Microchemical Journal 160 (2021) 105618

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Chromium speciation in organic fertilizer by cloud point extraction and optimization through experimental Doehlert design as support for legislative aspects

Ivero Pita de Sá [a,b], Gilberto Batista de Souza [a], Ana Rita de Araujo Nogueira [a,*]

[a] Embrapa Pecuária Sudeste, São Carlos, São Paulo State 13560-970, Brazil
[b] Group of Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, São Carlos, São Paulo State 13565-905, Brazil

ARTICLE INFO

Keywords:
Cr(VI)
Organic fertilizer
Cloud point extraction
Experimental design
Spectrophotometry
Flame atomic absorption spectrometry

ABSTRACT

Brazilian and EU legislation establishes 2 mg kg^{-1} as the maximum limit of Cr(VI) in organic fertilizers. A procedure involving chromium speciation was developed. With this approach, the interference caused by humic acid present in the samples was circumvented and met the legislation requirement. The procedure is based on the adjustment of the alkaline extraction (USEPA 3060A), and cloud point extraction (CPE) applied to determine Cr (VI) traces. The issues that directly affect Cr(VI) extraction in organic fertilizers, such as temperature, sample mass, and extractor volume were studied. Moreover, the parameters that influence CPE were evaluated using the Doehlert matrix. Under optimized conditions, the use of 0.2 g of sample and 10 mL of the extractor solution was defined. For the Cr(VI) separation and preconcentration (CPE), 2% (m v^{-1}) NaCl, 0.3% (v v^{-1}) Triton X-100, and 0.05% (m v^{-1}) 1,5 diphenylcarbazide were used. UV–Vis spectrophotometry or flame atomic absorption spectrometry (FAAS) were used as detection techniques. They provided quantification limits of 1.38 and 1.82 μg g^{-1}, respectively. The accuracy of the procedure was evaluated by analyzing certified reference materials (NIST 2701, Hexavalent Chromium in Contaminated Soil and NIST 695, Trace Elements in Multi-Nutrient Fertilizer) and analysis of spiked samples. The proposed procedure is suitable for the determination of Cr(VI) in organic fertilizers, meeting the requirements of normative instruction (NI) n. 7 (04/2016) of the Brazilian Ministry of Agriculture, Livestock and Supply and Regulation (EU) 2019/1009 of the European Parliament.

1. Introduction

The population growth is directly linked to agricultural production. In this scenario, fertilizers, minerals, and organics play a vital role in developing plants by providing the soil with the necessary nutrients. Organic fertilizers are produced with raw materials from the industrial, urban, and rural sectors. Due to the diverse types of fertilizers from these different segments, the chemical characterization of these materials is of fundamental importance as it provides data on nutrients and possible contaminants [1,2].

A category of chromium (Cr)-containing fertilizers is produced using byproducts of tanning and industrial process. These fertilizers contain Cr in the trivalent form, considered an essential trace element in human and animal nutrition. Furthermore, it also can be present in the extremely toxic Cr(VI), found in its various chemical forms ($Cr_2O_7^{2-}$, H_2CrO_4, and CrO_4^{2-}). Because of its high mobility, it presents acute toxicity to living organisms and potential carcinogenicity to humans. Therefore, several guidelines and regulations deal with its determination and control of Cr contained in fertilizers. Legislations frequently are controversial, because it is not based in scientific and experimental shreds of evidence and usually not distinguish the oxidation state of Cr, considering both Cr(III) and Cr(VI) as hazardous and toxic [3]. Otherwise, European Union, United States, and other countries such as Italy and Brazil have legislations in which the maximum admissible levels of Cr in organic fertilizers are referred to Cr(VI).

Regarding these guidelines, EU Regulation 2019/1009 (EUR-Lex, 2019) [4] and normative instruction (NI) n.7 of the Ministry of Agriculture, Livestock and Supply [5] indicates 2 mg kg^{-1} of dry mass as the maximum allowed in organic fertilizer. Alkaline extraction (USEPA 3060A) [6] and measurement by the colorimetric procedure with 1,5 diphenylcarbazide (DFC) for the determination of Cr(VI) (USEPA 7196) [7] is the indicative method by the Brazilian regulation. Otherwise,

---

* Corresponding author.
E-mail address: ana.nogueira@embrapa.br (A.R. de Araujo Nogueira).

https://doi.org/10.1016/j.microc.2020.105618
Received 12 September 2020; Received in revised form 7 October 2020; Accepted 7 October 2020
Available online 12 October 2020

despite the relative selectivity, alkaline extraction of Cr(VI) also extracts dark-colored humic compounds, making colorimetric determination difficult [3,8–10].

Humic compounds consist of humic and fulvic acids and humine, which are mainly composed of organic matter. Zhang et al. (2017) developed a study to evaluate the mechanism of interaction of humic acids with Cr(VI). The authors found that the carboxylic and phenolic groups of this compound reduce and complex Cr(VI) to Cr(III) in acidic medium. The mechanism of interaction occurs in 3 stages (i) Cr(VI) adsorption by carbonyl and ester groups; (ii) reduction from Cr(VI) to Cr (III) by phenol and polysaccharides; and (iii) complexation of Cr(III) reduced by carboxylic groups [11].

