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European Union Reference Laboratory for Feed Additives

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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

Dossier related to:	**FAD-2011-0044 - CRL/110017**
Name of Feed Additive:	**Seleno hydroxy analogue of methionine**
Active Agent (s):	**Hydroxy methyl seleno butanoic acid (HMSeBA)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Piotr Robouch (EURL-FA)**
Report checked by: Date:	**Dijana Mitić (EURL-FA)** **13/07/2012**
Report approved by: Date:	**Christoph von Holst** **13/07/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Hydroxy-Methyl-Seleno-Butanoic-Acid* (HMSeBA), under the category/functional group 3(b) 'nutritional additives'/'compounds of trace elements' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *HMSeBA* for all animal species and categories.

The *feed additive* is a preparation consisting of a minimum of 5 % R,S-2-hydroxyl-4 methylseleno butanoic acid ($C_5H_{10}O_3Se$) and a maximum of 95 % colloidal silica carrier. The organo seleno compounds consist of 99 % HMSeBA monomer and 1% dimers; thus corresponding to 40 % *total selenium* in HMSeBA or to 2 % *total selenium* in the *feed additive*. The preparation, marketed as a liquid or a powder forms, is intended to be incorporated into *premixtures*, compound *feedingstuffs* or *water* to obtain a maximum *total selenium* dosage of 0.25 mg/L *water* or 0.5 mg/kg *feedingstuffs,* thus complying with legal requirements; no minimum dose was proposed by the Applicant.

For the determination of HMSeBA in the active substance (HMSeBA *per se*) and in the *feed additive* (preparation) the Applicant submitted a validated and further verified method, based on High Performance Liquid Chromatography coupled to UV detection at 220 nm (HPLC-UV). The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.21 to 1.3 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.83 to 1.3 %; and – a recovery rate (R_{Rec}) ranging from 89 to 102 %. Based on the experimental evidence provided the EURL recommends for official control the validated and further verified gradient HPCL-UV method for the determination of HMSeBA in the *active substance* and in the *feed additive*.

For the determination of *total selenium* in the *feed additive* and in the *active substance* the Applicant submitted the validated and further verified method developed by the UT2A laboratory - already evaluated and recommended by the EURL - based on microwave digestion using nitric acid and hydrogen peroxide (HNO_3/H_2O_2) followed by inductively coupled plasma mass spectrometry (ICP-MS), for which the following performance characteristics were reported: - R_{rec} ranging from 94 to 95 %; and - RSD_{ip} ranging from 1.5 to 2.5 %. However, an alternative method has already been evaluated and recommended by the EURL, based on inductively coupled plasma atomic emission spectrometry (ICP-AES) for which the following performance characteristics were reported: - R_{rec} ranging from 99 to 105 %; - RSD_r ranging from 1.1 to 2.7 %; and - RSD_{ip} ranging from 1.5 to 2.5 %.

For the determination of *total selenium* in *premixtures* and *feedingstuffs* the Applicant submitted the CEN standard method EN 16159:2012 – already recommended in previous

EURL reports - based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO_3/H_2O_2. The following performance characteristics are reported for feed samples: - RSD_r ranging from 3.4 to 10 %; - a relative standard deviation for *reproducibility* (RSD_R) ranging from 15 to 23 %; and - a limit of quantification of 0.125 mg/kg, clearly below the maximum legal limit of 0.5 mg Se /kg feed. For the determination of *total selenium* in *premixtures*, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the abovementioned HGAAS method.

The Applicant did not submit any methods for the determination of *total selenium* in *water* However, the EURL already recommended the method approved by the National Institute for Occupational Safety and Health (NIOSH) and described in their Manual of Analytical Methods (NMAM), based on ICP-AES, for which a limit of detection (LOD) of 0.02 mg/L is 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.

KEYWORDS
Seleno hydroxy analogue of methionine, Hydroxy methyl seleno butanoic acid (HMSeBA), *total selenium*, nutritional additives, all species, trace elements

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *Hydroxy-Methyl-Seleno-Butanoic-Acid* (HMSeBA), under the category/functional group 3(b) 'nutritional additives'/'compounds of trace elements' according to the classification system of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of *HMSeBA* for all animal species and categories [1].

