EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.DG.D.5/CvH/ZE/AG/ARES(2012)859207

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-2010-0153 - CRL/100229 FAD-2010-0247 - CRL/100337
Feed additives:	Sodium ethyl 4-hydroxybenzoate (E215) Methyl 4-hydroxybenzoate (E218)
Active Substance(s):	Sodium ethyl 4-hydroxybenzoate Methyl 4-hydroxybenzoate
Rapporteur Laboratory:	European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium
Report prepared by:	Zigmas Ezerskis (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 13/07/2012
Report approved by: Date:	Christoph von Holst 13/07/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *sodium ethyl 4-hydroxybenzoate (E215)* and *methyl 4-hydroxybenzoate (E218)*, under the category / functional group 1(a) 'technological additives'/'preservatives', 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 pets (i.e. E218, FAD-2010-0247). and for cats & dogs (i.e. E215, FAD-2010-0153).

According to the Applicants *sodium ethyl 4-hydroxybenzoate (E215)* and *methyl 4-hydroxybenzoate (E218)* are white crystalline powders with a minimum purity of 98 %. Both *feed additives* are intended to be incorporated into *feedingstuffs*, with no proposed minimum or maximum levels.

For the determination of *sodium ethyl 4-hydroxybenzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph (01/2008:2134), where identification is based on the determination of the melting point; infrared absorption spectrophotometry; thin-layer chromatography; reaction for sodium; and color reaction with aminopyrazolone and potassium ferricyanide solutions; while quantification is based on the potentiometric titration method. For the determination of *methyl 4-hydroxybenzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph (07/2010:0409), where identification is based on the determination of the melting point; infrared absorption spectrophotometry and thin-layer chromatography, while quantification is based on high performance liquid chromatography (HPLC) with UV detection at 272 nm. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia Monographs for the determination of *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in the *feed additives*.

The Applicants did not provide any analytical method for the determination of *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in *feedingstuffs*. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in *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
sodium ethyl 4-hydroxybenzoate (E215), *methyl 4-hydroxybenzoate (E218)*, technological additives, preservatives, pets, cats and dogs

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 ethyl 4-hydroxybenzoate (E215)* and *methyl 4-hydroxybenzoate (E218)*, under the category / functional group 1(a) 'technological additives'/'preservatives' [1, 2], 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 pets (i.e. E218, FAD-2010-0247) [3] and for cats & dogs (i.e. E215, FAD-2010-0153) [4].

According to the Applicants, *sodium ethyl 4-hydroxybenzoate* (E215) and *methyl 4-hydroxybenzoate* (E218) are white crystalline powders with a minimum purity of 98 % [5, 6].

Sodium ethyl 4-hydroxybenzoate (E215) is intended to be incorporated directly into *feedingstuffs*, with no proposed minimum or maximum levels. However, a typical inclusion level of 435 mg/kg *feedingstuffs* is suggested by the Applicant [5].

Methyl 4-hydroxybenzoate (E218) is intended to be incorporated into *feedingstuffs*, with no proposed minimum or maximum levels. However, typical inclusion levels of the *feed additive* in *feedingstuffs* are to be defined by the veterinarian [6].

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 ethyl 4-hydroxybenzoate (E215)* and *methyl 4-hydroxybenzoate (E218)*, 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 [7].

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

For the determination of *sodium ethyl 4-hydroxybenzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph [8], where:

- identification is based on: a) the determination of the melting point; b) infrared absorption spectrophotometry; c) thin-layer chromatography; d) color reaction with aminopyrazolone and potassium ferricyanide solutions; and e) reaction for sodium; while
- quantification is based on the potentiometric titration method. 0.15 g of sample is dissolved in anhydrous acetic acid and titrated with 0.1 M perchloric acid. One ml of 0.1 M perchloric acid is equivalent to 18.82 mg of *sodium ethyl 4-hydroxybenzoate*.

For the determination of *methyl 4-hydroxybenzoate* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph [9], where:

- identification is based on: a) the determination of the melting point; b) infrared absorption spectrophotometry; c) thin-layer chromatography; while
- quantification is based on high performance liquid chromatography (HPLC) with UV detection at 272 nm.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia Monographs (01/2008:2134 and 07/2010:0409) for the determination of *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in the *feed additives*, respectively.

For the determination of *methyl 4-hydroxybenzoate* in a preparation (simulating a commercial feed supplement containing methylhydroxybenzoate, lanthanum-carbonate and d-*alpha*-tocopheryl-acetate) the Applicant (FAD-2010-0247) submitted a single laboratory validated method based on reversed phase high performance liquid chromatography (RP-HPLC) with UV detection at 256 nm. The RP-HPLC method is suitable for the quantification of *methyl 4-hydroxybenzoate* in the product meant to be sprayed on the pet food. However, the Applicant

did not provide any data or experimental method for the determination of *methyl 4-hydroxybenzoate* included in pet food / *feedingstuffs*.

Sodium ethyl 4-hydroxybenzoate is one of the ingredients of the pet food manufacturing process. However, the Applicant did not provide any data or the experimental method for the determination of *sodium ethyl 4-hydroxybenzoate* in the granulated or canned pet food.

Due to the lack of experimental evidence, the EURL cannot evaluate nor recommend any method for official control to determine *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in pet food / *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 two European Pharmacopoeia Monographs (01/2008:2134 and 07/2010:0409) for the determination of *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in the *feed additives*, respectively.

The Applicants did not provide any analytical method for the determination *of sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in *feedingstuffs*. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *sodium ethyl 4-hydroxybenzoate* and *methyl 4-hydroxybenzoate* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *sodium ethyl 4-hydroxybenzoate* in the *feed additive*:

- Potentiometric titration method (Monograph 01/2008:2134 of the European Pharmacopoeia)

For the determination of *methyl 4-hydroxybenzoate* in the *feed additive*:

- High performance liquid chromatography (HPLC) with UV detection at 272 nm (Monograph 07/2010:0409 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 ethyl 4-hydroxybenzoate (E215)* and *methyl 4-hydroxybenzoate (E218)* 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. 1831/0010-2012
[2] +Application, Reference SANCO/G1: Forw. Appl. 1831/0011-2012
[3] *Application, Proposal for Register Entry – Annex A
[4] +Application, Proposal for Register Entry – Annex A
[5] *Technical dossier, Section II: Identity, characterisation and conditions of use; methods of analysis
[6] +Technical dossier, Section II: Identity, characterisation and conditions of use; methods of analysis
[7] 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
[8] European Pharmacopoeia Monograph 6.0, 01/2008:2134
[9] European Pharmacopoeia Monograph 7.0, 07/2010:0409

*Refers to Dossier No. FAD-2010-0153

+Refers to Dossier No. FAD-2010-0247

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)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- RIKILT - Instituut voor Voedselveiligheid, Wageningen (NL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)