

# DIFLUBENZURON TECHNICAL PRE-CONCENTRATE (900 G/KG)

Specification WHO/SIT/25  
Approved 25 September 1989

## 1. Specification

### 1.1 Material

The material shall consist of diflubenzuron together with related manufacturing compounds and shall be in the form of an off-white, fine powder, free from visible extraneous impurities or added modifying agents except for the stabilizer.

### 1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

#### 1.2.1 *Diflubenzuron content (g/kg basis)*

The diflubenzuron content shall be declared (not less than 900 g/kg) and, when determined by the method described in section 2.1, the content obtained shall not differ from that declared by more than  $\pm 25$  g.

#### 1.2.2 *Wet sieving*

Not less than 995 g/kg of the material shall pass through a 45  $\mu\text{m}$  sieve when tested by the method described in section 2.2.

### 1.3 Packing and marking of packages

The diflubenzuron pre-concentrate 900 g/kg shall be packed in suitable clean containers, as specified in the order. All packages shall bear, durably and legibly marked on the container the following:

Manufacturer's name  
Diflubenzuron 900 g/kg pre-concentrate to specification WHO/SIT/25  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notice:

Use as a pre-concentrate to manufacture diflubenzuron formulations only. Keep out of reach of children. Do not contaminate food or drinking water. Avoid excessive skin contact.

## 2. Methods of determining chemical and physical properties

### 2.1 Diflubenzuron content

#### 2.1.1 Outline of method

The sample is dissolved in 1,4-dioxane and linuron is added as internal standard. The diflubenzuron content is determined by high performance liquid chromatography using a reverse-phase column and a mixture of acetonitrile-1,4 dioxane-water as mobile phase.

#### 2.1.2 Special apparatus

*Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and equipped with a pumping system able to maintain a pressure of 25 MPa, a UV spectrophotometer detector able to measure UV absorbance at 254 nm and a recorder.

*Liquid chromatographic column.* The column should be a stainless steel tube, 25 cm length and 4.6 mm internal diameter, packed with  $\leq 10$  mm C<sub>8</sub> bonded silicagel (Zorbax C<sub>8</sub> or equivalent)<sup>1</sup>.

*Filter.* Acrodisc Disposable Filter Assembly 1,2  $\mu$ m. Gelman or equivalent.

#### 2.1.3 Special reagents

*Diflubenzuron standard.* Analytical grade of known purity.

*Internal standard.* Linuron. Select a batch which, when chromatographed under the conditions given in section 2.1.5, gives no peak with a similar retention time to diflubenzuron peak.

*Acetonitrile.* HPLC grade

*1,4-Dioxane.* HPLC grade

*Water,* pure. HPLC grade or bi-distilled.

*Solvent mixture.* Acetonitrile-water: 45 + 55 (v/v).

*Mobile phase.* Degassed mixture of acetonitrile, 1,4-dioxane and water:  
45 + 10 + 45 (v/v).

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<sup>1</sup> Instead of Zorbax TM<sub>BP</sub>-C8 the following supports can be used:

- Spherisorb ODS (RP 18) 5  $\mu$ m
- Bondapak C<sub>18</sub> (RP 18) 10  $\mu$ m
- Zorbax TM<sub>BP</sub>-ODS (RP 18) 7  $\mu$ m

#### 2.1.4 Preparation of standard solutions

*Internal standard solution.* Weigh a 25 mg quantity of linuron into a 100 ml volumetric flask, dissolve, dilute to volume with acetonitrile and mix well.

*Diflubenzuron calibration solution.* Weigh (to the nearest 0.01 mg) about 50 mg of diflubenzuron standard into a 100 ml volumetric flask. Add 50 ml of 1,4-dioxane and dissolve by heating in a water bath at 80°C for thirty minutes. Swirl the flask occasionally. Add 40 ml of 1,4-dioxane, cool, dilute to volume with 1,4-dioxane and mix well. Pipette 5 ml of this solution into a 50 ml volumetric flask. Add by pipette 5 ml of internal standard solution. Dilute to volume with the solvent mixture and mix well.

