

Title

Bioequivalence study of two low molecular weight heparins in healthy volunteers

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Aim:

The aim of the present study is to determine if Clexane and Enoxaparin (Hangzhou Jiuyuan Gene Engineering Co., Ltd) can be regarded as bioequivalent at the dose level administered in healthy subjects.

Design of the study:

The study protocol was approved by the Ethics Committee of First Affiliated Hospital of College of Medicine, Zhejiang University, China. A single subcutaneous injection (pre filled syringes) of Enoxaparin, a LMWH (Low Molecular Weight Heparin) Sodium (60mg. Batch No. 7440, Clexane®) from Aventis Pharma and a LMMH Sodium (60 mg Batch No. 20060902) from Hangzhou Jiuyuan Gene Engineering Co., Ltd, China were given in a randomized, cross over design to 22 healthy subject with a minimum 7-day washout period.

The Anti-Xa activities of the pre filled syringes were measured and qualified with quality certification in Hangzhou Jiuyuan Gene Engineering Co., Ltd.

One week prior to and during the study the subjects were asked not to take any drugs that might interfere with hemostasis. On the day of administration of drugs the subjects were asked to be fasting. All the subjects were housed in the department of clinical pharmacology night prior to the study day and till 24 hours after administration of drug. The subjects were randomised to allocate one of the treatments.

In this study Clexane® was considered as reference drug and Enoxaparin produced by Jiuyuan as the test drug.

Venous blood samples for the determination of pharmacokinetic profiles were drawn from antecubital veins, predose and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 24 hours after the injection. The blood was collected into vacutainer tubes (VACUETTE®) containing 3.2% sodium citrate. Plasma was obtained by centrifugation at 2500 g for 15 minutes at +4°C. Plasma was divided in aliquots and stored at -70°C until analysis.

Healthy Subjects:

22 healthy male subjects between age of 19 to 23 years with a mean body weight 64.06 ± 5.92 kg. (range 54 to 75 kg) entered the study after giving informed consent. The study group was divided as Group-1 (G1) and Group-2 (G2).

Before the study began, all the subjects were evaluated for general good health on the basis of medical history, physical examination, electrocardiogram, routine biochemical and hematologic test. The clinical investigations of all the subjects who participated in the study were within the normal limits.

The subjects with the risk factors and contraindications like allergy or thrombocytopenic abnormalities were excluded from this study.

Laboratory Analysis:

Plasma sample: To determine if Clexane and Enoxaparin produced by Jiuyuan can be regarded as bioequivalent at the dose levels 60mg/0.6 ml in healthy subjects, the biological markers studied were Anti-Xa activities, Anti-IIa activities, activated partial thromboplastin time (APTT) and Heptest® plasma activities.

Anti-Xa Activity:

Anti-Xa activity in plasma was determined by heparin kit (No.0020009400) obtained from Instrumentation Laboratory Company, Lexington, USA.

The heparin kit is an assay based on a synthetic chromogenic substrate and on Factor Xa inactivation. Heparin levels in patient plasma are measured automatically on Sysmex CA-7000 Coagulation Systems, Japan.

Assay Calibration was carried out using LMWH reference standard (EP standard) with a known Anti-Xa activity. Calibration curves were constructed for Anti-Xa assay using a pool of platelet poor plasma (collected from 12 healthy male volunteers), spiked with increasing amount of LMWH EP standard. The measured absorbance was converted to Log absorbance and percent inhibition calculated for each standard. The percent inhibition of the plasma samples were also calculated similarly and the Anti-Xa activity of the plasma samples calculated from the Calibration curves. The Anti-Xa activity is expressed as IU/ml.

Other Tests:

Anti-IIa activity, APTT and Heptest were performed using commercial kits according to the instructions of the Manufacturers.

Anti-IIa kit (No.820) was obtained from American Diagnostica Inc. Stamford, USA; APTT kit (No. B4218-2) were obtained from Dade Behring; USA. Heptest kit (No. 830) was obtained from American Diagnostica Inc. Stamford, USA.

The clotting assays APTT and Heptest for plasma sample results are given as clotting time in seconds. The measurements of these assays were also carried out on Sysmex CA-7000 Coagulation Systems, Japan.

Parameter Analyzed:

The pharmacodynamic parameter of anti-Xa activity was analyzed and used to compare the bioequivalence between Clexane and Enoxaparin produced by Jiuyuan.

Results:

In the present investigation we have compared the bioequivalence of two low molecular weight heparin preparation in 22 healthy male human subjects with mean age 21.10 ± 1.02 (years); mean height 1.73 ± 0.05 (meters); mean weight 64.06 ± 5.93 (kilograms) (Table 16).

The pharmacodynamic parameter anti-Xa activity was used as a measure to compare the bioequivalence. Individual plasma anti-Xa activity obtained with reference and test at predose and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 24 hours after administration as show Table 1A and 1B. Additional parameters like anti-IIa activity, Heptest and APTT are shown in Table 11A and 11B, Table 12A and 12B, and Table 13A and 13B respectively. Peak activity (C_{max}) and area under the curve (AUC) and $t_{1/2}$ values of anti Xa activity are shown in Table 2A, 2B. Mean Anti Xa activity time profile is shown in Figure 1.

The peak anti-Xa activity was 0.883 ± 0.098 IU/ml with test while was 0.855 ± 0.109 IU·mL⁻¹ with reference product. The AUC_{0-t} was 7.984 ± 1.306 and 7.740 ± 1.312 IU·mL⁻¹·h with test and reference respectively. The mean ratio of AUC_{0-t} was 103.2% with 90% confidence interval (CI) of 100.7%~105.8% and the ratio lies between 80%~125%. The mean ratio of C_{max} was 103.5% with 90% confidence interval (CI) of 100.4%~106.7% and the ratio lies between 70.0%~143.0%. In conclusion, the test formulation of LMWH injection is bioequivalent with reference LMWH injection.

