



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

Subject: Addendum to EURL evaluation report

Reference: EURL evaluation report related to FAD-2010-0335 – Botanically Defined Flavourings Group BDG 09 - Zingiberales (JRC F.5/CvH/ZE/AS/Ares (2018)5225574) issued on 11/10/2018

In the EURL report mentioned above [1] analytical methods exclusively for *cardamom oil* were evaluated. *Cardamom oil* is one of nine feed additives submitted as group application of feed additives under the dossier FAD 2010-0335 (*botanically defined flavourings group 09 (BDG 09)*). In the addendum of this report, the EURL will evaluate the suitability of analytical methods for official control of seven from the nine *feed additives*, namely *turmeric oil*, *turmeric oleoresin*, *turmeric extract*, *turmeric tincture*, *ginger oil*, *ginger oleoresin* and *ginger tincture*. For the ninth *feed additive* of this grouped application, namely *ginger extract*, the European Commission had accepted the request from the Applicant to withdraw the application [2] for this *feed additive*. Therefore, the analytical methods for *ginger extract* were not evaluated in this addendum.

According to the updated information from the Applicant:

- *Turmeric oil* is a liquid, consisting of 40 to 60 % of *ar-turmerone* and 5 to 15 % of *beta-turmerone* as phytochemical markers; the content of the markers is expressed as the relative individual peak areas in the chromatogram [3].
- *Turmeric oleoresin* is a viscous liquid, consisting of 20 to 35 % (w/w) of total *curcuminoids* (curcumin, demethoxycurcumin and bis-demethoxycurcumin) as a phytochemical marker [4,5].
- *Turmeric extract* is a powder consisting of a minimum of 90 % (w/w) of total *curcuminoids* (curcumin, demethoxycurcumin and bis-demethoxycurcumin) as a phytochemical marker [6].
- *Turmeric tincture* is a liquid consisting of 0.04 to 0.09 % (w/v) total *curcuminoids* (curcumin, demethoxycurcumin and bis-demethoxycurcumin) as phytochemical marker [7].
- *Ginger oil* is a liquid consisting of 29 to 40 % of *alpha-zingiberene*, 8 to 14 % of *beta-sesquiphellandrene* and 5 to 12 % of *ar-curcumene* as phytochemical markers; the content of the markers is expressed as the relative individual peak areas in the chromatogram [8,9].

- *Ginger oleoresin* is a viscous liquid consisting of 0.5 to 8 % (w/w) of total *gingerols* and 3 to 6 % (w/w) of total *shogaols* as phytochemical markers [10].
- *Ginger tincture* is a liquid consisting of 0.08 to 0.13 % (w/v) of total *gingerols* and 0.02 to 0.04 % (w/v) of total *shogaols* as phytochemical markers [11].

The *feed additives* are intended to be incorporated into *feedingstuffs* or drinking *water* through *flavouring premixtures* [12,13] with no proposed minimum or maximum levels in *feedingstuffs* or *water* [12]. However, the Applicant suggested the typical maximum inclusion level of the *feed additive* of 25 mg /kg *feedingstuffs* [13].

Turmeric oil

For the identification of the phytochemical markers: *ar-turmerone* and *beta-turmerone* in the *feed additive*, the Applicant submitted a method based on gas chromatography coupled to mass spectrometry (GC-MS) using retention time locking (RTL) methodology, while the quantification of the markers is performed using gas chromatography coupled to flame ionisation detection (GC-FID) [14]. The proposed method is based on a generic ISO 11024 standard method on general guidance on chromatographic profiles for essential oils [15].

Before the analysis, the inlet pressure of the GC-MS system is adjusted in a way to match a retention time of 6.7 or 8.3 min for polar and non-polar columns, respectively, by using the reference substance for RTL – limonene. By making the proper adjustment to the inlet pressure, the retention times can closely match those of the corresponding reference databases. Then, the sample (with or without preparation) is used for chromatographic analysis. The phytochemical markers are identified by using the reference database/libraries of retention times and of mass spectra of flavourings, available from the Applicant on request. The quantification of the phytochemical markers is performed by GC-FID using relative areas of the markers versus the sum of the areas of all individual components in the chromatogram [14].

