



Science For A Better Life



Prothioconazole

DAPA Collaboration Trial

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Participants



BASF

BCS (FT Analytik Monheim)

BCS (RT Analytik Monheim)

BVL

Currenta (Dormagen)

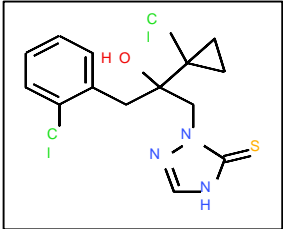
Syngenta

Eurofins

Agroscope CH



Prothioconazole

| | |
|--------------------|---|
| Common name: | Prothioconazole |
| Chemical name: | IUPAC (RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione |
| | CAS 3H-1,2,4-Triazole-3-thione, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro- |
| CAS No.: | 178928-70-6 |
| Trade names: | Proline, Prosaro, others |
| Structure: |  |
| Molecular Formula: | C ₁₄ H ₁₅ Cl ₂ N ₃ O S |
| Molar Mass: | 344.26 g/mol |
| Melting Point: | 139-144.5 °C |
| Solubility: | Aceton, Ethylacetat > 250 g/L; Xylene 8g/L (all at 20°C) |
| Description: | white to beige powder |
| Activity: | Fungicide (class: triazolinthione) |

Active substance



Outline of the Method and Samples

The homogenized sample containing Prothioconazole is dissolved in solvent mixture acetonitrile / purified water followed by active ingredient determination (g/kg) using gradient reversed phase high performance liquid chromatography incorporating UV detection at 254 nm with an external standard calibration.

Samples:

Prothioconazole **technical**, batch purity ≥ 970 g/kg

Prothioconazole, Emulsion Concentrate (**EC** 250 g/L; **250 g/L Prothioconazole**)

Prothioconazole, Flowable concentrate for seed treatment (**FS** 100 g/L; **100 g/L Prothioconazole**)

Prothioconazole, Flowable concentrate for seed treatment (**FS** 258 g/L; 250g/L **Pencycuron**
+ 8 g/L **Prothioconazole**)

Prothioconazole, Suspension Concentrate (**SC** 480 g/L; **480 g/L Prothioconazole**)



Outline of the Method

Method Parameters:

Column: Agilent Zorbax Extend C18, 50 mm x 4.6 mm, particle size 3,5 μm
(worldwide available)

Column temperature: 40 °C

Eluent & rinsing gradient:

| Time [min] | 10 mMol Phosphoric acid / 1000 mL water [%v/v] | ACN/THF/MeOH 10/5/5 [%v/v/V] | Flow rate [mL/min] |
|------------|--|------------------------------|--------------------|
| 0.0 | 50 | 50 | 2.0 |
| 2.7 | 50 | 50 | 2.0 |
| 2.71 | 05 | 95 | 3.0 |
| 3.6 | 05 | 95 | 3.0 |
| 3.61 | 50 | 50 | 3.0 |
| 4.0 | 50 | 50 | 2.0 |
| 4.4 | 50 | 50 | 2.0 |