Anti-Xa Activity:

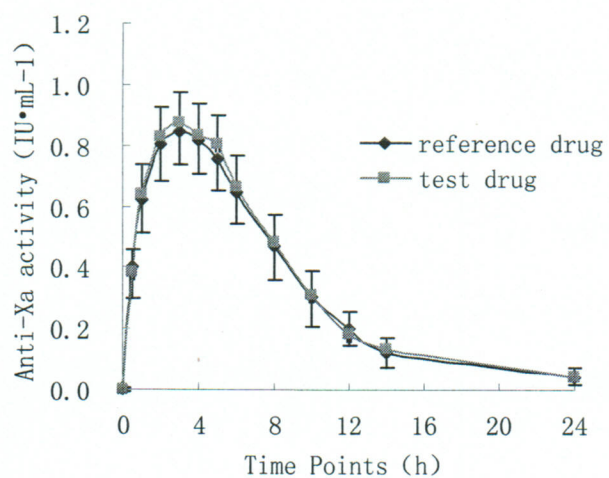


Figure 1 Comparison of anti-Xa activity between reference and test following subcutaneous injection of enoxaparin sodium in healthy male subjects (mean±SD)

Table- 1A Individual plasma anti-Xa activity following single subcutaneous injection of reference drug in 22 healthy male subjects (IU·mL⁻¹)

Volunteer	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1 A	0	0.417	0.657	0.883	0.901	0.9	0.795	0.641	0.459	0.272	0.167	0.095	0.033
G1 B	0	0.336	0.587	0.794	0.901	0.824	0.81	0.717	0.609	0.454	0.299	0.192	0.042
G1 C	0	0.264	0.449	0.632	0.764	0.768	0.746	0.646	0.452	0.275	0.163	0.1	0.035
G1 D	0	0.167	0.406	0.592	0.697	0.744	0.71	0.602	0.528	0.368	0.211	0.145	0.027
G1 E	0	0.372	0.603	0.767	0.803	0.81	0.785	0.701	0.54	0.368	0.224	0.127	0.037
G1 F	0	0.364	0.557	0.761	0.783	0.752	0.801	0.612	0.419	0.236	0.117	0.073	0.015
G1 G	0.008	0.327	0.547	0.711	0.81	0.691	0.611	0.621	0.396	0.254	0.166	0.098	0.033
G1 H	0.005	0.414	0.583	0.734	0.837	0.761	0.685	0.552	0.409	0.272	0.177	0.113	0.06
G1 I	0	0.367	0.647	0.928	0.941	0.954	0.903	0.774	0.621	0.391	0.239	0.155	0.054
G1 J	0.04	0.349	0.634	0.859	0.938	0.942	0.881	0.789	0.669	0.456	0.286	0.229	0.069
G1 K	0	0.396	0.708	0.914	0.929	0.879	0.826	0.691	0.497	0.313	0.157	0.097	0.005
G2 A	0	0.398	0.563	0.712	0.751	0.737	0.647	0.532	0.371	0.197	0.22	0.09	0.077
G2 B	0	0.488	0.63	0.827	0.854	0.844	0.685	0.56	0.386	0.218	0.319	0.074	ND
G2 C	0	0.405	0.582	0.761	0.833	0.802	0.751	0.599	0.458	0.29	0.132	0.104	0.064
G2 D	0	0.284	0.464	0.629	0.654	0.638	0.605	0.529	0.396	0.215	0.207	0.089	0.016
G2 E	0	0.538	0.863	1.063	1.141	1.111	1.073	0.923	0.74	0.5	0.209	0.218	0.06
G2 F	0	0.311	0.565	0.668	0.709	0.688	0.633	0.548	0.35	0.211	0.128	0.089	0.015
G2 G	0.007	0.569	0.799	0.996	0.916	0.924	0.761	0.673	0.41	0.272	0.172	0.095	0.058
G2 H	0.013	0.467	0.664	0.801	0.828	0.825	0.732	0.691	0.532	0.488	0.184	0.123	0.052
G2 I	0.009	0.57	0.83	0.962	0.928	0.863	0.756	0.603	0.358	0.22	0.234	0.111	0.053
G2 J	0.015	0.529	0.738	0.864	0.915	0.833	0.778	0.696	0.483	0.33	0.282	0.147	0.073
G2 K	0	0.422	0.638	0.772	0.765	0.68	0.651	0.481	0.277	0.186	0.153	0.068	0.026
Mean	0.004	0.398	0.623	0.801	0.845	0.817	0.756	0.645	0.471	0.308	0.202	0.120	0.041
SD	0.009	0.101	0.116	0.124	0.107	0.109	0.108	0.102	0.113	0.098	0.057	0.045	0.023
RSD(%)	209.1	25.5	18.5	15.4	12.7	13.4	14.3	15.7	24.1	31.8	28.1	37.5	54.8

ND (not detected)

Table- 1B Individual plasma anti-Xa activity following single subcutaneous injection of test drug in 22 healthy male subjects (IU·mL-1)

