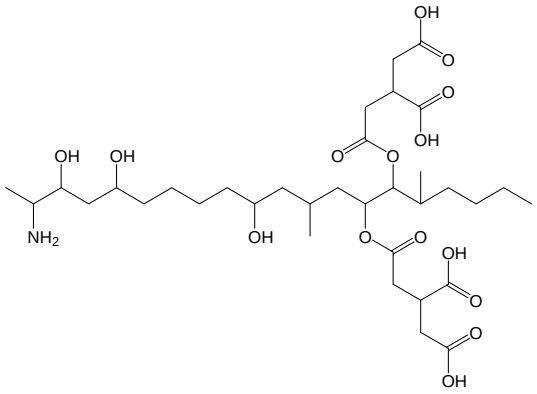
Fumonisin

Fumonisin B_1

Fumonisin B_2

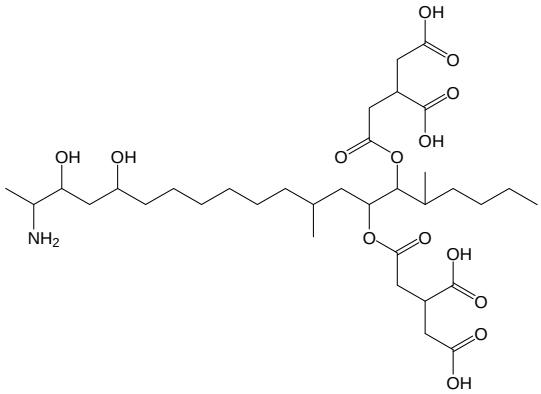
Fumonisin B_3

Fumonisin B_1



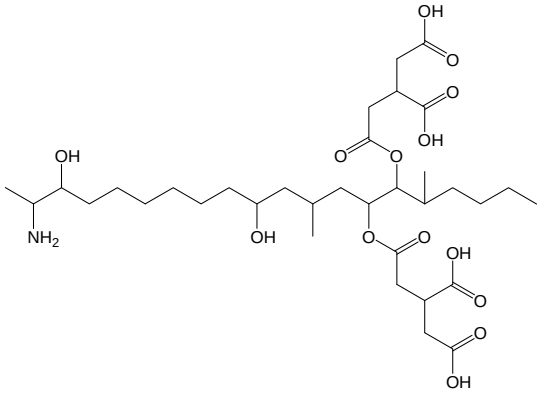
$C_{34}H_{59}NO_{15}$ MW: 721.8
CAS No.: 116355-83-0

Fumonisin B_2



$C_{34}H_{59}NO_{14}$ MW: 705.8
CAS No.: 116355-84-1

Fumonisin B_3



$C_{34}H_{59}NO_{14}$ MW: 705.8
CAS No.: 136379-59-4

[Summary of fumonisin]

Fumonisins are mycotoxins that was discovered in 1988 and that are produced by molds of genus *Fusarium*, and is the pathogen of equine liquefactive necrosis in white matter and porcine pulmonary edema. Its relationship with human esophageal cancer has also been suggested.

Fumonisin B_1 and B_2 were first discovered and their structures were determined. Moreover, 4 types of fumonisins (B_3, B_4, A_1 and A_2) have been separated and their structures determined. Among these, natural contamination is often found for fumonisins B_1, B_2 and B_3. As for fumonisin B_1 in surveillance conducted by FAMIC, it was detected in 96% (0.09-6.8 mg/kg) of corn in 2007 and 100% (0.09-1.8 mg/kg) of corn in 2008.

As for fumonisins in feeds, there has also been a report of contamination at a high concentration. Monitoring of the contamination status is an urgent task to secure the safety of feeds. In addition, the standard value for food (corn and corn products) in the United States is 2-4 mg/kg (content of fumonisin B_1, B_2 and B_3), and the standard value in EU for unprocessed corn is 4 mg/kg (content of B_1 and B_2).

[Methods listed in the Feed Analysis Standards]

1 Simultaneous analysis of fumonisin by liquid chromatography/ mass spectrometry [Feed Analysis Standards, Chapter 5, Section 1 15.1, 16.1 and 17.1]

Analyte compounds: Fumonisin B_1, B_2 and B_3 (3 components)

Scope of application: Feeds

A. Reagent preparation

Fumonisin mixture standard solution. Weigh accurately 10 mg each of fumonisin B_1 [$C_{34}H_{59}NO_{15}$], fumonisin B_2 [$C_{34}H_{59}NO_{14}$] and fumonisin B_3 [$C_{34}H_{59}NO_{14}$], put each of them in a 50- mL volumetric flask, respectively, dissolve by the addition of acetonitrile- water (1:1), and further add the same solvent to each volumetric flask up to the graduation line to prepare the fumonisin B_1, B_2 and B_3 standard stock solutions (1 mL each of these solutions contains 0.2 mg as fumonisin B_1, B_2 and B_3, respectively.).

