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

(Prepared 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. CCMAS42 therefore established an electronic working group (EWG) chaired by the United States of America, working in English, under the following terms of reference (TORs):
 - 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;
 - 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;
 - 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
 - discuss if the different determination schemes (i.e. separate or combined) could have an impact on the precision and accuracy of the methods.
3. No EWG was eventually constituted. However, Australia and the United States of America (USA) progressed the work in accordance with the TORs described in paragraph 2.
4. This report is therefore based on the activity by following CCMAS42 (2023). The report also contains the following Appendices:
 - i. Appendix 1: Provisions for nitrates (INS 251, 252) and nitrites (INS 249, 250) in the Step process or adopted (ingoing and residual use levels) based on CX/FA 21/52/7 Appendix 5 Annex 2, including information on the lowest proposed residual levels¹ for representative provisions in dairy (cheese), meat, and seafood, and highlighting the relevant sections in the *General Standard for Food Additives* (CXS 192-1995), most notably p. 183; and
 - ii. Appendix 2: Summary of comments on available test methods for nitrates and nitrites found in CX/FA 21/52/7 Appendix 5 Annex 1.

¹ the use of the term “lowest proposed residual level” suggests a desired method LOQ, not a maximum specification limit.

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

5. 'Residual ML (mg/kg)' in CX/FA 21/52/7 Appendix 5, Annex 2 provided a suggested LoQ for the methods and not the 'ML' CCMAS would require for method performance criteria development. The only Subcategory (commodity) listed with an adopted ML provision in CXS 192-1995 was '01.6.2.1 (*Ripened cheese, includes rind*)'. To obtain the other potential subcategory ML values, those proposed in the 'Comments submitted on GSFA Appendix 5: Provisions for nitrates (INS 251, 252) and nitrites (INS 249, 250) in the Step process or adopted (ingoing and residual use levels)' (CX/FA 21/52/7 Appendix 5) were used. The potential ML and corresponding performance criteria have been provided in Appendix 1 below, noting that there could be a mis-assigned subcategory number for 'processed cheese'.
6. To meet the requested CCFA requirements, a method would need to meet all the specified method performance criteria (including LoQ), and it would also need to have a LoQ less than the lowest 'Residual ML (mg/kg)' in CX/FA 21/52/7 Appendix 5, Annex 2 for the food matrix concerned.

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

7. The methods listed in CX/FA 21/52/7 Appendix 5 Annex 1 have been collated with freely available performance data and have also been listed below in Appendix 2 (apart from EU – 'Methods for the quantitative determination of nitrite alone' which included another 16 suggested method line items). Appendix 2 covers 59 method suggestions from 10 Member Countries, or Member Organization, or SDO, with some repetition (18 cases where the method submission was repeated by another member or organization).
8. 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' (7 cases). This left 34 methods for consideration against the method 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. Note only one method had 'Fish and fish 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'.
9. 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 method 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. 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.
10. 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 most likely met the criteria and was collaboratively trialed, this method was preferably listed in contrast to a 'single-laboratory validated method'. So, it might be useful to supplement the method list with more recently published methods, as the most recently published methods would be from 2018 (i.e. 5-6 years old) and it is hoped that more standardized methods would be updated.
11. Interestingly the article Zhong et al 2021² states that their Data extraction criteria applied was: a) the Griess reaction-based spectrophotometric method was used as the reference method, and b) the reliability and applicability of methods ranked as Griess assay (e.g., Association of Official Analytical Chemists method 993.03) > chromatographic methods (ion chromatography and other reversed-phase liquid chromatography (LC), LC-MS, gas chromatography (GC), GC-MS) > spectrofluorimetric methods > capillary electrophoresis > Chemiluminescence [Wang et al 2017³]. They also only list < 10 references

² Zhong et al 2021 Develop. Food Comp Database Assessing NO₃ & NO₂ Intake from Animal-based Foods Mol. Nutr. Food Res. 2022, 66, 2100272

³ Wang et al 2017 Methods for the detection and determination of nitrite and nitrate-A review Talanta 2017, 165

for Nitrate and Nitrite mg/kg in 'Fish and seafood products'. To see how methods with reduced LoD/LoQ could be used, a study by Lacumin et al 2019⁴ to determine the level of the "natural" nitrite and nitrate concentration in raw meat, salt and sugna (soft pork fat) used to produce San Daniele dry cured ham (SDDCH) and in SDDCH (PDO), could be referred to. 'Natural' means that both compounds were not deliberately added by the producers during the ham production. The data obtained highlighted that the concentration of nitrite and nitrate in SDDCH PDO must be considered natural when it was, respectively, less than 4 and 22 mg/kg of the ripened product, irrespective of the ripening time (14–19 months).

