Prothioconazole

HPLC Method

CIPAC Collaborative Trial according to CIPAC Information Sheet No 311

by

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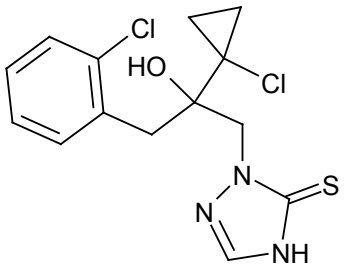
PROTHIOCONAZOLE
745

Chemical name:	(RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2- hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
ISO common name:	Prothioconazole
CAS-No.:	178928-70-6
Structure:	
Molecular mass:	344.3 g/mol
Empirical formula:	C14 H15 Cl2 N3 O S
m.p.:	140.3 °C
b.p.:	487 °C ± 50 °C (calculated)
Solubility:	ethyl acetate 215 g/L, acetone >280 g/L; dichlormethane 89 g/L; acetonitrile 69 g/L; water 22,5 mg/L at pH=7 (all at 20°C)
Description:	White to light beige crystalline powder

PROTHIOCONAZOLE
745/TC/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.

Take at least 100 g. Grind the sample thoroughly in a mortar.

2 Identity tests

2.1 HPLC. Use the HPLC method described below. The relative retention time of prothioconazole in the sample solution should not deviate by more than 2% from that of the calibration solution.

2.2 UV spectrometry. Record the UV spectrum during the HPLC determination. The UV spectrum obtained from the sample should not differ significantly from that of the standard. (Fig. 1)

2.3 Infrared. Prepare by direct application pure prothioconazole and the sample on an ATR unit. Scan from 4000 to 550 cm^{-1}. The spectrum produced by the sample should not differ significantly from that of the standard. (Fig. 2)

3 Prothioconazole

OUTLINE OF THE METHOD.

The content of prothioconazole (g/kg) is determined by reversed phase high performance liquid chromatography using UV detection at 254 nm and external standard calibration.

3.1 Determination of Prothioconazole by reversed phase HPLC

REAGENTS

Prothioconazole reference standard of known content
Acetonitrile (HPLC grade)
Methanole (e.g. Chromasolv, <99.9%) [RE 47]
Tetrahydrofurane (e.g. Chromasolv Plus, <99.9%)
Phosphoric acid 85 % (puriss. p.a.)
Purified water (HPLC grade) [RE 130]
L-Cysteine hydrochloride monohydrate
Eluent A: 10 mMol phosphoric acid in 1 L purified water
Eluent B: acetonitrile / tetrahydrofurane / methano 50/25/25 [v/v/v]
Calibration solutions C1 and C2. Weigh in duplicate (to the nearest 0.01 mg) approximately 50 mg (*s* in mg) of the prothioconazole reference standard into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate to each flask and suspend in 50 mL acetonitrile. Place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water

and mix thoroughly (calibration solutions C1, C2, chromatogram of C1 see Fig. 3).

APPARATUS

High performance liquid chromatograph equipped with an injection system capable to inject 3 µL and an UV spectrophotometric detector operated at 254 nm.

Column, stainless steel, 50 x 4.6 (i.d.) mm, packed with Zorbax Extend C18; 3.5 µm or equivalent with the same selectivity.

Electronic integrator or data system

Ultrasonic bath

Centrifuge

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Temperature 40 °C

Injection volume 3 µL

Detector wavelength 254 nm

Mobile phase and Flow rate

Time [min]	10 mMol phosphoric acid in 1 L purified water	Acetonitrile/ tretrahydrofurane/ methanole 50/25/25 [v/v/v]	Flow rate [mL/min]
0.0	50	50	2
2.7	50	50	2
2.71	05	95	3
3.6	05	95	3
3.61	50	50	3
4.0	50	50	2
4.4	50	50	2

Retention time approximately 2.0 minutes

(b) System equilibration. Pump sufficient mobile phase through the column to equilibrate the system. Inject 3 µL portions of the calibration solution C1 (see below) and repeat the injections until retention times and peak areas deviate by less than ± 1 % from the mean for three successive injections.

