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European Union Reference Laboratory for Feed Additives

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

Dossier related to:	FAD-2010-0112 - CRL/100139 FAD-2010-0198 - CRL/100173 FAD-2010-0263 - CRL/100240 FAD-2010-0265 - CRL/100170 FAD-2010-0307 - CRL/100369
Feed additive:	Niacin (Nicotinic acid) Niacinamide (Nicotinamide)
Active Substance(s):	Niacin (Nicotinic acid) Niacinamide (Nicotinamide)
Rapporteur Laboratory:	European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium
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Report revised by: Date:	Piotr Robouch (EURL-FA) 21/09/2011
Report approved by: Date:	Christoph von Holst 27/09/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1)[1,2] (new use in water) and 10(2)[1-5] for *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* under the category/functional group 3(a) "nutritional additives"/"vitamins, pro-vitamins and chemically well defined substances having similar effect", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories.

According to the Applicants the products are white granular crystals or crystalline powder, containing a minimum of 99[3] % *Niacin* and a minimum of 98.5[3] % *Niacinamide*. The *feed additive* is intended to be processed in *premixtures* or added directly into *feedingstuffs* or in *water*. However, the Applicants did not specify any maximum or minimum concentration of *Niacin* and *Niacinamide* in *feedingstuffs* or *water*, as set in the previous regulations.

For the determination of *Niacin* and *Niacinamide* in the *feed additive*, the Applicants propose various methods including the internationally recognised European Pharmacopoeia methods (Ph. Eur. 6th Edition, monograph 0459 for the determination of *Niacin* and monograph 0047 for the determination of *Niacinamide*). Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia methods to determine *Niacin* and *Niacinamide* in the *feed additive*.

For the determination of *Niacin* and *Niacinamide* in *premixtures*, the Applicants propose two methods based on ion-pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV), namely (1) the method developed by VDLUFA (Association of German Agricultural Analytical and Research Institutes) and (2) a single-laboratory validated and further verified method submitted by one of the Applicants. The VDLUFA method was ring-trial validated with a *Niacin* and *Niacinamide* content ranging from 3665 to 15540 mg/kg *premixtures*. The following performance characteristics were reported for Niacin and Niacinamide: (i) a relative standard deviation for *repeatability* (RSD_r) ranging from 3.49 to 7.58 %, and (ii) a relative standard deviation for *reproducibility* (RSD_R) ranging from 4.02 to 15.4 %.

The above mentioned single-laboratory validated and further verified method is also suitable for the determination *Niacin* and *Niacinamide* in *feedingstuffs.* The performance characteristics of this method were recalculated by the EURL using the experimental data provided by the Applicant, to obtain:

[1] FAD-2010-0112; [2] FAD-2010-0263; [3] FAD-2010-0198; [4] FAD-2010-0265; [5] FAD-2010-0397

* For *premixtures* samples with contents ranging from 0.4 to 40 g/kg, for Niacin and Niacinamide:
  - RSD_r ranging from 0.5 to 0.8 %;
  - RSD_{ip} (*intermediate precision*) ranging from 4.9 to 6.7 %;
  - R_{Rec} (recovery rate) ranging from 96.9 to 106.1 %, and

* For *feedingstuffs* samples with contents ranging from 8 to 800 mg/kg:
  - RSD_r ranging from 4.1 to 5.4 %;
  - RSD_{ip} ranging from 4.1 to 11.1 %;
  - R_{Rec} ranging from 95.7 to 107.0 %, and
  - LOD and LOQ of 2 and 8 mg/kg, respectively.

For the determination of *Niacin* and *Niacinamide* in *water* the Applicant provided data, upon request of the EURL, proving that the method for *premixtures* and *feedingstuffs* is also applicable when analysing *Niacin* and *Niacinamide* in *water*, without any sample preparation except a simple dilution.

Based on the above considerations and the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method submitted by the Applicant, based on ion-pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV), to determine *Niacin* and *Niacinamide* in *premixtures*, *feedingstuffs* and *water,* within the concentration range covered by the experimental data. Additionally, the EURL recommends for official control the VDLUFA (Method Bd. III, 13.9.1) using RP-HPLC-UV, to determine *Niacin* and *Niacinamide* in *premixtures*.

