JRC.F.5/CvH/MGH/AS/Ares

Subject: Addendum to the EURL evaluation report

References:

FAD-2012-0027 – Monimax® (JRC.D.5/SFB/CvH/SB/mds/Ares(2014)360749)

Upon the publication of a new multi-analyte ring-trial validated method EN 17299 [1] for the analysis of coccidiostats the EURL, considered appropriate to include this standard method within the recommended methods of analysis for official control for the above-mentioned *feed additive* dossier.

This addendum aims to provide an up-to-date EURL recommendations, including all the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [2] which will allow Member States official control laboratory full flexibility regarding the selection of method of analysis (single-analyte or multi-analyte method).

The recommendations included in this addendum apply for the *feed additives* containing *monensin sodium* and *nicarbazin* as active substances that have been already evaluated by the EURL and/or are currently authorised by the related Regulations.

The EURL has developed and fully validated a multi-analyte method based on high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the determination of the various coccidiostats, including *monensin sodium* and *nicarbazin*, in *compound feeds.*

According to the method the coccidiostats are extracted with a mixture of acetonitrile:methanol:water. The obtained extracts are centrifuged and supernatants are filtered. The analysis of samples is conducted by reversed-phase LC-MS/MS. The quantification of the detected target analytes is performed using a multi-level standard addition approach [1].

This method has been ring-trial validated for *monensin sodium* and *nicarbazin* in different feed matrices at additive and at cross-contamination levels and published as CEN standard (EN 17299) [1].

Based on the obtained performance characteristics and the scope of the method in terms of matrices, the EURL considers the multi-analyte ring-trial validated EN 17299 method based

on high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) fit for purpose for the determination of *monensin sodium* and *nicarbazin* in *compound feeds*.

Recommended text for the registry entry (analytical methods) (replacing the previous recommendations)

For the quantification of *monensin sodium* in the *feed additive*:

- High Performance Liquid Chromatography using post-column derivatisation coupled with photometric detection (HPLC-PCD-UV-Vis)

For the quantification of *monensin sodium* in *premixtures*:

- High Performance Liquid Chromatography using post-column derivatisation coupled with photometric detection (HPLC-PCD-UV-Vis) – EN ISO 14183

For the quantification of *monensin sodium* in *compound feeds*:

- High Performance Liquid Chromatography using post-column derivatisation coupled with photometric detection (HPLC-PCD-UV-Vis) – EN ISO 14183 or
- High performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) – EN 17299

For the determination of *monensin sodium* in *tissues*:

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

For the quantification of *nicarbazin* in the *feed additive*:

- High Performance Liquid Chromatography coupled with spectrophotometric detection (HPLC-UV)

For the quantification of *nicarbazin* in *premixtures*:

- High Performance Liquid Chromatography coupled with spectrophotometric detection (HPLC-UV) - EN ISO 15782

For the quantification of *nicarbazin* in *compound feed*:

- High Performance Liquid Chromatography coupled with spectrophotometric detection (HPLC-UV) - EN ISO 15782 or
- High Performance Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS) – EN 17299

For the quantification of *nicarbazin (*as *4,4'-dinitrocarbanilide (DNC))* in chicken *tissues*:

- High Performance Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS)

References

[1] EN 17299:2019 Animal feedingstuffs: Methods of sampling and analysis – Screening and determination of authorised coccidiostats at additive and 1 % and 3 % cross-contamination level, and of non-registered coccidiostats and of one antibiotic at sub-additive levels, in compound feed with High Performance Liquid Chromatography – Tandem Mass Spectrometry detection (LC-MS/MS)

[2] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisations of feed additives, OJ L 133 22.5.2008, p. 1

Addendum
- Prepared by María José González de la Huebra
- Reviewed and approved by Zigmas Ezerskis and Christoph von Holst (EURL-FA), respectively, Geel, 26/01/2023

EUROPEAN COMMISSION
DIRECTORATE GENERAL
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Directorate D: Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.D.5/SFB/CvH/SB/mds/Ares

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

Monimax®
(FAD-2012-0027; CRL/120018)

European Union Reference Laboratory
Feed Additives

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-2012-0027 - CRL/120018**
Name of Product:	***Monimax®***
Active Agent (s):	**Monensin sodium and Nicarbazin**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Stefano Bellorini**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **30/10/2014**
Report approved by: Date:	**Christoph von Holst** **30/10/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Monimax®*, under article 4(1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *chickens for fattening* and *chickens reared for laying*. *Monimax®* consists of 80g/kg of *monensin sodium* and 80g/kg of *nicarbazin* complemented by starch for granulation, wheat meal and calcium carbonate.

