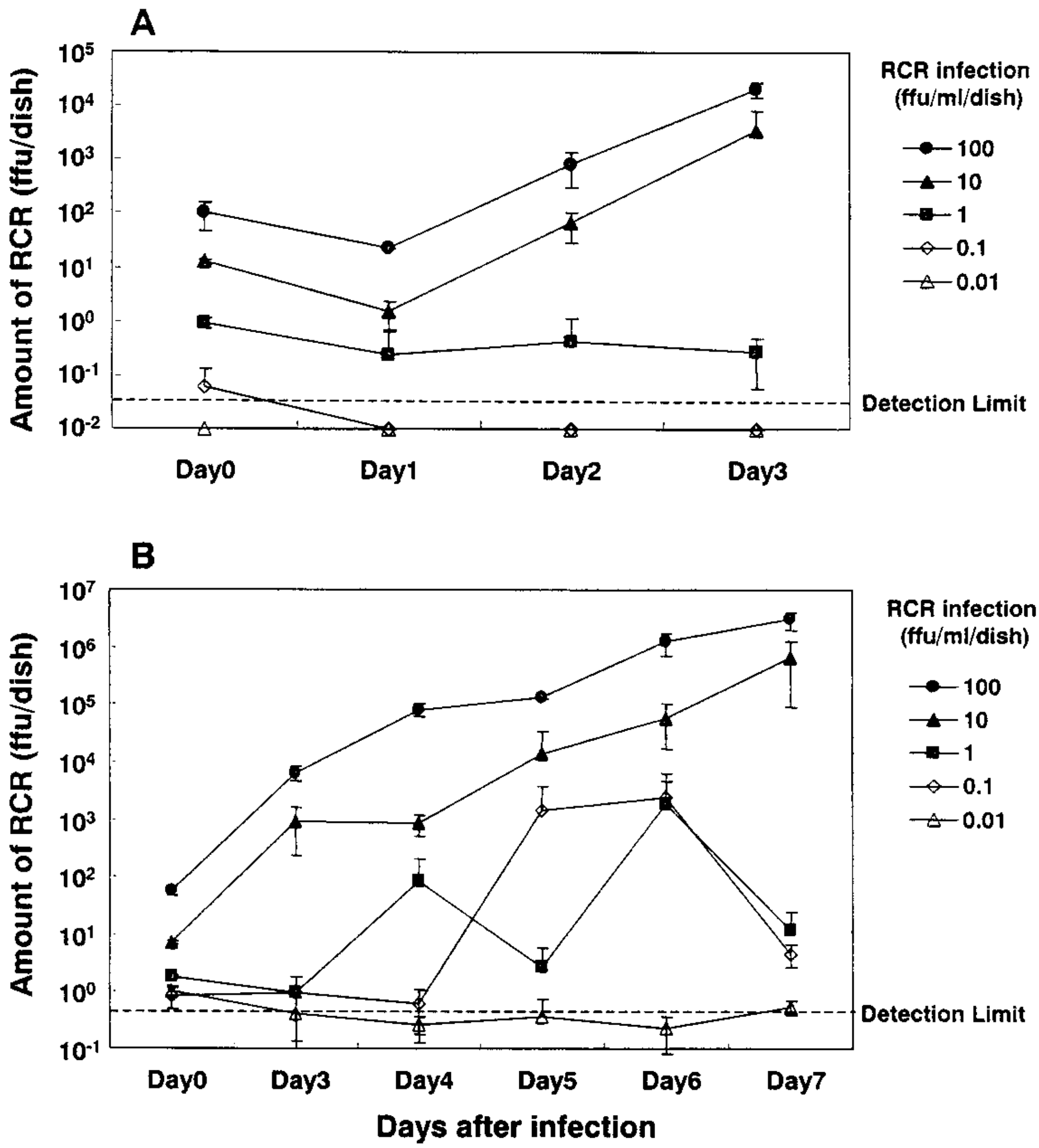

Fig.9 RCR growth curve in Mus dunni cells.
Mus dunni cells were infected with serial log dilution of RCR standard. Culture supernatant were harvested at indicated time, and RCR were concentrated by PEI-magnetic beads. Viral genome RNA were extracted and amount of RCR were determined by real-time quantitative RT-PCR.
Data were the mean ± S.D. (n=3)

Table 5 Comparison of direct S+L- assay and infectivity RT-PCR with PEI-beads concentration in detecting RCR

RCR Infection (ffu/ml/dish)	PG4(S+L-) assay	Infectivity RT-PCR and PEI-beads concentration				
	Day7	Day3	Day4	Day5	Day6	Day7
100	+ 6/6	+ 3/3	+ 3/3	+ 3/3	+ 3/3	+ 3/3
10	± 3/6	+ 3/3	+ 3/3	+ 3/3	+ 3/3	+ 3/3
1	± 1/6	± 1/3	± 1/3	± 1/3	+ 3/3	± 1/2
0.1	- 0/6	- 0/3	- 0/3	+ 3/3	± 2/3	+ 3/3
0.01	- 0/6	- 0/3	- 0/3	- 0/3	- 0/3	- 0/3

Serial dilutions of RCR in medium were evaluated in the direct PG4(S+L-) assay and infection of Mus dunni cells combined with PEI-beads concentration and reat-time quantitative RT-PCR.

Table 6 Comparison of direct S+L- assay and infectivity RT-PCR with PEI-beads concentration in detecting RCR spiked in retrovirus vector

RCR Infection (ffu/ml/dish)	PG4(S+L-) assay	Infectivity RT-PCR and PEI-beads concentration		
	Day7	Day5 Beads fraction	Day5 Culture sup 0.1ml	Day10 Beads fraction
100	+ 5/5	+ 5/5	+ 5/5	+ 5/5
10	± 4/5	+ 5/5	+ 5/5	+ 5/5
3	± 2/5	+ 5/5	+ 5/5	+ 5/5
1	± 1/5	± 2/5	- 0/5	± 3/5
0.3	- 0/5	- 0/5	- 0/5	± 1/5
0.1	- 0/5	- 0/5	- 0/5	- 0/5
0.01	- 0/5	- 0/5	- 0/5	- 0/5

Serial dilutions of RCR in ΨCRIP-LEGFP1 retrovirus vector sample were evaluated in the direct PG4(S+L-) assay and infection of Mus dunni cells combined with PEI-beads concentration and reat-time quantitative RT-PCR.

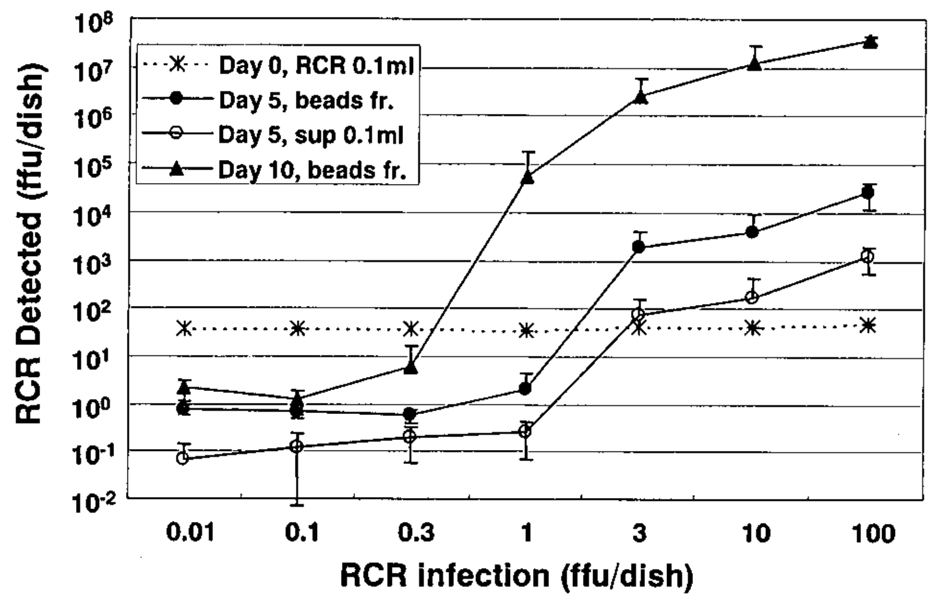

Fig.10 Detection of RCR in retroviral vector sample by infectivity RT-PCR and PEI-magnetic beads concentration.

Mus dunni cells were infected with serial log dilution of RCR standard in ΨCRIP-LEGFP1 retroviral vector sample. Cell culture supernatant were harvested on day5 and 10, and RCR were concentrated by PEI-magnetic beads. Viral genome RNA were extracted from beads fraction (day5, 10), untreated culture supernatant (day 5),and RCR solution (day 0),and amount of RCR of each sample was determined by real-time quantitative RT-PCR.
Data were the mean ± S.D. of (n=5)

