syngenta

Analytical Development
& Product Chemistry

Syngenta Crop Protection AG
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Emamectin benzoate (MK244)

Determination of Physical-Chemical Properties

Statement on the Fitness for Purpose of the Analytical Methods

The determination of the octanol / water partition coefficient, water solubility and vapour pressure has been performed under GLP and reported as study number PMLMK244001 (reference 1).

This statement summarises and demonstrates the fitness for purpose of the analytical methods used.

For the determination of the octanol / water partition coefficient, six determinations of the coefficient: three at one concentration and three at one-tenth this concentration, at pH 5, 7 and 9, were made.

For the determination of the water solubility, triplicate determinations of the solubility were made at pH 5, 7 and 9.

The determination of the vapour pressure was performed in triplicate using the gas saturation method.

Emamectin benzoate consists of two homologues: B1b and B1a.

Under the conditions used in the analytical methods the two homologues were not separated.

Reference 1

Study Title: **Determination of Physical-Chemical Properties of MK-244**
Study Number: **PMLMK244001**
Study Date: **17 December 1992**

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1 OCTANOL / WATER PARTITION COEFFICIENT

The octanol / water partition coefficient for emamectin benzoate was determined, using aqueous buffer solutions, at pH 5, 7 and 9, by the "shake-flask" method. At each pH, triplicate determinations, using two stock solutions of emamectin benzoate, differing by about a factor of ten, were made. After equilibration, the concentrations of emamectin benzoate in the two phases were determined by HPLC analysis.

All aliquots from the two, clear, separated non-miscible phases were so diluted that the final concentration of emamectin benzoate in all test solutions were comparable to those of the three reference solutions

The average log P values at pH 5, 7 and 9 were 3.0, 5.0 and 5.9 respectively.

Experiment at pH 5

Two stock solutions of emamectin benzoate in octanol were prepared: their concentrations were 8.2305 mg/ml (for runs 1, 2 and 3) and 0.9877 mg/ml (for runs 4, 5 and 6).

Three reference solutions were prepared with concentrations of 0.05240 µg/ml, 0.5240 µg/ml and 5.240 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999994	Y´ = 274485.0 * X - 359.277

Partition Coefficient and log P

Run	Partition Coefficient	log P
1	**1088.4**	**3.03681**
2	**1185.3**	**3.0738**
3	**1259.9**	**3.1003**
4	**602.8**	**2.780**
5	**718.3**	**2.856**
6	**852.2**	**2.931**
Average		**3.0**
Standard Deviation		**± 0.1**

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Recovery Data

Run	Recovery
1	95.2
2	95.4
3	96.7
Average	95.8
Standard Deviation	± 0.7
4	103.7
5	103.7
6	106.5
Average	104.6
Standard Deviation	± 1.3

Experiment at pH 7

Two stock solutions of emamectin benzoate in octanol were prepared: the concentrations were 8.3156 mg/ml (for runs 71, 72 and 73) and 0.9979 mg/ml (for runs 74, 75 and 76).

Three reference solutions were prepared with concentrations of 0.05240 µg/ml, 0.5240 µg/ml and 5.240 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999991	Y´ = 272517.5 * X - 436.023

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Partition Coefficient and log P

Run	Partition Coefficient	log P
71	6495.12	4.8126
72	103384.5	5.0145
73	63947.0	4.8058
74	96635.5	4.9851
75	216696.2	5.3356
76	171692.1	5.2348
Average		5.0
Standard Deviation		± 0.2

Recovery Data

Run	Recovery
71	103.2
72	98.3
73	96.0
Average	99.2
Standard Deviation	± 3.0
74	101.3
75	105.6
76	101.8
Average	102.9
Standard Deviation	± 2.4

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Experiment at pH 9 (stock solution of emamectin benzoate in octanol: 9.0896 mg/ml)

Three reference solutions were prepared with concentrations of 0.0517 µg/ml, 0.5176 µg/ml and 5.176 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 276951.8 * X + 3980.388

Partition Coefficient and log P

Run	Partition Coefficient	log P
91	**9.7513×10^5**	**5.98906**
92	**1.07937×10^6**	**6.03317**
93	**5.2830×10^6**	**6.7229**
Average		**6.2**
Standard Deviation		**± 0.3**

