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Aflatoxins

Application Note

Analysis of Aflatoxin B1, B2, G1, G2 by LC-MS/MS in cereals using AFFINIMIP® SPE Aflatoxins

Food testing

Introduction

This application note describes an efficient solid phase extraction (SPE) method for the cleanup and analysis of aflatoxin G2, G1, B2, B1 in cereals (barley and basmati rice) using **AFFINIMIP® SPE Aflatoxins** cartridges. The toxin concentrations tested were 1 µg/kg.

Mycotoxins are toxic compounds that are naturally produced by certain types of molds (fungi). Molds that can produce mycotoxins grow on numerous foodstuffs such as cereals, dried fruits, nuts, and spices. Mold growth can occur either before or after harvest; during storage; and on/in the food itself often under warm, damp, and humid conditions. Most mycotoxins are chemically stable and survive food processing [1].
Hundreds of mycotoxins have been identified. Some of them are actively monitored due to a potential or confirmed immediate or long-term concern about human health. For example, aflatoxins, among the most common mycotoxins detected in crops, have been found to be genotoxic and carcinogenic, and therefore, are regulated. E.U. has defined a Minimum Required Performance Limits (MRPLs) of 4µg/kg for the sum of Aflatoxin B1, B2, G1, G2 in cereals (Commission Regulation (EC) No 2023/915).

Compound	Abbreviation	CAS Number
Aflatoxin G2	AG2	7241-98-7
Aflatoxin G1	AG1	1165-39-5
Aflatoxin B2	AB2	7220-81-7
Aflatoxin B1	AB1	1162-65-8

Table 1. List of the tested aflatoxins..

Proceeding of the experiment

Sample preparation

Weight 10 g of crushed cereals into a 50mL centrifuge tube. Add 20 mL of acetonitrile/water/formic acid (79.9/20/0.1; v/v/v). Homogenize by manual agitation, then sonicate for 30 min. Homogenize by manual agitation and centrifuge at 4,000 rpm for 10 minutes. Dilute 5mL of the supernatant with 45mL of ultrapure water to form the loading solution.

Purification with a 3 mL AFFINIMIP® SPE Aflatoxins cartridge

EQUILIBRATION

1. 3 mL 2% acetic acid (in methanol)
2. 3 mL acetonitrile
3. 3 mL ultrapure water

LOADING

6 mL of loading solution at a rate of 0.5-1 mL/min

WASHING

1. 2 mL methanol/water (25/75; v/v)
2. Dry cartridge for 3 minutes by applying full vacuum.

ELUTION

3 mL Acetonitrile

Analysis

20µL of acetic acid is added to the elution (to stabilize the aflatoxins during evaporation). The elution is then evaporated to dryness under nitrogen stream at 45°C for 30 minutes. The residue is then dissolved in 1mL of Methanol/ 5mM ammonium acetate (in water) +0.5% acetic acid (50/50; v/v) prior to analysis.

The analytes were simultaneously analysed by LC-MS/MS. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

		Barley			Rice (Basmati)		
Compound	Spike level (µg/kg)	[C] in blank (µg/kg)	% Recovery	% RSD (n = 3)	[C] in blank (µg/kg)	% Recovery	% RSD (n = 3)
Aflatoxin G2	1	ND	104	1	ND	97	4
Aflatoxin G1	1	ND	109	1	ND	97	5
Aflatoxin B2	1	ND	100	3	ND	105	2
Aflatoxin B1	1	ND	97	4	ND	106	2

Table 2. Recovery obtained for tested analytes, and corresponding concentrations. The same cleanup procedure was repeated several times (n) for each matrix, from which the percent relative standard deviation (% RSD) was calculated to determine reproducibility of the method. (ND = Not detected).

LC Conditions			MS Conditions				
LC Dionex U3000			Qtrap 4000 ESI+ MS/MS				
Column : SilactHPLC – LC.A 150*2.1mm at 30°C			Curtain gas: 25				
			CAD: Medium				
Injection volume : 20µL			IS: 5500V				
T° sampler : 10°C			Temperature: 500°C				
Flow rate : 0.2mL/min			GS1/GS2: 50/50				
Time (min)	**Solvent A**	**Solvent B**	**Analyte**	**Retention time (min)**	**Q1**	**Q3**	**CE (V)**
0	85%	15%	Aflatoxin G2	8.72	331.1	189.1	57
1	85%	15%			331.1	313.0	35
6	5%	95%	Aflatoxin G1	8.90	329.1	243.1	39
14	5%	95%			329.1	200.1	57
15	85%	15%	Aflatoxin B2	9.15	315.1	287.1	37
22	85%	15%			315.1	259.1	41
Solvent A : 5mM ammonium acetate (in water) + 0.5% acetic acid **Solvent B** : 5mM ammonium acetate (in methanol) + 0.5% acetic acid			Aflatoxin B1	9.27	313.2	285.2	33
					313.2	241.2	53

Table 3. LC-MS/MS conditions for tested analytes.

Conclusion

AFFINIMIP® SPE Aflatoxins has been successfully used for the enrichment and cleanup of aflatoxin G2, G1, B2, B1 in barley and basmati rice. The method has shown excellent performances with recoveries from 97% to 109% and a good repeatability.

References

1. World Health Organization website: mycotoxins, May 9 2018

Product reference

- **AFFINIMIP® SPE Aflatoxins**

Catalog number: **FS120-03** for 50 cartridges 3mL

- **SilactHPLC – LC.A** 150x2.1cm 3µm

Catalog number: **C18LCP-150.2.1** for 1pc