

이온 크로마토그래피를 이용한 화장품 중 중금속 동시분석

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Simultaneous Determination of Heavy Metals in Cosmetic Products by Ion Chromatography

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요약: 화장품 중 불순물로서 미량의 중금속이 함유되어 있는 경우 이들의 피부흡착이 알려지기를 일으킬 수가 있다. 이에 중금속으로부터 안전한 화장품인지를 신속하고 정확하게 판단하는 것이 중요하여 색소원료와 화장품 중 미량의 중금속들(Pb^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Cd^{2+} 및 Mn^{2+})을 동시에 분석할 수 있는 이온크로마토그래피 분석법을 개발하였다. 8종의 중금속들은 이온교환 컬럼(IonPac CS5A)으로 잘 분리되었고, post-column 장치와 UV 분광기로 검출하였다. 0.1 ~ 1000 $\mu g/mL$ 농도범위에서 8종의 중금속들의 검량선은 선형적이었고($r^2 > 0.999$), 검출한계는 제품이 안전한지를 판단할 수 있는 $\mu g/L$ 수준이었다. 피크들의 머무름 시간과 면적의 상대표준편차는 0.21 %과 0.24 %이고, 회수율은 97 ~ 104 %이다. 이 결과들은 개발된 분석방법이 화장품 중의 미량의 중금속들을 신속하고 정확하게 분석할 수 있다는 것을 보여준다. 본 분석방법은 22개 화장품과 11개 색소원료 중의 중금속들의 함량을 분석하는데 활용하였다.

Abstract: No matter how small amount of heavy metals it may be cause skin allergies through percutaneous adsorption when existing in cosmetic products as impurities. In order to develop a highly sensitive method for simultaneous determination of Pb^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Cd^{2+} , and Mn^{2+} in coloring agents and cosmetic products with rapidity and accuracy, we carried out the determination on ion chromatography. All of these metals are well separated through a bifunctional ion-exchange column (IonPac CS5A) and detected by post-column reaction and spectrophotometric detection. The calibration graphs are linear ($r^2 > 0.999$) in the range 0.1 ~ 1000 $\mu g/mL$. Detection limits for 200 μL of sample solution are at the level of $\mu g/L$, which is sufficient for judging whether the product is safe or not. The relative standard deviations (RSDs) of the retention time and the peak area are less than 0.21 and 1.24 %, respectively. The recovery rates are 97 ~ 104 %. The new method was applied to analyze the amount of heavy metals which were contained in 22 cosmetic products and 11 coloring agents.

Keywords: heavy metals, cosmetic products, ion chromatography, simultaneous determination, coloring agents

1. Introduction

Many cosmetic products contain heavy metals such as lead, arsenic, mercury, cobalt, and nickel as ingredients or impurities. Recent research has reported

that these metals can easily cause many types of skin problems[1,2]. The use of some heavy metals in cosmetics has been controversial due to the biological accumulation of those metals and their toxicity in the human body.

In most countries, it is legally prohibited to use lead, arsenic and mercury in skin cosmetic products, but

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there are no specific rules on other heavy metals, such as cobalt, nickel and copper[3]. It is also reported that those metals can cause allergic contact dermatitis or other skin problems[4,5]. To provide customers with safe cosmetics, it is necessary to control the amount of heavy metals used in cosmetic products. In this study, a fast and accurate analysis method for quality control of 8 water soluble heavy metals such as Pb^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Cd^{2+} , and Mn^{2+} in cosmetic products using Ion chromatography (IC) and post-column reaction will be proposed. The new method will be effective in the determination of water soluble heavy metals in complex matrices such as cosmetic products.

ICP-MS, ICP, GF-AAS and Flame AAS are some examples of heavy metal analysis techniques. ICP-MS is one of the most favored analysis techniques because it is fast and highly sensitive in metal analysis. However, ICP-MS cannot achieve simultaneous analysis of trace metals such as Fe, Cu, Zn, and Ni because of the interference from polyatomic ion which is obtained by the reaction of Ar, N, O, H, and so on [1,6,7]. Also, ICP-MS, GF-AAS, and Flame AAS are not suitable for analysis of trace water soluble metals because of the interference of residual organic materials in the test sample.

Ion chromatography (IC) is a proper method for the separation and determination of water soluble trace heavy metals in complex matrices because it does not have any interference by residual organic materials. It is useful for quality control of cosmetic products since it is an inexpensive and accurate technique in view of instrumentation costs and maintenance.

