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NUMERIC PERFORMANCE CRITERIA FOR NITRATE AND NITRITE IONS IN CERTAIN FOOD MATRICES

(Prepared by the EWG co-chaired by Australia and the United States of America)

BACKGROUND

1. CCFA, in its 51st session (2019), agreed to take a risk management approach that would establish both ingoing and residue levels for nitrates and nitrites in the *General standard for food additives* (GSFA) (CXS 192-1995). In its 52nd session (2021), CCFA requested CCMAS to:
 - i. establish criteria for the detection of nitrate and nitrite ions in a variety of food matrices, specifically dairy (cheese), meat, and seafood; and
 - ii. provide information on available methods for detection that met the established criteria, and in addition whether the method can detect both ions and if so whether the method detects each ion separately or only in combination.
2. CCMAS42 (2023), in response to this request, noted that further consideration was needed to provide a reply to CCFA.
3. Despite planning to do so, no EWG was eventually constituted. However, Australia and the United States of America (USA) progressed the work and prepared the document CX/MAS 24/43/8.
4. CCMAS43 noted that further work was needed to respond to CCFA's questions and agreed to continue its efforts on this matter, so re-established the EWG, with the following terms of reference:
 - a. to establish numeric performance criteria for the determination of nitrate and nitrite ions in the food matrices listed in CX/FA 21/52/7 Appendix 5, Annex 2 including adopted MLs in the GSFA and the lowest proposed residual levels;
 - b. to review the methods in CX/FA 21/52/7 Appendix 5, Annex 1 and determine if these methods meet the numeric performance criteria established for the matrices in CX/FA 21/52/7 Appendix 5, Annex 2 for both adopted maximum levels (MLs) in the GSFA and lowest proposed residual levels;
 - c. to discuss if the methods determine both nitrate and nitrite ions and if so, whether the methods detect each ion separately or only in combination; and
 - d. to discuss if the different determination schemes (i.e. separate or combined) could have an impact on the precision and accuracy of the methods.
5. Following CCMAS43, an EWG was established, and two rounds of consultation have since been conducted. The list of EWG participants is provided in Appendix 4. Comments were received from the European Union and IDF. The USA and Australia, as co-Chairs of the EWG, subsequently further developed the document.
6. This report has expanded on the discussion paper provided to CCMAS43 and includes the activity following CCMAS43 to remedy the previous deficiencies and fully respond to CCFA's questions. The report contains the following Appendices:
 - i. Appendix 1: numeric performance criteria for adopted MLs listed in the GSFA with example methods from Appendix 3 which meet these criteria.
 - ii. Appendix 2: numeric performance criteria for the lowest proposed residual MLs as provided in CX/FA 21/52/7 Appendix 5 Annex 2, with example methods from Appendix 3 which meet these criteria.

- iii. Appendix 3: List of methods in CX/FA 21/52/7 Appendix 5, Annex (plus a recently published method) to which a review has been undertaken and the method validation data summarized for assessment against the numeric performance criteria.
- iv. Appendix 4: List of participants.

DISCUSSION

Establish numeric performance criteria for the determination of nitrate and nitrite ions in the food matrices listed in CX/FA 21/52/7 Appendix 5, Annex 2 as well as for adopted MLs in the GSFA

- 7. "Residual ML (mg/kg)" in CX/FA 21/52/7 Appendix 5, Annex 2 provided MLs for numeric performance criteria development. The only Subcategory (commodity) listed in Annex 2 with an adopted ML provision in CXS 192-1995 was '01.6.2.1 (Ripened cheese, includes rind)'. It is noted that there could be a mis-assigned subcategory number for processed cheese'.
- 8. For the adopted maximum levels in CXS 192–1995 (Revision 2024), numeric performance criteria and examples of applicable methods are given in Appendix 1. Similarly, the numeric performance criteria and examples of applicable methods that meet those criteria for the lowest 'Residual ML (mg/kg)' in CX/FA 21/52/7 Appendix 5, Annex 2 in the food matrix listed are given in Appendix 2.

Review the methods in CX/FA 21/52/7 Appendix 5, Annex 1 and determine if these methods meet the numeric performance criteria established for the matrices in CX/FA 21/52/7 Appendix 5, Annex 2 as well as adopted MLs in the GSFA

- 9. The methods listed in CX/FA 21/52/7 Appendix 5 Annex 1 have been collated with available performance data and listed in Appendix 3. Appendix 3 covers 74 method suggestions from 10 Member Countries, One Member organization, or standards development organisations (SDOs), plus one method recently published, with some repetition (18 cases where the method submission was repeated by another Member or organization).
- 10. Where method submissions did not have food within their scope, or was only a summary of methods, they were considered as 'not suitable for further review' (13 cases). This left 43 methods for consideration against the numeric performance criteria (of which 14 were standardized methods by AOAC, NMKL, ISO, IDF or EN) for varying food scopes, including singularly or in some cases, combinations of: meat and meat products, baby foods, vegetable and vegetable products, milk and milk products, fruits, and shellfish. Only one method had 'Fish and fishery products' in the scope and in this case "shellfish (mussels and clams)". Many methods involved 'colorimetry/ Griess reaction' {some automated with segmented flow analysis (SFA) or flow injection analysis (FIA)} and measured 'NO₂ & NO_x (NO₃ by subtraction)'; while another larger group included 'HPLC' with various detectors and measured 'NO₂ & NO₃ individually'.
- 11. It should be noted that only a limited number of methods published prior to 2005 provide a complete validation data set for comparison with the numeric performance criteria. Methods published between 1995-2005 tended to provide either LOD or LOQ and precision data. Prior to 1995, publication of validation data is rare and standardized methods since 2010 tend to include collaborative trial data (an exception is NMKL No. 194:2013). Published method research articles typically only provide single-laboratory validation but may compare results with standard methods or utilize a certified reference material in their validation.
- 12. With foods where nitrite/nitrate is used as an 'additive', there has been a push to lower 'residual' levels in the food where it did not increase the reporting of botulism. This led to more studies of various foods' endogenous level and lower LOQ methods generally. Where a standardized method met the criteria and was collaboratively trialed, this method was preferably listed in contrast to a 'single-laboratory validated method'. Only in the absence of a multi-laboratory validated method (MLV) should a single-laboratory validated (SLV) method be considered. Where MLV and SLV methods are listed for determination of nitrite or nitrate in a food subcategory, the SLV methods are only listed for information.
- 13. Methods which meet the respective criteria have been included in Appendix 1 and Appendix 2. In some cases, like the 'Nitrite 09.3.3 (Salmon substitutes, caviar, and other fish roe products)' had a very low LOD/LOQ criteria, which might be achievable with newer methodologies but probably not with existing 'standardized' methods and no published method with appropriate SLV could be found from the methods listed in Appendix 3 or recent literature searches and so have "**Multi-laboratory validation** – not available; **Single-laboratory validation** – not available."

Discuss if the methods determine both nitrate and nitrite ions and if so, whether the methods detect each ion separately or only in combination

- 14. For the latter discussion on impact on the precision and accuracy of the methods, it was necessary to expand the number of method variations, as some measure Nitrate ion but assume Nitrite is inconsequential or measure Nitrite and a 'total Nitrite plus Nitrate'(NO_x), so each method has been assigned one of the following five variations in Appendix 3 in response to this question.

- NO₂ & NO₃ individually,
 - NO_x only,
 - NO₂ only,
 - NO₃ only,
 - NO₂ & NO_x (NO₃ by subtraction).
15. As indicated above, methods utilizing 'HPLC' with various detectors, ion chromatography (IC) and capillary electrophoresis (CE) typically measure 'NO₂ & NO₃ individually'.
16. Some methods utilizing 'colorimetry/ Griess reaction' were automated with segmented flow analysis (SFA) or flow injection analysis (FIA) to measure 'NO₂' and would then have a separate test portion to undergo reduction (Cd or V(III), or Zn columns) to measure NO_x (as NO₂), with the nitrate concentration calculated by subtraction and application of a factor. In other cases, the level of nitrite was assumed to be low and thus the test portion would undergo reduction, measuring NO_x but reporting as Nitrate concentration.

