Published in final edited form as:
J Agric Food Chem. 2010 April 14; 58(7): 4065–4070. doi:10.1021/jf9039028.

A Rapid Analytical Method for Determination of Aflatoxins in Plant-Derived Dietary Supplement and Cosmetic Oils

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Abstract

Consumption of edible oils derived from conventional crop plants is increasing because they are generally regarded as more healthy alternatives to animal based fats and oils. More recently there has been increased interest in the use of alternative specialty plant-derived oils, including those from tree nuts (almonds, pistachios and walnuts) and botanicals (borage, evening primrose and perilla) both for direct human consumption (e.g. as salad dressings) but also for preparation of cosmetics, soaps, and fragrance oils. This has raised the issue as to whether or not exposure to aflatoxins can result from such oils. Although most crops are subject to analysis and control, it has generally been assumed that plant oils do not retain aflatoxins due to their high polarity and lipophobicity of these compounds. There is virtually no scientific evidence to support this supposition and available information is conflicting. To improve the safety and consistency of botanicals and dietary supplements, research is needed to establish whether or not oils used directly, or in the formulation of products, contain aflatoxins. A validated analytical method for the analysis of aflatoxins in plant-derived oils is essential, in order to establish the safety of dietary supplements for consumption or cosmetic use that contain such oils. The aim of this research was therefore to develop an HPLC method applicable to a wide variety of oils from different plant sources spiked with aflatoxins, thereby providing a basis for a comprehensive project to establish an intra- and inter-laboratory validated analytical method for analysis of aflatoxins in dietary supplements and cosmetics formulated with plant oils.

Keywords

Aflatoxin; *Aspergillus* spp; oils; borage; evening primrose; perilla; almond; walnut; cottonseed; peanut; soy

INTRODUCTION

Plant edible oils are important ingredients of the diet, generally regarded as more healthy alternatives to animal based fats and oils. Major crops used in culinary practice for this purpose are canola (rape seed), corn, cotton, peanuts and soy. More recently there has been increased interest in the use of alternative specialty plant-derived oils, including those from tree nuts (almonds, pistachios and walnuts) and botanicals (borage, evening primrose and perilla) both for direct human consumption (e.g. as salad dressings) but also for preparation of cosmetics, soaps, fragrance oils and aromatherapy. Whereas the large volume edible oils

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SAFETY
Aflatoxins are classified as hepatotoxins and carcinogens and should be handled with appropriate precautions.

are commonly used in cooking at high temperatures, the specialty oils are most often used as purchased, without heating.

Aflatoxins (Figure 1) are common contaminants, highly regulated in both domestic and export markets, of tree nuts, cotton, corn and peanuts and much less so in soybeans. Current tolerance levels set by the European Community for most food products are 2 ppb aflatoxin B_1 and 4 ppb total aflatoxins, although edible oils are not specifically addressed (1). With few exceptions (primarily spices) aflatoxins are routinely analyzed for only in major food crops and not in botanical products. Moreover, it has generally been assumed that aflatoxins are not sequestered in plant oils due to their high polarity and lipophobicity. There is virtually no scientific evidence to support this supposition and information is scattered. Mustard oil, used for cooking in northern India, was analyzed spectrophotometrically and 33 of 100 samples were found to contain aflatoxins at levels of 55–87 ppb (2). An early unpublished study of peanut oil in China showed that 48% of 1172 samples were positive for aflatoxin B_1 (3), and a more recent long term survey in Fujian province found 66% of 323 samples aflatoxin- positive, with 71 samples exceeding the Chinese tolerance level of 20 ppb aflatoxin B_1 (4). Aflatoxin contamination of olive oil has received the most attention but the results have been contradictory. Analysis of 50 Greek olive oils demonstrated the presence of aflatoxin B_1 in 72% of the samples but the highest level detected was 0.05 ppb (5), well below the EU regulation of 2 ppb. Earlier work had reported aflatoxin levels in Spanish olive oils, and in a selection of both Greek and Spanish oils, of 13–155 ppb and 5–10 ppb, respectively (6,7). More recently, total aflatoxin levels of 0.006–0.04 ppb were found in 46% of 28 Sicilian olive oil samples examined (8), and of 20 experimental and 15 commercial samples analyzed by liquid chromatography-mass spectrometry only three of the latter were contaminated, but below the method quantification limits for individual aflatoxins (9). An HPLC method with fluorescence detection developed for simultaneous analysis of aflatoxin B_1 and ochratoxin A showed that only three of 30 olive oil samples from southern Italy and Morocco contained aflatoxin B_1 at 0.5–2.4 ppb, whereas 80% contained ochratoxin A (10). A recent study has shown aflatoxins to be present in 15 out of 20 crude rice bran oil samples, whereas 6 out of 20 refined rice bran oil samples were positive (11). The average aflatoxin B_1 levels were measured at 618 ppb in the crude oil and 20 ppb in the refined oil; however, these values must be regarded with some caution since the aflatoxin was determined spectrophotometrically after extraction from a TLC plate (12), a method that is not comparable with the accuracy of current HPLC techniques.

Certain regulatory agencies have recently inquired as to the safety of nut oil products with respect to aflatoxin contamination. Preliminary research in our laboratory has established that the oil extracted with hexane from reject almond samples can contain significant quantities of aflatoxins; however, the oil aflatoxin content did not correlate well with the aflatoxin level in the original nut samples. In view of the relative insolubility of aflatoxins in non-polar media, it appears that final aflatoxin levels are more likely to correlate with oil quality; i.e. highly oxidized oils would be more polar and thus more likely to dissolve aflatoxin. Similarly, the variable composition of oils from different plant sources may influence the degree of aflatoxin solubility. In commercial samples, it is likely that differences in aflatoxin levels can be due to the method used to isolate the oil (pressing or solvent extraction) and subsequent refining of the crude oil which may remove all or part of the contamination (13,14). However, whereas bulk oils for cooking undergo considerable refining, botanical oils generally are subject to minimal processing.

