EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory
Feed Additives

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

Subject: Addendum of the EURL evaluation report

Reference: FAD-2010-0221 Botanically defined flavourings group BDG 02 - Apiales and Austrobaileyales (JRC.F.5/CvH/MGH/AS/Ares(2022)7541548)

Following the priorities expressed by EFSA, the EURL evaluated the methods of analysis provided by the Applicant for nine *feed additives* from the group, namely: *dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture* and *ferula assa-foetida oil* from the grouped application *botanically defined flavourings group 02 (BDG 02) - Apiales and Austrobaileyales*, and recently issued a partial EURL evaluation report [1].

In this addendum the EURL evaluated the new supplementary information provided by the Applicant related to the methods of analysis proposed for official control for the remaining eleven *feed additives* of the group application mentioned above and namely: *celery seed oil, caraway seed oil, coriander oil, taiga root tincture, fennel oil, common ivy extract (sb), ginseng tincture, anise oil, anise star oil, anise star terpenes* and *omicha tincture.*

The recommendations included of this addendum for the eleven *feed additives* listed above, together with the ones for the other nine *feed additives* stated in the previous report [1] complete the EURL evaluation for the grouped application of the *botanically defined flavourings group 02 (BDG 02) - Apiales and Austrobaileyales.*

Due to the intrinsic nature of the *BDG 02*, the accurate quantification of the *feed additives* in *premixtures* and *compound feed* is not achievable experimentally. Furthermore, the Applicant did not provide any experimental data to determine the *feed additives* in *water*. Therefore, the EURL cannot evaluate or recommend any method for official control to quantify the *feed additives* in *premixtures*, *compound feed* and *water.*

The corresponding phytochemical marker(s) for the eleven *feed additives* subject of this addendum of the EURL evaluation report are presented in Table 1, as stated in the respective analytical methods proposed for each of the *feed additives*.

Table 1. Specifications for additives belonging to the botanically defined group BDG 02 subject of this addendum to the original evaluation report [1, 2]

ID number	Additive	Plant species	Description	Phytochemical marker
API004a1	Celery seed oil	*Apium graveolens* L.	Brownish yellow to amber liquid	Limonene
API006a	Caraway seed oil	*Carum carvi L.=Apium carvi L.*	Clear, colourless to yellowish liquid	Carvone
				Limonene
API008a	Coriander oil	*Coriandrum sativum* L.	Clear, colorless to yellowish liquid	Linalool
API011f	Taiga root tincture	*Eleutherococcus senticosus* Rupr. Et Maxim.=Acantho	Brown green powder	Eleutheroside B
				Eleutheroside E
API012a	Fennel oil	*Foeniculum vulgare* Mill.	Pale yellow, clear liquid	Trans-anethole
				Fenchone
API013c	Common ivy extract (sb)	Hedera helix L.	Clear, greenish brown to brown liquid	Hederacoside C
API015f	Ginseng tincture	Panax ginseng C. A. Mey.	Light yellow liquid	Ginsenoside-Rg1
				Ginsenoside-Rb1
API017a	Anise oil	Pimpinella anisum L.	Colourless to yellowish clear liquid	Trans-anethole
AUS001a	Anise star oil	Illicium verum, Anisum stellatum	Clear, colourless to yellowish liquid	Trans-anethole
AUS001h	Anise star terpenes	Illicium verum, Anisum stellatum	Colourless to pale yellow liquid	Trans-anethole
AUS002f	Omicha tincture	Schisandra chinensis (Turcz.) Baill	Brownish liquid	Schisandrin
				Schisandrin A

For *celery seed oil, caraway seed oil, coriander oil, fennel oil, anise oil, anise star oil* and *anise star terpenes* the Applicant proposed the quantification of their respective phytochemical markers, by gas chromatography coupled with flame ionisation detection (GC-FID) methods, based on different available ISO standards or European Pharmacopeia monographs while for the *taiga root tincture, common ivy extract (sb)* and *omicha tincture* the Applicant proposed the quantification of their respective phytochemical markers by high performance liquid chromatography coupled with photometric detection (HPLC-UV) methods based on different European Pharmacopeia monographs.

Celery seed oil

According to the Applicant, *celery seed oil* is an essential oil obtained by steam distillation of the seeds from the plant species *Apium graveolens* L. with a content of *limonene* (phytochemical marker) ranging from 35 to 79 % (expressed as the relative individual peak area in the chromatogram) [3].

For the quantification of *limonene* in *celery seed oil* the Applicant proposed a GC-FID method based on the standard ISO 3760 – Oil of celery seed (*Apium graveolens* L.) [4].

Furthermore, the description of the product stated in the ISO 3760 standard corresponds to the one presented by the Applicant in the proposed consolidated specifications [3].

The Applicant provided experimental data for the analysis of the phytochemical marker (*limonene)* in seven batches of *celery seed oil* from two different geographical origin applying the method described in ISO 3760 leading to average contents which are the range of *limonene* stated in the ISO 3760 standard. In addition, the Applicant provided a typical chromatogram of *celery seed oil* demonstrating a good separation of the marker [5].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 3760 standard for the quantification of (*limonene)* (phytochemical marker) in *celery seed oil*.

Caraway seed oil

According to the Applicant, *caraway seed oil* is obtained from *Carum carvi L.=Apium carvi L.)* and have *limonene* and *carvone* as phytochemical markers [2].

For the determination of the phytochemical markers *limonene* and *carvone* in the *feed additive* (*caraway seed oil*) the Applicant submitted a GC-FID method based one described in the European Pharmacopeia monograph 01/2008:1817 for Caraway oil [6].

The Applicant provided experimental data for the analysis of the phytochemical markers *limonene* and *carvone* in one batch of *caraway seed oil* applying the method described in the European Pharmacopeia monograph 01/2008:1817 for Caraway oil leading to a *limonene* and *carvone* (phytochemical markers) contents of 44 and 54 %, respectively (expressed as the relative individual peak area in the chromatogram) [7]. In addition, the Applicant presented a typical chromatogram of *caraway seed oil* demonstrating a good separation of the markers [7].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the European Pharmacopoeia monograph 01/2008:1817 for Caraway oil, for the quantification of *limonene* and *carvone* (phytochemical markers) in *caraway seed oil*.

Coriander oil

According to the Applicant, *coriander oil* is an essential oil obtained by steam distillation of the fruits from *Coriandrum sativum* L. with a content of *linalool* (phytochemical marker) ranging from 65 to 78 % (expressed as the relative individual peak area in the chromatogram) [8].

For the quantification of *linalool* in *coriander oil* the Applicant proposed a GC-FID method based on the standard ISO 3516 – Oil of coriander fruits (*Coriandrum sativum* L.) [9].

Furthermore, the description of the product and the range of *linalool* stated in the ISO 3516 standard correspond to the range of the phytochemical marker as declared by the Applicant in the proposed consolidated specifications [8].

In addition, the Applicant presented a typical chromatogram of *coriander oil* demonstrating a good separation of the marker [10]. Moreover, the Applicant analysed the phytochemical marker (*linalool*) in five different batches of *coriander oil* leading to a content ranging from 67 to 76 % [8].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 3516 standard for the quantification of *linalool* (phytochemical marker) in *coriander oil*.

