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The study focuses on validating reference methods such as ICP-OES and ICP-MS for detecting ultra-trace levels of chromium in groundwater, where concentrations are typically very low. Additionally, it verifies a hyphenated technique, IC-ICP-MS, for determining naturally occurring Cr(VI) in tested waters. The validation process involved various chromium analysis variants, including isotopes ^{52}Cr and ^{53}Cr in ICP-MS and IC-ICP-MS techniques, along with specific emission lines in the ICP-OES technique. Statistical data processing revealed that the achieved limits of quantification for Cr in different techniques ranged from 0.053 µg/L to 1.3 µg/L, with the associated measurement uncertainty estimated between 14% and 19% (at a coverage factor k = 2, 95%). For speciation analysis, it was possible to quantitatively determine Cr(VI) at concentrations as low as 0.12 µg/L, with the measurement uncertainty ranging between 10% and 14%. The Kruskal-Wallis test indicated that for the 14 water samples analysed, there was no statistically significant difference in the results obtained using different analytical techniques ($p > 0.05$). The geochemical modelling approach applied enhances the understanding of chromium speciation in water samples, verifying the accuracy of speciation analysis and identifying specific ion forms in which Cr(III) and Cr(VI) occur. In the analysed water samples, the concentration of Cr(VI) ranges between 0.13 and 35 µg/L, with the primary form identified as the oxoanion CrO_4^{2-}. Importantly, statistical tests demonstrated no statistically significant differences between the total chromium concentration in water and the concentration of Cr(VI), indicating that the entire concentration of total chromium corresponds to Cr(VI) speciation.

Keywords Hexavalent chromium, IC-ICP-MS, Hyphenated techniques, Geochemical modelling, Speciation analysis, Method validation

Chromium (Cr) is a transition metal from block d and the 6th group of the periodic table (atomic number 24). 13 different isotopes of chromium are known, but stable are only four — ^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr[1,2]. In the lithosphere, chromium exists in concentrations even at 100 mg/kg, which places it as the 24th most abundant element in Earth's crust[3,4]. Chromium is a trace element in the hydrosphere of the Earth and its concentration in natural water is usually below 1 µg/L[3,5]. The most common speciation of chromium in water is: Cr(III), which exists mostly as Cr^{3+} or $CrOH^{2+}$, and Cr(VI) presents as CrO_4^{2-} and $Cr_2O_7^{2-}$[6,7]. In small amounts, chromium(III) is essential for life and plays an important role in the metabolism of glucose, some proteins, and fats. It is also a component of some enzymes and stimulates the activity of others[8,9]. While Cr(VI) compounds have toxic, mutagenic, and carcinogenic effects on humans, especially during chronic exposure[10–14]. Increased chromium concentrations in groundwater may result from natural weathering processes of basaltic, mafic and ultramafic rocks[15]. However, the increase of chromium concentrations in natural water has predominantly been associated with human and industrial activities, such as tanneries, galvanising plants, automotive and aviation industry plants, and chrome ore processing plants[16]. The worldwide regulations established guideline values or maximum permissible concentrations of total chromium in drinking water at different levels: U.S. EPA – 100 µg/L[17], WHO – 50 µg/L[18] whereas in EU the parametric value of 25 µg/L shall be met, at the latest, by 12 January 2036 and until that date it is set as 50 µg/L[19]. Accurate determination of the chromium content in natural water samples is crucial, especially when these samples naturally contain its toxic form, Cr(VI). Regulations introduced under the Community action in water policy[20] led to the establishment of technical specifications for analysing and monitoring the chemical status of water[21]. These regulations aim to ensure the comparability of results across laboratories in member countries, with quality management systems based on ISO 17025 standards[22]. Methods used for monitoring water quality[21] must adhere to minimum criteria for results, considering principles of

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measurement uncertainty and the limit of quantification for each monitored contaminant. To achieve result comparability, laboratories should not only meet standard requirements for testing and calibration but also participate in proficiency tests and use certified reference materials. In environmental studies related to assessing water quality, minimum criteria include a maximum measurement uncertainty of 50% at k = 2, 95%, and a limit of quantification at 30% of the relevant quality standards. For water intended for human consumption, the maximum acceptable uncertainty level, estimated at the parametric value of total chromium at 50 μg/L, is 30%[19]. An alternative to measurement uncertainty and quantification limit can be the minimum analysis characteristics, including trueness, precision, and detection limit, expressed as percentages of the parametric value. In Poland, for total chromium concentrations in water intended for consumption, these values are set at 10 %. Parametric values for Cr(III) or toxic forms of Cr(VI) are not established in most countries. In some states of the USA such as California and New Jersey, the environmental quality criteria for Cr(VI) are set at 20 and 70 μg/L, respectively[17,23].

Due to the typically low chromium concentrations in natural water, appropriate analytical techniques should be applied to obtain reliable and valid results. The most commonly used methods for total chromium determination are: inductively coupled plasma optical emission spectrometry (ICP-OES)[24], inductively coupled plasma mass spectrometry (ICP-MS)[25], flame atomic absorption spectrometry (FAAS)[26], electrothermal atomic absorption spectrometry (ET-AAS)[27], energy-dispersive X-ray fluorescence spectrometry (EDXRF)[28], total reflection X-ray fluorescence spectrometry (TXRF)[29], UV-Vis[30]. The same techniques may be used for Cr(III) and Cr(VI) species measurements after appropriate sample pretreatment, e.g. solid phase extraction (SPE)[31]. Hyphenated techniques are also successfully applied for chromium speciation analysis. The most commonly used are high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS)[32], ion chromatography combined with inductively coupled plasma mass spectrometry (IC-ICP-MS)[33], and ion chromatography (IC) with UV-Vis detection[3].

When only the total concentration of chromium is determined by analytical techniques, the calculation of several species concentrations of this element may be performed using geochemical modelling. The most popular programs such as PHREEQC[34] and Geochemist's Workbench[35] with appropriate thermodynamic databases[36] are used to perform calculations, the results of which are used in various studies[37–43]. To perform correct calculations and reflect the natural state of the tested object, field measurements of pH, oxidation-reduction potential (E_H) and temperature are necessary[44].

The primary objectives of this study are as follows: (i) To conduct a comparative analysis of two widely employed analytical techniques, ICP-MS and ICP-OES, for quantifying total chromium concentrations in water samples; (ii) To assess the strengths and limitations of ion chromatography in separating chromium speciation (especially Cr(VI)), and to explore the capabilities of the combined IC-ICP-MS approach for water sample analysis, (iii) To employ geochemical modelling in calculating chromium species distribution, based on the total chromium determinations, and subsequently comparing the modelling results with concentrations obtained through IC-ICP-MS techniques.

