

# Application solution of ion chromatography in food analysis and determination

## - Nitrate and nitrite

### Foreword

Nitrosamine is one of the three most recognized carcinogens in the world, the other two are aflatoxins and benzo[a]pyrene. Nitrosamine is formed by nitrite and secondary amine in protein and is widely distributed in nature. The content of nitrosamine in salted fish, dried shrimps, beer, bacon and sausage is high. Too long placing time for filling with meat and vegetable can also produce nitrite. Nitrite and nitrate are common inorganic salts in daily diet and drinking water. It is generally believed that excessive intake of these substances may lead to methemoglobinemia and produce carcinogenic nitrosamines in the body. Nitrate and nitrite are the ionic pollutants in GB 2762-2017 named "National Food Safety Standard -Limit of pollutants in food". GB 5009.33-2016 named "National Food Safety Standards for the Determination of Nitrite and Nitrate in Food" is to standardize the determination of these two substances, and ion chromatography as the first method is included in the standard.



### Implementation standard

GB 2762-2017 named "National Food Safety Standard -Limit of pollutants in food" limit 13 kinds of pollutants including lead, cadmium, mercury, arsenic, tin, nickel, chromium, nitrite, nitrate, benzo[a]pyrene, N-Nitrosodimethylamine, Polychlorodiphenyl s, 3-chloro-1,2-propanediol. among them the ionic pollutants are nitrite and nitrate only and The determination methods are different according to the food category. Beverages are determined according to the method stipulated in GB 8538, and other foods are determined according to the method specified in GB 5009.33.

GB 5009.33-2016 named "National Food Safety Standards for the Determination of Nitrite and Nitrate in Food" is suitable for the determination of nitrite and nitrate in foods and include three main methods: ion chromatography (first method), Spectrophotometric method and ultraviolet spectrophotometry (determination of nitrate in vegetables and fruits). Colorimetric method based on spectrophotometric method is the most widely used, but the operation of this method is cumbersome, time-consuming, reagent-consuming, and in this method the determination of nitrate

content is by cadmium column reduction. Ion chromatography, as a new chromatographic method, is simple, rapid, accurate and can simultaneously determine nitric acid ions and nitrite ions.

## Reagents and standards

1. Acetic acid ( $\text{CH}_3\text{COOH}$ )
2. Potassium hydroxide (KOH)

## Configuration and chromatographic conditions

- IC type: CIC-D160 (built-in eluent generator)
- IC column: SH-AC-5
- Guard column: SH-G-1
- Eluent: 10.0mM KOH
- Flow rate: 0.8mL/min
- Sample size: 50 $\mu$ L
- Determination method: suppressed conductivity method
- Pre-treatment column: C18 column, Ag column, Na column, H column, graphitized carbon black column.

## Pre-treatment

### Dairy product:

① Accurately weigh 10g dairy products; ② Add 80ml ultra pure water and mix evenly; ③ Ultrasonic extraction for 30 minutes; ④ Add 2mL acetic acid solution with 3% concentration; ⑤ Place for 20 minutes at 4 $^{\circ}$ C; ⑥ Remove and recover to room temperature, make ultra pure water volume to scale; ⑦ Take supernatant and centrifuge for 15 minutes at a speed of 10000 revolutions/min; ⑧ The solution is filtered by activated C18 column, Ag column, Na column and needle filter membrane; ⑨ Eluent is centrifuged by a ultrafiltration cup, and then tested on the instrument;

### Pickled fish, pickled meat and other salted products:

① Accurately weigh 2g and stir evenly; ② Add 80ml ultra pure water and mix evenly; ③ Ultrasonic extraction for 30 minutes; ④ vibrate for 5 minutes in a thermostat water bath at 75 $^{\circ}$ C; ⑤ Remove and recover to room temperature, make ultra pure water volume to scale; ⑥ Take supernatant and centrifuge for 15 minutes at a speed of 10000 revolutions/min; ⑦ The solution is filtered by activated C18 column, Ag column, Na column and needle filter membrane; ⑧ Eluent is centrifuged by a ultrafiltration cup, and then tested on the instrument;

## Fruits, vegetables, fish, meat, eggs and their products:

①Accurately weigh 5g and stir evenly;②Add 80ml ultra pure water and mix evenly;③Ultrasonic extraction for 30 minutes;④vibrate for 5 minutes in a thermostat water bath at 75℃;⑤Remove and recover to room temperature, make ultra pure water volume to scale;⑥The supernatant is filtered with filter paper;⑦The solution is filtered by graphitized carbon black column which is activated and then tested on the instrument.

