Subject: Addendum to the EURL evaluation report

References:

FAD-2010-0356 + FAD-2010-376 (*propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700* (mixture containing *sodium benzoate*, *propionic acid* and *sodium propionate*)) – JRC.DG.D.6/CvH/GB/mdS/Ares(2011)722225

Upon the recent publication of new ring-trial validated methods EN 17294 [1] and EN 17298 [2] for the analysis of organic acids in feed additives, premixtures, feed materials, compound feed and water, the EURL under the frame of article 5 of Regulation (EC) No 378/2005 [3] considered appropriate to perform a new evaluation of the methods of analysis for official control of *propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700* in the *feed additives*, *premixtures*, *feedingstuffs* and *water* in the frame of the above-mentioned *feed additive* dossiers. In this line, aiming to recommend the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [4], the EURL also updates in this amendment the relevant methods for the metals (*sodium* and *calcium*) and *ammonium*.

For the determination of *propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700* (as total *propionic acid*) in the *feed additives*, *premixtures*, *feedingstuffs* and *water* the EURL evaluated ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) [1]. This method is designed for the determination of formic, lactic, propionic, citric, fumaric, malic and acetic acids and their salts (as total individual acids) in feed additives, premixtures, feed materials, compound feed and water [1].

According to the method, 5 g of sample is mixed with 100 ml of water and the mixture is stirred for 60 min (or sonicated for 30 min). The resulting extract is filtered using ash free paper filter or centrifuged at 5000 *g* for 3 min. The filtrate or the supernatant after the dilution is filtered through mebrane filter before the chromatographic analysis. The individual analytes are detected by ion conductivity detection and the quantification is performed using an external standard calibration curve prepared from the standard solutions of the above-mentioned acids [1].

Table 1. The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method [1] for the quantification of total *propionic acid* in *premixtures*, *feedingstuffs* (feed material, complimentary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	33115 – 208263	2683 – 12205	995
RSD_r, %	1.3 – 2.6	1.3 – 3.4	1.3
RSD_R, %	6.1 – 15.4	4.9 – 8.9	4.2
Reference	[1]		

RSD_r and RSD_R: relative standard deviations for *repeatability and reproducibility, respectively*.

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method for the quantification of total *propionic acid* in *premixtures*, *feedingstuffs* (feed material, complimentary feed and compound feed) and *water* are presented in Table 1. In addition, a limit of quantification (LOQ) of 200 mg for *propionic* acid /kg *feedingstuffs* is reported [1].

Based on the performance characteristics available and the scope of the method in terms of matrices, the EURL recommends for official control the ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) for the determination of *propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700* (as total *propionic acid*) in the *feed additives*, *premixtures*, *feedingstuffs* and *water*.

For the determination of *sodium benzoate* (as total *benzoic acid*) in the *feed additive* (*E700*), *premixtures*, *feedingstuffs* and *water* the EURL evaluated the ring-trial validated EN 17298 method based on high performance liquid chromatography (HPLC) coupled to spectrophotometric (UV) detection at 230 nm [2]. This method is designed for the determination of benzoic and sorbic acids and their salts (as total individual acids) in feed additives, premixtures, feed materials, compound feed and water (only for benzoic acid).

Table 2. The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17298 method [2] for the quantification of total *benzoic acid* in *premixtures*, *feedingstuffs* (feed material, complimentary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	60121 – 90577	870 – 12668	204
RSD_r, %	1.8	1.0 – 2.5	0.8
RSD_R, %	3.0 – 3.2	1.6 – 5.5	3.1
Reference	[2]		

RSD_r and RSD_R: relative standard deviations for *repeatability and reproducibility, respectively*.

According to the EN 17298 method, 5 g of the sample is mixed with 100 ml of the extraction solution containing acetate buffer (pH 4.6) and methanol (60:40, v/v), and treated in ultrasonic bath for 30 min. The resulting extract is filtered using ash free paper filter or centrifuged at 5000 *g* for 3 min. The filtrate or the supernatant after the dilution with the extraction solution is filtered through membrane filter (0.45 µm) before the chromatographic analysis. The individual analytes are detected by spectrophotometry (UV) at 230 nm and the quantification is performed using an external standard calibration curve prepared from the standard solutions of the above-mentioned acids [2].

