CHEMINOVA

Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

Phone (+45) 96 90 96 90
Fax (+45) 96 90 96 91
www.cheminova.com
CVR-No. DK 12 76 00 43

ANALYTICAL METHOD AM 443.2

Determination of Omethoate (CAS No. 1113-02-6) and Isodimethoate (CAS No. 3344-11-4) in Dimethoate technical, Dimethoate concentrates and EC formulations

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Author: Elsa V. Sørensen — Analytical Dept. / EVS — February 20, 2002 — Date

Checked and approved by: Elsa V. Sørensen — Head of Analytical Dept. / EVS — February 20, 2002 — Date

Summary

Analytical method for determination of two impurities in Dimethoate technical, Dimethoate concentrates and formulations.

The analytical method is validated by Cheminova A/S. The validation project is conducted in accordance with the OECD principles of Good Laboratory Practice and FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The analytical method has undergone independent laboratory validation (ILV) by Institut Fresenius, Germany and Budapest Plant Health and Soil Conservation Station, Hungary.

A prerequisite for the use of this test method is that the person carrying it out has appropriate training in physical chemical testing/analysis technique, is familiar with the safe handling of chemicals and testing equipment, and has been informed about general laboratory guidelines relating to safety precautions and accident prevention and strictly observes these.

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Principle

Reverse phase liquid chromatography (HPLC) using ODS2 column, UV detection and quantitation by external standard method.

Precision, Accuracy and Limit of Quantitation (Lowest validated fortification)

When used in the concentration level interval mentioned below the accuracy and precision are:

Dimethoate technical	CAS No.	Concentration level in test sample [w/w%]	Mean value for Accuracy in terms of recovery, [%]	Maximum values for Precision, RSD (n=6), [%]	Lowest valited fortificati on (test sample) [ppm]
Omethoate	1113-02-06	< 0.1	117	1.33	240
Omethoate	1113-02-06	0.1 - 0.24	104	1.60	
Isodimethoate	3344-11-4	< 0.1	107	1.4	420
Isodimethoate	3344-11-4	0.1 - 0.39	105	1.3	

Dimethoate technical concentrations	CAS No.	Concentration level in test sample [w/w%]	Mean value for Accuracy in terms of recovery, [%]	Maximum values for Precision, RSD (n=6), [%]	Lowest validated fortificati on (test sample) [ppm]
Omethoate	1113-02-06	< 0.1	110	4.1	140
Omethoate	1113-02-06	0.1 - 0.16	100	0.78	
Isodimethoate	3344-11-4	< 0.1	107	2.5	350
Isodimethoate	3344-11-4	3.8	101	0.66	

Dimethoate EC formulations	CAS No.	Concentration level in test sample [w/w%]	Mean value for Accuracy in terms of recovery, [%]	Maximum values for Precision, RSD (n=6), [%]	Lowest validated fortificati on (test sample) [ppm]
Omethoate	1113-02-06	< 0.1	92	2.9	150
Omethoate	1113-02-06	0.1 - 0.17	94	4.3	
Isodimethoate	3344-11-4	< 0.1	108	1.4	460
Isodimethoate	3344-11-4	3.6	102	1.5	

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The method is considered satisfactory according to the recommended level of precision described in EPA's Pesticide Assessment Guidelines* which is -

- not greater than 10% for ingredients measured in the sample in a concentration from 0.1 w/w% up to 1.0 w/w%, and
- not greater than 20% for ingredients measured in the sample in a concentration less than 0.1 w/w%.

The method is also meeting the requirements outlined in EUROPEAN COMMISION, SANCO/3030/99, rev. 4, 11/07/00 working document.

* Reference: Pesticide Assessment Guidelines, Subdivision D: Product Chemistry
prepared by G. J. Beusch et al. Washington D.C.
U.S. Environmental Protection Agency (EPA)
Office of Pesticide and Toxic Substances
Springfield VA
National Technical Information Service (NTIS), October 1982,
(PB83-153890)
(EPA 540/9-82-018)
p. 61.

