



Efficiency of Various Solvents in the Extraction of Aflatoxin from Naturally Contaminated Cottonseed Meal, Corn Gluten Meal, Corn Gluten Feed, and Dried Distillers Grain

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Abstract

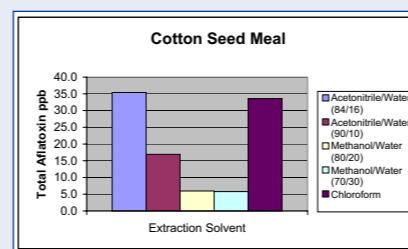
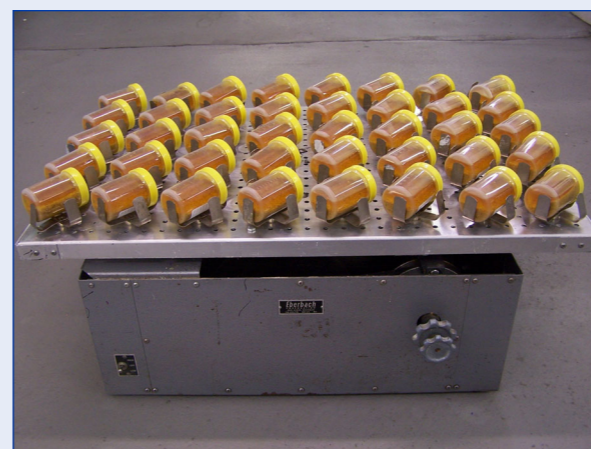
An evaluation was conducted to compare the extraction efficiency of aflatoxins from naturally contaminated cottonseed meal, corn gluten meal, corn gluten feed, and dried distillers grain using various solvents. The solvents compared were acetonitrile/water (84/16), acetonitrile/water (90/10), methanol/water (80/20), methanol/water (70/30), and chloroform. These solvents were chosen because they are commonly used for HPLC and test kit analysis of aflatoxins. Each naturally contaminated matrix was finely ground, well homogenized, and extracted using the various solvents in replicates of 10 and analyzed by HPLC using AOAC method #994.08 substituting the Kobra cell for the TFA derivative. The data demonstrates that the extraction efficiency of various solvents is matrix dependent with acetonitrile/water (84/16) consistently exhibiting the best extraction of aflatoxin. Solvents containing methanol/water should not be used for the extraction of aflatoxin from cottonseed meal. These solvents containing methanol/water at ratios of 80/20 and 70/30 only extracted 17% of the aflatoxin from cottonseed meal compared to the acetonitrile/water (84/16) solvent. This comparison of extraction solvents demonstrates the importance of the evaluation of naturally contaminated matrices when validating new methodology.

Procedure

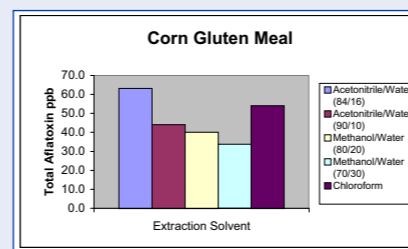
- Dried distillers grain, corn gluten meal, corn gluten feed, and cotton seed meal naturally contaminated with aflatoxin were finely ground and well homogenized
- Each sample was extracted with various extraction solvents, including acetonitrile/water (84/16), acetonitrile/water (90/10), methanol/water (80/20), methanol/water (70/30) and chloroform.
- Each commodity was extracted with each extraction solvent in replicates of 10 for 1 hour on a reciprocating shaker (180 rpms)
- The samples were analyzed for aflatoxins by HPLC using AOAC method 994.08 substituting the Kobra cell for the TFA derivative.
- Due to the differences in extraction solvents, the following method modifications were used:
 - Acetonitrile/water (84/16) and (90/10) – no modifications
 - Methanol/water (80/20) and (70/30) – 1ml of extract was combined with 1ml of acetonitrile before the solid phase purification step.
 - Chloroform - 2 ml portions of each extract was evaporated and redissolved in acetonitrile/water prior to the solid phase purification step.



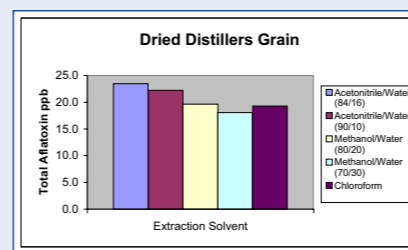
Cotton Seed Meal									
Acetonitrile/Water (84/16)	Acetonitrile/Water (90/10)	Methanol/Water (80/20)	Methanol/Water (70/30)	Chloroform					
Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)					
31.2	15.5	6.4	6.3	32.8					
34.9	18.9	5.5	5.6	35.7					
36.1	16.1	6.3	5.1	32.1					
37.5	17.2	5.4	5.4	32.2					
36.5	16.1	5.1	5.4	34.5					
35.1	16.3	5.6	5.4	29.5					
38.8	16.7	6.2	6.8	39.4					
38.3	16.2	6.7	6.0	28.6					
33.9	15.5	6.4	5.8	37.1					
36.9	18.7	6.0	6.0	33.9					
Mean	35.4	Mean	16.9	Mean	6.0	Mean	5.8	Mean	33.6
Std Dev	1.8	Std Dev	1.3	Std Dev	0.5	Std Dev	0.5	Std Dev	3.3
% CV	5.1	% CV	7.9	% CV	8.9	% CV	8.8	% CV	9.8



