Journal of Microbiology, Biotechnology and Food Sciences

Chilaka et al. 2012/13 : 2 (3) 833-849

REGULAR ARTICLE

COMPARATIVE PROFILING OF DIFFERENT ANALYTICAL METHODS FOR FUMONISIN DETECTION IN MAIZE

Cynthia Adaku Chilaka[*1, 2], *Suretha De Kock*[2] *and Michael Francis Dutton*[1]

Address: Cynthia Adaku Chilaka,

[1]Food, Environment and Health Research Group, Faculty of Health Science, University of Johannesburg, Doornfontein Campus, 2028 Gauteng, P.O Box 17011, South Africa

[2]Department of Food Technology, Faculty of Science, University of Johannesburg, Doornfontein Campus, 2028 Gauteng, P.O Box 17011, South Africa

*Corresponding author: adaku80@yahoo.com

ABSTRACT

Fumonisin is one of the most important *Fusarium* mycotoxin which is often associated with maize and maize-based products worldwide. It has been associated with various diseases in humans and animals. Fumonisin contamination was evaluated from forty samples of South Africa commercial maize by thin layer chromatography (TLC), VICAM immunoaffinity column/high performance liquid chromatography (VICAMIAC/HPLC), VICAM immunoaffinity column/fluorometer (VICAMIAC/fluorometer), enzyme linked immuno-sorbent (ELISA) and lateral flow test in comparison to high performance liquid chromatography (HPLC). Strong anion exchange (SAX) cartridge was used for cleanup of extracts prior to detection by TLC and HPLC while VICAM immunoaffinity column was used for cleanup prior to VICAMIAC/HPLC and VICAMIAC/fluorometer. Result on TLC showed that 45% of the samples were contaminated with fumonisin B_1. Result on the other methods showed that 100% of the samples were contaminated with total fumonisins (fumonisin B_1 + fumonisin B_2 + fumonisin B_3). The contamination levels of total fumonisins on HPLC (64 – 1035 ppb), VICAMIAC/HPLC (7 – 803 ppb) and VICAMIAC/fluorometer (177 – 1097 ppb) were significantly ($P \leq 0.05$) lower than ELISA (190 – 2450 ppb) and lateral flow test (350 – 2700 ppb). Alternatively, using the linear regression analysis on the

results, it was observed that HPLC and lateral flow method showed a close relationship with a regression coefficient of 0.824. Percentage recoveries were 97%, 72%, 91%, 50% and 85% for HPLC, VICAMIAC/HPLC, VICAMIAC/fluorometer, ELISA and lateral flow test, respectively.

Keywords: Fumonisins, Maize, Food analysis, Chromatography, Immuno-assay

INTRODUCTION

Maize is one of the major crops grown in South Africa and has been utilised in the production of varieties of food products for humans as well as for the production of animal feeds. However, the crop and its products are reported to be susceptible to fumonisin contamination **(Shephard *et al*., 1996)** which occur mainly as a result of *Fusarium* infection of the crop in the field and may continue in store if environmental conditions are favourable **(de Vries *et al*., 2003)**. In addition, the occurrence of this toxin has been reported in other commodities such as rice, wheat, spelt, barley, peanut, fig, pistachio as well as raisin **(Kushiro *et al*., 2007; Trucksess and Scott, 2008)**. Fumonisins (FBs) are foodborne carcinogenic mycotoxins produced by *F. verticillioides*, *F. proliferatum* and closely related species. High levels of FBs especially fumonisin B_1 (FB_1) in maize and maize-based products have been reported in Africa and other parts of the world **(Shephard *et al*., 1996)**. **Shephard *et al*. (2000)** revealed significantly high levels (1615 – 6115 ppb) of FBs in maize from Mazandaran Province in Iran with an increased oesophageal cancer. Further studies have also shown high range (17900 – 154900 ppb) of FB_1 in maize from China Provinces (Linxian County and Cixian County) with high record of oesophageal cancer **(Chu and Li, 1994)**. Rheeder *et al*. (1992) also reported a range of 110 to 117520 ppb of FB_1 in maize from Transkei region of South Africa. Furthermore high levels of FB_2 (22960 ppb) in naturally contaminated maize samples have been reported in Transkei, Eastern Cape Province in South Africa **(Rheeder *et al*., 1992)**. Co-occurrence of FB and other *Fusarium* mycotoxins such as zearalenone and trichothecenes (deoxynivalenol and nivalenol) as well as *Aspergillus* mycotoxins such as aflatoxins in food and feed commodities have also been reported **(D'Mello *et al*., 1999)**. Such co-occurrences may be more detrimental than individual mycotoxins as a result of their synergistic effects **(D'Mello *et al*., 1999)**.

Fumonisin B_1 has been linked to several human and animal diseases worldwide as a result of its hepatotoxicity, nephrotoxicity, immune stimulation and suppression, liver and kidney tumours and developmental abnormalities **(Harrison *et al*., 1990; Voss *et al*., 2001)**. In animals, studies have shown that ingestion of high level of FB_1 contaminated feed causes equine leukoencephalomalacia (ELEM) in horses **(Marasas, 1996)**, hepatocarcinogenesis in rats **(Gelderblom *et al*., 2001)** and porcine pulmonary oedema (PPE) in pigs **(Harrison *et al*., 1990)**. Human maternal consumption of high concentrations of FB_1 during the early stages of pregnancy is assumed to increase the risk of neural tube defects of the brain and spinal cord **(Missmer *et al*., 2006)**. **Bhat *et al*. (1997)** also reported an outbreak of abdominal pain and diarrhoea in humans in India as a result of consumption of mouldy maize and sorghum contaminated with FB_1.

