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# Improved fluorometric and chromatographic methods for the quantification of fumonisins $B_1$, $B_2$ and $B_3$

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**Abstract**

Fumonisins pose serious health risks to humans and livestock, making their detection imperative in foods and feedstuffs. This detection can be accomplished quickly, precisely and accurately in a two-step chromatographic process. In the first step, fumonisins are extracted from a sample and isolated on an immunoaffinity column. In the second step, fumonisins are converted to fluorescent derivatives and quantified either through high-performance liquid chromatography (HPLC) or by fluorometer. These methods offer significant improvements in performance compared to earlier technology: limits of detection as low as 0.016 μg/g with HPLC-based detection and 0.25 μg/g for fluorometer-based detection; greater assay linearity (with HPLC, $r=0.997$; with fluorometer, $r=0.998$); larger immunoaffinity column capacity (77% recovery at 12.5 μg) and extended assay range (0–10 μg/g) for both methods. The percentage recovery of fumonisins over the entire assay range averaged 83% for both the fluorometer and HPLC methods. Precision studies were performed for both the fluorometer and the HPLC methods. The average coefficient of variation was 14% for the fluorometer method and 8.3% for the HPLC method. As a result of the efficient separation, the improved HPLC method offers the advantage of precise individual quantification of $FB_1$, $FB_2$ and $FB_3$. The two methods were compared using 33 naturally or artificially contaminated corn samples. Linear regression analysis demonstrated an excellent correlation ($r=0.996$) between the two techniques. Higher recoveries of fumonisins were obtained using this HPLC method than with the official AOAC method. 

*Keywords:* Affinity adsorbents; Food analysis; Sample handling; Fumonisins; Mycotoxins

## 1. Introduction

Fumonisins are a group of structurally related mycotoxins produced by *Fusarium moniliforme, F. proliferatum* and other Fusarium species [1–4]. Over the past several years, numerous reports have emphasized the importance of assessing animal and human exposure to these toxins. Fumonisin $B_1$ ($FB_1$) is the most abundant fumonisin and commonly found in corn [5]. $FB_1$ is known to cause leukoencephalomalacia in horses [6,7], pulmonary edema syndrome and hydrothorax in pigs [8,9], liver cancer in rats [10], decreased body weight in chickens [11] and reduced growth in channel catfish [12]. In humans, consumption of $FB_1$ contaminated corn has been associated with esophageal cancer [13–16].

Sensitive, accurate and reproducible analytical methods for the detection of fumonisins in foods and feeds are essential to assess exposure. Since their initial discovery and characterization in 1988 [17] significant progress has been made in the analytical technology used to detect fumonisins. The current methods include thin-layer chromatography [17–19], gas chromatography (GC) [20,21], capillary GC [18,21], GC–mass spectrometry [18,20,22], high-

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performance liquid chromatography (HPLC) [17,23], HPLC coupled with immunoaffinity column, and enzyme-linked immunosorbent assays (ELISA) [24,25]. However, most of these techniques do not meet current field demands because they are time consuming, require extremely expensive instrumentation, lack sensitivity or they are not suited to high throughput screening.

This paper describes much-improved methods for immunoaffinity isolation and HPLC or fluorometric detection of fumonisins. These methods are available in kit form and were so obtained for use in this research, as FumoniTest–HPLC or FumoniTest–fluorometer (Vicam, Watertown, MA, USA). Significant performance gains are obtained whether detection of fluorescent fumonisin derivatives is accomplished by HPLC or by fluorometer. FumoniTest–HPLC offers the ability to resolve and quantify different fumonisins independently, while FumoniTest–fluorometer reports total fumonisins.