Discuss if the different determination schemes (i.e. separate or combined) could have an impact on the precision and accuracy of the methods

17. Methods that determine nitrate and nitrite in a range of matrices (commodities) might no longer be appropriate if the maximum levels allowable were significantly reduced from what was required historically. Most of the standardized methods were published either in or before 2006, with two exceptions NMKL No. 194:2013 and EN 12014-2:2018, so the majority were developed prior to recent technological advances in commercially available instrumentation (including FIA/SFA's, HPLC's, IC and CEs in that period) and improvements supporting collaborative trials and reporting of method performance. We might expect improved LOD/LOQ if these standards were updated with newer instrumentation and collaborative trials. However, it should also be noted that as lower ML and consequently LOD/LOQs are sought, the various interferences would become more evident (and every technique would have them - either from the matrix, the chemistries, or equipment involved) and might require additional steps or hybrid techniques to mitigate the interference. There might also be acceptance of a compromise technique which would not be the most sensitive but would give satisfactory performance, practicability, and applicability under normal laboratory conditions, over all or most commodities.
18. For example, it has been found that the HPLC, IC and Griess reaction analysis could give similar results for aqueous solutions. However, fat/protein would deplete the effectiveness of a HPLC column and the spectrometry cell for colorimetry, but while deproteinization of the extract Carrez solution would work well with Griess Reaction based analyses, the Carrez solution was not recommended as a clarifying solution for the ion chromatographic methods. The Griess reaction parameters had been well studied by Mohamed et al 2008¹. Merino et al. 2000² reported that the Carrez solution was not recommended as a clarifying solution for the ion chromatographic methods, and also studied the rate of depletion of nitrite and nitrate spikes in ham. The study found that the pH of the matrix at the time of the addition of nitrite/nitrate had the greatest influence on the stability of these ions; concluding the higher the pH, the better the recovery.
19. The Croitoru 2012³ study highlighted potential interferences in the Griess reaction quantitation and not just from the red color of fruits and vegetables, e.g. beetroot, showing that there were substances able to mimic the Griess reaction, so it could no longer be considered specific for nitrite anion. Spectrophotometric measurements were to be employed with care, with the formation of substances other than the expected azo dye so suggesting a chromatographic separation would solve problems raised by such interferences (which would be more apparent at lower concentrations).
20. For the spectrophotometry methods using the Griess reaction for nitrite and a parallel Griess reaction with Cd, V(III) or Zn reduction to quantitate NO_x and the nitrate concentration by subtraction, although well-established, the results were most accurate if nitrite and NO_x determinations were completed in parallel with automated FIA/SFA instrumentation for stability and reproducibility along with an in-line reduction column. For ISO 6635 and Cd-column methods, it has been shown that iron, copper, chloride or other metals above 1 mg L⁻¹ or organic material in a sample could decrease conversion efficiency by coating the active surface of the reductor

¹ Mohamed et al. 2008 Modification of AOAC Method 973.31 for Determination of Nitrite in Cured Meats JAOACI VOL. 91, NO. 4, p820-7

² Liquid Chromatographic Determination of Residual Nitrite/Nitrate in Foods: NMKL Collaborative Study, Journal of AOAC INTERNATIONAL, Volume 83, Issue 2, 1 March 2000, Pages 365-376

³ Croitoru MD, 2012. Nitrite and nitrate can be accurately measured in samples of vegetal and animal origin using an HPLC-UVVIS technique. Journal of Chromatography B, 911, 154-161

element. Cd has been classified as a suspected cancer agent (Carranzo, 2012⁴ ; Ferreira et al., 1998⁵), but this risk could be mitigated (but not eliminated) with the in-line reduction columns. We could also expect the relative measurement uncertainty for mid-range Nitrite and NO_x concentrations to be similar, but as a subtraction the calculated mid-range nitrate concentrations would have a higher combined standard uncertainty.

21. In the Griess Reaction with Cd-column methods, the reduction reaction would be mainly dependent on the mass transport of nitrate to the Cd/Cu surface; the reducing efficiency of the Cd/Cu mixture must be carefully verified. When the solutions were too alkaline or the metal surface was too inactive, only a partial reduction of nitrate to nitrite would take place. When solutions were too acidic or contained very electronegative metals, nitrate might be reduced further than nitrite. Both situations would result in an incomplete conversion of nitrate to nitrite and a consequent decrease in recovery and result bias (Cruz and Martins Loução, 2002⁶, Beheshti et al 2023⁷).
22. Beheshti et al 2023¹¹ compared three methods, BS EN 12014-2 (HPLC UV/Vis), ISO 6635, and Cd column, for measuring nitrate in 11 important vegetables with a wide range from low (such as tomato and watermelon) to very high levels of nitrate (such as lettuce, spinach, and celery). The merits and limitations of each technique from different aspects were evaluated.
23. For IC with conductometric detection, it was difficult to measure low nitrite concentrations in the presence of bulk chloride (Butt et al 2001⁸). Various techniques were available to mitigate these problems e.g. Aggrawal et al. 2020⁹ subjected sample extracts to a series of clean up steps before analysis by an IC system with a Dionex OnGuard II RP cartridge removing hydrophobic substances such as aromatic hydrocarbons from samples. The Dionex OnGuard II Ag/H cartridge layered the resins from both the Dionex OnGuard II Ag and Dionex OnGuard II H cartridges. A Dionex OnGuard II Ag cartridge would remove chloride, bromide, and iodide from samples, while in this application a Dionex OnGuard II H cartridge would trap any silver that might have leached from the Ag cartridge and other cations found in the sample. The method showed good precision with RSDs <0.2% and <5% (n=9) for retention time and peak area, respectively. The recoveries from meat homogenate and slurried spinach sample ranged from 89 to 100%. A recent method development and validation by Afanda et al., 2025¹⁰ using two-dimensional IC(2D-IC) with inline filtration and SPE purification following microwave assisted extraction for simultaneous chloride, nitrite and nitrate showed good sensitivity (Nitrite = 0.2, Nitrate = 0.8 mg/kg), precision (Nitrite and Nitrate Inter-assay %RSD = 0.34-1.4; 0.60-1.4), Mean recovery Nitrite = 92-102%; Nitrate = 97 – 104%). However, this method was only validated for a series of preserved and unpreserved meats, but appears adaptable for other food matrices. In this article several alternative methods suitable for meats but not listed in CX/FA 21/52/7 Appendix 5, Annex 1 are also provided and compared in Table 2.
24. Coviello et al. 2020¹¹ gave a good summary stating that during the last 15 years, numerous methods have been reported in the literature for the separation and detection of nitrite and (or) nitrate based on spectrophotometric, chemiluminescent, electrochemical, chromatographic, capillary electrophoretic, spectrofluorimetric, and electro chemiluminescent techniques. However, spectrophotometric methods are subject to various interferences and lack of selectivity. As far as chromatographic methods are concerned, gas chromatography would need a derivatization reaction of both nitrite and nitrate, and liquid chromatography would hide the risk of oxidation of nitrites, mainly when an acid medium is used. Ion chromatography (IC) coupled with conductivity detection (CD) would offer good reproducibility and high sensitivity and selectivity.

CONCLUSION

25. It should be remembered that CCMAS is providing numeric performance criteria, and any collaboratively validated method which could meet that criteria with the commodity and provision that can be utilized.

⁴ Carranzo, I.V. (2012) Standard Methods for examination of water and wastewater, Anales de hidrología médica. Universidad Complutense de Madrid, Vol.5 No.2 p 185

⁵ Ferreira AM, Rangel AO, Lima JL (1998) Flow injection systems for elemental soil analysis determinations. Communications in soil science and plant analysis 29.3-4: 327-360

⁶ Cruz C., Martins Loução M. (2002) Comparison of methodologies for nitrate determination in plants and soils. J Plant Nutr. 25:1185–1211

⁷ Beheshti et al. (2023) Comparison of three methods for determination of nitrate content in different vegetables. Preprint available at <https://www.researchsquare.com/article/rs-3071274/v1>

⁸ Butt et al. (2001), Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography Talanta 55 (4) p789-97

⁹ Aggrawal et al. (2020), Simultaneous determination nitrate and nitrite in spinach and meat by ion chromatography Thermo Fisher Appl. note an73450

¹⁰ Afanda, et al.(2025) Analysis of Chloride, Nitrite and Nitrate in Processed Meat using Microwave Extraction and Two-Dimensional Ion Chromatography." Journal of Food Composition and Analysis: 107323

¹¹ Coviello et al. (2020) Validation of an analytical method for nitrite and nitrate determination in meat foods for infants by ion chromatography with conductivity detection. Foods 9.9 (2020): 1238.

26. As stated above, there are some standardized methods which meet some of the criteria in Appendix 1 & 2 though not all, however with updates to the existing techniques, the compliance rate using standardized methods could be increased significantly. The '09.3.3 (Salmon substitutes, caviar, and other fish roe products)' was notable as no validated methods with sufficient sensitivity were identified. To try to achieve a single analytical system to determine nitrite and nitrate individually and meet all the current commodity and provisions would be challenging but not necessarily unachievable and with validation for cheeses and fish the Afanda et al., (2025)¹⁰ looks a good prospect.
27. Also, with an appropriate range of certified reference materials and other QA and QC, along with appropriate extract clean-up, determination schemes based on 2D-IC/UV, IC/CD, spectroscopy with automated FIA/SFA, or CE could provide available and practical techniques for routine control with the required LOD/LOQ, precision and accuracy.

