A rapid method for testing of long-term alcohol abuse based on IE-UHPLC

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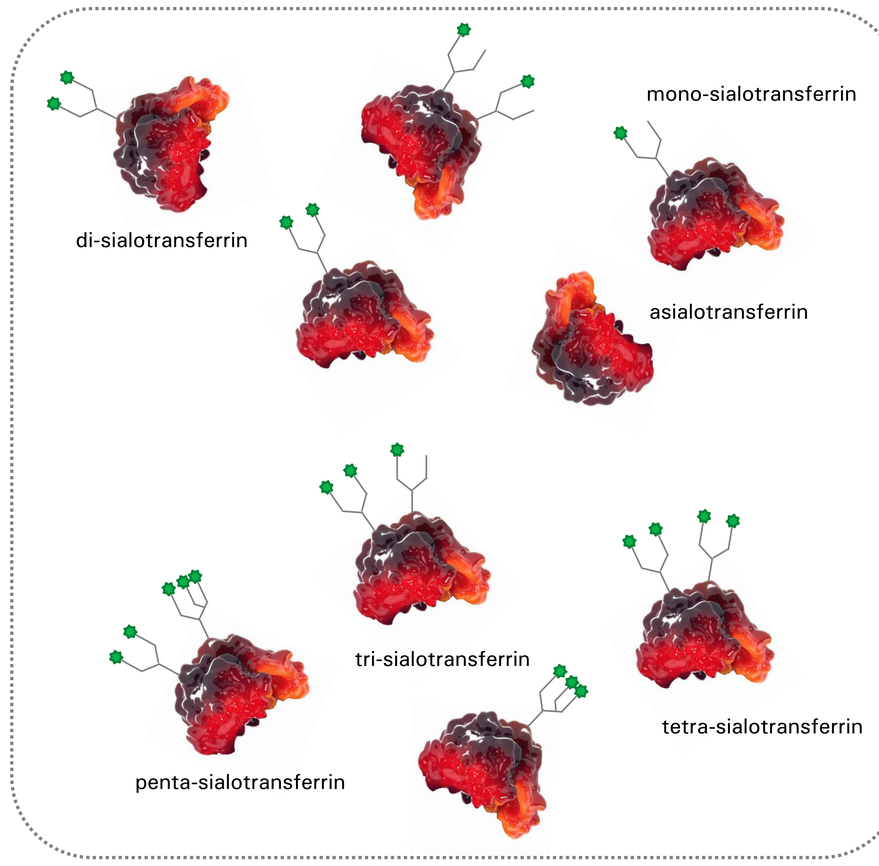
Ethanol abuse

Acetaldehyde, which is a metabolite of **ethanol**, inhibits the glycosyltransferases

Glycosylation of **Transferrin** occurs in the liver

glycosyltransferase



Sialytion variants

carbohydrate deficient transferrin (**CDT**)

The concentration of CDT in blood serum increases due to long-term alcohol abuse

tetra-sialotransferrin is the most abundant form in serum of a healthy donor

Protein glycans may contain sialinic acid as a negatively charged end-cap. Different sialytion variants of transferrin can be discriminted by AE-HPLC. Transferrin has 2 binding sites for iron ions and thus selectively absorbs UV-light at 460 nm.

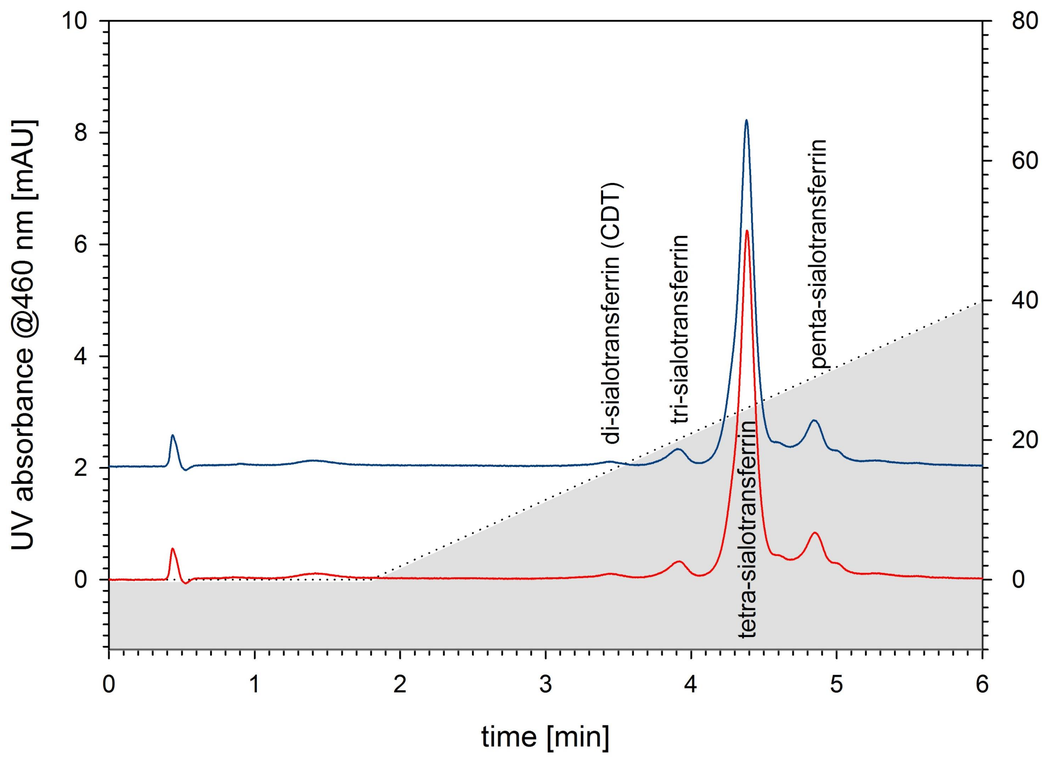
In 2003, Helander et al. published a method based on a Q-type PS-DVB stationary phase. Serum samples are iron-saturated with ferric nitrilotriacetic acid. Lipoproteins are precipitated with dextrane sulfate and calcium chloride. The different glycoforms of transferrin are separated within 30 min.

The relative amount of CDT compared to the total transferrin concentration in serum can be quantified by the area under the curve of the UV460 signal.

Transfer from **IE-HPLC** to **IE-UHPLC**

Material & Method

- TSKgel Q-STAT 4.6 mm ID x 10 cm L, Q-type ligands immobilized to a polymethacrylate backbone
- Buffer A: 50 mM Tris/HCl, pH 7.4
- Buffer B: 50 mM Tris/HCl + 250 mM ammonium acetate, pH 7.4
- Gradient: from 0-40 % B in 4.2 min
- Injection volume: 100 µl
- Flow rate: 1.0 mL/min
- Specific UV absorbance @460 nm
- Requires use of UHPLC or dead-volume optimized HPLC system



Conclusions

- ✓ Different sera samples from healthy donors could be analyzed with reproducible results
- ✓ Analysis time could be reduced from 30 min to 6 min.
- ✓ The separation is conducted with a simple linear gradient combining two buffers
- ✓ Similar or higher resolution of TSKgel Q-STAT compared to the original separation
- ✓ Fast mass-transfer due to non-porous particles

Literature

Helander A, Husa A, Jeppsson JO. Improved HPLC Method for Carbohydrate-deficient Transferrin in Serum. Clin Chem. 2003;49(11):1881–90.