A Rapid iMethod™ Test for Quantification of Aflatoxins in Food

iMethod™ Test for Quantitation of Aflatoxins in Food Version 1.0 for Cliquid® Software

Aflatoxins are a class of mycotoxins; they are secondary metabolites of certain fungi which attack many agricultural commodities. Food products contaminated with aflatoxins include cereals, rice, oilseeds, spices, tree nuts, and milk of animals. Aflatoxins are mainly produced by the fungal species Aspergillus flavus and A. parasiticus before or during harvesting, or because of improper feed storage. Aflatoxin B1, B2, G1, and G2 are major members among 18 different types of aflatoxins identified and aflatoxin M1 is the oxidative metabolic products of aflatoxin B1 produced by animals and appear in milk, urine and feces.

The aflatoxins have acutely hepatotoxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic effects and the main target organ for toxicity and carcinogenicity is the liver. Due to this, national and international authorities have established maximum residue levels and control measures for these compounds in food. There are many methods available for determining aflatoxins in different matrices, however, liquid chromatography/tandem mass spectrometry (LC/MS/MS) is often a more sensitive, accurate, and reliable confirmatory and quantitative procedure compared to immunochemical methods.

The following description outlines the instrument requirements and expected results obtainable from the Phenomenex iMethod™ Test for Aflatoxin quantification when using an AB SCIEX API 3200™ or 3200 QTRAP® instrument. The method included is for the quantitation analysis of aflatoxins B1, B2, G1, and G2 in food samples. Calibration is performed using a series of solutions mixed of aflatoxins B1, B2, G1, and G2 with known concentration. The method uses aflatoxin M1 as internal standard to correct for sample and instrument variability.

Sample preparation is based upon extraction by methanol / water followed by solid phase extraction. More in-depth sample preparation, and instrument parameter information is included as part of the standard operating procedure provided with the method. Solvents, standards and any supplies required for sample preparation are not.

The separation consists of the use of a methanol / ammonium acetate mixture with formic acid with separation on a Phenomenex Luna PFP HPLC column. An example chromatogram of the separation achieved is shown below in figure 1.

