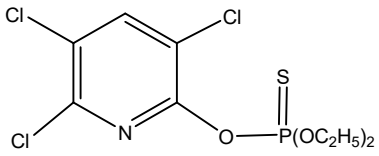
Chlorpyrifos
221

HPLC method

CIPAC Full Scale Collaborative Trial

CHLORPYRIFOS

221



ISO common name	Chlorpyrifos
Chemical name	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate(IUPAC) *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate(CA; 2912-88-2)
Empirical formula	$C_9H_{11}Cl_3NO_3PS$
RMM	350.6
m.p.	42-43.5°C
v.p.	27mPa at 25°C
Solubility	In water 1.4 mg/L at 25°C; In bezene 7900,actone 6500,chloroform 6300, carbon disulphide 5900, diethyl ether 5100,xylene 5000, isoctanol 790, methanol 450(all in g/kg, 25°C)
Stability	Rate of hydrolysis increases with pH, and in the presence of copper and possibly of other metals that can form chelates; DT_{50} 1.5d(water, pH8, 25°C) to 100d (phosphate buffer, pH7, 15°C)
Description	The pure material is colourless crystals, with a mild mercaptan odour
Formulation	Emulsifiable concentrates (EC)

CHLORPYRIFOS TECHNICAL

221/TC/M/-

1 Sampling. Take at least 100 g.

2. Identity test

2.1 HPLC. Use the reversed phase HPLC method below. The relative retention time of the Chlorpyrifos peak in the sample solution should not deviate by more than 1.5% from that of the calibration solution.

2.2 Infrared. Prepare potassium bromide discs for the technical sample and Chlorpyrifos reference substance. A typical potassium bromide disc should contain a sample prepared in the 0.15-0.35% by weight range. Scan the discs from 4000 to 600 cm^{-1}. The spectrum from the sample should not differ significantly from that of the reference substance.

2.3 GC-MS

PROCEDURE

(a) Preparation of sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain about 50 mg of Chlorpyrifos into a 10-mL glass bottle. Add chloroform to dissolve it and mix thoroughly.

(b) Chromatographic conditions (typical)

Gas chromatographic column quartz, 30m x 0.25 mm (i.d.), coated with diphenyl polysiloxane 95/5(e.g. TG-1701MS), film thickness 0.25μm

Injection system	
Injetor	Split injection
Split ratio	20:1
Gas flow(He) rate(ml/min)	2.0
Temperature	
Oven program	80℃ hold for 3min
	Gradient 10℃/min to 180℃,hold for 5min, 30℃/min to 250℃,hold for 5min
Injector	260
Injection volume	1μl
Ion source	EI, 70ev
Source temperature	280℃
Quad temperature	160℃
Range of mass number	30～700
Run time	25.3 min
Retention times	Chlorpyrifos about 19.8 min

The spectrum produced from the sample should not differ significantly from that of the standard.

3 Chlorpyrifos

OUTLINE OF METHOD Chlorpyrifos is determined by reversed phase high performance liquid chromatography using UV detection at 290 nm and external standardisation.

REAGENTS

Acetonitrile HPLC grade
Glacial acetic acid HPLC grade
Water HPLC grade
Chlorpyrifos standard of known content. Store refrigerated.

Calibration solutions. Weigh in duplicate about 100 mg (to the nearest 0.1 mg) of Chlorpyrifos standard (s mg) into separate volumetric flasks (100 ml). Add acetonitrile to the mark and place the flask in an ultrasonic bath for 5 min. Allow to cool to ambient temperature. Mix thoroughly. (calibration solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a detector suitable for operation at 290 nm (UV-detection) and an injection system capable of injecting 5 µl.

Liquid chromatographic column stainless steel, 250 x 4.6 mm (i.d.), Agilent Extend-C_{18}, 5 µm, or equivalent with the same selectivity.