Volunteer Code	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1 A	0	0.402	0.696	1.01	1.035	0.938	0.906	0.785	0.519	0.326	0.129	0.125	0.059
G1 B	0	0.449	0.696	0.778	0.845	0.823	0.789	0.656	0.539	0.402	0.112	0.191	0.076
G1 C	0	0.298	0.558	0.778	0.766	0.742	0.698	0.587	0.361	0.211	0.193	0.094	0.054
G1 D	0	0.273	0.487	0.709	0.851	0.782	0.766	0.64	0.471	0.307	0.14	0.119	0.018
G1 E	0	0.333	0.637	0.854	0.974	0.912	0.93	0.752	0.569	0.372	0.341	0.136	0.044
G1 F	0	0.261	0.49	0.774	0.785	0.832	0.787	0.588	0.42	0.234	0.137	0.085	ND
G1 G	0	0.35	0.597	0.828	0.84	0.793	0.781	0.575	0.412	0.289	0.168	0.131	0.048
G1 H	0.024	0.382	0.639	0.74	0.77	0.725	0.675	0.621	0.488	0.304	0.216	0.129	0.056
G1 I	0.004	0.378	0.723	0.896	0.912	0.954	0.889	0.761	0.565	0.394	0.211	0.198	0.074
G1 J	0	0.471	0.75	0.929	1.054	1.006	0.936	0.767	0.611	0.417	0.197	0.196	0.066
G1 K	0	0.313	0.582	0.807	0.881	0.86	0.822	0.648	0.432	0.245	0.114	0.102	0.009
G2 A	0	0.347	0.578	0.767	0.787	0.722	0.699	0.589	0.401	0.235	0.141	0.081	0.026
G2 B	0	0.393	0.629	0.746	0.81	0.821	0.823	0.613	0.435	0.262	0.147	0.089	0.006
G2 C	0.009	0.412	0.64	0.823	0.902	0.818	0.78	0.68	0.562	0.387	0.234	0.157	0.06
G2 D	0	0.266	0.496	0.744	0.779	0.734	0.686	0.574	0.373	0.231	0.132	0.089	0.026
G2 E	0	0.454	0.8	1.054	1.098	1.074	1.04	0.92	0.696	0.499	0.362	0.219	0.057
G2 F	0	0.327	0.462	0.682	0.736	0.668	0.669	0.626	0.451	0.255	0.131	0.083	0.019
G2 G	0.003	0.455	0.788	0.836	0.899	0.813	0.835	0.659	0.464	0.292	0.198	0.136	0.069
G2 H	0.013	0.542	0.783	0.835	0.864	0.85	0.768	0.65	0.45	0.295	0.205	0.12	0.072
G2 I	0.003	0.457	0.67	0.875	0.942	0.853	0.839	0.731	0.528	0.355	0.242	0.166	0.084
G2 J	0	0.393	0.669	0.911	0.951	0.933	0.86	0.747	0.544	0.362	0.218	0.156	0.073
G2 K	0	0.43	0.672	0.83	0.789	0.738	0.635	0.442	0.308	0.174	0.108	0.1	0.013
Mean	0.003	0.381	0.638	0.828	0.876	0.836	0.801	0.664	0.482	0.311	0.185	0.132	0.046
SD	0.006	0.075	0.100	0.092	0.100	0.101	0.102	0.100	0.091	0.080	0.068	0.042	0.026
RSD(%)	229.6	19.6	15.7	11.1	11.5	12.1	12.7	15.0	18.8	25.7	36.9	31.6	57.5

ND (not detected)

Table- 2A Pharmacokinetic parameters of plasma anti-Xa activity following single subcutaneous injection of reference drug

Volunteer Code	T _{1/2} h	C _{max} IU·mL ⁻¹	T _{max} h	AUC _(0-t) IU·mL ⁻¹ ·h	AUC _(0-∞) IU·mL ⁻¹ ·h	AUC _(0-t) /AUC _(0-∞)	MRT h
G1 A	2.636	0.901	3	7.673	7.798	0.984	6.144
G1 B	3.222	0.901	3	9.099	9.294	0.979	7.230
G1 C	2.718	0.768	4	6.903	7.040	0.981	6.638
G1 D	2.977	0.744	4	7.253	7.369	0.984	7.150
G1 E	2.606	0.810	4	8.066	8.205	0.983	6.750
G1 F	2.173	0.801	5	6.672	6.719	0.993	5.855
G1 G	2.911	0.810	3	6.715	6.854	0.980	6.455
G1 H	3.156	0.837	3	7.185	7.458	0.963	6.804
G1 I	2.904	0.954	4	9.258	9.484	0.976	6.864
G1 J	3.263	0.942	4	10.005	10.329	0.969	7.454
G1 K	2.344	0.929	3	7.855	7.871	0.998	5.836
G2 A	3.009	0.751	3	6.767	7.102	0.953	6.964
G2 B	2.525	0.854	3	7.057	7.326	0.963	5.819
G2 C	2.229	0.833	3	7.389	7.594	0.973	6.716
G2 D	2.832	0.654	3	6.060	6.125	0.989	6.432
G2 E	2.193	1.141	3	11.195	11.385	0.983	6.862
G2 F	2.756	0.709	3	6.086	6.146	0.990	6.076
G2 G	2.636	0.996	2	8.060	8.281	0.973	6.217
G2 H	2.012	0.828	3	8.363	8.514	0.982	6.832
G2 I	3.615	0.962	2	7.878	8.155	0.966	6.291
G2 J	3.429	0.915	3	8.693	9.054	0.960	7.000
G2 K	2.755	0.772	2	6.049	6.152	0.983	5.849
Mean	2.768	0.855	3.182	7.740	7.921	0.978	6.556
SD	0.423	0.109	0.733	1.312	1.355	0.011	0.490
RSD(%)	15.3	12.8	23.0	17.0	17.1	1.2	7.5

Table- 2B Pharmacokinetic parameters of plasma anti-Xa activity following single subcutaneous injection of test drug

