SPECIFICATION & TEST PROCEDURE

ACETYLSALICYLIC ACID Ph.Eur

Molecular Formula : $C_9H_8O_4$ **CAS Registry No. : [50 – 78 – 2]**

Molecular Weight : 180.2 **Reference : EP 8.0**

Other Names : 2 – (Acetyloxy) benzoic acid.

TESTS		**SPECIFICATIONS**
1. Appearance	:	White or almost white, crystalline powder or colourless crystals.
2. Solubility	:	Slightly soluble in water, freely soluble in ethanol (96 percent).
3. Melting point	:	About 143 ºC (Instantaneous Method)
4. Identification	:	First identification : A, B. Second identification : B, C, D.
A. IR absorption spectrum	:	The absorption maxima in the spectrum of sample corresponds in position and relative size to similarly recorded spectrum of Aspirin WRS.
B. Melting point of precipitate	:	156 º to 161 º C
C. Colour test.	:	The colour becomes blue.
D. Reaction of Salicylates	:	The solution gives reaction (a) of Salicylates.
5. Appearance of solution	:	The solution is clear and colourless.

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ACETYLSALICYLIC ACID Ph.Eur

TESTS		**SPECIFICATIONS**
6. Related Substances (%)		
Impurity A – 4-Hydroxybenzoic acid	:	NMT 0.15
Impurity B – 4-Hydroxyisophthalic acid	:	NMT 0.15
Impurity C – Salicylic acid	:	NMT 0.15
Impurity D – Acetylsalicylsalicylic acid	:	NMT 0.15
Impurity E – Salicylsalicylic acid	:	NMT 0.15
Impurity F – Acetylsalicylic anhydride	:	NMT 0.15
Unspecified Impurity	:	NMT 0.05
Total Impurities	:	NMT 0.25
7. Heavy metals (ppm)	:	Maximum 20
8. Loss on drying (%, w/w) (Determined on 1.000g by drying in vacuo)	:	Maximum 0.5
9. Sulphated Ash (%, w/w, determined on 1.0g)	:	Maximum 0.1
10. Assay (%, w/w, dried substance)	:	99.5 to 101.0

3. MELTING POINT

About 143ºC (Instantaneous Method)

3.1 APPARATUS AND REAGENTS

3.1.1 Melting point apparatus (Instantaneous)

3.2 PROCEDURE

3.2.1 Heat the block at a suitably rapid rate to a temperature about 10 °C below the presumed melting temperature, then adjust the heating rate to about 1°C per minute.

3.2.2 At regular intervals drop a few particles of powdered substance onto the block in the vicinity of the thermometer bulb, cleaning the surface after each test.

3.2.3 Record the temperature t_1 at which the substance melts instantaneously for the first time in contact with the metal. Stop heating.

3.2.4 During cooling drop a few particles of the substance at regular intervals on the block, cleaning the surface after each test.

3.2.5 Record the temperature t_2 at which the substance ceases to melt instantaneously when it comes in contact with the metal.

3.2.6 Calculate the instantaneous melting point of the substance using the expression $(t_1 + t_2) / 2$

4. IDENTIFICATION

First identification : A, B.

Second identification : B, C, D.

4 A. IR absorption spectrum :

Infrared absorption spectrum comparison with acetylsalicylic acid CRS or WRS.

4.A.1. APPARATUS AND REAGENTS

4.A.1.1. Analytical Balance
Mortar & Pestle
Hydraulic pellet press
IR Spectrophotometer
Hot air oven

4.A.1.2. Potassium bromide, finely powdered
Aspirin WRS

4.A.2 PROCEDURE

4.A.2.1 Transfer 1 to 2 mg of Aspirin WRS to mortar.

4.A.2.2 Mix it with 300 to 400 mg of Potassium bromide, finely powdered and dried at 250 º C for one hour previously.

4.A.2.3 Grind the mixture carefully with the pestle and spread it uniformly in a suitable die.

4.A.2.4 Compress it to a pressure of about 800 MPa (8 t. cm^{-2}) in a Hydraulic pellet press, so that a uniform disc is formed.

