R-BIOPHARM
RHÔNE LTD

Mycotoxins: a potential hazard

A complete range of analytical options

Worldwide presence
- Provider of choice in the spice and herb industry
- Global support

Validated methods
- Products used in collaborative trials
- Confidence in correct results first time around

Innovative
- Leading the way in multi-toxin analysis
- Optimising workflow by offering automated clean-up

More information:

https://r-b.io/spices

11 /2021

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The spice industry

What are spices and herbs?

A spice is a seed, fruit, root, bark or other plant substance primarily used for flavouring or colouring food. Herbs are the leaves, flowers, or stems of plants. This means that you can have two very different flavours and products from the same plant. For example, fenugreek or coriander, both are sold as a spice or as a green herb.

Culinary use typically distinguishes spices from herbs however both are primarily used in the food industry for flavouring but also as ingredients in cosmetics and medicinal products.

A spice may be presented in several forms -

- fresh
- whole dried
- pre-ground dried

Generally, spices are dried and ground into a powder for convenience. The flavor of a spice is derived in part from compounds (volatile oils) that oxidize or evaporate when exposed to air. Grinding a spice greatly increases its surface area and so increases the rates of oxidation and evaporation. Thus, the flavour is maximized by storing a spice whole and grinding when needed.

Some flavour elements in spices are soluble in water; many are soluble in oil or fat. As a general rule, the flavours from a spice take time to infuse into the food so spices are added early in preparation. This contrasts to herbs which are usually added late in preparation.

Due to recent requirements to reduce the salt content in food, many manufacturers are adding spices and herbs to products to compensate and to improve the overall flavour and taste of the food. Therefore, there has been an increase in the testing and analysis of products on the market.

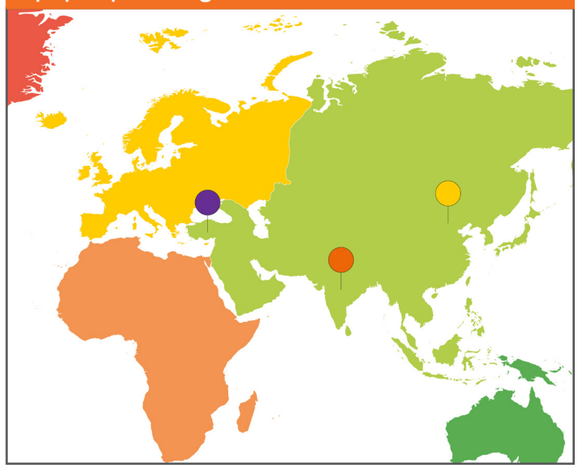
Production of spices

The increasing interest in international ethnic cuisines combined with the healthy living trend has led to an increase in the import and export of spices and herbs. Between 2014 and 2017, European import values for spices and herbs grew every year by 11 % on average to a value of €2.7 billion.

India accounts for over 70 % of the global spice product with exports from India increasing by over 80 % in a 20 year period and is expected to reach $18 billion by 2020. Over 50 different types of spices are produced by India. Other spice producing countries include Bangladesh, Turkey, China and Pakistan.

Pepper is the most valuable spice in the global trade with capsicum trade rising to higher annual tonnage however to a lesser commercial value.

Top spice producing countries



Process

Potential mycotoxin contamination

There are several key stages throughout the production process where fungal growth is favoured which could result in mycotoxin contamination.

1. Plantation

- Spices are part of the food category, regulations from local Government should be met.
- In India, this includes a trade license from Food Safety and Standards Authority of India (FSSAI).

Caution: Insect activity should be limited in the field as this can increase the potential for growth of moulds.

2. Harvest

- Spices are harvested manually using pruners, knives or scissors.
- Leaves should be free of insect damage and harvested after the morning dew has evaporated.

Caution: If produce is harvested when wet and stored incorrectly this can increase the potential for growth of moulds.

Optional qualitative screening test could be conducted at this point

3. Cleaning & drying

- Cleaned using magnetic destoner.
- Unground spices are cleaned to remove impurities such as stones, dust and dirt.
- Sample is dried either in sunlight or using steam sterilisation and natural pasteurisation. The quality will depend on how well the spice has been dried.

Caution: If produce has been insufficiently cleaned and dried, this can increase the potential for moulds to grow.

4. Roasting & grinding

- Once dried they are roasted. This helps to give the colour, aroma and taste.
- Grinding is performed by machine to produce a powder.
- Roasting is buyer specific.

Caution: Moulds may be have the potential to grow if material has been packaged in a warm, humid environment. If packing is unable to be carried out on site, care should be taken during storage and transportation to avoid favoured conditions for moulds. .

Quantitative final testing is required

5. Sieving & grading

- Metal detector is mandatory at this stage

6. Packing & distribution

- Powdered spice is packaged.
- Packaging must ensure that product is protected and preserved during distribution.

Caution: Inadequate packaging can increase the potential for growth of moulds.

Further qualitative testing may be conducted at port of entry

Mycotoxins in spices and herbs

What are mycotoxins?

Mycotoxins are toxic secondary metabolites produced by mould. They are produced under specific conditions of moisture and temperature and are generally associated with diseased or mouldy crops. However, not all fungi can produce mycotoxins. Even those with the ability to produce mycotoxins may not produce them all of the time. Growth of the mycotoxin depends on temperature, pH, humidity and the presence of plant substrates. The formation of mycotoxins depends on regional and seasonal environmental conditions, such as food availability, moisture content in substrate and surrounding air, temperature, pH value and interaction with other fungus. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high.

Mycotoxins can be divided into different groups due to their appearance at different stages between the development of the crop, harvest and storage and toxicity. Therefore, mycotoxin contamination can be separated into primary, secondary and carry over contamination. Primary contamination means that the commodity in the field is already infested by the fungus. Secondary contamination occurs during storage and the carry over refers to a mouldy commodity being eaten by an animal which passes the toxin on in the form of eggs, milk and meat.

In order to monitor levels from plantation to final quality control checks, it is essential that the method selected for detection of mycotoxins are able to accurately meet the legislative requirements and demands of the laboratory.

Legislation and Official Methods

Legislation

Regulations are in place which outline the maximum residue limits for various contaminants including mycotoxins. In Europe, these are covered by document EC 1181/2006 and 1137/2015.

Aflatoxin	**Maximum level (ppb)**	
Commodity	**B1**	**Total**
Certain species of spices: *Capsicum spp.* (dried fruits whole or ground including chillies, chilli powder, cayenne and paprika) *Piper spp.* (fruits including black and white pepper), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Curcumalonga* (turmeric).	5	10

Ochratoxin * Level under discussion	**Maximum level (ppb)**
Commodity	
Following species of spices: *Piper spp.* (fruits including black and white pepper), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Curcumalonga* (turmeric).	15
Capsicum spp. (dried fruits whole or ground including chillies, chilli powder, cayenne and paprika).	20
Mixtures of spices containing one of the above mentioned spices.	15
Liquorice, liquorice root, ingredient for herbal infusion.	20
Liquorice extract for use in food, some beverages and confectionery.	80
Foods containing liquorice for final consumer.	20*
Herbs, tea and herbal infusions.	10*
Sunflower and pumpkin seeds.	10*

Official methods

There are a number of official methods for the analysis of aflatoxin and ochratoxin in spices which recommend or stipulate the use of an immunoaffinity column. Some of the methods that exist for spices are outlined here in the table. These official methods have criteria with regards to immunoaffinity columns and products selected must meet these strict specifications. In all cases, the immunoaffinity columns from R-Biopharm meet required criteria.

Method	Matrices	Extraction	Specifications	
			Capacity	Recovery
CEN EN 14123 for AFT	• Nuts • Dried fruit • Spices	80 % methanol	≥100 ng AFT B1	≥80 % for AFT B1, B2 and G1 ≥60 % for G2
CEN EN 17424 for AFT	• Spices (not paprika)	acetonitrile : methanol : water (40:35:25 v/v/v)	≥100 ng AFT B1	not defined
AOAC 999.07 for AFT	• Peanut butter • Pistachio paste • Fig paste • Paprika	80 % methanol	≥100 ng AFT B1	≥80 % for AFT B1, B2 and G1 ≥60 % for G2
CEN EN 17250 for OTA	• Paprika • Chilli • Black & white pepper • Nutmeg • Spice mix • Liquorice • Cocoa	methanol : sodium bicarbonate (50:50 v/v)	≥100 ng AOTA	≥85 % for OTA
AOAC 2008.02 for AFT and OTA	• Ginseng, ginger	methanol : 0.5 % sodium bicarbonate (70:30 v/v)	≥100 ng Total AFT ≥ 100 ng OTA	≥80 % for AFT B1, B2, G1, G2 and OTA

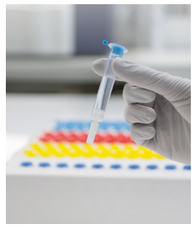
Analytical methods and controls

Flexible options

Currently the only reliable way to determine mycotoxin content and ensure compliance to regulatory control measures is by following sampling and analysis guidelines. Any samples taken should be representative of the lot and should be prepared by an accredited method.

Methods for mycotoxin determination in spices typically include chromatography with HPLC as this has been recognised as a reliable means of detecting aflatoxin and ochratoxin at the required levels. However, qualitative methods are also available. It is important that the selected method demonstrates high performance and reliability and is suitable for the analysis of the wide variety of spices available.

Our portfolio gives you the flexibility to choose the product that is best suited to your particular laboratory requirements. Choose from simple screening tests or from a complete range of immunoaffinity columns with both single and multi-toxin options. To complete our portfolio a range of immunoaffinity cartridges are available for automated analysis suitable for laboratories that require high throughput and improved workflow.

Screening tests	Immunoaffinity columns	Immunoaffinity cartridges	Standards	Accessory products
				

Rapid screening cards

Qualitative detection of aflatoxins and ochratoxin

An important measure for reducing the mycotoxin risk is to assess the levels throughout the production process. At an early stage in the process rapid and easy-to-use tests may be more convenient.

The easy-to-handle card tests from R-Biopharm are a suitable, fast option for the analysis of mycotoxins in spices. These versatile tests are available for aflatoxin and ochratoxin and are validated for many spices and herbs at legislative levels.

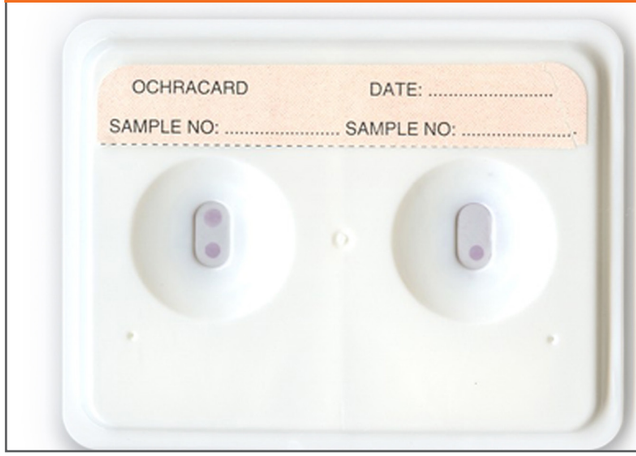
The clean-up column provided with the test kit ensures that all coloured pigments are removed from the sample extract enabling the extract to be applied to the port of the test card without issues from interfering components. This ensures accurate results are obtained which correlate well to HPLC results. Samples can be screened at various levels to suit the individual requirements.