The above mentioned above GC method was verified by analysing in duplicate two batches of *turmeric oil* [16-25]. The performance characteristics are presented in Table 1.

Based on the experimental evidence available the EURL recommends for official control the method mentioned above based on GC-MS using RTL methodology together with the GC-FID based on generic ISO 11024 standard method for the determination of the phytochemical markers: *ar-turmerone* and *beta-turmerone* in the *feed additive (turmeric oil)*.

Table 1. Performance characteristics of the GC-FID method for the quantification of the phytochemical markers (*ar-turmerone* and *beta-turmerone*) in the *feed additive* (*turmeric oil*)

	<i>Feed additive (batch no 1)</i>		<i>Feed additive (batch no 2)</i>	
	<i>ar-turmerone</i>	<i>beta-turmerone</i>	<i>ar-turmerone</i>	<i>beta-turmerone</i>
Content, % (relative area)	32.3	6.8	39.2	12.6
RSD _r , %	0.1	0.4	0.1	0.2
RSD _{ip} , %	0.2	0.5	0.1	0.2
Reference	[24]	[25]	[24]	[25]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively.

Alternatively (for example in the case of unavailability of the RTL systems), the EURL recommends for official control the identification of the phytochemical markers in the *feed additive* by GC-MS using the standard substances of the phytochemical markers instead of the RTL methodology. The Applicant should provide control laboratories with the standard substances in case they are not available commercially. In addition, the EURL recommends for official control the quantification of the phytochemical markers also by GC-MS in the total ion monitoring mode.

Turmeric oleoresin

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive*, the Applicant submitted the internationally recognised FAO JECFA monograph dedicated for “Turmeric Oleoresin” [26]. The quantification of total *curcuminoids* is based on spectrophotometry using the specific absorbance ($E_{1\%, 1\text{ cm}} = 1607$) of the curcumin standard in ethanol at 425 nm as described under method II of the JECFA monograph [26].

The Applicant applied this method for the quantification of total *curcuminoids* in four batches of the *feed additive* where the content of total *curcuminoids* was ranging from 23.5 to 32.2 % (w/w) [4].

The EURL recommends for official control the JECFA method mentioned above based on spectrophotometry using the specific absorbance of curcumin for the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive* (*turmeric oleoresin*).

Turmeric extract

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive* the Applicant submitted the internationally recognised FAO JECFA monograph “Curcumin” [27]. The quantification of total *curcuminoids* is based on spectrophotometry using specific absorbance ($E_{1\%, 1\text{ cm}} = 1607$) of a curcumin standard in ethanol at 425 nm as described in the monograph [27].

The Applicant applied this method for the quantification of total *curcuminoids* in one duplicate sample of the *feed additive* and a RSD_r of 0.8 % was derived for an average content of total *curcuminoids* of 86.8 % (w/w) [28]. In addition, the Applicant applied this method for the quantification of total *curcuminoids* in another four batches of the *feed additive* and a RSD_r of 0.7 % was derived for an average content of total *curcuminoids* of 95.7 % (w/w) [29].

Based on the experimental evidence available the EURL recommends for official control the JECFA method mentioned above based on spectrophotometry using specific absorbance of curcumin for the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive (turmeric extract)*.

Turmeric tincture

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive*, the Applicant submitted the method [30,31] based on European Pharmacopeia monograph “Turmeric Javanese” (01/2008:1441) [32]. The quantification of total *curcuminoids* as dicinnamoyl methane derivatives is based on spectrophotometry at 530 nm after the derivatisation with borates in the presence of oxalic acid [30,31].

The sample (1 ml) is diluted with glacial acetic, boric and oxalic acids and heated in a water bath at 90 °C for 30 min. After the cooling down and dilution with acetic acid, the mixture is shaken and filtered. The absorbance of the filtrate is measured at 530 nm. The standard solutions of curcumin are treated in the same way as the samples. The quantification of total *curcuminoids* is performed using an external calibration with curcumin as a standard substance [30,31].

