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**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-2010-0375
CRL/100249

Feed additive: Sodium Benzoate

Active Substance(s): Sodium Benzoate

Rapporteur Laboratory: European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium

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Date: 30/05/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *sodium benzoate* under the category / functional group 1(k) of 'technological additives'/silage additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for bovines, sheep, pigs, poultry, rabbits, horses, goats.

According to the Applicant, the *feed additive* is a white, odourless, solid substance. The *feed additive* consists of *sodium benzoate*, with a minimum purity of 99.5 %. The *feed additive* is intended to be used in *silage*, with a proposed maximum level of 2.4 g/kg *silage*.

For the determination of *sodium benzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph (01/2008:0123), where: - identification is based on the sodium and on the benzoate reactions, while - quantification is based on the titration of *sodium benzoate* with 0.1 M perchloric acid, using naphtholbenzein as an indicator.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia Monograph to determine *sodium benzoate* in the *feed additive*.

For the quantification of *sodium benzoate* in *silage* the Applicant submitted a single-laboratory validated and further verified method, based on high performance liquid chromatography with UV detection (HPLC-UV). The following performance characteristics were reported for *sodium benzoate* concentrations – ranging from 215 to 2620 mg/kg: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.6 % to 12 %; - a *recovery* rate (R_{rec}) ranging from 103 to 109 %; and - a limit of quantification (LOQ) of 125 mg/kg *silage*.

Based on the performance characteristics presented, the EURL considers the single laboratory validated and further verified HPLC-UV method suitable for the determination of *sodium benzoate* in *silage*. However, the unambiguous determination of the content of *sodium benzoate* added to *silage* cannot be performed satisfactorily due to intrinsic properties of *silage* matrice. Therefore, the EURL cannot recommend this method for official control to determine added *sodium benzoate* in *silage*.

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

sodium benzoate, technological additives, silage additives, bovines, sheep, pigs, poultry, rabbits, horses, goats

1. BACKGROUND

In the current application authorisation is sought under article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *sodium benzoate* under the category / functional group 1(k) 'technological additives'/silage additives' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for bovines, sheep, pigs, poultry, rabbits, horses, goats [2].

According to the Applicant, the *feed additive* is a white, odourless, solid substance [3] consisting of *sodium benzoate*, with a minimum purity of 99.5 % [2].

The *feed additive* is intended to be used in *silage* [3] with a proposed maximum level of 2.4 g/kg *silage* [2].

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 *sodium benzoate*, 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, PAHs and dioxins) are available from the respective European Union Reference Laboratories [4].

Description of the analytical methods for the determination of the active substance in feed additive and silage

For the determination of *sodium benzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph [5], where:

- identification is based on the sodium and on the benzoate reactions, further described in the methods of analysis section of the European Pharmacopoeia (01/2005:20301); while
- quantification is based on the titrimetric method. The assay requires the extraction of 0.25 g sample in anhydrous acetic acid, heated to 50 °C if necessary, and the titration with 0.1 M perchloric acid, using naphtholbenzein as an indicator. One ml of 0.1 M perchloric acid is equivalent to 14.41 mg of sodium benzoate.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia Monograph (01/2008:0123) to determine *sodium benzoate* in the *feed additive*.

For the quantification of *sodium benzoate* in *silage* the Applicant submitted a single-laboratory validated and further verified method, based on high performance liquid chromatography with UV detection (HPLC-UV) [6].

The sample is extracted with an acetate buffer/methanol mixture, after addition of an internal standard and filtered before measurement. *Sodium benzoate* is quantified using an internal standard calibration by spectrophotometry at 235 nm after reversed phase chromatography.

The following performance characteristics were reported for *sodium benzoate* concentrations ranging from 215 to 2620 mg/kg [6]:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.6 % to 12.4 %;
- a *recovery rate* (R_{rec}) ranging from 103 to 109 %; and
- a limit of quantification (LOQ) of 125 mg/kg *silage*.

Based on the performance characteristics presented, the EURL considers the single laboratory validated and further verified HPLC-UV method submitted by the Applicant suitable for the determination of *sodium benzoate* in *silage*. However, the unambiguous determination of the content of *sodium benzoate* added to *silage* cannot be performed satisfactorily due to intrinsic properties of *silage* matrice. Therefore, the EURL cannot recommend this method for official control to determine added *sodium benzoate* in *silage*.

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 Pharmacopoeia Monograph (01/2008:0123) for the determination of *sodium benzoate* in the *feed additive*.

The EURL considers the single-laboratory validated and further verified HPLC-UV method submitted by the Applicant suitable for the determination of *sodium benzoate* in *silage*. However, the unambiguous determination of the content of *sodium benzoate* added to *silage* cannot be performed satisfactorily due to intrinsic properties of *silage* matrice. Therefore, the EURL cannot recommend this method for official control to determine added *sodium benzoate* in *silage*.

Recommended text for the register entry (analytical method)

For the determination of *sodium benzoate* in the *feed additive*:

- Titrimetric method (Monograph 01/2008:0123 of the European Pharmacopoeia)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *sodium benzoate* 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/G1: Forw. Appl. 1831/0025-2012
 - [2] *Application, Proposal for Register Entry – Annex A
 - [3] *Technical dossier, Section II: Identity, characterisation and conditions of use; 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] European Pharmacopoeia Monograph 6.0, 01/2008:0123
 - [6] *Technical dossier, Section II – Annex II_24
- * Refers to Dossier No. FAD-2010-0375

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European 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:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- 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)
- RIKILT - Instituut voor Voedselveiligheid, Wageningen (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)