Recovery Data

Run	Recovery
91	**98.0**
92	**96.4**
93	**98.1**
Average	**97.5**
Standard Deviation	**± 0.8**

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Experiment at pH 9 (stock solution of emamectin benzoate in octanol: 1.2388 mg/ml)

Three reference solutions were prepared with concentrations of 0.04976 µg/ml, 0.4976 µg/ml and 4.976 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 240963.0 * X + 4092.055

Partition Coefficient and log P

Run	Partition Coefficient	log P
94	**3.1715×10^5**	**5.5013**
95	**3.0659×10^5**	**5.4866**
96	**3.4821×10^5**	**5.5418**
Average		**5.51**
Standard Deviation		**± 0.02**

Recovery Data

Run	Recovery
91	**97.9**
92	**92.4**
93	**101.7**
Average	**97.3**
Standard Deviation	**± 3.8**

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Partition Coefficient and log P - six results

Run	log P
91	5.98906
92	6.03317
93	6.7229
94	5.5013
95	5.4866
96	5.5418
Average	5.9
Standard Deviation	± 0.4

Accuracy (specificity)

High-purity reagents and octanol were used for the preparation of the mutually-saturated buffer solutions and octanol.

High-purity reagents were used for the dilution of the separated phases.

The method is sufficiently sensitive and reproducible.

It is specific for emamectin benzoate and no interference from other components would be expected. The repeatability figures also indicate no interference occurred

Acceptable linearity has been demonstrated for emamectin benzoate

Acceptable recovery has been obtained for emamectin benzoate

The method has been shown to be specific for the determination of emamectin benzoate, no interference was observed.

Accuracy (specificity, recovery, linearity) and precision (repeatability) were acceptable.

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2 WATER SOLUBILITY

The solubility in water (using aqueous buffer solutions) of emamectin benzoate was determined at pH *5,* 7 and 9 by the flask method. After equilibration, the concentrations of emamectin benzoate were determined by HPLC analysis.

All aliquots from the clear, saturated solutions were so diluted that the final concentration of emamectin benzoate in all test solutions was comparable to those of the three reference solutions

The average water solubility at pH 5, 7 and 9 was 320 mg/l, 24 mg/l and 0.1 mg/l respectively.

Experiment at pH 5

Three separate quantities of emamectin benzoate were weighed and transferred to three glass tubes. A known volume of the buffer solution was added to each tube and then the tubes were sealed and placed in a water bath maintained at the test temperature. On successive days. one tube was removed from the bath for analysis.

One day one, four reference solutions were prepared with concentrations of 0.003161 µg/ml, 0.3161 µg/ml, 3.161 µg/ml and 31.61 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999969	Y´ = 267288.7 * X - 20089.17

One day two, four reference solutions were prepared with concentrations of 0.005040 µg/ml, 0.05040 µg/ml, 0.5040 µg/ml and 5.040 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999983	Y´ = 280757.6 * X + 5183.376

One day three, the four reference solutions prepared for day two were re-used.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999950	Y´ = 288661.5 * X + 7994.205

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Water Solubility

Run	Solubility / [mg/l]
51	352.72
52	299.3
53	317.91
Average	320
Standard Deviation	± 27

Experiment at pH 7

The experiment at pH 7 was performed concurrently with that at pH 5. Consequently, only the solubility results are given in this section

Water Solubility

Run	Solubility / [mg/l]
71	26.081
72	20.60
73	25.175
Average	24
Standard Deviation	± 2.9

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Experiment at pH 9

The experiment at pH 9 was performed using a procedure similar to that used at pH 5 and at pH 7.

On day one, three reference solutions were prepared with concentrations of 0.05240 µg/ml, 0.5240 µg/ml and 5.240 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999995	Y´ = 272775.4 * X - 486.722

On day two, three reference solutions were prepared with concentrations of 0.05189 µg/ml, 0.5189 µg/ml and 5.189 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 273715.9 * X + 2133.5

On day three, three reference solutions were prepared with concentrations of 0.05253 µg/ml, 0.5253 µg/ml and 5.253 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 269584.0 * X + 3511.833

Water Solubility

Run	Solubility / [mg/l]
91	**0.034**
92	**0.2795**
93	**0.002183**
Average	**0.11**
Standard Deviation	**± 0.15**

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Accuracy (Recovery)

To ensure that the solvents were and remained saturated with respect to emamectin benzoate, an excess of the test substance was added to the water prior to equilibration. Therefore, it is not possible to present recovery data based upon determinations of test samples as their method of preparation, in accordance with the test guidelines, prevents a knowledge of exactly how much test item has been added to and dissolved in each solvent. However, based upon the good precision of the results there is no evidence to suggest that the recovery of emamectin benzoate was insufficient.