A purple spot must appear at the control site to indicate that the test is valid. A purple spot at the sample site shows that the contamination is less than selected screening level of the card. No colour at the sample site indicates contamination at a higher level than the selected screening level.

Methods available for the analysis of –

- black tea
- green tea
- turmeric
- paprika
- black pepper
- white pepper
- nutmeg
- chilli powder
- herbal teas (jasmine, peppermint)
- parsley
- oregano

OCHRACARD – a positive result



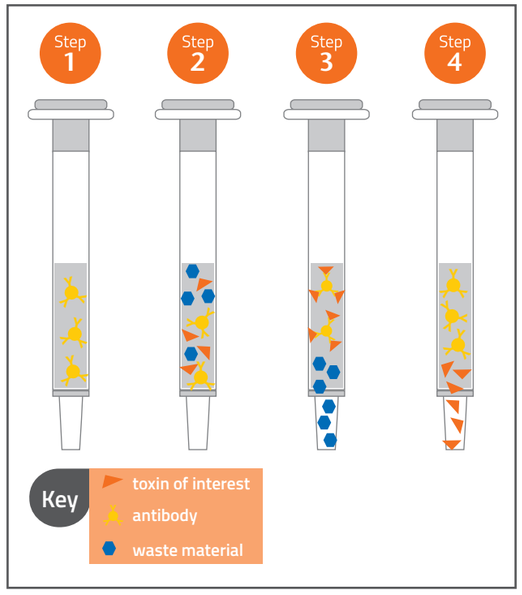
Immunoaffinity clean-up columns

Immunoaffinity column principle

Spices and herbs are generally considered to be complex matrices to analyse due to components within the sample resulting in matrix effects. The matrix effects can have a considerable effect on the quality of the results obtained, specifically with issues of interfering components on the chromatography.

Immunoaffinity clean-up methods are highly specific as the mycotoxin of interest is isolated and concentrated from the sample. This makes the method particularly suitable for the analysis of complex samples such as spices and herbs. All interfering components are removed from the sample which means that no matrix effects are observed. This ultimately leads to cleaner and more accurate chromatography and added confidence in your results.

Not all spices and herbs are the same and as a result the same extraction method may not apply to all samples. It is for this reason that R-Biopharm have a number of extraction methods covering a range of spices and herbs to ensure optimum performance.



Immunoaffinity clean-up columns

Aflatoxin analysis

AFLARHONE® and AFLARHONE® WIDE columns have been specially designed by R-Biopharm for the analysis of spices and herbs.

- Specific antibody ensuring excellent recoveries for all aflatoxins, including aflatoxin G2 which often can exhibit low recoveries with complex samples such as spices.
- Reduced volume of gel within the column ensuring that product is cost effective.
- The implementation of our recommended methods ensures optimum performance. However, the columns can also be used in conjunction with Official CEN or AOAC methods or with methods from Governing bodies such as Food Safety and Standards Authority of India (FSSAI) or from Trade Associations such as the American Spice Trade Association (ASTA).
- The columns meet all Official Method specifications with regards to capacity giving you the added confidence that results will be accurate and not be under-reported.
- Columns are available in both narrow and wide format to suit individual customer preference.

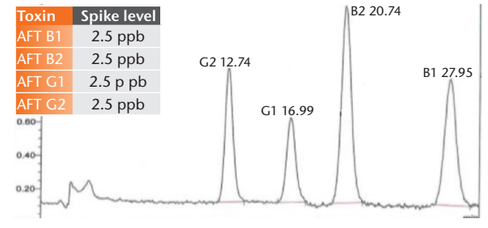
For all spices except cinnamon and cumin

- Weigh 25 g of sample and 5 g of sodium chloride
- Add 100 ml of AcN : MeOH : H_2O (40:35:25 v/v/v) and blend
- Filter the sample or centrifuge
- Dilute 2 ml of filtrate with 118 ml of10 % Tween 20 in PBS
- Pass 30 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 20 ml of PBS
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of water
- Inject 100 µl

For cinnamon and cumin

- Weigh 25 g of sample and 5 g of sodium chloride
- Add 100 ml of 75 % acetonitrile and blend
- Filter the sample or centrifuge
- Dilute 2 ml of filtrate with 58 ml of10 % Tween 20 in PBS, adjust pH to 7.4
- Pass 15 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 20 ml of PBS
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of water
- Inject 100 µl

Toxin	Spike level
AFT B1	2.5 ppb
AFT B2	2.5 ppb
AFT G1	2.5 p pb
AFT G2	2.5 ppb



Black pepper analysis using AFLARHONE® WIDE

Results obtained with AFLARHONE® WIDE, spiked at 10 ppb total AFT (n=3)

	% recovery (% RSD)				
	AFT B1	AFT B2	AFT G1	AFT G2	Total AFT
Paprika	84.5 (2.3)	90.9 (0.6)	84.8 (0.7)	90.9 (0.6)	84.5 (2.3)
Cumin	100.8 (3.4)	109.1 (2.0)	100.16 (1.1)	112.3 (0.9)	105.6 (1.2)
Curry powder	92.5 (4.2)	98.6 (2.8)	93.1 (2.7)	101.8 (3.4)	96.5 (3.3)
Mustard seeds	73.3 (1.5)	84.8 (1.7)	73.9 (1.3)	84.5 (0.0)	79.1 (0.9)
White pepper	84.5 (6.3)	90.6 (4.8)	78.7 (10.0)	86.1 (2.8)	85.0 (5.8)
Black pepper	80.6 (7.4)	88.3 (1.1)	73.6 (3.8)	85.1 (1.7)	81.9 (3.0)
Ground ginger	86.1 (2.3)	94.08 (2.7)	79.4 (1.9)	89.6 (3.4)	87.3 (1.4)
Root ginger	88.0 (3.8)	92.5 (2.2)	82.6 (2.9)	87.4 (1.1)	87.6 (2.0)
Cinnamon	92.8 (3.6)	97.9 (2.6)	87.7 (5.6)	94.1 (2.7)	93.1 (2.6)

Immunoaffinity columns

Ochratoxin analysis

OCHRARHONE® and OCHRARHONE® WIDE columns have again been specifically designed by R-Biopharm for the analysis of spices and herbs to ensure excellent recoveries are obtained and that customers are confident in results obtained.

