EMCR Environmental Monitoring & Contaminants Research
https://emcr-journal.org/
DOI: https://doi.org/10.5985/emcr.20200005
Environmental Monitoring and Contaminants Research Vol.1, pp.47–53, 2021
Technical Note

Development of an analytical method for fumonisin B_1 in porcine tissues and body fluids using UHPLC-MS/MS

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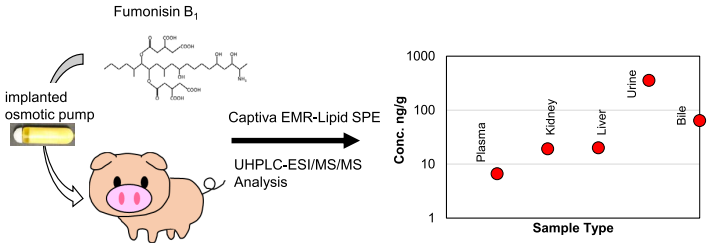
[Received October 21, 2020; Accepted February 5, 2021]

ABSTRACT



High-precision analytical methods to detect fumonisin B_1 (FB_1) in a limited range of biological samples have recently been reported. Here an analytical method for FB_1 for use in a wider range of tissue and body fluid samples, such as the plasma, kidney, liver, urine, and bile was developed. Pigs were used in this investigation and administered with FB_1 via osmotic pumps. Samples were extracted with a formic acid/acetonitrile/water mixture, purified on a phospholipid removal solid-phase extraction column, and analyzed by ultra-high performance liquid chromatography-electrospray ionization/tandem mass spectrometry (UHPLC-ESI/MS/MS). The mean recovery of FB_1 in the plasma, kidney, liver, and urine ranged from 80%–115%, and the corresponding relative standard deviation (RSD) was below 10%. The matrix effects resulted in ionization suppression in the bile samples, but these effects were stable and constant, and could be accounted for; the mean recovery and RSD for the bile samples was 10% and < 10%, respectively. Using this analytical method, FB_1 was precisely detected in pigs and its levels decreased in the order of urine > bile > kidney = liver > plasma, which implied fast excretion. The detection and quantitation limits (1 and 3 ng g^{-1}, respectively) alongside the other results indicated that this method was suitable for the analysis of FB_1 in various types of biological samples.

Key words: Fumonisin B_1; pig; tissue; body fluid; LC-MS/MS; matrix effect

INTRODUCTION

Fumonisin B_1 (FB_1) is one of the major mycotoxins produced by *Fusarium* fungi and mainly contaminates corn and corn-derived products (WHO, 2000). In Japan, FB_1-producing fungi and FB_1 have been widely detected in forage grains and animal feeds (Uegaki et al., 2015, 2018). Among the various fumonisins identified, FB_1 is the most harmful to animals (EFSA, 2018) and it exhibits highly species-specific toxicities. For example, FB_1 causes pulmonary edema in pigs, hepatic and renal damage in poultry, and leukoencephalopathy in horses, furthermore it also has teratogenic effects on the neural tubes of human neonates (WHO, 2000; EFSA, 2018). Consequently, in Japan, the identified tolerable daily intake of FB_1 by humans is 2 g kg^{-1} body weight (FSCJ, 2018). In 2020, the Ministry of Agriculture, Forestry and Fisheries of Japan limited the combined content of three fumonisins (FB_1 + FB_2 + FB_3) in livestock and poultry feed to 4 mg kg^{-1} (FAMIC, 2020).

Pharmacokinetic knowledge of substances, particularly their distributions in tissues, improves our understanding of their toxicities to living organisms. Due to the low bioavailability and short half-life of FB_1, its concentrations are extremely low (compared to the exposure levels) in animal tissues but high in the liver and kidneys (Norred et al., 1993; Prelusky et al., 1994; WHO, 2000; Dilkin et al., 2003; Meyer et al., 2003; Fodor et al., 2006, 2008). However, the research condition–dependent inconsistencies regarding the fate of FB_1 in animals suggest that further considerations, especially for highly accurate and precise analytical methods, are required.

The high sensitivity and selectivity of liquid chromatography-tandem mass spectrometry (LC-MS/MS) makes it a popular choice for the accurate analysis of various substances. However, the remaining biological matrices in the final extracts strongly influence detection sensitivity and should therefore be removed during pretreatment (Matuszewski et al., 2003; Van Eeckhaut et al., 2009). To date, numerous methods have been developed for FB_1 quantitation in animal tissues, blood, and feces, including the extraction and removal of proteins with acetonitrile/water or methanol/water mixtures, extraction and purification by the QuEChERS method, liquid–

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liquid partitioning with hexane, and further purification on octadecylsilane, strong anion exchange, Oasis HLB and phospholipid removal solid-phase extraction (PLR-SPE) columns (Meyer et al., 2003; Riley and Voss, 2006; Fodor et al., 2008; Gazzotti et al., 2011; Devreese et al., 2012; Flores-Flores and González-Peñas et al., 2017; Souto et al., 2017; De Baere et al., 2018). However, these methods have shown low and sample-type dependent irregularities in FB_1 recovery rates, or in some cases, they have high accuracy but are only suitable for specific sample types, such as plasma or urine. Therefore, they may not be suitable for the precise understanding of the fate of FB_1 in animals in exposure experiments. More importantly, the structural features of FB_1 allow it to easily form coordination bonds and be converted to monomethyl and dimethyl esters in methanolic solutions at room temperature (WHO, 2000).

