


# Matrix Effect Assessment of an Ion Chromatographic Method to Determine Inorganic Anions in Wastewater

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**Abstract** Inorganic anion monitoring is essential for bioreactor operation and is related for pollution control or energy and products recovery. However, there is a lack of studies validating methods for inorganic anions analyses in conditions compatible to those in bioreactor operations treating different types of wastewater. This paper provides a systematic statistical study and matrix-effect assessment for sugarcane vinasse, leachate, sewage and synthetic sewage. Sample preparation consisted of only a filtration and sample dilution.  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  were determined in a Dionex ICS 5000® equipped with a chemical conductivity suppressor. Calibration curves were linear and well-adjusted between 2.5 and 50  $\text{mg L}^{-1}$  for all the anions in all the tested matrices, except  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  in vinasse. A calibration range for  $\text{PO}_4^{3-}$  in all tested matrices was 5.0 to

100  $\text{mg L}^{-1}$ , whereas a range from 5.0  $\text{mg L}^{-1}$  to 50  $\text{mg L}^{-1}$  was obtained for  $\text{SO}_4^{2-}$  in vinasse. All the anions yielded recoveries in the range of 85–115% for all the tested matrices. Relative standard deviations lower than 10 and 2% were achieved for peak areas and retention times, respectively. A signal enhancement was observed for all the tested matrices and all the anions. The matrix effect level varied from  $-1.7$  ( $\text{NO}_2^-$  in vinasse) to  $-33.9\%$  ( $\text{Cl}^-$  in leachate). Sewage was the less affected matrix, while leachate gave higher matrix effects. Validation results and the matrix effect assessment showed that a simple sample preparation is suitable for multi-elemental analyses of inorganic anions for complex environmental samples.

**Keywords** Inorganic anion determination · Complex matrices · Standard addition method · Environmental chemistry

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## 1 Introduction

Ion chromatography (IC) is a well-established method to analyse inorganic anions in water samples (Jackson 2006). By and large, IC is traditionally used for monitoring inorganic anion concentrations in water in order to ensure that environment samples present the quality standards required by legislation. The Environment Protection Agency (EPA) validated an IC method to analyse various anions in drinking water, surface water, groundwater, mixed domestic and industrial wastewaters (USEPA 1993). Since then, many papers have been

published validating IC methodologies for different environmental samples, such as drinking water (Jackson et al. 1998; Miskaki et al. 2007; Lopez-Moreno et al. 2010), well-water (Lopez-Moreno et al. 2010), brines (Singh et al. 1996), high ionic water sample (Neele et al. 2002), coal mine and river water sample (Michalski et al. 2012).

A great deal of effort has gone into validating IC methods for inorganic anions in water, but there are few studies addressing different types of wastewater (Gade 1993; Jackson et al. 2001), and even in the EPA document, no further specification was presented regarding wastewater samples. In an extensive review, Michalski (2006) listed 67 studies in which IC was applied to anion determination in aqueous samples. Among these, only eight studies addressed IC use for wastewater characterisation, which is nearly 12% of the listed studies. Thus, IC is used for wastewater monitoring, but there is a lack of studies validating these methods for specific matrices. Validation and matrix-effect assessment studies are mandatory for wastewater applications, since these streams are complex regarding their chemical compositions.

Wastewaters can be classified as complex matrices, which are characterised by a high ionic strength and/or large disparities between analyte ion concentrations and other chemical species in the same sample (Paull and Nesterenko 2005). Due to its complexity, the matrix effect is likely to occur when analytical methods are used for wastewater. The matrix effect is broadly defined as the interference that other components other than the target analyte in the method response (Kruve et al. 2015). It is usually caused by compound co-elution with the analyte, which can either reduce or increase the response (signal suppression or signal enhancement, respectively) (Matuszewski et al. 2003; Kruve et al. 2015). There is a claim that IC is highly matrix independent (Neele et al. 2002), but the matrix effect in anion analyses using IC techniques was extensively investigated for cleaner matrices, such as drinking water and groundwater (Miskaki et al. 2007; Lopez-Moreno et al. 2010). Yet, to date, there are no studies addressing the matrix-effect in anion analyses using IC techniques adopted for wastewater samples.

Furthermore, validating IC methods used for wastewater needs to be reviewed to ensure that the method is still appropriate. On one hand, IC technology is being constantly modernised, with new generation suppressors, as well as high-selectivity and high-capacity

columns (Michalski 2006). On the other hand, IC methods are usually used for wastewater characterisation or for environmental control purposes, and law requirements tend to become stricter. Additionally, other IC applications for monitoring anions have emerged from new technologies and prospects for wastewater treatment. Among these, the biorefinery approach is gaining attention. According to this proposal, wastewater treatment plants can be considered as an industry, which is able to transform sustainable organic waste management into a spectrum of marketable products (Poggi-Varaldo et al. 2014). Given this context, monitoring anions is an important parameter for operating biological reactors treating wastewater, as some anions in excess, such as nitrite, can be toxic to microorganisms or can interfere in the target processes (Philips et al. 2002). High concentrations of sulphate, for example, could impair energy generation (methanogenesis) when sugarcane vinasse is used as feedstock (Kiyuna et al. 2017). Another approach of applying IC to wastewater is related to the possibility of nutrient recovery, such as phosphate (García et al. 2017) and elemental sulphur (Camiloti et al. 2016). Hence, it is critical to validate the IC method to analyse inorganic anions in conditions compatible to those in biological reactor operations.

Besides the compatibility of using the method with the samples conditions, it is highly desirable for analytical methods to be carried out quickly, adopting simple and automated sample pre-treatment procedures. These characteristics aim to save operator labour and time, and also minimise reagents and material wasted (Lopez-Moreno et al. 2010). Moreover, in the case of complex matrices, it is desirable for the accuracy of the method to be improved, taking into account matrix effects, widely recognised as an important source of error when analysing environmental samples. One of the advantages of using IC for inorganic anion determination is that it provides a simultaneous multi-elemental analysis, minimising the time required for the measurements (Michalski 2006). Nevertheless, environmental samples are usually considered too difficult to deal with, requiring complex pre-treatment steps (Jackson 2006). Technological advances in IC equipment, such as pre-columns (Michalski 2006), tend to minimise sample pre-treatment requirements and increase method accuracy. In this paper, sample preparation consisted of pre-filtration and dilution.

So far, IC application, method validation, and matrix-effect assessment for complex environmental matrices,

such as sugarcane vinasse, leachate, domestic and synthetic sewage, have not yet been examined in depth. In this paper, we studied the analyses of chloride, nitrite, nitrate, phosphate and sulphate in domestic wastewater, synthetic sewage (common substrates used for research purposes), sugarcane vinasse and leachate in an IC system equipped with a last generation chemical conductivity suppressor. Therefore, the main contribution of this paper is the systematic statistical study and matrix-effect assessment carried out for five inorganic anions in different wastewaters matrices submitted to simple pre-treatment.

## 2 Material and Methods

### 2.1 Instruments

A Dionex ICS 5000® (California, USA) system consisting of an AG23 guard column ( $4 \times 50$  mm), an AS 23 ( $4 \times 250$  mm) analytical column, a self-regeneration chemical suppression system, and a conductivity detector was used. The chemical suppression device was AERS 500 4 mm, and its current was set at 25 mA, while the conductivity detector was a CD20. The column temperature was set at 35 °C, and the conductivity detector was 30 °C.

Injection was made through an AS autosampler device, and the injection loop volume was set at 10  $\mu$ L. All parts of the IC, such as the injection valve, high-pressure pump, conductivity detector, suppressor module, peristaltic pump and autosampler were fully controlled by the *Chromeleon Chromatography Management 7.1* program.

Isocratic elution (at a flow rate of 1.0 mL min<sup>-1</sup>) was used, and an analytical grade (> 99%) sodium carbonate (Aldrich) and sodium hydrogen carbonate (Sigma-Aldrich—Steinheim, Germany) were diluted in ultrapurified water to prepare the eluent at a concentration of 4.5 and 0.8 mM, respectively.

### 2.2 Chemicals

All the chemicals included in this study were purchased at an analytical grade (purity > 99%). Sodium chloride (NaCl) and sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) were acquired from Sigma-Aldrich (Steinheim, Germany), while sodium nitrite (NaNO<sub>2</sub>), sodium nitrate (NaNO<sub>3</sub>) and potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>) were acquired from

Mallinckodt Backer (New Jersey, USA). The water used was ultrapurified with a Milli-Q water purification system from Millipore (Bedford, MA, USA), which conductivity was 0.6  $\mu$ S.

### 2.3 Sample Preparation

Individual standard stock solutions (1000 mg L<sup>-1</sup>) were prepared in deionised water and stored at 4 °C in the dark for a maximum of 3 days. Calibration standard solutions were prepared both in water and in the matrix at seven concentration levels: 2.5, 5.0, 10.0, 20.0, 40.0 and 50.0 mg L<sup>-1</sup> (chloride, nitrite, nitrate and sulphate) and 5.0, 10.0, 20.0, 40.0 and 100.0 mg L<sup>-1</sup> (phosphate). Calibration standard solutions were prepared by independent dilution from the standard stock solution. Two types of control samples were analysed: Blank samples were constituted by ultra-purified water, while the “zero” concentration level refers to matrix samples in which no standard solution was spiked. Four matrices were tested as follows:

1. Synthetic complex wastewater that mimics sewage. This lab-made wastewater is often used for research purposes since it does not present composition variation. The sample was prepared as described by Camiloti et al. (2013).
2. Domestic sewage was collected at the entrance of the wastewater treatment plant from the neighbourhood near Campus 2 at the University of São Paulo (EESC/USP).
3. Sugarcane vinasse, which is a by-product of ethanol production, rich in carbohydrates (COD may be 100-fold higher than in sewage) and in sulphate (Fuess and Garcia 2015). Sugarcane vinasse sample was collected from a distillery in Brazil centre-south region (Sao Martinho distillery) during the 2015/2016 harvest. Sugarcane vinasse was kept refrigerated at  $4 \pm 2$  °C.
4. Solid waste leachate: the sample was collected from a deactivated landfill (21°57' S and 47°55' W) in São Carlos, São Paulo (Brazil), in March 2016. Sampling was made from the leachate lagoon.

The mentioned matrices are used in biological reactors for treatment and have high ionic strength (Table 1) that could influence anion determination by ion chromatography (Jackson et al. 2001; Jackson 2006). To evaluate the interferences of the analytes contained in

the four different matrices used in this study, calibration curves were built for each of them. Besides inorganic anion profile, chemical organic demand (COD) was also assessed for the matrices following the spectrophotometric method (APHA 2005). Electrical conductivity was determined with the aid of a Digimed DM-31 conductivity electrode. COD concentration and the electrical conductivity of each tested matrix are presented in Table 1.

Matrix samples were prepared by spiking standard solutions in the matrix. The standard calibration volume varied according to the concentration level, and the matrix volume spiked varied according to the dilution rate applied for each matrix.

Synthetic wastewater and sewage were diluted 10 times, while a 100-time dilution rate was applied to sugarcane vinasse and solid waste leachate. The unique sample preparation procedure consisted of filtering samples in a 1.2- $\mu\text{m}$  glass fibre membrane followed by a 0.22  $\mu\text{m}$  cellulose acetate membrane. To conduct the same procedure in all cases, a single Chromafil® GF/PET (Macherey-Nagel, GE) syringe filter was used for each sample and all the samples were filtered (including those in ultra-purified water) (Jackson 2006).

## 2.4 Validation Study

The performance characteristics studied were linearity, detection/quantification limits, recovery, precision

(repeatability) and the matrix effect. Resolution and asymmetry were also evaluated according to the European Pharmacopoeia (EP) calculations.

### 2.4.1 Detection and Quantification Limits

A visual method was used to assess the limit of detection (LOD). Different known concentrations were added to deionised water. The lower concentration, which gave a visually observed response, was considered the minimum level at which the analyte could be reliably detected (Thompson et al. 2002; Araujo 2009; Kruve et al. 2015). The tested concentrations were 0.2, 0.5, 1.0, 2.5 and 5.0  $\text{mg L}^{-1}$  for all the tested anions. Each concentration level was injected in triplicate.

The limit of quantification (LOQ) was defined as the lowest concentration measured with a confidence degree that was systematically measured. Relative standard deviation regarding a peak area lower or equal to 10% (Thompson et al. 2002; Lopez-Moreno et al. 2010) and 2%, regarding retention time, was adopted as the confidence degree (Villagrán et al. 2004).