#### 2.1.5 Operating conditions for high-performance liquid chromatography

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperatures	Ambient. Avoid temperature fluctuations of more than 2°C.
Flow rate	1.3 ml. min <sup>-1</sup> .
Detector sensitivity	0.128 AUFS (Absorbance Unit Full Scale).
Wavelength	254 nm.
Injection volume	20 µl (loop type injector).
Chart speed	1 cm. min <sup>-1</sup> .
Retention times	
Linuron	5.2 min.
Diflubenzuron	7.0 min.

#### 2.1.6 System suitability test

Diflubenzuron samples can contain small amounts of p,p'-bis-(4-chlorophenyl)-urea (DIFUR)<sup>2</sup>. This compound elutes just after diflubenzuron. For good quantitative results, the column should give proper resolution for these compounds (R>1). The resolution can be determined by injection of 20 µl of a solution which contains:

4.2 mg diflubenzuron and 1.8 mg DIFUR dissolved in 100 ml solvent mixture.

$$\text{Determine the resolution } R_s \text{ as follows: } R_s = \frac{2d}{W_1 + W_2}$$

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<sup>2</sup> Request for a sample of DIFUR should be addressed to the manufacturer.

where  $d$  = distance between the peak maxima  
 $W_1$  = peak width of diflubenzuron at the base-line  
 $W_2$  = peak width of DIFUR at the base-line

If necessary, the resolution can be improved by a slight increase or decrease of the water content in the mobile phase.

### 2.1.7 *Sample preparation and analysis*

Homogenize the sample and weigh (to the nearest 0.1 mg) a quantity of sample containing about 1 g of diflubenzuron into a 200 ml volumetric flask. Add 150 ml of 1,4-dioxane and heat in a water bath at 80°C for thirty minutes, swirling the flask occasionally. Add 40 ml of 1,4-dioxane, cool, dilute to volume with 1,4-dioxane and mix well. Pipette 10 ml of this solution into a 100 ml volumetric flask, dilute to volume with 1,4-dioxane and mix well.

Pipette 5 ml of the diluted solution into a 50 ml volumetric flask. Add by pipette, 5 ml of internal standard solution, dilute to volume with the solvent mixture and mix well. Filter a portion of this solution through a 1.2 mm porosity filter Gelman Acrodisc or equivalent, and retain the filtrate for the analysis.

Pump mobile phase through the column until the system is equilibrated (flat base-line). Inject 20 µl of the calibration solution and adjust the operating conditions to give peak heights of 60-80% full scale and a retention time between 6.0 and 8.0 min for diflubenzuron. Allow 12 minutes between injections.

$$r = \frac{\text{area (or height) of diflubenzuron peak}}{\text{area (or height) of internal standard peak}}$$

Repeat the injection of the calibration solution and calculate the response ratio: Response ratios must agree within  $\pm 1\%$ . Average duplicate response ratios obtained by calibration solution injections. The response ratio is approximately 1.7.

Inject duplicate aliquots of sample solution. Average duplicate response ratios for sample solution. Response ratios must agree within  $\pm 1\%$ . If not, repeat the analysis starting with calibration solution injections.

Reinject calibration solution twice. Average response ratios of two calibration solution injections immediately preceding and following sample injections. These must agree within  $\pm 1\%$ . If not, repeat the analysis.

### 2.1.8 Calculation

$$\text{Diflubenzuron content (g / kg)} = \frac{r_2 \times w_1 \times P \times V}{r_1 \times w_2}$$

- where
- $r_1$  = average response ratio for calibration solution
  - $r_2$  = average response ratio for sample solution
  - $w_1$  = mass of diflubenzuron standard in the calibration solution (g)
  - $w_2$  = mass of sample taken (g)
  - $P$  = purity of diflubenzuron standard (g/kg)
  - $V$  = dilution factor of the sample (=20)

## 2.2 Wet sieving

### 2.2.1 Special reagent

*Nonionic wetting agent.* Prepare a solution containing about 2 g or 2 ml of the nonionic wetting agent in 100 ml of water.

### 2.2.2 Procedure

Prepare a 45  $\mu\text{m}$  sieve about 20 cm in diameter by freeing it from any film, grease, or other water-repellent material. Weigh (to the nearest 10 mg) 10 g of sample into a 250 ml beaker and add 100 ml of water containing the wetting agent. Allow to stand for 30 seconds, then stir with a glass rod by hand for 30 seconds at not more than four revolutions per second, making no deliberate attempt to break up any lumps. Transfer the slurry immediately to the sieve, rinsing with water.

Wash the material on the sieve with an oscillating, moderately vigorous spray of water, using a rubber hose with an internal diameter of 10 mm delivering 4-5 litres of water per minute. Continue the washing for 10 minutes, directing the water from the circumference of the sieve towards the centre and keeping the end of the hose not more than 5 cm from the surface of the sieve. Transfer the residue to a tared Gooch crucible, dry at 60<sup>0</sup>C, and weigh to constant weight. From this weight and the weight of the original sample taken, express the mass of the material passing through the sieve in g/kg.

# DIFLUBENZURON WATER- DISPERSIBLE POWDER

Specification WHO/SIF/47  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical diflubenzuron pre-concentrate together with filler(s) and any other necessary formulants and shall be in the form of white to yellowish brown powder that wets out readily on stirring into water. The pre-concentrate diflubenzuron used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/25.

### 1.2 Chemical and physical requirements

The material sampled from any part of the consignment (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

#### 1.2.1 *Diflubenzuron content (g/kg basis)*

The content of diflubenzuron determined by the method described in section 2.1, shall not differ from the nominal content by more than the following amounts:

<i>Nominal content</i>	<i>Tolerance permitted</i>
Up to 500 g/kg	± 5% of the nominal content
Above 500 g/kg	± 25 g/kg

The average content of all samples taken shall not be lower than the nominal content.

#### 1.2.2 *Acidity or alkalinity*

The acidity or alkalinity of the powder, determined by the method described in WHO/M/3, shall not be higher than 2 g/kg calculated as H<sub>2</sub>SO<sub>4</sub> or 2 g/kg calculated as NaOH.

#### 1.2.3 *Sieving after heat stability treatment*

Not less than 98% of the powder after the heat stability treatment (section 2.4) shall pass through a 75 µm sieve when tested by the method described in WHO/M/4.R1.

#### 1.2.4 *Suspensibility*

*In standard hard water after heat stability treatment.* When tested by the method described in section 2.2, a minimum of 60% of the diflubenzuron (6 g/l) shall be in suspension 30 minutes after agitating a suspension containing 10 g/l of diflubenzuron prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.4.

#### 1.2.5 *Wettability*

When tested by the method described in section 2.3, the powder shall be wetted in less than 2 minutes.

#### 1.2.6 *Heat stability*

The powder after treatment as described in section 2.4, shall comply with the requirements of section 1.2.1 and 1.2.2 of this specification.

### **1.3 Packing and marking of packages**

The diflubenzuron water-dispersible powder shall be packed in suitable, clean drums, as specified in the order. The drums shall contain a lining or bag of polyethylene or equivalent, with a nominal thickness of 0.1 mm. The lining or bag shall be hermetically sealed after filling.

All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name  
Diflubenzuron water-dispersible powder to specification WHO/SIF/47  
Diflubenzuron content ... g/kg  
Batch or reference number, and date of test  
Net weight of contents  
Date of formulation

and the following minimum cautionary notice:

Keep out of reach of children. Do not contaminate food or drinking water. Do not breath powder or spray mist. Do not eat or smoke while spraying. Wash hands thoroughly with soap after spraying and before eating or smoking.

## 2. Methods of determining chemical and physical properties

### 2.1 Diflubenzuron content

#### 2.1.1 Outline of method

The active ingredient contained in the sample is dissolved in 1,4-dioxane and linuron is added as internal standard. The diflubenzuron content is determined by high performance liquid chromatography using a reverse-phase column and a mixture of acetonitrile-1,4 dioxane-water as mobile phase.

