Supelco®
Analytical Products

Application Note

# Nitrite in Dried Meat

## Photometric determination using the Griess method subsequent to extraction

## Introduction

Nitrite, in the form of sodium nitrite (E250) and potassium nitrite (E249), is widely used in meat processing to ensure microbiological safety, stabilize cured color, and enhance flavor. Its inhibitory effect against *Clostridium botulinum* is considered critical for consumer protection, and for this reason nitrite has been authorized as a food additive under European legislation, including Regulation (EC) No 1333/2008 and its subsequent amendments.[1]

The concern with nitrite use arises from its potential to form *N*-nitroso compounds under certain conditions, many of which are recognized as carcinogenic.[2] As a result, residual nitrite levels in cured and dried meat products are tightly regulated and continuously monitored.

Several analytical techniques, including electrochemical and chromatographic methods, have been explored for nitrite determination, yet spectrophotometric approaches remain predominant due to their simplicity, cost-effectiveness, and suitability for routine analysis.[2,3] The photometric Griess method, in which extracted nitrite is converted into a stable azo dye,[3] provides a reliable reference for routine quality control and regulatory monitoring of nitrite in meat products.

## Experimental

### Method

Nitrite is extracted from dried meat using a potassium hydrogen phthalate solution. Then in acidic solution, nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically.

### Measuring Range

| | | |
|---|---|---|
| Spectroquant® Nitrite Cell Test (**1.14547**): | Test kit | 0.03–2.30 mg/L $NO_2$- |
| | Method | 0.60–46.0 mg/kg $NO_2$- |
| | | 0.90–69.0 mg/kg $NaNO_2$ |
| Spectroquant® Nitrite Test **1.14776**: | Test kit | 0.007–3.28 mg/L $NO_2$- |
| | Method | 0.14–65.6 mg/kg $NO_2$- |
| | | 0.21–98.4 mg/kg $NaNO_2$ |

### Applicable Sample

Dried Meat

### Reagents, Instruments and Materials

#### Reagent & Test Kits

- Spectroquant® Nitrite Cell Test (**1.14547**) or
- Spectroquant® Nitrite Test (**1.14776**)
- Potassium hydrogen phthalate for analysis (**1.04874**)
- MQuant® pH-indicator strips pH 0 - 6.0 (**1.09351**)
- Sulfuric acid 0.5 mol/L Titripur® (**1.09072**)
- Sodium hydroxide solution 1 mol/L Titripur® (**1.09137**)
- Water for analysis (**1.16754**)

#### Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)



MERCK

- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)
- Spectroquant® Colorimeter Move 100 (**1.73632**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

### Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

### Instrument Accessories

- Rectangular cells 10 mm (**1.14946**) or
- Rectangular cells 20 mm (**1.14947**) or
- Rectangular cells 50 mm (**1.14944**)

**Note:** Rectangular cells are only necessary if the Spectroquant® Nitrite test **1.14776** is used.

## Other Reagents and Accessories

- Analytical balance
- Volumetric flask, 100 mL
- Schliff Erlenmeyer flask, 250 mL
- Knife or scissors
- Mixer or electric coffee mill
- Centrifuge
- Standard laboratory glassware (e.g., glass beakers) and pipettes
- Folded filters

## Analytical Procedure

### Reagent preparation

Weigh 2.0 g potassium hydrogen phthalate and transfer it into a 100 mL volumetric flask. Fill up to the mark using water for analysis.

### Sample preparation

Cut down the sample with a knife or with scissors and ground it in a mixer or an electric coffee-mill to a fine, fibrous powder. In a closed Schliff-Erlenmeyer flask stir 5 g of the grounded product with 95 mL water for analysis and 5 mL potassium hydrogen phthalate solution 2% for 2 hours. Then separate the suspension in the centrifuge at 4000 turns per minute for 15 minutes. Filter the supernatant solution through a folded filter (=pretreated sample)

## Using Cat. No. 1.14547: Procedure and measurement

For more information on the measurement see the packaging insert of the test

### Procedure

- Pipette 5.0 mL pretreated sample into a reaction cell, close the cell tightly, and shake **vigorously until the reagent is completely dissolved.**
- **Leave to stand for 10 min (reaction time),** then measure the sample in the photometer.

### Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16-mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

## Using Cat. No. 1.14776: Procedure and measurement

For more information on the measurement see the packaging insert of the test

### Procedure

- Pipette 5.0 mL pretreated sample into a test tube,
- Add 1 level microspoon (in the cap of the $NO_2$-1 bottle) and shake **vigorously for 1 min until the reagent is almost completely dissolved. The pH must be in the range of pH 2.0–2.5.** Check with MQuant® pH-indicator strips. Adjust, if necessary, with sodium hydroxide solution or sulfuric acid.
- **Leave to stand for 10 min (reaction time),** then fill the sample into the cell, and measure the sample in the photometer.

**Note:** For measurement in the 50 mm cell both the sample volume as well as the quantity of reagent

$NO_2$-1 must be doubled. Alternatively, the semi-microcell **Cat. No. 1.73502** can be used. It is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.

### Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- If a 50 mm cell is used, it is recommended to perform the reagent blank one time for each measurement series. To do this tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- After the reagent blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

### Calculation

Nitrite content in mg/kg $NO_2$ = analysis value in mg/L $NO_2^-$ x 20

Nitrite content in mg/kg $NaNO_2$ = analysis value in mg/L $NO_2^-$ x 30

## Analytical quality assurance

Analytical Quality Assurance (AQA is recommended before each measurement series.

To check the photometric measurement system (test reagent, measurement device, handling) and the mode of working, the nitrite standard solution (see section 5 of the respective test kit instruction) can be used.

Sample-dependent interferences (matrix effects) can be determined by means of standard addition.

To view additional notes, visit
**Sigmaaldrich.com/qa-test-kits**

## References


1. Regulation (EC) No 1333/2008 on food additives **https://eur-lex.europa.eu/eli/reg/2008/1333/oj/eng**
2. Casoni D, Badiu RR, Frenţiu TI. Spectrophotometric determination and assessment of potential health risk of nitrite from meat and processed meat products. Stud. UBB Chem. **2009**;2:265-77. DOI: **10.24193/subbchem.2019.2.22**
3. Oliveira SM, Lopes TI, Rangel AO. Spectrophotometric determination of nitrite and nitrate in cured meat by sequential injection analysis. Journal of food science. **2004**;69(9):C690-5. DOI: **10.1111/j.1365-2621.2004.tb09917.x**


## Featured Products

| Description | Cat. No. |
|---|---|
| **Reagent and Test Kits** | |
| Spectroquant® Nitrite Cell Test | **1.14547** |
| Spectroquant® Nitrite Test | **1.14776** |
| **Instruments and Accessories** | |
| Spectroquant® VIS Spectrophotometer Prove 100 plus | **1.73026** |
| Rectangular cells 10 mm | **1.14946** |
| Rectangular cells 20 mm | **1.14947** |
| Rectangular cells 50 mm | **1.14944** |
| **Reagents** | |
| Potassium hydrogen phthalate for analysis EMSURE® Reag. Ph Eur | **1.04874** |
| MQuant® pH-indicator strips pH 0–6.0 | **1.09531** |
| Water for analysis EMSURE® | **1.16754** |

## Related Products

| Description | Cat. No. |
|---|---|
| **Instruments and Accessories** | |
| Spectroquant® UV/VIS Spectrophotometer Prove 300 plus | **1.73027** |
| Spectroquant® UV/VIS Spectrophotometer Prove 600 plus | **1.73028** |
| Spectroquant® colorimeter Move 100 | **1.73632** |
| Semi-microcells 50 mm | **1.73502** |
| **Reagents** | |
| Nitrite standard solution, 0.200 mg/L $NO_2$-N | **1.25041** |
| Sulfuric acid 0.5 mol/L Titripur® | **1.09072** |
| Sodium hydroxide solution 1 mol/L Titripur® | **1.09137** |

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