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Extraction and Quantification of Hexavalent Chromium in Eyeshadow Toy Cosmetics Using Ultrasonic Nebulization Mass Spectrometry and Anion Exchange Chromatography: Strategies to Prevent Chromium Species Interconversion

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ABSTRACT

Hexavalent chromium is a potent carcinogen occasionally detected in cosmetics as a trace impurity originating from raw materials or manufacturing processes. Its presence in children's toy eyeshadows poses serious health concerns due to potential dermal or mucosal absorption. Accurate Cr (VI) quantification is hampered by interconversion with Cr (III), especially during extraction and chromatographic analysis. This study presents a robust and sensitive analytical method for selective Cr (VI) determination in complex cosmetic matrices using ion chromatography coupled with inductively coupled plasma mass spectrometry, enhanced by ultrasonic nebulization. An optimized extraction protocol was developed employing a weakly alkaline buffer containing 2 mM tetrabutylammonium hydroxide to stabilize Cr (VI) and prevent reduction. The final method achieved a limit of detection of 0.3 µg/L and limit of quantification of 0.9 µg/L. Recovery experiments demonstrated that more than 99% of Cr (VI) could be efficiently extracted after two mild extraction cycles, whereas Cr (III) exhibited poor recoverability, even when subjected to harsh extraction conditions. Consequently, Cr (III) was quantified by subtracting Cr (VI) from total chromium, determined after microwave-assisted digestion. Method validation using in-house prepared reference materials confirmed satisfactory trueness (11-15%) and precision (CV ≤ 7.9%). Application to seven commercial toy eyeshadows revealed Cr (VI) levels below the regulatory limit (1.0 mg/kg), although total chromium concentrations indicated the prevalent presence of Cr (III). This methodology provides a reliable and regulatory-compliant approach for Cr (VI) speciation in cosmetic products, supporting consumer safety, particularly in paediatric populations.

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Introduction

Chromium is extensively used in personal care products (PCPs) and cosmetic formulations – such as eyeshadows, nail polishes, and lipsticks – alongside other metallic elements and pigments, contributing to distinctive characteristics including coloration, glittering, and bronzing effects [1-8]. PCPs represent a fundamental component of daily routines for millions of individuals worldwide [9,10]. Their increasing popularity has led to a substantial expansion in production and diversification of applications, particularly in contexts involving children, such as theatrical performances, masquerade events, and recreational activities that require the use of costume makeup [10-12].

Chromium exists in several oxidation states, each associated with distinct toxicological profiles [13-15]. According to the International Agency for Research on Cancer (IARC), metallic chromium and trivalent chromium [Cr (III)] are classified as Group 3 carcinogens, indicating limited evidence of carcinogenicity in humans and allowing for controlled use in cosmetics [8,16,17]. Conversely, hexavalent chromium [Cr (VI)] is classified as a Group 1 carcinogen, recognized for its mutagenic and carcinogenic potential, and is therefore subject to strict regulatory control to minimize human exposure [16,18-21]. Chromium-containing products may pose a risk of dermal absorption through contact with skin, lips, or the ocular mucosa [11,22,23]. The thin and highly permeable periocular skin, often exposed to eye cosmetics, is particularly vulnerable to percutaneous absorption, increasing the likelihood of irritative or allergic reactions [10,11,13,22,24]. Furthermore, biological variables such as sex, body weight, and fat distribution significantly modulate the physiological response to Cr exposure [25].

Eye cosmetics – including eyeshadows, eyeliners, and mascaras – typically consist of pigment dispersions formulated in emulsions, oils, or waxes, resulting in complex and highly variable matrices [1,6]. Despite adherence to Good Manufacturing Practices (GMP), impurities originating from raw materials, active substances, or processing residues may lead to contamination by heavy metals [9,11,14,22,23,26-33]. The absence of legally mandated impurity disclosure on cosmetic labels leaves consumers unaware of potential health risks [4,13-15,27]. This underscores the critical importance of the accurate identification and quantification of chromium species to ensure both consumer safety and regulatory compliance.

The safety of cosmetic products within the European Union is governed by Regulation (EC) No. 1223/2009, which permits the presence of metals only as "technically unavoidable traces" under strict toxicological evaluation [8]. The Scientific Committee on Consumer Products (SCCP) periodically reviews the safety of restricted substances. According to current literature, the maximum allowable concentration for total chromium is 1.0 mg/kg. If this threshold is exceeded, speciation studies become mandatory. For individual species, the maximum permissible levels are set at 1.0 mg/kg for Cr (VI) and 5.0 mg/kg for Cr (III) [16,20].

Reliable chromium speciation in cosmetics requires robust and sensitive analytical methodologies. As observed by Shang et al, the complex composition of cosmetic matrices poses considerable analytical challenges, especially due to potential interactions between analytes and background substances that may introduce interferences and compromise analytical accuracy [29]. Therefore, meticulous sample preparation is essential [2]. Conventional techniques – such as liquid-liquid and solid-liquid extraction – are commonly applied but typically involve multiple processing steps (e.g., agitation, heating, sonication, centrifugation, filtration), which may induce chemical alterations [29,34,35]. In particular, these methods may inadvertently modify the redox state of chromium species, emphasizing the need for speciation protocols that minimize analyte transformation and preserve the integrity of native species [32,36-38].

Alkaline extraction methods, such as the U.S. EPA Method 3060A, are frequently employed in speciation studies. This protocol utilizes a basic solution to selectively solubilize Cr (VI) while preserving the stability of oxyanionic species [16,39]. These oxyanionic species, including chromate and dichromate, are thermodynamically stable in alkaline conditions but can be readily reduced in acidic environments, where redox-active agents such as organic matter or certain transition metals may promote their conversion to Cr (III), thus compromising the reliability of speciation analyses. The use of an alkaline medium is therefore essential to minimize redox interconversion during extraction and preserve analytical integrity.

Quantification is typically achieved by ion chromatography (IC) coupled with spectrophotometric detection or inductively coupled plasma mass spectrometry (ICP-MS), the latter being preferred for trace-level analysis due to its superior sensitivity and specificity [7,19,37,40]. However, Cr determination via ICP-MS may suffer from polyatomic spectral interferences, particularly from ions with the same mass-to-charge ratio (m/z) as $^{52}Cr^{+}$, such as $^{40}Ar^{12}C^{+}$ or $^{35}Cl^{16}O1H^{+}$. These interferences necessitate the use of Dynamic Reaction Cell (DRC) technology to mitigate such effects through chemical reactions or collision-based ion suppression [40-42].

While chromium speciation has been extensively studied in environmental samples [36-38] and textile products, its investigation in cosmetic matrices remains markedly limited [43,44]. This knowledge gap is largely attributable to the intrinsic difficulties of developing selective extraction procedures capable of preventing interconversion between Cr (III) and Cr (VI). Only a few studies have successfully addressed the selective determination of chromium species in cosmetic formulations. Kang et al. proposed a method employing a cation-exchange column with ammonium acetate as the eluent, using pyridine-2,6-dicarboxylic acid (PDCA) to stabilize Cr (III) and post-column derivatization with 1,5-diphenylcarbazide (DPC) for Cr (VI) detection [34]. Analogous chromatographic approaches were adopted by Wüster et al., Senofonte et al., and Bruzzoniti et al. to achieve Cr speciation in complex matrices [40,45-46].

Despite these advancements, Perez et al. emphasized the significant lack of research focusing on chromium species in children's face products [47]. This deficit is especially alarming given the frequent presence of reducing organic compounds – such as antioxidants, preservatives, or botanical extracts – at elevated concentrations in such formulations. These substances can promote the reduction of Cr (VI) to Cr (III) during the extraction phase, thereby compromising the accuracy of analytical determinations. Such redox transformations may lead to a systematic underestimation of the toxicologically relevant Cr (VI) fraction, ultimately hampering risk assessment and regulatory enforcement.

