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## Preface

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## Preface

### International Conference on Chemical and Material Engineering (ICCME) 2020

International Conference on Chemical and Material Engineering (ICCME) is an annual conference organized by Universitas Diponegoro, Indonesia. The theme of ICCME 2020 is *“Role of Chemical and Material Engineering in Ensuring Food, Water and Energy for Sustainable Development Goals (SDGs)”*. The event is designed to emphasize advances and new findings in chemical and material science & technology and their impacts on Sustainable Development Goals (SDGs). The conference will bring together scholars, leading researchers, and experts from diverse backgrounds and applications areas in Science.

The Covid 19 pandemic has forced and taught us to hold international conferences online.

Alhamdulillah, with the permission of Allah SWT, the ICCME 2020 conference can be held online.

We inform you that there are 8 keynote speakers from 7 countries (from Indonesia, Malaysia, Brunei, India, Iran, and Vietnam). However, Prof. Masaru Watanabe (Tohoku University, Japan) could not make a presentation due to other activities.

Meanwhile, there were 179 papers and presenters from 5 countries (from Austria, Japan, Saudi Arabia, Malaysia, and Indonesia). From these papers, 8 papers were selected to be published in International Journal of Renewable Energy Development (IJRED), 5 papers in ASEAN Journal of Chemical Engineering (AJChE), and 139 papers in IOP Conference journals. All journals are indexed by Scopus.

On this good occasion. We apologize if the preparation and implementation of ICCME 2020 is still lacking. This is because we all work from home, making it difficult to coordinate directly. Thank you to all the committees who work responsibly and complement each other.

ICCME 2020 event was published on youtube. The link is available form

<https://www.youtube.com/watch?v=sjMfHbVU55g>

<https://www.youtube.com/watch?v=wKPLaPBxRwI>

Finally, Welcome to join online ICCME 2020. Hopefully it will be useful and increase our collaboration in the fields of education and research, especially in Chemical and Material Engineering.

Prof. Dr. Ir. Didi Dwi Anggoro, M.Eng

ICCME 2020 CHAIRPERSON



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## Peer review declaration

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## Peer review declaration

All papers published in this volume of *IOP Conference Series: Materials Science and Engineering* have been peer reviewed through processes administered by the Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

- **Type of peer review: Single-blind**
- **Conference submission management system: Google Form Conference Registration System**
- **Number of submissions received: 189 papers**
- **Number of submissions sent for review: 166 papers**
- **Number of submissions accepted: 148 papers**
- **Acceptance Rate (Number of Submissions Accepted / Number of Submissions Received X 100): 78,307**
- **Average number of reviews per paper: 13 reviews**
- **Total number of reviewers involved: 13 reviewers**
- **Any additional info on review process: no**
- **Contact person for queries: [iccme2020@live.undip.ac.id](mailto:iccme2020@live.undip.ac.id)**



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## Analysis of Red Colorants and Heavy Metals in Lipstick at Traditional Market in Surabaya

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# Analysis of Red Colorants and Heavy Metals in Lipstick at Traditional Market in Surabaya

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**Abstract.** Lipstick is classified as decorative cosmetics, generally, with many colours' variance attracting the consumers. However, red is the favourites amongst them. Protecting the consumer's health, lipstick should meet certain requirements which is registered by (National Agency of Drug and Food Control (Badan Pengawas Obat dan Makanan/BPOM). Regulation of BPOM No.HK.03.1.23.08.11.07517 of 2011 regulates prohibited red colorants such Rhodamine B. While at regulation of BPOM No.HK.03.1.23.07.11.6662 regulates a maximum level of heavy metals concentration. This study was conducted to determine the presence of Rhodamine B, Red Allure, and Amaranth as well as Pb and Cd in illegal brands of red lipstick at PXXX Market in Surabaya. The red colorants were identified with Thin Layer Chromatography (TLC)-densitometry while the metals, lead (Pb) and cadmium (Cd), were analysed with Inductively Coupled Plasma Spectrometer (ICPS). A validation method both for TLC-densitometry and ICPS were performed previously prior the analysis; meet the requirements for linearity, accuracy, precision as well as limit of detection (LOD) and limit of quantification (LOQ). Six illegal brands of lipsticks were identified at PXXX Market in Surabaya. One of them was qualitatively detected contains only Rhodamine B, 2 samples contain both Rhodamine B and Red Allure while the rest of 3 brands were contain Red Allure. None of them contain Amaranth. Hence, a quantitative analysis of Red Allure was further conducted. The Red Allure content in the identified red lipsticks was 34.19-187.88 mg/kg, below the maximum allowable content. In case of heavy metals, Pb and Cd. None of the lipstick's samples contain both Pb and Cd.

