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EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

European Union Reference Laboratory
Feed Additives

JRC.D.5/FSQ/CvH/DM/ago/ARES(2012)

EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0185 - CRL/100181** **FAD-2010-0214 - CRL/100066**
Name of Feed Additive:	**Vitamin C** **L-ascorbic acid (E 300)** **Sodium L-ascorbate (E 301)** **Calcium L-ascorbate (E 302)** **6-Palmityl-L-ascorbic acid (E 304)** **Ascorbyl monophosphate calcium sodium** **Ascorbyl monophosphate sodium**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (CRL-FA)** **Geel, Belgium**
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Date:	**12/10/2012**
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Date:	**24/10/2012**

EXECUTIVE SUMMARY

In the current joint application, authorisation is sought for six forms of *Vitamin C* under Articles 4(1) for *L-ascorbic acid (E 300)*[1,2] and *Ascorbyl monophosphate sodium*[1] under the category/functional group 3(a) "nutritional additives/vitamins, pro-vitamins and chemically well defined substances having a similar effect", and under Article 10(2) under the category/functional group 3(a) for *L-ascorbic acid (E 300)*[1,2] and *Ascorbyl monophosphate calcium sodium* [1,2] and under 1(b) "technological additives/antioxidants" for *L-ascorbic acid*[1], *Sodium L-ascorbate (E 301)*[1], *Calcium L-ascorbate (E 302)*[1] and *6-Palmityl-L-ascorbic acid (E 304)*[1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicants:

- *L-ascorbic acid* is white crystal or crystalline powder with a minimum purity of 99 %;
- *Sodium L-ascorbate* and *Calcium L-ascorbate* are white to yellowish crystalline powders with a minimum purity of 99 %;
- *6-Palmityl-L-ascorbic acid* is a white to yellowish white crystalline powder with a minimum purity of 98 %;
- *Ascorbyl monophosphate sodium* is a white powder with a minimum purity of 95%; and
- *Ascorbyl monophosphate calcium sodium* is beige to cream coloured compound.

Specifically, authorisation is sought for the use of the six forms of *Vitamin C* for all animal species and categories. The *feed additives* are intended to be incorporated to *feedingstuffs* directly or through *premixtures*. Additionally, *L-ascorbic acid* and *ascorbyl monophospate sodium* are to be used directly in *water*. No minimum or maximum concentration levels of the *feed additives* in *feedingstuffs* or *water* are recommended, similar to what was previously set in the regulation. However, typical concentrations in *feedingstuffs* range from 50-400 mg/kg.

For the determination of *active substances* in the *feed additives* the EURL recommends for official control four European Pharmacopoeia methods for the characterisation of *L-ascorbic acid; Sodium L-ascorbate; Calcium L-ascorbate* and *6-Palmityl-L-ascorbic acid* (Monographs 0253, 1791; 1182 and 0807, respectively), and the single-laboratory validated and further verified reversed phase High Performance Liquid Chromatography (RP-HPLC) method, submitted by the Applicant, for the determination of *ascorbyl monophosphate* in the *feed additives* (*ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*).

[1] FAD-2010-0185 [2] FAD-2010-0214

Additionally the EURL recommends for official control the ring-trial validated CEN methods EN ISO 6869, based on Atomic Absorption Spectrometry (AAS) or EN 15510, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), for the quantification of *total calcium* and *total sodium* in the relevant *feed additives.*

For the quantification of *L-ascorbic acid*, *Sodium L-ascorbate* and *Calcium L-ascorbate* in *premixtures* and *feedingstuffs* the Applicants submitted a single-laboratory validated and further verified titrimetric method. The following performance characteristics were reported:

– for *premixtures*: - a *recovery rate* (R_{rec}) of 105%; - a relative standard deviation for *repeatability* (RSD_r) ranging from 3.5 to 3.9 %; and - a relative standard deviation for *intermediate precision* (RSD_{ip}) of 4.1%;

– for *feedingstuffs*: - R_{rec} ranging from 82 to 103 %; - RSD_r ranging from 2.7 to 10.1 %; - RSD_{ip} ranging from 5.4 to 10.1 %; and a limit of quantification (LOQ) of 40 mg/kg *feedingstuffs.*

For the determination of *ascorbyl monophosphate* in *premixtures* and *feedingstuffs* (containing *ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*) the Applicants submitted a single-laboratory validated and further verified reversed phase High Performance Liquid Chromatography coupled to UV detection at 254 nm (RP-HPLC-UV). The following performance characteristics were reported:

– for *premixtures*: - R_{rec} ranging from 98 to 101 %; - RSD_r ranging from 0.6 to 2.2 %; and - RSD_{ip} ranging from 0.87 to 2.42 %;

– for *feedingstuffs*: - R_{rec} ranging from 100 to 105 %; - RSD_r ranging from 0.15 to 6.7 %; - RSD_{ip} ranging from 0.3 to 6.7 %; and LOQ = 28 mg/kg *feedingstuffs.*

Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified titrimetric method and RP-HPLC-UV methods, for the determination of *L-ascorbic acid*, *Sodium L-ascorbate*, *Calcium L-ascorbate* and/or *ascorbyl monophosphates* (originating from *ascorbyl monophosphate sodium* and *ascorbyl monophosphate calcium sodium*) in *premixtures* and *feedingstuffs.*

For the determination of *L-ascorbic acid* in *water* the Applicants proposed two internationally recognised methods: (i) the AOAC 967.21 titrimetric method developed for the determination of ascorbic acid in vitamin preparations and juices; and (ii) the ring-trial validated High Performance Liquid Chromatography coupled to UV detection at 265 nm (HPLC-UV) CEN method (EN 14130) developed for determination of vitamin C in foodstuffs. Based on the performance characteristics presented and the rationale that water is a simpler matrix than

juices and foodstuffs, the EURL recommends for official control the AOAC and the CEN methods for the determination of *L-ascorbic acid* in *water*.

The Applicant (FAD-2010-0185) did not provide any analytical methods for the determination of *6-Palmityl-L-ascorbic acid* in *premixtures* and *feedingstuffs,* or the determination of *ascorbyl monophosphate sodium* in *water.* Therefore the EURL cannot evaluate nor recommend any methods for official control to determine *6-Palmityl-L-ascorbic acid* in *premixtures* and *feedingstuffs* or *ascorbyl monophosphate sodium* 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

Vitamin C, L-ascorbic acid (E 300), Sodium L-ascorbate (E 301), Calcium L-ascorbate (E 302), 6-Palmityl-L-ascorbic acid (E 304), Ascorbyl monophosphate sodium, Ascorbyl monophosphate calcium sodium, nutritional additive, vitamins, technological additive, antioxidants, all animal species and categories

1. BACKGROUND

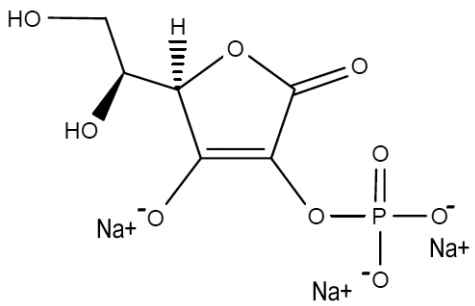
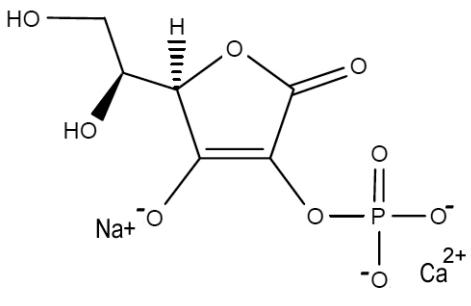
In the current joint application, authorisation is sought for six forms of *Vitamin C* under Articles 4(1) for *L-ascorbic acid (E 300)*[1,2](new use in water) and *Ascorbyl monophosphate sodium*[1] (new feed additive) under the category/functional group 3(a) "nutritional additives/vitamins, pro-vitamins and chemically well defined substances having a similar effect"[1, 2], and under Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) under the category/functional group 3(a) for *L-ascorbic acid (E 300)*[1,2] and *Ascorbyl monophosphate calcium sodium* [1,2] and under 1(b) "technological additives/antioxidants" for *L-ascorbic acid*[1], *Sodium L-ascorbate (E 301)*[1], *Calcium L-ascorbate (E 302)*[1] and *6-Palmityl-L-ascorbic acid (E 304)*[1] [1, 2], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

[1] FAD-2010-0185 [2] FAD-2010-0214

According to the Applicants:

- *L-ascorbic acid* is white crystal or crystalline powder with a minimum purity of 99 % [3,4];
- *Sodium L-ascorbate* and *Calcium L-ascorbate* are white to yellowish odourless crystalline powders with a minimum purity of 99 % [3];
- *6-Palmityl-L-ascorbic acid* is a white to yellowish white crystalline powder with a minimum purity of 98 % [3];
- *Ascorbyl monophosphate sodium* is a white powder, freely soluble in water, with a minimum purity of 95% [3]; and
- *Ascorbyl monophosphate calcium sodium* is beige to cream coloured compound [3].