### 2.4.2 Linearity

The linearity of the response was studied by using matrix-matched calibration solutions prepared by spiking standard stock extracts at seven concentration levels for all the five matrices. Concentration levels ranged

**Table 1** Physicochemical characterisation of the tested matrices

Parameters	Tested matrices			
	Synthetic wastewater	Sewage	Sugar cane vinasse	Solid waste leachate
<i>COD (mg O<sub>2</sub> L<sup>-1</sup>)</i>	<i>436.1 ± 32.3</i>	<i>564.3 ± 35.7</i>	<i>32,640.0 ± 255.0</i>	<i>1519.0 ± 76.0</i>
<i>Electrical conductivity (mS cm<sup>-1</sup>)</i>	<i>0.8</i>	<i>0.6</i>	<i>13.6</i>	<i>8.8</i>
pH	7.5–7.9	7.0–8.0	3.7–4.6	7.4–7.7
Chloride (mg L <sup>-1</sup> )	30–160	~0.0	59.4–1219.0	230.0–2305.0
Nitrite (mg L <sup>-1</sup> )	0.0	0.0	0.0	0.0
Nitrate (mg L <sup>-1</sup> )	4.2–4.6	0–2	0.0	22.4–35.1
Phosphate (mg L <sup>-1</sup> )	8.0–10.2	3.0–9.0		
Sulphate (mg L <sup>-1</sup> )	0.0		200–1400	7–42
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	592.0–620.0	100.0–200.0	0.0	3670.0–11,200.0
References	Chiu et al. (2007) Moura et al. (2012)	Tchobanoglous et al. (2003) Von Sperling (2007)	Moraes et al. (2015) Christofoletti et al. (2013) Fuess and Garcia (2015) Dos Santos et al. (2013)	Kjeldsen et al. (2002) Lacerda et al. (2014) Naveen et al. (2016)

COD and electrical conductivity were obtained by direct measurements (italic)

from 2.5 to 50 mg L<sup>-1</sup> for the anions, except phosphate, whose levels ranged from 5.0 to 100 mg L<sup>-1</sup>. Different concentration levels were injected randomly, but each matrix was injected into a separate batch. Calibration curves were prepared in triplicate, and matrix samples with no analyte addition were also analysed in triplicate. A blank (i.e., ultra-purified water) was injected at every 10 samples to ensure no occurrence of a carry-over effect.

Calibration curves were determined using the least squares method. The significance of linearity was assessed through the analysis of variance (test  $F$ ) and the lack of adjustment test. Calibration curves were considered linear when linearity was significant ( $F > F_{\text{critical}}$ ) and lack of adjustment was non-significant ( $F < F_{\text{critical}}$ ) for a significance level of 95% (Villagrán et al. 2004). The coefficient of determination ( $r^2$ ) was also calculated as the quotient  $SQ_{\text{reg}}/SQ_{\text{t}}$ .

#### 2.4.3 Recovery

Recovery studies were determined in all investigated matrices, i.e., synthetic sewage, domestic wastewater, vinasse and leachate. The samples were spiked with the analytes at three concentration levels—2.5, 20.0 and 50.0 mg L<sup>-1</sup>—and three replicates for the “blank” sample and spiked sample ( $n = 12$ ) for each matrix were performed.

The standard addition method was used for three matrices: domestic wastewater (chloride), sugarcane vinasse (chloride and sulphate) and solid waste leachate

(chloride and sulphate). The standard addition is used when the sample contains the analyte of interest (Miller and Miller 2010). The recovery was calculated by Eq. 1 and the recovery by standard addition was calculated by Eq. 2. An acceptable recovery should fall between 85 and 115% (USEPA 1993; Villagrán et al. 2004).

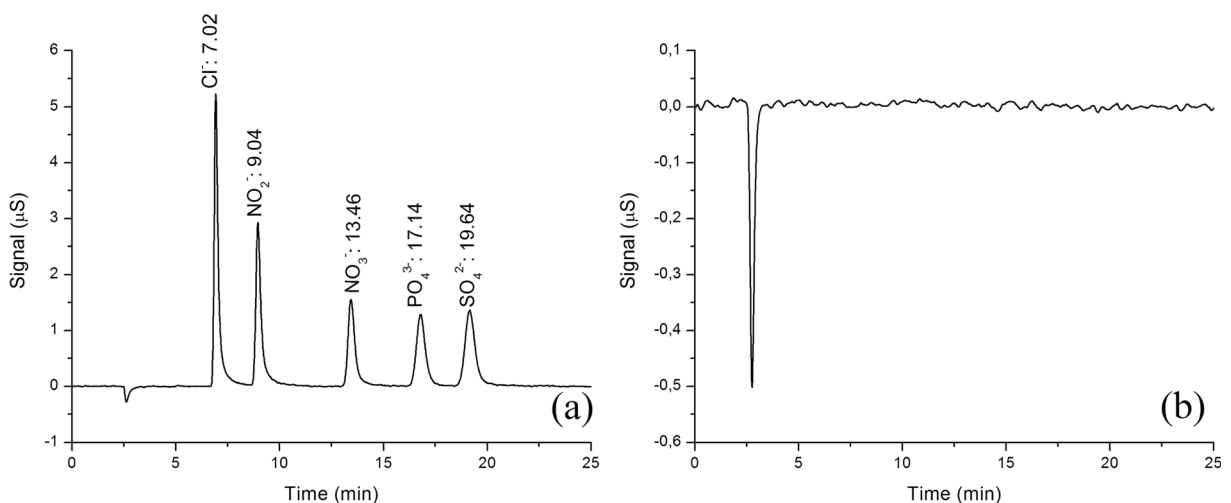
$$R(\%) = \frac{\text{measured concentration}}{\text{theoretical concentration}} \times 100 \quad (1)$$

$$R_{\text{sa}}(\%) = \frac{\text{measured concentration} - \text{blank}}{\text{added concentration}} \times 100 \quad (2)$$

#### 2.4.4 Precision and Sample Stability

Chromatographic method precision was determined as the relative standard deviation (RSD), obtained from injected triplicates of spiked matrices during the same day (repeatability). RSD was calculated for the peak area and retention time.

