Identification of the first fumonisin mycotoxins with three acyl groups by ESI-ITMS and ESI-TOFMS following RP-HPLC separation: palmitoyl, linoleoyl and oleoyl EFB1 fumonisin isomers from a solid culture of Fusarium verticillioides

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Running head: New fumonisins with three acyl groups

Keywords: *Fusarium*, mycotoxin, fumonisin isomers, EFB1, ITMS, TOFMS
Abstract

The aim of this study was to apply RP-HPLC/ESI–ITMS and RP-HPLC/ESI–TOFMS to investigate and characterize six new higher molecular weight fumonisins (three pairs of isomers) extracted from a *Fusarium verticillioides*-infected solid rice culture. The ITMS and ITMS$^2$ spectra clearly indicated the $m/z$ values (960, 984 and 986) of the protonated molecules and the FB$_1$ toxin-like structures of these compounds, respectively. Moreover, the data evaluation software of the TOFMS equipment unambiguously demonstrated the exact masses of the protonated molecules and the suggested empirical formulae ($C_{50}H_{89}NO_{16}$, $C_{52}H_{89}NO_{16}$ and $C_{52}H_{91}NO_{16}$) of the new fumonisins, with mass accuracy in the range between 0.1 and -1.1 ppm. Subtraction of the empirical formula of FB$_1$ toxin ($C_{34}H_{59}NO_{15}$) from these formulae and correction for the mass of water split-off from the fumonisin molecule during ester formation resulted in the empirical formulae of the fumonisin backbone esterifying agents (fatty acids): $C_{16}H_{32}O_2$ (palmitic acid, PA), $C_{18}H_{32}O_2$ (linoleic acid, LA) and $C_{18}H_{34}O_2$ (oleic acid, OA). We denoted the new compounds as esterified FB$_1$ (EFB$_1$) toxins, with the suggested names EFB$_1$ PA, iso-EFB$_1$ PA, EFB$_1$ LA, iso-EFB$_1$ LA, EFB$_1$ OA and iso-EFB$_1$ OA. The total amount of these new compounds comprised 0.1% of the FB$_1$ concentration, which may be rated as significant when it is considered that these new components are significantly more apolar than earlier-described fumonisins, and their uptake into and toxicity elicited in the various tissues of living organisms may therefore also be significantly different from those of other fumonisins.

INTRODUCTION

Fumonisins are structurally related toxic secondary metabolites of fungi, produced primarily by *Fusarium* species (Rheeder et al. 2002), especially *F. verticillioides* and *F.*
proliferatum (Seifert et al. 2003), which are worldwide pathogens of maize (Shephard et
al. 1996). However, in recent papers the production of some fumonisin B (FB) analogues
by Aspergillus niger has also been reported (Frisvad et al. 2007; Noonim et al. 2009;
Logrieco et al. 2009; Mogensen et al. 2010). Fumonisins can cause severe diseases in
animals, e.g. leucoencephalomalacia in horses (Kellerman et al. 1990) and pulmonary
oedema and hydrothorax in pigs (Colvin et al. 1992; Fazekas et al. 1998; Zomborszky-
Kovács et al. 2002), and they can produce toxic effects in chickens (Ledoux et al. 1992)
and turkeys (Weibking et al. 1993). Important data have also been published on the
nephrotoxicity (Riley et al. 1994), hepatotoxicity and hepatocarcinogenicity (Gelderblom
et al. 1991) of FB\textsubscript{1} toxin in laboratory experimental rodents. Although not yet
conclusively proved, the consumption of fumonisin-contaminated maize and maize-based
foods (at levels of 1-10 mg kg\textsuperscript{-1}) has been linked with the promotion of human primary
liver cancer (Ueno et al. 1997) and statistically with human oesophageal cancer in rural
areas in different countries (Rheeder et al. 1992; Yoshizawa et al. 1994; Doko et al. 1995;
Shephard et al. 2000). Fumonisins are regarded by the International Agency for Research
on Cancer (IARC) as group 2B carcinogens to humans (IARC 2002).