The *feed additive* (Selisseo® 2 % Se - former Selest® 5 % HMSeBA) is a preparation consisting of a minimum of 5 % R,S-2-hydroxyl-4 methylseleno butanoic acid ($C_5H_{10}O_3Se$) and other Se-based compounds and a maximum of 95 % amorphous precipitated silica carrier. The organo seleno compounds consist of 99 % HMSeBA monomer and 1% dimers [2a]. This would correspond to 40 % *total selenium* in HMSeBA [M_{Se}/M_{HMSeBA} = 78.96/197.09] or to 2 % *total selenium* in the *feed additive* [5% of 40%].

HMSeBA results of a chemical reaction between selenium, methyl-lithium and hydroxybutyrolactone. When mixed with the silica carrier, the preparation is a white odourless powder. The liquid form is an odourless pale yellow solution. The preparation is

intended to be incorporated into *premixtures*, compound *feedingstuffs* or *water* to obtain a maximum *total selenium* dosage of 0.25 mg/L *water* [3] or 0.5 mg/kg *feedingstuffs* [3] thus complying with legal requirements; no minimum dose was proposed by the Applicant.

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. The methods of analysis submitted in connection with *Hydroxy-Methyl-Seleno-Butanoic-Acid* (HMSeBA) and their suitability to be used for official controls in the frame of the authorisation were evaluated for the two dossiers of concern.

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, salmonella, mycotoxins and dioxins) are available from the respective European Union Reference Laboratories [4].

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

Hydroxy-Methyl-Seleno-Butanoic-Acid (HMSeBA)

For the determination of HMSeBA in the active substance (HMSeBA *per se*) and in the *feed additive* (preparation) the Applicant developed, validated and further verified two methods, based on High Performance Liquid Chromatography coupled to UV detection at 220 nm (HPLC-UV), using external calibration.

At first the Applicant developed an HPLC method with isocratic elution for the determination of the HMSeBA monomer [5]. 50 mg of the HMSeBA sample are dissolved in 100 ml of distilled water and shaken for homogenisation. An aliquot is then analysed by HPLC-UV. The following performance characteristics were reported, in the frame of the validation and verification studies [6]:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.21 to 0.76 %;

- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.37 to 0.67 %; and
- a recovery rate (R_{Rec}) ranging from 97.6 to 99.8 %.

While the Applicant discarded this method due to the fact that it can only determine the HMSeBA monomer, the EURL considers this method satisfactory for the determination of the active substance, consisting of 99 % HMSeBA monomer.

Nevertheless, the Applicant developed an alternative method based on gradient HPLC-UV for the determination of total HMSeBA (monomer + dimers) in the *feed additive* [7]. One gram of the feed additive sample is dissolved in 50 ml KH_2PO_4/CH_3CN buffer (pH = 2.5) solution and homogenised for 30 minutes with a magnetic stirrer at room temperature. The solution is then filtered; the filtrate is diluted in water (1:1 v:v) and homogenised. An aliquot is then analysed by HPLC-UV. The following performance characteristics were reported, in the frame of the validation [8] and verification [9] studies: - RSD_r ranging from 0.21 to 1.3 %; - RSD_{ip} ranging from 0.83 to 1.3 %; and - R_{Rec} ranging from 89 to 102 %. Furthermore, the experimental results confirm that HMSeBA used is present mainly in the monomer form (98 to 99 %).

Based on the experimental evidence provided the EURL recommends for official control the validated and further verified gradient HPCL-UV method for the determination of HMSeBA (monomer + dimer) in the *active substance* (HMSeBA *per se*) and in the *feed additive* (preparation).

Total selenium

For the determination of *total selenium* in the *feed additive* (i.e. preparation) and in the *active substance* (HMSeBA) the Applicant submitted the validated and further verified method developed by the UT2A laboratory [2b] - already evaluated and recommended by the EURL [10] - based on microwave digestion using nitric acid and hydrogen peroxide (HNO_3/H_2O_2), followed by inductively coupled plasma mass spectrometry (ICP-MS). The following performance characteristics were reported: - R_{rec} ranging from 94 to 95 %; and - RSD_{ip} ranging from 1.5 to 2.5 %. An alternative/additional method has already been evaluated and recommended by the EURL, based on inductively coupled plasma atomic emission spectrometry (ICP-AES) for which the following performance characteristics were reported [11]: - R_{rec} ranging from 99 to 105 %; - RSD_r ranging from 1.1 to 2.7 %; and - RSD_{ip} ranging from 1.5 to 2.5 %.

