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Supplementary Information

Systematic analysis of enoxaparins from different sources with online one and two-dimensional chromatography

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Abstract

Enoxaparin, one of the most important low molecular weight heparins (LMWHs) is widely used as a clinical anticoagulant. Different production processes and animal sources of its precursor (unfractionated heparin) can result in the structural diversity of enoxaparin. In this study, 38 lots of enoxaparin prepared at different times, from different providers and animal sources were systematically analyzed. SEC and SAX were used to analyze the oligosaccharide dispersity and structural compositions (disaccharide domains) of enoxaparins by size and charge, respectively. The results provide clues whether the structural variations in enoxaparin, observed in oligosaccharide mapping and/or disaccharide analysis, are attributable to differences in animal sources of its heparin precursor or enoxaparin production processes based on times or brands. The representative enoxaparins were fingerprinted with online multiple heart-cut two-dimensional liquid chromatography-mass spectrometry (MHC-2DLC-MS). The profiles in MHC-2DLC-MS showed the detailed structural information of enoxaparins. In addition, the binding capacities to antithrombin III (AT) of these 38 lots of enoxaparins were detected using surface plasmon resonance (SPR) with competitive inhibition mode. The results showed the glycan size distribution of an enoxaparin is more related to its production process. The disaccharide composition, sequence and the variety of glycans of an enoxaparin are more related to its AT binding-based anticoagulant activity.

Keywords: enoxaparin, size distribution, disaccharide composition, PCA, MHC-2DLC-MS