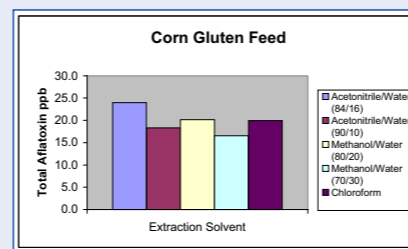
Corn Gluten Meal									
Acetonitrile/Water (84/16)	Acetonitrile/Water (90/10)	Methanol/Water (80/20)	Methanol/Water (70/30)	Chloroform					
Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)					
61.0	44.0	39.1	29.8	52.0					
61.9	44.1	39.9	32.3	56.4					
61.5	41.2	40.5	33.8	52.8					
63.5	43.6	41.1	34.6	57.8					
61.1	44.8	39.7	36.0	52.8					
64.1	43.8	40.5	34.4	50.6					
63.7	44.2	40.1	34.1	51.4					
64.6	44.5	40.2	33.1	59.1					
65.3	45.2	39.9	34.6	55.7					
65.3	45.4	40.1	34.9	51.6					
Mean	63.2	Mean	44.1	Mean	40.1	Mean	33.8	Mean	54.0
Std Dev	1.7	Std Dev	1.1	Std Dev	0.5	Std Dev	1.7	Std Dev	3.0
% CV	2.7	% CV	2.6	% CV	1.3	% CV	5.1	% CV	5.5



Dried Distillers Grain									
Acetonitrile/Water (84/16)	Acetonitrile/Water (90/10)	Methanol/Water (80/20)	Methanol/Water (70/30)	Chloroform					
Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)					
22.5	19.7	19.2	19.9	18.8					
22.3	22.1	19.1	17.6	17.7					
21.7	22.7	19.7	18.9	19.3					
22.8	22.7	19.1	17.6	18.6					
23.5	22.2	19.5	17.2	20.3					
24.1	22.2	20.6	18.5	21.3					
23.6	22.1	19.7	17.5	20.1					
24.8	22.8	19.2	18.0	19.7					
23.7	23.2	19.6	17.5	18.8					
25.8	22.5	20.7	18.0	19.4					
Mean	23.5	Mean	22.2	Mean	19.6	Mean	18.1	Mean	19.3
Std Dev	1.2	Std Dev	0.6	Std Dev	0.6	Std Dev	0.8	Std Dev	1.0
% CV	5.2	% CV	4.3	% CV	3.0	% CV	4.5	% CV	5.3



Corn Gluten Feed									
Acetonitrile/Water (84/16)	Acetonitrile/Water (90/10)	Methanol/Water (80/20)	Methanol/Water (70/30)	Chloroform					
Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)	Total Aflatoxins (ppb)					
25.4	18.1	17.1	16.2	21.0					
24.8	18.6	19.2	16.5	20.9					
22.5	18.6	18.6	14.1	21.3					
24.7	18.9	22.3	16.2	20.8					
24.3	17.9	19.5	15.3	19.0					
24.0	19.2	23.3	18.9	22.5					
25.1	17.5	19.4	18.2	19.3					
20.0	17.5	19.0	18.8	20.9					
25.2	18.2	20.7	15.1	18.2					
23.5	18.6	22.6	16.3	18.6					
Mean	24.0	Mean	19.3	Mean	20.2	Mean	16.6	Mean	20.0
Std Dev	1.6	Std Dev	0.8	Std Dev	2.0	Std Dev	1.6	Std Dev	1.9
% CV	6.9	% CV	3.1	% CV	9.9	% CV	9.7	% CV	9.6



Conclusions

- The efficiency of organic solvents in the extraction of aflatoxin from naturally contaminated products can be matrix dependent.
- This comparison of extraction solvents demonstrates the importance of the evaluation of naturally contaminated matrices when validating new methodology.
- Cotton Seed Meal**
 - Acetonitrile/water (84/16) and Chloroform were the most efficient extraction solvents
 - Acetonitrile/water (90/10), methanol/water (80/20, and methanol/water (70/30) should not be used for the extraction of aflatoxin from cotton seed meal
- Corn Gluten Feed**
 - Acetonitrile/water (84/16) was the most efficient extraction solvent
 - Chloroform, Acetonitrile/water (90/10), methanol/water (80/20), and methanol/water (70/30) had similar extraction efficiencies
- Dried Distillers Grain**
 - Acetonitrile/water (84/16) and acetonitrile/water (90/10) were the most efficient extraction solvents
 - Chloroform, methanol/water (80/20), and methanol/water (70/30) had similar extraction efficiencies
- Corn Gluten Meal**
 - Acetonitrile/water (84/16) and Chloroform were the most efficient extraction solvents
 - Acetonitrile/water (90/10), methanol/water (80/20), and methanol/water (70/30) should not be used for the extraction of aflatoxin from corn gluten meal

Aflatoxin Extraction Solvent Efficiencies					
	Acetonitrile/Water (84/16)	Acetonitrile/Water (90/10)	Methanol/Water (80/20)	Methanol/Water (70/30)	Chloroform
Corn Gluten Feed	100%	76%	84%	69%	83%
Dried Distillers Grain	100%	94%	83%	77%	82%
Corn Gluten Meal	100%	70%	63%	53%	85%
Cottonseed Meal	100%	48%	17%	16%	95%