Before use, mix a certain amount of fumonisin B_1, B_2 and B_3 standard stock solutions, dilute accurately with acetonitrile- water (1:1), to prepare several fumonisin mixture standard solutions that contain 1-1,000 ng as fumonisin B_1, B_2 and B_3, respectively, in 1 mL.

B. Quantification

Extraction. Weigh 20.0 g of an analysis sample, transfer it to a 200- mL stoppered Erlenmeyer flask, add 100 mL of methanol- water (3:1), and extract by shaking for 15 minutes. Transfer the extract to a stoppered centrifuge tube, centrifuge at 1,500×*g* for 5 minutes, to obtain supernatant to be a sample solution to be subjected to column treatment.

Column treatment. Wash a trimethylaminopropylsilyl silica gel minicolumn (500 mg) [Note 1] sequentially with 8 mL of methanol and 8 mL of methanol- water (3:1).

Load accurately 10 mL of the sample solution on the minicolumn, elute until the liquid level reaches the upper end of packing, [1] then add 8 mL of methanol- water (3:1) and 8 mL of methanol sequentially to the cartlidge and elute similarly.

Place a 50- mL recovery flask under the minicolumn. Add 14 mL of methanol- acetic acid (99:1) to the minicolumn to elute fumonisin B_1, B_2 and B_3. Concentrate the eluate under vacuum in a water bath at 40°C or less to be almost dried up, and then dry up by nitrogen gas flow. Dissolve the residue by the addition of accurately 1 mL of acetonitrile- water (1:1), centrifuge at 5,000×*g* for 5 minutes, to obtain supernatant to be a sample solution to be subjected to measurement by liquid chromatography- mass spectrometry.

Measurement by liquid chromatography- mass spectrometry. Inject 5 μL each of the sample solution and respective mixture standard solutions to a liquid chromatograph- mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

Column: Octadecylsilyl silica gel column (2.1 mm in inner diameter, 150 mm in length, particle size 5 μm) [Note 2]

Eluent: 0.1 % formic acid solution- acetonitrile (3:1) → 5 min → (1:1) (retained 3 min) →2 min→ (3:1)

Flow rate: 0.2 mL/min
Column oven temperature: 40°C
Detector: Quadrupole mass spectrometer Note 3
Ionization method: Electrospray ionization (ESI) (positive ion mode)
Fragmentor voltage: 220 V
Nebulizer pressure: N_2 (340 kPa)
Dryer gas: N_2 (10 L/min, 350 °C)
Capillary voltage: 3,000 V
Monitor ion: *m/z* 722 (fumonisin B_1), 706 (fumonisin B_2 and B_3)

Calculation. Obtain peak heights or peak areas from the resulting selected ion monitoring chromatograms [2] to prepare a calibration curve, and calculate the amounts of fumonisin B_1, B_2 and B_3 in the sample.

Note 1 Bond Elut LRC SAX (Varian) or equivalents.
2 ZORBAX Eclipse XDB-C18 (Agilent Technologies) or equivalents.
3 Example conditions for Agilent 1100 MSD SL (Agilent Technologies)

<<Summary of analysis method>>
This is a simultaneous analysis method to extract fumonisin B_1, B_2 and B_3 in feeds with methanol - water (3:1), purify with a strongly basic anion exchange minicolumn, and quantitate by a liquid chromatograph- mass spectrometer.

Quantitation of fumonisin by liquid chromatography usually requires derivatization; however, this method does not need derivatization because a mass spectrometer is used as a detector.

The flow sheet of the analysis method is shown in Figure 5.3.7-1.

