HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ONLINE/OFFLINE HYPHENATED TECHNIQUES: APPLICATION TO THE SPECIATION ANALYSIS OF CHROMIUM - A REVIEW

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Abstract
Researchers have established that the physiological effect of metals in biological systems depend largely on the specie of the metals and not on total concentration. Thus. prioritizing chemical speciation analysis over total concentration of analytes in the monitoring metal species/toxicity) trend in modern research in metal analysis. The recent interest in chromium speciation analysis, despite its numerous industrial applications, is kindled by the established physiological effects of Cr(III) and Cr(VI), which represents the "good" and the "bad" sides of the element respectively. Consequently, the sensitivity of detectors and the efficiency of separation processes, influence the quality of analytical results of chromium speciation analysis. This review focuses on the HPLC hyphenated systems and techniques developed for the speciation analysis of chromium in the last few decades. From this review, speciation analysis of chromium was carried out in biological, environmental and food samples. Most research focus on environmental samples. The HPLC-ICP-MS system was preferred for the speciation analysis. Derivatization was employed prior to preconcentration and HPLC separation process. One of the challenges in hyphenation is observed where the detector in ICP-MS especially in situations where organic solvents are involved due to the formation of argon carbide which interfere with analytical result through matrix effect. There seems to be prospect in the online hyphenation to flame atomic absorption spectrometer (FAAS). This is useful in cases where organic solvents are required as eluents or sample preparation

Keywords: HPLC, Hyphenation, hyphenates, Offline, Speciation, Chromium

Introduction

Attention is drawn to the speciation analysis of chromium in the last few decades due to the contrasting behavior of Cr(III) and Cr(VI) in biological system. Although Cr(III) is an essential element to humans, there is currently little or no records of nutritional benefits of chromium to plants (Cervantes *et al.*, 2001; Ghani, 2011). Nonetheless, Cr(III) plays a crucial role in the metabolism of glucose and lipids, which is important in the management of diabetes, (Baralkiewicz *et al.*, 2013; Kotas and Stasicka, 2000; Krejpcio, 2001; Pechova and Pavlata, 2007 ; M. Safari *et al.*, 2013; Shanker *et al.*, 2005(a); Vercoutere and Cornelis, 1995). It also aids the synthesis of nucleic and amino acids in mammals and some organisms thus, enabling the formation of DNA which is responsible for carrying and transferring genetic information to generations in all living organisms, (Kieber *et al.*, 2002; Krejpcio, 2001; Mulware, 2013).

In contrast, hexavalent chromium is a class I human carcinogen and has been linked to various health effects (Bielicka *et al.*, 2005; Langard and Costa, 2007; Lou *et al.*, 2013; Shadreck and Mugadza, 2013; Uddin *et al.*, 2007; Unceta *et al.*, 2010). For instance, on exposure to Cr(VI by steel industry workers, severe hemorrhage, cancer and death were reported, (Maher *et al.*, 2012; Shadreck and Mugadza, 2013). Toxicity of Cr(VI) to fish , (Kennedy, 2011), and plants, (Rodriguez *et al.*, 2012) have also been documented. The uptake of Cr(VI by plants have been linked to a reduction of bud sprouting and percentage germination, (Peralta *et al.*, 2001; RuscittiI *et al.*, 2011), shorten roots, stunted growth and yield reduction (Akinci and Akinci, 2010; Shanker *et al.*, 2005(b); Shanker *et al.*, 2005(a)).

Notable applications of Cr are in leather tanning (Unceta *et al.*, 2010), wood preservation, (Kieber *et al.*, 2002), artistic and anticorrosion paints, electroplating, and steel alloy and stainless steel welding, (Langard and Costa, 2007; Stern, 1982; USGS, 2012). Other industrial applications include metal plating, refractory, and metallurgy, (Kotas and Stasicka, 2000; Pariser, 2013; Unceta *et al.*, 2010). Study revealed that, human activities generate and discharge an estimated 75,000 tons of chromium into the atmosphere in addition to 54,000 tons from natural sources, a third of which is hexavalent chromium (Kieber *et al.*, 2002). This makes exposure to Cr species inevitable as Cr contaminated wastes are generated and deposited into the environment. In addition, it prompts the need for continuous monitoring of Cr species in the environment, food and other matrices. For effective monitoring, methods have been and are being developed for the speciation analysis of chromium.

The toxicity of a metal to organisms has long been based on its total concentration in a sample. But currently, research findings have established that the distinct effects of a metal in biological systems depend on its chemical forms (species). Therefore, analysts, prioritize chemical speciation analysis of metals over their total concentration for monitoring harmful chemical species (Cornelis *et al.*, 2003; Maher *et al.*, 2012).

A *Chemical specie* is a specific form of an element, -isotopic, ionic, complex, or molecular form. The term *Speciation,* on the other hand describes, (i), the distribution of *chemical species* of an element amongst defined chemical species in a system or, (ii) the grouping of analyte species in a matrix according to physical or chemical properties such as size, solubility, bonding, and reactivity. Nevertheless, *speciation analysis* refers to the series of analytical activities aimed to identify and/or quantify individual chemical species of an element in a matrix, (Cornelis *et al.*, 2003; Maher *et al.*, 2012). Chemical speciation analysis is crucial in risk assessment studies to monitor the toxicity of chemical species in food substances, ecosystem, and human occupational and environmental exposure (Grotti *et al.*, 2014; Maher *et al.*, 2012).

Since the premier linking of a gas chromatograph (GC) to a time-of-flight-mass spectrometer (TOF-MS) in the late 1950s, (Gohlke, 1959; Purcaro *et al.*, 2012), hyphenates or hyphenated techniques are preferred in chemical analyses for their speed and high degree of automation (Grotti *et al.*, 2014; Phalke and Kavade, 2013). Hyphenation (coupling/combination), being the "joining" of two or more instruments for analysis, makes seemingly independent instruments function simultaneously in harmony, (Patel *et al.*, 2010; Phalke and Kavade, 2013). A common hyphenated system is a separator-detector system, which provides a closed-system environment for good sample throughput, and reduced analyte contamination, thus enhances the quality of analytical result.

Recently, coupling is not limited to online analysis, but can be applied offline especially if the analyte, when separated does not pass through further rigorous sample treatment before analysis by the detector. Such offline procedure of analysis may be termed "offline combination, offline coupling, or offline combination". In this case, the separator and detector systems need not be in one place or function simultaneously since they are not linked directly. Offline coupling, in some instances gives room for little modification of the analyte to improved analytical result (Sumida *et al.*, 2005). In general, an efficient separator-detector system is powerful in unravelling the chemical profile of complex matrices (Phalke and Kavade, 2013). The choice of a hyphenated/combined system is a function of the type of analyte, sample, or desired outcome.

Previously, Komorowicz and Baralkiewicz (2011), reviewed the application of HPLC-ICP-MS for arsenic speciation. While–Maher *et al.* (2012) focused on metalloids speciation, Grotti *et al.* (2014) paid attention to elemental speciation analysis with HPLC-ICP-MS system only. In their article, Vogiatzis and Zachariadis (2014) dwelled on separation systems hyphenated to ICP-MS only. In another paper, Patel *et al.* (2010) were particular on the application of hyphenated systems in pharmacy. Nevertheless, Purcaro *et al.* (2012) focused on the general application of LC-GC-MS.
This review is specific on the application of both online and offline hyphenated systems for the speciation analysis of chromium in the last few decades.

Speciation of chromium

Hyphenated systems for chromium speciation
Figure 1, depicts the extend of the application of typical hyphenated systems for the speciation analysis of chromium in the review where the HPLC-ICP-MS system was preferred. Although ICP-MS is expensive, its preference as a detector is informed by its sensitivity, high speed of analysis with simultaneous multi-element determination as well as isotopic detection capability (Thomas, 2008); and ease of hyphenation, (Stanislawska *et al.*, 2013), in addition to compatibility with various sample preparation methods (Unceta *et al.*, 2010). The UV-Vis and DAD were next in the line of application and focused on ES and FS analysis. HPLC-AES systems were least utilized owing to online hyphenation difficulty. Low-level application of AAS, being a common instrument might also be attributable to the difficulty of coupling (online) to HPLC. Berndt (1988) made a landmark achievement by demonstrating the possibility of coupling FAAS or ICP-OES with a HPLC via a hydraulic high-pressure nebulizer (HHPN) system and compare signal from the analysis of Pb^+ through pneumatic nebulizer (PN) and HHPN. The HHPN was better due the discharge of more exploitable aerosol (aerosol reaching the plasma or flame) of the sample up to 40% to 45% as compared to 10% to 20% from a pneumatic nebulizer.
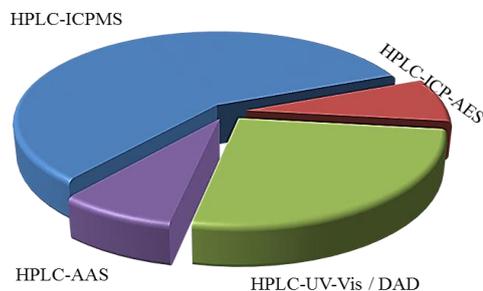
Figure 1: Common hyphenated systems for chromium speciation

