

Neosolaniol

[Methods listed in the Feed Analysis Standards]

1 Simultaneous analysis of mycotoxins by liquid chromatography/ tandem mass spectrometry [Feed Analysis Standards, Chapter 5, Section 1 9.1]

Analyte compounds: Aflatoxin B₁, aflatoxin B₂, aflatoxin G₁, aflatoxin G₂, sterigmatocystin, zearalenone, T-2 toxin, deoxynivalenol, nivalenol, neosolaniol and fusarenon-X (11 components)

Scope of application: Feeds

A. Reagent preparation

- 1) Mycotoxin standard stock solutions. Weigh accurately 1 mg each of aflatoxin B₁ [C₁₇H₁₂O₆], aflatoxin B₂ [C₁₇H₁₄O₆], aflatoxin G₁ [C₁₇H₁₂O₇], aflatoxin G₂ [C₁₇H₁₄O₇], sterigmatocystin [C₁₈H₁₂O₆] and zearalenone [C₁₈H₂₂O₅]; 5 mg each of T-2 toxin [C₂₄H₃₄O₉] and neosolaniol [C₁₉H₂₆O₈]; and 10 mg each of deoxynivalenol [C₁₅H₂₀O₆], nivalenol [C₁₅H₂₀O₇] and fusarenon-X [C₁₇H₂₂O₈]. Put each of them in a 50- mL amber volumetric flask, respectively, and dissolve by the addition of acetonitrile. Add the same solvent to each volumetric flask up to the graduation line to prepare the standard stock solutions of mycotoxins (1 mL each of these solutions contains 20 µg respectively as aflatoxin B₁, aflatoxin B₂, aflatoxin G₁, aflatoxin G₂, sterigmatocystin and zearalenone; 100 µg respectively as T-2 toxin and neosolaniol; and 200 µg respectively as deoxynivalenol, nivalenol and fusarenon-X.).
- 2) Mycotoxin mixture standard solution. Transfer 1 mL each of the aflatoxin B₁ and aflatoxin B₂ standard stock solutions, 2 mL of the zearalenone standard stock solution, 3 mL each of the aflatoxin G₁ and aflatoxin G₂ standard stock solutions, 10 mL each of the sterigmatocystin, deoxynivalenol and fusarenon-X standard stock solutions, 20 mL each of the T-2 toxin and neosolaniol standard stock solutions and 30 mL of the nivalenol standard stock solution to a 200- mL amber volumetric flask, add 32 mL of water and mix, and add acetonitrile up to the graduation line to prepare the mycotoxin mixture standard stock solution (1 mL of

this solution contains 0.1 μg respectively as aflatoxin B₁ and aflatoxin B₂; 0.2 μg as zearalenone; 0.3 μg respectively as aflatoxin G₁ and aflatoxin G₂; 1 μg as sterigmatocystin; 10 μg respectively as deoxynivalenol, fusarenon-X, T-2 toxin and neosolaniol; and 30 μg as nivalenol.).

Before use, dilute accurately a certain amount of the mycotoxin mixture standard stock solution with acetonitrile- water (21:4) to be a series of dilutions in the range between 10- to 200-fold, then dilute a certain amount of the dilutions with acetic acid (1:100) to be accurately 2-fold to prepare the mycotoxin mixture standard solutions.

B. Quantification

Extraction. Weigh 50 g of an analysis sample, transfer it to a 300- mL stoppered amber Erlenmeyer flask, add 100 mL of acetonitrile- water (21:4), and extract by shaking for 60 minutes. ^{Note 1} Transfer the extract to a stoppered centrifuge tube, centrifuge at 650 $\times g$ for 5 minutes, to obtain supernatant to be a sample solution to be subjected to column treatment.

Column treatment. Load 10 mL of the sample solution to a multifunctional column (for mycotoxin pretreatment), ^{Note 2} and discard the first 4 mL of the eluate. ^[1] Place a 10- mL amber test tube under the column, and collect the following 2 mL of the eluate. Transfer accurately 1 mL of the eluate to another 10- mL amber test tube, and dilute by the addition of accurately 1 mL of acetic acid (1:100). ^[2] ^[3] Transfer a certain amount of this solution to a plastic centrifuge tube (capacity: 1.5 mL), centrifuge at 5,000 $\times g$ for 5 minutes, to obtain supernatant to be a sample solution to be subjected to analysis by liquid chromatography- tandem mass spectrometry.

