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Determination of fumonisins in maize by HPLC with ultraviolet detection of *o*-phthaldialdehyde derivatives

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Abstract Fumonisin is a mycotoxin that is produced by various *Fusarium* species and occurs naturally in maize and maize-based foods. Fumonisin is carcinogenic, causing liver cancer in rats, and is associated with oesophageal cancer and neural tube defects in humans. Analytical methods for individual fumonisin analogues in maize rely on reversed-phase high-performance liquid chromatographic (HPLC) separation after suitable extraction and clean-up. As fumonisins lack a useful chromophore or fluorophore, HPLC detection is achieved by suitable derivatization and sensitive, specific fluorescence detection. A widely used and validated method involves extract clean-up on strong anion exchange solid phase extraction cartridges and pre-column derivatization with *o*-phthaldialdehyde (OPA). However, many laboratories requiring infrequent fumonisin analysis are only equipped with HPLC with ultraviolet (UV) detection. A HPLC system equipped with both UV and fluorescence detectors connected in series was used to determine the extent to which UV offers an alternative to fluorescence detection in the above analytical method. Comparison of the detection systems using fumonisin standards indicated that fluorescence is about 20-times more sensitive than UV. Analysis of maize samples with differing fumonisin contamination levels indicated that, at fumonisin B₁ levels above 1,000 µg/kg, the two detection systems were comparable and gave repeatabilities equal or less than 10% on six replicate analyses. Although a sensitive fumonisin analysis requires fluorescence detection, UV may offer an alternative in certain circumstances.

Keywords Fumonisin · Fluorescence detector · UV detector · Diode array detector · OPA · HPLC

Introduction

Fumonisin is a mycotoxin that is produced by *Fusarium* species, primarily *F. verticillioides* and *F. proliferatum* (Shephard et al. 1996). The main naturally occurring fumonisin analogues are fumonisin B₁ (FB₁), B₂ (FB₂), and B₃ (FB₃), with FB₁ being the most abundant. Fumonisin causes various diseases in animals such as leukoencephalomalacia in horses (Kellerman et al. 1990), pulmonary oedema in pigs (Harrison et al. 1990), and liver cancer in rats (Gelderblom et al. 1991). Fumonisin has been linked to oesophageal cancer (Rheeder et al. 1992) and neural tube defects in humans (Marasas et al. 2004). The International Agency for Research on Cancer has evaluated FB₁ as a group 2B carcinogen (possibly carcinogenic in humans) (IARC 2002).

The determination of fumonisins in maize is widely achieved using a validated method involving methanol-water extraction, strong anion exchange (SAX) clean-up and derivatization with *o*-phthaldialdehyde (OPA) prior to separation by reversed-phase HPLC with fluorescence detection (Sydenham et al. 1996). The derivatization of fumonisins is necessary as they lack a suitable chromophore or fluorophore. Although a number of fluorogenic derivatizing reagents have been investigated, OPA remains widely used for sensitive and specific analysis of fumonisins as it yields strongly fluorescent derivatives easily separated by HPLC. Other derivatization reagents that have been used for fumonisin analysis include maleic anhydride and fluorescamine. Maleyl derivatives have been reported to give detection limits of 10 µg/kg which is too high for

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Table 1 Limits of detection (LOD) and of quantitation (LOQ) for HPLC determination of OPA derivatives of fumonisin standards using fluorescence (FLD) or diode array (DAD) detectors

		Amount (ng) injected into the HPLC column		
		FB ₁	FB ₂	FB ₃
LOD (s:n=3)	FLD	1.0	1.2	1.2
	DAD	22	20	20
LOQ (s:n=10)	FLD	2.3	2.7	2.7
	DAD	37	48	56

natural samples, and fluorescamine derivatives have the disadvantage of forming two FB₁ peaks (Arranz et al. 2004).

On occasion, laboratories equipped with HPLC and ultraviolet (UV) detectors seek to undertake limited fumonisin determinations without the purchase of further instrumentation in the form of a fluorescence detector. The aim of this work was to investigate the degree to which the fumonisin method of Sydenham et al. (1996) could be used with UV detection of the fumonisin-OPA derivatives.

