
Betaine anhydrous
(FAD-2014-0031; CRL/140003)

Dossier related to: FAD-2014-0031 - CRL/140003

Name of Additive: Betaine anhydrous

Active Agent (s): Betaine

Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium

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EXECUTIVE SUMMARY

In the current application authorisation is sought for Betaine anhydrous, under article 4, for the category/functional group 3(a) ‘nutritional additives’/‘vitamins, pro-vitamins and chemically well defined substances having similar effect’, according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species and categories. The feed additive is produced from the genetically modified sugar beet K7-1 (KM-ØØØH71-4). According to the Applicant, Betaine anhydrous is to be marketed in two forms: as a brown liquid with a minimum content of 32 % Betaine anhydrous; or a free flowing powder with a minimum purity of 91 %. The liquid form is intended to be sprayed directly on feedingstuffs only, while the solid form is to be added into feedingstuffs directly or through premixtures. The Applicant proposed minimum contents of Betaine anhydrous in feedingstuffs of 0.1 g/kg for poultry and pigs and 1 g/kg for fish, while for other animal species and categories minimum daily doses "per head" were proposed ranging from 2 to 10 g per head/day. No maximum concentrations in feedingstuffs were specified.

For the quantification of Betaine anhydrous in the feed additive, premixtures and feedingstuffs the Applicant submitted an official ICUMSA's method (International Commission for Uniform Methods of Sugar Analysis) based on High Performance Liquid Chromatography with Refractive Index detector (HPLC-RI). This method is intended for the quantification of betaine in beet molasses. However, no experimental data were provided by the Applicant to demonstrate the applicability of the ICUMSA method for the quantification of Betaine anhydrous in feed additive, premixtures and feedingstuffs.

Nevertheless, the EURL previously evaluated several analytical methods in the frame of the "Betaine anhydrous and related compounds" dossiers, including a Betaine anhydrous produced from the same genetically modified sugar beet K7-1 (KM-ØØØH71-4). This feed additive is presently authorised by Commission Implementing Regulation (EU) 2015/1060 of 2 July 2015. The conclusions of the EURL included therein are considered valid for the purpose of the current application.

"For the quantification of betaine in feed additive, premixtures and feedingstuffs, Applicant submitted a single-laboratory validated and further verified method based on HPLC-RI. The following performance characteristics were reported:

- a precision (repeatability and intermediate precision) ranging from 0.1 to 0.8 % for the feed additive, or from 4.3 and 8.6 % for premixtures and feedingstuffs;
- a recovery rate (R_{Rec}) ranging from 94 to 107 % for premixtures and feedingstuffs; and a limit of quantification (LOQ) of 70 mg/kg feedingstuffs."

(extract from the EURL report on Betaine, 2011)
Based on the performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method, using HPLC-RI, to quantify Betaine anhydrous (expressed as total betaine) in the feed additives, premixtures, feedingstuffs within the concentration range covered by the experimental data.

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

Betaine anhydrous, nutritional additives, vitamins, pro-vitamins and chemically well defined substances having similar effect, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought for Betaine anhydrous, under article 4 (authorisation of a new product), for the category/functional group 3(a) ‘nutritional additives’/vitamins, pro-vitamins and chemically well defined substances having similar effect’, according to Annex I of Regulation (EC) No 1831/2003 [1,2]. Authorisation is sought for all animal species and categories [1,3].

The feed additive is produced from the genetically modified sugar beet H7-1 (KM-ØØØH71-4) [4,5]. According to the Applicant, Betaine anhydrous will be marketed as preparation in two forms [2,6-8]:

- as a brown aqueous liquid with a minimum content of 32 % (Vistabet Liquid); and
- as a free flowing light to yellow-brown crystalline powder with a minimum purity of 91 % (cf. Vistabet 91, Vistabet 96, and Vistabet 97).

The liquid form is intended to be sprayed directly on feedingstuffs only, while the solid form is to be added into feedingstuffs directly or through premixtures [3]. The Applicant proposed minimum contents of Betaine anhydrous in feedingstuffs of 0.1 g/kg for poultry and pigs and 1 g/kg for fish, while for other animal species and categories minimum daily doses "per head" were proposed ranging from 2 to 10 g per head/day. No maximum concentrations in feedingstuffs were specified [2,3].