Volunteer Code	T _{1/2} h	C _{max} IU·mL ⁻¹	T _{max} h	AUC _(0-t) IU·mL ⁻¹ ·h	AUC _(0-∞) IU·mL ⁻¹ ·h	AUC _(0-t) /AUC _(0-∞)	MRT h
G1 A	1.992	1.035	3	8.783	8.952	0.981	6.444
G1 B	1.765	0.845	3	8.598	8.791	0.978	7.256
G1 C	3.135	0.778	2	6.796	7.040	0.965	6.635
G1 D	2.285	0.851	3	7.210	7.269	0.992	6.424
G1 E	2.755	0.974	3	9.042	9.217	0.981	6.822
G1 F	2.475	0.832	4	6.650	6.953	0.956	5.822
G1 G	3.091	0.840	3	7.491	7.705	0.972	6.692
G1 H	3.141	0.770	3	7.588	7.842	0.968	6.966
G1 I	2.815	0.954	4	9.423	9.723	0.969	7.192
G1 J	2.450	1.054	3	9.830	10.063	0.977	6.972
G1 K	2.081	0.881	3	7.174	7.201	0.996	5.903
G2 A	2.601	0.787	3	6.636	6.733	0.986	6.107
G2 B	2.556	0.823	5	7.040	7.062	0.997	5.871
G2 C	3.073	0.902	3	8.639	8.905	0.970	7.057
G2 D	2.669	0.779	3	6.445	6.545	0.985	6.229
G2 E	3.367	1.098	3	11.186	11.463	0.976	7.056
G2 F	2.243	0.736	3	6.471	6.532	0.991	6.238
G2 G	3.256	0.899	3	8.260	8.584	0.962	6.798
G2 H	3.082	0.864	3	8.134	8.454	0.962	6.739
G2 I	3.554	0.942	3	9.003	9.434	0.954	7.193
G2 J	3.032	0.951	3	9.023	9.342	0.966	6.990
G2 K	2.646	0.830	2	6.219	6.269	0.992	5.741
Mean	2.730	0.883	3.091	7.984	8.185	0.976	6.598
SD	0.473	0.098	0.610	1.306	1.376	0.013	0.489
RSD(%)	17.3	11.0	19.7	16.4	16.8	1.3	7.4

Table- 3 Cmax ratio and relative bioavailability (F%)

Volunteer	C _{max} (IU·mL ⁻¹)			AUC _(0-∞) (IU·mL ⁻¹ ·h)			AUC _(0-∞) (IU·mL ⁻¹ ·h)		
	T	R	T/R	T	R	F (%)	T	R	F (%)
G1 A	1.035	0.901	1.149	8.783	7.673	114.464	8.952	7.798	114.796
G1 B	0.845	0.901	0.938	8.598	9.099	94.491	8.791	9.294	94.588
G1 C	0.778	0.768	1.013	6.796	6.903	98.454	7.040	7.040	100.003
G1 D	0.851	0.744	1.144	7.210	7.253	99.404	7.269	7.369	98.645
G1 E	0.974	0.810	1.202	9.042	8.066	112.107	9.217	8.205	112.338
G1 F	0.832	0.801	1.039	6.650	6.672	99.674	6.953	6.719	103.493
G1 G	0.840	0.810	1.037	7.491	6.715	111.556	7.705	6.854	112.423
G1 H	0.770	0.837	0.920	7.588	7.185	105.613	7.842	7.458	105.146
G1 I	0.954	0.954	1.000	9.423	9.258	101.782	9.723	9.484	102.523
G1 J	1.054	0.942	1.119	9.830	10.005	98.251	10.063	10.329	97.419
G1 K	0.881	0.929	0.948	7.174	7.855	91.336	7.201	7.871	91.483
G2 A	0.787	0.751	1.048	6.636	6.767	98.053	6.733	7.102	94.811
G2 B	0.823	0.854	0.964	7.040	7.057	99.763	7.062	7.326	96.394
G2 C	0.902	0.833	1.083	8.639	7.389	116.928	8.905	7.594	117.263
G2 D	0.779	0.654	1.191	6.445	6.060	106.362	6.545	6.125	106.861
G2 E	1.098	1.141	0.962	11.186	11.195	99.922	11.463	11.385	100.688
G2 F	0.736	0.709	1.038	6.471	6.086	106.322	6.532	6.146	106.290
G2 G	0.899	0.996	0.903	8.260	8.060	102.478	8.584	8.281	103.663
G2 H	0.864	0.828	1.043	8.134	8.363	97.253	8.454	8.514	99.289
G2 I	0.942	0.962	0.979	9.003	7.878	114.280	9.434	8.155	115.687
G2 J	0.951	0.915	1.039	9.023	8.693	103.796	9.342	9.054	103.183
G2 K	0.830	0.772	1.075	6.219	6.049	102.810	6.269	6.152	101.890
Mean	0.883	0.855	1.038	7.984	7.740	103.414	8.185	7.921	103.585
SD	0.098	0.109	0.085	1.306	1.312	6.865	1.376	1.355	7.227
RSD(%)	11.0	12.8	8.2	16.4	17.0	6.6	16.8	17.1	7.0

Anti-IIa Activity:

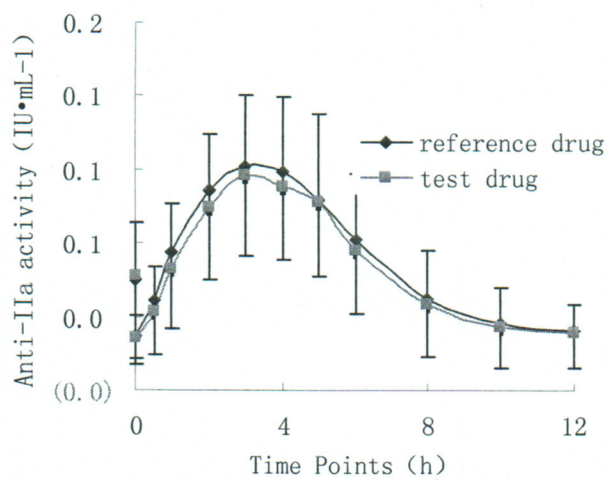


Figure 2 Comparison of anti-IIa activity between reference and test following subcutaneous injection of enoxaparin sodium in healthy male subjects (mean±SD)

Table- 4A Individual plasma anti-IIa activity following single subcutaneous injection of reference drug in 22 healthy male subjects (IU·mL⁻¹)