To improve the safety and consistency of botanicals and dietary supplements, research is needed to establish whether or not oils used directly, or in the formulation of products, contain aflatoxins. A validated analytical method for the analysis of aflatoxins in plant-derived oils is therefore necessary, in order to establish the safety of dietary supplements for

consumption or cosmetic use that contain such oils. The aim of this research was therefore to acquire representative source materials to be used in the development and validation of an analytical method for the analysis of aflatoxins in vegetable oil based botanical dietary supplements; and, to develop an HPLC method applicable to a wide variety of oils from different plant sources spiked with aflatoxins. Successful attainment of these objectives would provide a basis for a more comprehensive project to establish an intra- and inter-laboratory validated analytical method for analysis of aflatoxins in dietary supplements and cosmetics formulated with plant oils.

MATERIALS AND METHODS

General

All glassware was acid washed with 2M sulfuric acid and thoroughly rinsed before use. All solvents used were HPLC grade (Fisher Scientific, Pittsburgh, PA), except for ACS grade benzene (Sigma-Aldrich Corp., St. Louis, MO) and ultrapure water which was prepared with a Barnstead NANOpure system (Thermo Fisher Scientific Inc., Waltham, MA). Plant oil samples were obtained from the following purveyors of oils for speciality uses: New Directions Aromatics (San Ramon, CA); Oak Court Creations (Minooka, IL); Garden of Wisdom (Prescott, AZ); Spectrum Naturals (Boulder, CO); and Mountain Rose Herbs (Eugene, OR). The eight oils consisted of borage, evening primrose, perilla, almond, walnut, cottonseed, peanut, and soy.

Preparation of aflatoxin standards

Aflatoxin standard solutions were prepared as detailed in AOAC 971.22 (18th Edition, 2005). Using this procedure individual solutions of aflatoxins B_1, B_2, G_1, and G_2 (Sigma-Aldrich) were prepared by dissolving approximately 1 mg of each aflatoxin with benzene:acetonitrile (98:2, v/v) in a 100 mL volumetric flask. The UV spectrum of each aflatoxin was recorded from 200 to 500 nm with a model 8453 UV/Vis diode array spectrophotometer (Agilent Technologies) using the dissolution solvent as the reference. The absorbance (A) at the maximum closest to 350 nm along with the molar absorptivity value (ε) from AOAC 971.22 and the molecular weight (MW) for each aflatoxin were used to determine the concentration of each standard.

Spiking of oil samples

The aflatoxin standards were used to prepare three solutions in benzene:acetonitrile (98:2, v/v) to spike oil samples at combined aflatoxin levels of approximately 2, 25, and 100ppb with a ratio of 4:1:4:1 for aflatoxins B_1, B_2, G_1, and G_2 (Figure 1), respectively. Edible oil samples (2g) were spiked in triplicate with 100 μL of aflatoxin standard solution, using a Wiretrol II micropipette (Drummond Scientific Co., Broomall, PA), for each of the three aflatoxin concentrations. A blank spiked with the solvent only and no aflatoxin was also prepared for each oil sample.

Preparation of oil samples for aflatoxin analysis

Each spiked and blank oil sample was dissolved in 6 mL hexanes and applied to a SPEC SI 30mg, 3mL silica solid phase extraction cartridge (Varian, Inc., Walnut Creek, CA) using a Visiprep vacuum manifold (Supelco, Bellefonte, PA). The cartridge was washed with 2 × 1 mL hexanes and the combined hexanes and unretained oil were discarded. Aflatoxins were eluted directly into a 1.8 mL autosampler vial (National Scientific Co., Rockwood, TN) with 1.0 mL MeOH:H_2O (9:1, v/v). Standards for each aflatoxin concentration were prepared in triplicate by adding an aliquot directly to an autosampler vial, removing the solvent under

N_2 at 40 °C, and adding 1.0 mL MeOH:H_2O (9:1, v/v). Standards and oil samples were analyzed for aflatoxin by HPLC.

HPLC analysis of aflatoxins

Samples were analyzed for aflatoxins using a model 1100 HPLC system consisting of a degasser, autosampler, and quaternary pump, and fluorescence detector (Agilent) equipped with a 250 mm × 4.6 mm i.d., 5μ, Inertsil ODS-3, column (GL Sciences, Inc., Torrance, CA). A starting mobile phase of 100% H_2O:CH_3CN:MeOH (45:25:30, v/v/v) was held for 2 min after injection, followed by a gradient to 100% MeOH over the next 8 min, with 100% MeOH held for 1 min. The column was re-equilibrated with the starting solvent for 4 min before the next injection. The injection volume was 20 μL and the flow rate was 1.0 mL/min. Fluorescence detection at 365 nm excitation and 455 nm emission was enhanced with a post-column photochemical reactor for enhanced detection ("PHRED") (Aura Industries Inc., New York, NY). Aflatoxin retention times were 7.8 min. for G_2, 8.3 min. for G_1, 8.8 min for B_2, and 9.4 min for B_1. Aflatoxin peaks were recorded and integrated using ChemStation software (Agilent). Detection limits were 0.2 ppb for B_1 and G_1 and 0.05 ppb for B_2 and G_2.

