

DONRHONE® WIDE

Product Code: P141/100

Immunoaffinity columns for use in conjunction with HPLC.
For *in vitro* use only.

P141/V1/01.11.12



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Test Principle

The procedure is based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform.

The columns contain a gel suspension of monoclonal antibody specific to the toxin of interest. Following extraction of the toxin the sample extract is filtered, diluted and passed through the immunoaffinity column. Any toxin which is present in the sample is retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxin is then released by the antibody following elution with solvent. The eluate is collected, evaporated and reconstituted prior to analysis by HPLC.

The total extraction and clean-up time takes approximately 20 minutes to perform. The result is improved clean-up and concentration of the toxins from food and feed samples giving a much cleaner chromatogram and therefore providing more accurate and sensitive detection. The columns also have the added advantage that they can be automated for large scale analysis of samples.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (HPLC Grade Methanol)
- Phosphate Buffered Saline (PBS)* (RP202)
- Deoxynivalenol Standard (Please refer to Preparation of Standards section)
- Acetic Acid
- Sodium Hydroxide (to pH filtrate if required)

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper (P66 / P67)*
- Glass Microfibre Filter Paper (P68)*
- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

* Available from R-Biopharm. Please contact your local R-Biopharm distributor for further information.

Hazards

Mycotoxins are very hazardous substances. Only laboratories equipped to handle toxic materials and solvents should perform analyses. Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis.

Flammable solvents should be stored in an explosion-proof cabinet. Use a chemical hood and protective equipment as applicable.

The columns contain 0.01 % (w / v) thimerosal. Skin or eye splashes should be washed immediately with quantities of water. Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Decontamination

Prior to disposal, excess standard solutions should be treated with at least one-tenth their volume of 5 % sodium hypochlorite. Labware and contaminated waste should be immersed in 5 % sodium hypochlorite solution for 30 minutes followed by the addition of 5 % acetone for 30 minutes. Flush with copious amounts of water before disposal. After decontamination labware should be thoroughly washed. Incinerate waste if regulations permit.

Storage & Shelf Life

The columns have an expiry of 18 months from date of manufacture if stored at 2 - 8 °C or 12 months from date of manufacture if stored at 21 - 25 °C. Do not freeze.

Ensure that the column has not dried out and contains buffer above the gel. It is important to note that the antibody included in the immunoaffinity column can be denatured by extreme temperature or pH change.

Sampling

A representative sample should be obtained by following one of the officially recognised sampling procedures. It is recommended that a minimum of 1 kg of representative sample is finely ground and a portion (10 - 50 g dependent on method used) of this is removed and extracted.

Sensitivity

The sensitivity is dependent on the final detection system employed by the analyst. However the test sensitivity may be improved if required by increasing the volume of sample passed through the immunoaffinity column.

Recoveries

If an analyst wishes to account for losses during extraction it is recommended that a spiked sample of the same commodity type as the material being tested be analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can then be used to correct the results obtained with the test sample.

Column Preparation

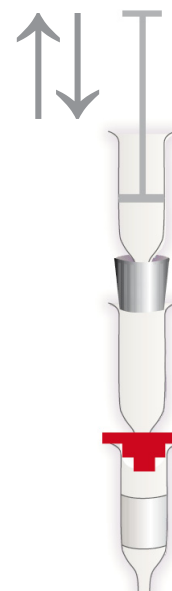
Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

Backflushing

Backflushing is carried out to increase the time the solvent is in contact with the antibody gel ensuring that all the toxin is eluted. Backflush by gently raising and lowering the syringe plunger during passage of the solvent through the column. This process will reverse the direction of flow of the eluant. This should be repeated 3 times.

Application Notes Available

Methods are available for all matrices covered by legislation as well as additional commodities. Please contact your local R-Biopharm distributor for further information.



Sample Preparation

• Cereal

This method has been tested on a number of cereals including wheat, barley, maize and cereal based products.

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 200 ml of water and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.
4. Filter again through glass microfibre filter paper.
5. Pass 2 ml of the filtrate (equivalent to 0.25 g of sample) through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxin by the antibody.
6. Wash the column by passing 10 ml of water through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.
7. Elute the toxin from the column at a flow rate of 1 drop per second using 1.5 ml of 100 % methanol and collect in a glass tube. Backflushing is recommended. Please refer to the Backflushing section for further information.
8. Evaporate the eluate to dryness under air at 60 - 70 °C.
9. Reconstitute with 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v). Vortex for 20 seconds.
10. Inject 100 µl of reconstituted eluate onto the HPLC system.