Table S-1. Structural composition results of 38 enoxaparin samples

Samples	Peak area%													
	ΔIVA	a	ΔIVS	ΔIIA	ΔIIIA	b	ΔIIS	ΔIIIS	c	ΔIA	ΔIS	d	e	f
Enox EP	2.97	0.72	3.52	3.91	1.83	1.26	10.58	6.41	1.81	1.18	60.63	1.83	1.32	1.37
Enox USP	3.03	0.89	3.55	4.14	1.84	1.09	10.84	6.38	1.84	1.19	59.84	1.88	1.39	1.42
Enox P1	2.33	0.36	3.70	4.13	1.29	1.79	11.23	5.94	2.46	1.57	59.09	2.14	1.91	1.94
Enox P2	2.29	0.33	3.48	4.50	1.41	1.74	11.11	5.98	2.55	1.65	58.84	2.16	1.91	1.93
Enox P3	1.58	0.32	4.12	1.37	0.58	1.90	6.83	13.59	2.44	0.19	60.41	0.77	2.42	3.14
Enox P4	2.21	0.43	2.57	4.63	1.34	1.90	11.21	6.01	2.41	1.60	59.64	2.24	1.86	1.84
Enox P5	2.11	0.32	3.24	4.08	1.36	1.54	11.29	5.81	2.22	1.67	61.49	1.84	1.34	1.58
Enox C1	2.60	0.45	3.35	4.01	1.57	2.26	10.98	6.36	1.75	1.09	60.34	1.60	1.43	1.35
Enox C2	2.73	0.60	3.22	4.08	1.70	1.00	10.84	6.71	1.79	1.17	60.94	1.63	1.42	1.33
Enox C3	2.24	0.32	3.58	4.35	1.49	3.57	11.72	6.21	1.65	1.10	58.53	1.65	1.40	1.21
Enox C4	2.08	0.25	3.45	4.34	1.50	2.84	11.76	6.37	1.71	1.13	59.31	1.57	1.41	1.23
Enox C5	2.71	0.48	3.26	4.17	1.70	1.15	10.85	6.68	1.79	1.15	60.78	1.65	1.43	1.34
Enox E1	2.15	0.28	3.06	4.35	1.36	2.04	11.49	6.60	1.53	1.74	60.24	2.49	1.22	1.34
Enox E2	2.93	0.78	3.10	3.79	1.97	0.74	10.56	7.06	1.47	1.54	61.32	2.08	1.19	1.37
Enox E3	3.17	0.74	3.48	4.34	1.89	0.73	10.94	6.42	1.78	1.21	60.16	1.55	1.25	1.27
Enox E4	3.12	0.82	3.46	4.10	1.91	0.76	10.76	6.42	1.83	1.23	60.41	1.68	1.24	1.28
Enox E5	3.11	0.79	3.50	4.30	1.87	0.83	10.72	6.47	1.81	1.24	60.26	1.52	1.25	1.27
Enox E6	3.17	0.90	3.56	4.11	1.82	0.85	10.50	6.55	1.88	1.11	60.30	1.70	1.35	1.42
Enox E7	2.89	0.80	3.47	4.27	1.80	1.04	10.81	6.39	1.82	1.19	60.20	1.57	1.32	1.35
Enox E8	2.73	0.83	3.53	4.09	1.78	1.25	12.29	6.24	1.89	1.17	58.78	1.65	1.34	1.36
Enox E9	2.81	0.76	3.29	4.26	1.82	0.60	12.16	6.44	1.88	1.22	59.23	1.82	1.37	1.35
Enox E10	2.95	0.98	3.39	4.18	1.85	0.76	11.77	6.48	1.84	1.18	59.31	1.69	1.37	1.35
Enox E11	2.79	0.79	3.41	4.02	1.82	1.04	11.53	6.29	1.90	1.17	59.81	1.74	1.39	1.43
Enox N1	2.28	0.30	3.11	4.46	1.66	1.53	11.10	6.71	1.39	1.56	61.32	1.28	1.06	0.92
Enox N2	2.17	0.32	3.00	4.13	1.60	1.55	10.98	6.60	1.42	1.57	61.99	1.58	1.13	0.96
Enox N3	2.21	0.23	3.07	4.06	1.61	1.94	11.11	6.54	1.40	1.57	61.60	1.77	1.11	0.95
Enox N4	2.23	0.25	3.04	4.10	1.61	1.77	11.09	6.57	1.41	1.57	61.71	1.77	1.12	0.95
Enox N5	2.22	0.28	3.06	4.19	1.62	1.70	11.07	6.61	1.41	1.57	61.66	1.60	1.11	0.95
Enox R1	3.92	0.10	3.83	4.12	1.69	1.03	11.02	6.33	1.55	1.15	60.33	2.12	0.90	1.37
Enox R2	4.06	0.10	3.90	4.48	1.72	1.12	11.82	6.32	1.74	1.16	58.56	1.95	0.88	1.42
Enox R3	4.11	0.11	3.93	4.33	1.72	1.14	11.82	6.37	1.71	1.14	58.53	2.10	0.99	1.37
Enox R4	2.04	0.18	1.31	2.10	0.61	0.72	9.82	6.11	1.90	0.54	70.14	1.91	0.63	1.96
Enox R5	1.16	0.13	1.49	1.79	0.81	0.76	9.06	5.47	2.54	0.56	71.75	1.59	0.69	2.15
Enox R6	4.04	0.37	3.96	2.11	1.19	0.36	8.98	18.39	1.83	0.16	53.56	0.85	1.21	2.77
Enox R7	0.84	0.08	1.17	0.76	0.07	2.38	7.92	4.86	1.89	0.06	75.98	0.61	1.32	1.99
Enox JF1	1.94	0.18	1.29	2.20	0.57	0.67	9.67	6.26	1.74	0.55	70.94	1.78	0.60	1.58
Enox JF2	3.47	0.21	3.75	1.80	1.08	0.30	9.42	17.82	1.48	0.19	55.63	0.76	1.25	2.56
Enox JF3	2.72	0.83	3.22	3.83	1.95	0.88	10.55	6.87	1.52	1.66	61.12	2.08	1.28	1.40

Table S-2. Anticoagulant activity (AT III) of LMWH samples determined by competition SPR

Samples	SPR	Samples	SPR	Samples	SPR	Samples	SPR
Enox USP	100	Enox E1	106	Enox N1	107	Enox R7	75
Enox P1	91	Enox E2	96	Enox N2	108	Enox JF1	114
Enox P2	88	Enox E3	105	Enox N3	105	Enox JF2	83
Enox P3	77	Enox E4	97	Enox N4	98	Enox JF3	105
Enox P4	102	Enox E5	105	Enox N5	107		
Enox P5	91	Enox E6	91	Enox R1	103		
Enox C1	98	Enox E7	98	Enox R2	98		
Enox C2	96	Enox E8	103	Enox R3	104		
Enox C3	92	Enox E9	97	Enox R4	118		
Enox C4	106	Enox E10	102	Enox R5	113		
Enox C5	91	Enox E11	107	Enox R6	87		

