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Reversed-phase liquid chromatography/electrospray ionization/mass spectrometry with isotope dilution for the analysis of nitrate and nitrite in water

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ABSTRACT

A new method was developed for the analysis of nitrate and nitrite in a variety of water matrices by using reversed-phase liquid chromatography/electrospray ionization/mass spectrometry in the negative ion mode. For this direct analysis method, nitrate and nitrite anions were well separated under the optimized LC conditions, detected by monitoring m/z 62 and m/z 46 ions, and quantitated by using an isotope dilution technique that utilized the isotopically labeled analogs. The method sensitivity, accuracy, and precision were investigated, along with matrix effects resulting from common inorganic matrix anions. The isotope dilution technique, along with sample pretreatment using barium, silver, and hydrogen cartridges, effectively compensated for the ionization suppression caused by the major water matrix anions, including chloride, sulfate, phosphate, and carbonate. The method detection limits, based on seven reagent water replicates fortified at 0.01 mg N/L nitrate and 0.1 mg N/L nitrite, were 0.001 mg N/L for nitrate and 0.012–0.014 mg N/L for nitrite. The mean recoveries from the replicate fortified reagent water and lab water samples containing the major water matrix anions, were 92–103% for nitrate with an imprecision (relative standard deviation, RSD) of 0.4–2.1% and 92–110% for nitrite with an RSD of 1.1–4.4%. For the analysis of nitrate and nitrite in drinking water, surface water, and groundwater samples, the obtained results were generally consistent with those obtained from the reference methods. The mean recoveries from the replicate matrix spikes were 92–123% for nitrate with an RSD of 0.6–7.7% and 105–113% for nitrite with an RSD of 0.3–1.8%.

1. Introduction

Nitrate and nitrite in water, agricultural products, and food are of concern due to their adverse effects on human and animal health. In the drinking water standards established by U.S. Environmental Protection Agency (EPA), the maximum contamination levels are 10 mg N/L for nitrate and 1 mg N/L for nitrite, respectively [1]. Numerous methods using various analytical techniques have been reported for the analysis of nitrate and nitrite in a wide variety of sample matrices [2–9].

Of the several techniques commonly used for water analyses, the ion selective electrode is the simplest, fastest, and least expensive technique for nitrate [2]. Spectrophotometry is another current technique widely used because of its satisfactory sensitivity, low cost, simplicity, and speed. Various spectrophotometric or colorimetric methods have been reported for the analysis of nitrate in water, such as ultraviolet [2], Brucine sulfate reaction [4], salicylate reaction [10], and the methods based on the reduction of nitrate to nitrite [2,3]. The spectrophotometric methods based on the Griess reaction have been used for the analysis of both nitrite and nitrate after reduction to nitrite by cadmium [3], hydrazine sulfate [11], and nitrate reductase [12]. The limits of detection (LODs) for nitrite at 1.5–8.0 mg N/L and nitrate at 1.2–6.0 mg N/L, depending on the diazotization-coupling and adsorption conditions, were achieved by coupling a pre-concentration technique with an ion-pairing adsorbent column [13]. LODs of 0.5 μg/L for nitrite and 2.5 μg/L for nitrate have been obtained from a flow injection analysis (FIA) spectrophotometric method based on the oxidation of naphthol green B by potassium bromate and cadmium reduction [14]. An air-segmented continuous FIA spectrophotometric method, coupled with nitrate reductase, provided method detection limits of 0.001 mg N/L for nitrite and 0.006 mg N/L for the sum of nitrate and nitrite [12].

Ion chromatography/conductivity detection (IC/CD) is another current technique widely used for the analysis of nitrate and nitrite in water. The reported sensitivity varies with the instrumental conditions, matrix complexity, and sample pretreatment techniques. In general, the IC/CD methods are less sensitive than the spectrophotometric methods based on the Griess reaction [15]. However, an LOD of 0.40 μg/L was recently obtained for nitrate after cadmium reduction prior to the suppressed IC analysis [16].

Other technologies have been applied to the analysis of nitrate and nitrite in water. Coupled with 100-fold solid phase extraction

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concentration enrichment and conversion of nitrate into nitrophenols, spectrophotometry, reversed-phase liquid chromatography (LC), and gas chromatography/mass spectrometry (GC/MS) provided LODs of 10, 6, and 3 μg/L for nitrate, respectively, in environmental water samples [17]. Reversed-phase LC has rarely been used for the analysis of nitrate and nitrite in water. An ion-pairing LC method provided LODs of 5 μg/L for nitrate and 10 μg/L for nitrite for the simultaneous determination of nitrate and nitrite in dew, rain, snow, and lake water samples [18]. Gas-phase chemiluminescence with FIA using a membrane separator provided LODs of 0.7 μg/L for nitrate and 0.35 μg/L for nitrite in water [19]. ^{15}N-labeled nitrate was used for the determination of nitrate in rainwater by using particle-induced ionization MS [20]. IC/isotope dilution MS was used for the analysis of nitrate in Antarctic snow samples [21]. ^{15}N-labeled nitrate and nitrite were determined by using ion-pairing LC/thermospray/MS in a nitrogen metabolism study [22] and by FT-IR in a natural water nitrogen uptake study [23].

Nitrate and nitrite anions were also detected by electrospray ionization/mass spectrometry (ESI/MS) with the use of dicationic reagents [24]. ESI/MS/MS has recently been used in studies involving multiply-charged metal nitrate ions [25], doubly-charged cluster ions of sodium and potassium nitrate [26], and characterization of ammonium nitrate [27]. A few anions in human amniotic fluids were studied by using IC/ESI/MS/MS that provided an LOD of 50 μg/L for nitrate [28]. Recently, reversed-phase LC/ESI/MS/MS has been successfully used for the analysis of inorganic oxyhalides, such as bromate and perchlorate, at sub-μg/L concentrations in aqueous samples without the use of ion-pairing agents [29–31].

In this work, a new reversed-phase LC/ESI/MS method has been developed for the analysis of nitrate and nitrite in various water matrices. The method uses isotope dilution based on isotopically labeled analogs of nitrate and nitrite. The nitrate and nitrite anions were well separated by the selected LC column under a moderate pH condition. Derivatization was not necessary for this reported method. The study was focused on the selection and optimization of reversed-phase LC conditions, the demonstration of method performance (sensitivity, accuracy, and precision), and the investigation of matrix interferences resulting from common inorganic anions. The reported method was also compared with the reference methods commonly used for drinking water compliance analysis by analyzing selected real world water samples.

2. Experimental

2.1. Standards and reagents

1.0 mg N/mL nitrate and nitrite standard stock solutions were obtained from Inorganic Ventures, Inc. (Lakewood, NJ) and AccuStandard, Inc. (New Haven, CT). Isotopically labeled analogs sodium nitrate ($Na^{15}N^{18}O_3$) and nitrite ($Na^{15}NO_2$) were obtained from Sigma–Aldrich (Milwaukee, WI), which were used as the internal standards for nitrate and nitrite, respectively. The optima grade methanol and glacial acetic acid were obtained from Fisher Scientific (St. Louis, MO). Reagent water (18.2 MΩ cm resistance) was obtained from a Milli-Q treatment unit (Millipore, Bedford, MA). All other neat chemicals, including sodium chloride, sodium sulfate, potassium phosphate (monobasic), and sodium carbonate, were purchased from Fisher Scientific.

2.2. Sample preparation

Water samples were collected in 100 mL precleaned plastic bottles, refrigerated, and analyzed within two days. In order to eliminate potentially high concentrations of common inorganic anions, the samples were pretreated by eluting an aliquot of approximately 2 mL through Dionex OnGuard®II cartridges (Sunnyvale, CA). The barium (Ba), silver (Ag), and hydrogen (H) OnGuard®II cartridges, were placed in series prior to sample treatment. The Ba cartridges were used to remove sulfate, phosphate, and carbonate anions. The Ag cartridges were used to remove chloride, phosphate, and carbonate anions. The H cartridges were used to remove excess metal ions. Based on the manufacturer-recommended procedures, the sample aliquot was eluted through the cartridges at a speed of approximately 1 mL/min. 1 mL sample was then fortified with the internal standards at a constant concentration.

2.3. Sample analysis and calibration

The separation was carried out using a Waters Alliance 2695 HPLC system (Milford, MA) with a Phenomenex Gemini C18 column (2.0 mm × 150 mm, 3 μm) (Torrance, CA). A Phenomenex SecurityGuard with a Gemini C18 guard column (2 mm × 4 mm) was used to protect the analytical column. The LC conditions were optimized to obtain satisfactory peak shapes and sufficient separation of nitrate and nitrite anions. The mobile phase was an isocratic flow of 10:90 of methanol/0.1% acetic acid in reagent water at a flow rate of 0.25 mL/min. The LC run time was set for 15 min. The column temperature was set to 40 °C. The injection volume was 25 μL.

The detection was carried out using a Waters Quattro micro API triple quadrupole mass spectrometer. The instrument was calibrated by using a mixed solution of sodium iodide and rubidium iodide. The instrument was run in negative ion mode and was optimized to obtain sufficient sensitivity and mass resolution. The key optimized conditions included 2.4 kV capillary voltage, 45 V cone voltage, 130 °C ion source block temperature, 400 °C desolvation temperature, 750 L/h desolvation gas flow, and 50 L/h cone gas flow. The LM and HM resolutions were set to 12 for each quadrupole. The data acquisition was set in a selected ion recording (SIR) mode. In the optimized final method, four ions were monitored, which included m/z 69 for $^{15}N^{18}O_3^-$, m/z 47 for $^{15}NO_2^-$, m/z 62 for both NO_3^- and NO_2^-, and m/z 46 for both NO_3^- and NO_2^-. The interchannel delay was set to 0.01 s. The dwell time was set to 0.5 s. The data acquisition time was set to 15 min.

The samples were analyzed with a new calibration curve on a daily basis. The calibration curve was obtained by analyzing a series of standard solutions containing nitrate at 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 7.5, and 10 mg N/L, and nitrite at 0.1, 0.5, 1, 2, 5, 7.5, and 10 mg N/L, respectively. Each standard solution contained the internal standards at a constant concentration of 1.0 mg ^{15}N/L. One measurement was made at each concentration level. The internal standards were quantified using an external calibration method, which was based on the peak area of $^{15}N^{18}O_3^-$(m/z 69) and $^{15}NO_2^-$(m/z 47). An isotope dilution calibration method based on the peak areas was used for the quantitation of nitrate (m/z 62 and/or m/z 46) and nitrite (m/z 62 and/or m/z 46).

The analysis batch could also include a continuing calibration check sample, analyzed at the end of the analysis batch to verify that the instrument was properly calibrated throughout the analysis, an external quality control sample analyzed before samples to verify that the calibration standards were properly prepared, a laboratory method blank analyzed before samples to demonstrate that there was no carryover from the standards and no interference from the sample processing hardware and/or solvents including laboratory reagent water, and matrix spike/matrix spike duplicate samples analyzed to examine any potential matrix effects.

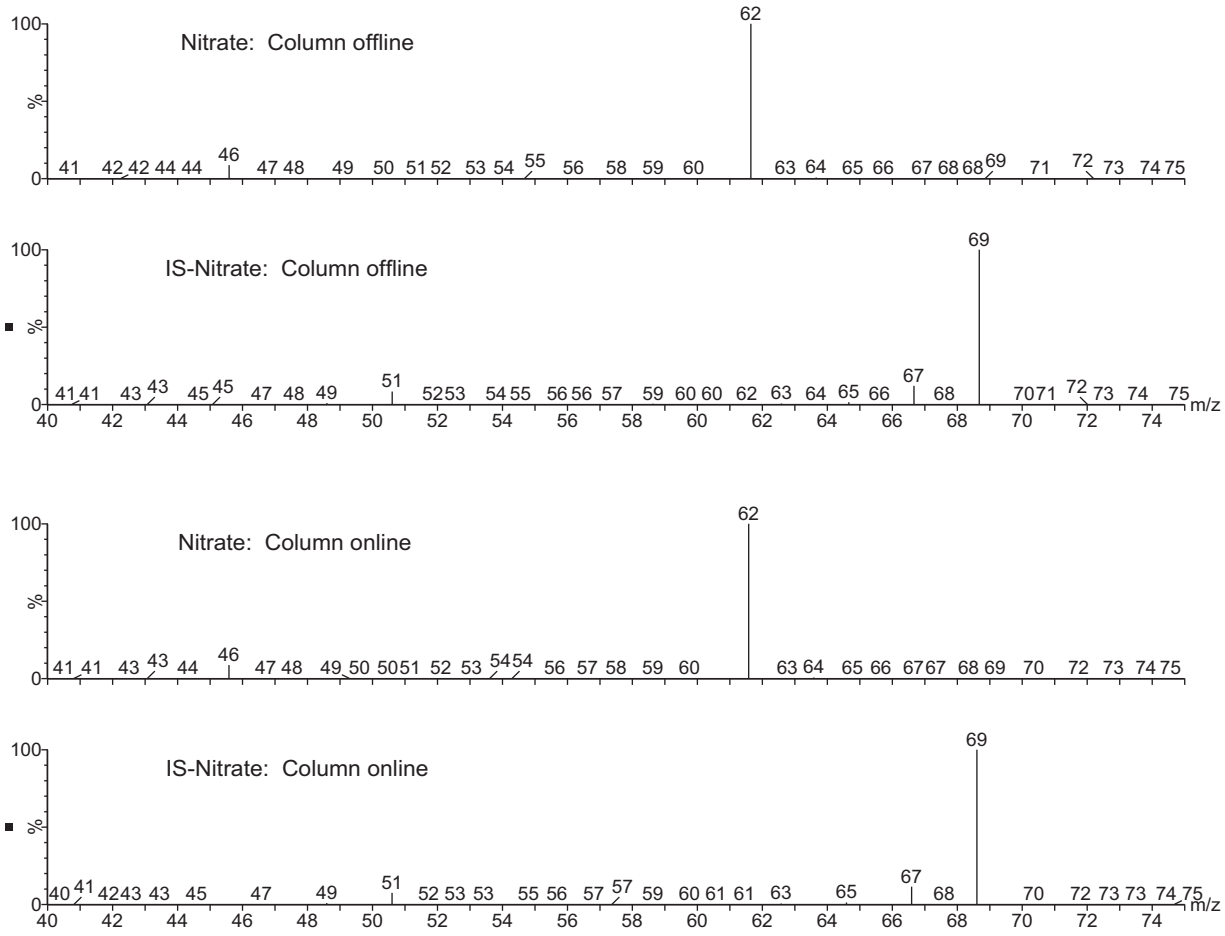

Fig. 1. ESI mass spectra of nitrate and labeled nitrate ($Na^{15}N^{18}O_3$). Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. *Y*-Axis: relative response.

3. Results and discussion

3.1. Reversed-phase LC/MS

The ESI mass spectra were initially investigated by infusing individual nitrite, nitrate, and the labeled analog standards. The mass spectra were further studied by analyzing the individual standards with and without an online column. As shown in Fig. 1, the nitrate standard results in two ions that are *m*/*z* 62 for NO_3^- and *m*/*z* 46 for NO_2^-, and the labeled nitrate standard results in two primary ions that are *m*/*z* 69 for $^{15}N^{18}O_3^-$ and *m*/*z* 51 for $^{15}N^{18}O_2^-$. The observed ion *m*/*z* 67 could result from $Na^{15}N^{18}O_2{}^{16}O$ that was probably present in the labeled nitrate standard as an impurity. This observation indicated that in the negative ESI process, both NO_3^- and $^{15}N^{18}O_3^-$ could form the lower mass ions by losing an ^{16}O and an ^{18}O, respectively. In addition, ions *m*/*z* 63 for $^{15}NO_3^-$ and *m*/*z* 47 for $^{15}NO_2^-$ forming from $^{15}NO_3^-$ losing an ^{16}O were also obtained for the labeled nitrate standard. These two ions are not shown in Fig. 1 due to their much lower ion counts. This can be rationalized to be due to the presence of $Na^{15}NO_3$ as the impurity in the $Na^{15}N^{18}O_3$ standard and the natural abundance of ^{15}N in nitrate.

As shown in Fig. 2, the column offline and online experiments result in different mass spectra for nitrite and ^{15}N-labeled nitrite. Without an online column, nitrite and labeled nitrite produced ions *m*/*z* 46 for NO_2^- and *m*/*z* 47 for $^{15}NO_2^-$, respectively. However, with an online column, both nitrite and labeled nitrite produced two ions. The nitrite standard resulted in ions *m*/*z* 46 for NO_2^- and *m*/*z* 62 for NO_3^-. The ^{15}N-labeled nitrite standard resulted in ions *m*/*z* 47 for $^{15}NO_2^-$ and *m*/*z* 63 for $^{15}NO_3^-$. This observation indicated that with the negative ESI, this particular chromatographic separation process resulted in the partial oxidation of NO_2^- and $^{15}NO_2^-$, which has not yet been fully understood. However, the experiments have demonstrated that the formation of NO_3^- from NO_2^- during the LC separation did not interfere with the accurate determination of nitrate because they were well separated, as shown in Fig. 3. Therefore, the formed NO_3^- could also be used as the quantitation ion for nitrite analysis.

Fig. 3 shows the chromatograms of nitrite, nitrate, and the internal standards in reagent water, which were obtained from the selected Gemini C18 column and the mobile phase of isocratic 10:90 methanol/0.1% acetic acid in reagent water at 0.25 mL/min. As shown in Fig. 3, nitrite and nitrate are well separated. The peaks appearing at 6.45 min include nitrite at 0.1 mg N/L (*m*/*z* 46 and *m*/*z* 62) and the labeled nitrite at 1.0 mg ^{15}N/L (*m*/*z* 47 and *m*/*z* 63). The peaks appearing at 9.90–9.94 min include *m*/*z* 62 and *m*/*z* 46 from nitrate at 0.1 mg N/L, *m*/*z* 69 and *m*/*z* 51 from the labeled nitrate at 1.0 mg ^{15}N/L, and *m*/*z* 63 and *m*/*z* 47 from $Na^{15}NO_3$ that could be present in the $Na^{15}N^{18}O_3$ standard. The separation was also confirmed by analyzing individual nitrite, nitrate, and the internal standards. In other words, in the absence of nitrate, nitrite at 0.1 mg N/L was detected only at approximately 6.45 min but no signal was detected at approximately 9.90–9.94 min, and vice versa. As shown in Fig. 3, *m*/*z* 69 and *m*/*z* 51 ions resulting from the $Na^{15}N^{18}O_3$ standard do not appear at 6.45 min.

