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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

XTRACT® Evolution-B

(FAD-2013-0010; CRL/130003)

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-2013-0010 - CRL/130003**
Name of Product:	**XTRACT® Evolution-B**
Active Agent (s):	**Carvacrol** **Cinnamaldehyde** **Capsicum oleoresin**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **14/04/2014**
Report approved by: Date:	**Christoph von Holst** **14/04/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *XTRACT® Evolution-B* under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening.

XTRACT® Evolution-B is a light to dark orange free-flowing powder preparation consisting of a hydrogenated rape seed oil carrier material, and containing the following active substances: a minimum of 4.6 %, 2.6 % and 2 % of *carvacrol*; *cinnamaldehyde*; and *capsicum oleoresin* (equivalent to measured *"capsaicin + dihydrocapsaicin"* content ranging from 0.06 to 0.24%), respectively.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* to obtain a dosage of 100 mg *XTRACT® Evolution-B* /kg *feedingstuffs*.

For the quantification of *carvacrol, cinnamaldehyde*, *capsaicin* and *dihydrocapsaicin* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified multi-analyte method based on gas chromatography with flame ionisation detection (GC-FID). The following performance characteristics were reported for the four mentioned above analytes: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 1.5 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.2 to 11.2 %; and - a *recovery* rate (R_{rec}) ranging from 99 to 104 %. Based on the experimental evidence available the EURL recommends this multi-analyte GC-FID method for the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive*.

Since the accurate quantification of *XTRACT® Evolution-B* content added to *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to determine *XTRACT® Evolution-B* 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

XTRACT® Evolution-B, carvacrol, cinnamaldehyde, capsaicin, dihydrocapsaicin, capsicum oleoresin, zootechnical additives, other zootechnical additives, chickens for fattening

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new *feed additive*) for *XTRACT® Evolution-B* under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives' according to the classification system of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening [1,2].

XTRACT® Evolution-B is a light to dark orange free-flowing powder preparation consisting of a hydrogenated rape seed oil carrier material [3], and containing the following active substances: a minimum of 4.6, 2.6 and 2 % of *carvacrol*; *cinnamaldehyde*; and *capsicum oleoresin* [2] (equivalent to measured *"capsaicin + dihydrocapsaicin"* content ranging from 0.06 to 0.24% [4]), respectively.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* to obtain a dosage of 100 mg *XTRACT® Evolution-B* /kg *feedingstuffs*.

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 particular dossier, the methods of analysis submitted in connection with *XTRACT® Evolution-B* 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, salmonella, mycotoxins and dioxins) are available from the respective European Union Reference Laboratories [5].

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

For the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified multi-analyte method based on gas chromatography with flame ionisation detection (GC-FID) [6].

The *XTRACT® Evolution-B* sample (6 g) is sonicated for 10 min after the addition of methyl tridecanoate internal standard and the adjustment to 50 ml with acetone. An aliquot of the extract is filtered and analysed by GC-FID. The quantification of the active substances is performed by matrix free calibration using solution standards containing the analytes of interest (combined with internal standard dilution for *carvacrol* and *cinnamaldehyde* only) [6].

The performance characteristics reported for the four analytes in the frame of the validation [7] and verification [8-11] studies are presented in Table 1. Furthermore, the Applicant reported similar limits of quantification (LOQ) of 0.3 g/kg *feed additive* for all four analytes [7].

Based on the experimental evidence available the EURL recommends for the official control the single-laboratory validated and further verified multi-analyte GC-FID method submitted by the Applicant for the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive*.

Table 1 The performance characteristics of the multi-analyte single laboratory validated (Val) and verified (Ver) method for the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive*

	carvacrol		cinnamaldehyde		capsaicin		dihydrocapsaicin	
	Val.	Ver.	Val.	Ver.	Val.	Ver.	Val.	Ver.
Concentration, g/kg	48.8	48.8 - 50.6	30.2	30.6 - 31.2	0.58	0.66 - 0.67	0.62	0.34 - 0.38
RSD_r, %	0.2		0.2		0.3	0.3 - 1.5	1.2	0.4 - 1.3
RSD_{ip}, %	0.2	0.2 - 0.4	0.3	0.3 - 0.4	2.0	0.7 - 5.1	2.0	2.5 - 11.2
R_{rec}, %	100	102 - 103	100	102 - 103	99	-	104	-
Reference	[7]	[8]	[7]	[9]	[7]	[10]	[7]	[11]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively;
R_{rec} - a *recovery* rate

Since the accurate determination of *XTRACT® Evolution-B* content added to *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine *XTRACT® Evolution-B* in *feedingstuffs*.

However, the Applicant submitted another single-laboratory validated and further verified method based on gas chromatography coupled with triple quadruple mass spectrometry (GC-MS/MS) for the quantification of the individual active substances: *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in *feedingstuffs* [12].

The *feedingstuffs* sample (17 g) is extracted with acetone using accelerated solvent extraction. An aliquot (0.5 ml) of the extract is cleaned using solid phase extraction (SPE). Internal standards (methyl tridecanoate or/and thymol, methylcinnamaldehyde, vanillylnonamide) are added to the SPE extract and further analysed by GC-MS/MS. Quantification is performed by matrix match calibration (using solution standards containing the analytes of interest) combined with the internal standards dilution [12]. For the quantification of *cinnamaldehyde* the SPE step was not applied.

While acceptable *precision* data were reported in the frame of the validation and verification studies [13-18] unacceptably low *recovery* rates for *cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in some of the feed samples were obtained. Hence, the EURL cannot recommend this multi-analyte method for official control to determine the four analytes of interest 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 single-laboratory validated and further verified multi-analyte method, based on gas chromatography with flame ionisation detection (GC-FID) for the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive*.

Since the accurate determination of *XTRACT® Evolution-B* content added to *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to determine *XTRACT® Evolution-B* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *carvacrol, cinnamaldehyde, capsaicin* and *dihydrocapsaicin* in the *feed additive*:

– Gas Chromatography with Flame Ionisation Detection (GC-FID)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *XTRACT® Evolution-B* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Ref.: SANCO/G1:Forw.Appl.1831/0007-2013
[2] *Application, Proposal for Register Entry - Annex A
[3] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition, Table II.2
[4] *Supplementary information – Memo-EURL question 20140226
[5] 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
[6] *Supplementary information – PR-RD_CH-57-2 - Analyse XT6930 (eng)_11032014
[7] *Technical dossier, Section II – Annex_II_102
[8] *Technical dossier, Section II – Annex_II_81
[9] *Technical dossier, Section II – Annex_II_82
[10] *Technical dossier, Section II – Annex_II_83
[11] *Technical dossier, Section II – Annex_II_84
[12] *Technical dossier, Section II – Annex_II_95
[13] *Technical dossier, Section II – Annex_II_90
[14] *Technical dossier, Section II – Annex_II_99
[15] *Technical dossier, Section II – Annex_II_91
[16] *Technical dossier, Section II – Annex_II_98
[17] *Technical dossier, Section II – Annex_II_92
[18] *Technical dossier, Section II – Annex_II_93

*Refers to Dossier no: FAD-2013-0010

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:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)[1]
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft, Nossen (DE)[2]

[1] Name and address according to Regulation (EC) No 885/2009: Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim

[2] Name and address according to Regulation (EC) No 885/2009: Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 – Landwirtschaftliches Untersuchungswesen, Leipzig