IMMUNOPREP® ONLINE AFLATOXIN

Product Code: P900/48, P900

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

P900/V14/0.07.05.21

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R-BIOPHARM
RHÔNE LTD

Contents

Test Principle

The online aflatoxin 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 aflatoxins B1, B2, G1 and G2. The immunoaffinity cartridge contains a monoclonal antibody that is specific for total aflatoxins coupled to a hydrophilic polymer that can withstand high pressure. This enables the cartridge to be incorporated directly online with the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST system.

The immunoaffinity cartridge offers highly specific, sensitive, rapid and automated analysis for total aflatoxins in a wide range of food and feed matrices. Using the aflatoxin 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. Cartridge reusability is optimised and controlled to ensure maximum performance and to minimise the possibility of interference or carryover.

Following extraction of the toxin from the sample with solvent, the extract 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 toxins are released from the antibody following online elution and the complete eluate from the cartridge is quantitatively analysed for total aflatoxins by HPLC.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with LC, e.g. MilliQ)
- Solvents (LC Grade Methanol and Acetonitrile)
- Sodium Chloride
- Ammonium Acetate
- Nitric Acid
- Potassium Bromide
- Sodium Hydroxide
- Glycolic acid ethoxylate lauryl ether (Laureth-11) - Mn 690
- Isopropanol
- Aflatoxin Standard (please refer to Preparation of Standards section)

Accessory Products

- Glass Microfibre Filter Paper
- KOBRA® CELL (K01)*

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

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.

Note: IMMUNOPREP® ONLINE AFLATOXIN cartridges must not be allowed to sit in position in the tray without buffer for more than 24 hours to prevent the antibody drying out. It is essential to run a standard through every cartridge on each day for correct calibration of samples.

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 24 months from date of manufacture if stored at 2 - 8 °C in buffer. It is advised when the cartridges are not in use to store them 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, organic solvent 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.

For optimal cartridge performance, aim to load sample containing a quantity of 0.025 ng up to 1 ng of total aflatoxin onto the cartridge. Do not exceed the quantity of 1 ng as this is close to the capacity of the cartridge.

Recoveries

In general recoveries of greater than 90 % for aflatoxin B1, B2, G1 and G2 are achieved providing the injected amount of toxin stays within the binding capacity (0.025 ng to 1 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 (total aflatoxin content in final extract greater than 1 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 reduce the chance of interference or carry-over, we would recommend to inject a blank (i.e. surfactant in water), standard, 12 test samples and then another standard 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 is 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 (20 mM Ammonium Acetate)

1. Add 1 litre of water to a flask.
2. Add and dissolve 1.54 g of ammonium acetate.
3. Check pH and then if necessary, adjust the pH to 6.8 - 7.0 using 1 M sodium hydroxide.

• Preparation of Wash Buffer 1 (20 mM Ammonium Acetate containing 6 % Acetonitrile and 4 % Methanol)

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

• Preparation of Wash Buffer 2 - Spices only (20 mM Ammonium Acetate containing 6 % Acetonitrile, 4 % Methanol and 1 % Tween 20)

1. Add 10 g of Tween 20 to a flask.
2. Add 900 ml of water and mix using a magnetic stirrer until fully dissolved.
3. Add and dissolve 1.54 g of ammonium acetate.
4. Add 60 mL of acetonitrile and 40 ml of methanol.
5. Adjust pH to 8.3 - 8.5 using 1 M sodium hydroxide.

• Preparation of Elution Buffer (50 mM Ammonium Acetate in Mobile Phase A)

1. Add 1 litre of mobile phase A to a flask.
2. Add and dissolve 3.85 g of ammonium acetate.
3. Adjust pH to 1.75 - 1.85 using concentrated nitric acid.

• Preparation of Dilution Buffer (5 % Laureth-11 w/v (pH 5.5))

1. Weigh 12.5 g of Laureth-11 into a flask.
2. Add 250 ml of water.
3. Mix using a magnetic stirrer until fully dissolved.
4. Adjust pH to 5.5 using 5 M sodium hydroxide (approx. 3 ml).

• Preparation of Mobile Phase A (Acetonitrile : Methanol : Water (15:35:50, v/v/v))

1. Add 1 litre of water to a flask.
2. Add 300 ml of acetonitrile and 700 ml of methanol.
3. Add 0.24 g of potassium bromide and 0.7 ml of 4 M nitric acid.

Note: RIDA®CREST System only: De-gas in a sonic bath for 30 minutes.

• Mobile Phase B (90 % Methanol)

• Autosampler Wash Solution (50% Methanol)

• Pump Seal Wash Solution

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

Sample Preparation

• Cereals

This method has been tested on a number of cereals including maize, rice and wheat.

1. Weigh 25 g of ground sample into a 500 ml flask or a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 84 % methanol and shake for 30 minutes or blend at high speed for 2 minutes.
3. Filter the sample through glass microfibre filter paper.
4. Dilute 1 ml of filtrate with 9 ml of dilution buffer.
5. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
6. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system or CHRONECT Symbiosis RIDA®CREST system .

• Nuts and Figs

This method has been tested on a number of nuts (including peanuts, hazelnuts and almonds) and dried figs.

1. Prepare a slurry sample by blending a mixture of 100 g of dry weight sample and 150 ml of water for 2 minutes at high speed.
2. Weigh 50 g of slurried sample into a 500 ml flask or a 1 litre capacity, solvent resistant blender jar..
3. Add 5 g of sodium chloride and 75 ml of methanol and shake for 30 minutes or blend at high speed for 2 minutes.
4. Filter the sample through glass microfibre filter paper.
5. Dilute 1 ml of filtrate with 9 ml of dilution buffer.
6. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
7. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system or CHRONECT Symbiosis RIDA®CREST system.

- **Spices**

This method has been tested on a number of spices including nutmeg, paprika, turmeric and black pepper.

Note: It is recommended to use the specified wash buffer and to select the recommended RIDA®CREST / CHRONECT Symbiosis RIDA®CREST hardware method in conjunction with spices. There is a specific application note available for mixed spices. Please contact your local R-Biopharm distributor for further information.

1. Add 25 g of ground sample and 5 g of sodium chloride into a 1 litre solvent resistant blender jar.
2. Add 100 ml of acetonitrile : methanol : water (40 : 35 : 25 v/v/v) and blend at high speed for 2 minutes.
3. Filter the sample through glass microfibre filter paper.
4. Dilute 0.5 ml of filtrate with 9.5 ml of dilution buffer.
5. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial. .
6. 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 1,000 ng/ml aflatoxin B1, B2, G1 and G2 stock solutions:

1. Ready-to-use AFLASTANDARD (P22 / P22A, 1,000 ng/ml) is available from R-Biopharm.

   or

1. Alternatively, crystalline powder of aflatoxins B1, B2, G1 and G2 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. This is then used to prepare a 1,000 ng/ml aflatoxin B1, B2, G1 and G2 stock solution.

   Note: The ratio of B1, B2, G1 and G2 may vary in each standard. Please note the correct ratio for the standard purchased.

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. 4 ppb):

1. Take 50 µl of 1,000 ng/ml total aflatoxin standard and make up to 1 ml with 84 % methanol (equivalent to 50 ng/ml).

2. Take 200 µl at 50 ng/ml and make up to 10 ml with 84 % methanol (equivalent to 1 ng/ml).

3. Direct injection: Take 1 ml at 1 ng/ml and make up to 10 ml with water (equivalent to 0.1 ng/ml).

   Cartridge injection: Take 1 ml at 1 ng/ml and make up to 10 ml with dilution buffer (equivalent to 0.1 ng/ml).

4. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml of low level standard onto the RIDA®CREST system or CHRONECT Symbiosis RIDA®CREST system.

High level standard (i.e. 15 ppb):

1. Take 50 µl of 1,000 ng/ml total aflatoxin standard and make up to 1 ml with 84 % methanol (equivalent to 50 ng/ml).

2. Direct injection: Take 75 µl at 50 ng/ml and make up to 10 ml with water (equivalent to 0.375 ng/ml).

   Cartridge injection: Take 75 µl at 50 ng/ml and make up to 10 ml with dilution buffer (equivalent to 0.375 ng/ml).

3. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml of high level standard onto the RIDA®CREST system or CHRONECT Symbiosis RIDA®CREST system.

Recommended RIDA®CREST Conditions - Cereals, Nuts and Figs

RIDA®CREST Conditions				
Derivatisation	KOBRA® CELL at 100 µA setting			
Analytical Column	InertSustain AQ C18 3 µm, 4.6 mm x 150 mm or equivalent			
Column Temperature	45 °C			
HPLC Pump (Line A1)	Mobile Phase A. Please refer to Preparation of Buffers section.			
HPLC Pump (Line B1)	Mobile Phase B. Please refer to Preparation of Buffers section.			
Gradient	Time (min)	% A1	% B1	Flow Rate (ml/min)
	0 - 2.0	100	0	0.4
	2.0 - 2.3	100	0	0.4
	2.3 - 8.0	100	0	1.2
	8.0 - 8.1	100	0	1.2
	8.1 - 9.0	0	100	1.2
	9.0 - 9.1	0	100	1.2
	9.1 - 10	100	0	1.2
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1B)	Wash Buffer 1. Please refer to Preparation of Buffers section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Buffers section.			
Recommended RIDA®CREST Conditions for Sample Analysis	Equilibration	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Conditioning	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of loading buffer. Or HPD flow 250 µl/min, volume 500 µl of loading buffer.		
	Cartridge Wash	HPD flow 2,000 µl/min, volume 6,000 µl of wash buffer 1.		
	Elution	HPD flow 800 µl/min, volume 800 µl of elution buffer.		
	Clamp Wash	HPD flow 5,000 µl/min, volume 2,000 µl of loading buffer.		
Fluorescence Detector	Excitation: 362 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 system.			
Elution Order	G2, G1, B2, B1			
Autosampler Wash Solution	50 % methanol			
Pump Seal Wash Solution	20 % isopropanol			

Recommended RIDA®CREST Conditions - Spices

RIDA®CREST Conditions				
Derivatisation	KOBRA® CELL at 100 µA setting			
Analytical Column	InertSustain AQ C18 3 µm, 4.6 mm x 150 mm or equivalent			
Column Temperature	45 °C			
HPLC Pump (Line A1)	Mobile Phase A. Please refer to Preparation of Buffers section.			
HPLC Pump (Line B1)	Mobile Phase B. Please refer to Preparation of Buffers section.			
Gradient	Time (min)	% A1	% B1	Flow Rate (ml/min)
	0 - 2.0	100	0	0.4
	2.0 - 2.3	100	0	0.4
	2.3 - 8.0	100	0	1.2
	8.0 - 8.1	100	0	1.2
	8.1 - 9.0	0	100	1.2
	9.0 - 9.1	0	100	1.2
	9.1 - 10	100	0	1.2
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1B)	Wash Buffer 1. Please refer to Preparation of Buffers section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1E)	Wash Buffer 2. Please refer to Preparation of Buffers section.			
Recommended RIDA®CREST Conditions for Sample Analysis	Equilibration	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Conditioning	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of loading buffer. Or HPD flow 250 µl/min, volume 500 µl of loading buffer.		
	Cartridge Wash	HPD flow 2,000 µl/min, volume 6,000 µl of wash buffer 2. HPD flow 2,000 µl/min, volume 2,000 µl of wash buffer 1.		
	Elution	HPD flow 800 µl/min, volume 800 µl of elution buffer.		
	Clamp Wash	HPD flow 5,000 µl/min, volume 2,000 µl of loading buffer.		
Fluorescence Detector	Excitation: 362 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 system.			
Elution Order	G2, G1, B2, B1			
Autosampler Wash Solution	50 % methanol			
Pump Seal Wash Solution	20 % isopropanol			

