637. Thiamethoxam

HPLC method

CIPAC Collaborative Trial according to CIPAC Information Sheet N^{o} 293

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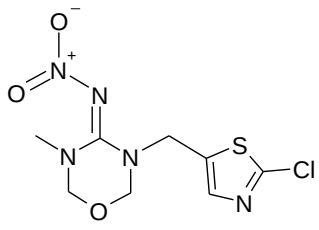
THIAMETHOXAM

637/TC/M/-



ISO Common Name:	Thiamethoxam
Chemical Name:	3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitro-amine
CAS-Number:	153719-23-4
Molecular mass:	291.7
Empirical formula:	$C_8H_{10}ClN_5O_3S$
m.p.	139.1°C
b.p.	Decomposition occurs at about 147 °C before boiling point is reached

1 Sampling. Take at least 100 g.

2 Identity test

2.1 HPLC. Use the reversed phase HPLC method described below in section 3.1. The relative retention time of the Thiamethoxam peak in the sample solution should not deviate by more than 2% from that of the calibration solution.

2.2 GC. Use the capillary GC method below. The relative retention time of the Thiamethoxam peak in the sample solution should not deviate by more than 2% from that of the calibration solution.

REAGENT
5ml water / 95ml acetone

APPARATUS
Gas chromatography equipped with flame ionisation detector.
Capillary column fused silica, length 30 m x 0.25 (i.d.) mm, film thickness: 0.5 μm, coated with crosslinked (5%-Phenyl)-methylpolysiloxane (DB-5 or equivalent).
Data system

PROCEDURE

(a)Gas chromatographic conditions (typical):

Column	Fused silica, length 30 m x 0.25 mm internal diameter. DB-5 or equivalent phase, with film thickness of 0.5 μm crosslinked (5%-Phenyl)-methylpolysiloxane
Injection system	
Injector	split injection
Injection volume	1 μl
Split ratio	1:20
Detector	flame ionisation
Temperatures	
Injection port	260 ºC
Detector	300 ºC
Oven programme	temp 1 60ºC, hold 1 min, ramp rate 20ºC/min temp 2 170ºC, hold 0 min, ramp rate 3ºC/min temp 3 200ºC, hold 0 min, ramp rate 20ºC/min temp 4 300ºC, hold 5 min,
Gas flow rates	
Column:	
Hydrogen (carrier)	2 ml/min (typically 11.7 psi at 60°C), run at constant flow
Detector:	
Air	300 ml/min
Hydrogen	30 ml/min
Nitrogen (make up)	30 ml/min
Retention times	
Thiamethoxam	18.3 min (approximate)

Sample preparation: Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 200 mg Thiamethoxam into a volumetric flask (100 ml). Add acetone (about 50 ml) and water (5 ml) and place the flask in an ultrasonic bath for 5 min. Allow to cool to ambient temperature and fill to the mark with acetone. Mix thoroughly.

Calibration solution: Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 200 mg Thiamethoxam into a volumetric flask (100 ml). Add acetone (about 50 ml) and water (5 ml) and place the flask in an ultrasonic bath for 5 min. Allow to cool to ambient temperature and fill to the mark with acetone. Mix thoroughly

3 Thiamethoxam

OUTLINE OF METHOD

Thiamethoxam content is determined (g/kg) using reversed phase high performance liquid chromatography incorporating UV detection at 230 nm with an external standard calibration.

3.1 Determination of Thiamethoxam by reversed phase HPLC

REAGENTS
Thiamethoxam reference standard of known content
Water HPLC grade
Acetonitrile HPLC grade
Phosphoric acid (85%) analytical grade
Eluent A: 0.1% v/v aqueous phosphoric acid
Eluent B: acetonitrile
Diluent: 0.1% v/v aqueous phosphoric acid / acetonitrile 1 + 4 (v/v)

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 45 - 55 mg of the Thiamethoxam reference standard (s mg) into separate volumetric flasks (50 ml). Add about 35 ml acetonitrile and 10 ml 0.1% v/v aqueous phosphoric acid and place the flasks in an ultrasonic bath for 5 minutes. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Transfer 25.0 ml of this solution in a 100 ml volumetric flask, add diluent (0.1% v/v aqueous phosphoric acid / acetonitrile, in a ratio of 1/4 (v/v)) and make up to the volume at room temperature (calibration solutions C_1 and C_2).