Due to the health risk of these toxins on humans and animals, there is need to monitor the safety of maize grains and its products. Thus, food legislation is put in place to serve as a tool to safeguard the health of food consumers and the economic interests of food and feed producers and traders **(van Egmond and Jonker, 2004)**. Setting up mycotoxin limits and regulation requires regular availability of toxicological data and data on the occurrence of mycotoxin concentrations in food commodities, and the knowledge of the distribution of mycotoxin concentrations within lots through availability of analytical methods **(van Egmond and Jonker, 2004)**. Although several methods have been developed for determination of FB, the most common and widely used and accepted definitive method is high-performance liquid chromatography (HPLC) **(Shephard *et al*., 1996)** which depends on the use of advanced instrumentation. This method has limitations, as it is expensive and requires trained technical staff to operate. In addition, the method does not give a rapid result which makes it difficult to be applied on a routine basis outside laboratory **(Sulyok *et al*., 2007)**. Simpler methods need to be developed which would be cheap, simple, quick and accurate, where necessary could be confirmed more precisely by HPLC over a longer period of time. If such a scheme could be developed and adopted as a general method of assessment, it would be possible to develop a general data model that describes the occurrence and levels of FB with respect to maize cultivars, climatic and seasonal effects and geographical location of maize production (M.F. Dutton, Personal Communication).

The present study aims to determine the efficacy of different analytical methods such as thin layer chromatography (TLC), VICAM immunoaffinity column / HPLC (VICAMIAC/HPLC), VICAM immunoaffinity column / fluorometer

(VICAMIAC/fluorometer), Ridascreen enzyme-linked immunosorbent assay (ELISA) and lateral flow test strip in comparison to HPLC in the detection of FB in maize.

MATERIAL AND METHODS

All chemicals were of analytical grade. Solvents used for chromatography were HPLC grade. Fumonisin B_1, FB_2, FB_2 standards were purchased from PROMEC, MRC, South Africa.

Sample material and preparation

Forty maize samples were randomly collected from several batches in two different commercial feed companies in Kwazulu Natal, South Africa in 2010. A total of twenty maize samples were collected from each of the feed company resulting to forty maize samples. The operational capacities of the companies are similar necessitating the equal sample collection. The collection source of maize for these companies covers all the provincial areas where maize is grown in South Africa. Maize samples were dry and without visible mould. Sampling was done randomly by taking samples from several locations in a batch and thoroughly mixed to obtain about 1kg giving a total collection weight of 40kg. Mixed maize samples were then milled using a clean, sterile commercial blender (Waring ®, Model 700G) and packaged in sealed sterile plastic bags The samples were then placed in a cool dry box and transported to Food, Environment and Health Research Group (FEHRG) laboratory, University of Johannesburg, South Africa for further analysis.

Extraction and clean-up of maize

i) Extraction and clean-up procedure by SAX columns was performed as described by Bennett and Richard (1994) with some modifications. Ten grams of finely ground maize sample was mixed in 30 ml of acetonitrile / water (50/50, v/v) and placed on a mechanical shaker for 60 min. The extract was filtered through Whatman No 4 filter paper. Aliquots (2 ml) of each extract was adjusted to pH between 5.9 and 6.5 with 1M acetic acid or 1M sodium hydroxide where necessary and further diluted with 4 ml of methanol/water (70/30, v/v) before loading onto a preconditioned [preconditioned with 5 ml methanol and washed with 5 ml methanol/water (70/30, v/v)] SAX column at a flow rate of 1 ml/min, while allowing column

not to dry out. The column was then washed with 5 ml methanol/water (70/30, v/v) and 5 ml of methanol. Fumonisins were eluted with 5 ml methanol/acetic acid (99/1, v/v) at flow rate of 1 ml/min and the eluate was evaporated to dryness at 50 ºC with a stream of nitrogen gas and stored at 4 ºC for further analyses.

ii) Maize samples were also extracted and cleaned with VICAM immuno-affinity clean-up according to VICAM instruction manual. Fifty grams of finely ground sample was mixed with 5 g of non iodized sodium chloride (NaCl) and 100 ml of methanol/water (80/20, v/v) and blended at high speed for 5 min using Waring ® Model 700G Blender. The sample was filtered through fluted filter paper and the extract (10 ml) was diluted with 40 ml phosphate buffer saline (PBS) and passed through a microfibre filter. Ten millilitres of the diluted extract was passed through the VICAM Fumonitest column and the column was washed with 10 ml PBS. Fumonisin was eluted by passing 1.5 ml of methanol through the column at flow rate of 1 ml/min and the eluate was collected in a glass cuvette. The eluate was transferred into an amber vial and dried under nitrogen gas at temperature of 50 ºC.