12. Methods which would 'likely' or 'possibly' meet the criteria have been included in Appendix 1 below but some work would still be needed to find a source of the additional validation data. 'Nitrite 09.3.3 (Salmon substitutes, caviar, and other fish roe products)' had a very low LoD/LoQ, which might be achievable with newer methodologies but probably not with existing 'standardized' methods.

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

13. Appendix 2 lists the 5 variations of 'NO₂ & NO₃ individually or combined':
 - NO₂ & NO₃ individually,
 - NO_x only,
 - NO₂ only,
 - NO₃ only
 - NO₂ & NO_x (NO₃ by subtraction).
14. As indicated above, methods utilizing 'HPLC' with various detectors and capillary electrophoresis (CE) typically measure 'NO₂ & NO₃ individually'.
15. 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

16. Methods that determine nitrate and nitrite in a range of matrices (commodities) might no longer be apropos 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 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.
17. For example, it has been found that the HPLC 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 parameters for Griess reaction parameters had been well studied by Mohamed et al 2008⁵. Merino et al. 2000⁶ 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

⁴ lacumin et al 2019 Natural levels NO₂ & NO₃ San Daniele dry cured ham PDO, & in meat, salt & sugna used for production. Food Control, Vol.100, p 257-261

⁵ Mohamed et al. 2008 Modification of AOAC Method 973.31 for Determination of Nitrite in Cured Meats JAOACI VOL. 91, NO. 4, p820-7

⁶ MERINO et al. 2000 LC Detn of Residual Nitrite-Nitrate in Foods, NMKL Collaborative Study_JAOACI Vol. 83, NO. 2, 2000

addition of nitrite/nitrate had the greatest influence on the stability of these ions; concluding the higher the pH, the better the recovery.

18. The Croitoru 2012⁷ 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).
19. 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 redactor element. Cd has been classified as a suspected cancer agent (Carranzo, 2012⁸; Ferreira et al., 1998⁹), 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.
20. 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 (Cruz and Martins Loução, 2002¹⁰, Beheshti et al 2023¹¹).
21. Beheshti et al 2023 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 figure of merits and limitations of each technique from different aspects were evaluated.
22. For IC with conductometric detection, it was difficult to measure low nitrite concentrations in the presence of bulk chloride (Butt et al 2001¹²). Various techniques were available to mitigate these problems e.g. Aggrawal et al. 2020¹³ 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%.
23. Coviello et al. 2020¹⁴ 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 electrochemiluminescent techniques. However, spectrophotometric methods

⁷ 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

⁸ Carranzo IV (2012) Standard Methods for examination of water and wastewater, *Anales de hidrología médica*. Universidad Complutense de Madrid, p 185

⁹ Ferreira AM, Rangel AO, Lima JL (1998) Flow injection systems for elemental soil analysis determinations. *Commun Soil Sci Plant Anal* 29:327–360

¹⁰ Cruz C, Martins Loução M (2002) Comparison of methodologies for nitrate determination in plants and soils. *J Plant Nutr* 25:1185–1211

¹¹ 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>

¹² Butt et al 2001, Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography *Talanta* 55 (4) p789-797

¹³ Aggrawal et al. 2020 Simultaneous detn nitrate and nitrite in spinach and meat by ion chromatography Thermo Fisher Appl. note an73450-en

¹⁴ Coviello et al. 2020 Valid. NO₂-NO₃ Detn in Meat Foods for Infants by IC with Cond. *Detn_Foods*. 2020 Sep 4;9(9)_1238

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. This is a very different outcome to the data extraction criteria used by Zhong et al 2021¹.

CONCLUSION

24. It should be remembered that CCMAS is providing method performance criteria, and any validated method which could meet that criteria with the commodity and provision can be utilized.
25. As stated above, there are some standardized methods which might meet the criteria (though further validation data will be required), however with updates to the existing techniques, the compliance rate using standardized methods could be increased significantly. 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.
26. Also, with an appropriate range of certified reference materials and other QA and QC, along with appropriate extract clean-up, determination schemes based on 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

- i. CCMAS43 is invited to consider the findings highlighted in this report in its discussion on the response to CCFA's requests; and:
 - a) whether to endorse the numeric performance criteria including example methods in Appendix 1 in the reply to CCFA;
 - b) decide if more work is needed to source and examine the validation data for the methods included as 'examples of applicable methods that meet the criteria' in Appendix 1; and
 - c) re-establish the EWG to review the methods in Appendix 2 in more detail and determine if the methods are suitable, and if the methods meet or exceed the numeric performance criteria.

