
Sacox®
(FAD-2012-0041; CRL/120032)
(FAD-2013-0029; CRL/130023)
(FAD-2013-0053; CRL/130037)

Dossier related to:  
- FAD-2012-0041 - CRL/120032  
- FAD-2013-0029 - CRL/130023  
- FAD-2013-0053 - CRL/130027  

Name of Product:  
-Sacox®-

Active Agent (s):  
-Salinomycin sodium-

Rapporteur Laboratory:  
-European Union Reference Laboratory for Feed Additives (EURL-FA)  
-Geel, Belgium-

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-Christoph von Holst  
-02/09/2014-
EXECUTIVE SUMMARY

Sacox® is a feed additive - belonging to the "Coccidiostats and other medicinal substances" group listed in Directive 70/524/EEC - currently authorized for chickens for fattening and reared for laying by Commission Regulations (EC) No 1463/2004 and No 1852/2003. In the current applications authorisation is sought under articles 10(2) and under article 13(3) of the Regulation (EC) No 1831/2003. Sacox® consists of salinomycin sodium (active substance) at concentrations of 120 and 200 g/kg (Sacox® 120 and Sacox® 200), silica dioxide as flowability enhancer and calcium carbonate as structure-forming agent and diluent. Sacox® is intended to be incorporated into feedingstuffs directly and/or through premixtures. The Applicant proposed a concentration of salinomycin sodium in feedingstuffs of 50 mg/kg for chickens reared for laying or ranging from 60 to 70 mg/kg for chickens for fattening. Furthermore the Applicant proposed two sets of MRLs for salinomycin in chicken tissues: 5 μg/kg in all wet tissues (as already established by Commission Regulation (EC) No 167/2008) or ranging from 12 to 145 μg/kg depending on the target tissues.

For the quantification of salinomycin sodium in premixtures and feedingstuffs the Applicant submitted the ring-trial validated method (EN ISO 14183) based on High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-UV-Vis). Furthermore, the Applicant adapted the EN ISO 14183 with minor experimental modifications and applied it to the feed additive (Sacox®) providing similar method performance characteristics. Based on the experimental evidence available the EURL recommends for official control the HPLC-UV-Vis method for the quantification of salinomycin in the feed additive, premixtures and feedingstuffs.

For the quantification of salinomycin in chicken tissues the Applicant submitted a single laboratory validated and further verified method based on reverse phase HPLC coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode using matrix matched standards (RP-HPLC-MS/MS), similar to the one developed and validated by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL, Berlin). The satisfactory performance characteristics provided by the Applicant for the four tissues of concern (i.e. muscle, kidney, skin/fat and liver) demonstrate that (i) the method proposed by the Applicant is equivalent to the BVL method, and (ii) the Applicant method is also applicable to kidney and skin/fat tissues. Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant or any equivalent analytical methods complying with the requirements set by Commission Decision 2002/657/EC to enforce the salinomycin MRLs in the relevant tissues.
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**

*Salinomycin sodium, Sacox®, coccidiostat, chickens for fattening & reared for laying*

**1. BACKGROUND**

*Sacox®* is a *feed additive* - belonging to the group "Coccidiostats and other medicinal substances" listed in Directive 70/524/EEC - currently authorized for chickens for fattening and reared for laying by Commission Regulation (EC) No 1463/2004 [1] and No 1852/2003 [2]. In the current applications authorisation is sought under articles 10(2)¹,² (authorisation of an existing product) [3,4] and under article 13(3)³ (modification of the existing authorisation) [5] of the Regulation (EC) No 1831/2003.

*Sacox®* consists of *salinomycin sodium* (active substance) at concentrations of 120 g/kg (*Sacox® 120*) and 200 g/kg (*Sacox® 200*), silica dioxide as flowability enhancer and calcium carbonate as structure-forming agent and diluent [6,7]. *Sacox®* is intended to be incorporated into *feedingstuffs* directly and/or through *premixtures*. The Applicant proposed a concentration of *salinomycin sodium* in *feedingstuffs* of 50 mg/kg for chickens reared for laying or ranging from 60 to 70 mg/kg for chickens for fattening [6,8].

