

Determination of hexavalent chromium in cosmetic products by ion chromatography and postcolumn derivatization

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Chromium hydroxide green [$\text{Cr}_2\text{O}(\text{OH})_4$] and chromium oxide green (Cr_2O_3) are colouring agents for use in cosmetic products. These colourants may contain chromium (VI), which cause skin allergies through percutaneous adsorption on the skin. Eye shadow is a representative cosmetic product in which significant colourants are used. We analysed the chromium (VI) in the eye shadows by ion chromatography and post column derivatization. We optimize conditions of chromium (VI) analysis in eye shadows. During the pretreatment procedure, there are no exchange of chromium (III) to chromium (VI). This method has a limit of quantification for chromium (VI) of $1.0 \mu\text{g l}^{-1}$, recovery rate of $100 \pm 3\%$ and analysis time less than 10 min. This result is 300 times more sensitive than the high-performance liquid chromatography method. We applied the optimized method to analyse 22 eye shadows and 6 colouring agents. 2 out of 22 of the products contained more than 5 mg l^{-1} . In our previous work, 5 mg l^{-1} of Cr represented a threshold level. There was much more Cr(VI) in the colouring agents. The Cr(VI) in one of the colouring agents was 97.6 mg l^{-1} .

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Hexavalent chromium [Cr(VI)] is a well-known skin contact allergen (1, 2). Cr(VI) is considered more toxic species than trivalent chromium (3). It is therefore important to consider the oxidation state of chromium when analysing it. There are conventional analytical procedures for the detection of Cr(VI) (4, 5) and 1,5-diphenyl carbazide (DPC) spectrometric method in particular is a very robust and common method of analysis (6–8). However, the colourimetric method easily interferes with coloured compounds (9, 10). It is thus important to study the detection of Cr(VI) samples, which are used on human skin and contain significant levels of interfering materials.

Eye shadow is a representative example of a group of cosmetic products in which significant colourants are used. Chromium hydroxide green [$\text{Cr}_2\text{O}(\text{OH})_4$] and chromium oxide green (Cr_2O_3) are allowed for use in cosmetic products listed in the EU Annex (4), part 1. However, these colouring agents may contain Cr(VI), which cause skin allergies through percutaneous adsorption on the skin (11, 12).

We reported on the Cr(VI) analysis method through HPLC, and on the safety evaluation of

chromium in makeup products (13). However, the limit of quantification (LOQ) of HPLC method is not very low (0.3 mg l^{-1}), and pre-column derivatization is time-consuming step. On the bases of the considerations of a previous study, we wish to propose an analytical method to determine of Cr(VI) in eye shadows by ion chromatography (IC).

The analysis of chromium ion based on an IC method is becoming increasingly popular and complementary to atomic adsorption spectrometry, inductively coupled plasma mass spectrometry, atomic emission spectrometry methods (14). IC offers an accurate, a simple and a sensitive method for determination of Cr(VI) by spectrophotometric postcolumn detection with DPC (Dionex Application Note 80, 2000).

The pretreatment method is simpler than those for HPLC and UV methods. We also utilized a postcolumn reaction with a colour reagent, which can reduce time and uncertainty. We also validated this method of analysis in terms of accuracy, linearity, recovery and LOQ. We applied the optimized method to analyse 22 eye shadows and 6 colouring agents.

The aim of this study was to develop a effective method to detect the Cr(VI) in the eye shadows. This method will be useful for the development of safe cosmetic products.

Materials and Methods

22 Samples were purchased in Seoul in March 2005. Blue and green eye shadows were selected from 6 companies. 6 colouring agents were purchased from international chemical company.

Instrumentation

The IC was Dionex ICS 2500 system (Dionex, Sunnyvale, CA, USA). It consists of GP50 pump, AS50 autosampler, EG50 eluent generator and ED50 detector. CHROMELEON software, version 6.60 was used to process all data. The column was Dionex IonPac CS5A analytical (4 × 250 mm) which was protected by a guard column [Dionex IonPac CG5A analytical (4 × 50 mm)].

Reagents

DPC, pyridine-2,6-dicarboxylic acid (PDCA), sulphuric acid, sodium phosphate dibasic and ammonium acetate were from Sigma-Aldrich (Broendby, Denmark). Sodium iodide, lithium hydroxide, chromium(III) and chromium(VI) standard solution 1000 mg l⁻¹ were purchased from Fluka (Buch, Switzerland). All solvents were analytical grade.

Processing of samples

Eye shadow samples and colouring agents were weighed (1 g) 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 450 g. After the solution was removed, this process was repeated. The samples were dried at 60 °C for 12 h. Dried sample with 10 ml of eluent solution (Table 1) were heated at 90 °C water bath for 30 min.

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 analysed by IC.

Methods of analysis

The samples were analysed through IC by using photodiode array (PDA). The operation condition was summarized in Table 1. This method utilizes a postcolumn reaction with a colour reagent (DPC).

Method validation

The recovery of Cr(VI) was tested by analysing colouring agents. Into the 8 ml, 9 ml and 9.5 ml of eluent solution, 1 g of colouring agent was added. The 10 mg l⁻¹ of Cr(VI) ion standard solution was spiked, 2 ml, 1 ml and 0.5 ml, respectively. The linearity of the method was evaluated with 0.05, 0.1, 0.5, 1, 5 and 10 mg l⁻¹ of Cr(VI). The precision was determined by multiple analysis of colouring agent. We prepared 5 samples with 1 g of colouring agent. These samples were analysed through the whole method. The precision of the method was calculated as the relative standard deviation (%RSD). LOQ is the lowest concentration, which can be determined with acceptable precision. In our study, 0.005, 0.0025 and 0.001 mg l⁻¹ of Cr(VI) solution were analysed.

Results

Method development for isolation of Cr(VI) in eye shadows – optimized method

Pretreatment method. A great challenge exists in Cr(VI) detection in cases of cosmetic products rich in organics, including high amounts of colouring agents, Cr(III), powder-coating materials, etc. We removed the hydrophobic coating materials which prevent elution of Cr(VI) of the analysed solution by ethanol-hexane (1:1, v/v) solution. Then Cr(III) was reacted with PDCA in the eluent (Table 1) at 90 °C.

Table 1. IC operation conditions for determination of water-soluble Cr(III) and Cr(IV)

System	Dionex ICS 2500
Column	Dionex IonPac CS5A analytical (4 × 250 mm), Dionex IonPac CG5A analytical (4 × 50 mm)
Eluent	2 mM PDCA, 2 mM Na ₂ HPO ₄ , 10 mM NaI, 50 mM CH ₃ CO ₂ NH ₄ , 2.8 mM LiOH in deionized water
Postcolumn reagent	2 mM DPC, 10% CH ₃ OH, 0.9 N H ₂ SO ₄ in deionized water
Volume of injection	200 µl
Detector wavelength	520 nm for Cr(VI)

PDCA, pyridine-2,6-dicarboxylic acid.



Fig. 1. Reaction on column and separation of Cr(III) and Cr(VI). DPC, 1,5-diphenyl carbazide; PDCA, pyridine-2,6-dicarboxylic acid.

Cr(III) standard solution was pretreated and analysed. In this sample, Cr(VI) was not detected. We also applied the same process to the Cr(VI) standard solution. In the same manner, Cr(III) was not detected. This result shows that our pretreatment method is suitable for Cr analysis.

Optimized analysis condition for Cr(VI) detection. The Cr(VI) ion forms $\text{Cr}(\text{PDCA})_2^-$ complex in eluent system and Cr(VI) ion exists chromate (Dionex Technical Note 26, 1991). The $\text{Cr}(\text{PDCA})_2^-$ complex and chromate were isolated through column. Cr(VI) form Cr(VI)-DPC complex by postcolumn reaction (Fig. 1). These 2 complexes are detected at 365 nm [for $\text{Cr}(\text{PDCA})_2^-$ complex], 520 nm [for Cr(VI)-DPC complex], respectively. We optimized the analysis conditions for Cr(VI) detection. Figure 2 shows typical chromatograms for Cr(VI).

Method validation

After optimizing the analytical conditions, the Cr(VI) detection method was validated in terms of linearity, recovery, precision and LOQ. The recovery of Cr(VI) was tested by analysing colouring agents. The Cr(VI) ion standard solution was spiked with 10 mg l^{-1} of Cr(VI) concentration, giving a mean recovery of $100 \pm 3\%$. The linearity of the method was evaluated with 0.05, 0.1, 0.5, 1 and 5 mg l^{-1} of Cr(VI). The calibration

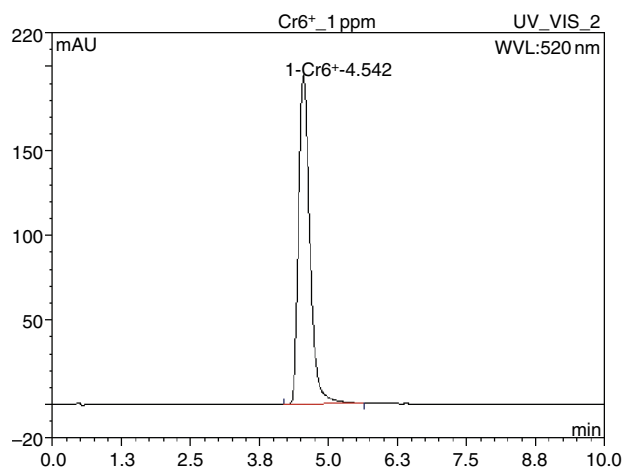


Fig. 2 Chromatogram of Cr(VI) standard solution (1 mg l^{-1}); flow rate = 1 ml min^{-1} ; $\lambda = 520 \text{ nm}$; injection volume = $200 \mu\text{l}$.

curve for Cr(VI) between 0.05 and 5 mg l^{-1} obtained with excellent linearity (Fig. 3). The precision was determined by multiple analysis of a colouring agent. 5 samples were analysed through the whole method. The precision of the method was calculated as 0.880% R.S.D.