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17298 method for the quantification of total *benzoic acid* in *premixtures*, *feedingstuffs* (feed material, complimentary feed and compound feed) and *water* are presented in Table 2. In addition, a limit of quantification (LOQ) of 200 mg for *benzoic* acid /kg *feedingstuffs* is reported [2].

Based on the performance characteristics available and the scope of the method in terms of matrices, the EURL recommends for official control the ring-trial validated EN 17298 method based on high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection for the determination of *sodium benzoate* (as total *benzoic acid*) in the *feed additive* (*E700*), *premixtures*, *feedingstuffs* and *water*.

For the determination of total *sodium*, *calcium* and *ammonium* in the *feed additives* (*propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700*) the EURL evaluated the three ring-trial validated methods, namely i) EN ISO 6869 based on atomic absorption spectrometry (AAS) [5]; ii) EN15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [6]; and iii) ISO 5664 based on distillation and titration of ammonia, respectively [7].

Based on the performance profile of these methods and given the fact that the above-mentioned methods have been recommended for the determination of *sodium*, *calcium* and *ammonium* in similar salts of formic acid [8], the EURL considers these recommendations also valid in the frame of this amendment.

Recommended text for the registry entry (analytical method) (replacing the previous recommendations)

For the determination of *propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700* (mixture containing *sodium benzoate*, *propionic acid* and *sodium propionate*) (as total *propionic* acid) in the *feed additives* (*propionic acid*, *sodium propionate*, *calcium propionate*, *ammonium propionate* and *E700*), *premixtures*, *feedingstuffs* and *water*:

- Ion chromatography with conductivity detection (IC-CD) – EN 17294

For the determination of *sodium benzoate* (as total *benzoic acid*) in the *feed additive* (*E700*), *premixtures*, *feedingstuffs* and *water*:

- High performance liquid chromatography with ultraviolet detection (HPLC-UV) – EN 17298

For the determination of total *sodium* and *calcium* in the *feed additives* (*sodium propionate*, *calcium propionate* and *E700* (mixture containing *sodium benzoate*, *propionic acid* and *sodium propionate*)):

- Atomic absorption spectrometry (AAS) – EN ISO 6869; or
- Inductively coupled plasma-atomic emission spectrometry (ICP-AES) – EN15510

For the determination of total *ammonium* in the *feed additive* (*ammonium propionate*):

- Distillation and titration – ISO 5664

References

[1] EN 17294 Animal feeding stuffs: Methods of sampling and analysis – Determination of organic acids by Ion Chromatography with Conductivity Detection (IC-CD) – Complementary element

[2] EN 17298 Animal feeding stuffs: Methods of sampling and analysis – Determination of benzoic and and sorbic acid by High Performance Liquid Chromatography (HPLC)

[3] Commission Regulation (EC) No 378/2005 of 4 March 2005 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, OJ L 059 5.3.2005, p. 8

[4] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisations of feed additives, OJ L 133 22.5.2008, p. 1

[5] ISO 6869:2000 – Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry

[6] EN 15510:2017 – Animal feeding stuffs: Methods of sampling and analysis – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES

[7] ISO 5664:1984 Water quality - Determination of ammonium - Distillation and titration method

[8] EURL evaluation report: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FormateGroup.pdf

---

Addendum
- Prepared by Zigmas Ezerskis
- Reviewed and approved by María José González de la Huebra and Christoph von Holst (EURL-FA), respectively, Geel, 27/05/2021

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.DG.D.6/CvH/GB/mds/ARES(2011)722225