1. Apparatus and parameters

Apparatus and parameters used by Cheminova A/S:

Liquid chromatograph:	Hewlett Packard HP 1100 equipped with binary eluent delivery system, autosampler and photodiodearray detector.
Data handling system:	HP Chemstation and HP laser Jet 4000 Printer.
Column:	Phenomenex Sphereclone ODS2, 5 µm, 120 mm x 4.6 mm.
Guard column:	Phenomenex Sphereclone ODS2, 5 µm, 50 mm x 4.6 mm.
Chemicals:	Acetonitrile, Lichrosolv®, Merck Art. 14291.
	Phosphoric acid, H_3PO_4, Merck. Art. 1.00573.
	KH_2PO_4, Merck Art. 1.04873.
Eluent:	Solvent A: Water, HPLC grade + phosphate buffer* pH 2.5 (9 + 1).
	Solvent B: Acetonitrile.

* 11.32 g of H_3PO_4 and 32.86 g of KH_2PO_4 in 1000 ml of HPLC grade water

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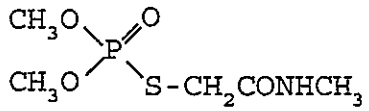
Gradient programme:	Time 0:	% B 15.
	Time 12:	% B 75.
	Time 17:	% B 75.
	Time 18:	% B 15.
	Stop time:	18 minutes.
	Post time:	5 minutes.
Flow programme:	Time 0:	0.75 ml/minute.
	Time 12:	0.75 ml/minute.
	Time 12.5:	2.0 ml/minute.
Oven temperature:	50°C.	
Signals:	Sample, Bw	Reference, Bw [nm]
	210, 10	550, 80
	215, 10	550, 80
	The photodiodearray might be used for checking peak purity.	
Detector slit size:	4 nm.	
Load:	15 µl of reference solution respectively test solution as per pos. 2.	
Runtime per chromatogram:	18 minutes.	
Quantitation:	Peak area.	
Recalibration:	Replace response factor.	
Spectrum:	Store all from 190 nm to 400 nm in steps of 2 nm.	

2. Preparation of reference solutions and test solutions

Reference materials: Compounds of known purity - as pure as possible.

Omethoate:



CAS No.: 1113-02-6.

CAS name: O,O-Dimethyl S-[2-(methylamino)-2-oxoethyl] phosphorothioate.

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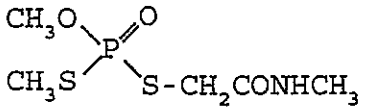
Isodimethoate (Iso-DMT):



CAS No.: 3344-11-4.

CAS name: Phosphorodithioic acid, O,S-Dimethyl S-[2-(methylamino)-2-oxoethyl] ester.

Reference solution:

Prepare an app. 1% solution of each reference material by weighing 0.1 g of reference material accurately to the fourth decimal place (a_1, a_2 g) into tared 12 ml sample bottles with screw cap. Add 10 ml of acetonitrile (Merck Art. 14291), weigh again (b_1 b_2 g) and mix well (stock solutions).

Weigh 100 µl aliquot of the stock solution of Omethoate and 500 µl of the stock solution of Iso-DMT accurately to the fourth decimal place (c_1, c_2 g) into a tared sample bottle with screw cap.

Tare the balance and add 9 ml of 10% acetonitrile/90% solvent A, weigh again (d_g) and mix well (Solution 1).

Weigh an aliquot of 7 ml of solution 1 (e_g) into a tared sample bottle. Add 7 ml of 10% CH_3CN/90% solvent A, weigh it (f_g) and mix well (Solution 2).

Weigh an aliquot of 2 ml of solution 2 into a tared sample bottle. Add 2 ml of 10% CH_3CN/90% solvent A, weigh it and mix well (Solution 3).

Weigh an aliquot of 1 ml of solution 2 into a tared sample bottle. Add 9 ml of 10% CH_3CN/90% solvent A, weigh it and mix well (Solution 4).

Inject from solution 1, 2, 3 and 4 into the liquid chromatograph.

Test solutions:

Technical material

0.3 g of technical Dimethoate are weighed accurately to the fourth decimal place (g_g) into a tared 12 ml sample bottle with screw cap. Add 10 ml of 10% CH_3CN/90% solvent A, weigh again (h_g) and mix well. This solution is injected into the HPLC.

