Challenge
Developing a reliable ISO 24384:2024 compliant method to quantify Cr(III) and Cr(VI) in water matrices.

Solution
Fast, sensitive, and accurate Cr(III) and Cr(VI) quantification according to ISO standard using PQLC and PlasmaQuant MS Q, separating both species in 8 minutes.

Intended audience
Labs, regulatory agencies, water treatment, industrial wastewater, and drinking water companies.

Determination of Chromium (III) and Chromium (VI) in Drinking Water, Surface Water, Groundwater, and Wastewater According to ISO 24384:2024

Introduction

Accurately determining chromium speciation is imperative for understanding its environmental and health impacts. Chromium exists in various chemical forms, with Cr(III) and Cr(VI) being the most significant in environmental contexts. While Cr(VI) is toxic, Cr(III) is often considered a dietary supplement, though its essentiality is debated. Therefore, strict adherence to regulatory standards for chromium in drinking water is crucial for public health and environmental safety.

Regulatory standards for chromium in drinking water vary worldwide, highlighting the necessity for precise chromium speciation. In the United States, the EPA mandates a maximum allowable level of 100 µg/L for total Cr, with California and New Jersey setting stringent limits for Cr(VI) at 0.02 µg/L and 0.07 µg/L, respectively, along with a maximum allowable level of 50 µg/L for total chromium.[1] Globally, the WHO recommends a provisional guideline value of 50 µg/L for total chromium in drinking water, while the EU sets a similar limit for water intended for human consumption which shall be 25 µg/L after January 2036.[2]

The methodology presents an advanced and reliable approach utilizing high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry to accurately quantify Cr(III) and Cr(VI) in water samples, meeting ISO 24384:2024 standard. The technique involves a chelating pretreatment that adeptly separates and detects low concentrations of both species across various water sources, establishing it as the most reliable and effective technique for determining Cr speciation.

The ISO 24384:2024 allows for the use of various types of mobile phases in combination with the appropriate type of LC column. The composition of the mobile phase is determined by the selected LC column. There are two

common approaches to performing chromium speciation, namely an Ion Pair Reverse Phase approach using a C-8 column and an Anion Exchange approach. The former provides a fast, robust method suitable for multielement speciation, while the latter involves utilizing an Anion Exchange on a short column to increase species retention and maintain peak shape and separation at larger retention volumes required for lower detection limits. In our method, the anion exchange approach was chosen for its ability to ensure better retention and resolution, especially at low detection limits.

Implementation of the HPLC-ICP-MS based methodology ensures confident achievement of accurate chromium speciation, providing essential information for regulatory compliance and risk assessment. By enabling determination of chromium and chromium concentrations in various water sources, including drinking water, surface water, groundwater, and wastewater samples, it facilitates better risk assessment and management strategies.

The method meets the requirements for legislative compliance and enables regulatory bodies to establish specific limits for individual chromium species, ensuring the safety and quality of drinking water. Additionally, the method contributes to the advancement of analytical strategies for chromium speciation analysis, offering a reliable approach for environmental monitoring and risk assessment.

Materials and Methods

The principle of the method described in ISO 24384:2024 is to separate Cr(III) and Cr(VI) using anion exchange chromatography. While Cr(VI) is already present in the form of anionic chromate in aqueous solution, Cr(III) is present as cation. Therefore, standard and sample solutions must be pretreated by adding 2,6-pyridinedicarboxylic acid (PDCA) or ethylenediaminetetraacetic acid (EDTA) for chelation of Cr(III) to form anionic Cr(III)-PDCA or Cr(III)-EDTA complexes, respectively. The chelation pretreatment is very sensitive to the pH and requires the sample solutions to be adjusted carefully to pH 6.9 ± 0.1[3][4]. After pretreatment, HPLC-ICP-MS is used to chromatographically separate and individually quantify Cr(III)-PDCA or Cr(III)-EDTA complexes and Cr(VI).

Instrumentation

pH and Temperature Monitor

A pH meter (DANOPLUS) was used for pH-adjustment of water samples within 6.9 ± 0.1 before chelating pretreatment.

