Tailored mycotoxin testing for your needs

- From on-site testing to automated high throughput testing
- LFD, ELISA, SPE, IAC, reusable cartridges
- Single or multi-toxin testing options
- Global provider for test kits and compatible equipment
- Certified reference material, standards and control material

https://r-b.io/mycotoxins

Challenges in mycotoxin analysis

Mycotoxins are an important global food safety issue. They are stable during processing and are therefore found in raw materials as well as in processed food and feed. In many countries mycotoxins are regulated with stringent limit values.

Regular mycotoxin screening is the foundation of protecting human and animal health. At each level in the food chain analytical testing helps to understand how to manage risks.

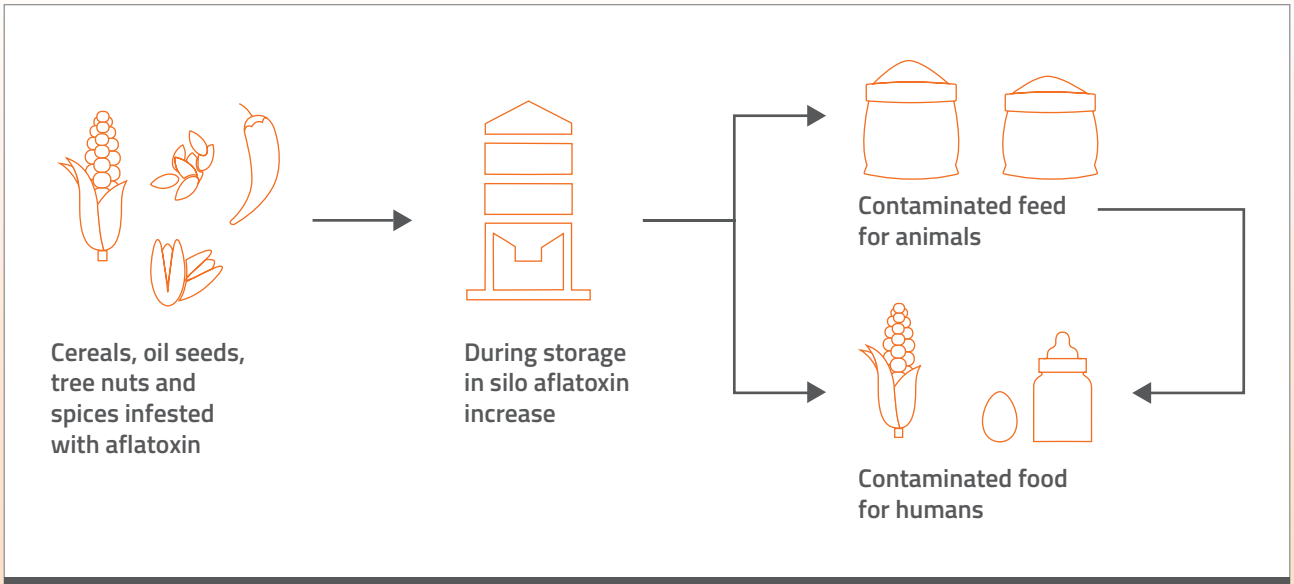


Figure 1: Aflatoxin in the food chain are a risk to human and animal health

Mycotoxin methods used depending on lab size

In today's technologically fast-moving world, lateral flow tests, ELISA, HPLC and LC/MS-MS are the most common testing methods. Different analytical approaches are used for the detection of individual or mixtures of mycotoxins depending on the lab equipment available (see figure next page).

Lateral flow tests in combination with the RIDA®SMART APP are very fast and easy for use in the field or testing of raw materials.

ELISA test kits are the ideal solution for a parallel measurement of multiple samples with incubation times of as low as 15 minutes for up to 42 samples.

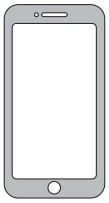
The main advantages of ELISA are that they are fast, inexpensive and giving reliable results. Automation of ELISA is possible using the e.g. Bolt™ or ThunderBolt®.

Reference methods for the quantitative and qualitative determination of mycotoxins are basically chromatographic systems such as HPLC and LC-MS/MS. However, these tests are time consuming, expensive and highly skilled technicians are needed to carry out the analysis.

With more than 30 years of experience in mycotoxin detection, the R-Biopharm Group provides the broadest range of mycotoxin testing solutions for the whole production process.

„Small“ laboratories

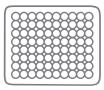
RIDA®QUICK lateral flow devices



Smartphone
- Google Pixel
- RIDA®SMART APP

Accessories
- Trilogy® Quality Control Material

„Midsize“ laboratories



- RIDASCREEN®FAST ELISA
- RIDASCREEN® ELISA

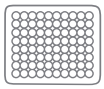
MTP reader
- RIDA®ABSORBANCE 96

- PuriTox solid phase columns
- PREP® immunoaffinity columns
- EASI-EXTRACT® immunoaffinity columns

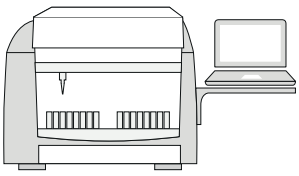
HPLC

Accessories
- Certified Trilogy® Reference Material
- Certified Trilogy® Liquid Standards
- Trilogy® Quality Control Material
- Trilogy® Dried/Liquid Analytical Standards

„Large“ laboratories

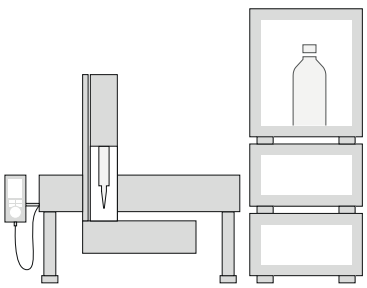


RIDASCREEN® ELISA



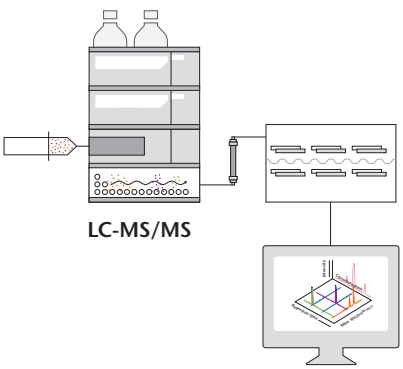
ELISA automate
- Bolt™/ThunderBolt®

IMMUNOPREP® ONLINE cartridges



HPLC automate
- RIDA®CREST

- PuriTox solid phase columns
- PREP® immunoaffinity columns
- EASI-EXTRACT® immunoaffinity columns



LC-MS/MS

Accessories
- Certified Trilogy® Reference Material
- Certified Trilogy® Liquid Standards
- Trilogy® Quality Control Material
- Trilogy® Dried/Liquid Analytical Standards

Mycotoxin test kits

The test systems can be used for screening purposes or to quantitivately detect all legislated mycotoxins either separately or in group.

R-Biopharm's products are a valuable tool to check if your food and feed comply with legislation.

RIDA®QUICK

Smartphone application for the quantitative analysis of lateral flow tests (RIDA®QUICK).

RIDA®QUICK lateral flow tests are immunochromatographic tests for the quantitative determination of mycotoxins with the innovative RIDA®SMART APP software.

RIDASCREEN®

Enzyme immunoassays (ELISAs) use the high specificity of antigen and antibody interaction to determine and quantify mycotoxins by photometric measurement.

PREP®, EASI-EXTRACT®, RIDA®

Use the high specificity of antigen and antibody interaction to isolate, purify and concentrate mycotoxins from many complex matrices prior to ELISA or chromatographic analysis.

Equipment for mycotoxin analysis

R-Biopharm develop matching applications for an even easier, faster and more efficient performance and analysis. ELISAs can be read with the innovative RIDA®Absorbance 96 or by a fully automated analyzer like the ThunderBolt®. The RIDA®CREST or RIDA®CREST ICE system enables the use of the IMMUNOPREP® ONLINE cartridges to be incorporated directly with HPLC, UHPLC or LC-MS/MS systems.
Everything for your analysis and performance just from one supplier.

RIDA®ABSORBANCE 96

Innovative microtiter plate photometer including RIDASOFT® Win.NET software.

ThunderBolt®

Fully automated device for ELISA analysis in microtiter plate format.

Automation of ELISA is recommended with the 1-microtiter plate analyser Bolt™ or the 2-microtiter plate analyser ThunderBolt®.

RIDA®CREST

IMMUNOPREP® ONLINE immunoaffinity cartridges are used together with the CHRONECT Symbiosis RIDA®CREST handling system to combine automated online sample preparation with quantitative analysis of the mycotoxin of interest.

Choose the best product for your workflow

Each analytical method for mycotoxin quantification offers its own set of advantages and drawbacks. In practice, a variety of methods for different purposes are used in order to balance between costs, sensitivity and ease of use.

Worldwide, many customers – including governmental and contract laboratories use R-Biopharm's efficient test kits, equipment and service.

Mill with small in-house lab

"We carry out approximately 10 mycotoxin analyses per week. We use lateral flow to check incoming goods directly on the lorries.
The time to result including sampling and sample preparation takes 20 min.

We use an aqueous extraction so there are no hazardous chemicals involved.
The reason why we use control material is that it gives us the assurance that our results can be trusted. By utilizing naturally contaminated control samples from Trilogy, we know our controls are exactly representative of the samples we test."

Equipment

Smartphone-based evaluation:

- RIDA®SMART APP – Mycotoxin Analyser SET (Art. No. ZRSAM1000-SET)

Mycotoxin lateral flow

- RIDA®QUICK DON RQS ECO (Art. No. R5911)

Mycotoxin quality control material

- Trilogy® QC Material Deoxynivalenol (DON) (Art. No. TQC-D100)

Large mill with in-house lab

"Working for a large mill means making rapid decisions for our logistic workflow in the warehouse. Sending cereal samples to an external lab and obtaining test results takes 5 days which is really too long for me! We have now set up our own in-house lab analyzing 1200 samples per year for DON, fumonisin and zearalenone.

We use mycotoxin lateral flows to check incoming goods directly on the lorries.
For internal quality control, we use mycotoxin and allergen FAST ELISA, the test procedure takes only 30 minutes. The RIDA® Absorbance 96 photometer is extremely fast and the reading takes only seconds. Our quality control material has been tailor made by Trilogy® based on our concentration requirements.
Some positive samples are confirmed by IAC/HPLC by an external lab.
We are currently setting up PCR GMO multiplex screening for 35S, NOS and FMV at the same time. For test kits and equipment, we have only one supplier, which is R-Biopharm. The test kits are well adapted for the equipment used and we are very satisfied with the technical support. We are currently thinking about automation of the ELISA with the Bolt™, a 1-microiter plate analyser."

Equipment

Smartphone-based evaluation:

- RIDA®SMART APP – Mycotoxin Analyser SET (Art. No. ZRSAM1000-SET)

ELISA microtiter plate reader:

- RIDA®ABSORBANCE 96 (Art. Nor. ZRA96FF)

qPCR thermocycler:

- RIDA®CYCLER (Art. No. ZRCYCLER)

Mycotoxin lateral flow

- RIDA®QUICK DON RQS ECO (Art. No. R5911)

Mycotoxin ELISA

- RIDASCREEN®FAST DON (Art. No. R5901)
- RIDASCREEN®FAST Fumonisin (Art. No. R5602)
- RIDASCREEN®FAST Zearalenon (Art. No. R5502)

Mycotoxin quality control material

- Trilogy® QC Material Multitoxin for Deoxynivalenol, Fumonisin and Zearalenone (Art. No. TQC-CUST)

Allergen ELISA

- RIDASCREEN®FAST Gliadin (Art. No. R7002)
- RIDASCREEN®FAST Soya (Art. No. R7102)

GMO PCR multiplex screening

- SureFood® GMO SCREEN 4plex 35S/NOS/FMV (Art. No. S2126)

Poultry farming – feed testing (simple matrix)

"The quality of the feed is a decisive factor in the development and health of poultry. We use certified farmers who deliver the feed to our own storehouses.
In our in-house lab, we use mycotoxin ELISA to test our feed consisting of raw materials and composite mixtures. 4000 samples for mycotoxins are tested per year. We use the FAST and the 'normal' ELISA depending on the limit of the quantification of the test kit; using ELISA, we have the results for 10 samples within 3 hours from our in-house lab. Next year, we plan to automate the ELISA lab work using the ThunderBolt®, a 2-microtiter plate analyzer. Some samples are also sent to an external lab using ELISA and HPLC for confirmation of results.
We use natural contaminated control material, single toxin and multi-toxin, from Trilogy. We were astonished that Trilogy® offers feed control material off the shelf; this way we ensure quality assurance. We prefer using natural contaminated over spiked control material, as it is a more realistic approach. Trilogy made our life easier in producing QC material based on our own feed, they formulated it to our requested concentration levels."

Equipment

ELISA microtiter plate reader

Mycotoxin ELISA

- RIDASCREEN®FAST DON (Art. No. R5901)
- RIDASCREEN® Ochratoxin A30/15 (Art. No. R1311)
- RIDASCREEN® Zearalenon (Art. No. R1401)
- RIDASCREEN® Aflatoxin B1 30/15 (Art. No. R1211)

Mycotoxin quality control material

- Trilogy® QC Material Deoxynivalenol (DON) (Art. No. TQC-D100)
- Trilogy® QC Material Multitoxin poultry feed (Art. No. TQC-TM100)

Feed customer (complex matrix)

"Brand reputation is important, and we pride ourselves on the quality of our results. In our well-equipped in-house lab, we use a mixture of HPLC and LC-MS/MS to conduct our mycotoxin analysis. At present, we currently analyze over 10,000 mycotoxin samples per year.

We use a mixture of the single and multi-toxin columns as these give us the flexibility to choose the toxin combinations of choice to suit our specific requirements. In general, we use the single toxin columns prior to HPLC detection, and we use the multi-toxin columns, occasionally in tandem, prior to LC-MS/MS detection.

We use the KOBRA® CELL to derivatise aflatoxins B1 and G1 followed by HPLC detection. Using this electrochemical cell ensures that we have best sensitivity and we comply with CEN and AOAC methods.

We use natural contaminated control material, single toxin and multi-toxin, from Trilogy. When required we can purchase specific, large batches of material for use in all of our global laboratories. In order to improve our general workflow going forward we are considering switching all analysis to multi-toxin columns, in particular using the 11+Myco MS-PREP® columns. We have been working closely with R-Biopharm for many years and through their consultative approach, we are confident that they are able to offer solutions that meet with our specific requirements."

Equipment

LC-MS/MS

HPLC including KOBRA® CELL (Art. No. RBRK01)

Mycotoxin immunoaffinity columns

- EASI-EXTRACT® AFLATOXIN (Art. No. RBRP70N)
- EASI-EXTRACT® ZEARALENONE (Art. No. RBRP90)
- OCHRAPREP® (Art. No. RBRP14B)
- DONPREP® (Art. No. RBRP50B)
- FUMONIPREP® (Art. No. RBRP31B)
- EASI-EXTRAC®T T-2 & HT-2 (Art. No. RBRP43B)
- AOF MS-PREP®(Art. No. RBRP115B)
- DZT MS-PREP® (Art. No. RBRP73B)

Mycotoxin quality control material

- Trilogy® QC Material Multi-toxin (Art. No. TQC-MT100)
- Trilogy® QC Material Complex Commodities Multi-toxin (Art. No. TQC-CC100)

State lab/federal lab

"Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. Special emphasis must be drawn to mycotoxin contamination of baby foods and infant formulas as babies and small children are the most susceptible population to the effects of these toxins. In our state lab, we test predominantly cereals for DON (3000 samples/year) and zearalenone (500 samples/year). We carry out screening with ELISA because many samples can be analyzed in little time. Due to the huge workload we use the ELISA automate ThunderBolt®. The changeover to automate was easy because we already used the normal ELISA with longer incubation times, which are suitable for automation. After screening with ELISA, positive samples are then confirmed by HPLC using immunoaffinity columns.

Quality assurance is our key priority this is why we use Trilogy® certified naturally contaminated reference materials. It allows a more realistic quality control than spiked samples, because mycotoxin extraction from spiked samples represent a rather artificial recovery compared to the extraction of samples naturally contaminated with mycotoxins. No other company offers a certified reference material for e.g. fumonisin that is naturally contaminated. By using certified using liquid standards methods can be validated to meet all of the ISO17025 requirements. Standards are available in fully constituted form, or in a dried “insitu” form. Liquid standards can be used as received. The dried standards can be stored for a slightly longer time and reconstituted as needed."

Equipment

2-microtiter plate automate:

- ThunderBolt® (Art. No. ZTB)

HPLC

Mycotoxin ELISA

- RIDASCREEN® Zearalenone (Art. No. R1401),
- RIDASCREEN® DON (Art. No. R5906),
- RIDASCREEN® Fumonisin (Art. No. R3401)

Mycotoxin Immunoaffinity columns for HPLC

- EASI-EXTRACT® ZEARALENONE (Art. No. RBRRP90)
- DONPREP® (Art. No. RBRP50B)
- FUMONIPREP® (50) (Art. No. RBRP31B)
- OCHRAPREP® (Art. No. RBRP14B)
- AFLAPREP (Art. No. RBRP07)
- EASI-EXTRACT® T2 & HT-2 (Art. No. RBRP 43B)

Mycotoxin certified reference material

- Certified Trilogy® Reference Material Zearalenonee (Art. No. CTRM-MT100)
- Certified Trilogy® Reference Material DON (Art. No. CTRM-D100)
- Certified Trilogy® Reference Material Fumonisin (Art. No. CTRM-F100)
- Certified Trilogy®Liquid Standards Zearalenone (Art. No. CTSL-422-5)
- Certified Trilogy®Liquid Standards Deoxynivalenol (Art. No. CTSL-383-5)
- Trilogy®Liquid Standard Fumonisin B1, B2 (Art. No. TSL-202-2)
- Trilogy® Quality Control Material

Retail for food supermarket

"Consumers in grocery stores want to know their food is safe. Therefore, we test in our in-house lab for mycotoxins and allergens.
Food is a complex matrix. Usually, higher-resolution MS instruments are impractical for routine testing or surveillance. However, using the 11+Myco MS-PREP® immunoaffinity column from R-Biopharm we have reduced time for sample preparation and still have great sensitivity: 11 mycotoxins in one run!
Depending on the food we screen for single or multi toxins. Some food e.g. cereals are only screened for DON and zearalenone to save time we determine them in tandem.
The major challenge is the sample preparation to reduce "matrix interference". The technical support we received was fantastic; we are now using these validated and efficient solutions for your mycotoxin screening.
For day to day use we employ the analytical liquid standards.
Allergens are determined depending on the parameter by PCR or by ELISA. The RIDA®CYCLER is very convenient as temperature templates are already pre-programmed, also the instrument is very small (15 x 15 x 13 cm).
The RIDA® Absorbance Reader is extremely handy, takes very little space (96 x 55 x 154 mm) and has no power supply cables so it can be easily moved"

Equipment

LC-MS/MS

ELISA microtiter plate reader

- RIDA®ABSORBANCE 96 (Art. No. ZRA96FF)

qPCR thermocycler

- RIDA®CYCLER (Art. No. ZRCYCLER)

Mycotoxin immunoaffinity columns for HPLC

- 11+Myco MS-PREP®(Art. No. RBRP128B)
- EASI-EXTRACT® ZEARALENONE (Art. No. RBRRP90)
- EASI-EXTRACT® T-2 &HT-2 (50) (Art. No. RBRP43P)
- DON PREP® (Art. No. RBRP50)

Mycotoxin Trilogy® material

- Certified Trilogy® Reference Material
- Certified Trilogy®Liquid Standards
- Trilogy® Quality Control Material
- Analytical Standard - Trilogy®Liquid Standard
- Analytical Standard - Trilogy®Dried Standard

Mycotoxin certified reference material

- Certified Trilogy® Reference Material Zearalenonee (Art. No. CTRM-MT100)
- Certified Trilogy® Reference Material DON (Art. No. CTRM-D100)
- Certified Trilogy® Reference Material Fumonisin (Art. No. CTRM-F100)
- Certified Trilogy®Liquid Standards Zearalenone (Art. No. CTSL-422-5)
- Certified Trilogy®Liquid Standards Deoxynivalenol (Art. No. CTSL-383-5)
- Trilogy®Liquid Standard Fumonisin B1, B2 (Art. No. TSL-202-2)
- Trilogy® Quality Control Material

Allergen PCR

- SureFood® ALLERGEN Mustard, Sesame, Fish, Crustaceans, Molluscs

Allergen ELISA

- RIDASCREEN®FAST Casein, Milk, Egg, Lysozyme, Gliadin, Soya, Hazelnut, Peanut, Mustard, Lupine, Almond, Cashew, Sesame

Baby food producer

"Babies are vulnerable consumers; therefore, infant formula is the most regulated food in the world. Contaminants like mycotoxins can have high concentration fluctuations in food. To obtain very low limit values for mycotoxins, we use different immunoaffinity columns. Sometimes we use them in tandem to save time especially when we must analyze the sample for a number of toxins. However, our sample numbers for aflatoxin are considerably higher than for the other toxins. In this case, we have implemented the automated system; CHRONECT Symbiosis RIDA®CREST. The system uses the IMMUNOPREP® ONLINE cartridges, which perform very much in the same way as an immunoaffinity column while allowing savings in time and labor therefore increasing our capacity.

