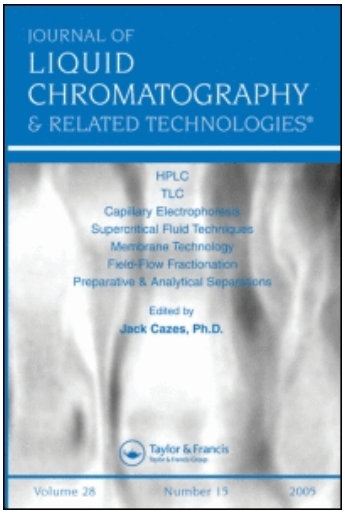

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

INVESTIGATION OF ALTERNATIVE REAGENTS TO 2-MERCAPTOETHANOL FOR THE PRE-COLUMN DERIVATIZATION OF FUMONISINS WITH O-PHTHALDIALDEHYDE, FOR HPLC ANALYSIS

Joerg Stroka[a]; Claudia Capelletti[a]; Andri Papadopoulou-Bouraoui[a]; Lea Pallaroni[a]; Elke Anklam[a]
[a] Joint Research Centre of the European Commission, Food Products Unit, Ispra, Italy

Online publication date: 24 July 2002

To cite this Article Stroka, Joerg , Capelletti, Claudia , Papadopoulou-Bouraoui, Andri , Pallaroni, Lea and Anklam, Elke(2002) 'INVESTIGATION OF ALTERNATIVE REAGENTS TO 2-MERCAPTOETHANOL FOR THE PRE-COLUMN DERIVATIZATION OF FUMONISINS WITH O-PHTHALDIALDEHYDE, FOR HPLC ANALYSIS', Journal of Liquid Chromatography & Related Technologies, 25: 12, 1821 — 1833
To link to this Article: DOI: 10.1081/JLC-120005876
URL: http://dx.doi.org/10.1081/JLC-120005876

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, No. 12, pp. 1821–1833, 2002

INVESTIGATION OF ALTERNATIVE REAGENTS TO 2-MERCAPTOETHANOL FOR THE PRE-COLUMN DERIVATIZATION OF FUMONISINS WITH O-PHTHALDIALDEHYDE, FOR HPLC ANALYSIS

Joerg Stroka, Claudia Capelletti, Andri Papadopoulou-Bouraoui, Lea Pallaroni, and Elke Anklam*

Joint Research Centre of the European Commission, Food Products Unit, I-21020 Ispra, Italy

ABSTRACT

This paper investigates the suitability of 37 reaction partners for the derivatization of fumonisins with o-phthaldialdehyde (OPA). All resulting fumonisin derivatives were compared for stability by UV-absorption. Subsequently, the relative fluorescence and retention times of derivatives found to be stable in UV-absorption were investigated by high performance liquid chromatography (HPLC), using well-established chromatographic conditions for fumonisin analysis. Several alternatives to 2-mercaptoethanol could be identified to be superior derivatization agents

*Corresponding author. E-mail: elke.anklam@jrc.it

DOI: 10.1081/JLC-120005876
1082-6076 (Print): 1520-572X (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

(3-mercapto-propionic acid, *N*-acetyl-cystein and thioglycerol). Those are especially valid when immunoaffinity column clean up is used prior to derivatization. The structure of the fumonisin B1-derivative obtained from the most suitable derivatization agent *N*-acetyl-cystein was verified by HPLC-MS.

INTRODUCTION

Fumonisin B_1 (FB_1) is the predominant and most toxic metabolite of the fumonisin group. It is mainly produced by the fungi *Fusarium moniliforme* (*verticilloides*) and *Fusarium proliferatum* and can occur in corn and corn derived products. The ingestion of fumonisin contaminated feed and food is associated with equine leukoencephalomalacia (ELEM) in horses,[1] pulmonary oedema in swine,[2] and oesophageal cancer in humans.[3–5] As fumonisins lack a chromophore (Fig. 1), direct detection by UV-absorption or fluorescence is not possible. However, this can be overcome by derivatization of the fumonisins prior to analysis by high performance liquid chromatography (HPLC).[6]

Commonly, the derivatization of fumonisins is performed with o-phthaldialdehyde (OPA) together with 2-mercaptoethanol (ME),[7] as proposed by Roth.[8–9] The reaction is specific to primary amino compounds according to the reaction given in Fig. 2.[10]

The main advantage of this derivatization reaction is that the reaction is easy to perform in aqueous solutions under ambient temperature, within a short