In addition, the reagent water blank study demonstrated that no significantly increased peak areas of nitrate and nitrite resulted

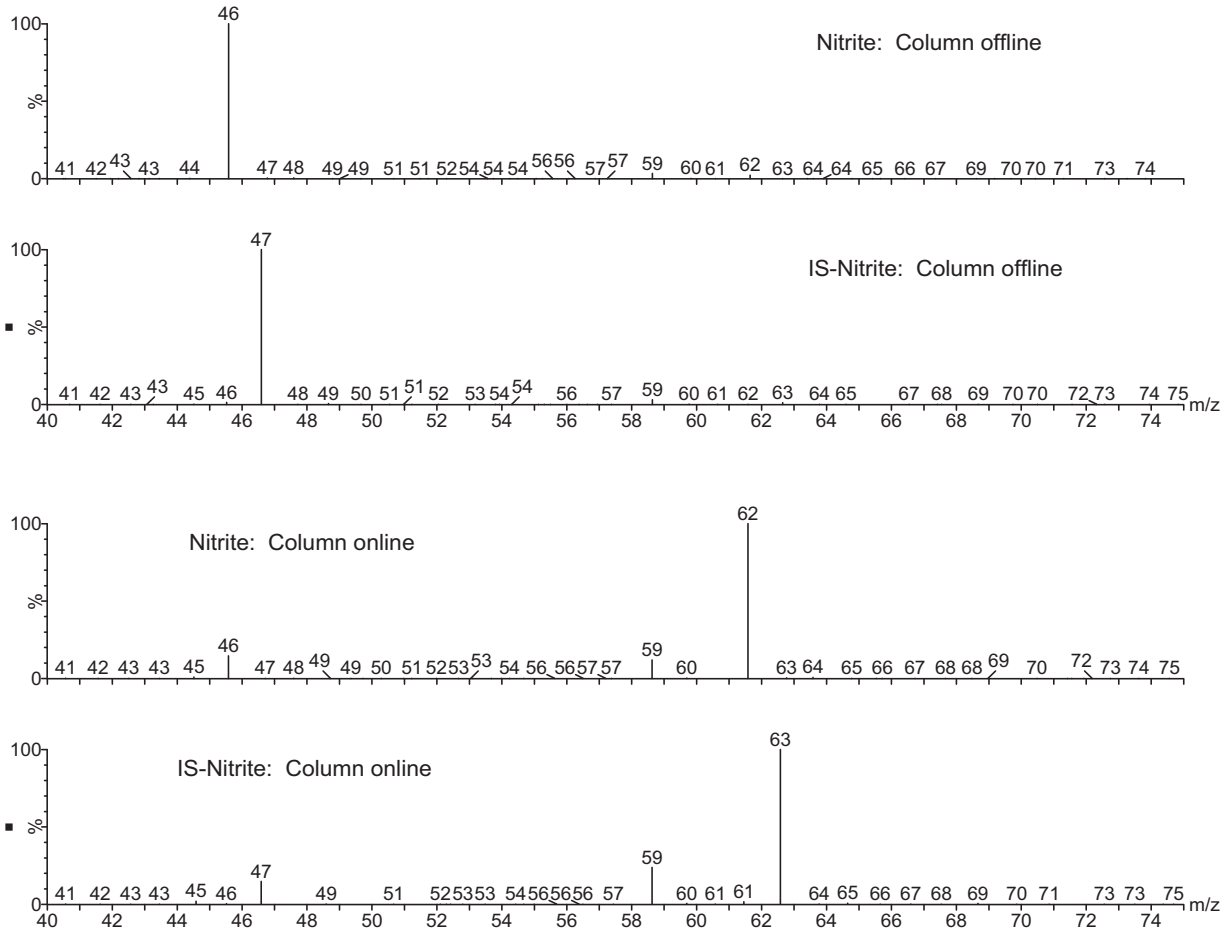

Fig. 2. ESI mass spectra of nitrite and labeled nitrite ($Na^{15}NO_2$). Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. *Y*-Axis: relative response.

from the contribution of the labeled analogs at the selected concentrations. It should be noted that column conditioning was critical for the separation of nitrate and nitrite. A batch-to-batch variation in retention time and peak shapes of nitrate and nitrite was often observed during the initial method development process. Frequent flushing of the column with reagent water, methanol, acetonitrile, and 0.1% acetic acid regenerated the column and resulted in sufficient separation, resolution, and reproducibility.

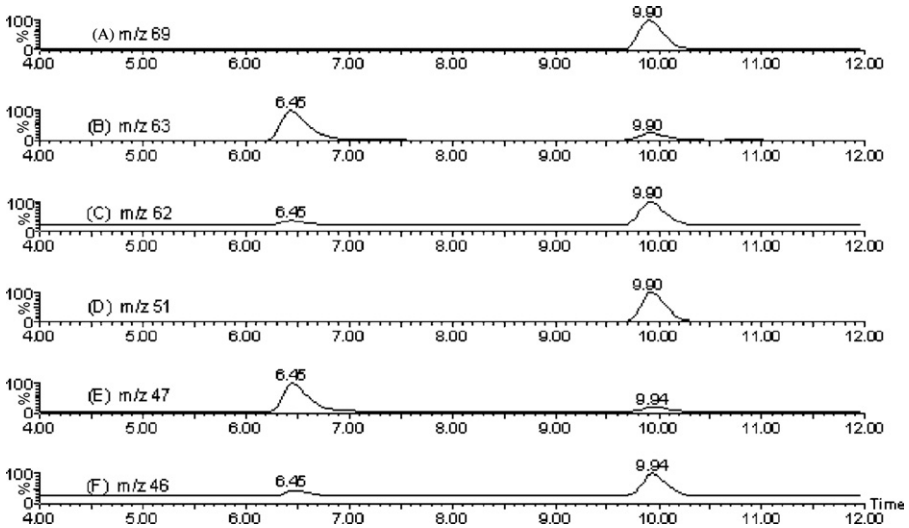

Fig. 3. Chromatograms of 0.1 mg N/L nitrate, 0.1 mg N/L nitrite, and 1.0 mg ^{15}N/L internal standards in reagent water using a Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. Retention time 6.45 min: nitrite (*m*/*z* 62 and *m*/*z* 46) and ^{15}N-labeled nitrite (*m*/*z* 63 and *m*/*z* 47); retention time 9.90–9.94 min: nitrate (*m*/*z* 62 and *m*/*z* 46) and ^{15}N- and ^{18}O-labeled nitrate (*m*/*z* 69, *m*/*z* 63, *m*/*z* 51, and *m*/*z* 47). *Y*-Axis: relative response.