Detector wavelength: 254 nm

Injection volume: 3 μL

Retention time: approx. 2.0 min

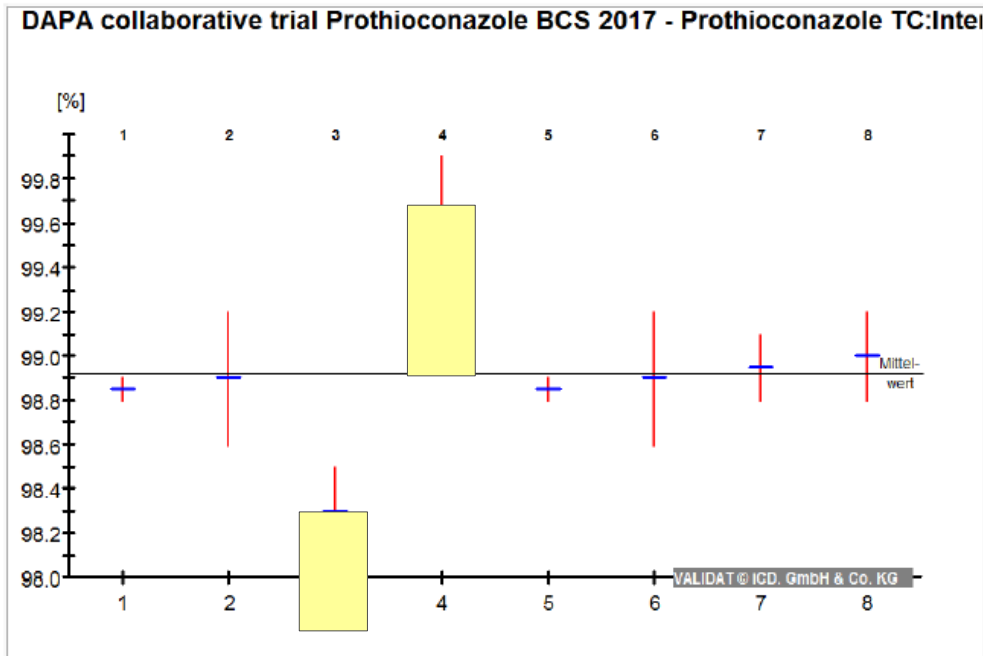
Certified Reference Material: AZ 17908, 99.8 %, Expiry date: 2017-03-12

General Remark



The analytical data reported on the consequent pages are presented in weight-%

Prothioconazole technical



Mean value: 98.92%

S_r : 0.31

S_R : 0.41

RSD(R): 0.42

Horwitz, RSD(R)_{max}: 2.00

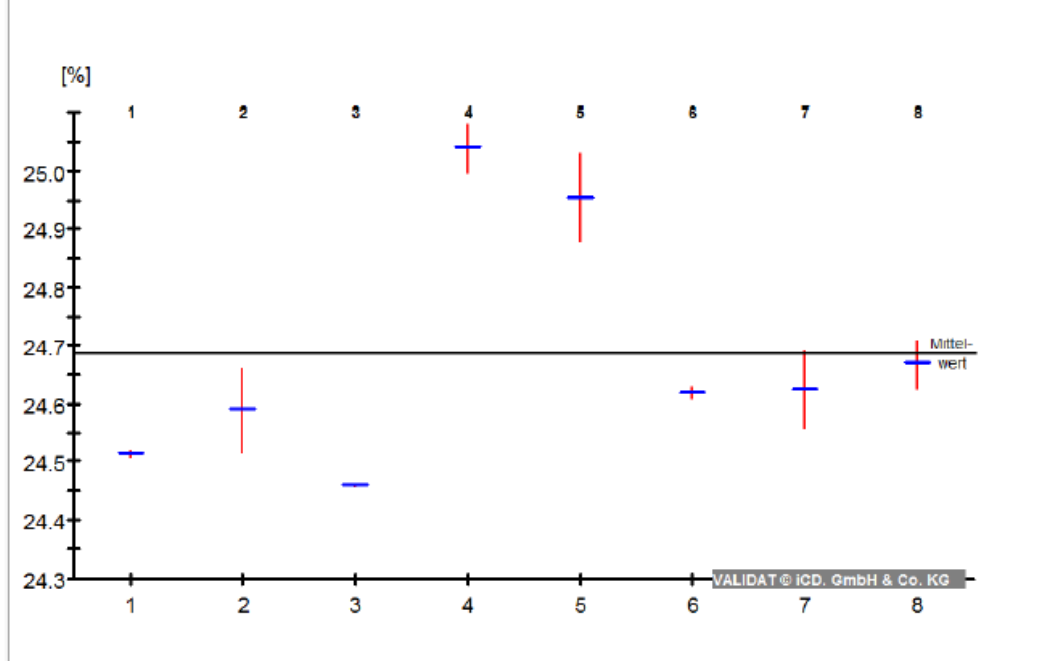
HorRat: 0.21

Outlier (Dixon): upper (lab 4)
lower (lab 3)

