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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 (3b818)

(FEED-2022-5010; CRL/220046)

European Union Reference Laboratory
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Dossier related to:	**FEED-2022-5010 - CRL/220046**
Name of Additive:	***Zinc-L-selenomethionine (3b818)***
Active Agent (s):	**Zinc-L-selenomethionine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **24/07/2023**
Report approved by: Date:	**Christoph von Holst** **24/07/2023**

EXECUTIVE SUMMARY

In the current application a modification of the current authorisation (3b818) is sought under article 13 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, the authorisation is sought for the use of the *feed additive* for all animal species and categories.

According to the current authorisation, the *feed additive* contains a minimum of 62 % (w/w) of *L-selenomethionine*, 24.5 % (w/w) of *selenium* and 19 % (w/w) of *zinc*. The modified authorisation is sought for a solid preparation (Availa® Se 4%, w/w) with a *selenium* content ranging from 1 to 46 g/kg of the preparation. The *feed additive* is intended to be incorporated into *feed* through *premixtures* with a proposed maximum *total selenium* content of 0.5 mg/kg *complete feed* or 0.2 mg/kg organic *selenium* supplemented via *complete feed*.

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 inductively coupled plasma-atomic emission spectrometry (ICP-AES). Furthermore, in the frame of former dossiers related to *feed additives* containing *selenium*, the EURL evaluated several alternative single-laboratory validated and further verified methods recommending 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*. Based on the performance characteristics available the EURL recommends for the official control the method of AOAC International (AOAC, 2006.03) and three single-laboratory validated and further verified methods (based on ICP coupled with AES or MS) mentioned above for the quantification of *total selenium* in the *feed additive*.

For the quantification of *total selenium* in *premixtures* and *compound feed* the Applicant did not propose any method for official control. Nevertheless, for the quantification of *total selenium* in *premixtures* and *compound feed*, the EURL found the ring-trial validated EN 17053 based on ICP-MS and designed for the "Determination of trace elements, heavy metals and other elements in feed". Furthermore, the EURL previously evaluated and recommended the CEN method EN 16159 based on hydride generation atomic absorption spectrometry (HGAAS). Based on the performance characteristics available the EURL

recommends for official control for the quantification of *total selenium* in *premixtures* and *compound feed* the ring-trial validated methods EN 17053 based on ICP-MS and the EN 16159 based on HGAAS.

For the quantification of *total zinc* in the *feed additive*, *premixtures* and *compound feed* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on ICP-AES. Furthermore, additional methods, formerly evaluated by the EURL in the frame of *zinc*-related dossiers, are considered fit-for-purpose for the current dossier. Therefore, based on the acceptable method performance characteristics, the EURL recommends for official control for the quantification of *total zinc* in the *feed additive* the ring-trial validated methods EN 15510 and EN 15621 based on ICP-AES or the ISO 6869 based on AAS. Furthermore, for the possible quantification of *total zinc* in *premixtures* and *compound feed*, the EURL considers these methods, together with the ring-trial validated method EN 17053 based on ICP-MS and the European Union method based on AAS (for *compound feed* only), fit-for-purpose

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

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

BACKGROUND

In the current application a modification of the current authorisation (3b818) is sought under article 13(3) 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, the 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*. The *feed additive* contains a minimum of 62 % (w/w) of *L-selenomethionine*, 24.5 % (w/w) of *selenium* and 19 % (w/w) of *zinc* [2,3]. While the authorised *feed additive* was formerly intended to be marketed as solid preparation of *Zinc-L-selenomethionine* with a *selenium* content ranging from 1 to 2 g/kg (Availa® Se 1000), now the authorisation is sought for a solid preparation (Availa® Se 4%, w/w) with a *selenium* content ranging from 1 to 46 g/kg of the preparation [2,3]. The *feed additive* is intended to be incorporated into *feed* through *premixtures* with a proposed maximum *total selenium* content

of 0.5 mg/kg *complete feed* or 0.2 mg/kg *organic selenium* supplemented via *complete feed* [2-4].

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 *Zinc-L-selenomethionine* 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)

Selenomethionine

In the frame of the current dossier the Applicant proposed for the determination of *selenomethionine* in the *feed additive* and *premixtures* a method based on high performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) [5].

Nevertheless, in the corresponding supporting documents, 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) [6,7]. Furthermore, the method was already evaluated and recommended in the frame of the former authorisation [3,8].

The sample (10 g) is added to 30 ml of an aqueous methanol solution (20 %, *v/v*) and 10 ml of NaOH solution (50 %, *w/v*). The mixture is then placed in a sonicator at 60 ºC 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.