Note: The EURL previously evaluated several analytical methods in the frame of the "Betaine anhydrous and related compounds" dossiers (cf. FAD-2010-0174; FAD-2010-0216 and FAD-2010-0253) [9]. The feed additive "Betaine anhydrous produced from genetically modified sugar beet K7-1 (KM-ØØØH71-4)" was recently authorised by Commission Implementing Regulation (EU) 2015/1060 of 2 July 2015 with the identification number "3a921" [10].
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 this particular dossier, the methods of analysis submitted in connection with Betaine anhydrous 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 [11].

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

For the quantification of Betaine anhydrous in the feed additive, premixtures and feedingstuffs the Applicant submitted the method developed by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) for the determination of betaine in beet molasses and to "all beet sugar processing products after the evaporation step" [12,13]. The method is based on High Performance Liquid Chromatography with Refractive Index detector (HPLC-RI) [13]. However, no experimental data were provided by the Applicant to demonstrate the applicability of the ICUMSA method for the quantification of Betaine anhydrous in feed additive, premixtures and feedingstuffs.

Nevertheless, the EURL previously evaluated several analytical methods in the frame of the SANCO group "Betaine anhydrous and related compounds" [9], including a Betaine anhydrous produced from the same genetically modified sugar beet K7-1 (KM-ØØØH71-4). This feed additive is presently authorised by Commission Implementing Regulation (EU) 2015/1060 [10]. The conclusions of the EURL included therein are considered valid for the purpose of the current application.
For the quantification of betaine anhydrous in the feed additive, premixtures and feedingstuffs, the Applicant submitted a single laboratory validated method and further verified by a second independent laboratory, based on HPLC-RI.

The feed additive samples (700 mg – 1 g) are diluted with water, filtered through 0.2 \( \mu \text{m} \) membrane filter to HPLC vials and analysis is performed using sodium based cation exchange column. For the determination of betaine in premixtures and feedingstuffs, the samples (1 g for premixtures and 10 g for feedingstuffs) are first diluted in water, ultrasonicated for 30 minutes and mixed with magnetic stirrer for 30 minutes. The extracted solution is then centrifuged for additional 10 minutes at 3500 rpm. Proteins and lipids are then removed by C18 solid phase extraction and eluate is filtered through 0.2 \( \mu \text{m} \) membrane filter to HPLC vials. The analysis is performed using calcium based cation exchange column. A chromatogram of the separated components is obtained by differential refractometry of the eluent. Calibration is performed using an external standard.

The following performance characteristics for the quantification of total betaine are derived from validation and verification studies:

- for the feed additive: a precision (repeatability and intermediate precision) ranging from 0.1 to 0.8 \%; and a recovery rate (R\text{rec}) ranging from 94 to 101 \%;
- for premixtures (samples with contents ranging from 6 to 1000 mg/g): a precision ranging from 4.3 to 6.9 \%; and R\text{rec} ranging from 94 to 102 \%;
- for feedingstuffs (samples with contents ranging from 0.7 to 100 mg/g): a precision ranging from 4.4 to 8.6 \%; R\text{rec} ranging from 94 to 107 \%; and a limit of quantification (LOQ) of 70 mg/kg feedingstuffs.

Based on the performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method, using HPLC-RI, to quantify Betaine anhydrous (expressed as total betaine) in the feed additives, premixtures, feedingstuffs within the concentration range covered by the experimental data.

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 High Performance Liquid Chromatography with Refractive Index detector (HPLC-RI) to quantify Betaine anhydrous (expressed as total betaine) in the feed additives, premixtures and feedingstuffs.

**Recommended text for the register entry (analytical method)**

For the quantification of Betaine anhydrous (expressed as total betaine) in the feed additive, premixtures and feedingstuffs:

– High Performance Liquid Chromatography method with Refractive Index detector (HPLC-RI)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Betaine anhydrous 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

[2] *Application, Proposal for Register Entry - Annex A
[3] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[4] *Technical dossier, Section II: 2.1.1 Name of the additive
[6] *Technical dossier, Section II: 2.1.5 Physical state of each form of the product
[8] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
[12] *Technical dossier, Section II: 2.6 Methods of analysis and reference samples
*Refers to Dossier no: FAD-2014-0031
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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)