

FIGURE 1

## Fab glycosylation species

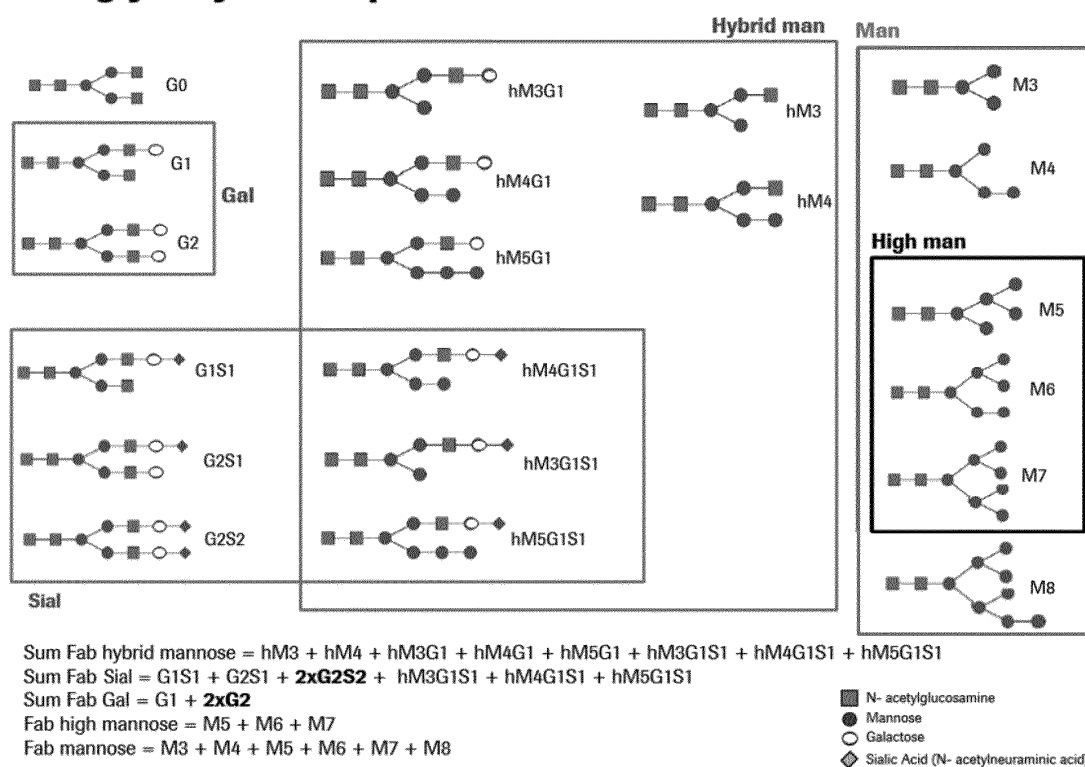


**Figure 1:** Fab Glycosylation Species - The species are summed up into several sum parameters as Sum Fab hybrid mannose (Hybrid man), Sum Fab Sial (Sial), Sum Fab Gal (Gal), Fab high mannose (High man) and Fab mannose (Man)

