Research Article

Determination of Nitrite and Nitrate in Water and Leafy Vegetable Samples using Ion Chromatography with Conductivity Detection

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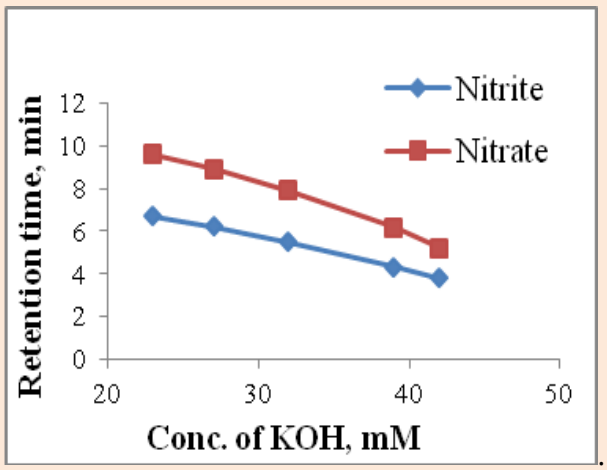
Abstract

Prolonged intake of nitrate from our diet generates gastric problems and causes cancer due to the formations of nitrosamines. Thus the primary aim of this study was to develop a new accurate, reliable and robust ion chromatography method for estimation of nitrite and nitrate. The newly developed method was applied to water and selected leafy vegetables collected from organic farming market and local vegetable market to measure levels of nitrate and nitrite. Nitrite and nitrate anions were separated on an ion Pac AS18 column by anion exchange technique. Potassium hydroxide was used as an eluent (39 mM at 1 mL/min) and detected by DS-6 suppressed conductivity detector. The calibration curve was linear over the range 1-10 ppm of nitrite and 1-100 ppm of nitrate and correlation coefficient was 0.999 for both nitrite and nitrate. The detection limits were 0.016 and 0.017 ppm for nitrite and nitrate respectively and the relative standard deviations for the two ions were less than 2 %. Higher nitrate and nitrite content were observed for vegetables sampled from local market. The ion chromatography based assay shown higher efficiency and better recovery for determination of nitrate and nitrite levels in water and leafy vegetables.

Keywords: Ion Chromatography, nitrite and nitrate, eluent, conductivity detector.



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Introduction

Today every country of the World is growing towards the global industrialization. The impact of this industrialization growth increases heavy pollution in the form of solid and liquid waste. The effect of nitrite and nitrate on living body is of great concern. Nitrate or nitrite contamination in water is most often due to contact with natural minerals, agricultural runoff or industrial processes. The concentration levels of nitrite and nitrate in water and soil environment are important indicators of water quality and are also associated with eutrophication and blooms [1, 2]. Ingested nitrites and nitrates also have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines. Nitrites can induce methemoglobinemia in humans, particularly in young infants. The harmful impact of nitrite is due to its interaction with blood pigment to produce methemoglobinemia. The consumption of high nitrate or nitrite levels from water prevents the transport of oxygen through the body and can result in sudden death in livestock or the possibility of abortion in pregnant animals [3, 4, 5].

The determination of nitrite and nitrate plays an important role in the analysis of water and vegetables. Several methods have been reviewed for the quantitative determination of nitrate and nitrite including ultraviolet and visible spectrophotometry, fluorimetry, electrochemistry, chemiluminescence, gas chromatography, liquid chromatography and capillary electrophoresis [6, 7]. HPLC is a widely used technique for determination of inorganic anions [8]. Determination of NO_2^- and NO_3^- anions from different matrices has been performed using ion chromatography (IC) by many investigators which involve separation of ions on an ion exchange column followed by suppressor column

and a conductivity detector. The EPA has approved IC methods 300.0 and B-1011 for the analysis of nitrite and nitrate in drinking water [9, 10].

The majorities of classical methods are much more time-consuming and laborious than ion chromatography and sometimes require the use of expensive and toxic reagents. Quantitative analysis of nitrite ions can be very difficult or impossible due to masking of nitrite peak by chloride ion peak. Similarly, retention times of bromide and phosphate ions are close to nitrate ions, thus, difficult to resolve them [11, 12, 13]. It becomes difficult to determine these ion concentrations accurately in some vegetable samples. Separation and determination of nitrate and nitrite ions by ion chromatography is carried out in anion-exchange columns or bifunctional column filled with a suitable exchanger and using a proper eluent, e.g. water solution of sodium carbonate and/or sodium hydro carbonate, sodium chloride and most often with conductometric or UV detection [14, 15].

