

LC-ICP-MS method for the determination of trivalent and hexavalent chromium in toy materials to meet European regulation EN71-3:2012 Migration of certain elements

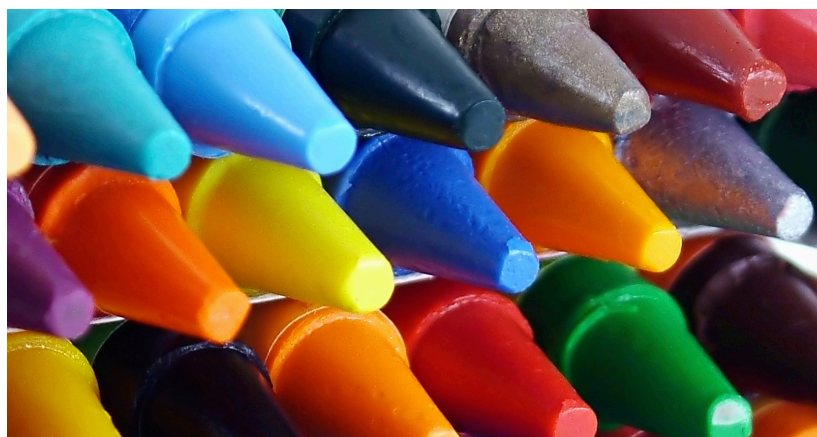
Application note

Materials

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Introduction

Chromium (Cr) is widely used in industrial processes such as plating, tanning and dyeing, and in manufactured products including steel and alloys. Cr has various oxidation states but normally exists as either trivalent Cr, Cr(III), or hexavalent Cr, Cr(VI). While Cr(III) is an essential trace nutrient for human health, Cr(VI) is well-known as a toxic substance. Cr(VI) is recognized as a human carcinogen and so the maximum contamination level of Cr(VI) is strictly regulated. For example, the European Union (EU) prohibits the use of Cr(VI) in electronic equipment under the Restriction of Hazardous Substances Directive (RoHS).

Similarly, the EU Toy Safety Directive (2009/48/EC) seeks to ensure the safety of children by minimizing their exposure to potentially hazardous or toxic toy products. The European Standard on the safety of toys (EN71)



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supports the requirements of EU Directive 2009/48/EC, and Part 3 of the Standard (EN71-3) covers the migration of certain elements from various categories of toy products, based on the assumption that toys may be chewed, sucked or swallowed. The most recent (2012) revision of EN71-3 came into force on 20 July 2013; since then all toys sold in the EU must comply with the revised Standard.

Toy materials and parts of toys are divided into three categories: Category I: Dry, brittle, powder-like or pliable materials; Category II: Liquid or sticky materials; Category III: Coatings and scraped-off materials. EN71-3 specifies migration limits for 17 elements aluminum, antimony, arsenic, barium, boron, cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, selenium, strontium, tin (and organotin compounds), and zinc. Cr has separate migration limits for Cr(III) and Cr(VI) as shown Table 1. Toy materials in Category II (liquid products that may be most easily swallowed) must meet the lowest limit of 5 ppb for Cr(VI). The sample preparation method specified in EN71-3 (see Figure 1) applies a total dilution factor of 500x. This means that the limit of Cr(VI) in the samples as measured is 10 ppt, so this application needs a very

sensitive method for Cr species. This note describes a method to measure the Cr species at the low level required, using the Agilent 7700x ICP-MS connected to an Agilent 1260 Bio-inert LC.

