

## ERDOSTEINE CAPSULES - ASSAY &amp; RELATED SUBSTANCES

## HPLC ASSAY AND RELATED SUBSTANCE

<b>Column</b>	Eurospher 100, C18, 25x0.46cm 5 $\mu$
<b>Mobile Phase</b>	Buffer pH 2.0*: Acetonitrile (88:12 v/v)
* Buffer pH 2	Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> ) - 0.68g
	Hepatane sulphonic acid - 1.01g
	Phosphoric acid (85%) - 4.6mL & Water - to 1000mL adjust pH to 2.0 with Sodium hydroxide (35% w/v)
<b>Flow rate</b>	1.0mL/min
<b>Sample volume</b>	10 $\mu$ L
<b>Detector</b>	UV at 220nm, AUFS 0.01
Mobile phase proportions and flow rate may be varied in order to achieve the required system suitability	
<b>ALL SOLVENTS USED MUST BE HPLC GRADE</b>	
<b>ALL SOLUTIONS MUST BE FRESH DAILY</b>	

STANDARD PREPARATION

Accurately weigh about 14mg of Erdosteine A.S. into a 50mL volumetric flask. Add about 35mL of mobile phase and sonicate to dissolve. Make up to volume with mobile phase. This is the standard solution.

SYSTEM SUITABILITY SOLUTION

Weigh about 6mg of Metabolite 1 into a 20mL volumetric flask. Dissolve in and make up to volume with standard solution.

<b>ED. NO: 04</b>	<b>Effective Date:</b> IAGIM DD/MM/2000	<b>APPROVED:</b> SI - 10862 ERDOSTEINE 300mg CAPSULES #02 ASSAY & RELATED SUBSTANCE FOR STABILITY STUDY			
<b>Ed. Status : Supcds 03</b>		<i>Anne</i> ANALYST	<i>Bella</i> SUPERVISOR	<i>Edanna</i> QC	<i>Carol</i> HEAD

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**SYSTEM SUITABILITY TEST**

Inject the System Suitability Solution. The retention time of the Erdosteine peak is about **6** minutes and of Metabolite 1 peak is about **7.5** minutes.

The resolution factor between these two peaks (calculated according to USP) should be not less than **2.5**.

The tailing factor of the Erdosteine peak (calculated according to USP) should be not greater than **1.5**.

A relative standard deviation, calculated for **5** replicate injections of standard preparation must be not more than **2.0%**.

**SAMPLE PREPARATION**

Weigh 20 capsules. Transfer as completely as possible the contents of the capsules to a suitable tared container and determine the average content weight per capsule.

Mix the combined contents and accurately weigh about 60mg of the powder into a 200mL volumetric flask. Add 150mL of mobile phase and sonicate for 15 minutes.

Make up to volume with mobile phase. Filter through a 0.45µ membrane filter.

**PROCEDURE**

Inject the Standard and Sample solutions into the chromatograph and determine the peak area of Erdosteine in each chromatogram with a suitable integrator.

**CALCULATION**

$$\frac{\text{Pk area smp} \times \text{Std wt}^* (\text{mg}) \times \text{Avg cap. cont. wt}(\text{mg}) \times 400}{\text{Pk area std} \times \text{smp wt}(\text{mg}) \times \text{Dose}(\text{mg} / \text{cap})} = \% \text{ Erdosteine of labeled claim}$$

\* Std wt is corrected in accordance with % Assay and % Water.

**CONTENT OF METABOLITE 1**

During the HPLC determination of Erdosteine in capsules, the evaluation of Metabolite 1 can be done from the same chromatogram.

$$\frac{\text{Pk area Met 1}}{\text{Pk area Erdosteine}} \times \text{RF}^* \times 100 = \% \text{ of Metabolite 1}$$

