Original: Japanese
Provisional translation

Analytical Method for Acetochlor (Agricultural Products)

1. Analytes

Acetochlor

Metabolites converted to EMA [2-ethyl-6-methylaniline] under basic conditions

Metabolites converted to HEMA [2-(1-hydroxyethyl)-6-methylaniline] under basic conditions

2. Applicable food

Grains, legumes, nuts, seeds, and corn (immature)

3. Instrument

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

4. Reagents

Use reagents listed in Section 3 of the General Rules, except the following.

Quaternary ammonium group-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg): A polyethylene column of 12−13 mm inside diameter packed with 150 mg of quaternary ammonium group-modified divinylbenzene-*N*-vinylpyrrolidone copolymer, or a cartridge equivalent to the specified one in separation capability.

Reference standard of acetochlor: Contains not less than 95% of acetochlor.

Reference standard of EMA: Contains not less than 97% of EMA.

Reference standard of HEMA: Contains not less than 95% of HEMA.

5. Procedure

1) Extraction

For grains, legumes, nuts and seeds, add 20 mL of water to 10.0 g of each sample and let stand for 30 min. For corn (immature), weigh 20.0 g of the sample.

Add 100 mL of methanol to the sample, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 20 mL (10 mL for immature corn) aliquot of the solution, concentrate at below 40°C, and remove the solvent.

2) Conversion to EMA and HEMA

Dissolve the residue obtained in 1) in 4 mL of methanol and transfer to a reaction vessel. Add 4 mL of 50% sodium hydroxide solution while cooling the reaction vessel on ice, seal tightly, and heat at 120°C for 4 hrs. After allowing the reaction vessel to cool to approximately room temperature, it should be cooled on ice. Open the reaction vessel and drop the ice-cooled reaction solution into 30 mL of pre-ice-cooled water. Wash the reaction vessel with 10 mL of water and

combine the washings.

3) Clean-up

Inject 2 mL of acetonitrile and 3 mL of water into a quaternary ammonium group-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg) sequentially and discard each effluent. After transferring the solution obtained in 2) to the cartridge, add 10 mL of water, and discard the effluent. Following this, add 4 mL of acetonitrile and water (7:3, v/v), add acetonitrile and water (7:3, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare EMA and HEMA standard solutions (acetonitrile and water [7:3, v/v]) of several concentrations, inject each solution into LC-MS/MS and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of EMA and HEMA in the test solution corresponding to 0.01 mg/kg (equivalent to acetochlor) in the sample results in 0.001 mg/L (equivalent to acetochlor) for each analyte.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of EMA and HEMA from the calibration curve made in 6. When calculating the concentration of acetochlor including metabolites converted to EMA and HEMA under basic conditions, use the following equation.

Concentration of acetochlor (including metabolites converted to EMA and HEMA under basic conditions) (ppm) = A × 1.995 + B × 1.784

A: Concentration of EMA (ppm)

B: Concentration of HEMA (ppm)

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 0.01 vol% formic acid and 0.01 vol% formic acid-acetonitrile solution (9:1, v/v) for 5 min, followed by a linear gradient to (1:19, v/v) in 15 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*)

EMA: Precursor ion 136, product ions 119, 117

HEMA: Precursor ion 152, product ions 119, 91

Injection volume: 3 μL

Expected retention time

EMA: 15 min

HEMA: 9 min

10 Limit of quantification

Acetochlor: 0.01 mg/kg

Metabolites converted to EMA under basic conditions: 0.01 mg/kg (equivalent to acetochlor)

Metabolites converted to HEMA under basic conditions: 0.01 mg/kg (equivalent to acetochlor)

