

# Simultaneous Detection of Nitrite and Nitrate in Celeries and Carrots

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**Abstract:** In this study, the anions nitrite ion ( $\text{NO}_2^-$ ) and nitrate ion ( $\text{NO}_3^-$ ) in celeries and carrots were detected with the technique of ion chromatography. A Dionex ICS-2000 ion chromatograph was used to detect the content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries and carrots.  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were separated on a DIONEX Ionpac AS19 A-4 mm ion-exchange column and an Iopac AG AS19 A-4 mm guard column. The eluant was suppressed with an anion electrical self-regenerating suppressor (ASRS 300) and the content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was detected with a conductivity detector. The detection limits were obtained for anions  $\text{NO}_2^-$  and  $\text{NO}_3^-$ . The present results showed that the ion chromatography was a suitable technique for the simultaneous determination of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in vegetables.

**Keywords:** Ion Chromatography,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , Celery, Carrot

## Introduction

Ion Chromatography (IC) is a popular method for anion analysis. With the technique of IC, a variety of parameters could be detected in one run (Tartari *et al.*, 1995; Marchetto *et al.*, 1995; Rey and Pohl, 1996). In addition, IC has high reliability and sensitivity. The reproducible data can be yielded under the same experimental conditions. For detecting inorganic ions and organic acids, the conductivity detector is usually combined with the method of chemical suppression (Buldini *et al.*, 1997a; 1997b; Hafez *et al.*, 1991). Nowadays, IC has been used in the studies of environment, food quality, water quality, chemical industry and life science (Ohta and Tanaka, 1999; Ding *et al.*, 2001; Tanaka *et al.*, 2001).

In the previous studies, nitrite and nitrate in the rain water samples were simultaneously determined by ion chromatography with the post-column ammonium derivatization (Niedzielski *et al.*, 2006). High performance ion chromatography was used to detect nitrite and nitrate in the foodstuffs, biological fluids and seawater (Santillana *et al.*, 1993; Tozer *et al.*, 1995; Horioka *et al.*, 2014). In addition, the kinetic studies were performed with ion-pair chromatography to detect nitrite and nitrate in rats (Stein *et al.*, 1988). In the saliva and foodstuffs, a non-suppressed ion chromatography was used to detect nitrate and nitrite with a bulk acoustic wave detector (Yu *et al.*, 2001).

In the food products or digestive system of human, nitrite could be converted to carcinogenic nitrosamines. Compared to nitrite, nitrate is more stable and less toxic. In the food products, nitrate is readily converted to nitrite by microorganisms. Thus the content of nitrite and nitrate in vegetables should be detected for their toxicity. It is necessary to analyze the content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in vegetables from the toxicological points. In this study, a Dionex ICS-2000 ion chromatograph with a conductivity detector was used to detect the anions  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries and carrots.

## Materials and Methods

### Instrument

A Dionex ICS-2000 ion chromatograph with a conductivity detector was used to detect  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries and carrots. Anions were separated by a DIONEX Ionpac AS19 A-4 mm ion-exchange column with an Iopac AG AS19 A-4 mm guard column. The eluant was suppressed with an anion electrical self-regenerating suppressor (ASRS 300, 4 mm I.D.) and the signals of ions were detected with a conductivity detector.

### Reagents

The water was purified with a Milli-Q system (Millipore). Then the purified water was used to prepare

the eluents, reagents, as well as standard solutions. Both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  standard solutions ( $1.0 \text{ g L}^{-1}$ ) were purchased from Shanghai standard solutions center (Shanghai, China).

### Treatment of Vegetable Samples

The celeries and carrots were collected from a local market in zibo (Shandong, China). About 5.0 g vegetable sample was weighed and ground with a mortar and pestle. Then the samples were put into a volumetric flask (50 mL) and the activated carbon ( $0.1 \text{ g L}^{-1}$ ) was added into the volumetric flask to decolorize the supernatant. Finally, the samples were oscillated for 15 min with an ultrasonic sound (59 kHz) and further centrifuged at 8000 r/min for 15 min. The final solutions of supernatants were filtered with a Dionex on Guard C18, followed by a  $0.22 \mu\text{m}$  Nylon filter.

### Anion Chromatographic Analysis

Isocratic elution was used for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  detection. About 30 mM potassium hydrate (KOH) was used as eluant and the velocity of flow was  $1.0 \text{ mL min}^{-1}$ . 25  $\mu\text{L}$  sample was injected and the run time was 16-25 min.

