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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**

Zinc-L-selenomethionine
(FAD-2016-0056; CRL/160044)



**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-2016-0056 - CRL/160044**

Name of Feed Additive: ***Zinc-L-selenomethionine***

Active Agent (s): **Zinc-L-selenomethionine**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
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Date: **15/05/2017**

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Date: **15/05/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Zinc-L-selenomethionine* under the category/ functional group (3b) "nutritional additives" / "compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories.

The *feed additive* (*Zinc-L-selenomethionine*) is a complexation product of zinc chloride and L-selenomethionine containing about 63 % of L-selenomethionine and about 20 % of zinc. It is to be marketed as a solid preparation, containing a minimum of 0.36 % of *Zinc-L-selenomethionine*, which corresponds to a minimum of 0.1 % of *selenium* and 0.05 % of *zinc*; it also contains calcium carbonate, silicon dioxide and vegetable oil as carriers. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* with a proposed maximum *total selenium* content of 0.5 mg/kg *feedingstuffs* to comply with legal requirements.

For the quantification of *Selenomethionine* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on high performance liquid chromatography with fluorescence detection (HPLC-FLD) after pre-column derivatisation with *ortho*-phthalaldehyde (OPA). Based on the acceptable performance characteristics presented the EURL recommends for official control this HPLC-FLD method to quantify *Selenomethionine* in the *feed additive*.

For the quantification of *total selenium* in the *feed additive* the Applicant submitted the official AOAC 2006.03 method designed for the analysis of elements in fertilisers, based on microwave digestion with nitric acid followed by inductively coupled plasma-optical emission spectrometry (ICP-OES). In the frame of other dossiers related to selenium containing feed additives the EURL evaluated several alternative single-laboratory validated and further verified methods, and recommended for official control either inductively coupled plasma-atomic emission spectrometry (ICP-AES), or inductively coupled plasma-mass spectrometry (ICP-MS) for the quantification of *total selenium* in the *feed additive*.

For the quantification of *total selenium* in *premixtures* and *feedingstuffs* the Applicant did not propose any method for official control. However, the EURL previously evaluated and recommended the CEN method EN 16159:2012 based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion with HNO₃/H₂O₂. For the quantification of *total selenium* in *premixtures*, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the HGAAS method mentioned above. Based on the performance characteristics available, the EURL recommends for official control the

CEN method EN 16159:2012 for the quantification of *total selenium* in *premixtures* and *feedingstuffs*.

For the quantification of *total zinc* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES). Two additional ring-trial validated methods were previously evaluated by the EURL in the frame of the Zinc group dossiers: EN 15621 based on ICP-AES after pressure digestion and the Community method based on atomic absorption spectrometry (AAS). Based on the performance characteristics available the EURL recommends for the official control these two CEN methods for the quantification of *total zinc* 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) is not considered necessary.

KEYWORDS

Zinc-L-selenomethionine, *selenium*, nutritional feed additives, all animal species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new *feed additive*) for *Zinc-L-selenomethionine* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories [1,2].

The *feed additive* (*Zinc-L-selenomethionine*) is a complexation product of zinc chloride and L-selenomethionine containing about 63 % of L-selenomethionine and about 20 % of zinc [3]. It is to be marketed as a solid preparation, containing a minimum of 0.36 % of *Zinc-L-selenomethionine*, which corresponds to a minimum of 0.1 % of *selenium* and 0.05 % of *zinc*; it also contains calcium carbonate, silicon dioxide and vegetable oil as carriers [2,3].

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* with a proposed maximum *total selenium* content of 0.5 mg/kg *feedingstuffs* [2,3] to comply with legal requirements [2,3].

Note: Indications of the % content of an element or a compound are expressed in terms of mass fraction.

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 *Zinc-L-selenomethionine* 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 [4].

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

Selenomethionine

For the quantification of *Selenomethionine* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on high performance liquid chromatography with fluorescence detection (HPLC-FLD) after pre-column derivatisation with *ortho*-phthalaldehyde (OPA) [5].

