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**Research Article**

# Analysis of $NO_2^-$ and $NO_3^-$ in Apples, Pears and Oranges with Ion Chromatography

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**Abstract:** Ion Chromatography (IC) can yield the precise and reproducible data if the experimental condition is kept constant. In this study, the anions $NO_2^-$ and $NO_3^-$ in apples, pears and oranges were determined with the technique of IC. A Dionex ICS-2000 ion chromatograph with a Dionex gradient pump, eluent degassing module and conductivity detector was used to detect $NO_2^-$ and $NO_3^-$. Anions were separated on a DIONEX Ionpac AS19 A-4 mm ion-exchange column, with an Iopac AG AS19 A-4 mm guard column and detected after suppression with ASRS 300 anion electrical self-regenerating suppressor. The results indicated that the technique of IC was suitable for the rapid, precise and accurate determination of $NO_2^-$ and $NO_3^-$ in apples, pears and oranges. In addition, the acceptable detection limits were obtained for $NO_2^-$ and $NO_3^-$ and the analysis time was significantly shortened with the technique of IC. The data will provide theories and rapid methods for the supervision of fruit quality.

**Keywords:** Apple, ion chromatography, $NO_2^-$, $NO_3^-$, orange, pear

## INTRODUCTION

Ion Chromatography (IC) has been developed into a popular method for anion analysis since the introduction of IC by Small *et al*. (1975). It is known that IC has high precision and low maintenance costs and many parameters can be determined in one run with the technique of IC (Tartari *et al*., 1995; Marchetto *et al*., 1995; Rey and Pohl, 1996). The conductivity detector combined with chemical suppression is suitable for the determination of inorganic ions and organic acids (Buldini *et al*., 1997a, 1997b; Hafez *et al*., 1991). IC has been widely used to analyze the quality of various waters (Ohta and Tanaka, 1999; Ding *et al*., 2001; Tanaka *et al*., 2001).

In the past, minor and trace elements, as well as some other water quality determinands, were mainly detected with the technique of analytical chemistry. The analytical chemistry techniques were used to ensure that the results were sufficiently reliable for the analysis of food quality. IC has high reliability and sensitivity and all anions of interest could be simultaneously detected in a shorter time with easy operation and sample preparation. In addition, IC can yield the precise and reproducible data if the experimental condition is kept constant. The anion analysis in fruit samples is important from the nutritional and toxicological point of view. In this study, the anions $NO_2^-$ and $NO_3^-$ in apples, pears and oranges were determined with a Dionex ICS-2000 ion chromatograph.

## MATERIALS AND METHODS

**Instrument:** A Dionex ICS-2000 ion chromatograph with a Dionex gradient pump, eluent degassing module and conductivity detector was used. Anions were separated on a DIONEX Ionpac AS19 A-4 mm ion-exchange column with an Iopac AG AS19 A-4 mm guard column. The signals were detected after suppression with ASRS 300 (4mm I.D.) anion electrical self-regenerating suppressor.

**Reagents:** All reagents, eluents and standard solutions were prepared using water purified with a Milli-Q system (Millipore). Both $NO_2^-$ and $NO_3^-$ standard solutions (1.0 g/L) were purchased from the standard solutions center in Shanghai, China.

**Treatment of fruit samples:** The apples, pears and oranges were purchased from a local market in zibo, Shandong province. For the ion chromatographic analysis, 5.0 g fruit sample was weighed and ground with a mortar and pestle. The samples were put into a volumetric flask (50 mL) and 0.1 g/L activated carbon was added into the volumetric flask to decolorize the supernatant. Finally, the samples were oscillated for 15 min with ultrasonic sound (59 kHz) and further centrifuged at 8000 r/min for 15 min. The final solutions of supernatants were filtered through 0.22 μm Nylon filters and Dionex On Guard C18 before analysis.

