



JRC.D.5/FSQ/CvH/PRO/ag/ARES(2012)214390

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

Dossier related to: FAD-2010-0337 – CRL/100148
FAD-2010-0371 – CRL/100295
FAD-2010-0402 – CRL/100302

Feed Additive Name: Cobalt Group

Active Substance(s): E 3
Cobaltous acetate, tetrahydrate
Cobaltous carbonate
basic Cobaltous carbonate, monohydrate
Cobaltous sulphate, heptahydrate

Rapporteur Laboratory: European Union Reference Laboratory
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Date: 24/02/2012

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EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate; Cobaltous sulphate, heptahydrate* under the category of "nutritional additives" functional group 3b (compounds of trace elements), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species.

The Applicants stated minimum total cobalt contents of 23% in *Cobaltous acetate, tetrahydrate*; 45% in *Cobaltous carbonate* and/or *basic Cobaltous carbonate, monohydrate*; and 20% in *Cobaltous sulphate, heptahydrate*.

The *feed additives* are intended to be incorporated into *premixtures, feedingstuffs* and *water*. Applicants (FAD-2010-0227 and FAD-2010-0371) suggested maximum levels of total cobalt of 2 mg/kg *feedingstuffs* and 1 mg/L *water* to comply with the limits set in Regulations (EC) No 1334/2003. Applicant (FAD-2010-0402) took into consideration the EFSA Scientific opinion recommending to reduce the maximum level of Cobalt to 1 mg/kg *feedingstuffs*.

For the identification of the various cobalt compounds in the *feed additives* the EURL recommends the general identification tests of acetates, carbonates and sulphates, described in the European Pharmacopoeia (Monograph 01/2008:20301). Additionally the EURL recommends crystallographic techniques, such as X-Ray diffraction, for the characterisation of crystalline structure of *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate* and *Cobaltous sulphate, heptahydrate*.

For the *determination* of total cobalt in the *feed additive, premixtures* and *feedingstuffs* the Applicants submitted internationally recognised ring trial validated methods EN 15510 and CEN/TS 15621. Both methods are based on inductively coupled plasma atomic emission spectroscopy (ICP-AES), with or without pressure digestion. Similar performance characteristics were reported for the two methods mentioned above: - a relative standard deviation for *repeatability* (RSD_r) ranging from 2.5 to 12 %; - a relative standard deviation for *reproducibility* (RSD_R) ranging from 11 to 26 %; and – a limit of quantification (LOQ) around 0.7 mg/kg *feedingstuffs*. Based on these performance characteristics the EURL recommends for official control the two CEN methods (EN 15510 and CEN/TS 15621), based on ICP-AES, to determine total cobalt content in the *feed additive, premixtures* and *feedingstuffs*. Furthermore, the EURL recommends the ring-trial validated CEN method EN ISO 11885, based on inductively coupled plasma optical (atomic) emission spectroscopy (ICP-AES) for the quantification of total cobalt in *water*.

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

Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate; Cobaltous sulphate, heptahydrate; nutritional additive; compounds of trace elements; all animal species and categories.

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) [1,3] and 10(2) [1,2] for *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate; Cobaltous sulphate, heptahydrate* under the category of "nutritional additives" functional group 3b (compounds of trace elements) [1-3], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species [1-6].

According to the Applicants:

- *Cobaltous acetate, tetrahydrate* ($\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4 \text{H}_2\text{O}$) are intense red crystals with a minimum content of 23% total cobalt [4];
- *Cobaltous carbonate* (CoCO_3) [6] and *basic Cobaltous carbonate, monohydrate* ($2 \text{CoCO}_3 - 3 \text{Co}(\text{OH})_2 \cdot \text{H}_2\text{O}$) [4,5] are red to pink crystalline powder with a minimum content of 45% total cobalt; and
- *Cobaltous sulphate, heptahydrate* ($\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$) is a dark pink to brown crystalline powder with a minimum content of 20% total cobalt [4].

Furthermore, Applicant (FAD-2010-0371) intends to market a film granulated preparation of *basic Cobaltous carbonate monohydrate* containing a minimum of 5% total cobalt [5,8].

The *feed additives* are intended to be incorporated into *premixtures, feedingstuffs* [7-9] and *water* [7]. Applicants (FAD-2010-0227 and FAD-2010-0371) suggested maximum levels of total cobalt of 2 mg/kg *feedingstuffs* and 1 mg/L *water* [7-9] to comply with the limits set in Regulations (EC) No 1334/2003. Applicant (FAD-2010-0402) took into consideration the EFSA Scientific opinion recommending to reduce the maximum level of Cobalt to 1 mg/kg *feedingstuffs* [10].

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 *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate; Cobaltous sulphate, heptahydrate*, 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 additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, dioxins and dioxin like PCBs) are available from the respective European Union Reference Laboratories [11].

Identification /Characterisation of the feed additive

For the identification of cobalt in the various *feed additives* Applicant (FAD-2010-0371) submitted a spectrophotometric method [12], based on the formation of a bright blue thiocyanate complex of divalent cobalt in acidic media.

According to the general identification tests described in the European Pharmacopoeia [13]:

- Ethyl acetate is liberated - recognizable by its characteristic odour - when an acetate salt (i.e. *Cobaltous acetate, tetrahydrate*) is warmed with sulphuric acid and alcohol.
- The presence of *Cobaltous carbonate* and *basic Cobaltous carbonate* (containing Cobaltous and carbonate Cobaltous hydroxide) can be verified with the effervescent degassing of CO₂, when diluted hydrochloric acid is added.
- A *Cobaltous sulphate* solution yields a white precipitate insoluble in hydrochloric or in nitric acid in the presence of barium chloride.

