Simultaneous Detection of Nitrite and Nitrate in Celeries and Carrots

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Abstract: In this study, the anions nitrite ion (NO$_2^-$) and nitrate ion (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 NO$_2^-$ and NO$_3^-$ in celeries and carrots. NO$_2^-$ and 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 NO$_2^-$ and NO$_3^-$ was detected with a conductivity detector. The detection limits were obtained for anions NO$_2^-$ and NO$_3^-$. The present results showed that the ion chromatography was a suitable technique for the simultaneous determination of NO$_2^-$ and NO$_3^-$ in vegetables.

Keywords: Ion Chromatography, NO$_2^-$, 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 NO$_2^-$ and 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 NO$_2^-$ and NO$_3^-$ in celeries and carrots.

Materials and Methods

Instrument

A Dionex ICS-2000 ion chromatograph with a conductivity detector was used to detect NO$_2^-$ and 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 NO$_2^-$ and NO$_3^-$ standard solutions (1.0 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 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 µm Nylon filter.

**Anion Chromatographic Analysis**

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

**Results**

**Separation Condition**

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

**Linearity**

To acquire the calibration of NO$_2^-$ and NO$_3^-$, three standard solutions (1.0, 10.0 and 20.0 mg L$^{-1}$) were used. The typical chromatogram for NO$_2^-$ and NO$_3^-$ standard solutions was obtained (Fig. 1). The calibration was linear for NO$_2^-$ ($y = 4.7033x-0.0062; r^2 = 0.999946$) and 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 NO$_2^-$ and 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 NO$_2^-$ and NO$_3^-$ was 3.56 and 5.21% (Table 2), respectively. The detection limit (3N/S) for NO$_2^-$ and NO$_3^-$ was calculated, which was 0.64 and 0.53 µg L$^{-1}$, respectively.

The suitable amount of NO$_2^-$ and NO$_3^-$ standard solutions were added into the real vegetable samples. The content of NO$_2^-$ and NO$_3^-$ were detected with the optimum experiment conditions. For NO$_2^-$ and NO$_3^-$, recovery was expressed as the mean percentage ratio between the measured amounts and the added ones. The recovery for NO$_2^-$ and NO$_3^-$ was 98.33±0.24% and 99.57±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 NO$_2^-$ and NO$_3^-$ in celeries and carrots can be seen in Table 3 and 4. The results indicated that both NO$_2^-$ and NO$_3^-$ were present in celeries and carrots. However, the concentration of NO$_2^-$ and NO$_3^-$ in celeries was higher than that of carrots.

Table 1. Optimum condition for IC

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

Table 2. Detection limit and precision for detection of anions

| Anion   | NO$_2^-$ | NO$_3^-$ |
|---------|----------|----------|
| R.S.D. (%)(n = 5) | 3.56    | 5.21     |
| Detection limit (µg/L) | 0.64    | 0.53     |
| The recovery (%) (n = 3) | 98.33±0.24 | 99.57±0.31 |
| R.S.D.: The relative standard deviation |          |

Table 3. The content of NO$_2^-$ and NO$_3^-$ in celery

| Sample | NO$_2^-$ (mg/kg) | 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±2.34 | 2073.57±19.60   |

Table 4. The content of NO$_2^-$ and NO$_3^-$ in carrot

| Sample | NO$_2^-$ (mg/kg) | 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±3.43 | 406.15±5.18     |
Fig. 1. The chromatogram for the standard solution of NO$_2^-$ and NO$_3^-$ (20.0 mg L$^{-1}$). The ion separation column was Ionpac AS19 A-4 mm. NO$_2^-$ and NO$_3^-$ 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$_2^-$ and NO$_3^-$ in celery. The ion separation column was Ionpac AS19 A-4 mm. NO$_2^-$ and NO$_3^-$ were detected with a conductivity detector. The injection volume was 25 µL and the eluant was 30 mM KOH.
Fig. 3. The chromatogram for NO$_3^-$ in carrot. The ion separation column was Ionpac AS19 A-4 mm. NO$_2^-$ and NO$_3^-$ were detected with a conductivity detector. The injection volume was 25 µL 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 NO$_2^-$ and 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 NO$_2^-$ and NO$_3^-$ in the vegetables. The suitable parameters for evaluating vegetables could be acquired with IC. There were high sensitivity and reproducibility for quantifying NO$_2^-$ and NO$_3^-$ in vegetables. In addition, there were acceptable detection limits for NO$_2^-$ and NO$_3^-$ analysis and the content of NO$_2^-$ and NO$_3^-$ could be analyzed in a shorter time.