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**Anion chromatographic analysis:** Isocratic elution was used for $NO_2^-$ and $NO_3^-$ determination with 30 mM potassium hydrate (KOH) as eluent (1.0 mL/min). The injection volume was 25 µL, the run time was set to 16-25 min.

## RESULTS AND DISCUSSION

**Separation condition:** The anions $NO_2^-$ and $NO_3^-$ were successfully separated by the optimum chromatographic conditions summarized in Table 1. Figure 1 shows the chromatogram of a standard solution (20.0 mg/L).

Table 1: Ion chromatographic separation condition

| | |
|---|---|
| KOH concentration | 30 mmol/L |
| Flow rate of eluent | 1.0 mL/min |
| 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 |

**Linearity:** Three standard solutions with increasing concentrations were used for calibration of $NO_2^-$ and $NO_3^-$ (1.0, 10.0 and 20.0 mg/L) determinations. The calibration was linear for $NO_2^-$ ($y = 4.7169x - 0.0094$; $r^2 = 0.999984$) and $NO_3^-$ ($y = 5.6179x - 0.2191$; $r^2 = 0.999147$) (Fig. 2 and 3). The typical chromatogram

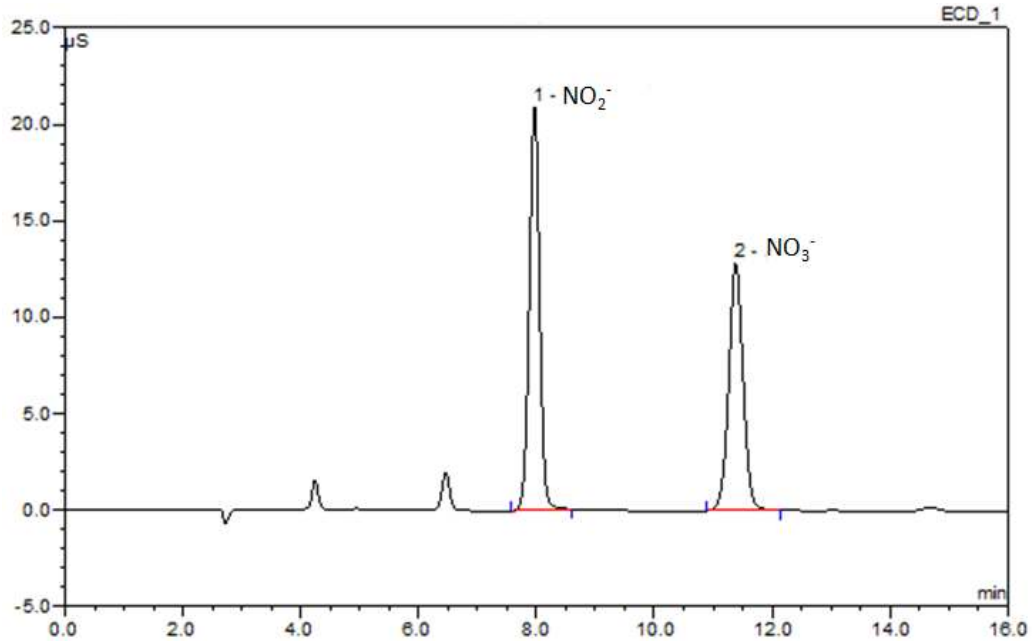


Fig. 1: The chromatogram for $NO_2^-$ and $NO_3^-$ standard solution (20.0 mg/L). Column, Ionpac AS19 A-4 mm; injection volume, 25 µL; conductivity detector; elution with 30 mM KOH

