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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

Phenylcapsaicin

(FEED-2022-4830; CRL/220010)

European Union Reference Laboratory
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Dossier related to:	**FEED-2022-4830 - CRL/220010**
Name of Product:	***Phenylcapsaicin***
Active Agent (s):	**Phenylcapsaicin**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**María José González de la Huebra** **19/01/2023**
Report approved by: Date:	**Christoph von Holst** **20/01/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *phenylcapsaicin* under the category/functional group 4(e) "zootechnical additives"/"Physiological condition stabilisers", according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for chickens for fattening.

According to the Applicant, the product contains as active substance not less than 98 % (w/w) *phenylcapsaicin*. The product is intended to be incorporated directly into *compound feed* at a level ranging from 10 to 15 mg / kg *complete feed*.

For the determination of *phenylcapsaicin* in the *feed additive* the Applicant proposed a single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography (HPLC) coupled to spectrophotometric (UV) detection. Based on the presented performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on HPLC coupled to UV detection for the determination of *phenylcapsaicin* in the *feed additive*.

For the determination of *phenylcapsaicin* in *compound feed* the Applicant proposed an analytical method based on HPLC coupled to UV detection (HPLC-UV). The analytical method was not supported by any validation and / or verification study and could not be considered fit for purpose for official control. However, in the frame of the current dossier, a second single-laboratory validated and further verified method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been presented for the "*determination of phenylcapsaicin in liquid and solid feed*". The following performance characteristics are reported: a relative standard deviation for repeatability (RSD_r) ranging from 1.1 to 7.2 %, a relative standard deviation for intermediate precision (RSD_{ip}) of 1.9 % and a recovery rate (R_{Rec}) ranging from 77 to 107 %. Furthermore, the Applicant reported a limit of detection (LOD) and a limit of quantification (LOQ) respectively of 0.03 and 0.1 mg / kg *compound feed*. Based on the performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the determination of *phenylcapsaicin* in *compound feed*.

The Applicant presented a single-laboratory validated and further verified method based on LC-MS/MS for the determination of *phenylcapsaicin* residues in animal tissues is provided. The following performance characteristics are reported for the analysis in liver in a range between $2x10^{-4}$ to 0.02 mg / kg: a RSD_r ranging from 0.1 to 4.4 %, a RSD_{ip} of 3.8 % and a R_{Rec} ranging from 100 to 119 %. Furthermore, the Applicant reported a LOQ of $2x10^{-4}$ mg/kg. However, the Applicant did not propose any maximum residue levels (MRL) of

phenylcapsaicin in tissues of chicken for fattening. Therefore, the EURL cannot evaluate the fitness for purpose of this analytical method.

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

Phenylcapsaicin, zootechnical additives, physiological condition stabilisers, chickens for fattening.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *phenylcapsaicin* under the category/functional group 4(e) "zootechnical additives"/"Physiological condition stabilisers", according to Annex I of Regulation (EC) No 1831/2003 [1-2]. The authorisation is sought for the use of the *feed additive* for chickens for fattening [2].

According to the Applicant, the chemically synthesised product is a dark brown viscous liquid analogue of the naturally occurring capsaicin [RF]. It contains as *active substance* not less than 98 % (w/w) *phenylcapsaicin* [3]. The product is intended to be incorporated directly into *compound feed* at a level ranging from 10 to 15 mg / kg [2]. The Applicant did not propose any maximum residue limits (MRLs) for *phenylcapsaicin* in chicken for fattening tissues (i.e. muscle, kidney, skin/fat and liver) [4].

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 *phenylcapsaicin* 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 *phenylcapsaicin* in the *feed additive* the Applicant proposed a single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography (HPLC) coupled to spectrophotometric (UV) detection [5,6].

The sample is diluted in acetonitrile, transferred in a vial and directly injected into HPLC for the chromatographic analysis. The analyte is detected at 240 nm and it is quantified by normalization procedure.

The Applicant obtained excellent performance characteristics from the tests performed in the frame of the supporting validation and verification studies (i.e. precision values corresponding to 0.02 % and a recovery rate of 100.1 %) [7,8].

Based on the presented performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on HPLC coupled to spectrophotometric UV detection for the determination of *phenylcapsaicin* in the *feed additive*.

For the determination of *phenylcapsaicin* in *compound feed* the Applicant proposed an analytical method based on HPLC coupled to UV detection (HPLC-UV) [5,9].

Samples, containing as internal standard capsaicin, are extracted with acetonitrile. The sample is cleaned up via dispersive solid phase extraction (dSPE – QuEChERS), filtered and analysed by HPLC-UV (240 nm) [9].