Inductively coupled plasma mass spectrometry

Total chromium concentration was determined using a PlasmaQuant MS Q ICP-MS, with Pt-tipped cones, and a Peltier-cooled Scott type spray chamber. The analysis was automated using an ASX-560 autosampler (Cetac Technologies) and an ASXPress Plus injection valve (Cetac Technologies) on the ICP-MS system. The PlasmaQuant MS Q system incorporates an integrated collision/reaction cell (iCRC) equipped with helium (He) collision mode and kinetic energy discrimination (KED) conducted by the ion mirror lenses, along with hydrogen (H_2) as a reactive gas. This configuration effectively manages common polyatomic ions, including those originating from EDTA of the HPLC mobile phase. Specifically, the system operates in reaction mode using hydrogen as the cell gas to eliminate interference from carbon- and chlorine-based ions on $^{52}Cr^+$. Additionally, the combination of hydrogen as a reaction gas with Boost ensures the effective reduction of interferences without compromising analyte sensitivity.

HPLC-ICP-MS

A PQ LC HPLC system, non-metal, PEEK (Analytik Jena GmbH+Co. KG) equipped with a Hamilton PRP-X100 anion exchange separation column mounted in a column oven S4115 was used to determine the Cr species in water samples. The outlet of the separation column was directly connected to a Micromist™ nebulizer 0.4 mL/min, and the PlasmaQuant MS Q was used as an element specific detector. The ICP-MS and HPLC operating conditions are given in Table 1. Cr was detected at *m/z* 52. Data were collected using single-ion monitoring (SIM) to ensure maximum sensitivity for Cr species. Each day, the ICP-MS was tuned with a 1 µg/L Cr(III) solution in 1% HNO_3 to achieve a signal of 5 kcps/ppb. The HPLC system was allowed to stabilize for 30 minutes, reaching a consistent pressure of 70 ± 2 bar before any injections were made. The data were processed using Clarity workstation.

Table 1: Optimized HPLC-ICP-MS operating parameters

Parameter (ICP-MS)	Specification	Parameter (HPLC)	Specification
Plasma Gas Flow	9.0 L/min	Anion Exchange HPLC Column	Hamilton PRP-X100, 4.1 x 50 mm, 5 µm
Auxiliary Gas Flow	1.50 L/min	Mobile Phase	10 mM HNO_3 + 0.5 mM EDTA, pH 7.0 ± 0.1
Sheath Gas Flow	0.00 L/min	Injection Volume	200 µL
Nebulizer Gas Flow	1.05 L/min	Flow Rate	1.0 L/min
Sampling Depth	5.0 mm	Gradient	Isocratic
Plasma RF Power	1.35 kW	Elution Times	Cr(III) – 4.2 min Cr(VI) – 6.7 min
Sample Introduction	Inert Kit, PFA	Total Elution Time	8 min
Cones	Pt-tipped cones	Column Temperature	30 °C
Rump Rate	100 rpm – blue/blue PVC pump tubing	HPLC Vials	Polypropylene, 1.5 mL uncapped
Stabilization Delay	0 s		
iCRC Gas Setting	H_2 150 mL/min		
Boost	2 V		
Dwell Time	^{52}Cr – 500 ms		
Measurement Mode	Peak Area – ^{52}Cr		

Reagents and standards

All the solutions were prepared using the following high purity reagents:

- Deionized water (>18.2 MΩ/cm, Millipore MilliQ)
- Nitric acid sub-boiled 69%
- Ammonium hydroxide 25%
- EDTA (ethylenediamine tetraacetic acid) free acid (≥99.5%, Ultrapure, VWR International)
- Cr(III) standard solution, 1000 mg/L in HNO_3 (PlasmaCAL, SCP Science)
- Cr(VI) standard solution, 1000 mg/L in H_2O, (*TraceCERT*, Supelco, Sigma-Aldrich Production GmbH)

Sample preparation

Sampling, preservation, and storage of samples

Samples were collected into rigorously cleaned bottles. Samples were filtered on-site using a 0.45 µm syringe filter, with the exception of the drinking water, which was obtained from a local supermarket. A portion of the sample was used to rinse the filter, and the required volume of the filtrate was collected. The filtered samples were stored at 3 ± 2 °C before sample preparation and measured within 24 hours for total Cr content.

pH-adjustment of water sample

The pH of the filtered water sample was adjusted to 6.9 ± 0.1 using either 25% (v/v) NH_4OH and/or 2 M HNO_3 solution. If the pH of the water sample fell within the range of 6.9 ± 0.1, no adjustment was necessary. Otherwise, the required amount of acid or alkali for pH adjustment was determined by measuring the pH of a 10 mL aliquot of the water sample and adding the appropriate solution incrementally. Once the desired pH was reached, 2 mL of 0.025 M EDTA solution was added to the water sample in a 20 mL volumetric flask, then filled up to the mark with water and gently swirled to mix.

