cooperation during the study, and to Mr. Debuda for caring for the animals.

REFERENCES

1. Worldwide Oilseed Forecast, JAOCS 57:585A (1981).
2. Vimal, O.P., and K.T. Naphade, J. Sci. Ind. Res. 39:198 (1980).
3. Development of minor and non-edible oils, Indian Central Oilseed Committee, Hyderabad, 1964, p. 5.
4. Report of the Subcommittee on Minor Oil Seeds, Ministry of Food and Agriculture, Government of India, New Delhi, 1971, p. 4.
5. Mitra, C.R., Sheh. Sandesh. 40:3 (1970).
6. Mitra, C.R., Khadi Gramodyog. 17:712 (1971).
7. Gandhi, V.M.; K.M. Cherian and M.J. Mulky, J. Oil Tech. Ass. India. 8:65 (1976).
8. Rukmini, C.; M. Vijayaraghavan and P.G. Tulpule, JAOCS 59:415 (1982).
9. Directorate of Non-edible Oils and Soap Industry, Non-edible Oils and Soap Industry, Review 1953-1973, 1974, p. 2.
10. Parmar, B.S.; K.L. Sahrawat and S.K. Mukherjee, J. Sci. Ind. Res. 35:608 (1976).
11. Khanna, R.N., and T.R. Seshadri, Curr. Sci. 33:644 (1964).
12. Mandal, B.; S. Ghosh Majumdar and C.R. Maity, Acta Alimentaria 13: in press (1984).
13. Mandal, B.; S. Ghosh Majumdar and C.R. Maity, Abstract of proceeding of ACBI, 1982, p. 146.
14. Official Standardized and Recommended Methods of Analysis by the Society of Analytical Chemists, 2nd edn., AOAC, London, 1973, p. 675.
15. Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1, 3rd edn., AOCS, Champaign, IL, 1973.
16. Mandal, B., and C.R. Maity, Ann. Natl. Acad. Med. Sci. (India). 19:194 (1983).
17. Mandal, B.; S. Ghosh Majumdar and C.R. Maity, Proc. Indian Natn. Sci. Acad. B48:596 (1982).
18. Carrol, K.K., J. Nutr. 64:399 (1958).
19. Folch, J.M.S.; M. Less and G.H.S. Stanley, J. Biol. Chem. 226:497 (1957).
20. Fiske, C., and Y.P. Subbarow, J. Biol. Chem. 66:375 (1925).
21. Novak, M., J. Lipid Res. 6:431 (1965).
22. Sperry, M.M., and J. Webb, J. Biol. Chem. 187:97 (1950).
23. Amenta, J.S., J. Lipid Res. 5:270 (1964).

[Received March, 1984]

Method of Analysis for Deoxynivalenol and Zearalenone from Cereal Grains

G.A. BENNETT, S.E. MEGALLA[1] and O.L. SHOTWELL, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

A method was developed to determine deoxynivalenol and zearalenone in corn, wheat, oats, rice and barley. The toxins are extracted with methanol/water (50:50, v/v) (2×) and partially purified by partitioning into ethyl acetate and then defatting with acetonitrile-petroleum ether. Toxins are isolated by silica gel column chromatography. Interfering materials are removed from the column with benzene; zearalenone is eluted with benzene/acetone (95:5, v/v), and after a column wash of chloroform/methanol (98:2, v/v), deoxynivalenol is eluted with chloroform/methanol (95:5, v/v). Zearalenone is quantitated by thin-layer chromatography and deoxynivalenol by gas-liquid chromatography of the trimethylsilyl derivative. The detection limit is about 0.02 μg/g for each toxin. Recoveries of added toxins varied with substrate and level of toxins. Recovery of deoxynivalenol ranged from 58% for 1 ppm in rice to 108% for 1 ppm in corn. Average recoveries for all levels (1, 2 and 5 ppm) ranged from 69% for barley to 89% for oats. Recovered zearalenone ranged from 40% for 5 ppm in wheat to 100% for 1 ppm in barley. Average recoveries for zearalenone at 1, 2 and 5 ppm varied from 53% for wheat to 87% for rice.

