

Determination of Lead Content in Red Colored Lipsticks from Mandalay Market by Flame Atomic Absorption Spectrophotometer

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Red colored lipstick is the most widely used cosmetic product. Although lipstick gives a lot of social, psychological and therapeutic benefits, it may harm the consumers. Because some lipsticks contain a considerable amount of heavy metal especially lead. Lead is being used in lipstick mainly for the pigments required to obtain needed colors. Lead accumulates in the body over time and lead-containing lipstick applied several times a day, every day, combined with lead in water and other sources, could add up to significant exposure levels. Therefore, this study was aimed to determine lead content in red colored lipsticks from market. This study was laboratory-based, analytical study by using 25 lipstick samples. Red colored lipsticks were bought from Mandalay Market by random sampling procedure and they were completely coded to avoid the bias. Then, lead content in coded samples was determined by Flame AAS according to International Conference on Harmonization (ICH) guideline. Lead contents of 88% of the lipsticks samples were more than specified limit (20 ppm) of Food and Drug Administration, United States. All of them, lead content was highest in counterfeit lipsticks group. Among the tested lipstick samples, lipstick with lowest lead content was LE-RL 01 (15.74 ppm) and the lipstick with highest lead content was CF-RL 01(60.09 ppm). In conclusion, lead contents of red colored lipsticks (22 out of 25) from market samples were higher than allowable limit (20 ppm).

Key words: Red color lipsticks, Lead, Flame AAS

INTRODUCTION

Lipstick is the most widely used cosmetic product. It is primarily used by women to enhance their attractiveness and femininity; however, there are products available for men as well. Products for men usually included uncolored sticks offering hydration and sun protection¹. Although lipstick gives a lot of social, psychological and therapeutic benefits, it may harm the consumers. Because some lipsticks contain a considerable amount of heavy metal especially lead. Lead is being used in lipstick mainly for the pigments required to obtain needed colors.²

Cosmetic industries claimed that they do not put lead in lipstick. It serves no purpose in cosmetics and is not used. Some lipsticks have a higher concentration of lead because the naturally occurring red and pink colors have more residual lead present. Lead also may be introduced from raw materials such as zinc oxide, titanium dioxide, ozokerite and petroleum-based ingredients are other sources of lead.³ One of the reasons that the manufactures do not remove naturally occurring lead from the colors used to make

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cosmetics is because it is chemically difficult to do but the levels of lead in lipstick are not harmful.⁴

However, there is no safe level for lead and it is a proven neurotoxin that can cause learning, language and behavioral problems such as lowered Intelligent Quotient (IQ), reduced school performance and increased aggression.⁵ Lead affects virtually every system in the body such as the reproductive, neurological, hematopoietic, hepatic, and renal systems. It is well established that lead can cross the placenta during pregnancy and has been associated with intrauterine fetal death, premature delivery and low birth weight. Maternal blood lead levels of approximately 10 µg/dl have been linked to increased risks of pregnancy hypertension, spontaneous abortion, and reduced offspring neurobehavioral development.⁶

Lead accumulates in the body over time and lead-containing lipstick applied several times a day, every day, combined with lead in water and other sources, could add up to significant exposure levels. When people lick their lips, eat and drink while wearing lipstick, or kiss someone who is wearing lipstick, lipstick's ingredients may be swallowed. According to the study done in US, amount of lipsticks that women averagely swallowed is approximately 4.5 pounds per life time.⁷

Some animal studies showed that bio-accumulation of lead (Pb) in blood of rats originated from lipstick sample after 12 weeks exposure. Oral exposure to lipstick cause significantly disposition of lead in the blood of rats.⁸ Due to the accumulative effect of lead in chronic exposure and neurotoxic nature, high lead level in lipsticks should not be ignored.⁷

Thus, United States Food and Drug Administration (USFDA) limits lead in color additives to maximum specified levels, typically no more than 20 ppm for color additives approved for used in cosmetics.⁹ In 2012, Health Canada released a Draft Guidance on Heavy Metal Impurities

in Cosmetics that outlines impurities limits in cosmetics for lead (10 ppm), arsenic (3 ppm), cadmium (3 ppm), mercury (3 ppm) and antimony (5 ppm).¹⁰ There is no limit for lead in lipstick for Myanmar. Thus, Department of Food and Drug Administration (DFDA) also applied the allowable limit of USFDA.