Detection and quantification of fumonisins

i) Thin Layer Chromatography

Maize samples were screened for fumonisin using TLC plate (20 x 20 cm) precoated with silica gel G. The dried extract from SAX column was re-dissolved with 200 µl of methanol and 20 µl of aliquot or FB_1 standard (concentration: 20 µg/ml) was spotted on a TLC plates, dried and a one and two-dimensional TLC was performed using butanol/water/acetic acid (12/5/3, v/v/v) (BWA). Plate was dried and derivatised with anisaldehyde reagent (70 ml CH_3OH, 5 ml concentrated sulphuric acid (H_2SO_4), 10 ml glacial acetic acid and 500 µl p-anisaldehyde (p- methoxy-benzaldehyde)) followed by heating for 1 min at 120 ºC.

ii) High Performance Liquid Chromatography

Fumonisins (FB_1, FB_2 and FB_3) were quantified by HPLC according to Shephard *et al*. (2000). Maize extracts from SAX column were reconstituted in 1 ml of methanol and 50 µl of extract or standard (concentration: 5, 10, 20 µg/ml) were transferred into HPLC vial and

derivatized with 250 µl o-phthaldialdehyde (OPA) (40 mg OPA in 1ml CH_3OH and diluted with 5 ml 0.1 M sodium tetraborate ($Na_2B_2O_4$) and 50 µl mercapthoethanol). The mixture (40µl) was injected into the HPLC at excitation and emission wavelengths of 335 and 440 nm, respectively within a minute of preparation due to the instability of OPA. The mobile phase (0.1 M sodium di-hydrogen phosphate/methanol (20/80, v/v) adjusted to pH 3.4) was run at a flow rate of 1 ml/min.

iii) VICAM

The dried extracts from VICAM immuno-affinity column were re-dissolved in 200 µl of HPLC grade methanol and quantified with HPLC and fluorometer method. The eluate for the fluorometer was mixed with 1 ml of developer A and B mixture in a cuvette and placed in a calibrated fluorometer (VICAM SERIES 4) to determine total fumonisins (FB_1+FB_2+FB_3) concentration after 240 s.

iv) Enzyme linked immuno-sorbent assay (ELISA)

Maize samples were assayed using Ridascreen fumonisin kit according to Ridascreen fumonisin manual. Briefly 5 g of each ground sample was mixed with 25 ml of 70% methanol, shaked for 3 min and filtered. The filtrate was diluted with distilled water to a ratio of 1/13, v/v and 50 µl of diluted extract was used for the analysis. Fifty microliter of the standard solution or sample was added into the microwells and 50 µl enzyme conjugate and 50 µl anti-fumonisin antibody solution added. The mixture was mixed gently and incubated for 30 min at room temperature. The mixture was poured out and the plates were washed three times with 250 µl of distilled water followed by addition of 100 µl substrate/chromogen, mixed and incubated for 15 min at room temperature in the dark. Thereafter 100 µl of stop solution was added, mixed gently and the absorbance read within 10 min at wavelength of 450 nm.

v) Lateral flow test

Maize samples were analysed using ROSA mycotoxin test kit for fumonisin (Charm Sciences INC, USA). Briefly 50 g of sample was mixed with 100 ml of 70% methanol and shake for 2 min and filtered. The extract (100 µl) was diluted with 1 ml fumonisin dilution

buffer. A second dilution was done by diluting 300 µl diluted extract with 1ml fumonisin dilution buffer and 300 µl of second diluted extract or standard was pipetted into the strip and incubated for 10 min before removing the strip and read on ROSA-M reader within 2 min after incubation.

Method validation

Validation of methods was done by determination of recovery rate and repeatability of the methods. Recovery analysis was determined in triplicates using the different analytical methods by spiking maize samples of known concentration with 50 µg/kg of FB standards (FB_1, FB_2 and FB_3).

Statistical analysis

Results obtained were analysed using a one-way analysis of variance (ANOVA) to derive mean values which were compared by significant difference (≤ 0.05) using all pairwise multiple comparison procedures (HOLM-Sidak method). Additionally, a linear regression analysis was performed between the results of VICAMIAC/fluorometer, VICAMIAC/HPLC, ELISA and lateral flow method and HPLC. Data were graphically represented with SigmaPlot and mean values were deemed to be significantly different if the level of probability was ≤ 0.05.

RESULTS

All the maize samples analysed were positive for total fumonisins (FB_1+FB_2+FB_3) on the different methods, except for the TLC which is often used for qualitative analysis. The HPLC analysis showed the occurrence of different ratios of fumonisins in the maize samples ranging from 8 – 892 ppb, 31 – 143 ppb and 0 – 242 ppb for FB_1, FB_2 and FB_3, respectively (Table 1 and Figure 1). The result revealed that FB_1, FB_2 and FB_3 occurrence were similar to that reported by Ross *et al*. (1992) and Nelson *et al*. (1993). Fumonisin B_1 was significantly different from FB_2 and FB_3 while a significant difference was also observed between FB_2 and FB_3 in the commercial maize samples from South Africa (Table 1).

The data on TLC analysis demonstrates the occurrence of FB_1 at 45 % incident rate in the maize samples (Figure 2). Data obtained on other methods used showed total FB

concentration ranging between 64 to 1035 ppb (HPLC), 7 to 803 ppb (VICAMIAC/HPLC), 177 to 1097 ppb (VICAMIAC/fluorometer), while result of ELISA and lateral flow method revealed much higher level of FB, 190 – 2450 ppb and 350 - 2700 ppb, respectively (Table 2). Table 2 and 3 showed that HPLC, VICAMIAC/HPLC and VICAMIAC/fluorometer had lower range and mean values of FB when compared to ELISA and Lateral flow method. Furthermore, no significant difference was observed between HPLC, VICAMIAC/HPLC and VICAMIAC/fluorometer on FB contamination. Significant difference was observed between HPLC and ELISA and Lateral flow method.

Using a simple linear regression analyses to compare the results of different methods, the highest correlation was observed between HPLC and lateral flow method with a regression coefficient of 0.824 followed by the correlation between HPLC and VICAMIAC/fluorometer method (regression coefficient = 0.764) while HPLC and VICAMIAC/HPLC had a regression coefficient of 0.503 (Figure 3). However, result of HPLC in comparison with result of ELISA gave a very low regression coefficient (r = 0.223) when compared to other methods (Figure 3).

