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Minicolumn Technique for the Detection of Deoxynivalenol in Agricultural Commodities

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Trichothecenes are a group of over 50 chemically related, naturally occurring sesquiterpenoids produced as secondary metabolites by fungi such as Fusarium, Trichoderma, Cephalosporium, Myrothecium etc. Recently, there has been lot of concern regarding the deleterious effects of trichothecenes in man and animals (Ueno, 1983). Deoxynivalenol (DON) is one of the trichothecenes with known biological effects and thus has assumed importance because of its world wide prevalence (Hesseltine, 1983). The toxic effects of DON include dermal toxicity, diarrhoea, abdominal cramps, throat irritation, gastro-intestinal disorders and immunosuppression. Further, at the molecular level DNA and protein synthesis inhibition has also been reported (Uneo, 1983). Realizing its importance as a contaminant in food, Safe limits of DON in foods ranging from 5 to 2000 ppb have been fixed in Canada, Romania and the USSR. However, the FDA, of the USA, has suggested an advisory limit of 300 ppb for DON in foods. The problem of DON is further enhanced by its prevalence in foods that have been processed (Scott et al. 1984). Several methods of analysis and detection using TLC, GLC and HPLC have been reported to quantify DON in foods and feeds (Pohland et al. 1984; Romer, 1984). These methods are time consuming, expensive and require elaborate laboratory facilities as well as skilled personnel. Several minicolumn chromatographic techniques for rapid screening of mycotoxins such as aflatoxins have been developed during the last decade (Holaday, 1981; Sashidhar et al. 1988).

The present communication describes the development of a simple, reliable method for screening and detection of DON by the Minicolumn technique (MCT).

MATERIALS AND METHODS

Five ml glass syringes of borosilicate glass (11 mm id) were obtained from Hindustan Syringes, India. Florisil (100-200 mesh) was obtained

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from Sigma Chemicals, St. Louis, USA. Column grade silica gel (60-120 mesh) was obtained from Acme Synthetic Chemicals, Bombay, India and DON standards from Wako Pure Chemicals, Tokyo, Japan. All other chemicals were of analytical grade.

A circular filter paper disc (Whatman No. 4) was put in the five ml glass syringes at the bottom. The syringes were then packed with a mixture of activated charcoal, neutral alumina, celite (7:5:3 previously mixed after weighing) upto a height of 3 mm. The material was tightly packed using a plunger of diameter smaller than that of the syringe. These are the cleanup columns.

DON from naturally contaminated maize, wheat, wheat flour (refined) and whole wheat folour along with uncontaminated samples (as confirmed by TLC, Anon, 1986) and spiked samples of the above (Std. DON 0.5 and 1 µg/gm in methanol) and were extracted with acetonitrile and water (84:16) in a 250 ml ehrlenmeyer flask on a wrist action shaker for 30 min (Trucksess et al. 1984). The extracts were filtered, flash evaporated and redissolved in 2 ml methylene chloride.

The analytical columns comprised of 5 ml glass syringes with a circular filter paper disc at the bottom and packed in the following order - finely powdered anhydrous sodium sulphate (2 mm) at the bottom, silica gel (5 mm), florisil (2 mm) filter paper disc (Whatman No. 4), anhydrous sodium sulphate (4 mm) and celite (2 mm) at the top. Each layer was tightly packed using a plunger of diameter smaller than that of the syringe. The columns were activated at 100^{o}C for 1 hr and stored in a dessicator (Fig. 1).

All the above extracts (1 ml) were placed onto the cleanup columns separately. Simultaneously, standard concentrations of DON (ranging from 100-1000 ng) in dichloromethane were also loaded onto another set of columns. The columns were allowed to drain by gravity. When the level of the solvent touched the packing of the column, 2 ml of ethylacetate was added and the solvent drained rapidly using the plunger. The drained solvent was directly collected into previously activated analytical columns. These were allowed to drain freely by gravity and 3 ml of cleaning solvent comprising of hexane and petroleum ether ($40-60^{o}$C) (9:1) was added. After freely draining, 0.5 ml of 20% aluminium chloride (w/v) in 10% absolute alcohol (v/v) was added and the plunger pressed rapidly draining the remaining solvent. The columns were placed in a hot air oven for 10 min at 140^{o}C. At room temperature they were viewed under long wave UV light (365 nm). The light blue fluorescent band was seen at the florisil layer interface and the fluorescent intensity compared with the standard set of DON columns (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ng).

Levels of DON in contaminated samples and spiked samples were compared with fluorescent intentsity in the standard columns (100-1000 ng). The following equation was then used for quantitation of the contaminated and spiked samples.

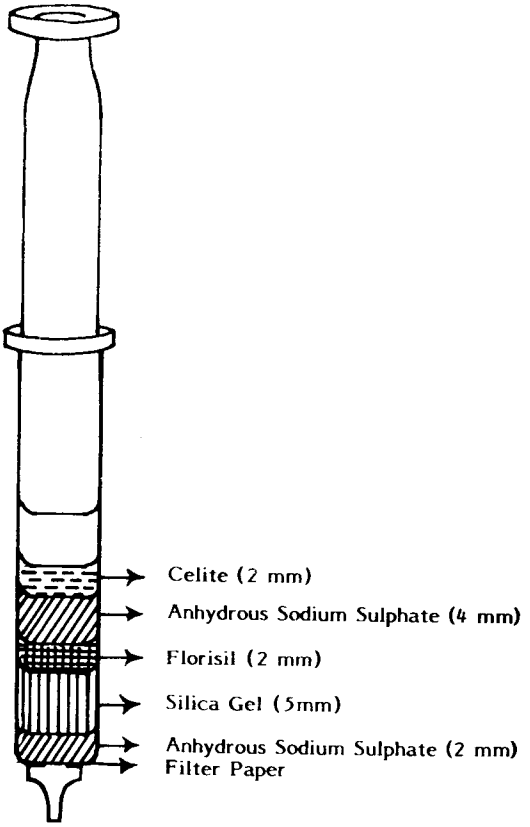

Fig. 1. MINI-COLUMN FOR DEOXYNIVALENOL

$$S_2 = \left[S_1 \, V_1 \, / \, V_2 \right] \quad (\mu g/ml)$$

Where,

S_1 = Standard DON concentration matched with the fluorescence of the sample

S_2 = Concentration of the sample fluorescent band matched with the standard DON band

V_1 = Volume of the standard DON loaded to the column (ml)

V_2 = Volume of the sample extract loaded onto the column (ml).

The remaining one ml extract in dichloromethane of each sample was passed through the cleanup column and taking appropriate aliquots, high pressure liquid chromatography (Shimadzu LC-6A, UV-Vis Spectrophotometric detector SPD-6AV) was done (Bennet et al. 1981).

RESULTS AND DISCUSSION

DON gives a characterstic blue fluorescent band under long wave UV when reacted with aluminium chloride. Earlier, aluminium

chloride has been used as a spray reagent to confirm the presence of DON in TLC analysis (Kamimura et al. 1981). This property has been put to use in the detection of DON in food grains. Unlike other mycotoxins such as aflatoxins, zearalenone, sterigmatocystin etc., DON does not fluoresce naturally (Romer, 1984) and requires a specific reaction resulting in the formation of a fluorescent compound (Ramakrishna and Bhat, 1987).

Table 1: Comparison of MCT and HPLC methods for detection of DON in naturally contaminated samples

Sample	MCT analysis (μg/gm)	HPLC analysis (μg/gm)*	Percent variation between MCT & HPLC methods
Wheat	1.2	0.86±0.08	40
Wheat flour (refined)	2.2	1.64±0.12	34
Wheat flour (whole)	0.5	0.35±0.04	43
Maize	1.6	1.27±0.10	26

*Samples were analysed in triplicates and the values expressed are mean ± SD.

The results of the MCT analysis when compared with the HPLC technique are depicted in Table 1. The percentage of variation between the methods was found to be 26-43%. The coefficient of variation of the method within the laboratory at two spiked levels (0.5 and 1 μg/ml) was found to be 22%. The minimum detection limit of 200 ng was observed in the contaminated samples. The control samples showed no fluorescence, possibly this could be due to the high temperature treatment required for the reaction (140^{o}C/10 min) where in the interfering substances become charred.

In the MCT, a single compact blue fluorescent band was observed because of the tight packing and the application of positive pressure. The cleaning solvent effectively removes the fat soluble interfering substances. The method also includes the confirmatory test since DON characterstically fluoresces blue after forming a derivative with aluminium chloride (Kamimura et al. 1981). The whole process of sample extraction and analysis takes 3 hours.

Next to aflatoxins, DON is a widely prevalent mycotoxin for which Safe limits have been fixed in various countries (van Egmond, 1987). Keeping this in view, routine screening of food grains is necessary. This method is simple, economically feasible and would be particularly useful in developing countries for the rapid screening of DON in

agricultural commodities. It serves as an important analytical tool for routine serveillance of DON in public health laboratories.

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