The sample (10 g) is added to 30 ml of an aqueous methanol solution (20 %, v/v) and 10 ml of NaOH solution (50 %, wt/v). The mixture is then placed in a sonicator at 60 °C for 1 h. After cooling, the mixture is adjusted to 100 ml with water, shaken for 5 min and filtered. An aliquot (1 ml) of the filtrate is combined with 1 ml of 0.05 M EDTA solution, adjusted up to 50 ml with 2.75 mM sodium tetraborate buffer (pH 7.2) and mixed. The derivatisation is performed during the autosampling procedure in a separate vial using a commercial OPA reagent. The analyte is detected by fluorescence measurements using excitation and emission wavelengths of 340 nm and 450 nm, respectively. The quantification of *Selenomethionine* is performed using calibration with external standard solutions treated in the same way as the sample [5].

The performance characteristics reported in the frame of the validation [5] and verification [6] studies are presented in Table 1.

Table 1 The performance characteristics of the methods for the determination of *Selenomethionine* in the *feed additive* and of *total selenium* in *feedingstuffs*

	<i>Selenomethionine</i>		<i>total Selenium</i>
	Feed additive		Feedingstuffs
	Validation	Verification	EN 16159
Method	HPLC-FLD		HGAAS
Mass fraction range, mg/kg	2890 - 2970	2430 - 2470	0.25-73.6
RSD _r , %	0.5 - 2.5	0.9 - 1.9	3.4 - 10
RSD _{ip} , %	2.5	1.9	15 - 23 ^(*)
R _{rec} , %	103	98	-
LOQ, mg/kg	50	-	0.125
Reference	[5]	[6]	[13]

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

Based on the experimental evidence provided, the EURL recommends the HPLC-FLD method mentioned above for the quantification of *Selenomethionine* in the *feed additive*.

In addition, the Applicant applied the HPLC-FLD method for the analysis of a batch of the *feed additive* sample diluted with calcium carbonate to mimic the sample of *premixtures*. Performance characteristics similar to those presented in Table 1 were reported for a *Selenomethionine* content ranging from 1435 to 1469 mg/kg diluted *feed additive* samples [5]: RSD_r ranging from 1.8 to 2.9 %; RSD_{ip} of 2.9 %; and R_{rec} of 99 %.

Total selenium

For the quantification of *total selenium* in the *feed additive* the Applicant submitted the official method of AOAC International (AOAC, 2006.03) designed for the analysis of several elements (including selenium) in fertilisers [7]. The method is based on inductively coupled plasma-optical emission spectrometry (ICP-OES) after microwave digestion with concentrated nitric acid [7]. In the frame of other dossiers related to selenium containing feed additives, the EURL evaluated and recommended three alternative single-laboratory validated and further verified methods [8-12]: i) an inductively coupled plasma-atomic emission spectrometry (ICP-AES) method with the following performance characteristics [8]: a relative standard deviation for *repeatability* (RSD_r) ranging from 1.1 to 2.7 %; a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.5 to 2.5 %; and a *recovery rate* (R_{rec}) ranging from 99 to 105 %; or ii) two methods based on microwave digestion with nitric acid and hydrogen peroxide (HNO₃/H₂O₂) followed by inductively coupled plasma-mass

spectrometry (ICP-MS) with similar performance characteristics [11, 12]: RSD_{ip} ranging from 2 to 7%; and R_{rec} ranging from 94 to 102 %.

Based on the performance characteristics available the EURL recommends for the official control the three single-laboratory validated and further verified methods mentioned above for the quantification of total selenium in the *feed additive*.

For the quantification of total selenium in *premixtures* and *feedingstuffs* the Applicant applied the AOAC method mentioned earlier for the analysis of *selenium* in mineral vitamin *premixtures* and ICP-MS for the analysis of *selenium* in *feedingstuffs* in the frame of homogeneity studies [3].

However, the EURL evaluated and recommended in the frame of previous dossiers related to selenium containing feed additives [8-12] the ring-trial validated method EN 16159:2012 based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion with HNO_3/H_2O_2 [13]. The performance characteristics of the method are presented in Table 1.

Furthermore, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the HGAAS method mentioned above to quantify total selenium in *premixtures*.

Based on the performance characteristics available the EURL recommends for the official control the ring-trial validated method EN 16159 based on HGAAS for the quantification of total selenium in *premixtures* and *feedingstuffs*.