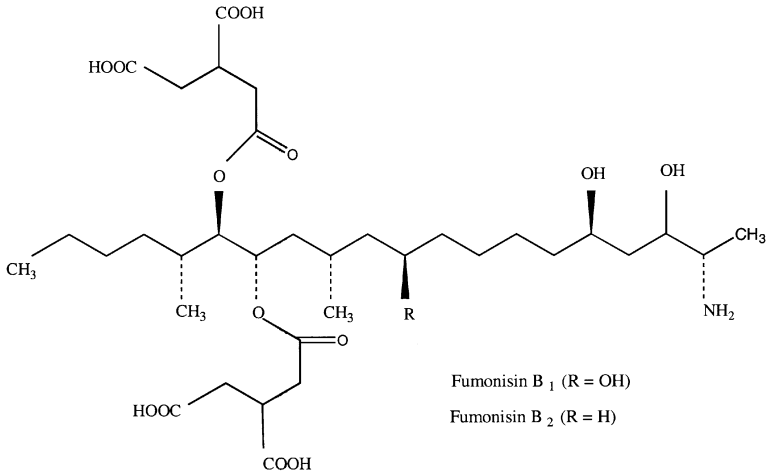

Figure 1. Structure of the two main fumonisins (FB_1 and FB_2).

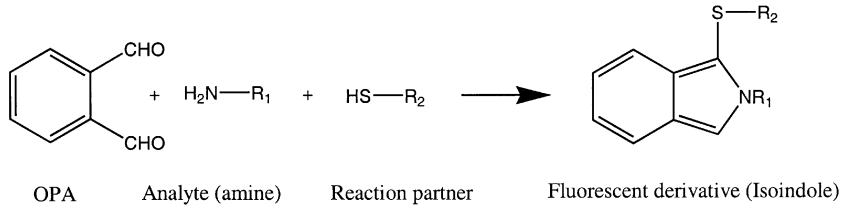

Figure 2. Reaction mechanism of the OPA derivatization for amines.

reaction time of a few minutes, and results in a highly fluorescent derivative. However, instability of the formed derivative is the main drawback.[7] Therefore, the derivatization must be performed under time controlled conditions. Other methods such as the derivatization with e.g., fluorescamine, 4-fluoro-7-nitrobenzofurazon, or 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ.Fluor®) have, therefore, been described with the aim of obtaining superior fumonisin derivatives, [6,7,11–14] while the derivatization with OPA and 2-ME is still the most common procedure for fumonisins due to its wide acceptance and easy handling.[15–17]

For amine- and amino-acid-analysis using OPA, several reactants other than 2-ME have been described to be superior in terms of stability and intensity of fluorescence of the formed pre-column derivatives.[10,18–25] Recently, an extensive and direct comparison of different reaction partners was made for the derivatization of various primary amino compounds in order to evaluate the stability, reaction time, and fluorescence.[26] The paper showed that the combination of the analyte and the reaction partner had a significant influence on stability and relative fluorescence of the formed derivative.

A similar pre-column approach for the derivatization of FB_1 has not been described yet, while a post column method for the derivatization with OPA has been described.[27] However, post column methods require precise auxiliary pumps for the reagent and consume more derivatization agent than pre-column methods.

In order to improve the simple OPA pre-column derivatization further, the aim of this work is the comparison of different pre-column reaction partners for OPA for HPLC derivatization of FB_1.

EXPERIMENTAL

UV Absorption Experiments

UV-absorption was measured using a Perkin-Elmer lambda-2 UV-spectrophotometer with 1 cm cuvettes and stopper. A fumonisin standard solution

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

(50 μL) containing 1 mg/mL FB_1 in acetonitrile-water (50 + 50) were added to 2.1 mL of the derivatization solution. This mixture was measured against the derivatization solution containing 50 μL of a blank acetonitrile-water (50 + 50) solution for a period of 3 hours in 5 min. intervals (36 cycles), at a wavelength of 335 nm. The first measurement cycle was performed directly after mixing of the solutions for 10 seconds.

The derivatization solution, each time, contained 0.114 mmol/mL reactant (thiol/mercaptan) and 0.114 mmol/mL OPA in a solution of methanol and borate buffer according to Solfrizzo.[15] Reactants with acidic properties were neutralized by addition of aliquot amounts of a NaOH solution (2 mol/L) to the borate buffer, to form the corresponding sodium salts. For drift measurement, a solution of potassium dichromate was measured against water over the same period of time as the samples.

HPLC Separation for Fluorescence

HPLC separation was performed according to a previously published method,[15] employing a HPLC pump (Kontron, Model: 420), an ABZ RP-18 column (Supelco, 5 μm, 15 cm × 4.6 mm), and fluorescence measurement with a scanning HPLC-fluorescence detector (Kontron, Model: SFM 25).

