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### PYRAOXYSTROBIN

964



*ISO common name* Pyraoxystrobin

*Chemical name* methyl (2E)-2-(2-{[3-(4-chlorophenyl)-1-methylpyrazol-5-yl]oxymethyl} phenyl)-3-methoxyacrylate (IUPAC)

methyl (αE)-2-[[[3-(4-chlorophenyl)-1-methyl-1H-pyrazol-5-yl]oxy]methyl] -α-(methoxymethylene)benzeneacetate (CA; 862588-11-2)

*Empirical formula* C22H21ClN2O4

*RMM* 412.87

*m.p.* 129.6°C

*v.p.* 1.22×10-8 Pa at 20°C

*Solubility* In water 0.03 mg/L at 20°C; high solubility in *N*,*N*-Dimethylformamide, acetone, ethyl acetate, toluene, acetonitrile

*Stability* Not stable in alcohol to heat

*Description* The pure material is a white, odourless solid

*Formulation* Suspension concentrates (SC)

### PYRAOXYSTROBIN technical

964/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 Pyraoxystrobin 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 pyraoxystrobin 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.

**3 Pyraoxystrobin**

OUTLINE OF METHOD Pyraoxystrobin is determined by reversed phase high performance liquid chromatography using UV detection at 280 nm and external standardisation.

REAGENTS

*Acetonitrile* HPLC grade

*Water* HPLC grade

*Pyraoxystrobin* reference standard of known content. Store refrigerated.

*Calibration solutions*. Weigh in duplicate about 40 mg (to the nearest 0.1 mg) of pyraoxystrobin reference standard (s mg) into separate volumetric flasks (100 ml). Add acetonitrile (about 80 ml) and place the flask in an ultrasonic bath for 15 min. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. (calibration solutions CA and CB).

APPARATUS

*High performance liquid chromatograph*  equipped with a detector suitable for operation at 280 nm (UV-detection) and an injection system capable of injecting 5 µl.

*Liquid chromatographic column*  stainless steel, 150 x 4.6 mm (i.d.), Agilent ZORBAX SB C18, 5 µm, or equivalent with the same selectivity.

*Electronic integrator or data system*

*Ultrasonic bath*

PROCEDURE

1. *Chromatographic conditions* (typical)

*Column temperature* 30°C

*Flow rate* 1.3 ml/min

*Detector wavelength* 280 nm

*Injection volume* 5 µl

*Mobile phase* acetonitrile - water, 600 + 400 (v/v)

*Retention time* pyraoxystrobin approximately 6.4 min

1. *Equilibration of the system.* Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µl portion of calibration solution CA until the response obtained from two consecutive injections deviate by less than 1.0%. Then inject 5 µl portion of calibration solution CB. The response factor for this solution should not deviate by more than 1.0% from that for calibration solution CA, otherwise prepare new calibration solutions.
2. *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 40 mg of pyraoxystrobin (*s* mg) into a volumetric flask (100 ml). Add acetonitrile (about 80 ml) and place the flask in an ultrasonic bath for 15 min. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. (sample solutions S1 and S2).
3. *Determination.* Inject in duplicate 5 μl portions of each sample solution bracketing them by injections of the calibration solutions as follows: calibration solution CA, sample solution S1, sample solution S1, calibration solution CB, sample solution S2, sample solution S2, calibration solution CA, and so on. Measure the relevant peak areas. Average the values of the duplicate sample injections. Calculate the mean values of the response factors of the calibration solution bracketing two sample solutions and use this value to calculate the pyraoxystrobin content of the bracketed samples.
4. *Calculation*. Determine the peak area of pyraoxystrobin 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 pyraoxystrobin content of the bracketed sample solutions. The pyraoxystrobin content is the mean value of two sample solutions.



Pyraoxystrobin content  g/kg

where:

*fi =* individual response factor

*f =* mean response factor

*Hs =* peak area of pyraoxystrobin in the calibration solution

*Hw =* peak area of pyraoxystrobin in the sample solution

*s* = mass of pyraoxystrobin reference standard in the calibration solution (mg)

*w =* mass of sample taken (mg)

*P* = purity of pyraoxystrobin reference standard (g/kg)