Materials and methods

Chemicals

Fumonisin (FB₁, FB₂, and FB₃) standards were isolated at the PROMEC Unit according to the method of Cawood et al. (1991) and a stock solution was prepared at a concentration of 250 µg/mL in acetonitrile-water (1:1, v/v). All other reagents were analytical grade from Merck (Darmstadt, Germany).

Sample preparation

The maize samples were extracted using the method of Sydenham et al. (1996). In brief, a milled maize sample (20 g) was homogenized in methanol-water (3:1, v/v; 100 mL) for 3 min. After centrifugation, an aliquot of the supernatant was cleaned-up using strong anion extraction (SAX) cartridges (Bond-Elut, Varian, Harbor City, CA, USA), which were preconditioned with methanol and methanol-water (3:1). After washing with methanol, the fumonisins were eluted with acetic acid-methanol (1:99, v/v; 10 mL), dried under nitrogen at 60°C and then stored at 4°C prior to analysis.

Chromatography

Dried-down samples were dissolved in methanol and an aliquot derivatized with OPA (Sydenham et al. 1996). The reversed-phase HPLC separation was performed on a Phenomenex (Torrance, CA, USA) Luna C18 5 µ particle size column (150×4.60 mm). The column was eluted

isocratically at a flow rate of 1 mL/min with methanol-0.1 M sodium dihydrogen phosphate (77:23; v/v) mobile phase adjusted to pH 3.35 with *o*-phosphoric acid. The HPLC instrument was configured with an Agilent (Waldbronn, Germany) 1100 series diode array detector (DAD) and an Agilent 1100 series fluorescence detector (FLD) connected in series. The sequence of the detectors was the DAD first followed by the FLD (to prevent overpressure, the fluorescence detector should always be the last module in the flow system.) Optimal UV detection was obtained at 335 nm, whereas the FLD was set at excitation wavelength 335 nm and emission wavelength 440 nm. Data was collected and analyzed by Agilent ChemStation software and quantification was achieved by comparison of peak areas with those of authentic fumonisin standards.

Results and discussion

The connection of dual detectors in series enables the relative responses of the two detectors to be compared without variations produced by repeat derivatizations and injections. Initial experiments were aimed at comparing responses for FB₁, FB₂, and FB₃ standards and estimating the respective limits of detection (LODs) and limits of quantitation (LOQs). LOD was calculated at three times the signal-to-noise ratio, whereas LOQ was calculated at ten times the signal-to-noise ratio. Results in Table 1 indicate

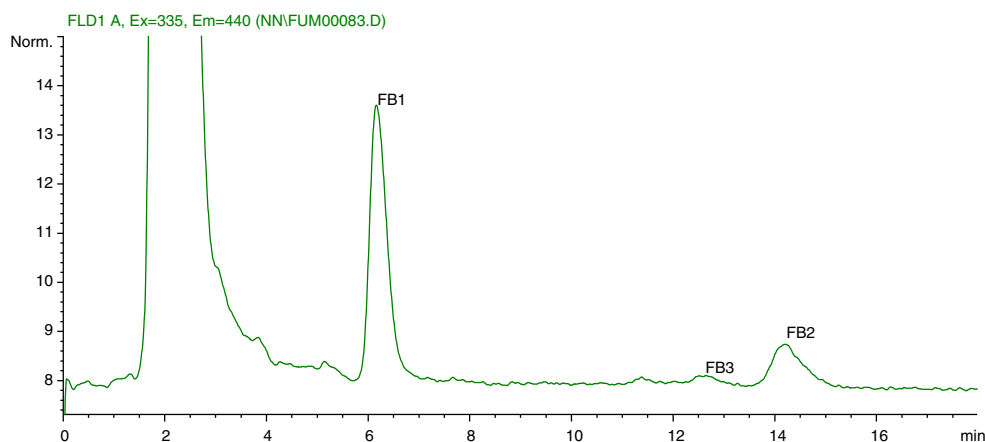
Table 2 Determination of fumonisins in two maize samples using fluorescence (FLD) and diode array (DAD) detectors

	Mean fumonisin (µg/kg)		
	FB ₁	FB ₂	FB ₃
Sample 1			
FLD	229±23 ^a	60±16	ND
DAD	455±367	ND	ND
Sample 2			
FLD	1190±95	325±11	48±7
DAD	1087±112	264±102	ND

ND Not detected (see Table 1)

^a Standard deviation for 6 replicates

Fig. 1 Naturally contaminated sample with 1,092 $\mu\text{g/kg}$, 322 $\mu\text{g/kg}$ and 43 $\mu\text{g/kg}$ levels of FB₁, FB₂, and FB₃ respectively. Analysis done by HPLC-FLD



that, as would be expected, diode array detection is less sensitive than fluorescence detection. Overall, based on both LOD and LOQ for all three fumonisin analogues, the fluorescence is about 20-times more sensitive based on the amount of fumonisin injected into the HPLC column. Also, due to their longer retention times and hence broader peak widths, FB₂ and FB₃ tend to have marginally greater LODs and LOQs.

