

## B. GENERAL TESTS

### Fats and Related Substances Tests

The Fats and Related Substances Tests are designed to determine the ester value, saponification value, acid value, and hydroxyl value of fats and related substances such as fatty acids, higher aliphatic alcohols, and fatty acid esters, excluding flavoring substances.

#### 1. Ester Value

The ester value is the number of mg of potassium hydroxide (KOH) required to saponify the esters in 1 g of a sample.

Hereinafter in the Monographs, such a specification as " 125 - 164 (Fats and Related Substances Tests) " indicates that when determined as directed in the procedure below, the ester value is 125 - 164.

**Procedure** Unless otherwise specified, determine the saponification value and the acid value, and calculate the ester value by the following formula:

$$\text{Ester value} = \text{Saponification value} - \text{Acid value}$$

#### 2. Saponification Value

The saponification value is the number of mg of potassium hydroxide (KOH) to saponify the esters in 1 g of the sample and neutralize the free acids in 1 g of a sample.

**Procedure** Unless otherwise specified, proceed as follows: Weigh accurately about 1 g of the sample, transfer into an Erlenmeyer flask, add 40 ml of ethanol, and dissolve while warming if necessary. Add 20 ml of ethanolic potassium hydroxide TS, accurately measured, equip the flask with a reflux condenser, and heat in a water bath for 30 minutes while shaking the flask occasionally. Cool, add a few drops of phenolphthalein TS, and immediately titrate excess potassium hydroxide with 0.5 mol/l hydrochloric acid. Perform a blank test, and calculate the saponification value by the formula

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}}$$

where a = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the blank test,

b = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the test.

#### 3. Acid Value

The acid value is the number of mg of potassium hydroxide (KOH) required to neutralize 1 g of a sample.

Hereinafter in the Monographs, such specification as " not more than 15 (Fats and Related Substances Tests) " indicates that when determined as directed in the follow-

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ing procedure, the acid value is not more than 15.

**Procedure** Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of the sample given in the table, according to the acid value of the table, and, unless otherwise specified, add 50 ml of an ethanol - ether mixture (1 : 1). Dissolve while heating if necessary, and use this solution as the test solution. Cool, add a few drops of phenolphthalein TS, titrate with 0.1 mol/l ethanolic potassium hydroxide until the pink color of the solution persists for 30 seconds, and calculate the acid value by the formula below. Before using the solvent, add 0.1 mol/l ethanolic potassium hydroxide to the solvent until its pink color persists for 30 seconds, using phenolphthalein TS as an indicator.

$$\text{Acid value} = \frac{\text{Volume (ml) of 0.1 Mol/l ethanolic potassium hydroxide consumed}}{\text{Weight (g) of the sample}} \times 5.611$$

**Table**

Acid value	Weight (g) of sample
Less than 5	10
5 to less than 15	5
15 to less than 50	3
50 to less than 120	1
Not less than 120	0.5

### 4. Hydroxyl Value

The hydroxyl value is the number of mg of potassium hydroxide (KOH) required to neutralize acetic acid combined to hydroxyl groups, when 1 g of a sample is acetylated under the following conditions.

Hereinafter in the Monographs, such a specification as “ 155 - 187 (Fats and Related Substances Tests), provided the acid value is assumed to be 0 ” indicates that when determined as directed in the following procedure by assuming the acid value to be 0, the hydroxyl value is 155 - 187.

**Procedure** Unless otherwise specified, proceed as follows: Weigh accurately 1 g of the sample, transfer into a round-bottom flask shown in the figure, and add 5 ml of acetic anhydride - pyridine TS, accurately measured. Place a small funnel on the neck of the flask, and heat for 1 hour while immersing the flask to the depth of about 1 cm from the bottom into an oil bath at 95 - 100 . Cool, add 1 ml of water, shake well, and heat for 10 minutes. After cooling, rinse the funnel and the neck of the flask with 5 ml of ethanol, and titrate with 0.5 mol/l ethanolic potassium hydroxide (indicator: 1 ml of phenolphthalein TS). Perform a blank test, and calculate the

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hydroxyl value by the formula

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}} + \text{acid value}$$

where a = volume (ml) of 0.5 mol/l ethanolic potassium hydroxide consumed in the blank test,

b = volume (ml) of 0.5 mol/l ethanolic potassium hydroxide consumed in the test.