Chelating pretreatment

After pH adjustment, all samples and standards were left at room temperature for 60 minutes to allow for EDTA chelation. It was observed that chelation between Cr(III) and EDTA occurred with high efficiency, negating the need to warm up the samples as suggested by ISO 24384:2024. After this time, samples were immediately injected into the optimized HPLC-ICP-MS system for chromium species identification.

Determination of total Cr
For total Cr measurements, a five-point external calibration curve was generated using a Cr(III) single-element standard covering a concentration range of 0.01 to 50 µg/L. Each filtered water sample was diluted two-fold to a final volume of 10 mL with a 1% (v/v) HNO_3 blank. Triplicate preparations were made for each sample. Total Cr content was determined using ICP-MS with scandium (Sc) at a concentration of 10 µg/L as the internal standard. A blank containing the reagents used for total Cr determination was run simultaneously with the sample preparations.

Mobile phase preparation
The mobile phase was prepared daily by adding 637 µL of HNO_3 (69%) and 5 mL of EDTA 0.1 M in deionized water. The pH was then adjusted with nitric acid and ammonium hydroxide to 7.0 ± 0.1, and the solution was filled up to 1000 mL with deionized water.

Conditioning of HPLC column
Before using the column each day, it is crucial to allow the mobile phase to flow through it for 30 minutes. At the end of each day's use, it is important to wash the column with a mixture of methanol and water in a ratio of 5/95 (v/v) for a period of 15 minutes. This step serves to remove any salts present in the system and inhibit bacterial growth.
For long-term storage, the column should be rinsed with pure water followed by a mixture of 40/60 (v/v) methanol/water. After rinsing, the column is capped to prevent it from drying out.

Determination of Cr(III) and Cr(VI) species
A series of mixed calibration solutions for Cr(III) and Cr(VI) ranging from 0.01 to 10 µg/L was prepared by diluting intermediate stock solutions of 1 mg/L of Cr(III) and 1 mg/L of Cr(VI) prepared in water. Each calibration solution was prepared directly in the HPLC PP vial (1 mL), where 100 µL of 0.025 M EDTA was added, followed by filling up to the 1 mL mark with mobile phase. To maintain the oxidation states of Cr(III) and Cr(VI), the chelating pretreatment was conducted within one hour after preparing the calibration standards. Following the chelating pretreatment, the calibration standards were injected into the HPLC-ICP-MS system, followed by the water samples prepared in the same manner as the standards.

Recovery test
Due to the unavailability of certified reference materials (CRM) for Cr(III) and Cr(VI) in water, and the need to assess accuracy in analyzing unknown samples susceptible to matrix effects (e.g., loss or redox conversion of Cr(VI) or Cr(III)), a recovery test was conducted. This test involved analyzing unspiked and spiked samples with a known target concentration, along with blanks and standards.

For the recovery test, 100 µL of a 100 µg/L standard solution of Cr(III) and Cr(VI), plus 100 µL of 0.025 M EDTA were added to 800 µL of various water in 1mL HPLC PP vials. The purpose of this test was to validate the method's performance in real water samples. Following this, analysis of the spiked and unspiked water samples, as well as the standard solution of the Cr species at 1 µg/L, was carried out.

Results and Discussion

Calibration curve, LODs and MDLs
Figure 1 shows overlaid chromatograms of eight calibration standards ranging from 0.01–10 µg/L for both Cr species, demonstrating baseline separation and accurate measurement at low concentrations in water samples. The method exhibited low background levels, robust Cr ion signal sensitivities, and stable retention times (RT). Individual Cr(III)-EDTA complexes and Cr(VI) were identified, well separated and eluted within eight minutes allowing short run times. Additionally, the calibration curves (Figure 2a and b) for quantifying Cr(III) and Cr(VI) species in samples were constructed by plotting peak area against analyte concentration, yielding a correlation coefficient higher than 0.9995 for both species. The slope of calibration curves for Cr(III) and Cr(VI) match very closely (0.3% difference), demonstrating that the species do not interconvert, and the complexation of Cr(III) with EDTA is quantitatively complete.

