

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

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## Introduction

Aflatoxins are metabolites produced by fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) in high humidity environment in crops such as maize nuts and processed food. Aflatoxin contamination in food is monitored with strict regulations worldwide due to high toxicity of the compounds [1]. Recently, several media reports revealed the exceed levels of aflatoxins found in peanut butters in the USA and Taiwan. Aflatoxins in food have been

analyzed by LC/MS/MS using various sample pre-treatment methods. We describe a high sensitivity LC/MS/MS method for quantitative analysis of aflatoxins in peanut butters using QuEChERS sample pre-treatment procedure [2], as opposed to the use of immunoaffinity column or other methods for sample pre-treatment which are more expensive and tedious. High sensitivity and good recoveries were achieved using this LC/MS/MS method.

## Experimental

A mixed standard of aflatoxin B1, B2, G2 and G2 was obtained from Supelco. A stock solution was prepared using methanol as the diluent, from which calibrant series and spiked samples were prepared. The QuEChERS kits were purchased from RESTEK. Two grams of peanut butter was first extracted with the extraction kits followed by cleaning up using dSPE tubes. The procedure was adjusted and optimized to obtain highest recovery. A

LCMS-8050 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A C18 column (Kinetex, 2.1 x 100mm, 1.7 $\mu$ m) was used for fast separation of aflatoxins using a gradient elution program. The method development and performance evaluation were carried out using spiked aflatoxins in peanut butter samples. Table 1 shows the analytical conditions on LCMS-8050.

Table 1: LC/MS/MS analytical conditions of aflatoxins on LCMS-8050

|                     |   |
|---------------------|---|
| Column              | Kinetex C18 (2.1mmI.D x 100mmI.D, 1.7 $\mu$ m)  |
| Flow rate           | 0.5 mL/min  |
| Mobile phase        | A: 5mM ammonium acetate in water with 0.1% FA<br>B: 5mM ammonium acetate in MeOH  |
| Oven temp.          | 40 °C   |
| Injection vol.      | 5 $\mu$ L   |
| Elution mode        | Gradient elution, B%: 5% (0 to 0.5 min) $\rightarrow$ 50% (4 to 5.5 min)<br>$\rightarrow$ 85% (6 to 7.5 min) $\rightarrow$ 5% (8.1 to 10 min) |
| Interface           | ESI   |
| MS mode             | Positive, MRM, 2 transitions for each compound  |
| Interface temp.     | 350 °C  |
| Block temp.         | 400 °C  |
| DL temp.            | 250 °C  |
| CID gas             | Ar (350 kPa)  |
| Nebulizing gas flow | 3.0 mL/min  |
| Drying gas flow     | 10.0 L/min  |
| Heating gas flow    | 10.0 L/min  |

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## Results and Discussion

### QuEChERS sample pre-treatment

Hexane was used in the procedure to remove fats, oils and non-polar components from the peanut butter samples. The extraction step was completed using Q-sep QuEChERS extraction salt packet (4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate). Dispersive SPE tube containing MgSO<sub>4</sub>, PSA and C18 was used in the clean-up process to remove remaining water, organic acid and non-polar components respectively. The process of the sample preparation is illustrated in Figure 1.

### Method Development

Automated MRM optimisation of aflatoxins was carried out using the LabSolutions workstation. The precursors of aflatoxins B1, B2, G1 and G2 were their protonated ions ( $m/z$ 313.1,  $m/z$ 315.1,  $m/z$ 329.1 and  $m/z$ 331.1, respectively). Two MRM transitions of every aflatoxin were chosen as quantifier and confirmation ion (Table 2). A peanut butter matrix free from aflatoxins was used as a "blank" and matrix for the preparation of post-spiked calibrants to build calibration curves. The blank and every post-spiked calibrant was injected thrice and the average area was calculated to obtain reliable results. A chromatogram of spiked sample is shown in Figure 2. Linear calibration curves were obtained for all four aflatoxin compounds. Good linearity with correlation coefficient ( $r^2$ ) greater than 0.999 across the range of 10 pg/mL - 10 ng/mL was obtained. The calibration curves of aflatoxins spiked in peanut butter matrix are shown in Figure 3.