Fumonisins are characterized by a 19-20 carbon atom long amino-polyhydroxyalkyl
chain, which is generally diesterified at the hydroxy groups on C-14 and C-15 with a
terminal carboxyl group of propane-1,2,3-tricarboxylic acid (tricarballylic acid, TCA)
(Rheeder et al. 2002), but in some minor fumonisins the esterification involves other
organic acids, such as \textit{cis}-aconitic acid, oxalsuccinic acid and oxalfumaric acid (Bartók et
al. 2006, 2008). Partially hydrolysed (e.g. PHFB\textsubscript{1}) and fully hydrolysed fumonisins (e.g.
HFB\textsubscript{1}) have also been described (Rheeder et al. 2002; Bartók et al. 2006, 2008). In
partially hydrolysed fumonisins, the backbone is monoesterified, while in fully
hydrolysed compounds (usually formed during nixtamalization of maize, baking and
frying of food products or by alkaline hydrolysis in the laboratory) there is no esterifying agent on the backbone. Depending on their chemical structures, the fumonisins can be classified into four main groups (FA, FB, FC and FP); the toxicologically most important ones being the FB analogues (Rheeder et al. 2002), primarily because the FB analogues, and especially FB$_1$ toxin (Fig. 1), can be found in the highest concentration in maize and maize-based food- and feedstuffs (Stack 1998), and the free primary amine group on C-2 contributes significantly to the toxicity of the FB analogues (Bolger et al. 2001; Lemke et al. 2001). The first fumonisins isolated and identified in 1988, were FB$_1$ and FB$_2$ toxin (Bezuidenhout et al. 1988). The amine group in the FA analogues is acetylated. The structure of the FC analogues is similar to that of the FB analogues, without the C-1 methyl group. The most recently described group of fumonisins comprises the FP analogues, in which a 3-hydroxypyridine moiety can be found on C-2 (Musser et al. 1996).

HPLC/ESI–MS and MS/MS techniques with different types and different combinations of mass analysers have become popular in fumonisin analysis. The most significant advantages of these hyphenated procedures for the analysis of fumonisins are their selectivity and sensitivity, and the fact that, in contrast with UV or fluorescence detection, there is no need for derivatization (pre- or post-column) (Zöllner and Mayer-Helm 2006).

The aim of this study was to apply reversed-phase high-performance liquid chromatography/electrospray ionization – ion trap and time-of-flight mass spectrometry (RP-HPLC/ESI–ITMS and RP-HPLC/ESI–TOFMS) to investigate and characterize six new, higher molecular weight FB analogues of fumonisins (three pairs of isomers with different $m/z$ values) extracted from a *Fusarium verticillioides*-infected solid rice culture.
This is the first report on the detection of fumonisins in which, besides two TCA moieties, there is a third esterifying agent in the molecule.

MATERIALS AND METHODS

Chemicals

The FB1 standard and methanol (MeOH, gradient grade) were purchased from Sigma-Aldrich Ltd. (Budapest, Hungary). Acetonitrile (MeCN, hypergrade) was from Merck Ltd (Budapest, Hungary). Purified water with a resistivity of 18 MΩ was delivered with a Nanopure II (Barnstead/Thermolyne Co., Dubuque, IA, USA) water purification device.

Culturing of Fusarium verticillioides and extraction of fumonisins produced

A Fusarium verticillioides isolate acquired from maize stalk (isolate no. Fv 16) was identified via its macro- and micromorphological characteristics and grown on a solid rice culture as described earlier (Bartók et al. 2006, 2010). It was demonstrated in those reports that this isolate has a good fumonisin-producing capability, yielding 18.27 mg g⁻¹ FB1 toxin. The freeze-dried culture material containing fumonisins was extracted with a mixture of MeOH/H₂O (75/25, v/v), using a high-speed homogenizer UltraTurrax T25 (IKA, Staufen, Germany) at 9500 rpm for 4 min. After extraction, and centrifugation (10000xg for 10 min), the supernatant was filtered through a PTFE membrane (0.2 µm) into an HPLC autosampler vial and was subsequently analysed with the instruments detailed below.