Taiga root tincture

According to the Applicant, *taiga root tincture* is a tincture obtained by extraction of dried roots of *Eleutherococcus senticosus* (Rupr. & Maxim.) with contents of *syringaresinol-di-O-glucoside* and *sinapyl alcohol 4-O-glucoside* (phytochemical markers) ranging from 0.0001 to 0.005 % [11]. According to the Applicant, constituents isolated from the roots of *Eleutherococcus senticosus* are often referred in literature as “eleutherosides” (e.g. *eleutheroside E* for *syringaresinol-di-O-glucoside* and *eleutheroside B* for *sinapyl alcohol 4-O-glucoside*) [11].

For the determination of *eleutheroside E* and *eleutheroside B* (phytochemical markers) in *taiga root tincture* the Applicant submitted a HPLC-UV method [12] based on the method described in the European Pharmacopeia monograph 01/2008:1419 for Eleutherococcus [13]. Additionally, the Applicant claimed the equivalence of both methods [12].

The *taiga root tincture* is extracted in a heated ultrasonic bath with a mixture of water and alcohol. The supernatant is then filtrated through paper and further microfiltrated before being injected into the HPLC system. *Eleutheroside B* and *eleutheroside E* (phytochemical markers) are determined by reversed-phase HPLC using a gradient elution and detected at 220 nm [12].

The contents of *eleutheroside B* and *eleutheroside E* are calculated on basis of a ferulic acid external calibration curve and applying the appropriate response factors as stated in the European Pharmacopeia monograph for Eleutherococcus [12-13].

Moreover, the Applicant analysed the phytochemical markers (*eleutheroside E* and *eleutheroside B*) in five different batches of *taiga root tincture* leading to average contents of 14.2 µg / ml (0.0014 %) and 10.6 µg / ml (0.0011 %), respectively [11].

Based on the experimental evidences provided, the EURL recommends for official control the HPLC-UV method based on the European Pharmacopeia monograph 01/2008:1419 for Eleutherococcus for the quantification of *eleutheroside E* and *eleutheroside B* (phytochemical markers) in *taiga root tincture*.

Fennel oil

According to the Applicant, *fennel oil* products (bitter and sweet) are essential oils obtained by steam distillation of the seeds and stems of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare (fennel oil bitter)* or from the seeds of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *dulce (fennel oil sweet)* with contents of *1-methoxy-4-(prop-1(trans)-enyl)benzene (trans-anethole)* and *fenchone* (phytochemical markers) ranging from 15 to 30 % and from 7 to 16 %, respectively for *fennel oil bitter*, and ranging from 60 to 82 % and from 1 to 20 %, respectively for *fennel oil sweet* (expressed as the relative individual peak area in the chromatogram) [14].

For the determination of *trans-anethole* and *fenchone* (phytochemical markers) in the *feed additive* (*fennel oil*) the Applicant proposed two GC-FID methods based on i) the international standard "ISO 17412 – Oil of bitter fennel (*Foeniculum vulgare* Mill. ssp. *vulgare var. vulgare* L.)" [15] for the *fennel oil bitter* and on ii) the French Norm “NF T 75-257 – Huille essentielle de fenouil doux (*Foeniculum vulgare* Miller spp. *vulgare* var. *dulce* (Miller) Thelung)” [16] for the *fennel oil sweet* [17].

In addition, the Applicant presented typical chromatograms of *fennel oil bitter* and *fennel oil sweet* demonstrating a good separation of the markers [17]. Moreover, the Applicant analysed the phytochemical markers (*trans-anethole* and *fenchone*) in five different batches of *fennel oil bitter* and *fennel oil sweet* leading to mean contents for *trans-anethole* and *fenchone* of 25 % and 13 % , respectively for *fennel oil bitter* and of 78 % and 5 % , respectively for *fennel oil sweet* [14].

Given the performance characteristics and data currently available, the EURL recommends for official control for the quantification of *trans-anethole* and *fenchone* (phytochemical markers) the GC-FID methods based on the ISO 17412 standard for *fennel oil bitter* and on the NF T 75-257 for *fennel oil sweet.*

Common ivy extract (sb)

According to the Applicant, *common ivy extract* is an extract obtained from *Hedera helix* L. having *hederacoside C* as phytochemical marker [2].

For the determination of *hederacoside C* in *common ivy extract* the Applicant submitted a HPLC-UV method based on the one described in the European Pharmacopeia monograph 01/2008:2148 for Ivi Leaf [18]. The Applicant applied the mentioned method to the *feed additive* (*common ivy extract)* and provided experimental evidences of their applicability [19].

The *common ivy extract* is prepared according to the Ph. Eur. procedure using ethanol as the extraction solvent. The obtained solution supernatant is then diluted with water before being injected into the HPLC system. *Hederacoside C* is determined by reversed-phase HPLC using a gradient elution and detected at 205 nm. The amount of *hederacoside C* is then calculated on basis of a *hederacoside C* external calibration curve [19].

Moreover, the phytochemical marker (*hederacoside C*) was analysed in one batch of *common ivy extract* leading to a content of 2.5 % [20].

Based on the experimental evidences provided, the EURL recommends for official control the HPLC-UV method based on the European Pharmacopeia monograph 01/2008:2148 for Ivi Leaf for the quantification of *hederacoside C* (phytochemical marker) in *common ivy extract*.

Ginseng tincture

According to the Applicant, *ginseng tincture* is obtained from *Panax ginseng C. A. Mey* having *ginsenoside-Rg1* and *ginsenoside-Rb1* as phytochemical markers [2].

For the determination of *ginsenosides* (*ginsenoside-Rg1* and *ginsenoside-Rb1)* in *ginseng tincture* the Applicant submitted a HPLC-UV method described in the European Pharmacopeia monograph 01/2008:1523 for Ginseng [21]. The Applicant applied the mentioned method to the *feed additive* (*ginseng tincture)* and provided experimental evidences of their applicability [22].

The sample is extracted in a heated ultrasonic bath with a mixture of water and alcohol. The supernatant is then filtrated through paper and further microfiltrated before being injected into the HPLC system. *Ginsenoside-Rg1* and *ginsenoside-Rb1* are separated and determined by reversed-phase HPLC using a gradient elution and detected at 203 nm. The contents of *ginsenoside-Rg1* and *ginsenoside-Rb1* are calculated on basis of their respective external standard calibration curves [22].

Moreover, the Applicant analysed the phytochemical markers (*ginsenoside-Rg1* and *ginsenoside-Rb1*) in one batch of *ginseng tincture* leading to average contents of 165.7 μg / ml and 113.9 μg / ml respectively [23].

Based on the experimental evidences provided, the EURL recommends for official control the HPLC-UV method based on the European Pharmacopeia monograph 01/2008:1523 for Ginseng for the quantification of *ginsenoside-Rg1 and ginsenoside-Rb1* (phytochemical markers) in *ginseng tincture.*

Anise oil

According to the Applicant, *anise oil* is an essential oil obtained by steam distillation of the fruits of from the plant species *Pimpinella anisum* L with a content of *1-methoxy-4-(prop-1(trans)-enyl)benzene (trans-anethole)* (phytochemical marker) ranging from 85 to 96 %, (expressed as the relative individual peak area in the chromatogram) [24].

