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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**

Plantago ovata L. husk: fleawort absolute
(FAD-2010-0327; CRL/100289)

**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**

Dossier related to: **FAD-2010-0327 - CRL/100289**

Name of Feed Additive: ***Plantago ovata L. husk: fleawort absolute***

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

Phytochemical marker **Verbacosides**

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Date: **29/10/2020**

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Date: **29/10/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 10(2) for the botanically defined *Plantago ovata L. husk: fleawort absolute* under the category / functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for cats and dogs.

The *feed additive* is a powder consisting of a mixture of chemical components naturally present, namely mucopolysaccharides. The Applicant specified *verbascosides* as a phytochemical marker for *Plantago ovata L. husk: fleawort absolute* without specifying a range of its mass fraction in the *feed additive*. In addition, the Applicant specified for the characterisation of the *feed additive* criteria for three parameters as defined by the European Pharmacopoeia Monograph 01/2008:1334, namely a minimum value of 40 for the swelling index, and maximum values of 12 % (w/w) for the loss on drying and 4 % (w/w) for total ash content.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant did not propose a minimum or a maximum level of the *feed additive*. However, a maximum content of 50 mg *feed additive* / kg *feedingstuffs* was suggested by the Applicant.

For the determination of the phytochemical marker (*verbascosides*) in the *feed additive* the Applicant submitted an in-house method based on high performance liquid chromatography coupled to diode array detection and triple quadrupole mass spectrometry (HPLC-DAD-MS/MS). However, neither validation nor verification data of the method were submitted by the Applicant. Furthermore, the Applicant did not specify the target content of *verbascosides* as a criterion in the *feed additive*.

Therefore, based on the available information, the EURL is not able to recommend for official control the HPLC-DAD-MS/MS or any other method for the determination of *verbascosides* in the *feed additive*.

For further characterisation of the *feed additive* the Applicant proposed the methods for the determination of the swelling index, loss on drying and total ash content, and presented acceptable data from the analysis of different batches of the *feed additive*. For the identification of the *feed additive* the Applicant did not submit proofs of the applicability of a more specific method based on thin-layer chromatography (TLC) as described in the mentioned European Pharmacopoeia Monograph.

Based on the available information, the EURL considers that the methods for the determination of the swelling index, loss on drying and total ash content are fit-for purpose

and might be recommended for official control for the characterisation of the *feed additive*, but only if combined with a more specific method, namely thin-layer chromatography (TLC), which was not demonstrated as fit-for-purpose.

As the unambiguous determination of the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate or recommend any method for official control for the determination of *Plantago ovata L. husk: fleawort absolute* in these matrices.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Plantago ovata L. husk: fleawort absolute, *swelling index*, verbascosides, sensory additives, flavourings compounds, cats and dogs

1. BACKGROUND

In the current application an authorisation is sought under Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for the botanically defined *Plantago ovata L. husk: fleawort absolute* under the category / functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the *feed additive* is sought to be used for cats and dogs [2-3].

The *feed additive* is a powder consisting of a mixture of chemical components naturally present, namely mucopolysaccharides [4]. The Applicant specified *verbascosides* as a phytochemical marker for *Plantago ovata L. husk: fleawort absolute* without specifying a range of its mass fraction in the *feed additive* [5]. In addition, certain criteria for the characterisation of the product such as the minimum value of 40 for the swelling index and maximum values of 12 % (w/w) for the loss on drying and 4 % (w/w) for the total ash content in the *feed additive* have been specified by the Applicant [4].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant did not propose a minimum or a maximum level of the *feed additive*. However, a maximum content of 50 mg *feed additive* / kg *feedingstuffs* was suggested by the Applicant [3,6].

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 *Plantago ovata L. husk: fleawort absolute* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the determination of the phytochemical marker (*verbascosides*) in the *feed additive* the Applicant submitted an in-house method based on high performance liquid chromatography coupled to diode array detection and triple quadrupole mass spectrometry (HPLC-DAD-MS/MS) [7]. However, neither validation nor verification data of this method were submitted by the Applicant for the determination of the *verbascosides* in the *feed additive*. Furthermore, the Applicant did not specify a target content of *verbascosides* as a criterion in the *feed additive*.

Therefore, the EURL is not able to recommend for official control the HPLC-DAD-MS/MS or any other method for the determination of *verbascosides* in the *feed additive*.

As the unambiguous determination of the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate or recommend any method for official control for the determination of *Plantago ovata L. husk: fleawort absolute* in these matrices.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

Evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For further characterisation of the *feed additive* the Applicant proposed the methods for the determination of the swelling index, loss on drying and total ash content [5] as described in the European Pharmacopoeia Monograph dedicated for 'Ispaghula husk (*Plantago ovata*

Forssk. P. Ispaghula Roxb.)' [8] and presented acceptable data from the analysis of different batches of the *feed additive* [4].

As the methods for the swelling index, loss on drying and total ash content were considered suitable for the characterisation but not specific enough to identify the *feed additive*, the EURL requested proofs of the applicability of a more specific method based on thin-layer chromatography (TLC) also described in the above mentioned monograph [9]. However, the Applicant did not submit any relevant information.

Based on the available information, the EURL considers that the methods for the determination of the swelling index, loss on drying and total ash content are fit-for purpose and might be recommended for official control for the characterisation of the *feed additive*, but only in combination with a more specific method, namely thin-layer chromatography (TLC), which was not demonstrated as fit-for-purpose.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL is not able to recommend for official control the in-house method based on high performance liquid chromatography coupled to diode array detection and triple quadrupole mass spectrometry (HPLC-DAD-MS/MS) for the determination of the phytochemical marker (*verbascosides*) in the *feed additive* as no validation or verification data of the method were submitted by the Applicant and no target content of *verbascosides* as a criterion in the *feed additive* was specified.

The EURL considers that the methods for the determination of the swelling index, loss on drying and total ash content are fit-for purpose and might be recommended for official control for the characterisation of the *feed additive*, but only together with a more specific method, namely thin-layer chromatography (TLC), which was not demonstrated as fit-for-purpose.

As the unambiguous determination of the *feed additive* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control for the determination of *Plantago ovata L. husk: fleawort absolute* in these matrices.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Plantago ovata L. husk: fleawort absolute* 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_FWD. APPL. 1831-0016-2018
 - [2] *Application form, Annex 1, Submission No. 1288800711695-1390
 - [3] *Application, Proposal for Register Entry – Annex A
 - [4] *Technical dossier, Section II: II.1.3. Qualitative and quantitative composition
 - [5] *Technical dossier, Section II: II.6. Method of analysis and reference samples
 - [6] *Technical dossier, Section II: II.5. Condition of use of the additives
 - [7] *Technical dossier, Section II: Annex II_6_01
 - [8] European Pharmacopoeia Monograph (corrected 6.0, 01/2008:1334)
 - [9] *Request for clock-stop, FAD-2010-0327, ref. Ares(2018)4761286 - 17/09/2018
- *Refers to Dossier no: FAD-2010-0327

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

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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- ¹Wageningen Food Safety Research (WFSR) (NL)
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- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)

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