Volunteer Code	Time Points (h)										
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h
G1 A	0	0.021	0.052	0.099	0.101	0.124	0.108	0.076	0.027	0.014	0.007
G1 B	0	0.000	0.017	0.047	0.069	0.064	0.059	0.052	0.019	0.003	ND
G1 C	0	0.000	0.000	0.022	0.043	0.047	0.036	0.021	ND	ND	ND
G1 D	0	0.001	0.003	0.024	0.034	0.040	0.041	0.036	0.017	0.001	-
G1 E	0.001	0.019	0.033	0.050	0.062	0.068	0.064	0.048	0.024	0.010	ND
G1 F	0	0.014	0.033	0.069	0.069	0.075	0.066	0.047	0.014	ND	ND
G1 G	0.031	0.041	0.073	0.098	0.121	0.114	0.098	0.083	0.049	0.031	0.019
G1 H	0.005	0.039	0.052	0.101	0.106	0.085	0.070	0.055	0.032	0.018	0.001
G1 I	0.024	0.055	0.090	0.135	0.166	0.181	0.166	0.139	0.086	0.052	0.034
G1 J	0.044	0.070	0.114	0.129	0.163	0.147	0.145	0.132	0.116	0.075	0.055
G1 K	0	0.008	0.031	0.089	0.094	0.087	0.069	0.048	0.012	ND	ND
G2 A	0.001	0.031	0.052	0.075	0.087	0.113	0.071	0.045	0.007	ND	ND
G2 B	0	0.000	0.029	0.055	0.069	0.066	0.041	0.003	ND	ND	ND
G2 C	0	0.000	0.015	0.045	0.090	0.068	0.068	0.033	ND	ND	ND
G2 D	0	0.000	0.005	0.034	0.027	0.034	0.029	0.026	0.007	ND	ND
G2 E	0	0.034	0.087	0.152	0.166	0.186	0.155	0.129	0.054	0.001	ND
G2 F	0	0.005	0.024	0.041	0.054	0.057	0.045	0.022	0.007	ND	ND
G2 G	0.021	0.069	0.118	0.153	0.174	0.138	0.115	0.085	0.033	0.022	0.026
G2 H	0	0.041	0.073	0.098	0.111	0.094	0.081	0.068	0.016	0.036	0.009
G2 I	0.032	0.103	0.152	0.215	0.192	0.192	0.145	0.093	0.047	0.036	0.032
G2 J	0.037	0.078	0.112	0.127	0.161	0.143	0.125	0.111	0.086	0.078	0.070
G2 K	0	0.008	0.043	0.089	0.085	0.052	0.048	0.012	ND	ND	ND
Mean	0.009	0.029	0.055	0.089	0.102	0.099	0.084	0.062	0.030	0.017	0.012
SD	0.015	0.030	0.042	0.049	0.049	0.048	0.042	0.040	0.032	0.025	0.020
RSD(%)	165.9	104.1	77.1	55.4	48.2	49.0	49.8	63.9	107.1	143.1	173.5

ND (not detected)

Table- 4B Individual plasma anti-IIa activity following single subcutaneous injection of test drug in 22 healthy male subjects (IU·mL⁻¹)

Volunteer		Time Points (h)									
Code	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h
G1 A	0	0.015	0.048	0.108	0.125	0.124	0.11	0.080	0.029	0.015	0.003
G1 B	0	0	0.027	0.061	0.055	0.057	0.043	0.026	ND	ND	ND
G1 C	0	0	0	0.029	0.052	0.034	0.026	0.005	ND	ND	ND
G1 D	0	0.003	0.01	0.031	0.052	0.043	0.045	0.019	0.007	ND	ND
G1 E	0	0.007	0.041	0.064	0.101	0.076	0.085	0.054	0.024	0.007	ND
G1 F	0	0.007	0.026	0.064	0.054	0.055	0.052	0.034	0.008	ND	ND
G1 G	0.018	0.045	0.063	0.117	0.122	0.14	0.139	0.078	0.041	0.027	0.024
G1 H	0.008	0.026	0.054	0.065	0.088	0.08	0.073	0.057	0.032	0.013	0.013
G1 I	0.026	0.05	0.106	0.135	0.155	0.152	0.142	0.116	0.088	0.045	0.041
G1 J	0.034	0.067	0.109	0.148	0.171	0.189	0.166	0.119	0.083	0.055	0.041
G1 K	0	0	0.026	0.062	0.083	0.064	0.071	0.031	ND	ND	ND
G2 A	0	0.017	0.033	0.076	0.101	0.078	0.076	0.052	0.015	ND	ND
G2 B	0	0	0.003	0.036	0.048	0.05	0.038	0.007	ND	ND	ND
G2 C	0	0	0	0.021	0.045	0.038	0.027	0.005	ND	ND	ND
G2 D	0	0	0.007	0.05	0.057	0.052	0.033	0.019	ND	ND	ND
G2 E	0	0.022	0.043	0.097	0.136	0.145	0.115	0.096	0.031	0.015	0.012
G2 F	0	0.001	0.014	0.038	0.052	0.04	0.038	0.026	0.008	ND	ND
G2 G	0.019	0.047	0.078	0.106	0.131	0.117	0.111	0.085	0.054	0.045	0.027
G2 H	0.005	0.055	0.085	0.094	0.121	0.106	0.096	0.063	0.027	0.011	0.005
G2 I	0.047	0.072	0.112	0.135	0.168	0.161	0.163	0.132	0.07	0.055	0.041
G2 J	0.021	0.049	0.08	0.143	0.145	0.152	0.14	0.116	0.072	0.05	0.039
G2 K	0.001	0.015	0.036	0.062	0.062	0.047	0.029	0.003	ND	ND	ND
Mean	0.008	0.023	0.046	0.079	0.097	0.091	0.083	0.056	0.027	0.015	0.011
SD	0.013	0.024	0.036	0.040	0.043	0.048	0.047	0.042	0.029	0.021	0.016
RSD(%)	165.3	108.1	79.3	49.9	44.5	53.2	56.6	75.1	109.6	134.2	144.3

ND (not detected)