4.A.2.5 Remove the disc carefully from the press and record the IR absorption spectrum between 4000 – 650 cm^{-1} (2.5 – 15.4 μm) using IR Spectrophotometer.

4.A.2.6 Prepare a disc of the substance to be examined by the same procedure as mentioned above and record the IR absorption spectrum of the sample under the same operational conditions.

4.A.2.7 The transmission minima (absorption maxima) in the spectrum obtained with the sample corresponds in position and relative size to those in the spectrum obtained with Aspirin WRS.

4 B. Melting point of precipitate:

To 0.2 g add 4 ml of dilute sodium hydroxide solution and boil for 3 min. Cool and add 5 ml of dilute sulphuric acid. A crystalline precipitate is formed. Filter, wash the precipitate and dry at 100 – 105°C. The melting point is 156°C to 161°C.

4.B.1. APPARATUS & REAGENTS

4.B.1.1 Analytical balance
Glass beaker, 100 ml
Filter assembly
Pipettes, 5 ml
Hot plate
Melting point apparatus
Hot air oven

4.B.1.2 Dilute Sodium hydroxide solution
Dilute Sulphuric acid.

4.B.2. PROCEDURE

4.B.2.1 Weigh about 0.2 g of substance in a 100 ml glass beaker; add 4 ml of dilute sodium hydroxide solution and boil for 3 minutes.

4.B.2.2 Cool and add 5 ml of dilute sulphuric acid. A crystalline precipitate is formed.

4.B.2.3 Filter, wash the precipitate with water and dry at 100 – 105°C.

4.B.2.4 Check the melting point of the dried precipitate. It is 156° to 161°C.

Note: Preserve the precipitate for test 4D.

4C Colour test:

In a test tube mix 0.1 g with 0.5 g of calcium hydroxide. Heat the mixture and expose to the fumes produced a piece of filter paper impregnated with 0.05 ml of nitrobenzaldehyde solution. A greenish-blue or greenish-yellow colour develops on the paper. Moisten the paper with dilute hydrochloric acid. The colour becomes blue.

4.C.1 APPARATUS & REAGENTS

4.C.1.1 Analytical balance
Test tube
Pipettes, 1 ml
Hot plate
Filter paper

4.C.1.3 Calcium hydroxide
Nitrobenzaldehyde solution
Dilute hydrochloric acid

4.C.2 PROCEDURE

4.C.2.1 Mix 0.1 g of test sample with 0.5 g of Calcium hydroxide in a test tube.

4.C.2.2 Heat the mixture on a Hot plate.

4.C.2.3 Expose to the fumes produced a piece of filter paper impregnated with 0.05 ml of nitrobenzaldehyde solution.

4.C.2.4 A greenish – blue or greenish – yellow colour develops on the paper.

4.C.2.5 Moisten the paper with dilute hydrochloric acid.

4.C.2.6 The colour becomes blue.

4D Reaction of Salicylates:

Dissolve with heating about 20 mg of the precipitate obtained in identification test B in 10 ml of water and cool. The solution gives reaction (a) of salicylates (2.3.1)

4.D.1 APPARATUS & REAGENTS

4.D.1.1Glass beaker, 100 ml
Pipettes, 1 ml, 10 ml
Hot Plate
Test tube.

4.D.1.2 Ferric chloride solution
Acetic acid 5M.

4.D.2 PROCEDURE

4.D.2.1 Take about 20 mg of precipitate obtained in identification test B

in 10 ml of water in a glass beaker and dissolve by heating on Hot plate.

4.D.2.2 Cool and take 1 ml of the solution in to a test tube and add 0.5 ml of Ferric chloride solution.

4.D.2.3 A violet colour is produced.

4.D.2.4 Add 0.1 ml of Acetic acid 5M to the above solution. The violet colour persists.

5 APPEARANCE OF SOLUTION

Dissolve 1.0 g in 9 ml of ethanol (96 per cent). The solution is clear (2.2.1) and colourless (Method II, 2.2.2).

