



# Efficiency of Various Solvents in the Extraction of Ochratoxin A from Naturally Contaminated Wheat and Corn

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## Abstract

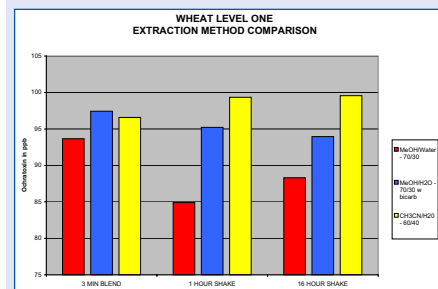
Ochratoxin A is a nephrotoxic and nephrocarcinogenic mycotoxin produced by several fungi of the *Aspergillus* and *Penicillium* species. This mycotoxin has been found in a wide variety of matrices including cereals, cereal products, coffee, spices, beer, wine, grape juice and raisins. An evaluation was conducted to compare the extraction efficiency of Ochratoxin A from naturally contaminated wheat and corn using methanol/water (70/30), methanol/water (70/30) with bicarb, and acetonitrile/water (60/40). These solvents were chosen because they are commonly used for HPLC and test kit analysis of ochratoxin A. The naturally contaminated wheat and corn were finely ground, well homogenized, and extracted using a 1 hour shake, 16 hour shake, and a 3 minute blend using methanol/water (70/30), methanol/water (70/30) with bicarb, and acetonitrile/water (60/40) in replicates of 3. The extracts were then analyzed by HPLC using AOAC method #2000.03. Data will be presented that compares the extraction efficiency of these three solvents using various extraction methods and extraction times.

## Introduction

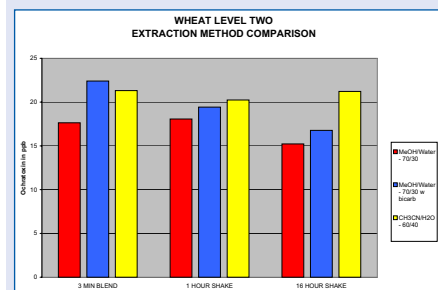
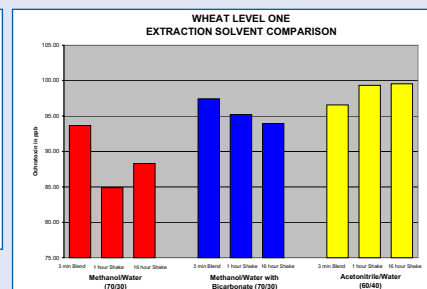
- Wheat and corn naturally contaminated with Ochratoxin, each at two different concentrations, were finely ground and well homogenized.
- Each sample was extracted with various extraction solvents, including acetonitrile/water (60/40), methanol/water (70/30), and methanol/water (70/30) with sodium bicarbonate.
- Each commodity was extracted with each extraction solvent in triplicate for 16 hours and 1 hour on a reciprocating shaker (180 rpms) and 3 minutes by blending.
- The samples were analyzed for Ochratoxin A by HPLC using AOAC method 2000.03.
- 25 g of each sample was extracted with 100 ml of the various solvents
- The samples were extracted by shaking and blending for various times.
- The extracts were filter then 4mls of each diluted with 44 mls PBS buffer.
- The entire amount was passed through an ochratoxin immunoaffinity column at 2 drops /second.
- Each column was rinsed with 5 ml of PBS followed by 10 ml of water.
- The bound ochratoxin was eluted with 4.0 ml of methanol.
- The methanol was evaporated and each sample was reconstituted with 1 ml of HPLC mobile phase.

### HPLC conditions:

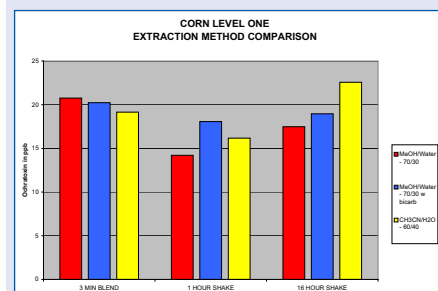
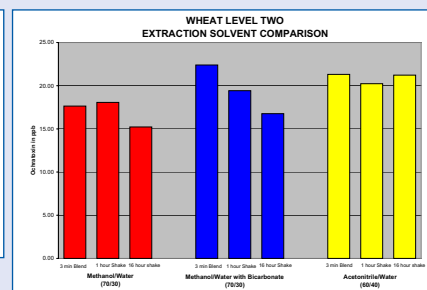
Detector – Fluorescence, excitation 333nm and emission 460 nm  
Column – Spheri-5, RP-18, 5um, 100mmX 4mm  
Mobile Phase – acetonitrile/water/acetic acid (45/54/1)  
Flow Rate – 2.0 ml/min  
Injection Volume – 150 ul



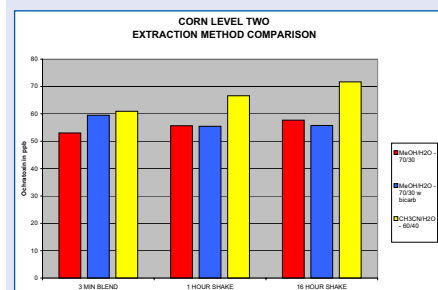
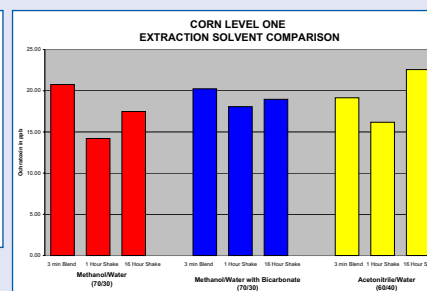
Extraction Method	Ochratoxin in ppb			
	Rep 1	Rep 2	Rep 3	Average
3min Blend Methanol/Water 70/30	98.76	90.26	91.93	93.65
1hour Shake Methanol/Water 70/30	90.62	80.37	83.70	84.90
16 hour Shake Methanol/Water 70/30	98.66	81.86	84.40	88.31
3min Blend Methanol/Water 70/30 with bicarb	111.29	95.83	85.17	97.43
1hour Shake Methanol/Water 70/30 with bicarb	100.36	98.64	86.63	95.21
16 hour Shake Methanol/Water 70/30 with bicarb	98.77	80.95	102.14	93.95
3min Blend Acetonitrile/Water 60/40	106.54	89.97	93.22	96.58
1hour Shake Acetonitrile/Water 60/40	96.24	97.63	104.16	99.34
16 hour Shake Acetonitrile/Water 60/40	111.07	93.33	94.27	99.56