Considering the above, we herein aimed to establish an easy and highly accurate technique for the detection of FB_1 in animal tissues and body fluids. Specifically, we developed a procedure for the UHPLC-ESI/MS/MS–based quantitation of FB_1 in exposed pig samples that were extracted with an acidic acetonitrile/water mixture to remove proteins and were then purified on a PLR-SPE column (Captiva EMR-Lipid column).

MATERIALS AND METHODS

CHEMICALS AND EQUIPMENT

The FB_1 standard, purchased from Fuji Film Wako Pure Chemicals (Osaka, Japan; 50 μg mL^{-1}, acetonitrile : water [1 : 1, v/v]), was diluted with acetonitrile : ultrapure water (1 : 1, v/v) to 1 or 5 μg mL^{-1}. LC/MS-grade acetonitrile, formic acid, and other organic solvents (Fuji Film Wako Pure Chemical, Osaka, Japan), a Captiva EMR-Lipid (3 mL, 300 mg, Agilent, CA, USA) cartridge column for purification, 0.20-μm Millex-LG (Merck Millipore, MA, USA) filter cartridges, polypropylene (PP) vials (700 μL, La-Pha-Pack, Langerwehe, Germany; or 300 μL, Waters, MA, USA), and PP centrifuge tubes (15 mL or 50 mL; Fukase Kasei, Kobe, Japan) were also used. For sample pretreatment, a CS-6KR centrifuge (Beckman Coulter, CA, USA) and an MMS-510 shaker (EYELA, Tokyo, Japan) were employed. The UHPLC-ESI/MS/MS system was comprised of an ACQUITY UPLC (Waters, MA, USA) instrument and an ACQUITY TQ detector (Waters, MA, USA).

SAMPLE COLLECTION

Samples were collected during a previous pig exposure experiment. Briefly, three five-week-old male LW strain pigs were exposed to an FB_1 solution (2 mL, 10 mg mL^{-1} in 9 : 1 (v/v) distilled water : DMSO, 20 mg head^{-1} week^{-1}) using a surgically implanted osmotic pump (ALZET® 2ML1) in the upper neck, behind the left ear (exposure group [T] animals: T–1, T–2, and T–3). The control group (n = 3) received a vehicle without FB_1 (control group [C] animals: C–1, C–2, and C–3). The average body weights of the T and C groups before implanting the osmotic pumps were 11.1 kg and 10.6 kg, respectively. They were fed twice a day with a commercial feed (Itochu, Ito Feed Company, Aichi, Japan) and water was provided *ad libitum*. Blood samples were collected one and three days after the onset of exposure. After seven days of administration, the animals were euthanized, and their organs and body fluids were sampled and stored below –20°C. Liver and kidney samples were homogenized before extraction. Blood was stored as plasma. The animal exposure experiment was conducted according to the guidelines for animal experiments of the National Institute of Animal Health (NIAH-NARO, Tsukuba, Japan). The protocol was approved by the Committee on the Ethics of Animal Experiments of the NIAH-NARO (Protocol No.: 17–054).

CHEMICAL ANALYSIS

The extraction method used was a drastically modified version of that described in previous studies (Souto et al., 2017; De Baere et al., 2018). Briefly, plasma, kidney, liver, bile (from gallbladder), and urine (from bladder) were thawed at 4°C, and 1-g specimens were transferred to 50-mL PP centrifuge tubes. The body fluid and tissue samples were supplemented with 1 mL and 1.5 mL of ultrapure water, respectively, vortexed for 20 s, and left to settle. Then, ice-cooled formic acid/acetonitrile (1.25%, v/v, 4 mL) was added to each sample, and the mixture was vortexed for 15 s, shaken for 15 min, and centrifuged at 3,750 rpm at 4°C for 10 min. The supernatant was transferred to a clean 15-mL PP centrifuge tube, and the residue was treated with above acidic acetonitrile (4 mL), suspended via 10-s ultrasonication, and then the above steps were repeated. The supernatant was combined with the first extract in the same 15-mL PP centrifuge tube, and the mixture was thoroughly vortexed. The tube was stored below –20°C overnight. The mixture was then shaken for 15 min, centrifuged as described above, and the supernatant was transferred to another 15-mL PP centrifuge tube as the final extract.