Quality assurance is crucial for baby food because it is tested at extremely low levels. This is why it is important to use certified reference material and certified liquid standards. Our materials were produced by Trilogy, a ISO 17034 accredited producer of certified materials. Claims of vitamin labels on baby food need to be confirmed by very accurate and sensitive testing carried out by reliable and accredited laboratories. For this, we use various EASI-EXTRACT® immunoaffinity columns from R-Biopharm and follow the associated AOAC methods. Food allergy is also a critical health issue, because a small amount of a food allergen may cause a fatal reaction. The gold standard for gluten testing is the RIDASCREEN® Gliadin test kit, which is the CODEX Alimentarius method. To ensure milk-free products we use the Casein ELISA."

Equipment

HPLC
LC-MS/MS
HPLC automate:
- CHRONECT Symbiosis RIDA®CREST (Art. No. ZRIDACREST)

ELISA microtiter plate reader

Mycotoxin immunoaffinity columns for HPLC

- AFLAPREP® M (Art. No. RBRDP04)
- DONPREP® (Art. No. RBRP50)
- FUMONIPREP® (Art. No. RBRP31B)
- OCHRAPREP® (Art. No. RBRP14)
- IMMUNOPREP® ONLINE AFLATOXIN (Art. No. RBRP900)

Mycotoxin Trilogy® material

- Certified Trilogy® Reference Material
- Certified Trilogy®Liquid Standards
- Trilogy® Quality Control Material
- Analytical Standard - Trilogy®Liquid Standard
- Analytical Standard - Trilogy®Dried Standard

Vitamin immunoaffinity columns for HPLC

- EASI-EXTRACT® Vitamin B12 (Art. No. RBRP80)
- EASI-EXTRACT® Biotin (Art. No. RBRP82B)
- EASI-EXTRACT® FOLIC ACID (Art. No. RBRP81B)

Vitamin microbiological test kit

- VitaFAST® Pantothenic Acid (Art. No. P1005)

Allergen ELISA

- RIDASCREEN® Gliadin (Art. No. R7001)
- RIDASCREEN®FAST Casein (Art. No. R4612)

Enzymatic test kit

- Roche Lactose/Galactose (Art. No. 10176303035)
- Roche Sucrose/D-Glucose/D-Fructose (Art. No. 10139041035)

Large contract lab

"Due to an increased sample volume for aflatoxins (> 10 mycotoxin samples/day) we have invested in the automated system; CHRONECT Symbiosis RIDA®CREST which is using online immunoaffinity cartridges. The cartridges are reusable (up to 15 times). This gives us a price advantage over other contract labs and our staff is able to carry out other tasks (walk-away). We also use the 11+Myco MS-PREP® multi-toxin columns for samples which require more than just aflatoxin to be analyzed. We find that even if we are just analyzing for 3 of the toxins, these columns still offer many benefits in terms of improvements to our workflow. From our experience it is necessary to use immunoaffinity clean-up to achieve good, reliable and consistent results. This is achieved using either the cartridges or columns as suitable extractions and clean-up methods are offered by both products. In addition, the use of both these products allowed us to manage the increasing demands while still maintaining quality results. We use the Certified Trilogy® Liquid Standards for method validation and instrument calibration purposes.

We switched to the Trilogy certified reference materials because they are naturally contaminated, unlike most competitors offering only spiked certified reference materials. The material has been ground to a fine consistency (30 mesh; 0.595 mm) and thoroughly homogenized to ensure uniform distribution of the analytes. The certificates give very exhaustive information. We also participate in the Trilogy® proficiency testing rounds because natural contaminated material is used.

Equipment

HPLC automate

- CHRONECT Symbiosis RIDA®CREST (Art. No. ZRIDACREST)

LC-MS/MS

Automatic analyzer for RIDA®CUBE

- RIDA®CUBE SCAN340/546 (Art. No. ZRCSO580)

Mycotoxin immunoaffinity cartridges

- IMMUNOPREP® ONLINE AFLATOXIN (Art. No. RBRP900)

Mycotoxin Immunoaffinity columns

- 11+Myco MS-PREP® (Art. No. RBRP128B)

Mycotoxin Trilogy® material

- Certified Trilogy® Reference Material
- Certified Trilogy®Liquid Standards
- Trilogy® Quality Control Material
- Analytical Standard - Trilogy®Liquid Standard
- Analytical Standard - Trilogy®Dried Standard

GMO PCR screening

- SureFood® GMO SCREEN 4plex 35S/NOS/FMV + IAC (Art. No. S2126),
- SureFood® GMO Plant PLUS(Art. No. S2049)

Enzymatic test kit

- Roche Sucrose/D-Glucose/D-Fructose (Art. No. 10139041035)
- Roche Lactose/Galactose (Art. No. 10176303035)
- Roche Maltose/Sucrose/D-Glucose (Art. No. 11113950035)

Quantitative lateral flow test (LFD)

In general, testing of raw ingredients can be done with on-site rapid test. Results are available in minutes and require only a minimal amount of training and equipment. While conventional *LFD* kits use an organic solvent, more environmentally-friendly kits use a water extraction to avoid organic solvent waste.

To remove subjectivity in the evaluation, digital reading of quantitative results is carried out with the RIDA®SMART APP.

It provides consistent interpretation of results. Further advantages of the RIDA®SMART APP are:

- Compact and portable
- Provides an easy interpretation of results
- Test results can be easily exported via WIFI for reporting and tracking
- Provides traceability and data storage

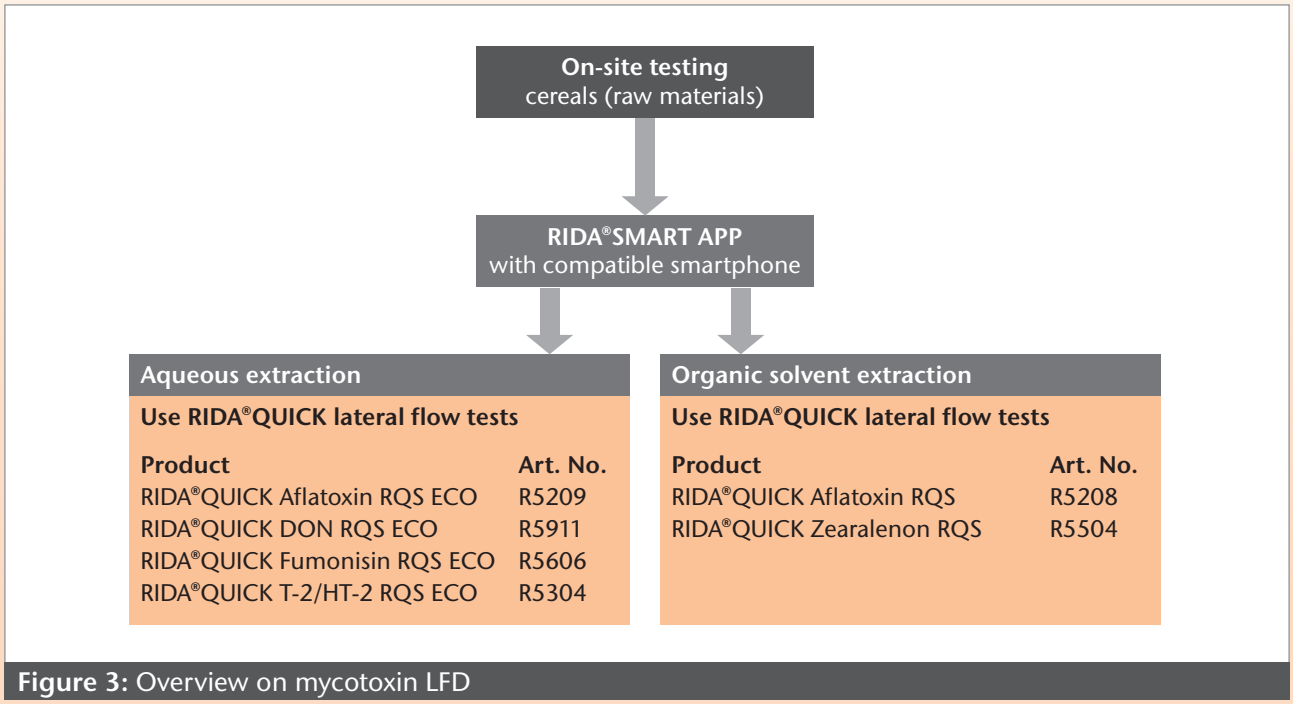


Figure 3: Overview on mycotoxin LFD

ELISA

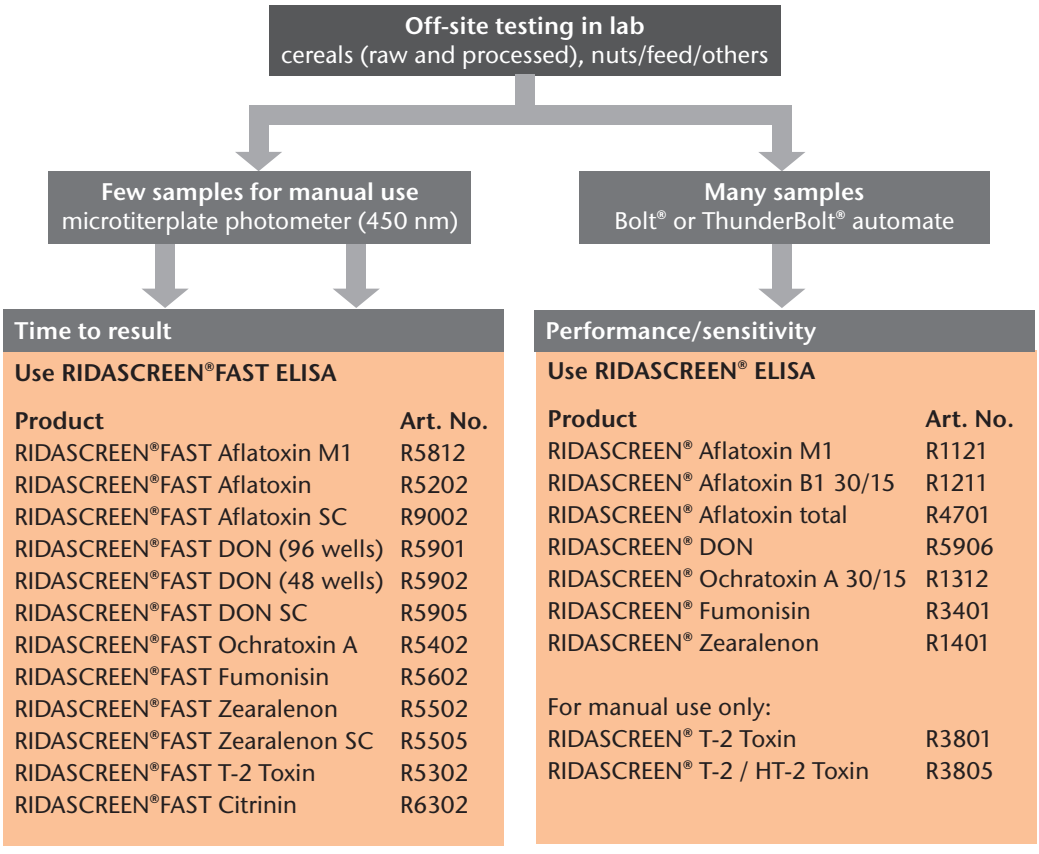
ELISA (enzyme-linked immunosorbent assays) test kits are accurate and easy to use. *ELISA* test kits are the ideal solution for a measurement of multiple samples. R-Biopharm offers two product lines for ELISA:

RIDASCREEN®FAST

Short incubation times of as low as 8 - 15 minutes, for mostly up to 19 samples in single determination in one run.

