



**EUROPEAN COMMISSION**  
JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements (Geel)  
**Standards for Food Bioscience**  
European Union Reference Laboratory Feed Additives - Authorisation

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

**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**

**BDG 20**  
*(FAD-2010-0354; CRL/100324)*





**EURL Evaluation Report on the Analytical Methods  
submitted in connection with the Application for the  
Authorisation of Feed Additives according to  
Regulation (EC) No 1831/2003**

Dossier related to: **FAD-2010-0354 – CRL/100324**

Feed additives: **Botanically defined flavourings from  
BDG20 - Lovage root oil, Hyssop oil and  
Sunflower extract**

Active Substance(s): **Lovage root oil, Hyssop oil and Sunflower  
extract**

Rapporteur Laboratory: **European Union Reference Laboratory  
for Feed Additives (EURL-FA)**

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Date: **28/04/2013**

## EXECUTIVE SUMMARY

The *Botanically Defined Flavourings - Group 20 (BDG 20)* is an application for which authorisation as *feed additive* is sought under the category/functional group 2(b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. In the current application submitted according to Article 4(1) and Article 10(2) of Regulation (EC) No 1831/2003, the authorisation for cats and dogs is requested. This grouped application concerns three flavouring compounds (Sunflower extract, Hyssop oil and Lovage root oil) derived from three botanical species. In order to characterise each *flavouring* compound the Applicant prescribed to monitor the following specific phytochemical markers: - oleic acid for Sunflower extract; - cis-3-Butylidene-4,5-dihydrophthalide for Lovage root oil; - and pinocamphone and isopinocamphone for Hyssop oil. *Flavouring compounds* are intended to be incorporated only into *feedingstuffs* or drinking water, through *premixture*. The Applicant suggested no minimum or maximum levels for the different flavouring compounds, but normal contents of *flavouring compounds* in *feedingstuffs* range up to from 0.1 ppm to 100 ppm.

For the identification of the phytochemical marker mentioned above, the Applicant submitted three dedicated standard methods based on gas chromatography (EN ISO 5508:1990; ISO 9841:2007 and ISO 11019:1998). Furthermore, the Applicant applied these methods to representative "oil extract" and "solid premixtures" containing the essential oils under investigation and provided chromatograms showing well resolved peaks of the respective phytomarkers. Based on the satisfactory experimental evidence provided, the EURL recommends for official control the international standard methods mentioned above for the qualitative identification of the three *flavouring compounds* of interest in the *feed additive* and *premixtures*, even though no performance characteristics were provided.

As the accurate determination of *Sunflower extract, Hyssop oil and Lovage root oil* in *feedingstuffs* is not achievable experimentally, the EURL does not recommend any methods for official control to determine *Sunflower extract, Hyssop oil and Lovage root oil* 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

Botanically Defined Flavourings - Group 20, flavouring compounds, sensory additives, cats and dogs.

## 1. BACKGROUND

The *Botanically Defined Flavourings - Group 20 (BDG 20)* is an application for which authorisation of three *feed additives* are sought under the category/functional group 2(b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1].

In the current application submitted according to Article 4(1) (new use in water) and Article 10(2) (re-evaluation of additives already authorised under Directive 70/524/EC) of Regulation (EC) No 1831/2003, the authorisation for cats and dogs is requested [1].

*Botanically defined flavourings* used as *feed additives* include oils, distillates, oleoresins, solvent based and water based extracts, concentrates, tinctures, absolutes and other preparation types, according to British and European Pharmacopeia [2]. This grouped application concerns *three flavouring compounds* (Sunflower extract, Hyssop oil and Lovage root oil) derived from three botanical species (*Helianthus annuus* L., *Hyssopus officinalis* L. and *Levisticum officinale* Koch, respectively). In order to characterise each *flavouring* compound the Applicant prescribed to monitor the following specific phytochemical markers [3]: - oleic acid for Sunflower extract; - cis-3-Butylidene-4.5-dihydrophthalide for Lovage root oil; - and pinocamphone and isopinocamphone for Hyssop oil.

*Flavouring compounds* are intended to be incorporated only into *feedingstuffs* or drinking water, through *premixture* [4]. The Applicant suggested no minimum or maximum levels for the different flavouring compounds [2], but normal contents of *flavouring compounds* in *feedingstuffs* range up to from 0.1 ppm to 100 ppm [4].

## 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 *Botanically Defined Flavourings – Group 20*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

### 3. EVALUATION

#### *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 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 premixtures.***

For the determination of the phytochemical marker *Oleic acid* in the *feed additive* and *premixture*, the Applicant submitted two internationally recognised ISO CEN methods:

- EN ISO 12966:2011 [6] describing the sample preparation, based on the formation of methyl ester by transmethylation with methanolic potassium hydroxide; and
- EN ISO 5508:1990 [7] providing the standard operating procedure for the determination of methyl esters of fatty acids by gas chromatography with flame ionisation detection (GC-FID).