Monimax® is intended to be incorporated in *feedingstuffs* through *premixtures* and it is not to be mixed with other coccidiostats. For each of the active substances the Applicant proposes a final concentration in *feedingstuffs* ranging from 40 to 50 mg/kg. Furthermore, the Applicant proposed Maximum Residue Limits (MRLs) in skin/fat, muscle, liver and kidney. As these MRLs are not set up by Commission Regulation (EC) No 37/2010, the correspondent methods of analysis have to be evaluated by the EURL.

For the quantification of *monensin sodium* in *premixtures* and *feedingstuffs* the Applicant submitted the multi-analyte ring-trial validated method EN ISO 14183 based on High Performance Liquid Chromatography with post-column derivatisation and VIS detection (HPLC-VIS). The Applicant applied this method for the analysis of the *feed additive* and presented performance characteristics similar to those reported for the EN ISO standard method. Based on the experimental evidence available the EURL recommends for official control the HPLC with post-column derivatisation and VIS detection for the quantification of *monensin sodium* in the *feed additive*, *premixtures* and *feedingstuffs*.

For the quantification of *nicarbazin* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method EN ISO 15782 based on HPLC coupled with UltraViolet (UV) detection. A similar method was used by the Applicant for the quantification of *nicarbazin* in the *feed additive*. Based on the performance characteristics presented the EURL recommends for official control the HPLC method with UV detection for the quantification of *nicarbazin* in the *feed additive*, *premixtures* and *feedingstuffs*.

For the quantification of *monensin sodium* and *nicarbazin* in target tissues (skin/fat, muscle, liver and kidney) the Applicant submitted a single-laboratory and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode (ESI) using matrix matched standards. Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs of *monensin sodium* and *nicarbazin* in the target *tissues*.

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

Monensin sodium, Nicarbazin, Monimax, coccidiostat, chickens for fattening, chickens reared for laying

1. BACKGROUND

In the current application authorisation is sought for *Monimax®*, under article 4(1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *chickens for fattening* and *chickens reared for laying* [1-2].

Monimax® is a green-brown granulated *feed additive* containing two active substances *monensin sodium* and *nicarbazin* [3]. The *feed additive* consists of 80g/kg of *monensin sodium* and 80g/kg of *nicarbazin* complemented by starch for granulation, wheat meal and calcium carbonate [2,4]. *Monensin sodium* is a fermentation-derived salt with a minimum *monensin* activity of 27 % [4]. *Nicarbazin* is a chemically synthesised product with a minimum purity of 95 %; it consists of an equimolecular crystalline complex of 4,4-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl-pyrimidine (HDP) [4,5].

Monimax® is intended to be incorporated in *feedingstuffs* through *premixtures* and it is not to be mixed with other coccidiostats [6]. For each of the active substances the Applicant proposes a final concentration in *feedingstuffs* ranging from 40 to 50 mg/kg [2,6].

Furthermore the Applicant proposed Maximum Residue Limits (MRLs) in skin/fat, muscle, liver and kidney as listed in Table 1 [2]. As these MRLs are not set up by Commission Regulation (EC) No 37/2010 [7], the correspondent methods of analysis have to be evaluated by the EURL.

Table 1. Maximum Residue Limits (MRLs) in the relevant foodstuffs of animal origin. All values are expressed in µg/kg of fresh material. DNC is used as the marker residue for *Nicarbazin*.