RECOMMENDATION

28. CCMAS44 is invited to consider the findings highlighted in this report in response to CCFA's requests; and:
- i. consider and agree on the numeric performance criteria for nitrates and nitrites in Appendix 1 and Appendix 2 and refer these to CCFA for their consideration;
 - ii. decide if more work is needed to source applicable methods for Appendices 1 & 2 where none could be identified from Appendix 3; and
 - iii. examine the validation data for the methods included in Appendix 3 and whether this information needs supplementation.

Appendix 1: Numeric performance criteria for the adopted MLs

Food Additive	Subcategory for which value was provided	Adopted Maximum Levels (CXS 192-1995)*	Calculated method performance criteria based on Maximum level (mg/kg)					Examples of applicable methods that meet the criteria
			Min Appl. Range (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision (RSD _R (%))	Recovery (%)	
01.6 (<i>Cheese and analogues</i>)								
Nitrate	01.6.2 (<i>Ripened cheese</i>)	35 mg/kg as residual NO ₃ ion.	25.2 - 44.8	3.5	7	18.7	80 – 110	Multi-laboratory validation - ISO 14673-3 IDF 189-3: 2004 Single-laboratory validation - ISO 14673-2 IDF 189-2: 2004[^]
08.0 (<i>Meat and meat products, including poultry and game</i>)								
Nitrite	08.2.2 (Heat-treated processed meat, poultry, and game products in whole pieces or cuts/)	80 mg/kg as residual NO ₂ ion.	60.1 – 99.9	8	16	16.5	80 - 110	Multi-laboratory validation - AOAC Method 973.31; NMKL 165: 2000 Ed.; Single-laboratory validation - Afanda et al., (2025); lammarino et al. 2013; Ferreira et al. (2008) for Ham; Siu et al., 1998 for Salami and Ham
Nitrite	08.3 (Processed comminuted meat, poultry, and game products)	80 mg/kg as residual NO ₂ ion.	60.1 – 99.9	8	16	16.5	80 - 110	Multi-laboratory validation - AOAC Method 973.31; NMKL 165: 2000 Ed.; Single-laboratory validation - Afanda et al., (2025); lammarino et al., 2013; Ferreira et al., (2008) for Ham; Siu et al., 1998 for Salami and Ham

Notes: * Maximum levels specification in CXS 192-1995 Revision 2024.

[^] In the absence of LOD or LOQ being specified in the method, the collaborative study report being unavailable at-this-time, and relying on in-house validation data, the validation designated as SLV, although the SLV status may be reviewed with additional data.

Appendix 2: Numerical performance criteria for the lowest proposed residual MLs for representative provisions in dairy (cheese), meat, and seafood as provided in CX/FA 21/52/7 Appendix 5 Annex 2.

Food Additive	Subcategory for which value was provided	Lowest Proposed Residual ML (mg/kg)	Notes	Calculated method performance criteria based on the Lowest Proposed Residual ML					Examples of applicable methods that meet the criteria
				Min Appl. Range (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision (RSD _R (%))	Recovery (%)	
01.6 (Cheese and analogues)									
Nitrate	01.6.2.1 (Ripened cheese, includes rind)	7	As NO ₃	4.5 – 9.5	0.7	1.4	23.9	80 – 110	Multi-laboratory validation - ISO 14673-3 IDF 189-3: 2004 Single-laboratory validation - ISO 14673-2 IDF 189-2: 2004 [^]
Nitrite	01.6.4 (Processed cheese) *(see note 1)	2	As NO ₂	1.1 – 2.9	0.2	0.4	28.8	80 – 110	Multi-laboratory validation – not available. Single-laboratory validation – not available.
08.0 (Meat and meat products, including poultry and game)									
Nitrate	Same residual proposed in multiple food categories including 08.2.1.1 (Cured (including salted) non-heat treated processed meat, poultry, and game products in whole pieces or cuts)	7	As NO ₃	4.5 – 9.5	0.7	1.4	23.9	80 – 110	Multi-laboratory validation – not available. Single-laboratory validation - Afanda, et al.,(2025); Ferreira et al., (2008) for Ham
Nitrite	08.2.1.3 (Fermented non-heat treated processed meat, poultry, and game products in whole pieces or cuts)	33	As NO ₂	23.6 – 42.4	3.3	6.6	18.9	80 – 110	Multi-laboratory validation - EN 12014-3:2005, NMKL 165: 2000 Ed.; AOAC Method 973.31; Single-laboratory validation - Afanda, et al., (2025), Ferreira et al., (2008) for Ham; Siu et al., 1998 for Salami, Ham
09.0 (Fish and fish products, including molluscs, crustaceans, and echinoderms)									
Nitrite	09.3.3 (Salmon substitutes, caviar, and other fish roe products)	4.4	As NO ₂	2.7 – 6.1	0.44	0.88	25.6	80 – 110	Multi-laboratory validation – not available. Single-laboratory validation – not available.

Notes 1. The subcategory doesn't match the description in Annex 2, as Food category No. 01.6.1 is 'Unripened cheese'; while Food Category No 01.6.4 is 'Processed Cheese'.

[^]. In the absence of LOD or LOQ being specified in the method, the collaborative study report being unavailable at-this-time, and relying on in-house validation data, the validation designated as SLV, although the SLV status may be reviewed with additional data).

Appendix 3. List of methods in CX/FA 21/52/7 Appendix 5, Annex (plus a recently published method) to which a review has been undertaken and the method validation data summarized for assessment against the numeric performance criteria.

Line #	Country	Method reference	Principle	Matrix Scope	NO ₂ & NO ₃ individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
1	Australia	QIS 12641 (based on Kirk, R.S. and R. Sawyer, "Pearson's Composition and Analysis of Food". 9 th Edition, Longman, New York, 1991	?						
2	Australia	Standard Methods for the Examination of Water and Wastewater - 4110 B.	Ion Chromat. with Chemical Suppression of Eluent Conductivity	Water / Wastewater Water (not suitable for further review)	NO ₂ & NO ₃ individually				
3	Australia	based on AOAC 973.31 see Chile Line 10.							
4	Brazil	NMKL 165 (nitrites and nitrates) 2000 Ed.	10 g sample + 0.5 g activated charcoal + 5 mL saturated borax + 50 mL water (80°C), placed in a boiling water bath 15 min extn. With IC-UV. Nitrite and/or nitrate in foodstuffs by ion chromatography.	meat and meat products, baby food, vegetables, and cheese.	NO ₂ & NO ₃ individually	1 mg/kg (NO ₂); 10 mg/kg NO ₃ (Merino et al. 2000)	5 and 25 mg/kg for nitrite and nitrate ions, respectively	RSD _R % 5.8 - 27.7 Nitrite, 5.6 - 21.1 Nitrate.	96–108% and 96–107% recovery for Nitrite and nitrate ions respectively.
5	Brazil	NMKL 194 (nitrites and nitrates). See EU line 50.							
6	Brazil	ISO 2918 (nitrites). See EU line 35.							
7	Brazil	ISO 3091 (nitrates)	Extraction of a test portion with hot water, precipitation of the proteins and filtration. Reduction of the extracted nitrates to nitrite by metallic cadmium. Development of a red colour by addition of sulphanilamide and N-I - naphthylethylenediamine dihydrochloride to the filtrate and photometric measurement at 538 nm.	meat and meat products	NO _x only. Requires ISO 2918 determination for NO ₂ subtraction and calculation of NO ₃ .			The difference between the results of two determinations carried out simultaneously or in rapid succession, by the same analyst, shall not be greater than 10 % of the mean value.	
8	Chile	Wootton M, Kok SH, Buckle KA (2006) Determination of nitrite and nitrate levels in meat and vegetable	A range of fresh and processed meat and vegetable products was analysed by HPLC for nitrite and nitrate contents.	vegetable products and most meats, provided	NO ₂ & NO ₃ individually				