FIGURE 2

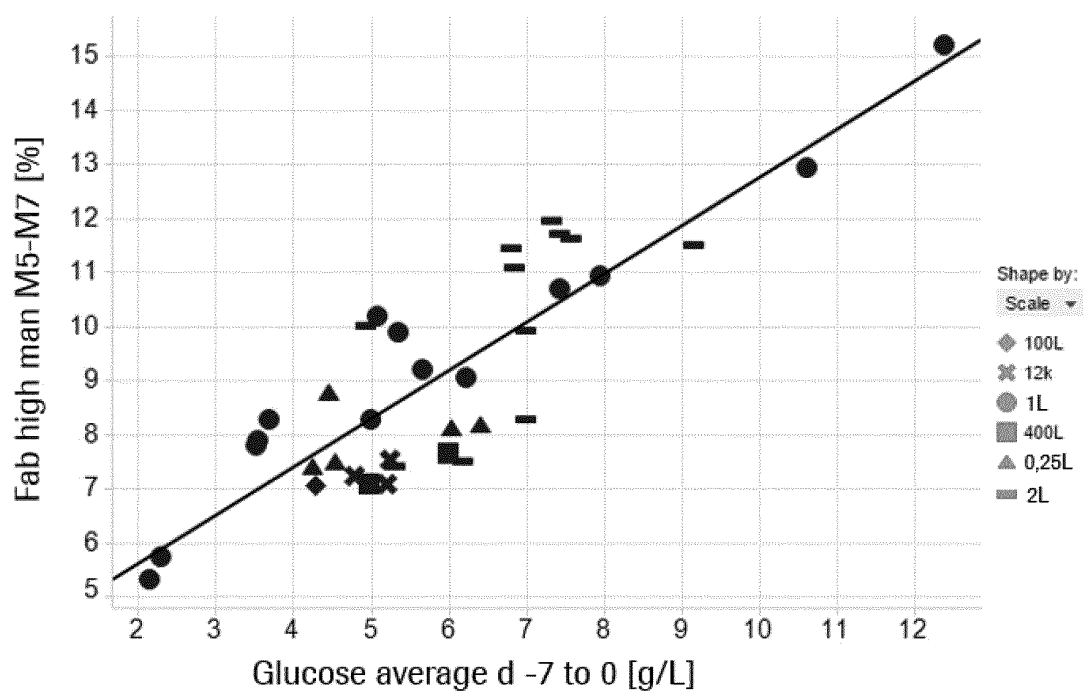


**Figure 2:** Fab high mannose in area% over average glucose concentration from day -7 to day 0 [g/L]. Available data from all representative runs of the given project shown (not only runs dedicated to glucose variations). Fermentation runs vary in scales, sites and minor process changes (e.g. agitation, aeration, cell bank, cell age). A glucose solution is added on demand via bolus or continuous addition. Analysis of the glycoforms from Protein A purified samples.

