

Article

New Green and Sustainable Tool for Assessing Nitrite and Nitrate Amounts in a Variety of Environmental Waters

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Abstract: This paper aims to provide improved selectivity and sensitivity with a short analysis time of about 10 min and low residues for quantitation of nitrite and nitrate in waters by liquid chromatography. Ion-pair formation and ion exchange retention mechanisms were considered. The optimized option was in-tube solid phase microextraction (IT-SPME) by means of a silica capillary of 14 cm length and 0.32 mm id, coupled online with a capillary anion exchange analytical column (Inertsil AX 150 × 0.5 mm id, 5 μm) and the use of their native absorbance. Precision of the retention times expressed as % relative standard deviation (RSD) were <1% for both, nitrite ($t_R = 5.8$ min) and nitrate ($t_R = 10.5$ min). Well, river, channel, lake, sea, tap and bottled waters and several matrices of a drinking water treatment plant were analysed, and no matrix effect was observed for all of them. Inorganic anions and several organic acids were tested as possible interferences and suitable selectivity was obtained. Precision expressed as % relative standard deviation (RSD) was between 0.9 and 3%. Low detection limits of 0.9 and 9 μg/L for nitrite and nitrate were obtained, respectively, and low residue generation near 100 μL per run was also achieved.

Keywords: nitrite; nitrate; waters; ion exchange; capillary LC; IT-SPME



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1. Introduction

The excessive use of products rich in nitrogen in several fields has generated an alteration of its cycle [1]. From some of them, and due to their solubility in water, nitrate and nitrite concentrations affect markedly the quality of drinking water [2] and contribute to eutrophication. When these compounds are ingested, they can generate N-nitrosamines that according to the International Agency for Research on Cancer (IARC) are probably carcinogenic in humans and were included in group 2A [3]. Current regulations on nitrate and nitrite content in drinking water are based on guidelines established by the World Health Organization (WHO) considering short-term effects such as methemoglobinemia and thyroid effects, but not long-term health effects [4].

European legislation established a maximum concentration of 50 mg/L for nitrate and 0.5 mg/L for nitrite [5] in drinking water, and in groundwater the limit value is 50 mg/L for nitrate [6], but their content in environmental waters is variable for both, nitrate and nitrite [7]. Various spectroscopic and electroanalytical techniques have been proposed for nitrate and/or nitrite determination each having its own merits and demerits [8,9]. However, conventional ion chromatography with electronic suppression of eluent conductivity and conductimetric detection is a standard method (method 4110 C) to measure nitrate and nitrite in water and wastewater chemical analysis [10], with limits of detection (LOD) around 0.1 mg/L. Ion-pair formation from adding to the mobile phase of an ion-pairing agent and using conventional reversed phase analytical columns were also proposed [11–13], but higher LODs than that achieved by method 4110 C are achieved.

A more recent work has proposed the determination of inorganic anions in seawater samples by ion chromatography with UV detection using a monolithic octadecylsilyl

column of 150×4.6 mm i.d. coated with dodecylammonium cation [14] using a flow rate of 400 $\mu\text{L}/\text{min}$ and a sample injection volume of 100 μL and achieving LODs of 0.9 $\mu\text{g}/\text{L}$ and 1.9 $\mu\text{g}/\text{L}$ for nitrite and nitrate, respectively, although both limits were estimated from signal/noise ratios ($S/N = 3$). Sedyohutomo et al. [15] described the utilization of triacontyl-bonded silica coated with imidazolium ions for capillary ion chromatographic determination of nitrite and nitrate in both river water and seawater. The dimensions of the column used was 100×0.32 mm i.d., 5 μm working at a flow rate of 4 $\mu\text{L}/\text{min}$ and the injected sample volume was 2 μL . In these conditions, the LODs were 0.14 and 0.16 mg/L for nitrite and nitrate, respectively.

A portable analyser using two-dimensional ion chromatography with UV light-emitting diode-based absorbance detection for nitrate monitoring within both saline and freshwaters was proposed by Fitzhenry et al. [16]. Two columns (50×4 mm, 9 μm) in tandem were used, the flow rate was 700 $\mu\text{L}/\text{min}$ and the injected sample volume was 195 μL . The LODs obtained in [16] were 20 and 10 $\mu\text{g}/\text{L}$ for nitrate in freshwater and marine water, respectively.

The miniaturization of analytical systems in order to achieve real information from samples, in as short as possible time while taking account of environmental and economic issues, is one of the main goals in modern analytical chemistry. In this sense, miniaturized liquid chromatography (LC) contributes in this way. The main advantages perceived in using miniaturized LC are related to the reduction of column id, which comes along with a reduction in mobile phase flow rate; this promotes reductions in both the solvent consumption and waste from the analysis. Although the sample dilution ratio for capillary (Cap)-LC is lower than that achieved in conventional HPLC, as shown before from dimensions of the analytical columns and flow rates of [15] vs. [14,16], respectively, as examples, sensitivity for several applications is unsatisfactory mainly due to the low injected sample volume [15]. A method that facilitates working with higher sample volumes without losses in resolution is the online in-tube solid-phase microextraction (IT-SPME) [17,18]. IT-SPME is based typically on the use of a fused-silica capillary tube packed or coated on its inner surface with an extractive phase. When the sample is passed through the capillary, the analytes are extracted and concentrated by adsorption/absorption onto or into the internal coating of the capillary. Then, the extracted analytes are desorbed by filling the capillary with a solvent, which are transferred to the capillary LC. Clean-up, preconcentration, separation, and detection of analytes can be carried out online by coupling the IT-SPME to LC.