RIDASCREEN®

Low limit of detection (LOD). With incubation times of e.g. 75 minutes for up to 42 samples in double determination. Due to the longer incubation times these test kits can be automated using the e.g. ThunderBolt®.



Time to result

Use RIDASCREEN®FAST ELISA

Product	Art. No.
RIDASCREEN®FAST Aflatoxin M1	R5812
RIDASCREEN®FAST Aflatoxin	R5202
RIDASCREEN®FAST Aflatoxin SC	R9002
RIDASCREEN®FAST DON (96 wells)	R5901
RIDASCREEN®FAST DON (48 wells)	R5902
RIDASCREEN®FAST DON SC	R5905
RIDASCREEN®FAST Ochratoxin A	R5402
RIDASCREEN®FAST Fumonisin	R5602
RIDASCREEN®FAST Zearalenon	R5502
RIDASCREEN®FAST Zearalenon SC	R5505
RIDASCREEN®FAST T-2 Toxin	R5302
RIDASCREEN®FAST Citrinin	R6302

Performance/sensitivity

Use RIDASCREEN® ELISA

Product	Art. No.
RIDASCREEN® Aflatoxin M1	R1121
RIDASCREEN® Aflatoxin B1 30/15	R1211
RIDASCREEN® Aflatoxin total	R4701
RIDASCREEN® DON	R5906
RIDASCREEN® Ochratoxin A 30/15	R1312
RIDASCREEN® Fumonisin	R3401
RIDASCREEN® Zearalenon	R1401
For manual use only:	
RIDASCREEN® T-2 Toxin	R3801
RIDASCREEN® T-2 / HT-2 Toxin	R3805

Figure 4: Overview on mycotoxin ELISA

The main advantages of ELISA are that it is fast for a high number of samples, low cost for set up and minimal training required. ELISA can be automated using the e.g. ThunderBolt®. Cost effectiveness for automation requires a minimum of 4 to 5 samples tested per ELISA run.
Results are measured with an ELISA plate reader, such as the RIDA® Absorbance 96:

- Innovative LED technology (96 wells are read simultaneously)
- Robust (no moveable parts inside)
- Small instrument (size: 96 x 55 x 154 mm)
- Power through PC connection (no extra power cord)
- Evaluation software RIDASOFT® Win.NET included

HPLC and LC-MS/MS

HPLC and *LCMSMS* are also reference methods if it comes to legal issues. The main advantages of High Performance Liquid Chromatography (HPLC) and LC-MS/MS are their high sensitivity, applicability to complex matrixes and accuracy. However, to obtain top-notch HPLC and LC-MS/MS performance, an optimized sample clean-up using solid phase or immunoaffinity columns are required.

For very high sample throughput, fully automated systems like CHRONECT Symbiosis RIDA®CREST using immunoaffinity cartridges can be used up to 15 times. With LC-MS/MS all regulated mycotoxins are detected as well as most common masked and emerging metabolites. It is also possible to analyse other parameters such as pesticides and veterinary drugs.

Table 1: Overview of different mycotoxins regulated in different food and feed

Commodity	Aflatoxin B1 and/or total	Ochratoxin A	Zearalenone	Fumonisins	Deoxy-nivalenol	T-2 & HT-2	Aflatoxin M1
Nuts and nut products	■						
Peanuts	■						
Dried fruit	■	■					
Spices	■	■					
Cereals	■	■	■		■	■	
Maize	■	■	■	■	■	■	
Baby foods	■	■	■	■	■		■
Animal feed	■	■	■	■	■	■	
Roast coffee		■					
Bread					■		
Pasta			■		■		
Maize oil			■				
Maize breakfast cereals				■	■		
Milk and milk products							■

The number of commodities and mycotoxins that are now covered under EU legislation is increasing. Table 1 provides a simplistic overview of the key mycotoxins regulated in specific food. For nuts, nut products and peanuts only aflatoxins are regulated, so a single mycotoxin IAC or SPE should be selected. Baby foods and animal feeds because they are composite in nature are regulated for all mycotoxins so in this case a multi-mycotoxin approach, e.g. using 11+Myco MS-PREP®, is recommended.

Table 2 gives an overview for the different immunoaffininy and solid phase columns available. More than 10 multi-toxin columns are available. However, single immunoaffinity columns can be connected in *tandem* (coupling of different immunoafffinity columns) to target different groups of mycotoxins, this offers even more flexibility.

Table 2: Product overview for immunoaffinity and solid phase columns

Product	Art No.	Format	Multi-toxin column	For use with HPLC	For use with LC-MS/MS	Cross reacts with modified mycotoxins
Immunoaffinity Columns						
AFLAOCHRA PREP®	P89/P89B	1 mL	✓	✓	✓	✓
AFLAPREP®	P07	1 mL		✓	✓	✓
AFLAPREP® M	P04	1 mL		✓	✓	✓
AFLAPREP® M WIDE	P124/P124B	3 mL		✓	✓	✓
AOF MS-PREP®	P115/P115B	3 mL	✓		✓	✓
AO ZON PREP®	P112/P112B	3 mL	✓	✓	✓	✓
DONPREP®	P50/P50B	3 mL		✓	✓	✓
DZT MS-PREP®	P73/P73B	1 mL	✓		✓	✓
EASI-EXTRACT® AFLATOXIN	RP71/RB70N	3 mL		✓	✓	✓
EASI-EXTRACT® CITRININ	P126/P126B	3 mL		✓	✓	
EASI-EXTRACT® STERIGMATOCYSTIN	P125/P125B	3 mL		✓	✓	
EASI-EXTRACT® T-2 & HT-2	P43/P43B	3 mL		✓	✓	✓
EASI-EXTRACT® ZEARALENONE	RB91/RP90	3 mL		✓	✓	✓
FUMONIPREP®	P31	3 mL		✓	✓	✓
OCHRAPREP®	P14/P14B	3 mL		✓	✓	✓
11+Myco MS-PREP	P128/128B	3 mL	✓		✓	✓
Solid Phase						
PuriTox Aflatoxin	P25	Syringe		✓		
PuriTox AflaZON	TC-M160	Syringe	✓	✓	✓	✓
PuriTox Trichothecene	TC-T220	Syringe	✓		✓	✓
PuriTox Total Myco-MS	TC-MT3000	Syringe	✓		✓	✓

Immunoaffinity columns (IAC)

IAC contain monoclonal antibodies making them highly selective. Further advantages are:

- Excellent sample clean-up, especially with complex commodities. This makes it possible to use solvent based standards with LC-MS/MS detection.
- Clean eluates mean that there is no requirement for matrix matched or isotopic labelled

Solid phase extraction columns (SPE)

SPE use selected solid adsorbents to bind interfering components and pigments, allowing the mycotoxins to be easily analyzed. Further benefits are:

- Non-specific clean-up, making this an ideal clean-up tool for the analysis of multi-toxins in simple matrices like cereals.
- Simple, fast and cost-effective clean-up providing an excellent screening test in conjunction with LC-MS/MS.
- Can be used in combination with matrix matched standards removing the requirement for expensive isotopic labelled standards.

Trilogy® naturally contaminated materials and mycotoxin standards

Trilogy® is a full service ISO 17025-accredited laboratory and accredited as a reference material producer according to ISO 17034. Trilogy® Analytical Laboratory is one of the few producers of certified, **naturally contaminated** reference materials and certified mycotoxin standards. Additionally, naturally contaminated quality control materials and analytical standards for daily quality assurance are available.

'Naturally contaminated reference material' is preferred over 'spiked reference material'. Natural contaminated reference material provides the most realistic testing scenario possible; spiked materials are easier to extract and might falsify sample results.

Certified natural contaminated reference material are available from Trilogy

Certified Trilogy® mycotoxin products (according to ISO 17034)

Certified mycotoxin standards

- Ready-to-use liquids
- Single toxin solutions available
- Metrological traceability

Certified reference materials

- Naturally contaminated
- Single and multitoxin options available
- Metrological traceability

Trilogy® quality control products for daily use

Quality control materials

- Naturally contaminated
- Single and multitoxin products available
- Cereals, corn, rice, and more
- Complex matrices like feed

Analytical standards

- Dried standard substances
- Ready-to-use standards, liquid
- Single and multitoxin options available

Contract and consulting services

Trilogy provides tailor made materials e.g. 2 kg to 800 kg formulated control material based on the customer food or feed samples. Trilogy® formulates the material to the requested concentration levels.
Trilogy provides a contract laboratory service (ISO/IEC 17025:2017) for the detection of natural toxins in food and feed. All testing services are carried out by highly trained scientific analysts using state-of-the-art equipment like LC-MS/MS and HPLC. Our typical mycotoxin analysis services includes:

• Aflatoxins (B1, B2, G1, G2)	• 3-Acetyl Deoxynivalenol
• Aflatoxin M1	• 15-Acetyl Deoxynivalenol
• Deoxynivalneol (DON, Vomitoxin)	• Diacetoxyscirpenol (DAS)
• Fumonisins (B1, B2, B3)	• Fusarenon-X
• Ochratoxin A	• HT-2 Toxin
• Patulin	• Neosolaniol
• Zearalenone	• Nivalenol
• T-2 Toxin	• Citrinin

Trilogy proficiency testing

Regular participation in proficiency testing is required for laboratories that want to comply with international standards (ISO) and (inter) national legislation. In addition to the competence of a laboratory, proficiency tests are also a basis for the integrity and trust of the customer, accreditation body, and management.

Proficiency tests are scheduled for Aflatoxin B1, B2, G1, G2, total Aflatoxins, OTA, DON, ZEA, T-2, HT-2, total T-2/HT-2, Fumonisin B1, B2, total Fumonisins and Aflatoxin M1.

For participation in proficiency testing register at *https://trilogylab.com/proficiency-testing/*.

Why R-Biopharm?

We offer the largest product portfolio for mycotoxin testing and we will be your expert partner offering a complete range of analyses, equipment, consultancy and proficiency testing. We have more than 30 years' experience in developing test kits. Our team of experts conducts ongoing research and development in order to provide innovative products and methodologies that will improve and optimize your workflow resulting in maximum throughput. We actively participate in decisive committees such as CEN, DIN and AOAC to continue to offer high quality products that complement each other to allow you to confidently meet the stringent quality standards.