FIGURE 3A & 3B

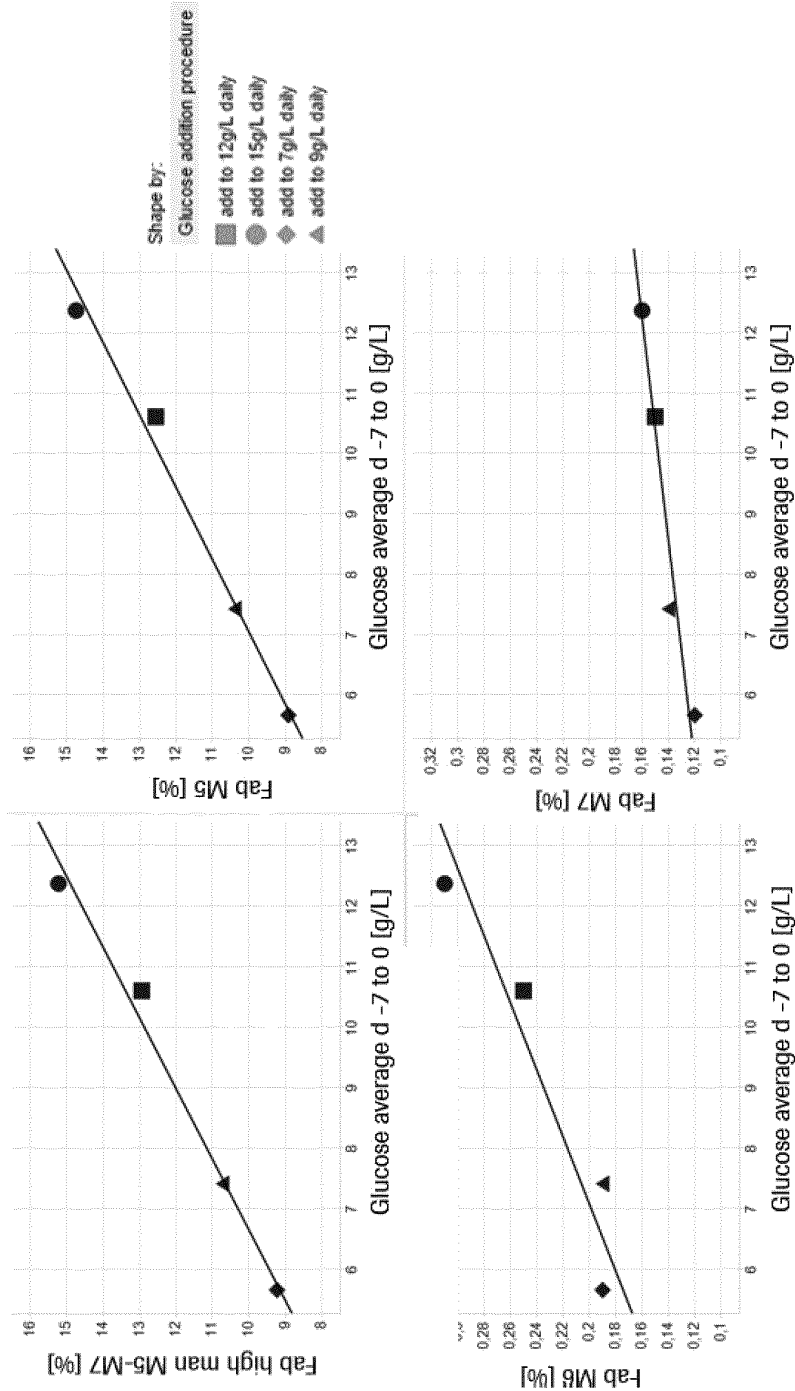


**Figure 3A:** Fab high mannose (M5-M7), Fab mannose 6 and Fab mannose 7 in area% over average glucose concentration from day -7 to day 0 [g/L]. Comparison of different glucose levels. One experimental setup dedicated for glucose variation, all other parameters are kept constant. Glucose is added on demand daily via bolus addition. Analysis of the glycoforms from Protein A purified samples.

FIGURE 3A &amp; 3B (CONT'D)

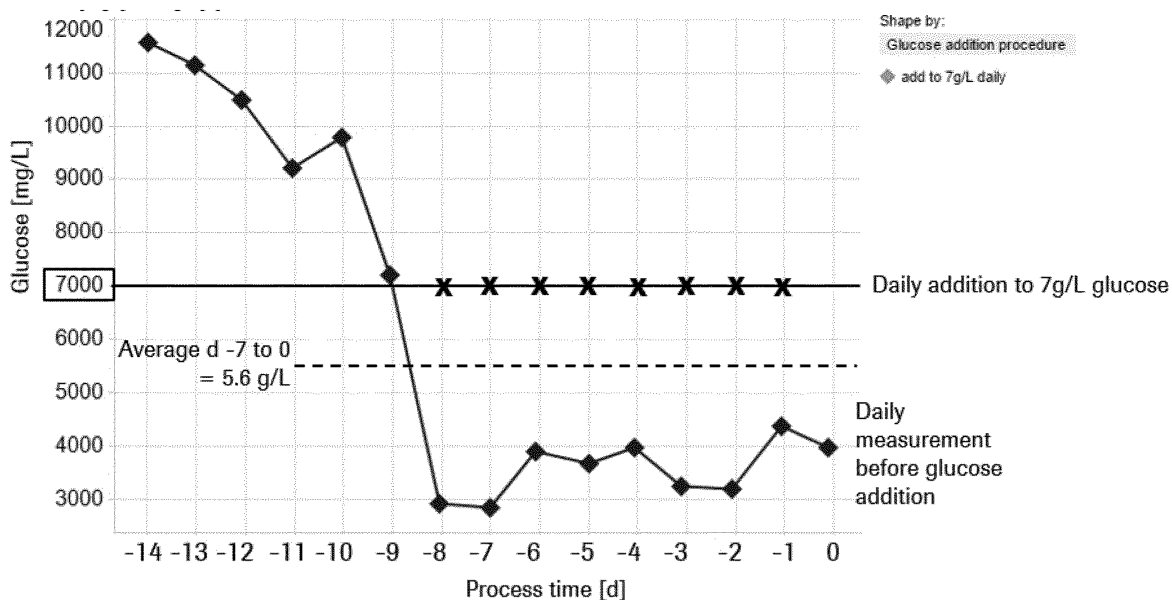


**Figure 3B:** Glucose concentration of daily samples (◆) are compared to the average concentration from day -7 to day 0 (dotted line ----) and shown over process time. Calculation is done with the described formula on the basis of the daily measured samples and the daily bolus addition (x). Daily samples were taken before the bolus addition.