## 1. Introduction

Lipstick is a decorative cosmetic contains oils, waxes, fats, aceto-glycerides, surfactants, antioxidants, and colorants. Some artificial colorants which approved to be used in lipsticks are such green-red no.17, DC red, red allure, and amaranth. The artificial is more preferred compare to natural colorants due to its attractive, long-lasting, strong intensity and cheap such rhodamine B. Rhodamine B is a favourite red colorant either in food or cosmetics as well as in cosmetics. Though it is prohibited due its potential to be carcinogenic and damage the liver [1], it is still be found in wide variety of products. Clean and guarantee safe of cosmetics is crucial for the health especially for part of the body which directly contacted with such skin, lips, hair, etc [2]. This coincide to the increase of consumer





awareness i.e. using healthy and quality of cosmetics. Commonly safety of the products follows by the quality. They do not affect the consumer health. Unfortunately, many cosmetics manufacturer designed break the consumer regulations. Using low-quality of materials for gaining more benefit, minimizing cost of the production process. Therefore, some hazardous contaminants found in low-quality or even unregistered cosmetic products. Textile colorants as well as heavy metals such lead (Pb) and cadmium (Cd) are the commonly found hazardous contaminants.

Rhodamine B was found in lipstick at market of Manado [3,4] as well as at market of North Jakarta [5]. Twenty-five samples of lipsticks were identified containing rhodamine B [5]. Red colorants in cosmetics widely used in lipsticks for giving red shades colour. Red allure, ponceau SX and carmoisine, the azo-red dyes, are the dominant red colorants founded in lipsticks [6]. Carmoisine 144  $\mu\text{g/g}$  detected presence in lip gloss though it is prohibited considering the Food and Drug Administration (FDA) of US regulation [6]. Moreover, Guerra et al [6] also found high content of paraben, ca.  $> 1000 \mu\text{g/g}$  beyond the permitted level in 24 of toothpaste in Spain. Quinoline was also detected in children toothpaste [6]. It is not allowed as food additive since toothpaste is easily swallowed by accident for children during brushing their teeth. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was applied for identifying preservatives and synthetic dyes in cosmetics [6].

Imported lipstick in Iran also reported contains lead beyond the allowable level, ca. 20 mg/kg [7]. Heavy metals are identified present in lipstick for such giving a dark brown colour from Cd [8]. Lead (Pb) and Cd are heavy metals which is common presents in lipsticks.

It is reported that 38.2  $\mu\text{g/g}$  of cadmium and 124.2  $\mu\text{g/g}$  of lead are detected respectively in pink and blue lipstick [9]. Lead and Cd were also reported found in low-quality cosmetics brought from an e-commerce platform in Pinduoduo, China [2]. Ultrasound assisted extraction (UAE) was applied for extracting the heavy metals from lipstick samples. While laser induced breakdown spectroscopy (LIBS) was for Pb and Cd analysis. The accuracy of LIBS analysis to the Pb and Cd content then compared to inductively coupled plasma-mass spectrometry ICP-MS [2]. High sensitivity of both Pb and Cd were shown by the limit of detection (LOD). The LOD for Pb and Cd by LIBS were 0.028 mg/kg and 0.016 mg/kg, respectively [2]. Though, the content both of Pb and Cd is low; both heavy metals is not allowed in presence in exported cosmetics product into Germany. Lead make the skin smoother, lighter even shinier while Cd give tanner shade colour. Other similar results were found in Jordan [10]. Content of several heavy metals, i.e. Cd, chromium (Cr), copper (Cu), nickel (Ni), and Pb, were analysed from mascara, eyeliner, eye pencils, kohl, face cream, powder, body cream, and lipstick. The highest concentration of Pb was found in face cream, powder, body cream [10]. While Cr was highest at mascara, lipstick. In case of lipstick and eye pencil contained Cu. Cosmetics products from Sudan have higher heavy metals concentration than those produced by both of Jordan and Syria [10]. It is seen that manufacturer also take a responsibility for producing clean and guarantee safe of cosmetics by applying a good manufacturing practice in flow of the production process. In case of local market, Arifiyana [11] identified the Pb content in both registered and unregistered brands of lipstick at DXX market in Surabaya. Surprisingly, two from six samples of registered brands of lipstick and four from six sample of unregistered brands did contain lead.