## Results

### Separation Condition

The chromatographic conditions for separating anions  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were shown in Table 1. The chromatogram of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was shown in Fig. 1, which was obtained after the injection of a standard solution ( $20.0 \text{ mg L}^{-1}$ ). It can be found that  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were successfully separated under these chromatographic conditions.

### Linearity

To acquire the calibration of  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , three standard solutions ( $1.0$ ,  $10.0$  and  $20.0 \text{ mg L}^{-1}$ ) were used. The typical chromatogram for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  standard solutions was obtained (Fig. 1). The calibration was linear for  $\text{NO}_2^-$  ( $y = 4.7033x - 0.0062$ ;  $r^2 = 0.999946$ ) and  $\text{NO}_3^-$  ( $y = 5.6148x - 0.2034$ ;  $r^2 = 0.999163$ ), respectively.

### Precision and Detection Limit

The linear relationship, reproducibility and sensitivity were good for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  under the separation conditions summarized in Table 1. The precision was acquired after one real sample was injected five times. The Relative Standard Deviation (RSD) for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was 3.56 and 5.21% (Table

2), respectively. The detection limit (3N/S) for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was calculated, which was  $0.64$  and  $0.53 \mu\text{g L}^{-1}$ , respectively.

The suitable amount of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  standard solutions were added into the real vegetable samples. The content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were detected with the optimum experiment conditions. For  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , recovery was expressed as the mean percentage ratio between the measured amounts and the added ones. The recovery for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was  $98.33 \pm 0.24\%$  and  $99.57 \pm 0.31\%$  (Table 2), respectively.

### Analysis of Vegetable Samples

The chromatogram of celery and carrot was shown in Fig. 2 and 3. The content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries and carrots can be seen in Table 3 and 4. The results indicated that both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were present in celeries and carrots. However, the concentration of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries was higher than that of carrots.

Table 1. Optimum condition for IC

KOH concentration	$30 \text{ mmol L}^{-1}$
Flow rate of eluent	$1.0 \text{ mL min}^{-1}$
The electric current of suppressor	75 mA
Chromatographic column	Ionpac AS19 A-4 mm ion-exchange column
Guard column	Iopac AG AS19 A-4 mm guard column

Table 2. Detection limit and precision for detection of anions

Anion	$\text{NO}_2^-$	$\text{NO}_3^-$
R.S.D. (%) (n = 5)	3.56	5.21
Detection limit ( $\mu\text{g/L}$ )	0.64	0.53
The recovery data (%) (n = 3)	$98.33 \pm 0.24$	$99.57 \pm 0.31$
R.S.D.: The relative standard deviation		

Table 3. The content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celery

Anion content	$\text{NO}_2^-$ (mg/kg)	$\text{NO}_3^-$ (mg/kg)
Samle 1	14.48	2055.76
Samle 2	11.70	2070.38
Samle 3	9.84	2094.56
Average	12.01	2073.57
Standard error	2.34	19.60
Anion content	$12.01 \pm 2.34$	$2073.57 \pm 19.60$

Table 4. The content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in carrot

Anion content	$\text{NO}_2^-$ (mg/kg)	$\text{NO}_3^-$ (mg/kg)
Samle 1	10.32	406.82
Samle 2	8.6	410.96
Samle 3	15.22	400.66
Average	11.38	406.15
Standard error	3.43	5.18
Anion content	$11.38 \pm 3.43$	$406.15 \pm 5.18$

