Application Note: 303

# Analysis of Vomitoxin (DON) and T-2 Toxin by LC/MS


Key Words

- Fungal Infections
- Pseudomolecular Ion Species
- LC/MS
- Finnigan™ Surveyor™ MSQ


## Chromatography and Mass Spectrometry Application Note


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### Introduction

Grain and grain products may be susceptible to a variety of fungal infections. *Fusarium* species fungi are known to produce a number of mycotoxins including Vomitoxin (DON) and T-2 Toxin. These mycotoxins may evolve pre-harvest in the field or post harvest during processing, storage and transportation. The presence of these mycotoxins can lead to human and animal toxicology and in the severest cases, death. The US FDA (United States Food and Drug Administration) has issued 'advisory' limits for Vomitoxin in grain and grain products as follows:

1 ppm–Finished wheat products for human consumption.
5 ppm–Grain and grain by-products destined for swine and other non ruminant species.
10 ppm–Grain and grain by-products for ruminating beef and feedlot cattle older than 4 months.

Additional restrictions on percentage make up of the diet are also advised.

*Fusarium* infections of grain products affect not only the vigor of the crop itself leading to lower yields, but also the quality of the crop as a feedstuff. The severity of effect of the mycotoxin contamination is to a large extent dependent on the age and species of the particular livestock. In particular, pigs are susceptible to even low levels of Vomitoxin in their feed.

### Goal

This report describes a simple LC/MS method for the determination of fungal mycotoxins. It will allow analysts to develop and optimize sample preparation, extraction and clean-up techniques for these important contaminants. The flexibility of simultaneous SIM and scanning acquisition will facilitate the development of quantitative and qualitative mothodologies.


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### Chromatographic Conditions

| | |
|---|---|
| LC Column: | 150×2.1 mm |
| Packing: | 5 µm C18 |
| Flow Rate: | 200 µL/min |
| Mobile Phase: | Water/Methanol |
| Gradient: | 80/20 to 20/80 in 10 min, held 5 min. |
| Injection Volume: | 10 µL |
| Column Temp: | 40 °C |

### Mass Spectrometer Conditions

| | |
|---|---|
| Instrument: | Surveyor MSQ |
| Ionization: | Electrospray |
| Polarity: | Positive |
| Needle Voltage: | 3 keV |
| Probe Temp: | 350 °C |
| Cone Voltage: | 90 eV |
| Scan Range: | 100 – 650 Da |
| Scan Rate: | 0.8 seconds per scan |
| Data Acquisition: | Centroid |
| Selected Ions: | *m/z* 319 (M+23) and 489 (M+23) |
| Dwell Time: | 100 ms |

