



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers & Reference Materials (Geel)
European Union Reference Laboratory for Feed Additives



JRC F.5/CvH/ZE/AS/Ares

**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
Feed Additive according to Regulation (EC) No 1831/2003**

Sodium nitrite
(FAD-2010-0378; CRL/100251)
(FAD-2010-0421; CRL/100343)



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Dossier related to: **FAD-2010-0378 - CRL/100251**
FAD-2010-0421 – CRL/100343

Name of Feed Additive: ***Sodium nitrite***

Active Agent (s): **Sodium nitrite**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
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Date: **25/02/2021**

EXECUTIVE SUMMARY

In the current group of applications an authorisation is sought under Article 10(2) for *sodium nitrite* under the category/functional group 1(k) ‘technological additives’ / ‘silage additives’ according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for pigs, bovines, poultry, sheep, goats, rabbits and horses.

According to the Applicants the *feed additive* consists of a minimum of 98.5 % (w/w) of *sodium nitrite* as active substance. The *feed additive* is intended to be used in *silage* through *premixtures* with proposed levels of *sodium nitrite* ranging from 100 to 900 mg/kg *silage*.

For the quantification of the active substance (*sodium nitrite*) in the *feed additive*, the Applicants proposed several methods, namely (i) the method based on redox titration with potassium permanganate described in the FAO JECFA monograph “sodium nitrite” for food additives, (ii) the ring-trial validated EN 26777 method based on spectrophotometry dedicated for the determination of the nitrite content in the frame of water quality control and (iii) the ring-trial validated method EN 12014-4 based on ion-exchange chromatography coupled to spectrophotometric detection (IEC-UV), which is dedicated to the determination of the nitrate and/or nitrite content in foodstuffs.

The above mentioned FAO JECFA and EN 26777 methods were successfully applied for the analysis of different batches and in the frame of stability studies of the *feed additive*.

Based on the overall available performance data, the EURL recommends for official control the above mentioned titrimetric method described in the FAO JECFA monograph “sodium nitrite” and the EN 26777 method based on spectrophotometry for the quantification of *sodium nitrite* in the *feed additive*.

In addition, given the acceptable performance data for the EN 12014-4 method and that the *feed additive* under the two applications contains no matrix components which might interfere with the quantification of *nitrite*, the EURL considers also the EN 12014-4 method recommendable for official control for the quantification of *sodium nitrite* in the *feed additive* after its appropriate dilution during the sample preparation.

For the quantification of the active substance (*sodium nitrite*) in *premixtures*, the Applicants proposed the above mentioned EN 26777 and EN 12014-4 methods. Furthermore, these methods were successfully used in the stability studies of several different *premixtures* containing *sodium nitrite*.

Based on the available performance data the EURL recommends for official control the above mentioned EN 26777 method based on spectrophotometry and the EN 12014-4 method based on IEC-UV for the quantification of *sodium nitrite* in *premixtures*.

For the quantification of *sodium nitrite* in *silage* the Applicants did not submit any method or data. Furthermore, according to the Applicant (FAD-2010-0378) *sodium nitrite* is not stable during the fermentation process of ensilaging. As a consequence, the accurate quantification of *sodium nitrite* added to *silage* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the quantification of *sodium nitrite* added to *silage*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Sodium nitrite, technological additives, silage additives, pigs, bovines, poultry, sheep, goats, rabbits and horses.

1. BACKGROUND

In the current group of applications an authorisation is sought under Article 10(2) (re-authorisation of an existing feed additive) for *sodium nitrite* under the category/functional group 1(k) 'technological additives' / 'silage additives' according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1-5]. Specifically, the authorisation is sought for the use of the *feed additive* for pigs, bovines, poultry, sheep, goats, rabbits and horses [1,3-5].

According to the Applicants the *feed additive* is a white solid substance [5,6] or white-to-slightly-yellow crystalline powder [7], consisting of a minimum of 98.5 % (w/w) [5,6] or 99.0 % (w/w) of *sodium nitrite* as active substance [6,7].