11. Explanatory note

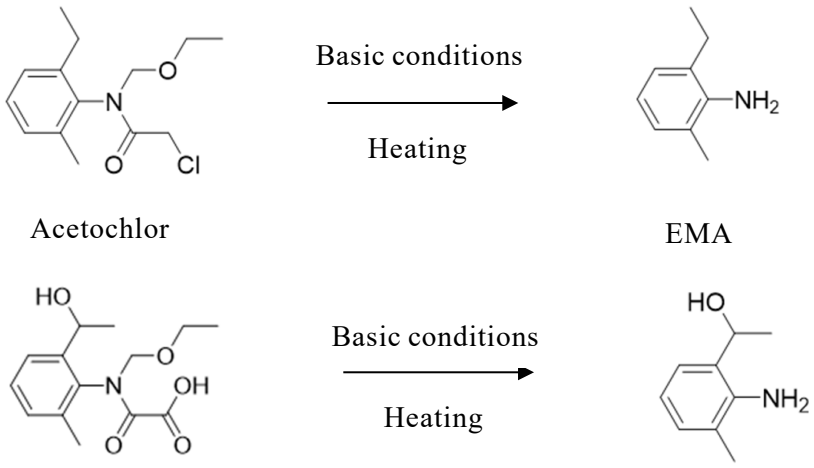
1) Outline of analytical method

The method consists of extraction of acetochlor and its metabolites from the sample with methanol, conversion to EMA and HEMA by heating under basic conditions, clean-up with a quaternary ammonium group-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS. In the method, EMA and HEMA are quantified respectively. When calculating the concentration of acetochlor including metabolites that are converted to EMA and HEMA under basic conditions, the concentration of EMA and HEMA is converted to the concentration of acetochlor by multiplying by a conversion factor respectively, and the sum of the concentration of acetochlor, EMA and HEMA is regarded as the analytical result of acetochlor.

2) Notes

i) When removing the solvent from the extracts, add about 10 mL of ethanol (about 5 mL each, about twice) to concentrate if water remains.

ii) To ensure that the conversion to EMA is sufficient, spike and recovery tests should be performed using the reference standard of acetochlor. The sufficient conversion of acetochlor to EMA is expected to result in conversion to HEMA as well. At the time of the analytical method development, the conversions to EMA and HEMA were confirmed using reference standards of acetochlor and 2-[(ethoxymethyl){2-(1-hydroxyethyl)-6-methylphenyl}amino]-2-oxoacetic acid, respectively.



Conversions to EMA and HEMA

iii) After re-dissolving the residue obtained from the extraction in methanol, it generates a slight heat when mixed with 50% sodium hydroxide solution, which may cause EMA and HEMA to volatilize. Therefore, 50% sodium hydroxide solution should be added while cooling the reaction vessel on ice.

iv) Airtight containers such as vials with aluminium seals are used in the conversion reaction.

v) After the conversion reaction, adding water to the reaction solution may cause intense heat generation, which may result in volatilization of EMA and HEMA. Therefore, the ice-cooled reaction solution should be added dropwise to the pre-ice-cooled water when diluting the reaction solution with water.

vi) After the conversion reaction, suspended materials may be generated when the reaction solution is diluted with water. In the clean-up process using a cartridge, inject the solution into the cartridge including the suspended materials. The flow velocity may be slowed down when suspended materials are injected first into the cartridge. Therefore, inject the solution with as few suspended materials as possible, then mix the remaining small amount of suspended materials in water used for the washing operation (about 2 mL each, about 3 times) to inject into the cartridge. The suspended materials dissolve during the process of mixing in water and injecting them into the cartridge. If the flow velocity is slow, suction may be used as necessary.

vii) When the analytical methods for EMA and HEMA using LC-MS/MS were developed, the following monitoring ions were used:

EMA:

for quantitative ions *(m/z)*: precursor ion 136, product ion 119

for qualitative ions (*m/z*): precursor ion 136, product ion 117

HEMA:

for quantitative ions *(m/z)*: precursor ion 152, product ion 119

for qualitative ions (*m/z*): precursor ion 152, product ion 91

viii) When the sensitivity is not sufficient in LC-MS/MS measurements for HEMA, the following monitoring ions should be used:

for quantitative ions *(m/z)*: precursor ion 134, product ion 115

for qualitative ions (*m/z*): precursor ion 134, product ion 119

ix) Food items used to develop the analytical method: soybean and corn (immature)

12. References

None

13. Type

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