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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-2010-0023
CRL/ 100003

Name of Feed Additive: Methionine

- DL-Methionine
- DL-Methionine sodium salt
- DL-Methionine protected with ethylcellulose
- DL-Methionine protected with copolymer vinylpyridine/styrene
- Hydroxy analogue of Methionine
- Calcium salt of hydroxy analogue of Methionine
- Isopropyl ester of the hydroxylated analogue of Methionine

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EXECUTIVE SUMMARY

In the current application authorisation is sought for seven forms of *methionine* under Articles 4(1) and 10(2), under the category/functional group 3(c) 'nutritional additives'/amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003.

According to the Applicant:

- *DL-methionine (DLM)* is intended for all animal species and categories with a minimum purity of 99%;
- *liquid sodium DL-methionine (DLM-Na salt)* is intended for all animal species and categories with a minimum purity of 40% of *DLM*;
- *DL-methionine protected with copolymer vinylpyridine/styrene (DLM-RP-copolymer)* is intended for dairy cows with a minimum purity of 74% of *DLM*;
- *DL-methionine protected with ethylcellulose (DLM-RP-ethyl cellulose)* is intended for ruminants with a minimum purity of 85% of *DLM*;
- *hydroxy analogue of methionine (HMTBa)* is intended for all animal species and categories with a minimum purity of 88%;
- *calcium salt of hydroxy analogue of methionine (HMTBa-Ca)* is intended for all animal species and categories with a minimum purity of 84% of *HMTBa*; and
- *isopropyl ester of the hydroxylated analogue of methionine (HMBi)* is intended for dairy cows with a minimum purity of 95%.

The *feed additives* are intended to be mixed either in *premixtures* or to be added directly to complete *feedingstuffs* or *water*. The Applicant proposed no minimum or maximum *methionine* concentrations in *feedingstuffs*.

For the determination of *methionine* in *feed additives* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer* the EURL identified the ring-trial validated ISO/CD 17180 method. The method applies an amino acid analyzer or reversed phase ion exchange high performance liquid chromatography equipment coupled with post-column derivatisation and spectrophotometric ultraviolet or fluorescence detection (RP-HPLC-UV/FD) for the determination of *methionine* content in commercial amino acid products. The following performance characteristics are reported for a *methionine* content ranging from 31 to 93 %:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.5 to 1.1 %; and
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 1.5 to 2.6 %.

Based on the performance characteristics presented, the EURL recommends for official control the ISO/CD 17180 method, based on ion exchange chromatography coupled with

post-column derivatisation and UV or fluorescence detection to determine *methionine* in *feed additives* containing *DLM*, *DLM-Na salt*, *DLM-RP-copolymer* or *DLM-RP-ethyl cellulose*.

For the determination of *methionine* in *premixtures* and *feedingstuffs* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*, the Applicant submitted the ring-trial validated Community method - Commission Regulation (EC) No 152/2009. The following performance characteristics were reported for the determination of *total methionine*:

- RSD_r ranging from 1.1 to 5.6 %;
- RSD_R ranging from 6.9 to 13%; and
- a limit of quantification (LOQ) of 0.25 g/kg.

Furthermore, upon request of the EURL, the Applicant submitted experimental data obtained applying the above mentioned Community method for the determination of the *methionine* in *water* containing *DLM* and *DLM-Na salt*. The EURL calculated the following performance characteristics from the experimental data provided by the Applicant for *methionine* concentrations ranging from 0.5 to 10 g/L:

- RSD_r ranging from 0.1 to 2.6%;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.3 to 2.6 %; and
- a *recovery rate* (R_{Rec}) ranging from 98.7 to 101.3%.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and UV or fluorescence detection to determine *methionine* in *premixtures*, *feedingstuffs* and *water* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*.

For the determination of *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca*, the Applicant submitted a single laboratory validated and further verified titrimetric method. The following correspondent performance characteristics were reported:

- RSD_r ranging from 0.1 to 0.8 %;
- RSD_{ip} ranging from 0.2 to 1.1 %; and
- R_{Rec} ranging from 99.7 to 101.1 %.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified titrimetric method to determine *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca*.

