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Institute for Reference Materials and Measurements  
**Community Reference Laboratory for Feed Additives**



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CRL Evaluation Report on the Analytical Methods submitted in  
connection with the Application for Authorisation as a Feed Additive  
according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2007-0050 CRL/070028
Name of Additive:	Zinc chelate of amino acids hydrate (Biokey Zn)
Active Substance(s):	Zinc (as chelate of amino acids hydrate)
Rapporteur Laboratory:	Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy
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## EXECUTIVE SUMMARY

In the current application authorisation is sought for *Zinc chelate of amino acids hydrate* (*Biokey Zn*) under the category 'nutritional additives', functional group 3(b), 'compounds of trace elements', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically authorisation is sought for *Biokey Zn* for all species, according to Article 4(1) of regulation (EC) No 1831/2003.

The product is intended to be marketed in a powder form containing 14 to 17 % Zinc chelate of amino acids hydrate as active agent. It is intended to be added to complete feed to supplement Zn for all species within legal limits for total zinc ranging from 150 to 250 mg/kg of *feedingstuffs* for all species.

For the determination of Zinc in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposed the ISO 11885:1998 standard method and the Lebensmittel-und Bedarfsgegenständegesetz (LMGB) method #35. These methods apply to water and food matrices while the applicant did not provide any experimental evidence to demonstrate the applicability of these methods to the matrices of relevance.

However, several official methods are available for the determination of total zinc in *premixtures* and *feedingstuffs*. The Community Method (Commission Regulation (EC) No 152/2009) for the determination of trace elements such as zinc in feed is based on the sample digestion by hydrochloric acid, followed by atomic absorption spectrometry (AAS) determination. Only one performance characteristic is provided: the limit of quantification (LOQ) of 20 mg/kg of *feedingstuffs*. The same experimental principles are applied in the ISO 6869 standard method, which was ring-trial validated on various matrices with total zinc concentrations ranging from 30 to 15000 mg/kg. The reported performance characteristics are:

- a relative standard deviation of repeatability ( $RSD_r$ ) ranging from 1.7 to 7.6 %;
- a relative standard deviation of reproducibility ( $RSD_R$ ) ranging from 3 to 15 %, and
- LOQ = 5 mg/kg of *feedingstuffs*.

Based on these acceptable performance characteristics the CRL recommends for official controls: - the Commission Regulation (EC) No 152/2009 and the ISO 6869 for the determination of total zinc in the *feedingstuffs* and - the ISO 6869 standard method for the determination of total zinc in *premixtures* and as well as in *feed additives*.

Further testing or validation is not considered necessary.

## KEYWORDS

Zinc, trace element, chelate, amino acids hydrate, nutritional additive, all species

## 1. BACKGROUND

*BioKey Zn* is a feed additive, for which authorisation is sought under the category 'nutritional additives', functional group 3(b), 'compounds of trace elements', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically authorisation is sought for *Biokey Zn* for all species, according to Article 10(2) of regulation (EC) No 1831/2003 [1].

The *product* to be marketed is a solid, free flowing, beige to light brown powder with a slight characteristic odour, containing 14% to 17% *Zinc chelate of amino acids hydrate* as active agent [2]. It is intended to be added to complete feed to supplement Zn for all species within legal limits set in Regulation (EC) No 1334/2003 for total zinc ranging from 150 to 250 mg/kg *feedingstuffs* [3].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *BioKey Zn* were evaluated for their suitability to be used for official controls.

### 3. EVALUATION

#### *Identification/Characterisation of the feed additive*

##### *Quantitative and qualitative 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, mercury, lead, dioxin and dioxin-like PCBs) are available at the respective Community Reference Laboratories [4].

##### *Description of the analytical methods for the determination of the active agent in the feed additive, premixtures and feedingstuffs*

For the determination of Zinc in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposed the ISO 11885:1998 standard method [5] and the Lebensmittel-und Bedarfsgegenständegesetz (LMGB) method §35 [6]. While the ISO method is applicable to raw, potable and waste waters, the LMBG method concerns the determination of trace elements in food matrices. The applicant did not provide experimental evidence demonstrating the applicability of these methods to the *feed additive*, *premixtures* and *feedingstuffs*.

However, several official methods are available for the determination of total zinc in *premixtures* and *feedingstuffs*: - the Community Method (EC) No 152/2009 [7], - ISO 6869 [8], - EN 15510 [9] and - VDLUFA VII.2.2.2.6 [10].