We formulated the following hypotheses:

1. The ICP-MS with a single quadrupole and a collision-reaction cell (CRC) can be utilized for ultra-trace chromium analysis in natural water samples, regardless of the monitored ^{52}Cr or ^{53}Cr isotope.
2. The utilization of ICP-OES techniques with axial iFR plasma viewing provides the ability to accurately determine chromium even at low concentrations.
3. The combined IC-ICP-MS technique demonstrates its effectiveness in accurately determining low concentrations of chromium speciation in water samples.
4. In the case of groundwater, employing geochemical modelling facilitates the reliable calculation of chromium speciation, producing valid results.

Experimental
Reagents
During the experiment, we verified standardized methods[45,46] for determining trace concentrations of chromium in water intended for human consumption, which naturally contains elevated levels of Cr. We employed a TraceCERT® multielement standard solution in 10% nitric acid, with a concentration of 10 mg/L Cr, sourced from Sigma-Aldrich (Missouri, USA), to create calibration standards for ICP-OES and ICP-MS techniques. In ICP-OES measurements, we used TraceCERT® 1000 mg/L Ge (Sigma-Aldrich, Missouri, USA) at an emission line of 265.118 nm as the internal standard, chosen for its lack of spectral interference with the element of interest. For the ICP-MS technique, we utilized a single solution of scandium (LGC Ltd, England) as the internal standard, monitoring the ^{45}Sc isotope. The certified reference materials such as fortified lake water TMDA 64.3 obtained from Environment and Climat Change Canada, and Hard Drinking Water UK — Metals obtained from LGC Standards were also used.

In the ion chromatographic system (IC) coupled to ICP-MS, a 100 mg/L Cr^{6+} single solution (LGC Ltd, England) was employed to construct a calibration curve for analysing Cr(VI) concentrations in water samples. Calibration standards and internal standard solutions were freshly prepared each day, with dilution as needed using ultrapure water with a resistivity of 18.2 MΩ·cm at 25 °C from the Merck Millipore Direct-Q 3 UV-R Purification System. For chromatographic separation of Cr(VI) the IonPac AG-7 Guard Column (2 × 50 mm) was used. Retention time of Cr(VI) speciation was ~ 35 s. Utilizing only a 5 cm guard column, the analysis time is significantly reduced, thereby increasing the number of samples that can be analysed in a day. With an injection volume of 50 μL, the guard column alone provides sufficient chromatographic resolution to successfully separate Cr(VI) from Cr(III). The guard column was not thermostated, and the analyses were conducted at the ambient laboratory temperature of 20–22 °C. The PFA-LC nebulizer used (Table 1) ensured zero dead volume. The

Parameter	ICP-OES	ICP-MS	IC-ICP-MS
Plasma gas flow [L/min]	13.5	15	15
Auxiliary gas flow [L/min]	0.5	0.8	0.8
Nebuliser gas flow [L/min]	0.45	1.025	1.025
Collision/Reaction Cell Gas (CRC)	—	He, 4.95 mL/min	
Nebuliser type	Concentric borosilicate glass	Concentric borosilicate glass	PFA-LC
Spray chamber	Quartz, cyclonic		
Injector	Quartz 2 mm ID	Quartz 2.5 mm ID	
Torch	Quartz		
RF Power	1250 W	1550 W	
Plasma viewing	Axial iFR	—	
Number of replicates	3	10	1
Elements emission line (ICP-OES) or isotope (ICP-MS) analysed	Cr – 267.716 nm Cr – 283.563 nm 40 s of exposure to the detector to enhance the limit of detection and obtain better precision at low concentrations	^{52}Cr and ^{53}Cr analysed in the kinetic energy discrimination (KED) mode (CRC to avoid interferences).	

Table 1. Operating conditions.

operating pressure of the chromatographic system ranged from 750 to 800 psi. The separation of chromium(VI) species from the water sample was carried out under isocratic elution conditions, with 100% of the mobile phase consisting of 0.4 M HNO_3 at a flow rate of 0.4 mL/min.

These standards and blanks were acidified using ultrapure 67% nitric acid (NORMATOM® Ultrapure for trace metal analysis, VWR, USA), with a ratio of 1 mL HNO_3 per 50 mL of standard. The same acid was utilized to prepare an eluent containing 0.4 M HNO_3, which served as the mobile phase in IC analysis of Cr(VI).

Chromium(III) calculation

The amount of chromium(III) in samples of natural water was calculated based on the difference between the determined concentration of total chromium and hexavalent chromium. This method is frequently used in environmental studies, especially when only one chromium speciation is determinable[47].

$$Cr_{tot} = Cr\,(III) + Cr\,(VI) \tag{1}$$

$$Cr\,(III) = \ Cr_{tot} - Cr\,(VI) \tag{2}$$

This approach assumes that the entire content of Cr(VI) has been measured. To determine whether there are statistically significant differences between the obtained results of Cr(VI) determinations and total chromium, the independent samples t-test was used.

Instrumental operating conditions

We determined total chromium using the Thermo Scientific iCAP RQ ICP-MS and iCAP PRO XP ICP-OES. Calibration, including wavelength adjustment and optimizing radio frequency power, was done with Thermo Scientific's blank and multielement solutions to maximize instrument sensitivity. In the ICP-MS, a Calibration Solution (CS) was employed to calibrate mass whenever peak width or alignment deviated from specifications. This CS was also used for detector calibration when sensitivity issues or other non-sample introduction system problems arose. Detector calibration involved adjusting voltages in the pulse-counting and analogue sections to maintain accurate cross-calibration. Furthermore, we conducted tuning of the ICP-MS whenever the sample introduction system was replaced or when the instrument did not meet the manufacturer's daily performance check requirements, ensuring accurate and reliable results for total chromium determination. The operating conditions of the ICP-OES and ICP-MS instruments are presented in Table 1.

Method validation parameters and interlaboratory comparisons

The rapid determination of toxic chromium speciation, such as Cr(VI), has been validated using a single-laboratory approach in accordance with Eurachem guide guidelines to confirm the suitability of a non-standardized method for a specific application and presenting its performance characteristics[48]. Standardized methods for total chromium determination in natural waters have been verified, following ISO 17025 guidelines for testing laboratories[22].

This study focused on establishing the working range for total chromium and Cr(VI) in water samples, crucial for accurate determination within acceptable uncertainty. The working range for total chromium was in the range from the estimated LOD to an upper limit of 50 µg/L, aligned with WHO guidelines for human consumption[18]. For Cr(VI), the upper tested working range was set at 5 µg/L, reflecting its lower concentration in the natural environment compared to Cr(III)[5]. Linearity within these ranges was evaluated through R^2 calculation. The study adopted the IUPAC-recommended confidence interval of 0.05 for the limit of detection and calculated the limit of quantification as the smallest concentration with a precision not exceeding 10%.

