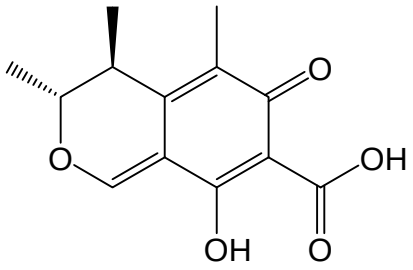
Citrinin



(3*R*,4*S*)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3*H*-isochromene-7-carboxylic acid

$C_{13}H_{14}O_5$ MW: 250.25 CAS No.: 518-75-2

[Summary of citrinin]

Citrinin is a mycotoxin that was discovered in 1931 from *Penicillium citrinum*, and is produced by many species in *Penicillium* and *Aspergillus*. In so-called the "poisoning by moldy rice" case that occurred in Japan in 1953-54, *Penicillium citrinum* was isolated from imported rice and it was confirmed that the metabolite of the mold was citrinin. Citrinin has renal toxicity, though the toxicity is milder than that of ochratoxin A

There have been small numbers of monitoring of citrinin in feed materials, and therefore it hardly has been detected, but there is a need for monitoring in the future.

[Methods listed in the Feed Analysis Standards]

1 Simultaneous analysis of ochratoxin A and citrinin by liquid chromatography [Feed Analysis Standards, Chapter 5, Section 1 19.1]

Analyte compounds: Ochratoxin A and citrinin (2 components)

Scope of application: Grains and formula feeds (except formula feeds for citrinin)

A. Reagent preparation
1) Ochratoxin A standard solution. Weigh accurately 5 mg of ochratoxin A [$C_{20}H_{18}NO_6Cl$], put in a 25- mL amber volumetric flask, dissolve by the addition

of methanol, and further add the same solvent up to the graduation line (1 mL of this solution contains 0.2 mg as ochratoxin A.). Moreover, dilute accurately a certain amount of this solution with methanol, to prepare the ochratoxin A standard stock solution that contains 1 μg as ochratoxin A in 1 mL.

2) Citrinin standard solution. Weigh accurately 5 mg of citrinin [$C_{13}H_{14}O_5$], put in a 25- mL amber volumetric flask, dissolve by the addition of methanol, and further add the same solvent up to the graduation line (1 mL of this solution contains 0.2 mg as citrinin.). Moreover, dilute accurately a certain amount of this solution with methanol, to prepare the citrinin standard stock solution that contains 1 μg as citrinin in 1 mL.

3) Mixture standard solution. Before use, mix a certain amount of ochratoxin A and citrinin standard stock solutions, dilute accurately with acetonitrile- water (1:1), to prepare several mixture standard solutions that contain 1-50 ng as ochratoxin A and citrinin, respectively, in 1 mL.

B. Quantification

Extraction. Weigh 25.0 g of an analysis sample, transfer it to a 200- mL stoppered Erlenmeyer flask, moisten by the addition of 100 mL of acetonitrile - hydrochloric acid - water (8:1:1), then leave at rest for 5 minutes, [Note 1] and further extract by shaking for 30 minutes. Filter the extract with filter paper (No. 5A), transfer accurately 5 mL of the filtrate to a 50- mL recovery flask, and concentrate under vacuum to be 1 mL or less at 40°C or less. Further, remove acetonitrile by a mild flow of nitrogen gas, [1] to be a sample solution to be subjected to purification.

Purification. Add about 0.5 g of sodium chloride to the sample solution, then add accurately 10 mL of ethyl acetate, mix well, transfer to a 10- mL test tube, and centrifuge at 1,500×*g* for 5 minutes. Transfer accurately 5 mL of the ethyl acetate layer (upper layer) to a 50- mL recovery flask, concentrate under vacuum 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 2 mL of acetonitrile- water (1:1). Transfer this solution to a filter cup with ultrafiltration membrane (molecular weight cutoff: 30,000) [Note 2] that is attached to a plastic centrifuge tube (capacity: 1.5 mL) in advance, centrifuge at 5,000×*g* for 15 minutes, to obtain filtrate to be a sample solution to be subjected to column treatment.

Liquid chromatography. [2] Inject 20 μL each of the sample solution and respective mixture standard solutions to a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength, 335 nm; emission wavelength, 480 nm)

Column: Octadecylsilyl silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm) Note 3[3][4]

Eluent: Acetonitrile- water/ 1 v/v% phosphoric acid (230:230:1)

Flow rate: 1.0 mL/min

Column oven temperature: 40°C

Calculation. Obtain peak heights or areas from the resulting chromatograms [5][6]to prepare a calibration curve, and calculate the amounts of ochratoxin A and citrinin in the sample.

Note 1 Leave at rest until it stops foaming.

2 Use Microcon YM-30 (Millipore) or equivalents.

3 Inertsil ODS-2 (GL Sciences) or equivalents.

<<Summary of analysis method>>

This is an analysis for ochratoxin A and citrinin in the case of grains, and for ochratoxin A in the case of formula feeds.

Mycotoxins in a sample are extracted with acetonitrile acidified with hydrochloric acid. After the solvent is replaced, the solution is passed through ultrafiltration filter (molecular weight cutoff: 30,000), and quantitated by a liquid chromatograph with a fluorescence detector.

The flow sheet of the analysis method is shown in Figure 5.3.9-1.