Given the high concentration of humic compounds in organic fertilizers, it is expected that Cr(VI) is at a low concentration level. However, for Cr(VI) contents to be adequately quantified, separation and preconcentration methods are required. In this sense, several methods are described in the literature for Cr(VI) preconcentration, such as solid-phase extraction (SPE), adsorption, precipitation and coprecipitation, dispersive liquid–liquid micro-extraction (DLLME), and cloud point extraction (CPE) [12,13].

A coprecipitation method called carrier element-free coprecipitation (CEFC) was proposed by Bulut et al. (2009) for Cr speciation in water and solid samples. A water-insoluble organic compound was used as coprecipitation agent, avoiding the need for carrier elements for separation. The contamination risks were minimized, and LOD of 0.7 μg L^{-1} of Cr(III) was obtained by flame atomic absorption spectrometry (FAAS) [14]. Ozbertan et al. (2020) explored a liquid-liquid microextraction (LLME) for Cr speciation in water samples. An ultrasonic-assisted extraction and Cr(VI)-DFC determination by spectrophotometry allows LOD of 0.76 μg L^{-1} and an enhanced factor of 50 [15]. These preconcentration procedures not considered the effect of the humic acid in the Cr(VI) reduction.

Cloud point extraction has been applying since 1976 for separation and preconcentration procedures [16]. This highlighting was its properties, such as low surfactant toxicity and low volatility compared to organic solvents, thus meeting the principles of green chemistry. Furthermore, CPE provides high preconcentration factors and good accuracy. Regarding the procedure principle, CPE occurs when nonionic surfactants, in aqueous solutions and quantities above the critical micellar concentration (CMC), are heated above a given temperature (cloud point), causing the solution to cloud over. Above cloud point by centrifuged, two liquid phases are formed i) one containing high surfactant concentration and the extracted species (rich phase), and ii) the aqueous phase containing a small surfactant concentration near CMC (poor phase) [16].

Considering the need for Cr(VI) determination without the interference of humic acids, we proposed the alkaline extraction followed by CPE for Cr(VI) determination in organic fertilizers by UV–Vis spectrophotometry or FAAS. The main parameters that influence Cr(VI) extraction and stability, such as sample mass and extraction solution volume for the alkaline extraction procedure and the CPE ideal conditions were evaluated.

2. Material and methods

2.1. Apparatus

A microwave oven with modified polyethylene bottles (polyfluoramide, PFA) (ETHOS 1, Milestone, Sorisole, Italy) was used for digested samples in the total Cr determination.

A UV–Vis spectrophotometer in a wavelength path at 545 nm (Femto 432, São Paulo, Brazil) with a micro-cuvette (1 mL), and a flame atomic absorption spectrometer (SpectrAA 800, Varian, Mulgrave, Australia) equipped with chromium hollow cathode lamp in 357.9 nm wavelength were used for Cr(VI) and total Cr determination.

Liquid chromatography coupled to inductively coupled mass spectrometry (LC-ICP-MS) (LC 1200 series and ICP-MS 7800, Agilent Technologies, Tokyo, JHS, Japan) equipped with an anion exchange column (G3268-80001, Agilent Technologies, Tokyo, JHS, Japan), an LC connection kit (G1833-65200, Agilent Technologies, Tokyo, JHS, Japan), He pressurized (99.9999%, White Martins-Praxair, Sertãozinho, São Paulo, Brazil), and high purity argon (99.999%, White Martins-Praxair, Sertãozinho, São Paulo, Brazil) was used for Cr(III)/Cr(VI) separation, and auxiliary, plasma generation and nebulization gas. Integrated method setup and sequence control of the combined LC-ICP-MS system was carried out from the ICP-MS MassHunter (MH) software package (Agilent Technologies, Tokyo, JHS, Japan). The instrumental operational parameters and monitored isotopes are presented in Table 1.

A centrifuge with capacity of 15 and 50 mL tubes (Excelsa II 206 BL, FANEM, São Paulo, Brazil) and a heating device (Dubnoff bath, NT232 Nova Técnica, São Paulo, Brazil) stabilized at 90–95 °C with continuous auto stirring were used during the sample preparation and extraction procedures.

2.2. Solutions

All dilutions and solutions were prepared with deionized water (Milli-QTM, Millipore, Bedford, MA, USA). The glassware and the polypropylene bottles used were decontaminated with 10% nitric acid (v v^{-1}) for 24 h and rinsed with water. Sub-distilled nitric acid (Synth, Diadema, SP, Brazil) and hydrogen peroxide 30% (v v^{-1}) (Sigma Aldrich, Germany) were used for acid decomposition.

The extraction solution was prepared with 0.5 mol L^{-1} NaOH plus 0.28 mol L^{-1} Na_2CO_3. This solution was stored in a tightly capped polyethylene bottle at 20–25 °C [6]. The pH of the extraction solution, ≥11.5, should be checked before the use. Phosphate buffer solution, 0.5 mol L^{-1} K_2HPO_4/KH_2PO_4 at pH 7, 1% (m v^{-1}) DFC stored in an amber bottle, and 4% (v v^{-1}) Triton X-100 surfactant solution were other used solutions [7].

