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Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the re-evaluation of an authorised additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2008-0052 CRL/080028
Product name:	Cycostat 66G
Active Substance(s):	Robenidine hydrochloride
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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Date:	21/08/2009

EXECUTIVE SUMMARY

Cycostat 66G (E 758) is a product already authorised as feed additive for use in rabbits for breeding and chickens, turkeys and rabbits for fattening, under the category 'coccidiostats and histomonostats' according to Annex I of Regulation (EC) No 1831/2003.

In the current application a re-evaluation according to Article 10 (2) of Regulation (EC) No 1831/2003 is sought for *Cycostat 66G* for rabbits for fattening and rabbits for breeding on the inclusion level ranging from 50 to 66 mg active substance per kilogram of feedingstuffs. The active substance of *Cycostat 66G* is robenidine hydrochloride.

Cycostat 66G contains 6.6 % of the active substance (robenidine hydrochloride), 4.0 % of calcium lignosulfonate (binder) and 89.4 % of calcium sulphate dehydrate (carrier).

The performance characteristics of the Community method based on reverse phase high performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection are: - a limit of quantification (LOQ) of 5 mg/kg; - a relative standard deviations for repeatability (RSD_r) ranging from 3.3 to 4.1 % and a relative standard deviation for intermediate reproducibility (RSD_R) ranges from 9.7 to 10.6 % for *feedingstuffs* for rabbits.

The applicant used the above mentioned Community method to determine robenidine hydrochloride in *feedingstuffs* and applied it also to *feed additive* and *premixtures*. The following method performance characteristics were reported:

- For *feed additive*: - LOQ = 150 mg/kg; - RSD_r = 3.4 %; - RSD_R = 4.4 % and – a recovery rate of 93 %;
- For *premixtures*: - limit of detection (LOD) of 119 mg/kg; - RSD_r = 3.1 %, - RSD_R = 7.2 % and - a recovery rate of 99 %;
- For *feedingstuffs*: - LOD = 0.5 mg/kg; - RSD_r = 2.6 %; - RSD_R = 3.1 % and - a recovery rate ranging from 94 to 98 %.

The reported performance characteristics confirm the applicability of the Community method in the frame of this authorisation, i.e. when robenidin hydrochloride is measured in *Cycostat 66G* or when robenidin hydrochloride is introduced in premixtures and feedingstuffs via *Cycostat 66G*. Therefore, the CRL recommends the Community method (Commission Regulation (EC) No 152/2009, Annex IV, Method E) for the determination of robenidine hydrochloride in the *feed*

additive, *premixtures* and *feedingstuffs* for official control purposes in the frame of the *Cycostat 66G* authorisation.

Further testing or validation is not considered necessary.

KEYWORDS

Cycostat 66G, coccidiostats, robenidine hydrochloride, rabbits

1. BACKGROUND

Cycostat 66G (E 758) is a product already authorised as feed additive for rabbits for breeding [1] and chickens, turkeys and rabbits for fattening [2] under the category 'coccidiostats and histomonostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorised inclusion level of active substance ranges from 50 to 66 mg/kg of feedingstuffs [3].

In the current application submitted according to Article 10 (2) of Regulation (EC) No 1831/2003 the re-evaluation of *Cycostat 66G* for rabbits for breading and fattening [4] is sought.

Cycostat 66G is a greyish product consisting of free flowing granules. The active substance of additive is robenidine hydrochloride of minimum purity of 97 %. The additive *Cycostat* contains 6.6 % of active substance, 4.0 % of calcium lignosulphonate and 89.4 % of calcium sulphate dihydrate [5].

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 the 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 *Cycostat 66G*, and their suitability to be used for official control in the frame of the authorisation, were evaluated.

3. EVALUATION

Quantitative and qualitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances (e.g. heavy metals) in the *additive* are available at the respective Community Reference Laboratories [6].

