AOAC Official Method 995.15
Fumonisins $B_1$, $B_2$, and $B_3$ in Corn
Liquid Chromatographic Method
First Action 1995
Final Action 1999

AOAC–IUPAC Method
(Applicable for determination of fumonisins in corn at ≥1 μg/g.)

Caution: Fumonisins are hepatotoxic and carcinogenic to rats; effects on humans are not fully known. Wear protective gloves to avoid skin contact with corn extracts. Any laboratory spillages should be washed with a 5% aqueous solution of commercial sodium hypochlorite followed by $H_2O$.

See Table 995.15 for results of the interlaboratory study supporting acceptance of the method.

A. Principle
Fumonisins are extracted from corn with methanol-$H_2O$ solution. Filtered extract is purified on strong anion exchange solid-phase extraction cartridge and fumonisins are eluted with acetic acid-methanol solution. Residue is dissolved in methanol and o-phthaldialdehyde (OPA)/2-mercaptoethanol is added to form fluorescent fumonisin derivatives, determined by reversed-phase liquid chromatography with fluorescence detection.

B. Apparatus
(a) Liquid chromatograph (LC).—LC pump delivering 1 mL/min constant flow rate, with injection system calibrated to deliver 10 μL.
(b) LC column.—(1) 150 × 4.6 mm id, C$_{18}$ reversed-phase, stainless steel, packed with 5 μm octadecyl silica (ODS) material, and (2) suitable corresponding reversed-phase guard column containing similar packing material. Maintain LC column oven at ambient temperature (ca 23°C).
(c) Fluorescence detector.—Variable wavelength; set at 335 nm (excitation) and 440 nm (emission).
(d) Tissue homogenizer.
(e) Solid-phase extraction (SPE) cartridges.—10 mL capacity; containing 500 mg silica-based strong anion exchange (SAX) sorbent (Varian Bond Elut® cartridge is suitable). Evaluate performance of individual batches of sorbent using certified or in-house reference materials.
(f) SPE manifold.
(g) Solvent evaporator.—To hold 4 mL capacity glass vials; operating at 60°C.
(h) Membrane filter.—Porosity 0.45 μm.

C. Reagents
(All reagents must be analytical grade, unless otherwise noted.)

Table 995.15 Interlaboratory study results for determination of fumonisins in corn by liquid chromatographic method

<table>
<thead>
<tr>
<th>Fumonisin analogue</th>
<th>Amount added, ng/g</th>
<th>Mean, ng/g</th>
<th>Recovery, %</th>
<th>$s_t$</th>
<th>$s_R$</th>
<th>RSD, %</th>
<th>RSD$_R$, %</th>
<th>$r^a$</th>
<th>$R^b$</th>
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$^a$ $r = 2.8 \times s_t$.
$^b$ $R = 2.8 \times s_R$.
$^c$ NB$_1$ = Corn naturally contaminated with fumonisin $B_1$ at a mean concentration of 4246 ng/g.
$^d$ NB$_2$ = Corn naturally contaminated with fumonisin $B_2$ at a mean concentration of 1234 ng/g.
$^e$ NB$_3$ = Corn naturally contaminated with fumonisin $B_3$ at a mean concentration of 373 ng/g.
(a) Methanol.
(b) Phosphoric acid.—Concentration >85%.
(c) 2-Mercaptoethanol.
(d) Acetonitrile-H₂O solution.—1:1 (v/v).
(e) Acetic acid-methanol solution (1 + 99).—Use glacial acetic acid when preparing solution.
(f) Sodium dihydrogen phosphate solution.—0.1M. Dissolve 15.6 g NaH₂PO₄×2H₂O in 1 L H₂O.
(g) Methanol-H₂O solution.—3 + 1 (v/v).
(h) Sodium hydroxide.—1M. Dissolve 40.0 g NaOH in 1 L H₂O.
(i) Disodium tetraborate solution.—0.1M. Dissolve 3.8 g Na₂B₄O₇·10H₂O in 100 mL H₂O.
(j) LC mobile phase.—methanol-0.1M NaH₂PO₄ (77 + 23; v/v), adjusted to apparent pH 3.3 with H₃PO₄.
(b) Filter mobile phase through membrane filter and pump at 1 mL/min flow rate. LC mobile phase composition may have to be adjusted to conform with individual LC column characteristics.
(k) o-Pthalialdehyde (OPA) reagent.—Dissolve 40 mg OPA in 1 mL methanol, (a), and dilute with 5 mL 0.1M Na₂B₄O₇. (i), add 50 µL 2-mercaptoethanol, (c), and mix. Store OPA reagent at room temperature in capped amber or aluminum foil-covered vial up to 1 week.
(l) Fumonisin standards.—Available from PROMEC, Medical Research Council, PO Box 19070, Tygerberg 7505, South Africa.
(m) Fumonisin standard solutions.—Prepare stock solution of individual fumonisin B1, fumonisin B2, and fumonisin B3 at concentrations of 250 µg/mL in acetonitrile-H₂O solution, (d). Transfer 100 µL aliquots of each stock solution to clean glass vial and add 200 µL acetonitrile-H₂O solution, (d), to yield standard working solution containing the 3 fumonisins analogues at individual concentrations of 50 µg/mL. Fumonisins stock and working standard solutions are stable up to 6 months at 4°C.

