

## Quantification of glycated hemoglobin as long-term control of diabetes

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### User Benefits

- ◆ Promising method for a routine control in diabetes treatment.
- ◆ Easy sample preparation.
- ◆ High-speed method without chromatography.

### Introduction

Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) facilitates a simple and quick analysis to obtain molecular weight information on diverse types of samples from small to macromolecules. Thus, there is a wide application range for MALDI-TOF MS instruments in R&D and quality control of synthetics and biological molecules.

MALDI-TOF MS is widely spread in high-throughput routine e. g. in microbial ID in clinics. As many MALDI applications go without time consuming chromatography step, MALDI enables high-speed analysis.

Long-term control of the glycaemic state of hemoglobin is the most important and reference tool for the management of diabetes. The Dutch diabetic association recommends monitoring the level of glycated hemoglobin (HbA1c) two to four times a year, depending on the type of diabetes (1).

Several procedures and numerous commercial instruments, based mainly on chromatographic separation methods, are currently available for the determination of HbA1c in blood samples.

MALDI can help to reduce analysis time and therefore increase the number of samples that can be analyzed per day and instrument.



Fig. 1 MALDI-8020 benchtop mass spectrometer

### Measurement Conditions and Samples

To validate the method, hemoglobin A1c standards (Biorad) were dissolved in 0.5 mL deionized water and diluted 1:1000. Real blood samples showed the capability of the method beyond standards. Samples were prepared with pre-coating method and Sinapinic acid as matrix.

The analysis was run on the bench-top MALDI-8020 mass spectrometer (Fig 1). SampleStation™ and AuraSolution™ software package enabled the automated high-throughput analysis:

With SampleStation a sample list with unique sample patient identifier can be created. The target will be recognized by the integrated barcode reader and the analysis is automatically run by AuraSolution software.

### Mass spectra of HbA1c standards

In the mass spectrum we observe two clusters of peaks, the singly charged ions around 15 kDa and the doubly charged ions around 7.5 kDa (Fig 2).

The intensity of the doubly charged molecules is six times higher than for the singly charged molecules. Next to the higher sensitivity a better resolution is observed for the doubly charged ions. Therefore, this cluster of peaks was used for further investigations.

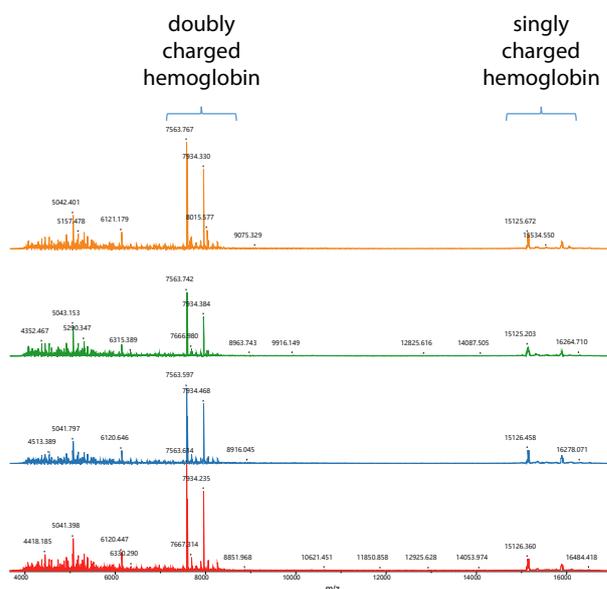


Fig. 2 Mass spectrum of HbA1c standards, ratio of glycosylated hemoglobin to non-glycosylated hemoglobin is decreasing from top to bottom

A more detailed view of this  $m/z$  range is shown in Fig 3:  $\alpha$ - and  $\beta$ -chain of hemoglobin can clearly be observed as well as the glycosylated equivalent of each.

