International
Standard

ISO 7158

First edition
2024-02

Meat and meat products — Determination of nitrite and nitrate content — Ion chromatography method

Viandes et produits carnés — Détermination des teneurs en nitrites et nitrates — Méthode de chromatographie ionique

Reference number
ISO 7158:2024(en)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products.*

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Meat and meat products — Determination of nitrite and nitrate content — Ion chromatography method

1 Scope

This document specifies a method for the determination of nitrite and nitrate in meat and meat products using ion chromatography.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Samples are extracted in water with interferences of protein and other substances removed by the addition of protein precipitators and purification columns. The analytes are separated by anion-exchange chromatography. Detection is performed using conductivity or UV/VIS detection with an external standard method.

5 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in CAC/GL 50-2004.

It is important that the laboratory-received sample is truly representative and has not been damaged or changed during transport or storage.

Start from a representative sample of at least 200 g. Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (7.2). Take care that the temperature of the sample does not exceed 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared sample. Close the container and store it in such a way that prevents deterioration and change in composition. Analyse the sample as soon as possible, but always within 24 h after homogenization.

7 Test method of Ion chromatography

7.1 Reagents and Materials

Only reagents of recognized analytical grade and only water of at least grade 1 purity as defined in ISO 3696 shall be used.

7.1.1 Potassium ferrocyanide (106 g/L), Weigh 106 g of potassium ferrocyanide, dissolve with water, dilute to 1 000 mL, and mix well.

7.1.2 Zinc sulphate (220 g/L), Weigh 220 g of zinc sulfate, dissolve with water, dilute to 1 000 mL, and mix well.

7.1.3 Potassium hydroxide (1 mol/L), weigh 5,6 g of potassium hydroxide, dissolve with water, dilute to 100 mL, and mix well.

7.1.4 Sodium nitrite ($NaNO_2$, CAS No.®[1]: 7632-00-0), standard reagent, alternatively, sodium nitrite standard solution with reference material certificate, may be used.

7.1.5 Sodium nitrate ($NaNO_3$, CAS No.®: 7631-99-4), standard reagent, alternatively, sodium nitrate standard solution with reference material certificate, may be used.

7.1.6 Nitrite standard stock solution (100 mg/L, as NO_2^-, the same herein below), accurately weigh 0,150 g of sodium nitrite which has been dried to constant weight at 104 °C ± 3 °C, dissolve with water and transfer into a 1 000-mL volumetric flask, adjust to 1 000 mL with water, and mix well. The stock solution may be used for a maximum of 2 weeks if stored in a refrigerator at 4 °C.

7.1.7 Nitrate standard stock solution (1 000 mg/L, as NO_3^-, the same herein below), accurately weigh 1,371 g of sodium nitrate which has been dried to constant weight at 104 °C ± 3 °C, dissolve with water and transfer into a 1 000-mL volumetric flask, adjust to 1 000 mL with water, and mix well. The stock solution may be used for a maximum of 2 weeks if stored in a refrigerator at 4 °C.

7.1.8 Sodium nitrate and sodium nitrite mixed standard intermediate solution, accurately pipette 1,0 mL of nitrite ion (NO_2^-) standard solution and nitrate ion standard solution (NO_3^-) respectively into a 100-mL volumetric flask, and adjust to 100 mL with water. Each 1 L of the obtained solution contains 1,0 mg of nitrite ion and 10,0 mg of nitrate ion. It is recommended to prepare the standard solutions on the day of use.

7.1.9 Sodium nitrate and sodium nitrite mixed standard working solutions, pipette sodium nitrate and sodium nitrite mixed standard intermediate solution, serially dilute with water to prepare a set of mixed standard working solutions with the recommended nitrite ion concentration of 0,02 mg/L, 0,04 mg/L, 0,06 mg/L, 0,08 mg/L, 0,10 mg/L, 0,15 mg/L, 0,20 mg/L and nitrate ion concentration of 0,2 mg/L, 0,4 mg/L, 0,6 mg/L, 0,8 mg/L, 1,0 mg/L, 1,5 mg/L and 2,0 mg/L. It is recommended to prepare the standard solutions on the day of use.

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[1] Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

7.2 Apparatus

The usual laboratory equipment and, in particular, the following.

7.2.1 Ion chromatograph, equipped with electrical conductivity detector and suppressor or ultraviolet detector. The loop size may vary between different instrumental setups and the analyte concentration in the sample. An autosampler with cooling is recommended to increasing sample stability and laboratory accuracy and efficiency.

7.2.2 Homogenising equipment, mechanical or electrical, capable of homogenising the test sample, including food grinder or high-speed rotational cutter.

7.2.3 Ultrasonic bath.

7.2.4 Analytical balances, with sensitivities of 0,1 mg and 1 mg.

7.2.5 Centrifuge, with 50 mL or 100 mL centrifuge tubes.

7.2.6 Syringe filter, with hydrophilic filter membrane of 0,22 μm.

7.2.7 Purification column, including C_{18} column, Ag column and Na column or equivalent columns.

7.2.8 Syringe, with nominal capacity of 1,0 mL and 2,5 mL, or other filtration with equivalent performance.

NOTE All glassware shall be soaked in 2 mol/L potassium hydroxide solution and water for 4 h, respectively, in turn, then rinsed with water for 3 to 5 times, and then dried before use.

