Application Note AN-U-080

肉制品中的亚硝酸盐和硝酸盐

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.

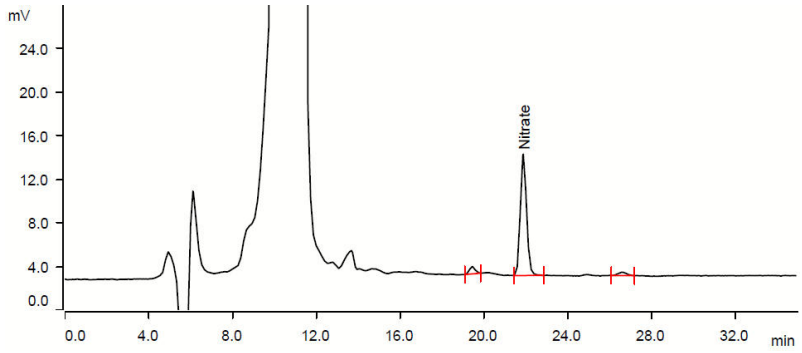

Figure 1. Chromatogram of a black blood sausage sample. Results: sodium nitrite <1.0 mg/kg, and sodium nitrate 22.5 mg/kg.

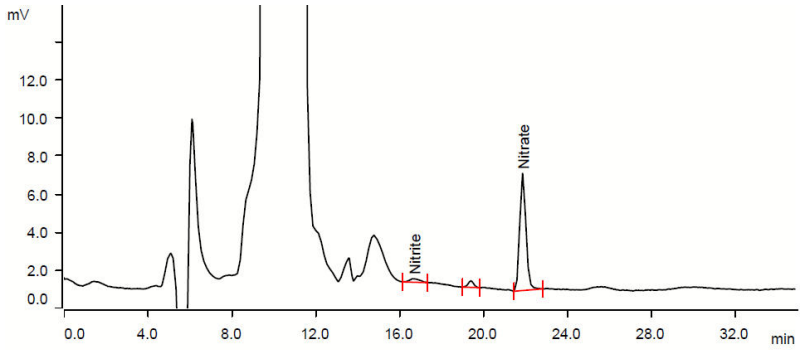

Figure 2. Chromatogram of a pork knuckle sample. Results: sodium nitrite 1.5 mg/kg, and sodium nitrate 9.6 mg/kg.

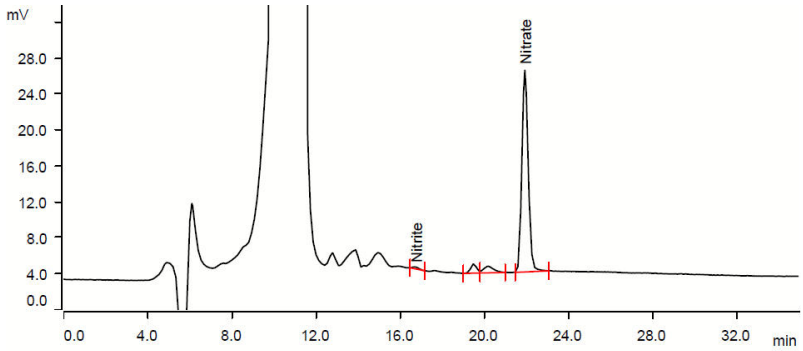

Figure 3. Chromatogram of a Chistorra sausage sample. Results: sodium nitrite <1.3 mg/kg, and sodium nitrate 49.4 mg/kg.

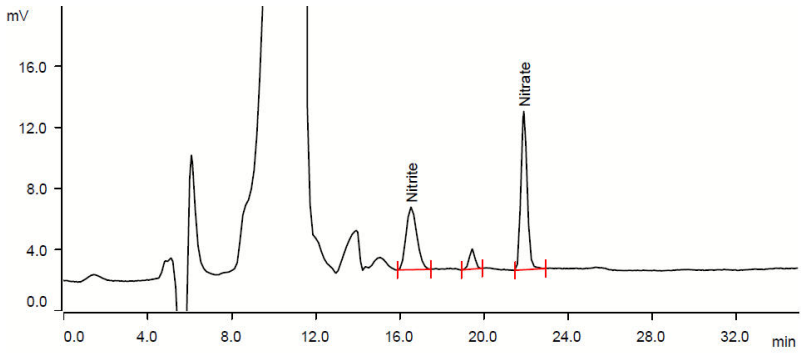

Figure 4. Chromatogram of a pork shoulder sample. Results: sodium nitrite 53.7 mg/kg, and sodium nitrate 20.0 mg/kg.

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 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 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.

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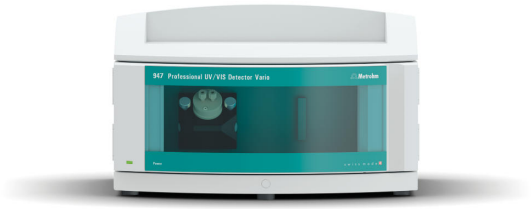
CONFIGURATION

930 Compact IC Flex Oven/SeS/Deg

930 Compact IC Flex Oven/SeS/Deg 是智能型 Compact 离子色谱仪器,带有**柱加热炉**、**序列抑制器**和内置**脱气装置**。可使用一台 800 Dosino 用于抑制器再生。该仪器可使用各种分离和检测方法。

典型的应用范围:

- 阴离子或阳离子测定,序列抑制法及电导检测



947 Professional UV/VIS Detector Vario SW

智能单波长 检测器,947 Professional UV/VIS Detector Vario SW,可对紫外光或可见光区域内的活性物质进行安全可靠的定量操作。可选择一种波长。

Metrosep A Supp 5 - 50/4.0

Metrosep A Supp 5 - 50/4.0 可在不超过 6 分钟之内分离这七种标准阴离子。即便是氟化物也可从进样尖峰分离,能够毫无问题地加以整合。此基于聚乙烯醇聚合物的色谱柱就如所有 A-Supp-5 系列色谱柱一样分离度高,由此具备出色的分离效率。Metrosep A Supp 5 - 50/4.0 色谱柱是短时间内解决简单分离任务的选择-并且无须放弃其极低的指示极限。

Metrosep A Supp 7 - 250/4.0

水处理中的副产物(消毒副产物)不仅可能危害健康,甚至可能致癌。因此羰基卤化物成为许多检测方法及标准的目标(例如 EPA 300.1 方法 B 部分、EPA 方法 317.0、EPA 方法 326.0)。首先针对那些饮用水臭氧化过程中由溴化物中产生的溴酸盐。Metrosep A Supp 7 - 250/4.0 是可以同时测定标准阴离子、羰基卤化物和二氯乙酸的高效分离柱。借助此柱甚至在低 µg/L 范围内也可准确可靠地测定这些离子。使用 5 µm 的聚乙烯醇聚合物使其达到高度指示灵敏度,其分离度高,由此实现优秀的分离性能和指示灵敏度。此外,还可通过更改温度来针对特殊应用要求调整分离工作。

919 IC Autosampler plus

919 IC Autosampler plus 满足实验室内处理中等样品量的要求。使用该仪器,可实现万通产品的各种离子色谱分析自动化。

800 Dosino

800 Dosino 驱动头,带有可用于智能型加液单元的读/写硬件设备。带固定电缆。

807 Dosing Unit 2 mL

807 Dosing Unit,带内置的数据芯片,2 mL 玻璃计量筒和遮光罩,可安装在有 ISO/ DIN 玻璃螺纹 GL 45 的试剂瓶上。FEP 管路连接、防扩散滴管头。