For fluorescence measurements, 50 μL of a FB1 standard solution containing 50 μg/mL FB_1 in acetonitrile-water (50 + 50) were added to 500 μL of the derivatization solution. Subsequently a 20 μL portion was injected for HPLC separation. Measurements were performed after 3 min of reaction time, using 335 nm and 440 nm for excitation and emission wavelength, respectively.

HPLC-Mass Spectrometry

Analysis was performed using an HPLC Agilent 1100 Series coupled to a mass spectrometric (MS) ion trap detector (LC/MSD ion trap Agilent Technologies, Milano, Italy) equipped with an electrospray interface (ESI).

The chromatographic separation was carried out in isocratic 70% methanol-30% acidified water (2% acetic acid) at a flow rate of 0.350 mL/min, on a C18 column (Nucleosil, 120Å pore size, 3 μm particle size, 125 mm length, 3 mm i.d., Machery-Nagel, Düren, Germany) at 35°C.

MS settings: nebulizer 50 psi, dry gas (N_2) 12 L/min, dry temperature 325°C, scan range 200–1100 m/z, all the other parameters were set automatically by the instrument, in order to optimise the detection of mass 900.

MS/MS and MS^n experiments were performed, isolating the target mass with a width of four mass units and applying 0.8 V fragmentation amplitude. The

fragmentation pattern of FB_1 was evaluated by direct infusion of a standard solution of 5 μg/mL FB_1 at 4 μL/min flow rate.

For confirmation of the derivative structure, a program was applied using the divert valve, in order to introduce only the eluate between 5 and 8 min into the MS detector to avoid salts used in the derivatization process from clogging the interface and entering the MS detector. Full scan acquisitions were performed, both in positive and in negative ionisation mode, while MS^n experiments were carried out in positive ionisation mode only.

Reagents

Methanol for HPLC separation; Acetic acid, Sodium dihydrogensulphate; 2-Mercaptobenzothiazole [149-30-4]; 2-Mercaptobenzimidazole [583-39-1]; 2-Mercaptobenzoxazol [2382-96-9]; 1-Mercapto-propan-2-ol [1068-47-9]; 6-Mercapto purine [28,128-19-0]; 2-Mercapto pyrimidine [1450-85-7]; 3-Mercapto-1,2,4-triazol [3179-31-5]; Thiomalic acid [70-49-5]; Sodium sulphite [7757-83-7] and Potassium dichromate were purchased from Merck-Eurolab (Milano, Italy). o-Phthaldialdehyde; two Mercaptoethanesulfonic acid (MESNA) [19,767-45-4]; 2-Mercapto-2-thiazolin [96-53-7]; Thioglycolic acid [68-11-1]; Thiolactic acid [79-42-5]; 3-Mercaptopropionic acid [107-96-0]; 3-Mercapto-1,2-propanediol [96-27-5]; 2-Mercaptobenzoic acid [147-93-3]; *N*-Acetyl-L-cysteine [616-91-1]; 2-Mercaptobenzothiazole [149-30-4]; 1-Thioglycerol [96-27-5]; 3-Mercapto-1-propansulfonic acid Na-salt [17,636-10-1]; 2-Mercapto-4(3H)-quinazolinone [13,906-09-7]; 2-Mercapto-nicotinic acid [38,521-46-9]; 3-Mercapto-2-butanol [54,812-86-1]; 4-Mercapto-1-butanol [14,970-83-3]; 6-Mercapto-1-hexanol [1633-78-9]; 2-Mercapto-ethyl-sulfide [3570-55-6]; 2-Mercapto-ethyl-ether [2150-02-9] were purchased from Sigma-Aldrich (Milano, Italy). 1-Mercapto-cytosin [333-49-3] and p-Thiocresol [106-45-6] were purchased from Fluka (Milano, Italy), while *N*,*N*-dimethyl-2-mercapto-ethyl-ammonium chloride (Thiofluor®) [13242-44-9] was purchased from Pickering Laboratories (Mountain View CA, USA).

RESULTS AND DISCUSSION

Reaction Kinetics by UV-Absorption

Measurements of mixtures of the investigated reactants with OPA and FB_1 by UV spectrometry supported the rapid decay found for 2-ME. From all reactants tested, only a few resulted in less rapid decay than 2-ME. Table 1 summarises the results of those reactants that indicated a better stability.

Table 1. UV-Absorption Decay of the Tested Reagents [Decay Rates for All Reactants Tested that Allowed the Spectrophotometric Measurement of the Derivative Against a Blank Solution of Acetonitrile : Water (50 : 50)]

Substance Tested (UV Absorption Kinetic)	% of Decay after 2 h, 50 min
2-Thioglycerol	0*
Thioflour®	0*
Sodium sulphite	0*
3-Mercapto-1,2,4-triazol	0*
2-Mercapto-ethyl-ether	7
2-Mercapto-ethyl-sulfide	14
Thiolactic acid Na salt	16
Thiosuccinic acid Na salt	24
3-Mercaptopropionic acid Na salt	26
MESNA	26
Thioglycolic acid Na salt	28
Thiomalic acid Na salt	29
3-Mercapto-propansulfonic acid Na salt	31
N-Acetyl cystein	33
3-Mercapto-2-butanol	87
1-Mercapto-2-propanol	89
2-Mercaptoethanol	94

*Decay rates reported with 0% were characterized by a steady and slight increase of absorbance after a period of decay. Thus the true decay of the derivative might be higher.

Measurements beyond a 3-hour period were not performed, due to the large number of reactants, as well as for practicability reasons. In some cases, the blank reaction mixture turned yellow after addition of solutions of acetonitrile, this is of interest since acetonitrile has been reported to be the most practicable solvent for preparation of fumonisin standard solutions.[28]

HPLC Analysis of the Fumonisin Derivatives

The fluorescence, after HPLC separation, was tested for reagents that showed good stability in the UV-absorption experiment. However, only a few of the substances from the UV-absorption experiments, resulted in a discrete baseline, resolved and interference free peak for FB_1 within an analysis time of less than

Table 2. Performance Parameter for Various Thiols for Fluorescence Detection After HPLC Separation

Substance	Fluorescence by Peak Area	Fluorescence by Peak Height	Decay Rate after 2.5 h (Peak Area)	Retention Time
2-ME	100%	100%	92%	6.5 min
N-AC	91%	104%	51%	5.6 min
3-MPA	131%	114%	41%	8.5 min
2-TG	112%	121%	67%	5.6 min
2-Mercapto ethyl ether	99%	43%	30%	16.5 min
Thio lactic acid	8%	7%	88%	8.0 min
Thioflour®	28%	47%	53%	3.5 min
3-Mercapto-2-butanol	84%	57%	84%	9.9 min

15 min, for the HPLC separation parameters chosen. Other separation systems were not evaluated, since the HPLC method selected here is commonly used in food control laboratories for fumonisin determination and has been subject to a recent international validation study,[15] in which the authors laboratory participated as testing partner. Thus, method performance parameters are available. Thus, an in-house revalidation of the method was not performed in this study, since only a single parameter was subject to change in this study, which is discussed here.

The relative fluorescence of all test mixtures was measured from the respective HPLC chromatograms of FB_1 standards. Both peak height and area were taken into account, since the latter reflects the absolute fluorescence independent of retention times, while the peak height is a more relevant parameter for HPLC (signal-to-noise). However, the peak area (total fluorescence) approach is interesting for direct fluorimetric measurements of clean fumonisin extracts subsequent to immunoaffinity clean-up.[29]

As can be seen, several alternatives to 2-ME revealed interesting properties as superior reagents. Particularly in the case of 3-mercapto propionic acid (3-MPA) and 2-thioglycerol (2-TG), the resulting fluorescence was higher than that of 2-ME. *N*-acetyl cystein (*N*-AC) being a crystalline and odourless substance of low toxicity, resulted in a similar high amount of fluorescence as 2-ME, while the better stability, as it has been observed in the UV-absorbance experiments, was verified for the HPLC-fluorescence in all three cases.

Regarding the optimal reaction time, it was observed that the formation of derivatives using *N*-AC and 3-MPA was not as rapid as applying 3-ME or 2-TG. Therefore, a reaction time of at least 5 min was allowed prior to injection for all reactants tested.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

As a result of the fluorescence experiments, the most suitable reactants with OPA were found to be 3-MPA, 2-TG, and *N*-AC. In order to assess the applicability of these three reactants for real food matrices, fortified corn material was analysed according to Ref. 15. The purified extracts were subsequently treated with the various derivatization mixtures (including 2-ME for comparison reasons) prior to HPLC analysis.

It can be seen that 3-MPA, 2-ME, 2-TG, and *N*-AC are equally suitable as pre-column derivatization reactants for methods employing 1AC for sample preparation, in view of analysis of fumonisins in complex food matrices (Fig. 3). Especially, *N*-AC (also used as food additive) seems to be the candidate substance of choice due to its low toxicity and the absence of odour, which should be of interest for analysts that know the odour problems occurring with 2-ME.

