
Extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE)  
(FAD-2017-0047; CRL/170032)

Dossier related to: FAD-2017-0047 - CRL/170032
Name of Product: Extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE)
Active Agent (s): Hops beta-acids
Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA) JRC Geel, Belgium
Report prepared by: Zigmas Ezerskis
Report checked by: Piotr Robouch (EURL-FA) Date: 12/01/2018
Report approved by: Christoph von Holst Date: 19/02/2018
EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for the extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE) 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. Specifically, authorisation is sought for the use of the feed additive for all animal species and categories.

According to the Applicant, the feed additive is a dark brown viscous liquid consisting of 38.5% to 41.5% hops beta-acids as active substance(s) and containing minor amounts of hops alpha-acids, hop oils, and propylene glycol. The maximum moisture content is 8%.

The feed additive is intended to be incorporated into feedingstuffs through premixtures with no proposed minimum or maximum limits. However, the Applicant suggested a maximum inclusion level of 20 mg of hops beta-acids/kg feedingstuffs.

For the quantification of hops beta-acids in the feed additive and premixtures the Applicant submitted a European Brewery Convention (EBC) ring-trial validated method. This method was originally designed for the determination of individual hops alpha- and beta-acids in all hops, in hop powder products and in all conventional hop extracts. This method is based on reversed phase high performance liquid chromatography coupled to UV detection (HPLC-UV) at 313 nm wavelength.

The Applicant applied this HPLC-UV method for the analysis of five batches of the feed additive containing 40% of hops beta-acids and obtained a relative standard deviation for repeatability (RSD_r) of 1.6%, which is in good agreement with the RSD, reported in the frame of the ring-trial validation study published by EBC for hop extracts (2.6 to 4.3%). Based on the acceptable performance characteristics available, the EURL recommends this method for official control to quantify hops beta-acids in the feed additive.

Furthermore, the Applicant applied the above mentioned HPLC-UV method for the analysis of premixtures sample consisting of sprayed BRHE on silica, and reported satisfactory performance characteristics derived from the validation and verification studies. While the sprayed silica is not representative of the various premixtures that may be used, this HPLC-UV method is considered fit-for-purpose for the quantification of hops beta-acids in silica based premixtures.

Since the accurate quantification of hops beta-acids from BRHE added in feedingstuffs is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to quantify hops beta-acids from BRHE added content 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

**KEYWORDS**

*Beta rich hop extract (BRHE), hops beta-acids, sensory additives, all animal species*

1. **BACKGROUND**

In the current application authorisation is sought under article 4(1) (new *feed additive*) for the *extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE)* 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. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories [1, 2].

According to the Applicant, the *feed additive* is a dark-brown viscous liquid consisting of 38.5% to 41.5% *hops beta-acids*, which contains various beta acids as active substances(s) with the main components: colupulone, lupulone and adlupulone, and traces of postlupulone, prelupulone and adprelupulone. In addition, the *feed additive* contains minor amounts of hops alpha-acids, hop oils, and propylene glycol. The maximum moisture content is 8% [3, 4].

The *feed additive* is intended to be incorporated into *feedingstuffs through premixtures* with no proposed minimum or maximum limits. However, the Applicant suggested a maximum inclusion level of 20 mg of *hops beta-acids*/kg *feedingstuffs* [3].

2. **TERMS OF REFERENCE**

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with the *extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE)* and their suitability to be used for official controls in the frame of the authorisation were evaluated.
3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [5].

For the identification/characterisation of the feed additive the Applicant submitted several methods of the European Brewery Convention to analyse (i) hops alpha- and beta-acids by HPLC-UV [6]; (ii) hop oils by steam distillation [7]; (iii) the individual components by GC-FID [8]; and (iv) the VDLUFA method based on GC-FID for the determination of propylene glycol [9]. The EURL considers these methods fit-for-purpose for the identification / characterisation of the feed additive.

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

For the quantification of the active substance (hops beta-acids) in the feed additive and premixtures the Applicant submitted a European Brewery Convention (EBC) ring-trial validated method [6]. This method was originally designed for the determination of individual hops alpha- and beta-acids in all hops, in hop powder products, and in all conventional hop extracts. The method is based on reversed phase high performance liquid chromatography coupled to UV detection (HPLC-UV) at 313 nm.

