IMMUNOPREP® ONLINE AFLATOXIN M1

Product Code: P904/48

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

P904/V8/20.07.22

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R-BIOPHARM RHÔNE LTD

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Test Principle

The online aflatoxin M1 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 aflatoxin M1. The immunoaffinity cartridge contains a monoclonal antibody that is specific for aflatoxins M1 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 aflatoxin M1 in a wide range of food and feed matrices. Using the aflatoxin immunoaffinity cartridge, the sample application, washing and elution are 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 evaporated, reconstituted, if necessary filtered and transferred to an autosampler vial. The obtained 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 goes to waste. Subsequently the toxin is released from the antibody following online elution and the complete eluate from the cartridge is quantitatively analysed for aflatoxin M1 by HPLC.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with LC, e.g. MilliQ)
- Solvents (LC Grade Methanol and Acetonitrile)
- Ammonium acetate
- Formic acid
- Hydrochloric acid
- Magnesium sulphate, anhydrous
- Sodium acetate, anhydrous
- Sodium hydroxide
- Trisodium citrate dihydrate
- Tris (hydroxymethyl) aminomethane
- Tween 20
- Aflatoxin M1 Standard (please refer to Preparation of Standards section)

Accessory Products

- Chromafil® Nylon PA Xtra 0.45 µm syringe filters

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 M1 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 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 (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 0.005 ng up to 1 ng of aflatoxin M1 onto the cartridge. Do not exceed the quantity of 1 ng as this is close to the suggested maximum working range of the cartridge.

Recoveries

In general recoveries of greater than 85 % for aflatoxin M1 is achieved providing the injected amount of toxin stays within the binding capacity (0.005 ng to 1 ng). 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 (aflatoxin M1 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 to reduce the chance of interference or carryover we would recommended to inject a blank (i.e. reconstitution buffer), standard, 12 test samples and then another standard (for bracketed calibration) through each cartridge (a total of 15 injections).

Preparation of Buffers

Note: When preparing buffers it is important to ensure that they are within the pH range specified. This is particularly important if analysing for AFT M2 as this analyte is extremely pH sensitive.

• Preparation of Loading Buffer (20 mM Ammonium Acetate)

1. Add 1 litre of water to a flask.
2. Add 1.54 g of ammonium acetate.
3. Adjust the pH to 7.4 using 1 M sodium hydroxide, if necessary.

• Preparation of Cartridge Wash Buffer (20 mM Ammonium Acetate, 25 mM Tris containing 12.5 % Methanol)

1. Add 875 ml of water to a flask.
2. Add 1.54 g of ammonium acetate and 3.02 g of tris (hydroxymethyl) aminomethane.
3. Add 125 ml of methanol.
4. Adjust pH to 8.5 using concentrated nitric acid.

• Preparation of Elution Buffer (Acetronitrile : Methanol : Water, containing 50 mM Ammonium acetate (10 : 26 : 64 v/v/v))

1. Add 640 ml of water to a flask.
2. Add 3.85 g of ammonium acetate.
3. Add 100 ml of 100 % acetonitrile and 260 ml of 100 % methanol.
4. Adjust pH to 2.0 using concentrated nitric acid.

• Mobile Phase A (Methanol : Water : Formic Acid (2 : 97.9 : 0.1, v/v/v))

1. Add 20 ml of methanol to a flask.
2. Add 979 ml of water and 1 ml of formic acid.
3. Degas in a sonic bath for 30 minutes.

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

1. Add 900 ml of acetonitrile into a flask.
2. Add 99 ml of water and 1 ml of formic acid.
3. Degas in a sonic bath for 30 minutes.

- **Reconstitution Buffer (Loading buffer, containing 10 % Methanol)**

1. Add 1.386 g of ammonium acetate to a flask.
2. Add 900 ml of water and 100 ml of methanol.
3. Adjust pH to 7.4 using 1 M of sodium hydroxide.

- **Autosampler Wash (50 % Methanol)**

- **Pump Seal Wash Solution (RIDA®CREST 20 % Isopropanol),**
  (CHRONECT Symbiosis RIDA®CREST system 10 % Isopropanol)

Sample Preparation

• Powdered Milk, Infant Milk Fromula, Milk, Cheese, Yoghurt and Cream

1. Dependent on the matrix to be analysed extract the appropriate quantity of sample according to the table below and add to centrifuge tube.

