

Validation of a chromogenic Factor IIa Assay for testing of Dalteparin Sodium and Dalteparin Injection

Introduction

Dalteparin anti-FIIa is a chromogenic assay intended for the quantitative determination of Dalteparin in purified solutions by measurement of factor IIa inhibition activity. The kit can be used for 100 test reactions as per microtiter plate protocol.

The inhibitory effect of anti-thrombin III (AT-III) on thrombin, factor IIa and other coagulation serine proteases in plasma is increased several thousand-fold by Dalteparin. This inhibition accounts for the anticoagulant effect of Dalteparin.

The quantitative determination of Dalteparin levels by the measurement of their anti-IIa activity is a necessary tool for monitoring treatment efficacy.

Presence of Dalteparin catalyzes the reaction between ATIII and α -Thrombin. The factor IIa inhibition test is the most useful assay covering the widest variety of Dalteparin preparations. In the assay, the rate of factor IIa inhibition is directly proportional to the Dalteparin concentration since both factor IIa and AT-III are in excess.

The residual factor IIa activity is inversely proportional to the Dalteparin concentration

Materials and Method:

Materials provided in lyophilized form are :

Materials	Amount of DI water for reconstitution (ml)	After Reconstitution 1:4 dilution
Human Anti-thrombin III Reagent	1ml	100 μ l of M.S + 400 μ l of buffer
Human Thrombin- α reagent	1m	100 μ l of M.S + 400 μ l of buffer reagent
Chromogenic Substrate	1ml	100 μ l of M.S + 400 μ l of H ₂ O

Note : M. Reagents Reconstituted Main stock M. Dalteparin Sodium EPRS

Dilution Buffer	20mM Tris, pH 7.4 and 150mM NaCl
Stop Solution	20% v/v Glacial Acetic Acid
Recommended Standard concentration (considering 1mg=100IU)	0.20 IU/ml, 0.15 IU/ml, 0.10 IU/ml, and 0.05 IU/ml

Assay Procedure :

Standard or Test Sample	50 μ l
Human Anti-thrombin III	50 μ l
Mix but do not allow bubbles to form. Incubate at 37°C, for 2 minutes	
Human thrombin α	50 μ l
Mix and incubate at 37°C, for exactly 2 minutes	
Chromogenic Substrate	50 μ l
Mix and incubate at 37°C, for 2 minutes	
Acetic Acid	50 μ l
Mix and measure the absorbance at 405nm	

Standard and Test Sample preparation :

Example - Standard Concentration 100 IU/ml (Main Stock) is to be diluted as per below table:

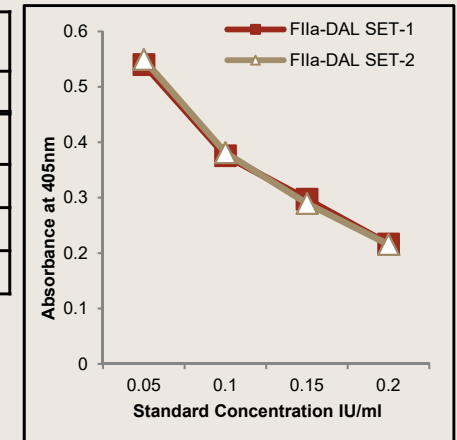
Sr.no	Concentration (IU/ml)	stock(μ l)	Diluent (μ l) (buffer pH7.4)	Total volume (μ l)
S1	10	50 μ l of Main Stock	450	500
S2	1	50 μ l of S1	450	500
S3	0.20	100 μ l of S2	375	500
S4	0.15	75 μ l of S2	400	500
S5	0.10	50 μ l of S2	450	500
S6	0.05	25 μ l of S2	475	500

Test Dilution – Test Sample Main Stock is of concentration 100IU/ml

Sr.no	Concentration (IU/ml)	stock(μ l)	Diluent (μ l) (buffer pH7.4)	Total volume (μ l)
T1	10	50 μ l of Main Stock	450	500
T2	1	50 μ l of T1	450	500
T3	0.20	100 μ l of T2	375	500
T4	0.15	75 μ l of T2	400	500
T5	0.10	50 μ l of T2	450	500
T6	0.05	25 μ l of T2	475	500

Result :

Standard concentration IU/ml	Absorbance at 405nm	
	Set-1	Set-2
0.05	0.5404	0.5495
0.1	0.3757	0.3813
0.15	0.2973	0.2896
0.2	0.2165	0.2155



Data Interpretation :

For each series, calculate the regression of the absorbance against log concentration of the sample solutions and the standard solutions.

Calculate the potency of the Dalteparin in IU of Anti-Factor IIa activity/ml using statistical methods for parallel-line assays.

The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the Anti-Factor IIa activity of the sample in mg.

Standard and Test Samples being serial diluted should pass the test for linearity and parallelism as the interpretation is done by extrapolating the data. We have used proprietary MS Excel software for the same based on DJ Finney algorithm.

Conclusion :

The assay kits manufactured by KRISHGEN BIOSYSTEMS are validated Chromogenic Assays for the determination of Dalteparin using anti-IIa activity in human plasma successfully met all standard assay-validation parameters and were suitable for use in bioequivalence studies.

Authors:

Ms Prajakta Ambre, Ms Trupti Bendigeri, Ms Jyoti Gupta, Dr Amitabha De - KRISHGEN BIOSYSTEMS R&D Laboratory