The aim of this study was to develop and validate ion chromatographic method for estimation of nitrite and nitrate in water and common leafy vegetables collected from organic farming market and local market. Vegetables from local market grown with excessive nitrogen fertilizer contain much more nitrate. Accumulation of nitrate in soil is due to low availability of phosphorus and potassium [16]. The excessive use of inorganic fertilizers combined with the mismanagement of natural resources results in perturbation of both local and global nitrogen cycles [17]. Thus present research results can contribute in awareness to the farmers that they should provide limited supply of nitrogenous fertilizer to their soil to protect human health. High nitrate concentrations initially present in vegetables can be decreased during the treatment of food by utilization of the ability of nitrates and nitrites to dissolve in water [18]. Novelty of work is that boil water method was used to extract nitrite and nitrate from leafy vegetables rather than simple washing and blending. After boiling, silver sulphate was used to remove chloride impurity in the form of silver chloride precipitate.

Experimental

Materials and methods

All chemicals used were of analytical grade. Respective salts obtained from Fisher Scientific India were used for preparation of anion standard solutions. Regenerating solution (0.2 N H_2SO_4) was prepared using concentrated sulfuric acid. Deionized water with a specific resistance of 18.2 MΩ.cm from milli-Q water purification system (Millipore, Bedford, USA) was used for preparation of all aqueous solutions. All standard solutions were stored in quartz glassware.

Nitrate and nitrite stock standard solutions (1000 ppm) were prepared in 1000 ml volumetric flask by dissolving potassium nitrate (0.1629 g) and sodium nitrite (0.1500 g) in milli-Q water. Standard stock solution was diluted to a series of concentrations containing 1, 2, 4, 6, 8, 10, 50 and 100 ppm of nitrite and nitrate and stored at 4 ^{0}C. Eluent stock solution (39 mM KOH) was prepared from 50 % (w/w) aqueous potassium hydroxide solution. The solutions were prepared freshly after every seven days.

Sample preparation for analysis

All leafy vegetable samples including spinach, coriander and fenugreek were brought from organic farming market and local market. The vegetable samples were cleaned carefully rinsed with tap water and then with deionized water. The samples were divided into smaller parts using a mixer. The high solubility of ionic species in water means that such solutes can often be removed from solid samples prior to IC analysis simply by aqueous extraction of the finely divided sample. For estimation of vegetables, 5 g vegetable slurry was weighed and 100 ml deionized water was added and kept for 5 min in a boiling water bath. After homogenizing in blender for 2 min, the volume was diluted to 250 ml after centrifuge. All samples were filtered through 0.45 µm membrane filter before use. Water was used as an extracting solution to avoid introduction of extraneous peaks into the final chromatogram [19]. The water samples collected for Ion Chromatographic (IC) analysis not required any sample pretreatment. Drinking-water samples were only filtered through a membrane filter to remove particulates [20].

Apparatus and Methodology

For IC sample analysis, a Dionex ICS-1100 (USA) ion chromatograph was used which contains a built-in Eluent Regeneration (ER) Controller and pre-plumbed tubing for operation in the RFIC-ER (Reagent-Free Ion

Chromatography with eluent regeneration) mode. RFIC-ER technology uses the suppressor to regenerate returning eluent. The instrument was equipped with an anion self-regenerating suppressor column, ASRS-Ultra 4 mm and a flow-through, temperature-condensed conductivity detector. External water mode was used for suppressor. Analyte chromatograms were plotted, and data was handled using a Chromeleon software (version 6.80 SR6a or later). Chromeleon also provides data acquisition and data processing functions. Ion Pac AS18 (250 mm × 4.0 mm) analytical column and Ion Pac AG18 (50 mm × 4.0 mm) guard column were used for this work.
The sample was loaded into the 25 µl sample loop via 1 mL hypodermic syringe. The sample was then injected into a flowing stream of 39 mM potassium hydroxide eluent with a flow rate 1 mL/min. The sample was pumped through two different ion exchange columns, then a conductivity suppressor device and into a conductivity detector.

