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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:	FAD-2007-0011 EFSA-Q-2007-094
Product name:	Mintrex® Mn
Active Substance(s):	Manganese (as manganese chelate of hydroxy analogue of methionine)
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Date:	30/11/2007

EXECUTIVE SUMMARY

Mintrex® Mn is a product for which authorisation is sought under the category "nutritional additives", functional group 3b "compounds of trace elements", according to the classification system of Annex I, of Regulation (EC) No 1831/2003. According to the applicant, *Mintrex® Mn* contains 13% of Manganese as chelate of hydroxyl analogue of methionine, 2-hydroxy-4-methylthiobutanoic acid (HMTBa) as active substance. *Mintrex®Mn* is also a source of methionine activity as HMTBa.

In the current application authorisation is sought for use of *Mintrex®Mn* for all animal species. *Mintrex®Mn* is intended to be added to complete feed to supplement Mn within legal limits for each species which are: fish 100 mg/kg, other species 150 mg/kg.

For the determination of Mn in the feed additive, premixtures and feedingstuffs for official control the CEN standard method EN 15510:2007, as proposed by the applicant, is recommended by the CRL.
The proposed methods for the determination of HMTBa are considered suitable for the intended purpose.

KEYWORDS
Mintrex®Mn, Manganese, 2-hydroxy-4-methylthiobutanoic acid (HMTBa), chelate, nutritional additive, all species.

BACKGROUND

Mintrex®Mn is a product for which authorisation is sought under the category "nutritional additives", functional group 3b "compounds of trace elements" according to Annex I of Regulation (EC) No 1831/2003. According to the applicant, *Mintrex®Mn* contains at least 13% Manganese as Mn-$(HMTBa)_2$ chelate and 76% of 2-hydroxy-4-methylthiobutanoic acid (HMTBa), the remaining is feed grade mineral oil (excipient) [1-2].

The intended use (*cf*. EFSA-Q-2007-098) of the current application is for all animal species, as a source of essential trace mineral Manganese. The feed additive is intended to be mixed in complete feed to supplement Mn within EU legal limits for each species which according to Regulation (EC) No 1334/2003 are: fish 100 mg/kg, other species 150 mg/kg [2]. Feed formulation should be adjusted to account for the methionine activity of HMTBa.

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 *Mintrex®Mn* dossier (EFSA-Q-2007-094) and their suitability to be used for official controls in the frame of the authorisation were evaluated.

EVALUATION

Description of the methods used for the determination of the criteria listed under item 2.5.1 of Commission Directive 2001/79/EC

Degree of purity – qualitative and quantitative composition of the impurities and toxic substance

Analytical methods for official control of undesirable substances, when required by EU legislation (i.e. aflatoxins, cadmium, lead, dioxins, and dioxins-like PCBs) are available at the competent official control laboratories.

Description of the analytical methods for the control of the active substance in the feed additive, premixtures and feedinstuffs

Determination of manganese

For the determination of manganese in the feed additive (*Mintrex®Mn*), premixtures and feed, the applicant proposed published CEN, ISO or AOAC methods.

Method 1

Mn was determined by flame atomic absorption spectrometry (FAAS) using an ISO method [3]. A test portion was dissolved in hydrochloric acid, after ashing in a muffle furnace at 550 ± 15 °C. The residue was dissolved with hydrochloric acid and diluted to the appropriate volume with water. The test solution was then aspirated into the air-acetylene flame of atomic absorption spectrometer. The determination of manganese was performed at 279,5 nm. The absorbance of the test solution was measured by comparison with the absorbance of calibration solutions of Mn. The limit of determination of the method was 5 mg/kg. The precision of this method was established by interlaboratory tests. Relative standard deviation for repeatability (RSD_r%) and relative standard deviation for reproducibility (RSD_R%) are reported in Table 1.

