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

JRC.DG.D.6/CvH/SB/ag/ARES(2011)1330303

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-2010-0078 CRL/100061
Feed Additive Name:	Propyl Gallate
Active Substance(s):	Propyl Gallate
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Stefano Bellorini (EURL-FA)
Report revised by:	Dijana Mitic (EURL-FA) Piotr Robouch (EURL-FA)
Date:	09/12/2011
Report approved by: Date:	Christoph von Holst 09/12/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Propyl Gallate* under Articles 4 and 10, category 'technological additives', functional group 'antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003. *Propyl Gallate (E310)* is already authorised as *feed additive* under Commission Directive 70/524/EEC. According to the Applicant the *feed additive* contains minimum 97% of *Propyl Gallate*. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is intended to be mixed in *premixtures* or added directly in complete *feedingstuffs* or *water*. The Applicant proposed 100 mg/kg as specific maximum level in the daily ration of *feedingstuffs* for the *feed additive* alone or together with Octyl Gallate (E311) and/or Dodecyl Gallate (E312).

For the determination of *Propyl Gallate* in the *feed additive* the Applicant proposed to apply the internationally recognised European Phamacopoeia ultraviolet-visible spectrophotometric method (Ph. Eur. 6.0, method 01/2008:1039). Even though no performance characteristics of this method are provided, the EURL considers this method suitable to determine *Propyl Gallate* in the *feed additive* within the frame of official control.

For the determination of *Propyl Gallate* in *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified multi-analyte Reversed Phase High Performance Liquid Chromatographic method with UltraViolet-Diode-Array Detection (RP-HPLC-UV(DAD)). The following correspondent performance characteristics were reported for concentrations in *premixtures* ranging from 5 to 120 g/kg and concentrations in *feedingstuffs* ranging from 35 to 226 mg/kg:

- a standard deviation for repeatability (RSD_r) ranging from 1.7 to 7%;
- a standard deviation for reproducibility (RSD_R) ranging from 3.0 to 12%;
- a recovery rate (R_{Rec}) ranging from 84.4 to 112%; and
- a limit of quantification (LOQ) of 6 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-UV(DAD) method, submitted by the Applicant, to determine *Propyl Gallate* in *premixtures* and *feedingstuffs*.

The Applicant provided no experimental data for the determination of *Propyl Gallate* in *water*. Therefore the EURL could not evaluate nor recommend a method for official control to determine *Propyl Gallate* in *water*.

The Applicant provided no experimental data for the determination of Octyl Gallate (E311) and/or Dodecyl Gallate (E312) in *feedingstuffs*. The EURL identified a method characterised by the "Association of Official Analytical Chemists" (AOAC 983.15) "Phenolic Antioxidants in oils, fats and butter oil". The method has been ring-trial validated and it is applicable in a range from 10 to 100 mg/kg. However the analytical method is specifically designed for the determination of antioxidants in food matrices. Further experiments would be necessary in order to eventually extend the scope of this method to different matrices such as *feedingstuffs*. Therefore the EURL could not evaluate nor recommend a method for official control to determine Octyl Gallate (E311) and/or Dodecyl Gallate (E312) 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
Propyl Gallate (*E310)*, technological additives, antioxidants, all animal species and categories, Octyl Gallate (E311), Dodecyl Gallate (E312).

1. BACKGROUND

In the current application authorisation is sought for *Propyl Gallate* under Articles 4 and 10, category 'technological additives', functional group 'antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1, 2]. *Propyl Gallate (E310)* is already authorised as *feed additive* under Commission Directive 70/524/EEC [3].

The *feed additive* is a white, or almost white, crystalline powder, odourless and slightly soluble in water, in fats, oils and freely soluble in ethanol and acetone [4-6].

The *feed additive* contains a minimum of 97% *Propyl Gallate* and it is produced by synthesis through esterification of Gallic acid with n-Propanol [2, 7, 8]. According to the Applicant *Propyl Gallate* is universally used as antioxidant for fats and oils in feed, food, cosmetics and pharmaceuticals [9]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is intended to be mixed in *premixtures* or added directly in complete *feedingstuffs* or *water*. Furthermore, the Applicant proposed a maximum level in the daily ration of 100 mg/kg for the *feed additive* alone or together with Octyl Gallate (E311) and/or Dodecyl Gallate (E312) [2, 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 *Propyl Gallate*, 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, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [11].

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

For the determination of *Propyl Gallate* in the *feed additive*, the Applicant proposed to apply the internationally recognised European Phamacopoeia methods [6, 12], where:

- infrared absorption spectrophotometric method (test B) is applied for the identification, and
- ultraviolet-visible spectrophotometric method (assay) for quantification.

No performance characteristics of these methods are provided. However, the EURL considers these methods suitable to determine *Propyl Gallate* in the *feed additive* within the frame of official control.