(c) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* in mg)

(containing approximately 50 mg of prothioconazole) into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate to each flask and suspend in 50 mL acetonitrile. Place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly (sample solutions S1, S2, chromatogram of S1 see Fig. 4)

(d) Determination. Inject in duplicate each sample solution and bracket a series of sample solution injections by injections of the calibration solution as follows: calibration solution C1, calibration solution C2, calibration solution C1, sample solution S1, sample solution S1, sample solution S2, sample solution S2, calibration solution C1 ... (C1, C2, C1, S1, S1, S2, S2, C1, …)

Determine the peak area of prothioconazole.

(e) Calculation. Calculate the response factors 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. They must not deviate by more than ± 1 % of the average, otherwise repeat the determination. Calculate the content of the sample solutions.

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

$$\text{prothioconazole content (g/kg)} = \frac{H_W \times f}{W}$$

Where:

fi = single response factor
f = average response factor
H_S = peak area of prothioconazole standard in the calibration solution
H_W = peak area of prothioconazole in the sample solution
s = weight of the prothioconazole standard in the calibration solution (mg)
w = weight of the sample (mg)
P = purity of the prothioconazole standard (g/kg)

PROTHIOCONAZOLE EMULSIFIABLE CONCENTRATE 745/EC/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.

Take at least 500 mL. Shake the sample well before weighing.

2 Identity tests.
2.1 HPLC. As for prothioconazole 745/TC/M/-
2.2 UV spectrometry. As for prothioconazole 745/TC/M/-

3 Prothioconazole.
Same approach as for prothioconazole 745/TC/M/-

3.1 Determination of prothioconazole by reversed phase HPLC
As for prothioconazole 745/TC/M/-
(Sample solutions S3, S4, chromatogram of S3 see Fig. 5)

PROTHIOCONAZOLE FLOWABLE CONCENTRATE FOR SEED TREATMENT 745/FS/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.

Take at least 500 mL. Shake the sample well before weighing.

2 Identity tests.
2.1 HPLC. As for prothioconazole 745/TC/M/-
2.2 UV spectrometry. As for prothioconazole 745/TC/M/-

3 Prothioconazole.
Same approach as for prothioconazole 745/TC/M/-

3.1 Determination of prothioconazole by reversed phase HPLC
As for prothioconazole 745/TC/M/- in addition:
APPARATUS
Centrifuge
PROCEDURE
(c) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* in mg) (containing approximately 50 mg of prothioconazole) into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate and dissolve in 5 mL purified water in order to suspend the sample. Then, add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Clarify a part of the solution by centrifugation or filtration prior to analysis (sample solutions S5, S6, chromatogram of S5 see Fig. 6).

PROTHIOCONAZOLE SUSPENSION CONCENTRATE 745/SC/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.

Take at least 500 mL. Shake the sample well before weighing.

2 Identity tests.
2.1 HPLC. As for prothioconazole 745/TC/M/-
2.2 UV spectrometry. As for prothioconazole 745/TC/M/-

3 Prothioconazole.
Same approach as for prothioconazole 745/TC/M/-

3.1 Determination of prothioconazole by reversed phase HPLC
As for prothioconazole 745/FS/M/-
(Sample solutions S7, S8, chromatogram of S7 see Fig. 7).

PROTHIOCONAZOLE FLOWABLE CONCENTRATE FOR SEED TREATMENT WITH PROTHIOCONAZOLE BELOW 1 % W/W 745/FS1/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.

Take at least 500 mL. Shake the sample well before weighing.

