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European Union Reference Laboratory for Feed Additives

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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

Echinachea angustifolia DC. extract and Echinachea purpurea (L.) Moench. extract

(FAD-2010-0332; CRL/100326)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2010-0332 – CRL/100326**
Name of Feed Additive(s):	***Echinachea angustifolia DC. extract and Echinachea purpurea (L.) Moench. extract***
Phytochemical marker(s):	**Echinacoside** **Total polyphenols** **Cichoric acid**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Stefano Bellorini** **28/10/2020**
Report approved by: Date:	**Christoph von Holst** **28/10/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 10(2) for the botanically defined *feed additives*, namely *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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 additives* are sought to be used for cats and dogs.

According to the Applicant, the phytochemical markers for the characterisation of the *feed additives* are *echinacoside* in *Echinachea angustifolia DC. extract* and *total polyphenols* in *Echinachea purpurea (L.) Moench. extract.* The minimum content of each marker in the *feed additives* is 4.0 % (w/w). In addition, the Applicant specified *cichoric acid* as a phytochemical marker for *Echinachea purpurea (L.) Moench. extract* without specifying a range of its mass fraction in the *feed additive*.

For the quantification of *echinacoside* in the *feed additive* (*Echinachea angustifolia DC. extract*) the Applicant proposed a method based on high performance liquid chromatography (HPLC) with UV detection at 330 nm described in the European Pharmacopoeia monograph (corrected 6.0, 01/2008:1821).

Based on the acceptable performance data of the method, the EURL recommends the HPLC-UV method described in the above mentioned European Pharmacopoeia monograph for the quantification of *echinacoside* in the *feed additive* (*Echinachea angustifolia DC. extract*).

For the quantification of *total polyphenols* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*) the Applicant submitted an in-house method based on spectrophotometry at 715 nm after derivatisation with Folin's-Denis reagent. However, neither validation nor verification data of the method were submitted by the Applicant.

Therefore, based on the available information the EURL is not able to recommend for official control the above mentioned in-house method based on spectrophotometry or any other method for the quantification of *total polyphenols* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

For further identification/characterisation of *Echinachea purpurea (L.) Moench. extract* the EURL recommends the HPLC-UV based method as proposed by the Applicant and described in the European Pharmacopoeia monographs (corrected 6.0, 01/2008:1823 and 01/2008:1824) for the qualitative analysis of *cichoric acid* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

As the unambiguous determination of the *feed additives* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate or recommend any

method for official control for the determination of *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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

Echinachea angustifolia DC. extract, Echinachea purpurea (L.) Moench. extract, echinacoside, total polyphenols, cichoric acid, 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 *feed additives*, namely *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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 additives* are sought to be used for cats and dogs [2,3].

Both *feed additives* are powdered herbal extracts consisting of mixtures of chemical components that are naturally present (namely phenols and polysaccharides) [4]. According to the Applicant, the phytochemical markers for the characterisation of the *feed additives* are *echinacoside* in *Echinachea angustifolia DC. extract* and *total polyphenols* in *Echinachea purpurea (L.) Moench. extract* [4,5]. The minimum content of each marker in the *feed additives* is 4.0 % (w/w) [4]. In addition, the Applicant specified *cichoric acid* as a phytochemical marker for *Echinachea purpurea (L.) Moench. extract* without specifying a range of its mass fraction in the *feed additive* [5].

The *feed additives* are intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposed no minimum or maximum level of the *feed additive*; however, a maximum content of 40 mg of each of the *feed additive*/kg *feedingstuffs* was suggested by the Applicant [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 *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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 quantification of *echinacoside* in the *feed additive* (*Echinachea angustifolia DC. extract*) the Applicant proposed a method based on high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection at 330 nm described in the European Pharmacopoeia monograph [7]. The HPLC-UV method is dedicated for the analysis of dried, whole or cut underground parts of *Echinachea angustifolia DC.* [7].

The Applicant analysed several different batches of the *feed additive* using the above mentioned HPLC-UV method and a relative standard deviation for batch-to-batch variability of 16.4 % was obtained [4].

Based on the acceptable performance data of the method, the EURL recommends the HPLC-UV method described in the above mentioned European Pharmacopoeia monograph for the quantification of *echinacoside* in the *feed additive* (*Echinachea angustifolia DC. extract*).