Limit of detection and limit of quantification were determined with the following equations:

$$LOD = 3 * \frac{\sigma\ BLANK}{b} \tag{3}$$

$$LOQ = k_Q * \frac{\sigma\ BLANK}{b} \tag{4}$$

where:

b – sensitivity (slope of the calibration curve) of the method derived from the linearity $y = bx + a$.

σ_{BLANK}– standard deviation from the repeated measurements of the blank.

k_Q– multiplier which reciprocal corresponds the selected quantifying RSD. The maximum allowable precision adopted for the purposes of this work is 10%, therefore k_Q= 10.

In light of ongoing discussions about reducing the parametric value for total chromium to 25 μg/L and establishing a separate parametric value for toxic Cr(VI), it is important to verify the applicability of these methods for accurately determining trace amounts of this element in such samples. Precision, measured by repeatability, and trueness, evaluated through bias assessment using certified reference materials and spiked samples, were essential components[48–51]. Measurement uncertainty, considering type A and type B approaches, was estimated through statistical distribution and probability density functions, encompassing sources like linearity ($u_{linearity}$), precision ($u_{precision}$), trueness ($u_{trueness}$), and random errors (u_{RE}), resulting in the calculation of expanded uncertainty (*U*)[52]. U is the product of the combined standard uncertainty ($u_c(y)$) and the coverage factor (k), which is typically $1.96 \approx 2$ for a 95% probability level.

To verify the reliability of the results obtained in this study, interlaboratory comparisons were conducted with independent accredited research laboratory, which analysed samples with unknown Cr(VI) concentrations using ion chromatography with spectrophotometric detection. For the interlaboratory comparisons, 20 L of drinking water were collected and prepared for laboratory testing, resulting in 8 samples. The first sample served as the matrix, and known concentrations of Cr(VI) were added to the remaining 7 samples using the reagents specified in Sect. 2.1. Each sample was prepared by the independent analyst not included in this study, in a 1-liter volumetric flask and then divided into two portions. One portion was transferred to bottles provided by the participating laboratory, containing $(NH_4)_2SO_4$ as a sample stabilizer and buffer between Cr(III) and Cr(VI) transitions, and to prevent Cr(VI) sorption on the walls of the container, especially at trace levels. The second portion was remained in the Authors' laboratory. The laboratories participating in this interlaboratory comparison were only aware of the expected concentration range of Cr(VI) in the water, not the true value.

Geochemical modelling of chromium species distribution

Geochemical modelling was employed to calculate the distribution of aqueous Cr(VI) species in the natural water samples used to validate Cr(VI) speciation by IC-ICP-MS. All calculations were performed using Geochemist's Workbench Professional software version 17.0.2, utilizing the thermodynamic database *thermo.dat*[35]. This database includes 46 elements, 47 basic species, 48 redox pairs, 551 aqueous species, and 624 minerals. In these calculations, activity coefficients for individual ions in simple solutions were determined using the Debye-Hückel limiting law[53]. However, this method has its limitations, specifically an ionic strength not exceeding 0.1 molal. Beyond this threshold, alternative approaches like the extended Debye-Hückel law (with B-dot correction by[54], Davies law, Pitzer equation, or other empirical models can be applied[35,43]. The choice of which law to apply depends on the molal concentration of the solution being examined. Generally, the more concentrated the solution, the less reliable the estimated activity coefficients become. Distribution of the Cr(VI) aqueous species was calculated using SpecE8 module and their stability diagram (based on pH, E_H and activity) was prepared in Act2 module of the Geochemist's Workbench software. For the calculations of Cr species, the total concentrations of Cr obtained with two analytical techniques were used and compared with the IC-ICP-MS speciation analysis results.

Results and discussion

Total chromium and Cr(VI) validation results

Linearity

First, linearity tests were performed to check whether the method was linear within the assumed range. To determine the linearity of the ICP-MS and ICP-OES techniques, 8 calibration standards were used, the concentration of which ranged from 0.010 μg/L to 50 μg/L. Linearity results are presented in Table 2.

As can be noted from the results, the ICP-MS technique allowed the creation of a calibration curve starting from 0.010 μg/L. The chromium ion counts for the blank sample differed significantly from the calibration standard STD-1. For determinations of ^{52}Cr, the difference exceeded 700 counts, while for ^{53}Cr, it was much lower, around 100 counts. This significant variance in detector counting results is linked to the natural abundance of the ^{53}Cr isotope in the environment, which is 9.50%, in contrast to the ^{52}Cr isotope, which is approximately 83.79%[55].

ICP-MS offers a strong advantage with its broad linear range and high sensitivity[56].

In this study, ICP-MS revealed excellent linearity within the tested range of 0.025 μg/L to 50 μg/L for both ^{52}Cr and ^{53}Cr isotopes (Supplementary Figure S1a). The technique showed sensitivity values of 42,680 cps/μg/L for ^{52}Cr and 5926 cps/μg/L for ^{53}Cr. The coefficient of determination (R^2) for both isotopes confirmed linearity, with a value of 0.9995. Similar results were obtained with IC-ICP-MS instrumentation (Table 3), which also showed a linear relationship within the range of 0.050 μg/L to 5 μg/L, considering the typical low concentrations of Cr in water (Supplementary Figure S1c). The R^2 values for both ^{52}Cr and ^{53}Cr isotopes were 0.9999 and 0.9998, respectively. The sensitivity of the hyphenated technique was comparable to ICP-MS alone.

Calibration standard	Cr concentration µg/L	ICP-MS [cps] ^{52}Cr	^{53}Cr	ICP-OES [cps] 267.716 nm	283.563 nm
Blank	0.000	1549	213	1.7	27.7
STD-1	0.010	2201	314	~Blank (points excluded from the calibration curve)	
STD-2	0.025	2764	384		
STD-3	0.050	5167	720		
STD-4	0.10	5942	822	2.0	34.5
STD-5	1.0	40,538	5652	5.0	44.2
STD-6	10.0	397,256	55,292	41.4	145.7
STD-7	25.0	1,029,558	143,376	101.7	318.3
STD-8	50.0	2,150,436	298,447	199.0	588.9

Table 2. Linearity of the ICP-MS and ICP-OES techniques. For ICP−OES, responses for STD-1−STD-3 were at the same level as in the blank samples. These standards were excluded from linearity tests.

Calibration standard	Cr(VI) concentration µg/L	IC-ICP-MS [cps] ^{52}Cr	^{53}Cr
Blank	0.000	223.3	34.3
STD-1	0.050	2825	463.1
STD-2	0.1	4049	526.4
STD-3	1.0	41,590	5004
STD-4	5.0	214,551	26,357

Table 3. Linearity of the hyphenated technique of IC-ICP-MS.