Matrices used for Cr speciation
The extend of application of the hyphenated system to various category of samples is summarised in **Figure 2**. The classes of matrices were biological, foods, and environmental samples. Biological samples (BS), include blood, fresh meat, blood plasma, urine, and eggs. Food samples, (FB) are drinking water, mineral water, cereals, chocolates, and beverages. Others are fruits, animal oils, dairy products, and sea products. Air and air particulate matter, river water, wastewater, soils, sediments, wood, leachate and welding fumes and certified reference materials (CRM), are environmental samples, (ES). Research focused on environmental samples followed by food samples analysis. The awareness of the danger associated with exposure to Cr(VI) viz a viz its industrial application could be responsible.
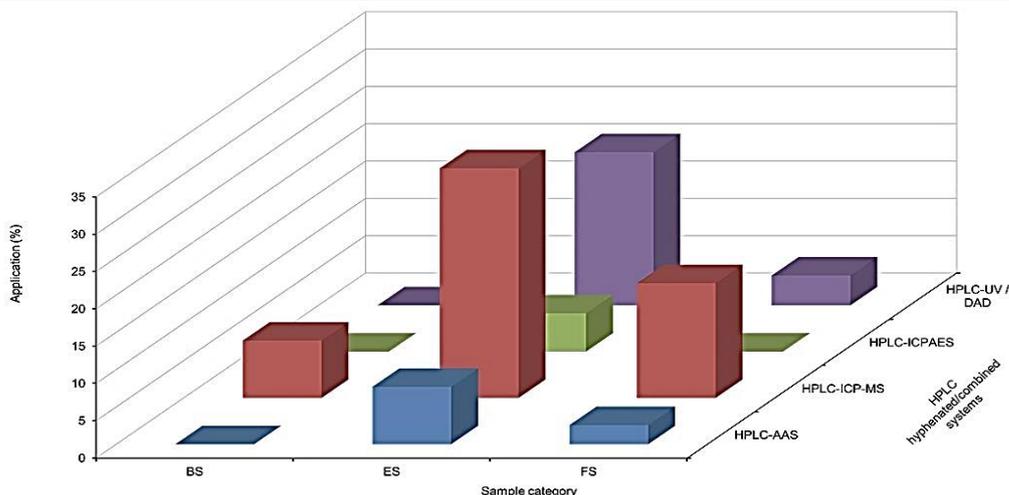
Figure 2: Sample categories used by the HPLC hyphenated systems for the speciation analysis of Cr. BS (Bio-samples), ES (Environmental samples), and FS (Food samples)

Hyphenated systems encountered for the speciation of chromium
The HPLC-HHPN-AAS system
Posta *et al.* (1993) hyphenated an AAS to HPLC via the HHPN, (HPLC-HHPN-AAS) system for online speciation analysis. In this method, they accomplish online derivatization via the ion pairing agent-tetrabutylammonium acetate (TBAA), which enables the speciation analysis of Cr species on C18 and chromium specific columns (**Figure 3**). Analysis of wastewater, river water and soil extract with the method gave limit of detection (LOD) of 0.03 μg/L and 0.02 μg/L of Cr(III) and Cr(VI) respectively and precision of 0.5% to 9.0%. In a similar research, Andrle *et al.* (1997) coupled HPLC-HHPN-ICP-MS in a study that used UV, GF-AAS (offline) and ICP-MS techniques for the speciation analysis of Cr in galvanic waste water. However, they observed that the LODs when coupled to ICP-MS were not good. The formation of ArC^+ was responsible for this observation due to high concentration of organic solvent, acetonitrile (67% v/v) in the eluent. This, they resolved by analyzing for ^{50}Cr and ^{53}Cr isotopes and increasing the RF power of the ICP to 1.5 kW.
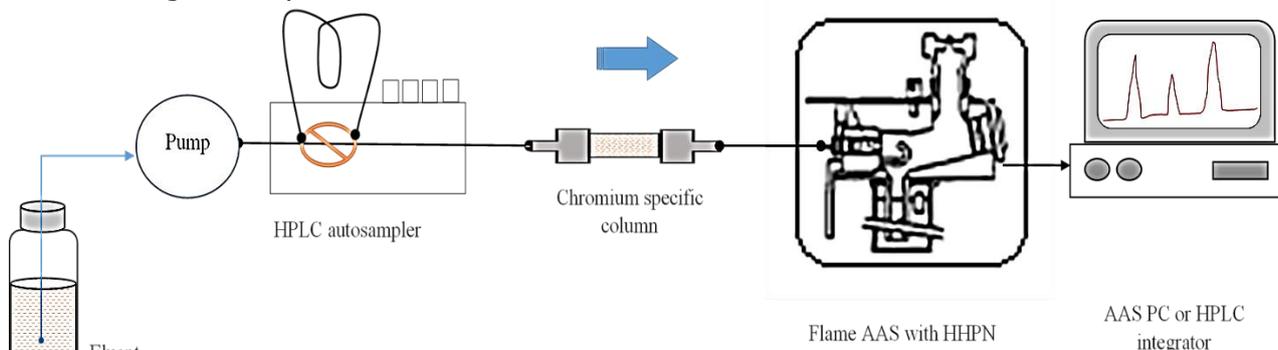
Figure 3: Set up for the online preconcentration and detection of Cr(III) and Cr(VI) species by AAS. Source: *Posta et al. (1993)*

The dual column system for the speciation of chromium
A dual column system based on the use of agarose-based adsorbents was introduced by Hashemi *et al.* (2004) for online preconcentration of Cr(III) and Cr(VI) and offline AAS analysis (**Figure 4**). The two columns, one 20 mm long was pack with iminodiacetic acid Novarose (IDA-Nov), while Q-Sepharose (an agarose-based anion exchanger) was packed in an 80 mm long column. The columns were pretreated with 10 ml each of DI water, 2 M HCl, and 0.1 M acetate buffer pH 5.5. In this method, the sample or test solution is pumped through the columns and fractions were collected and analyzed with AAS. Optimum conditions of preconcentration were obtained by pumping 80 ml sample buffered with 0.1 M acetate buffer at pH 3.0 at 3.0 mL/min sample flow rate and eluting the preconcentrate with 6.5 ml 2 M HCl. The LOD of both chromium species was 7.7 μg/L and preconcentration factor of 12 (Hashemi *et al.*, 2004).
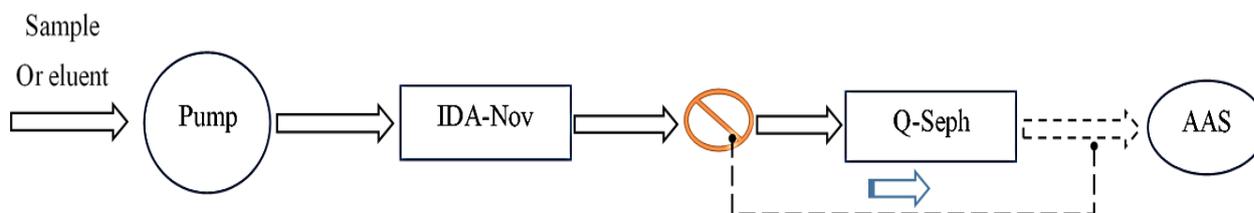
Figure 4: Online preconcentration of Cr(III) and Cr(VI) species by dual columns combined with AAS. Source: *Hashemi et al. (2004)*