#### 2.1.2 Special apparatus

*Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and equipped with a pumping system able to maintain a pressure of 25 MPa, a UV spectrophotometer detector able to measure UV absorbance at 254 nm and a recorder.

*Liquid chromatographic column.* The column should be a stainless steel tube, 25 cm length and 4.6 mm internal diameter, packed with  $\leq 10 \mu\text{m}$  C<sub>8</sub> bonded silicagel (Zorbax C<sub>8</sub> or equivalent)<sup>1</sup>.

*Filter.* Acrodisc Disposable Filter Assembly 1,2  $\mu\text{m}$ . Gelman or equivalent.

#### 2.1.3 Special reagents

*Diflubenzuron standard.* Analytical grade of known purity.

*Internal standard.* Linuron. Select a batch which, when chromatographed under the conditions given in section 2.1.5, gives no peak with a similar retention time to diflubenzuron peak.

*Acetonitrile.* HPLC grade.

*1,4-Dioxane.* HPLC grade.

*Mobile phase.* Degassed mixture of acetonitrile, 1,4-dioxane and water:  
45 +10 +45 (v/v).

*Water,* pure. HPLC grade or bi-distilled.

*Solvent mixture.* Acetonitrile-water: 45 + 55 (v/v)..

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<sup>1</sup> Instead of Zorbax TM<sub>BP</sub>-C8 the following supports can be used:

- Spherisorb ODS (RP 18) 5  $\mu\text{m}$
- Bondapak C<sub>18</sub> (RP 18) 10  $\mu\text{m}$
- Zorbax TM<sub>BP</sub>-ODS (RP 18) 7  $\mu\text{m}$

#### 2.1.4 Preparation of standard solution

*Internal standard solution.* Weigh a 25 mg quantity of linuron into a 100 ml volumetric flask, dissolve, dilute to volume with acetonitrile and mix well.

*Diflubenzuron calibration solution.* Weigh (to the nearest 0.01 mg) about 50 mg of diflubenzuron standard into a 100 ml volumetric flask. Add 50 ml of 1,4-dioxane and dissolve by heating in a water bath at 80°C for thirty minutes. Swirl the flask occasionally. Add 40 ml of 1,4-dioxane, cool, dilute to volume with 1,4-dioxane and mix well. Pipette 5 ml of this solution into a 50 ml volumetric flask. Add by pipette 5 ml of internal standard solution. Dilute to volume with the solvent mixture and mix well.

#### 2.1.5 Operating conditions for high-performance liquid chromatography

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperatures	Ambient. Avoid temperature fluctuations of more than 2°C.
Flow rate	1.3 ml. min <sup>-1</sup> .
Detector sensitivity	0.128 AUFS (Absorbance Unit Full Scale).
Wavelength	254 nm.
Injection volume	20 µl (loop type injector).
Chart speed	1 cm. min <sup>-1</sup> .
Retention times	
Linuron	5.2 min.
Diflubenzuron	7.0 min.

#### 2.1.6 System suitability test

Diflubenzuron samples can contain small amounts of p,p'-bis-(4-chlorophenyl)-urea (DIFUR)<sup>2</sup>. This compound elutes just after diflubenzuron. For good quantitative results, the column should give proper resolution for these compounds (R>1).

The resolution can be determined by injection of 20 µl of a solution which contains: 4.2 mg diflubenzuron and 1.8 mg DIFUR dissolved in 100 ml solvent mixture.

Determine the resolution  $R_s$  as follows:  $R_s = \frac{2d}{W_1 + W_2}$

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<sup>2</sup> Request for a sample of DIFUR should be addressed to the manufacturer.

where  $d$  = distance between the peak maxima  
 $W_1$  = peak width of diflubenzuron at the base-line  
 $W_2$  = peak width of DIFUR at the base-line

If necessary, the resolution can be improved by a slight increase or decrease of the water content in the mobile phase.

