# UHPLC for determination of difenoconazole on treated barley seeds

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## 1. Objectives

Seed treatment with fungicides and insecticides are used to protect crops against pests and pathogens. To assure a good efficacy, each seed and so each plant has to be correctly treated.

Good quality seed treatment with plant protection products means that the average concentration of active substance on seeds has to be as close as possible to the target rate and that the distribution of the active substance on individual seeds has to be uniform.

An analytical method was developped and validated to determine difenoconazole on treated barley seeds using Ultra High Performance Liquid Chromatography with UV Diode Array Detection (UHPLC-DAD).

## 2. Material and method

**Sample preparation**

Weighting 10 g sample
or 1 single seed

**Extraction**

Average loading
100 mL acetonitrile / 0.1% $H_3PO_4$ in water (80/20, v/v)
Ultrasonication for 45 minutes
Single seed analysis
2 mL acetonitrile / 0.1 % $H_3PO_4$ in water (80/20, v/v)
Ultrasonication for 45 minutes

**Chromatographic conditions**

**Apparatus : Waters Acquity UPLC™**
Column : Waters Acquity UPLC™ BEH C18,
100 x 2.1 mm i.d., 1.7 µm particle size
Injection volume : 5 µL
Flow rate : 1.0 mL/min
Column temperature : 50 °c
Detection : 220 nm

**Mobile phase for chromatographic determination**

| Time (min.) | 0.1 % aqueous phosphoric acid (% v/v) | Acetonitrile (% v/v) |
|---|---|---|
| 0.00 | 65 | 35 |
| 0.05 | 65 | 35 |
| 1.15 | 33 | 67 |
| 1.20 | 5 | 95 |
| 1.60 | 5 | 95 |
| 1.65 | 65 | 35 |

## 3. Results of validation

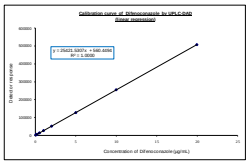
Validation of the analytical method

Every method used to analyse treated seeds has to be validated on its specificity (blank, untreated seeds), repeatability (of injections, of the method), linearity , accuracy (efficacy of extraction, recoveries), LOQ, stability of a.s. during analytical phase.

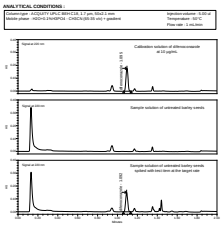
*Specificity*
no interference at the retention time of difenoconazole

*Repeatability of injections*
6 injections of a 5 µg/mL standard solution (RSD = 0.1%)

*Linearity of the detector response* ($R^2$ = 1.0000)



*Examples of chromatograms*



*Repeatability of the method*

*Accuracy*
*Efficacy of extraction*

| Specimen code | Sample No | Extraction value (g / 100 kg seed) (*) | Re-extraction value (g / 100 kg seed) (*) | (%) of the extraction |
|---|---|---|---|---|
| Treated barley seeds (Se 17) | 1 | 8.79 | 0.14 | 1.55 % |
| | 2 | 9.22 | 0.16 | 1.73 % |
| | 3 | 8.78 | 0.14 | 1.58 % |
| | 4 | 9.16 | 0.15 | 1.66 % |
| | 5 | 8.95 | 0.15 | 1.65 % |
| | 6 | 9.17 | 0.14 | 1.56 % |
| **Mean** | | **9.01** | | |
| Standard deviation | | 0.20 | | |
| Relative standard deviation | | 2.21 % | | |
| Confidence interval (**) | | 9.01 ± 0.21 | | |

(*) Each result is the mean of 2 chromatographic injections.
(**) Student t-test with a probability of 95 %.

*Recoveries*

| Specimen | Fortification level (g / 100 kg seed as difenoconazole) | Extraction date | Injection date | Recovery (*) (%) |
|---|---|---|---|---|
| Barley seeds (Se 15) | 8.44 | 29/06/2010 | 02/07/2010 | 97 |
| | 8.41 | 29/06/2010 | 02/07/2010 | 96 |
| | 8.48 | 29/06/2010 | 02/07/2010 | 97 |
| | 8.45 | 29/06/2010 | 02/07/2010 | 97 |
| | 8.48 | 29/06/2010 | 02/07/2010 | 97 |
| **Mean** | | | | **97** |
| Standard deviation | | | | 0.34 |
| Relative standard deviation | | | | 0.35 % |
| Confidence interval (**) | | | | 97 ± 0.42 |

(*) Each result is the mean of 2 chromatographic injections.
(**) Student t-test with a probability of 95 %.

*LOQ*
0.05 g a.s. / 100 kg seeds

*Stability of a.s. during analytical phase*
minimum 10 days in the seeds final extract solution and at ambient temperature

## 4. Conclusion

- The UHPLC-DAD method described here yields satisfactory validation results.
- UHPLC permits to analyse a maximum of samples in a minimum of time with a good accuracy and precision.
- The advantages of UHPLC are :
  - a better resolution (separation more efficient)
  - a faster chromatographic run
  - a better sensitivity (sharper and higher peaks)
  - less solvent used for mobile phase

in comparison with classical HPLC

## 5. Acknowledgement

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