**Conclusion**

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

**References**

Buldini, P.L., D. Ferri and J. Lal Sharma, 1997a. Determination of some inorganic species in edible vegetable oils and fats by ion chromatography. J. Chromatogr. A, 789: 549-555. DOI: 10.1016/S0021-9673(97)00822-4
Buldini, P.L., S. Cavalli and A. Trifiro, 1997b. State-of-the-art ion chromatographic determination of inorganic ions in food. J. Chromatogr. A, 789: 529-548. DOI: 10.1016/S0021-9673(97)00963-1
Ding, M., K. Tanaka, W. Hu, K. Hasebe and P.R. Haddad, 2001. Simultaneous ion-exclusion chromatography and cation-exchange chromatography of anions and cations in environmental water samples on a weakly acidic cation-exchange resin by elution with pyridine-2,6-dicarboxylic acid. Analyst, 126: 567-570. DOI: 10.1039/B100104N
Hafez, A.A., S.S. Goyal and D.W. Rains, 1991. Quantitative determination of total sulfur in plant tissues using acid digestion and ion-chromatography. Agron. J., 83: 148-153. DOI: 10.2134/agronj1991.000219620083000100034x

Horoka, Y., S. Kurata and K. Ito, 2014. Simultaneous and selective determination of nitrite, nitrate and phosphate ions in seawater by ion chromatography. Bunseki Kagaku, 63: 657-663. DOI: 10.2116/bunsekikagaku.63.657

Marchetto, A., R. Mosello, G.A. Tartari, H. Muntau and M. Bianchi et al., 1995. Precision of ion chromatographic analyses compared with that of other analytical techniques through intercomparison exercises. J. Chromatogr. A, 706: 13-19. DOI: 10.1016/0021-9673(95)00039-P

Niedzielski, P., I. Kurzyca and J. Siepak, 2006. A new tool for inorganic nitrogen speciation study: Simultaneous determination of ammonium ion, nitrite and nitrate by ion chromatography with post-column ammonium derivatization by nessler reagent and diode-array detection in rain water samples. Anal. Chim. Acta, 577: 220-224. DOI: 10.1016/j.aca.2006.06.057

Ohta, K. and K. Tanaka, 1999. Simultaneous determination of common mono- and divalent cations in natural water samples by conductimetric detection ion chromatography with an unmodified silica gel column and oxalic acid/18-crown-6 as eluent. Anal. Chim. Acta, 381: 265-273. DOI: 10.1016/S0003-2670(98)00720-X

Rey, M.A. and C.A. Pohl, 1996. Novel cation-exchange stationary phase for the separation of amines and of six common inorganic cations. J. Chromatogr. A, 739: 87-97. DOI: 10.1016/0021-9673(96)00042-8

Santillana, M.I., E. Ruiz, M.T. Nieto and M. De Alba, 1993. High performance ion chromatography determination of nitrite and nitrate in foodstuffs. J. Liquid Chromatography, 16: 1561-1571. DOI: 10.1080/10826079308020973

Stein, C., H.G. Classen and G. Schwedt, 1988. Kinetic studies on nitrite and nitrate in rats by ion-pair chromatography. Clinica Chimica Acta, 175: 167-173. DOI: 10.1016/0009-8888(88)90006-X

Tanaka, K., K. Ohta, P.R. Haddad, J.S. Fritz and A. Miyanaga et al., 2001. High-performance ion-exclusion/cation-exchange chromatography of anions and cations in acid rain waters on a weakly acidic cation-exchange resin. J. Chromatogr. A, 920: 239-245. DOI: 10.1016/S0021-9673(01)00578-7

Tartari, G.A., A. Marchetto and R. Mosello, 1995. Precision and linearity of inorganic analyses by ion chromatography. J. Chromatogr. A, 706: 21-29. DOI: 10.1016/0021-9673(94)01222-Z

Tozer, G.M., V.E. Prise, P. Wardman and M. Stratford, 1995. Nitric oxide in biological fluids: Analysis of nitrite and nitrate by high-performance ion chromatography. J. Chromatogr. A, 706: 437-442. DOI: 10.1016/0021-9673(95)00078-2

Yu, B.S., P. Chen, L.H. Nie and S.Z. Yao, 2001. Simultaneous determination of nitrate and nitrite in saliva and foodstuffs by non-suppressed ion chromatography with bulk acoustic wave detector. Anal. Sci., 17: 495-498. DOI: 10.2116/analsci.17.495