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		products by high performance liquid chromatography. J Sci Food Agric 36:297-304		satisfactory results, but some meat products subject to matrix interference.					
9	Chile	McMullen SE, Casanova JA, Gross LK, Frank J, Schenck FJ (2005) Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. J AOAC Int 88:1793-1796	Nitrate and nitrite were separated on a hydroxide-selective anion exchange column using online electrolytically generated high-purity hydroxide eluant and detected using suppressed conductivity detection.	fruit and vegetable baby foods.	NO2 & NO3 individually				
10	Chile	AOAC Method 973 (should be 973.31)	5g meat/40ml 80°C water to 500mL extn. - Colorimetry (NED - Griess reaction).	Cured meat, including in canned corned beef and luncheon meat	NO ₂ only.	1.13 mg/kg Est. (Mohamed et al. 2008)	3.77 mg/kg Est. (Mohamed et al. 2008)	NA	78- 85% (Mohamed et al. 2008)
11	Chile	Merino et al (2000) JAOACI Vol. 83, No.2, validation of NMKL 165 (nitrites and nitrates) see Brazil line 4.							
12	Chile	Wootton et al (2006) see Chile Line 8.							
13	Chile	Pandurangappa et al. (2011) Quantification of Nitrite/Nitrate in Food Stuff Samples Using 2-Aminobenzoic Acid as a New Amine in Diazocoupling Reaction. Food Anal. Methods (2011) 4:90-99.	The proposed method is based on the diazotization of nitrite with 2-aminobenzoic acid and its subsequent coupling with N-(1-naphthyl) ethylenediamine dihydrochloride in aqueous medium to form an azo dye.	vegetable, fruit juice, and milk powder	NO2 & NOX (NO3 by subtraction)	0.056, 0.062, 0.078 µg ml ⁻¹ in Tomato, Orange and Moosambi respectively (values may be in food juice diluted in water).	1.07, 1.80, 2.30 µg ml ⁻¹ in Tomato, Orange and Moosambi respectively.	RSD = 1.16-2.02%	99.3 – 103% spike recovery
14	Chile	Chou et al (2003) A High Performance Liquid Chromatography Method for Determining Nitrate and Nitrite Levels in Vegetables. Journal of Food and Drug Analysis, Vol. 11, No. 3, 2003, Pages 233-238	Fifty mL of deionized (DI) water was added to the well-homogenized sample weighed 1g in a 100 mL volumetric flask. The flask in 80°C bath for 20 min., shaken and cooled, and diluted to 100 mL with DI water. Portion filtered through a 0.45 µm syringe filter. Analysis by HPLC-UV (213 nm), Luna	vegetables	NO2 & NO3 individually	5 mg/kg	NA	Repeated trials all obtained CV values less than 1.5%,	The recoveries of nitrate and nitrite spiked were in the range of 96.6 ~ 108.7% and 98.9 ~ 105.7%, respectively

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			C18 HPLC column (5 µm, 250 × 4.6 mm i.d.), mobile phase 0.01 M octylammonium orthophosphate of aqueous 30% (v/v) methanol of pH 7.0 for the mobile phase at flow rate of 0.8 mL/min, injection volume 10 µL.						
15	Chile	Connolly et al (2001) Rapid determination of nitrate and nitrite in drinking water samples using ion interaction liquid chromatography. Analytica Chimica Acta 441 53-62		Water (not suitable for further review)					
16	Chile	Ferreira et al. (2008) Quantification of residual nitrite and nitrate in ham by reverse-phase high performance liquid chromatography/diode array detector. Talanta 74:1598-1602	RP-HPLC/diode array detection for nitrites and nitrates in ham. Using a HyPurity C18, 5 µm column & gradient elution with 0.01M n-octylamine and 5mM tetrabutyl-ammonium hydrogensulphate to pH 6.5.	Ham (cooked and dried)		Nitrites and nitrates with 0.019 and 0.050 mg/kg, respectively.		Coefficients of variation lower than 2.89% and 5.47% were obtained for nitrite and nitrate, respectively (n = 6).	Recoveries of residual nitrite/nitrate ranged between 93.6% and 104.3%.
17	Chile	UNE-EN 12014-1: 1997. Food products. Determination of nitrate and / or nitrite content. Part 1: General. (see EU Line 26)							
18	Chile	UNE-EN 12014-1 / AI: 2001. Food products. Determination of nitrate and / or nitrite content. Part 1: General. (see Chile line 17 & EU Line 26)	Note: this provides only amendments to UNE-EN 12014-1: 1997; it is till only a summary of EN methods for foodstuffs. So not suitable for review inclusion.						
19	Chile	UNE-EN 12014-2: 2018. Food products. Determination of nitrate and / or nitrite content. Part 2: Method by high performance ion exchange liquid chromatography (HPLC / IC) for the determination of nitrate content in vegetables and							

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		horticultural products. (see EU Line 27)							
20	Chile	UNE-EN 12014-3: 2006. Food products. Determination of nitrate and / or nitrite content. Part 3: Spectrometric determination of nitrate and nitrite content in meat products after enzymatic reduction of nitrate to nitrite. (see EU Line 30)							
21	Chile	UNE-EN 12014-4: 2006. Food products. Determination of nitrate and / or nitrite content. Part 4: Method by ion chromatography (IC) for the determination of nitrate and nitrite content in meat products. (see EU Line 31)							
22	Chile	UNE-EN 12014-5: 1997. Food products. Determination of nitrate and / or nitrite content. Part 5: Enzymatic determination of nitrate content in food based on vegetables, for children and babies. (see EU Line 27)	Also see similar principle used by R-BIOPHARM Enzymatic BioAnalysis https://food.r-biopharm.com/wp-content/uploads/2012/06/roche_ifu_nitrate_10905658035_en-v9_2019-11.pdf						
23	Chile	UNE-EN 12014-7: 2000 Food products. Determination of nitrate and / or nitrite content. Part 7: Continuous flow method for the determination of nitrate content in vegetables and products derived from vegetables after reduction with cadmium. (see EU line 29)							

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
24	Colombia	AOAC 973.31-1996(1997) (see Chile line 10)	Nitrites in cured meat – colorimetric method	Cured meat, including in canned corned beef and luncheon meat					
25	Colombia	AOAC 935.48-1996	Nitrates and Nitrites in meat – Xylenol method. Nitrate ion react with 2,4-xylenol in sulfuric acid, steam-distilled and measured at 450 nm. Nitrite is oxidise to nitrate with potassium permanganate and determined by difference.	Meat	NO2 & NO3 individually				
26	EU	EN 12014-1 1997. Revision-A1 (1999). Foodstuffs — Determination of nitrate and/or nitrite content — Part 1: General considerations. European Committee for Standardization (CEN)	Note: this is only a summary of EN methods for foodstuffs. So not suitable for review inclusion. Note: EN decided to not consider any methods involving the use of open sources of spongy cadmium on the grounds of its potential threat to the environment. As a result, the only methods available for inclusion in this standard were vertical methods for the particular substance of interest.						
27	EU	EN 12014-2 1997. Foodstuffs — Determination of nitrate and/or nitrite content — Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products. European Committee for Standardization (CEN). (This reference has been replaced by EN 12014-2:2018, with lower LOQ, extensively revalidated and precision data in Annex B), inclusion of iceberg lettuce verified by interlaboratory testing, update of HPLC/IC-conditions and chromatograms in Annex A.	For Liquid samples, e.g. vegetable juice – shake and filter. For Solid samples, e.g. leaf vegetable and Pasty samples, e.g. mashed vegetables, homogenize and weigh approximately 10 g of material add approximately 400 ml of hot water (approximately 80°C) & place into a boiling water bath for 15 min. & dilute to 500 ml. Filter. For low nitrate, make appropriate adjustments, to the initial test portions and volumetric ratios but check modified method performance. The determination is performed either by reverse-phase HPLC and UV detection, or by IC and conductivity or UV detection.	Vegetables and vegetable products including Liquid samples e.g. vegetable juice; Solid samples, e.g. leaf vegetable; Pasty samples, e.g. mashed vegetables	NO2 & NO3 individually (Laboratory experience has shown that this analytical method is also suitable for the determination of nitrite in other matrices; however, this has not been validated in the interlaboratory test scheme cited here).		25 mg/kg nitrate. Note, modifications allowed for low concentrations but require performance checks. Beheshti et al 2023 reports LOQ of 0.2 mg/kg for nitrate	naturally contaminated Beetroot juice, Puréed carrots, and Iceberg lettuce repeatability of 7.2, 12.7 & 6.26%; and Reproducibility of 26.3%, 46.2% & 19.6% respectively.	