## 2. Experimental

### 2.1. Reagents and samples

$FB_1$, $FB_2$ and $FB_3$ were obtained from Dr. Robert Eppley, US Food and Drug Administration, Division of Natural Products, Washington, DC, USA. Stock standard solutions were prepared in distilled water (Milli-Q) at 2–5 mg/ml. Working standard solutions were prepared (1 μg/μl and 50 ng/μl) in distilled water as a 5:2:1 ratio $FB_1$, $FB_2$, and $FB_3$ and stored at 4°C until use. $^{14}$C-labelled $FB_1$ was purchased from Dr. David Miller, Eastern Cereal and Oil Seeds Research Center, Ottawa, Canada at specific activity of 1 mCi/mmol. Analytical grade methanol, acetone, acetonitrile, acetic acid, *o*-phosphoric acid (>85% purity), *o*-phthaldialdehyde (OPA), 2-mercaptoethanol, sodium phosphate monobasic, sodium bicarbonate, sodium chloride, Tween-20 and disodium tetraborate were obtained from either Sigma (St. Louis, MO, USA) or Fisher Scientific (Pittsburg, PA, USA). FumoniTest calibration standards, FumoniTest immunoaffinity columns, and developers were obtained from Vicam. The kits were used according to the instructions supplied by the manufacturer. Clean corn samples were obtained from a local store. Naturally contaminated samples were obtained from Dr. Mary Trucksess, US Food and Drug Administration, Division of Natural Products, Washington, DC, USA and Dr. Eric Sydenham, South African Medical Research Council, Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa.

### 2.2. Apparatus

For liquid chromatography, the Waters Alliance 2690 system with auto addition was used, with a $C_{18}$ Nova-Pak column of a stainless steel, 3.9×150 mm, 4 μm spherical particle (Waters, Milford, MA, USA). The mobile phase was isocratic and composed of methanol–0.1 *M* sodium phosphate monobasic (3.45 g $NaH_2PO_4 \cdot H_2O$ in 250 ml distilled water) (77:23, v/v), adjusted to pH 3.3 with *o*-phosphoric acid, and filtered through a 0.22-μm filter membrane (Micron Separations, Westboro, MA, USA). The mobile phase was degassed and pumped at a flow-rate of 0.8 ml/min. Fluorescence was detected with a Waters 474 scanning fluorescence detector at 335 nm excitation and 440 nm emission with slit widths of 12 nm. Chromatographic data was analyzed with a Digital 575 Venturis computer equipped with MILLENNIUM 2010 chromatography manager system. For fluorometry, a Series-4 Fluorometer V1.0 (Vicam) was used after calibration with FumoniTest calibration standards.

### 2.3. HPLC and fluorometer methods

Samples (50 g) were extracted in a blender jar (Waring, New Hartford, CT, USA) by adding 5 g NaCl, 100 ml methanol–water (80:20, v/v) and blending for either 5 min (HPLC method) or 1 min (fluorometer method). Extracts were filtered through a fluted filter paper and 10-ml aliquots were diluted with 40 ml of either phosphate buffered saline (PBS) (HPLC method) or PBS–0.1% Tween-20 (fluorometer method). Diluted extracts were then filtered through either a 1.0-μm (FumoniTest–fluorometer) or 1.5-μm (FumoniTest–HPLC) glass microfiber filter, 10 ml (1 g sample equivalent) were applied to the FumoniTest column. The columns were then washed either with 10 ml 1×PBS (FumoniTest–HPLC) or 10 ml PBS–0.1% Tween-20 followed by

10 ml PBS (FumoniTest–fluorometer) and eluted with either 1.5 ml HPLC grade methanol (HPLC method) or 1.0 ml methanol (fluorometer method) into a clean glass cuvette.

### 2.4. Fumonisins derivatization and analysis

For HPLC analysis, samples were dried using a concentrator (SpeedVac from Savant), resuspended in 200 μl methanol–water (50:50, v/v) and transferred to 250 μl screw cap (Waters) vials. Injections were made by programming the auto addition feature of the Waters Alliance system so that 45 μl of reagent A and B mixture were collected, followed by 10 μl of the sample and another 45 μl of reagent A and B mixture. A delay time of 1 min was observed prior to each injection. For the fluorometer method, 1.0 ml Developer A/Developer B mixture was added to the 1 ml sample eluate, mixed and read in the fluorometer after a 240-s delay time.