Method 2

In the AOAC method [4], the sample (1 g) was dry-ashed at 500°C for 2 h; after cooling the ashes were treated with nitric acid. Nitric acid excess was evaporated on hot plate and the sample was returned to furnace and ashed for 1h a 500 °C. The residue was dissolved in hydrochloric acid and diluted to 50 ml with water. Zn was determined by inductively coupled plasma emission spectroscopy (ICP-AES). The determination of Mn was performed at 2576 Å. The concentration of manganese was determined using external calibration.

Method 3

Manganese was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a European Committee for Standardisation (CEN) method [5]. A test portion of the sample was digested under pressure with nitric acid and hydrogen peroxide, then the digestion solution was diluted to the appropriate volume with water. The test solution was then aspirated into the plasma and measured the emission of manganese was determined.

The determination of manganese was performed at 257,6 nm. The concentration of manganese was determined using external calibration or standard addition technique. The LOQ of the method was 3 mg/kg. The precision of this method was established by an interlaboratory test. Relative standard deviation for repeatability (RSD_r%) and relative standard deviation for reproducibility (RSD_R%) are reported in Table 1.

Table 1: performance profiles of the methods

		Premixtures	Feed
FAAS [3]	RSD_r%	2,8	2,0
	RSD_R%	12,1	13,4
ICP-AES [5]	RSD_r%	4,6	4,1
	RSD_R%	9,0	6,8

Determination of HMTBa

For the determination of HMTBa in the feed additive (*Mintrex®Mn*) and premixtures, an in house method [6] which performed a titration with bromide/bromate was proposed by the applicant. The method was based on a red-ox reaction: bromate reacted with bromide under acid conditions to produce bromine. The bromine liberated under acid conditions oxidizes organic sulphates present in the sample. All titrations were performed in triplicate with the average being the reported value. If the range of the three titrations exceeded 0,4% in measured assay, the analysis was suspect and additional samples were analysed. Fourier transform infra-red spectrometry (FT-IR) [7] and crystal X-ray diffraction [8] studies were performed to demonstrate that the carboxylate group of HMTBa was coordinated to manganese.

This method is considered suitable for the intended purpose.

For the determination of HMTBa in feedingstuffs, a high performance liquid chromatography (HPLC) in house validated method [9] is proposed by the applicant. An amount of sample was shaken with an extraction solution (1/9 acetonitrile/water), following extraction, an aliquot of the sample was shaken with potassium hydroxide and then added with concentrated phosphoric acid. After centrifugation a portion of the supernatant was analysed by reversed phase chromatography using an ultraviolet detector at 214 nm. Standards for calibration are prepared by supplementing a typical commercial starter diet of corn, soy bean meal, vitamin-

mineral premix, and fat with different levels of HMTBa. A calibration curve was generated by plotting peak area counts against the amount of HMTBa present in these standards, which were extracted and treated according to the sample preparation procedure. The recovery was 97% with a relative standard deviation of 3,7 %. The method is considered suitable for the intended purpose.

CONCLUSIONS AND RECOMMENDATIONS

For the determination of Mn in the feed additive, premixtures, and feedingstuffs for official control the CEN standard method EN 15510:2007 is recommended by the CRL.

The proposed methods for the determination of HMTBa are considered suitable for the intended purpose.

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

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.

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of *Mintrex®Mn* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

REFERENCES

The dossier provided by the applicant is divided into various documents structured according to the Annex of Commission Directive 2001/79/EC, containing the following files:

[1] Technical dossier, Annex II 2.1.3

[2] Annex III Register Entry

[3] BS EN ISO 6869:2001, BS 5766-25:2001
[4] AOAC Official Method 985.01
[5] CEN Standard EN 15510:2007
[6] Technical dossier, Annex II 2.5.1.1
[7] Technical dossier, Section II 2.2.4.1
[8] Technical dossier, Annex II 2.2.4.2
[9] Technical dossier, Annex II 2.5.1.2

RAPPORTEUR LABORATORY

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