Matrix	Volume of Sample Extracted	Volume of Water at 50 °C Used During Extraction
Milk	10 g	0 ml
Milk Powder	4 g	10 ml
Infant Milk Powder	1.4 g	10 ml
Cheese	5 g	10 ml
Yoghurt	5 g	10 ml
Cream	5 g	10 ml

2. According to the table above add the required volume of water at 50 °C and mix sample vigorously. Allow to cool to room temperature.

3. Add 20 ml of 100 % acetonitrile and mix well by hand.

4. Weigh 4 g of magnesium sulphate, 1 g of sodium acetate and 0.5 g of sodium citrate and mix well by hand before adding the mixture to centrifuge tube containing sample. Alternatively, add Supel™ QuE Citrate Extraction Tube salts.

5. Mix at 2,000 rpm for 60 minutes using a shaker.

6. Centrifuge at 4,000 rpm for 10 minutes.

7. Transfer 2 ml of the acetonitrile layer (top layer) into a glass tube.

8. Add one drop of Tween 20.

9. Evaporate to dryness under nitrogen at 60 °C.

10. Reconstitute with 2 ml of reconstitution buffer.

11. If cloudy (e.g. cheese), pass the reconstituted sample through a Chromafil® Nylon PA Xtra 0.45 µm syringe filter.

12. Transfer 1.5 ml of the filtrate into an amber autosampler vial.

13. Depending on the sensitivity of the fluorescence detector, inject 1 ml onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST system.

Preparation of Standards

Preparation of 1,000 ng/ml aflatoxin M1 stock solutions:

1. A crystalline powder of aflatoxin M1 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 M1 stock solution.

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**

1. Take 50 µl of 1,000 ng aflatoxin M1 standard and make up to 10 ml with acetronitrile (equivalent to 5 ng/ml).

2. Standard 1: Take 100 µl at 5 ng/ml and make up to 10 ml with reconstitution buffer (equivalent to 0.05 ng/ml).

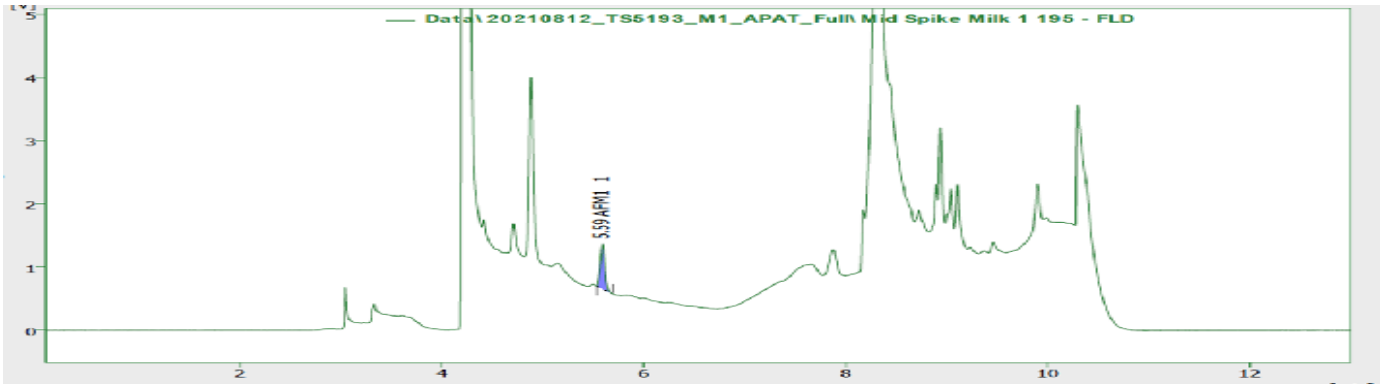
3. Inject 1 ml of standard 1 onto the RIDA®CREST or CHRONECT Symbiosis RIDA®CREST system.