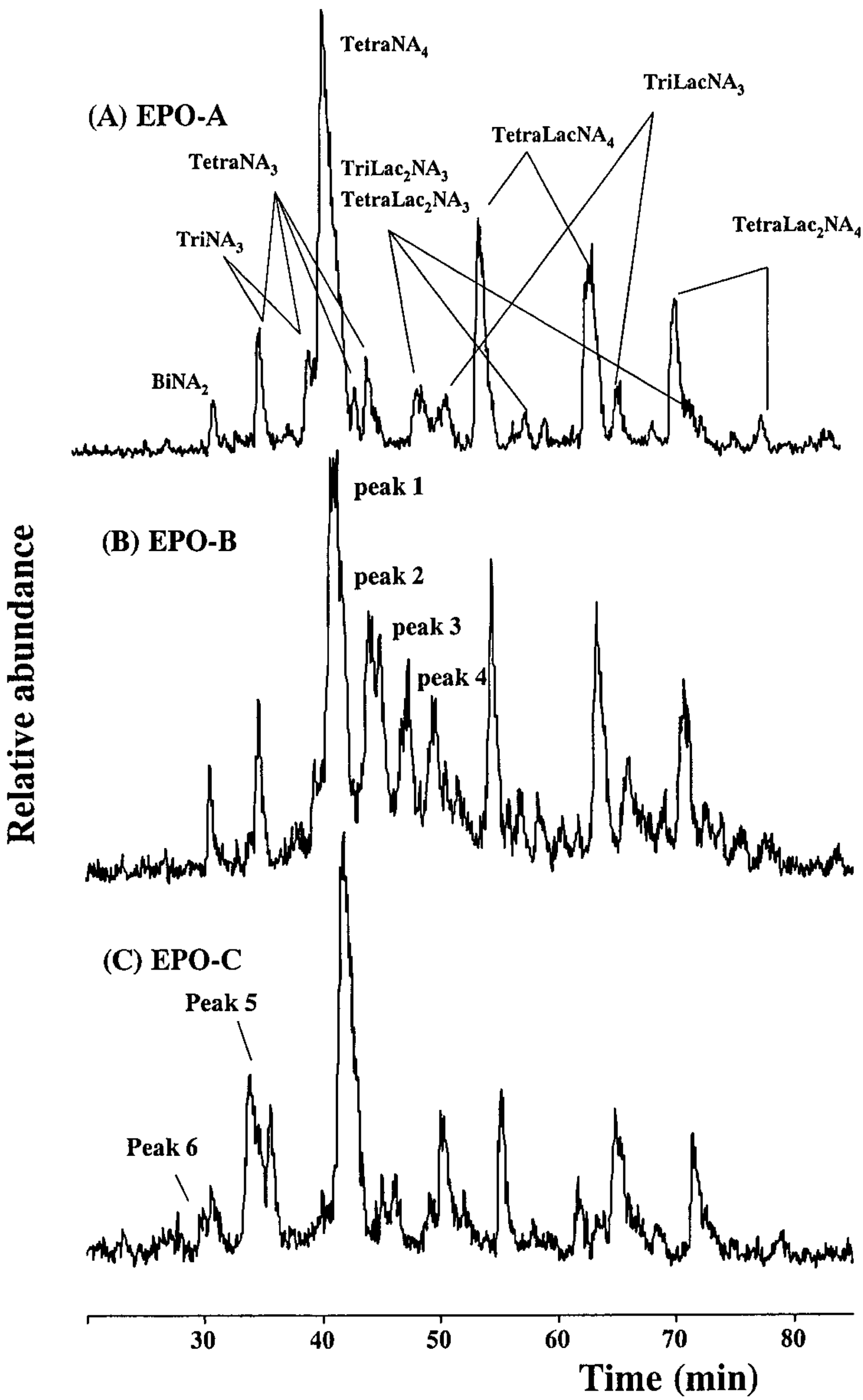

Fig. 11 Sugar mapping of EPO-A, B and C by LC/MS

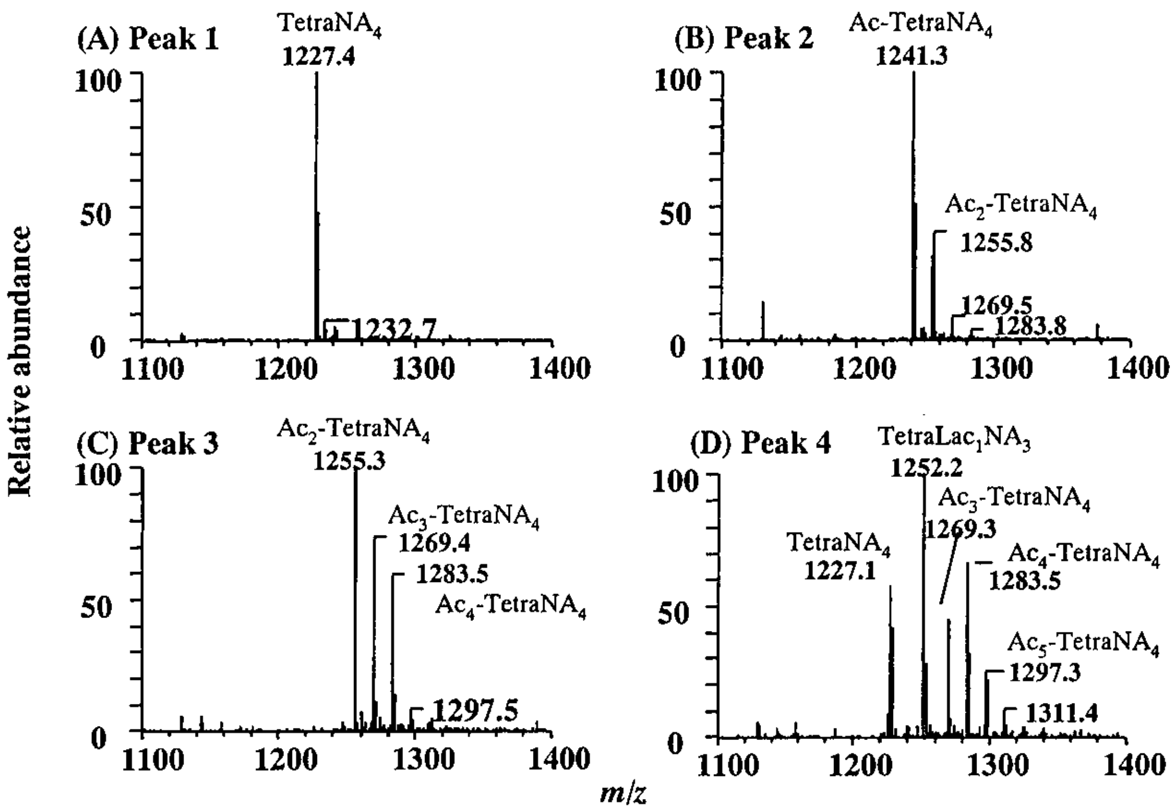

Fig. 12 Mass spectra of peaks 1-4 in Fig. 11B

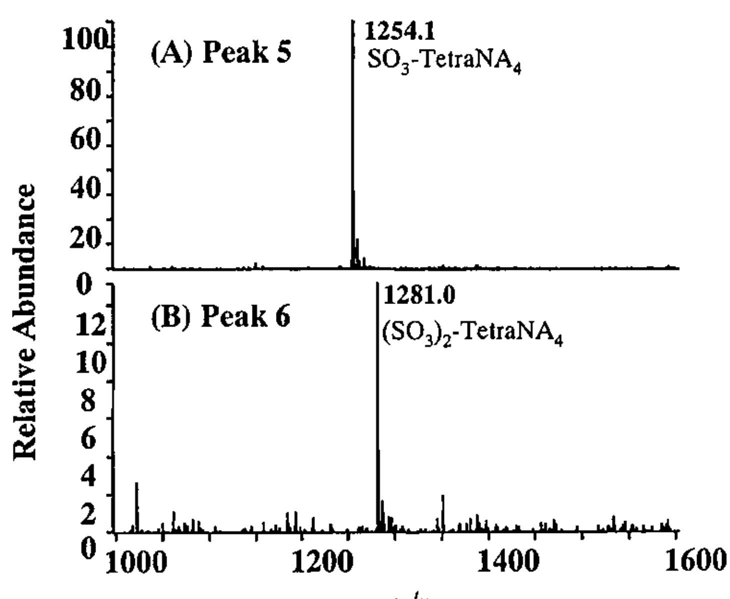

Fig. 13 Mass spectra of peaks 5, 6 in Fig. 11C

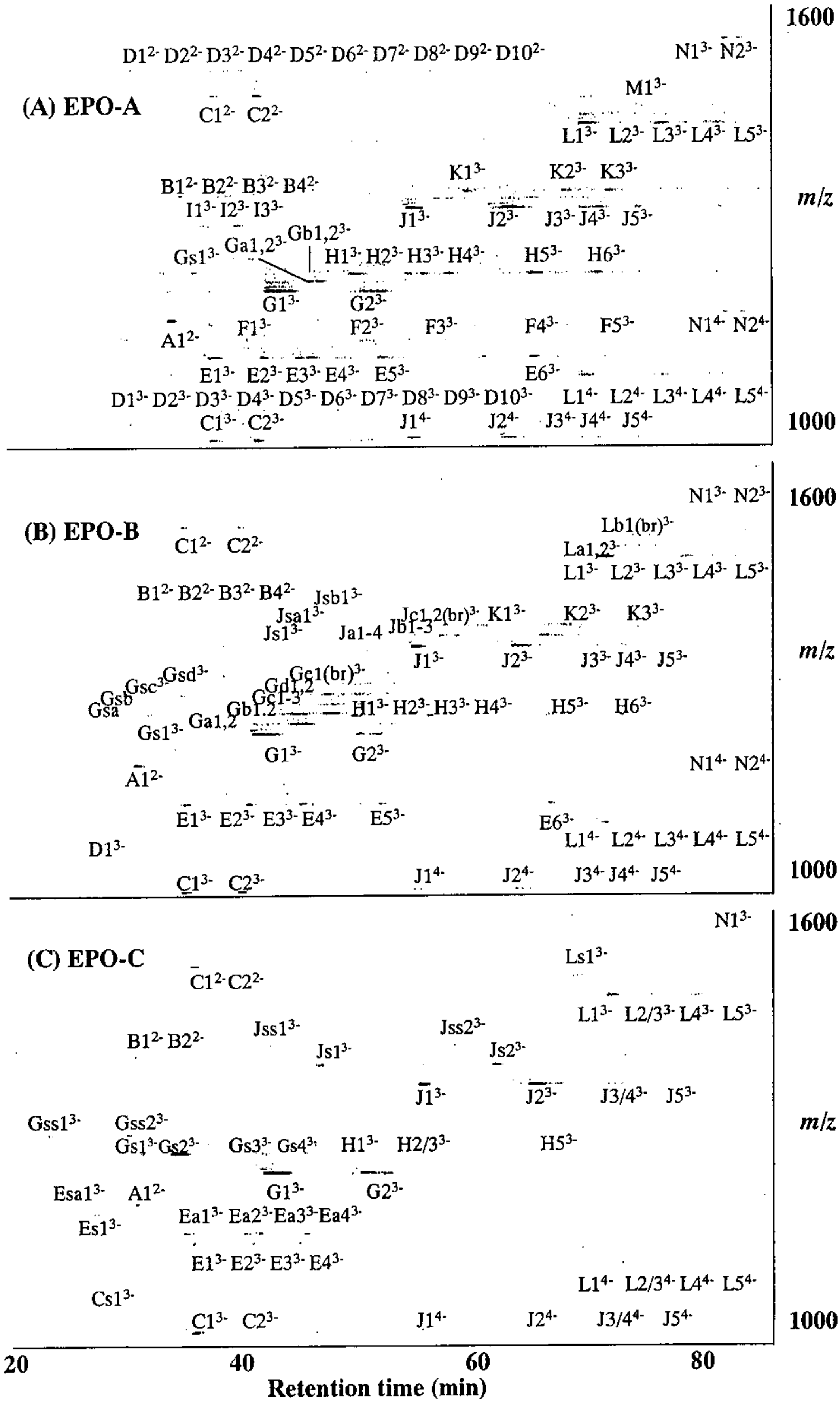

Fig.14 2-D sugar maps of EPO-A, -B, and -C

Table 7

Carbohydrate structures of A1·N2 in Fig. 4, and their theoretical masses and observed m/z values