## 3. Results and discussion

The use of an immunoaffinity column for the isolation of fumonisins from corn, and their subsequent detection by HPLC (FumoniTest–HPLC) or fluorometer (FumoniTest–fluorometer) methods was initially described by Hansen et al. [26]. The performance of the FumoniTest column has been comprehensively evaluated by Ware et al. [27] and used for the determination of fumonisins in canned and frozen sweet corn [5], corn gluten meal and corn gluten feed [28], milk [29] and beer [1]. The aim of the present study was to determine which factors affect performance of these kits in corn and corn based feeds.

The importance of extraction time was investigated using a corn sample naturally contaminated with $FB_1$, $FB_2$ and $FB_3$. Amounts (50 g) were extracted in 5 g NaCl and 100 ml methanol–water (80:20, v/v) by blending for either 1 or 5 min. Blending for 5 min extracted 15% more $FB_1$, $FB_2$ and $FB_3$ than a 1-min blending. A 5-min blending in methanol–water (75:25 v/v) was also found to be effective. A 1-min extraction time was retained in the FumoniTest–fluorometer method because adjustments can be made for lower recoveries while speed of analysis is maintained.

To increase recovery from the affinity columns, the ability of different organic solvents to completely elute fumonisins was examined. Corn samples were experimentally contaminated with 1 μg/g $FB_1$ and applied to the FumoniTest immunoaffinity columns. Columns were then eluted with 100% methanol, methanol–water (80:20, v/v), 100% acetonitrile, 100% acetone, or 100% acetic acid were used to elute bound toxin. The bound $FB_1$ was completely removed by 100% methanol whereas 80% methanol eluted 26% and the other solvents less than 5% of the bound material. When a secondary 100% methanol elution was performed following primary elution with the other organic solvents, the remaining toxin was recovered, confirming the other solvents inability to elute $FB_1$ from the immunoaffinity columns. These results were confirmed using corn or PBS experimentally contaminated with radiolabelled $FB_1$ ($^{14}$C-$FB_1$). Recoveries of the radiolabelled toxin were 94%, 26% and 0% using either 100% methanol, 80% methanol and 100% acetonitrile indicating that 100% methanol is the optimum elution solvent.

Since the fluorescence intensity generated by the reaction of OPA, 2ME and fumonisins is time dependent, the stability of OPA (Developer A)–2ME (Developer B) derivatives under the current assay conditions was examined. A solution of $FB_1$ (5 μg) standard was derivatized with HPLC Developer A and B mixture and injected manually into the HPLC system over a period of 0.5–27 min. Maximum fluorescence was obtained within 30 s of derivatization of $FB_1$, followed by a sharp drop (15%) within 5 min. Therefore, a 1-min delay time was chosen for derivatization of both manually and auto injected samples. This delay allows enough time for manual injections to be processed and proved to be more precise (R.S.D.=4%) than the 1-s delay time (R.S.D.=20%) achieved using the auto addition capability of the Waters Alliance HPLC System. Using the fluorometer Developer A and B mixture, the kinetics of the reaction time were different from HPLC developers. Fluorescence changed by 48% within 2 min and by only 1.6% for the next 8 min. Maximum fluorescence was not achieved until a full 2 min had elapsed. Consequently, a 4-min reaction time was selected to ensure that complete deri-

vatization would occur under different assay conditions. The different kinetics of the FumoniTest–HPLC and FumoniTest–fluorometer reactions can be explained by different developer concentrations, since Developer B (2-ME) concentration is lower in the fluorometer method to prevent interfering background fluorescence. Differences in stability of the developers is probably not a factor in the differences observed since both, the HPLC and the fluorometer Developer A and B formulations, when mixed, can be stored at room temperature for up to 5 days without significant change in performance.

