3-Acetyldeoxynivalenol

15-Acetyldeoxynivalenol

[Methods listed in the Feed Analysis Standards]

1 Simultaneous analysis of trichothecene mycotoxin by gas chromatography [Feed Analysis Standards, Chapter 5, Section 1 11.1 and 12.1]

Analyte compounds: 3-Acetyldeoxynivalenol, 15-acetyldeoxynivalenol, deoxynivalenol, nivalenol and fusarenon-X (5 components)

Scope of application: Feeds

A. Reagent preparation

1) 3-Acetyldeoxynivalenol standard stock solution. Put 1 mg of 3-acetyldeoxynivalenol [$C_{17}H_{22}O_7$][1] in a 5- mL amber volumetric flask, dissolve by the addition of acetonitrile, and further add the same solvent up to the graduation line to prepare the 3-acetyldeoxynivalenol standard stock solution (1 mL of this solution contains 0.2 mg as 3-acetyldeoxynivalenol.).
2) 15-Acetyldeoxynivalenol standard stock solution. Put 1 mg of 15-acetyldeoxynivalenol [$C_{17}H_{22}O_7$][1] in a 5- mL amber volumetric flask, dissolve by the addition of acetonitrile, and further add the same solvent up to the graduation line to prepare the 15-acetyldeoxynivalenol standard stock solution (1 mL of this solution contains 0.2 mg as 15-acetyldeoxynivalenol.).
3) Deoxynivalenol standard stock solution. Put 1 mg of deoxynivalenol [$C_{15}H_{20}O_6$][1] in a 5- mL amber volumetric flask, dissolve by the addition of acetonitrile, and further add the same solvent up to the graduation line to prepare the deoxynivalenol standard stock solution (1 mL of this solution contains 0.2 mg as deoxynivalenol.).
4) Nivalenol standard stock solution. Put 1 mg of nivalenol [$C_{15}H_{20}O_7$][1] in a 5 mL amber volumetric flask, dissolve by the addition of acetonitrile, and further add the same solvent up to the graduation line to prepare the nivalenol standard stock solution (1 mL of this solution contains 0.2 mg as nivalenol.).

5) Fusarenon-X standard stock solution. Put 1 mg of fusarenon-X [$C_{17}H_{22}O_8$][1] in a 5 mL amber volumetric flask, dissolve by the addition of acetonitrile, and further add the same solvent up to the graduation line (1 mL of this solution contains 0.2 mg as fusarenon-X.).

6) Mixture standard stock solution. Mix a certain amount of each of the 3-acetyldeoxynivalenol standard stock solution, 15-acetyldeoxynivalenol standard stock solution, deoxynivalenol standard stock solution, nivalenol standard stock solution and fusarenon-X standard stock solution, and dilute accurately with acetonitrile to prepare mixture standard stock solution that contains 10 μg as each mycotoxin in 1 mL.

7) Derivatization reagent. Note 1 *N*-Trimethylsilylimidasol[2]- *N*,*O*-bis (trimethylsilyl) acetamide[2]- trimethylchlorosilane [2] (3:3:2) (prepare before use.)

B. Quantification

Extraction. Weigh 25.0 g of an analysis sample, transfer it to a 200- mL stoppered Erlenmeyer flask, add 100 mL of acetonitrile- water (21:4), and extract by shaking for 60 minutes. Note 2. Transfer the extract to a 10- mL centrifuge tube, centrifuge at 650×g for 5 minutes, to obtain supernatant to be a sample solution to be subjected to column treatment.

Column treatment. Transfer the sample solution to a multifunctional column (for trichothecene mycotoxins pretreatment), Note 3 and discard the first 3 mL of eluate. [3] Transfer accurately 2 mL of the following 3 mL of eluate [4] to a 50 - mL recovery flask to be a sample solution to be subjected to derivatization.

Derivatization. Concentrate the sample solution under vacuum in the water bath at 50°C or less to be almost dried up, and then dry up by nitrogen gas flow. [5] Add 0.1 mL of the derivatization reagent to the residue, seal the recovery flask that contained the sample solution, and leave at rest at room temperature for 15 minutes. Dissolve the residue by the addition of accurately 1 mL of 2,2,4-trimethylpentane, and further add 1 mL of water, and shake for 5 minutes. Transfer the whole amount of this solution to a 10- mL or smaller test tube, shake, and then leave at rest, to obtain the 2,2,4-trimethylpentane layer (upper layer) to be a sample solution to be subjected to gas chromatography.