Experimental

The Agilent 7700x ICP-MS was connected to an Agilent 1260 Bio-inert LC. The collision/reaction cell (CRC) of the 7700 uses helium (He) mode to remove spectral interferences such as $^{40}\text{Ar}^{12}\text{C}$, $^{34}\text{S}^{18}\text{O}$, $^{37}\text{Cl}^{16}\text{O}$ and $^{35}\text{Cl}^{16}\text{OH}$ that would interfere with the low level measurements of the Cr isotopes. He mode enables the 7700 to achieve the required low DL for the Cr species, and also allows independent optimization of the chromatographic method without having to compromise the chromatography to ensure that the Cr species peaks are resolved from peaks formed from carbon, sulfur and/or chlorine compounds. To measure the two Cr species, cationic Cr(III), which exists as $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$ in solution, is converted to the anion $[\text{Cr}^{3+}\text{-EDTA}]^{-1}$ by chelation with EDTA. The two Cr species can then be effectively separated using an anion exchange column. Integrated method setup and sequence control of the combined LC-ICP-MS system was carried out from the ICP-MS MassHunter (MH) software package.

Table 1. Categories of toy materials and the respective migration limits of Cr species

Toy Material	Category 1	Category 2	Category 3
Coatings of paints, varnishes, lacquers, printing inks, polymers, foams and similar coatings			√
Polymeric and similar materials, including laminates, whether textile reinforced or not, but excluding other textiles			√
Paper and paper board			√
Textiles, whether natural or synthetic			√
Glass, ceramic, metallic materials			√
Other materials whether mass colored or not			
Compressed paint tablets, materials intended to leave a trace or similar materials in solid form appearing as such in the toy	√		
Pliable modeling materials, including modeling clays and plaster	√		
Liquid paints, including finger paints, varnishes, lacquers, liquid ink in pens and similar materials in liquid form appearing as such in the toy.		√	
Migration limits from toy materials (mg/kg)			
Chromium (III)	37.5	9.4	460
Chromium (VI)	0.02	0.005	0.2

ICP-MS

The Agilent 7700x ICP-MS with the standard Micromist nebulizer was used for Cr measurement. The outlet of the LC column was connected directly to the ICP-MS nebulizer. Cobalt (Co) was introduced as the internal standard (ISTD) via the on-line ISTD kit (G3280-60590). Point-to-point ISTD correction was applied to each chromatogram, providing a simple and effective way to correct for signal drift. Peak integration was performed by the auto-integrator of the ICP-MS MassHunter software. The operating conditions are shown in Table 2. The plasma conditions were optimized to give the maximum sensitivity for Cr with an oxide ratio for CeO^+/Ce^+ of less than 0.5% in He collision mode. The ion lens voltages were optimized by the auto-tuning functions of ICP-MS MassHunter.

HPLC

An Agilent 1260 Infinity Bio-inert HPLC was used to meet the very low Cr detection limit requirement: G5611A Bio-inert Quaternary pump and G5667A Bio-inert High-Performance Auto sampler. The bio-inert HPLC replaces all materials that contact with sample or mobile phase with inert materials, eliminating deterioration of bio-samples such as protein, and avoiding metal background leaching from components

of the HPLC. The Bio-inert LC is highly suitable for metal speciation work where the lowest possible DL is required.

An anion exchange column, Agilent Bio WAX 5 μm (5190-2488), 4.6 x 50 mm, 5 μm , PEEK guard, was used for separation of Cr species at ambient temperature. Glass vials fitted with polypropylene vial caps (5182-0540) were used. Prior to use, the vials were cleaned with dilute nitric acid and thoroughly rinsed with ultrapure water (UPW). It should be noted that rubber, plastic and even trace organic residues can easily cause reduction of Cr species when the sample comes into contact with them. Table 2 shows the operating parameters of the HPLC.

Reagents

Cr(III) and Cr(VI) were prepared from $\text{Cr}(\text{NO}_3)_3$ and K_2CrO_4 respectively, purchased from KANTO Chemicals (Tokyo, Japan). High purity Na-EDTA was purchased from DOJINDO Laboratories (Kumamoto, Japan) and was neutralized with ammonia (aq) after it was dissolved and diluted. Semiconductor grade HCl and ammonia (aq), (Tama pure AA-100, purchased from Tama Chemicals, Kanagawa, Japan), were used for the sample preparation.