RESULTS AND DISCUSSION

Numerous methods for analysis of aflatoxins in foodstuffs have been developed, primarily for solid samples. Analysis in oils presents an entirely different matrix that could potentially complicate clean-up of samples prior to analysis. In the context of this study, namely to develop methodology to analyze aflatoxins in dietary supplements and cosmetics formulated with plant oils, suitable for intra- and inter-laboratory collaborative studies (15), HPLC using fluorescence detection was selected as the most suitable. The technique has been adopted for routine aflatoxin analysis in foodstuffs in many commercial laboratories, the equipment is relatively inexpensive and adaptable to analysis of multiple samples, and operator training is minimal. More sophisticated techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been applied to olive oils but although suitable for confirmatory analysis the sensitivity was less than that of HPLC-fluorescence detection (9). Furthermore, the equipment is expensive and requires considerable operator expertise.

The experimental design for this study was therefore to obtain from commercial speciality botanical oil suppliers samples of representative herbal and nut oils, together with selected samples of more common vegetable oils. The botanical and nut oils consisted of borage, evening primrose, perilla, almond and walnut. Samples of each oil were obtained from three different suppliers except for perilla oil, for which only two samples were found to be commercially available. The vegetable oils were cottonseed (two samples), peanut (one sample), and soy (three samples). Although these oils are of the type used for cooking they are sold by speciality suppliers not for this purpose but rather as carriers or base oils for cosmetics preparation, massage or aromatherapy diluents. In all, 20 oil samples were obtained, representing a diversity of phytochemical types. The oils were then spiked in triplicate with aflatoxins at low, intermediate and high levels (approximately 2, 25 and 100 ppb total, respectively), covering a typical range of aflatoxin concentrations found in contaminated agricultural products. The spiked samples were then subjected to a clean-up procedure, and finally analyzed for percent recovery by HPLC with fluorescence detection. The aflatoxin mixture consisted of aflatoxins B_1, B_2, G_1, and G_2 (Figure 1) in the ratio 4:1:4:1, respectively. This represented typical contamination of foodstuffs, in which *Aspergillus flavus* produces the B group aflatoxins and *A. parasiticus* produces both B and G group aflatoxins. Each oil was also spiked with solvent alone as a blank to establish that the oils were not contaminated with aflatoxin prior to the procedure.

The crucial step in the analytical procedure was anticipated to be clean-up to retain aflatoxins but eliminate the large quantity of oil prior to HPLC separation. Monoclonal antibody-based affinity chromatography columns such as AflaTest are routinely used for analysis of aflatoxins in agricultural products but these require the use of methanol/water or methanol alone, and it was felt that it would be difficult to develop a solvent system capable of eluting the oils while retaining the aflatoxins. Sobolev (16) has shown that simple, rapid pre-chromatographic clean-up of ground tree nuts, peanuts, corn and rice can be achieved using a minicolumn packed with Florisil. In this procedure the column was sequentially eluted with methanol/water, methanol, and chloroform/methanol, before desorption of aflatoxins with acetone/water/formic acid. It appeared unnecessary to resort to a series of eluents for oil analysis since the oil itself should elute readily with a non-polar solvent such as hexane. However, the ultimate aim of this work was to develop methodology that could be used for an inter-laboratory study and the in-house preparation of Florisil minicolumns (16) was deemed to be a potential point where discrepancies could occur because of variability between product lots and packing techniques. We therefore sought for commercial products that would have the advantages inherent in Florisil, namely simple, fast and inexpensive, but would be generally available and consistent in quality and performance. A 30 mg silica-based solid phase extraction (SPE) cartridge was selected and proved to be suitable. A 6 mL solution of the individual oils in hexane was applied to the cartridge; oils and other non-polar contaminants eluted with 2 × 1 mL hexanes; and the aflatoxins directly eluted into an HPLC autosampler vial with 1.0 mL MeOH:H_2O (9:1). This minimal volume meant that the eluant did not need to be concentrated, or the sample evaporated and redissolved, prior to analysis.

HPLC analysis was achieved using a reversed-phase octadecyl silica (ODS) column with gradient elution from H_2O:CH_3CN:MeOH (45:25:30) to 100% MeOH. Detection was by fluorescence at 455 nm with 365 nm excitation. Fluorescence detection of aflatoxins requires derivatization, either pre- or post-column. Typically, pre-column derivatization is performed by treatment of the sample with trifluoroacetic acid in hexane (17). However, this method requires evaporation of the solvent from the sample, treatment with derivatizing reagent, and redissolving the sample. These additional steps can be avoided by post-column photochemical derivatization (18). An added advantage is that a collaborative study of the photochemical technique showed no significant differences from other post-column derivatization methods (19), and the use of additional chemicals is avoided. All of the aflatoxins eluted with good resolution in under 10 minutes and the total analysis time, including re-equilibration of the column was 14 minutes. No extraneous peaks were observed from 7.5–10.0 minutes, the region of the chromatogram in which the four aflatoxins eluted. A representative analysis of cold-pressed borage oil (sample 9), spiked at the three different total aflatoxin levels, is shown in Figure 2. The chromatogram for the lowest spiking level of 2.1 ppb total aflatoxins is shown in Figure 3A. The peaks for each aflatoxin are well resolved, with the levels of individual aflatoxins being 0.9 ppb for aflatoxins B_1 and G_1, and 0.2 ppb for aflatoxins B_2 and G_2; these levels are well below that required by the most stringent EU regulation of 2 ppb for aflatoxin B_1. For comparison, chromatograms for the medium (25 ppb) and high (100 ppb) spiking levels in evening primrose oil (sample 11) and perilla oil (sample 15), respectively, are shown in Figures 3B and 3C.