EC 250 (250 g/L Prothioconazole)



DAPA collaborative trial Prothioconazole BCS 2017 - Prothioconazole EC 250



Mean value: 24.684 %

S_r : 0.067

S_R : 0.211

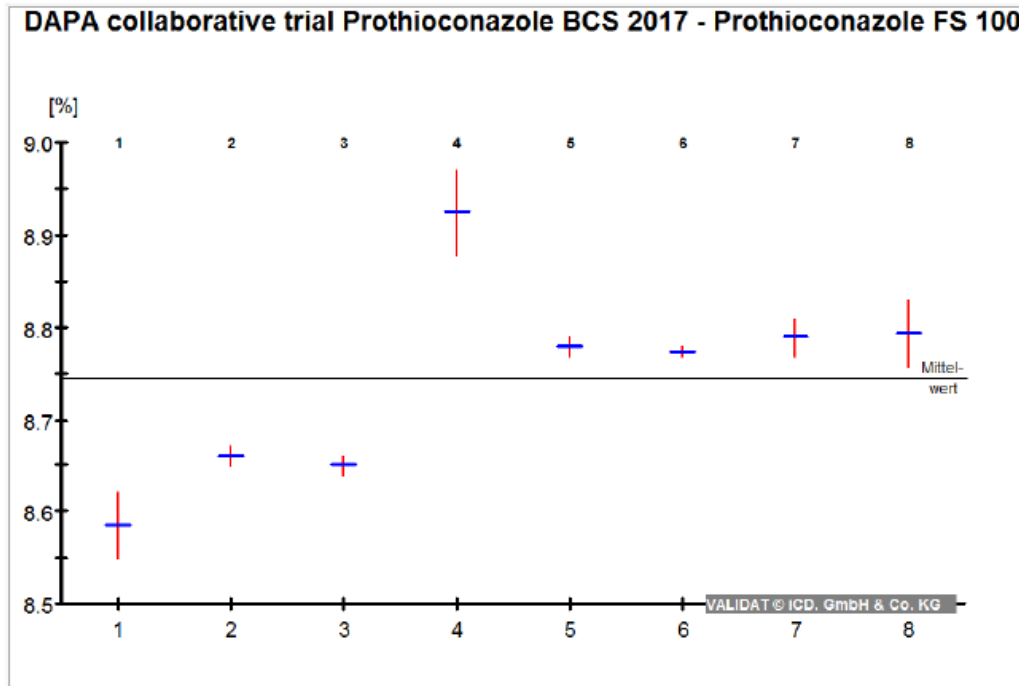
RSD(R): 0.85

Horwitz, RSD(R)_{max}: 2.47

HorRat: 0.34

Outlier (Dixon): no

FS 100 (100 g/L Prothioconazole)