For the determination of *trans-anethole* (phytochemical marker) the *feed additive* (*anise oil*) the Applicant proposed a GC-FID method based on the international standard "ISO 3475 – Essential oil of aniseed (*Pimpinella anisum* L)" [25].

The Applicant provided experimental data for the analysis of the phytochemical marker (*trans-anethole)* in five different batches of *anise oil* applying the method described in ISO 3475 leading to an averaged content of 92.6 % [26], which is within the range of *trans-anethole* stated in the ISO 3475 standard.

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 3475 standard for the quantification of *trans-anethole* (phytochemical marker) in *anise oil.*

Anise star oil

According to the Applicant, *anise star oil* is an essential oil obtained from *Illicium verum, Anisum stellatum* with a content of *1-methoxy-4-(prop-1(trans)-enyl) benzene (trans-anethole)* (phytochemical marker) ranging from 80 to 95 %, (expressed as the relative individual peak area in the chromatogram) [2].

For the determination of the phytochemical marker *1-methoxy-4-(prop-1(trans)-enyl) benzene (trans-anethole)* in the *feed additive* (*anise star oil*) the Applicant proposed a GC-FID method based on the international standard "ISO 11016 Oil of star anise, Chinese type (*Illicium verum* Hook. f.)" [27].

The Applicant provided experimental data for the analysis of the phytochemical marker (*trans-anethole)* in one batch of *anise star oil* applying the method described in ISO 11016 leading to an averaged content of 88.9 % (expressed as the relative individual peak area in the chromatogram), which is within the ranged stated in the ISO 11016 [27]. Additionally the Applicant provided a typical chromatogram of *anise star oil* demonstrating a good separation of the marker [28].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 11016 standard for the quantification of *trans-anethole* (phytochemical markers) in *anise star oil.*

Anise star terpenes

According to the Applicant, *anise star terpenes* is obtained from *Illicium verum, Anisum stellatum* having *1-methoxy-4-(prop-1(trans)-enyl) benzene (trans-anethole)* as phytochemical marker [2].

For the determination of the phytochemical marker *1-methoxy-4-(prop-1(trans)-enyl) benzene (trans-anethole)* in the *feed additive* (*anise star terpenes*) the Applicant proposed a GC-FID method based on the international standard "ISO 11016 Oil of star anise, Chinese type (*Illicium verum* Hook. f.)" [27].

The Applicant provided experimental data for the analysis of the phytochemical marker (*trans-anethole)* in three batches of *anise star terpenes* applying the GC-FID method leading to a *trans-anethole* content ranging from 7.3 to 8.5 % as well as a typical chromatogram of *anise star oil* demonstrating a good separation of the marker [29].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 11016 standard for the quantification of *trans-anethole* (phytochemical markers) in *anise star terpenes.*

Omicha tincture

According to the Applicant, *omicha tincture* is obtained from *Schisandra chinensis (Turcz.) Baill* having *schisandrin* and *schisandrin A* as phytochemical markers [2].

For the determination of *schisandrin* and *schisandrin A (deoxysandrin)* in *omicha tincture* the Applicant submitted a HPLC-UV method described in the European Pharmacopeia monograph 01/2009:2428 for Schisandra fruit [30].–The Applicant applied the mentioned method to the *feed additive* (*omicha tincture)* and provided experimental evidences of their applicability [31].

The sample is extracted in a heated ultrasonic bath with a mixture of water and alcohol. The supernatant is then filtrated through paper and further microfiltrated before being injected into the HPLC system. *Schisandrin* and *schisandrin A (deoxysandrin)* are separated and determined by reversed-phase HPLC using a gradient elution and both are detected at 250 nm. The amounts of *schisandrin* and *schisandrin A (deoxysandrin)* are calculated on basis of their respective standard calibration curves [31].

Moreover, the Applicant analysed the phytochemical markers (*schisandrin* and *schisandrin A*) in one batch of *omicha tincture* leading to contents of 7.9 µg / ml and 479.5 µg / ml, respectively [32].

Based on the experimental evidences provided, the EURL recommends for official control the HPLC-UV method based on the European Pharmacopeia monograph 01/2009:2428 for Schisandra fruit for the quantification of *schisandrin* and *schisandrin A* (phytochemical markers) in *omicha tincture*.

Recommended text for the registry entry (analytical method)

For the determination of *limonene* (phytochemical marker) in the *feed additive (celery seed oil), limonene* and *carvone* (phytochemical markers) in the *feed additive (caraway seed oil), linalool* (phytochemical marker) in the *feed additive (coriander oil), trans-anethole* and *fenchone* (phytochemical markers) in the *feed additive (fennel oil)* and *trans-anethole* (phytochemical marker) in the *feed additives (anise oil, anise star oil* and *anise star terpenes)*:

– gas chromatography coupled to flame ionisation detection (GC-FID)

For the determination of *eleutheroside B* and *eleutheroside E* (phytochemical markers) in the *feed additive* (*taiga root tincture), hederacoside C* (phytochemical marker) in the *feed additive (common ivy extract (sb)), ginsenoside-Rg1* and *ginsenoside-Rb1* (phytochemical markers) in the *feed additive (ginseng tincture)* and *schisandrin and schisandrin A* (phytochemical markers) in the *feed additive (omicha tincture)*:

– high performance liquid chromatography coupled with photometric detection (HPLC-UV)

References

[1] EURL Report FAD-2010-0221 Botanically defined flavourings group BDG 02 - Apiales and Austrobaileyales JRC.F.5/CvH/MGH/AS/ Ares(2022)7541548

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

[3] Supplementary information: 2022-04-08-SIn_reply_celery_seed_oil.pdf

[4] ISO 3760:2002 – Oil of celery seed (Apium graveolens L.)

[5] Supplementary information: 2022_04_08_EURL_appendix_celery_seed_oil.pdf

[6] European Pharmacopoeia monograph 01/2008:1817 for Caraway oil,

[7] Supplementary information: 20220617_Conf_EURL_appendix_caraway_seed_oil.pdf

[8] Supplementary information: 2020-11-27-SIn-reply-coriander-oil.pdf

[9] ISO 3516:1997 – Oil of coriander fruits (*Coriandrum sativum* L.)

[10] Supplementary information: 2020-11-27_EURL_appendix_coriander_oil.pdf

[11] Supplementary information: 20220822_SIn_reply_taiga_root_tincture.pdf

[12] Supplementary information: SIN2012-Annex_I_HPLC_B02-2_Description.pdf

[13] European Pharmacopoeia monograph 01/2008:1419 for Eleutherococcus,

[14] Supplementary information: : 20210907_SIn_reply_fennel_oil.pdf

[15] ISO 17412 – Oil of bitter fennel (Foeniculum vulgare Mill. ssp. vulgare var. vulgare L.

