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EURL 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-2010-0030 EURL/ 090043 FAD-2010-0073 EURL/ 100057
Name of Feed Additive:	Pantothenic acid
Active Agent (s):	Calcium-D-Pantothenate D-Panthenol
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

In the current application authorisation is sought for *Pantothenic acid* under Articles 4(1) and 10(2), under the category of 'nutritional additives' functional group 3(a), 'vitamins, pro-vitamins and chemically well defined substances having a similar effect' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Pantothenic acid* for all animal species and categories. The *feed additive* to be registered is in the form of two active components: *Calcium-D-Pantothenate* and *D-Panthenol*. *Calcium-D-Pantothenate* is a white to almost white, spray dried or crystalline powder, slightly hygroscopic, freely soluble in water, with a minimum content of 98%. It is intended to be used in *premixtures* and *feedingstuffs*. *D-Panthenol* is highly viscous transparent or yellowish liquid at room temperature, freely soluble in water, with a minimum purity of 98%; it is intended to be used only in *water*. No minimum or maximum concentrations of the *feed additive* in *feedingstuffs* or *water* are proposed by the Applicants.

For the determination of *Calcium-D-Pantothenate* in the *feed additive* the Applicants (FAD-2010-0030 and 2010-0073) proposed the European Pharmacopoeia method, based on potentiometric titration with perchloric acid and identification by specific optical rotation. The EURL considers this method suitable to be used within the frame of official control.

For the determination of *Calcium-D-Pantothenate* in *premixtures* and *feedingstuffs* the Applicant (FAD-2010-0073) proposed a single laboratory validated and further verified method, based on Reverse Phase High-Performance Liquid Chromatography coupled to a single-quadrupole mass selective detector (RP-HPLC-MS). The following performance characteristics were reported:

- for *premixtures*: - a relative standard deviation of *repeatability* (RSD_r) ranging from of 1.7 to 2.4%; and - a *recovery* rate (R_{Rec}) ranging from 103 to 106%;

- for *feedingstuffs* (containing the *Calcium-D-Pantothenate* at a concentration from 30 to 60 mg/kg): - RSD_r ranging from of 2 to 4.7%; and - R_{Rec} ranging from 120 to 124%.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified method using RP-HPLC-MS, submitted by the Applicant, to determine *Calcium-D-Pantothenate* in *premixtures* and *feedingstuffs.*

For the determination of *D-Panthenol* in the *feed additive* the Applicants (FAD-2010-0030 and 2010-0073) proposed the European Pharmacopoeia method, based on titration with perchloric acid and potassium hydrogen phthalate and identification by specific optical rotation and infrared spectroscopy. The EURL considers this method suitable to be used within the frame of official control.

For the determination of *D-Panthenol* in *water* the Applicant (FAD-2010-0073) proposed a single laboratory validated and further verified method, using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), coupled to UV detector. The following performance characteristics were reported in water containing *D-Panthenol* at 10 mg/L:

- RSD_r ranging from of 0.27 to 0.95%;
- a relative standard deviation of *intermediate precision* (RSD_{int}) ranging from of 0.3 to 0.97%;
- R_{Rec} ranging from 99 to 101%;
- a limit of detection (LOD) and a limit of quantification (LOQ) of 0.9 mg/L and 3 mg/L, respectively.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method, submitted by the Applicant, to determine *D-Panthenol* in *water*.

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
Pantothenic acid, Calcium-D-Pantothenate, D-Panthenol, nutritional additives, all species, vitamins.

1. BACKGROUND

In the current application authorisation is sought for a feed additive *Pantothenic acid* under Articles 4(1) (new use in water) and 10(2) (re-evaluation of the already authorised additive under council directive 70/524/EEC), under the category of 'nutritional additives' functional group 3(a), 'vitamins, pro-vitamins and chemically well defined substances having a similar effect' according to Annex I of Regulation (EC) No 1831/2003 [1, 2]. Specifically,

authorisation is sought for the use of *Pantothenic acid* for all animal species and categories. The *feed additive* to be registered is in the form of two active components: *Calcium-D-Pantothenate* and *D-Panthenol* [3-5]. According to the Applicants (FAD-2010-0030 and 2010-0073) *Calcium-D-Pantothenate* is a white to almost white, spray dried or crystalline powder, freely soluble in water and glycerol, with a minimum content of 98% [6, 7]. It is intended to be used in *premixtures* and *feedingstuffs*. *D-Panthenol* is highly viscous transparent or yellowish liquid at room temperature, freely soluble in water, methanol and ethanol, with a minimum purity of 98%; it is intended to be used only in *water* [6, 7]. No minimum or maximum concentrations of the *feed additive* in *feedingstuffs* or *water* are proposed by the Applicants [3-5], furthermore no limits were set in previous regulation [8]. However, normal contents for *Calcium-D-Pantothenate* range from 8 to 50 mg/kg in *feedingstuffs* and for *D-Panthenol in water* from 10 to 20 mg/L, respectively [9, 10].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 (EFSA) for each application or group of applications. For these dossiers, the methods of analysis submitted in connection with *Pantothenic acid*, 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

Qualitative and quantitative composition of impurities in the 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 [11].

