IMMUNOPREP® ONLINE ZEARALENONE

Product Code: P903

Online immunoaffinity cartridges for use in conjunction with a RIDA®CREST or CHRONECT Symbiosis RIDA®CREST system.
For *in vitro* use only.

P903/V3/20.07.22

www.r-biopharm.com

R-BIOPHARM RHÔNE LTD

Contents

Test Principle

The online zearalenone immunoaffinity cartridge is used in conjunction with the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST system, combining automated online sample application with quantitative analysis of zearalenone. The immunoaffinity cartridge contains a monoclonal antibody that is specific for zearalenone coupled to a hydrophilic polymer that can withstand high pressure. This enables the cartridge to be incorporated directly online with the HPLC system part of the RIDA®CREST or CHRONECT RIDA®CREST Symbiosis system.

The immunoaffinity cartridge offers highly specific, sensitive, rapid and automated analysis for zearalenone in a variety of cereals, including wheat, oats, maize and other grain based products in food and feed. Using the zearalenone immunoaffinity cartridge, the sample application, washing and elution is performed online for a specified number of analyses before the cartridge is automatically removed and replaced with a new cartridge. This level of reuse has been found to offer optimum cartridge performance and prevent interference or carryover.

Following extraction of the toxins from the sample with solvent, the extract is centrifuged, the supernatant is filtered, diluted and transferred to an autosampler vial. The diluted extract is injected onto the immunoaffinity cartridge and any toxin present in the sample is retained by antibody in the cartridge. Unbound material is then removed by washing the cartridge and sending the resulting wash to waste. Subsequently the toxin is released from the antibody following online elution and the complete eluate from the cartridge is quantitatively analysed for zearalenone by HPLC.

Reagents Not Provided

- Zearalenone Standard (please refer to Preparation of Standards section)
- Ammonium acetate
- Tris(hydroxymethyl)aminomethane
- Formic acid
- Hydrochloric acid
- Sodium hydroxide
- Sodium chloride
- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- IPA
- Distilled / Deionised Water (suitable for use with LC, e.g. Milli-Q)
- Tween 20

Accessory Products

- Glass Microfibre Filter Paper

Cartridge Handling

Please refer to the Cartridge Handling Instructions included in the kit for details on how to handle the cartridges and store them for short periods of time.

Recommended Methods and Application Notes

Methods are available for all matrices covered by Legislation as well as additional commodities. Deviation from the methods described in our Instructions For Use may not result in optimum results. However, it is possible as part of the validation process that R-Biopharm Rhône can support customer specific methods. 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.

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 cartridges have an expiry of 12 months from date of manufacture if stored at 2 – 8 °C in buffer. It is advised when the cartridges are not in use for periods of more than 24 hours then they should be stored in the buffer supplied at 2 – 8 °C. This will ensure optimum shelf life and keep the immunoaffinity packing in the cartridge hydrated. Do not freeze. For further information please refer to the Cartridge Handling Instructions.

It is important to note that the antibody included in the immunoaffinity cartridge 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 (5 - 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.

For optimal cartridge performance, aim to load a sample containing a quantity of 0.05 ng up to 25 ng of zearalenone onto the cartridge. Do not exceed the quantity of 25 ng as this is close to the suggested maximum working range of the cartridge.

Recoveries

In general recoveries of greater than 80 % for zearalenone are achieved providing the injected amount of toxin stays within the binding capacity (0.05 ng to 25 ng) of the immunoaffinity cartridge. Please note the capacity decreases if higher flow rates are used during sample loading. In addition, the ratio of solvent to dilution buffer should not be increased. For highly contaminated samples (a zearalenone content in the final extract of >25 ng/ml), it is recommended to further dilute the extract with the appropriate dilution buffer.

Recommended Re-Usability

It is essential to run a standard through a cartridge each day of analysis for correct calibration of samples and to correct for recovery. To offer optimum cartridge performance and to reduce the chance of interference or carryover we would recommended to inject a blank (i.e. 6 % acetonitrile, 4 % methanol containing 20 mM Ammonium Acetate pH 8.3 - 8.5), standard, 12 test samples and then another standard (for bracketed calibration) through each cartridge (a total of 15 injections).

Cartridge Preparation

Cartridges should be at ambient temperature before use. Prior to use, the antibody is activated by conditioning the cartridge with loading buffer. This can be automatically programmed as part of the sample clean-up program.

Preparation of Buffers

When preparing buffers it is important to ensure that they are within the pH range specified.

- **Preparation of Loading Buffer (0.02% Tween 20 containing 20 mM Ammonium acetate)**

1. Weigh 0.2 g of Tween 20 into a 1 litre bottle.
2. Add 1 litre of water.
3. Add and dissolve 1.54 g of ammonium acetate.
4. Check pH and, if necessary, adjust the pH to 6.8 - 7.0 using 1 M sodium hydroxide.