The samples were kept in the auto sampler and were injected at 2, 4, 6, 8, 12 and 24 h to evaluate the injector stability. Storage stability was studied at -20 °C. A sample in the medium level of the curve range was injected after being subjected to freezing and defreezing after 2, 4, 6, 8, 12 and 24 h. Injector and storage stabilities were assessed only for deionised water.



**Fig. 1** a Typical chromatogram for the target anions. b Typical chromatogram obtained for a blank sample

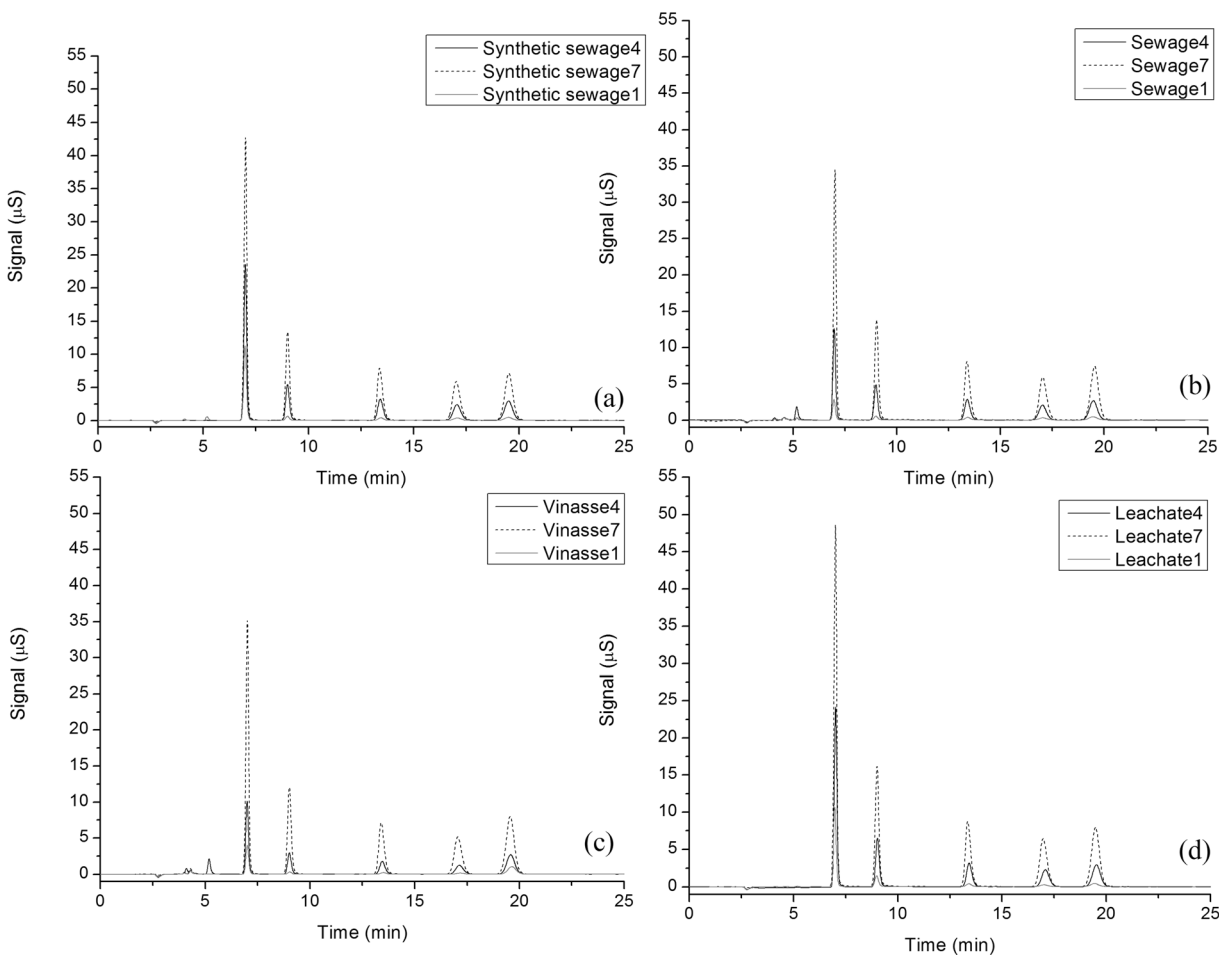
**Table 2** Retention times (min) of the target anions in the investigated matrices

	Chloride	Nitrite	Nitrate	Phosphate	Sulphate
Water	7.02 ± 0.01	9.04 ± 0.01	13.46 ± 0.02	17.14 ± 0.03	19.64 ± 0.04
Synthetic sewage	7.01 ± 0.01	9.01 ± 0.01	13.42 ± 0.03	17.06 ± 0.03	19.52 ± 0.02
Domestic wastewater	7.00 ± 0.02	9.00 ± 0.01	13.40 ± 0.02	17.03 ± 0.02	19.49 ± 0.03
Vinasse	7.01 ± 0.01	9.02 ± 0.01	13.45 ± 0.04	17.11 ± 0.05	19.59 ± 0.03
Leachate	7.00 ± 0.02	9.00 ± 0.01	13.39 ± 0.03	17.01 ± 0.04	19.47 ± 0.03

### 2.4.5 Matrix Effect

The matrix effect assessment was made by comparing the slopes of the analytical curves prepared in matrix and in ultra-purified water, represented by the percentage of signal enhancement or suppression

(C(%)). The matrix effect was considered low when between the range of  $-20\% < C(\%) < 20\%$ , medium for the range  $-50\% < C(\%) < -20\%$  or  $20\% > C(\%) > 50\%$  and high when lower than  $-50\%$  ( $C(\%) < -50$ ) or higher than 50 ( $C(\%) > 50$ ) (Economou et al. 2009).