MRLs	Skin/Fat	Muscle	Liver	Kidney
Monensin sodium	25	8	8	8
DNC	4000	4000	15000	6000

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 for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Monimax®* 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 [8]

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

Monensin sodium

For the quantification of *monensin sodium* in *premixtures* and *feedingstuffs* the Applicant submitted the multi-analyte ring-trial validated method EN ISO 14183 based on High Performance Liquid Chromatography with post-column derivatisation and Visible detection (HPLC-VIS) [9,10]. The method consists in the extraction of *monensin* using methanol/water (90/10) followed by mechanical shaking for 1 h and filtration. The active substance is then determined by reversed-phase HPLC using post-column derivatisation with vanillin, and detection at 520 nm. According to *Campbell & Nayeri*, potential interferences in the determination of *monensin sodium* cannot be expected [11]. The performance characteristics reported for the EN ISO method are:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 5.2 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.8 % to 13 %;
- a recovery rate (R_{Rec}) ranging from 91 to 107 % (calculated by the EURL); and
- a limit of quantification (LOQ) of 1 mg/kg *feedingstuffs.*

The Applicant applied the above mentioned EN ISO method for the quantification of *monensin sodium* in the *feed additive.* The sample preparation consists in adding methanol/water (90/10) to a grinded and homogenised subsample followed by the extraction

of *monensin* via ultrasonic bath for 30 minutes. The extract is filtered through a double filter paper and directly injected. The *active substance* is then determined by HPLC-VIS at 520 nm. The following performance characteristics were recalculated by the EURL based on the experimental data provided by the Applicant [12-14]:

- RSD_r ranging from 0.6 to 1.4 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.0 to 2.0 %; and
- R_{Rec} ranging from 98 to 101 %

Based on the performance characteristics available the EURL recommends for official control the HPLC method with post-column derivatisation and VIS detection for the quantification of *monensin sodium* in the *feed additive*, *premixtures* and *feedingstuffs*.

Nicarbazin

For the quantification of *nicarbazin* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on HPLC coupled with UltraViolet detection (UV) [10,15]. The sample is grinded and homogenised. A subsample is transferred in a 100ml volumetric flask where 40ml of acetonitrile/methanol (50/50) are added for extraction. After 30 min sonication the solution is brought to volume with the mobile phase acetonitrile/water (65:35). The extract is sonicated once again; an aliquot is diluted, filtered and directly injected into the chromatographic system. The *active substance* is then determined by reversed-phase HPLC and detected at 350 nm. The following performance characteristics were recalculated by the EURL based on the experimental data reported by the Applicant [14]:

- a RSD_r ranging from 0.6 to 0.8 %
- a RSD_{ip} ranging from 1.2 to 1.5 %
- a R_{Rec} ranging from 99% to 101.7%

Based on the performance characteristics available the EURL recommends for official control the single laboratory validated and further verified method based on HPLC-UV for the quantification of *nicarbazin* in the *feed additive*.

For the quantification of *nicarbazin* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method EN ISO 15782 based on HPLC coupled with UV detection [10,16]. *Nicarbazin* is assayed using DNC as target compound. The mass fraction of *nicarbazin* in the test sample is calculated via a specific equation. The method consists in the preliminary milling and mixing of the *feedingstuffs* sample followed by the shaking-extraction of the analyte using acetonitrile/methanol/water. *Premix* samples are not milled and no water is used for solvent extraction. After dilution and filtration the solution is directly injected into

the chromatographic system. DNC moiety of *nicarbazin* is then determined by reversed-phase HPLC and detected at 350 nm. Even though this wavelength is not very specific, no potential interferences were observed and reported in the frame of the single laboratory validation preceding the EN ISO ring trial [17]. The following performance characteristics were reported for the EN/ISO method [14,16,17]:

- RSD_r ranging from 2.6 to 10 %;
- RSD_R ranging from 4.8 to 12 %;
- R_{Rec} ranging from 91 to 108 %; and
- LOQ = 20 mg/kg *feedingstuffs.*

Based on the performance characteristics available the EURL recommends for official control the ring-trial validated EN ISO 15782 method, based on HPLC-UV detection for the quantification of *nicarbazin* in *premixtures* and *feedingstuffs*.