The HPLC ICP-MS/AES system for the speciation of chromium

The separation and preconcentration of Cr species in seawater coupled with ICP-MS/AES techniques was investigated, (**Figure 5**). In their research, Posta *et al.* (1996), used an ion pairing agent, 0.005 M tetrabutylammonium bromide at pH 3.0 for the preconcentration and separation processes. The hydrophobic part of the pairing agent interacts strongly with the C18 stationary phase while the hydrophilic positive charged part tends to hold back the Cr(VI) anion. On pumping the sample, Cr(III) passes through the C18 column faster due to non-pairing with the eluent and none interaction with the C18 stationary phase. Conversely, Cr(VI) passes with delay as a result of the effect of the pairing agent. The pairing effect is due to the attraction of the Cr(VI) anions by the pairing agent and the interaction of the hydrophobic part of the agent with the C18 particles in the column. This way, both Cr species reach the plasma at different times, and Cr(III) being the first. The study indicate that the IC P-AES analysis was better and give LOD 4.6 ng/mL Cr(III) and 3.7 ng/mL Cr(VI) and the precision was 2% to 3% respectively. Nevertheless, Cr(VI) was determined as ^{50}Cr isotope in the ICP-MS analysis, due to isobaric interference of the pairing agent and the LOD of 0.12 ng/ml Cr(VI) was obtained.
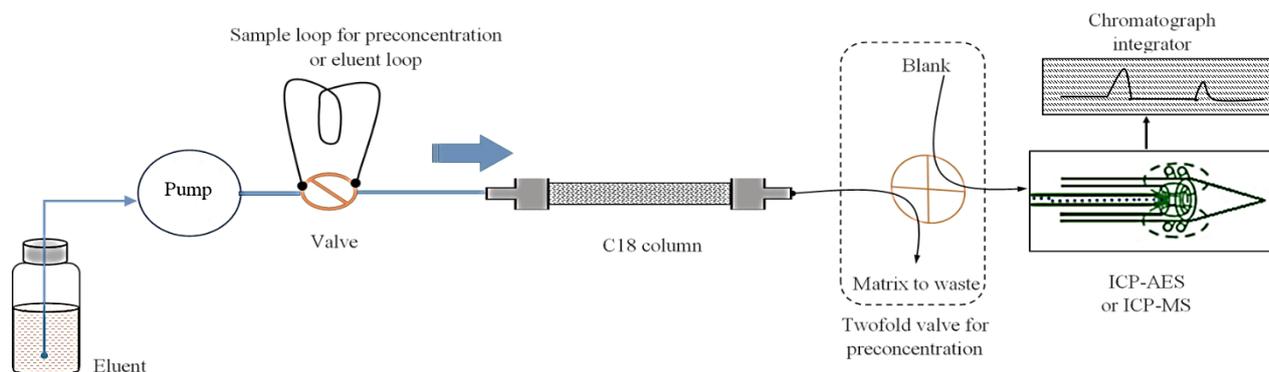
Figure 5: Schematic diagram of the online separation and preconcentration of Cr species hyphenated to ICP-MS/AES. Source: *Posta et al. (1996)*

The automated system for total-and-speciation system for chromium analysis

In Figure 6, Quarles *et al.* (2019) deveoped a single platform for the automated chromium speciation analysis in drinking water, wastewater, industrial waters, and automated total metals detection for recipient waters, sludges, soils, organic waste, ashes, biological samples, and paint. Samples were analysed using a prepFAST IC system for Cr(III) and Cr(VI) speciation analysis and the results were compared to those from the conventional HPLC analysis. The sensitive of this method was about 43times compare to the literature as the limits of detection for Cr(VI) and Cr(III) using the prepFAST IC and ICP-MS combination are 7 ng/L and 12 ng/L, respectively.

The principle of this robust system employed a 50 x 4 mm ion exchange column (prepFAST IC, Elemental Scientific) attached to a switchable speciation column valve that allows the bypass of the column while in total metals mode or inline in speciation mode with respect to the ICP-MS. In the total metals mode, the diluted sample is introduced via the sample directly into the ICP-MS for the duration of the ICP-MS method analysis time (230 s). But in the speciation mode of analysis, the diluted sample is introduced via the loop into the anion exchange column based on an adjustable injection time of 60 s which allow for the separation of the Cr(III) and Cr(VI) ions before the ICP-MS detection.
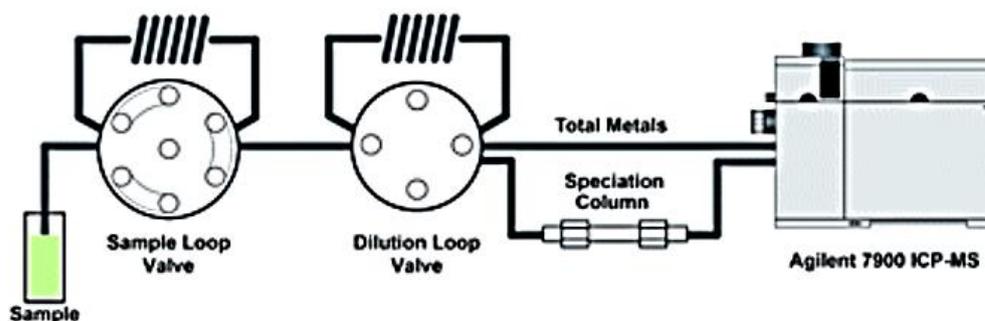
Figure 6: Fully automated total metals and chromium speciation single platform introduction system for ICP-MS. Source : (Quarles et al., 2019)

The operational and analytical features of hyphenated systems for chromium speciaition analysis

Table 1 gives the features of the separation process of Cr species by HPLC, whereas **Table 2** presents analytical features of the speciation analysis. Reports were based on optimal conditions as applied to real samples except otherwise stated.

From **Table 1**, derivatization of Cr(III) and/or Cr(VI) species was extensively used before chromatography, and ligands such as APDC (Andrle *et al.*, 1997; Hossain *et al.*, 2005; M. Safari *et al.*, 2013) and EDTA (Marcinkowska *et al.*, 2016; Marcinkowska *et al.*, 2017) were used. The chemical modification of Cr species was also achieved during chromatography in-situ via the introduction of ion pairing agents in eluent such as tetrabutylammonium acetate (TBAA) and tetrabutylammonium bromide (TBAB) (Posta *et al.*, 1996; Posta *et al.*, 1993). Both methods of derivatization modify the properties of one or both Cr species to enhance the separation process. Analytical and separation characteristics varied without a peculiar pattern within or between sample categories.

Nonetheless, **Table 2** gave the analytical features of Cr speciation analysis. Analyte recovery were between 80% to 120% which is in agreement with analytical standard (EMEA, 1995, 2011), calibration linearity were mostly in the µg/L and mg/L level depending on the technique sensitivity. The calibration coefficients were >0.9, indicating precision of measurements and the LODs were in the ng/L and ug/L region which indicated high sensitivity of the techniques.

Table 1: Chromatographic features of the hyphenated system encountered for the speciation analysis of chromium

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp (ºC)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (µL)	Elution time (min)	Reference
HLCL-prepFASC IC-ICP MS	drinking water, waste water, industrial waters, and recipient water	-	Agilent 1290 Infnity, USA	prepFAST IC (50 mm x 10 mm)	-	10 ppm Tm; 2.3% NH_4NO_3; pH = 1.97	0.3	300	5	(Quarles *et al.*, 2019)
HPLC-UV-Vis	Tap water, River water and Mineral water	APDC /Dual electromembrane extraction (DEME)	Agilent 1200 series HPLC, USA	Eclipse XDB-C18 (150 mm x 4.6 mm, 5 µm)	-	Methanol:Water, (75:25 v/v);	1.0		<14	(Meysam Safari *et al.*, 2013)
HPLC-DAD	Wastewater	APDC / 1-Butyl-3-methylimidiazollium hexaflourophosphate ($[C_4MIM][PF_6]$) 1-Butyl-3-methylimidazolium hexafluorophosphate	Agilent 1100 series, G1313A HPLC pump, USA	waters C18-AR (250 mm x 4.6 mm, 5 µm)	-	Methanol: ACN:water (53:14:33)	NS	20	25	(Ying *et al.*, 2011)
HPLC-UV-Vis	Sediment	1-(2-thiazolylazo)-2-naphthol (TAN) / CPE with Triton X-114	Waters 1525 binary pump , USA	Waters ODS C18 (150 mm x 4.6 mm, 5 µm)	-	Methanol:Water (61:39 v/v) and 4.5 mM/L cetyltrimethyl ammonium bromide CTMAB buffered with acetate buffer, pH 5.5	0.8	-	40	(L. L. Wang *et al.*, 2010)
HPLC-ICP-MS	Drinking water	EDTA /ion pair in eluent	PerkinElmer series 200 HPLC , Canada	Perkin Elmer C8 (33 mm x 4.6 mm, 3 µm)	25	0.8 mM/L TBAH and 0.6 mM/L EDTA, pH 6.9.	1.2	50	<2.0	(Baralkiewicz *et al.*, 2013)
HPLC-ICP-MS	Work place air	EDTA/ion pair in eluent	PerkinElmer 200 HPLC series, USA	Perkinelmer RP-C8 (3 mm x 3 mm) ; Hamilton PRP-X100 (150 mm x 4.6 mm, 5 µm)	25	1 mM/L TBAH + 0.6 mM/L EDTA, pH 7.2 – 7.4; 5% methanol, 50 mM/L ammonium acetate, + 10 mM/L sodium perchlorate, pH 9.0	1.4	200	<3.0	(Stanislawska *et al.*, 2013)

