Original: Japanese
Provisional translation

Analytical Method for Alachlor
(Animal Products)

1. Analytes

Alachlor

Metabolites converted to DEA [2,6-diethylaniline] by hydrolysis

Metabolites converted to HEEA [2-ethyl-6-(1-hydroxyethyl) aniline] by hydrolysis

2. Applicable food

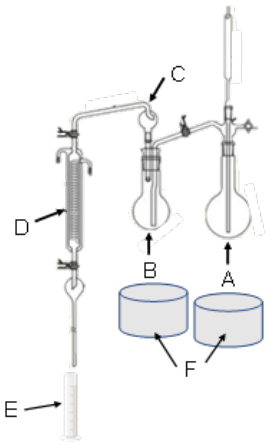
Animal products

3. Instruments

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

Steam distillation apparatus: The apparatus is made of glass, and its outline is shown in the following diagram.

A: 1,000 mL round-bottom flask (for steam generation)
B: 500 mL round-bottom flask (for distillation)
C: Distillation trap
D: Condenser
E: 100 mL graduated cylinder
F: Heating mantles



4. Reagents

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

Nitrogen-containing polar group-modified methacrylate-styrene-divinylbenzene copolymer cartridge (200 mg): A polypropylene tube of 12−13 mm inside diameter packed with 200 mg of nitrogen-containing polar group-modified methacrylate-styrene-divinylbenzene copolymer, or a cartridge equivalent to the specified one in separation capability.

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

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

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

5. Procedure

1) Extraction

Add 50 mL of methanol to 10.0 g of the sample, homogenize, centrifuge at 3,000 rpm for 10 min, and collect the supernatant. Add 25 mL of methanol to the residue, homogenize, centrifuge as

described above, and collect the supernatant. Combine the resulting supernatants and add methanol to make exactly 100 mL. Take exactly a 50 mL aliquot of the solution into a round-bottom flask (for distillation) and concentrate to about 3 mL at below 40°C.

2) Hydrolysis

Add 50 mL of 50 w/v% sodium hydroxide solution to the concentrated solution obtained in 1). After adding 1−2 drops of silicone defoamers and boiling stones, attach the round-bottom flask (for distillation) immediately to a steam distillation apparatus. Separately, cool the condenser to below 10°C and immerse the lower end of the adapter with a trap in a 100 mL graduated cylinder (ice-cooled) containing 10 mL of water (trapping solution). In addition, prepare 1,000 mL of water in a round-bottom flask (for steam generation), add boiling stones, and then attach to the steam distillation apparatus and heat the water to 100°C in advance. Heat the round-bottom flask (for distillation) to 100°C, hydrolyze for 30 min, and then start steam distillation. Steam distill until the distillate reaches 75 mL (volume combined with the trapping solution), and confirm that the distillate is neutral with pH test paper. Add 2 vol% triethylamine to the collected distillate to make 100 mL.

3) Clean-up

Inject 5 mL each of acetonitrile and water into a nitrogen-containing polar group-modified methacrylate-styrene-divinylbenzene copolymer cartridge (200 mg) sequentially and discard each effluent. Transfer the solution obtained in 2) to the cartridge, add 5 mL of water, and discard the effluent. Then, add 10 mL of acetonitrile, concentrate the eluate to about 1 mL at below 40°C, add water and methanol (3:2, v/v) to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare each stock standard solution by dissolving the reference standard of DEA and HEEA in methanol, respectively. Mix each stock standard solution appropriately, dilute with water and methanol (3:2, v/v), and prepare standard solutions of several concentrations. Inject each standard solution into LC-MS/MS respectively and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of DEA and HEEA in the test solution corresponding to 0.002 mg/kg (equivalent to alachlor) in the sample results in 0.001 mg/L (equivalent to alachlor).

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of DEA and HEEA from the calibration curve made in 6. When calculating the concentration of alachlor including metabolites converted to DEA and HEEA by hydrolysis, use the following equation.

Concentration of alachlor (including metabolites converted to DEA and HEEA by hydrolysis) (ppm) = A × 1.808 + B × 1.633

A: Concentration of DEA (ppm)

B: Concentration of HEEA (ppm)

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.0 mm inside diameter, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 0.1 vol% formic acid and 0.1 vol% formic acid-methanol solution (11:9, v/v) for 1 min, followed by a linear gradient to (1:49, v/v) in 13 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*)

DEA: Precursor ion 150, product ions 105, 77

HEEA: Precursor ion 166, product ions 148, 118

Injection volume: 5 μL

Expected retention time

DEA: 8 min

HEEA: 4 min

10. Limit of quantification

Alachlor (converted to DEA by hydrolysis): 0.002 mg/kg (equivalent to alachlor)