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
28	EU	EN 12014-5 1997. Foodstuffs — Determination of nitrate and/or nitrite content — Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants. European Committee for Standardization (CEN).	Nitrate is reduced by reduced nicotinamide-adenine dinucleotide phosphate (NADPH) to nitrite in the presence of the enzyme nitrate reductase (NR). The amount of NADPH oxidized during the reaction is stoichiometric to the amount of nitrate. The decrease in NADPH is measured by means of its light absorbance at 340 nm.	Vegetable containing foods for babies and infants	NO3 only.	NA	50 mg/kg nitrate ion	See EN 12014-5: 1997 Annex B Precision data	NA
29	EU	EN 12014-7 1998. Foodstuffs — Determination of nitrate and/or nitrite content — Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after Cadmium reduction. European Committee for Standardization (CEN)	Homogenise frozen vegetable, weigh 40g, add 35g extraction buffer (only needed if nitrite is to be detn.) & 325g water. Homogenise and filter. Test portion extracted with water, filtered. Portion of nitrate ions diffuses in dialyzing unit of continuous flow system, into slightly alkaline buffer solution and reduced to NO2 by Cd column. NO2 ion quantitated colorimetrically after Griess reaction & absorbance measurement between 520-540nm.	Vegetables and vegetable products (precision data provided for beetroot (2), lettuce (3), endive, spinach).	NOx only (unless nitrite quantified and subtracted from NOx to quantify Nitrate. (see EN 12014-7: 2000 Annex E Nitrite analysis).	NA	50 mg/kg nitrate ion	See EN 12014-7: 2000 Annex C Precision data (acceptance criteria repeatability and reproducibility ≤5%).	NA. Note-absorbance maxima of beetroot extract & diazotized NO2 solutions are nearly identical. Thus, NO3 or NO2 values corrected for blank absorption for beetroot extracts & increasing MU.
30	EU	EN 12014-3 2005. Foodstuffs — Determination of nitrate and/or nitrite content — Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite. European Committee for Standardization (CEN)	Weigh, 10 g of the homogenized sample. Add about 50 ml of water and homogenize, & sodium hydroxide to adjust pH-value to 8,0 to 8,5. Heat the flask for 15 minutes in a boiling water bath, shaking several times. Cool, add 4 ml each of Carrez solutions No. 1 and No. 2, shaking & dilute to 200mL, filter. Nitrite in extract treated with sulfanilamide and NED. The formed red compound is measured spectrometrically at λ=540 nm. Nitrate extract is converted into nitrite by nitrate reductase. The treated as above for nitrite, the sample nitrate content is	meat products	NO2 & NOX (NO3 by subtraction)		1.4 mg/kg for sodium nitrite and 3.6 mg/kg for sodium nitrate. Or 0.93 mg/kg as Nitrite ion, and 2.6 as Nitrate ion. (Note 2) Applicable for total nitrite and nitrate content from 5 mg/kg up to 125 mg/kg calculated as sodium nitrite.	See EN 12014-3: 2006 Annex A Precision data in 2002 interlab. test, RSD _r = 5.7 - 8.1% RSD _R = 9.5-14.0% for NaNO ₂ /kg (2 meats, precision for high nitrite unacceptable at 30.7%). RSD _r = 4.3 – 7.8% RSD _R = 9.1 – 10.7% for NaNO ₃ /kg (3 meats).	NA

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			calculated from the difference between the spectrometric measurements.						
31	EU	EN 12014-4 2005. Foodstuffs — Determination of nitrate and/or nitrite content — Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products. European Committee for Standardization (CEN).	Weigh, 10g homogenized sample. Add 50 ml of water at 50 °C to 60 °C and mix. Add 50 ml acetonitrile, mix gently, cool to room temperature and dilute to 200mL with water. Filter with fluted filter paper and then 0.45 µm membrane filter. Prepare blank replacing the test portion by 10 ml of water. The nitrate and nitrite contents determined by ion-exchange chromatography (IC) and ultraviolet (UV) detection at a wavelength of 205 nm, isocratic Lithium borate gluconate buffer solution /acetonitrile mobile phase at 1 ml/min, ≥40 µL injection.	meat products, vegetables and baby food. (Nitrite in meat products greater than 40 mg/kg)	NO2 & NO3 individually	The limit of detection (LOD) for nitrate was 10 mg/kg (EFSA 2017)	50 mg/kg nitrate ion; 40 mg/kg as nitrite ion	See EN 12014-4: 2006 Annex B Precision data.	NA
32	EU	EN ISO 14673-1:2004. Milk and milk products — determination of nitrate and nitrite contents - Part 1: method using cadmium reduction and spectrometry. ISO, Geneva. See IDF line 72							
33	EU	EN 14673-2:2004. Milk and milk products — determination of nitrate and nitrite contents — Part 2: method using segmented flow analysis (routine method). ISO, Geneva. See IDF line 73							
34	EU	EN 14673-3:2004. Milk and milk products — determination of nitrate and nitrite contents — Part 3: method using cadmium reduction and flow injection analysis with in-line dialysis (routine method). ISO, Geneva. See IDF line 74							

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
35	EU	ISO 2918:1975 Meat and meat products -- Determination of nitrite content (Reference method).	Extraction of a test portion with hot water, precipitation of the proteins and filtration. In the presence of nitrite, development of a red colour by the addition of sulphanilamide and N-1-naphthylethylenediamine dihydrochloride to the filtrate and photometric measurement at 538 nm.	meat and meat products	NO2 only.	No validation data.			
36	EU	ISO 4099:1984 Cheese -- Determination of nitrate and nitrite contents -- Method by cadmium reduction and Photometry (withdrawn and replaced by ISO 14673-1:2004 IDF 189-1:2004; ISO 14673-2:2004 IDF 189-2:2004; & ISO 14673-3:2004 IDF 189-3:2004, thus not suitable for further review)							
37	EU	Butt et al 2001, Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography. Talanta 55 (4), 789-797	Normal phase ion-pair HPLC has been used for simultaneous separation of nitrite and nitrate using tetraethylammonium (TEA)+ as ion-pairing reagent and UV detector. The performance of the proposed method is compared with ion chromatography for quantification of the anions in food samples, such as spinach and lettuce.	spinach and lettuce	NO2 & NO3 individually	NA	NA	The developed method is capable of analyzing nitrite and nitrate in the presence of 50-fold concentration of chloride and sulfate with a maximum RSD of 10%, with shorter analysis time.	NA
38	EU	Siu et al 1998, Ion chromatographic determination of nitrate and nitrite in meat products. Journal of Chromatography A 804, 157-160	Ten grams of ham or salami, deionized water added to a final volume of 100mL. Homogenized in a blender heated and the temperature was maintained between 70°C and 80°C for 15 min. Cooled to room temp and centrifuged at 4960 g for 10 mins. Supernatant was removed and filtered successively with Whatman No. 2 & GF/A filters, and then through the 1.2 µm and 0.2 µm Acrodisc filters. Nitrate and nitrite were separated using Dionex	Meat products: Salami, Ham.	NO2 & NO3 individually	Detection limits for nitrate and nitrite from the extracts, 0.5 mg/kg and 0.3 mg/kg for the meat sample), respectively	Est. Nitrate and Nitrate 1.5mg/kg; 0.9 mg/kg respectively.	%RSD Nitrate 1.0-2.9% (N=5), Nitrite 2.3-1.7% (N=5).	Meat spike recoveries were between 90% and 100% for nitrate, and between 90% and 105% for nitrite.

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			DX500 with AD20 UV-Vis detector, isocratic conditions with an IonPac AG11guard column and AS11 analytical column—5 mM sodium hydroxide for 10 min, followed by a column wash with 100 mM sodium hydroxide for 5 min, and equilibration with 5 mM sodium hydroxide for 10 min. The injection volume was 25 µl and eluent flow-rate was 1 ml /min. Analytes were detected using UV detection at 225 nm.						
39	EU	McMullen et al 2005. Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. Journal of AOAC International 88, 1793-1796 See Chile line 9							
40	EU	Stalikas et al 2003 Ion chromatographic method for the simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection. Journal of Chromatography A 1002, 237-241	IC with post-column IFD to determine simultaneously nitrite and nitrate, at an emission wavelength of 355 nm after excitation at 270 nm.	Salami		Not specified, but this reviewer estimates LOD at ≈5mg/kg based on peak size & RSDs.			Recoveries from fortified samples salami compared to AOAC method was 95% Nitrate (N=3) and 96% Nitrite (N=3).
41	EU	Merino et al. 2000. Liquid chromatographic determination of residual nitrite/nitrate in foods: NMKL collaborative study. Journal of AOAC International 83, 365-375. See Brazil line 4 and Chile line 11.							
42	EU	Di Matteo V and Esposito E, 1997. Methods for the determination of nitrite by high performance liquid chromatography with electrochemical detection.	This is a review of other papers where three different papers included either Fish and cured meats, or Meat products, or Meat. But all with different method parameters and only provided						