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

Calcium-D-Pantothenate

Calcium-D-Pantothenate is a white to almost white powder, slightly hygroscopic and freely soluble in water. A maximum loss on drying of 3 % is determined on 1 g by drying in an oven at 105°C, as described in the European Pharmacopoeia – Monograph 0470 [12, 13].

For the determination of *Calcium-D-Pantothenate* in the *feed additive* both Applicants (FAD-2010-0030 and 2010-0073) proposed the European Pharmacopoeia method – Monograph 0470 [12, 13], based on potentiometric titration with perchloric acid and identification by specific optical rotation. No performance characteristics of this method are provided. However, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *Calcium-D-Pantothenate* in *premixtures* the Applicant (FAD-2010-0030) proposed two microbiological methods (US pharmocopeia USP XXIX, M91 method and AOAC 45.2.05 method 945.74), using *Lactobacillus plantarum* ATCC 8014 as test organism [14, 15]. These methods are used for mixture of vitamins and are not suitable for *premixtures*.

The second Applicant (FAD-2010-0073) proposed a single laboratory validated and further verified HPLC-MS method [16], for the determination of *Calcium-D-Pantothenate* in *premixtures*. The sample (5g) is extracted with water at 37°C for 20 min. After cooling to room temperature, the extract is shaken and filtrated through PVDF filter or centrifuged for three minutes at 13000 rpm. The filtered sample extracts and calibration solutions, prepared depending on the expected concentration of pantothenic acid in samples, are transferred into HPLC vials and internal standard solution (isotope labelled calcium pantothenate) is added. The samples are then analysed by Reverse Phase High-Performance Liquid Chromatograph (RP-HPLC) mass spectrometry. The method performance characteristics derived from the validation and verification studies [16] are presented in Table 1.

For the determination of *Calcium-D-Pantothenate* in *feedingstuffs* the Applicant (FAD-2010-0030) claims that the microbiological methods suggested for *premixtures* could be applied, but did not submit any data [17].

Table 1: Method performance characteristics for the determination of *Pantothenic acid* in *premixtures* (PM), *feedingstuffs* (FS) and *water*. Target values for *Calcium-D-Pantothenate* content ranging from 2 to 4 g/kg for *premixtures* and from 30 to 60 mg/kg for *feedingstuffs*. Target values for *D-Panthenol* at 10 mg/L.

Pantothenic acid		RSD_r (%)		R_{Rec} (%)		RSD_{int} (%)	
		Validation	Verification	Validation	Verification	Validation	Verification
Calcium-D-Pantothenate	PM	2.4 [16]	1.7 [16]	103 [16]	106 [16]	np	np
	FS	4.7 [16]	2.0 [16]	120 [16]	124 [16]	np	np
D-Panthenol	Water (HPLC Method)	0.27 [22]	0.95 [22]	99-101 [22]	99 [22]	0.3 [22]	0.97 [22]
	Water (Vanillin Method)	1.13 [21]	np	101 [21]	np	np	np

RSD_r, RSD_{int} - relative standard deviation for *repeatability* and *intermediate precision*,
R_{Rec} – recovery rate
np – not provided

However, the second Applicant (FAD-2010-0073) proposed a single laboratory validated and further verified HPLC-MS method [16] for the determination of *Calcium-D-Pantothenate* in *feedingstuffs*. It is the same method used for the determination of *Calcium-D-Pantothenate* in *premixtures*. The method performance characteristics derived from the validation and verification studies [16] are presented in Table 1.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-MS method, submitted by the Applicant, to determine *Calcium-D-Pantothenate* in *premixtures* and *feedingstuffs*.

D-Panthenol

D-Panthenol is a colourless or slightly yellowish, viscous hygroscopic liquid, very soluble in water. A maximum loss on drying of 1 % is determined by drying in a vacuum with diphosphorous pentoxide, as described in the European Pharmacopoeia – Monograph 20232 [18].

For the determination of *D-Panthenol* in the *feed additive* both Applicants (FAD-2010-0030 and 2010-0073) proposed the European Pharmacopoeia method – Monograph 0761 [19, 20], based on titration with perchloric acid and potassium hydrogen phthalate and identification by specific optical rotation and infrared spectroscopy. No performance characteristics of this method are provided. However, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *D-Panthenol* in *water* the Applicant (FAD-2010-0030) proposed an in house colorimetric method with vanillin [21]. The samples are subjected to alkaline hydrolysis with 0.5 N sodium hydroxide in a boiling water bath for 1 hour and then neutralized with 0.5 N sulphuric acid. Duquenois reagent (containing vanillin) and McIlvane buffer are then added. The colour developed is measured with spectrophotometer at 406 nm. The method was partially validated and performance characteristics derived from the validation [21] are presented in Table 1. Furthermore, a limit of detection (LOD) and a limit of quantification (LOQ) of 10 mg/L and 50 mg/L in *water* were determined by the Applicant [21]. However, no verification data was provided.