Standard stock solutions for the calibration curves and standard addition tests were prepared from 1000 mg L^{-1} stock solutions of Cr (Fluka, Buchs St. Gallen, Switzerland) and 1000 mg L^{-1} Cr(VI) prepared from potassium dichromate (Synth, Diadema, SP, Brazil) after successive dilutions with water. Standard solutions of 15 to 250 μg L^{-1} and 20 to 1000 μg L^{-1} Cr(VI) for UV–Vis and FAAS respectively were prepared. A volume of 10 mL of 7% (m v^{-1}) NaCl solution, adjust pH to 1.5 ± 0.5 with HNO_3 solution, was used in the CPE method.

2.3. Samples

Six samples of organic fertilizers (OF) were analyzed, two of them

Table 1
Instrumental parameters applied in Cr speciation by LC-ICP-MS.

Instrumental parameters	Operational conditions
Plasma power (W)	1550
Sampling depth (mm)	8
Carrier gas (L min^{-1})	1.1
Makeup gas (L min^{-1})	0
Option gas (L min^{-1})	0
Collision and reaction cell He gas flow (mL min^{-1})	4.5
Nebulizer	Micromist
Spray chamber	Scott type, double-pass
Isotope	^{52}Cr, ^{59}Cr
LC conditions	
Mobile phase	25 mmol ammonium sulfate and 1 mmol sodium hydroxide, pH 8.0 ± 0.2
Flow rate (mL min^{-1})	0.8
Injection volume (μL)	50
Acquisition parameters	
Acquisition	Time-Resolved Analysis (TRA)
Dwell time (s)	1.0 for $m/z = 52$
Run Time (s)	300

from tannery, were supplied by the Federal Agricultural Laboratories (LFDA Brazilian official laboratories), (OF1 and OF2). Three samples provided by the Brazilian Association of Inputs for Sustainable Agriculture (INPAS, Brazilian association), identified as batch substrate 270913, and batch soil conditioners PT300712 and 2505 (OF3, OF4, and OF5). Other evaluated sample was a sewage sludge from a water and sewage treatment plant (OF6).

Certified Reference Materials, Hexavalent chromium in contaminated soil (National Institute for Standard and Technology - NIST 2701) and Trace Elements in Multi-Nutrient Fertilizer (NIST 695) and analysis of spiked samples were used to evaluate the accuracy of the proposed method.

2.4. Sample preparation

2.4.1. Digestion method for the total Cr determination

For sample digestion, 250 mg of organic fertilizer was weighed directly into PFA microwave digestion flasks, in triplicate. Then, 6.0 mL of HNO_3 (7.0 mol L^{-1}) and 2.0 mL of H_2O_2 (30% v v^{-1}) were added. The heating program was (1) 15 min at 120 °C, (2) 20 min until reaching 220 °C, and (3) 20 min at 220 °C. After digestion, the solutions were diluted to 50 mL with water.

2.4.2. Cr(VI) alkaline extraction

The Cr(VI) alkaline extraction was based on the USEPA 3060A [6], with modification. Approximately 200 mg of sample was weighed into 25 mL conical flasks. Then, 10 mL of the alkaline extractor solution (0.5 mol L^{-1} NaOH + 0.28 mol L^{-1} Na_2CO_3) pH ≥ 11.5, 100 mg $MgCl_2 \cdot 6H_2O$, and 250 μL of 0.5 mol L^{-1} phosphate buffer at pH 7 were added. The mixture was stirred for at least 60 min at 90–95 °C. After cooling, the extract was transferred to a 50 mL conical flask and centrifuged for 5 min at 3000 rpm.

2.4.3. Cloud point extraction method

The supernatant of the extract of item 2.4.2 was transferred to another 50 mL conical flask containing 1.0 g NaCl. The pH was adjusted to 1.5 ± 0.5 with 5.0 mol L^{-1} HNO_3, the volume adjusted to 50 mL, and the solution was centrifuged for 5 min at 3000 rpm. About 10 mL of the extract was transferred into a 15 mL conical flask, and then 500 μL of DFC (1% m v^{-1}) and 750 μL Triton X-100 surfactant (4% v v^{-1}) were added. The solution was heated in a thermostatic bath at 55–60 °C for 15 min and then centrifuged for 5 min at 3000 rpm. The supernatant was discarded, and the surfactant rich phase was diluted with 200 μL ethanol.

2.4.4. LC-ICP-MS analysis

A comparative study was carried out between the developed procedure, based on EPA 3050A, and the chromatographic method ISO 17075-2:2017 (IULTCS/IUC 18-2:2017), developed for chemical determination of Cr(VI) content in leather [17]. This comparison was made to check the possibility of interconversion of Cr species. Basically, in 17075-2:2017 Cr(VI) is extracted in phosphate buffer pH 7.0–8.0 at room temperature. In the present proposal, Cr(VI) is extracted in the alkaline medium (pH ≥ 11.5) at 90–95 °C, and afterward, the pH is also adjusted to 7.0–8.0 to preserve de chromatographic column. Aliquots of the extracted were filtrate and analyzed by LC-ICP-MS.