To further investigate the applicability of the proposed alternatives for the simultaneous determination of FB_1 and FB_2, blank extracts were fortified with both fumonisins and, subsequently, measured by HPLC-fluorescence. In the case of *N*-AC and 3-MPA, the peak decay ratio of the FB_1 to FB_2 peaks remained

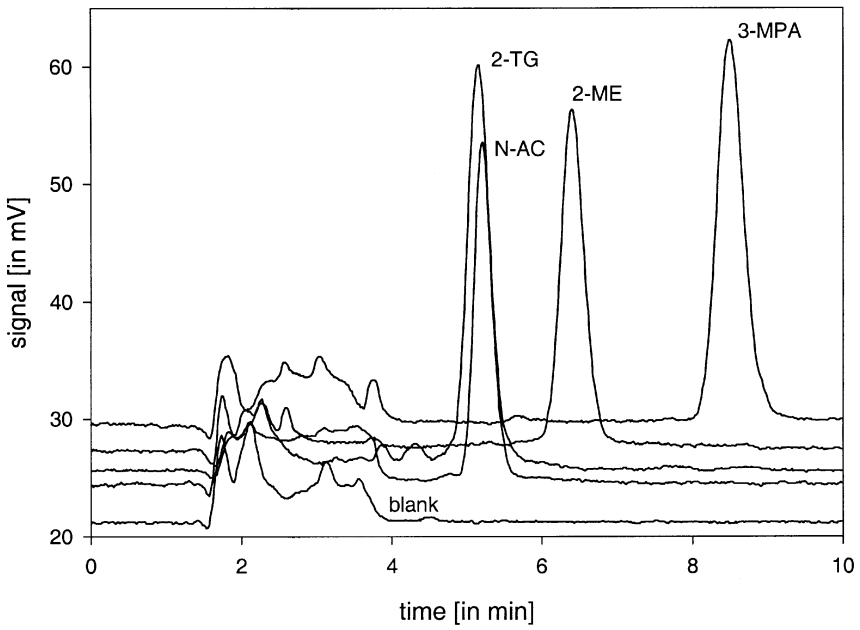

Figure 3. Chromatograms of fumonisin B derivatives with different reactants and OPA from corn extracts after IAC clean-up. The resulting peaks were free of any interferences. The shown blank is derived from the derivatization with 2-ME. Blanks from the other reactants showed a similar interference free baseline in the regions of the peaks.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

constant over the time period of 2.5 hours (multiple injection), while for 2-TG it was observed that the ratio (FB_1/FB_2) decreased to about half the value within this time. This indicates that the decay of the FB_2 derivative is slower compared to FB_1. Thus, the proposed alternatives are also applicable for the determination of both fumonisins.

Due to its superior stability, the here discussed derivatization reagents may also be applied in thin layer chromatography (TLC) as prospective alternatives for pre- and post development derivatization (e.g., spraying agents).[30–31]

Confirmation of the *N*-Acetyl Cystein–Fumonisin B_1 Derivative by HPLC-MS

The confirmation of the structure of the derivative of FB_1 was performed only for *N*-AC, revealing it to be the most suitable compound for substituting 2-ME. Under an electrospray interface (ESI) condition, FB_1 is detected as a protonated ion $[M+H]^+$ 722.5 m/z in the positive mode. Collision induced dissociation (CID) of the protonated ion, provided characteristic fragments due to the loss of tricarballylic acid groups (TCA) side chains and the subsequent loss of one to four molecules of water $[M+H–nH_2O]^+$. The fragmentation of $[M+H]^+$ 722.5 m/z yielded three main product ions: $[M+H–H_2O]^+$ 704.5 m/z, $[M+H–TCA–H_2O]^+$ 528.3 m/z, and $[M+H–2TCA–H_2O]^+$ 352.4 m/z (Fig. 4).

The derivative formed between FB_1, OPA, and *N*-AC was analysed both in positive and negative ionisation mode, showing a signal at $[M+H]^+$ 983.5 m/z and $[M\text{-}H]^-$ 981.5 m/z, respectively. A molecular weight of 982.5 corresponds to the theoretical one, calculated according to the proposed reaction.

MS/MS experiments were carried out in order to confirm the nature of the peak. Fragmentation of the parent ion $[M+H]^+$ 983.5 m/z showed a unique product ion $[M+H\text{-}163]^+$ 820.5 m/z, resulting from the neutral loss of 163, which is *N*-AC. A further fragmentation of the $[M+H–(N\text{-AC})]^+$ 820.5 m/z, resulted mainly in the loss of one molecule of water $[M+H–(N\text{-AC})–H_2O]^+$ 802.5 m/z and of one or two TCA groups, $[M+H–(N\text{-AC})–TCA]^+$ 644.3 m/z and $[M+H–(N\text{-AC})–2TCA]^+$ 468.5.4 m/z (Fig. 4b). As have been already shown in the fumonisin fragmentation pattern, minor product ions were formed due to the subsequent loss of one to three molecules of water. Further fragmentation MS^4 of $[M+H–(N\text{-AC})–H_2O]^+$ 802.5 m/z, showed losses of one or both TCA side chains and of water molecules.