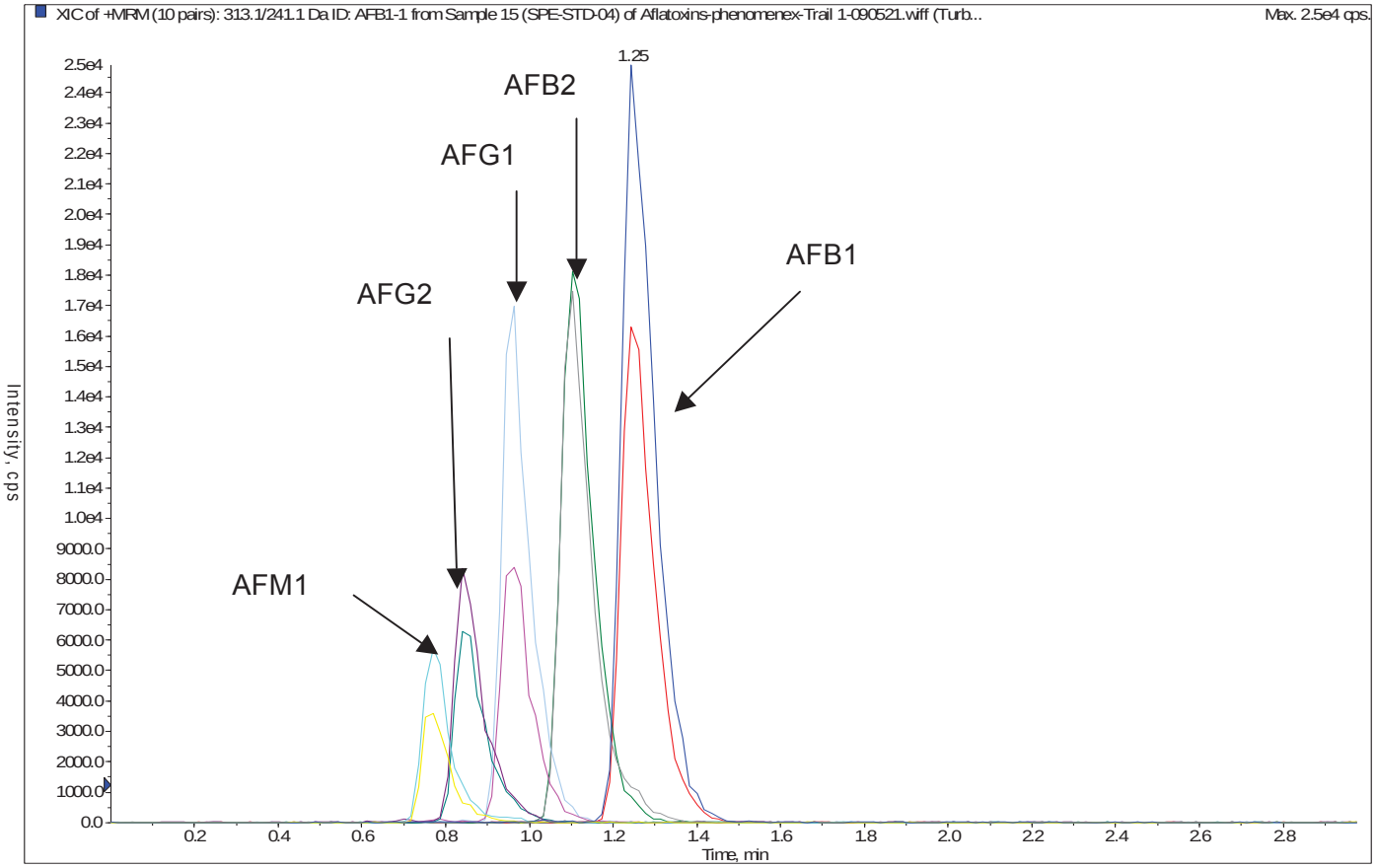


Figure 1: Chromatogram of a 100 ng/ml aflatoxin mix run on a 3200 QTRAP® LC/MS/MS System.

Results

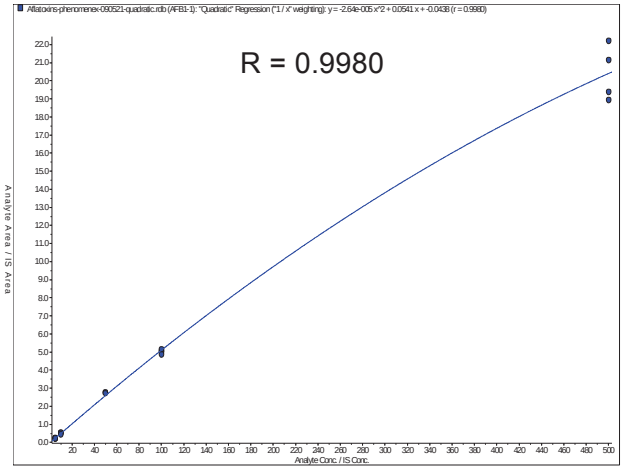
This method was evaluated using both 5 non-extracted calibration standards with relevant concentration at 5, 10, 50, 100, and 500 ng/mL and 5 spiked calibration standards with relevant concentration at 5, 10, 50, 100, and 500 ng/mL. % CV and S/N values for the target analytes were obtained using two replicates at 50 ng/mL with 2 injections for each (n=4).

With non-extracted standards, the accuracy is 88 to 110% and % CV values are 0.2 to 7.0. With spiked standards, the accuracy is from 96 to 106% and % CV values are 1.4 to 11.1. The estimated detection limits for each analyte are more than sufficient to allow the analytical method to be used for either confirmation or quantification.

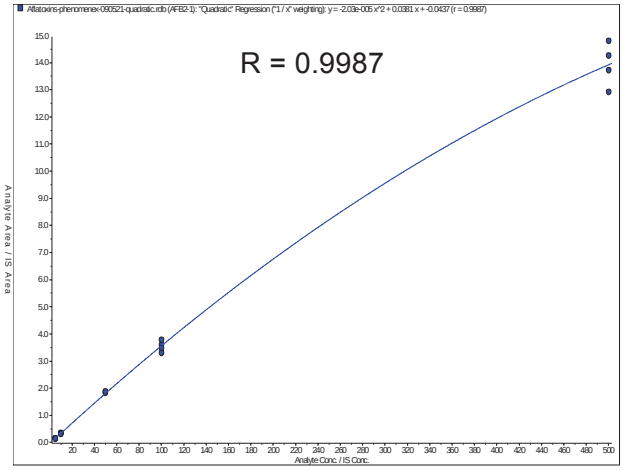
The following chromatogram and calibration curves are representative of the performance obtained on the instrument using the method described here, and may not be representative of performance on any other instrument.

