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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

Preparation of hydroxy analogue of Methionine (HMTBa) and calcium salt of HMTBa

(FAD-2016-0030; CRL/160014)

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-2016-0030 - CRL/160014**
Name of Product:	**Preparation of hydroxy analogue of Methionine (HMTBa) and calcium salt of HMTBa**
Active Agent (s):	**Hydroxy analogue of Methionine (HMTBa)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC, Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (EURL-FA)** **11/11/2016**
Report approved by: Date:	**Christoph von Holst** **11/11/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for the *preparation of hydroxy analogue of Methionine (HMTBa) and calcium salt of HMTBa* under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species. The preparation (*feed additive*) is a light beige powder, consisting of a minimum of 88% of *HMTBa* and minimum of 8% of *calcium* in the *feed additive*. The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures* with no minimum or maximum dose proposed. However, the Applicant suggested typical inclusion levels ranging from 0.2 to 4 g of the *feed additive* /kg complete *feedingstuffs*.

The Applicant submitted two single-laboratory validated and further verified methods based on: (i) a potentiometric titration after a redox reaction for the quantification of *HMTBa* in the *feed additive*; and (ii) Reversed Phase High Performance Liquid Chromatography coupled with UV detection at 214 nm (RP-HPLC-UV) for the quantification of *HMTBa* in *premixtures* and *feedingstuffs* containing the *preparation*.

The following performance characteristics were reported for the above mentioned titrimetric method: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.1 to 0.2 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) of 0.2 %; and - a *recovery* rate (R_{rec}) ranging from 100 to 101 %.

The performance characteristics reported for the above mentioned RP-HPLC-UV method for the mass fractions of HMTBa in *premixtures* and *feedingstuffs* ranging from 0.16 to 6.6 g/kg, were as follows: - a RSD_r ranging from 0.4 to 2.7 %; - a RSD_{ip} ranging from 1.6 to 2.7 %; and - a R_{rec} ranging from 89 to 99 %.

Furthermore, both methods were previously recommended by the EURL in the frame of report FAD-2010-0023.

Based on the performance characteristics presented, the EURL recommends for official control these two methods for the quantification of *HMTBa* in the *feed additive*, *premixtures* and/or *feedingstuffs*.

The Applicant did not submit any method for the quantification of *calcium* in the *feed additive*, however the EURL identified three suitable CEN methods: EN ISO 6869:2000, based on Atomic Absorption Spectrometry (AAS), EN 15510:2007 or EN 15621:2012, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after dissolution of 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) is not considered necessary.

KEYWORDS

Hydroxy analogue of Methionine (HMTBa), Preparation of HMTBa and calcium salt of HMTBa, nutritional additives, amino acids, their salts and analogues, all animal species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new *feed additive*) for the *preparation of hydroxy analogue of Methionine (HMTBa) and calcium salt of HMTBa* under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues' according to the classification system of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species [1,2].

The *preparation (feed additive*) is a light beige powder, consisting of minimum of 88% of *HMTBa* [2,3] and minimum of 8% of *calcium* [3].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures* with no minimum or maximum dose proposed [2]. However, the Applicant suggested typical inclusion levels ranging from 0.2 to 4 g of the *feed additive* /kg complete *feedingstuffs* [3].

Note: The methods for the quantification of HMTBa in feed additives, premixtures and feedingstuffs were previously evaluated in the frame of the dossier FAD-2010-0023 [4].

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 *Preparation of HMTBa and calcium salt of HMTBa* 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 [5].

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

For the quantification of *HMTBa* in the *feed additive* (*preparation of HMTBa and calcium salt of HMTBa*), the Applicant submitted a single-laboratory validated and further verified method based on potentiometric titration after a redox reaction. Bromate reacts with bromide under acidic condition to produce bromine, which oxidizes sulfur present in the *feed additive* [6]. The Applicant applied this method for the quantification of *HMTBa* in the *preparation of HMTBa and calcium salt of HMTBa*, and presented performance characteristics [7] similar to those reported in the frame of the dossier FAD-2010-0023 [4] (Table 1), thus confirming the suitability of the above mentioned titrimetric method.

For the quantification of *HMTBa* in *premixtures* and *feedingstuffs* containing the *preparation*, the Applicant submitted another single-laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled with UV detection at 214 nm (RP-HPLC-UV) [8].