Parameter		ICP-MS ^{52}Cr	^{53}Cr	ICP-OES 267.716 nm	283.563 nm	IC-ICP-MS ^{52}Cr	^{53}Cr
Replicates/run		10	10	3	3	1	1
$\bar{x}$	cps	1540	220.8	1.31	33.68	748.1	119.5
s	cps	224.7	53.4	0.52	1.08	540.5	63.4
b	cps/µg/L	42,680	5926	3.96	11.19	42,883	5260
LOD	µg/L	0.016	0.027	0.39	0.291	0.038	0.036
LOQ	µg/L	0.053	0.090	1.31	0.969	0.126	0.121

Table 4. Estimated LOD and LOQ values for ICP-MS and ICP-OES techniques.

In contrast, ICP-OES had intensities for the first three calibration standards at the blank level due to differences in the measurement process and lower sensitivity compared to ICP-MS, resulting in higher detection and quantification limits[24,57]. Therefore, the ICP-OES working range was evaluated from 0.10 µg/L to 50 µg/L. Both chromium emission lines showed excellent linearity, with R^2 coefficients of 0.9999 for 267.716 nm wavelength and 0.9997 for 283.563 nm (Supplementary Figure S1b). The sensitivity for these emission lines was 4 cps/µg/L for 267.716 nm and 11.118 cps/µg/L for 283.563 nm.

LOD and LOQ estimation

To assess the limits of detection (LOD) and quantification (LOQ) for ICP-MS, ICP-OES and IC-ICP-MS, 10 blank samples for each technique were used, performing 10, 3 and 1 replicates during analysis.

Based on the signal from chromium obtained during the analysis of blank samples the calculated detection limits were as follows: ICP-MS had limits of 0.016 µg/L and 0.027 µg/L, while ICP-OES had limits of 0.39 µg/L and 0.29 µg/L. For IC-ICP-MS, LOD and LOQ values were slightly higher than direct ICP-MS measurements but remained similar for both chromium isotopes (Table 4).

Fiket et al.[58] reported an ICP-MS instrument detection limit (IDL) of 0.010 µg/L for ^{52}Cr in He gas mode, while Bityukova & Petersell[59] noted the same value during multielemental analysis of bottled mineral waters. In contrast, Birke et al.[60] reported a practical detection limit (PDL) of 0.014 µg/L and a reporting detection limit of 0.2 µg/L. Measurements of ^{52}Cr in standard mode without a collision-reaction cell may suffer from polyatomic interferences with m/z = 52, such as $^{40}Ar^{12}C^+$, $^{35}Cl^{16}O^1H^+$, $^{37}Cl^{15}N^+$, $^{34}S^{18}O^+$, $^{36}S^{16}O^+$, $^{38}Ar^{14}N^+$, $^{36}Ar^{15}N^1H^+$, and

$^{35}Cl^{17}O^{+}$[61]. Additionally, KED measurements with helium as a collision gas need optimization to avoid sample matrix interference, particularly with high chlorine content, leading to too high recovery[56].

For the ICP-OES technique, the estimated limit is an order of magnitude higher (Table 4). LOQ values for ICP-MS were 0.053 μg/L for ^{52}Cr and nearly twice as high for ^{53}Cr, at 0.090 μg/L. In contrast, ICP-OES quantification limits were 1.3 μg/L at a wavelength of 267.716 nm and 1 μg/L at 283.563 nm. IC-ICP-MS revealed the quantification limit at the level of 0.126 and 0.121 μg/L for ^{52}Cr and ^{53}Cr respectively.

Trueness and precision

The validation of our methods involved the analysis of certified reference materials in various water matrices to determine total chromium concentration. These materials were diluted according to Tables 5 and 6 to cover the working range between the quantification limit and the upper point of calibration curve.

Our analysis revealed that during ICP-OES instrumentation, the poorest recovery was observed at the 0.5 μg/L level for the LGC reference material, ranging from 84.9 to 139.1% for the 267.716 nm and 283.563 nm emission wavelengths. The results obtained for the 267.716 nm line might be acceptable for measurements, but the measurement precision was unacceptable as it exceeded 30%. For the second emission line, the precision was less than 7%, but the recovery value significantly exceeded the expected values (Supplementary Figure S2, Table 5). In the case of the Lake Water reference material, at the concentration level of 0.283 μg/L, the analysis results were below the detection limit (Table 5). For both chromium isotopes, the recovery for the LGC reference material at a concentration level of 0.050 μg/L was notably higher, at 120% and 119%, respectively. However, for lake water reference material, the recoveries were lower and did not exceed 110% (Table 5).

The precision of the measurements was at a very high level, ranging from 0.2% (ICP-OES at 283.563 nm for 50 μg/L LGC RM) to 6.6% (ICP-MS for ^{52}Cr at 0.0566 TMDA 64.3 RM). For determinations below the limit of quantification (LOQ), the precision ranged from 4.4 to 30.5% (Tables 5 and 6). Both validated techniques can be effectively used to determine the total chromium content in water, whether at the level of several dozen nanograms (ICP-MS) or micrograms per litre (ICP-OES). Previous comparative research has also demonstrated the advantages of these two techniques for determining chromium content in environmental and industrial samples, including mineral water samples and toy samples after Cr(VI) extraction[62], silicate materials[55], water samples following Cr(III) separation by montmorillonite[63], and crude oil post-microwave digestion[64].

Analyte	Reference material	Cr total μg/L	Verification results	Chromium concentration in CRM after dilution 0.025 μg/L	0.050 μg/L	0.5 μg/L	5 μg/L	25 μg/L	50 μg/L
^{52}Cr	Hard Drinking Water UK—Metals	50 ± 1.9	Result	< LOD	**0.060**	0.569	4.89	24.27	45.26
			R [%]		**120.2**	113.9	97.8	97.1	90.5
			RSD [%]		**4.4**	2.6	2.1	2.4	2.6
^{53}Cr			Result	< LOD	**0.060**	0.582	4.98	24.85	45.86
			R [%]		**119.1**	116.5	99.6	99.4	91.7
			RSD [%]		**7.2**	2.0	2.4	2.8	2.4
Cr at 267.716 nm			Result	< LOD	< LOD	**0.425**	4.62	23.79	49.55
			R [%]			**84.9**	92.5	95.2	99.1
			RSD [%]			**30.5**	5.7	0.3	0.4
Cr at 283.563 nm			Result	< LOD	< LOD	**0.696**	5.03	24.07	49.87
			R [%]			**139.1**	100.6	96.3	99.7
			RSD [%]			**6.5**	2.8	0.5	0.2