On the other hand, in the context of current chemistry, the requirements of sustainable and green chemistry also have to be considered. The concept “sustainable” first appeared in the 70’s, and is connected to the idea of linking economic development with the preservation of natural ecosystems [19]. Later on, green chemistry emerged from the concern about environmental contamination caused by pollution from chemical industry in 1990s. These interests were expressed by Anastas and Warner in 1998, who established a list of 12 principles as a guide for a good practice in green chemistry [20]. In this context, there is an intersection zone between the mentioned subjects, greenness and sustainability, both included into the wider concept of suitable chemistry [21]. A goal of this paper was also to contribute in this direction [22,23].

This paper demonstrates the feasibility of the online coupling of IT-SPME and ion-exchange capillary liquid chromatography (IE-CapLC) with diode array detection for the first time. The selected analytes were nitrite and nitrate considering their relevant influence in health and environmental scenarios. The paper demonstrates the achievements of this couple for improving parameters in the literature indicated in previous paragraphs such detection limits (LODs), time analysis, and applicability to a variety of water matrices with also different levels of both analytes and trueness, besides decreasing waste and without sample treatment.

2. Materials and Methods

2.1. Materials and Reagents

Ultra-pure water was obtained for a Nanopure II system (Barnstead, NH, USA). Acetonitrile (99.8%) and methanol (96%) grade LC-MS were supplied by VWR Chemicals (Randnor, PA, USA). Potassium nitrate (99%), potassium nitrite (97%), sodium sulfate (99%), potassium biphosphate (98%), citric acid (99%), and orthophosphoric acid (85%) were purchased from Panreac (Barcelona, Spain). Hexadecyltrimethylammonium hydroxide (TBA-OH, 99%), tetrabutylammonium chloride (TBA-Cl, 99%), and hexadecyltrimethylammonium bromide (HTAB, 99%), gallic acid (97.5%), benzoic acid (99.5%), chlorogenic acid (95%), salicylic acid (99%), and phthalic acid (99.5%) were obtained from Sigma-Aldrich (Darmstadt, Germany).

2.2. Equipment and Columns

A capillary LC Agilent 1260 infinity series (Agilent, Waldbronn, Germany) with an injection valve rheodyne 7725i and coupled online in in-valve mode to IT-SPME was used. For this purpose, the loop of the six-port injection valve was substituted by the extractive IT-SPME capillary [17,18]. Several IT-SPME capillaries were tested: fused silica capillary of 0.32 mm id and 15 cm length (Supelco, Bellefonte, Pennsylvania USA) and 50% Diphenyl-50% dimethyl polysiloxane, bonded and crosslinked phase capillary: TRB 50 (Teknokroma, Barcelona, Spain) with the same dimensions and 3 μm of film thickness. A photodiode array detector (DAD, Hewlett-Packard 1040M series II) with a cell volume of 80 nL was employed. The analytical columns tested were: ZORBAX SB-C18 150 \times 0.5 mm id, 5 μm (Agilent Technologies Spain), ZORBAX SB-C18 35 \times 0.5 mm id, 5 μm (Agilent technologies Spain), and Inertsil AX 150 \times 0.5 mm id, 5 μm (GL Sciences Tokyo, Japan). The data were acquired and processed by the Agilent HPLC ChemStation Software. Signals were recorded in the range of 210–400 nm and monitored at 220 nm.

The processed volume of standards or samples was 25 μL and all of them were loaded manually into the IT-SPME system using a 100 μL precision syringe at a flow rate of 50 $\mu\text{L}/\text{min}$. Then, the valve was changed to the injection position, so that the analytes retained in the capillary were desorbed with the mobile-phase and transferred to the analytical column for separation and detection. The chromatographic run was carried out with the valve in the injection position.

2.3. Optimization of Chromatographic Conditions

Ion pair capillary liquid chromatography (IP-CapLC) technique was studied employing the capillary reversed analytical columns and several ion pairing agents: TBH-OH, TBH-Cl or HTAB at levels 1, 5, 10 mM in the mobile phase (see Table 1) and 1 mg/L standard solution of nitrate and mixtures of nitrate and sulfate and nitrate and phosphate.

Table 1. Mobile phases for IP-CapLC method, flow rate 8 $\mu\text{L}/\text{min}$. Ionic pairing agent solutions at pH = 3.1 reached with HCl containing 20% of methanol (MeOH) and MeOH compositions.

Time (min)	Mobile Phase %					
	TBH-OH	MeOH	TBH-Cl	MeOH	HTAB	MeOH
0	100	0	100	0	50	50
2	50	50	80	20		
4	40	60	40	60		
8	30	70	30	70		
10	20	80	20	80		
12	100	0	100	0		

For ion-exchange capillary liquid chromatography (IE-CapLC) a column Interstil AX was employed and different compositions of acetonitrile and water at pH 3.1 reached with phosphoric acid were evaluated in the mobile phase and also ionic strength was varied by adding NaCl or KH_2PO_4 . Three flow rates were tested (8, 10 and 12 $\mu\text{L}/\text{min}$). The figures

of merit: linearity ($n = 5$), LODs, limits of quantitation (LOQs), selectivity and matrix effect were evaluated. Nitrite and nitrate concentrations were assayed up to 1 and 2.5 mg/L, respectively, for obtaining the linear calibration graph. Precision was estimated at LOQ ($n = 3$) and 0.1 ($n = 4$) and 1 ($n = 4$) mg/L for both nitrite and nitrate and the matrix effect at the latter levels of concentrations.

LODs and LOQs were obtained by injecting decreasing values of analyte concentration until obtaining signal/noise ratios of 3 and 10 times, respectively.

2.4. Analysis of Water Samples

Natural water samples that were collected in different places in the vicinity of Valencia (well, river, channel, sea and lake) and drinking waters such as tap water obtained in the municipality of Burjassot (Valencian community) and several bottled commercial waters. Samples from a potable water treatment plant provided by the municipality of Gandia (Valencian community) collected in three stages of the treatment process of diminishing nitrate concentration (input, mid-process and output) were also analysed. The samples were passed through nylon filters with a pore size of 0.22 μm and/or diluted if necessary.

A study of confirmation was carried out by using the native absorbance of nitrate measured with a CARY UV Visible spectrophotometer model G6860AAR (Agilent Technologies, Santa Clara, CA, USA) with a quartz cuvette of 1 cm path length. Nitrate analysis was performed by measuring the sample directly at a wavelength of 220 nm, the values obtained were interpolated on a calibration line up to 50 mg/L. For nitrite, the Griess technique from PDMS sensors embedding the reagents [24] was employed. A Griess sensor was placed in a vial containing 0.5 mL of the sample and 0.5 mL of citric acid (330 mM), left to stand for 8 min and the absorbance was measured at a wavelength of 540 nm, the values were interpolated on a calibration graph up to 1.5 mg/L.

3. Results and Discussion

3.1. Assessment of Chromatographic Performance

Two mechanisms were evaluated for the direct analysis of nitrite and nitrate; the first one was ionic pair chromatography with a capillary C18 column (IP-CapLC), and the second one was anion exchange capillary liquid chromatography with an anionic column (IE-CapLC). In both cases, the IT-SPME silica capillary was used. The IT-SPME capillary column was used as the loop of the injector valve (in-valve, in-tube SPME), and the analytes are extracted in the IT-SPME capillary during sample loading, and then transferred to the capillary analytical column with the mobile phase by changing the valve to the injection position. The sensitivity can be improved by flushing a sample volume higher than that of the capillary internal volume through the capillary [17,18].

To study the chromatographic conditions in the IP-CapLC method, three ion pairing agents were evaluated. TBH-OH and TBH-Cl were analysed in gradient mode (see Table 1) and HTAB in isocratic mode. The more suitable responses were obtained working with concentrations of 5 mM for TBH-OH and TBH-Cl and 1 mM for HTAB. The achieved retention times (t_R) for nitrate were: 18.6, 11.2 and 4.7 min for TBH-OH, TBH-Cl and HTAB, respectively, as Figure 1 shows.

Mixtures of nitrate and sulfate and/or phosphate were assayed and overlapped peaks were obtained in all cases (Figure 1). Bearing in mind the obtained results for IP-CapLC, ion-exchange capillary liquid chromatography (IE-CapLC) was assayed. The selected column contained diethylamino group, which is a strong base ($pK_a \approx 10.6$). Z. Kadlecová et al. [25] found that the anion-exchange retention gradually diminishes above pH 5, although it is fully charged below $pH < 9$. The authors indicated a possible mechanism that is deprotonation of residual silanols of uncapped sorbent, which can result in the formation of zwitter-ions and decrease in diethylamino surface charge. The mobile phases were adjusted at pH 3.1 with orthophosphoric acid. Methanol was discarded as a modifier as used in IP-CapLC because assaying a mixture 60:40 methanol:water as the mobile phase, nitrate remained in the analytical column for more than 200 min. Acetonitrile was employed

instead of methanol and water/acetonitrile ratios of 70:30, 55:45, and 50:50 were evaluated in the mobile phase and also the effect of adding NaCl or KH_2PO_4 .

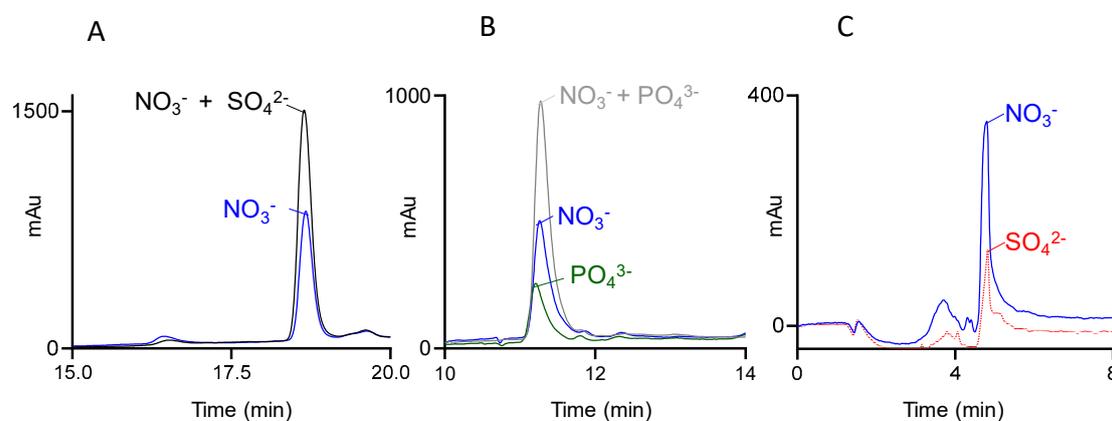


Figure 1. Chromatograms obtained by the IP–CapLC method for the standard solutions, using the three ion pairing agents tested: (A) TBH–OH (1 mg/L nitrate and 0.5 mg/L Nitrate + 0.5 mg/L Sulfate), (B) TBH–Cl (1 mg/L Nitrate, 0.5 mg/L Nitrate + 0.5 mg/L Phosphate and 0.25 mg/L Phosphate) and (C) HTA–Br (1 mg/L Nitrate and 0.5 mg/L Sulfate).