**PYRAOXYSTROBIN suspension concentrates**

964/SC/M/-

**1 Sampling.** Take at least 600 ml.

**2. Identity test**

**2.1 HPLC.** As for pyraoxystrobin technical 964/TC/M/2.1

**2.2 HPLC-MS**

PROCEDURE

1. *Preparation of sample.* Homogenise the sample by vigorous shakingWeigh (to the nearest 0.1 mg) sufficient sample to contain about 20 mg of pyraoxystrobin into a volumetric flask (50 ml). Add acetonitrile (about 40 ml) and place the flask in an ultrasonic bath for 15 min. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter through a 0.45 µm PTFE filter prior to injection.
2. *Chromatographic conditions* (typical)

*Liquid chromatographic column*  stainless steel, 150 x 4.6 mm (i.d.), Agilent ZORBAX Extend C18, 5 µm, or equivalent with the same selectivity.

*Column temperature* 30°C

*Flow rate* 1.0 ml/min

*Injection volume* 10 µl

*Mobile phase* acetonitrile - water, 500 + 500 (v/v)

*Detector* MS

Ionisation mode +ESI

Full scan mode 100 - 1000 m/z

*Total run time* 25 min

*Retention time* pyraoxystrobin approximately 15 min

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

**3 Pyraoxystrobin**

As for pyraoxystrobin technical 964/TC/M/3 except

1. *Preparation of sample.* Homogenise the sample by vigorous shaking. Prepare sample solutions in duplicate for each sample.Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 40 mg of pyraoxystrobin (*s* mg) into a volumetric flask (100 ml). Add acetonitrile (about 80 ml) and place the flask in an ultrasonic bath for 15 min. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter an aliquot of each prepared solution through a 0.45 µm PTFE filter prior to analysis (sample solutions S1 and S2).

**4 Suspensibility**

REAGENTS AND APPARATUS. As for pyraoxystrobin technical 964/TC/M/- and MT 184.

PROCEDURE

1. *Preparation of suspension and determination of sedimentation.* MT184.
2. *Determination of pyraoxystrobin in the bottom 25 ml of suspension.* After removal of the top 225 ml of suspension transfer the remaining 25 ml to a volumetric flask (100 ml) with acetonitrile (50 ml). Place the flask in an ultrasonic bath for 15 min. Allow to cool to ambient temperature and fill to the mark with acetonitrile. Mix thoroughly. Filter through a 0.45 µm filter prior to analysis. Determine the mass of pyraoxystrobin (*Q* g) by 964/TC/M/3.
3. *Calculation*

Suspensibility%

where:

*c* = mass of pyraoxystrobin in the sample taken for the preparation of the suspension (g)

*Q* = mass of pyraoxystrobin in the bottom 25 ml of suspension (g)