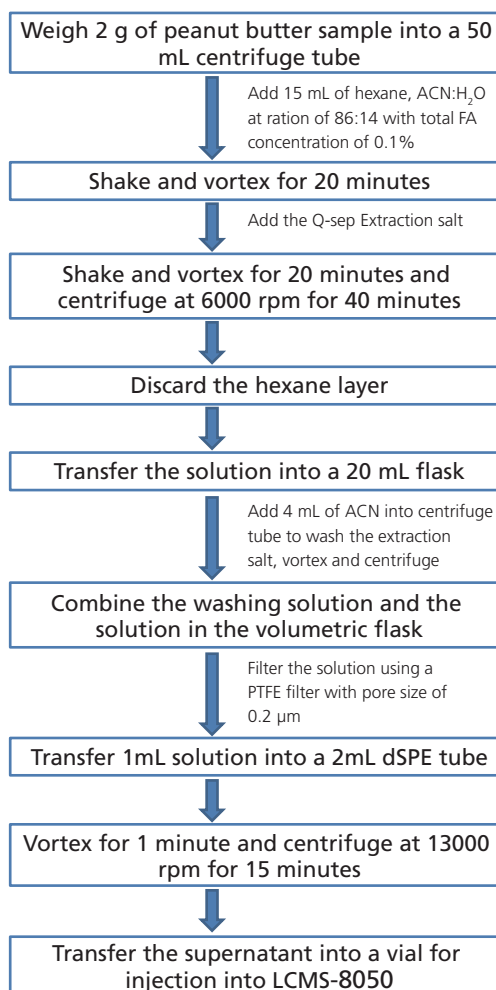


Figure 1: Flow chart of sample pretreatment for aflatoxins in peanut butter by modified QuEChERS method.

Table 2: LC/MS/MS analytical conditions of LCMS-8050 for aflatoxins

| Compound     | MRM (m/z)    | CID Voltage (V) |     |     |
|--------------|--------------|-----------------|-----|-----|
|              |              | Q1              | CE  | Q3  |
| Aflatoxin B1 | 313.1>241.0* | -15             | -37 | -26 |
|              | 313.1>213.0  | -15             | -46 | -22 |
| Aflatoxin B2 | 315.1>259.1* | -15             | -30 | -28 |
|              | 315.1>287.0  | -15             | -27 | -20 |
| Aflatoxin G1 | 329.1>243.0* | -30             | -27 | -27 |
|              | 329.1>200.0  | -30             | -42 | -21 |
| Aflatoxin G2 | 331.1>245.0* | -16             | -33 | -25 |
|              | 331.1>189.0  | -16             | -42 | -19 |

\*MRM transitions used as quantifiers.

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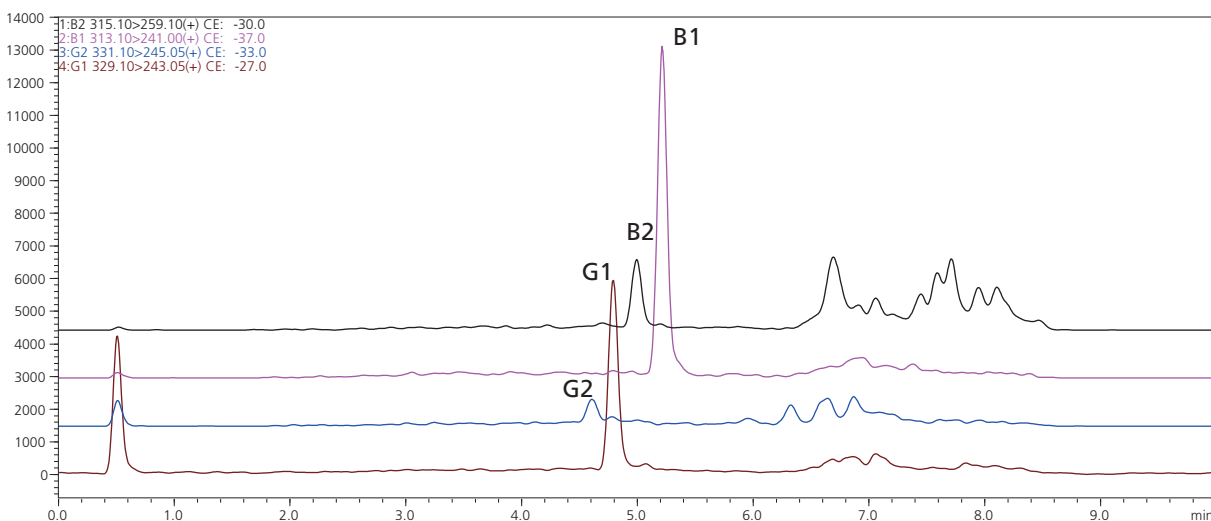


