SPECIFICATIONS & TEST PROCEDURES

ASPIRIN USP

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

Molecular Weight : 180.16 **Reference : USP 39**

Other Names : Benzoic acid, 2 - (acetyloxy) - Salicylic acid acetate, Acetyl salicylic acid.

TESTS	**SPECIFICATIONS**
1. Description	: White crystals, commonly tabular or needle-like, or white, crystalline powder. Is odorless or has a faint odor. Is stable in dry air, in moist air it gradually hydrolyzes to salicylic and acetic acids.
2. Solubility	: Freely soluble in alcohol; soluble in chloroform and in ether; sparingly soluble in absolute ether; slightly soluble in water.
3. Identification	:
A. Colour test	: Colour change to violet red by the addition of ferric chloride TS to a heated and cooled sample with water.
B. IR absorption	: The IR absorption spectrum of the sample exhibits maxima only at the same wavelengths as that of a similar preparation of Aspirin WRS.
4. Loss on drying (% w/w)	: Not more than 0.5
5. Readily carbonizable substances	: The solution of 500 mg sample dissolved in 5 mL of H_2SO_4 has no more colour than Matching fluid Q.
6. Residue on ignition (% w/w)	: Not more than 0.05
7. Substances insoluble in Sodium carbonate TS	: A solution of 500 mg sample in 10 mL of warm sodium carbonate TS is clear.
8. Chloride (% w/w)	: Not more than 0.014

SPECIFICATIONS & TEST PROCEDURES
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TEST	SPECIFICATIONS
9. Sulfate (% w/w)	: Not more than 0.04
10. Heavy metals (µg per g)	: Not more than 10
11. Limit of free salicylic acid (% w/w)	: Not more than 0.10
12. Assay (% w/w, On dried basis)	: Not less than 99.5 and Not more than 100.5.

ADDITIONAL TESTS:

13. Related Substances (%)

Salicylic acid	: Not more than 0.10
Any unknown Impurity	: Not more than 0.05
Sum of impurities	: Not more than 0.25

14. Alternate Test For Sulphates As Per IP : Not more than 0.04

SPECIFICATIONS & TEST PROCEDURES
ASPIRIN USP

1. DESCRIPTION

1.1 Place 5gm of sample on the watch glass, observe physically the colour of sample, nature of the substance and extraneous matter present.

1.2 Observe for any lumps or non-homogeneity.

1.3 For odour, examine the sample immediately after opening the bag.

1.4 If any odour is noticeable, open container and re-examine after 15 minutes.

1.5 If the odour is still discernible, the sample does not comply with the description odorless.

2. SOLUBILITY

APPARATUS AND REAGENTS:

2.1 Analytical balance
Stopper Conical flask: 100 ml, 250 ml.
Volumetric flask,1000 ml
Measuring cylinder: 100 ml
Ethanol
Ether
Absolute ether
Distilled water.

2.2 PROCEDURE

2.2.1 Freely soluble: Take 1.0 gm of the material in 100 ml of stopper flask and add 10 ml of ethanol shake vigorously for one minute and keep aside for 15 minutes. The material should completely dissolve.

2.2.2 Soluble: Take 1.0 gm of each material in two separate 100 ml of stopper flask and add 30 ml of chloroform in one flask and 30 ml of ether in another flask shake vigorously for one minute and keep aside for 15 minutes. The material should completely dissolve.

2.2.3 Sparingly soluble: Take 1.0 gm of the material in 250 ml of stopper flask and add 100 ml of absolute ether shake vigorously for one minute and keep aside for 15 minutes. The material should completely dissolve.

2.2.4 Slightly soluble: Take 1.0 gm of the material in 1000 ml of volumetric flask and add 1000 ml of distilled water shake vigorously for one minute and keep aside for 15 minutes. If sample has not been completely dissolved repeat shaking for two minutes and keep aside for 15 minutes. The material should completely dissolve.

3. IDENTIFICATION

A. Colour Test:

Heat the sample with water for several minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

3.A.1. APPARATUS AND REAGENTS

3.A1.1 Test tube
Hot plate
Pair of tongs

3.A1.2 Water, distilled
Ferric chloride TS

3.A.2. PROCEDURE

3.A2.1 Take approximately about 0.5 g of sample into a test tube and add approximately about 10 ml of water. Heat the solution on a hot plate for about 3 minutes.

3.A2.2 Cool it and add 1 or 2 drops of Ferric chloride TS.

3.A2.3 A violet red color should be produced.

B. Infrared absorption:

Infrared absorption spectra comparison of sample and Aspirin WRS.

