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A feasibility study for producing an egg matrix candidate reference material for the polyether ionophore salinomycin

Rosana Gomes Ferreira [a,*], Mychelle Alves Monteiro [a], Mararlene Ulberg Pereira [a], Rafaela Pinto da Costa [a], Bernardete Ferraz Spisso [a], Veronica Calado [b]

[a] National Institute for Quality Control in Health/Oswaldo Cruz Foundation (INCQS/FIOCRUZ), Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ, Brazil
[b] Chemistry School, Federal University of Rio de Janeiro (UFRJ), Av. Horácio Macedo 2030, Technology Centre, University City, Rio de Janeiro, RJ, Brazil

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ABSTRACT

The aim of this work was to study the feasibility of producing an egg matrix candidate reference material for salinomycin. Preservation techniques investigated were freeze-drying and spray drying dehydration. Homogeneity and stability studies of the produced batches were conducted according to ISO Guides 34 and 35. The results showed that all produced batches were homogeneous and both freeze-drying and spray drying techniques were suitable for matrix dehydrating, ensuring the material stability. In order to preserve the material integrity, it must be transported within the temperature range of −20 up to 25 °C. The results constitute an important step towards the development of an egg matrix reference material for salinomycin is possible.

1. Introduction

Sodium salinomycin is authorized in the European Union under the frame of the Regulation (EC) No 1831/2003 as a coccidiostat feed additive [1]. It is allowed for use in chickens for fattening with a maximum content of the active ingredient in feed of 70 mg kg^{-1} and a withdrawal period of one day, for laying hens (up to 12 weeks of age) with a maximum content of 50 mg kg^{-1} and no withdrawal period [2].

Since 2009, the European Union established the value of 3 μg kg^{-1} of salinomycin in egg as a Maximum Tolerance Limit [3]. Currently, in Australia, the maximum limit for SAL in egg is 0.02 mg kg^{-1} [4].

In Brazil, the salinomycin polyether ionophore is authorized by the Ministry of Agriculture, Livestock and Food Supply (MAPA) as an anticoccidial feed additive in broilers, replacement pullets and quails to help in coccidiosis prevention, with the restrictions of not to use in laying hens and chickens over 16 weeks of age [5]. There is a considerable consumer health risk from coccidiostats exposure if it is used in laying hens, because of it the use is not authorized [6].

In Brazil, the Normative Instruction No. 17 of May 29, 2013 included in the analytical scope the coccidials as lasalocid, maduramycin, monensin, salinomycin and semduramycin in the monitoring subprogram and residues and contaminants controls in eggs. A reference limit for taking a regulatory action, for these analytes in eggs, was set as 10 μg kg^{-1} [7].

In 2010, Spisso and coworkers have studied the occurrence of antibiotic residues in 100 eggs sample of different brands, collected in Rio de Janeiro municipality. This study revealed residues of maduramicin, monensin, narasin, salinomycin and semduramycin, respectively, at 1%, 3%, 5%, 21% and 4% of the samples, and samples with 2% of non-compliance rate for salinomycin, according to the values advised by the European Union [8].

The analytical quality comprovation, about food safety, is important for Public Health and Exportation of these products. In addition, consumers are requiring more and more that kind of assurance [9].

Reference materials (RM) represent tools for quality control in accredited laboratories or for those who aspire accreditation by ISO/IEC 17025 [10]. There is a shortage of reference materials in the field of veterinary drug residues in animal products, which complicates the control and surveillance of these products. Guidelines on the preparation of reference materials are available in ISO Guides 31, 34 and 35 [9,11–13].

A feasibility study for producing a reference material, allows

* Corresponding author.
E-mail addresses: rosana.ferreira@incqs.fiocruz.br, rosanagf.01@gmail.com (R.G. Ferreira).

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the evaluation of what is the best way for preparation, treatment and processing of the matrix and establishes the best analytical method and statistical techniques to be used in evaluation of homogeneity, stability and characterization study of the material [9].

It is known that reference materials must be sufficiently homogeneous and stable and having similar characteristics to the actual samples. That's why is so important, to ensure the preservation of perishable matrices like shelled eggs (that suffer deterioration because of various species of organisms) which produce putrefactions. Thus, pasteurization, freeze-drying and spray drying processes helps on eggs preservation [9].

Pasteurization process of liquid eggs must be done, before drying, because of the possible presence of *Salmonella*. Thus, in this work, pasteurized eggs samples were used in this work.

The aim of this work was to study the feasibility for the production of an egg matrix candidate reference material for salinomycin, and to establish a method for preservation of the analyte and the matrix, ensuring the homogeneity and stability of the material.

2. Materials and methods

2.1. Chemicals

Standards of salinomycin (SAL) and sodium salt of nigericin (NIG) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sodium acetate (NaOAc) Suprapur®, acetonitrile (ACN) of high-performance liquid chromatography (HPLC) grade and Suprapur® formic acid (FOA) were acquired from Merck (Darmstadt, Germany).