Mean value: 8.745 %

S_r : 0.036

S_R : 0.110

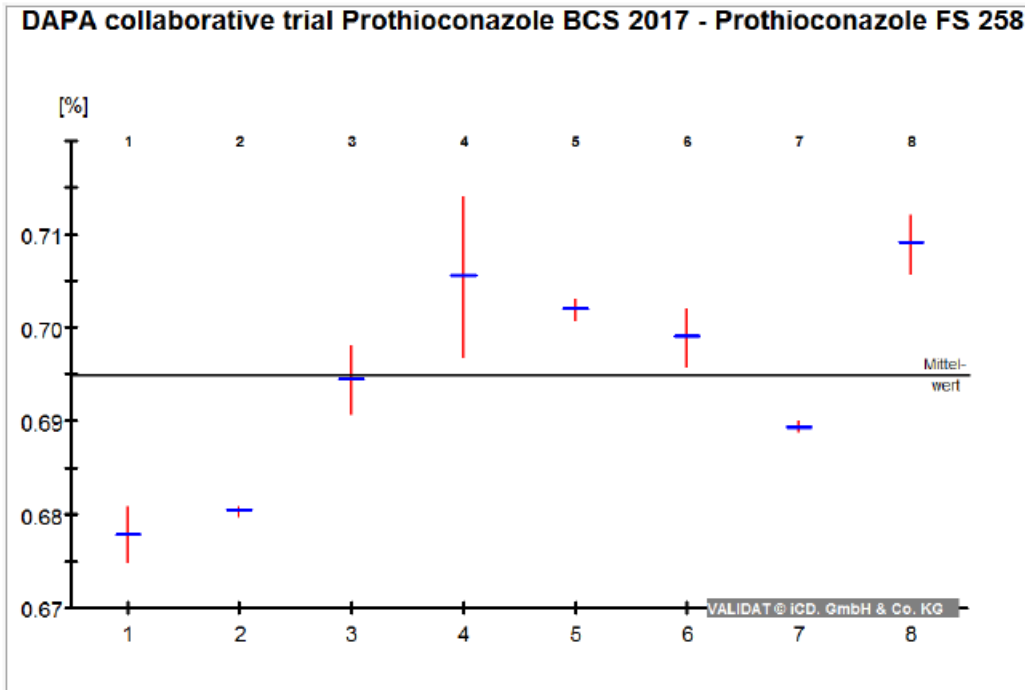
RSD(R): 1.26

Horwitz, RSD(R)_{max}: 2.87

HorRat: 0.44

Outlier (Dixon): no

FS 258 (8 g/L Prothioconazole)



Mean value: 0.6948 %

S_r : 0.0053

S_R : 0.0119

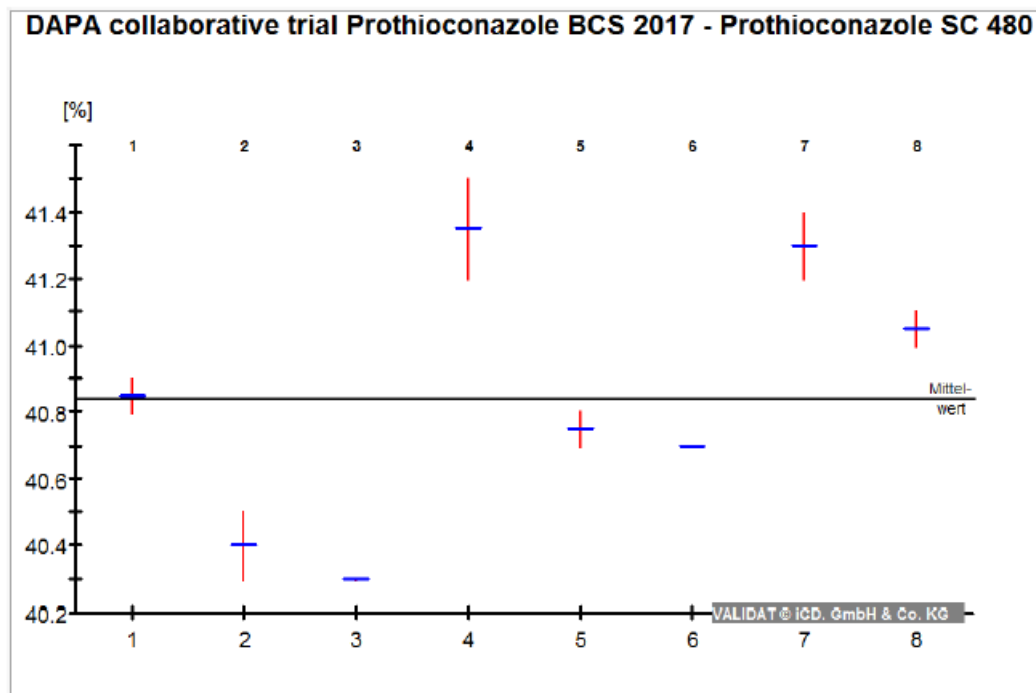
RSD(R): 1.72

Horwitz, RSD(R)_{max}: 4.23

HorRat: 0.41

Outlier (Dixon): no

SC 480 (480 g/L Prothioconazole)