To address these analytical challenges and mitigate Cr species interconversion, the present study proposes an innovative methodology derived from the protocol developed by Kang et al. [34]. The original clean-up step was re-evaluated and replaced with a targeted pre-treatment aimed at removing interfering organic matter while preserving analyte stability. A cationic ion-pairing reagent was introduced to stabilize Cr (VI) through complex formation during pre-treatment. Furthermore, the incorporation of an ultrasonic nebulizer minimized matrix-induced interferences and reduced the risk of contamination in the IC/ICP-MS system, thereby improving analytical precision. The chromatographic conditions were also optimized to prevent carryover and ensure compliance with regulatory detection limits. The proposed method, combining anion-exchange chromatography with ICP-MS detection, achieved a limit of detection (LoD) of 0.3 µg/L and a limit of quantification (LoQ) of 0.9 µg/L, offering a sensitive and reliable solution for Cr (VI) quantification in cosmetic matrices.

Materials and Methods

Chemicals and Materials

Milli-Q grade water (resistivity 18.2 MΩ·cm) was produced using a Millipore Synergy water purification system coupled with a Millipore RiOs-Essential-5 deionization unit (Merck, Darmstadt, Germany).

A certified potassium dichromate solution (1.000 g/L as Cr, Sigma-Aldrich, USA) was used for the preparation of Cr (VI) standard solutions. A certified chromium (III) nitrate solution for atomic absorption (1.000 g/L as Cr in 3% HNO_3, Solutions Plus Inc., Cincinnati, OH, USA) was used for the preparation of Cr (III) standard solutions.

A bronze-coloured powdered eyeshadow, manufactured in Europe and declared chromium-free by the producer, was used as a matrix for method optimization trials. Prior to use, the eyeshadow was tested by DRC-ICP/MS to confirm the absence of detectable chromium, thereby validating its suitability as a blank cosmetic matrix. Known amounts of Cr (VI) and/or Cr (III) standard solutions were spiked into this matrix to evaluate analytical performance under controlled conditions.

High-purity nitric acid (fuming HNO_3, 100%, Merck, Darmstadt, Germany) and certified reference standard solutions of Cr and Ir (1.000 g/L, Accu Trace Reference Standards, Accu Standard, New Haven, CT, USA) were used for the preparation of intermediate dilutions and final analytical solutions for total chromium and internal standard (IS), respectively.

The selection of ^{193}Ir as internal standard was based on established criteria for accurate and reproducible quantification in ICP-MS. A suitable IS should possess chemical and physical properties similar to those of the analyte and must be absent from the sample matrix to avoid endogenous interference. While iridium does not perfectly match all these ideal characteristics for chromium, ^{193}Ir was selected as a practical and effective compromise, offering acceptable similarity in ionization behaviour and plasma response. Its absence in the cosmetic matrix, combined with high signal stability and low susceptibility to common interferences, makes it a suitable and reliable internal reference for both total and speciated chromium determinations.

Currently, no certified reference materials (CRMs) specifically formulated for powder-based eyeshadows are commercially available for use in the development and validation of analytical methods. Therefore, two internal reference materials (IRMs) were prepared in-house by doping a chromium-free commercial powder-based eyeshadow matrix with known quantities of either Cr (III) or Cr (VI). The doping procedure was performed using the wet doping method via incipient wetness impregnation followed by rotary mixing, ensuring a uniform surface distribution of the spiked analytes across the powdered matrix. The chromium-free blank cosmetic matrix was used as the base material. Prior to doping, the powder was dried at 45°C for 24 hours to remove residual moisture and sieved to a particle size ≤ 100 µm using a nylon mesh sieve (150 mesh/inch, equivalent to 100 µm), manufactured by Eastar Filtration Industry Co., Ltd. (Hebei, China), composed of a nylon monofilament mesh screen with a plastic frame. For each IRM, 2.00 g of dried eyeshadow powder was weighed into a clean glass mortar. To reach the target concentrations of 5.0 mg/kg Cr (III) and 1.0 mg/kg Cr (VI), respectively, 400.0 µL of a 25 mg/L Cr (III) solution or a 5 mg/L Cr (VI) solution were added to the corresponding powder aliquots. The addition was carried out using a micropipette, delivering the solution in successive small aliquots (20-25 µL), uniformly distributed over the powder surface in concentric circular patterns. During dosing, the powder was continuously mixed using a PTFE pestle to promote uniform wetting and prevent localized oversaturation. After the complete addition of the standard solution, the moistened powders were transferred into 15-mL polyethylene tubes and homogenized using a rotary mixer at 60 rpm for 30 min to ensure full homogenization. The doped powders were subsequently dried in glass dishes in a ventilated oven at 45°C for 24 hours. After drying, they were sieved again through the same nylon mesh sieve (150 mesh/inch) to restore uniform particle size and break any residual aggregates. The IRMs were stored in airtight polypropylene vials under dry nitrogen at 4°C until use. Subsamples taken from each IRM batch were subjected to homogeneity and recovery testing, confirming relative standard deviations ≤ 7% and recovery rates within 90-110% of the expected values.

2,6-pyridinedicarboxylic acid (PDCA, Merck, Darmstadt, Germany), ammonium dihydrogen phosphate (Merck, Darmstadt, Germany), ammonium acetate (Merck, Darmstadt, Germany), ammonium hydroxide (Carlo Erba, Milan, Italy), and tetrabutylammonium hydroxide (TBAOH, Fluka AG, Buchs, Switzerland) were accurately weighed and dissolved in Milli-Q grade water to prepare the extractant solution. The purity of each reagent used in the analytical workflow was verified by DRC-ICP/MS to ensure the absence of chromium contamination.

Sodium iodide, originally proposed by Kang et al, was replaced with ammonium iodide. The rationale behind the introduction of iodide – specifically in the form of NH_4I – into the analytical procedure will be discussed in detail in the following section [34]. The NH_4I reagent was prepared starting from a 2.00 M NaI solution and a 0.50 M NH_4AcO solution, using a cation exchange process. A total of 20.0 g of strong cation exchange resin (Dowex 50Wx8, H^+ form, strongly acidic, 100–200 mesh, Merck, Darmstadt, Germany) was packed into an empty 30 mL polyethylene SPE cartridge, equipped with two pre-inserted polyethylene frits. The resin was first conditioned by washing with 300 mL of 1.00 M nitric acid to remove any residual chromium potentially released from the material; the eluate was monitored with DRC-ICP/MS to ensure complete elimination of total chromium. The resin was then rinsed with 100 mL of Milli-Q grade water. Subsequently, the resin was saturated with ammonium ions by eluting 150 mL of 0.50 M ammonium acetate through the cartridge until the eluate reached a pH of 6.6, indicating complete ion exchange from H^+ to NH_4^+. The resin was again rinsed with 100 mL of Milli-Q grade water and gently dried. Finally, 10 mL of 2.00 M NaI solution were passed through the NH_4^+-form resin. The resulting eluate, containing ammonium iodide, was collected and used as the final reagent. The solution was subsequently analysed by DRC-ICP/MS to confirm the absence of chromium contamination.

Ethanol and n-hexane were used in preliminary clean-up tests of the cosmetic matrix. To verify the absence of chromium contamination in these organic solvents, a control procedure was performed: 100 mL of each solvent was evaporated to dryness in an oven at 60°C, and the residue was reconstituted with 10 mL of Milli-Q grade" water acidified with 1% (v/v) HNO_3. The resulting solutions were analysed by DRC-ICP/MS to confirm the absence of detectable chromium.

Nitric fuming acid, hydrofluoric acid (38-40%, Merck, Darmstadt, Germany), and hydrogen peroxide (30%, Romil Ltd., Cambridge, UK) were used for the microwave-assisted digestion of real samples prior to total chromium determination.

Instrumentation and General Conditions

A Domel Vibromix vortex mixer (Železniki, Slovenia) was used for the preliminary homogenization of powdered cosmetic samples placed in 15-mL polyethylene tubes. For complete homogenization of the cosmetic matrix spiked with standard solutions, a Turbula T2F rotary mixer (Muttenz, Switzerland) was employed.