The Applicant proposed two sets of MRLs for *salinomycin* in chicken *tissues*: 5 μg/kg in all wet tissues (i.e. muscle, kidney, skin/fat and liver) as already established by Commission Regulation (EC) No 167/2008 [9], or ranging from 12 to 145 μg/kg (12 μg/kg for muscle; 35 μg/kg for kidney; 140 μg/kg for liver; and 145 μg/kg for skin/fat) [6]. These MRLs are not covered by the Commission Regulation (EC) No 37/2010 [10], and need to be evaluated by the EURL.

**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

¹ FAD 2012-0041 ; ² FAD 2013-0029; ³ FAD 2013-0053
methods of analysis submitted in connection with Sacox® 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 [11].

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

For the quantification of salinomycin sodium in premixtures and feedingstuffs the Applicant submitted the ring-trial validated method (EN ISO 14183) [12], based on High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-UV-Vis).

This method can distinguish between the main substances of the narasin, salinomycin and monensin ionophores. The detection principle of post-column derivatisation coupled to spectrophotometric detection (HPLC-UV-Vis) is specific. Potential interferences in the determination of salinomycin, caused by other components of the feed additive, premixtures of feedingstuffs, are not expected [13].

Salinomycin sodium is extracted using methanol:water (90:10) with mechanical shaking for 1 h, filtered and subjected to analysis without further clean-up. The target analyte is determined by reverse-phase HPLC using post-column derivatisation with vanillin and detection at 520 nm [12].

This method was ring-trial validated for broiler feedingstuffs and premixtures at a mean salinomycin content of 68.4 and 1000 mg/kg, respectively. The following performance characteristics were reported [12]:

- a relative standard deviation for repeatability (RSDr) ranging from 2.5 to 3.5 %;
- a relative standard deviation for reproducibility (RSDR) ranging from 2.7 to 5.5 %; and
- a limit of quantification (LOQ) of 2 mg/kg.

For the quantification of salinomycin sodium in the feed additive (Sacox®) the Applicant applied the standard method mentioned above adapting the sample preparation step [14]. The performance characteristics derived from the validation and verification studies [15-19] were recalculated by the EURL leading to precisions (repeatability and intermediate precision) ranging from 0.4 to 0.8 % [20].
These performance characteristics are in good agreement with those reported in the EN ISO 14183 standard and demonstrate the applicability (extension of the scope) of the EN ISO 14183 method to the feed additive (Sacox®).

Based on the performance characteristics available, the EURL recommends for official control the HPLC-PCD-UV-Vis method for the quantification of salinomycin in feed additive, premixtures and feedingstuffs.

Methods of analysis for the determination of the residues of the additive in food.

For the quantification of salinomycin in poultry tissues the Applicant submitted a single-laboratory validated [21-23] and further verified method (in kidney, skin/fat, muscle and liver) [24-30] based on reverse phase HPLC coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode using matrix matched standards (RP-HPLC-MS/MS).

The minced tissue is extracted with acetonitrile, vortex mixed for 30 s, placed in an ultrasonic bath for 10 min and centrifuged for another 10 min at 4 °C. The supernatant is then evaporated until dryness under a gentle stream of nitrogen at 40 ± 5 °C. The dry residue is then dissolved back in acetonitrile vortex mixed for 20 s, placed in an ultrasonic bath for 2 min and vortex mixed again for another 20 s. The resulting solution is filtered through a nylon filter before injection in the HPLC-MS/MS system [21-23].

A similar method has been previously developed and validated by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL, Berlin) for the determination of salinomycin in two target tissues (muscle and liver). The EURL already evaluated and recommended this method in the frame of previous narasin dossiers [31].

The Applicant validated and further verified the RP-HPLC-MS/MS method at the MRL level of 5 μg/kg in wet tissues (i.e. kidney, liver, skin/fat and muscle) thus complying with the requirements defined in "The rules governing medicinal products in the European Union" and in International Guidances (VICH GL49) [32]. Three salinomycin levels i.e. MRL/2; MRL and 2MRL were investigated for each target tissue [21-30]. Four identification points were set for salinomycin using one parent and two daughter ions. Quantification is based on the transition m/z 773 > 431 while confirmation is based on the transition m/z 773 > 531 complying thus with the confirmatory requirements set by of Commission Decision 2002/657/EC [33].