Measurement by liquid chromatography-tandem mass spectrometry. Inject 10 μL each of the sample solution and respective mixture standard solutions to a liquid chromatograph- tandem mass spectrometer to obtain selected reaction monitoring chromatograms.

Example of measurement conditions

(Liquid chromatography part)

Column: Octadecylsilyl silica gel column (4.6 mm in inner diameter, 150 length, particle size 5 μm) ^{Note 3}

Eluent : 10 mmol/L ammonium acetate solution- acetonitrile (9:1) (1 min retention) \rightarrow 19 min \rightarrow 10 mmol/L ammonium acetate solution- acetonitrile (1:4) (15 min retention)

Flow rate: 0.2 mL/min
 Column oven temperature: 40 °C
 (Tandem mass spectrometry part^{Note 4})
 Ionization method: Electrospray ionization (ESI)
 Ion source temperature: 120 °C
 Desolvation temperature: 300 °C
 Capillary voltage: Positive 4.0 kV, negative 1.5 kV
 Cone voltage: As shown in the table below
 Collision energy: As shown in the table below
 Monitor ion: As shown in the table below

Table: Monitor ion conditions for mycotoxins

Name of mycotoxin	Measurement mode	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Cone voltage (V)	Collision energy (eV)
Aflatoxin B ₁	+	313	241	40	35
Aflatoxin B ₂	+	315	243	40	35
Aflatoxin G ₁	+	329	214	40	35
Aflatoxin G ₂	+	331	217	40	35
Sterigmatocystin	+	325	281	40	35
T-2 toxin	+	484	305	20	15
Neosolaniol	+	400	305	15	15
Zearalenone	-	317	175	40	25
Deoxynivalenol	-	355	295	10	10
Nivalenol	-	371	281	10	15
Fusarenon-X	-	353	263	25	15

Calculaionn. Obtain peak areas from the resulting selected reaction monitoring chromatograms^[4] to prepare a calibration curve, and calculate the amount of respective mycotoxins in the sample.

Note 1 When the analysis sample absorbs the extraction solvent and cannot be shaken, use 150 mL of the extraction solvent.

2 MultiSep 226 AflaZon+ (Romer Labs) or equivalents.

3 ZORBAX XDB-C18 (Agilent Technologies) or equivalents.

4 Example conditions for Quattro micro API Mass Analyzer (Waters).

<<Summary of analysis method>>

This is a simultaneous analysis method to extract aflatoxin B₁, B₂, G₁ and G₂, sterigmatocystin, zearalenone, T-2 toxin, neosolaniol, deoxynivalenol, nivalenol and fusarenon-X in feeds with acetonitrile- water (21:4), purify with a multifunctional cleanup (MFC) column, and quantitate by a liquid chromatograph- tandem mass spectrometer.

The accuracy of this method is currently inferior to individual analysis methods of respective mycotoxins by LC or LC-MS (or similar simultaneous analysis methods of mycotoxins); therefore if the analysis result is over the standard value, the result needs to be confirmed by individual analysis methods.

The flow sheet of the analysis method is shown in Figure 5.3.1-1.

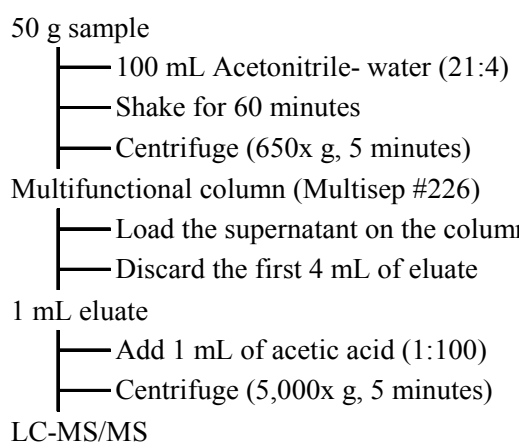


Figure 5.3.1-1 Flow sheet of the simultaneous analysis method for mycotoxins by liquid chromatography- tandem mass spectrometry

References: Rie Fukunaka, Hisaaki Hiraoka: Research Report of Animal Feed, 31, 2 (2006)