Phase separation of the extracted fraction from the residual solid material was carried out using an ALC-4236 CWS centrifuge.

A PerkinElmer Lambda-35 UV-VIS spectrophotometer was employed during the preliminary matrix clean-up trials to assess the concentration and nature of the extracted organic substances containing chromophoric functional groups. UV absorption spectra of the cosmetic extracts were recorded over the 190-300 nm wavelength range, using quartz cuvettes with a 10 cm optical path length. The spectra were acquired with a scan speed of 15 nm/min and a spectral slit width of 0.50 nm.

The determination of total chromium in both standard solutions and acid-digested cosmetic samples was performed using a PerkinElmer NexION 300D ICP-MS spectrometer (Waltham, MA, USA), operating in DRC mode. The instrument was equipped with platinum sampler and skimmer cones, and operated with a dwell time of 50 ms/amu across all measurements. The detector was set to dual mode, enabling a broad dynamic range suitable for both low and high concentrations. A Teledyne Cetac U6000AT+

ultrasonic nebulizer (Omaha, NE, USA) was employed for continuous aerosol generation, connected to a PerkinElmer ESI-SC4-DX autosampler via one channel of a three-channel peristaltic pump integrated into the ICP-MS system. The IS solution was delivered through a second pump channel and continuously mixed with the sample flow immediately prior to nebulization using a PEEK T-connector, with the outlet directly connected to the ultrasonic nebulizer. Ammonia was used as the reaction gas to suppress potential isobaric interferences. The reaction cell was operated with an auxiliary gas flow of 50 mL/min, a resolution parameter *q* (*RPq*) of 0.75 V, and a reaction parameter *a* (*RPa*) of 0.00 V, which were selected based on previously published optimized conditions [40].

The quantification of hexavalent chromium in both standard solutions and matrix extracts was performed using the same ICP-MS spectrometer, operated in standard mode with the same ultrasonic nebulizer and platinum cones, and maintaining a dwell time of 50 ms/amu. For these analyses, the detector was operated in pulse mode, given the lower concentration range and narrower dynamic requirements associated with IC-ICP/MS determinations. The nebulizer was alternately connected either to the HPLC system or to the peristaltic pump via an IDEX MXP9900-000 2-position/6-port PEEK switching valve (Northbrook, IL, USA). One channel of the peristaltic pump continuously delivered 1% HNO_3 solution for system rinsing. The switching valve managed the fluid routing among the HPLC system, peristaltic pump, ultrasonic nebulizer, and waste outlet (Figure 1), according to the following configuration:

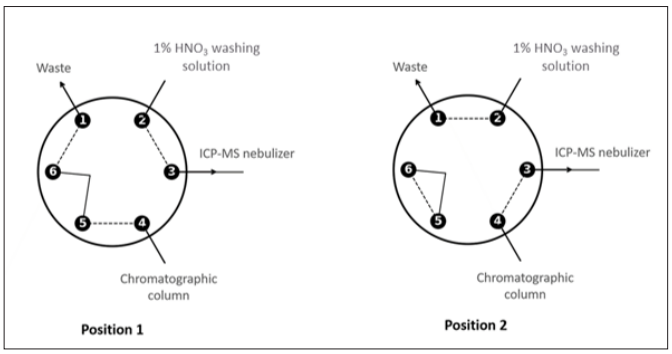

Figure 1: Schematic Representation of the Fluidic Connections to the dex mxp9900-000 2-Position/6-port Peek Switching Valve in its two Operational Positions (Position 1 and Position 2). Dashed Lines Represent Internal Connections within the Valve, while Solid Lines Indicate External Fluidic Pathways

- **Position 1:** Between chromatographic runs, the flow of HNO_3 was routed to the ultrasonic nebulizer for system flushing, and the HPLC effluent was directed to waste.
- **Position 2:** During a chromatographic run, the flow from the HPLC column was directed to the ultrasonic nebulizer, while the HNO_3 stream was diverted to waste.

This configuration was specifically implemented to minimize matrix load from the HPLC eluent on the plasma torch and the sample introduction system, thereby prolonging torch lifetime and enhancing instrument sensitivity. In both operational modes, the IS solution was delivered via a second channel of the peristaltic pump at 0.40 mL/min and was continuously mixed with the analytical flow (1.00 mL/min) – originating either from the HPLC system or the autosampler – using a PEEK T-connector. The outlet of the T-connector was directly connected to the ultrasonic nebulizer, ensuring on-line mixing of the IS with either the HPLC eluent or HNO_3 rinse solution immediately prior to aerosol generation.

The HPLC system consisted of an eluent reservoir, a PerkinElmer Series-200 3-channel vacuum degasser (Shelton, CT, USA), a Shimadzu LC-10AD reciprocating pump (Kyoto, Japan), an injection port, a Dionex IonPac CG5A guard column (4 × 50 mm), and a Dionex IonPac CS5A analytical column (4 × 250 mm) (Thermo Fisher Scientific Inc., USA). The injection port comprised a 6-port Rheodyne PEEK manual-injection valve connected to a 200-µL PEEK sample loop, a polyethylene syringe (needle removed) for manual sample loading, a waste outlet, and a short (2 cm) Tygon tubing (Figure 2).

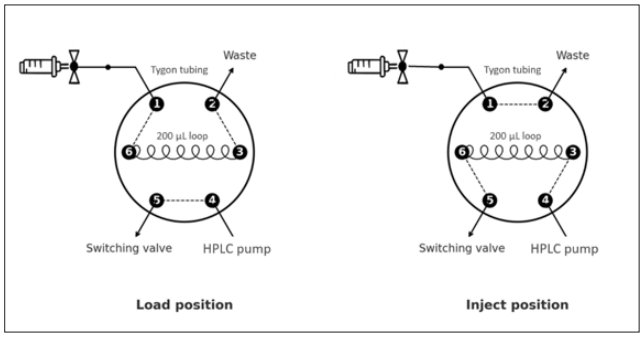

Figure 2: Schematic Representation of the Fluidic Connections to the 6-port Rheodyne PEEK Manual-Injection Valve in its two Operational Positions (Load Position and Inject Position). Dashed Lines Indicate Internal Connections within the Valve, while Solid Lines Represent External Fluidic Pathways. The Coil Represents the Injection Loop, which is Internally Mounted Between Ports 3 and 6. Port 1 is Connected to the Syringe Via a Polyethylene ball Valve and Tygon Tubing, Forming the Sample Loading Line

This tubing was connected on one side to the injection valve and on the other to a polyethylene ball valve equipped with a female Luer-lock fitting, which ensured secure attachment of the syringe. The use of Tygon tubing and a polyethylene interface prevented chromium contamination arising from stainless-steel needle during the injection of the extractant solution. Such contamination was observed in preliminary tests in which the extractant solution – used as a procedural blank free of chromium – was introduced using a stainless-steel needle, leading to detectable and variable levels of hexavalent chromium in the injected solution.

All other fluidic connections were made using PEEK tubing with 0.10-mm inner diameter.

To remove suspended particulates, prevent contamination of the analytical system, and avoid obstruction of chromatographic and nebulization pathways, 0.1-µm PVDF syringe filters (Millipore, MA, USA) were mounted at the outlet of the polyethylene syringes and used to filter the samples directly during manual injection into the sample loop.

Before each analytical session, the ICP-MS instrument was tuned in standard mode using a tuning solution containing Be, Ce, Fe, In, Li, Mg, Pb, and U, each at 1.00 mg/L in 1% HNO_3. Daily performance was verified against manufacturer specifications, and mass calibration was performed using a multi-element external standard.

Data acquisition and processing for ICP-MS analyses were performed using PerkinElmer NexION software (version 1.5, Shelton, CT, USA), while chromatographic data acquisition and integration were carried out using PerkinElmer Chromera software (version 4.1.2.6410, Shelton, CT, USA).

For quantitative analysis, the stable isotopes of chromium (^{50}Cr, ^{52}Cr, and ^{53}Cr) were initially considered, based on the polyatomic interference data reported in the ICP-MS Interference Table (Table 1) published by May and Wiedmeyer [48-54].