Methods of analysis for the determination of the residues of the additive in food.

Monensin sodium

For the quantification of *monensin sodium* in target tissues (skin/fat, muscle, liver and kidney) the Applicant submitted a single laboratory and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode (ESI) using matrix matched standards [10,18].

Minced tissue is spiked with an appropriate amount of nigericin sodium as internal standard (and the analyte, *monensin sodium*, preparing matrix-matched calibrators). The sample is vortex mixed. Acetonitrile is added to the tissue samples for a second vortex-homogenisation followed by ultrasonic bath. Subsequently, the sample is centrifuged for 10 min at 4000 rpm and 4ºC. The supernatant is transferred into a clean tube and evaporated under nitrogen. The dried residue is reconstituted in 1ml acetonitrile, vortex mixed, sonicated and vortex mixed once again. Finally the extract is filtered and transferred into a vial for being injected in the RP-HPLC-MS/MS system. *Monensin sodium* is detected by MS/MS (positive mode) after ionisation by ESI.

The target matrices (skin/fat, muscle, liver and kidney) of various species (including chicken and turkeys) were investigated at different *monensin* concentrations [18,19]. The method was further verified by a second independent laboratory [20-23]. Three *monensin sodium* levels (i.e. MRL/2; MRL and 2MRL) were investigated for each target *tissue*. Four identification points were set for *monensin sodium* using one parent and two daughter ions. Quantification is based on the transition m/z 693.3 > 675.3 while confirmation is based on the transition m/z 693.3 > 461.3 to comply with the confirmatory requirements set by Commission Decision

2002/657/EC [24,25]. The performance characteristics derived from the validation and verification studies are presented in Table 2. Furthermore the Applicant reported an LOQ of 0.5 µg/kg for muscle, liver, kidney and skin/fat tissues.

Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs for *monensin sodium* in the target *tissues*.

Table 2. Performance characteristics of analytical method for the quantification of *monensin sodium residues* in tissues [14,18-23].

Tissues		µg/kg (#)	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
			Valid	Verif	Valid	Verif	Valid(*)	Verif
Chicken	Muscle [18,22]	4	1.4	---	1.4	---	100.6	---
		8	4.7	6.8-7.8	4.6	8.3	94.2	92
		16	3.8	---	6.3	---	106.6	---
	Liver [18,21]	4	5.3	---	6	---	96.3	---
		8	5.6	2.9-7.9	12.6	5.3	98.7	102
		16	8.3	---	15	---	98.9	---
	Skin/fat [18,23]	12.5	2	---	6.5	---	99.3	---
		25	3.2	4.0-4.7	4.8	4.2	97.5	97.2
		50	1.9	---	3.5	---	96.3	---
	Kidney [18,20]	4	4.3	---	6.8	---	94.8	---
		8	6.8	3.7-7.0	5	6.5	98.1	99
		16	5.2	---	2.8	---	100.9	---
Turkey	Muscle [19]	4	4.2	---	6.9	---	97.4	---
		8	3.1	---	7.4	---	93.3	---
		16	6.4	---	11.1	---	96.3	---
	Liver [19]	4	1.6	---	5.7	---	105.5	---
		8	1.6	---	4.4	---	96.9	---
		16	2.3	---	2.3	---	105	---
	Skin/fat [19]	12.5	3.2	---	6.8	---	96.9	---
		25	5.2	---	5.1	---	103.4	---
		50	5.1	---	2.6	---	100.2	---
	Kidney [19]	4	4.3	---	3.5	---	106.8	---
		8	4.6	---	4.1	---	104.2	---
		16	3.9	---	6.8	---	106.3	---

RSD_r; RSD_R and RSD_{ip}: relative standard deviation for *repeatability*, *reproducibility* and *intermediate precision*, respectively;
R_{Rec}: *recovery* rate;
Fortified level; (*) Recalculated by the EURL [14].

