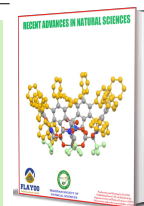


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Two-step one-pot speciation of chromium as Cr-APDC-and-NaEDTA complexes followed by ETA-AAS analysis

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ABSTRACT

A two-step preconcentration method involving solid-and-liquid-phase extraction procedures has been proposed for the speciation of chromium in a one-pot system. The method used sodium ethylenediaminetetraacetic acid (NaEDTA) and ammonium pyrrolidinedithiocarbamate (APDC). The Cr(VI)-APDC was extracted with ethyl acetate and digested with nitric acid. while the Cr(III)-EDTA complex ion was adsorbed onto Al₂O₃ (neutral grade) with the aid of a tetrabutylammonium bromide (TBAB) pairing agent and desorbed with a hydrochloric acid solution. Sample splitting was not required. The ZEE nit 650P, electrothermal atomization atomic absorption spectrometer, (ETA-AAS) was employed for the analysis. The concentration of the chromium (III) in the samples ranged from 1.83±0.00 µg L⁻¹ to 106.28±0.21 µg L⁻¹, while <LOD to 19.85±0.12 µg L⁻¹ was the range of Cr(VI) concentration in the samples. Recovery from spiked samples was between 83% and 117% Cr(III) and 79% and 99% Cr(VI). Precision (n = 6), was 1.87% for Cr(III) and 7.67% for Cr(VI). The limits of detection (LODs), calculated by the Aspect LS software (3σ, n = 11) were 0.42 µg L⁻¹ and 0.62 µg L⁻¹ for Cr(III) and Cr(VI) respectively. The mean total chromium (n = 3), from the certified reference material (CRM), LGC6019 was 0.74±0.61 µg L⁻¹, (certified value = 0.78±0.20 µg L⁻¹). The preconcentration factor: ratio of the Cr(III) content of CRM before and after preconcentration was 3.57. Parameters such as pH, time, temperature, and the amount of adsorbent were optimized by the isolation method. The method was validated and applied for the speciation analysis of chromium in water samples.

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1. INTRODUCTION

The emphasis on chemical speciation analysis of metals rather than their total concentration in samples emerged from the knowledge that biological systems respond differently to chem-

ical forms of metal [1–3]. Thus, the physiological response of biological organisms to tri-and-hexavalent chromium represents the beneficial and the harmful roles associated with the element respectively [4, 5].

The trivalent Cr being essential to humans plays a vital role in the metabolism of glucose and lipids, [2, 5–7], and the formation of deoxyribonucleic acid (DNA) in mammals [1, 5]. Con-

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trariwise, hexavalent chromium is a class 1 human carcinogen [8–10]. Instances of contact with Cr(VI) through inhaling the dust, dermal contact, and ingestion of Cr(VI) contaminated substances have been linked to nasal septum, asthma, and inflammation of the larynx and liver. Humans and experimental animals have developed dermatitis, and skin ulceration, and shown mutagenic and genotoxic effects on exposure to hexavalent chromium [8, 9, 11–13].

Due to its numerous industrial applications in wood preservation, leather tanning, electroplating, and steel alloy production, exposure to hexavalent Cr is unavoidable [14–17]. When discharged into the environment, the atmosphere and aquatic systems serve as means of long-range chromium transport and distribution [9, 11].

The ammonium 1-No Suggestions Available (APDC), has been widely employed in the speciation analysis of chromium via derivatization. Sample preparation has been strategic to minimize or eliminate redox reactions, which may convert the labile Cr(VI) to Cr(III) [1, 18]. The Cr(VI)-APDC reaction yields the tris(1-No Suggestions Available)chromium(III), or [Cr(III)(PDC)₃] and [Cr(III)(PDC)₂(OPDC)] [9, 19]. Likewise, ethylenediaminetetraacetic acid (EDTA) has been employed as a ligand for the chemical speciation of chromium and it selectively chelates Cr(III) compared to Cr(VI) (Figure 1) [20–22]. This has enabled the speciation analysis of chromium in tap water and drinking water [23], human blood, urine, plasma and erythrocytes [24], wastewater [22], surface water, groundwater and tap water [25], as well as workplace air [26].

From the literature, ion pairing agents such as tetraethylammonium (TEA) and tetrabutylammonium, (TBA) bromides, chlorides and hydroxides have been employed in conjunction with EDTA derivatization for the speciation analysis of chromium by chromatography [27, 28]. Other authors have used the cloud point extraction (CPE), based on isopropyl 2-[(No Suggestions Available)disulfonyl] ethane-aided speciation of Cr in human blood [29]. Shirkanloo *et al.* [4] used Triton-X45 and graphene in a CPE for the speciation of Cr in water samples by the ETA-AAS technique and the concentration of each Cr specie was based on difference with the total concentration. Chromium in inhaled human breath condensate was analysed by micro liquid chromatography coupled to inductively coupled plasma mass spectroscopy (μ LC-ICP-MS) hyphenated system [2].

The literature indicated that using a combination of liquid and solid phase preconcentration techniques in one-pot speciation analysis of the element is rare. But, Honma [19] described a one-pot two-step procedure which used APDC and diisobutyl ketone (DIBK). It relied on the rate of reaction of the Cr species with the ligand, (which is very slow with Cr(III)) at room temperature. The Cr(VI)-PDC was taken into the DIBK within a few minutes of the reaction, and the Cr(III)-PDC was extracted with a fresh portion of DIBK after a long time.

From other reports, EDTA has been employed in conjunction with APDC [30, 31] and diethyldithiocarbamate (DDTC), for the speciation analysis of chromium where the EDTA functioned as a masking agent only. The concentration of Cr(III) was based on the difference with the total concentration and a redox step and sample splitting were required [32, 33]. Fasihi *et al.* [32] used 1-(2-pyridylazo)-2-naphthol as a complexing agent, while 4-

hydroxy-2-[(E)-(4-sulfonato-1-naphthyl) diazenyl] naphthalene-1-sulfonate (azorubine) was employed by Tuzen *et al.* [34] for the analysis of Cr in water and beverages samples. In a related research, Khoshmaram and Mohammadi [35] employed 1,5 diphenyl carbazide for the speciation of Cr in surface and ground-water samples.