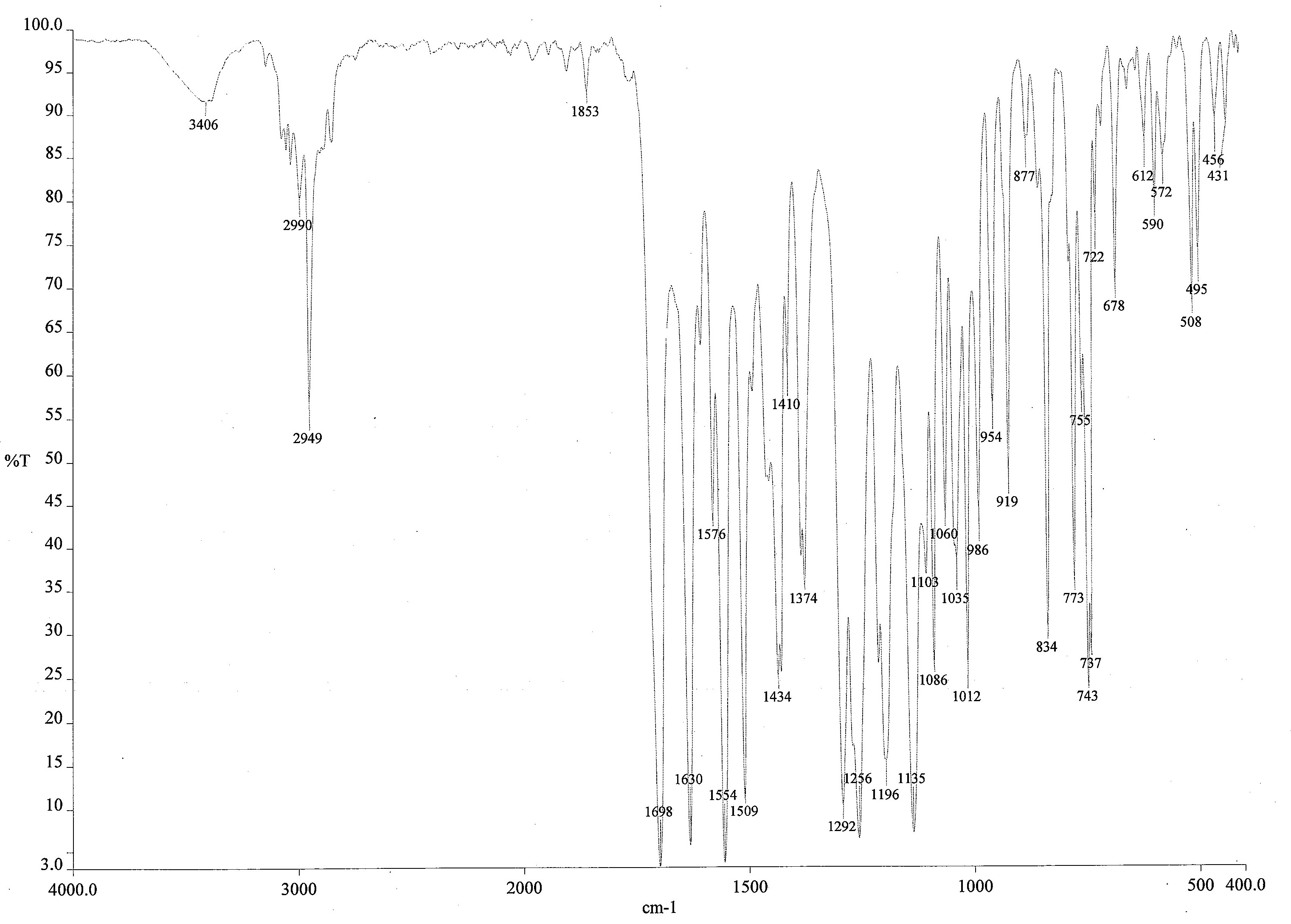


Fig.1 Infrared spectrum of pyraoxystrobin

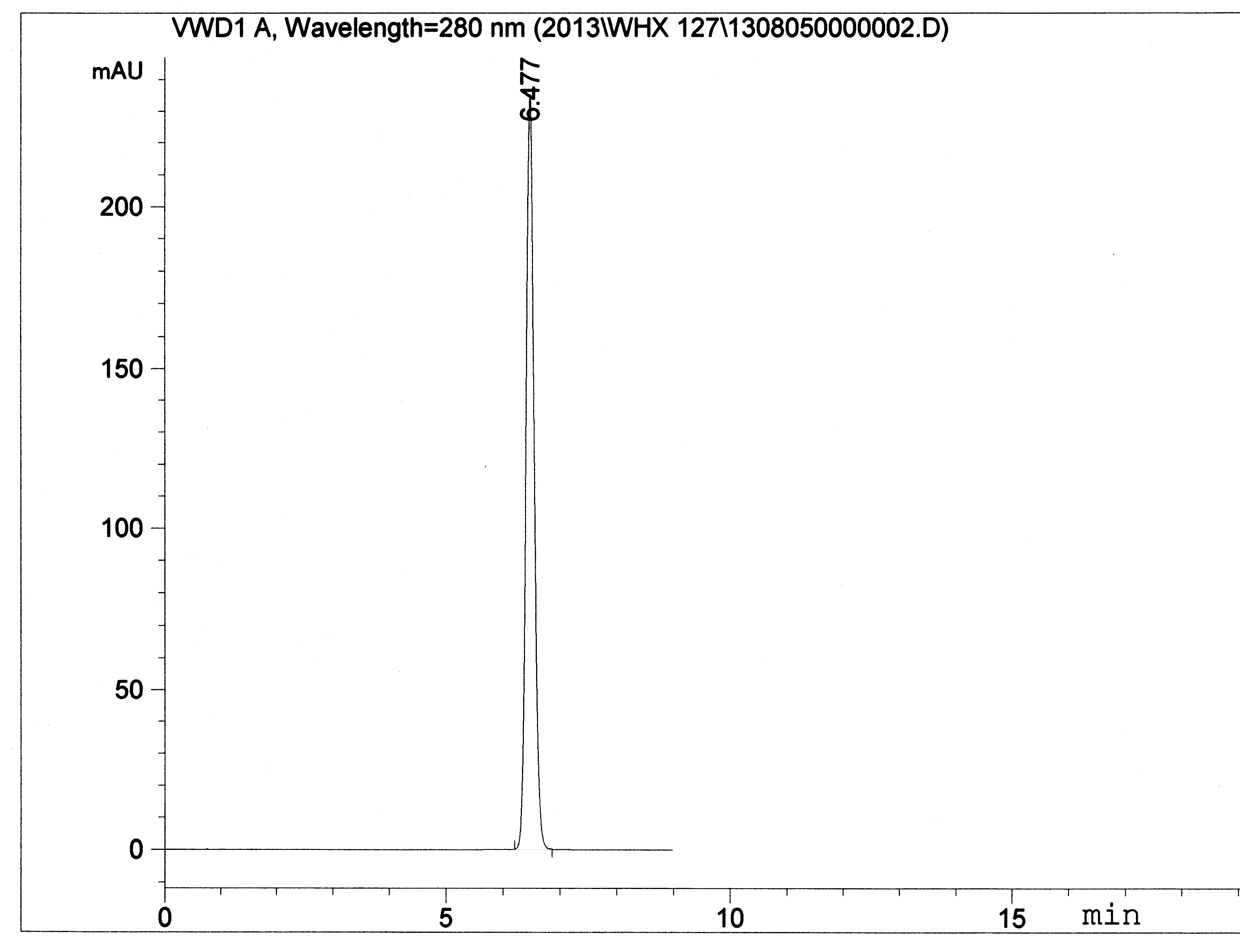


