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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

Disodium 5'-guanylate (GMP) produced by fermentation with *Corynebacterium ammoniagenes KCCM 10530 and Escherichia coli K12 KFCC 11067*

(FAD-2019-0085; CRL/190047)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2019-0085 - CRL/190047**
Name of Product:	***Disodium 5'-guanylate (GMP)*** **produced by fermentation with** ***Corynebacterium ammoniagenes KCCM 10530*** **and** ***Escherichia coli K12 KFCC 11067***
Active Agent (s):	***Disodium 5'-guanylate***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **04/09/2020**
Report approved by: Date:	**Christoph von Holst** **04/09/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *disodium 5'-guanylate (GMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM 10530* and *Escherichia coli K12 KFCC 11067* under the category/functional group 2(b) 'Sensory additives' / 'flavouring compounds' 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 all animal species and categories.

The *feed additive* consists of a minimum of 97 % (w/w) of *disodium 5'-guanylate (GMP)* as an active substance, which is produced by fermentation with the strains of *Corynebacterium ammoniagenes KCCM 10530* and *Escherichia coli K12 KFCC 11067*.

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and in *water* for drinking with proposed maximum levels of 50 mg *GMP*/kg *feedingstuffs*.

For the identification of *GMP* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph "disodium 5'-guanylate", which is comprised of various fit-for-purpose tests based on measuring solubility, absorbance signals in spectrophotometric measurements, presence of sodium, ribose and organic phosphate.

The EURL recommends for official control the above mentioned FAO JECFA monograph for the identification of *GMP* in the *feed additive*.

For the determination of *GMP* in the *feed additive*, *flavouring premixtures* and *water* the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to UV detection (HPLC-UV).

The following performance characteristics were reported in frame of the validation study for the determination of *GMP* content in the aqueous solution of the *premixtures* ranging from 7.8 to 182 mg/L: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.3 to 0.6 %; and a *recovery* rate (R_{rec}) of 100 %. The lowest tested level of *GMP* in water (7.8 mg/L) was assigned by the Applicant as a limit of quantification (LOQ). In addition, the Applicant demonstrated proper selectivity of the method by submitting the chromatograms of *flavouring premixtures*, containing *GMP* and disodium 5'-inosinate.

Based on the available performance characteristics, the EURL recommends for official control the single-laboratory validated HPLC-UV method submitted by the Applicant for the determination of *disodium 5'-guanylate (GMP)* in the *feed additive, flavouring premixtures* and *water*.

The Applicant did not provide any method for the determination of *GMP* in *feedingstuffs*, therefore the EURL could not evaluate nor recommend any method for official control to determine *GMP* in *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

Disodium 5'-guanylate (GMP), sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *disodium 5'-guanylate (GMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM 10530* and *Escherichia coli K12 KFCC 11067* under the category/functional group 2(b) 'Sensory additives' / 'flavouring compounds' according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for the use of the *feed additive* for all animal species and categories [2]. *Disodium 5'-guanylate (GMP)*, produced by RNA hydrolysis, is already authorised as a sensory additive, flavouring compound under Commission Implementing Regulation (EU) 2018/238 [3].

The *feed additive* consists of a minimum of 97 % (w/w) of *disodium 5'-guanylate (GMP)* as an active substance which is produced by fermentation with the strains of *Corynebacterium ammoniagenes KCCM 10530* and *Escherichia coli K12 KFCC 11067* [4]. The production strains are deposited in the Korean Culture Centre of Microorganisms (KCCM) and Korean Federation of Culture Collections (KFCC) under the accession numbers KCCM 10530 and KFCC 11067, respectively [4].

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and in *water* for drinking with recommended maximum levels of 50 mg *GMP*/kg *feedingstuffs* [5], which is in align with provisions of the above mentioned Commission Implementing Regulation (EU) 2018/238 [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 *disodium 5'-guanylate (GMP)* 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 determination of *GMP* in the *feed additive*, *flavouring premixtures* and *water* the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to UV detection (HPLC-UV) [6].

