Update on rapid testing of mycotoxins

by Carol Donnelly, R-Biopharm Rhône Ltd, G20 0XA, Scotland.

Aflatoxin and ochratoxin A were some of the first mycotoxins of interest and are produced by the fungi Aspergillus when foods such as nuts, cereals, dried fruit, herbs, spices and coffee are stored under adverse conditions of temperature and humidity.

Over the years, legislation for mycotoxins in food and feed has increased to incorporate additional matrices and toxins, including many of the commonly occurring Fusarium mycotoxins. This increase in legislation has placed pressure on laboratories to use screening methods and to perform multiple mycotoxin analysis on an ever-increasing range of commodities.

The result of this is an increased demand for faster and less labour intensive tests to allow more analyses to be performed in a shorter time period.

Currently there are a wide variety of tests available for the detection of mycotoxins. These include 'traditional' methods such as thin layer chromatography and more rapid tests such as, enzyme immunoassays including cards or lateral flow devices commonly known as dipstick tests.

Rida Quick lateral flow tests have been developed for the qualitative and quantitative determination of a range of mycotoxins. Using the Rida Quick kits for qualitative analysis the test result is read visually, the appearance of a control band ensures that the test is valid or has been run correctly, whilst the appearance of a test band shows a positive toxin result (see Fig. 1).

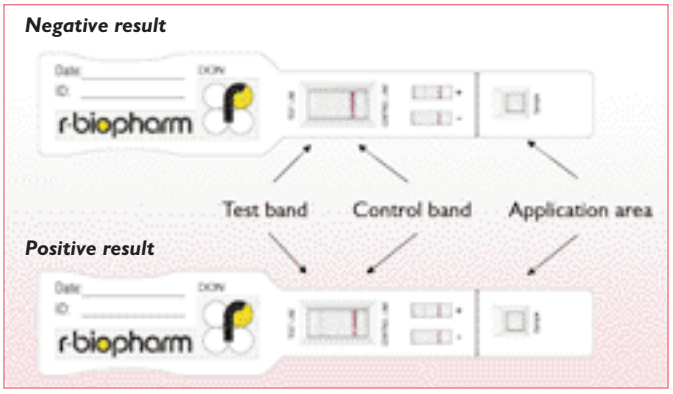

Fig. 1. Overview of the Rida Quick procedure.

To obtain a quantitative mycotoxin result with Rida Quick, the strips are read using a Rida Quick Scan reading device.

Rida Quick Scan is calibrated for each new batch of Rida Quick lateral flow tests by scanning a barcode that is present on each Quality Control certificate or on individual tests using a barcode scanning device. The Rida Quick Scan equipment is then used to read the test strip and to provide quantitative results in minutes (see Table 1 for results obtained with three different batches of Rida Quick DON and Rida Quick Scan compared to HPLC results).

For smaller laboratories who need to analyse more complex matrices without the requirement for reading equipment, membrane card tests such as Aflacard B1 and Aflacard Total can be used for screening of aflatoxin B1 or total aflatoxins at various screening levels.

The cards take only 10 minutes to run and application notes are available allowing analysts to screen at various levels ranging from 2ppb to 30ppb depending on the commodity of interest.

The development of a coloured spot with the control in Aflacard B1 and Aflacard Total confirms that the test has been performed correctly and the appearance of a coloured spot with the test sample indicates that the sample is below the selected limit and can be released.

A special clean-up column allows the test to work on various pigmented and complex food and feed matrices.

Tables 2 and 3 show a comparison of Aflacard B1 and Aflacard Total screening results with HPLC results for a variety of commodities using Aflaprep immunoaffinity columns.

Aflacard has also been approved officially in Japan for screeing of aflatoxins in medicinal herbs. Ochracard is also available for qualitative determination of ochratoxin A in cereals, coffee, chocolate and spices.

For food and feed companies running large numbers of analysis R-Biopharm have developed the largest range of ELISA test formats for mycotoxins to meet the growing demands of the market. ELISAs are important for those analysts who require quantitative analysis of mycotoxins, but who do not have an HPLC or LC-MSMS.

However, one drawback of using quantitative ELISAs is the need to run several standards alongside test samples in order to obtain accurate results. Quantitative ELISAs are normally quite labour intensive involving the preparation and addition of numerous standards, increasing the chance of pipetting mistakes and adding to the overall cost per test.

In response to this problem, R-Biopharm developed the Ridascreen Fast Sc mycotoxin ELISAs.

These ELISAs include a single control but still enable the analysts to achieve a fully quantitative result. Each batch of the Ridascreen Fast SC kits are internally QC tested using a complete range of standards.

The specific results and standard curve obtained during the quality control process are included in a certificate of conformity placed in each kit. Using the Rida Soft Win software, the customer chooses the appropriate programme and enters the standard OD values from the batch certificate followed by the OD values obtained in their laboratory with their single control and test samples.