APPARATUS
High performance liquid chromatograph equipped with a detector suitable for operation at 230 nm (UV-detection) and an injection system capable to inject 5 µl.
Liquid chromatographic column stainless steel, 75 x 4.6 mm (i.d), packed with Nucleodur C_{18} ec (3 µm) Macherey-Nagel or equivalent with the same selectivity.
Electronic integrator or data system
Ultrasonic bath

PROCEDURE

(a) Chromatographic conditions (typical):

Column temperature	40°C
Flow rate	1.5 ml/min
Detector wavelength	230 nm
Injection volume	5 µl
Retention time	approximately 2.6 minutes

Gradient program:

time [minutes]	0.1 % aqueous phosphoric acid [%]	acetonitrile [%]
0	90	10
4	30	70
10	5	95
11	5	95
11.1	90	10
14	90	10

(b) Equilibration of the system. Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µl portions of the calibration solution C_1 and repeat the injections until retention times and peak areas deviate by less than $\pm$ 1 % from the mean for three successive injections.

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 45 - 55 mg of Thiamethoxam tech. (w mg) into a volumetric flask (50 ml). Add about 35 ml acetonitrile and 10 ml 0.1 % v/v aqueous phosphoric acid and place the flasks in an ultrasonic bath for 5 minutes. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Transfer 25.0 ml of this solution in a 100 ml volumetric flask, add diluent (0.1% v/v aqueous phosphoric acid / acetonitrile, in a ratio of 1/4 (v/v)) and make up to the volume at room temperature (sample solutions S_1 and S_2).

(d) Determination. Inject 5 µl portions of the second calibration solution (C_2) for two successive injections. The mean response factor for this solution should deviate by no more than 1% from those for the first calibration solution (C_1) (see paragraph (b) *Equilibration of the system*), otherwise the calibration solutions should be prepared again.

Inject in duplicate 5 µl portions of each sample solution (S_1, S_2, …, etc.) bracketing them by duplicate injections of the calibration solution (C_1) using the following sequence:

$$C_1, C_1, S_1, S_1, S_2, S_2, C_1, C_1\ldots$$

Determine the peak area of Thiamethoxam.

(e) Calculation. Calculate the response factors (*f*) from the calibration solutions bracketing the injections of the sample solutions. Average the response factors of the calibration solutions preceding and following the sample solution injections. These must agree within ± 1 % of the average otherwise repeat the determination. Calculate the content of the sample solutions.

$$f_i = \frac{s \times P}{H_s}$$

$$\text{Thiamethoxam content} = \frac{H_w \times f}{w} \text{ [g/kg] } (M)$$

where:

f_i = single response factor
f = average response factor
H_s = peak area of Thiamethoxam in the calibration solution
H_w = peak area of Thiamethoxam in the sample solution
s = mass of Thiamethoxam reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of Thiamethoxam reference standard (g/kg)

THIAMETHOXAM WATER DISPERSIBLE GRANULE
637/WG/M/-

1 Sampling. Take at least 100 g.

2 Identity test

2.1 HPLC. As for Thiamethoxam technical 637/TC/M/2.1

2.2 GC As for Thiamethoxam technical 637/TC/M/2.2

3 Thiamethoxam
Same approach as for Thiamethoxam technical 637/TC/M/3

3.1 Determination of Thiamethoxam by reversed phase HPLC
As for Thiamethoxam technical 637/TC/M/3.1 except

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter.

