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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-2010-0157** **CRL/ 100127**
Name of Feed Additive:	**Sodium saccharin**
Active Substance(s):	**Sodium saccharin**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report revised by: Date:	**Piotr Robouch, Roberto Molteni (EURL-FA)** **11/04/2011**
Report approved by: Date:	**Christoph von Holst** **11/04/2011**

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *sodium saccharin* 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. According to the Applicant, the active substance of the *feed additive* is *sodium saccharin* with purity above 98 %. Specifically, authorisation is sought for the use of the *feed additive* for suckling and weaned piglets, pigs for fattening, bovines and calves for rearing and fattening. The *feed additive* is intended to be used via *premixtures* in *feedingstuffs* and *water* at a maximum level of 150 mg/kg.

For the determination of *sodium saccharin* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method 01/2008:0787, based on potentiometric titration with 0.1 M perchloric acid. No performance characteristics of this method are provided. However, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *sodium saccharin* in *feedingstuffs* and *water* the Applicant proposed a single laboratory validated and further verified High-Performance Liquid Chromatography with UV detection (HPLC-UV) method. The following performance characteristics were reported for *feedingstuffs*:

- a relative standard deviation of *repeatability* (RSD_r) ranging from of 2.7 to 11%;
- a relative standard deviation for *intermediate precision* (RSD_{int}) ranging from 6.6 to 18%
- a *recovery* rate (R_{Rec}) ranging from 82 to 100%; and
- a limit of detection (LOD) and a limit of quantification (LOQ) of 0.1 and 0.3 mg/kg, respectively.

Furthermore the Applicant applied the method, upon request from the EURL, to determine the method performance characteristics in *water* and reported an RSD_r ranging from 0.1 to 1.1 %. Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified HPLC-UV method, submitted by the Applicant, to determine *sodium saccharin* in *feedingstuffs* and *water*.

For the determination of *sodium saccharin* in *premixtures* the Applicant did not provide sufficent validation data, therefore the EURL cannot evaluate nor recommend any method for official control to determine *sodium saccharin* in *premixtures*.

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

KEYWORDS
Sodium saccharin, sensory additive, flavouring compounds, pigs, suckling and weaned piglets, pigs for fattening, bovines, calves for rearing and fattening

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of additives already authorised under council directive 70/524/EEC) for *sodium saccharin* under the category/functional group 2(b) "sensory additives"/"flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant, the active substance of the *feed additive* is *sodium saccharin* with purity above 98 % [2, 3]. It is a white to almost white crystalline powder, freely soluble in water [4]. Specifically, authorisation is sought for the use of the *feed additive* for suckling and weaned piglets, pigs for fattening, bovines and calves for rearing and fattening [2]. The *feed additive* is intended to be used via *premixtures* in *feedingstuffs* and *water* at a maximum level of 150 mg/kg [2].

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 *sodium saccharin*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Qualitative and quantitative composition of impurities in the additive

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

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

For the determination of *sodium saccharin* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method 01/2008:0787 [6], based on potentiometric titration with 0.1 M perchloric acid. No performance characteristics of this method are provided. However, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *sodium saccharin* in *feedingstuffs* and *water* the Applicant proposed a single laboratory validated and further verified High-Performance Liquid Chromatography with UV detection (HPLC-UV) method [7]. The feedingstuff samples, 10 g of feed mixed with 5 g of diatomaceous earth, are analysed in triplicates. First the samples are extracted in 2 extraction cycles by pressurized liquid extraction (PLE), using a solution made of 50% acetonitrile/50% deionised water at 80°C and 100 bar. The extracts obtained are collected, appropriately diluted and purified on a solid phase extraction (SPE) column. Finally the purified solutions are injected three times in HPLC and detection is carried out at 202 nm. The method performance characteristics derived from all the validation and verification studies [8-11] are presented in Table 1. Furthermore, a limit of detection (LOD) and a limit of quantification (LOQ) of 0.1 mg/kg and 0.3 mg/kg were determined by the Applicant [11].