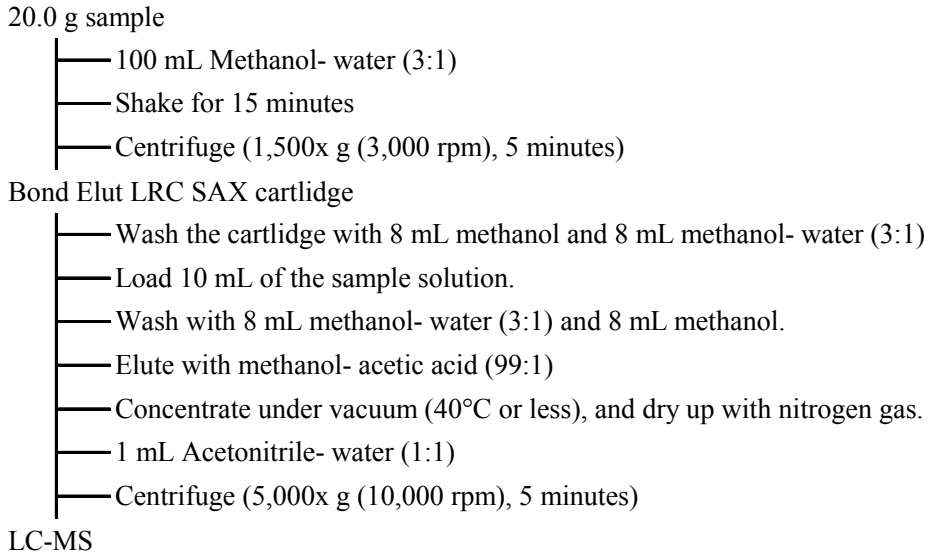


Figure 5.3.7-1 Flow sheet of the simultaneous analysis method for fumonisin by liquid chromatography- mass spectrometry

References:Koji Aoyama and Eiichi Ishiguro: Research Report of Animal Feed, **31**, 118 (2006)
History in the Feed Analysis Standards [28] New

<<Analysis method validation>>

- Spike recovery and repeatability

Name of spiked component	Sample type	Spike concentration (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Fumonisin B_1	Formula feed for adult chickens	300~3,000	3	72.9~102.9	5.2
	Formula feed for calves during lactation	300~3,000	3	80.6~106.3	11.3
	Corn	300~3,000	3	78.6~89.7	10.2
	Barley	300~3,000	3	70.5~74.4	2.3
Fumonisin B_2	Formula feed for adult chickens	150~1,500	3	70.6~92.8	3.0
	Formula feed for calves during lactation	150~1,500	3	75.6~108.7	9.1
	Corn	150~1,500	3	76.9~88.3	4.9
	Barley	150~1,500	3	64.9~71.1	9.1
Fumonisin B_3	Formula feed for adult chickens	60~600	3	70.0~90.7	5.3
	Formula feed for calves during lactation	60~600	3	78.2~110.0	10.3
	Corn	60~600	3	75.0~75.6	5.2
	Barley	60~600	3	70.1~72.1	3.7

- Collaborative study

Name of analyzed component	Sample type	Number of laboratories	Spike concentration (μg/kg)	Spike recovery (%) (measured value (μg/kg))	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
Fumonisin B_1	Chicken formula feed	11	1,000	80.5	4.3	13.6	0.85
	Chicken formula feed	11	Natural contamination	(218)	9.9	19.2	0.95
Fumonisin B_2	Chicken formula feed	11	400	72.0	6.2	10.0	0.54
	Chicken formula feed	11	Natural contamination	(59)	9.7	15.7	0.71
Fumonisin B_3	Chicken formula feed	11	150	74.8	7.0	11.6	0.55
	Chicken formula feed	11	Natural contamination	(24)	11.3	19.4	0.88

- Lower limit of quantification: 2 μg/kg for respective mycotoxins (*SN* ratio: 10)
- Lower limit of detection: 0.6 μg/kg for respective mycotoxins (*SN* ratio: 3)

<<Notes and precautions>>

[1] Elute at around 1 drop/s using a suction manifold or by pressurized flow; be careful to avoid drying packing.

[2] Examples of selected ion monitoring (SIM) chromatograms are shown in Figure 5.3.7-2.