The limit of detection (LOD) of fumonisins using the two methods was then determined. For this study, LOD was defined as the smallest amount of $FB_1$, $FB_2$ and $FB_3$ which could be reproducibly and accurately detected. Since fumonisin-free samples were unavailable, LOD was determined by experimentally contaminating (Table 1) corn extracts which had previously been depleted of fumonisins using FumoniTest affinity columns. The LOD values for the FumoniTest–fluorometer and FumoniTest–HPLC methods were calculated to be at least 0.25 μg/g (92% recovery, 26% R.S.D.) and 0.016 μg/g (81% recovery, 3.8% R.S.D.) respectively.

Assay linearity was determined using $FB_1$, $FB_2$ and $FB_3$ experimentally contaminated corn samples ranging from 0.016 to 10 μg/g for FumoniTest–HPLC and 0.25 to 10 μg/g for FumoniTest–fluorometer. Assay linearity was determined by linear regression analysis with $r$ values of 0.997 and 0.998 for FumoniTest–HPLC and FumoniTest–fluorometer, respectively.

Column capacity was estimated by analyzing experimentally contaminated PBS solutions rather than corn samples to control for toxin loss during extraction. Recoveries from the 10 and 12.5 μg/g solutions using FumoniTest–HPLC were 89 and 77%, respectively, indicating that the chosen assay range (0–10 μg/g) is well within the capacity of the column to recover fumonisins. $FB_1$, $FB_2$ and $FB_3$ are recovered equally well. Results obtained with FumoniTest–HPLC are the average of the individual determinations of the three fumonisin analogues.

Table 2
Percentage recovery and precision of total fumonisins in corn using FumoniTest–fluorometer method

| Added (μg/g) | Average (μg/g) | S.D. (μg/g) | R.S.D. (%) | Recovery (%) |
|---|---|---|---|---|
| 0.25 | 0.23 | 0.060 | 26 | 92 |
| 0.5 | 0.38 | 0.10 | 26 | 76 |
| 1.0 | 0.78 | 0.080 | 10 | 78 |
| 2.5 | 2.2 | 0.17 | 7.7 | 88 |
| 5.0 | 4.1 | 0.37 | 9.0 | 82 |
| 7.5 | 6.5 | 0.70 | 11 | 87 |
| 10 | 7.9 | 0.48 | 6.1 | 79 |

Average percentage recovery was calculated based on many determinations ($n$=12–18). The FumoniTest–fluorometer test was calibrated to match HPLC results, i.e., the results are reported without correction for column recovery.

Percentage recovery using FumoniTest–fluorometer was determined using corn samples experimentally contaminated with 0.25 to 10 μg/g $FB_1$, $FB_2$ and $FB_3$. Table 2 shows the average percentage recovery obtained from six independent experiments, using two different corn samples, performed on 6 different days, by three different operators. The average percentage recovery varied from 76 to 92% depending on the level of contamination with an overall (0.25–10 μg/g range) average of 83%. Percentage

Table 1
Limit of detection of total fumonisins in corn extract using FumoniTest–fluorometer and FumoniTest–HPLC methods

| FumoniTest–fluorometer | | | FumoniTest–HPLC | | |
|---|---|---|---|---|---|
| Added (μg/g) | Detected (μg/g) | Recovery (%) | Added (μg/g) | Detected (μg/g) | Recovery (%) |
| 0.25 | 0.23 | 92 | 0.016 | 0.013 | 81 |
| 0.50 | 0.38 | 76 | 0.032 | 0.029 | 91 |
| 1.0 | 0.78 | 78 | 0.080 | 0.075 | 94 |

Corn extracts containing no detectable fumonisins were experimentally contaminated with varying amounts of fumonisins and analyzed using either FumoniTest–fluorometer or FumoniTest–HPLC method. Results are the average of at least two determinations for each data point. The FumoniTest–fluorometer test was calibrated to match HPLC results, i.e, results are reported without correction for column recovery.

