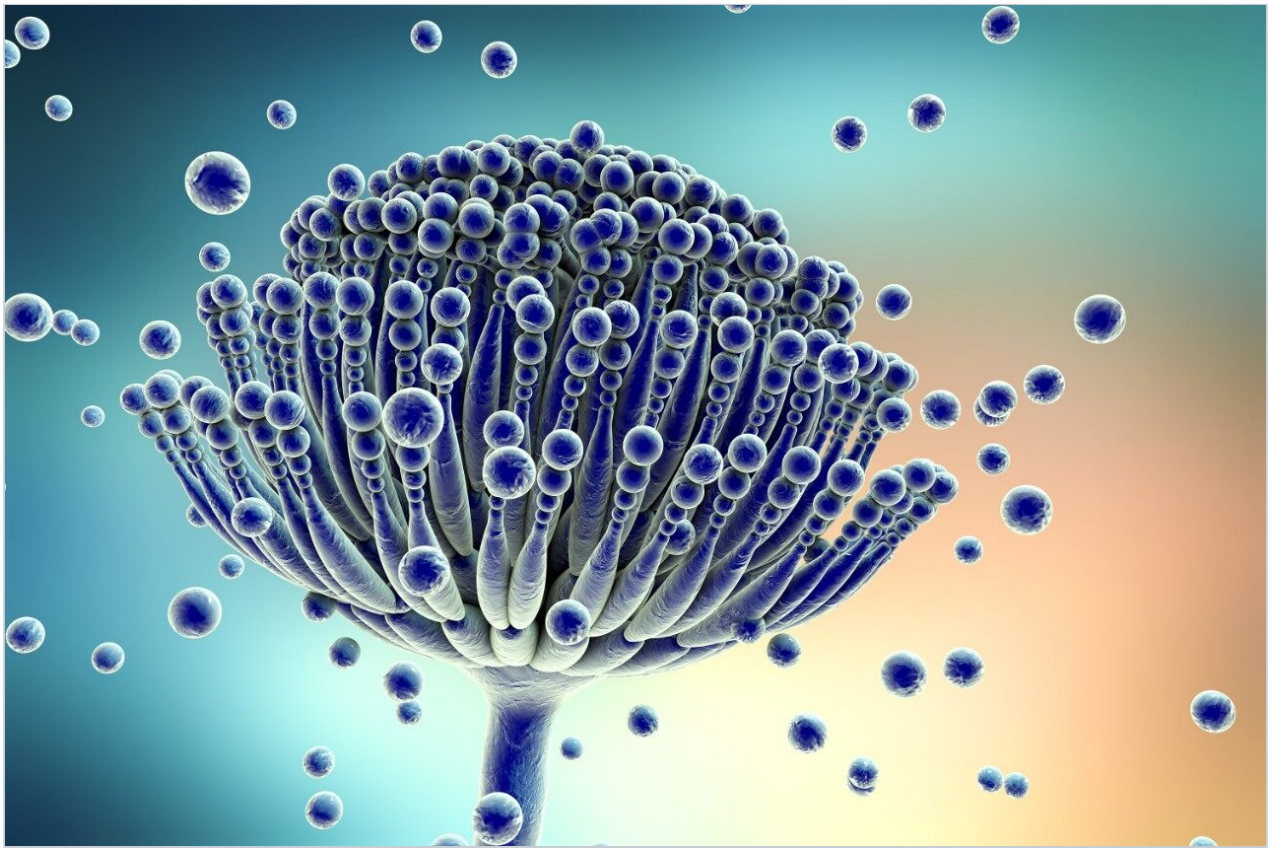
Waters™

アプリケーションノート

# Rapid Multi-Mycotoxin Analysis using ACQUITY UPLC and Quattro Premier XE

Andre de Kok, Martien Spanjer, Jos Scholten, Peter Rensen, Gordon Kearney

Dutch Food and Consumer Product Safety Authority, Waters Corporation



## Abstract

This application note describes an extended multi-mycotoxin method for 25 contaminants in a variety of

sample types which not only meets the requirements for analysis of regulated compounds, but also includes a range of other compounds of concern.

## Introduction

Many agricultural crops are susceptible to colonization by molds and fungi. Stress during plant growth or poor post-harvest storage conditions allow fungal species to infect a variety of commodities, often leading to unacceptable taste, odor, or appearance. It is also possible for some fungal infestations to produce toxic secondary metabolites that have the potential to contaminate both animal feed and food intended for human consumption. These secondary metabolites are known generally as mycotoxins.

There are various classes of mycotoxins, produced by several species of mold, and some of the most important in terms of food safety are the aflatoxins, tricothecenes, ochratoxins and fumonisins. The aflatoxins, for example, first rose to notoriety in 1960, when they caused the deaths of thousands of turkeys on farms in the UK. The bird feed had been made with peanut meal, imported from Brazil, which had been contaminated with the mold *Aspergillus flavus*. This incident highlighted the dangers posed by these compounds, dangers exacerbated by the global nature of modern agricultural trade.[1,2]

It is possible for foodstuffs to be contaminated with a range of mycotoxins from more than one class. The consumption of mycotoxins can have long-term adverse effects on health, so both human foodstuffs and animal feed must be routinely monitored for their presence. The aflatoxins, ochratoxin A, the fumonisins and tricothecenes such as deoxynivalenol are legislated against in many countries. Rapid, sensitive, and accurate analysis may be carried out for these compounds using immunoaffinity test kits. Immunoaffinity sample preparation is also appropriate for chromatography based analysis where the maximum sensitivity and selectivity is required.[3] In addition, a single analytical method able to target a variety of mycotoxin classes in a range of agricultural produce is desirable in order to obtain more comprehensive information on the range of contaminants that are present in human food. Such a multi-mycotoxin method is appropriate for laboratories testing food for consumption in the European Union, where the range of contaminants legislated against is the most extensive in the world.

