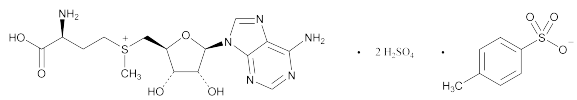
Ademetionine Disulfate Tosylate



$C_{22}H_{34}N_6O_{16}S_4$ 766.80
S-(Adenosyl)-L-methionine disulfate tosylate.
(3*S*)-5′-[(3-Amino-3-carboxypropyl)methylsulfonio]-5′-deoxyadenosine, disulfate-methylbenzenesulfonate [*29908-08-0*].

» Ademetionine Disulfate Tosylate is the disulfate–tosylate mixed salt of a mixture of diastereoisomers of the ademetionine ions. It contains not less than 95.0 percent and not more than 105.0 percent of ademetionine ($C_{15}H_{23}N_6O_5S^+$), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a refrigerator.

USP Reference standards ⟨11⟩**—***USP Ademetionine Disulfate Tosylate RS. USP S-Adenosyl-L-Homocysteine RS.*

Labeling—Label it to indicate the minimum content, in percentage, of *S,S*-isomer.

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of ademetionine in the *System suitability solution,* as obtained in the *Assay.*

pH ⟨791⟩**:** between 1.0 and 2.0, in an aqueous solution (1 in 20).

Water, *Method Ia* ⟨921⟩: not more than 3.0%.

Heavy metals, *Method I* ⟨231⟩**:** not more than 0.002%.

Isomeric ratio—

Buffer A—Transfer 4.2 g of citric acid monohydrate and 2.03 g of sodium dihydrogen phosphate dihydrate to a 1-L volumetric flask, dissolve in and dilute with water to volume, and mix.

Mobile phase—Transfer 4.0 g of sodium dodecyl sulfate and 440 mL of acetonitrile to a 1-L volumetric flask, dilute with *Buffer A* to volume, and mix.

Standard solution—Dissolve an accurately weighed quantity of USP Ademetionine Disulfate Tosylate RS in water to obtain a solution having a known concentration of 1.0 mg per mL.

Test solution—Transfer about 100 mg of Ademetionine Disulfate Tosylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard solution,* and record the peak response as directed for *Procedure:* the resolution, *R,* between the *S,S*-isomer and the *R,S*-isomer is not less than 1.0; and the relative retention times are about 0.94 and 1.0 for the *R,S*- and *S,S*-isomers, respectively.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Identify the peaks of the *S,S*- and *R,S*-isomers in the chromatogram of the *Test solution* by comparison with the chromatogram of the *Standard solution,* and calculate the percentage of the *S,S*-isomer by the formula:

$$100[r_{SS} / (r_{SS} + r_{RS})]$$

in which r_{SS} and r_{RS} are the areas of the peaks corresponding to the *S,S*-isomer and the *R,S*-isomer, respectively, in the *Test solution*. Not less than 60% and not less than the labeled amount of the *S,S*-isomer is found.

Change to read:

Content of sulfate—

Mobile phase—Prepare a solution of 8.0 mM sodium carbonate and 1.0 mM sodium bicarbonate in water.

Standard solution—Dissolve an accurately weighed quantity of potassium sulfate in water to obtain a solution having a known sulfate concentration of about 0.18 mg per mL.

Test solution—Transfer about 50 mg of Ademetionine Disulfate Tosylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an ion detector with suppressed conductivity and a 4.0-mm × 25-cm column that contains 7-µm packing •L74.• (RB 1-Mar-2010) The column temperature is maintained at 30°. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 8200 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area of the sulfate peak. Calculate the percentage of sulfate in the portion of Ademetionine Disulfate Tosylate taken by the formula:

$$10{,}000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the *Standard solution; W* is the weight, in mg, of Ademetionine Disulfate Tosylate taken to prepare the *Test solution;* and r_U and r_S are the peak responses obtained for sulfate in the chromatograms of the *Test solution* and the *Standard solution,* respectively. The content of sulfate is not less than 23.5% and not more than 26.5%.

Assay—

Buffer—Transfer 10 mL of glacial acetic acid to a 1-L volumetric flask, add 500 mL of water, and mix. To the flask add 2.06 g of sodium 1-hexanesulfonate, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed solution of *Buffer* and acetronitrile (85 : 15).

System suitability solution—Dissolve accurately weighed quantities of USP Ademetionine Disulfate Tosylate RS and USP *S*-Adenosyl-L-Homocysteine RS in water to obtain a solution having concentrations of about 400 µg of each per mL.

Standard preparations—Dissolve an accurately weighed quantity of USP *S*-Adenosyl-L-Homocysteine RS in water to obtain *Standard preparation A,* having a known concentration of about 400 µg per mL. Dilute portions of *Standard preparation A* to obtain *Standard preparation B* and *Standard preparation C,* having known concentrations of about 200 µg per mL and 80 µg per mL, respectively.

Assay preparation—Transfer about 20 mg of Ademetionine Disulfate Tosylate, accurately weighed, to a 50-mL volumetric flask, add 40 mL of water, and stir for 30 minutes; then dilute with water to volume, and mix. Transfer 1.0 mL of the solution to a 1.5-mL microcentrifuge tube, and centrifuge for 1 minute. Use a portion of the supernatant as the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-

mm × 15-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution* and *Standard preparation B,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.68 for *S*-adenosyl-L-homocysteine and 1.0 for ademetionine disulfate tosylate; the resolution, *R*, between *S*-adenosyl-L-homocysteine and ademetionine disulfate tosylate is not less than 1.5; the tailing factor is not more than 1.5 for *Standard preparation B;* and the relative standard deviation for replicate injections is not more than 2.0% for *S*-adenosyl-L-homocysteine in *Standard preparation B*.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparations* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area of the *S*-adenosyl-L-homocysteine peak in all three solutions and the ademetionine disulfate tosylate peak in the *Assay preparation.* Plot a calibration curve of the peak area of the *Standard preparations* versus the corresponding *S*-adenosyl-L-homocysteine concentration, in mg per mL, and draw the straight line best fitting the three points. From the calibration curve, and using the peak area of ademetionine from the chromatogram obtained with the *Assay preparation,* determine the concentration, *C,* in mg per mL, of ademetionine as *S*-adenosyl-L-homocysteine in the *Assay preparation.* Calculate the quantity, in mg, of ademetionine ($C_{15}H_{23}N_6O_5S^+$) in the portion of Ademetionine Disulfate Tosylate taken by the formula:

$$50C(399.44/384.41)$$

in which 399.44 and 384.41 are the molecular weights of ademetionine and *S*-adenosyl-L-homocysteine, respectively.