The Applicant applied this method for the quantification of total *curcuminoids* in six batches of the *feed additive* produced from two different suppliers of raw material and a RSD_r ranging from 2 to 15 % was derived for an average the content of total *curcuminoids* ranging from 0.05 to 0.07 % (w/v) [33].

Based on the experimental evidence available the EURL recommends for official control the spectrophotometric method based on European Pharmacopeia monograph “Turmeric

Javanese” (01/2008:1441) for the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive (turmeric tincture)*.

Ginger oil

For the determination of the phytochemical markers: *alpha-zingiberene*, *beta-sesquiphellandrene* and *ar-curcumene* in the *feed additive (ginger oil)*, the Applicant submitted the same GC-MS and GC-FID methods as for the determination of markers in *turmeric oil* [14].

The GC method mentioned above was verified by analysing eleven batches of the *ginger oil* from two different geographical regions [8,9,14]. The performance characteristics are presented in Table 2.

Based on the experimental evidence available the EURL recommends for official control the method based on GC-MS using RTL methodology together with the GC-FID based on the generic ISO 11024 standard method for the determination of the phytochemical markers: *alpha-zingiberene*, *beta-sesquiphellandrene* and *ar-curcumene* in the *feed additive (ginger oil)*.

Alternatively (for example in the case of unavailability of the RTL systems), the EURL recommends for official control the identification of the phytochemical markers in the *feed additive* by GC-MS using the standard substances of the phytochemical markers instead of RTL methodology. The Applicant should provide control laboratories the standard substances in case they are not available commercially. In addition, the EURL recommends for official control the quantification of the phytochemical markers also by GC-MS in the total ion monitoring mode.

Table 2. Performance characteristics of the gas chromatographic method [14] for the quantification of the phytochemical markers (*alpha-zingiberene*, *beta-sesquiphellandrene* and *ar-curcumene*) in *ginger oil*

	<i>alpha-zingiberene</i>	<i>beta-sesquiphellandrene</i>	<i>ar-curcumene</i>
Content, % (relative area)	33.8 – 39.2	9.2 – 11.2	8.5 – 11.0
RSD _r , %	1.8 – 5.9	3.2 – 6.6	2.9 – 5.5
Reference	[8,9,14]		

RSD_r: a relative standard deviation for *repeatability*.

Ginger oleoresin

For the quantification of the phytochemical markers total *gingerols* and total *shogaols* in the *feed additive*, the Applicant submitted the internationally recognised ring-trial validated ISO 13685 method [34]. The method is based on reversed phase high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection and is dedicated for the analysis of *gingerols* and *shogaols* in ginger and its oleoresins [34].

The sample (0.5 g) is dissolved in methanol and the resulting solution is further analysed by HPLC. The analytes are detected at 280 nm. The quantification is performed by an external standard calibration using nonanoic acid vanillylamide (NVA) as the standard substance. For the calculation of the mass fraction of each individual gingerol ([6]-G, [8]-G and [10]-G) or shogaol ([6]-S, [8]-S and [10]-S) the corresponding response factors for NVA and for the individual analytes are applied [34].

The Applicant applied this ISO standard for analysis of six *feed additive* samples and a RSD_r ranging from 3.1 to 8.2 % was derived for the content of total *gingerols* ranging from 1.0 to 7.4 % (w/w); and a RSD_r ranging from 1.2 to 8.7 % was obtained for the content of total *shogaols* ranging from 4.0 to 5.7 % (w/w) [10]. This is in agreement with the performance characteristics derived in the frame of the collaborative trial for the quantification of total *gingerols* and total *shogaols* [34].

Based on the experimental evidence available the EURL recommends for official control the ISO 13685 standard method for the quantification of the phytochemical markers: total *gingerols* and total *shogaols* in the *feed additive (ginger oleoresin)*.

Ginger tincture

For the quantification of the phytochemical markers total *gingerols* and total *shogaols* in the *feed additive*, the Applicant submitted the ISO 13685 method [34]. The Applicant applied the mentioned above method for the analysis of five batches of the *ginger tincture* and a relative standard deviations for *repeatability* of 8.4 and 9.6 % was derived for an average content of total *gingerols* and total *shogaols* of 0.1 and 0.03 % (w/v), respectively [11].