Table 3
Percentage recovery of total fumonisins in corn using FumoniTest–HPLC method

| Added (μg/g) | Average (μg/g) | S.D. (μg/g) | R.S.D. (%) | Recovery (%) |
|---|---|---|---|---|
| 0.016 | 0.013 | 0.00050 | 3.8 | 81 |
| 0.032 | 0.029 | 0.0030 | 10 | 91 |
| 0.080 | 0.073 | 0.0065 | 8.9 | 91 |
| 0.16 | 0.15 | 0.0010 | 1.0 | 94 |
| 0.25 | 0.20 | 0.027 | 14 | 80 |
| 0.32 | 0.29 | 0.010 | 3.4 | 91 |
| 0.50 | 0.39 | 0.039 | 10 | 78 |
| 1.0 | 0.72 | 0.096 | 13 | 72 |
| 2.5 | 2.1 | 0.14 | 6.7 | 84 |
| 5.0 | 4.2 | 0.36 | 8.6 | 84 |
| 7.5 | 6.1 | 0.71 | 12 | 81 |
| 10 | 7.4 | 0.69 | 9.3 | 74 |

Average percentage recovery was calculated based on several determinations ($n$=2–5). Results are reported without correction for column recovery.

recovery using FumoniTest–HPLC was determined using $FB_1$, $FB_2$ and $FB_3$ experimentally contaminated corn samples ranging from 0.016 to 10 μg/g and varied between 72 and 94% with an overall average of 83% (Table 3).

The precision of the FumoniTest–fluorometer method in the 0.25–10 μg/g range was examined by performing six independent experiments on different days, using two different corn samples, by three different operators. At least 12 data points were used to calculate the mean, standard deviation and % R.S.D. A good precision (≤11% R.S.D.) in the 1–10 μg/g range was obtained (Table 2). At lower levels (0.25 and 0.5 μg/g) FumoniTest–fluorometer was less precise (average of 26% R.S.D.). The precision of the FumoniTest–HPLC was also determined using three different experimentally contaminated corn samples. Experiments were performed in triplicate, by two different operators, on 3 different days (Table 3). For the 0.016–10 μg/g range, the precision was 8.3% and in contrast with FumoniTest–fluorometer there was no difference in precision between high and low values in the assay range. Similar precision was attained using naturally contaminated corn samples.

In order to compare the HPLC and fluorometer detection methods directly, a study was performed using 33 corn samples (17 naturally contaminated samples and 16 experimentally contaminated samples). The results were analyzed by linear regression analysis and a correlation coefficient of 0.996 was obtained indicating an excellent correlation between the two methods. The significant differences between the previous and reformatted FumoniTest methods are summarized in Table 4.

Several other important changes were incorporated into the improved FumoniTest including using a 1.0-μm size glass microfiber filter to eliminate background fluorescence in the FumoniTest–fluorometer method, using a single column for both HPLC and fluorometer methods with increased capacity, eliminating a water wash step which resulted in greater precision and producing of developers with

Table 4
Comparison between previous and improved FumoniTest–fluorometer and FumoniTest–HPLC methods

| | FumoniTest | | | |
|---|---|---|---|---|
| | Previous | | Improved | |
| | HPLC | Fluorometer | HPLC | Fluorometer |
| Limit of detection (μg/g) | 0.050[b] | 1.0 | 0.016 | 0.25 |
| Assay linearity | N.D. | 0.996 | 0.997 | 0.998 |
| Column capacity (μg/g) | 2.5[a,c] | N/A | 10 | N/A |
| Assay range (μg/g) | 0–5.0 | 0–5.0 | 0–10 | 0–10 |
| Average recovery over assay range (%) | 59% | ND | 83% | 83% |
| Average R.S.D. over assay range (%) | 21 | ND | 8.3 | 14 |
| Fumonisins detected | $B_1$ | $B_1$, $B_2$, $B_3$ | $B_1$, $B_2$, $B_3$ | $B_1$, $B_2$, $B_3$ |

[a] From Ref. [33].
[b] From Ref. [26].
[c] From Ref. [28].
N.D.=not done; N/A=not applicable.

