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# OPTIMIZATION OF KIELDAHL DIGESTION PROCEDURE FOR DETERMINATION OF MERCURY IN LIPSTICK BY BOX-HUNTER DESIGN

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**ABSTRACT**

*This article describes the application of response surface methodology to the development of a procedure for mercury determination by cold vapor atomic absorption spectroscopy (CV-AAS) in lipstick samples after digestion by Kieldahl method. A Box-Hunter matrix was used to find optimal conditions for the procedure through a response surface study. Three variables "time, temperature, volume of $HNO_3$ acid" were regarded as factors in the optimization study. In optimized condition, the linearity range was 0.2 ÷ 15 µg/L while the limit of detection (LOD) and limit of quantification (LOQ) were 0.17 and 0.57 ppb, respectively. This method presented good precision (RSD ≤ 8.2%) and good recovery (81%-109%). This method was applied to determine mercury in seven lipstick samples and the highest mercury content was 0.229 ± 0.016 mg/kg.*

***Keywords:*** mercury, lipstick, CV-AAS, Box-Hunter design.

## 1. Introduction

Most of the cosmetic products used by both male and female has been shown to contain various types of heavy metals in different proportions (Ahmed et al., 2017). Lipsticks are usually available as compressed powders, anhydrous creams, emulsions, gel, ingots, which are frequently used by women of all ages. Lipsticks contain particles of heavy metals especially Hg as impurities in the pigments, added during the manufacturing process or released by the metallic devices (fake products). Mercury is a toxic element because of its high volatility, mobility and ability to accumulate in various organisms. It causes serious health problems, for examples, allergic reactions, skin irritation or neurotoxic manifestations, cardiovascular, immune and reproductive systems (Ababneh & Al-Momani, 2018; Ahmed et al., 2017; Moyer, Nixon, & Ash, 1999).

Lipstick is becoming more popular with many women of all ages. long-term exposure to mercury accumulation due to frequenly using of lipstrick is higher than that of other cosmetic products on skin. Therefore, determination of the mercury content in the lipstick is extremely necessary. Many studies have been made to determine mercury in cosmetics. Agorku (2016) and his colleagues used the CV-AAS to determine the amount of

mercury in 62 skin toning creams and cosmetic soaps. Mean concentration of total mercury were 0.098 ± 0.082 and 0.152 ± 0.126 μg/g, respectively. The levels of mercury and hydroquinone in these analyzed products were below acceptable limit of 1.0 μg/g according to the US Food and Drug Administration. In the study of Fuad A. Ababneh and Idrees F. Al-Momani (2018), who investigated 112 cosmetics in Jordan; Hg was least-frequently detected, presented in 29/112 (25.9%) and at >3 ppm in 15/112 (13.4%) products. The highest proportion of Hg was observed in skin lightening creams (mean concentration 1.008 ppm). Heavy metals were found in cosmetic products of different countries markets in Khyber Pakhtunkhwa, Pakistan, Caribbean, Banja Luka (Jelić, Antunović, & Đermanović, 2017; Mohammed, Mohammed, & Bascombe, 2017; Ullah et al., 2017). In Vietnam, some authors have identified banned substances in cosmetics. Lê Thị Hương Hoa (2013) determined organic substances as tretinoin, hydroquinone, hydrocortisone acetate, dexamethasone, betamethasone, betamethasone, 17-valerate, triamcinolone, coloring and heavy metals (arsenic, cadmium, lead and mercury). However, most of the authors did not use experiment design to optimize the procedure for determination mercury in lipstick samples.

The main aim of this study was to propose the procedure for the determination of Hg in lipsticks by CV-AAS method after using Box-Hunter design to archive optimized condition.

## 2. Experimental

### *2.1. Instrumentation*

Atomic absorption spectroscopy was performed by AA Spectrometer iCE 3000 series (Thermo Fisher Scientific) instrument with VP100 mercury/hydride system. This system is a manual accessory for high-sensitivity determination of mercury and metal elements by hydride generation, graphite furnace or flame atomic absorption (AA) spectroscopy.