The sample (50 mg) is dissolved in a volume of 100 ml of water. An aliquot of the aqueous solution is diluted 5 times before the chromatographic analysis. The analyte is detected by UV at 254 nm wavelength. The quantification of *GMP* is performed by using an external calibration with *GMP* as a standard substance [6].

The following performance characteristics were reported in frame of the validation study for the determination of the *GMP* content in the aqueous solution of *flavouring premixtures* ranging from 7.8 to 182 mg/L: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.3 to 0.6 % and a *recovery* rate (R_{rec}) of 100 % [6]. The lowest tested level of *GMP* in water (7.8 mg/L) was assigned by the Applicant as limit of quantification (LOQ). In addition, the Applicant demonstrated a proper selectivity of the method by submitting the chromatograms of *flavouring premixtures*, containing *GMP* and disodium 5'-inosinate [6].

The above mentioned HPLC-UV method is very similar to another HPLC-UV method specified in Commission Implementing Regulation (EU) 2018/238 [3] for official control of *GMP*. Since this method has been applied in a different laboratory compared to the laboratory of the current application, the EURL concluded that sufficient transferability of the HPLC-UV method has been demonstrated.

Based on the available performance characteristics, the EURL recommends for official control the single-laboratory validated HPLC-UV method submitted by the Applicant for the

determination of *disodium 5'-guanylate (GMP)* in the *feed additive, flavouring premixtures* and *water*.

The Applicant did not provide any method for the determination of *GMP* in *feedingstuffs*, therefore the EURL could not evaluate nor recommend any method for official control to determine *GMP* in *feedingstuffs*.

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 *GMP* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph "disodium 5'-guanylate" [7]. The monograph specifies fit-for-purpose identification tests based on solubility, absorbance signals in spectrophotometric measurements, presence of sodium, ribose and organic phosphate [7].

The EURL recommends for official control the above mentioned FAO JECFA monograph for the identification of *GMP* in the *feed additive*.

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 (i) the FAO JECFA monograph "disodium 5'-guanylate" for the identification of *disodium 5'-guanylate (GMP)* in the *feed additive*; and (ii) the single-laboratory validated HPLC-UV method submitted by the Applicant for official control for the determination of *disodium 5'-guanylate (GMP)* in the *feed additive, flavouring premixtures* and *water*.

The Applicant did not provide any method for the determination of *GMP* in *feedingstuffs*, therefore the EURL could not evaluate nor recommend any method for official control to determine *GMP* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification of *disodium 5'-guanylate (GMP)* in the *feed additive*:

– FAO JECFA monograph "disodium 5'-guanylate"

For the determination of *disodium 5'-guanylate (GMP)* in the *feed additive*, *flavouring premixtures* and *water*:

– High performance liquid chromatography coupled to UV detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *disodium 5'-guanylate (GMP)* produced by fermentation with *Corynebacterium ammoniagenes KCCM80161* 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. 1831-0025-2020

[2] *Application, Application Form – Annex 1 – Submission No. 1575380641321-2484

[3] Commission Implementing Regulation (EU) 2018/238 of 15 February 2018 concerning the authorisation of disodium 5′-ribonucleotides, disodium 5′-guanylate and disodium 5′-inosinate as feed additives for all animal species, OJ L 53, 23.2.2018

[4] *Technical dossier, Section II, II.1. Identity of the additive

[5] *Technical dossier, Section II, II.5. Condition of use of the additive

[6] *Supplementary information: CJE_IMP_HPLC_190830

[7] FAO JECFA Combined Compendium of Food Additive Specifications, "Disodium 5'-guanylate", Monograph No. 1 (2006) http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-165.pdf (last visited 15/07/2020)

*Refers to Dossier no: FAD-2019-0085

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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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
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