Table 1: Common Interferences in Chromium Determination by ICP-MS

Isotope	Abundance (%) *	Interferences	
		Isobaric	Polyatomic
^{50}Cr	4.3	$^{50}V^+$, $^{50}Ti^+$	$^{34}S^{16}O^+$, $^{36}Ar^{14}N^+$, $^{35}Cl^{15}N^+$, $^{36}S^{14}N^+$, $^{32}S^{18}O^+$, $^{33}S^{17}O^+$
^{52}Cr	83.8	–	$^{40}Ar^{12}C^+$, $^{35}Cl^{16}O^1H^+$, $^{36}Ar^{16}O^+$, $^{37}Cl^{15}N^+$, $^{34}S^{18}O^+$, $^{36}S^{16}O^+$, $^{38}Ar^{14}N^+$, $^{36}Ar^{15}N1H^+$, $^{35}Cl^{17}O^+$
^{53}Cr	9.5	–	$^{37}Cl^{16}O^+$, $^{38}Ar^{15}N^+$, $^{38}Ar^{14}N^1H^+$, $^{36}Ar^{17}O^+$, $^{36}Ar^{16}O1H+$, $^{35}Cl^{17}O^1H^+$, $^{35}Cl^{18}O^+$, $^{36}S^{17}O^+$, $^{40}Ar^{13}C^+$
^{54}Cr	2.4	$^{54}Fe^+$	$^{37}Cl^{16}O^1H^+$, $^{40}Ar^{14}N^+$, $^{38}Ar^{15}N^1H^+$, $^{36}Ar^{18}O^+$, $^{38}Ar^{16}O^+$, $^{36}Ar^{17}O^1H^+$, $^{37}Cl^{17}O^+$, "$^{19}F_2{}^{16}O+$"

Isotopic abundance of naturally occurring chromium as reported by IUPAC [55].

In DRC-ICP/MS mode, employed for total chromium determinations and operated in dual detection mode, the ^{52}Cr and ^{53}Cr isotopes were selected to maximize sensitivity and ensure accurate quantification across a broad concentration range. In contrast, in IC-ICP/MS mode, used for Cr (VI) speciation and operated in pulse detection mode, the ^{50}Cr and ^{53}Cr isotopes were preferred to avoid signal saturation and to maintain linearity at trace levels. Consequently, the ^{52}Cr isotope was excluded from acquisition in this mode due to its high natural abundance, which frequently led to signal saturation under the tested concentration conditions.

Each sample and standard solution was analysed in duplicate by IC-ICP/MS and in triplicate by DRC-ICP/MS to evaluate repeatability and ensure analytical robustness.

Microwave-assisted acid digestion of the cosmetic samples was performed using a Milestone MLS-1200 MEGA system (FKV, Milan, Italy), equipped with a six-vessel rotor. One of the six vessels was designated as a control vessel for the real-time monitoring of temperature and pressure during digestion. This vessel was loaded with the blank cosmetic matrix and the same reagent volumes used in the analytical vessels, thereby simulating actual sample conditions and ensuring accurate and representative process control.

Optimization of the Chromatographic Separation

A systematic optimization of the chromatographic separation conditions was performed to achieve reliable and selective resolution of chromium species in complex matrices. The optimization process involved the following experimental stages:

Eluent Composition and pH Optimization

The chromatographic eluent was prepared with the following base composition: 2 mM PDCA, 50 mM NH_4AcO, 2 mM $NH_4H_2PO_4$, 10 mM NH_4I, and NH_4OH as the pH modifier.

A series of tests was conducted to evaluate the effect of pH variation in the range of 6.0 to 7.0 on the retention behaviour of Cr (III) and Cr (VI). To this end, 10 mL of individual standard solutions of Cr (III) and Cr (VI), each at a concentration of 10 µg/L, were injected separately under identical chromatographic conditions for each tested pH value. All standard solutions were freshly prepared in the eluent adjusted to pH 6.0, in order to ensure consistent matrix composition and ionic strength across all tests. This approach was specifically adopted to avoid kinetic effects associated with the in-line complexation of Cr (III) with PDCA during injection, thereby ensuring that Cr (III) was already present in its complexed form prior to chromatographic separation.

Effect of Iodide Concentration

The influence of iodide concentration on the chromatographic separation of Cr (III) and Cr (VI) was evaluated by varying the amount of NH_4I in the eluent across a range of 0 to 30 mM, while keeping the other components constant. The eluent composition was as follows: 2 mM PDCA, 50 mM NH_4AcO, 2 mM $NH_4H_2PO_4$, 2.8 mM NH_4OH, and NH_4I in the range 0 - 30 mM, with the pH adjusted to 6.6. To isolate the effect of iodide introduced through the eluent and avoid unwanted redox reactions, NH_4I was deliberately omitted from the preparation of standard solutions. Individual standard solutions of Cr (III) and Cr (VI), each at a concentration of 10 µg/L, were prepared in iodide-free eluent and 10 mL of each were injected separately under each test condition.

Sample Injection Volume Optimization

A series of tests was carried out to determine the minimum sample volume required to achieve complete and reproducible transfer of the injected solution into the injection loop, while avoiding losses in a valve-tubing system operated without a needle.

Sample volumes of 3.0 - 5.0 mL were tested using a 10 µg/L standard solution of Cr (VI) prepared in iodide-free eluent. The eluent composition used during the test runs was 2 mM PDCA, 50 mM NH_4AcO, 2 mM $NH_4H_2PO_4$, 2.8 mM NH_4OH, and 20 mM NH_4I, with the pH adjusted to 6.6. Peak intensity was monitored to evaluate the completeness and reproducibility of loop filling for each tested volume.

Evaluation of Pre-Injection Filters

The effect of syringe filtration on Cr (III) and Cr (VI) recoveries was assessed using a selection of 0.45 µm syringe filters (Millipore, MA, USA) composed of different membrane materials: polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE), glass microfiber (GMF), and mixed cellulose ester (MCE).

Each filter type was tested by injecting 4.0 mL of standard solutions of Cr (III) or Cr (VI) at a concentration of 10 µg/L, prepared in iodide-free eluent, under the same experimental conditions described in previous sub Section 2.3.3 Filtration effects were evaluated by comparing peak areas in filtered versus unfiltered samples for each analyte. In the case of Cr (VI), particular attention was paid to possible adsorption phenomena or redox-induced

reduction to Cr (III) upon contact with the filter material. Cr (III) standards were tested in parallel to monitor any potential losses due to interactions with the membrane.

Development of the Extraction Method: Experimental Attempts and Optimization Pathway

A series of extraction experiments was carried out in an attempt to develop a robust protocol for the selective isolation of Cr (III) and Cr (VI) from a complex cosmetic matrix, aiming to maximize analyte recovery, minimize matrix interference, and prevent redox interconversion.

Preliminary clean-up strategies targeting matrix-derived organic compounds included an organic solvent-based approach, tested in parallel with a procedure that did not involve any clean-up step. The aqueous extractant solution used to recover the analytes from the cosmetic matrix was formulated to match the final chromatographic eluent, with the deliberate exclusion of ammonium iodide to avoid potential reduction of Cr (VI) prior to chromatographic separation. Optimization of the extractant composition involved varying the concentrations of PDCA and TBAOH, as well as adjusting the pH using NH_4OH or HNO_3. All IC-ICP/MS analyses were performed using a fixed injection volume of 4.0 mL.

The specific test conditions – relating to clean-up strategies, extractant formulation, and extraction procedures – are detailed in the following sub-sections.

Exploratory Clean-up Attempt Using Organic Solvents

Initial attempts to remove potentially-interfering organic substances were carried out using a mixture of n-hexane and ethanol (1:1, v/v) as suggested by Kang et al [34]. For each test, 100 mg of cosmetic matrix was treated with 2.5 mL of the solvent mixture, followed by 15 min of sonication and 20 min of centrifugation at 2000 rpm. The supernatant was evaporated to dryness under nitrogen, reconstituted in 5 mL of Milli-Q grade water, and analysed by UV spectrometry to monitor the removal kinetics of organic matter. Solid residues from each centrifugation cycle were subjected to successive extractions using the same procedure.