3.2. Calibration curves

An isotope dilution technique was used to generate the calibration curves for nitrate and nitrite. The isotopically labeled nitrate and nitrite are the ideal internal standards for the quantitative analysis of nitrate and nitrite and can provide the ultimate compensation for instrumental performance variations and matrix interferences, primarily ionization suppression. The linear ranges were from 0.01 to 10 mg N/L with a linear regression correlation coefficient (r) of 0.9998 for nitrate and from 0.1 to 10 mg N/L with a linear regression correlation coefficient (r) of 0.9995 for nitrite. The calibration curve slopes were 1.158 for nitrate with a Y-axis intercept of −0.03047 and 1.049 for nitrite with a Y-axis intercept of 0.0009696.

3.3. Effects of common matrix anions

Chloride, sulfate, phosphate, and carbonate are typically considered the major common inorganic matrix anions that are often present at relatively high concentrations and potentially interfere with the analyses of other anions in drinking water. It is particularly important to investigate the effects of these anions on the LC/ESI/MS analysis of nitrate and nitrite because they can affect the chromatographic separation and can result in significant ionization suppression if they are not well separated from the target anions. In order to investigate the effects of these anions, a series of reagent water solutions, containing nitrate, nitrite, the internal standards, and these matrix anions at varying concentrations were studied.

First, coexisting common inorganic matrix anions affected the retention times and peak shapes of nitrate and nitrite. As shown in Table 1, the retention times of nitrate and nitrite, both present at 0.1 mg N/L, gradually decrease with the increase in the concentrations of chloride, sulfate, phosphate, and carbonate anions. However, more peak shifting was observed for nitrate. As shown in Fig. 4 in the presence of 50 mg/L chloride, sulfate, phosphate as P, and carbonate anions, broadening peaks are obtained for nitrate at 0.1 mg N/L (m/z 62 and m/z 46) appearing at approximately 9.2 min and nitrite at 0.1 mg N/L (m/z 46 and m/z 62) appearing at approximately 6.2 min, compared with Fig. 1 for reagent water. More severe peak broadening was observed for nitrate. The retention time shifting and peak broadening could significantly affect the detection and quantitation of nitrate and nitrite. Fig. 4 also shows that the peak shapes and retention times of nitrate and nitrite are quite consistent with the corresponding internal standards. Therefore, the isotope dilution technique could at least partially compensate for the negative impacts of these coexisting common matrix anions.

Table 1
Retention times (t_R) of nitrate and nitrite at 0.1 mg N/L in the presence of common anions.

Anion Conc (mg/L)	Nitrate t_R (min)				Nitrite t_R (min)			
	Cl^-	PO_4^{3-} [a]	SO_4^{2-}	CO_3^{2-}	Cl^-	PO_4^{3-} [a]	SO_4^{2-}	CO_3^{2-}
0	9.96	9.96	9.96	9.96	6.49	6.49	6.49	6.49
10	9.82	9.96	9.96	NS[b]	6.49	6.49	6.49	NS[b]
20	9.82	9.96	9.86	NS[b]	6.42	6.49	6.49	NS[b]
50	9.54	9.93	9.68	NS[b]	6.39	6.45	6.42	NS[b]
75	8.95	9.82	9.33	NS[b]	6.32	6.42	6.39	NS[b]
100	8.81	9.68	8.91	9.93	6.24	6.35	6.28	6.42
200	ND[c]	9.68	8.14	9.89	ND[c]	6.21	6.18	6.35
300	ND[c]	9.58	NS[b]	NS[b]	ND[c]	5.89	NS[b]	NS[b]
500	ND[c]	9.19	7.30	9.44	ND[c]	ND[c]	6.07	6.14
750	ND[c]	ND[c]	6.39	NS[b]	ND[c]	ND[c]	6.03	6.00
1000	ND[c]	ND[c]	5.37	8.91	ND[c]	ND[c]	ND [c]	5.89

[a] The concentration was described as mg P/L.
[b] Not studied.
[c] The retention time could not be determined.

Secondly, coexisting common inorganic matrix anions affected recoveries of nitrate and nitrite. Figs. 5 and 6 show the effects of chloride, sulfate, phosphate, and carbonate anions on the recoveries of nitrate and nitrite at 0.1 mg N/L. The internal standards, both $^{15}NO_2^-$ and $^{15}N^{18}O_3^-$, were present at 1.0 mg ^{15}N/L. As shown in Fig. 5, in the studied concentration ranges, sulfate anion (10–1000 mg/L) does not significantly affect the recoveries of 0.1 mg N/L nitrate. However, the recoveries of nitrate gradually decreased with the increase in the concentrations of chloride (10–100 mg/L), carbonate (100–1000 mg/L), and phosphate (75–500 mg P/L) anions. As shown in Fig. 6, in the studied concentration ranges, sulfate (10–500 mg/L), phosphate (10–200 mg P/L), and carbonate (100–1000 mg/L) anions do not significantly affect the recoveries of 0.1 mg N/L nitrite. However, the recoveries of nitrite slightly decreased with the increase in the concentrations of chloride anion (10–100 mg/L). The obtained recoveries of nitrite were only 62% in the presence of sulfate anion at 750 mg/L and 35% in the presence of phosphate anion at 300 mg P/L, respectively.

