Notification (draft)

Analytical Method for Nitrofurantoin, Furazolidone and Furaltadone

(Targeted to Agricultural, Animal and Fishery Products)

The target compounds to be determined:

1-aminohydantoin for nitrofurantoin.

3- amino -2-oxazolidone for furazolidone.

3-Amino-5-morpholinomethyl-2-oxazolidinone for furaltadone.

1. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

2. Reagents

Use the reagents listed in Section C ***Reagent/Test Solution, Etc.***, Part II ***Food Additives***, except the following. Reagents designated as "special grade" in this section must meet the requirements for "special grade" specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Acetonitrile prepared for liquid chromatography.

Porous diatomaceous earth cartridge (to hold 20 mL of solution): A polyethylene tube of 20-30 mm in inside diameter packed with granular porous diatomaceous earth prepared for column chromatography (to hold 20 mL of solution), or a cartridge equivalent to the specified one in separation capability.

o-Nitrobenzaldehyde: *o*-Nitrobenzaldehyde (special grade)

Water: Water prepared for liquid chromatography.

3. Reference standard

Reference standard of 3-amino-2-oxazolidone: Contains not less than 99% of 3-amino -2-oxazolidone. Decomposition point of the standard is 65–67ºC.

Reference standard of 1-aminohydantoin hydrochloride: Contains not less than 90% of 1-aminohydantoin hydrochloride. Decomposition point of the standard is 201–205ºC.

Reference standard of 3-amino-5-morpholinomethyl-2-oxazolidinone: Contains not less than 99% of 3-amino-5-morpholinomethyl-2-oxazolidinone. Decomposition point of the standard is 115–120ºC.

4. Procedure

1) Extraction

Homogenize sample, and then weigh 5.00 g of the sample, add 70 mL of 0.1 mol/L

hydrochloric acid, homogenize, and centrifuge at 2500 rpm for 5 minutes. Collect the supernatant, and add 0.1 mol/L hydrochloric acid to make exactly 100 mL.

2) Derivatization

Collect 10 mL of the solution obtained in 1), add 0.4 mL of 0.05 mol/L *o*-nitrobenzaldehyde dimethyl sulfoxide solution, and let stand at 37°C for 16 hours. Add 5 mL of 0.1 mol/L dipotassium hydrogen phosphate solution, add 0.8 mL of 1 mol/L sodium hydroxide solution, and adjust pH 7-8. Some residue may appear in the solution, in this case, centrifuge at 2,500 rpm for 5 minutes, and collect the supernatant.

3) Clean-up

Transfer the solution obtained in 2) to the porous diatomaceous earth cartridge (to hold 20 mL of solution). Let stand the cartridge for 5 minutes, and then transfer 100 mL of ethyl acetate, collect the eluate to a vacuum rotary evaporator flask, and remove ethyl acetate at below 40°C. Dissolve the residue in acetonitrile/toluene (1:1, v/v), and use this solution as the test solution.

5. Measurement

1) Qualification

Perform the test under the measurement conditions described below. The result shall agree with those obtained for the reference standards under the procedure described in 4.b and 4.c.

Measurement conditions

Column packing: Octadecylsilanized silica gel (2-5 μm in particle diameter).

Column: A stainless tube of 2.0-6.0 mm in inner diameter, 100-250 mm in length.

Column temperature: 40ºC

Mobile phase: Linear gradient from acetic acid (1:4, v/v) to (4:1, v/v) in 15 minutes. Adjust the flow rate to elute the derivatives of 3-amino-2-oxazolidinone at about 12 minutes.

2) Quantification

Quantify using peak-height or peak-area method, on the basis of the result obtained using the measurement conditions described in 1).