2.5. ASSAYS

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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 *ethanol (96 per cent) R* and *light petroleum R3*, previously neutralised with *0.1 M potassium hydroxide* or *0.1 M sodium hydroxide*, unless otherwise specified, using 0.5 mL of *phenolphthalein solution R1* as indicator. If necessary, heat to about 90 °C to dissolve the substance to be examined. When the substance to be examined has dissolved, titrate with *0.1 M potassium hydroxide* or *0.1 M sodium hydroxide* until the pink colour persists for at least 15 s (*n* mL of titrant). When heating has been applied to aid dissolution, maintain the temperature at about 90 °C during the titration.

$$I_A = \frac{5.610n}{m}$$

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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 acid 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 prescribed in the monograph. Add the quantity of *acetic anhydride solution R1* stated in Table 2.5.3.-1 and attach the air condenser.

Table 2.5.3.-1

Presumed value I_{OH}	Quantity of sample (g)	Volume of acetylating reagent (mL)
10 - 100	2.0	5.0
100 - 150	1.5	5.0
150 - 200	1.0	5.0
200 - 250	0.75	5.0
250 - 300	0.60 or 1.20	5.0 or 10.0
300 - 350	1.0	10.0
350 - 700	0.75	15.0
700 - 950	0.5	15.0

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 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*).

$$I_{OH} = \frac{28.05\,(n_2 - n_1)}{m} + I_A$$

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*. Swirl 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*).

$$I_{OH} = \frac{5.610\,(n_1 - n_2)}{m}$$

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:

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

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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 2.5.4.-1

Presumed value I_I	Quantity of sample (g)
less than 20	1.0
20 - 60	0.5 - 0.25
60 - 100	0.25 - 0.15
more than 100	0.15 - 0.10

Introduce the prescribed quantity of the substance to be examined (*m* g) into a 250 mL flask fitted with a ground-glass stopper and previously dried or rinsed with *glacial acetic acid R*, and dissolve it in 15 mL of *chloroform R* unless otherwise prescribed. Add very slowly 25.0 mL of *iodine bromide solution R*. Close the flask and keep it in the dark 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 thiosulfate*, shaking vigorously until the yellow colour is almost discharged. Add 5 mL of *starch solution R* and continue the titration adding the *0.1 M sodium*