Recommended CHRONECT Symbiosis RIDA®CREST Conditions

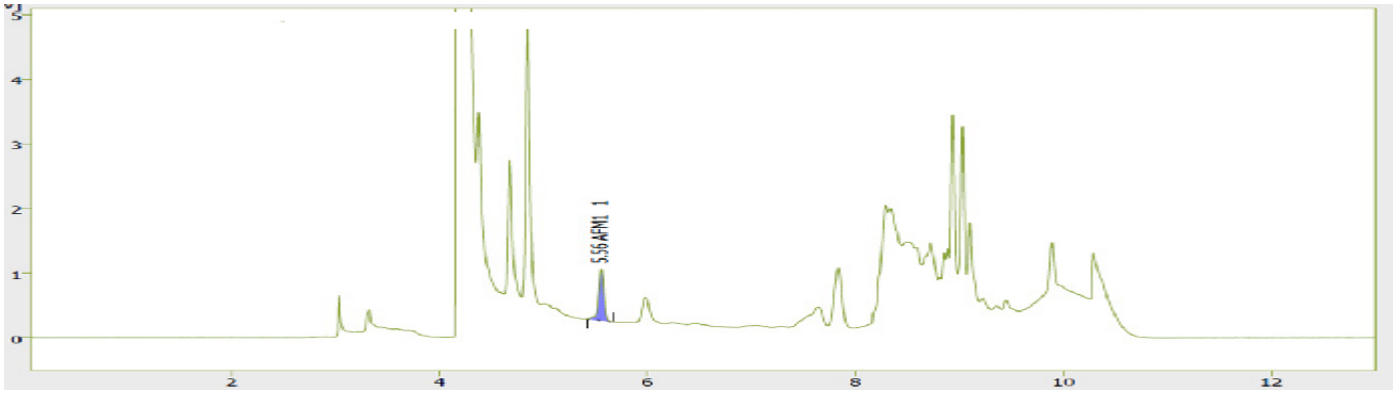
CHRONECT Symbiosis RIDA®CREST Conditions				
Analytical Column	Kinetex Phenyl-Hexyl, 2.6 µm, 4.6 mm x 150 mm or equivalent			
HPLC Pump 1 (Line A1)	Mobile Phase A			
HPLC Pump 2 (Line B1)	Mobile Phase B			
Gradient HPLC	Time (min)	% A1	% B1	Flow Rate (ml/min)
	Initial	100	0	1.0
	0.01	100	0	0.1
	2.00	100	0	0.1
	2.10	60	40	0.8
	7.00	60	40	0.8
	7.10	0	100	1.0
	9.00	0	100	1.0
	9.01	100	0	1.0
	12.00	100	0	1.0
HPD1 (Line 1A)	Loading Buffer			
HPD1 (Line 1B)	Wash Buffer			
HPD1 (Line 1C)	Elution Buffer			
HPD1 (Line 1D)	10 % methonal (cleaning only)			
Direct injection Autosampler Wash Cycle	Pre-wash 1	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
	Post wash 1	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
	Post-wash 2	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
IPO injection Autosampler Wash Cycle	Pre-wash 1	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
	Post wash 1	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
	Post-wash 2	Draw 50 µl/sec, volume 1 ml of wash 1, eject 200 µl/sec		
Direct injection				
Gain	Time (mins)			
X4	0 - 6.99			
X16	7.00 - 11.99			
X4	12.00			
IMMUNOPREP® ONLINE injection				
Gain	Time (mins)			
X4	0 - 5.44			
X16	5.45 - 11.99			
X4	12.00			
Note: Gain split time only required when assessing both M1 and M2. Gain split time may vary depending on mobile phase, detector settings used				
Fluorescence Detector	Excitation: 355 nm			
	Emission: 430 nm			
Column Heater	Maintain guard and analytical columns at 45 °C			
Injection Volume	1 ml			
Recommended conditions for sample analysis	Conditioning	HPD Flow 5 ml/min, volume 1 ml of Loading Buffer		
	Equilibration	HPD Flow 5 ml/min, volume 1 ml of Loading Buffer		
	Sample Application	HPD Flow 1 ml/min, volume 1 ml of Loading Buffer		
	Cartridge Wash	HPD Flow 2 ml/min, volume 6 ml of Wash Buffer		
	Cartridge Elution	HPD Flow 0.3 ml/min, volume 0.6 ml of Elution Buffer		
	Clamp Wash	HPD Flow 5 ml/min, volume 2 ml of Loading Buffer		

