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JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of a new Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0015** **CRL/090045**
Product Name:	**Chemically defined flavourings from Flavouring Group 01 – Straight-chain primary aliphatic alcohols/aldehydes/ acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes**
Active Substance(s):	**Eighty six chemically defined flavourings from Chemical Group 01 – *CDG01***
Rapporteur Laboratory:	**Community Reference Laboratory for Feed Additives (CRL-FA)**
Report prepared by:	**Piotr Robouch (CRL-FA)**
Report revised by: Date:	**Roberto Molteni (CRL-FA)** **03/08/2010**
Report approved by: Date:	**Christoph von Holst** **10/08/2010**

EXECUTIVE SUMMARY

The *Chemically Defined Flavourings - Group 01* (*CDG01, Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes*), in this application comprises 86 substances, for which authorisation as feed additives is sought under the category "sensory additives", functional group 2(b) "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 all species is requested. The flavouring compounds of interest have a purity ranging from 92% to 99.5% (80% for Hexadecanoic acid).

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water*. The Applicant suggested no minimum or maximum levels for the different flavouring compounds in *feedingstuffs*.

For the identification of volatile chemically defined flavouring compounds *CDG01* in the *feed additive,* the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method, using Retention Time Locking (RTL), which allows a close match of retention times on GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database of RTL spectra. The Applicant maintained two FLAVOR2 databases/libraries (for retention times and for MS spectra) containing data for more than 409 flavouring compounds. These libraries were provided to the CRL. The Applicant provided the typical chromatogram for the *CDG01* of interest.

In order to demonstrate the transferability of the proposed analytical method (relevant for the method verification), the Applicant prepared a model mixture of flavouring compounds on a solid carrier to be identified by two independent expert laboratories. This mixture contained twenty chemically defined flavourings belonging to twenty different chemical groups to represent the whole spectrum of compounds in use as feed flavourings with respect to their volatility and polarity. Both laboratories properly identified all the flavouring compounds in all the formulations. Since the substances of *CDG01* are within the volatility and polarity range of the model mixture tested, the Applicant concluded that the proposed analytical method is suitable to determine qualitatively the presence of the substances from *CDG01* in the *mixture of flavouring compounds.*

Based on the satisfactory experimental evidence provided, the CRL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of)

flavouring compounds of interest (*) the GC-MS-RTL (Agilent specific) method submitted by the Applicant.

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *feedingstuffs* and *water*, no methods could be evaluated. Therefore the CRL is unable to recommend a method for the official control to identify the *active substance(s)* of interest (*) in *feedingstuffs* or *water*.

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.

(*) Full list provided in the CRL evaluation report available from the CRL website.

KEYWORDS
Chemically Defined Flavourings - Group 01, sensory additives, all species.

1. BACKGROUND

The *Chemically Defined Flavourings - Group 01* (*CDG01*) is a grouped application for which authorisation as feed additive is sought under the category "sensory additives", functional group 2(b) "flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. The *CDG01* application contains 86 flavouring compounds (listed in Table 1) belonging to the group - described in Annex I of Commission Regulation (EC) No 1565/2000 [2] as - " *Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes* ".

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 all species is requested [1].

The flavouring compounds of interest are produced by different routes of manufacturing, providing a purity ranging from 92% to 99.5% (80% for Hexadecanoic acid) [3]. *Mixtures of flavouring* compounds are usual prepared as liquid (diluted in an appropriate solvent, such as propane-1,2-diol) or solid (with an inorganic carrier, such as silicic acid + calcium carbonate) formulations.

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water* [4]. The Applicant suggested no minimum or maximum levels for the different flavouring compounds [3], but normal contents of single flavouring compounds in *feedingstuffs* range from 0.1 to 100 mg/kg [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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL 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 *Chemically Defined Flavourings – Group 01,* 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 Community Reference Laboratories [5].