**Fig. 2** Chromatograms obtained for **a** synthetic sewage, **b** sewage, **c** vinasse and **d** leachate. The chromatograms were obtained at low (1), medium (4) and high (7) level concentrations. Low,

medium and high range levels correspond to 5.0, 40.0 and 100.0  $\text{mg L}^{-1}$  for phosphate. For the other anions, range levels are 2.5 (low), 20 (medium) and 50 (high)  $\text{mg L}^{-1}$

### 3 Results and Discussion

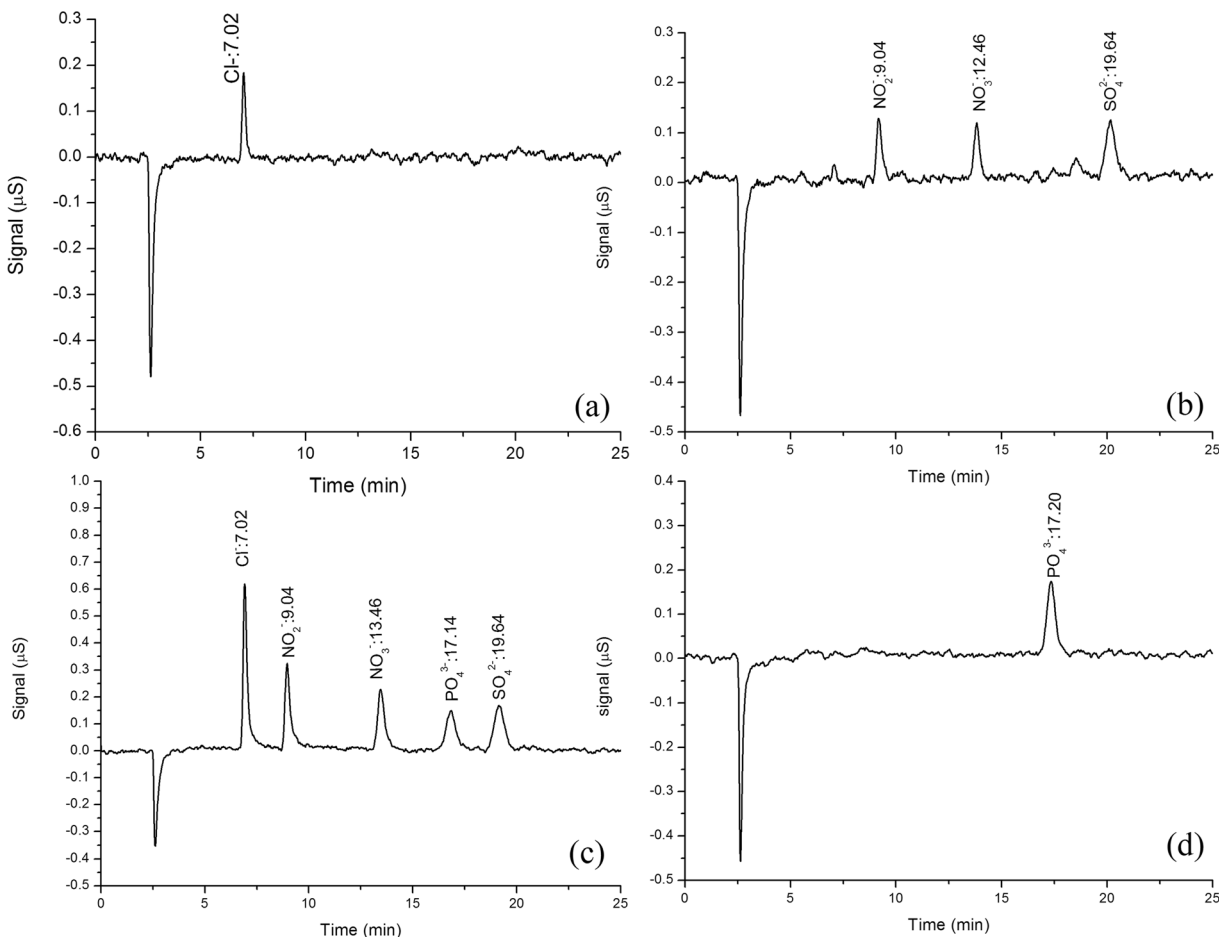
A typical chromatogram obtained in ultra-purified water is shown in Fig. 1. The retention times for the peaks of interest in all the five investigated matrices are shown in Table 2. Average resolution and asymmetry were 6.69 and 1.14 (EP), respectively. We did not observe distortion of the baseline in the concentration range tested. The chromatograms observed in high ionic matrices, fortified with higher anion concentration, were characterised by good resolution (Fig. 2). Neither resolution decline nor peaks broadening were observed in samples with high anion concentration. These effects were previously reported (Neele et al. 2002) as related to high ionic strength matrices in IC but were not observed in the wastewater matrices tested. This difference could be due to the exchange capacity of the columns.

#### 3.1 Limits of Detection and Quantification

LOD obtained according to the visual method was  $0.5 \text{ mg L}^{-1}$  for chloride, nitrite, nitrate and sulphate (Fig. 3a, b). For phosphate, the LOD was  $2.5 \text{ mg L}^{-1}$  (Fig. 3c).

LOQ obtained with  $\text{RSD} \leq 10\%$  for the peak and  $\leq 2\%$  retention time shift were  $2.5 \text{ mg L}^{-1}$  for chloride, nitrate, nitrite and sulphate, and  $5.0 \text{ mg L}^{-1}$  for phosphate (Fig. 3d).

Both LOD and LOQ obtained by visual method are higher than those described elsewhere (USEPA 1993; Neele et al. 2002; Lopez-Moreno et al. 2010). However, these authors calculated the LOD and LOQ by multiplying the standard deviation of the lower standard by 3 (to obtain LOD) or 10 (to obtain LOQ). Neither of them utilised the visual method, which gives a stricter



**Fig. 3** Chromatograms obtained at LOD and LOQ concentrations of **a**  $0.5 \text{ mg L}^{-1}$  (LOD) for  $\text{Cl}^-$ , **b**  $0.5 \text{ mg L}^{-1}$  (LOD) for  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ , **c**  $2.5 \text{ mg L}^{-1}$  LOQ for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and LOD for  $\text{PO}_4^{3-}$ , and **d**  $5.0 \text{ mg L}^{-1}$  (LOQ) for  $\text{PO}_4^{3-}$

response. Additionally, EPA (1993) and Neele et al. (2002) used analytical columns characterised by low exchange capacity ( $20 \mu\text{eq L}^{-1}$ ).

### 3.1.1 Linearity

Calibration curves in water were linear for all anions tested in the range of  $2.5\text{--}50.0 \text{ mg L}^{-1}$  for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ . Interval calibration range for  $\text{PO}_4^{3-}$  went from  $5.0$  to  $100 \text{ mg L}^{-1}$ . This range is compatible with the range presented by Michalski et al. (2012), who utilised an analytical column whose capacity was  $210 \mu\text{eq L}^{-1}$ , and applied IC for coal mine water and for a water river samples highly contaminated with industrial waste. Hence, obtaining

linear calibration curves in  $2.5\text{--}50 \text{ mg L}^{-1}$  is convenient for inorganic anions analyses in wastewater samples.