For the determination of *HMTBa* in *premixtures* and *feedingstuffs* containing *HMTBa* or *HMTBa-Ca*, the Applicant submitted a single laboratory validated and further verified method based on RP-HPLC-UV. The following correspondent performance characteristics were

reported for concentrations in *premixtures* and *feedingstuffs* ranging from 5 to 80 g/kg and from 0.14 to 4 g/kg, respectively:

- RSD_r ranging from 0.7 to 2.2 %;
- RSD_{ip} ranging from 1.7 to 8.3 %;
- R_{Rec} ranging from 96 to 108 %; and
- LOQ of 0.2 and 0.08 g/kg *feedingstuffs* for *HMTBa-Ca* and *HMTBa*, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method to determine *HMTBa* in *premixtures* and *feedingstuffs* containing *HMTBa* or *HMTBa-Ca*.

The Applicant provided no experimental data for the identification of *HMTBa* in *water*. Therefore the EURL cannot evaluate nor recommend a method for official control to determine *HMTBa* content in *water*.

For the determination of *HMBi* in the *feed additive* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified analytical method based on RP-HPLC-UV. The following performance characteristics were reported:

- RSD_r ranging from 0.2 to 3.7 %;
- RSD_{ip} ranging from 0.4 to 4.6 %;
- R_{Rec} ranging from 94 to 104.7 %; and
- LOQ of 2.3 g/kg in *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method to determine *HMBi* in the *feed additive* and in *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

Methionine, DL-Methionine (*DLM*), liquid sodium DL-Methionine (*DLM-Na salt*), DL-Methionine protected with copolymer vinylpyridine/styrene (*DLM-RP-copolymer*), DL-Methionine protected with ethylcellulose (*DLM-RP-ethyl cellulose*), Hydroxy analogue of Methionine (*HMTBa*), Calcium salt of hydroxy analogue of Methionine (*HMTBa-Ca*), Isopropyl ester of the hydroxylated analogue of Methionine (*HMBi*), nutritional additives, amino acids, all animal species and categories, dairy cows, ruminants.

1. BACKGROUND

Different forms of *methionine* were already authorised as *feed additive* without any restrictions [1-5]. In the current application authorisation is sought under Articles 4(1) (new use) and 10(2) (re-evaluation of the already authorised feed additive) for seven forms of *methionine* under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003 [6]. According to the Applicant the following forms are produced through synthesis from petrochemical raw materials [6-9]:

- *DL-methionine (DLM, C₅H₁₁NO₂S)*, intended for all animal species and categories, is a solid white to yellowish powder with a minimum purity of 99%;
- *liquid sodium DL-methionine (DLM-Na salt, C₅H₁₁NO₂S•Na)*, intended for all animal species and categories, is a light brown liquid with a minimum purity of 40% of *DLM*;
- *DL-methionine protected with copolymer vinylpyridine/styrene (DLM-RP-copolymer)*, intended for dairy cows, is a solid product in beadlets with a minimum purity of 74% of *DLM*;
- *DL-methionine protected with ethylcellulose (DLM-RP-ethyl cellulose)*, intended for ruminants, is a solid white product in form of pellets with a minimum purity of 85% of *DLM*;
- *hydroxy analogue of methionine (HMTBa, C₅H₁₀O₃S)*, intended for all animal species and categories, is an amber/dark liquid with a minimum purity of 88%;
- *calcium salt of hydroxy analogue of methionine (HMTBa-Ca, C₅H₁₀O₃S₂Ca)*, intended for all animal species and categories, is a solid granular powder with a minimum purity of 84% of *HMTBa*; and
- *isopropyl ester of the hydroxylated analogue of methionine (HMBi, C₈H₁₆O₃S)*, intended for dairy cows, is a light brown liquid product with a minimum purity of 95%.

Table 1: Proposed mode of use of the different forms of *methionine* in *feed additive* (FA), *premixtures* (PM), *feedingstuffs* (FS) and *water* (W)

	FA	PM	FS	W
DLM	X	X	X	X
DLM-Na salt	X	X	X	X
DLM-RP-copolymer	X	X	X	
DLM-RP-ethyl cellulose	X	X	X	
HMTBa	X		X	X
HMTBa-Ca	X	X	X	
HMBi	X		X	

The *feed additives* are intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs* or *water* as presented in Table 1 [10-11]. The Applicant proposed no minimum or maximum *methionine* concentrations in *feedingstuffs* [7].

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 this dossier, the methods of analysis submitted in connection with seven forms of *methionine* (*DL-Methionine*, *liquid sodium DL-Methionine*, *DL-Methionine protected with copolymer vinylpyridine/styrene*, *DL-Methionine protected with ethylcellulose*, *Hydroxy analogue of Methionine*, *Calcium salt of hydroxy analogue of Methionine*, *Isopropyl ester of the hydroxylated analogue of Methionine*), 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 [12].