- **Preparation of Wash Buffer (10% Acetonitrile containing 20 mM Tris, 20 mM Ammonium Acetate)**

1. Add 900 ml of water and 100 ml of acetonitrile into a 1 litre bottle.
2. Add and dissolve 1.54 g of ammonium acetate and 2.42 g of tris(hydroxymethyl)aminomethane.
3. Adjust pH to 8.3 - 8.5 using concentrated hydrochloric acid.

- **Preparation of Elution Buffer (50 % Acetonitrile, 10 % Methanol containing 20 mM Ammonium Acetate)**

1. Add 200 mL of water, 250 ml of acetonitrile and 50 mL of methanol into a 500 ml bottle.
2. Add and dissolve 0.77 g of ammonium acetate.

- **Preparation of Dilution Buffer (6% Acetonitrile, 4% Methanol containing 20 mM Ammonium Acetate)**

1. Add 900 ml of water, 60 ml of acetonitrile and 40 ml of methanol into a 1 litre bottle.
2. Add and dissolve 1.54 g of ammonium acetate.
3. Adjust pH to 8.3 - 8.5 using 1M sodium hydroxide.

- **Mobile Phase A (Methanol : Water : Formic Acid (2 : 98 : 0.1 v/v/v))**

- **Mobile Phase B (Acetonitrile : Water : Formic Acid (90 : 9.9 : 0.1 v/v/v))**

- **Autosampler Wash (80 % Acetonitrile)**

- **Pump Seal Wash Solution**

- RIDA®CREST System: 20 % Isopropanol
- CHRONECT Symbiosis RIDA®CREST Syetem: 10 % Isopropanol

Sample Preparation

• Cereal

1. Weigh 25 g of sample into a 1 litre capacity flask.
2. Add 5 g of sodium chloride and 125 ml of 80 % methanol.
3. Shake vigorously for 60 minutes.
4. Centrifuge at 4,000 rpm for 5 minutes.
5. Dilute 1 ml of supernatant with 7 ml of dilution buffer.
6. Filter the sample through glass microfibre filter paper.
7. Transfer 1.5 ml of diluted filtrate into an amber autosampler vial.
8. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System.

• Animal Feed

1. Weigh 50 g of sample into a 1 litre capacity flask.
2. Add 5 g of sodium chloride and 150 ml of 90 % acetonitrile.
3. Shake vigorously for 60 minutes.
4. Centrifuge at 4,000 rpm for 5 minutes.
5. Dilute 1 ml of supernatant with 79 ml of dilution buffer.
6. Filter the sample through glass microfibre filter paper.
7. Transfer 1.5 ml of diluted filtrate into an amber autosampler vial.

• Beer

1. Degas 20 ml of beer in ultrasonic bath for 30 minutes.
2. Dilute 1 ml of sample with 7 ml of dilution buffer.
3. Filter the sample through glass microfibre filter paper.
4. Transfer 1.5 ml of diluted filtrate into an amber autosampler vial.
5. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System.

Preparation of Standards

Preparation of 0.4 mg/ml zearalenone stock solution:

1. Dissolve 10 mg zearalenone powder in 25 ml methanol (note: stock should be kept at -20°C).

Calibration Standard

The diluted standard solution should be prepared fresh on the day of analysis and used within a 24 hour period. It is essential to run a standard through every cartridge on each day for correct calibration of samples.

Examples of how to prepare calibration standards (can be modified according to legislative requirements or contamination levels):

- **For Routine Analysis**

Low level standard (i.e. 75 ppb)

1. Take 125 µl of 0.4 mg/ml zearalenone stock solution and make up to 10 ml with methanol (equivalent to 5000 ng/ml).
2. Take 1000 µl at 5000 ng/ml and make up to 10 ml with methanol (equivalent to 500 ng/ml).
3. Take 37.5 µl at 500 ng/ml, add 1 ml methanol and make up to 10 ml with dilution buffer (equivalent to 1.875 ng/ml).
4. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml of low level standard onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System.