3.B.1. APPARATUS AND REAGENTS

3.B1.1 Analytical balance
Mortar & Pestle
Hydraulic pellet press
FT / IR Spectrophotometer

3.B1.2 Potassium bromide, finely powdered.
Aspirin WRS.

3.B.2. PROCEDURE

3.B2.1 Transfer 1 to 2 mg of Aspirin WRS to mortar and pestle.

3.B2.2 Mix it with 0.3 to 0.4 g of dried, finely powdered potassium bromide.

3.B2.3 Grind the mixture carefully and spread it uniformly in a suitable die.

3.B2.4 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.

3.B2.5 Keep the disc in Infrared spectrophotometer sample compartment and record the spectrum between 3800 cm^{-1} and 650 cm^{-1} (2.6 μm to 15 μm).

3.B2.6 Repeat the procedure from step 1 to 5 to record the spectrum of the test sample by taking the sample instead of Aspirin WRS

3.B2.7 The IR absorption spectrum of the sample exhibits maxima only at the same wavelengths as that of a similar preparation of Aspirin WRS.

4. LOSS ON DRYING

Dry it over silica gel for 5 hours: it loses not more than 0.5% of its weight.

4.1 APPARATUS AND REAGENTS

4.1.1 Analytical Balance
Shallow weighing bottle, glass stoppered
Desiccator, blue silica gel, self-indicating

4.2 PROCEDURE

4.2.1 Take a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes in the desiccator containing silica gel in blue colour.

4.2.2 Ensure that the colour of the silica gel is pure blue in colour; otherwise dry the silica gel till pure blue colour appears.

4.2.3 Weigh and record the empty weight of the bottle with lid (W_1).

4.2.4 Put about 1 g of the substance in the bottle, replace the lid, and accurately weigh and record the weight of bottle with contents (W_2).

4.2.5 By gentle, side-wise shaking, distribute the material as evenly as practicable to a depth of about 5 mm and not more than 10 mm.

4.2.6 Place the loaded bottle in the desiccator, removing the stopper and leaving it also in the desiccator.

4.2.7 Dry the material for 5 hours and then open the desiccator, close the bottle promptly with stopper.

4.2.8 Weigh and record the weight of bottle with contents (W_3).

4.3 CALCULATION

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

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

5. READILY CARBONIZABLE SUBSTANCES

Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than Matching Fluid Q.

5.1 APPARATUS AND REAGENTS

5.1.1 Analytical Balance
Nessler Cylinders, 50 ml
Pipettes, 1 ml, 5 ml
Glass rods.

5.1.2 Cobaltous Chloride CS
Cupric Sulphate CS
Ferric Chloride CS
Distilled water
Sulfuric acid

5.2 PROCEDURE

5.2.1 Dissolve 500 mg of finely powdered sample by adding in small portions in 5 ml of Sulfuric acid in a Nessler cylinder.

5.2.2 Stir the mixture with a glass rod until solution is complete. Allow the solution to stand for 15 minutes.

5.2.3 Prepare a matching fluid Q by mixing 0.2 ml of Cobaltous Chloride CS, 0.3 ml of Ferric Chloride CS, 0.1 ml of Cupric Sulphate CS and 4.4 ml of water in another nessler cylinder, just before testing.

5.2.4 View the fluids transversely against a white background.

5.2.5 The colour of the sample solution shall not be more than the colour of the matching fluid Q.

6. RESIDUE ON IGNITION

Moisten the sample of about 1 g with Sulfuric acid, heat gently at a temperature as low as practicable until the material is thoroughly charred. Ignite at 600±50 ^{o}C until the residue is completely incinerated and then cool. The residue on ignition should not be more than 0.05%.

6.1 APPARATUS AND REAGENTS

6.1.1 Analytical Balance
Silica or Porcelain or Platinum crucible
Hot plate
Desiccator containing silica gel desiccant
Muffle Furnace
Pipette, 2 ml.

6.1.2 Sulfuric acid

6.2 PROCEDURE

6.2.1 Ignite a Silica or Porcelain or Platinum crucible at 600 ± 50 º C for 30 minutes. Cool in a desiccator containing silica gel desiccant. Weigh accurately and record the weight of crucible (W_1).

6.2.2 Weigh accurately 1 g of the test sample in the crucible. Record the weight of crucible with contents (W_2).

6.2.3 Moisten the sample with 1 ml of Sulfuric acid. Heat gently at a

temperature as low as practicable until the substance is thoroughly charred.