For all spices except mustard seeds, black and white pepper

- Weigh 5 g of sample
- Add 200 ml of 1 % sodium bicarbonate
- Filter the sample or centrifuge
- Dilute 20 ml of filtrate with 20 ml of PBS, adjust to pH 7.4 and filter through GMF paper
- Pass 10 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 20 ml of 0.01 % Tween 20 in PBS
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of water
- Inject 100 µl

For mustard seeds

- Weigh 5 g of sample
- Add 200 ml of 60 % methanol and blend
- Filter the sample or centrifuge
- Dilute 20 ml of filtrate with 20 ml of PBS, adjust to pH 7.4 and filter through GMF paper
- Pass 10 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 20 ml of 0.01 % Tween 20 in PBS
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of water
- Inject 100 µl

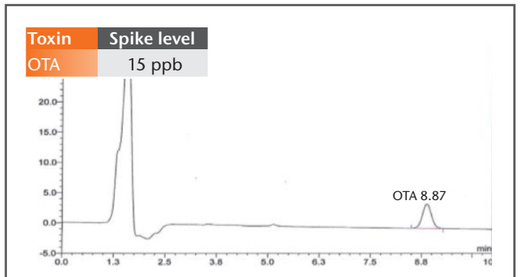
For black and white pepper

- Weigh 12.5 g of sample
- Add 100 ml of 60 % methanol : 3 % sodium bicarbonate (50:50 v/v) and blend
- Filter the sample through a glass microfibre filter paper twice
- Dilute 16 ml of filtrate with 200 ml of PBS and 4 ml of 2 % Tween 20 in water
- Pass 10 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 1 ml of 2 % in Tween 20 in water followed by 10 ml of water
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of acetic acid
- Inject 100 µl

For herbs and herbal tea

- Weigh 5 g of sample
- Add 200 ml of 1 % sodium bicarbonate
- Filter the sample or centrifuge
- Dilute 20 ml of filtrate with 20 ml of PBS, adjust to pH 7.4 and filter through GMF paper
- Pass 10 ml of filtrate (equivalent to 0.125 g of sample) through the column
- Wash with 20 ml of 0.01 % Tween 20 in PBS
- Elute with 1.5 ml of 100 % methanol followed by 1.5 ml of water
- Inject 100 µl

Toxin	Spike level
OTA	15 ppb



Mustard analysis using OCHRARHONE® WIDE

Results obtained with OCHRARHONE® WIDE, spiked at 15 ppb (n=3)

	% recovery (% RSD)
Paprika	80.4 (1.9)
Cumin	89.4 (18.3)
Curry powder	77.4 (0.7)
Mustard seeds	99.4 (2.7)
Mustard flour	78.1 (6.1)
White pepper	71.7 (12.3)
Black pepper	78.1 (1.6)
Ground ginger	81.4 (10.6)
Root ginger	88.3 (4.3)
Cinnamon	78.3 (6.2)
Oregano	85.4 (1.8)
Lemongrass	109.0 (0.6)
Coriander	85.5 (2.9)
Black tea	97.1 (1.5)
Rosehip & hibiscus tea	78.3 (6.2)

Immunoaffinity columns

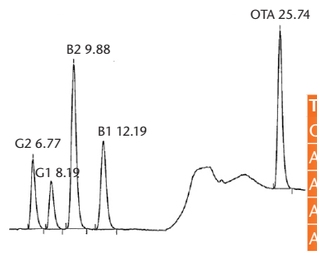
Multi-toxin analysis

Legislation exists for both aflatoxin and ochratoxin in spices therefore, multi-mycotoxin columns present an opportunity to manage increasing demands while still maintaining quality results.

As more and more samples are having to be analysed for more than one mycotoxin, it makes sense to try and harmonise methods reducing time spent on extractions and ultimately the cost of consumables.

For all spices

- Weigh 25 g of sample and 5 g of sodium chloride
- Add 100 ml of 80 % methanol and blend at high speed
- Filter the sample or centrifuge
- Dilute 4 ml of filtrate with 36 ml of10 % Tween 20 in PBS, adjust to pH 7.4 and filter through GMF paper
- Pass 10 ml of filtrate (equivalent to 0.25 g of sample) through the column
- Wash with 20 ml of PBS
- Elute with 1 ml of 100 % methanol followed by 1 ml of water
- Inject 100 µl



Toxin	Spike level
OTA	15 ppb
AFT B1	2.5 ppb
AFT B2	2.5 ppb
AFT G1	2.5 ppb
AFT G2	2.5 ppb

Paprika analysis using AFLAOCHRA PREP®

Results obtained with AFLAOCHRA PREP®, spiked at 10 ppb for total AFT and 15 ppb OTA (n=3)

	% recovery (% RSD)					
	AFT B1	AFT B2	AFT G1	AFT G2	Total AFT	OTA
Paprika	82.8 (4.6)	86.8 (3.0)	78.3 (3.1)	82.9 (1.8)	82.7 (3.1)	86.0 (2.3)
Cumin	80.6 (0.9)	95.5 (1.8)	76.0 (2.0)	84.3 (2.0)	81.6 (1.6)	97.3 (2.8)
Curry powder	76.0 (1.2)	86.9 (0.5)	74.0 (0.8)	81.2 (1.0)	79.5 (0.2)	94.2 (1.8)
Mustard seeds	96.2 (4.9)	101.8 (5.1)	88.4 (2.6)	95.2 (2.5)	95.4 (3.8)	94.5 (5.2)
Mustard flour	96.0 (1.6)	99.0 (2.7)	86.2 (2.6)	91.6 (2.3)	93.2 (2.1)	97.5 (3.4)

The future is automation

Numerous high-throughput food and feed companies are moving towards automated solutions to improve work flow and increase productivity.