Recommended CHRONECT Symbiosis RIDA®CREST Conditions – Cereals, Nuts and Figs

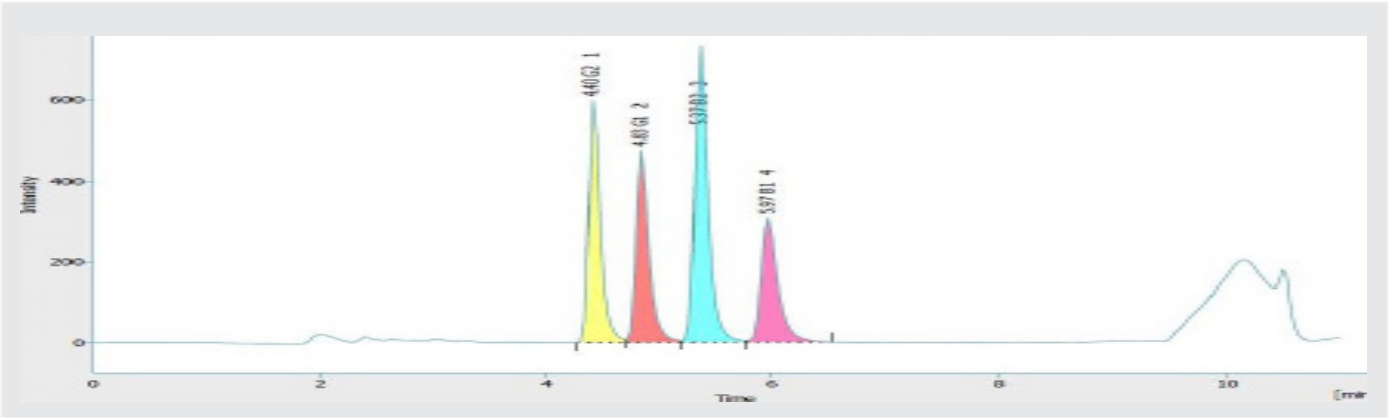
CHRONECT Symbiosis RIDA®CREST Conditions				
Derivatisation	KOBRA® CELL at 100 µA setting			
Analytical Column	InertSustain AQ C18 3 µm, 4.6 mm x 150 mm or equivalent			
Column Temperature	50 °C			
HPLC Pump (Line A1)	Mobile Phase A. Please refer to Preparation of Buffers section.			
HPLC Pump (Line B1)	Mobile Phase B. Please refer to Preparation of Buffers section.			
Gradient	Time (min)	% A1	% B1	Flow Rate (ml/min)
	0 - 2.0	100	0	0.3
	2.0 - 2.3	100	0	0.3
	2.3 - 8.0	100	0	1.1
	8.0 - 8.1	100	0	1.1
	8.1 - 9.0	0	100	1.1
	9.0 - 9.1	0	100	1.1
	9.1 - 10	100	0	1.1
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1B)	Wash Buffer 1. Please refer to Preparation of Buffers section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Buffers section.			
Recommended RIDA®CREST Conditions for Sample Analysis	Equilibration	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Conditioning	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of loading buffer. Or HPD flow 250 µl/min, volume 500 µl of loading buffer.		
	Cartridge Wash	HPD flow 2,000 µl/min, volume 6,000 µl of wash buffer 1.		
	Elution	HPD flow 800 µl/min, volume 800 µl of elution buffer.		
	Clamp Wash	HPD flow 5,000 µl/min, volume 2,000 µl of loading buffer.		
Fluorescence Detector	Excitation: 362 nm			
	Emission: 455 nm			
Data Control System	CHRONOS and Clarity™ or from preferred supplier			
Injection Volume	Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the CHRONECT Symbiosis RIDA®CREST system.			
Elution Order	G2, G1, B2, B1			
Autosampler Wash Solution	50 % methanol			
Pump Seal Wash Solution	10 % isopropanol			