Specifically, authorisation is sought for the use of the six forms of *Vitamin C* for all animal species and categories. The *feed additives* are intended to be incorporated to *feedingstuffs* directly or through *premixtures*. Additionally, *L-ascorbic acid* and *ascorbyl monophosphate sodium* are to be used directly in *water*. No minimum or maximum concentration levels of the *feed additives* in *feedingstuffs* or *water* are recommended [5,6], similar to what was previously set in the regulation [7]. However, typical concentrations in *feedingstuffs* range from 50-400 mg/kg [8].

	
Ascorbyl monophosphate sodium	*Ascorbyl monophosphate calcium sodium*

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 the *Vitamin C*, 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) are available from the respective European Union Reference Laboratories [9].

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

Feed additive

For the characterisation of *ascorbic acid* in the *feed additive*, both Applicants submitted the European Pharmacopoeia method (Monograph 0253) [10], where:

- identification is based on infrared absorption spectrophotometry, pH and ultraviolet and visible absorption spectrophotometry; while
- quantification is based on titration, in aqueous acidic conditions, with 0.05 M iodine. 1 mL of 0.05 M iodine is equivalent to 8.81 mg of ascorbic acid.

For the characterisation of *sodium ascorbate* in the *feed additive*, Applicant (FAD-2010-0185) submitted the European Pharmacopoeia method (Monograph 1791) [11], where:

- identification is based on specific optical rotation and infrared absorption spectrophotometry; while
- quantification is based on titration, in aqueous acidic conditions, with 0.05 M iodine. 1 mL of 0.05 M iodine is equivalent to 9.91 mg of *sodium ascorbate*.

For the characterisation of *calcium ascorbate* in the *feed additive*, Applicant (FAD-2010-0185) submitted the European Pharmacopoeia method (Monograph 1182) [12], where:

- identification is based on specific optical rotation and infrared absorption spectrophotometry; while
- quantification is based on titration, in aqueous acidic conditions, with 0.05 M iodine. 1 mL of 0.05 M iodine is equivalent to 10.66 mg of *calcium ascorbate*.

For the characterisation of *6-Palmityl-L-ascorbic acid* in the *feed additive*, Applicant (FAD-2010-0185) submitted the European Pharmacopoeia method (Monograph 0807) [13], where:

- identification is based on specific optical rotation and infrared absorption spectrophotometry; while
- quantification is based on titration, in non acidic conditions, with 0.05 M iodine. 1 mL of 0.05 M iodine is equivalent to 20.73 mg of *ascorbyl palmitate*.

Even though no performance characteristics are provided, the EURL recommends for official control the four European Pharmacopoeia methods mentioned above for the determination of *L-ascorbic acid, Sodium L-ascorbate, Calcium L-ascorbate* and *6-Palmityl-L-ascorbic acid* in the *feed additives.*

For the quantification of *total calcium* and *total sodium* in the *feed additive*, the EURL recommends two ring-trial validated methods - EN ISO 6869:2000, based on Atomic Absorption Spectrometry (AAS) after dilution in hydrochloric acid [14], and - EN 15510:2007, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after dilution in hydrochloric acid [15], for which relative precisions ranging from 4 to 25 % were reported.

For the determination of *ascorbyl monophosphate* in the *feed additives* (*ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*), the two Applicants submitted the same single-laboratory validated [16-19] and further verified [20-25] method. The method is based on reversed phase High Performance Liquid Chromatography (RP-HPLC) coupled to Variable Wavelength Detector (VWD), using external calibration. Samples (60 mg) are dissolved in water and treated with ultrasonic for 1 minute. After dilution in water, the samples are heated at 60° C for 5 minutes, cooled and filtrated. The samples are then analysed by HPLC using an external calibration. The ascorbyl monophosphate content can be further expressed as ascorbic acid equivalent. The performance characteristics reported are presented in Table 1.