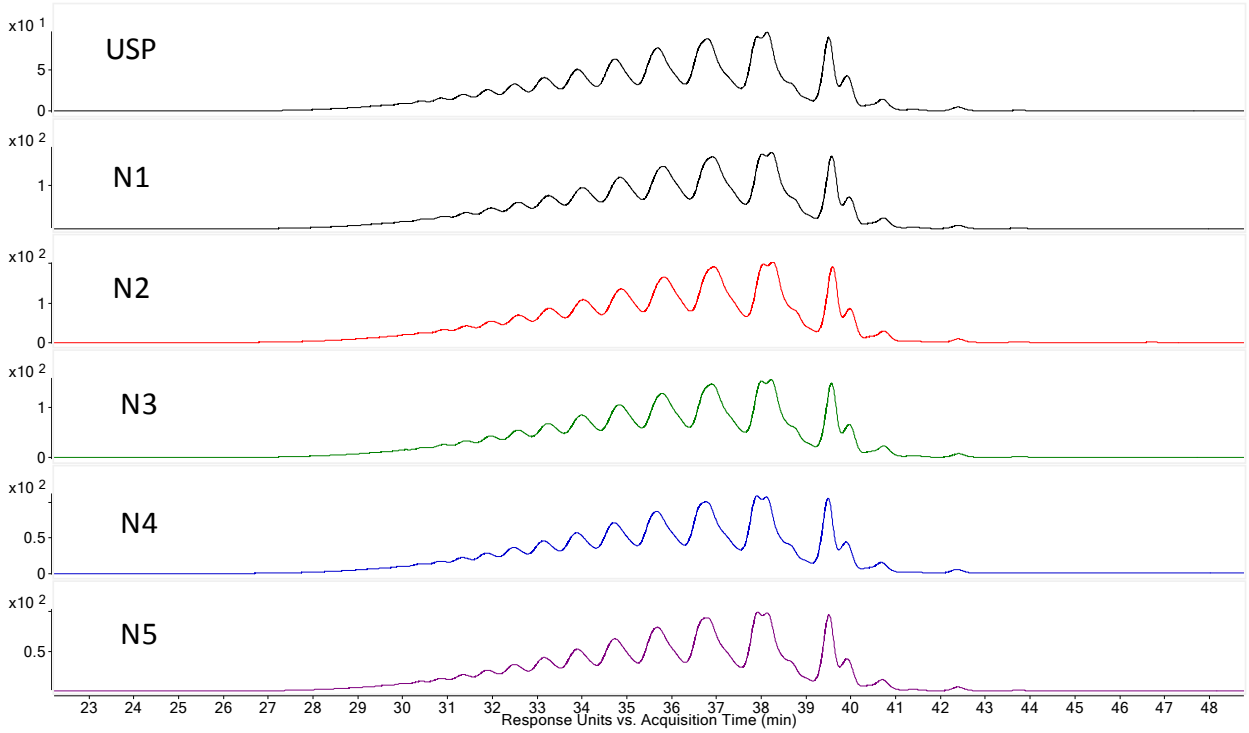

Figure S1. The chromatographic profiles of enoxaparin N batch and enoxaparin standards (USP)