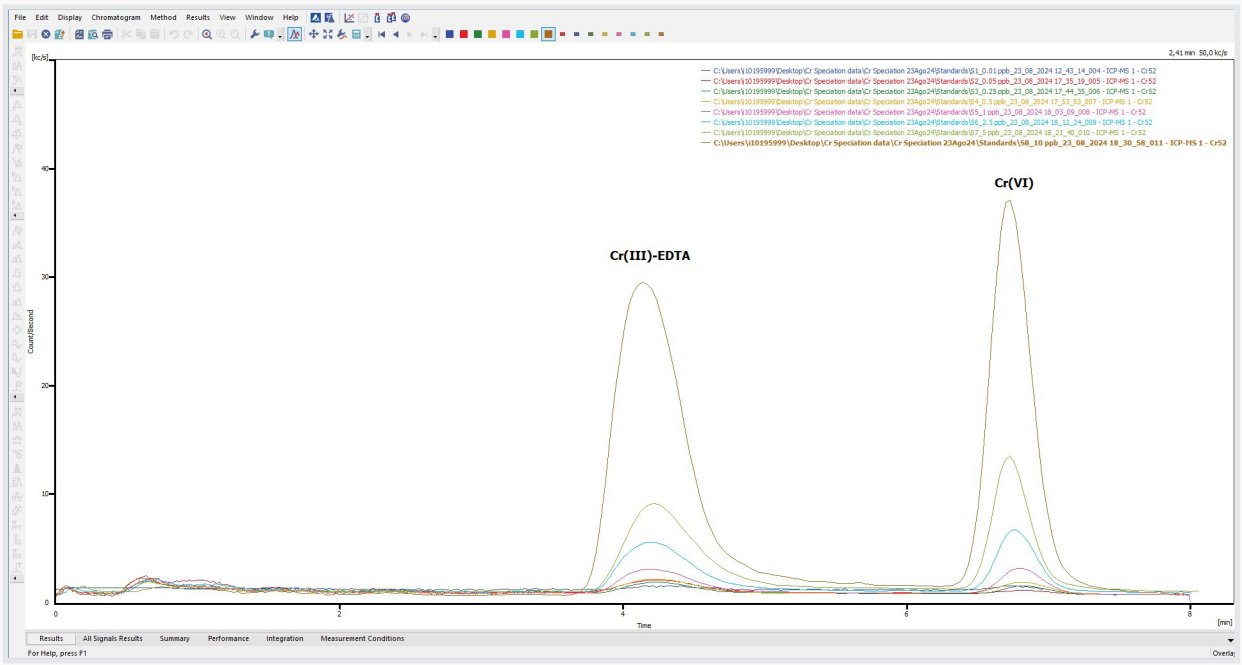

Figure 1: Overlaid chromatograms of eight calibration standards ranging from 0.01 up to 10 µg/L.