5.1 APPARATUS AND REAGENTS

5.1.1 Analytical balance
Nessler Cylinders, 50 ml
Pipettes, 10 ml.

5.1.2 Reference solution B9
Ethanol (96 %).

5.2 PROCEDURE

5.2.1 Dissolve 1 g of sample in 9 ml of ethanol (96 %) in a nessler cylinder.

5.2.2 In another nessler cylinder take 10 ml of ethanol (96 %).

5.2.3 After 5 minutes, compare the solutions of nessler cylinders in diffused daylight, viewing vertically against a black background.

5.2.4 The test solution is considered ***clear*** if its clarity is the same as that of ethanol (96 %).

5.2.5 Prepare reference solution B9 in another nessler cylinder and compare the test solution with it in diffused daylight, viewing vertically against a white background.

5.2.6 The test solution is considered ***colourless*** if it is not more intensely coloured than reference solution B9.

6 RELATED SUBSTANCES

By liquid chromatography. Prepare the solutions immediately before use.

6.1 APPARATUS AND REAGENTS

6.1.1 A High performance Liquid Chromatograph - Shimadzu VP series.

A stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel for chromatography R (5 µm).

Analytical Balance
Filter assembly with 0.45µ membrane filter
Volumetric flasks, 10ml, 50 ml, 100 ml
Pipettes, 1 ml, 5 ml.

6.1.2 Phosphoric acid
Acetonitrile
Water

6.2.PREPARATION OF MOBILE PHASE:

6.2.1 Mix thoroughly Phosphoric acid, Acetonitrile and Water in the ratio of 2 : 400 : 600 (V/V/V).

6.2.2 Filter through 0.45µ membrane filter.

6.3 CHROMATOGRAPHIC CONDITIONS:

Flow rate	:	1.0 ml / min.
Detection	:	At 237 nm
Injection	:	10 µl
Run time	:	50 min.

6.4 PREPARATION OF SOLUTIONS:

Test solution: Dissolve 0.100 g of the substance in Acetonitrile and dilute to 10 ml with the same solvent.

Reference solution (a): Dissolve 50 mg of Salicylic acid in the mobile phase and dilute to 50 ml with the mobile phase.
Dilute 1 ml of this solution to 100 ml with the mobile phase.

Reference solution (b): Dissolve 10 mg of Salicylic acid in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of this solution add 0.2 ml of test solution and dilute to 100 ml with the mobile phase.

Stock solution: Weigh accurately 25 mg of each impurity and transfer carefully to 100 ml volumetric flask. Dissolve in and dilute to the mark with Acetonitrile.
Impurity Mix Solution: Dilute 1 ml of stock solution to 10 ml with Acetonitrile.

Reference solution (c): Dissolve with the aid of ultrasound the contents of a vial of acetylsalicylic acid for peak identification CRS (Containing impurities A, B, D, E and F) in 1.0ml of Acetonitrile.

Identification of impurities: Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C. Use the chromatogram obtained with acetylsalicylic acid for peak identification CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, D, E and F.

Note: As the new lot available for peak identification CRS. Inject separately reference solution C and impurity mix solution. Comparison will be made for both the chromatogram once to identify the peaks due to A, B, D, E and F. This activity will be done once in three years or if new lot arrival in the market whichever is earlier and also if any chemical expires that is used in the preparation of impurity mix stock solution. Basing on the outcome of results it is concluded that the impurity mix solution will be injected in place of peak identification CRS at the end of every analysis.

Impurity	Relative Retention times
4-Hydroxybenzoic acid	0.7
4-Hydroxyisophthalic acid	0.8
Salicylic acid	1.3
Acetylsalicylsalicylic acid	2.3
Salicylsalicylic acid	3.2
Acetylsalicylic anhydride	6.0

6.5 EVALUATION OF SYSTEM SUITABILITY:

6.5.1 Inject 10µl of Acetonitrile solution into the chromatograph and record the chromatogram up to 50 minutes. Examine the mobile phase for any extraneous peaks and disregard corresponding peaks observed in the chromatogram of the test solution.

6.5.2 Inject 10µl of reference solution (b) into the chromatograph and record the chromatogram up to 20minutes. The resolution between the peaks due to Acetylsalicylic acid and Impurity C is not less than 6.0.