The use of HPLC, coupled to a Waters Quattro Ultima Tandem Quadrupole Mass Spectrometer has been reported previously for multi-mycotoxin analysis.[4,5] Using UltraPerformance Liquid Chromatography (UPLC), it is possible to expand the method while significantly reducing the analysis time and increasing sensitivity.

This note describes an extended multi-mycotoxin method for 25 contaminants in a variety of sample types which not only meets the requirements for analysis of regulated compounds, but also includes a range of

other compounds of concern. The method uses a simple, generic sample preparation method followed by Waters ACQUITY UPLC separation and detection with a Waters Quattro Premier XE Tandem Quadrupole Mass Spectrometer.

*Waters ACQUITY UPLC System with Quattro Premier XE Mass Spectrometer.*

# Experimental

## Sample Preparation

- 25 g of ground sample is mixed with 100 mL 80:20 acetonitrile/water for 2 hours.
- Extracts are filtered and diluted 4 fold with water.
- 20 µL of extract is injected for LC-MS/MS analysis.

## LC Conditions

| | |
|---|---|
| Column: | ACQUITY UPLC BEH $C_{18}$ 1.7 µm; 2.1 x 100 mm Column |
| Mobile phase A: | 0.1% formic acid in water |

| | |
|---|---|
| Mobile phase B: | 0.1% formic acid in acetonitrile |
| Flow rate: | 0.4 mL/min |

*Mobile phase gradient is shown in Table 1.*

| Time (min) | % A | %B |
|---|---|---|
| Initial | 90 | 10 |
| 3 | 90 | 10 |
| 10 | 30 | 70 |
| 10.1 | 10 | 90 |
| 12 | 10 | 90 |
| 12.1 | 90 | 10 |
| 15 | 90 | 10 |

*Table 1. Mobile phase gradient.*

## MS Conditions

The eluent from the column was directed into the electrospray source of a Quattro Premier XE Tandem Quadrupole Mass Spectrometer operated in positive ionization, multiple reaction monitoring (MRM) mode. Tables 2a and 2b show the two MRM transitions monitored for each compound. The monitoring of two transitions allows the presence of a mycotoxin contaminant to be confirmed.

## Acquisition and Processing Methods

The data were acquired and processed using Waters MassLynx Software.

| | Parent Ion (m/z) | Product Ion (m/z) | Cone Voltage (V) | Collision Voltage (V) |
|---|---|---|---|---|
| Aflatoxin B1 | 313 | 241 | 50 | 37 |
| | 313 | 285 | 50 | 23 |
| Aflatoxin B2 | 315 | 259 | 50 | 30 |
| | 315 | 287 | 50 | 26 |
| Aflatoxin G1 | 329 | 243 | 40 | 25 |
| | 329 | 283 | 40 | 25 |
| Aflatoxin G2 | 331 | 245 | 50 | 30 |
| | 331 | 257 | 50 | 30 |
| Ochratoxin A | 404 | 239 | 25 | 22 |
| | 406 | 241 | 25 | 22 |
| Deoxynivalenol | 297 | 249 | 20 | 10 |
| | 297 | 231 | 20 | 13 |
| Fumonisin B1 | 722 | 334 | 50 | 40 |
| | 722 | 352 | 50 | 40 |
| Fumonisin B2 | 706 | 336 | 50 | 40 |
| | 706 | 318 | 50 | 40 |
| Nivalenol | 313 | 295 | 13 | 8 |
| | 313 | 175 | 13 | 20 |
| Diacetoxyscirpenol | 367 | 307 | 15 | 10 |
| | 367 | 289 | 15 | 10 |
| T2 Toxin | 467 | 305 | 10 | 9 |
| | 467 | 245 | 10 | 9 |
| HT2 Toxin | 425 | 263 | 15 | 12 |
| | 425 | 105 | 15 | 40 |

*Table 2a. Two MRM transitions monitored for each compound.*

| | Parent Ion (m/z) | Product Ion (m/z) | Cone Voltage (V) | Collision Voltage (V) |
|---|---|---|---|---|
| 3-acetyl-DON | 339 | 231 | 20 | 12 |
| | 339 | 213 | 20 | 12 |
| 15-acetyl-DON | 339 | 231 | 20 | 12 |
| | 339 | 279 | 20 | 10 |
| Zearalenone | 319 | 187 | 20 | 10 |
| | 319 | 185 | 20 | 23 |
| Penicillic acid | 171 | 125 | 18 | 12 |
| | 171 | 153 | 18 | 7 |
| Fusarenon X | 355 | 247 | 15 | 13 |
| | 355 | 268 | 30 | 27 |
| Ergotamine | 582 | 208 | 30 | 42 |
| | 582 | 208 | 30 | 28 |
| Roquefortin | 390 | 193 | 30 | 19 |
| | 390 | 322 | 30 | 19 |
| β-Zearalanone | 323 | 305 | 15 | 7 |
| | 323 | 277 | 15 | 15 |
| α-Zearalanone | 323 | 305 | 15 | 7 |
| | 323 | 277 | 15 | 15 |
| Citrinin | 251 | 205 | 28 | 24 |
| | 251 | 191 | 28 | 24 |
| Zearalanone | 321 | 303 | 18 | 13 |
| | 321 | 285 | 18 | 13 |
| Cyclopiazonic acid | 337 | 196 | 20 | 26 |
| | 337 | 182 | 20 | 20 |
| Sterigmatocystin | 325 | 281 | 50 | 36 |
| | 325 | 253 | 50 | 39 |