Electronic integrator or data system

Ultrasonic bath

PROCEDURE

(a) Chromatographic conditions (typical)

Column temperature	25℃
Flow rate	1.0 ml/min
Detector wavelength	290 nm
Injection volume	5 µl
Mobile phase	acetonitrile – water – glacial acetic acid , 820 + 175 + 5 (v/v)
Retention time	Chlorpyrifos approximately 8. 1 min

(b) Equilibration of the system. Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µl portion of calibration solution C_A until the response obtained from two consecutive injections deviate by less than 1.0%.

(c) Preparation of sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 100mg of Chlorpyrifos (*s* mg)

into a volumetric flask (100 ml). Add acetonitrile to the mark and place the flask in an ultrasonic bath for 5 min. Allow to cool to ambient temperature. Mix thoroughly. (sample solutions S_1 and S_2).

(d) Determination. Inject 5 µl portion of calibration solution C_B. The response factor for this solution should not deviate by more than 1.0% from that for calibration solution C_A, otherwise prepare new calibration solutions. Inject in duplicate 5 µl portions of each sample solution bracketing them by injections of the calibration solutions as follows: C_A, S_1, S_1, C_B, S_2, S_2, C_A, and so on. Measure the relevant peak areas.

(e) Calculation. Determine the peak area of Chlorpyrifos and calculate the mean value of response factors from the calibration solutions bracketing the injections of the sample solutions and use this value for calculating the Chlorpyrifos content of the bracketed sample solutions. The Chlorpyrifos content is the mean value of two sample solutions.

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

$$\text{Chlorpyrifos content} = \frac{f \times H_w}{w} \quad \text{g/kg}$$

where:

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

CHLORPYRIFOS EMULSIFIABLE CONCENTRATES

221/EC/M/-

1 Sampling. Take at least 100 ml.

2. Identity test

2.1 HPLC. As for Chlorpyrifos technical 221/TC/M2.1

2.2 GC-MS. As for Chlorpyrifos technical 221/TC/M2.3

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As for Chlorpyrifos technical 221/TC/M except

(c) Preparation of sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (w mg) to contain about 100 mg of Chlorpyrifos (s mg) into a volumetric flask (100 ml). Add acetonitrile (about 80 ml) and place the flask in an

ultrasonic bath for 5 min . Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly.(sample solutions S_1 and S_2).