The Applicant applied the two methods mentioned above to the "sunflower extract" and to a "solid premixtures" containing sunflower oil. The chromatograms provided clearly show a well resolved oleic acid peak [8,9].

For the determination of the phytochemical markers *pinocamphone* and *isopinocamphone* in the *feed additive* and *premixture*, the Applicant submitted the internationally recognised ISO CEN method (ISO 9841:2007) dedicated to the analysis of oil of hyssop by gas chromatography with flame ionisation detection (GC-FID) [10]. The Applicant applied the above mentioned method to the "Hyssop essential oil" and to a "solid premixtures" containing hyssop oil. The chromatograms provided clearly show well resolved pinocamphone and isopinocamphone peak [11,12].

For the identification of the phytochemical marker *Cis-3-Butyldilidene-4,5-dihydrophthalide* in the *feed additive* and *premixture*, the Applicant submitted the internationally recognised ISO CEN method (ISO 11019:1998) dedicated to the analysis of lovage root oil by gas chromatography with flame ionisation detection (GC-FID). [13]. The Applicant applied the above mentioned method to the "lovage root oil" and to a "solid premixtures" containing lovage root oil. The chromatograms provided clearly show a well resolved cis-3-Butyldilidene-4.5-dihydrophthalide peak [14,15].

Based on the satisfactory experimental evidence provided, the EURL recommends for official control the four international standard methods mentioned above for the qualitative identification of the three *flavouring compounds* of interest in the *feed additive* and

*premixtures*, even though no performance characteristics were provided. However, some National Reference Laboratories (NRLs) questioned the specificity of the oleic acid marker selected for the *sunflower extract*.

As the accurate determination of *Sunflower extract*, *Hyssop oil* and *Loverage root oil* in *feedingstuffs* is not achievable experimentally, the EURL does not recommend any methods for official control to determine *Sunflower extract*, *Hyssop oil* and *Loverage root oil* 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 three internationally accepted standard methods for the identification of phytochemical marker in the *feed additive* and *premixtures* containing:

- (i) Sunflower extract: EN ISO 5508:1990 for oleic acid;
- (ii) Lovage root oil: ISO 11019:1998 for cis-3-Butylidene-4.5-dihydrophthalide;
- (iii) Hyssop oil: ISO 9841:2007 for pinocamphone and isopinocamphone

##### ***Recommended text for the register entry (analytical method)***

For the identification of *Oleic acid* in the *feed additive* and *premixture*:

Gas-chromatography with flame ionisation detection (GC-FID) - EN ISO 5508:1990

For the identification of *pinocamphone* and *isopinocamphone* in the *feed additive* and *premixture*:

Gas-chromatography with flame ionisation detection (GC-FID) - ISO 9841:2007

For the determination of *Oleic acid* in the *feed additive* and *premixture*:

Gas-chromatography with flame ionisation detection (GC-FID) - ISO 11019:1998

## 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 020 (BDG 20)* 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/D/2 Forw. Appl. 1831/00181 (10367)/-2010
- [2] \* Technical dossier, Section II – Annex II 05
- [3] \* Application, Proposal for Register Entry – Annex A
- [4] \* Technical dossier, Section II – 2.5. Conditions of use of the additive
- [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, GC-B20-1 - Annex\_I\_EN\_ISO\_12966-2\_2011  
*Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids* [nb: this standard supersedes EN ISO 5509:2000]
- [7] \* Supplementary information, GC-B20-1 - Annex\_II\_EN\_ISO\_5508\_1990 – *Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acid*
- [8] \* Supplementary information, GC-B20-1 - Annex\_III\_GC\_B20-1\_Matrix
- [9] \* Supplementary information, GC-B20-1 - Annex\_IV\_GC\_B20-1\_Premixture
- [10] \* Supplementary information, GC-B20-2 - Annex\_I\_NF\_ISO\_9841\_2007
- [11] \* Supplementary information, GC-B20-2 - Annex\_III\_GC\_B20-2\_Matrix
- [12] \* Supplementary information, GC-B20-2 - Annex\_IV\_GC\_B20-2\_Premixture
- [13] \* Supplementary information, GC-B20-3 - Annex\_I\_NF\_EN\_ISO 11019\_1998
- [14] \* Supplementary information, GC-B20-2 - Annex\_III\_GC\_B20-3\_Matrix
- [15] \* Supplementary information, GC-B20-2 - Annex\_IV\_GC\_B20-3\_Premixture

\*Refers to Dossier No. FAD-2010-0354



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## **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:

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- Fødevarestyrelsen, Ringsted (DK)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)