For sewage, synthetic sewage and leachate calibration curves were linear in the same range as water. In vinasse samples, calibration curves were linear in the range of  $5.0\text{--}50 \text{ mg L}^{-1}$  for chloride and sulphate, probably due to the high concentrations of such anions in the matrix. For the other anions (nitrite, nitrate and phosphate), calibration curves were linear in the same range described for water ( $2.5$  to  $50 \text{ mg L}^{-1}$  for nitrite and nitrate or  $5.0$  to  $50 \text{ mg L}^{-1}$  for phosphate). ANOVA showed that the analyte calibration curves presented linearity in all matrices with well-adjusted models without lack-of-fit (Table 3).

**Table 3** Linearity and lack of adjustment tests for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  for five different matrices

		Curve	$r^2$	Linearity test		Lack of adjustment test	
				$F$	Ftab	$F$	Ftab
Chloride	Water	$0.08647x - 0.06405$	0.9909	1643.9	4.6	2.3	3.5
	Sewage	$0.09278x + 0.2058$	0.9839	1340.8	4.3	2.6	2.8
	Synthetic sewage	$0.10952x + 1.3811$	0.9935	3367.8	4.3	1.6	2.7
	Vinasse (*)	$0.0966x + 0.7812$	0.9964	4200.8	4.5	1.9	3.3
	Leachate	$0.11567x + 1.5268$	0.9924	2758.2	4.3	2.2	2.8
Nitrite	Water	$0.05244x - 0.0442$	0.9827	1568.4	4.5	2.7	3.2
	Sewage	$0.05495x - 0.0428$	0.9878	1555.1	4.4	2.2	3.0
	Synthetic sewage	$0.05915x - 0.415$	0.9939	6209.5	4.4	1.6	3.0
	Vinasse	$0.053x - 0.0433$	0.9940	11,201.1	4.4	2.0	3.0
	Leachate	$0.0633x + 0.1699$	0.9930	6517.7	4.3	2.4	2.7
Nitrate	Water	$0.0450x - 0.0194$	0.9902	1408.6	4.6	3.0	3.5
	Sewage	$0.04654x - 0.0238$	0.9876	1507.2	4.4	2.2	3.0
	Synthetic sewage	$0.0507x - 0.0247$	0.9935	5818.4	4.4	1.6	3.0
	Vinasse	$0.04616x - 0.0381$	0.9981	9447.4	4.4	1.6	3.0
	Leachate	$0.05381x - 0.0382$	0.9955	6695.7	4.5	1.7	3.3
Phosphate	Water	$0.02205x - 0.0245$	0.9869	1119.0	4.5	1.8	3.3
	Sewage	$0.02318x + 0.0054$	0.9782	1978.7	4.3	1.2	2.7
	Synthetic sewage	$0.02565x + 0.0053$	0.9941	7381.1	4.3	1.9	2.7
	Vinasse	$0.02308x - 0.0336$	0.9982	10,190.4	4.4	1.4	3.2
	Leachate	$0.02707x - 0.0465$	0.9964	4880.4	4.4	2.4	3.2
Sulphate	Water	$0.05947x - 0.0228$	0.9861	2136.5	4.5	2.2	3.3
	Sewage	$0.06112x + 0.0503$	0.9888	1955.6	4.3	1.7	2.7
	Synthetic sewage	$0.06659x + 0.0174$	0.9943	7753.0	4.3	1.8	2.7
	Vinasse(*)	$0.06288x + 0.6417$	0.9965	4365.0	4.5	2.0	3.4
	Leachate	$0.06980x + 0.0338$	0.9948	7749.5	4.3	2.1	2.8

\*Calibration curve range from  $5.0$  to  $50 \text{ mg L}^{-1}$



### 3.1.2 Recovery

Recovery was assessed in low-, medium- and high-range levels. Table 4 shows trueness calculated for each of the tested matrices. All the target anions yielded recoveries in the range of 85–115% for all tested matrices in the three range levels of the calibration curve. The highest recovery was observed for nitrite in domestic sewage in low level concentration (114%). Nitrite in low level concentration was also the anion for which we observed the worst recovery—91% in vinasse calibration curve. Nitrite, nitrate and phosphate in vinasse were the anions which yielded the lowest recoveries, compared with the same parameter in other matrices. Recoveries for chloride and nitrate in low level concentration for water are higher than expected. Since the calibration curve in water was prepared in ultrapure water, without any compound, it was expected recoveries to be nearer to 100%. Carry over effect could explain higher recoveries, but this effect was not seen in any blank sample injected during the validation procedures. Besides, phosphate and sulphate recoveries are in the expected range for low-level concentration in water. Nevertheless, chloride and nitrate recoveries are within the acceptable range (USEPA 1993; Villagrán et al. 2004).

### 3.1.3 Precision

Method repeatability was evaluated as relative standard deviation (RSD) at three different levels of the range (low, medium and high). Precision was assessed for the peak area (Table 5) and for retention time (Table 6). It can be observed in Table 5 that the RSD of peak areas were lower than 10% for all the target anions in all the five matrices. In case of water samples, higher RSD was observed in medium level concentration for chloride, nitrite, nitrate and sulphate, but higher RSD was expected for low level concentration. Regarding the other matrices, higher RSD was observed in low level for all tested concentrations only in leachate. No trend was observed in RSD for the other matrices. Nonetheless, RSD of peak areas was lower than 10% for all the target anions in all the five matrices. Therefore, precision regarding peak areas is within the range recommended by Thompson et al. (2002), which indicates that this method fits for inorganic anion analyses in different types of wastewater samples.