No	Carbohydrate structure[a]	Theoretical mass[b]	Observed m/z								
			EPO-A			EPO-B			EPO-C		
			M^{2-}	M^{3-}	M^{4-}	M^{2-}	M^{3-}	M^{4-}	M^{2-}	M^{3-}	M^{4-}
A1	BiNA$_2$	2372.2	1185.0			1184.9			1185.3		
B1	Bi Lac$_1$NA$_2$, TriNA$_2$[c]	2737.5	1367.9			1367.5			1367.9		
B2	Bi Lac$_1$NA$_2$, TriNA$_2$	2737.5	1367.3			1367.8			1368.7		
B3	Bi Lac$_1$NA$_2$, TriNA$_2$	2737.5	1367.9			1367.4					
B4	Bi Lac$_1$NA$_2$, TriNA$_2$	2737.5	1367.5			1367.4					
C1	TriNA$_3$	3028.8	1513.3	1008.7		1513.4	1008.5		1513.1	1008.5	
C2	TriNA$_3$	3028.8	1513.1	1008.5		1513.5	1008.5		1513.4	1009.1	
Cs1	Ac-TriNA$_3$	3070.8							nd	1035.6	
D1	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$[d]	3102.9	1551.0	1033.4		1550.8	1033.5				
D2	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1549.8	1033.9							
D3	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.4	1033.0							
D4	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.8	1033.8							
D5	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1549.7	1033.2							
D6	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.6	1033.8							
D7	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.4	1033.3							
D8	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.2	1033.6							
D9	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1551.0	1033.4							
D10	BiLac$_2$NA$_2$, TriLac$_1$NA$_2$, TetraNA$_2$	3102.9	1550.3	1032.9							
E1	TriLac$_1$NA$_3$, TetraNA$_3$[e]	3394.1		1130.3			1130.4			1130.5	
E2	TriLac$_1$NA$_3$, TetraNA$_3$	3394.1		1130.3			1130.2			1130.2	
E3	TriLac$_1$NA$_3$, TetraNA$_3$	3394.1		1130.3			1130.7			1130.6	
E4	TriLac$_1$NA$_3$, TetraNA$_3$	3394.1		1130.4			1130.5			1130.4	
E5	TriLac$_1$NA$_3$, TetraNA$_3$	3394.1		1130.6			1130.3				
E6	TriLac$_1$NA$_3$, TetraNA$_3$	3394.1		1130.6			1130.4				
Ea1	Ac-TriLac$_1$NA$_3$, Ac-TetraNA$_3$	3436.1								1144.0	
Ea2	Ac-TriLac$_1$NA$_3$, Ac-TetraNA$_3$	3436.1								1144.0	
Ea3	Ac-TriLac$_1$NA$_3$, Ac-TetraNA$_3$	3436.1								1144.1	
Ea4	Ac-TriLac$_1$NA$_3$, Ac-TetraNA$_3$	3436.1								1144.2	
Es1	SO$_3$-TriLac$_1$NA$_3$, SO$_3$-TetraNA$_3$	3474.2								1157.2	
F1	TriLac$_2$NA$_2$, TetraLac$_1$NA$_2$[f]	3468.2		1154.9							
F2	TriLac$_2$NA$_2$, TetraLac$_1$NA$_2$	3468.2		1155.3							
F3	TriLac$_2$NA$_2$, TetraLac$_1$NA$_2$	3468.2		1155.5							
F4	TriLac$_2$NA$_2$, TetraLac$_1$NA$_2$	3468.2		1155.4							

F5	TriLac$_2$NA$_2$, TetraLac$_1$NA$_2$	3468.2	1155.4					
G1	TetraNA$_4$	3685.4	1227.4		1227.4		1227.5	
G2	TetraNA$_4$	3685.4	1227.4		1227.1		1227.3	
Ga1	Ac-TetraNA$_4$	3727.4	1241.5		1241.3			
Ga2	Ac-TetraNA$_4$	3727.4	1241.5		1241.3			
Gb1	Ac$_2$-TetraNA$_4$	3769.5	1256.2		1255.8			
Gb2	Ac$_2$-TetraNA$_4$	3769.5	1255.5		1255.3			
Gc1	Ac$_3$-TetraNA$_4$	3811.5			1269.5			
Gc2	Ac$_3$-TetraNA$_4$	3811.5			1269.3			
Gc3	Ac$_3$-TetraNA$_4$	3811.5			1269.3			
Gd1	Ac$_4$-TetraNA$_4$	3853.5			1283.5			
Gd2	Ac$_4$-TetraNA$_4$	3853.5			1283.8			
Gd3	Ac$_4$-TetraNA$_4$	3853.5			1283.5			
Ge1br	Ac$_5$-TetraNA$_4$	3895.6			1297.7			
Gs1	SO$_3$-TetraNA$_4$	3765.5					1253.9	
Gs2	SO$_3$-TetraNA$_4$	3765.5	1254.5		1254.4		1254.1	
Gs3	SO$_3$-TetraNA$_4$	3765.5					1253.9	
Gs4	SO$_3$-TetraNA$_4$	3765.5					1254.0	
Gss1	(SO$_3$)$_2$-TetraNA$_4$	3845.5					1280.7	
Gss2	(SO$_3$)$_2$-TetraNA$_4$	3845.5					1281.0	
Gsa1	SO$_3$-Ac-TetraNA$_4$	3807.5			1268.1			
Gsb1	SO$_3$-Ac$_2$-TetraNA$_4$	3849.5			1282.2			
Gsc1br	SO$_3$-Ac$_3$-TetraNA$_4$	3891.6			1296.5			
Gsd1br	SO$_3$-Ac$_4$-TetraNA$_4$	3933.6			1310.0			
H1	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$[g]	3759.5	1252.2		1252.2		1252.8	
H2	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$	3759.5	1252.2		1252.4		1252.5	
H3	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$	3759.5	1252.1		1252.4			
H4	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$	3759.5	1252.2		1252.6			
H5	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$	3759.5	1252.2		1252.5		1252.0	
H6	TriLac$_2$NA$_3$, TetraLac$_1$NA$_3$	3759.5	1252.1		1252.1			
I1	TetraLac$_2$NA$_2$	3833.5	1276.8					
I2	TetraLac$_2$NA$_2$	3833.5	1276.9					
I3	TetraLac$_2$NA$_2$	3833.5	1276.3					
J1	TetraLac$_1$NA$_4$	4050.7	1349.1	1011.8	1349.2	1011.7	1349.1	1011.8
J2	TetraLac$_1$NA$_4$	4050.7	1349.0	1011.9	1349.0	1011.7	1349.1	1011.7
J3	TetraLac$_1$NA$_4$	4050.7	1348.7	1011.1	1348.9	1011.1	1349.2	1011.2
J4	TetraLac$_1$NA$_4$	4050.7	1349.3	1011.3	1349.6	1011.8		
J5	TetraLac$_1$NA$_4$	4050.7	1349.2	1011.4	1349.4	1011.5	1349.3	1011.0
Ja1	Ac-TetraLac$_1$NA$_4$	4092.7	1363.2	1021.9	1363.2	1021.9		
Ja2	Ac-TetraLac$_1$NA$_4$	4092.7	1363.3	1022.0	1363.3	1022.1		
Ja3	Ac-TetraLac$_1$NA$_4$	4092.7	1363.8	nd	1363.0	1022.9		

Ja4	Ac-TetraLac$_1$NA$_4$	4092.7	1363.0	1022.9	1363.5	1022.1		
Jb1	Ac$_2$-TetraLac$_1$NA$_4$	4134.8			1377.5	1032.9		
Jb2	Ac$_2$-TetraLac$_1$NA$_4$	4134.8			1377.2	1031.7		
Jb3	Ac$_2$-TetraLac$_1$NA$_4$	4134.8			1377.6	1032.8		
Jc1br	Ac$_3$-TetraLac$_1$NA$_4$	4176.8			1391.2	nd		
Jc2br	Ac$_3$-TetraLac$_1$NA$_4$	4176.8			1391.9	nd		
Js1	SO$_3$-TetraLac$_1$NA$_4$	4130.8			1375.8	1032.1	1375.8	1031.5
Js2	SO$_3$-TetraLac$_1$NA$_4$	4130.8					1375.8	1031.9
Jss1	(SO$_3$)$_2$-TetraLac$_1$NA$_4$	4210.8					1402.5	1052.0
Jss2	(SO$_3$)$_2$-TetraLac$_1$NA$_4$	4210.8					1402.6	1051.5
Jsa1	SO$_3$-Ac-TetraLac$_1$NA$_4$	4172.8			1390.6	nd		
Jsb1	SO$_3$-Ac$_2$-TetraLac$_1$NA$_4$	4172.8			1404.1	1052.3		
K1	TetraLac$_2$NA$_3$	4124.8	1373.9		1374.1			
K2	TetraLac$_2$NA$_3$	4124.8	1374.1		1374.3			
K3	TetraLac$_2$NA$_3$	4124.8	1374.4		1374.2			
L1	TetraLac$_2$NA$_4$	4416.1	1470.9	1103.1	1470.9	1103.1	1470.9	1103.3
L2	TetraLac$_2$NA$_4$	4416.1	1470.4	1102.5	1471.0	1103.1	1470.9	1103.2
L3	TetraLac$_2$NA$_4$	4416.1	1470.9	1103.2	1471.3	1102.9		
L4	TetraLac$_2$NA$_4$	4416.1	1471.5	1102.9	1471.6	1103.4	1471.0	1102.3
L5	TetraLac$_2$NA$_4$	4416.1	1470.7	1103.3	1471.2	1103.2	1471.2	1103.1
La1	Ac-TetraLac$_2$NA$_4$	4458.1			1485.2	1113.2		
La2	Ac-TetraLac$_2$NA$_4$	4458.1			1485.6	1113.7		
Lb1br	Ac$_2$-TetraLac$_2$NA$_4$	4500.2			1499.1	nd		
Ls1	SO$_3$-TetraLac$_2$NA$_4$	4496.2					1498.3	1123.4
M1	TetraLac$_3$NA$_3$	4490.1	1495.6					
N1	TetraLac$_3$NA$_4$	4781.4	1592.8	1194.4	1592.9	1194.1	1593.0	1195.2
N2	TetraLac$_3$NA$_4$	4781.4	1593.5	1194.1	1592.5	1194.7	1593.3	1195.6

