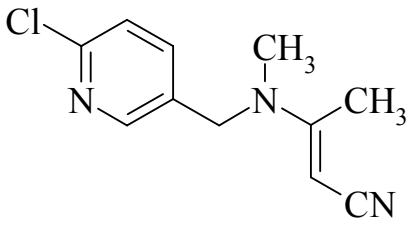
ACETAMIPRID
649



ISO common name	Acetamiprid
Chemical name	*(E)*-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methyl-acetamidine (IUPAC); (*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N*′-cyano-*N*-methyl-ethanamidine (CA; *135410-20-7*)
Empirical formula	$C_{10}H_{11}ClN_4$
RMM	222.7
m.p.	98.9 °C
v.p.	less than 1×10^{-6} Pa (25 °C)
Solubility	In water: 4.25 g/l; acetone: >200 g/l; acetonitrile: > 200 g/l; xylene: 35 g/l; methanol: > 200 g/l; all at 25 °C
Description	White powder
Stability	Stable at room temperature
Formulations	Wettable powders, water soluble powders, water soluble granules, soluble concentrates and emulsifiable concentrates.

ACETAMIPRID TECHNICAL
*649/TC/(M)/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use one of the HPLC methods below. The retention time of acetamiprid for the sample solution should not deviate by more than 0.2 min from that of the calibration solution (Fig. 2 and 3).

2.2 Infrared. Prepare potassium bromide discs from the sample and from pure acetamiprid. Scan the discs from 4000 to 400 cm^{-1}. The spectrum obtained from the sample should not differ significantly from that of the standard (Fig. 1).

3 Acetamiprid

3.1 Reversed phase HPLC method

OUTLINE OF METHOD Acetamiprid is determined by high performance liquid chromatography on a column (C_{18}) using acetonitrile + water + 10% phosphoric acid solution as mobile phase, UV detection at 246 nm and coumarin as internal standard.

REAGENTS

Acetonitorile HPLC grade
Water HPLC grade
Acetamiprid analytical standard of known purity
Coumarin internal standard. Must not show a peak with the same retention time as acetamiprid.
Phosphoric acid 85%
Phosphoric acid solution 10% Weigh 11.8 g of phosphoric acid 85% into volumetric flask (100 ml) and fill to 100 ml with water. Mix thoroughly.
Diluting solvent I acetonitrile - water, 50 + 50 (v/v)
Diluting solvent II acetonitrile - water, 6 + 4 (v/v)
Mobile phase acetonitrile - water - phosphoric acid solution 10%, 250 + 750 + 1 (v/v/v)
Internal standard solution Weigh into a volumetric flask (500 ml) coumarin (15.0 g), add acetonitrile (approximately 400 ml). Place the flask in an ultrasonic bath for 5 min and swirl it occasionally. Allow to cool to room temperature, make up to volume with acetonitrile and mix thoroughly.

* CIPAC method 2005. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

Calibration solution Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) approximately 100 mg of acetamiprid analytical standard (*s* mg). Add by pipette internal standard solution (20 ml), make up to volume with diluting solvent *I* and mix thoroughly. Ensure that the acetamiprid has dissolved completely. Transfer by pipette 400 µl of this solution into a sample vial and add diluting solvent *I* (10 ml). Mix thoroughly (Solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with an automatic loop injector (5 µl) and a UV spectrophotometric detector operated at 246 nm
Column stainless steel, 150 × 4.6 mm (i.d.), packed with Symmetry C18 (5 µm), or equivalent
Column oven
Electronic integrator or *data system*
Ultrasonic bath

PROCEDURE

(a) Operating conditions (typical):

Stationary phase	stainless steel, 150 × 4.6 mm (i.d.), packed with Symmetry C_{18} (5 µm)
Mobile phase	acetonitrile - water - phosphoric acid 10%, 250 + 750 + 1 (v/v/v)
Flow rate	1.0 ml/min
Column temperature	40 °C
Injection volume	5 µl
Detector wavelength	246 nm
Retention time	acetamiprid: about 5 min coumarin: about 8 min

(b) Linearity check. Before conducting the determination, check the linearity of the detector response by injecting 5 µl of solutions with acetamiprid concentrations 0.5, 1 and 2 times that of the calibration solution.

(c) System equilibration. Prepare two calibration solutions. Inject 5 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 1%. Then inject 5 µl portions of the second solution. The response factor for this solution should not deviate by more than 1% from that for the first calibration solution. Otherwise prepare new calibration solutions.