2 Identity tests.
2.1 HPLC. As for prothioconazole 745/TC/M/-
2.2 UV spectrometry. As for prothioconazole 745/TC/M/-

3 Prothioconazole.
Same approach as for prothioconazole 745/TC/M/-

3.1 Determination of prothioconazole by reversed phase HPLC
As for prothioconazole 745/FS/M/- except
REAGENTS
Calibration solutions C3 and C4. Weigh in duplicate (to the nearest 0.01 mg) approximately 50 mg (*s* in mg) of the prothioconazole reference standard into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate to each flask and suspend in 50 mL of acetonitrile. Place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Then transfer 10 mL of each solution into a separate volumetric flask (100 mL), make up the flask with acetonitrile / purified water 50 / 50 % v/v to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile / purified water 50 / 50 %v/v and mix thoroughly (calibration solutions C3, C4, chromatogram of C3 see Fig. 8).

PROCEDURE
(c) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* in mg) (containing approximately 5 mg of prothioconazole) into separate volumetric flasks (100 mL). Add approximately 5 mg of

L-cysteine hydrochloride monohydrate and dissolve in 5 mL purified water in order to suspend the sample. Then, add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Clarify a part of the solution by centrifugation (sample solutions S9, S10, chromatogram of S9 see Fig. 9).

Fig. 1 UV-Spectrum of prothioconazole

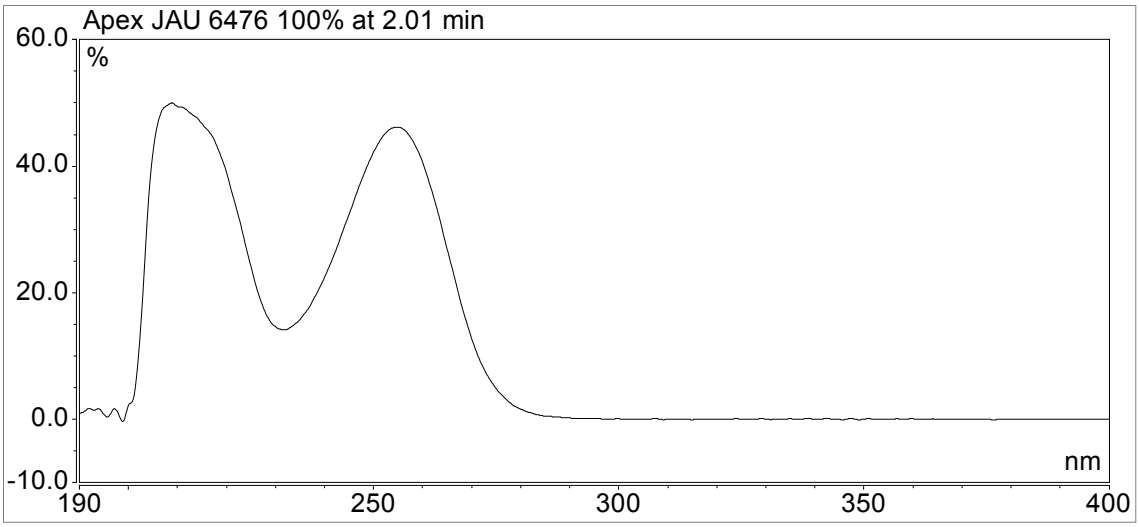


Fig. 2 Infrared Spectrum of prothioconazole

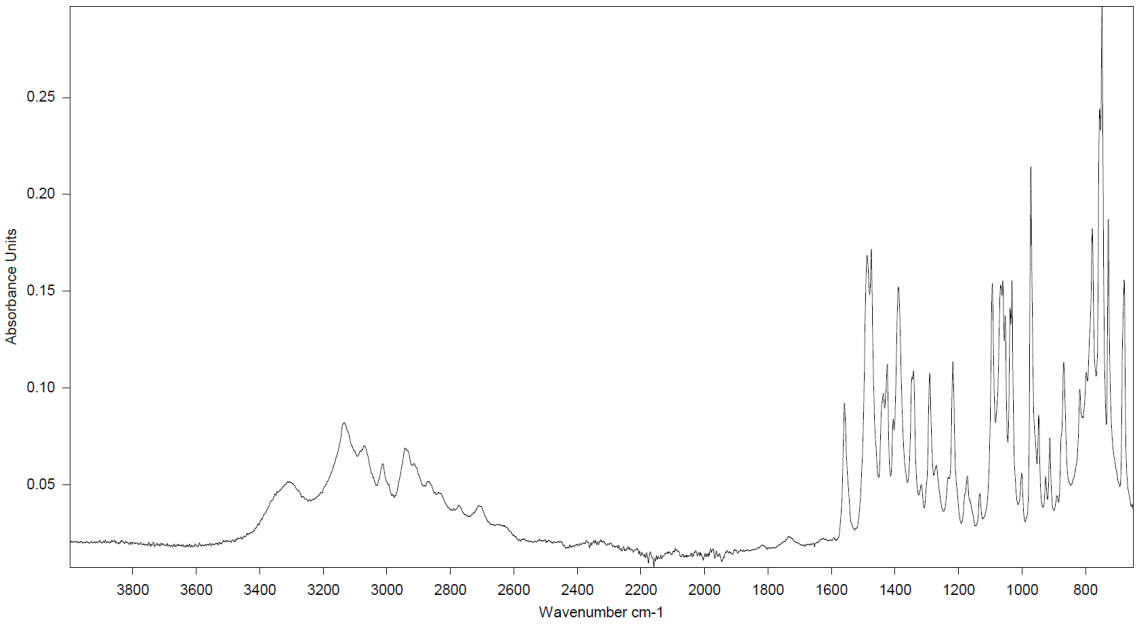


Fig. 3 Analytical Standard prothioconazole (C1)

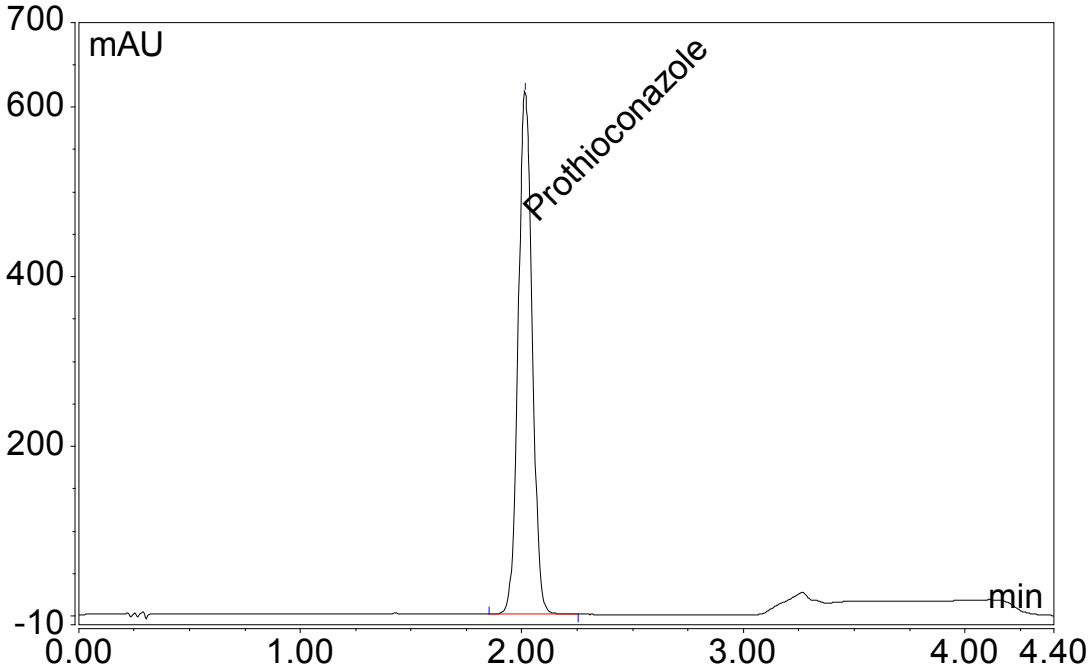


Fig. 4 Technical Material TC (S1)