Example HPLC Chromatograms for CHRONECT Symbiosis RIDA®CREST

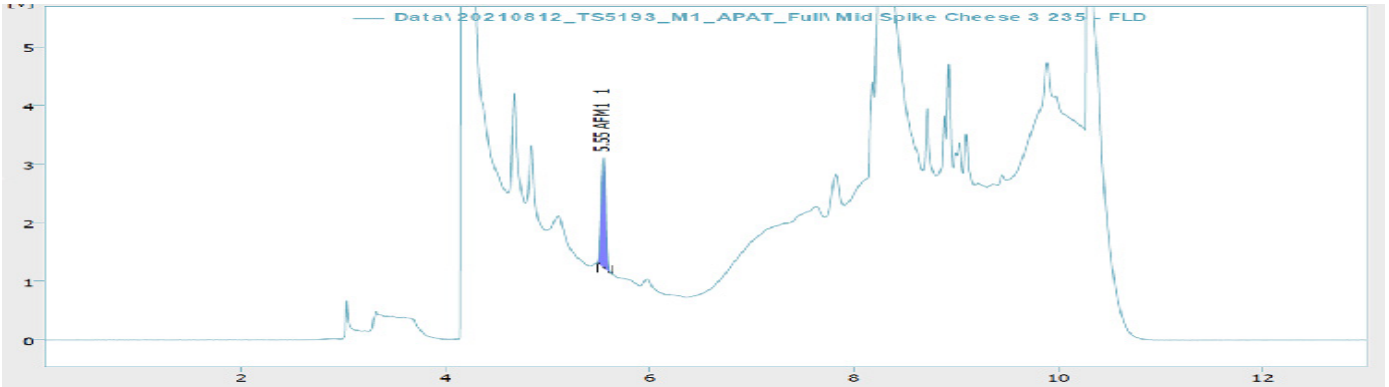
- **Example HPLC Chromatogram for Milk (Spiked at 0.05 ppb)**



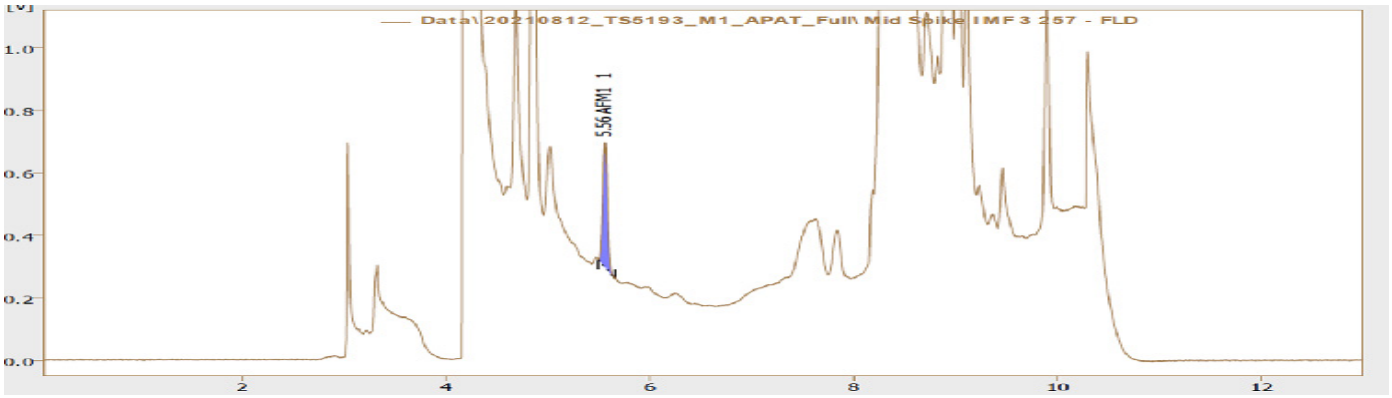
- **Example HPLC Chromatogram for Milk Powder (Spiked at 0.05 ppb)**