Derivatization of standard stock solution. Transfer accurately 1 mL of the mycotoxin mixture standard stock solution to a 50- mL recovery flask, and dry up by nitrogen gas flow. Add 0.1 mL of the derivatization reagent to the residue, seal the recovery

flask, and leave at rest at room temperature for 15 minutes. Dissolve the residue by the addition of accurately 5 mL of 2,2,4-trimethylpentane, [Note 4] and further add 1 mL of water, and shake for 5 minutes. Transfer the whole amount of this solution to a 10 mL or smaller test tube, shake, and then leave at rest. Dilute the 2,2,4-trimethylpentane layer (upper layer) accurately with the same solvent to prepare several standard solutions that contain 0.01-1 μg respectively as respective mycotoxins in 1 mL to be subjected to gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution and respective standard solutions to a gas chromatograph, [Note 5] to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column [Note 6]:Fused silica capillary column (35 % diphenyl- 65 % dimethylpolisiloxane coating, 0.25 mm in inner diameter, 30 m in length, 0.25 μm in membrane thickness)

Carrier gas: He (1.5 mL/min)

Make-up gas: N_2 (40 mL/min)

Sample introduction: Splitless (60 s)

Injector temperature: 250 °C

Column oven temperature: 80 °C (retained 1 minute) → elevation by 20 °C/min → 180 °C → elevation by 5 °C/min → 300 °C (retained 10 minutes)

Detector temperature: 300 °C

Calculation. Obtain peak heights from the resulting chromatograms [6] to prepare a calibration curve, and calculate the amounts of mycotoxins in the sample.

Note 1 Use the reagent that can sufficiently derivatize mycotoxins to be quantitated.

2 In the case of samples like bran that tends to be pasty, weigh 25.0 g of a sample, transfer it to a 300- mL stoppered Erlenmeyer flask, add 150 mL of acetonitrile- water (21:4), and extract by shaking for 60 minutes.

3 Autoprep MF-T 1500 (Showa Denko), MultiSep 227 Trich+ (Romer Labs) or equivalents.

4 Use reagents for residual pesticide analysis or equivalents.

5 Use a insert treated with silane for the sample injector. Make sure that this insert does not affect the quantitation value.

6	Make sure that the peaks can be sufficiently separated from contaminant peaks.

<<Summary of analysis method>>

This is a simultaneous analysis method to extract trichothecene mycotoxins (Group B, 5 components) in feeds with acetonitrile- water (21:4), purify with a multifunctional cleanup (MFC) column, derivatize, and then quantitate by a gas chromatograph.

The flow sheet of the analysis method is shown in Figure 5.3.5-1.

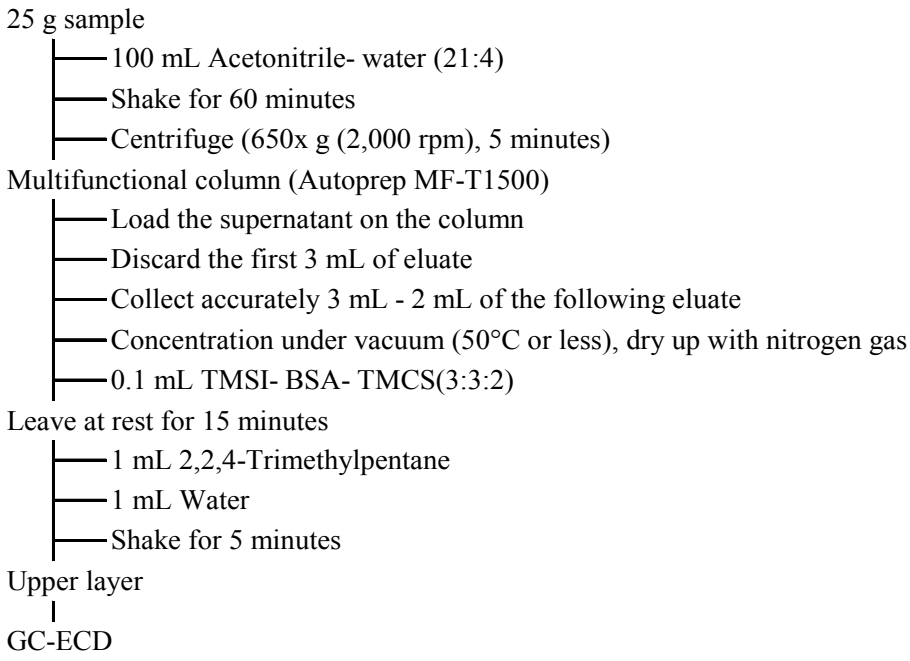

Figure 5.3.5-1 Flow sheet of the simultaneous analysis method for trichothecene mycotoxins (type B) in feeds

References: Yuji Shirai: Research Report of Animal Feed, **28**, 7 (2003)

History in the Feed Analysis Standards [26] 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)
Deoxynivalenol	Chicken formula feed	100~1,000	3	90.8~99.4	10.6
	Pig formula feed	100~1,000	3	93.2~96.8	11.4
	Milo	100~1,000	3	94.2~99.6	2.2
	Barley	100~1,000	3	92.8~98.7	3.6
Nivalenol	Chicken formula feed	100~1,000	3	95.3~105.2	4.0
	Pig formula feed	100~1,000	3	93.5~99.7	8.1
	Milo	100~1,000	3	96.1~96.3	0.7
	Barley	100~1,000	3	85.8~92.4	4.3
3-Acetyldeoxynivalenol	Chicken formula feed	100~1,000	3	95.0~96.5	4.2
	Pig formula feed	100~1,000	3	96.6~99.2	7.6
	Milo	100~1,000	3	93.2~95.7	6.0
	Barley	100~1,000	3	92.3~99.1	3.2
15-acetyldeoxynivalenol	Chicken formula feed	100~1,000	3	98.6~103.7	6.7
	Pig formula feed	100~1,000	3	97.9~98.3	6.2
	Milo	100~1,000	3	92.8~94.7	3.6
	Barley	100~1,000	3	94.2~97.1	3.2
Fusarenon-X	Chicken formula feed	100~1,000	3	94.6~98.1	4.5
	Pig formula feed	100~1,000	3	96.1~99.8	5.3
	Milo	100~1,000	3	92.6~97.0	2.4
	Barley	100~1,000	3	91.4~101.0	2.5