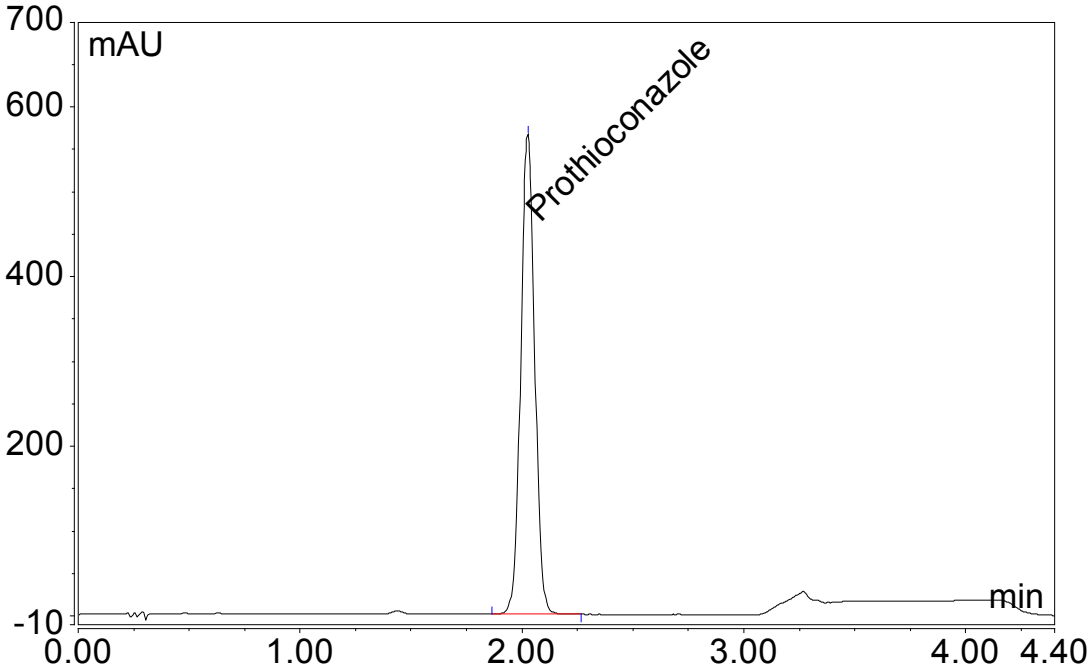


Fig. 5 Emulsifiable Concentrate EC (S3)