Nicarbazin

For the quantification of *DNC* (target compound for *Nicarbazin*) in target tissues (skin/fat, muscle, liver and kidney) the Applicant submitted a single laboratory and further verified method based on RP-HPLC-MS/MS in ESI mode using matrix matched standards [10,26].

Minced tissue is spiked with an appropriate amount of of 4,4-dinitrocarbanilide-D8 or 1,3-Bis(4-nitrophenyl)urea-D8 as internal standard (and the analyte, DNC, preparing matrix-matched calibrators). The sample is vortex mixed. Acetonitrile is added to the tissue samples for a second vortex-homogenisation followed by ultrasonic bath. The sample is then centrifuged for 10 min at 4000 rpm and 4ºC. The supernatant is transferred into a clean tube and evaporated under nitrogen. The dried residue is reconstituted in 1ml acetonitrile, vortex mixed, sonicated and vortex mixed once again. The extract is filtered and transferred into a vial to be injected in the RP-HPLC-MS/MS system. *DNC* is detected by MS/MS (positive mode) after ionisation by ESI.

The target matrices (skin/fat, muscle, liver and kidney) of various species (including chicken and turkeys) were investigated at different *DNC* concentrations [26,27]. The method was further verified by a second independent laboratory [28]. Three *DNC* levels (i.e. MRL/2; MRL and 2MRL) were investigated for each target *tissue*. Four identification points were set for *DNC* using one parent and two daughter ions. Quantification is based on the transition m/z 301.0 > 136.9 while confirmation is based on the transition m/z 301.0 > 106.7 complying thus with the confirmatory requirements set by of Commission Decision 2002/657/EC [25,29].

The performance characteristics derived from the validation and verification studies are presented in Table 3. Furthermore the Applicant reported an LOQ of 1000 µg/kg for muscle, liver, kidney and skin/fat tissues.

Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs for *DNC* in the target *tissues*.

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 3. Performance characteristics of analytical method for the determination of *DNC residues* in food [14,26-28].

Tissues		µg/kg (#)	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
			Valid	Verif	Valid	Verif	Valid(*)	Verif
Chicken	Muscle [26,28]	2000	3.0	---	2.6	---	99.6	---
		4000	5.0	5.6-6.4	2.2	6.2	100.8	106.7
		8000	2.2	---	4.3	---	97.9	---
	Liver [26,28]	7500	2.6	---	2.6	---	105.3	---
		15000	3.8	6.1	3.5	6.9	99.9	95.8
		30000	4.6	---	6.8	---	92.2	---
	Skin/fat [26,28]	2000	1.8	---	2.6	---	96.4	---
		4000	2.5	3.1-6.2	1.4	4.7	98.2	99.9
		8000	1.6	---	1.0	---	98.9	---
	Kidney [26,28]	3000	2.2	---	2.3	---	99.4	---
		6000	0.9	7.0-7.8	2.4	7.4	98.6	93.8
		12000	1.3	---	2.4	---	99.3	---
Turkey	Muscle [27]	2000	2.2	---	4.2	---	101.6	---
		4000	2.3	---	4.6	---	100.9	---
		8000	1.7	---	1.1	---	102.3	---
	Liver [27]	7500	0.9	---	2.3	---	98.9	---
		15000	2.7	---	3.6	---	95.0	---
		30000	3.9	---	1.0	---	106.4	---
	Skin/fat [27]	2000	2.1	---	1.2	---	101.4	---
		4000	1.1	---	1.2	---	102.3	---
		8000	0.8	---	0.9	---	103.6	---
	Kidney [27]	3000	1.3	---	1.6	---	98.7	---
		6000	1.0	---	1.7	---	99.3	---
		12000	1.5	---	1.8	---	99.3	---

RSD_r; RSD_R and RSD_{ip}: relative standard deviation for *repeatability*, *reproducibility* and *intermediate precision*, respectively;
R_{Rec}: *recovery* rate;
Fortified level; (*) Recalculated by the EURL [14]

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

− the single-laboratory validated and further verified method based on on High Performance Liquid Chromatography (HPLC) with post-column derivatisation and Visible (VIS) detection for the quantification of *monensin sodium* in the *feed additive*;

− the ring-trial validated EN ISO 14183 method, based on HPLC with post-column derivatisation and VIS detection for the quantification of *monensin sodium* in *premixtures* and *feedingstuffs*;

− the single-laboratory validated and further verified method based on HPLC with UV detection for the quantification of *nicarbazin* in the *feed additive*;

− the ring-trial validated EN ISO 15782 method, based on HPLC-UV detection for the determination of *nicarbazin* in *premixtures* and *feedingstuffs*;

− the single-laboratory validated and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to triple quadrupole mass spectrometer (RP-HPLC-MS/MS) - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the quantification of *monensin sodium* in chicken and turkey *tissues*; and

− the single laboratory validated and further verified method based on RP-HPLC-MS/MS proposed by the Applicant - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the quantification of *4,4-dinitrocarbanilide (DNC)* in chicken and turkey *tissues.*

Recommended text for the register entry (analytical method)

For the quantification of *monensin sodium* in the *feed additive*:

– High Performance Liquid Chromatography using post-column derivatisation coupled to Visible detection (HPLC-VIS)

For the quantification of *monensin sodium* in *premixtures* and *feedingstuffs*:

– High Performance Liquid Chromatography using post-column derivatisation coupled to Visible detection (HPLC-VIS) - EN ISO 14183

For the quantification of *nicarbazin* in the *feed additive*:

– High Performance Liquid Chromatography using post-column derivatisation coupled to UltraViolet detection (HPLC-UV)

For the quantification of *nicarbazin* in *premixtures* and *feedingstuffs*:

– High Performance Liquid Chromatography using post-column derivatisation coupled to UltraViolet detection (HPLC-UV) - EN ISO 15782

For the quantification of *monensin sodium* and *nicarbazin* in *tissues*:

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Monimax*® 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, Reference SANCO/G1: Forw. Appl. 1831/0044-2012

[2] *Application, Proposal of Registry Entry – Annex A

[3] *Technical dossier, Section II: 2.1 Identity of the additive

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

[5] *Technical dossier, Section II: 2.1.4 Purity

[6] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[7] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

[8] 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

[9] EN ISO 14183:2008 *Animal feedingstuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatography method using post-column derivatisation* (ISO 14183:2005)

[10] *Technical dossier, Section II, 2.6 Methods of analysis and reference samples

[11] Harold Campbell et Gita Nayeri, *Determination of Monensin, Narasin and Salinomycin in mineral premixes, supplements and animal feeds by liquid chromatography and post-column derivatization: collaborative study*, J. of AOAC Int., 89, 5, 1229 – 1242, 2006

[12] *Technical dossier, Section II, Annexes, Reference II.37

[13] *Technical dossier, Section II, Annexes, Reference II.51

[14] EURL FA data recalculated

[15] *Technical dossier, Section II, Annexes, Reference II.38

[16] * EN ISO 15782:2009 *Animal feedingstuffs – Determination of nicarbazin. High-performance liquid chromatographic method* (ISO 15782:2009)

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[18] *Technical dossier, Section II, Annexes, Reference II.55

[19] *Supplementary information, 2011-fl-11 mon in turkey

[20] *Supplementary information, CRLFA-verification-form_monensin_broiler_kidney.doc

[21] *Supplementary information, CRLFA-verification-form_monensin_broiler_liver.doc

[22] *Supplementary information, CRLFA-verification-form_monensin_broiler_muscle.doc

[23] *Supplementary informaton, CRLFA-verification-form_monensin_broiler_skin_fat.doc

[24] *Supplementary information, July 2014, 2011-FL-02 amendment 2 (monensin chicken tissue).pdf

[25] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

[26] *Technical dossier, Section II, Annexes, Reference II.57

[27] *Technical dossier, Section II, Annexes, Reference_II_57 TUR

[28] *Technical dossier, Section II, Annexes, Reference_II_56[1].pdf

[29] *Supplementary information, 2011-FL-08 amendment 2 (DNC chicken tissue).pdf

*Refers to Dossier no: FAD-2012-0027

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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