Determination of Aflatoxins in Various Botanicals by Liquid Chromatography with Fluorescence Detection

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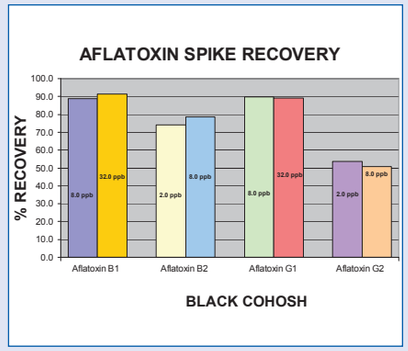
Abstract

An evaluation was performed on various botanical matrices for the determination of Aflatoxin B1, B2, G1, G2 using the HPLC methodology described in AOAC #999.07. The botanical samples selected were oat fiber, dandelion root powder, ginger root powder, ginseng root extract, black cohosh root powder, and milk thistle extract. Each sample type was evaluated to insure the cleanup was adequate for HPLC analysis and also to insure adequate spike recovery and precision for each Aflatoxin. Each of the sample types were analyzed by AOAC #999.07 both unspiked and spiked with known amounts of Aflatoxins in triplicate using 2 different concentrations of Aflatoxin B1, B2, G1, and G2. All samples were extracted with methanol/water (82/20), purified using an immunoaffinity column, and analyzed by HPLC using a post column derivatization (Kobra cell) prior to fluorescence detection. The HPLC analysis of Aflatoxin B1, B2, G1, and G2 using the AOAC #999.07 gave acceptable cleanup, accuracy and precision for oat fiber, dandelion root powder, ginger root powder, ginseng root extract, and black cohosh root extract botanicals. The milk thistle extract required a method modification in order to remove HPLC matrix interferences. An additional solid phase sample extract cleanup step was added prior to the immunoaffinity column cleanup procedure for the analysis of the milk thistle. With this method modification, the cleanup, accuracy, and precision data for the milk thistle was acceptable for the Aflatoxins B1 and G1.

BLACK COHOSH ROOT

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.8	85	28.5	89	1.4	70	6.3	79
Rep 2	7.8	97	28.8	90	1.7	84	5.9	74
Rep 3	6.7	84	30.4	95	1.4	68	6.6	83
Average	7.1	88.7	29.2	91.3	1.5	74.0	6.3	78.7
Std Dev	0.6		1.0		0.2		0.4	
% CV	8.6		3.5		11.5		5.6	

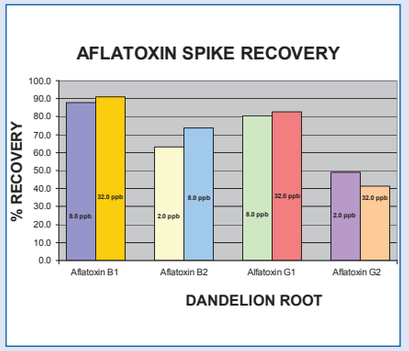
	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.7	86	27.8	87	1.1	55	4.2	52
Rep 2	7.8	97	28.2	88	1.2	59	3.9	49
Rep 3	6.8	86	29.7	92	0.8	42	4.1	51
Average	7.1	89.7	28.6	89.0	1.1	53.7	4.1	50.7
Std Dev	0.6		1.0		0.2		0.2	
% CV	8.2		3.5		14.3		3.8	



DANDELION ROOT

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	7.0	88	28.5	89	1.2	61	6	75
Rep 2	6.8	85	30.4	95	1.3	64	6.2	77
Rep 3	7.2	90	28.5	89	1.3	64	5.5	69
Average	7.0	87.7	29.1	91.0	1.3	63.0	5.9	73.7
Std Dev	0.2		1.1		0.1		0.4	
% CV	2.9		3.8		4.6		6.1	

