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Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

Feed Additives

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

Dossier related to:	FAD-2009-0005 CRL/080040
Name of Additive:	Sodium Benzoate (Protural)
Active Substance(s):	*Sodium benzoate*
Rapporteur Laboratory:	National Veterinary Institute (SVA), Uppsala, Sweden
Report prepared by:	Erik Nordkvist (SVA)
Report revised by:	Sulhattin Yasar (CRL-FA) Piotr Robouch (CRL-FA) 21/10/2009
Report approved by:	Giuseppe Simone (CRL-FA)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for *Sodium Benzoate (Protural)* under the category 'zootechnical additives', functional group 4(d), 'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically authorisation is sought to use *Protural* for weaned piglets. It is intended to be marketed in the form of a white, odourless, non-dust forming granule. Its active agent is *sodium benzoate* with a minimum purity of 99.4%. It is intended to be incorporated into *premixtures* and/or complete *feedingstuffs* for weaned piglets to obtain a recommended dosage of *sodium benzoate* of 4 g/kg in *feedingstuffs*.

For the determination of *sodium benzoate* in the *feed additive* the applicant proposed a potentiometric method for the intended purpose. For official control the CRL recommends the European Pharmacopoeia's titrimetric method [Monograph 01/2008:0123] to determine sodium benzoate in the *feed additives*.

For the determination of *sodium benzoate* in the *premixtures* and *feedingstuffs* the applicant proposed a method, based on ion chromatograph (IC) coupled to Ultraviolet (UV) detection. The method was in-house validated with acceptable performance characteristics. However, the verification of the IC-UV method was missing as the second laboratory applied a different method based on High Performance Liquid Chromatography (HPLC) coupled to UV detection, instead of the IC-UV method. According to the opinion of experts from National Reference Laboratories the application of IC-UV in the frame of official control could be difficult due to the instrumentation required. However, other methods based on HPLC-UV are routinely used by National Reference Laboratories for official control for the determination of *benzoic acid* in the *premixtures* and the *feedingstuffs*. Such a method was developed and single-laboratory validated at the Austrian Agency for Health and Food Safety (AGES) on *premixtures* and *feedingstuffs* samples containing *benzoic acid* at a concentration range of 5 to 10 g/kg. The reported performance characteristics were: - a limit of detection (LOD) of 0.4 g/kg, - a limit of quantification (LOQ) of 1.25 g/kg, - a relative standard deviation for repeatability (RSD_r) ranging from 2.3 to 4.9%, - a relative standard deviation for intermediate precision (RSD_R) ranging from 4.2 to 6.9% and - a recovery rate (RR) ranging from 96 to 101%.

Based on these acceptable performance characteristics the CRL recommends the HPLC-UV method developed and validated by AGES to determine *sodium benzoate* in the *premixtures* and *feedingstuffs* for official controls.

Further testing or validation is not considered necessary.

KEYWORDS

Sodium Benzoate, Zootechnical Additive, Weaned Piglets

1. BACKGROUND

Sodium Benzoate (*Protural*) is a feed additive, for which authorisation is sought under the category 'zootechnical additives', functional group 4(d), 'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the applicant the active agent of *Protural* is *sodium benzoate* with a minimum purity of 99.4% [1].

The *product* is available in the form of a white, odourless, non-dust forming granule [2]. It is intended to be incorporated into *premixtures* and/or complete *feedingstuffs* for weaned piglets to obtain a recommended dosage of *sodium benzoate* of 4 g/kg of *feedingstuffs* [1].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *Protural* were evaluated for their suitability to be used for official controls.

3. EVALUATION

Identification/Characterisation of the feed additive

Quantitative and qualitative composition of impurities in the additive

Since the additive consists solely of *sodium benzoate* with a minimum purity of 99.4%, no analytical methods were submitted by the applicant to monitor impurities. The remaining impurity (0.6%) is water [3].

Methods of analysis of the active substance in the feed additive, premixtures, and feedingstuffs

For the determination of *sodium benzoate* in the *feed additive* the applicant proposed a potentiometric method. For official control the CRL recommends the European Pharmacopoeia's titrimetric method [Monograph 01/2008:0123] to determine *sodium benzoate* in the *feed additives*. The assay requires the extraction of 0.25 g sample in anhydrous acetic acid, heated to 50 ^{o}C if necessary, and the titration with 0.1 M perchloric acid until a green colour is obtained. One ml of 0.1 M perchloric acid is equivalent to 14.41 mg sodium benzoate [4].