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 45 - 55 mg Thiamethoxam (w mg, equal to 180 – 220 mg Thiamethoxam formulation WG 25) into a volumetric flask (50 ml). Suspend with 10 ml 0.1% v/v aqueous phosphoric acid. Add about 35 ml acetonitrile and place the flasks in an ultrasonic bath for 5 minutes. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter solutions through a 0.45 µm filter Transfer 25.0 ml of this solution in a 100 ml volumetric flask, add diluent (0.1% v/v aqueous phosphoric acid / acetonitrile, in a ration of 1/4 (v/v)) and make up to the volume at room temperature (sample solutions S_1 and S_2).

4 Suspensibility

REAGENTS AND APPARATUS as for 637/TC/M/- and MT 184.

add at:
APPARATUS

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter

PROCEDURE

(a) Preparation of suspension and determination of sedimentation. MT 184.

(b) Determination of Thiamethoxam in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension, transfer the remaining 25 ml quantitatively into a volumetric flask (100 ml) with acetonitrile (about 50 ml). Place the flask in an ultrasonic bath for 5 minutes. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Dilute 10.0 ml of the solution with the eluent to a volume of 100 ml. Mix thoroughly. Clear the suspension by filtration through a 0.45 µm filter prior to injection. Determine the mass (*Q* g) of Thiamethoxam according to 637/WG/M/3.1, using a calibration solution with the appropriate final concentration of Thiamethoxam standard.

(c) Calculation

$$\text{Suspensibility} = \frac{111(c - Q)}{c} \%$$

where:
c = mass of Thiamethoxam in the sample taken for the preparation of the suspension (g)
Q = mass of Thiamethoxam in the bottom 25 ml of suspension (g)

THIAMETHOXAM FLOWABLE CONCENTRATE
637/SC/M/-

1 Sampling. Take at least 100 g.

2 Identity test

2.1 HPLC. As for Thiamethoxam technical 637/TC/M/2.1

2.2 GC As for Thiamethoxam technical 637/TC/M/2.2

3 Thiamethoxam
Same approach as for Thiamethoxam technical 637/TC/M/3

3.1 Determination of Thiamethoxam by reversed phase HPLC
As for Thiamethoxam technical 637/TC/M/3.1 except

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter.

(c) Sample preparation. Homogenize sample thoroughly. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 45 - 55 mg Thiamethoxam (w mg, equal to 210 – 250 mg Thiamethoxam formulation SC 240) into a volumetric flask (50 ml). Suspend with 10 ml 0.1% v/v aqueous phosphoric acid. Add about 35 ml acetonitrile and place the flasks in an ultrasonic bath for 5 minutes. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter solutions through a 0.45 µm filter. Transfer 25.0 ml of this solution in a 100 ml volumetric flask, add diluent (0.1% v/v aqueous phosphoric acid / acetonitrile in a ratio of 1/4 (v/v)) and make up to the volume at room temperature (sample solutions S_1 and S_2).

THIAMETHOXAM FLOWABLE CONCENTRATE FOR SEED TREAMENT
637/FS/M/-

1 Sampling. Take at least 100 g.

2 Identity test

2.1 HPLC. As for Thiamethoxam technical 637/TC/M/2.1

2.2 GC As for Thiamethoxam technical 637/TC/M/2.2

3 Thiamethoxam
Same approach as for Thiamethoxam technical 637/TC/M/3

3.1 Determination of Thiamethoxam by reversed phase HPLC
As for Thiamethoxam technical 637/TC/M/3.1 except

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter.

(c) Sample preparation. Homogenize sample thoroughly. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 45 - 55 mg Thiamethoxam (w mg, equal to 130 – 160 mg Thiamethoxam formulation FS 350) into a volumetric flask (50 ml). Suspend with 10 ml 0.1% v/v aqueous phosphoric acid. Add about 35 ml acetonitrile and place the flasks in an ultrasonic bath for 5 minutes. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter solutions through a 0.45 µm filter. Transfer 25.0 ml of this solution in a 100 ml volumetric flask, add diluent (0.1% v/v aqueous phosphoric acid / acetonitrile in a ratio of 1/4 (v/v)) and make up to the volume at room temperature (sample solutions S_1 and S_2).

