$$\text{Aflatoxin, ng/g} = A \times (T_v/L_v) \times (1/W) = A \times 40$$

where W = weight of commodity represented by eluate, A = ng aflatoxin in eluate injected, T_v = final eluate volume (2000 µL), and L_v = eluate injected (50 µL). Add concentrations of the 4 aflatoxins to obtain total aflatoxin concentration.

[*Note:* Soak all laboratory glassware in 10% solution of household bleach, which generally contains 5.25% NaOCl, before reusing or discarding. *See* **990.32J** (*see* 49.2.16) for further details on decontamination.]

Reference: *JAOAC* **74**, 81(1991).

Revised: March 2002

49.2.18A

AOAC Official Method 2005.08 Aflatoxins in Corn, Raw Peanuts, and Peanut Butter

Liquid Chromatography with Post-Column Photochemical Derivatization

First Action 2005

(The study is a change of method proposal for **991.31** (*see* 49.2.18) and **999.07** (*see* 49.2.29) and the modification (from TLC to LC) for **970.45** (*see* 49.2.09).)

[The photochemical reactor for enhanced detection (PHRED) is applicable to determination of aflatoxins in test extracts of corn and peanuts when using **991.31** (*see* 49.2.18), **999.07** (*see* 49.2.29), or **970.45** (*see* 49.2.09) with LC. Although no significant difference exists for the PHRED in comparison to other post-column methods with peanuts, a slightly high bias is obtained with corn when compared with the iodine or Kobra cell possibly due to higher recovery.]

Caution: Mycotoxins are toxic substances. Perform manipulations in a hood wherever possible, taking particular precautions, such as using a glove box, when toxins are in dry form because of their electrostatic nature and tendency to disperse. Swab any accidental spills and all glassware and waste materials with 5% NaOCl bleach. Use UV glasses if there is exposure to any direct or reflected UV light from the light source. Aflatoxins must be handled with extreme caution because they are known to be carcinogens. Use hypochlorite bleach for cleaning glassware and when disposing of waste materials.

A. Principle

Post-column derivatization of aflatoxins can increase detectability and/or selectivity of responses for the HPLC detector. By performing the derivatization photochemically, the derivative structures B_{2a} and G_{2a} are apparently obtained, providing the enhanced signals for the B_1 and G_1 aflatoxins without effect on the B_2 and G_2 aflatoxins.

B. Apparatus

Note: Evaluate any leakage of UV light from source equipment and if detected, provide shielding or protective glasses during use. To prevent leakage of the knitted reactor coil, do not overtighten the connection. If leakage occurs, disconnect power to the photochemical reactor before inspecting the unit.

Equipment noted is not restrictive; equivalent systems can be substituted.

(a) *LC system.*—SP 8700 XR pump, SP 4200 computing integrator, SP 8780 autosampler, and SP WINNER software (Spectra-Physics Analytical, San Jose, CA, USA) with Kratos FS 970 LC fluorometer set to provide 365 nm excitation and 435 nm emission.

(b) *Column.*—Beckman Ultrasphere C18, 150 × 4.6 mm with 5 µm particle size (No. 235330; Alltech Associates, Inc., Deerfield, IL, USA; www.alltechweb.com/US/Home.asp).

(c) *PHRED photochemical reactor.*—With low-pressure mercury lamp and knitted reactor coils, preferably KRC-25-25 with a 25 m × 0.25 mm id coil (AURA Industries, New York, NY, USA; www.aura-inc.com).

(d) *Silanized vials.*—Four mL, amber with Teflon-lined screw caps (No. 72680; Alltech).

(e) *Pipet.*—Class A, volumetric, 2 mL.

(f) *Replacement plastic grooved ferrules.*—No. ZGF1PK-10 (Valco Instrument Co. Inc., Houston, TX, USA; www.vici.com/profiles/prof_val.php).

C. Reagents

(a) *Degassed mobile phase.*—40% Methanol in 60% water (v/v), or a suitable mixture of methanol, acetonitrile, and water that results in baseline separation of the aflatoxins. *Note*: Unless otherwise specified, use only analytical grade reagents. One of the solvent systems indicated for the photochemical system permits the elimination of acetonitrile. This can be advantageous for some laboratories. Any combination of methanol, water, and acetonitrile can be used so long as the mobile phase provides baseline separation of the aflatoxins.

(b) *Injection solvent.*—Same as that used in mobile phase.

(c) *Aflatoxin standards.*—Supelco Inc. (Bellefonte, PA, USA; www.sigma-aldrich.com), or other suppliers.

D. Fluorescence Detector Conditions

Use detector parameters which are applicable for the available equipment in accordance with the manufacturer's recommendations. (Conditions found optimum in one laboratory for fluorescence detector: Excitation, 365 nm; emission, 435 nm; filter mode, resistor-capacitor circuit (RC) response setting of slow; digital filter, 3 or 5 s; gain, 10 or above; attenuation, 1 or as needed.)

E. Standards

To establish a standard curve, prepare 5 concentrations of a mixed aflatoxin standard containing from 0.1 to 1.0 ng toxin per 20 µL injection solvent. Evaporate these individual extracts to dryness under nitrogen in silanized vials. Subsequently, reconstitute each of the extracts with 2.0 mL injection solvent and stir with a Vortex mixer for at least 2 min.

F. Analysis

Prepare test extracts by any of the officially recognized AOAC aflatoxin extraction methods using post-column derivatization such as **991.31** (*see* 49.2.18) and **999.07** (*see* 49.2.29), or the non-LC method **970.45** (*see* 49.2.09). Dry to a film in silanized vials and store frozen at –20°C until ready for evaluation.

(*1*) Reconstitute test extracts with 2.0 mL injection solvent and stir with a Vortex mixer for at least 2 min.

(*2*) Using a 1 mL/min flow rate of the mobile phase and 20 µL injections of standards and extracts, begin with the use of a fres

preparation of aflatoxin standards and confirm that the equipment operation is providing the expected standard values for aflatoxins B_1, B_2, G_1, and G_2. Adjust flow rate if necessary to effect best separation.

(*3*) Inject 20 µL of each test extract, recording both peak height and area data. If concentration is found to be below 5 ng/g, re-inject 100 µL which will increase sensitivity by a factor of 5.

(*4*) Calculate concentrations using the equations in **991.31** (*see* 49.2.18), **999.07** (*see* 49.2.29), or derived from

$$\text{Aflatoxin, ng/g} = A \times (T/I) \times (1/W)$$

where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (µL), I = volume eluate injected into LC (µL), W = mass (g) of commodity represented by final extract.

The use of at least a 3-point calibration curve is preferable but a single-point calibration can be used if the response has previously been shown to be linear and injections of the standard have been made throughout the run to demonstrate the detector response is within acceptable limits. For example, the 0.5 ng/20 µL may be used for the calibration after the curve is verified. The useful life of the UV bulb is approximately 3000 h, or until a consistent decrease of response (i.e., 10%) is noticed by a change of the B_1/B_2 peak area ratio for the aflatoxin standard.

Reference: *J. AOAC Int.* **89**, 678(2006).