For the determination of *Propyl Gallate* in *premixtures* and *feedingstuffs* the Applicant submitted a multi-analyte Reversed Phase High Performance Liquid Chromatographic method with UltraViolet-Diode-Array Detection (RP-HPLC-UV(DAD)) [12]. The analytical method was single laboratory validated and further verified [13-16]. The method consists of an extraction of the *active substance* from the matrix using methanol (together with ascorbic acid for *premixtures*). The extract is further diluted with methanol and mixed thoroughly. The supernatant is filtered and injected into a gradient reversed-phase HPLC system. The *Propyl*

Gallate content is determined via UV-DAD adjusted at 285 nm (reference wavelength of 390 nm). The concentration of *Propyl Gallate* is determined using an external calibration curve. The following correspondent performance characteristics were reported for concentrations in *premixtures* ranging from 5 to 120 g/kg [13, 14] and concentrations in *feedingstuffs* ranging from 35 to 226 mg/kg [15, 16]:

- a standard deviation for repeatability (RSD_r) ranging from 1.7 to 7%;
- a standard deviation for reproducibility (RSD_R) ranging from 3.0 to 12%;
- a recovery rate (R_{Rec}) ranging from 84.4 to 112%; and
- a limit of quantification (LOQ) of 6 mg/kg [15].

Furthermore, this multi-analyte technique allows the determination of others synthetic antioxidants such as Ethoxyquin (only in *premixtures*), t-butyl-4-hydroxyanisole (BHA) and 2,6-di-t-butylhydroxytoluene (BHT).

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-UV(DAD) method, submitted by the Applicant, to determine *Propyl Gallate* in *premixtures* and *feedingstuffs*.

Moreover, as above mentioned, the Applicant proposed 100 mg/kg as specific maximum level in the daily ration of *feedingstuffs* for the *feed additive* alone or together with Octyl Gallate (E311) and/or Dodecyl Gallate (E312) [2, 10]. Nevertheless the Applicant provided no experimental data for the determination of Octyl Gallate (E311) and/or Dodecyl Gallate (E312) in *feedingstuffs*. However, the EURL identified a method characterised by the "Association of Official Analytical Chemists" (AOAC 983.15) "Phenolic Antioxidants in oils, fats and butter oil". The method has been ring-trial validated and it is applicable in a range from 10 to 100 mg/kg. *Propyl Gallate,* Octyl Gallate, Dodecyl Gallate and a series of other antioxidants are extracted into acetonitrile, concentrated and finally diluted with 2-propanol. The compounds under analysis are separated and determined by HPLC-UV at 280 nm. However the analytical method is specifically designed for the determination of antioxidants in food matrices. Further experiments would be necessary in order to eventually extend the scope of this method to different matrices such as *feedingstuffs*. Therefore the EURL could not evaluate nor recommend a method for official control to determine Octyl Gallate (E311) and/or Dodecyl Gallate (E312) in *feedingstuffs.*

The Applicant provided no experimental data for the determination of *Propyl Gallate* in *water*. Therefore the EURL could not evaluate nor recommend a method for official control to determine *Propyl Gallate* 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the European Pharmacopoeia 6.0, method 01/2008:1039, using ultraviolet-visible spectrophotometry to determine *Propyl Gallate* in *feed additive*;
- a single laboratory validated and further verified method using RP-HPLC with UV-DAD detection to determine *Propyl Gallate* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *Propyl Gallate* in *feed additive*:

- Spectrophotometry at 275 nm (Ph. Eur. 6.0, method 01/2008:1039)

For the quantification of *Propyl Gallate* in *premixtures* and *feedingstuffs*:

- Reversed Phase High Performance Liquid Chromatography coupled to UltraViolet-Diode-Array Detection (RP-HPLC-UV(DAD), 285 nm)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Propyl Gallate* 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/D/2 Forw. Appl. 1831/059-2010
[2] *Application, Proposal of Register Entry – Annex A
[3] Council Directive 70/524/EEC concerning additives in feedingstuffs - List of authorised additives in feedingstuffs (2004/C50/01)
[4] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
[5] *Technical dossier, Section II: 2.2 Characterisation of the active substance
[6] European Pharmacopoeia 6.0, method 01/2008:1039

[7] *Technical dossier, Section II: 2.3.1 Active substance(s)/agent(s)
[8] *Technical dossier, Section II: 2.1.4 Purity
[9] *Technical dossier, Section II: 2.1.2 Proposal for classification
[10] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[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] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substance
[13] *Technical dossier, Section II; Annex: Antoxiac_Method of Analysis and Validation_Premix
[14] *Technical dossier, Section II; Annex: Antoxiac_Method verification_Premix-PG
[15] *Technical dossier, Section II; Annex: Antoxiac_Method of Analysis and Validation_Feed
[16] *Technical dossier, Section II; Annex: Antoxiac_Method verification_Feed-PG
* Refers to Dossier No. FAD-2010-0078

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:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Rikilt - Instituut voor Voedselveiligheid, Wageningen (NL)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Państwowy Instytut Weterynaryjny, Puławy (PL)