SOP # S-270-03-0601

ANALYTICAL METHOD PROCEDURES

Total Number of Pages 6.

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

Changes. Editorial and Caution

CHROMATOGRAPHIC CONDITIONS:

Column & Packing : Beckman, Ultrasphere ODS, 5µ, 25×0.46cm column.

Mobile phase and flow : Run gradient program as per table:

Table 01. GRADIENT MATRIX

No	Time (minutes)	Solution A[1] (expressed in %)	Solution B[2] (expressed in %)	Flow rate mL/min
1	0	100	0	1.0
2	10	100	0	1.0
3	12	100	0	1.5
4	18	25	75	1.5
5	30	25	75	1.5
6	35	100	0	1.5
7	40	100	0	1.0
8	45	100	0	1.0

KEY

1 Solution A - buffer phosphate pH = 2[3] / Acetonitrile (88:12v/v)

2 Solution B - buffer phosphate pH = 2[3] / Acetonitrile (40:60v/v)

3 **Preparation of Phosphate Buffer pH = 2 :**

Dissolve 0.68g of potassium phosphate monobasic (KH_2PO_4), 1.01g of Sodium heptansulphonate in 970mL of water, add 26.75mL of 25% phosphoric acid, adjust the pH to 2.0 with 20% sodium hydroxide and dilute to 1000mL with water.

CAUTION : The analytical determination should be carried-out as rapidly as possible. Standard and Sample solutions should be prepared promptly and injected immediately. Optimally an auto-sampler with refrigeration can be used.

IAGIM Scientific Committee e-mail: research@iagim.org

SOP # S-270-03-0601

ANALYTICAL METHOD PROCEDURES

Total Number of Pages 6.

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

CAUTION - ALL SOLVENTS USED MUST BE OF HPLC GRADE

Column & Packing	:	Beckman, Ultrasphere ODS, 5µ, 25 × 0.46cm column
Detector & Path	:	UV at 220nm, Path = 10mm cell
Injection Volume	:	10µL
Auto-sampler washing liquid	:	Acetonitrile / water (50:50v/v)
Diluent	:	Solution A*

Flow rate and mobile phase proportions may be varied in order to achieve the required system suitability parameters and spectrum.

STANDARD SOLUTION PREPARATION

- Accurately weigh about 18mg of Erdosteine A.S. into a 50mL volumetric flask. Add about 35mL of diluent and sonicate to dissolve fully. Make up to volume with Diluent. This solution is labeled the Standard Solution.

SYSTEM SUITABILITY SOLUTION

- Weigh about 6mg of Metabolite 1 into a 20mL volumetric flask. Add 10mL of standard solution and sonicate to dissolve. Make up to volume with standard solution.

SYSTEM SUITABILITY TEST

- 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.
- Inject the system suitability solution in the gradient mode.

SYSTEM SPECIFICATIONS

- 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 **1.7**.
- The relative standard deviation, calculated for **5** replicate injections of the standard preparation must be not more than **2.0%.**

IAGIM Scientific Committee e-mail: research@iagim.org

SOP # S-270-03-0601

ANALYTICAL METHOD PROCEDURES

Total Number of Pages 6.

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

SAMPLE SOLUTION PREPARATION - CONSTITUTED SUSPENSION

- Analyze 3 product containers individually as follows:
- Constitute the suspension by adding 60mL of purified water into the bottle (by weight). Place the bottle on it's flat side and shake mechanically for 10 minutes.
- Transfer 1mL by weight into a 100mL volumetric flask, add 60mL of diluent and sonicate for 10 minutes.
- Make up to volume with diluent. Immediately centrifuge a portion of the above solution at 3500 rpm for 10 minutes. The clear supernatant solution is labeled as the TEST SAMPLE.
- Determine the suspension's density utilizing a calibrated pycnometer of 10mL (according to Standard Pharm. Eur. Method).

PROCEDURE

- Inject Solution A (diluent) to detect any system peaks.
- Prior to any injection of Standard Solution, - clean the column by injecting the diluent solution. Now Inject the Standard and Samples Solutions. Determine the peak areas for Active Erdosteine and Metabolite1 in each chromatogram by use of a suitable integrator.

CALCULATION

For Assay of Erdosteine

$$\frac{\text{Peak area Smp}}{\text{Peak area Std}} \times \frac{\text{Std wt * (mg)}}{50} \times \frac{100 \times \text{Density (mg / mL)}}{\text{Smp wt (mg)}} \times \frac{100}{\text{Dose (mg / mL)}} = \%\ \text{Erdosteine of labeled claim}$$

For Assay of Metabolite 1

$$\frac{\text{Peak area Met1} \times \text{RF **}}{\text{Peak area Std}} \times \frac{\text{Std wt * (mg)}}{50} \times \frac{100 \times \text{Density (mg / mL)}}{\text{Smp wt (mg)}} \times \frac{100}{\text{Dose (mg / mL)}} = \%\ \text{Metabolite 1 of labeled claim of Erdosteine}$$

* Std wt - is corrected according to % Assay and % water.

** RF = 4.0 - response factor for calculation of Metabolite 1 =

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

CAUTION : ADDITIONAL EXCIPIENT PEAK

Where the artificial sweetner Nutrasweet Aspartam™ is used in the end product formula, an excipient placebo peak appears at a RRT of approximately 1.4

SOP # S-270-03-0601

ANALYTICAL METHOD PROCEDURES

Total Number of Pages 6.

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

ACCEPTANCE CRITERIA

In any case, the difference between the results should be less than 8%.