6.5.3 Inject 10µl of reference solution (a) into the chromatograph and record the chromatogram up to 20 minutes.

6.6 PROCEDURE:

6.6.1 Inject 10µl of test solution into the chromatograph and record the chromatogram up to 50 minutes. Disregard any peaks other than known impurities with an area less than 0.30 times the area of the principal peak in the chromatogram obtained with reference solution (a).

6.6.2 Inject 10µl of impurity mix solution into the chromatograph and record the chromatogram until all the impurities are eluted for identification of impurities.

6.7 CALCULATION:

Calculate the percentage of all the impurities using the following formula

$$\text{Impurity \%} = \frac{\text{Area of the impurity} \times 0.1}{\text{Area of Salicylic acid peak in Ref. Solution (a)}}$$

7 HEAVY METALS

Dissolve 1.0 g in 12 ml of acetone and dilute to 20 ml with water. 12 ml of this solution complies with test B for heavy metals (20 ppm Maximum). Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting lead standard solution (100 ppm Pb) with a mixture of 6 volumes of water and 9 volumes of acetone.

7.1 APPARATUS AND REAGENTS

7.1.1 Analytical Balance
Measuring Cylinders, 10 ml, 25 ml
Nessler Cylinders, 50 ml
Pipette, 1 ml, 2 ml, 10 ml

7.1.2 Acetone
Thioacetamide Solution
Buffer solution (pH 3.50)
Lead standard solution (1 ppm Pb)

7.2 PROCEDURE

7.2.1 Sample Solution: Weigh 1 g of sample and dissolve in 12 ml of acetone in a nessler cylinder. Dilute it to 20 ml with water.

7.2.2 Test Solution: Take 12 ml of the sample solution into another nessler cylinder. Add 2 ml of buffer solution pH 3.50. Mix and add 1.20 ml of Thioacetamide solution. Mix immediately.

7.2.3 Reference Solution: (Standard) take 10 ml of standard lead solution (1ppm Pb) and 2 ml of above sample solution into another nessler cylinder. Add 2

ml of buffer solution pH 3.50. Mix and add 1.20 ml of Thioacetamide solution. Mix immediately.

7.2.4 Blank Solution: Take 10 ml of acetone and 2 ml of above sample solution into another nessler cylinder. Add 2 ml of buffer solution pH 3.50. Mix and add 1.20 ml of Thioacetamide solution. Mix immediately.

7.2.5 Examine the above three solutions after 2 minutes.

7.2.6 The reference solution should show a slight brown colour when compared to the blank solution. If not the test is invalid.

7.2.7 Any brown colour produced in the test solution should not be more intense than that in the reference solution.

8 LOSS ON DRYING

Maximum 0.5 per cent, determined on 1.000 g by drying in vacuo.

8.1 APPARATUS AND REAGENTS

8.1.1 Analytical Balance
Vacuum Desiccator, with fresh Diphosphorus pentoxide
Weighing bottle, stoppered.

8.2 PROCEDURE

8.2.1 Weigh accurately a stoppered weighing bottle, which is previously dried for about 30 minutes at a pressure of 1.5 kPa to 2.5 kPa (vaccum of 740 to 750 mm of Hg) at room temperature in a desiccator containing Diphosphorus pentoxide.

8.2.2 Note down the empty weight of the bottle (W_1).

8.2.3 Take 1.000g of test sample into the weighing bottle, close the stopper and weigh accurately. Note down the weight of bottle with sample (W_2).

8.2.4 Distribute the sample as evenly as practicable by gentle sidewise shaking. Place the loaded bottle in a desiccator containing Diphosphorus pentoxide, remove the stopper and leave it also in desiccator. Dry the sample for about 5 hours at a pressure of 1.5 kPa to 2.5 kPa (vaccum of 740 to 750 mm of Hg) at room temperature.

8.2.5 Remove the bottle after drying, close the stopper and weigh accurately. Note down the weight of bottle with contents (W_3).