(d) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) approximately sufficient sample to contain 100 mg (*w* mg) of acetamiprid. Add by pipette internal standard solution (20.0 ml), diluting solvent *I* (approximately 60 ml) and mix thoroughly. Ensure that the sample has dissolved completely. Make up to volume with diluting solvent *I* and mix thoroughly. Transfer by pipette 400 µl of this solution into a sample vial and add dilution solvent *II* (10 ml). Mix thoroughly (solutions S_A and S_B).

(e) Determination. Inject 5 µl of each sample solution bracketing them by injections of calibration solutions as follows: C_A, S_A, S_B, C_B, ….

(f) Calculation. Calculate the mean value of response factors bracketing the two injections of a sample and use this value for calculating the acetamiprid content of the bracketed sample injections.

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

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

where:

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

The acetamiprid content is the mean value of two sample solutions.

Repeatability r = 29 g/kg at 1000 g/kg active ingredient content
Reproducibility R = 29 g/kg at 1000 g/kg active ingredient content

*3.2 Normal phase HPLC method

OUTLINE OF METHOD Acetamiprid is determined by high performance liquid chromatography on a CN column using *n*-heptane + ethanol as mobile phase, UV detection at 254 nm and *p*-nitroaniline as internal standard.

REAGENTS

n-Heptane HPLC grade
Ethanol HPLC grade
Ethyl acetate HPLC grade
Acetamiprid analytical standard of known purity
p-Nitroaniline internal standard. Must not show a peak with the same retention time as acetamiprid.
Mobile phase n-heptane - ethanol, 70 + 30 (v/v)
Internal standard solution. Weigh *p*-nitroaniline (10.0 g) into a flask (500 ml), add ethyl acetate (approximately 400 ml). Place the bottle in an ultrasonic bath for 5 min and swirl it occasionally. Allow to cool to room temperature and fill to 500 ml with ethyl acetate. Ensure that the *p*-nitroaniline has dissolved completely. Mix thoroughly.
Note: p-Nitroaniline is toxic. Avoid inhalation and eye and skin contact. Use adequate protection during handling.
Calibration solution. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) approximately 100 mg of acetamiprid analytical standard (*s* mg) into a volumetric flask (100 ml). Add by pipette internal standard solution (20.0 ml) and dilute to volume with ethyl acetate. Mix thoroughly. Ensure that the acetamiprid has dissolved completely. Transfer by pipette 400 µl of this solution into a sample vial, and add ethyl acetate (10 ml). Mix thoroughly (Solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a UV spectrophotometric detector operated at 254 nm
Column stainless steel, 250 × 4.0 mm (i.d.), packed with LiChrosorb CN (5 µm), or equivalent
Column oven
Electronic integrator or *data system*
Ultrasonic bath

* Tentative CIPAC method 2004. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

PROCEDURE

(a) Operating conditions (typical):

Stationary phase	250 × 4.0 mm (i.d.), packed with LiChrosorb CN (5 µm)
Mobile phase	*n*-heptane - ethanol , 70 + 30 (v/v)
Flow rate	1.0 ml/min
Column temperature	35 °C
Injection volume	5 µl
Detector wavelength	254 nm
Retention time	acetamiprid: about 9 min *p*-nitroaniline: about 5 min

(b) Linearity check. Before conducting the determination, check the linearity of the detector response by injecting 5 µl of solutions with acetamiprid concentrations 0.5, 1 and 2 times that of the calibration solution.

(c) System equilibration. Prepare two calibration solutions. Inject 5 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 1%. Then inject 5 µl portions of the second solution. The response factor for this solution should not deviate by more than 1% from that for the first calibration solution, otherwise prepare new calibration solutions.

(d) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain 100 mg (*w* mg) of acetamiprid. Add by pipette internal standard solution (20.0 ml) and make up to volume with ethyl acetate. Mix thoroughly. Transfer by pipette 400 µl of this solution into a sample vial and add ethyl acetate (10 ml). Mix thoroughly (Solutions S_A and S_B).

(e) Determination. Inject 5 µl of each sample solution bracketing them by injections of calibration solutions as follows: C_{A1}, S_{A1}, S_{B1}, C_{B1} ….

(f) Calculation. Calculate the mean value of response factors bracketing the two injections of a sample and use this value for calculating the acetamiprid content of the bracketed sample injections.