In addition, a second aliquot of the same cosmetic matrix, spiked with 500 ng of Cr (III), was allowed to equilibrate at room temperature for 24 hours before being processed using this clean-up protocol to evaluate the possible co-extraction of chromium under these conditions. The resulting aqueous phase was acidified to 1% (v/v) with HNO_3 and analysed by DRC-ICP/MS to determine the total chromium content extracted through this procedure.

Exploratory Extraction Attempt without a Preliminary Clean-up: Recoveries and Associated Matrix Effects

To evaluate the extraction efficiency of Cr (III) and Cr (VI) in the absence of any preliminary matrix clean-up, an exploratory procedure was conducted on a cosmetic sample artificially spiked with known quantities of the target species.

Specifically, 200 ng of Cr (III) or Cr (VI) were spiked into 100 mg of cosmetic matrix, and the mixtures were allowed to rest at room temperature for 24 hours to ensure equilibration between the spiked analytes and the matrix components. Each aliquot of the spiked matrix was dispersed in 5.0 mL of extractant solution consisting of PDCA 2.0 mM, AcONH4 50 mM, $NH_4H_2PO_4$ 2.0 mM, and NH_4OH 2.8 mM. The resulting suspension was treated in an ultrasonic bath for 15 min to promote analyte desorption and homogenization, followed by centrifugation at 2000 rpm for 20 min. The supernatant was then filtered through a two-stage filtration system consisting of PVDF syringe filters with pore sizes of 5.0 µm and 0.45 µm, connected in series. The filtrate was collected in a 15 mL polypropylene tube and diluted 2-fold with Milli-Q grade water. The solid residue resulting from this first extraction cycle was then subjected to two additional extraction steps under identical conditions, each time using 5.0 mL of fresh extractant solution. The resulting supernatants were individually filtered and collected in separate polypropylene tubes.

All three extracts obtained from each sample were analysed separately by IC-ICP/MS to quantify Cr (III) and Cr (VI), and to assess both the recovery performance and the potential matrix effects associated with the absence of a clean-up step.

Effect of TBAOH on the Stability of Hexavalent Chromium

To evaluate the effect of TBAOH on the stability and recovery of Cr (VI) in the presence of a complex cosmetic matrix, a dedicated extraction study was carried out.

A total of 200 ng of Cr (VI) was spiked into 100 mg of cosmetic matrix, and the mixture was allowed to equilibrate for 24 hours at room temperature to ensure interaction between the analyte and the matrix components. The equilibrated matrix was then dispersed in 5.0 mL of extractant solution composed of 2 mM PDCA, 2 mM $NH_4H_2PO_4$, 50 mM NH_4AcO, 2.8 mM NH_4OH, and variable concentrations of TBAOH (0.0, 0.1, 1.0 or 2.0 mM). The resulting suspension was treated in an ultrasonic bath for 15 min and subsequently centrifuged at 2000 rpm for 20 min. The supernatant was then filtered through a two-stage PVDF syringe filtration system, consisting of filters with 5.0 µm and 0.45 µm pore sizes, connected in series. The filtrate was collected in a 15 mL polypropylene tube. The solid residue from the first extraction was subjected to a second extraction cycle under identical conditions, using an additional 5.0 mL of fresh extractant solution containing the same TBAOH concentration. The second supernatant was filtered as described above and combined with the first extract.

The resulting solution was analysed by IC-ICP/MS to assess the recovery of Cr (VI) as a function of the TBAOH concentration in the extractant, and to investigate the potential stabilizing effect of TBAOH on the hexavalent chromium species under the given extraction conditions.

Exploratory Extraction Attempt for Complete Recovery of Cr (III) Via Extractant pH Optimization

To evaluate the influence of extractant pH on the recovery of Cr (III) from a complex cosmetic matrix, an exploratory extraction procedure was carried out under controlled conditions.

A total of 200 ng of Cr (III) was spiked into 100 mg of cosmetic matrix, and the sample was allowed to equilibrate for 24 hours at room temperature to promote interaction between Cr (III) and the organic components of the matrix. The equilibrated matrix was then dispersed in 5.0 mL of extractant solution containing PDCA at a concentration of 100 mM, increased from the standard 2 mM to enhance the formation of stable Cr (III) - PDCA complexes. The extractant also included the same buffering components described in the previous sub-section, and the pH was adjusted to values ranging from 6.1 to 1.0 using concentrated HNO_3. For each test condition, two successive extractions were performed at room temperature using 5.0 mL of extractant solution per

cycle, following the same procedure detailed in sub-section"The Identification and Quantification of Cr (VI) were Performed by IC/ICP-. The resulting supernatants were combined, and the pooled extract was analysed by DRC-ICP/MS to determine the total amount of chromium recovered under each pH condition.

In a subsequent trial, aimed at evaluating the maximum extractive efficiency under strongly acidic and thermal conditions, an additional aliquot of cosmetic matrix (100 mg) was spiked with 200 ng of Cr (III) and equilibrated for 24 hours at room temperature. The sample was then subjected to four successive extractions at pH 1.0, using the same extractant composition and volume (5.0 mL), but performing each extraction step at 90°C for 30 min. After each extraction cycle, the suspension was centrifuged and the supernatant was filtered through the same two-stage PVDF syringe filtration system (5.0 µm followed by 0.45 µm). Each extract was collected in a separate polypropylene tube, diluted 2-fold with Milli-Q grade water, and analysed individually by DRC-ICP/MS to monitor the progressive release of Cr (III) from the matrix under aggressive extraction conditions. All other experimental parameters were kept constant with respect to the previous trial described in this section.

Final Optimized Procedure for Sample Extraction and Analysis

Given the challenges in reliably recovering Cr (III), the final procedure adopted includes selective extraction and quantification of Cr (VI) using two successive extraction steps under mild conditions (pH 7, ambient temperature), followed by total chromium determination via acid mineralization. Cr (III) concentrations are calculated by difference between total chromium and Cr (VI).

The selective extraction of Cr (VI) from the cosmetic matrix was performed using an optimized extractant solution composed of 2 mM PDCA, 2 mM $NH_4H_2PO_4$, 50 mM NH_4AcO, 2.8 mM NH_4OH, and 2 mM TBAOH.

Eyeshadow samples were finely ground and homogenized using a vortex mixer, then dispersed in 5 mL of the optimized extractant solution. The suspension was subjected to ultrasonic treatment in an ultrasonic bath for 15 min and subsequently centrifuged at 2000 rpm for 20 min. The resulting supernatant was filtered through a two-stage filtration system consisting of PVDF syringe filters with pore sizes of 5.0 µm and 0.45 µm, connected in series. The filtrate was collected in a 15 mL polypropylene tube. The solid residue from the first extraction was subjected to a second extraction cycle under identical conditions, using an additional 5 mL of fresh extractant solution. The resulting supernatant was filtered as before and combined with the initial extract. Prior to instrumental analysis, the combined extract was diluted to a final volume of 20 mL with extractant solution.

The Identification and Quantification of Cr (VI) were Performed by IC/ICP-MS

Application of the Optimized Procedure to Real Samples

The optimized extraction procedure was applied to a commercially available cosmetic-toy palette consisting of seven distinct powder-based eyeshadows, all manufactured in China.

Sample preparation was carried out according to the conditions described in the previous sub-section. The resulting extracts were analysed for hexavalent chromium using IC/ICP-MS. For each sample, three replicate aliquots were subjected to IC/ICP-MS analysis. Quantification of Cr (VI) was performed by standard addition method, using a three-point calibration curve that included the unspiked sample.

In parallel, the total chromium content was determined following microwave-assisted acid digestion of the same eyeshadow samples. Approximately 100 mg of homogenized powder were accurately weighed and transferred into inert PTFE digestion vessels compatible with the microwave mineralisation system. The digestion protocol consisted of three consecutive treatments, the operational parameters of which are summarized in Table 2.