Recommended CHRONECT Symbiosis RIDA®CREST Conditions - Spices

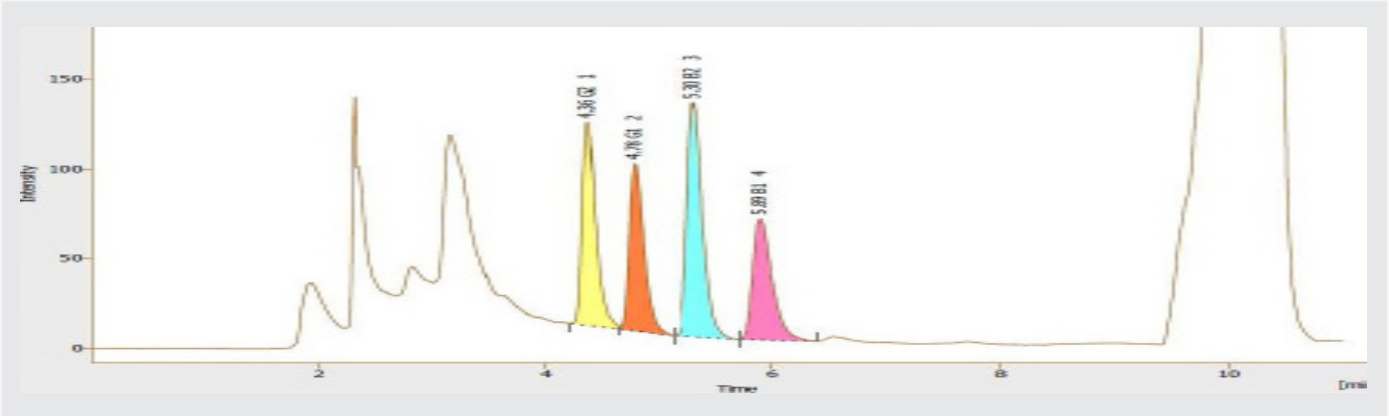
CHRONECT Symbiosis RIDA®CREST Conditions				
Derivatisation	KOBRA® CELL at 100 µA setting			
Analytical Column	InertSustain AQ C18 3 µm, 4.6 mm x 150 mm or equivalent			
Column Temperature	50 °C			
HPLC Pump (Line A1)	Mobile Phase A. Please refer to Preparation of Buffers section.			
HPLC Pump (Line B1)	Mobile Phase B. Please refer to Preparation of Buffers section.			
Gradient	Time (min)	% A1	% B1	Flow Rate (ml/min)
	0 - 2.0	100	0	0.3
	2.0 - 2.3	100	0	0.3
	2.3 - 8.0	100	0	1.1
	8.0 - 8.1	100	0	1.1
	8.1 - 9.0	0	100	1.1
	9.0 - 9.1	0	100	1.1
	9.1 - 10	100	0	1.1
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1B)	Wash Buffer 1. Please refer to Preparation of Buffers section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1E)	Wash Buffer 2. Please refer to Preparation of Buffers section.			
Recommended RIDA®CREST Conditions for Sample Analysis	Equilibration	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Conditioning	HPD flow 5,000 µl/min, volume 1,000 µl of loading buffer.		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of loading buffer. Or HPD flow 250 µl/min, volume 500 µl of loading buffer.		
	Cartridge Wash	HPD flow 2,000 µl/min, volume 6,000 µl of wash buffer 2. HPD flow 2,000 µl/min, volume 2,000 µl of wash buffer 1.		
	Elution	HPD flow 800 µl/min, volume 800 µl of elution buffer.		
	Clamp Wash	HPD flow 5,000 µl/min, volume 2,000 µl of loading buffer.		
Fluorescence Detector	Excitation: 362 nm			
	Emission: 455 nm			
Data Control System	CHRONOS and Clarity™ or from preferred supplier			
Injection Volume	Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the CHRONECT Symbiosis RIDA®CREST system.			
Elution Order	G2, G1, B2, B1			
Autosampler Wash Solution	50 % methanol			
Pump Seal Wash Solution	10 % isopropanol			