Furthermore, recovery values in triplicate were calculated to determine the accuracy of the methods. It was observed that HPLC (97%), VICAMIAC/fluorometer (91%) and lateral flow method (85%) gave the highest recovery rate when compared to VICAMIAC/HPLC (72%) and ELISA (50%) methods.

Table 1 Fumonisins contamination in maize samples using HPLC

Fumonisin	Sample (%)	Ranges (ppb)	Mean (ppb)	**Sig.**
FB_1	100	8 - 892	331 (±163)	0.000*
FB_2	100	31 - 143	78 (±30)	0.000*
FB_3	95	0 - 242	46 (±48)	0.000*

Values are average of 40 samples, * the mean difference is significant at ≤ 0.05 level, Values in parathesis are ±standard deviation, sig. - significant difference

Table 2 Comparative study of total fumonisins detection in South African commercial maize with different analytical methods

Analytical methods	% Incident	Range (ppb)	Mean (ppb)
HPLC	100	64-1035	455 (±201)
VICAMIAC/HPLC	100	69-907	338 (±201)
VICAMIAC/Fluorometer	100	177-1097	473 (±204)
ELISA	100	190-2450	1337 (±795)
Lateral flow method	100	350-2700	1050 (±485)

Mean values are average of 40 samples, Values in parathesis are ±standard deviation

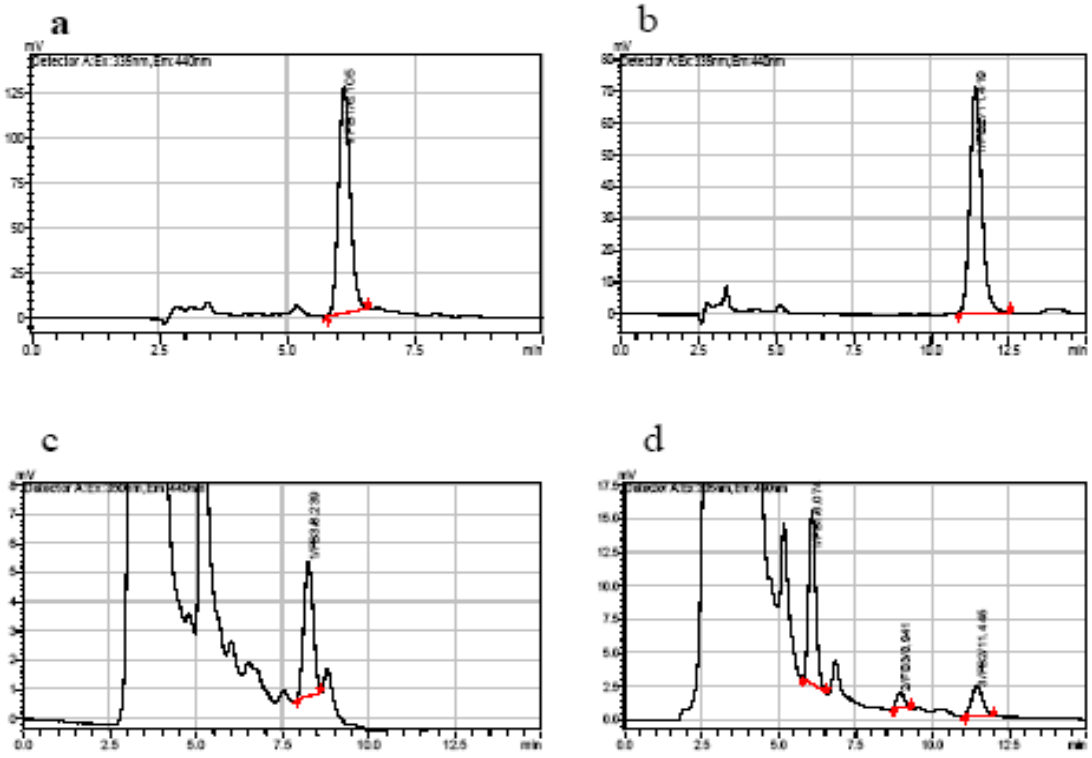

Figure 1 Chromatogram of fumonisin on HPLC at 40 µl injection (a) fumonisin B_1 standard (10 µg/ml), (b) fumonisin B_2 standard (10 µg/ml), (c) fumonisin B_3 standard (10 µg/ml) and (d) fumonisin B_1, B_2 and B_3 in maize