6.2.4 Cool, moisten again the residue with 1 ml of Sulfuric acid, heat gently until white fumes are no longer evolved, and ignite at 600 ± 50 ° C until the residue is completely incinerated.

6.2.5 Ensure that flames are not produced at any time during the procedure.

6.2.6 Cool the crucible in a desiccator containing silica gel desiccant and weigh accurately.

6.2.7 If the amount of the residue so obtained exceeds the limit specified, repeat the moistening with sulfuric acid, heating and igniting as before, using a 30-minute ignition period, until the percentage of residue complies with the limit.

6.2.8 Weigh accurately and record the weight of crucible with residue (W_3).

6.3 CALCULATION

Calculate the residue on ignition present in the sample using the following formula

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

7. SUBSTANCES INSOLUBLE IN SODIUM CARBONATE TS

A solution of 500 mg in 10 mL of warm sodium carbonate TS is clear.

7.1 APPARATUS AND REAGENTS

7.1.1 Analytical Balance
Nessler Cylinder, 50 ml
Hot plate
Glass rod
Measuring Cylinder, 10 ml.

7.1.2 Sodium Carbonate TS

7.2 PROCEDURE

7.2.1 Take 500 mg of test sample into a nessler cylinder and add 10 ml of warm Sodium Carbonate TS.

7.2.2 Stir the solution with a glass rod. The solution shall be clear.

8. CHLORIDE

Boil 1.5 g with 75 mL of water for 5 minutes, cool, add sufficient water to restore the original volume, and filter. A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

8.1 APPARATUS AND REAGENTS

8.1.1 Analytical Balance
Hot plate
Beaker, 250 ml
Nessler Cylinders, 50 ml
Measuring Cylinders, 100 ml
Measuring Pipette, 25 ml
Graduated Pipettes, 1 ml
Glass funnel
Filter paper, whatman 42
Glass rods

8.1.2 Nitric acid
Silver nitrate TS
Hydrochloric acid, 0.020 N.

8.2 PROCEDURE

8.2.1 Boil 1.5 g of test sample with 75 ml of water for 5 minutes. Cool, add sufficient water to restore the original volume and filter.

8.2.2 Pipette 25 ml of the filtrate into a nessler cylinder, add 1 ml each of nitric acid and silver nitrate TS.

8.2.3 Pipette 0.10 ml of 0.020 N hydrochloric acid into another nessler cylinder and add sufficient water to make 25 ml. Add 1 ml each of nitric acid and silver nitrate TS.

8.2.4 Mix the contents of the two cylinders with two separate glass rods and allow to stand for 5 minutes protected from direct sunlight.

8.2.5 After 5 minutes, any turbidity produced in the sample solution should not be more than that turbidity produced in the standard solution.

9. SULFATE

Dissolve 6.0 g in 37 mL of acetone, and add 3 mL of water. Titrate potentiometrically with 0.02 M lead per chlorate, prepared by dissolving 9.20 g of lead per chlorate in water to make 1000 mL of solution, using a pH meter capable of a minimum reproducibility of ±0.1 mV and equipped with an electrode system consisting of a lead-specific electrode and a silver-silver chloride reference glass-sleeved electrode containing a 1 in 44 solution of tetraethyl ammonium per chlorate in glacial acetic acid: not more than 1.25 mL of 0.02 M lead per chlorate is consumed (0.04%).
[NOTE — After use, rinse the lead-specific electrode with water, drain the reference electrode, flush with water, rinse with methanol, and allow to dry.]

9.1 APPARATUS AND REAGENTS

9.1.1 Analytical Balance
Measuring Cylinders, 50 ml
Graduated Pipettes, 5 ml
Potentiometer.

9.1.2 0.02 M Lead per chlorate
Acetone
Distilled Water.

9.2 PROCEDURE

9.2.1 Weigh 6.0 g of sample and dissolve in 37 ml of acetone. Add 3 ml of water and titrate with 0.02 M lead per chlorate potentiometrically.

9.2.2 Not more than 1.25 ml of 0.02 M lead per chlorate should be consumed.

10. HEAVY METALS

Dissolve 2 g in 25 mL of acetone, and add 1 mL of water. Add 1.2 mL of thioacetamide - glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, and allow to stand for 5 minutes: any color produced is not darker than that of a control made with 25 mL of acetone and 2 mL of Standard Lead Solution, treated in the same manner. The limit is 10 µg per g.
The colour of the Monitor, Prepared with 25 mL of a solution prepared as directed for Test Preparation and 2 mL of Standard Lead Solution, treated in the same manner is equal to or darker than that of the control.

