



# Analysis of Multiple Mycotoxins in Animal Feed

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# Mycotoxins and Regulatory Limits

FDA Center for Veterinary Medicine (CVM) focuses on 5 major mycotoxins in the US:

- ❑ Action levels – aflatoxins  
[http://www.fda.gov/ora/compliance\\_ref/cpg/cpgvet/cpg683-100.html](http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg683-100.html)
- ❑ Guidance levels – fumonisins  
<http://www.fda.gov/cvm/fumonisin.htm>
- ❑ Advisory levels – Deoxynivalenol (DON)  
<http://www.cfsan.fda.gov/~dms/graingui.html>
- ❑ No action, guidance or advisory levels for ochratoxin A (OTA) or zearalenone (ZON) have been established by the FDA in animal feeds.

## Advisory levels for Deoxynivalenol in livestock feed

| Class of Animal  | Feed Ingredients & Portion of Diet                        | DON Levels in Grains & Grain By-products and (Finished Feed) |         |
|--|---|--|---------|
| Ruminating beef and feedlot cattle older than 4 months | Grain and grain by-products not to exceed 50% of the diet | 10 ppm   | (5 ppm) |
| Chickens   | Grain and grain by-products not to exceed 50% of the diet | 10 ppm   | (5 ppm) |
| Swine  | Grain and grain by-products not to exceed 20% of the diet | 5 ppm  | (1 ppm) |
| All other animals                                      | Grain and grain by-products not to exceed 40% of the diet | 5 ppm  | (2 ppm) |

# Mycotoxin Action Limits (OTSC Regulations)

- ❑ **Fumonsins:** total Level of Fumonisin (FB<sub>1</sub>+FB<sub>2</sub>+FB<sub>3</sub>) are over 5 ppm

grain, oilseeds, processed grain, and oilseed meal containing fumonisin above 5 parts per million (ppm) except that with proper labeling as approved by the Office of the Texas State Chemist and targeted for animal species as follows: <20 ppm for swine and catfish not to exceed 50% of diet; <30 ppm for breeding ruminants, breeding poultry and breeding mink not to exceed 50% of diet; <60 ppm for ruminants > 3 months old being raised for slaughter, and mink being raised for pelt production not to exceed 50% of diet; <100 ppm for poultry being raised for slaughter not to exceed 50% of diet; all other species or classes of livestock and pet animals <10 ppm not to exceed 50% of diet except equids and rabbits which should not exceed 5 ppm and 20% of diet; >100 ppm requires a blending permit issued by the Office of the Texas State Chemist. (§61.61 Poisonous or Deleterious Substances)

- ❑ **Aflatoxins:** total Aflatoxins (AFB<sub>1</sub>+AFB<sub>2</sub>+AFG<sub>1</sub>+AFG<sub>2</sub>) are over 20 ppb

grain, oilseeds, processed grain and oilseed meals containing aflatoxin B1, B2, G1, G2 above 20 parts per billion (ppb) individually or total except that with proper labeling as approved by the Office of the Texas State Chemist as follows: <50 ppb may be distributed when destined for wildlife; <100 ppb may be distributed when destined for breeding cattle and breeding goats not used in production of milk for human consumption, breeding swine, mature poultry, and sheep; <200 ppb may be distributed when destined for finishing swine (more than 100 lbs. body weight); <300 ppb may be distributed when destined for finishing cattle in confinement; grain containing >300 to <500 ppb requires a blending permit issued by the Office of the Texas State Chemist; aflatoxin >500 ppb in grain and >300 ppb in oilseed, processed grain, and oilseed meal may not enter commerce and a record of disposition shall be submitted to the Office of the Texas State Chemist.

(§61.61 Poisonous or Deleterious Substances)

- ❑ **Other mycotoxins including DON, OTA and ZON**

No action limit has been established yet.

# Analytical Methods

## □ Immunoassays

Pros: High throughput, Semi-quantitative

Cons: Low sensitivity, Sample purification required, Poor linearity.

**(AOAC OMA 994.01-ZON, 986.17-DON)**

## □ HPLC/UV or Fluorescence Methods

Pros: Matrix effect independent, acceptable sensitivity, robust

Cons: Time consuming, Error-prone cleanup steps involved in sample preparation.