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Concentrates

Weigh an appropriate amount of concentrates to give 300 mg of Dimethoate accurately to the fourth decimal place (g_g) into a tared 12 ml sample bottle with screw cap. Add 10 ml of 10% CH_3CN/90% solvent A, weigh again (h_g) and mix well. This solution is injected into the HPLC.

Dilute the test sample appropriate if the area of one impurity exceeds the area of the strongest calibration solution.

Formulations

Weigh 0.5 g of EC formulations accurately to the fourth decimal place (g_g) into a tared 12 ml sample bottle with screw cap. Add 10 ml of 10% CH_3CN/90% solvent A, weigh again (h_g) and mix well.

If the solutions look cloudy (milky) centrifugation might be used. Injection from the milky solution should not cause problems a washing step is ending the chromatographic run.

Dilute the test sample appropriate if the area of one impurity exceeds the area of the strongest calibration solution.

3. System check

Check the chromatographic system as follows to ensure it is suitable for the intended purpose:

Repeatability
Inject reference solution 2 at least three times or so many times that the areas of Omethoate and Isodimethoate do not differ more between two successive measurements than 5.0% relative.

Linearity
Inject reference solution 1, Solution 3, Solution 4, Solution 2 and measure the areas of Omethoate and Isodimethoate. Calculate the response factor, r_f for Omethoate and Isodimethoate (see pos. 2 and 6) in reference solution 1, solution 2, solution 3 and solution 4.

The linearity is acceptable if the response factors in solution 1, solution 2, solution 3 and solution 4 do not differ more from the mean than: ± 5.0% relative. Alternatively calculate the linear regression coefficients (r^2). Accepted linearity for $r^2 > 0.98$.

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Carry over

Inject a blank sample after reference solution 2 from above and measure the areas of Omethoate and Isodimethoate. The "carry over" from the previous injection is acceptable if the measured areas of Omethoate and Isodimethoate in the blank are less or equal to 2.0% relative of the measured areas of the compounds in reference solution 2.

4. Chromatographic procedure

After the system check has been successfully completed proceed as follows.

Place the samples in the magazine of the autosampler according to the injection sequence below. Enter all information about the samples such as name, dilution, position etc. into the data handling system. Print out the sequence list and check manually, if the position of the samples corresponds with the sequence list. Print out the method including all parameters.

The following injection sequence is used:

Sol. 2 , T_1 , T_2, T_3, T_4 , T_5, T_6 , T_7, T_8 Sol. 2 T_9 ---- T_{16} Sol. 2, T_{17} --- etc.

Where T : Test solutions (1,2,3,..,n).

Recalibration after all Sol. 2 measurements (Replace response factor).

Always end the sequence with a reference solution 2.

N.B. Only one injection from each vial.

5. Retention times

Omethoate: 3.7 minutes.

Isodimethoate: 4.8 minutes.

(Dimethoate: 7.7 minutes).

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6. Calculations

External standard method.

Example:

Measure the peak area of Omethoate both from the reference solution and the test solution.

Determine the weight per cent as follows:

$$\% \text{ w/w of Omethoate} = \frac{\text{Area Omethoate in testsolution} \times h \times r_f}{g}$$

r_f is found by means of the reference solution 2:

$$r_f = \frac{\text{Purity (\% w/w) of Omethoate (ref. material)} \times a \times c \times e}{\text{Area Omethoate in Sol. 2} \times b \times t \times f}$$

$$t = c_1 + c_2$$

The data handling system will calculate according to the equations described above, when the dilution of the test samples and the concentration of 100% pure reference material in the reference solution (solution 2) are entered.

$$\text{Dilution of sample} = \frac{h}{g}$$

Concentration of Omethoate in solution 2 =

$$\frac{a \times c \times e \times \text{purity (\% w/w) of Omethoate (ref. material)}}{b \times t \times f}$$

7. Validation

Validation of this method by Cheminova A/S is described in GLP Project No. ILV 443.2.

The method has undergone independent Laboratory validation (ILV) by Institut Fresenius, Germany and Budapest Plant Health and Soil Conservation Stadion, Hungary.