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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:	EFSA-Q-2005-117
Name of Additive:	*Alkosel®/Selsaf*
Active Substance:	Selenium
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 *Alkosel®/Selsaf* under the category/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 *Alkosel®/Selsaf* as a source of selenium for all animal species. *Alkosel®/Selsaf* is an inactivated Selenium enriched yeast *(Saccharomyces cerevisiae)* 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)* to adjust the selenium content. The final product is an inactivated whole cell yeast containing minimum 2000 mg/kg of total selenium of which 2% are residual inorganic selenium. At least 60% of the total organic selenium is in the form of selenomethionine. The active substance in *Alkosel®/Selsaf* is selenium (Se). *Alkosel®/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.

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

For the determination of the active substance in *Alkosel®/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 determination of the active substance (total selenium) in premixtures and feedingstuffs also two methods based on FAAS or ICP-AES are proposed. Since information on a complete validation study performed on the target feed was not available, the suitability of this method for official control purposes cannot 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 fully ring trial validated at relevant concentrations of the active substance in relevant matrices. The method and the results from the related interlaboratory study are presented in the method collection of the “Association of German Agricultural Analytical and Research Institutes” (VDLUFA, Germany). The obtained method performance characteristics for this method are considered acceptable, since the relative between-laboratory reproducibility standard deviation (RSD_R) for a premixture containing 112 mg/kg of Se was 7.3 % and the between-laboratory RSD_R for a feedingstuff matrix containing 0.48 mg/kg of Se was 7.4 %. However, the validated method includes different options for the mineralisation procedure and also for the type of

instrumentation, since either Zeeman graphit furnace Atomic Absorption Spectrometry (AAS) or Hydrid AAS can be used for the final measurement of Se. Therefore, the laboratory has to select a specific analytical procedure based on these options and must demonstrate that the method performance criteria as obtained in the ring trial can be met. The VDLUFA method shows different limits of quantification depending on the specific analytical procedure selected, but they are all sufficiently below the legal limit of 0.5 mg Se /kg feed and therefore acceptable for the purpose of analysis.

For the determination of selenium in target tissues and animal products the applicant proposed the same methods as described for the quantification of selenium in premixtures and feeds, but without submitting corresponding validated data. Validated methods are available in the literature such as a recently published method aiming at the determination of selenium in chicken meat and using inductively coupled plasma mass spectrometry (ICP-MS) for analysis. The obtained method performance characteristics included a limit of detection (LOD) of 83 μg/kg, a relative recovery rate ranging from 97 to 100 % and a relative standard deviation of about 3 %. (*J. Anat. At. Spectrom., 2004, 19, 1361-1369*). However, since there are no legal limits for the active substance in animal products fixed by the European legislation, the suitability for official control purposes cannot be evaluated.

Further testing or validation is not considered necessary.

KEYWORDS

ALKOSEL®, SELSAF, Selenium enriched yeast (*Saccharomyces cerevisiae)*, nutritional additive, all species

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1. BACKGROUND

Alkosel®/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 which 2% are residual inorganic selenium. The intended use (*cf*. EFSA-Q-2005-117) of the current application is to enhance the selenium enriched yeast (*Saccharomyces cerevisiae)* for all animal species, by mixing the feed additive into premixtures or feedingstuffs.

Alkosel®/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 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 suitability of the control methods and validation studies submitted in connection with *Alkosel®/Selsaf, cf.* EFSA-Q-2005-117, were evaluated.

3. EVALUATION

The numbering system under this point refers to that of Section II of the Annex of Commission Directive 2001/79/EC (2.5 Control methods). The method protocols and corresponding validation data are given in Section II of the dossier.

Description of the methods used for the determination of the criteria listed (cf. pt. 2.5.1 of Commission Directive 2001/79/EC)

Determination of the yeast strain

Identification of the strain was performed by Polymerase Chain Reaction (PCR) and the method is considered suitable for the intended purpose.

Determination of the chemical form (s) of selenium

The identification of the chemical form(s), known as "speciation" of selenium was performed by two different methods. The first method involved a treatment of the sample with various solutions for the separation of the Se-species and a mixture of HNO_3/H_2O_2 for the determination of total Selenium. Se-species were determined by High Performance Liquid Chromatography coupled to inductively coupled plasma mass spectrometry (HPLC ICP-MS). The second method involved an enzymatic digestion using protease XIV. The digestate was analysed by cation exchange HPLC for separation of the positively charged selenium species (seleno amino acids), and the selenium-selective detection was carried out on-line by ICP-MS of ^{80}Se. Both methods showed that selenomethionine was the dominating species detected in the yeast. Selenomethionine accounted for between 60 and 76% of the selenium extracted after proteolysis. These methods are quite laborious but useful to get information on Se-speciation and thus information on the fraction of organic Se.

Stability of the additive

The applicant performed studies according to the International Committee on Harmonization (ICH) guidelines for total selenium and selenomethionine. Stability was evaluated at room temperature and in accelerated condition. An inductively coupled plasma atomic emission spectrometry (ICP-AES) method was used for the detection of total selenium, while liquid chromatography after amino acid release by hydrolysis was utilise to determinate selenomethionine. These methods are considered suitable for the purpose.

Stability during the preparation and storage of the feed

Studies of stability of the additive during preparation and storage of premixtures and during storage of feed were performed. For total selenium analysis an ICP-AES method was used, while the analysis of selenomethionine was performed by liquid chromatography. The methods are considered suitable for this purpose.

Quantitative analysis of active substance (total selenium content) in the feed additive

For the determination of the active substance (total selenium) in selenium enriched yeast either flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) methods are proposed.

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. Concerning the ICP-AES method, the sample is digested with nitric acid/ hydrochloric acid in closed vessels in a microwave oven. Selenium is quantified either by applying the standard addition technique or by utilising 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.

Description of the qualitative and quantitative analytical methods for routine control of the active substance in premixtures and feedingstuffs cf. pt. 2.5.2 of Commission Directive 2001/79/EC).

Total selenium content in premixtures is performed either by FAAS or by ICP-AES method applying the same method as mentioned above. However, since information on a complete validation study performed on premixtures and feedingstuffs were not available, the suitability of this method for official control purposes cannot be evaluated.

For official control regarding the determination of the active substance in premixtures and in feedingstuffs, the CRL recommends a fully ring trial validated analytical method that has been conducted at relevant concentrations of the active substance in relevant matrices. The method and the results from the related interlaboratory study are presented in the method collection of the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany) [1]. The obtained method performance characteristics for this method are considered acceptable, since the relative between-laboratory reproducibility standard deviation (RSD_R) for a premixture containing 112 mg/kg of Se was 7.3 % and the between-laboratory RSD_R for a feedingstuff matrix containing 0.48 mg/kg of Se was 7.4 %. However, the validated method includes different options for the mineralisation procedure and also for the type of instrumentation, since either Zeeman graphit furnace Atomic Absorption Spectrometry (AAS) or Hydrid AAS can be used for the final measurement of Se. Therefore, the laboratory has to select a specific analytical procedure based on these options and must demonstrate that the method performance criteria as obtained in the ring trial can be met. The VDLUFA method shows different limits of quantification depending on the specific analytical procedure selected, but they are all sufficiently below the legal limit of 0.5 mg Se /kg feed and therefore acceptable for the purpose of analysis.

Description of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products (cf. pt. 2.5.3 of Commission Directive 2001/79/EC).

For the determination of selenium in target tissues and animal products the applicant proposed a method measuring selenium either with FAAS of with ICP-AES. However, a complete validation of the method is missing and therefore the suitability of the method for the intended purpose cannot be evaluated. Alternatively, another method can be applied which has been in-house validated and published in a peer reviewed journal and which utilised ICP-MS [2]. A closed vessel microwave mineralization procedure is performed for chicken meat samples with a mixture of nitric acid/hydrogen peroxide. Quantification is based on standard addition of known amounts of Se. The obtained method performance characteristics included a limit of detection (LOD) of 83 μg/kg, a relative recovery rate ranging from 97 to 100 % and a relative standard deviation of about 3.%. Since the performance characteristics are considered acceptable, the method is considered suitable for the detection of selenium at the concentration level, at which the validation was conducted. However, since there are no legal limits for the active substance in animal products fixed by the European legislation, the suitability for official control purposes cannot be evaluated.

CHECKLIST

		Y	N	N/A	Comments
1.1	Is/Are the method(s) mentioned on Premixtures accompanied by information on:				
	- Sampling Method used		**x**		Not sufficient validation data on the target matrix
	- Percentage Recovery		**x**		
	- Specificity		**x**		
	- Accuracy		**x**		
	- Precision		**x**		
	- Limits of detection		**x**		
	- Limits of quantification		**x**		
	- Validation procedure used				
1.2	Is/Are the method(s) mentioned on Feedingstuffs accompanied by information on:				
	- Sampling Method used		**x**		Not sufficient validation data on the target matrix
	- Percentage Recovery		**x**		
	- Specificity		**x**		
	- Accuracy		**x**		
	- Precision		**x**		
	- Limits of detection		**x**		
	- Limits of quantification		**x**		
	- Validation procedure used				
2.1	Is/Are the method(s) mentioned on Target tissues accompanied by information on:				
	- Sampling Method used		**X**		Not sufficient validation data on the target matrix
	- Percentage Recovery		**X**		
	- Specificity		**X**		
	- Accuracy		**X**		
	- Precision		**X**		
	- Limits of detection		**X**		
	- Limits of quantification		**X**		
	- Validation procedure used				

4. CONCLUSIONS AND RECOMMENDATIONS

The evaluation of the analytical methods related to the dossier of the additive ALKOSEL®, SELSAF revealed that the information on a complete validation study were not available. Therefore, the CRL proposes for the detection of total Selenium in feedingstuffs the procedures recommended by the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, German). This method is also suitable for official control purposes.

For the determination of selenium in target tissues and animal product a validated method is reported in scientific literature and could be proposed for official control purposes. However, since there are no legal limits for the active substance in animal products fixed by the European legislation, the suitability for official control purposes cannot be evaluated.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of ALKOSEL®, SELSAF have been sent to the Community Reference Laboratory for feed additives authorisation.

6. REFERENCES

[1] VDLUFA Methodenbuch III, 1993, Selen 11.6.1

[2] R. Bou at all. “Validation of mineralization procedures for the determination of selenium, zinc, iron and copper in chicken meat and feed samples by ICP-AES and ICP-MS” *J. Anat. At. Spectrom., 2004, 19, 1361-1369*

7. RAPPORTEUR LABORATORY

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