Many global companies are also interested in harmonising and standardising methods within and between different sites to enable improved sharing and transfer of methods and data in an effort to increase efficiency.

R-Biopharm have developed a range of unique and innovative immunoaffinity cartridges, IMMUNOPREP® ONLINE, for mycotoxins and vitamins, which are automated via the CHRONECT Symbiosis RIDA®CREST system.

The system is suitable for connection to fluorescence, UF or MS detectors.

Market factors influencing change to automation

- Time pressure on laboratories is increasing
- Demands to increase throughput
- Demands to decrease overheads
- Staff costs are increasing
- Improve quality of results to meet ISO 17025 requirements
- Increased regulations and safety standards
- Standardisation and harmonise methods between sites
- Bring testing in-house

automation presents an opportunity to manage these factors

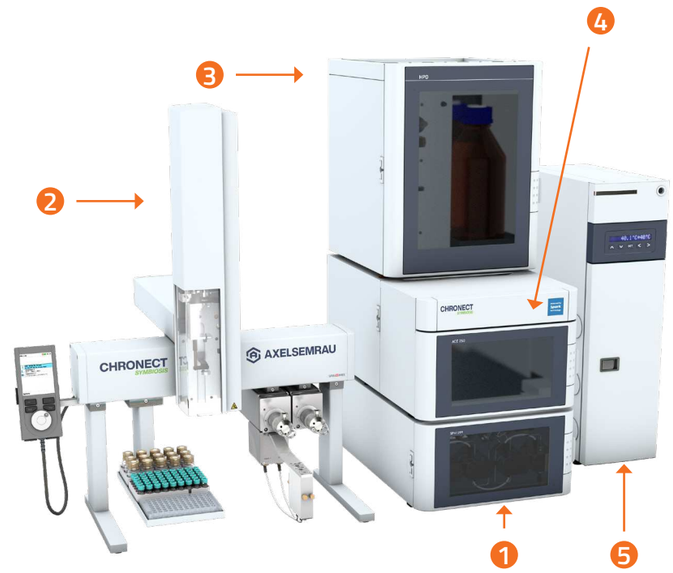
Automated immunoaffinity clean-up

The equipment: CHRONECT Symbiosis RIDA®CREST

- The IMMUNOPREP® ONLINE cartridges are used in combination with the CHRONECT Symbiosis RIDA®CREST system
- CHRONECT Symbiosis RIDA®CREST is a UHPLC system for connection to the users own fluorescence, UV or MS detector
- The CHRONECT Symbiosis RIDA®CREST system contains two modules (ACE and HPD) for handling and processing the cartridges
- Flexibility to use as a general UHPLC for other analytes and for analysis of eluates following off-line immunoaffinity column clean-up

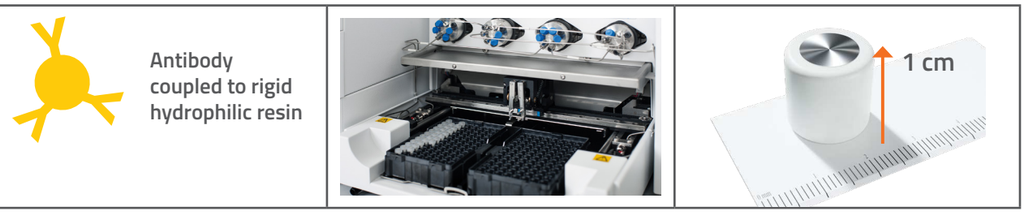
Module	Specification
1. Gradient pump	SPH1299 UHPLC pump
2. Autosampler	CTC-PAL LSI
3. HPD (High Pressure Dispenser)	1x syringe, 1x valve, 2x SSH for mixing
4. ACE (Automated Cartridge Exchanger)	Dual clamp, 4x valve (UHPLC)
5. Column oven	Mistral cool (5 - 90 °C)
Software	CHRONOS

1. Pump
2. Autosampler
3. HPD
4. ACE
5. Column oven



IMMUNOPREP® ONLINE description

- The monoclonal antibody is bound to a rigid hydrophilic polymer, which is packed into the small cartridge
- Automation and use of the IMMUNOPREP® ONLINE cartridge is performed using the CHRONECT Symbiosis RIDA®CREST system



The disposable: IMMUNOPREP® ONLINE cartridges

- A patented technology
- Monoclonal antibody bound to a rigid polymer
- Can withstand high pressures of HPLC or LC-MS/MS
- Cartridges are processed and samples are analysed directly online
- Combines benefits of immunaoffinity clean-up with automation

Re-usability of IMMUNOPREP® ONLINE cartridges	Multiple sample injections	Quality Control each cartridge
15 injections	15 samples	▪ Possible to QC each cartridge using a standard, blank or reference sample ▪ Emergency samples can be added mid-run