RP-HPLC/ESI–ITMS and RP-HPLC/ESI–TOFMS analyses of fumonisins

The instrumental parameters were described in detail recently (Bartók et al. 2010). Briefly, the fumonisins extracted from the solid rice culture material were analysed by
RP-HPLC/ESI–ITMS and also RP-HPLC/ESI–TOFMS at two research locations, where different Agilent (Santa Clara, CA, USA) HPLC systems (1090 Series II with DR5 binary pump and 1200 Series with binary pump) were attached to the mass spectrometers. Since the dead volume of the flow path of the 1200 Series HPLC system applied is higher than that of the 1090 Series II HPLC, the pulse damper from the 1200 Series pump was removed in order to decrease the retention time differences for the fumonisins on the two HPLC systems. A YMC-Pack J’sphere ODS H80 (YMC Europe GmbH, Dinslaken, Germany) (250 mm x 2.1 mm, 4 µm) HPLC column and a flat (24% B to 40% B in 79 min, then increased to 100% B in 15 min) linear binary gradient of H₂O (A) and MeCN (B) (each supplemented with 0.1% (v/v) formic acid) were applied at a flow rate of 0.2 ml min⁻¹ for the separation of the compounds. Mass spectrometric detection was performed with a Varian (Palo Alto, CA, USA) 500MS IT, and on Agilent 6210 TOF mass spectrometers. Both mass spectrometers were equipped with ESI ion sources and operated in positive ion mode. Relative quantitation (% of FB₁) of the new compounds was performed by ESI-ITMS, using an external standard (ESTD) method in a 5 order of magnitude concentration range of an FB₁ calibration standard dissolved in MeCN/H₂O 50/50 (v/v). The quantitative evaluation of the new compounds by using ESTD calibration of FB₁ provided only approximate data, because the polarities of the detected new fumonisins are very different from that of FB₁; naturally, the ionization of the new compounds is assumed, but is not known to be the same as that for FB₁.

RESULTS AND DISCUSSION

Thanks to their outstanding sensitivity in full scan mode, mass spectrometers equipped with IT mass analysers are eminently suitable for the detection and partial identification of new secondary metabolites (e.g. mycotoxins) and their isomers (Josephs 1996; Bartók
et al. 2006, 2008, 2010). As illustrated by the MS$^2$ spectrum of FB$_1$ toxin (Fig. 2), the product ion spectra of the molecular ions ([M+H]$^+$) of fumonisins are highly characteristic; in other words, it is easy to decide almost at a glance whether or not a given product ion spectrum is produced by a fumonisin. In most fumonisins, the backbone is esterified by two organic acid molecules (most often TCA), and accordingly the product ion spectra contain three groups of fragment ions (indicated by 1, 2 and 3 in Fig. 2). The first group of fragment ions consists of the dehydrated molecular ions, the second is the [M+H–TCA]$^+$ group and the third is the [M+H–2TCA]$^+$ group. It is characteristic of the product ion spectra of the fumonisins that, within a given product ion group, there is a difference of 18 Da between the masses of the neighboring ions, corresponding to the splitting-off of one H$_2$O molecule from the larger molecular or fragment ion. The difference between the m/z values of the corresponding ions of the neighboring product ion groups yields the molar mass of the organic acid esterifying the backbone (TCA or, in the case of a few minor fumonisins, cis-aconitic acid, oxalfumaric acid or oxalsuccinic acid) (Bartók et al. 2006, 2008). In the course of our earlier HPLC/ESI–ITMS studies these characteristics indicated that isolates of Fusarium verticilliodes produce as yet unpublished fumonisins of higher molecular mass. Under the given experimental conditions, these fumonisins were eluted from the HPLC column at close to 100% MeCN, demonstrating that these components are considerably more apolar than other fumonisins so far described. The m/z values of the fragment ions in the MS$^2$ spectra, including the hydrocarbon backbone at m/z 299, revealed that the structures of these new fumonisins are highly similar to that of FB$_1$ toxin, except for the presence of additional functional groups. We assumed that the new substituents might be carboxylic acids esterifying the free OH groups of the fumonisin backbone, carboxylic acids that had not been identified on the fumonisin backbone. We were earlier unable to identify these
fumonisins with our ITMS (lack of exact mass measurement). However, component identification following RP-HPLC separation was achieved by using an ESI-MS instrument equipped with a TOF mass analyser suitable for exact mass measurement. Three research groups had previously mentioned application of the TOFMS technique in fumonisin research, but neither of them utilized the procedure for the analysis of fumonisin isomers (Lemke et al. 2001; Senyuva and Gilbert 2008; Mogensen et al. 2010).