Mean value: 40.84%

S_r : 0.11

S_R : 0.39

RSD(R): 0.96

Horwitz, RSD(R)_{max}: 2.29

HorRat: 0.42

Outlier (Dixon): no

Summary of the results



| | Mean value | RSD(R) | HorRat | all results included |
|----------------------------------|--------------------|-------------------|-------------|----------------------|
| Prothioconazole technical | 98.92 | 0.42 | 0.21 | yes |
| EC 250 (250 g/L Prothioconazole) | 24.6 | 0.85 | 0.34 | yes |
| FS 100 (100 g/L Prothioconazole) | 8.75 | 1.26 | 0.44 | yes |
| FS 258 (8 g/L Prothioconazole) | 0.69 | 1.72 | 0.41 | yes |
| | HorRat value/range | | | |
| SC 480 (480 g/L Prothioconazole) | 40.84 | 0.96 | 0.42 | yes |
| | HorRat ≤ 1 | Recommended range | | |

Summary



8 participating laboratories

Remarks / deviations:

- Lab 1 all samples filtrated through 0.45 μm filter
- Lab 2 - / -
- Lab 3 all samples filtrated by syringe filters
- Lab 4 Day 1: retention time changes between 2.2 and 2.3 min within the sequence.
Day 2: retention time was constant, because the runtime was changed from 5 to 8 min.; naming of calibration solutions inconsistent (calibration solution I versus C1 and C1v)
- Lab 5 In the absence of a centrifuge, the samples were filtered through Chromafil disposable filters 0.2 μm
- Lab 6 - / -
- Lab 7 first sequence interrupted over night, FS 258 samples were still slightly turbid after centrifuged
- Lab 8 Standard injection volume: 5 μl instead of 3 μl , all samples filtrated through 0.2 μm RC filter, C2 on day 1 is not prepared correctly

No additional comments regarding the method

Next step: CIPAC collaboration trial



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Many thanks to the participants of the DAPA
collaboration trial!



Chromatograms



Fig. 1 Analytical Standard I - Prothioconazole

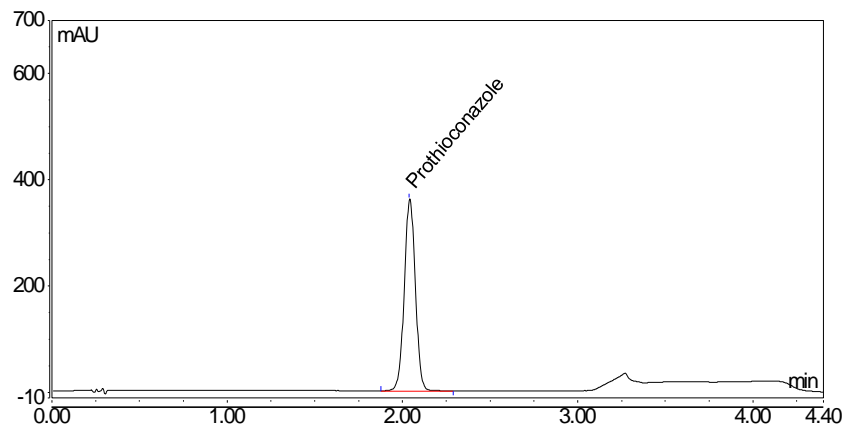


Fig. 2 Prothioconazole, technical (TC)

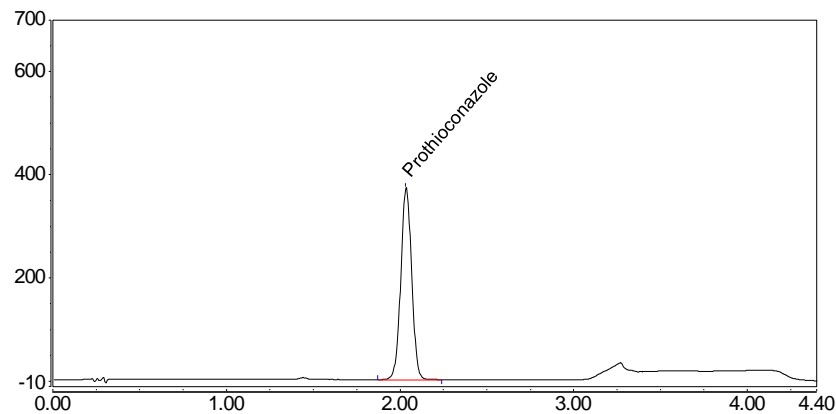


Fig. 3 Prothioconazole EC 250 g/L

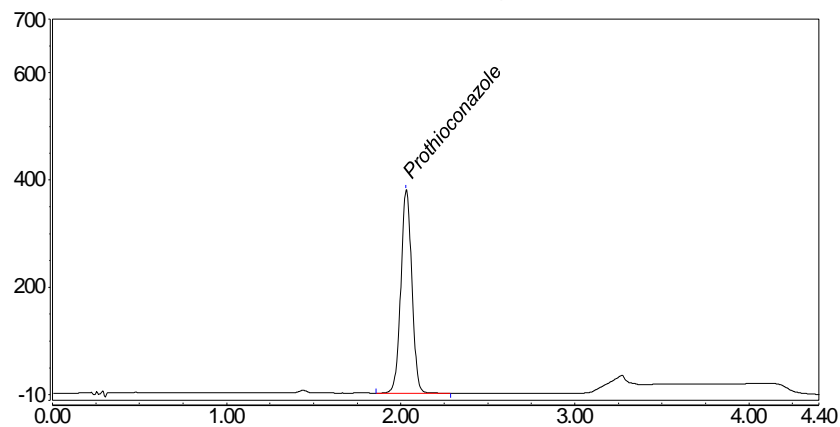
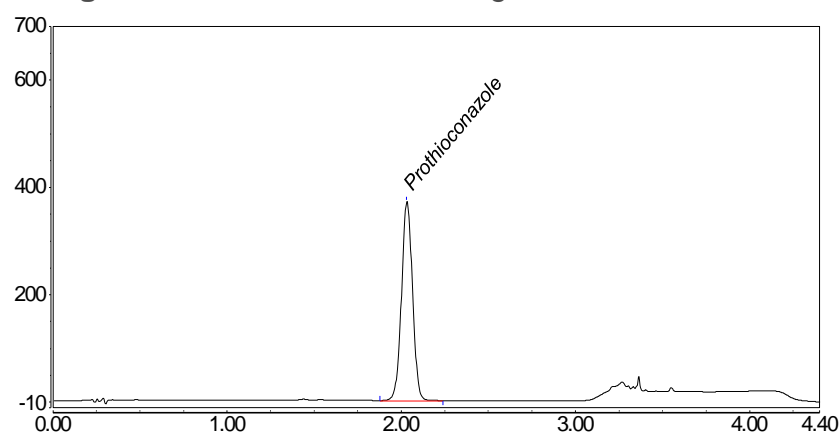


Fig. 4 Prothioconazole FS 100 g/L



Typical Chromatograms



Fig. 5 Prothioconazole SC 480 g/L

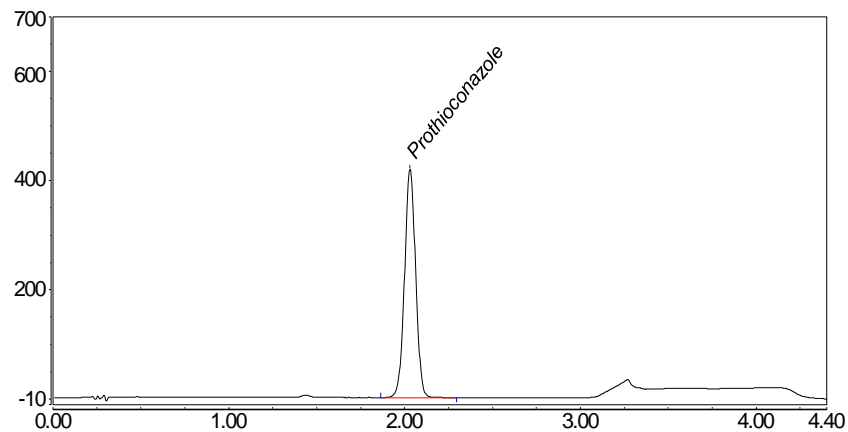


Fig. 6 Prothioconazole FS 258 g/L (8g/L Prothioconazole)