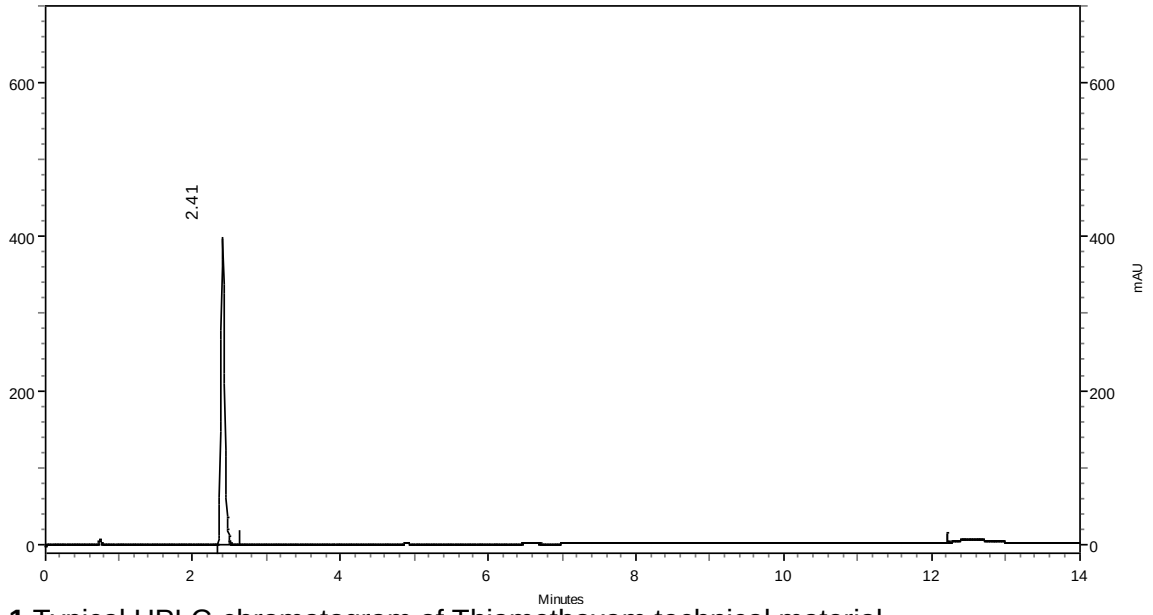


Fig 1 Typical HPLC-chromatogram of Thiamethoxam technical material

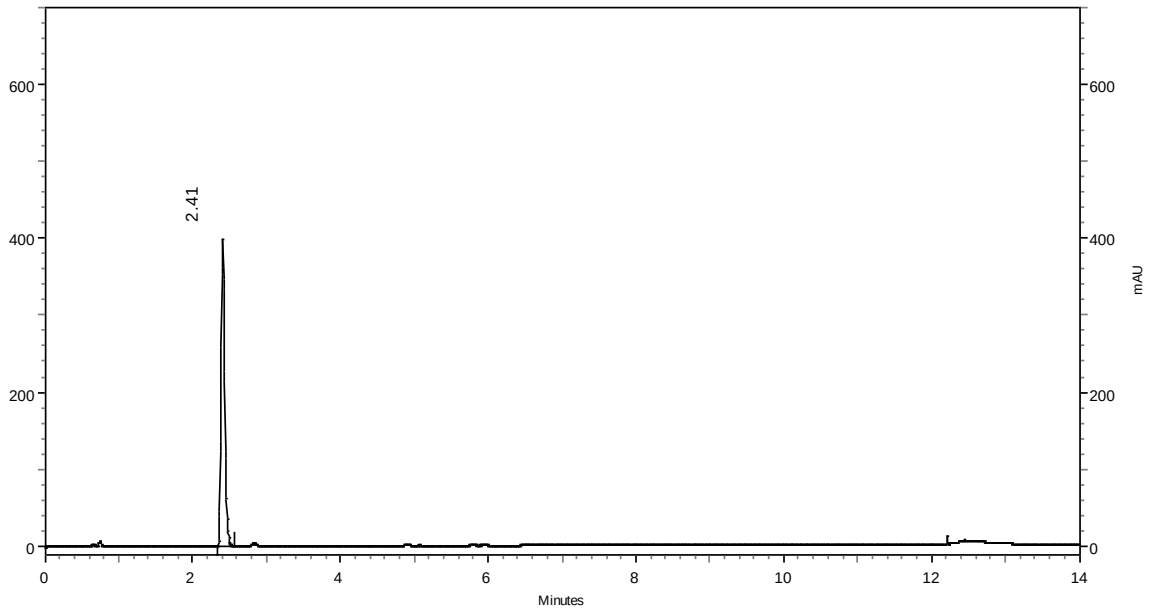


Fig 2 Typical HPLC-chromatogram