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The Evaluation of ICP OES for the Determination of Potentially Toxic Elements in Lipsticks: Health Risk Assessment

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Abstract

This study aimed to optimize and validate the inductively coupled plasma optical emission spectrometric method (ICP OES) for the simultaneous determination of eleven potentially toxic elements (Al, Cd, Cr, Co, Cu, Ni, Pb, Fe, Sb, Mn, and Zn) in lipstick samples. The method was evaluated by applying the standard addition method. The recoveries for all elements in lipsticks were between 90% and 110%, except for Cd and Pb they were <90% and >110%, respectively. The health risk assessment was determined by calculating the average daily intake (ADD), hazard quotient (HQ), and hazard index (HI). The highest mean value for ADD was for Fe (4.8×10^{-1} mg kg^{-1} day^{-1}), and the lowest was for Co (9.3×10^{-6} mg kg^{-1} day^{-1}). There was no significant toxic health risk for any of the elements (HQ < 1), except for Fe (HQ < 3) which indicates a potential health risk. Based on PCA, all potentially toxic elements have been classified in the three groups. The first group includes Fe, the second includes Al, and all other elements belong to the third group. The cluster analysis of the elements provided the identical grouping that was obtained on the basis of PCA. Two separate clusters were obtained when cluster analysis was applied to the analyzed samples. The first cluster contained the only sample that was brown. The second cluster was divided into two sub-clusters. The first sub-cluster included the samples belonging to category I regarding the price, while the second sub-cluster included the samples belonging to category II and III regarding the price.

Keywords: Trace elements; makeup; inductively coupled plasma optical emission spectrometry; method development; health risk assessment

1. Introduction

Cosmetic products play an important role in human lives, being a part of routine body care.[1] During the recent decades, these products have been used by an increasing number of people, which has influenced the increase in their production. Cosmetics include skin care creams, lotions, powders, perfumes, lipsticks, nail polish, eye makeup, hair dyes, deodorants, baby products, bathing oils, and many other types of products. Some cosmetics are benign, but others have been investigated as a possible cause of cancer. The most important are those which are directly applied to human skin, because they may produce local effects on human skin, such as allergy, irritation, sensitization, or photoreactions.[2–4] These effects are linked to the presence of harmful chemicals in cosmetics. Potentially toxic elements are some of them. The sources of elements in cosmetics are raw materials which naturally contain them, the water used, the coated apparatuses during the cosmetics production, and the metal compounds used during the manufacturing of cosmetics.[5]

Toxic elements are widely diffused in colored makeup products such as lipsticks. Some of the studies conducted have shown that lipsticks contain potentially toxic elements such as Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, and Zn.[6–13] These potentially toxic elements can be classified as particularly toxic elements such as Pb, Cd, Ni, As, Sb, Al, and Hg, and toxic trace elements that are essential but dangerous in excessive amounts, such as Cr, Fe, Cu, Mn, Zn, and Co.[5] Lipstick consumers are exposed to potentially toxic elements only in small amounts, but they expose themselves for a prolonged period of wearing time, which makes it significant in developing chronic health risk or skin damages. Nickel, chromium, cobalt, and cadmium may sensitize the immune system and produce an allergic reaction due to their cumulative effects.[14–16] It was reported that cosmetic products must contain less than 5 mg kg^{-1}, and preferably levels below 1 mg kg^{-1} of elements such as chromium, cobalt, and nickel, in order to reduce the risk of eczema or allergic reactions.[17,18] Also, the U.S. Food and Drug Administration together with the Campaign for Safe Cosmetics[19] has conducted a study and found that 61% of the 33 brands of lipsticks contained lead, with levels ranging up to 0.65 mg kg^{-1}. This research by the U.S. Food and Drug Administration[20] also found lead in all lipstick samples tested, with levels ranging from 0.09 mg kg^{-1} to 3.06 mg kg^{-1}. The Health Canada[21] reported that 81% of the lipstick samples they tested on lead had levels ranging from 0.079 to 0.84 mg kg^{-1}, and one of them contained lead in the concentration of 6.3 mg kg^{-1}. Also, some elements such as As, Sb, Cd, Pb, Cr, and Hg are banned as intentional ingredients in cosmetics in Canada.[22]

Most studies determined the content of potentially toxic elements in cosmetic products, but only a couple of them determined the content of some elements and their impact on human health. Volpe et al.[7] determined the content of Pb using flame-atomic absorption spectrometry (F-AAS), and contents of Cd, Cr, Co, and Ni by ICP OES in eyeshadow samples. Ullah et al.[8] used F-AAS for the determination of Pb, Cd, Cu, Co, Fe, Cr, Ni, and Zn in samples of shampoo, talc powders, lipstick, surma, and cream available in the Pakistan market. Bocca et al.[9] reviewed the concentrations of Sb, As, Cd, Cr, Co, Hg, Ni, and Pb in cosmetics, including lipstick samples, using different techniques. Piccinini et al.[10] evaluated microwave acid digestion for the determination of the content of Pb in lip products (lipsticks and lip glosses) of different color and price using ICP-MS, and Ziarati et al.[11] evaluated the wet digestion method for the determination of Pb and Cd by flame emission spectrophotometer. Zakaria and Ho[12] evaluated the potential health risks due to the daily ingestion of Pb, Cd, and Cr in lipsticks, while Batista et al.[13] developed ICP OES method for determination of Cd, Co, Cr, Cu, and Ni and the graphite furnace atomic absorption spectrometry (GF AAS) method for the determination of Pb in lipsticks.

Because of the importance of the control and monitoring of toxic elements in cosmetics, our primary objective was to develop an effective and sensitive inductively coupled plasma atomic emission (ICP OES) method which may be applicable for a simultaneous analysis of potentially toxic elements in lipsticks. The second one was to apply the chemometric techniques of principal component analysis (PCA) and cluster analysis (CA) to the results obtained from the ICP OES determination of eleven potentially toxic elements in marketed lipsticks to assess whether there is a similarity regarding their element contents as well as to evaluate the possibility of potential health risk due to the daily ingestion of toxic elements in lipsticks among lipstick users.

2. Experimental

2. 1. Chemicals

Ultra-scientific (U.S.A.) ICP multi-element standard solutions of about 20.00 ± 0.10 mg L^{-1} were used as a stock solution for calibration. Nitric acid (65%) (Merck, Darmstadt, Germany) and hydrogen peroxide (30%) (Fluka, Buchs, Switzerland) were both of the analytical grade.

2. 2. Instrumentation

The thermo Scientific iCAP 6000 inductively coupled plasma atomic emission spectrometer with an Echelle optical design (52.91 grooves mm^{-1}, 63.5° blaze angle) and a change injection device solid-state detector (RACID86) was used to analyze the lipstick samples. The nebulizer was glass concentric. The iTEVA operating software for iCAP 6000 series was used to control all functions of the instrument. The microwave digestion system ETHOS 1 was used for performing a digestion (Milestone, Bergamo, Italy).

