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Research Article

A method validation and analysis of lead content in lipstick products sold in e-commerce using atomic absorption spectrophotometer (AAS)

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ABSTRACT

Lipstick is the most widely cosmetic that used to beautify the appearance of the face. Lipstick that safe to use has an amount of lead metal less than 20 ppm. This study aims to ensure that the analytical method used has been validated, to determine the metal content of lead in lipsticks that sold in e-commerce, to determine the average metal content of lead using an Atomic Absorption Spectrophotometer (AAS) and to determine the safety of the lipstick. The methods used to validation are determine linearity, accuracy, precision, LoD, and LoQ. Samples were obtained from e -commerce with criteria that had price less than IDR 17,500, had sales history at least 10,000 units and had shipments from within the country. Sample preparation was carried out by wet digestion method, then analyzed qualitatively by tube test and analyzed quantitatively by measuring its absorption using AAS. The results obtained from this study are the linearity of validation parameter is 0.9959, the percent accuracy is entirely in the range 80-110%, the percent precision is less than 11% and the LoD and LoQ numbers are 0.630 ppm and 1.908 ppm. The average levels of lead contained in lipsticks that sold in e-commerce for brands A to F are 33.285±0.918 ppm; 33.576±0.918 ppm; 35.029±0.918 ppm; 38.372±1.836 ppm; 39.390±0.918 ppm; and 32.994±0.918 ppm. Lipstick brands A, B, C, D, E, and F contain more than 20 ppm lead metal so they are not safe for daily use.

1. INTRODUCTION

Lipstick is a cosmetic that used to give color and an artistic touch to the lips so that it can improve aesthetics in facial makeup (Adliani, Nazliniwaty & Purba, 2012). This reason makes lipstick one of the most popular cosmetic products for most women. Currently, it is very easy to get these lipsticks, which can be searched by online. The development of technology can ease Indonesian people to get information and insight about products and goods needed in everyday life (Saimima, 2013). There are so many e-commerce that used to trade daily necessities such as: Shopee, Lazada, Tokopedia, and others (Suleman, 2018).

Lipstick that sold in e-commerce cannot be guaranteed safety because it is not known what ingredients are contained in the product. Based on the provisions regulated by Badan Pengawas Obat dan Makanan (2011), safe lipstick does not contain harmful contaminants such as lead in excess of 20 ppm. Lead metal is often added to cosmetics to give better pigmentation to the lips and make lipstick last longer. The red pigment in lipstick is

obtained from lead tetraoxide (Pb_3O_4) compound. In the human's body, excess lead will become a neurotoxin that has been proven to cause low IQ levels and cause behavioral problems such as aggressiveness. Therefore, it is necessary to conduct research to determine the amount of lead metal in lipsticks that sold in e-commerce so it can be determined whether the lipstick can used in everyday life.

Several previous studies suggest that the heavy metal lead contained in some cosmetic products include eyebrow pencils which are sold in the shopping center of Surabaya, lipsticks that are sold in Kiaracondong Market, and lipsticks which are sold in Pasar Raya Padang City. However, there is a weakness from previous studies, namely that sampling is carried out only in certain areas. To correct these shortcomings, this research was conducted by taking samples from a wide area, namely online shopping places. The criteria for the sample to be taken are the lipsticks that are most in demand by e-commerce users and have been sold a lot.

Validation of analytical methods is the act of determining parameters based on laboratory experiments to prove that these parameters meet the requirements for their use. Validation is critical to reliably unite the strength, purity, quality and potency of the finished product. Sometimes it is necessary to transfer metrology from one laboratory to another. This activity allows the use of a method by many people in different laboratories and on different instruments, allowing for greater reductions in reproducibility. These problems can be predicted and avoided by validating analytical methods (Shrivastava & Gupta, 2011).

In the manufacture of lipstick, there are many ingredients that are used to analyze the presence of lead metal that needs to be carried out in research using selective analytical methods. In addition, the method used must also be sensitive because the number of samples used is not large, so it requires a method that is very sensitive to slight changes in a concentration analysis. Based on this thought, the method that is suitable to be used to analyze lead metal in lipstick samples is the Atomic Absorption Spectrophotometry (AAS) method. This method is suitable because it has high performance, prefers metal content, is relatively simple to perform, and has little interference.

2. MATERIALS AND METHODS

Materials and Tools

The materials used in this study were lipstick samples with various brands to be tested, HNO_3 65% pa, HCl 37% pa, $Pb(NO_3)_2$ standard solution, KI, NaOH, HCl, NH_4OH, aquadestilata, and aquabidestilata.

The tools used in this study were Shimadzu Atomic Absorption Spectrophotometer (AAS) type AA-6880F, fume hood, analytical balance, hot plate, micro pipette, Whatman No. 42 filter paper, as well as laboratory glassware.

Methods

Validation of Analysis Method

1000 ppm $Pb(NO_3)_2$ was put into a 10 mL volumetric flask as much as 100 µL then added with aquabidestilata to the mark so that a concentration of 10 ppm was obtained. The 10 ppm $Pb(NO_3)_2$ stock solution obtained was taken 1 mL; 2 mL; 3 mL; 4 mL; 5 mL; and 6 mL and put into a 10 mL volumetric flask and then added with aquabidestilata to the limit mark and then measure the absorption using an AAS at wavelength 283.3 nm. From the linear regression equation, the linearity, LoD, and LoQ parameters can be determined. To determine accuracy and precision, so add 3 mL; 4 mL; and 5 mL of 10 ppm $Pb(NO_3)_2$ stock solution into a 10 mL volumetric flask and add aquabidestilata to the limit. Then the absorption was measured at wavelength 283.3 nm and calculate the percent accuracy and precision.

Sampling

Samples were obtained from Shopee, Lazada, and Tokopedia with criteria had a price of less than IDR 17,500, had sales history at least 10,000 units, had shipments from within the country and had the same color series much in demand by the public.

Sample Preparation by Wet Destruction

Sample preparation by wet digestion method with a mixture of nitric acid solution (HNO_3 65%) and

hydrochloric acid (HCl 37%) in a ratio of 1:3 or called aquaregia solution. Samples (±2 grams) were weighed in a beaker glass, then added aquaregia solution. Digest with the stove until the brown smoke disappears and the digestion is stopped when a clear solution is obtained. The sample solution that has been digested is then put into a 10 ml volumetric flask and then added with aquabides to the mark and homogenized, then filtered with whatman no. 42 filter paper and put into the volumetric flask.

Qualitative Analysis of Lead Metal Content

The solution that obtained from the destruction of samples A, B, C, D, E, and F was put into four test tubes, then added with 0.1 N NaOH solution; HCl 0.1 N solution; NH4OH 6 N solution; and KI solution.

Determination of Lead Metal Content in Samples

The $Pb(NO_3)_2$ with a concentration of 10 ppm was made in series by taking a solution of 1 mL; 5 mL; 6 mL; 7 mL; 8 mL; and 9 mL which was put into a 10 mL volumetric flask and then diluted with aquabidestilata to the mark. Then the absorption was measured using an AAS at a wavelength of 283.3 nm. After that, it was continued by measuring the absorption of the sample solution from the destruction of samples A, B, C, D, E, and F at the same wavelength to measure the levels of lead contained therein.

3. RESULTS AND DISCUSSIONS

Validation

Linearity

The results obtained in the linearity test are presented in **Table 1**. This linearity test produces a relation coefficient (r) of 0.9959 which means that the standard curve measured is linear because r > 0.99 which is in accordance with what is recommended for a good analytical method (Miller & Miller, 2018). From the standard curve obtained, the standard curve equation y = 0.0027371(x) + 0.00052 which shows a slope (b) of 0.0027371 and an intercept (a) of 0.00052. The graphic of linearity test are presented in **Figure 1**. This linear standard curve shows a correlation which states that there is a proportional relationship between concentration (x) and absorbance (y). This means that the standard curve equation can be used to determine the value or sample content (Windaryati, Pranjono & Banawa, 2013).

Accuracy

AOAC (Association of Official Analytical Chemist) sets a good percent accuracy for a unit concentration of 1 ppm is 80-110% (González & Herrador, 2007). The accuracy test was carried out with a minimum of three concentration levels with three replications. The results obtained in the accuracy test are presented in **Table 2**.

In this study, the accuracy obtained was 98.81%; 102.12%; and 98.01% at the three concentration levels. All percent accuracy is in the range of 80-110%, so it can be said that this AAS method has good validity for the test of lead heavy metal.

Precision

AOAC (Association of Official Analytical Chemist) sets a good percent precision for a unit concentration of 1 ppm which is less than 11% (González & Herrador, 2007). The results obtained in the precision test are presented in **Table 3**.

The precision test was carried out on the same sample as the accuracy test. In this study, the precision obtained was 8.39%, 3.23% and 9.58% at the three concentration levels. All percent precision is less than 11%, it can be said that the AAS method has good validity for the test of lead heavy metal.

