FOOD CHEMICAL CONTAMINANTS

Use of Multitoxin Immunoaffinity Columns for Determination of Aflatoxins and Ochratoxin A in Ginseng and Ginger

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Conditions were optimized for the simultaneous, alkaline, aqueous methanol extraction of aflatoxins (AFL), i.e., B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2), and ochratoxin A (OTA) with subsequent purification, isolation, and determination of the toxins in ginseng and ginger. Powdered roots were extracted with methanol–0.5% $NaHCO_3$ solution (7 + 3). After shaking and centrifugation, the supernatant was diluted with 100 mM phosphate buffer containing 1% Tween 20 and filtered through glass microfiber filter paper. The filtrate was then passed through an immunoaffinity column, and the toxins were eluted with methanol. The AFL were separated and determined by reversed-phase liquid chromatography (RPLC) with fluorescence detection after postcolumn UV photochemical derivatization. OTA was separated and determined by RPLC with fluorescence detection. Recoveries of AFL added at 2–16 ng/g and OTA added at 1–8 ng/g to ginseng were 72–80 and 86–95%, respectively. Recoveries of AFL and OTA added to ginger were similar to those for ginseng. A total of 39 commercially available ginger products from 6 manufacturers were analyzed. Twenty-six samples were found to be contaminated with AFL at 1–31 ng/g and 29 samples, with OTA at 1–10 ng/g. Ten samples contained no AFL or OTA. Ten ginseng finished products were also analyzed; 3 contained AFL at 0.1 ng/g and 4 contained OTA at levels ranging from 0.4 to 1.8 ng/g. LC/tandem mass spectrometry with multiple-reaction monitoring of 3 collisionally induced product ions from the protonated molecular ions of OTA, AFB_1, and AFG_1 was used to confirm the identities of the toxins in extracts of the finished products.

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Interest in the use of botanicals as dietary supplements or as alternative medicines appears to be increasing. According to a survey in 2000, Americans use supplements to feel good, prevent or overcome illness, increase longevity, or lose weight (1). Three in 10 Americans take herbal supplements, and about 9% of those use ginseng.

Some botanical supplements such as garlic, ginger, and turmeric have been used as food and condiments for centuries. They are generally regarded as safe for consumption but may be contaminated with mold toxins such as aflatoxins (AFL; 2, 3). Ochratoxin A (OTA) or AFL have been found in botanicals such as ginseng, ginger, licorice, turmeric, and kava-kava in the United States (4), Spain (5), Thailand (6), India (7), China (8), and other countries, whereas fumonisins have been found in medicinal wild plants in South Africa (9) and in herbal tea and medicinal plants in Turkey (10). Zearalenone has been identified in ginseng root (11). The levels of contamination in most cases are considered to be below the levels of concern for health (4–11); however, the toxicological effects of long-term low-level exposure to carcinogenic toxins such as AFL, i.e., B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2), and OTA are largely unknown.

Many methods for the detection and measurement of AFL and OTA in grains and nuts, such as thin-layer chromatography, liquid chromatography (LC), enzyme-linked immunosorbent assays, and LC/mass spectrometry (MS), have been reported. However, validated methods have not been reported for these toxins in botanicals. Because AFL and OTA can occur together in some agricultural commodities (12–14), we used a multitoxin immunoaffinity column (IAC) packed with antibodies specific for AFL and OTA coupled with LC to determine their levels simultaneously. Results were compared with those obtained by using single-toxin IACs.

Experimental

Plant Material

Finely ground ginseng (*Panax quinquefolius*) for the recovery study was purchased from Schumacher Ginseng (Marathon, WI). Finely ground ginger (*zingiber officinale*) for the recovery study was purchased from McCormick

Table 1. Recovery of total AFL from ginger and ginseng[a]