TRIS (N,N',N" -tris[(p-chlorobenzylidene)amino]guanidine) and AZIN (bis-(4-chlorobenzylidene) hydrazine) impurities can be monitored using the RP-HPLC method submitted by the applicant [7] for the determination of the active substance, while solvent residues (i.e. as methanol, isopropanol) can be analysed using FID-GC (gas chromatography with flame ionisation detector).

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

The applicant used the Community method, based on a reverse phase high performance liquid chromatography (RP-HPLC) assay coupled to UV detection measuring at 317 nm, to determine robenidine hydrochloride in *feedingstuffs* [8] and applied it also to *feed additive* [9] and *premixtures* [10].

Approximately or 5 g of feed additives or premixtures or 15 g of feedingstuffs are accurately weighed into a 250 ml conical flask, extracted by shaking with 100 ml of acidic methanol for 60 minutes. After filtration, purification on aluminium-oxide column and appropriate dilution if necessary the clear solution is injected into the HPLC apparatus. External standard calibration is used for the quantification of robenidine hydrochloride.

The following acceptable performance characteristics were reported:

- For *feed additive* [9]*:* - LOQ = 150 mg/kg; - RSD_r = 3.4 % (as recalculated by CRL [11]); - RSD_R = 4.4 % and – a recovery rate of 93 %.
- For *premixtures* [10]: - LOD = 119 mg/kg; - RSD_r = 4.1 %, - RSD_R = 7.2 % and - a recovery rate of 99 %.
- For *feedingstuffs* [8] *:* - LOD = 0.5 mg/kg; - RSD_r = 2.6 %; - RSD_R = 3.1 % (as recalculated by CRL [11]) and - a recovery rate ranging from 94 to 98 %.

The following acceptable performance characteristics of the Community method [12] are: - LOQ = 5 mg/kg; - RSD_r ranging from 3.3 to 4.1 % and - RSD_R ranges from 9.7 to 10.6 % for feedingstuffs for rabbits.

The performance characteristics reported by applicant are in agreement, thus confirming the applicability of the Community method in the frame of this authorisation, i.e. when robenidin hydrochloride is measured in *Cycostat 66G* or when robenidin hydrochloride is introduced in premixtures and feed via *Cycostat 66G*. Therefore, the CRL recommends the Community method (Commission Regulation (EC) No 152/2009, Annex IV, Method E) [12] for the determination of robenidine hydrochloride in the *feed additive*, *premixtures* and *feedingstuffs* for official control purposes in the frame of the *Cycostat 66G* authorisation.

Further testing or validation is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the Community method (Commission Regulation (EC) No 152/2009, Annex IV, Method E) for the determination of robenidin in the *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation is not considered necessary.

Recommended text for the register entry, fourth column (analytical method)

Determination of robenidine hydrochloride by reverse phase high performance liquid chromatography coupled to ultraviolet spectrometry (HPLC/UV) according to Commission Regulation (EC) 152/2009, Annex IV, Method E.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of robenidine hydrochloride, have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] Commission Regulation (EC) No 2430/1999 of 16 November 1999 linking the authorisation of certain additives belonging to the group of coccidiostats and other medicinal substances in feedingstuffs to persons responsible for putting them into circulation

[2] Commission Regulation (EC) No 1800/2004 of 15 October 2004 concerning the authorisation for 10 years of the additive Cycostat 66G in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances

[3] * Annex III. Proposal of Register entry

[4] * SANCO/D/2 Forw. Appl. 1831/003-2008

[5] * Section II.3, Manufacturing process, including any specific processing procedures

[6] 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 Community Reference Laboratories, Official Journal of the European Union L 136. 24.5.2006.

[7] * Section II, Annex II.2.1, section 3.2.S.4.3

[8] * Section II – Annex II.6.3 ″Determin robenidine feed"

[9] * Supplementary information – Validation of analytical method for determination of robenidine in Cycostat 66G

[10] * Section II – Annex II.6.1 " Determin robenidine premixes"

[11] * Supplementary information – MiniTab calculation performed by CRL

[12] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, Annex IV, part E

* Refers to Dossier No: FAD-2008-0052

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community 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.

8. ACKNOWLEDGEMENTS

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- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig, Deutschland
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