Results and discussion

Optimization of eluent concentration and flow rate

The two ion exchange columns, a precolumn or guard column and a separator column are packed with an anion exchange resin. Ions are separated into discrete bands based on their affinity for the exchange sites of the resin. Then the ions with eluent flow through anion suppressor unit, where the hydroxide eluent is converted to water and the analyte ions converted to corresponding acids. The separated ions with the eluent pass through the conductivity detector where the conductance due to NO_2^- and NO_3^- ions was recorded. Quantitation was accomplished by measuring the peak area and comparing it to a calibration curve generated from known standards.

Separation of NO_2^- and NO_3^- can be improved by adjusting the eluent concentration and flow rate. Different concentrations (23, 27, 32, 39, 42 mM) of the KOH eluent at different flow rates (0.5, 1, 1.5 ml/min) were tried in order to achieve separation of nitrite peak from nitrate peak on running IC chromatograms. As shown in **Figure 1** the 39 mM KOH gives proper resolution. On 23, 27, & 32 mM KOH higher resolution was achieved but retention time becomes long and 42 mM KOH gives less resolution. **Figure 2** shows that 1 ml/min was the preferred flow rate. At 0.5 ml/min flow rate retention time was long and at 1.5 ml/min flow rate, proper resolution was not achieved. So the 39 mM KOH and 1 ml/min flow rate was considered as optimum condition.

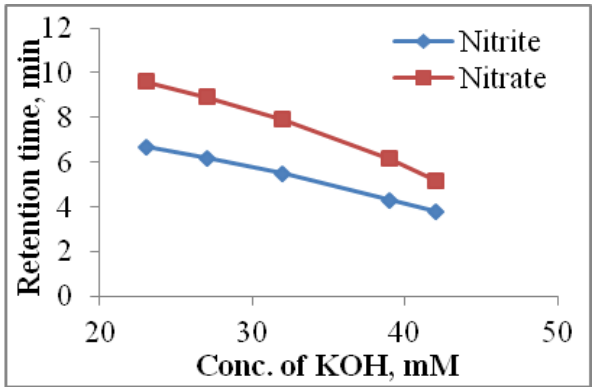

Figure 1 Effect of various concentration of KOH eluent on retention times of nitrite and nitrate

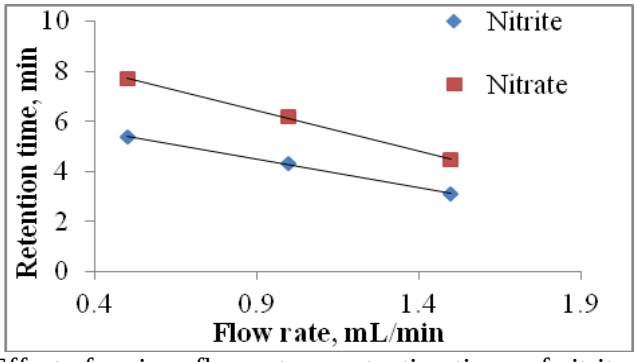

Figure 2 Effect of various flow rate on retention times of nitrite and nitrate

Development of IC method for nitrite and nitrate estimation

The developed IC method using 39 mM KOH as eluent was applied on water and vegetable samples. The prepared sample solutions were sonicated and filtered through 0.45 µm membrane filter before injection and a flow rate of 1.0 ml/min was applied. The other related IC conditions of the method were as described previously. The total analytical time of the method for one sample analysis was within 12 min. The retention times of nitrite and nitrate were 4.307±0.02 and 6.171±0.03 min, respectively. **Figure 3** shows the resolved peaks of both the analytes on the developed IC method.