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

Dossier related to:	FAD-2010-0356 CRL/100049 FAD-2010-0376 CRL/100253
Name of Feed Additive:	-
Active Substance(s):	E 280 (propionic acid), E 281 (sodium propionate), E 282 (calcium propionate), E 284 (ammonium propionate) and E 700 (mixture containing sodium benzoate, propionic acid and sodium propionate)
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Gerhard Buttinger (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 01/07/2011
Report approved by: Date:	Christoph von Holst 04/07/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *propionic acid*[1]*, sodium propionate*[1]*, calcium propionate*[1], *ammonium propionate*[1] *and E700*[2], under the category of "technological additives" functional group 1(a) "preservatives"[1,2] and 1(k) "silage additives" (except *calcium propionate*)[1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant (FAD-2010-0356[1]) *propionic acid (E280), sodium propionate (E281),* and *calcium propionate (E282)* have minimum purities of 98%, while *ammonium propionate (E284)* has a minimum purity of 19 % in a propionic acid matrix. The feed additive E700 related to application FAD-2010-376 is an aqueous solution containing three active substances, namely (1) sodium benzoate at 140 g/kg, (2) propionic acid at 370 g/kg and (3) sodium propionate at 110 g/kg.

Specifically, authorisation is sought by the Applicant[1] for the use of the various *feed additives* (propionic acid, sodium propionate, calcium propionate, ammonium propionate) for all animal species and categories. The *feed additives* are intended to be used in *premixtures*, *feedingstuffs, water* and *silage* (except *calcium propionate*). The Applicant[1] did not propose any minimum or maximum concentration; similarly to what was set in previous regulation. However, the recommended levels are ranging between 0.1 to 6 g/L for *water,* 0.1 to 40 g/kg for *feed* and 1 to 10 g/kg for *silage*.

Specifically, authorisation is sought by the Applicant[2] for the use of the *feed additive E700* for pigs, bovines, poultry, sheep, goats, rabbits and horses. The *feed additive* is intended to be used in *premixtures* and *feedingstuffs*. The Applicant[2] proposes a maximum concentration for the *feed additive* of 10000 mg/kg in complete feedingstuffs and a concentration ranging from 3000 to 22000 mg/kg in cereal.

For the quantification of *ammonium propionate* in the *feed additive* the Applicant[1] proposed to combine two methods: - a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) for the determination of total propionate (expressed as *total propionic acid*); and - an indirect titration with sulphuric acid and sodium hydroxide for the determination of ammonia. No performance characteristics were provided. However, the EURL recommends for official control to combine titration and HPLC-RI methods for the indirect determination of *ammonium propionate* in the *feed additive*.

For the quantification of *propionic acid, sodium propionate, calcium propionate and ammonium propionate* (expressed as *total propionic acid*) in *feed additive*, *premixtures, feedingstuffs* and *water* Applicant[1] proposed a ring-trial validated method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV).

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

This method does not distinguish between *propionic acid* and its salts. The following performance characteristics were reported for the HPLC-RI method:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2 to 17 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 7 to 23 %;
- a recovery rate ranging from 98 to 101 %; and
- a limit of quantification (LOQ) of 0.07 g *propionic acid*/kg *feedingstuffs.*

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-RI to determine *propionic acid, sodium propionate* and *calcium propionate* and *ammonium propionate* (expressed as *total propionic acid*) in *feed additive*, *premixtures, feedingstuffs* and *water*.

For the quantification of *propionic acid, sodium propionate and ammonium propionate* in *silage* the Applicant[1] did not provide any analytical method or experimental data. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *propionic acid, sodium propionate* and *ammonium propionate* in *silage*.

E700 is a mixture of three active substances; it contains of *sodium benzoate, propionic acid* and *sodium propionate.* Therefore, the characterisation of *E700* is derived from the quantification of (i) benzoate, (ii) total propionate and (iii) total sodium amounts. For the determination of *sodium benzoate* in *E700*, *premixtures* and *feedingstuffs* the Applicant[2] proposed a single laboratory validated and further verified method, based on high performance liquid chromatography with UV detection (HPLC-UV). The reported precisions range between 0.4 and 5.4 %. Based on the acceptable performance characteristics presented, the EURL recommends for official control the HPLC-UV method to determine *sodium benzoate* in *E700,* within the concentration range covered by the experimental data. For the determination of total propionate in *E700*, the EURL recommends for official control the above mentioned ring trial validated method, based on ion-exclusion HPLC-RI. For the determination of total sodium in *E700*, the EURL recommends for official control the internationally agreed EN ISO 6869:2000 method, based on atomic absorption spectrometry.