We recently verified the usefulness of exact mass measurement by TOFMS during the identification of new FB$_1$ isomers (Bartók et al. 2010). Since the HPLC equipment used in these TOFMS measurements was different from that used in earlier ITMS analyses, slightly different retention times were obtained for the same components. As shown in Tables 1 and 2, however, the retention times (relative to FB$_1$ toxin) of the same components separated in the two different HPLC systems were highly similar. The differences between the retention times relative to FB$_1$ lay in the range 0.085-0.088. After full scan analysis by RP-HPLC/ESI–TOFMS, the data-processing software (Mass Hunter) of the equipment was asked to produce both the total ion and the extracted ion chromatograms (EICs) at m/z values of 960, 984 and 986 (Fig. 3). Nevertheless, similarly to RP-HPLC/ESI–ITMS, the total ion chromatogram obtained by RP-HPLC/ESI–TOFMS did not display these minor components in addition to the main components (FB$_1$, FB$_2$, FB$_3$ and FB$_4$). TOFMS EICs (Fig. 3) and ITMS EICs (chromatogram not shown), however, unambiguously indicated six new fumonisins in the retention time range 90.623-91.928 min and 89.324-90.569 min, respectively, i.e. two components at each of three different m/z values. The leading suggestions of the Mass Hunter software for all six fumonisins were the following empirical formulae (unprotonated) and the corresponding exact mass values ([M+H]$^+$): C$_{50}$H$_{89}$NO$_{16}$ (960.62541); C$_{52}$H$_{89}$NO$_{16}$ (984.62541) and C$_{52}$H$_{91}$NO$_{16}$ (986.64106). Subtraction of the empirical formula of FB$_1$
toxin \((C_{34}H_{59}NO_{15})\) from these empirical formulae, followed by addition of the \(H_2O\) removed from a OH group of the fumonisin backbone and the organic acid molecules in the course of esterification, yielded the empirical formulae of the organic (fatty) acids esterifying the third OH group of the backbone of the FB\(_1\) toxin, i.e. \(C_{16}H_{32}O_2\) (palmitic acid, PA), \(C_{18}H_{32}O_2\) (linoleic acid, LA) and \(C_{18}H_{34}O_2\) (oleic acid, OA). With regard to the currently accepted nomenclature (FB\(_1\), PHFB\(_1\) (partially hydrolysed FB\(_1\)) and HFB\(_1\) (fully hydrolysed FB\(_1\))), the new fumonisins were named esterified FB\(_1\) (EFB\(_1\)) toxins. The abbreviation at the end of the name (PA, LA or OA) indicates the organic acid, esterifying the third OH group of the fumonisin backbone. Thus, the suggested names of the six new identified fumonisins are EFB\(_1\) PA, iso-EFB\(_1\) PA, EFB\(_1\) LA, iso-EFB\(_1\) LA, EFB\(_1\) OA and iso-EFB\(_1\) OA. The mass accuracy (based on the measured and the calculated mass values) of the new EFB\(_1\) toxins was in the range between 0.1 and -1.1 ppm. Data relating to the RP-HPLC/ESI–TOFMS measurements are listed in Table 1.