In Myanmar, there are a lot of counterfeit cosmetics and cosmetics imported without notification to DFDA and they are widely distributed and available in market.¹¹⁻¹³ Therefore, this study was designed to investigate the lead level in red colored lipsticks including counterfeit lipsticks available from markets by using Flame Atomic Absorption Spectrophotometer (AAS) with objectives of providing valuable information to prevent chronic subtle lead poisoning, to support the function of Myanmar DFDA and to give health education to public for awareness of lead in lipsticks.

MATERIALS AND METHODS

Sample size determination

Sample size for this study was calculated by using the formula,

$$n = Z_{\alpha/2}^2 \sigma^2 / M^2$$

n = sample size

$Z_{\alpha/2}^2$ = Z score confident interval

σ = Standard deviation

M = Margin of error (precision).¹⁴

According to this formula and results obtained from pilot study, 25 lipsticks were bought from Mandalay market and were used for this study.

Sampling procedure and coding the samples

Twenty-five red colored lipsticks were bought from the wholesalers such as Zay-Cho Market, Yee-Shin Cosmetic Store and Kyi-Pyar Cosmetic Store. Sampling procedure was carried out on 3 different places. Samples were collected by random sampling and sampling form was filled completely and immediately after sampling.

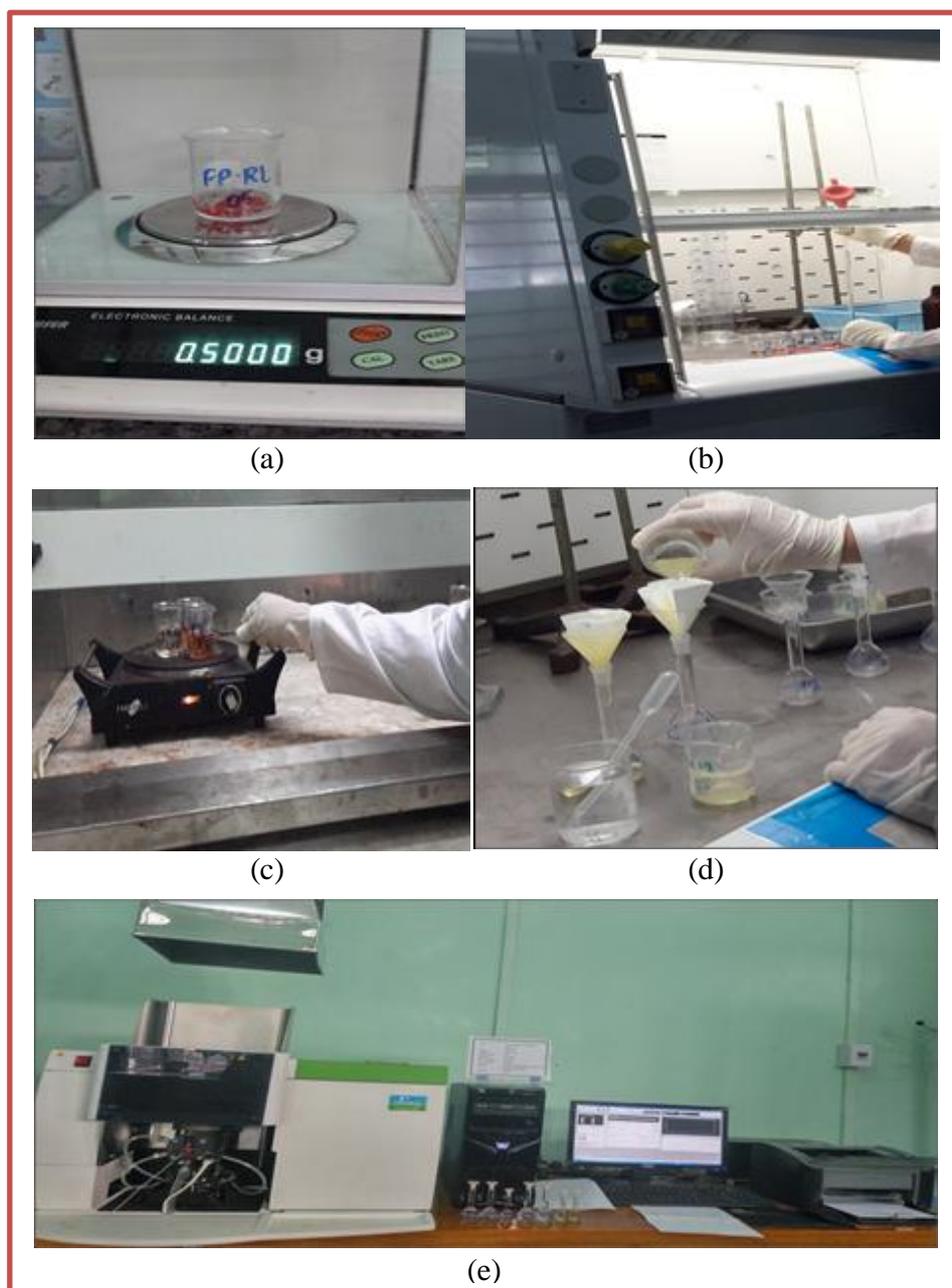


Fig. 1. Determination of lead content in red colored lipsticks by Flame AAS (a) Weighing of sample (b) Addition of tri-acid mixture (c) Digestion of the samples (d) Filtering of digested samples (e) Determination of lead content in samples by using Flame AAS

In sampling forms, product information such as trade name, color code, product presentation, distributor, manufacturer, manufacturing date, expiry date, unit price, quantity collected were mentioned as described in the labels of the lipsticks. The term “Do not mention” was filled, when the product’s label did not describe the information concerned about the product. Sampling forms also included the detail outlets information and comment on the