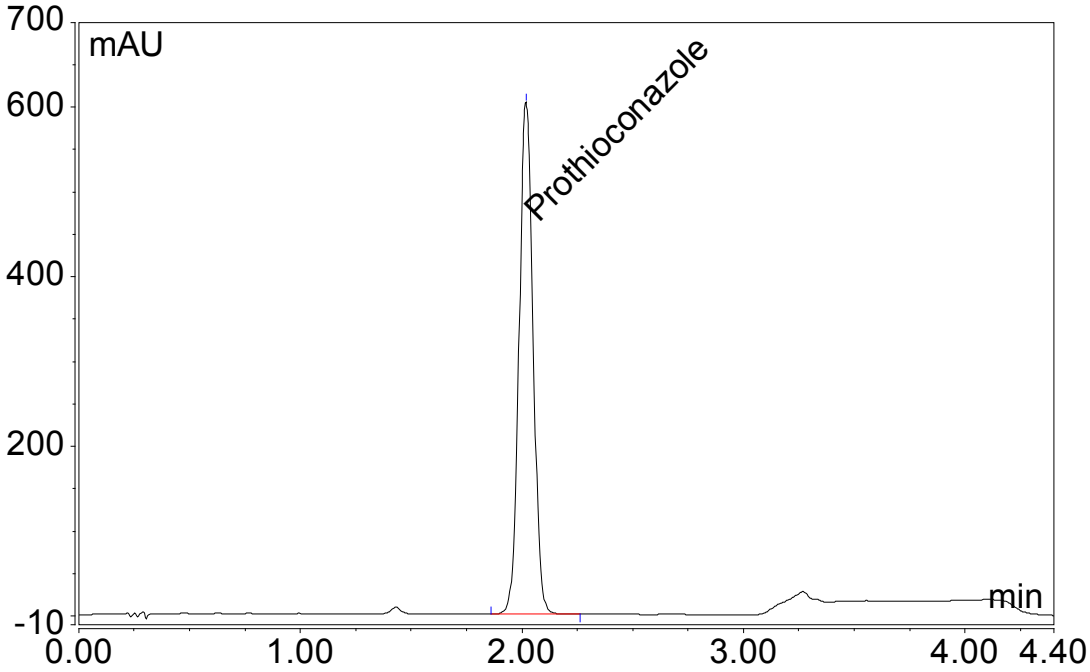


Fig. 6 Flowable Concentrate For Seed Treatment FS (S5)

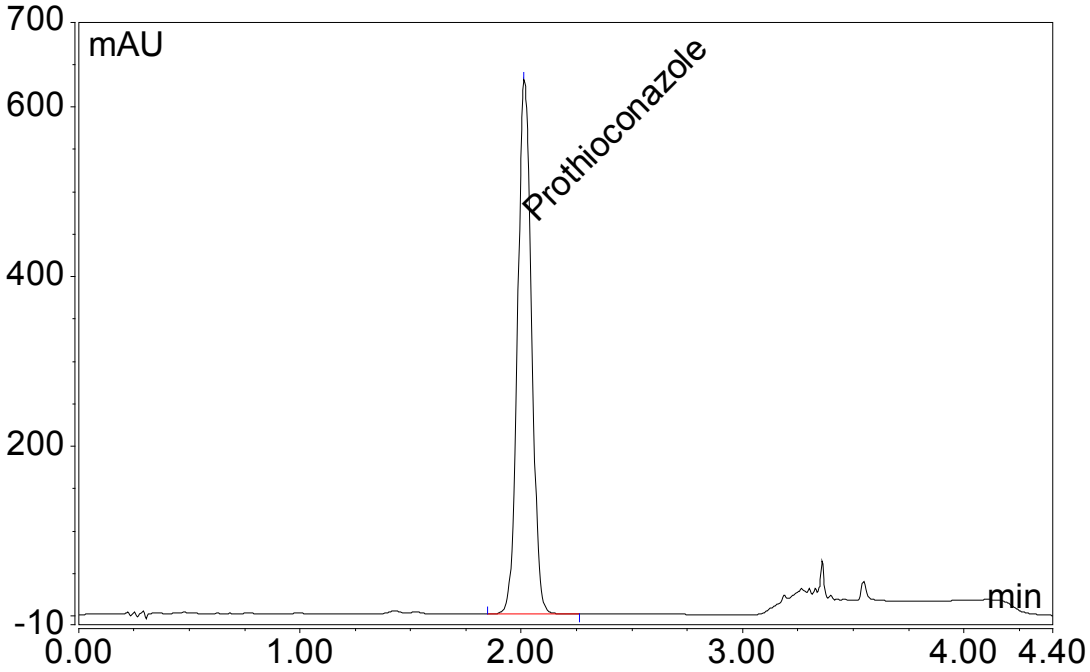


Fig. 7 Suspension Concentrate SC (S7)