- 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
Deoxynivalenol	Milo	8	400	105.2	4.1	6.2	0.34
	Pig formula feed	8	Natural contamination	(503)	4.7	10.3	0.58
Nivalenol	Milo	8	400	95.4	4.5	6.1	0.33
	Pig formula feed	8	Natural contamination	(56.7)	8.4	14.7	0.67
3-Acetyldeoxynivalenol	Milo	8	400	107.3	5.9	6.6	0.36
15-acetyldeoxynivalenol	Milo	8	400	105.4	5.1	7.3	0.40
	Pig formula feed		Natural contamination	(89.3)	8.4	17.3	0.79
Fusarenon-X	Milo	8	400	106.1	5.4	6.1	0.33

- Lower limit of quantification: 10 μg/kg in a sample for each mycotoxin

<<Notes and precautions>>

[1] Standards are commercially available from Sigma-Aldrich, etc. Also, Mycotoxin Mixture 2 (B-trichothecene) (mixture solution of 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, deoxynivalenol and nivalenol) is commercially available from Kanto Chemical.

[2] Commercially available from GL Sciences, Tokyo Chemical Industry, and Sigma-Aldrich, etc.

[3] Recovery of nivalenol is low in the fraction of 0-3 mL eluate.

[4] Contaminants that interfere the quantitation of mycotoxins may be eluted in the fraction of eluate over 7 mL.

[5] If water remains, it turns cloudy by the addition of derivatization.

[6] An example of chromatograms is shown in Figure 5.3.5-2.

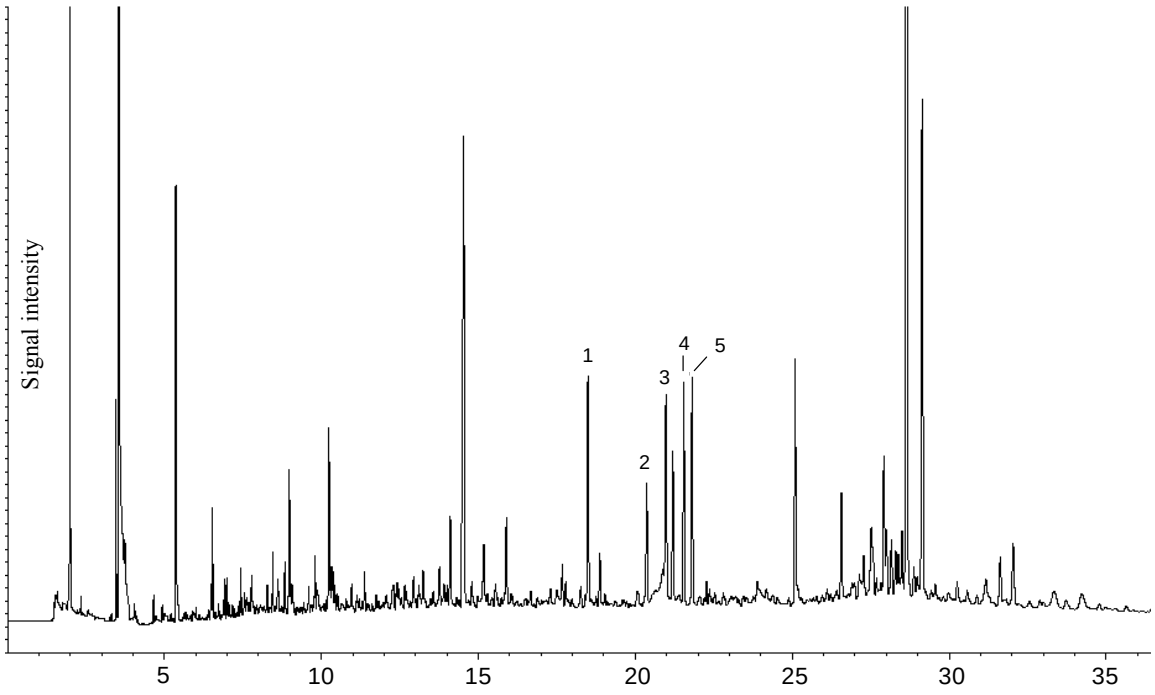

Figure 5.3.5-2 Chromatogram of a pig formula feed spiked with an amount equivalent to 100 μg/kg as respective mycotoxins

Peak name

1 Deoxynivalenol 4 3-Acetyldeoxynivalenol

2 Nivalenol 5 15-Acetyldeoxynivalenol

3 Fusarenon-X