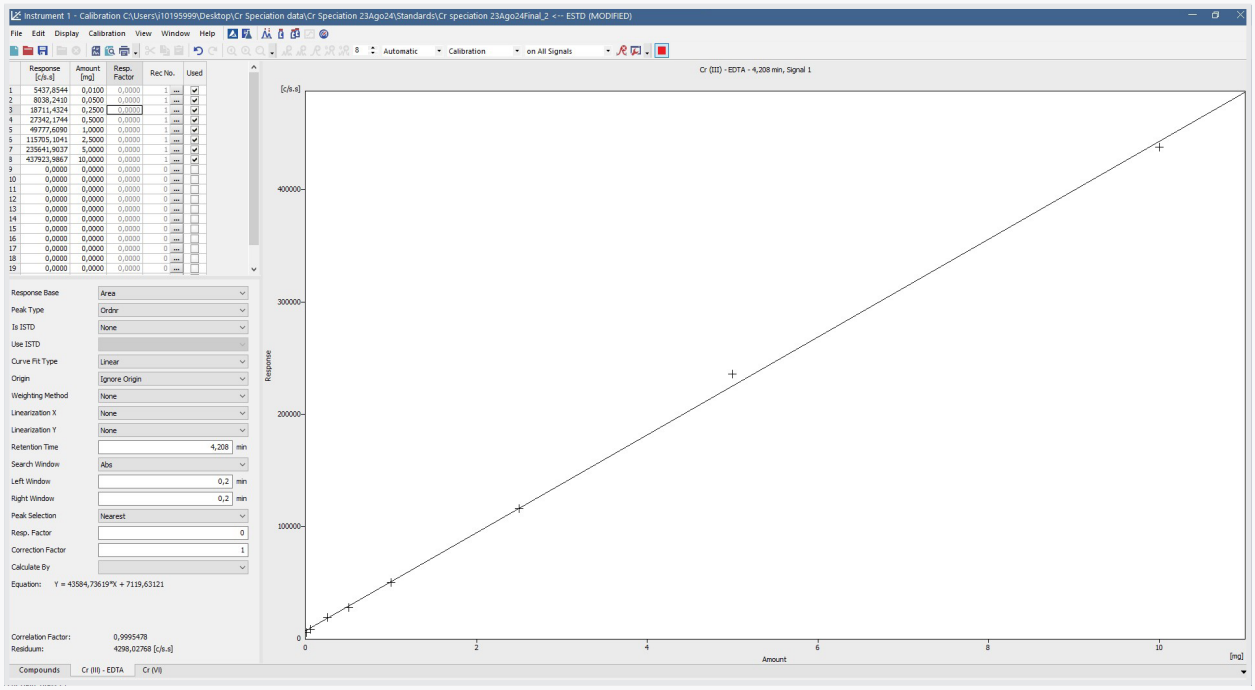

Figure 2a: Calibration curve of Cr(III)

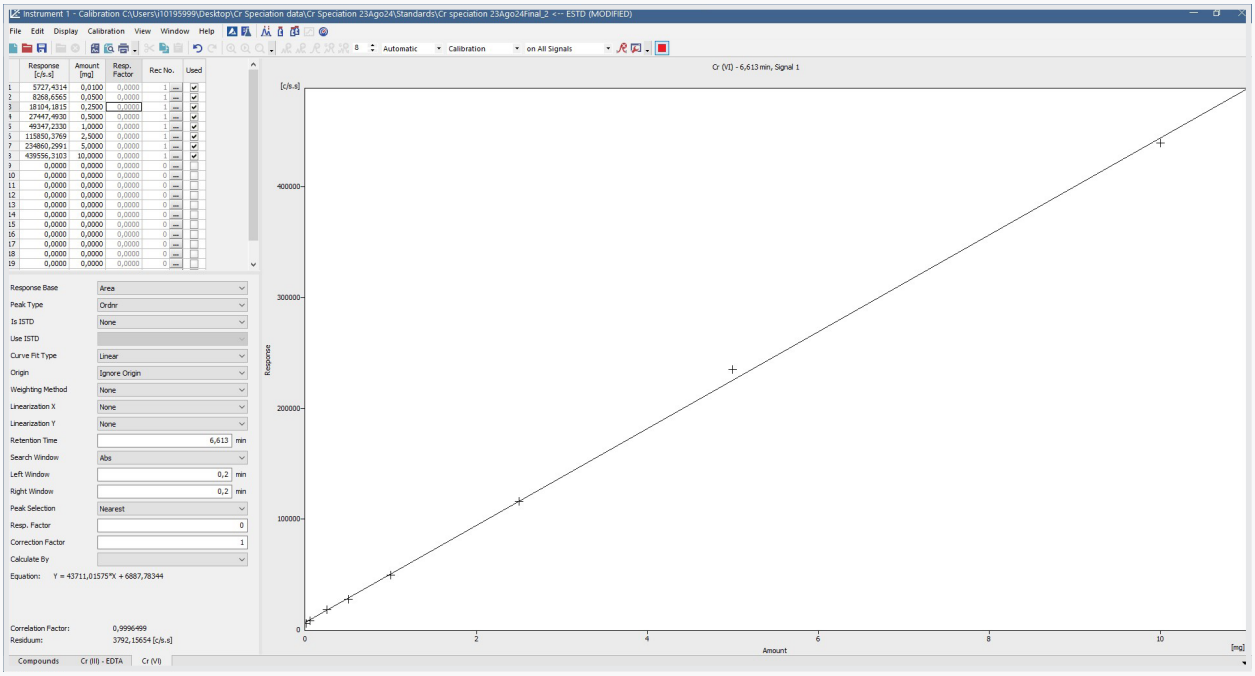

Figure 2b: Calibration curve of Cr(VI)

The chromatograms in Figure 3 display the 10 ng/L standard and the blank containing the mobile phase. Both peaks are distinctly visible above baseline and can be accurately measured, demonstrating that the detection limits for each species are below 10 ng/L under these conditions.
While ISO 24384:2024 does not mandate labs to obtain or report a method detection limit (MDL), it serves as a valuable performance indicator and may be required by various regulatory bodies involved in compliance monitoring. Hence, this study provides 0.01 µg/L for both Cr species as detection limits.

