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# Determination of Alternariol in Tomato Products and Diacetoxyscirpenol in Corn by Immunoassay

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## Abstract

Competitive enzyme-linked immunosorbent assays (ELISA) for the detection of the mycotoxins Alternariol (AOH) and Diacetoxyscirpenol (DAS) were developed. Rabbit polyclonal antibodies were produced and used for the development of these assays. The lower limit of quantification (LLOQ) for AOH and DAS in each ELISA was 0.2 ppb and 0.1 ppb, respectively. The AOH assay calibration curve ranges from 0.2 ppb to 20 ppb whereas the DAS assay has a calibration range of 0.1 ppb to 10 ppb. Extraction of AOH residues from various tomato products used methanol followed by centrifugation. DAS extraction from corn used an 80% methanol solution followed by filtration. After dilution of the liquid extracts, each ELISA was run for a total of 70 minutes. The percent recovery from AOH spiked ketchup was determined to be within the range of 107 – 120%. Low ppb levels of AOH residues were found in some of the commercial ketchup and moldy samples tested. Fresh tomatoes were below the LLOQ. Recovery of DAS from spiked ground corn (50 ppb) was determined to be 82%. Both ELISA kits are stable at least 6 months when stored at 4 °C. The results demonstrate that the immunoassay kits developed in this study can be used as rapid and accurate tools for determination of Alternariol and Diacetoxyscirpenol in various food products.

## Introduction

Alternariol (AOH) is a mycotoxin produced by one of the *Alternaria* species as a secondary metabolite. These metabolites include alternariol monomethyl ether, altenuene, tentoxin and tenuazonic acid. *Alternaria* species are ubiquitous pathogens and common fungal contaminants in decaying fruits and vegetables. Natural occurrences of AOH have been reported in various fruits, processed fruit products such as apple juice, tomato products, wheat and other grains, sunflower seeds and pecan. Soft-skinned vegetables and fruits such as tomatoes are especially susceptible to invasion by *Alternaria* species. Analytical methods such as liquid chromatography (LC) and gas chromatography (GC) have been developed for determination of AOH. However, those instrument based methods are expensive, and sample cleanup procedures are time consuming. Currently, there are no regulations for the presence of AOH in food or feed.

Diacetoxyscirpenol is one of the type A trichothecene mycotoxins produced by *Fusarium* species as secondary metabolites. Trichothecenes are inhibitors of protein and DNA synthesis. Acute and sub-acute toxicity is characterized by feed refusal and weight loss in animals and by increased susceptibility to infectious diseases. Type A trichothecenes have been reported to have extremely toxic effects on skin and mucous surfaces. Gas chromatography has been used for simultaneous detection of type A and B trichothecenes. However, a derivatization step is generally necessary to turn mycotoxins into volatile compounds for these detection methods. HPLC-UV method also has been used involving pre-column derivatization due to lack of characteristic UV absorption. These instrumental analyses are expensive and cumbersome. In this study, competitive ELISAs were developed and evaluated allowing for simple and accurate determination of AOH and DAS in tomato products and corn grain samples.

## Methodology

**Polyclonal antibody production:**

In the development of these assays, AOH or DAS was coupled to a protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. The antiserum was collected monthly.

**Sample preparation:**

❖ **For AOH ELISA**

1. Weigh 5 g into a clean glass vial.
2. Add 50 mL of 80% Methanol in water.
3. Vortex for 2 minutes.
4. Transfer 1 mL into a microcentrifuge tube.
5. Centrifuge for 3 minutes at 6,000 rpm.
6. Dilute the supernatant with deionized water.
7. Sample extract is ready for testing.

❖ **For DAS ELISA**

1. Weigh 10 g in a clean glass vial.
2. Add 50 mL of 80% Methanol in water.
3. Shake vigorously for 3 minutes.
4. Filter (Fisher P5) extract.
5. Dilute the filtered extract with deionized water.
6. Sample extract is ready for testing.

**Assay protocol:**

1. Add 50 µL of HRP conjugate to each well and add 50 µL of calibrator or sample extract.
2. Add 50 µL of antibody solution to the wells and mix gently for 30 seconds. Incubate the wells for 30 minutes.
3. Dump the well contents and wash 5 times with deionized water.
4. Add 100 µL of substrate solution into each well and incubate for 30 minutes.
5. Add 100 µL of stop solution and measure absorbance at 450 nm – 650 nm.
6. The sample concentration is calculated from the calibration curve multiplied by the total dilution factor used during the sample extract preparation.