[16] NF T 75-257 – Huille essentielle de fenouil doux (Foeniculum vulgare Miller spp. vulgare var. dulce (Miller) Thelung

[17] Supplementary information: 20210907_EURL_appendix_fennel_oil.pdf

[18] European Pharmacopoeia monograph 01/2008:2148 for Ivi Leaf,

[19] Supplementary information: SIN2012- Annex_II_HPLC_B02-4_Matrix_Premix.pdf

[20] Supplementary information: 2009-16-12_DrSchaetteGmbH_CoA-1_API013c_Common ivy extract.pdf

[21] European Pharmacopoeia monograph 01/2008:1523 for Ginseng

[22] Supplementary information: SIN2012 Annex_II_HPLC_B02-3_Matrix tincture.pdf

[23] Supplementary information: 2010-01-02_Greencoat Ltd_CoA-1_API.015_Ginseng tincture.pdf

[24] Supplementary information: 20210218_SIn_reply_anise_oil.pdf

[25] ISO 3475:2020– Essential oil of aniseed (Pimpinella anisum L)

[26] Supplementary information: 2022-11-02-EURL_appendix_anise_oil.pdf

[27] ISO 11016:1999 – Oil of star anise, Chinese type (Illicium verum Hook. f.)

[28] Supplementary information: 2022-11-02-EURL_appendix_anise_star_oil.pdf

[29] Supplementary information: 2022-11-02-EURL_appendix_anise_star_terpenes.pdf

[30] European Pharmacopoeia monograph 01/2009:2428 for Schisandra fruit

[31] Supplementary information: SIN2012 Annex_II_HPLC_B02-5_Matrix tincture.pdf

[32] Supplementary information: 2010-01-02_Greencoat Ltd_CoA-1_AUS.002_Schisandra tincture.pdf

*Refers to Dossier no: FAD-2010-0221

Acknowledgements

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

- Prepared by María José González de la Huebra
- Reviewed and approved by Stefano Bellorini and Christoph von Holst (EURL-FA), respectively, Geel, 13/12/2022

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)
Food and Feed Compliance

European Union Reference Laboratory
Feed Additives

JRC F.5/CvH/MGH/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 02 (Apiales and Austrobaileyales)

(FAD-2010-0221; CRL/100174)

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-2010-0221 - CRL/100174**
Name of Product(s) / Feed Additive(s):	***Dill herb oil, dill tincture, dong quai tincture, celery seed oil, caraway seed oil, coriander oil, cumin oil, taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), ginseng tincture, parsley tincture, anise oil, anise tincture, ferula assa-foetida oil, anise star oil, anise star tincture, anise star terpenes and omicha tincture from botanically defined flavourings Group (BDG 02) - Apiales and Austrobaileyales***
Phytochemical Marker (s):	**Carvone, ferulic acid, chlorogenic acid, limonene, linalool, cuminaldehyde, alpha-pinene, anethole, fenchone, anisaldehyde, apiole, myristicin, sec-butyl propenyl disulfide, eleutheroside B, eleutheroside E, hederacoside C, ginsenoside, schisandrin and schisandrin A**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Stefano Bellorini** **27/10/2022**
Report approved by: Date:	**Christoph von Holst** **27/10/2022**

EXECUTIVE SUMMARY

In the current grouped application an authorisation is sought under Articles 4 and 10 for *dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, ferula assa-foetida oil, anise star oil, anise star tincture, anise star terpenes* and *omicha tincture* from *botanically defined flavourings group 02 (BDG 02) - Apiales and Austrobaileyales*, under the category/functional group 2(b) 'sensory additives'/flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003 for all animal species. The application for nine *feed additives* included in the original dossier namely: *dill seed extract, celery seed extract (oleoresin), celery tincture, caraway oleoresin/extract, opoponax oil, ajowan oil, parsley oil, taiga root extract (sb)* and *hares ear tincture* has been withdrawn since the original submission and thus will not be subject of any EURL evaluation.

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

Following the priorities expressed in the EFSA request, this EURL report will focus exclusively on the evaluation of the suitability of analytical methods for official control of nine of the above listed *feed additives*, namely: *dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture* and *ferula assa-foetida oil.*

For the determination of the phytochemical markers *carvone* in the *dill herb oil*; *alpha-pinene* and *cuminaldehyde* in *cumin oil* and *(E)-sec-butyl propenyl disulfide* in *ferula assa-foetida oil* the Applicant proposed different gas chromatography coupled with flame ionisation detection (GC-FID) methods based on relevant ISO standard methods and provided experimental evidences for the suitability of the proposed methods to the mentioned *feed additives*. Based on the evidences provided the EURL recommends for official control the proposed GC-FID methods for the determination of the phytochemical markers *carvone* in the *dill herb oil*; *alpha-pinene* and *cuminaldehyde* in *cumin oil* and *(E)-sec-butyl propenyl disulfide* in *ferula assa-foetida oil.*

For the determination of the phytochemical markers *ferulic acid* and *chlorogenic acid* in the *dong quai tincture* the Applicant proposed a single-laboratory validated and further verified method based on high performance liquid chromatography coupled with diode array detection

(HPLC-DAD). The Applicant provided validation and verification studies demonstrating satisfactory performance characteristics. Based on the experimental evidences provided, the EURL recommends for official control the single-laboratory validated and further verified method based on HPLC-DAD for the quantification of the phytochemical markers *ferulic acid* and *chlorogenic acid* in *dong quai tincture.*

For the characterisation of the *feed additives* namely *anise tincture, star anise tincture, dill tincture, fennel tincture* and *parsley tincture* the Applicant proposed a spectrophotometry method based on the European Pharmacopoeia monograph for the determination of *total polyphenols* and high performance thin layer chromatography (HPTLC) methods for the determination of the *total flavonoids* content in the *feed additives.*

Furthermore, the Applicant also provided additional high performance thin layer chromatography (HPTLC) methods for the determination of *anethole* and *anisaldehyde* in *anise tincture; anethole* in *star anise tincture; carvone* in *dill tincture; anisaldehyde* in *fennel tincture* and *myristicin* and *apiole* in *parsley tincture.*

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of *anise tincture, star anise tincture, dill tincture, fennel tincture* and *parsley tincture.*

Due to the intrinsic nature of the *BDG 02*, the accurate quantification of the *feed additives* in *premixtures* and *feedingstuffs* is not achievable experimentally. Furthermore, the Applicant did not provide any experimental data to determine the *feed additives* in *water*. Therefore, the EURL cannot evaluate or recommend any method for official control to quantify the *feed additives* in *premixtures*, *feedingstuffs* and *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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, ferula assa-foetida oil, anise star oil, anise star tincture, anise star terpenes and *omicha tincture* from *botanically defined flavourings group 02 (BDG 02), apiales and austrobaileyales*, sensory additives, flavouring compounds, all animal species.

1. BACKGROUND

In the current grouped application an authorisation is sought under Articles 4(1) (new product and new use in water) and 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, ferula assa-foetida oil, anise star oil, anise star tincture, anise star terpenes* and *omicha tincture* from *botanically defined flavourings group 02 (BDG 02) - Apiales and Austrobaileyales*, under the category/functional group 2(b) 'sensory additives'/flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003. These authorisations are sought for all animal species [1,2].

