

Evaluation of Automated Quantitative Analysis of the Doubly Charged Glycated β -Haemoglobin by MALDI-TOF MS

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1. Overview

This approach opens a new world to time and cost-effective analysis of HbA1c within the clinical chemistry

2. Introduction

Long-term control of the glycemic state of haemoglobin is the most important and reference tool for the management of diabetes. The Dutch diabetic association recommends monitoring the level of glycated haemoglobin (HbA1c) two to four times a year, depending on the type of diabetes¹.

Several procedures and numerous commercial instruments, based mainly on chromatographic separation methods, are currently available for the determination of HbA1c in blood samples. In this study we have developed a method for automated quantification of HbA1c with MALDI-TOF. ^{2,3}

All MALDI analysis were performed with a MALDI-8020 benchtop MALDI-TOF mass spectrometer (Shimadzu Corporation, Japan). Batch analysis was performed using Sample station and Aurasolution.



Compact benchtop design

- Fast
 - 200 Hz
 - Rapid sample introduction
- Low maintenance
 - UV laser-based source cleaning
- Quiet operation

Figure 2 MALDI-8020 benchtop MALDI-TOF mass spectrometer.

4. Results

4-1 Mass spectrum

In the mass spectrum we observe two clusters of peaks, the single charged for the α chain of haemoglobin with and without glycation at 15125.93 Da and 15287.92 Da, for the β chain of haemoglobin at 15868.98 Da and 16033.00 Da respectively. The double charged molecules for the α - and β chain of haemoglobin with and without glycosylation are found at 7563.67 Da, 7934.42 Da, 7644.75 Da and 8015.95 Da, respectively. Next to the glycosylated form of haemoglobin a matrix peak is detected in the mass spectrum, which can be associated to a sinapinic acid matrix adduct of the α - and β chains of haemoglobin (see figure 3 and 4).

The intensity of the doubly charged molecules are six times higher than for the singly charged molecules. Next to the higher sensitivity a better resolution is observed for the doubly charged molecule. Therefore it was decided to use the doubly charged molecules for constructing the calibration curve.

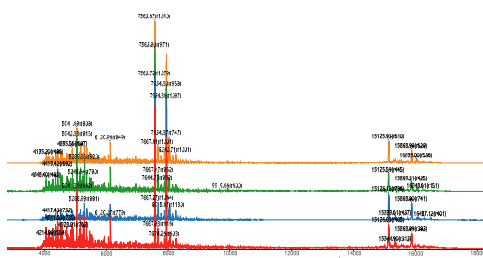


Figure 3 Full mass chromatogram of (glycated) haemoglobin, showing single and double charged molecules.

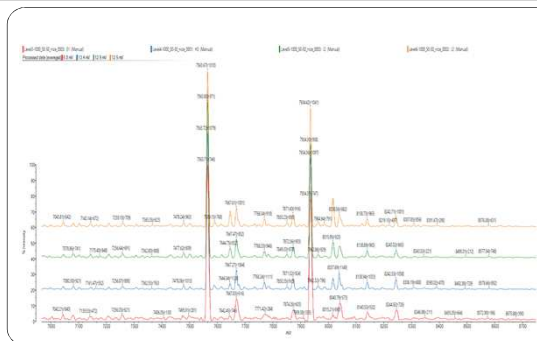


Figure 4 Zoomed mass chromatograms of the double charged α - and β (glycated) haemoglobin chain for four levels.

4-2 Linearity

The calibration curve consists of five different levels of %HbA1c, traceable to NGSP. Mass spectrometric peak areas from the MH_2^{2+} β Hb and glycated MH_2^{2+} β Hb were determined to calculate the ratio between the glycated and non-glycated form of haemoglobin.

Linear regression analysis results were $Y = 1.3613x - 5.1456$ with a correlation coefficient of $r^2 = 0.9993$. The linear range was from 4.7 to 19% IFCC (corresponding to 27 – 184 mmol/mol). (see figure 5)
Precision around the cut off value (7% IFCC; 53 mmol/mol) is 3.5%, determined over five successive measurements.

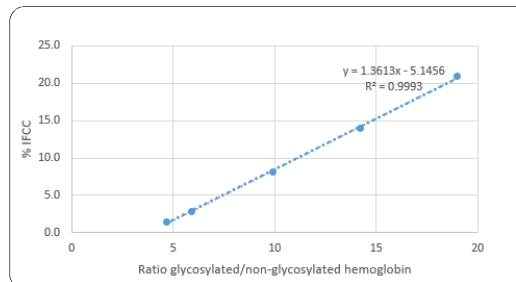


Figure 5 Representative calibration curve (Lyphocheck haemoglobinA1c) ranging from 4.7% to 19% IFCC (27 – 184 mmol/mol).

4-3 Method comparison of real blood sample

MALDI TOF-MS analysis was finally compared with a conventional chromatographic separation method by analyzing 20 real patient blood samples with different concentrations describing the complete measuring range. The method correlation was evaluated through Passing-Bablock regression (see figure 6).

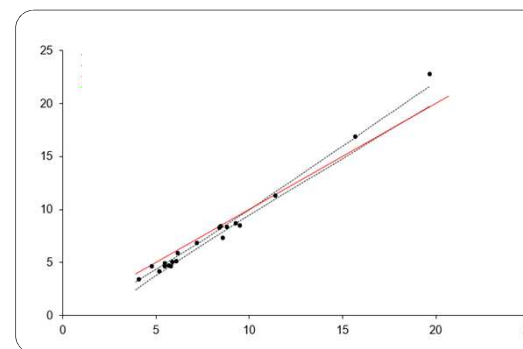


Figure 6 Passing Bablock regression analysis for comparison of MALDI TOF-MS method with a conventional chromatographic method.

3. Methods

Commercially available lyophilized haemoglobin A1c standards and whole blood samples were used for this experiment. Standards were dissolved in 0.5 mL water and then 1000 times diluted before spotting on the MALDI target.

The MALDI target was prepared with the dried droplet technique after precoating. The target was precoated with 0.5 μ L Sinapinic acid dissolved to 40 mg/mL in 50/50/0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid. After a few seconds the droplet was removed. Next 0.5 μ L of the diluted sample was applied to the target, directly followed by 0.5 μ L of the same matrix as used for precoating.

5. Conclusions

This method allows for fast screening of the HbA1c levels without time consuming chromatography and is opening doors to simple, automated, routine analysis within the clinical laboratory.

¹ <https://www.diabetesfonds.nl>

² Biorccio A., Urbani A. & Massoud R. A quantitative method for the analysis of glycated and glutathiolated hemoglobin by matrix-assisted desorption ionization-time of flight mass spectrometry. Anal. Biochemistry 336 (2005), 279 – 288

³ Yeboah F.K. & Yaylayan V.A., Analysis of glycated proteins by mass spectrometric techniques: qualitative and quantitative aspects. Nahrung/Food (2001) 45 (3), 164 - 171

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