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp ($^\circ$C)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (μL)	Elution time (min)	Reference
HPLC-ICP-MS	Chromate workers' urine	EDTA	Waters 600E HPLC pump, USA	Hamilton PRP-1, (150 mm x 4.6 mm, 5 μm)	-	2.0 mM/L TEA, pH 3.5	1.5	-	<2.5	(H. J. Wang *et al.*, 2010)
HPLC-ICP-MS	River sediments pore water	EDTA	HPLC model and conditions were not specified	-	-	-	-	-	-	(Burbridge *et al.*, 2012)
HPLC-ICP-MS	Water reservoir and Sediments	NaEDTA ion pairing	PerkinElmer 200LC HPLC pump, USA	IonPac AS-7 (200 mm x 4 mm, 10 μm); IonPac AG-7 (50 mm x 4 mm, 10 μm)	35	1 mM/L phtalic acid, 10 mM/L $NaEDTA_2$, pH 4.5; 1 M ammonium nitrate, pH 40.8 M nitric acid (gradient elution).	1.2	80	-	(Jablonska-Czapla *et al.*, 2014)
HPLC-ICP-MS	Surface waters, Ground water and Tap water	EDTA	Agilent 11000 series HPLC pump, Palo Alto, CA	Dionex As-11250 mm x 4.1 mm	25	20 mM NaOH ; Dionex AMMS suppressor: 36 mM H_2SO_4	1.25; Dionex AMMS suppressor flow: 1.4	35	180 s	(Bednar *et al.*, 2009)
HPLC-ICP-MS	Ambient air particles	-	CDionex GS50 HPLC pump , , Bannockburn, IL	IonPac CG5A IC (50 mm x 4 mm)	-	sodium bicarbonate solution	-	100	<2.5	(Menga *et al.*, 2011)
HPLC-ICP-MS	Tap water and drinking water	EDTA	Shimadzu LC10Ai, and Shimatzu CTO-20A, pump, Kyoto, Japan.	Agilent part number G 3268Å 30 mm x4.6 mm id.	40	5 mM pH 7, Na_2EDTA.	-	-	<300 s	(Catalani *et al.*, 2015)
HPLC-ICP-MS	Blood, Urine, Blood plasma, and Erythrocytes.	EDTA monovettes /, Triton-X-100.	Agilent 1200 Series HPLC, Germany.	Hamilton, PRP X 100, (250 x 4 mm, 10 μm)	-	20 mM NH_4HCO_3, 8 mM CH_3COONa, 2.4 mM $NaNO_3$ and 1 % (V/V) ethanol, pH 8.9.	1.5	50	-	(Heitland *et al.*, 2017)

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp (°C)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (μL)	Elution time (min)	Reference
HPLC-ICP-MS	Wastewater	EDTA online preconcentration with	Agilent 1200, USA	C18 RP (50 mm x 4.6 mm i.d, 5 μm)		10 mM TBAH, pH 3.5	1.0		<100 s	(Jia et al., 2016)
HPLC-ICP-MS	Bottled drinking water	NaEDTA/ion pairing	PerkinElmer Series 200 or 225 system. Ontario, Canada	PRP-X100 (4.6 mm x 150 mm)	20	3 mM EDTANa$_2$, pH 4.6; 36 mM NH$_4$NO$_3$ pH 9.0	1.5	100	<8.0	(Marcinkowska et al., 2016)
HPLC-ICP-MS	Wastewater, Surface water and Wood leachate	EDTA and TBAH Ion pair	PerkinElmer serials 200 HPLC system, USA.	PerkinElmer Spheri-5 RP-18 (220 x 4.6 mm)	-	5% (v/v) methanol/0.5 mM EDTA þ 1 mM TBAH, pH 7.2	1	100	<6.0	(Hu et al., 2016)
HPLC-ICP-MS	Welding fume	NaEDTA/ anion resin (polystyrene/ divenyl benzene)	Agilent 1200 HPLC system, Tokyo, Japan	Q HR 5/5 Uppsala,(5 x 50 mm, 10 μm);	-	-	-	-	~400 s	(Scancar et al., 2015)
HPLC-ICP-MS	Oils from buffalo, cow, and fish	EDTA, TBAB/ion pairing	Hitachi Model CM-5110 HPLC pump	Perkin Elmer C8 (3.0 mm i.d. × 33 mm, 3 μm)	-	0.5 mM TBAB and 0.3 mM EDTA in 1% methanol (pH 6.9)	1.0	100	<1.5	(Lin et al., 2016)
HPLC-ICP-MS	Bottled drinking water	NaEDTA/ion pairing	PE series 200 HPLC pump, Ontario, Canada.	Hamilton PRP-×100	20	3 mM EDTANa$_2$ pH 4.6, 36 mM NH$_4$NO$_3$ pH 9.0	1.2	100	~7.0	(Marcinkowska et al., 2017)
HPLC-ICP-MS	Drinking water	No chelates/Anion exchange	PE series 200 HPLC pump, Ontario, Canada.	Hamilton PRP-X100 (4.6 mm x 150 mm, 5 μm)	Ambient	Procedure A: 22 mM (NH$_4$)$_2$HPO$_4$, 25 mM NH$_4$NO$_3$ Procedure B: 22 mM (NH$_4$)$_2$HPO$_4$, 65 mM NH$_4$NO$_3$, pH of eluent 9.2;	1.4	100	<4.0	(Marcinkowska et al., 2015)
HPLC-ICP-MS	Dairy products, Cereals, Chocolate, Beverages, Vegetables, Fruits, Eggs,	No chelates/Anion exchange	Dionex ICS-3000 HPLC system. Sunnyvale,CA,USA	Superdex-200 HR 10/30 (300×10 mm i.d.), (size exclusion) and	-	: 0.085 M HNO$_3$ and 0.35 M HNO$_3$	1	100	~ 30	(Vacchina et al., 2015)

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp (ºC)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (µL)	Elution time (min)	Reference
	Meat and Sea products.			CS5A (250×4 mm i.d.), (ion exchange).						
HPLC-UV-Vis	synthetic wastewater	APDC/ no preconcentration	Tosoh Corp RP-HPLC–UV system, Tokyo, Japan.	TSK GEL ODS-120A C18 (15 × 4.6 cm, 5 µm)	-	Acetonitrile:water system (2:1, v/v)	0.6	-	<10	(Hossain et al., 2005)
HPLC-UV-Vis	CRM 544	APDC /SPE	Biotrnik BT8100 LC, Germany	LiChrospher 60 RP-8 (125mm X 4mm, 10 µm)	-	Acetonitrile:water system (2:1, v/v)	1.5	-	<12	(Bittner and Broekaert, 1998)
HPLC-UV-Vis	CRM 544	APDC /SPE	Biotrnik BT 8100 LC, Germany	LiChrospher 100 RP-8 (125mm X 4mm, 5 µm)	-	Acetonitrile:water system (2:1, v/v)	1.0	-	<12	(Bittner and Broekaert, 1998)
HPLC-UV-Vis	Galvanic waste water	APDC /SPE	BT 8100 HPLC pumps, Biotronik, Malntal, FRG.	LiChrosorb RP-18, (250mm x 4 mm, 5 µm)	34	Acetonitrile: H20 (67 : 33)% (v/v)	0.8	20	<13.5	(Andrle and Broekaert, 1993)
HPLC-UV-Vis	Galvanic waste waters	APDC /continuous flow and SPE	Eppendorf-Biotronic HPLC pump, Maintal, Germany	LiChrospher 60 RP-8 (125mm X 4mm, 5 µm)	25	aceto-nitrile:water (67:33)%,(v:v)	0.4			(Andrle et al., 1997)
HPLC-GF-AAS (offline)	Galvanic waste waters	APDC/fractions collection	Eppendorf-Biotronic HPLC pump, Maintal, Germany.	LiChrospher 60 RP-8 (125mm X 4mm, 5 µm)	25	aceto-nitrile:water (67:33)%,(v:v)	0.4	-	<12	(Andrle et al., 1997)
HPLC-HHPN/ICP-MS	Galvanic wastewater	APDC/SPE (C18 adsorbent)	Eppendorf-Biotronic HPLC pump, Berlin, Germany.	LiChrospher 60 RP-8 (125mm X 4mm, 5 µm) HHPN;orifice: 10 µm; Pressure:240 bar		Accetonitrile:water (67:33)%,(v:v)	0.5	20	<10	(Andrle et al., 1997)

