APPLICATION NOTE

HPLC-ICP-MS

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# Speciation of Hexavalent Chromium in Children's Toys According to EN 71-3 Category II with the NexSAR HPLC-ICP-MS

## Introduction

Testing for harmful metal(loid)s that may be included in raw materials, processes, paints and additives used in the manufacturing processes of children's toys has been mandated by the regulation EN 71-3. This regulation recommends specific testing methods and maximum allowable concentrations of leachable metals and metalloids such as Al, As, B, Ba, Cd, Co, Cr (III), Cr (VI), Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, organotin, Sr and Zn in parts of toys, with the exclusion of general packaging materials. Tests on these toys are separated into three different categories, with an associated method of analysis for each element.[1] The maximum permissible metal content for each type of toy is further restricted depending on the potential path of exposure in children.

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One of the metals regulated by EN 71-3, chromium, is often used in industrial processes due to its anti-corrosion, wear, and heat-resistant properties. As the use of this element in industrial processes grew, so did the reports on the adverse effects of certain chromium compounds on human health. In the current day, chromium as pure metal has no known adverse health effects and trivalent chromium is known to be an essential micronutrient. Conversely, hexavalent chromium has both acute and chronically toxic effects and is a known carcinogen.[2]

As such, hexavalent chromium is strictly regulated in toy products to levels <5 ppb, where the method requires that the instrumentation being used is capable of achieving detection limits of <10 ppt due to the 500x dilution factor which is applied to the sample extracts.[1] Since the regulatory requirement distinguishes between the various forms of chromium, it is essential to separate and quantitatively analyze Cr (VI), where the recommended analytical instrumentation to do this is HPLC-ICP-MS. It is essential in such characterizations that the system being used has a metal-free fluid path so that it does not contribute toward the chromatographic baseline for chromium, since this would adversely affect the limits of detection and quantification.

Apart from the need for a metal-free and inert fluid path in such applications, there are several other factors which may affect the accurate quantification of Cr. Since mass spectrometric techniques are based upon *m/z*, carbon from the mobile phase or sample may form an $^{40}Ar^{12}C$ molecular (polyatomic) interference on $^{52}Cr$, leading to inaccurate recoveries and raised baselines for Cr in HPLC-ICP-MS applications. This can be effectively dealt with through the use of pure reaction gases, such as $NH_3$, and a quadrupole-based reaction cell which is able to selectively interact with and eliminate this interference, enabling the accurate measurement of chromium concentrations at low-ppt levels.[3]

## Review of EN 71-3: Migration of Hexavalent Chromium in Toy Samples

As shown in Table 1, the migration of elements from toys can be divided into the following categories:

- Category I: Dry, brittle, powder-like or pliable materials;
- Category II: Liquid or sticky materials;
- Category III: Scraped-off materials.

In this work, we report a method for the measurement of hexavalent Cr in three different Category II materials, including blue paint, paint and slime which were evaluated in accordance with the method EN 71-3 Category II. The analysis was performed using a PerkinElmer NexSAR™ HPLC-ICP-MS solution, which is comprised of an inert NexSAR Speciation Analysis Ready HPLC system coupled to a NexION® ICP-MS.

*Table 1.* Categories under which materials of interest fall and the limit of Cr (VI) in these categories add according to EN 71-3.

| Toy Material | Category I | Category II | Category III |
| --- | --- | --- | --- |
| Coatings of paints, varnishes, lacquers, printing inks, polymers, forms and similar coatings | | | X |
| Polymeric and similar materials, including laminates, whether textile-reinforced or not, but excluding other textiles | | | X |
| Paper and paper board | | | X |
| Textiles, whether natural or synthetic | | | X |
| Glass, ceramic, metallic materials | | | X |
| Wood, fiber board, hard board, bone, leather and other solid materials | X | | |
| Compressed paint tablets, materials intended to leave a trace, or similar materials in solid form appearing as such in the toy | X | | |
| Pliable modeling materials, including modeling clays and plaster | X | | |
| Liquid paints, including finger paints, varnished, lacquers, liquid ink in pens, and similar materials in liquid form appearing as such in the toy (e.g. slimes, bubble solution) | | X | |
| Glue sticks | | X | |
| **Limit of Cr (VI)** | **0.02 mg/L** | **0.005 mg/L** | **0.2 mg/L (0.053 mg/L from Nov 2019)** |

## Experimental

Ammonium nitrate (75 mmol, pH 7) was used as a mobile phase for the separation of chromium species and was prepared from $HNO_3$ (Ultrapur-100 KANTO, Portland, Oregon, USA) and $NH_3OH$ (25% Suprapur® Merck, Burlington, Massachusetts, USA). For the extraction of hexavalent chromium from the sample, HCl (0.07 M, Ultrapur-100 KANTO) was used as specified by the EN 71-3 standard.

