EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

Amendment to CRL report (D08/FSQ/CVH/DG/D (2007) 19586) from 29 August 2007 on the dossier EFSA-Q-2007-050 (CreAmino)

Based on the proposed register entry regarding the dosage (minimum content for the active substance in complete feedingstuffs of 300 mg/kg), the CRL-FA recommend in the above mentioned report a single-laboratory validated method which has been proposed by the applicant. The method is based on ion chromatography (IC) method coupled to ultraviolet-visible (UV-VIS) and was considered suitable for the official control of active substance (guanidinoacetic acid) in complete feedingstuffs. With the present amendment the CRL-FA confirms the validity of this statement also for the modified target content of the active substance in complete feedingstuff, with 600 mg/kg for the minimum and 1200 mg/kg for the maximum concentration.

Geel, 04/03/2009

D08/FSQ/CVH/DG/D (2007) 19586

CRL Evaluation Report on the Analytical Methods submitted in connection with Section II – 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2007-0003** **EFSA-Q-2007-050**
Product name:	**CreAmino™**
Active Substance(s):	**Guanidinoacetic acid**
Rapporteur Laboratory:	**ÚKZÚZ, NRL RO Praha, The Czech Republic**
Report prepared by:	**Jaroslava Petrová (NRL RO Praha)**
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Report approved by:	**Christoph von Holst (CRL-FA)**
Date:	**24.08.2007**

EXECUTIVE SUMMARY

In the current application authorisation is sought for guanidinoacetic acid (GAA) under the category 'nutritional additives', functional group 'amino acids, their salts and analogues', according to the classification system of Annex I of Regulation (EC) No 1831/2003.

The applicant intends to place GAA on the market as a granular product containing at least 96% of GAA and at maximum 1% of starch as granulation agent under the trade name *CreAmino*™. The product is intended being mixed into *feedingstuffs* for broilers and turkeys at a minimum concentration of 300 mg/kg and at a recommended concentration of 600 mg/kg of GAA in complete *feedingstuffs*.

For the determination of the active substance in the *product* and in *feedingstuffs,* the same single-laboratory validated method is proposed, which is based on ion chromatography (IC) method coupled to ultraviolet-visible (UV-VIS) detection operated at a wavelength of 200 nm. Method performance characteristics were determined by conducting replicate analyses of *CreAmino*™ and two types of feed samples containing the active substance at different concentrations. The limit of detection (LOD) and limit of quantification (LOQ) was 20 mg/kg and 55 mg/kg, respectively. The relative standard deviation for repeatability (RSD_r) was 0.1% for the feed additive and varied from 0.77% to 2.86% for the feed samples depending on the concentration of the active substance. The values for the recovery rate were in all cases above 97%. Also the ruggedness of the method was confirmed by analysing feed samples of different composition on different instruments and days. The performance characteristics are considered acceptable and therefore the method is suitable for official control purposes within the frame of the authorisation.

For the determination of the active substance in *premixtures,* the applicant proposed a different method which has also been single-laboratory validated and which is based on high performance liquid chromatography (HPLC) coupled to ultraviolet-visible (UV-VIS) detection operated at a wavelength of 210 nm. The validation of the method was performed on typical premixtures with supplementation levels of the active substance at 1, 5, 25 and 50%, respectively. The obtained values for the recovery rate varied between 96 and 108% and the obtained values for the RSD_r ranged from 0.6 to 1.4%. All methods have also been successfully tested by a second independent analytical laboratory. The performance characteristics are considered acceptable and therefore the method is considered suitable for the intended purpose.

For the determination of GAA in *muscle tissues*, *liver* and *kidney* an IC method is proposed which is based on the same principle as the method for the determination of GAA in the *product* and in the *feedingstuffs*. The method was single-laboratory validated on the target tissues, obtaining acceptable values for the recovery rate and the precision. The limit of

quantification (LOQ) was 0,8 mg/kg in *muscle tissues* and 1,6 mg/kg in *liver* and *kidney*. Since the applicant did not propose Maximum Residue Levels of GAA in the target tissues, the CRL is unable to comment on the suitability of the proposed method for official control purposes.