Sample preparation

The sample preparation procedure is given in Figure 1. The procedure follows EN71-3, which simulates gastric digestion as would occur in the case when a child swallows toy material; it is NOT a method for the total extraction of chromium species. The extraction (migration) solutions obtained were stabilized with EDTA and ammonia (aq). The addition of ammonia to neutralize the solution preserves the chromium species extracted from toy materials for several hours with no species inter-conversion or loss by precipitation. We confirmed that both Cr species were preserved for at least 24 hours after the sample preparation, if the solution was neutralized at $\text{pH} = 7 \pm 0.5$. Calibration standards were also prepared by the same sample preparation method.

Table 2. LC-ICP-MS operating parameters

ICP-MS conditions	
RF power	1550 W
Sampling depth	8.0 mm
Carrier gas	1.10 L/min
Makeup gas	0 L/min
Option gas	0 L/min
Cell gas	He at 4.3 mL/min
Octopole bias	-18 V
KED	+3 V
HPLC conditions	
Mobile phase	75 mM HNO_3 adjusted to $\text{pH} = 7$ with ammonia (aq)
Flow rate	0.8 mL/min
Injection volume	100 μL
Acquisition parameters	
Acquisition	Time Resolved Analysis (TRA)
Dwell time	1.0 s for $m/z = 52$ and 0.1 s for $m/z = 59$
Run time	160 s

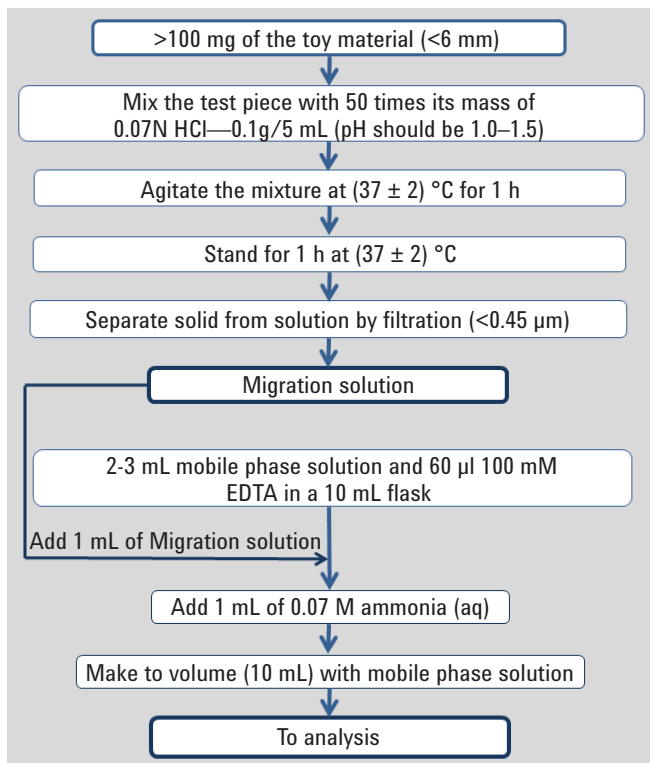


Figure 1. Sample preparation flow (total sample dilution is 500x)

Results and discussion

Figure 2 shows overlaid chromatograms of the calibration standards, and the Cr(VI) calibration plot. The chromatograms correspond to the calibration standards containing 5 ppt, 10 ppt, 20 ppt, 50 ppt and 100 ppt Cr(VI) and 10 times higher concentrations of Cr(III). The Cr species were completely resolved at retention times of 0.85 and 1.67 min. for Cr(III) and Cr(VI), respectively.