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		Journal of Chromatography A 789 (1-2), 213-219	LoD, no precision or accuracy. Thus, not considered suitable for further review.						
43	EU	Iammarino et al. 2013. Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: results of five years of official controls and monitoring. Food Chemistry, 140, 763-771	in-house validated ion chromatographic method with electrochemical detection. In this work the results obtained from 5 years of official controls and monitoring focused on tracing quantifiable amounts of nitrites and nitrates in 1785 samples: 200 fresh meats (beef, pork, horse and chicken), 1195 meat products (fresh meat preparations, cured meats and other meat products), 180 dairy products ("mozzarella" cheeses, short and lengthy maturation cheeses), 120 shellfishes (mussels and clams) and 90 leafy vegetables (fresh and frozen spinaches and lettuces). Analyses performed from 2007 to 2011 using a validated ion chromatography with conductivity detection method.	fresh meats (beef, pork, horse and chicken); meat products (fresh meat preparations, cured meats and other meat products); dairy products ("mozzarella" cheeses, short and lengthy maturation cheeses); shellfish & leafy vegetables.	NO2 & NO3 individually	Nitrate = 3.2 mg/kg. Nitrite = 1.5 mg/kg	Nitrate = 9.6 mg/kg; Nitrite = 4.5 mg/kg	Intermediate precision Nitrite @ 75, 150, 225 mg/kg = 2.6, 3.0 & 2.4%; Nitrate @125, 250, 375 mg/kg = 3.6, 2.6 & 3.9%	Nitrite mean recovery = 98.7%; Nitrate mean recovery = 98.3%
44	EU	Croitoru MD, 2012. Nitrite and nitrate can be accurately measured in samples of vegetal and animal origin using an HPLC-UVNIS technique. Journal of Chromatography B, 911, 154-161	HPLC-UV/VIS method, for matrices including vegetal samples based on a pre-column derivatization of nitrite anion using the Griess reaction and direct determination of nitrate using its UV absorbance. A chromatographic process with detection at two wavelengths allows the determination of both anions in one run (23 min with column re-equilibration included).	vegetables and fruits were tested: white & red potatoes, red & white onion, garlic, beetroot, carrot, parsley, celery, parsnip, tomatoes, cucumbers, cabbage, red cabbage, lettuce, radish, different types of apples, oranges, kiwi, strawberries, lemon, and tangerine.	NO2 & NO3 individually	In the case of vegetables, the limits of detection 0.2 mg/kg. For the vegetables and fruits were tested, no interferences were recorded and only about 5% of the samples fall under the LODs.	0.6 mg/kg	Using the spike studies described under recovery with 5 samples for every type of product tested), the coefficients of variations recorded for every concentration level did not exceed 5%.	For accuracy, a spiked samples technique used. The content of nitrate and nitrite present in samples before spiking subtracted from final values using potato, onion & apple samples. Recovery range 97.20 and 103.55% for nitrate and 98.29 and 104.05% for nitrite.

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
45	EU	Oztekin et al 2002. Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis. Food Chemistry 76, 103-106	Meat products (10 g) were weighed in a beaker. DI water (150 ml) added, blended & suspension incubated 15min at 50°C. Cooled & diluted to 250 ml, filtered with filter paper & 0.45-mm cellulose acetate filter disc, internal standard (KSCN) added and made to volume. Separations with a commercial CE injection system with an on-column variable UV-Visible detector at 210 nm. The fused silica capillaries 75 mm I.D., capillary length was 75 cm, and the length to the detector was 60 cm. Washing for 2 min with 0.1 mol/l HCl and 2 min with buffer between runs was applied.	Cured meat products and fresh vegetables - Salami, Ham, Turkey sausage, Sausage, Spinach, Parsley, Dill, Leek	NO2 & NO3 individually	LOD for nitrite corresponding to a signal/noise ratio of three is 0.105 µg/ml and for nitrate is 0.099 µg/ml. Corresponds to 4 mg/kg nitrite in real samples.		Reproducibility of the method for six successive injections of turkey sausage sample, give 4.51 %RSD for NO2, and 2.54 %RSD for NO3.	Four cured meat products and four vegetables, with recovery data for nitrite 94-103% and nitrate 92-106%.
46	EU	Ensafi et al., 2004. Simultaneous spectrophotometric determination of nitrite and nitrate by flow injection analysis. Analytical Sciences 20 (12), 1749-1753	For the beef sausage, 5.0 g of sample was mixed and homogenized. The mixed sample digested carefully by heating of solution content in water for 2 h. The mixture was filtered (Whatman No. 1) and diluted to 100 ml. Analysis by a flow injection spectrophotometric method for simultaneous determination of nitrite and nitrate based on the catalytic effect of nitrite on the oxidation of Pyrogallolsulfonephthalein with bromate in acidic media.	Water and sausage samples	NO2 & NOX (NO3 by subtraction)	The detection limits for nitrite were 1.6 ng ml ⁻¹ and for nitrate were 3.0 ng ml ⁻¹ (S/N = 3). LOD provided only for water samples, not the sausage.		The relative standard deviation (n = 10) was 2.8% and 3.7% at 0.080 µg ml ⁻¹ nitrite and nitrate, respectively.	To validate the accuracy of the proposed method, the results were compared with a Griess standard method using increasing concentrations 94-103% NO2 recovery, & 110-94% NO3 recovery.
47	EU	Casanova et al., 2006. Use of Griess reagent containing vanadium(III) for post-column derivatization and simultaneous determination of nitrite and nitrate in baby food. Journal of AOAC International 89 (2), 447-451	Ion chromatographic/ spectrophotometric method (Griess reaction and spectrophotometric detection @ 535 nm) and AOAC Method 993.03 yielded comparable results for various baby foods that contained incurred nitrate. The reduction of nitrate to nitrite was accomplished by the online post-column addition of VCl ₃ rather than by using Cd. Nitrate and nitrite ions were extracted from the fruit and vegetable	baby food – including Carrots, Spinach, Green beans, Banana, Squash, Mango, Grapes.	NO2 & NO3 individually	LOD not specifically stated.	≤4 mg/kg. LOQ not specifically stated.	Spike recoveries for 4 baby food (sample N=5) Nitrite 1.3% - 19.4% RSD; Nitrate 2.4% - 7.9	Recoveries of NOx from various fortified baby food fruits and vegetables (25–400 ppm) ranged from 82 to 107%.

Line #	Country	Method reference	Principle	Matrix Scope	NO ₂ & NO ₃ individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			baby foods with 50–60°C water or with buffer, followed by clarification with acetonitrile and centrifugation.						
48	EU	Andrade et al. 2003. A flow-injection spectrophotometric method for nitrate and nitrite determination through nitric oxide generation. Food Chemistry 80, 597-602.	Method is based on the reduction of nitrite and nitrate to nitric oxide, with subsequent reaction with iron (II) and thiocyanate in an acid medium, forming FeSCNNO+. The absorbance of the complex, with a maximum at 460 nm, is proportional to the nitrite and nitrate concentrations.	vegetables and meat products	NO ₂ & NO _x (NO ₃ by subtraction)	For a sample of 5.0 g, the determination limit of the method was 20 and 13 mg kg ⁻¹ of nitrate and nitrite, respectively.	NA	Precision comparable to reference spectrophotometric method (AOAC reference method for the determination of nitrate in foodstuffs).	Accuracy comparable to reference spectrophotometric method (AOAC reference method for the determination of nitrate in foodstuffs).
49	EU	Kazemzadeh et al., 2001. Sequential flow injection spectrophotometric determination of nitrite and nitrate in various samples. Analytica Chimica Acta 442, 319-326	Method based on the reaction of nitrite with safranin O to form a diazonium salt which caused the reddish-orange dye colour of the solution to be changed to blue in acidic media, and which absorbs at 520 nm. The injected sample in the flow injection system is split in two streams. One of the streams is transported through a reductor microcolumn containing copperised cadmium, where nitrate is reduced to nitrite. The two streams are then mixed and treated with the appropriate reagents.	Meat, flour, cheese etc.	NO ₂ & NO _x (NO ₃ by subtraction)	Detection limits (3σ) of 0.5 and 3 ng ml ⁻¹ were obtained for nitrite and nitrate, respectively (presumably for the sample in solution)	NA		
50	EU	NMKL (Nordic Committee on Food Analysis), 2013. Determination of nitrate and/or nitrite in foodstuffs and water by spectrophotometry after zinc reduction and Griess reaction. NMKL No. 194. This method has only been evaluated in a single laboratory validation study, but over several days, and with satisfactory results available in the method.	Spectrophotometric method for the determination of nitrate/nitrite content in foodstuffs and water after zinc reduction and Griess reaction. (The Griess reaction is specific for nitrite. Analysis of nitrate by this reaction requires chemical or enzymatic reduction of nitrate to nitrite prior to the diazotization reaction (Tsikas D. 2007)	vegetables (lettuce), meat products, baby food, dairy product (milk) and surface water - method validated for above foods	NO ₂ & NO _x (NO ₃ by subtraction)	5 mg/kg (meat products – EFSA 2017 p 16)			