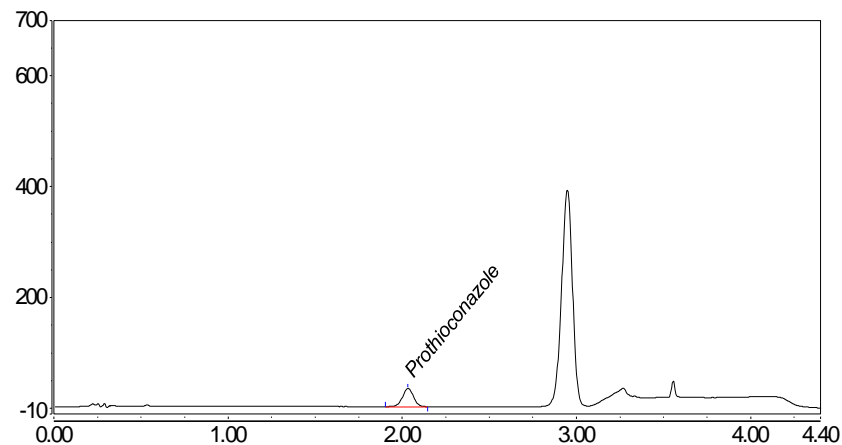
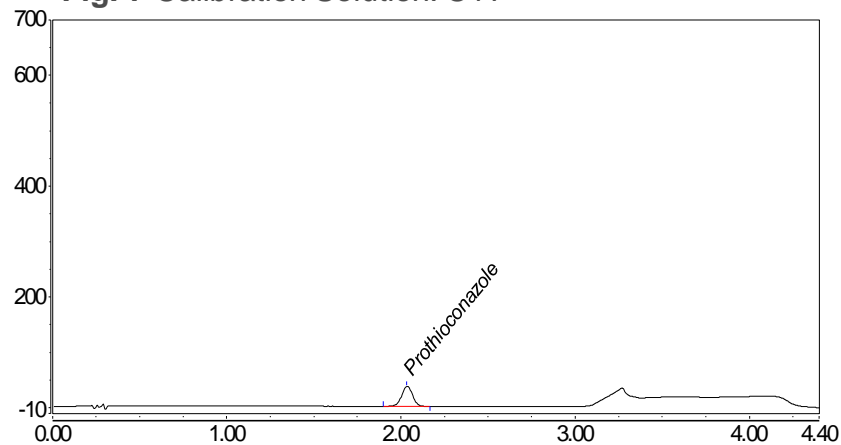
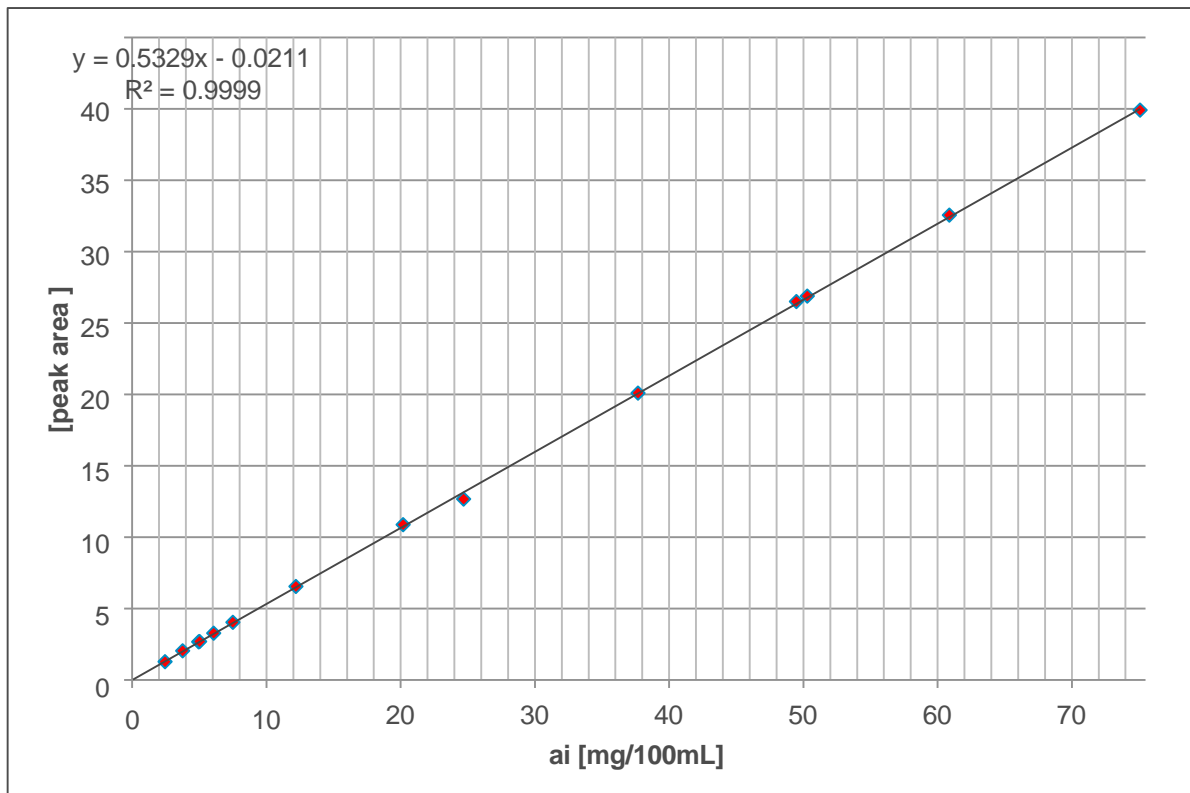