Root	IAC	% Tween in 50 mM PBS	AFL added, ng/g	Recovery, %	SD, %[b]	RSD_r, %[c]	HorRat, within-laboratory[d]
Ginger	Multitoxin (AflaochraTest with 20% more antibodies)	0.1	16	60.0	1.61	2.68	0.1
			8	69.1	1.29	1.86	0.1
			4	75.3	2.74	3.63	0.2
			2	62.1	1.25	2.01	0.1
			0	ND[e]			
		1.0	16	87.1	5.42	6.22	0.3
			8	91.8	1.47	1.60	0.1
			4	87.0	5.07	5.82	0.2
			2	90.1	1.00	1.11	0.04
			0	ND			
Ginseng	Multitoxin (AflaochraTest with 20% more antibodies)	0.1	16	103	10.79	10.47	0.5
			8	101	6.61	6.55	0.3
			4	84.3	2.50	2.97	0.1
			2	99.7	2.37	2.37	0.1
			0	ND			
		1.0	16	88.9	2.92	3.29	0.2
			8	84.5	2.16	2.55	0.1
			4	81.6	2.05	2.52	0.1
			2	90.7	1.02	1.33	0.05
			0	ND			
Ginger	Aflatest P	1.0	16	85.2	0.92	1.08	0.1
			8	86.8	4.92	5.67	0.3
			4	83.6	1.4	1.67	0.1
			2	77.8	7.42	9.53	0.4
			0	ND			

[a] $n = 4$.
[b] SD = Standard deviation.
[c] RSD_r = Within-laboratory relative standard deviation.
[d] Within-laboratory HorRat = RSD_r/(predicted relative standard deviation between laboratories × 0.66), value of <1.33 is acceptable.
[e] ND = None detected; i.e., AFB_1 at <0.1 ng/g.

(Baltimore, MD). All of the finished products were purchased locally from grocery stores and health and nutrition supplements stores.

Apparatus

(**a**) *Orbital shaker*.—DS-500E (VWR International, Bridgeport, NJ).

(**b**) *Centrifuge tube*.—50 mL, polypropylene.

(**c**) *Refrigerated centrifuge*.—Allegra X-22R (VWR International).

(**d**) *Glass microfiber filter paper*.—11 cm (No. 934AH; Whatman, Inc., Clifton, NJ).

(**e**) *IACs*.—Aflatest P column, wide-bore AflaochraTest column, OchraTest column, and wide-bore AflaochraTest column custom-packed with 20% more antibodies than are found in the commercially available product (Vicam, Watertown, MA).

(**f**) *12-Position pump stand manifold (with 10 mL syringe)*.—Vicam.

(**g**) *Column reservoir*.—15 mL polypropylene (Alltech Associates, Deerfield, IL).

(**h**) *LC system*.—Waters Model 2690 Alliance separate system (Waters, Milford, MA) and Waters Model 2475 fluorescence detector. LC operating conditions: flow rate, 0.8 mL/min for AFL and 1.0 mL/min for OTA; detector set at excitation wavelength (Ex) 365 nm and emission wavelength (Em) 445 nm for AFL, Ex 333 nm and Em 460 nm for OTA; column for AFL, Waters Cat. No. AQ12S031546WT, YMC

Table 2. Recovery of OTA from ginger and ginseng[a]

Root	IAC	% Tween in 50 mM PBS	OTA added, ng/g	Recovery, %[a]	SD, %[b]	RSD_r, %[c]	HorRat, within-laboratory[d]
Ginger	Multitoxin (AflaochraTest with 20% more antibodies)	0.1	8	81.1	3.32	4.09	0.2
			4	83.8	4.89	5.83	0.2
			2	87.9	1.28	1.45	0.1
			1	73.2	4.15	5.67	0.2
			0	ND[e]			
		1.0	8	58.5	0.79	1.36	0.1
			4	60.8	2.02	3.32	0.1
			2	61.3	0.86	1.41	0.1
			1	67.4	1.2	1.78	0.1
			0	ND			
Ginseng	Multitoxin (AflaochraTest with 20% more antibodies)	0.1	8	77.9	5.89	7.56	0.4
			4	71.9	7.28	10.12	0.4
			2	88.9	6.02	6.77	0.3
			1	81.1	2.04	2.51	0.1
			0	ND			
		1.0	8	75.6	5.32	7.03	0.3
			4	84.6	2.16	2.55	0.1
			2	79.5	1.37	1.72	0.1
			1	87.5	3.25	3.71	0.1
			0	ND			
Ginger	Single-toxin (OchraTest)	0.1	8	90.8	0.55	0.6	0.03
			4	81.1	1.48	1.82	0.1
			2	83.1	2.76	3.33	0.1
			1	79.0	0.91	1.15	0.04
			0	ND			
Ginseng	Single-toxin (OchraTest)	0.1	8	76.3	1.16	1.52	0.1
			4	73.7	0.87	1.18	0.05
			2	81.4	1.3	1.6	0.1
			1	85.0	3.14	3.69	0.1
			0	ND			

[a] $n = 4$.
[b] SD = Standard deviation.
[c] RSD_r = Within-laboratory relative standard deviation.
[d] Within-laboratory HorRat = RSD_r/(predicted relative standard deviation between laboratories × 0.66), value of <1.33 is acceptable.
[e] ND = None detected; i.e., AFB_1 at <0.1 ng/g.