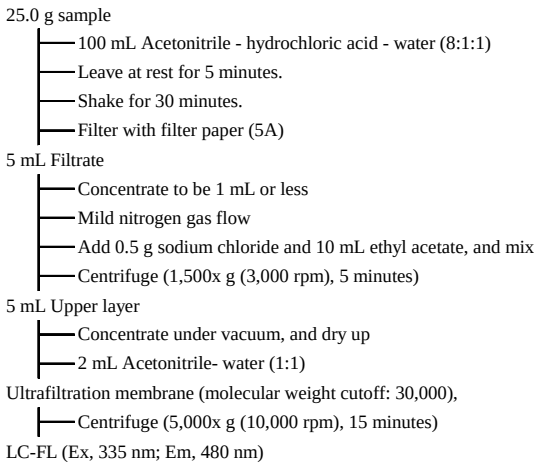


Figure 5.3.9-1 Flow sheet of the simultaneous analysis method for ochratoxin

A and citrinin

References: Koji Aoyama, Kaori Morifuji, Eiichi Ishiguro: Research Report of Animal Feed, **29**, 11 (2004)

History in the Feed Analysis Standards [27] 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)
Ochratoxin A	Formula feed for adult chickens	10~100	3	96.3~97.1	12.6
	Formula feed for pork pig fattening	10~100	3	98.6~103.4	13.6
	Corn	10~100	3	105.6~106.5	8.9
	Barley	10~100	3	98.2~103.8	10.2
Citrinin	Formula feed for adult chickens	100~400	3	85.0~86.2	3.0
	Formula feed for pork pig fattening	100~400	3	87.5~88.7	3.0
	Corn	100~400	3	77.2~90.8	12.5
	Barley	100~400	3	83.0~84.0	7.4

- 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
Ochratoxin A	Pig formula feed	11	20	100.4	3.3	7.9	0.36
	Milo	11	Natural contamination	(18.6)	20.6	23.5	1.07
Citrinin	Pig formula feed	11	200	83.6	6.1	11.8	0.58

- Lower limit of quantification: 5 μg/kg for ochratoxin A (Spike recovery 93.9~100.7 %, relative standard deviation 23.3 % or less, *SN* ratio 10), citrinin 20 μg/kg (Spike recovery 90.0~94.9 %, relative standard deviation 19.2 % or less, *SN* ratio 10)

<<Notes and precautions>>

[1] Recovery of citrinin becomes poorer if it is dried up in this step. When it happens to be dried up, add about 1 mL of 6 mol/L hydrochloric acid before analysis.

[2] Conduct measurement after removing methanol by sufficiently flowing the eluent, etc., when methanol remains in the column. The citrinin peak may become broad if methanol remains.

[3] In addition to Inertsil ODS-2, Shodex C18M 4E (Showa Denko) can be used.

[4] The order of elution of ochratoxin A and citrinin may vary even if the same column is used.

[5] Examples of chromatograms are shown in Figure 5.3.9-2.

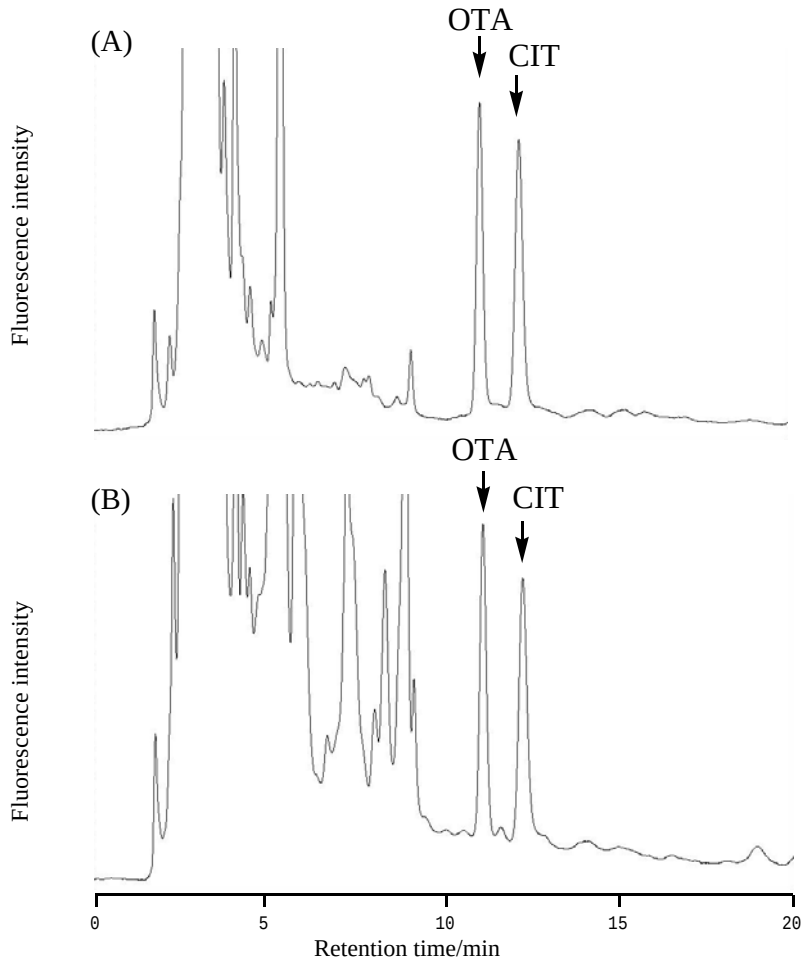


Figure 5.3.9-2 Chromatograms of ochratoxin A and citrinin
(OTA, peak of ochratoxin A; CIT, peak of citrinin.)

LC conditions are according to the Example of measurement conditions. The column is Inertsil ODS-2 (GL Sciences).

(A) Barley (spiked with amounts equivalent to 400 µg/kg as citrinin, and 100 µg/kg as ochratoxin A)

(B) Formula feeds for pork pig fattening (spiked with amounts equivalent to 400 µg/kg as citrinin, and 100 µg/kg as ochratoxin A)

[6] Peaks can be identified by changing the acid concentration in the eluent, emission wavelength, etc.