High level standard (i.e. 3,000 ppb)

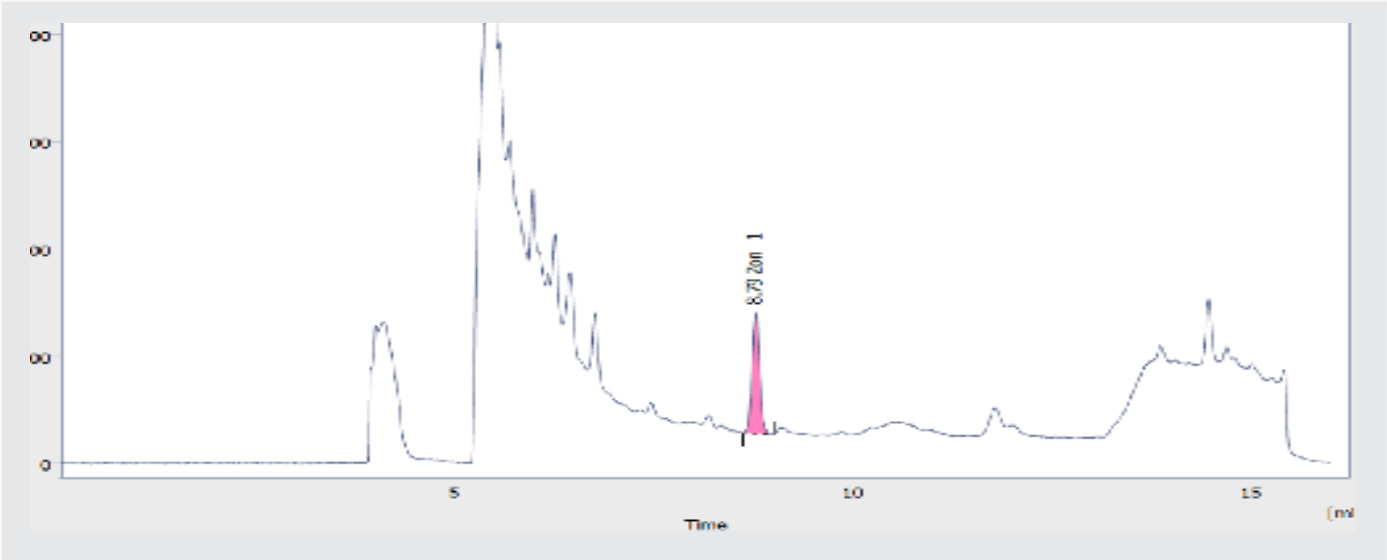
1. Take 125 µl of 0.4 mg/ml zearalenone stock solution and make up to 10 ml with methanol (equivalent to 5000 ng/ml).
2. Take 25 µl, add 1 ml methanol and make up to 10 ml with dilution buffer (equivalent to 12.5 ng/ml).
3. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml of high level standard onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System.

Recommended RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System Conditions

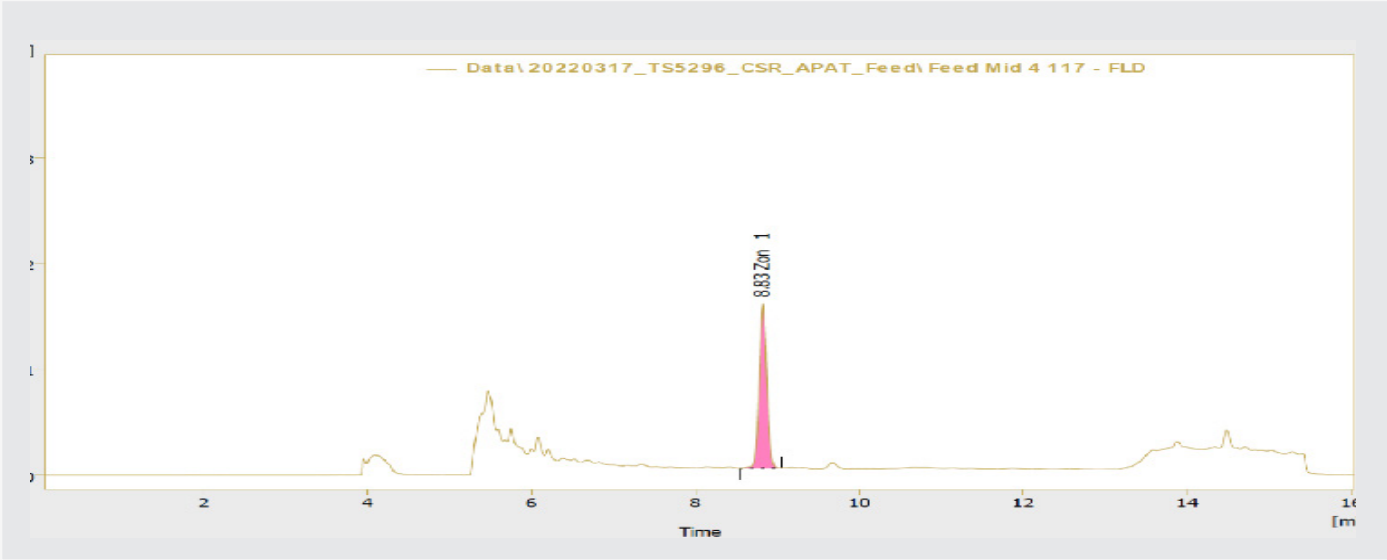
RIDA®CREST or CHRONECT Symbiosis RIDA®CREST Conditions				
Analytical Column	InertSustain AQ C18 3 µm, 4.6 mm x 150 mm or equivalent			
Column Temperature	50 °C			
HPLC Pump 1 (Line A1)	**Mobile Phase A:** Please refer to Preparation of Buffers section.			
HPLC Pump 2 (Line B1)	**Mobile Phase B:** Please refer to Preparation of Buffers section.			
Gradient	Time (min)	% A1	% B1	Flow Rate (ml/min)
	0.00	100	0	0.2
	2.00	100	0	0.2
	4.00	35	65	1.0
	12.00	30	70	1.0
	12.10	0	100	1.0
	14.00	0	100	1.0
	14.10	100	0	1.0
	16.00	100	0	1.0
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Loading Buffer section.			
HPD1 (Line 1B)	Wash Buffer. Please refer to Preparation of Wash Buffer section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Elution Buffer section.			
Recommended RIDA®CREST Conditions for Sample Analysis	Equilibration	HPD flow 1,000 µl/min, volume 1,000 µl of loading buffer.		
	Conditioning	HPD flow 1000 µl/min, 1,000 µl of loading buffer.		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of loading buffer.		
	Cartridge Wash	HPD flow 1,000 µl/min, volume 6,000 µl of wash buffer.		
	Elution	HPD flow 400 µl/min, volume 600 µl of elution buffer.		
	Clamp Wash	HPD flow 1,000 µl/min, volume 2,000 µl of loading buffer.		
Fluorescence Detector	Excitation: 274 nm			
	Emission: 455 nm			
Data Control System	Clarity™ or from preferred supplier			
Injection Volume	Depending on the sensitivity of the fluorescence detector, inject 0.5 -1 ml onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST System.			