Table 1. Standard curve for validation

Concentration (ppm)	Absorbance
1	0.0026
2	0.0065
3	0.0092
4	0.0116
5	0.0138
6	0.0169

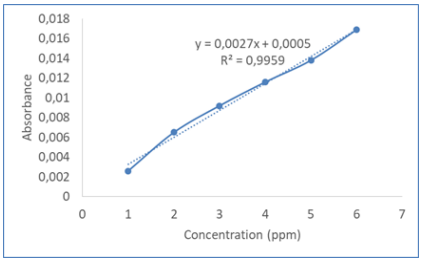

Figure 1. Graph of standard curve equation for validation

Table 2. Accuracy parameter validation data

C theory (ppm)	Absorbance	C realized (ppm)	C Average (ppm)	Accuracy (%)
3	0.0081	2.77	2.96	98.81
	0.0084	2.88		
	0.0094	3.24		
4	0.0120	4.19	4.09	102.12
	0.0118	4.12		
	0.0113	3.94		
5	0.0154	5.44	4.90	98.01
	0.0134	4.71		
	0.0130	4.56		

Table 3. Precision parameter validation data

C theory (ppm)	Absorbance	C realized (ppm)	C Average (ppm)	SD	Precision (%)
3	0.0081	2.77	2.96	0.249	8.39
	0.0084	2.88			
	0.0094	3.24			
4	0.0120	4.19	4.09	0.132	3.23
	0.0118	4.12			
	0.0113	3.94			
5	0.0154	5.44	4.90	0.469	9.58
	0.0134	4.71			
	0.0130	4.56			

Table 4. Sentivity parameter validation data

C (ppm)	Absorbance	y'	y-y'	$(y-y')^2$
1	0.0026	0.003257	-0.0006571	0.00000043178
2	0.0065	0.005994	0.0005058	0.00000025583
3	0.0092	0.008731	0.0004687	0.00000021968
4	0.0116	0.011468	0.0001316	0.00000001732
5	0.0138	0.014206	-0.0004055	0.00000016443
6	0.0169	0.016943	-0.0000426	0.00000000182
Amount				**0.00000109086**

Sensitivity (Limit of Detection and Limit of Quantitation)

The results obtained in the sensitivity test are presented in **Table 4**.

$$Sb = \sqrt{(\sum(y-y')^2/n-2} = (0.00000109086/4) = 0.000522221 \quad (1)$$

$$LoD = 3.3 \times (Sb/slope) = 3.3 \times (0.000522221 \times 0.0027371 = 0.63 \text{ ppm} \quad (2)$$

$$LoQ = 10 \times (Sb/slope) = 10 \times (0.000522221 \times 0.0027371 = 1.908 \text{ ppm} \quad (3)$$

Based on these results, the LoD and LoQ values are 0.63 ppm and 1.91 ppm respectively. The LoD results show the sensitivity of the method as indicated by the LoD value that falls within the optimum working range of the Flame Atomic Absorption Spectrometry tool, which is 0.01-2 ppm (Agilent Technologies, 2017).

Qualitative Analysis

The samples obtained from the destruction were analyzed qualitatively and quantitatively. In the qualitative analysis, a test tube was carried out with the results as presented in **Table 5**.

From the data, it was stated that only samples C, D, E and F gave rise to a white precipitate when 0.1 N NaOH reagent that added. This indicated means that the samples contained lead metal by the reaction:

$$Pb^{2+} + 2OH^- \rightarrow Pb(OH)_2 \downarrow \text{ (white precipitate)}$$

On added 0.1 N HCl, all samples gave rise to a white precipitate. This indicated means that the samples contained lead metal by the reaction:

$$Pb^{2+} + 2Cl^- \rightarrow Pb(Cl)_2 \downarrow \text{ (white precipitate)}$$

Table 5. Data from qualitative analysis

Sample	Absorbance	+ NaOH	+ HCl	+ NH_4OH	+ KI	Presence of lead metal
A1	0,0003	-	+	-	-	Yes
A2	0,0004	-	+	-	-	Yes
B1	0,0004	-	+	-	-	Yes
B2	0,0005	-	+	-	-	Yes
C1	0,0009	+	+	+	-	Yes
C2	0,0010	+	+	+	-	Yes
D1	0,0020	+	+	+	-	Yes
D2	0,0022	+	+	+	-	Yes
E1	0,0024	+	+	+	+	Yes
E2	0,0025	+	+	+	+	Yes
F1	0,0002	+	+	-	-	Yes
F2	0,0003	+	+	-	-	Yes

Table 6. Standard curve data for quantitative analysis

Concentration (ppm)	Absorbance
1	0,0026
5	0,0798
6	0,0964
7	0,1074
8	0,1253
9	0,1410