**(AOAC OMA 985.18-ZON, 2000.03-OTA)**

## □ HPLC/MS Methods

Pros: Robust, Sensitive, High throughput, no extra cleanup.

Cons: Expensive instrumentation

**AOAC OMA ?**

# Sample Preparation and Analysis



Sample



Extraction  
ACN:Water 84:16



Addition of  
Internal Standard



Filtration/Dilution

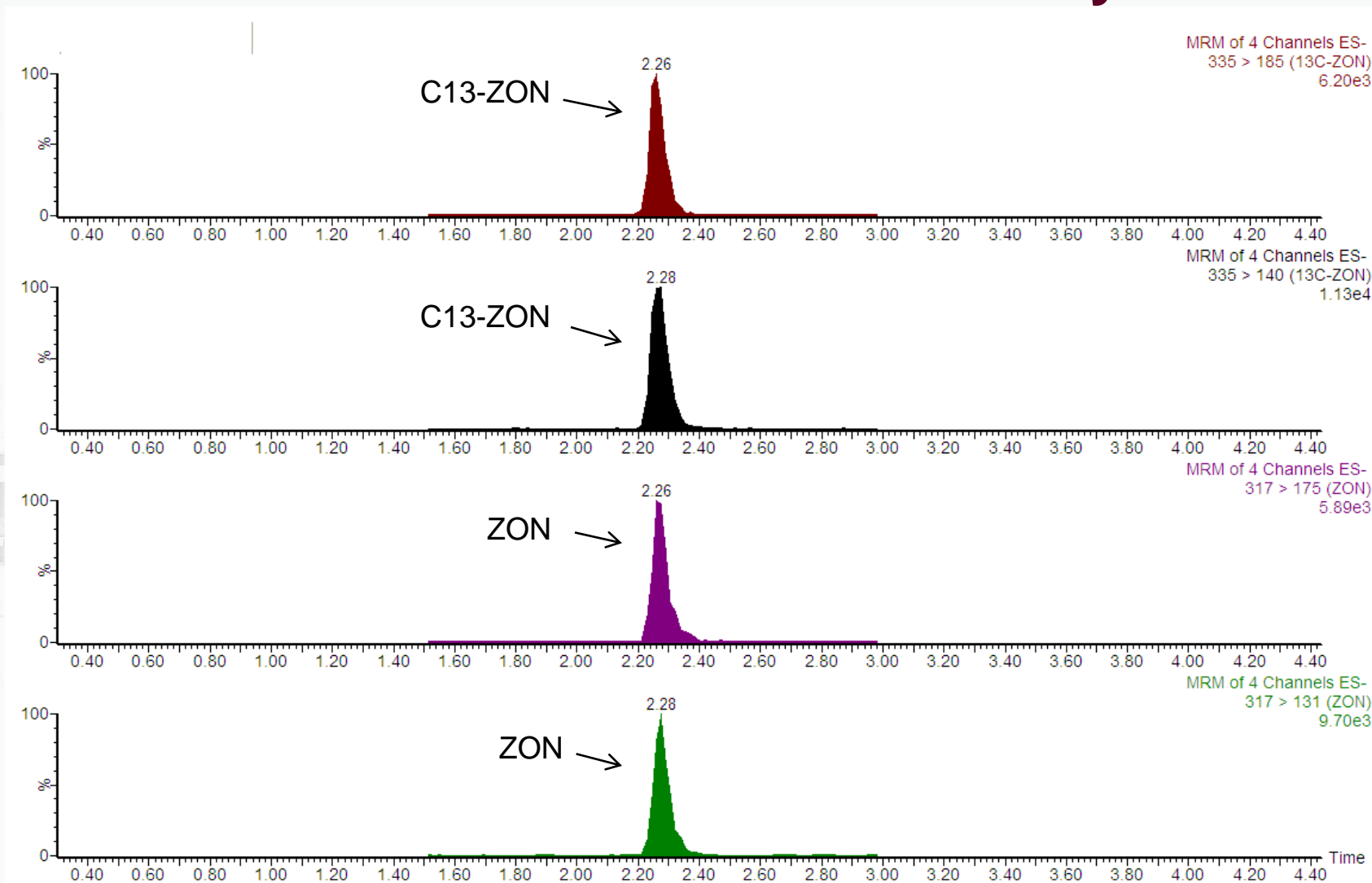


LC/MS  
Analysis

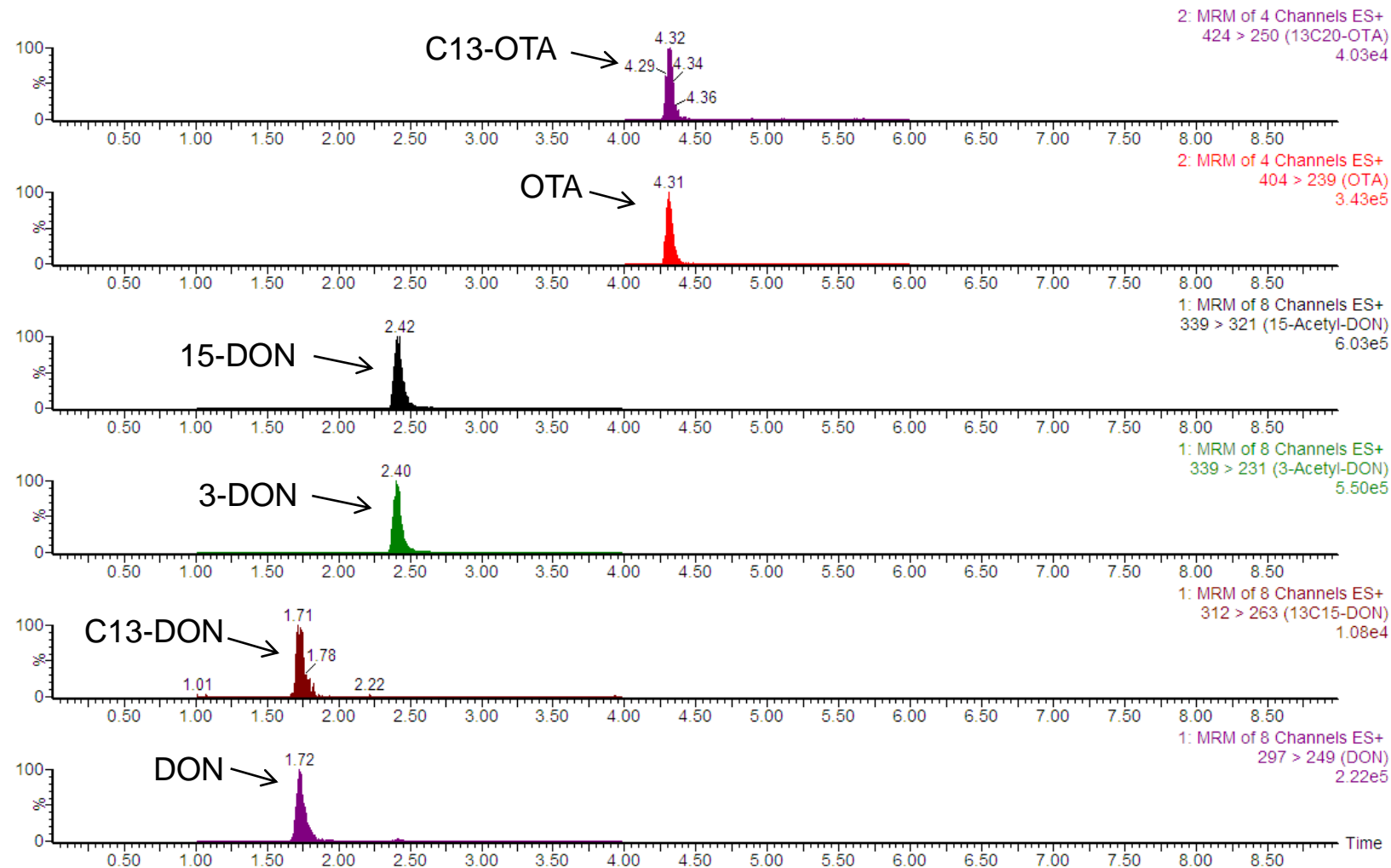




# LC/MS/MS Method for ZON Analysis



# LC/MS/MS Method for DON and OTA Analysis





# Limit of Quantitation (LOQ)

| Analyte | Maximum Tolerable Levels in Complete Feed (ppb)* | LOQ of HPLC method (ppb) | LOQ of LC/MS method (ppb) |
|---------|--|--------------------------|---------------------------|
| DON     | 2000   | 200                      | 75                        |
| OTA     | 5  | 1                        | 0.5                       |
| ZON     | 200  | 10                       | 2                         |

\* Based on FDA guidance or EC regulation.

