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63.2.d Vo 1107/2009

Physicochemical parameters

[redacted]epMV, CH2 strain, isolate 1906

Additional tests 'Suspensibility' and 'Spontaneity of Dispersion'

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

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PRODUCT NAME:

[redacted]PEPMV, CH2 STRAIN, ISOLATE 1906

FORMULATION TYPE:

The product is manufactured [redacted]

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PHYSICOCHEMICAL PARAMETERS TESTED

The presented analyses were performed in completion of the physicochemical studies presented in (1) the 3 batch analyses report for physicochemical parameters performed by Scientia Terrae (18/07/2012) and (2) Physical and chemical properties and low temperature storage stability of PMV-01, Study number 22973 performed by CRA-W, Gembloux (17/7/2012). Suspensibility tests were performed in the 3 batch study in 1% and 10% concentrations. Here, suspensibility tests and analyses were performed on the lowest and highest concentrations of use, more specifically 4L in 300L water or 1.3% and 8L in 160L water or 5%. In addition, the physicochemical parameter 'spontaneity of dispersion' was determined for the highest concentration of use 5%.

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Table 1: Parameters determined in this study

General quality parameter	**Method**	**Concentration**	**Criteria**
1. Concentration of PepMV CH2 strain isolate 1906	RT-qPCR (Gutiérrez-Aguirre et al, 2009)	100%	> $5*10^5$ genome copies per µl [redacted]
SC-specific quality parameter	**Method**		**Requirement**
Suspensibility	ST-LAB-11 adapted from CIPAC 161	1,3% and 5%	[redacted]
Spontaneity of dispersion	ST-LAB-12 adapted from CIPAC MT 160	5%	[redacted]

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1. PRODUCTION SPECIFICATIONS

Production date: [redacted]

Batch number: [redacted]

Production location: [redacted]

2. TESTING METHODS USED

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]

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.

Suspensibility

The suspensibility was determined according to method ST-LAB-11 which was especially developed for this analysis and that was adopted from CIPAC MT 161 (see annex 1).

Spontaneity of dispersion

The spontaneity of dispersion was determined according to method ST-LAB-12 which was especially developed for this analysis and that was adopted from CIPAC MT 160 (see annex 2).

General remark:

Due to the sensitivity of the virus for high temperatures, the tests were carried out with standard water temperatures 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. Duplicate analyses from the same sample result in C_T-values that differ maximum [redacted]

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[redacted]

3. RESULTS

3.1. GENERAL QUALITY PARAMETERS

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

Parameter (unit)	[redacted]
Concentration of PepMV CH2 strain isolate 1906 (genome copies per µl)*	[redacted]

Conclusion:

The batch meets the product-specific quality control criterium presented in Table 1.

3.2. SC-SPECIFIC PARAMETERS

3.2.1. 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	1,3% dilution	1,3% dilution	5% dilution	5% 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]				

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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	1.3% dilution	1.3% dilution	5% dilution	5% 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]is means that no significant sedimentation of the virus particles to the lower layer of the dilution occurs in the dilution range to be used for spraying.

3.2.2. Spontaneity of dispersion test

The spontaneity test was carried out according to method ST-LAB-12, adapted from CIPAC MT 160. The results are summarized in tables 6 (C_T values) and 7 (virus genome copies per µl).

Table 6: Results of the spontaneity of dispersion test expressed (C_T-values)

Concentration of PepMV CH2 strain isolate 1906 (C_T value)		
Batch number	5% dilution	5% dilution
	C_T of total volume	C_T of lower 10 volume %
[redacted]		

Table 7: Results of spontaneity of dispersion test expressed in genome copies per µl

Concentration of PepMV CH2 strain isolate 1906 (genomes copies)		
Batch number	5% dilution	5% dilution
	C_T of total volume	C_T of lower 10 volume %
[redacted]		

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[redacted]

4. OVERALL CONCLUSION

Based on these analyses, it can be concluded that the product meets the suspensibility and spontaneity of dispersion criteria for suspension concentrates (SC).

Report finalized on 28th September 2012. All tests and analyses were performed following internal quality standards.

List of Annexes:

Annex 1: ST-LAB-11-2 (Suspensibility)

Annex 2: ST-LAB-12 (Spontaneity of dispersion)

Study directors:

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[redacted]

Signatur[redacted]

[redacted]

Signature [redacted]

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Determination of suspensibility of PepMV-CH2-1906 (ST-LAB-11-2)

[redacted]

[redacted]

[redacted]

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Determination of suspensibility of PepMV-CH2-1906 (ST-LAB-11-2)

[redacted]

[redacted]

[redacted]

[redacted]

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Determination of spontaneity of dispersion of PepMV-CH2-1906 (ST-LAB-12)

[redacted]