Gas Chromatographic Determination of Deoxynivalenol in Wheat

GLENN A. BENNETT, ROBERT D. STUBBLEFIELD, GAIL M. SHANNON, and ODETTE L. SHOTWELL
U.S. Department of Agriculture, Agricultural Research Service, Northern Regional Research Center, Peoria, IL 61604

Modifications to a published method are described for the determination of deoxynivalenol (DON) in wheat by gas chromatography with electron capture quantitation of the heptafluorobutyrate derivative. In the modified method, DON is extracted by shaking the sample with methanol–water on a wrist-action shaker, followed by filtration through rapid flow paper. One concentration step is eliminated, and a hexane wash is incorporated to remove toluene from the silica gel column. Recoveries of DON from wheat samples spiked at 0.1, 0.5, and 1.0 ppm ranged from 77.3 to 86.3% and averaged 81.5%.

Methods development for mycotoxins is an evolutionary process whereby procedures from various methods are combined or modified to improve assays or to reduce analysis time. Scott et al. (1) developed a sensitive method for the determination of deoxynivalenol (DON) in wheat by gas chromatography with electron capture (GC–EC) detection and confirmation by mass spectrometry (MS) to assess the extent of contamination in the 1980 and 1981 Canadian wheat crop. This method has a low detection limit (10 ppb), is reliable (coefficient of variation 10%), gives satisfactory recoveries (57–86%), and has been tested on a large number of naturally contaminated samples. A subsequent evaluation (2) of currently available methodology for trichothecenes concluded that GC–EC with MS confirmation is the best technique to date for determining these toxins.

In 1982, some of the hard red winter wheat harvested in eastern Nebraska and Kansas was visibly damaged (scabby) with Fusarium sp. and suspected of being contaminated with DON (3, 4). To expedite analysis of a large number of wheat samples, we modified the Scott procedure (1) to increase the number of samples an analyst may prepare each day. This communication describes the revised method currently used in this laboratory.

METHOD

Reagents and Materials

(a) Solvents.—HPLC grade methanol, ethyl acetate, n-hexane, methylene chloride, toluene, acetone, acetonitrile.
(b) Ammonium sulfate.—30% solution.
(c) Celite.—Hyflo Super-Cel diatomaceous earth.
(d) Silica gel.—E. Merck 60, 0.063–0.200 mm particle size, containing 1% water. Activate by drying 1 h at 105°C. Add 1 mL water/100 g, seal, shake until thoroughly mixed, and store 15 h in airtight container.
(e) Deoxynivalenol.—Working solutions of 1 and 2 Jg/mL toluene-acetonitrile (95 + 5) prepared from stock solution of 0.10 mg crystalline deoxynivalenol/mL acetonitrile. Available from Myco-Lab Co., PO Box 321, Chesterfield, MO 63170.
(f) N-Heptafluorobutyrylimidazole (HFBI).—1 g ampules (Pierce Chemical Co., Rockford, IL 61105).
(g) Sodium bicarbonate.—5% aqueous solution.
(h) Sodium sulfate.—Anhydrous, granular (Mallinckrodt Chemical Co., Paris, KY).
(i) Potassium chloride.—5% aqueous solution.

Apparatus

(a) Shaker.—Burrell wrist-action shaker.
(b) Filter paper.—Rapid flow, S&S 588 (Carl Schleicher and Schuell Co., Keene, NH).
(c) Heating block.—Model 2090 Temp-Block module heater (Lab-Line Instruments, Inc., Melrose Park, IL).
(d) Vial-shaking device.—Vortex tube mixer, or equivalent.
(e) Chromatographic column.—Glass, 50 × 1.3 cm od with Teflon stopcock.
(f) Micro vials.—2 mL, with septum caps (Regis Chemical Co., Morton Grove, IL).
(g) Gas chromatograph.—Bendix 2500, with 3 ft × 2 mm id glass column packed with 3% OV-101 on 100–120 mesh Gas-Chrom Q, and electron capture detector (63Ni) and electron capture linearizer (Tracor Model 114556). Packard 800

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chromatograph with a 6 ft x 4 mm id glass column packed with 3% OV-1; 3H detector.

Auto dispensers.—Eppendorf with 500 and 50 μL disposable tips, or equivalent.

Sample Preparation
Grind sample (ca 1 kg) to pass 20 mesh screen and blend 15 min in Hobart planetary mixer.

Extraction
Add 250 mL methanol-water (1+1) to 500 mL extraction flask (Erlenmeyer with Teflon-lined screw cap) containing 50 g sample and 25 g Celite. Extract 30 min by shaking on wrist-action shaker. Filter through rapid flow paper and collect 25 mL 5% aqueous potassium chloride. Shake vigorously 3 times with ethyl acetate (100 mL) and combine ethyl acetate fractions in 500 mL Erlenmeyer flask. Add 35 g sodium sulfate and swirl for 3-4 min. (If lumps form, add additional 10 g sodium sulfate.) Decant solution through funnel containing glass wool plug into 500 mL round-bottom flask. Rinse sodium sulfate with ethyl acetate (100 mL) and add ethyl acetate rinse to round-bottom flask. Evaporate to dryness under reduced pressure on rotary evaporator at ca 50°C.