3. Results and discussion

3.1. Method optimization

3.1.1. Alkaline extraction method

The United States Environmental Protection Agency (USEPA) describes the 3060A procedure for Cr(VI) extraction in sediment, soil, sludge, and similar materials, such as organic fertilizers [6]. However, the application of this procedure without appropriate adjustments does not provide satisfactory data for the determination of this analyte in organic fertilizers due to humic compounds. As an alternative to overcome this limitation, the main parameters involved in Cr(VI) extraction in the method 3060A were optimized.

In the speciation analyses, the oxi/reduction among the species needs to be avoided. The amount of Cr(VI) extracted would be expected to be proportional to the mass of the sample. However, this ratio was not observed for organic fertilizer samples, as shown in Fig. 1. The suppression of the Cr(VI) signal occurs with the increase of sample mass. This fact is associated with the presence of humic substances, which are concomitantly extracted, hence reducing Cr(VI) to Cr(III). Signal drifts of 66% for sample OF1 and 100% for sample OF2 were observed comparing the obtained signals with 0.2 g and 1.0 g. These results emphasize the non-compliance of procedure 3060A for organic fertilizer, who recommend using 2.5 g of sample mass; thus, all Cr(VI) could be reduced during the extraction step. In this sense, as required to reduce the sample amount, the volume of the extraction solution (NaOH/Na_2CO_3) for Cr(VI) extraction was also considered. The volumes of 10, 20, and 50 mL were evaluated, and the obtained results were statistically comparable. Based on these results, a volume of 10 mL of extraction solution and 0.2 g of organic fertilizer was established for further studies.

Extraction temperature was studied in a range of 60 to 90 °C to evaluate the possibility of Cr(III) oxidation. Amounts of 500 μg L^{-1} of Cr (VI) were added in samples of organic fertilizer (OF1) and the CRM (hexavalent chromium in contaminated soil, NIST 2701). The obtained recoveries showed that temperature is not a critical factor in the oxi/reduction process, as shown in Fig. 2.

3.1.2. Cloud point extraction method

The UV–Vis determination of Cr(VI) with DFC is sensitive, simple, and easy to implement [7]. The procedure is based on the reaction between Cr(VI) and DFC, producing a complex with intense violet coloration in an acidic medium. The reaction is represented by the Eq. (1):

$$2CrO_4^{-2} + 3C_{13}H_{14}N_4O + 8H^+ \ [Cr(C_{13}H_{12}N_4O)_2]^+ + C_{13}H_{12}N_4O + 8H_2O + Cr(III) \quad (1)$$

CPE was used to improve the detection limits of the procedure, aiming to support the EU and Brazilian legislation, which determine 2 mg kg^{-1} Cr(VI) as the maximum allowed [5]. In this circumstance, the CPE procedure developed for the Cr(VI) determination in water was adapted to the investigated matrix (Sussuline and Arruda, 2006) [18].

The proposed procedure was responsible for developing a rich phase formed in the upper part of the system, indicating the possible occurrence of a change in the micelle structure. An experiment was conducted

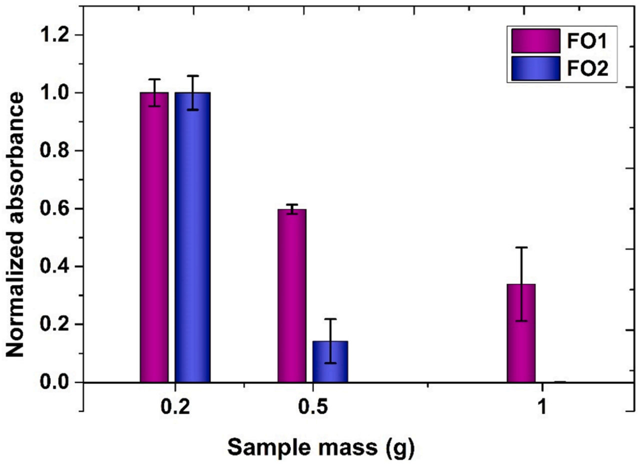

Fig 1. Sample mass evaluation of Cr(VI) extraction from tannery residue (OF1 and OF2) organic fertilizers. Spectrophotometric determination (n = 3).

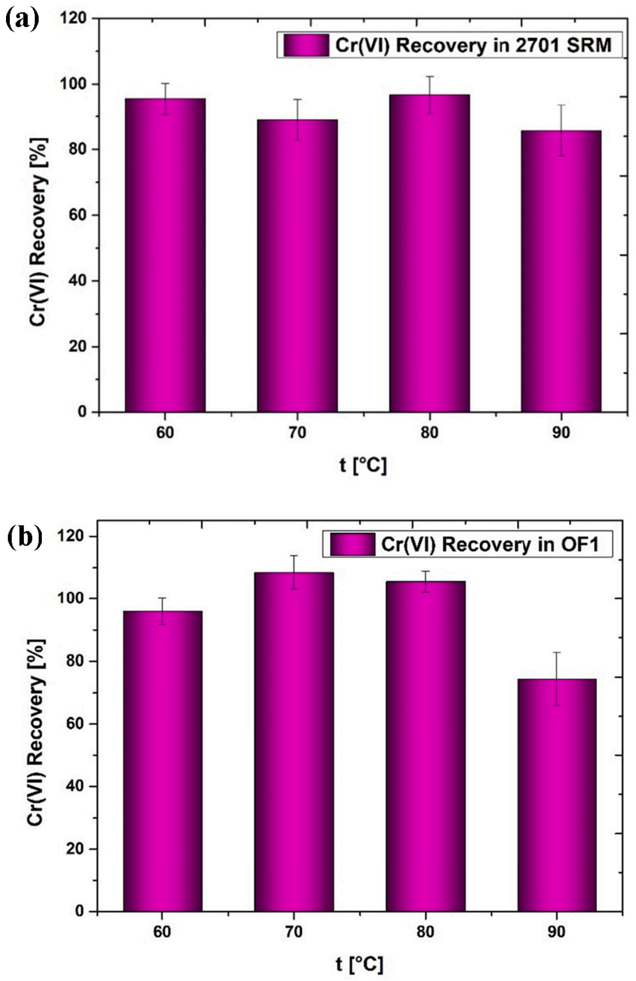

Fig 2. Temperature dependence of Cr(VI) from (a) CRM 2701 and (b) organic fertilizer (n = 3).

to identify the cause of micelle destabilization and establish the best condition for Cr (VI) extraction in organic fertilizer. It involved the possible destabilization factors such as NaCl concentration and temperature, which directly influence micelle formation. In this study, a Doehlert's experimental design was applied, and the results are shown in Fig. 3. At concentrations above 8% (m v^{-1}) NaCl, there is a drastic suppression of the analytical signal, evidenced by the alteration of micelles. On the other hand, in the studied range, the temperature did not interfere in forming the micelles. This method adjustment was indispensable due to the differences between the matrices. During the extraction phase in fertilizer samples, a large amount of electrolytes is present. The addition of 2% (m v^{-1}) NaCl in the samples proved to be enough to stabilize the micelles in the temperature range of 55–60 °C.

It is also essential to highlight the dark color of the extracts provided by the humic compounds present in the organic fertilizer samples. In acidic medium, the heaviest organic acids are precipitated and can be centrifuged to be separated. The final solution is a pale yellow, characteristic of the lighter fraction, such as fulvic acids. The addition of DFC in this medium is responsible for an intense violet color, even with a low amount of Cr(VI). Although fulvic acid absorbs radiation at 545 nm, Cr (VI) can be detected in the medium. The CPE procedure has to be applied in the presence and absence of the DFC complexant to avoid the sample original color interference. The difference between absorbances is relative to the Cr(VI) present in the sample. By using this strategy, it is possible to obtain results without interference during the determination of Cr(VI) in organic fertilizer by UV–Vis. On the other side, when FAAS is used as a detection technique, this correction is not required.

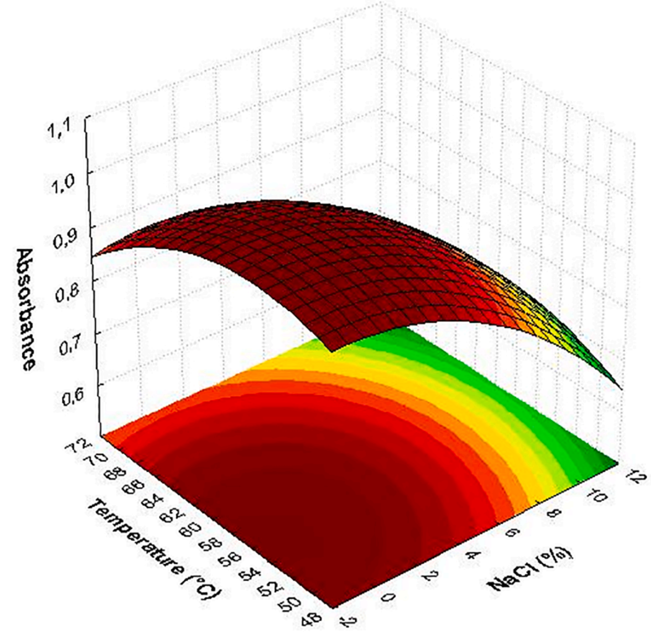

Fig 3. Chromium extract from organic fertilizer (OF6) sample with the ISO 1701-2 and alkaline extraction, and 25 μg L^{-1} Cr(VI) standard by LC-ICP-MS.

3.1.3. *Analytical parameters*

The analytical parameters were evaluated with the optimized conditions according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations [19]. The detection limit was defined as LOD = 3 s/S, where s is the standard deviation obtained by ten analytical blank signals, and S is the slope of the calibration curve. The limit of quantification (LOQ) was calculated as 10 s/S. The enrichment factor was defined as the ratio of the slope of the calibration curve for the CPE method to that of the calibration curve without preconcentration. The precision of the procedure was estimated by the relative standard deviation of 10 measurements at the smallest and highest concentration of the calibration curve, and these values were less than 7.0%. The obtained values for the parameters are summarized in Table 2.

3.1.4. *Interference and matrix effect*

Several ions are described in the literature as potentially interfering in Cr(VI) determination by DFC procedure. In this study, different concentrations of potentially interfering ions were added to solutions containing 100 μg L^{-1} of Cr(VI) and then were submitted to the CPE method. The recovery results for Cr(VI) in the presence of the evaluated ions are shown in Table 3. Other ions as alkali metals and alkaline earth metals and anions were previously proved not to interfere [15,17]. Furthermore, ions as Na(I), Mg(II), and phosphate were used during the extraction step. The obtained results indicate that the procedure can be applied to determine Cr(VI) in organic fertilizer samples.