Improved reproducibility
- High precision
- Automation removes variability

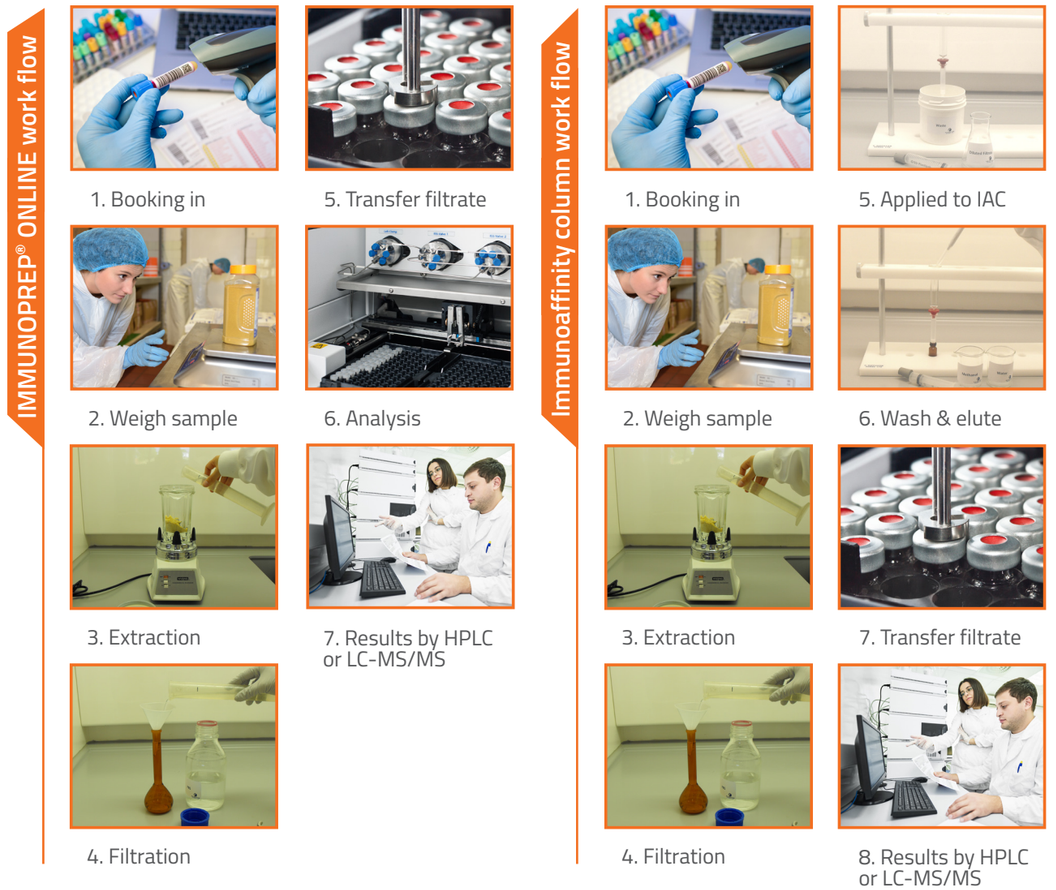
Improved performance
- Pre-programmed, optimised and validated methods

Improved productivity
- Reduction in bench time

Robust methods
- Confidence in results first time around

Improved quality
- Standard or blank can be loaded between sets

Comparision of immunoaffinity column and cartridge clean-up



Clean-up with IMMUNOPREP® ONLINE cartridges provides -

Benefits of immunoaffinity clean-up
- High selectivity
- Concentration of analyte from sample matrix
- Removal of interfering components

Combined with benefits of automation
- Time and labour savings throughout process
- Increased throughput
- Improved traceability and reproducibility
- Improved Quality Control

r-biotube®

Results:
IMMUNOPREP® ONLINE AFLATOXIN

Sample extraction method
- Weigh 25 g of sample and 5 g of sodium chloride

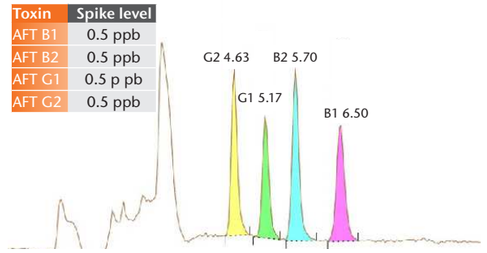
For individual spices:
- Add 100 ml of acetonitrile : methanol : water (40:35:25 v/v/v) and shake for 30 minutes

For mixed spices:
- Add 100 ml of 84 % methanol and shake for 30 minutes
- Filter through GF/A

For individual spices:
- Dilute 0.5 ml of filtrate with 9.5 ml of dilution buffer 1

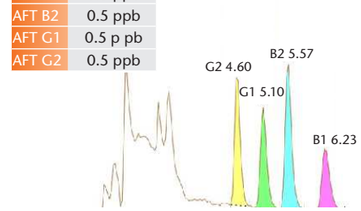
For mixed spices:
- Dilute 0.5 ml of filtrate with 9.5 ml of dilution buffer 2
- Inject 1 ml onto the CHRONECT Symbiosis RIDA®CREST system

Toxin	Spike level
AFT B1	0.5 ppb
AFT B2	0.5 ppb
AFT G1	0.5 p pb
AFT G2	0.5 ppb



Turmeric analysis using IMMUNOPREP® ONLINE AFLATOXIN

Toxin	Spike level
AFT B1	0.5 ppb
AFT B2	0.5 ppb
AFT G1	0.5 p pb
AFT G2	0.5 ppb



Black pepper analysis using IMMUNOPREP® ONLINE AFLATOXIN

Results obtained with IMMUNOPREP® ONLINE AFLATOXIN (n=3)

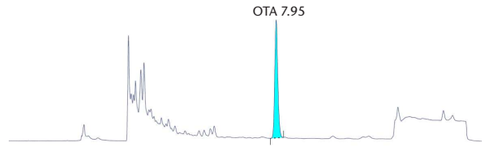
* Not reported due to high natural contamination in sample