Table 1 shows the percent recovery and standard deviation for aflatoxins B_1, B_2, G_1, and G_2, spiked into 20 oil samples from eight plant sources commonly used in dietary supplement and cosmetics at total levels of 2, 25 and 100 ppm. None of the blank (unspiked) oil samples showed the presence of aflatoxins, indicating absence of natural aflatoxin contamination. Recoveries for total aflatoxin were excellent, ranging from 86.4–100.0% at 2 ppb, 84.6–104.9% at 25 ppb, and 87.4–106.5% at 100 ppb. At 2 ppb total, the recoveries of aflatoxins

B_1, B_2, and G_2 were close to 100% for all oil matrices but aflatoxin G_1 recoveries were somewhat lower, generally around 90%. This may reflect the fundamental structural differences between the two classes, with the B group having a cyclopentenone ring and the G group a pyranone ring. Such differences could affect the relative solubilities of the individual aflatoxins in non-polar solvents and therefore the overall recovery. However, a similar trend, with aflatoxin G_1 having lower recovery than the other aflatoxins was not evident at the medium (25 ppb) and high (100 ppb) spiking levels, although the B group aflatoxins showed a better overall recovery than the G group aflatoxins.

There were no obvious differences between aflatoxin recovery from the oils from different plant sources. However, the number of samples was limited to no more than three of any type. Furthermore, when specified, the method used for obtaining oils was either expeller-or cold-pressed; only one oil (#13, cottonseed) was solvent extracted. It will require a much larger number of samples to determine whether or not there is any relationship between oil source and extraction method and ability to sequester aflatoxins. Similarly, the degree of oxidation may influence aflatoxin accumulation and we are currently undertaking an investigation of laboratory-extracted almond oils in an attempt to correlate peroxide values with aflatoxin solubility.

This study has led to the successful development of a simple, reliable method for determination of aflatoxins in edible oils. The method should provide a basis for an intra- and inter-laboratory validated analytical method for analysis of aflatoxins in dietary supplements and cosmetics formulated with plant-derived oils. Previous investigations have resulted in conflicting reports regarding the potential for aflatoxins to be present at significant levels in edible oils (4–10). However, this study also demonstrates that aflatoxins are capable of being retained in such oils, a matter of some concern with their increasing use in a minimally processed form. Aflatoxin contamination of bulk oils used in cooking is probably minimal due to subsequent refining processes employed post-extraction. In fact the method developed in this study demonstrates that aflatoxins can be selectively retained by a silica-based cartridge, and bulk absorbents such as Florisil (16) might therefore be capable of removing aflatoxins in a commercial process. However, it should be recognized that the use of absorbents as a means of aflatoxin decontamination could also result in the removal of desirable constituents and a change in the physical and organoleptic properties of the oil.

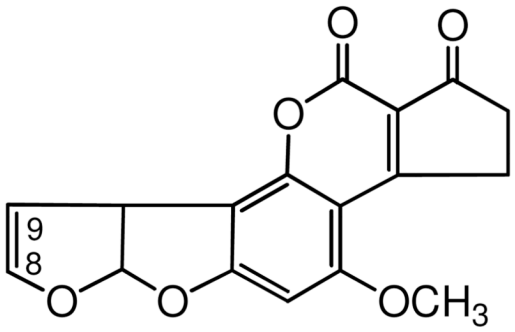
Acknowledgments

We thank the National Institutes of Health, Office of Dietary Supplements, for financial support of this work through Agreement No. Y1-OD-8619-01. The advice and encouragement of Dr. Joseph M. Betz, Director, ODS Dietary Supplements Methods and Reference Materials Program, are gratefully acknowledged.

LITERATURE CITED

1. European Commission (EC). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union 2006;L 364:5–24.
2. Sahay SS, Prasad T. The occurrence of aflatoxins in mustard and mustard products. Food Addit. Contam. A 1990;7:509–513.
3. Li F-Q, Li Y-W, Wang Y-R, Luo X-Y. Natural occurrence of aflatoxins in Chinese peanut butter and sesame paste. J. Agric. Food Chem 2009;57:3519–3524. [PubMed: 19338351]
4. Zhang JS, Zhang SJ. A nine-year surveillance of aflatoxin B_1 in peanut oil. Hai Xia Yu Fang Yi Xue Za Zhi 1999;5:52–53.
5. Daradimos E, Marcaki P, Koupparis M. Evaluation and validation of two fluorometric HPLC methods for the determination of aflatoxin B_1 in olive oil. Food Addit. Contam. A 2000;17:65–73.
6. Gracian J, Arevalo G. Presencia de aflatoxinas en los productos del olivar. Grasas y Aceitas 1980;31:167–171.