Based on the experimental evidence available the EURL recommends for official control the ISO 13685 standard method for the quantification of the phytochemical markers: total *gingerols* and total *shogaols* in the *feed additive (ginger tincture)*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

Recommended text for the registry entry (analytical method)

For the quantification of the phytochemical markers: *ar-turmerone* and *beta-turmerone* in the *feed additive (turmeric oil)* and *alpha-zingiberene*, *beta-sesquiphellandrene* and *ar-curcumene* in the *feed additive (ginger oil)*:

- Gas chromatography coupled to mass spectrometry (GC-MS) (full scan mode) using Retention Time Locking (RTL) methodology (or standard substances of the phytochemical markers) with (or without) gas chromatography coupled to flame ionisation detection (GC-FID) based on the ISO 11024 standard method

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive (turmeric oleoresin)*:

- Spectrophotometry – FAO JECFA Combined Compendium of Food Additive Specifications, “Turmeric Oleoresin”, monograph No. 1 (2006)

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive (turmeric extract)*:

- Spectrophotometry – FAO JECFA Combined Compendium of Food Additive Specifications, “Curcumin”, monograph No. 1 (2006)

For the quantification of the phytochemical marker (total *curcuminoids*) in the *feed additive (turmeric tincture)*:

- Spectrophotometry (based on European Pharmacopeia monograph “Turmeric Javanese” (01/2008:1441))

For the quantification of the phytochemical markers: total *gingerols* and total *shogaols* in the *feed additives (ginger oleoresin and ginger tincture)*:

- High performance liquid chromatography (HPLC) with spectrophotometric (UV) detection – ISO 13685

References

- [1] FAD-2010-0335, Botanically Defined Flavourings Group BDG 09 – Zingiberales, Ref. JRC F.5/CvH/ZE/AS/Ares(2018)5225574
<https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0335-cardamom-oil.pdf>
- [2] *Supplementary information – Partial withdrawal of applications for various Botanically Defined Groups from FEFANA ASBL – Ares(2019)1299322
- [3] *Supplementary information – 2018-08-08_SIn_Reply_Turmeric_Oil
- [4] *Supplementary information – 2019 05-31 Appendix_EURL_Turmeric_OR
- [5] *Supplementary information – 2019-07-31 SIn reply Turmeric oleoresin
- [6] *Supplementary information – Turmeric_extract_ZIN002c_SIn_Reply_final

- [7] *Supplementary information – Turmeric_tincture_ZIN002f_SIn_Reply_final
- [8] *Supplementary information – 2018-08-08_SIn_reply_Ginger_oil
- [9] *Supplementary information – 2018-08-30_SIn_reply_Ginger_oil_rev
- [10] *Supplementary information – 2019-03-15 SIn reply_Ginger oleoresin
- [11] *Supplementary information – SIn reply_Ginger tincture_revised_ pages17-21
- [12] *Application, Proposal for Register Entry – Annex A
- [13] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis
- [14] *Supplementary information – Annex_I_Sin_Reply_Ginger_oil_Batch_data
- [15] ISO 11024:1998 – *Essential oils - General guidance on chromatographic profiles*
- [16] *Supplementary information – BDG09-ZIN002a Turmeric oil
C15013727_1_Phytomarkers_190531
- [17] *Supplementary information – BDG09-ZIN002a Turmeric oil
C15013727_1_Phytomarkers_190604
- [18] *Supplementary information – BDG09-ZIN002a Turmeric oil
C15013727_2_Phytomarkers_190531
- [19] *Supplementary information – BDG09-ZIN002a Turmeric oil
C15013727_2_Phytomarkers_190604
- [20] *Supplementary information – BDG09-ZIN002a Turmeric oil
SE180230_1_Phytomarkers_190531
- [21] *Supplementary information – BDG09-ZIN002a Turmeric oil
SE180230_1_Phytomarkers_190604
- [22] *Supplementary information – BDG09-ZIN002a Turmeric oil
SE180230_2_Phytomarkers_190531
- [23] *Supplementary information – BDG09-ZIN002a Turmeric oil
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- [24] *Supplementary information – Turmeric oil - ar-Turmerone_eurl-fa-technical-guide_sensory-additives
- [25] *Supplementary information – Turmeric oil - beta-Turmerone_eurl-fa-technical-guide_sensory-additives
- [26] FAO JECFA Combined Compendium of Food Additive Specifications, “Turmeric Oleoresin”, monograph No. 1 (2006)
http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-484.pdf (last visited on 20/01/2020)
- [27] FAO JECFA Combined Compendium of Food Additive Specifications, “Curcumin”, monograph No. 1 (2006)
http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-140.pdf (last visited on 20/01/2020)
- [28] *Supplementary information – Annex II spec B09-1 protocols analytical results
- [29] *Supplementary information – Annex_II_Turm Extr_Batch_to_batch