As it can be seen in Table 2 the percentage of water had a great influence on the retention time of nitrate for ion-exchange. The influence of the presence of chloride in the mobile phase on t_R of nitrate (36.6 min vs. 46 min without chloride) was not significant compared to the results provided by dihydrogen phosphate (t_R 14 min vs. 46 min without dihydrogen phosphate). The best chromatographic conditions were achieved with mobile phases containing this last anion, which produces the elution of nitrate in short times. The selected conditions are given in Table 2, and it can be seen the retention time for nitrate was 10.5 min. Lower concentrations of dihydrogen phosphate (20 mM and 10 mM) increased t_R of nitrate and they were discarded.

Table 2. Optimization of mobile phase in IE–CapLC. For all mobile phases pH = 3.1; ¹ 130 mM concentration. * Optimal conditions. For more explanations see text.

Percentage % Water-Acetonitrile	Salt ¹	Flow Rate $\mu\text{L}/\text{min}$	t_R Nitrate (min)
70–30	-	8	75
55–45	-	8	57
50–50	-	8	46
50–50	NaCl	8	36.6
50–50	KH_2PO_4	8	14
50–50	KH_2PO_4	10	12
* 50–50	KH_2PO_4	12	10.5

Two capillaries with phases with different polarity and with the same internal volume of 12 μL were tested for IT–SPME: fused silica and TRB 50. A sample clean-up step was not necessary after in-valve processing of the samples, which were directly transferred to the analytical column by switching the valve to the injection position as mentioned above. The chromatographic run was achieved in the inject mode of the IT–SPME valve. The chromatographic profile was the same for both IT–SPME capillaries, as it can be seen in Figure 2A; however, similar areas were obtained, although the processed concentrations were 0.1 and 0.5 mg/L of nitrate for silica and TRB 50 capillaries, respectively. The most polar capillary of silica provided a higher level of preconcentration than TRB 50 for a processed volume of 25 μL in accordance with the polar nature of the analytes: nitrite and nitrate. The silica capillary achieved a factor of a preconcentration of five in reference to TRB 50, considering that their internal volumes were the same.

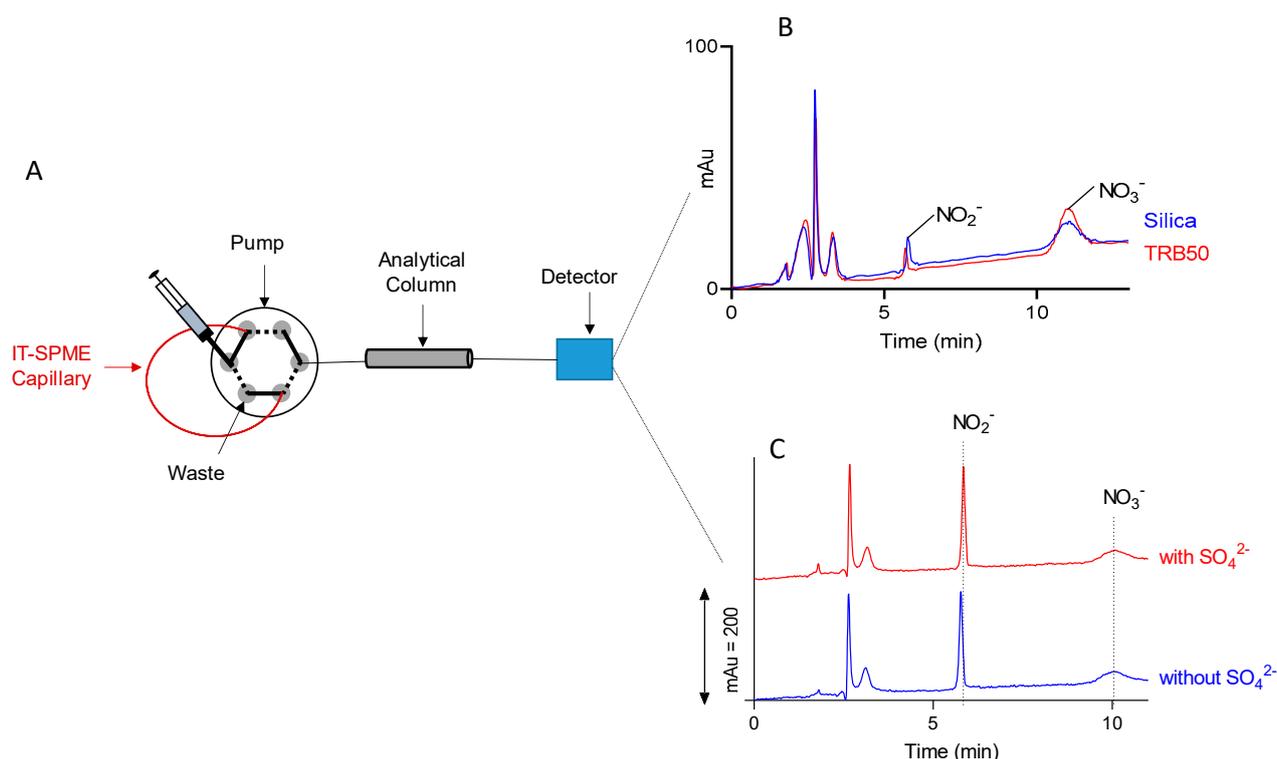


Figure 2. (A) Scheme of the IT-SPME-CapLC-DAD. (B) Influence of the nature of IT-SPME capillary: silica vs. TRB 50 for nitrate concentrations of 0.1 and 0.5 mg/L, respectively. (C) Chromatograms obtained by IE-CapLC for mixtures of standard solutions containing 0.5 mg/L of nitrite and 0.1 mg/L of nitrate and 0.5 mg/L of nitrite, 0.1 mg/L of nitrate and 0.5 mg/L of sulfate. The retention times for nitrite and nitrate are 5.8 and 10.5 min, respectively.