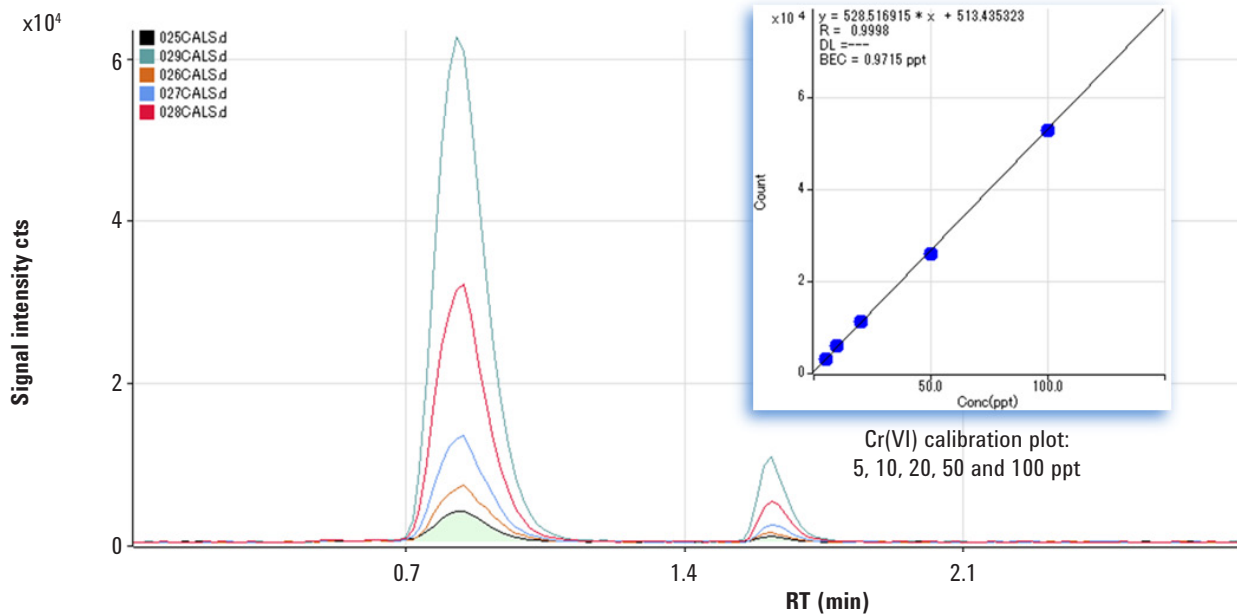


Figure 2. Overlaid chromatograms of Cr(III) and Cr(VI) calibration standards and Cr(VI) calibration plot

A summary of the method validation and analysis results of three toy materials is given in Table 3.

Table 3. Summary of the method and analysis

			Cr(III)	Cr(VI)
Retention time		min	0.95	1.67
DL	in solution	ppt	3.1	2.0
	in material	µg/kg	1.6	1.0
MDL	in solution	ppt	8.6	2.1
	in material	µg/kg	4.3	1.1
Washout			< 1/10000	< 1/10000
Quantified value	sample 1	µg/kg	78.6 ± 4.2	3.0 ± 0.7
	sample 2		15.1 ± 0.9	<D.L.
	sample 3		50.8 ± 1.3	<D.L.
Spike recovery	sample 1	%	98.0	111.2
	sample 2		97.2	98.1
	sample 3		102.7	96.2
Stability over 50 samples	sample 1	RSD %	2.4	2.9
	sample 2		2.9	4.6
	sample 3		1.4	4.3

DL and MDL

Figure 3 shows overlaid chromatograms of 10 replicate analyses of the level 1 (lowest level) calibration standard: 50 ppt Cr(III) and 5 ppt Cr(VI). From the S/N of the chromatogram, Detection Limits (DLs) in solution were determined to be 3.1 and 2.0 ppt for Cr(III) and Cr(VI), respectively. Peak-to-peak noise was measured over the range shown in the figure, and DLs were calculated as the concentration giving S/N = 3. The DLs achieved are sufficiently low to meet the requirements of EN71-3 for category 2 toy material, which has the lowest migration limit. Method Detection limits (MDLs) were also calculated from the 10 chromatograms. The MDLs were defined as concentration equivalent to 3σ of the quantified values of 10 analyses (Table 3).

