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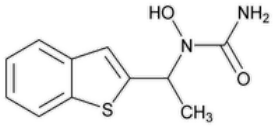
Zileuton



$C_{11}H_{12}N_2O_2S$ 236.29

Urea, *N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxy-, (±)-.

(±)-1-(1-Benzo[*b*]thien-2-ylethyl)-1-hydroxyurea CAS RN®: 111406-87-2; UNII: V1L22WVE2S.

» Zileuton contains not less than 98.5 percent and not more than 101.5 percent of $C_{11}H_{12}N_2O_2S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at room temperature.

USP Reference standards ⟨11⟩—

USP Zileuton RS

USP Zileuton Related Compound A RS

N-(1-Benzo-[*b*]thien-2-ylethyl)urea;

Also known as 1-[1-(Benzo[*b*]thiophen-2-yl)ethyl]urea.

$C_{11}H_{12}N_2OS$ 220.29

USP Zileuton Related Compound B RS

2-(Benzo[*b*]thien-2-oyl)benzo[*b*]thiophene;

Also known as Bis(benzo[*b*]thiophen-2-yl)methanone.

$C_{17}H_{10}OS_2$ 294.39

USP Zileuton Related Compound C RS

1-Benzo-[*b*]thien-2-ylethanone;

Also known as 1-(Benzo[*b*]thiophen-2-yl)ethan-1-one.

$C_{10}H_8OS$ 176.23

Identification—

A: *Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K*.

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

Specific rotation ⟨781S⟩: between −0.5° and +0.5°.

Test solution: 10 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩: not more than 1.5%.

Residue on ignition ⟨281⟩: not more than 0.2%.

Specific surface area, *Method I* ⟨846⟩—Outgas a portion of the test sample, about 100 mg, at 90° for 1 hour at ambient pressure using 0.001 mole fraction of krypton in helium as the adsorbate gas: between 0.9 and 3.1. m^2 per g.

Change to read:

▲**Arsenic ⟨211⟩, *Procedures, Procedure 2*▲** (CN 1-Jun-2023) **:** 2 µg per g.

Limit of boron—

Sulfuric acid solution—Carefully add 50 mL of sulfuric acid to 450 mL of water, and mix.

Standard solution—Prepare a solution in *Sulfuric acid solution* having a concentration of about 2.0 µg of boron per mL. Use of a commercially prepared boron ICP standard solution is recommended.

Test solution—Accurately weigh approximately 1.0 g of Zileuton into a 125-mL conical flask. Add 1 to 1.5 mL of sulfuric acid, and digest in a fume hood on a hot plate until charring begins. Add 2 mL of nitric acid to the cooled sample to aid digestion, and heat until brown fumes are not evolved. Cautiously add 30 percent hydrogen peroxide, dropwise, allowing the reaction to subside, and heating between drops. Add the first few drops very slowly with sufficient mixing in order to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the sample from caking on glass exposed to the

heating unit. Maintain oxidizing conditions at all times during the digestion by adding small quantities of the 30 percent hydrogen peroxide, whenever the mixture turns brown or darkens. Approximately 1 to 2 mL of nitric acid can be added, if necessary, which will create a refluxing effect to wash down any particles adhering to the neck of the flask. Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Transfer the solution to a 25-mL volumetric flask using about 7 mL of water. Repeat the washing twice more, and combine the washings in the volumetric flask. Dilute with water to volume, and mix.

Procedure—The inductively coupled plasma-atomic emission spectrometer is set up with wavelength of 249.7 nm, RF power of 1.25 KW, argon torch flow of about 13 L per minute, argon nebulizer flow of about 1 L per minute, and argon auxillary flow of about 0.5 L per minute. Analyze the *Standard solution* and the *Test solution,* using *Sulfuric acid solution* as the blank. Calculate the quantity, in µg of boron per g, in the portion of Zileuton taken by the formula:

$$25C/W,$$

where C is the concentration, in µg per mL, of boron in the *Test solution* determined from the instrument; and W is the weight, in g, of the Zileuton: not more than 10 µg per g is found.

Limit of pyridine—

Standard solution—Dissolve an accurately weighed quantity of pyridine, approximately 250 mg, in dimethyl sulfoxide, and dilute with dimethyl sulfoxide to 50 mL. Transfer 5 µL to a 100-mL sealed headspace vial.

Test solution—Transfer about 100 mg of Zileuton, accurately weighed, to a vial, add 5.0 mL of dimethyl sulfoxide and 1 g of anhydrous sodium sulfate, and seal with a septum and crimp cap. Heat the sealed vial at 80° for 60 minutes.

Chromatographic system (see *Chromatography ⟨621⟩*)—[Note—The use of a headspace apparatus is allowed.] The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused silica analytical column coated with a 3.0-µm G43 stationary phase. The carrier gas is helium with a linear velocity of about 35 cm per second. The injection port and detector temperatures are maintained at 140° and 260°, respectively. The column temperature is programmed according to the following steps. It is maintained at 40° for 20 minutes, then increased rapidly to 240°, and maintained at 240° for 20 minutes. Inject the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 15%.