Analyte	Reference material	Cr total μg/L	Verification results	Chromium concentration in CRM after dilution 0.0283 μg/L	0.0566 μg/L	0.283 μg/L	2.83 μg/L	28.3 μg/L	56.6 μg/L
^{52}Cr	Fortified Lake Water – TMDA 64.3	283 ± 17	Result	< LOD	0.061	0.302	2.84	27.06	52.32
			R [%]		108.1	106.6	100.5	95.6	92.4
			RSD [%]		6.6	4.2	4.5	4.6	5.8
^{53}Cr			Result	< LOD	**0.062**	0.306	2.87	27.47	53.24
			R [%]		**109.5**	108.0	101.3	97.1	94.1
			RSD [%]		**8.0**	5.3	4.6	5.0	6.0
Cr at 267.716 nm			Result	< LOD	< LOD	< LOD	2.72	27.99	56.86
			R [%]				96.1	98.9	100.5
			RSD [%]				3.3	3.4	1.4
Cr at 283.563 nm			Result	< LOD	< LOD	< LOD	3.07	27.65	55.70
			R [%]				108.5	97.7	98.4
			RSD [%]				4.1	2.6	1.6

Table 5. Results of chromium determination in hard drinking and fortified Lake Water reference material. Results below the limit of quantification, but higher than limit of detection are marked in bold.

Analyte	Estimated standard uncertainty				Combined standard uncertainty	Expanded uncertainty
	u	*LGC*	*TMDA 64.3*	Mean u	$u_c(y)$	U (k = 2, 95%)
^{52}Cr	$u_{linearity}$	0.000091	0.000091	0.000091	0.070	13.91
	$u_{trueness}$	0.041	0.066	0.053		
	$u_{precision}$	0.028	0.051	0.040		
	u_{RE}	0.020	0.020	0.020		
^{53}Cr	$u_{linearity}$	0.00060	0.00060	0.00060	0.074	14.83
	$u_{trueness}$	0.042	0.068	0.055		
	$u_{precision}$	0.033	0.058	0.046		
	u_{RE}	0.020	0.020	0.020		
Cr at 267.716 nm	$u_{linearity}$	0.031	0.031	0.031	0.068	13.68
	$u_{trueness}$	0.040	0.065	0.052		
	$u_{precision}$	0.021	0.027	0.024		
	u_{RE}	0.020	0.020	0.020		
Cr at 283.563 nm	$u_{linearity}$	0.017	0.017	0.017	0.062	12.42
	$u_{trueness}$	0.039	0.067	0.053		
	$u_{precision}$	0.012	0.028	0.020		
	u_{RE}	0.02	0.020	0.020		

Table 6. Estimated measurement uncertainties (k = 2, 95%) for particular ICP-MS and ICP-OES techniques.

The precision and trueness of the IC-ICP-MS technique were calculated based on spiked natural water samples with 1 µg/L of Cr(VI) certified reference material. As can be observed in the chromatograms (Supplementary Figure S3), both ^{52}Cr and ^{53}Cr retention times are very similar, indicating minimal variability in the retention time of the chromatographed substance. This results in a very good average precision of 3.6% for chromium-52 and 5.7% for chromium-53.

Regarding trueness, expressed as recovery, it ranged from 98.5 to 108.3% for ^{52}Cr, with an average of 103.5%, and from 93.3 to 109.5% for ^{53}Cr, with an average of 101%. The obtained results show that the IC-ICP-MS technique allows the determination of Cr(VI) in natural water samples with satisfactory precision and trueness.

Uncertainty

For the calculation of the expanded uncertainty U (k = 2, 95%), the standard uncertainties derived from linearity, trueness, precision and random errors (*RE*) were estimated. The expanded uncertainty was estimated at each analysed level of chromium concentration in the matrix reference materials. Our study revealed that all the relative expanded uncertainty values for ICP-MS and ICP-OES techniques are very similar. For particular isotopes or the emission lines uncertainty was averaged based on the measurements performed (Table 6). For the ICP-MS technique, uncertainty estimated for ^{52}Cr is about 13.9% and for ^{53}Cr is equal to 14.8%. The highest uncertainty estimated for the ICP-OES measurement was for 267.716 nm emission line and reached almost 14%. It can be stated that both techniques in the assumed working range are suitable chromium analysis in natural water samples. Calculated validation parameters met the criteria set for analytical methods for analysing the concentration of chromium in water in terms of assessing its chemical status ($U < 50\%$) as well as its intended use for human consumption ($U < 30\%$).

The estimation of uncertainty resulting from chromatographic separation followed a similar approach as with previous techniques, involving the consideration of four distinct components in the uncertainty budget. To assess recovery, the estimation was based on the analysis of the spiked samples with addition of 1 µg/L of certified Cr(VI) reference material. In the case of IC-ICP-MS, the primary focus was on evaluating the impact of chromatographic separation of Cr(VI) on the overall uncertainty. The estimation outcomes are presented in Table 7.

The expanded uncertainty for the coupled technique was found to be 9.7% for the ^{52}Cr isotope and 13.6% for the^{53}Cr isotope. Therefore, it can be concluded that, when compared to ICP-MS in isolation, chromatographic separation does not significantly affect the final result uncertainty, as there was no observable increase in uncertainty based on the validation studies.

Results of interlaboratory comparisons

The results of the interlaboratory comparisons show very good agreement between the results obtained in the Authors' laboratory and those from independent accredited research laboratory. The analyte recovery obtained using the IC-ICP-MS technique in this study ranges from 98.8 to 109.9%. In the case of the second laboratory participating in the comparisons, the recovery ranged from 93.2 to 101.4% (Table 8). The Pearson correlation coefficient (r) for the two sets of results is 1.000. The IC-ICP-MS technique used for measurements can be considered reliable for determining Cr(VI) in water samples.

Chromium in natural waters — instrumentation and speciation modelling

All the above-mentioned analytical techniques were employed to determine chromium in natural water samples (NWS) collected from sources near the Zlatibor massif in Serbia. This massif is predominantly composed of

Monitored isotope	Estimated relative standard uncertainty u		Combined relative standard uncertainty $u_c(y)$	Expanded uncertainty [%] U (k = 2, 95%)
^{52}Cr	$u_{linearity}$	0.0000020	0.048	**9.66**
	$u_{trueness}$	0.026		
	$u_{precision}$	0.036		
	u_{RE}	0.020		
^{53}Cr	$u_{linearity}$	0.000035	0.068	**13.58**
	$u_{trueness}$	0.031		
	$u_{precision}$	0.057		
	u_{RE}	0.020		

Table 7. Estimated measurement uncertainties (k = 2, 95%) for IC-ICP-MS hyphenated technique.