8.3 CALCULATION

Calculate the Loss on drying of the sample using the following formula

$$\frac{(W_2 - W_3) \times 100}{(W_2 - W_1)}$$

9 SULPHATED ASH

Maximum 0.1 per cent, determined on 1.0 g.

9.1 APPARATUS AND REAGENTS

9.1.1 Analytical Balance
Silica / platinum / porcelain / quartz crucible
Muffle Furnace
Pipette, 2 ml
Hot Plate
Desiccator containing Silica gel desiccant.

9.1.2 Sulphuric acid.

9.2 PROCEDURE

9.2.1 Ignite a silica / platinum / porcelain / quartz crucible at 600 ± 50 °C for 30 minutes in a muffle furnace.

9.2.2 Allow the crucible to cool in a desiccator over silica gel desiccant.

9.2.3 Weigh accurately the dried empty crucible and note down the weight (W_1).

9.2.4 Place 1.0 g of the sample in the crucible and weigh accurately. Note down the weight of crucible with contents (W_2).

9.2.5 Moisten the substance with 1 ml of sulphuric acid, heat gently at low temperature until the substance is thoroughly charred.

9.2.6 Cool, moisten the residue with a few drops of sulphuric acid; heat gently until white fumes are no longer evolved.

9.2.7 Ignite at 600 ± 50 °C until the residue is completely incinerated.

9.2.8 Ensure that fumes are not produced at any time during the procedure.

9.2.9 Allow the crucible to cool in the desiccator over silica gel desiccant and

weigh accurately.

9.2.10 If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with sulphuric acid and ignition, as previously, for 30-minute periods until the percentage of residue complies with the prescribed limit.

9.2.11 Allow cooling in the desiccator over silica gel and weigh accurately.

9.2.12 Note down the final weight of the crucible with residue (W_3).

9.3 CALCULATION

Calculate the Sulphated ash content in the sample using the following formula

$$\frac{(W_3 - W_1) \times 100}{(W_2 - W_1)}$$

10 ASSAY

In a flask with a ground-glass stopper, dissolve 1.000 g in 10 ml of ethanol (96 %). Add 50.0 ml of 0.5 M sodium hydroxide. Close the flask and allow to stand for 1 h. Using 0.2 ml of phenolphthalein solution as indicator, titrate with 0.5 M hydrochloric acid. Carry out a blank titration.
1 ml of 0.5 M sodium hydroxide is equivalent to 45.04 mg of $C_9H_8O_4$.

10.1 APPARATUS AND REAGENTS

10.1.1 Balance, Analytical
Burette, 50 ml
Cylinders, Measuring, 10 ml
Flask with a ground-glass stopper, 500 ml

10.1.2 Phenolphthalein solution indicator.
0.5 M Sodium hydroxide solution
0.5 M Hydrochloric acid solution
Ethanol (96 %)

10.2 PROCEDURE

10.2.1 Take two flasks with ground – glass stoppers; dissolve in each, accurately weighed 1 g of sample in 10 ml of ethanol (96 %).

10.2.2 Add 50.0 ml of 0.5 M sodium hydroxide solution. Close the flask and allow to stand for 1 hour.

10.2.3 In another flask with a ground – glass stopper, take 10 ml of ethanol (96 %) and 50.0 ml of 0.5 M sodium hydroxide solution. (Blank). Close the flask and allow to stand for 1 hour.

10.2.4 Using phenolphthalein solution as indicator, titrate the two samples and blank with 0.5 M hydrochloric acid solution.

10.3 CALCULATION

Calculate the assay of the sample on dried basis using the following formula

$$\frac{(BV - TV) \times N \times F \times 100 \times 100}{W \times 0.5 \times (100 - LOD)}$$

Where

BV = Titre volume of 0.5 M hydrochloric acid solution for Blank.

TV = Titre volume of 0.5 M hydrochloric acid solution for the sample.

N = Normality of 0.5 M hydrochloric acid solution.

F = 0.04504. (Gram equivalent of Aspirin to 1 ml of 0.5 M Sodium Hydroxide)

W = Weight of the sample.

LOD = Loss on drying %.