2. Materials and Methods

2.1. Materials

4-(2-Pyridylazo)resorcinol (PAR), pyridin-2,6-dicarboxylic acid (PDCA), formic acid, potassium sulfate, 2-dimethylaminoethanol, ammonium hydroxide and sodium bicarbonate were purchased from Sigma-Aldrich (Broendby, Denmark). Metal ion standard solutions of (1000 mg/L) of lead (II), Iron (II), copper (II), nickel (II), zinc (II), cobalt (II), cadmium (II), and man-

ganese (II) were purchased from Fluka (Buch, Switzerland). All solvents used in this study were analytical grade.

2.2. Instrumentation

Determination of the metal ions were carried out by ion chromatography, Dionex ICS 2500 system (Dionex, Sunnyvale, CA, USA) consisting of GP50 pump, AS50 autosampler, PC10 Post-column pneumatic Delivery Package and PDA-100 photodiode array detector. Chromeleon software, version 6.60 was used to process all the data obtained. The column was Dionex IonPac CS5A (4 × 250 mm) analytical which was protected by a guard column (4 × 50 mm, Dionex IonPac CG5A, USA).

2.3. Sample Preparation

One gram of samples were weighed and placed in 15 mL polypropylene centrifuge tubes with 10 mL of ethanol-hexane (1 : 1, v/v) solution. The mixtures were sonicated for 15 min, followed by centrifuge for 20 min at 2,000 rpm. After the solution was decanted, this process was repeated 2 times in a low. The sample were dried at 60 °C for 12 h. Dried sample with 10 mL of eluent solution were heated 90 °C water bath for 1 h. After cooling the samples to room temperature, they were sonicated for 15 min. The solution was filtered on a PVDF membrane filter (0.45 mm) and then analyzed by IC.

2.4. Method of Analysis

The samples were analyzed through IC by using PDA. The operation condition was summarized in Table 1. This method utilized a post-column reaction with a color reagent (PAR).

2.5. Method Validation

The specificity could be tested with photodiode array detector. To check the precision of the method, multiple replicate analysis of a standard solution was performed. The relative standard deviations (RSDs) of the retention time and the peak area were calculated as the reproducibility of data. The linearity of the method was

Table 1. Operation Conditions of Ion Chromatography for Determination of Water-soluble Pb^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Cd^{2+} , and Mn^{2+}

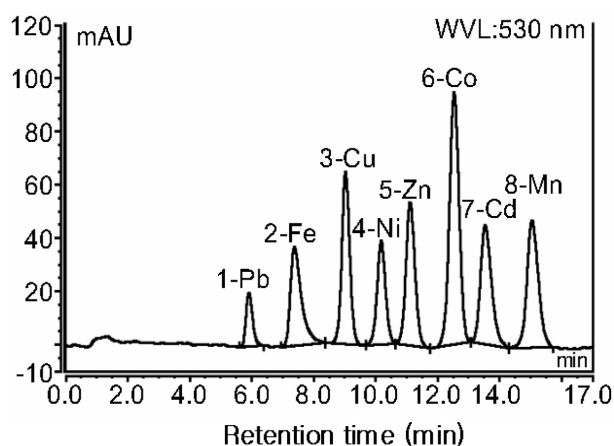
System	Dionex	ICS	2500
Column	IonPac CS5A Analytical (4 × 250 mm) IonPac CG5A Guard (4 × 50 mm)		
Eluent	PDCA 7.5 mM Potassium hydroxide 66 mM Formic acid 74 mM Potassium sulfate 5.6 mM		
Post-column reagent	PAR 0.65 mM 2-Dimethylaminoethanol 1.0 M Ammonium hydroxide 0.5 M Sodium bicarbonate 0.3 M		
Flow rate	0.7 mL/min		
Reagent flow rate	0.35 mL/min		
Mixing device	375- μ L Knitted reaction coil		
Column of injection	100 μ L		
Detector wavelength	530 nm		

investigated in the range of 0.1 ~ 1000 μ g/mL for the metals tested. The result for the linearity of the method was evaluated in the range 0.1 ~ 1000 μ g/mL for the test metals. The recovery rate was obtained from the spiking test, which added standard solution with constant concentration to the analyzing cosmetic product in order to check the accuracy of the test. The detection limit (signal-to-noise ration of 3 : 1) of this method was defined according to IUPAC and the ACS[8].

3. Results

3.1. Chromatographic Separation

Hydrated and weakly complexed heavy metals could be separated as cations on a cation exchange column. If weak organic acids such as oxalic, citric and tartaric acid were used as chelating agent in the eluent, the net charge on the metal would be reduced, because the chelating agents would be anionic state in solution above their pK_a 's. The selectivity of the separation was actually due to the different degrees of association between the metals and the chelating agents that were producing different net charges on the metal complexes.

**Figure 1.** Chromatogram of standard metals. Peaks (μ g/mL) : Pb = 10, Fe = 0.25, Cu = 0.5, Ni = 0.5, Zn = 0.5, Co = 0.5, Cd = 2.5, Mn = 1.0 (530 nm).

