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EURL 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

Guanidinoacetic acid

(FAD-2011-0043 ; CRL/110016)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2011-0043** **CRL/110016**
Feed Additive:	**Guanidinoacetic acid**
Active Substance(s):	**Guanidinoacetic acid**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Date:	**23/04/2013**
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Date:	**26/04/2013**

EXECUTIVE SUMMARY

Guanidinoacetic acid (GAA) is already authorised as feed additive for chickens for fattening. In the current application authorisation is sought under Article 4(1) for *guanidinoacetic acid* under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for *all animal species*.

For the determination of *GAA* in the *feed additive* and *feedingstuffs* the Applicant proposed a single laboratory validated and further verified method based on Ion Chromatography coupled to UltraViolet detection (IC-UV). The performance characteristics recalculated by EURL, based on the experimental data provided by the Applicant, are :

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.11 to 4.2 %;
- a *recovery* rate (R_{Rec}) ranging from 96.4 to 99.1 %; and
- a limit of quantification (LOQ) in the *feedingstuffs* of 50 mg/kg.

For the determination of *GAA* in *premixtures* the Applicant proposed a single laboratory validated method based on High Performance Liquid Chromatography coupled to UltraViolet (HPLC-UV), which is very similar to the method applied for the feed additive and feedingstuffs. The Applicant reported RSD_r values ranging from 0.6 to 1.4% and R_{Rec} values ranging from 96 to 108%.

Based on the performance characteristics presented, the EURL recommends for official control (1) the single laboratory validated and further verified method based on ion chromatography coupled to ultraviolet detection to determine *guanidinoacetic acid* in the *feed additive* and *feedingstuffs* and (2) the single laboratory validated to determine *guanidinoacetic acid* in the *premixture.*

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
Guanidinoacetic acid (GAA), nutritional additives, amino acids, their salts and analogues, all animal species and categories.

1. BACKGROUND

Guanidinoacetic acid (GAA) is already authorised as feed additive for chickens for fattening [1] with a minimum and maximum content in complete feed of 600 mg/kg. In the current application authorisation is sought under Article 4(1) (authorisation of a new use of a feed additive already authorised) for *GAA* under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for *all animal species* [2,3].

According to the Applicant, *guanidinoacetic acid* is a crystalline chemically synthesised product with a minimum purity of 98% [1,4].

GAA is intended to be incorporated in *premixtures* and/or in *feedingstuffs* [5]. While no minimum or maximum *GAA* concentrations in *feedingstuffs* are proposed [2], the Applicant mentions a recommended dosage of 600 mg *GAA* /kg in complete feed [5].

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 (EFSA) for each application or group of applications. For this dossier, the methods of analysis submitted in connection with *guanidinoacetic acid* 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, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [6].

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

For the determination of *GAA* in the *feed additive* and *feedingstuffs* the Applicant proposed a single laboratory validated and further verified method based on Ion Chromatography coupled to UltraViolet detection (IC-UV) [7-9]. According to the Applicant this method is applicable for the determination of *GAA* in the pure substance as well as for feed supplemented with GAA at a level ranging from 100 to 5000 mg /kg [8].

Feed additive samples (50 ± 5 mg) are dissolved in water for extraction in an ultrasonic bath and an aliquot of the solution is injected in the IC system. Similarly, grinded *feedingstuffs* samples (10 ± 1 g), are dissolved in deionised water in an ultrasonic bath; after filtration, an aliquot is injected in the IC system for analysis. The chromatographic system is coupled with UV detector measuring at 200 nm. The *GAA* concentration is determined using external calibration [8]. This single laboratory validated method was further verified by a second independent laboratory [9]. The performance characteristics were recalculated by EURL, based on the experimental data provided by the Applicant [10] for the *feed additive*:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.11 to 0.16 %;
- a *recovery* rate (R_{Rec}) of 97.3 [8],

and for samples containing 470 to 1500 mg GAA / kg *feedingstuffs:*

- RSD_r ranging from 0.5 to 4.2 %
- R_{Rec} ranging from 96.3 to 99.1 %.

Additionally, the Applicant reported a limit of detection (LOD) and quantification (LOQ) of 20 and 50 mg/kg *feedingstuffs*, respectively.

For the determination of *GAA* in *premixtures* the Applicant proposed a single laboratory validated method based on High Performance Liquid Chromatography coupled to UltraViolet (HPLC-UV) [7,11]. The samples are extracted with water using an ultrasonic bath, filtered and injected for analysis without further purification into HPLC system. *GAA* is detected by UV detector at 210 nm and quantified against calibration standards prepared in water. The method was single-laboratory validated for *premixtures* containing 1 to 50% *GAA*, to derive: - RSD_r ranging from 0.64 to 1.4% and - R_{Rec} ranging from 96% to108% [11]. While the performance characteristics provided are satisfactory only a partial verification by a second independent laboratory was provided [9]. Nevertheless, the EURL does not foresee any problem when using the method due to its very simple design.

Based on the performance characteristics presented, the EURL recommends for official control (1) the single laboratory validated and further verified method based on ion

chromatography coupled to ultraviolet detection to determine *guanidinoacetic acid* in the *feed additive* and *feedingstuffs* and (2) the single laboratory validated to determine *guanidinoacetic acid* in the *premixture.*

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 single laboratory validated and further verified method based on ion chromatography coupled to ultraviolet detection to determine *guanidinoacetic acid* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *guanidinoacetic acid* in the *feed additive*, *premixtures* and *feedingstuffs*:

- ion chromatography coupled with ultraviolet detection (IC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *guanidinoacetic acid* 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] Commission Regulation (EC) No 904/2009 of 28 September 2009 concerning the authorisation of guanidinoacetic acid as a feed additive for chickens for fattening, O.J. L 256/28, 29.09.2009

[2] *Application, Proposal of Registry Entry – Annex A

[3] *Application/Ref: SANCO/G1: Forw.Appl.1831/0111-2011

[4] *Technical dossier, Section II: 2.1 Identity of the additive

[5] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[6] 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.

[7] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance

[8] *Technical dossier, Section II: Annex_II_26_GAA AM.pdf.

[9] *Supplementary information, Verification_Report.pdf
[10] *Additional Information – Precision data as recalculated by the EURL
[11] *Technical dossier, Section II: Annex_II_28_GAA_Premix_AM.pdf
* Refers to Dossier No FAD-2011-0043

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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

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- Central Institute for Supervising and Testing in Agriculture (CZ)
- Service Commun des Laboratoires Laboratoire Rennes (FR)