Azinphos-methyl

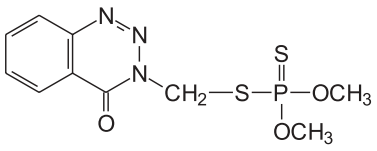
Method number	1
Application	Air analysis
Analytical principle	High performance liquid chromatography
Completed in	September 1997

Summary

To determine the levels of azinphos-methyl in the air, measured air volumes are drawn through Tenax adsorption tubes by a battery-operated pump. The adsorbed substance is extracted with acetonitrile and determined using a UV detector after liquid chromatographic separation. Quantitative evaluation is carried out using a calibration curve. The peak areas obtained using calibration standards are plotted against the azinphos-methyl concentrations.

Precision:	Standard deviation (rel.):	s = 1.6%
	Mean variation:	u = 4.0%
	for 5 activated carbon tubes each loaded with 26 µg azinphos-methyl	
Limit of quantification:	25 µg/m^3 azinphos-methyl for a sampled air volume of 720 L	
Recovery:	$\bar{\eta}$ = 98.3 or 98.7% at concentrations of 26 or 300 µg/m^3	
Sampling recommendation:	Sampling time:	6 hours
	Sampled air volume:	720 L

Azinphos-methyl [CAS No. 86-50-0]



Azinphos-methyl, a non-systemic insecticide introduced in 1955, has a broad spectrum of action and may be applied to numerous crops.

Azinphos-methyl is a colourless, crystalline solid with a melting point of 73 °C and a molecular weight of 317.1.
Azinphos-methyl has a MAK value of 0.2 mg/m^3 (2001) – measured as the inhalable aerosol fraction. Because of the danger of absorption through the skin, azinphos-methyl has been marked with an "H" in the List of MAK and BAT Values [1].

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Contents

1 General principles

To determine the levels of azinphos-methyl in the air measured air volumes are drawn through Tenax adsorption tubes by a battery-operated pump. The adsorbed substance is extracted with acetonitrile and determined with a UV detector after liquid chromatographic separation. Quantitative evaluation is carried out using a calibration curve. The peak areas of the calibration standards are plotted against the azinphos-methyl concentrations.

2 Equipment, chemicals and solutions

2.1 Equipment

Adsorption tube type NIOSH, Tenax® (e. g. Günther Karl OHG, Gau-Algesheim, Germany Order No. GK-26-35-03-GO)
Sampling pump (e. g. HI FLOW Sampler, model HFS 113 A, Gilian Instruments, New Jersey, USA)
Gasmeter
Thermometer
Barometer
High performance liquid chromatograph with gradient pump system and UV detector (220 nm)
Glass cutter
10 mL and 25 mL volumetric flasks
50 µL, 100 µL and 250 µL precision syringes
20 mL flanged vials with Teflon-coated butyl rubber stoppers
Laboratory shaker

2.2 Chemicals

Azinphos-methyl (e. g. Ehrenstorfer, Augsburg, Germany)
Acetonitrile, suitable for HPLC gradient elution
Water, suitable for HPLC

2.3 Solutions

Stock solution: To prepare the stock solution, 25 mg azinphos-methyl is weighed exactly into a 25 mL volumetric flask. Then the flask is filled to the mark with acetonitrile (1 g/L).

Elution solution: To prepare the elution solution 1 mL of the stock solution is pipetted into a 10 mL volumetric flask containing approximately 5 mL acetonitrile. Then the flask is filled to the mark with acetonitrile (100 mg/L).

Calibration standards: From this elution solution calibration standards containing 0.1–10 mg/L of azinphos-methyl are prepared by diluting with acetonitrile. The following pipetting scheme is used (Table 1):

Table 1. Pipetting scheme for the preparation of the calibration standards.