The *feed additive* is intended to be used in *silage* through *premixtures* [8,9] with proposed levels of *sodium nitrite* ranging from 100 to 900 mg/kg *silage* [8,9].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the

methods of analysis submitted in connection with *sodium nitrite* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of the active substance (*sodium nitrite*) in the *feed additive*, the Applicants proposed [10,11] several methods, namely (i) the method based on redox titration with potassium permanganate described in the FAO JECFA monograph "sodium nitrite" for food additives [12], (ii) the ring-trial validated EN 26777 method based on spectrophotometry dedicated for the determination of nitrite content in the frame of water quality control [13], and (iii) the ring-trial validated method EN 12014-4 based on ion-exchange chromatography coupled to spectrophotometric detection (IEC-UV), which is dedicated to the determination of the nitrate and/or nitrite content in foodstuffs [14].

According to the titrimetric JECFA method, the dried sample (1 g) is dissolved in a total volume of 100 ml of water. An aliquot (10 ml) of the latter solution is mixed with 50 ml of 0.1 N potassium permanganate solution, 100 ml of water and 5 ml of sulfuric acid. The mixture is warmed up to 40 °C for 5 min for the accomplishment of the redox reaction between the permanganate and the nitrite. Then, 25 ml of 0.1 N oxalic acid solution is added to react with the excess of the permanganate from the above mentioned reaction; the mixture is heated up to 80 °C to complete the redox reaction between the permanganate and oxalic acid. The excess of oxalic acid from the latter reaction is titrated with 0.1 N potassium permanganate solution. The calculation of the amount of *sodium nitrite* is based on the stoichiometry of the two above mentioned redox reactions [12]. Furthermore, the Applicant (FAD-2010-0421) applied this titrimetric method for the analysis of a few batches of the *feed additive* demonstrating excellent precision [7].

According to the EN 26777 method, a solution of the *nitrite* sample of 40 ml (with a mass concentration of *nitrite* of 0.8 mg/l) is reacting with 1 ml of colouring reagent containing 40 g / l of 4-aminobenzene sulphonamide and 2 g / l of N-(1-naphthyl)-1,2-diaminoethane dihydrochloride in an aqueous solution of 1.5 M ortho-phosphoric acid at pH 1.9. The formed pink-coloured derivatisation product is detected by spectrophotometry at 540 nm. The quantification of the *nitrite* content is performed by using an external standard calibration curve prepared from *sodium nitrite* standard solutions, which were treated in the same way as the sample [13].

In the frame of a ring-trial validation study for the quantification of the *sodium nitrite* content in sewage effluent, sea and river waters and standard aqueous solutions of *sodium nitrite*, the following performance characteristics have been reported at levels ranging from 0.1 to 5.3 mg/l: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.1 to 1.2 % and relative standard deviation for *reproducibility* (RSD_R) ranging from 0.1 to 1.4 % [13]. In addition, one Applicant (FAD-2010-0378) used the EN 26777 method in the frame of the stability studies of 10 different batches of the *feed additive* and an RSD_r ranging from 0.1 to 0.3 % was reported [15].

According to the EN 12014-4 method, a sample of 10 g is mixed thoroughly with water at 50 to 60 °C. After the addition of acetonitrile, the mixture is cooled down to room temperature, diluted with water and filtered for the chromatographic analysis. The analyte is detected at 205 nm and the quantification is performed via an external calibration using standard calibration solutions of *sodium nitrite* [14].

The following performance characteristics of the EN 12014-4 method have been reported in the frame of three ring-trial validation studies for the quantification of the *nitrite* content in meat-based products such as corned beef, sausages, salami, paté and baby food for the mass fraction of nitrite ranging from 39 to 161 mg/kg foodstuffs: an RSD_r ranging from 3.8 to 4.0 % and an RSD_R ranging from 6.9 to 17.1 % [14].

Given the above mentioned performance characteristics and that the *feed additive* under the two applications does not contain matrix components which might interfere with the quantification of *nitrite*, the EURL considers also the EN 12014-4 method suitable for the quantification of *sodium nitrite* in the *feed additive* after its appropriate dilution during the sample preparation.

Based on the overall available performance data, the EURL recommends for official control the above mentioned titrimetric method described in FAO JECFA monograph “sodium nitrite”, the EN 26777 method based on spectrophotometry and the EN 12014-4 method based on IEC-UV for the quantification of *sodium nitrite* in the *feed additive*.

For the quantification of the active substance (*sodium nitrite*) in *premixtures*, the Applicants proposed [10,11] the above mentioned EN 26777 [13] and EN 12014-4 [14] methods. Furthermore, the EN 26777 method was used for the stability studies of three different *premixtures* with *sodium nitrite* contents ranging from 104 to 245 g/kg *premixtures* and an RSD_r ranging from 1.4 to 3.8 % was reported [15].

In addition, the Applicant (FAD-2010-0421) applied the EN 12014-4 method for another type of *premixture* sample in the frame of the stability studies and an RSD_r of 3.3 % was obtained for the quantification of the *sodium nitrite* content of 46 g/kg *premixture* [16].

Based on the available performance data the EURL recommends for official control the above mentioned EN 26777 method based on spectrophotometry and the EN 12014-4 method based on IEC-UV for the quantification of *sodium nitrite* in *premixtures*.

For the quantification of *sodium nitrite* in *silage* the Applicants did not submit any method or data. Furthermore, according to the Applicant (FAD-2010-0378) *sodium nitrite* is not stable during the fermentation process of ensilaging [15]. As a consequence, the accurate quantification of *sodium nitrite* added to *silage* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the quantification of *sodium nitrite* added to *silage*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the identification of the *feed additive*, the fit-for-purpose qualitative tests for sodium and nitrite are specified in the above mentioned JECFA monograph [12].

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control for the quantification of *sodium nitrite* in the *feed additive* and *premixtures*: (i) the method based on a redox titration with potassium permanganate described in the FAO JECFA monograph "sodium nitrite" (only for the *feed additive*); (ii) the EN 26777 method based on spectrophotometry; and (iii) the EN 12014-4 method based on ion-exchange chromatography coupled to spectrophotometric detection (IEC-UV).

The accurate quantification of *sodium nitrite* added to *silage* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the quantification of *sodium nitrite* added to *silage*.

Recommended text for the register entry (analytical method)

For the quantification of *sodium nitrite* in the *feed additive* and *premixtures*:

- Titration with potassium permanganate – FAO JECFA monograph “sodium nitrite” (only for the *feed additive*); or/and
- Spectrophotometry; or/and
- Ion-exchange chromatography coupled to spectrophotometric detection (IEC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *sodium nitrite* have been sent to the European Union Reference Laboratory for Feed Additives. The dossiers have been made available to the EURL by EFSA.

6. REFERENCES

- [1] *Application, Reference SANCO/G/1_FWD. APPL. 0074-2011
- [2] +Application, Reference SANCO/G/1_FWD. APPL. 0119-2011
- [3] *Application, Annex 1 – Submission number 1288694971165-1332
- [4] +Acceptance of the valid application referring to Sodium nitrite (E 250) (Safesil) for pigs, poultry, bovines, ovines, goats, rabbits and horses, Ref. KL/vk – OC-2020-24121701
- [5] *Application, Proposal for Register Entry – Annex A
- [6] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition (active sub-stance/agent, other components, impurities, batch to batch variation)
- [7] +Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition of the additive (active substance/agent, other components, impurities, batch to batch variations)
- [8] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
- [9] +Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
- [10] *Technical dossier, Section II: 2.6. Methods of analysis and reference samples
- [11] +Technical dossier, Section II: 2.6. Methods of analysis and reference samples
- [12] FAO JECFA Combined Compendium of Food Additive Specifications, “sodium nitrite”, Monograph No. 1 (2006)
http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-417.pdf (last visited 14/01/2021)
- [13] EN 26777:1993 – Water quality - Determination of nitrite - Molecular absorption spectrometric method (ISO 6777:1984)

[14] EN 12014-4 – 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

[15] *Technical dossier, Section II: 2.4.1 Stability

[16] +Technical dossier, Section II – Annex 2.4.1.2

*Refers to Dossier no: FAD-2010-0378

+ Refers to Dossier no: FAD-2010-0421

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Istituto Superiore di Sanità. Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Roma (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Wageningen Food Safety Research¹ (WFSR) (NL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

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