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
51	EU	Chung et al. 2011. Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. Food Additives and Contaminants, 4, 34-41	Levels of nitrate and nitrite were determined by ion chromatography and flow injection analysis, respectively.	vegetables - leafy, legumes, root and tuber, and fruiting	NO2 (by FIA) & NO3 (by IC) individually	Nitrite 0.8 mg/kg, Nitrate 4 mg/kg.	NA	NA	NA
52	EU	Leth et al., 2008. Nitrite and nitrate content in meat products and estimated intake in Denmark from 1998 to 2006. Food Additives and Contaminants, 25, 1237-1245	Nitrite and nitrate were extracted from the samples by mixing 5 min with hot water (70°C). Protein was precipitated with addition of Carrez solution I and II and the suspension was filtrated. The filtrate was injected in a FIA system, where sulphanilamide and N-(1-naphthyl)-Ethylenediammonium-chloride were added to the carrier stream to react with nitrite to form a violet azo colour which was measured spectrophotometrically at 540 nm. For nitrate, after injection, the carrier stream went through a Cd-column where nitrate was reduced to nitrite which subsequently reacted to form the azo colour.	Liver paste; paté; fatty meat intended to be used in sandwiches; lean meat intended to be used for sandwiches; salami; sausages for dinner; medium fat and fat pork meat for dinner	NO2 & NOX (NO3 by subtraction)	Detection limit for NaNO2 was 3 mgkg ⁻¹ and for NaNO3 5mgkg ⁻¹ .		Article reporting a survey where for QA/QC reference material of freeze-dried dill and a recovery experiment for both nitrate and nitrite. Depending on sample type and concentration level an average recovery of 100% for nitrate and 95% for nitrite was found and RSD% between 2 and 4% for NO2 & between 2 and 5% for NO3.	
53	EU	Koupparis et al., (1982) Determination of nitrite in waters by using a stopped-flow analyzer. Analyst 107, 1309-1315.	Automatic kinetic methods for the determination of nitrite in waters with a stopped-flow analyser are described. The methods are based on the diazotisation of sulphanilamide, the product being coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye, which is measured at 540 nm. The sample throughput for routine analysis can be up to 360 samples per hour in the range 0.025–2.00 mg/L of nitrite-nitrogen	Water (not suitable for further review)	NO2 only				
54	EU	Liang et al., (1994) Catalytic spectrophotometric determination of nitrite using the chlorpromazine	Kinetic methods. In a 0.2 mol l ⁻¹ acetic acid–6.6 × 10 ⁻³ mol l ⁻¹ oxalic acid medium at 20 °C, 5 × 10 ⁻³ mol l ⁻¹ chlorpromazine hydrochloride (CPH)	Rain and river water (not suitable for further review).	NO2 only				

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		hydrogen-peroxide redox reaction in acetic-acid. Medium. Analyst 119, 2113-2117	was oxidized by $5 \times 10^{-2} \text{ mol l}^{-1} \text{ H}_2\text{O}_2$ with the reaction being catalysed by nitrite ions. The maximum absorbance is proportional to the nitrite concentration; this allows the determination of nitrite in the range 0.003–1.500 mg/L.						
55	EU	Ensafi et al., (1999) Ultra-trace analysis of nitrite in food samples by flow injection with spectrophotometric detection. Fresenius Journal of Analytical Chemistry 363 (1), 131-133	Spectrophotometry. A flow injection system with spectrophotometric detection is proposed for the determination of low levels of nitrite based on its catalytic effect on the oxidation of gallocyanine(GC) by bromate in acidic media. The dye reacts to a colorless compound, thus decrease in absorbance of GC at 530 nm. Sample prep.- 5g sample, 50mL hot water(80°C) blended. Dilute to 300mL, heat steam bath for 2hrs, then dilute to 500mLs, filter and analyses against aqueous standards.	Food samples. Validated only on sausage.	NO2 only	0.01 µg/mL of Nitrite. Based on article should correspond to 1 mg Nitrate ion/kg sausage		The relative standard deviation for n = 10 of 0.040, 0.080 and 0.200 µg/mL of nitrite was 3.3, 2.8 and 2.1%, respectively.	Compared to results from Fiddler et al., (1984) J. Assoc. Off. Anal. Chem 67: p525-8 for three sausage samples range 3.0-4.4 mg/kg 101, 101, 99% recovery.
56	EU	Ghasemi et al., (2004) Kinetic spectrophotometric determination of nitrite based on its catalytic effect on the oxidation of methyl red by bromate. Analytical Letters 37 (10), 2205-2214.	Spectrophotometry. In acidic solution, methyl red (MR) is oxidized by bromate to form a colorless compound, with reaction accelerated by trace nitrite & measured by absorbance at 520 nm. nitrite can be determined in the range of 0.05–1.2 mg/kg.	Meat products	NO2 only	0.045 mg /kg		The standard deviation is 0.06 for six replications at 0.3 mg/kg.	
57	EU	Fang et al., (2002) Flow injection determination of nitrite in food samples by dialysis membrane separation and photometric detection. International Journal of Environmental Analytical Chemistry 82 (1), 1-6	Spectrophotometry. On-line dialysis preconcentration determination of nitrite by injection method. Nitrite penetrating dialysis membrane, is diazotised with sulphanilamide to form a diazonium action, which is subsequently coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a stable purple azo dye, the absorbance of which is measured at 525 nm. Calibration curve was between 0.00125 mg/L and 3.2 mg/L.	method applied to determination of nitrite in food samples, including milk, sausage and fruit juices, with satisfactory results	NO2 only	4.5 ng/ml = 0.0045 mg/L		15 samples can be analyzed per hour with a relative precision of ca.2.8%	

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
58	EU	Chen et al., (1999) Flow-injection catalytic spectrophotometric determination of trace amounts of nitrite. Analytical Letters 32 (14), 2887-2897	Spectrophotometry. A flow-injection catalytic spectrophotometric method based on its catalytic effect on potassium bromate oxidation of acridine orange in phosphoric acid medium. Monitored by measuring the decrease in absorbance of acridine orange at 491.5 nm coupling with the stopped-flow technique.	Waters and food samples	NO2 only	2.2 ng/mL = 0.0022 mg/L		Up to 30 samples can be analyzed per hour with a relative precision of ca. 1.9%.	
59	EU	Ensafi et al., (1994) Selective kinetic spectrophotometric determination of nitrite in food and water. Analytical Letters 27 (1), 169-182	Spectrophotometry. Method based on its Nitrite reaction with Nile blue 2B in acidic medium and monitored spectrophotometrically at 595 nm for nitrite in the range 0.005 - 1.100 mg/L.	trace amounts of nitrite in sausage and water	NO2 only	0.001 mg/L		Ten replicates of 0.020 mg/L RSD=1%	
60	EU	He et al., (2007) Chemiluminescence microflow injection analysis system on a chip for the determination of nitrite in food. Food Chemistry 101 (2), 667-672	Chemiluminescence. Using microflow injection analysis (μ FIA) system on a chip. Nitrite is sensed by the chemiluminescence (CL) reaction of luminol with ferricyanide that is the product of the reaction of ferrocyanide with nitrite in acidic medium. The linear range of the nitrite concentration is 8–100 μ g L ⁻¹	Food	NO2 only	0.004 mg/L		Nine replicates of 0.050 mg/L, RSD = 4.1%	
61	EU	Li et al., (2003) Spectrofluorimetric determination of total amount of nitrite and nitrate in biological sample with a new fluorescent probe 1,3,5,7-tetramethyl-8-(3',4'-diaminophenyl)-difluoroboradiaza-s-indacence. Talanta 61 (6), 797-802	Fluorescence. A new synthesized fluorescent probe, 1,3,5,7-tetramethyl-8-(3',4'-diaminophenyl)-difluoroboradiaza-s-indacence (TMDABODIPY), has been used to detect nitrite. When TMDABODIPY reacted with nitrite, a weak fluorescent triazole formed in 0.2 mol l ⁻¹ HCl. The fluorescence quenching intensity linear over a nitrite concentration of 0.0025–0.02 mg/L.	Human serum and urine (not suitable for further review).	NO2 only	0.00002 mg/L			91.83-101.80% in human serum and urine
62	EU	Jie et al., (1999) A fluorescence quenching method for the determination of nitrite with indole. Microchemical Journal 62 (3), 371-376	Fluorescence. The method is based on the reaction between nitrite and the fluorescent indole to form a compound which has no fluorescence in acidic medium. Measured in 1 cm quartz cell with excitation and emission wavelengths of 285 and 350 nm, respectively, range 0.01–0.6 mg/L	Water and food samples	NO2 only	0.0025 mg/L			

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
63	EU	Jie et al., (1994) Fluorometric-determination of nitrite using a new reagent system. Analytical Letters 27, 1001-1008	Fluorescence. Method based on the reaction of nitrite with tyrosine to form a highly fluorescent compound in alkaline medium. The calibration graph is linear in the concentration range of nitrite 0.004-0.20 mg/L.	water and food samples	NO2 only	0.0002 mg/L			
64	EU	Jie et al., (1993) Fluorimetric determination of nitrite. Talanta 40 (7), 1009-1011	Fluorescence. Method based on the reaction of nitrite with tryptophan to form a highly fluorescent compound in alkaline medium. Appears to be prelude to Jie et al. 1994 with another publisher (not suitable for further review)	water and food samples	NO2 only				
65	EU	Gao Q, (2002) Uric acid-hexacyanoferrate (III)-luminol chemiluminescence system for the determination of trace nitrite. Chinese Journal of Analytical Chemistry 30 (7), 812-814.	Fluorescence. In acidic medium that NO2- can oxidize [Fe(CN)(6)](4-) to [Fe(CN)(6)](3-) based on the uric-[Fe(CN)(6)](3-)-luminol chemiluminescence system, the new method for the determination of nitrite was developed. The linear range for the determination of NO2- was 0.001 mg/L to 10 mg/L.	Water and food samples	NO2 only	0.00005 mg/L		RSD for the determination of samples (n=11) was 1.8% - 3.2%.	Between 95.7% - 105.8%.
66	EU	Kazemzadeh A, (2005) Development of new optical nitrite detector. Asian Journal of Chemistry 17 (2), 767-773	Optical sensor technology. A detector developed by immobilizing a direct indicator dye in an optical sensing film was fabricated by binding pyrogallol red to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. Nitrite can be determined for the range 0.006-1.50 mg/mL.	States potential application to food (not suitable for further review).	NO2 only	0.000001 mg/L			
67	EU	Kazemzadeh et al., (2005) Optical nitrite sensor based on chemical modification of a polymer film. Spectrochim Acta A Mol Biomol Spectrosc 61 (8), 1871-1875	Optical sensor technology. A detector developed by immobilizing a direct indicator dye in an optical sensing film for food and environmental monitoring. This sensor was fabricated by binding galloyanine to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. Nitrite can be determined for the range 0.008–1.50 mg/L.ml with 3 δ detection limits of 1 ng/ml. The method is easy to perform and uses acetylcellulose as a carrier.	for food and environmental monitoring.	NO2 only	1 ng/mL or 0.000001 mg/L			