A typical interference observed in IC is ionic character displacement (USEPA 1993). It occurs when

**Table 4** Recoveries of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  in five different samples

Matrix	Anion	Recovery		
		Low	Medium	High
Water	Chloride	112.5	98.2	102.1
	Nitrite	104.8	97.8	106.2
	Nitrate	107.2	101.5	103.0
	Phosphate	98.9	105.7	97.0
	Sulphate	99.3	101.2	97.3
Synthetic sewage	Chloride	<i>103.6</i>	<i>103.5</i>	<i>100.1</i>
	Nitrite	103.0	101.4	100.3
	Nitrate	103.8	101.6	100.4
	Phosphate	111.5	101.0	100.9
	Sulphate	108.3	101.4	100.5
Domestic wastewater	Chloride	<i>103.3</i>	<i>99.1</i>	<i>103.4</i>
	Nitrite	113.7	99.8	102.2
	Nitrate	102.4	100.5	102.0
	Phosphate	108.3	101.4	100.5
	Sulphate	112.7	99.7	101.8
Vinasse	Chloride	<i>106.4</i>	<i>93.6</i>	<i>99.9</i>
	Nitrite	90.8	96.2	99.8
	Nitrate	93.2	96.4	99.7
	Phosphate	94.4	95.9	100.1
	Sulphate	<i>98.6</i>	<i>95.3</i>	<i>99.2</i>
Solid waste leaching	Chloride	<i>106.8</i>	<i>98.1</i>	<i>101.5</i>
	Nitrite	<i>111.9</i>	<i>97.1</i>	<i>101.5</i>
	Nitrate	104.3	95.6	100.3
	Phosphate	98.5	97.4	101.3
	Sulphate	<i>105.3</i>	<i>98.5</i>	<i>101.4</i>

Low, medium and high range levels correspond to 5.0, 40.0 and 100.0  $\text{mg L}^{-1}$  for phosphate. For the other anions, range levels are 2.5 (low), 20 (medium) and 50 (high)  $\text{mg L}^{-1}$ . Italicized values indicate calculations made according to the standard addition method

retention time shifts due to the influence of high ionic strength matrices. Shifts in retention time were reported by other authors testing cleaner matrices (Neele et al. 2002). However, data summarised in Table 6 shows that RSD of retention times was lower than 2% for all the target anions in all the tested matrices, indicating that retention time shifts did not occur.

Regarding auto-sampler stability, RSD of peak areas ranged from a minimum of 1.2% (for  $\text{PO}_4^{3-}$ ) to a maximum of 1.3% (for  $\text{Cl}^-$ ) over the 24 h the sample was maintained in the autosampler device. RSD of peak areas from the storage stability tests ranged from a

**Table 5** Relative standard deviation of peak areas of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  at three different levels of the calibration curve range in five different matrices

Anion	Water	Synthetic sewage	Sewage	Vinasse	Leachate
Chloride	L: 6.3	L: 5.5	L: 6.9	L: 7.7	L: 7.9
	M: 9.2	M: 1.9	M: 4.7	M: 5.8	M: 3.0
	H: 5.3	H: 2.4	H: 7.7	H: 3.1	H: 4.0
Nitrite	L: 4.2	L: 2.6	L: 8.4	L: 5.1	L: 7.8
	M: 8.5	M: 2.1	M: 4.6	M: 4.7	M: 3.7
	H: 5.3	H: 2.0	H: 7.3	H: 3.0	H: 4.0
Nitrate	L: 1.9	L: 1.3	L: 4.2	L: 5.4	L: 5.3
	M: 7.9	M: 1.7	M: 4.4	M: 7.5	M: 2.8
	H: 5.2	H: 1.9	H: 7.4	H: 2.9	H: 3.7
Phosphate	L: 2.8	L: 6.4	L: 4.0	L: 3.0	L: 7.5
	M: 0.8	M: 2.0	M: 4.9	M: 5.0	M: 3.6
	H: 5.2	H: 2.2	H: 8.2	H: 3.2	H: 3.8
Sulphate	L: 3.4	L: 3.2	L: 5.9	L: 1.9	L: 7.1
	M: 9.9	M: 2.3	M: 4.4	M: 5.1	M: 3.6
	H: 5.2	H: 1.7	H: 8.2	H: 2.8	H: 3.8

*L* low level, corresponds to 2.5 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 5.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$ ; *M* medium level, corresponds to 20.0 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 40.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$ ; *H* high level, corresponds to 50.0 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 100.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$

minimum of 1.4% (for  $\text{PO}_4^{3-}$ ) to a maximum of 2.0% (for  $\text{SO}_4^{2-}$ ).

Linearity, recovery and precision parameters indicate that the method configurations tested are suitable for the analyses of inorganic anions in

different types of wastewater as matrix. This might be interesting for laboratories where various complex matrices are monitored, since the maintenance of the same operational conditions save time and labour.

**Table 6** Relative standard deviation of retention times of  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  at three different levels of the calibration curve range in five different matrices

Anion	Matrix				
	Water	Sewage	Synthetic sewage	Vinasse	Leachate
Chloride	L: 0.10	L: 0.06	L: 0.06	L: 0.05	L: 0.06
	M: 0.03	M: 0.05	M: 0.03	M: 0.05	M: 0.21
	H: 0.07	H: 0.16	H: 0.00	H: 0.00	H: 0.08
Nitrite	L: 0.07	L: 0.04	L: 0.08	L: 0.06	L: 0.04
	M: 0.07	M: 0.04	M: 0.02	M: 0.02	M: 0.18
	H: 0.07	H: 0.13	H: 0.02	H: 0.04	H: 0.06
Nitrate	L: 0.05	L: 0.05	L: 0.09	L: 0.07	L: 0.04
	M: 0.08	M: 0.04	M: 0.03	M: 0.04	M: 0.13
	H: 0.10	H: 0.06	H: 0.04	H: 0.03	H: 0.04
Phosphate	L: 0.09	L: 0.07	L: 0.14	L: 0.08	L: 0.06
	M: 0.11	M: 0.06	M: 0.03	M: 0.06	M: 0.23
	H: 0.17	H: 0.12	H: 0.07	H: 0.04	H: 0.09
Sulphate	L: 0.14	L: 0.06	L: 0.15	L: 0.13	L: 0.07
	M: 0.15	M: 0.05	M: 0.03	M: 0.04	M: 0.18
	H: 0.16	H: 0.11	H: 0.06	H: 0.03	H: 0.10

*L* low level, corresponds to 2.5 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 5.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$ ; *M* medium level, corresponds to 20.0 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 40.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$ ; *H* high level, corresponds to 50.0 mg L<sup>-1</sup> for  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  and to 100.0 mg L<sup>-1</sup> for  $\text{PO}_4^{3-}$