History in the Feed Analysis Standards [29] 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)
Aflatoxin B ₁	Corn	1~4	3	98.6~106.0	6.2
	Cattle formula feed	1~4	3	96.2~99.5	7.8
Aflatoxin B ₂	Corn	1~4	3	101.4~105.5	6.4
	Cattle formula feed	1~4	3	94.2~100.8	7.5
Aflatoxin G ₁	Corn	3~12	3	98.7~103.0	4.9
	Cattle formula feed	3~12	3	93.4~100.4	7.3
Aflatoxin G ₂	Corn	3~12	3	100.3~103.0	5.8
	Cattle formula feed	3~12	3	97.4~101.3	9.1
Sterigmatocystin	Corn	10~40	3	97.5~109.3	15.1
	Cattle formula feed	10~40	3	99.6~101.4	6.2
Zearalenone	Corn	2~8	3	99.8~102.4	14.0
	Cattle formula feed	2~8	3	105.9~109.3	9.8
T-2 toxin	Corn	100~400	3	102.7~103.0	8.6
	Cattle formula feed	100~400	3	100.1~108.1	10.7
Deoxynivalenol	Corn	100~400	3	104.4~106.2	7.7
	Cattle formula feed	100~400	3	96.4~103.9	9.9
Nivalenol	Corn	300~1,200	3	99.6~106.6	11.3
	Cattle formula feed	300~1,200	3	91.8~101.8	12.5
Neosolaniol	Corn	100~400	3	101.8~110.3	13.0
	Cattle formula feed	100~400	3	91.1~92.6	12.4
Fusarenon-X	Corn	100~400	3	97.9~106.2	8.3
	Cattle formula feed	100~400	3	104.6~110.2	12.2

• Collaborative study

Name of analyzed component	Sample type	Number of laboratories	Spike concentration ($\mu\text{g}/\text{kg}$)	Spike recovery (%) (measured value ($\mu\text{g}/\text{kg}$))	Intra-laboratory repeatability RSD_t (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
Aflatoxin B ₁	Corn	6	4	97.1	6.0	23.2	1.05
	Cattle formula feed	6	4	89.7	12.3	36.3	1.65
Aflatoxin B ₂	Corn	6	4	100.0	7.9	26.2	1.19
	Cattle formula feed	5	4	99.1	3.5	35.2	1.60
Aflatoxin G ₁	Corn	6	12	86.3	6.3	41.4	1.88
	Cattle formula feed	5	12	82.0	5.1	47.1	2.14
Aflatoxin G ₂	Corn	6	12	93.8	5.7	28.5	1.30
	Cattle formula feed	6	12	85.3	17.1	37.1	1.69
Sterigmatocystin	Corn	6	40	113.3	7.0	11.6	0.53
	Cattle formula feed	5	40	113.9	7.0	17.4	0.79
Zearalenone	Corn	6	8+Natural contamination	(16.2)	13.0	14.6	0.66
	Cattle formula feed	6	8+Natural contamination	(27.9)	19.0	36.1	1.64
T-2 toxin	Corn	6	400	108.7	2.6	13.8	0.75
	Cattle formula feed	5	400	107.4	3.6	17.9	0.97
Deoxynivalenol	Corn	6	400+Natural contamination	(444.3)	4.5	5.6	0.31
	Cattle formula feed	5	400	112.8	5.2	17.6	0.96
Nivalenol	Corn	5	1,200	86.7	9.9	14.9	0.96
	Cattle formula feed	6	1,200	61.7	27.6	23.9	1.54
Neosolaniol	Corn	5	400	109.6	1.4	13.1	0.71
	Cattle formula feed	6	400	83.3	17.9	30.0	1.63
Fusarenon-X	Corn	5	400	104.4	6.2	11.3	0.62
	Cattle formula feed	4	400	105.6	5.8	5.8	0.32

- Lower limit of quantification: 1 $\mu\text{g}/\text{kg}$ for aflatoxin B₁, B₂, G₁ and G₂, sterigmatocystin and zearalenone; 8 $\mu\text{g}/\text{kg}$ for T-2 toxin and neosolaniol; 40 $\mu\text{g}/\text{kg}$ for deoxynivalenol; 60 $\mu\text{g}/\text{kg}$ for nivalenol; and 80 $\mu\text{g}/\text{kg}$ for fusarenon-X (*SN* ratio)
- Lower limit of detection: 0.3 $\mu\text{g}/\text{kg}$ for aflatoxin B₁, B₂, G₁ and G₂, sterigmatocystin and zearalenone; 2.4 $\mu\text{g}/\text{kg}$ for T-2 toxin and neosolaniol; 12 $\mu\text{g}/\text{kg}$ for deoxynivalenol; 18 $\mu\text{g}/\text{kg}$ for nivalenol; and 24 $\mu\text{g}/\text{kg}$ for fusarenon-X (*SN* ratio)

<<Notes and precautions>>

[1] Recovery of sterigmatocystin, zearalenone, T-2 toxin, deoxynivalenol, nivalenol and fusarenon-X is low in the fraction of 0-4 mL eluate.