In this research, a proposed method that utilized NaEDTA and APDC for the speciation analysis of Cr has been described. The NaEDTA served as both a precursor to the preconcentration of Cr(III) and as a masking agent. The Cr(III) was separated as the Cr(III)-EDTA by SPE via the ion pairing principle and the Cr(VI) was preconcentrated as the Cr(VI)-APDC complex by LPE. Sample splitting and a redox step were not required. Hence, the proposed method may find application in microanalysis where the sample is in small quantity. Parameters such as the effect of temperature, reaction time, pH, effects of metal ions and the amounts of adsorbent, TBA, and ligands, were studied. The method was validated and applied to water samples.

2. MATERIALS AND METHOD

2.1. INSTRUMENTATION

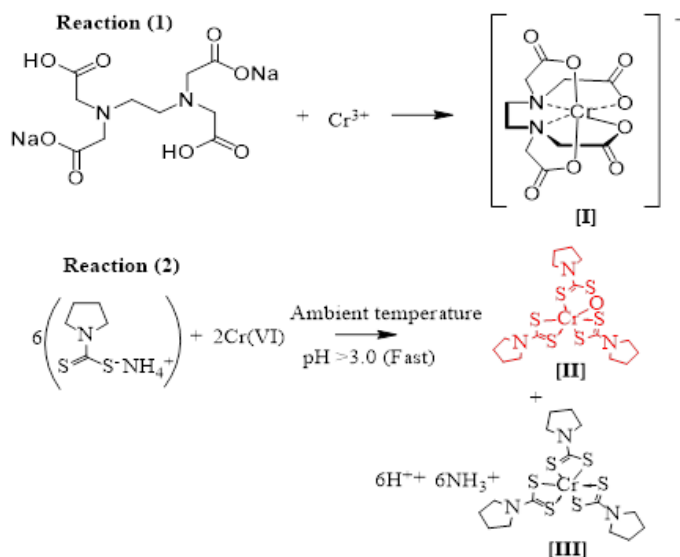
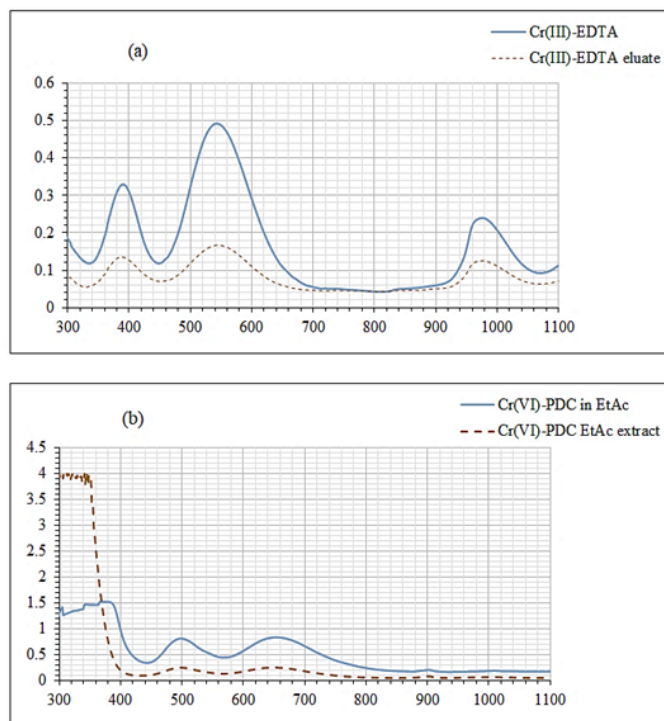
The analysis of chromium was achieved with the ZEE nit 650P GF-AAS (Analytik Jena, Germany), equipped with an MPE 60 auto-sampler, a transversely heated graphite tube atomizer, and a Zeeman-2-way background correction system. The radiation source was a 4.0 mA current from a Cr hollow cathode lamp (Analytik Jena AG, Germany). The analysis was performed on the liquid platform set up, 357.90 nm analytical line, slit width 0.8 nm, and PMT 300 v. The drying temperature and time were 80°C – 110°C, and 50 s respectively, Pyrolysis temperature and time were respectively 350°C – 1300°C, and 30 s, atomization temperature and time were 2300°C, and 5s correspondingly, clean up temperature and time were 2450°C, and 4 s, in that order and spectral measurements were performed within 5 s of atomization. The Fisherbrand FB70155 (Fisher Scientific, UK) pump and SPE set were used for the solid phase preconcentration process. The pH meter was the Metler-Toledo pH meter, (FiveEasy F20, Switzerland), and the shaking-water bath was the Julabo SW22 (Julabo GmbH, Germany) while centrifugation was achieved by the KUBOKU 4200 centrifuge, (Kuboku, Japan).

2.2. REAGENTS AND CHEMICALS

The standard stock solution (200 μ g L⁻¹) for analysis was prepared from Cr(III) for ICP-MS (999 \pm 4 mg L⁻¹ Trace CERT), and Cr(VI) for ICP-MS 1000 \pm 2 mg L⁻¹ trace CERT (Sigma-Aldrich, USA). The stock for external calibration of the ETA-AAS was prepared from chromium(III) nitrate standard for AAS (Spectrosol, APS chemical, Australia). The calibration was achieved via the auto-dilution setup of the Aspect LS software. The 0.5% (w/v) dithiocarbonate solution was prepared from ammonium pyrrolidinedithiocarbamate (APDC) (99.0% Trace metal basis, Sigma-Aldrich, USA). The disodium ethylenediaminetetraacetic acid (Na₂EDTA) was obtained from Ajax Chemicals (Unilab, Ajax Chemical, Australia) from where the 0.5% (w/v) solution was prepared. A 5% (v/v) hydrochloric acid solution was prepared from HCl (37% pure, Merck Darmstadt, Germany). The ethyl acetate (HPLC grade) was obtained from Fisher Scientific, (Leics, UK). While NaOH (BioXtra >98%,

Table 1. The effect of metals in the 50 µg L⁻¹ Cr(III) and Cr(VI) each spiked with 2.5 µg L⁻¹, 7.5 µg L⁻¹ and 15.0 µg L⁻¹ multielement standard XVI, Merck.

MS XVI (µg L ⁻¹)	Spiked (µg L ⁻¹)		Mean found (µg L ⁻¹) n = 3		Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
2.50	50.00	50.00	51.01	49.35	102.02	98.70
7.50	50.00	50.00	53.07	49.71	108.14	99.42
15.0	50.00	50.00	56.20	33.45	114.40	79.90
		MIN	51.01	33.45	102.02	79.90
		MAX	56.20	49.71	114.40	99.42

**Figure 1.** Reaction (1): Cr(III)-EDTA reaction gives [Cr(EDTA)]⁻, [I]; Reaction (2): Cr(VI)-APDC reaction gives, [Cr(III)-(PDC)₂(OPDC)], [II] and [Cr(III)(PDC)₃], [III].**Figure 2.** The UV-Vis spectra (a) extracts of Cr(III)-EDTA from test tubes (A) and (C), and (b) extracts of Cr(VI)-PDC from test tubes (B) and (C).