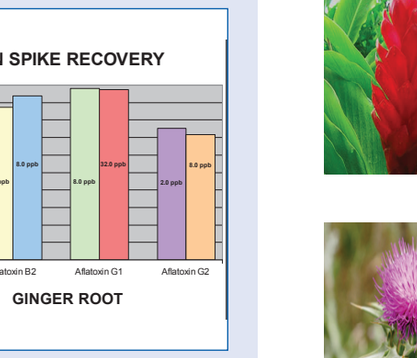
	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.1	76	24.6	77	0.9	47	3.3	41
Rep 2	6.6	83	28.2	88	0.9	47	3.5	44
Rep 3	6.6	82	26.6	83	1.1	53	3.1	39
Average	6.4	80.3	26.5	82.7	1.0	49.0	3.3	41.3
Std Dev	0.3		1.8		0.1		0.2	
% CV	4.5		6.8		11.9		6.1	



GINGER ROOT

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	8.0	100	33.9	106	1.7	86	8.4	105
Rep 2	7.7	96	31.0	97	1.7	85	7.0	87
Rep 3	8.3	104	30.7	96	1.8	91	7.1	89
Average	8.0	100.0	31.9	99.7	1.7	87.3	7.5	93.7
Std Dev	0.3		1.8		0.1		0.8	
% CV	3.8		5.5		3.3		10.4	

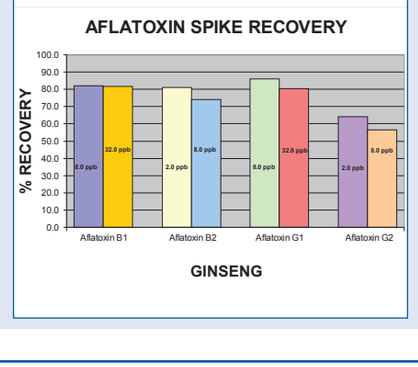
	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	7.8	97	33.3	104	1.4	70	6.2	78
Rep 2	7.4	92	30.1	94	1.5	76	5.3	66
Rep 3	8.4	105	30.1	94	1.6	80	5.7	71
Average	7.9	98.0	31.2	97.3	1.5	75.3	5.7	71.7
Std Dev	0.5		1.8		0.1		0.5	
% CV	6.4		5.9		6.7		7.9	



GINSENG ROOT

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	5.9	74	25.6	80	1.4	73	6.0	75
Rep 2	6.7	84	25.6	80	1.5	77	5.7	71
Rep 3	7.0	88	27.2	85	1.9	93	6.1	76
Average	6.5	82.0	26.1	81.7	1.6	81.0	5.9	74.0
Std Dev	0.6		0.9		0.3		0.2	
% CV	8.7		3.5		16.5		3.5	

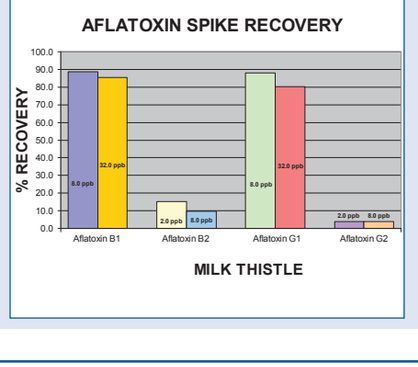
	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.2	79	25.6	80	1.2	59	4.5	56
Rep 2	6.7	84	25.0	78	1.3	63	4.2	53
Rep 3	7.6	95	26.6	83	1.4	70	4.8	60
Average	6.8	86.0	25.7	80.3	1.3	64.0	4.5	56.3
Std Dev	0.7		0.8		0.1		0.3	
% CV	10.4		3.1		7.7		6.7	



MILK THISTLE

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	7.2	90	25.9	81	0.4	20	0.8	10
Rep 2	7.2	90	28.8	90	0.2	12	0.7	9
Rep 3	6.7	86	26.9	85	0.3	14	0.8	10
Average	7.0	88.7	27.2	85.3	0.3	15.3	0.8	9.7
Std Dev	0.3		1.5		0.1		0.1	
% CV	4.1		5.4		33.3		7.5	