Fig.2 HPLC chromatogram of pyraoxystrobin standard

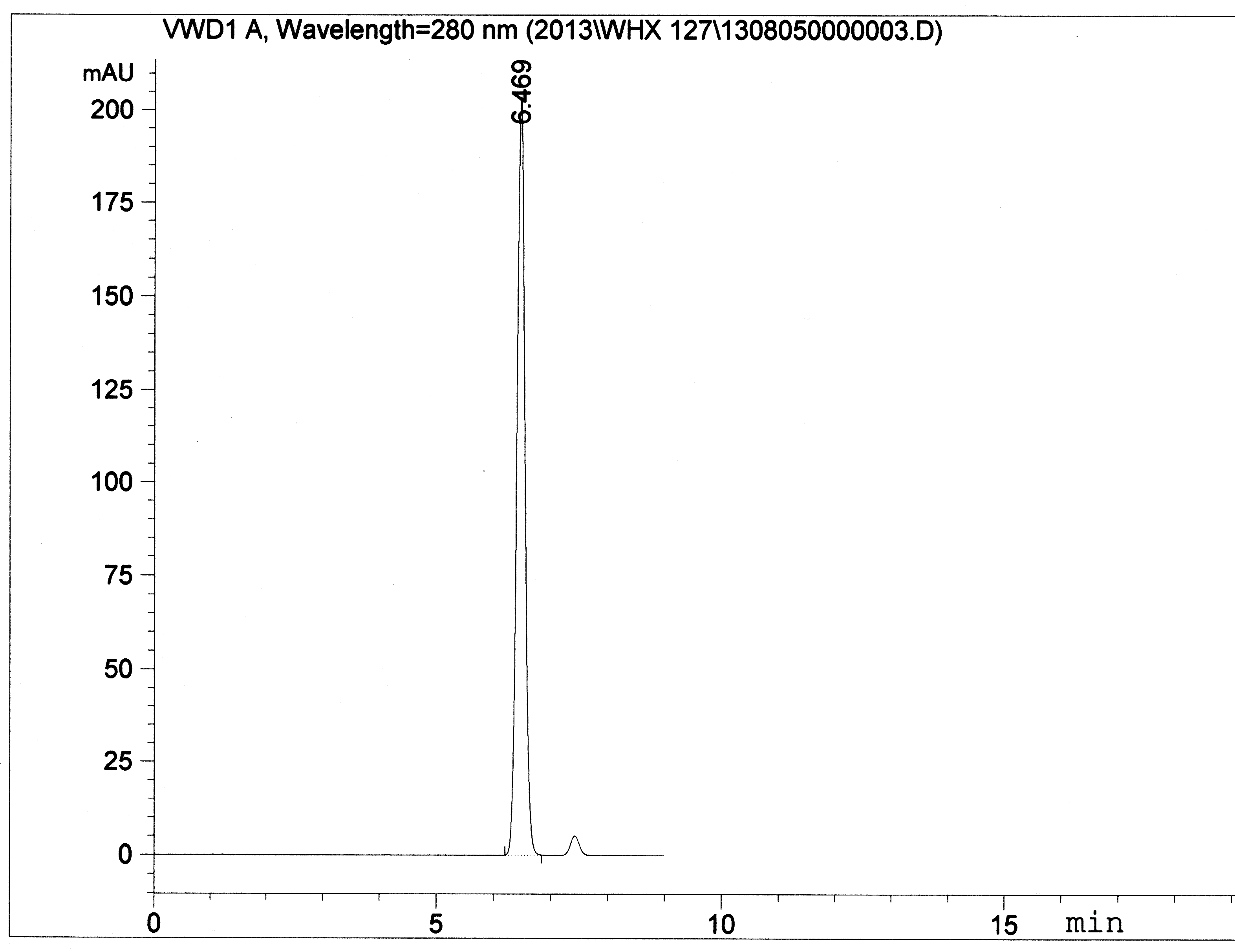