**D. Extraction and Cleanup**

Grind corn laboratory sample to yield material of such size that ca 90% is retained between 500–250 µm mesh screens. Weigh 50 g test portion into 250 mL plastic centrifuge bottle. Add 100 mL methanol-H₂O solution, (g), and homogenize 3 min at 60% full speed setting. Alternatively, blender may also be used at the same speed settings, but period of 5 min should be allowed for complete extraction.

Centrifuge mixture 10 min at 500 × g and filter supernate through fluted filter paper. Filtrate should have apparent pH ca 5.8. If necessary, adjust pH to 5.8–6.5 with 1M NaOH (only 2–3 drops should be required).

Fit SPE cartridge to SPE manifold. Condition cartridge by washing successively with 5 mL methanol, (a), followed by 5 mL methanol-H₂O solution, (g). Apply 10 mL filtered extract to cartridge, while maintaining flow rate ≤2 mL/min. Wash cartridge with 5 mL methanol-H₂O solution followed by 3 mL methanol. Do not let cartridge dry. Elute fumonisins with 10 mL acetic acid-methanol solution, (e), at flow rate ≤1 mL/min. (Note: It is critical that flow rate does not exceed 1 mL/min.) Collect eluate in 20 mL glass collection vial.

Sequentially transfer aliquot of eluate to 4 mL glass vial, while evaporating solvent to dryness under stream of N at ca 60°C. Rinse collection vial with 1 mL methanol and add rinsing solvent to 4 mL vial, washing sides of vial to concentrate residue at its base. Evaporate additional methanol to dryness to ensure that all acetic acid has evaporated. Dried residues may be retained up to 1 week at 4°C prior to LC analysis.

**E. Derivatization and LC Analysis**

(a) Preparation of standard derivative.—Transfer 25 µL fumonisin standard working solution, (m), to base of small test tube. Add 225 µL OPA reagent, (k), mix, and inject 10 µL into LC system within 1 min after addition of OPA reagent.

(Note: It is critical to adhere to reproducible times between addition of OPA reagent and injection into LC system. Fluorescence of OPA-fumonisin begins to decrease after 2 min.)

(b) Detector and recorder response.—Adjust sensitivity settings of fluorescence detector so fumonisin B₁ standard-OPA derivative yields at least 80% detector response.

(c) Corn extracts.—Redissolve residue from D in 200 µL methanol. Transfer 25 µL solution to base of small test tube and add 225 µL OPA reagent. Mix and inject 10 µL solution into LC system within 1 min of adding OPA reagent. All fumonisin peaks should be on scale. Peak identity should be confirmed by comparison of retention times in extracts with those observed for individual fumonisin standard.

If fumonisin chromatographic peaks exceed those of fumonisin standard solution, make additional dilutions of extracts with methanol and repeat derivatization with OPA reagent.

**F. Calculations**

Calculate fumonisin present in aliquot injected (F; ng) into LC system using peak areas for each fumonisin analogue, as follows:

\[
F \text{ (ng)} = \frac{P_u}{P_f} \times S
\]

where \(P_u\) = individual fumonisin peak area of test solution; \(P_f\) = individual fumonisin peak area of standard solution; \(S\) = amount of individual fumonisin standard injected into LC system (50 ng/fumonisin analogue) [based on concentration in fumonisin standard working solution, (m)].

Calculate concentration of fumonisin present in corn (C; ng/g), as follows:

\[
C \text{ (ng/g)} = \frac{F \times V_i \times D}{V \times W}
\]

where \(V_i\) = total volume of derivatized solution, 250 µL; \(D\) = any dilution factor which may have been used; \(V_i\) = injection volume, 10 µL; \(W\) = test portion equivalent weight, 0.625 g.

CAS-116355-83-0 (Fumonisin B₁)
CAS-116355-84-1 (Fumonisin B₂)
CAS-136379-59-4 (Fumonisin B₃)
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