Original: Japanese

Provisional Translation

Analytical Method for 1-Methylcyclopropene (Agricultural Products)

1. Analyte

1-Methylcyclopropene

2. Instruments

Gas chromatograph-flame ionization detector (GC-FID)

Gas chromatograph-mass spectrometer (GC-MS)

Extraction apparatus: Use the apparatus shown in the figure below.

Volume of 2.2 L, inside diameter of approximately 9 cm, and height of approximately 35 cm (upper part of height 20 cm, lower part of height 20 cm and joint part of height 5 cm)

A: Sampling valve

B: Fixing nut

C: Lid

D: O-ring

E: Head space

F: Fixing bolt

G: Blender cup

H: Blender blades

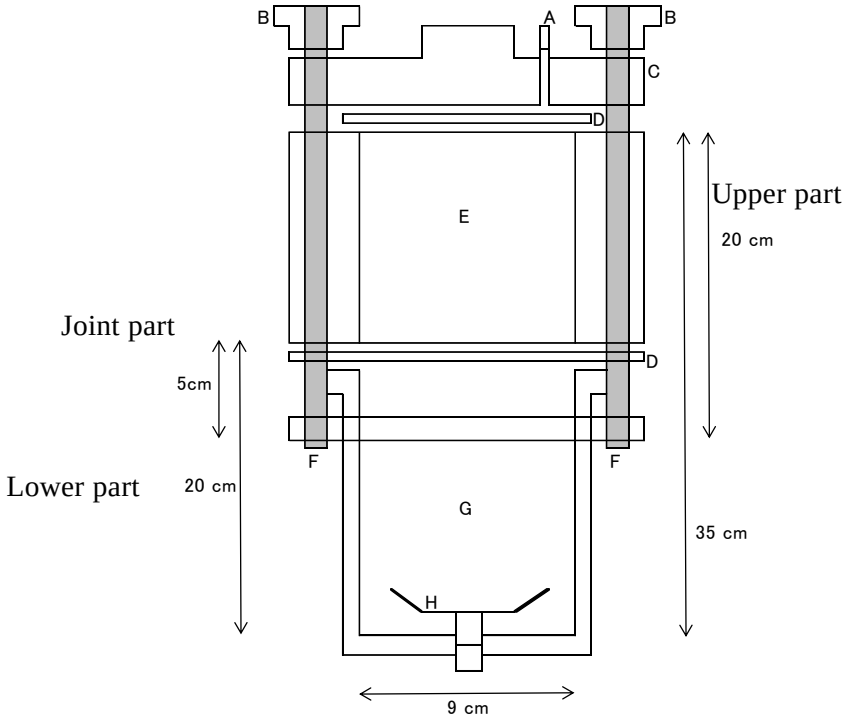

Fig. Overview of the extraction apparatus

3. Reagents

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

Basic ammonia sulfate solution: Add 50 mL of aqueous ammonia to 1 L of saturated ammonium sulfate solution.

Reference standard of isobutylene: Contains not less than 99% of isobutylene.

4. Procedure

Weigh 200 g of chopped sample, and transfer to the extraction apparatus.

Add 250 mL of basic ammonium sulfate solution, fasten the lid tightly with the fixing nut,

homogenize for 5 minutes. Let stand for 30 minutes and use the gas in the head space of the extraction apparatus as the test gas.

5. Calibration curve

Dilute the reference standard of isobutylene with air to make 1000 μL/L, and use the gas as the standard gas. Dilute the standard gas with air to prepare several concentrations for a calibration curve. Inject each standard gas to GC, and make a calibration curve by peak-height or peak-area method. Regard the concentration of isobutylene standard gas as equivalent to the concentration of 1-methylcyclopropene standard gas. When the test gas is prepared following the above procedure, a sample containing 0.01 mg/kg of 1-methylcyclopropene gives a test gas of 0.515 μL/L (at 24°C) in concentration.

6. Quantification

Take the head space gas from the sampling valve of the extraction apparatus, inject to GC, and calculate the concentration of 1-methylcyclopropene from the calibration curve made in **5**. Use the following equation to calculate the content of 1-methylcyclopropene in the sample. Assume that the specific gravity of the sample is 1 g/cm^3, and use the equation of state of ideal gas, $PV = nRT$, for the calculation.

A: Concentration of 1-methylcyclopropene calculated from the calibration curve (μL/L)

P: Partial pressure of 1-methylcyclopropene in the head space (atm)

V: Volume of the head space after extraction (L)

n: Amount of 1-methylcyclopropene in the head space (mol)

R: Gas constant 0.08206

T: Room temperature (K)

$P = A \times 1 \text{ atm} = A \times 10^{-6}$

V = Volume of the extraction apparatus − Volume of basic ammonium sulfate solution − volume of the sample

= 2.2 − 0.25 − 0.2= 1.75

$n = (P \times V) / (0.08206 \times T)$

1-methylcyclopropene content (mg/kg)

= (n × molecular weight of 1-methylcyclopropene × 10^3) / weight of the sample (kg)

= ((PV/0.08206T) × 54.1 × 10^3) / 0.2

= (A/T) × 5.77

7. Confirmation

Confirm using GC-MS.

