Application Note AN-U-080

Nitrite and nitrate in meat products

Robust routine analysis with ion chromatography

Nitrite and nitrate salts are used as preservatives for meat and meat products. They are labeled on foods as E 249–E 252. These so-called curing salts prevent bacteria growth, stabilize the color of the meat, and enhance its flavor. Nitrate salts (E 251, E 252) have a low toxicity. However, long-term exposure is of concern, as the lower gut reduces nitrate to nitrite, which is a precursor of nitrosamines (classified as carcinogenic) [1]. Nitrite itself is classified as probably carcinogenic to humans. The MPL (maximum permitted levels) after the manufacturing process vary for nitrite (E 249, E 250) between 50–180 mg/kg [2], and for nitrate between 150–300 mg/kg [3], depending on the product. The European Commission limits nitrate and nitrite salts in processed meat to less than 150 mg/kg [4].

Classical HPLC-UV methods often suffer from asymmetric peaks, low reproducibility on retention times, and poor sensitivity. Other analytical methods such as spectrophotometric or automated discrete analysis methods show interferences depending on different meat matrices, making this kind of analysis difficult for laboratories where a wide variety of food and beverage products need to be analyzed.

Ion chromatography with UV detection offers a robust and universal method for quality control of nitrite and nitrate in different meat matrices.

SAMPLE PREPARATION

Various meat products like pork knuckle, pork shoulder, black blood sausage, and Chistorra sausage were investigated. The same sample preparation worked for all tested meat products.

Samples were treated with *Carrez* precipitation to remove fats and proteins. The amount of *Carrez* reagent is adjusted to the fat and protein content of the sample type. For example, a freshly chopped meat sample (5 g) was treated with *Carrez* solutions (2.5 mL *Carrez* I + 2.5 mL *Carrez* II) and diluted to 100 mL with ultrapure water (UPW). After centrifugation (5000 rpm) and filtration (0.45 µm), 10 mL of the solution was further diluted with UPW to 50 mL (5-fold dilution). For consistent results, standard solutions were also prepared with *Carrez* reagents.

EXPERIMENTAL

Samples (50 µL) were injected into the IC system after Inline Ultrafiltration. Two columns with different properties (Metrosep A Supp 7 - 250/4.0 and Metrosep A Supp 5 - 50/4.0) were used in series to avoid co-elution of nitrite with organic components. Analytes were separated by isocratic anion exchange chromatography with a carbonate/methanol eluent (3.6 mmol/L Na_2CO_3 + 15% methanol) and a flow rate of 0.7 mL/min (Table 1, Figures 1–4). A column temperature of 52 °C further improved the resolution of the nitrite peak. Sequential suppression reduced the background noise to enable sensitive UV/VIS detection (205 nm). Quantification was performed over a range of 0.02–2.00 mg/L for nitrite, and 0.05–5 mg/L for nitrate.

Table 1. Summary of IC method parameters.

Columns	Metrosep A Supp 7 - 250/4.0 + Metrosep A Supp 5 - 50/4.0
Eluent	3.6 mmol/L Na_2CO_3 + 15% methanol
Flow	0.7 mL/min
Temp	52 °C
Injection	50 µL
Detection	UV 205 nm

Sample concentrations were calculated for sodium nitrate and sodium nitrite. In order to keep the system clean from any organic contaminations, the sample flow path was rinsed with methanol/UPW (1:1 v/v) after each analysis and the suppressor was regenerated with a mixture of sulfuric acid (500 mmol/L), oxalic acid (100 mmol/L), and acetone (20% v/v).

RESULTS

Figures 1–4 show exemplary chromatograms for different tested meat samples. The nitrite concentration varied from not detectable to 54 mg/kg and the nitrate concentration was between 10–50 mg/kg. During these tests, nitrite exceeded the critical limit of 50 mg/kg in only one sample (pork shoulder), whereas nitrate was always measured well within the allowed concentration limit [4]. Long-term studies in quality control laboratories of meat manufacturers have proven that this IC method is a robust and precise enough for routine analysis of nitrite and nitrate.

This universal analytical method is also suitable for beverage and vegetable samples. A wide variety of food and beverage samples were evaluated, showing symmetric peaks, high reproducibility of the concentration values, and negligible interferences from matrix compounds. Limits of quantification were well below 5 mg/kg for sodium nitrite and sodium nitrate in all tested samples.