FIGURE 4A & 4B

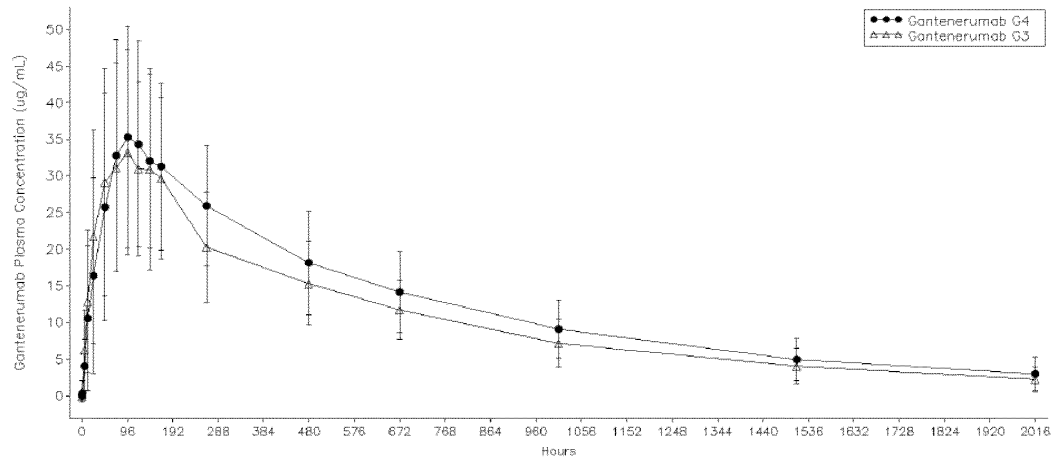


**Figure 4A:** Mean (± SD) Gantenerumab Plasma Concentrations vs Time by Treatment – Linear Scale – for Gantenerumab produced by G3 and G4 processes

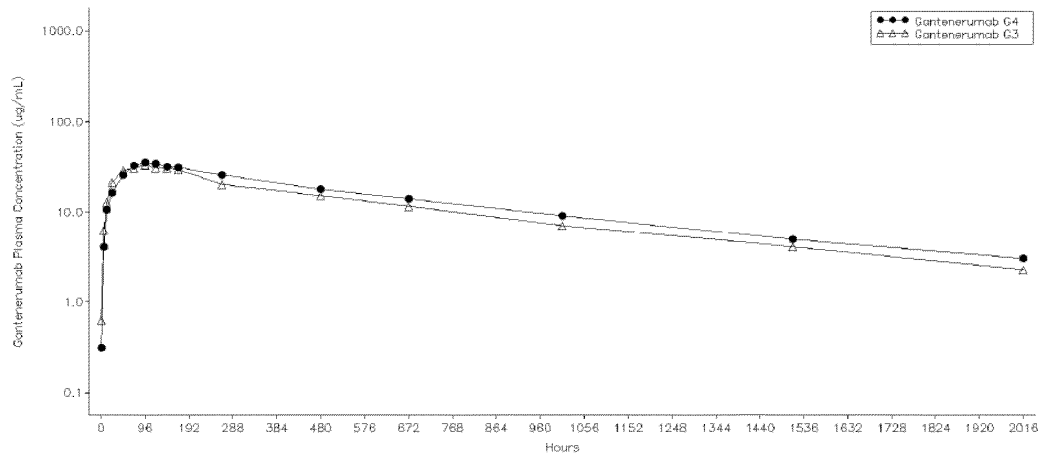


**Figure 4B:** Mean (± SD) Gantenerumab Plasma Concentrations vs Time by Treatment – Semi-log Scale – for Gantenerumab produced by G3 and G4 processes