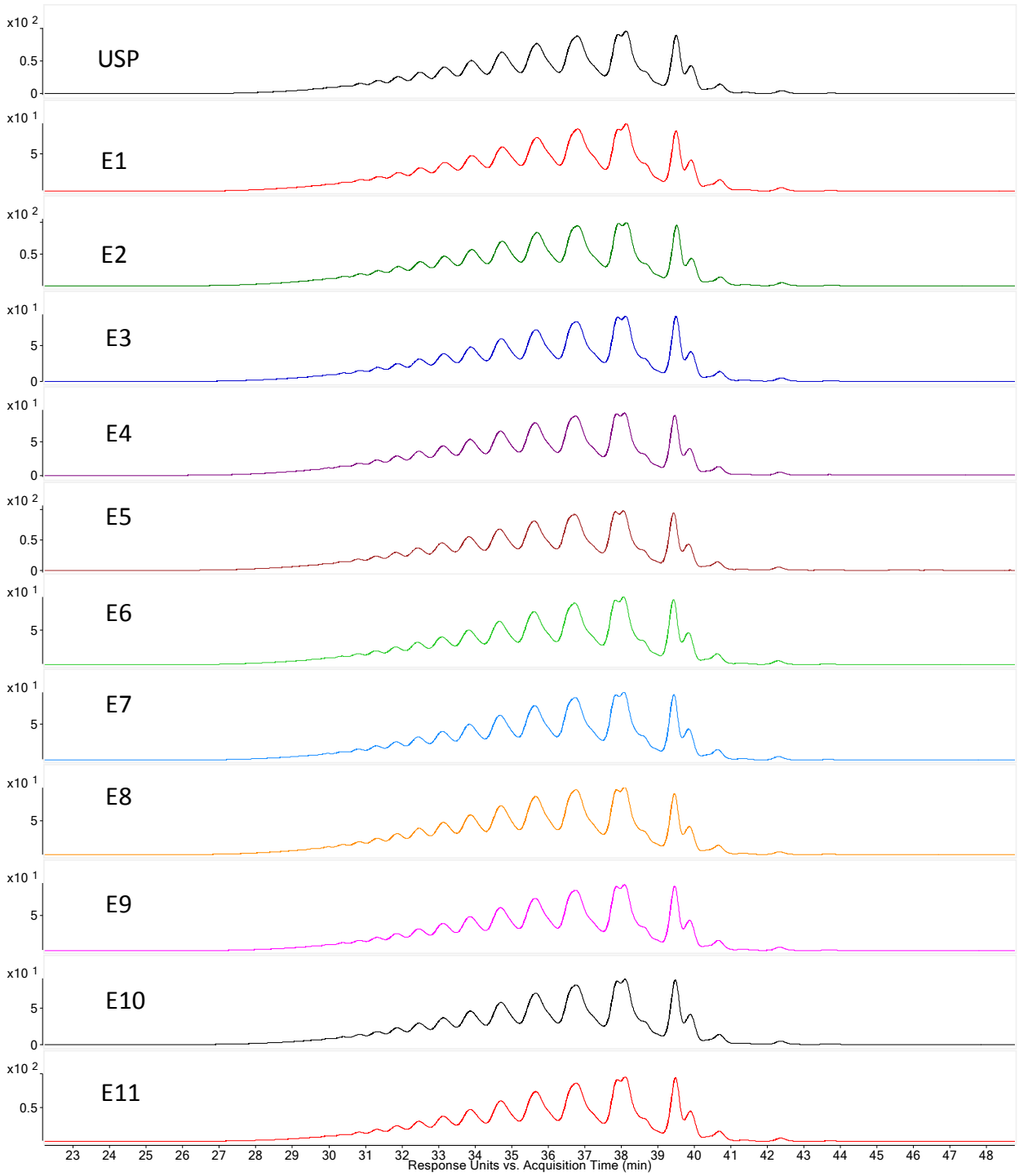

Figure S2. The chromatographic profiles of enoxaparin E batch and enoxaparin standards (USP)

