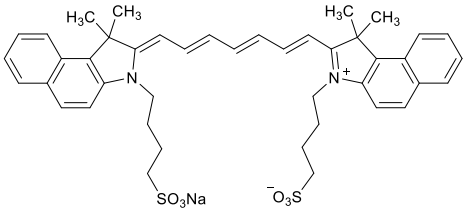
Indocyanine Green

インドシアニングリーン



$C_{43}H_{47}N_2NaO_6S_2$: 774.96

4-(2-{(1*E*,3*E*,5*E*,7*Z*)-7-[1,1-Dimethyl-3-(4-sodiosulfonatobutyl)-1,3-dihydro-2*H*-benzo[*e*]indol-2-ylidene]hepta-1,3,5-trien-1-yl}-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium-3-yl)butane-1-sulfonate [*3599-32-4*].

Indocyanine Green, when dried, contains not less than 90.0% and not more than 100.0% of indocyanine green ($C_{43}H_{47}N_2NaO_6S_2$), and not more than 5.0% of sodium iodide.

Description Indocyanine Green occurs as a dark green-blue powder.

It is soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

It is hygroscopic.

Melting Point: about 230 ℃.

Identification **(1)** Determine the absorption spectrum of a solution of Indocyanine Green in methanol (1 in 500,000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indocyanine Green RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Indocyanine Green as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum or the spectrum of Indocyanine Green RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with a solution of Indocyanine Green (1 in 200) as directed under Flame Coloration Test *<1.04>* (1): a yellow color appears.

pH *<2.54>* Dissolve 0.1g of Indocyanine Green in 20 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity **(1)** Clarity and color of solution－Dissolve 25 mg of Indocyanine Green in 50 mL of water: the solution is clear and dark green-blue.

(2) Related substances－Dissolve 25 mg of Indocyanine Green, previously dried, in 25 mL of methanol. To 4 mL of this solution add methanol to make 20 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.4 to indocyanine green obtained from the sample solution is not larger than 1/2 times the peak area of indocyanine green from the standard solution, and the total area of the peaks other than the peak of indocyanine green from the sample solution is not larger than 3/5 times the peak area of indocyanine green from the standard solution.

Operating conditions －

Detector: An ultraviolet absorption photometer (wavelength: 216 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with sodium hydroxide TS, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of indocyanine green is about 20 minutes.

Time span of measurement: About 2 time as long as the retention time of indocyanine green, beginning after the peak of sodium iodide having the relative retention time of about 0.08 to indocyanine green.

System suitability －

Test for required detectability: Pipet 2 mL of the standard solution, add methanol to make exactly 50 mL. Confirm that the peak area of indocyanine green obtained with 10 μL of this solution is equivalent to 3 to 5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of indocyanine green are not less than 8,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of indocyanine green is not more than1.0%.

(3) Sodium iodide Weigh accurately about 0.2 g of Indocyanine Green, previously dried, dissolve in 100 mL of

water, add 1 mL of nitric acid, and titrate <*2.50*> with 0.01 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.01 mol/L silver nitrate VS = 1.499 mg of NaI

Loss on drying <*2.41*> Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 70℃, 5 hours).

Assay Weigh accurately about 25 mg each of Indocyanine Green and Indocyanine Green RS, both previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add methanol to make exactly 25 mL. Pipet 2 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, at 785 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of Indocyanine Green ($C_{43}H_{47}N_2NaO_6S_2$)
$= M_S \times A_T / A_S$

M_S: Amount (mg) of Indocyanine Green RS taken

Containers and storage Containers－Tight containers.
Storage－Light-resistant, not exceeding −20℃.

Add the following to 9.01 Reference Standards (1):

Indocyanine Green RS