## Mycotoxin Screening in 2011 (June-October)

|   | DON (ppm) | OTA (ppb) | ZON (ppb) | AF (ppb) | FB (ppm) |
|---|-----------|-----------|-----------|----------|----------|
| FDA Guidance                            | 2         | -         | -         | 20       | 5        |
| EU Regulation                           | 1.25      | 5         | 200       | 20       | 5        |
| Number of Violations                    | 0         | 17        | 0         | 82       | 38       |
| Percentage of Violations in all samples | 0         | 6%        | 0         | 31%      | 14%      |

266 regulatory samples (maize) were analyzed in total.

Note: The results for DON and OTA are preliminary because the screening method was not validated yet at that time, and methanol/water but not acetonitrile/water solvent were applied for extraction.

# Mycotoxin Analysis in Maize 2011

| Sample ID | DON (ppm) | OTA (ppb) | ZON (ppb) | AF (ppb) | FB (ppm) |
|-----------|-----------|-----------|-----------|----------|----------|
| RS1       | 0         | 43        | 0         | 161      | 5        |
| RS2       | 0         | 22        | 0         | 158      | 4        |
| RS3       | 0         | 17        | 0         | 246      | 0        |
| RS4       | 0         | 13        | 0         | 140      | 0        |
| RS5       | 0         | 10        | 0         | 33       | 3        |
| RS6       | 0         | 23        | 0         | 369      | 0        |
| RS7       | 0         | 15        | 0         | 207      | 0        |
| RS8       | 0         | 13        | 0         | 34       | 2        |
| RS9       | 0         | 36        | 0         | 495      | 7        |
| RS10      | 0         | 26        | 0         | 101      | 3        |
| RS11      | 0         | 38        | 0         | 95       | 10       |
| RS12      | 0         | 52        | 0         | 223      | 15       |
| RS13      | 0         | 10        | 0         | 0        | 0        |
| RS14      | 37        | 24        | 0         | 137      | 1        |
| RS15      | 52        | 9         | 0         | 118      | 0        |
| RS16      | 0         | 23        | 0         | 120      | 0        |
| RS17      | 0         | 44        | 0         | 126      | 6        |

17 out of 266 samples were found with AF levels higher than 100 ppb. 13 of these 17 samples have OTA higher than 5 ppb.

# Mycotoxin Analysis in Cottonseed 2012

| Sample ID | DON (ppm) | OTA (ppb) | ZON (ppb) | AF (ppb) | FB (ppm) |
|-----------|-----------|-----------|-----------|----------|----------|
| RS1       | 0         | 56        | 0         | 73       | 0        |
| RS2       | 0         | 146       | 0         | 301      | 0        |
| RS3       | 0         | 0         | 0         | 66       | 0        |
| RS4       | 0         | 0         | 0         | 10       | 0        |
| RS5       | 0         | 221       | 0         | 328      | 0        |
| RS6       | 0         | 90        | 0         | 80       | 0        |
| RS7       | 0         | 0         | 0         | 29       | 0        |
| RS8       | 0         | 0         | 0         | 33       | 0        |
| RS9       | 0         | 0         | 0         | 0        | 0        |
| RS10      | 0         | 0         | 0         | 0        | 0        |

Ten regulatory samples (cottonseed) were analyzed with validated methods.

**Most of the samples with OTA are observed to contain aflatoxins.**

# Summary

- LC/MS/MS methods for quantifying deoxynivalenol, ochratoxin A and zearalenone in animal feed were established.
- Among 266 maize samples tested in 2011, 17 samples (6%) were found with OTA levels higher than 5 ppb.
- Among 10 cottonseed samples analyzed in 2012, 4 samples were found with OTA levels higher than 5 ppb.
- Most of the samples with OTA are observed to contain aflatoxins.

# Acknowledgement

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