Sample Preparation

• Animal Feed

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 200 ml of water and blend at high speed for 2 minutes.
3. Centrifuge the sample at 4,000 rpm for 10 minutes.
4. Filter the supernatant through a glass microfibre filter paper.
5. Pass 2 ml of the filtrate (equivalent to 0.25 g of sample) through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxin by the antibody.
6. Wash the column by passing 10 ml of water through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.
7. Elute the toxin from the column at a flow rate of 1 drop per second using 1.5 ml of 100 % methanol and collect in a glass tube. Backflushing is recommended. Please refer to the Backflushing section for further information.
8. Evaporate the eluate to dryness under air at 60 - 70 °C.
9. Reconstitute with 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v). Vortex for 20 seconds.
10. Inject 100 µl of reconstituted eluate onto the HPLC system.

Preparation of Standards

1. Crystalline powder of Deoxynivalenol can be purchased. Contact your local R-Biopharm distributor for further information. The powder is reconstituted as per the instructions provided and left overnight in the dark at room temperature to give a stock concentrate.
2. Dilute the solution with 100 % acetonitrile to give a concentration of 100 µg/ml.

Calibration Curve

It is recommended to run at least a 3 - 6 point calibration curve. In constructing a suitable curve the levels of the calibration standards should bracket or include the range of expected results.

To prepare a six point calibration curve:

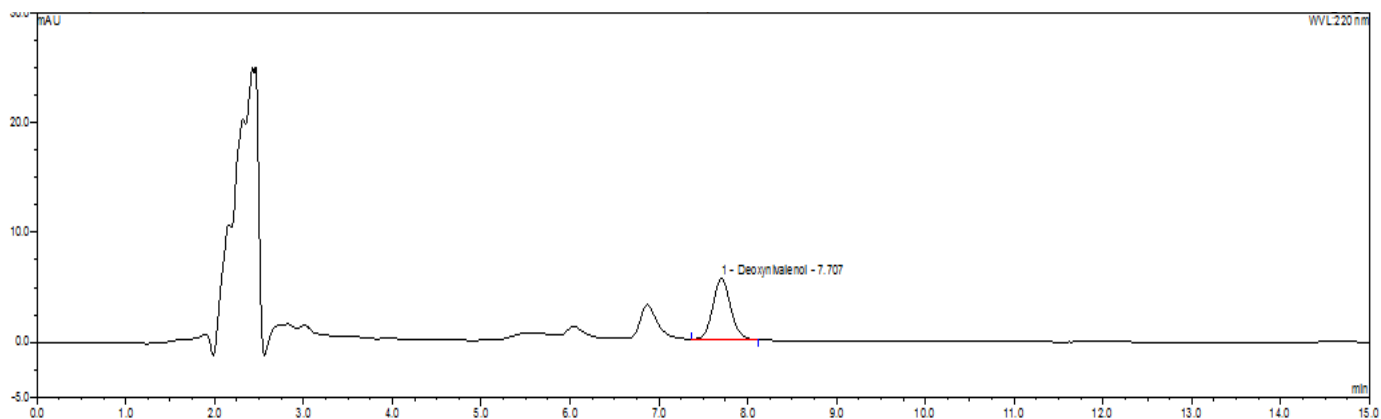
1. Add 200 µl of reconstituted crystalline standard to 800 µl of 100 % acetonitrile (equivalent to 20,000 ng/ml).
2. Evaporate 200 µl of the solution to dryness under air at 60 - 70 °C.
3. Standard 6: Reconstitute with 2 ml of 15 % methanol (equivalent to 2,000 ng/ml).
5. Standard 5: Take 1 ml of 2,000 ng/ml and add 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v) (equivalent to 1,000 ng/ml).
6. Standard 4: Take 1 ml of 1,000 ng/ml and add 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v) (equivalent to 500 ng/ml).
7. Standard 3: Take 1 ml of 500 ng/ml and add 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v) (equivalent to 250 ng/ml).
8. Standard 2: Take 1 ml of 250 ng/ml and add 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v) (equivalent to 125 ng/ml).
8. Standard 1: Take 1 ml of 125 ng/ml and add 1 ml of 0.01 % acetic acid : methanol (85 : 15 v/v) (equivalent to 62.5 ng/ml).
9. Inject 100 µl of each solution onto the HPLC system.

