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

Benzoic acid
(FAD-2017-0009; CRL/170001)

**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2017-0009 - CRL/170001**

Name of Feed Additive: ***Benzoic acid***

Active Agent (s): **Benzoic acid**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

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Date: **10/07/2017**

Report approved by: **Christoph von Holst**
Date: **11/07/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *benzoic acid* under the 'category' / 'functional group' 1(j) 'technological additives' / 'acidity regulator' according to the classification system of Annex I of Regulation (EC) No 1831/2003 for pigs for fattening. *Benzoic acid* is a *feed additive* currently authorized as a chemically defined flavouring for all animal species and as zootechnical additive for porcine and poultry species. The *feed additive* consists of *benzoic acid* white crystalline flakes with a minimum purity of 99.8 %. It is intended to be incorporated directly in *feedingstuffs* or through *premixtures* at maximum inclusion levels of 10 g/kg of complete *feedingstuffs*.

For the characterisation quantification of *benzoic acid* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted an analytical method based on reversed phase High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV) similar to the one described in the ISO 9231:2008 standard.

The EURL evaluated several dossiers related to *feed additives* containing *benzoic acid*, and recommended for official control the European Pharmacopoeia method (01/2008.0066) for the characterisation of *benzoic acid (feed additive)*, and the ISO 9231:2008 method for the quantification of *benzoic acid* in *premixtures* and *feedingstuffs* leading to relative precisions (repeatability and intermediate precision) ranging from 0.4 to 1.9 % for *feedingstuffs* and *premixtures* containing 3-5 g/kg and 50-100 g/kg, respectively, and a limit of quantification (LOQ) of 0.5 g/kg *feedingstuffs*. The Applicant applied the above mentioned HPLC-UV method in the frame of the stability confirming thus its suitability.

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

Benzoic acid, technological additives, acidity regulator, pigs for fattening.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *benzoic acid* under the 'category' / 'functional group' 1(j) technological additives' / 'acidity regulator' according to the classification system of Annex I of Regulation (EC) No 1831/2003 for pigs for fattening [1][2].

Benzoic acid is a *feed additive* currently authorized as a chemically defined flavouring for all animal species by Commission Implementing Regulation (CIR) (EU) No 2017/63 [3], and as zootechnical additive for porcine and poultry species by CIR (EC) No 1730/2006 [4] CIR (EC) No 11385/2007 [5], CIR (EU) No 2016/900 [6] and CIR (EU) No 2015/1426 [7].

The *feed additive* consists of *benzoic acid* white crystalline flakes with a minimum purity of 99.8 % [2][8].

The *feed additive* is intended to be incorporated directly in *feedingstuffs* or through *premixtures* at maximum inclusion levels of 10 g/kg of complete *feedingstuffs* [2][9].

Note: The EURL previously evaluated and recommended analytical methods for the quantification of *benzoic acid* in the frame of several dossiers [10][11].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 *benzoic acid* 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 (arsenic, cadmium, lead, mercury, dioxin-like PCBs and dioxins) are available from the respective European Union Reference Laboratories [12].

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

For the quantification of *benzoic acid* in the *feed additive, premixtures and feedingstuffs* the Applicant submitted an analytical method based on reversed phase High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV) [13] - similar to the one described in the ISO 9231:2008 standard.

Furthermore, the EURL previously evaluated several dossiers where the *feed additives* consisted of *benzoic acid* [10][11], and recommended for the characterisation of *benzoic acid* the European Pharmacopoeia method (01/2008.0066) [14], in which identification is based on melting point and solubility tests, while quantification is based on acid/basic titration with 0.1 M sodium hydroxide.

The experimental data provided in the frame of dossier FAD-2010-0029 [10] demonstrated the applicability of the ISO 9231:2008 standard method for the quantification of *benzoic acid* in *premixtures and feedingstuffs*. The sample is extracted with a 0.1 M sodium hydroxide solution and cleared from lipids and proteins by precipitation with Carrez solution [10][13]. The solution is filtered and directly used for the determination via high performance liquid chromatography with UV detection (HPLC-UV) at 227 nm, using external calibration. The following performance characteristics were reported: (i) relative precisions (repeatability and intermediate precision) ranging from 0.4 to 1.9 % for *feedingstuffs* and *premixtures* containing 3-5 g/kg and 50-100 g/kg, respectively; and (ii) a limit of quantification (LOQ) of 0.5 g/kg *feedingstuffs* [10]. The Applicant did not provide any validation or verification data, but applied the proposed method in the frame of the stability tests (for the *feed additive, premixtures and feedingstuffs*) [15] thus proving also the applicability of the HPLC-UV method to the *feed additive*.

