

ZONRHONE® WIDE

Product Code: P118/100

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

P118/V1/03.04.13



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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 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 toxin 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 or Acetonitrile)
- Phosphate Buffered Saline (PBS)* (RP202)
- Zearalenone Standard (Please refer to Preparation of Standards section)
- Sodium Hydroxide (to pH filtrate if required)

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper (P66 / P67)*
- 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. Please note that the ratio of solvent to phosphate buffered saline (PBS) should be maintained.

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

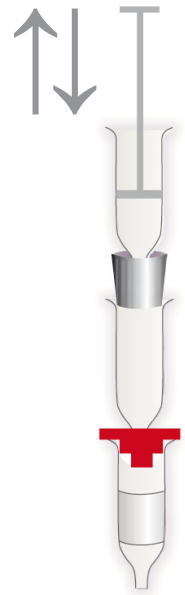
Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

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 and Animal Feed

This method has been tested on a number of cereal and animal feed samples 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 125 ml of 75 % acetonitrile 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. Dilute 20 ml of filtrate with 80 ml of phosphate buffered saline (PBS) solution.
5. Adjust to around pH 7.4 using 2 M sodium hydroxide.
6. Pass 25 ml of the diluted filtrate (equivalent to 1 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.

Note: It is important to control the flow rate to ensure that the filtrate doesn't pass through the column too quickly.

7. Wash the column by passing 10 ml of PBS through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.
8. Elute the toxin from the column at a flow rate of 1 drop per second using 1 ml of 100 % acetonitrile and collect in a 5 ml amber glass vial. Backflushing is recommended. Please refer to the Backflushing section for further information.

Note: Ensure that the solvent is in contact with the antibody gel for approximately 30 seconds to allow complete elution.

9. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.
10. Inject 100 μ l onto the HPLC system.

Preparation of Standards

Preparation of 1,000 ng/ml zearalenone stock solutions:

1. Ready-to-use ZEASTANDARD SOLUTION (P44 / P44A, 1,000 ng/ml) is available from R-Biopharm.

or

1. Alternatively, crystalline powder of Zearalenone 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 solution.

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 include the range of expected results. The diluted standard solutions should be prepared fresh on the day of use and used within a 24 hour period.

To prepare a four point calibration curve:

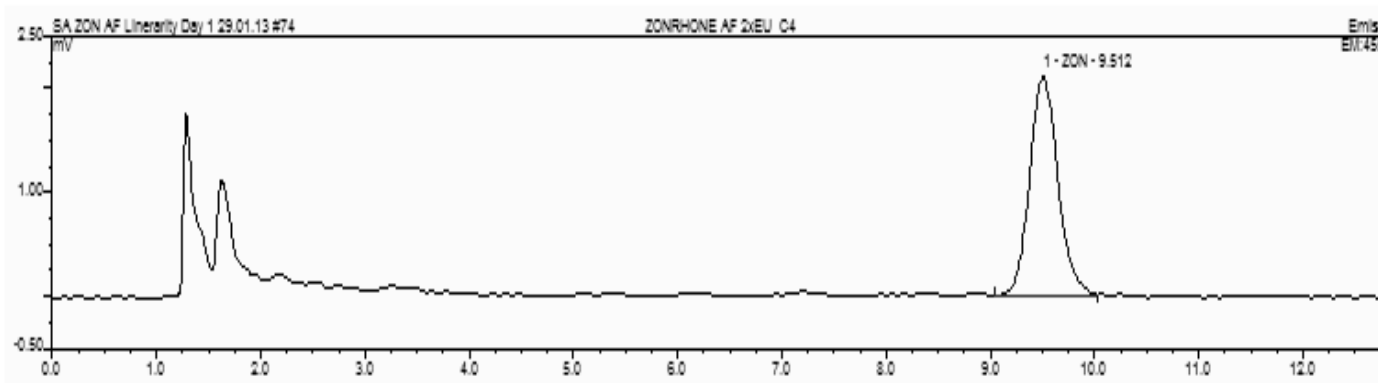
1. Dilute the stock solution with 100 % acetonitrile to give a concentration of 1,000 ng/ml. This standard solution is stable for 6 months at 2 - 8 °C.
2. Take 1.8 ml of 1,000 ng/ml zearalenone solution and add 1.2 ml of 100 % acetonitrile (equivalent to 600 ng/ml).
3. Standard 4: Take 2 ml of 600 ng/ml and add 2 ml of water (equivalent to 300 ng/ml).
4. Standard 3: Take 2 ml of 300 ng/ml and add 2 ml of 50 % acetonitrile (equivalent to 150 ng/ml).
5. Standard 2: Take 2 ml of 150 ng/ml and add 2 ml of 50 % acetonitrile (equivalent to 75 ng/ml).
6. Standard 1: Take 2 ml of 75 ng/ml and add 2 ml of 50 % acetonitrile (equivalent to 37.5 ng/ml).
7. Inject 100 µl of each standard onto the HPLC system.

Recommended HPLC Conditions

HPLC Conditions	
Guard Cartridge	Inertsil ODS-3 5 µm, 4 mm x 10 mm (Hichrom) or equivalent
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent
Mobile Phase	Acetonitrile : Water : Methanol (46 : 46 : 8 v/v/v) Prepare fresh on day of use.
HPLC Pump	To deliver mobile phase
Flow Rate	1.0 ml per minute
Fluorescence Detector	Excitation: 274 nm Emission: 455 nm
Column Heater	Maintain guard and analytical columns at 40 °C
Integrator / Data Control System	From preferred supplier
Injector	Autosampler / Rheodyne valve
Injection Volume	100 µl

Typical HPLC Trace for Analysis of Zearalenone Using ZONRHONE® WIDE Immunoaffinity Columns

- Animal Feed



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