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

Mycotox Res (2009) 25:225–228
DOI 10.1007/s12550-009-0031-1

ORIGINAL PAPER

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

**Ncediwe Ndube · Liana van der Westhuizen · Gordon S. Shephard**

Received: 24 August 2009 / Revised: 16 October 2009 / Accepted: 19 October 2009 / Published online: 11 November 2009


**Abstract** Fumonisins are mycotoxins that are produced by various *Fusarium* species and occur naturally in maize and maize-based foods. Fumonisins are carcinogenic, causing liver cancer in rats, and are 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 $B_1$ 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.

N. Ndube (✉) · L. van der Westhuizen · G. S. Shephard
PROMEC Unit, Medical Research Council,
P.O. Box 19070, Tygerberg 7505, South Africa
e-mail: ncediwe.ndube@mrc.ac.za

**Keywords** Fumonisins · Fluorescence detector · UV detector · Diode array detector · OPA · HPLC

## Introduction

Fumonisins are mycotoxins that are produced by *Fusarium* species, primarily *F. verticillioides* and *F. proliferatum* (Shephard et al. 1996). The main naturally occurring fumonisin analogues are fumonisin $B_1$ ($FB_1$), $B_2$ ($FB_2$), and $B_3$ ($FB_3$), with $FB_1$ being the most abundant. Fumonisins cause 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). Fumonisins have 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 $FB_1$ 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

**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 $FB_1$ 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 ($FB_1$, $FB_2$, and $FB_3$) 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 $FB_1$, $FB_2$, and $FB_3$ 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

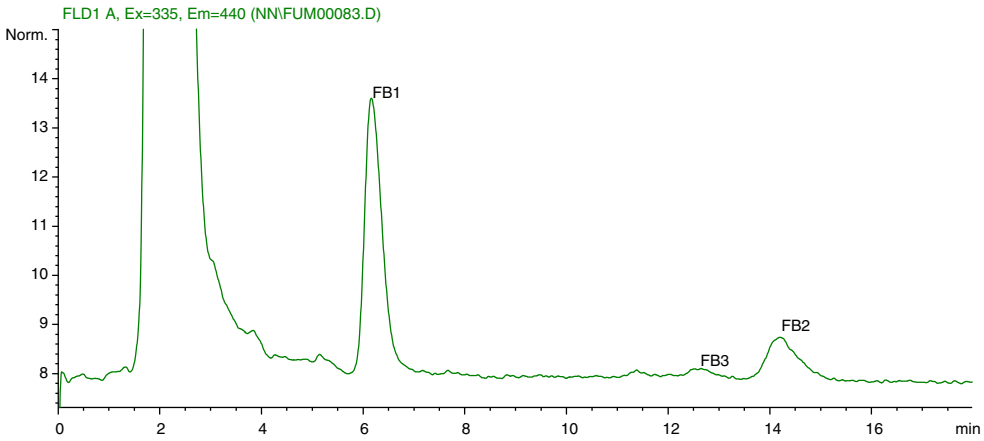


**Fig. 1** Naturally contaminated sample with 1,092 µg/kg, 322 µg/kg and 43 µg/kg levels of $FB_1$, $FB_2$, and $FB_3$ respectively. Analysis done by HPLC-FLD

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, $FB_2$ and $FB_3$ 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 $FB_1$, $FB_2$, and $FB_3$, 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 $FB_1$ (229 µg/kg as measured by FLD) and $FB_2$ (60 µg/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%. $FB_3$ levels were below the LODs for both detectors, as was $FB_2$ for the DAD. The second maize sample was contaminated with $FB_1$ at levels above 1,000 µg/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 $FB_2$, 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 $FB_1$, the major fumonisin analogue, at moderate levels of contamination and could find use in analyzing maize samples in situations where levels above 1,000 µg/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 $FB_1$ levels in maize above 1 mg/kg, UV detection is comparable with fluores-