Table 3: Product overview of the different test kits for different mycotoxin analytes

	RIDASCREEN®	RIDA®QUICK	Rhône	RIDA® EASI-EXTRACT® PREP® IMMUNOPREP®	PuriTox EASIMIP®	Rhône TRILOGY®	TRILOGY®
	ELISA	Lateral flow	Test cards	Immuno-affinity columns	Clean-up columns	Standards	Reference material
Mycotoxins							
Aflatoxins • Total • B1 • M1	● ● ●	●	● ●	● ●	●	● ● ●	●
Citrinin	●			●		●	
DON	●	●		●	●	●	●
Fumonisins	●	●		●	●	●	●
Multi-Toxin				●	●	●	●
Ochratoxin A	●		●	●	●	●	●
Patulin					●	●	
Sterigmatocystin				●			
T-2 Toxin	●			●	●	●	●
T-2 & HT-2 Toxin	●	●		●	●	●	●
Trichothecenes					●	●	●
Zearalenone	●	●		●	●	●	●

Questions?

Please contact us for more information about mycotoxin testing:

02/2021

R-Biopharm AG • An der neuen Bergstraße 17, 64297 Darmstadt, Germany • E-mail: info@r-biopharm.de • https://food.r-biopharm.com/

Further resources – overview

Content overview for further reading

- Lateral flow device (*LFD*)
- Flowchart for an indirect competitive ELISA (*ELISA*)
- Solid phase extraction column (*SPE*)
- Immunoaffinity column (*IAC*)
- Coupling of different immunoaffinity columns (*IAC tandem*)
- Analytical principle of high perfomance liquid chromatography (*HPLC*)
- Analytical principle of liquid chromatography coupled to tandem mass spectrometry (*LC-MS/MS*)
- EU mycotoxin legislation (please see extra document)

A playlist of videos is available at *https://r-b.io/mycotoxinvideos*

- Sampling: the key to reliable analysis
- RIDA®SMART APP – the smart way to test for mycotoxins
- ELISA analysis: which equipment do you need? RIDA®ABSORBANCE 96
- How reference material makes your mycotoxin analysis more reliable (mycotoxin reference materials)
- Multi-mycotoxin testing: a mycotoxin rarely comes alone
- RIDA®CREST: making mycotoxin analysis easy
- Automated mycotoxin analysis with IMMUNOPREP®ONLINE and RIDA®CREST

Lateral flow device

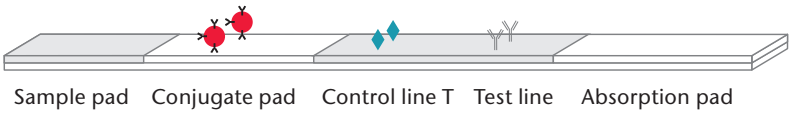
e.g. RIDA®QUICK Aflatoxin RQS (Art. No. R5208)

RIDA®QUICK Aflatoxin RQS (Art. No. R5208) is an immunochromatographic test for quantitative analysis of aflatoxin in corn (maize). The evaluation is carried out with the RIDA®SMART APP software (Art. No. ZRSAM1000).

The immunochromatographic test in the form of a test strip is based on an antigen-antibody reaction. A specific anti-aflatoxin antibody detects aflatoxin in the sample. During incubation of the test strip, a band pattern forms that is used to determine the concentration of aflatoxin.
The appearance and the intensity of the test and control line depend on the aflatoxin concentration of the sample. The test line increases as the aflatoxin concentration increases. The control line weakens as the aflatoxin concentration in the sample increases. The functioning of the test is verified by a control line. The test strip is evaluated using the RIDA®SMART APP software.

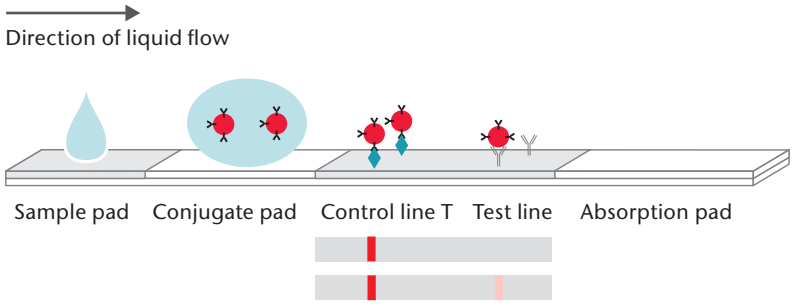
The flowchart below is an oversimplification but explains the major principles.

Virgin strip



The conjugate pad contains beads coated with specific aflatoxin antibodies, the control line is coated with aflatoxin and the test line with non-specific antibody.

Uncontaminated sample

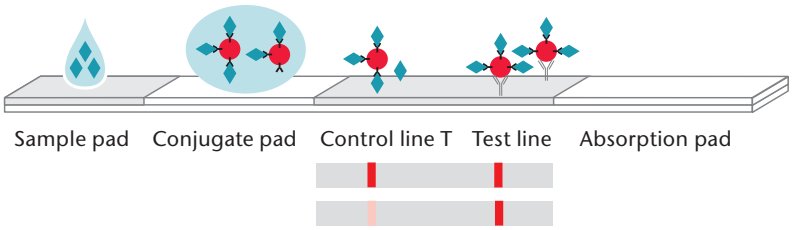


When an uncontaminated sample is added to the sample pad a chromatographic process begins moving the molecules along the test strip towards the reaction area.

First the liquid from the sample picks up the beads and moves them along to the control and test line. The specific antibodies of the beads will bind to the aflatoxin on the control line.

Beads not bound by the control line are bound by the test line. Negative samples should give a red control line and no or a weak test line.

Contaminated sample



When an aflatoxin containing sample is added then the aflatoxin will bind to the specific antibodies on the beads.

Beads that have free aflatoxin binding sites will bind at the control line.

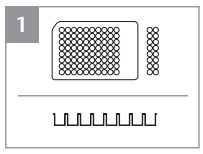
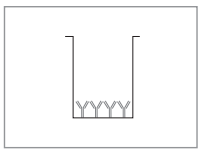
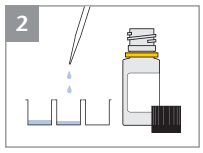
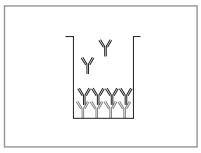
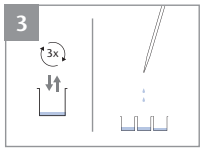
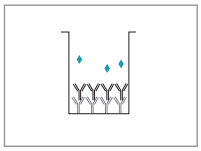
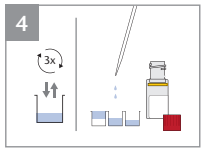
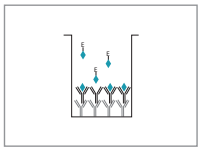
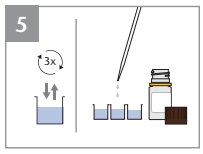
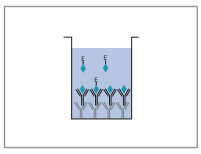
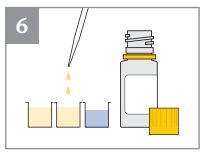
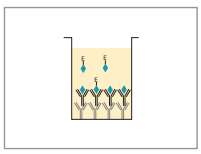
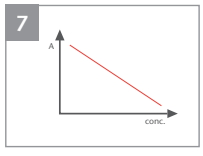
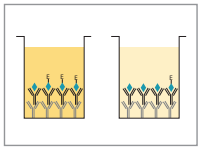
At the test line the bead binds to the non-specific antibody.

09/2020

Flowchart – indirect competitive ELISA

e.g. RIDASCREEN® Aflatoxin M1 (Art. No. R1121)

The indirect competitive ELISA operates on the basis of competition between analyte in the sample and conjugate ('aflatoxin M1 conjugated to an enzyme') for a limited number of specific binding sites on the anti-aflatoxin M1 antibodies. In the case of RIDASCREEN® Aflatoxin M1 a specific antibody needs to be added to the wells. Most indirect competitive ELISAs are already coated with specific antibody.

Test procedure	Test principle
1  Place the required number of microwell strips in the frame.	 Microwells are coated with unspecific antibodies (capture antibodies).
2  Add 100 µL of antibody solution.	 Specific antibody solution (anti-aflatoxin M1 antibodies) is added.
15 min incubation at room temperature	
3  Wash 3 times with washing buffer. Add 100 µL of standard solution or prepared sample.	 Standards and samples are added to their respective wells.
30 min incubation at room temperature	
4  Wash 3 times with washing buffer. Add 100 µL of enzyme conjugate.	 Conjugate is added. Free aflatoxin from the sample/standard and enzyme conjugated aflatoxin M1 compete for the antibody binding sites.
15 min incubation at room temperature in the dark	
5  Wash 3 times with washing buffer. Add 100 µL substrate/chromogen.	 Substrate/chromogen is added. The substrate reacts with the enzyme from the conjugate and a color change from red to blue takes place.
15 min incubation at room temperature in the dark	
6  Add 100 µL of stop solution.	 H_2SO_4 stops the substrate/chromogen reaction.
7  Results are read in a MTP reader at 450 nm.	 The antigen of interest (aflatoxin M1) is inversely proportional to the color in the well. The more yellow colour the less target aflatoxin M1 is present in the sample.

07/2020

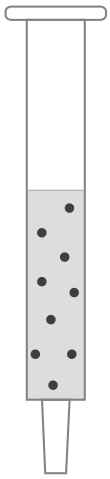
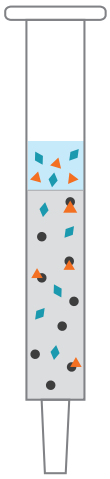
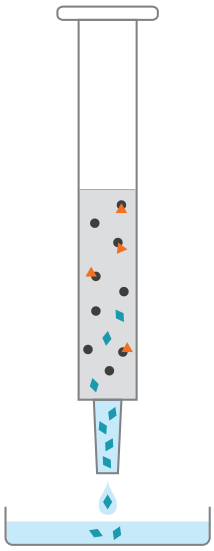
Solid phase extraction column (SPE)

e.g. PuriTox Total Myco-MS (Art. No. TC-MT3000)

Direct injection of complex matrices can result in matrix effects being observed which can manifest as ion suppression or enhancement therefore, some form of sample clean-up is required. SPE columns are a cost effective way to clean-up cereal and cereal based products prior to analysis with LC-MS/MS.

Some SPE columns like PuriTox Total Myco-MS are also suitable for multi-toxin analysis. PuriTox Total Myco-MS is used for sample clean-up prior to the analysis of total aflatoxins, ochratoxin A, DON, 3-acetyl DON, 15-acetyl DON, ZON, T-2, HT-2, FB1, FB2 and FB3 using LC-MS/MS.