The EURL does not recommend for official control any methods for the determination of *E700* (mixture of three active substances) in *premixtures* and *feedingstuffs.*

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

KEYWORDS
Propionic acid, sodium propionate, calcium propionate, ammonium propionate, sodium benzoate, technological additive, preservatives, silage additives, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) and 10(2) for *propionic acid*[1]*, sodium propionate*[1]*, calcium propionate*[1], *ammonium propionate*[1] *and E700*[2], under the category of "technological additives" functional group 1(a) "preservatives"[1,2] and 1(k) "silage additives" (except *calcium propionate*)[1], according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1, 2].

According to the Applicant[1]:

- *propionic acid (E280)* is a clear liquid with pungent odour and a minimum purity of 99.5 %;
- *sodium propionate (E281)* is a white or colourless hygroscopic crystal with a minimum purity of 98.5 %;
- *calcium propionate (E282)* is a white powder or crystals in the mono or trihydrate form with minimum purity of 98 %; and
- *ammonium propionate (E284)* is a clear to pale liquid with faint odour of ammonia with a minimum purity of 19 % [1,3].

The *feed additive E700*[2] contains three active substances, namely (1) sodium benzoate at 140 g/kg, (2) propionic acid at 370 g/kg and (3) sodium propionate at 110 g/kg [2, 4].

Specifically, authorisation is sought by the Applicant[1] for the use of the various *feed additives* for all animal species and categories [1]. The *feed additives* are intended to be used in *premixtures*, *feedingstuffs, water* and *silage* (except *calcium propionate*) [3]. The Applicant did not propose any minimum or maximum concentration [3]; similarly to what was set in previous regulation [5]. However, the recommended levels are ranging between 0.1 to 6 g/L for *water,* 0.1 to 40 g/kg for *feed* and 1 to 10 g/kg for *silage* [6].

Specifically, authorisation is sought by the Applicant[2] for the use of the *feed additive E700* for pigs, bovines, poultry, sheep, goats, rabbits and horses [2]. The *feed additive* is intended to be used in *premixtures* and *feedingstuffs* [4]. The Applicant[2] proposes a maximum concentration of 10000 mg/kg complete feedingstuffs and a concentration ranging from 3000 to 22000 mg/kg cereal [4]; corresponding to what was set in two Commission Regulations [7, 8], authorising the feed additive.

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *propionic acid, sodium propionate, calcium propionate, ammonium propionate* and *E700 (*containing the active substances *propionic acid, sodium propionate* and *sodium benzoate*), 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

For the identification of *sodium propionate* in *feed additive* the EURL recommends the methods described in the European Pharmacopoeia monograph 2041 [9], based on infrared absorption spectrophotometry and selective precipitation reactions of sodium.

For the identification of *calcium propionate* in *feed additives* the EURL recommends the methods described in the USP Food Chemicals Codex (FCC) monograph 'calcium propionate' [10], based on a selective precipitation reaction of calcium, flame coloration and the formation of an alkaline residue after low temperature ignition.

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury and dioxins) are available from the respective European Union Reference Laboratories [11].

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

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

Propionic acid, sodium propionate, calcium propionate, ammonium propionate

For the quantification of *ammonium propionate* in the *feed additive*, consisting of a minimum of 19 % *ammonium propionate* in a propionic acid matrix, Applicant[1] proposed to combine two methods:

- a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) [12] (described below) for the determination of total propionate;
- an indirect titration with sulphuric acid and sodium hydroxide after distillation of the ammonia [12] for the determination of ammonia.