Total zinc

For the *quantification* of total zinc in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [14]. For an organic *feedingstuffs*, the test sample is ashed and dissolved in hydrochloric acid, while for a mineral compound feed, the test sample is wet digested with hydrochloric acid.

Two additional methods were previously evaluated and recommended by the EURL in the frame of the Zinc group dossier [15]: the ring-trial validated EN 15621 method based on ICP-AES after pressure digestion [16] and the Community method based on atomic absorption spectrometry (AAS) [17]. The Community method was further ring-trial validated by the UK Food Standards Agency [18], using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets.

The performance characteristics reported for the three methods mentioned above are summarised in Table 2.

Table 2: Performance characteristics for the quantification of *total zinc* in *premixtures* and *feedingstuffs*

	EN 15510 [14]	EN 15621 [16]	UK FSA [18]
Method	ICP-AES	ICP-AES	AAS
Content (mg/kg)	27.4 – 3826	26.6 – 3618	93 – 199
RSD _r (%)	1.7 – 8.8 ^(*)	1.5 – 5.4	1.0 – 6.1
RSD _R (%)	5.0 – 19 ^(*)	2.7 – 22	4.1 – 9.5
LOQ (mg/kg)	3	1	20

RSD_r and RSD_R: relative standard deviation for *repeatability* and *reproducibility*;

LOQ: limit of quantification;

(*) the largest precision values were obtained for mineral mixes.

Based on the performance characteristics available the EURL recommends for the official control the two CEN ring-trial methods based on ICP-AES for the quantification of *total zinc* in the *feed additive*. Furthermore, these two methods and the Community method are considered suitable for the quantification of *total zinc* in *premixtures* and *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) is not considered necessary.

Note: inductively coupled plasma-optical emission spectrometry (ICP-OES) is equal to inductively coupled plasma-atomic emission spectrometry ICP-AES.

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 fluorescence detection (HPLC-FLD) to quantify *Selenomethionine* in the *feed additive*;
- the single-laboratory validated and further verified methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS) to quantify *total selenium* in the *feed additive*;
- the CEN ring-trial validated method based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (EN 16159:2012) to quantify *total selenium* in *premixtures* and *feedingstuffs*; and
- the CEN ring-trial validated methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) (EN 15510 and EN 15621) for the quantification of *total zinc* in the *feed additive*.

Furthermore, the EURL considers EN 15510, EN 15621 and Community methods suitable for the quantification of *total zinc* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *Selenomethionine* in the *feed additive*:

- high performance liquid chromatography with fluorescence detection (HPLC-FLD)

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

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

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

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

For the quantification of *total zinc* in the *feed additive*:

- inductively coupled plasma-atomic emission spectrometry (ICP-AES) – EN 15510; or
- inductively coupled plasma-atomic emission spectrometry after pressure digestion, (ICP-AES) – EN 15621

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Zinc-L-selenomethionine* 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. 1831/0039-2016
- [2] *Application, Proposal for Register Entry – Annex A
- [3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis
- [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-55
- [6] *Supplementary information – CRL-FA Verification form_Availa Se_TeLA_March 2017
- [7] AOAC Official Method 2006.03 Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers, Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry

- [8] # FAD-2009-0010 – JRC.DDG.D.6/CvH/PRO/MDS/ARES(2010)175099
- [9] # FAD-2011-0028 – JRC.DG.D.5/CvH/PRO/AG/ARES(2012)612832
- [10] # FAD-2011-0044 – JRC.DG.D.5/CvH/PRO/AG/ARES(2012)856822
- [11] # FAD-2012-0042 – JRC.D.5/SFB/CvH/ZE/mds/Ares(2013)2041882
- [12] # FAD-2009-0029+FAD-2010-0044 – JRC.DG.D.6/CvH/PRO/AG/ARES(2011)255176
- [13] EN 16159:2012 – *Animal feedingstuffs: - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide)*
- [14] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*
- [15] #Zinc Group – JRC.DG.D.6/CvH/PR/mds/ARES(2011)1156545
- [16] 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*
- [17] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed – Annex IV-C
- [18] Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010

*Refers to Dossier no: FAD-2016-0056;

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

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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