Method Validation for Moisture Content in Combined Live Newcastle Disease and Infectious Bronchitis Vaccines by Vacuum Oven Method

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Abstract

Background: The appropriate moisture content is critical for the viability of the vaccine, which affects to vaccine efficacy. The ASEAN standard for animal vaccines, the moisture content in freeze dried vaccines must not exceed 4%. Moisture content in freeze-dried vaccines is commonly determined using Karl Fischer titration and vacuum drying. Currently, the Veterinary Biologics Assay and research Center uses a vacuum oven method to determine moisture content, under conditions of 60±3°C, vacuum < 25 millibar, at least 3 hours drying time and room relative humidity (RH) < 45%. Therefore, this study conducted a validation of the vacuum oven method for moisture content by reducing the drying time from 3 to 2 hours to time-saving and cost-effective method for determining the moisture content in freeze dried live vaccines.

Objectives: To validate the vacuum oven method for moisture content determination in freeze dried live vaccines at Veterinary Biologics Assay and research Center.

Methods: Ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine were tested using vacuum oven at 60°C, 24 millibar, RH < 45% for duration time 2 and 3 hours. For precision, 100 µL of DW was dispensed in to weighed container and weighed. Samples were dried in vacuum oven at 60°C, 24 millibar, RH < 45% and reweighed. Weight difference before and after drying was used to calculate % moisture and the acceptance was determined using the F-test. Accuracy was tested by adding 100 µL of DW to moisture-free combined live Newcastle disease and infectious bronchitis vaccine and determine the acceptance of the method using %Recovery. Method validation was performed using ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine, acceptance of the method variability was assessed using the F-test and t-test.

Results: At 2 and 3 hours, the precision average moisture content was showed 101.23% and 101.39% with Fcal of 0.79. Accuracy average moisture content was 100.04±1.05% and 100.05±1.34% and %R were 101.23 and 101.39. The method validation average moisture content was 3.09±0.68% and 3.00±0.44% with Fcal=1.54 and tcal=0.26 (n=10, Fcrit=3.18, tcrit=2.10, p=0.05). This results within the acceptable range according to AOAC (1993) and method met the validation of Fcal<Fcrit and tcal<tcrit at 95% confidence level.

Conclusions: Validation results of the moisture content in combined live Newcastle disease and Infectious Bronchitis vaccines by vacuum oven method at temperature of 60°C, vacuum 24 millibar and drying time of 2 hours can be used as a reliable method for determining moisture content in combined live Newcastle disease and infectious bronchitis vaccines.

Keywords: Method validation, Moisture content determination, Combined live Newcastle disease and infectious bronchitis vaccines, Vacuum oven method

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