Recommended HPLC Conditions

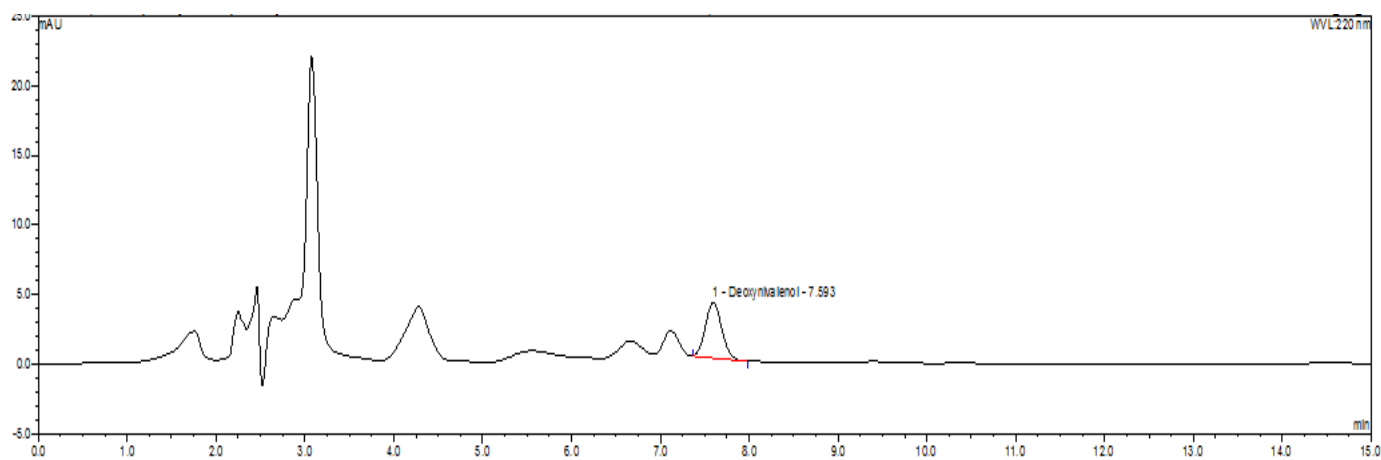
| HPLC Conditions | |
|----------------------------------|--|
| Guard Cartridge | AQUASIL C18 3 µm, 4 mm x 10 mm or equivalent |
| Analytical Column | AQUASIL C18 3 µm, 4.6 mm x 150 mm or equivalent |
| Mobile Phase | 0.01 % Acetic Acid in Water : Methanol (85 : 15 v/v) Prepare fresh on day of use. |
| HPLC Pump | To deliver mobile phase |
| Flow Rate | 0.8 ml per minute |
| UV Detector | 220 nm |
| Column Heater | Maintain guard and analytical column at 45 °C |
| Integrator / Data Control System | From preferred supplier |
| Injector | Autosampler / Reodyne valve |
| Injection Volume | 100 µl |

Typical HPLC Trace for Analysis of Deoxynivalenol Using DONRHONE® Immunoaffinity Columns

- Cereal



- Animal Feed



Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 and ISO 13485 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to our customers:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

Warranty

R-Biopharm Rhône Ltd makes no warranty of any kind, express or implied, except that all products made by R-Biopharm Rhône Ltd are made with materials of suitable quality. If any materials are defective, R-Biopharm Rhône Ltd will provide a replacement product. The user assumes all risk and liability resulting from the use of R-Biopharm Rhône Ltd products and procedures. R-Biopharm Rhône Ltd shall not be liable for any damages, including special or consequential damages, loss or expense arising directly or indirectly from the use of R-Biopharm Rhône Ltd products or procedures.

R-Biopharm Rhône Ltd
Block 10 Todd Campus
West of Scotland Science Park
Acre Road, Glasgow G20 0XA
www.r-biopharm.com