Table 7. Data from quantitative analysis of samples A to F

Sample	Absorbance	C (ppm)	C Average (ppm)	SD	RSD (%)	C ± LE (ppm)
A1	0.0003	33.14	33.29	0.206	0.62	33.29±0.92
A2	0.0004	33.43				
B1	0.0004	33.43	33.58	0.206	0.61	33.58±0.92
B2	0.0005	33.72				
C1	0.0009	34.88	35.03	0.206	0.59	35.03±0.92
C2	0.0010	35.17				
D1	0.0020	38.08	38.37	0.411	1.07	38.37±1.84
D2	0.0022	38.66				
E1	0.0024	39.24	39.39	0.206	0.52	39.39±0.92
E3	0.0025	39.53				
F1	0.0002	32.85	32.99	0.206	0.62	32.99±0.92
F2	0.0003	33.14				

On added 6 N NH_4OH, only samples C, D and E gave rise to a white precipitate. This indicated means that the samples contained lead metal by the reaction:

$$Pb^{2+} + 2NH_3 + 2H_2O \rightarrow Pb(OH)^2 \downarrow + 2NH^{4+} \text{ (white precipitate)}$$

On added KI solution only sample E gave rise to a yellow precipitate. This indicated means that the samples contained lead metal by the reaction:

$$Pb^{2+} + 2^{I-} \rightarrow Pb(I)_2 \downarrow \text{ (yellow precipitate)}$$

According to Maria (2009), about the principle of AAS instumentation, if light with a resonant wavelength was passed through a flame containing the atoms that suitable, then some of the light will be absorbed and the amount of absorption will be directly proportional to the number of atoms in the ground state in a flame. From the absorbance data that obtained, all samples have an absorption of lead cathode lamp with a wavelength of 283.3 nm, it showed that all samples contain lead metal. However, in the test tube some samples showed negative results. This is because the amount of lead metal in the samples is very small so it cannot be detected by conventional methods such as in the test tube.

Quantitative analysis

The concentration of samples that obtained from the destruction were measured using Atomic Absorption Spectrophotometry at a wavelength of 283.3 nm. Windaryati et al., (2013), stated that calibration curves should always be carried out at all times when performing sample analysis. The measurement of the sample content in

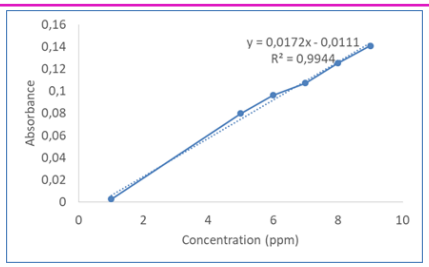

Figure 2. Graph of standard curve equation for quantitative analysis

this study was carried out at a different time from the implementation of the method validation so standard curve was made again with the same procedure. The standard curve data for the quantitative analysis of the samples that obtained were shown in **Table 6**. **Figure 2** shows the shape of the standard curve obtained, the standard curve equation y = 0.0172x - 0.0111 which shows the slope (b) of 0.0172 and the intercept (a) of -0.0111.

Table 7 shows the average concentrations measured in samples A, B, C, D, E and F respectively at 33.29 ppm; 33.58 ppm; 35.03 ppm; 38.37 ppm; 39.39 ppm; and 32.99 ppm. Lipstick brands A to F that studied did not safety for daily use because the lead content in them exceeded the provisions set by BPOM, namely < 20 ppm. Percentage precision of samples A, B, C, D, E and F respectively 0.62%; 0.61%; 0.59%; 1.07%; 0.52%; and 0.62%. Based on the references used, namely (González & Herrador, 2007), AOAC determined a good percent precision for a unit concentration of 10 ppm is less than 7.3% so it can be concluded that the measurement results of these six samples have good analytical procedures accuracy.

4. CONCLUSIONS

Based on the results of the study that linearity of validation parameter is 0,9959, the percent accuracy is entirely in the range 80-110%, the percent precision is less than 11% and the LoD and LoQ numbers are 0.630 ppm and 1.908 ppm so that the analytical method used has been validated. All of the samples are contained of lead metal. The average levels of lead contained in lipsticks that sold in e-commerce for brands A to F are 33,285±0,918 ppm; 33,576±0,918 ppm; 35,029±0,918 ppm; 38,372±1,836 ppm; 39,390±0,918 ppm; and 32,994±0,918 ppm. Lipstick brands A, B, C, D, E and F contain more than 20 ppm lead metal so they are not safe for daily use.

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