Our TOFMS measurements confirmed that the signals of the new fragment ion groups (4, 5 and 6) in the MS\(^2\) spectra obtained by ITMS are due to the presence of the third organic acid on the fumonisin backbone (Fig. 4). The experimental data obtained by ITMS allow conclusions that complement the fragmentation pathways published earlier (Bartók et al. 2006, 2010). Figure 5a-c depicts theoretically possible pathways for the fragmentation of the newly detected fumonisins. Of these theoretically possible reaction pathways, dominant routes can be suggested by comparison of the \(m/z\) values of the MS\(^2\) spectra shown in Figs 4 and 5a-c. No experiments were performed to address fragmentations depending on the CID energy, and it is therefore not possible to distinguish the roles of kinetic and thermodynamic parameters at this point. It is currently assumed that, the main fragmentation pathways can be characterized by the abundances of the ions in the product ion spectra. The experimental data mentioned above draw
attention to various features regarding the dominant fragmentation pathways. Firstly, it is immediately apparent that the fragmentation patterns (abundance systems) of the MS\(^2\) spectra of the molecular ions of the newly detected fumonisins differ markedly from those of previously published fumonisins (Fig. 4): for the new fumonisins, there are six groups of fragment ions instead of the three characteristic of the MS\(^2\) spectra of the molecular ions ([M+H]\(^+\)) of most fumonisins (Figs 2 and 4). Secondly, from the six groups of fragment ions, the ions (m/z values) of groups 4, 5 and 6 are also present in the groups of fragment ions of the mass spectra of the FB\(_1\) toxin and its isomers (1-3 in Fig. 2) (Bartók et al. 2010). The explanation seems obvious: in the MS fragmentation of EFB\(_1\)-type fumonisins, the fatty acid or its ketene form is split off the molecule before either of the TCAs, and two of the ions characteristic of the MS\(^2\) spectrum of FB\(_1\) toxin, i.e. the ions at m/z 704 and m/z 722, are produced. From this step on, the theoretical MS fragmentation scheme of the EFB\(_1\) components is identical with that of FB\(_1\) toxin. Thirdly, it is of diagnostic value that the m/z values of the hydrocarbon backbones detected at the end of the fragmentation in the MS\(^2\) spectra of the molecular ions of the newly detected fumonisins (299 Da) are identical with the m/z value of the hydrocarbon backbone in the mass spectra of FB\(_1\) toxin and its isomers. From the predominant directions of fragmentation, the example of a fragmentation previously unknown among fumonisins is shown in Fig. 6 for EFB\(_1\) LA (C-10 is the presumed esterification site for the LA). The fragmentation patterns are similar for the other EFB\(_1\)s and for the iso-EFB\(_1\) components. It follows from the structures of the new esterified fumonisins that groups modifying the backbone are split off in the course of fragmentation, i.e. C-O and C-N bond scission leads to the elimination of H\(_2\)O, organic acids (TCA, LA, OA or PA) and NH\(_3\). From the product ions formed as a result of fragmentation, it is clear that TCA can also be eliminated in a stepwise manner, while H\(_2\)O is released with the concomitant
formation of the corresponding anhydride (TCAD 158 Da, Fig. 7). The splitting-off of TCA and the new fatty acids (LA, OA or PA) esterifying the backbone in the form of their ketene (TCAK 158 Da, LAK 262 Da, OAK 264 Da and PAK 238 Da, Fig. 7) was also observed, as can be seen in the theoretical fragmentation pathways (Fig. 5a-c) and the MS<sup>2</sup> spectra (Fig. 4). The MS<sup>2</sup> spectra permit, the following general observations: (i) the ions produced by the fragmentation of LA, OA or PA were most abundant; (ii) the product ions with the lowest abundance were those formed by the splitting-off of 2TCA; (iii) the sequence of the groups of fragment ions according to abundances is 4>2>5>6>1≥3 for EFB<sub>1</sub> and 4>5>6>1≥2>3 for iso-EFB<sub>1</sub>. The characteristic data (retention time, retention time relative to FB<sub>1</sub>, quantity relative to FB<sub>1</sub> and MS<sup>2</sup> spectral information on the molecular ions) for the EFB<sub>1</sub> compounds, obtained by RP-HPLC/ESI–ITMS, are detailed in Table 2. The relative quantities (% of FB<sub>1</sub>) of the new fumonisins were in the range 0.003–0.036%. Their total amount was 0.1% of the FB<sub>1</sub> concentration, which may be rated as significant when it is considered that these new components are much more apolar than earlier-described fumonisins, and their uptake into and toxicity elicited in the various tissues of living organisms may therefore also be significantly different from those of other fumonisins.

The experimental results presented in this article clearly revealed that the parallel use of two hyphenated techniques (RP-HPLC/ESI–ITMS and RP-HPLC/ESI–TOFMS) was essential for identification of new type of fumonisins with three acyl groups, i.e. there are situations when a single hyphenated technique is not sufficient for the identification of a new compound. Of course, neither procedure could determine the relative and absolute configurations of the new fumonisins. In order to elucidate the structures of these toxins, other spectroscopic techniques, such as NMR, XRD and ORD, must be used. However,
to perform these structural examinations and to examine the toxicity of these compounds, the most abundant EFB\textsubscript{1} isomers should first be isolated.