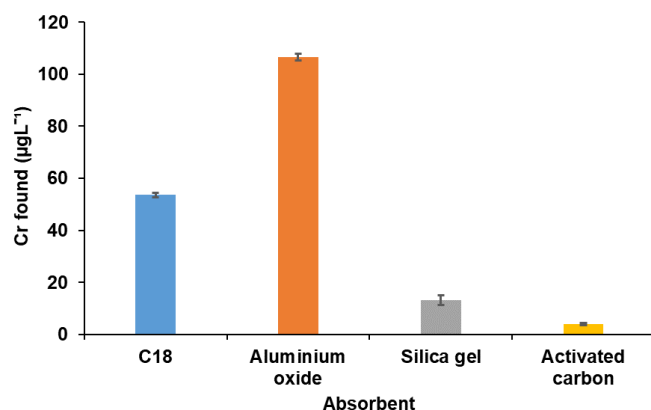
Sigma-Aldrich, USA) and HNO₃ (65% Merck Darmstad, Germany) were used for the pH adjustment. A 0.3% (w/v) solution of the pairing agent was prepared from Tetrabutylammonium bromide (TBAB) (97%, Sigma-Aldrich, USA). The adsorbent, Aluminium oxide (WN-6. Neutral activity grade super 1), was obtained from Sigma-Aldrich (S-A, USA).

2.3. SAMPLES

Samples of wastewater were obtained from the wastewater treatment plant of the Sungai Buloh Hospital, Selangor, Malaysia. Tap and drinking water samples were from Bangunan Makmal Kimia of the University Malaya, Kuala Lumpur. Samples were collected in 1 L high-density polyethylene (HDPE) containers and stored as recommended by the USEPA [9, 13].

2.4. THEORY AND PRINCIPLE OF THE METHOD

This proposed method relied on the selectivity of the NaEDTA for trivalent chromium which served to mask the species in the first step [36]. Thus, providing the route for the hexavalent chromium-APDC complexation, (Figure 1) [9]. The Cr(VI)-PDC is sparingly soluble in aqueous media and was taken by LPE extraction into ethyl acetate and digested with HNO₃ before ETA-AAS analysis. The Cr(III)-EDTA was then adsorbed

**Figure 3.** Comparison of adsorbents for the adsorption of 100 µg L⁻¹ chromium at optimum conditions.

onto an adsorbent (Al₂O₃, neutral grade) with the aid of the ion-pairing agent, tetrabutylammonium (TBA) ion (R₄N⁺). The

Table 2. Accuracy and mass balance study of the LPE-and-SPE preconcentration method.

Spiked Cr(III)	$(\mu\text{g L}^{-1})$ Cr(VI)	Mean found $(\mu\text{g L}^{-1})$ (n =3)		Recovery (%)		Total Cr spiked	Mass balance $(\mu\text{g L}^{-1})$	
		Cr(III)	Cr(VI)	Cr(III)	Cr(VI)		Mean found (speciation) (n =3)	Mean found (ETA-AAS) (n=3)
10	10	8.40	9.39	83.96	93.91	20	17.79	18.84
20	20	19.49	19.42	97.45	97.10	40	38.91	39.01
50	50	41.11	42.55	82.22	85.10	100	83.66	85.10
100	100	98.55	100.21	98.55	100.21	200	198.76	197.45
	MIN	8.40	9.39	82.22	97.10		17.79	18.84
	MAX	98.55	100.21	98.55	100.21		198.76	197.45

Table 3. Comparison of the LODs from other methods with the present method.

Matrix	Chelating agents or adsorbent	Technique	LOD	Reference
Wastewater, drinking and tap water	Ammonium pyrrolidinedithiocarbamate (APDA)/ethylenediaminetetraacetic acid (EDTA)	ETA-AAS	0.42 $\mu\text{g L}^{-1}$ Cr(III); 0.62 $\mu\text{g L}^{-1}$ Cr(VI)	This work
Waster automotive water and artificial seawater	Ammonium pyrrolidinedithiocarbamate (APDA)/diisobutyl ketone (DIBK)	AAS	10 $\mu\text{g L}^{-1}$ Cr	[19]
Water (river, well, spring, and tap)	HTSRTL	UV-Vis	0.4 $\mu\text{g L}^{-1}$ Cr(VI)	[10]
Wastewater	APDC and preconcentrate with $[\text{C}_4\text{MIM}][\text{PF}_6]$	HPLC-DAD	1.9 $\mu\text{g L}^{-1}$ Cr(III); 1.0 $\mu\text{g L}^{-1}$ Cr(VI)	[39]
River water and blood samples	Chromabond NH2	GF-AAS	0.105 $\mu\text{g/L}$ Cr	[11]
Sediment	1-(2-thiazolylazo)-2-naphthol (TAN)	HPLC-UV-Vis	7.5 $\mu\text{g L}^{-1}$ Cr(III); 3.5 $\mu\text{g L}^{-1}$ Cr(VI)	[27]
Tap, river and mineral water	APDC	HPLC-UV-Vis	5.1 $\mu\text{g L}^{-1}$ Cr(III); 2.8 $\mu\text{g L}^{-1}$ Cr(IV)	[1]
Drinking water	EDTA	HPLC –ICP-MS	0.09 $\mu\text{g L}^{-1}$ Cr(III); 1.0 $\mu\text{g L}^{-1}$ Cr(VI)	[20]
Water samples	-	GF-AAS	0.29 $\mu\text{g L}^{-1}$ Cr(III); 0.87 $\mu\text{g L}^{-1}$ Cr(VI)	[15]
Workplace air	EDTA	HPLC –ICP-MS	0.4 $\mu\text{g L}^{-1}$ Cr(III); 0.6 $\mu\text{g L}^{-1}$ Cr(VI)	[26]
tap water	1-(2-pyridylazo)-2-naphthol	FAAS	0.4 ng mL ⁻¹	[32]
Chromate workers' urine	TEA containing EDTA	HPLC-ICP-MS	0.03 $\mu\text{g L}^{-1}$ Cr	[27]
environmental water samples	Magnetic graphene oxide coated with Fe3O4–SiO2–GO–AAIL	ICP-OES	0.406 L ⁻¹ Cr(III); 0.186 L ⁻¹ Cr(VI)	[40]
Wastewater	EDTA	ICP-MS	0.0041 $\mu\text{g L}^{-1}$ Cr(III); 0.0068 $\mu\text{g L}^{-1}$ Cr(VI)	[22]
Welding fumes of stainless steel	DPC/Acid digestion	UV-Vis/FAAS	NA	[16]
beverages and vegetables	4-hydroxy-2-[(E)-(4-sulfonate-1-naphthyl) diazenyl] naphthalene-1-sulfonate (azorubine)	FAAS	0.03 $\mu\text{g L}^{-1}$ Cr(VI)	[34]
Human blood and urine	APDC	FAAS	2.4 $\mu\text{g L}^{-1}$ Cr(VI)	[18]
Supplement	EDTA/ tetrabutylammonium hydroxide (TBAH)	HPLC –ICP-MS	0.02 $\mu\text{g L}^{-1}$ Cr(III)	[14]
Surface and ground Water	1,5 diphenyl carbazide	GF-AAS	0.9 Cr(VI)	[35]
Exhaled breath condensate	EDTA	μLC -ICP-MS	were 0.007 $\mu\text{g L}^{-1}$ Cr(III); 0.002 $\mu\text{g L}^{-1}$ Cr(VI)	[2]
Water	-	surface-enhanced Raman scattering (SERS)	0.1 mg L ⁻¹ Cr(VI)	[21]
Exhaled breath condensate	EDTA	μLC -ICP-MS	0.006 $\mu\text{g L}^{-1}$ Cr(III); 0.002 Cr(VI) $\mu\text{g L}^{-1}$	[2]
Tap, bottled and seawater	2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (TRIS)	HR CS AAS	5 ng L ⁻¹ Cr	[29]