*Table 2b. Two MRM transitions monitored for each compound.*

## Results and Discussion

Figure 1 shows the chromatogram obtained from this multimycotoxin method with nivalenol eluting first at a retention time of 1.1 minutes, and cyclopiazonic acid eluting last, at a retention time of 9.3 minutes. Peak widths range from approximately 7 seconds wide at base for some early-eluting components (eluting during the isocratic portion of the chromatographic method) to approximately 4.5 seconds wide at base for some that were better retained. Figure 2 shows chromatograms for the four aflatoxin compounds. In this study the method was validated for the matrix pistachio nut and Figures 3-8 show calibration curves obtained for the aflatoxins, ochratoxin A and DON. The red lines are obtained from a matrix matched set of calibration standards and the blue lines are obtained from a set of solvent standards.

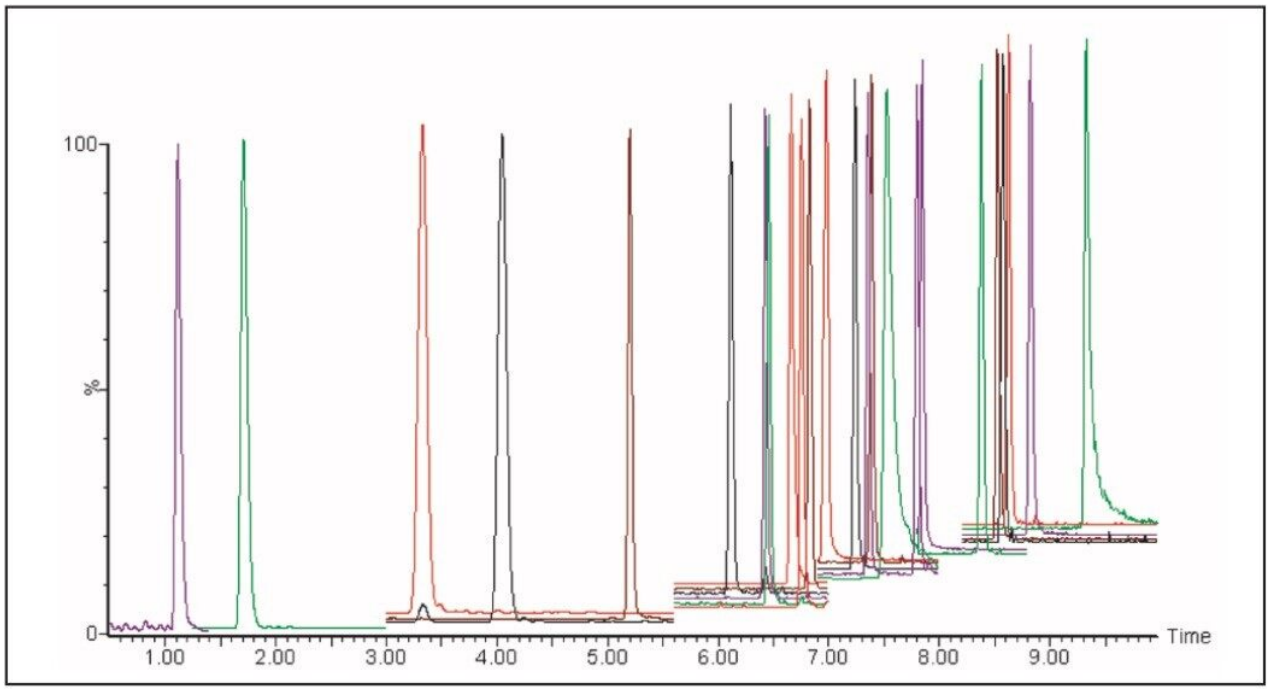



*Figure 1. Chromatograms for all 25 mycotoxins.*

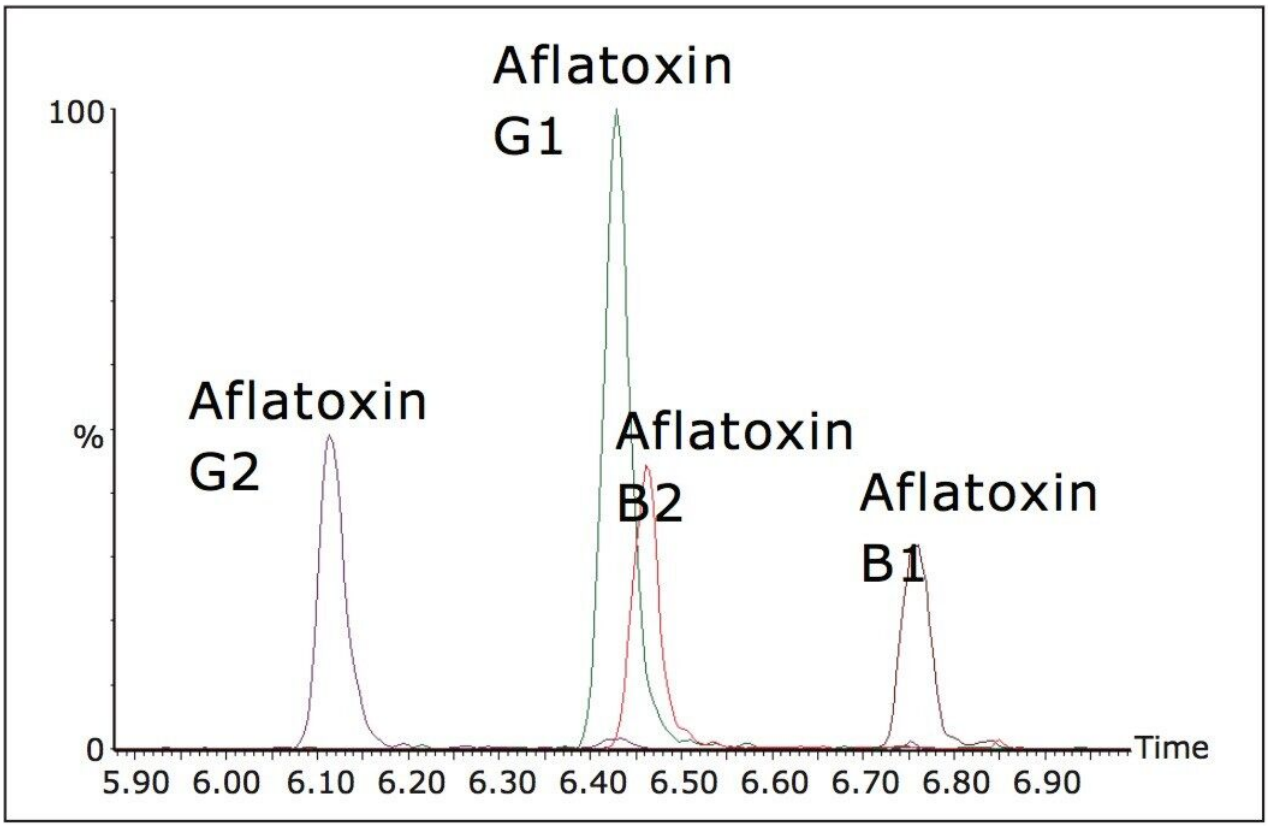



*Figure 2. Chromatograms for the 4 aflatoxins.*

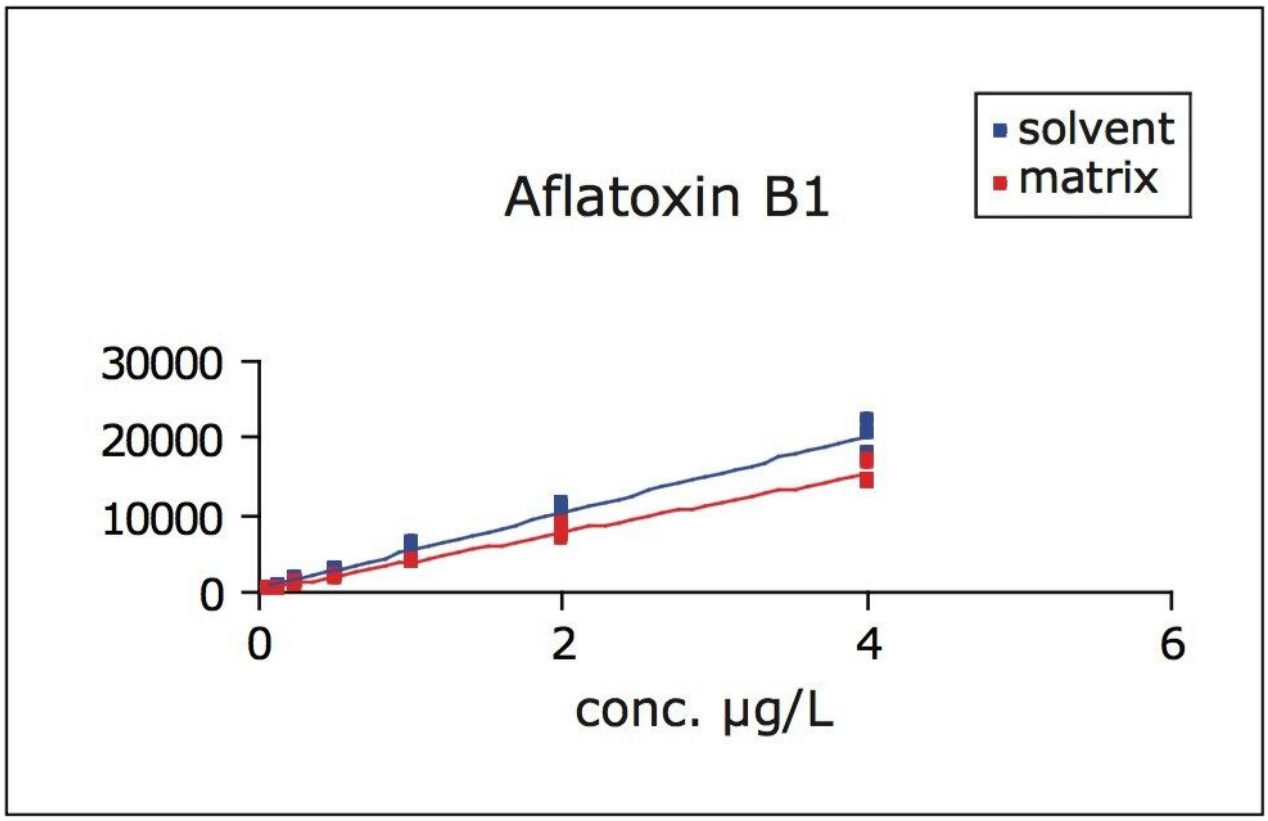



*Figure 3. Matrix-effect aflatoxin B1.*

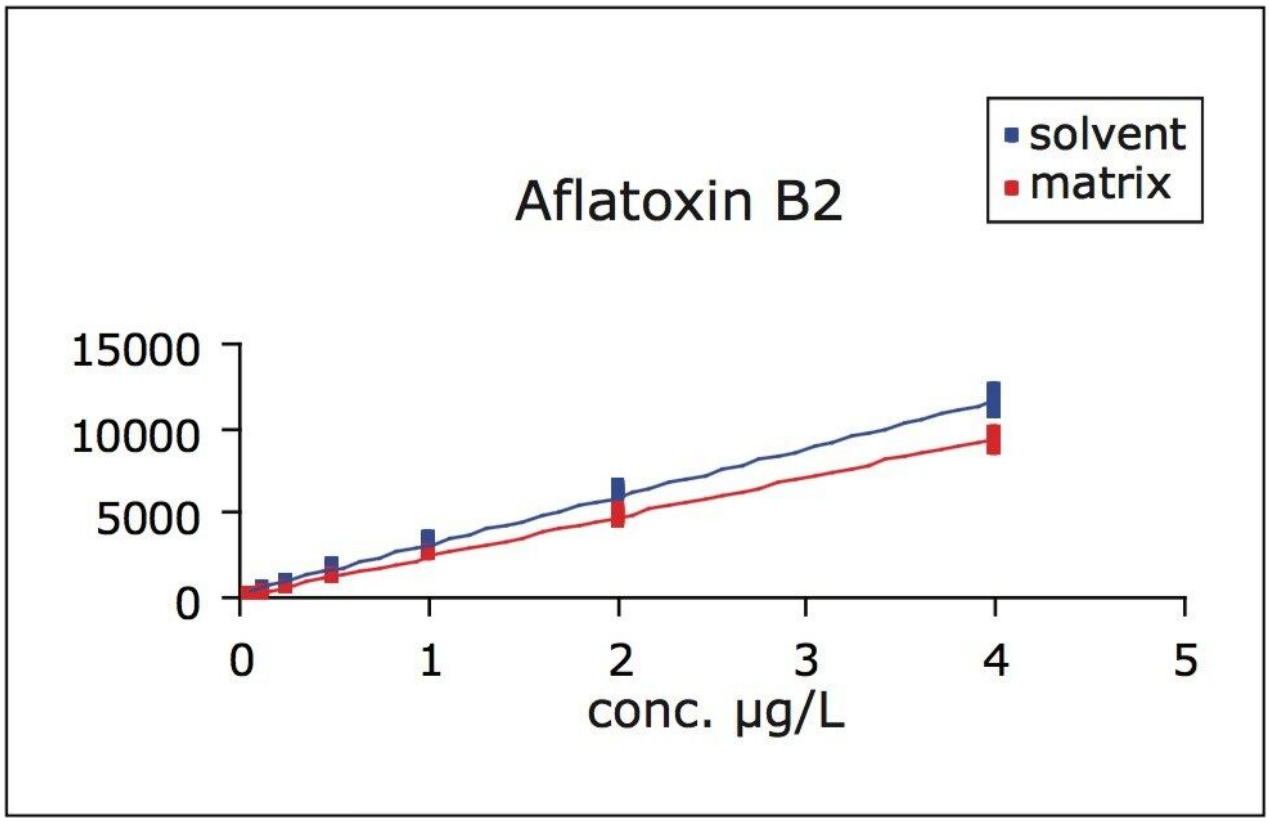



*Figure 4. Matrix-effect aflatoxin B2.*

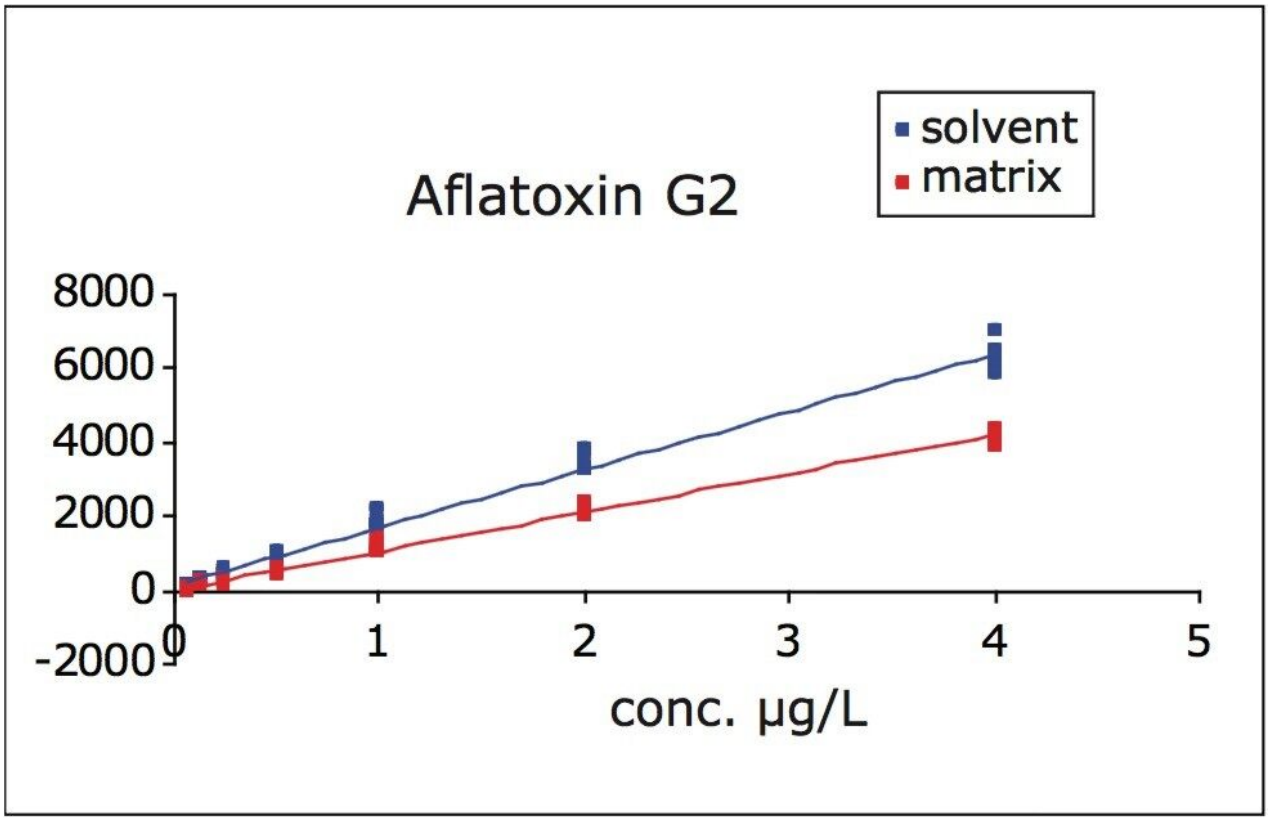



*Figure 5. Matrix-effect aflatoxin G1.*

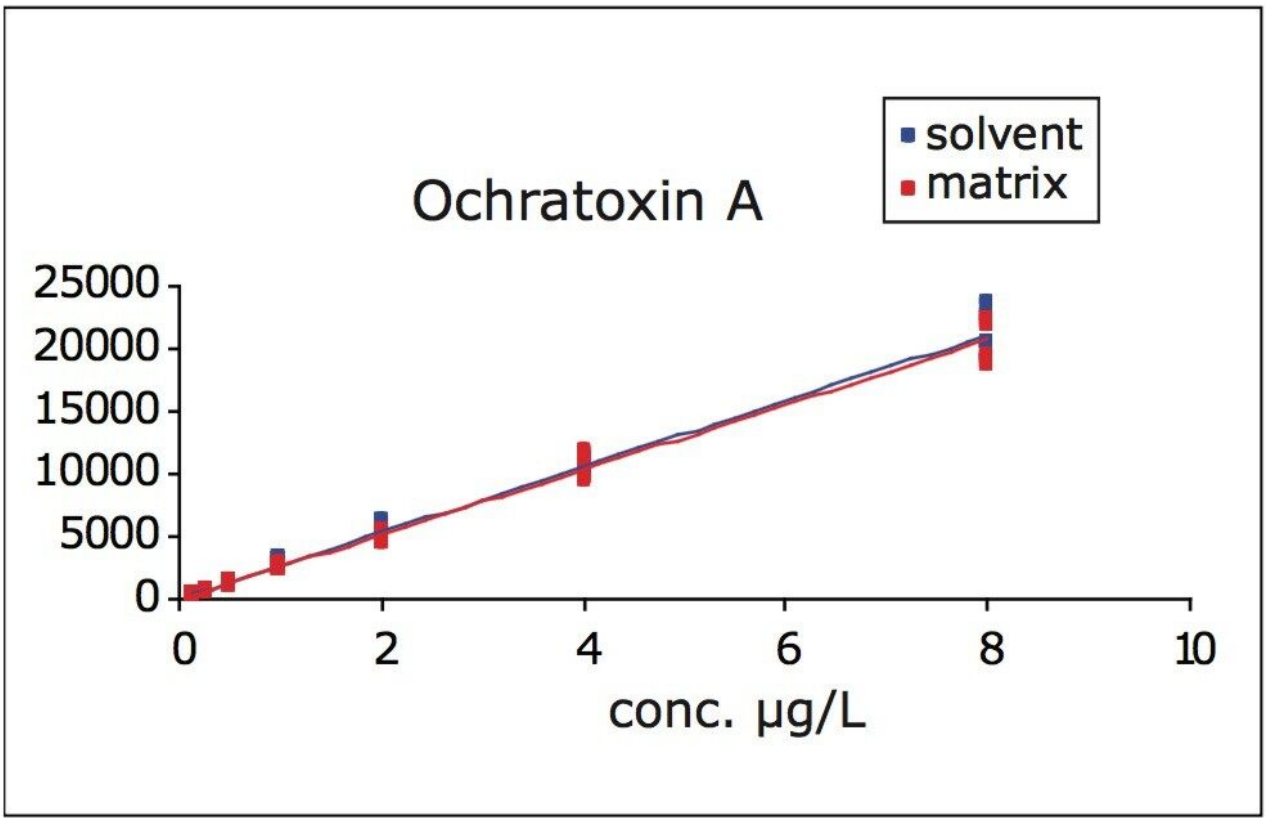



*Figure 6. Matrix-effect aflatoxin G2.*