products. The samples were divided into five groups according to prices of sample lipsticks. These five groups were counterfeit, fair price, regular price, less expensive and expensive lipsticks. Counterfeit lipsticks mean lipsticks produced by imitation of real one, popular brand and sold with very cheap price. Fair price means the unit price of lipstick between 1000 kyats to 2500 kyats. Regular price means the unit price of lipstick between 2600 kyats to 5000 kyats.

Less expensive means the unit price of lipstick between 5000 kyats to 10,000 kyats. Expensive means the unit price of lipstick between 11000 kyats to 32500 kyats.

Each group contained five red colored lipsticks. Counterfeit group was coded as CF-RL, fair price group was coded as FP-RL, regular price was coded as RP-RL, less expensive group was coded as LE-RL and expensive group was coded as EX-RL.

Method validation

Determination of lead content in samples was conducted by Flame AAS (Fig. 1). Before the study, the proposed Flame AAS method for determination of lead content was validated in accordance with International Conference on Harmonization (ICH) guideline. The selected parameters for method validation are linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). Minimum requirement for correlation coefficient (R^2) is 0.995. Accuracy was measured by % recovery. Acceptable range for the ppm level determination of heavy metal is 80 to 120% recovery. The precision of the analytical method was assessed by intra-day and inter-day precision. The precision is expressed as percentage of relative standard derivatives (% RSD).

All the glassware to be used were thoroughly cleaned and immersed in 20% nitric acid for overnight. Then, they were dried in temperature controlled oven at 70-80°C for at least 2 hours. This procedure must be carried out to prevent the lead contamination from glassware used.