No.	Interlaboratory comparisons samples	Cr(VI) spike [μg/L]	Present work: IC-ICP-MS [μg/L]	Present work: Recovery [%]	Independent Accredited Laboratory: IC with spectrophotometric detection [μg/L]	Independent Accredited Laboratory: Recovery [%]
1	Water matrix	0.00	**0.091**	—	< 0.4	—
2	Range: 0.5–1 μg/L Cr(VI)	0.50	0.592	100.0	< 0.8*	—
3	Range: 0.5–5 μg/L Cr(VI)	1.00	1.08	98.8	0.98	98.0
4	Range: 1–5 μg/L Cr(VI)	2.50	2.84	109.9	2.33	93.2
5	Range: 1–10 μg/L Cr(VI)	5.00	5.41	106.3	4.86	97.2
6	Range: 1–25 μg/L Cr(VI)	10.00	10.07	99.8	9.65	96.5
7	Range: 5–50 μg/L Cr(VI)	25.00	26.21	104.5	24.7	98.8
8	Range: 25–100 μg/L Cr(VI)	50.00	53.25	106.3	50.7	101.4

Table 8. Results of the interlaboratory comparisons. The Cr(VI) concentration measured in the matrix was subtracted from the results of samples 2–8. *Matrix interference as reported by the laboratory taking part in the organised ILC

ultramafic rocks, with the presence of Upper Cretaceous formations, Triassic carbonate rocks, and Tertiary lake sediments[65]. Water flowing through diverse geological formations within the massif may be enriched with chromium, which naturally occurs in the rocks of these geological formations. Residents often capture and use these waters as drinking water. The groundwater examined was characterised by the pH in the range from 7.26 to 9.39. The electrical conductivity, which is an approximation for total dissolved solids, varied between 0.224 mS/cm and 0.708 mS/cm. According to mineralisation, the tested waters can be classified as fresh waters (TDS < 1 g/L)[66]. The results of the total chromium concentration analysis indicate that chromium levels in the 14 groundwater samples tested range from 0.1 to 35 μg/L (Table 9). Among the results obtained using the ICP-OES technique, 3 of them fell below the limit of detection, and also 3 below the quantification limit estimated during method verification. In contrast, for the ICP-MS technique, each result was above the LOQ value.

A correlation analysis using PS IMAGO PRO 9 software was performed to assess the consistency of results between these two techniques. The Pearson correlation coefficient (r) obtained for the comparison of the selected emission lines and the two Cr isotopes monitored during ICP-MS analysis revealed positive correlation with $r(13) = 0.999$, $p < 0.001$ (two-tailed) to $r(13) = 1.000$, $p < 0.001$ (two-tailed).

During chromatographic separation, only chromium(VI) compounds were identified in the tested water samples, with a retention time of approximately 35 s. Since no other forms of chromium were detected during the speciation analysis, the total chromium content obtained by two reference methods was compared to the IC-ICP-MS technique (Fig. 1a, b). When comparing the results obtained using the ICP-OES and IC-ICP-MS techniques (Fig. 1a), it can be seen that the results obtained for individual emission lines closely align with the results obtained for the two chromium isotopes monitored during the IC-ICP-MS analysis (see Fig. 1a). In the case of the ICP-MS technique (Fig. 1b), slightly higher chromium concentration results were obtained with the IC-ICP-MS technique for concentrations ranging between 25 μg/L and 35 μg/L. This difference may suggest a memory effect of the analytical column after the analysis of high Cr(VI) content leading to too high recovery for these samples.

The Kruskal-Wallis test, non-parametric one-way analysis of variance for independent variables, was applied to confirm that the results were not statistically different. The test results confirmed that there are no statistically significant differences in the chromium concentrations in the tested natural water samples (independent variables) obtained using all instrumental techniques (grouping variable) – $p > 0.05$. Since the difference between results is insignificant, the results of Cr concentrations for each technique were averaged to calculate the analyte recovery based on the analysis of the spiked natural water samples with Cr(VI) certified reference material. The

Sample name	T	pH	γ_{25}	pe	ICP-OES		ICP-MS		IC-ICP-MS	
$n=14$	[°C]	[–]	[mS/cm]	[—]	267.716 nm	283.563 nm	^{52}Cr	^{53}Cr	^{52}Cr	^{53}Cr
NWS-1	10.5	9.39	0.388	4.66	1.77	2.10	1.91	1.96	2.06	1.99
NWS-2	18.6	7.28	0.536	7.67	< LOD	< LOD	0.175	0.175	0.215	0.202
NWS-3	10.7	7.26	0.224	7.66	< LOD	< LOD	0.132	0.134	0.130	0.129
NWS-4	15.5	8.35	0.435	8.11	14.42	14.63	13.08	13.35	13.87	13.66
NWS-5	15.2	7.40	0.708	13.27	25.85	26.11	23.28	23.45	26.12	26.72
NWS-6	13.1	8.42	0.453	8.57	10.14	10.16	10.11	10.10	11.35	11.56
NWS-7	13.2	8.92	0.553	10.45	8.75	8.86	7.89	7.92	9.72	10.05
NWS-8	15.7	7.77	0.333	9.85	< LOQ	< LOQ	0.634	0.646	0.687	0.599
NWS-9	12.6	8.27	0.346	6.75	< LOD	< LOD	0.246	0.249	0.194	0.168
NWS-10	14.1	8.11	0.474	8.73	24.18	24.23	22.16	22.16	24.21	25.53
NWS-11	12.6	8.50	0.398	7.44	12.94	13.16	11.70	11.80	13.77	14.56
NWS-12	12.5	8.40	0.342	6.70	35.33	35.02	31.77	32.27	34.23	35.61
NWS-13	10.5	7.81	0.275	6.94	< LOQ	< LOQ	0.653	0.675	0.621	0.634
NWS-14	15.4	7.35	0.468	7.78	< LOQ	< LOQ	0.571	0.579	0.617	0.488

Table 9. Results of total chromium and chromium speciation analysis in natural water samples [µg/L] and field measurement parameters used for speciation modelling. **T** – temperature of water sample on outflow; $\boldsymbol{\gamma_{25}}$– electrical conductivity; **pe** – electron activity calculated based on the oxidation–reduction potential E_H of water samples measurement in the field.

