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Application Note

Analysis of 11 regulated mycotoxins by LC-MS/MS in cereals using AFFINIMIP® SPE Multimyco LCMSMS

Food testing

Introduction

This application note describes an efficient solid phase extraction (SPE) method for the cleanup and analysis of 11 mycotoxins in cereals (barley and small spelts) using **AFFINIMIP® SPE Multimyco LCMSMS** cartridges. Aflatoxin B1, B2, G1, G2, fumonisin B1, B2, ochratoxin A, deoxynivalenol, zearalenone, toxin T2, and HT2 toxin were extracted and analyzed simultaneously. The toxin concentrations tested varied from 1.25 to 100 µg/kg, depending on the mycotoxin.

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. Other mycotoxins such as fumonisins, ochratoxin A, zearalenone, nivalenol, deoxynivalenol, T2, HT2 toxin, and patulin also fall under regulations[2] given concerns over their effects on health.

Compound	CAS Number	Category
Deoxynivalenol	DON	51481-10-8
Aflatoxin G2	AG2	7241-98-7
Aflatoxin G1	AG1	1165-39-5
Aflatoxin B2	AB2	7220-81-7
Aflatoxin B1	AB1	1162-65-8
Fumonisin B1	FB1	116355-83-0
HT2 toxin	HT2	26934-87-2
T2 toxin	T2	21259-20-1
Fumonisin B2	FB2	116355-84-1
Ochratoxin A	OTA	303-47-9
Zearalenone	ZON	17924-92-4

Table 1. List of the tested mycotoxins.

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 70mL of ultrapure water to form the loading solution.

Purification with a 3 mL AFFINIMIP® SPE Multimyco LCMSMS 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 (10/90; v/v)
2. Dry cartridge for 3 minutes by applying full vacuum.

ELUTION

- 3 mL methanol

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			Small spelts		
Compound	Spike level (µg/kg)	[C] in blank (µg/kg)	% Recovery	% RSD (n = 3)	[C] in blank (µg/kg)	% Recovery	% RSD (n = 3)
Deoxynivalenol	100	ND	77	4	53.2	78	7
Aflatoxin G2	2.5	ND	110	12	ND	115	1
Aflatoxin G1	1.25	ND	109	14	ND	89	8
Aflatoxin B2	3.75	ND	106	5	ND	98	10
Aflatoxin B1	3.75	ND	102	6	ND	97	4
Fumonisin B1	50	ND	96	7	ND	74	5
HT2 toxin	50	ND	96	2	ND	97	1
T2 toxin	5	ND	103	6	ND	104	3
Fumonisin B2	37.5	ND	88	5	ND	84	8
Ochratoxin A	5	ND	98	11	ND	98	5
Zearalenone	25	ND	111	5	ND	101	5

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).

Cleanliness comparison

A solution for “dilute and shoot” was prepared to get a visual comparison of cleanliness with **AFFINIMIP® SPE**. The cereals extracted were small spelts using the same extraction described above. 400µL of the supernatant from extraction was diluted with 600µL of ultrapure water. The dilution factor was chosen to obtain concentrations in the same range of those obtained with **AFFINIMIP®SPE Multimyco LCMSMS**.

Figure 1. Comparison between Dilute and shoot (left), barley after **AFFINIMIP®SPE Multimyco LCMSMS** (center), and small spelt (right) after **AFFINIMIP®SPE Multimyco LCMSMS**

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%	Deoxynivalenol	7.08	297.1	249.2	17
					297.1	203.2	23
1	85%	15%	Aflatoxin G2	8.72	331.1	313.0	35
					331.1	189.1	57
6	5%	95%	Aflatoxin G1	8.90	329.1	243.1	39
					329.1	200.1	57
14	5%	95%	Aflatoxin B2	9.15	315.1	259.1	41
					315.1	287.1	37
15	85%	15%	Aflatoxin B1	9.27	313.2	241.2	53
					313.2	285.2	33
22	85%	15%	Fumonisin B1	9.46	722.4	334.3	55
					722.4	352.4	53
Solvent A : 5mM ammonium acetate (in water) + 0.5% acetic acid **Solvent B** : 5mM ammonium acetate (in methanol) + 0.5% acetic acid			HT2 toxin	9.73	442.3	215.1	19
					442.3	263.2	19
			T2 toxin	10.15	484.3	215.1	29
					484.3	185.1	31
			Fumonisin B2	10.25	706.4	336.3	53
					706.4	318.3	55
			Ochratoxin A	10.27	404.1	239.1	35
					404.1	102.2	105
			Zearalenone	10.85	319.2	187.1	29
					319.2	283.1	19

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

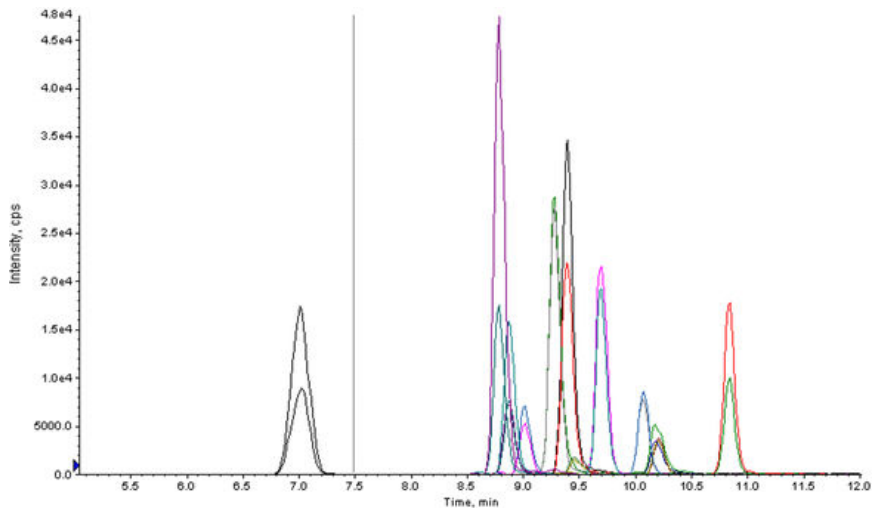

Figure 2. Typical chromatogram obtained during LC-MS/MS analysis. (Deoxynivalenol; Aflatoxin B1, B2, G1, G2; ochratoxin A; zearalenone; fumonisin B1, B2; T2 Toxin; HT2 toxin).

Conclusion

AFFINIMIP® SPE Multimyco LCMSMS has been successfully used for the enrichment and cleanup of 11 regulated mycotoxins in barley and small spelts. The method has shown excellent performances with recoveries from **74% to 115%** and a good repeatability.
The extracts obtained after **AFFINIMIP®SPE Multimyco LCMSMS** are clear while the dilute and shoot sample was yellowish and cloudy, demonstrating the effectiveness of the clean-up.

References

1. World Health Organization website: mycotoxins, May 9 2018
2. European Commission. (2006c). Commission regulation (EC) 1881/2006. Official Journal of the European Union, L 364, 5e24.

Product reference

- **AFFINIMIP® SPE Multimyco LCMSMS**

Catalog number: **FS118-03-NG** for 50 cartridges 3mL

- **AFFINIMIP® SPE Multimyco LCMSMS**

Catalog number: **FS118-03B-200-NG** for 50 cartridges 6mL

- **HPLC column: SilactHPLC – LC.A 150x2.1cm 3µm**

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