EUROPEAN COMMISSION
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European Union Reference Laboratory for Feed Additives

JRC.D.5/FSQ/CvH/CMP/ag/ARES(2012)138974

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

Dossier related to:	FAD-2010-0196 CRL/100007
Name of Additive:	Inositol
Active Substance(s):	Inositol
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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Report revised by: Date:	Piotr Robouch (EURL-FA) 07/02/2012
Report approved by: Date:	Christoph von Holst 07/02/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Inositol* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories. According to the Applicant, *Inositol* is an odourless white crystalline powder with a minimum purity of 97 %. The *feed additive* is intended to be incorporated in *premixtures* and *feedingstuffs.* The Applicant did not specify any minimum or maximum concentrations of *Inositol* in *feedingstuffs*, however, the following doses were recommended: from 350–500 mg /kg for salmonids and a maximum of 1000 mg/kg for laying hens.

For the quantification of *Inositol* in the *feed additive, premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on the microbiological activity analysis. The following performance characteristics were reported for *feed additive, premixtures* and *feedingstuffs*:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1.9 to 9.5 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.2 to 16.2 %;
- a recovery rate (R_{rec}) ranging from 82 to 119 %; and
- a limit of detection and quantification (LOD and LOQ) of 4 and 8 mg/kg, respectively.

Additionally, for the identification of *Inositol,* the EURL identified the internationally recognised European Pharmacopoeia method (Ph. Eur. 01/2008:1805), based on liquid chromatography and infrared absorption spectrophotometry. Even though no performance characteristics are provided the EURL considers this method to be suitable within the frame of official control.

Based on these performance characteristics, the EURL recommends for official control the European Pharmacopeia method (monograph 1805) based on liquid chromatography for the identification of *Inositol* in *feed additive* and the single-laboratory validated and further verified method based on microbiological activity analysis for the quantification of *Inositol* in *feed additive, premixtures* and *feedingstuffs*.

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

Inositol, nutritional additive, pro-vitamins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use) and 10(2) (re-evaluation of the additive already authorised under provisions of Council Directive 70/524/EEC) for *Inositol* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2].

According to the Applicant, *Inositol* is produced by chemical synthesis with a minimum purity of 97% [3]. The *feed additive* is intended to be incorporated in complete or complementary *feedingstuffs* through *premixtures*. The Applicant did not specify any minimum or maximum concentrations of *Inositol* in *feedingstuffs*, however, the following doses were recommended: from 350–500 mg /kg for salmonids and a maximum of 1000 mg/kg for laying hens [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 (EFSA) for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Inositol*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive
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, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [5].

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

For the quantification of *Inositol* in the *feed additive, premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated [6] and further verified [7] method based on microbiological activity analysis.

In order to quantify bio available (total) *Inositol*, consisting of endogenous/bound inositol and added/free *Inositol*, feed samples need to be first hydrolysed. 2 g of samples are transferred into hydrolysis tubes and 40 ml of HCl is added. The samples are then placed in a drying cabinet for 10 hours at 125 °C. After cooling the samples are filtered and an aliquot is transferred to a volumetric flask where phosphate buffer is added and pH adjusted to 4.5. The extract is filtered and diluted.

Saccharomyces carlsbergensis (ATCC 90980, DSM 70424) is inoculated in liquid malt agar extract, containing all required nutrients for growth, except *Inositol*. The standard solution containing *Inositol* or the hydrolysed sample is then added to the microorganism growth solution. The principle of the method is based on the growth of the microorganism in the presence of *Inositol*, inducing an increase on turbidity monitored by spectrophotometry. After an incubation period of 15 hours at 32 °C, turbidity is analysed in a 2 cm cuvette using a photometer at 540-560 nm and compared to calibration solutions with known *Inositol* concentrations.

The performance characteristics are listed in Table 1. Furthermore the Applicant reported a limit of detection and of quantification (LOD and LOQ) of 4 and 8 mg/kg *feedingstuffs.*

Table 1: Performance characteristics for the determination of *Inositol* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS)

	Conc. (g/kg)	RSD_r (%)		RSD_{ip} (%)		R_{rec} (%)	
		Validation [6]	Verification [7]	Validation [6]	Verification [7]	Validation [6]	Verification [7]
FA	960-990	1.9	4.3	1.2	8.1	100-101	102
PM	15-120	4.8	3.1	7.3	4.7	103-119	104
FS	0.8-0.9	4.4	9.5	16.2	14.3	82-92	83

RSD_r and RSD_{ip}= relative standard deviation for *repeatability* and *intermediate precision*;
R_{rec} = recovery rate; Conc= Concentration

Additionally, the EURL identified the internationally recognised European Pharmacopoeia method (Ph. Eur. 01/2008:1805) [8] based on liquid chromatography and infrared absorption spectrophotometry for the identification of *Inositol* in the *feed additive*. Even though no performance characteristics are provided the EURL considers this method to be suitable within the frame of official control.

Based on these performance characteristics, the EURL recommends for official control the European Pharmacopeia method (Ph. Eur. 01/2008:1805) based on liquid chromatography and infrared absorption spectrophotometry for the identification of *Inositol* in *feed additive* and the single-laboratory validated and further verified method based on microbiological activity analysis for the quantification of *Inositol* in *feed additive, premixtures* and *feedingstuffs*.

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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the European Pharmacopeia method (Ph. Eur. 01/2008:1805) based on liquid chromatography and infrared absorption spectrophotometry for the identification of *Inositol* in *feed additive;* and the single-laboratory validated and further verified method based on microbiological activity analysis for the quantification of *Inositol* in *feed additive, premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification of *Inositol* in *feed additive*:

- Liquid Chromatography and infrared absorption spectrophotometry (Ph. Eur. 01/2008:1805)

For the quantification of *Inositol* in the *feed additive, premixtures* and *feedingstuffs:*

- Single laboratory validated and verified Microbiological activity analysis for the quantification of *Inositol* in the *feed additive, premixtures* and *feedingstuffs*

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5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Inositol* 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. 1831/00115-2010
[2] *Application, Proposal for Register Entry – Annex A
[3] *Technical dossier, Section II, 2.2.1.1. Chemical substances
[4] *Technical dossier, Section II, 2.5.1. Proposed mode of use 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, Annex_II_12
[7] *Technical Dossier, SectionI,Annex_II_VITAC inositol(…) verification
[8] European Pharmacopoeia Ph. Eur. 01/2008:1805

* Refers to Dossier No. FAD-2010-0196

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Italy
- Plantedirektoratet, Laboratorium for Foder og Gødning, Denmark
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Slovenia
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)