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

For the identification of volatile chemically defined flavouring compounds *CDG01* (cf. Table 1) in the *feed additive,* the Applicant submitted a qualitative multi-analyte gas-chromatography mass-spectrometry (GC-MS) method [6], using Retention Time Locking (RTL) [7] methodology for which a patent is owned by Agilent Technology [8]. The Applicant does not mention about similar RTL system from company other than Agilent.

RTL allows a close match of retention times on Agilent GC-MS. By making an adjustment to the inlet pressure, the retention times can be closely matched to those of a reference chromatogram. It is then possible to screen samples for the presence of target compounds using a mass spectral database. The Applicant maintained two FLAVOR2 database/libraries (for the retention times and for MS spectra) containing data for more than 409 flavouring compounds (including those listed in Table 1) [8]. These libraries were provided to the CRL.

At first a GC-MS system suitability check is performed using an equal-weight mixture of Linalool, Acetophenone, Benzyl Acetate, Benzyl Alcohol, Hydroxycitronellal. The obtained characteristics of the chromatogram - related to quantitative compositions, peak shapes and elution order - should be comparable with those of the reference chromatogram [9].

Retention times of d-limonene [6] are measured at five inlet pressures (nominal pressure and nominal pressure ±10% and ±20%, respectively) [7] to construct the calibration curve "retention time" *vs.* "inlet pressure". The "nominal" inlet pressure is then interpolated using the Agilent GC-RTL software and the retention time of d-limonene of the "reference" chromatogram (8.3 or 6.7 min for non-polar or polar columns, respectively). This "nominal" inlet pressure is finally used when analysing the samples of interest with an Agilent GC-MS. The retention times of the peaks detected in the chromatograms are compared to those of the reference chromatogram to identify the various compounds detected, using the FLAVOR2 screener database. Further confirmation is performed using the FLAVOR2 mass spectral library [8].

Two sample preparation protocols are described. Solid samples of *mixture of flavouring compounds* are extracted with the Soxhlet or with the Accelerated Solvent Extractor (80%/20% hexane/acetone mixture). The extract is evaporated at vacuum to 50 mL. The solution is filtered on a 0.45 µm nylon filter and injected in the GC-MS [6] at constant "nominal" inlet pressure. Liquid samples of *mixture of flavouring compounds* are diluted (1:1) with acetone and injected in the GC-MS [6] at constant "nominal" inlet pressure. The Applicant provided the typical chromatogram for the *CDG01* of interest (cf. Fig II.2-13 [4]).

In order to demonstrate the transferability of the proposed analytical method (relevant for the method verification), the Applicant prepared a model mixture of flavouring compounds on a solid carrier (containing silicic acid and calcium carbonate) to be identified by two independent expert laboratories. This mixture contained twenty chemically defined flavourings belonging to twenty different chemical groups to represent the whole spectrum of compounds in use as feed flavourings with respect to their volatility and polarity. Both laboratories properly identified all the flavouring compounds in all the formulations [10, 11]. Since the substances of *CDG01* are within the volatility and polarity range of the model mixture tested, the Applicant concluded that the proposed analytical method is suitable to determine qualitatively the presence of the substances from *CDG01* in the *mixture of flavouring compounds.*

Based on the satisfactory experimental evidence provided, the CRL recommends for official control for the qualitative identification in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest (listed in Table 1) the GC-MS-RTL (Agilent specific) method submitted by the Applicant.