If sufficiently strong chelating agents were used in high concentration, the net charge of the metal complexes could be negative. These anionic metal complexes were separated by anion exchange on the IonPac CS5A column, which was allowing metals to be separated as cations and anions on a single column[9]. The common chelating agent was oxalic acid. But, oxalic acid did not elute the iron. Using this chelating agent, cadmium and manganese were eluted together. Therefore, Fe^{2+} , Cd^{2+} , and Mn^{2+} could not simultaneously be determined from oxalic acid[10,11]. In this study, PDCA chelating agent was used for metal separation. PDCA forms strong anionic complexes with most metal ions ; thus the separation of metals with PDCA on the IonPac CS5A column was an anion-exchange separation. Figure 1 showed that all metals were simultaneously separated well with better resolution. If this method could be applied in cosmetic samples, it would improve the accuracy of determining toxic metals in the quality control process.

3.2. Optimization of Spectrophotometric Detection

Because most hydrated and weakly complexed metals would be precipitated in a suppressor, therefore, it could not be detected by conductivity. Also, with a few exceptions, heavy metals could not be detected by direct UV absorbance. Therefore, the metal complexing agent was added to post-column to form a light-ab-

Table 2. Relative Standard Deviations (RSDs), Linear Ranges, Correlation Coefficients and Detection Limits

Metal	RSD (%)		Linear range ($\mu\text{g/mL}$)	Correlation coefficients (r^2)	Detection limit ($\mu\text{g/L}$)
	Retention time (min)	Peak area			
Pb	0.210	1.02	2.5 ~ 40.0	0.9986	41
Fe	0.166	1.13	0.1 ~ 1000	0.9995	3.4
Cu	0.214	1.08	0.1 ~ 10	0.9992	5.5
Ni	0.184	0.99	0.1 ~ 40	0.9997	6.1
Zn	0.16	1.04	0.2 ~ 1000	0.9992	5.8
Co	0.137	1.16	0.2 ~ 200	0.9991	3.4
Cd	0.144	1.24	0.8 ~ 12.5	0.9988	19
Mn	0.072	0.98	0.2 ~ 100	0.9995	9.8

sorbing complex (Dionex Application Note 10, 2000). A number of metal complexing agents were often used for the determination of heavy metals with high sensitivity, e. g., dithizone, PAR, and 8-hydroxyquinoline-5-sulfonate (HQS)[12]. However, dithizone and HQS could not be used under the same conditions for different metals. Consequently, they were not suitable for the simultaneous detection of metals in chromatographic analysis. PAR was used as derivatizing agent because it could produce stable and colorful derivatives with the most metals under the same conditions. Optimum flow rate and concentration combination of PDCA and PAR with stable baseline and high sensitivity could low the detection limits, so this could be accepted as an satisfactory process for safe cosmetic products.

3.3. Validation of the Method

Distinct peaks with good separation were obtained using Ion chromatography. The specificity of the each metal was validated by the evaluation of the peak homogeneity from the UV spectrum of the photodiode array detector. The method showed a good linearity ($r^2 > 0.999$) with the test metals for concentration ranging from 0.1 to 1000 $\mu\text{g/mL}$. When 200 μL of sample was injected, the detection limits (signal-to-noise ratio 3 : 1) of this method were 41, 3.4, 5.5, 6.1, 5.8, 3.4, 19, and 9.8 $\mu\text{g/L}$ for Pb^{2+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Cd^{2+} , and Mn^{2+} , respectively. The reproducibility was de-

Table 3. Concentrations and Recoveries of Metals Spiked in Eye Shadow

Metals	Concentration ($\mu\text{g/mL}$)	Spiked ($\mu\text{g/mL}$)	Found ($\mu\text{g/mL}$)	Recovery (%)
Pb	-	50	50.56	101.1
Fe	75.7	5	80.31	99.5
Cu	2.55	5	7.38	97.7
Ni	5.76	5	11.04	102.6
Zn	50.34	5	55.82	100.9
Co	0.26	5	5.34	101.5
Cd	-	5	4.88	97.6
Mn	17.83	5	23.65	103.6

termined by multiple analysis of a standard solution. The relative standard deviations (RSDs) of the retention time and the peak area were less than 0.21 and 1.24 % respectively. The linearity, the detection limits and the relative standard deviations were summarized in Table 2. The recovery rates were 97 ~ 104 %, shown in Table 3.