## Test

### Making of standard curve

Standard series working solution were injected into ion chromatograph to obtain chromatogram map of standard working fluids of various concentrations. The corresponding peak heights or peak areas were determined. The standard curves were drawn with the concentration of standard working fluids as abscissa and the peak heights or peak areas as ordinates.

### Determination of sample solution

The blank and sample solution were injected into the ion chromatograph, and the blank's and sample solution's peak height or peak area were obtained. According to the standard curve, the concentration of nitrite ion or nitrate ion in the test solution was obtained.

## Standard curve

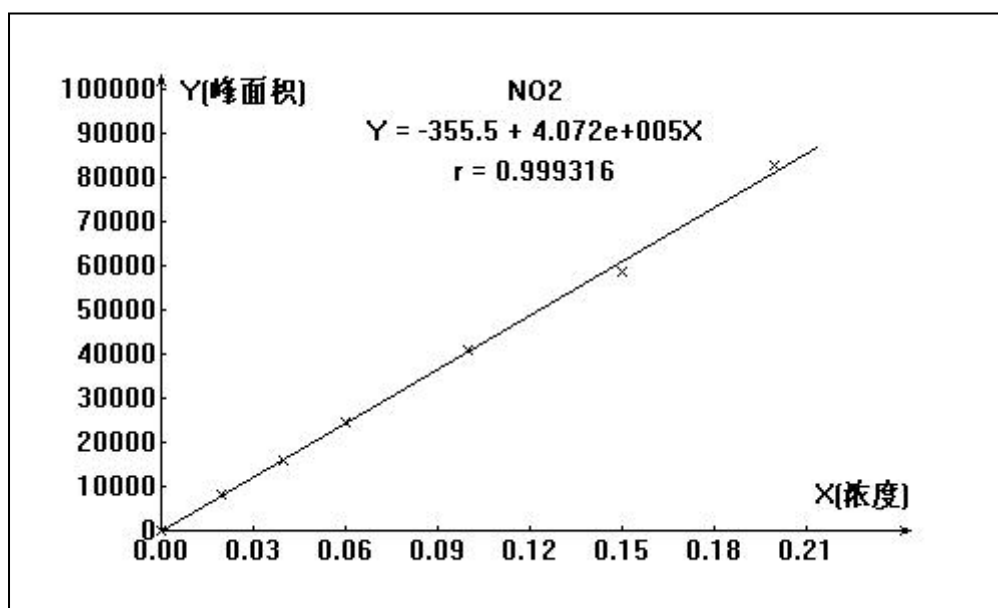


Figure 1 standard curve of nitrite(0-200ppb)