Example HPLC Chromatograms for CHRONECT Symbiosis RIDA®CREST

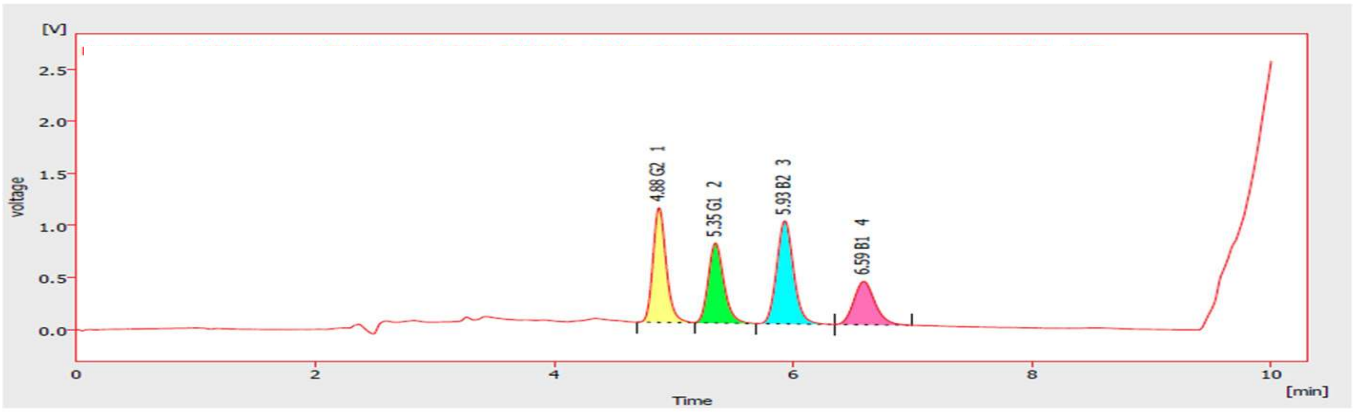
- **Maize (Spiked at 40 ppb total aflatoxin)**



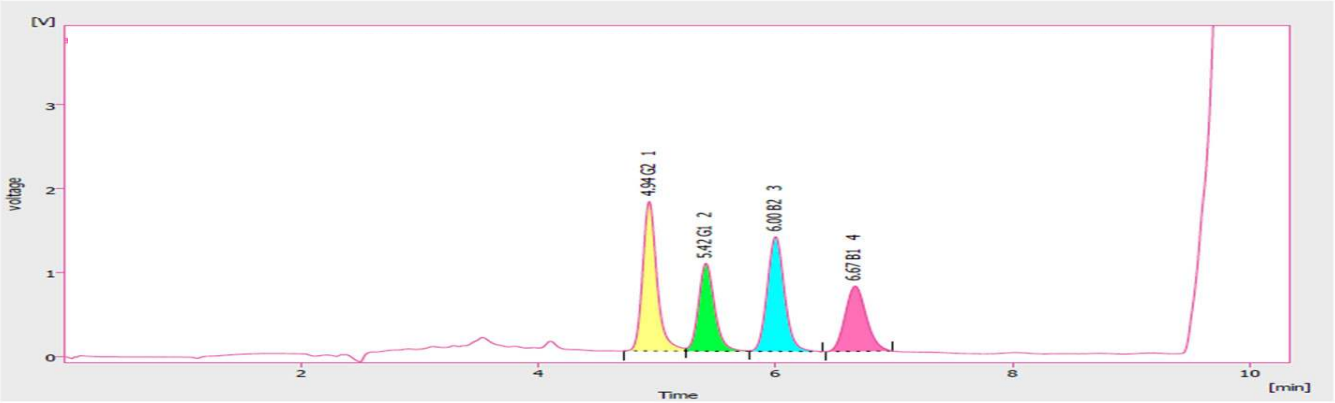
- **Curry Powder (Spiked at 10 ppb total aflatoxin)**



- **Peanuts (Spiked at 5 ppb total aflatoxin)**



- **Turmeric (Spiked at 10 ppb total aflatoxin)**



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.
- 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.

Acknowledgement

R-Biopharm Rhône Ltd would like to acknowledge Scarlett Biselli and her team at Eurofins, Hamburg, for their assistance during the development of this product.

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.

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