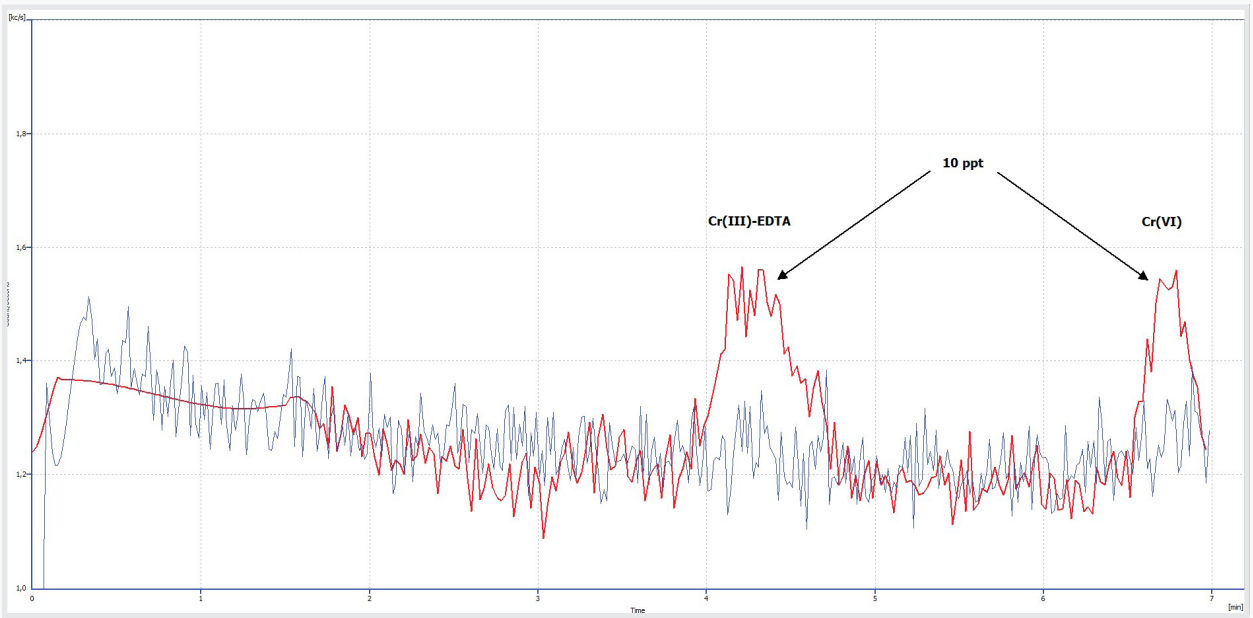

Figure 3: Comparison of the chromatograms showing 10 ng/L of both chromium species and the blank

Total Cr and Cr species concentrations

Table 2 shows the Cr total concentrations obtained in the filtered water samples by ICP-MS, Cr(III) and Cr(VI) species concentration in the water samples analyzed and recoveries obtained comparing both techniques HPLC-ICP-MS vs. ICP-MS. According to the results, recoveries from 97% to 116% were achieved demonstrating the accuracy of the results, which agree with the ISO/FDIS 23484:2024 criterium of 80% up to 120%, indicating an efficient chelating procedure.

Table 2: Concentration of total Cr obtained by ICP-MS and Cr species obtained by HPLC-ICP-MS, and recoveries in water samples. The recovery was calculated as the sum of Cr(III) and Cr(VI) divided by the concentration of total Cr. LOD of 10 ng/L.

Water sample type	Sample ID	Concentration [µg/L]			Recovery [%]
		Cr(III)	Cr(VI)	Total Cr	
Drinking Water	DW1	<LOD	0.023	0.021	109
	DW2	<LOD	0.116	0.102	114
Surface water	SW1	<LOD	0.078	0.067	116
	SW2	0.020	0.010	0.026	115
Groundwater	GW1	<LOD	0.125	0.124	101
	GW2	0.025	0.146	0.176	97
Wastewater	WW1	1.19	<LOD	1.201	99
	WW2	0.069	0.199	0.242	111

Accuracy

To assess the accuracy of the results, all samples were spiked with 1 mg/L of both chromium species. The recoveries for the spikes were within 10% of the target values (as illustrated in Figure 4), affirming the accuracy of the method. Due to the presence of organic matter or high Fe concentration in the sample matrix, it is important to note that wastewater sample WW1 did not exhibit detectable amounts of Cr(VI) after being spiked with 1 mg/L of Cr(III) and Cr(VI). This can be attributed to the reducing environment created by these factors, which lead to quantitative reduction of Cr(VI) to Cr(III). This effect was taken into account when calculating the recovery of spiked Cr species.