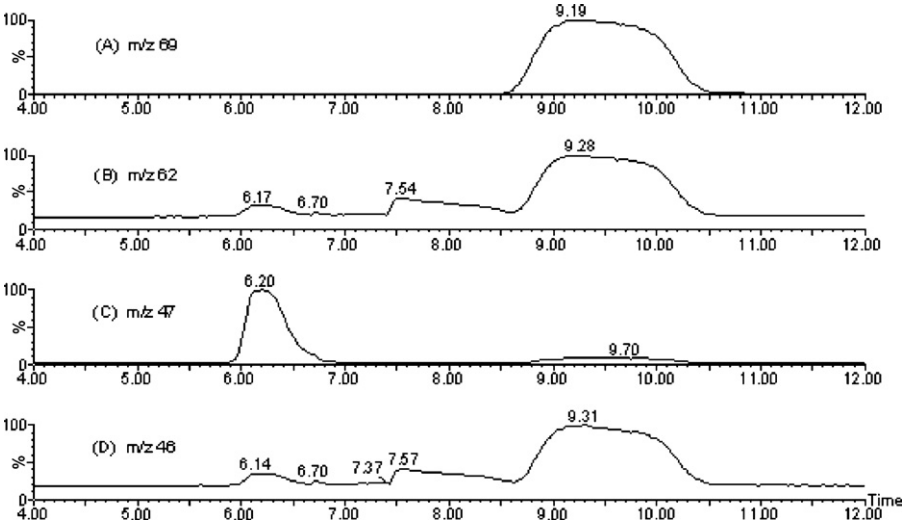

Fig. 4. Chromatograms of 0.1 mg N/L nitrate, 0.1 mg N/L nitrite, and 1.0 mg ^{15}N/L internal standards in lab water A using a Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. Lab water A contained 50 mg/L of chloride, sulfate, phosphate as P, and carbonate anions. Retention time 6.1–6.2 min: nitrite (m/z 62 and m/z 46) and ^{15}N-labeled nitrite (m/z 47); retention time 9.2–9.3 min: nitrate (m/z 62 and m/z 46) and ^{15}N- and ^{18}O-labeled nitrate (m/z 69 and m/z 47). Y-Axis: relative response.

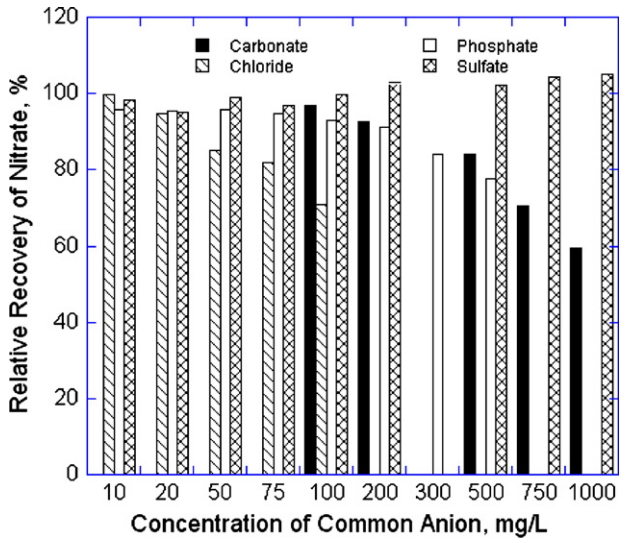

Fig. 5. Effects of chloride, sulfate, phosphate, and carbonate anions on the recoveries of 0.1 mg N/L nitrate using a Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. The internal standard ^{15}N- and ^{18}O-labeled nitrate was at 1.0 mg ^{15}N/L.

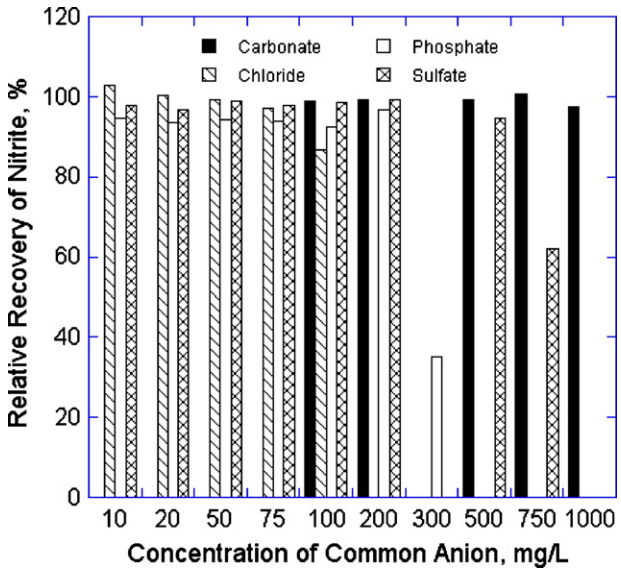

Fig. 6. Effects of chloride, sulfate, phosphate, and carbonate anions on the recoveries of 0.1 mg N/L nitrite using a Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. The internal standard ^{15}N-labeled nitrite was at 1.0 mg ^{15}N/L.

As a result, in order to accurately determine nitrate and nitrite in water, it was necessary to remove these common matrix anions when present at relatively high concentrations.

Fig. 7 shows the chromatograms of nitrate, nitrite, and the internal standards in the presence of 1000 mg/L of chloride, sulfate, phosphate as P, and carbonate anions after pretreatment with the Ba, Ag, and H cartridges. As shown in Fig. 7, the removal of these matrix anions significantly improves the peak shapes of nitrate and nitrite. Compared with Fig. 2, sharper peaks were obtained for ions (m/z 62 and m/z 46) of nitrate and nitrite, both at 0.1 mg N/L. Their peak shapes and retention times were also consistent with the corresponding internal standards $^{15}N^{18}O_3^-$ (m/z 69) at 1.0 mg ^{15}N/L and $^{15}NO_2^-$ (m/z 47), both at 1.0 mg ^{15}N/L, respectively. Therefore, the cartridge pretreatment technique, along with the isotope dilution technique, could significantly reduce the effects of these coexisting common inorganic matrix anions, which could subsequently improve the method performance (sensitivity, accuracy, and precision).

Moreover, unknown organic acids and other organic compounds may be present in finished drinking water and source water. However, these are generally present at much lower concentrations, and should result in less ionization suppression in the analysis of nitrate and nitrite. Under the optimized LC column and mobile phase condition, many organic species will not be able to elute from the column. In order to minimize the effects of potentially accumulated organic species, the column was flushed with pure methanol for 30 min after each analysis batch. As long as the internal standards pass the quality control criteria, they should be able to effectively compensate for the ionization suppression and other associated effects caused by unknown organic species.

