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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**

**Great mullein tincture
(Verbascum thapsus L.)
(FAD-2010-0350; CRL/100350)**

**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-2010-0350 - CRL/100350**

Name of Product: ***Great mullein tincture (Verbascum thapsus L.)***

Active substance(s): **total polyphenols, total phenolic acids**

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

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Date: **13/08/2019**

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Date: **13/08/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for the botanically defined *great mullein tincture* 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 all animal species and categories.

The *feed additive* is a mixture of naturally occurring chemical components, including total polyphenols and total phenolic acids as major constituents. The *feed additive* is intended to be incorporated directly into *feedingstuffs* alone or in combination with other flavouring substances (flavouring *premixtures*). The Applicant did not propose any minimum or maximum content of *great mullein tincture* in *feedingstuffs*.

The Applicant did not indicate any phytochemical marker and did not provide any corresponding method, but other methods aiming at the identification/characterisation of the *feed additive*.

The Applicant characterised the *feed additive* by determination of: loss on drying and ash content (gravimetry); total polyphenols (spectrophotometry); and total phenolic acids (high performance thin layer chromatography - HPTLC).

Furthermore, the Applicant has provided as reference a typical HPTLC profile of *great mullein tincture*'s phenolic acids with the aim to provide a tool for the unambiguous identification of the *feed additive*. A detailed description of the characteristics of the profile has been included.

For the identification/characterisation of the *feed additive* the EURL considers the methods based on gravimetry, spectrophotometry and high performance thin layer chromatography (HPTLC) proposed by the Applicant as fit-for-purpose.

Furthermore, the Applicant did not provide experimental data or analytical method for the determination of *great mullein tincture* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

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

Great mullein tincture, sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new additive) for the botanically defined *great mullein tincture* 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 [1-3]. Specifically, the *feed additive* is sought to be used for all animal species and categories [3,4].

The *feed additive* is a dark brown hydroalcoholic extract (75 % water and 25 % ethanol) of *great mullein* (*Verbascum thapsus L.*) aerial parts obtained after maceration, containing a mixture of naturally occurring chemical components.

The Applicant selected in the original dossier, as requested by chapter 2.2.1.1 of Annex II of Commission Regulation (EU) No 429/2008, total flavonoids as phytochemical marker [5,6]. Nevertheless, lately, the Applicant modified the description of the *feed additive*, removing total flavonoids as phytochemical marker and generically indicating total polyphenols and total phenolic acids as major constituents [7-9].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* alone or in combination with other flavouring substances (flavouring premixtures). The Applicant did not propose any minimum or maximum content of *great mullein tincture* in *feedingstuffs* [10].

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 *great mullein tincture* 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)

The Applicant did not provide, in the dossier or as supplementary information, any method for the determination of the phytochemical marker(s) [8,11,12].

Furthermore, the Applicant did not provide experimental data or analytical method for the determination of *great mullein tincture* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

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)

An 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)

The Applicant characterised the *feed additive (great mullein tincture)* by the determination of loss on drying and ash content, total polyphenols content and total phenolic acids content [8, 13].

For the determination of loss on drying and ash content in the *feed additive* the Applicant used gravimetric methods, while for the determination of total polyphenols the Applicant applied a spectrophotometric method based on the European Pharmacopoeia [14,15].

For the determination of total phenolic acids content in the *feed additive* the Applicant proposed for the official control a high performance thin layer chromatography (HPTLC) method which is recognised in the French Pharmacopoeia [14,16,17].

Furthermore, applying strictly the conditions indicated in the standard operating procedure, the Applicant has provided as reference a typical HPTLC profile of *great mullein tincture's* phenolic acids with the aim to offer a tool for the unambiguous identification of the *feed additive*. A detailed description of the characteristics of the profile has been included [17].

In the frame of the batch to batch variability, the Applicant has provided the result of the analysis of five different lots of the *feed additive (great mullein tincture)* characterised by applying the methods above. These analyses led to average values of 2.8 % for the loss on drying, 0.3 % for the ash content, 0.2 % of total polyphenols and 0.09 % of total phenolic acids [8,13,18].

For the identification/characterisation of the *feed additive* the EURL considers the above mentioned methods based on gravimetry, spectrophotometry and HPTLC 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 recommends for the characterisation of *feed additive (great mullein tincture)* the gravimetric methods for the determination of loss on drying and the ash content; the spectrophotometric method for the determination of total polyphenols; and the method based on high performance thin layer chromatography (HPTLC) for the determination of total phenolic acids.

Recommended text for the register entry (analytical method)

For the characterisation of *great mullein tincture*:

- gravimetric method for the determination of loss on drying and the ash content
- spectrophotometric method for the determination of total polyphenols content
- high performance thin layer chromatography (HPTLC) method for the determination of total phenolic acids

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *great mullein tincture* 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. 1831-0011-2011. FAD 2010-0350
- [2] *Application form, Annex I, Update III- 15 05 2018.pdf, Ref. Ares(2018)2523298 - 15/05/2018
- [3] *Application, Proposal for Register Entry – Annex A
- [4] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.5.1 Proposed mode of use in animal nutrition
- [5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives, O.J. L 133/1, 22.5.2008 - 2.2.1.1. Chemical substances
- [6] *Technical dossier, Section II: 2.6.1.2. Analysis of the tincture Mugwort
- [7] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.1.3.1 Description of the additive

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- [8] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.1.3.4 Active substances
 - [9] Supplementary information - Request_info_flavonoidsVSphenolic_acids.pdf
 - [10] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.5.1 Proposed mode of use in animal nutrition
 - [11] Supplementary information, cs_fad-2010-0350_Denseflower.pdf, Ref. Ares(2018)1724642 - 28/03/2018
 - [12] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.6 Methods of analysis and reference samples
 - [13] Supplementary information - 190312_NEWSection II_Identity.pdf - Section II: 2.1.3.3 Analytical data and batch to batch variability
 - [14] Supplementary information – Annex_II_3_Methods_of_analysis.pdf
 - [15] European Pharmacopoeia, Chapter 2.8.14 Determination of tannins in herbal drugs
 - [16] Supplementary information – Annex_II_10_Detailed report of phenolic acids quantification.pdf
 - [17] Supplementary information – 190312_NEWSection II_Identity.pdf - Section II: 2.2.1.5 Proposal for official controls of great mullein tincture
 - [18] Supplementary information – Annex_II_4_Results of analysis.pdf
- *Refers to Dossier no: FAD-2010-0350

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:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
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