The consumer National Agency of Drug and Food Control (Badan Pengawas Obat dan Makanan/BPOM) of Indonesia through BPOM regulation No. 12 of 2019 regulates the contaminations in cosmetics including microbial and heavy metals contamination. The allowable level of lead (Pb) and cadmium (Cd) in cosmetics are, respectively,  $\leq 20 \text{ mg/kg}$  or  $\text{mg/L}$  and  $\leq 5 \text{ mg/kg}$  or  $\text{mg/L}$ . Prior the distribution, cosmetic products must fulfil the requirements by BPOM. A distribution permission should be issued. Without a legal permission, meaning that the product did not meet the requirements; it unregistered or illegally distributed.

Hence, study for identifying the safety product of unregistered products of lipstick especially focus on red colorants and Pb and Cd content in Surabaya need to be conducted. Safety assessment of cosmetic products need to be conducted regularly; provide assurance of safety and security of consumers. The PXXX market is chosen as a studied area due it remarkable crowded market in East

area of Surabaya. Thin Layer Chromatography (TLC) coupled with densitometry method and Inductively Coupled Plasma Spectrometer (ICPS), respectively, applied for both identification and quantification of the red colorants of the lipstick and for Pb and Cd content for fulfilling the required accuracy by BPOM,. Moreover, the validation methods of ICPS covering selectivity, linearity, accuracy, prediction, limit of detection (LOD) and limit of quantification (LOQ) parameters were previously conducted prior the quantitative analysis of heavy metals.

## 2. Experimental Section

### 2.1. Materials

Standard compound of allure red, rhodamine B, and amaranth in high purity grade were purchase from Sigma Aldrich (India) while standard solutions of  $\text{Cd}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$  of 1000.0 ppm from Merck (Darmstadt, Germany). All solvent such methanol, ammonia, n-hexane, and ethyl acetate in p.a. grade from Fulltime Chemical (China). Concentrated  $\text{HNO}_3$  and concentrated  $\text{H}_2\text{O}_2$  in p.a. grade Merck (Darmstadt, Germany). Whereas Silica Gel 60 F254 for qualitative analysis of colorants by thin layer chromatography form Merck (Darmstadt, Germany). In case for filtration, quantitative filter paper ash less grade 41 and nylon filter membrane (in 0.45 and 0.2  $\mu\text{m}$  porosity) both purchased from Whatman (Germany). Inductively Coupled Plasma Spectrometer (ICPS)-OES ICAP 6200 (Thermo scientific) was used for analysing the heavy metals content.



**Figure 1.** Six lipsticks sample were studied.

### 2.2. Lipsticks Sample

Lipsticks sample which meet the requirements criteria were sampled and analysed. The criteria's are (1) it doesn't have a distribution permission from BPOM or illegally distributed; (2) red colour of lipstick; (3) sell in range of price of 10,000-35,000 IDR; and (4) at least sell at two different shop at PXXX market in Surabaya. Hence, 6 illegal lipsticks with different brand (SK, RV, LN, LM, MB, and RE) are collected from 4 cosmetic shops (**Fig. 1**).

### 2.3. Red Colorant Analysis

**2.3.1. Sample preparation.** The lipstick samples were separated from the package, accurately weighed 0.2 grams, and added subsequently with 2 mL of methanol in ultra-sonication bath (**Sonica Ultrasonic Cleaners 1200 S3, Soltec**) until it well dissolved, ca. approximately 2-3 mins. Furthermore, a 5.0 mL of n-hexane was added for fat extraction and vortexed. Two phases are formed, i.e. upper-hexane phase and lower-methanol phase. The hexane phase which rich in fat is separated by pipetting the

upper phase and leaving the methanol phase. Hexane addition was repeated three times, resulting a fat-free of methanol phase. The obtained methanol phase was filtered using a 0.45  $\mu\text{m}$  membrane filter, resulting a clear colorant extract. The filtrate was collected in 5.0 mL of volumetric flask, added methanol up to mark level of the flask, and finally kept in a vial for further used of analysis.