10.1 APPARATUS AND REAGENTS

10.1.1 Analytical Balance
Nessler Cylinders, 50 ml
Measuring Cylinders, 25 ml
Pipettes, 1 ml, 2 ml
Glass rods.

10.1.2 Acetone, AR
Thioacetamide- glycerin base TS
Standard Lead solution, *10 µg per g*
Acetate buffer, pH 3.5.

10.2 PROCEDURE

10.2.1 Standard preparation: Into a 50 ml Nessler Cylinder pipette 2 ml of Standard Lead Solution and add 25 ml of Acetone and 1 ml of water.

10.2.2 Test preparation: Dissolve 2 g of Sample in 25 ml of acetone in a 50 ml Nessler Cylinder and add 1 ml of water.

10.2.3 Monitor Preparation: Prepare again Test Preparation into a third 50 ml Nessler Cylinder and add 2 ml of Standard Lead Solution.

10.2.4 To each of the above three preparations add 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, and allow to stand for 5 minutes, and view downward over a white surface.

10.2.5 The colour of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation and the colour of the solution from the Monitor Preparation is equal to or darker than that of the solution from the Standard Preparation.

11. LIMIT OF FREE SALICYLIC ACID

Dissolve 2.5 g in sufficient alcohol to make 25.0 mL. To each of two matched color-comparison tubes add 48 mL of water and 1 mL of a freshly prepared, diluted ferric ammonium sulfate solution (prepared by adding 1 mL of 1 N hydrochloric acid to 2 mL of ferric ammonium sulfate TS and diluting with water to 100 mL). Into one tube pipette 1 mL of a standard solution of salicylic acid in water, containing 0.10 mg of salicylic acid per mL. Into the second tube pipette 1 mL of the 1 in 10 solution of Aspirin. Mix the contents of each tube: after 30 seconds, the color in the second tube is not more intense than that in the tube containing the salicylic acid (0.1%)

11.1 APPARATUS AND REAGENTS

11.1.1 Analytical Balance,
Volumetric Flask, 25 ml
Nessler Cylinders, 50 ml
Graduated Pipettes, 1 ml, 2 ml

11.1.2 Alcohol
Diluted ferric ammonium sulphate solution
Standard solution of salicylic acid (0.01 % w/v)

11.2 PROCEDURE

11.2.1 Dissolve 2.5 g of sample in alcohol and make upto 25 ml in a volumetric flask.

11.2.2 To each of two matched nessler cylinders add 48 ml of water and 1 ml of freshly prepared, diluted ferric ammonium sulphate solution.

11.2.3 Into one nessler cylinder pipette 1 ml of standard solution of salicylic acid.

11.2.4 Into the second nessler cylinder pipette 1 ml of 1 in 10 solution of Aspirin. Mix the contents of each cylinder.

11.2.5 After 30 seconds, the colour in the second cylinder is not more intense than that in the cylinder containing the salicylic acid.

12. ASSAY

Place about 1.5 g of Aspirin, accurately weighed, in a flask, add 50 mL of 0.5 N sodium hydroxide, and boil the mixture gently for 10 minutes. Add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N Sulfuric acid VS. Perform a blank determination.
Each mL of 0.5 N sodium hydroxide is equivalent to 45.04 mg of $C_9H_8O_4$.

12.1 APPARATUS AND REAGENTS

12.1.1 Analytical Balance
Conical Flask, 500 ml
Burette, 50 ml
Water bath / Hot plate

12.1.2 Phenolphthalein TS
0.5 N Sodium hydroxide
0.5 N Sulfuric acid VS.

12.2 PROCEDURE

12.2.1 Take two 500 ml conical flasks and weigh accurately about 1.5 g of sample into each flask and add 50 ml of 0.5 N sodium hydroxide.

12.2.2 Into another 500 ml conical flask take 50 ml of 0.5 N sodium hydroxide (Blank).

12.2.3 Place all the three flasks on a water bath / hot plate and boil the mixture gently for 10 minutes.

12.2.4 Add phenolphthalein TS and titrate the excess sodium hydroxide with 0.5 N Sulfuric acid VS in all the three flasks.

12.3 CALCULATIONS

Calculate the assay of the Aspirin sample 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 Sulfuric acid VS for Blank.

TV = Titre volume of Sulfuric acid VS for the sample.

N = Normality of Sulfuric acid VS.

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 %.

ADDITIONAL TESTS

13. RELATED SUBSTANCES

By liquid chromatography. Prepare the solutions immediately before use.