[a] All carbohydrates contain fucosylated trimannosylcore. Bi, biantennary; Tri, triantennary; Tetra, tetraantennary; NA, NeuAc; Lac, N-acetyllactosamine. [b] average mass value. [c-g] isomers

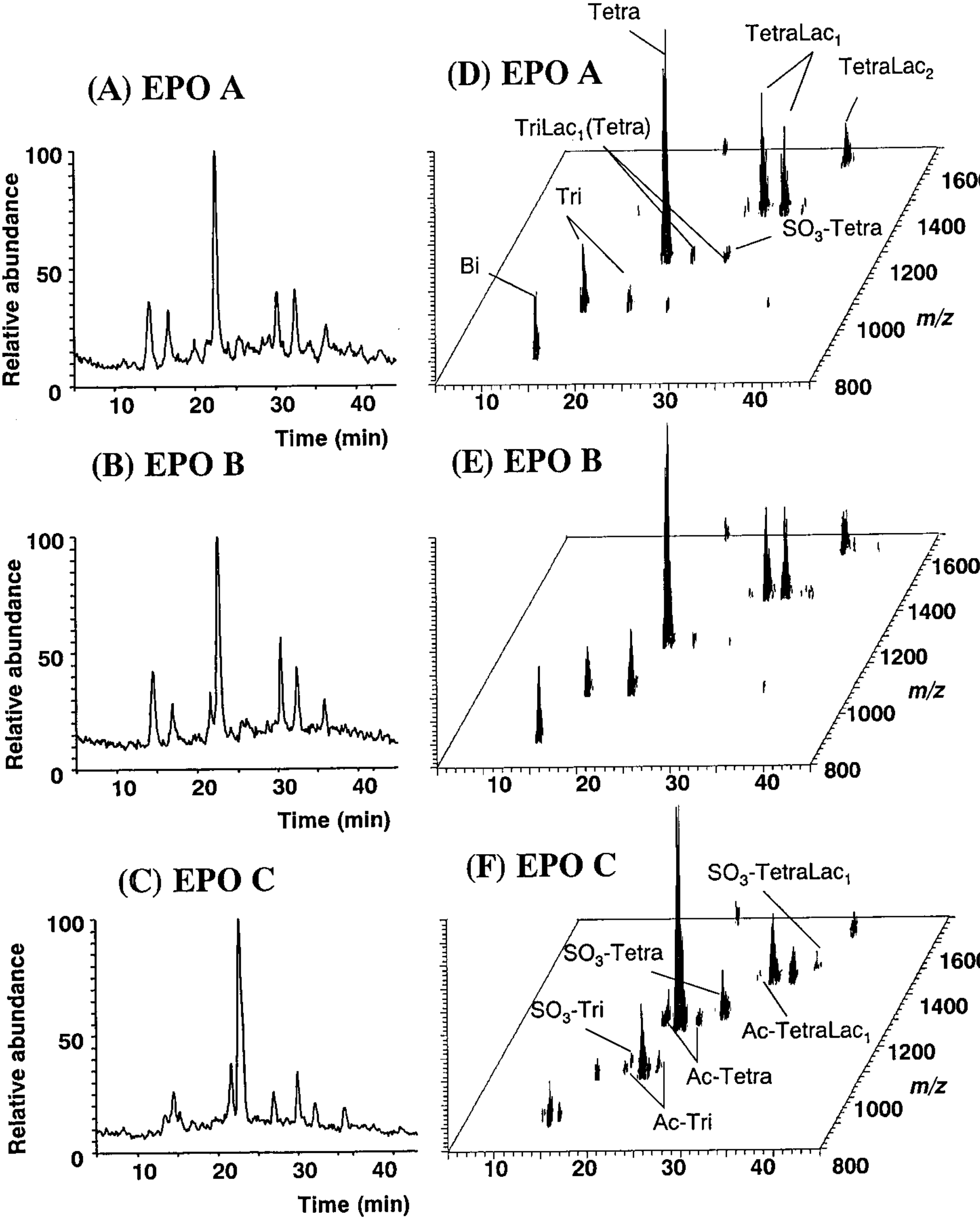

Fig.15 Sugar mapping of asialo-EPO-A, -B, and -C

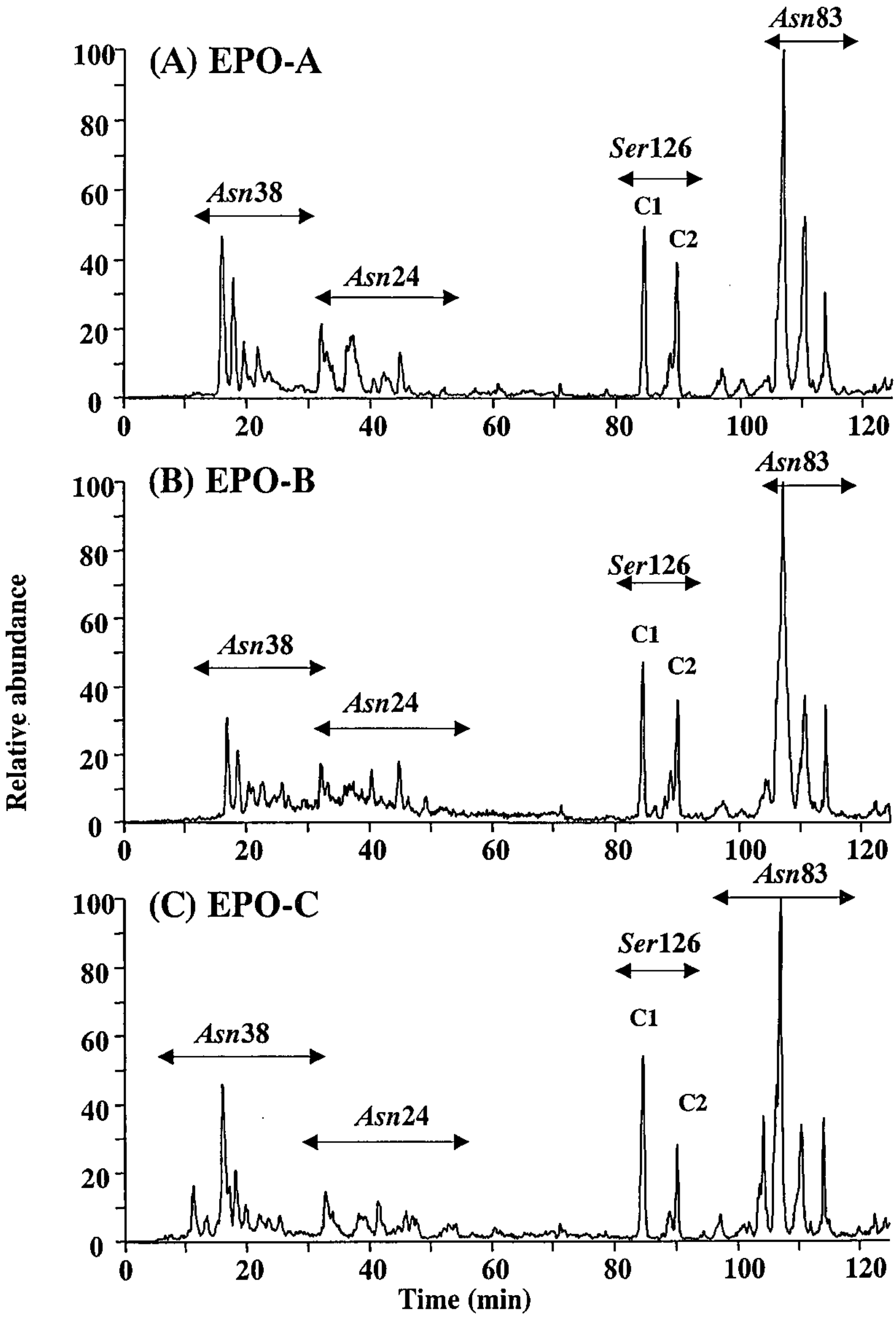

Fig. 16 glycopeptide mapping of EPO-A, -B, and -C

Table 8 Peak number, glycoforms, and theoretical and observed m/z of N38 glycopeptides in EPO-A, B and C digests

Peak	Glycoform	Charge state	Theoretical m/z Mono	Theoretical m/z Average	Observed m/z EPO-A	Observed m/z EPO-B	Observed m/z EPO-C
A1	Tetra-NA$_4$-SO$_2$	4-	1,119.1	1,119.8			1,119.3*
	Tetra-Lac$_1$-NA$_4$-SO$_2$	4-	1,210.4	1,211.1			1,210.3
A2	Tetra-NA$_4$-SO$_1$	4-	1,099.1	1,099.8		1,099.5	1,099.8*
A3	Tetra-Lac$_1$-NA$_4$-SO$_1$	4-	1,190.4	1,191.1		1,190.6	1,190.9*
A4	Tetra-Lac$_2$-NA$_4$-SO$_1$	4-	1,281.7	1,282.4			1,281.5*
A5	Tetra-NA$_4$ [1)]	4-	1,079.2	1,079.8	1,079.1*	1,079.4*	1,079.3*
A6	Tetra-NA$_4$ [2)]	4-	1,079.2	1,079.8			1,079.2*
A7	Tetra-Lac$_1$-NA$_4$	4-	1,170.4	1,171.1	1,170.3*	1,170.3*	1,170.2*
A8	Tetra-Lac$_2$-NA$_4$	4-	1,261.7	1,262.4	1261.6*	1,261.8*	1,262.1*
A9	Tri-NA$_3$ [3)]	3-	1,220.5	1,221.2	1,220.8*	1,221.0	
	Tetra-Lac$_2$-NA$_4$	4-	1,261.7	1,262.4	1,261.9	1,261.7	1,262.1
	Tetra-Lac$_3$-NA$_4$	4-	1,353.0	1,353.8	1,352.8	1,352.9	1,353.4*
	Tetra-NA$_4$-Ac$_1$	4-	1,089.7	1,090.3	1,089.8	1,089.7*	
A10	Tetra-NA$_3$ (or Tri-Lac$_1$-NA$_3$) [4)]	3-	1,342.2	1,342.9	1,341.5*	1,342.5*	1,342.6*
	Tetra-Lac$_1$-NA$_4$-Ac$_1$ [5)]	4-	1,180.9	1,181.6		1,181.0	
A11	Tetra-Lac$_1$-NA$_3$ (or Tri-Lac$_2$-NA$_3$)	3-	1,463.9	1,464.7	1,464.1	1,463.9*	1,463.7
		4-	1,097.7	1,098.3	1,097.4*	1,097.6	1,097.6
	Tetra-Lac$_1$-NA$_3$-Ac$_1$ (or Tri-Lac$_2$-NA$_3$-Ac$_1$)	3-	1,477.9	1,478.7			1,478.1
	Tetra-NA$_3$-Ac$_1$	3-	1,356.2	1,356.9			1,356.3*
A12	Tetra-Lac$_2$-NA$_3$	4-	1,188.9	1,189.6	1,189.1*	1,189.3	
	Tri-NA$_3$ [6)]	3-	1,220.5	1,221.2		1,220.5	1,220.7*
	Tetra-NA$_4$-Ac$_2$ [7)]	3-	1,467.2	1,468.0		1,467.6*	
A13	Tetra-NA$_3$ (or Tri-Lac$_1$-NA$_3$) [8)]	3-	1,342.2	1,342.9			1,342.9*
	Tetra-NA$_3$-Ac$_1$(or Tri-Lac$_1$-NA$_3$-Ac$_1$)	3-	1,356.2	1,356.9	1,356.3*	1,356.8*	
	Tetra-Lac$_3$-NA$_3$	4-	1,280.2	1,281.0	1,280.1		
	Tri-NA$_3$	3-	1,220.5	1,221.2			1,220.7*
	Tri-NA$_2$	3-	1,123.4	1,124.1	1,123.4		
	Tetra-Lac$_1$-NA$_4$-Ac$_1$ [9)]	4-	1,180.9	1,181.6	1,181.0	1,180.9	
	Tetra-Lac$_1$-NA$_4$-Ac$_2$ [10)]	4-	1,191.4	1,192.1		1,191.4*	
A14	Tetra-NA$_2$ (or Tri-Lac$_1$-NA$_2$)	3-	1,245.1	1,245.8	1,245.4*	1,245.6*	1,245.3*
A15	Tetra-NA$_2$ (or Tri-Lac$_1$-NA$_2$)	3-	1,245.1	1,245.8	1,245.4*	1,245.5	1,245.0*
	Tetra-NA$_2$-Ac$_1$ (or Tri-Lac$_1$-NA$_2$-Ac$_1$)	3-	1,259.1	1,259.9			1,258.9
	Tetra-NA$_2$-Ac$_2$ (or Tri-Lac$_1$-NA$_2$-Ac$_2$)	3-	1,273.1	1,273.9			1,273.2
	Tetra-Lac$_1$-NA$_2$ (or Tri-Lac$_2$-NA$_2$)	3-	1,366.9	1,367.6	1,366.5	1,367.5	1,367.4
	Tetra-NA$_4$-Ac$_2$ [11)]	4-	1,100.2	1,100.8		1,100.1*	
	Tetra-NA$_4$-Ac$_3$	4-	1,110.7	1,111.3		1,110.4	
	Tetra-Lac$_1$-NA$_4$-Ac$_2$ [12)]	4-	1,191.4	1,192.1		1,191.6	
A16	Tetra-Lac$_2$-NA$_2$	3-	1,488.6	1,489.4	1,489.4*		
	Tetra-Lac$_1$-NA$_2$ (or Tri-Lac$_2$-NA$_2$)	3-	1,366.9	1,367.6	1,367.6		

All carbohydrates contain a fucosylated core. *, base peak in the mass spectra of each HPLC peak in Fig.2A; Mono, monoisotopic;
Bi, bianntenary; Tri, trianntenary; Tetra, tetraanntenary; NA, N-acetylneuramic acid, Lac, N-acetyllactosamine; Ac, acetyl; SO, sulphate;
[1)] and [2)], [3)] and [6)], [4)] and [8)], [5)] and [9)], [7)] and [11)], [10)] and [12)], isomer.

Table 9 Peak number, glycoforms, and theoretical and observed m/z of N24 glycopeptides in EPO-A, B and C diges

Peak	Glycoform	Charge state	Theoretical m/z Mono	Theoretical m/z Average	Observed m/z EPO-A	Observed m/z EPO-B	Observed m/z EPO-C
B1	Tetra-NA$_4$-SO$_1$	4-	1,357.0	1,357.8			1,357.4*
B2	Tetra-Lac$_1$-NA$_4$-SO$_1$	4-	1,448.3	1,449.1			1,448.4
	Tetra-Lac$_2$-NA$_4$-SO$_1$	4-	1,539.5	1,540.5			1,540.2
B3	Tetra-NA$_4$	4-	1,337.0	1,337.8	1,337.4*	1,337.2*	1,337.4*
B4	Tetra-Lac$_1$-NA$_4$	4-	1,428.3	1,429.1	1,428.5*	1,428.7*	1,428.3*
B5	Tetra-Lac$_2$-NA$_4$	4-	1,519.6	1,520.5	1,519.8*	1,520.1*	1,519.9*
B6	Tetra-Lac$_3$-NA$_4$	4-	1,610.8	1,611.8	1,611.6	1,611.1	1,610.8*
	Tetra-NA$_4$-Ac$_1$	4-	1,347.5	1,348.3	1,347.9*	1,347.8*	1,347.6
	Tri-NA$_3$-SO$_1$	4-	1,192.9	1,193.7			1,193.4*
	Tetra-Lac$_2$-NA$_4$-Ac$_1$	4-	1,530.1	1,531.0			1,530.0
B7	Tri-NA$_3$[1)]	4-	1,172.9	1,173.6	1,173.1*	1,173.3*	1,173.2*
B8	Tetra-NA$_3$ (or Tri-Lac$_1$-NA$_3$)[2)]	4-	1,264.2	1,265.0	1,264.6*	1,264.1*	1,264.0
	Tetra-NA$_3$-Ac$_1$ (or Tri-Lac$_1$-NA$_3$-Ac$_1$)[3)]	4-	1,274.7	1,275.5			1,274.8*
	Tetra-Lac$_1$-NA$_3$ (or Tri-Lac$_2$-NA$_3$)	4-	1,355.5	1,356.3	1,356.0	1,355.5	1,355.4
	Tetra-Lac$_1$-NA$_3$-Ac$_1$(or Tri-Lac$_2$-NA$_3$-Ac$_1$)	4-	1,366.0	1,366.8			1,366.0
B9	Tetra-Lac$_2$-NA$_3$	4-	1,446.8	1,447.6	1,446.7*	1,447.0	1,446.9
	Tetra-Lac$_2$-NA$_3$-Ac$_1$	4-	1,457.3	1,458.1			1,457.0
	Tetra-Lac$_3$-NA$_3$	4-	1,538.1	1,539.0	1,538.2	1,538.3	1,538.4
	Tetra-NA$_3$-Ac$_1$ (or Tri-Lac$_1$-NA$_3$-Ac$_1$)	4-	1,274.7	1,275.5			1,275.1*
	Tetra-NA$_3$-Ac$_2$ (or Tri-Lac$_1$-NA$_3$-Ac$_2$)	4-	1,285.2	1,286.0			1,285.5
	Tetra-Lac$_1$-NA$_3$-Ac$_1$ (or Tri-Lac$_2$-NA$_3$-Ac$_1$)	4-	1,366.0	1,366.8			1,365.7
	Tetra-Lac$_1$-NA$_3$-Ac$_2$ (or Tri-Lac$_2$-NA$_3$-Ac$_2$)	4-	1,376.5	1,377.3			1,376.4
	Tetra-NA$_4$-Ac$_2$[4)]	4-	1,358.0	1,358.8		1,358.2*	
B10	Tri-NA$_3$[5)]	4-	1,172.9	1,173.6	1,173.0*	1,173.4*	1,172.8*
	Tri-NA$_3$-Ac$_1$	4-	1,183.4	1,184.1			1,183.3
	Tetra-NA$_3$ (or Tri-Lac$_1$-NA$_3$)[6)]	4-	1,264.2	1,265.0			1,264.3
	Tetra-NA$_3$-Ac$_1$ (or Tri-Lac$_1$-NA$_3$-Ac$_1$)[7)]	4-	1,274.7	1,275.5	1,274.8		1,275.3
	Tetra-Lac$_1$-NA$_3$-Ac$_1$ (or Tri-Lac$_2$-NA$_3$-Ac$_1$)	4-	1,366.0	1,366.8	1,365.9		
B11	Tri-NA$_2$[8)]	3-	1,467.2	1,468.1	1,467.3*	1,467.4	1,467.1
	Tri-NA$_2$-Ac$_1$	4-	1,110.7	1,111.3			1,110.6
	Tri-SA$_2$-Ac$_2$	4-	1,121.2	1,121.8		1,121.7	
	Tri-NA$_3$-Ac$_1$	4-	1,183.4	1,184.1		1,183.8	
	Tetra-NA$_2$ (or Tri-Lac$_1$-NA$_2$)	4-	1,191.4	1,192.2	1,191.2	1,191.8	1,191.7
	Tetra-NA$_2$-Ac$_1$ (or Tri-Lac$_1$-NA$_2$-Ac$_1$)	4-	1,201.9	1,202.7			1,201.6*
	Tetra-NA$_2$-Ac$_2$ (or Tri-Lac$_1$-NA$_2$-Ac$_2$)	4-	1,212.4	1,213.2			1,213.0
	Tetra-NA$_4$-Ac$_2$[9)]	4-	1,358.0	1,358.8		1,358.0*	
B12	Tetra-NA$_2$ (or Tri-Lac$_1$-NA$_2$)	4-	1,191.4	1,192.2	1,191.3*	1,192.1	
	Tetra-NA$_2$-Ac$_1$ (or Tri-Lac$_1$-NA$_2$-Ac$_1$)	4-	1,201.9	1,202.7			1,202.0
	Tetra-NA$_2$-Ac$_2$ (or Tri-Lac$_1$-NA$_2$ -Ac$_2$)	4-	1,212.4	1,213.2			1,212.3*
	Tri-NA$_3$-Ac$_1$	4-	1,183.4	1,184.1		1,183.7*	
	Tetra-Lac$_1$-NA$_2$ (or Tri-Lac$_2$-NA$_2$)	4-	1,282.7	1,283.5	1,282.8		
	Tetra-Lac$_1$-NA$_2$-Ac$_2$ (or Tri-Lac$_2$-NA$_2$-Ac$_2$)	4-	1,303.7	1,304.5			1,303.5
	Tetra-Lac$_2$-NA$_2$	4-	1,374.0	1,374.8	1,374.2		
	Tetra-Lac$_3$-NA$_2$	4-	1,465.3	1,466.2	1,465.8		
B13	Bi-NA$_2$	3-	1,345.5	1,346.3	1,345.8*	1,345.4*	1,345.9*
	Bi-NA$_2$-Ac$_1$	3-	1,359.5	1,360.3			1,359.8
B14	Tri-NA$_2$[10)]	4-	1,100.2	1,100.8	1,100.0*	1,100.8*	1,099.9
	Tri-NA$_2$-Ac$_1$	4-	1,110.7	1,111.3			1,110.7*
	Tri-NA$_3$-Ac$_2$	4-	1,193.9	1,194.7		1,194.5	
B15	Bi-NA$_2$-Ac$_1$	3-	1,359.5	1,360.3		1,359.3*	
	Tri-NA$_3$-Ac$_2$	4-	1,193.9	1,194.7		1,194.5	
B16	Bi-NA$_1$	3-	1,248.5	1,249.2	1,248.3*	1,248.2*	1,248.3
	Bi-NA$_1$-Ac$_1$	3-	1,262.5	1,263.2			1,262.8*
	Tri-NA$_3$-Ac$_2$	4-	1,193.9	1,194.7		1,193.8	
B17	Bi-NA$_2$-Ac$_2$	3-	1,373.5	1,374.3		1,374.0*	
	Tri-NA$_1$-Ac$_2$	3-	1,398.2	1,399.0			1,398.1*

All carbohydrates contain a fucosylated core. *, base peak in the mass spectra of each HPLC peak in Fig. 2B; Mono, monoisotopic; Bi, bianntenary; Tri, trianntenary; Tetra, tetraanntenary; NA, N-acetylneuramic acid; Lac, N-acetyllactosamine; Ac, acetyl; SO, sulphate; 1) and 5), 2) and 6), 3) and 7), 4) and 9), 8) and 10), isomer .

Table 10 Peak number, glycoforms and theoretical and observed m/z of *N*83 glycopeptides in EPO-A, B and C digests.

Peak	Glycoform	Charge state	Calculated m/z		Observed m/z		
			Mono	Average	EPO-A	EPO-B	EPO-C
D1	Tetra-Lac2-NA4-SO2	5-	1,443.6	1,444.4			1443.9*
	Tetra-Lac2-NA3-SO2	5-	1,385.4	1,386.2			1,386.3
D2	Tetra-NA4-SO2	4-	1,622.2	1,623.1			1622.6*
	Tetra-Lac1-NA4-SO2	4-	1,713.4	1,714.4			1,714.5
	Tetra-NA3-SO2	4-	1,549.4	1,550.3			1,549.7
D3	Tetra-Lac1-NA4-SO1	4-	1,693.4	1,694.4			1694.1*
	Tetra-Lac1-NA4-SO1-Gc1	4-	1,697.4	1,698.4			1,698.6
	Tetra-Lac2-NA4-SO1	4-	1,784.7	1,785.8			1,784.7
	Tetra-Lac2-NA4-SO1-Ac1	4-	1,795.2	1,796.3			1,795.1
	Tetra-Lac2-NA4-SO1-Gc1	4-	1,788.7	1,789.8			1,788.8
	Tetra-Lac3-NA4-SO1	5-	1,500.6	1,501.5			1,501.0
	Tetra-Lac1-NA3-SO1	4-	1,620.7	1,621.6			1,620.8
	Tetra-Lac2-NA3-SO1	4-	1,712.0	1,713.0			1,712.6
D4	Tetra-NA4-SO1	4-	1,602.2	1,603.1			1602.7*
	Tetra-NA4-SO1-Ac1	4-	1,612.7	1,613.6			1,612.9
	Tetra-NA4-SO1-Gc1	4-	1,606.2	1,607.1			1,607.1
	Tetra-Lac1-NA4-SO1	4-	1,693.4	1,694.4			1,694.1
	Tetra-NA3-SO1	4-	1,529.4	1,530.3			1,530.3
D5	Tetra-Lac2-NA4	4-	1,764.7	1,765.8	1764.8*	1764.9*	1765.4*
	Tetra-Lac2-NA4-Ac1	4-	1,775.2	1,776.3	1,775.3	1,775.3	
	Tetra-Lac2-NA4-Ac2	4-	1,785.7	1,786.8		1,785.9	
	Tetra-Lac2-NA4-Ac3	4-	1,796.2	1,797.3		1,797.2	
	Tetra-Lac2-NA4-Ac4	4-	1,806.8	1,807.8		1,807.3	
	Tetra-Lac2-NA4-Gc1	4-	1,768.7	1,769.8	1,769.7	1,768.7	1,769.1
	Tetra-Lac1-NA4	4-	1,673.5	1,674.4	1,673.6	1,674.1	1673.6*
	Tetra-Lac1-NA4-Ac1	4-	1,684.0	1,684.9			1,684.3
	Tetra-Lac1-NA4-Gc1	4-	1,677.5	1,678.4			1,677.9
	Tetra-Lac3-NA4	4-	1,856.0	1,857.1	1,856.5	1,856.2	1,856.7
	Tetra-Lac3-NA4-Ac1	4-	1,866.5	1,867.6		1,866.7	
	Tetra-Lac4-NA4	4-	1,947.3	1,948.4	1,947.7		1,947.8
D6	Tetra-NA4	4-	1,582.2	1,583.1	1582.6*	1582.9*	1582.8*
	Tetra-NA4-Ac1	4-	1,592.7	1,593.6	1,593.5	1,593.7	1,592.8
	Tetra-NA4-Ac2	4-	1,603.2	1,604.1		1,603.9	
	Tetra-NA4-Ac3	4-	1,613.7	1,614.6		1,613.9	
	Tetra-NA4-Ac4	4-	1,624.2	1,625.1		1,624.4	
	Tetra-NA4-Ac5	4-	1,634.7	1,635.6		1,635.4	
	Tetra-NA4-Ac6	4-	1,645.2	1,646.2		1,645.6	
	Tetra-NA4-Ac7	4-	1,655.7	1,656.7		1,656.3	
	Tetra-NA4-Ac8	4-	1,666.2	1,667.2		1,667.1	
	Tetra-NA4-Gc1	4-	1,586.2	1,587.1	1,586.9	1,587.2	1,586.7
	Tetra-NA4-Ac1-Gc1	4-	1,596.7	1,597.6		1,598.0	
	Tetra-Lac1-NA4	4-	1,673.5	1,674.4	1,674.1	1,673.4	1,673.7
	Tetra-Lac1-NA4-Ac1	4-	1,684.0	1,684.9	1,684.6	1,684.0	
	Tetra-Lac1-NA4-Ac2	4-	1,694.5	1,695.4		1,694.4	
	Tetra-Lac1-NA4-Ac3	4-	1,705.0	1,706.0		1,705.2	
	Tetra-Lac1-NA4-Ac4	4-	1,715.5	1,716.5		1,715.8	
	Tetra-Lac1-NA4-Ac5	4-	1,726.0	1,727.0		1,726.8	
	Tetra-Lac1-NA4-Ac6	4-	1,736.5	1,737.5		1,737.0	
	Tetra-Lac1-NA4-Ac7	4-	1,747.0	1,748.0		1,747.7	
	Tetra-Lac1-NA4-Gc1	4-	1,677.5	1,678.4		1,677.7	
	Tetra-Lac1-NA4-Ac1-Gc1	4-	1,688.0	1,688.9		1,687.6	
	Tetra-Lac2-NA4	4-	1,764.7	1,765.8	1,764.7	1,765.4	1,764.9
D7	Tetra-Lac1-NA3 (or Tri-Lac2-NA3)	4-	1,600.7	1,601.6	1601.2*	1601.0*	1600.9*
	Tetra-Lac1-NA3-Ac1 (or Tri-Lac2-NA3-Ac1)	4-	1,611.2	1,612.1	1,612.1	1,611.7	1,611.3
	Tetra-Lac1-NA3-Ac2 (or Tri-Lac2-NA3-Ac2)	4-	1,621.7	1,622.6		1,622.2	1,621.7
	Tetra-Lac1-NA3-Ac3(or Tri-Lac2-NA3-Ac3)	4-	1,632.2	1,633.1		1,632.4	1,632.4
	Tetra-Lac1-NA3-Gc1	4-	1,604.7	1,605.6		1,604.9	1,604.8
	Tetra-Lac2-NA3	4-	1,692.0	1,692.9	1692.0*	1,692.4	1,691.8
	Tetra-Lac2-NA3-Ac1	4-	1,702.5	1,703.5	1,703.0	1,702.9	1,702.4
	Tetra-Lac2-NA3-Ac2	4-	1,713.0	1,714.0		1,713.5	1,713.1
	Tetra-Lac2-NA3-Ac3	4-	1,723.5	1,724.5		1,723.7	1,723.7
	Tetra-Lac2-NA3-Gc1	4-	1,696.0	1,696.9			1,696.0
	Tetra-Lac3-NA3	4-	1,783.2	1,784.3	1,784.4	1,784.0	1,784.1
	Tetra-Lac3-NA3-Ac1	4-	1,793.8	1,794.8		1,794.5	1,794.7
	Tetra-Lac3-NA3-Ac2	4-	1,804.3	1,805.3		1,805.1	

Peak	Glycoform	Charge state	Mono	Average	A	B	C
	Tetra-Lac4-NA3	4-	1,874.5	1,875.6	1,875.1		1,874.8
D8	Tetra-NA3 (or Tri-Lac1-NA3)	4-	1,509.4	1,510.3	1509.9*	1509.5*	1509.5*
	Tetra-NA3-Ac1 (or Tri-Lac1-NA3-Ac1)	4-	1,519.9	1,520.8	1,520.1	1,520.0	1,519.7
	Tetra-NA3-Ac2 (or Tri-Lac1-NA3-Ac2)	4-	1,530.4	1,531.3		1,530.7	
	Tetra-NA3-Ac3 (or Tri-Lac1-NA3-Ac3)	4-	1,540.9	1,541.8		1,541.5	
	Tetra-NA3-Ac4 (or Tri-Lac1-NA3-Ac4)	4-	1,551.4	1,552.3		1,552.4	
	Tetra-NA3-Ac5 (or Tri-Lac1-NA3-Ac5)	4-	1,561.9	1,562.8		1,561.9	
	Tetra-NA3-Gc1 (or Tri-Lac1-NA3-Gc1)	4-	1,513.4	1,514.3		1,513.1	1,514.1
	Tetra-NA3-Ac1-Gc1 (or Tri-Lac1-NA3-Ac1-Gc1)	4-	1,523.9	1,524.8		1,524.2	
	Tetra-Lac1-NA3 (or Tri-Lac2-NA3)	4-	1,600.7	1,601.6	1,601.7	1,600.8	1,601.1
	Tetra-Lac1-NA3-Ac1 (or Tri-Lac2-NA3-Ac1)	4-	1,611.2	1,612.1	1,611.7	1,611.7	1,611.9
	Tetra-Lac1-NA3-Ac2 (or Tri-Lac2-NA3-Ac2)	4-	1,621.7	1,622.6		1,622.2	
	Tetra-Lac1-NA3-Ac3(or Tri-Lac2-NA3-Ac3)	4-	1,632.2	1,633.1		1,632.4	
	Tri-NA3	4-	1,418.1	1,418.9	1,418.8	1,418.7	1,418.6
	Tri-NA3-Ac1	4-	1,428.6	1,429.5		1,429.4	
	Tri-NA3-Ac2	4-	1,439.1	1,440.0		1,439.5	
	Tri-NA3-Ac3	4-	1,449.6	1,450.5		1,450.0	
	Tri-NA3-Ac4	4-	1,460.1	1,461.0		1,461.0	
D9	Tetra-NA2 (or Tri-Lac1-NA2)	4-	1,436.6	1,437.5	1437.0*	1437.4*	1,436.7
	Tetra-NA2-Ac1 (or Tri-Lac1-NA2-Ac1)	4-	1,447.1	1,448.0	1,447.5	1,448.0	1,447.4
	Tetra-NA2-Ac2 (or Tri-Lac1-NA2-Ac2)	4-	1,457.6	1,458.5		1,458.8	1,457.6
	Tri-NA2	4-	1,345.3	1,346.1	1,345.4	1,345.4	1,345.2
	Tri-NA2-Ac1	4-	1,355.8	1,356.6		1,355.8	1,356.0
	Tetra-Lac1-NA2 (or Tri-Lac2-NA2)	4-	1,527.9	1,528.8	1,527.8	1,528.9	1,527.5
	Tetra-Lac1-NA2-Ac1 (or Tri-Lac2-NA2-Ac1)	4-	1,538.4	1,539.3		1,539.3	1,538.5
	Tetra-Lac1-NA2-Ac2 (or Tri-Lac2-NA2-Ac2)	4-	1,548.9	1,549.8			1,549.6
	Tetra-Lac2-NA2	4-	1,619.2	1,620.1	1,620.5	1,619.9	1,620.4
	Bi-NA2	4-	1,254.1	1,254.8			1254.8*

All carbohydrates contain fucosylated core. *, base peak in the mass spectra of each peak in Fig. 2D; Mono, monoisotopic; Bi, bianntenary; Tri, trianntenary; Tetra, tetraanntenary; NA, N-acetylneuramic acid, Lac, *N*-acetyllactosamine; Ac, acetyl; Gc, glycolyl; SO, sulphate.

Table 11 Peak number, glycoforms, and theoretical and observed m/z of O126 glycopeptides in EPO-A, B and C digest

Peak	Glycoform	Charge state	Theoretical m/z		Observed m/z		
			Mono	Average	A	B	C
C1	NA2-Gal-GalNAc	2-	1,391.1	1,391.9	1,391.1	1,392.0	1,391.5
C2	NA-Gal-GalNAc	2-	1,245.6	1,246.3	1,246.0	1,245.6	1,245.7

Peak C1 and C2 was shown in Fig. 1. NA, N-acetylneuramic acid; Mono, monoisotopic.

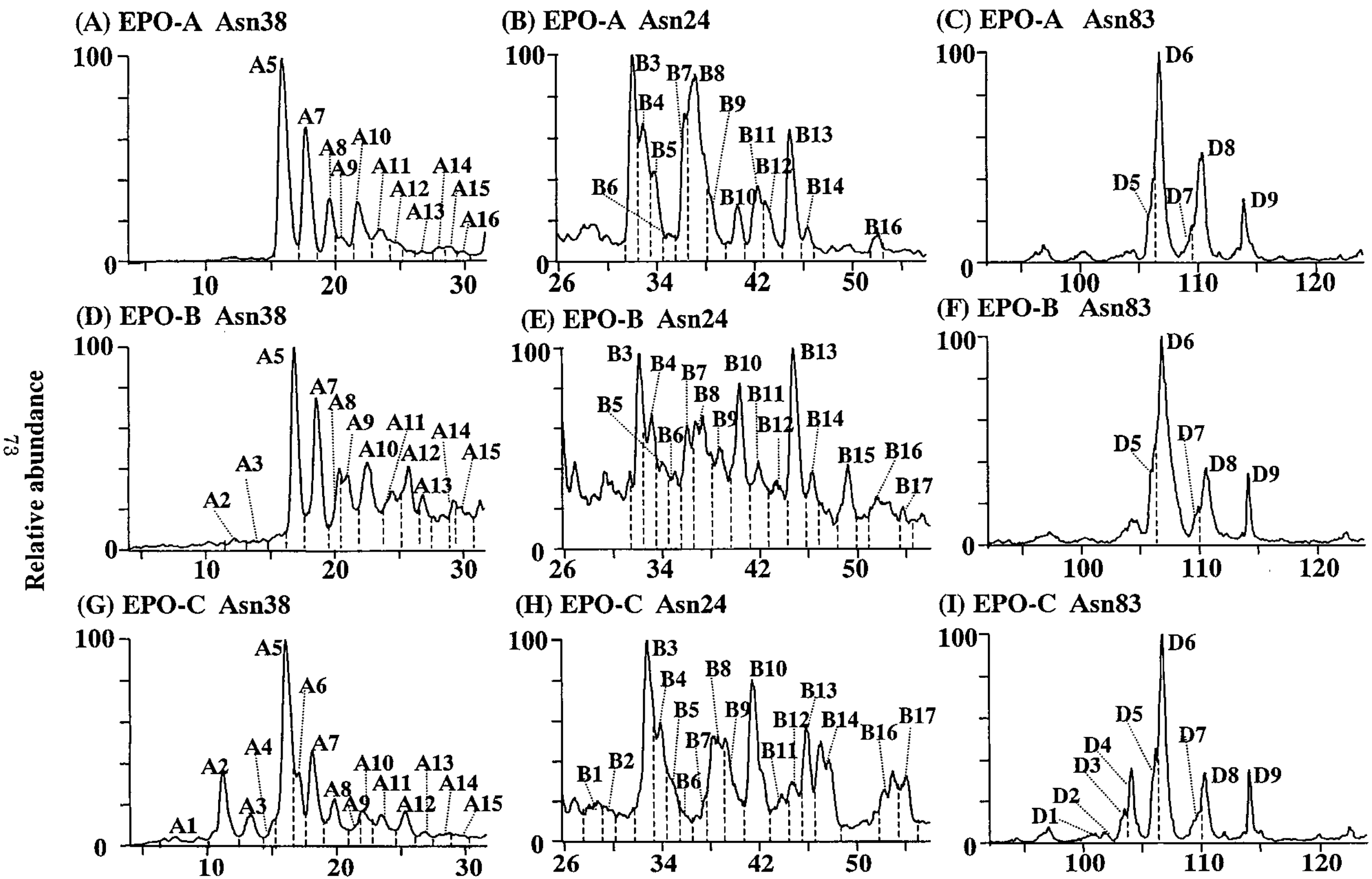

Fig.17 Expanded views of glycopeptide maps of N38, N24, and N83 glycopeptides in EPO-A, -B, and -C

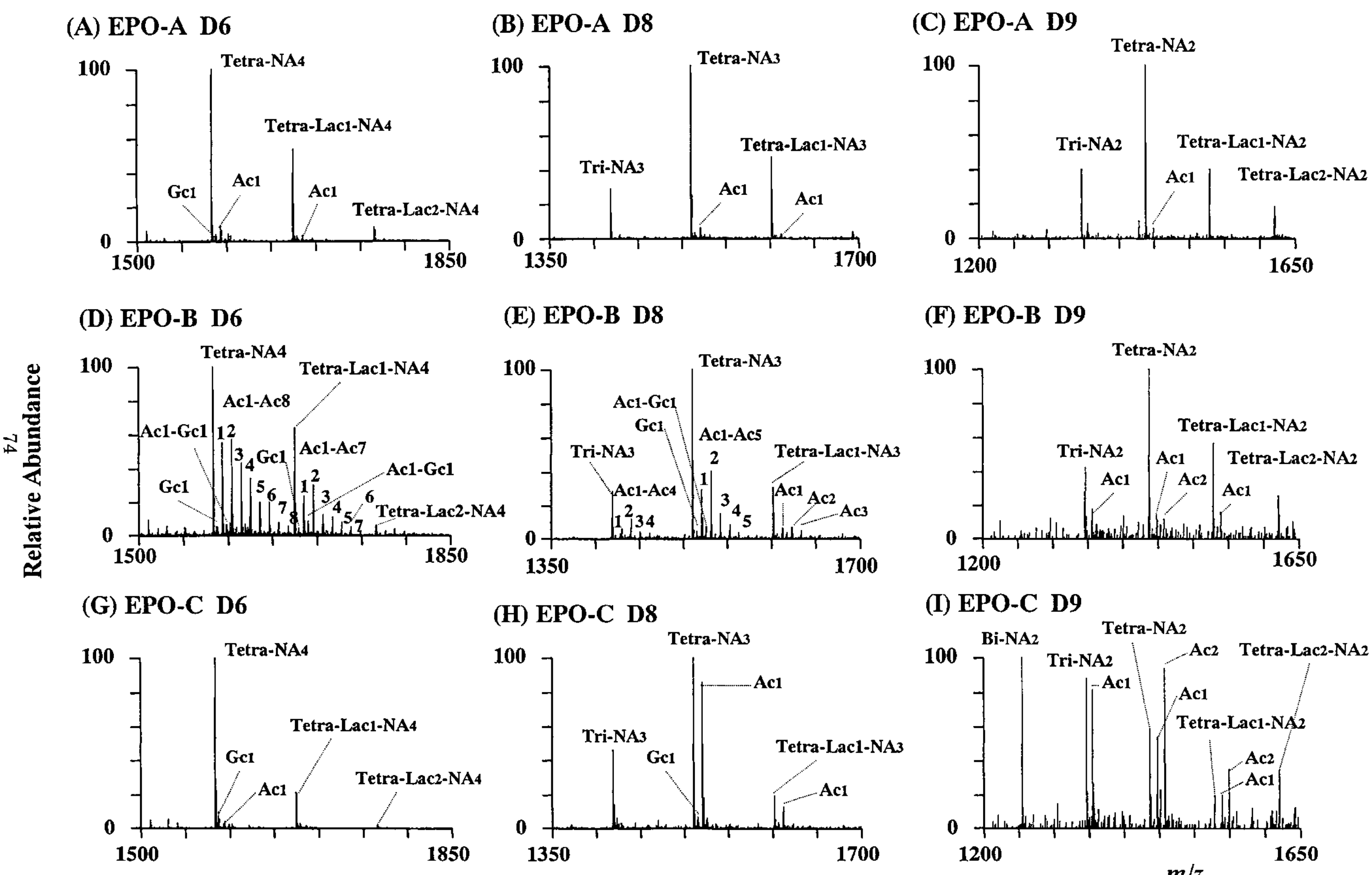

Fig. 18 Mass spectra of peaks D6, D8, and D9 in Fig. 17

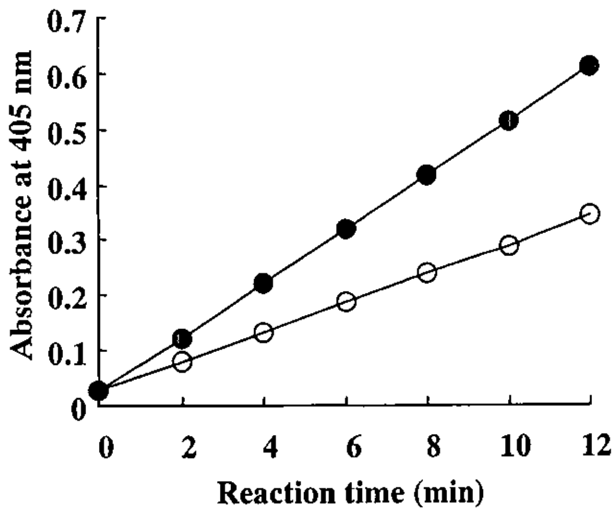

Fig.19 Time course of formation of *p*-nitroaniline from S-2366 by uh-TM in the standard method.
The uh-TM reference material was used in the experiment at concentrations of 2 uh-TM units/ml (○) and 4 uh-TM units/ml (●).

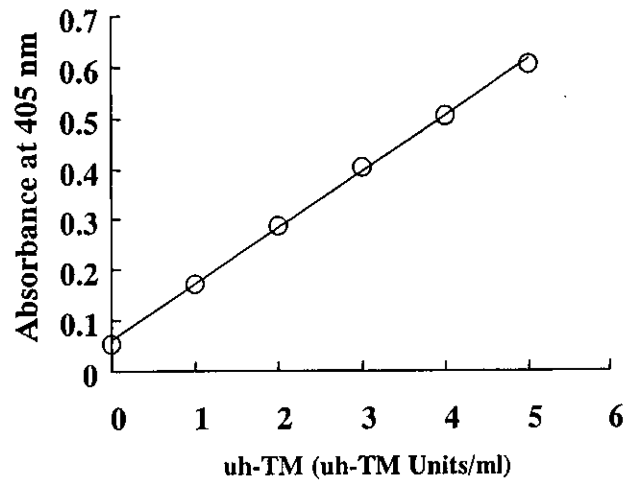

Fig. 20 Dose-response curve of uh-TM in the standard method.
The activity of uh-TM is expressed in the specific units (uh-TM units) of the original method of measurement.

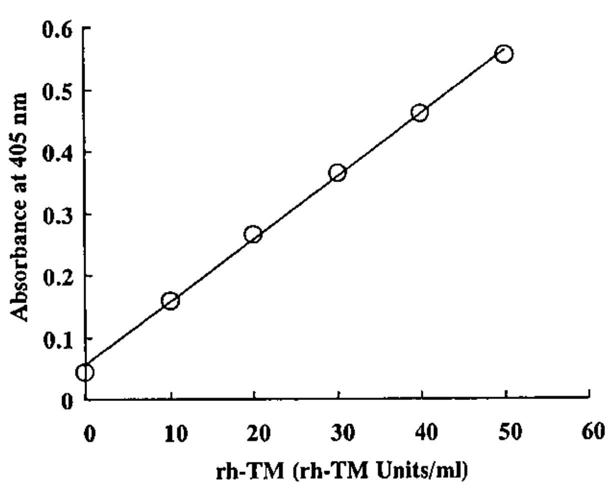

Fig.21 Dose-response curve of rh-TM in the standard method.
The activity of rh-TM in the standard method. The activity of rh-TM is expressed in the specific units (rh-TM units) of the original method of measurements.

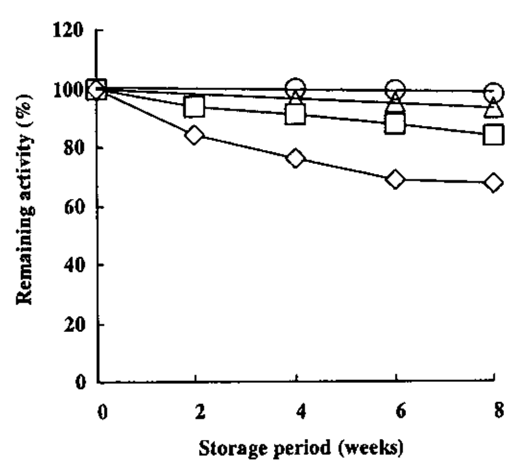

Fig.22 Effect of heat treatment on the stability of the Japanese reference standard for h-TM.
The Japanese reference standard was stored at 40℃ (○),60℃(△),70℃(□), and 80℃(◇) for various periods.

Table 12 Intra-batch precision of h-thrombin in measurements of the activity of the uh-TM reference material by the standard method

h-thrombin (Lot no.)	uh-TM activity (uh-TM Units/vial)	
A	98.6	Average=99.0
B	102	S.D.=2.87
C	96.3	CV (%)=2.90

h-Protein C (Lot F) was used in the experiment.

Table 13 Intra-batch precision of h-protein C in measurements of the activity of the uh-TM reference material by the standard method

h-protein C (Lot no.)	uh-TM activity (uh-TM Units/vial)	
D	112	Average=107
E	109	S.D.=7.03
F	98.6	CV (%)=6.57

h-Thrombin (Lot A) was in the experiment.

Table 15 Analysis of variance

Factor	Square sum (S)		Degree of Freedom (ψ)		Variance (V)	
Concentration	S_{conc}	3096.35	ψ_{conc}	4	Vconc	774.09
Laboratory	S_R	9953.01	ψ_R	10	V_R	995.30
Experiment	S_{RW}	1225.1	ψ_{RW}	30	V_{RW}	40.84
Residual	S_r	642.26	ψ_r	90	V_r	7.14
Total	S_T	14916.72	ψ_T	134		

Various parameters were calculated from the results shown in Table 3. Similarly, intra-sample pecision, inter-day precision, and inter-laboratory precision (CV) were calculated to be 1.30%, 1.63% and 5.02%, respectively, from the variance values. The confidence interval of σ_{IM}, intermediate precision (standard deviation) with 95% confidence coefficient, was calculated to be $8.0 \leqq \sigma_{IM} \leqq 18.4$.

Table 14 Measurement of the activity of the Japanese reference standard for h-TM by the standard method

Dilution factor	Laboratory	Exp. 1	Exp. 2	Exp. 3
x 200	A	223.3	231.7	218.3
		218.3	235.0	226.7
		226.7	235.0	218.3
	B	203.3	211.7	208.3
		201.7	216.7	210.0
		198.3	213.3	205.0
	C	210.0	206.7	203.3
		203.3	206.7	203.3
		205.0	208.3	200.0
x 100	A	219.2	220.8	211.7
		216.7	219.2	220.8
		225.0	222.5	225.8
	B	195.8	205.8	201.7
		195.8	207.5	207.5
		198.3	208.3	196.7
	C	202.5	202.5	197.5
		197.5	205.8	200.8
		200.8	210.0	198.3
x 66.7	A	218.4	212.9	215.1
		217.3	216.2	216.2
		221.8	218.4	220.1
	B	196.8	204.0	202.9
		197.9	205.1	197.9
		196.8	203.4	199.5
	C	199.5	200.7	194.0
		197.3	200.1	196.8
		196.2	199.5	197.3
x 50.0	A	215.0	211.3	213.8
		215.0	211.3	213.8
		212.9	218.8	210.0
		214.6	210.8	215.8
	B	195.0	201.7	202.5
		195.0	202.5	196.7
		190.8	201.3	200.0
	C	195.0	197.5	191.7
		193.8	197.1	195.4
		193.3	197.9	191.7
x 40.0	A	213.0	209.3	204.0
		210.3	214.0	208.0
		213.3	211.7	210.3
	B	189.3	195.7	196.0
		189.7	195.7	194.0
		190.3	195.3	196.7
	C	195.0	192.7	190.3
		191.3	195.0	192.0
		192.3	195.0	190.0
Average			205.4	