Application Note: 377

Analysis of Mycotoxins in Various Cattle Forages and Food Matrices with the TSQ Quantum Discovery MAX

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Key Words

- TSQ Quantum Discovery MAX
- Food and Environmental
- H-SRM
- Quantitation

Introduction

Mycotoxins are toxic metabolites produced by certain species of fungi that can infect and colonize on various agricultural crops in the field and during storage. Environmental factors such as temperature and humidity influence the occurrence of these toxins on grains, nuts and other commodities susceptible to mold infestation. In addition, any crop that is stored for more than a few days is a target for mold growth and mycotoxin formation.

Most mycotoxins are relatively stable compounds that are not destroyed by food processing or cooking. Although the generating organisms might not survive processing, the toxin can still be present. Mycotoxins pose a potential threat to human and animal health through the ingestion of contaminated food products. Mycotoxins can have both chronic and acute effects on human and animal health. They can be teratogenic, mutagenic, or carcinogenic in susceptible animal species. They are linked to various diseases in domestic animals, livestock, and humans in many parts of the world. Most mycotoxins are toxic in very low concentrations and therefore require sensitive and reliable methods for their detection.

This application note describes an LC-MS/MS method for the determination of mycotoxins in various cattle forages. Using this method it is possible to simultaneously measure the following 12 mycotoxins within 12 minutes: Nivalenol (NIV), Deoxynivalenol (DON), Aflatoxin G1, Aflatoxin G2, Aflatoxin B1, Aflatoxin B2, Fumonisin B1, Fumonisin B2, Diacetoxyscripenol (DAS), T2-Toxine, Ochratoxin A, and Zearalenon (ZEN). See Figure 1.

The Thermo Scientific TSQ Quantum Discovery MAX triple quadrupole system has been evaluated for round-the-clock analysis of different mycotoxins. Multiple samples with different matrices (cattle forages, food matrices) have been analyzed.

Goal

To demonstrate that the TSQ Quantum Discovery MAX™, with its H-SRM capabilities and H-ESI source, is ideally suited for the rigorous demands of high-throughput analyses of mycotoxins in various matrices.

Experimental Conditions

Sample Preparation

The samples analyzed were various extracts of cattle forages and food products. The following sample extraction procedure, adapted from TLR International Laboratories, was used. To begin, 25 g of grounded sample was dissolved in 100 mL of acetonitrile:water (80:20 v/v). The extract was then mixed for two hours. Afterwards, the extracts were filtered and diluted four times with water. The resulting solution was acetonitrile:water 20:80 v/v.

HPLC

HPLC analysis was performed using the Thermo Scientific Surveyor HPLC System. Each 20 µL sample was injected directly onto a Thermo Scientific Hypersil GOLD 100 × 2.1 mm, 5 µm analytical column. A gradient LC method used mobile phases A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in water) at a flow rate of 0.3 mL/min. The gradient is described in Figure 2.

Mass Spectrometry

MS analysis was carried out on a TSQ Quantum Discovery MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe. The MS conditions were as follows:
Ion source polarity: Positive ion mode
Spray voltage: 4000 V
Vaporizer temperature: 300 °C
Sheath gas pressure (N2): 30 units
Auxiliary gas pressure (N2): 30 units
Ion transfer tube temperature: 350 °C
Scan Type: SRM

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The MS conditions and the H-SRM transitions were obtained by automatic optimization with the auto-tune software. Figures 3 and 4 show two examples of the collision energy optimization. Figure 5 summarizes all of the H-SRM transitions that were used.

Two product ions were measured for all compounds; one was used as the quantifier ion and the other was used as the qualifier ion. In this way, the ion ratio confirmation was done as an identity confirmation. See Figure 5 for further details.