Sulpizi et al. [26] gave a detailed understanding of the molecular behaviour of the silica–water interface, indicating that the silanols determine the surface acidity and modulate the water properties. These authors showed how the silanols' orientation and their hydrogen bond properties are responsible for an amphoteric behavior of the surface, which can explain the results obtained here. TRB 50 is a slightly polar stationary phase containing phenyl into methylpolysiloxane, i.e., $-\text{CH}_3$ groups with phenyl groups, $-\text{C}_6\text{H}_5$, providing π - π , dipole–dipole, and dipole-induced dipole interactions and moderate amounts of hydrogen bonding. Figure 2B shows the chromatograms obtained for mixtures of nitrite and nitrate without and with sulfate. This anion did not interfere due to it does not absorb at 220 nm.

3.2. Figures of Merit

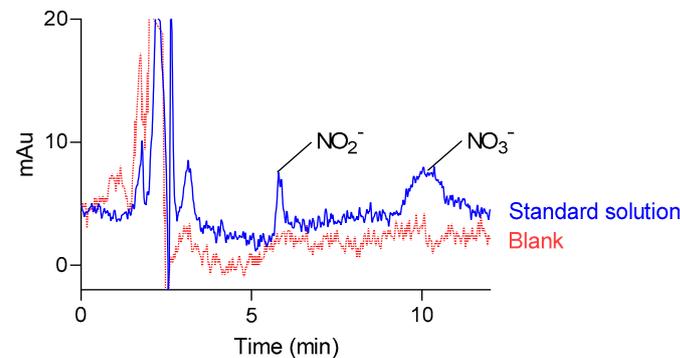
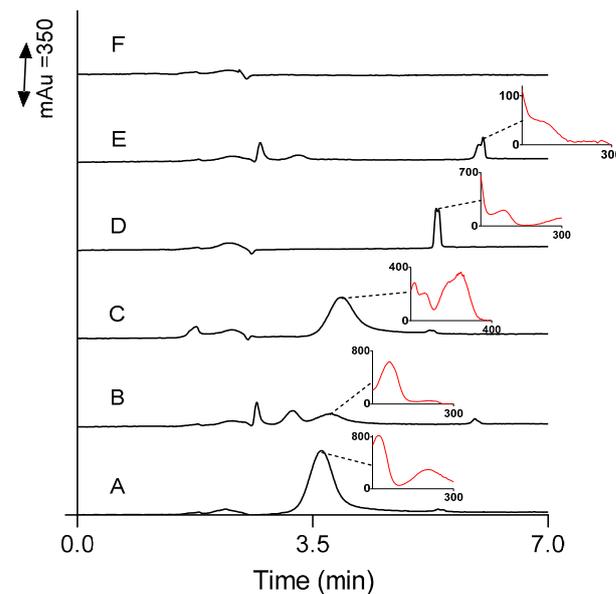
Linearity, LODs, LOQs and precision were studied for IT-SPME-IE-CapLC-DAD. Suitable linearity was obtained for nitrite and nitrate as Table 3 shows. The obtained LOQs permit the quantification of a wide variety of environmental and drinking waters. Figure 3 shows the chromatogram obtained for the LOQs. Precision was evaluated from % RSD for LOQs and by injecting four replicates of the 0.1 and 1.0 mg/L standard solutions and satisfactory values were obtained (see Table 3). Precision of the retention times expressed as % RSD was <1%, $n = 20$ and 100 μL per run of wastes were generated.

Several organic acids assayed as possible interferents (Figure 4), with pKa between 2.93 and 5.4 [27–31], gave retention times different to those obtained by nitrite and nitrate (see Figure 2B). Retention time for nitrite (5.8 min) was between those presented by salicylic (5.3 min) and phthalic (6.0 min); this latter was the closest one but at the working pH value of the mobile phase, phthalic acid is protonated. On the other hand, its UV absorption spectra are easily distinguishable from that provided by nitrite.

Table 3. Analytical parameters obtained with the proposed method IT-SPME-IE-CapLC-DAD.

Anion	Linearity ¹ $b_1 \pm s_{b1}$	$b_0 \pm s_{b0}$	$Y = b_1x + b_0$ R^2	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$); %RSD (n = 3)	% RSD
nitrite	1011 \pm 45	64 \pm 6	0.993	0.9	3; 6	1.8 ² ; 3 ³
nitrate	1669 \pm 100	115 \pm 40	0.996	9	30; 5	0.9 ² ; 2 ³

Notes: ¹ mg/L n = 5, Established at a concentration of ² 0.1 and ³ 1 mg/L n = 4.

**Figure 3.** Limits of quantification for nitrite and nitrate (3 and 30 $\mu\text{g/L}$, respectively) by IT-SPME-IE-CapLC-DAD.**Figure 4.** Chromatograms and spectra of organic acids: gallic ($\text{pK}_a = 4.2$, trace A), benzoic ($\text{pK}_a = 4.1$, trace B), chlorogenic ($\text{pK}_a = 3.6$, trace C), salicylic ($\text{pK}_a = 2.9$, trace D), phthalic ($\text{pK}_a = 5.4$, trace E) and sulfate (trace F) using the optimized conditions for nitrite and nitrate determination by IT-SPME-IE-CapLC-DAD. Phthalic and salicylic acids were assayed at 1 mg/L, chlorogenic, benzoic and gallic acids at 3.5, 0.5 and 1.7 mg/L, respectively.