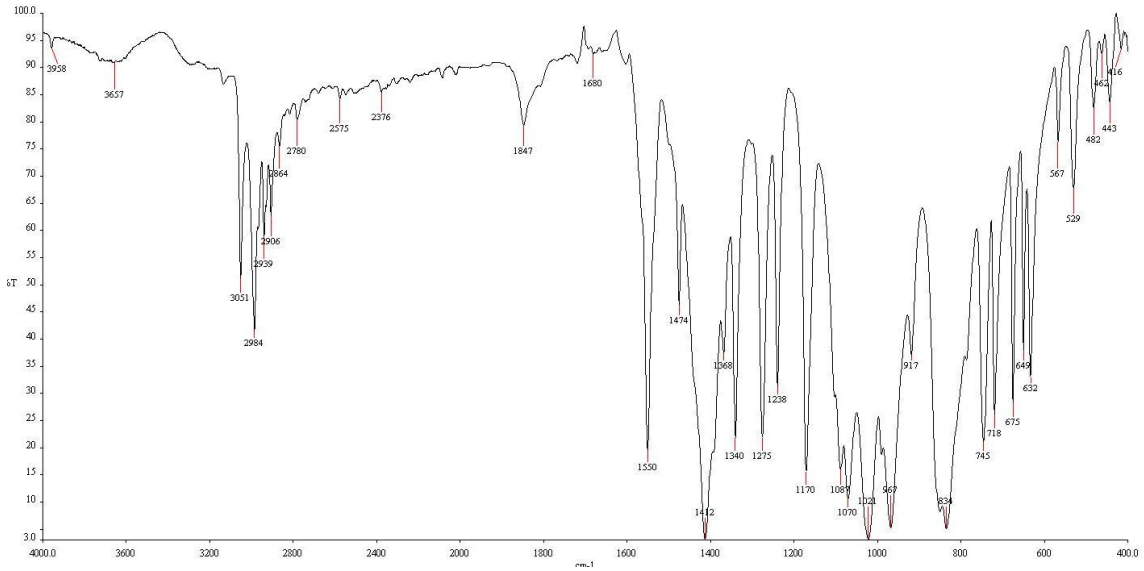

Fig.1 Infrared spectrum of Chlorpyrifos

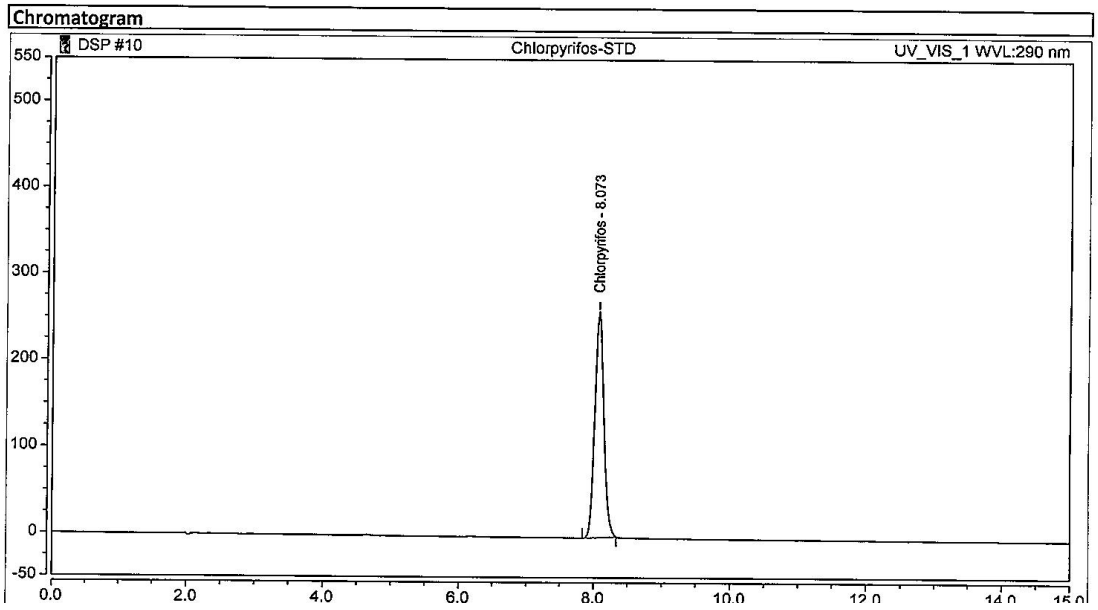

Fig.2 HPLC chromatogram of Chlorpyrifos standard