7.3 Procedures

7.3.1 General

If it is required to check whether the repeatability requirement is met, two individual determinations should be performed.

7.3.2 Extraction and clarification

Weigh out 5 g of the sample, place it in a 200 mL volumetric flask, add 50 mL of water, and dissolve the sample in the ultrasonic bath (or equivalent technique). Ultrasonic dissolution is recommended to be performed at room temperature with a frequency 40 kHz. For protein precipitation add 5 mL of the 106 g/L potassium ferrocyanide solution, shake well, and then add 5 mL of the 220 g/L zinc sulfate solution. Add water to the 50 mL scale, shake well, and extract the sample for 30 min.

Afterwards the solution needs to be centrifuged for at least 3 min (operating at a radial acceleration of about 5 000 g) and the supernatant is ready for later use.

Take about 15 mL of the ready-to-use solution mentioned above, filter with a syringe filter with hydrophilic filterable membrane of 0,22 μm and pass through C_{18} column, Ag column and Na column in sequence, and eliminate 7 mL of the initial filtrate. Collect the subsequent eluent for determination later.

The solid phase extraction column shall be activated before applied following the manufactuers' instructions. For example, the activation process of C_{18} column (1,0 mL), Ag column (1,0 mL) and Na column (1,0 mL) is as follows: 5 mL of methanol and 10 mL of water passes through the column and let it stand for activation for 30 min before use.

7.3.3 Chromatographic conditions

7.3.3.1 Chromatographic column: with selectivity to hydroxides, anionic exchange column compatible to gradient elution with divinyl benzene-ethylstyrene copolymer matrix, alkanol-based quaternary ammonium salt functional group with high capacity, 4 mm × 250 mm (with a protection column of 4 mm×50 mm), or other ion chromatographic columns with equivalent performance.

7.3.3.2 Eluent. Potassium hydroxide solution: with concentration of 6 mmol/L to 70 mmol/L; with recommended elution gradient of 6 mmol/L for 30 min, 70 mmol/L for 5 min, 6 mmol/L for 5 min; and flow rate of 1,0 mL/min, or other elution gradient with equivalent performance. See Figures A.1 and A.2 for typical chromatograms.

7.3.3.3 Suppressor.

7.3.3.4 Detector: electrical conductivity detector, with temperature of detector cell at 35 °C; or

UV spectrophotometric detector, with detection wavelength of 226 nm.

7.3.3.5 Injection volume: 50 µL (the injection volume can be adjusted to obtain optimum chromatography and sensitivity following the manufactuers' instructions.)

7.3.4 Determination

7.3.4.1 Plotting for standard curve

Respectively inject the series of standard working solutions into the ion chromatograph to obtain the chromatogram of the standard working solution at each concentration. Determine the corresponding peak height (µS) or peak area. Plot the standard curve with the concentration of standard working solution as abscissa and peak height (µS) or peak area as ordinate.

7.3.4.2 Determination of test sample solution

Inject the blank and test sample solution into the ion chromatograph to obtain the peak height (µS) or peak area of blank and test sample solution. Achieve the concentration of nitrite ion and nitrate ion in the solution to be tested based on the standard curve.

7.4 Calculation

The contents of nitrite ion and nitrate ion in the test sample shall be calculated according to Formula (1):

$$X=\frac{(\rho-\rho_0)\times V\times f\times 1\,000}{m\times 1\,000} \tag{1}$$

where

X is the content of nitrite or nitrate ion in test sample, mg/kg;

ρ is the concentration of nitrite or nitrate ion in test sample solution used for determination, mg/L;

ρ_0 is the concentration of nitrite or nitrate ion in reagent blank solution, mg/L;

V is the volume of test sample solution, mL;

f is the dilution ratio of test sample solution;

1 000 is the conversion coefficient;

m is the sampling amount of test sample, g.

Multiply the obtained content of nitrite ion in the test sample by 1,5 to get the nitrite content (as sodium nitrite). Multiply the obtained content of nitrate ion in the test sample by 1,37 to get the nitrate content (as sodium nitrate).

Keep two significant figures for the result.

7.5 Precision

See Annex B for a summary of the statistical results of this test. Statistical parameters are expressed in accordance with ISO 5725-2.