### 2.1.7 *Sample preparation and analysis*

Homogenize the sample and weigh (to the nearest 0.1 mg) a quantity of sample containing about 1 g of diflubenzuron into a 200 ml volumetric flask. Add 150 ml of 1,4-dioxane and heat in a water bath at 80°C for thirty minutes, swirling the flask occasionally. Add 40 ml of 1,4-dioxane, cool, dilute to volume with 1,4-dioxane and mix well. Pipette 10 ml of this solution into a 100 ml volumetric flask, dilute to volume with 1,4-dioxane and mix well.

Pipette 5 ml of the diluted solution into a 50 ml volumetric flask. Add by pipette, 5 ml of internal standard solution, dilute to volume with the solvent mixture and mix well. Filter a portion of this solution through a 1.2 µm porosity filter Gelman Acrodisc or equivalent, and retain the filtrate for the analysis.

Pump mobile phase through the column until the system is equilibrated (flat base-line). Inject 20 µl of the calibration solution and adjust the operating conditions to give peak heights of 60-80% full scale and a retention time between 6.0 and 8.0 min for diflubenzuron. Allow 12 minutes between injections.

$$r = \frac{\text{area (or height) of diflubenzuron peak}}{\text{area (or height) of internal standard peak}}$$

Repeat the injection of the calibration solution and calculate the response ratio: Response ratios must agree within ± 1%. Average duplicate response ratios obtained by calibration solution injections. The response ratio is approximately 1.7.

Inject duplicate aliquots of sample solution. Average duplicate response ratios for sample solution. Response ratios must agree within ± 1%. If not, repeat the analysis starting with calibration solution injections.

Reinject calibration solution twice. Average response ratios of two calibration solution injections immediately preceding and following sample injections. These must agree within ± 1%. If not, repeat the analysis.

### 2.1.8 Calculation

$$\text{Diflubenzuron content (g / kg)} = \frac{r_2 \times w_1 \times P \times V}{r_1 \times w_2}$$

where

- $r_1$  = average response ratio for calibration solution
- $r_2$  = average response ratio for sample solution
- $w_1$  = mass of diflubenzuron standard in the calibration solution (g)
- $w_2$  = mass of sample taken (g)
- $P$  = purity of diflubenzuron standard (g/kg)
- $V$  = dilution factor of the sample (=20)

## 2.2 Suspensibility

### 2.2.1 Outline of method

A suspension of known concentration of diflubenzuron in standard hard water is prepared, poured into a 250 ml graduated cylinder, maintained at a constant temperature, and allowed to remain undisturbed for 30 minutes. The top 9/10 ths are drawn off and the content of diflubenzuron in the bottom 1/10 th is determined, so allowing to evaluate the active ingredient mass still in suspension after 30 minutes.

### 2.2.2 Special apparatus

1. A 250 ml graduated cylinder with ground-glass stopper and a distance of 20-21.5 cm between the bottom and the 250 ml calibration mark.
2. A glass tube, about 40 cm long and about 5 mm in internal diameter, pointed at one end to an opening of 2-3 mm, the other end being connected to a suitable source of suction.

### 2.2.3 Special reagents

*Standard hard water.* Dissolve 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in distilled water and make up to one litre. This provides water with a hardness of 342 mg/l, calculated as calcium carbonate. Check the hardness by method WHO/M/26 and correct if appropriate.

#### 2.2.4 Procedure

Weigh accurately into a 100 ml beaker an amount of the sample to form 250 ml of a suspension containing 10 g/l of diflubenzuron. Add a volume of hard water at  $30 \pm 1^{\circ}\text{C}$  equal to at least twice the mass of the sample taken. Allow to stand for 30 seconds and then stir by hand for 30 seconds with a glass rod, 4-6 mm in diameter, at not more than four revolutions per second, making no deliberate attempt to break up any lumps. Then immediately transfer the mixture quantitatively to the 250 ml graduated cylinder, using hard water at  $30 \pm 1^{\circ}\text{C}$  for rinsing, and again avoiding mechanical disintegration of any lumps. Immediately add sufficient hard water at  $30 \pm 1^{\circ}\text{C}$  to bring the volume up to the 250 ml mark, insert the stopper, and invert the cylinder end-over-end 30 times at a rate of one complete cycle every two seconds. During agitation the cylinder must be thermally insulated from the hands to maintain the prescribed temperature of the suspension. This operation should be carried out as smoothly as possible, keeping the axis of rotation fixed. Allow the graduated cylinder to stand for 30 minutes in a water-bath at  $30 \pm 1^{\circ}\text{C}$ , care being taken that the bath is free from vibrations.