The determinations were alternatively performed by FAAS. In this

Table 2
Analytical parameters developed for the CPE-Cr(VI) method.

Parameters	UV–Vis	FAAS
Calibration curve	Absorbance = 0.0041 $_{\pm 0.0006}$ [μg/L] −0.019 $_{\pm 0.102}$	Absorbance = 0.0010 $_{\pm 0.0001}$ [μg/L] + 0.059 $_{\pm 0.0002}$
LOD (μg/g)	0.41	0.55
LOQ (μg/g)	1.38	1.82
Work range	1.50–250 μg/L	1.85–1000 μg/L
Enrichment factor	5	10
Precision	5.5%	7.0%

Table 3
Interference studies of ions in the determination of Cr(VI) by UV–Vis and FAAS.

Ions	Rate	% Recovery (UV–Vis)	% Recovery (FAAS)
Hg (II)	1:100	102	115
Mo(V)	1:100	99	91
Fe(III)	1:100	92	97
V (V)	1:100	97	84
Ni(II), Cd(II), Pb(II), Mn(VII), Co(II)	1:50	99	99

procedure, the matrix effect was evaluated by calibration curves for Cr (VI) by external calibration with 2%, 5%, 7%, and 10% NaCl (m v^{-1}), and matrix matching calibration by using prepared extracts from fertilizer samples. Both procedures underwent previously described CPE, with concentrations varying from 20 to 500 μg L^{-1} Cr(VI). No significant differences were found between slopes of the external calibration curve with 7% (m v^{-1}) NaCl and the matrix matching curve, which was considered similar and had no critical matrix effect. Therefore, Cr(VI) determinations can be performed by external calibration.

3.1.5. *Method validation*

The accuracy of the CPE method for the Cr(VI) determination was evaluated with CRMs hexavalent chromium in contaminated soil (NIST 2701) and Trace Elements in Multi-Nutrient Fertilizer (NIST 695), and analysis of spiked samples.

Student's *t*-test was applied to compare the obtained and certified values. There was no significant difference since the calculated *t* values 3.94, 0.45, and 0.66 obtained from CPE-FAAS, CPE-UV–VIS, and FAAS were lower than the critical t value (4.3) in the analysis of Cr(VI) and total Cr. Recoveries from 94 to 101% were obtained for total Cr and Cr (VI) by FAAS and UV–Vis in the CRMs, as can be observed in Table 4. In the analysis of spiked samples, 500 μg L^{-1} Cr(VI) was added to the organic fertilizer samples, and the alkaline extraction procedure was employed. Recovery values from 82 to 90% were obtained for OF1, OF2, OF4, and OF5. Otherwise, for OF3 and OF6 samples, quantitative recoveries were not obtained, probably because of the too high content of humic compounds in these samples, which reduced the Cr(VI), even using a low amount of sample. Similar results were observed by Krüger and collaborators when analyzing organic fertilizers from sewage sludge ash [20].

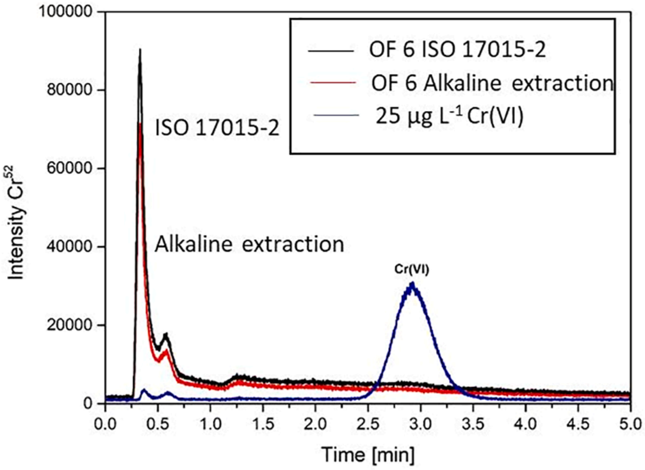

Fig 4. Doehlert experimental design response surface for temperature versus NaCl (% m v^{-1}) in CPE method optimization.

3.1.6. *LC-ICP-MS comparative procedure*

The comparison between the developed procedure, with initial extraction at pH ≥ 11.5 at 90–95 °C and the chromatographic method ISO 17075-2:2017 [17], extraction at pH 7.0–8.0 and room temperature is presented in Fig. 4. It is possible to observe the no occurrence of oxi/reduction during the extraction procedures.