The precision of nitrite contents measurement method should be quoted as follows: Repeatability standard deviation: $S_r = 0{,}022\ 8\ m$; Reproducibility standard deviation: $S_R = 0{,}069\ 1\ m$

The precision of nitrate contents measurement method should be quoted as follows: Repeatability standard deviation: $S_r = 0{,}027\ 9\ m$; Reproducibility standard deviation: $S_R = 0{,}069\ 2\ m$

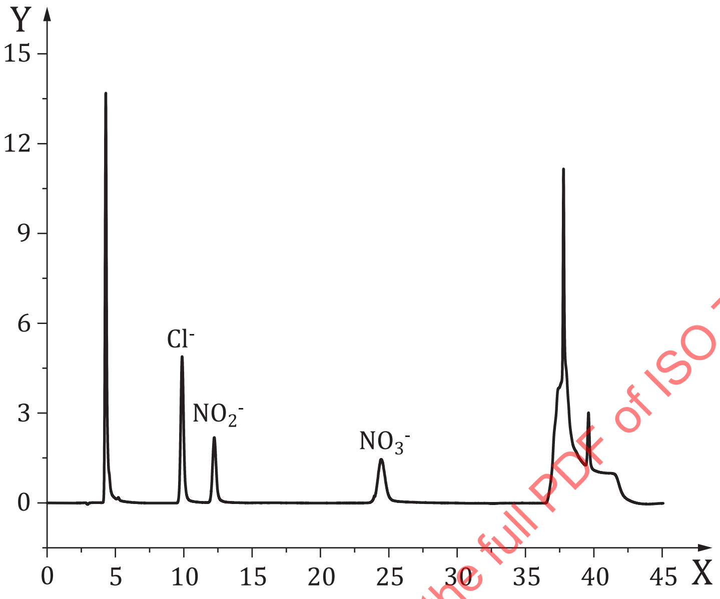
7.6 Limits of detection (LOD) and limits of quantification (LOQ)

The average blank value (n = 20) plus 3 times the standard deviation of the blank is taken as the detection limit and the average blank value (n = 20) plus 10 times the standard deviation of the blank is taken as the determination limit.

The limits of detection of nitrite and nitrate are 1 mg/kg and 2 mg/kg, respectively. The limits of quantification of nitrite and nitrate are 4 mg/kg and 6 mg/kg, respectively.

Annex A
(Informative)

Chromatogram

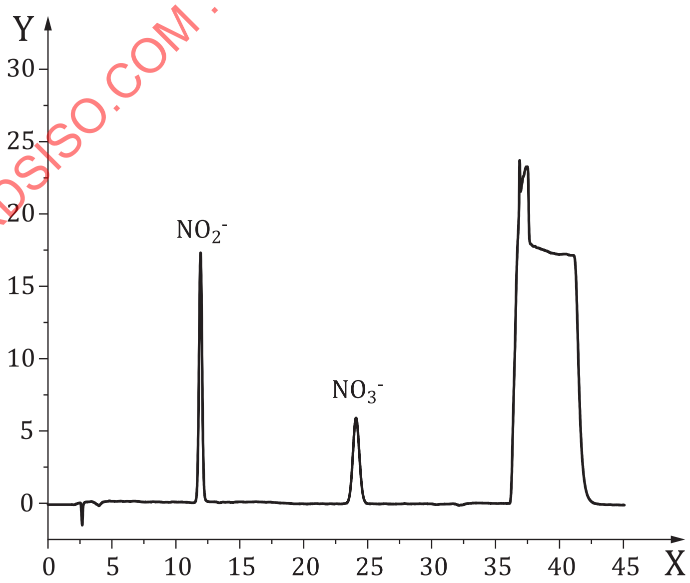


Key

X time (min)

Y value (µS)

Figure A.1 — Chromatogram with CD detector



Key

X time (min)

Y value (mAU)

Figure A.2 — Chromatogram with UV detector

Annex B
(Informative)

Validation study

A collaborative study was conducted during the period June to August 2022, with the participation of 10 laboratories. The study was performed on five samples of meat and meat products. Sample A-C represent porcine with different spiked amounts. Sample D-E represent chicken and ham respectively with different spiked amounts. Each sample was analysed in duplicate. The results were evaluated in accordance with ISO 5725-2. The statistical data are given in Tables B.1 and B.2 after calculation. No anomalous values have been determined applying Mandel's h and k statistics, Cochran tests and Grubbs tests.

Table B.1 — Summary of statistical results (nitrite content)

	Sample A	Sample B	Sample C	Sample D	Sample E
Number of participating laboratories, N	10	10	10	10	10
Number of laboratories after anomalous elimination data, n	10	10	10	10	10
Mean, mg/kg	39,625	21,995	4,364	4,133 5	5,421 5
Repeatability, sr, mg/kg	0,98	0,66	0,018	0,019	0,067
CV,r (%) (sr × 100/average)	2,47	3,00	0,41	0,46	1,24
Reproducibility, sR, mg/kg	3,01	1,09	0,047	0,031	0,047
CV,R (%) (sR × 100/average)	7,60	4,96	1,08	0,75	0,87

Table B.2 — Summary of statistical results (nitrate content)

	Sample A	Sample B	Sample C	Sample D	Sample E
Number of participating laboratories, N	10	10	10	10	10
Number of laboratories after anomalous elimination data, n	10	10	10	10	10
Mean, mg/kg	30,765	21,145	26,09	10,939 5	37,645
Repeatability, sr, mg/kg	0,40	0,27	0,84	0,50	1,47
CV,r (%) (sr × 100/average)	1,30	1,28	3,22	4,57	3,90
Reproducibility, sR, mg/kg	1,87	1,11	2,72	0,88	2,34
CV,R (%) (sR × 100/average)	6,08	5,25	10,43	8,04	6,22