Fig.3 HPLC chromatogram of pyraoxystrobin TC

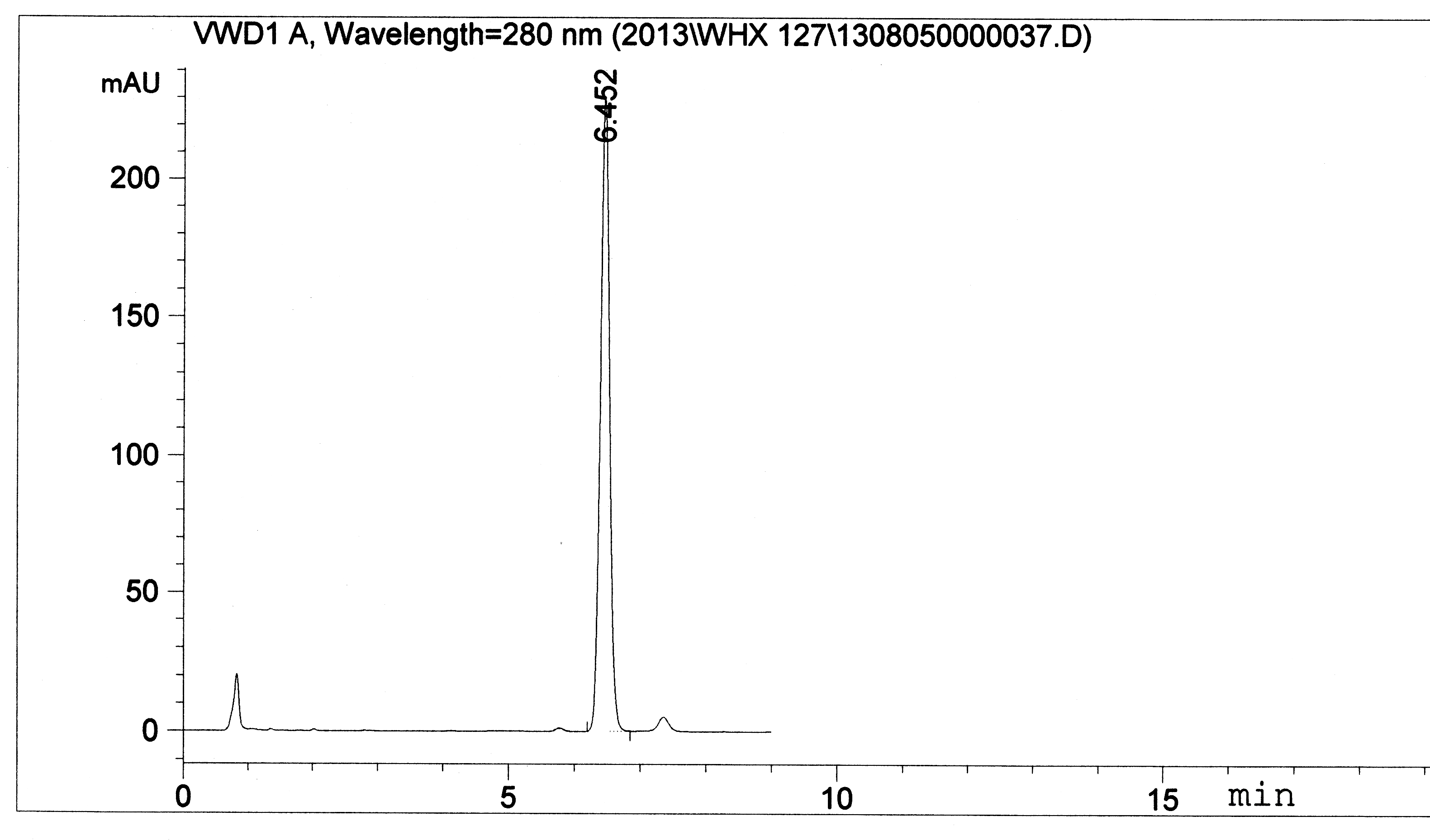