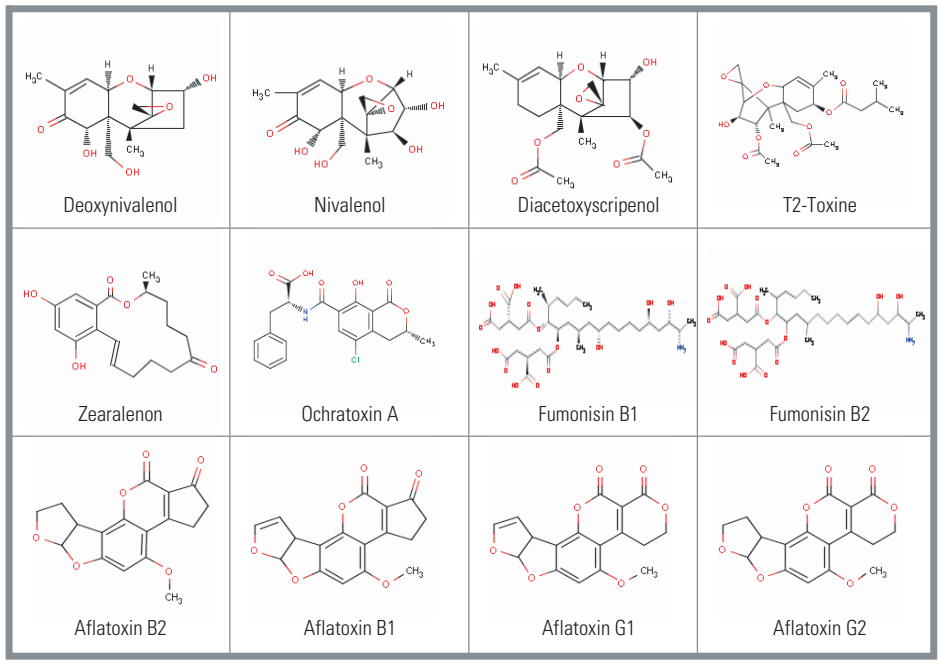
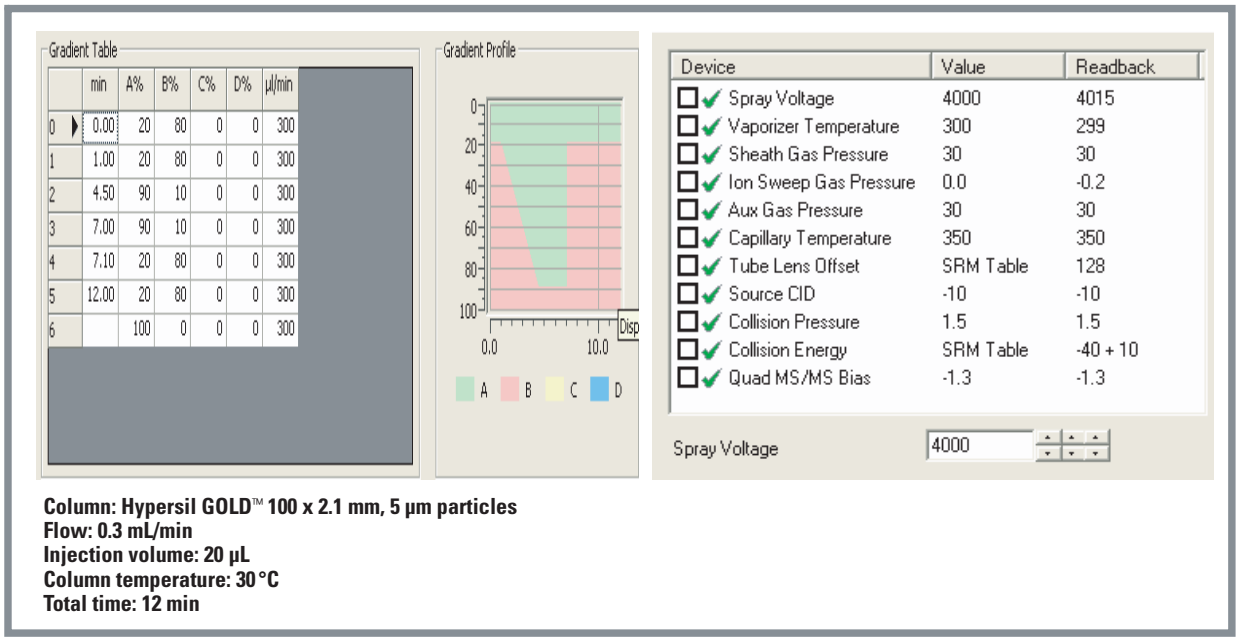


