TOSOH

Application Note

AN IE-UHPLC METHOD FOR TESTING OF LONG-TERM ALCOHOL ABUSE

Carbohydrate deficient transferrin can be quantified using TSKgel Q-STAT

TRANSFERRIN IS A BIOMARKER

Transferrin is one of the most abundant plasma proteins in humans. The majority of transferrin is produced in the liver. It has two binding sites for $Fe3^{+}$ ions and plays an important role in the metabolism of iron. In human plasma, different glycosylation forms of transferrin are present, which may carry sialinic acid end-caps. In healthy humans, tetrasialo-transferrin is the most abundant transferrin. Long-term alcohol abuse leads to inhibition of glycosyltransferases in the liver. As a consequence, the relative concentration of disialo-, monosialo-, and asioalo-transferrin increases. Thus, the relative abundance of carbohydrate deficient transferrin (CDT) is used as a biomarker. A simplified illustration of the different glycoforms of Transferrin is shown in Figure 1.

At neutral pH, sialinic acid is a charged residue, which allows separation of the different sialo forms of transferrin based on charge. Anion exchange chromatography is a well-established method to quantify the relative plasma concentration of CDT[1] With the published method, a single run can be accomplished within 30 minutes.

METHOD TRANSFER TO UHPLC

In the recent years, many routine applications have been transferred from HPLC to UHPLC. Adoption of UHPLC can improve a given separation and/or reduce the required analysis time. In biochromatography, the term UHPLC mainly refers to the use of low dead volume systems and small particles with excellent mass transfer properties. Based on UHPLC technology, separation of CDT from tetrasialo-transferrin could be accomplished in 6 min. Representative chromatograms of plasma samples from healthy donors are shown in Figure 2.

GLYCOSILATION OF TRANSFERRIN

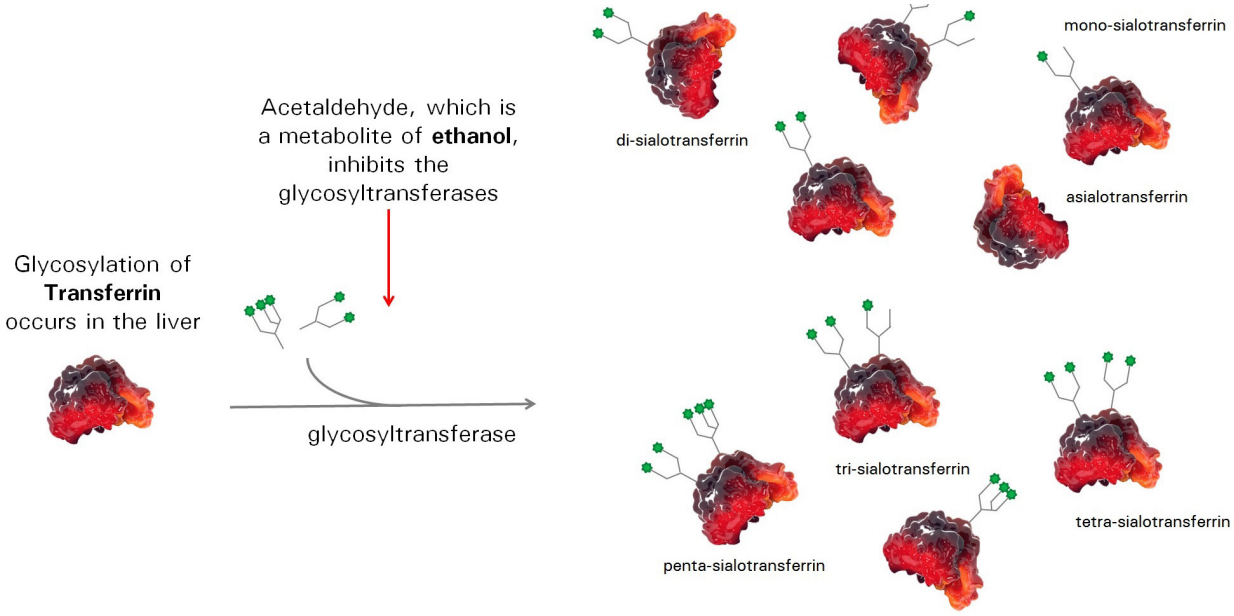

Figure 1

Glycosylation of Transferrin leads to various different glycosylated forms. The attached glycan structures may be end-capped by sialinic acid. The resulting charge variants can be distinguished by anion exchange chromatography.

SAMPLE PREP

The sample preparation includes a lipoprotein precipitation step and iron-saturation of transferrin. 4 mL of a 10 mM nitrilo triacetic acid tri-sodium + 10 mM iron-(III) chloride solution at pH 6.5 were mixed with 2 mL of a 1.4 % (w/v) solution of dextran sulfate and 2 mL of a 0.7 mM solution of magnesium chloride directly prior to use. 50 µl of this reagent mix were added to 125 µl of plasma and thoroughly mixed. After addition of 700 µl distilled water, samples were incubated at 4 °C for 1 hour. Samples were centrifuged at 6400 g for 10 min. The supernatant was used for injection.

CONCLUSIONS

The presented method based on TSKgel Q-STAT reduces the required analysis time from 30 min to 6 min. This may lead to a fivefold increase of sample throughput compared to the conventional anion exchange method based on HPLC.

REFERENCES

[1] A. Helander, A. Husa, J.O. Jeppsson, Improved HPLC Method for Carbohydrate-deficient Transferrin in Serum, Clin. Chem. 49 (2003) 1881–1890. doi:10.1373/clinchem.2003.023341.

SEPARATION OF PLASMA SAMPLES

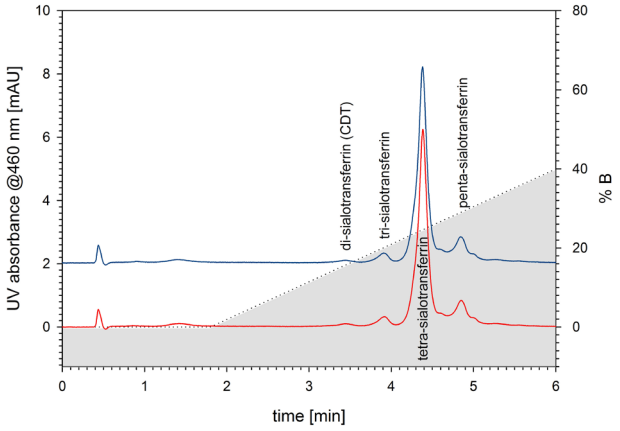

Figure 2

Figure 2 shows two plasma samples from healthy donors being separated on TSKgel® Q-STAT (4.6 mm ID x 10 cm L, P/N 0021961) at 1.0 mL/min. 100 µl of the samples were injected in 50 mM Tris/HCl, pH 7.4. The sialo-transferrin variants were separated in a 0-40 % B gradient in 4.2 min. Buffer B consisted of 50 mM Tris/HCl, pH 7.4 + 250 mM ammonium acetate. Transferrin specifically absorbs UV light at 460 nm.