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp (ºC)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (µL)	Elution time (min)	Reference
HPLC-UV-Vis HPLC-UV-AAS (offline)	Tap water, river water and wastewater	iminodiacetic acid (IDA), tris(2-aminoethyl)amine (TREN) and dipicolylamine (DPA) / Q-Sepharose and IDA adsorbent.	EYLA RP-1000 pump, Japan.	8mm x 5.7 mm i.d (for Q-Sepharose) adsorbent and 20 mm x 5.7 mm i.d for ID aadsorbent adsorbent	-	2M HCl at pH 3.0	3.5 to 4.0	-	-	(Hashemi et al., 2004)
HPLC-HHPN-AAS	Drinking water, Wastewater, Soil extract	tetrabutylammonium acetate (TBAA) / ion pairing		Knauer modified C18 type, Cr column (5 cm, 4.6-mm, ID,	-	20% methanol and 0.00015 M tetrabutylammonium acetate (TBAA) ; pH 3-3.3	2.5	-	<3	(Posta et al., 1993)
HPLC-UV-Vis	Drinking water, surface water and ground water	diphenylcarbazide (DPC)/ C18 adsorbent.	Dionex 2000i/SP HPLC pump, Sunnyvale, CA USA,	C18 (100mm x 6 mm, i.d)	-	20% (v/v) Acetonitrile and 0.006 M sulphuric acid.	1.0		<10	(Padaruskas et al., 1998)
HPLC-ICP-AES (offline)	River water, tap water and waste water	iminodiacetate chelating resin	ALITEA, Sweden and SPETEC, Germany pumps	PTFE tubing (5cm×2mm i.d.) packed with resin	-	2 M Nitric acid	1.0	-	<10	(Sumida et al., 2005)
HPLC-UN-ICP-AES	Seawater	Ammonium acetate ion pairing	Perkin Elmer 10 LC pump, Norwalk, CT, USA.	Lichrospher 100-RP 18 (125 mm x 4.6 mm i.d. , 5 µm)		TBABr, 0.004 NH3, 0.0001 M acetate and methanol (40%, v/v)	1.2	100		(Posta et al., 1996)
HPLC-UN-ICP-MS	Seawater	Ammonium acetate ion pairing	Perkin Elmer, Series 10 LC pump, Norwalk, CT, USA	Lichrospher 100-RP 18 (125 mm x 4.6 mm i.d. , 5 µm)		TBAB, 0.004 NH3 0.0001 M acetate and methanol (40%, v/v	1.2	100		(Posta et al., 1996)
HPLC-HHPN-ICP-MS	Tap water	Anion exchange resin (poly(styrene–divinylbenzene) with ($-NR_3$)$^+$	Knauer, dual-piston HPLC pump, Berlin, Germany.	Dionex IonPac-AG5 (50 mm x 4 mm i.d, 15 µm)	-	0.3 M Nitric acid pH 0.5 and 1.0 M nitric acid pH 0.0	1.2 ml/ min	100	<10	(Barnowski et al., 1997)

Hyphenated system	Matrix	Derivatization / preconcentration of Cr	HPLC model	Column	Col. Temp (ºC)	Eluent	Eluent flowrate (mL/min)	Sample injection volume (µL)	Elution time (min)	Reference
HPLC-IC-ICP-MS	Lake water	TMA group on Polymethylacrylate Anion exchange resin and sulphonic group on Polybutldiene maleic anhydride silca cation resin	LKB 2249 gradient pump	IC-Pak Anion (trimethyl Ammonium group); cation: Guard-Pak CM/D (3.9 mm x 5 mm, 5 µm) and IC-Pak CM/D (3.9 mmx 150 mm) with sulfonic Acid group	-	0.4 mM to 40 mM nitric acid	0 to 20 (gradient); 1.0 to 2.5 (isochratic)		<10	(Mari Pantsar-Kallio and Manninen, 1996)
HPLC-IC-ICP-AES	Spiked DI water and CRM	NaEDTA/anion exchange resin	Dionex DX-300, metal-free HPLC system., CA, USA	Dionex IonPac AG7 and Dionex IonPac AS7 (250 mm × 4 mm, 10 µm)		250 mM $(NH4)_2SO_4$ 100 mM NH_4OH pH 9.2	1.5	100	<4.0	(Byrdy et al., 1995)
HPLC-IC-ICP-MS	Spiked DI water and CRM	NaEDTA/anion exchange resin	Dionex DX-300, metal-free HPLC system. CA, USA	Dionex NG1 and Dionex lonPac AS7(250 mm × 4 mm, 10 µm)	-	35 mM $(NH_4)_2SO_4$ pH 9.2 with NH_4OH	2.9	100	<4.0	(Byrdy et al., 1995)

EDTA = Ethylenediaminetetraacetic acid, NaEDTA = Sodium ethylenediaminetetraacetic acid, APDC = Ammoniumpyrrolidinedithiocarbamate, SPE = Solid phase extraction, CRM = Certified reference material, TBAB or TBABr   = Tetrabutylammonium bromide, TBAH = Tetrabutylammonium hydroxide

Table 2: Analytical features of Cr speciation analysis process

Hyphenated system	Detector model	Detector condition	Recovery (%)	Calibration Linearity	Coefficient	LOD	LOQ	RSD/CV (%)	CF	Matrix	Reference
HPLC-prepFAST IC-ICP MS	Agilent 7900, USA	RFP: 1.6 kW; PGF: 18 L/min; NGF: 1.0 L/min; AGF: 1.2 L/min; RGF: (NH_3), 0.5 mL/min; CGF: 2 L/min; RF: 1.8 V Rejection parameter; 0.7 m/z: 52 (Cr)	-	0.010–100 000 µg/L	0.9846	7 ng/ L(Cr(III) and 12 ng /L Cr(VI)	-	-	-	drinking water, waste water, industrial waters, and recipient water	(Quarles et al., 2019)
HPLC-UV-Vis	Agilent UV detector (G1314B), USA .	Wavelength: 254 nm	96.4 to 104.0 Cr(III) and 97.9 to 101.3 Cr(VI)	10 to 500 µg/L Cr(III); 20 to 500 µg/L Cr(VI)	0.9979, Cr(III) ; 0.9981, Cr(VI)	5.1 µg/L Cr(III) ; 2.8 µg/L Cr(IV)	-	9.8 to 13.7	21.8 to 33	Tap water, River water and Mineral water	(Meysam Safari et al., 2013)
HPLC-DAD	Hewlett Packard 1100 seires DAD, USA	Wavelength: 254 nm	Cr(III) , Cr(VI).	25 µg/mL to 200 µg/mL	0.9977 (Cr(III)); 0.9978 Cr(VI)	1.9 µg/L Cr(III) and 1.0 µg/L Cr(VI)	.4 µg/L Cr(III) ; 3.4 µg/L Cr(VI	-	-	Waste water	(Ying et al., 2011)
HPLC-UV-Vis	Waters 2487 Dual wavelength absorbance system, USA	Wavelength: 490 nm	96. 4 to 140 Cr(III); and 97.9 to 101.3 Cr(VI)	50 µg/L to 5000 µg/L Cr(III) and Cr(VI)	-	7.5 µg/L Cr(III) and 3.5 µg/L Cr(VI)	-	4.7 Cr(III) ; 2.7 Cr(VI)	45 Cr(III); 40 Cr(VI)	Sediment	(L. L. Wang et al., 2010)
HPLC-ICP-MS	PerkinElmer Elan DRC II ICP-MS, Canada	RFP: 1050 W; PGF: 15 L/min; NGF: 0.88 L/min; AGF: 1.2 L/min; RGF: (NH_3), 0.5 mL/min; Rejection parameter; 0.7 m/z: 52 (Cr)	100 to 115 Cr(III) and 93 to 106 Cr(VI)	0.3 TO 10 µg/L Cr(III) and Cr(VI)	0.9999 Cr(III) and Cr(VI)	0.09 µg/L Cr(III); 0.10 µg/L Cr(VI)	0.28 Cr(III) ; 0.30 µg/L Cr(VI)	15 Cr(III) ; 1.6 Cr(VI)	-	Drinking water	(Baralkiewicz et al., 2013)

HPLC-ICP-MS	PerkinElmer SCIEX ELAN DRC-e ICP-MS, USA	RFP: 1100 W; PGF: 15 L/min; RG: methane, 1.0 mL/min; Dwell time:1000 ms	NA, Cr(III); and 98.4 to 99.2 ,Cr(VI)	Cr(III): 2.5 to 20 µg/L; Cr(VI): 2.5 to 50 µg/L	0.999, Cr(III); 0.998, Cr(VI)	0.4 mg/L Cr(III); 0.6 µg/L Cr(VI)	1.0 µg/L Cr(III); 1.2 µg/L Cr(VI).	-	-	Work place air	(Stanislawska et al., 2013)
HPLC-ICP-MS	Thermo X7 ICP-MS, USA	RFP: 1200 W; PGF: 13.0 L/min; AGF; 0.7 L/min; NGF: 0.76 L/min; Mode: time resolved analysis; Dwell time: 100 ms; m/z: ^{52}Cr	NA, Cr(III) ;94.8 to 105 µg/mL, Cr(VI)	-	-	0.03 µg/L	-	-	-	Chromate workers' urine	(H. J. Wang et al., 2010)
HPLC-ICP-MS	ICP-MS model specified	-	76 to 140 Cr(III) and 0 to 90 Cr(VI) without EDTA; and 1.3 to 180 Cr(III) and 0.0 to 7.4 with EDTA	-	-	0.1 µg/L to 0.8 µg/L Cr(VI);	0.33 µg/L to 2.7 µg/L Cr(VI	-	-	River sediments pore water	(Burbridge et al., 2012)
HPLC-ICP-MS	PerkinElmer Elan 6100-e ICP-MS, USA	RFP: 1125 W; PGF: 15.0 L/min; NGF: 0.76-0.82 L/min; Analysis mode: peak-hopping Dwell time: 250 ms; m/z: ^{52}Cr	102.56 Cr(III); 101.49 Cr(VI); As(III), As(V) and Sb(III), Sb(V).	1 µg/L to 2 5 µg/L, Cr(III) and Cr(VI) ; 0.5 µg/L to 5 µg/L others.	0.9991 to 0.9999	0.11 µg/L, Cr(III); 0.17 µg/L Cr(VI); 0.18 µg/L As(III); 0.22 µg/L As(V); 0.009 µg/L Sb(III); 0.17 µg/L Sb(V).	-	1.6 Cr(III); 2.1 Cr(VI	-	Water reservoir and Sediments	(Jablonska-Czapla et al., 2014)
HPLC-ICP-MS	PerkinElmer Elan DRC II ICP-MS, Waltham, MA	RFP: 1250 W; NGF: 0.85 L/min; Data aquisit: 70 s; Dwell time: 200 ms; Dwell time: 300 ms m/z (std mode): 52, 53, 77 and 82;	99 to 114, Cr(III); 97 to 114, Cr(VI). Se(IV), Se(VI)	0 to 100 µg/L Cr(III) and Cr(VI)	>0.999 Cr(III) and Cr(VI) Se	0.7 to 1.5 µg/L Cr(III); 0.6 t0 0.8 µg/L Cr(VI).	-	0.00 to 0.27 Cr(III); 0.03 to 0.32 Cr(VI	-	Surface waters, Ground water and Tap water	(Bednar et al., 2009)