Each final extract (3 mL) was loaded on a Captiva EMR-Lipid cartridge and allowed to elute by gravity flow. When the liquid level reached the top surface of the adsorbent, 20% ultrapure water/acetonitrile (v/v, 0.7 mL) was added and allowed to elute by gravity flow. The remaining solvent in the cartridge was eluted at a reduced pressure of 10 inHg (~34 kPa). The combined eluates were nearly dried under a gentle N_2 stream at 45°C. The residue was redissolved in 0.1% formic acid/50% acetonitrile/ultrapure water (v/v/v, 500 μL) via thorough vortexing and ultrasonication. The samples were filtered before injection. FB_1 quantitation was performed by UPLC-ESI/MS/MS in accordance with the conditions stated in Table 1.

QUALITY CONTROL AND QUALITY ASSUNACE

The linearity, instrumental detection limit (IDL), and instrumental quantitation limit (IQL) were determined basically according to the method of Ministry of the Environment, Japan (MoE 2016). An eight-point calibration curve (2, 4, 10, 20, 50, 100, 250, and 500 ng mL^{-1} in 0.1% formic acid/50% acetonitrile, v/v) was prepared using either 1 or 5 μg mL^{-1} FB_1 standard solutions. The linearity and detection limits were determined by analyzing 2–10 ng mL^{-1} standards eight times and 20–500 ng mL^{-1} standards four times (calibration curve). In addition, standard solutions of 20, 50, 100, and 500 ng mL^{-1} were concurrently measured (daily validation) before and after FB_1 analysis in the actual samples.

The IDL and IQL were calculated using following equations (MoE, 2016).

Table 1 UHPLC-ESI/MS/MS CONFIGURATION AND PARAMETERS FOR FB_1 ANALYSIS

UHPLC: Waters ACQUITY UPLC system
- Solvent A: 0.1% formic acid/ultrapure water
- Solvent B: 0.1% formic acid/acetonitrile
- Wash A: 0.1% formic acid/20% acetonitrile/80% ultrapure water
- Wash B: 0.1% formic acid/(acetonitrile: isopropanol: ultrapure water = 1 : 1 : 1)
- Flow rate: 0.4 mL min^{-1}
- Gradient: liner gradient, solvent A: 80%–45% (5 min) –10% (7 min) –5% (7.1–9.75 min) –80% (10.5–12 min)
- Column: ACQUITY UPLC BEH C18 (1.7 μm, 2.1 × 100 mm; Waters)
- Column temperature: 40°C
- Sample temperature: 5°C
- Injection vol: 5 μL

ESI/MS/MS: Waters ACQUITY TQ detector
- Ionization: ESI positive
- Source temperature: 150°C
- Desolvation temperature: 400°C
- Cone gas flow: 50 L/h
- Desolvation gas flow: 800 L/h
- Capillary voltage: 3 kV
- Detection: MRM
  - Quantification: 722.4→334.4 (Cone: 52 V, Collision: 40 eV)
  - Identification: 722.4→352.4 (Cone: 52 V, Collision: 35 eV)

$$IDL = t_{n-1,0.05} \times \sigma_{n-1,I} \times 2$$
$$IQL = 10 \times \sigma_{n-1,I}$$

Where $t_{n-1,0.05}$ is the t-value (one side) with a risk factor of 5% and a degree of freedom of $n-1$, and $\sigma_{n-1,I}$ is the standard deviation obtained when measuring eight times of the 2-ng mL^{-1} standard solution used to construct the calibration curve.

The method detection limit (MDL) and method quantification limit (MQL) were calculated by multiplying IDL and IQL by the parameters at the time of pretreatment and the instrumental measurements, respectively. The procedural blank was performed without a sample.

Matrix effects (MEs) were estimated as follows. The final extract of each control animal sample (180 μL) was mixed with an FB_1 standard solution (20 μL, 1.0 μg mL^{-1}) and quantified (FB_1 concentration of the final solution: 100 ng mL^{-1}). Two sample sets were prepared for each tissue and body fluid. MEs were calculated as follows:

$$ME\ (\%) = [(A_m - A_b) - A_s] / A_s \times 100$$

where A_m is the FB_1 peak area of the ME confirmation sample, A_b is the peak area of the actual sample used for the ME confirmation, and A_s is the peak area of the standard solution (100 ng mL^{-1}).

The FB_1 recovery test was carried out as follows. Each control group tissue or body fluid (1 g) was placed in a 50-mL PP centrifuge tube and spiked with the FB_1 standard solution (50 μL of 1.0 μg mL^{-1} or 100 μL of 5.0 μg mL^{-1}) to afford concentrations of 50 ng g^{-1} (low) and 500 ng g^{-1} (high). The mixtures were thoroughly vortexed and allowed to stand for 2 h before analysis. In addition, T–2 samples from the treated group were analyzed twice for repeatability of the results in actual samples.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Methanol was not used in the method developed in this study, and as much as possible, metal and glass apparatus were avoided during sample extractions. Lipids, phospholipids (PLs), fatty acids, etc., have large MEs in ESI/MS/MS analysis (Van Eeckhaut et al., 2009) and in some cases adsorb into the apparatus. In fact, De Baere et al. (2018) reported that analytical problems can be attributed to the presence of high amounts of PLs in the deproteinized sample. Consequently, purification by Captiva EMR-Lipid column was conducted, as it has high removal efficiency for lipids including PLs and a stable recovery rate for compounds that easily form coordinate bonds. The Captiva EMR-Lipid column also has a maximum efficacy at an acetonitrile : water ratio of 8 : 2 (v : v). Therefore, extraction/protein precipitation was conducted in two steps using acetonitrile: water (2 : 1 v/v) and acetonitrile, and the extract was applied directly to the column. The extract was also acidified with formic acid as acidification optimizes the extraction of fumonisins (Sulyok et al., 2006; Mol et al., 2008). As mentioned above, this method is relatively easy as it does not require the extract to be redissolved in different solvents prior to the SPE clean-up.