ODS-AQ S-3, 4.6 × 150 mm; column for OTA, Beckman Cat. No. 235335, Utrasphere, 4.6 × 250 mm, 5 μm, C-18 (Beckman Instruments, Inc., Fullerton, CA).

(**i**) *Postcolumn derivatization system for AFL*.—PHRED cell (postcolumn photochemical derivatization cell; AURA Industries, New York, NY).

(**j**) *LC/MS/MS system*.—Agilent 1100 series liquid chromatograph (Agilent, Wilmington, DE) coupled to a MicroMass Quattro Premier mass spectrometer (Waters).

Column: YMC ODS-AQ S-3, 2 × 150 mm, 3 μm. LC separation: mobile phase gradient with mobile phase A, methanol–acetonitrile–water (25 + 15 + 60) + 0.1 mL formic acid, and mobile phase B, acetonitrile–formic acid (100 + 0.1); flow rate, 0.2 mL/min; gradient program: 100% A, hold for 20 min; a linear increase to 80% B over 10 min; hold at 80% B for 5 min; re-equilibrate to initial conditions (total run time, 55 min). MS/MS operating conditions: positive ion electrospray ionization mode; capillary voltage, 3 kV; cone

Table 3. Recovery of OTA and total AFL from ginger and ginseng, using commercially available AflaochraTest columns[a]

Root	1% Tween in 100 mM PB	OTA added, ng/g	AFL added, ng/g	Recovery, %	SD, %[b]	RSD_r, %[c]	HorRat, wthin-laboratory[d]
Ginger		8		86.8	3.72	4.28	0.20
		4		92.3	4.86	5.26	0.22
		2		92.3	2.55	2.76	0.10
		1		82.3	3.00	3.64	0.12
		0		ND[e]			
			16	78.0	4.02	5.15	0.26
			8	83.2	5.16	6.21	0.29
			4	80.5	1.48	1.84	0.08
			2	73.5	1.88	2.55	0.10
			0	ND			
Ginseng		8		88.3	4.89	5.54	0.26
		4		95.2	2.10	2.21	0.09
		2		86.3	2.75	3.18	0.12
		1		89.1	1.16	1.31	0.04
		0		ND			
			16	74.6	1.45	1.95	0.09
			8	78.3	1.92	2.45	0.10
			4	72.1	3.17	4.39	0.16
			2	80.2	2.86	3.57	0.12
			0	ND			

[a] $n = 4$.
[b] SD = Standard deviation.
[c] RSD_r = Within-laboratory relative standard deviation.
[d] Within-laboratory HorRat = RSD_r/(predicted relative standard deviation between laboratories × 0.66), value of <1.33 is acceptable.
[e] ND = None detected; i.e., AFB_1 at <0.1 ng/g.

voltage, 30 V; extractor, 2 V; RF lens, 0 V; source temperature, 120°C; desolvation temperature, 250°C; cone gas (nitrogen) flow rate, 11 L/h; desolvation gas (nitrogen) flow rate, 600 L/h; Q1 low- and high-mass resolution, 13 V; Q1 ion energy, 1 V; entrance lens, 1 V; collision energy, 35 V; exit lens, 0 V; Q3 low- and high-mass resolution, 15 V; Q3 ion energy, 1 V.

Reagents

(**a**) *Solvents and reagents*.—LC grade methanol and acetonitrile; ACS grade acetic acid, monosodium phosphate, disodium phosphate, sodium chloride, and trifluoroacetic acid; phosphate-buffered saline, pH 7.4 (PBS; Sigma P-3813, Sigma-Aldrich, St. Louis, MO); and Tween 20 (Sigma P-5927).

(**b**) *PBS solution, 10 mM*.—Dissolve 1 package PBS powder in 1 L water.

(**c**) *PBS soultion, 50 mM*.—Dissolve 1 package PBS powder in 200 mL water.

(**d**) *Phosphate buffer (PB) solution, 100 mM*.—Dissolve 4.66 g monosodium phosphate and 8.69 g disodium phosphate in 800 mL water, adjust to pH 7.4 with 2 N sodium hydroxide, and dilute to 1 L with water.

(**e**) *AFL*.—A6636, A9887, A0138, and A0263 (Sigma-Aldrich).