Finally, the matrix effects of the reversed-phase LC/ESI/MS method on the analysis of nitrate and nitrite in waters will primarily

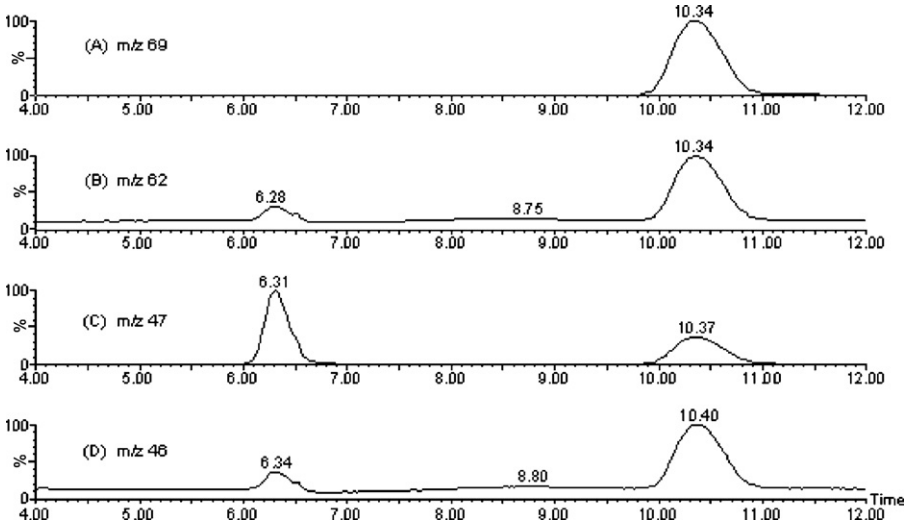

Fig. 7. Chromatograms of 0.1 mg N/L nitrate, 0.1 mg N/L nitrite, and 1.0 mg ^{15}N/L internal standards in lab water B using a Phenomenex Gemini C18 column (2.1 mm × 150 mm, 3 μm) and 10:90 of methanol/0.1% acetic acid in reagent water at 0.25 mL/min as the mobile phase. Lab water B contained 1000 mg/L of chloride, sulfate, phosphate as P, and carbonate anions, which was pretreated with the Ba, Ag, and H cartridges. Retention time 6.1–6.2 min: nitrite (m/z 62 and m/z 46) and ^{15}N-labeled nitrite (m/z 47); retention time 9.2–9.3 min: nitrate (m/z 62 and m/z 46) and ^{15}N- and ^{18}O-labeled nitrate (m/z 69 and m/z 47). *Y*-Axis: relative response.

Table 2
Method detection limit data ($n = 7$).

Matrix	Mass (m/z)	Spike Conc (mg N/L)	Nitrate			Nitrite		
			Mean Rec (%)	MDL (mg N/L)	PtP S/N[a]	Mean Rec (%)	MDL (mg N/L)	PtP S/N[a]
Reagent water	62	0.01	106	0.001	70	ND[b]	ND[b]	ND[b]
	46	0.01	106	0.001	56	ND[b]	ND[b]	ND[b]
	62	0.1	NC[c]	NC[c]	NC[c]	102	0.014	13
	46	0.1	NC[c]	NC[c]	NC[c]	107	0.012	13
Lab water A[d]	62	0.1	86	0.011	84	103	0.011	11
	46	0.1	90	0.011	65	98	0.005	11
Lab water B[e]	62	0.1	97	0.015	142	101	0.012	23
	46	0.1	99	0.009	114	100	0.013	16

[a] Peak-to-peak signal/noise ratio.
[b] Not determined because the spike concentration was too low.
[c] Not calculated because the spike concentration was too high.
[d] Lab water A containing 50 mg/L of Cl^-, PO_4^{3-} as P, SO_4^{2-}, and CO_3^{2-} anions.
[e] Lab water B containing 1000 mg/L of Cl^-, PO_4^{3-} as P, SO_4^{2-}, and CO_3^{2-} anions, which was pretreated with the Ba, Ag, and H cartridges.

depend on how well they are separated from each other and from interferences as well as concentration levels of interferences. When different LC columns and mobile phases are used, coexisting common matrix anions and organic species may cause different extent of retention time shifting, peak broadening, and ionization suppression, which will subsequently be associated with the sensitivity, accuracy, and precision of the nitrate and nitrite analyses.

3.4. Sensitivity, accuracy, and precision

Method sensitivity, accuracy, and precision were investigated by analyzing replicate laboratory fortified blanks (LFBs) in both reagent water and lab water prepared by dissolving the inorganic chemicals into reagent water. In lab water A, the concentrations of chloride, sulfate, phosphate as P, and carbonate anions were 50 mg/L. In lab water B, the concentrations of chloride, sulfate, phosphate as P, and carbonate anions were 1000 mg/L, which was much higher than the concentrations of these common anions that were usually detected in drinking water samples at this laboratory. The LFBs in lab water B were pretreated with the Ba, Ag, and H cartridges prior to analysis. The method detection limit (MDL), as defined in the U.S. Federal Code of Regulations, is based upon the precision of replicate injections for an analyte [32]. The MDLs in this paper were calculated based on the measurement of seven replicate LFBs spiked with nitrate at 0.01–0.1 mg N/L and nitrite at 0.1 mg N/L and were calculated from 3.14 times the standard deviation of the analyses (3.14 is the Student's t-value for the 99% confidence level with $n - 1$ degrees of freedom).

Table 2 indicates that the calculated MDLs for nitrate are 0.001 mg N/L for reagent water, 0.011 mg N/L for lab water A, and 0.009–0.015 mg N/L for lab water B, depending on the selected quantitation masses. The calculated MDLs for nitrite were 0.012–0.014 mg N/L for reagent water, 0.005–0.011 mg N/L for lab water A, and 0.012–0.013 mg N/L for lab water B, which was also dependent on the selected quantitation ions. Good recoveries were obtained for both nitrate and nitrite at such low concentration levels. The mean recoveries were 86–106% for nitrate at 0.01 mg N/L in reagent water and at 0.1 mg N/L in the lab waters, and 98–107% for nitrite at 0.1 mg N/L in reagent water and the lab waters.