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

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

where:

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

The acetamiprid content is the mean value of two sample solutions.

Repeatability r = 47 g/kg at 1001 g/kg active ingredient content
= 26 g/kg at 996 g/kg active ingredient content
Reproducibility R = 47 g/kg at 1001 g/kg active ingredient content
= 38 g/kg at 996 g/kg active ingredient content

ACETAMIPRID WETTABLE POWDERS
*649/WP/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests

2.1 HPLC. As for acetamiprid technical **649/**TC/(M)/2.1.

2.2 Infrared. Extract the sample with a suitable solvent, filter and evaporate the solvent with a stream of clean dry air. Proceed as for acetamiprid technical **649/**TC/(M)/2.2.

* CIPAC method 2005. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

3 Acetamiprid. As for acetamiprid technical **649/**TC/(M)/3.1 except:
(d) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 100 mg (*w* mg) of acetamiprid into a volumetric flask (100 ml). Add by pipette internal standard solution (20.0 ml) and diluting solvent *I* (approximately 60 ml). Place the flask in an ultrasonic bath for 10 min and swirl it occasionally. Allow to cool to room temperature, make up to volume with diluting solvent *I* and mix thoroughly. Filter the solution through a 0.45 µm filter. Transfer by pipette 400 µl of filtrate into a sample vial and add diluting solvent *II (*10 ml). Mix thoroughly (solutions S_A and S_B).

Repeatability r = 35 g/kg at 711 g/kg active ingredient content
Reproducibility R = 36 g/kg at 711 g/kg active ingredient content

ACETAMIPRID WATER SOLUBLE POWDERS
*649/SP/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for acetamiprid technical **649/**TC/(M)/2.1.
2.2 Infrared. Extract the sample with a suitable solvent, filter and evaporate the solvent with a stream of clean dry air. Proceed as for acetamiprid technical **649/**TC/(M)/2.2.

3 Acetamiprid. As for acetamiprid technical **649/**TC/(M)/3.1 except:
(d) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 100 mg (*w* mg) of acetamiprid into a volumetric flask (100 ml). Add by pipette internal standard solution (20.0 ml) and dilute with diluting solvent *I* (approximately 60 ml). Place the flask in an ultrasonic bath for 10 min and swirl it occasionally. Allow to cool to room temperature. Ensure that the sample has dissolved completely. Make up to volume with diluting solvent *I* and mix thoroughly. Transfer by pipette 400 µl of this solution into a sample vial and add diluting solvent *II* (10 ml). Mix thoroughly (Solutions S_A and S_B).

Repeatability r = 7.5 g/kg at 204 g/kg active ingredient content
Reproducibility R = 11 g/kg at 204 g/kg active ingredient content

* CIPAC method 2005. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

ACETAMIPRID WATER SOLUBLE GRANULES
*649/SG/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for acetamiprid technical **649/**TC/(M)/2.1.
2.2 Infrared. Extract the sample with a suitable solvent, filter and evaporate the solvent with a stream of clean dry air. Proceed as for acetamiprid technical **649/**TC/(M)/2.2.
3 Acetamiprid. As for acetamiprid technical **649/**TC/(M)/3.1 except:
(d) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 100 mg (*w* mg) of acetamiprid into a volumetric flask (100 ml). Add by pipette internal standard solution (20.0 ml) and dilute with diluting solvent *I* (approximately 60 ml). Place the flask in an ultrasonic bath for 10 min and swirl it occasionally. Allow to cool to room temperature. Ensure that the sample has dissolved completely. Make up to volume with diluting solvent *I* and mix thoroughly. Transfer by pipette 400 µl of this solution into a sample vial and add diluting solvent *II* (10 ml). Mix thoroughly (Solutions S_A and S_B).

Repeatability r = 8.7 g/kg at 312 g/kg active ingredient content
Reproducibility R = 14 g/kg at 312 g/kg active ingredient content

ACETAMIPRID SOLUBLE CONCENTRATES
*649/SL/(M)/-

1 Sampling. Take at least 500 ml.

2 Identity tests
2.1 HPLC. As for acetamiprid technical **649/**TC/(M)/2.1.
2.2 Infrared. Evaporate the solvent. Proceed as for acetamiprid technical **649/**TC/(M)/2.2.