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) is not considered necessary.

KEYWORDS
Niacin (Nicotinic acid), Niacinamide (Nicotinamide), nutritional additive, vitamins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 4(1)[1,2] (new use in water) and 10(2)[1-5] (re-evalutation of *feed additives* already authorized under provisions of Council Directive 70/524/EEC) for *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* under the category/functional group 3(a) "nutritional additives"/"vitamins, pro-vitamins and chemically well defined substances having similar effect" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories [1].

According to the Applicants the products are white granular crystals or crystalline powder, containing a minimum of 99[3] % *Niacin* and a minimum of 98.5[3] % *Niacinamide* [2-6]. The *feed additive* is intended to be processed in *premixtures* or added directly into *feedingstuffs* or in *water*. However, the Applicants did not specify any maximum or minimum concentration of *Niacin* and *Niacinamide* in *feedingstuffs* or *water* [2-6], as set in the previous regulations [7].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 these particular dossiers, the methods of analysis submitted in connection with *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, mycotoxins, PAHs and dioxins) are available from the respective European Union Reference Laboratories [8].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the determination of *Niacin* and *Niacinamide* in the *feed additive*, the Applicants propose two internationally recognised European Pharmacopoeia methods [9,10]:

- Monograph 0459 for the determination of Niacin, based on a titration with 0.1 M sodium hydroxide using phenolphthalein as indicator. In this assay 1 mL of 0.1 M sodium hydroxide is equivalent to 12.31 mg of $C_6H_5NO_2$.
- Monograph 0047 for the determination of Niacinamide, based on a titration with 0.1 M perchloric acid, using crystal violet solution as indicator. In this assay 1 mL of 0.1 M perchloric acid is equivalent to 12.21 mg of $C_6H_6N_2O$.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia methods (Ph. Eur. 6th Edition, monographs 0459 and 0047) to determine *Niacin* and *Niacinamide* in the *feed additive*.

The Applicants proposed three methods for the determination of *Niacin* and *Niacinamide* in *premixtures*, *feedingstuffs* and *water*: - the AOAC colorimetric method [11,12]; - the VDLUFA ring-trial validated method for *premixtures* [13], and - the single-laboratory validated and further verified chromatographic method [14].

The AOAC Official colorimetric method [11,12] only provides the standard operating procedure with no further performance characteristics.

The VDLUFA chromatographic Method Bd. III, 13.9.1 [13] is based on ion-pair Reversed Phase High Performance Liquid Chromatograpy (RP-HPLC) coupled to an UV detector measuring at 261 nm. *Premixture* sample is extracted with a mixture of diethylenetriaminepentaacetic acid (titriplex V) and methanol and subjected without further clean-up to HPLC. The target analytes are quantified against external calibration. The method was ring-trial validated with *Niacin* and *Niacinamide* content ranging from 3665 to 15540 mg/kg *premixtures.* The following performance characteristics were reported representing both analytes: (i) a relative standard deviation for *repeatability* (RSD_r) ranging from 3.49 to 7.58 %, and (ii) a relative standard deviation for *reproducibility* (RSD_R) ranging from 4.02 to 15.4 %.

The single-laboratory validated [14,15] and further verified [16] method is based on the samples extraction with diluted phosphoric acid and separation by ion-pair Reversed Phase

[1] FAD-2010-0112; [2] FAD-2010-0263; [3] FAD-2010-0198; [4] FAD-2010-0265; [5] FAD-2010-0307

High Performance Liquid Chromatography (RP-HPLC), using a UV detector at 264 nm. *Niacin* and *Niacinamide* are quantified against external calibration. [14]. The relative standard deviation of *repeatability* (RSD_r) and relative standard deviation of *intermediate precision* (RSD_{ip}) were recalculated by EURL [17] using the experimental data provided by the Applicant [16]. The *recovery* rate (R_{Rec}) and limit of detection/quantification (LOD/LOQ) were reported by the Applicant [15,16]. The performance characteristics are*:*

For *premixtures* samples with contents ranging from 0.4 to 40 g/kg:

- RSD_r ranging from 0.5 to 0.8 %;
- RSD_{ip} ranging from 4.9 to 6.7 %;
- R_{Rec} ranging from 96.9 to 106.1 %, and

For *feedingstuffs* samples with contents ranging from 8 to 800 mg/kg:

- RSD_r ranging from 4.1 to 5.4 %;
- RSD_{ip} ranging from 4.1 to 11.1 %;
- R_{Rec} ranging from 95.7 to 107.0 %, and
- LOD and LOQ of 2 and 8 mg/kg, respectively.