**2.3.2. Qualitative analysis.** Qualitative analysis of red colorant was conducted by Thin Layer Chromatography (TLC). Prior the analysis, mobile phase optimization of TLC separation was carried out for best separating of rhodamine B (CI. No. 45170), amaranth/acid red 27 (CI. No. 16185), and red allure/red allure AC (CI. No. 16035). Referring BPOM No. HK.03.1.23.08.11.07331 of 2011 about analysis methods of cosmetics, six different mobile phase systems are recommended. They are: dichloromethane as system A; a mixture of ethyl acetate-methanol-(ammonia 25%-water (3:7, v/v) = 15: 3 : 3 (w/w/w) as system B; a mixture of ethanol-water-iso-butanol-ammonia 25% = 31: 32: 40: 1 (w/w/w/w) as system C; a mixture of isopropanol-ammonia 25% = 100: 25 (w/w) as system D; a mixture of n-butanol-ethanol-water-glacial of acetic acid = 600: 10: 20: 0.5 (w/w/w/w) as system E, and a mixture of ethyl acetate-n-butanol-ammonia 25 % = 20: 55:25 (w/w/w) as system F. **Therefore, only six combinations of mobile phase referred by BPOM were studied as it is suggested on HK.03.1.23.08.11.07331 of 2011.** Qualitative analysis of red colorant was subsequently conducted after the optimum mobile phase was obtained by spotting 5.0  $\mu\text{L}$  the sample (obtained from **subsection 2.3.1**) into silica gel of TLC plate.

Quantification analysis was further conducted only for red colorants which qualitatively identified by TLC. A calibration curve of red colorant standard should be established prior the quantification. A wide range concentration of red colorant standard, ca. 20-80 ppm, was made by serial dilution from 1000 ppm of standard compound solution stock of red colorants.

#### 2.4. Heavy Metals Analysis

Heavy metals analysis was conducted using Inductively Coupled Plasma Spectrometer (ICPS)-OES ICAP 6200 (Thermo scientific).

**2.4.1. Sample preparation.** Lipstick sample should be destructed prior analysed into ICPS. Lipstick samples were accurately weighed  $\pm 1.5$  grams, digested with 5 mL of concentrated  $\text{HNO}_3$  in beaker glass at 80  $^\circ\text{C}$  during 15-30 mins. This destruction process was repeated twice. The final solution of destruction then allowed to cool to room temperature. A 10 mL of 30%  $\text{H}_2\text{O}_2$  was gradually added until it became a clear solution at 80  $^\circ\text{C}$ , cooled till reached a room temperature. Afterward, a filtration step was conducted using Whatman filter paper No. 41 and directly transferred to a 50.0 mL of volumetric flask. A 2%  $\text{HNO}_3$  was added, reaching the marked level of volumetric flask, homogenized, and filtered with 0.2  $\mu\text{m}$  Whatman filter paper. The obtained sample solution was ready to measure with ICPS-OES ICAP 6200.

#### 2.4.2. Validation method of analysis.

- a) **Selectivity.** Selectivity is expressed by selecting a wavelength of the heavy metals which give smallest interference and best sensitivity. The wavelengths selection was based on the interference and sensitivity data in the online library of ICPS for vendor.
- b) **Linearity.** Linearity is shown as a linear regression curve ( $y = a + bx$ ) between response ( $y$ ) from the analysis instrument and analyte concentrations ( $x$ ) with linear correlation value ( $r$ ) = 0.9999 [9-10].

Calibration curve both of Pb and Cd was conducted with in concentration range of 0-800 ppb. Standard compound of Pb were 0, 30, 60, 100, 200, 400, 500, and 800 ppb; while 0, 30, 60, 100, 400, 500, 600, and 800 ppb for Cd. Standard stock solution both for Pb and Cd was made at 1000

ppm. Hence, a serial dilution using this standard stock solution is conducted to have the desired concentration.

- c) **Limit of Detection (LOD) and Limit of Quantification (LOQ).** The lowest concentration of analyte which give a significant response compared to blank is accepted as limit of detection (LOD). While limit of quantification (LOQ) is expressed as the lowest concentration of analyte that can be determined with certain precision and accuracy of the applied analysis method. Both LOD and LOQ were calculated as follow [12]:

$$LOD = \frac{3s_{y/x}}{s} \quad (1)$$

$$LOQ = \frac{10s_{y/x}}{s} \quad (2)$$

While  $s$  is a standard deviation of blank and  $s_{y/x}$  is standard deviation residual.

- d) **Accuracy.** How close is the analysis results with the actual value, is the definition of accuracy [13,14]. Accuracy can be determining with % recovery of analyte. Spiked method with internal standard addition was applied in this study. Spiked sample was prepared as follows: lipstick samples were accurately weighed  $\pm 1.5$  grams and placed in beaker glass of 100.0 mL. Each of lipstick matrices, i.e. lipstick samples, was added a stock solution of standard compound in different volume. Having, a varieties of spiked lipstick samples with different concentration of standard compound. A digestion step was conducted by adding 10 mL of concentrated  $\text{HNO}_3$  (80 °C) for 15-30 mins. This was repeated twice.