Figure 1: Structures of 12 mycotoxins



Column: Hypersil GOLD™ 100 x 2.1 mm, 5 µm particles
Flow: 0.3 mL/min
Injection volume: 20 µL
Column temperature: 30 °C
Total time: 12 min

Figure 2: LC/MS conditions

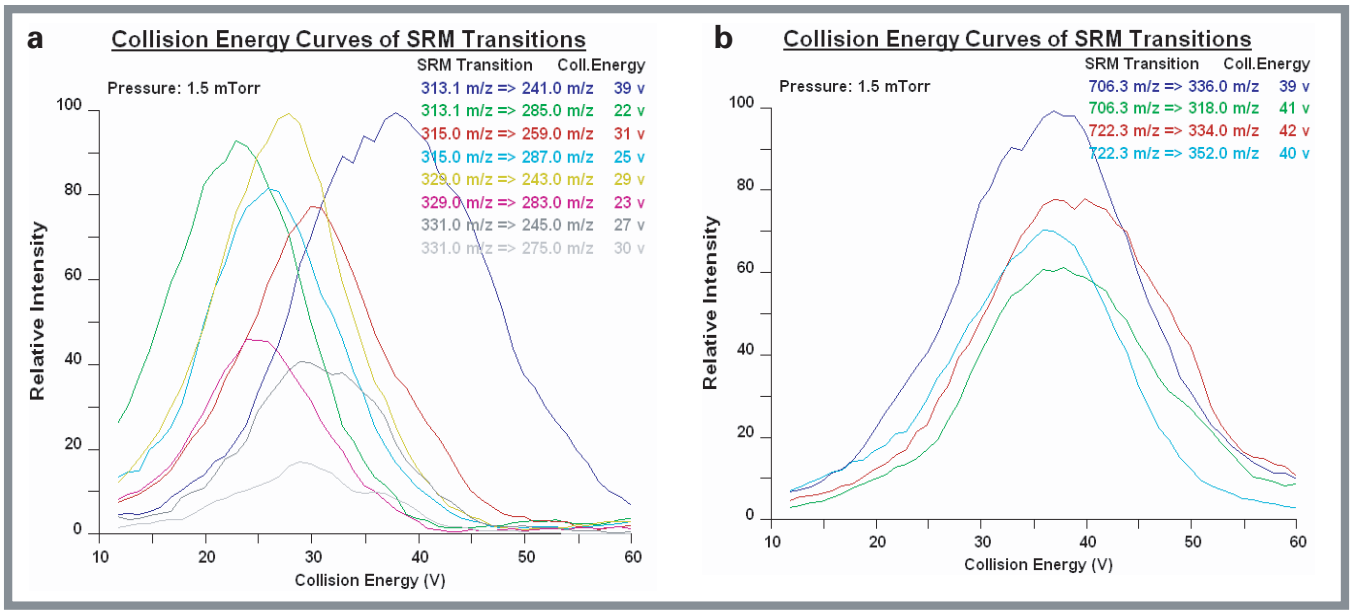


Figure 3: Optimization of collision energies of Aflatoxin A1/B1/G2

Figure 4: Optimization of collision energies of Fumonsin B1/B2

Compound	Precursor	Product Ion (Quan)	Product Ion (Qual)	RT	Conc Range (ppb)
Aflatoxin B1	313	241	285	4.8	0.1–100
Aflatoxin B2	315	259	287	4.5	0.1–100
Aflatoxin G1	329	243	283	5.0	0.1–100
Aflatoxin G2	331	245	275	4.8	0.1–100
Fumonisin B1	722	334	352	4.4	0.1–1000
Fumonisin B2	706	336	318	4.8	0.1–1000