		m/z reaction cell: 52, 53, 78 and 80. RGF: methane at 0.7 L/min. RPq: 0.65									
HPLC-ICP-MS	VG Instruments VG PlasmaQuad PQIII Q- ICP-MS system, Cheshire, UK	RFP: 1450 W; Reflected power: 1.6 W; PGF: 12 L/min; AGF: 0.88 L/min NGF: 0.99 L/min Sample delivery rate: 1.25 mL/min; Dwell time: 300 ms; Isotopes: ^{50}Cr, ^{52}Cr, ^{53}Cr.	36 Cr(III) , 57 Cr(VI) (pre-sampling spiked); 31 Cr(III) , 67 Cr(VI) (post sampling spiked)	-	-	0.08 ng/m^3	-	15 Cr(III) ; 11 Cr(VI) (pre-sampling spike); 19 Cr(III) , 14 Cr(VI) (post sampling spiked)	-	Ambient air particles	(Menga et al., 2011)
HPLC-ICP-MS	Perkin Elmer Sciex Elan DRC II ICP-MS, Waltham, USA	RFP: 1100 W PGF: 15 L/min Monitored ion m/z 52Cr+ Dwell time: 1000 ms RGF: (NH3),: 0.6 L/min	85 to 116 Cr(III); 80 to 107.5 Cr(VI).	0.1–5 µg/L Cr(III) and Cr(VI)	0.998	0.1 µg/L Cr(III) and Cr(VI);	-	5.7 Cr(III), and 5.4 Cr(VI)	-	Tap water and Drinking water	(Catalani et al., 2015)
HPLC-ICP-MS	Agilent 7700x ICP-MS, Waldbronn, Germany	RFP : 1500 W; PGF: 15 L/min; IGF: 1 L/min ; NGF, 1.2 L/min.	NA	0.0 to 20 µg/L.	NA	0.05 Cr(VI) in urine; 0.1 Cr(VI) in blood; 0.1 Cr(VI) in plasma; 0.25 µg/L Cr(VI) in erythrocytes	-	-	-	Blood, Urine, Blood plasma and Erythrocytes.	(Heitland et al., 2017)
HPLC-ICP-MS	Agilent 7500 Cx ICP-MS, USA.	RFP: 1200 W; NGF: 0.80 L/min; AGF: 0.70 L/min; PGF: 15.0 L/min; Sampler diam: 1.0 mm; Skimmer diam Nickel: 0.4 mm; CGF: 3.5 mL/min;	85 to 115 Cr(III) and Cr(VI).	0.01 to 10 ng/mL Cr(III) and Cr(VI).	0.9992 Cr(III); 0.9995 Cr(VI).	0.0058 ng/mL Cr(III); 0.0041 ng/mL Cr(VI).	-	4.3 Cr(III); 3.6 Cr(VI)	105 Cr(III); 128 Cr(VI).	Wastewater	(Jia et al., 2016)

		Isotopes: ^{52}Cr; Dwell time : 100 ms; Acquisition mode :Time resolved analysis									
HPLC-ICP-MS	PE Sciex ELAN 6100 DRC II, Ontario, Canada	RFP: 1050 W, 1100 W; NGF: 0.86 L/ min, 0.92 L min; AGF: 1.375 L /min; PGF: 14.5 L/ min; Sampler and skimmer cones Pt Lens voltage: 7.5 V e 10 V Detector mode: Dual; Data collection mode: ^{91}As O,^{52}Cr,^{121}Sb; Scan mode: Peak hopping; Dwell time: 250 DRC (O) flow: 0.55 L /min; Rpq: 0.55; Rpa: 0.	98 Cr(VI); 91 to 110 others.	0.5 to 10 µg/L Cr(VI) . and others	0.9999 Cr(VI); 0.9994 to 0.9999 others.	0.098 µg/L Cr(VI); 0.067 µg/L As(III); 0.068 µg/L As(V); 0.083 µg/L Sb(III); and 0.038 µg/L , Sb(V).	0.29 µg/L Cr(VI); 0.11 to 0.25 µg/L others	1.7 to 2.4	-	Bottled drinking water	(Marcinkowska *et al.*, 2016)
HPLC-ICP-MS	Perkin Elmer ELAN DRC e ICPMS systems. USA	RFP: 1350 W; Spray chamber: Quartz cyclonic spray chamber NGF: 0.9, 1.05 mL/ min; Lens voltage : 7.0, 9.0; RGF, (O$_2$): 0.8 mL/ min; RPq: 0.7; RPa: 0 Acquisition time :15 min	86.2 to 116.5 Cr(III); 91.4 to 107.0 Cr(VI)	-	-	0.9 µg/L Cr(VI);	-	-	-	Wastewater, Surface water and Wood leachate	(Hu *et al.*, 2016)

HPLC-ICP-MS	Agilent 7700x ICP MS, Tokyo, Japan	Sampler Nickel and skimmer cones orifices: 1.0 and 0.4 mm; Data processing: peak area;	NA: Cr; 98 to 103 others	LOQ to 100 ng/mL all analytes	0.998	0.02 ng/mL Cr.; 0.1 ng/mL to 0.2 ng/mL others;	-	<2 Cr; 2 to 3 others	-	Welding fume	(Scancar et al., 2015)
HPLC-ICP-MS	Perkin-Elmer SCIEX ELAN 6100 DRCII, Concord, Canada	RFP: 1300 W; PGF: 15 L /min; AGF: 1.325 L /min; NGF: 0.97 L /min; Dwell time: 100 ms; Sweeps/Reading :5; Reading/Replicate: 300; Replicates: 1; Isotopes: ^{50}Cr, ^{52}Cr, ^{53}Cr RGF: (NH_3) 0.6 mL/ min; Rpq: 0.65 Rpa: 0 AFV: 175 V	103 to 195 Cr(III), 0.95 Cr(VI).	0.2 to 10 ng/mL Cr(III) and Cr(VI).	0.9994 Cr(III) and Cr(VI).	0.045 ng/mL Cr(III), 0.052 ng/mL Cr(VI).	-	-	-	Oils from buffalo, cow, and fish	(Lin et al., 2016)
HPLC-ICP-MS	PE Sciex ELAN 6100 DRC II, Ontario, Canada.	RFP: 1100–1150 W NGF (Ar) : 0.89–0.91 L /min; AGF: (Ar) 1.20 L/ min; PGF: (Ar) 16 L /min; Sampler and skimmer cones (Pt) Lens voltage : 8.5–9.5 V; Detector mode: Dual; Data collection mode : ^{91}AsO, ^{52}Cr, ^{121}Sb; Scan mode: Peak hopping; Dwell time: 250;	127.6 Cr(VI), 84.4 to 124.8 others; Ext. cal with matrix matching: 129.0 Cr(VI) , 75.8 to 128.0 others; 96.3 Std. addition: Cr(VI), 84.1 to 115.0 others Sb As and Cr	0.5 to 10 µg/L Cr(VI); 0.1 to 10 µg/L others. Ext. cal. with matrix matching: 0.5 to 10 µg/L; 0.1 to 10 µg/L others. Std. addition: 0.5 to 5.0 µg/L Cr(VI); 0.1 to 10 µg/L others *	0.9994 Cr(VI), 0.9991 to 0.9999 others; Ext. cal. with matrix matching: 0.9992 Cr(VI), 0.9893 to 1.000 others; Std. addition: 1.0000 Cr(VI), 0.9993 to 0.9999 others.	0.098 µg/L Cr(VI), 0.038 µg/L to 0.083 µg/L others;	0.29 µg/L Cr(VI), 0.20 µg/L to 0.25 µg/L others	1.8 Cr(VI) , 2.5 to 5.6 others	-	Bottled drinking water	(Marcinkowska et al., 2017)