of Thiamethoxam formulation WG 25

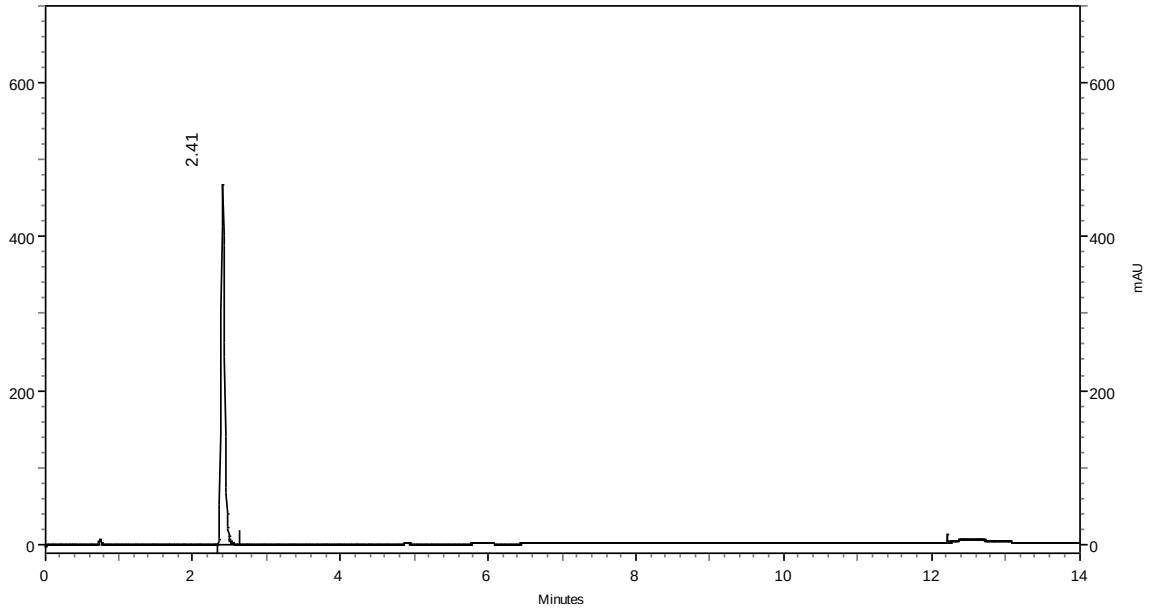


Fig 3 Typical HPLC-chromatogram of Thiamethoxam in formulation SC 240

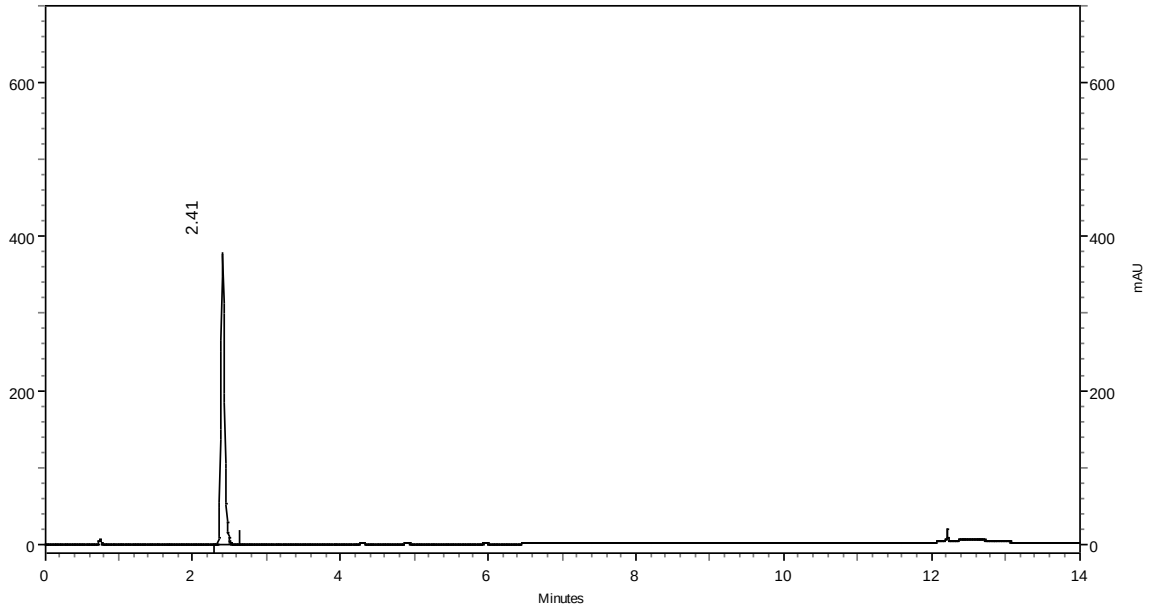