2. 3. Samples

Fourteen lipstick samples (L1–L14) were purchased in local markets in Serbia. The lipstick samples were classified by price in three categories varied from "cheap" (category I) to „expensive" (category III). Every sample label contained the origin country of manufacture and was stored at room temperature until the analysis.

2. 4. Sample Preparation

The sample preparation was carried out using a microwave digester according to the method of Zakaria and Ho[12] with slight modification. 0.2 g of lipstick was weighed in a microwave vessel and 6 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) were added. The conditions were as follows: 1800 W, 90 bar, with the temperature program: heating to 130 °C in 15 min (held for 20 min), then to 200 °C in 15 min (held for 20 min). After the second step of the programme, the vessels were cooled to 50 °C for 10 min. The extracts were filtered and then diluted with 0.5% HNO_3 to the final volume of 25 mL. The procedure was carried out in triplicate. A blank was prepared in the same way. The plastic containers used for storing the samples were cleaned to avoid the contamination of the samples with the traces of any elements. The containers were treated with 20% HNO_3 and washed with ultra-pure water 0.05 µS cm^{-1} (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany).

2. 5. Operating Plasma Condition

Before the metal analysis, the operating parameters were conducted to check the instrument performance. The following ICP OES instrument performance tests should be performed: RF power, nebulizer gas flow rate, torch gas flow rate, viewing height and sample introduction rate. RF power, viewing height, and nebulizer gas flow rate are three of the parameters that have high influence on the analytical characteristics of ICP plasma.[23] In the present study, using a 27.12 MHz ICP, RF power, and nebulizer argon flow rate were varied under the constant values of other plasma conditions.[24,25] Also, both viewing modes (axial/radial) were considered in this study. In order to eliminate the memory effect, the delay time for washing between samples and signal measurement was set to 30 s. Mermet[26] reported that plasma robust conditions can be represented by a Mg II 280.270 nm/Mg I 285.213 nm ratio (Mg II/Mg I) higher than 10. Under robust plasma conditions matrix effects as well as other interferences are mainly assigned to the aerosol transport.

2. 6. The selection of Analytical Lines and the Evaluation of Matrix Effect (ME)

Prior to the analysis, the line selections were performed. The spectral interferences and matrix effect in both axial and radial view modes for a total of 44 lines recommended by the ICP OES spectrometer library, corresponding to 11 determined elements, were checked. The analytical lines were evaluated according to the ratio of the slope of the calibration curve and slope of the standard addition method line (slope$_{cal}$/slope$_{sam}$).

A standard addition method was used to overcome the matrix effect.[23,26,27] A portion of the sample was spiked at a different concentration levels of the standard according to U.S. Food and Drug Administration[28] (from 0.1 mg L^{-1} to 1.0 mg L^{-1} for trace elements). The increase in signal was to the standard that was added, and the original signal was due to the analyte only. A ratio of two elemental signals was used to calculate the matrix effect.[29]

2. 7. Validation

The instrument was calibrated at a four-point calibration curve. The linearity of each element was tested ranging from 0 mg kg^{-1} to 5 mg kg^{-1}. The calibration curve linearity for each element was evaluated by the coefficient of determination (R^2). Each sample of lipstick was analyzed in triplicate in order to gain a more precise estimation of the data. A method blank was carried throughout the entire sample preparation and analytical process.

The detection (LOD) and quantification (LOQ) limits were calculated with three and ten times of the residual standard deviation of the regression line (3σ and 10σ criterion), divided with a slope of the calibration curve.[30] Both limits were expressed in ng g^{-1}.

The signal-to-background ratio (SBR) is also a figure of merit that can be correlated with the limit of detection.

$$SBR = \frac{I_{standard} - I_{blank}}{I_{blank}} \tag{1}$$

All signals were measured in the presence of some degree of background. A quantitative measure of the background level is called the background equivalent concentration (BEC), and was calculated by the following formula:

$$BEC = \frac{c_{standard}}{SBR} \tag{2}$$

where $I_{standard}$ and I_{blank} are emission intensities for the multielemental standard and blank solutions, $c_{standard}$ is the concentration of the multielemental standard solution (2 mg L^{-1}).

The recovery test was evaluated by spiking three replicates of each lipstick sample with the element standard. 2 mL of 62.5 mg L^{-1} of Al and Fe, and 2 mL of 6.25 mg L^{-1} of Mn, Ni, Sb, Cu, Zn, Cr, Cd, Co, and Pb were added to the lipstick samples. The samples were prepared as is described in the Sample preparation part.

2. 8. Human Health Risk Assessment

Regarding health risk assessment, risk level was determined using the average daily intake dose (ADD, mg kg^{-1} day^{-1}) of ingestion, hazard quotient (HQ) and hazard index (HI).

ADD is used to quantify the oral exposure dosage for deleterious substances.[31] This dose of the elements was calculated using the following equation:[12,32]

$$ADD = \frac{c \times IR \times ED \times EF}{BW \times AT} \times CF \tag{3}$$

where *c* is the concentration of elements found in the tested lipstick samples (mg kg^{-1}); *IR* is the intake rate (40 mg day^{-1});[33] *ED* is the exposure duration (35 years); *EF* is the exposure frequency (260 days year^{-1}); *BW* is the average body weight for an adult (57.9 kg);[12] *AT* is the average exposure time for non-carcinogenic effects (365 days years^{-1} × number of exposure years (35)); *CF* is the conversion factor (10^{-3}).

To assess the health risk associated with a potential toxic element, the hazard quotient and hazard index can be calculated using the following equations:[12,34]

$$HQ = ADD/RfD \tag{4}$$

$$HI = \sum HQ \tag{5}$$

where *RfD* is the oral reference dose (mg kg^{-1} day^{-1}), based on the US EPA database. The RfD value is regarded as an estimate of a daily exposure to the human population that is likely to be without a significant risk of harmful effects during a lifetime.[35]

If value $HQ \leq 1$ then no adverse health effects (no risk); if $HQ \geq 1$ then adverse health effects and $1 < HQ \leq 5$ (low risk); $5 < HQ \leq 10$ (medium risk); $HQ \geq 5$ (high risk).[36] The HI index represents the sum of the HQ index and shows the influence of toxic elements on human health.

