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

Coenzyme Q10

(FAD-2012-0031; CRL/110028)

European Union Reference Laboratory
Feed Additives

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-2012-0031- CRL/110028**
Name of Product	***Kaneka Q10***
Active Substance:	**Coenzyme Q10**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium**
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Date:	**04/02/2014**
Report approved by:	**Christoph von Holst**
Date:	**04/02/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Kaneka Q10* under the category/functional group 3(a) "nutritional additives" / "vitamins, provitamins and chemically well-defined substances having similar effect", according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for all animal species. *Kaneka Q10* is a yellow to orange crystalline powder containing at least 98% of *coenzyme Q10. Kaneka Q10* is intended to be used in *premixtures* and *feedingstuffs*. The Applicant specified no maximum or minimum concentration of *coenzyme Q10* in *feedingstuffs* but recommend a maximum dosage of 100 mg/kg for all animal species.

For the determination of *coenzyme Q10* in the *feed additive* the Applicant submitted High Performance Liquid Chromatography method with UV detector (HPLC-UV) described in the Japanese Pharmacopoeia. The EURL recommends this method for official control to determine *coenzyme Q10* in the *feed additive.* For the determination of *coenzyme Q10* in *premixtures* and *feedingstuffs* the Applicant submitted a High Performance Liquid Chromatography method with UV detector (HPLC-UV) based on the Japanese Pharmacopoeia. Based on the satisfactory experimental evidence provided, the EURL recommends for official control the above mentioned HPLC-UV method for the determination of *coenzyme Q10* in *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. Based

KEYWORDS

Kaneka Q10, *coenzyme Q10*, nutritional additives, vitamins, pro-vitamins and chemically well-defined substances having similar effect, all animal species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *Kaneka Q10* under the category/functional group 3(a) "nutritional additives" / "vitamins, provitamins and chemically well-defined substances having similar effect", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for the use of the *feed additive* for all animal species [2].

Kaneka Q10 is a yellow to orange crystalline powder containing at least 98% of *coenzyme Q10* (also called ubidecarenone) [3]. It is intended to be used in *premixtures* and *feedingstuffs*. The Applicant specified no maximum or minimum concentration of *coenzyme Q10* in *feedingstuffs* but recommended a dosage of 100 mg/kg for all animal species [3].

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 for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Kaneka Q10,* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Qualitative and quantitative composition of impurities in the feed additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive such as heavy metals (arsenic, cadmium, lead and mercury), dioxins, microbiological agents and mycotoxins are available from the respective European Union Reference Laboratories [4].

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

For the determination of *coenzyme Q10* in the *feed additive* the Applicant proposed the Japanese Pharmacopoeia method [5]. Identification is based on colour reaction in the presence of potassium hydroxide and infrared spectrophotometry while quantification is based on High Performance Liquid Chromatography with UV detector (HPLC-UV) method at 275 nm. The EURL recommends this method for official control to determine the *coenzyme Q10* in *the feed additive.*

Furthermore, the Applicant applied this HPLC-UV method for the determination of the *coenzyme Q10* in *premixtures* and *feedingstuffs* and provided a detailed description of the sample preparation [6]. In order to prevent degradation of the *coenzyme Q10* amber glassware is used and the sample preparation is to be carried out in room temperature (below 25°C). Pelleted feed samples should be milled before extraction. A sample (1 g, *premixtures* or

feedingstuffs) is extracted with n-hexane (7.5 ml) and methanol in 50 ml volumetric flask. An aliquot (1 ml) is filtered and analysed with HPLC-UV. Quantification is performed by using external calibration with coenzyme Q10 standard solutions. The performance characteristics which were obtained in the frame of validation and verification studies [7, 8, 9, 10] are presented in Table 1. Furthermore, the Applicant determined a limit of quantification (LOQ) of 8.6 mg/kg [7].

Based on the satisfactory experimental evidence available the EURL recommends for official control the method based on the Japanese Pharmacopoeia submitted by the Applicant for the determination of *coenzyme Q10* in *premixtures* and *feedingstuffs*.

Table 1: Performance characteristics of analytical method for the determination of *coenzyme Q10* in *premixtures* (PM) and *feedingstuffs* (FS). The *coenzyme Q10* content is indicated between parenthesis.

	RSD_r (%)		RSD_{ip} (%)		R_{rec} (%)	
	Val	**Ver**	**Val**	**Ver**	**Val**	**Ver**
PM [10] (10 g/kg)	2.3	6.8	4.0	7.0	95.1 *	93.1 #
FS [9] 100 mg/kg	1.8	2.9	1.8	3.1	80.1	92.0

RSD_r and RSD_{ip}: relative standard deviation for repeatability and intermediate precision, R_{rec}: recovery rate
* recalculated using [8 Table 4.3] # recalculated using [10 table 3-3]

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 Japanese Pharmacopeia method using High-Performance Liquid Chromatography with UV detector submitted by the Applicant for the determination of *coenzyme Q10* in the *feed additive* and HPLC-UV method based on the Japanese Pharmacopeia for the determination of *coenzyme Q10* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

Determination of *coenzyme Q10* in the *feed additive*:

- High-Performance Liquid Chromatography with UV detector (HPLC-UV) - Japanese Pharmacopeia monograph 'Ubidecarenone'

Determination of *coenzyme Q10* in *premixtures* and *feedingstuffs*:

- High-Performance Liquid Chromatography with UV detector (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference sample of *Kaneka Q10* 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] *Reference SANCO/D/2 Forw. Appl. 1831/0067-2012

[2] *Application, Proposal for Register Entry

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; methods of analysis

[4] 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

[5] Technical dossier, Section II – Annex 2.6.1.a, Japanese Pharmacopoeia monograph on Ubidecarenone

[6] Supplementary information - 2.6 CoQ10 analytical method (Premix and Feed)

[7] Supplementary information - Verification Report (CoQ10 Feed)_KANEKA

[8] Supplementary information - Verification Report (CoQ10 Premix)_KANEKA

[9] Supplementary information - Verification JRC validation CRL guidance 2008 feed(6)

[10] Supplementary information - 2.6 Verification JRC validation CRL guidance 2008 premix

*Refers to Dossier no: FAD-2012-0031

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the 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:

- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)