$[\text{Cr}(\text{EDTA})]^-$ ion pairs with R_4N^+ from the quaternary ammonium ion [37]. The pairing results in an electrically neutral pseudo compound of R_4N^+ and $[\text{Cr}(\text{EDTA})]^-$. The SPE adsorption is based on the attraction between the Al_2O_3 adsorbent and the electrically neutral pseudo compound, $\text{R}_4\text{N}^{(+)}[\text{Cr}(\text{EDTA})]^-$, which is deposited on the adsorbent by dispersion forces of interaction [37]. The $[\text{Cr}(\text{EDTA})]^-$ complex is then desorbed by a strong HCl solution and analysed for Cr(III) by the ETA-AAS.

2.5. METHOD DEVELOPMENT

Preliminary study

The preliminary study of the SPE and LPE steps was made by the UV-Vis (Shimadzu UV-1800, Japan) and aimed to ascertain the specificity of the NaEDTA and APDC to Cr(III) and Cr(VI) respectively in the mixture. The premier investigation involved three test tubes. In test tubes, (A) and (B) were 2 mL each of

Table 4. The relative matrix effect of the fortified samples and HCl and HNO₃ solution.

Sample/ Solvent	Calibration characteristic			
	Slope (Abs $\mu\text{g}^{-1} \text{L}^{-1}$)	R ²	Char. conc. $\mu\text{g L}^{-1} (1\%A)^{-1}$	RME (%)
DI water	0.0079	0.9955	0.5505	100.00
HW 1	0.0080	0.9999	0.5464	99.25
HW 2	0.0082	0.9999	0.5311	96.47
HW 3	0.0079	0.9999	0.5508	100.00
HW 4	0.0078	0.9999	0.5629	102.19
HW 5	0.0078	0.9989	0.5623	102.19
DW	0.0078	0.9999	0.5580	101.41
TW	0.0204	0.9930	0.2135	103.55
HCl	0.0209	0.9955	0.2091	105.68 [#]
HNO ₃	0.0199	0.9969	0.2196	100.66 [#]
MIN	0.0078	0.9930	0.2091	96.47
MAX	0.0210	0.9999	0.5629	105.68

HW = Hospital Wastewater, DW = Drinking Water, TW = Tap Water. RME(%) = Percentage Relative Matrix Effect, [#] = Reagent.

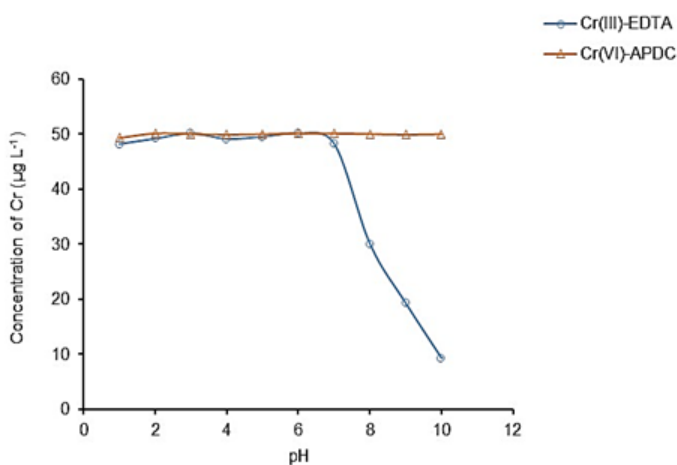


Figure 4. The effect of pH on the complexation of 50 $\mu\text{g L}^{-1}$ of each species Cr(III) and Cr(VI). Cr(III)-EDTA: 4 mL EDTA, at 60 °C for 20 min, 3 mL TBAB solution and $1.0 \pm 0.2 \text{ mL min}^{-1}$ flow rate and 300 mg Al_2O_3 adsorbent and desorbed with HCl solution), Cr(VI)-APDC (4 mL APDC, 5 mL ethyl acetate and digest in HNO_3 solution).

1000 mg L^{-1} Cr(III) and Cr(VI) standards respectively. Equal volumes (1 mL each) of the same standard solutions were dispensed into a test tube (C). Excess APDC standard solution was dispensed into a test tube (B) and allowed for 10 minutes then extracted with ethyl acetate and the UV-Vis spectra were determined. Test tubes (A) and (C) were treated with excess NaEDTA standard solution in a water bath at 60 °C until the purple colour was fully developed and allowed to cool. The UV-Vis spectrum of the mixture from test tube (A) (Cr(III)-EDTA), was obtained, but test tube (C) was further treated with excess APDC and extracted with ethyl acetate and the UV-Vis spectrum from the extract (Cr(VI)-PDC), was obtained. Excess TBAB solution was added to the residue from the test tube (C) containing Cr(III)-EDTA complex and diluted to 40 mL with deionized water. The pH was adjusted to pH 4.0 and loaded onto the Al_2O_3 adsorbent for solid-phase preconcentration.