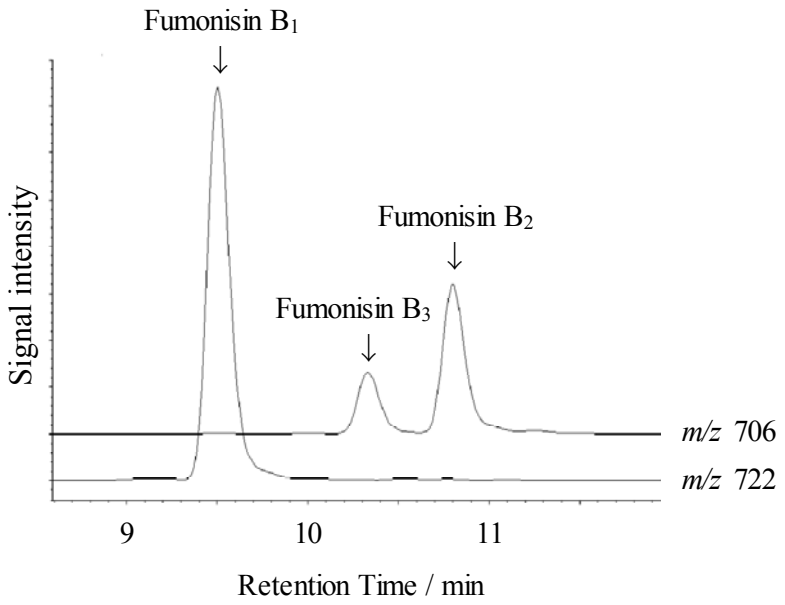


Figure 5.3.7-2 SIM chromatogram of a naturally contaminated formula feed.
Measurement conditions are according to the Example of measurement conditions.
The column used is Agilent Technologies ZORBAX Eclipse XDB-C18.

2 Simultaneous analysis of fumonisin B_1 and B_2 by liquid chromatography [Feed Analysis Standards, Chapter 5, Section 1 15.2 and 16.2]

Analyte compounds: Fumonisin B_1 and B_2 (2 components)

Scope of application: Formula feeds and corn

A. Reagent preparation

Fumonisin mixture standard solution. Weigh accurately 10 mg each of fumonisin B_1 [$C_{34}H_{59}NO_{15}$][1] and fumonisin B_2 [$C_{34}H_{59}NO_{14}$], [1] put each of them in a 20- mL volumetric flask, respectively, dissolve by the addition of methanol, and further add the same solvent to each volumetric flask up to the graduation line to prepare the fumonisin B_1 and B_2 standard stock solutions (1 mL each of these solutions contains 0.5 mg as fumonisin B_1 and B_2, respectively.).

Before use, mix a certain amount of fumonisin B_1 and B_2 standard stock solutions, dilute accurately with methanol- water (3:1), to prepare several fumonisin mixture standard solutions that contain 1-8 μg as fumonisin B_1 and B_2, respectively, in 1 mL.

B. Quantification

Extraction. Weigh 20.0 g of an analysis sample, transfer it to a 200- mL stoppered Erlenmeyer flask, add 100 mL of methanol- water (3:1), and extract by shaking for 15 minutes. Filter the extract with filter paper (No. 5A), Note 1 to obtain filtrate to be a sample solution to be subjected to column treatment.

Column treatment. Wash a trimethylaminopropylsilyl silica gel minicolumn (500 mg) Note 2 sequentially with 8 mL of methanol and 8 mL of methanol- water (3:1).

Load accurately 10 mL of the sample solution on the minicolumn, elute until the liquid level reaches the upper end of packing, then add 8 mL of methanol- water (3:1) and 8 mL of methanol sequentially to the cartlidge and elute similarly.

Place a 50- mL recovery flask under the minicolumn. Add 14 mL of methanol- acetic acid (99:1) to the minicolumn to elute fumonisin B_1 and B_2. Concentrate the eluate under vacuum in a water bath at 40°C or less to be almost dried up, and then dry up by nitrogen gas flow. [2] Dissolve the residue by the addition of accurately 1 mL of methanol - water (3:1), filter with membrane filter (pore size 0.5 μm or less), to be a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 μL each of the sample solution and respective fumonisin mixture standard solutions to a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength, 340 nm; emission wavelength, 450 nm)

Column: Branched polyfluoroalkylsilyl silica gel column (4.6 mm in inner diameter, 30 mm in length, particle size 5 μm) Note 3[3]

Eluent: Methanol- anhydrous trifluoroacetic acid solution (0.1 v/v%) (1:1)

Reaction solution Note 4: Fluorescent labeling test solution. Dissolve 0.4 g of *o*-phthalaldehyde [4] and 0.5 g of *N*-acetyl-L-cysteine in 5 mL of methanol, and fill up to be 500 mL with a borate buffer (dissolve 24.7 g of boric acid and 12.3 g of sodium hydroxide in water to be 1 L, and adjust pH to 9.9-10.1 with a sodium hydroxide solution (30 w/v%).).