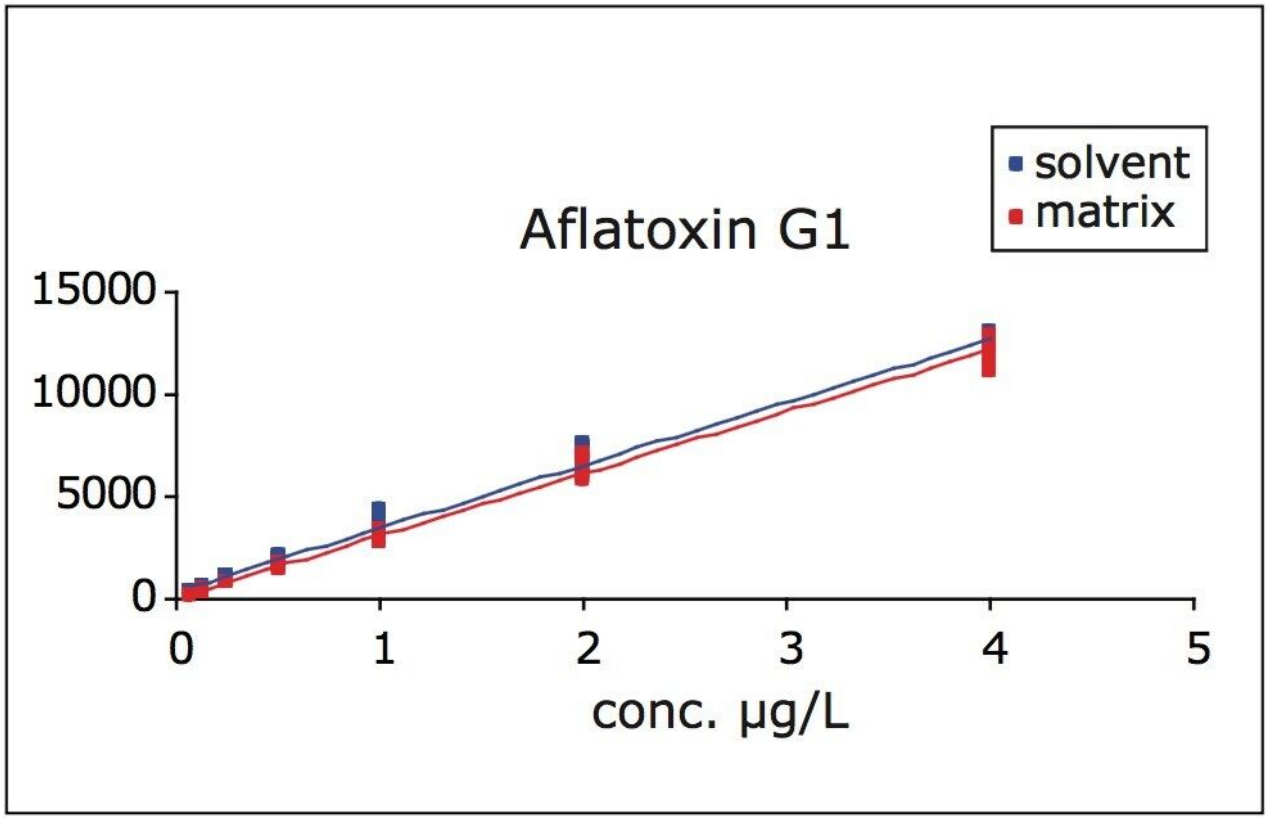



*Figure 7. Matrix-effect ochratoxin A.*

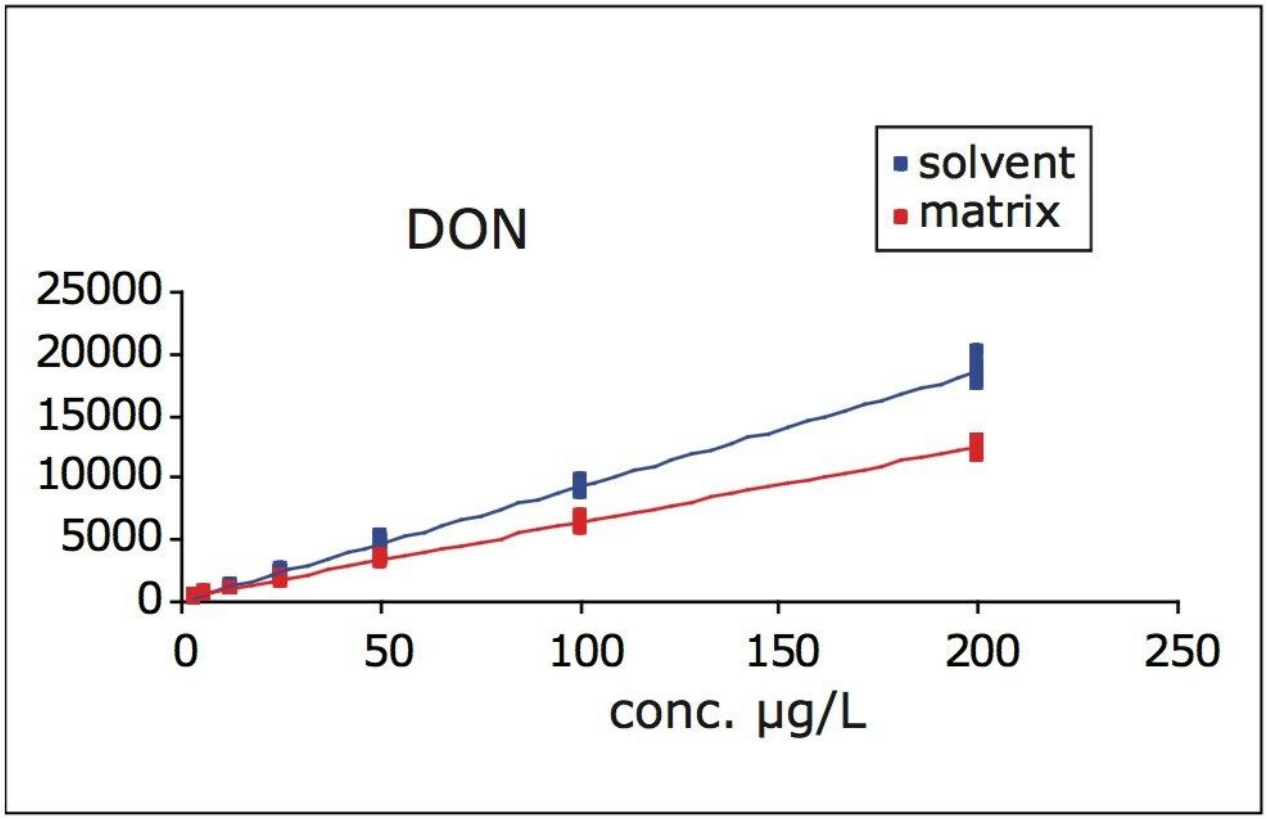



*Figure 8. Matrix-effect DON.*

Figure 8 shows the highest level of matrix suppression obtained for any of the analytes in this method; the signal for deoxynivalenol is suppressed by approximately 34% in the pistachio matrix. From these figures, it is clear that ion suppression in this matrix (for the mycotoxins tested) varies from almost absent to clearly present. Such matrix effects can be reduced or eliminated by the use of SPE sample cleanup and this may be investigated in future work. These six mycotoxins were chosen because they are presently subject