2. 9. Statistical Analysis

Statistical multivariate methods such as principal component analysis (PCA) and cluster analysis (CA) were used for the classification of samples based on the metal content. PCA and CA were performed using a statistical package running on a computer (Statistica 8.0, StatSoft, Tulsa, Oklahoma, USA). The Tukey's test was used (significant level of $p < 0.05$) for the determination of the difference between the mean concentration of metal in the analyzed lipsticks.[37]

3. Results and Discussion

3. 1. The Optimization of Plasma Operating Conditions

The Mg II/Mg I line intensity ratio was selected to evaluate the ICP operating conditions and the plasma robustness.[26] Using a 27.12 MHz ICP, the RF power was varied from 750 W to 1350 W with the intervals of 200 W. The other plasma conditions were constant: flush pump rate 50 rpm, analysis pump rate 50 rpm, nebulizer gas flow rate 0.5 L/min, coolant gas flow rate 12 L/min, auxiliary gas flow rate 0.7 L/min, dual (axial/radial) viewed plasma mode and sample uptake delay 30 s. The highest Mg II/Mg I ratio was obtained for the RF power of 1150 W and was found to be 10.35 and 11.42 for the axially and radially viewed, respectively. As expected, the results showed a higher Mg II/Mg I ratio for the radially viewed configuration (Table 1).[24,25]

Using an RF power of 1150 W, nebulizer gas flow was varied from 0.5 mL/min to 1.5 mL/min in intervals of 0.5 mL/min. The highest Mg II/Mg I ratio of 10.35 and 11.42 for axially and radially viewed ICP OES, respectively, was at the nebulizer gas flow of 0.5 L min^{-1} (Table 1). This value was selected for further proceedings.

3. 2. The Selection of Analytical Lines

The best lines experimentally found in both axial and radial plasma viewing modes after the study of ratio slope$_{cal}$/slope$_{sam}$ and matrix effect, are shown in Table 2. A final selection of wavelength lines also took into consideration the accuracy obtained for each line and spectral interferences. As it can be seen in Table 2, the slopes of both kinds of lines were statistically comparable, which indicates the lack of the matrix effects. The ME data up to 9.5% indicate that the method of the evaluation of the matrix effect generates reliable results.

3. 3. Validation

On the basis of the calibration curve of each metal, the selected wavelengths of the analyte lines, coefficient of

Table 1. Results (mean ± SD, $n = 3$) obtained for RF power and nebulizer gas flow rate in axial/radial mode

Viewing mode	RF power (W)	Relative intensity ± SD (280.270 nm)	RSD (%)	Relative intensity ± SD (285.213 nm)	RSD (%)	Intensity ratio Mg II/Mg I
Axial	750	318233 ± 4550	1.43	75120 ± 1092	1.45	4.24
	950	832600 ± 6038	0.73	109697 ± 953	0.87	7.59
	1150	1155400 ± 7779	0.67	111658 ± 872	0.78	10.35
	1350	1413317 ± 19909	1.41	141050 ± 1275	0.90	10.02
Radial	750	18685 ± 273	1.46	2454 ± 6	0.24	7.61
	950	30189 ± 509	1.69	3147 ± 35	1.11	9.59
	1150	42177 ± 377	0.89	3694 ± 27	0.73	11.42
	1350	45005 ± 435	0.97	3961 ± 30	0.76	11.36

Viewing mode	Nebulizer gas flow (l/min)	Relative intensity ± SD (280.270 nm)	RSD (%)	Relative intensity ± SD (285.213 nm)	RSD (%)	Intensity ratio Mg II/Mg I
Axial	0.5	1155400 ± 7779	0.67	111658 ± 872	0.78	10.35
	1.0	1029000 ± 9337	0.91	102286 ± 1057	1.03	10.06
	1.5	698000 ± 6902	0.99	70950 ± 395	0.56	9.84
Radial	0.5	42177 ± 377	0.89	3694 ± 27	0.73	11.42
	1.0	39209 ± 452	1.15	3573 ± 45	1.26	10.97
	1.5	32041 ± 280	0.87	3094 ± 26	0.84	10.36

Table 2. Analyte line selected with the ratio $slope_{cal}/slope_{sam}$, matrix effect (ME), BEC and SBR, as well as coefficient of determination (R^2), LOD and LOQ of the calibration for each metal determination. Plasma view mode: axial.

Element	λ (nm)	$Slope_{cal}/Slope_{sam}$	ME (%)	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})	BEC (ng g^{-1})	SBR
Al	396.152	0.976	−2.4	0.9995	85	280	109.3	18.3
Cd	226.502	1.056	5.6	0.9999	8	25	26.9	74.3
Co	228.616	1.078	7.8	0.9999	2	5	3.5	563
Cr	283.563	0.905	−9.5	0.9997	30	100	36.5	54.8
Cu	324.754	1.019	1.9	1	25	80	15.2	131.2
Fe	259.940	1.011	1.1	0.9998	25	80	63.1	31.7
Mn	257.610	0.982	−1.8	0.9997	5	15	20.5	97.7
Ni	231.604	0.983	−1.7	0.9998	20	68	17.1	116.6
Pb	220.353	0.958	−4.2	1	11	36	15.9	125.9
Sb	231.147	1.033	3.3	0.9999	17	55	14.2	104.5
Zn	213.856	0.981	−1.9	0.9998	5	15	16.8	119.3

determination, limit of detection and limit of quantification are shown in Table 2. The four-point calibration curve showed good linearity over the concentration range from 0 to 5 mg kg^{-1}, where correlation coefficients ranged from 0.9995 to 1. Table 2 also shows the parameters of merit (BEC, SBR, LOD, LOQ) obtained under robust conditions

Table 3. Spiked concentration[a] (mg kg^{-1}) of elements in lipstick samples and recovery test (n = 3)