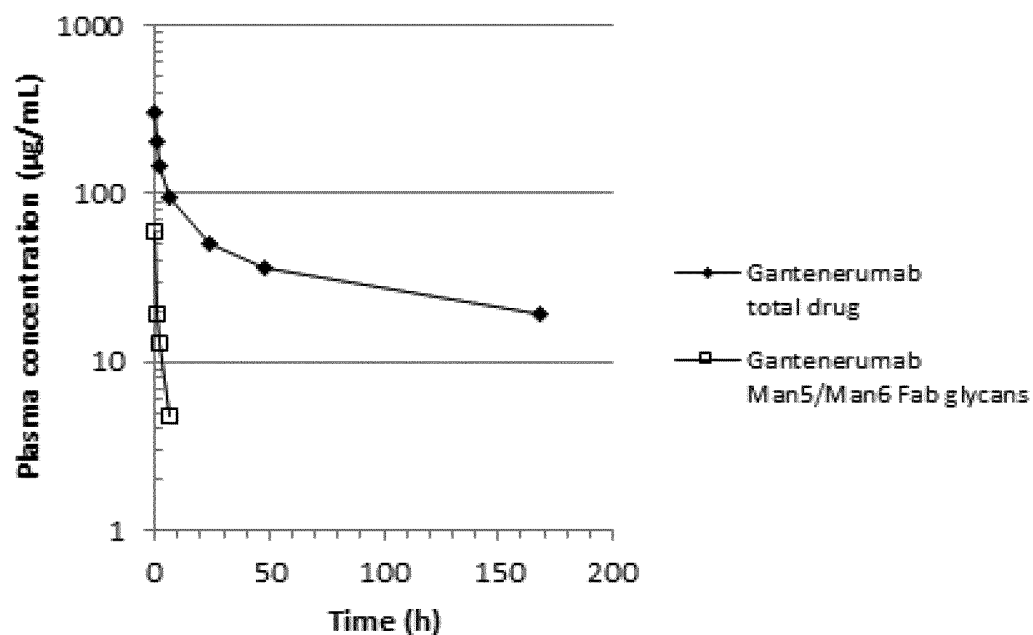
**FIGURE 5**

**Figure 5:** Plasma concentrations of total Gantenerumab and Gantenerumab with Man5/Man6 Fab glycans were determined following intravenous administration of Gantenerumab to rats (for determination of Gantenerumab Man5/Man6 Fab glycans plasma concentrations see Example 4 / Rat, intravenous Gantenerumab injection study 3)

FIGURE 6A & 6B

| %                |     |     |      |      |      |       |       |         |         |         |     |     |     |     |     |    |          |
|------------------|-----|-----|------|------|------|-------|-------|---------|---------|---------|-----|-----|-----|-----|-----|----|----------|
| Sample           | G1  | G2  | G1S1 | G2S1 | G2S2 | hM4G1 | hM5G1 | hM3G1S1 | hM4G1S1 | hM5G1S1 | M3  | M4  | M5  | M6  | M7  | M8 | Sum M5-8 |
| G4               |     |     |      |      |      |       |       |         |         |         |     |     |     |     |     |    |          |
| process-produced | 1.4 | 4.7 | 4.1  | 25.9 | 26.7 | 1.6   | 1.7   | 5.4     | 4.3     | 6.5     | 0.7 | 0.4 | 4.6 | 0.3 | 0.5 | nd | 5.4      |

Figure 6A: the % of each Gantenerumab glycoform in the product of the G4 process (data from bulk samples, i.e. fully purified)

| Glycoform        | Relative % in G4 process | Relative % in G3 process |
|------------------|--------------------------|--------------------------|
| Man5             | 4.6                      | 9.5                      |
| Man6             | 0.3                      | 2.5                      |
| Man7             | 0.5                      | 0.7                      |
| Man8             | nd                       | 0.1                      |
| Sum of Man5-Man8 | 5.4                      | 12.8                     |

Figure 6B: comparison of the concentration of mannose glycoforms produced in the G4 process and the G3 process materials (data from bulk samples, i.e. fully purified).

**FIGURE 7**

|                                                                            | Rat SC        |                | Rat IV             |                    | Rat IV         |                | Human sc       |                |
|----------------------------------------------------------------------------|---------------|----------------|--------------------|--------------------|----------------|----------------|----------------|----------------|
|                                                                            | G3            | G4             | G1                 | G2                 | G3             | G4             | G3             | G4             |
| Administration Route                                                       | single sc     | single sc      | single iv dose     | single iv dose     | single iv dose | single iv dose | single dose sc | single dose sc |
| Dose                                                                       | 20 mg/kg      | 20 mg/kg       | 6 mg/kg            | 6 mg/kg            | 20 mg/kg       | 21 mg/kg       | 600 mg/kg      | 600 mg/kg      |
| Fab Man5+Man6                                                              | 12%           | 5.1 %          | 3.1 %              | 10 %               | 12 %           | 4.9 %          | 12%            | 5.1 %          |
| Fab Man5-7                                                                 | 12.7 %        | 5.2%           | nd                 | nd                 | 12.7 %         | 5.4 %          | 12.7 %         | 5.2%           |
| Fab Man5-8                                                                 | 12.8%         | 5.3 %          | nd                 | nd                 | 12.8%          | n.a.           | 12.8%          | 5.3 %          |
| Animals per group                                                          | n=6           | n=6            | n=14               | n=14               | n=14           | n=13           | n=57           |                |
| AUC <sub>0-inf</sub> (mean +/-sd) [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ] | 7610 +/- 1080 | 17400 +/- 6140 | 5810 +/- 908       | 4670 +/- 297       | 22200 +/- 5330 | 33300 +/- 5690 | 18235.7        | 21476.1        |
| AUC <sub>0-672</sub> (mean +/-sd) [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ] | 7400 +/- 910  | 14500 +/- 3520 | 5360 +/- 684       | 4250 +/- 269       | 18100 +/- 3760 | 25900 +/- 3830 | nd             | nd             |
| Clearance (mean +/-sd) [ $\text{ml}/(\text{min}\cdot\text{kg})$ ]          | nd            | nd             | 0.0187 +/- 0.00292 | 0.0226 +/- 0.00145 | nd             | nd             | nd             | nd             |
| Clearance (mean +/-sd) [ $\text{ml}/(\text{day}\cdot\text{kg})$ ]          | nd            | nd             | nd                 | nd                 | 22.2 +/- 5.4   | 15.1 +/- 2.6   | nd             | nd             |
| V <sub>ss</sub> [ $\text{ml}/\text{kg}$ ]                                  | nd            | nd             | 240 +/- 33.3       | 336 +/- 35.5       | 329 +/- 59.9   | 255 +/- 18.6   | nd             | nd             |
| c <sub>max</sub> [ $\mu\text{g}/\text{ml}$ ]                               | 30.7 +/- 1.9  | 44.4 +/- 5.2   | 127 +/- 11.3       | 86.3 +/- 24.3      | 363 +/- 77.3   | 431 +/- 92.7   | 31.2           | 32.8           |