Chlorpyrifos

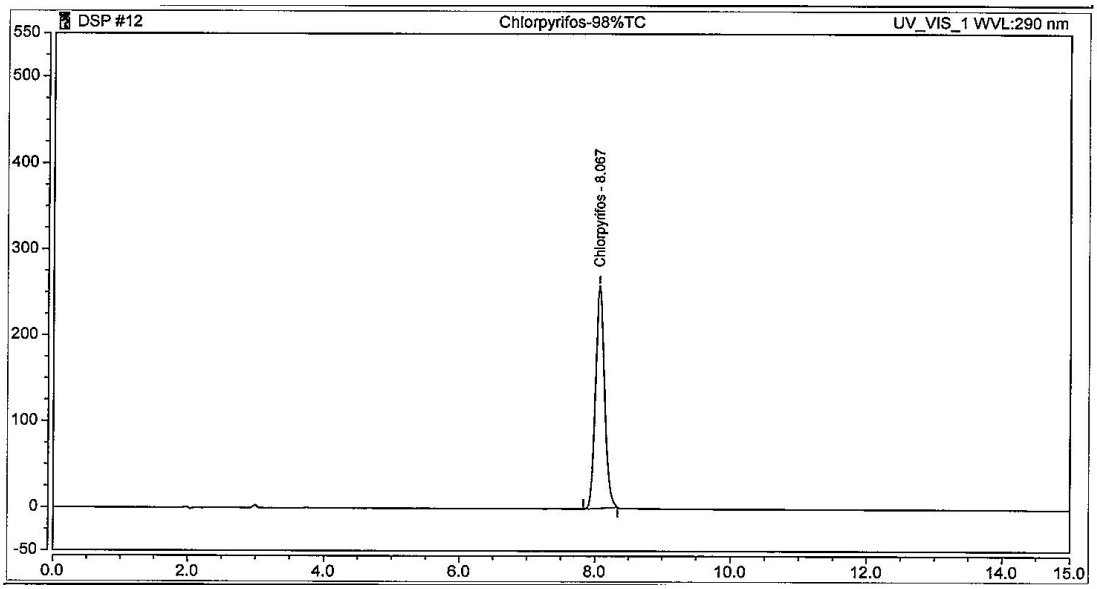

Fig.3 HPLC chromatogram of Chlorpyrifos TC

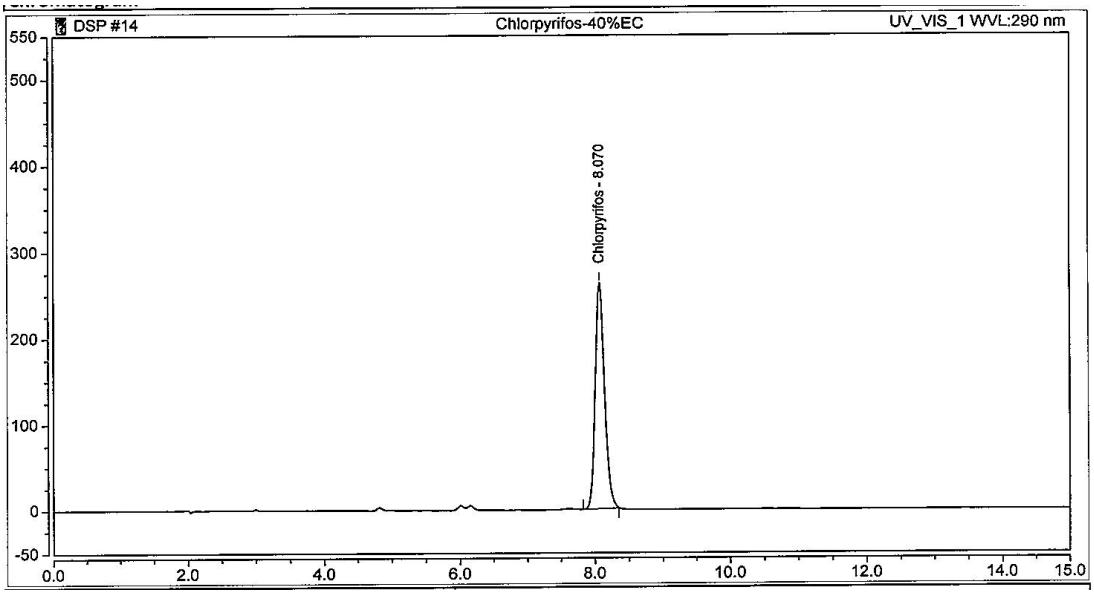

