



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 Na₂CO₃ + 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 ₂ CO 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 guarantees optimal resolution of nitrite 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 Journal 15(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.

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CONFIGURATION

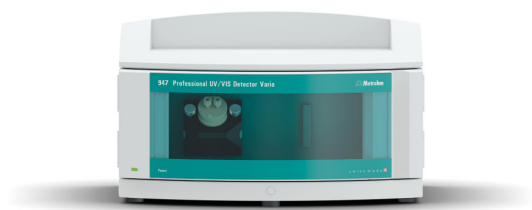


930 Compact IC Flex Oven/SeS/Deg

Le 930 Compact IC Flex Oven/SeS/Deg est l'appareil CI compact intelligent avec un **four à colonne**, **suppression séquentielle** et un **dégazeur** intégré. On peut utiliser un 800 Dosino pour la régénération du supprimeur. L'appareil peut être utilisé avec n'importe quelles méthodes de séparation et de détection.

Domaines d'application typiques :

- Déterminations d'anions ou de cations avec suppression séquentielle et détection de conductivité



947 Professional UV/VIS Detector Vario SW

Le 947 Professional UV/VIS Detector Vario SW, un détecteur intelligent à longueur d'onde unique, permet une quantification sûre et fiable des substances actives dans le domaine ultraviolet ou visible. Une longueur d'onde peut être sélectionnée.



Metrosep A Supp 5 - 50/4,0

La Metrosep A Supp 5 - 50/4,0 sépare les sept anions standard en moins de 6 minutes. Même le fluorure reste séparé du pic de temps mort et se laisse parfaitement intégrer. Cette colonne basée sur un polymère à base d'alcool polyvinylique se distingue, comme toutes les autres colonnes de la famille A Supp 5, par un nombre élevé de plateaux et, par conséquent, par une excellente performance de séparation. La Metrosep A Supp 5 - 50/4,0 est la colonne de choix lorsqu'il s'agit de résoudre de simples tâches de séparation en peu de temps – sans renoncer à des limites de sensibilité très basses.



Metrosep A Supp 7 - 250/4.0

Les produits secondaires du traitement de l'eau (disinfection by-products) sont soupçonnés d'être non seulement nocifs pour la santé, mais aussi cancérigènes. C'est la raison pour laquelle les oxohalogénures sont devenus l'objet de nombreuses recherches et de normes (p. ex. EPA 300.1 partie B, EPA 317.0, EPA 326.0). Il s'agit en premier lieu du bromate, produit du bromure lors de l'ozonisation de l'eau potable. La colonne Metrosep A Supp 7 - 250/4.0 est une colonne de séparation haute performance pour la détermination en parallèle des anions standard, des oxyhalogénures et de l'acide dichloracétique. Avec cette colonne, ces ions peuvent être déterminés jusque dans la gamme inférieure des µg/L de manière sûre et précise. La sensibilité de détection élevée est atteinte par l'utilisation d'un polymère à base d'alcool polyvinylique de 5 µm, permettant d'obtenir un nombre de plateaux très élevé et, par conséquent, d'excellentes propriétés de séparation et de détection. En plus, par modification de la température, il est possible d'adapter la séparation aux exigences spécifiques de l'application.



919 IC Autosampler plus

Le 919 IC Autosampler plus satisfait les exigences des laboratoires ayant un nombre moyen d'échantillons à traiter. Il permet l'automatisation de tous les systèmes de chromatographie ionique de Metrohm.



800 Dosino

Le Dosino 800 est un moteur de burette avec système de lecture/écriture pour les Unités de distribution intelligentes. Avec câble attenant (150 cm).



807 Dosing Unit 2 mL

807 Dosing Unit avec puce électronique de données intégrée avec cylindre en verre de 2 mL et protection contre la lumière, pouvant être installée sur une bouteille à réactifs avec filetage en verre ISO/DIN GL 45, connexion tubulaire FEP, pointe anti-diffusion. Connexion tubulaire FEP, pointe anti-diffusion.