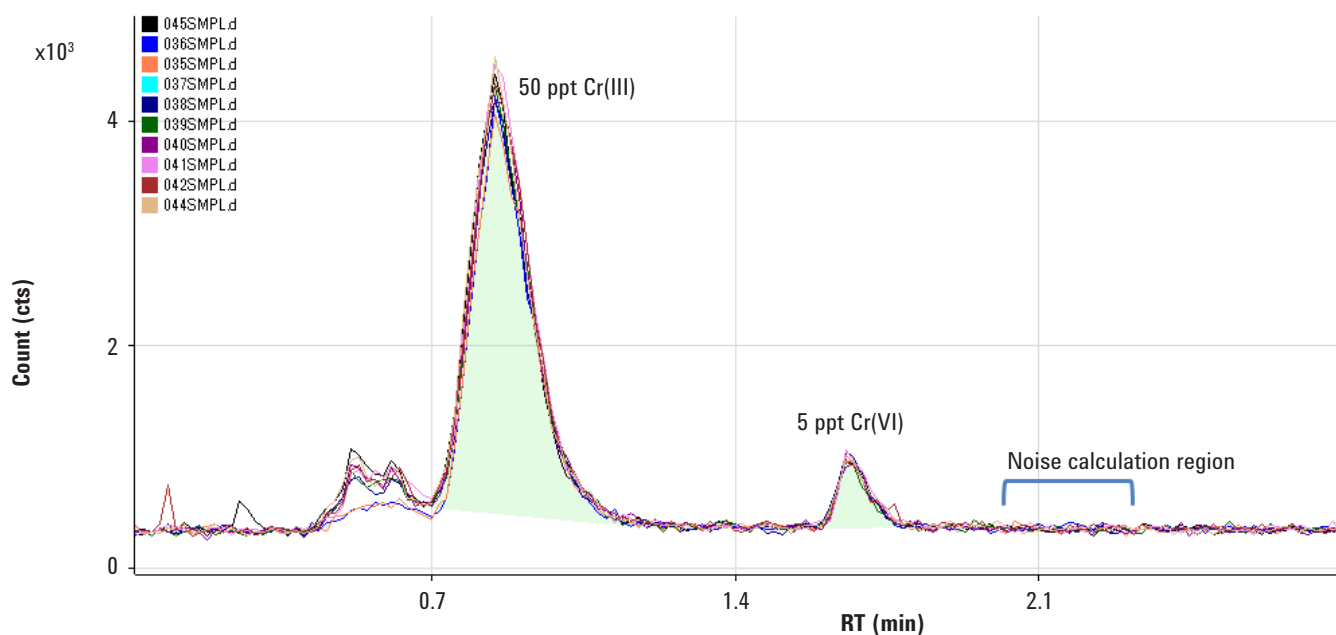


Figure 3. 10 replicate chromatograms of level 1 standard: 50 ppt Cr(III) and 5 ppt Cr(VI)

Influence of peak tail of high concentration Cr(III)

Figure 3 shows what looks like an excellent separation of the two Cr species, but the concentration of Cr(III) is normally far higher than that of Cr(VI). This means that trace Cr(VI) needs to be separated well enough to be accurately quantified in the presence of a high concentration of Cr(III). Figure 4 shows a chromatogram of a solution containing 0.1 ppb Cr(VI) with 200 ppb Cr(III). In the right expanded chromatogram, the peak of the 0.1 ppb Cr(VI) can be easily recognized. It is well integrated to give a measured concentration of 0.097 ppb (97% recovery), with an error (calculated from the SD of 10 replicates) of ± 0.003 ppb. This demonstrates that Cr(VI) can be accurately quantified, even in the presence of 2000 times higher concentration of Cr(III).

Washout test

Since a relatively low DL is required for Cr(VI), carryover after introduction of a high concentration sample is of concern. To check the washout performance of

the LC-ICP-MS method, a preparation blank sample was analyzed immediately before and after a solution containing 100 ppb of each Cr species. Measured concentrations in the blank were found to be less than the DL for both Cr species.

Toy material sample analysis: Quantification, spike-recovery test and stability

Three types of toy materials were analyzed: Crayon (sample 1), Bubble liquid (sample 2) and Soft Plastic (sample 3). These materials are categorized into EN71-3 category 1, 2 and 3, respectively. They were prepared by the procedure described in Figure 1 and then analyzed using the LC-ICP-MS method described above. The determined concentrations of Cr species in each sample are summarized in Table 3.

