MALATHION

DETERMINATION OF ISOMALATHION IN MALATHION TC, UL, DP AND EW

(Adapted from Cheminova analytical method VAM 005-03)

Principle

Isomalathion is separated by reversed-phase HPLC and determined by UV-absorption, with external standardization.

Chemicals

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

Water, HPLC grade (solvent A).

Isomalathion, 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 of 75% v/v acetonitrile/water, weigh again (b g) and mix well (stock solution).

Weigh accurately 180 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 2 ml of Solution 1 (e g.) into a tared sample bottle. Add 6 ml of 75% v/v acetonitrile/water, weigh again (f g) and mix well (Solution 2).

Weigh an aliquot of 1 ml of Solution 2 into a tared sample bottle. Add 2 ml of 75% v/v acetonitrile/water, weigh again and mix well (Solution 3).

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

Solutions 1, 2, 3 and 4 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 Sphereclone ODS2, 5 µm, 120 mm x 4.6 mm, or equivalent.

Guard column, Phenomenex Sphereclone ODS2, 5 µm, 50 mm x 4.6 mm, or equivalent.

Typical operating parameters

Gradient and flow programme	time (min.)	% B	flow (ml/min.)
	0.0	40	1.0
	8.0	40	1.0
	9.0	95	2.0
	12.0	95	2.0
	12.5	40	1.0
Stop time:	14 min.		
Post time:	1 min.		
DAD stop time:	8 min.		
Column temperature:	50ºC.		
Signals	sample, 200 nm (8 nm bandwidth) interference check, 225 nm (8 nm bandwidth) reference (360 nm (100 nm bandwidth)		
Slit width	4 nm		
Spectrum	190-400 nm in 2 nm steps		
Injection volume	25 µl		
Integration	peak area		
Typical retention time	6.3 min (isomalathion)		

System suitability checks

Repeatability

Inject Solution 2 at least three times or until the peak area obtained from isomalathion does not differ by more than 5.0% between two successive measurements.

Linearity

Inject Solutions 1, 2, 3 and 4 and measure the peak areas of the isomalathion. 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 >0.998.

Carry over

Inject a blank solution after Solution 2 and measure the peak area obtained for isomalathion. 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

Technical material

Weigh accurately 0.3 g malathion TC (g g) into a tared 12 ml sample bottle with a screw cap. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. This solution is injected into the HPLC.

EW formulations

Weigh accurately sufficient EW to contain about 0.06 g malathion (g g) into a tared 12 ml sample bottle. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution if it appears cloudy and transfer 1-2 ml of the upper liquid to an autosampler vial.

DP formulations

Weigh accurately sufficient DP to contain about 0.3 g of malathion (g g) into a tared 12 ml sample bottle. Add 10 ml of 75% v/v acetonitrile/water, weigh again (h g) and mix well. Sonicate the solution in an ultrasonic bath for 10 minutes. Approximately half-way through the period, handshake the mixture vigorously. Centrifuge the solution and transfer 1-2 ml of the upper liquid to an autosampler vial.

All test solutions

If the area of the isomalathion peak observed exceeds that obtained from the most concentrated calibration solution, dilute the solution accordingly.

HPLC analysis

Inject the test and standard solutions in the following sequence:

Solution 2 , T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, Solution 2, T_9, ….T_{16} Solution 2, 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 2 measurement and end the sequence with an injection of Solution 2.

Sequential injections of Solution 2 should produce peak areas within 5% of each other. Examine spectra or wavelength ratios obtained across the isomalathion peaks detected, to ensure that there is no significant interference from other components.

Calculation

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

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

$$\text{isomalathion (g/kg)} = \frac{\text{peak area of isomalathion in test solution} \times h \times r_f}{g}$$

where: g and h are the weights (g) measured in the preparation of the test solutions, described above;
r_f is the response factor, determined from Solution 2 as follows:

$$r_f = \frac{\text{purity (g/kg) of isomalathion (ref. material)} \times a \times c \times e}{}$$

peak area isomalathion in Solution 2 x b x d x f

where: a, b, c, d, e and f are the weights (g) measured in the preparation of Solution 2, described above.

Determine the isomalathion content of formulation samples, in % w/w of the malathion content, as follows:

$$\text{isomalathion (\% w/w of malathion)} = \frac{\text{isomalathion (g/kg)} \times 100}{\text{malathion content (g/kg)}}$$