3.4. Analysis of Samples

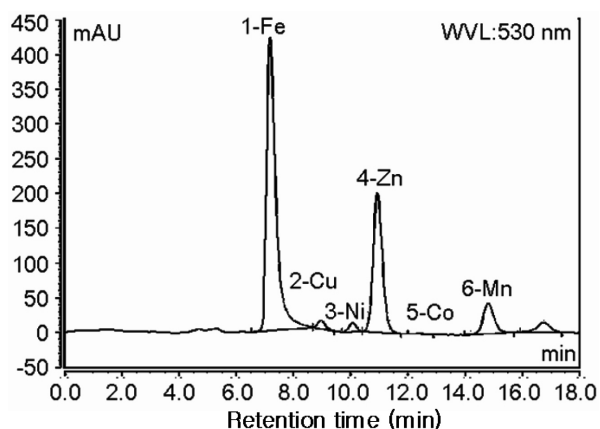
As a validation of the analytical technique, coloring agents and cosmetic products could be analyzed. Figure 2 showed the chromatogram of all the metals from coloring agent. The concentrations of the metals in coloring agents and cosmetic products were presented in Table 4, Table 5, respectively.

Lead and cadmium which can cause serious problem of safety on the skin were not detected in the coloring agent and cosmetic product. Copper, nickel, cobalt, and manganese were not detected in all of the cosmetic products except some of coloring agents. It means that all the cosmetic products on the test for skin purpose are safe for heavy metals except some coloring agents. However, in order to prevent cosmetic products from contamination of heavy metals, which were originated from raw materials, the quality control process for heavy metals is definitely necessary in the production of cosmetic products.

Table 4. Determination of Metals in Coloring Agents

Samples	Concentration ($\mu\text{g/mL}$)							
	Pb	Fe	Cu	Ni	Zn	Co	Cd	Mn
Ultramarine pink	-	75.7	2.5	5.7	50.3	0.3	-	17.8
Ultramarine blue	-	156.3	1.8	-	100.9	0.5	-	34.9
Cobalt blue	-	2.2	0.4	-	21.6	177.9	-	0.8
Chromium oxide green	-	30.2	0.4	-	27.1	-	-	8.4
Unipure green	-	-	0.9	1.2	28.5	-	-	-
I.O. black	-	719.9	-	-	13.8	-	-	80.1
Mapico black	-	789.8	-	-	17.0	-	-	7.7
Mapico red	-	666.8	6.6	-	103.3	2.9	-	67.7
Soft-TEX red	-	616.9	-	-	16.6	-	-	9.4
Mapico yellow	-	541.2	-	4.5	22.9	7.2	-	16.9
Socovit brown	-	-	-	19.5	31.1	4.5	-	27.5

* Above were the average concentration values of 3 times in injection

**Figure 2.** Chromatogram of ultramarine pink coloring agent (530 nm).

4. Conclusion

We have developed a simultaneous determination method of water soluble trace heavy metals in cosmetic products and coloring agents. The proposed techniques show that ion chromatography with an IonPac CS5A column and a post-column reaction system can be used for fast and accurate determination of water soluble heavy metals in cosmetic samples. Consequently, this proposed method for the simultaneous determination of heavy metals was proven to be fast and sensitive due to rare interference, which can improve the quality

Table 5. Determination of Metals in Cosmetic Products

Samples	Concentration ($\mu\text{g/mL}$)							
	Pb	Fe	Cu	Ni	Zn	Co	Cd	Mn
Eye shadow pink	-	3.7	2.5	-	679.3	-	-	3.9
Eye shadow blue	-	66.9	-	-	715.8	-	-	-
Eye shadow yellow	-	7.3	0.3	-	508.2	-	-	1.7
Eye shadow purple	-	2.2	-	-	181.3	-	-	-
Make up base A	-	1.3	-	-	173.2	-	-	-
Make up base B	-	0.5	-	-	59.4	-	-	-
Make up base C	-	-	-	-	62.7	-	-	-
Foundation A	-	0.8	-	-	116.7	-	-	-
Foundation B	-	2.2	-	-	32.6	-	-	-
Foundation C	-	8.4	0.5	-	88.7	-	-	-
Glose A	-	1.1	-	-	25.6	-	-	-
Glose B	-	2.9	2.6	-	26.9	-	-	-
Glose C	-	0.6	-	-	26.6	-	-	-
Lip stick A	-	2.4	0.4	-	24.1	-	-	-
Lip stick B	-	-	-	-	17.5	-	-	-
Lip stick C	-	2.0	-	-	23.5	-	-	-
Mascara A	-	55.7	-	-	27.8	-	-	-
Mascara B	-	52.5	-	-	57.3	-	-	-
Mascara C	-	224.1	-	-	68.3	-	-	-
Eye liner A	-	342.5	-	-	118.0	-	-	-
Eye liner B	-	115.7	-	-	33.2	-	-	9.6
Eye liner C	-	683.1	-	-	78.2	-	-	-

Above were the average concentration values of 3 times injection.

control process for safe cosmetic products.

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