Ochratoxin	404	239	221	5.6	0.1–1000
Zearalenon	319	187	185	5.7	0.1–100
Deoxynivanlenol	297	249	231	1.2	0.1–100
Diacetoxyscripenol	367	307	289	4.4	0.1–100

Figure 5: H-SRM transitions

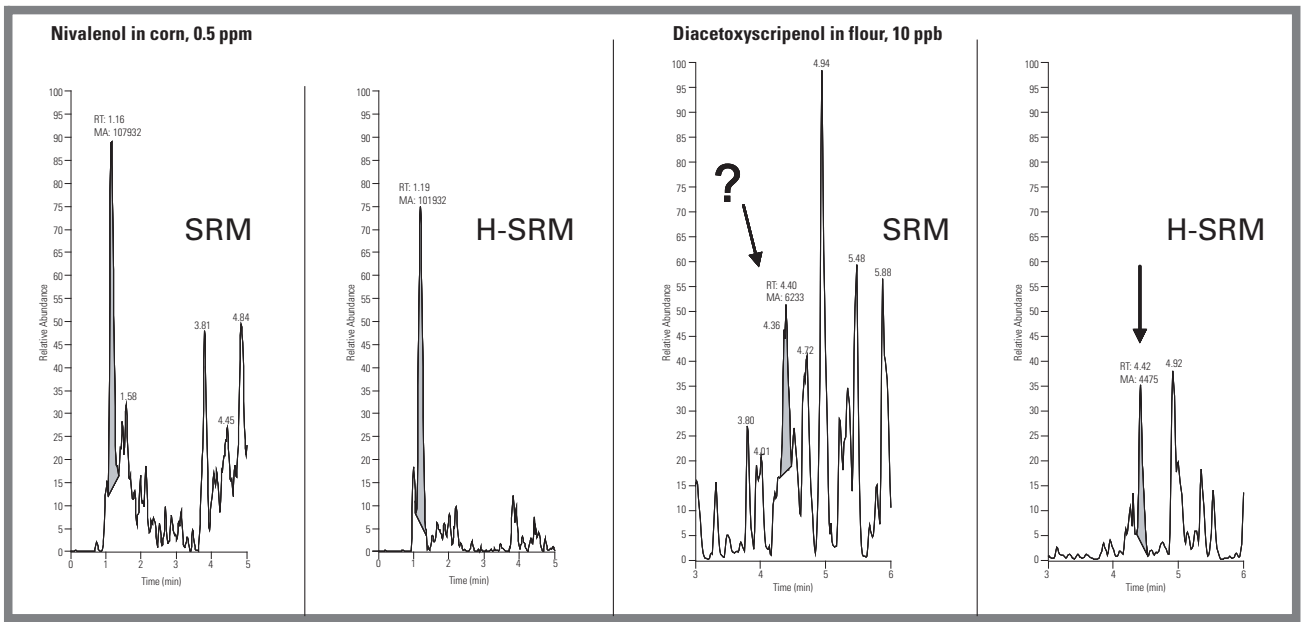


Figure 6: Comparison of SRM and H-SRM data for two samples

Results and Discussion

The TSQ Quantum Discovery MAX offers the unique capability of highly selective reaction monitoring (H-SRM). Setting the resolution of Q1 at 0.1 FWHM helps to decrease the background noise and eliminate isobaric interferences. This improves the signal-to-noise ratio and results into a lower limit of quantification. Figure 6 compares SRM and H-SRM data for two samples.