Nevertheless, the analytical method proposed by the Applicant is not supported by any validation and/or verification study. Therefore, the EURL cannot consider the above mentioned method suitable for the official control of the product in *compound feed.*

However, in the frame of the current dossier, a second single-laboratory validated and further verified method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been presented for the "*determination of phenylcapsaicin in liquid and solid feed*" [10].

Liquid and sieved solid samples are supplemented with capsaicin as internal standard and appropriately diluted with acetonitrile. Solid samples are centrifuged and supernatant is cleaned up via dSPE. The extract and/or the diluted liquid samples are filtered before injection in the LC system. *Phenylcapsaicin* is determined by MS/MS using electrospray ionisation in positive mode (ESI+) and quantified by standard addition [10].

The following performance characteristics are reported in the frame of the corresponding validation and verification studies for the analysis of liquid and solid *compound feed* samples [10,11]:

- a relative standard deviation for repeatability (RSD_r) ranging from 1.1 to 7.2 %;
- a relative standard deviation for intermediate precision (RSD_{ip}) of 1.9 %; and
- a recovery rate (R_{Rec}) ranging from 77 to 107 %.

Furthermore, the Applicant reported a limit of detection (LOD) and a limit of quantification (LOQ) respectively of 0.03 and 0.1 mg / kg solid samples [11].

Based on the performance characteristics, the EURL recommends for official control the single laboratory validated and further verified method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the determination of *phenylcapsaicin* in *compound feed*.

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)

Even if the Applicant did not propose any maximum residue limits (MRLs) for *phenylcapsaicin* in chicken for fattening tissues (i.e. muscle, kidney, skin/fat and liver), a single-laboratory validated and further verified method based on LC-MS/MS for the determination of *phenylcapsaicin* residues in animal tissues is provided [5,12].

The analytical method is similar to the method recommended for the determination of *phenylcapsaicin* in *compound feed* [10]. Tissues are minced and homogenised with the addition of water. Subsamples are supplemented with capsaicin as internal standard and appropriately diluted with acetonitrile before centrifugation. Supernatant is cleaned up via dSPE, filtered and injected in the LC system. *Phenylcapsaicin* is determined by MS/MS using electrospray ionisation in positive mode (ESI+) and quantified by standard addition [12].

The following performance characteristics are reported in the frame of the corresponding validation and verification studies for the analysis in liver in a range between $2x10^{-4}$ and 0.02 mg / kg [13,14]:

- a RSD_r ranging from 0.1 to 4.4 %,
- a RSD_{ip} of 3.8 %; and
- a R_{Rec} ranging from 100 to 119 %.

Furthermore, the Applicant reported a LOQ of $2x10^{-4}$ mg / kg [13].

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 single-laboratory validated and further verified method based on HPLC coupled to spectrophotometric UV detection for the determination of *phenylcapsaicin* in the *feed additive*; and (ii) the single laboratory validated and further verified method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the determination of *phenylcapsaicin* in *compound feed.*

Recommended text for the register entry (analytical method)

For the determination of *phenylcapsaicin* in the *feed additive*:

- High performance liquid chromatography coupled to spectrophotometric detection (HPLC-UV)

For the determination of *phenylcapsaicin* in *compound feed*:

- High performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Phenylcapsaicin* 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] Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – https://webgate.ec.europa.eu/esfc/#/applications/6730 https://open.efsa.europa.eu/questions/EFSA-Q-2022-00355
[2] *Technical dossier, Section II: aXichem_Sect_II_Conditions of Use
[3] *Technical dossier, Section II: aXichem_Sect_II_Identification and characterisation Rev1 25Aug2022, 2.1.3 Qualitative and Quantitative Composition
[4] *Scientific Summary, Section III: 3.2 Studies Concerning the Safety of Use of the Additive for Consumers
[5] *Technical dossier, Section II: aXichem_Sect_II_Methods of Analysis Rev1 25Aug2022
[6] *Technical dossier, Section II: Annex II-9 -Method of analysis_chromatographic purity Phenylcapsaicin
[7] *Technical dossier, Section II, Annex: Validation of MOA additive-Hwasun-English
[8] *Technical dossier, Section II, Annex: Lab verification report Q&Q feed additive
[9] *Technical dossier, Section II: Annex II-10 - Measurement of Phenylcapsaicin in feed results
[10] *Technical dossier, Section II: Annex II-11.1 - Method Validation-phenylcapsaicin
[11] *Technical dossier, Section II, Annex: Lab verification report Q&Q feed
[12] *Technical dossier, Section II: Annex II-11.3 - Method of analysis
[13] *Technical dossier, Section II, Annex: Annex II-11.2 - Measurement of Phenylcapsaicin in tissue
[14] *Technical dossier, Section II: Lab verification report Q&Q chicken liver

*Refers to Dossier no: FEED-2022-4830

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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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)