Fig.4 HPLC chromatogram of pyraoxystrobin SC

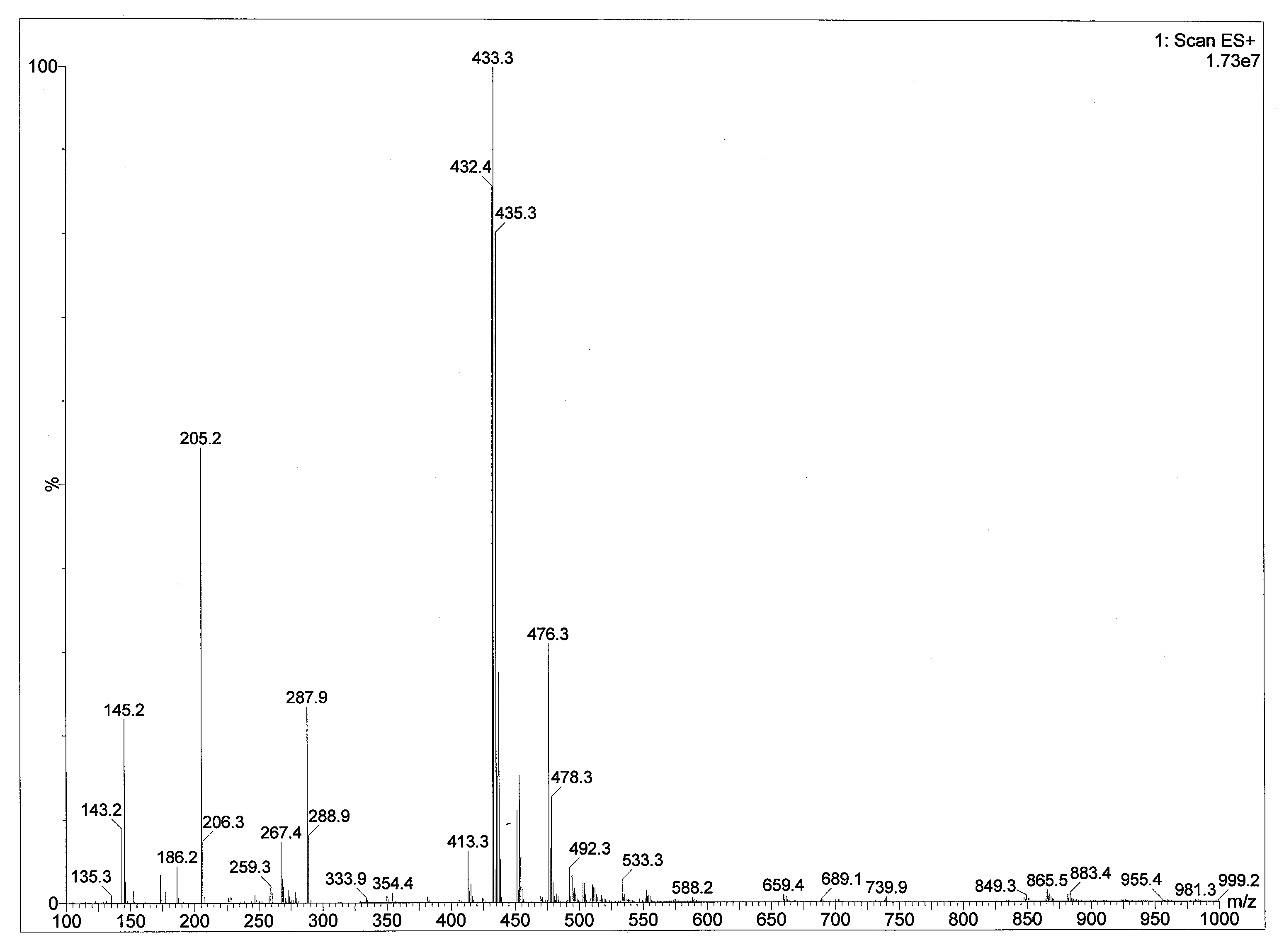


Fig.5 HPLC-MS spectrum of