The flavouring preparations described in this dossier have a natural origin (botanically defined) and are derived from plant species belonging to the botanical order “Apiales and Austrobaileyales” [4]. The application for nine *feed additives* included in the original dossier namely: *dill seed extract* [5], *celery seed extract (oleoresin)* [5], *celery tincture, caraway oleoresin/extract* [5], *opoponax oil* [5], *ajowan oil* [6], *parsley oil* [7], *taiga root extract (sb)* [8] and *hares ear tincture* [9] has been withdrawn since the original submission, and thus will not be subject of any EURL evaluation.

The *feed additives* are intended to be incorporated into *feedingstuffs* or into *water* directly or through flavouring *premixtures* with no proposed minimum or maximum levels [10]. However, the Applicant suggested the typical maximum inclusion level of the *feed additives* of 25 mg/kg *feedingstuffs* [10].

Following the priorities expressed in the EFSA request, this EURL report will focus exclusively on the evaluation of the suitability of analytical methods for official control of nine of the above listed *feed additives*, namely: *dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture* and *ferula assa-foetida oil.*

The corresponding phytochemical marker(s) for the nine *feed additives* subject of this EURL evaluation report are presented in Table 1, as stated in the respective analytical methods proposed for each of the *feed additives*.

Table 1. Specifications for additives belonging to the botanically defined group BDG 02 subject of this evaluation report [3]

ID number	Additive	Plant species	Description	Phytochemical marker
API001a1	Dill herb oil	*Anethum graveolens* L.	Very light yellow to light yellow, liquid	Carvone
API001f	Dill tincture*	*Anethum graveolens* L	Brown liquid	Carvone
API003f	Dong quai tincture	*Angelica sinensis* (Oliv.) Diels	Brownish liquid	Ferulic acid
				Chlorogenic acid
API009a	Cumin oil	Cuminum cyminum L.	Pale brown, liquid	Cuminaldehyde
				Alpha-pinene
API012f	Fennel tincture*	Foeniculum vulgare Mill.	Brown liquid	Anisaldehyde
API016f	Parsley tincture*	Petroselinum sativum Hoffm.= P. crispum Mill.= P.	Brown liquid	Apiole
				Myristicin
API017f	Anise tincture*	Pimpinella anisum L.	Brown liquid	Anethole
AUS001f	Star anise tincture*	Illicium verum, Anisum stellatum	Brown to yellow liquid	Anethole
API019a	Ferula Assa-foetida oil	Ferula assa-foetida L, extract	Colourless to pale yellow green liquid	(E) sec-butyl propenyl disulfide

*characterisation of the *feed additive*

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 *botanically defined flavourings group BDG 02 - Apiales and Austrobaileyales* 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)

Dill herb oil

Table 2. Performance characteristics of the GC-FID method for the quantification of the phytochemical marker (*carvone*) in the *feed additive* (*dill herb oil*) [12].

	Carvone	
	Batch 1	**Batch 2**
Content, % (relative area)	42.2	42.2
[a]RSDr, %	0.04	0.08
[a]RSDip, %	0.20	0.28

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

[a)]Recalculated by EURL [14]

According to the Applicant *dill herb oil* is an essential oil obtained by steam distillation of the fresh herb (including stalks and leaves harvested in early ripening stages) of *Anethum graveolens* L. with a content of *carvone* (phytochemical marker) ranging from 28 to 45 % (expressed as the relative individual peak area in the chromatogram) [11].

For the quantification of *carvone* in *dill herb oil* the Applicant proposed a gas chromatography coupled with flame ionisation detection (GC-FID) method based on the generic ISO 11024:1998 standard for "Essential oils: General guidance on chromatographic profiles" [12].

The Applicant verified the above mentioned method for the analysis of the phytochemical marker (*carvone)* following the "EURL–FA Validation and verification technical guide for Sensory feed Additives – flavouring compounds 2(b) from botanical origin" [13]. Table 2 shows a summary of the relevant performance characteristics obtained in the verification study. The precision values (relative standard deviations for *repeatability* and for *intermediate precision)* recalculated by the EURL [14] from the verification study, ranged from 0.04 to 0.28 % for the determination of *carvone* in *dill herb oil* [12].

Based on the experimental evidences provided the EURL recommends for official control the GC-FID method based on the generic ISO 11024 standard for the quantification of *carvone* (phytochemical marker) in *dill herb oil*.

Dill tincture

According to the Applicant, *dill tincture* is a hydro alcoholic extract of the seeds of *Anethum graveolens* L. containing *total polyphenols, total flavonoids* and *carvone* as major constituents [15].

For the determination of *total polyphenols* the Applicant proposed a spectrophotometry method based on the European Pharmacopoeia monograph [16].

Table 3. Batch to batch results for *dill tincture* [18]

Batch	total polyphenols (gallic acid equivalent)		total flavonoids (rutin equivalent)		Carvone (phytochemical marker)	
	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)
1	205	0.0199	132	0.0136	22	0.0023
2	229	0.0222	97	0.0100	19	0.0019
3	232	0.0225	129	0.0133	29	0.0030
4	284	0.0276	149	0.0153	26	0.0027
5	322	0.0313	160	0.0164	49	0.0051

The *dill tincture* is diluted and mixed with the Folin-Ciocalteu's reagent and the obtained reaction products are measured at 760 nm. The *total polyphenols* content is then quantified with an external calibration using a gallic acid standard and expressed as gallic acid equivalents [16].

For the determination of *total flavonoids* and *carvone* (phytochemical marker) the Applicant proposed high performance thin layer chromatography (HPTLC) methods [17]. For the *total flavonoids* rutin is used as external calibration standard and the *total flavonoids* content is therefore expressed as rutin equivalents [17].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*dill tincture*) characterised by applying the methods mentioned above [18]. A summary of the results are shown in Table 3. These analyses led to average values of 0.0247 % of tincture for the *total polyphenols*, 0.0137 % of tincture for the *total flavonoids* and 0.0030 % of tincture for *carvone* (phytochemical marker) [18].

Furthermore, the Applicant proposed the use of the HPTLC profiles of *dill tincture flavonoids* as a tool for the unequivocal identification of the *feed additive* [15].

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of the *feed additive (dill tincture).*

Dong quai tincture

According to the Applicant *dong quai tincture* is a tincture obtained by extraction of dried roots of *Angelica sinensis* (Oliv.) Diels with a content of *4-hydroxy-3-methoxycinnamic acid* (*ferulic acid*) and *chlorogenic acid* (phytochemical markers) ranging from 0.001 to 0.01 % and from 0.0001 to 0.01 %, respectively [19].

For the determination of *ferulic acid* and *chlorogenic acid* in *dong quai tincture* the Applicant submitted the general high performance liquid chromatography coupled to photometric detection (HPLC-UV) method for polar phenolics [20].

According to the procedure provided by the Applicant, a filtrated *dong quai tincture* extract is directly injected into the HPLC system. The phytochemical markers (*ferulic acid* and *chlorogenic acid*) are then separated by reversed-phase HPLC using a gradient elution. *Chlorogenic acid* is eluted at 12.4 min *ferulic acid* is eluted at 23.9 min. Both phytochemical markers are quantified at 320 nm by using external standard curves [20].