It should be noted that lower fortification concentration levels were not studied for the measurement of MDLs because of the concentration levels of nitrate and nitrite resulting from the mobile phase and injector rinsing solvents. As shown in Table 2, the peak-to-peak signal/noise ratios (PtP S/N) of nitrate and nitrite resulting from the fortified concentrations are larger than 10, which indicates that the real LODs should be much lower than the studied concentration levels, particularly for nitrate. The relatively large PtP S/N ratios resulting from nitrate anion in lab water B could be rationalized in part as being due to the nitrate levels present in the chloride, sulfate, phosphate, and carbonate salts used to prepare lab water B.

The percent mean recovery and relative standard deviation (RSD) were measured based on four replicate LFBs in reagent water and lab water. Nitrate and nitrite were spiked at different concentrations. Similarly, the LFBs in lab water B were pretreated with the Ba, Ag, and H cartridges prior to analysis. As shown in Table 3, nitrate has a mean recovery of 92–103% with an RSD of 0.4–2.1% and nitrite has a mean relative recovery of 92–110% with an RSD of 1.1–4.4% for all the measurements depending on the spiking levels and water matrices.

Table 3
Method accuracy and precision data ($n = 4$).

Matrix	Mass (m/z)	Spike Conc (mg N/L)	Nitrate		Nitrite	
			Mean Rec (%)	RSD (%)	Mean Rec (%)	RSD (%)
Reagent water	62	0.1	97	1.8	102	4.4
	46	0.1	97	0.7	107	3.6
	62	1.0	96	0.5	110	1.8
	46	1.0	96	0.4	99	1.4
	62	10.0	92	1.4	92	1.6
	46	10.0	92	1.6	96	1.1
Lab water A[a]	62	1.0	99	0.7	103	1.1
	46	1.0	98	1.7	98	1.8
Lab water B[b]	62	1.0	103	1.4	101	3.1
	46	1.0	102	2.1	96	2.6

[a] Lab water containing 50 mg/L of Cl^-, PO_4^{3-} as P, SO_4^{2-}, and CO_3^{2-} anions.
[b] Lab water containing 1000 mg/L of Cl^-, PO_4^{3-} as P, SO_4^{2-}, and CO_3^{2-} anions, which was pretreated with the Ba, Ag, and H cartridges.

Table 4
Nitrate and nitrite results of real world water samples ($n = 3$) and matrix spikes ($n = 4$).

Sample	EPA 353.2	EPA 300.0	LC/ESI/MS			
	Mean Conc (mg N/L)	Mean Conc (mg N/L)	Conc (mg N/L)	Mean Conc (mg N/L)	Spike Rec (%)	Mean RSD (%)
Nitrate						
Drinking water A	<0.1	<0.5	0.04	1.0	103	0.6
Drinking water B	1.02	0.98	1.01	1.0	99	1.2
River water A	1.49	1.39	1.44	1.0	123	0.7
River water B	1.07	1.01	1.03	1.0	109	1.0
Well water A	16.2	14.6	16.1	1.0	92	7.7
Well water B	0.30	0.26	0.27	1.0	99	2.0
Nitrite						
Drinking water A	<0.01	<0.5	<0.1	1.0	109	1.5
Drinking water B	<0.01	<0.5	<0.1	1.0	108	1.8
River water A	0.01	<0.5	<0.1	1.0	112	1.2
River water B	<0.01	<0.5	<0.1	1.0	110	0.3
Well water A	<0.01	<0.5	<0.1	1.0	113	0.4
Well water B	<0.01	<0.5	<0.1	1.0	105	1.0

Tables 2 and 3 indicate that without the need for sample pretreatment, nitrate and nitrite at 0.1 mg N/L or higher concentrations can be directly analyzed by the reported method, as long as the presence of the matrix anions is at 50 mg/L or less. The results also indicate that with pretreatment, nitrate and nitrite at 0.1 mg N/L or higher concentrations can be quantitatively analyzed by the reported method in the presence of these common matrix anions at concentrations of 1000 mg/L or less.

3.5. Water sample studies

Two finished drinking water samples, two river water samples, and two groundwater samples were selected to evaluate the performance of the described LC/ESI/MS method in a side-by-side comparison with EPA Methods 353.2 and 300.0. Three replicates of each sample were used to measure the mean concentrations of nitrate and nitrite. Four replicate matrix spikes fortified at 1.0 mg N/L for each sample matrix were analyzed to evaluate the method accuracy and precision. As shown in Table 4, the concentrations of nitrate and nitrite resulting from the reported LC/ESI/MS method are basically consistent with those resulting from the reference methods. 0.04 mg N/L of nitrate in drinking water A was detected by the LC/ESI/MS method, but was not detected by the comparison methods because it was lower than the minimal reporting levels. For nitrite, the new LC/ESI/MS method was slightly less sensitive than EPA Method 353.2 but more sensitive than EPA Method 300.0. Nitrite was only detected at 0.01 mg N/L in river water sample A by EPA Method 353.2 but was not detected in any of the other samples. As shown in Table 3, the reported LC/ESI/MS method also demonstrated good accuracy and precision for matrix spikes, depending on the water sample matrices. For nitrate, a mean recovery of 92–123% was obtained with an RSD of 0.6–7.7%. For nitrite, a mean recovery of 105–113% was obtained with an RSD of 0.3–1.8%.

4. Conclusions

This paper demonstrates a new method for the analysis of nitrate and nitrite in finished drinking water, surface water, and groundwater. Nitrate and nitrite anions were well separated under optimized reversed-phase LC conditions within 12 min and were specifically detected by negative ESI/MS. The two ions (m/z 62 and m/z 46), in conjunction with isotope dilution, provided additional specificity to the analysis of nitrate and nitrite. The isotope dilution approach, along with the cartridge pretreatment technique used for the removal of high concentrations of common inorganic matrix anions, was effective for compensation of instrumental performance variations and matrix effects, primarily ionization suppression, peak broadening, and retention time shifting. Satisfactory accuracy and precision were obtained for all the studied water matrices, including real world water samples. This new analytical method is capable of meeting the sensitivity requirements for drinking water compliance analysis for nitrate and nitrite.

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