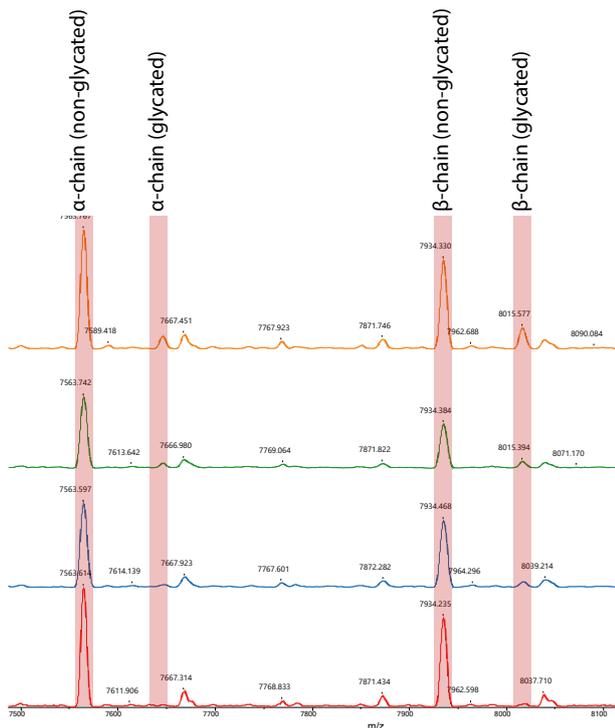


Fig. 3 Peaks representing the doubly charged glycosylated and non-glycosylated hemoglobin ( $\alpha$ - and  $\beta$ -chain). Ratio of glycosylated to non-glycosylated hemoglobin is decreasing from top to bottom.

### Quantification

HbA1c is defined as the ratio between glycosylated hemoglobin and non-glycosylated hemoglobin. Depending on the local conventions this ratio is given in % or in mmol/mol.

So  $[M+2H]^{2+}$  of the glycosylated and non-glycosylated  $\beta$ -chain of hemoglobin were used to determine the HbA1c value.

Linear regression of the hemoglobin A1c standard showed a correlation coefficient of  $r^2=0.9993$  within the range of 4.7-19% or 27-184 mmol/mol (Fig 4).

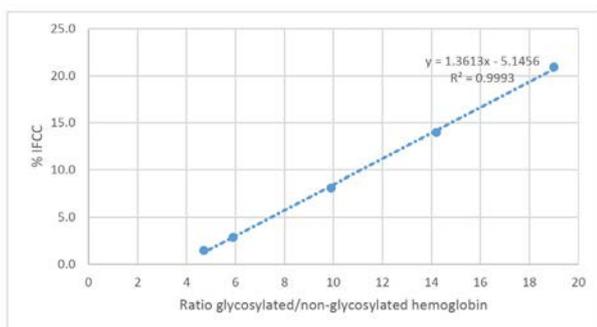


Fig. 4 Calibration curve with linear regression and correlation coefficient.

### Method transfer to real blood samples

The MALDI method was applied on real blood samples as well. A number of 20 real patient samples were analyzed with the MALDI-8020 and with conventional chromatographic system to get a comparison with this well-established method.

Obviously, there is a close correlation between both techniques in this clinically relevant concentration range (Fig 5).

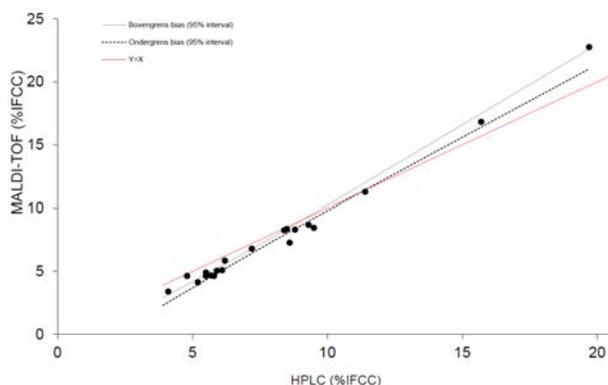


Fig. 5 Correlation between the HbA1c analyzed by MALDI-TOF MS compared to conventional chromatographic technique.

### Conclusion

This application shows another example of MALDI-TOF MS in clinical environment. With the increasing number of possible analysis the capacity of a MALDI instrument can be used efficiently.

With a time efficient sample preparation this method can be established as a quick analysis tool to reduce measurement time for this routine control in diabetes treatment.

### References

- (1) <https://www.diabetesfonds.nl>
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- (3) Yeboah F.K. & Yaylayan V.A., Analysis of glycosylated proteins by mass spectrometric techniques: qualitative and quantitative aspects. *Nahrung/Food* (2001) 45 (3), 164 - 171

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