The sample is extracted in solvent, filtered and passed through the device. The analytical principle is based on adsorbent material to which interfering components adsorb and thereby removing it from the sample. The R-Biopharm column does not require conditioning prior to use, no wash steps are required and toxins are eluted in one simple step without any evaporation steps. The result is reduced background interference therefore improving the accuracy of results.

SPE columns are ready to use	Sample extract is applied to SPE	Clean-up and release of mycotoxins
		
The SPE column contains adsorbent material.	The mycotoxin sample is extracted with acetonitrile The extract is applied to the SPE and the interfering components stick to the adsorbent material.	The eluate from the SPE is collected and injected onto the LC-MS/MS.

● Absorbent material ◆ Antigen ▲ Interfering substance

11/2020

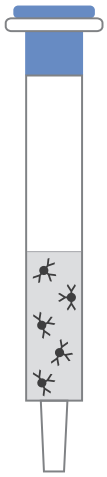
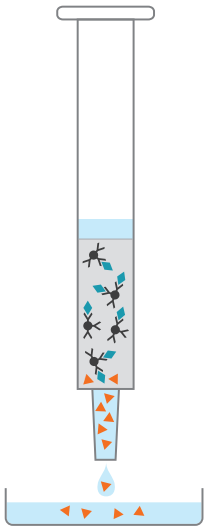
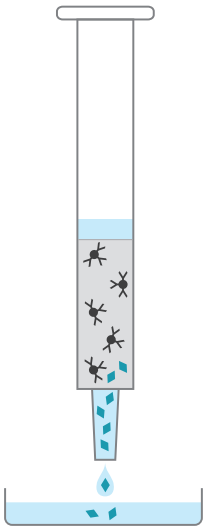
Immunoaffinity column (IAC)

e.g. EASI-EXTRACT® AFLATOXIN (Art. No. RBRRP71/RBRRP70N)

Immunoaffinity columns are used in conjunction with HPLC or LC-MS/MS. Methods are found to be highly specific as the mycotoxin of interest is isolated and concentrated from the sample. The sample matrix is completely removed and no issues are observed with matrix effects, making them particularly suitable for the analysis of complex samples or for when particularly low LODs are required as immunoaffinity clean-up removes all interfering components from the sample ensuring cleaner chromatograms.

Mycotoxin immunoaffinity columns contain a gel suspension of monoclonal antibodies specific to the mycotoxin/s of interest. The use of a monoclonal antibody makes the column highly specific for the target mycotoxin and offers improved sensitivity.

Following extraction of the toxin the sample extract is filtered, diluted and passed through the immunoaffinity column. Any toxin that is present in the sample is bound by the antibody within the gel suspension. The column is washed to remove unbound material and the toxin is then released by the antibody following elution with solvent. The eluate is then be analyzed by HPLC or LC-MS/MS.

IAC ready-to-use	Extracted sample	Washing with PBS buffer	Elution with methanol
			
	The sample containing aflatoxin is extracted and diluted before applying to the column. The antibodies bind to the aflatoxin in the column.	The column is washed where any waste material is removed.	The solvent denatures the antibody and releases aflatoxin.

Absorbent material Antigen Interfering substance

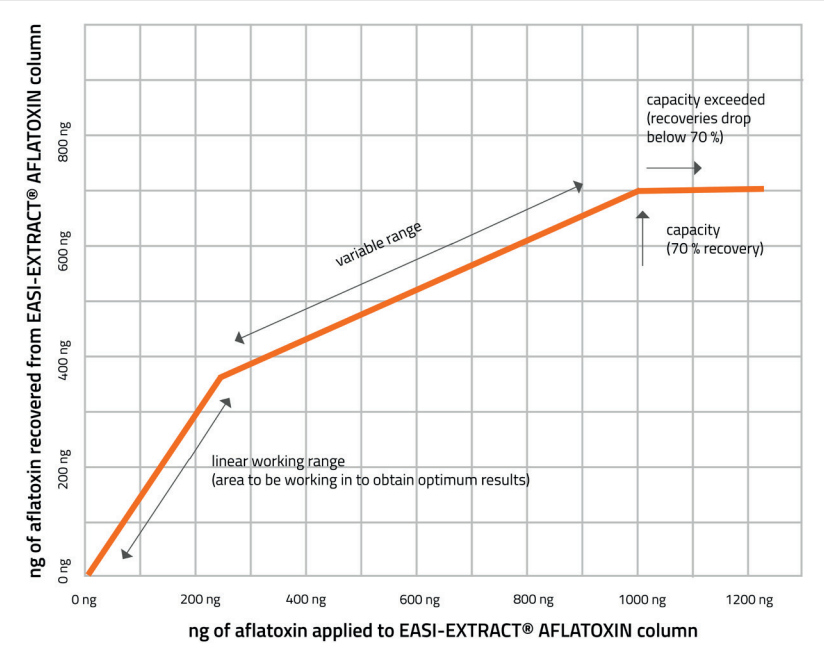
Immunoaffinity column (IAC)

e.g. EASI-EXTRACT® AFLATOXIN (Art. No. RBRRP71/RBRRP70N)

For detection of aflatoxin by HPLC, derivatization is required. Derivatisation is a technique used to transform a chemical compound into a product of a similar chemical structure. For aflatoxin, the chemical structures of aflatoxin B1 and G1 are changed to a more fluorescent from, increasing the signal for detection. Derivatisation can be carried out in a number of ways however R-Biopharm can offer the original electrochemical device; the KOBRA® CELL (Art. No. RBRK01) (*flyer, instruction manual*).

When selecting a product it is important to know the capacity of the column to ensure that you will be within the working range of the column when following the selected method. R-Biopharm define the capacity of a column as the amount of analyte in nanograms applied to the column which will result in a recovery of 70 % or more.

Figure 1: Capacity of an immunoaffinity column. When plotting data in the form of amount of toxin recovered versus the amount of toxin applied to an immunoaffinity column, three distinct regions within the graph can be observed. The first is the linear working range of the immunoaffinity column, for EASI-EXTRACT® AFLATOXIN it is advised that should be up to 350 ng. The second area is the variable region which approaches the capacity of the column, toxin applied to the column should not exceed 700 ng. The last area is the plateau region where capacity of the column is exceeded.



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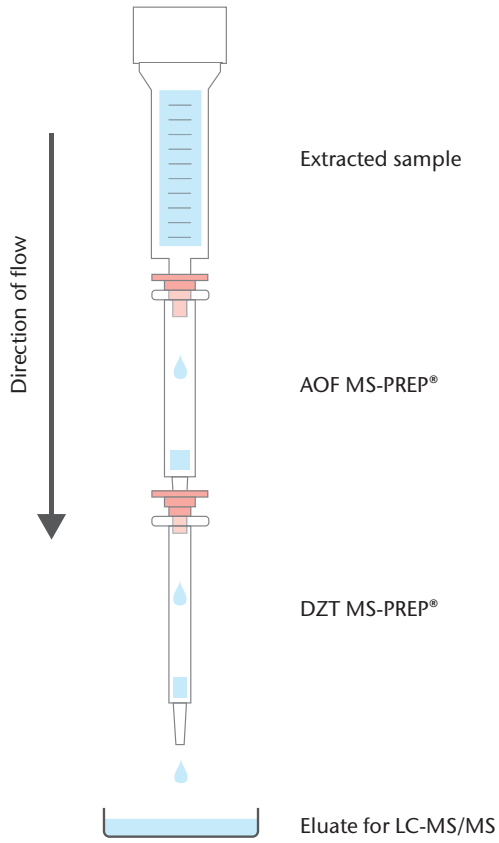
Coupling of different immunoaffinity columns (IAC)

For greater flexibility two IACs can be used in tandem

To have increased flexibility and save time, different immunoaffinity columns can be coupled in tandem for the simultaneous analysis of mycotoxins. This provides selective clean-up, based on targeted mycotoxins known to co-occur in specific matrices. After the sample is extracted, the IAC for aflatoxins+ochratoxin A+fumonisins (AOF MS PREP®, Art. No. RBRP115) is combined with the IAC for deoxynivalenol+zearalenone+T-2/HT-2 toxins (DZT MS-PREP, Art. No. RBRP73). The equipment used to stack the IACs is the column rack (Art. No. CR1) and the items in the Accessory Pack (Art. No. AP01). The eluate is then analyzed by LC-MS/MS under MRM mode with positive polarity.

- To connect the columns in tandem column adapters (see figure) are used (these are supplied as part of the Accessory Pack, Art. No. AP01).
- Remove the top cap from the AOF MS-PREP column and firmly attach the to a glass syringe barrel using an adapter and place in an immunoaffinity column rack (Art. No. CR1) or clamp stand.
- Remove bottom cap and discard.
- Remove the top cap from the DZT MS-PREP column and discard. Firmly attach a column adapter and connect below the AOF MS-PREP column.
- Remove the bottom cap and discard. It is important to ensure that the columns are connected in this way to ensure a proper flow of the sample through the column.



Publications of interest

ML0937: Current methods for mycotoxin analysis and innovative strategies for their reduction in cereals. An overview – detection and detoxification techniques for mycotoxins in cereals, *Shanakat et al*, 2018
ML0845: The use of immunoaffinity columns connected in tandem for selective and cost-effective mycotoxin clean-up prior to multi-mycotoxin liquid chromatographic tandem mass spectrometric analysis in food matrices, *Wilcox et al*, 2015

12/2020

Analytical principle of High Perfomance Liquid Chromatography (HPLC)

Chromatography is an analytical technique by which different analytes of a food sample are separated and thereafter quantified.

After injecting the sample extract via the auto-sampler, the sample migrates through the analytical column and is separated. The purple analyte moves faster through the column than the orange analyte which has a higher affinity for the stationary phase. The affinity of the analyte molecules depend on adsorption, partition, ion exchange etc.

The principle of HPLC can be summarized as follows:

- The separation is based on the distribution of the analyte between a mobile phase (eluent) and a stationary phase (packing material of the column). The sample components that display strong interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in rate separates the various analytes for detection.
- Under the same conditions, the time between the injection of an analyte into the column and the elution of that analyte is constant.