This method was single-laboratory validated [21] and further verified [22]. In the frame of the validation and verification studies [21, 22] the Applicant reported relative standard deviations for repeatability (RSD_r) ranging from 0.7 to 1.5 % (for *ferulic acid*) and from 0.6 to 1.8 % (for *chlorogenic acid*) and relative standard deviations for *intermediate precision* (RSD_{ip}) of 1.3 to 2.4 % (for *ferulic acid*) and from 1.0 to 1.6 % (for *chlorogenic acid*) as well as and recovery rates of 91 % and 99 % for *chlorogenic acid* and *ferulic acid*, respectively [23]. Furthermore the Applicant stated a limit of quantification (LOQ) of 0.15 and 0.08 µg /ml of the tincture for *chlorogenic acid* and *ferulic acid*, respectively [23] and provided typical chromatograms for *dong quai tincture* that demonstrate the lack of potential interferences for the determination of *chlorogenic acid* and *ferulic acid* in *dong quai tincture* [20].

Moreover, the Applicant analysed the phytochemical marker (*chlorogenic acid* and *ferulic acid*) in 5 different batches of *dong quai tincture* leading to a contents ranging from 0.001 to 0.006 % for *chlorogenic acid* and from 0.003 to 0.007 % for *ferulic acid* [19].

Based on the experimental evidences provided, the EURL recommends for official control the high performance liquid chromatography coupled with diode array detection (HPLC-DAD) method described above for the quantification of *chlorogenic acid* and *ferulic acid* (phytochemical markers) in *dong quai tincture*.

Cumin oil

According to the Applicant *cumin oil* is an essential oil obtained by distillation of the seeds of the plant species *Cuminum cyminum* L with a content of *pin-2(3)-ene (alpha-pinene)* and of *4-isopropylbenzaldehyde (cuminaldehyde)* (phytochemical markers) ranging from 0.3 to 2.0 % and from 15 to 46 %, respectively (expressed as the relative individual peak area in the chromatogram) [24].

For the determination of *cuminaldehyde* and *alpha-pinene* (phytochemical markers) in the *feed additive* (*cumin oil*) the Applicant proposed a gas chromatography coupled to flame ionisation detection (GC-FID) method based on the international standard "ISO 9301 – Oil of cumin seed (*Cuminum cyminum* L.)" [25].

Furthermore, the description of the product and the ranges of *cuminaldehyde* and *alpha-pinene* stated in the ISO 9301 standard correspond to the range of the phytochemical marker as declared by the Applicant in the proposed consolidated specifications [24].

The Applicant provided experimental data for the analysis of both phytochemical markers (*cuminaldehyde* and *alpha-pinene)* in one batch of *cumin oil* applying the method described in ISO 9301 [26].

Given the performance characteristics and data currently available, the EURL recommends for official control the GC-FID method based on the ISO 9301 standard for the quantification of *cuminaldehyde* and *alpha-pinene* (phytochemical markers) in *cumin oil*.

Fennel tincture

According to the Applicant, *fennel tincture* is a hydro alcoholic extract obtained from the seeds of *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* containing. *total polyphenols*, *total flavonoids* and *anysaldehyde* as major constituents [27].

For the determination of *total polyphenols* in *fennel tincture* the Applicant proposed the spectrophotometry method based on the European Pharmacopoeia monograph already described for the *dill tincture* [16].

For the determination of *total flavonoids* and *anisaldehyde (*phytochemical marker) in *fennel tincture* the Applicant proposed high performance thin layer chromatography (HPTLC) methods [28]. For the *total flavonoids* rutin is used as external calibration standard and the *total flavonoids* content is therefore expressed as rutin equivalents [28].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*fennel tincture*) characterised by applying the methods mentioned above [29]. A summary of the results are shown in Table 4. These analyses led to average values of 0.0586 % of tincture for the *total polyphenols*, 0.0052 % of tincture for the *total flavonoids* and 0.0035 % of tincture for *anisaldehyde (*phytochemical marker) [29].

Furthermore, the Applicant proposed the use of the HPTLC profiles of *fennel tincture flavonoids* as a tool for the unequivocal identification of the *feed additive* [27].

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of the *feed additive (fennel tincture).*

Table 4. Batch to batch results for *fennel tincture* [29]

Batch	total polyphenols (gallic acid equivalent)		total flavonoids (rutin equivalent)		Anisaldehyde (phytochemical marker)	
	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)
1	739	0.0725	63.69	0.0065	34.81	0.0035
2	469	0.0460	59.94	0.0061	29.05	0.0030
3	584	0.0572	53.01	0.0054	29.41	0.0030
4	602	0.0590	45.57	0.0047	36.12	0.0037
5	596	0.0583	35.05	0.0036	43.92*	0.0045*

*value above the authorised range deviation

Parsley tincture

According to the Applicant, *parsley tincture* is a hydro alcoholic extract of the seeds of *Petroselinum crispum (Mill.)* Nyman ex A.W. Hill. containing *total polyphenol* and *total flavonoids* as major constituents [30].

The Applicant proposed to characterise the *parsley tincture* by the determination of *total polyphenols* and *total flavonoids* (active substances) and stated *myristicin* and *apiole* markers of the *feed additive* [30]

For the determination of *total polyphenols* in *parsley tincture* the Applicant proposed the spectrophotometry method based on the European Pharmacopoeia monograph already described for the *dill tincture* [16].

For the determination of *total flavonoids, myristicin* and *apiole* (phytochemical markers) the Applicant proposed high performance thin layer chromatography (HPTLC) methods [31]. For the *total flavonoids* rutin is used as external calibration standard and the *total flavonoids* content is therefore expressed as rutin equivalents [31].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*parsley tincture*) characterised by applying the methods mentioned above [33]. A summary of the results are shown in Table 5. These analyses led to average values of 0.0198 % of tincture for the *total polyphenols*, 0.0085 % of tincture for the *total flavonoids* and 0.0032 % of tincture for *apiole. Myristicin* was detected only in one of the batches leading to a value of 0.0014 % of tincture [33].

Furthermore, the Applicant proposed the use of the HPTLC profiles of *parsley tincture flavonoids* as a tool for the unequivocal identification of the *feed additive* [30].

Table 5. Batch to batch results for *parsley tincture* [33]

Batch	total polyphenols (gallic acid equivalent)		total flavonoids (rutin equivalent)		Apiole (phytochemical marker)		Myristicin (phytochemical marker)	
	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)
1	195	0.0196	114.15	0.0118	20.59	0.0021	ND*	ND*
2	208	0.0202	109.11	0.0112	21.66	0..0022	ND*	ND*
3	209	0.0217	112.89	0.0117	53.94	0.0056	13.67	0.0014
4	189	0.0184	40.07	0.0041	298.2	0.0031	ND*	ND*
5	199	0.0193	36.58	0.0038	30.57	0.0032	ND*	ND*

*Not Detected

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of the *feed additive (parsley tincture).*

Anise tincture

According to the Applicant, *anise tincture* is a hydro alcoholic extract of the seeds of *Pimpinella anisum* L. containing *total polyphenols, total flavonoids* as major constituents [33].

The Applicant proposed to characterise the *anise tincture* by the determination of *total polyphenols* and *total flavonoids,* (active substances) and *anethol*e and *anisaldehyde* (phytochemical markers) [33].

For the determination of *total polyphenols* in *anise tincture* the Applicant proposed the spectrophotometry method based on the European Pharmacopoeia monograph and already described for the *dill tincture* [16].