to EU law; nevertheless, the presence of DON in a pistachio sample would not normally be expected. Ochratoxin A, however, can be found in pistachio nuts. The figures presented clearly indicate that the matrix effect depends on the analyte, which makes it obligatory to determine ion suppression for every single separate matrix-mycotoxin combination. Validation in peanut and cornflake matrix has been published before.

## Conclusion

The method described is applicable to the enforcement of action levels for regulated substances such as the

aflatoxins in agricultural produce and foodstuffs. It is also applicable to the monitoring of various mycotoxin contaminants of emerging concern. It allows the determination of multiple contaminants per sample, which may ultimately enable a more strategic picture to be obtained of exposure to these compounds from the human diet.

## References


1. European Mycotoxin Awareness Network web site: http://www.mycotoxins.org/ <http://www.mycotoxins.org/>
2. The International Society for Mycotoxicology (ISM) web site: http://www.mycotox-society.org/ <http://www.mycotox-society.org/>
3. VICAM web site: http://www.vicam.com/products/mycotoxin.html <http://www.vicam.com/products/mycotoxin.html>
4. MC Spanjer, PM Rensen and JM Scholten, Multimycotoxin analysis by LC-MS/MS in a Single Sample Extract, Proceedings of the XIth International IUPAC Symposium on Mycotoxins and Phycotoxins, May 17-21 2004, Bethesda, Maryland, USA. Wageningen Academic Publishers, Nov 2006.
5. MC Spanjer, PM Rensen and JM Scholten, Multi-mycotoxin Analysis: the LC-MS Approach, Proceedings of the Third Conference of the World Mycotoxin Forum, 10-11 November 2005. Wageningen Academic Publishers, Nov 2006.
6. MC Spanjer, JM Scholten, and PM Rensen, 2003. Single Run LC-MS/MS Analysis of Mycotoxins Subject to Actual and Upcoming EU Legislation in one Sample Extract. Poster presentation at the Second World Mycotoxin Forum, 17-18 February 2003, Noordwijk, NL.


## Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

MassLynx MS Software <https://www.waters.com/513662>

720001996, August 2007