QUALITY ASSURANCE AND QUALITY CONTROL

Despite the likelihood of carryover when FB_1 is injected into the LC-ESI/MS/MS system at a high concentration (Tamura et al., 2011), no carryover was observed when the 500-ng mL^{-1} FB_1 samples were analyzed (Fig. 1e). The relationship between the concentration of the injected standard

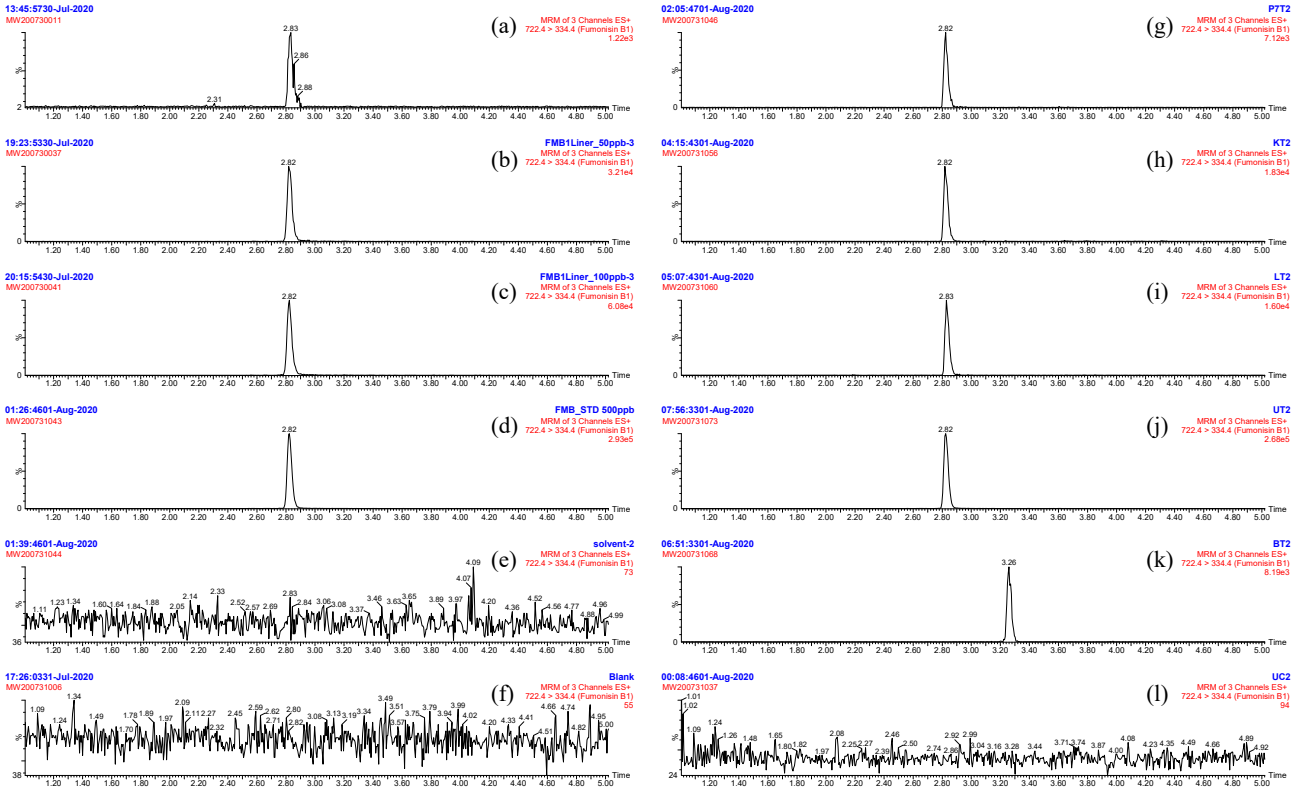

Fig. 1 Chromatograms of (a–d) FB_1 standard solutions at concentrations of 2, 50, 100, and 500 ng mL^{-1}, respectively; (e) solvent blank injected after standard solution of 500 ng mL^{-1}; (f) procedure blank; (g–k) plasma, kidney, liver, urine, and bile of T–2; (l) urine of C–2.