The final solution of allowed to cool to room temperature and gradually added 5 mL of 30%  $\text{H}_2\text{O}_2$  while it boiled, cooled till reached a room temperature. Subsequently, a filtration step was conducted using Whatman filter paper No. 41 and directly transferred to a 100.0 mL of volumetric flask. Add distillate water up to the marked level of volumetric flask, homogenized, and filtered using 0.2  $\mu\text{m}$  Whatman filter paper. The filtrate of sample solution was ready to measure with ICPS-OES ICAP 6200.

The resulted intensity from ICPS then determined as total intensity. Using a linear calibration curve from linearity, a total concentration of heavy metal either for Pb or Cd was measured. However, it is included the heavy metal concentration which was spiked previously in the lipstick matrices. Therefore, to know the real value of heavy metal concentration of sample; a blank sample with similar weight of lipstick sample should be treated similarly as above and measured it intensity. Finally, the acceptable range of % recovery value was referred to AOAC [15].

$$\% \text{ recovery} = \frac{(C_F - C_A)}{C_A^*} \times 100\% \quad (3)$$

$C_F$  = concentration of analyte resulted from analysis.

$C_A$  = real concentration of analyte.

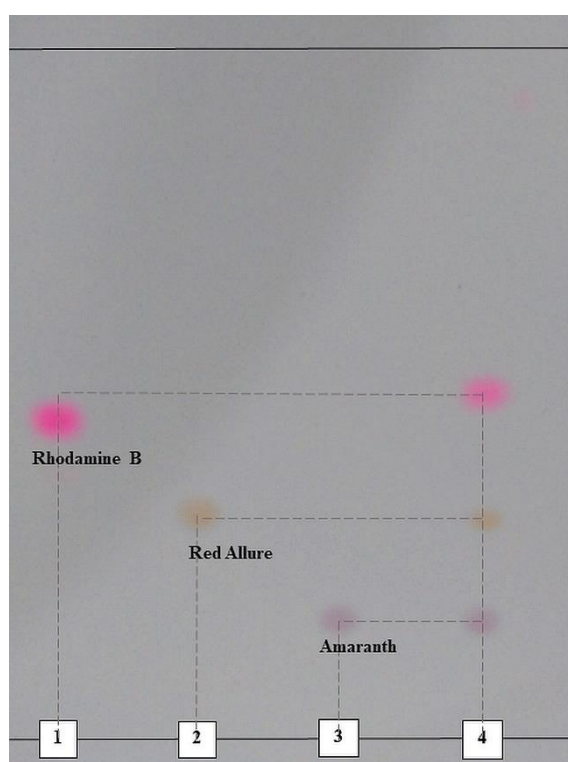
$C_A^*$  = spiked concentration of analyte.

- e) **Precision.** Method analysis precision indicates the degree of conformity between individual test results when the procedure is repeatedly applied to a homogeneous sample, meaning a repeatability or reproducibility. Precision is expressed by coefficient of variation (KV) or relative standard deviation, where  $KV = \text{SD}/\bar{x} \times 100$  (SD = Standard Deviation and  $\bar{x}$  = average level).

### 3. Results and Discussion

#### 3.1. Mobile Phase Optimization for Thin Layer Chromatography (TLC) Analysis

Six different combinations of mobile phase were studied, as it is referred to HK.03.1.23.08.11.07331 of 2011 by BPOM, for colorants identification in cosmetics by TLC (subsection 2.3.1). The mobile phase mixture may well separate three of different red colorants, i.e. rhodamine B, amaranth, and allure red. Combination mixture of ethyl acetate-methanol-(ammonia 25%-water (3: 7, w/w) = 15: 3: 3 (w/w/w) gave the best separation of the red colorants (Fig. 2).



**Figure 2.** Spot visualization for successfully separation of rhodamine B, amaranth, and red allure using a mixture of ethyl acetate-methanol-(ammonia 25%-water (3: 7, w/w) = 15: 3: 3 (w/w/w) as mobile phase by Thin Layer Chromatography (TLC).

Spot of rhodamine B standard = 1, red allure standard = 2, amaranth standard = 3, and a mixture of rhodamine B, red allure, and amaranth standard = 4.