\*\*RF= 4.0 - Response factor for calculation of Metabolite 1 =

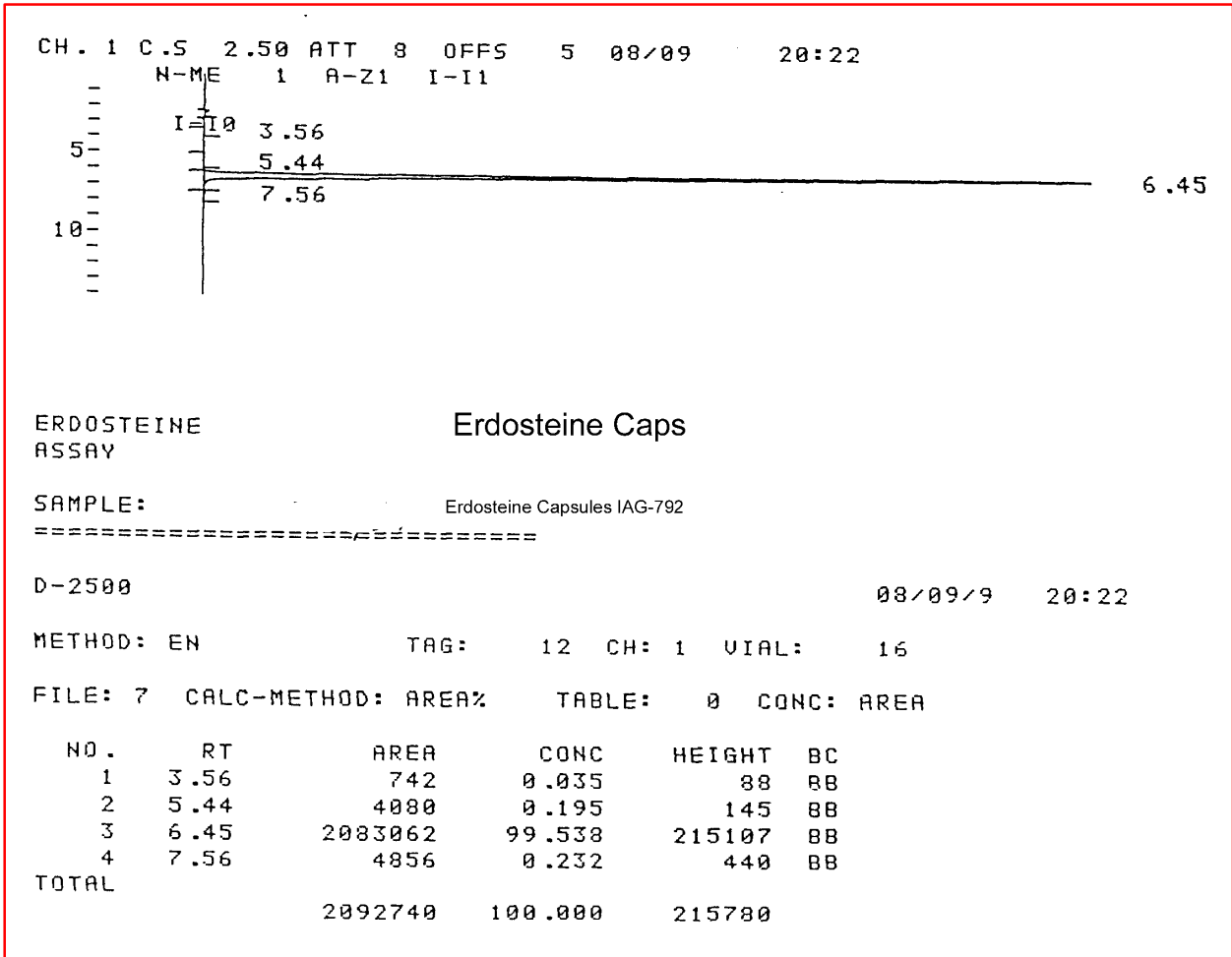
$$\left( \frac{\text{Absorptivity of Erdosteine}}{\text{Absorptivity of Metabolite 1}} = 4.0 \right)$$

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**ANALYTICAL METHOD  
PROCEDURES**

**ERDOSTEINE CAPSULES - ASSAY & RELATED SUBSTANCES**

**TYPICAL CHROMATOGRAM**



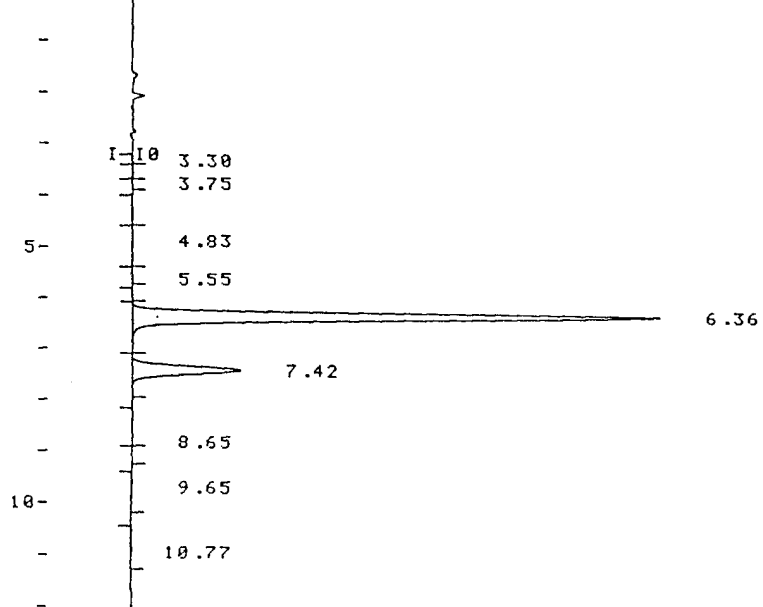
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**ANALYTICAL METHOD  
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**SYSTEM SUITABILITY GRAPH**

CH. 1 C.S 10.00 ATT 9 OFFS 5 08/09/9 17:35  
N-ME 1 A-Z1 I-11



$$R = \frac{2 \times (7.42 - 6.36)}{0.30 + 0.30} = 3.5$$

$$T = \frac{0.30}{2 \times 0.12} = 1.25$$

ERDOSTEINE  
ASSAY

**System Suitability**

SAMPLE: =====

D-2500 08/09/9 17:35

METHOD: EN TAG: 1 CH: 1 VIAL: 11

FILE: 7 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	HEIGHT	BC
1	3.30	1332	0.036	228	88
2	3.57	6327	0.171	920	88
3	3.75	3552	0.096	645	88
4	4.07	1504	0.041	154	88
5	4.83	3910	0.106	245	88
6	5.55	1014	0.027	108	88
7	5.92	1127	0.031	146	88
8	6.36	2954467	80.024	312006	88
9	7.42	706609	19.139	63980	88
10	8.65	3009	0.008	116	88
11	9.03	2644	0.072	255	88
12	9.65	4798	0.130	370	88
13	10.77	1675	0.045	85	88
TOTAL					
PEAK REJ :		3691968	100.000	379258	
INJ-VOL :		250			
		10.00			

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