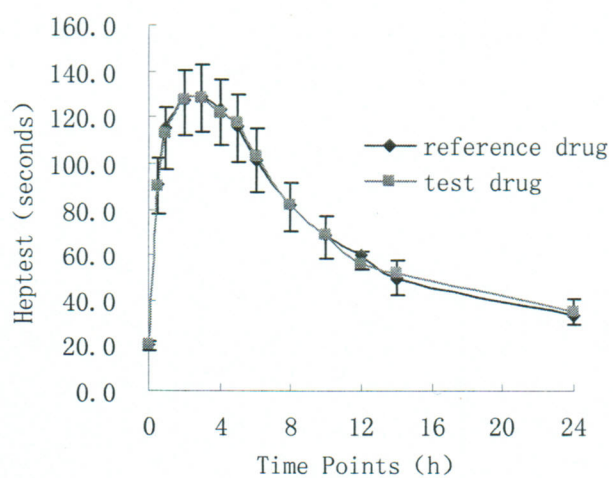


Figure 3 Comparison of Heptest between reference and test following subcutaneous injection of enoxaparin sodium in healthy male subjects (mean $\pm$ SD)

Table- 5A Individual heptest following single subcutaneous injection of reference drug in 22 healthy male subjects (second)

Volunteer Code	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1A	21.0	84.2	140.2	157.8	147.6	150.4	136.8	118.0	84.8	67.8	63.2	51.4	36.0
G1B	26.4	92.8	117.8	131.2	133.4	129.4	125.8	113.6	102.6	88.8	72.4	63.4	44.4
G1C	20.2	77.8	88.2	117.8	128.4	120.8	121.0	100.2	81.2	66.4	61.0	45.0	31.0
G1D	18.0	59.0	81.4	100.0	104.8	105.0	103.8	87.8	78.4	64.2	56.2	48.8	29.8
G1E	19.8	85.4	108.2	113.2	122.6	118.6	114.2	104.4	90.0	77.4	63.4	51.4	34.6
G1F	19.2	84.0	104.6	118.4	124.4	111.2	107.8	98.8	81.6	66.0	54.2	45.8	31.0
G1G	20.0	84.0	101.4	123.2	121.0	110.8	100.0	88.4	82.8	66.2	55.4	45.6	30.0
G1H	21.4	88.4	114.4	122.2	121.6	109.6	104.6	92.8	81.2	66.0	54.6	47.0	35.8
G1I	20.6	87.0	116.6	131.8	137.0	133.0	128.4	112.0	85.0	69.2	61.8	51.8	32.6
G1J	25.2	107.4	129.2	138.4	150.0	147.6	136.2	110.4	93.2	92.8	72.8	71.0	42.4
G1K	20.4	97.6	135.6	145.8	144.6	130.2	126.4	114.6	83.8	69.8	56.4	48.0	29.0
G2A	18.0	112.4	133.2	138.8	134.6	137.4	129.6	113.0	83.8	66.8	69.2	48.6	31.8
G2B	17.8	87.0	110.4	124.0	131.6	119.4	112.2	96.4	71.6	60.2	65.6	46.6	29.4
G2C	18.0	91.0	102.4	118.0	118.6	122.8	101.6	82.6	70.4	59.6	48.6	44.4	34.2
G2D	ND	77.4	93.2	105.0	104.4	98.6	95.2	89.6	72.2	57.6	56.0	41.8	30.0
G2E	20.8	96.2	144.2	158.6	159.4	154.6	153.6	140.0	112.8	88.8	58.0	60.0	41.2
G2F	23.2	79.2	91.4	109.0	101.0	107.8	95.2	88.4	70.8	57.6	55.4	47.4	32.0
G2G	18.2	100.0	121.2	130.2	125.2	109.2	106.6	83.8	74.0	59.0	54.6	43.4	29.2
G2H	18.8	99.6	112.6	128.2	121.2	119.4	110.6	106.4	72.4	84.6	56.4	46.2	33.0
G2I	22.2	113.6	143.2	150.2	137.4	132.4	115.4	101.6	71.8	63.2	57.2	47.0	35.2
G2J	22.6	105.8	122.2	123.0	139.0	134.0	120.6	105.2	86.2	68.4	64.6	52.0	36.6
G2K	18.2	90.0	121.8	128.0	127.6	110.0	104.2	86.2	67.6	59.6	60.0	44.0	27.0
Mean	20.5	90.9	115.2	127.9	128.9	123.3	115.9	101.6	81.7	69.1	59.9	49.6	33.5
SD	2.4	12.8	18.0	15.7	14.8	15.4	15.1	14.0	11.0	10.6	6.2	7.0	4.6
RSD(%)	11.7	14.0	15.6	12.3	11.5	12.5	13.0	13.8	13.5	15.4	10.4	14.1	13.6

ND (not detected)

Table- 5B Individual heptest following single subcutaneous injection of test drug in 22 healthy male subjects (second)