3.3. Analysis of Waters

To evaluate the applicability of the developed method, several real water samples including natural and drinking water and samples from a potable water treatment collected during the nitrate removal process (inlet, mid-process, and outlet water) were analysed. Representative chromatograms obtained are given in Figure 5.

The chromatograms obtained for samples, shown in Figure 5, maintained the shape of the peaks, the resolution, and the retention times compared to the standard solutions of nitrite and nitrate (see Figures 2 and 3). The inserts of Figure 5 contained the spectra obtained at the maximum of the chromatographic peak, which permit the identification

of the analytes. Spiked samples provided % recoveries near to $100 \pm 5\%$ and then, the matrix effect was not present. Table 4 shows the obtained values for nitrite and nitrate in the analysed waters. A study of confirmation was carried out by analysing samples with the Griess method for nitrite and UV spectrophotometry for nitrate, and the results are also included in Table 4.

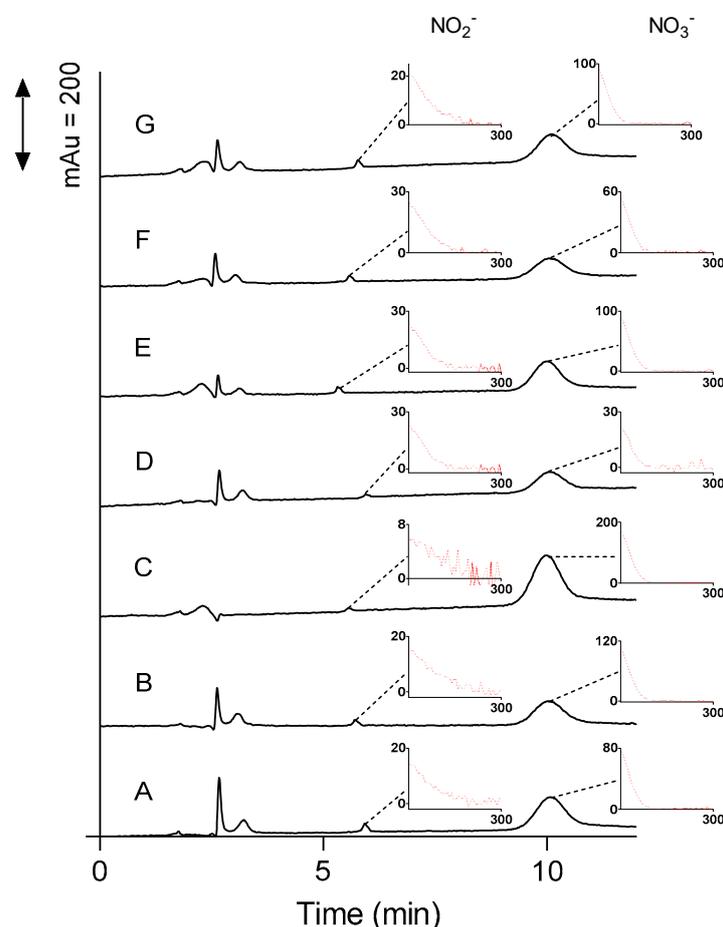


Figure 5. Chromatograms and spectra obtained at maximum of each chromatographic peak for several waters by IT–SPME–IE–CapLC–DAD. Natural water: well (trace G), river (trace F), and lake (trace E) diluted 1/40, 1/50 and 1/500, respectively, drinking water: tap (trace D) diluted 1/50 and undiluted bottled (trace C) and water treatment plant: inlet (trace A) and outlet (trace B), both diluted 1/50. The retention times for nitrite and nitrate are 5.8 and 10.5 min, respectively.

In reference to nitrite determination by the Griess method, a river water sample presented interference and its concentration could not be obtained. On the other hand, only samples containing nitrite concentrations higher than 0.03 mg/L could be quantified by the Griess method. However, the proposed IT-SPME-EI-CapLC-DAD provided results for all samples, as can be seen in Table 4.

In the case of nitrate, the proposed method provided quantitative results for all samples, which presented concentrations between 1.56 and 93 mg/L. Biased results were obtained for the sea water assayed from UV-spectrophotometry at 220 nm and this method did not permit quantifying nitrate below 9 mg/L. Table 4 shows that when the Griess and UV spectrophotometry methods could be applied, the results were statistically similar to those provided by the proposed method.

Table 4. Nitrite and nitrate concentration in real water samples (natural water, drinking water and water treatment plant) (n = 4). For more explanations see text.

Sample		Nitrite (mg/L)		Nitrate (mg/L)	
		Cap-ionLC	Griess Method ¹	Cap-ionLC	UV Spectrophotometry ²
Natural water	Well	0.4 ± 0.1	-	54.4 ± 0.6	55.1 ± 0.7
		0.20 ± 0.05	-	19.8 ± 0.6	21.5 ± 0.7
	River	0.20 ± 0.05	Not applicable ³	53 ± 1	51 ± 1
		0.30 ± 0.09	-	36.5 ± 0.5	31 ± 1
		0.40 ± 0.09	0.4 ± 0.1	93 ± 1	102 ± 1
		0.30 ± 0.09	-	40.3 ± 0.6	41.3 ± 0.8
Channel	0.30 ± 0.03	-	30.8 ± 0.4	30.5 ± 0.3	
lake	0.40 ± 0.03	-	13.3 ± 0.5	14 ± 0.7	
sea	0.30 ± 0.03	-	4.3 ± 0.3	Not applicable ³	
Drinking water	tap	0.05 ± 0.01	<LOQ	12.9 ± 0.5	12.6 ± 0.4
		0.09 ± 0.01	-	17.4 ± 0.9	17 ± 1
		0.10 ± 0.01	-	19.5 ± 0.1	18.0 ± 0.2
	bottled	0.010 ± 0.003	<LOD	2.23 ± 0.01	<LOD
		0.030 ± 0.005	≈LOD	1.56 ± 0.06	<LOD
		<LOD	-	2.19 ± 0.03	<LOD
Water treatment plant	inlet	0.9 ± 0.1	1.0 ± 0.2	55.8 ± 0.6	56.0 ± 0.7
	half process	0.7 ± 0.1	-	37.9 ± 0.6	37.1 ± 0.4
	outlet water	0.10 ± 0.01	-	14.7 ± 0.6	13 ± 1

Notes: ¹ PDMS Griess sensor [24], LOD 0.01 mg/L, ² direct UV spectrophotometry at 220 nm, LOD 3 mg/L. ³ Not applicable due to interference.

4. Conclusions

In the present work, a novel method for the quantification of nitrite and nitrate in a variety of waters with variable concentrations was proposed. The ion exchange mechanism provided better results than ion-pair formation with respect to analysis time, selectivity and trueness. The IT-SPME-IE-CapLC-DAD analytical procedure was applied for water analysis, decreasing the amount of waste generation by minimizing the amount of solvents employed, 100 µL/run instead of 10 mL/run for conventional liquid chromatography, which uses flow rates around 1 mL/min and considering a retention time for nitrate of around 10 min. A goal of this work was also to demonstrate that minimizing sample treatment by online IT-SPME by using a fused silica capillary, and miniaturizing the system can remarkably improve the greenness and sustainability of an analytical method, maintaining or even improving its figures of merit as detection limits and applicability to different water matrices with different levels of nitrite and nitrate, between 0.01–0.9 mg/L and 1.56–93 mg/L, respectively. The proposed IT-SPME-IE-CapLC-DAD approach proposed here for the first time is a good choice for the direct analysis of environmental waters. The quantitative performance of the proposed method is suitable in terms of linearity and precision, LODs, and selectivity, besides the absence of the matrix effect for both anions. The precision of the retention times is also remarkable for standards and samples.