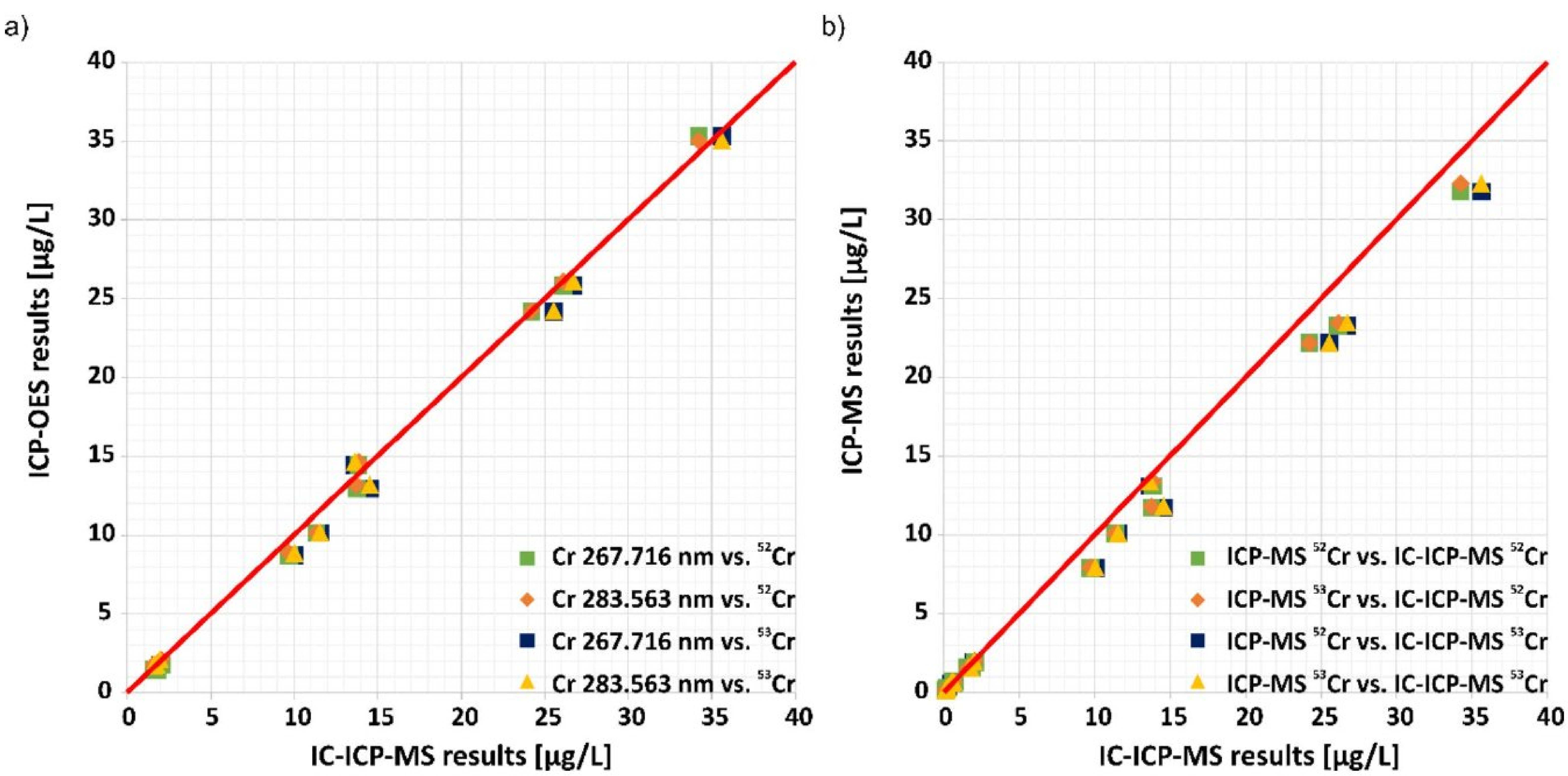

Fig. 1. Comparison of (a) ICP-OES and (b) ICP-MS vs. IC-ICP-MS results.

average analyte recovery ranged from 94.8 to 112.9% for IC-ICP-MS/ICP-OES and from 73.1 to 125.1% for IC-ICP-MS/ICP-MS.

The determinations of both total chromium and chromium(VI) in water samples showed that the concentrations obtained from these analyses are very close to each other. This may indicate that there is only one form present in the water, namely Cr(VI), and the concentration of the other speciation, Cr(III), is insignificant from the analytical perspective. Therefore, the independent samples t-test was applied to check this theory. The grouping variables were the analytical techniques, and the equivalence of groups was checked within them using the $\chi 2$ test and the homogeneity of variance was assessed using Levene's test. The tests indicated that the compared groups are equivalent ($\chi 2$ (2, $N=36$) $=2.000$; $p=0.368$) and the variances within them are homogeneous (p for Levene's test >0.05). When comparing both group 1, which consisted of measurements obtained by ICP-MS, and group 2, consisting of results obtained by IC-ICP-MS, as well as between the results obtained by ICP-OES and IC-ICP-MS, no statistically significant differences in the mean chromium concentration were observed ($p>0.05$). This suggests that the total chromium in water samples corresponds to the entire Cr(VI) concentration, and

Cr(III) may be treated as negligible. These findings are also supported by the results of geochemical modelling conducted for this study.

In the modelling of aqueous chromium species, it was found that in 14 samples, the predominant form of chromium is Cr(VI), mainly in the form of the chromium oxoanion CrO_4^{2-} (Fig. 2). In 9 samples (NWS-4–NWS-12), CrO_4^{2-} constituted over 90% of the total calculated speciation of chromium(VI) in the analysed water samples. For 4 samples (NWS-1, NWS-2, NWS-13 and NWS-14), this percentage ranged from almost 88% to nearly 90%. In the case of sample NWS-1, the concentration of CrO_4^{3-} was approximately 9% of the total Cr. Chromium in this anion exists in the +5 valence state, and this speciation is unstable in water, undergoing disproportionation between Cr(III) and Cr(VI)[67]. For NWS-3 sample, Cr(VI) comprised approximately 64%, while Cr(III) accounted for 26% of the total chromium concentration in the water. The migration of Cr(III) and Cr(VI) species and their transformation in the aquatic environment is strictly dependent on redox processes, including changes in oxidation-reduction conditions and the presence of redox pairs such as NO_2/NO_3, Fe(II)/Fe(III) or Mn(II)/Mn(IV), presence of clay minerals, Fe and Mn oxides and organic matter content. Manganese compounds may also be responsible for the transformation of Cr(III) to Cr(VI)[5].