Methanol (MeOH) for UV/HPLC chromatography was from JT Baker (Phillipsburg, NJ, USA).

Ultra pure water provided by a Milli-Q system from Merck Millipore (Bedford, MA, USA) was used with resistivity of 18 MΩ cm^{-1} at 25 °C.

Standard stock solutions (1000 mg L^{-1}) of salinomycin and nigericin were prepared by dissolving the compounds in methanol. These solutions were stored at −70 °C.

Working solutions of several concentrations were freshly prepared by dilutions of the standard stock solutions with methanol.

2.2. Instrumentation

A high dispersion Kinematica Polytron PT 6100 (Eschbach, Germany), an Ultra-Turrax® T25 Basic disperser from IKA®-WERKE (Deutschland, Germany), a freeze dryer model K105 from Liotop (São Carlos, São Paulo, Brazil) and a mini Spray Dryer B-290 model of Büchi (Flawil, Switzerland) were used for the production of the candidate reference material.

The vacuum checking inside each bottle was carried out by using a Tesla coil from Harvard Apparatus 2–12–8 (Holleston, MA, USA).

For determining the concentration of salinomycin in the samples, a LC-MS/MS system, composed of a high performance liquid chromatograph Shimadzu Prominence (Kyoto, Japan), and a tandem mass spectrometer API5000 ABSCIEX (Fostercity, CA, USA) were used, with Turbolonspray® interface. The Analyst® 1.4.2 software was used for system control, acquisition and analysis of data. The liquid chromatograph configuration consisted of a LC-20CE quaternary pump, a DGU-20A5 membrane degasser, a SIL-20AC autosampler, a CTO-20AC column oven and a CBM-20A controller.

An ACE C18 analytical column (50 mm × 2.1 mm, 3 μm particle size, 100 Å), with a guard column of the same material (Advanced Chromatography Technologies, Aberdeen, Scotland), was used.

Residual moisture and total solids were determined in the samples using a drying oven FANEM (Guarulhos, São Paulo, Brazil).

Statistical evaluation of data was performed by Excel® and Statistica® 12.0 software (StatSoft, USA).

2.3. Samples

2 kg of refrigerated pasteurized eggs (Tapiratiba, São Paulo, Brazil) were used for the production of the candidate reference material.

Fresh organic eggs (large red type) (Rural Itiropina, São Paulo, Brazil) were used as blank samples in the assays for determining salinomycin concentrations.

Incurred egg sample previously analyzed containing about 5 μg kg^{-1} of salinomycin residues.

2.4. Incurred and spiked samples

A incurred egg sample was analyzed and found about 5 μg kg^{-1} of salinomycin residues. This sample was used to compare with the spiked sample and to check if the analytical behaviors are the same. So, the spiked sample was prepared in order to obtain a concentration of 5 μg kg^{-1} of salinomycin in the final product.

10 mL of SAL solution at 1 μg mL^{-1} were added to 2 kg of pasteurized whole egg previously analyzed and confirmed salinomycin free. The material was shaken with the aid of a glass rod and homogenized for 1 h using a high dispersion mixer at 3000 rpm speed. The spiked material was analyzed to determine if salinomycin concentration was at the desired level, according to the method described in item 2.7. Samples were injected in duplicate. Comparison of salinomicym concentrations of incurred and spiked samples were carried out by *t*-test and *F*-test. The spiked samples moisture was measured in triplicate by loss of drying in an oven at 103 °C. The process yield was calculated.

The spiked products were distributed in approximately 130 g portions into 4 plastic containers, such as a "Tupperware" and freezed at −70 °C for 12 h to be subsequently freeze dried. The remaining product was also stored at −70 °C for testing on spray dryer. The freeze-drying process efficiency was calculated.

2.5. Freeze-drying of the spiked sample

The four spiked sample portions were freeze dried, providing one batch. Freeze-drying conditions were as follows: initial temperature −9 °C, final temperature −102 °C, vacuum 0.044 mm Hg and time period of 40 h.

The freeze-dried material was manually crushed with a mortar and a pestle. Then, it was homogenized using a rotary evaporator with stirring at 25 rpm and vacuum at 600 mmHg for 1 h at room temperature. 0.8 g portions were distributed into 10 mL amber glass bottles under dehumidification condition.

Bottles were weighted and subsequently sealed with aluminum seals using freeze-drier closure frasks system under vacuum. Three bottles (representing the beginning, the middle and the end of the weighing) were separated to measure the moisture and the total solids, in triplicate, by loss drying in an oven at 105 °C.

2.6. Spray drying of the spiked sample

600 mL of the spiked sample were diluted with ultra pure water in the proportion of 30:70, v/v, in a 2000 mL volumetric flask. Three 400 mL aliquots of the diluted sample were separated for spray drying. The aliquot volume was defined according to the maximum capacity of the equipment by process. Each aliquot was homogenized in an Ultra-Turrax® T 25 Basic disperser at a speed