(**f**) *AFL standard solutions*.—(*1*) *Stock standard solutions*.—Prepare for each of the 4 AFL at 10 μg/mL in acetonitrile according to revised AOAC Method **971.22** (15). (*2*) *Intermediate mixed standard solution 1 containing total AFL at 400 ng/mL*.—Add appropriate volume of each stock standard solution to the same volumetric flask to obtain the following concentrations after dilution to volume with acetonitrile: AFB_1 at 200 ng/mL, AFB_2 at 50 ng/mL, AFG_1 at 100 ng/mL, and AFG_2 at 50 ng/mL. This is the spiking solution for the recovery study. (*3*) *Intermediate mixed standard solution 2 containing total AFL at 8 ng/mL*.—Add 100 μL intermediate mixed standard solution 1 to a 5 mL volumetric flask, and dilute to volume with methanol–water (1 + 1). (*4*) *Working standard solution containing total AFL at 8, 4, 2, 1, and 0.5 ng/mL*.—Make serial dilutions of intermediate mixed standard solution 2 with methanol–water (1 + 1).

(**g**) *OTA*.—Isolated from cultures and purified in the U.S. Food and Drug Administration laboratory. Purity was > 95%.

Table 4. AFL and OTA found in finished ginger products

Manufacturer	Lot[a]	OTA, ng/g	AFL, ng/g
1	1A	1.4	1.9
	1A	1.4	1.4
	1A	1.0	1.7
	1A	1.5	1.6
	1A	1.2	0.6
	1A	1.6	2.9
	1A	1.5	2.5
	1B	3.6	3.2
	1C	0.5	ND[b]
	1C	0.5	ND
	1C	0.5	ND
2	2D	5.3	13.8
	2D	4.3	13.7
3	3E	7.7	24.0
	3E	7.8	21.2
4	4F	1.6	1.1
5	5G	2.3	9.3
	5G	2.5	10.2
	5G	1.8	9.3
	5G	1.8	12.3
	5G	1.9	10.8
	5H	10.3	23.0
	5H	7.6	19.4
	5H	8.4	22.8
	5H	9.1	19.3
	5H	7.5	19.6
	5H	7.2	23.0
	5H	8.6	19.8
5	5I	5.0	31.2
6	6J (1)[c]	ND	ND
	6K (4)[c]	ND	ND
	6L (3)[c]	ND	ND
	6M (2)[c]	ND	ND

[a] Number identifies manufacturer, and the letter identifies lot. Multiple entries with the same number and letter represent single analyses of multiple samples from the same lot.

[b] ND = None detected; <0.1 ng/g.

[c] Value in parentheses is the number of samples from the same lot.

(**h**) *OTA standard solutions*.—(*1*) *Stock standard solution*.—Prepare OTA solution at approximately 100 μg/mL in benzene–acetic acid (99 + 1) according to AOAC Method **973.37** (15). Pipet 1 mL of the 100 μg/mL OTA solution into a 4 mL vial, and evaporate to dryness under a stream of nitrogen. Dissolve the dried film in 3 mL methanol. Determine the absorbance at 333 nm. Use the molar absorptivity of 6330 to calculate the OTA concentration, which should be approximatly 30 μg/mL. (*2*) *Intermediate standard solution 1 containing OTA at 200 ng/mL*.—Add appropriate volume of stock standard solution to a 25 mL volumetric flask, and dilute to volume with methanol. This is the spiking solution for the recovery study. (*3*) *Intermediate standard solution 2 containing OTA at 4 ng/mL*.—Add 100 μL intermediate standard solution 1 to a 5 mL volumetric flask, and dilute to volume with methanol–water (1 + 1). (*4*) *Working standard solution containing OTA at 4, 2, 1, 0.5, and 0.25 ng/mL*.—Make serial dilutions of intermediate standard solution 2 with methanol–water (1 + 1).

(**i**) *Mobile phase*.—(*1*) *For AFL postcolumn derivatization with PHRED cell*.—Water–methanol–acetonitrile (600 + 250 + 150). (*2*) *For OTA*.—Acetonitrile–water–acetic acid (50 + 50 + 1).