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			The reagents used for activating the cellulose support are inexpensive, non-toxic and widely available. Appears to be replicate of previous entry with another publisher. (not suitable for further review)						
68	EU	Fang et al., (2005) A dip-and-read test strip for the determination of nitrite in food samples for the field screening. Analytical Letters 38 (11), 1803-1811	Dipstick technology. Method for field screening of nitrite in aqueous samples based on the diazo-coupling reaction between the nitrite and the Griess reagents. The test strip has a circular sensing zone that contains two layers: the Griess reagents act as the sensing reagent and is immobilized in the bottom layer; the top layer is a cellulose acetate membrane that can be used as a dialysis membrane to remove the matrix from the sample, which can enhance the selectivity of this method. When the test strip was directly dipped into the samples, a color change of the test strip was observed, and the intensity of color that appears on the test strip is proportional to the concentration of nitrite in the range from 0.50 to 25 mg/L in food samples.;	Food samples	NO2 only	Under the experimental conditions, as low as 0.20 mg/L nitrite can be observed			
69	Indonesia	Egan, H., Kirk, R.S. and Sawyer, R. 1981. Pearson's Chemical Analysis of Foods. 8 th Edition, Churchill Livingstone, London, NY	analyze nitrate and nitrite is Spectrophotometric.						
70	Malaysia	Determination of Nitrite and Nitrate in Meat using Ion Chromatography (Methrohm): US Environmental Protection Agency, (EPA Method 9056A), Rev. 1, Nov. 2000	IC-Cond, sequential determination of anions including nitrate (NO ₃), nitrite (NO ₂), in aqueous samples, such as drinking water, wastewater, aqueous extracts of solids, and the collection solutions from the bomb combustion of solid waste samples.	Not specifically for food so would need to review Malaysia method validation in food matrices. Thus, not considered suitable for further review.					

Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
71	Thailand	Analysis of residual nitrates and nitrites in food is High Performance Liquid Chromatography (HPLC) Method - UV detector (Journal of Food and drug Analysis, Vol.11, No.3, 2003, p233-238) see Chile line 14							
72	IDF	ISO 14673-1 IDF 189-1: 2004 — Milk and milk products — Determination of nitrate and nitrite — Part 1: Method using cadmium reduction and spectrometry	A test portion is dispersed in warm water, with precipitation of the fat and proteins, then filtration. The nitrate ions are reduced to nitrite ions in a portion of the filtrate by means of copperized cadmium. A red colour is developed in portions of both unreduced filtrate and the reduced solution, by addition of sulfanilamide and N-1-naphthyl ethylenediamine dihydrochloride. Spectrometric measurements are carried out at a wavelength of 538 nm. May be performed using automatic equipment, in particular by segmented flow analysis (SFA) or flow injection analysis (FIA)	Milk and milk products - whole and partly skimmed and skimmed dried milk; hard, semi-hard and soft cheeses; processed cheese; whey cheese, caseins and caseinates and dried whey	NO2 & NOX (NO3 by subtraction)		Est. Nitrite = 0.56 mg/kg; Nitrate = 4.6 mg/kg (infant formula & milk powder)	See ISO 14673-1 IDF 189-1: 2004 section 11 for precision	
73	IDF	ISO 14673-2 IDF 189-2: 2004 — Milk and milk products — Determination of nitrate and nitrite — Part 2: Method using segmented flow analysis (Routine method)	A test portion is suspended in water. Part of the suspension is transferred to the analyser for dialysis. The nitrate ions are reduced to nitrite. The nitrite content is determined by a segmented flow analysis and spectrometric detection. Any nitrite present is determined as nitrate. A correction for the nitrite present can be applied after determination of the nitrite content.	Milk and milk products - milk, cheese, and liquid and dried milk products and infant foods.	NO2 & NOX (NO3 by subtraction)	Accessing the collaborative study report prior to year 2000 has proved challenging, but in-house validation data has reported a LOD for cheese of 0.5 mg/kg.	Accessing the collaborative study report prior to year 2000 has proved challenging, but in-house validation data has reported a LOQ for cheese of 1 mg/kg	Reproducibility - For samples with a nitrate content <100 mg/kg: 15 mg/kg; for samples with a nitrate content ≥ 100 mg/kg: 20 % of the arithmetic mean of the results.	
74	IDF	ISO 14673-3 IDF 189-3: 2004 — Milk and milk products — Determination of nitrate and nitrite — Part 3: Method using cadmium	A test portion is suspended in a warm extraction buffer solution. Fat is separated by centrifuging and rapid cooling. Analyses are made of small	Milk and milk products - hard, semi-hard and soft cheeses of various ages,	NO2 & NOX (NO3 by subtraction)	The detection limits of the method are 0,5 mg of nitrate ions	Accessing the collaborative study report prior to year	See ISO 14673-3 IDF 189-3: 2004 section 11 for precision. Reproducibility -	


Line #	Country	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		reduction and flow injection analysis with in-line dialysis (Routine method) Note: This eWG received information that while this methods is widely used, the equipment is not commercially available anymore.	portions of the de-fatted solution by flow injection analysis (FIA). Inline dialysis is used to remove protein and remaining fat. The nitrate ions are reduced to nitrite ions by cadmium. The nitrite ions are reacted with sulfanilamide and N-1-naphthyl ethylenediamine dihydrochloride to give a red coloured azo dye. The colour is measured in a flow cell at maximum absorption of the dye at 540 nm with reference to the absorption measured at 620 nm.	and processed cheese; whey powder, milk powder and milk-based infant food.		per kilogram and 1,0 mg of nitrite ions per kilogram, respectively.	2000 has proved challenging	for samples with a nitrate content <100 mg/kg: 15 mg/kg; for samples with a nitrate content ≥100 mg/kg: 20 % of the arithmetic mean of the results.	
75		Afanda et al., (2025) Analysis of chloride, nitrite and nitrate in processed meat using microwave extraction and two-dim. IC, J. Food Comp. Anal., Vol.141,107323	Samples were extracted by microwave-assisted aqueous digestion, combined with automated inline solid phase extraction (SPE). Analysis by two-dimensional ion chromatography (2D-IC) method employing suppressed conductivity (chloride) and UV=210nm (nitrite and nitrate) detection	Uncured ham, Pork Loin Joint, Pork Fillet, Pork medallion, Mince pork, Bacon, Beef, Turkey breast, Lamb Mince meat, Rashers, Salami, Chorizo, Bacon Eyseloin/joints	NO2 & NO3 individually	Nitrite = 0.07 mg/kg; Nitrate = 0.27 mg/kg	Nitrite = 0.2 mg/kg; Nitrate = 0.8 mg/kg	Nitrite and nitrate Intra-assay %RSD = 1.0-1.9, 0.61-1,4 Nitrite and Nitrate Inter-assay %RSD = 0.34-1.4; 0.60-1.4	Nitrite mean recovery = 92-102%; Nitrate mean recovery = 97 – 104%. FAPAS inter-laboratory proficiency test results obtained with the method were satisfactory as z scores of – 1.1 and 0.3 were achieved for nitrite and nitrate, respectively


Index:

IC-Cond.: Ion Chromatography with Chemical suppression of eluent conductivity

Colorimetry – NED: Colorimetric method - diazotization-coupling reaction of sulfanilamide with N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Griess reaction.

NA – Not available.

 This reference not considered suitable for further review

 This reference repeated and thus consolidated under one referred line row

Note 2. To convert: mg sodium nitrite (NaNO₂)/kg food sample x 46.005/68.995 = mg NO₂ ion / kg food sample.

To convert: mg sodium nitrate (NaNO₃)/kg food sample x 62.005/84.995 = mg NO₃ ion / kg food sample.

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