Volume of the elution solution µL	Final volume of the calibration standards mL	Concentration of the calibration standards mg/L
10	10	0.1
100	10	1.0
200	10	2.0
500	10	5.0
1000	10	10.0

3 Sample collection and preparation

The adsorption tube is opened at both ends and connected to the inlet of the pump with a short tube. The adsorption tube contains two adsorption phases separated by cotton wool; the larger phase (100 mg Tenax) is closest to the inlet of the tube during air sampling. The second, smaller adsorption phase (50 mg Tenax) serves to check for possible breakthrough of the substance during sampling. For sampling, air is drawn through the adsorption tube at a flow rate of 2 L/min for six hours. After sampling, the air sample volume is noted. In addition, the parameters important for determining the concentration such as temperature and air pressure at the site of sampling must be determined. The closed tubes should be stored in a refrigerator until sample preparation is performed.
For preparation, the collection phase and the control phase are each transferred separately to 20 mL flanged vials; the cotton wool separating the two phases and the upper piece of cotton wool at the tube inlet are analysed together with the collection phase.
10 mL acetonitrile (collection phase) and 5 mL acetonitrile (control phase) are added to the flanged vials and the vials are closed. The substance is extracted from the adsorption material by shaking the tube on a laboratory shaker for 10 minutes. 20 µL of the acetonitrile solution is then injected without further processing into the high performance liquid chromatograph for separation.
In each analysis series a reagent blank is prepared and analysed in the same way.

4 Operating conditions for high performance liquid chromatography

Precolumn:	Material:	RP-18 LiChrospher 100
	Length:	4 mm
	Internal diameter:	4 mm
	Particle size:	5 µm

Column:	Material:	RP-18 LiChrospher 100
	Length:	25 cm
	Internal diameter:	4 mm
	Particle size:	5 µm
Column temperature:	40 °C	
Solvent:	A = water	
	B = acetonitrile	
Gradient:	0 minutes	45% B
	2 minutes	45% B
	8 minutes	70% B
	10 minutes	70% B
	11 minutes	45% B
	14 minutes	45% B
Flow rate:	1.0 mL/min	
Detector:	UV detector	
Detection wavelength:	220 nm	
Injection volume:	20 µL	

Under the high performance liquid chromatographic conditions described above, the retention time was about 9.3 minutes (see Figure 1).

5 Analytical determination

Under the conditions described above, 20 µL of the acetonitrile solution is injected into the high performance liquid chromatograph using an injection loop. After high performance liquid chromatographic separation of the azinphos-methyl from the other components collected on the Tenax and extracted with acetonitrile, the azinphos-methyl is detected by the UV detector.

6 Calibration

20 µL of each of the calibration standards (see Section 2.3) is injected into the high performance liquid chromatograph and detected by the UV detector. To draw the calibration curve, the measured peak areas or heights are corrected by subtraction of the reagent blank values and then plotted against the azinphos-methyl concentrations used in mg/L (see Figure 2).
Each solution is analysed twice, and the mean value is used in the calculation.

7 Calculation of the analytical result

Using the peak areas or heights obtained after subtraction of the reagent blank values, the azinphos-methyl concentration in mg/L acetonitrile is read from the calibration curve. If analysis of the control phase reveals an azinphos-methyl concentration of more than 10% of the total azinphos-methyl concentration (from the collection phase and control phase), a breakthrough has occur. In this case sampling must be repeated under different conditions (e.g. a lower air sample volume).
The concentration by weight ρ (mg of azinphos-methyl per m^3 of air) in the sample air is calculated according to the following equation:

$$\rho = \frac{a \cdot b}{V_Z \cdot \eta} \tag{1}$$

At 20 °C and 1013 hPa:

$$\rho_0 = \rho \cdot \frac{273 + t_a}{293} \cdot \frac{1013}{p_a} \tag{2}$$

The corresponding concentration by volume – independent of the variables pressure and temperature – is given by:

$$\sigma = \rho_0 \cdot \frac{V_m}{M} = \rho \cdot \frac{273 + t_a}{293} \cdot \frac{1013}{p_a} \cdot \frac{24.1}{317.1} \tag{3}$$

$$\sigma = \rho \cdot \frac{273 + t_a}{p_a} \cdot 0.263$$

For t_a = 20 °C and p_a = 1013 hPa:

$$\sigma = \rho \cdot 0.076 \ \frac{mL}{mg} \tag{4}$$

where:

- a is the azinphos-methyl concentration in acetonitrile in mg/L taken from the calibration curve
- b is the volume of acetonitrile used for extraction in L
- V_z is the air sample volume in m^3
- η is the recovery
- t_a is the temperature of the ambient air in °C
- p_a is the air pressure of the ambient air in hPa
- ρ is the azinphos-methyl concentration in the ambient air in mg/m^3 at t_a and p_a
- ρ_o is the azinphos-methyl concentration in the ambient air in mg/m^3 at 20 °C and 1013 hPa

M is the molecular weight of azinphos-methyl
V_m is the molecular volume in L/mole
σ is the azinphos-methyl concentration in the ambient air in mL/m^3

8 Reliability of the method

8.1 Precision

To determine the precision, 5 Tenax adsorption tubes were each loaded with 26 µg azinphos-methyl in dissolved form and subjected to the preparation procedure described above. The standard deviation (rel.) was s = 1.6% and the mean variation u = 4.0%.