Table 5
Comparison between FumoniTest–HPLC and AOAC-HPLC methods

| Sample | FumoniTest[a]–HPLC (μg/g) | AOAC-HPLC (μg/g) | Difference[b] (%) |
|---|---|---|---|
| 1 | 1.07 | 0.97 | 10.3 |
| 2 | 1.12 | 0.64 | 75.8 |
| 3 | 3.58 | 2.1 | 70.5 |
| 4 | 1.00 | 0.79 | 27.2 |
| 5 | 4.40 | 3.08 | 43.0 |
| 6 | 3.62 | 2.42 | 49.8 |
| 7 | 0.06 | 0.09 | −27.8 |
| 8 | 1.98 | 1.37 | 44.9 |
| 9 | 3.12 | 2.05 | 52.4 |
| 10 | 4.22 | 2.41 | 75.3 |
| 11 | 5.42 | 3.91 | 38.6 |
| Average | 2.69 | 1.80 | 41.8 |

[a] Results are reported without correction for column recovery.
[b] Percentage difference represents the percentage by which the FumoniTest–HPLC results are higher than the AOAC results.

greater shelf life. The new FumoniTest–HPLC method also enables the detection of $FB_1$, $FB_3$ and $FB_2$ with approximate retention times of 5.5, 11.5 and 12.5 min at 25°C, respectively, and their accurate quantification. No attempts were made to separate and/or quantify minor fumonisin metabolites ($FB_4$ and $FC_1$).

An HPLC method originally developed by Shephard et al. [30], improved by Sydenham et al. [23] and adopted by the Association of Analytical Chemistry (AOAC) as the official method for the determination of $FB_1$, $FB_2$ and $FB_3$ in corn (method 995.15) [31] was used for comparison with FumoniTest–HPLC. The 12 naturally contaminated corn samples were processed using the AOAC-HPLC method (performed in Dr. Sydenham' laboratory) and FumoniTest-HPLC method. Greater recoveries (overall 42% higher) were obtained using the FumoniTest–HPLC method (Table 5). When the results were compared on a percentage basis, one AOAC-HPLC result varied by more than two standard deviations from the percentage mean and was removed from the comparison. Our findings are in accordance with the results obtained by Scott et al. [32] indicating that the current FumoniTest kit has better recovery than the AOAC HPLC method.

In conclusion, we have demonstrated that the FumoniTest methods for the detection of fumonisins in corn and corn based feeds are reliable, accurate and sensitive. As a result of these studies, the FumoniTest kits (HPLC and fluorometer) have been significantly improved and will be the subject of a collaborative study to be conducted in the near future by the AOAC.

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## References

[1] P.M. Scott, G.A. Lawrence, J. Food Prot. 58 (1995) 1379.
[2] W.P. Norred, J. Toxicol. Environ. Health 38 (1993) 309.