Samples	Al[b] c_{sr} ± SD	RSD[c]	Recovery[d]	Cd c_{sr} ± SD	RSD	Recovery	Cr c_{sr} ± SD	RSD	Recovery	Co c_{sr} ± SD	RSD	Recovery	Cu c_{sr} ± SD	RSD	Recovery	Ni c_{sr} ± SD	RSD	Recovery	Pb c_{sr} ± SD	RSD	Recovery
L1	79 ± 1	1.3	97.5	0.49 ± 0.01	2.0	88.8	1.43 ± 0.02	1.4	96.6	0.49 ± 0.01	2.1	97.4	1.74 ± 0.05	2.9	93.8	0.78 ± 0.01	1.3	97.5	1.31 ± 0.04	3.1	88.5
L2	254 ± 1	0.4	98.8	0.55 ± 0.02	3.6	85.3	1.59 ± 0.03	1.9	96.4	0.61 ± 0.02	3.3	96.0	8.2 ± 0.1	1.2	91.5	1.07 ± 0.02	1.9	96.4	1.58 ± 0.03	1.9	87.8
L3	164 ± 3	1.8	97.6	0.49 ± 0.01	2.1	88.5	1.39 ± 0.02	1.4	97.2	0.52 ± 0.01	1.9	95.7	1.88 ± 0.05	2.7	94.2	0.84 ± 0.01	1.2	96.5	1.20 ± 0.04	3.3	88.9
L4	26 ± 1	3.8	96.3	0.67 ± 0.02	3.0	111.4	1.43 ± 0.02	1.4	97.3	0.50 ± 0.01	2.0	96.9	1.01 ± 0.02	2.0	94.9	0.72 ± 0.01	1.4	97.3	1.11 ± 0.04	3.6	88.1
L5	226 ± 1	0.4	98.3	0.50 ± 0.01	2.0	97.9	1.42 ± 0.02	1.4	102.9	0.54 ± 0.02	3.7	96.1	2.16 ± 0.04	1.8	91	0.84 ± 0.01	1.2	95.4	2.02 ± 0.05	2.5	87.8
L6	361 ± 4	1.1	99.4	0.56 ± 0.01	1.8	85.6	1.77 ± 0.03	1.7	95.7	0.52 ± 0.01	1.9	98.3	1.35 ± 0.03	2.2	92.3	0.89 ± 0.02	2.2	94.7	1.37 ± 0.04	2.9	113.2
L7	112 ± 1	0.9	96.5	0.54 ± 0.01	1.9	84.8	2.43 ± 0.04	1.6	95.3	1.00 ± 0.02	2.0	95	2.22 ± 0.04	1.8	91.8	1.77 ± 0.03	1.7	97.2	17.33 ± 0.6	3.5	85.8
L8	113 ± 1	0.9	97.4	0.55 ± 0.01	1.8	85.4	1.76 ± 0.02	1.1	95.6	0.51 ± 0.01	1.9	98.5	1.51 ± 0.04	2.6	92.1	0.84 ± 0.02	2.4	97.7	1.63 ± 0.05	3.1	88.1
L9	139 ± 1	0.7	97.9	0.54 ± 0.01	1.8	88.1	1.54 ± 0.01	0.6	96.9	0.57 ± 0.01	1.8	97.8	1.16 ± 0.03	2.6	95	0.80 ± 0.01	1.3	98.8	1.06 ± 0.03	2.8	88.3
L10	488 ± 3	0.6	99.2	0.47 ± 0.01	2.1	87.7	1.37 ± 0.01	0.7	97.2	0.52 ± 0.01	1.9	95.6	2.02 ± 0.03	1.5	90.7	0.72 ± 0.01	1.4	97.3	0.92 ± 0.02	2.2	87.6
L11	9.02 ± 0.05	0.5	96.5	0.48 ± 0.01	2.1	87	1.40 ± 0.01	0.7	97.2	0.74 ± 0.01	1.3	95	1.93 ± 0.02	1.0	91.1	1.34 ± 0.02	1.5	103.9	1.21 ± 0.04	3.3	89
L12	190 ± 1	0.5	97.9	1.20 ± 0.03	2.5	85.1	3.47 ± 0.04	1.2	94.8	0.88 ± 0.02	2.3	97.8	3.77 ± 0.05	1.3	90	1.41 ± 0.02	1.4	96.6	7.00 ± 0.08	1.1	86.7
L13	432 ± 2	0.5	99.1	0.57 ± 0.02	3.5	87.6	1.71 ± 0.02	1.2	96.6	0.49 ± 0.01	2.0	98.8	2.19 ± 0.04	1.8	92	1.29 ± 0.02	1.6	97.7	1.35 ± 0.03	2.2	87.1
L14	262 ± 2	0.8	99.6	0.55 ± 0.01	1.8	88	4.94 ± 0.05	1.0	95.4	0.62 ± 0.01	1.6	96.6	5.11 ± 0.07	1.4	93.7	1.84 ± 0.03	1.6	95.3	6.76 ± 0.07	1.0	86.3

Fe c_{sr} ± SD	RSD	Recovery	Sb c_{sr} ± SD	RSD	Recovery	Mn c_{sr} ± SD	RSD	Recovery	Zn c_{sr} ± SD	RSD	Recovery
75 ± 1	1.3	94.9	6.34 ± 0.2	3.2	94.6	1.23 ± 0.01	0.8	93.2	3.59 ± 0.02	0.6	95
659 ± 2	0.3	99.5	7.75 ± 0.1	1.3	94.5	2.83 ± 0.05	1.8	90.4	21.7 ± 0.1	0.5	98.8
93 ± 1	1.1	95.9	1.44 ± 0.07	4.9	93.5	0.94 ± 0.01	1.1	94.9	3.07 ± 0.01	0.3	95.2
145 ± 1	0.7	98.0	2.05 ± 0.08	3.9	92.8	2.96 ± 0.07	2.4	90.5	6.15 ± 0.04	0.6	94.7
21.4 ± 0.3	1.4	95.5	1.03 ± 0.05	4.9	93.6	1.41 ± 0.03	2.1	93.4	3.37 ± 0.02	0.6	94.3
974 ± 13	1.3	99.8	8.9 ± 0.1	1.1	91.6	1.99 ± 0.03	1.5	94.3	9.68 ± 0.05	0.5	96.9
548 ± 3	0.5	98.9	7.08 ± 0.08	1.1	95	3.27 ± 0.04	1.2	91.1	6.18 ± 0.06	1.0	95
617 ± 2	0.3	101.0	7.8 ± 0.1	1.3	94.8	6.79 ± 0.02	0.3	94.1	13.8 ± 0.1	0.7	97.9
360 ± 3	0.8	100.5	4.54 ± 0.04	0.9	93.4	3.24 ± 0.04	1.2	93.9	4.85 ± 0.03	0.6	94.2
9.4 ± 0.1	1.1	94.9	5.05 ± 0.04	0.8	92.7	0.96 ± 0.01	1.0	92.3	2.41 ± 0.02	0.8	94.7
22.8 ± 0.3	1.3	96.6	3.26 ± 0.07	2.2	92.3	0.74 ± 0.01	1.3	91.4	1.84 ± 0.02	1.1	94.7
3816 ± 17	0.4	99.0	6.9 ± 0.1	1.4	94.5	5.85 ± 0.04	0.7	96.8	14.3 ± 0.1	0.7	97.3
802 ± 4	0.5	99.5	9.2 ± 0.2	2.3	95.6	1.82 ± 0.02	1.1	91.9	12.3 ± 0.1	0.8	97
50 ± 0.4	0.8	97.5	6.97 ± 0.08	1.1	95.3	1.25 ± 0.01	0.8	91.9	6.71 ± 0.02	0.3	94.8

[a] Mean (c_{sr}) ± standard deviation (SD) of three replicates, [b] Spiked concentrations for Al and Fe are 62.5 mg L^{-1} (2 mL), while for other elements are 6.25 mg L^{-1} (2 mL), [c] Relative standard deviation (%), [d] Results obtained from recovery test (%)

and using an axially viewed configuration. High SBR values and low BEC values were obtained.

The results obtained by the standard addition method and the recovery experiments for lipstick samples are shown in Table 3. In the tested samples, the recovery for all elements was between 90% and 110%, except for Cd and Pb, they were < 90% and > 110%. The element concentrations precision ranged from 0.3% to 4.9%. Lower RSD values were obtained for Zn, higher RSD values were obtained for Pb and Sb. Nevertheless, in all cases, the accuracy and precision were within the acceptable recoveries and RSD percentages obtained from the Horwitz function[38–41] and from the AOAC Peer-Verified Methods (PVM) program on the analyte level.[42] According to Horwitz and AOAC PVM RSDs, the maximum RSD values acceptable for the analyte level of 100 μg kg^{-1} are 22.6% and 15%; for 1 mg kg^{-1} they are 16% and 11%; for 10 mg kg^{-1} they are 11.3% and 7.3%; for 100 mg kg^{-1} they are 8% and 5.3%, respectively, and so on. The results obtained clearly demonstrate that this type of digestion and ICP measurements are suitable for all elements.

3. 4. The Concentration of Elements in Lipsticks

Potentially toxic elements concentrations, expressed as milligram per kilogram (mg kg^{-1}), in the lipstick samples investigated are shown in Table 4. The overall (n = 14) mean concentration of potentially toxic elements was: 202 ± 2 mg kg^{-1} for Al; 0.160 ± 0.003 mg kg^{-1} for Cd; 1.55 ± 0.02 mg kg^{-1} for Cr; 0.129 ± 0.004 mg kg^{-1} for Co; 2.31 ± 0.02 mg kg^{-1} for Cu; 0.61 ± 0.01 mg kg^{-1} for Ni; 3.25 ± 0.08 mg kg^{-1} for Pb; 584 ± 3 mg kg^{-1} for Fe; 5.00 ± 0.09 mg kg^{-1} for Sb; 2.20 ± 0.02 mg kg^{-1} for Mn, and 7.62 ± 0.04 mg kg^{-1} for Zn. Based on the mean concentrations, the potentially toxic metal contents were arranged in the following decreasing order: Fe > Al > Zn > Sb > Pb > Cu > Mn > Cr > Ni > Cd > Co.

The concentrations of Al, Cd, Cr, Co, Cu, Ni, Pb, Fe, Sb, Mn, and Zn in all analyzed lipsticks were in the interval of 4.35–487 mg kg^{-1}, 0.038–0.914 mg kg^{-1}, 0.88–4.68 mg kg^{-1}, n.d.–0.556 mg kg^{-1}, 0.56–8.5 mg kg^{-1}, 0.24–1.43 mg kg^{-1}, 0.55–19.7 mg kg^{-1}, 4.9–3850 mg kg^{-1}, 0.6–9.1 mg kg^{-1}, 0.31–6.72 mg kg^{-1}, 1.44–21.5 mg kg^{-1}, respectively. The concentration ranges of Cd, Cr, Co, Cu, Ni, Pb, Fe, Zn in lipsticks found by Ullah et al.[8] were: 0.2–0.43 mg kg^{-1}, n.d.–0.77 mg kg^{-1}, 0.3–0.872 mg kg^{-1}, 0.026–6.036 mg kg^{-1}, 0.696–1.610 mg kg^{-1}, 2.58–11.33 mg kg^{-1}, 258–1164 mg kg^{-1}, 0.696–1.610 mg kg^{-1}, respectively. Zakaria and Ho[12] found Cd, Cr, and Pb in concentration ranges n.d.–0.33 mg kg^{-1}, 0.24–2.50 mg kg^{-1}, 0.77–15.44 mg kg^{-1}, respectively. Liu, Hammond, and Rojas-Cheatham[43] found Al in lipsticks in the concentration interval of 4.448–27.032 mg kg^{-1}, while Al-Qutob, Alatrash, and Abol-Ola[44] found it in the concentration range 10.98–694.5 mg kg^{-1}. As can be seen, the obtained results are consistent with the results obtained by other authors. Aluminum is added to cosmetics as white pigments in colored cosmetics. The EC Regulation[45] allowed some compounds of Al as colorants in cosmetics. According to the U.S. low (FD&C Act)[46] color additives can contain lead in lipsticks as an impurity up to 10 mg kg^{-1}. On the other hand, the EC Regulation[45] banned Pb and its compounds as intentional ingredients in cosmetics. Also, The EC Regulation[45] banned the use of Cd, Ni, Sb, and Co as metallic ions or salts in the preparation of cosmetic formulations. The Health Canada sets out a list of banned or limited ingredients in cosmetics, where some elements such as As, Sb, Cd, Pb, Cr, and Hg and its compounds are banned. Also, the Health Canada determined appropriate limits for As, Cd, and Hg (3 μg g^{-1}), for Pb (10 μg g^{-1}), and for Sb (5 μg g^{-1}), as impurities in cosmetic products, while Germany set a limit for Cd as an impurity of 5 mg kg^{-1}.[47,48] The Cr(III) oxide green and Cr(III) hydroxide green are allowed for use as colorants in cosmetic products.[49] The EU

Table 4. Element contents* (mg kg^{-1}) in lipstick samples

Samples[b]	Country of production	Color	Price category	Al c_{sr} ± SD	Cd c_{sr} ± SD	Cr c_{sr} ± SD	Co c_{sr} ± SD
L1	China	orange	I	76 ± 1	0.054 ± 0.002^{c}	0.98 ± 0.03^{b}	n.d.^{a}
L2	Slovenia	pink	II	252 ± 1^{b}	0.145 ± 0.007^{efg}	1.15 ± 0.02^{c}	0.131 ± 0.002^{g}
L3	France	orange	I	163 ± 4	0.050 ± 0.002^{bc}	0.93 ± 0.02^{a}	0.041 ± 0.002^{cd}
L4	Turkey	red	I	22 ± 1	0.099 ± 0.005	0.97 ± 0.01^{b}	0.019 ± 0.001^{ab}
L5	London	purple	I	225 ± 2	0.071 ± 0.002^{c}	0.88 ± 0.02^{a}	0.064 ± 0.005^{ef}
L6	Serbia	red	III	358 ± 5	0.155 ± 0.002^{g}	1.35 ± 0.04^{d}	0.030 ± 0.002^{c}
L7	Poland	pink	II	111 ± 1^{a}	0.133 ± 0.002^{e}	2.05 ± 0.03	0.556 ± 0.007
L8	Poland	pink	II	111 ± 1^{a}	0.144 ± 0.002^{ef}	1.34 ± 0.04^{d}	0.022 ± 0.000^{b}
L9	Ireland	red	III	137 ± 1	0.114 ± 0.007^{d}	1.09 ± 0.04^{c}	0.081 ± 0.006^{f}
L10	Turkey	violet	I	487 ± 2	0.038 ± 0.002^{a}	0.91 ± 0.01^{a}	0.049 ± 0.002^{de}
L11	Poland	orange	I	4.35 ± 0.04	0.047 ± 0.004^{ab}	0.94 ± 0.05^{ab}	0.28 ± 0.01
L12	China	brown	II	189 ± 1	0.914 ± 0.005	3.16 ± 0.05	0.400 ± 0.007
L13	Germany	red	II	431 ±2	0.151 ± 0.005^{fg}	1.27 ± 0.02	n.d.^{a}
L14	Serbia	pink	I	258 ± 2^{b}	0.124 ± 0.002^{de}	4.68 ± 0.02	0.14 ± 0.01^{g}