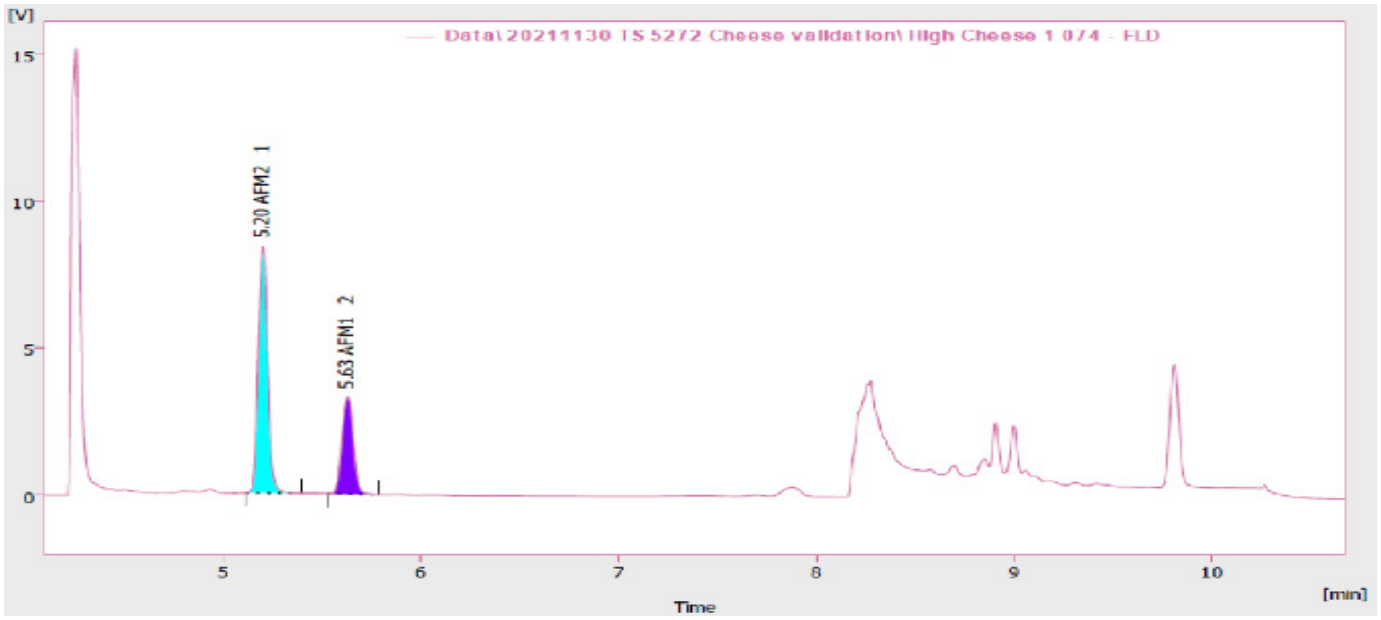
- **Example HPLC Chromatogram for Cheese (Spiked at 0.25 ppb)**



- **Example HPLC Chromatogram for Infant Milk Formula (Spiked at 0.025 ppb)**



- **Example HPLC Chromatogram for Cheese (Spiked at 0.5 ppb aflatoxin M1 and M2)**