Figure 2: MRM chromatograms of aflatoxins spiked in peanut butter sample (B1 and G1 at concentration of 100 pg/mL; B2 and G2 at concentration of 30 pg/mL).

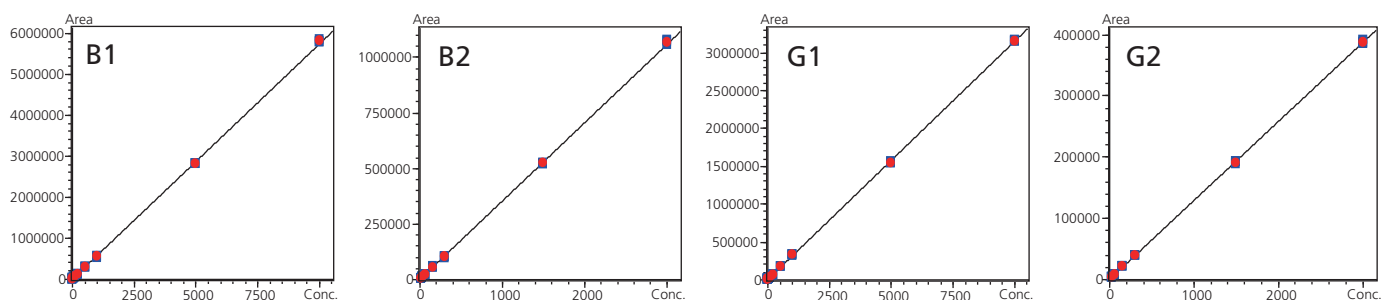


Figure 3: Calibration curves of aflatoxins B1, B2, G1 and G2 in peanut butter matrix.

Table 3: LOD, LOQ and repeatability of aflatoxin spiked samples at different concentrations

| Compound | Concentration range (pg/mL) | %RSD (n=6) |        |        |        |         |         | LOQ  | LOD | r2     |
|----------|-----------------------------|------------|--------|--------|--------|---------|---------|------|-----|--------|
|          |                             | 15 ppt     | 30 ppt | 50 ppt | 60 ppt | 100 ppt | 200 ppt |      |     |        |
| B1       | 10 - 10000                  |            |        | 2.71   |        | 2.22    | 1.20    | 4.6  | 1.5 | 0.9995 |
| B2       | 11 - 3000                   | 7.73       | 7.51   |        | 2.96   |         |         | 8.7  | 2.9 | 0.9997 |
| G1       | 10 - 10000                  |            |        | 2.21   |        | 1.82    | 1.21    | 4.2  | 1.4 | 0.9995 |
| G2       | 30 - 3000                   |            | 12.12  |        | 9.10   |         |         | 22.4 | 7.4 | 0.9995 |

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

## Method Performance Evaluation

As shown in Table 4, the LOD and LOQ of aflatoxins in peanut butter matrix are lower than 7.4 pg/mL and 22.4 pg/mL respectively. The repeatability of the method was evaluated using using spiked samples at lower concentrations. The peak area %RSD of aflatoxins were found to be lower than 7.5% except for G2 with %RSD of 12.1%.

The matrix effect was evaluated by using the average of spiked samples injected thrice at different concentrations. The recoveries of aflatoxins were determined by using a duplicate set of samples at different concentrations. Each duplicate was obtained from the average of three injections. The results are shown in Table 4.

