Application Note

Food & Beverages

Nitrate and Nitrite

Introduction

Nitrate (NO_3^-) and nitrite (NO_2^-) are naturally occurring ions that are part of the nitrogen cycle [1,2]. Vegetables and cured meat are in general the main sources of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Both anions are also found in soil and water due to use of inorganic fertilizers in agriculture [3]. Furthermore, nitrate and nitrite are approved additives (E249 - E252) in food products such as salami, ham and cheese [4]. These additives are added in processed food, as preservatives, antimicrobial agents and as color fixatives. Cured meat products may contain up to 150 mg/kg nitrate or nitrite additives in the EU [5]. For ground water the EU directive 2006/118/EC sets a limit of 50 mg/L for nitrates. The dietary intake of nitrite & nitrate is associated with some potential health risks [6]. Nitrate can be converted into nitrite by bacteria in the mouth. Nitrite can oxidize haemoglobin into methaemoglobin, which in excess reduces the ability of red blood cells to bind and transport oxygen to the body. Furthermore, nitrite may be transformed into nitro samines in the body, some of which are carcinogenic. In 2002, the Acceptable Daily Intake (ADI) was set by the WHO to 0.07 mg and 3.7 mg per kg of body weight per day (mg/kg bw/day) for nitrite and nitrate, respectively. Various analytical techniques for the determination of nitrite and nitrate are developed over the past decades [7]. This note demonstrates the fast and simple analysis of nitrate and nitrite in food products and water using the ALEXYS LC-UV analyzer.

Method

The ALEXYS HPLC-UV analyzer consist of a P6.1L pump, AS 6.1L autosampler, CT 2.1 column thermostat and UVD 2.1L detector. The UVD 2.1L is a single wavelength UV detector equipped with a 10 mm path length flow cell (wavelength range 190—750 nm). The P 6.1L pump has an integrated dual channel vacuum degasser and solvent selection valve, allowing step-gradient chromatography. The LC-UV conditions are listed in table 1 and are adapted from ref [8].

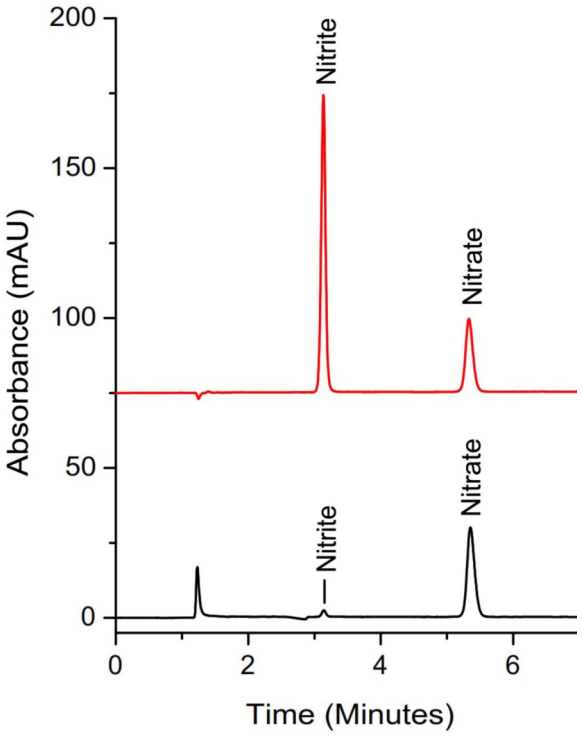

Fig. 1. *Example chromatograms of a 25 µL injection of a standard mix of 4.6 mg/L nitrite and 6.2 mg/L nitrate in water (red) and a surface water sample (black).*

Table 1. LC-EC conditions

HPLC	ALEXYS LC-UV Analyzer
Column	Thermo Scientific™ Dionex™ IonPac™ AS11, 250 x 4 mm ID, 13 µm analytical column, with Thermo Scientific™ Dionex™IonPac™ AG11, 50 x 4 mm, 13 µm guard column
Mobile phase (MP)	MP A: 5 mM NaOH MP B: 100 mM NaOH
Flow rate	1 mL/min
Step gradient	0—10 min MP A (isocratic elution) 10—15 min MP B (wash/regeneration) 15—30 min MP A (equilibration)
Backpressure	About 65 bar
Injection volume	25 µL (partial loop fill), samples cooled at 5°C
Temperature	35°C
Flow cell	10 mm path length, 1/16”, 10 µL volume (pn A4061XB)
Wavelength	225 nm
Filter	0.2 sec (time constant)

Isocratic separation was achieved using an 250 x 4 mm ID anion exchange column at 35°C, in combination with a 5 mM NaOH eluent. After elution of the anions, the column is flushed for 5 minutes with 100 mM NaOH. The anions are detected at a wavelength of 225 nm.