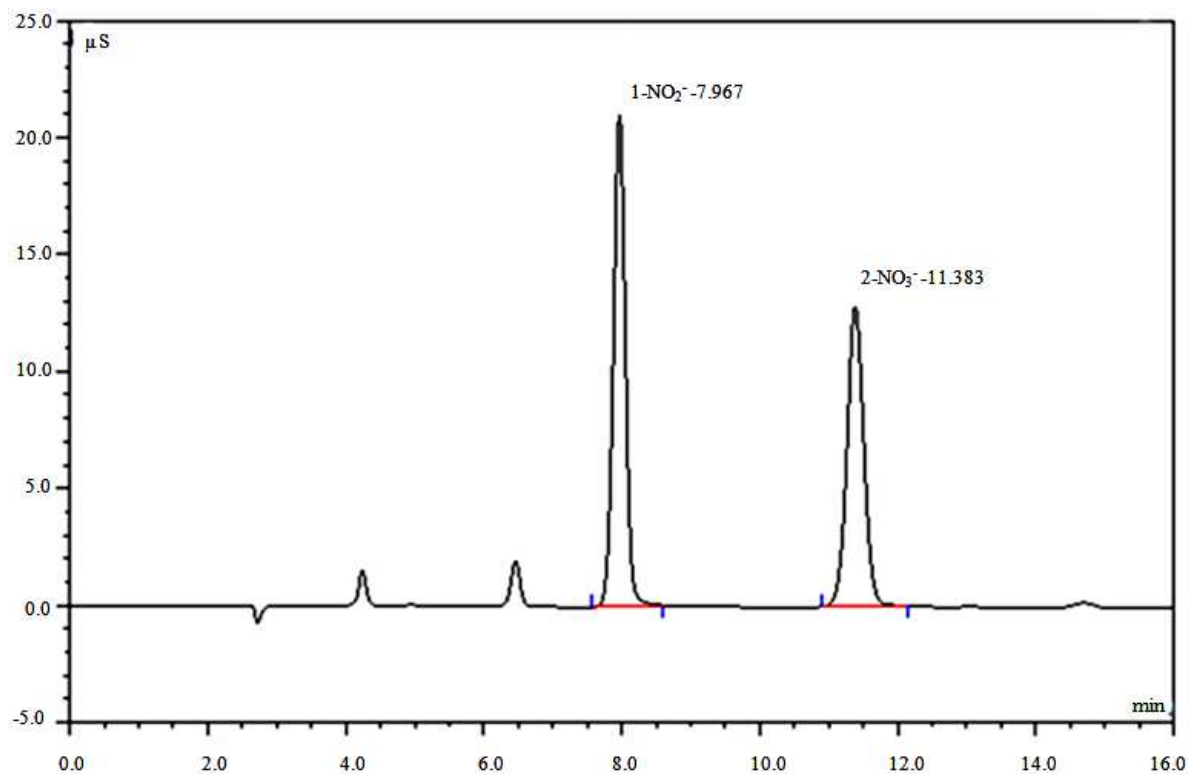


Fig. 1. The chromatogram for the standard solution of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> (20.0 mg L<sup>-1</sup>). The ion separation column was Ionpac AS19 A-4 mm. NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were detected with a conductivity detector. The injection volume was 25 μL and the eluant was 30 mM KOH

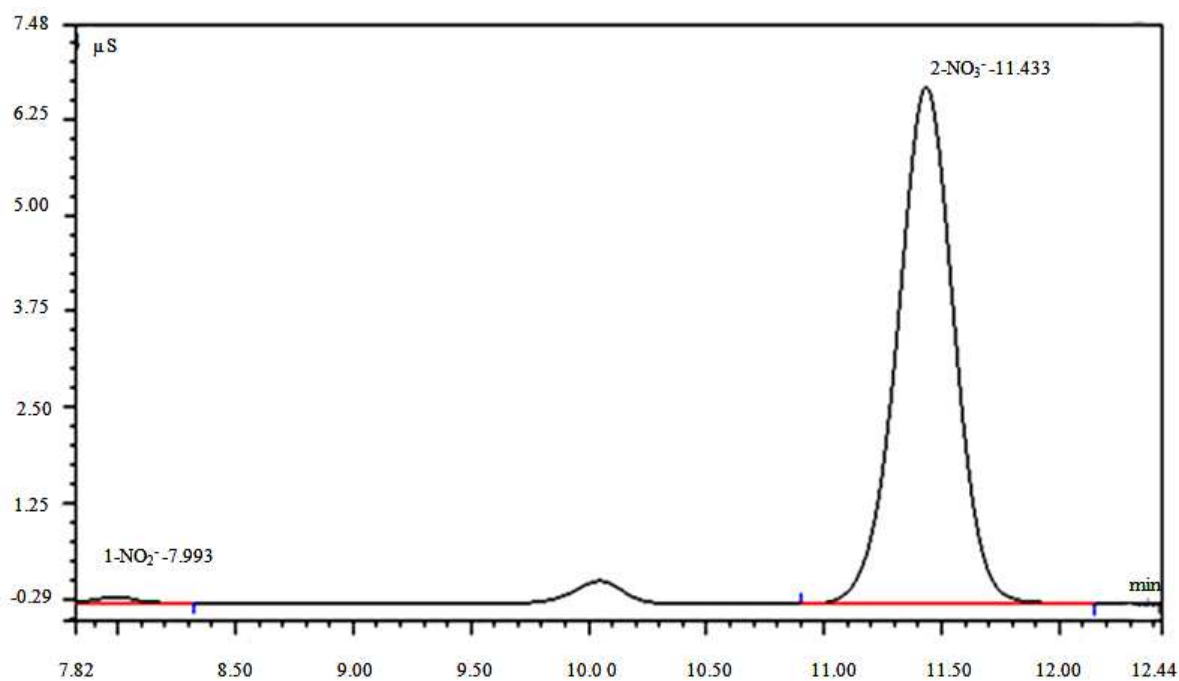


Fig. 2. The chromatogram for NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in celery. The ion separation column was Ionpac AS19 A-4 mm. NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were detected with a conductivity detector. The injection volume was 25 μL and the eluant was 30 mM KOH

