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JOINT RESEARCH CENTRE

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

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FINAL REPORT

CRL Evaluation Report on the Analytical Methods submitted in connection with Section II, 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2008-0005 EFSA-Q-2008-381 CRL/080017
Product name:	Selsaf
Active Substance(s):	Selenium enriched yeast (*Saccharomyces caerevisiae* CNCM I-3399)
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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Date:	12/12/2008
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Date:	12/12/2008

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Selsaf* under the category/functional group (3/b), nutritional additives/compounds of trace elements, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use *Selsaf* as a source of selenium for all animal species. *Selsaf* is an inactivated Selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) product, containing high levels of the essential trace element selenium. The inactivated and dried Selenium enriched yeast product is blended with non viable dehydrated yeast (*Saccharomyces cerevisiae* CNCM I-3399) to adjust the selenium content. The final product is an inactivated whole cell yeast containing minimum 2000 mg/kg of total selenium with a maximum of 2% of residual inorganic selenium. At least 60% of the total organic selenium is in the form of seleno-methionine. *Selsaf* is added to the feedingstuffs to obtain a concentration of total Se up to 0.5 mg/kg.

The active substance is measured as total selenium regardless of its chemical form, i.e. independently of whether it is present as organically-bound or inorganic Se.

For the determination of the active substance in *Selsaf* either flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) methods are proposed by the applicant. Since both methods are based on well known principles, they are considered suitable for the determination of selenium in the feed additive.

For the determination of the active substance (total selenium) in premixtures and feedingstuffs the same two methods (FAAS and ICP-AES) are proposed. Since information on a complete validation study performed on the target feed was not provided, the suitability of these methods for official control purposes cannot be evaluated.

However, for official control regarding the determination of the active substance in premixtures and feedingstuffs, the CRL recommends an analytical method that has been ring-trial validated in the relevant matrices at the relevant concentrations of the active substance. The method and the results from the related inter-laboratory study are presented in the method collection of the “Association of German Agricultural Analytical and Research Institutes” (VDLUFA, Germany). The method for the determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion – based on the extraction with 65% nitric acid and 30% H_2O_2. The following method performance characteristics are reported: - a reproducibility relative standard deviation (RSD_R) of 7.3 % for a pre-mixture containing 112 mg/kg of Se; RSD_R = 7.4 % for a feedingstuffs containing 0.48 mg/kg of Se and the limits of quantification are clearly below the legal limit of 0.5 mg Se /kg

feed and therefore acceptable for the purpose of analysis. This VDLUFA method is currently being adopted as a CEN standard.

No further testing or validation is required.

KEYWORDS

Selsaf, selenium enriched yeast (*Saccharomyces cerevisiae)*, nutritional additive, all species

1. BACKGROUND

Selsaf is a feed additive belonging to the category nutritional additives and the functional group ”compounds of trace elements”(3/b). It contains an inactivated whole cell yeast (*Saccharomyces cerevisiae*) containing minimum 2000 mg/kg of total selenium of with a maximum of 2% residual inorganic selenium. At least 60% of the total organic selenium is in the form of seleno-methionine. In the current application (cf. EFSA-Q-2008-381) the selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) is intended as a source of selenium in an organic form for all animal species.

Selsaf is added to the feedingstuffs obtaining a concentration of the feed additive in the feed of 250 mg/kg which corresponds to a concentration of selenium in the feedingstuffs of 0.5 mg/kg.

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 the 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 (EFSA) for each application. For this particular dossier, the methods of analysis, submitted in connection with *Selsaf* (EFSA-Q-2008-381), and their suitability to be used for official controls in the frame of authorisation, were evaluated.

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 impurities in the *additive* (e.g. heavy metals or other undesirable substances) are available at the respective Community Reference Laboratories [1].

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

Methods capable of measuring selenium methionine (SeMet), - the dominant component of organically-bound selenium, are missing, therefore organic and inorganic sources of selenium can not be distinguished. As consequence, the active substance is measured as total selenium regardless of its chemical form, i.e. independently of whether it is present as organically-bound or inorganic Se.

For the determination of the active substance (total selenium) in the *feed additive* (selenium enriched yeast) two methods are proposed using either flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) [2]. The FAAS method includes a digestion of the sample with a mixture of nitric acid/hydrochloric acid and the amount of selenium is determined comparing the signal of the sample with those of the standard solutions. In the ICP-AES method, the sample is digested with nitric acid and hydrochloric acid in closed vessels in a microwave oven. Selenium is quantified either by applying the standard addition technique or by the internal standard method to compensate for matrix effects. Since both methods are based on well known principles, they are considered suitable for the determination of selenium in the *feed additive*.

Total selenium content in *premixtures* is performed either by FAAS or by ICP-AES method applying the method mentioned above [2]. However, since insufficient validation data were provided the suitability of this method for official control purposes could not be evaluated.

For official control regarding the determination of the active substance in *premixtures* and *feedingstuffs*, the CRL recommends an analytical method that has been ring-trial validated at relevant concentrations of the active substance in relevant matrices. The method and the results from the related inter-laboratory study are presented in the method collection of the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany) [3]. The method for the determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion – based on the extraction with 65% nitric acid and 30% H_2O_2. The following method performance characteristics are reported: - a reproducibility relative standard deviation (RSD_R) of 7.3 % for a pre-mixture containing 112 mg/kg of Se; RSD_R = 7.4 % for a feedingstuffs containing 0.48 mg/kg of Se and the limits of quantification are clearly below the legal limit of 0.5 mg Se /kg feed. This method is therefore recommended by the CRL-FA for official control in the frame of this authorisation. Furthermore, this VDLUFA method is currently being adopted as a CEN standard [4].

No further testing or validation is required.

4. CONCLUSIONS AND RECOMMENDATIONS

For official control of the total selenium content in *feedingstuffs* around the legal limit of 0.5 mg Se /kg of complete feedingstuffs - the CRL recommends the Draft CEN method "Animal feeding stuffs: - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (extraction with 65% nitric acid and 30% hydrogen peroxide)".

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

Determination of total selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (extraction with 65% nitric acid and 30% hydrogen peroxide).

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Selsaf 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] 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 Community reference laboratories, Official Journal of the European Union L 136.

[2] Section 2 - Appendix 2.7: FAAS and ICP methods

[3] VDLUFA Methodenbuch III, 1993, Selen 11.6.1

[4] CEN-method DRAFT: "*Animal feeding stuffs: - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (extraction with 65% nitric acid and 30% hydrogen peroxide)*"
CEN/TC 327 WI 00327058:2007 (version March 2007).

* Refers to Dossier No: FAD-2008-0005

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community Reference Laboratory for Feed Additives, IRMM, Geel, Belgium.

8. ACKNOWLEDGEMENTS

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- Plantedirektoratet Laboratorium, Lyngby, Danemark
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