SPECIFICATION & TEST PROCEDURE
ASPIRIN IP

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

Molecular weight : 180.2 Reference : I.P-Addendum 2015

Other names : 2 – Acetoxybenzoic acid.

Description : Colourless crystals or white, crystalline powder; odourless or almost odourless.

Solubility : Freely soluble in ethanol (95%); soluble in chloroform and in ether; slightly soluble in water.

	TESTS		**SPECIFICATIONS**
1.	Identification	:	Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if Test A is carried out. A. IR spectrum of the sample is concordant with that of Aspirin WRS. B. A deep violet colour with ferric chloride solution. C. On heating with sulphuric acid, odour of ethyl acetate perceptible.
2.	Appearance of solution	:	A 1 % w/v solution in ethanol (95%) is clear and not more intensely coloured than reference solution BS8.
3.	Clarity of solution in alkali	:	A 5% w/v solution in warm 5% w/v solution of sodium carbonate is clear.

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	TESTS		**SPECIFICATIONS**
4.	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
5.	Arsenic	:	Sample stain shall not be more intense than Standard stain. (2 ppm)
8.	Heavy metals	:	Not more than 10 ppm
8.	Chlorides	:	Not more than 430 ppm
8.	Sulphates	:	Not more than 650 ppm
9.	Readily Carbonisable substances	:	A 10% solution in H_2SO_4(94.5 – 95.5%w/w); any colour produced is not more than that of reference solution BYS4.
10.	Sulphated Ash	:	Not more than 0.1%
11.	Loss on drying	:	Not more than 0.5%
12.	Assay (By HPLC)	:	NLT 99.5 % & NMT 100.5 %

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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

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if Tests A is carried out.

Test A : IR spectrum of the sample is concordant with that of Aspirin WRS.

3.1.A APPARATUS AND REAGENTS

3.1.A.1 Analytical Balance,
Mortar & Pestle
Hydraulic pellet press
IR Spectrometer
Potassium bromide,
Aspirin WRS

3.2.A PROCEDURE

3.2.A.1 Triturate about 1 mg of the Aspirin WRS with approximately 300 mg of dry finely powdered potassium bromide IR

3.2.A.2 Grind the mixture thoroughly; spread it uniformly in a suitable die and Compress under vacuum at a pressure of about 800 MPa.

3.2.A.3 Mount the resultant disc in a suitable holder in the spectrometer sample compartment and record the spectrum between 4000 cm-1 and 625 cm-1(2.5 μm to 16 μm).

3.2.A.4 Repeat the procedure from step 1.2.A.1 to 1.2.A.3 to record the spectrum of the material under test for taking sample instead of Aspirin WRS.

Test B :

3.1.B APPARATUS & REAGENTS

3.1.B.1 Analytical Balance,
Glass beaker, 100 ml
Filter assembly,
Pipettes, 2 ml, 5 ml, 10 ml,
Hot plate,
Nessler Cylinder, 50 ml
Sodium hydroxide solution,
Dilute Sulphuric acid,
Ferric chloride test solution.

3.2.B PROCEDURE

3.2.B.1 Boil about 0.50 gm of Sample with 10 ml of sodium hydroxide solution for 3 minutes in 100ml glass beaker.

3.2.B.2 Cool and add 10 ml of dilute sulphuric acid. A white crystalline precipitate is produced and the odour of acetic acid is perceptible.

3.2.B.3 Filter, dissolve the precipitate about 2.0ml of water and add ferric chloride test solution in a nessler cylinder. A deep violet colour is produced

Caution: Preserve the filtrate for Test C.

Test C

3.1.C APPARATUS & REAGENTS

3.1.C.1 Nessler Cylinders, 50 ml
Pipettes, 5 ml,
Water bath,
Ethanol, 95%
Sulphuric acid.

3.2.C PROCEDURE

3.2.C.1 Take the filtrate obtained in Test B in a nessler cylinder; add 3 ml of ethanol, 95% and 3 ml of sulphuric acid.

3.2.C.2 Warm this mixture on a water bath and the odour of ethyl acetate is perceptible.

4. APPEARANCE OF SOLUTION

4.1 APPARATUS AND REAGENTS

4.1.1 Analytical Balance,
Measuring Cylinders, 100 ml
Pipettes, 10 ml
Nessler Cylinders, 50 ml
Reference solution BS8

4.2 PROCEDURE

4.2.1 Dissolve 1 gm of Sample in 10 ml of ethanol (95 %) in a nessler cylinder.

4.2.2 In another nessler cylinder take 10 ml of ethanol (95 %).

4.2.3 After 5 minutes compare the contents of nessler cylinders against a black background by viewing under diffused light down the vertical axis of the nessler cylinders.

4.2.4 The solution is considered ***clear*** if its clarity is the same as that of water or of the solvent used.

4.2.5 The solution not more intensely coloured than reference solution BS8.

5. CLARITY OF SOLUTION IN ALKALI

5.1 APPARATUS AND REAGENTS

5.1.1 Nessler Cylinders, 50 ml
Water bath,
Analytical Balance,
Measuring Cylinders, 10 ml
Sodium Carbonate

5.2 PROCEDURE

5.2.1 Take about 0.5 g of Sample in a nessler cylinder and add 10 ml of warm 5 % w/v Sodium Carbonate solution. Dissolve the sample by shaking the nessler cylinder.

5.2.2 In another nessler cylinder take 10 ml of warm 5 % w/v Sodium Carbonate solution.

5.2.3 The solution is considered clear if its clarity is the same as that of the solvent used.

6. RELATED SUBSTANCES

By liquid chromatography. Prepare the solutions immediately before use.

6.1 APPARATUS AND REAGENTS

6.1.1 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
HPLC
Filter assembly with 0.45µ membrane filter
Volumetric flasks, 10ml, 50 ml, 100 ml
Pipettes, 1 ml, 5 ml.
OrthoPhosphoric acid
Acetonitrile
Water

6.2 PREPARATION OF MOBILE PHASE:

6.2.1 Mix thoroughly Phosphoric acid, Acetonitrile and Water in the ratio of

0.2: 40: 60 (V/V/V).

6.2.2 Filter through 0.45µ membrane filter and sonicate for 5 minutes.

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.

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 two principle peaks 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.

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

6.6 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. ARSENIC

7.1 APPARATUS AND REAGENTS

A wide mouthed bottle capable of holding about 100 ml is fitted with a rubber bung through which passes a glass tube. The lower part of the tube is drawn to an internal diameter of 1.0 mm, and 15 mm from its tip is lateral orifice 2 to 3 mm in diameter. When the tube is in position in the stopper the lateral orifice should be at least 3 mm below the lower surface of the stopper. The upper end of the tube as a perfectly flat surface at right angles to the axis of the tube. A second glass tube of the same internal diameter and 30 mm long, with similar flat surface, is placed in contact with the first and is held in position by two spiral springs or clips. In to the lower tube insert 50 to 70 mg of lead acetate cotton, loosely packed, or small plug of cotton and a rolled piece of lead acetate paper weighing 50 to 70 mg. Between the flat surfaces of the tubes place a disc or a small square of Mercuric chloride paper large enough to cover the orifice of the tube (15 mm X 15 mm).

7.1.1 Analytical Balance,
Silica crucible
Measuring Cylinders, 10 ml, 25 ml, 50 ml
Filter papers
Pipettes, 2 ml, 5 ml
Hot plate
Arsenic solution, Strong, AsT
Arsenic solution, dilute, AsT
Bromine solution, AsT
Brominated Hydrochloric acid, AsT
Cotton wool
Potassium iodide, 1M
Mercuric chloride paper
Sodium carbonate, anhydrous,
Stannous chloride solution, AsT
Stannated Hydrochloric acid, AsT
Zinc,

7.2 PROCEDURE

7.2.1 Mix 5g of Sample and 3g of anhydrous Sodium Carbonate in a Silica crucible. Add 10 ml of Bromine solution and mix thoroughly.

7.2.2 Evaporate to dryness on a hot plate, gently ignite, and dissolve the cooled residue in 16 ml of Brominated Hydrochloric acid, AsT and 45 ml of water.

7.2.3 Remove the excess of bromine with 2 ml of Stannous chloride, AsT (Test solution)

7.2.4 Two sets of glass tubes are to be prepared as mentioned in the apparatus. One is meant for substance under examination and other is for standard stain.

7.2.5 In one bottle put the test solution, and 5 ml of Stannated Hydrochloric acid, add 5 ml of 1M Potassium iodide. If any yellow colour appears, add 2 to 3 drops of stannous chloride solution to remove the colour of bromine and keep the bottle aside.

7.2.6 In second bottle, add 45 ml of water, 1 ml dilute arsenic solution and 5 ml of 1M potassium iodide. Add 2 to 3 drops of stannous chloride solution to remove the yellow colour of bromine. Add 5 ml of Stannated Hydrochloric acid and keep the bottle aside.

7.2.7 Add 10 g of Zinc to both the bottles and put the prepared glass tubes quickly in position.

7.2.8 The action is allowed to proceed for 40 minutes.

7.2.9 The yellow stain produced on the mercuric chloride paper for the test solution is not more intense than the standard stain.

7.2.10 The comparison shall be made **immediately** after the test in daylight.

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8. HEAVY METALS

8.1 APPARATUS AND REAGENTS

8.1.1 Analytical Balance,
Measuring Cylinders, 25 ml
Nessler Cylinders, 50 ml
Pipettes, 1 ml, 10 ml
Acetone,
Hydrogen sulphide solution
Lead standard solution (20 ppm Pb)

8.2 PROCEDURE

8.2.1 Dissolve 2.0g of sample in 25 ml of Acetone, add 1 ml of water and 10 ml of Hydrogen sulphide solution in a nessler cylinder.

8.2.2 Prepare a standard solution in another nessler cylinder by using 25 ml of acetone, 1 ml of Lead standard solution (20 ppm) and 10 ml of Hydrogen sulphide solution.

8.2.3 Any colour produced with the sample is not more intense than that of standard

9. CHLORIDES

9.1 APPARATUS AND REAGENTS

9.1.1 Analytical Balance,
Glass Beaker, 250 ml,
Nessler Cylinders, 50 ml
Measuring Cylinders, 25 ml, 100 ml,
Glass Funnel
Filter papers,
Pipettes, 1 ml, 2 ml, 10 ml,
Hot plate
Dilute Nitric acid
Silver Nitrate, 0.1M,
Chloride standard solution (25 ppm)

9.2 PROCEDURE

9.2.1 Boil 1.75 g of Sample in 250 ml of glass beaker with 75ml of distilled water on hot plate for 5 minutes and cool. Filter the solution through glass funnel having a filter paper. Add sufficient distilled water to restore the original volume of 75 ml of test solution.
Caution: Preserve the filtered solution for sulphate test.

9.2.2 Transfer 25 ml of test solution into a 50 ml nessler cylinder. Add 10 ml of dilute Nitric acid, and dilute to 50 ml with distilled water and add 1 ml of 0.1 M Silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes.

9.2.3 In another cylinder place 10 ml of Chloride standard solution (25 ppm) and add 10 ml of dilute Nitric acid. Dilute to 50 ml with distilled water and add 1 ml of 0.1M Silver nitrate. Stir immediately with a glass rod and allow to stand for 5minutes.

9.2.4 The opalescence formed with the test sample is not more intense than that of the Standard.

10. SULPHATES

10.1 APPARATUS AND REAGENTS

10.1.1 Nessler Cylinders, 50 ml,
Measuring Cylinders, 25 ml,
Pipettes, 1 ml, 2 ml, 10 ml
Barium chloride solution, 25% w/v,
Ethanolic Sulphate standard solution (10 ppm SO_4)
Sulphate standard solution (10 ppm SO_4)
Acetic acid 5M

10.2 PROCEDURE

10.2.1 Take 10 ml of the filtrate obtained in test for chloride into a nessler cylinder and add 1 ml of a 25.0 % w/v solution of Barium chloride.

10.2.2 Add 1.5 ml of ethanolic Sulphate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute. Add 0.15 ml of 5M acetic acid and add sufficient distilled water to produce 50 ml. Stir immediately with a glass rod and allow to stand for 5 minutes.

10.2.3 In another nessler cylinder, transfer 1 ml of a 25.0% w/v solution of Barium chloride and add 1.5 ml of ethanolic Sulphate standard

solution, mix and allow to stand for 1 minute.

10.2.4 Add 15 ml of Sulphate standard solution (10 ppm SO_4), 0.15 ml of 5M Acetic acid and add sufficient distilled water to produce 50 ml. Stir immediately with a glass rod and allow to stand for 5 minutes.

10.2.5 The opalescence formed is compared against a black background with the test sample is not more intense than that of the Standard.

11. READILY CARBONISABLE SUBSTANCES

11.1 APPARATUS AND REAGENTS

11.1.1 Analytical Balance,
Nessler Cylinders, 50 ml
Pipettes, 1ml, 5ml, 10ml
Volumetric flask, 100ml
Sulphuric acid (94.5 % to 95.5 %w/w),
Hydrochloric acid (1%w/v),
Ferric chloride CS,
Cobaltous chloride CS,
Cupric sulphate CS

11.2 PROCEDURE

11.2.1 Weigh about 0.5 g of the sample under examination in a nessler cylinder and add 5 ml of Sulphuric Acid (containing 94.5% to 95.5% w/w Sulphuric acid) dissolve the material by shaking the nessler cylinder.

