2.5. ASSAYS

01/2005:20501

2.5.1. ACID VALUE

The acid value \( I_A \) is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the free acids present in 1 g of the substance. Dissolve 10.00 g of the substance to be examined, or the quantity prescribed (\( m \) g) in 50 ml of a mixture of equal volumes of alcohol \( R \) and ether \( R \), previously neutralised with 0.1 M potassium hydroxide, unless otherwise specified, using 0.5 ml of phenolphthalein solution \( R1 \) as indicator. When the substance to be examined has dissolved, titrate with 0.1 M potassium hydroxide until the pink colour persists for at least 15 s (\( n \) ml of 0.1 M potassium hydroxide).

\[
I_A = \frac{5.610n}{m}
\]

01/2005:20502

2.5.2. ESTER VALUE

The ester value \( I_E \) is the number that expresses in milligrams the quantity of potassium hydroxide required to saponify the esters present in 1 g of the substance. It is calculated from the saponification value \( I_S \) and the acid value \( I_A \):

\[
I_E = I_S - I_A
\]

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2.5.3. HYDROXYL VALUE

The hydroxyl value \( I_{OH} \) is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the alcohol combined by acylation in 1 g of the substance.

METHOD A

Introduce the quantity of the substance to be examined shown in Table 2.5.3.1 (\( m \) g) into a 150 ml acetylation flask fitted with an air condenser, unless another quantity is shown in Table 2.5.3.1 (\( n \) ml of 0.1 M potassium hydroxide). Close the flask and allow to cool. Add 5 ml of water about 2.5 cm above the level of the liquid in the flask. Heat the flask in a water-bath for 1 h. Shaking frequently add sufficient pyridine \( R \) to clear it, noting the volume added. Shake the flask and replace in the water-bath for 10 min. Withdraw the flask and allow to cool. Rinse the condenser and the walls of the flask with 5 ml of alcohol \( R \), previously neutralised to phenolphthalein solution \( R1 \). Titrate with 0.5 M alcoholic potassium hydroxide using 0.2 ml of phenolphthalein solution \( R1 \) as indicator (\( n_1 \) ml of 0.5 M alcoholic potassium hydroxide). Carry out a blank test under the same conditions (\( n_2 \) ml of 0.5 M alcoholic potassium hydroxide).

\[
I_{OH} = \frac{2.0 \times 0.5 (n_2 - n_1)}{m} + I_A
\]

METHOD B

Introduce the prescribed quantity of the substance to be examined (\( m \) g) into a perfectly dry 5 ml conical flask fitted with a ground-glass or suitable plastic stopper and add 2.0 ml of propionic anhydride reagent \( R \). Close the flask and shake gently to dissolve the substance. Allow to stand for 2 h unless otherwise prescribed. Remove the stopper and transfer the flask and its contents into a wide-mouthed 500 ml conical flask containing 25.0 ml of a 9 g/l solution of aniline \( R \) in cyclohexane \( R \) and 30 ml of glacial acetic acid \( R \). Stir the contents of the flask, allow to stand for 5 min, add 0.05 ml of crystal violet solution \( R \) and titrate with 0.1 M perchloric acid until an emerald-green colour is obtained (\( n_1 \) ml of 0.1 M perchloric acid). Carry out a blank test under the same conditions (\( n_2 \) ml of 0.1 M perchloric acid).

\[
I_{CH} = \frac{5.610(n_1 - n_2)}{m}
\]

To take account of any water present, determine this (\( y \) per cent) by the semi-micro determination of water (2.5.12). The hydroxyl value is then given by the equation:

\[
I_{OH} = (\text{hydroxyl value as determined}) - 31.1y
\]

01/2005:20504

2.5.4. IODINE VALUE

The iodine value \( I_I \) is the number that expresses in grams the quantity of halogen, calculated as iodine, that can be fixed in the prescribed conditions by 100 g of the substance.

When the monograph does not specify the method to be used, method A is applied. Any change from method A to method B is validated.

METHOD A

Unless otherwise prescribed, use the following quantities (Table 2.5.4.1) for the determination.

<table>
<thead>
<tr>
<th>Presumed value ( I_I )</th>
<th>Quantity of sample (g)</th>
<th>Volume of acetylation reagent (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 100</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>100 - 150</td>
<td>1.5</td>
<td>5.0</td>
</tr>
<tr>
<td>150 - 200</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>200 - 250</td>
<td>0.75</td>
<td>5.0</td>
</tr>
<tr>
<td>250 - 300</td>
<td>0.60 or 1.20</td>
<td>5.0 or 10.0</td>
</tr>
<tr>
<td>300 - 350</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>350 - 700</td>
<td>0.75</td>
<td>15.0</td>
</tr>
<tr>
<td>700 - 950</td>
<td>0.5</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Heat the flask in a water-bath for 1 h keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask and allow to cool. Add 5 ml of water \( R \) through the upper end of the condenser. If a cloudiness appears add sufficient pyridine \( R \) to clear it, noting the volume added. Shake the flask and replace in the water-bath for 30 min unless otherwise prescribed, shaking frequently. Add 10 ml of a 100 g/l solution of potassium iodide \( R \) and 100 ml of water \( R \). Titrate with 0.1 M sodium anhydride solution \( R1 \).