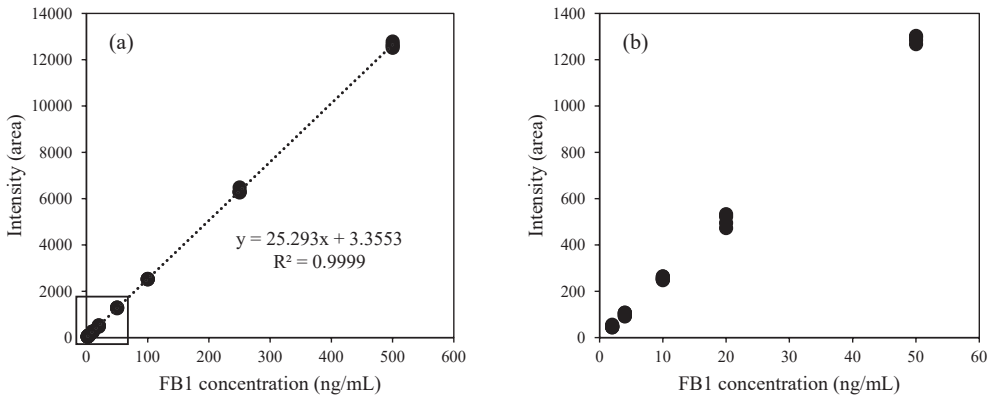

Fig. 2 Calibration curves and linearity of the FB_1 standard (precursor ion *m/z* 722.4, product ion *m/z* 334.4) determined for (a) the full range of examined FB_1 concentrations (2–500 ng mL^{-1}) and (b) FB_1 concentrations of 2–50 ng mL^{-1} [enlarged view of the square in (a)].

and the quantification ion sensitivity (peak area) exhibited good linearity in the concentration range of 2–500 ng mL^{-1} (Fig. 2). The variation (relative standard deviation, RSD) at each concentration was less than 10%, the intensity ratio of the quantification ions to the identification ions was 1.08 ± 0.0624 (RSD: 5.76%), and the retention time deviation was stable at < 0.02 min (Table 2). The daily linearity confirmation during the actual sample analysis had an error within the acceptable limit (< 20%) set by the MoE (2016), and the ion ratios were equal (Table 2). The retention time deviation was close to that of the calibration curve.

The IDL and IQL are shown in Table 2. The related MDL and MQL were determined as 1 and 3 ng g^{-1}, respectively. In addition, during the actual sample analysis, the signal-to-noise ratio (s/n) near the FB_1 peak was estimated. However, the concentration calculated from the three times s/n was lower than the MDL. The MQL in this method is comparable to those reported previously (Meyer et al., 2003; Fodor et al., 2008; Gazzotti et al., 2011; Devreese et al., 2012; De Baere et al., 2018), and no FB_1 peak was detected in the procedural blank (Fig. 1f).

The results of the MEs, spiked recovery test, and repeatability test are shown in Table 3. Of the MEs, except for the bile samples, only a single urine sample showed some en-

Table 2 RETENTION TIME, INTENSITY RATIO, AND VARIATION OF EACH POINT ON THE CALIBRATION CURVE AND THEIR DAILY VALIDATIONS[a]

STD conc. (ng mL^{-1})	2	4	10	20	50	100	250	500
Calibration curve								
RT (min)[b]	2.83	2.83	2.83	2.82	2.82	2.82	2.82	2.82
Intensity ratio[c]	1.09 ± 0.0730	1.12 ± 0.0847	1.06 ± 0.0715	1.07 ± 0.0532	1.06 ± 0.0284	1.07 ± 0.0142	1.07 ± 0.0163	1.08 ± 0.0137
RSD (%)[d]	8.12	6.49	2.43	5.34	1.23	0.404	1.57	0.903
IDL (ng mL^{-1})	0.6							
IQL (ng mL^{-1})	2							
Daily validation of pre-sample injection								
RT (min)[b]	—	—	—	2.82	2.82	2.82	—	2.82
Intensity ratio[e]	—	—	—	1.05	1.09	1.06	—	1.05
Variation (%)[f]	—	—	—	−11.3	−3.15	−8.75	—	−8.41
Variation (%)[g]	—	—	—	3.92	14.2	11.6	—	7.27
Daily validation of post-sample injection								
RT (min)[b]	—	—	—	2.82	2.82	2.82	—	2.82
Intensity ratio[e]	—	—	—	1.08	1.07	1.05	—	1.06
Variation (%)[f]	—	—	—	−14.8	−16.0	−18.8	—	−14.8

[a]Calibration curve and daily validation: see text. [b]Retention time. [c]Intensity ratio of quantifier ion to qualifier ion (average ± standard deviation). [d]Relative standard deviation of area of quantifier ions. [e]Intensity ratio of quantifier ion to qualifier ion. [f]Variation (%) of concentrations calculated from peak area of daily validation and calibration curve to setting concentration. [g]Variation (%) of peak area of daily validation for pre- and post-sample injection.