Table 1 presents the performance characteristics reported in the frame of the validation and verification studies together with those reported by BVL. Additionally, BVL reported LOD of 2.3 μg /kg and recoveries ranging from 90.3 to 109 %.
Table 1. Performance characteristics for the quantification of salinomycin residues in chicken tissues obtained in the frame of the validation (Val.) and verification (Ver.) studies [21-30], compared to those reported by the European Union reference Laboratory Pharmacologically Active Substances (BVL).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Conc. (μg/kg)</th>
<th>RSD_r (%)</th>
<th>RSD_ip (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>BVL</td>
<td>0.75-2.75</td>
<td>14-28</td>
</tr>
<tr>
<td></td>
<td>Val.</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Ver.</td>
<td>5</td>
<td>11-15</td>
</tr>
<tr>
<td>Liver</td>
<td>BVL</td>
<td>0.75-2.75</td>
<td>14-28</td>
</tr>
<tr>
<td></td>
<td>Val.</td>
<td>2.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Ver.</td>
<td>5</td>
<td>5-10</td>
</tr>
<tr>
<td>Kidney</td>
<td>Val.</td>
<td>2.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Ver.</td>
<td>5</td>
<td>5.2-13</td>
</tr>
<tr>
<td>Skin/Fat</td>
<td>Val.</td>
<td>2.5</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Ver.</td>
<td>5</td>
<td>2.9-4.4</td>
</tr>
</tbody>
</table>

RSD_r; RSD_ip: relative standard deviation for repeatability and intermediate precision

The satisfactory performance characteristics obtained by the Applicant for liver and muscle tissues demonstrate that the BVL method was equivalent to the one proposed by the Applicant. Additionally the satisfactory results provided by the Applicant for kidney and skin/fat tissues further demonstrate the applicability - and therefore extension of scope - of the Applicant method to these two additional tissues.

Even though the Applicant did not provide data for the second set of MRLs (i.e. 145 μg/kg for skin/fat, 140 μg/kg for liver, 35 μg/kg for kidney and 12 μg/kg for muscle [5]), the EURL considers the submitted HPLC-MS/MS method suitable for official control to enforce salinomycin at higher MRLs in the target tissues.

Consequently, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC [33] for the enforcement of salinomycin levels in chicken tissues.

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 (i) the HPLC-PCD-UV-Vis method for the quantification of salinomycin in the *feed additive, premixtures and feedingstuffs* and (ii) the RP-HPLC-MS/MS single laboratory validated and further verified method proposed by the Applicant - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the quantification of salinomycin in *chicken tissues*.

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

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

- High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis)

For the quantification of salinomycin in *premixtures and feedingstuffs*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis) - EN ISO 14183

For the quantification of salinomycin in *tissues*:

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Sacox® 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


[7] Technical dossier, Section II: 2.1 Identity of the additive
[8] Technical dossier, Section II: 2.5 Conditions of use
[14] Technical dossier, Section II, References, Reference II.43 – SOP FA
[16] Technical dossier, Section II, References, Reference II.58 – Verif FA 120
[18] Technical dossier, Section II, References, Reference II.54 – Verif FA 120
[19] Technical dossier, Section II, References, Reference II.55 - Verif FA 200
[20] Supplementary Information, EUURL_ANOVA_calculation.pdf
[21] Technical dossier, Section II, References, Reference II.47 – Valid tissues
[22] Technical dossier, Section II, References, Reference II.56 – Valid tissues
[23] Supplementary Information, validation muscle.pdf
[26] Technical dossier, Section II, References, Reference II.50 – Verif skin/fat
[27] Technical dossier, Section II, References, Reference II.57 – Verif FA 120
[28] Technical dossier, Section II, References, Reference II.58 – Verif kidney
[29] Technical dossier, Section II, References, Reference II.59 - Verif skin/fat
[30] Supplementary Information, verification muscle.pdf
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

The following National Reference Laboratories contributed to this report:

- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim¹ (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus² (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)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft. Nossen³ (DE)

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– RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
– Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
– Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
– Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarni inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)