INTRODUCTION

Fusarium graminearum, a common colonist of cereal grains, produces both zearalenone and deoxynivalenol in corn and mixed feed (1,2). This coexistence of zearalenone and deoxynivalenol could pose a serious threat to animals, especially swine, due to estrogenic and feed refusal effects. Procedures have been published for the individual determination of deoxynivalenol and zearalenone which are both specific and sensitive (3-6). Also some information is available on the simultaneous determination of several *Fusarium* mycotoxins in grains and feeds (7,8). A more rapid procedure which utilizes commonly available chemicals and equipment was required to analyze inoculated cereal grains for the co-production of deoxynivalenol and zearalenone by various *Fusarium* species. Vesonder et al. (9) have determined the production of both toxins by 16 *Fusarium* isolates on corn, but recoveries of the toxins by the method used were not reported. We have developed a screening method for the determination of both toxins in the same extract and have tested the procedure on inoculated and spiked samples of corn, rice, oats, wheat and barley. This paper describes the method of analysis and reports the recoveries of both toxins from spiked substrates.

[1] Present address: Department of Botany, Assiut University, Assiut, Egypt.

MATERIALS AND METHODS

Spiked Substrates

Samples (50 g) of toxin-free, ground, blended corn, wheat, oats, rice and barley were individually spiked with deoxynivalenol and zearalenone to contain 1.0, 2.0 and 5.0 ppm of each toxin. Triplicate samples of the grains were prepared at these three levels. The toxins (in acetonitrile) were added to the samples with a Hamilton syringe (100 μL), and solvent was allowed to evaporate ca. 2 hr before extraction.

Extraction

A modified procedure of Scott et al. (3) was used to extract deoxynivalenol and zearalenone from the spiked substrates and inoculated substrates: blend substrate with 250 mL methanol/water (1:1, v/v) for 5 min. Transfer suspension to centrifuge bottles and centrifuge for 5 min at 5,000 rpm. Decant centrifugate and re-extract sediment with second 250 mL solvent. Repeat centrifugation and combine centrifugates. Add 100 mL saturated sodium chloride to extract and partition with 100 mL ethyl acetate (3×). Add ca. 60 g anhydrous sodium sulfate to the combined ethyl acetate fractions and swirl to remove traces of water. Filter, evaporate solvent on a rotary evaporator and dissolve residue in acetonitrile (50 mL). Transfer to separatory funnel and defat with 50 mL petroleum ether (2×). Remove acetonitrile under vacuum and dissolve residue in methylene chloride for column chromatography.

Column Chromatography

Prepare silica gel column as described by Eppley (1) for the isolation of zearalenone. Transfer extract to column quantitatively with methylene chloride and allow extract to move into column. Wash column with 100 mL benzene and discard. Elute zearalenone with 200 mL benzene/acetone (95:5, v/v), evaporate solvent and save residue for thin-layer chromatography. Toluene may be used in place of benzene. Wash column with 100 mL chloroform/methanol (98:2, v/v) and discard. Elute deoxynivalenol with 250 mL chloroform/methanol (95:5, v/v), evaporate solvent and save residue for gas chromatography.

Quantitation

Dissolve residue containing zearalenone in 500 μL benzene and assay by thin-layer chromatography as described by Shotwell et al. (4), using chloroform/ethanol (95:5, v/v) as developing solvent. Zearalenone was quantitated by fluorodensitometry by using excitation wavelength of 327 nm and measuring emission at 455 nm.

Dissolve deoxynivalenol residue in 1.0 mL acetone and transfer 200 μL to 2-dram vial with septum cap. Evaporate solvent under N_2 and add 100 μL Tri-Sil TBT (Pierce Chemical Co.). Heat sealed vial for 1 hr at 60 C. Cool to room temperature and inject 1 or 2 μL into gas chromatograph equipped with glass column (4 ft × 2 mm) packed with 3% OV-1 on Gas Chrom Q 100/120 mesh and flame ionization detector. Program temperature of column from 175-250 C at 5 C/min (150-250 C for corn). Maintain injection port and detector at 210 C and 250 C, respectively. Measure retention times and peak areas with an electronic integrator (Hewlett/Packard Model 3370B or equivalent).

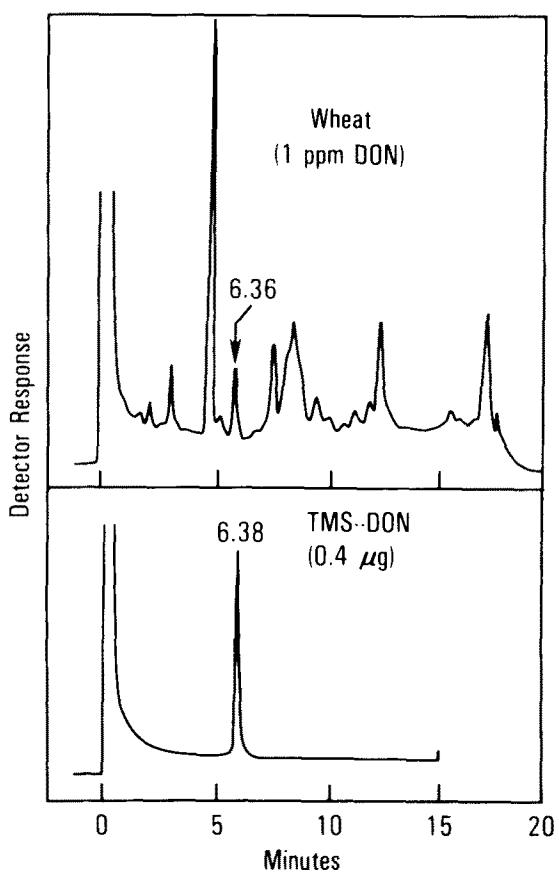

FIG. 1. Gas-liquid chromatograms of TMS derivatives of deoxynivalenol standard (lower) and of deoxynivalenol isolated from wheat (1 ppm). Conditions as described in text.

RESULTS AND DISCUSSION

Figure 1 is a gas chromatogram of the TMS derivative of standard deoxynivalenol (lower pattern) and deoxynivalenol recovered from wheat containing 1.0 ppm toxin. All grains spiked at levels of 1.0 ppm or higher were easily analyzed. By adjusting the attenuation of the flame ionization detector to 5×10^{-12} AFS (amps full scale), 0.2 ppm deoxynivalenol could be detected in wheat, rice and corn. Barley contained significantly more interfering materials, as shown in Figure 2, and the detection limit by this method was about 0.5 ppm deoxynivalenol. Table I summarizes the recovery of deoxynivalenol and zearalenone from five different cereal grains spiked to contain 1, 2 and 5 ppm toxin. The recovery of deoxynivalenol from corn, oats and wheat averaged 82%. However, the recovery of toxin

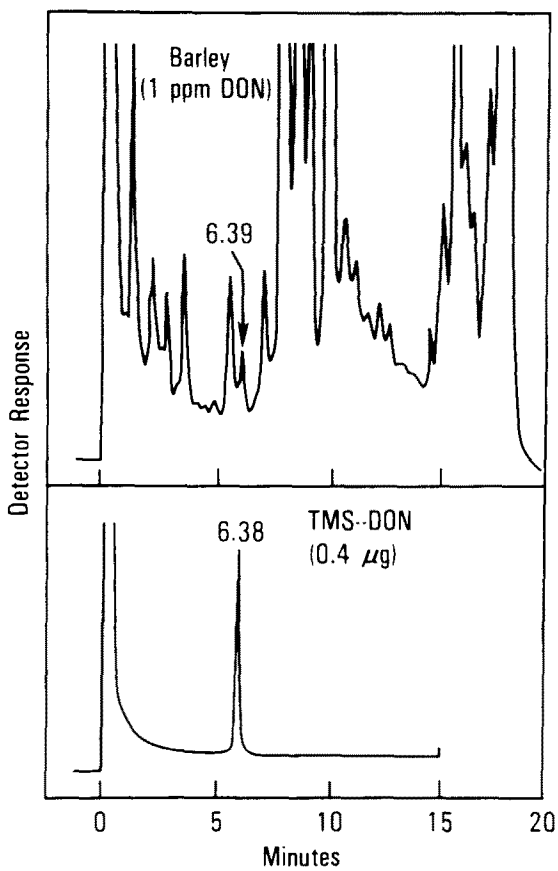

FIG. 2. Gas-liquid chromatograms of TMS derivatives of deoxynivalenol standard (lower) and of deoxynivalenol isolated from barley (1 ppm). Conditions as described in text.

TABLE I

Recovery of Deoxynivalenol (DON) and Zearalenone (F-2) Added to Cereal Grains

	% Recovery[a]							
	1 ppm		2 ppm		5 ppm		Average, %	
Substrate	DON	F-2	DON	F-2	DON	F-2	DON	F-2
Corn	108	70	73	85	76	65	86	73
Wheat	97	70	80	50	67	40	81	53
Rice	58	80	67	85	84	95	70	87
Oats	100	75	90	67	78	56	89	66
Barley	65	100	67	60	76	55	69	72

[a]Average of three determinations by GLC of TMS derivative of DON and by TLC of zearalenone.