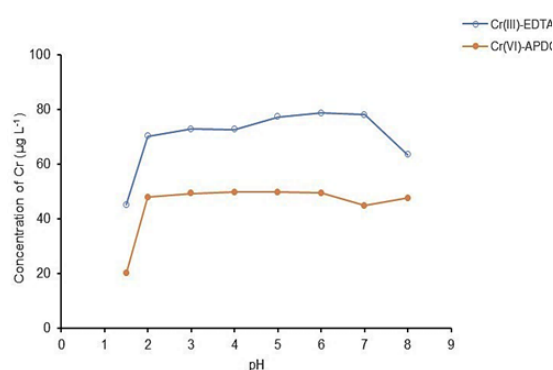


Figure 5. The effect of pH on the preconcentration of 100 $\mu\text{g L}^{-1}$ Cr(III)-EDTA and 50 $\mu\text{g L}^{-1}$ Cr(VI)-APDC complexes: Cr(III)-EDTA (4 mL EDTA, at 60 °C for 20 min, 3 mL TBAB solution and $1.0 \pm 0.2 \text{ mL min}^{-1}$ flow rate and 300 mg Al_2O_3 adsorbent and desorbed with HCl solution); Cr(VI)-APDC (4 mL APDC, 5 mL ethyl acetate, and digest in HNO_3 solution).

The purple-coloured Cr(III)-EDTA complex was eluted with 5% (v/v) HCl into a test tube and the UV-Vis spectrum was obtained. The UV-Vis spectra of Cr(III)-EDTA, from test tubes (A) and (C) were compared. Similarly, the spectra of the ethyl acetate extracts containing Cr(VI)-PDC, from test tubes (B) and (C) were compared (Figure 2).

Parameters were optimized by the isolation method (one parameter varied at a time), and determinations were made by the ETA-AAS. An optimum condition became a constant parameter while optimizing the next parameter. Variable amounts of Cr(III) and Cr(VI) species standards were used for the optimization [36, 38]. Since the SPE tank and vacuum pump were not equipped with the facility to control the flow rate of samples, the SPE system was manually calibrated and the flow rate was fixed at $1.0 \pm 0.2 \text{ mL min}^{-1}$ and used through the solid phase preconcentration process.

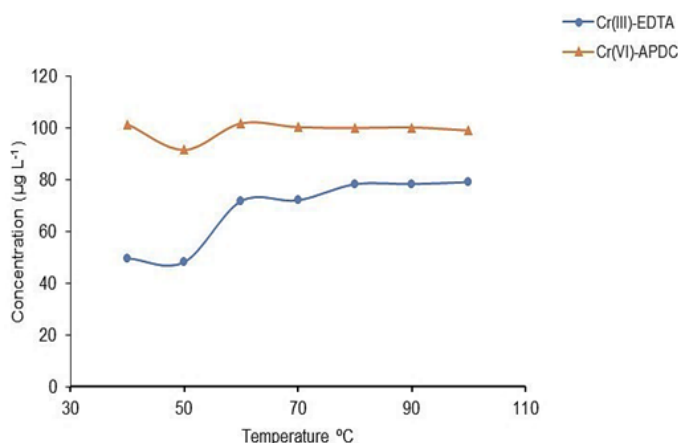


Figure 6. Effect of temperature on the formation of Cr(III)-EDTA and Cr(VI)-PDC complexes. Cr(III)-EDTA: (4 mL EDTA, pH 5 to pH 6, heat for 20 min, 3 mL TBAC solution and 1.0 ± 0.2 mL min⁻¹ flow rate and 300 mg Al₂O₃ adsorbent and desorbed with HCl solution); Cr(VI)-APDC (4 mL APDC, 5 mL ethyl acetate and digest in HNO₃ solution).

Adsorbents efficiency

Four adsorbents were compared to study the efficiency of the solid phase preconcentration of the Cr-EDTA complex. The result of the comparison presented in Figure 3 indicated that the Al₂O₃ was a better adsorbent of the Cr-EDTA complex.

2.6. SPECIATION ANALYSIS OF CHROMIUM PROCEDURE

Chromium species were determined by the ETA-AAS using the setup outlined under Instrumentation. The external calibration method was used for the instrument calibration. A 30 mL portion of the dilute sample, 40 mL CRM or 20 mL of suitable Cr standard, was placed in a 50 mL HDPE centrifuge tube. 2 mL of 0.3% (v/w) Na₂EDTA solution was added and the pH was adjusted with NaOH and HNO₃ to between pH 5.0 and pH 6.0 using the Metler-Toledo pH meter. The tube was capped loosely and placed in the Julabo SW22 shaking water bath at 80°C for 10 minutes. The mixture was cooled to room temperature in the open air, and then 2.5 mL of 0.5% (w/v) APDC solution was added and allowed to stand for 3 min. A 3 mL portion of ethyl acetate was added and covered securely before shaking the mixture vigorously and centrifuged for 3 min at 4500 rpm with the KUBOKU centrifuge. The organic phase was pipetted into a 25 mL conical flask containing 10 mL, 5% (v/v) HNO₃ covered with a watch glass and then digested on a hot plate at ≤ 90 °C. The digest was transferred into a 15 mL HDPE test tube and made up to 10 mL with deionized water before analysis for Cr(VI) concentration.

The aqueous phase was treated with 3.5 mL 0.3% (w/v) TBAB solution and diluted to 40 mL with deionized water (except the CRM), before adjusting the pH (pH 5 to pH 6), with HNO₃ and NaOH solutions. The mixture was then loaded onto a 3 mL SPE cartridge holding 500 mg Al₂O₃ at about 1.0 ± 0.2 mL min⁻¹. The Cr-EDTA complex was eluted with 6 mL 5% (v/v) HCl solution into a 15 mL centrifuge tube and made up to 10 mL with deionized water before ETA-AAS analysis.