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

For the determination of *methionine* in the *feed additives* containing *DLM*, *DLM-Na salt* or *DLM-RP-ethyl cellulose*, the Applicant submitted a single laboratory validated titrimetric method [13]. For the determination of *methionine* in *DLM-RP-copolymer* the Applicant suggested to digest the coating by the action of an acid and apply High Performance Liquid Chromatography with UltraViolet detection (HPLC-UV) at 205 nm [14].

The EURL identified instead the ring-trial validated ISO/DIS 17180 method [15]. This multi-analyte technique applies for the determination of *methionine* content in commercial amino acid products, using an amino acid analyzer or HPLC equipment. The method does not

distinguish between the salts and the amino acid enantiomers and it applies for products containing more than 10% of amino acid. *Methionine* is extracted with diluted hydrochloric acid and diluted with sodium citrate buffer. After addition of norleucine as internal standard, the amino acids are separated by ion exchange chromatography. *Methionine* is determined colorimetrically after post-column derivatisation with ninhydrine and UV detection at 440 and 570 nm or by fluorescence detection (FD) after post column reaction with ortho-phthaldialdehyde (OPA). The following performance characteristics are reported for a *methionine* content ranging from 30.6 to 93.3 %:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.5 to 1.1 %; and
- a standard deviation for *reproducibility* (RSD_R) ranging from 1.5 to 2.6 %.

Based on the performance characteristics presented, the EURL recommends for official control the ISO/CD 17180 method, based on ion exchange chromatography coupled with post-column derivatisation and UV or fluorescence detection to determine *methionine* in *feed additives* containing *DLM*, *DLM-Na salt*, *DLM-RP-copolymer* or *DLM-RP-ethyl cellulose*.

For the determination of *methionine* in *premixtures* and *feedingstuffs* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*, the Applicant submitted the ring-trial validated Community method [13, 16]. This method applies for the determination of *free* and of *total* (peptide-bound and free) amino acids, using an amino acid analyzer or HPLC equipment. The method does not distinguish between the salts and the amino acid enantiomers. The *free* amino acids are extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography and determined by post column derivatisation with ninhydrin and photometric detection at 570 nm. For the determination of the *total methionine*, the sample must be oxidised to methionine sulphone prior to hydrolysis. Oxidation is performed at 0° C with a performic acid/phenol mixture. Excess oxidation reagent is decomposed with sodium disulphite. Methionine sulphone is hydrolysed with hydrochloric acid (6 mol/L) for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography and determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm. The analytical result is expressed as mass fraction of *methionine* calculated from the measured methionine sulphone and using a conversion factor (149.21 g/mol). The Community method was ring trial validated using four different matrices listed in Table 2. This method was further ring-trial validated by twenty-three laboratories, resulting in the CEN EN ISO 13903:2005 method [17]. The corresponding performance characteristics are listed in Table 2. Furthermore, a limit of quantification of 0.25 g/kg *feedingstuffs* was reported in the CEN/ISO standard.

Table 2: Method performance characteristics obtained in the frame of two different ring-trial validation exercises for the determination of *total methionine* in *premixtures* and *feedingstuffs*.

Intercomparison study	Matrix	Methionine g/kg	RSD _r (%)	RSD _R (%)
Commission Regulation (EC) No 152/2009 [16] <i>study carried out in 1990</i>	mixed pig feed	3.3	3.4	7
	broiler compound	5.1	3.1	10.9
	protein concentrate	12	2.2	13
	premix	90.2	2.4	6.9
ISO 13903:2005 [17] <i>study carried out in 1994</i>	poultry meal	11.7	2.1	12
	broiler finisher feed	5.3	1.1	7.6
	broiler starter feed	6.2	2.1	10.2
	corn	1.8	5.6	11.7
	fishmeal	16.1	1.9	9.7

RSD_r, RSD_R - relative standard deviation for *repeatability* and *reproducibility*, respectively

For the determination of the *methionine* in *water* containing *DLM* and *DLM-Na salt*, the Applicant submitted, upon request of the EURL, experimental data [11, 18, 19] obtained applying the ring trial validated Community method [16]. The EURL calculated the following performance characteristics from the experimental data provided by the Applicant for *methionine* concentrations ranging from 0.5 to 10 g/l [20]:

- RSD_r ranging from 0.1 to 2.6%;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.3 to 2.6%; and
- a *recovery rate* (R_{Rec}) ranging from 98.7 to 101.3%.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine the *methionine* in *premixtures*, *feedingstuffs* and *water* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*.