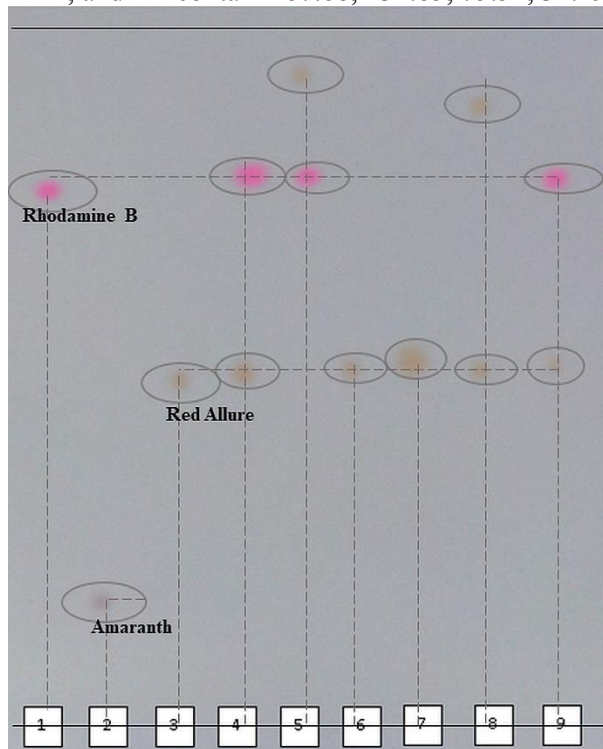
#### 3.2. Qualitative Analysis of Red Colorants

A qualitative test of red colorants was carried out by thin layer chromatography (TLC) using a mixture of ethyl acetate-methanol-(ammonia 25%-water (3: 7, w/w) = 15: 3: 3 (w/w/w) as mobile phase; comparing the retardation value ( $R_f$ ) value between standard compounds of red colorants and sample (Fig. 2).

It is shown from TLC plate that three of lipsticks sample, i.e. SK, RV, and RE, contain rhodamine B, a prohibited red colorant (Fig. 3 and Table 1). Hence, it should not be distributed and sell. None of the lipsticks sample contain amaranth and five samples, i.e. SK, LN, LM, MB, and RE, contain red allure as its red colorant. Both amaranth and red allure are permitted to use in all types of cosmetic [16].

Furthermore, quantification of red allure contained in the lipsticks sample was conducted using TLC-densitometer at  $\lambda = 474$  nm. A calibration curve should be established prior the quantification. A serial concentrations of red allure standard, i.e. 20, 30, 40, 50, 60, and 80 ppm, was applied giving a linier calibration curve of  $y = 1570.79 + 75.22x$  ( $r = 0.98$ ). Considering the correlation value ( $r$ ) < 0.999, the relative process standard deviation value ( $V_{x0}$ ) should be calculated [12]. Assuring that the

linear correlation between response area (y) and red allure concentration (x) is truly achieved [9]. Those, giving  $V_{x0} = 4.51\%$  below 5%, the acceptable accuracy test. Lipsticks sample of SK, LN, LM, MB, and RE contain 187.88, 132.85, 75.52, 34.19, and 104.71 mg/kg of red allure, respectively.



**Figure 3.** Spot visualization of lipsticks sample by TLC separation (**right**).

Spot of rhodamine B standard = 1, amaranth standard = 2, red allure standard = 3, lipstick sample SK = 4, lipstick sample RV = 5, lipstick sample LN = 6, lipstick sample LM = 7, lipstick sample MB = 8, and lipstick sample RE = 9.

**Table 1.** Qualitative analysis of red colorants in red lipstick samples.

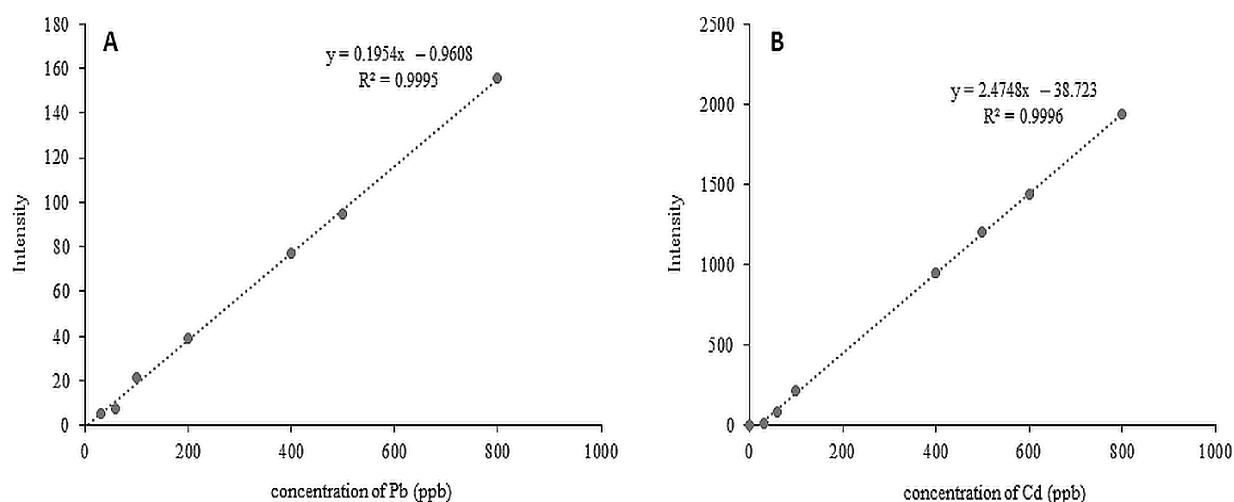
Sample code (Fig.3 code)	Red Colorants		
	Rhodamine B	Red Allure	Amaranth
SK (4)	+	+	-
RV (5)	+	-	-
LN (6)	-	+	-
LM (7)	-	+	-
MB (8)	-	+	-
RE (9)	+	+	-

