SHIMADZU

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Determination of mycotoxins in dry feed

Laudicéia Alves de Oliveira[1], Marcelo Viana de Moraes[2], Edivaldo Domingues Velini[2], Ichiro Hirano[1] , Marcos Albieri Pudenzi [1], Marília Santoro Cardoso[1]
(1) Shimadzu do Brazil Ltda; (2) Bioativa, Brazil.

1. Introduction

- Mycotoxins, secondary metabolites of some fungus species are present in several type of food and are toxic for humans and animals, even though for animal nutrition system this toxins are ignored. However, several studies have been observed that mycotoxins can affect beef cattle and lamb performance, and there are not safe levels of this toxins for small animals, like dogs and cats, once these dry feed are the principal nutrition source for these animals. Nacional programs for official control of feed determine levels of Aflatoxins (B1, B2), Ochratoxin A, Deoxynivalenol, Zearalenone, and Fumonisins (B_1 and B_2) **(Fig 1).**
- So the aim of this work was to develop a method to determination of mycotoxin in dry feed using LCMS.

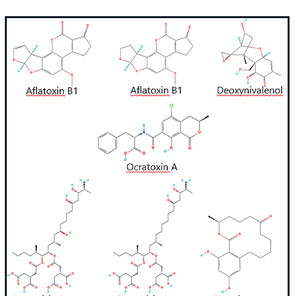


Figure 1- Molecular structure of the mycotoxins analyzed

2. Methods

Mycotoxins were extracted using 0.8g of dry feed solubilized in 2mL of water under agitation for 10 minutes, followed by 5 minutes in ultrasound. After this step, 2mL of Acetonitrile with 1% acetic acid was added following the previous stirring and ultrasound steps. Subsequently, 0.8g of MgSO4 and 0.2g of NaCl were added and vortexed for 2 minutes. 1mL of supernatant was removed and centrifuged at 4,600 rpm, -4ºC, for 15 min. The post-centrifugation supernatant was placed in a vial for injection into the LCMS.

- The analyzes were performed using the liquid chromatograph, NexeraXR with high pressure gradient, and the mass spectrometer LCMS-8060NX (Shimadzu Corporation, Japan) **(Fig 2).**

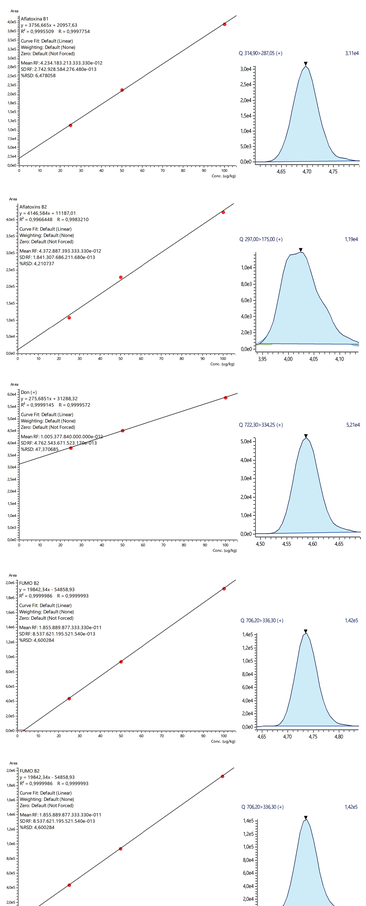
Figure 2 - Nexera LC-40D XR HPLC coupled to the LCMS-8060NX

Column	Shimpack FC-ODS (150 x 2 mm, 3 um) P/N 228-40511-95
Mobile Phase	A – Acetic Acid 0,1% in H2O; B – Acetic Acid 0,1% in methanol
Time Program	0 a 2 min = 10% B; 2 a 4 min = 95% B; 4 a 8min = 95%; 8,01 a 12 = 10% B.
Flow	0,4 mL/min
Column oven temperature	30° C
Injection Volume	2,5 µL
DL Temperature	150 ° C
Heat Block Temperature	200 ° C
Interface Temperature	200 ° C
Nebulizer Gas Flow	3 L/min
Drying Gas Flow	10 L/min
Heating Gas Flow	10 L/min

Table 1 – Analytical Conditions

3. Results

- The analyzes got matrix curves with excellent linearity (R^2>0.99) and sensitivity for the first point of the curve (25µg/Kg), as shown in **Figure 3**. For this method, a quick and effective extraction method was used that does not require cleaning steps.-up. Excellent results were obtained with recovery above 70% for all compounds, reaching quantification limits 100x lower than those required by Brazilian and international regulations



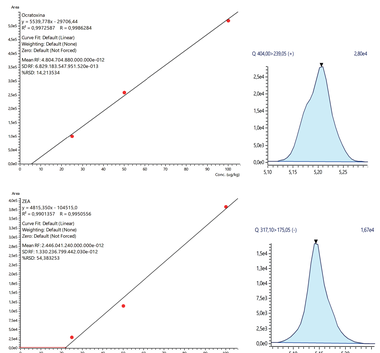
Aflatoxin B1

Aflatoxin B2

Deoxynivalenol

Fumonisin B1

Fumonisin B2



Ocratoxin

Zearalanone

Figure 3 – Calibration curve and peak at the first concentration level

- The chromatogram at first point of the analytical curve is shown in **Figure 5**

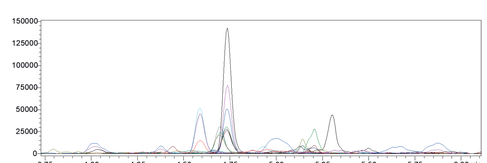


Figure 5 - Chromatogram at the first point of the calibration curve

4. Conclusion

- Using the LCMS-8060NX and a simple sample preparation method, it was possible to obtain satisfactory results for the determination of mycotoxins in dry feed to meet the needs determined by regulatory departments.