Table 2: Summary of the Operational Parameters Applied in the Microwave-Assisted Digestion Protocol

Treatment	Max P (bar)	Max T (°C)	Chemicals introduced in the vessel	Step	Power (W)	Time (min)
1	20	150	10 mL HNO_3 + 1 mL HF	A	400	4
				B	250	5
				C	150	6
2	25	160	None	A	400	5
				B	280	5
				C	190	10
3	10	155	2 mL H_2O_2	A	400	5
				B	200	5
				C	155	20

The digestion mixture consisted of HNO_3, HF, and H_2O_2, as detailed in the same table. The same acid mixture was used to prepare matrix-matched blanks for calibration and quality control. Quantification of total chromium was performed by external calibration, using standard solutions prepared in the same acid matrix as the digested samples. Measurements were performed using DRC-ICP/MS.

For each sample, the results obtained from the analysis of two isotopes and the replicates were averaged to ensure accuracy and precision.

Results and Observations

Effect of Eluent Composition on the Chromatographic Separation of Cr (III) and Cr (VI)

The chromatographic separation of trivalent and hexavalent chromium species was efficiently achieved using a Dionex IonPac CS5A column, characterized by a bifunctional stationary phase that incorporates both cation- and anion-exchange functionalities. This dual mechanism arises from the presence of sulfonic and alkanol quaternary ammonium groups in a 1:2 molar ratio, which proved essential for enabling the differential retention of Cr (III) and Cr (VI) based on their charge state and coordination chemistry.

Cr (III) was resolved into two distinct peaks at retention times of 1.7 and 4.6 min (Figure 3), corresponding respectively to a weakly retained monocomplex, $[Cr(PDCA)(H_2O)_3]^+$, and a strongly retained bis-complex, $[Cr(PDCA)_2]^-$. The greater retention of the latter is attributable to its anionic character, which promotes stronger electrostatic interactions with the anion-exchange sites on the column. In contrast, Cr (VI) appeared as a single peak with a retention time ranging from 8.1 to 9.8 min, depending on the eluent pH. This behaviour is explained by the pH-dependent speciation of Cr (VI): as the pH increases, the equilibrium shifts from $HCrO_4^-$ to CrO_4^{2-}, the latter being more strongly retained due to its higher charge density and enhanced interaction with the quaternary ammonium groups.

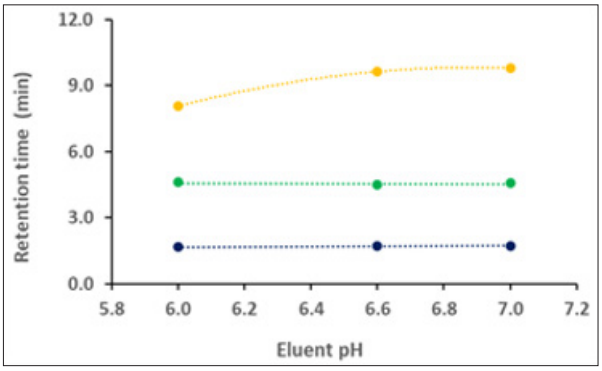

Figure 3: Retention Times of Cr (III) and Cr (VI) Chromatographic Peaks Recorded During the Optimization of the Eluent pH. ●: First Cr (III) Peak; ●: Second Cr (III) peak; ●: Cr (VI) peak

To maximize resolution and retention selectivity, a systematic optimization of the eluent composition was carried out. The base mobile phase consisted of 2 mM PDCA, 50 mM NH_4AcO, 2 mM $NH_4H_2PO_4$, and 10-20 mM NH_4I, with the pH finely adjusted using NH_4OH. The influence of eluent pH was investigated within the 6.0-7.0 range (Figure 3), revealing that pH 6.6 yielded optimal conditions in terms of retention time. At this pH, Cr (III) was predominantly present in its bis-complexed form, as confirmed by the progressive disappearance of the monocomplex peak (Figure 4), while Cr (VI) was almost exclusively in the form of CrO_4^{2-}. These conditions ensured maximal separation between the species and prevented the precipitation of $Cr(OH)_3$, which typically occurs at pH values near to 8.5. In parallel, the effect of iodide concentration in the eluent was investigated across a 0-30 mM range (Figure 5).

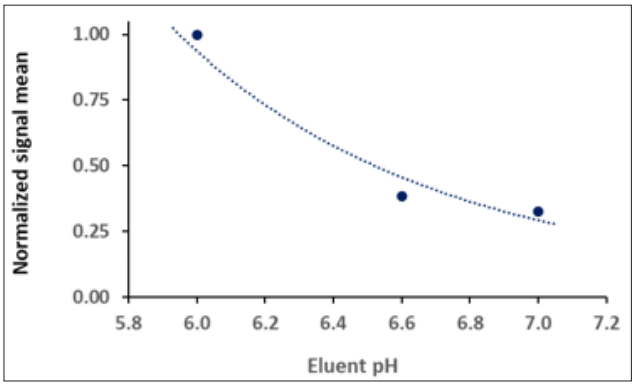

Figure 4: Effect of Eluent pH on the Normalized Signal Intensity of the First Cr (III) Peak

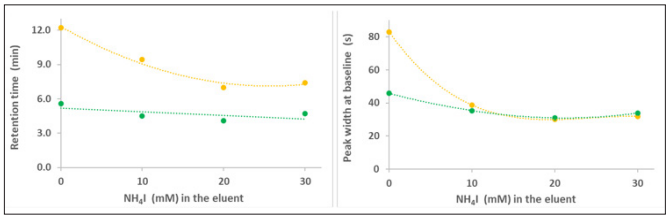

Figure 5: Effects of NH_4I Concentration in the Eluent on the Retention Times and Baseline Peak Widths of the Second Cr (III) Peak and the Cr (VI) Peak

Increasing the NH_4I concentration progressively reduced the retention time and peak width of Cr (VI), reaching a plateau at 20 mM. This behaviour is attributed to iodide acting as a highly competitive anion that modulates ion-exchange equilibria at the quaternary ammonium sites. Unlike other common counterions, I^- is large, poorly hydrated, and highly mobile in the stationary phase, allowing it to displace retained CrO_4^{2-} effectively without promoting redox reduction of Cr (VI) during the chromatographic separation. Notably, the retention behaviour of Cr (III) was only marginally affected by iodide concentration, reflecting the greater stability and stronger interaction of Cr (III)-PDCA complexes with the stationary phase.

In summary, the interplay between eluent pH, iodide concentration, and stationary phase chemistry was critical in achieving the selective separation of Cr (III) and Cr (VI). The final eluent conditions – 2 mM PDCA, 50 mM NH_4AcO, 2 mM $NH_4H_2PO_4$, 2.8 mM NH_4OH, and 20 mM NH_4I at pH 6.6 – ensured optimal resolution, peak symmetry, and analyte stability for downstream quantification by IC-ICP/MS.

Optimization of Sample Injection Volume and Filtration Medium

A minimum injection volume of 4.0 mL was required for complete loop filling without loss (Figure 6). Among the tested syringe filter materials, PVDF and PTFE membranes showed no Cr (VI) loss; however, PVDF was preferred due to superior hydrophilicity and ease of handling. Glass microfiber and mixed cellulose ester filters caused analyte losses of 17% and 11%, respectively (Figure 7).