8.2 Recovery rate

To determine the recovery, defined amounts of the substance in dissolved form were added to each of 5 Tenax adsorption tubes. Care was taken that the solution was added to the middle of the tube and that the spiked volume did not exceed 50 µL. The solvent was removed by drawing air through the tube (2 L/min) for about ten minutes. The tubes were then prepared and analysed as described in Sections 3 and 4. Table 2 shows the resulting recovery values.

Table 2. Recovery η.

Concentration mg/m^3	Recovery η %	Mean recovery: $\bar{\eta}$ %
0.026	96.5–101	98.3
0.300	97.1–102	98.7

8.3 Quantification limit

Under the given analytical conditions the quantification limit of the procedure is 25 µg/m^3.
By a slight modification of the method, described below, the quantification limit can be lowered to 0.5 µg/m^3. During sample preparation the collection and control phases are mixed separately each with 3 mL acetonitrile and the substance is extracted from the adsorption material by shaking the tubes for 30 minutes on a laboratory shaker. Before liquid chromatographic separation 30% water is added to the extract. 200 µL of the acetonitrile/water solution is then injected directly into the high performance liquid chromatograph.

8.4 Shelf-life

To check the shelf-life, Tenax tubes were spiked with defined amounts of azinphos-methyl. The spiked amount was equivalent to about 0.05 mg/m^3 for an air sample volume of 720 L. The solvent was removed by drawing air through the tube (2 L/min) for about ten minutes. The tubes were then stored in either a refrigerator (approx. +1 to +6 °C) or a deep-freeze (approx. –22 to –25 °C). After storage for 11 days the adsorption tubes were prepared and analysed as described in Sections 3 and 4 in the form of a repeated determination. Under both kinds of storage conditions recovery was > 99 %.

9 Discussion of the method

The method described for determining azinphos-methyl in air consist of two steps. In the first step the substances in the air are deposited in the collection system and in a second step extracted from it. Tenax has proved to be an ideal collection phase. Tenax adsorption tubes are also suitable for collecting gaseous and particle-bound residues of other pesticides from the air [2]. Care must be taken when determining the recovery that the solution is added to the middle of the tube and that the spiked volume is 10–50 µL. Smaller volumes increase the inaccuracy of the dose, larger volumes lead, however, to increased substance losses during removal of the solvent. Recovery was found to be adequate with "worst case" climatic conditions (T = 35 °C; relative humidity = 80 %).

Instruments used:
Liquid chromatograph HP 1050 with UV detector and autosampler

10 References

[1] *Deutsche Forschungsgemeinschaft*. MAK- und BAT-Werte-Liste 2001. Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Mitteilung 37. WILEY-VCH Verlag, Weinheim

[2] *Riegner K, Schmitz JR* (1994). Erzeugung einer Testatmosphäre und Abscheidung gasförmiger und partikelgebundener Pflanzenschutzmittelrückstände aus Luft auf Tenax®-Sammelröhrchen. Pflanzenschutz-Nachrichten Bayer 47: 161–176