For the determination of *sodium benzoate* in the *premixtures* and *feedingstuffs* the applicant proposed a method, based on extraction, separation and detection of sodium benzoate by ion chromatograph (IC) coupled to Ultraviolet (UV) detection. The assay protocol is described as follows: 1 gram of sample is extracted in water at room temperature for 30 min. The extract is filtered, diluted to a suitable concentration and analysed by IC-UV at 260 nm [5]. The method is *in-house* validated on the premix and feed samples, and the following performance characteristics were reported [5]: - a limit of detection (LOD) of 0.01 g/kg, - a limit of quantification (LOQ) of 0.05 g/kg, - a relative standard deviation for repeatability (RSD_r) ranging from 6.3 to 7.0% and - a recovery rate (RR) ranging 93 to 100%. No proper verification study was provided as the applicant reported data obtained by a second laboratory applying a different method HPLC (High Performance Liquid Chromatography) coupled to UV detection , instead of IC-UV. Therefore, the CRL could not evaluate the suitability of the applicant IC-UV method for official controls.

According to the opinion of experts from the National Reference Laboratories the application of IC-UV within the frame of official control may be difficult due to the instrumentation required. However, other methods based on HPLC-UV are routinely used by National Reference Laboratories

to determine *benzoic acid* in *premixtures* and *feedingstuffs* for official control. Such a method was developed and single-laboratory validated at the Austrian Agency for Health and Food Safety (AGES) for *premixtures* and *feedingstuffs* samples containing *benzoic acid* at a concentration range of 5 to 10 g/kg. The assay protocol is described as follows: 1.0 g of sample is extracted in diluted sulphuric acid, followed by filtration and detection by UV-VIS detector at 240 nm [6]. The reported performance characteristics were [7]: - LOD = 0.4 g/kg, - LOQ = 1.25 g/kg, - RSD_r ranging from 2.3 to 4.9%, - a relative standard deviation for intermediate precision (RSD_R) ranging from 4.2 to 6.9% and - RR ranging from 96 to 101%.

Based on these acceptable performance characteristics the CRL recommends the above mentioned HPLC-UV method to determine *sodium benzoate* in the *premixtures* and *feedingstuffs* for official controls.

4. CONCLUSIONS AND RECOMMENDATIONS

For the determination of *sodium benzoate* in the *feed additive* a titrimetric method [Monograph 01/2008:0123] described in the European Pharmacopoeia is recommended for official controls.

For the determination of *sodium benzoate* in the *premixtures* and *feedingstuffs* the in-house validated HPLC-UV method developed by the AGES is recommended for official controls.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

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

Titrimetric method (Monograph 01/2008:0123 of the European Pharmacopoeia).

For the determination of *sodium benzoate* in the *premixtures* and *feedingstuffs*:

HPLC method with UV-detection.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of Protural have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] * Proposal for Register entry (Annex A)

[2] * Technical dossier, Section 2.3.1

[3] * Technical dossier, Section 2.1.5

[4] European Pharmacopoeia [Monograph 01/2008:0123 corrected 6]. Sodium Benzoate Assay in: European Pharmacopoeia (2008) 6th Edition, Directorate for the Quality of Medicines within the Council of Europe, Strasbourg, Volume 2, pp. 2890-2891.

[5] * Technical dossier, Section 2.6.1 Annex II.4.

[6] * Supplementary Information: HPLC_UV_ Method by AGES_englisch.pdf: Determination of Benzoic and Sorbic Acid in Feedingstuffs using HPLC. Austrian Agency for Health and Food Safety GmbH (AGES) Institute for Feedingstuffs, Vienna Spargelfeldstraße, Department of Feed Analysis.

[7] * Supplementary Information: Validation Data_ Benzoic Acid_CRL.pdf

* Refers to Dossier No: FAD-2009-0005

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the National Veterinary Institute (SVA), Uppsala, Sweden. 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.

8. ACKNOWLEDGEMENTS

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