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**References**


Bolger M, Coker RD, DiNovi M, Gaylor D, Gelderblom WCA, Olsen M. in: Safety evaluation of certain mycotoxins in food, Edited by the Fifty-sixth meeting of the Joint


Seifert KA, Aoki T, Baayen RP, Brayford D, Burgess LW, Chulzes S, Gams W, Geiser D, deGruyter J, Leslie JF, Logrieco A, Marasas WFO, Nirenberg HI, Odonnell K,


**Figure 1.** Structure of FB$_1$ toxin.
41x14mm (600 x 600 DPI)
Figure 2. Product ion spectra (CID) of protonated molecules ([M+H]^+) of FB1 with product ion groups (1-3) indicated. The left and right doubled arrows indicate the mass difference of 176 Da (loss of one molecule of TCA) between the corresponding product ions of the neighbouring product ion groups.

68x53mm (600 x 600 DPI)
Figure 3. Total ion and extracted ion chromatograms (EICs, enlarged, at m/z 984.62, 960.62 and 986.64) obtained by RP-HPLC/ESI–TOFMS on an extract of a solid rice culture infected with *Fusarium verticillioides*. The asterisks (* and **) indicate the isotope peaks of EFB$_1$ LA (1) and iso-EBF$_1$ LA (2) at an m/z value of 986.64.

109x74mm (600 x 600 DPI)
Figure 4. Product ion spectra (CID) of protonated molecules ([M+H]^+) of esterified FB1 (EFB1) isomers: EFB1 LA (1), iso-EFB1 LA (2), EFB1 PA (3), iso-EFB1 PA (4), EFB1 OA (5), iso-EFB1 OA (6) by ESI–ITMS². The product ion groups (1-6) are indicated in the MS² spectrum of [M+H]^+ at m/z 984 of EFB1 LA (1).

214x243mm (600 x 600 DPI)
Figure 5a-c. Theoretically possible fragmentation (CID) pattern characteristic only of protonated molecules ([M+H]+) of esterified FB1 (EFB1) isomers. (a) EFB1 LA (1), iso-EFB1 LA (2); (b) EFB1 PA (3), iso-EFB1 PA (4); (c) EFB1 OA (5), iso-EFB1 OA (6). The vertical, horizontal and diagonal arrows indicate the loss of H2O (18 Da); TCA or TCAK (158 Da), PAK (238 Da), LAK (262 Da), OAK (264 Da) and TCA (176 Da), PA (256 Da), LA (280 Da), OA (282 Da) from the molecular and product ions, respectively. The m/z values underlined were not observed in the mass spectra. See details in the text, and for abbreviations, see Fig. 7.
Figure 6. Assumed structures of some characteristic ions formed in the CID fragmentation processes of protonated molecules of EFB$_1$ isomers. C-10 is the presumed esterification site for the fatty acids.
Figure 7. Acids conjugated to the fumonisin backbone and structures of compounds possibly formed in the CID fragmentation processes of protonated molecules of EFB$_1$ isomers.
Table 1. Characteristic data obtained by RP-HPLC/ESI-TOFMS on new esterified FB₁ (EFB₁) isomers.

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<th>Empirical formula (unprotonated)</th>
<th>Exact mass of [M+H]+ (calculated)</th>
<th>Exact mass of [M+H]+ (measured)</th>
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<td>iso-EFB₁ PA (4)</td>
<td>91.670</td>
<td>6.890</td>
<td>Cₕ₀H₈₀NO₁₆</td>
<td>960.6254</td>
<td>960.6255</td>
<td>0.1</td>
</tr>
<tr>
<td>EFB₁ OA (5)</td>
<td>91.732</td>
<td>6.895</td>
<td>C₅₀H₈₀NO₁₆</td>
<td>986.6411</td>
<td>986.6412</td>
<td>0.1</td>
</tr>
<tr>
<td>iso-EFB₁ OA (6)</td>
<td>91.928</td>
<td>6.909</td>
<td>Cₕ₀H₈₀NO₁₆</td>
<td>986.6411</td>
<td>986.6412</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 2. Characteristic data obtained by RP-HPLC/ESI-ITMS on new esterified FB$_1$ (EFB$_1$) isomers (for abbreviations, see Fig. 7).