### 3.3. Validation of Heavy Metals Analysis

A validation method of heavy metals analysis of lead (Pb) and cadmium (Cd) in lipsticks matrices sample using Inductively Coupled Plasma Spectrometer (ICPS) was previously conducted.

**3.3.1. Selectivity.** Considering the highest sensitivity and smallest interference, Pb metal was analysed at 220.353 nm while CD at 228.802 nm.

**3.3.2. Linearity.** Calibration curve both of Pb and Cd was conducted with in concentration range of 0-800 ppb (**Fig. 4**). Standard compound of Pb were 0, 30, 60, 100, 200, 400, 500, and 800 ppb; while 0, 30, 60, 100, 400, 500, 600, and 800 ppb for Cd. Giving  $y = 0.19x - 0.96$  ( $r = 0.9995$ ) and  $y = 2.47x - 38.72$  ( $r = 0.9996$ ), respectively, for Pb and Cd. Both heavy metals have linear correlation between its response of intensity (y) and concentration (x).



**Figure 4.** Calibration curve both of Pb (A) and Cd (B) using Inductively Coupled Plasma Spectrometer (ICPS).

3.3.3. *Limit of Detection (LOD) and Limit of Quantification (LOQ).* Following the calculation of Yuwono and Indrayato [9], the LOD and LOQ both of Pb and Cd are shown at **Table 2**.

**Table 2.** LOD and LOQ of both Pb and Cd (range concentration of 0-800 ppb) analysed by Inductively Coupled Plasma Spectrometer (ICPS)-OES ICAP 6200.

Heavy metals	LOD (ppb)	LOQ (ppb)
Lead (Pb)	39.88	109.99
Cadmium (Cd)	59.44	125.11

3.3.4. *Accuracy.* Accuracy of both heavy metals are expressed as % recovery (**Table 4**) and calculated using equation (3). Spiked method with internal standard addition was applied in this study. Lipsticks sample was used as matrices in this study due to a difficulty finding an artificial matrix for mimicking the real matrices. Therefore, a correlation between weight of lipstick sample/matrices concentration and ICPS intensity should be initially performed (**Table 3** and **4**). Furthermore, the intensity of heavy metals in blank matrices was calculated using the correlation shown at **Table 3** and **4**.

**Table 3.** Correlation between weight of lipstick (g) and intensity of Cd.

Weight of lipstick (g)	Intensity*	Matrices concentration (ppb)
1.5067	1.1068	16.09
1.5071	1.4426	16.23
1.5076	1.1849	16.38

\*intensity was read from ICPS analysis result

**Table 4.** Correlation between weight of lipstick (g) and intensity of Pb.

Weight of lipstick (g)	Intensity*	Matrices concentration (ppb)
1.5067	1.6016	13.11
1.5071	1.6367	13.29

1.5076	1.6408	13.33
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\*intensity was read from ICPS analysis result

**Table 5.** Percentage recovery of lead (Pb).

Added concentration of standard compound (ppb)	Weight of lipstick matrices (g)	Total intensity*	Total concentration (ppb)	Matrices concentration (ppb)	% recovery
400	1.5073	79.37	411.04	13.29	99.44%
	1.5071	79.51	411.14	13.29	99.46%
	1.5073	79.56	411.75	13.29	99.62%
500	1.5072	97.56	504.11	13.29	98.16%
	1.5070	98.25	504.11	13.29	98.89%
	1.5073	92.72	504.11	13.29	98.33%
800	1.5071	156.30	804.67	13.29	98.92%
	1.5073	157.16	809.07	13.29	99.47%
	1.5070	156.83	807.38	13.29	99.26%

\*Total intensity = concentration of standard in matrices + added concentration of standard compound

The % recovery required is in range of 80-110% due the analyte levels of 0.0001% [15]. Hence, the accuracy of lead meets the requirements. A similar protocol for having cadmium accuracy was conducted; gave a 97.12-98.84 % recovery of cadmium. An accuracy requirements level of cadmium is also fulfilled.