Furthermore the Applicant provided data, upon request of the EURL, proving that the method for *premixtures* and *feedingstuffs* is also applicable for the analysis of *Niacin* and *Niacinamide* in *water*, without any sample preparation except a simple dilution [18].

Based on the above considerations and the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method submitted by the Applicant, based on ion-pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV), to determine *Niacin* and *Niacinamide* in *premixtures*, *feedingstuffs* and *water,* within the concentration range covered by the experimental data. Additionally, the EURL recommends for official control the VDLUFA method, based on ion-pair RP-HLC-UV, to determine *Niacin* and *Niacinamide* in *premixtures*, within the concentration range investigated.

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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 0459) using titration with sodium hydroxide for the determination of *Niacin (Nicotinic acid)* in the *feed additive*;
- the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 0047) using titration with perchloric acid for the determination of *Niacinamide (Nicotinamide)* in the *feed additive*;
- Ion pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV) for the determination of *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* in *premixtures*, as per VDLUFA Bd.III, 13.9.1 method and the single-laboratory validated and further verified method submitted by the Applicant;
- the single-laboratory validated and further verified method submitted by the Applicant, based on ion-pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV) for the determination of *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* in *feedingstuffs* and *water.*

Recommended text for the register entry (analytical method)

For the determination of *Niacin (Nicotinic acid)* in the *feed additive*:

Titration with sodium hydroxide from European Pharmacopoeia
(Ph. Eur. 6th edition, monograph 0459)

For the determination of *Niacinamide (Nicotinamide)* in the *feed additive*:

Titration with perchloric acid from European Pharmacopoeia
(Ph. Eur. 6th edition, monograph 0047)

For the determination of *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* in *premixtures*, *feedingstuffs* and *water*:

Ion pair Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Niacin (Nicotinic acid)* and *Niacinamide (Nicotinamide)* 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 SANCO/D/2 Forw. Appl. 1831/SANCO D2/MGR/eu (2011)303290
[2] *Application, Proposal for Register Entry – Annex A
[3] +Application, Proposal for Register Entry – Annex A
[4] ±Application, Proposal for Register Entry – Annex A
[5] #Application, Proposal for Register Entry – Annex A
[6] ⊗Application, Proposal for Register Entry – Annex A
[7] Official Journal of the European Union, C 50 of 25.2.2004, p. 1, *List of the authorised additives in feedingstuffs (1) published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs*
[8] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
[9] *Technical Dossier, Section II, Annex II 1 01 Pharmacopoeia 6th Ed.: Monograph 0459
[10] *Technical Dossier, Section II, Annex II 1 02 Pharmacopoeia 6th Ed.: Monograph 0047
[11] ±Technical dossier, Section II – Annex 2.6.2.01. AOAC Official Method 961.14
[12] ⊗Technical dossier, Section II – Annex II.5 AOAC Official Method 961.16
[13] +Technical dossier, Section II – Annex 2.6.01 Method of analysis VDLUFA 13.9.1.
[14] *Technical Dossier, Section II, Annex II 6 04 Niacin & Niacinamide
[15] *Technical Dossier, Section II, Annex II 6 05 Validation NS-NSA premix-feed
[16] *Technical Dossier, Section II, Annex II 6 06 Verification NS-NSA premix-feed
[17] *Supplementary information – Precision data as recalculated by the EURL
[18] *Supplementary information provided by the Applicant upon request EURL

* Refers to Dossier No. FAD-2010-0112
+ Refers to Dossier No. FAD-2010-0263
± Refers to Dossier No. FAD-2010-0198
Refers to Dossier No. FAD-2010-0265
⊗ Refers to Dossier No. FAD-2010-0307

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Reference Laboratory for Feed Additives, IRMM, 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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)