Volunteer	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1A	23.0	101.6	128.4	155.6	153.6	151.8	143.8	126.0	98.8	77.8	56.0	60.0	39.2
G1B	21.8	99.8	124.0	133.2	131.2	123.8	127.6	116.2	97.4	86.8	59.2	66.8	49.4
G1C	17.2	79.2	114.2	127.2	115.4	122.8	118.8	97.4	71.6	62.8	53.6	45.4	32.2
G1D	18.8	68.6	91.6	103.0	105.0	110.4	105.2	92.4	75.2	67.0	53.2	49.0	32.8
G1E	19.6	83.6	115.0	135.6	140.4	129.2	133.0	119.4	94.8	77.6	70.4	55.0	37.8
G1F	20.6	75.8	101.0	123.4	117.6	114.0	111.0	100.0	76.2	66.3	52.4	48.7	29.8
G1G	20.2	83.0	110.2	130.8	121.8	117.0	116.2	94.0	76.6	67.0	56.4	49.2	32.2
G1H	21.8	96.0	117.0	115.2	127.2	109.6	107.4	103.0	81.8	71.4	51.8	51.0	32.4
G1I	19.4	86.8	114.4	123.4	125.6	121.4	118.4	103.6	83.8	70.4	57.0	53.0	34.4
G1J	25.0	93.8	117.6	120.2	135.8	134.6	124.4	109.6	91.2	71.2	59.2	56.0	43.6
G1K	20.0	89.4	115.6	140.0	141.8	134.8	131.6	110.0	83.0	68.2	49.8	48.4	29.0
G2A	18.8	98.6	130.6	136.0	148.0	133.8	123.6	113.6	88.4	73.6	58.6	51.0	32.2
G2B	22.0	111.6	109.2	115.2	114.2	115.8	113.8	95.0	65.0	63.6	55.2	47.8	32.2
G2C	18.6	83.0	105.0	125.8	122.8	110.4	108.8	98.4	70.6	65.6	57.8	49.8	35.2
G2D	19.2	75.4	91.2	115.0	113.0	101.0	96.4	86.2	67.4	58.0	50.6	47.4	31.0
G2E	20.8	92.2	126.8	149.8	154.0	147.4	140.0	119.6	89.4	81.6	63.0	58.8	40.8
G2F	19.4	82.8	95.0	109.2	117.0	107.4	106.2	97.6	80.8	66.6	56.4	49.4	30.8
G2G	18.0	84.4	105.0	119.8	116.4	107.0	105.0	86.2	78.0	62.8	53.4	46.8	31.6
G2H	18.8	105.8	121.0	116.4	132.6	122.4	114.6	100.6	78.4	68.6	52.6	48.2	33.0
G2I	24.0	113.0	128.0	146.6	148.0	140.6	127.0	118.4	93.0	76.0	65.2	57.4	44.2
G2J	21.8	84.6	105.4	130.6	126.4	130.0	112.6	105.8	85.0	68.2	60.0	54.8	38.8
G2K	18.2	98.0	110.0	132.4	113.8	101.8	99.8	80.0	70.2	56.0	45.2	42.8	29.2
Mean	20.3	90.3	112.6	127.5	128.3	122.1	117.5	103.3	81.7	69.4	56.2	51.7	35.1
SD	2.0	11.8	11.6	13.3	14.3	14.3	12.7	12.2	9.8	7.4	5.5	5.6	5.5
RSD(%)	9.9	13.1	10.3	10.4	11.2	11.7	10.8	11.8	12.0	10.6	9.8	10.8	15.6

ND (not detected)

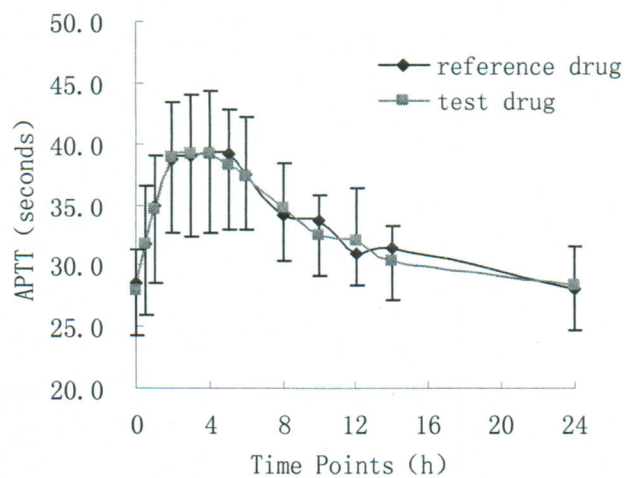


Figure 4 Comparison of APTT between reference and test following subcutaneous injection of enoxaparin sodium in healthy male subjects (mean $\pm$ SD)

Table- 7A Individual APTT following single subcutaneous injection of reference drug in 22 healthy male subjects (second)

Volunteer Code	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1A	28.1	30.9	34.1	40.2	36.1	38.3	36.4	39.7	32.3	31.8	30.2	36.2	28.8
G1B	24.1	24.8	30.1	32.5	31.0	34.1	33.5	34.7	32.7	29.7	28.1	28.0	24.9
G1C	26.5	27.8	29.3	33.8	33.9	34.6	35.0	33.8	32.8	32.8	31.1	30.5	26.9
G1D	29.2	31.5	32.0	37.3	36.2	35.3	39.1	39.8	39.4	36.0	35.2	34.5	29.6
G1E	23.9	25.1	26.3	29.8	28.8	28.5	30.0	30.8	28.4	26.8	24.9	25.6	21.4
G1F	30.2	32.6	34.7	39.6	39.8	39.8	39.0	38.9	34.7	33.2	31.3	30.6	29.1
G1G	30.9	32.5	33.0	36.8	38.6	40.9	41.9	41.3	37.8	36.9	31.5	32.6	30.3
G1H	32.2	27.2	29.7	35.4	31.6	33.0	32.7	30.2	31.0	32.4	32.8	31.7	28.9
G1I	25.7	29.6	32.2	35.8	36.8	36.6	36.0	35.6	33.2	31.3	30.1	31.5	26.3
G1J	26.3	30.2	31.8	35.8	36.6	36.0	36.3	35.8	31.8	31.1	30.8	28.1	26.2
G1K	29.0	32.6	38.2	41.1	41.0	39.7	39.6	39.8	35.6	32.8	30.2	29.3	29.3
G2A	36.3	42.3	44.7	52.2	51.6	54.7	50.3	45.5	41.3	44.3	29.7	38.9	33.7
G2B	29.2	32.1	38.0	40.0	40.1	39.4	38.1	37.8	34.1	34.2	28.7	29.7	28.1
G2C	24.0	26.3	28.4	32.8	32.3	33.2	33.2	33.4	28.0	28.4	33.4	27.8	23.1
G2D	20.1	20.4	25.5	27.2	30.6	29.2	32.5	29.0	28.2	27.9	32.2	21.8	23.7
G2E	29.1	33.8	40.1	44.7	46.8	47.4	50.6	45.9	38.0	37.2	26.5	39.3	30.6
G2F	31.1	32.7	37.8	39.0	41.9	41.3	41.2	38.4	33.6	35.2	36.2	31.0	29.7
G2G	35.2	43.5	51.8	51.2	54.1	47.1	49.0	43.4	37.1	39.6	31.2	34.8	33.7
G2H	36.2	43.1	43.6	42.6	44.2	50.7	50.2	40.9	39.6	42.5	31.5	37.8	31.2
G2I	26.4	32.4	34.7	39.9	39.6	38.1	35.3	33.3	29.6	28.9	35.0	28.6	24.7
G2J	24.8	31.8	32.9	40.3	41.2	39.1	40.2	37.8	35.4	31.7	30.6	30.0	25.6
G2K	29.1	36.0	42.1	43.6	45.9	47.4	43.1	39.6	37.0	35.9	31.5	33.2	30.6
Mean	28.5	31.8	35.0	38.7	39.0	39.3	39.2	37.5	34.2	33.7	31.0	31.4	28.0
SD	4.2	5.8	6.5	6.1	6.7	6.7	6.2	4.6	3.8	4.5	2.6	4.3	3.3
RSD(%)	14.6	18.2	18.5	15.6	17.1	17.1	15.7	12.4	11.2	13.5	8.5	13.7	11.6

