
Axtra® PHY 20000 TPT2
(FAD-2015-0048; CRL/150029)

Dossier related to: FAD-2015-0048 - CRL/150029
Name of Product: Axtra® PHY 20000 TPT2
Active Agent (s): 6-phytase (EC 3.1.3.26)
Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA)
Geel, Belgium
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Date: 19/05/2016
Report approved by: Christoph von Holst
Date: 23/05/2016
EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Axtra® PHY 20000 TPT2*, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for several animal species. The active agent of *Axtra® PHY 20000 TPT2* is 6-phytase (EC 3.1.3.26), produced by fermentation of *Trichoderma reesei*. According to the Applicant, *Axtra® PHY 20000 TPT2* is a dry formulation with a guaranteed minimum enzyme activity of 20000 FTU/g. It is intended to be used in premixtures and/or complete feedingstuffs to obtain 6-phytase activities of 250 FTU/kg feedingstuffs. The Applicant expresses the phytase enzymatic activity in FTU/g units, where "one phytase unit (FTU) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C".

For the quantification of phytase in the feed additive, premixtures and feedingstuffs, the Applicant submitted a single-laboratory validated and further verified colorimetric methods similar to the EN ISO 30024 standard method. Based on the experimental data available, the EURL recommends for official control these colorimetric methods for the quantification of phytase activity 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.

KEYWORDS

6-phytase, *Axtra® PHY 20000 TPT2*, zootechnical additives, digestibility enhancers, chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor poultry species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species
1. BACKGROUND

In the current application authorisation is sought for Axtra® PHY 20000 TPT2, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor poultry species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species [1,2].

The active agent of Axtra® PHY 20000 TPT2 is 6-phytase (EC 3.1.3.26), produced by fermentation of a Trichoderma reesei [3]. The Applicant expresses the phytase enzymatic activity in FTU/g units, where "one FTU is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C" [3]. This definition is in agreement with the phytase activity unit defined in the EN ISO 30024 [4].

According to the Applicant, Axtra® PHY 20000 TPT2 is a dry formulation with a guaranteed minimum enzyme activity of 20000 FTU/g [2,3]. It is intended to be used in premixtures and/or complete feedingstuffs to obtain 6-phytase activities of 250 FTU/kg feedingstuffs [2,5].

Note: The analytical methods for the determination of a different formulation of Axtra® PHY in relevant matrices of the target species were already evaluated by the EURL in the frame of the (FAD-2013-0049) dossier [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 Axtra® PHY 20000 TPT2 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

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [7].

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

For the quantification of the phytase activity in the feed additive [8], premixtures [9] and feedingstuffs [10], the Applicant submitted a single-laboratory validated and further verified methods already evaluated by the EURL [6] similar to the ring-trial validated ISO method (EN ISO 30024) [4].

Phytase is incubated with sodium phytate, resulting in the release of inorganic phosphate and forming a yellow complex with an acidic molydate/vanadate reagent [4]. Based on the satisfactory experimental evidence presented (Table 1) [8] the EURL recommends for official control this single-laboratory validated and further verified method for the quantification of phytase activity in the feed additive.

For the quantification of phytase activity in feedingstuffs the Applicant applied the proposed method to feed samples containing Axtra® PHY 20000 TPT2 [10]. Furthermore, the Applicant quantified the phytase activity in premixture samples containing Axtra® PHY 20000 TPT2 [9] by first diluting the samples with heat treated whole grain wheat flour or maize and then analysing them as feedingstuffs samples.

The experimental data provided in the frame of the stability studies [8,9,10] were used by the EURL to calculated relative standard deviations (for repeatability and for intermediate precision) ranging from 0.5 to 14.4% [11,12,13]. The results obtained with the different matrices are summarized in Table 1 and demonstrate the applicability of the proposed method to the feed matrices containing the new formulation.

Additionally, in the frame of a previous dossier related to the liquid formulation of the same feed additive [6] the Applicant obtained similar performance characteristics for the in-house method and the EN ISO 30024 standard method when analysing mash feed fortified with their product [14], and therefore demonstrated the equivalence of the two methods.

Based on the satisfactory experimental evidences presented in Table 1 [9,10] the EURL recommends for official control the method submitted by the Applicant to quantify phytase in the feed additive and the EN ISO 30024 method to the determination of phytase in premixtures and feedingstuffs samples containing the feed additive under investigation.
Table 1: Performance characteristics of analytical methods for the determination of phytase in feed additive (FA), premixtures (PM) and feedingstuffs (FS)

<table>
<thead>
<tr>
<th>Method</th>
<th>Matrix</th>
<th>Activity</th>
<th>RSDₙ (%)</th>
<th>RSDᵢₚ (%)</th>
<th>RSDᵦ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danisco</td>
<td>FA [8]</td>
<td>26640-30215 (FTU/g)</td>
<td>1.0-4.4</td>
<td>2.7-4.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PM [9]</td>
<td>136802-149287 (FTU/kg)</td>
<td>4.3-8.1</td>
<td>6.1-14.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FS (Mash) [10]</td>
<td>346-397 (FTU/kg)</td>
<td>0.5-11.3</td>
<td>9.1-11.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FS (Pellets) [10]</td>
<td>406-477 (FTU/kg)</td>
<td>7.5-10.8</td>
<td>7.5-17.6</td>
<td>-</td>
</tr>
<tr>
<td>EN ISO 30024</td>
<td>FS [4]</td>
<td>500-1500 (FTU/kg)</td>
<td>2.2-10.6</td>
<td>3.3-12.7</td>
<td>5.4-15.0</td>
</tr>
</tbody>
</table>

RSDₙ, RSDᵢₚ, and RSDᵦ: relative standard deviation for repeatability, intermediate precision and reproducibility.

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 single-laboratory validated and further verified colorimetric method for the quantification of phytase activity in the feed additive and the EN ISO 30024 colorimetric method for the quantification of phytase activity in premixtures and feedingstuffs.

**Recommended text for the register entry (analytical method)**

For the quantification of phytase activity in the feed additive:
- colorimetric method based on the enzymatic reaction of phytase on phytate

For the quantification of phytase activity in premixtures and feedingstuffs:
- colorimetric method based on the enzymatic reaction of phytase on phytate – EN ISO 30024

One phytase unit (FTU) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at 37°C and pH 5.5

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Axtra® PHY 20000 TPT2 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

[2] *Application, Proposal for Register Entry – Annex A
[3] *Technical dossier, Section II: II.1 Identity of the additive
[5] *Technical dossier, Section II: II.5 Conditions of use of the additive
[12] *Supplementary information, eurl_anova_axtraphy2000tpt2_pm.pdf
[14] *Supplementary information, Annex_II_29
*Refers to Dossier no: FAD-2015-0048

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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 sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
Laboratorio Arbitral Agroalimentario. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid (ES)

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