Table 1. Retention Time Locked for the flavouring compounds of *CDG01* [3], and d-limonene

FL-no	CAS-no	EU Register name	RTL polar (min)	RTL non-polar (min)
02.004	71-36-3	Butan-1-ol	5.37	2.23
02.005	111-27-3	Hexan-1-ol	10.30	4.59
02.006	111-87-5	Octan-1-ol	16.00	9.30
02.007	143-08-8	Nonan-1-ol	19.00	12.30
02.008	112-53-8	Dodecan-1-ol	27.17	21.70
02.021	111-70-6	Heptan-1-ol	13.34	6.73
02.024	112-30-1	Decan-1-ol	21.90	15.50
02.040	71-41-0	Pentan-1-ol	7.69	3.24
02.078	64-17-5	Ethanol	2.94	1.90
05.001	75-07-0	Acetaldehyde	1.95	1.85
05.002	123-38-6	Propanal	2.29	1.90
05.003	123-72-8	Butanal	2.78	2.10
05.005	110-62-3	Pentanal	3.35	2.70
05.008	66-25-1	Hexanal	4.60	3.40
05.009	124-13-0	Octanal	8.75	7.30
05.010	112-31-2	Decanal	14.50	13.27
05.011	112-54-9	Dodecanal	20.40	19.77
05.025	124-19-6	Nonanal	11.55	10.20
05.031	111-71-7	Heptanal	6.39	5.10
05.034	112-44-7	Undecanal	17.62	16.50
06.001	105-57-7	1,1-Diethoxyethane	2.72	2.94
08.001	64-18-6	Formic acid	26.33	1.85
08.002	64-19-7	Acetic acid	15.18	2.10
08.003	79-09-4	Propionic acid	16.75	2.56
08.005	107-92-6	Butyric acid	19.08	3.45
08.007	109-52-4	Valeric acid	22.00	5.05
08.009	142-62-1	Hexanoic acid	24.60	7.33
08.010	124-07-2	Octanoic acid	30.06	13.18
08.011	334-48-5	Decanoic acid	34.70	19.33
08.012	143-07-7	Dodecanoic acid	39.10	25.30
08.013	112-80-1	Oleic acid	59.50	39.00
08.014	57-10-3	Hexadecanoic acid	48.80	35.50
08.016	544-63-8	Tetradecanoic acid	43.50	30.40
08.028	111-14-8	Heptanoic acid	27.40	10.21
08.029	112-05-0	Nonanoic acid	32.50	16.66
09.001	141-78-6	Ethyl acetate	2.70	2.20
09.002	109-60-4	Propyl acetate	3.28	2.76
09.004	123-86-4	Butyl acetate	4.38	3.50
09.006	142-92-7	Hexyl acetate	8.40	7.59
09.007	112-14-1	Octyl acetate	13.88	13.61

Table 1 (continued)

FL-no	CAS-no	EU Register name	RTL polar (min)	RTL non-polar (min)
09.008	143-13-5	Nonyl acetate	16.79	16.85
09.009	112-17-4	Decyl acetate	19.90	20.17
09.010	112-66-3	Dodecyl acetate	25.30	26.11
09.022	112-06-1	Heptyl acetate	10.97	10.61
09.023	79-20-9	Methyl acetate	2.39	1.90
09.037	140-88-5	Ethyl acrylate	3.56	2.49
09.038	623-42-7	Methyl butyrate	3.42	2.80
09.039	105-54-4	Ethyl butyrate	3.93	3.50
09.042	109-21-7	Butyl butyrate	7.00	7.40
09.044	540-18-1	Pentyl butyrate	9.44	10.17
09.045	2639-63-6	Hexyl butyrate	12.30	13.17
09.046	110-39-4	Octyl butyrate	18.00	19.32
09.059	110-38-3	Ethyl decanoate	18.45	19.74
09.060	123-66-0	Ethyl hexanoate	7.31	7.22
09.061	626-77-7	Propyl hexanoate	9.50	10.13
09.065	540-07-8	Pentyl hexanoate	15.01	16.16
09.066	6378-65-0	Hexyl hexanoate	17.64	19.13
09.069	106-70-7	Methyl hexanoate	6.40	5.54
09.072	109-94-4	Ethyl formate	2.36	1.87
09.093	106-30-9	Ethyl heptanoate	9.90	10.30
09.099	106-33-2	Ethyl dodecanoate	24.14	25.39
09.104	124-06-1	Ethyl tetradecanoate	29.27	31.06
09.107	123-29-5	Ethyl nonanoate	15.54	16.50
09.111	106-32-1	Ethyl octanoate	12.65	13.06
09.121	105-37-3	Ethyl propionate	3.12	2.72
09.134	554-12-1	Methyl propionate	2.71	2.46
09.147	539-82-2	Ethyl valerate	5.35	5.10
09.148	591-68-4	Butyl valerate	9.73	10.08
09.191	2396-83-0	Ethyl hex-3-enoate	9.18	7.43
09.192	111-62-6	Ethyl oleate	38.81	39.56
09.193	628-97-7	Ethyl hexadecanoate	33.80	36.00
09.248	623-70-1	Ethyl trans-2-butenoate	5.85	4.14
09.274	627-90-7	Ethyl undecanoate	21.57	22.77
09.409	7452-79-1	Ethyl 2-methylbutyrate	4.01	4.25
09.413	97-62-1	Ethyl isobutyrate	3.21	3.16
09.447	108-64-5	Etyhl isovalerate	4.22	4.36
09.449	109-19-3	Butyl isovalerate	7.75	8.83
09.462	556-24-1	Methyl isovalerate	3.82	3.43
09.478	2349-07-7	Hexyl isobutyrate	10.20	11.83
09.483	868-57-5	Methyl 2-methylbutyrate	3.60	3.46
09.499	25415-62-7	Pentyl isovalerate	10.26	11.64