### *2.2. Reagents and standards*

Analytical grade nitric acid (65%, Merck, Germany) and perchloric acid (70%, Merck, Germany) were used for sample preparation. Calibration standards for mercury were prepared each day from the certified standard stock solution (1000 ppm-Merck KgaA, Germany) in the range from 0.2 to 15 ppb. All the solutions were prepared in deionized double-distilled water.

### *2.3. Sample preparation*

Lipstick samples were purchased between winter 2016 and January 2017 from local markets and shops in Tan Binh, Go Vap, district 5 (labelled from M1 to M7). All samples were homogenized and kept in a clean, dry container and stored at room temperature until analysis.

Mercury was extracted from lipsticks with $HNO_3$ and $HClO_4$ solution (to degrade the organic and inorganic matter). 500 milligrams of each sample was weighted in an analytical balance and transferred to the glass vessels. A solution of 10 mL 65% $HNO_3$ was added to each vessel, shaken and then added 10 mL 70% $HClO_4$. It was boiled in Kjeldahl digestion system on fume hood until no more brown fume was observed. After cooling samples, they were added 5 mL 25g/L $KMnO_4$ solution and excessive $KMnO_4$ was reduced by 50 g/L $NH_2OH.HCl$ solution. The solution was passed through quantitative filter papers (Whatman No 42) in 50 ml volumetric flask and diluted with double-distilled water. The samples were stored in a refrigerator until analysis. Digestions were replicated three times to ensure high accuracy and precision.

## 3. Results and discussion

### *3.1. Setting of experimental conditions*

To study the conditions for analysis of mercury, some parameters were supplied by the manufacturer and some factors are optimized such as carrier gas flow rate, the height of T cell, concentration of HCl and $NaBH_4$ solution at constant mercury concentration 6 ppb. The results are shown in table 1.

***Table 1.*** *Operational working conditions*

| Parameter | Value |
|---|---|
| Wavelength | 253.7 nm |
| HCl solution flow rate | 0.7 ml/min |
| $NaBH_4$ solution flow rate | 1.6 ml/min |
| Sample channel flow rate | 7.5 ml/min |
| Height of T cell | 10.5 mm |
| Carrier gas flow rate | 35 ml/min |
| Concentration of $NaBH_4$ solution | 0.55% (m/v) |
| Concentration of HCl solution | 1.1 M |

### *3.2. Evaluation of linearity range, limit of detection (LOD) and limit of quantification (LOQ)*

In this study, calibration curve method was used to determine the parameters such as the linearity range, LOD and LOQ. Twelve standard solutions were run in triplicate ranges from 0.2 to 15 µg/L. The calibration curves of the Absorbance of mercury were constructed against the concentration of the standard solutions of mercury for the linear regression equation and correlation coefficient. The results obtained for correlation coefficient was approximately 0.9999 ensured the standard quality of calibration curve.

LOD and LOQ were estimated by the standard deviation of regression line, $S_y$, and slope, b. The value of them were LOD = $3S_y/b$ = 0.17 ppb while LOQ = $10S_y/b$ = 0.57 ppb. Figure 1 shows these results.

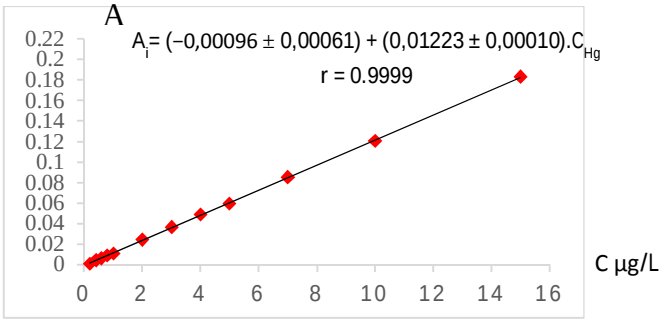


***Fig 1.*** *Calibration cure of mercury*

### *3.3. Optimization of lipstick digestion*

*3.3.1. Optimization of the procedure by univariate methodology*

In order to optimize the digestion sample, a survey of Jackelin lipstick (M1) was conducted: accurately weighs 500 milligrams of the sample, breaks the sample using the Kjeldahl system and modify the following factors to find the optimal condition, including the volume of $HClO_4$ (purified) solution, 25 g/L $KMnO_4$ solution and $HNO_3$ (purified) solution. The results are shown in Figure 2. It was seen that peak height increased with increasing $HNO_3$ volume and reached maximum at 10 mL. Therefore, the volume of 10 mL was selected as an optimum condition. Use concentrated $HClO_4$ and $KMnO_4$ to break the sample together with $HNO_3$ acid, the destruction occurs completely, high absorbance and good repeatability. Increasing the volume of these two solutions, the absorbance changes insignificantly, the volume of 5 mL in each solution was chosen because of saving the material.

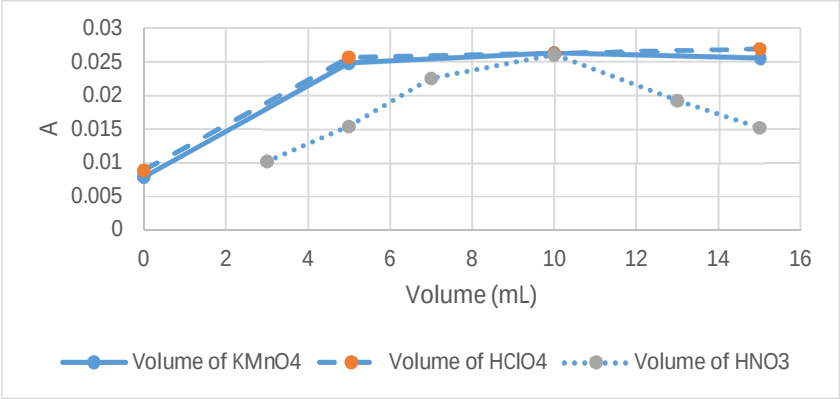


***Fig 2.*** *Effect of oxidizing agent of lipstick digestion*

The volume of 50 g/L $NH_2OH.HCl$ solution (just enough, excess 1 mL or excess 2 mL), temperature (from $240^0$C to $470^0$C ), digestion time (60÷120 minutes) were also surveyed. In the sample treatment stage, hydroxylamine chloride solution is used to reduce the excess $KMnO_4$. With a small amount solution, the absorption signal affected insignificantly. However, overuse may reduce $Hg^{2+}$ in the sample causing loss and lead to errors for measurement. Therefore, the amount of enough $NH_2OH.HCl$ solution was used to remove the color of $KMnO_4$ solution. The optimum temperature level and digestion time chosen for this experiment were $350^0$C and 100 minutes because it gave the highest signal.

*3.3.2. Optimization of the procedure by Box-Hunter design*

In univariate methodology, three factors affecting significantly on the absorbance were temperature, digestion time and volume of nitric acid solution. Therefore, Box-Hunter design was used to optimize the digestion procedure (Nguyen Canh, 2004). The significant variables like digestion time, temperature level and volume of nitric acid were designated $x_1$, $x_2$ and $x_3$, respectively. The low, middle, and high levels of each variable were designated as -1, 0 and +1, respectively, and given in Table 2. The actual design of experiments in given in Table 3.

***Table 2.*** *The levels of variables chosen for the trials*

| **Variables** | **Level** | | |
|---|---|---|---|
| | High | Middle | Low |
| | +1 | 0 | -1 |
| $x_1$: digestion time (min) | 120 | 100 | 80 |
| $x_2$: temperature ($^o$C) | 410 | 355 | 300 |
| $x_3$: volume of nitric acid (mL) | 13 | 10 | 7 |

