determined as repeatability and intermediate-precision. Repeatability defined as %CV value from 5 measurements of same honey sample in same day. Intermediate-precision defined as %CV value from 4 measurements of same honey sample in different days.

Accuracy Test

Accuracy of four analysis methods determined as recovery percentage of standard addition. Standard solution of lead which equal to 5.0µg. 10.0µg and 20.0µg lead were added to same honey sample. Then, sample determined with same procedures.

Result and Discussion

Lead Content in the Samples

Analysis result of lead content in honey samples with 4 different analysis method shown in **Table** 1 and visualized in **Figure** 2. Based on significance tsting between methods with t test, there was no significant difference of average analysis result from 4 analysis methods. Base on f test, there was no significant difference of analysis result variance between method A (standard method), method B and method C. There was significant difference of analysis result variance between method A (standard method) and method D, but method D has significantly better precision than method A because method D has smaller variance value than method A.

Table 1. Analysis result of lead content with 4 different analysis methods

Method	Lead Content in Honey Samples(µg/g)									$\bar{x}$	%CV
	Repeatability					Intermediate-Precision					
A	0.8161	0.7630	0.8389	0.7112	0.8169	0.8143	0.7934	0.7582	0.8186	0.7923	5.11
B	0.8366	0.8186	0.7906	0.8024	0.7470	0.8020	0.8121	0.7635	0.7773	0.7944	3.54
C	0.8172	0.8006	0.8193	0.8167	0.8121	0.8103	0.8189	0.7599	0.7432	0.7998	3.53
D	0.7920	0.8004	0.8001	0.7599	0.7990	0.8115	0.8114	0.7534	0.7930	0.7912	2.63

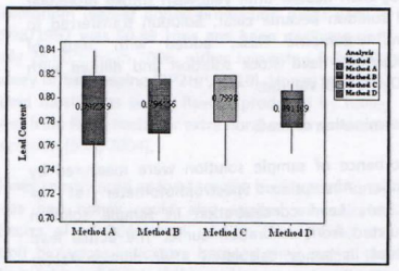

Figure 2. Final result comparison between methods

Analytical Performance of Test Methods

Linearity and Working Range

There was no distinctive difference between 2 calibration method, therefore, both can be used (figure 3). However, calibration without matrices correction has better residual correlation coefficient and homogeneity than calibration with matrices correction, therefore, only calibration without matrices correction was used for calculation in this research. High content of organic compound in calibration with matrices correction caused blockage at burner head and gave higher residual value at 3.0000-5.0000µg/mL standard concentration.

Limit of Detection and Limit of Quantitation

Limit of detection of method A, B, C and D were 0.1128µg/mL, 0.1074µg/mL, 0.1767µg/mL and 0.1310µg/mL respectively. Comparison of 4 analysis methods shown in figure 4. Method B has lowest limit of detection and method C has highest limit of detection. Limit of quantitation of method A, B, C and D were 0.2883µg/mL, 0.1909µg/mL, 0.4721µg/mL and 0.2026µg/mL respectively. Comparison of 4 analysis methods shown in Figure 5. Method B has lowest limit of quantitation and method C has highest limit of quantitation.

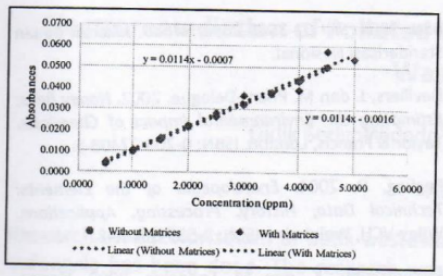

Figure 3. Comparison of calibration curve

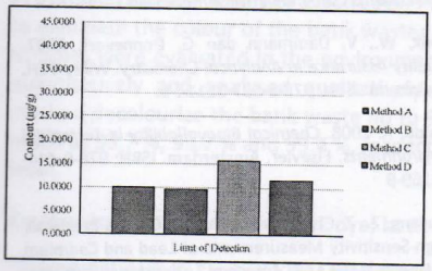

Figure 4. Comparison of limit of detection

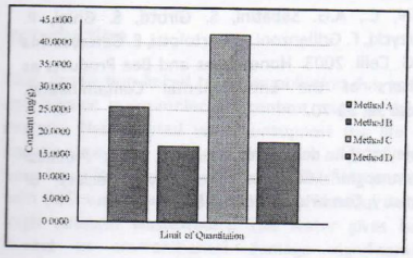

Figure 5. Comparison of limit of quantitation

Precision Test

Repeatability of method A, B, C and D, defined as %CV, were 6.56%, 4.24%, 0.92% and 2.19% respectively. Method C has best repeatability and method A has worst repeatability. Intermediate-precision of method A, B, C and D, defined as %CV, were 3.03%, 2.52%, 4.43% and 3.00% respectively. Method B has best intermediate-precision and method C has worst intermediate-precision. Precision of all methods could be classified as good because still far below %CV Horwitz value (±16%). Comparison of repeatability and intermediate for 4 methods shown in **Figure 6**.

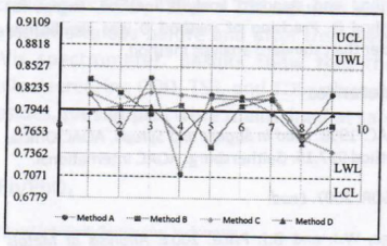

Figure 6. Control chart of analysis result

Accuracy Test

Average accuracy of method A, B, C and D, defined as percent of standard addition recovery, were 99.79% (99.44 – 100.13%), 99.77% (99.55 – 100.01%), 99.93% (99.41 – 100.48%) and 99.65% (98.41 – 101.90%) respectively. Method C has best accuracy (closest value to 100%) and method D has worst accuracy. Precision of all methods could be classified as good because all recovery result still within range 80 – 110%. Comparison of standard addition recovery for 4 methods shown in **Figure 7**.

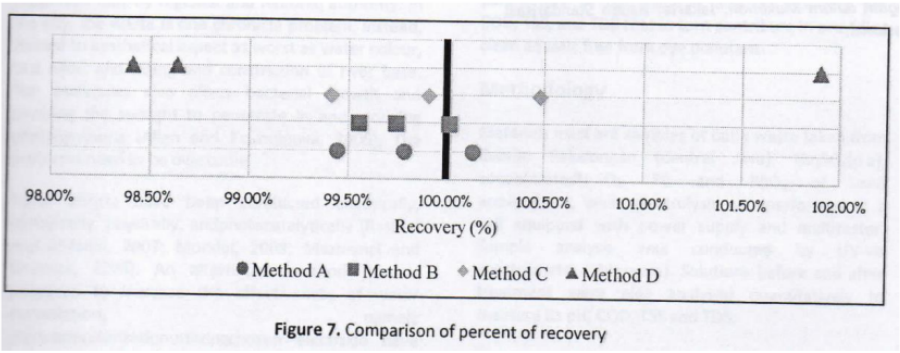

Figure 7. Comparison of percent of recovery

Conclusion

The 4 tested analysis method can be used to analyse lead content in natural honey sample. Analytical performance parameter, such as linearity, working range, limit of detection, limit of quantitation, precision and accuracy are still acceptable based on current regulation. Lead content in natural honey sample which analysed with method A, B, C and D were found at 0.7923 ± 0.0311μg/g, 0.7944 ± 0.0216μg/g, 0.7998 ± 0.0218μg/g and 0.7912 ± 0.0160μg/g respectively. Significance testing result showed that there were no significant difference s in precision and accuracy between developed analysis method and standard analysis method, except for method D. Precision of method D was significantly better than standard analysis method.

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