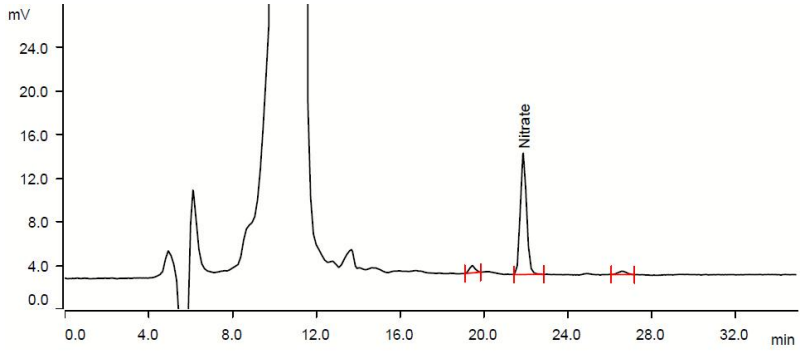

Figure 1. Chromatogram of a black blood sausage sample. Results: sodium nitrite <1.0 mg/kg, and sodium nitrate 22.5 mg/kg.

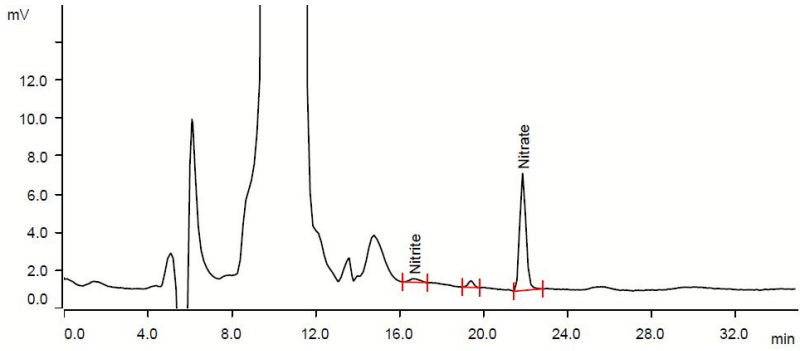

Figure 2. Chromatogram of a pork knuckle sample. Results: sodium nitrite 1.5 mg/kg, and sodium nitrate 9.6 mg/kg.

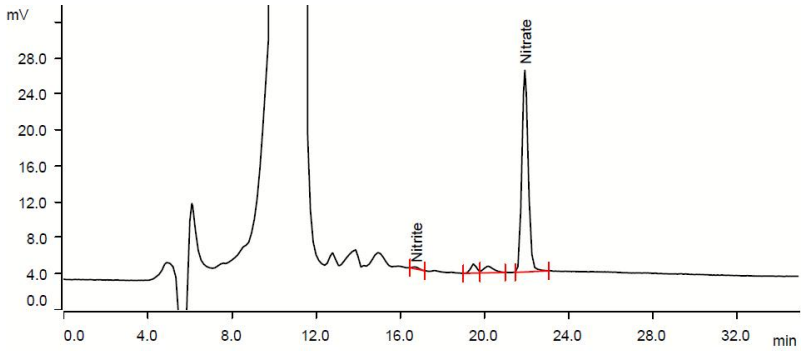

Figure 3. Chromatogram of a Chistorra sausage sample. Results: sodium nitrite <1.3 mg/kg, and sodium nitrate 49.4 mg/kg.

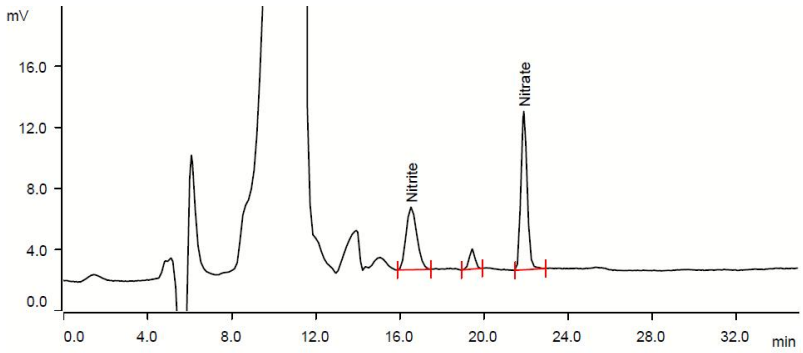

Figure 4. Chromatogram of a pork shoulder sample. Results: sodium nitrite 53.7 mg/kg, and sodium nitrate 20.0 mg/kg.

