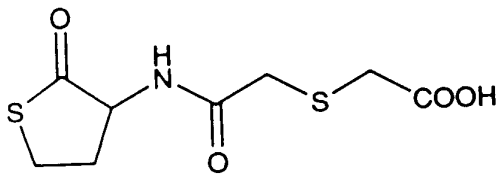
ERDOSTEINE - MONOGRAPH.

STRUCTURAL FORMULA



(±1S-(2-[N-3-(2-oxotetrahydro thienyl)]acetamido)-thioglycolic acid)

$C_8H_{11}NO_4S_2$ M.W. = 249.307

DESCRIPTION

Color : White to ivory white

Appearance : Microcrystalline powder

SOLUBILITY

Slightly soluble in methanol, ethanol, acetone, water.

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IDENTIFICATION

1. **Infrared Spectrum**

   The infrared spectrum of a nujol homogeneous dispersion of the test material, exhibits maxima only at the same wavelengths as that of a similar preparation of an Erdosteine A.S.

2. **HPLC**

   The retention time of the major peak in the chromatogram of the Sample preparation corresponds to that of the Standard Preparation, obtained as directed in the Assay.

PURITY TESTS

1. Appearance of solution

   A test solution (1.0% w/v in Methanol) is clear and colorless, according to EPV.6.1.

2. Loss on drying

   Determined on a sample of about 1.0g, exactly weighed, in oven at 105°C up to constant weight, according to USP Method <731>.

   Specification: Not more than 1.0%

3. Residue on ignition

   Determined on a sample of about 1.0g, exactly weighed, according to USP Method <281>.

   Specification: Not more than 0.2%

4. Heavy metals

   Determined on a sample of about 2g, exactly weighed, according to USP Method II <231>.

   Specification: Not more than 0.001%

5. **Chromatographic purity by HPLC (Limit Test)**

The potential related substances most likely to be present are as follows:

- Homocysteine thiolactone (raw material)
- Homocysteine (coming from homocysteine thiolactone)
- N-chloro-acetyl-homocysteine thiolactone (RV 142) (synthesis intermediate)
- Thiodiglycolic acid (degradation impurity during synthesis)
- N-thiodiglycolyl homocysteine (Metabolite 1, corresponding to the Erdosteine cycle opening)
- Thioglycolic acid: (raw material)
- S-{2-[N-3-(2-oxotetrahydro thienyl] acetamido}-N(carboxymethyl thioacetyl)-homocysteine (RV 201) (synthesis secondary product)
- Bis N-(2-oxo-3-tetrahydrothienylthiodiglycolylamide (EP 21506) (Synthesis secondary product). (The presence of two peaks in the graph is due to the fact that EP 21506 is a mixture of 4 diastereoisomers).

- **THIS TEST SHOULD BE CARRIED-OUT AS RAPIDLY AS POSSIBLE.**
- **STANDARD SOLUTIONS SHOULD BE PREPARED PROMPTLY AND PROTECTED FROM LIGHT.**
- **THE MOBILE PHASE USED FOR MAKING DILUTIONS MUST BE EQUAL TO THE ONE PASSING ON THE COLUMN.**
- **THE USE OF AN AUTOSAMPLER WITH REFRIGERATION IS RECOMMENDED.**

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HPLC Conditions

Column	: Eurospher 100 C18, 25 x 0.40cm, 5µ
Column Temperature	: 30°C
Mobile Phase	: Acetonitrile:Solution A* (11:89 v/v)
Flow Rate	: 1.0mL/min
Detector	: UV at 220nm, AUFS 0.005
Sample Value	: 20 µL

* Solution A - Buffer solution pH 2.0:

Dissolve 0.68g Potassium dihydrogen phosphate (KH_2PO_4) and 1.01g Heptane sulphonic acid Sodium salt in about 500mL water. Add 26.8mL of a 25% (w/v) Phosphoric acid and make up to 1L with water. Adjust to pH 2.0 with a 10N Sodium hydroxide solution.

Mobile phase proportions and flow rate may be varied in order to achieve the required system suitability.

ALL SOLVENTS USED MUST BE OF HPLC GRADE

Impurities stock solutions' and standard preparation:

Homocysteine Solution

Accurately weigh about 18mg ± 0.4mg Homocysteine into a 50mL volumetric flask. Dissolve in and make up to volume with mobile phase.

Homocysteine Thiolactone Solution

Accurately weigh about 18 ± 0.4mg Homocysteine Thiolactone into a 50 mL volumetric flask. Dissolve in and make up to volume with mobile phase.

Thioglycolic acid Solution (Mercaptuacetic acid)

Accurately weigh about 18mg ± 0.4mg Thioglycolic acid into a 50mL volumetric flask. Dissolve in and make up to volume with mobile phase.

Thiodiglycolic acid Solution

Accurately weigh about 18mg ± 0.4mg Thiodiglycolic acid into a 50mL volumetric flask. Dissolve in and make up to volume with mobile phase.

RV 142 Solution

Accurately weigh about 12mg ± 0.3mg RV 142 A.S. into a 25mL volumetric flask. Dissolve in and make up to volume with mobile phase.

Impurities Diluted Solution

Pipet 1mL each of the impurities stock solutions into a 25mL volumetric flask and make up to volume with mobile phase.

Erdosteine stock solution

Accurately weigh about 12mg ± 0.3mg Erdosteine A.S. into a 10mL volumetric flask. Dissolve in and make up to volume with mobile phase.

Standard solution

Pipet 1mL of the Impurities Diluted Solution and 4mL of Erdosteine Stock Solution into a 50mL volumetric flask and make up to volume with mobile phase.

The resulting concentrations of the impurities in this solution as percent of Erdosteine concentration are as follows:

Homocysteine 0.3%

Homocysteine thiolactone 0.3%

Thioglycolic acid 0.3%

Thiodiglycolic acid 0.3%

RV 142 0.4%

System suitability test

Inject the standard solution and run the chromatogram up to 25 minutes. The peaks elute in the following order:

PEAK ORDER	RRT
Thiodiglycolic acid	0.52
Thioglycolic acid	0.60
Homocysteine	0.76
Homocysteine thiolactone	0.81
Erdosteine	1.0
RV 142	1.26

Typical retention time of the Erdosteine peak is about 6.2 minutes.

The resolution factor between Homocysteine thiolactone and Erdosteine peaks and between Erdosteine and RV 142 should be no less than 2.

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Sample Solution preparation

Accurately weigh about 30mg ± 0.6mg Erdosteine test material into a 25mL volumetric flask. Dissolve in and make up to volume with mobile phase. Pipet 4mL of this solution into a 50mL volumetric flask and make up to volume with mobile phase.

Procedure

Inject mobile phase (blank run). Inject the standard and sample solutions and run the chromatogram up to 25 minutes. Determine the peak areas in each solution using a suitable integrator.

Acceptance criteria

Subtract any blank peak from the sample solution chromatogram. No peak area in the sample solution chromatogram should be greater than that of the corresponding peak due to impurities in the standard solution chromatogram. Calculate any other impurities such as RV 201 (RRT = 2.08), Metabolite 1 (RRT = 1.15) and EP 21506 (double peak at RRT = 3.23) by means of area normalization. If no peak is detected at the corresponding RRT, report it as "Not detected" or "Less than the specified detection limit".

Specification

IMPURITIES	SPECIFICATIONS (UPPER LIMIT)	DETECTION LIMITS
Homocysteine	0.3%	0.25%
Homocysteine thiolactone	0.3%	0.15%
Thioglycolic acid	0.3%	0.15%
Thiodiglycolic acid	0.3%	0.15%
RV 142	0.4%	0.1%
RV 201	0.5%	0.1%
EP 21506	0.5%	0.02%
Metabolite 1	0.5%	0.01%
Single unknown impurity	0.2%	
Total	1.5%	

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ASSAY BY HPLC

This test should be carried-out as rapidly as possible.
Standard solutions should be prepared promptly and protected from light. The mobile phase used for making dilutions must be equal to the one passing on the column. The use of an autosampler with refrigeration is recommended.

HPLC Conditions

The same as for Chromatographic Purity (p.4).

Standard Solution preparation

Accurately weigh about 30mg Erdosteine A.S. into a 25mL volumetric flask, add about 20mL mobile phase and sonicate to dissolve. Make up to volume with mobile phase (standard stock solution). Dilute 4mL of this solution to 50mL with mobile phase.

System suitability solution

Weigh about 6mg Metabolite 1 into a 50mL volumetric flask. Dissolve in and make up to volume with mobile phase. Pipet 3mL of this solution and 2mL of the Erdosteine standard stock solution into a 25mL volumetric flask and make up to volume with mobile phase.

System suitability test

Inject the system suitability solution. The retention time of the Erdosteine peak is about 6.2 minutes. Metabolite 1 elutes at relative retention time of 1.15 related to Erdosteine. The resolution factor between these two peaks (calculated according to USP) should be not less than 1.5. A relative standard deviation calculated for 5 standard replicate injections must be not more than 2.0.

Sample Solution preparation

Accurately weigh about 30mg Erdosteine test material into a 25mL volumetric flask, add about 20mL of mobile phase and sonicate to dissolve. Make up to volume with mobile phase. Pipet 4mL of this solution into a 50mL volumetric flask and make up to volume with mobile phase.

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Procedure

Inject the standard and sample solutions into the chromatograph and determine the peak area of Erdosteine in each chromatogram.

Calculation

1. Assay of Erdosteine:

$$\frac{\text{Pk area smp x Std wt * (mg) x 100}}{\text{Pk area std x smp wt * *(mg)}} = \text{\% Assay calculated on dry basis}$$

2. Assay of Metabolite 1:

$$\frac{\text{Pk area smp x Std wt * (mg) x Rf * * * (mg) x 100}}{\text{Pk area std x Smp wt * *(mg)}} = \text{\% Assay of Metabolite 1 calculated on dry basis}$$

* std wt - is corrected according to % Water and % Assay

** smp wt - is corrected according to % Water

*** RF = 4.0, response factor for calculation of Metabolite 1 $= \left(\frac{\text{Absorptivity of erdosteine}}{\text{Absorptivity of metabolite1}}\right) = 4.0$

Specification

98.0% - 102.0% Erdosteine assay, calculated on dry basis.

PARTICLE SIZE (This test is performed only for micronized active drug substance)

Apparatus : Computerized inspection system

Method : By volume distribution

Specifications : 100% less than 40µ