11.2.2 Take volumetric flask,100ml prepare the reference solution BYS4 by adding a mixture of 6.0 ml of Ferric chloride CS, 2.5 ml of Cobaltous chloride CS, 1.0 ml of Cupric sulphate CS and 90.5 ml of 1% w/v Hydrochloric acid solution.

11.2.3 Take 5ml of the above BYS4 solution in another nessler cylinder and compare with the test solution.

11.2.4 Any colour produced with the test solution is not more intense than that of reference solution BYS4

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12. SULPHATED ASH

12.1 APPARATUS AND REAGENTS

12.1.1 Analytical Balance,
Silica Crucible,
Muffle Furnace,
Pipette, 2 ml
Hot plate,
Desiccator
Sulphuric acid

12.2 PROCEDURE

12.2.1 Heat a silica crucible to redness in a muffle furnace for 10 minutes.

12.2.2 Allow the crucible to cool to room temperature in the muffle furnace itself and transfer the crucible to a desiccator.

12.2.3 Take the weight of the empty crucible (W_1)

12.2.4 Transfer to the crucible 1g of the substance being examined and weigh the crucible and contents (W_2) accurately and note.

12.2.5 Ignite, gently at first, on hot plate until the substance is thoroughly charred.

12.2.6 Cool, moisten the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved.

12.2.7 Ignite at 800 ± 25 °C, until all black particles have disappeared.

12.2.8 Allow crucible to cool to room temperature and note the weight (W_3).

12.3 CALCULATION

12.3.1 The amount of Sulphated ash present in substance is expressed by

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

13. LOSS ON DRYING

13.1 APPARATUS AND REAGENTS

13.1.1 Analytical Balance,
Glass weighing Bottle,
Desiccator

13.2 PROCEDURE

13.2.1 Take the empty weight of the glass weighing bottle (W_1)

13.2.2 Weigh accurately 1 gm of test sample into the weighing bottle and note down the weight (W_2).

13.2.3 Distribute the sample sidewise, and place the loaded bottle in a desiccator filled with Phosphorous pentoxide,

13.2.4 Remove the stopper and leave it also in the desiccator.

13.2.5 Keep the sample for 5 hours, and reweigh the weighing bottle (W_3).

13.2.6 Note the difference in loss in weight of the substance and calculate the percentage.

13.3 CALCULATIONS

13.3.1 The Loss on drying present in substance is expressed by

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

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14. ASSAY (Determine by Liquid chromatography)

14.1 APPARATUS AND REAGENTS

14.1.1 A stainless steel column 15cm long and 4.6mm in internal diameter packed with octadecylsilane bonded to porous silica (5 µm).
Analytical Balance
HPLC
Filter assembly with 0.45µ membrane filter
Volumetric flasks, 25ml, 50 ml, 100 ml
Pipettes, 25 ml.
OrthoPhosphoric acid
Acetonitrile
Water

14.2 PREPARATION OF MOBILE PHASE:

14.2.1 A Mixture of Water, Acetonitrile and Phosphoric acid, in the ratio of 600:400: 2 (V/V/V).

14.2.2 Filter through 0.45µ membrane filter and sonicate for 5 minutes.

14.3 CHROMATOGRAPHIC CONDITIONS:

Flow rate	:	1.0 ml / min.
Spectrophotometer	:	At 245 nm
Injection volume	:	10 µl
Run time	:	12 min.

14.4 PREPARATION OF SOLUTIONS:

Test solution: Dissolve 50mg of the substance under examination in the mobile phase and dilute to 50.0ml with the mobile phase. Dilute 25.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution: A 0.05% w/v solution of Aspirin WRS in the mobile phase.

The retention time of the principal peak is about 4.0 minutes

Inject 10µl of mobile phase into the chromatograph and record the chromatogram up to 12 minutes. Examine the mobile phase for any extraneous peaks observed in the chromatogram of the test solution.

Inject 10µl of reference solution separately six times into the chromatograph and record the chromatograms.

Calculate the % RSD for peak responses (area) and retention time by processing the chromatograms and also test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 percent.

14.5 PROCEDURE:

Inject 10µl of test solution into the chromatograph and record the chromatogram up to 12 minutes.

14.6 CALCULATION:

Calculate the percentage of the Assay with reference to the dried substance using the following formula

$$\frac{\text{Test area x Std. Conc. Weight x Assay of std. x 100}}{\text{Std. average area x Test conc. weight x (100-LOD)}}$$