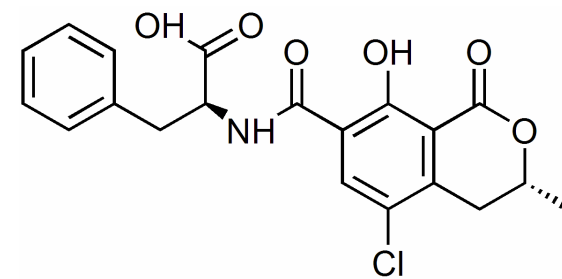
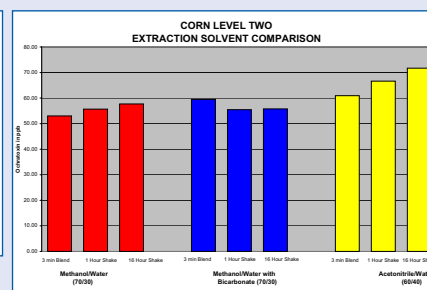
Extraction Method	Ochratoxin in ppb			
	Rep 1	Rep 2	Rep 3	Average
3min Blend Methanol/Water 70/30	17.71	18.03	17.19	17.64
1hour Shake Methanol/Water 70/30	17.92	21.40	14.89	18.07
16 hour Shake Methanol/Water 70/30	14.88	14.95	15.84	15.22
3min Blend Methanol/Water 70/30 with bicarb	18.06	25.56	23.59	22.40
1hour Shake Methanol/Water 70/30 with bicarb	18.85	17.43	22.00	19.43
16 hour Shake Methanol/Water 70/30 with bicarb	18.45	17.99	13.86	16.77
3min Blend Acetonitrile/Water 60/40	20.48	23.34	20.11	21.31
1hour Shake Acetonitrile/Water 60/40	17.98	20.62	22.11	20.24
16 hour Shake Acetonitrile/Water 60/40	22.76	18.78	22.13	21.22



Extraction Method	Ochratoxin in ppb			
	Rep 1	Rep 2	Rep 3	Average
3min Blend Methanol/Water 70/30	19.19	21.39	21.69	20.76
1hour Shake Methanol/Water 70/30	14.87	15.18	12.57	14.21
16 hour Shake Methanol/Water 70/30	15.86	21.45	15.12	17.48
3min Blend Methanol/Water 70/30 with bicarb	23.19	20.17	17.34	20.23
1hour Shake Methanol/Water 70/30 with bicarb	18.05	18.24	17.91	18.07
16 hour Shake Methanol/Water 70/30 with bicarb	18.31	19.01	19.56	18.96
3min Blend Acetonitrile/Water 60/40	20.25	18.08	19.11	19.15
1hour Shake Acetonitrile/Water 60/40	16.04	17.26	15.22	16.17
16 hour Shake Acetonitrile/Water 60/40	26.34	21.20	20.17	22.57



Extraction Method	Ochratoxin in ppb			
	Rep 1	Rep 2	Rep 3	Average
3min Blend Methanol/Water 70/30	50.82	52.29	55.98	53.03
1hour Shake Methanol/Water 70/30	53.74	58.32	54.95	55.67
16 hour Shake Methanol/Water 70/30	57.42	56.61	59.12	57.72
3min Blend Methanol/Water 70/30 with bicarb	66.17	55.80	56.55	59.51
1hour Shake Methanol/Water 70/30 with bicarb	58.26	54.82	53.30	55.46
16 hour Shake Methanol/Water 70/30 with bicarb	52.77	60.42	54.15	55.78
3min Blend Acetonitrile/Water 60/40	60.81	64.47	57.59	60.96
1hour Shake Acetonitrile/Water 60/40	70.09	56.95	72.82	66.62
16 hour Shake Acetonitrile/Water 60/40	75.01	75.33	64.77	71.70



Ochratoxin A



Naturally Contaminated Reference Materials

## Conclusions

Overall the three solvent systems and the three methods of extraction were similar in efficiencies. Our specific findings were:

### WHEAT

- The three extraction solvents evaluated, when used with a three minute blend, were statistically equivalent for both wheat samples.
- Acetonitrile/Water 60/40 exhibited a statistically significant higher extraction efficiency than methanol/water 70/30 for a 1 hour shake on the higher contaminated ochratoxin sample.
- Acetonitrile/Water 60/40 exhibited a statistically significant higher extraction efficiency than methanol/water 70/30 and methanol/water 70/30 with bicarb for a 16 hour shake on the lower contaminated ochratoxin sample.

### CORN

- The three extraction solvents evaluated, when used with a three minute blend, were statistically equivalent for both corn samples.
- Acetonitrile/Water 60/40 exhibited a statistically significant higher extraction efficiency than methanol/water 70/30 and methanol/water 70/30 with bicarb for a 16 hour shake on the higher contaminated ochratoxin sample.

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