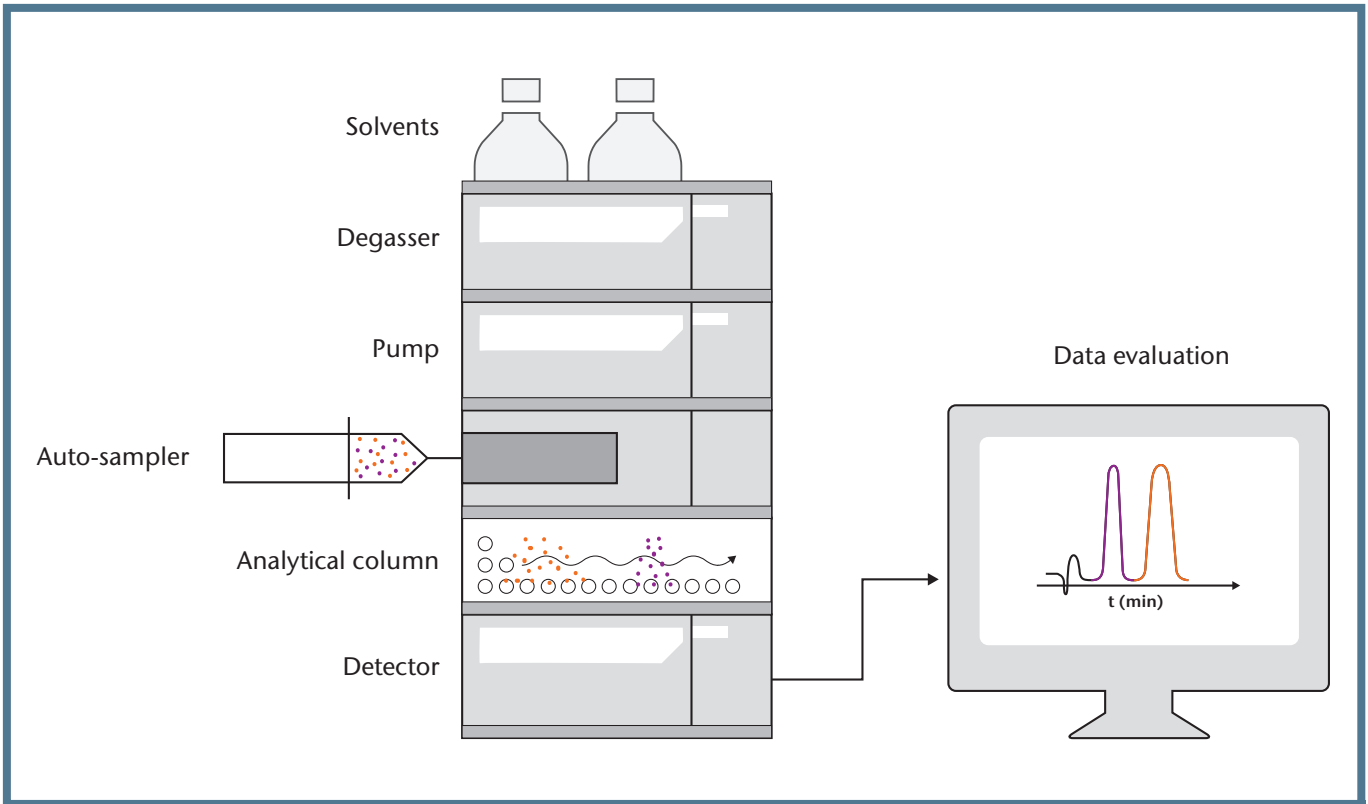


Figure 1: Principle and system configuration of HPLC

Clean-up prior to HPLC detection

In order to obtain good, reliable results consistently the sample matrix should be removed. It is therefore necessary to ensure that a suitable sample extraction and clean-up method are employed.

Typically, immunoaffinity columns are used prior to HPLC detection. Single and multi-toxin column options are available as well as automated cartridges. If an immunoaffinity clean-up method is employed, methods are found to be highly specific as the mycotoxin of interest is isolated and concentrated from the sample. The sample matrix is completely removed and no issues are observed with matrix effects as all interfering components are removed from the sample.

HPLC chromatograms

The chromatogram is the amplified response plotted against time. Components such as the injection solvent are not retained within the column and elute at the 'dead time', this is seen on the chromatogram as the 'solvent front' (see Figure 2).

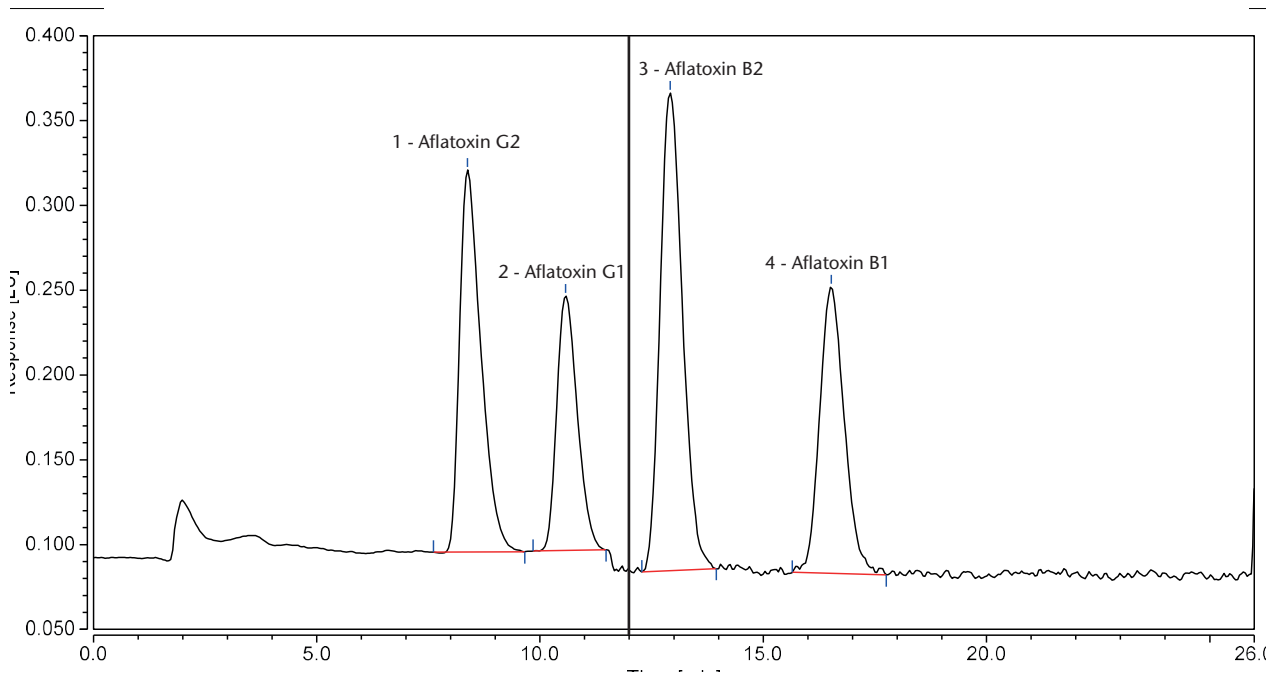


Figure 2: Chromatogram using AFLARHONE® WIDE (Art. No. RBRP116). A wheat sample was spiked at a total concentration of 10 ppb (2.5 ppb for each individual aflatoxin). The solvent front is at a retention time of 2 min and found individual peaks are obtained, one for each aflatoxin. Aflatoxin B1 has the longest retention time with the peak showing at 16 min. All four peaks are well separated and return to baseline. No interfering components are present on the chromatogram. In this instance, the wavelength changes in the middle of the run and is indicated by the vertical line.

Peaks generated should be Gaussian shaped (see Figure 3). The retention times provide the qualitative aspect of the chromatogram. The retention time of a compound should be the same under identical chromatographic conditions.

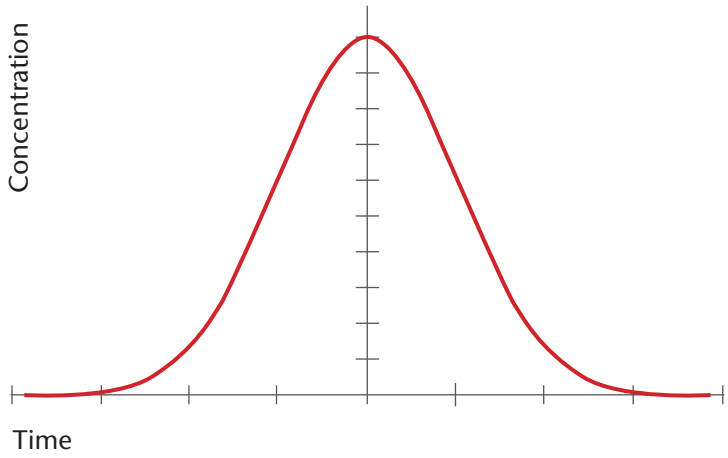


Figure 3: In HPLC, Gaussian shaped peaks are obtained. The peak height or peak area is related to the quantity of analyte. For determination of the actual amount of analyte, the area or height is compared against standards of known concentration.

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Analytical principle of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) combines chromatography and multiple quadrupole mass spectrometers. LC-MS/MS is highly specific to the structure of the compounds of interest and therefore provides a high degree of selectivity and sensitivity.

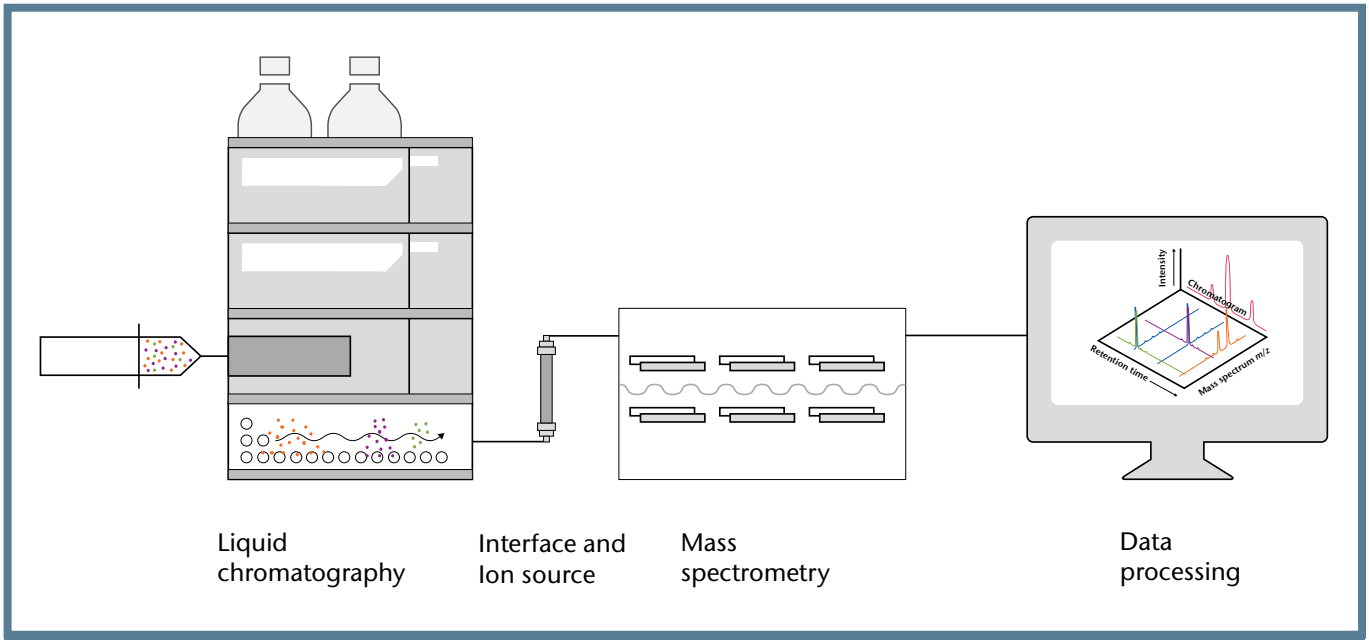

Figure 1: Principle and system configuration of LC-MS/MS. The chromatographic system first separates the different analytes, concentrating the amount of each single analyte. In the mass spectrometer the molecules are ionized and separated based on their mass/charge ratio.