Samples	Cu c_{sr} ± SD	Ni c_{sr} ± SD	Pb c_{sr} ± SD	Fe c_{sr} ± SD	Sb c_{sr} ± SD	Mn c_{sr} ± SD	Zn c_{sr} ± SD
L1	1.35 ± 0.01^{a}	0.30 ± 0.01^{b}	0.98 ± 0.04	74 ± 1	6.2 ± 0.2^{a}	0.82 ± 0.01^{b}	3.28 ± 0.01^{a}
L2	8.50 ± 0.1	0.61 ± 0.01	1.30 ± 0.05^{d}	657 ± 2	7.7 ± 0.1^{b}	2.63 ± 0.01^{c}	21.5 ± 0.1
L3	1.50 ± 0.06^{ab}	0.37 ± 0.01^{de}	0.85 ± 0.05^{bc}	92 ± 2	1.04 ± 0.06	0.49 ± 0.02^{a}	2.73 ± 0.02
L4	0.56 ± 0.01	0.24 ± 0.01^{a}	0.76 ± 0.05^{ab}	143 ± 1	1.71 ± 0.07	2.77 ± 0.06^{c}	5.99 ± 0.02^{b}
L5	1.87 ± 0.05^{c}	0.38 ± 0.01^{e}	1.80 ± 0.04	17.4 ± 0.2	0.60 ± 0.02	1.01 ± 0.01	3.07 ± 0.01^{a}
L6	0.96 ± 0.01	0.44 ± 0.01	0.71 ± 0.05^{ab}	971 ± 12	9.2 ± 0.2^{c}	1.61 ± 0.03	9.49 ± 0.06
L7	1.92 ± 0.01^{c}	1.32 ± 0.02	19.7 ± 0.4	549 ± 4	6.95 ± 0.06^{a}	3.09 ± 0.04^{d}	6.00 ± 0.05^{b}
L8	1.14 ± 0.01	0.36 ± 0.02^{cd}	1.35 ± 0.04^{d}	606 ± 1	7.7 ± 0.1^{b}	6.72 ± 0.01	13.6 ± 0.1
L9	0.72 ± 0.01	0.31 ± 0.02^{bc}	0.70 ± 0.04^{ab}	353 ± 3	4.36 ± 0.03	2.95 ± 0.02^{cd}	4.65 ± 0.02
L10	1.73 ± 0.01	0.24 ± 0.01^{a}	0.55 ± 0.04^{a}	4.9 ± 0.1	4.95 ± 0.04	0.54 ± 0.01^{a}	2.05 ± 0.02
L11	1.62 ± 0.02^{b}	0.79 ± 0.02^{f}	0.86 ± 0.03^{c}	18.6 ± 0.3	3.03 ± 0.09	0.31 ± 0.01	1.44 ± 0.01
L12	3.69 ± 0.01	0.96 ± 0.02	7.57 ± 0.06	3850 ± 17	6.8 ± 0.03^{a}	5.54 ± 0.05	14.2 ± 0.1
L13	1.88 ± 0.03^{c}	0.82 ± 0.01^{f}	1.05 ± 0.05	801 ± 5	9.1 ± 0.2^{c}	1.48 ± 0.01	12.17 ± 0.09
L14	4.95 ± 0.01	1.43 ± 0.02	7.33 ± 0.08	46.3 ± 0.1	6.81 ± 0.04^{a}	0.86 ± 0.01^{b}	6.58 ± 0.01

*Mean ± standard deviation, $n = 3$; n.d. – not detected; values with different letters within the columns are statistically different at $p < 0.05$ by Tukey's test

banned Cr(VI) which can still be in these coloring agents.[45,49] Moreover, as opposed to the other elements, Cr is not limited as a cosmetic's impurity. Also, some countries (Germany and Canada) adopted the national limits to define the maximum allowable amount of Sb as an impurity in cosmetics (5–10 mg kg^{-1}).[47,48]

Concentrations of analyzed potentially toxic elements in lipsticks were below reported limits, but it was needed to evaluate the possibility of potential health risk (ADD, HQ, HI, and RfD) due to daily exposure of these elements through lipstick consummation.

3. 5. Health Risk Assessment

The oral reference doses (RfD) for Co, Cu, Cr, Cd, Fe, Ni, Zn, and Mn are 3×10^{-4}, 4×10^{-2}, 3×10^{-3}, 1×10^{-3}, 7×10^{-1}, 2×10^{-2}, 3×10^{-1}, 1.4×10^{-1}, respectively.[50–54] Lead, chromium, and cadmium belong to toxic and potentially carcinogenic substances. According to US EPA, it is inappropriate to develop an RfD value for inorganic lead because the degree of uncertainty about the health effects of lead is quite low.[12,55]

The mean daily intake of potentially toxic elements for adults (ADD) and potential toxic health risk effects (HQ and HI) are given in Table 5. Since As, Pb, Cd, and Cr are classified by the US EPA[50–52] as being carcinogenic agents, HQ of Cd and Cr were used to calculate HI.

The ADD values of Co, Cu, Fe, Ni, Zn, Mn, Cr, and Cd varied from n.d. to 2.7×10^{-4}; 2.8×10^{-4} to 4.2×10^{-3}; 1.9×10^{-3} to 4.8×10^{-1}; 1.2×10^{-4} to 7.0×10^{-4}; 7.1×10^{-4} to 1.1×10^{-2}; 1.5×10^{-4} to 3.3×10^{-3}; 3.7×10^{-4} to 2.3×10^{-3}; 1.9×10^{-5} to 7.6×10^{-5}, respectively. The mean concentrations for daily intake decrease in the following order: Fe > Zn > Cu~Mn > Cr > Ni > Co > Cd.

The obtained ADD values were lower than the RfDs, which indicated that there would not be any adverse health effects. A study by Zakaria and Ho[12] also reported that there was no significant health risk due to the exposure of

Table 5. Health risk assessment for the exposure to Co, Cu, Fe, Ni, Zn, Mn, Cr, and Cd in the lipstick samples