The separation and detection of hexavalent chromium in the material specified in Table 2 was performed with a NexSAR Inert HPLC (PerkinElmer, Inc., Shelton, Connecticut, USA) coupled to a NexION 2000 ICP-MS (PerkinElmer, Inc.) using the parameters specified in Table 3 and Table 4. To ensure a low chromatographic baseline for $^{52}Cr$ and allow its detection at a low-ppt level, Universal Cell Technology™ with pure ammonia gas was used, thereby effectively removing the polyatomic interferences on $^{52}Cr$.

Calibration standards for Cr (VI) were prepared from a stock solution of chromium ($Cr^{6+}$, 10 mg/L, Inorganic Ventures, Christiansburg, Virginia, USA) in the mobile phase with concentrations ranging from 0.010 to 0.160 µg/L (n=6).

Three different Category II materials (blue paint, paint and brown slime prior to treatment) were spiked with 0.020 µg/L hexavalent chromium to cover the lower range of the calibration as recommended by EN 71-3 and the spike recoveries determined to evaluate the accuracy of the method.

## Results

Hexavalent chromium eluted at approximately 1.9 minutes, demonstrating that high sample throughput can be achieved despite the complex matrix of these samples. Good linearity across the measured range was achieved (0.9999), exceeding the required correlation coefficient of 0.99 as defined by EN 71-3 (Figure 1). The retention time of the calibration standards (Figure 2) was the same irrespective of the Cr (VI) concentration. This demonstrates the ability of the pump to deliver consistent flow rates and is indicative of the robustness of the method over a wide linear dynamic range. The 10 ppt standard (Figure 2) had a signal-to-noise (S/N) of 27, providing a theoretical method detection limit (MDL) of 1.1 ppt for this method, demonstrating how essential the metal-free fluid path of the NexSAR Inert HPLC is for determining ultra-trace concentrations of Cr.

*Table 3.* NexSAR Inert HPLC System Conditions.

| Parameter | Value |
|---|---|
| Column | Hamilton PRP-X-100 4.1 X 50 mm, 5 µm |
| Mobile Phase | 75 mM $NH_4NO_3$ (from $HNO_3$+$NH_4OH$) Adjusted to pH 7.0 |
| Flow Rate | 0.8 mL/min |
| Separation Scheme | Isocratic |
| Injection Volume | 100 µL |
| Column Temperature | Ambient |

*Table 4.* NexION ICP-MS Instrument Conditions.

| Parameter | Value |
|---|---|
| Nebulizer | MEINHARD® Plus Glass Type C |
| Spray Chamber | Glass Cyclonic, 4 °C |
| RF Power | 1600 W |
| Injector | 2.0 mm Quartz Injector |
| Nebulizer Gas Flow Rate | 1.02 mL/min |
| Mode | Reaction Mode, $NH_3$, 0.7 mL/min |
| RPq | 0.65 |
| Dwell Time | 999 ms |

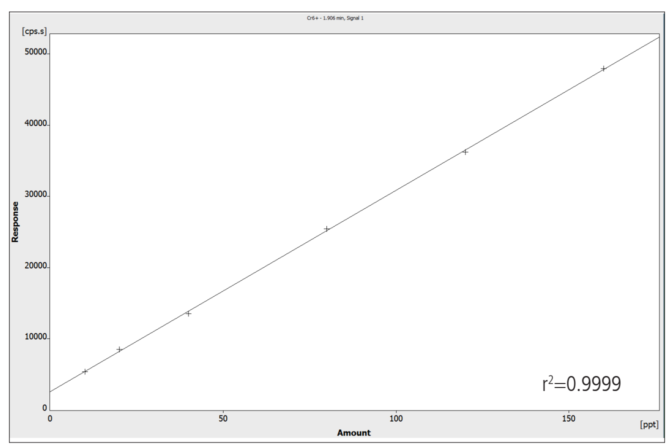
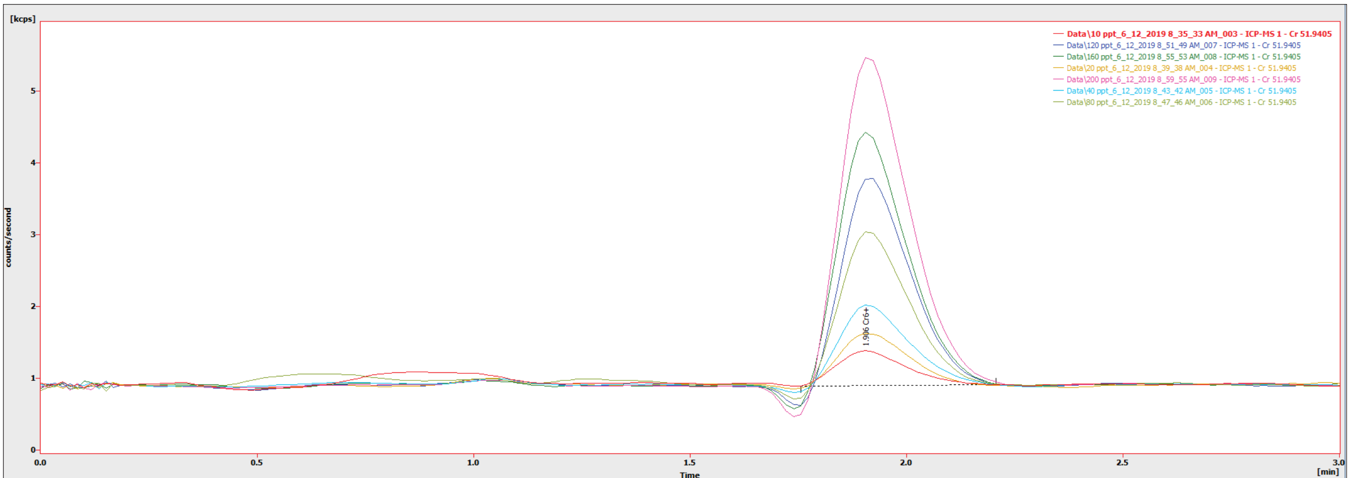