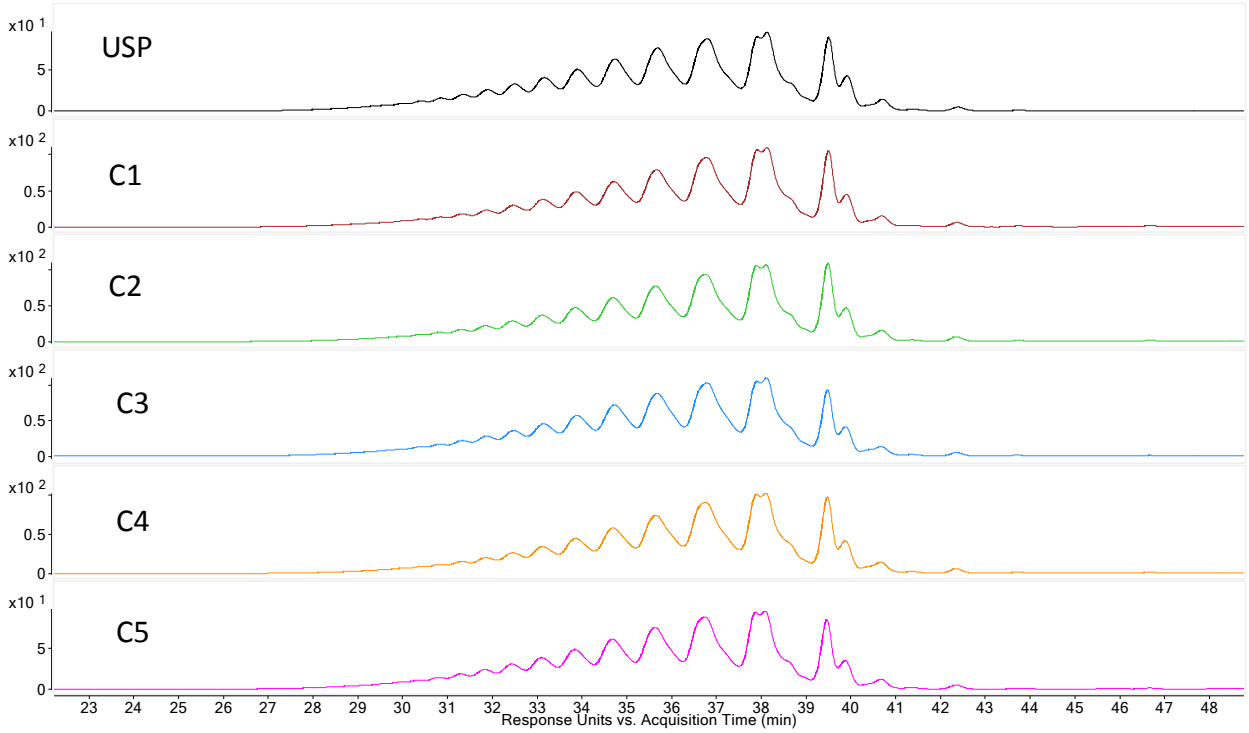

Figure S3. The chromatographic profiles of enoxaparin C batch and enoxaparin standards (USP)

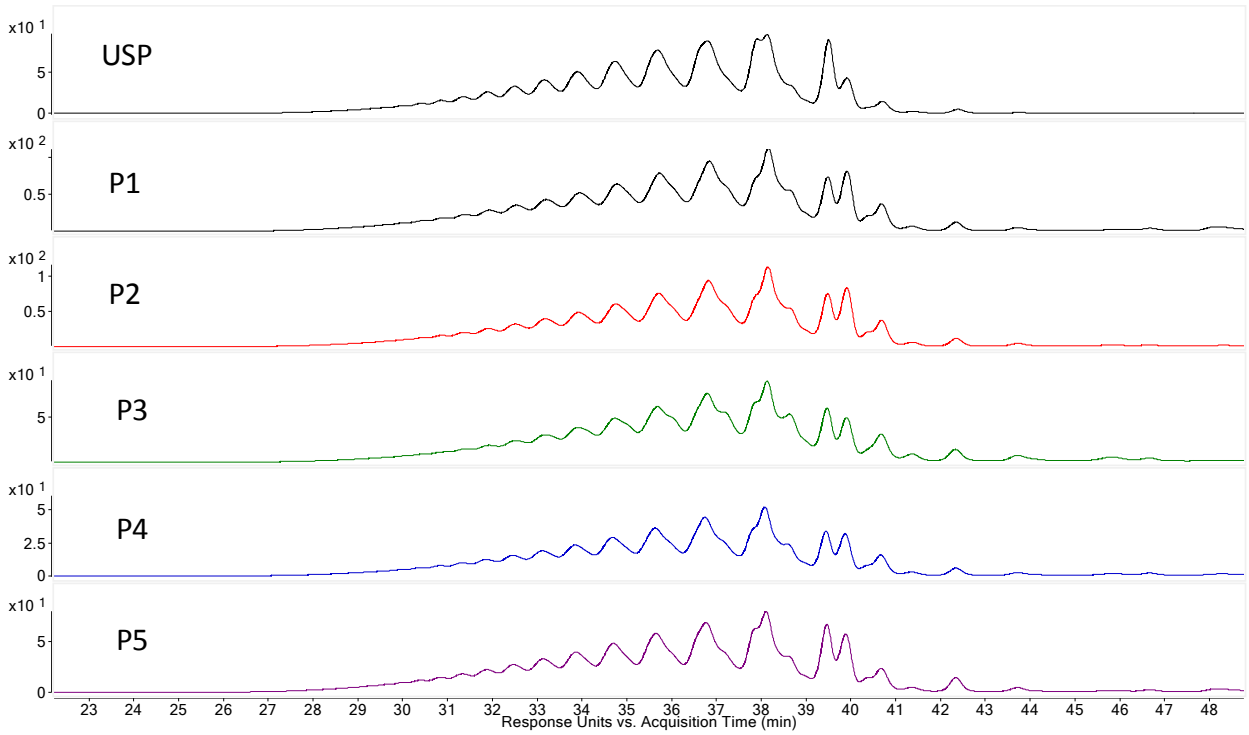

Figure S4. The chromatographic profiles of enoxaparin P batch and enoxaparin standards (USP)