<table>
<thead>
<tr>
<th>Compound name and m/z (italics) of [M+H]$^+$</th>
<th>Retention time (min)</th>
<th>Retention time relative to FB</th>
<th>Relative quantity (% of FB$_1$)</th>
<th>m/z values (italics) and relative abundances (%) of product ions of [M+H]$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFB, LA (1)</td>
<td>984</td>
<td>89.324 6.897 0.036</td>
<td>966 948 930 826 808 790 772 722 704 688 668 650 632 614 596 546 528 510 492 370 352 334 316 299</td>
<td>17.9 21.8 0.7 2.0 16.1 52.9 0.9 0.7 100 823 16.6 1.6 5.4 8.6 5.4 5.7 23.5 36.7 7.2 0.8 20.1 31.2 5.4 3.2</td>
</tr>
<tr>
<td>iso-EFB, LA (2)</td>
<td>984</td>
<td>89.542 6.914 0.010</td>
<td>966 948 930 826 808 790 772 722 704 688 668 650 632 614 596 546 528 510 492 370 352 334 316 299</td>
<td>4.7 14.1 0 0.8 2.6 9.0 0.6 0 100 54.3 9.8 0.7 1.0 2.0 2.0 4.1 20.9 19.5 6.6 3.2</td>
</tr>
<tr>
<td>EFB, PA$^+$ (3)</td>
<td>980</td>
<td>90.150 6.962 0.015</td>
<td>942 924 906 802 784 766 748 722 704 686 668 650 632 614 596 572 546 528 510 492 370 352 334 316 299</td>
<td>33.0 21.6 1.1 1.1 31.4 70.3 2.5 1.8 93.1 100 21.7 1.6 8.3 7.0 1.2 10.4 36.2 40.8 9.6 1.4</td>
</tr>
<tr>
<td>iso-EFB, PA$^+$ (4)</td>
<td>980</td>
<td>90.323 6.976 0.003</td>
<td>942 924 906 802 784 766 748 722 704 686 668 650 632 614 596 572 546 528 510 492 370 352 334 316 299</td>
<td>3.2 11.6 4.8 2.0 9.3 12.4 0 0 0 8 100 75.4 14.4 0 2.8 3.6 0 4.5 42.8 26.3 3.1</td>
</tr>
<tr>
<td>EFB, OA$^*$ (5)</td>
<td>986</td>
<td>90.395 6.981 0.027</td>
<td>968 950 932 828 810 792 774 722 704 686 668 650 634 616 598 546 528 510 492 370 352 334 316 299</td>
<td>28.8 22.8 0 2.6 24.1 74.4 5.3 2.2 99.5 100 28.8 1.1 3.3 9.3 0.2 1.1 32.8 40.4 6.9 1.2</td>
</tr>
<tr>
<td>iso-EFB, OA$^*$ (6)</td>
<td>986</td>
<td>90.569 6.995 0.008</td>
<td>968 950 932 828 810 792 774 722 704 686 668 650 634 616 598 546 528 510 492 370 352 334 316 299</td>
<td>5.8 12.2 2.8 2.0 4.5 6.6 0.9 0 100 45.1 10.9 0.7 0.4 6.5 0.2 3.2 39.9 15.6 1.2 7.0 25.6 13.7 2.1 0.7</td>
</tr>
</tbody>
</table>

$^a$ m/z 626 [M+H – TCA – TCAK]$^+$ 3.1%; $^b$ m/z 626 [M+H – TCA – TCAK]$^+$ 1.5%; $^c$ m/z 474 [M+H – TCA – OA – 3H$_2$O]$^+$ 0.9%; $^d$ m/z 388 [M+H – 2TCAK – OA]$^+$ or [M+H – TCAK – TCA – OA]$^+$ 1.4%; $^e$ m/z 474 [M+H – TCA – OA – 3H$_2$O]$^+$ 0.2%; $^f$ m/z 388 [M+H – 2TCAK – OA]$^+$ or [M+H – TCAK – TCA – OA]$^+$ 0.4%; characteristic m/z values and relative abundances (%), in brackets) of MS$^2$ spectrum of FB; toxin ([M+H]$^+$) at m/z 722) according to Bartók et al. 2010: 704 (43.6); 686 (100); 668 (18.1); 650 (0.4); 564 (3.3); 546 (28); 528 (92.2); 510 (31.5); 492 (5.7); 388 (0.9); 370 (16.7); 352 (52.9); 334 (28.7); 316 (3.9); 299 (1.9)