

Determination of Aflatoxins and Ochratoxin A in Various Spices by Liquid Chromatography with Fluorescence Detection

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Abstract

Liquid Chromatography methods were developed and evaluated to determine aflatoxins and ochratoxin A in chili pepper, paprika, and red pepper. Aflatoxins were extracted with acetonitrile/water (84/16), purified using a solid phase cleanup column, and analyzed by liquid chromatography with fluorescence detection using the Kobra cell for post column derivatization. Ochratoxin A was extracted with methanol/water (70/30), purified using an immunoaffinity column, and analyzed by liquid chromatography with fluorescence detection. Accuracy and precision data for both methods will be presented. Samples of chili pepper, paprika, and red pepper from various suppliers were purchased from retail stores from several parts of the United States and analyzed for aflatoxins and ochratoxin A. All 12 samples of chili pepper were non detect for aflatoxins (detection limit 1 ppb) with 6 positive for ochratoxin A (detection limit 1 ppb) ranging from 1.6 to 8.3 ppb. For the paprika, 1 out of 9 samples was positive for aflatoxin B1 at 1.1 ppb with 5 samples positive for ochratoxin A ranging from 1.1 to 5.4 ppb. For the red pepper, 8 out of 9 samples were positive for aflatoxin B1 ranging from 2.9 to 27.7 ppb with all 9 samples positive for ochratoxin A ranging from 2.0 to 11.8 ppb.

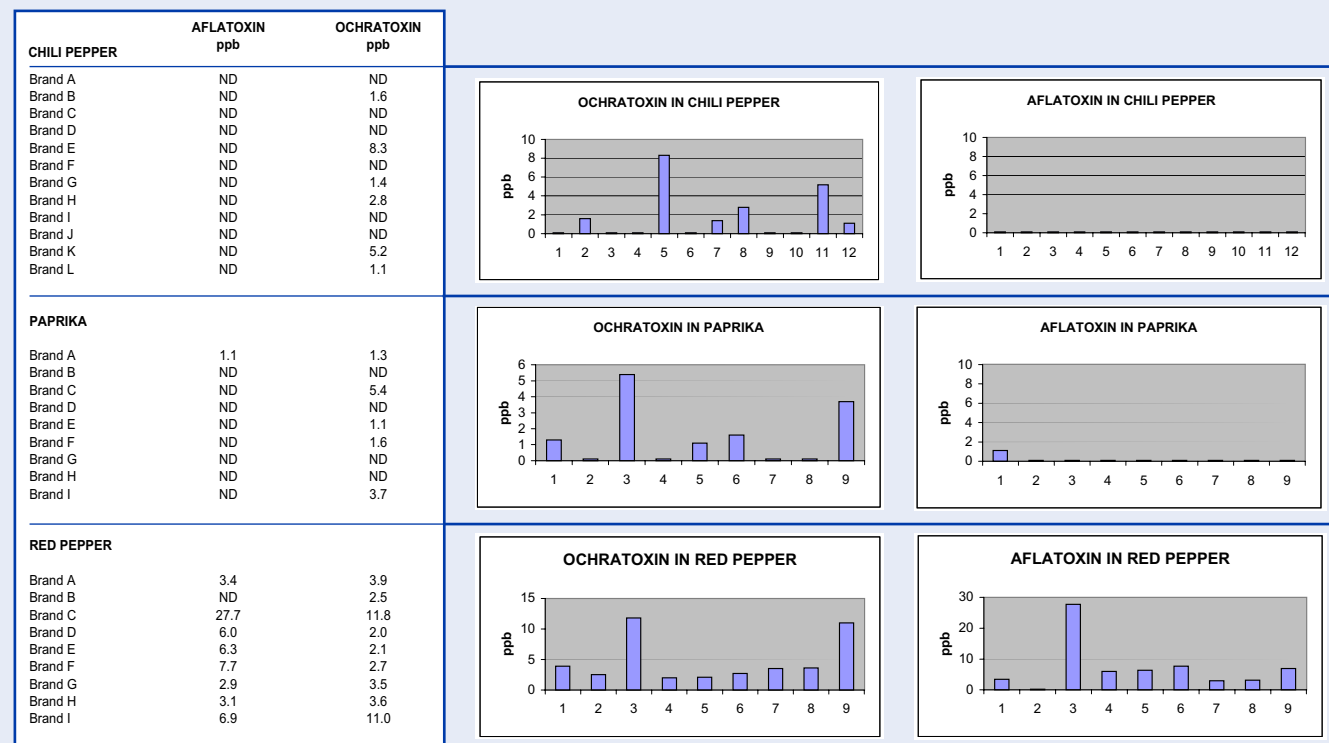
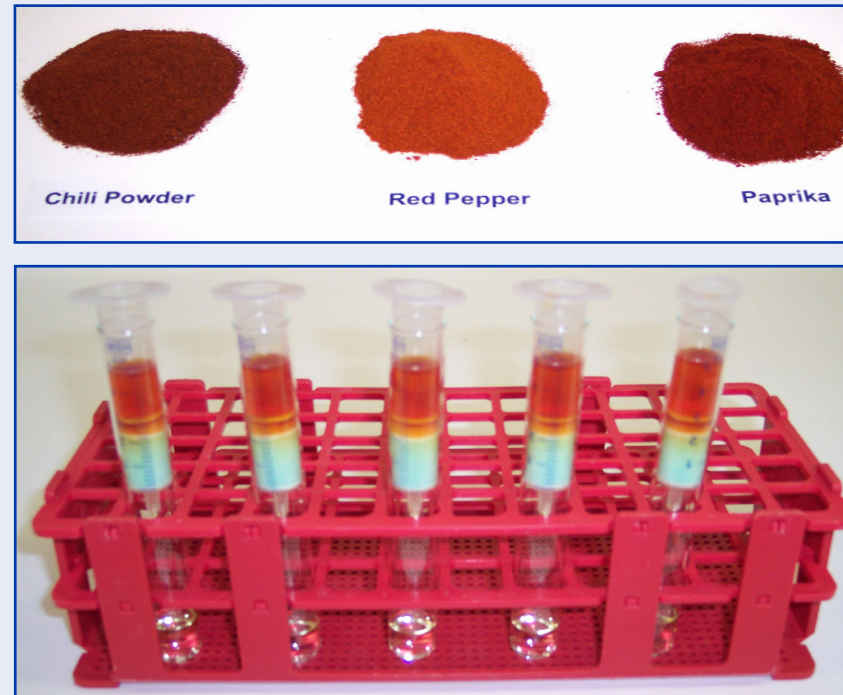
Aflatoxin Procedure

- Samples of chili pepper, paprika, and red pepper (5 g) were extracted with 20 mls acetonitrile/water (84/16) for 1 hour using a reciprocal shaker.
- The extracts were filtered and purified using a Puritox cleanup column.
- The purified extracts were diluted with water (200ul / 888ul water) and 200ul injected into the HPLC.
- HPLC conditions:
 - Fluorescence detector - excitation 360nm, emission 440 nm
 - Flow rate - 2.0 ml/minute
 - Mobile phase - water/methanol/acetonitrile/nitric acid/ potassium bromide (2500ml/500ml/500ml/360ul/1.2g)
 - Column - Perkin Elmer, Spheri-5, RP - 18, 5 um, 100 x 4.6 mm
 - Derivative - post column Kobra cell

Ochratoxin Procedure

- Samples of chili pepper, paprika, and red pepper (5 g) were extracted with 20 mls methanol/water (70/30/) with 1% sodium bicarbonate for 1 hour using a reciprocal shaker.
- 5 mls of extract were diluted with 20 mls of PBS with 0.01% Tween 20 and filtered using glass fiber filter paper.
- 15 mls of diluted extract were purified using an immunoaffinity column with 15mls of water wash.
- The column was eluted with 2.0 mls of methanol and the solvent evaporated.
- The sample was reconstituted with 0.5 ml of mobile phase and 150ul injected into the HPLC.
- HPLC conditions:
 - Fluorescence detector - excitation 333 nm, emission 460 nm
 - Flow rate - 1.5 ml/minute
 - Mobile phase - water/acetonitrile/acetic acid (2420/1800/20)
 - Column - Perkin Elmer, Spheri-5, RP - 18, 5 um, 100 x 4.6 mm

Results



Conclusions

- The HPLC methods described can accurately quantify Aflatoxin and Ochratoxin A in chili pepper, paprika, and red pepper
 - Chili pepper average spiked recoveries
 - Aflatoxin – 5 ppb (86.0 %), 20 ppb (93.0 %)
 - Ochratoxin – 5 ppb (78.0 %), 20 ppb (77.5 %)
 - Paprika average spiked recoveries
 - Aflatoxin – 5 ppb (96.0 %), 20 ppb (90.5 %)
 - Ochratoxin – 5 ppb (72.0 %), 20 ppb (78.0 %)
 - Red pepper average spiked recoveries
 - Aflatoxin – 5 ppb (92.0 %), 20 ppb (96.5 %)
 - Ochratoxin – 5 ppb (78.0 %), 20 ppb (76.5 %)
- Chili pepper – 12 samples
 - All 12 samples were non detect (1 ppb) for Aflatoxin
 - 6 samples were positive for Ochratoxin A ranging from 1.6 to 8.3 ppb
- Paprika - 9 samples
 - 1 sample was positive for Aflatoxin at 1.1 ppb
 - 5 samples were positive for Ochratoxin A ranging from 1.1 to 5.4 ppb
- Red pepper - 9 samples
 - 8 samples were positive for Aflatoxin ranging from 2.9 to 27.7 ppb
 - All 9 samples were positive for Ochratoxin A ranging from 2.0 – 11.8 ppb

AFLATOXIN - HPLC PERCENT RECOVERY DATA

PAPRIKA		REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE								
5.0 PPB		4.6	4.9	4.9	4.8	96.0	0.2	3.6
20.0 PPB		18.5	16.6	19.2	18.1	90.5	1.3	7.4

CHILI PEPPER

	REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE							
5.0 PPB	4.1	4.1	4.8	4.3	86.0	0.4	9.3
20.0 PPB	18.3	18.6	18.9	18.6	93.0	0.3	1.6

RED PEPPER

	REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE							
5.0 PPB	4.9	4.6	4.4	4.6	92.0	0.3	5.4
20.0 PPB	19.8	19.0	19.2	19.3	96.5	0.4	2.2

OCHRATOXIN - HPLC PERCENT RECOVERY DATA

PAPRIKA		REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE								
5.0 PPB		3.4	3.6	3.8	3.6	72.0	0.2	5.6
20.0 PPB		15.0	16.4	15.5	15.6	78.0	0.7	4.5

CHILI PEPPER

	REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE							
5.0 PPB	3.8	3.6	4.3	3.9	78.0	0.4	9.2
20.0 PPB	14.2	15.1	17.1	15.5	77.5	1.5	9.6

RED PEPPER

	REP_1	REP_2	REP_3	MEAN	MEAN REC %	SD	RSD %
SPIKE							
5.0 PPB	3.8	4.0	3.9	3.9	78.0	0.1	2.6
20.0 PPB	15.2	16.8	14.0	15.3	76.5	1.4	9.2