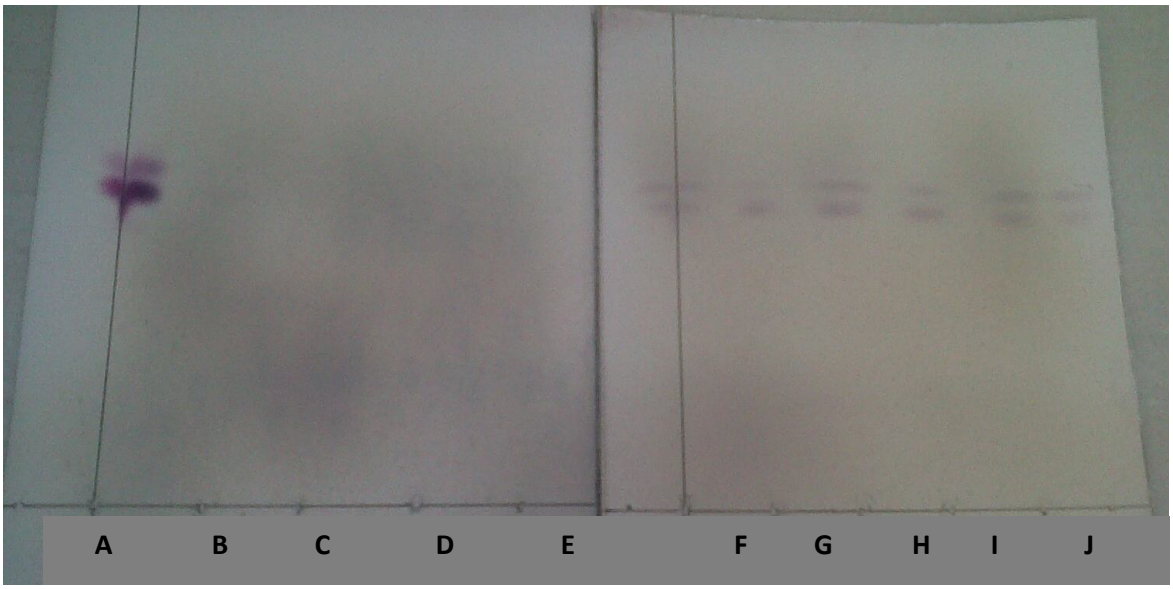

Figure 2 One dimensional TLC silica plate showing FB_1 standard (A), uncontaminated samples (B-E) and contaminated maize samples (F-K).

Table 3 Results of HPLC, VICAMIAC/HPLC, VICAMIAC/fluorometer, ELISA, lateral flow method in South African commercial maize showing significant different

(a) Methods	(b) Methods	Sig.
HPLC	VICAMIAC/HPLC	0.243
	VICAMIAC/Fluorometer	0.850
	ELISA	0.000*
	Lateral flow	0.000*
VICAMIAC/HPLC	HPLC	0.243
	VICAMIAC/Fluorometer	0.175
	ELISA	0.000*
	Lateral flow	0.000*
VICAMIAC/Fluorometer	HPLC	0.850
	VICAMIAC/HPLC	0.175
	ELISA	0.000*
	Lateral flow	0.000*
ELISA	HPLC	0.000*
	VICAMIAC/HPLC	0.000*
	VICAMIAC/Fluorometer	0.000*
	Lateral flow	0.004*
Lateral flow	HPLC	0.000*
	VICAMIAC/HPLC	0.000*
	VICAMIAC/Fluorometer	0.000*
	ELISA	0.004*

Values are average of 40 samples, * the mean difference is significant at ≤ 0.05 level, sig. - significant difference

