application note

Automated Glycosylated Hemoglobin (HbA1c) Analysis for Diabetes Monitoring

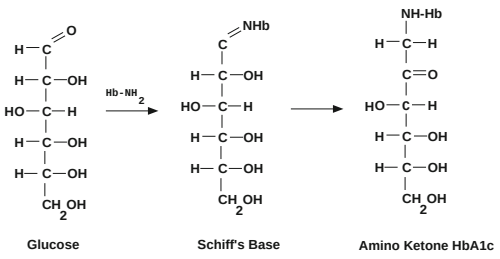
Abstract

A rapid, sensitive and automated method for the determination of glycosylated hemoglobin HbA1c is described. In the assay, HBA1c is separated from HBA0 with excellent resolution, allowing for accurate quantitation. HbA1c is also resolved from other 'minor' hemoglobins: HbF and HbA3, eliminating any of their possible interference. The labile Schiff base precursor of HbA1c, which can elevate test results and produce inaccurate quantitation, is also removed in the sample preparation. Chromatography is based on the proven cation-exchange technique, using a binary gradient, with a column lifespan exceeding 1000 runs. Each analysis is completed within 8 minutes, with the generation of a fully validated report at the completion of each analysis. Up to 160 samples can be batched for unattended analysis. Reproducibility of the assay is excellent with an RSD of 1.2% for retention time and 1.6% RSD for peak area. The system also offers automatic setup/shutdown sequence and intelligent diagnostics to ensure optimal performance and protection of precious samples.

Keywords:
HbA1c, Glycosylated Hemoglobin, DIABETES, Cation-exchange

'..HBA1c is separated from HBA_0 with excellent resolution, allowing for accurate quantitation...'

Hemoglobin A1c is a minor component of the adult hemoglobin. HbA1c is formed by the reaction of glucose with the terminal amino group of a valine residue of HbA0 as follows.



A labile Schiff's base (labile HbA1c) is initially formed in the biochemical process, followed by its subsequent chemical rearrangement to the corresponding stable HbA1c, an amino ketone. Since HbA1c is irreversibly bound to the red blood cells, its blood concentration offers a measure of the average blood glucose level for the past 4 to 6 weeks. In fact, the determination of HbA1c in a single blood sample has been considered as a more reliable indication of an individual's glucose tolerance as compared with alternate conventional assays.1

In the case of diabetic patients, HbA1c can be 2-3 times higher than normal. An accurate quantitation of the HbA1c level thus provides a reliable measure of the long-term metabolic control in the subjects, allowing clinicians to make the appropriate adjustments to treatment.

In addition, diabetes affects some 0.2% of pregnant women and gestational diabetes affects about 2% of pregnant women. Both forms of diabetes can be fatal to the baby unless the conditions are detected early and precautions are taken. Diabetes is also associated with pregnancy-induced hypertension in the mother and congenital defects in the newborn. These problems can most often be avoided if a diabetic woman receives the appropriate treatment and advice before conception. In this regard, the measurement of HbA1c has provided a very effective means for the desired diagnosis and monitoring of diabetes.

A proven cation exchange methodology for HbA1c analysis has been chosen for our system.2 A strong cation exchange column is adopted with a binary LiCl gradient using sodium malonate as a buffer. The column resin does not shrink or swell, and has been designed for the specific HPLC of biomolecules. The gradient generates little noise in its formation, excluding the need to employ complex multi-wavelength detection as is in some existing systems. The pH of the buffer has been optimised to provide maximum difference in the electrophoretic mobility and net charge among various different hemoglobins. This

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results in exceptional separation with well-defined peaks, making accurate, reproducible integration of peak areas possible. The labile HbA1c Schiff base precursor is also removed in the sample preparation, eliminating its possible interference in the assay. Typical separation of HBA1c is illustrated in Figure 1.

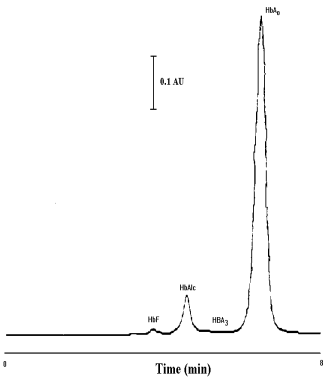

Figure 1 *HPLC Separation of HbAlc*

The reproducibility of the analysis is 1.2% RSD for retention time and 1.6% RSD for peak area based on ten consecutive analyses. Each separation is completed within 8 minutes, with automatic re-equilibration of the column at the end of the run for the next analysis. With good laboratory practice, such as the filtration of all buffers before use, each column can be used for more than 1000 runs.

Automation of the hemoglobin analyser is accomplished by control of the LC1150 Quaternary Gradient HPLC Pump and the robotic functions of LC1650 Advanced Autosampler via the WinChrom Chromatography Data Management System. The system utilises a comprehensive set of validation parameters, enabling single and group validation during analyses. Validation of results includes calculation of criteria such as variance on peak area/height, retention time, plate count, resolution and other commonly used parameters. Command sequence has been pre-programmed, but can also be easily modified, to allow the execution of different analytical routines depending on the validation results obtained. This ensures optimal performance of the analyser and avoids wastage of analysis time and precious samples during unattended operations. Multi-tasking of the management system also permits the execution of other software programs, e.g., for report preparation, while analyses are being processed.

The LC1650 Advanced Autosampler has a sample capacity of 160. A programmed sequence can also be conveniently interrupted for priority samples. The LC1150 Quaternary Gradient HPLC Pump has exceptionally low delay (dwell) volume and provides rapid on column gradient formation. Increased reliability is ensured with the dual in-series pistons arrangement utilising only two check valves. In addition, the configuration of the analyser offers maximum flexibility in allowing easy modification of the existing system for the analyses of other biochemicals such as catecholamines and amino acids.

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GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

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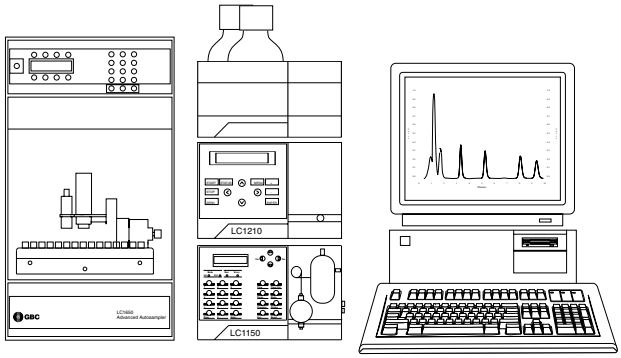
References

1. D.E Goldstein, *N. Engl. J. Med.*, 310, (1984), 284.

2. J-O. Jeppsson, P. Jerntorp, G. Sundkvist, H. Englund and V. Nylund, *Clin. Chem.*, 32(10), (1986), 1867.



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