No performance characteristics were provided. However, the EURL recommends for official control to combine titration and HPLC-RI methods for the indirect determination of *ammonium propionate* in the *feed additive*.

For the quantification of *propionic acid, sodium propionate, calcium propionate and ammonium propionate* (as *total propionic acid* content) in *feed additive*, *premixtures, feedingstuffs* and *water* Applicant[1] proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) [12]. This method does not distinguish between *propionic acid* and its salts.

The sample is extracted with 0.005 M sulphuric acid at a pH ranging from 2 to 3.5. The solution is then centrifuged or filtered and used for the HPLC measurement. After ion-exclusion chromatography, *propionate* is quantified as propionic acid by spectrophotometry at 217 nm or by the refractive index, using external calibration.

The following performance characteristics for the quantification of total propionate, expressed as *total propionic acid*, were derived from the single-laboratory validation study [12]:

- a relative standard deviation for *repeatability* ranging from 2 % to 17 % (for concentrations down to 0.01 mg/kg);
- a limit of quantification (LOQ) of 0.07 g *propionic acid*/kg *feedingstuffs;* and
- a *recovery* rate (R_{rec}) ranging from 98 to 101% when RI detection is used; or ranging from 70 to 114% when UV detection is used. The Applicant did not explain whether the large scatter observed during the experiments with UV detection was due to potential interferences from the matrix, which was not evidenced using the RI detection.

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

Furthermore, the HPLC-UV/RI method was ring trial validated with five laboratories and a relative standard deviation for *reproducibility* (RSD_R) ranging from 7.2 % to 23 % was determined for *premixtures* and *feedingstuffs* containing 5 to 50 g *propionic acid*/kg, respectively [12].

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-RI method to determine *propionic acid, sodium propionate* and *calcium propionate* and *ammonium propionate* (expressed as *total propionic acid*) in *feed additive*, *premixtures, feedingstuffs* and *water*.

For the quantification of *propionic acid, sodium propionate and ammonium propionate* in *silage* the Applicant[1] did not provide any analytical method or experimental data. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *propionic acid, sodium propionate* and *ammonium propionate* in *silage*.

E700

E700 is a mixture of three active substances; it consists of 140 g *sodium benzoate,* 370 g *propionic acid* and 110 g *sodium propionate* per kilogram of product. Therefore, the characterisation of E700 is derived from the quantification of (i) benzoate, (ii) total propionate and (iii) total sodium amounts.

For the quantification of *sodium benzoate* in *E700*, *premixtures* and *feedingstuffs* the Applicant[2] proposed a single laboratory validated and further verified method, based on high performance liquid chromatography with UV detection (HPLC-UV) [13].

The sample is extracted, after addition of an internal standard, with an acetate buffer/methanol mixture. The solution is filtered and directly used for HPLC measurements. *Sodium benzoate* is quantified using an internal standard calibration, after reversed phase chromatography, by spectrophotometry at 235 nm.

The method performance characteristics are summarized in Table 1. Based on the acceptable performance characteristics presented, the EURL recommends for official control the HPLC-UV method to determine *sodium benzoate* in *E700,* within the concentration range covered by the experimental data (200 mg/kg up to 220 g/kg).

Table 1. Validation and verification data for *sodium benzoate* in *feed additive*, *premixtures* and *feedingstuffs* for contents ranging from 200 mg/kg up to 220 g/kg [13]

	E700		premixture		feedingstuff	
	validation	verification	validation	verification	validation	verification
LOQ [mg/kg]					25	7.5
RSD_r [%]	2.0	0.44	2.1	0.38	4.3	5.4
RSD_{ip} [%]	2.0	0.48	2.5	0.38	5.9	5.4

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

For the quantification of total propionate in *E700*, the EURL recommends for official control the above mentioned ring trial validated method, based on ion-exclusion HPLC-RI.

For the quantification of total sodium in *E700*, the EURL recommends for official control the internationally agreed EN ISO 6869:2000 method [14], based on atomic absorption spectrometry.