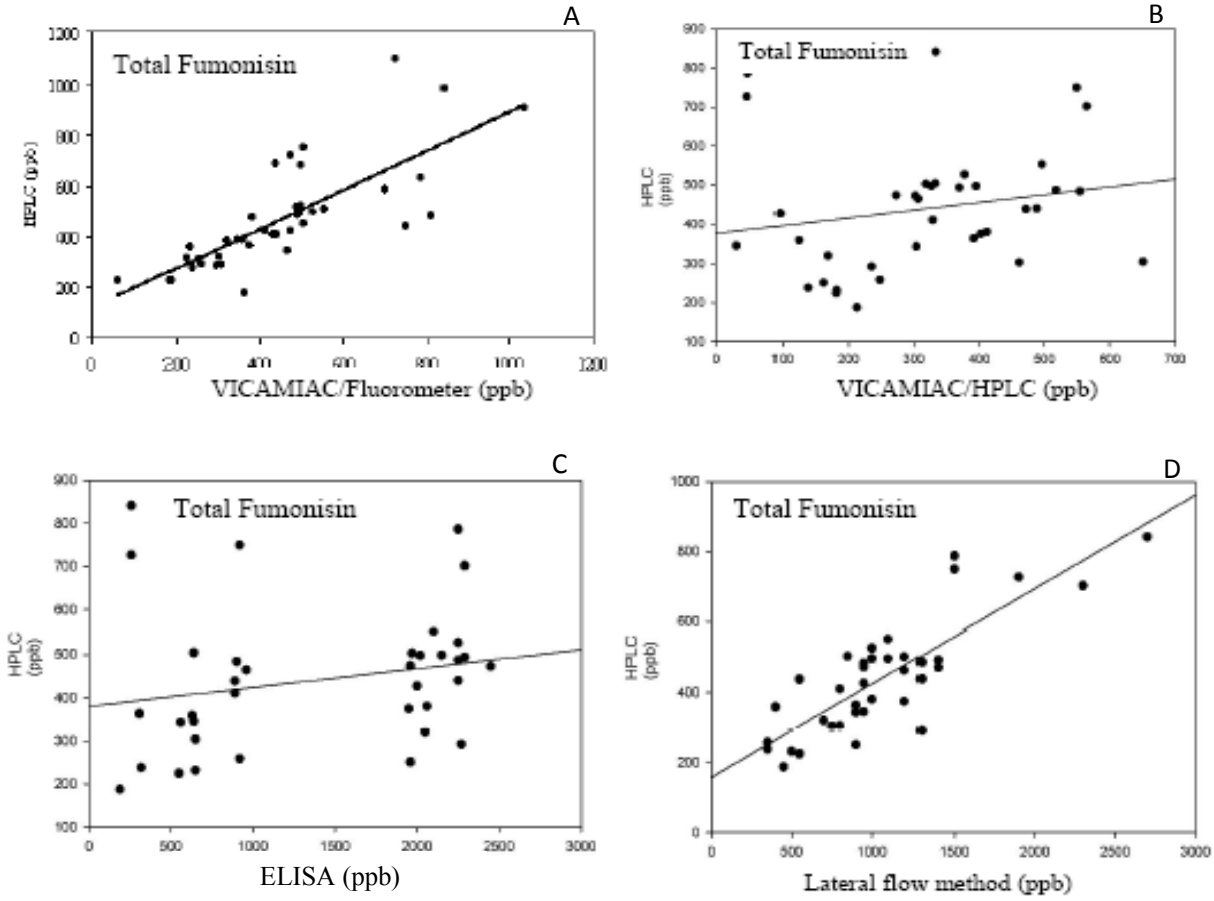

Figure 3 Linear regression curve (A) HPLC versus VICAMIAC/Fluorometer (r = 0.764), (B) HPLC versus VICAMIAC/HPLC (r = 0.503), (C) HPLC versus ELISA (r = 0.223) and (D) HPLC versus lateral flow method (r = 0.824)

DISCUSSION

The present study showed high contamination of South African commercial maize with fumonisins (FB_1, FB_2 and FB_3). Such studies on maize and maize-based products from South Africa and other parts of the world have been reported **(Sydenham *et al.*, 1990; Rheeder *et al.*, 1992; Shephard *et al.*, 2000)**. Studies in South Africa have associated FB contamination in maize with high incidence rate of human oesophageal and liver cancer **(Sydenham *et al.*, 1991; Ueno *et al.*, 1997)**. High contamination of maize with fumonisins may be attributed to its susceptibility to the principal producers (*F. verticillioides* and *F. proliferatum*) of these toxins **(Sydenham *et al.*, 1990; Dutton, 1996)**. The TLC result showed a lower incidence rate when compared to the other methods (HPLC, VICAMIAC/HPLC, VICAMIAC/fluorometer, ELISA and lateral flow test). This may be attributed to higher detection limit of TLC method (detection limit for FB on TLC: 0.5 mg/g) when compare with

the other methods **(Sydenham *et al.*, 1990).** In addition, the percentage variation of the results between TLC and other methods may be attributed to poor sensitivity and precision of TLC **(Pascale and Visconti, 2008)** which is often the reason why TLC is used as a preliminary screening method to confirm the presence of FB_1 in samples while methods such as HPLC is used to determine the levels of the toxin present in sample.

High performance liquid chromatography and VICAM methods (VICAMIAC/HPLC and VICAMIAC/fluorometer) results showed no significant difference. This result is in agreement with previously reported data by **Meister (1999)** using SAX cartridges and immuno-affinity column on maize and maize products. **Nilufer and Boyacioglu (2002)** also reported a similar correlation ($r = 0.978$) in their study between HPLC and fluorometer method. However, there was a significant difference ($P < 0.05$) between HPLC, ELISA and Lateral flow results in the present study. The ELISA and Lateral flow results showed high fumonisins (FB_1+FB_2+FB_3) contamination in maize samples when compared to HPLC. Similar variation between HPLC and ELISA was reported by **Pestka *et al.* (1994)** and **Sydenham *et al.* (1996)**. The high FB contamination of ELISA and Lateral flow methods could be attributed to cross-reactivity with related mycotoxins as well as matrix interference **(Sydenham *et al.*, 1996; Pascale and Visconti, 2008; Molinelli *et al.*, 2009)** since sample clean-up procedure was not inclusive in ELISA and lateral flow extraction methods. **Sharman and Gilbert (1991)** reported the importance of immuno-affinity clean-up procedure in aflatoxin analysis, which not only gave clean extracts but removed the interfering substances and also ease aflatoxin determination. Furthermore, the extraction solvent may also affect the performance of the immunoassay. **Molinelli *et al.* (2009)** and **Anfossi *et al.* (2010)** observed that the use of organic solvents such as aqueous methanol led to co-extraction of fatty materials in the samples which may interfere in the assay. This factor may have contributed to high levels of FB obtained in ELISA and Lateral flow method since extraction solvent was methanol. In addition, it has been reported that some antibodies take longer time to equilibrate and failure to attend to the equilibration stage may lead to variation in the results **(Meneely *et al.*, 2011)**. This may have contributed to the variation observed between the methods, HPLC, VICAM methods, ELISA and lateral flow method.

Another possible explanation for the low FB values in the maize samples on HPLC and VICAM methods, as compared to ELISA and lateral flow methods could be due to the limited capacity of the immuno-affinity columns. Immuno-affinity column has specific capacity and in the case of highly contaminated samples, it may be saturated with the antibody binding and this may lead to loss of some toxins resulting in lower concentrations in

the samples **(Shephard, 1998; Cigic and Prosen, 2009)**. Furthermore, **Molinie *et al.* (2005)** reported recoveries rate as low as 54.4 % for FB which arose during the purification of the samples through the immuno-affinity columns. They concluded that some unknown co-extractants may have blocked the antibody sites thereby reducing the trapping efficiency for FB.

