Scientia Terrae

10.1.c Wob
juncto 63.2.d
Vo 1107/2009

3 batch analysis report – physicochemical parameters

[redacted] PepMV, CH2 strain, isolate 1906

Content

Scientia Terrae *performs research based on the on this moment leading scientific views and knowledge. Since the PPP analysed in this study is a plant virus to be used for crossprotection or vaccination purposes, and no such products have previously been registered as PPP in Europe, no specific guidelines or reference protocols could be followed. Scientia Terrae used all its relevant knowledge and expertise to perform this study. The study was conducted with uttermost care, following internal quality standards. Scientia Terrae will not accept any responsibility for possible damage which is directly or indirectly the consequence of analyses, judgments or recommendations made in this report.*

1. STUDY DETAILS

PRODUCT NAME:

[redacted] PEPMV, CH2 STRAIN, ISOLATE 1906

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FORMULATION TYPE:

The production process is described in detail in the technical dossier (Tier II, Document M-MPCA, IIM 1.4.3 and IIM 4.2.8).

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[redacted]

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PHYSICOCHEMICAL PARAMETERS TESTED

The goal of the presented 3-batch analysis was to verify the reproducibility of the production process and the consistency of the formulated product for a set of 5 general quality parameters and for 2 quality parameters that are specific for SC formulations (Table 1). [redacted] testing of suspensibility and wet sieve residue were deemed the most critical parameters and therefore relevant for testing. [redacted]

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Table 1: Quality control criteria as defined in the Annex 2 dossier

General quality parameter	Method	Range (unit)	
		From	To
Viscosity	ST-LAB-03 adapted from OECD 114 (Brookfield LV-DVE)	2 cP	4 cP
pH	ST-LAB-01 adapted from CIPAC MT 75 (telqel, 20°C)	5,4	6
Relative density	CIPAC MT 3.2.1 (20°C)	1,001	1,005
Colour	Visual assessment	dark green	brownish green
Concentration of PepMV CH2 strain isolate 1906	RT-qPCR (Gutiérrez-Aguirre et al, 2009)	> $5*10^5$ genome copies per µl [redacted]	
SC-specific quality parameter	**Method**	**Requirement**	
Wet sieve residue	ST-LAB-07 adapted from CIPAC MT 59.3	max 2% residue on 75 µm (200 mesh) sieve	
Suspensibility*	ST-LAB-11 adapted from CIPAC 161	difference in C_T value < 3	

* suspensibility is determined only for this 3 batch analysis and will not be determined for each production batch

PRODUCTION SPECIFICATIONS

Production date: [redacted]

Batch numbers: [redacted]

[redacted]

[redacted]

Production location: [redacted]

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2. TESTING METHODS USED

Viscosity

The viscosity of the formulation was determined on a Brookfield rotational viscosity meter (LV-DVE) according to method ST-LAB-03 (see annex 1).

pH

The pH of the formulation (undiluted, telqel) was determined on a Hanna pH/mV-meter equipped with a glass electrode and temperature probe, after calibration with two buffers (pH 7 and pH 4), according to method ST-LAB-01 (see annex 2).

Relative density

The relative density was determined according to CIPAC MT 3.2.1 with a capillary-stoppered pyknometer.

Concentration of PepMV CH2 strain isolate 1906

A TaqMan RT-qPCR assay was used to determine the concentration of PepMV virus particles in the formulation (Gutiérrez-Aguirre I. et al., 2009). This method measures the amount of viral genetic material (RNA) and results in C_T-values (threshold detection cycles). Note that the C_T-value is inversely correlated with the virus concentration: the higher the C_T-value, the lower the virus concentration. [redacted] The standard curve and the details of the method are provided in the technical dossier (Tier II, Document M-MCPA, IMM 1.4.1).

Reference :

Gutiérrez-Aguirre I. et al., 2009. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162 : 46-55.

Wet sieve residue

The residue over a 75 µm (200 mesh) sieve was determined by wet sieving according to ST-LAB-07 (see annex 3).

Suspensibility

The suspensibility was determined according to method ST-LAB-11 which was especially developed for this 3 batch analysis and that was adopted from CIPAC MT 161 (see annex 4).

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Due to the sensitivity of the virus for high temperatures, the test was carried out with standard water C of 20°C instead of 30°C. Due to the specific nature of the formulation, with the virus particles being the actual active ingredients, the TaqMan RT-qPCR method must be used to measure the concentrations and concentration differences of the active ingredient. The reproducibility of the TaqMan RT-qPCR method was verified in standardized dilution series (see also the technical dossier Tier II, Document M-MPCA, IIM 1.4.1). [redacted]

3. RESULTS

3.1. GENERAL QUALITY PARAMETERS

Table 2: Measurements of general quality control parameters on 3 batches

Parameter (unit)	18102011_1	18102011_2	18102011_3	Mean
Viscosity (cP)	[redacted]			
pH				
Relative density				
Colour				
Concentration of PepMV CH2 strain isolate 1906 (genome copies per µl)*				

Conclusion:

All three batches meet the product-specific quality control criteria presented in Table 1. The number of viral genome copies per µl and the fysicochemical parameters were not significantly different in all three batches. These results confirm that the production process is consistent and reproducible.

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3.2. SC-SPECIFIC PARAMETERS

3.2.1. Wet sieving

The results of the wet sieve test over a 75 µm sieve are indicated in table 3.

Table 3: Dry weight (100°C) of the wet sieve residues

Batch number	Dry weight of sieve residue (grams)	Sample weight (grams)	% (m/m) of formulation
[redacted]			

For all three batches [redacted]

Conclusion:

All three batches meet the 'wet sieve residue' (CIPAC MT 59.3) criterion for SC formulations (less than 2% m/m over 75 µm).

3.2.2. Suspensibility test

The suspensibility test was carried out according to method ST-LAB-11, adapted from CIPAC MT 161. The results are summarized in tables 4 (C_T values) and 5 (virus genome copies per µl).

Table 4: Results of the suspensibility test expressed (C_T-values)

Concentration of PepMV CH2 strain isolate 1906 (C_T value)				
Batch number	10^{-1} dilution	10^{-1} dilution	10^{-2} dilution	10^{-2} dilution
	C_T of total volume	C_T of lower 10 volume %	C_T of total volume	C_T of lower 10 volume %
[redacted]				

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Table 5: Results of suspensibility test expressed in genome copies per µl

Concentration of PepMV CH2 strain isolate 1906 (genome copies)				
Batch number	10^{-1} dilution	10^{-1} dilution	10^{-2} dilution	10^{-2} dilution
	C_T of total volume	C_T of lower 10 volume %	C_T of total volume	C_T of lower 10 volume %
[redacted]				
[redacted]				
[redacted]				

[redacted] This means that no significant sedimentation of the virus particles to the lower layer of the dilution occurs in the dilution to be used for spraying.

4. OVERALL CONCLUSION

Based on these analyses on three different batches, it can be concluded that the production process is reproducible, the product formulation is consistent and the formulation type meets the criteria for suspension concentrates (SC).

Report finalised on 18th July 2012. All tests and analyses were performed following internal quality standards.

List of Annexes:

Annex 1: ST-LAB-03 (viscosity)

Annex 2: ST-LAB-01 (pH)

Annex 3: ST-LAB-07 (Wet sieve residue)

Annex 4: ST-LAB-11 (Suspensibility)

Study directors:

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Signature

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