The derivative does not provide the same fumonisin product ions because of the strength of the binding formed between fumonisin and OPA, nevertheless, occurrence of the same neutral losses as fumonisin clearly confirmed the proposed structure.

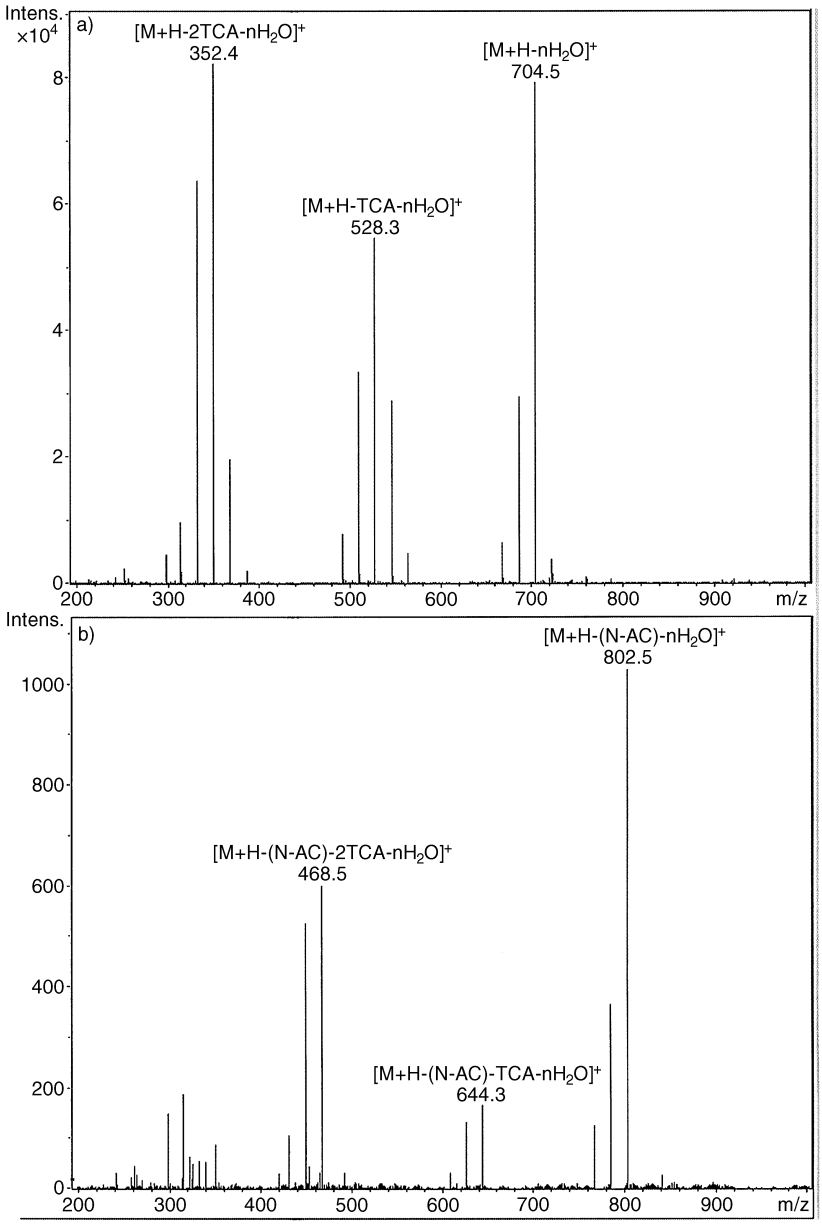

Figure 4. Fragmentation spectra of FB1 and FB1–OPA–*N*-AC derivative: Spectra a) is the resulting MS^2 of FB1 $[M+H]^+$ 722.5 m/z. Spectra b) is the MS^3 of FB1–OPA–*N*-AC $[M+H]^+$ 983.5 $m/z \rightarrow [M+H]^+$ 820.5 m/z.