APPENDIX I

From Annex 2 information on the lowest proposed residual levels for representative provisions in dairy (cheese), meat, and seafood as provided by Members of the CCFA EWG on the General Standard for Food Additives in response to requests for comment to the first and second circulars plus method performance criteria based on the adopted maximum limits or proposed ML from GSFA Appendix 5 in the step process.

Food Additive	Subcategory for which value was provided	Residual ML (mg/kg)	Notes	Adopted Maximum limits (CXS 192-1995) or <u>proposed ML from GSFA App.5: Provisions for nitrates & nitrites in the Step process or adopted.</u>	Based on the Adopted Maximum limits (CXS 192-1995) or proposed ML from GSFA					
					Min Appl. Range (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision (RSD _R (%))	Recovery (%)	Examples of applicable methods that meet the criteria
01.6 (Cheese and analogues)										
Nitrate	01.6.2.1 (Ripened cheese, includes rind)	7	As NO ₃	35 mg/kg as residual NO ₃ ion.	25.2 - 44.8	3.5	7	18.7	80 – 110	Most likely ISO 14673-3 IDF 189-3 ; possibly ISO 14673-2 IDF 189-2 , and lammarino et al. 2013 (Note 2)
Nitrite	01.6.44 (Processed cheese) <i>*(see note 1)</i>	2	As NO ₂	<u>20 mg/kg as residual NO₂ ion.</u>	13.9 - 26.1	2	4	18.4	80 – 110	Most likely ISO 14673-3 IDF 189-3 ; possibly ISO 14673-2 IDF 189-2 , and lammarino et al. 2013 (Note 2)
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	<u>1600 mg/kg as residual NO₃ ion.</u>	1347 - 1853	160	320	10.5	95 – 105	Most likely EN 12014-3 2005 ; possibly Siu et al 1998 ; lammarino et al. 2013 or NMKL No.194:2013 (Note 2: check precision & recoveries).
Nitrite	08.2.1.3 (Fermented non-heat treated processed meat, poultry, and game products in whole pieces or cuts)	33	As NO ₂	<u>130 mg/kg as residual NO₂ ion.</u>	100 - 160	13	26	15.4	90 – 107	Most likely EN 12014-3 2005 ; possibly Siu et al 1998 ; lammarino et al. 2013 or NMKL No.194:2013 (Note 2: check precision & recoveries).
09.0 (Fish and fish products, including molluscs, crustaceans, and echinoderms)										
Nitrate		None reported								
Nitrite	09.3.3 (Salmon substitutes, caviar, and other fish roe products)	4.4	As NO ₂	<u>5 mg/kg as residual NO₂ ion.</u>	3.1 - 6.9	0.5	1.0	25.1	80 – 110	Possibly lammarino et al. 2013 (Note 2)

Note 1: In the dot point above 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'. Neither 'Unripened cheese' or 'Processed Cheese' has an adopted 'Nitrite (as NO₂)' specification in STAN 192-1995 Revision 2021.

Note 2: require 'full article', as 'free access' information doesn't provide sufficient validation data.

APPENDIX II

For Review: methods in CX/FA 21/52/7 Appendix 5, Annex 1 with the validation data currently available.

Line #	Member/ Observer	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 extrn. 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	RSDR % 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	

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
								10 % of the mean value.	
8	Chile	Wootton M, Kok SH, Buckle KA (2006) Determination of nitrite and nitrate levels in meat and vegetable products by high performance liquid chromatography. J Sci Food Agric 36:297-304	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 satisfactory results, but some meat products subject to matrix interference.	NO2 & NO3 individually				
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. (LOD & LOQ calculated as per APHA?)	RSD = 1.16-2.02%	99.3 – 103% spike recovery
14	Chile	Chou et al (2003) A High Performance Liquid Chromatography Method for	Fifty mL of deionized (DI) water was added to the well-homogenized sample weighed 1g in a 100 mL	vegetables	NO2 & NO3 individually	5 mg/kg	NA	Repeated trials all	The recoveries of nitrate

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		Determining Nitrate and Nitrite Levels in Vegetables. Journal of Food and Drug Analysis, Vol. 11, No. 3, 2003, Pages 233-238	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 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.					obtained CV values less than 1.5%,	and nitrite spiked were in the range of 96.6 ~ 108.7% and 98.9 ~ 105.7%, respectively
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 tetrabutylammonium hydrogeno-sulphate 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							

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		performance ion exchange liquid chromatography (HPLC / IC) for the determination of nitrate content in vegetables and 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)							
24	Colombia	AOAC 973.31-1996(1997) (see Chile line 10)	Nitrites in cured meat – colorimetric method	Cured meat, including in canned					

