Acetonitrile extraction: for the analysis of multi-toxins in animal feeds

11⁺Myco MS-PREP® Art. No.: P128

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Introduction

EU regulations for mycotoxins are complex with varying limits applied to specific commodities. This has resulted in an increasing trend in multi-mycotoxin analysis within the food and feed industry. As a result, there has been greater demand for multi-toxin immunoaffinity columns to effectively remove sample matrix from complex commodities such as animal feeds to ensure compliance with EU method performance criteria. This study summaries the validation of a new acetonitrile extraction prior to clean-up using a multi-toxin immunoaffinity column; 11⁺Myco MS-PREP® for animal feeds such as silage and forage. A multi-toxin dried distillers grains (DDGS) reference material (Trilogy Analytical Lab, USA) was also included to assess the accuracy and reliability of this clean-up method. Samples were extracted and passed through n=3 replicate IAC and calculated contamination, % recovery and % RSD were reported. For the reference material, the calculated contamination was corrected for recovery prior to reporting. Solvent-based calibration standards were used for quantification throughout and were compared against matrix-matched calibration standards to assess matrix effects.

Method

1. Weigh 3 g of sample into a centrifuge tube..
2. Add 25 ml of acetonitrile : water : formic acid (79:20:1, v/v/v) and shake for 30 minutes.
3. Filter or centrifuge.
4. Dilute 2 ml of filtrate with 98 ml of PBS.
5. Pass 30 ml of diluted filtrate through the column.
6. Wash qith 10 ml of 0.1 % Tween 20 in PBS followed by 10 ml of 20 mM ammonium acetate.
7. Elute the toxin using 1 ml of 100 % methanol followed by 1 ml of water
8. Pass 1.5 ml of water through the column and collect in the same vial.
9. Inject 25 µl onto the LC-MS/MS system.

Results and discussion

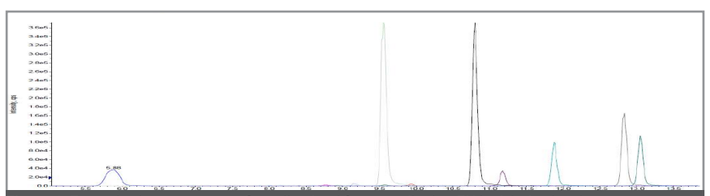

Figure 1: Example chromatogram of small animal forage spiked at EU legislative levels

Table 1: Summary of results for samples spiked at EU legislative levels

	Mean recovery (%) / (% RSD)			
Analyte	**Silage**	**Forage**	**Small Animal Forage**	**DDGS**
Aflatoxin B1	83.1 (3.6)	80.2 (6.0)	75.1 (7.3)	N/A
Aflatoxin B2	85.7 (3.6)	94.1 (9.6)	83.3 (5.1)	N/A
Aflatoxin G1	81.4 (3.0)	84.3 (6.0)	80.8 (6.3)	98.1 (4.1)
Aflatoxin G2	87.2 (11.6)	94.0 (7.0)	76.8 (5.3)	94.3 (3.2)
Ochratoxin A	82.2 (4.3)	86.0 (4.0)	83.7 (4.5)	94.3 (3.4)
Fumonisin B1	83.7 (6.1)	84.6 (3.1)	80.8 (1.9)	N/A
Fumonisin B2	88.9 (4.3)	86.7 (3.4)	86.5 (5.5)	N/A
Deoxynivalenol	89.4 (2.0)	85.4 (1.7)	87.0 (0.8)	N/A
Zearalenone	102.1 (3.6)	103.0 (1.5)	98.6 (1.4)	N/A
T-2	86.7 (1.2)	87.6 (3.5)	83.1 (5.3)	91.4 (0.9)
HT-2	90.7 (3.9)	87.5 (3.1)	89.7 (2.8)	97.7 (8.6)

Table 2: Summary of matrix effects

	% difference in areas between solvent and matrix-matched calibration standards	
Analyte	**Silage**	**Forage**
Aflatoxin B1	0.7	4.0
Aflatoxin B2	4.2	8.2
Aflatoxin G1	8.5	2.0
Aflatoxin G2	4.2	4.5
Ochratoxin A	4.5	2.7
Fumonisin B1	10.4	2.2
Fumonisin B2	7.2	4.7
Deoxynivalenol	0.1	5.7
Zearalenone	4.7	6.2
T-2	6.1	4.2
HT-2	5.6	2.5

Table 3: Summary of calculated contamination of DDGS reference material

	Calculated Concentration (ppb) / (%RSD)	
Analyte	**Calculated contamination**	**Assigned Reference Value**
Aflatoxin B1	18.8 (4.8)	17.5 (N/A)
Aflatoxin B2	5.8 (0.4)	1.1 (N/A)
Aflatoxin G1	N/A	Not reported
Aflatoxin G2	N/A	Not reported
Ochratoxin A	N/A	Not reported
Fumonisin B1	9297 (1.3)	8400 (N/A)
Fumonisin B2	2997 (2.2)	3000 (N/A)
Deoxynivalenol	2341 (3.6)	2700 (N/A)
Zearalenone	170 (1.3)	146 (N/A)
T-2	N/A	Not reported
HT-2	N/A	Not reported

Conclusion

- Matrix effects were <10% for all analytes, demonstrating excellent clean-up with 11⁺Myco MS-PREP®.
- Excellent recoveries were obtained for all spiked animal feed samples and ranged from 75 to 103 % with % RSD generally <10 % demonstrating an accurate and reliable method that complies with EU method performance criteria (EC 401/2006).
- For the reference material, values were consistent with the certified concentrations.
- Chromatography was acceptable with a single peak in the relevant chromatogram for each analyte.
- From previous validation work, the acetonitrile extraction method has also been shown to be acceptable for animal feed pellets and cereal based feeds. Therefore is suitable for the control of 11 regulated mycotoxins using a simple, single sample extraction and clean-up prior to LC-MS/MS detection.

More infomation:

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