	Spike level (ppb)	% recovery (% RSD)			
		AFT B1	AFT B2	AFT G1	AFT G2
Cumin	2	87.8 (2.1)	89.4 (1.3)	91.0 (2.5)	95.1 (0.8)
	10	87.3 (1.0)	87.3 (0.5)	89.2 (0.3)	90.8 (1.3)
Chilli	2	NR*	91.1 (1.2)	92.8 (1.1)	94.7 (1.8)
	10	104.0 (0.1)	83.7 (0.2	84.9 (0.3)	84.1 (0.8)
Coriander	2	97.1 (1.0)	93.5 (0.9	94.3 (1.4)	95.7 (2.0)
	10	87.5 (0.7)	87.2 (0.8)	88.1 (0.6)	89.3 (0.9)
Turmeric	2	83.1 (3.7)	87.7 (2.0)	90.6 (1.2)	92.6 (1.1)
	10	79.1 (0.9)	81.0 (0.1)	82.1 (0.2)	87.2 (0.8)
Ginger	2	104.5 (2.0)	90.4 (0.6)	92.6 (0.8)	92.3 (1.1)
	10	96.0 (1.8)	93.2 (1.6)	90.7 (1.4)	89.7 (2.8)
Black pepper	2	82.6 (3.3)	82.4 (3.9)	78.6 (1.3)	76.7 (0.7)
	10	90.6 (1.2)	90.6 (1.2)	86.6 (0.6)	86.1 (0.7)
Mixed spice	2	112.1 (7.5)	117.1 (6.3)	117.4 (3.8)	98.0 (0.7)
	10	84.9 (0.7)	78.0 (0.9)	81.1 (2.2)	78.0 (0.0)
Garam masala	0.5	81.1 (3.3)	83.0 (1.8)	71.8 (3.7)	69.9 (3.2)
	2.5	80.2 (2.1)	81.5 (1.1)	64.8 (2.6)	70.0 (2.2)

Results:
IMMUNOPREP® ONLINE OCHRATOXIN

Sample extraction method
- Weigh 5 g of sample
- Add 20 ml of acetonitrile : methanol (60:40 v/v) and shake for 60 minutes
- Centrifuge at 4,000 rpm for 10 minutes
- Filter through glass micro filter paper
- Measure 9 ml and 1 & sodium bicarbonate into a suitable container and add 1 ml of filtrate

For spices & liquorice:
- Pass 2 ml of filtrate through two nylon 0.2 µm syringe filters in tandem and collect in amber autosampler vial
- Dilute 0.7 ml of sample filtrate with 0.7 ml of 3 % Tween 20 in water in an amber autosampler vial
- Inject 1 ml onto the CHRONECT Symbiosis RIDA®CREST system



Liquorice analysis using IMMUNOPREP® ONLINE OCHRATOXIN

Results obtained with IMMUNOPREP® ONLINE OCHRATOXIN (n=3)

	Spike level (ppb)	% recovery (% RSD)
Black pepper	7.5	87.3 (1.1)
	15	79.9 (0.7)
	30	90.3 (3.4)
Turmeric	7.5	90.7 (5.2)
	15	88.8 (3.4)
	30	98.2 (1.5)
Mixed spice	7.5	80.5 (1.0)
	15	78.0 (0.9)
	30	89.3 (2.4)
Liquorice	7.5	77.3 (0.9)
	15	84.2 (7.4)
	30	98.0 (2.5)

CHRONECT Symbiosis RIDA®CREST with IMMUNOPREP® ONLINE

Combined benefits

- Re-usable immunoaffinity cartridges
- Pre-programmed applications for a variety of analytes
- Automated cartridge clean-up and concentration of analyte, suitable for testing complex matrices
- Flexible equipment allows for automated direct injection when required
- An automated platform for a growing number of analytes

Improved laboratory work flow

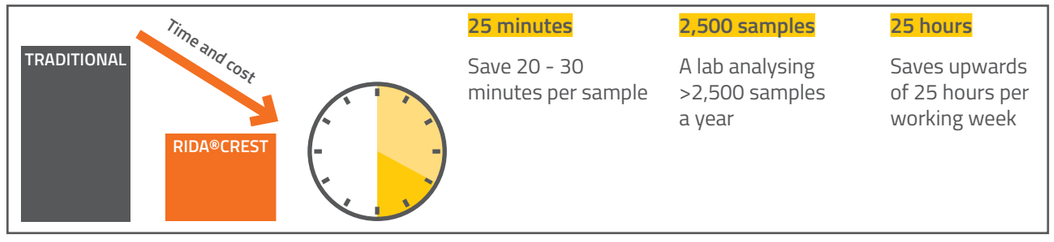
- Two cartridges are run simultaneously, reducing analytical time and increasing throughput
- Automation of the cartridge application online reduces handling time at the bench
- Flexible programs allow emergency samples to be added mid-run

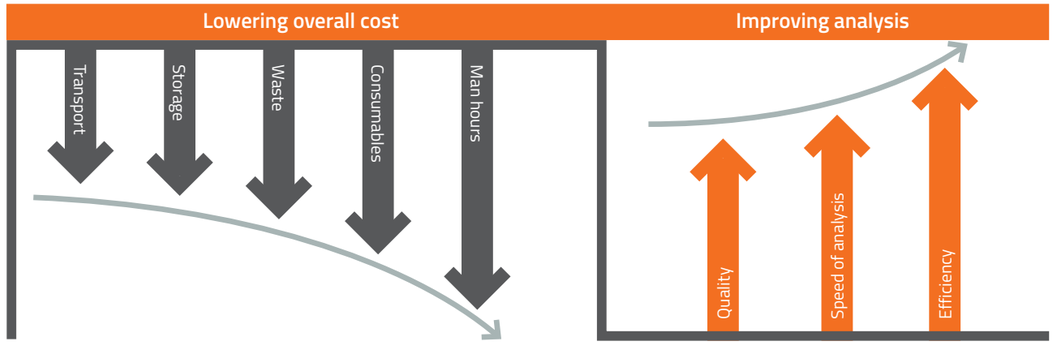
Enhanced quality performance

- Unique, automated device that can include online quality control
- Reproducible and accurate results used in combination with your chosen detector
- Monoclonal antibodies improve selectivity and sensitivity resulting in better limits of detection and quantification
- Exceeds CEN and AOAC Method Performance Criteria for mycotoxins
- Ensuring the best results are achieved first time