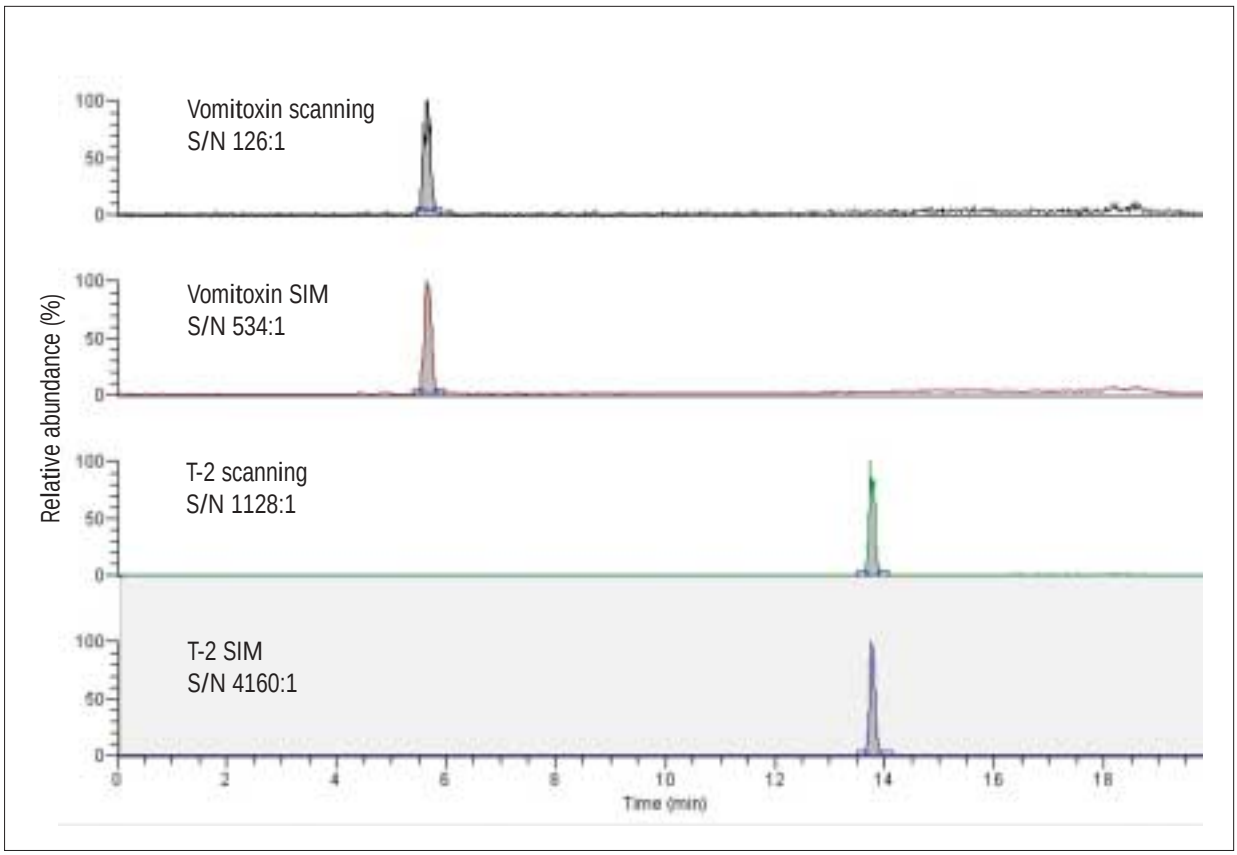


Figure 1. SIM and Scanning Mass Chromatograms can be run simultaneously.

## Results and Discussion

Figure 1 shows the unique capability of the Surveyor MSQ to collect selected ion monitoring (SIM) and scanning data from the same run. The improvement in signal to noise from the scanning (upper) to the selected ion (lower) acquisitions is shown on the pseudomolecular ion traces for both Vomitoxin (DON) and T-2 Toxin respectively. This allows low level quantitative data and qualitative survey data to be obtained from the same injection.

The full-scan mass spectral data obtained from this run is shown in Figure 2. Pseudomolecular ion species, presumably sodium ion adducts, were observed at [M+23] for both the Vomitoxin (upper) and the T-2 Toxin (lower).

The calibration data from 5-500 pg/µL for Vomitoxin is shown in Figure 3. The selected ion monitoring chromatogram for the 5 pg/µL standard shows a signal to noise ratio of 28 to 1 and the correlation co-efficient ($R^2$) was 0.9994. For T-2 Toxin a similar calibration line was observed (not shown) giving a signal to noise of 7 to 1 for the 5 pg/µL standard level and a correlation coefficient ($R^2$) of 0.9982.