Extraction and IAC Cleanup

(**a**) *Multitoxin IAC (wide-bore AflaochraTest column)*.—(*1*) *Extraction*.—Weigh 5 g test sample in a 50 mL centrifuge tube. Add 1 g NaCl and 25 mL methanol–0.5% $NaHCO_3$ solution (70 + 30). Mix on a Vortex mixer until sample particles and extraction solvent are well mixed. Shake mixture at 400 rpm for 10 min. Centrifuge at 7000 rpm (*g* value = 5323 mm/s^2) at 10°C for 10 min. Pipet 7 mL supernatant into a 50 mL centrifuge, add 28 mL 100 mM PB containing 1% Tween 20 mix, and filter through glass microfiber paper. Collect 25 mL filtrate (equivalent to 1 g test sample) in a 25 mL graduated cylinder, and proceed immediately with IAC chromatography. (*2*) *Column chromatography*.—Attach IAC to pump stand manifold and connect a reservoir to the IAC. Pass filtrate through the IAC, and wash column with 5 mL 10 mM PBS solution, followed by 5 mL water. Let solutions pass through column by gravity. Use syringe to pass air through column for 1 s to dry column. Elute AFL and OTA from the IAC with two 1 mL portions of methanol, and collect eluate in a 3 mL volumetric flask; dilute to volume with water.

(**b**) *Single-toxin IAC for AFL (Aflatest P column)*.—The procedure is similar to that for the multitoxin IAC. Exceptions: Extract with methanol–10 mM PBS containing 1% Tween 20 (8 + 2), shake mixture for 30 min, dilute with 10 mM PBS containing 1% Tween 20, and wash with 10 mL water.

(**c**) *Single-toxin IAC for OTA (OchraTest column)*.—The procedure is similar to that for the multitoxin IAC. Exceptions: Extract with methanol–1% $NaHCO_3$ solution (7 + 3), dilute with 10 mM PBS containing 0.1% Tween 20, and wash with 5 mL 10 mM PBS, followed by 5 mL water.

LC Analysis

(**a**) *For OTA*.—Inject 50 μL reagent blank (50% methanol), working OTA standard solutions, or test sample into the LC column. Identify the OTA peak in the chromatogram of the test sample by comparing the retention time (ca 8.7 min) with those of standards. For some ginger finished products, an interference peak appeared in the chromatogram at 8.3 min. It was necessary to use less

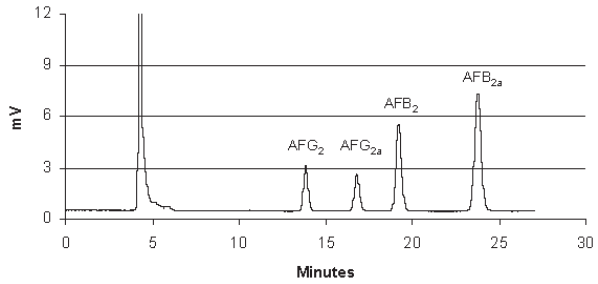

Figure 1. Liquid chromatogram of AFL in a ginger product.

acetonitrile in the mobile phase, i.e., acetonitrile–water–acetic acid (47 + 53 + 1), to obtain baseline separation of the OTA peak from the interference peak. OTA eluted at 12–13 min. Construct the standard curve. Determine the concentration of OTA in the test solution by comparison with the standard curve. If the test sample gives a response that is out of the range of the standard curve, dilute with acetonitrile–water, or concentrate, and reinject to bring the sample response within the range of the standard curve.

(**b**) *For AFL.*—Inject 50 µL reagent blank (50% methanol), AFL working standard solutions, or test sample into the LC column. Identify the AFL peaks in the chromatogram of the test sample by comparing the retention time with those of standards. AFL elute in the order of AFG_2, AFG_{2a} (derivative of AFG_1), AFB_2, and AFB_{2a} (derivative of AFB_1), with retention times between about 14 and 24 min, and must be baseline resolved. Construct the standard curve for each AFL, and determine the concentration of each AFL in the test solution by comparison with the standard curves.

Calculations

Plot peak area (response, *Y*-axis) of each toxin (OTA and each AFL) standard versus concentration (ng/mL, *X*-axis) and determine slope (S) and *Y*-intercept (a). Calculate level of toxin in sample by using the equation shown below, where R is the peak area in the chromatogram of the test solution, V is the final volume (mL) of the injected test solution, F is the dilution factor (F is 1 when V is 3 mL), and W is 1 g, weight of the test sample passed through the IAC. The total AFL concentration is the sum of the concentrations of the 4 AFL.

$$\text{Toxin, ng/g} = ([(R - a)/S] \times V/W) \times F$$

LC/MS/MS Analysis

Use the following detection parameters: multiple reaction monitoring (MRM) of 3 collisionally induced decomposition product ions per compound of interest (9 total reactions); AFG_1: *m*/*z* 215, 243, and 283 coming from the decomposition of the protonated molecular ion *m*/*z* 329; AFB_1: *m*/*z* 214, 241, and 269 coming from the decomposition of the protonated molecular ion *m*/*z* 313; OTA: *m*/*z* 239, 341, and 358 coming from the decomposition of the protonated molecular ion *m*/*z* 404.