pyraoxystrobin standard

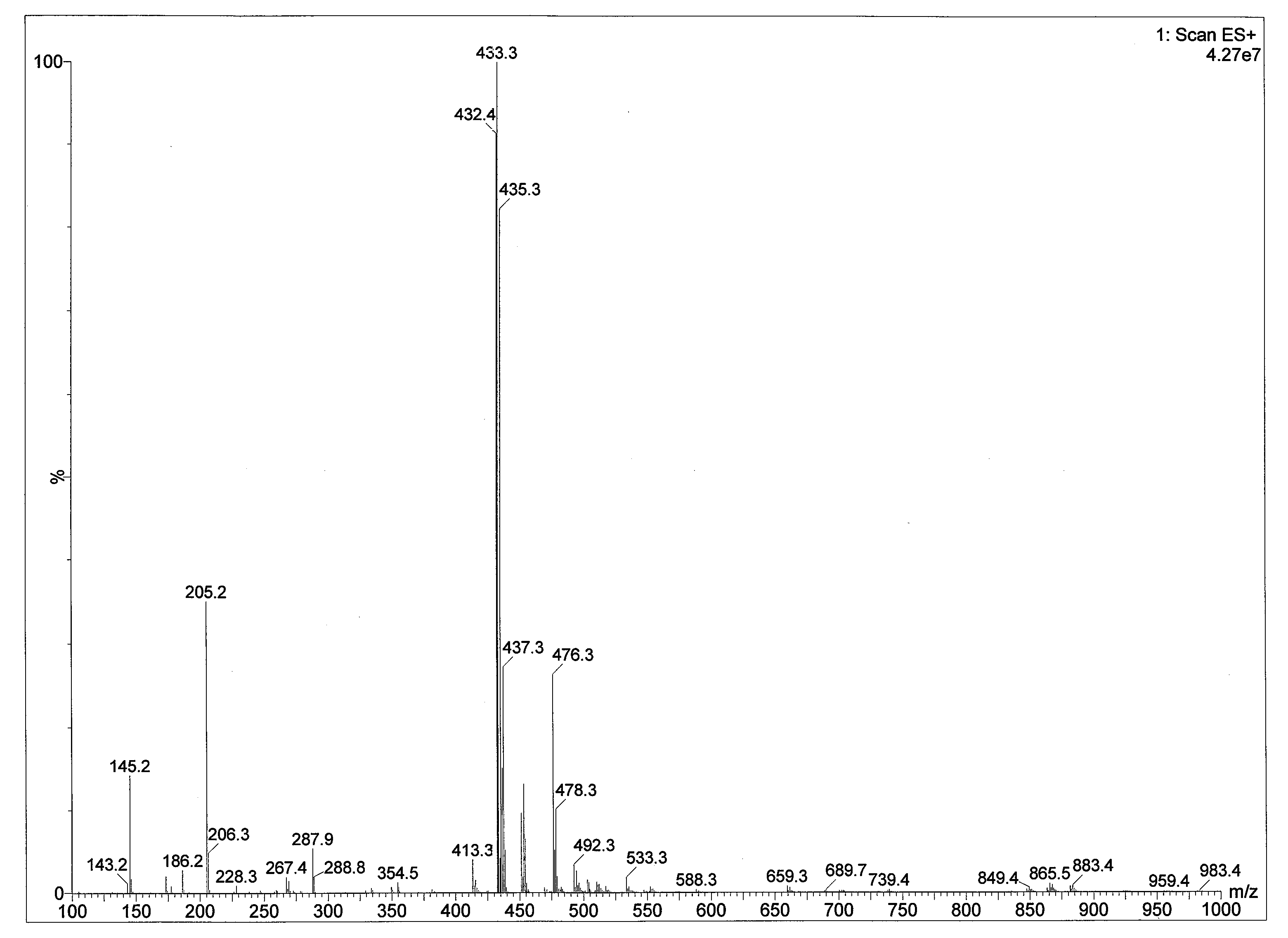


Fig.6 HPLC-MS spectrum of pyraoxystrobin SC