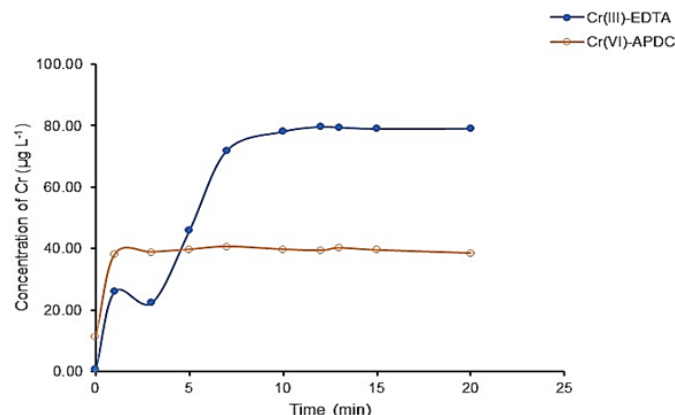


Figure 7. Time of reaction for 40 µg L⁻¹ Cr(VI) and 100 µg L⁻¹ Cr(III) species. Cr(III)-EDTA: (4 mL EDTA, pH 5 to pH 6, 80°C, 3 mL TBAC solution and 1.0 ± 0.2 mL min⁻¹ flow rate and 300 mg Al₂O₃ adsorbent and desorbed with HCl solution); Cr(VI)-APDC: (4 mL APDC, 5 mL ethyl acetate and digest in HNO₃ solution).

3. RESULTS AND DISCUSSION

3.1. UV-VIS ANALYSIS OF THE COMPLEXES

The spectral analysis of extracts, eluates, and contents from test tubes (A), (B) and (C) clarified the feasibility of the one-pot speciation analysis of Cr(III) and Cr(VI) by EDTA and APDC chelating agents due to the similarity of the spectra, (Figure 2). The proposed mechanism of the chromium - EDTA reaction has been reported by Cerar [36] and a study on the general ligand-metal behaviour during complex formation was reported by Eigen and Wilkins [41].

3.2. THE EFFECT OF PH ON THE CHROMIUM-EDTA-AND-APDC COMPLEXES FORMATION

Even though the chromium-EDTA-and-APDC complexation is well known, studying the effect of pH on the formation was important because a combination of complexation agents was involved in this study. It was studied using standards of the species fortified in DI water and treated at various pH before preconcentration at pH 5. Figure 4 depicts the effect of pH on the formation of the Cr(III)-EDTA and Cr(VI)-APDC complexes. The Cr(III)-EDTA complexation was affected at higher pH values probably due to the continuous replacement of the water molecules in the [Cr(H₂O)₆]³⁺ by OH⁻ and the subsequent formation of solid [Cr(OH)₃(H₂O)₃] complex that makes the OH⁻ replacement difficult as well as deplete the concentration of reactive Cr(III) species in the solution, thus accounting for the low preconcentration of the analyte for complexation.

3.3. THE EFFECT OF PH ON THE CHROMIUM-EDTA-AND-APDC COMPLEXES PRECONCENTRATION

The pH is significant in the preconcentration of Cr(III)-EDTA as it plays a major role in determining the magnitude and stability of the charge of the Cr-EDTA complex ion [36]. The role of pH in the preconcentration techniques was studied by treating mixed analytes in DI water at the optimum pH of formation and other optimized conditions, followed by preconcentration at different

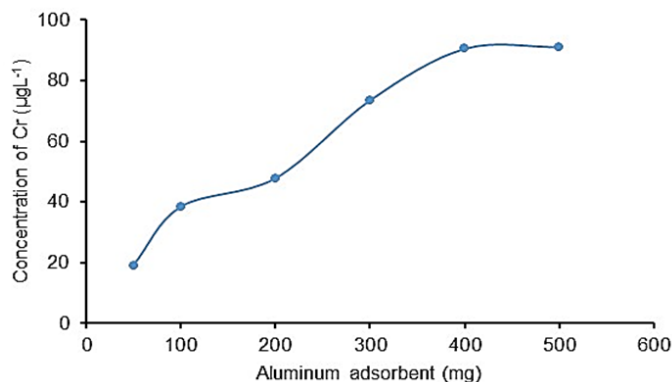


Figure 8. Amount (mg) of Al_2O_3 adsorbent for the adsorption of Cr(III)-EDTA from $100 \mu\text{g L}^{-1}$ Cr(III) standard. (2 mL EDTA, 80°C , 10 min, pH 5, 3.5 mL TBAC solution and $1.0 \pm 0.2 \text{ mL min}^{-1}$ flow rate, adsorbent and 6 mL HCl solution).

pH values. The effect of pH on preconcentration techniques as presented in Figure 5 indicated that solid-phase preconcentration of the Cr-EDTA seems unaffected.

At pH 5 to pH 7, similar behaviour was observed in previous research [36, 40]. Earlier studies revealed that under slightly acidic conditions, the monovalent $[\text{Cr}(\text{EDTA})]^-$ ion predominates [36]. The data also indicate that the univalent $[\text{Cr}(\text{EDTA})]^-$ ion is favourably paired with the equally univalent $(\text{R}_4\text{N})^+$ ion of the TBAB. Nevertheless, the preconcentration of the Cr-APDC complex was relatively stable between pH 2 to pH 6. Hence pH 5 to pH 6 was chosen for both the liquid and solid phase preconcentration of the Cr complexes.

3.4. THE EFFECT OF TEMPERATURE ON THE CHROMIUM-EDTA-AND-APDC COMPLEXATION

From the preliminary studies and the literature, the formation of the Cr(III)-EDTA complex is slow at room temperature while Cr(VI)-APDC forms instantaneously under the same condition. This observation made it necessary to study the effect of temperature on the complexation process. The standards of Cr species fortified in DI water and kept at pH 5 to pH 6 were treated in a water bath at temperatures ranging from 40°C to 100°C and the liquid-and-solid phase preconcentration processes were carried out at the same pH range. The concentration-temperature curve (Figure 6), showed that 80°C is the optimum temperature for Cr-EDTA complexation. On the other hand, the Cr(VI)-APDC complexation seems unaffected by temperature in this study.

3.5. THE EFFECT OF TIME ON THE CR(III)-EDTA AND CR(VI)-APDC COMPLEXATION

The standards of chromium species were mixed, and treated at optimum temperature (80°C), and pH, (pH 5 to pH 6), at various times, and then preconcentrated with the LPE-and-SPE procedures before analysis with the ETA-AAS. The formation of Cr-PDC complexes was fast while the Cr-EDTA formation took 10 min to complete under the conditions as shown in Figure 7.