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Data Availability Statement: Data are included in the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Karlović, I.; Posavec, K.; Larva, O.; Marković, T. Numerical groundwater flow and nitrate transport assessment in alluvial aquifer of Varaždin region, NW Croatia. *J. Hydrol. Reg. Stud.* **2022**, *41*, 101084. [[CrossRef](#)]
2. Beeckman, F.; Motte, H.; Beeckman, T. Nitrification in agricultural soils: Impact, actors and mitigation. *Curr. Opin. Biotechnol.* **2018**, *50*, 166–173. [[CrossRef](#)]
3. International Agency for Research on Cancer (IARC). World health organization international agency for research on cancer. *Iarc. Monogr. Eval. Carcinog. Risks Hum.* **2010**, *94*, 1–464.
4. Picetti, R.; Deeney, M.; Pastorino, S.; Miller, M.R.; Shah, A.; Leon, D.A.; Dangour, A.D.; Green, R. Nitrate and nitrite contamination in drinking water and cancer risk: A systematic review with meta-analysis. *Environ. Res.* **2022**, *210*, 112988. [[CrossRef](#)]
5. European Parliament. Directive 2015/1787/EU: Amending Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Off. J. Eur. Union* **2015**, *L260*, 6–17.
6. European Parliament. Directiva 2006/118/CE: On the protection of groundwater against pollution and deterioration. *Off. J. Eur. Union* **2006**, *L372*, 19–31.
7. European Parliament. Directive 2013/39/EC: Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* **2013**, *L226*, 1–17.
8. Wang, Q.-H.; Yu, L.-J.; Liu, Y.; Lin, L.; Lu, R.-G.; Zhu, J.-P.; He, L.; Lu, Z.-L. Methods for the detection and determination of nitrite and nitrate: A review. *Talanta* **2017**, *165*, 709–720. [[CrossRef](#)] [[PubMed](#)]
9. Singh, P.; Singh, M.K.; Beg, Y.R.; Nishad, G.R. A review on spectroscopic methods for determination of nitrite and nitrate in environmental samples. *Talanta* **2018**, *191*, 364–381. [[CrossRef](#)] [[PubMed](#)]
10. APHA–AWWA–WEF. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
11. Moldoveanu, S.C.; David, V. *Retention Mechanisms in Different HPLC Types in Essentials in Modern HPLC Separations*; Elsevier: Amsterdam, The Netherlands, 2013. [[CrossRef](#)]
12. Soleimani, M.; Yamini, Y.; Rad, F.M. A Simple and High Resolution Ion-Pair HPLC Method for Separation and Simultaneous Determination of Nitrate and Thiocyanate in Different Water Samples. *J. Chromatogr. Sci.* **2012**, *50*, 826–830. [[CrossRef](#)]
13. Khan, S.S.; Riaz, M. Determination of UV active inorganic anions in potable and high salinity water by ion pair reversed phase liquid chromatography. *Talanta* **2014**, *122*, 209–213. [[CrossRef](#)] [[PubMed](#)]
14. Horioka, Y.; Kusumoto, R.; Yamane, K.; Nomura, R.; Hirokawa, T.; Ito, K. Determination of Inorganic Anions in Seawater Samples by Ion Chromatography with Ultraviolet Detection Using Monolithic Octadecylsilyl Columns Coated with Dodecylammonium Cation. *Anal. Sci.* **2016**, *32*, 1123–1128. [[CrossRef](#)] [[PubMed](#)]
15. Sedyohutomo, A.; Suzuki, H.; Fujimoto, C. The Utilization of Triacetyl-Bonded Silica Coated with Imidazolium Ions for Capillary Ion Chromatographic Determination of Inorganic Anions. *Chromatography* **2021**, *42*, 119–126. [[CrossRef](#)]
16. Fitzhenry, C.; Jowett, L.; Roche, P.; Harrington, K.; Moore, B.; Paull, B.; Murray, E. Portable analyser using two-dimensional ion chromatography with ultra-violet light-emitting diode-based absorbance detection for nitrate monitoring within both saline and freshwaters. *J. Chromatogr. A* **2021**, *1652*, 462368. [[CrossRef](#)]
17. Moliner-Martinez, Y.; Herráez-Hernández, R.; Verdú-Andrés, J.; Molins-Legua, C.; Campíns-Falcó, P. Recent advances of in-tube solid-phase microextraction. *TrAC Trends Anal. Chem.* **2015**, *71*, 205–213. [[CrossRef](#)]
18. Moliner-Martinez, Y.; Ballester-Caudet, A.; Verdú-Andrés, J.; Herráez-Hernández, R.; Molins-Legua, C.; Campíns-Falcó, P. In-tube solid-phase microextraction in Solid-Phase Extraction. In *Handbooks in Separation Science*; Poole, C.F., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; Chapter 14; pp. 387–427. [[CrossRef](#)]
19. Fowkes, M.R. Planetary forecast: The roots of sustainability in the radical art of the 1970s. *Third Text* **2009**, *23*, 669–674. [[CrossRef](#)]
20. Gałuszka, A.; Migaszewski, Z.; Namiesnik, J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends Anal. Chem.* **2013**, *50*, 78–84. [[CrossRef](#)]
21. Horváth, I.T. Introduction: Sustainable Chemistry. *Chem. Rev.* **2018**, *118*, 369–371. [[CrossRef](#)]
22. Ballester-Caudet, A.; Campíns-Falcó, P.; Pérez, B.; Sancho, R.; Lorente, M.; Sastre, G.; González, C. A new tool for evaluating and/or selecting analytical methods: Summarizing the information in a hexagon. *TrAC Trends Anal. Chem.* **2019**, *118*, 538–547. [[CrossRef](#)]
23. Ballester-Caudet, A.; Navarro-Utiel, R.; Campos-Hernández, I.; Campíns-Falcó, P. Evaluation of the sample treatment influence in green and sustainable assessment of liquid chromatography methods by the HEXAGON tool: Sulfonate-based dyes determination in meat samples. *Green Anal. Chem.* **2022**, *3*, 100024. [[CrossRef](#)]
24. Hakobyan, L.; Monforte-Gómez, B.; Moliner-Martínez, Y.; Molins-Legua, C.; Campíns-Falcó, P. Improving Sustainability of the Griess Reaction by Reagent Stabilization on PDMS Membranes and ZnNPs as Reductor of Nitrates: Application to Different Water Samples. *Polymers* **2022**, *14*, 464. [[CrossRef](#)] [[PubMed](#)]
25. Kadlecová, Z.; Kalíková, K.; Folprechtová, D.; Tesařová, E.; Gilar, M. Method for evaluation of ionic interactions in liquid chromatography. *J. Chromatogr. A* **2020**, *1625*, 461301. [[CrossRef](#)] [[PubMed](#)]

26. Sulpizi, M.; Gageot, M.-P.; Sprik, M. The Silica–Water Interface: How the Silanols Determine the Surface Acidity and Modulate the Water Properties. *J. Chem. Theory Comput.* **2012**, *8*, 1037–1047. [[CrossRef](#)]
27. Beltrán, J.; Sanli, N.; Fonrodona, G.; Barrón, D.; Özkan, G.; Barbosa, J. Spectrophotometric, potentiometric and chromatographic pKa values of polyphenolic acids in water and acetonitrile–water media. *Anal. Chim. Acta* **2003**, *484*, 253–264. [[CrossRef](#)]
28. Borgo, L. Evaluation of buffers toxicity in tobacco cells: Homopiperazine-1,4-bis (2-ethanesulfonic acid) is a suitable buffer for plant cells studies at low pH. *Plant Physiol. Biochem.* **2017**, *115*, 119–125. [[CrossRef](#)]
29. Geiser, L.; Henchoz, Y.; Galland, A.; Carrupt, P.-A.; Veuthey, J.-L. Determination of pKa values by capillary zone electrophoresis with a dynamic coating procedure. *J. Sep. Sci.* **2005**, *28*, 2374–2380. [[CrossRef](#)]
30. Hollingsworth, C.A.; Seybold, P.G.; Hadad, C.M. Substituent effects on the electronic structure and pKa of benzoic acid. *Int. J. Quantum Chem.* **2002**, *90*, 1396–1403. [[CrossRef](#)]
31. Kabir, F.; Katayama, S.; Tanji, N.; Nakamura, S. Antimicrobial effects of chlorogenic acid and related compounds. *J. Korean Soc. Appl. Biol. Chem.* **2014**, *57*, 359–365. [[CrossRef](#)]

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