Table 3 MATRIX EFFECTS, RECOVERY RATES, AND REPEATABILITY OF FB_1 IN PORCINE TISSUES AND BODY FLUIDS

Sample type	Matrix effect[a]	Recovery (50 ng g^{-1})[b]	Recovery (500 ng g^{-1})[b]	Repeatability in the actual sample[c]
	%	%	%	%
Plasma	2.58, 7.38	91.1 ± 2.32 (2.54)	89.0 ± 1.60 (1.80)	10.3
Kidney	10.8, 11.2	84.1 ± 2.69 (3.19)	93.5 ± 1.40 (1.50)	8.38
Liver	15.0, 19.0	87.2 ± 3.14 (3.60)	93.5 ± 2.09 (2.23)	4.88
Urine	5.84, 34.0	112 ± 4.16 (3.73)	99.3 ± 5.83 (5.88)	3.68
Bile	−90.6, −87.3	9.44 ± 0.23 (2.38)	10.4 ± 0.35 (3.37)	8.38

[a]Data indicates matrix effects of two samples. [b]Data indicates the average ± standard deviation (relative standard deviation) of triplicate analysis. [c]Variation of duplicate analytical results.

hancement of ionization, with the error otherwise not exceeding 20%. The recovery of this analytical method determined by the spiked recovery test equaled 84.1%–112% (RSD: < 10%), except for the bile samples. When conducting pharmacokinetic (distribution) studies of FB_1, an accurate method is required to analyze FB_1 residues in various tissues and body fluids. However, previous studies reported accurate analytical methods only for a limited number of sample types (Gazzotti et al., 2011; Devreese et al., 2012; Flores-Flores and González-Peñas et al., 2017; Souto et al., 2017; De Baere et al., 2018). In previous papers, methods were introduced to analyze FB_1 in various tissues and body fluids; however, the recovery rate was either low overall or the accuracy was poor in specific tissues (Meyer et al., 2003; Fodor et al., 2008). Although further analysis of unexamined tissues is required, our analytical method, which showed good results for the plasma, urine, liver, and kidney, is likely to be useful for studying the distribution of FB_1 in animals.

An ionization suppression of ~90% was detected for bile samples, and a corresponding recovery rate of 10% was obtained in the spike recovery test (Table 3). However, there was no clear correlation between recovery and spiking level, and the variation (RSD) was constant at < 10%. In both tests, the retention time for the FB_1 during bile sample analysis was stable at ~3.25 min, i.e., it shifted compared to that observed for the standard solutions and other sample types (Fig. 1). These

results suggested that the poor recovery (~10%) of the bile samples was caused by the ME-induced suppression of ionization. As the quantification limit increases in conditions with strong ionization suppression, the corresponding matrix should be removed via a suitable pretreatment. However, as the retention time, recovery, and degree of ion suppression were stable, we concluded that FB_1 in bile could be analyzed. with a certain accuracy Based on the above results, the FB_1 concentration in the bile was calculated by multiplying the measured value by 10 in this study, as it is a common way to estimate concentrations at low and constant recovery.

Duplicate analysis of the T–2 samples (plasma: only day-7 after administration) indicated that these variations were small in all sample types examined (Table 3). This indicates a high level of repeatability in the actual sample analysis.

When actual samples were analyzed, the intensity ratio of the quantification ion to the identification ion for plasma day-7 of T–1 (ion ratio: 1.30) exceeded that of the standard solution (ion ratio: 1.08) by 20.8%. Except for this sample, the error was < 20% for all samples (ion ratio: 1.06 ± 0.0727, range: 0.875–1.20).

DISTRIBUTION OF FB_1 IN PIGS

After seven days of administration, the osmotic pumps were removed and confirmed to contain no residual FB_1 solution. This affirmed that the total FB_1 exposure volume was 20 mg head^{-1} week^{-1}. The FB_1 concentrations in the tissues and body fluids of the exposed and control animals, showed that it was not detected in any of the control animals (MDL: < 10 for bile samples, < 1 for other samples; Table 4). In general, for all individuals of the exposure group, the levels of FB_1 were the highest in the urine at concentrations 2–20 times higher than those in the kidneys and liver (Table 4, Fig. 1). In contrast, FB_1 in the bile was detected for T–2 and T–3, and the observed levels exceeded those in the other organs and plasma. In previous studies, Prelusky et al. (1994) reported that the FB_1 levels in pig plasma were significantly decreased during the first 60 min after a single intravenous injection, which was ascribed to its rapid distribution/excretion. They also noted that a single intravenous injection of ^{14}C-FB_1 into a biliary cannulated pig resulted in rapid excretion from the urine and bile, while the bile excretion continued to be gradually eliminated for 24–36 h after administration. In addition, Souto et al. (2017) found a significant positive correlation between the daily exposure to FB_1 and plasma concentrations in pigs fed an FB_1 mixed diet. The high concentrations in the urine and bile in this study also suggested that FB_1 was excreted at a high rate.

FB_1 levels in the kidney and liver of group T exceeded those in the plasma, in line with previous reports in which the kidneys and liver were shown to be the major organs of residual FB_1 accumulation in pigs (Prelusky et al., 1994; Meyer et al., 2003; Fodor et al., 2006, 2008).