Based on the available information, the EURL recommends for official control the European Pharmacopoeia method (01/2008.0066) [14] for the characterisation of *benzoic acid (feed additive)* and the reversed phase HPLC-UV method based on the ISO 9231:2008 standard [16] for the quantification of *benzoic acid* in the *feed additive, premixtures and 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 the European Pharmacopoeia method (01/2008.0066) for the characterisation of *benzoic acid*, and the reversed phase HPLC-UV method based on the ISO 9231:2008 standard for the quantification of *benzoic acid* in the *feed additive, premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of *benzoic acid (feed additive)*:

- European Pharmacopoeia monograph (01/2008.0066)

For the quantification of *benzoic acid* in the *feed additive, premixtures* and *feedingstuffs*:

- Reversed phase High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *benzoic acid* 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 SANTE:E5: Forw. Appl. 1831/0006-2017
- [2] *Application, Proposal for Register Entry – Annex A
- [3] Commission Implementing Regulation (EU) No 2017/63 of 14 December 2016 concerning the authorisation of benzyl alcohol, 4-isopropylbenzyl alcohol, benzaldehyde, 4-isopropylbenzaldehyde, salicylaldehyde, p-tolualdehyde, 2-methoxybenzaldehyde, benzoic acid, benzyl acetate, benzyl butyrate, benzyl formate, benzyl propionate, benzyl hexanoate, benzyl isobutyrate, benzyl isovalerate, hexyl salicylate, benzyl phenylacetate, methyl benzoate, ethyl benzoate, isopentyl benzoate, pentyl salicylate and isobutyl benzoate as feed additives for all animal a species and of veratraldehyde and gallic acid as feed additives for certain animal species
- [4] Commission Regulation (EC) No 1730/2006 of 23 November 2006 concerning the authorisation of benzoic acid (VevoVitall) as a feed additive
- [5] Commission Regulation (EC) No 1138/2007 of 1 October 2007 concerning the authorisation of a new use of benzoic acid (VevoVitall) as a feed additive
- [6] Commission Implementing Regulation (EU) No 2016/900 of 8 June 2016 concerning the authorisation of benzoic acid as a feed additive for sows (holder of authorisation DSM Nutritional Product Sp. z o. o.)
- [7] Commission Implementing Regulation (EU) No 2015/1426 of 25 August 2015 concerning the authorisation of the preparation of benzoic acid, thymol, eugenol and piperine as a feed additive for chickens for fattening, chickens reared for laying, minor

- poultry species for fattening and reared for laying (holder of the authorisation DSM Nutritional Product)
- [8] *Technical dossier, Section II: 2.2 Characterisation of the active substance
- [9] *Technical dossier, Section II: II.5 Conditions of use of the additive
- [10] EURL Evaluation Report FAD 2010-0029:
<https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0029.pdf>
- [11] EURL Evaluation Reports:
https://ec.europa.eu/jrc/sites/jrcsh/files/updated_finrep_fad-2016-0078_benzoic_acid.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0147-benzoic_acid_corr.pdf
- [12] 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
- [13] *Technical dossier, Section II: Annex II.12; II.13 & II.14
- [14] European Pharmacopoeia monograph (Ph.Eur. 7.0/2008:0066)
- [15] *Technical dossier, Section II: Annex II.28; II.30 & II.31
- [16] ISO 9231, Milk and milk products. Determination of the benzoic and sorbic acid contents
- *Refers to Dossier no: FAD-2017-0009

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Fødevarestyrelsens Laboratorie Ringsted (DK)
- Laboratorio Arbitral Agroalimentario. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid (ES)

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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
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 - RIKILT Wageningen UR, Wageningen (NL)
 - Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors (FI)