[3] P.E. Nelson, A.E. Desjardins, R.D. Plattner, Ann. Rev. Phytopathol. 31 (1993) 233.
[4] C.W. Bacon, P.E. Nelson, J. Food Prot. 57 (1994) 514.
[5] M.W. Trucksess, M.E. Stack, S. Allen, N. Barrion, J. AOAC Int. 78 (1995) 705.
[6] T.S. Kellerman, W.F.O. Marasas, P.G. Thiel, W.C.A. Gelderblom, M.E. Cawood, J.A.W. Coetzer, Onderstepoort J. Vet. Res. 57 (1990) 269.
[7] W.F.O. Marasas, T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel, J.J. Van der Lugt, Onderstepoort J. Vet. Res. 55 (1988) 197.
[8] B.M. Colvin, L.R. Harrison, Mycopathologia 117 (1992) 79.
[9] L.R. Harrison, B.M. Colvin, J.T. Greene, L.E. Newman, J.R. Cole, J. Vet. Diagn. Invest. 2 (1990) 217.
[10] W.C.A. Gelderblom, N.P.J. Kriek, W.F.O. Marasas, P.G. Theil, Carcinogenesis 12 (1991) 1247.
[11] Y. Espada, R. Ruiz de Gopegui, C. Cuadradas, F.J. Cabanes, Avian Diseases, (1994) 454.
[12] M.H. Li, S.A. Raverty, E.H. Robinson, J. World Aquacult. Soc. 25 (1994) 513.
[13] J.P. Rheeder, W.F.O. Marasas, P.G. Thiel, E.W. Sydenham, G.S. Shephard, D.J. Van Schalkwyk, Phytopathology 82 (1992) 353.
[14] E.W. Sydenham, P.G. Thiel, W.F.O. Marasas, G.S. Shepard, D.J. Van Schalkwyk, K.R. Koch, J. Agr. Food Chem. 38 (1990) 1900.
[15] P.G. Thiel, W.F.O. Marasas, E.W. Sydenham, G.S. Shephard, W.C.A. Gelderblom, Mycopathologia 117 (1992) 3.
[16] W.P. Norred, K.A. Voss, J. Food Prot. 57 (1994) 522.
[17] W.C.A. Gelderblom, K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar, N.P.J. Kriek, Appl. Environ. Microbiol. 54 (1988) 1806.
[18] R.D. Plattner, W.P. Norred, C.W. Bacon, K.A. Voss, R. Peterson, D.D. Shackelford, D. Weisleder, Mycologia 82 (1990) 698.
[19] P.F. Ross, L.G. Rice, R.D. Plattner, G.D. Osweiller, T.M. Wilson, D.L. Owens, H.A. Nelson, J.L. Richard, Mycologia 114 (1991) 129.
[20] M.A. Jackson, G.A. Bennet, Appl. Environ. Microbiol. 56 (1990) 2296.
[21] E.W. Sydenham, W.C.A. Gelderblom, P.G. Thiel, W.F.O. Marasas, J. Agr. Food Chem. 38 (1990) 285.
[22] K.A. Voss, R.D. Plattner, C.W. Bacon, W.P. Norred, Mycopathologia 112 (1990) 81.
[23] E.W. Sydenham, G.S. Shepard, P.G. Thiel, J. AOAC Int. 79 (1992) 688.
[24] J.I. Azcona-Oliveira, M.M. Abouzied, R.D. Plattner, J.J. Pestka, J. Agr. Food Chem. 40 (1992) 531.
[25] E. Usleber, M. Straka, G. Terplan, J. Agr. Food Chem. 42 (1994) 1392.
[26] T.J. Hansen, N.A. Zabe, P.L. Skipper, presented at the 106th Annual AOAC International Meeting, Cincinnatti, OH, 1992.
[27] G.M. Ware, A.S. Umrigar, A.S. Carman, S.S. Kuan, Anal. Lett. 27 (1994) 693.
[28] J.G. Keng, R. Bernetti, presented at the 109th AOAC International Annual Meeting and Exposition, Opyland Hotel, Nashville, TN, 1995.
[29] P.M. Scott, D.B. Delgado, H.L. Prelusky, H.L. Trenholm, J.D. Miller, J. Environ. Sci. Health B 29 (1994) 989.
[30] G.S. Shephard, E.W. Sydenham, P.G. Thiel, W.C.A. Gelderblom, J. Liq. Chromatogr. 13 (1990) 2077.
[31] E.W. Sydenham, G.S. Shepard, P.G. Theil, S. Stockenstrom, P.W. Snijman, D.J. Van Schalkwyk, J. AOAC Int. 79 (1996) 688.
[32] P.M. Scott, M.W. Trucksess, J. AOAC Int. 80 (1997) 941.
[33] N. Zabe, K. Kolak, T. Hansen, B. Jackson, presented at the 109th AOAC International Annual Meeting, Opryland Hotel, Nashville, TN, 1995.