3.6. EFFECT OF THE AMOUNT OF SOLVENTS, LIGANDS, AND PAIRING AGENTS ON THE RECOVERY OF CR(III) AND CR(VI)

From the preliminary study, the NaEDTA and APDC are specific with respect to Cr(III) and Cr(VI) species respectively. However, a study has shown that they are complex with other metals as well [36]. Besides, real sample compositions are heterogeneous, particularly in terms of metal content. For this reason, the optimization of the amounts of solvent, ligands, and pairing agent was particularly important to ensure significant analytes recovery in real samples despite competition. The optimization process was achieved by varying the volume of these reagents one at a time and computing the recoveries. In Figure S1 (Supplementary), the recovery of Cr(VI) was stable from 1.5 mL ethyl acetate. Therefore, 3 mL to 4 mL ethyl acetate was used in the subsequent preconcentration of Cr(VI)-PDC. On the other hand, the recovery of eluted Cr-EDTA was relatively stable from 6 mL HCl as shown in Figure S3 (Supplementary). For subsequent elution, 6 mL to 10 mL (5% v/v) HCl was chosen for the elution of the Cr(III)-EDTA complex. While 2 mL (0.5% w/v) EDTA and 2.5 mL (0.5% w/v), APDC were sufficient for the complexation of $100 \mu\text{g L}^{-1}$ each of Cr(III) and Cr(VI) respectively as shown in Figures S2 and S4 (supplementary) and were used as optimum amounts of ligands in further sample preparation. Nevertheless, 4 mL of TBAB (0.3% w/v), was the optimal volume for pairing $100 \mu\text{g L}^{-1}$ Cr(III)-EDTA for the SPE preconcentration, Figure S5 (Supplementary).

3.7. EFFECT OF METAL IONS ON THE RECOVERY OF CR(III) AND CR(VI)

Metal ions of Cu, Fe, Co, and Ni among others, chelate EDTA and APDC [36, 40], which makes it necessary to study the effect of other metals that may compete for the ligands. A multielement standard XVI (MS XVI) (Merck, Darmstadt, Germany), made of 21 elements from different parts of the periodic table at equal concentration was used. Though it is not expected that the metal ions be present in equal amounts naturally, the composition of MS XVI placed it close to the real environment. To accomplish studying the effects of metals, portions of DI water containing $50 \mu\text{g L}^{-1}$ each of Cr(III) and Cr(VI), were spiked with $2.5 \mu\text{g L}^{-1}$, $7.5 \mu\text{g L}^{-1}$ and $15.0 \mu\text{g L}^{-1}$ of MS XVI and treated as in the procedure. The recovery studies (Table 1), were between 102% and 114% Cr(III) and 80% to 99% Cr(VI). The recoveries were within the acceptable range (80% to 120%) despite the presumed competition for the ligands [9]. Nevertheless, the observed increase in recovery of Cr(III) with increasing concentration of MS XVI is presumably a response to the presence of Cr(III) in the MS XVI standard. In a real application, the wastewater samples were diluted before sample preparation and the results were calculated appropriately.

3.8. EFFECT OF ADSORBENT, Al_2O_3 DOSAGE ON THE RECOVERY OF CR(III) AND CR(VI)

The effect of the adsorbent dosage was studied and the result is presented in Figure 8 where it can be seen that 400 mg adsorbent was preferred for the adsorption of Cr species.

4. METHOD VALIDATION

Table 5. Chromium content and recovery study from water and wastewater samples.

Sample	Speciation		Recovery		Found ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Cr(III)- EDTA	Cr(VI)- APDC	Added ($\mu\text{g L}^{-1}$) Spiked ($\mu\text{g/l}$)		Cr(III)- EDTA	Cr(VI)- APDC	Cr(III)- EDTA	Cr(VI)- APDC
HW1	31.43±0.68	10.19±0.00	50.00		43.50	48.31	96.62	87.00
HW2	106.28±0.21	19.46±0.12	50.00		39.85	57.16	114.32	79.70
HW3	27.46±0.50	13.18±0.08	50.00		43.45	47.00	94.00	86.90
HW4	24.33±0.23	13.09±0.08	50.00		43.16	46.71	93.42	86.32
HW5	44.84±0.10	19.85±0.12	50.00		43.97	58.55	117.10	87.94
DW	2.28±0.03)	<LOD	50.00		43.35	41.77	83.54	86.70
TW	1.83±0.00)	<LOD	50.00		44.24	45.86	91.72	88.48
CRM	2.64±0.31)	<LOD	50.00		49.55	43.60	87.20	99.10
MIN	1.83±0.00)	<LOD			39.85	41.77	83.54	79.70
MAX	106.28±0.21	19.85±0.12			49.55	58.55	117.10	99.10

4.1. ACCURACY, MASS BALANCE AND PRECISION STUDIES

The accuracy of the method was studied by monitoring percentage recovery. Four concentration levels, ($10 \mu\text{g L}^{-1}$, $20 \mu\text{g L}^{-1}$, $50 \mu\text{g L}^{-1}$, and $100 \mu\text{g L}^{-1}$), of the analytes in DI water, were subjected to treatment and analysis as described in the procedure and the accuracy was calculated for each level using Equation (1). The result in Table 2 showed that recoveries from the analysis fell within the acceptable range of 80% to 120% [9].

The mass balance compares the sum of chromium recovered from speciation analysis with the total determined from a fortified sample containing an equal concentration of the analyte mixture as that used for the speciation analysis. To determine the total concentration, the APDC complex of the mixture was pre-concentrated in ethyl acetate, digested with HNO_3 and diluted to a volume equal to that used for speciation analysis before the ETA-AAS analysis. The results in Table 2, showed agreement between the sum from the speciation analysis and the total Cr determined.

$$\text{Accuracy (\%)} = \frac{C_f (\mu\text{gL}^{-1})}{C_s (\mu\text{gL}^{-1})} \times 100 \quad (1)$$

The method precision was studied following the method described by Narola *et al.* [42]. Six portions of each of the DI water samples fortified with $100 \mu\text{g L}^{-1}$ Cr(III) and $50 \mu\text{g L}^{-1}$ Cr(VI), were subjected to sample treatment and analysis as in the procedure to obtain six replicate determinations. The RSD% was calculated using Equation (2). The result in Table S1 (Supplementary) recorded 1.87% and 7.67% precision for Cr(III) and Cr(VI) respectively and agree with the literature of $\leq 10.00\%$ relative standard deviation [20].

$$\text{RSD (\%)} = \frac{\text{SD } (\mu\text{g L}^{-1})}{\text{Mean concentration } (\mu\text{g L}^{-1})} \times 100 \quad (2)$$

4.2. LOD AND LOQ

The limits of detection and quantitation represent the concentration of analytes detectable without and with accuracy respectively [7, 20]. The LOD and LOQ were determined following the fortified blank method [43–45]. Deionized water was

spiked with a very low concentration of analytes and passed through sample preparation. The ZEE nit 650P GF-AAS was programmed to analyse and calculate the limits. Eleven replicates were made from where the standard deviation (σ), and the limits were calculated by the Aspect LS software. By this software, the LODs were 3σ ($0.42 \mu\text{g L}^{-1}$ and $0.62 \mu\text{g L}^{-1}$) Cr(III) Cr(VI) and the LOQs were 9σ ($1.27 \mu\text{g L}^{-1}$ and $1.87 \mu\text{g L}^{-1}$) for Cr(III) and Cr(VI) respectively, (Table 3).