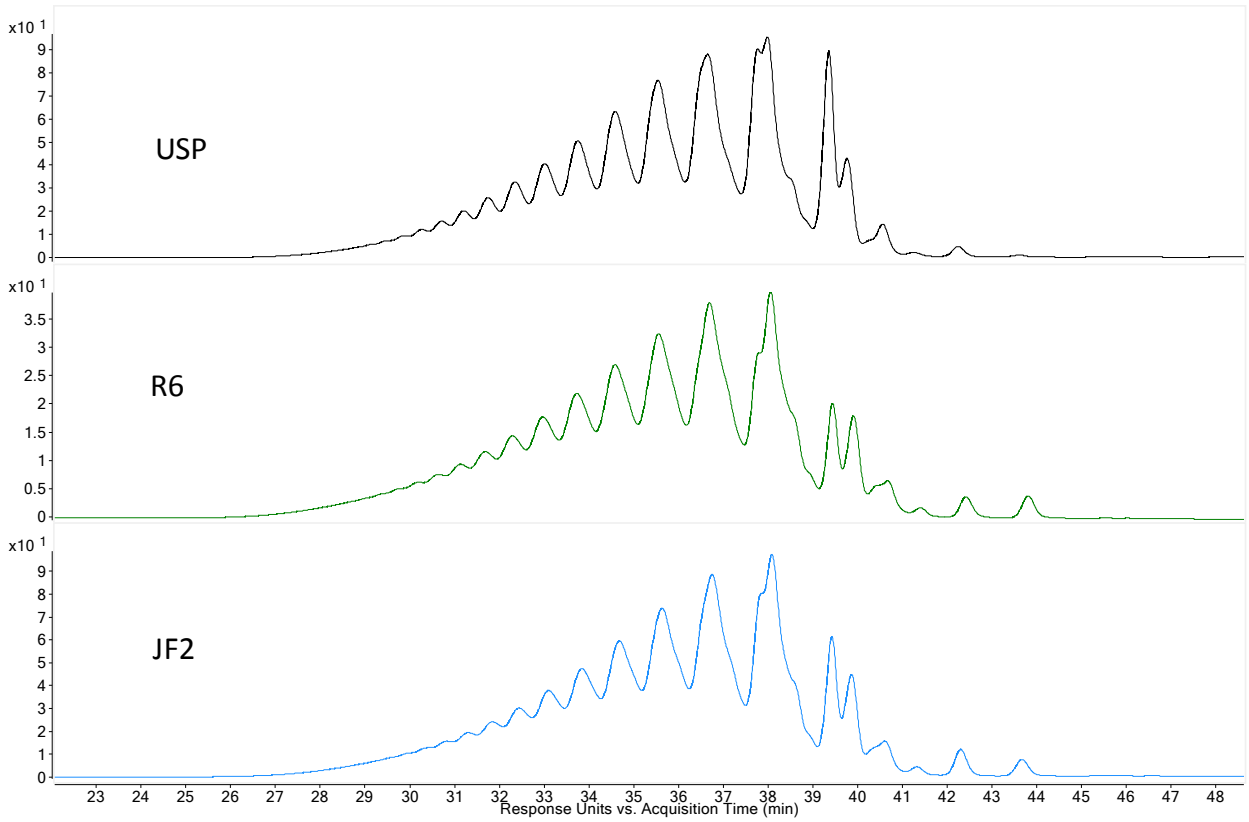

Figure S5. The chromatographic profiles of enoxaparin from bovine lung (R6, JF2) and enoxaparin standards (USP)