For the determination of *total selenium* in *premixtures* and *feedingstuffs* the Applicant submitted the CEN standard method EN 16159:2012 [12] – already recommended in previous EURL reports - based on Hydride Generation Atomic Absorption Spectrometry (HGAAS)

after microwave digestion with HNO_3/H_2O_2. The following performance characteristics are reported for feed samples:

- RSD_r ranging from 3.4 to 10 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 15 to 23 %; and
- a limit of quantification of 0.125 mg/kg, clearly below the maximum legal limit of 0.5 mg Se /kg feed.

For the determination of *total selenium* in *premixtures*, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the abovementioned HGAAS method.

The Applicant did not submit any methods for the determination of *total selenium* in *water* However, the EURL already recommended [13] the method approved by the National Institute for Occupational Safety and Health (NIOSH) and described in their Manual of Analytical Methods (NMAM) [13], based on ICP-AES, for which a limit of detection (LOD) of 0.02 mg/L is 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

- the single laboratory validated and further verified method, using high performance liquid chromatography coupled to UV detection at 220 nm (HPLC-UV) to determine *HMSeBA* (monomer + dimer) in the *feed additive* and in the *active substance;*
- two alternative laboratory validated and further verified methods, based on (i) inductively coupled plasma atomic emission spectrometry (ICP-AES) or (ii) inductively coupled plasma mass spectrometry (ICP-MS) to determine *total selenium* in the *feed additive* and in the *active substance;*
- the CEN ring trial validated method (EN 16159:2012), using hydride generation atomic absorption spectrometry (HGAAS) to determine *total selenium* in *premixtures* and *feedingstuffs*; and
- the NIOSH method based on ICP-AES for the determination of *total selenium* in *water.*

Recommended text for the register entry (analytical method)

For the determination of *Hydroxy-Methyl-Seleno-Butanoic-Acid* in the *feed additive*:

- high performance liquid chromatography coupled to UV detection at 220 nm (HPLC-UV)

For the determination of *total selenium* in the *feed additive*:

- inductively coupled plasma mass spectrometry (ICP-MS), or
- inductively coupled plasma atomic emission spectrometry (ICP-AES)

For the determination of *total selenium* in *premixtures* and *feedingstuffs:*

- hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (EN 16159:2012)

For the determination of *total selenium* in *water:*

- inductively coupled plasma atomic emission spectrometry (ICP-AES)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Hydroxy-Methyl-Seleno-Butanoic-Acid* (HMSeBA) 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/Ref:SANCO/D/2:Forw.Appl.1831/00123-2011
[2a] *Technical dossier, Section II – Identity
[2b] *Technical dossier, Section II 2.6.1.1.a
[3] *Application, Annex A
[4] 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
[5] * Technical dossier, Section II – Annex II 2-6-4
[6] * Technical dossier, Section II – Annex II 2-6-3
[7] * Technical dossier, Section II – Annex II 2-6-5
[8] * Technical dossier, Section II – Annex II 2-6-6
[9] * Technical dossier, Section II – Annex II 2-6-7
[10] #FAD-2010-0044 - JRC.DG.D.6/CvH/PRO/AG/ARES(2011)255176
[11] #FAD-2009-0010 - JRC.DDG.D.6/CvH/PRO/MDS/ARES(2010)175099
[12] EN 16159:2012 - "Animal feeding stuffs: - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide)"
[13] #FAD-2010-0028 - JRC.DG.D.5/CvH/PRO/AG/ARES(2012)612832

*Refers to Dossier no: FAD-2011-0044

#http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports/

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:

- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha, CZ
- Fødevarestyrelsen, Ringsted, DK
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino, IT
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena, DE
- Państwowy Instytut Weterynaryjny, Puławy, PL
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin, PL
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim, DE