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
				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 interlaborato		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 #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
					ry test scheme cited here).				
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 dializing 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 $\leq 5\%$).	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	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,	meat products	NO2 & NOX (NO3 by subtraction)		for total nitrite and nitrate content from 5 mg/kg up to 125 mg/kg calculated as	See EN 12014-3: 2006 Annex A Precision data	NA

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		Committee for Standardization (CEN)	shaking & dilute to 200mL, filter. Nitrite in extract treated with sulfanilamide and NED. The formed red compound is measured spectrometrically at $\lambda=540$ nm. Nitrate extract is converted into nitrite by nitrate reductase. The treated as above for nitrite, the sample nitrate content is calculated from the difference between the spectrometric measurements.				sodium nitrite.		
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 μ m 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 μ L 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 57							
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 58							

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
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 59							
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 (<i>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</i>)							
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	NA

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
								with a maximum RSD of 10%, with shorter analysis time.	
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 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.	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		%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.
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	IC with post-column IFD to determine simultaneously nitrite and	Salami		Not specified,			Recoveries from

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection. Journal of Chromatography A 1002, 237-241	nitrate, at an emission wavelength of 355 nm after excitation at 270 nm.			but this reviewer estimates LOD at ≈5mg/kg based on peak size & RSDs.			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. Journal of Chromatography A 789 (1-2), 213-219	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 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	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	The LOD for nitrate was 3.2 mg/kg. (EFSA 2017 p17)			Acceptable agreement in the comparative performance of both methods was reported. (EFSA 2017 p17)

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			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.						
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.
45	EU	Oztekin et al 2002. Simultaneous determination of nitrite and nitrate in meat products and vegetables by	Meat products (10 g) were weighed in a beaker. DI water (150 ml) added, blended & suspension incubated 15min at 50°C. Cooled &	Cured meat products and fresh vegetables - Salami, Ham, Turkey	NO2 & NO3 individually	LOD for nitrite corresponding to a		Reproducibility of the method for six	Four cured meat products and four

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		capillary electrophoresis. Food Chemistry 76, 103-106	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.	sausage, Sausage, Spinach, Parsley, Dill, Leek		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.		successive injections of turkey sausage sample, give 4.51 %RSD for NO2, and 2.54 %RSD for NO3.	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	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	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% -	Recoveries of NOx from various fortified

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO ₂ & NO ₃ individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		AOAC International 89 (2), 447-451	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 foods with 50–60°C water or with buffer, followed by clarification with acetonitrile and centrifugation.					19.4% RSD; Nitrate 2.4% - 7.9	baby food fruits and vegetables (25–400 ppm) ranged from 82 to 107%.
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	Spectrophotometric method for the determination of nitrate/nitrite content in foodstuffs and water after	vegetables (lettuce), meat products, baby food, dairy product	NO ₂ & NO _x (NO ₃ by subtraction)	5 mg/kg (meat products –			

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
		nitrite in foodstuffs and water by spectrophotometry after zinc reduction and Griess reaction. NMKL No. 194	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(Tsikis D. 2007)	(milk) and surface water - method validated for above foods		EFSA 2017 p 16)			
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	

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO2 & NO3 individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
								and 5% for NO3.	
53	EU		Didn't review 16 articles for 'Methods for the quantitative determination of nitrite alone'.						
54	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.						
55	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 (NO3), nitrite (NO2), 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.					
56	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							
57	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	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)			See ISO 14673-1 IDF 189-1: 2004 section 11 for precision	

Line #	Member/ Observer	Method reference	Principle	Matrix Scope	NO ₂ & NO ₃ individually or combined	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Precision	Recovery
			segmented flow analysis (SFA) or flow injection analysis (FIA)						
58	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.	NO ₂ & NO _X (NO ₃ by subtraction)			See ISO 14673-2 IDF 189-2: 2004 section 11 for precision	
59	IDF	ISO 14673-3 IDF 189-3: 2004 — Milk and milk products — Determination of nitrate and nitrite — Part 3: Method using cadmium reduction and flow injection analysis with in-line dialysis (Routine method)	A test portion is suspended in a warm extraction buffer solution. Fat is separated by centrifuging and rapid cooling. Analyses are made of small portions of the de-fatted solution by flow injection analysis (FIA). In-line 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.	Milk and milk products - hard, semi-hard and soft cheeses of various ages, and processed cheese; whey powder, milk powder and milk-based infant food.	NO ₂ & NO _X (NO ₃ by subtraction)	The detection limits of the method are 0,5 mg of nitrate ions per kilogram and 1,0 mg of nitrite ions per kilogram, respectively.		See ISO 14673-3 IDF 189-3: 2004 section 11 for precision.	


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