7. Toussaint G, Lafaverges F, Walker EA. The use of high pressure liquid chromatography for determination of aflatoxin in olive oil. Arch. Inst. Pasteur Tunis 1977;3–4:325–334.
8. Finoli C, Vecchio A, Planeta D. Mycotoxin occurrence in extra virgin olive oils and in olives. Industrie Alimentari 2005;44:506–514.
9. Cavaliere C, Foglia P, Guarino C, Nazzari M, Samperi R, Laganà A. Determination of aflatoxins in olive oil by liquid chromatography–tandem mass spectrometry. Anal. Chim. Acta 2007;596:141–148. [PubMed: 17616251]
10. Ferracane R, Tafuri A, Logrieco A, Galvano F, Balzano D, Ritieni A. Simultaneous determination of aflatoxin B_1 and ochratoxin A and their natural occurrence in Mediterranean virgin olive oil. Food Addit. Contam. A 2007;24:173–180.
11. Jayaraman P, Kalyanasunderam I. Natural occurrence of aflatoxins and toxigenic fungi in rice bran and de-oiled bran. Indian J. Sci. Technol 2009;2:35–37.
12. Nabney J, Nesbitt BF. A spectrophotometric method for determining the aflatoxins. Analyst 1965;90:155–160.
13. Parker WA, Melnick D. Absence of aflatoxins from refined vegetable oils. J. Am. Oil Chem. Soc 1996;43:635–638. [PubMed: 5981571]
14. Mahjoub A, Bullerman LB. A method for aflatoxin B_1 determination in olives. Rev. Fr. Corps Gras 1990;37:245–246.
15. Trucksess MW, Stack ME, Nesheim S, Page SW, Albert RH, Hansen TJ, Donahue KF. Immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization for determination of aflatoxins in corn, peanuts and peanut butter: collaborative study. J. Assoc. Off. Anal. Chem 1991;74:81–88. [PubMed: 2026580]
16. Sobolev VS. Simple, rapid, and inexpensive cleanup method for quantitation of aflatoxins in important agricultural products by HPLC. J. Agric. Food Chem 2007;55:2136–2141. [PubMed: 17319689]
17. Mahoney N, Molyneux RJ. Phytochemical inhibition of aflatoxigenicity in *Aspergillus flavus* by constituents of walnut (*Juglans regia*). J. Agric. Food Chem 2004;52:1882–1889. [PubMed: 15053524]
18. Joshua H. Determination of aflatoxins by reversed-phase high-performance liquid chromatography with post-column in-line photochemical derivatization and fluorescence detection. J. Chromatogr. A 1993;654:247–254.
19. Waltking AE, Wilson D. Liquid chromatographic analysis of aflatoxin using post-column photochemical derivatization: collaborative study. J. AOAC Int 2006;89:678–692. [PubMed: 16792067]



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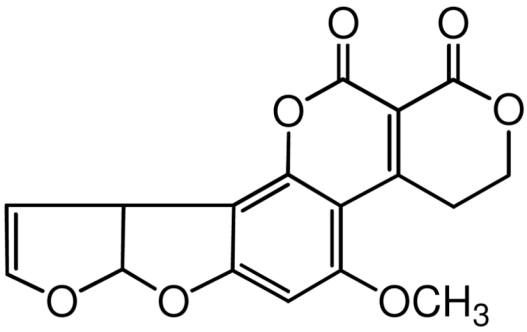

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Figure 1.
Chemical structures of major aflatoxins B_1, **1**, and G_1, **2**; minor aflatoxins B_2 and G_2 are the 8,9-dihydro derivatives of **1** and **2**, respectively.