Further testing or validation is not considered necessary.

KEYWORDS

CreAmino™, Guanidinoacetic acid (GAA), creatine precursor, nutritional feed additive, broilers, turkeys

1. BACKGROUND

Guanidinoacetic acid *(CreAmino™)* is a product for which authorisation is sought as feed additive under the category 'nutritional additives', functional group 'amino acids, their salts and analogues', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active substance is guanidinoacetic acid (GAA). According to the applicant *CreAmino*™ contains at least 96% of the active substance [1].

The intended use (*cf.* EFSA-Q-2007-050) of the current product is to substitute and satisfy the nutritional demand of creatine in vegetarian diets for broilers and turkeys. The minimum dosage proposed by the applicant is 300 mg/kg of GAA in complete *feedingstuffs* [1] and the recommended dosage is 600 mg/kg of GAA in complete *feedingstuffs* [2].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with guanidinoacetic acid (*CreAmino™)* and their suitability to be used for official controls in the frame of authorisation were evaluated.

3. EVALUATION

The numbering system under this point refers to that of Section II of the Annex of Commission Directive 2001/79/EC (2.5 Control methods).

Description of the methods used for the determination of the criteria listed in Section II of the Annex point 2.5.1 of Commission Directive 2001/79/EC

Determination of the purity of the product

CreAmino™ is granulated product with particles in shape of round bowls which contains GAA at a minimum concentration of 96% and water and starch at a maximum concentration

of 1%. The product also contains traces of glycine with a maximum concentration of 1.5%, dicyandiamide with a maximum concentration 0.5% and cyanamide with a maximum concentration of 0.05% [3]. The applicant proposes methods based on ion chromatography coupled to ultraviolet detection (IC-UV) for the determination of glycine [4] and cyanamide [5] and a high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) method for the determination of dicyandiamide [6].

Quantitative analysis of active substance (GAA) in CreAmino™

For the determination of GAA in the product *CreAmino™,* the applicant proposed a method based on IC-UV measuring at 200 nm [7]. 50 mg of the product are dissolved in water and an aliquot of the solution is injected into the IC. The method was single-laboratory validated, obtaining a recovery rate of about 98% and a relative standard deviation for repeatability (RSD_r) of 0.1%. The method has also been successfully tested by a second independent analytical laboratory [10]. Based on acceptable performance characteristics, this method is considered suitable for official control purposes.

Quantitative analysis of active substance (GAA) in feedingstuffs

For the determination of GAA in *feedingstuffs,* the applicant proposed the same IC method [7] as for the determination for the active substance in the *product.* However, the sample amount was adapted to account for the lower GAA concentration in *feedingstuffs* compared to the *product*. 10 g of a feed sample are extracted with water using an ultrasonic bath. The resulting suspension is filtered and an aliquot of the solution is injected into IC system. The target analyte in the *feedingstuffs* is quantified against calibration standards prepared in water.

Method performance characteristics were determined by single-laboratory validation, analysing *feedingstuffs* containing the target at various concentrations ranging from 470 mg/kg to 2000 mg/kg. The obtained values for the recovery rate varied between 97 and 100% and the values for the RSD_r ranged from 0.77 to 2.9%. The limit of detection (LOD) and limit of quantification (LOQ) was 20 mg/kg and 55 mg/kg, respectively. The chromatogram shows a single peak of GAA without interferences, thus confirming sufficient selectivity of the method. Also the ruggedness of the method was demonstrated by analysing feed samples of different composition on different instruments and days. The method has also been successfully tested by a second independent analytical laboratory [10]. Based on acceptable performance characteristics, the method is considered suitable for official controls within the frame of the authorisation.