Additionally, the second laboratory, which performed the verification study, encountered a low recovery using the Applicant's method [11] and a large values for the intermediate precision (Table 1). In consequence, the second laboratory recommended a slight modification to the method. The proposed modifications concerned the increase of PLE extraction cycles from 2 to 4. Therefore, the second laboratory validated the slightly modified method obtaining 6.6 – 8.5 % for the RSD_{int} and 92 – 100% for the recovery [9, 10].

Table 1: Method performance characteristics for the determination of *sodium saccharin* in *feedingstuffs* (FS), applying the original method. Target values for *sodium saccharin* content ranging from 25 to 500 mg/kg for *feedingstuffs.* The applicant conducted validation experiments of a slightly modified method, obtaining a better performance profile (see text)

Sodium saccharin in FS	RSD_r (%)	RSD_{int} (%)	R_{rec} (%)
Validation	2.7 – 11 [8-10]	6.6 – 8.5 [9,10]	76 – 100 [8-10]
Verification	5 [11]	18 [11]	95 [11]

RSD_r,RSD_{int} - relative standard deviation for *repeatability* and *intermediate precision*, R_{Rec} – recovery rate

For the determination of *sodium saccharin* in *water*, the PLE step is not needed [12]. The samples are filtrated, injected in HPLC and analyzed. Furthermore upon request from the EURL, the Applicant applied the method, to determine the method performance characteristics in *water* and reported an RSD_r ranging from 0.1 to 1.1 % [13].

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified HPLC-UV method, submitted by the Applicant, to determine *sodium saccharin* in *feedingstuffs* and *water*. For the *feedingstuffs* the EURL recommends the slightly modified method, using a higher number of extraction cycles.

For the determination of *sodium saccharin* in *premixtures* the Applicant did not provide enough experimental data, therefore the EURL cannot evaluate nor recommend any method for official control to determine *sodium saccharin* in *premixtures*.

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:

- the European Pharmacopoeia method 01/2008:0787 using potentiometric titration with perchloric acid for the determination of *sodium saccharin* in the *feed additive*, and

– the single laboratory validated and further verified method using High-Performance Liquid Chromatography with UV detection (HPLC-UV) to determine *sodium saccharin* in *feedingstuffs* and *water*.

For the determination of *sodium saccharin* in *premixtures* the Applicant did not provide enough experimental data, therefore the EURL cannot evaluate nor recommend any method for official control to determine *sodium saccharin* in *premixtures*.

Recommended text for the register entry (analytical method)

For the determination of the *sodium saccharin* in the *feed additive*:

– potentiometric titration with perchloric acid (Pharm. Eur. 01/2008:0787)

For the determination of *sodium saccharin* in *feedingstuffs* and *water*:

– High-Performance Liquid Chromatography with UV Detection (HPLC-UV).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

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

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/0100/2010
[2] *Application, Proposal for Register Entry - Annex A
[3] *Application, Appendix to Description and Conditions of Use of the Additive
[4] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis
[5] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
[6] *Technical dossier, Section II - Annex_II_12: Ph_Eur_Na-Sacch
[7] *Technical Dossier, Section II – Annex _II_26_Sodium saccharin in feedingstuffs
[8] *Technical Dossier, Section II – Annex _II_27_Sodium saccharin in feed-method validation
[9] *Technical Dossier, Section II – Annex _II_28_Validation saccharinate HEVA-SO

[10] *Technical Dossier, Section II – Annex _II_29_Complement to validation saccharinate HEVS-SO
[11] *Technical Dossier, Section II – Annex _II_30_Sodium saccharin_verification report
[12] *Supplementary information, 2011-03-02_FFAC-Na-saccharin additional information
[13] *Supplementary information, Sodium saccharin in water
* Refers to Dossier No. FAD-2010-0157

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

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

8. ACKNOWLEDGEMENTS

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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
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