Results and Discussion

Use of Multitoxin Columns for Root Materials

Use of multitoxin IACs for purification and isolation has proven to be very challenging. When toxins were added to the diluted methanol–water test sample extracts immediately before the extracts were passed through the IAC, recoveries of AFL and OTA were almost 100%. When toxins were added to powdered roots that were allowed to stand for 1 h before extraction, recoveries of added toxins were <30%. Subsequently, the method was modified. The extraction solvent was changed to methanol–1% $NaHCO_3$ solution to increase the recoveries of the added OTA. PBS was used as the diluting solvent to neutralize the $NaHCO_3$. A surfactant such as Tween 20 was added to the PBS to reduce the nonspecific binding of polyphenolic compounds in the botanicals to the antibodies. It was found that recoveries of the added toxins depended on the concentration of the sodium bicarbonate in the extraction solvent, the concentration of the dilution buffer, and the amount of Tween 20 in the dilution buffer. When the $NaHCO_3$ concentration was <0.3%, the recoveries of the added OTA were about 40%, and when the $NaHCO_3$ concentration was >1%, the recoveries of the added AFL were <50%. Therefore, various concentrations of PBS for dilution were used: 10, 25, and 50 mM. Recoveries of AFL and OTA were <40 and 60%, respectively, when 10 mM PBS was used. Recoveries were >60% when 25 or 50 mM PBS was used. Recoveries of the toxins also depended on the concentration of Tween 20 in the PBS. The optimized conditions were determined to be extraction with methanol–1% $NaHCO_3$ solution (7 + 3) and dilution with 50 mM PBS containing 1% Tween. Recoveries of AFL and OTA added at 8 ng/g to ginseng were >80%, but recoveries from ginger were lower. Custom packed IACs containing 10 and 20% more antibodies than are found in the commercially available columns were used. The columns containing 20% more antibodies gave better recoveries of the added toxins than did the other columns.

The above procedure was further modified in order to improve the recoveries of OTA added to ginger. The concentration of the PB was increased to 100 mM, and the PB contained 1% Tween 20, but without sodium chloride. The recoveries of the AFL and OTA added to ginseng and ginger

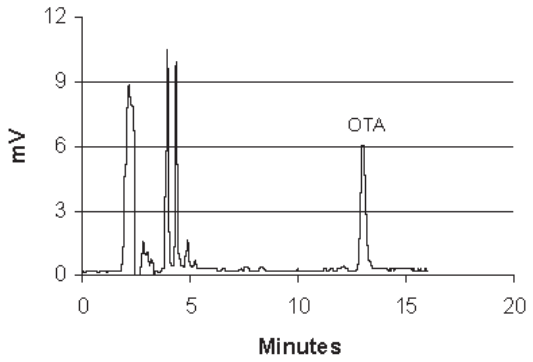

Figure 2. Liquid chromatogram of OTA in a product.

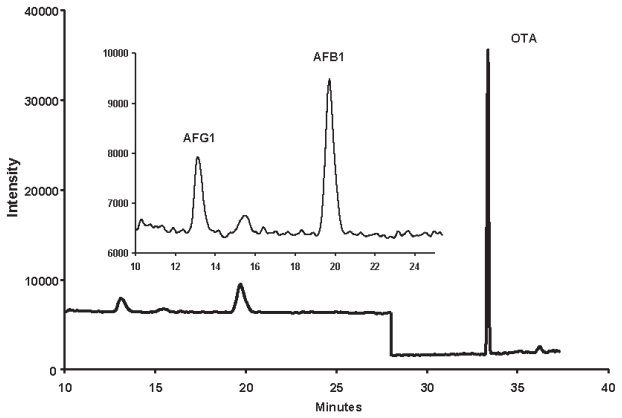

Figure 3. LC/MS/MS MRM total ion chromatogram of AFL and OTA in a ginger product.

were about 70–80%. The same procedure was repeated with the regular commercially available AflaochraTest columns; recoveries of the 2 toxins added to ginger and ginseng were similar to those obtained with columns packed with 20% more antibodies. This indicated that the effects of using custom-packed columns on recoveries of the toxins were minimal. It was found that performing IAC purification and isolation immediately after dilution and filtration was crucial. Recoveries of both toxins were reduced by >20% when the diluted test sample extracts were kept at room temperature for 1 h before IAC chromatography.