3 **Acetamiprid.** As for acetamiprid technical **649/**TC/(M)/3.1 except:
(d) Preparation of sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) approximately 100 mg (*w* mg) of acetamiprid into a volumetric flask (100 ml). Add by pipette internal standard solution (20.0 ml) and diluting solvent *I*

* CIPAC method 2005. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

(approximately 60 ml). Mix thoroughly. Make up to volume with diluting solvent *I* and mix thoroughly. Transfer by pipette 400 µl of this solution into a sample vial and add dilution solvent *II* (10 ml). Mix thoroughly (solutions S_A and S_B).

Repeatability r = 5.3 g/kg at 205 g/kg active ingredient content
Reproducibility R = 8.1 g/kg at 205 g/kg active ingredient content

ACETAMIPRID EMULSIFIABLE CONCENTRATES
*649/EC/(M)/-

1 Sampling. Take at least 500 ml.

2 Identity tests
2.1 HPLC. As for acetamiprid technical **649/**TC/(M)/2.1.
2.2 Infrared. Evaporate the solvent. Proceed as for acetamiprid technical **649/**TC/(M)/2.2.

3 Acetamiprid. As for acetamiprid technical **649/**TC/(M)/3.2

Repeatability r = 1.0 g/kg at 30.9 g/kg active ingredient content
= 1.0 g/kg at 31.2 g/kg active ingredient content
= 0.7 g/kg at 32.3 g/kg active ingredient content
Reproducibility R = 1.3 g/kg at 30.9 g/kg active ingredient content
= 1.2 g/kg at 31.2 g/kg active ingredient content
= 0.9 g/kg at 32.3 g/kg active ingredient content

* CIPAC method 2005. Prepared by the Japanese PAC (JAPAC). Chairman: N Tamori. Based on a method supplied by Nippon Soda Co Ltd, Japan.