The FB_1 concentrations in the analyzed samples varied greatly among the individuals. For instance, T–2 had the highest FB_1 accumulation, followed by T–1 and T–3. It was noted that pigs might have large individual variations for the elimination of certain chemicals because of the half-lives which may reflect inherent variabilities in pig metabolism (Guruge et al., 2016). Interestingly, individual differences in residual concentrations of FB_1 differed in the tissue and body fluids. The maximum and minimum concentration differences for the FB_1 in the kidney (ratio: 4.0) and liver (ratio: 2.8) were smaller than those in the plasma (ratio: 8–> 15), bile (ratio: > 18), and urine (ratio: 32). This might reflect the differences in the residual patterns of the FB_1 in each tissue and body fluid. Specifically, individual differences were low in the kidney and liver, which are the main FB_1 residual organs. On the contrary, since FB_1 has a short half-life in the body, the residues in the plasma, urine, and bile may reflect individual differences in distribution and excretion rates.

In conclusion, the analytical method developed to determine FB_1 in biological samples is expected to improve our understanding and evaluation of the risks posed by this toxin in animals and humans.

ACKNOWLEDGMENTS

The authors thank Dr. N. Oyama-Okubo (IVFS-NARO) who supported during the LC-ESI/MS/MS management, Mr. M. Shoji and Ms. C. Yamamoto for animal caring, Dr. H. Wasada (Kumamoto Prefectural Central Livestock Hygiene Service Center, Japan), Dr. A. Sakuma (Miyagi Prefectural Sendai Livestock Hygiene Service Center, Japan), and Dr. R. Uegaki (NIAH-NARO) for their support during the experiments.

Table 4 FB_1 CONCENTRATIONS (ng g^{-1} wet wt) IN PORCINE TISSUES AND BODY FLUIDS

Group		Control group			Exposure group		
Pig ID		C–1	C–2	C–3	T–1	T–2	T–3
Plasma	1 day	< 1[a]	< 1[a]	< 1[a]	9.0	7.7	< 1[a]
	3 days	—[b]	—[b]	—[b]	4.1	17	2[c]
	7 days	—[b]	—[b]	—[b]	4.9	15	< 1[a]
Kidney		< 1[a]	< 1[a]	< 1[a]	14	35	8.7
Liver		< 1[a]	< 1[a]	< 1[a]	18	31	11
Urine		< 1[a]	< 1[a]	< 1[a]	380	670	21
Bile		< 10[a]	< 10[a]	< 10[a]	< 10	180[d]	12[cd]

[a]concentration lower than MDL. [b]not analyzed. [c]concentration lower than MQL. [d]analytical concentrations × 10 (see text).

REFERENCES

De Baere, S., Croubels, S., Novak, B., Bichl, G., Antonissen, G., 2018. Development and validation of a UPLC-MS/MS and UPLC-HR-MS method for the determination of fumonisin B1 and its hydrolysed metabolites and fumonisin B2 in broiler chicken plasma. *Toxins* 10, 62. doi: 10.3390/toxins10020062.

Devreese, M., De Baere, S., De Backer, P., Croubels, S., 2012. Quantitative determination of several toxicological important mycotoxins in pig plasma using multi-mycotoxin and analyte-specific high performance liquid chromatography-tandem mass spectrometric methods. *J. Chromatogr. A* 1257, 74–80. doi: 10.1016/j.chroma.2012.08.008.

Dilkin, P., Zorzete, P., Mallmann, C.A., Gomes, J.D., Utiyama, C.E., Oetting, L.L., Corrêa, B., 2003. Toxicological effects of chronic low doses of aflatoxin B1 and fumonisin B1-containing Fusarium moniliforme culture material in weaned piglets. *Food Chem. Toxicol.* 41, 1345–1353. doi: 10.1016/s0278-6915(03)00137-6.

Van Eeckhaut, A., Lanckmans, K., Sarre, S., Smolders, I., Michotte, Y., 2009. Validation of bioanalytical LC-MS/MS assays: evaluation of matrix effects. *J. Chromatogr. B* 877, 2198–2207. doi: 10.1016/j.jchromb.2009.01.003.

EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen, H.K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Nebbia, C.S., Petersen, A., Rose, M., Roudot, A.C., Schwerdtle, T., Vleminckx, C., Vollmer, G., et al., 2018. Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. *EFSA J.* 16, 5242. doi: 10.2903/j.efsa.2018.5242.

FAMIC (Food and Agricultural Materials Inspection Center, Japan), 2020. About Regulation Value of Dietary Harmful Materials. FAMIC, Saitama. http://www.famic.go.jp/ffis/feed/r_safety/r_feeds_safety22.html#mycotoxins (accessed 28 September 2020)

Flores-Flores, M.E., González-Peñas, E., 2017. An LC-MS/MS method for multi-mycotoxin quantification in cow milk. *Food Chem.* 218, 378-385. doi: 10.1016/j.foodchem.2016.09.101.