Example HPLC Chromatograms for CHRONECT Symbiosis RIDA®CREST System

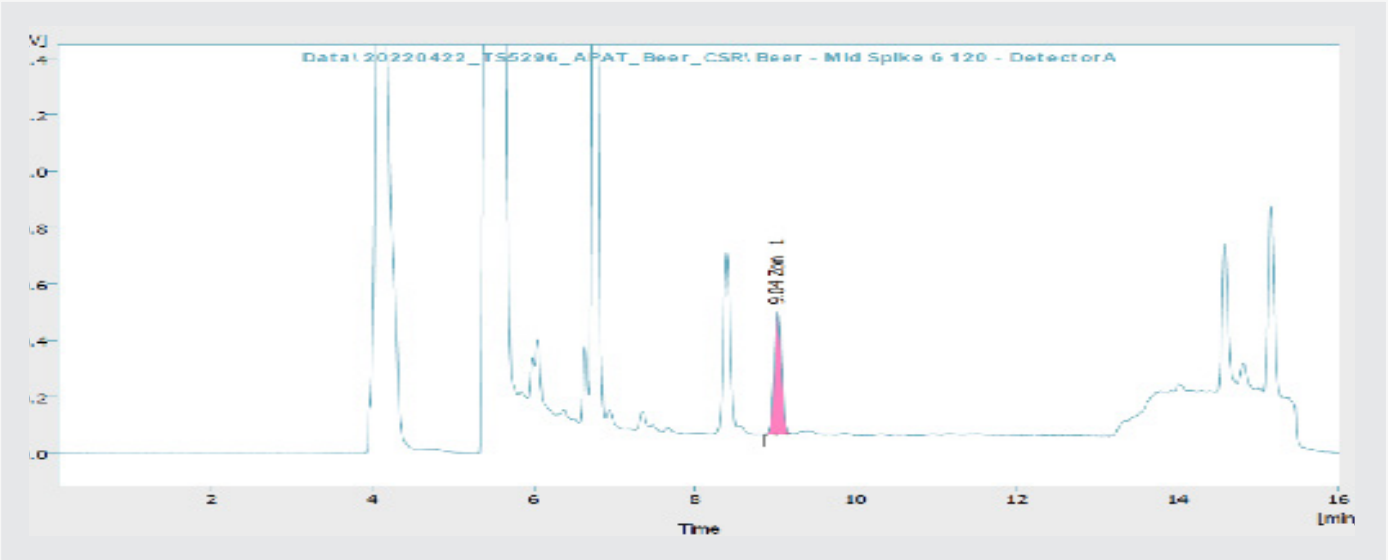
- **Oatmeal (Spiked at 50 ppb)**



- **(Animal Feed spiked at 2,000 ppb)**



- **Beer (Spiked at 20 ppb)**



Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 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.
- 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