Table 1 Performance characteristics of the single-laboratory validated and further verified HPLC-FLD method for the determination of *selenomethionine* in the *feed additive*

HPLC-FLD	Validation [6]	Verification [7]
Mass fraction (mg/kg)	2890 - 2970	2430 - 2470
RSD_r (%)	0.5 - 2.5	0.9 - 1.9
RSD_{ip} (%)	2.5	1.9
R_{rec} (%)	103	98
LOQ (mg/kg)	50	-

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*.

The quantification of *selenomethionine* is performed using calibration with external standard solutions treated in the same way as the sample [6]. The method was validated and verified analysing the original *feed additive* preparation. The performance characteristics reported in the frame of the validation and verification studies, as recalculated by the EURL, are presented in Table 1 [8].

In addition the Applicant, in the frame of "batch to batch variation" studies, applied the HPLC-FLD method to the analysis of *selenomethionine* in the new preparation (Availa® Se 4%) obtaining satisfactory results [2].

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

Total selenium

For the quantification of *total selenium* in the *feed additive* the Applicant submitted the ring-trial validated official method of AOAC International (AOAC, 2006.03) designed for the analysis of several elements (including *selenium*) in fertilisers [5,9]. The method is based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) after microwave digestion with concentrated nitric acid [9]. In addition the Applicant, in the frame of "batch to batch variation" studies, applied the ICP-AES method to the analysis of *selenium* in the new preparation Availa® Se 4% obtaining satisfactory results [2].

Furthermore, in the frame of other dossiers related to *feed additives* containing *selenium*, the EURL evaluated and recommended three alternative single-laboratory validated and further verified methods [8]:

- an inductively coupled plasma-atomic emission spectrometry (ICP-AES) method with the following performance characteristics: RSD_r ranging from 1.1 to 2.7 %, RSD_{ip} ranging from 1.5 to 2.5 % and a *recovery* rate (R_{rec}) ranging from 99 to 105 %; or
- two methods based on microwave digestion with nitric acid and hydrogen peroxide (HNO_3/H_2O_2) followed by inductively coupled plasma-mass spectrometry (ICP-MS) with similar performance characteristics: 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 method of AOAC International (AOAC, 2006.03) and 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 *compound feed* the Applicant did not present any method for official control [5].

Nevertheless, for the quantification of *total selenium* in *premixtures* and *compound feed*, the EURL found the ring trial validated EN 17053:2018 based on ICP-MS and designed for the "Determination of trace elements, heavy metals and other elements in feed" [10].

The sample is digested with concentrated nitric acid under pressure. The elemental *selenium* is detected by ICP-MS at mass-to-charge (m/z) of 78 and/or 80 and/or 82. The quantification of the analyte is performed using an external standard calibration or standard additions [10]. Furthermore, in the frame of homogeneity studies, the Applicant presented analysis performed applying the ring-trial validated EN 17053 to the analysis of *selenium* in mineral *premixtures* containing Availa® Se 4% [11].

Moreover, the EURL evaluated and recommended [8] in the frame of previous dossiers related to *feed additives* containing *selenium*, 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 [12]. The following performance characteristics were presented: RSD_r ranging from 3.4 to 10 and relative standard deviation for reproducibility RSD_R ranging from 15 to 23 % [12]. Furthermore, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the EN 16159 method to quantify *total selenium* in *compound feed* [8].

Based on the performance characteristics available the EURL recommends for official control for the quantification of *total selenium* in *premixtures* and *compound feed* the ring-trial validated methods EN 17053 based on ICP-MS and the EN 16159 based on HGAAS.

Total zinc

For the *quantification* of *total zinc* in the *feed additive*, *premixtures* and *compound feed* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [5,13].

The test sample is ashed and dissolved in hydrochloric acid (in the case of organic *compound feed*) or wet digested with hydrochloric acid (in the case of mineral compounds). The elemental *zinc* is detected by ICP-AES at selected specific wavelengths (206.200 and/or 213.856 nm) and the quantification is performed using an external standard calibration or standard additions [13].

Furthermore, additional methods were previously evaluated by the EURL in the frame of *zinc*-related dossiers [8,14]. For the quantification of *total zinc* in the *feed additive*, *premixtures* and *compound feed*, the EURL evaluated for official control:

- the ring-trial validated EN 15621 method based on ICP-AES where the sample is digested under pressure with the mixture of nitric acid and hydrogen peroxide (or only using nitric acid) [15]; and
- the ring-trial validated method ISO 6869 based on AAS where the sample is ashed and dissolved in hydrochloric acid (in the case of organic *compound feed*) or wet digested with hydrochloric acid (in the case of mineral compounds). The analyte is detected by an air-acetylene flame AAS at selected specific wavelength (213.8 nm). The quantification is performed using an external standard calibration curve [16].