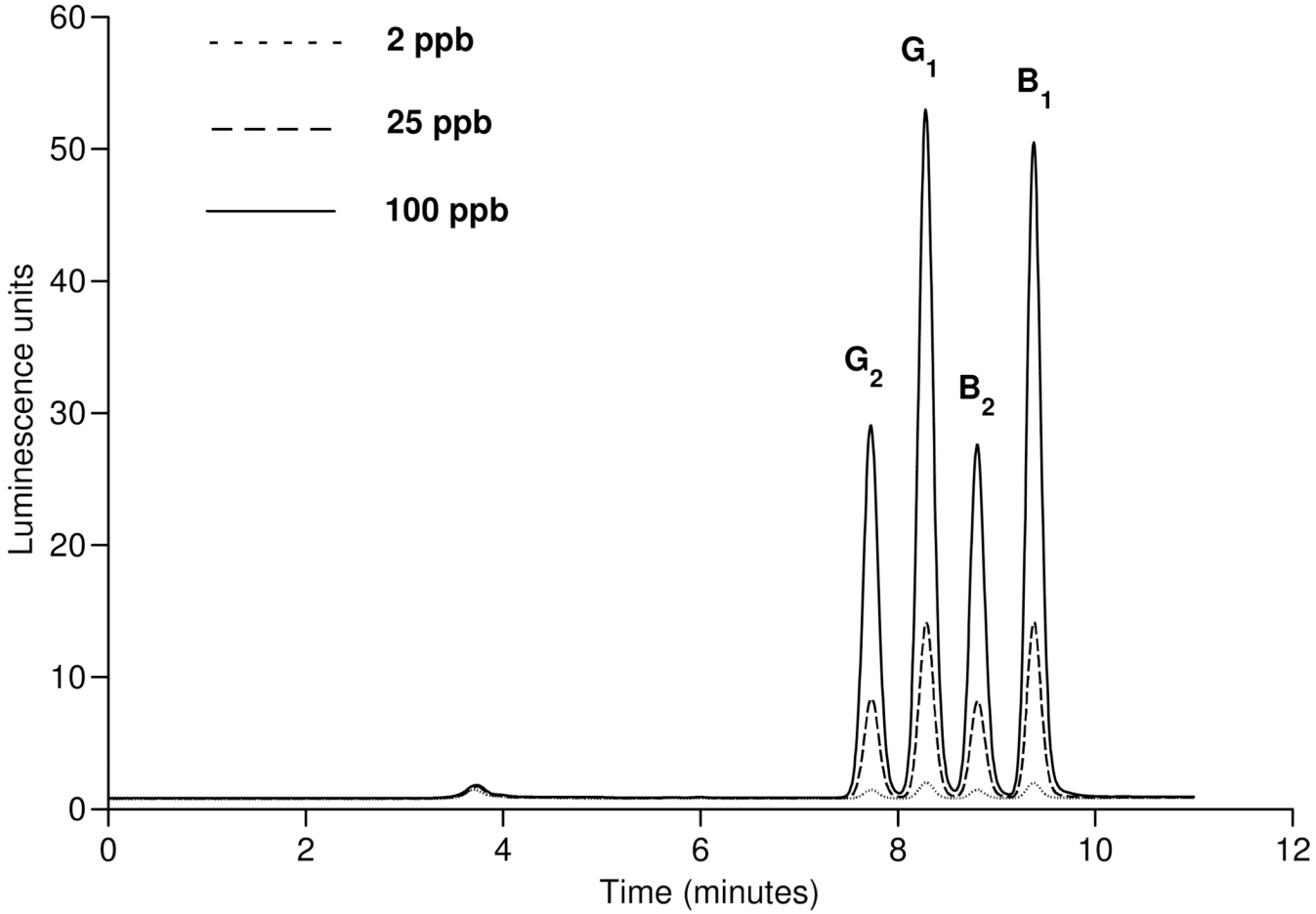

Figure 2.
HPLC analysis of cold-pressed borage oil (sample #9) spiked at ~2 ppb; ~25 ppb; and ~100 ppb total aflatoxins.

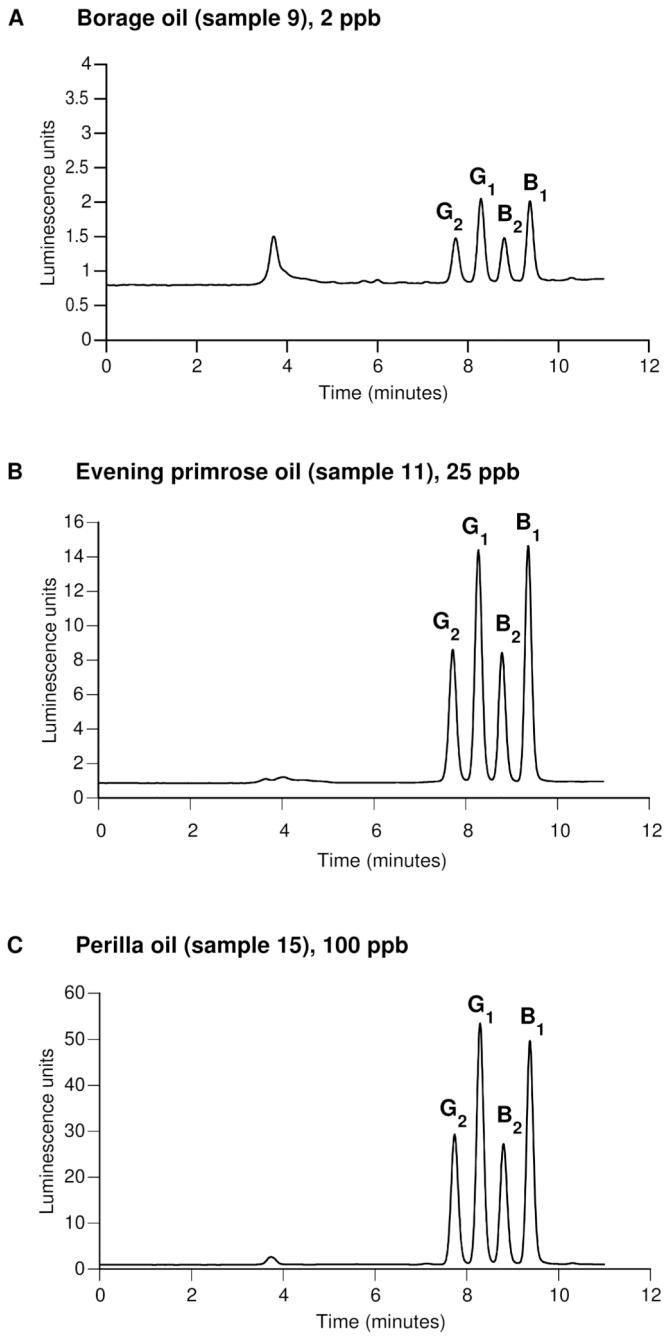

Figure 3.

A. HPLC analysis of cold-pressed borage oil (sample #9) spiked at ~2 ppb; **B**. HPLC analysis of cold-pressed evening primrose oil (sample #11) spiked at ~25 ppb; and, **C**. HPLC analysis of cold-pressed perilla oil (sample #15) spiked at ~100 ppb.

Table 1

HPLC Analysis of Individual and Total Aflatoxin Recovery from Twenty Different Edible Oils Spiked at Three Concentrations.

Sample #/Source (Extraction method)	Aflatoxin--low level spiking (~2ppb total) average ppb % recovery (% SD)					Aflatoxin--medium level spiking (~25ppb total) average ppb % recovery (% SD)					Aflatoxin--high level spiking (~100ppb total) average ppb % recovery (% SD)				
	G2	G1	B2	B1	Total	G2	G1	B2	B1	Total	G2	G1	B2	B1	Total
Aflatoxin Std.	**0.2**	**0.9**	**0.2**	**0.9**	**2.2**	**2.2**	**9.6**	**2.5**	**10.3**	**24.6**	**8.9**	**38.4**	**9.9**	**41.1**	**98.3**
	100.0 (1.3)	100.0 (0.8)	100.0 (1.5)	100.0 (1.7)	100.0	100.0 (1.1)	100.0 (0.6)	100.0 (1.2)	100.0 (1.1)	100.0	100.0 (0.6)	100.0 (0.9)	100.0 (0.5)	100 (0.04)	100.0
#1/Almond	**0.2**	**0.7**	**0.2**	**0.9**	**2.0**	**1.8**	**7.5**	**2.3**	**9.6**	**21.2**	**6.9**	**29.8**	**9.5**	**39.7**	**85.9**
(n.s.)	100.0 (1.7)	77.8 (3.8)	100.0 (3.1)	100.0 (1.7)	90.9	81.8 (3.0)	78.1 (3.1)	92.0 (0.7)	93.2 (0.6)	86.2	77.5 (3.1)	77.6 (1.8)	96.0 (1.2)	96.6 (0.7)	87.4
#2/Almond	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.0**	**8.8**	**2.4**	**9.9**	**23.1**	**8.4**	**36.2**	**10.1**	**42.1**	**96.8**
(n.s.)	100.0 (3.0)	88.9 (1.6)	100.0 (3.0)	100.0 (2.5)	95.5	90.9 (2.8)	91.7 (2.7)	96.0 (2.0)	96.1 (1.6)	93.9	94.4 (1.0)	94.3 (0.6)	102.0 (1.5)	102.4 (1.2)	98.5
#3/Almond	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.0**	**8.6**	**2.4**	**9.8**	**22.8**	**8.1**	**35.2**	**10.0**	**41.8**	**95.1**
(c.p.)	100.0 (3.0)	88.9 (4.2)	100.0 (2.9)	100.0 (2.6)	95.5	90.9 (4.7)	89.6 (4.0)	96.0 (6.3)	95.1 (5.6)	92.7	91.0 (2.1)	91.7 (0.5)	101.1 (2.9)	101.7 (2.6)	96.7
#4/Walnut	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.1**	**9.0**	**2.4**	**10.1**	**23.6**	**8.5**	**36.5**	**10.3**	**42.8**	**98.1**
(n.s.)	100.0 (3.0)	88.9 (1.6)	100.0 (3.1)	100.0 (9.3)	95.5	99.5 (2.0)	93.8 (1.9)	96.0 (2.7)	98.1 (2.8)	95.9	95.5 (1.3)	95.1 (1.1)	104.0 (1.4)	104.1 (1.3)	99.8
#5/Walnut	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.0**	**9.0**	**2.4**	**10.2**	**23.6**	**8.3**	**36.1**	**10.0**	**41.6**	**96.0**
(n.s.)	100.0 (3.0)	88.9 (2.4)	100.0 (2.8)	100.0 (2.5)	95.5	90.9 (1.5)	93.8 (1.2)	96.0 (1.4)	99.0 (1.2)	95.9	93.3 (3.1)	94.0 (2.4)	101.0 (2.4)	101.2 (2.0)	97.7
#6/Walnut	**0.2**	**0.7**	**0.2**	**0.8**	**1.9**	**1.7**	**7.6**	**2.2**	**9.3**	**20.8**	**7.1**	**31.8**	**9.3**	**39.7**	**87.9**
(e.p.)	100.0 (1.8)	77.8 (1.0)	100.0 (3.2)	88.9 (2.7)	86.4	77.3 (0.6)	79.2 (0.4)	88.0 (0.4)	90.3 (0.5)	84.6	79.8 (3.0)	82.8 (2.2)	93.9 (2.1)	96.6 (1.2)	89.4
#7/Borage	**0.2**	**0.9**	**0.2**	**0.9**	**2.2**	**2.1**	**9.3**	**2.4**	**10.0**	**23.8**	**8.8**	**38.3**	**10.0**	**41.6**	**98.7**
(n.s.)	100.0 (1.4)	100.0 (0.8)	100.0 (1.5)	100.0 (2.5)	100.0	95.5 (3.8)	96.9 (4.1)	96.0 (3.9)	97.1 (4.0)	96.7	98.9 (1.7)	99.7 (1.6)	101.0 (1.3)	101.2 (1.3)	100.4
#8/Borage	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.0**	**8.9**	**2.3**	**9.7**	**22.9**	**8.1**	**36.2**	**9.6**	**40.6**	**94.5**
(n.s.)	100.0 (3.0)	88.9 (0.8)	100.0 (1.5)	100.0 (0.8)	95.5	90.9 (0.8)	92.7 (0.7)	92.0 (0.8)	94.2 (0.5)	93.1	91.0 (1.7)	94.3 (2.6)	97.0 (2.5)	98.8 (2.1)	96.1
#9/Borage	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.2**	**9.5**	**2.4**	**10.1**	**24.2**	**9.1**	**39.4**	**10.3**	**42.6**	**101.4**
(c.p.)	100.0 (4.2)	88.9 (2.3)	100.0 (3.0)	100.0 (3.3)	95.5	100.0 (0.4)	99.0 (0.2)	96.0 (0.5)	98.1 (0.5)	98.4	102.2 (0.6)	102.6 (0.6)	104.0 (0.8)	103.6 (0.7)	103.2
#10/Eve. primrose	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.0**	**8.8**	**2.3**	**9.7**	**22.8**	**8.4**	**37.5**	**9.9**	**41.6**	**97.4**
(n.s.)	100.0 (1.3)	88.9 (0.8)	100.0 (2.8)	100.0 (1.7)	95.5	90.9 (0.7)	91.7 (1.1)	92.0 (1.8)	94.2 (1.8)	92.7	94.4 (1.4)	97.7 (1.1)	100.0 (1.5)	101.2 (1.4)	99.1
#11/Eve. primrose	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.3**	**9.5**	**2.5**	**10.4**	**24.7**	**8.8**	**38.2**	**10.0**	**42.5**	**99.5**
(c.p.)	100.0 (2.9)	88.9 (3.2)	100.0 (4.4)	100.0 (4.1)	95.5	104.5 (1.4)	99.0 (1.4)	100.0 (1.1)	101.0 (1.1)	100.4	98.9 (2.5)	99.5 (2.1)	101.0 (2.1)	103.4 (2.2)	101.2
#12/Eve. primrose	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.1**	**8.9**	**2.4**	**9.9**	**23.3**	**8.9**	**39.8**	**10.6**	**45.4**	**104.7**

Sample #/Source (Extraction method)	Aflatoxin--low level spiking (~2ppb total) average ppb % recovery (% SD)					Aflatoxin--medium level spiking (~25ppb total) average ppb % recovery (% SD)					Aflatoxin--high level spiking (~100ppb total) average ppb % recovery (% SD)				
	G2	**G1**	**B2**	**B1**	**Total**	**G2**	**G1**	**B2**	**B1**	**Total**	**G2**	**G1**	**B2**	**B1**	**Total**
(c.p.)	100.0 (2.9)	88.9 (2.5)	100.0 (1.5)	100.0 (2.6)	95.5	95.5 (3.2)	92.7 (5.7)	96.0 (0.3)	96.1 (1.0)	94.7	100.0 (1.1)	103.6 (1.7)	107.1 (3.8)	110.5 (2.0)	106.5
#13/Cottonseed	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.2**	**9.4**	**2.4**	**10.2**	**24.2**	**8.7**	**37.9**	**10.2**	**42.8**	**99.6**
(s.e.)	100.0 (1.4)	88.9 (1.6)	100.0 (3.0)	100.0 (1.7)	95.5	100.0 (2.8)	97.9 (2.7)	96.0 (2.9)	99.0 (2.8)	98.4	97.8 (0.3)	98.7 (0.6)	103.0 (0.8)	104.1 (0.6)	101.3
#14/Cottonseed	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.1**	**9.1**	**2.4**	**10.3**	**23.9**	**8.4**	**36.5**	**9.9**	**41.4**	**96.2**
(n.s.)	100.0 (0.0)	88.9 (0.8)	100.0 (1.5)	100.0 (0.9)	95.5	95.5 (3.9)	94.8 (3.7)	96.0 (2.8)	100.0 (2.4)	97.2	94.4 (1.1)	95.1 (1.7)	100.0 (2.7)	100.7 (2.8)	97.9
#15/Perilla	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.3**	**9.8**	**2.4**	**10.4**	**24.9**	**9.1**	**39.4**	**9.9**	**42.0**	**100.4**
(c.p.)	100.0 (2.9)	88.9 (1.6)	100.0 (1.6)	100.0 (1.7)	95.5	104.5 (2.7)	102.1 (2.1)	96.0 (2.8)	101.0 (3.0)	101.2	102.2 (1.7)	102.6 (1.3)	100.0 (1.0)	102.2 (0.6)	102.1
#16/Perilla	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.2**	**9.5**	**2.4**	**10.1**	**24.2**	**8.8**	**38.6**	**10.0**	**41.6**	**99.0**
(n.s.)	100.0 (2.9)	88.9 (0.8)	100.0 (1.6)	100.0 (1.7)	95.5	100.0 (1.1)	99.0 (0.8)	96.0 (0.1)	98.1 (0.7)	98.4	98.9 (2.6)	100.5 (2.1)	101.0 (2.1)	101.2 (2.1)	100.7
#17/Peanut	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**1.9**	**8.8**	**2.4**	**10.4**	**23.5**	**7.7**	**35.1**	**9.6**	**40.6**	**93.0**
(e.p.)	100.0 (3.3)	88.9 (1.7)	100.0 (1.6)	100.0 (1.7)	95.5	86.4 (1.0)	91.7 (0.9)	96.0 (1.3)	101.0 (1.8)	95.5	86.5 (0.4)	91.4 (0.7)	97.0 (1.8)	98.8 (2.3)	94.6
#18/Soy	**0.2**	**0.8**	**0.2**	**0.8**	**2.0**	**2.0**	**8.9**	**2.4**	**10.0**	**23.3**	**7.9**	**35.5**	**9.7**	**40.4**	**93.5**
(e.p.)	100.0 (3.4)	88.9 (1.8)	100.0 (1.4)	88.9 (2.7)	90.9	90.9 (1.1)	92.7 (0.6)	96.0 (0.6)	97.1 (0.9)	94.7	88.8 (1.5)	92.4 (1.0)	98.0 (1.6)	98.3 (1.7)	95.1
#19/Soy	**0.2**	**0.9**	**0.2**	**0.9**	**2.2**	**2.3**	**9.8**	**2.6**	**10.8**	**25.5**	**8.9**	**39.1**	**10.4**	**43.1**	**101.5**
(n.s.)	100.0 (1.4)	100.0 (1.5)	100.0 (1.4)	100.0 (0.8)	100.0	104.5 (3.1)	102.1 (2.5)	104.0 (2.2)	104.9 (2.2)	103.7	100.0 (0.4)	101.8 (0.2)	105.1 (0.6)	104.9 (0.6)	103.3
#20/Soy	**0.2**	**0.8**	**0.2**	**0.9**	**2.1**	**2.2**	**9.6**	**2.5**	**10.3**	**24.6**	**8.6**	**37.6**	**10.0**	**41.7**	**97.9**
(e.p.)	100.0 (1.5)	88.9 (0.8)	100.0 (1.4)	100.0 (0.9)	95.5	100.0 (1.1)	100.0 (0.8)	100.0 (0.6)	100.0 (0.6)	100.0	96.6 (1.5)	97.9 (1.4)	101.0 (1.6)	101.5 (1.4)	99.6

Extraction Methods: n.s.: not specified; c.p.: cold pressed; e.p.: expeller pressed; s.e.: solvent extracted