Renewal Assessment Report

Lecanicillium muscarium Ve6

- Mycotal -

Volume 3MP – B.5 Analytical methods

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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B.5 Analytical methods

Note to reader:
Information from the original DAR and/or addenda to the DAR is highlighted grey.

B.5.1 Methods for the analysis of the preparation

Information from the original DAR

The notifier performed quality control tests of each MYCOTAL batch at regularly basis during storage. Spore viability was tested in spore germination tests and efficacy in bio-assays on whiteflies.

B.5.1.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation

New data 2016

An analytical method has been developed and validated for the determination of the content of active ingredient *L. muscarium* Ve6 in the formulated product MYCOTAL.

The following analytical method validation for the determination of the active substance has not previously been reviewed and is provided in support of this assessment.

Reference:

Report:	KMP 5.1/01, Coranelli, S. (2014)
Title:	Analytical method validation for the determination of the active ingredient content in the formulated product MYCOTAL
Document No:	Study BT062/14
Guidelines:	SANCO/3030/99 rev.4. (11/07/00)

Material:

Mycotal, Batch No. 86M10, containing *Lecanicillium muscarium* strain 19.79, content: 1×10^{10} spores/g

Reference item: MYCOTAL - Slurry, Batch No. 86M16-SL, containing *Lecanicillium muscarium* strain 19.79, content: 2×10^{10} spores/g

Blank Formulation: Blank formulation MYCOTAL

Method: The active ingredient content was determined by the following method:

a) An appropriate amount of test item is dispersed in Triton-saline to obtain a suspension with a dilution factor of 10^{-1}.

b) the suspension is shaken for 1 h at room temperature at 110 rpm

c) Further dilutions are obtained by adding 9 mL of saline solution to 1 mL of the previously diluted sample.

d) 0.02 mL of appropriate dilutions are put in Petri dishes filled with SDA growth media (Sabouraud Dextrose Agar). Each dilution is plated in duplicate.

e) The inoculated plates are incubated at 23°C for 3-4 days.

f) After incubation the number of grown colonies is registered and the number of CFU per gram of product, of CFU per mL of aqueous dilution is calculated with the following formula:

[CFU/mL] = (dilution) × (mean number of colonies counted in two plates)/0.02

The method was validated with regard to specificity, linearity, precision and accuracy.

For the validation of the method in aqueous dilutions (dispersions), the samples were prepared using Standard Water D.

Validation Data:

The following validation of the analytical method for the determination of the active substances in the product has not previously been reviewed and is provided in support of this assessment.

Linearity:

The linearity of the response was determined by analysing five dispersions of reference item (technical powder) at different concentrations in Triton-saline (0.5 g/L Triton, 9.0 g/L NaCl). The concentrations were chosen in order to obtain a calibration curve over a range appropriate to cover the nominal concentration on the active ingredient in the formulated product (0.1, 0.2, 0.5, 1.5, 5.0%). The dispersions were further diluted to allow for an easy determination of the colony forming units. For the determination of the CFU, 0.02 mL of the dispersions with dilution factor from 10^{-1} to 10^{-3} were inoculated on Petri dishes with SDA in duplicate and incubated at 23°C for 3-4 days. Afterwards, colonies were counted and the number of CFU per g calculated. The number of CFU per L of each solution was represented graphically together with the linear regression curve and the regression coefficient (R^2), both calculated by means of the least scares method.

Results validation:

The results were linear within the range between 0.1 and 50 g/L. The equation of the calibration line (n=5) was found to be $y = 2 \times 10^8 x + 9 \times 10^8$ (correlation coefficient 0.9915). Where x is the concentration in g/L. The method was accepted because $R^2 > 0.98$

Precision

Five independent weightings of 1 g each of the test item (formulated product) were performed and diluted to 100 mL (dilution (10^{-2}) with Triton-saline. The dispersions were further diluted to allow for an easy determination of the colony forming units. For the determination of the CFU, 0.02 mL of the dispersions with dilution factor from 10-5 to 10^{-8} were inoculated on Petri dishes with SDA in duplicate and incubated at 23°C for 3-4 days. Afterwards, colonies were counted and the number of CFU per g calculated.

Results Precision:

For the validity for precision the value of % RSD should be ≤ 2.68%. The mean value was 8.25×10^8, the standard deviation (SD) = 1.77×10^8, and the % relative standard deviation (%RSD) = 2.1%. The method was accepted because CV% ≤ 2.68

Accuracy

It was estimated by the examination of 2 dispersions containing a known quantity of the active ingredient corresponding to the dispersions L2 and L4 of the linearity test. The dispersions were prepared adding a known quantity of reference item (technical product) to the blank formulation in order to obtain a concentration of active ingredient corresponding to the formulated product (4.8% *Lecanicillium muscarium* strain 19.79 in MYCOTAL).

Results accuracy:

Recovery of CFU was 97.83% and 96.95% in sample one and two, respectively. Therefore the mean recovery was 97.39%. The method was accepted because mean % recovery is within 97.0 - 103.0%.

Table 5.1-1: Summary of validation of the method for the determination of *L. muscarium* Ve6

Reference	Linearity	Precision product	Accuracy
KMP 5.1/01	5 solutions of the reference item (technical product) prepared at different concentrations. In the formulated product: $y = 2 \times 10^8 x + 9 \times 10^8$ ($R^2 = 0.9915$). Method accepted.	5 independent solutions prepared by diluting 5 independent weightings of formulated product and analysed. In the formulated product: %RSD = 2.1 Method accepted.	2 solutions containing a known quantity of the a.i. were prepared. The samples consisted of blank formulation to which a known quantity of reference item (technical product) was added. In the formulated product: Mean recovery = 97.39% Method accepted.

Comments:

The analytical method is suitable and reliable for the determination of the number of CFU of *Lecanicillium muscarium* strain 19.79 in the formulated product MYCOTAL because the method satisfies the requirements given by the SANCO/3030/99 rev. 4 guideline concerning linearity, precision and accuracy.

B.5.1.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform

See Volume 3MA B.5.1.2.

B.5.1.3 Methods to identify any contaminating micro-organisms of the preparation

See Volume 3MA B.5.1.7.

B.5.1.4 Methods for the determination of relevant impurities or metabolites in the manufactured material

See Volume 3MA B.5.1.6.

B.5.1.5 Methods used to determine the storage stability and shelf life of the preparation

See Volume 3MA B.5.1.8.

B.5.2 Methods to determine and quantify residues (viable or non-viable)

Information from the original DAR

Residues of the active micro-organism are determined by plating samples onto malt agar extract or selective medium (Rose bengal chloramphenicol agar). Colonies can be identified by morphological identification methods (see addendum 2009 B.5.1.5)..

A method to determine non-viable residues is not necessary (see Volume 3MA B.7).

B.5.3 References relied on

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
KMA 5.1/01	Coranelli, S.	2014	ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF THE ACTIVE INGREDIENT CONTENT IN THE FORMULATED PRODUCT MYCOTAL Koppert, BT062/14 Biotecnologie BT Srl, Fraz. Pantalla, Italy GLP: yes Published: no	Y New data for new formulation, not previously submitted nor evaluated	KBS