## Results

**Figure 1. Calibration curves.**

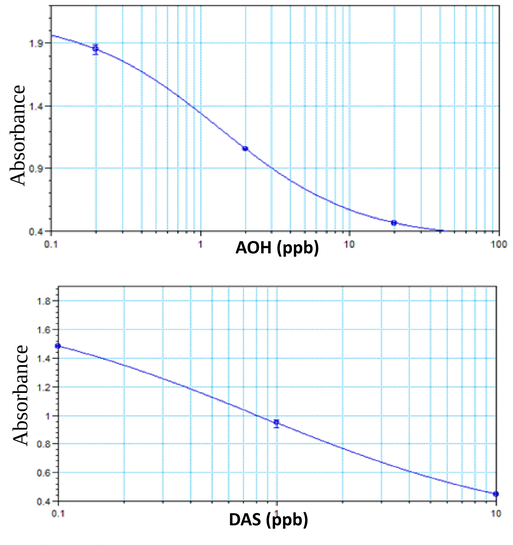


**Table 1. OD data of AOH and DAS calibrators.**

| AOH Calibrator Conc. | OD | Average OD ± SD | %RSD | %B0 | Matrix Conc. (X50) |
|---|---|---|---|---|---|
| Blank | 2.107<br>2.108 | 2.107 ± 0.000 | 0 | 100 | ND |
| 0.2ppb | 1.825<br>1.880 | 1.852 ± 0.039 | 2.1 | 88 | 10 ppb |
| 2 ppb | 1.055<br>1.061 | 1.058 ± 0.004 | 0.4 | 50 | 100 ppb |
| 20 ppb | 0.470<br>0.463 | 0.466 ± 0.005 | 1.1 | 22 | 1.0 ppm |

| DAS Calibrator Conc. | OD | Average OD ± SD | %RSD | %B0 | Matrix Conc. (X50) |
|---|---|---|---|---|---|
| Blank | 1.836<br>1.781 | 1.808 ± 0.039 | 2.1 | 100 | ND |
| 0.1 ppb | 1.488<br>1.482 | 1.485 ± 0.004 | 0.2 | 82 | 5 ppb |
| 1 ppb | 0.925<br>0.963 | 0.944 ± 0.027 | 2.8 | 52 | 50 ppb |
| 10 ppb | 0.446<br>0.452 | 0.449 ± 0.005 | 1.0 | 25 | 500 ppb |

**Table 2. Recovery of AOH and DAS spiked in ketchup samples and corn.**

| Mycotoxin | Matrix | Spike | Measured (ppb) | Recovery (%) |
|---|---|---|---|---|
| AOH | Ketchup A | Blank | 29.6 | --- |
| | | 50 ppb | 89.4 | 120 |
| | Ketchup B | Blank | 12.9 | --- |
| | | 50 ppb | 66.3 | 107 |
| | Ketchup C | Blank | 14.1 | --- |
| | | 50 ppb | 71.9 | 116 |
| DAS | Corn Grain | Blank | 0.20 | --- |
| | | 50 ppb | 41.2 | 82 |

## Results

**Table 3. AOH found in commercial tomato ketchup samples.**

| Ketchup Sample | AOH (ppb) |
|---|---|
| A | 24.3 |
| B | 12.9 |
| C | 22.4 |
| D | 14.2 |
| E | 22.6 |
| F | 13.8 |

## Discussion

Competitive immunoassays were developed for determination of AOH and DAS in various processed tomato products and corn (grain) respectively. The tomato products tested here using the AOH ELISA were found to contain low levels of AOH residues. Tables 2 & 3 contain results from ketchup testing where results ranged from 13-30 ppb. Similar levels (ppb) of AOH were also found in other tomato products such as pizza sauce, condensed tomato soup and pasta sauce (data not shown). Prelle *et al*. found AOH residues in various tomato products with levels of 4.0 – 6.8 ppb. Scott *et al.* found that 70 out of 83 grain samples tested positive for the presence of AOH residues up to levels of 63 ppb. The AOH levels found in these products could be from concentration and accumulation occurring during food processing, however further analysis is needed to confirm the residue concentrations.

Trace levels of DAS were found in a composite corn sample (0.2 ppb, below LLOQ). This sample was used for evaluation of the spike and recovery data (Table 2). 82% of the DAS residues were recovered when a 50 ppb (ng/g) spiked sample was extracted and quantified. The DAS assay developed is sensitive enough to meet the current regulations for this compound which is less than 200 ppb in grain (Israel), and in swine and poultry feed at 2 ppm and 1 ppm respectively (Canada). It was found that both immunoassay kits are stable for more than 12 months when stored at 4 °C. There are few if any commercially available ELISA kits for analysis of these 2 toxins. These results indicate that the immunoassays developed in this study can be used as rapid and accurate tools for the determination of AOH in various tomato products and DAS in grain samples.

## References

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