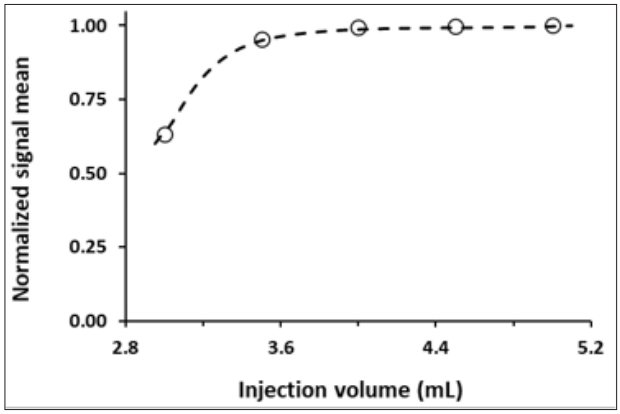

Figure 6: Effect of Injection Volume on the Normalized Signal Mean of the Injected Standard Solution

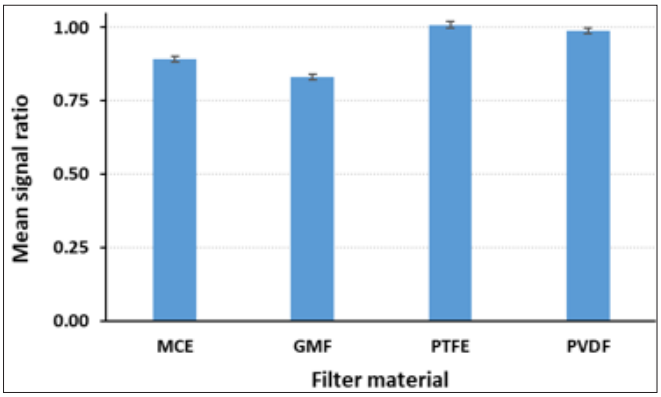

Figure 7: Effect of 0.45-µm Filter Material on the Normalized Signal Mean of the Injected Standard Solution

Development and Optimization of Extraction Protocol

Initial attempts to remove organic interferences from the cosmetic matrix by means of a clean-up step using a 1:1 (v/v) n-hexane/ethanol mixture revealed persistent matrix retention of UV-absorbing organic compounds. Spectrophotometric analysis of the first three extracts showed two characteristic absorption maxima, both progressively decreasing in intensity with each extraction cycle (Figure 8).

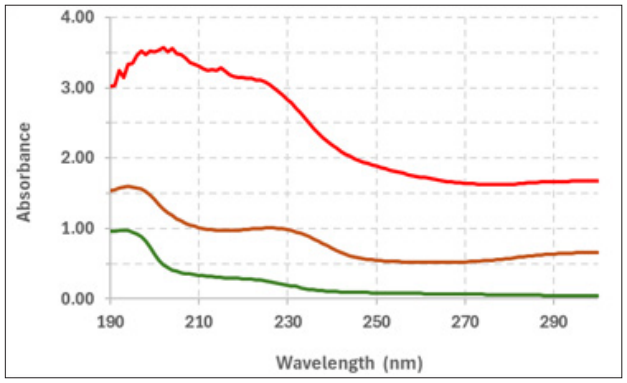

Figure 8: UV Absorption Spectra of Three Consecutive Extracts Obtained During the Initial Clean-up Attempts. Red Line: First Extract; Brown Line: Second Extract; Green Line: Third extract

The decay of these absorbance signals followed apparent first-order kinetics. As shown in Figure 9, linear regression of the logarithmic plots of absorbance versus extraction number allowed extrapolation of the number of extraction steps required to reduce the residual organic content to an acceptable absorbance threshold of 0.01. According to this model, ten sequential extractions were necessary to completely eliminate the compound responsible for the first absorption maximum, and six for the second. Moreover, the clean-up process induced a 9.6% loss of Cr (III) during the first two extractions (Figure 10), suggesting partial co-extraction of the analyte, likely due to complexation with endogenous organic acids released from the matrix. These findings confirmed the impracticality of the organic solvent-based clean-up protocol, owing to both its inefficiency and the significant loss of one of the target analytes.

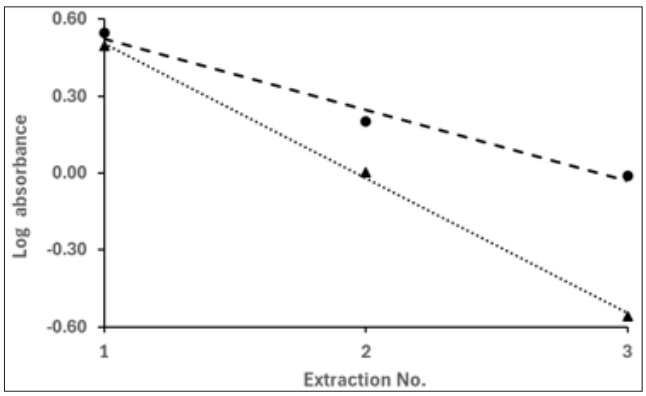

Figure 9: Effect of Extraction Number on the Absorbance Values of the Two Maxima Identified in the UV Spectra Shown in Figure 8. ●: Maximum at 192-203 nm; ▲: Maximum at 222-226 nm

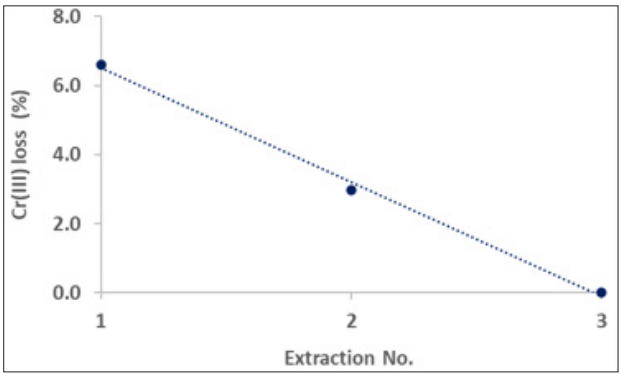

Figure 10: Cr (III) Loss at each Extraction Step During the Initial Matrix Clean-up Attempts

A simplified protocol omitting the clean-up step was therefore explored. Using ultrasonic-assisted extraction and PVDF filtration, this method yielded excellent Cr (VI) recovery – 95% after two extraction steps – indicating that Cr (VI) is weakly bound to the matrix. In contrast, Cr (III) was poorly recovered: only 13.3% was extracted after three consecutive cycles (Table 3).

Table 3: Recovery Rates of Cr (III) and Cr (VI) Obtained in Initial Extraction Tests in the Absence of a Preliminary Clean-up Step

Extraction No.	Recovery (%) of Cr(III)	Recovery (%) of Cr(VI)
1	4.6	78.9
2	4.4	16.1
3	4.3	0.0
Total	13.3	95.0

This suggests strong binding of Cr (III) to matrix constituents, possibly via chelation by organic acids or slow complexation kinetics.

To improve the stabilization and recovery of Cr (VI), 2 mM TBAOH was added to the extractant. This modification enhanced Cr (VI) recovery without introducing interferences in Cr (III) determination, confirming the compatibility of TBAOH under mild extraction conditions (Figure 11).

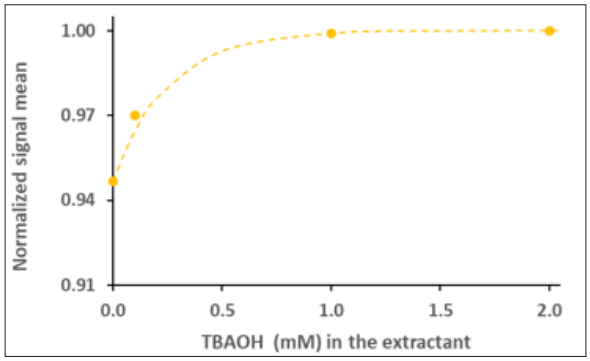

Figure 11: Effect of TBAOH Concentration in the Extractant on the Normalized Signal Mean of Cr (VI)

Given the limited Cr (III) recoveries, a dedicated experiment was conducted to investigate the effect of extractant pH on its release from the matrix. The extraction protocol used a high concentration of PDCA (100 mM) to promote complex formation, and the pH of the extractant was varied between 6.1 and 1.0. As illustrated in Figure 12, Cr (III) recovery increased progressively with decreasing pH, indicating that acidic conditions favour the neutralization of matrix-bound acids and facilitate Cr (III) desorption. Nevertheless, even at pH 1.0, only 40% of the spiked Cr (III) was recovered after two successive extraction steps. These findings demonstrate that neither increased ligand concentration nor pH reduction is sufficient, on its own, to fully release Cr (III) from the matrix.