Clean-up prior to LC-MS/MS detection

In order to obtain good, reliable results consistently the sample matrix should be removed. It is therefore necessary to ensure that a suitable sample extraction and clean-up method are employed. There are several options available to laboratories:

- The first is to use **direct injection** or 'dilute and shoot' methods. However, it should be noted that these methods are the most basic form of clean-up and will result in some matrix effect being observed. These will need to be corrected for by using isotopic labeled standards and potentially also matrix matched standards. Direct injection methods are quick and therefore may be used for the screening of samples. However, sensitivity may not meet specific requirements (i.e. EC No. 401/2006 and 2002/657/EC) and results should therefore be confirmed with another method. Over time issues with the LC-MS/MS system may be observed due to the build-up of dirt which can result in ion suppression or enhancement leading to inaccurate results.

- **Solid phase columns** are often used however again are considered as a basic form of clean-up. As a result, these are most commonly used for the analysis of simple commodities like cereal samples and are not recommended for complex or highly pigmented samples or for where low LODs are required. SPE methods are again quick however ion suppression and enhancement effects can still be observed. These issues can be overcome to some degree by the use of matrix matched and/or isotopic labeled standards. These methods could still be considered as screening methods unless isotopic standards are used correctly (i.e. used to spike the sample prior to extraction and taken through the full procedure).

- **Immunoaffinity columns** (IAC) are often used. Due to the nature of LC-MS/MS, multi-toxin columns are generally preferred therefore R-Biopharm have developed a unique range of immunoaffinity columns Using IAC, the sample matrix is completely removed and no matrix effects are observed resulting in very low LODs. In addition, injecting clean samples onto the LC-MS/MS means less down time of the system and fewer technical issues.

LC-MS/MS detection

During chromatography, compounds are separated based on their affinity to the stationary phase. The separated components are then fed into the mass spectrometer which produces ions of the individual analytes. An ion of a particular mass is selected in the first stage of a tandem mass spectrometer. Next, in the second mass spectrometer stage a fragmentation reaction takes place, in Q3 an ion product of the fragmentation reaction of the precursor ion is selected for detection (see Figure 2).

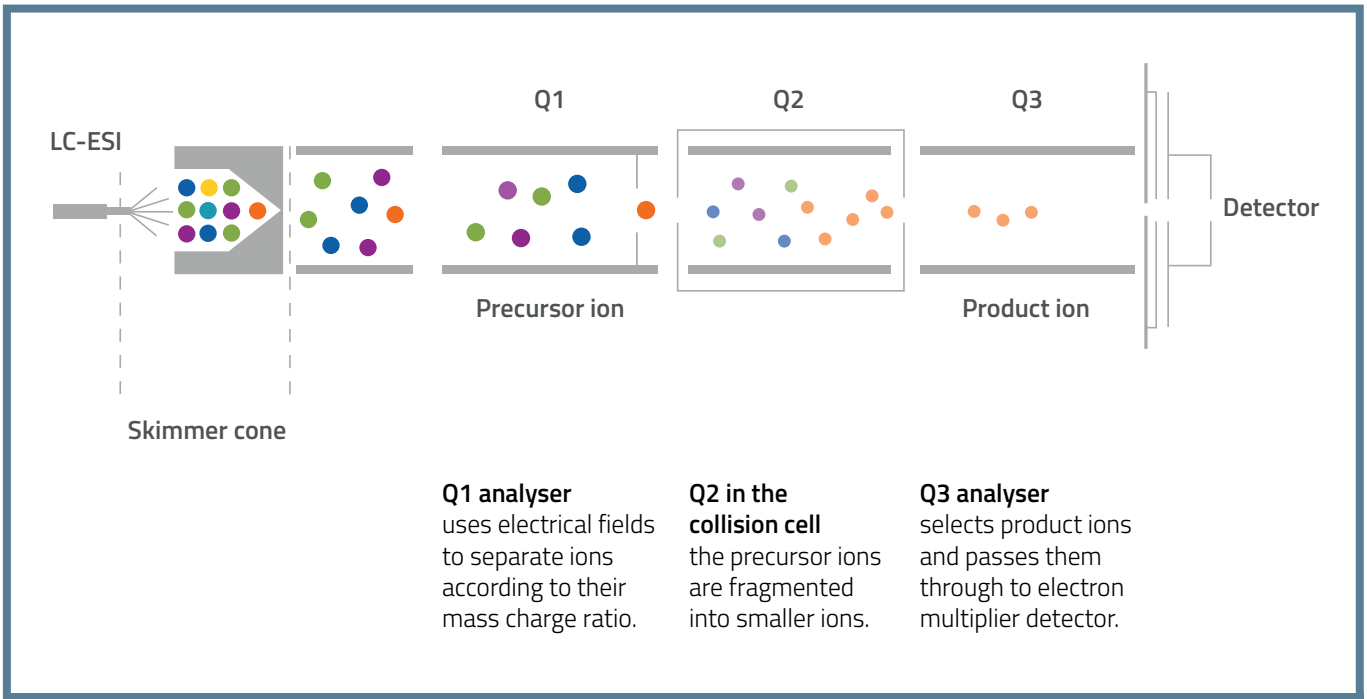

Figure 2: Tandem Mass Spectrometer (MS/MS). After separation by LC, the components are introduced to the MS/MS via the skimmer cone. In Q1 (analyser), the individual analytes are ionised to from "precursor ions" before entering Q2 (collision cell). Here, they are fragmented and a 'product ion' is created. Q3 (2nd analyser) selects only certain product ions to pass through to the detector.

Table 1 details the LC retention time of each analyte, as well as the mass of the "precursor" ions and two resulting "product" ions. One product ion is used to quantify analytes, and a second is used to qualify analytes.

Instrument Setting

Time segment (min)	Toxin	Precursor ion (m/z)	Precursor ion (m/z)	Dwell time (s)	Cone voltage (V)	Collision voltage (eV)
3.0 - 6.0	DON	297.01 [M+H]+	249.10 (Quantifier) 231.08 (Qualifier)	0.661	24 24	10 12
6.5 - 9.5	AFT G2	331.01 [M+H]+	245.13 (Quantifier) 189.07 (Qualifier)	0.105	48 48	32 40
6.5 - 9.5	AFT G1	329.01 [M+H]+	243.06 (Quantifier) 199.88 (Qualifier)	0.105	50 50	28 44
6.5 - 9.5	AFT B2	315.07 [M+H]+	287.12 (Quantifier) 259.14 (Qualifier)	0.105	56 56	26 30
6.5 - 9.5	AFT B1	313.00 [M+H]+	284.93 (Quantifier) 241.10 (Qualifier)	0.105	52 52	22 40
6.5 - 9.5	FUM B1	772.39 [M+H]+	334.39 (Quantifier) 352.40 (Qualifier)	0.105	52 52	40 38
8.5 - 10.5	FUM B2	706.39 [M+H]+	336.40 (Quantifier) 318.39 (Qualifier)	0.105	56 56	40 42
9.5 - 11.0	HT-2	442.21 $[M+NH_4]+$	263.16 (Quantifier) 251.10 (Qualifier)	0.272	18 18	12 14
9.5 - 11.5	T-2	484.21 $[M+NH_4]+$	305.14 (Quantifier) 245.12 (Qualifier)	0.272	26 26	14 14
10.5 - 13.0	OTA	403.9 [M+H]+	239.0 (Quantifier) 358.10 (Qualifier)	0.428	32 32	22 14
10.5 - 12.5	ZON	319.11 [M+H]+	283.17 (Quantifier) 187.10 (Qualifier)	0.256	22 22	12 20

With LC-MS/MS individual ion chromatograms are obtained as well as total ion chromatograms (see Figure 3).

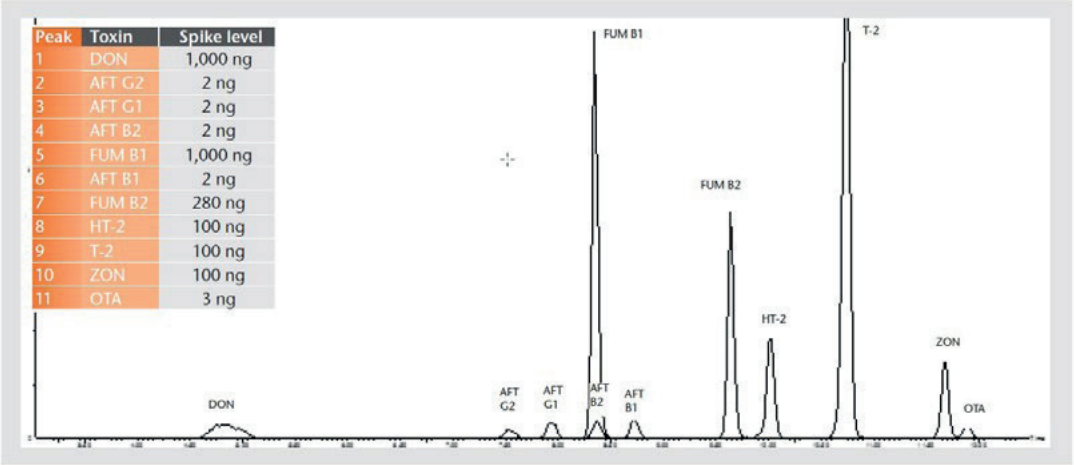

Figure 3: A total ion chromatogram is a chromatogram created by summing up intensities of all mass spectral peaks belonging to the same scan.

Benefits of LC-MS/MS analysis

LC-MS/MS has recently become more popular for the quantitative determination of mycotoxins as it offers several advantages such as improved accuracy, precision and better selectivity. By combining HPLC and MS the benefits of both techniques can be utilised.
LC-MS/MS is an expensive option in terms of capital and running costs and requires skilled personnel to set-up. However, more laboratories are turning to this technique in order to analyse samples for multiple mycotoxins in a single run as the data generated and time saved using single extractions and runs for multiple toxins can outweigh the high costs associated with routine use of such systems.

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