The uncertainties of measurements of Cr(VI) content in CRM (NIST 2701) by using the developed procedure and the chromatographic method ISO 17075-2:2017[17] were evaluated following the Guide to the Expression of Uncertainty in Measurement (2008), NIST Technical Note 1297 [21], and similarly to the scheme presented by Leśniewska et al. (2016) [22]. The parameters included in the model equation were identified as components significantly contributing to the measurement results. Based on their own standard uncertainties and the law of propagation of uncertainty, the combined standard uncertainty of analyte content in the fraction of the CRM, u($C_{Cr(VI)}$), was evaluated according to the Eq. (2):

$$u_c\left(C_{Cr(VI)}\right)/C_{Cr(VI)} = \sqrt{\left(u(m_s)/m_s\right)^2 + \left(u(V_e)/V_e\right)^2 + \left(u(cal)\right)^2 + \left(u(R)/R\right)^2 + \left(u(f)/f\right)^2 + u\left(repeat_{ext.}\right)^2} \tag{2}$$

Where u(ms), u(Ve), u(cal), u(R), u(f), and u(repeat$_{ext.}$) denote standard uncertainties of mass of sample, volume of extract, calibration, recovery, dilution factor and repeatability of the extraction process, respectively.

The obtained expanded uncertainty (U) of measurements of Cr(VI) content in CRM (NIST 2701, reference value of 551.2 ± 34.5 μg g^{-1} Cr (VI)), was 535.7 ± 40.3 μg g^{-1} Cr(VI); U = 8.5% (k = 2) in the solution extract with ISO 17075–2:2017 (pH 7.0–8.0, at room temperature). The U obtained with developed procedure (pH ≥ 11.5, 90 95 °C) was 540.2

Table 4
Evaluation of the accuracy of CPE-Cr(VI) method by UV–Vis and FAAS in CRM (NIST 2701 and NIST 695) by FAAS, results expressed in μg g^{-1} (mean ± standard deviation, n = 3) and the recoveries (%).

Sample	Certified value mg kg^{-1}		Found value mg kg^{-1}		
	Total Cr	Cr(VI)	Total Cr^{a}	Cr(VI)^{b}	Cr(VI)^{c}
CRM 2701	–	551.2 ± 34.5	–	531 ± 7 (96)	559 ± 31 (101)
CRM 695	244 ± 6	–	230 ± 13 (94)	–	–

Determination by: ^{a}FAAS; ^{b}CPE-FAAS; ^{c}CPE-UV–VIS.

Table 5
Application of the procedure to determine total Cr and Cr(VI) in organic fertilizer samples by developed separation and preconcentration method (mean ± standard deviation, n = 3).

Sample	Total Cr^{a} mg kg^{-1}	Cr(VI)^{b} μg g^{-1}	Cr(VI)^{c} μg g^{-1}
OF1	20146 ± 1275	346 ± 35	403 ± 39
OF2	28928 ± 799	1001 ± 69	940 ± 77
OF3	379 ± 11	<1.82	<1.38
OF4	73 ± 2	<1.82	<1.38
OF5	42 ± 2	<1.82	<1.38
OF6	162 ± 2	<1.82	<1.38

Determination by: ^{a}FAAS; ^{b}CPE-FAAS; ^{c}CPE-UV–VIS.

± 33.6 µg g^{-1} Cr(VI); U = 7.6% (k = 2). These results not present statistic difference (Tukey, $P > 0.05$).

3.1.7. Cr(VI) in organic fertilizers

The obtained mass fractions of total Cr and Cr(VI) in organic fertilizers are presented in Table 5.

In the OF1 and OF2 samples, the amount of Cr(VI) was higher than the maximum allowed, 2 µg g^{-1} Cr(VI), indicating that these materials cannot be used as organic fertilizer. As the obtained values for these samples are above 19 µg g^{-1}, the FAAS LOQ, we performed direct determination in the alkaline extract without the preconcentration step. The results were similar to the obtained with the CPE method. In the other evaluated organic samples, Cr(VI) mass fraction was below the LOD of the developed method.

4. Conclusion

A procedure based on alkaline extraction followed by CPE to determine Cr(VI) in organic was optimized by a Doehlert experimental design. With the defined extraction conditions, the limitations related to the presence of humic compounds for most of the samples were overcome, providing precise and accurate results. An LC-ICP-MS comparative study with different extractions procedures was carried out to evaluate the possibility of interconversion of Cr species. No significant difference was observed, confirming that the procedure is suitable for the determination of Cr (VI) in organic fertilizers. Minimization of reagents, excellent extraction efficiency, and low-cost analysis instruments were the main characteristics of the procedure. It can be performed by UV–Vis spectrophotometry or by FAAS. Moreover, the developed analytical suitable to support the Brazilian and European legislation. Some kinds of organic compounds still showed interference due to the high concentration of humic compounds. This troublesome implies that procedures with better extraction efficiency need to be developed. Hence smaller amounts of samples can be analyzed, presenting less interference without losing adequate sensitivity for Cr(VI) determination.

CRediT authorship contribution statement

Ivero Pita de Sá: Investigation, Methodology, Writing - original draft. **Gilberto Batista de Souza:** Validation, Visualization. **Ana Rita de Araujo Nogueira:** Conceptualization, Supervision, Investigation, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the Research Foundation of São Paulo (FAPESP, grants 2015/14488-0 and 2018/26145-9), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 308178/2018-1) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES – Finance Code 001). This is a contribution of the National Institute of Advanced Analytical Science and Technology (INCTAA).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2020.105618.

References

[1] R.P. Singh, Organic Fertilizers: Types, Production and Environmental Impact, Nova Science Publishers, New York, NY, 2012.