There was high correlation between the HPLC and VICAM method. This may be attributed to the clean-up procedure that was carried out on the extracts used for these analyses. Recovery values were calculated in triplicates to determine the accuracy of the method. HPLC gave the highest recovery rate followed with the VICAMIAC/fluorometer and VICAMIAC/HPLC which may be attributed to the clean-up procedure which agrees with the previous report on FB in animal feeds from South Africa **(Mwanza, 2007)**. However, an advantage of HPLC over fluorometer is that HPLC method gives the concentration of individual FB (FB_1, FB_2 and FB_3) as seen in this study while VICAM/fluorometer gave the total FB. On the other hand, HPLC and ELISA showed low correlation (r = 0.223). Further analysis was done using ELISA on spiked samples to determine the percentage recovery and the result obtained gave 50 % recovery. The low recovery in this study is not in agreement with the previous study by **Ueno *et al.* (1997)**. The variation may be attributed to the method of analysis used in their work. On the other hand, the linear regression analysis between HPLC and lateral flow method yielded a good correlation. Such correlation has been reported between LC-MS/MS and lateral flow method for FB detection in raw maize and maize products **(Anfossi *et al.*, 2010)**. Furthermore, a study by **Molinelli *et al.* (2009)** reported the recovery efficacy on lateral flow method similar to what was observed in this study (85 %). The authors determined the extraction efficiencies using different solvent ratio, methanol/water (60:40, 70:30, 80:20, v/v) on lateral flow assay. The recoveries of the assay ranged from 79.8 - 80.3 % and were further confirmed by LC-MS/MS method.

Pascale and Visconti (2008) reported that ELISA and lateral flow method are easy methods of analyses with inexpensive equipment, simple sample preparation, easy to used and rapid and can be used in the field for easy monitoring. On the other hand, the VICAM methods requires clean up procedure and derivatization, need for expensive equipments such as HPLC or fluorometer for quantification of the mycotoxin and specialist expertise. Due to the high values (almost double the results of HPLC and the VICAM method) of the result obtained from ELISA and lateral flow method in the present study, it can be concluded that these methods (ELISA and lateral flow method) are better used as screening method considering their rapid, easy application and require no expert to operate. However an

immunoaffinity column clean-up step may be incorporated in the extraction procedure of ELISA and Lateral flow method to eliminate matrix interference, and give more reliable and accurate result compare to HPLC.

Acknowledgments: University of Johannesburg, South Africa is greatly acknowledged for financing this research. The contribution of the members of Food, Environment and Health Research Group and Department of Food Technology, University of Johannesburg, South Africa are highly appreciated. We also thank the feed companies in South Africa for providing us with the maize.

REFERENCES

ANFOSSI, L. – CALDERARA, M. – BAGGIANI, C. – GIOVANNOLI, C. – ARLETTI, E. GIRAUDI, G. 2010. Development and application of a quantitative lateral flow immunoassay for fumonisins in maize. In *Analytica Chimica Acta,* vol. 682, no. 1-2, 2010, p. 104-109.

BENNETT, G. A. – RICHARD, J. L. 1994. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. In *Journal of the Association of Official Analytical Chemists International,* vol. 77, no. 2, 1994, p. 501-106.

BHAT, RV. – SHETTY, PH. – AMRUTH, RP. – SUDERSHAN, RV. 1997. A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. In *Clinical Toxicology,* vol. 35, no. 3, 1997, p. 249-255.

CHU, FS. – LI, GY. 1994. Simultaneous Occurrence of Fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. In *Applied and Environmental Microbiology,* vol. 60, no. 3, 1994, p. 847-852.

CIGIĆ, IK. – PROSEN, H. 2009. An overview of conventional and emerging analytical methods for the determination of mycotoxins. In *International Journal of Molecular Sciences,* vol. 10, no. 1, 2009, p. 62-115.

D' MELLO, JPF. – PLACINTA, CM. – MACDONALD, AMC. 1999. *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. In *Animal Feed Science and Technology,* vol. 80, no. 3-4, 1999, p. 183-205.

DE VRIES, JW. – TRUCKSESS, MW. – JACKSON, LS. 2003. Mycotoxins and Food safety. In *Trends Food Science and Technology,* vol. 14, 2003, p. 111-115.

DUTTON, MF. 1996. Fumonisins, mycotoxins of increasing importance: Their nature and their effect. In *Pharmacology and Therapeutics,* vol. 70, no. 2, 1996, p. 137-161.

GELDERBLOM, WCA. - ABEL, S. – SMUTS, CM. – MARNEWICK, J. – MARASAS, WFO. – LEMMER, ER. – RAMLJAK, D. 2001. Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. In *Environmental Health Perspectives,* vol. 109, no. 2, 2001, p. 291-299.

HARRISON, LR. – COLVIN, BM. – GREENE, JT. – NEWMAN, LE. – COLE, JR. 1990. Pulmonary oedema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. In *Journal of Veterinary Diagnostic Investigation,* vol. 2, no. 3, 1990, p. 217-221.

KUSHIRO, M. – ZHENG, Y. – SAGOU, Y. – TANAKA, K. – NAGATA, T. 2007. Liquid chromatographic determination of fumonisins B_1, B_2 and B_3 in rice. In *Mycotoxins,* vol. 57, no. 2, 2007, p. 95-104.

MARASAS, WFO. 1996. Fumonisins: history, world-wide occurrence and impact. In *Advances in Experimental Medicine and Biology,* vol. 392, 1996, p. 1-17.

MEISTER, U. 1999. Effect of extraction and extract purification on the measurable fumoni sin content of maize and maize products. Test on the efficiency of acid extraction and use of immunoaffinity columns. in *Mycotoxin Research,* vol. 15, no. 1, 1999, p. 13-23.

MENEELY, JP. - RICCI, F. - VAN EGMOND, HP. – ELLIOTT, CT. 2011. Current methods of analysis for the determination of trichothecene mycotoxins in food. In *Trends in Analytical Chemistry,* vol. 30, no. 2, 2011, p. 192-203.

MISSMER, SA. – SUAREZ, L. – FELKNER, M. – WANG, E. – MERRILL, JR AH. – ROTHMAN, KJ. – HENDRICKS, KA. 2006. Exposure to fumonisins and occurrence of neural tube defects along the Texas-Mexico Border. In *Environmental Health Perspectives,* vol. 114, no. 2, 2006, p. 237-241.