Additionally the EURL recommends crystallographic techniques, such as X-Ray diffraction, for the characterisation of crystalline structure of *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate* and *Cobaltous sulphate, heptahydrate*.

Furthermore, all Applicants suggested [7-9] to quantify the total cobalt content (described in the next section) for the characterisation of the above mentioned products.

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

For the *determination* of total cobalt in the *feed additive, premixtures and feedingstuffs* the Applicants submitted the internationally recognised ring trial validated method EN 15510 [14], based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). For the determination of total cobalt, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic feedingstuffs) or wet digested with hydrochloric acid (in the case of mineral compounds). The following performance characteristics were reported [14] for a complete feed for pigs, a complete feed for sheep, a rock phosphate, a mineral premix and two different mineral mixtures, where the total cobalt content ranged from 0.75 mg/kg to 19.9 g/kg:

- a relative standard deviation of *repeatability* (RSD_T) ranging from 3 to 12 %
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 12 to 26 %; and
- a limit of quantification (LOQ) equal to the lowest concentration investigated of 0.75 mg/kg *feedingstuffs*, suitable for the monitoring of maximum limits set by the legislation.

An alternative CEN ring-trial validated method (CEN/TS 15621) [15], based on ICP-AES after pressure digestion, was submitted by the Applicants for the determination of total cobalt in the *feed additive, premixtures and feedingstuffs*. The total cobalt concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported [15] for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total cobalt content ranged from 0.67 to 30.7 mg/kg:

- RSD_T ranging from 2.5 to 5.3 %
- RSD_R ranging from 11 to 19 %; and
- LOQ equal to the lowest concentration investigated of 0.67 mg/kg *feedingstuffs*, suitable for the monitoring of maximum limits set by the legislation.

Based on these acceptable method performance characteristics, the EURL recommends for official control the two CEN methods (EN 15510 and CEN/TS 15621) - based on ICP-AES - to determine total cobalt content in the *feed additive, premixtures and feedingstuffs*.

For the quantification of total cobalt in *water* the EURL identified the ring trial validated CEN method EN ISO 11885 [16], based on inductively coupled plasma optical (atomic) emission spectroscopy (ICP-AES). The total cobalt concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported for a total cobalt content ranging from 19 to 76 µg/L: - RSD_r ranging from 1.6 to 2.5 %; - RSD_R ranging from 3.9 to 5.5 %; and – LOQ = 10 µg/L.

Based on the method performance characteristics presented, the EURL recommends for official control the CEN method (EN ISO 11885) based on ICP-AES to quantify total cobalt content in the *water*.

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:

- the European Pharmacopoeia monograph (01/2008:20301) for the identification of acetate, carbonate and sulphate functional groups in the *feed additives*;
- X-Ray diffraction for the characterisation of cobalt crystalline products;
- two ring-trial validated CEN methods (EN 15510 and/or CEN/TS 15621) - based on ICP-AES - for the determination of total cobalt content in the *feed additive, premixtures and feedingstuffs*; and
- the ring-trial validated EN ISO 11885 method, based on ICP-AES, to quantify total cobalt content in the *water*.

Recommended text for the register entry (analytical method)

For the identification of *acetate, carbonate and sulphate* in *feed additive*:

- European Pharmacopoeia Monograph 1/2008:20301

For the crystallographic characterisation of *feed additives*:

- X-Ray diffraction

For the quantification of *total cobalt* content in the *feed additive, premixtures* and *feedingstuffs*:

- EN 15510 - inductively coupled plasma optical (atomic) emission spectrometry (ICP-AES);

or

- CEN/TS 15621 - inductively coupled plasma optical (atomic) emission spectrometry (ICP-AES) after pressure digestion;

For the quantification of *total cobalt* content in *water*:

- EN ISO 11885 - inductively coupled plasma optical (atomic) emission spectrometry (ICP-AES).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate;* and *Cobaltous sulphate, heptahydrate* 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] ^a Application, Reference SANCO/D/2 Forw. Appl. 1831/00147/10247/2010
- [2] ^b Application, Reference SANCO/D/2 Forw. Appl. 1831/7162-2010
- [3] ^c Application, Reference SANCO/D/2 Forw. Appl. 1831/0014/2011
- [4] ^a Application, Proposal for Register Entry – Annex A.
- [5] ^b Application, Proposal for Register Entry – Annex A
- [6] ^c Application, Proposal for Register Entry – Annex A
- [7] ^a Technical dossier, Section II – Identity; Conditions of use of the additive
- [8] ^b Technical dossier, Section II – Identity; Conditions of use of the additive
- [9] ^c Technical dossier, Section II – Identity; Conditions of use of the additive
- [10] EFSA Journal 2009, 7(12):1383
- [11] 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
- [12] ^b Technical dossier, Section II – Annex_II_23.2.6.1.a
- [13] European Pharmacopoeia Monograph 01/2008:20301 - *Identification reactions of ions and functional groups*
- [14] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*

- [15] CEN/TS 15621:2007 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*
- [16] EN ISO 11885:2009 – *Water quality – Determination of selected elements by inductively coupled plasma optical (atomic) emission spectrometry (ICP-OES – ICP-AES)*

^a Refers to Dossier No. FAD-2010-0337

^b Refers to Dossier No. FAD-2010-0371

^c Refers to Dossier No. FAD-2010-0402

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:

- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)