**Figure 7:** Pharmacokinetic data comparing results obtained with Gantenerumab produced according to the methods herein (G4 process: highmannose low) with Gantenerumab produced according to previous methods (G3 process: highmannose high, G1 and G2 processes).

Rot, 2010

FIGURE 8A & 8B  
pK study, rat G2 material, *Fc glycosylation*

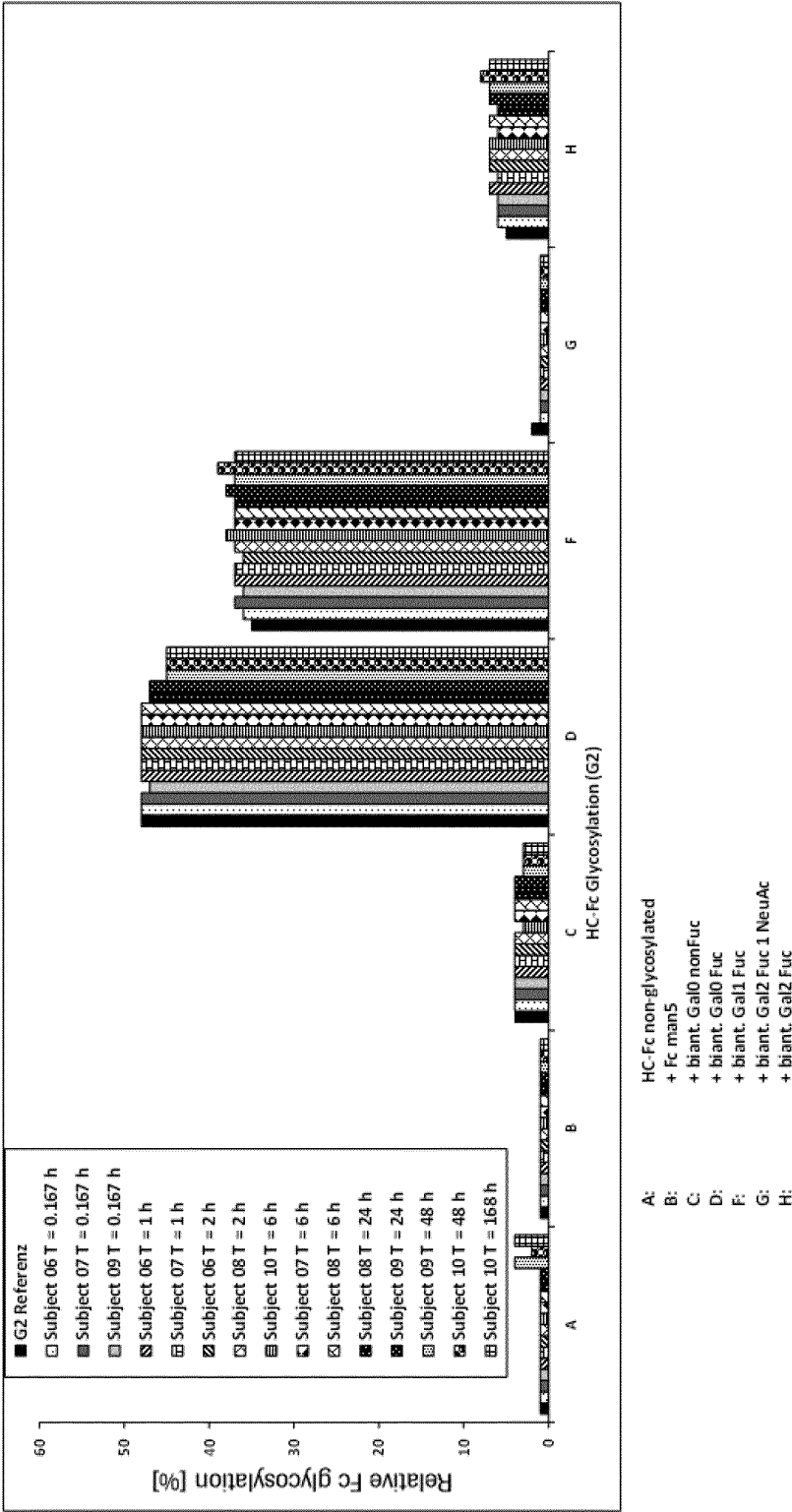


FIGURE 8A & 8B CONTINUED

pK study, rat G2 material, Fab glycosylation