For the determination of *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca*, the Applicant submitted a single laboratory validated and further verified titrimetric method based on potentiometric titration followed by oxidation reduction reaction [13, 21, 22]. Bromate reacts with bromide under acidic condition to produce bromine, which oxidizes *methionine* sulphur present in the *feed additive*. The performance characteristics reported for the determination of *HMTBa* content in the *feed additives* are listed in Table 3.

Table 3: Method performance characteristics obtained in the frame of single laboratory validation and verification studies for the determination of *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca* [21-22].

Feed Additive	RSD _r (%)	RSD _{ip} (%)	R _{Rec} (%)
HMTBa	0.1 - 0.4	0.2 - 0.4	100.2 - 100.3
HMTBa-Ca	0.3 - 0.8	0.7 - 1.1	99.7 - 101.1

RSD_r, RSD_R - relative standard deviation for *repeatability* and *intermediate precision*, respectively; R_{Rec} – recovery rate.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified titrimetric method to determine *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca*.

For the determination of *HMTBa* in *premixtures* and *feedingstuffs* containing *HMTBa* or *HMTBa-Ca*, the Applicant submitted a single laboratory validated and further verified method based on Reversed Phase HPLC coupled with UV detection at 214 nm (RP-HPLC-UV) [13, 23-25]. The *active substance* is extracted using a water/methanol solution. The polymers contained in the extracts are hydrolysed with an alkaline reaction. After centrifugation, the supernatant is filtered and injected in a gradient RP-HPLC-UV system and detected at 214 nm. The performance characteristics reported are listed in Table 4. Furthermore, the Applicant reported LOQs of 0.08 and 0.2 g/kg *feedingstuffs* for *HMTBa* and *HMTBa-Ca*, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method to determine *HMTBa* in *premixtures* and *feedingstuffs* containing *HMTBa* or *HMTBa-Ca*.

Table 4: Method performance characteristics obtained in the frame of single laboratory validation and verification studies for the determination of *HMTBa* in *premixtures* (PM) and *feedingstuffs* (FS) containing *HMTBa* or *HMTBa-Ca* [23-25].

Feed Additive	Matrix	Range (g/kg)	RSD _r (%)	RSD _{ip} (%)	R _{Rec} (%)
HMTBa	FS	0.14 - 3.9	0.7 - 6.2	2.5 - 8.3	87 - 108
HMTBa-Ca	PM	5 - 80	1.5 - 1.9	1.7 - 3.2	96
	FS	0.25 - 4	2.2	2.1 - 3.5	99 - 103

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

For the determination of *HMTBa* in *water*, the Applicant suggested the above mentioned single laboratory validated and further verified RP-HPLC-UV method [13], providing no experimental data. Hence, the EURL cannot evaluate nor recommend a method for official control to determine *HMTBa* in *water*.

For the determination of *HMBi* in the *feed additive* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified analytical method based on RP-HPLC-UV [26-31]. The method consists of an extraction of the *active substance* using a dilution buffer (pH 7) for the *feed additive* or a 'water:acidified methanol' (50:50) solution for *feedingstuffs*. *HMBi* is determined by injecting the filtered extract into HPLC coupled with UV detection at 210 nm. The reported performance characteristics are listed in Table 5. Furthermore, the Applicant reported a LOQ of 2.3 g/kg *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method to determine *HMBi* in the *feed additive* and in *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.

Table 5: Method performance characteristics obtained in the frame of single laboratory validation and verification studies for the determination of *methionine* content in *HMBi* in *feed additive* (FA) and *feedingstuffs* (FS) [26-31].

Feed Additive	Matrix	Range (g/kg)	RSD _r (%)	RSD _{ip} (%)	R _{Rec} (%)
HMBi	FA	-	0.18 - 2.2	0.39 - 1.6	94.0 - 100.4
	FS	3.5 - 39	2.2 - 3.7	3.0 - 4.6	97.0 - 104.7

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

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the analytical methods listed in Table 6.

For the determination of *HMTBa* in *water*, the Applicant suggested the above mentioned single laboratory validated and further verified RP-HPLC-UV method [13], providing no experimental data. Hence, the EURL cannot evaluate nor recommend a method for official control to determine *HMTBa* in *water*.

Table 6: EURL's recommendations for official control (FAD-2010-0023).