DEA (including metabolites converted to DEA by hydrolysis): 0.002 mg/kg (equivalent to alachlor)

HEEA (including metabolites converted to HEEA by hydrolysis): 0.002 mg/kg (equivalent to alachlor)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of alachlor and its metabolites from the sample with methanol, hydrolysis to DEA and HEEA by heating in sodium hydroxide solution, collecting DEA and HEEA by steam distillation, clean-up with a nitrogen-containing polar group-modified methacrylate-styrene-divinylbenzene copolymer cartridge, and quantification and confirmation using LC-MS/MS. In the method, DEA and HEEA are quantified respectively. When calculating the concentration of alachlor including metabolites hydrolyzed to DEA and HEEA in sodium hydroxide solution, the concentration of DEA and HEEA is converted to the concentration of acetochlor by multiplying by a conversion factor respectively, and the sum of the concentration of DEA and HEEA is regarded as the analytical result of alachlor.

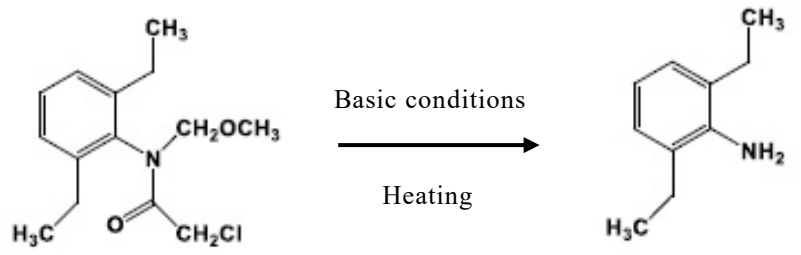
The concentration of DEA includes alachlor and the metabolites that are converted to DEA by hydrolysis.

2) Notes

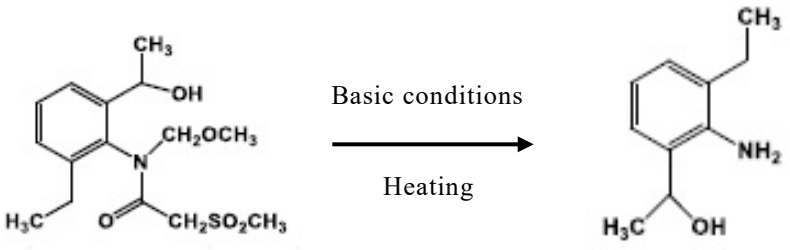
i) For the centrifuge used during the development of this analytical method, 3,000 rpm corresponds

to approximately 2,130 × g.

ii) Spike and recovery tests should be conducted using alachlor reference standard to ensure adequate conversion to DEA. An adequate conversion from alachlor to DEA is expected to result in a conversion to HEEA as well.



Alachlor DEA



N-(2-Ethyl-6-(1-hydroxyethyl)phenyl)-*N*-(methoxymethyl)-2-(methylsulfonyl)acetamide HEEA

Conversions to DEA and HEEA

N-(2-Ethyl-6-(1-hydroxyethyl)phenyl)-*N*-(methoxymethyl)-2-(methylsulfonyl) acetamide is shown as an example of a metabolite that is converted to HEEA.

iii) Ensure that the tip of the adapter with a trap is immersed in the trapping solution (the tip should be attached to a silicone tube to prevent damage and connect a glass tube to the end of the silicone tube). The distillation rate should be approximately 5−10 mL/min.

iv) To reduce the influence of matrix carryover in the sample in LC-MS/MS measurements, it is recommended to wash the cartridge by increasing the methanol concentration in the mobile phase after DEA is eluted.

v) The round-bottom flask (for distillation) should be inspected for cracks or damage before use, as 50 w/v% sodium hydroxide solution is used in distillation. In addition, the glass needs to be replaced with a new one as necessary after about 10 times of use even if there are no cracks or breaks since it tends to deteriorate with repeated use.

vi) While the pH test paper confirms the distillate to be neutral, when it becomes basic, the target compound may not be adequately collected and should be retested.

vii) As DEA is highly volatile, care should be taken to avoid volatilization during hydrolysis and steam distillation operations in “5. Procedure-2) Hydrolysis”. In addition, ensure that laboratory equipment is not contaminated.

viii) When the analytical methods for DEA and HEEA using LC-MS/MS were developed, the following monitoring ions were used:

DEA:

for quantitative ions *(m/z*): precursor ion 150, product ion 105

for qualitative ions (*m/z*): precursor ion 150, product ion 77

HEEA:

for quantitative ions *(m/z*): precursor ion 166, product ion 148

for qualitative ions (*m/z*): precursor ion 166, product ion 118

ix) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, milk and chicken egg.

12. References

None

13. Type

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