4.3. MATRIX EFFECT

Analytes signals are often enhanced or suppressed by the presence of in the matrix (endogenous substances), substances introduced into the matrix (exogenous substances) or substances within the analysis environment called the extraneous substances. The matrix effect affects the quality of analytical results. The extent of suppression or enhancement of the signal was studied by the calibration slope method as described by Lehotay *et al.* [46] and Leito [47] with some modifications and reported as a percentage relative matrix effect RME(%). The RME% compared the slopes of the matrix-matched samples or the reagents (used for digestion or elution), to that of the fortified DI water which is often used for dilution purposes (Equation (3)). This method for studying the ME was suitable due to the non-availability of the commercial Cr-EDTA and Cr-APDC complexes. The Aspect LS software was programmed to calibrate by dilution and $0 \mu\text{g L}^{-1}$, $10 \mu\text{g L}^{-1}$, $20 \mu\text{g L}^{-1}$, and $30 \mu\text{g L}^{-1}$ were prepared by programmed dilution from $40 \mu\text{g L}^{-1}$ matrix-matched stock solutions and fortified DI water. The solutions were prepared in DI water, HCl (5%), HNO_3 (10%) and filtered samples diluted five times. The RME(%) above 100% indicated signal enhancement and below 100% showed signal suppression.

$$\text{RME (\%)} = \frac{m_{(\text{Spiked} \vee \text{solvent})}}{m_{(\text{SpikedDIwater})}} \times 100 \quad (3)$$

The RME% as presented in Table 4 was between 96% and 105% representing signal enhancement and suppression between -5% and +5% respectively, ($<10\%$ in both cases). This may be indicative of the non-detrimental effect of the RME on the overall analytical result. The sensitivity of the instrument as measured by the characteristic concentration was between $0.2 \mu\text{g L}^{-1}$

(1%A)⁻¹ and 0.5 µg L⁻¹ (1%A)⁻¹. The calibration curves of the fortified matrix-matched samples, digestion and elution reagents as well as fortified DI water are shown in Figure S6 (Supplementary).

5. APPLICATION

5.1. SPECIATION ANALYSIS OF CHROMIUM

The result of the speciation analysis of chromium by the method is present in Table 5 from where the concentration of Cr(III) as Cr(EDTA) ranges from 1.83 µg L⁻¹ to 106 µg L⁻¹, while that of Cr(VI) as Cr(PDC)₃ and Cr(PDC)₃(OPDC) was between <LOD (0.62 µg L⁻¹) to 19.85 µg L⁻¹. Studies have shown that redox reaction may be triggered in the presence of organic matter and some metals especially Mn and Fe resulting in the inter-conversion of Cr(VI) and Cr(III) which more often than not favour the Cr(VI) to Cr(III) conversion [22]. This could be responsible for the general higher amounts of Cr(III) compared to Cr(VI) species in the wastewater samples.

From the literature, Table 3, the LOD of Cr(III) ranged from 0.0041 µg L⁻¹ to 7.7 µg L⁻¹, while that of Cr(VI) is between 0.002 µg L⁻¹ and 3.5 µg L⁻¹. Although analysts employed different techniques for the determination of Cr species, the LODs (0.423 µg L⁻¹ Cr(III) and 0.624 µg L⁻¹ Cr(VI)), from this work indicates that the sensitivity of the method and instrument compared favourably with the literature.

The certified value of Cr in the CRM is 0.78 µg L⁻¹ Cr(III), the amount of the Cr(III) found after sample treatment was higher. That could be attributed to the preconcentration process as the species was harnessed into 10 mL from a 40 mL sample before analysis. Thus, the preconcentration factor (PF), calculated as the ratio of Cr(III) content of CRM after and before preconcentration is 3.57, (Equation (4)). Due to the non-availability of the CRM of the species, the PF determination could not be applied to Cr(VI).

$$PF = \frac{C_{(Preconcentrated)}}{C_{(Certified)}} \times 100, \quad (4)$$

where C = Concentration (µg L⁻¹).

The percentage recoveries of the analytes from the fortified samples were between 83% and 117% Cr(III) and 80% and 99% Cr(VI). The fortified CRM (GC6019), recorded 87% Cr(III) and 99% Cr(VI) recovery. Speciation of chromium in water samples by various methods has shown similar recoveries [1, 2, 38].

6. CONCLUSION

The selectivity of EDTA for Cr(III) and the solubility of Cr(VI)-APDC complex were exploited for the speciation of Cr(III) and Cr(VI) in this proposed method. The ETA-AAS analysis revealed that the LOD of was Cr(III) 0.423 µg L⁻¹ and that of Cr(VI) was 0.624 µg L⁻¹. The recovery of Cr(III) was between 83% and 117% and that of Cr(VI) was 79 and 99%, indicating that the accuracy of the method is within the analytically acceptable limit. The relative matrix effect was ±5% and the R² from the study of the matrix effect ranged from 0.99298 to 0.99997, further confirming the reliability of detection. The total chromium (0.74 µg L⁻¹), determined from the raw CRM (LG6019), agreed with the certified value of 0.78±0.20 µg L⁻¹

of the CRM. Due to the non-availability of the Cr(VI) reference material, the preconcentration factor, ratio of the Cr(III) content of CRM before and after preconcentration was 3.57. The proposed method requires no redox reaction (to convert one species Cr to the other), this saves both reagents and the cost of analysis as well as possible matrix contamination. More so, this proposed method needs no sample splitting and, therefore, may be applied at the micro-scale level with different detection techniques similar to the ETA-AAS. Hence, the proposed method is comparable to earlier methods of chromium speciation involving a two-step procedure.

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