Maximum (%) - Minimum (%) ≤ 8%

REPORTING

Report area percent of any additional peak up to 12 minutes (ignore system / placebo peaks).

REPORTING NOTES on DL and QL:

In the absence of a Metabolite-1 peak at RRT = 1.2 (related to Erdosteine), proceed to report: - Peak Less than 0.1% (DL) Limit of Detection

For peak between 0.1% - 0.25% Report less than 0.25% (QL - Limit of Quantitation).

The analytical determination should be carried-out as rapidly as possible. Standard and Sample solutions should be prepared promptly and injected immediately. Optimally an auto-sampler with refrigeration can be used.

Tips & Traps…

Not only does a analytical drug researcher need to keep a strict record of every detail of the product development assay - for both the advances and the failures of the experimental procedures but the laboratory equipment used to obtain those results needs to be trusted year-in and year-out with each and every assay.

Analytical equipment needs to be installed correctly, calibrated, cleaned and maintained on a routine basis - thus insuring both accurate **and** precise analytical test results all the time

Written SOPs must demonstrate that analysts are following a key rule of a good researcher namely that : The analytical procedures must be carefully described so that they can be replicated by others, whenever and wherever, needed.

Testing procedures and methods should be designed in close cooperation with the plant production laboratory facilities, to insure an eventual smooth transfer of technical data (TD) and key documentation as required in future TD transfers.

Photo curtsey of IAGIM Analytical Laboratory

Edition No: 03	Effective Date:	APPROVED	info@iagim.org			
Ed. Status : SUPERSEDES 02	IAGIM Jan 200Y	_____ Department	_____ R &D	_____ RA	_____/_____ QC / QA	

SOP # S-270-03-0601

ANALYTICAL METHOD PROCEDURES

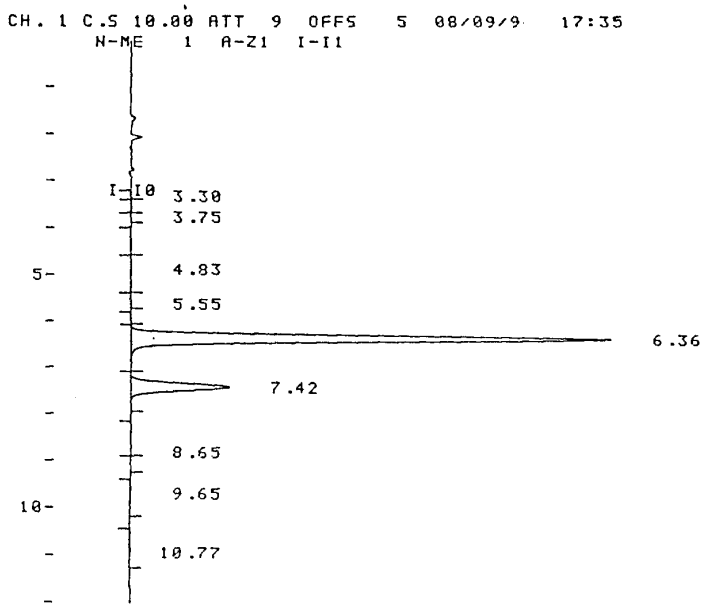
Total Number of Pages 6.

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

In order to achieve the required conditions for system suitability, adjustments are permissible in the flow rate and in the ratio of the component of the mobile phase for HPLC (up to 10%) Any exceptions to these changes must be recorded in the method.

SYSTEM SUITABILITY CHROMATOGRAM



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

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

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

ERDOSTEINE SUSPENSION 175mg/5mL
ASSAY & IMPURITIES & DEGRADATION PRODUCTS

ERDOSTEINE POWDER FOR ORAL SUSPENSION 3.5% #04 Containing: 175 mg Erdosteine per 5mL (3.5g per bottle) 10mg Sodium Benzoate per 5mL (200 mg per bottle) ASSAY OF ERDOSTEINE AND DETERMINATION OF IMPURITIES & DEGRADATION PRODUCTS FOR STABILITY STUDIES

PROCEDURAL NOTES

1 For assay calculation, peak responses may be rounded off to at least 4 digits.

2 A Standard Solution (S1 or S2) must be injected every 6 injections or every 3 hours, whichever is the shorter, or as stated in the specific method in order to demonstrate the on-going system repeatability. The CV for all the control (standard) injections must not be more than 2.0%.

3 The measurement of standard solutions

3.1 Two independent standard solutions (S_1, S_2) are prepared and then one of the standard solutions (S_1) is injected into the chromatographic system 5 times unless otherwise required in the specific method.

3.2 Standard Deviation of the Standard Solution (S_1) responses:

$$S = \sqrt{\frac{\Sigma(Xi - \overline{X})^2}{n-1}}$$

and if the

$$CV = \frac{S\text{x}100}{\overline{X}}$$

where:

Xi = Each measurement (X_1, X_2..X_5)

$\overline{X}$ = Average

n = no. of measurements

is 2.0% or smaller, the system is considered to be precise.

3.3 If CV> 2.0% proceed as follows:
Evaluate the 5 responses obtained for a suspected outlier according to the Dixon Q-test (see SOP Rejecting Outliers by Dixon Q-test):

$$Q = \frac{Xi - Xj}{X\,\text{max} - X\,\text{min}}$$

Xi = “suspected” response (usually Xmin or Xmax)
Xj = the closest response to the suspected outlier
Xmin = the smallest response; Xmax = the largest response

3.4 If Q > 0.64 (for 5 measurements only), the suspected outlier is discarded, the standard solution is injected once again and the average of the 5 injections is calculated.