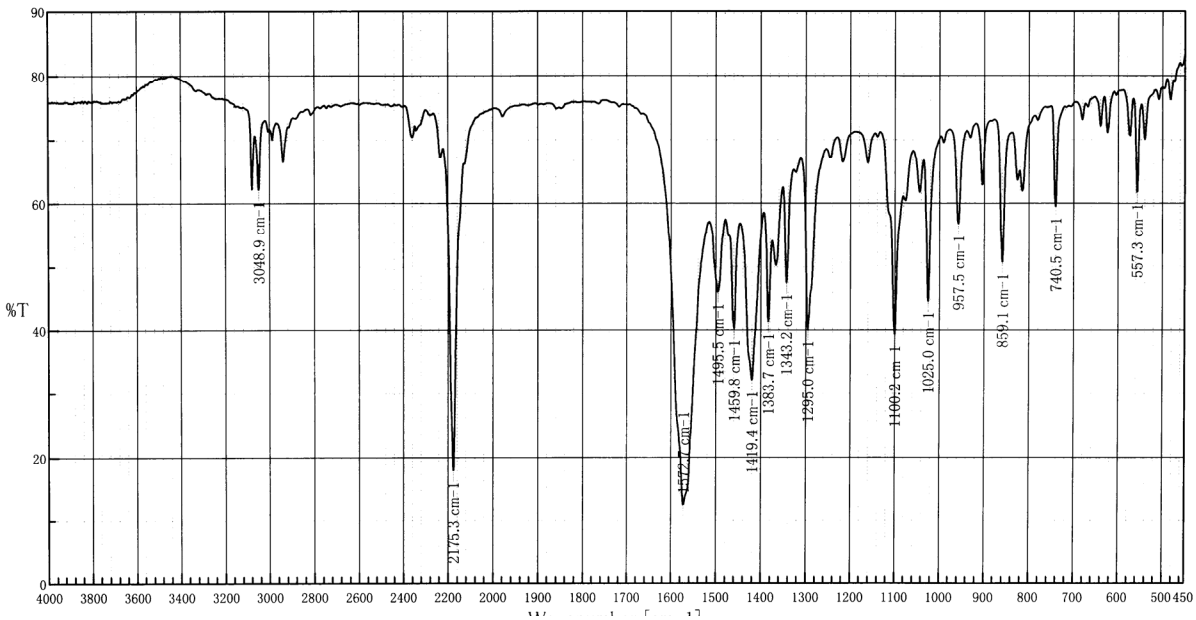


Fig. 1 IR spectrum of acetamiprid

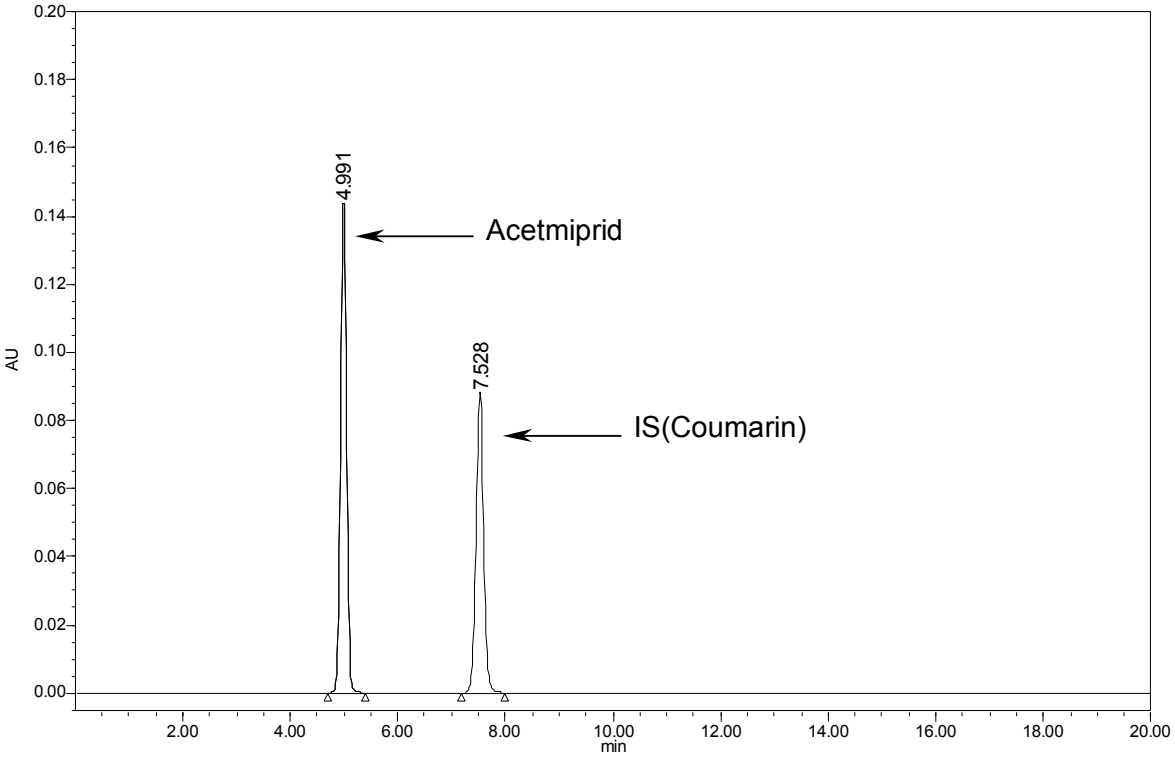


Fig. 2 Reversed phase chromatogram of acetamiprid TC