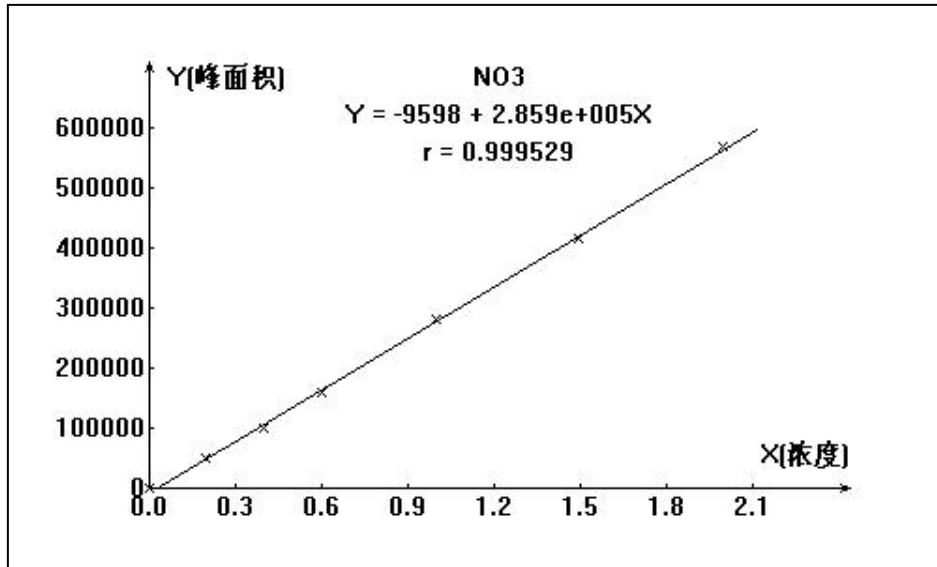
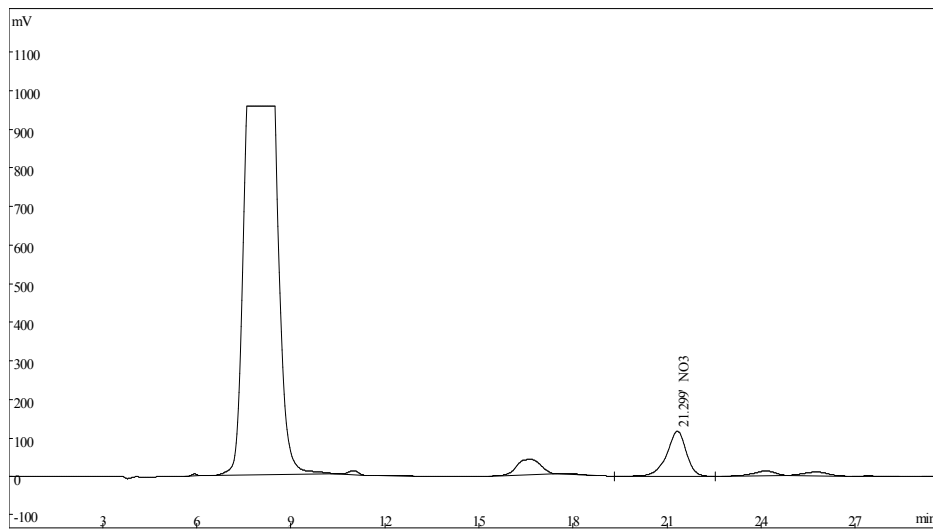


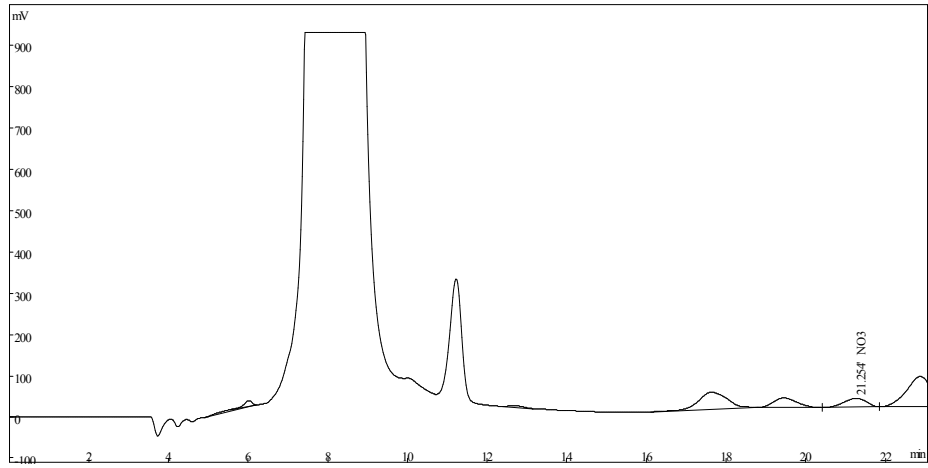
Figure 2 standard curve of nitrate(0-2000ppb)