Eco-friendly

- Less solvents used and discarded to waste
- A re-usable product means less storage and transport costs
- A mini cartridge means less plastic and waste





Derivatisation using a KOBRA® CELL

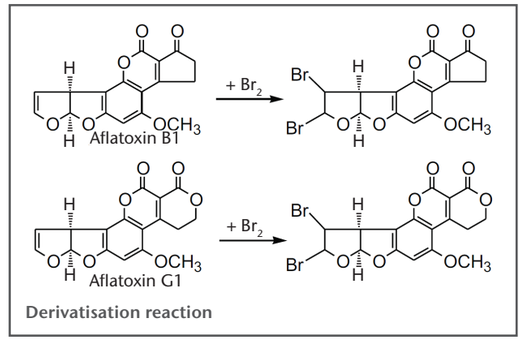
Confirmation of the presence of aflatoxins in a sample by HPLC requires derivatisation of aflatoxins B1 and G1.

Derivatisation is a technique used to transform a chemical compound into a product of a similar chemical structure. A specific functional group of the compound is involved and transforms the parent compound to an alternative derivative of a different chemical composition. The resulting new chemical properties can then be used for quantification or separation. During derivatisation the chemical structures of aflatoxin B1 and G1 are changed to a more fluorescent form, increasing the fluorescent signal in each case for detection by HPLC.

The KOBRA® CELL is an electrochemical cell connected to an HPLC system downstream from the HPLC column and in line with the column effluent and the fluorescence detector. The KOBRA® CELL generates a reactive form of bromine for derivatisation of aflatoxins B1 and G1, resulting in enhanced fluorescence and thus more sensitive detection.

The aflatoxins and the mobile phase enter the KOBRA® CELL and the electrochemical reaction occurs generating the reactive form of bromine. The reaction between the reactive bromine and the aflatoxins must take place before the derivatised aflatoxins enter the fluorescence detector. Hence the length of the reaction coil post KOBRA® CELL is critical. A minimum reaction time of 4 seconds is required.

The KOBRA® CELL is expected to amplify the signal of aflatoxin G1 and B1 by approximately 37 and 21 times respectively.



Derivatisation reaction

Mycotoxins: a potential hazard

A complete range of analytical options

Product	Description	No. of tests/amount	Art. No.:
	Test cards		
AFLACARD	Qualitative detection of aflatoxin B1 at various screening levels	20 determinations	RBRP27
AFLACARD TOTAL	Qualitative detection of total aflatoxins at various screening levels	20 determinations	RBRP38
OCHRACARD	Qualitative detection of ochratoxin A at various screening levels	20 determinations + 20 immunoaffinity columns	RBRP48
	Single toxin immunoaffinity columns		
AFLAPREP®	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	10 columns (1 ml format) 50 columns (1 ml format) 500 columns (3ml format)	RBRDP07 RBRP07 RBRP07/500
EASI-EXTRACT® AFLATOXIN	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	10 columns (3 ml format) 50 columns (3 ml format) 500 columns (3ml format)	RBRRP71 RBRRP70N RBRRP70N/500
AFLARHONE®	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	25 columns (1 ml format) 100 columns (1 ml format)	RBRP56/25 RBRP56/100
AFLARHONE® WIDE	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	25 columns (3 ml format) 100 columns (3 ml format) 500 columns (3ml format)	RBRP116/25 RBRP116/100 RBRP116/500
OCHRAPREP®	Immunoaffinity columns for the sample clean-up prior to the analysis of ochratoxin A using HPLC or LC-MS/MS	10 columns (3 ml format) 50 columns (3 ml format) 500 columns (3ml format)	RBRP14 RBRP14B RBRP14/500
OCHRARHONE®	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	25 columns (1 ml format) 100 columns (1 ml format)	RBRP59/25 RBRP59/100
OCHRARHONE® WIDE	Immunoaffinity columns for the sample clean-up prior to the analysis of aflatoxin B1, B2, G1 and G2 using HPLC or LC-MS/MS	25 columns (3 ml format) 100 columns (3 ml format)	RBRP119/25 RBRP119/100
	Multi-toxin		
AFLAOCHRA PREP®	Immunoaffinity columns for the sample clean-up prior to the analysis of total aflatoxins and ochratoxin A using HPLC or LC-MS/MS	10 columns (1 ml format) 50 columns (1 ml format)	RBRP89 RBRP89B
	Immunoaffinity cartridges		
IMMUNOPREP® ONLINE AFLATOXIN	Online immunoaffinity cartridges used in conjunction with the CHRONECT Symbiosis RIDA®CREST handling system for the automated clean-up and analysis of aflatoxins B1, B2, G1 and G2 with HPLC	48 cartridges 96 cartridges	RBRP900/48 RBRP900
IMMUNOPREP® ONLINE OCHRATOXIN	Online immunoaffinity cartridges used in conjunction with the CHRONECT Symbiosis RIDA®CREST handling system for the automated clean-up and analysis of ochratoxin A with HPLC	48 cartridges 96 cartridges	RBRP901/48 RBRP901

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