Recovery of AFL and OTA from Spiked Roots

Table 1 shows data from the recovery study of AFL added to ginger and ginseng, using 0.1 and 1% Tween 20 in 50 mM PBS as the diluting solvents for the extracts. The recoveries of total AFL added to ginseng at 2–16 ng/g (AFB_1:AFB_2:AFG_1:AFG_2 = 2:0.5:1:0.5) were 84–103% when 0.1% Tween was used and 82–91% when 1% Tween was used with the multitoxin column. Recoveries of AFB_1 and AFG_1 were >80% under both conditions. The same procedures were used for ginger, and recoveries of total AFL were 60–75 and 87–92%, when 0.1 and 1% Tween, respectively, were used. For AFB_1, recoveries were <60 and >90%, respectively, under the same conditions. Tween concentration had no effect on AFG_1 recoveries from ginger (>80%). Only 1% Tween was used to evaluate the single-toxin IAC, Aflatest P, because results from our previous study showed that this single-toxin column worked well for ginseng extract diluted with PBS containing 0.1% Tween (4). Recoveries of AFL added to ginger at the same spiking levels were 78–87%, which are similar to those obtained when the multitoxin columns were used. The within-laboratory HorRat values were ≤0.5 for all cases. Within-laboratory HorRat values of <1.3 indicated acceptable method performance (10).

Table 2 shows data from the recovery study of OTA added to ginger and ginseng (final extract for both OTA and AFL). OTA was added to ginger and ginseng at 1–8 ng/g. Recoveries of OTA from ginseng were 72–89% when 0.1% Tween was used and 76–88% when 1% Tween was used with the multitoxin columns. For ginger, recoveries of OTA were 73–88 and 59–67% when 0.1 and 1% Tween, respectively, were used. OTA recoveries from ginseng were the same when 0.1 and 1% Tween were used in 50 mM PBS as the dilution buffer, but OTA recoveries from ginger were reduced from 80 to 60% when the Tween concentration was increased from 0.1 to 1%. Without Tween 20, recoveries were <50% at AFL levels of 2–8 ng/g and OTA levels of 2–4 ng/g. For the single-toxin IAC, OchraTest, OTA recoveries from ginger and ginseng were 79–91 and 74–85%, respectively. These results demonstrate that higher recoveries of OTA were obtained with single-toxin columns than with multitoxin columns. The within-laboratory HorRat values (16) were ≤0.4 in all cases.

Table 3 shows data from a recovery study of AFL and OTA, using 100 mM PB containing 1% Tween 20 for dilution. AFL was added to ginger and ginseng at 2–16 ng/g, and the ranges of recoveries were 73–83 and 72–80%, respectively. OTA was added to ginger and ginseng at 1–8 ng/g, and the ranges of recoveries were 82–92 and 86–95%, respectively. The within-laboratory HorRat values were <0.3 in all cases.

The limits of detection (LODs) of the multitoxin or single-toxin method were 0.1 ng/g for AFB_1 and 0.1 ng/g for OTA. The LODs were based on a signal-to-noise ratio of >5 to 1 for AFB_1 at 0.025 ng/mL or OTA at 0.025 ng/mL. The lowest limits of quantitation (LOQs) with recovery data were 1 ng/g for AFB_1 and 1 ng/g for OTA. The LOQs could be much lower if the spiking levels were lower and the final extracts were evaporated and then diluted to a small volume. In our experiments, we used 3 mL volumetric flasks to collect the IAC eluates and dilution to volume before LC analysis in order to save time and avoid toxin degradation from overheating during evaporation.