*Figure 1.* Linear regression of calibration standards for Cr (VI) (0.010-0.160 µg/L, n=6).

*Table 2.* Category II Materials Evaluated and Methods Employed for the Determination of Hexavalent Chromium with the NexSAR HPLC-ICP-MS System.

| Sample Type | Sample Preparation | Mass Used | Acid Used and Volume |
|---|---|---|---|
| Blue Color Paint | 0.1 g | $^{52}Cr$ | 5 mL of HCl (0.07 M) |
| Paint | 0.1 g | $^{52}Cr$ | 5 mL of HCl (0.07 M) |
| Brown Slime | 0.1 g | $^{52}Cr$ | 5 mL of HCl (0.07 M) |




Summary Table

| | | Sample ID | Sample | Sample Amount | Cr6+ Reten. Time [min] | Response | Amount [ppt] | Amount% [%] | Peak Type | Compound Name | S/N | signal/ASTMnoise |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Data\10 | ICP-MS 1 - Cr | 10 ppt | | 0.000 | 1.906 | 5377.352 | 9.866 | 100.0 | Ordnr | Cr6+ | 27 | 0 |

*Figure 2.* Overlay of calibration standards (0.010-0.160 µg/L, n=6) in the mobile phase at pH 7 where the S/N of the 10 ppt standard is outlined in red.

Figures 3-4 and Figures 5-6 respectively show the chromatograms for un-spiked and spiked paint samples from two different sources. Both paint samples (Figure 3 and 5) had concentrations of Cr (VI) below the method detection limits (S/N < 3). Both samples had good spike recoveries, with percentage recovery ranging between 113.5% and 132% for the blue paint and paint samples respectively (Figures 4 and 6) for the 0.020 µg/L spiked concentration of hexavalent chromium. In the brown slime sample used in this test, hexavalent chromium was evaluated at a concentration of 0.004 µg/L (Figure 7), and the recovery of the spiked concentrations in the samples was 114% (Figure 8).

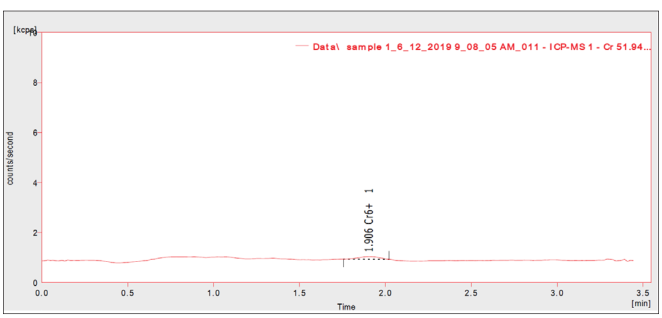



*Figure 3.* Chromatogram of un-spiked blue color paint sample.

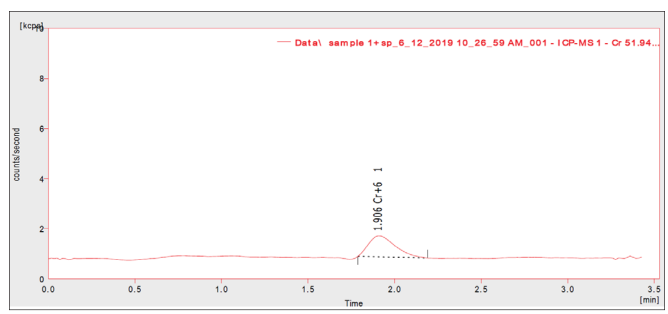



*Figure 4.* Chromatogram of blue color paint sample with 0.020 µg/L standard spike.

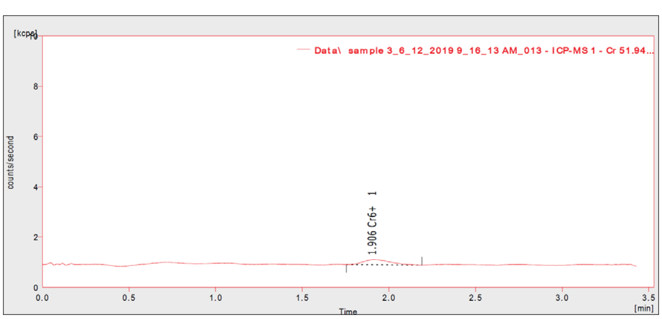



*Figure 5.* Chromatogram of un-spiked paint sample.

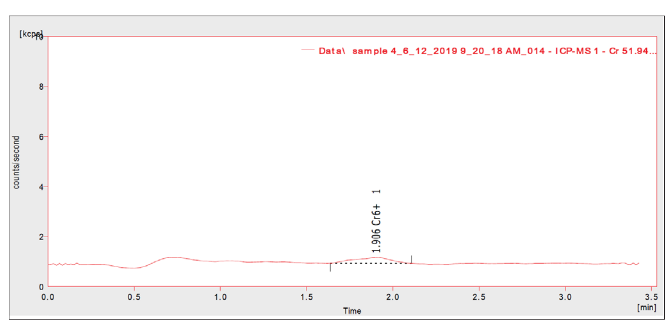



*Figure 6.* Chromatogram of paint sample with 0.02 µg/L standard spike.

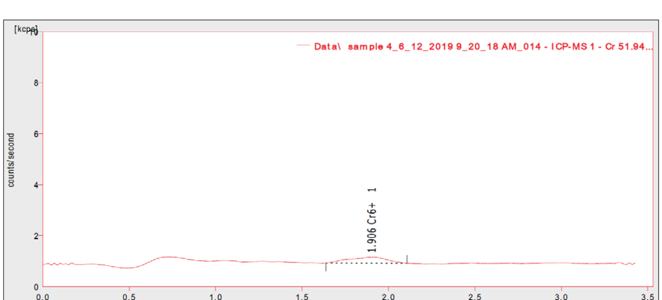



*Figure 7.* Chromatogram of un-spiked brown slime sample.

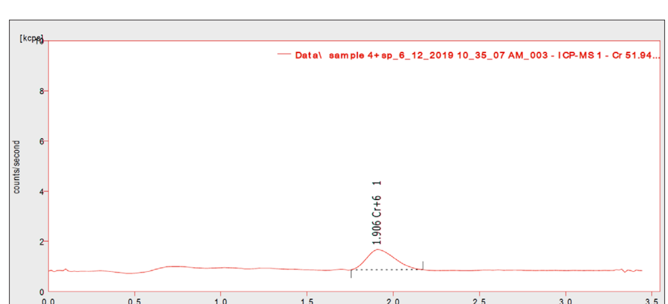



*Figure 8.* Chromatogram of brown slime sample with 0.020 µg/L standard spike.

## Conclusion

This study evaluated the hexavalent chromium content in Category II materials in accordance with the regulation EN 71-3 using a NexSAR Inert HPLC coupled to a NexION ICP-MS. The results showed that the low concentrations of hexavalent chromium outlined by the EN 71-3 Category II method could be easily and accurately quantified using the proposed speciation solution. Of the samples analyzed, hexavalent chromium was found to be lower than the regulated limit for Cr VI in Category II materials in toys following EN 71-3. The accurate characterization and quantification of low concentrations of hexavalent chromium with a method detection limit of 1.1 ppt was made possible by two factors:

- The metal-free and inert fluid path of the NexSAR HPLC Speciation Analysis Ready system;
- The ability to use pure reaction gases in a true quadrupole ICP-MS which was able to selectively control reactions taking place in the cell and actively eject interfering ions.

## References


1. EN 71-3:2019: Safety of Toys-Part 3 Migration of Certain Elements.
2. Baruthio F., Toxic effects of chromium and its compounds, *Biol Trace Element Res.*, 1992(32), 145-153.
3. Neubauer K., Reuter W., Perrone P., 2012, Chromium Speciation in Water by HPLC/ICP-MS, PerkinElmer Application Note.


## Consumables Used

| Component | Description | Part Number |
|---|---|---|
| HPLC Vials | HPLC tested plastic vials, 1.5 mL PP | N9301736 |
| PEEK Tubing | Yellow, 0.007" ID, 1/16" OD (5 feet) | N9302678 |
| PEEK Fittings | Fingertight for 1/16" OD PEEK tubing | 09920513 |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