

## B. GENERAL TESTS

### Flavoring Substances Tests

#### 1. Alcohol Content

The alcohol content is the quantity of alcohols contained in a sample.

**Procedure** Unless otherwise specified, proceed as directed in Method 1 or Method 2, as appropriate.

**Method 1** Measure exactly 10 ml of the sample, transfer into a 100-ml flask, and add 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate. Connect the flask to an air condenser, and boil the mixture gently on a hot-plate for 1 hour. Allow to cool for 15 minutes, and add 50 ml of water. Heat the mixture in a water bath for 15 minutes while shaking occasionally. Cool, transfer the mixture into a separating funnel, and remove the aqueous layer. Wash the oil layer with anhydrous sodium carbonate solution (1 : 8) until the washings become alkaline, wash further with sodium chloride solution (1 : 10) until the washings become neutral. Transfer the oil layer into a dry container, add about 2 g of anhydrous sodium sulfate, shake well, allow to stand for about 30 minutes, and filter. Weigh accurately the specified quantity of the acetylated oil thus obtained, and determine the ester value as directed under Ester Value in the Flavoring Substances Tests. This ester value, also referred to as acetyl value, is calculated by the formula

$$\text{Acetyl value} = \frac{(a - b) \times 28.05}{\text{Weight(g) of acetylated oil}}$$

Calculate the alcohol content by the formula

$$\begin{aligned} \text{Alcohol content} &= \frac{\text{Molecular weight of alcohol} \times (a - b) \times 0.5}{[\text{Weight (g) of acetylated oil} - 0.02102(a - b)] \times 1,000} \times 100(\%), \\ &= \frac{\text{Acetyl value} \times \text{Molecular weight of alcohol}}{561.1 - (0.4204 \times \text{Acetyl value})} (\%), \end{aligned}$$

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

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

**Method 2** Weigh accurately the specified quantity of the sample, transfer into a 200-ml flask with a ground-glass stopper, and add 5 ml of acetic anhydride-pyridine TS, exactly measured. Moisten the ground-glass joint with 2 to 3 drops of pyridine, stopper lightly, and heat in a water bath for 1 hour. Cool, and add 10 ml of water to wash the stopper and the inside of the flask. Stopper tightly, shake well, and cool to ordinary temperature. Wash the ground-glass joint and the inside of the flask with 5 ml of neutralized ethanol, and titrate with 0.5 mol/l ethanolic potassium hydroxide TS. Confirm the end point using a indicator ( 2 to 3 drops of cresol red - thymol blue TS) or a potentiometer. Perform a blank test in the same manner as for the test solution, and calculate the alcohol content by the formula

$$\text{Alcohol content} = \frac{\text{Molecular weight of alcohol} \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1,000} \times 100(\%),$$

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

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

### 2. Aldehyde and Ketone Content

Aldehyde and Ketone Content is obtained using the property that aldehyde or ketone reacts to hydroxylamine (NH<sub>2</sub>OH).

**Procedure** Unless otherwise specified, proceed directed in Method 1 or Method 2, as appropriate.

**Method 1** Weigh accurately the specified quantity of a sample, add 50 ml of 0.5 mol/l hydroxylamine hydrochloride, exactly measured. Either shake it well, and allow to stand for the time specified in the individual monograph, or gently boil it with a reflux condenser, in a water bath for the time specified in the individual monograph, and cool to room temperature. Titrate the liberated acid with 0.5 mol/l ethanolic potassium hydroxide. Confirm the end point, using a potentiometer, or, macroscopically by checking the change in the color of the solution to green-yellow. Perform a blank test, make necessary correction, and calculate the content by the formula

Aldehyde and Ketone content

$$= \frac{\text{Molecular weight of aldehyde or ketone} \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1,000} \times 100(\%),$$

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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 test.

**Method 2** Weigh accurately the specified quantity of a sample, add 75 ml of hydroxylamine TS, exactly measured. Either shake it well, and allow to stand for the time specified in the individual monograph or gently boil it, equipped with a reflux condenser, in a water bath for the time specified in the individual monograph, and cool to room temperature, and titrate excess hydroxylamine with 0.5 mol/l hydrochloride acid. Confirm the end point, using a potentiometer, or, macroscopically by checking the change in the color of the solution from purple to green-yellow. Perform a blank test, make necessary correction, and calculate the content by the formula

Aldehyde and Ketone content

$$= \frac{\text{Molecular weight of aldehyde or ketone} \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1,000} \times 100(\%)$$

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. Ester Value

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

Hereinafter in the Monographs, such a specification as "not more than 3.0 (5 g, Flavoring Substances Tests)" indicates that when determined by weighing about 5 g of the test substance and proceeding as directed in the following procedure, the ester value is not more than 3.0.

**Procedure** Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of a sample, transfer into a 200-ml flask, add 10 ml of ethanol and 3 drops of phenolphthalein TS, and neutralize with potassium hydroxide solution (1 250). Add 25 ml of 0.5 mol/l ethanolic potassium hydroxide, exactly measured, equip the flask with a reflux condenser, and gently boil in a water bath for 1 hour. Cool, and titrate the excess potassium hydroxide with 0.5 mol/l hydrochloride acid. Confirm the end point using a indicator ( 2 to 3 drops of phenolphthalein TS) or a potentiometer. Perform a blank test in the same manner, and calculate the ester value by the formula

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

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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.

### 4. Ester Content

The content of ester of monobasic acid is obtained by performing the test as directed under Ester Value in Flavoring Substances Tests and then calculating by the formula

$$\begin{aligned} \text{Ester content} &= \frac{\text{Molecular weight of ester} \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1,000} \times 100 (\%), \\ &= \frac{\text{Ester value} \times \text{Molecular weight of ester}}{561.1} (\%), \end{aligned}$$

where a and b are the a and the b, respectively, indicated in Ester Value.

### 5. Halogenated Compounds

Halogenated Compounds are identified by utilizing the flame coloration of copper chloride.

**Procedure** Use a copper gauze of 1.5 cm in width, 5 cm in length, and about 1 mm in mesh, which is wound around the end of a copper wire. Heat the copper gauze in a colorless flame of the burner until no green color is observed in the flame, cool, and repeat this procedure several times. After cooling, moisten the copper gauze with 2 drops of the sample, and burn. Repeat this procedure three times, and burn the gauze in the outer edge of a colorless flame adjusted to the height of about 4 cm. No green color develops in the flame.

### 6. Saponification Value

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

**Procedure** Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of a sample, and transfer into a 200-ml flask. Add 25 ml of 0.5 mol/l ethanolic potassium hydroxide, exactly measured, equip the flask with a reflux condenser, and gently boil in a water bath for 1 hour. Cool, and titrate the excess alkali with 0.5 mol/l hydrochloride acid. Confirm the end point using a indicator (1ml of phenolphthalein TS) or a potentiometer. Perform a blank test in the same manner, and calculate the saponification value by the formula

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

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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.

### 7. 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 a specification as "not more than 6.0 (Flavoring Substances Tests)" indicates that when determined as directed in the following procedure, the acid value is not more than 6.0.

**Procedure** Unless otherwise specified, proceed as follows: To about 10 g of a sample, accurately weighed, add about 50 ml of neutralized ethanol, dissolve by warming if necessary, and add several drops of phenolphthalein TS. Titrate while shaking occasionally with 0.1 mol/l potassium hydroxide, using a microburet until a light pink color persists for 30 seconds, or a potentiometer. Calculate the acid value by the following formula

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

### 8. Phenol Content

The phenol content is the quantity of alkali hydroxide-soluble substances contained in a sample.

**Procedure** Unless otherwise specified, proceed as follows: Transfer 10 ml of a sample, exactly measured, into a 150-ml cassia flask, and add 75 ml of 1 mol/l potassium hydroxide divided into 3 portions while shaking well. Shake well for 5 minutes, allow to stand for 30 minutes, and gradually add 1 mol/l potassium hydroxide to raise the insoluble oil layer to the graduated part of the cassia flask. Allow to stand for 1 hour, read the volume of the insoluble oil (ml), and calculate the content by the formula

$$\text{Phenol content} = 10 \times [10 - \text{Volume (ml) of insoluble oil}] (\% \text{ vol}).$$