13.1 APPARATUS AND REAGENTS

13.1.1 A High performance Liquid Chromatograph .

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.

13.1.2 Phosphoric acid
Acetonitrile
Water

13.2 PREPARATION OF MOBILE PHASE:

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

13.2.2 Filter through 0.45µ membrane filter.

13.3 CHROMATOGRAPHIC CONDITIONS:

Flow rate	:	1.0 ml / min.
Detection	:	At 237 nm
Injection	:	10 µl
Run time	:	40 min. (7 times the retention time of Aspirin)

13.4 PREPARATION OF SOLUTIONS:

Salicylic acid solution : Transfer accurately weighed 50 mg of salicylic acid WRS to a 50 ml volumetric flask. Dissolve in and dilute to 50 ml with the mobile phase.

Test solution: Transfer accurately weighed 100 mg of sample to a 10 ml volumetric flask. Dissolve in and dilute to 10 ml with acetonitrile.

Reference solution (a): Dilute 1 ml of Salicylic acid solution to 100 ml with the mobile phase.

Reference solution (b): Transfer 1 ml of Salicylic acid solution and 0.2 ml of test solution to a 100 ml volumetric flask and dilute to 100 ml with the mobile phase.

Reference solution (c): Dilute 5 ml of reference solution (a) to 10 ml with the mobile phase.

Impurity Mix Solution: Dilute 1 ml of stock solution to 10 ml with Acetonitrile.

[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.]

13.5 EVALUATION OF SYSTEM SUITABILITY:

13.5.1 Inject 10µl of acetonitrile solution into the chromatograph and record the chromatogram up to 40 minutes. Examine the mobile phase for any extraneous peaks and disregard corresponding peaks observed in the chromatogram of the test solution.

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

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

13.5.4 Inject 10µl of reference solution (c) into the chromatograph and record the chromatogram up to 20minutes.
Determine the signal to noise ratio - Limit NLT10.

13.6 PROCEDURE:

13.6.1 Inject 10µl of test solution into the chromatograph and record the chromatogram up to 40 minutes. Disregard any peaks other than known impurities with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a).

13.6.2 Inject 10µl of impurity mix solution (0.025%) into the chromatograph and record the chromatogram until all the impurities are eluted for checking the retention time of impurities.

13.7 CALCULATION:

Calculate the percentage of all the impurities using the following formula

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

Where

Impurity	Response Factor (RF)
4-Hydroxybenzoic acid	1.30
4-Hydroxyisophthalic acid	1.24
Salicylic acid	1.00
Acetylsalicylsalicylic acid	1.15
Salicylsalicylic acid	0.93

ALTERNATE TEST FOR SULPHATES AS PER IP

14. SULPHATE

Weigh accurately 1.75 g of Aspirin in 250 ml of glass beaker and add 75 ml of distilled water. Boil the solution on hot plate for 5 minutes and cool. Add sufficient distilled water to restore the original volume of 75 ml test solution. Filter the solution through glass funnel having a whatman filter paper No.42. 10 ml of the filtrate complies with the limit test for sulphate (0.04 %).

14.1 APPARATUS AND REAGENTS

14.1.1. Analytical Balance
Glass Beaker, 250 ml
Cylinders, Measuring, 100 ml
Cylinders, Nessler, 50 ml
Pipettes, Graduated, 1 ml, 2 ml, 10 ml
Glass rods
Glass Funnel
Whatman filter paper No.42
Hot plate

14.1.2 Barium chloride solution, 25% w/v,
Ethanolic, Sulphate standard solution (10 ppm SO_4)
Sulphate standard solution (10 ppm SO_4)
Acetic acid 5M

14.2 PROCEDURE

14.2.1 Take a mixture of 1 ml of a 25.0% w/v solution of Barium chloride and 1.5 ml of ethanolic Sulphate standard solution into two nessler cylinders. Allow them to stand for 1 minute.

14.2.2 Transfer 10 ml of the test filtrate obtained and 0.15 ml of 5M acetic acid to one of the above nessler cylinders and add sufficient distilled water to produce 50 ml. Stir immediately with a glass rod and allow to stand for 5 minutes.

14.2.3 Transfer 9.2 ml of Sulphate standard solution (10 ppm SO_4) and 0.15 ml of 5M Acetic acid to another nessler cylinder and add sufficient distilled water to produce 50 ml. Stir immediately with a glass rod and allow to stand for 5 minutes.

14.2.4 View transversely against a black background. Any opalescence produced in the test solution should not be more intense than that produced in the Sulphate standard solution.