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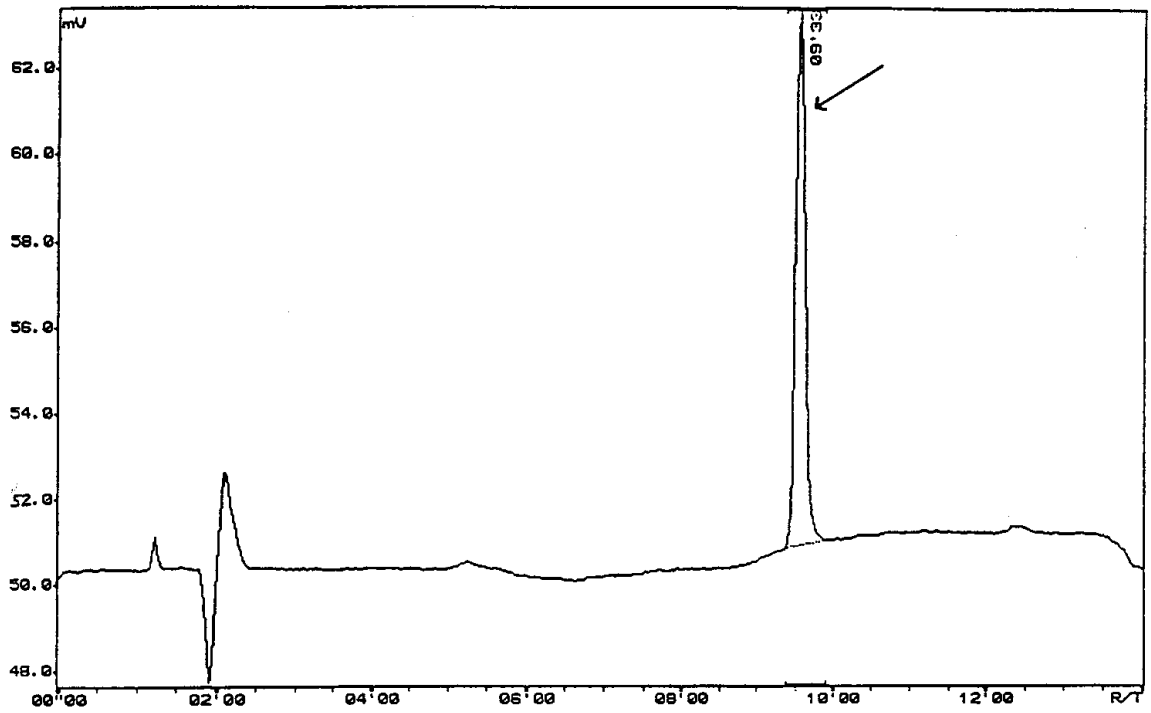

Fig. 1. HPLC chromatogram of a standard spiked with 2 mg azinphos-methyl per litre.

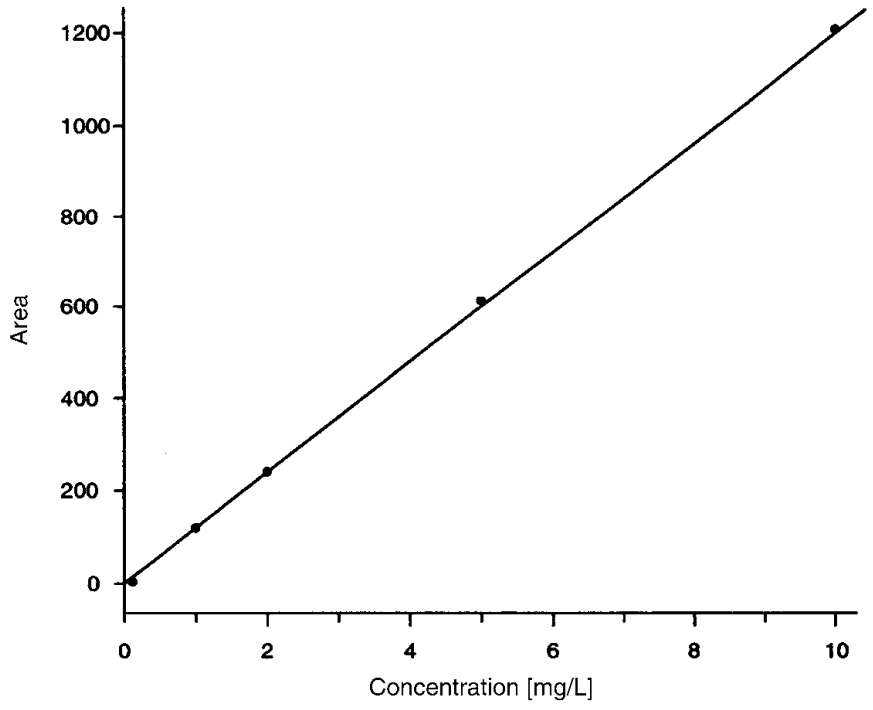

Fig. 2. Example of a calibration curve.