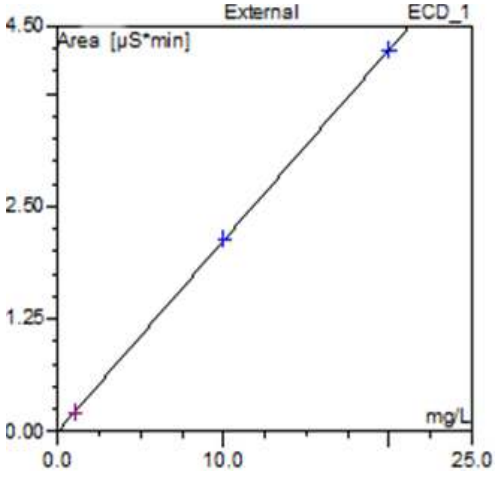


Fig. 2: The standard curve for $NO_2^-$ with $NO_2^-$ standard solution (1.0, 10.0 and 20.0 mg/L)

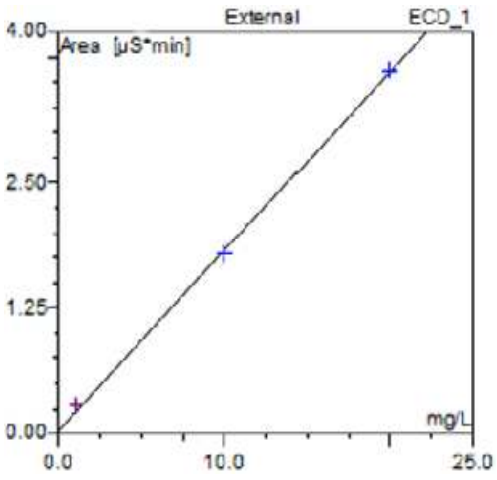


Fig. 3: The standard curve for $NO_3^-$ with $NO_3^-$ standard solution (1.0, 10.0 and 20.0 mg/L)

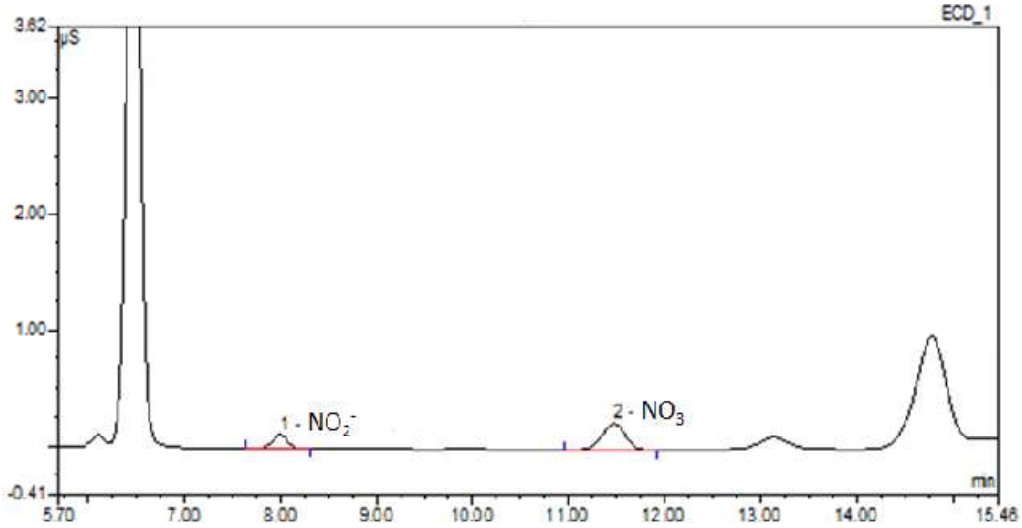


Fig. 4: The chromatogram for $NO_2^-$ and $NO_3^-$ in apples. Column, Ionpac AS19 A-4 mm; injection volume, 25 µL; conductivity detector; elution with 30 mM KOH