Conclusions

The analytical techniques selected for testing, such as ICP-OES and ICP-MS, showed that these techniques have a very wide range of linearity – ICP-MS from several dozens of ng/L to several dozens of µg/L. The lower linearity range in ICP-OES is slightly higher (µg/L to several dozen µg/L), because this technique does not have the same high sensitivity compared to ICP-MS. The results showed that depending on the selected chromium isotope monitored in ICP-MS ^{52}Cr or ^{53}Cr) or the selected emission line (267.716 nm and 283.563 nm) in ICP-OES, the obtained results of detection and quantification limits may differ significantly (ng /L in ICP-MS to µg/L in ICP-OES). This is influenced by the natural abundance of chromium in the environment, the sensitivity of the analytical techniques themselves and the possibility of interference occurrence, especially in water with high total dissolved solids. The estimated validation parameters showed that both techniques can be useful for the analysis of total chromium content in natural water samples. The uncertainty associated with the precision errors of the determinations was a maximum of 6%, and the error associated with the correctness of the determinations

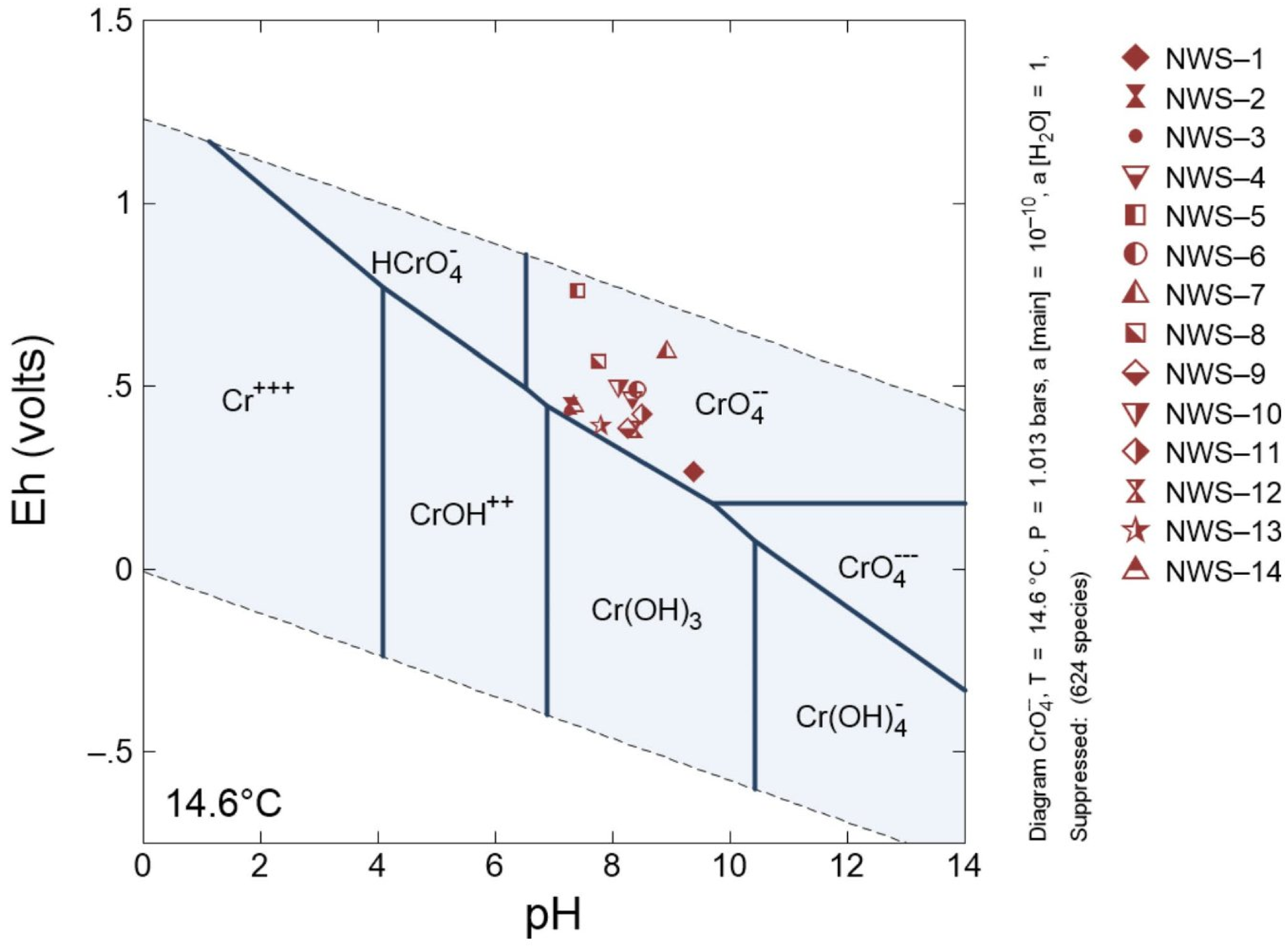

Fig. 2. Pourbaix plot for aqueous Cr species distribution in the modelled system.

was a maximum of 7%. This resulted in measurement uncertainty of 14–15% for the ICP-MS technique and 12 and 14% for the ICP-OES technique. The IC-ICP-MS technique was also validated for the determination of ultra-traces of Cr(VI) in natural water samples. Validation of the hyphenated technique showed that it is fast (~ 35 s of Cr(VI) retention time), precise (maximum RSD equal to 6% for ^{53}Cr) and characterized by very good recovery regardless of the monitored isotope. Speciation analysis of the waters selected for testing showed that they contained only the toxic form of chromium — Cr(VI). The results of the speciation analysis were positively correlated with the results of the analysis of total chromium content ($r > 0.999$), confirming the accuracy of the analyses performed. Moreover, the results of statistical analysis using the Kruskal-Wallis test did not show statistically significant differences in the results obtained with all analytical techniques used during the research ($p > 0.05$). The reliability of the results obtained using the IC-ICP-MS technique was confirmed through interlaboratory comparisons organized by the Authors, which demonstrated very good agreement between the results from the two research laboratories.

Moreover, geochemical modelling of aquatic chromium species was carried out to verify whether there were any changes in the form of chromium in water from the moment of sample collection to the analysis. For 14 samples, full consistency of results was obtained confirming that the chromium in water is hexavalent, mainly in the form of the oxoanion CrO_4^{2-}. Any difference between instrumentation and geochemical modelling may result from errors during measurements important for modelling parameters such as E_H, pH or temperature, or from more complicated processes occurring in the water-rock system, for which a discrepancy was obtained[35]. It should be taken into account that only chromium found in water was taken into account for modelling. At this stage, the possibility of the presence of mineral phases containing chromium in the system was not considered. Nevertheless, geochemical modelling can be successfully used to assist in the overall evaluations of chromium speciation analysis and to check whether the transformation of Cr(VI) to Cr(III) speciation or inversely occurred from the moment of sample collection to the moment of analysis, in particular when the sample for analysis is not preserved.

Data availability

All data generated or analysed during this study are included in this published article.

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Conceptualization – PR, KW, EK, VRV Methodology – PR, KW, EK Validation – PR, KW Formal analysis – PR Investigations – PR Writing – original draft – PR, KW Writing – Review & Editing – KW, EK, PR, VRV Visualization – PR, KW, EK.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
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