For the quantification of *total polyphenols* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*) the Applicant submitted an in-house method based on spectrophotometry at 715 nm after derivatisation with Folin's-Denis reagent [8].

The EURL has requested the Applicant to present the validation and verification data of the in-house method [9], as indicated in the EURL-FA Technical guide for botanical flavouring compounds [10], however no supplementary information was received from the Applicant.

Therefore, the EURL is not able to recommend for official control the above mentioned in-house method based on spectrophotometry or any other method for the quantification of *total polyphenols* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

As the unambiguous determination of the *feed additives* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate or recommend any method for official control for the determination of *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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)

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)

For further identification/characterisation of *Echinachea purpurea (L.) Moench. extract* the Applicant proposed the quantification of another phytochemical marker, namely *cichoric acid* using a method based on high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection at 330 nm described in the European Pharmacopoeia monographs [11,12]. The HPLC-UV method is dedicated for the analysis of dried, whole or cut flowering aerial [11] and underground parts [12] of *Echinachea purpurea (L.) Moench*.

The Applicant presented no data, which would demonstrate the applicability of the HPLC-UV method to quantify *cichoric acid* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*). Furthermore, the Applicant did not specify the range of mass fractions of *cichoric acid* in the *feed additive* [5].

However, the EURL recommends the above mentioned HPLC-UV method for the qualitative analysis of *cichoric acid* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

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 official control (i) the method based on high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection described in the European Pharmacopoeia monograph (corrected 6.0, 01/2008:1821) for the quantification of the phytochemical marker (*echinacoside*) in the *feed additive* (*Echinachea angustifolia DC. extract*); and (ii) the method based on high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection described in the European Pharmacopoeia monographs (corrected 6.0, 01/2008:1823 and 01/2008:1824) for the qualitative analysis of the phytochemical marker (*cichoric acid*) in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

As the Applicant did not provide validation and verification data for the quantification of the phytochemical marker (*total polyphenols*) in the *feed additive Echinachea purpurea (L.) Moench. extract*, the EURL is not able to recommend for official control the in-house method from the Applicant based on spectrophotometry or any other method for the quantification of the phytochemical marker *total polyphenols* in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*).

As the unambiguous determination of the *feed additives* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate or recommend any method for official control for the determination of *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* in these matrices.

Recommended text for the register entry (analytical method)

For the quantification of the phytochemical marker (*echinacoside*) in the *feed additive* (*Echinachea angustifolia DC. extract*):

- High performance liquid chromatography (HPLC) with spectrophotometric (UV) detection – European Pharmacopoeia monograph (corrected 6.0, 01/2008:1821)

For the qualitative analysis of phytochemical marker (*cichoric acid*) in the *feed additive* (*Echinachea purpurea (L.) Moench. extract*):

- High performance liquid chromatography (HPLC) with spectrophotometric (UV) detection – European Pharmacopoeia monographs (corrected 6.0, 01/2008:1823 or 01/2008:1824)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Echinachea angustifolia DC. extract* and *Echinachea purpurea (L.) Moench. extract* 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. 1831-0020-2018
[2] *Application form, Annex I, Submission No. 1288799980597-1384
[3] *Application, Proposal for Register Entry – Annex A
[4] *Technical dossier, Section II: I.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] European Pharmacopoeia monograph (corrected 6.0), 01/2008:1821

[8] *Technical dossier, Section II – Annex_II_6_01

[9] *Request for clock-stop, FAD-2010-0332, ref. Ares(2018)3918506 - 24/07/2018

[10] EURL –FA Validation and verification technical guide for Sensory feed Additives - flavouring compounds 2(b) from botanical origin https://ec.europa.eu/jrc/sites/jrcsh/files/eurl-fa-technical-guide_sensory-additives.doc

[11] European Pharmacopoeia monograph (corrected 6.0), 01/2008:1823

[12] European Pharmacopoeia monograph (corrected 6.0), 01/2008:1824

*Refers to Dossier no: FAD-2010-0332

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:

- [1]Wageningen Food Safety Research (WFSR) (NL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)

[1] Name and address according to COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.