**3.3.5. Precision.** Using the % recovery obtained in the accuracy for each concentration of spiked heavy metals, a variance coefficient (KV) was calculated (**Table 6**). Giving the overall variance coefficient (KV) is 0.44% of lead; meet the requirement KV level is 11%. While the overall variance coefficient (KV) is 0.50% of cadmium. Hence, the developed method analysis both for Pb and Cd by ICPS is precise.

Consequently, a quantitative analysis of Pb and Cd by ICPS was conducted on six lipsticks sample, i.e. SK, RV, LN, LM, MB, and RE. Fortunately, none of them contain either Pb or Cd.

#### 4. Conclusion

Three different red colorants, i.e. rhodamine B, red allure, and amaranth, in lipstick was successfully well separated by Thin Layer Chromatography (TLC) using a mixture of ethyl acetate-methanol-(ammonia 25%-water (3: 7, w/w) = 15: 3: 3 (w/w/w) as mobile phase. Moreover, a development method to analyse lead (Pb) and cadmium (Cd) with spiked method of internal standard addition using Inductively Coupled Plasma Spectrometer (ICPS) was meet all the required requirements of validation. Pb was selectively analysed at 220.353 nm 228.802 nm for Cd. It gave a great linear correlation ( $r = 0.9995-0.9996$ ), a good range of accuracy ca. 98.92-99.44% and 97.12-98.84% recovery respectively for Pb and Cd as well as it precision. Which shown by variance coefficient (KV) is 0.44% and 0.50%, respectively, for Pb and Cd.

Quantitative analysis of six lipsticks (SK, RV, LN, LM, MB, and RE) sampled in PXXX market in Surabaya shown that none of illegal brand of lipsticks contains amaranths, five lipsticks (SK, LN, LM, MB, and RE) contain red allure, and three lipsticks (SK, RV, and RE) contain a prohibited red



colorant, rhodamine B. Hence, should be attracted from market and not distributed. Moreover, none of them contain Pb and Cd.

## References

- [1] BPOM. 2015. Regulation of the Head of the Food and Drug Administration of the Republic Indonesia No. 18/201, No. HK.031.1.23.08.11.07517 concerning Technical Requirements for Cosmetics Materials. Jakarta: BPOM
- [2] Liu Y, Chu Y, hu Z, Zhang S, Ma S, Khan M S, Chen F, Zhang D, Guo L and Lau C 2020 *J. Microc* **158** 105322
- [3] Tangka J, Pojoh J A and Hasan H A M 2012 *JIF* **3** 70
- [4] Mamoto L V, Fatimawali and Citraningtyas G 2013 *Pharmacon JIF* **2** 61
- [5] Jusnita N 2016 *Ind. Nat. Res. Pharmaceutical J.* **1** 1
- [6] Guerra E, Alvarez-Rivera G, Llompert M and Garcia-Jares C 2018 *Talanta* **188** 251
- [7] Ziarati P, Moghimi S, Arbabi-Bidgoli S and Qomi M 2012 *Int. J. Chem. Eng. App.* **3** 6
- [8] Khalid A, Bukhari I H, Riaz M, Rehman G, Munir S, Bokhari T H, Rasool N, Zubair M and Qu A 2013 *IJBPAS* **2** 5
- [9] Ayodele O E, and Auremi O I 2014 *Int. J. Pharma. Res. Allied Sci.* **3** 45
- [10] Massadeh A M, El-khateeb M Y and Ibrahim S M 2017 *J. Puhe* **149** 130
- [11] Arifiyana D 2018 *J. Pharma. Sci.* **3** 1
- [12] Yuwono M and Indrayanto G 2005 *Excipients Rel. Method.* **32** 243
- [13] UNDOC 2009
- [14] USP 40 2017
- [15] AOAC, 2016, *Official Methods of Analysis 2016, Guidelines for Standard Method Performance Recruitment*, Appendix F, 9.
- [16] BPOM. 2014. Regulation of the Head of the Food and Drug Administration of the Republic Indonesia No. 17/ 2014 on Amendments to the Regulation of the Head of the Food and Drug Administration No. HK.03.1.23.07.11.6662, 2011 concerning Requirements for Microbial and Heavy Metal Contamination in Cosmetics. Jakarta: BPOM.