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**EURL Evaluation Report on the Analytical Methods  
submitted in connection with the Application for the  
Authorisation of Feed Additives according to  
Regulation (EC) No 1831/2003**

Dossier related to: FAD-2010-0254 CRL/100232  
Product Name: -  
Active Substance(s): Methionine-zinc  
Rapporteur Laboratory: European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium  
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## EXECUTIVE SUMMARY

In the current application authorisation is sought under article articles 4(1) and 10(2) for *methionine-zinc* under the categories of "nutritional additives" functional group 3b (compounds of trace elements) and functional group 3c (amino acids, their salts and analogues), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* in group 3b for all categories and species and in group 3c for ruminants.

According to the Applicant *methionine-zinc* is a white or almost white crystalline powder with a minimum content of 78% DL-methionine and a maximum content of 18.5 % total zinc.

The *feed additive* is intended to be used in *premixtures*, *feedingstuffs*, complementary feed or in feed supplements. The Applicant suggested maximum levels ranging from 150 to 250 mg total zinc/kg *feedingstuffs* depending on the species of concern, as set in the previous legislation.

For the quantification of *methionine* in *feed additive*, the Applicant proposed the ring-trial validated VDLUFA method. This method is the basis of the internationally recognised methods ISO CD 17180 and AOAC 999.13. This method applies for the determination *methionine* content in commercial amino acid products and premixtures, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The method does not distinguish between the salts and the amino acid enantiomers. The following performance characteristics were reported in the ISO CD 17180 standard for products with a methionine content between 9.1 % and 93 %:

- a relative standard deviation of *repeatability* ( $RSD_T$ ) ranging from 0.5 to 1.6 % and
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) ranging from 1.5 to 2.6 %.

Based on the performance characteristics presented, the EURL recommends for official control the ISO CD 17180 method, based on ion exchange chromatography coupled with post-column derivatisation and photometric or fluorescence detection to quantify *methionine* in *feedadditive*.

For the *determination* of total zinc in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a ring-trial validated VDLUFA method. The EURL identified instead two alternative CEN ring trial validated methods:

- EN 15510, based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The following performance characteristics were reported: -  $RSD_T$  ranging from 1.7 to 8.8 %; -  $RSD_R$  ranging from 5.0 to 19 %; and - a limit of quantification (LOQ) of 3 mg/kg.

- CEN/TS 15621, based on ICP-AES after pressure digestion. The total zinc concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg: - RSD<sub>T</sub> ranging from 1.5 to 5.4 %; - RSD<sub>R</sub> ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs*.

Furthermore, the EURL reminds that a Community method is available for the determination of total zinc in *feedingstuffs*, with limited performance characteristics provided. However, the UK Food Standards Agency recently organised of a ring-trial based on the above mentioned Community method, and reported precisions (RSD<sub>T</sub> and RSD<sub>R</sub>) for *feedingstuffs* ranging from 1.0 to 9.5 %.

Based on the acceptable method performance characteristics presented, the EURL recommends for official control the CEN methods EN 15510 or CEN/TS 15621 to determine total zinc content by ICP-AES in the *feed additive* and *premixtures*. As for the determination of total zinc content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

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**

*methionine-zinc*, all categories and species, compounds of trace elements, amino acids their salts and analogues

## **1. BACKGROUND**

In the current application authorisation is sought under articles 4(1) and 10(2) for *methionine-zinc* under the categories of "nutritional additives" functional group 3b (compounds of trace elements) and functional group 3c (amino acids, their salts and analogues) [1].

Specifically, authorisation is sought for the use of the *feed additives* in group 3b for all categories and species and in group 3c for ruminants [1, 2].

According to the Applicant, *methionine-zinc* is a white or almost white crystalline powder with a minimum content of 78 % DL-methionine and a maximum content of 18.5 % *total zinc* [2, 3], similar to the characteristics published in the previous legislation [4].

The *feed additive* is intended to be used in *premixtures*, *feedingstuffs*, complementary feed or in feed supplements [3]. The Applicant suggested maximum levels ranging from 810 to 1350 mg of *methionine-zinc* /kg *feedingstuffs*. Knowing that the product contains a minimum of 18.5% of zinc, this is then equivalent to maximum levels ranging from 150 to 250 mg *total zinc*/kg *feedingstuffs*, as set in the previous legislation [4].

## 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 *methionine-zinc*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

## 3. EVALUATION

### *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 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 *methionine* in *feed additive*, the Applicant submitted the ring-trial validated VDLUFA method [6]. This method is the basis of the internationally recognised methods ISO/CD 17180 [7] and AOAC 999.13 [8]. This method applies for the determination of *methionine* content in commercial amino acid products and premixtures, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The method does not distinguish between the salts and the amino acid enantiomers.

**Table 1:** Method performance characteristics obtained in the frame of ring-trial validation exercises [7] for the determination of *methionine* in the *feed additive* and *premixtures*.