MOLINELLI, A. – GROSSALBER, K. – KRSKA, R. 2009. A rapid lateral flow test for the determination of total type B fumonisins in maize. In *Analytical and Bioanalytical Chemistry,* vol. 395, no. 5, 2009, p. 1309-1316.

MOLINIE, A. – FAUCET, V. – CASTEGNARO, M. - PFOHL-LESZKOWICZ, A. 2005. Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B1: development of a method for simultaneous extraction of ochratoxin A and citrinin. In *Food Chemistry,* vol. 92, no. 3, 2005, p. 391-400.

MWANZA, M. 2007. An investigation in South African domesticated animals, their products and related health issues with reference to mycotoxins and fungi. Master Dissertation, University of Johannesburg.

NELSON, PE. – DESJARDINS, AE. – PLATTNER, RD. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry and significance. In *Annual Review Phytopathology,* vol. 31, 1993, p. 233-252.

NILUFER, D. – BOYACIOGLU, D. 2002. Comparative study of three different methods for the determinationof aflatoxins in tahini. In *Journal of Agricultural and Food Chemistry,* vol. 50, 2002, p. 3375-3379.

PASCALE, M. – VISCONTI, A. 2008. Mycotoxin detection methods. In: Leslie JF, Bandyopadhyay R, Visconti A (eds), *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade,* Cromwell Press, UK, p. 165-183.

PESTKA, JJ. - AZCONA-OLIVERA, JI. – PLATTNER, RD. – MINERVINI, F. – DOKO, MB. – VISCONTI, A. 1994. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS, and HPLC. In *Journal of Food Protection,* vol. 57, no. 2, 1994, p. 169-172.

RHEEDER, JP. – MARASAS, WFO. – THIEL, PG. – SYDENHAM, EW. – SHEPHARD, GS. - VAN SCHALKWIJK, DJ. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. In *Phytopathologia,* vol. 82, no. 3, 1992, p. 353-357.

ROSS, PF. – RICE, LG. – OSWEILER, GD. – NELSON, PE. – RICHARD, JL. – WILSON, TM. 1992. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. In *Mycopathologia,* vol. 117, no. 1-2, 1992, p. 109-114.

SHARMAN, M. – GILBERT, MJ. 1991. Automated aflatoxin analysis of food and animal feeds using immunoaffinity column clean-up and high-performance liquid chromatographic determination. In *Journal of Chromatography,* vol. 543, no. 1, 1991, p. 220-225.

SHEPHARD, GS. 1998. Chromatographic determination of fumonisin mycotoxins. In *Journal of Chromatography A,* vol. 815, no.1,1998, p. 31-39.

SHEPHARD, GS. – MARASAS, WFO. – LEGGOTT, NL. – YAZDANPANAH, H. – RAHIMIAN, H. – SAFAVI, N. 2000. Natural occurrence of fumonisins in corn from Iran. In *Journal of Agricultural and Food Chemistry,* vol. 48, no. 5, 2000, p. 1860-1864.

SHEPHARD, GS. – THEIL, PG. – STOCKENSTROM, S. – SYDENHAM, EW. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. In *Journal of the Association of Official Analytical Chemists International,* vol. 79, no. 3, 1996, p. 671-687.

SULYOK, M. - KRSKA, R. - SCHUHMCHER, R. 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantative screening of moldy food samples. *Analytical and Bioanalytical Chemistry*, vol. 389, no. 5, 2007, p. 1505-1523.

SYDENHAM, EW. – SHEPHARD, GS. – THEIL, PG. – BIRD, C. – MILLER, BM. 1996. Determination of fumonisins in corn: Evaluation of competitive immunoassay and HPLC techniques. In *Journal of Agricultural and Food Chemistry,* vol. 44, no. 1, 1996, p. 159-164.

SYDENHAM, EW. – SHEPHARD, GS. – THEIL, PG. – MARASAS, WFO. – STOCKENSTRÖM, S. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. In *Journal of Agricultural and Food Chemistry,* vol. 39, no. 11, 1991, p. 2014-2018.

SYDENHAM, EW. – THEIL, PG. – MARASAS, WFO. – SHEPHARD, GS. - VAN SCHALKWYK, DJ. – KOCH, KR. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, Southern Africa. In *Journal of Agricultural and Food Chemistry,* vol. 38, no. 10, 1990, p. 1900-1903.

TRUCKSESS, MW. – SCOTT, PM. 2008. Mycotoxins in botanicals and dried fruits: A review. In *Food Additives and Contaminants,* vol. 25, no. 2, 2008, p. 181-192.

UENO, Y. – IIJIMA, K. – WANG, SD. – SUGIURA, Y. – SEKIJIMA, M. – TANAKA, T. – CHEN, C. – YU, SZ. 1997. Fumonisins as a possible contributory risk factor for primary liver cancer: A 3-year study of corn harvested in Heimen, China, by HPLC and ELISA. In *Food and Chemical Toxicology,* vol. 35, 1997, p. 1143-1150.

VAN EGMOND, HP. – JONKER, MA. 2004. Current regulations governing mycotoxin limits in food. In: Magan N, Olsen M (eds), *Mycotoxins in Food – Detection and Control*, Boca Raton, Fla., CRC Press, New York, p 49-66.

VOSS, KA. – RILEY, RT. – NORRED, WP. 2001. An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium verticillioides.* In *Environmental Health Perspectives,* vol. 109, no. 2, 2001, p. 259-266.