AM-0240-0200-03
Total Pages: 4

ANALYTICAL METHOD PROCEDURES

ANALYTICAL METHOD
ASSAY AND RELATED SUBSTANCE DETERMINATION
HPLC Determination of 300mg Erdosteine Capsules

ASSAY of the MONTH

This assay is suitable for Erdosteine Capsules 300 mg as well as Erdosteine Suspension 175mg/5mL. No Erdosteine HPLC stability Indicating Assay has yet been published to date in the USP 24 / NF 19 or Pharmacopeial Forum 27 No 1 - 2.

HPLC ASSAY AND RELATED SUBSTANCE

Column	Eurospher 100, C18, 25 x 0.40cm 5µ
Mobile Phase	Buffer pH 2.0*: Acetonitrile (88 **:** 12 v/v)
* Buffer pH 2	Potassium dihydrogen phosphate (KH_2PO_4) - 0.68g
	Hepatane sulphonic acid - 1.01g
	Phosphoric acid (85%) - 4.6mL & Water to 1000mL adjust pH to 2.0 with Sodium hydroxide 10N (35% w/v)
Flow rate	1.0mL / min
Sample volume	10µL
Detector	UV at 220nm, AUFS 0.01

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

ALL SOLVENTS USED MUST BE HPLC GRADE

ALL SOLUTIONS MUST BE FRESH DAILY

THIS ASSAY IS CURRENTLY NOT AVAILABLE IN THE USP24 / NF19 or PHARMACOPEIAL FORUM

STANDARD SOLUTION 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 solution is labeled as 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.

AM-0240-0200-03
Total Pages: 4

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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 SOLUTION PREPARATION

■ Weigh 20 capsules units. 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

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

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

CONTENT OF METABOLITE 1

During the HPLC assay determination of Erdosteine active in capsules, the evaluation of Erdosteine 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 =

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

ED. N0: 04	Effective Date: IAGIM	APPROVED: IAG-04 ERDOSTEINE 300mg CAPSULES #04 HPLC ASSAY AND RELATED SUBSTANCE TEST INDICATED FOR STABILITY PROFILES			
Ed. Status : Supcds 03	10/01/2001	Anne	Bella	Edanna	Carol
		ANALYST	SUPERVISOR	QC	HEAD

AM-0240-0200-03
Total Pages: 4

ANALYTICAL METHOD PROCEDURES

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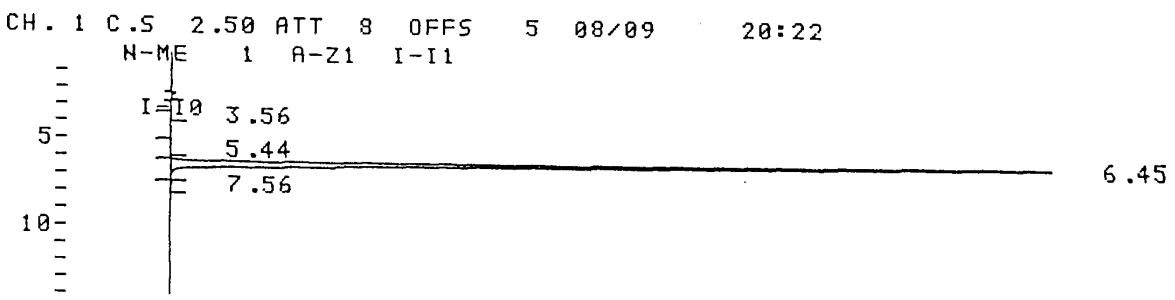
ASSAY AND RELATED SUBSTANCE DETERMINATION
HPLC Determination of 300mg Erdosteine Capsules

TYPICAL CHROMATOGRAM



ERDOSTEINE
ASSAY

Erdosteine Caps

SAMPLE: Erdosteine Capsules IAG-792

D-2500 08/09/9 20:22

METHOD: EN TAG: 12 CH: 1 VIAL: 16

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

NO.	RT	AREA	CONC	HEIGHT	BC
1	3.56	742	0.035	88	BB
2	5.44	4080	0.195	145	BB
3	6.45	2083062	99.538	215107	BB
4	7.56	4856	0.232	440	BB
TOTAL		2092740	100.000	215780	

SYSTEM SUITABILITY SET-UP

Inject the system suitability solution as specified in the method, at the beginning of each analysis and whenever a significant change is made in the system, i.e. mobile phase, column, detector, etc. Record the chromatogram and calculate the system suitability parameters.

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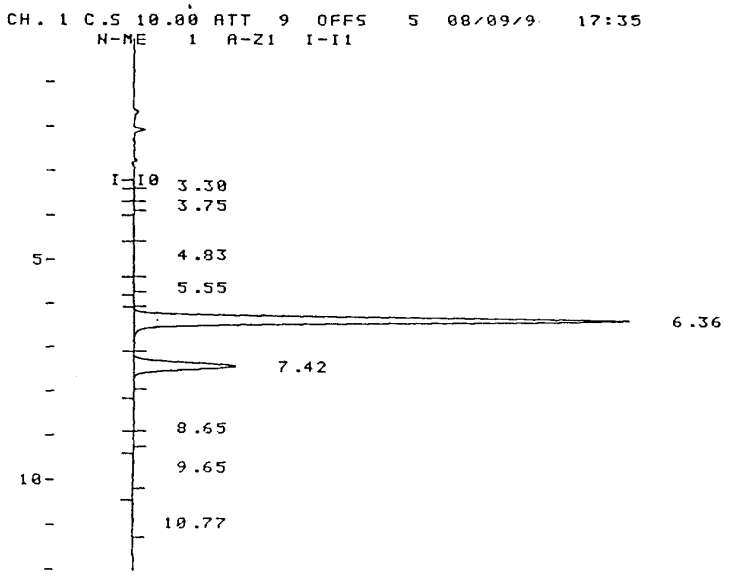
SYSTEM SUITABILITY GRAPH



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

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

ERDOSTEINE
ASSAY
SAMPLE:System Suitability

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	BB
2	3.57	6327	0.171	920	BB
3	3.75	3552	0.096	645	BB
4	4.07	1504	0.041	154	BB
5	4.83	3910	0.106	245	BB
6	5.55	1014	0.027	108	BB
7	5.92	1127	0.031	146	BB
8	6.36	2954467	80.024	312006	BB
9	7.42	706609	19.139	63980	BB
10	8.65	3009	0.082	116	BB
11	9.03	2644	0.072	255	BB
12	9.65	4798	0.130	370	BB
13	10.77	1675	0.045	85	BB
TOTAL		3691968	100.000	379258	

PEAK REJ : 250
INJ-VOL : 10.00

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