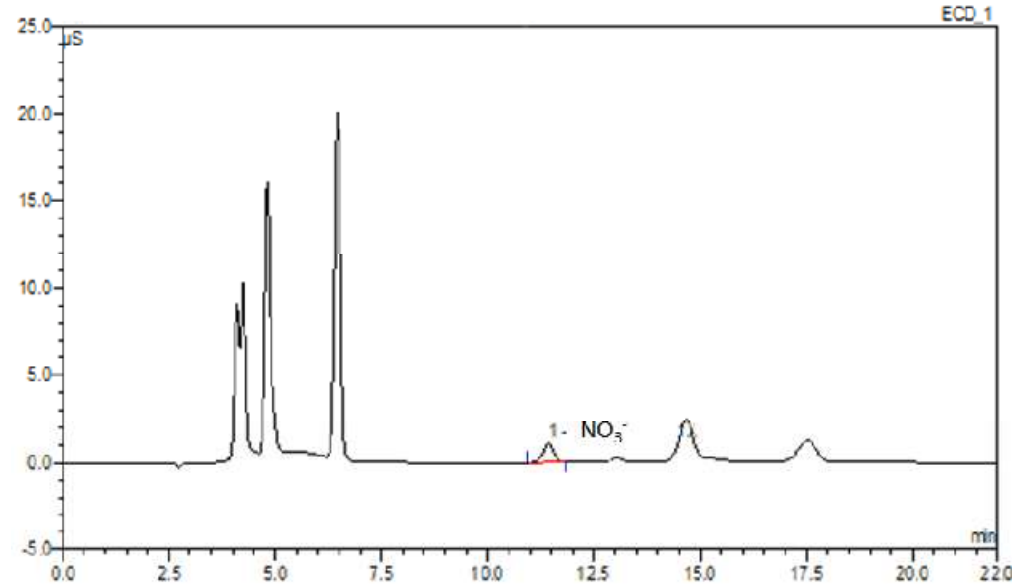


Fig. 5: The chromatogram for $NO_3^-$ in pears. Column, Ionpac AS19 A-4 mm; injection volume, 25 µL; conductivity detector; elution with 30 mM KOH

Table 2: Precision and detection limit data for determination of anions

| Anion | $NO_2^-$ | $NO_3^-$ |
|---|---|---|
| R.S.D.(%)(n = 5) | 4.72 | 6.38 |
| Detection limit (µg/L) | 0.636 | 0.534 |
| The recovery data (n = 3) | 101.26±0.31 | 99.71±0.41 |

Table 3: The content of $NO_2^-$ and $NO_3^-$ in apples

| Anion content | $NO_2^-$ (mg/kg) | $NO_3^-$ (mg/kg) |
|---|---|---|
| Samle 1 | 11.70 | 44.52 |
| Samle 2 | 13.06 | 36.32 |
| Samle 3 | 14.42 | 47.65 |
| Average | 13.06 | 42.83 |
| S.D | 1.360 | 5.85 |
| Anion content | 13.06±1.36 | 42.83±5.85 |

obtained for $NO_2^-$ and $NO_3^-$ standard solutions are shown in Fig. 1.

Table 4: The content of $NO_2^-$ and $NO_3^-$ in pears

| Anion content | $NO_2^-$ (mg/kg) | $NO_3^-$ (mg/kg) |
|---|---|---|
| Samle 1 | — | 269.30 |
| Samle 2 | — | 283.13 |
| Samle 3 | — | 292.18 |
| Average | — | 281.53 |
| Standard deviation | — | 11.520 |
| Anion content | — | 281.53±11.52 |

**Precision and detection limit:** Under the optimum experiment conditions, $NO_2^-$ and $NO_3^-$ showed good linear relationship, sensitivity and reproducibility. Repeating five times, the precision of the analysis of one real sample was calculated. From Table 2, it can be seen that the Relative Standard Deviation (R.S.D.) for $NO_2^-$ and $NO_3^-$ is 4.72% and 6.38%, respectively. The