[2] Ionization of aflatoxin B₁, B₂, G₁ and G₂, T-2 toxin, neosolaniol,

deoxynivalenol, nivalenol and fusarenon-X is enhanced by the addition of acetic acid to the solution to be injected.

[3] Ion suppression of sterigmatocystin and zearalenone is prevented by diluting to be 2-fold.

[4] Examples of selected reaction monitoring (SRM) chromatograms are shown in Figure 5.3.1-2.

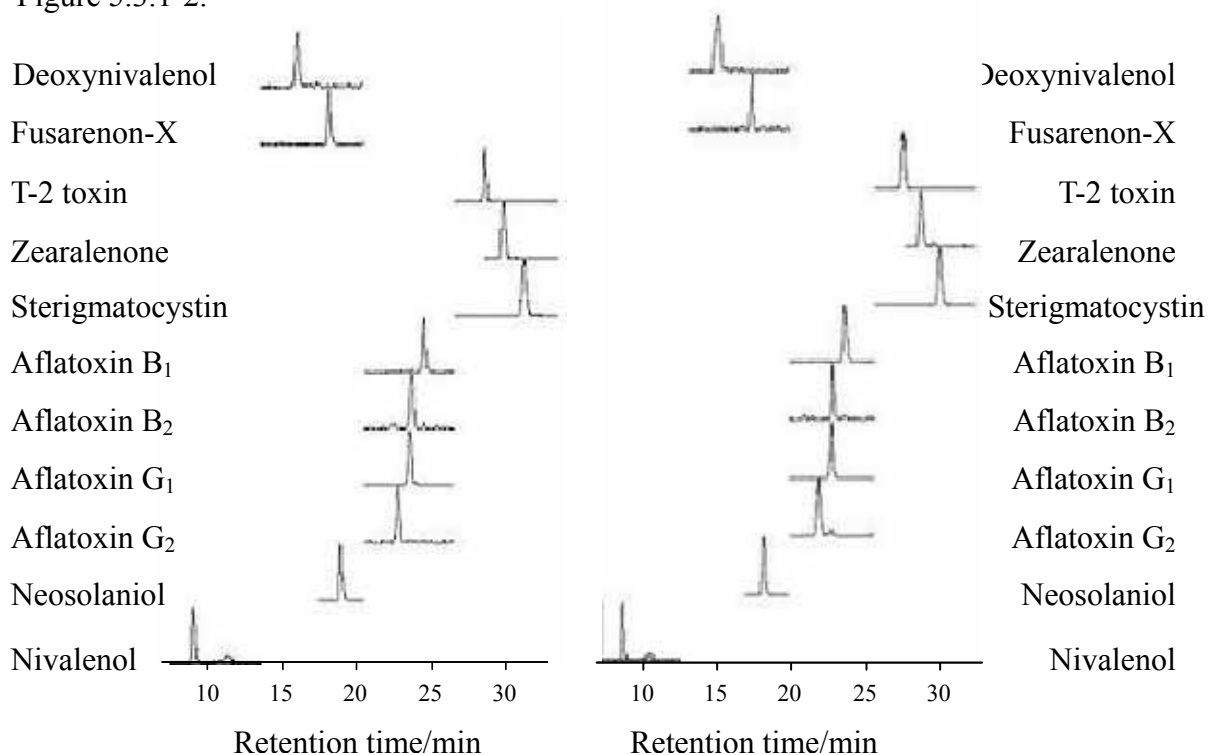


Figure 5.3.1-2 SRM chromatograms of mycotoxin-spiked formula feeds and corn (Left) formula feeds; (Right) corn

Spike concentration: 200 µg/kg for deoxynivalenol, fusarenon-X and T-2 toxin; 4 µg/kg for zearalenone; 20 µg/kg for sterigmatocystin; 2 µg/kg for aflatoxin B₁ and B₂; 6 µg/kg for aflatoxin G₁ and G₂; 200 µg/kg for neosolaniol; and 600 µg/kg for nivalenol.