[2] K. Moller, U. Schulteiß, Chemical characterization of commercial organic fertilizers, Arch. Agron. Soil Sci. 61 (2014) 989–1012, https://doi.org/10.1080/03650340.2014.978763.

[3] C. Ciavatta, C. Manoli, L. Cavani, C. Franceschi, P. Sequi, Chromium-containing organic fertilizers from tanned hides and skins: a review on chemical, environmental, agronomical and legislative aspects, J Environ Prot Ecol 3 (2012) 1532–1541, https://doi.org/10.4236/jep.2012.311169.

[4] Regulation (EU) 2019/1009 of the European Parliament and of the Council of June 5 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (Text with EEA relevance). https://eur-lex.europa.eu/eli/reg/2019/1009/oj. [accessed on October 30, 2019].

[5] Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. IN n. 7 de 12 de abril de 2016. Diário Oficial da União. Brasil.

[6] Official Methods of Analysis. United States Environmental Protection Agency, Method 3060A, in: Alkaline Digestion for Hexavalent Chromium. 199https://www.epa.gov/sites/production/files/2015-12/documents/3060a.pdf [accessed on October 3, 2020].

[7] Official Methods of Analysis (1992) United States Environmental Protection Agency, Method 7196A, in: Chromium Hexavalent (Colorimetric). https://www.epa.gov/sites/production/files/2015-12/documents/7196a.pdf [accessed on October 3, 2020].

[8] R.J. Vitale, G.R. Mussoline, K.A. Rinehimer, Environmental Monitoring of Chromium in Air, Soil, and Water, Regul. Toxicol. Pharmacol. 26 (1997) 80–85.

[9] A.D. Dayan, A.J. Paine, Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000, Hum. Exp. Toxicol. 20 (2001) (1985) 439–451, https://doi.org/10.1191/096032701682693062.

[10] M. Sager, About Chromium (VI) Extraction from Fertilizers and Soils, Econ. Environ. Geol. 38 (2005) 657–662.

[11] J. Zhang, L. Chen, H. Yin, S. Jin, F. Liu, H. Chen, Mechanism study of humic acid functional groups for Cr(VI) retention: Two-dimensional FTIR and 1^{3C} CP/MAS NMR correlation spectroscopic analysis, Environ. Pollut. 225 (2017) 86–92, https://doi.org/10.1016/j.envpol.2017.03.047.

[12] W.I. Mortada, Recent developments and applications of cloud point extraction: A critical review, Microchem. J. 157 (2020), https://doi.org/10.1016/j.microc.2020.105055, 105055.

[13] Y.G.A. El-Reash, H.A. Tantawy, E. Abdel-Latif, W.I. Mortada, Micelle-mediated extraction for speciation and spectrophotometric determination of iron using a newly synthesized salicylic acid derivative, Microchem. J. 158 (2020), 105280, https://doi.org/10.1016/j.microc.2020.105280.

[14] V.N. Bulut, D. Ozdes, C. Bekircan, A. Gundogdu, C. Duran, M. Soylak, Carrier element-free coprecipitation (CEFC) method for the separation, preconcentration and speciation of chromium using an isatin derivative, Anal. Chim. Acta 632 (2009) 35–41, https://doi.org/10.1016/j.aca.2008.10073.

[15] N. Ozkantar, M. Soylak, M. Tuzen, Ultrasonic-assisted supramolecular solvent liquid-liquid microextraction for inorganic chromium speciation in water samples and determination by UV-vis spectrophotometry, At. Spectrosc. 41 (1) (2020) 43–50.

[16] M.A. Bezerra, M.A.Z. Arruda, S.L.C. Ferreira, Cloud point extraction as a procedure of separation and pre-concentration for metal determination using spectroanalytical techniques: a review, Appl. Spectrosc. Rev. 40 (2005) 269–299, https://doi.org/10.1080/05704920500230880.

[17] JCGM 100:2008; Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf [accessed on September 7, 2020].

[18] A. Sussulini, M. A. Z. Arruda. Determinação de cromo(VI) por espectrometria de absorção atômica com chama após a extração e pré-concentração no ponto nuvem. Eclet. Quím. 31 (2006) 73-80. https://www.scielo.br/pdf/eq/v31n1/29332.pdf [accessed on September 7, 2020].

[19] M. Thompson, S.L.R. Ellison, R. Wood, Harmonized guidelines for singlelaboratory validation of methods of analysis, Pure Appl. Chem. 74 (2002) 835–855, https://doi.org/10.1351/pac200274050835.

[20] O. Krüger, F. Fiedler, C. Adam, C. Vogel, R. Senz, Determination of chromium (VI) in primary and secondary fertilizer and their respective precursors, Chemosphere. 182 (2017) 48–53, https://doi.org/10.1016/j.chemosphere.2017.05.011.

[21] B.N. Taylor, C.E. Kuyatt, Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); https://www.nist.gov/pml/nist-technical-note-1297. [accessed on September 7, 2020].

[22] B. Leśniewska, M. Krymska, E. Świerad, J. Wiater, B. Godlewska-Żyłkiewicz, An ultrasound-assisted procedure for fast screening of mobile fractions of Cd, Pb and Ni in soil. Insight into method optimization and validation, Environ Sci Pollut Res. 23 (2016) 25093–25104, https://doi.org/10.1007/s11356-016-7745-3.