The feed additive sample (0.5 g) is dissolved by sonication in 40 ml of methanol; adjusted with methanol up to 100 ml. The solution is filtered for further HPLC analysis [6]. The sample of premixtures (10 g) is mixed with 20 ml of methanol, 100 ml of diethyl ether, and 40 ml of 0.1 M HCl solution; the mixture is shaken for 40 min. After the separation of the extract phases, 5 ml of supernatant ether phase is adjusted with methanol up to 50 ml, mixed and filtered for further chromatographic analysis [10]. The quantification of the main components of beta-acids (colupulone, lupulone and adlupulone) is performed spectrophotometrically by external calibration using the International Calibration Extract (ICE) hop standard, with known mass fractions of hops alpha- and beta-acids. The total content of hops beta-acids is expressed as the sum of the main components of beta-acids [6, 10].
Table 1  Performance characteristics of the HPLC-UV method for the quantification of hops beta-acids content in premixtures (BRHE sprayed silica)

<table>
<thead>
<tr>
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<th>Premixtures</th>
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<tbody>
<tr>
<td></td>
<td>Validation</td>
</tr>
<tr>
<td>Mass fraction, %</td>
<td>16.5</td>
</tr>
<tr>
<td>RSD_r, %</td>
<td>0.6 – 1.2</td>
</tr>
<tr>
<td>RSD_ip, %</td>
<td>1.2</td>
</tr>
<tr>
<td>R_rec, %</td>
<td>97</td>
</tr>
<tr>
<td>Reference</td>
<td>[10]</td>
</tr>
</tbody>
</table>

RSD_r and RSD_ip: relative standard deviations for repeatability and intermediate precision, respectively; R_rec - recovery rate.

The Applicant applied this HPLC-UV method for the analysis of five batches of the feed additive containing 40% of hops beta-acids and reported a relative standard deviation for repeatability (RSD_r) of 1.6%, which is in good agreement with the RSD_r (ranging from 2.6 to 4.3%) reported by EBC [6].

Based on the acceptable performance characteristics presented, the EURL recommends for official control the European Brewery Convention (EBC) ring-trial validated method based on reversed phase HPLC-UV for the quantification of hops beta-acids in the feed additive.

Furthermore, the Applicant applied the HPLC-UV method mentioned above for the analysis of hops beta-acids in premixture samples consisting of sprayed beta rich hop extract (BRHE) on silica [10]. The performance characteristics reported in the frame of the validation [10] and verification [11] studies are shown in Table 1. Satisfactory performance characteristics were obtained analysing "simple" samples that may not represent most of the premixtures used. Nevertheless, based on the experimental evidence available, the EURL considers this method fit-for-purpose for the quantification of hops beta-acids in silica based premixtures.

Since the accurate quantification of hops beta-acids from BRHE added in feedingstuffs is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.
4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the European Brewery Convention (EBC) ring-trial validated method based on reversed phase high performance liquid chromatography with UV detection (HPLC-UV) for the quantification of hops beta-acids in the feed additive.

Since the accurate quantification of hops beta-acids from BRHE added in feedingstuffs is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control.

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

For the quantification of hops beta-acids in the feed additive:

- reversed phase high performance liquid chromatography with UV detection (HPLC-UV) – ring-trial validated European Brewery Convention method 7.7

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of extract from supercritical carbon dioxide extraction of Humulus lupulus L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE) 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 SANTE_E5_FWD. APPL. 1831-0039-2017
[2] *Application Form (Annex I), 16/08/2017
[3] *Application, Proposal for Register Entry – Annex A
[4] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis
[6] *Technical dossier, Section II – Annex II_95
[7] European Brewery Convention method 7.10 – Hop Oil Content of Hops and Hop Products
[10] *Technical dossier, Section II – Annex II_96
*Refers to Dossier no: FAD-2017-0047

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:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Instytut Zootechniki – Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- RIKILT Wageningen UR, Wageningen (NL)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)