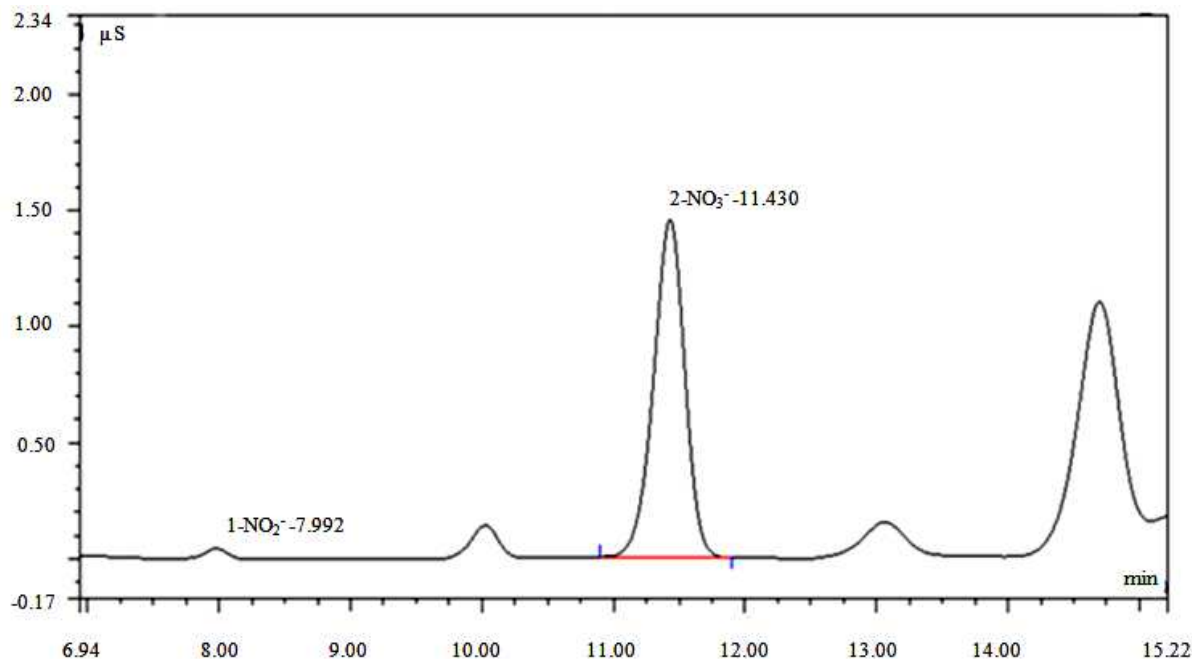


Fig. 3. The chromatogram for  $\text{NO}_3^-$  in carrot. The ion separation column was Ionpac AS19 A-4 mm.  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were detected with a conductivity detector. The injection volume was 25  $\mu\text{L}$  and the eluant was 30 mM KOH

## Discussion

In the previous study, high performance ion chromatography was used to detect nitrite and nitrate in the foodstuffs, biological fluids and seawater (Santillana *et al.*, 1993; Tozer *et al.*, 1995; Horioka *et al.*, 2014). In this study, the anions  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in celeries and carrots were determined with the technique of IC and a conductivity detector. The results showed that IC was suitable for the accurate detection of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the vegetables. The suitable parameters for evaluating vegetables could be acquired with IC. There were high sensitivity and reproducibility for quantifying  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in vegetables. In addition, there were acceptable detection limits for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  analysis and the content of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  could be analyzed in a shorter time.

## Conclusion

IC was suitable for the accurate detection of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the vegetables. There were high sensitivity and reproducibility for quantifying  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in vegetables.

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## Author's Contributions

**Dongwu Liu:** Analyzed the data and wrote the paper.

**Jinye Niu and Ling Kong:** Performed the experiments.

**Zhiwei Chen:** Conceived and designed the experiments.

## Conflict of Interest

The author declares that they have no conflict of interest.

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