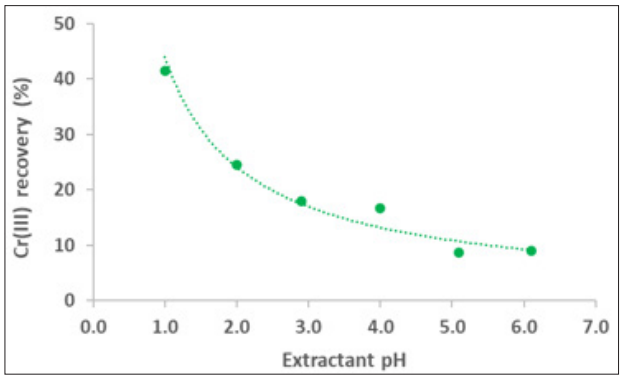

Figure 12: Effect of Extractant pH on Cr (III) Recovery from Two Consecutive Pooled Extracts

To further assess the extractive efficiency under more aggressive conditions, an additional experiment was performed using four consecutive extractions at pH 1.0 and elevated temperature (90°C). Each extraction was conducted for 30 min using 5.0 mL of extractant, and individual extracts were analysed to monitor the release profile of Cr (III). As shown in Figure 13, Cr (III) recovery increased gradually with each extraction, yielding a cumulative recovery of only 78% after fourth steps. The application of heat did not significantly enhance desorption compared to extractions performed at room temperature, confirming the kinetic inertness and strong matrix retention of Cr (III).

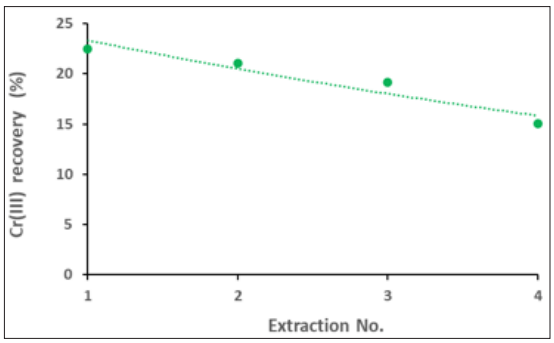

Figure 13: Effect of Extraction Number on Cr (III) Recovery from Single-Step Extractions Performed at pH = 1.0

These results collectively indicate that Cr (III) is substantially more difficult to extract than Cr (VI), likely due to strong complexation with matrix components and slow ligand exchange kinetics. Even under strongly acidic and thermal conditions, complete recovery of Cr (III) could not be achieved, necessitating alternative strategies for its accurate quantification.

Final Analytical Protocol and Application to Real Samples

The final protocol comprised selective Cr (VI) extraction at pH 6.6 using two extraction cycles (Figure 14), followed by total chromium determination via microwave-assisted mineralization. Cr (III) was quantified by subtraction.

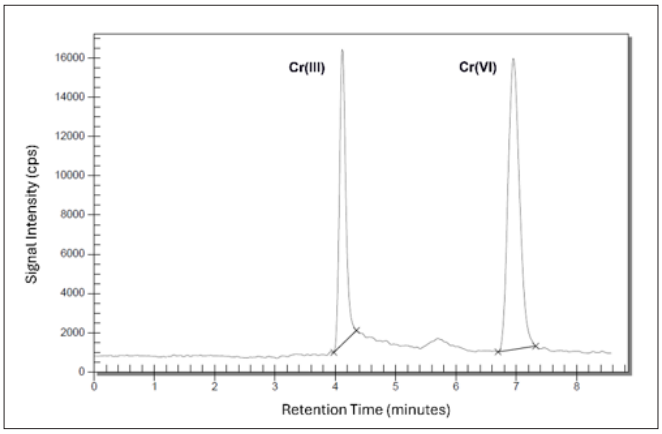

Figure 14: IC-ICP/MS Chromatograms of Two Consecutive Pooled Extracts from a Cr (VI)-Free Real Sample Spiked with 50 ng of Cr (VI). The First Peak Corresponds to Cr (III) and Accounts for 8.3% of the Total Cr (III) Originally Present in the Sample. The Second Peak, Corresponding to Cr (VI), Demonstrates a Recovery of 99.9% of the Initially Added Amount

The method demonstrated a LoD of 0.3 µg/L and a LoQ of 0.9 µg/L.

Method validation using two IRMs is summarized in Table 4, confirming trueness (11-15%) and precision (7.1-7.9%).

Table 4: Certified Concentrations of Cr (III) and Cr (VI) in Two Internal Reference Materials and Corresponding Trueness and Precision Results Obtained During Method Validation

IRM No.	Species	Certified concentration (mg/kg)	Uncertainty (mg/kg)	Method	Mean measured value (mg/kg)	Bias (%)	CV (%)
1	Cr(III)	5.00	0.32	DRC-ICP/MS	5.53	11	7.1
2	Cr(VI)	1.00	0.07	DRC-ICP/MS	0.85	15	7.9
2	Cr(VI)	1.00	0.07	IC-ICP/MS	1.12	12	7.3

Application to real commercial eyeshadow samples revealed Cr (VI) concentrations below regulatory limits. Total chromium was higher, confirming the presence of Cr (III) and reinforcing the need for speciation analysis (Table 5).

Table 5: Analytical Results for Cr (VI) and Total Cr in the Examined Real Samples

Sample description	Total Cr		Cr (VI)	
	Mean mg/Kg	SD mg/Kg	Mean mg/Kg	SD mg/Kg
Light-pink eyeshadow	6.3	2.5	0.05	0.03
Blue eyeshadow	7.8	2.5	0.02	0.03
Green eyeshadow	6.8	3.3	0.03	0.03
Yellow eyeshadow	5.3	2.5	0.04	0.03
Violet eyeshadow	4.5	0.9	0.01	0.04
Pink eyeshadow	6.1	2.7	< 0.01	0.03
Orange eyeshadow	6.9	2.9	0.22	0.04

Discussion and Conclusions

The results indicate that selective and sensitive speciation of chromium in complex cosmetic matrices is achievable using ion chromatography coupled with ICP-MS, provided that experimental parameters are rigorously optimized. The bifunctional resin of the CS5A column enabled concurrent anion and cation exchange, crucial for separating Cr (III)-PDCA complexes and CrO_4^{2-}. The chromatographic behaviour of Cr (VI) was especially sensitive to eluent pH, confirming that electrostatic interactions drive retention mechanisms. Iodide played a dual role as both a displacing counterion and a selectivity enhancer. Its high affinity for quaternary ammonium groups facilitated efficient elution of CrO_4^{2-} without inducing its reduction – an essential requirement for accurate Cr (VI) quantification. The choice of NH_4I over NaI mitigated spectral and physical interferences common in ICP-MS due to alkali metals [56,57].

Filtration studies underscored the importance of membrane selection. PVDF filters provided reliable performance, avoiding redox transformations and sample losses, which were significant for other materials.

Despite intensive efforts, Cr (III) proved challenging to extract quantitatively from the cosmetic matrix. Even under acidic and thermal stress, organic ligands in the matrix appeared to sequester Cr (III) tightly, limiting complexation with PDCA. These findings are consistent with literature on the kinetic inertness of Cr (III) and its affinity for organic moieties [58]. In contrast, Cr (VI) demonstrated robust recoveries under mild, near-neutral conditions. TBAOH addition stabilized Cr (VI), preventing reduction during extraction. The optimization of extractant composition and extraction parameters yielded a reproducible, sensitive, and environmentally compatible method.

Validation through certified reference materials confirmed the method's suitability for trace-level determinations in regulatory contexts. The application to toy cosmetics highlights the potential public health relevance of the procedure, particularly given children's heightened susceptibility to Cr (VI) exposure.

In conclusion, the developed method satisfies analytical, regulatory, and toxicological requirements for Cr speciation in complex cosmetic products. Future work may focus on improving Cr (III) recovery via ligand exchange acceleration or matrix deactivation strategies.

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