Analysis of Finished Products

Twenty-six containers of finished ginger products from 5 manufacturers were purchased from local grocery stores. Products were in powdered form and weighed from 49 to 227 g/container. In addition, 5 bottles of ginger capsules (60 capsules/bottle, 625 mg/capsule) and eight 1 lb bags of

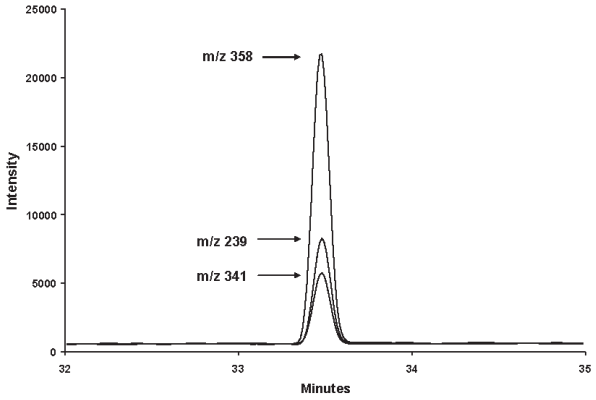

Figure 4. LC/MS/MS MRM chromatograms (3 ions) of OTA found in a ginseng product.

ginger powder were purchased from a botanical supplier. Results of the analyses are shown in Table 4. Products from only 1 manufacturer were found to be free of AFL and OTA. All of the other samples were found to be contaminated with OTA at levels of 1–10 ng/g, and 28 products contained AFL at levels of 1–31 ng/g. All ginger capsules (lot 5G) and ginger powder (lots 5H and 5I) from the same botanical supplier were contaminated with AFL at levels of 9–31 ng/g and OTA at levels of 2 and 5–10 ng/g, respectively. The finding that the AFG_1 level was about 1–3 ng/g higher than that of AFB_1 was unexpected because AFB_1 is usually the major AFL found in most grains, nuts, and other agricultural commodities.

A total of 10 ginseng products was purchased from herbal supply stores, grocery stores, and drug stores. Three products were found to contain AFB_1 at about 0.1 ng/g, and 4 products were found to contain OTA at levels ranging from 0.4 to 1.8 ng/g. The remaining samples contained no AFL or OTA (<0.1 ng/g).

Liquid Chromatograms of Roots

Liquid chromatograms of AFL, obtained by using AflaochraTest and Aflatest P columns in analyses of ginseng, ginger, kava-kava, turmeric, and licorice, were similar. Figure 1 shows a liquid chromatogram of AFL found in powdered ginger root from a botanical supplier. There are no matrix interference peaks, and the 4 AFL are well resolved from each other. Liquid chromatograms of OTA, obtained by using AflaochraTest and OchraTest columns in analyses of corresponding roots, were also similar. Figure 2 shows OTA in the same ginger sample. For LC determination of both AFL and OTA, there were high response solvent front peaks. In most cases, there were no peaks other than those of the solvent front and the toxins. The use of a delayed data integration program (i.e., integration delay for 5 min) could give better toxin peak scaling at levels of <2 ng/g.

LC/MS/MS Analysis

LC/MS/MS with MRM was used to confirm the identities of the toxins. The 3 collisionally induced product ions from the protonated molecular ion of each standard of OTA, AFB_1, and AFG_1 were compared with the MRM traces of the sample extracts. The retention times for AFG_1, AFB_1 and OTA were about 12.5, 18.5, and 33.5 min, respectively. Figure 3 shows the LC/MS/MS MRM total ion chromatogram of AFL and OTA in ginger naturally contaminated with the toxins. The standards and the samples gave the same molecular ions and induced product ions (AFG_1, $[M+H]^+$ ion at *m*/*z* 329, MRM product ions at *m*/*z* 243, 215, and 283; AFB_1, $[M+H]^+$ ion at *m*/*z* 313, MRM product ions at *m*/*z* 241, 269, and 214; OTA, $[M+H]^+$ ion at *m*/*z* 404, MRM product ions at *m*/*z* 358, 239, and 341), which confirmed the presence of the toxins in finished ginger products of manufacturer 1 and manufacturer 5 (both ginger powder and ginger capsules). No LC/MS/MS analysis of the remaining ginger products was performed because the liquid chromatograms were similar to those for which peak identity had been confirmed. Figure 4 shows 3 LC/MS/MS MRM ion chromatograms of OTA in ginseng naturally contaminated with OTA.

Conclusions

The commercially available wide-bore IAC packed with anti-AFL and -OTA antibodies is suitable for the detection of AFL and OTA at levels of >0.1 ng/g in ginseng and ginger. The single-toxin columns for AFL or OTA are suitable for detection of AFL or OTA. For certain roots such as ginger, the recoveries of added toxin and the levels of OTA contamination detected in naturally contaminated ginger were higher with the single-toxin column than with the multitoxin columns. The multitoxin column reduces solvent use, time of analysis, and cost. Both the extraction conditions and the type of root affect the recoveries of added toxins. This analytical method should be evaluated for each type of root under investigation.

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