Quantitative analysis of active substance (GAA) in premixtures

The applicant proposed a HPLC-UV method for the detection of the active substance in *premixtures* [8]. The method protocol foresees that samples are extracted with water using an ultrasonic bath, filtered and injected for analysis into HPLC system. The active substance is detected by UV detector at 210 nm. The target analyte in the *premixtures* is quantified against

calibration standards prepared in water. The method has been single-laboratory validated by analysing *premixtures* in triplicates at various levels of the target analytes ranging from 10 to 500 g/kg *premixtures*. The obtained values for the RSD_r varied from 0.64 to 1.4% and the obtained values for the recovery rate varied from 96% to108%. Also the ruggedness of the method was demonstrated by analysing feed samples of different composition on different instruments and days. The method has also been successfully tested by a second independent analytical laboratory [10].

Based on acceptable performance characteristics the method is considered suitable for the intended purpose.

Description of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products listed in Section II of the Annex point 2.5.3 of Commission Directive 2001/79/EC

The applicant proposed a HPLC-UV method for the determination of GAA and creatine and an IC method for the determination of creatinine in *muscle tissues*, *liver* and *kidney* of broilers and turkeys [9]. The target analytes are extracted from the *muscle tissues* into deionised water using an ultrasonic bath. In case of *liver* and *kidney*, a methanol water mixture is used as extracting agent. The resulting solution is centrifuged, filtered and injected for analysis. The methods were single-laboratory validated obtaining the following results. The limit of quantification (LOQ) for GAA was 0.8 mg/kg for *muscles* and 1.6 mg/kg for *liver* and *kidney*. For creatine, the LOQ was 66 mg/kg in *muscles* and 26 mg/kg in *liver* and *kidney*. For creatinine, the LOQ was 10 mg/kg in *muscles* and 4 mg/kg in *liver* and *kidney*. The obtained values for the RSD_r for the three target analytes were below 6% and the values for the recovery rate ranged from 84 to 104%. These values are considered acceptable. Since the applicant did not propose Maximum Residue Levels for GAA or the other substances in the target tissues, the CRL is unable to comment on the suitability of the proposed method for official control purposes.

4. CONCLUSIONS AND RECOMMENDATIONS

For the quantification of the guanidinoacetic acid in various matrices, the applicant proposes single laboratory-validated chromatographic methods based on well known principles and demonstrating acceptable performance characteristics. They are considered suitable for official control purposes in the frame of authorisation.

Further testing or validation is not considered necessary.

Recommended text for the register entry, fourth column (composition, chemical formula, description, analytical method)

Ion chromatography (IC) with UV detection (λ = 200 nm).

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

According to the requirements of Regulation (EC) No 1831/2003, reference samples of *CreAmino*™ have been sent to the Community Reference Laboratory for Feed Additives.
The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] FAD-2007-0003_GAA(CreAmino)_Annex_III.

[2] Section II. Chapter 2.2.2 Classification by category and functional group.

[3] Section II. Chapter 2.3.7 Purity.

[4] Annex of section II: AnnexII_15. Determination of the glycine content of Guanidinoacetic acid (GAA).

[5] Annex of section II: AnnexII_17. Determination of the cyanamide content of Guanidinoacetic acid (GAA).

[6] Annex of section II: AnnexII_16. Determination of the dicyandiamide content of Guanidinoacetic acid (GAA).

[7] Annex of section II: AnnexII_20. Determination of Guanidinoacetic acid in the pure substance, CreAmino™, and feed by ion chromatography.

[8] Section II. Annex II, No. 21 Determination of GAA in pre-mixtures by HPLC.

[9] Section II. Annex II, No. 22 Determination of Guanidinoacetic acid, Creatine, and Creatinine in muscle tissue, liver, and kidney of broiler and turkey by ion chromatography and HPLC.

[10] Supplementary information received upon request of the CRL on 23 July 2007.

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was ÚKZÚZ, NRL RO Praha, the Czech Republic.