For the determination of *total flavonoids, anethol*e and *anisaldehyde* (phytochemical markers) the Applicant proposed high performance thin layer chromatography (HPTLC) methods [34]. For the *total flavonoids* rutin is used as external calibration standard and the *total flavonoids* content is therefore expressed as rutin equivalents [34].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*anise tincture*) characterised by applying the methods mentioned above [35]. A summary of the results are shown in Table 6. These analyses led to average values of 0.0414 % of tincture for the *total polyphenols*, 0.0145 % of tincture for the *total flavonoids* 0.0003 % of tincture for *anethol*e and 0.0009 % of tincture for *anisaldehyde* (phytochemical markers) [35].

Table 6. Batch to batch results for *anise tincture* [35]

Batch	total polyphenols (gallic acid equivalent)		total flavonoids (rutin equivalent)		Anethole (phytochemical marker)		Anisaldehyde (phytochemical marker)	
	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)
1	417	0.0431	161.8	0.0167	5.347	0.0005	8.273	0.0008
2	429	0.0443	169.6	0.0175	9.222	0.0009	11.45	0.0012
3	466	0.0477	174.2	0.0178	1.922	0.0002	12.15	0.0012
4	347	0.0357	92.6	0.0095	1.224	0.0001	8.278	0.0008
5	352	0.0362	103.3	0.0106	0.289	< 0.0001	5.783	0.0006

Furthermore, the Applicant proposed the use of the HPTLC profiles of *anise tincture flavonoids* as a tool for the unequivocal identification of the *feed additive* [33].

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of the *feed additive (anise tincture).*

Star anise tincture

According to the Applicant, *star anise tincture* is a hydro alcoholic extract of star anise fruits from *Illicium verum* Hook.f. containing *total polyphenols* and *total flavonoids* as major constituents [36].

The Applicant proposed to characterise the *star anise tincture* by the determination of *total polyphenols* and *total flavonoids* (active substances) and *anethol*e (phytochemical marker) [36].

Table 7. Batch to batch results for *star anise tincture* [38]

Batch	total polyphenols (gallic acid equivalent)		total flavonoids (rutin equivalent)		Anethole (phytochemical marker)	
	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)	(mg/L)	(% of tincture)
1	2107	0.2174	228.63	0.0236	15.44	0.0016
2	2054	0.2113	209.68	0.0216	14.53	0.0015
3	3015	0.3131	225.62	0.0234	40.44	0.0042
4	2739	0.2832	234.94	0.0243	43.83	0.0045
5	2590	0.2690	209.44	0.0217	121.9	0.0127

For the determination of *total polyphenols* in *star anise tincture* the Applicant proposed the spectrophotometry method based on the European Pharmacopoeia monograph already described for the *dill tincture* [16].

For the determination of *total flavonoids* and *anethol*e (phytochemical marker) the Applicant proposed high performance thin layer chromatography (HPTLC) methods [37]. For the *total flavonoids* rutin is used as external calibration standard and the *total flavonoids* content is therefore expressed as rutin equivalents [37].

The Applicant has provided the result of the analysis of five different batches of the *feed additive* (*star anise tincture*) characterised by applying the methods mentioned above [38]. A summary of the results are shown in Table 7. These analyses led to average values of 0.2588 % of tincture for the *total polyphenols*, 0.0229 % of tincture for the *total flavonoids* and 0.0049 % of tincture for *anethol*e (phytochemical marker) [38].

Furthermore, the Applicant proposed the use of the HPTLC profiles of *star anise tincture flavonoids* as a tool for the unequivocal identification of the *feed additive* [36].

Based on the experimental evidences provided the EURL recommends for official control the above mentioned methods based on spectrophotometry and high performance thin layer chromatography (HPTLC) for the characterisation of the *feed additive (star anise tincture).*

Ferula assa-foetida oil

According to the Applicant *ferula assa-foetida oil* is an essential oil obtained by steam distillation of the gum resin from *Ferula assa-foetida* L.with a content of *(E)-sec-butyl propenyl disulfide* (phytochemical marker) ranging from 8 to 25 % (expressed as the relative individual peak area in the chromatogram) [39].

For the quantification of *(E)-sec-butyl propenyl disulfide* (phytochemical marker) in *ferula assa-foetida oil* the Applicant proposed a gas chromatography coupled with flame ionisation detection (GC-FID) method based on a generic ISO 11024:1998 standard for "Essential oils: General guidance on chromatographic profiles" [40].

The Applicant verified the above mentioned method for the analysis of *(E)-sec-butyl propenyl disulfide* (phytochemical marker) following the "EURL–FA Validation and verification technical guide for Sensory feed Additives – flavouring compounds 2(b) from botanical origin" [13]. Table 8 shows a summary of the relevant performance characteristics obtained in the verification study. The precision values (relative standard deviations for *repeatability* and for *intermediate precision*), recalculated by the EURL [41] from the verification study ranged from 0.64 to 1.24 % for the determination of *(E)-sec-butyl propenyl disulfide* in *ferula assa-foetida oil* [40].

Table 8. Performance characteristics of the GC-FID method for the quantification of the phytochemical marker (*(E)-sec-butyl propenyl disulfide*) in the *feed additive* (*ferula assa-foetida oil*) [40].

	(E)-sec-butyl propenyl disulfide	
	Batch 1	**Batch 2**
Content, % (relative area)	20.9	16.5
[a]RSDr, %	0.64	1.24
[a]RSDip, %	0.7	1.24

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and for *intermediate precision, respectively.*
[a)]Recalculated by EURL [41]

Based on the experimental evidences provided the EURL recommends for official control the GC-FID method based on the generic ISO 11024 standard for the quantification of *(E)-sec-butyl propenyl disulfide* in *ferula assa-foetida oil.*

The *feed additives* described in this dossier have a natural origin (botanically defined) and are derived from plant species belonging to the botanical order "Apiales and Austrobaileyales". Consequently, due to their intrinsic nature, the accurate quantification of the *feed additives* in *premixtures* and *feedingstuffs* is not achievable experimentally. Furthermore, the Applicant did not provide any experimental data to determine the *feed additives* in *water*. Therefore, the EURL cannot evaluate or recommend any method for official control to quantify the *feed additives* in *premixtures*, *feedingstuffs* and *water.*

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)

The evaluation of corresponding methods of analysis is not relevant for the present application.