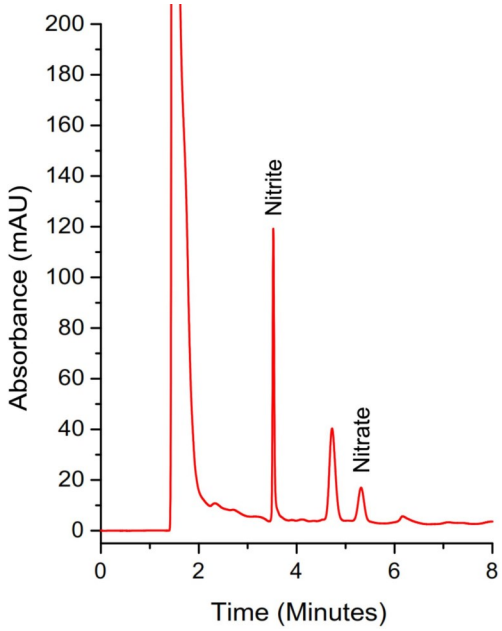

Fig. 2. *Chromatogram of a salami sample (25 µL injection volume).*

Example chromatograms of an 25 µL injection of a 100 µM standard mix and a real surface water sample are shown in 1. The surface water sample was filtered using a 0.2 µm PTFE 25 mm disc filter. Both compounds are eluted within 6 minutes. The Limit of Detection (LOD) for nitrite and nitrate calculated from the response of the standard mix are 21 and 42 µg/L, respectively. The LOD concentration for nitrate was approximately a factor 80 lower than the actual concentration found in a worked up salami sample (see next paragraph) and a factor 200 lower for nitrite. The repeatability was assessed using standards. RSD's (peak area) of 1.5 and 1.7 % (n=10) were obtained for a 0.5 mg/L standard for nitrite and nitrate. The linearity was investigated in the concentration range of 50 µg/L—50 mg/L. In this conc. range the linearity is excellent and correlation coefficients for peak area were better than 0.999 for both anions.

In figure 2 an example chromatogram of a 25 µL injection of a salami sample is shown. The sample preparation of the salami sample was performed in the following manner: 2.5 gram of salami in 25 mL DI water was homogenized with a turax for 5 minutes. The test tube with homogenate was kept at 70 °C in a water bath for 15 minutes. After cooling down, the homogenate was centrifuged at 3000 x g for 10 minutes and the supernatant collected. Subsequently, the supernatant was filtered over a 0.2 µm 25 mm PES (polyethersulfone) disc filter and 25 µL injected into the LC system. The salami sample was spiked with the anions for peak identification.

The amount of nitrite and nitrate in the sample were quantified based on the calibration curve. The calculated contents of nitrite and nitrate in the salami sample were 41 mg/kg and 30 mg/kg product, respectively.

References

1. Wiki, Nitrite, https://en.wikipedia.org/wiki/Nitrite
2. Wiki, Nitrate, https://en.wikipedia.org/wiki/Nitrate
3. World Health Organization (WHO), *Nitrite and Nitrate in drinking water,* https://www.who.int/water_sanitation_health/dwq/chemicals/nitratenitrite2ndadd.pdf
4. R.C. Sprong et. Al., *Intake assessment of the food additives nitrites (E 249 and E 250) and nitrates (E 251 and E 252), RIVM report 2-16-0208*, https://www.rivm.nl/bibliotheek/rapporten/2016-0208.pdf
5. Food safety Authority of Ireland, *Guidance document for Nitrate/Nitrite sampling of Meat products*, https://www.fsai.ie/publications_nitrate_nitrite_sampling.html
6. EFSA, *Nitrites and nitrates added to food*, https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/nitrates-nitrites-170614.pdf
7. Q.-H. Wang et al, *Methods for the detection and determination of nitrite and nitrate: A review.* Talanta, (2017)165, 709–720.
8. Dionex , *Determination of nitrate and nitrite in meat using HPAEC*, application note 112

Ordering information

180.0077UV	ALEXYS HPLC-UV analyzer Consisting of P 6.1L pump, AS 6.1L autosampler, UVD 2.1L UV

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