Sample	Co		Cu		Fe		Ni	
	ADD	HQ	ADD	HQ	ADD	HQ	ADD	HQ
L1	n.d.	n.d.	6.6×10^{-4}	0.017	3.6×10^{-2}	0.052	1.5×10^{-4}	0.007
L2	6.4×10^{-5}	0.215	4.2×10^{-3}	0.105	3.2×10^{-1}	0.462	3.0×10^{-4}	0.015
L3	2.0×10^{-5}	0.067	7.4×10^{-4}	0.018	4.5×10^{-2}	0.065	1.8×10^{-4}	0.009
L4	9.3×10^{-6}	0.031	2.8×10^{-4}	0.007	7.0×10^{-2}	0.100	1.2×10^{-4}	0.006
L5	3.1×10^{-5}	0.104	9.2×10^{-4}	0.023	8.6×10^{-3}	0.012	1.9×10^{-4}	0.009
L6	1.5×10^{-5}	0.049	4.7×10^{-4}	0.012	4.8×10^{-1}	0.683	2.2×10^{-4}	0.011
L7	2.7×10^{-4}	0.912	9.4×10^{-4}	0.024	2.7×10^{-1}	0.386	6.5×10^{-4}	0.032
L8	1.1×10^{-5}	0.036	5.6×10^{-4}	0.014	3.0×10^{-1}	0.426	1.8×10^{-4}	0.009
L9	4.0×10^{-5}	0.133	3.5×10^{-4}	0.010	1.7×10^{-1}	0.248	1.5×10^{-4}	0.008
L10	2.4×10^{-5}	0.080	8.5×10^{-4}	0.021	2.4×10^{-3}	0.003	1.2×10^{-4}	0.006
L11	1.4×10^{-4}	0.459	8.0×10^{-4}	0.020	9.1×10^{-3}	0.013	3.9×10^{-4}	0.019
L12	2.0×10^{-4}	0.656	1.8×10^{-3}	0.045	1.9×10^{-3}	2.707	4.7×10^{-4}	0.024
L13	n.d.	n.d.	9.2×10^{-4}	0.023	3.9×10^{-1}	0.563	4.0×10^{-4}	0.020
L14	6.9×10^{-5}	0.230	2.4×10^{-3}	0.061	2.3×10^{-2}	0.033	7.0×10^{-4}	0.035

Sample	Zn		Mn		Cr		Cd		HI[a]
	ADD	HQ	ADD	HQ	ADD	HQ	ADD	HQ	
L1	1.6×10^{-3}	0.005	1.6×10^{-3}	0.005	4.8×10^{-4}	0.161	2.7×10^{-5}	0.027	0.188
L2	1.1×10^{-2}	0.035	1.1×10^{-2}	0.035	5.7×10^{-4}	0.189	7.1×10^{-5}	0.071	0.260
L3	1.3×10^{-3}	0.004	1.3×10^{-3}	0.004	4.8×10^{-4}	0.152	2.5×10^{-5}	0.025	0.177
L4	2.9×10^{-3}	0.010	2.9×10^{-3}	0.010	4.1×10^{-4}	0.138	4.9×10^{-5}	0.049	0.187
L5	1.5×10^{-3}	0.005	1.5×10^{-3}	0.005	3.7×10^{-4}	0.125	3.5×10^{-5}	0.035	0.160
L6	4.7×10^{-3}	0.016	4.7×10^{-3}	0.016	5.7×10^{-4}	0.191	7.6×10^{-5}	0.076	0.267
L7	2.9×10^{-3}	0.010	2.9×10^{-3}	0.010	8.7×10^{-4}	0.291	6.5×10^{-5}	0.065	0.356
L8	6.7×10^{-3}	0.022	6.7×10^{-3}	0.022	5.7×10^{-4}	0.190	7.1×10^{-5}	0.071	0.261
L9	2.3×10^{-3}	0.008	2.3×10^{-3}	0.008	4.6×10^{-4}	0.153	5.6×10^{-5}	0.056	0.209
L10	1.0×10^{-3}	0.003	1.0×10^{-3}	0.003	3.8×10^{-4}	0.128	1.9×10^{-5}	0.019	0.147
L11	7.1×10^{-4}	0.002	7.1×10^{-4}	0.002	4.0×10^{-4}	0.132	2.3×10^{-5}	0.023	0.155
L12	7.0×10^{-3}	0.023	7.0×10^{-3}	0.023	1.6×10^{-3}	0.518	4.5×10^{-5}	0.450	0.968
L13	6.0×10^{-3}	0.020	6.0×10^{-3}	0.020	6.2×10^{-4}	0.208	7.4×10^{-5}	0.074	0.282
L14	3.2×10^{-3}	0.011	3.2×10^{-3}	0.011	2.3×10^{-3}	0.768	6.1×10^{-5}	0.061	0.829

[a]H I = ΣHQ(Cr)+ HQ(Cd)

Pb, Cd, and Cr in lipsticks from Malaysia, USA, Korea, France, and the United Kingdom.

The HQ risk value for Fe in one lipstick sample was 2.707 and indicates the potential of an adverse effect to human health, but low risk. The HQ values for other elements were below 1, indicating an acceptable level and no significant toxic health risk for lipstick users. The order of severity of the heavy metal total health risk is Fe > Cr > Co > Cd > Cu > Ni ~ Zn > Mn. The HI values for all lipsticks were below 1, which suggested that none of the analyzed potential toxic elements may pose a health risk.

3. 6. Chemometric Techniques for Correlation Analysis

To understand the connection between lipstick samples and potentially toxic elements contents, chemometric techniques PCA and CA were used. The obtained results for PCA are given in Fig. 1.

Two significant principal components are extracted based on the Kaiser criterion.[56] The first principal component (PC1) (with an eigenvalue of 10.51) explained 75.08% of the variance and the second principal component (PC2) (with an eigenvalue of 3.46) explained 24.72% of the variance. The first two PCs are enough to explain 99.80% of the pattern variation. Based on PCA, all potentially toxic elements have been classified in three fully separated groups. Iron is located on the negative side of PC1 and on the negative side of PC2 and it is a major contributor to PC1. Aluminum is located on the negative side of the PC1 and positive side of PC2 and it is a major contributor to PC2. Other elements (Sb, Cd, Cr, Co, Cu, Ni, Pb, Zn, Mn) are located on the positive side of PC1 and the near-zero values of PC2. Such grouping of elements is probably based on the origin of elements in lipstick. The most abundant element

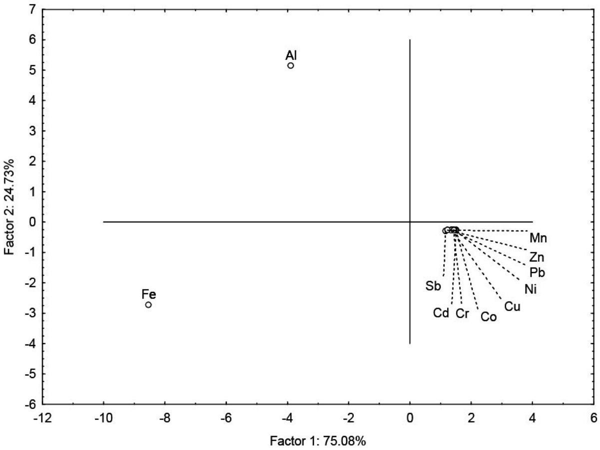

Figure 1. Principal component analysis of element contents in lipstick samples

in lipsticks is Fe, as a result of using metal-based pigments in color cosmetics such as orange, red, yellow, and black.[45] Aluminum is the light metal and adds as a pigment in lipsticks.[45] Other elements may be added as pigments or derivatives of oil such as mineral oils, paraffin, silicones.[9]

Cluster analysis (CA), as a multivariate method, is also used to classify elements and lipsticks in clusters based on their similarities.[57,58] Ward´s method with Euclidean distance was used to adopt measures.[58] The linkage distance was given as D_{link}/D_{max}, which represents the quotient between the linkage distances for a particular case divided by the maximal linkage distance.[57,58]

The dendrogram of the cluster analysis of the analyzed elements is presented in Fig. 2 and shows three separated clusters at $(D_{link}/D_{max}) \times 100 < 50$.