Flow rate: Eluent 1.0 mL/min; reaction solution 0.5 mL/min

Temperature: column oven 50 °C; reaction vessel 50 °C

Calculation. Obtain peak areas from the resulting chromatograms [5] to prepare a calibration

curve, and calculate the amounts of fumonisin B_1 and B_2 in the sample.

Note 1 If necessary, transfer the filtrate to a 50- mL stoppered centrifuge tube, centrifuge at 1,500×*g* for 5 minutes, to obtain supernatant to be a sample solution to be subjected to minicolumn chromatography.
2 Bond Elut LRC SAX (Varian) or equivalents.
3 Fluofix 120E (Wako Pure Chemicals) or equivalents.
4 The eluate from the column is fluorescent-labeled by the addition of the reaction solution in a reaction coil (0.5 mm in inner diameter, 2 m in length), and immediately sent to the fluorescence detector.

<<Summary of analysis method>>
This is a simultaneous analysis method to extract fumonisin B_1 and B_2 in feeds with methanol- water (3:1), purify with a strongly basic anion exchange minicolumn, and quantitate by post-column fluorescent derivatization and liquid chromatography. For the quantitation of fumonisin B_3, it is required to use 8. Simultaneous analysis of fumonisin by liquid chromatography/ mass spectrometry in this section.

The flow sheet of the analysis method is shown in Figure 5.3.8-1.

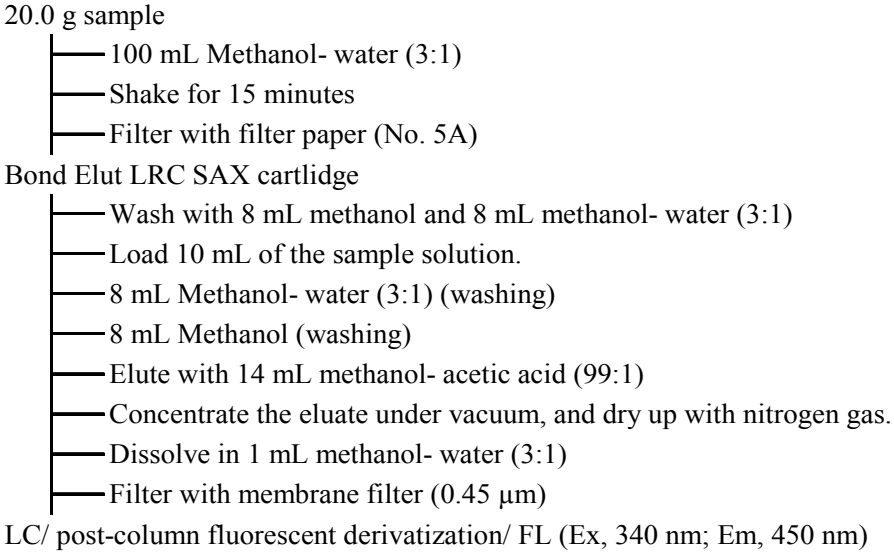


Figure 5.3.8-1 Flow sheet of the analysis method for fumonisin B_1 and B_2

References:Hiroshi Hibino, Yuji Shirai, and Eiichi Ishiguro: Research Report of Animal Feed, **24**, 27 (1999)
Hiroshi Hibino and Yukie Ishida: Research Report of Animal Feed, **22**, 1 (1997)
History in the Feed Analysis Standards [21] New

<<Analysis method validation>>
Spike recovery and repeatability

Name of spiked component	Sample type	Spike concentration (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Fumonisin B_1	Formula feed for adult chickens	500~2,000	3	86.7~93.0	3.7
	Formula feed for pork	500~2,000	3	90.3~97.3	3.9
	Formula feed for dairy	500~2,000	3	88.3~95.7	4.2
	Corn	1,000~4,000	3	94.7~99.3	9.0
Fumonisin B_2	Formula feed for adult chickens	500~2,000	3	81.7~94.0	6.6
	Formula feed for pork	500~2,000	3	87.7~92.3	2.9
	Formula feed for dairy	500~2,000	3	85.0~93.0	7.9
	Corn	1,000~4,000	3	88.7~93.0	7.5

Collaborative study

Name of analyzed component	Sample type	Number of laboratories	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
Fumonisin B_1	Formula feed for middle-aged chicks	6	1,000	85.0	4.8	2.7	0.17
Fumonisin B_2	Formula feed for middle-aged chicks	6	1,000	83.0	3.6	6.6	0.41

<<Notes and precautions>>

[1] Commercially available from Sigma-Aldrich, Kanto Chemical, Wako Pure Chemicals, etc.