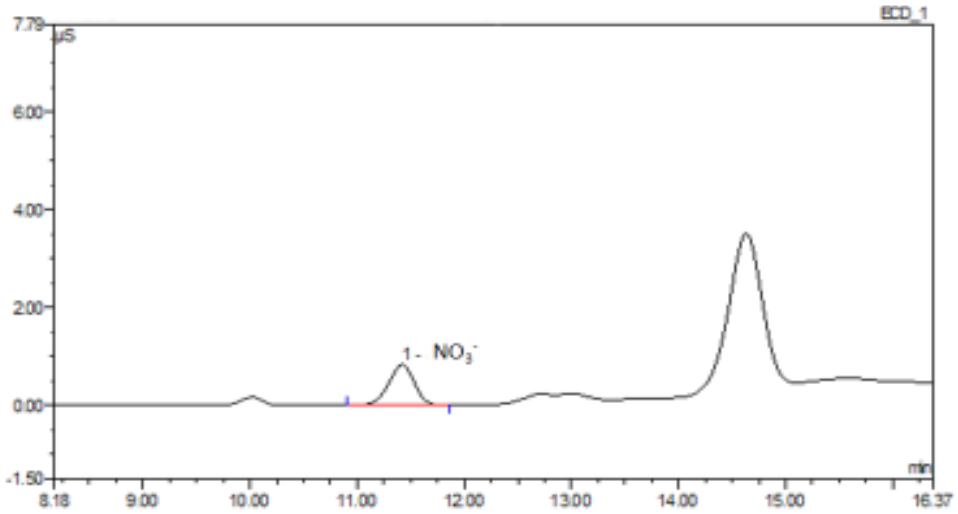


Fig. 6: The chromatogram for $NO_3^-$ in oranges. Column, Ionpac AS19 A-4 mm; injection volume, 25 µL; conductivity detector; elution with 30 mM KOH

Table 5: The content of $NO_2^-$ and $NO_3^-$ in oranges

| Anion content | $NO_2^-$ (mg/kg) | $NO_3^-$ (mg/kg) |
|---|---|---|
| Samle 1 | — | 208.73 |
| Samle 2 | — | 215.74 |
| Samle 3 | — | 231.90 |
| Average | — | 218.79 |
| Standard deviation | — | 11.880 |
| Anion content | — | 218.79±11.88 |

detection limit for the proposed method was calculated (3N/S) for anion determinations. The calculated detection limits for $NO_2^-$ and $NO_3^-$ were 0.636 and 0.534 µg/L, respectively.

The suitable amount of $NO_2^-$ and $NO_3^-$ standard solutions (5.0 mg/L) were added into the real fruit samples and the mixtures were analyzed using the proposed procedure. Recovery was expressed for each component as the mean percentage ratio between the measured amounts and the added ones. As shown in Table 2, the recovery data for $NO_2^-$ and $NO_3^-$ are 101.26±0.31% and 99.71±0.41%, respectively.

**Analysis of fruit samples:** All samples were pretreated according to the methods described and analyzed under the optimum conditions summarized in Table 1. The chromatogram of apple, pear and orange was shown in Fig. 4 to 6. The determination results of $NO_2^-$ and $NO_3^-$ in apples, pears, and oranges can be seen in Table 3 to 5. The results indicate that both $NO_2^-$ and $NO_3^-$ are present in apples. However, there are only $NO_3^-$ in pears and oranges, no $NO_2^-$ is detected.

## CONCLUSION

IC has been developed for the determination of inorganic ions and organic acids. Chromatography can yield the precise and reproducible data if the experimental condition is kept constant. In this study, the anions $NO_2^-$ and $NO_3^-$ in apples, pears and oranges were determined with the technique of IC. The results indicated that the technique of IC was suitable for the rapid, precise and accurate determination of $NO_2^-$ and $NO_3^-$ in fruit samples and IC can be used to provide suitable parameters for discrimination among different kinds of fruits. The analytical method proposed shows a high sensitivity and reproducibility and has the advantage of quantifying the anions $NO_2^-$ and $NO_3^-$ in fruits. IC offers a wide dynamic range and allows the simultaneous determination of anions of interest in a short time. In addition, the acceptable detection limits were obtained for $NO_2^-$ and $NO_3^-$ and the analysis time was significantly shortened with the technique of IC.

## ACKNOWLEDGMENT

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