Procedure—Using a heated gas-tight syringe separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution* and the *Test solution* into the gas chromatograph, and record the peak responses. Calculate the quantity, in ppm, of pyridine in the portion of Zileuton taken by the formula:

$$100(r_U/r_S)(W_S/W_U)$$

in which r_U and r_S are the peak responses for pyridine in the *Test solution* and the *Standard solution,* respectively; W_S is the weight, in mg, of pyridine used to prepare the *Standard solution;* and W_U is the weight, in mg, of Zileuton: not more than 100 ppm is found.

Chromatographic purity—[Note—For *Test 1* and *Test 2,* the *System suitability solution,* the *Standard solution,* and the *Test solution* are to be refrigerated at or below 5° immediately after preparation and during analysis using a refrigerated autosampler. The solutions are stable at or below 5° for about 36 hours.]

TEST 1—

Buffer solution—Prepare as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (82:18). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

System suitability solution—Dissolve accurately weighed quantities of USP Zileuton RS and USP Zileuton Related Compound A RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 5 µg of each USP Reference Standard per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Zileuton RS in acetonitrile to obtain a solution having a known concentration of about 10 µg per mL.

Test solution—Transfer about 125 mg of Zileuton, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system—Prepare as directed in the *Assay,* except to use a flow rate of 2.2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between zileuton and zileuton related compound A is not less than 1.5; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Zileuton taken by the formula:

$$100F(C_S/C_U)(r_i/r_S)$$

in which *F* is the relative response factor for each impurity, which is 1.0 for any peak with a relative retention time of 0.5, 0.7, 1.2, 1.6, 3.2, or 3.4, and is 1.2, 1.4, and 1.7 for peaks with relative retention times of 0.8, 2.1, and 2.8, respectively; C_S is the concentration, in mg per mL, of USP Zileuton RS in the *Standard solution;* C_U is the concentration, in mg per mL, of zileuton in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for zileuton obtained from the *Standard solution:* not more than 0.1% of any individual impurity with a relative retention time of 0.8, 1.6, or 2.1 is found; not more than 0.10% of any individual impurity with a relative retention time of 0.7, 3.2, or 3.4 is found; not more than 0.20% of any individual impurity with a relative retention time of 0.5 or 1.2 is found; and not more than 0.07% of any individual impurity with a relative retention time of 2.8 is found.

TEST 2—

Perchloric acid solution—Dissolve 5.0 mL of perchloric acid in 1000 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of *Perchloric acid solution* and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Standard stock solution—Dissolve an accurately weighed quantity of USP Zileuton Related Compound B RS in acetonitrile to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.

System suitability solution—Dissolve an accurately weighed quantity of USP Zileuton Related Compound C RS in acetonitrile to obtain a solution having a known concentration of about 10 µg per mL. Transfer 5.0 mL of this solution and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Standard solution—Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Test solution—Proceed as directed for *Test solution* under *Test 1*.

Chromatographic system—Prepare as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between zileuton related compound B and zileuton related compound C is not less than 20. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Zileuton taken by the formula:

$$100(C_S/C_U)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Zileuton Related Compound B RS in the *Standard solution;* C_U is the concentration, in mg per mL, of zileuton in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for zileuton related compound B obtained from the *Standard solution:* not more than 0.1% of any individual impurity is found; and not more than 0.7% of total impurities is found, the results for *Test 1* and *Test 2* being added.

Assay—

[NOTE—The *Standard preparation* and the *Assay preparation* are to be refrigerated at or below 5° immediately after preparation and during analysis using a refrigerated autosampler. The solutions are stable at or below 5° for about 36 hours.]

Buffer solution—Dissolve 7.7 g of ammonium acetate and 0.25 g of acetohydroxamic acid in about 900 mL of water in a 1000-mL volumetric flask, adjust with perchloric acid to a pH of 2.0, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (72:28). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Internal standard preparation—Transfer about 30 mg of methylparaben, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Standard stock preparation—Dissolve an accurately weighed quantity of USP Zileuton RS in acetonitrile to obtain a solution having a known concentration of about 1 mg per mL.

Standard preparation—Transfer 5.0 mL of the *Standard stock preparation* and 4.0 mL of the *Internal standard preparation* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Assay preparation—Transfer about 100 mg of Zileuton, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer 5.0 mL of this solution and 4.0 mL of the *Internal standard preparation* to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *CHROMATOGRAPHY ⟨621⟩*)—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between zileuton and methylparaben is not less than 5.0; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 0.6%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the quantity, in mg, of $C_{11}H_{12}N_2O_2S$ in the portion of Zileuton taken by the formula:

$$1000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Zileuton RS in the *Standard preparation;* and R_U and R_S are the peak area ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ZILEUTON	Documentary Standards Support	SM52020 Small Molecules 5
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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