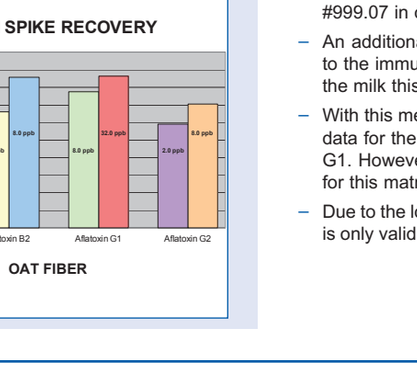
	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	7.2	90	24	75	0.12	6	0.4	5
Rep 2	7.0	88	27.8	87	0.08	4	0.2	3
Rep 3	6.8	86	25.3	79	0.04	2	0.3	4
Average	7.0	88.0	25.7	80.3	0.08	4.0	0.3	4.0
Std Dev	0.2		1.9		0.04		0.1	
% CV	2.9		7.5		50.0		33.3	



OAT FIBER

	Aflatoxin B1 Spike 8.0 ppb		Aflatoxin B1 Spike 32.0 ppb		Aflatoxin B2 Spike 2.0 ppb		Aflatoxin B2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.1	76	27.2	85	1.5	75	6.8	85
Rep 2	5.8	73	28.8	90	1.2	62	6.9	86
Rep 3	6.7	84	28.2	88	1.2	61	6.9	86
Average	6.2	77.7	28.1	87.7	1.3	66.0	6.9	85.7
Std Dev	0.5		0.8		0.2		0.1	
% CV	7.4		2.9		13.3		0.8	

	Aflatoxin G1 Spike 8.0 ppb		Aflatoxin G1 Spike 32.0 ppb		Aflatoxin G2 Spike 2.0 ppb		Aflatoxin G2 Spike 8.0 ppb	
	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec	Result ppb	%Rec
Rep 1	6.2	77	26.2	82	1.3	66	5.5	69
Rep 2	5.8	72	28.5	89	1.2	61	5.4	70
Rep 3	6.6	83	28.2	88	1.0	50	5.6	72
Average	6.2	77.3	27.6	86.3	1.2	59.0	5.5	70.3
Std Dev	0.4		1.3		0.2		0.1	
% CV	6.5		4.5		13.1		1.8	



Procedure

- Each of the 6 samples were extracted as received and also spiked with known amounts of Aflatoxins in triplicate using 2 different concentrations of Aflatoxin B1, B2, G1, and G2
- The toxin concentrations used were 8.0 ppb and 32.0 ppb for Aflatoxin B1 and G1 and 2.0 ppb and 8.0 ppb for Aflatoxin B2 and G2.
- The spiked and unspiked samples were then analyzed using AOAC method # 999.07.
- All samples were extracted with methanol/water (80/20), purified using an Aflatoxin immunoaffinity column, and analyzed by HPLC using a post column derivatization (Kobra cell) prior to fluorescence detection per the AOAC method.

Method Modification for Additional Sample Purification:

- The methanol/water extract (3ml) is mixed with acetonitrile (3ml)
- The diluted extract is passed through a solid phase cleanup column (Trilogy TC-A100)
- The purified extract (2.5 ml) is mixed with PBS containing Tween 20 (10 ml) and passed through the immunoaffinity column.
- Continue with the AOAC #999.07 procedure.


Black Cohosh Plant


Dandelion Plant


Red Ginger Plant


Ginseng Plant


Milk Thistle Plant


Oat Plant

Conclusions

- The HPLC analysis of Aflatoxin B1, B2, G1, and G2 using the AOAC method #999.07 gave acceptable cleanup, accuracy and precision results for the following evaluated commodities:
  - oat fiber
  - dandelion root powder
  - ginger root powder
  - ginseng root extract
  - black cohosh root extract
- The milk thistle extract required a modification to the AOAC method #999.07 in order to remove the HPLC matrix interferences.
- An additional solid phase sample extract cleanup was added prior to the immunoaffinity column cleanup procedure for the analysis of the milk thistle extract.
- With this method modification, the cleanup, accuracy, and precision data for the milk thistle extract was acceptable for Aflatoxin B1 and G1. However, the recoveries for the Aflatoxin B2 and G2 were low for this matrix.
- Due to the low Aflatoxin B2 and G2 recoveries, this modified method is only valid if the milk thistle sample contains no Aflatoxin B1 or G1.