The calibration curves were generated by dilutions in acetonitrile:water 20:80 v/v. Figure 7 presents the linear fit calibration curves for five mycotoxins using H-SRM.

The calibration curves have R^2 values that are greater than 0.998, which indicate excellent linear fits over the dynamic range.

The mycotoxin levels found in the various matrices were in the expected range. For example, in a QC-sample used as an internal control, Aflatoxin B1 was expected on a level of 5 ppb (in extract). The detected amounts (ppb in solution) are presented in Table 1. This level for Aflatoxin B1, is subjected to EU legislation as the low limit of quantification.

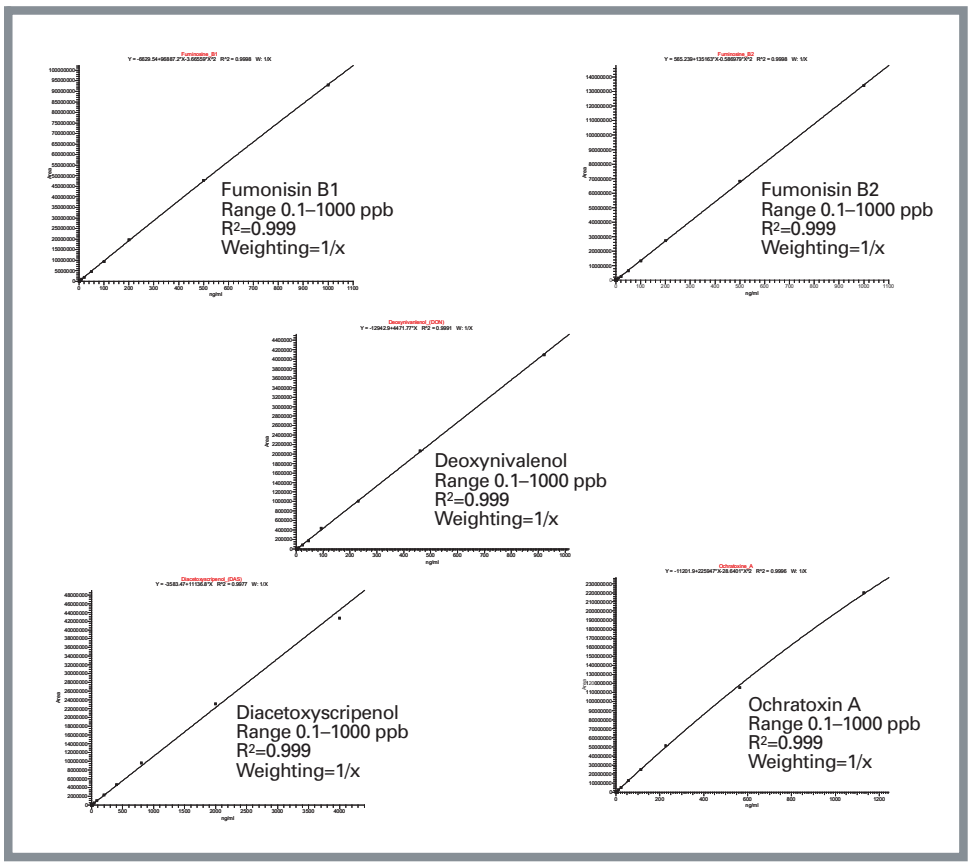


Figure 7: Calibration curves for five mycotoxins

Sample	Detected Amount (ppb)
Sample-01	1.19
Sample-02	1.28
Sample-03	1.43
Sample-04	1.25
Sample-05	1.15
Sample-06	1.37
Average	1.28
RSD	0.1
RSD%	8.3%
Average in Extract	**5.11**

Table 1. Detected Amounts of Aflatoxin B1 in Solution

For the analysis of mycotoxins in various matrices, the heated electrospray ionization (H-ESI) probe provides significant advantages. The dual desolvation zone design increases the ionization efficiency and helps to get rid of the clustering solvents. (See Figure 8.) This leads to higher signals with better %RSDs. The H-ESI probe also handles higher LC flows (up to 1 mL/min) without losing ionization efficiency. This helps to speed up the method without the need to split the LC flow. Figures 9 and 10 describe the increased sensitivity of the H-ESI probe with two samples of mycotoxins.