A dilution of *premixtures* with corn meal is performed [9]. *HMTBa* is extracted from the diluted premixture and feed samples using a water/methanol solution. After alkaline hydrolysis with potassium hydroxide, the reaction mixture is acidified with ortho-phosphoric acid, centrifuged and the aliquot of the supernatant after filtration is injected into a gradient RP-HPLC system with UV detection at 214 nm [8].

The Applicant applied this method for the quantification of *HMTBa* in *premixtures* and *feedinsgtuffs* containing the *preparation* and presented performance characteristics [9,10] similar to those reported in the frame of the dossier FAD-2010-0023 [4] (Table 1), thus confirming the suitability of this method.

Based on the performance characteristics available, the EURL recommends for official control the titrimetric method to quantify *HMTBa* in the *feed additive* and the RP-HPLC-UV method to quantify *HMTBa* in *premixtures* and *feedingstuffs* containing the *preparation of HMTBa and calcium salt of HMTBa*.

Table 1: Method performance characteristics obtained in the frame of single-laboratory validation and verification studies for the quantification of *HMTBa* in the *feed additives*, *premixtures* and *feedingstuffs*.

	Feed Additive		Premixtures		Feedingstuffs	
FAD no.	**2010-0023**	**2016-0030**	**2010-0023**	**2016-0030**	**2010-0023**	**2016-0030**
Content, g/kg	-	-	5 – 80	6.22 – 6.60	0.14 – 4.0	0.16 – 0.35
RSD_r, %	0.1 – 0.8	0.1 – 0.2	1.5 – 1.9	1.2 – 2.7	0.7 – 6.2	0.4 – 2.6
RSD_{ip}, %	0.2 – 1.1	0.2	1.7 – 3.2	1.7 – 2.7	2.1 – 8.3	1.6 – 2.6
R_{rec}, %	100 – 101	100 – 101	96	89 – 93	87 – 108	92 – 99
Reference	4	7	4	9	4	10

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{rec} - a *recovery* rate.

The Applicant did not submit any method for the quantification of *calcium* in the *feed additive*, however the EURL identified three CEN methods fit for the purpose: EN ISO 6869:2000, based on Atomic Absorption Spectrometry (AAS) [11]; EN 15510:2007 [12] or EN 15621:2012 [13], based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after dissolution of the *feed additive*. The relative standard deviations for *repeatability* and *reproducibility* ranging from 4 to 11 % were reported.

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 two single-laboratory validated and further verified methods, based on: (i) titrimetry for the quantification of *HMTBa* in the *preparation of HMTBa and calcium salt of HMTBa*; and (ii) Reversed Phase High Performance Liquid Chromatography coupled with UV detection (RP-HPLC-UV) for the quantification of *HMTBa* in *premixtures* and *feedingstuffs* containing the *preparation*.

Recommended text for the register entry (analytical method)

For the quantification of *HMTBa* in the *feed additive*:

- Titrimetry, potentiometric titration after a redox reaction

For the quantification of *HMTBa* in *premixtures* and *feedingstuffs*:

- Reversed Phase High Performance Liquid Chromatography coupled with UV detection (RP-HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *preparation of hydroxy analogue of Methionine (HMTBa) and calcium salt of HMTBa* 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: Forw. Appl. 1831/0028-2016

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II

[4] #EURL Evaluation Report – JRC.D.5/FSQ/CvH/SB/ag/Ares(2012)240861

[5] 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

[6] *Technical dossier, Section II – Annex_II_6_1

[7] *Technical dossier, Section II– Annex_II_6_3

[8] *Technical dossier, Section II– Annex_II_6_4

[9] *Technical dossier, Section II– Annex_II_6_5

[10] *Technical dossier, Section II– Annex_II_6_6

[11] EN ISO 6869:2000 – *Animal feedingstuffs - Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc - Method using atomic absorption spectrometry*

[12] EN 15510:2007 – *Animal feedingstuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*

[13] EN 15621:2012 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*

*Refers to Dossier no: FAD-2016-0030;

#Refers to Dossier no: FAD-2010-0023

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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- Laboratorio Arbitral Agroalimentario. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid (ES)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)