Should excessive flocculation occur during the test, accompanied by the appearance of transparent liquid, the material is unsatisfactory. At the end of the 30 minutes settling period, insert the glass tube into the cylinder and, with a minimum of disturbance, withdraw during 10-15 seconds by means of the suction tube nine-tenths of the suspension, i.e. 225 ml. This is achieved by maintaining the tip of the glass tube just below the sinking top level of the suspension. Discard the suspension withdrawn.

Determine the mass ( $m_1$  g) of diflubenzuron in the bottom one-tenth of suspension from the suspensibility test by transferring it quantitatively with water to a tared evaporating basin (a g), remove the water by heating on a boiling water-bath, dry in an oven at  $100^{\circ}\text{C}$  for 30 minutes, cool and reweigh (b g).

The mass of the residue in the basin ( $c$  g) = ( $b - a$ ). Homogenize the residue. Depending on the mass  $c$ , take an aliquot (9 g) (weighed to the nearest 0.1 mg) containing about 0.5 g diflubenzuron and transfer into a 100 ml volumetric flask. Continue as in 2.1.7 here above by "adding 150 ml of 1,4-dioxane...".

#### 2.2.4 Calculation

Mass ( $m_1$  g) of diflubenzuron in the retained one-tenth of the suspension

$$m_1 = \frac{r_2 \times w_1 \times c \times P}{r_1 \times q \times 1000}$$

where:  $r_1$  = average response ratio for calibration solution  
 $r_2$  = average response for sample solution  
 $w_1$  = mass of diflubenzuron standard in the calibration solution (g)  
 $q$  = mass of the aliquot of sample taken in the residue (g)  
 $c$  = mass of the residue (g)  
 $P$  = purity of diflubenzuron standard (g/kg)

From the value obtained in section 2.1.8 for the content of diflubenzuron, calculate the mass of diflubenzuron ( $m_2$ ) in the initial sample taken for the suspensibility test.

$$\text{Suspensibility (\%)} = \frac{(m_2 - m_1) \times 111.1}{m_2}$$

where  $m_1$  = mass (g) of diflubenzuron found in the retained one tenth of the suspension  
 $m_2$  = mass (g) of diflubenzuron in the initial sample

### 2.3 Wettability

#### 2.3.1 Apparatus

250 ml beaker, internal diameter  $6.5 \pm 0.5$  cm, height  $9.0 \pm 0.5$  cm

#### 2.3.2 Reagent

*Standard hard water.* Dissolve 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in distilled water and make up to one litre. This provides water with a hardness of 342 mg/l, calculated as calcium carbonate. Check the hardness by method WHO/M/26 and correct if appropriate.

### 2.3.3 Procedure

Pour 100 ml of standard hard water into the beaker. Weigh (to the nearest 0.1 g) 5 g of powder, taking care that it remains in a non-compacted state. Add all the powder at once, by dropping it on the water, from a position level with the rim of the beaker, without undue agitation of the liquid surface. When the powder is added start the stop watch and note the time taken for it to become completely wetted. A film of fine particles remaining on the surface may be neglected. Report the time to the nearest second required for complete wetting of the powder.

## 2.4 Heat stability treatment

Fill a 50 ml<sup>3</sup> wide-mouthed glass bottle to within 1 cm of the top of the sample. Seal the bottle with a phenolic plastic cap having a soft liner which will ensure a good seal. Turn the cap firmly to assure a tight seal and place in a forced-draught oven maintained at  $54 \pm 1^{\circ}\text{C}$  for 3 days. At the end of the heating period, remove from the oven and allow to come to room temperature before removing the cap.

After completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

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<sup>3</sup> 100 ml if a larger quantity of the sample is required.