Column Chromatography
Prepare silica gel column as described by Scott et al. (1) and transfer extract residue directly from round-bottom flask to column with 3 mL methylene chloride. Rinse round-bottom flask with 2 additional 3 mL washes and add rinses to column. Wash column with 30 mL acetone-toluene (5+95) and with 20 mL hexane and discard washes. Elute DON with 50 mL methylene chloride-methanol (95+5) and evaporate eluate to dryness on rotary evaporator. Transfer residue to 4 mL vial with methylene chloride, evaporate solvent under nitrogen, and seal vial with Teflon-lined screw cap. Store in refrigerator for analysis.

Derivatization
Redissolve residue from column in 4 mL toluene-acetonitrile (95+5) and mix on Vortex mixer. Transfer 500 μL sample solution (0.5 μg) and standard DON solution (4 μL or 2 μL) to separate Teflon-lined screw-cap vials (15 x 45 mm). Add 50 μL HFBI reagent to each solution with disposable pipet and seal vials tightly. Agitate on Vortex mixer and heat 1 h at 60°C. Cool vial to room temperature and add 1.0 mL 5% sodium bicarbonate. Agitate vigorously on Vortex mixer for 1 min to form fine emulsion. Let phases separate (5-10 min) and carefully transfer 50 μL of upper phase to 2.0 mL vial, with septum cap, containing 950 μL n-hexane. Sample concentration is 100 μg sample equivalent/μL and standard concentration is 100 or 50 pg/μL.

Gas Chromatography and Quantitation
Construct a standard curve each day to determine electron capture detector response to DON standard. GC conditions were as follows: Bendix 2500, 3 ft x 2 mm column at 160°C. Inlet and 63Ni detector at 210 and 280°C, respectively. Carrier gas, argon-methane (95+5) at 60 mL/min. Electron capture linearizer at 4 or 8X attenuation. Packard chromatograph with 3H detector. Carrier gas, nitrogen at 50 mL/min. Inlet and detector at 210 and 220°C, respectively. Column, 180°C isothermal. Electrometer at 1 X 10^-9 or 3 X 10^-9 AFS. Increase column temperature to 200°C after 15-20 injections to clean columns. Record precise volume of sample and standard injected by withdrawing syringe plunger into barrel so that lower meniscus is at 1.0 μL mark. Record total volume in syringe. Inject and again withdraw plunger and measure volume remaining in syringe. Difference is volume injected. Determine concentration of DON in sample from formula:

\[
\text{Deoxynivalenol, ppm = } \left( \frac{\text{peak height sample/peak height std}}{\text{pg std injected/μg sample injected}} \right) \times \text{pg std injected/μg sample injected}
\]

Results and Discussion
The revised procedure was tested on naturally contaminated and spiked wheat samples. Table 1 shows the results obtained by 4 analysts on the same naturally contaminated wheat sample. Analyst 4 used the procedure described here. The other analysts used the Scott method (1), but the extracts were prepared by shaking and filtering instead of by blending and centrifuging. The additional modifications described and used by analyst 4 reduced analysis time and gave essentially the same results obtained by the other analysts. Percent recoveries of DON from spiked wheat are shown in Table 2. Average recovery for the 3 levels tested was 81.5%. The modified procedure was evaluated by comparing assay results...
Table 1. Results of intralaboratory assay on one naturally contaminated wheat sample by modified procedure

<table>
<thead>
<tr>
<th>Analyst</th>
<th>Deoxynivalenol, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.49</td>
</tr>
<tr>
<td>2</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>1.33</td>
</tr>
<tr>
<td>4</td>
<td>1.55(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Scott method (1) except extraction was done by shaking 30 min on wrist-action shaker.
\(^b\) Obtained with modifications described in text.

Table 2. Recovery of deoxynivalenol from spiked wheat samples by modified procedure

<table>
<thead>
<tr>
<th>Sample</th>
<th>DON added, ppm</th>
<th>Rec., %</th>
<th>CV, (^b) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>77.3</td>
<td>7.44</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>81.0</td>
<td>7.63</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>86.3</td>
<td>7.91</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>81.5</td>
<td>7.66</td>
</tr>
</tbody>
</table>

\(^a\) Triplicate analyses.
\(^b\) CV = coefficient of variation.

with those obtained on identical samples by outside laboratories which used the Scott method as published (1). A comparison of our results and results obtained by 2 different outside laboratories is presented in Table 3. The DON level in sample 2 was reported only to be greater than 14.5 ppm.

The Scott method was modified to increase the number of samples an analyst could prepare for quantitation per day. Vigorous shaking of ethyl acetate-aqueous phase containing potassium chloride unexpectedly enhanced phase separation. A hexane wash of the silica column removed residual toluene and reduced dry-down time for the DON fraction. The use of disposable pipets saved considerable time in preparing solutions for derivatization. The modifications permitted each analyst to prepare 6–8 samples per day to the step where the extract is ready for derivatization and quantitation.

REFERENCES

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(4) Bertelsen, A. (1982) *Feedstuffs* 54(29), 1