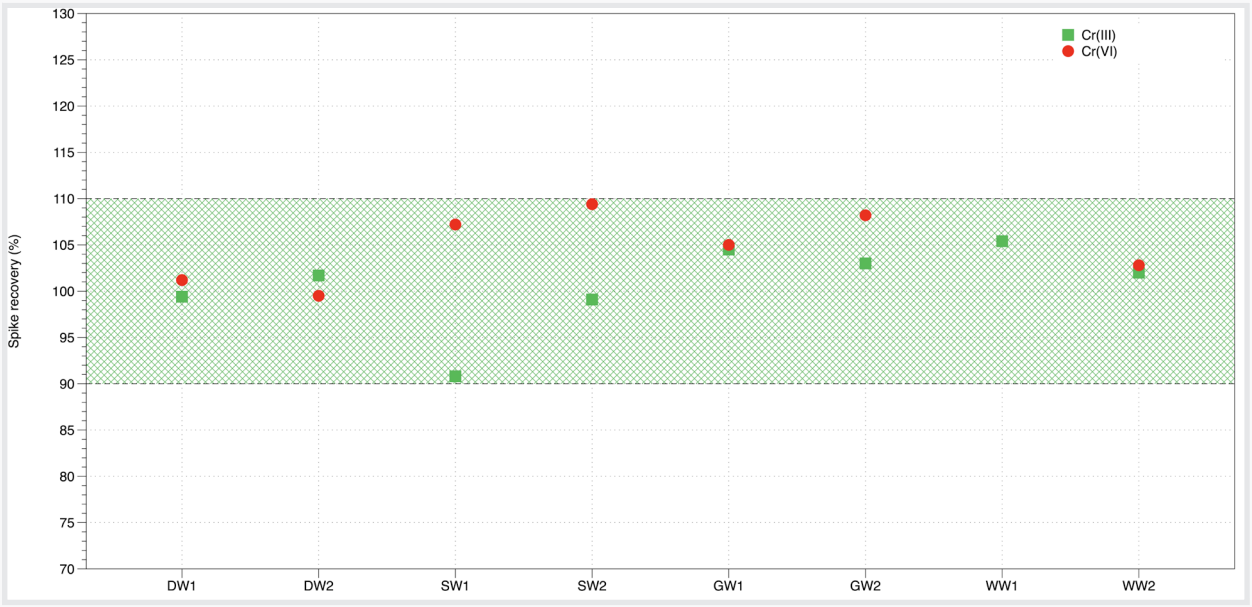

Figure 4: Recoveries of 1 µg/L of both Chromium species on all water samples tested. Due to reducing conditions, the sample WW1 did not contain detectable amounts of Cr(VI), and spiked Cr(VI) was quantitatively reduced to Cr(III) before analysis

Quality control (QC) – Long-term stability

Continuous analysis of water samples over 8-hour period was conducted, with a check standard (QC – 1 µg/L) measured every 30 minutes to assess long-term stability. Figure 5 illustrates the plot of the check standard, showing variations of less than 6% for both species indicating stability in the coupled HPLC-ICP-MS system (Figure 5).

Figure 5: Long-term stability of QC – 1 µg/L over 8-hours sequence

Repeatability – Short-term stability

To evaluate short-term stability, the methodology was tested by analyzing one of the water samples (DW2) nine times within a 90-minute period. The resulting Cr(VI) concentrations are depicted in Figure 6, with all readings normalized to the initial measurement. With concentrations showing a variation of less than ± 5%, this affirms the method's short-term stability.