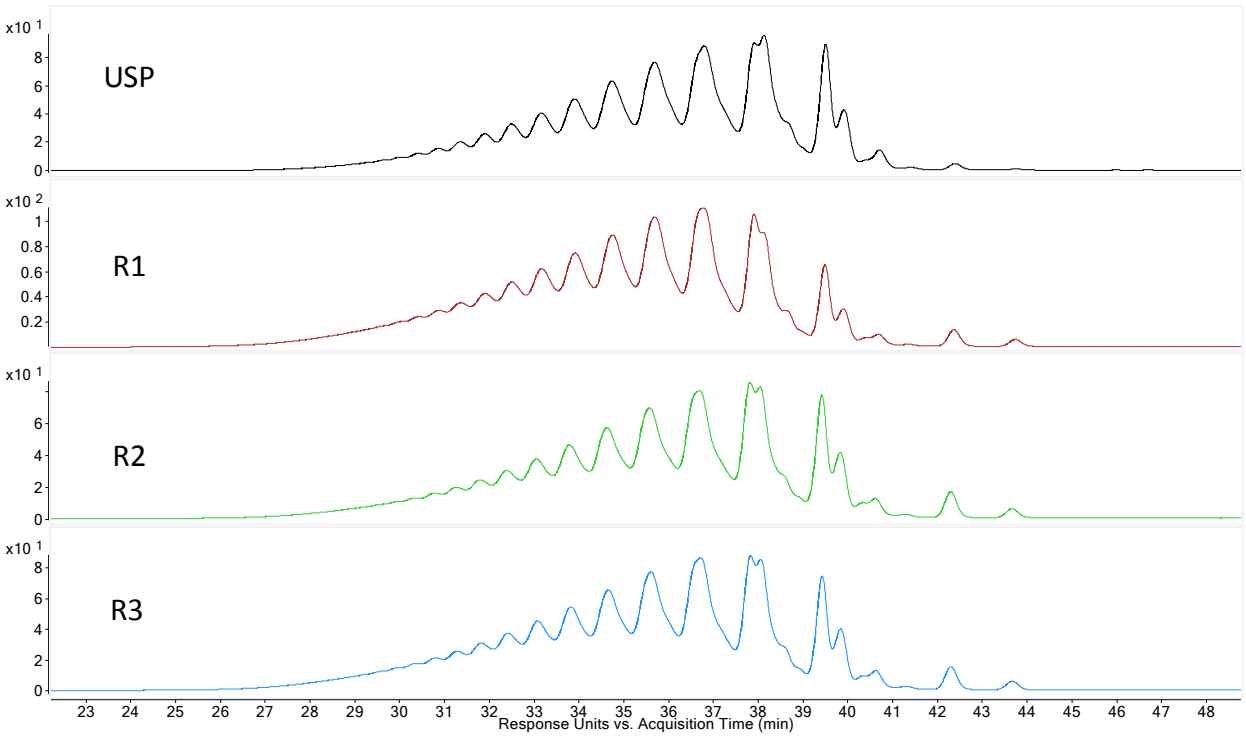

Figure S6. The chromatographic profiles of enoxaparin R batch and enoxaparin standards (USP)