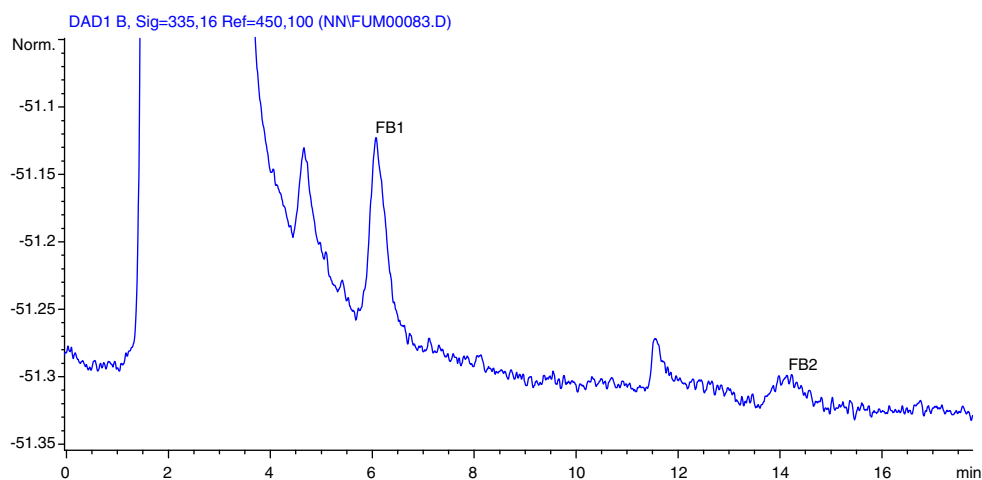
The second stage of this comparison between detectors was to compare analytical results on two maize samples of differing fumonisin levels obtained from the former Transkei region of South Africa. These analyses were each conducted with six replicates so as to compare mean results for FB₁, FB₂, and FB₃, as well as the repeatability of the results achieved at different levels of fumonisin contamination, as obtained with the two detection systems (Table 2). The first sample was contaminated with low levels of FB₁ (229 $\mu\text{g/kg}$ as measured by FLD) and FB₂ (60 $\mu\text{g/kg}$). However, the result from DAD showed an overestimation by 100%, and whereas fluorescence detection achieved an acceptable repeatability of 10% relative standard deviation (EC 2006), the repeatability on DAD was an unacceptable 81%. FB₃ levels were below the LODs for both detectors,

as was FB₂ for the DAD. The second maize sample was contaminated with FB₁ at levels above 1,000 $\mu\text{g/kg}$ and both detectors achieved comparable results with repeatabilities of 8 and 10% for the FLD and DAD, respectively (Figs. 1 and 2). Although both detectors could quantify FB₂, the repeatability on DAD was a high 39% as opposed to the 3% achieved with FLD. These results indicate that UV detection is viable for the determination of FB₁, the major fumonisin analogue, at moderate levels of contamination and could find use in analyzing maize samples in situations where levels above 1,000 $\mu\text{g/kg}$ are to be expected.

Conclusion

Although HPLC fumonisin analysis has been performed by fluorescence detection of OPA derivatives since 1990 (Shephard et al. 1990), this is the first work to explore the possible use of UV detection of these derivatives. As expected, UV detection is less sensitive than fluorescence. However, this work indicates that at FB₁ levels in maize above 1 mg/kg, UV detection is comparable with fluores-

Fig. 2 Naturally contaminated sample with 968 $\mu\text{g/kg}$ and 321 $\mu\text{g/kg}$ levels of FB₁ and FB₂, respectively. Analysis done by HPLC-DAD



cence, thus defining a possible niche area for this application.

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