The second Applicant (FAD-2010-0073) proposed a single laboratory validated and further verified HPLC-UV method [22], for the determination of *D-Panthenol* in *water*. The samples are shaken and treated in the ultrasonic bath for 10 minutes at room temperature. After appropriate dilution, the samples are centrifuged for 3 minutes at 14000 rpm and analysed by RP-HPLC-UV at 210 nm, using solutions of D-Panthenol with a defined concentration as an external standard. The method is applicable for the determination of *D-Panthenol* with a concentration higher than 3 mg/L in water and the performance characteristics derived from the validation and verification studies [22] are presented in Table 1. Furthermore, a limit of detection (LOD) and a limit of quantification (LOQ) of 0.9 mg/L and 3 mg/L in *water* were determined by the Applicant [22].

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-UV method, submitted by the Applicant, to determine *D-Panthenol* in *water*.

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 European Pharmacopoeia Monograph 0470 using drying in an oven at 105°C to determine loss on drying in *Calcium-D-Pantothenate*;

- the European Pharmacopoeia Monograph 0470 using potentiometric titration with perchloric acid and identification by specific optical rotation to determine *Calcium-D-Pantothenate* in *feed additive*;

- a single laboratory validated and further verified method using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) coupled to a single-quadrupole mass selective detector, to determine *Calcium-D-Pantothenate* in *premixtures* and *feedingstuffs*;

- the European Pharmacopoeia Monograph 20232 using drying in a vacuum with diphosphorous pentoxide to determine loss on drying in *D-Panthenol*;

- the European Pharmacopoeia Monograph 0761 using titration with perchloric acid and potassium hydrogen phthalate and identification by specific optical rotation and infrared spectroscopy to determine *D-Panthenol* in *feed additive;* and

- a single laboratory validated and further verified method, using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) coupled to UV detector to determine *D-Panthenol* in *water*.

Recommended text for the register entry (analytical method)

For the determination of *Calcium-D-Pantothenate* in *feed additive*:

- potentiometric titration with perchloric acid and identification with specific optical rotation (European Pharmacopoeia – Monograph 0470)

For the determination of *Calcium-D-Pantothenate* in *premixtures* and *feedingstuffs*:

- a single laboratory validated and verified Reverse Phase High-Performance Liquid Chromatography coupled to mass selective detector (RP-HPLC-MS)

For the determination of *D-Panthenol* in *feed additive*:

- titration with perchloric acid and potassium hydrogen phthalate, specific optical rotation and infrared spectroscopy (European Pharmacopoeia – Monograph 0761)

For the determination of *D-Panthenol* in *water*:

- a single laboratory validated and verified Reverse Phase High-Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Pantothenic acid/Calcium-D-Pantothenate and Pantothenic acid/D-Panthenol* 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/Ref:SANCO/D/2:Forw.Appl.1831/0056-2010

[2] +Application/Ref:SANCO/D/2:Forw.Appl.1831/0025-2010

[3] *Application, (Annex A), FAD-2010-0073_DescriptAdd_Calcium-d-pantothenate

[4] *Application, (Annex A), FAD-2010-0073_DescriptAdd_D-panthenol

[5] +Application, Annex A, Proposal for register entry

[6] *Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; methods of analysis, 2.1.3. Qualitative and quantitative composition

[7] +Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; methods of analysis, 2.1.3. Qualitative and quantitative composition

[8] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs

[9] *Technical dossier, Section II, Annex_II_30_AWT_2002

[10] +Technical dossier, Section II, Ref 2.5.01

[11] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[12] *Technical dossier, Section II, Annex_II_31_PhEur

[13] +Technical dossier, Section II, Annex 2.01

[14] +Technical dossier, Section II, Annex 2.17

[15] +Technical dossier, Section II, Annex 2.19

[16] *Technical dossier, Section II, Annex_II_33_Analytics_Ca-D-pantothenate

[17] +Technical dossier, Section II, 2.6.1 Methods of analysis for the active substance/additive

[18] European Pharmacopeia, 6th Edition, 01/2008:20232

[19] *Technical dossier, Section II, Annex_II_32_PhEur_D-panthenol_2008

[20] +Technical dossier, Section II, Annex 2.02

[21] +Technical dossier, Section II, Annex 2.18

[22] *Technical dossier, Section II, Annex_II_34_Analytics_D -panthenol_2010

*Refers to Dossier no: FAD-2010-0073

+Refers to Dossier no: FAD-2010-0030

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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)