CONCLUSION

Several alternatives to 2-ME could be identified for the derivatization of FB_1 and FB_2 with OPA. Especially, 3-mercapto-propionic acid (3-MPA), *N*-acetyl-cystein (*N*-AC), and 2-thioglycerol (2-TG) demonstrated to be superior derivatives, when compared to 2-ME. Considering properties such as odour and toxicity of the reactants, *N*-AC was identified as the most suitable alternative, especially when fumonisins are monitored routinely in enforcement or research laboratories. Due to the better derivative stability of all alternatives proposed, higher method robustness can be expected. None of the discussed alternatives requires a change in the final method design, as it is commonly used for the determination of fumonisins with OPA and 2-ME.

REFERENCES

1. Kellerman, T.S.; Marasas, W.F.O.; Thiel, P.G.; Gelderblom, W.C.A.; Cawood, M.E.; Coetzer, J.A.W. Leukoencephalomalacia in Two Horses Induced by Oral Dosing of Fumonisin B_1. Onderstepoort J. of Vet. Res. **1990**, *57*, 269–275.
2. Harrison, L.R.; Colvin, B.M.; Greene, J.T.; Newman, L.E.; Cole, J.R. Jr. Pulmonary Edema and Hydrothorax in Swine Produced by Fumonisisn B_1, A Toxic Metabolite of *Fusarium moniliforme.* J. Vet. Diag. Invest. **1990**, *2*, 217–221.
3. Rheeder, J.P.; Marasas, W.F.O.; Thiel, P.G.; Sydenham, E.W.; Shephard, G.S.; van Schalkwyk, D.J. Fusarium Moniliforme and Fumonisins in Corn in Relation to Human Esophageal Cancer in Transkei. Phytopathol. **1992**, *82*, 353–357.
4. Gelderblom, W.C.A.; Kriek, N.P.J.; Marasas, W.F.O.; Thiel, P.G. Toxicity and Carcinogenicity of the *Fusarium moniliforme* Metabolite, Fumonisn B_1 in Rats. Carcinogen. **1991**, *12*, 1247–1251.
5. Chu, F.S.; Li, G.Y. Simultaneous Occurrence of Fumonisin B1 and Other Mycotoxins in Moldy Corn Collected from the Peoples Republic of China in Regions with High Incidences of Esophageal Cancer. Appl. Environ. Microbiol. **1994**, *60*, 847–852.
6. Shephard, G.S. Chromatographic Determination of the Fumonisin Mycotoxins (review) J. Chromatogr. A **1998**, *815*, 31–39.
7. Sydenham, E.W.; Shephard, G.S. Chromatographic and Allied Methods of Analysis for Selected Mycotoxins. In *Progress in Food Contaminant Analysis*; Gilbert, J., Ed.; ISBN: 0-7514-0337-7.
8. Roth, M. Fluorescence Reaction for Amino Acids. Anal. Chem. **1971**, *43*, 880–882.

9. Roth, M.; Hampai, A. Column chromatography of amino acids with fluorescence detection. J. Chromatogr. **1973**, *83*, 353.
10. Frister, H.; Meisel, H.; Schlimme, E. OPA [phthalaldehyde] Method Modified by Use of NN-Dimethyl-2-mercaptoethylammonium Chloride as Thiol Component. Fres. Z. Anal. Chem. **1988**, *330*, 631–633.
11. Velazquez, C.; Llovera, M.; Plana, J.; Canela, R. Effect of Solvents on the Fumonisins Analysis by High-performance Liquid Chromatography with AccQ.Fluor as the Derivatizing Reagent. J. Chromatogr. A **2000**, *870*, 469.
12. Ware, G.M.; Francis, O.; Kuan, S.S.; Umrigar, P.; Carman, A.; Carter, L.; Bennet, G.A. Determination of Fumonisin B1 in Corn by High Performance Liquid Chromatography with Fluorescence Detection. Anal. Lett. **1993**, *26*, 1751–1770.
13. Scott, P.M.; Lawrence, G.A. Liquid Chromatographic Determination of Fumonisins with 4-Fluoro-7-nitrobenzofurazan. J. AOAC **1992**, *75*, 829–834.
14. Scott, P.M.; Lawrence, G.A.; Analysis of Beer for Fumonisins. *J. Food Prot.* **1995**, *58*, 1379–1382.
15. Solfrizzo, M.; De Girolamo, A.; Visconti, A.; Sizoo, E.; van Egomond, H.P. In *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millenium.* de Koe, W. J., Samson, R.A., van Egomond, H.P., Gilbert, J., Sabino, M., Eds.; W. J. de Koe: Wageningen, NL.; 87–94.
16. Shephard, G.S.; Sydenham, E.W.; Thiel, P.G.; Gelderblom, W.C.A. Quantitative Determination of Fumonisin B_1 and B_2 by High-performance Liquid Chromatography with Fluorescence Detection. J. Chromatogr. **1990**, *13*, 2077–2087.
17. Sydenham, E.W.; Shephard, G.S.; Thiel, P.G. Liquid Chromatographic Determination of Fumonisins B_1, B_2 and B_3 in Food and Feeds. J. AOAC **1992**, *75*, 313–318.
18. Beljaars, P.R.; van Duk, R.; Bisschop, E.; Spiegelenberg, W.M Liquid-chromatographic Determination of Free Glutamic Acid in Soup, Meat Products and Chinese Foods: an Inter-laboratory Study. J. AOAC Internat. **1996**, *79*, 697–702.
19. Bruckner, H.; Wittner, R.; Godel, H. Automated Enantioseparation of Amino Acids by Derivatisation with o-Phthaldialdehyde and n-Acylated Cysteines. J. Chromatogr. **1989**, *476,* 73–82.
20. Jegorov, A.; Triska, J.; Trnka, T.; Cerny, M. Separation of Alpha-amino-acid Enantiomers by Reversed-phase high-performance Liquid Chromatography after Derivatization with Phthalaldehyde and A Sodium Salt of 1-thio-beta-D-glucose. J. Chromatogr. **1988**, *434*, 417–422.
21. Fiorino, A.; Frigo, G.; Cucchetti, E. Liquid-chromatographic Analysis of Amino- and Imino-acids in Protein Hydrolysates by Post-column