Fodor, J., Meyer, K., Riedlberger, M., Bauer, J., Horn, P., Kovacs, F., Kovacs, M., 2006. Distribution and elimination of fumonisin analogues in weaned piglets after oral administration of Fusarium verticillioides fungal culture. *Food Addit. Contam.* 23, 492–501. doi: 10.1080/02652030500544964.

Fodor, J., Balogh, K., Weber, M., Miklós, M., Kametler, L., Pósa, R., Mamet, R., Bauer, J., Horn, P., Kovács, F., Kovács, M., 2008. Absorption, distribution and elimination of fumonisin B1 metabolites in weaned piglets. *Food Addit. Contam.* 25, 88–96. doi: 10.1080/02652030701546180.

FSCJ (Food Safety Commission of Japan), 2018. Fumonisins (natural toxins and mycotoxins). *Food Saf.* 6, 160–161. doi: 10.14252/foodsafetyfscj.2018006s.

Gazzotti, T., Zironi, E., Lugoboni, B., Barbarossa, A., Piva, A., Pagliuca, G., 2011. Analysis of fumonisins B1, B2 and their hydrolysed metabolites in pig liver by LC-MS/MS. *Food Chem.* 125, 1379–1384. doi: 10.1016/j.foodchem.2010.10.009.

Guruge, K.S., Noguchi, M., Yoshioka, K., Yamazaki, E., Taniyasu, S., Yoshioka, M., Yamanaka, N., Ikezawa, M., Tanimura, N., Sato, M., Yamashita, N., Kawaguchi, H., 2016. Microminipigs as a new experimental animal model for toxicological studies: comparative pharmacokinetics of perfluoroalkyl acids. *J. Appl. Toxicol.* 36, 68–75. doi: 10.1002/jat.3145.

Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M., 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* 75, 3019–3030. doi: 10.1021/ac020361s.

Meyer, K., Mohr, K., Bauer, J., Horn, P., Kovács, M., 2003. Residue formation of fumonisin B1 in porcine tissues. *Food Addit. Contam.* 20, 639–647. doi: 10.1080/0265203031000119043.

MoE (Ministry of the Environment, Japan), 2016. Guidebook for the Environmental Monitoring of Chemicals (ver. FY2015). MoE, Tokyo. https://www.env.go.jp/chemi/kurohon/tebiki/mat01.pdf (accessed 23 November 2020) (in Japanese)

Mol, H.G., Plaza-Bolaños, P., Zomer, P., de Rijk, T.C., Stolker, A.A., Mulder, P.P., 2008. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. *Anal. Chem.* 80, 9450–9459. doi: 10.1021/ac801557f.

Norred, W.P., Plattner, R.D., Chamberlain, W.J., 1993. Distribution and excretion of [^{14}C] fumonisin B1 in male Sprague-Dawley rats. *Nat. Toxins* 1, 341–346. doi: 10.1002/nt.2620010604.

Prelusky, D.B., Trenholm, H.L., Savard, M.E., 1994. Pharmacokinetic fate of ^{14}C-labelled fumonisin B1 in swine. *Nat. Toxins* 2, 73–80. doi: 10.1002/nt.2620020205.

Riley, R.T., Voss, K.A., 2006. Differential sensitivity of rat kidney and liver to fumonisin toxicity: organ-specific differences in toxin accumulation and sphingoid base metabolism. *Toxicol. Sci.* 92, 335–345. doi: 10.1093/toxsci/kfj198.

Souto, P.C.M.C., Jager, A.V., Tonin, F.G., Petta, T., Di Gregório, M.C., Cossalter, A.M., Pinton, P., Oswald, I.P., Rottinghaus, G.E., Oliveira, C., 2017. Determination of fumonisin B1 levels in body fluids and hair from piglets fed fumonisin B1-contaminated diets. *Food Chem. Toxicol.* 108, 1–9. doi: 10.1016/j.fct.2017.07.036.

Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R., 2006. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* 20, 2649–2659. doi: 10.1002/rcm.2640.

Tamura, M., Uyama, A., Mochizuki, N., 2011. Development of a multi-mycotoxin analysis in beer-based drinks by a modified QuEChERS method and ultra-high-performance liquid chromatography coupled with tandem mass spectrometry. *Anal. Sci.* 27, 629–635. doi: 10.2116/analsci.27.629.

Uegaki, R., Tohno, M., Yamamura, K., Tsukiboshi, T., Uozumi, S., 2015. Natural occurrence of mycotoxins in forage maize during crop growth in Japan: case study. *Mycotoxin Res.* 31, 51–56. doi: 10.1007/s12550-014-0210-6.

Uegaki, R., Tsunoda, A., 2018. Mycotoxin contamination in Japanese domestic feed. *Food Saf.* 6, 96–100. doi: 10.14252/foodsafetyfscj.2017030.

WHO (World Health Organization), 2000. Fumonisin B1. Environmental Health Criteria 219. WHO, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc219.htm (accessed 27 January 2021)