Matrix	RP-HPLC-UV/FD	HPLC	Titrimetry	RP-HPLC-UV
Analytical Method	ISO/DIS 17180	Reg Com 152/2009	validated & verified	validated & verified
DLM	FA	PM, FS and W	-	-
DLM-Na salt	FA	PM, FS and W	-	-
DLM-RP-copolymer	FA	PM and FS	-	-
DLM-RP-ethyl cellulose	FA	PM and FS	-	-
HMTBa	-	-	FA	FS
HMTBa-Ca	-	-	FA	PM and FS
HMBi	-	-	-	FA and FS

FA – *feed additive*; PM – *premixtures*; FS – *feedingstuffs*; W - *water*

Recommended text for the register entry (analytical method)

For the determination of *methionine* in *feed additives* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*:

- ion exchange chromatography coupled with post-column derivatisation and photometric or fluorescence detection (HPLC-UV/FD) - ISO/DIS 17180.

For the determination of *methionine* in *premixtures*, *feedingstuffs* and *water* containing *DLM*, *DLM-Na salt*, *DLM-RP-ethyl cellulose* or *DLM-RP-copolymer*:

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (HPLC/UV) - Commission Regulation (EC) No 152/2009 (Annex III, F).

For the determination of *HMTBa* in *feed additives* containing *HMTBa* or *HMTBa-Ca*:

- titrimetry, potentiometric titration followed by oxidation reduction reaction

For the determination of *HMTBa* in *premixtures* and *feedingstuffs* containing *HMTBa* or *HMTBa-Ca*:

- High-Performance Liquid Chromatography and photometric detection (HPLC-UV)

For the determination of *HMBi* in the *feed additive* and *feedingstuffs*:

- High-Performance Liquid Chromatography and photometric detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of the seven forms of *methionine* 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] Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition
- [2] Commission Directive 89/520/EEC of 6 September 1989 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition
- [3] Commission Directive 93/26/EEC of 4 June 1993 amending Council Directive 82/471/EEC concerning certain products used in animal nutrition
- [4] Commission Directive 93/26/EEC of 4 June 1993 amending Council Directive 82/471/EEC concerning certain products used in animal nutrition
- [5] Commission Directive 2003/104/EC of 12 November 2003 authorising isopropyl ester of the hydroxylated analogue of methionine
- [6] *Application/Ref:SANCO/D/2:Forw.Appl.1831/0030-2010
- [7] *Application, Proposal of Registry Entry - Annex A
- [8] *Technical dossier, Section II: 1.3 Qualitative and quantitative composition
- [9] *Technical dossier, Section II: 1.2 Proposal for classification
- [10] *Application, Proposal of Registry Entry – Appendix to Annex 1
- [11] *Supplementary Information, 2011-05-03_AMAC Methionine_Answer to EFSA and EURL.pdf, pag.54-55
- [12] 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
- [13] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substances
- [14] *Technical dossier, Section II: Annex_II_106_Methionine in DLM-RP-Copolymers.pdf
- [15] Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acids products and premixtures; ISO/DIS 17180

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- [16] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009
 - [17] Animal feeding stuffs – Determination of amino acids content; CEN EN ISO 13903:2005
 - [18] *Supplementary Information, Annex 6_DLM_Stability in water.pdf
 - [19] *Supplementary Information, Annex 9_DLM Na salt_Stability in water.pdf
 - [20] *Supplementary Information, Water DLM&salt.xls
 - [21] *Technical dossier, Section II: Annex_II_103_Method, validation and verification for HMTBa a.pdf
 - [22] *Technical dossier, Section II: Annex_II_614_Method, validation and verification for HMTBa-C.pdf
 - [23] *Technical dossier, Section II: Annex_II_612_Method, validation and verification for HMTBa d.pdf
 - [24] *Technical dossier, Section II: Annex_II_615_Method, validation and verification for HMTBa-C.pdf
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 - [26] *Supplementary Information, HMBi 2010VAL007 Validation Preparation.pdf
 - [27] *Supplementary Information, HMBi S11-00182 Verification Preparation.pdf
 - [28] *Supplementary Information, HMBi 2011 11 03 - Evaluation Preparation.pdf
 - [29] *Supplementary Information, HMBi 2010VAL008 Validation In-feeds.pdf
 - [30] *Supplementary Information, HMBi S11-00050 Verification In feeds.pdf
 - [31] *Supplementary Information, HMBi 2011 11 03 - Evaluation In-feeds.pdf
- *Refers to Dossier no: FAD-2010-0023

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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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Kmetijski inštitut Slovenije, Ljubljana (SI)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (AT)
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