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: (i) the single-laboratory validated and further verified methods based on HPLC-DAD for the determination *ferulic acid* and *chlorogenic acid* in the *dong quai tincture,* (ii) the GC-FID methods based on the relevant ISO standards for the determination of *carvone* in the *dill herb oil*; *alpha-pinene* and *cuminaldehyde* in *cumin oil* and *(E)-sec-butyl propenyl disulfide* in *ferula assa-foetida oil*; (iii) the spectrophotometric method for the determination of *total polyphenols* in *anise tincture, star anise tincture, dill tincture, fennel tincture* and *parsley tincture* (iv) the high performance thin layer chromatography (HPTLC) methods for the determination of *total*

flavonoids in *dill tincture, fennel tincture, parsley tincture, anise tincture* and *star anise tincture; carvone* in *dill tincture; anisaldehyde* in *fennel tincture; myristicin* and *apiole* in *parsley tincture; anethole* and *anisaldehyde* in *anise tincture* and *anethole* in *star anise tincture.*

Recommended text for the register entry (analytical method)

For the determination of *carvone* (phytochemical marker) in the *feed additive (dill herb oil)*; *alpha-pinene* and *cuminaldehyde* (phytochemical markers) in the *feed additive* (*cumin oil*) and *(E)-sec-butyl propenyl disulfide* (phytochemical marker) in the *feed additive (ferula assa-foetida oil)*:

- gas chromatography coupled to flame ionisation detection (GC-FID)

For the determination of *ferulic acid* and *chlorogenic acid* (phytochemical markers) in the *feed additive* (*dong quai tincture*):

- high performance liquid chromatography coupled with diode array detection (HPLC-DAD)

For the characterisation of the *feed additive (dill tincture)*:

- spectrophotometry for the determination of *total polyphenols*
- high performance thin-layer chromatography (HPTLC) for the determination of *total flavonoids* and *carvone*

For the characterisation of the *feed additive (fennel tincture)*:

- spectrophotometry for the determination of *total polyphenols*
- high performance thin-layer chromatography (HPTLC) for the determination of *total flavonoids* and *anisaldehyde*

For the characterisation of the *feed additive (parsley tincture)*:

- spectrophotometry for the determination of *total polyphenols*
- high performance thin-layer chromatography (HPTLC) for the determination of *total flavonoids, myristicin* and *apiole*

For the characterisation of the *feed additive (anise tincture)*:

- spectrophotometry for the determination of *total polyphenols*
- high performance thin-layer chromatography (HPTLC) for the determination of *total flavonoids, anethole* and *anisaldehyde*

For the characterisation of the *feed additive (star anise tincture)*:

- spectrophotometry for the determination of *total polyphenols*
- high performance thin-layer chromatography (HPTLC) for the determination of *total flavonoids* and *anethole*

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *BDG 02 - Apiales and Austrobaileyales* 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. 00128/(10129)/1831/-2010

[2] * Annex I – submission number 1288003294124-1110

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

[4] *Technical dossier, Sect_II_Identity.pdf : introduction

[5] *Supplementary information: withdrawal_partial_EC-1.pdf "Partial withdrawal of applications for various Botanically Defined Groups"; Ref. Ares(2019)1299322 - 26/02/2019

[6] *Supplementary information: withdrawal_partial_EC-2.pdf "Partial withdrawal by FEFANA ASBL concerning the feed additive Trachyspermum ammi (L.) Sprag. et Turr.: Ajowan oil, Botanically defined flavourings from BDG 02 - Apiales/Austrobaileyales "; Ref. Ares (2021)4247018 - 30/06/2021

[7] *Supplementary information: withdrawal_partial_EC-3.pdf "Partial withdrawal by FEFANA ASBL concerning the feed additive Petroselinum sativum Hoffm. = P. crispum Mili. = P. hortense L.: Parsley leaf oil CAS 8000-68-8 FEMA 2836 CoE 326 EINECS 281-677-1 / Parsley seed oil CAS 8000-68-8 CoE 326 EINECS 281-677-1 Botanically defined flavourings from BDG 02 - Apiales/Austrobaileyales" Ref. Ares (2021)4246999 - 30/06/2021

[8] Supplementary information: withdrawal_partial_EC-4.pdf "Partial withdrawal by FEFANA ASBL concerning the feed additive Eleutherococcus senticosus Rupr. et Maxim. = Acanthopanax s. Harms: Taiga root extract (sb), Botanically defined flavourings from BDG 02 - Apiales/Austrobaileyales" Ref. Ares (2021) 247002 - 30/06/2021

[9] Supplementary information: withdrawal_partial_EC-5.pdf "Partial withdrawal by FEFANA ASBL concerning the feed additive Bupleurum rotundifolium L.: Hare's ear tincture, Botanically defined flavourings from BDG 02 - Apiales/Austrobaileyales"; Ref. Ares(2021)4539609 – 13/07/2021

[10] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition

[11] Supplementary information: 2021-10-15-SIn_reply_dill_herb_oil.pdf

[12] Supplementary information: 2021-10-29-EURL_appendix_dill_herb_oil.pdf

[13] EURL-FA Validation and verification technical guide for Sensory feed Additives – flavouring compounds 2(b) from botanical origin

[14] Supplementary information: eurl_anova_dill-herb-oil.pdf

[15] Supplementary information: dill-tincture-section II_Identity-oct20.pdf

[16] European Pharmacopoiea, Chapter 2.8.14 Determination of tannins in herbal drugs

[17] Supplementary information: dill-tincture-Annex_II_2_Methods of analysis.pdf

[18] Supplementary information: dill-tincture-Annex_II_3_Results of analysis.pdf

[19] Supplementary information: 20210907_SIn_reply_dong_quai_tincture.pdf

[20] Supplementary information: BDG02 - EURL_appendix_dong_quai_tincture -Annex_I-IAB-Method-Description-Matrix-tinctures-1.pdf

[21] Supplementary information: BDG02 - EURL_appendix_dong_quai_tincture-Annex_III_IAB_Method_Validation_Report.pdf

[22] Supplementary information: BDG02 - EURL_appendix_dong_quai_tincture -Annex IVa IAB Method Verification Study Reports.pdf

[23] Supplementary information: BDG02 - EURL_appendix_dong_quai_tincture-Annex_IVb_IAB Method_Verification Comparison Reports.pdf

[24] Supplementary information: 20210708_SIn_reply_cumin_oil.pdf

[25] ISO 9301 – Oil of cumin seed (*Cuminum cyminum* L.)

[26] Supplementary information: 20210708_EURL_appendix_cumin_oil.pdf

[27] Supplementary information: fennel-tincture-section II_Identity-oct20.pdf

[28] Supplementary information: fennel-tincture-Annex_II_2_Methods of analysis.pdf

[29] Supplementary information: fennel-tincture-Annex_II_3_Results of analysis.pdf

[30] Supplementary information: parsley-tincture-section II_Identity-oct20.pdf

[31] Supplementary information: parsley-tincture-Annex_II_2_Methods of analysis.pdf

[32] Supplementary information: parsley-tincture-Annex_II_3_Results of analysis.pdf

[33] Supplementary information: anise-tincture-section II_Identity-oct20.pdf

[34] Supplementary information: anise-tincture-Annex_II_2_Methods of analysis.pdf

[35] Supplementary information: anise-tincture -Annex_II_3_Results of analysis.pdf

[36] Supplementary information: star_anise-tincture -section II_Identity-oct20.pdf

[37] Supplementary information: star_anise-tincture-Annex_II_2_Methods of analysis.pdf

[38] Supplementary information: star_anise-tincture-Annex_II_3_Results of analysis.pdf

[39] Supplementary information: 20210713_SIn_reply_ferula assa-foetida_oil.pdf

[40] Supplementary information: 20210713_EURL-appendix-ferula assa-foetida-oil.pdf

[41] Supplementary information: eurl_anova_asafoetida-oil.pdf

*Refers to Dossier no: FAD-2010-0221

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.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Wageningen Food Safety Research (WFSR)[1] (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.