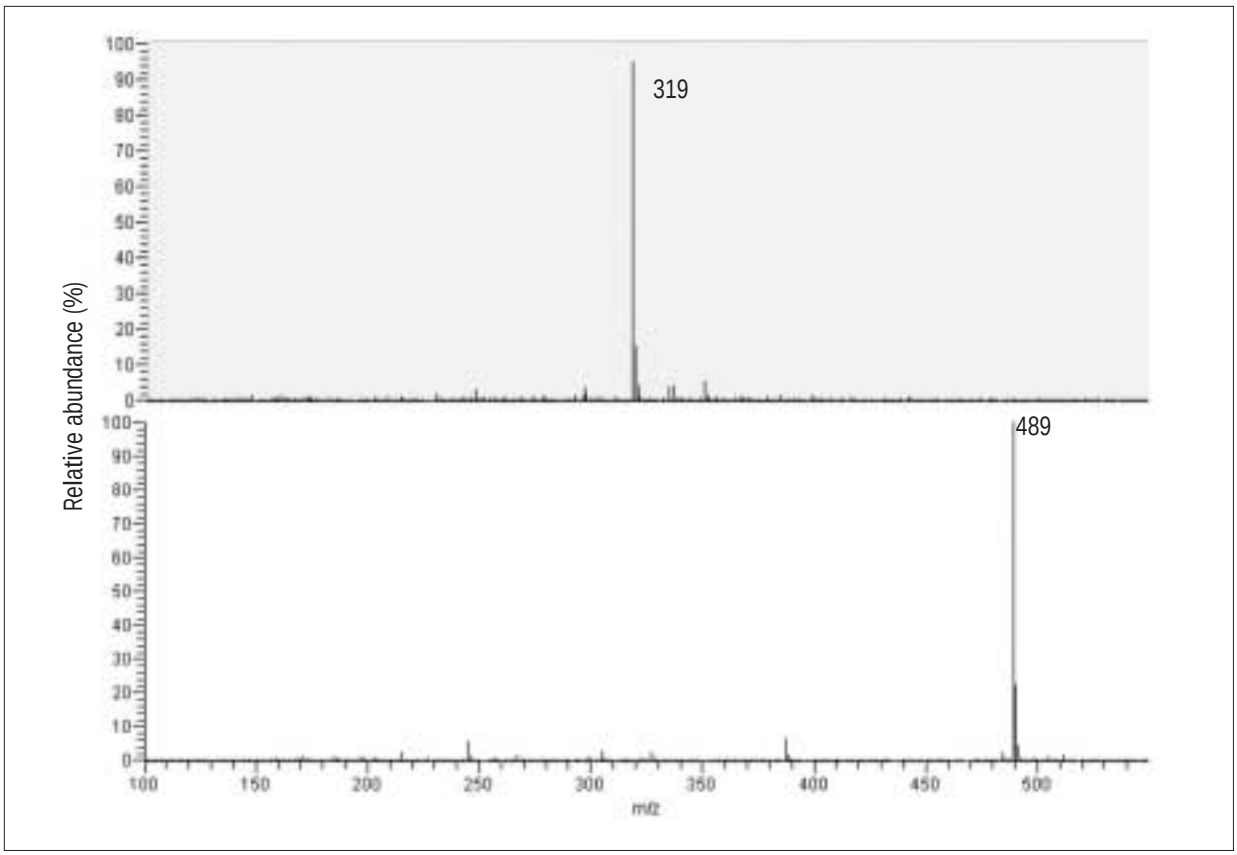


Figure 2. Vomitoxin (upper) and T-2 Toxin (lower) mass spectra

## Conclusions

A simple LC/MS method for the separation and analysis of these important fungal metabolites is shown. The data show excellent linearity and sensitivity for these compounds that would readily allow determination on contamination to below ppm levels in grain samples when combined with a suitable extraction technique. The unique capability of the Surveyor MSQ to acquire both SIM and scanning data from the same sample injection is demonstrated

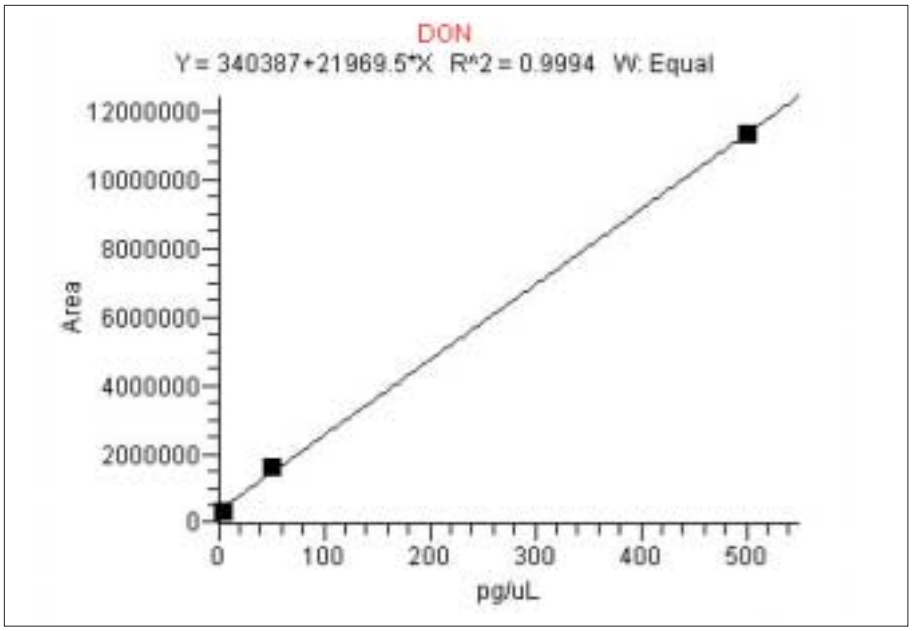


Figure 3. Vomitoxin calibration data

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