HT-2 toxin

[Methods listed in the Feed Analysis Standards]

1 One-component analysis method by liquid chromatography/ mass spectrometry [Feed Analysis Standards, Chapter 5, Section 1 7.1]

A. Reagent preparation

HT-2 toxin standard solution. Weigh accurately 1 mg of HT-2 toxin [$C_{22}H_{32}O_8$], put in a 5-mL amber volumetric flask, dissolve by the addition of acetonitrile, and add the same solvent up to the graduation line (1 mL of this solution contains 0.2 mg as HT-2 toxin.). Moreover, dilute accurately a certain amount of this solution with acetonitrile to prepare the HT-2 toxin standard stock solution that contains 25 μg as HT-2 toxin in 1 mL.

Before use, dilute accurately a certain amount of the standard stock solution with water/ methanol/ acetonitrile (18:1:1) to prepare several HT-2 toxin standard solutions that contain 0.005-1 μg as HT-2 toxin in 1 mL.

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. Note1 Transfer the extract to a stoppered centrifuge tube, centrifuge at 1,000×g for 5 minutes, to obtain supernatant as a sample solution to be subjected to column treatment.

Column treatment. Load the sample solution on a multifunctional column (for trichothecene mycotoxins pretreatment), Note 2 and discard the first 3 mL of eluate. Place a 10-mL test tube under the column, and collect 3 mL of the following eluate.[1] Transfer accurately 2 mL of the eluate to a 50-mL recovery flask, concentrate under vacuum in the water bath at 50°C or less to be almost dried up, and further dry up by nitrogen gas flow.

Dissolve the residue by accurately adding 1 mL of water/ methanol/ acetonitrile (18:1:1). Transfer a part of this solution to a plastic centrifuge tube[2] (volume: 1.5 mL), centrifuge at 5,000×g for 5 minutes, to obtain supernatant as a sample solution to be subjected to measurement by a liquid chromatograph/ mass spectrometer.

Measurement by liquid chromatography/ mass spectrometry. Inject 5 μL each of the sample solution and respective HT-2 toxin standard solutions to a liquid chromatograph/ mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

(Liquid chromatography part)

Column: Octadecylsilyl silica gel column (3.0 mm in inner diameter, 250 mm in length, particle size 5 μm) Note 3 [3]

Eluent[4]: 10 mmol/L ammonium acetate solution -methanol (4:1) → 15 min → methanol (5 min retention)

Flow rate: 0.5 mL/min

column oven temperature: 40°C

(Mass spectrometry part, Example 1)

Detector: Quadrupole mass spectrometer Note 4

Ionization method: Atmospheric Pressure Photo ionization (APPI) (negative ion mode)

Fragmentor voltage: 100 V

Nebulizer pressure: N_2 (380 kPa)

Dryer gas: N_2 (7.0 L/min, 350°C)

Vaporizer temperature: 300°C

Capillary voltage: 1,500 V

Monitor ion [5]: *m/z* 483

(Mass spectrometry part, Example 2)

Detector: Quadrupole mass spectrometer Note 5

Ionization method: Atmosphere pressure chemical ionization (APCI) (negative ion mode)

Nebulizer gas: N_2 (2.5 L/min)

Interface temperature : 400°C

Heat block temperature : 200°C

C D L temperature : 200°C

Monitor ion[5]: *m/z* 483

Calculation. Obtain peak heights or areas from the resulting selected ion monitoring chromatograms[6] to prepare a calibration curve, and calculate the amount of HT-2 toxin in a sample.

Note 1 When the analysis sample absorbs the extraction solvent and cannot be

shaken, use a 300-mL stoppered Erlenmeyer flask and 150 mL of the extraction solvent.

2 MultiSep 227 Trich+ (Romer Labs) or equivalents

3 ZORBAX Eclipse XDB-C18 (Agilent Technologies; the retention time of HT-2 toxin is about 13 minutes under these measurement conditions) or equivalents

4 Example conditions for Agilent 1100 Series MSD SL (Agilent Technologies)

5 Example conditions for LCMS-2010EV (Shimadzu)

<<Summary of analysis method>>

In this method, HT-2 toxin in a feed is extracted with aqueous acetonitrile, purified with a multifunctional cleanup (MFC) column MultiSep 227 Trich+, and quantitated by a liquid chromatograph/ mass spectrometer. Procedures in this method are the same as Section 3 4 Simultaneous analysis of trichothecene mycotoxins by liquid chromatography/ mass spectrometry in this chapter, therefore it is possible to conduct continuous analysis by both methods using the same sample solution.

The flow sheet of the analysis method is shown in Figure 5.1.7-1.

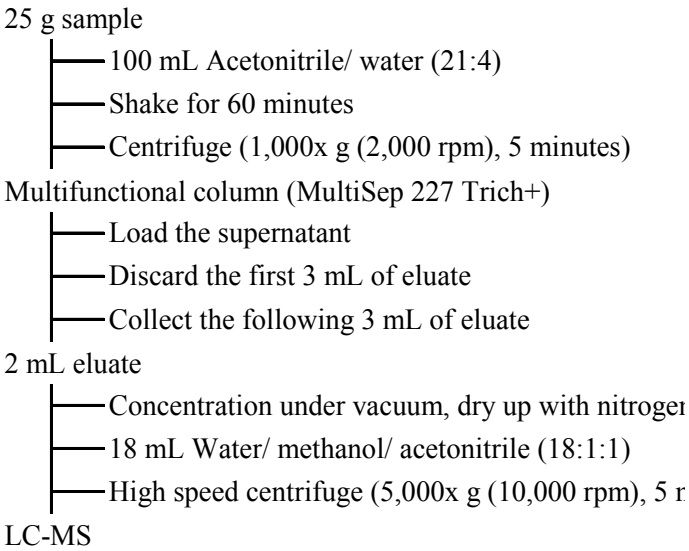

Figure 5.1.7-1 Flow sheet of the analysis method for HT-2 toxin

References: Takayuki Ishibashi, Masatoshi Yoshimura, and Daisaku Makino: Research Report of Animal Feed, 32, 13 (2007)