Fig 4 Typical HPLC-chromatogram of Thiamethoxam in formulation FS 350

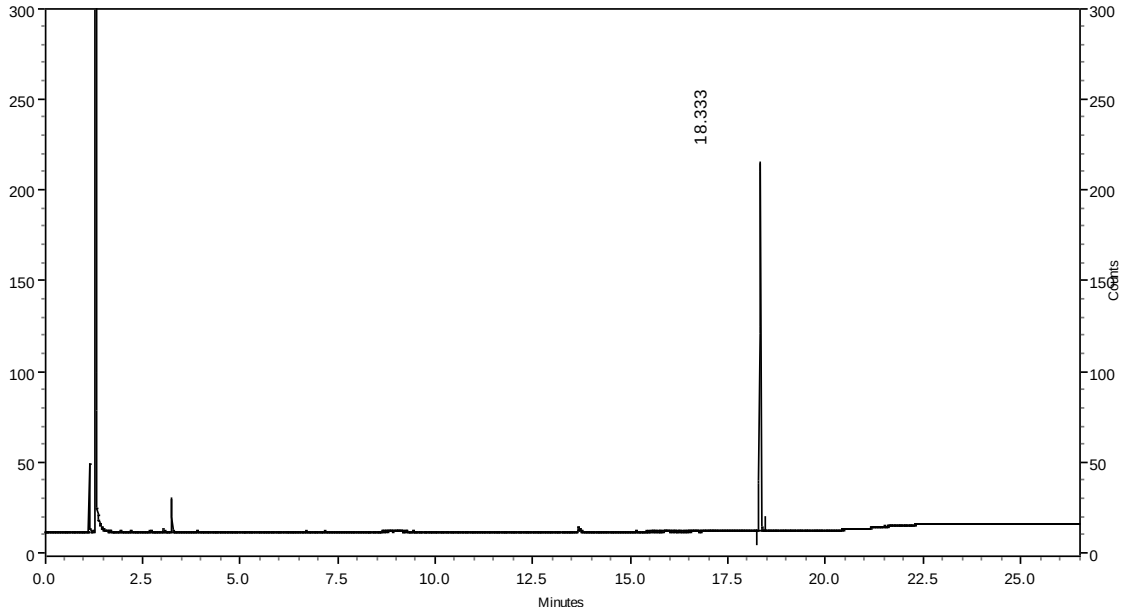


Fig 5 Typical GC-chromatogram of Thiamethoxam technical material