CONCLUSION

The described sample preparation and the chromatographic method worked for all tested meat products. The presented IC method with two separation columns guaranties optimal resolution of nitrate and nitrate from interfering matrix peaks and thus sensitive analysis for quality control even in complex matrices (LOQ <5 mg/kg for meat products). This method is already established in certain food laboratories as a standard method for quality control, exhibiting high accuracy and reproducibility independent from the food matrix.

Inline Ultrafiltration makes this method even more suitable for fast and time-saving routine analysis because sample preparation is straightforward and does not require costly sample preparation cartridges as in some traditional methods. As any interfering matrix is either removed by Inline Ultrafiltration or is well resolved on the analytical column, this method shows superior analytical performance for determining nitrite and nitrate in meat samples when compared to classical HPLC-UV.

Nitrite and nitrate are directly quantified, which is an advantage over traditional methods where the sum parameter of total nitrogen is determined (e.g., AOAC Official Method 935.48 or 993.03).

REFERENCES

1. Wang, P. et al. (2002), Nitric Oxide Donors: Chemical Activities and Biological Applications, Chemical Reviews 102 (4): 1091–1134.
2. EFSA (European Food Safety Authority) (2017), Re-evaluation of potassium nitrite (E 249) and sodium nitrite (E 250) as food additives, EFSA Journal 15(6):4786.
3. EFSA (European Food Safety Authority) (2017), Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives, EFSA Journal15(6):4787.
4. European Commission (2011) Decision No 1129/2011/EC of 11 November 2011, amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Off J Eur Union L295 1-177.

Internal reference: AW IC ES6-0010-042020

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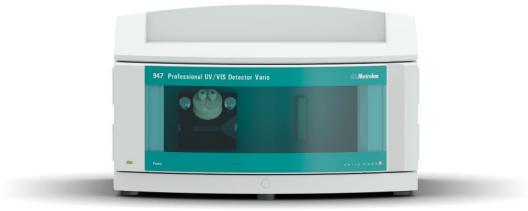
CONFIGURATION

930 Compact IC Flex Oven/SeS/Deg

The 930 Compact IC Flex Oven/SeS/Deg is the intelligent Compact IC instrument with column oven, sequential suppression and built-in degasser. An 800 Dosino can be used for the regeneration of the suppressor. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Anion or cation determinations with sequential suppression and conductivity detection



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Metrosep A Supp 5 - 50/4.0

The Metrosep A Supp 5 - 50/4.0 separates the seven standard anions in less than 6 minutes. Even fluoride is still separated from the injection peak and can be integrated perfectly. Like all columns in the A Supp 5 product range, the column based on a polyvinyl alcohol polymer is characterized by high plate numbers and therefore by outstanding separating efficiency. The Metrosep A Supp 5 - 50/4.0 is the column of choice when simple separation tasks need to be solved in a short time – and that without having to sacrifice very low limits of detection.

Metrosep A Supp 7 - 250/4.0

Disinfection byproducts from water treatment are suspected not only of being health hazards but even of being carcinogenic. Oxyhalides have therefore become the subject of many investigations and standards (e.g., EPA 300.1 Part B, EPA 317.0, EPA 326.0). Of primary concern is bromate, which forms from bromide during the ozonization of drinking water. The Metrosep A Supp 7 - 250/4.0 is a high-performance separation column for the parallel determination of standard anions, oxohalides and dichloroacetic acid. With this column, these ions are determined with certainty and precision down to the lower µg/L range. The high detection sensitivity is achieved through the use of the 5 µm polyvinyl alcohol polymer, with which extremely high plate numbers and thus outstanding separation and detection properties are achieved. In addition, the separation can be adapted to the specific requirements of the application by modifying the temperature.

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800 Dosino

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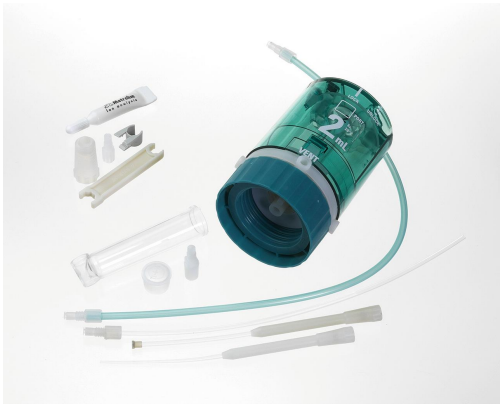

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