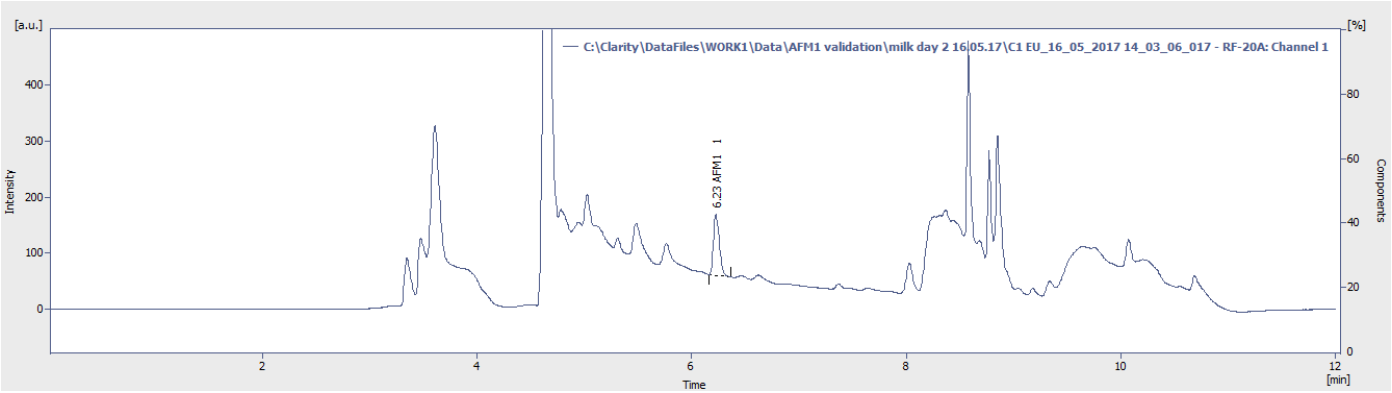


Recommended RIDA®CREST Conditions

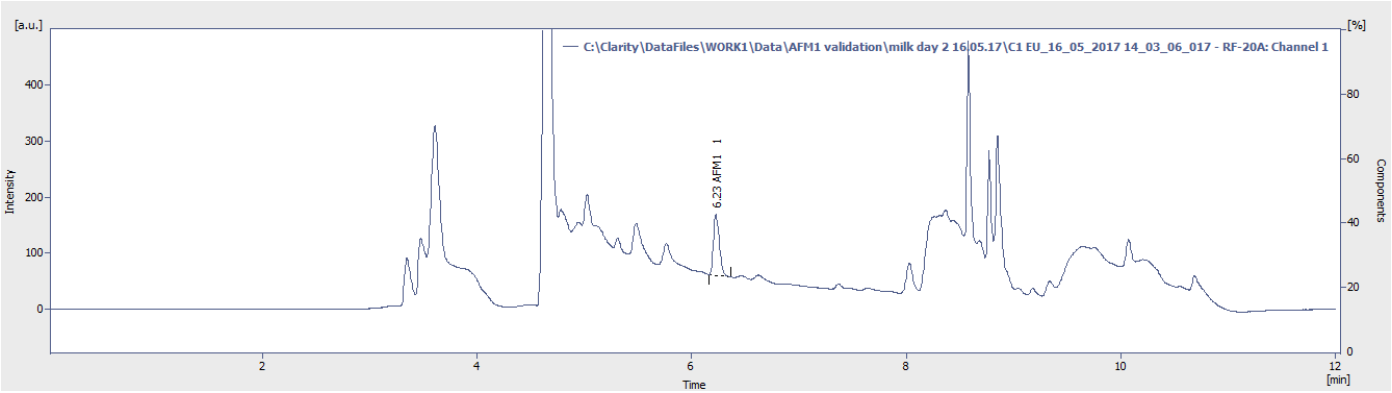
RIDA®CREST Conditions				
Analytical Column	Kinetex Phenyl-Hexyl, 2.6 µm, 4.6 mm x 150 mm or equivalent			
HPLC Pump 1 (Line A1)	Mobile Phase A			
HPLC Pump 2 (Line B1)	Mobile Phase B			
Gradient HPLC	Time (min)	% A1	% B1	Flow Rate (ml/min)
	Initial	100	0	1.0
	0.01	100	0	0.1
	2.00	100	0	0.1
	2.10	60	40	0.8
	7.00	60	40	0.8
	7.10	0	100	1.0
	9.00	0	100	1.0
	9.01	100	0	1.0
	12.00	100	0	1.0
HPD1 (Line 1A)	Loading Buffer			
HPD1 (Line 1B)	Wash Buffer			
HPD1 (Line 1C)	Elution Buffer			
HPD1 (Line 1D)	10 % methonal (cleaning only)			
Fluorescence Detector	Excitation: 355 nm			
	Emission: 430 nm			
Column Heater	Maintain guard and analytical columns at 40 °C			
Injection Volume	1 ml			
Recommended conditions for sample analysis	Conditioning	HPD Flow 5 ml/min, volume 1 ml of Loading Buffer		
	Equilibration	HPD Flow 5 ml/min, volume 1 ml of Loading Buffer		
	Sample Application	HPD Flow 1 ml/min, volume 1 ml of Loading Buffer		
	Cartridge Wash	HPD Flow 2 ml/min, volume 6 ml of Wash Buffer		
	Cartridge Elution	HPD Flow 0.3 ml/min, volume 0.6 ml of Elution Buffer		
	Clamp Wash	HPD Flow 5 ml/min, volume 2 ml of Loading Buffer		

