Acidity. To 1.25 g, finely powdered, add 25 ml of *carbon dioxide-free water R*. Heat at 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances. Examine by thin layer chromatography (*2.2.27*), using *TLC silica gel GF_{254} plate R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a). Dissolve 20 mg of *sulfamethoxypyridazine CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dilute 2.5 ml of test solution (b) to 50 ml with *acetone R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *dilute ammonia R1*, 9 volumes of *water R*, 30 volumes of *2-propanol R* and 50 volumes of *ethyl acetate R*. Dry the plate at 100-105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals (*2.4.8*). 1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay of primary aromatic amino-nitrogen (*2.5.8*), using 0.2500 g, determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 28.03 mg of $C_{11}H_{12}N_4O_3S$.

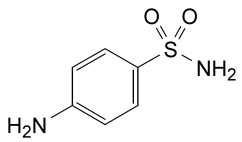
STORAGE

Protected from light.

01/2008:1571
corrected 6.0

SULFANILAMIDE

Sulfanilamidum



$C_6H_8N_2O_2S$ M_r 172.2
[63-74-1]

DEFINITION

Sulfanilamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-aminobenzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White or yellowish-white crystals or fine powder, slightly soluble in water, freely soluble in acetone, sparingly soluble in alcohol, practically insoluble in methylene chloride. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 164.5 °C to 166.0 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sulfanilamide CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid*. Dilute 1 ml of the solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S. To 2.5 g add 50 ml of *carbon dioxide-free water R*. Heat at about 70 °C for about 5 min. Cool in iced water for about 15 min and filter.

Acidity. To 20 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution (a). Dissolve 20 mg of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b). Dissolve 0.10 g of the substance to be examined in 0.5 ml of *concentrated ammonia R* and dilute to 5 ml with *methanol R*. If the solution is not clear, heat gently until dissolution is complete.

Reference solution (a). Dissolve 20 mg of *sulfanilamide CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b). Dilute 1.25 ml of test solution (a) to 50 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Reference solution (c). Dissolve 20 mg of the substance to be examined and 20 mg of *sulfamerazine CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Apply to the plate 5 µl of each solution. Develop over a path corresponding to two-thirds of the plate height using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (b), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Heavy metals (*2.4.8*). 12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.140 g and determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 17.22 mg of $C_6H_8N_2O_2S$.

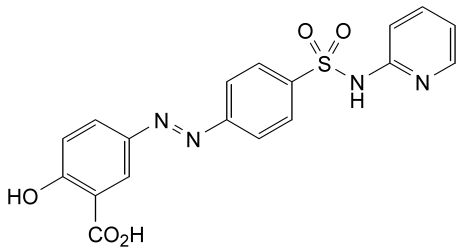
STORAGE

Store protected from light.

01/2008:0863
corrected 6.0

SULFASALAZINE

Sulfasalazinum



$C_{18}H_{14}N_4O_5S$ M_r 398.4
[599-79-1]

DEFINITION

2-Hydroxy-5-[2-[4-(pyridin-2-ylsulphamoyl)phenyl]diazenyl]-benzoic acid.

Content: 97.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: bright yellow or brownish-yellow, fine powder.

Solubility: practically insoluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *sulfasalazine CRS*.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *dilute ammonia R3*.

Reference solution (b). Dissolve 1.0 mg of *sulfasalazine derivative for resolution CRS* in 10.0 ml of reference solution (a). Dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: in a 1000 ml volumetric flask dissolve 1.13 g of *sodium dihydrogen phosphate R* and 2.5 g of *sodium acetate R* in 900 ml of *water R*; adjust to pH 4.8 with *glacial acetic acid R* and dilute to 1000 ml with *water R*;
- *mobile phase B*: mobile phase A, *methanol R* (10:40 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60 → 45	40 → 55
15 - 25	45	55
25 - 60	45 → 0	55 → 100
60 - 65	0	100
65 - 67	0 → 60	100 → 40
67 - 77	60	40

Flow rate: 1 ml/min.

Detection: spectrophotometer at 320 nm.

Injection: 20 µl.

Relative retention with reference to sulfasalazine: impurity H = about 0.16; impurity I = about 0.28; impurity C = about 0.80; impurity F = about 0.85; impurity G = about 1.39; impurity E = about 1.63; impurity B = about 1.85; impurity D = about 1.90; impurity A = about 2.00.

System suitability: reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to sulfasalazine and sulfasalazine derivative for resolution.

Limits:
- *impurities A, B, C, D, E, F, G, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak with a retention time less than 6 min (due to impurities H and J).

Impurities H and J. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dissolve 5.0 mg of *salicylic acid R* (impurity H) and 5.0 mg of *sulfapyridine CRS* (impurity J) in *dilute ammonia R3* and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 100.0 ml with *dilute ammonia R3*.

Column:
- *size*: *l* = 0.25, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mobile phase B (described in the test for related substances), mobile phase A (described in the test for related substances) (30:70 *V/V*).

Flow rate: 1 ml/min.