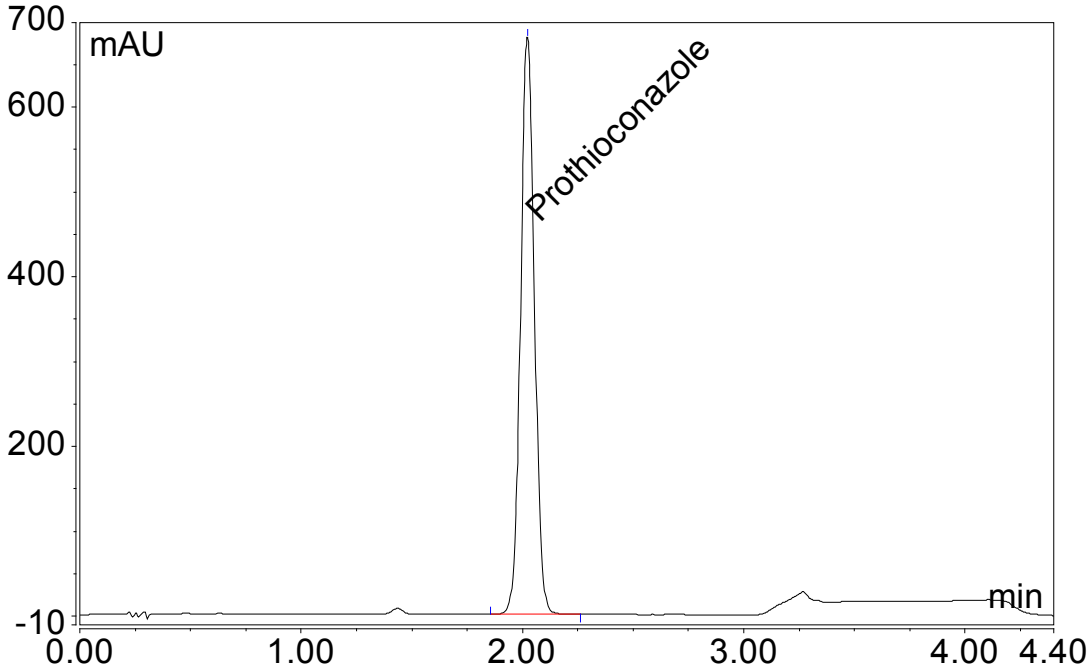


Fig. 8 Analytical Standard for FS formulation with prothioconazole below 1 % w/w (C3)

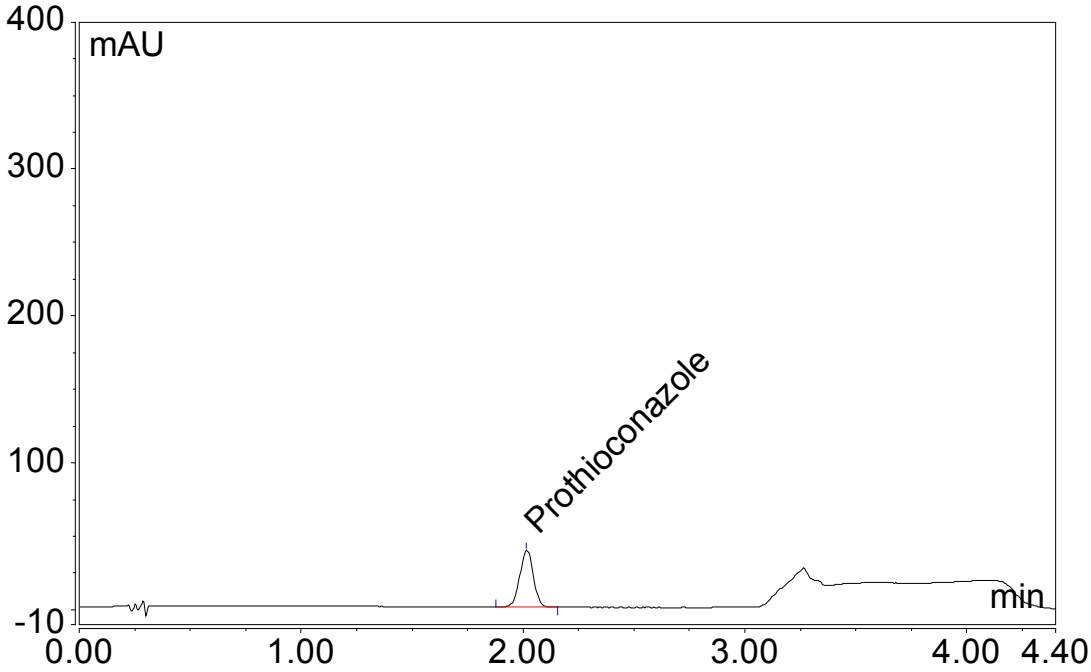


Fig. 9 Flowable Concentrate For Seed Treatment FS with Prothioconazole below 1% w/w (S9)