- [30] *Supplementary information – Annex_I_SPEC_B09-1_Description
- [31] *Supplementary information – Annex_VI_Turm_Tinct_Curcuminoids_Method
Photometer
- [32] European Pharmacopoeia monograph, 01/2008:1441
- [33] *Supplementary information – Annex_Ia_Turm_Tinct_Photometer_Dicinnamoyl
methane derivatives
- [34] ISO 13685:1997 – *Ginger and its oleoresins - Determination of the main pungent compounds (gingerols and shogaols) - Method using high performance liquid chromatography*

Acknowledgments

The following National Reference Laboratories contributed to this addendum:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- ¹Wageningen Food Safety Research (WFSR) (NL)

Addendum

- Prepared by Zigmās Ezerskis
 - Reviewed and approved by María José González de la Huebra and Christoph von Holst (EURL-FA), respectively, Geel, 21/02/2020
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¹ Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.



EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Feed Additives

 Ref. Ares(2018)5225574 - 11/10/2018

JRC F.5/CvH/ZE/AS/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**

Botanically Defined Flavourings Group BDG 09 - Zingiberales
(FAD-2010-0335; CRL/100212)

**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-2010-0335 - CRL/100212**

Name of Feed Additive: **cardamom oil (this EURL report), turmeric oil, turmeric oleoresin, turmeric extract, turmeric tincture, ginger oil, ginger oleoresin, ginger extract and ginger tincture from botanically defined flavourings group 09 (BDG 09) – Zingiberales**

Phytochemical marker (s): ***terpineol acetate (this EURL report), beta-turmerone, curcumin, zingiberene and gingerol(s)***

Rapporteur Laboratory: **European Union Reference Laboratory for Feed Additives (EURL-FA)
JRC Geel, Belgium**

Report prepared by: **Zigmas Ezerskis**

Report checked by: **Maria Jose Gonzalez de la Huebra**
Date: **11/10/2018**

Report approved by: **Christoph von Holst**
Date: **11/10/2018**

EXECUTIVE SUMMARY

In the current grouped application authorisation is sought under articles 4(1) and 10(2) for *cardamom oil, turmeric oil, turmeric oleoresin, turmeric extract, turmeric tincture, ginger oil, ginger oleoresin, ginger extract* and *ginger tincture* from *botanically defined flavourings group 09 (BDG 09)* as the *feed additives* under the category/functional group (2b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additives* for all animal species and categories.

In this report the EURL will focus exclusively on the evaluation of the suitability of analytical methods for official control of one of the nine above listed *feed additives*, namely *cardamom oil*.

According to the Applicant, the *feed additive (cardamom oil)* is a transparent colourless to pale yellow fluid liquid with characteristic odour, consisting of 30 to 42 % (expressed as a relative area) of *terpineol acetate* as a phytochemical marker.

The *feed additive* is intended to be incorporated into *feedingstuffs* or drinking water through *flavouring premixtures* with no proposed minimum or maximum levels in *feedingstuffs* or water. However, the Applicant suggested the typical maximum inclusion level of the *feed additive* of 25 mg /kg *feedingstuffs*.

For the determination of *terpineol acetate* (phytochemical marker) in *cardamom oil*, the Applicant submitted an in-house developed multi-analyte method, where the identification is based on gas chromatography coupled to mass spectrometry (GC-MS), using the special "retention time locking (RTL)" methodology, and the quantification is performed using GC coupled to flame ionisation detection (FID).