Technique	Instrument	Parameters	Recovery	Linear range	Correlation	LOD	LOQ			Sample	Reference
		DRC gas (O₂) flow rate: 0.55 L /min; Rpq: 0.55 Rpa :0									
HPLC-ICP-MS	PE Sciex ELAN 6100 DRC II	RFP: 1100W; NGF: (Ar) (0.86 – 0.92) L /min; AGF: (Ar) 1.2 L/ min PGF: (Ar) 15.0 L /min; Sampler and skimmer Cones: Pt; Lens voltage: (7.5– 10.0) V; Detector mode: Dual; Data collection mode: ^{91}AsO, ^{52}Cr; Scan mode: Peak hopping; Dwell time : 250; DRC gas (O2) flow: 0.45 L /min; Rpq: 0.45; Rpa: 0	Method A: 96 102 Cr(VI), and 96 to 103 others. Method B: 96 to 100 Cr(VI) and 94 to 101 others	Method A: 0.5 µg/L to 10µg/L Cr(VI), and others. Method B: 5 µg/mL to 50 µg/L Cr(VI) and others.	Method A: 0.9994 Cr(VI); 0.9995 and 0.9992 others Method B: 0.9999 Cr(VI) ; 0.9997 and 0.9999 others.	Method A: 0.073 µg/L Cr(VI), 0.09 µg/L and 0.16 µg/L others. Method B: 0.15 µg/mL Cr(VI) ; 0.062 µg/L and 0.140 µg/L, others.	Method A: 0.22 µg/L Cr(VI), 0.27 µg/L and 0.49 µg/L others. Method B: 0.46 µg/mL Cr(VI) ; 0.019 µg/L and 0.43 µg/L, others	Method A: 7.6 Cr(VI), and 4.2 7.4 others. Method B: 2.0 Cr(VI) and 2.3 to 2.4 others	-	Drinking water	(Marcinkowska et al., 2015)
HPLC-ICP-MS	ELAN 6100 DRC II ICP-MS system. Concord, ON, Canada.	Operation mode: standard (with no reaction gas ans with reaction gas(NH₃)	94 to 99 (lower level); 92 to 103 (higher level)	0 µg/L to 20 µg/L	>0.995	1 µg/L to 10 ug/kg;	3 µ/L to 30 µ g/kg; c: 4 to 8;	-	-	Dairy products, Cereals, Chocolate, Beverages, Vegetables, Fruits, Eggs, Meat and Sea products.	(Vacchina et al., 2015)
HPLC-UV-Vis	UV-8020 UV system, Tokyo, Japan	Wavelength: 254 nm	-	3 to 5000 µg/L Cr(VI); 5 to 3000 µg/L Cr(III)	-	4.5 µg/L Cr(III); 2.2 µg/L Cr(VI)	-	4 Cr(III); <2 Cr(VI)	-	Synthetic wastewater	(Hossain et al., 2005)

Technique	Instrument	Parameters	Recovery	Linear range	Correlation	LOD		RSD		Sample	Reference
HPLC-UV-Vis	BT 8200 UV system, Germany	-	84.2 (total Cr)	1.0 µg/L to 300 µg/L Cr(III) and Cr(VI).	0.999 Cr(III) an Cr(VI)	0.2 µg/L Cr(III); 0.06 µg/L Cr(VI).	-	2.8 Cr(III); 2.8 Cr(VI).	-	CRM 544	(Bittner and Broekaert, 1998)
HPLC-UV-Vis	BT 8200 UV system, Germany	-	87.7 (total Cr)	1.0 µg/L to 300 µg/L Cr(III) and Cr(VI).	0,997 Cr(III) and Cr(VI).	0.2 µg/L Cr(III); 0.1 µg/L Cr(VI).	-	3.4 Cr(III); 4.5 Cr(VI).	-	CRM 544	(Bittner and Broekaert, 1998)
HPLC-UV-Vis	BT 8200 Spectrophotometer, Biotronik, Malntal, FRG.	Wavelength: 259 nm	-	5 µg/L to 5000 µg/L Cr(III) and Cr(VI).	-	2.4 µg/L Cr(III); 2.1 µg/L Cr(VI)	-	-	-	Galvanic wastewater	(Andrle and Broekaert, 1993)
HPLC-UV-Vis	Eppendorf-Biotronic UV detector, Duisburg, Germany	Wavelength: 254 nm	-	-	-	0.1 µg/L Cr(III) and Cr(VI)	-	2.8 Cr(III) and Cr(VI)	-	Galvanic wastewater	(Andrle et al., 1997)
HPLC-GA-AAS (offline)	Perkin-Elmer HGA 500 GF-AAS, Oberlingen, Germany.	Temp: (°C) ;Ram(S); hold time(S); Ar flow (mL/min). Drying step 1: 90; 5; 30; 300. Drying step 2: 120; 5; 10; 300. Charring step: 1000; 5; 20; 300. Atomization step: 2250; 0; 5; 0. Heating step: 2700; 1; 5; 300. Cr HCL at 10 mA. Deuterium background correction	-	-	-	0.6 µg/L Cr(III) and Cr(VI)	-	3.2 Cr(III); 2.2 Cr(VI).	-		(Andrle et al., 1997)
HPLC-HHPN-ICP-MS	Plasma Quad PQ2 Turbo Plus ICP-MS system, Winsford, UK)	RFP: 1500 W; Reflected power: 25 W; AGF : 1.15 L/min; IGF: 0.85 L/min.; PGF: 15 L/min; RGF: (O_2), 0.08 L/min;	-	-	-	0.2 µg/L Cr(III); 0.1 µg/L Cr(VI)	-	5.0 Cr(III); 4.0 Cr(VI).	-	Galvanic wastewater	(Andrle et al., 1997)

		Sampling distance: 10 mm; Dwell time: 655.36 ms; Acquisition time: 750 s									
HPLC-UV-AAS (offline)	Shimadzu AA-670 AAS, Japan; Jeneway 3020 Spectronic 20D spec., USA.	-	100 Cr(III) (IDA adsorbent adsorbent; 100 Cr(VI) (Q-Sepharose adsorbent)	-	-	7.7 µg/L Cr(VI)	-	1.2 Cr(III) and 1.3 Cr(VI)	12	Tap water, River water and Wastewater	(Hashemi et al., 2004)
HPLC-HHPN-AAS	Varian SpectrAA 400, Darmstadt, Germany	Wavelength: 357.8 nm; Slit width: 0.7; nm; slightly reduced air/acetylene flame:, Measuring height, 8 mm above the burner slit.		-	-	0.03 µg/mL Cr(III); 0.02 µg/mL Cr(VI).	-	0.5 to 4.8 Cr(III); 1.2 to 9.0 Cr(VI) other samples; 1.0 Cr(III) and 2.0 Cr(VI) (drinking water)	-	Drinking water, Wastewater, Soil extract	(Posta et al., 1993)
HPLC-UV-Vis	Laboratomi LCD 2563 UV-Vis, Prague, Czech Republic.	UV-Vis. Wavelength: 546 nm	96.1 to 101 Cr(VI)	0.05 ng/mL to 2.5 ng/mL	0.998	0.02 ng/mL Cr(VI)	-	< 4.0 Cr(VI) (samples); 1.5 to 4.5 Cr(VI) (standards)	-	Drinking water, Surface water and Ground water	(Padarauskas et al., 1998)
HPLC-ICP-AES (offline)	SII VISTA-PRO ICP-AES system, Seiko, Japan	RFP: 1.2kW; PGF: 15 L/min; AGF: 1.5 L/min; NGF: 0.75 L/min; Sample uptake rate: 1.0 mL/min; Nebulizer: Concentric nebulizer; Torch: One-piece low flow extended torch in the axial view mode ;	99.0 and 103 Cr(III) and 98.0 and 99.0 Cr(VI) 94 and 96 Cr(VI)	0.2 µg/L to 2.0 µg/L	-	0.08 µg/L Cr(III) and 0.15 µg/L Cr(VI).	-	-	-	River water, Tap water and Waste water	(Sumida et al., 2005)

		Measurement mode: Time scan mode; Emission line: 267.716 nm									
HPLC-UN-ICP-AES	JY 38 VHR, Longjumeau, France)	RFP: 2.2 kW Induction coil: 3 coils, 32 mm o.d., 30 mm height, Torch: INSA, demountable; PGF: 16.5 L/min, coating 0.2 I/mm, aerosol 0.6 I/min; Monochromator: HR 1000 M; Spectra line: (Cr(II)) 267.7 nm	94 and 96 Cr(VI)	-	-	4.6 ng/mL Cr(III) and 3.7 ng/mL Cr(VI).	-	2 to 3, Cr(III) and Cr(VI)	-	Seawater	(Posta *et al.*, 1996)
HPLC-ICP-MS	Perkm-Elmer Sciex Elan 5000, USA	Nebulizer: Cross flow type, with Ryton condensation chamber of the Scott type Interface: Sampler and skimmer cones in Pt-Rh ahoy, i.d. 1 mm; RFP: 1.0 kW; PGF: 16 l/mm, auxiliary 0.9 I/min, aerosol 1.0 I/min; Replicate time: l000 ms, Dwell time: 1000 ms; Resolution: 0.9-0.6 amu; Scanning mode: Peak hopping;	91 and 96 Cr(VI)	-	-	0.12 ng/mL, Cr(VI)	-	3.2, Cr(VI)	-	Seawater	(Posta *et al.*, 1996)