Intercomparison study	Matrix	<i>Methionine</i> (%)	RSD <sub>r</sub> (%)	RSD <sub>R</sub> (%)
ISO/CD 17180	premix	26.55	1.56	1.85
	premix	11.26	1.24	1.84
	premix	14.21	0.93	1.92
	premix	32.04	0.5	2.59
	premix	30.59	1.06	2.5
	premix	9.1	0.92	1.48
	premix	8.91	1.17	1.89
	premix	19.09	0.99	1.73
	premix	18.65	0.68	1.53
	DL-Methionine	93.27	0.85	1.52

*RSD<sub>r</sub>*, *RSD<sub>R</sub>* - relative standard deviation for *repeatability* and *reproducibility*, respectively

Methionine is extracted with diluted hydrochloric acid. After addition of internal standard, the solution is diluted with citric acid buffer and filtered. The amino acid is separated by ion exchange chromatography and determined by post-column derivatisation with ninhydrin and photometric detection at 440 and 570 nm or with OPA and fluorescence detection.

The ISO method was ring trial validated using ten different matrices listed in Table 1, with a methionine content ranging from 9 to 93%, thus including the methionine content in the product (of 78%). The performance characteristics reported for the determination of *methionine* [7] are listed in Table 1.

Based on the performance characteristics presented, the EURL recommends for official control the ISO/CD 17180 method, based on ion exchange chromatography coupled with post-column derivatisation and photometric or fluorescence detection to quantify *methionine* in the *feed additive*.

For the determination of total zinc in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the VDLUFA method (Methodenbuch, Band III, method 11.5.2). The EURL recommends instead two ring trial validated CEN methods based on inductively coupled plasma atomic emission spectroscopy (ICP-AES): EN 15510 [9] and CEN/TS 15621 [10].

For the determination of total zinc according to EN 15510, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic feedingstuffs) or wet digested with hydrochloric acid (in the case of mineral compounds). The following performance characteristics were reported [9] for a complete feed for pigs, a complete feed for sheep, a

rock phosphate, a mineral premix and two different mineral mixtures, where the total zinc content ranged from 27.4 to 3826 mg/kg:

- a relative standard deviation of *repeatability* ( $RSD_r$ ) ranging from 1.7 to 8.8 %<sup>(\*)</sup>
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) ranging from 5.0 to 19 %<sup>(\*)</sup>;
- a limit of quantification (LOQ) of 3 mg/kg.

(\*) the highest precision values were obtained for mineral mixes

Alternatively, the CEN ring-trial validated method (CEN/TS 15621) is based on ICP-AES after pressure digestion. The total zinc concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported [10] for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg: -  $RSD_r$  ranging from 1.5 to 5.4%; -  $RSD_R$  ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs*.

Furthermore, the EURL reminds that a Community method is available for the determination of total zinc in *feedingstuffs* [11]. The sample is brought into solution in hydrochloric acid after destruction of organic matter, if any. Zinc is then determined after appropriate dilution by AAS. No method performance characteristics are reported in the Regulation, except an LOQ of 20 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently organised a ring-trial [12] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets. Precisions ( $RSD_r$  and  $RSD_R$ ) ranging from 1.0 to 9.5 % were reported for samples containing total zinc levels ranging from 93 to 199 mg/kg *feedingstuffs*.

Based on these acceptable method performance characteristics the EURL recommends for official control the CEN methods (EN 15510 or CEN/TS 15621) to quantify total zinc content by ICP-AES in the *feed additive* and *premixtures*. As for the quantification of total zinc content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

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.

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#### 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the ISO/CD 17180 method, using ion exchange chromatography coupled to post-column derivatisation and photometric or fluorometric detection, to quantify *methionine* in *feed additive*
- the CEN methods EN 15510 and CEN/TS 15621 for the quantification of total zinc content by ICP-AES in the *feed additive* and *premixtures*;
- the Community method based on AAS and the above mentioned CEN method (EN 15510 or CEN/TS 15621) for the quantification of total zinc in the *feedingstuffs*;

##### ***Recommended text for the register entry (analytical method)***

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

- ISO/CD 17180: ion exchange chromatography method with post-column derivatisation and photometric or fluorometric detection:

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

- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES), or
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

For the quantification of total zinc in the *feedingstuffs*:

- Regulation (EC) No 152/2009 - Atomic Absorption Spectrometry (AAS); or
- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES); or
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *methionine-zinc* 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 SANCO/D/2: Forw. Appl. 1831/00146/(10185)/2010
  - [2] \*Application, Proposal for Register Entry – Annex A
  - [3] \*Technical dossier, Section II
  - [4] Commission Directive 88/485/EEC amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition
  - [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, Annexes: Annex 2.12\_Method of analysis Methionine\_VDLUFA (*i.e.* Methodenbuch, Band III, method 4.11.6)
  - [7] ISO/CD 17180 – *Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures*
  - [8] AOAC Official Method 999:13 – Lysine, Methionine and Threonine in Feed Grade Amino Acids and Premixes
  - [9] 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*
  - [10] CEN/TS 15621:2007 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*
  - [11] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (cf. Annex IV-C)
  - [12] \*Supplementary Information - Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010
- \* Refers to Dossier No. FAD-2010-0254



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## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European 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

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- Laboratori Agroalimentari, Departament d’Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils (ES)
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