The quantification of added *E700* in *premixtures* and *feedingstuffs* is only possible when identical matrix samples without added E700 are available, thus allowing corrections for endogenous benzoate, propionate and sodium present in the feed; such cases are not frequent. When the blank (untreated) feed is *not* available, an estimate of "*E700-equivalent*" could be roughly estimated monitoring the total propionate (P) and benzoate (B) concentrations, for which a theoretical ration (P/B) of 3.24 (= [370+84]/140) is expected. However, since the contribution of the various active substances of E700 from other sources than E700 cannot be determined, the EURL does not recommend for official control any methods for the determination of *E700* in *premixtures* and *feedingstuffs.*

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- to combine titration and HPLC-RI methods for the indirect quantification of *ammonium propionate*[1] in the *feed additive*;
- the ring trial validated method based on ion-exclusion HPLC-RI method to quantify *propionic acid*[1]*, sodium propionate*[1], *calcium propionate*[1] and *ammonium propionate*[1] (expressed as *total propionic acid*) in *feed additive*, *premixtures, feedingstuffs* and *water*;
- for the characterisation of the *E700*[2] composition, to combine (i) the single laboratory validated and further verified method using HPLC-UV for quantification of *sodium benzoate;* (ii) the ring trial validated method based on ion-exclusion HPLC-RI method to quantify *sodium propionate* (expressed as *total propionic acid*); and (iii) the EN ISO 6869:2000 standardised atomic absorption spectrometry method to quantify *total sodium* .

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

For the quantification of *propionic acid, sodium propionate and ammonium propionate* in *silage* the Applicant did not provide any analytical method or experimental data. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *propionic acid, sodium propionate and ammonium propionate* in *silage*.

Recommended text for the register entry (analytical method)

E280, E281, E282, E284:

For the quantification of the *ammonium propionate* in the *feed additive*:

- (1) ion exclusion High Performance Liquid Chromatography with refractive index detection (HPLC-RI) – for the determination of total propionate; and
  (2) titration with sulphuric acid and sodium hydroxide – for the determination of ammonia.

For the quantification of *propionic acid, sodium propionate, calcium propionate and ammonium propionate* as *total propionic acid* in *feed additive, premixtures, feedingstuffs* and *water*:

- ion exclusion High Performance Liquid Chromatography with refractive index (HPLC-RI)

E700:

For the quantification of *E700* in *feed additive*:

- (1) reversed phase chromatography with UV detection (HPLC-UV) - for the determination of benzoate; and
  (2) ion exclusion High Performance Liquid Chromatography with refractive index (HPLC-RI) – for the determination of total propionate; and
  (3) atomic absorption spectrometry, AAS (EN ISO 6869) – for the determination of total sodium.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *propionic acid, sodium propionate, calcium propionate, ammonium propionate* and *E700* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/00149/ (10177)/2010

[2] +Application, Reference SANCO/D/2 Forw. Appl. 1831/(00169) (10365)-2010

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

[4] +Application, Proposal for Register Entry - Annex A

[5] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs

[6] *Technical dossier, Section II: Identity, characterisation and conditions of use

[7] COMMISSION REGULATION EC/1876/2006 of 18 December 2006 concerning the provisional and permanent authorisation of certain additives in feedingstuffs

[8] COMMISSION REGULATION EC/757/2007 of 29 June 2007concerning the permanent authorisation of certain additives in feedingstuffs

[9] European Pharmacopoeia Monograph 2041

[10] USP Food Chemicals Codex Monograph 'propionic acid'

[11] 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

[12] *Technical dossier, Section II – Annex II_5

[13] +Technical dossier, Section II - Annex_II_5

[14] EN ISO 6869:2000 Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry

* Refers to Dossier No. FAD-2010-0356

+ Refers to Dossier No. FAD-2010-0376

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

[1] FAD-2010-0356

[2] FAD-2010-0376 (E700)

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby, DK
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino, IT
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha, CZ
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien, AT

[1] FAD-2010-0356
[2] FAD-2010-0376 (E700)