		Optimization: at masses ^{103}Rh, ^{24}Mg and ^{208}Pb; CEM: - 3.0 kV Analytical masses: Cr. ^{52}Cr, ^{53}Cr, ^{54}Cr									
HPLC-HHPN-ICP-MS	ICP-MS system I (self-developed)—	RFP: 1200 W; AGF: 0.75 L/min; PGF: 25 L/min.	100.8 Cr(III); 87.0 Cr(VI)	1.0 µg/L to 100 µg/L, Cr(III) and Cr(VI)	0.9988 Cr(III); 0.9979, Cr(VI).	0.1 µg/L Cr(III); 0.2 µg/L Cr(VI).	-	≈5	-	Tap wter CRM (NIST 1643c,)	(Barnowski *et al.*, 1997)
HPLC-IC-ICP-MS	Fisons Plasma Quad PQ II + ICP-MS system	RFP:1.35 k W; PGF:13.5 L/min; Intermediate gas flow: 0.90 L/min; NGF: 0.80 L/min; Spray chamber temperature: 4 C: Dwell time: 500 ms; Total acquisition time: 650 s; m/z: 52 Detector voltage, JIT 1: 3.45 mV; Detector voltage, JIT 2: 2.9 mV	-	0.0 µg/L to 500 µg/L	-	0.3 µg/L Cr(III); 0.5 µg/L Cr(VI)	-	5.94 to 8.34, Cr(III); 1.92 to 6.41 Cr(VI)	-	Lake water	(Mari Pantsar-Kallio and Manninen, 1996)
HPLC-IC-ICP-AES	Plasma-Therm HFS-2500D system, NJ USA	RFP: 1.35 kW; Forward Reflected: <5W; AGF: 1.0 L/min; CGF: 16 L/min; NGF: 1.0 L/min; Spray chamber: double-pass; Nebuliser: Concentric.	-	10 ppm to 50 ppm Cr(III); 25 ppm to 100 ppm Cr(VI)	0.9987 Cr(III); 0.9996 Cr(VI).	0.1 µg/L Cr(III); 0.2 µg/L Cr(VI).			-	Spiked DI water and CRM	(Byrdy *et al.*, 1995)
HPLC-IC-ICP-MS	VG Plas-maQuad PQII + system, Cheshire, UK.	RFP: 1.45 kW; Forward Reflected: <5 W; AGF: 1.2 L/min; CGF: 12 L/min; NGF: 1.0 L/min;	-	3 ppb to 600 ppb Cr(III) ; 5 ppb to 1000 ppb Cr(VI) (peak are) and 3 ppb to 600	0.9998 Cr(III); 0.9990 Cr(VI) (peak are) and 0.9999 Cr(III); 0.9990	0.3 ng Cr(III); 0.3 ng Cr(VI) (peak are) and 0.04 ng Cr(III);	-	4 Cr(III) ; 3 Cr(VI) (peak are) and 4 Cr(III) ; 4 Cr(VI) (Peak height)	-	Spiked DI water and CRM	(Byrdy *et al.*, 1995)

Spray chamber: double-pass; Nebulizer: Concentric	ppb Cr(III) ; 5 ppb to 1000 ppb Cr(VI) (Peak height)	Cr(VI) (Peak height)	0.1 ng Cr(VI) (Peak height)

AFV = Axial field voltage, RFP = Rf power, NGF = Nebulizer gas flow, AGF = Auxiliary/Aerosol gas flow, RGF = Reaction gas flow, CGF = Carrier gas flow, RG = Reaction gas, PGF = Plasma gas flow , IGF = Intermediate gas flow, RG = Reaction gas

Matrix effect associated with the HPLC hyphenated systems
Matrix effect interfare with analytes signal by enhancing or suppressing the signal leading to poor analytical accuracy, reproducibility, and linearity. It is caused by the components of matrix co-extracted with the analyte. (Silverstrol *et al.*, 2013). Many authors have proposed post-extraction spike addition and post-column infusion as methods of dealing with matrix effect in LC related analysis. In addition, cleanup, standard addition and the use of internal standards have been suggested, (Cappiello *et al.*, 2008).

Matrix effect in ICP analysis
For instance, in the ICP instrumentation, the aerosol (the quality of which depends on surface tension and viscosity of the matrix), passes quickly through the plasma. Within this time, the plasma must dry, breakup, dissociate, atomized, and ionize the analytes to 100% mono-charged ions as possible. However, matrices influence the efficiency of the ICP process by modifying (i) the energy demand and side diffusion, (ii) the electron-ion recombination and displacement of ions particularly in ICP-MS analysis, and (iii) the ambipolar diffusion rates of collision and effect of space load, (Chan and Hieftje, 2006; Fraser and Beauchemin, 2001; Gregoire, 1987; Lehn *et al.*, 2003) . Marcinkowska *et al.* (2015) used a dynamic reaction cell (DRC) in a HPLC-DRC-ICP-MS to remove interference during the speciation analysis of Cr(VI), As(III) and As(V) in water samples. Likewise, a standard solution of EDTA was added to samples prior to the speciation analysis Se and Cr in surface, ground and tap water samples (Bednar *et al.*, 2009).
In their analysis of Cr in galvanic wastewater by HPLC-UV, HPLC-GFAAS (offline) and HPLC-HHPPN-ICP-MS, Andrle *et al.* (1997) noted poor quality of ICP-MS analysis compare to the other detectors. This was attributed to the high concentration of argon carbide, due to a high amount of organic solvent used for the chromatography which clogged the detector interface. The problem was resolved by determining Cr as ^{50}Cr and ^{53}Cr isotopes because the power of detection of ^{50}Cr and ^{53}Cr is low due to low relative abundance of the isotopes 4.4% and 9.5% respectively, which led to better result. The researchers also increase the power of the ICP to 1.5 kW and add oxygen to the aerosol gas to lower the formation and interference of argon carbide. Similarly, the detection of ^{53}Cr isotope was preferred due to high background interference by SO^+ from eluent (Byrdy *et al.*, 1995).

Matrix effect in UV and DAD analysis
In the HPLC-UV-Vis, and DAD analysis, co-eluted matrix component, and analyte come under the same electromagnetic radiation. Sallustio *et al* observed this phenomenon and chose to use longer elution time to ensure complete elution of *sirolimus, tacrolimus* and *everolimus* from human blood samples in addition to spiking the blank, standard reference materials and samples, (Sallustio *et al.*, 2011).
Cleanup is the major way of eliminating matrix effect in HPLC UV and DAD method. In most cases, derivatization has led to reduction of the co-elution due to unique properties of complexes. In many if not all analysis in this review, derivatization is carried out prior to separation or in situ as in the case of using an ion pairing mobile phases.

Challenges and prospects of HPLC hyphenated systems for Cr speciation analysis
A typical example of the challenges in hyphenation is observed where ICP-MS is the detector specially if organic solvents are involved in sample preparation or as eluent. This is due to the formation of argon carbide which interfere with analytical result by clogging the ICP-MS nozzle. The other challenge is the co-elution which interfere with analytes signal in UV-Vis detector system. Nevertheless, the hplc hyphenated systems are are versatile and found application in many fields including Cr speciation analysis. There seems to be prospect in the online hyphenation to flame atomic absorption spectrometer (FAAS). This is useful in cases where organic solvents are required as eluents or for sample preparation.

Conclusion
The HPLC hyphenated systems played crucial a role in the speciation analysis of Cr(III) and Cr(VI) from environmental, bio-samples, and food samples. The HPLC was found to be compatible with ICP-MS and, UV-Vis for online analysis. Nonetheless, online preconcentration and analysis with AAS detector was successful with the aid of HHPN.

The HPLC-ICP-MS system was preferred. Its preference is likely due to its sensitivity, high speed of analysis, and isotopic detection capability. In terms of isotopic detection capability, the ^{50}Cr or ^{53}Cr isotopes were determined instead of the ^{52}Cr to avoid isobaric interference due to high carbon concentration in the analysis mixture which form argon carbide

Derivatization was employed to modify the properties of one or both Cr species. Pre-analysis modification was largely used and involves the reaction of the Cr species with a ligand prior to analysis, but then using the ion pairing agents during the chromatographic process constitute the in-situ derivatization. This chemical modification plays a key role in preconcentration during sample preparation, improved chromatographic separation process, and the selective detection and quantitation of the individual Cr species thus enhance the quality of analytical result

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