For the quantification of *total zinc* in *premixtures* and *compound feed*, the EURL evaluated for official control the above-mentioned ring-trial validated EN 17053 based on ICP-MS where the sample is digested with concentrated nitric acid under pressure. The elemental *zinc* is detected by ICP-MS at mass-to-charge (m/z) of 66 and/or 68. The quantification of the analyte is performed using an external standard calibration or standard additions [10]. Furthermore, experiments for the determination of *Zinc* in *premixtures* were presented in the frame of the "homogeneity studies" thus demonstrating the applicability of the method to the new preparation [11].

Moreover, for the quantification of *total zinc* in *compound feed*, the EURL evaluated for official control the European Union method based on atomic absorption spectrometry (AAS) where the sample is ashed and dissolved in hydrochloric acid (in the case of organic *compound feed*) or wet digested with hydrochloric acid (in the case of mineral compounds) [17]. Other methods of digestion such as a microwave pressure may be used provided they have been demonstrated giving similar results. The analyte is detected by an air-acetylene flame AAS at selected specific wavelength (213.8 nm). The quantification is performed using an external standard calibration curve [17].

Table 2: Performance characteristics for the quantification of *total zinc* in *premixtures* and *compound feed.*

	EN 15510 [13]	EN 15621 [15]	ISO 6869 [16]	EN 17053 [10]	UK FSA [18]
Method	**ICP-AES**	**ICP-AES**	**AAS**	**ICP-MS**	**AAS**
Mass fraction (mg/kg)	27.4 – 3826	127 – 10310	29 – 14600	55 – 6286(**)	93 – 199
RSD_r (%)	1.7 – 8.8(*)	2.1 – 3.5	1.7 – 7.6	2.3 – 5.5	1.0 – 6.1
RSD_R (%)	5.0 – 19(*)	5.5 – 13.2	3.3 – 15.0	5.2 – 11.3	4.1 – 9.5
LOQ (mg/kg)	3	1	5	5	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 premixes; (**) based on dry weight.

The method was further ring-trial validated by the UK Food Standards Agency (FSA) using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets [18]. The performance characteristics reported for the five methods mentioned above are summarised in Table 2.

Based on the acceptable method performance characteristics, the EURL recommends for official control for the quantification of *total zinc* in the *feed additive* the ring-trial validated methods EN 15510 and EN 15621 based on ICP-AES or the ISO 6869 based on AAS. Furthermore, for the possible quantification of *total zinc* in *premixtures* and *compound feed*, the EURL considers these methods, together with the ring-trial validated method EN 17053 based on ICP-MS and the European Union method based on AAS (for *compound feed* only), fit-for-purpose.

Note: When applying the recommended methods for the quantification of *zinc* in the *feed additive*, the EURL suggests to perform an appropriate additional dilution of the samples after the digestion in a way that the mass fraction of the analyte would fit the ring-trial validation ranges of the methods.

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.

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)

An evaluation of corresponding methods of analysis is not relevant for the present application.

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

- 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 ring-trial validated method AOAC, 2006.03 or the single-laboratory validated and further verified methods both 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 ring-trial validated method EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) or the CEN ring-trial validated method EN 16159 based on hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion to quantify *total selenium* in *premixtures* and *compound feed*;
- the ring-trial validated method EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) or the ring-trial validated EN 15621 method based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) or the ring-trial validated method ISO 6869 based on atomic absorption spectrometry (AAS) to quantify *total zinc* in the *feed additive.*

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 *compound feed*:

- inductively coupled plasma-mass spectrometry (ICP-MS) – EN 17053; or
- hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion – EN 16159

For the determination 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; or
- atomic absorption spectrometry (AAS) – ISO 6869

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] *Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – https://webgate.ec.europa.eu/esfc/#/applications/6870 https://open.efsa.europa.eu/questions/EFSA-Q-2022-00857

[2] *Technical dossier, Section II: Conditions of use

[3] Commission Implementing Regulation (EU) 2019/49 of 4 January 2019 concerning the authorisation of sodium selenite, coated granulated sodium selenite and zinc-L-selenomethionine as feed additives for all animal species

[4] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[5] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance

[6] *Technical dossier, Section II: II.6.2 Covance Validation Report_Availa Se 1000_conf

[7] **Supplementary information – CRL-FA Verification form_Availa Se_TeLA_March 2017

[8] EURL report: https://joint-research-centre.ec.europa.eu/publications/fad-2016-0056_en

[9] 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 Final Action 2009

[10] EN ISO 17053:2018 Animal feeding stuffs: Methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)

[11] *Technical dossier, Section II: II.4.2 Zinpro Study_The homogeneity of Availa Se 4_ in a premix_conf

[12] 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)

[13] 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

[14] EURL report: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0072_en

[15] 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

[16] ISO 6869:2000 Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry

[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: FEED-2022-5010
**Refers to Dossier no: FAD-2016-0056

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

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- [1]Wageningen Food Safety Research (WFSR) (NL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)

[1] Name and address according to COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.