Fig.4 HPLC chromatogram of Chlorpyrifos EC

Chlorpyrifos

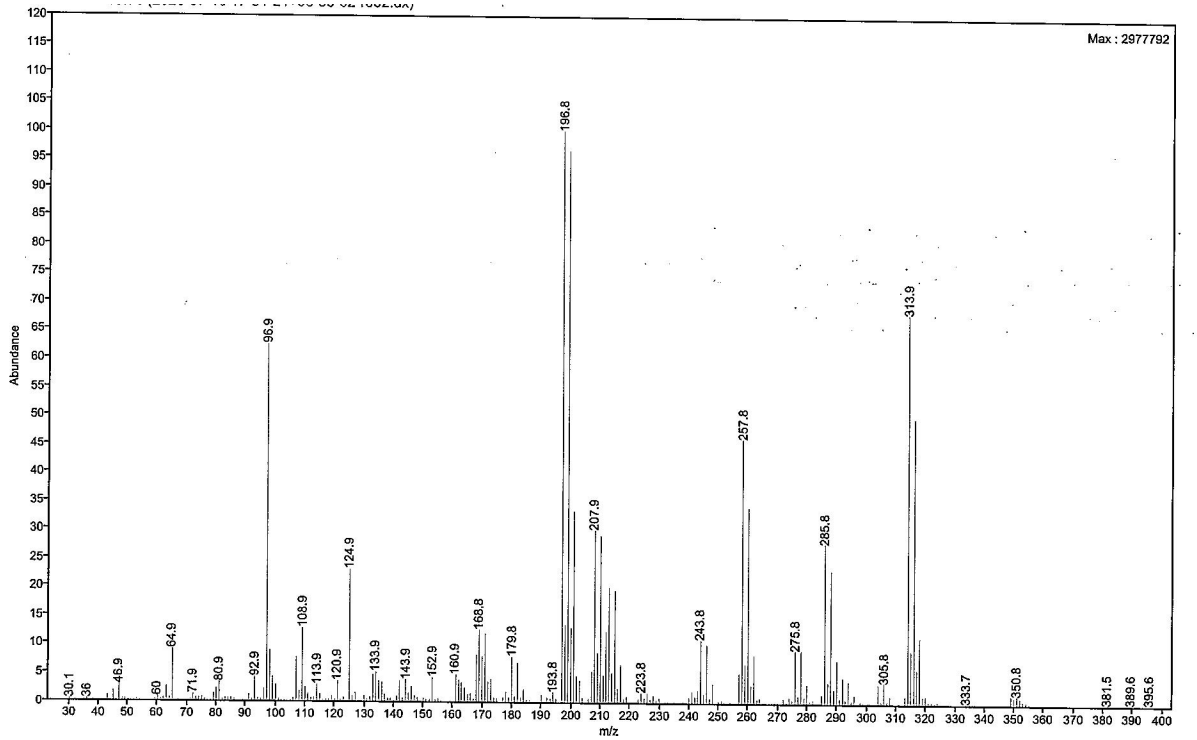

Fig.5 GC-MS spectrum of Chlorpyrifos standard