The first cluster contained Fe, the second cluster contained Al and the third cluster involved Sb, Cd, Cr, Co, Cu, Ni, Pb, Zn, and Mn. It can be seen that the CA of the elements provided the identical grouping obtained on the basis of PCA.

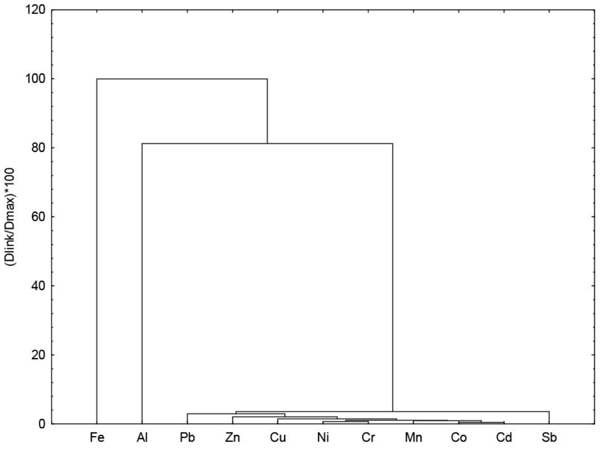

Figure 2. The dendrogram of the cluster analysis of elements based on their concentrations

The cluster analysis was applied to the analyzed samples using Ward's method, with Euclidian distances as the criterion for forming clusters of samples. Two separate clusters were obtained (Fig. 3).

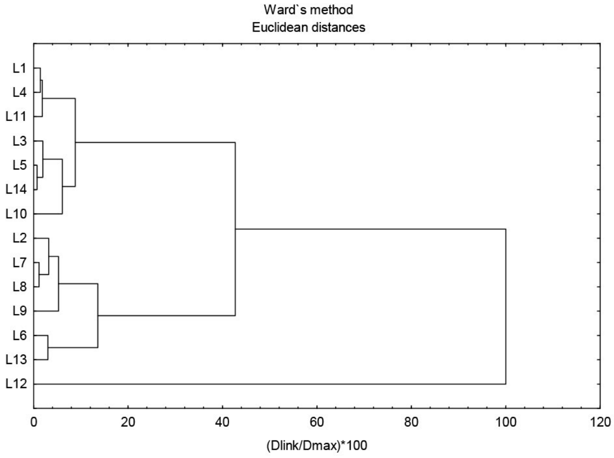

Figure 3. The dendrogram of the cluster analysis of lipsticks

The first cluster contained sample L12, which is the only one in brown color. The second cluster is divided into two sub-clusters. The first sub-cluster included samples L1 and L4 (88-Euclidean distances)/L11 which showed a close association with samples L5 and L14 (45-Euclidean distances)/L3 and L10. All lipsticks in this sub-cluster belong to category I regarding the price. The second sub-cluster included samples L7 and L8 (73-Euclidean distances)/L2 and sample L9 which showed a close association with samples L6 and L13 (185-Euclidean distances). All lipsticks in this sub-cluster belong to category II and III regarding the price.

4. Conclusion

The optimization and validation of the ICP OES method permitted an accurate and precise determination of Al, Cd, Cr, Co, Cu, Ni, Pb, Fe, Sb, Mn, and Zn in lipsticks. The recoveries for all elements in lipsticks were between 90% and 110%, except for Cd and Pb they were < 90% and > 110%, respectively. The element concentrations precision ranged from 0.3% to 4.9%. Lower RSD values were obtained for Zn, higher RSD values were obtained for Pb and Sb. Results for spike experiments in lipsticks have confirmed the suitability of the whole analytical procedure. In all samples, the concentrations of potentially toxic elements (Pb, Cd, and Cr) were within the level permitted in cosmetics. Only the sample L7 contained a higher concentration of lead compared to the acceptable and safe concentration given by the Health Canada and FDA (< 10 mg kg^{-1}), but lower than the permissible limit subscribed by German Federal Government (< 20 mg kg^{-1}) and EU which banned lead. The concentrations of other potentially toxic elements did not exceed the permissible limits. The HQ and HI values for Co, Cu, Fe, Ni, Zn, Mn, Cr, and Cd were less than 1, except HQ value for Fe (HQ < 3). The obtained results indicate that there is no risk to human health. Based on the comparison of element concentration, the examined elements were classified into three groups by the PCA interpretation. The first group contains Fe, the second group includes Al and the third group contains the other analyzed elements (Sb, Cd, Cr, Co, Cu, Ni, Pb, Zn, and Mn). CA confirms the results obtained by PCA. The cluster analysis separated the analyzed samples into two clusters regarding the price and color.

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Povzetek

Namen te študije je bil optimizirati in validirati metodo induktivno sklopljene plazme z optično emisijsko spektrometrijo (ICP OES) za hkratno določevanje enajstih potencialno strupenih elementov (Al, Cd, Cr, Co, Cu, Ni, Pb, Fe, Sb, Mn in Zn) v vzorcih rdečila za ustnice. Metodo smo evalvirali z uporabo metode standardnega dodatka. Izkoristki so bili za vse elemente v rdečilih med 90 % in 110 %, razen za Cd in Pb, za katera sta izkoristka bila <90 % in >110 %. Oceno zdravstvenega tveganja smo naredili z izračunom povprečnega dnevnega vnosa (ADD), kvocienta tveganja (HQ) in indeksa tveganja (HI). Najvišja povprečna vrednost za ADD je bila za Fe ($4{,}8 \times 10^{-1}$ mg kg^{-1} dan^{-1}) in najnižja za Co ($9{,}3 \times 10^{-6}$ mg kg^{-1} dan^{-1}). Za nobenega od elementov nismo ugotovili pomembnejšega zdravstvenega tveganja (HQ < 1), razen za Fe (HQ < 3), kar kaže na potencialno zdravstveno tveganje. Glede na PCA smo vse potencialno strupene elemente klasificirali v tri skupine. V prvi skupini je Fe, v drugi je Al, vsi ostali elementi pa so v tretji skupini. Analiza skupkov (CA) je za elemente pokazala enako grupiranje, kot je bilo opaženo s PCA. Če smo CA uporabili za analizirane vzorce, smo opazili dva ločena skupka. Prvi skupek je vseboval samo en vzorec, ki je bil rjave barve. Drugi skupek se je delil na dva podskupka. Prvi podskupek je vseboval vzorce iz kategorije I glede na ceno, drugi podskupek pa vzorce iz kategorij II in III glede na ceno.