Acceptable recovery has been obtained for emamectin benzoate

Accuracy (specificity)

High-purity reagents and octanol were used for the preparation of the mutually-saturated buffer solutions and octanol.

High-purity reagents were used for the dilution of the separated phases.

The method is sufficiently sensitive and reproducible.

It is specific for emamectin benzoate and no interference from other components would be expected. The repeatability figures also indicate no interference occurred

Acceptable linearity has been demonstrated for emamectin benzoate

Acceptable recovery has been obtained for emamectin benzoate

The method has been shown to be specific for the determination of emamectin benzoate, no interference was observed.

Accuracy (specificity, recovery, linearity) and precision (repeatability) were acceptable.

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3 VAPOUR PRESSURE

The vapour pressure of emamectin benzoate was determined with the gas saturation method using nitrogen gas and a C-18 adsorbent. A HPLC method was used to determine the adsorbed emamectin benzoate after having been washed from the adsorbent.

All aliquots prepared were so diluted that the final concentration of emamectin benzoate in all test solutions was comparable to those of the three reference solutions

The vapour pressure was determined to be 3.9×10^{-6} Pa at 21 °C

Experiment at flow rate 37.5 ml/min

Three reference solutions were prepared with concentrations of 0.05253 µg/ml, 0.5253 µg/ml and 5.232 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 269584.0 * X + 3511.833

Experiment at flow rate 30.2 ml/min

Three reference solutions were prepared with concentrations of 0.04976 µg/ml, 0.4976 µg/ml and 4.976 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999999	Y´ = 240963.0 * X + 4092.055

Experiment at flow rate 87.0 ml/min

Three reference solutions were prepared with concentrations of 0.04154 µg/ml, 0.4154 µg/ml and 4.154 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999909	Y´ = 244556.5 * X + 9469.166

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Vapour Pressure

Emamectin benzoate adsorbed / [µg]	Flow rate / [ml/min]	Vapour Pressure / [10^{-6} Pa]
0.04315	37.5	2.05
0.1097	30.2	6.12
0.1930	87.0	3.59
	Average	3.9
	Standard Deviation	± 2.1

Accuracy (Recovery)

The efficiency of the C-18 adsorbent for emamectin benzoate and the recovery of emamectin benzoate from the adsorbent were verified as described.

A 5.0 ml aliquot of a solution of emamectin benzoate.in methanol of concentration 28.09 µg/ was passed through one cartridge of the C-18 adsorbent. The eluant was collected and analysed. The adsorbent was rinsed with a 2.0 ml aliquot of a 0.2 % solution of phosphoric acid in methanol. This was subsequently collected and also analysed.

Three reference solutions were prepared with concentrations of 0.05040 µg/ml, 0.5040 µg/ml and 5.040 µg/ml.

Regression analysis is summarised below

	Correlation Coefficient (r^2)	Regression line Y´
Emamectin benzoate	0.999967	Y´ = 284166.9 * X + 8097.5

The amount of emamectin benzoate adsorbed by the C-18 adsorbent was 99. 9 %

The recovery (= amount of emamectin benzoate subsequently rinsed from the C-18 adsorbent) was 98.3 %

Acceptable recovery has been obtained for emamectin benzoate

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Accuracy (specificity)

High-purity reagents were used for the determination of emamectin benzoate

The method is sufficiently sensitive and reproducible.

It is specific for emamectin benzoate and no interference from other components would be expected. The repeatability figures also indicate no interference occurred

Acceptable linearity has been demonstrated for emamectin benzoate

Acceptable recovery has been obtained for emamectin benzoate

The method has been shown to be specific for the determination of emamectin benzoate, no interference was observed.

Accuracy (specificity, recovery, linearity) and precision (repeatability) were acceptable.

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