Ref. Ares(2015)729537 - 20/02/2015

EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate D: Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.D.5/SFB/CvH/ZE/mds/Ares

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

Sodium Selenite

(FAD-2010-0104; CRL/100149)
(FAD-2010-0362; CRL/100303)
(FAD-2010-0369; CRL/100296)

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-2010-0104 - CRL/100149** **FAD-2010-0362 - CRL/100303** **FAD-2010-0369 - CRL/100296**
Name of Feed Additive:	**Sodium Selenite**
Active Agent (s):	**Sodium Selenite**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **17/02/2015**
Report approved by: Date:	**Christoph von Holst** **19/02/2015**

EXECUTIVE SUMMARY

In the current applications authorisation is sought under article 10(2) for *sodium selenite* 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 categories and species. According to two of the Applicants (FAD-2010-0104 and FAD-2010-0362) the *feed additive* is a white powder containing at least of 98% of *sodium selenite* (based on anhydrous weight), which corresponds to a minimum content of 45% *selenium* and 13% *sodium*. Applicant FAD-2010-0369 intends to market a film granulated preparation of *sodium selenite* containing a minimum of 1% of *total selenium*. All *feed additives* are intended to be incorporated into *feedingstuffs* through *premixtures* with a maximum level of 0.5 mg *total selenium* /kg *feedingstuffs*.

For the characterisation of *sodium selenite* in the *feed additive* containing at least 98% of *sodium selenite* Applicant FAD-2010-0362 submitted the European Pharmacopoeia Monograph 1677, where identification is based on specific reactions involving ascorbic acid, barium chloride and sodium ions; while quantification is based on redox titration with potassium iodide, sodium thiosulfate and iodine solutions.

For the quantification of *total selenium* in the *feed additive* containing at least 98% of *sodium selenite* Applicant FAD-2010-0104 submitted a single-laboratory validated method based on conversion of sodium selenite to selenium and its further quantification by gravimetry; while Applicant FAD-2010-0369 applied a quantification assay derived from the European Pharmacopoeia method - based on redox titration with potassium iodide and sodium thiosulfate.

Additionally, two alternative single-laboratory validated and further verified methods based on (1) inductively coupled plasma atomic emission spectrometry (ICP-AES) and (2) inductively coupled plasma mass spectrometry (ICP-MS) - submitted in the frame of the dossiers FAD-2009-0010 and FAD-2012-0042 - were previously evaluated and recommended by the EURL - in the frame of other selenium dossiers - for the quantification of *total selenium* in *feed additives*.

Based on the information available, the EURL recommends for official control the titrimetric method described in the European Pharmacopoeia and/or the equivalent gravimetric method submitted by the Applicant for the characterisation of the *feed additive* containing at least 98% of *sodium selenite;* while ICP-MS or ICP-AES methods are recommended to quantify *total selenium* in the film granulated preparation (*feed additive*).

For the quantification of *total sodium* in the *feed additive*, containing at least 98% of *sodium selenite*, the EURL recommends for the official control two internationally recognised ring-trial validated methods: (i) EN ISO 6869:2000, based on Atomic Absorption Spectrometry (AAS) after dissolving in hydrochloric acid; and (ii) EN 15510:2007, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after dissolving in hydrochloric acid, for which relative precisions ranging from 4 to 27 % were reported.

For the quantification of *total selenium* in *premixtures* and *feedingstuffs* two Applicants (FAD-2010-0362 and FAD-2010-0369) suggested several CEN and AOAC methods, while the Applicant FAD-2010-0104 submitted the EN 16159:2012 method based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO_3/H_2O_2. This method was already evaluated and recommended by the EURL in the frame of previous selenium dossiers.

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

Sodium Selenite, Selenium, nutritional additives, compounds of trace elements, all animal species and categories

1. BACKGROUND

In the current applications authorisation is sought under article 10(2) (re-evaluation of the already authorised additives under provisions of Council Directive 70/524/EEC) for *sodium selenite* 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 [1-3]. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species [1-6].

According to two of the Applicants (FAD-2010-0104 and FAD-2010-0362) the *feed additive* is a white powder containing at least of 98% of *sodium selenite* (based on anhydrous weight) [7,8], which corresponds to a minimum content of 45% *selenium* and 13% *sodium* [4,5], while Applicant FAD-2010-0369 intends to market a film granulated preparation of *sodium selenite* containing a minimum of 1% of *total selenium* [9].

All *feed additives* are intended to be incorporated into *feedingstuffs* through *premixtures* with a maximum level of 0.5 mg *total selenium* /kg *feedingstuffs* [4-6].

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 *sodium selenite* 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 and dioxins) are available from the respective European Union Reference Laboratories [10].

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

For the characterisation of *feed additive* containing at least 98% of *sodium selenite* Applicant FAD-2010-0362 submitted the European Pharmacopoeia Monograph 1677 [11], where:

- identification is based on specific reactions involving ascorbic acid, barium chloride and sodium ions; while
- quantification is based on redox titration with potassium iodide, sodium thiosulfate and iodine solutions.

In addition, for the quantification of *total selenium* in the *feed additive* containing at least 98% of *sodium selenite* Applicant FAD-2010-0104 submitted a single-laboratory validated method based on conversion of sodium selenite to selenium and its further quantification by gravimetry [12] for which a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.05 to 0.16% was reported in the frame of the validation study using *feed additive* samples containing 45.6 % *total selenium* [13]. Applicant FAD-2010-0369 applied instead a quantification assay derived from the European Pharmacopoeia method - based on redox titration with potassium iodide and sodium thiosulfate [14].

Two alternative single-laboratory validated and further verified methods based on (1) inductively coupled plasma atomic emission spectrometry (ICP-AES) and (2) inductively coupled plasma mass spectrometry (ICP-MS) - submitted in the frame of the dossiers FAD-2009-0010 and FAD-2012-0042 - were previously evaluated and recommended by the EURL [15-18].

The following performance characteristics were reported for the two methods:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1.1 to 2.9 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.5 to 2.9 %; and
- a *recovery* rate (R_{rec}) ranging from 99 to 105 %.

Based on the performance characteristics available, the EURL recommends for official control the European Pharmacopoeia, or the equivalent gravimetric method submitted by the Applicant for the characterisation of the *feed additive* containing at least 98% of *sodium selenite*, while methods based on ICP-MS or ICP-AES are recommended for official control to quantify *total selenium* in the film granulated preparation (*feed additive*).

For the quantification of *total sodium* in the *feed additive*, containing at least 98% of *sodium selenite*, the EURL identified two internationally recognised ring-trial validated methods: (i) EN ISO 6869:2000, based on Atomic Absorption Spectrometry (AAS) after dissolving in hydrochloric acid [19]; and (ii) EN 15510:2007, based on Inductively Coupled Plasma

Atomic Emission Spectrometry (ICP-AES) after dissolving in hydrochloric acid [20], for which relative precisions ranging from 4 to 27 % were reported for *total sodium* content ranging from 0.1 to 237 g/kg of feed related matrices.

For the quantification of *total selenium* in *premixtures* and *feedingstuffs* Applicants (FAD-2010-0362 and FAD-2010-0369) [8,9] suggested the EN 14627:2005 method for determination of *total selenium* by hydride generation AAS in foodstuffs; and several AOAC methods:

939.09 - selenium in food by titrimetry;
974.15 - selenium in human and pet food by fluorometry;
986.15 - selenium in human and pet food by atomic absorption spectrophotometry;
996.16 – selenium in feeds and premixes by fluorometry; and
996.17 – selenium in feeds and premixes by continuous hydride generation atomic absorption,

while Applicant FAD-2010-0104 [7] submitted the CEN standard method EN 16159:2012 [21], originally developed by the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany) [22]. This method was previously evaluated and recommended for official control by the EURL for the determination of *total selenium* in *premixtures* and *feedingstuffs*, in the frame of other selenium dossiers.

The CEN method for the quantification of *total selenium* is based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO_3/H_2O_2. The following performance characteristics are reported for *total selenium* content ranging from 0.25 to 74 mg/kg feed:

- a relative standard deviation for *repeatability* (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.

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 European Pharmacopoeia monograph 1677 and/or the equivalent gravimetric method submitted by the Applicant to characterise the *feed additive* containing at least 98% of *sodium selenite*;
- the single-laboratory validated and further verified methods based on ICP-MS or ICP-AES methods for the quantification of *total selenium* in the film granulated preparation (*feed additive*);
- the ring trial validated CEN methods EN ISO 6869, based on Atomic Absorption Spectrometry (AAS) or EN 15510, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), to quantify *total sodium* in the *feed additives* (containing at least of 98% of *sodium selenite*); and
- the CEN ring trial validated method (EN 16159:2012), using hydride generation atomic absorption spectrometry (HGAAS) to quantify *total selenium* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of *sodium selenite*:

- Titrimetry - European Pharmacopoeia Monograph 01/2008:1677, and/or
- Gravimetry

For the quantification of *total selenium* in the film granulated preparation:

- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

For the quantification of *total sodium* in *sodium selenite:*

- Atomic Absorption Spectrometry (AAS) - EN ISO 6869:2000; or
- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) - EN:15510:2007

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

- Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion - EN 16159:2012

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *sodium selenite* 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] [a] Application, Reference SANCO/G1: Forw. Appl.1831/0038-2014
[2] [b] Application, Reference SANCO/G1: Forw. Appl.1831/0039-2014
[3] [c] Application, Reference SANCO/G1: Forw. Appl.1831/0040-2014
[4] [a] Application, Proposal for Register Entry – Annex A
[5] [b] Application, Proposal for Register Entry – Annex A
[6] [c] Application, Proposal for Register Entry – Annex A
[7] [a] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis
[8] [b] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis
[9] [c] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis
[10] 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
[11] European Pharmacopoeia Monograph 01/2008:1677
[12] [a] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis – Annex_II_20
[13] [a] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis – Annex_II_21
[14] [c] Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis – Annex_II_27
[15] [#]FAD-2009-0010 – JRC.DDG.D.6/CvH/PRO/MDS/ARES(2010)175099
[16] [#]FAD-2012-0042 – JRC.D.5/SFB/CvH/ZE/mds/Ares(2013)2041882
[17] [#]FAD-2011-0028 – JRC.DG.D.5/CvH/PRO/AG/ARES(2012)612832
[18] [#]FAD-2011-0044 – JRC.DG.D.5/CvH/PRO/AG/ARES(2012)856822
[19] 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
[20] 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
[21] 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)
[22] VDLUFA Methodenbuch III (2003), 11.6.1 – Selen

[a] Refers to Dossier no: FAD-2010-0104
[b] Refers to Dossier no: FAD-2010-0362
[c] Refers to Dossier no: FAD-2010-0369
[#] https://ec.europa.eu/jrc/en/eurl/feed-additives/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:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)[1]
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft, Nossen (DE)[2]

Name and address according to Regulation (EC) No 885/2009:
[1] Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim
[2] Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 – Landwirtschaftliches Untersuchungswesen, Leipzig