Derivatization with Phthalaldehyde and 3-Mercaptopropionic ACID. J. Chromatogr. **1989**, *476*, 83–92.

22. Euerby, M.R.; Nunn, P.B.; Partridge, L.Z. Resolution of Neuroexcitatory Non-protein Amino-acid Enantiomers by High-performance Liquid Chromatography Utilizing Pre-column Derivatization with Phthalaldehyde Chiral Thiols. Application to omega-N-oxylyldiamino-acids. J. Chromatogr. **1989**, *466*, 407–414.
23. Euerby, M.R.; Partridge, L.Z.; Rajani, P. Resolution of Lombricine Enantiomers by High-performance Liquid Chromatography Utilizing Pre-column Derivatization with Phthalaldehyde Chiral Thiols. J. Chromatogr. **1988**, *447*, 392–397.
24. Rowlett, R.; Murphy, J. Convenient Spectrophotometric Method for Kinetic Analysis of the Enzymic Hydrolysis of N-Acyl-peptides Using Phthalaldehyhe. Anal Biochem. **1981**, *112*, 163–169.
25. Buck, R.H.; Krummen, K. High-performance Liquid-chromatographic Determination of Enantiomeric Amino-acids and Amino-alcohols After Derivatization with Phthalaldehyde and Various Chiral Mercaptans: Application to Peptide Hydrolysates. J. Chromatogr. **1987**, *387*, 255–265.
26. Dai, F.; Burkert, V.P.; Singh, H.N.; Hinze, W.L. Update and Evaluation of the Effectiveness of Different Thiols and Micellar Media in Roth's Fluorimetric Method for the Determination of Primary Amino Compounds. Microchem. J. **1997**, *57*, 166–198.
27. Miyahara, M.; Akiyama, H.; Toyoda, M.; Saito, Y. New Procedure for Fumonisins B_1 and B_2 in Corn and Corn Products by Ion Pair Chromatography with o-Phthaldialdehyde Post Column Derivatization and Fluorimetric Detection. J. Agric Food Chem. **1996**, *44*, 842–847.
28. Visconti, A.; Doko, M.B.; Bottalico, C.; Schurer, B.; Boenke, A. Stability of Fumonisins (FB_1 and FB_2) in Solution. Food Add Contamin. **1994**, *11*, 427–431.
29. Duncan, K.; Scott, K.; Kruger, S.; Zabe, N.; Kohn, B.; Prioli, R. Improved Fluorometric and Chromatographic Methods for the Quantification of Fumonisins B1 B2 and B3. J. Chromatogr. **1998**, *815*, 41–45.
30. Preis, A.R.; Vargas, E.A.; A Method for Determining Fumonisin B1 in Corn Using Immuoaffinity Column Clean-up and Thin-layer Chromatography/Densitometry. Food Add Contam. **2000**, *17*, 463–468.
31. Shelby, R.A.; Rottinghaus, G.E.; Minor, H.C.; Comparison of Thin-layer Chromatography and Competitive Immunoassay Methods for Detecting Fumonisins on Maize. J Agric Food Chem. **1994**, *42*, 2064–2067.

Received March 1, 2002
Accepted April 10, 2002
Manuscript 5811