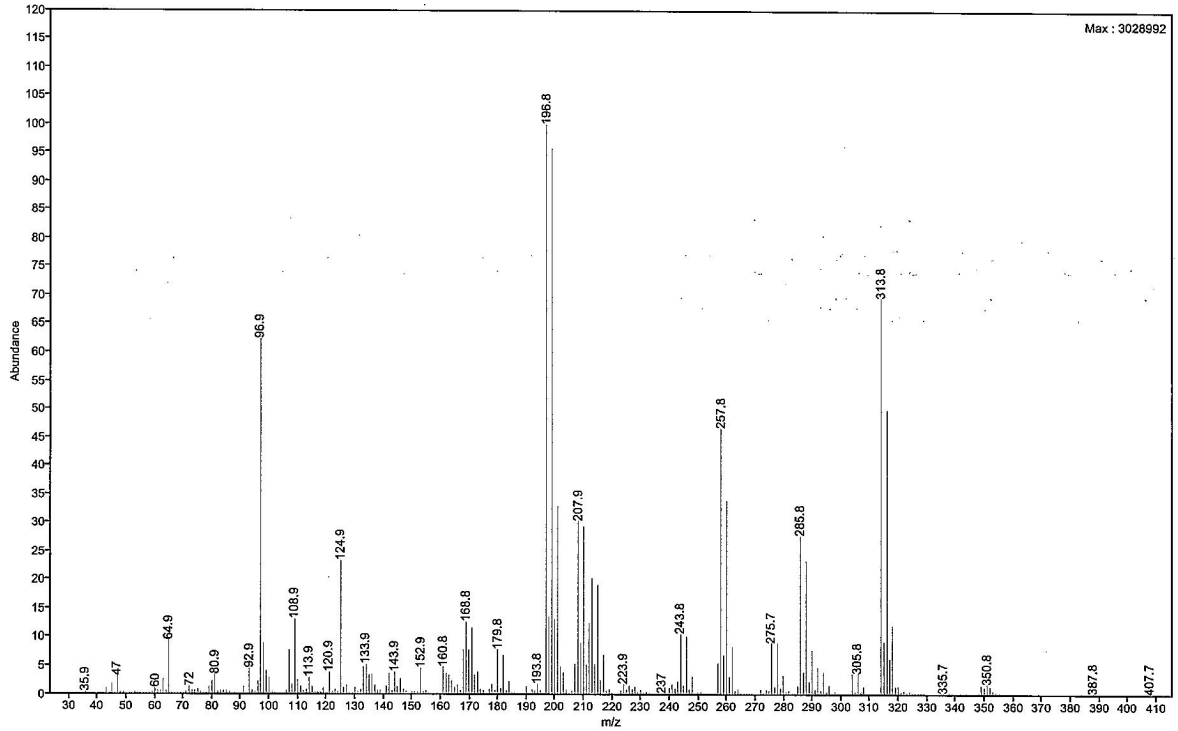

Fig.6 GC-MS spectrum of Chlorpyrifos TC

Chlorpyrifos

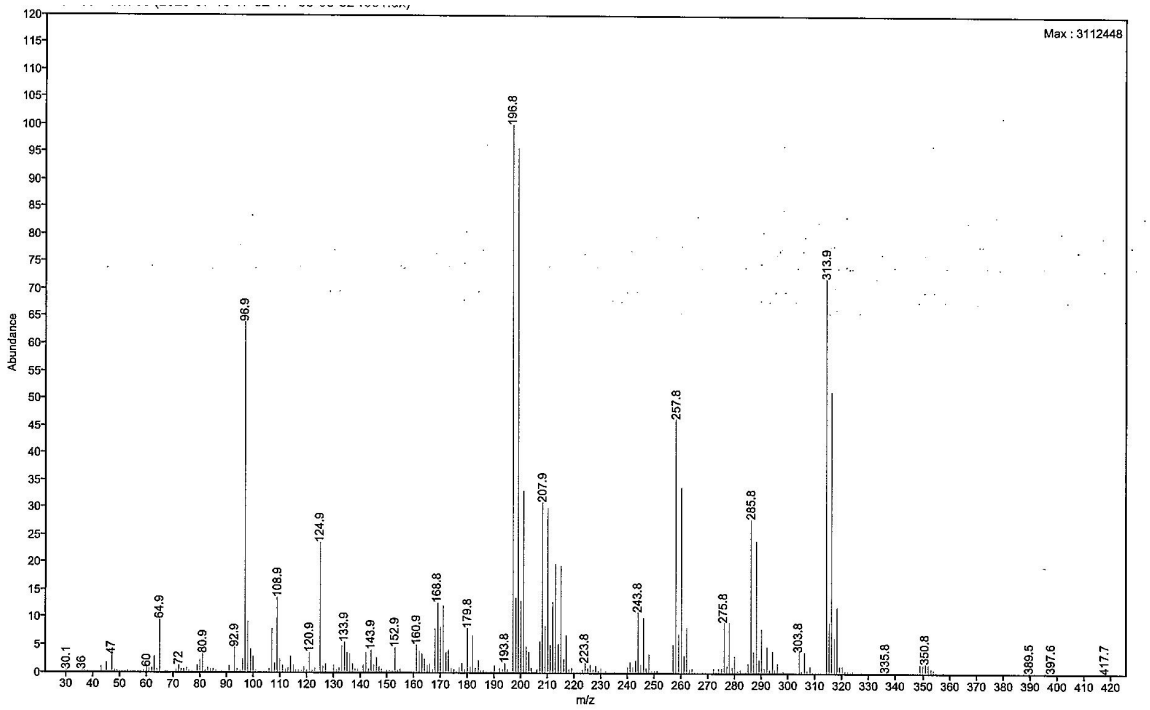

Fig.7 GC-MS spectrum of Chlorpyrifos EC