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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

3-Nitrooxypropanol
(preparation of minimum of 10 % of 3-nitrooxypropanol)

(FAD-2019-0057; CRL/190024)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2019-0057 - CRL/190024**
Name of Product:	**3-Nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol)**
Active Agent (s):	**3-Nitrooxypropanol**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**María José González de la Huebra** **14/02/2020**
Report approved by: Date:	**Christoph von Holst** **14/02/2020**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *3-nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol)* under the category / functional group 4(c) 'zootechnical additives' /'substances which favourably affect the environment', according to Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for dairy cows and cows for reproduction, dairy sheep and ewes for reproduction, dairy goats and goats for reproduction, other ruminants for milk production and reproduction.

The *feed additive* is a white powder preparation containing a minimum of 10 % (w/w) of *3-nitrooxypropanol* as an active substance. The *feed additive* also contains propylene glycol and silicon dioxide as carriers.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant proposed levels of *3-nitrooxypropanol* ranging from 53 to 88 mg/kg complete *feedingstuffs*.

For the quantification of the *3-nitrooxypropanol* content in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography (HPLC) coupled to spectrophotometric (UV) detection.

The following performance characteristics were reported by the Applicant in the frame of the validation and verification studies for the quantification of *3-nitrooxypropanol* content:

- in the *feed additive*: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 1.0 %; a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.3 to 1.0 %; and a *recovery* rate (R_{rec}) ranging from 100 to 101 %.
- in *premixtures* (2870 to 17390 mg/kg): a RSD_r ranging from 0.4 to 1.1 %; a RSD_{ip} ranging from 0.8 to 1.5 %; and a R_{rec} ranging from 100 to 101 %.
- in *feedingstuffs* (29 to 132 mg/kg): a RSD_r ranging from 0.6 to 5.2 %; a RSD_{ip} ranging from 1.0 to 5.2 %; a R_{rec} ranging from 98 to 101 %; and a limit of quantification (LOQ) ranging from 8 to 14 mg of *3-nitrooxypropanol* /kg *feedingstuffs*.

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified reversed phase HPLC-UV method for the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs*.

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

3-Nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol), zootechnical additives, substances which favourably affect the environment, dairy cows and cows for reproduction, dairy sheep and ewes for reproduction, dairy goats and goats for reproduction, other ruminants for milk production and reproduction

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a feed additive) for *3-nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol)* under the category / functional group 4(c) 'zootechnical additives' /'substances which favourably affect the environment', according to Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for the use of the *feed additive* for dairy cows and cows for reproduction, dairy sheep and ewes for reproduction, dairy goats and goats for reproduction, other ruminants for milk production and reproduction [2].

The *feed additive* is a white powder preparation containing a minimum of 10 % (w/w) of *3-nitrooxypropanol* as an active substance. The *feed additive* also contains propylene glycol and silicon dioxide as carriers [3].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [3]. The Applicant proposed levels of *3-nitrooxypropanol* ranging from 53 to 88 mg/kg complete *feedingstuffs* [2,3].

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 *3-nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol)* 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 *3-nitrooxypropanol* content in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography (HPLC) coupled to spectrophotometric (UV) detection [4-7].

According to the method, the samples of the *feed additive* (50 to 150 mg) and *premixtures* (0.8 to 1.2 g) are extracted in two steps with acetonitrile and water at 40 ^{o}C. The samples of *feedingstuffs* (9 to 11 g) are extracted with a mixture containing acetonitrile, water and formic acid at 55 ^{o}C when analysing mash and pelleted feed or only with acetonitrile at 50 ^{o}C when analysing cattle feed. The samples are centrifuged and filtered for further HPLC analysis. The analyte is detected at 210 nm. The quantification is performed by an external calibration using *3-nitrooxypropanol* as a standard substance [4-7].

The performance characteristics reported by the Applicant in the frame of the validation [8,9,10] and verification [11-15] studies for the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs* are presented in Table 1.

In addition, a limit of quantification (LOQ) of 8 to 14 and 10 mg of *3-nitrooxypropanol* /kg *feedingstuffs* (mash, pelleted feed and total mixed ration) were reported by the Applicant and the 2nd laboratory, respectively [12].

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified reversed phase HPLC-UV method for the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs*.

Table 1 The performance characteristics of the single-laboratory validated and verified HPLC-UV method for the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs*

	Feed additive		***Premixtures***		***Feedingstuffs***	
	Validation	**Verification**	**Validation**	**Verification**	**Validation**	**Verification**
Mass fraction, mg/kg	111730 – 112260	108000 – 111667	2870 – 17390	5412 – 5520	29 – 132	41 – 129
RSD_r, %	0.2 – 0.4	0.5 – 1.0	0.4 – 1.1	0.9 – 1.1	0.6 – 1.9	1.5 – 5.2
RSD_{ip}, %	0.3 – 0.6	1.0	0.8 – 1.5	1.1	1.0 – 3.4	1.9 – 5.2
R_{rec}, %	100 – 101	101	100 – 101	101	98 – 101	98 – 101
Reference	[8]	[11,12]	[9]	[12,13]	[10]	[12,14,15]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{rec} - a *recovery* rate.

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)

The evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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 the single-laboratory validated and further verified reversed phase HPLC-UV method from the Applicant for the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *3-nitrooxypropanol* in the *feed additive*, *premixtures* and *feedingstuffs*:

- reversed phase high performance liquid chromatography with spectrophotometric detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *3-Nitrooxypropanol (preparation of minimum of 10 % of 3-nitrooxypropanol)* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANTE_E5_FWD. APPL. 1831-0058-2019

[2] *Application, Annex I – submission number 1567085276730-2440

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis

[4] *Technical dossier, Section II – Annex_II_007

[5] *Technical dossier, Section II – Annex_II_046

[6] *Technical dossier, Section II – Annex_II_049

[7] *Technical dossier, Section II – Annex_II_052

[8] *Technical dossier, Section II – Annex_II_043

[9] *Technical dossier, Section II – Annex_II_047

[10] *Technical dossier, Section II – Annex_II_050

[11] *Technical dossier, Section II – Annex_II_044

[12] *Technical dossier, Section II – Annex_II_045

[13] *Technical dossier, Section II – Annex_II_048

[14] *Technical dossier, Section II – Annex_II_051

[15] *Technical dossier, Section II – Annex_II_054

*Refers to Dossier no: FAD-2019-0057

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

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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- [1]Wageningen Food Safety Research (WFSR) (NL)

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.