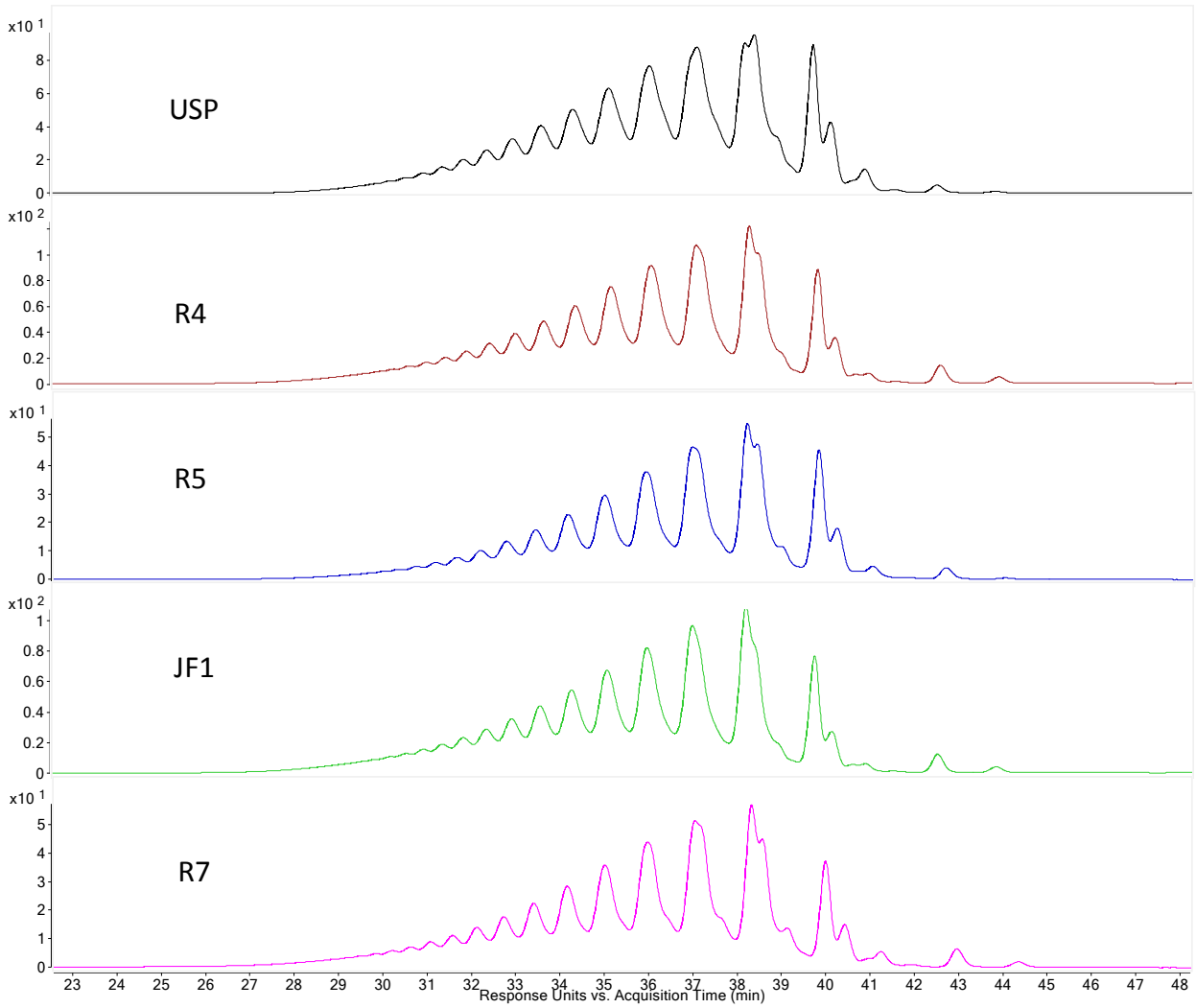

Figure S7. The chromatographic profiles of ovine-sourced (R4, R5, JF1), bovine lung-sourced (R7) enoxaparin and enoxaparin standards (USP)

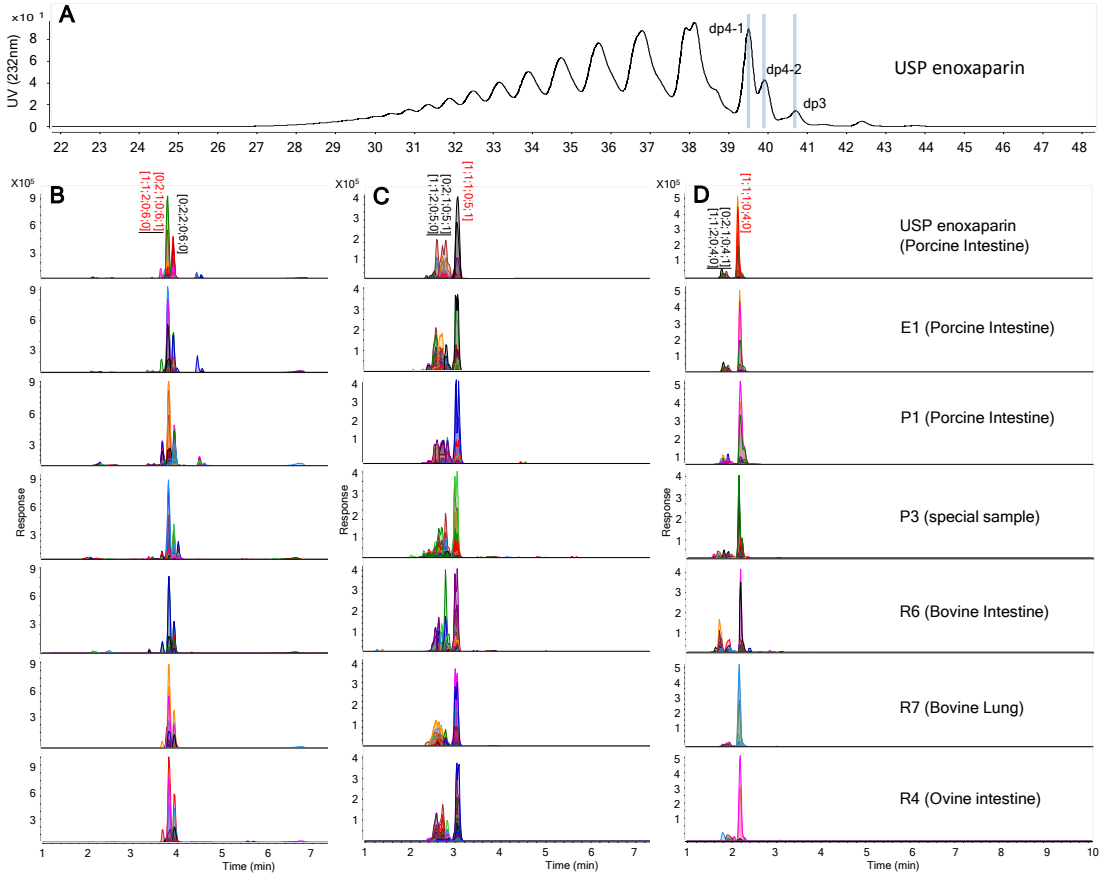

Figure S8. ECC of dp4-1, 4-2 and 3 of enoxaparins based on MHC-2DLC-MS results

A, [1]D chromatogram of USP enoxaparin; B, ECCs of dp4-1 of USP, E1, P1, P3, R4, R6 and R7; C, ECCs of dp4-2 of USP, E1, P1, P3, R4, R6 and R7; D, ECCs of dp3 of USP, E1, P1, P3, R4, R6 and R7