History in the Feed Analysis Standards [31] New

<<Analysis method validation>>

- Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Corn	15~200	3	101.2~110.7	6.9
Barley	15~200	3	100.6~107.3	7.3
Formula feed for adult chickens	15~200	3	98.0~112.7	8.7
Formula feed for beef cattle fattening	15~200	3	103.2~113.3	8.6

- Collaborative study

Sample type	Number of laboratories	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Intra-laboratory repeatability RSD_R (%)	HorRat
Chicken formula feed	7	200	106.2	2.9	8.5	0.42
Barley	7	200	103.6	2.6	9.6	0.47

- Lower limit of quantification: 15 μg/kg (Spike recovery and relative standard deviation)
- Lower limit of detection 5 μg/kg (*SN* ratio)

<<Notes and precautions>>

[1] HT-2 toxin recovery is low in the fraction of 0-1 mL eluate. The first 3 mL of eluate is discarded and the following 3 mL of eluate is collected here according to the procedures of the original analysis method (Section 3 4 in this chapter).

[2] Make sure that there is no background ion to interfere with the quantitation of HT-2 toxin.

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

[4] By linear gradient. The conditions are matched to those of the eluent in the original analysis method (Section 3 4 in this chapter).

[5] The acetate adduct ion of HT-2 toxin $[M+CH_3COO]^-$ is used as the monitor ion. The mass spectrum of HT-2 toxin is shown in Figure 5.1.7-2.

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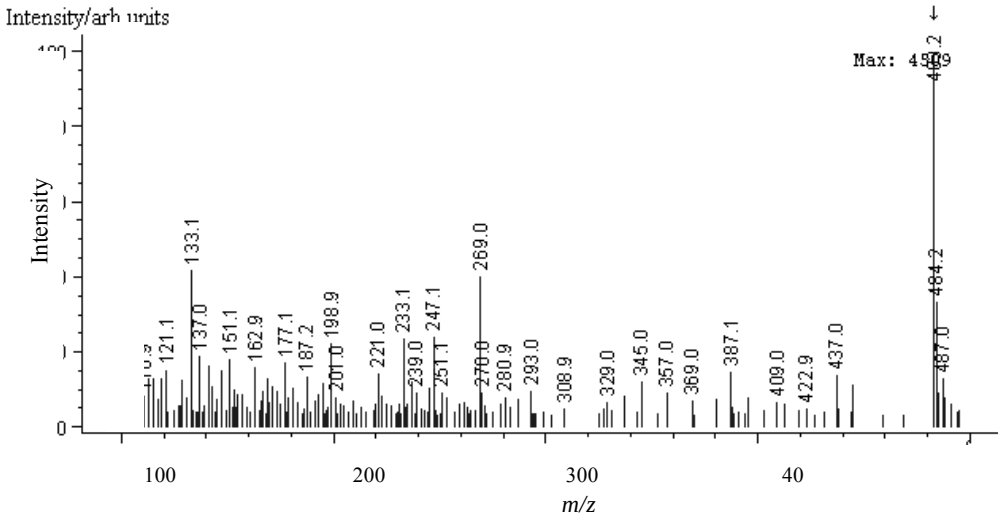

Figure 5.1.7-2 Mass spectrum of HT-2 toxin
(The arrow indicates the acetate adduct ion of HT-2 toxin $[M+CH_3COO]^-$.)

[3] An example of selected ion monitoring (SIM) chromatograms is shown in Figure 5.1.7-3.

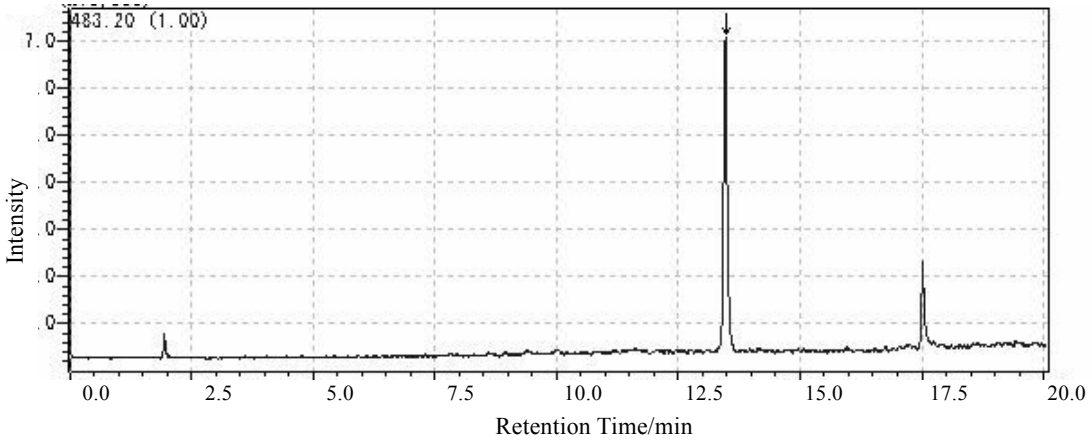

Figure 5.1.7-3 SIM chromatogram of a formula feed for adult chickens spiked with an amount equivalent to 200 µg/kg as HT-2 toxin
(The arrow indicates the peak of HT-2 toxin.)
Measurement conditions were as shown in Example of measurement conditions.
Column used was Agilent Technologies ZORBAX Eclipse XDB-C18.