***Table 3.*** *The Box-Hunter design for the optimization of digestion*

| **N** | **$x_0$** | **$x_1$** | **$x_2$** | **$x_3$** | **y** |
|---|---|---|---|---|---|
| 1 | +1 | -1 | -1 | -1 | 0,005 |
| 2 | +1 | 1 | -1 | -1 | 0,016 |
| 3 | +1 | -1 | 1 | -1 | 0,017 |
| 4 | +1 | 1 | 1 | -1 | 0,013 |
| 5 | +1 | -1 | -1 | 1 | 0,013 |
| 6 | +1 | 1 | -1 | 1 | 0,006 |
| 7 | +1 | -1 | 1 | 1 | 0,017 |
| 8 | +1 | 1 | 1 | 1 | 0,009 |
| 9 | +1 | -1,682 | 0 | 0 | 0,016 |
| 10 | +1 | 1,682 | 0 | 0 | 0,022 |
| 11 | +1 | 0 | -1,682 | 0 | 0,008 |
| 12 | +1 | 0 | 1,682 | 0 | 0,017 |

| | | | | | |
|---|---|---|---|---|---|
| 13 | +1 | 0 | 0 | -1,682 | 0,015 |
| 14 | +1 | 0 | 0 | 1,682 | 0,016 |
| 15 | +1 | 0 | 0 | 0 | 0,030 |
| 16 | +1 | 0 | 0 | 0 | 0,033 |
| 17 | +1 | 0 | 0 | 0 | 0,030 |
| 18 | +1 | 0 | 0 | 0 | 0,031 |
| 19 | +1 | 0 | 0 | 0 | 0,029 |
| 20 | +1 | 0 | 0 | 0 | 0,029 |

In a system involving three significant independent variables $x_1$, $x_2$ and $x_3$ the mathematical relationship of the response con these variables can be approximated by the quadratic (second degree) polynomial equation:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_{33}^2 \quad (1)$$

where y is the estimate response, $b_0$ is constant, $b_1$, $b_2$ and $b_3$ are linear coefficients, $b_{12}$, $b_{23}$ and $b_{31}$ are interaction coefficients between the three factors, $b_{11}$, $b_{22}$ and $b_{33}$ are quadratic coefficients. A multiple regression analysis was done to obtain the coefficients and the equation can be used to predict the response. As a result, we obtained set of coefficients below.

$b_0 = 0,0304$;

$b_1 = 0.0002$; $b_2 = 0,0023$; $b_3 = -0,0003$;

$b_{12} = -0,002$; $b_{13} = -0,0028$; $b_{23} = -0,0003$

$b_{11} = -0,0046$; $b_{22} = -0,0069$; $b_{33} = -0,0058$

Using T-test, we rejected some coefficients which affect insignificantly. Finally, the equation below explains the relationship of the three variables and analytical response.

$$y=0,0304+0,0023x_2-0,002x_1x_2-0,0028x_1x_3-0,0046x_1^2-0,0069x_2^2-0,0058x_3^2 \quad (2)$$

The critical point in the surface response was found by solving the set of three equations below.

$$\begin{cases} \frac{\partial y}{\partial x_1} = -0,0092x_1 - 0,002x_2 - 0,0028x_3 = 0 \\ \frac{\partial y}{\partial x_2} = -0,002x_1 - 0,0138x_2 + 0,0023 = 0 \\ \frac{\partial y}{\partial x_3} = -0,0028x_1 - 0,0116x_3 = 0 \end{cases} \Leftrightarrow \begin{cases} x_1 = -0,0405 \\ x_2 = 0,1725 \\ x_3 = 0,0098 \end{cases}$$

After that, the value of the variables was transferred to condition of experiments. As a result, the optimal condition was digestion time of 100 minutes, temperature of approximately $360^0$C and volume of nitric acid solution of 10 mL.

### *3.4. Method validation*

To survey the repeatability of the measurement, the standard solution of mercury was analyzed at three concentrations 1.0 ppb, 5.0 ppb and 10.0 ppb with the same optimal condition. Measurement of each sample was repeated 10 times, the value of relative standard deviation (RSD) ranged from 1.4 to 8.2 %. So, the method had good precision.

Method validation and quality control were ensured using method blanks, spiked recoveries. Spiked recoveries were determined using samples spiked with 1.0 µg/L; 1.5 µg/L and 2.0 µg/L mercury. The recoveries are given in Table 4.

***Table 4.*** *Recovery of lipstick samples*

| Sample | | Spike | Recovery |
|---|---|---|---|
| **Name** | **Shade/ Production country** | | |
| JACKELIN (M1) | Red Thailand | 1.0 µg/L | 87 |
| | | 1.5 µg/L | 85.2 |
| | | 2.0 µg/L | 101 |
| RIMMEL (M2) | Red United Kingdom | 1.0 µg/L | 88 |
| | | 1.5 µg/L | 106 |
| | | 2.0 µg/L | 95.8 |
| AFTER 90 (M3) | Pink Thailand | 1.0 µg/L | 95 |
| | | 1.5 µg/L | 93.4 |
| | | 2.0 µg/L | 92.5 |
| IN THE NUDE (M4) | Brown USA | 1.0 µg/L | 104 |
| | | 1.5 µg/L | 96.7 |
| | | 2.0 µg/L | 106 |
| MAC (M5) | Blue Russian | 1.0 µg/L | 81 |
| | | 1.5 µg/L | 100 |
| | | 2.0 µg/L | 91.2 |
| BLACK UP (M6) | Red orange Korea | 1.0 µg/L | 88.2 |
| | | 1.5 µg/L | 80.1 |
| | | 2.0 µg/L | 94.5 |
| LUVSKIN (M7) | Orange pink Korea | 1.0 µg/L | 109 |
| | | 1.5 µg/L | 105 |
| | | 2.0 µg/L | 103 |

Base on the results obtained, this method is suitable for the analysis mercury in lipstick samples.

### *3.5. Application*

The presence of mercury in seven samples of lipsticks were examined by atomic absorption spectroscopy. The Content of mercury element was very low. Two samples M4, M6 were under the limit of detection of analytical methods, others detected from 0.075 ±

0.012 to 0.229 ± 0.016 mg/kg. None of the lipstick samples exceeded the maximum limit which proposed by the law of Ministry of Public Health, ASEAN (1,0 mg/kg).

## 4. Conclusion

In this paper, a reliable method for the determination of mercury in lipstick samples by CV-AAS technique was illustrated. Furthermore, the method was optimized by Box-Hunter design and validated by using various validation tools, repeatability, LOD, LOQ, recoveries and found satisfactory result after applying on the real samples. Therefore, this method is suitable for the determination of mercury in lipstick samples.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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# TỐI ƯU HÓA QUY TRÌNH XỬ LÍ MẪU KIELDAHL CHO VIỆC XÁC ĐỊNH THỦY NGÂN TRONG SON MÔI BẰNG QUY HOẠCH THỰC NGHIỆM BOX-HUNTER

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## TÓM TẮT

*Bài báo này mô tả ứng dụng của phương pháp bề mặt đáp ứng đối với việc phát triển quy trình xác định thủy ngân trong mẫu son môi bằng kĩ thuật quang phổ hấp thụ nguyên tử kết hợp với hóa hơi lạnh sau khi đã xử lí mẫu theo phương pháp Kieldahl. Ma trận Box-Hunter được sử dụng để tìm điều kiện tối ưu cho quy trình phân tích thông qua nghiên cứu bề mặt đáp ứng. Ba thông số để nghiên cứu tối ưu hóa là thời gian, nhiệt độ và thể tích dung dịch acid nitric. Trong điều kiện tối ưu, khoảng tuyến tính của phương pháp là 0,2÷15 µg/L; giới hạn phát hiện (LOD) và giới hạn định lượng (LOQ) lần lượt là 0.17 và 0,57 ppb. Phương pháp này cũng cho độ chụm tốt (RSD≤ 8,2%) và hệ số thu hồi tốt (81%-109%). Phương pháp trên được áp dụng để xác định thủy ngân trong bảy mẫu son môi và giá trị hàm lượng thủy ngân cao nhất là 0,229 ± 0,016 mg/kg.*

***Từ khóa:*** thủy ngân, son môi, phổ hấp thụ nguyên tử kết hợp hóa hơi lạnh, quy hoạch thực nghiệm Box-Hunter.