Fig. 7 Calibration Solution: C1v



Linearity I



| a.i. mg / 100 mL (x) | peak area (y) |
|----------------------|---------------|
| 2.47 | 1.2667 |
| 3.77 | 2.0267 |
| 4.95 | 2.6487 |
| 5.03 | 2.6845 |
| 7.51 | 4.0190 |
| 20.2 | 10.851 |
| 24.7 | 12.653 |
| 37.7 | 20.066 |
| 49.5 | 26.495 |
| 50.3 | 26.859 |
| 60.9 | 32.531 |
| 75.1 | 39.895 |

Analytical standard solution: 50 mg / 100 mL

Typical Chromatograms



| Sample | Sample Preparation | Sample Weight |
|--|---------------------------------------|-------------------------------------|
| Prothioconazole technical Prothioconazole, EC 250 g/L | grind (homogenize) 10 g shake well | 50 mg / 100 mL* 200 mg / 100 mL* |

Dissolve the sample in 50 mL acetonitrile, (ultrasonication for 15 min) and fill up with purified water.

| | | |
|-----------------------------|------------|-------------------|
| Prothioconazole, FS 100 g/L | shake well | 570 mg / 100 mL* |
| Prothioconazole, FS 258 g/L | shake well | 675 mg / 100 mL** |
| Prothioconazole, SC 480 g/L | shake well | 120 mg / 100 mL* |

Dissolve the sample in 10 mL purified water, add 50 mL acetonitrile (ultrasonication for 15 min) and fill up with purified water.

To each sample, approx. 5 mg of L-Cysteine Hydrochloride Monohydrate is added.

* - Analytical standard solution I (50 mg / 100 mL)

** - Analytical standard solution II

(10 mL of calibration solution I / 100 mL (acetonitrile / purified water 50 / 50 %v/v))