The Community Method for the determination of trace elements such as zinc in feed is based on the sample digestion by hydrochloric acid, followed by an atomic absorption spectrometry (AAS) determination. Only one performance characteristic is reported: the limit of quantification (LOQ) of 20 mg/kg of *feedingstuffs* [7].

The same experimental principles are applied in the ISO 6869 standard method [8], as follows: a test portion of 1 to 5 g of the prepared test sample is dissolved in hydrochloric acid, if necessary after ashing in a muffle furnace at  $(550 \pm 15) ^\circ\text{C}$ . Any silica compounds present are removed by precipitation and filtration. The precipitate is dissolved in hydrochloric acid, diluted and aspirated into the air-acetylene flame of the atomic absorption spectrometer. Absorbances are measured at 213.8 nm and compared with the absorbance of calibration solutions. Dry ashing must be applied for samples containing organic matter (i.e. *feedingstuffs*), while only wet

digestion/decomposition is to be used for samples containing little or no organic matter (i.e. *feed additives*, *premixtures* or *mineral feedingstuffs*).

The ISO 6869 method was ring-trial validated on various matrices (i.e. barley, milk powder, *premixtures* containing inorganic and organic matter silage and mixed feeds) with total zinc concentrations ranging from 30 to 15000 mg/kg. The method is applicable for the determination of total zinc in the *feed additives* since the assay protocol is suitable to make appropriate dilution after the digestion process of *feed additive* samples containing up to 17% zinc.

The reported performance characteristics are [8]:

- a relative standard deviation of repeatability ( $RSD_r$ ) ranging from 1.7 to 7.6 %;
- a relative standard deviation of reproducibility ( $RSD_R$ ) ranging from 3 to 15 %, and
- LOQ = 5 mg/kg of *feedingstuffs*.

These performance characteristics are considered acceptable.

Both EN 15510 [9] and VDLUFA VII.2.2.2.6 [10] methods are based on the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method for the determination of zinc in *premixtures* and *feedingstuffs*. The instrumentation used is different from the one recommended in the Community Method. Furthermore, the performance characteristics reported are not significantly better than those of ISO 6869.

Therefore, the CRL recommends for official controls: - the Commission Regulation (EC) No 152/2009 and the ISO 6869 for the determination of total zinc in the *feedingstuffs* and - the ISO 6869 standard method for the determination of total zinc in *premixtures* and as well as in *feed additives*.

Further testing or validation is not considered necessary.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official controls: - the Commission Regulation (EC) No 152/2009 and the ISO 6869 for the determination of total zinc in the *feedingstuffs* and - the ISO 6869 standard method for the determination of total zinc in *premixtures* and as well as in *feed additives*.

Further testing or validation is not considered necessary.

***Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)***

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

ISO 6869 - Atomic Absorption Spectrometry

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

Commission Regulation (EC) No 152/2009 and the ISO 6869

#### 5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of Biokey Zn have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

## 6. REFERENCES

- [1] \* Application, Reference SANCO/D/2 Forw. Appl. 1831/002-2008
- [2] \* Technical dossier, Section II: identity, characterisation and condition of use of the additive.
- [3] \* Application, Annex III. Description and Condition of Use of the Additive as Proposed by the applicant
- [4] Commission Regulation (EC) No 776/2006 amending Annex VII to Commission Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community reference laboratories, OJ L 136 (23 May 2006)
- [5] \* Technical Dossier, Annex\_II.5.1\_metal\_ISO.pdf
- [6] \* Technical Dossier, Annex\_II.5.1\_metal\_LMBG.pdf
- [7] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, OJ L 136 (27 Jan 2009)
- [8] ISO 6869 - Animal feeding stuffs -- Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc - Method using atomic absorption spectrometry
- [9] EN 15510 - Animal feeding stuffs. Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES
- [10] VDLUFA Methodenbuch VII 2.2.2.6 (2003); Bestimmung von ausgewählten Elementen in pflanzlichem Material und Futtermitteln mit ICP-OES

\* Refers to Dossier No: FAD-2007-0050

## **7. RAPPORTEUR LABORATORY**

The Rapporteur Laboratory for this evaluation was the Centro di Riferenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy. 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.

## **8. ACKNOWLEDGEMENTS**

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- Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands
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