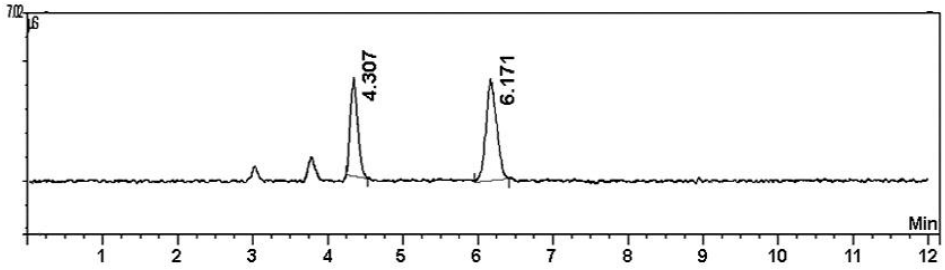

Figure 3 A resolved peak of nitrite and nitrate

The linearity of the standard curve

Figures 4 and **5** provided the standard curves of nitrite and nitrate. Linearity was obtained over the tested concentration range of 1, 2, 4, 6 and 10 and 1, 5, 10, 50 and 100 ppm of nitrite and nitrate, respectively. The linear regression equations of nitrite and nitrate standard curves were calculated as y = 0.204x + 0.002 (R^2 = 0.999) and y = 0.191x + 0.033 (R^2 = 0.999) resolutions. The correlation coefficients were both greater than 0.999, which showed a very good linearity within the range receptive to nitrite and nitrate. y is the value of peak area and x is the value of various concentrations of standard solutions.

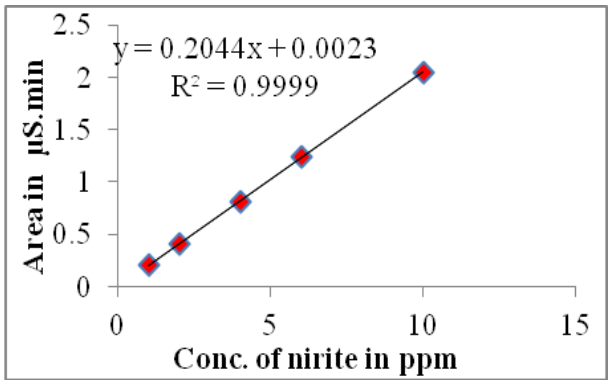

Figure 4 Standard curve of nitrite

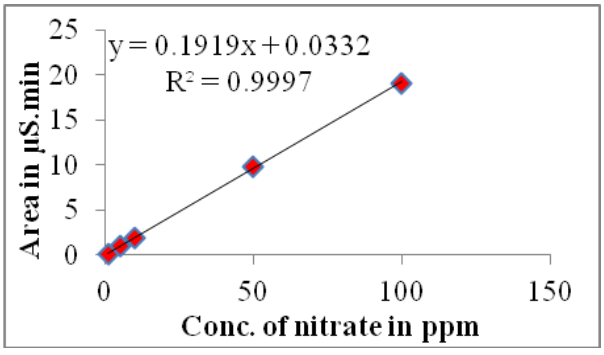

Figure 5 Standard curve of nitrate

Reproducibility

Reproducibility of the measurements was evaluated by intra-day and inter-day analysis calculated from the results of 3 replicates and illustrated by the coefficient of variations (% RSD), as shown in **Tables 1** and **2**. Repeated trails were carried and the obtained % RSD values are observed less than 2.0, pointing out high degrees of reproducibility.

Table 1 Reproducibility results of intra-day and inter-day analysis for nitrite (n=3)

Conc. of Nitrite in ppm	Intra-day	Inter-day
	% RSD	% RSD
1	0.69	1.48
2	0.35	1.63
4	0.30	0.20
6	1.72	1.38
10	0.57	0.95

Table 2 Reproducibility results of intra-day and inter-day analysis for nitrate (n=3)

Conc. of Nitrate in ppm	Intra-day	Inter-day
	% RSD	% RSD
1	1.31	0.74
5	0.19	0.17
10	0.71	1.14
50	0.63	0.42
100	1.19	1.48

Recovery

The recoveries of nitrite and nitrate in the study are shown in **Tables 3** and **4**. The recoveries of nitrite for five concentrations (1, 2, 4, 6 and 10 ppm) into vegetable samples were in the range of 99.34 to 100.19 % and nitrate for five concentrations (1, 5, 10, 50 and 100 ppm) into vegetable samples were in the range of 98.06 to 99.96 %. The average recoveries of nitrite and nitrate were 99.82 % and 99.92 % respectively, indicating that the method is quite accurate.