Table 1 (continued)

FL-no	CAS-no	EU Register name	RTL polar (min)	RTL non-polar (min)
09.507	10032-15-2	Hexyl 2-methylbutyrate	12.71	14.56
09.512	77-93-0	Triethyl citrate	38.40	26.55
09.519	15706-73-7	Butyl 2-methylbutyrate	7.40	8.46
09.529	10032-13-0	Hexyl isovalerate	13.31	14.76
09.549	2177-77-7	Methyl 2-methylvalerate	4.92	4.69
01.045	5989-27-5	d-Limonene (standard)	8.33	6.70

FL-no: EU Flavour Number; RTL: Retention Time Locked

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *feedingstuffs* and *water*, no methods could be evaluated. Therefore the CRL is unable to recommend a method for the official control to identify the *active substance(s)* of interest (cf. Table 1) in *feedingstuffs* or *water*.

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

The CRL recommends for official control the Agilent specific method submitted by the Applicant, for the identification of the 86 *flavouring compounds* of the *CDG01* in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest.

The Applicant provided no experimental data for *feedingstuffs* and *water*, therefore the CRL is unable to recommend a method for the identification of the 86 *flavouring compounds* of the *CDG01* in *feedingstuffs* and *water*.

Recommended text for the register entry (analytical method)

For the identification of 86 *flavouring compounds* in mixtures of flavourings:

Gas-chromatography mass spectrometry with retention time locking (GC-MS-RTL)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Chemically Defined Flavourings – Group 01* (*CDG01*) have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/013-2010

[2] Commission Regulation (EC) No 1565/2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council

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

[4] *Technical dossier, Section II – Sect_II_Identity.pdf: 2.1. Identity of the additives - 2.5. Conditions of use of the additive – 2.6. Method of analysis and reference samples

[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] *Technical dossier, Section II – Annex_II_05_FFAC 2008 GCMS method.pdf "GC/MS method for the identification and assay of feed flavourings"

[7] *Technical dossier, Section II – Annex_II_07_RTL Lock.pdf

[8] *Technical dossier, Section II – Annex_II_06_Flavour RTL.pdf

[9] *Technical dossier, Section II – Annex_II_04_Methods assay.pdf

[10] #Supplementary Information – Analytical report Pancosma.pdf

[11] #Supplementary Information – Analytical report Phytosynthese.pdf

* Refers to Dossier No. FAD-2010-0015

Refers to Dossier No. FAD-2009-0050

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community 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

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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)