Table 4: Matrix effects of the MRM method for aflatoxins in spiked peanut butter samples

| Concentration (pg/mL) | Matrix effect (%) |       | Concentration (pg/mL) | Matrix effect (%) |       |
|-----------------------|-------------------|-------|-----------------------|-------------------|-------|
|                       | B1                | G1    |                       | B2                | G2    |
| 50                    | 78.35             | 80.63 | 60                    | 72.31             | 70.14 |
| 100                   | 71.88             | 71.24 | 150                   | 73.81             | 74.72 |

Table 5: Recoveries of aflatoxins in spiked peanut butter samples

| Compound | Recovery (%) |       |          |       |          |       |           |       |
|----------|--------------|-------|----------|-------|----------|-------|-----------|-------|
|          | 30 pg/mL     |       | 50 pg/mL |       | 60 pg/mL |       | 200 pg/mL |       |
|          | Dup 1        | Dup 2 | Dup 1    | Dup 2 | Dup 1    | Dup 2 | Dup 1     | Dup 2 |
| B1       |              |       | 70.24    | 74.00 |          |       | 81.52     | 80.49 |
| B2       | 65.22        | 71.20 |          |       | 87.40    | 85.12 |           |       |
| G1       |              |       | 90.92    | 95.11 |          |       | 77.47     | 79.38 |
| G2       | 79.97        | 79.58 |          |       | 87.76    | 86.52 |           |       |

## Analysis of aflatoxins in actual samples

Three peanut butter samples from local supermarket were analysed using the established MRM method. The results showed that aflatoxins were found in two of the samples (Table 6). While the aflatoxins in sample M is within the

EU regulatory limits (sum of aflatoxins below 4 µg/kg), the aflatoxin B1 amount in sample UL exceeds the allowed concentration (aflatoxin B1 below 2 µg/kg).

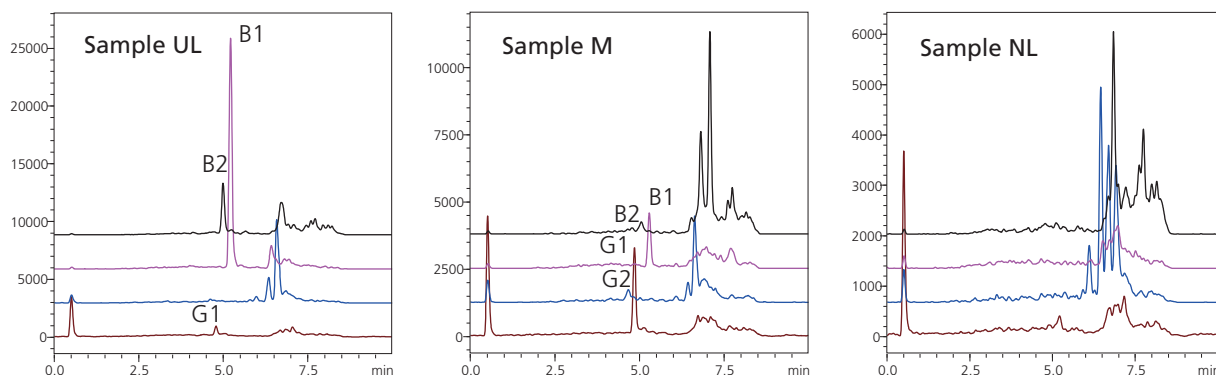


Figure 4: Chromatograms of peanut butter samples.

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

Table 6: The amount of aflatoxins found in peanut butter samples from supermarket

| Sample | Concentration (ng/g) |              |              |              |
|--------|----------------------|--------------|--------------|--------------|
|        | B1                   | B1           | G1           | G2           |
| UL     | 2.09                 | 0.79         | 0.03         | Not detected |
| M      | 0.16                 | 0.06         | 0.50         | 0.18         |
| NL     | Not detected         | Not detected | Not detected | Not detected |

## Conclusions

A high sensitivity LC/MS/MS method with QuEChERS for sample pre-treatment was established using Shimadzu LCMS-8050 system. The QuEChERS sample preparation method was proven effective and easy to operate. The method performance including sensitivity, linearity,

repeatability, matrix effect and recovery were carried out and the results confirm that the method is feasible and reliable for determination of aflatoxins in peanut butter samples.

## References

- (1) Pereira, V.; Fernandes, J.; Cunha, S. *Trends in Food Science & Technology* 2014, 36, 96-136.
- (2) Liu, Y.; Han, S.; Lu, M.; Wang, P.; Han, J.; Wang, J. *Journal of Chromatography B* 2014, 970, 68-76.