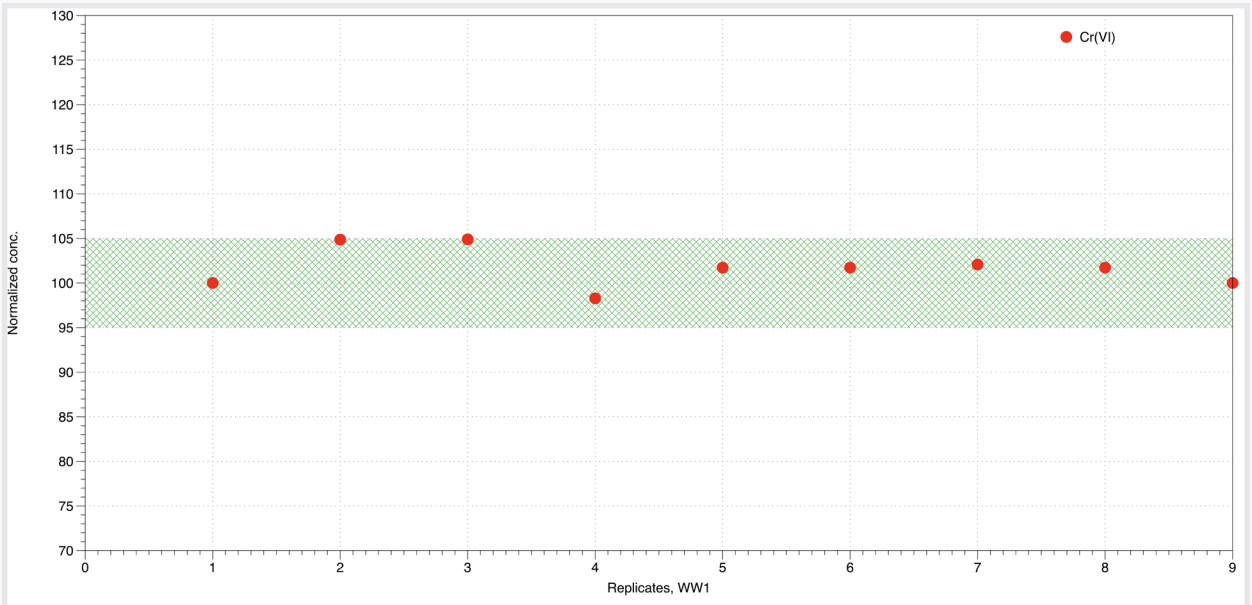

Figure 6: Repeated analysis of DW2 over 90 minutes. The concentration of Cr(III) was below LOD

Summary

This application note showcases the successful determination of Cr(III) and Cr(VI) species in diverse water samples using a PQ LC system coupled to the PlasmaQuant MS Q. Moreover, the HPLC method developed for Cr speciation in various water types with ICP-MS detection adheres to ISO 24384:2024 standards, enabling rapid separation of both Cr species within a mere eight minutes on a Hamilton PRP-X100 column, and a straightforward, rapid, and reliable sample preparation protocol was employed, ensuring minimal interconversion and loss of Cr species. The remarkable sensitivity of the PlasmaQuant MS Q ensures precise measurement of both Cr species at low concentrations. Method Detection Limits (MDLs) calculated comply with regulatory standards and fell within the low ng/L range, underlining the sensitivity and effectiveness of the approach for precise Cr speciation analysis, meeting the growing demand for swift and routine measurement of toxic Cr(VI) by regulatory bodies.

Figure 7: PlasmaQuant MS Q

Recommended device configuration

Table 3: Overview of devices, accessories, and consumables

Article	Article number	Description
Inductively Coupled Plasma Mass Spectrometry		
PlasmaQuant MS Q	818-08011-2	The PlasmaQuant MS Q is a high-performance ICP-MS, capable of measuring over 75 elements in a single measurement, from ultra-trace to major levels.
Cetac ASXPress Plus (or equivalent)	810-88017-0	The switching valve for Rapid Sample Introduction Accessory accelerates sample and rinsing processes and thus ensures both a high sample throughput and efficient washout behavior.
Cetac ASX-560 autosampler	810-88015-0	The Cetac Technologies ASX-560, next generation autosampler with integrated rinse function is sleek and durable by design.
HPLC		
PQ LC – HPLC System, nonmetal, PEEK	810-88409-0	The PQ LC system is a versatile and customizable solution, with a quaternary gradient pump, optional vacuum degasser, and micro- or analytical pump head.
HPLC column PRP-X-100,	418-88253-0	The Hamilton PRP-X100 is a highly stable and inert ion chromatography column designed for separating ions at varying concentrations. It offers reliable performance and selectivity, making it suitable for a wide range of analytical applications.

References

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[3] Shigeta, K., Fujita, A., Nakazato, T. and Tao, H., *A ROBUST METHOD FOR THE DETERMINATION OF CR(III) AND CR(VI) IN INDUSTRIAL WASTEWATERS BY LIQUID CHROMATOGRAPHY-INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY COMBINED WITH A CHELATING PRETREATMENT WITH 2,6-PYRIDINEDICARBOXYLIC ACID*. Analytical Sciences. 2018, 34, pages 925–932
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Version 1.0 · Author: RuSa
en · 01/2025