Table 1: Performance characteristics for the determination of *ascorbyl monophosphate salts* in *feed additive (FA), premixtures (PM)* and *feedingstuffs (FS)*

	Concentration (mg/kg)	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
		Validation	Verification	Validation	Verification	Validation	Verification
FA	(250 -750) 10^3	1.2 [17]	0.2 [20] #	1.2 [17]	0.6 [20] #	101 [17]	100 [20]
PM	(1-40) 10^3	0.6 – 2.2 [34]	0.7 - 1.1 [40]#	0.9 – 2.4 [34]	1.1 [40] #	100 – 101 [34]	98 [40] #
FS	50 – 500	0.2 – 1.0 [35]	4.5 - 6.7 [40]#	0.3 – 1.2 [35]	6.7 [40] #	100 [35]	105 [40] #

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*, respectively.
R_{Rec}: a recovery rate; # recalculated by the EURL

Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified RP-HPLC-VWD method, for the determination of *ascorbyl monophosphate* in the *feed additives* (*ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*).

Premixtures and Feedingstuffs

For the determination of *L-ascorbic acid* in *premixtures* and *feedingstuffs* both Applicants submitted the same single-laboratory validated [26-29] and further verified [30,31] titrimetric method, developed by DSM. Additionally, Applicant (FAD-2010-0185) proposed to apply the same method for the determination of *Sodium L-ascorbate* and *Calcium L-ascorbate* in *premixtures* and *feedingstuffs.* The samples are treated first with dichloromethane in ultrasonic bath to dissolve any fat-soluble coating, then extracted in aqueous 5% oxalic acid solution. The extracts are then titrated with 2,6-dichlorophenolindophenol (DPI) via potentiometric end-point detection using a double platinum pin electrode. Calibration is done by titration of the standard solution (where 1 mL contains 0.5 mg ascorbic acid). The performance characteristics reported are presented in Table 2. Furthermore, the Applicant reported a limit of quantification (LOQ) of 40 mg/kg *feedingstuffs* [22], thus allowing the quantification of *L-ascorbic acid* at 50 mg/kg levels with an acceptable measurement uncertainty.

Table 2: Performance characteristics for the determination of *L-ascorbic acid* in *premixtures (PM)* and *feedingstuffs (FS)*

	Concentration (mg/kg)	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
		Validation [27]	Verification [30]	Validation [27]	Verification [30]	Validation [27]	Verification [30]
PM	(8.9 – 20) 10^3	3.5	3.9	-	4.1	-	105
FS	40 – 461.2	2.7 - 10.1	5.2	10.1	5.4	82 - 94	103

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*, respectively.
R_{Rec}: a recovery rate

In addition, Applicant (FAD-2010-0214) submitted the ring-trial validated CEN method (EN 14130) [43] based on High Performance Liquid Chromatography coupled to UV detection at 265 nm (HPLC-UV). This CEN method was developed for the determination of vitamin C in foodstuffs. Several materials were tested (such as orange juice, liquid and powder-dried soup, powdered milk, breakfast cereal and fruits baby food) and satisfactory performance characteristics were reported. The EURL requested the Applicant to test the EN 14130 method on feed samples in order to confirm the applicability of this method to feed matrices (i.e. extension of scope). As the Applicant did not provide this experimental evidence the EURL cannot recommend the EN 14130 method for official control of feed samples.

The EURL recommends instead for official control the single-laboratory validated and further verified titrimetric method, for the determination of *L-ascorbic acid, Sodium L-ascorbate* and *Calcium L-ascorbate* in *premixtures* and *feedingstuffs.*

For the determination of *ascorbyl monophosphate* in *premixtures* and *feedingstuffs* (containing *ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*) both Applicants submitted the same single-laboratory validated [32-39] and further verified [40, 41] HPLC method, developed by DSM. The method is based on reversed phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV), using external calibration. The samples are extracted in 50 mL of phosphate buffer (pH = 3.0) on a magnetic stirrer for 20 minutes at room temperature. After appropriate dilution, the samples are centrifuged and the supernatant is analysed by HPLC using UV detection at 254 nm. The ascorbyl monophosphate content can be further expressed as ascorbic acid equivalent. The performance characteristics reported are presented in Table 1. Furthermore, the Applicants reported a limit of quantification (LOQ) of 28 mg/kg *feedingstuffs* [40], which is well below the recommended concentration levels.

Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified RP-HPLC-UV method, for the determination of *ascorbyl monophosphate* in *premixtures* and *feedingstuffs* (containing *ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*).

Applicant (FAD-2010-0185) did not provide any analytical method for the determination of *6-Palmityl-L-ascorbic acid* in *premixtures* and *feedingstuffs*. Therefore the EURL cannot evaluate nor recommend any method for official control to determine the active substance in the respective matrices.

Water

For the determination of *L-ascorbic acid* in *water* the Applicants proposed two internationally recognised methods: (i) the AOAC 967.21 titrimetric method [42] developed for the determination of ascorbic acid in vitamin preparations and juices; and (ii) the ring-trial validated CEN method (EN 14130) [43] based on High Performance Liquid Chromatography coupled to UV detection at 265 nm (HPLC-UV), developed for determination of vitamin C in foodstuffs (including orange juice, liquid soup and powdered milk). Based on the performance characteristics presented and the rationale that water is a simpler matrix than juices and foodstuffs, the EURL recommends for official control the AOAC and the CEN methods for the determination of *L-ascorbic acid* in *water*.

Applicant (FAD-2010-0185) did not provide any analytical method for the determination of *ascorbyl monophosphate sodium* in *water,* therefore the EURL cannot evaluate nor recommend any method for official control.

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 titration methods described in the European Pharmacopoeia monographs (0253; 1791; 1182 and 0807) to determine *L-ascorbic acid, Sodium L-ascorbate, Calcium L-ascorbate* and *6-Palmityl-L-ascorbic acid* in the *feed additives;*
- the ring trial validated CEN methods EN ISO 6869, based on Atomic Absorption Spectrometry (AAS) or EN 15510, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), for the quantification of *total calcium* and *total sodium* in the *feed additives*;
- the single-laboratory validated and further verified reversed phase High Performance Liquid Chromatography (RP-HPLC) method, for the determination of *ascorbyl monophosphate* in the *feed additives* (*ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium);*
- the single-laboratory validated and further verified titrimetric method, for the determination of *L-ascorbic acid, Sodium L-ascorbate* and *Calcium L-ascorbate* in *premixtures* and *feedingstuffs;*

– the single-laboratory validated and further verified reversed phase High Performance Liquid Chromatography coupled to UV detection (RP-HPLC-UV) method, for the determination of *ascorbyl monophosphate* in *premixtures* and *feedingstuffs* (containing *ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*)*;* and

– the AOAC 967.21 titrimetric method or the ring-trial validated CEN method (EN 14130), based on High Performance Liquid Chromatography coupled to UV Detection (HPLC-UV), for the determination of *L-ascorbic acid* in *water.*

Applicant (FAD-2010-0185) did not provide any analytical methods for the determination of *6-Palmityl-L-ascorbic acid* in *premixtures* and *feedingstuffs,* or for the determination of *ascorbyl monophosphate sodium* in *water.* Therefore the EURL cannot evaluate nor recommend any methods for official control to determine these active substances in the respective matrices.

Recommended text for the register entry (analytical method)

For the quantification of *total calcium* and *total sodium* in the *feed additives*:

- Atomic Absorption Spectrometry, AAS (EN ISO 6869:2000); or
- Inductively Coupled Plasma Atomic Emission Spectrometry, ICP-AES (EN:15510:2007)

For the determination of *L-ascorbic acid* in the *feed additive*:

- Titrimetry - European Pharmacopoeia monograph (Ph.Eur. 01/2011:0253)

For the determination of *Sodium L-ascorbate* in the *feed additive*:

- Titrimetry - European Pharmacopoeia monograph (Ph.Eur. 01/2011:1791)

For the determination of *Calcium L-ascorbate* in the *feed additive*:

- Titrimetry - European Pharmacopoeia monograph (Ph.Eur. 01/2008:1182)

For the determination of *6-Palmityl-L-ascorbic acid* in the *feed additive*:

- Titrimetry - European Pharmacopoeia monograph (Ph.Eur. 01/2008:0807)

For the quantification of *ascorbyl monophosphate* in the *feed additives* (*ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*):

- High Performance Liquid Chromatography coupled to VWD detector

For the quantification of *L-ascorbic acid*, *Sodium L-ascorbate* and *Calcium L-ascorbate* in *premixtures* and *feedingstuffs*:

- Titrimetry

For the quantification of *ascorbyl monophosphate* in *premixtures* and *feedingstuffs* (containing *ascorbyl monophosphate sodium* or *ascorbyl monophosphate calcium sodium*):

- High Performance Liquid Chromatography coupled to UV detection at 254 nm (HPLC-UV)

For the quantification of *L-ascorbic acid* in *water*:

- Titrimetry (AOAC 967.21); or
- High Performance Liquid Chromatography coupled to UV detection at 265 nm (EN 14130:2003)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Vitamin C* 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] [a,b]Application/Ref:SANCO/D2: Group Forward Slip Ares(2011)255263

[2] [a]Supplementary Information, EURL supplementary information request on Vitamin C

[3] [a]Technical dossier, Section II: Identity, Characterisation and Conditions of use of the Additive; Methods of Analysis.

[4] [b]Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition

[5] [a]Application, Proposal for Register Entry – Annex A

[6] [b]Application, Proposal for Register Entry – Annex A

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

[8] [b]Technical Dossier, Section II, Ref_2_2_01_AWT_Vitamins in Animal Nutrition

[9] 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

[10] European Pharmacopoeia Monograph 01/2011:0253 – Ascorbic acid

[11] European Pharmacopoeia Monograph 01/2011:1791 – Sodium ascorbate

[12] European Pharmacopoeia Monograph 01/2008:1182 – Calcium ascorbate

[13] European Pharmacopoeia Monograph 01/2008:0807 – Ascorbyl palmitate

[14] EN ISO 6869:2000 - *Animal feedingstuffs - Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc - Method using atomic absorption spectrometry*

[15] EN 15510:2007 – *Animal feedingstuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*

[16] [a]Supplementary Information, Annex DSM EURL 1 - 2-98 DSM 2010
[17] [a]Supplementary Information, Annex DSM EURL 2 - 2-99 DSM 2010
[18] [b]Supplementary Information, Annex_F_Description of the analytical method_Feed additive (2)
[19] [b]Supplementary Information, Annex_G_Validation study report_Laboratory 1
[20] [a]Supplementary Information, Annex DSM EURL 3 - Verification Study Report Rovimix Stay-C 35 + Stay-C 50
[21] [a]Supplementary Information, Annex DSM EURL 4 - Verification Report Rovimix Stay-C 35 + Stay-C 50
[22] [a]Supplementary Information, Annex DSM EURL 5 - Method Stay-C - Appendix 2012
[23] [b]Supplementary Information, Annex_H_Verification study report_Laboratory 2
[24] [b]Supplementary Information, Annex_I_Verification study report_Comparison Laboratory 1 vs Laboratory 2
[25] [b]Supplementary Information, Annex_J_Review of the operating procedure
[26] [a]Technical Dossier, Section II, Annex 2-85 Etheve et al 2010
[27] [a]Technical Dossier, Section II, Annex 2-86 Hoffmann et al 1993
[28] [b]Supplementary Information, Annex_A_Description of the analytical method_Feed_Premixtures
[29] [b]Supplementary Information, Annex_B _Validation study report_Feed_Premixtures
[30] [a]Supplementary Information, Annex 2-87 Schaefer 2010
[31] [b]Supplementary Information, Annex_C_Verification study report_Feed_Premixtures
[32] [a]Technical Dossier, Section II, Annex 2-88 Schaefer et al 2010
[33] [a]Technical Dossier, Section II, Annex 2-90 Schaefer et al 2010
[34] [a]Technical Dossier, Section II, Annex 2-89 Schaefer and Kessler 2010
[35] [a]Technical Dossier, Section II, Annex 2-91 Schaefer and Kessler 2010
[36] [b]Technical Dossier, Section II, Annex_II_44
[37] [b]Technical Dossier, Section II, Annex_II_45
[38] [b]Technical Dossier Section II, Annex_II_46
[39] [b]Technical Dossier Section II, Annex_II_47
[40] [a]Technical Dossier, Section II, Annex 2-92 Schaefer and Evans 2010
[41] [b]Technical Dossier Section II, Annex_II_48
[42] AOAC method 967.21: Ascorbic acid in Vitamin Preparations and Juices
[43] EN 14130:2003 – *Foodstuffs – Determination of vitamin C by HPLC*

[a] Refers to Dossier no: FAD-2010-0185

[b] Refers to Dossier no: FAD-2010-0214

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.

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