Using injections of $200 \mu\text{l}$, the method has a LOQ for Cr(VI) of 0.001 mg l^{-1} , which is sufficient for determination of safe cosmetic products (13). This result is 300 times more sensitive than the HPLC method. The precision at the level of 0.001 mg l^{-1} was calculated as 0.978% R.S.D.

Analysis of Cr(VI) in eye shadows and colouring agents

The study covered 22 eye shadows from 6 brands and 6 colouring agents from 2 companies. The concentrations of the chromium ion in the eye shadows are summarized in Table 2. 2 out of 22 of the products contained more than 5 mg l^{-1} . There was much more Cr(VI) in the colouring agents. The concentration of Cr(VI) was surprisingly high in one colouring agent. The Cr(VI) in one of the colouring agents from Company A was 97.6 mg l^{-1} (Table 3).

Discussion

We developed a pretreatment and analysis method for Cr(VI) in cosmetic products. Cr(III) can be exchanged to Cr(VI) through redox reactions (15) during the pretreatment process. Critical points are the removing of the matrix, including powder-coating materials, and the minimization of exchange of Cr(III) to Cr(VI).

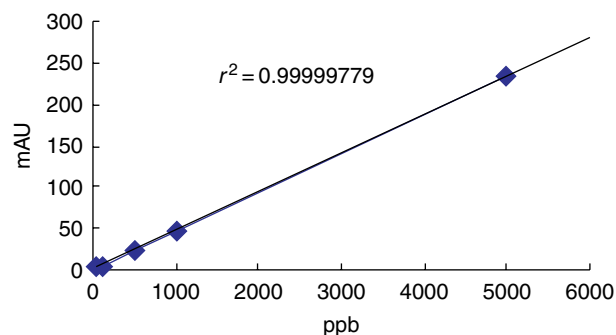


Fig. 3. The calibration curve for Cr(VI) detection. The calibration curve was obtained by least-square linear regression with the coefficient of correlation being 0.9999.

Table 2. Concentrations of Cr(VI) in eye shadows

Manufacturer	Product name	Concentration of Cr(VI) ($\mu\text{g l}^{-1}$)
B	04 apple green	1275.7
B	07 space blue	634.86
B	118 light aqua	464.08
B	106 oriental green	124.51
A	KH203	118.59
C	141	25.30
B	119 aqua blue	16.97
D	51 khaki green	7.83
D	13 satin blue	6.76
A	GA01	n.d.
A	BL02	n.d.
A	BL203	n.d.
A	BL20	n.d.
E	GR630	n.d.
E	BL524	n.d.
E	BL535	n.d.
E	BL568	n.d.
D	6 khaki	n.d.
D	4 sky blue	n.d.
F	8424 bobby blue	n.d.
F	8431 blue star	n.d.
C	135	n.d.

n.d. not detected.

Through the whole method, we confirmed that Cr(III) was not exchanged to Cr(VI).

Also, we optimized and validated the method in terms of linearity, recovery, precision and LOQ through IC. Through the analysis of Cr(VI), critical points are the pH of eluent and flow rate of eluent and postcolumn reagent.

The pH values of the sample and eluent systems are critical to the efficiency of the separation. The high pH values cause inhibition of the $\text{Cr}(\text{PDCA})_2^-$ complex formation, and low pH values cause a marked conversion of chromate ion to dichromate ion, which can be harmful to the column. The pH of the system and samples is chosen as 6.7 to allow optimum separation and detection (Dionex Technical Note 26. 1991).

The fast flow of postcolumn reagent causes noise that decreases sensitivity of detection. The too-slow flow of postcolumn reagent causes ineffective formation of Cr(VI)-DPC complex that causes decreasing sensitivity of Cr(VI) detection. 1.1 ml min^{-1} of eluent flow and 0.3 ml min^{-1} of postcolumn reagent flow is the best condition for

Cr(VI) detection. This ratio gives us a sensitive and accurate results for Cr(VI) analysis.

We applied the optimized method to analyse 22 eye shadows and 6 colouring agents. Despite the fact that the regulations relating to cosmetic products do not permit Cr(VI) in cosmetic products sold in the EU, the Cr(VI) in one of the colouring agents was 97.6 mg l^{-1} . 2 out of 22 of the products contained more than 5 mg l^{-1} . 5 mg l^{-1} is the lowest level for safety on skin, according to the findings of our previous work. The threshold for allergic reactivity to Cr(VI) is 5 mg l^{-1} . 15% of positive allergic responses were appeared at the level of 5 mg l^{-1} (13). We hope that our method of discerning Cr(VI) by IC will be helpful for producing skin-safe cosmetic products.

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Table 3. Concentrations of Cr(VI) in colouring agents

Manufacturer	Lot number	Concentration of Cr(VI) (mg l^{-1})
1	i	2.6
1	ii	97.6
1	iii	1.5
1	iv	20.1
1	v	1.73
2	1	0.165

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