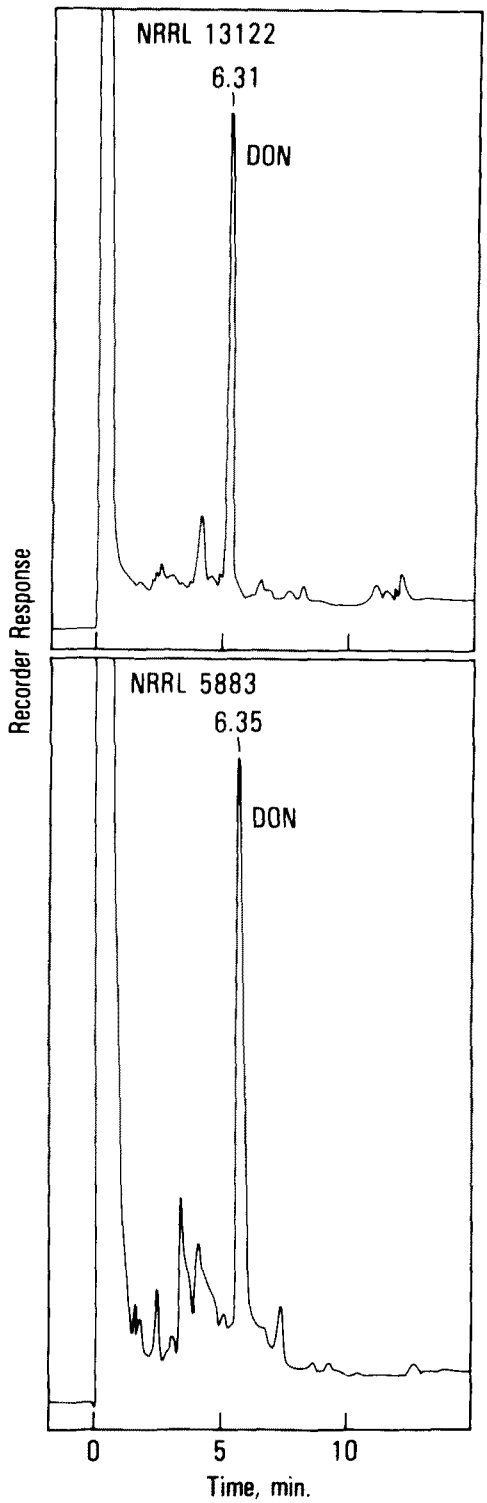

FIG. 3. Gas-liquid chromatograms of TMS derivatives of deoxynivalenol isolated from corn inoculated with *Fusarium graminearum* NRRL 13122 (upper) and with *F. graminearum* NRRL 5883 (lower).

tended to decrease as the level increased from 1 to 5 ppm. This suggests that some toxin is being lost in the extraction process, clean up steps or derivatization step. Increasing the proportion of methanol in the extraction solvent did not increase the amount of deoxynivalenol extracted. Also, no toxin could be detected in the column fraction that preceded the deoxynivalenol elution or in an additional 100 mL fraction that followed deoxynivalenol. The recovery from barley and rice (69 and 70%, respectively) was consistently lower than recoveries from the other substrates. Rice yielded a cleaner extract than the other grains, so the loss of toxin from this matrix could not be explained from "masking" by interfering materials.

The efficiency of the method to isolate deoxynivalenol from inoculated corn is shown in Figure 3. As determined by this method, the quantity of toxin produced by *F. graminearum* NRRL 13122 was 44.5 ppm and by *F. graminearum* NRRL 5883 was 20.4 ppm. The levels of toxins encountered in inoculated substrates could easily be detected by the method described. Also, the procedure may be used to isolate quantities of deoxynivalenol from highly contaminated substrates.

Zearalenone, determined by thin-layer chromatography, was less efficiently extracted by methanol/water (1:1, v/v) than was deoxynivalenol. The average recovery for all three levels ranged from 53% (wheat) to 87% (rice). A higher concentration of methanol (60, 70 and 80%) improved the recovery of zearalenone but also increased the quantity of materials that interfered with the analysis of deoxynivalenol. The procedure described here for the analysis of deoxynivalenol and zearalenone in the same extract was developed to screen inoculated cereal grains for the production of these toxins. Fifty isolates of *Fusarium* spp. from contaminated corn were inoculated on rice and corn, and toxin production was determined by the method described (results to be published elsewhere).

REFERENCES

1. Mirocha, C.J.; S.V. Pathre and C.M. Christensen, "Advances in Cereal Sci. and Technol." Vol. III, edited by Y. Pomeranz, Am. Assoc. Cereal Chem. (1980), pp. 185-186.
2. Marasas, W.F.O.; L. Leistner; G. Hofmann and C. Eckardt, Eur. J. Appl. Microbiol. Biotechnol. 7:289 (1979).
3. Scott, P.M.; P.Y. Lau and S.R. Kanhere, J. Assoc. Off. Anal. Chem. 64:1364 (1981).
4. Shotwell, O.L.; M.L. Goulden, and G.A. Bennett, Ibid. 59:666 (1976).
5. Eppley, R.M., JAOCS 56:824 (1979).
6. Ware, G.M., and C.W. Thorpe, J. Assoc. Off. Anal. Chem. 61:1058 (1978).
7. Kamimura, H.; M. Nishijima; K. Yasuda; S. Saito; A. Ibe; T. Nagayama; H. Ushiyama and Y. Naoi, Ibid. 64:1067 (1981).
8. Rittinghaus, G.E.; B. Olesen and G.D. Osweiler, Am. Assoc. Veterinary Laboratory Diagnosticians, 25th Annual Proceedings, 477 (1982).
9. Vesonder, R.F.; J.J. Ellis and W.H. Rohwedder, Appl. Environ. Microbiol. 42:1132 (1981).
10. Eppley, R.M., J. Assoc. Off. Anal. Chem. 51:74 (1968).

[Received December 15, 1983]