Example HPLC Chromatograms for RIDA®CREST

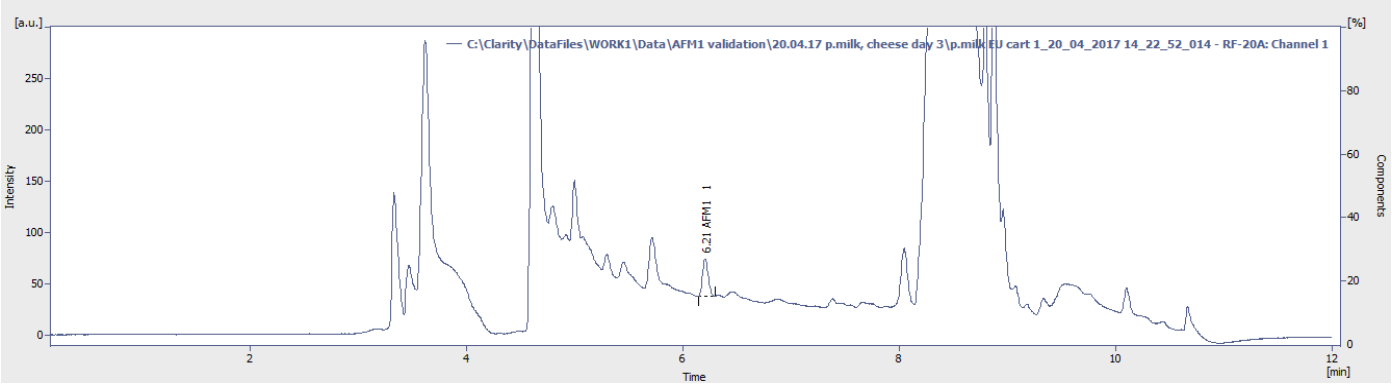
- **Example HPLC Chromatogram for Milk (Spiked at 0.05 ppb)**



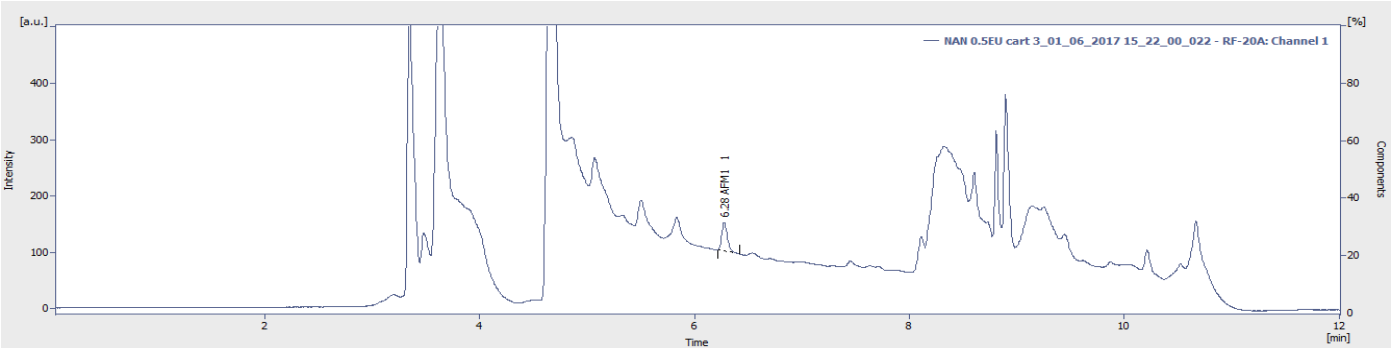
- **Example HPLC Chromatogram for Powdered Milk (Spiked at 0.05 ppb)**



- **Example HPLC Chromatogram for Cheese (Spiked at 0.05 ppb)**



- **Example HPLC Chromatogram for Cheese (Spiked at 0.0125 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.
- 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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