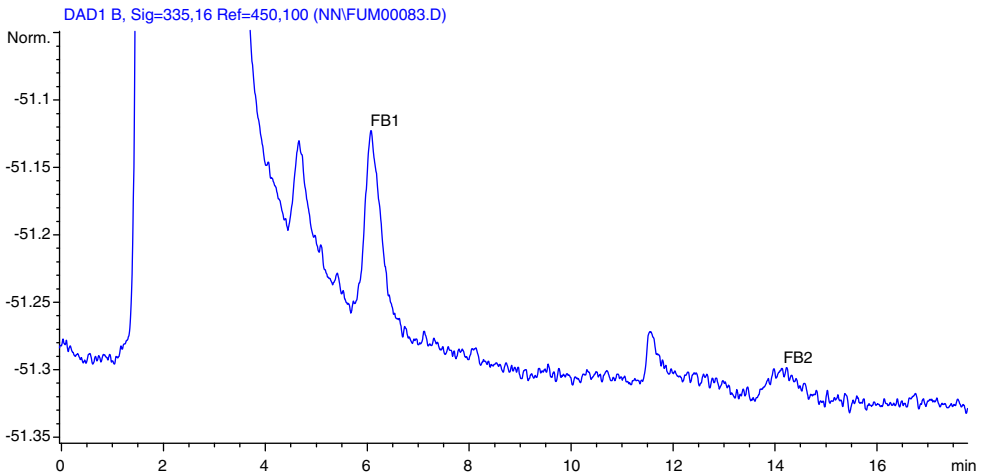


**Fig. 2** Naturally contaminated sample with 968 µg/kg and 321 µg/kg levels of $FB_1$ and $FB_2$, respectively. Analysis done by HPLC-DAD

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

**Acknowledgement** The authors acknowledge research incentive funding received by GSS from the National Research Foundation, Pretoria, South Africa.

## References

Arranz I, Baeyens WRG, Van der Weken G, Saefer S, Van Peteghem C (2004) Review: HPLC determination of fumonisin mycotoxin. Crit Rev Food Sci Nutri 44:196–203

Cawood ME, Gelderblom WCA, Vleggaar R, Behrend Y, Thiel PG, Marasas WFO (1991) Isolation of fumonisin mycotoxins: a quantitative approach. J Agric Food Chem 39:1985–1962

EC, European Commission (2006) Laying down the methods of sampling and analysis for the official control of the levels of mycotoxin in foodstuff. Commission Regulation EC No 401/2006

Gelderblom WCA, Kriek NPJ, Marasas WFO, Thiel PG (1991) Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisins $B_1$, in rats. Carcinogenesis 12:1247–1251

Harrison LR, Colvin BM, Green JT, Newman LE, Cole JR (1990) Pulmonary edema and hydrothorax in swine produced by fumonisin $B_1$, a toxic metabolite of *Fusarium moniliforme*. J Vet Diagn Res 2:217–221

IARC, International Agency for Research on Cancer (2002) Fumonisin $B_1$. In: IARC monographs on the evaluation of the carcinogenic risks to humans: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene, vol. 82. IARC, Lyon, France, pp 301–366

Kellerman TS, Marasas WFO, Thiel PG, Gelderblom WCA, Cawood M, Coetzer JAW (1990) Leukoencephalomalacia in two horses induced by oral dosing of fumonisins $B_1$. Onderstepoort J Vet Res 57:269–275

Marasas WFO, Riley RL, Hendricks KA, Stevens VL, Sadler TW, Gelineau-Van Waes J, Missmer SA, Cabrera J, Torres O, Gelderblom WCA, Allegood J, Martinez C, Maddox J, Miller JD, Starr L, Sullards MC, Roman AW, Voss KA, Wang E, Merrill AH Jr (2004) Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. J Nutr 134:711–716

Rheeder JP, Marasas WFO, Thiel PG, Sydenham EW, Shephard GS, Van Schalkwyk DJ (1992) *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82:353–357

Shephard GS, Sydenham EW, Thiel PG, Gelderblom WCA (1990) Quantitative determination of fumonisins $B_1$ and $B_2$ by high-performance liquid chromatography with fluorescence detection. J Liq Chromatogr 13:2077–2087

Shephard GS, Thiel PG, Stockenström S, Sydenham EW (1996) Worldwide survey of fumonisin contamination of corn and corn-based products. J AOAC Int 79:671–687

Sydenham EW, Shephard GS, Thiel PG, Stockenström S, Snijman PW, Van Schalkwyk DJ (1996) Liquid chromatographic determination of fumonisins $B_1$, $B_2$, and $B_3$ in corn: IUPAC/AOAC interlaboratory collaborative study. J AOAC Int 79:688–696