A spike recovery test was done using the migration solutions. Cr(III) and Cr(VI) were spiked into the solutions to give a concentration of 1 ppb and 0.1 ppb, respectively. Spikes were added after the solutions were

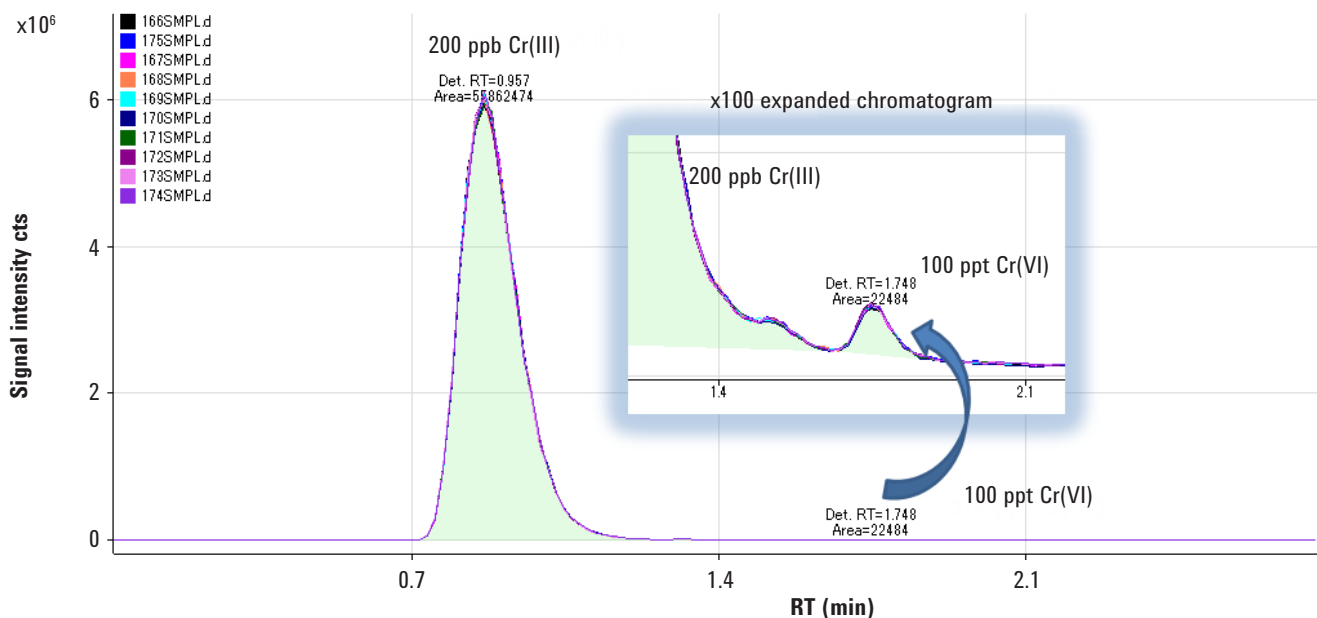


Figure 4. Separation and accurate measurement of 0.1 ppb Cr(VI) in the presence of 200 ppb Cr(III)

neutralized by ammonia, to avoid the reduction of Cr(VI). The result of the spike recovery test is shown in Table 3, confirming good recovery for both Cr species. Using the spiked migration samples, the long term stability was checked, and the analytical result of 50–60 samples for each type of toy material are shown in Figure 5. The plot shows the measured concentration of Cr species relative to the average concentration throughout the run. Sample to sample run time is about 4 minutes and the total run time was 3–4 hours for each toy material sample. Good stability was confirmed, demonstrating that neither species inter-conversion between the Cr species nor loss by precipitation occurred in any of the three toy samples.