Table 3 Recovery results for nitrite analysis (n=3)

Spiked level	% Average recovery	% RSD
1	99.98	0.92
2	100.19	0.42
4	99.94	0.16
6	99.67	0.48
10	99.34	1.39
Average	99.82	0.67

Table 4 Recovery results for nitrate analysis (n=3)

Spiked level	% Average recovery	% RSD
1	99.96	0.58
5	98.96	0.59
10	98.06	0.72
50	99.20	0.79
100	98.40	0.93
Average	98.92	0.73

Comparison with other analytical techniques

Comparison of present method for estimation of nitrite and nitrate with previously developed methods is summarized in **Table 5**. The detection used in current method is suppressed conductivity detector which is common and sufficiently sensitive as compared to other detectors used in ion chromatography. The use of suppressor with conductivity detector reduces the conductivity produced by eluent ions and enhances the conductivity produced by analyte of interest. AG18 guard column increases the sensitivity of detector and gives lower detection limits as well as avoids interference of other ions. EG 40 cartridge protect the column from metal contamination. Also the common run time for previous developed methods is about 15 min but in present method the run time is about 12 min. The method is directly applicable for determination of nitrite and nitrate from the water and vegetable samples. Among all the available methods IC method is having distinct advantage that it is very fast, sensitive and can separate nitrate and nitrite ions with proper resolution in presence of interfering ions.

Table 5 Comparison of Ion Chromatography methods for nitrite and nitrate estimation

Sample matrix	Column	Eluent	Detector	Detection limit ppm	Reference
Water	Dionex AS11	48-50 mM KOH	Conductivity	< 1 for nitrate & 0.3 for nitrite	[21]
Drinking water	Capillary	100 mM KOH	UV	< 1 for nitrate	[22]
Vegetables	IC-PAK ion exchange	1.3 mM sodium gluconate/1.3 mM borax buffer	Conductivity	0.63 for nitrate	[23]
Mineral water	Ion Pac AG4A & AS4A	15 mM $NaHCO_3$ & 1.3 mM Na_2CO_3	Conductivity with suppressor	< 0.012 for both nitrite & nitrate	[24]
Vegetables	AG18 & AS18	39 mM KOH	Conductivity with suppressor	0.016 for nitrite & 0.017 for nitrate	(Present method)

Determination of nitrite and nitrate in water and vegetable samples

The results for nitrite and nitrate analysis of the selected vegetables from organic farming market and local market and water samples showed that the nitrite and nitrate contents varied significantly in the range of 0-10 ppm and 0-44 ppm, respectively (**Tables 6** and **7**). Higher concentrations of nitrite and nitrate were found in vegetables obtained from local market as compared to samples from organic farming market. The samples from organic farming market showed significantly lower concentrations of nitrite and nitrate.

Table 6 Average nitrite and nitrate levels in vegetable samples from organic farming market and local vegetable market (n=3)

Sample	Organic Farming Market				Local Vegetable Market			
	Nitrite (ppm)	% RSD Nitrite	Nitrate (ppm)	% RSD Nitrate	Nitrite (ppm)	% RSD Nitrite	Nitrate (ppm)	% RSD Nitrate
Spinach leaves	7.8	0.62	38.5	0.01	9.4	1.21	43.6	0.45
Coriander leaves	4.7	0.52	18.4	0.01	6.2	0.73	21.3	0.03
Fenugreek leaves	2.6	1.02	25.9	0.01	4.8	0.56	19.7	0.02

Table 7 Average nitrite and nitrate levels in water samples (n=3)

Sample	Nitrite (ppm)	% RSD Nitrite	Nitrate (ppm)	% RSD Nitrate
Tap water	0.43	1.10	1.54	0.23
Well water	0.12	1.24	0.23	0.13

The nitrate amounts in some samples have reached hazardous levels. The wide ranges and large variations in nitrite and nitrate levels for the same vegetables purchased from different organic farming and local market were not surprising because boiling of vegetables can extract the nitrate concentration by almost 80 %. Nitrate concentration decreases to large extent when sufficient amount of boiling water is used for washing the vegetables before cooking.

Conclusion

The developed analytical method is rapid, simple and sensitive for determining nitrate and nitrite in vegetable samples. The Ion Pac AS18 column provides ideal selectivity for the separation of nitrite and nitrate from matrix components which are eluted mostly within analysis run time. This method eliminates the need for time consuming and costly sample pretreatment using reverse phase or ion-exchange cartridges and protein precipitating reagents. Recoveries of nitrite and nitrate were greater than 99 %. Hence this method may be applied for estimation of nitrite and nitrate in water and other environmental samples. Extraction of nitrite and nitrates from vegetables using boiled water before cooking may reduce the harmful impacts of nitrite and nitrate on human health.

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