**Table 7** Matrix effect calculated for domestic wastewater, synthetic sewage, vinasse and leachate

	Sewage	Synthetic sewage	Vinasse	Leachate
Chloride	-7.4%	<b>-25.7%</b>	-11.7%	<b>-33.9%</b>
Nitrite	-4.8%	-12.8%	-1.7%	<b>-20.6%</b>
Nitrate	-3.4%	-12.7%	-2.6%	<b>-19.5%</b>
Phosphate	-5.1%	-16.3%	-4.7%	<b>-22.4%</b>
Sulphate	-2.7%	-11.9%	-5.7%	-17.4%

In bold, medium matrix effect

### 3.2 Matrix Effect

A signal enhancement was observed for all the tested matrices and all the anions. The matrix effect level varied from -1.7% (nitrite in vinasse) to -33.9% (chloride in leachate) (Table 7). Chloride was the anion which showed the highest matrix effect for all the tested matrices, while leachate was the matrix which showed the highest matrix effect for all the tested anions. Overall, nitrate was the anion least affected by the matrix effect and sewage was the matrix which showed the least matrix effect for the tested analytes.

Although vinasse was the matrix which presented the highest electrical conductivity (Table 1), it was not the matrix which gave higher matrix effect. For vinasse, the higher matrix effects were observed for chloride, but still in the range considered as a low matrix effect. In fact, it seems that alkalinity has a higher impact over the equipment response. Comparing the observed response for leachate and vinasse (since the same dilution rate was applied for both matrices), the former presents higher alkalinity, while its electrical conductivity was lower. The observed matrix effects were higher for leachate in all the tested anions (Table 7).

We expected the matrix effect of sewage and synthetic sewage to be similar since the latter aims to mimic the former. The higher matrix effect for synthetic sewage is

probably related to the chemical compounds added to the mimic sewage composition. The protocol for this lab-made wastewater (Camiloti et al. 2013) requires the addition of  $\text{NaHCO}_3$ , as a source of alkalinity. The addition of  $\text{HCO}_3^-$  could explain the higher matrix effect when compared with sewage. It is likely that carbonates influence the signal response, since the eluent is a carbonate-based solution.

These results indicate that simple dilution is an effective procedure to inorganic anion analyses of high ionic strength matrices (Neele et al. 2002). Moreover, our study is in accordance with Michalski et al. (2012), who reported that analytical columns with high exchange capacity are suitable for the analysis of complex matrices.

### 3.3 Anion Profile in the Tested Matrices

Some of the tested matrices contained the target anions. In such cases, the addition standard method was applied, as previously mentioned. Table 8 shows that the anion concentration calculated in case the analyte was present in the matrix. The confidence limits varied from 7.75% (chloride for domestic sewage) to 12.5% (sulphate for vinasse). In almost all cases, the observed concentrations are within the range described in the literature (Table 1), although the waste characterisation in the references mentioned earlier used methods other than IC. The exception was nitrite in leachate. This anion is usually not present in leachate samples (Table 1), but it was observed in our sample. Leachate was collected from landfill and kept without refrigeration for a few days before it was used for validation procedures, providing conditions for partial ammonia oxidation occurrence. Nonetheless, in most cases, the anion concentrations for the analytes which are present in some matrices are followed with the expected values, which hint on favour of the described method.

**Table 8** Inorganic anion concentration in case the analyte was present in the matrix

Anion	Synthetic wastewater	Domestic sewage	Vinasse	Leachate
Chloride ( $\text{mg L}^{-1}$ )	130 ± 16	21.9 ± 1.7	809 ± 62.5	1320 ± 145
Nitrite ( $\text{mg L}^{-1}$ )	ND	ND	ND	270 ± 32
Sulphate ( $\text{mg L}^{-1}$ )	ND	ND	1021 ± 12.8	ND

ND non-detected

## 4 Applications

The IC method described in this paper has been used to determine anions concentrations for all the tested matrices previously described. One application was nitrite and nitrate removal monitoring in a continuous up-flow structured-bed reactor subjected to recirculation and intermittent aeration (SBRRIA) (Santos et al. 2016). The SBRRIA was fed with synthetic wastewater similar to sewage. The authors aimed to study the influence of COD/N ration on the simultaneous nitrogen and carbon removal process. They concluded that the system achieved acceptable removal efficiencies of organic matter and nitrogen. Nitrogen removal was achieved through the integration of nitrification, heterotrophic denitrification and anammox processes (Santos et al. 2016).

Another application of the IC method was to investigate sulphur recovery in an Internal Silicone Membrane Reactor (ISMR). Valdés et al. (2016) studied an innovative microaerobic reactor configuration with bubble-free aeration, aiming to recovery elemental sulphur. The ISMR was fed with synthetic sewage, and sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) was added. IC method was used to monitor sulphate concentrations in the system.

IC was used to assess the influence of sulphate on COD removal and methane production using sugarcane vinasse as feedstock (Kiyuna et al. 2017). Sulphate is one of the main interfering compounds on anaerobic digestion and is present in sugarcane vinasse. Distilleries usually add sulphuric acid to prevent microbial contamination and enhance fermentation control in ethanol production (Fuess and Garcia 2015). Hence, vinasse, which is the wastewater generated in ethanol production, is sulphate-rich. A study carried out by Kiyuna et al. (2017) shed light on the influence of this anion in energy recovery.

The IC method was also used in nitrite and nitrate monitoring of an effluent of leachate nitrification. Martins et al. (2017) evaluated the ammonium adsorption in zeolites and its regeneration via nitrification. The authors concluded that nitrite was the main product of regeneration, hinting that anammox-based processes could be used further to achieve complete nitrogen removal from leachate.

Therefore, the anion analysis via the IC method presented was effective for monitoring various bio-processes, targeting both wastewater treatment and the recovery of energy and products.

## 5 Conclusions

The matrix-effect assessment showed that IC is efficient and reliable for inorganic anion analyses in different types of wastewaters using a simple dilution and filtration as sample preparation. Since operational conditions were the same for all the tested matrices, this method seems to be a good option for multi-elemental analyses in various high-ionic wastewaters, allowing to save time and labour. This method was successfully used to monitor inorganic anions found in complex wastewater samples and can be utilised for bioreactor monitoring.

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### Compliance with Ethical Standards

**Conflict of interest** The authors declare that they do not have any conflict of interest.

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