However, the EURL identified the equivalent ISO 4733 standard method based on GC-FID for the determination of *terpineol acetate* in *cardamom oil*.

Based on the available performance profile, the EURL recommends for official control the ISO 4733 standard method based on gas chromatography coupled to flame ionisation detection (GC-FID) for the determination of *terpineol acetate* (phytochemical marker) in *cardamom oil*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Terpineol acetate, cardamom oil, sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current grouped application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *cardamom oil, turmeric oil, turmeric oleoresin, turmeric extract, turmeric tincture, ginger oil, ginger oleoresin, ginger extract* and *ginger tincture* from *botanically defined flavourings group 09 (BDG 09)* as the *feed additives* under the category/functional group (2b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for the use of the *feed additives* for all animal species and categories [2].

In this report the EURL will focus exclusively on the evaluation of the suitability of analytical methods for official control of one of the nine above listed *feed additives*, namely *cardamom oil*.

According to the Applicant, the *feed additive (cardamom oil)* is a transparent colourless to pale yellow fluid liquid with characteristic odour, consisting of 30 to 42 % (expressed as a relative area) of *terpineol acetate* as a phytochemical marker [3,4]; in addition, *cardamom oil* contains the others components such as alpha-pinene, sabinene, myrcene, limonene, 1,8-cineole, linalool, linalyl acetate, terpinen-4-ol, alpha-terpineol, trans-nerolidol and methyleugenol [3,4].

The *feed additive* is intended to be incorporated into *feedingstuffs* or drinking water through *flavouring premixtures* [2, 3] with no proposed minimum or maximum levels in *feedingstuffs* or *water* [2]. However, the Applicant suggested the typical maximum inclusion level of the *feed additive* of 25 mg /kg *feedingstuffs* [3].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 the *botanically defined flavourings group*

09 (BDG 09) - Zingiberales and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the determination of *terpineol acetate* (phytochemical marker) in *cardamom oil*, the Applicant submitted an in-house developed multi-analyte method, where the identification is based on gas chromatography coupled to mass spectrometry (GC-MS), using the special "retention time locking (RTL)" methodology, and the quantification is performed using GC coupled to flame ionisation detection (FID) [5].

However, the EURL identified the equivalent ISO 4733 standard method based on GC-FID for the determination of *terpineol acetate* in *cardamom oil* [6].

The sample is injected into the GC system without dilution. The individual components (phytochemical marker – *terpineol acetate* included) are identified by separation on two columns, namely a non-polar and a polar capillary column. Finally, the quantification of the phytochemical marker is performed with FID and expressed as the chromatographic relative area of the phytochemical marker (in %) [6].

Based on the available performance profile, the EURL recommends for official control the ISO 4733 standard method based on gas chromatography coupled to flame ionisation detection (GC-FID) for the determination of *terpineol acetate* (phytochemical marker) in *cardamom oil*.

For the identification of *terpineol acetate* (phytochemical marker) in *flavouring premixtures*, the Applicant submitted the above mentioned GC-MS-RTL method [5].

However, no experimental data or analytical method were provided for the quantification of *cardamom oil* in *premixtures* and *feedingstuffs* as the unambiguous determination of *cardamom oil* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of *cardamom oil* in *premixtures* and *feedingstuffs*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not considered necessary by the EURL.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the ISO 4733 standard method based on gas chromatography coupled to flame ionisation detection (GC-FID) for the determination of *terpineol acetate* (phytochemical marker) in the *feed additive (cardamom oil)*.

Recommended text for the register entry (analytical method)

For the determination of *terpineol acetate* (phytochemical marker) in the *feed additive (cardamom oil)*:

- gas chromatography coupled with flame ionisation detection (GC-FID) – ISO 4733

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *botanically defined flavourings group 09 (BDG 09) - Zingiberales* 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_D2_FWD. APPL. 1831/0127/10181/2010
- [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] *Supplementary information – Sin_letter_reply_Cardamom_oil_BDG09
- [5] *Technical dossier, Section II – Annex_II_7
- [6] ISO 4733:2004 – *Oil of Cardamom [Elettaria cardamomum (L). Maton]*

*Refers to Dossier no: FAD-2010-0335

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

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