Conclusions

A method has been developed using an Agilent 7700x ICP-MS connected to an Agilent 1260 Bio-inert LC for the speciation of Cr (separation and determination of Cr(III) and Cr(VI)) in toy material to comply with the new limits specified in EN71-3:2012. The European Standard EN71-3 supports the goals of the EU Toy Safety Directive 2009/48/EC, and requires that the method is able to accurately determine Cr(VI) at 10 ppt in the measured solution following sample preparation. The method developed proved to have single digit ppt DL for both Cr species, and was validated to demonstrate the performance required for the application.

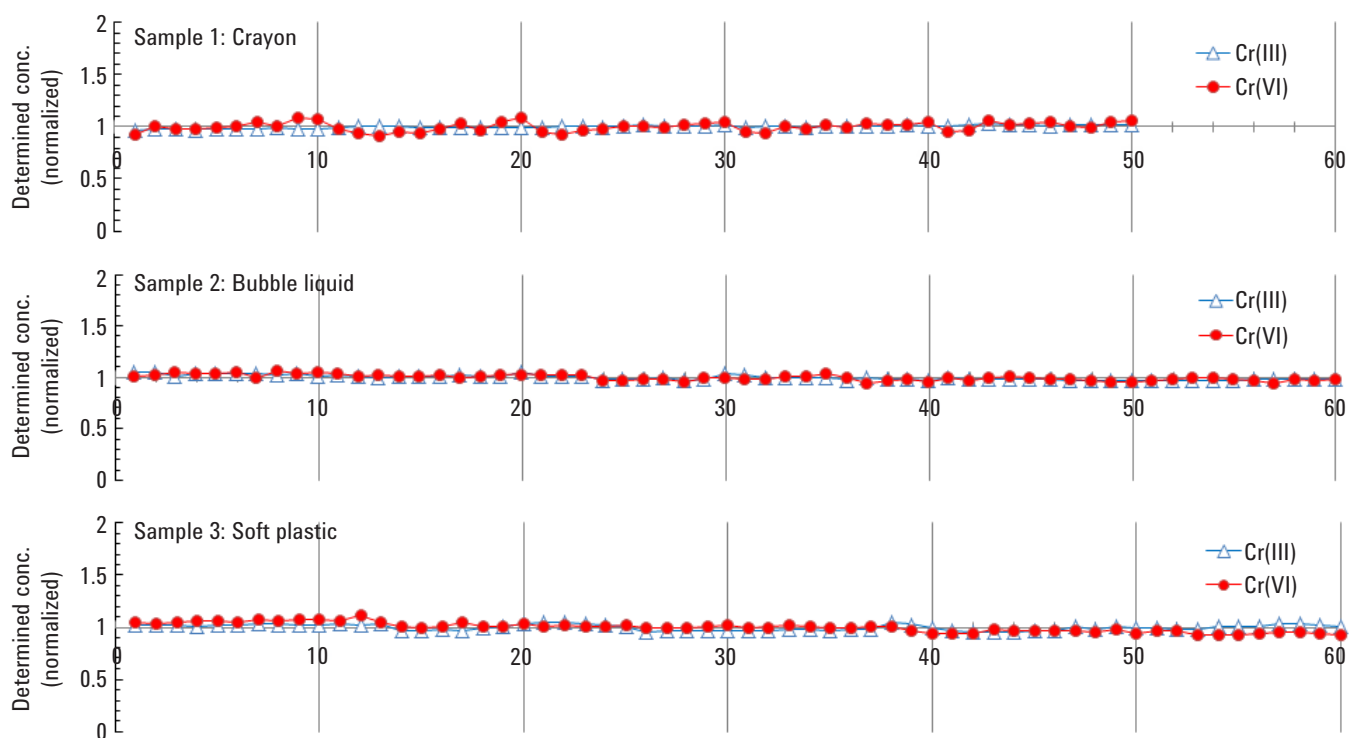


Figure 5. Signal stability of Cr(III) and Cr(VI) over 50 samples (200 minutes). Migration solutions of three toy samples were spiked with 1 ppb Cr(III) and 0.1 ppb Cr(VI) prior to analysis.

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