ND (not detected)

Table- 7B Individual APTT following single subcutaneous injection of test drug in 22 healthy male subjects (second)

Volunteer	Time Points (h)												
	0	0.5h	1h	2h	3h	4h	5h	6h	8h	10h	12h	14h	24h
G1A	19.7	24.2	28.1	31.4	32.7	33.3	32.2	31.0	30.2	29.6	37.6	27.8	26.7
G1B	23.5	28.8	31.2	35.2	34.7	35.0	34.6	32.4	32.3	28.8	34.8	29.3	24.3
G1C	28.2	31.8	34.9	39.6	38.7	36.8	38.8	36.6	32.3	32.0	26.3	28.8	27.2
G1D	30.6	33.9	39.1	43.4	42.2	43.8	41.2	39.5	38.3	35.0	24.9	31.2	29.4
G1E	23.8	30.0	28.6	31.6	31.6	30.5	30.9	30.4	28.6	26.9	36.0	25.7	23.9
G1F	31.2	39.7	41.3	46.0	43.3	43.9	43.9	41.5	38.0	37.4	31.2	33.9	34.2
G1G	29.1	32.3	32.2	40.1	40.0	43.7	42.3	41.7	37.0	32.0	33.4	30.3	29.3
G1H	29.1	28.5	30.6	38.8	41.8	35.3	33.7	32.3	36.0	30.9	34.4	32.9	27.2
G1I	26.3	32.8	36.5	40.9	42.6	44.9	39.4	35.8	36.5	32.7	26.1	29.3	27.4
G1J	26.9	32.3	35.0	39.3	40.2	40.1	41.2	44.1	34.6	32.9	32.1	30.6	27.2
G1K	29.3	35.1	39.6	42.6	45.1	42.7	41.9	39.3	35.4	33.5	32.6	31.0	31.1
G2A	32.8	35.6	42.2	42.9	45.0	48.5	47.0	46.2	43.0	38.7	40.9	37.8	35.1
G2B	28.2	29.3	31.8	37.2	37.2	36.8	37.5	35.9	34.8	33.5	29.1	28.9	27.4
G2C	24.0	25.7	30.0	32.2	30.9	30.1	30.8	30.2	28.7	26.8	25.6	26.6	23.6
G2D	25.7	23.6	29.0	33.4	34.4	32.5	31.4	31.6	30.4	29.3	27.2	25.9	24.2
G2E	30.0	31.2	35.1	38.5	40.3	41.5	39.6	39.7	36.4	30.3	37.1	30.9	28.5
G2F	28.0	30.4	33.6	38.4	36.3	38.9	38.8	39.3	35.0	34.5	31.7	30.9	30.9
G2G	32.8	35.3	40.2	42.7	43.2	42.2	42.1	45.4	37.1	38.0	37.6	34.1	32.3
G2H	33.7	45.6	40.5	48.7	51.0	47.5	44.7	40.5	39.5	37.4	29.8	33.8	31.8
G2I	26.2	30.5	34.5	37.6	38.0	39.0	36.8	36.0	31.6	30.1	29.8	28.9	25.4
G2J	26.1	28.8	32.1	34.9	35.6	37.6	36.2	34.6	33.5	30.2	30.9	29.3	24.7
G2K	29.1	31.1	34.4	39.4	38.6	38.6	37.2	37.8	36.0	33.8	34.2	30.6	30.1
Mean	27.9	31.7	34.6	38.9	39.2	39.2	38.3	37.4	34.8	32.5	32.0	30.4	28.3
SD	3.4	4.9	4.4	4.6	4.9	5.2	4.6	4.8	3.6	3.4	4.4	2.9	3.3
RSD(%)	12.2	15.4	12.6	11.7	12.6	13.2	12.1	12.9	10.3	10.5	13.8	9.5	11.8

ND (not detected)

Safety information:

No adverse event was observed during the study.

Conclusion:

The mean ratio of AUC_{0-t} was 103.2% with 90% confidence interval (CI) of 100.7%~105.8% and the ratio lies between 80%~125%. The mean ratio of C_{max} was 103.5% with 90% confidence interval (CI) of 100.4%~106.7% and the ratio lies between 70.0%~143.0%. T_{max} difference between the test formulation and reference formulation is not significant after nonparametric test. In conclusion, the test formulation of LMWH injection (Enoxaparin produced by Jiuyuan) is bioequivalent with reference LMWH injection (Clexane).