8. Measurement conditions

Example

1) GC

Detector: FID

Column: styrene-divinylbenzene porous polymer, 0.25 mm in inside diameter, 25 m in

length and 3 μm in film thickness

Column temperature: 50°C (0 min) - 20°C/min - 170°C (0 min) - 50°C/min - 275°C (0 min)

Injection port temperature: 75°C

Detector temperature: 295°C

Carrier gas: Helium

Injection volume: 500 μL

Expected retention time: 1-Methylcyclopropene 5 min, isobutylene 5 min

2) GC-MS

Column: styrene-divinylbenzene porous polymer, 0.25 mm in inside diameter, 25 m in length and 3 μm in film thickness

Column temperature: 50°C (0 min) - 20°C/min - 270°C (0 min)

Injection port temperature: 75°C

Carrier gas: Helium

Ionization mode (ionization energy): EI (70 eV)

Major monitoring ions (*m/z*): 54, 39, 27

Injection volume: 500 μL

Expected retention time: 5 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory notes

1) Outline of analytical method

The method consists of grinding the sample with basic ammonium sulfate solution, measurement of 1-methylcyclopropene that is generated in the gas phase using GC-FID, indirect quantification using the calibration curve for isobutylene, and confirmation using GC-MS. This analytical method was evaluated for fruits (persimmon and pear) only, and whether this method is applicable to other agricultural products (grains, legumes, nuts and seeds, vegetables, tea leaves etc.) has not been evaluated. Moreover, note that in the example calculation given above, the specific gravity of the sample is assumed to be 1 g/cm^3.

2) Notes

i) 1-Methylcyclopropene is gas at normal temperature; therefore, a well-sealed extraction apparatus should be used, and all procedures including sample preparation should be performed quickly.

ii) When preparing samples, cutting the samples very finely may lead to loss of analyte. Chop into chunks but not into thin strips.

iii) 1-Methylcyclopropene cannot be used as a reference standard for the calibration curve because it is unstable in air. For this reason, isobutylene, which has almost the same retention time in GC and sensitivity in FID, is use as an alternative standard.

iv) Use a gas-tight syringe when preparing gas for the calibration curve and GC measurement.

v) When preparing gas for a calibration curve, let some air into a Tedlar bag first and put isobutylene into the bag. An example procedure for making a calibration curve is as follows.

Take 0.8 mL of isobutylene using a gas-tight syringe, and add it to a Tedlar bag which has filled with 800 mL of air in advance to prepare 998.40 µL/L (When purity of isobutylene is 99.94 %) standard gas. Take 0.20, 0.41, 0.80, 4.0, and 8.0 mL aliquots of the standard gas using a gas-tight syringe, and add each gas to a Tedlar bag which has filled with 800 mL of air in advance to prepare 0.250, 0.511, 0.997, 4.97, and 9.89 µL/L gases, respectively, for a calibration curve. Inject these gases to GC, and make a calibration curve by peak-height or peak-area method.

vi) A recovery test should be performed using 1-methylcyclopropene, which is generated by dissolving 1-methylcyclopropene formulation into water, as spike gas. The concentration of generated 1-methylcyclopropene should be calculated from the calibration curve using isobutylene standard gas, and actual measured value should be used for the calculation. An example of the recovery test is as follows.

Summary of the procedure: Dissolving 80 mg of 3.3% 1-methylcyclopropene formulation into 3 mL of water in a sealed container with 250 mL capacity will generate 4814 µL/L (theoretical value) of 1-methylcyclopropene gas. Use this gas as spiking gas. When spiking concentration to 200 g of sample is 0.01 mg/kg, assuming actual measured concentration of spiking gas is 4814 µL/L and room temperature is 24°C, the spiked amount of 1-methylcyclopropene is 0.187 mL as calculated using the following equations.

Calculation example

a) Concentration of 1-methylcyclopropene in spike gas

Equation 1: Number of moles of 1-methylcyclopropene in spiking gas (mol)

$3.3\ (\%) \times 80\ (mg) \times 10^{-5}/54.1 = 4.880 \times 10^{-5}\ (mol)$

Equation 2: Volume of 1-methylcyclopropene in spiking gas (µL)

$4.880 \times 10^{-5}\ (mol) \times 22.4 \times 10^{6}\ (\mu L) \times (273 + 24) / 273 = 1189\ (\mu L)$

Equation 3: Concentration of 1-methylcyclopropene in spiking gas (µL/L)

$1189\ (\mu L) / (250\ (mL) - 3\ (mL)) \times 1000 = 4814\ (\mu L/L)$

b) Spike amount of 1-methylcyclopropene spiking gas when the spike concentration to 200 g of sample is 0.01 mg/kg

Equation 1: Weight of 1-methylcyclopropene required for spiking 0.01 mg/kg (g)

Spiking concentration (mg/kg) × sample weight (kg) / 1000

$= 0.01 \times 0.2 / 1000 = 2.00 \times 10^{-6}\ (g)$

Equation 2: Number of moles of 1-methylcyclopropene required for spiking 0.01 mg/kg (mol)

n_A = weight of 1-methylcyclopropene (g) / molecular weight

$= 2.00 \times 10^{-6} / 54.1 = 3.70 \times 10^{-8}\ (mol)$

Equation 3: Partial pressure of 1-methylcyclopropene in spiking gas (atm)

P = 4814 (μL/L) × 1 (atm) = 4.814 ×10^{-3} (atm)

Equation 4: Number of moles of 1-methylcyclopropene in 1 mL of spiking gas (mol/mL)

$n_B = PV/RT$ = 4.814 ×10^{-3} × 0.001 / 0.08206 / 297 = 1.98 ×10^{-7} (mol/mL)

Equation 5: Required amount of spiking gas

Required amount (mL) = n_A (mol) / n_B (mol/mL)

= 3.70 ×10^{-8} (mol) / 1.98 ×10^{-7} (mol/mL)

= 0.187 (mL)

11. References

1) Overview of the sealed container used for the development of analytical method for 1-methylcyclopropene

2) A summary of an agricultural chemical, 1-methylcyclopropene

12. Type

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