.

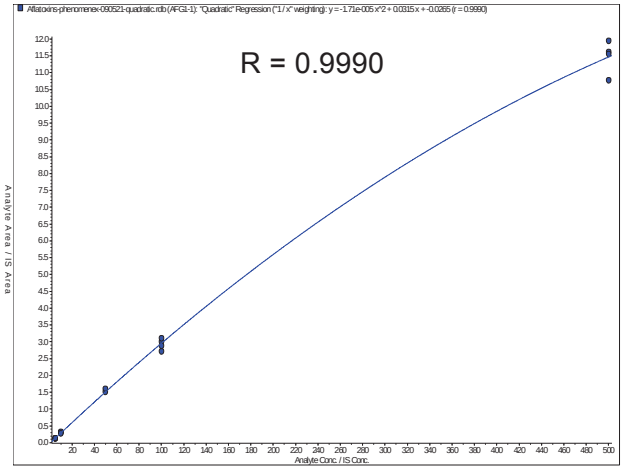
AFB1



AFB2



AFG1



AFG2

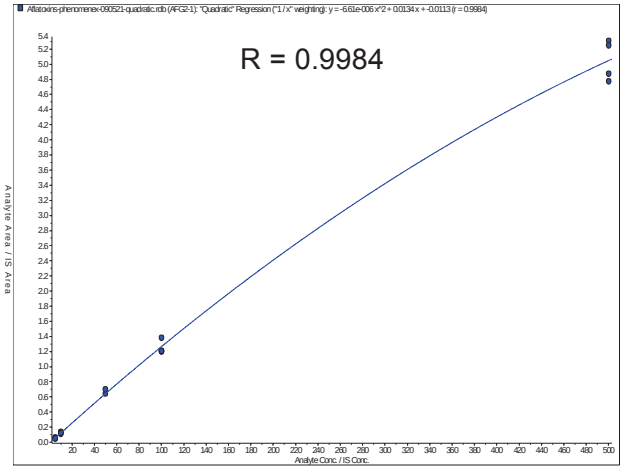


Figure 2: Representative calibration curves for primary quantitation ions of AFB1, AFB2, AFG1, and AFG2 for the concentration range of 5 to 500 ng/ml. Instrument calibration was performed in the quadratic mode with 1/x weighting.

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples; the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance, and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

Representative Signal-to-Noise Ratios at 50 ng/ml (Spiked Samples with SPE)

Analyte	S/N*	% CV	Recovery**
Aflatoxin B1	275.9	5.0	141.7
Aflatoxin B2	302.2	4.7	144.3
Aflatoxin G1	224.5	3.2	132.8
Aflatoxin G2	180.0	6.5	136.4

* Signal-to-noise (S/N) is the peak height divided by the noise measured at three standard deviations of the noise.
** Recovery was obtained using non-extracted standard calibration curve.

Table 1: The following table represents the recovery and % CV value of spiked sample with SPE at concentration 50ng/ml, prepared with SPE on Florisil cartridges, including the % CV estimates from 4 replicate samples, with 2 injections for each sample (n=8).

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP® or API 3200™) LC/MS/MS System
- A Shimadzu Prominence 20A LC System with Reservoir tray and bottles, System controller CBM-20A, 100 µL mixer, 2 Isocratic pumps LC-20AD, 3 Channel degasser Autosampler SIL-20AC, Column oven CTO-20AC
- Aflatoxin Standards (www.sigmaaldrich.com)
- Aflatoxin Internal Standards (www.sigmaaldrich.com)
- LC/MS-Grade Water, Methanol, Acetone, Ammonium Acetate, and Formic Acid
- Phenomenex Luna 3u 100 PFP A 50 x 2 mm HPLC column
- Phenomenex Strata Florisil (FL-PR) cartridges
- Pipettes and standard laboratory glassware

Please note that the Phenomenex Luna PFP HPLC column is required but not included with this iMethod™ Test. This method can also be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated to reflect the configuration used.

Important Note

The purchase and use of certain of the chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ procedure.

Legal Acknowledgements / Disclaimers

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

For Research Use Only. Not for use in diagnostic procedures.

Publication number: 17602110-01

Headquarters
110 Marsh Drive | Foster City CA 94404 USA
Phone 650-627-2600
www.absciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices