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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

Kelforce
(FAD-2013-0022; CRL/130016)

Corrected Version

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-2013-0022 - CRL/130016**
Name of Product:	***Kelforce***
Active Agent (s):	**L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA-Na_4)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **24/02/2014**
Report approved by: Date:	**Christoph von Holst** **25/02/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Kelforce (GLDA-Na_4)*, for the category/functional 4(c)(d) "zootechnical additives"/"substances ~~with~~ which favourably affect the environment"/"other zootechnical additives" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for *chicken for fattening*. *Kelforce* active substance is *L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA-Na_4)*. The product is intended to be marketed in a liquid form containing at least ~~47.7~~ 47.4% of *GLDA-Na_4* and less than 50% water (*GLDA-Na_4 L)*, and in a solid form containing at minimum 30% of *GLDA-Na_4* and at maximum 36% silicic acid and ~~34~~ 35% water (*GLDA-Na_4 P)*.

The *feed additive* is intended to be included into *premixtures* and/or *feedingstuffs* with a maximum *feed additive* content of 2110 or 3333 mg/kg *feedingstuffs* for the liquid or solid preparation, respectively.

For the determination of *GLDA-Na_4* in the *feed additive* and *premixtures*, the Applicant submitted an in-house validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to spectrophotometric detection (RP-HPLC-UV), while for the determination of *GLDA-Na_4* in the *feedingstuffs* the Applicant submitted an in-house validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to mass spectrometry detection (RP-HPLC-MS). The experimental evidence provided, allows the EURL to recommend for official control the RP-HPLC-UV method for the determination of *GLDA-Na_4* in the *feed additive* and *premixtures* and the RP-HPLC-MS method for the determination of *GLDA-Na_4* 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

L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA-Na_4), *Kelforce*, "zootechnical additives"/"substances with favourably affect the environment"/"other zootechnical additives", *chicken for fattening.*

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *Kelforce (GLDA-Na_4)*, for the category/functional 4(c)(d) "zootechnical additives"/"substances ~~with~~ which favourably affect the environment"/"other zootechnical additives" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, authorisation is sought for the use of the *feed additive* for *chicken for fattening* [1].

According to the Applicant, the active agents in the products is *L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA-Na_4)* [1-3]. The product is intended to be marketed in the form of 1) an aqueous solution of *GLDA-Na_4* (hereinafter *GLDA-Na_4 L)* and 2) *GLDA-Na_4* adsorbed onto a silicic acid, precipitated and dried (hereinafter *GLDA-Na_4 P).*

The *GLDA-Na_4 L* product contains at least ~~47.7~~ 47.4% of *GLDA-Na_4* and less than 50% water, while the *GLDA-Na_4 P* product contains at minimum 30% of *GLDA-Na_4* and at maximum 36% silicic acid and ~~34~~ 35% water [3].

The *feed additive* is intended to be included into *premixtures* and/or *feedingstuffs* with a maximum *feed additive* content of 2110 or 3333 mg/kg *feedingstuffs* for the liquid or solid preparation, respectively [2]. Assuming that *Kelforce* is the *feed additive* included, this would lead to a maximum *GLDA-Na_4* content of 1000 mg/kg *feedingstuffs* [3].

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 *Kelforce* 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 [4]

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

For the determination of *GLDA-Na_4* in the *feed additive* and *premixtures* the Applicant submitted an in-house validated [5] and further verified method [6] based on Reversed Phase High Performance Liquid Chromatography coupled to spectrophotometric detection (RP-HPLC-UV) [7].

The sample is extracted with 75 ml of water (for the *feed additive*) or a copper acetate solution (for the *premixtures*). The mixture is agitated for 1 h and make up to 100 ml with water (for *feed additive*) or a copper acetate solution (for the *premixtures*) followed by further homogenisation. Let the solution stand until obtaining a clear supernatant. If necessary the mixture can be centrifuged and further diluted to fall within the calibration curve before being analysed by RP-HPLC-UV at 290 nm [7].

For the determination of *GLDA-Na_4* in the *feedingstuffs* the Applicant submitted an in-house validated [8] and further verified [9] method based on Reversed Phase High Performance Liquid Chromatography coupled to mass spectrometry detection (RP-HPLC-MS) [10].

The ground *feedingstuffs* is extracted with 40 ml of water by shaking the mixture vertically for 2 h followed by 10 min centrifuge. An aliquot of the obtained supernatant is then mixed with a TFA (2,2,2 trifluoroacetic acid) solution and further centrifuged for another 10 min. The obtained supernatant is then filtered and analysed by RP-HPLC-MS (m/z 264.070) [10].

The performance characteristics calculated from the validation and in the verification reports submitted by the Applicant [5,6,8,9] are summarised in Table 1. Furthermore the Applicant reported a limit of quantification (LOQ) of 2.0 mg/kg *feedingstuffs* [8].

Table 1. Performance characteristics of analytical method for the determination of *GLDA-Na_4* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS), as recalculated by the EURL, based on the experimental data provided in the validation (Val) and verification (Ver) reports.

Matrices	Concentration (g/100 g)	RSDr (%)		RSDip (%)		R_{Rec} (%)	
		Val	Ver	Val	Ver	Val	Ver
FA *Powder [5,6]*	37.7	0.37	1.60	0.37	2.96	100	106
FA *Liquid [5,6]*	47.0-47.5	0.75	2.77	0.75	2.84	100	104
PM [5,6]	0.97	1.20	2.37	1.30	5.19	99.0	106
FS [8,9]	0.01	5.99	1.99	5.99	2.04	99.1	97.7

RSD_r: relative standard deviation for *repeatability* (%); RSD_{ip}: relative standard deviation for *intermediate precision* (%); R_{Rec}: *recovery* rate (%);

Based on the performance characteristics presented, the EURL recommends for official control the in-house validated and further verified method based on RP-HPLC-UV for the determination of *GLDA-Na_4* in the *feed additive* and *premixtures* and the in-house validated and further verified method based on RP-HPLC-MS for the determination of *GLDA-Na_4 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the in-house validated and further verified method based on RP-HPLC-UV for the determination of *GLDA-Na_4* in the *feed additive* and *premixtures* and the in-house validated and further verified method based on RP-HPLC-MS for the determination of *GLDA-Na_4* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *GLDA-Na_4* in *feed additive and premixtures*:

- Reversed-Phase High Performance Liquid Chromatography coupled to spectrophotometric detection at 290 nm (RP-HPLC-UV)

For the determination of *GLDA-Na_4* in *feedingstuffs*:

- Reversed-Phase High Performance Liquid Chromatography coupled to mass spectrometry detection (RP-HPLC-MS).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Kelforce* 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. 1831/0018-2013

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II, Identity, characterisation and conditions of use of the additive; methods of analysis

[4] 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

[5] *Technical dossier, Section II: Annex_II_38

[6] *Technical dossier, Section II: Annex_II_44

[7] *Technical dossier, Section II: Annex_II_42

[8] *Technical dossier, Section II: Annex_II_39

[9] *Technical dossier, Section II: Annex_II_45

[10] *Technical dossier, Section II: Annex_II_43

*Refers to Dossier no: FAD-2013-0022

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

The following National Reference Laboratories contributed to this report:

- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft. Nossen[2] (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT))

[1] Name and address according to Regulation (EC) No 885/2009: Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby

[2] Name and address according to Regulation (EC) No 885/2009: Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig