Testing for Aflatoxins in Corn and Peanut Butter using QuEChERS Cleanup and LC Fluorescence

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Introduction

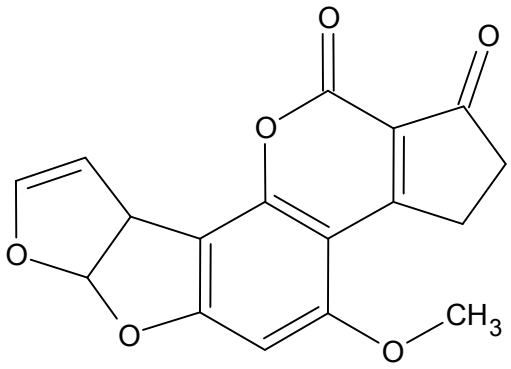
QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged, Safe and is used as a dispersive SPE (dSPE) method. It is widely used for cleanup of samples during analysis of pesticides in fruits and vegetables.

We employed the new effective lipid-removal sorbent (Z-Sep$^+$) in combination with another common SPE sorbent (Alumina N) to achieve the desired cleanup results during analysis of aflatoxins.

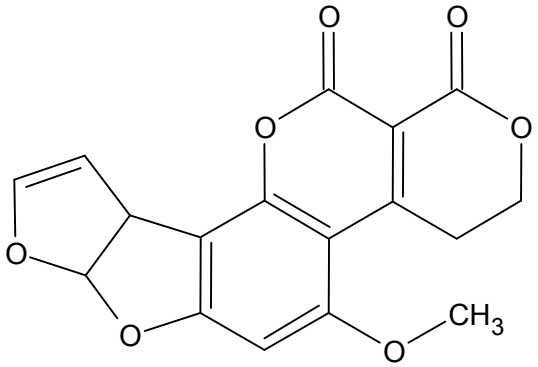
We compared the cleanup using QuEChERS procedure to that of using Immunoaffinity SPE. Two methods of detection were used – TFA pre-column off-line derivatization and bromine post-column derivatization.

The method was tested for two different samples – one was peanut butter paste (certified aflatoxins free, from Trilogy Laboratories), the other one was corn meal (purchased in the grocery store).

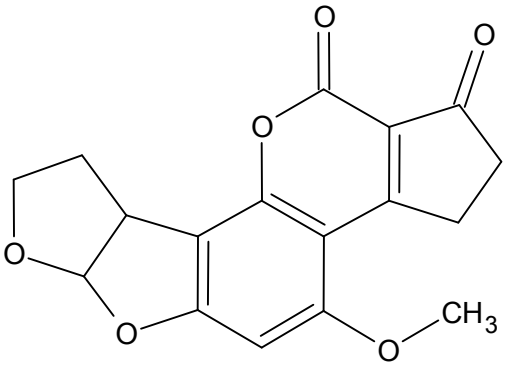
Structures of Aflatoxins



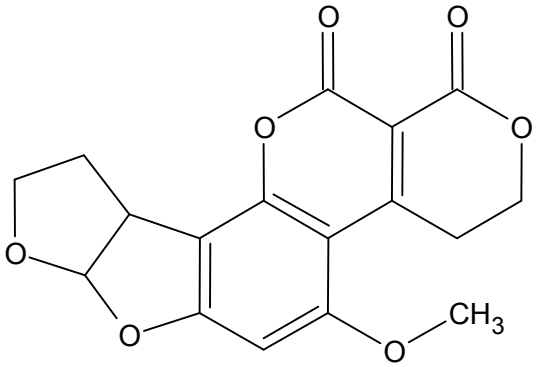
Aflatoxin B_1



Aflatoxin G_1



Aflatoxin B_2



Aflatoxin G_2

Experimental

Extraction

25 g of the sample (peanut butter or corn meal) blended for 3 min. with 100 mL of 86:14 acetonitrile:water. The resulting solution was filtered using paper filter. The extract was spiked with aflatoxins G_1, B_1, G_2, B_2 at 50 ng/g total concentration.

Cleanup using QuEChERS

The extract was first mixed with concentrated formic acid (added to reach 1% final total concentration). Then mixed with 600 mg of total sorbents in a 2 mL centrifuge tube. The mixture was shaken for 1 min. and centrifuged.

Cleanup using Immunoaffinity Columns

The extract was diluted to less then 5% acetonitrile using phosphate buffered saline. Then 18 mL of the resulting solution was loaded into the Afla Star™ column from Romer Labs®. The column was rinsed using 20 mL of water and eluted using acetonitrile. For comparison purpose, the acetonitrile was evaporated to dryness, and the extract was reconstituted into 1 mL of 84-16 acetonitrile:water solvent.

Experimental (contd.)

Derivatization Using Trifluoroacetic Acid (TFA)

200 µL of the eluent was transferred to a 5 mL reaction vial and 880 µL of a derivatization agent (70:20:10 water:TFA:acetic acid) was introduced. The mixture was shaken by hand, and reacted at 65 °C for 9 minutes then cooled to room temperature prior to HPLC analysis.

LC Conditions for TFA-derivatives:

instrument:	Hitachi Elite LaChrom
column:	Discovery® C18, 15 cm x 4.6 mm, 5 µm particles
flow rate:	2.0 mL/min.
temp.:	35 °C
det.:	360/440 nm FL
injection:	99 µL

Post-column Derivatization

KOBRA CELL (100 µA) was used with the following LC mobile phase: potassium bromide, nitric acid in 74:13:13, water:acetonitrile:methanol.

Results

Table 1. Recoveries of Aflatoxins from Peanut Butter using Cleanup by QuEChERS (n=3)

Derivatization	G_1	B_1	G_2	B_2
TFA	91%(11)	88%(1)	91%(1)	88%(1)
Br_2	105%(13)	98%(4)	91%(3)	94%(1)

Table 2. Recoveries of Aflatoxins from Corn Meal Using Cleanup by QuEChERS (n=3)

Derivatization	G_1	B_1	G_2	B_2
TFA	91%(2)	93%(1)	104%(1)	91%(1)
Br_2	92%(2)	97%(2)	95%(4)	96%(1)

Results (contd.)

Table 3. Recoveries of Aflatoxins from Corn Meal and Peanut Butter Using Cleanup by Immunoaffinity Columns and TFA Derivatization (n=3)

Sample	G_1	B_1	G_2	B_2
Peanut butter	87%(2)	95%(3)	93%(3)	93%(6)
Corn meal	102%(4)	98%(4)	99%(4)	100%(3)

Figure 1. Aflatoxin Standard (in solvent) Derivatized with TFA for Fluorescence Detection

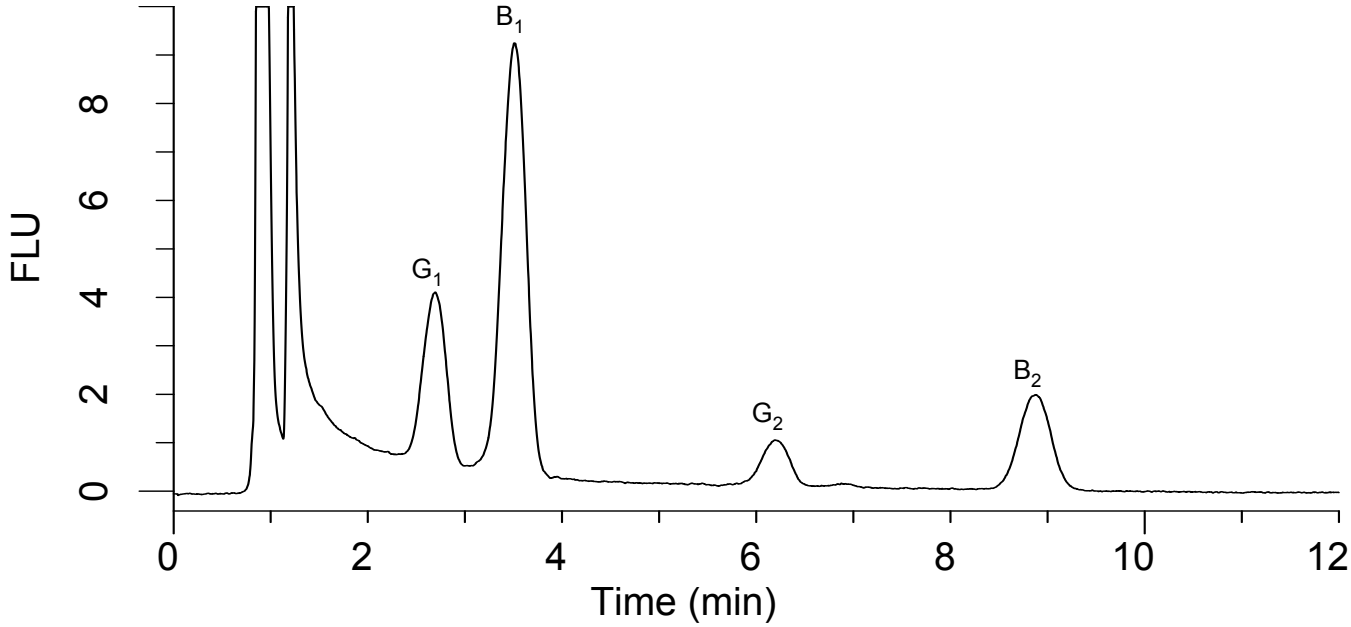


Figure 2. Aflatoxins from Peanut Butter (A) and Corn Meal (B) Derivatized with TFA for Fluorescence Detection and cleaned using QuEChERS

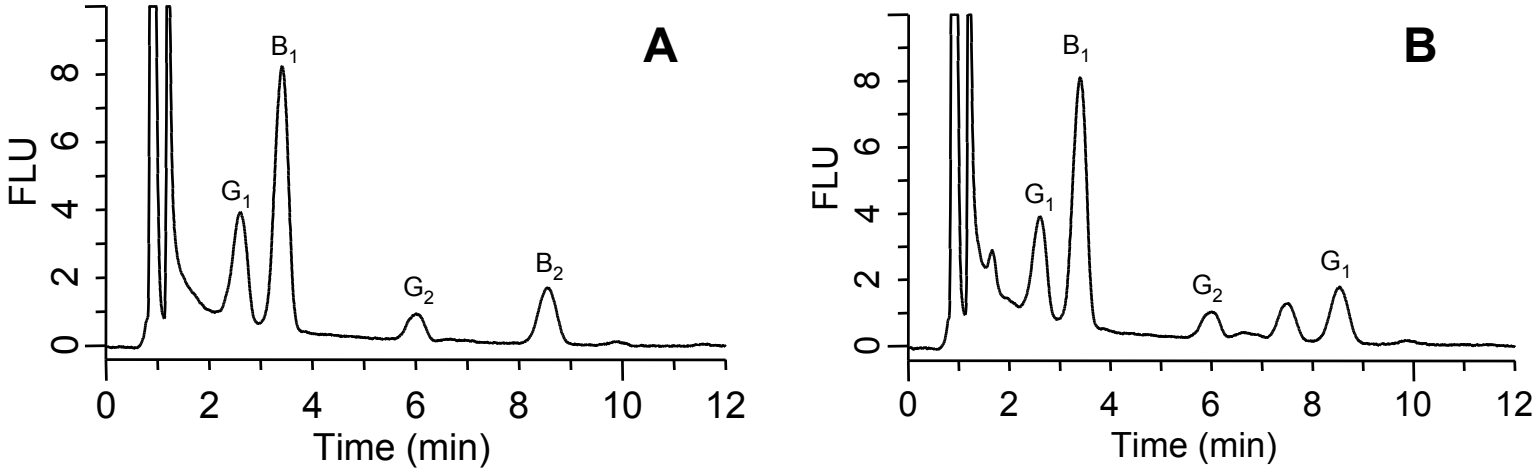


Figure 3. Aflatoxins from Peanut Butter (A) and Corn Meal (B) Derivatized with TFA for Fluorescence Detection and cleaned using Immunoaffinity Columns

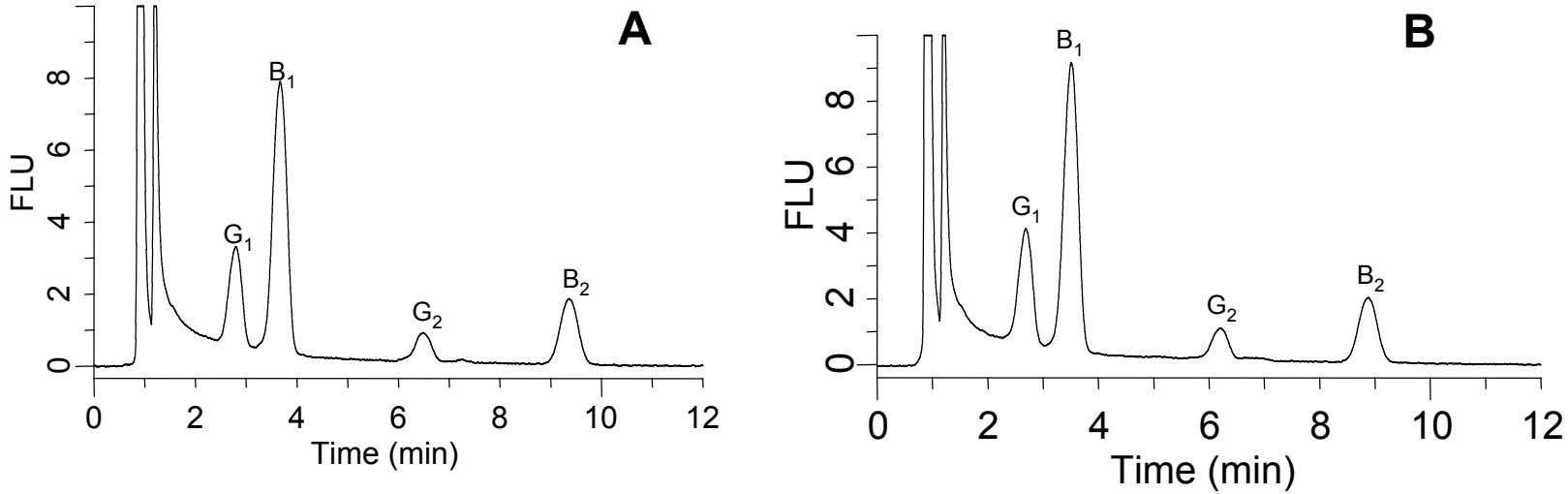
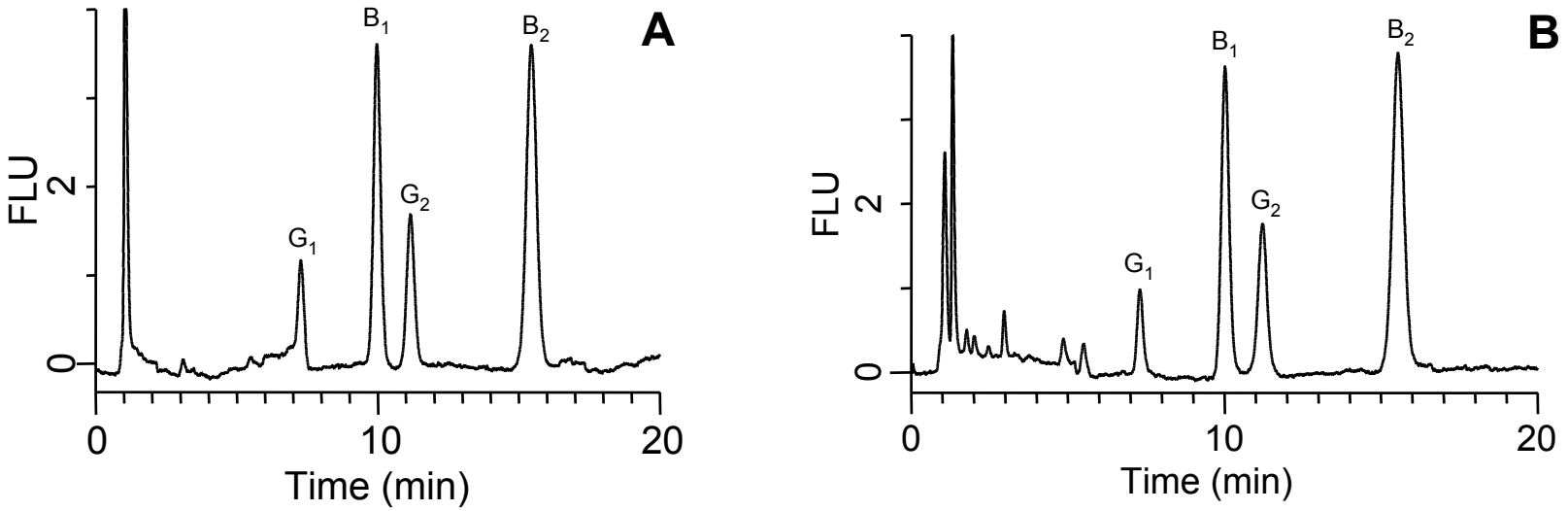


Figure 4. Aflatoxins from Peanut Butter (A) and Corn Meal (B) Post-column Derivatized with Bromine for Fluorescence Detection and cleaned using QuEChERS



Discussion

Development of a Mix of SPE Phases for QuEChERS Method

Several common sorbents were tested to achieve the desired cleanup along with the Z-Sep$^+$. These included: silica, alumina, florisil. Alumina N gave the best cleanup results in mixture with Z-Sep$^+$ and was used for the developed method.

The peanut butter was used as a sample during method development. Corn was analyzed later using the same method.

Cleanup using Z-Sep$^+$-Alumina mix was compared to that of using C18-Alumina mix. The values for background signal at 2 min. were compared in TFA-derivatized samples. The background was significantly lower when using Z-Sep$^+$-Alumina mix (data not shown in this poster).

Discussion (contd.)

Recovery and Cleanup

The recoveries of aflatoxins spiked into extracts of peanut butter and corn were very good when a new QuEChERS cleanup was used. They compare well to the recoveries obtained by using immunoaffinity cleanup columns.

The reproducibility of the method was excellent for 50 ppb spiked extracts using both TFA and bromine derivatizations.

The cleanup for peanut butter samples produced no background peaks. The corn samples cleaned by QuEChERS and derivatized with TFA contained one or two background peaks that did not interfere with aflatoxins quantitation. When bromine post-column derivatization was used – no background peaks were observed for corn samples.

The QuEChERS method was tested for 2 other mycotoxins – deoxynivalenol (DON) and zearalenone (ZON). These compounds were retained too strongly on the Z-Sep$^+$ and could not be quantitatively recovered.

Conclusions

The new QuEChERS cleanup method performed well for the analysis of aflatoxins B_1, B_2, G_1, G_2 in peanut butter and corn samples.

The recoveries of spiked aflatoxins using QuEChERS cleanup were comparable to those obtained using immunoaffinity cleanup method.

QuEChERS cleanup was faster than the cleanup using immunoaffinity columns, there was no need for sample dilution, loading 20 mL of sample and washing with 20 mL of water.

References

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