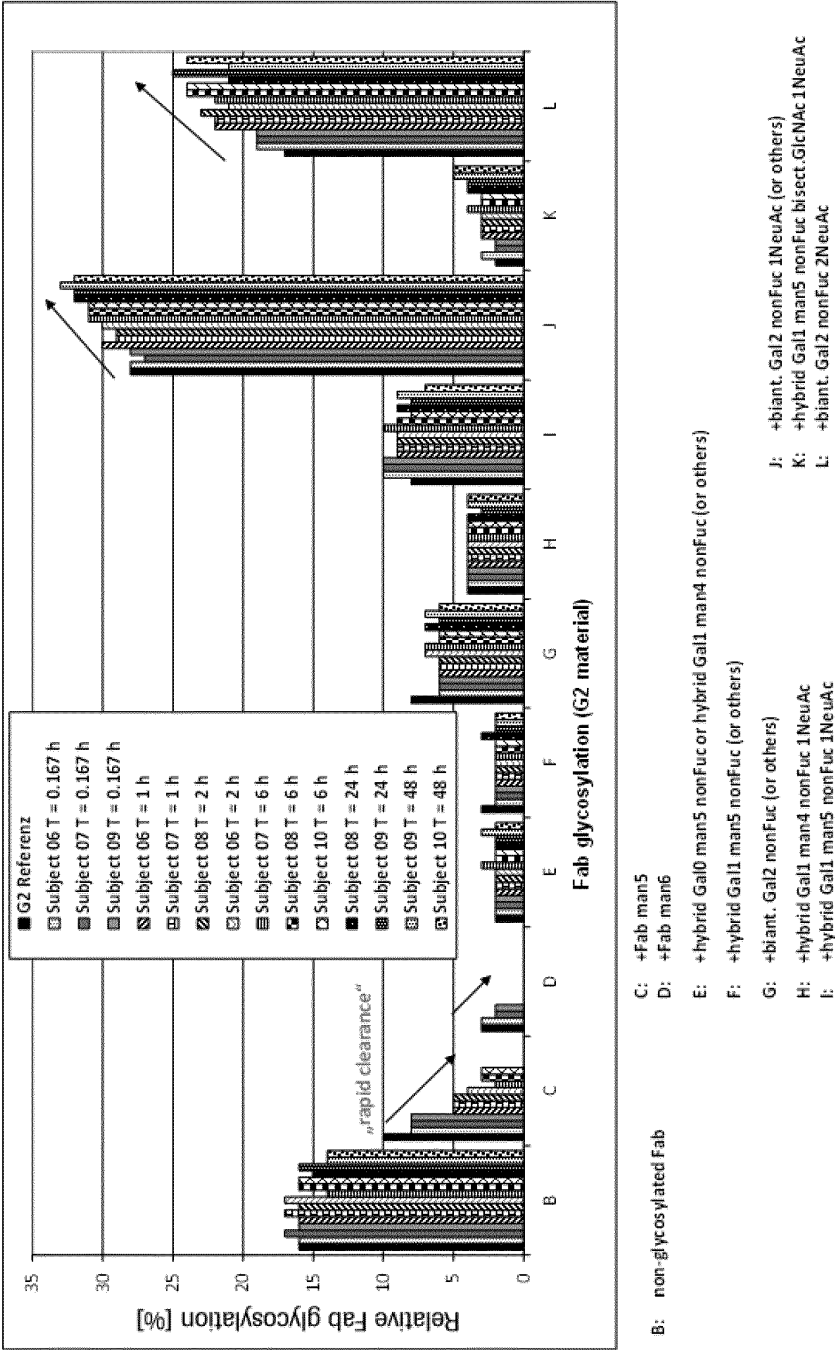


FIGURE 9

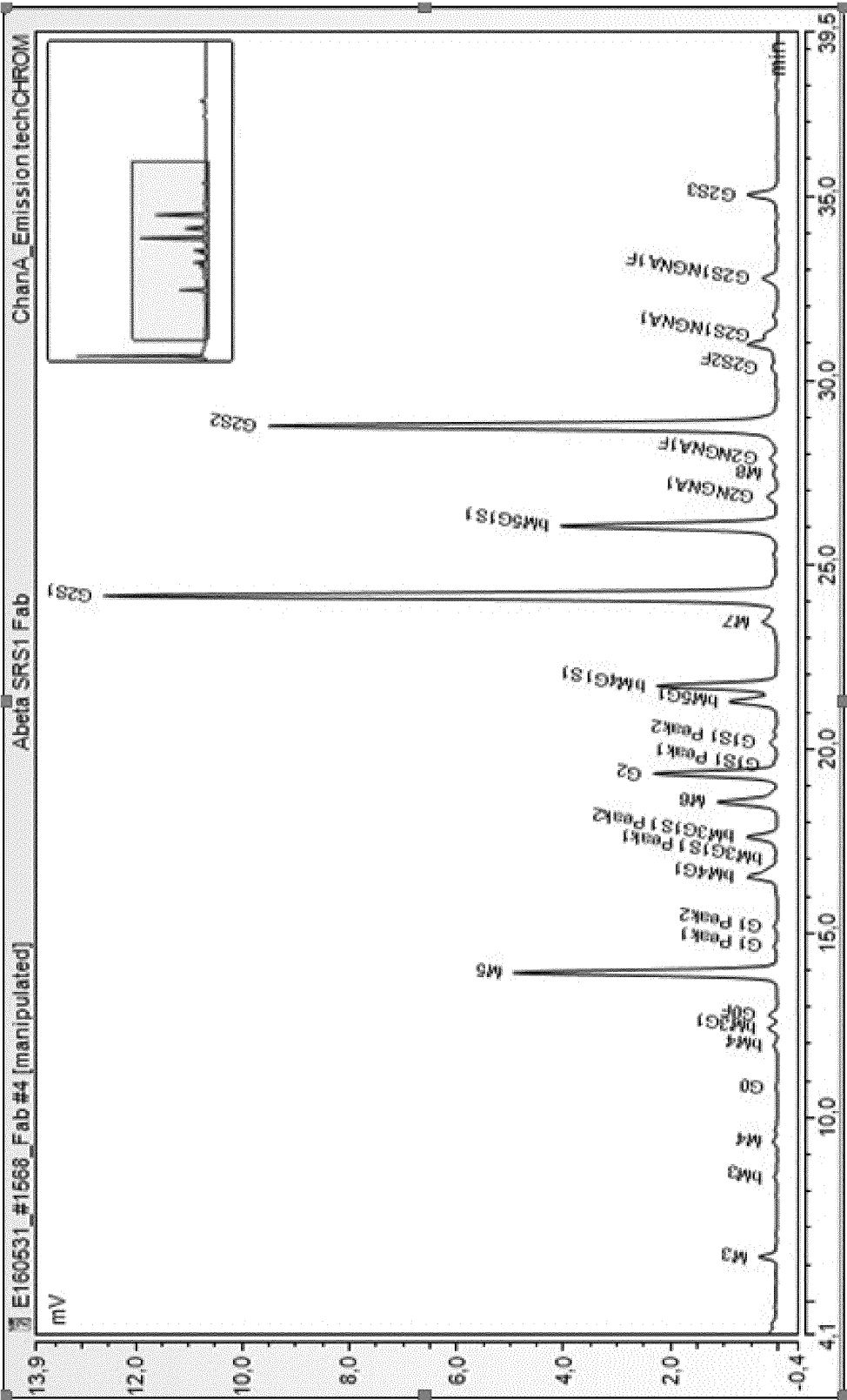


Figure 9: chromatogram showing elution time for Gantenerumab Fab glycoforms from which the percentage of glycoforms can be calculated