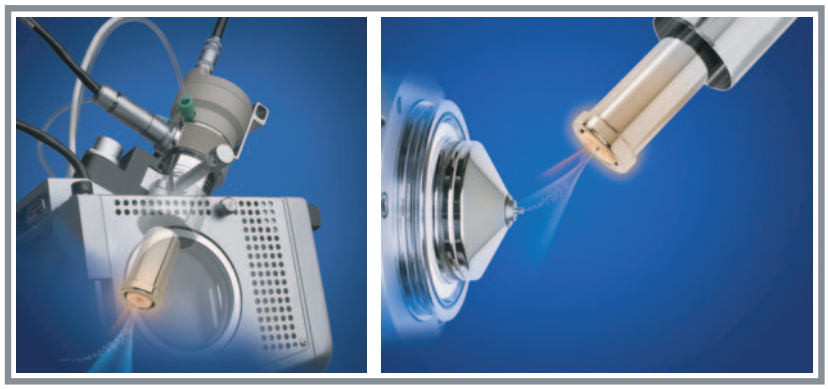


Figure 8: H-ESI – Heated Electrospray Ionization probe

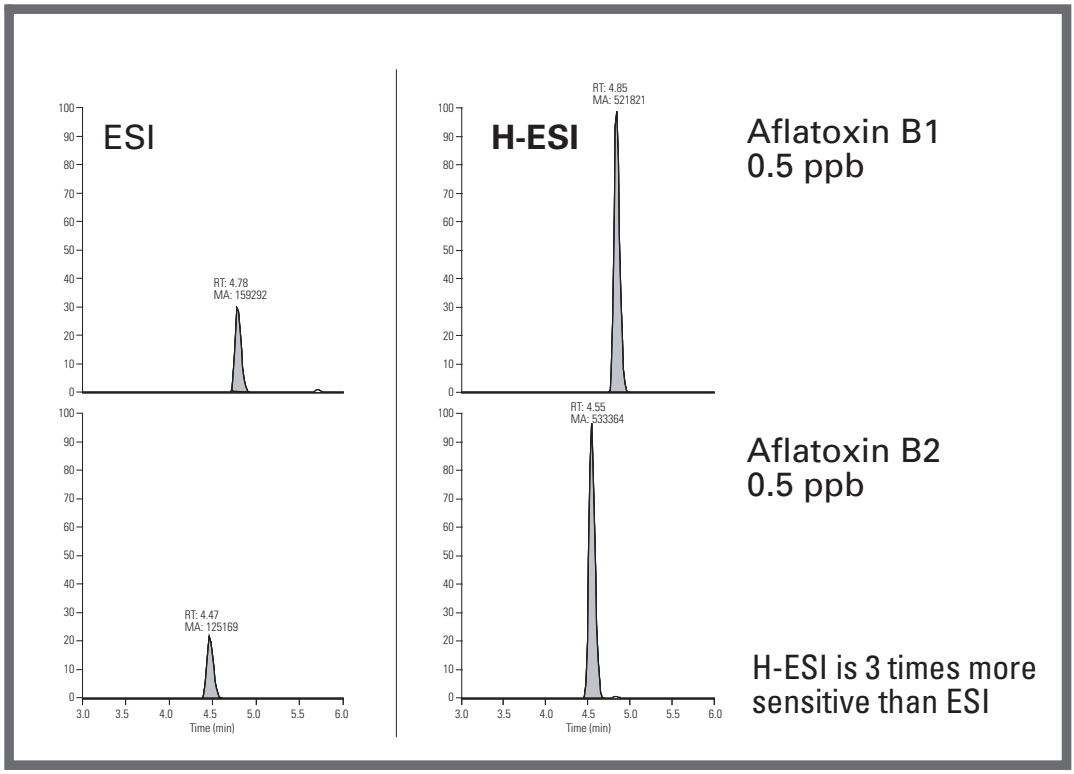


Figure 9: Increased sensitivity with the H-ESI probe in Aflatoxin data

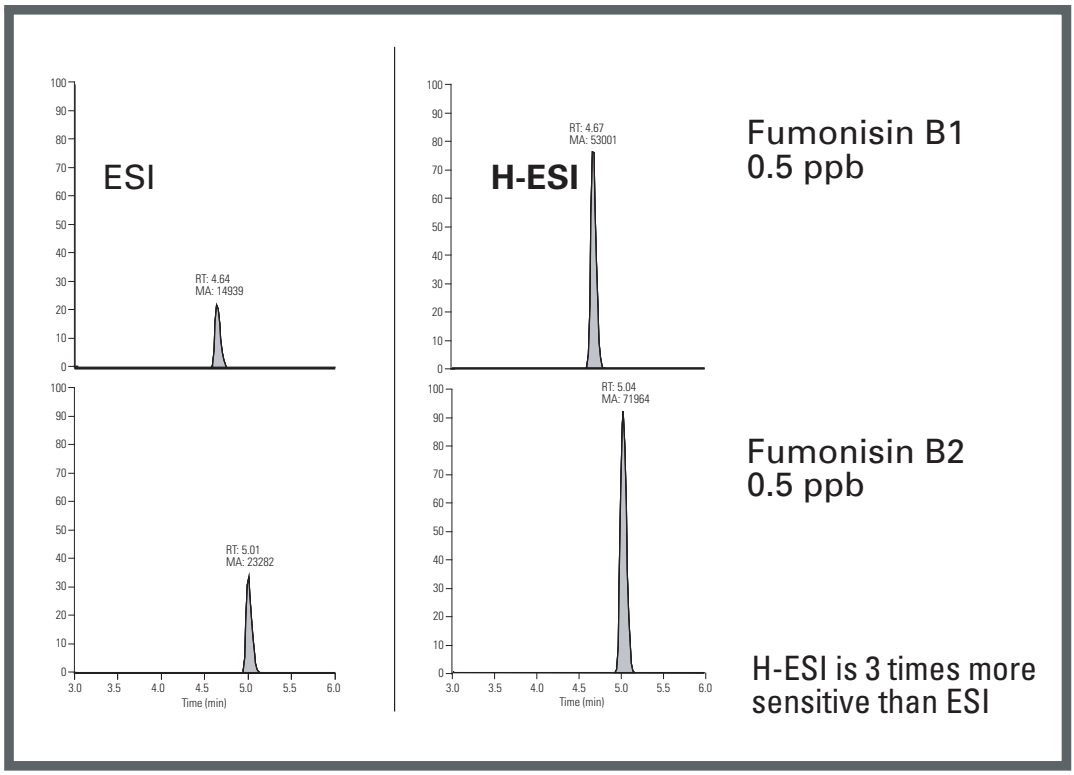


Figure 10: Increased sensitivity with the H-ESI probe in Fumonisin data

Conclusion

LC-MS/MS is a major technique for all kinds of environmental safety and food control laboratories. The TSQ Quantum Discovery MAX is the workhorse of the TSQ Quantum series for round the clock productivity. Matrix effects are always an issue with LC-MS/MS methods. However, this application note shows that the TSQ Quantum Discovery MAX can help overcome these effects with its unique features of H-SRM and H-ESI. The results presented here were obtained without extensive preparation. A wide range of matrices were analyzed and excellent results were obtained.

Acknowledgements

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