Sample lipsticks from the market were analyzed according to the group. Lipstick amount 0.5000 g was accurately weighed on a digital balance. The lipstick was digested with mixture of nitric acid, sulphuric acid and perchloric acid (70:7:23) and then heated on hotplate (85°C *ca.*) until white fume was obtained. The digestion was carried out at low temperature at first, followed by increase in temperature. The digest was cooled and quantitative amount

of de-ionized water was added and was filtered into 25 ml volumetric flask. The final volume was adjusted volumetrically with double de-ionized water.²

Sample solutions were freshly prepared by acid digestion method. The resultant solutions were injected into Flame AAS and lead contents were determined at optimized condition by proposed method. All the measurements were triplicate and the results were expressed as part per million (ppm).

RESULTS

Method validation

The standard curve for lead determination was linear over a range of 0.1 ppm to 1.2 ppm (0.1, 0.3, 0.6, 0.9, 1.2 ppm). A correlation coefficient (R^2) was 0.99997 and % recovery for lead content determination (n=5) was 94.96±6.78. Thus, % recovery was within the range for percent of ppm level determination of heavy metal. % RSD was not more than 10%. The lower limit of detection (LLOD) was 0.0257 mg/l (0.0257 ppm) and lower limit of quantification (LLOQ) was 0.078 mg/l (0.078 ppm). According to the ICH, ISO and USP guidelines, the results obtained from the parameters for method validation of Flame AAS showed that the proposed analytical method was a reliable one with less technical errors.

Quantitative determination of lead content in sample lipsticks from market

Sample lipsticks from the market were analyzed according to the group. Sample solutions were freshly prepared by digestion method. The resultant solutions were injected into Flame AAS and lead contents were determined at optimized condition. The lead content of 88% (22/25) of the lipsticks sample from market was more than specified limit (20 ppm). All of them, lead content of counterfeit lipsticks group was highest. The lowest lead concentration was detected in LERL-01 (15.74 ppm) and the highest lead concentration was detected in CFRL-01 (60.09 ppm) and all the lead

contents are shown in Table 1. Lead contents of red colored lipsticks (22 out of 25) from market samples were higher than allowable limit (20 ppm).

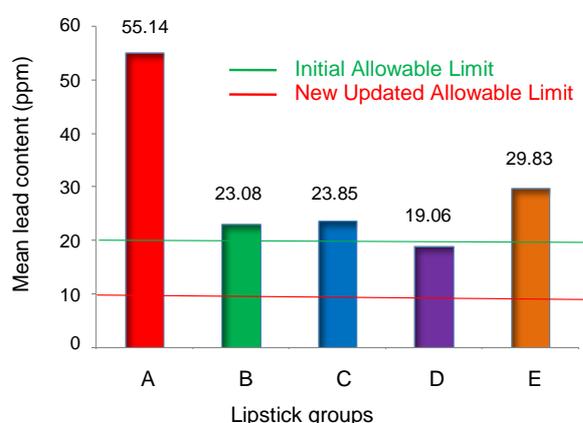
Table 1. Lead contents and standard deviations of red colored lipsticks from Mandalay market

No.	CF-RL Mean±SD (ppm)	FP-RL Mean±SD (ppm)	RP-RL Mean±SD (ppm)	LE-RL Mean±SD (ppm)	EX-RL Mean±SD (ppm)
01	60.09 ±3.27	22.73 ±1.39	25.33 ±0.98	15.74 ±0.37*	33.77 ±1.00
02	56.58 ±3.85	21.60 ±0.58	17.53 ±0.60*	19.93 ±0.53	36.19 ±0.83
03	55.71 ±2.26	24.07 ±0.70	25.92 ±1.11	23.18 ±2.57	38.31 ±1.87
04	53.72 ±4.07	24.95 ±0.07	24.88 ±1.56	21.81 ±1.28	20.32 ±0.29
05	44.59 ±0.72	22.57 ±0.90	25.57 ±1.48	17.66 ±0.58*	21.55 ±0.63

Specified allowable limit=20 ppm

*=Within allowable limit

CF-RL=Counterfeit group, FP-RL=Fair price group, RP-RL=Regular price group, LE-RL=Less expensive group, EX-RL=Expensive group



A=Counterfeit, B=Fair price, C=Regular price, D=Less expensive price, E=Expensive price

Fig. 2. Mean lead content of red colored lipsticks according to group

The mean lead contents in lipstick for counterfeit group was 55.14 ± 6.15 ppm, for fair price group was 23.08 ± 1.17 ppm, for regular price group was 23.85 ± 3.35 ppm, for less expensive group was 19.06 ± 3.02 ppm and expensive group was 29.89 ± 8.30 ppm, respectively (Fig. 2). Counterfeit group was the highest mean lead content.

DISCUSSION

Lead was detected in all samples tested. Only 3 out of 25 tested lipstick samples were within the allowable limit (20 ppm). The lowest lead content was found in sample code LERL-01 (15.74 ± 0.35 ppm) and the highest lead content was found in sample code CFRL-01 (60.09 ± 3.38 ppm). In counterfeit group, all the sample lipsticks had high level of lead.

The difference in lead content was mainly due to the presence of color additives. The reason for high lead content may also be due to quality of raw materials, non-compliance with Good Manufacturing Practices (GMP) and intentionally lead was added to obtain the desired color. The high content of lead in lipsticks can threaten the health of consumers.

Lead was detected in all sample lipsticks ranging from 15.74 to 60.09 ppm. USFDA also found lead in all the 400 samples of lipstick that it tested, with levels ranging from 0.09 to 3.06 ppm.¹⁵ Health Canada found that 81% of the samples of lipstick that it tested for lead had levels ranging from 0.079 to 0.84 ppm, and that one lipstick contained 6.3 ppm.^{16, 17} A study in Nepal by Center for Public Health and Environmental Development (CEPHED) found that most common and widely used eight branded lipsticks in Nepalese market contained higher level of lead content ranged from 30 ppm to 145 ppm.²

According to the results obtained from the present study, lead contents of lipsticks from Mandalay Market were higher than allowable limit while US Market and Canada Market samples were within the allowable limit. Although lipsticks from Mandalay Market have high lead contents, those were relatively lower than that of Nepal Market samples.

Recently, United States FDA released a "Draft guidance for industry: Lead in cosmetic lip products and externally applied cosmetics: Recommended maximum level".

This guidance provides a recommended maximum level of 10 parts per million (ppm) for lead as an impurity in cosmetic lip products and externally applied cosmetics. USFDA has concluded that a recommended maximum level of 10 ppm for lead as an impurity in cosmetic lip products and externally applied cosmetics would not pose a health risk. USFDA consider the recommended maximum lead level to be achievable with the use of good manufacturing practices and to be consistent with the 10 ppm maximum lead level for similar products recommended by other countries.¹⁸ According to this guideline, the mean lead contents of all lipstick samples from market were more than the recommended maximum level (10 ppm).

This study was conducted on only 25 red colored lipsticks by using Flame AAS. Various colors of lip products (lip gloss, lip tint, matte, etc.) should also be analyzed for further study by using microwave assistance digestion and inductively couple plasma mass spectrometry (ICP-MS).

Conclusion

According to initial specified allowable limit, 88% (22/25) of the sample lipsticks from market had higher lead content than specified allowable limit (20 ppm). However, according to new update limit, all red colored lipsticks from market sample had higher lead content than allowable limit (10 ppm). Thus, all the detailed results obtained from this study were officially reported to Myanmar DFDA to initiate the necessary action for public awareness upon the high lead content in lipsticks.

Therefore, regular lipstick users must be aware this fact and should remove the lipsticks before eating anything, avoid using long lasting lipsticks, reduce the lipstick application frequency and use antioxidant colorless lipsticks before application of colored lipsticks.

Competing interests

The authors declare that they have no competing interests.

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