Once this data has been entered, a full standard curve will be produced, which should be compared to the batch certificate to confirm that it

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Table 1. Comparison of three batches of Rida Quick DON and Rida Quick Scan results to HPLC results.

Commodity	Mean obtained with three different batches of Rida Quick DON (ppm)	HPLC value
Wheat	<0.5	0.3
	0.83 ± 0.05	0.95
	1 ± 0.15	1.13
	1.17 ± 0.18	1.12
	4.16 ± 0.6	4.7
	>5	7.8

Commodity	Screening level (ppb)	Aflacard B1 results	HPLC value (ppb)
Peanuts	2	Negative	1.2
		Positive	2.6
Maize	2	Positive	3.3
		Negative	1.8
Walnut	2	Positive	3.4
Paprika	2	Positive	3.2
Animal feed	2	Negative	0.3
Apricots	2	Negative	1.3
Rice	2	Negative	1.5

Table 2. Comparison of Aflacard B1 results to HPLC results.

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meets the recommended specifications. Provided the standard curve is satisfactory, quantitative test results will be automatically produced for each of the test samples in parts per billion (ppb). The Ridascreen Fast SC are suitable for quantitative analysis of clean commodities and can be used in conjunction with solid phase clean-up columns or immunoaffinity columns to test more complex matrices such as spices, feed and coffee.

For more experienced and better equipped laboratories wanting to follow official methods a wide range of immunoaffinity columns are available for individual or multi-mycotoxins, which allow legislative levels to be easily reached quantitatively by HPLC or LC-MSMS.

Immunoaffinity columns are officially recognised as the method of choice for compliance with specific mycotoxin regulations however, there is a growing requirement for columns, which can offer multi-mycotoxin analysis in conjunction with either HPLC or LC-MSMS using a single extraction method.

R-Biopharm has developed a number of new multi-mycotoxin immunoaffinity columns including Aflaochra Prep And Easi-Extract T-2 and Ht-2 for use with HPLC and DZT MS-PREP for the simultaneous detection of deoxynivalenol, zearalenone, T-2 and HT-2 by LC-MSMS. The immunoaffinity column procedure is based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform.

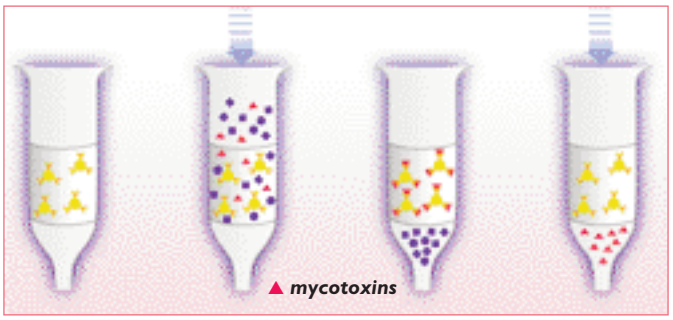
The columns contain a gel suspension of monoclonal antibodies specific to the toxin or toxins of interest. Following extraction, the sample extract is filtered, diluted and passed through the immunoaffinity column where the relevant toxins are retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxin is then released by the antibody following elution with solvent. The eluate is collected prior to analysis by HPLC or LC-MSMS (see Fig. 2).

The advantage of these new immunoaffinity columns is that only one sample preparation method is required for quantifying all mycotoxins in a single run, therefore having greater sample throughput and a reduction in the use of solvents and consumables. The development of faster, accurate tests enables easier and more thorough monitoring of foods by the supplier. If such testing is used in conjunction with preventative measures at all stages in the production process, contamination risk to the consumer will be significantly reduced.

Commodity	Screening level (ppb)	Aflacard Total results	HPLC value (ppb)
Maize	4	Negative	3.1
Paprika	4	Positive	7.9
Black pepper	4	Negative	3.3
Turmeric	4	Negative	2.9
Peanuts	4	Positive	4.4
Cocoa	4	Positive	4.2
Animal feed	5	Positive	7.1

Table 3. Comparison of Aflacard Total results to HPLC results.

Fig. 2. Overview of immunoaffinity column procedure.



Immunoaffinity columns can also be combined with automated systems such as the Aspec system to process and run the columns automatically, reducing labour and enabling samples to be run overnight. R-Biopharm Rhone are also developing a range of on line, multi use mycotoxin columns to reduce handling and to speed up analysis, whilst lowering the cost per analysis.

R-Biopharm have over 20 years experience in the development of various test formats, methods and services for the detection of mycotoxins. The company plans to continue to develop tests for other mycotoxins ahead of legislation, providing the complete mycotoxin solution for both large and small food companies. ■

✉ info@r-biopharmrhone.com