[2] Evaporate completely until the odor of acetic acid disappears.

[3] The column to be used only needs to be one that uses packing treated by corresponding endcapping.

[4] A product of approximately 99% purity is commercially available from Sigma-Aldrich.

[5] An example of chromatographs is shown in Figure 5.3.8-2.

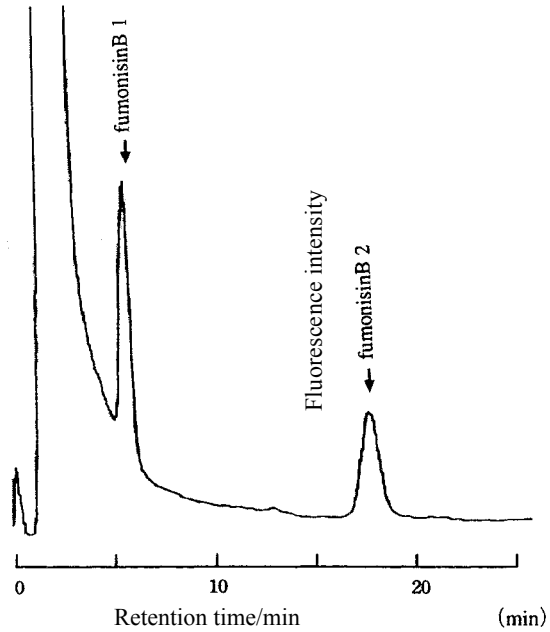


Figure 5.3.8-2 Chromatogram of a formula feed for adult chickens spiked with an amount equivalent to 1.0 mg/kg as fumonisin B_1 and B_2

Measurement conditions
Detector: Fluorescence detector (excitation wavelength, 340 nm; emission wavelength, 450 nm)
Column: Neos (distributed by Wako Pure Chemicals) Fluofix 120E (4.6 mm in inner diameter, 30 mm in length, particle size 5 μm)
Eluent: Methanol - anhydrous trifluoroacetic acid (0.1 v/v%) (1:1)
Reaction solution: Fluorescent labeling test solution. Dissolve 0.4 g of *o*-phthalaldehyde and 0.5 g of *N*-acetyl-L-cysteine in 5 mL of methanol, and fill up to be 500 mL with a borate buffer (dissolve 24.7 g of boric acid and 12.3 g of sodium hydroxide in water, and adjust pH to 9.9-10.1 with a sodium hydroxide solution (30 w/v%), and fill up to be 1 L.)
Flow rate: Eluent 1.0 mL/min; reaction solution 0.5 mL/min
Temperature: column oven 50 °C; reaction vessel 50 °C
Reaction coil: 0.5 mm in inner diameter, 2 m in length

[Other analysis methods]

3 ELISA

Products manufactured by Neogen (available from AR Brown, Kikkoman, etc.), R-Biopharm Rhône (available from AZmax, etc.), etc. are available in Japan from several distributors.

Table 5.1.15-1 shows major kits commercially available now in Japan, and their summaries.

Table 5.1.15-1 ELISA kits for fumonisin analysis commercially available in Japan

Item		Lower limit of detection	Analysis time	Shelf life of the kit	Applicable samples	Notes
Qualification kit	Agri-Screen® for Fumonisin	0.2 ppm	20 minutes	6 months	Corn, cottonseed, milo, peanut, rice, soybean, wheat etc.	Neogen, for total fumonisin
Quantitation kit	Veratox® for Fumonisin	0.2 ppm	20 minutes	6 months	Corn, rice, flour etc.	Neogen AOACI method (2001.06) USDA/GIPSA method (2001-102)
	RIDASCREEN® FAST Fumonisin	0.22 ppm	25 minutes	6-9 months	Grains, feeds	R-Biopharm Rhône FGIS, GIPSA approved
	AgraQuant® FUM	0.2 ppm	60 minutes	6-9 months	Grains	Romer Labs
	Max Signal™ Fumonisin	10 ppb	60 minutes	6-9 months	Grains, feeds, nuts and seeds, milk	BIOO Scientific
	Charm ROSA Fumonisin	0.05 ppm	60 minutes	6-9 months	Grains, feeds	Charm Sciences