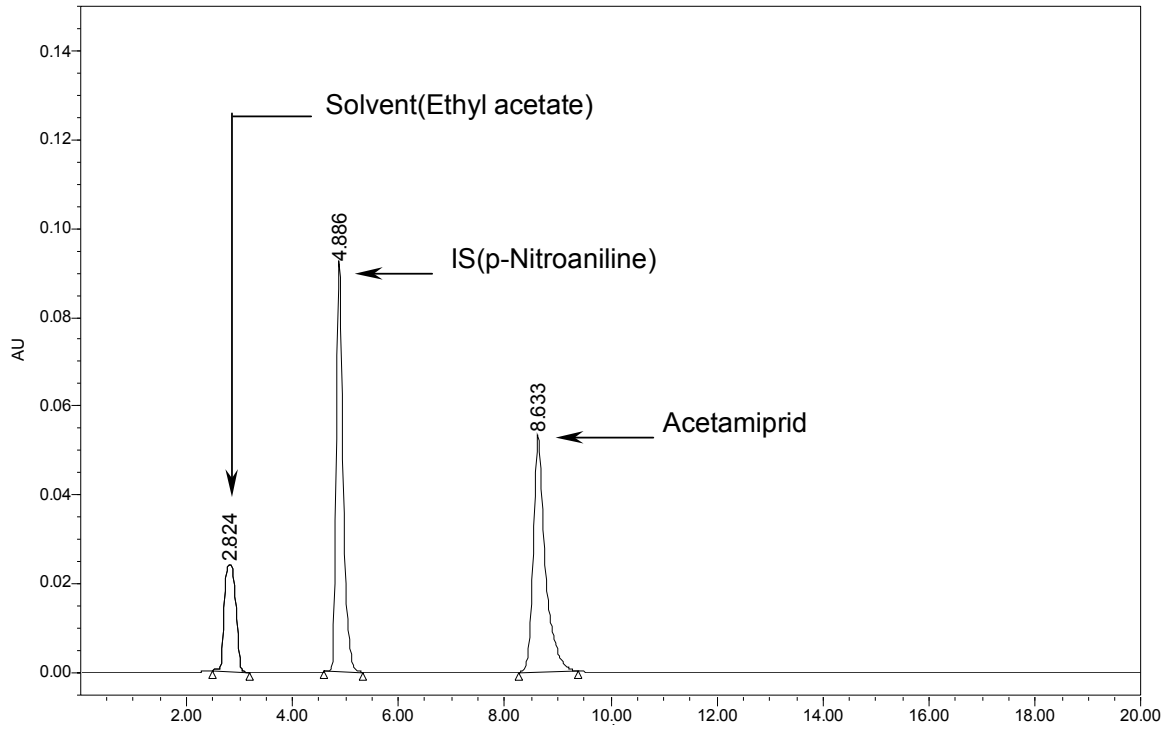


Fig. 3 Normal phase chromatogram of acetamiprid TC

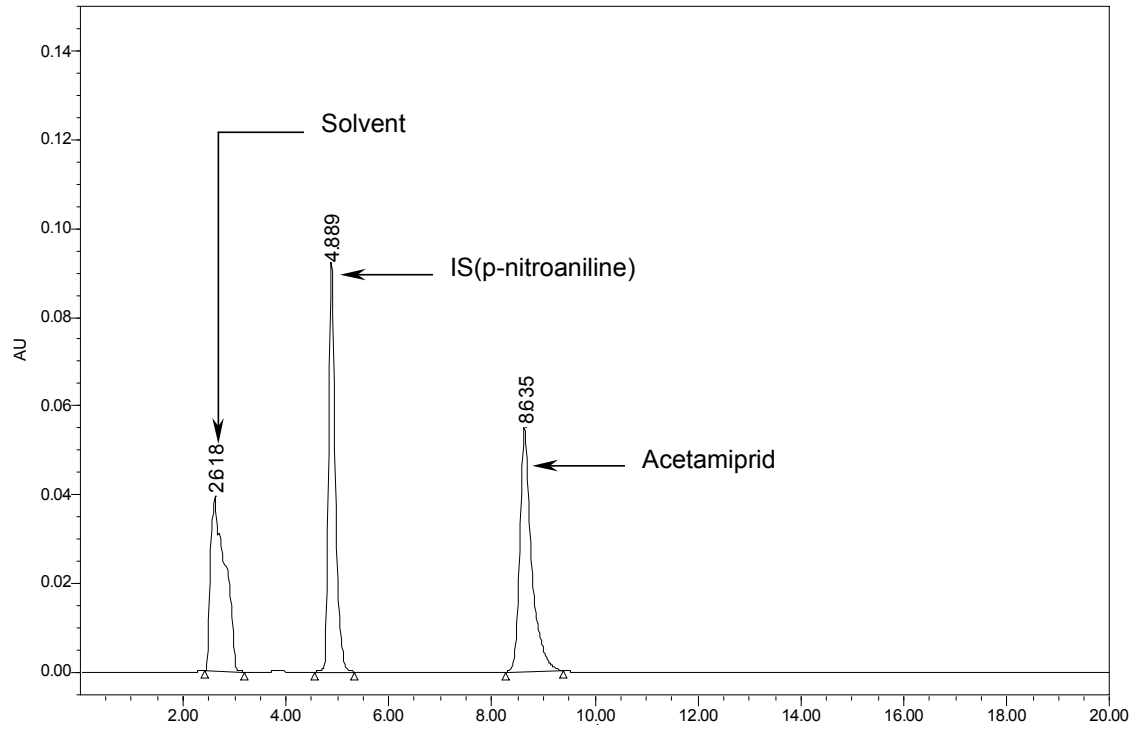


Fig. 4 Normal phase chromatogram of acetamiprid EC