MALATHION

DETERMINATION OF MALAOXON IN MALATHION DP

(Adapted from Cheminova analytical method VAM 208-01)

Principle

Malaoxon is separated by reversed-phase HPLC, detected by UV absorption and determined by and external standardization.

Chemicals

Acetonitrile, Lichrosolv®, Merck Art.14291, or equivalent. (solvent B)

Water, HPLC grade (solvent A).

Malaoxon, reference standard, as pure as practicable.

Prepare an approximately 1% solution of the reference material by weighing accurately about 0.1 g (a g) into a tared 12 ml sample bottle with screw cap. Add 10 ml acetonitrile, weigh again (b g) and mix well (stock solution).

Weigh accurately 50 µl of the stock solution (c g) into a tared sample bottle with screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (d g) and mix well (Solution 1).

Weigh an aliquot of 5 ml of Solution 1 (e g.) into a tared sample bottle. Add 5 ml of 75% v/v acetonitrile/water, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 5 ml of Solution 2 (g g) into a tared sample bottle. Add 5 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well (Solution 3).

Weigh an aliquot of 2 ml of Solution 3 (i g) into a tared sample bottle. Add 3 ml of 75% v/v acetonitrile/water, weigh again (j g) and mix well (Solution 4).

Weigh an aliquot of 1 ml of Solution 4 (k g) into a tared sample bottle. Add 1.5 ml of 75% v/v acetonitrile/water, weigh again (l g) and mix well (Solution 5).

Solutions 1, 2, 3, 4 and 5 are injected into the liquid chromatograph.

Apparatus

HPLC system, equipped with binary eluent delivery system, autosampler, photodiodearray detector and data handling system.

Analytical column, Phenomenex Prodigy ODS2, 5 µm, 150 mm x 4.6 mm, or equivalent. Two columns are connected in series to form a column of 300 mm length.

Guard column, Phenomenex Prodigy ODS2, 5 µm, 30 mm x 4.6 mm, or equivalent.

Typical operating parameters

Gradient programme	time (min.)	% B
	0.0	35
	10.0	35
	10.5	95
	15.5	95
	16.0	35
Flow rate	1.5 ml/min.	
Stop time:	20 min.	
Post time:	10 min.	
DAD stop time:	15 min.	
Column temperature:	50ºC.	
Signals	sample, 215 nm (8 nm bandwidth) interference check, 230 nm (8 nm bandwidth) reference (400 nm (100 nm bandwidth)	
Slit width	8 nm	
Spectrum	190-400 nm in 2 nm steps	
Injection volume	50 µl	
Integration	peak area	
Typical retention times	11.1 min (malaoxon) 14.1 (malathion)	

System suitability checks

Lamp test

Check the lamp intensity and the wavelength calibration of the detector (holmium oxide check) as described in the operating manual for the liquid chromatograph and make sure they meet the defined criteria.

Repeatability

Inject Solution 2 at least three times or until the peak area obtained from malaoxon does not differ by more than 10% between two successive measurements.

Linearity

Inject Solutions 1, 2, 3, 4 and 5 and measure the peak areas of the malaoxon. Having calculated the concentrations of the solutions from the weights measured, calculate the linear regression coefficient (r^2) of the calibration curve, which should be >0.98.

Carry over

Inject a blank solution after Solution 2 and measure the peak area obtained for malaoxon. The "carry over" from the previous injection is acceptable if ≤2.0% of solution 2.

Interference

Ensure that there is clear baseline separation between the isomalathion and malathion in a test solution.

Preparation of test solutions

DP formulations

Weigh accurately 2 g malathion DP (m g) into a tared 12 ml sample bottle with a screw cap. Tare the bottle and add 4 ml of 75% v/v acetonitrile/water, weigh again (n g) and sonicate the sample for 10 min. Centrifuge the solution for 5 min. and weigh accurately an aliquot of 0.5 ml of the clear liquid (o g) into a tared 12 ml sample glass. Add 1.5 ml of 65% v/v acetonitrile/water, weigh again (p g) and mix well.

Prepare duplicate test solutions for each test sample. These solutions are injected into the HPLC.

If the area of the malaoxon peak observed exceeds that obtained from the most concentrated calibration solution, dilute the solutions accordingly, using 75% v/v acetonitrile/water.

HPLC analysis

Inject the test and standard solutions in the following sequence:

Solution 3 , T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, Solution 3, T_9, ….T_{16} Solution 3, T_{17}… etc. Where T_1….T_n are test solutions 1 to n and only one injection is made from each vial. Recalculate the response factor after each Solution 3 measurement and end the sequence with an injection of Solution 3.

Sequential injections of Solution 3 should produce peak areas within 5% of each other. Examine spectra or wavelength ratios obtained across the malaoxon peaks detected, to ensure that there is no significant interference from other components.

Calculation

Measure the peak areas of malaoxon both from the reference solution and the test solution.

Determine the malaoxon content of the test sample, in g/kg, as follows:

$$\text{malaoxon (g/kg)} = \frac{\text{peak area of malaoxon in test solution} \times n \times p \times r_f}{m \times o}$$

where: m, n, o and p are the weights (g) measured in the preparation of the test solutions, described above;
r_f is the response factor, determined from Solution 3 as follows:

$$r_f = \frac{\text{purity (g/kg) of malaoxon (ref. material)} \times a \times c \times e \times g}{\text{peak area of malaoxon in Solution 3} \times b \times d \times f \times h}$$

where: a, b, c, d, e, f, g and h are the weights (g) measured in the preparation of Solution 3, described above.