## Test spectrum

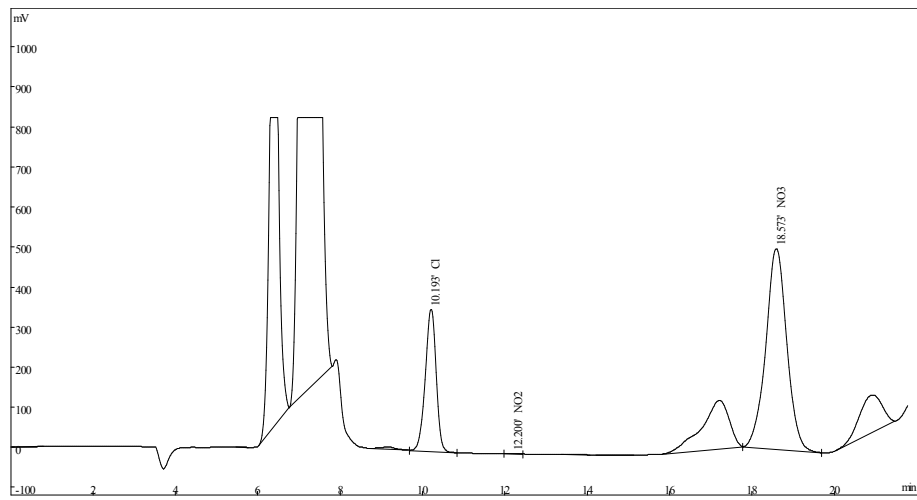
1. A brand of dairy products



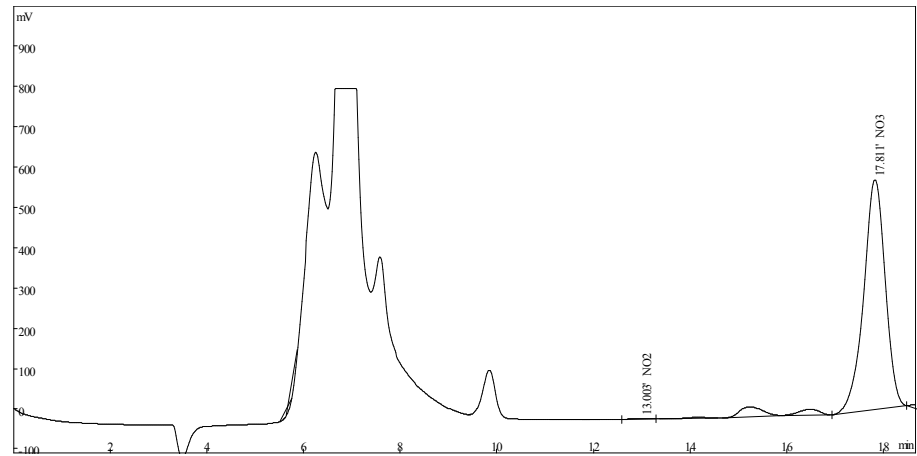
2. A brand of dairy products



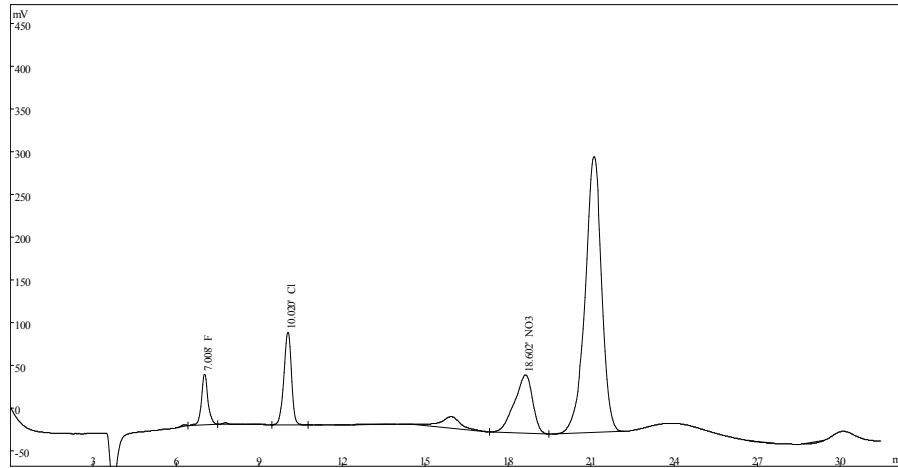
3. A brand of ham



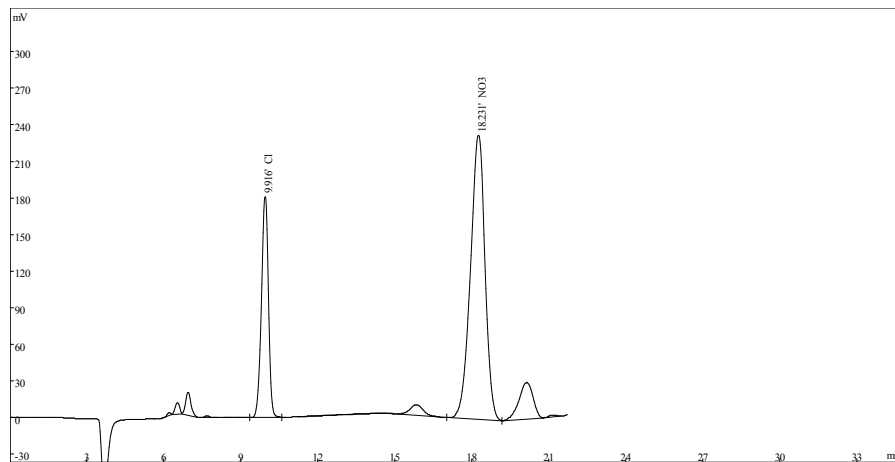
4. A brand of ham



5. vegetable samples(spinach)



## 6. vegetable samples(ruccola salad)



## Conclusion

In this solution, nitrate and nitrite in food were determined by ion chromatography with hydroxyl system and suppressed conductivity method. The results of the determination met the complete requirements, and the precision was high. The pretreatment was simple, rapid, low cost and no complicated steps and expensive reagents were needed. The external influence of the process was small, and it was suitable for large quantities of samples.

## Product introduction



## CIC-D160 IC

CIC-D160 ion chromatograph is the first hydrogen-oxygen ion chromatograph made in China. It is equipped with bipolar conductance detector which greatly improves the detection ability, stability of the instrument, and brings the best usage experience to the users. Its built-in eluent generator can generate the required concentration of eluent on line by pure water and possesses the function of gradient elution which can determine complex samples which isocratic elution cannot. Now it is widely used in the environment, food, chemical industry, power, electronics, mining and metallurgy and other fields.

- Built-in eluent generator: No need to configure the eluent and possesses the function of gradient elution;
- Built-in circulating 3D constant temperature technology which ensures the accuracy and reliability of the experimental data;
- Built-in low-pressure degassing technology to eliminate bubble interference for more stability;
- Self-regenerating electrolytic micro-membrane suppressor which has high pressure resistance, small dead volume, and responsive signal is high;
- Equipped with intelligent automatic injection system for large sample volumes, which features automatic dilution to save labor and time;
- Observatory intelligent workstation which is configured with integrated control, compatibility for a variety of instruments, and customized images.
- Perfect after-sale support to solve the worries of users

## Ion Chromatographic Column



AS the first domestic developer and manufacturer of Ion chromatographic column, Sheng Han have the technology of the development and production of three kinds of Ion chromatographic column including ion exchange chromatographic column, ion exclusion chromatographic column and ion pair chromatographic column. At the same time, Sheng Han have also successfully developed and produced hydroxyl system of Ion chromatographic column in large scale ranking second in the world, which broken the monopoly of imported brands in the high-end ion chromatographic column field more than ten years. The use of domestic ion chromatography can reduce the cost of operation and maintenance of users by about 35%.