HPLC Analysis of Glyburide

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The fast analysis of glyburide was demonstrated using a LC/MS friendly buffer on a narrow-bore ZirChrom®-PBD column. The use of elevated temperature for the analysis reduces the eluent viscosity, thereby allowing higher flow rates and shorter analysis times, with only modest back pressure. Despite the fast analsis time, the retention factor of the analyte is preserved at the high flow rates, allowing the elution of potential matrix interferences before the analyte of interest.

Introduction

The structure of glyburide, an antihypoglycemic drug from the family of sulfonylureas, is shown in Figure 1. When taken in an oral dosage form, glyburide is effective in reducing high blood glucose levels.

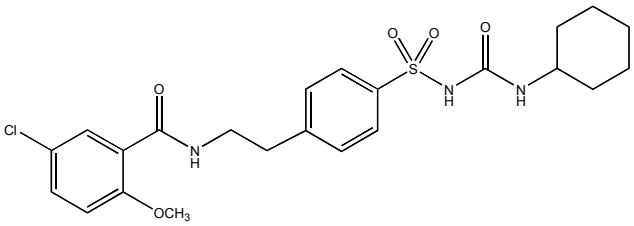


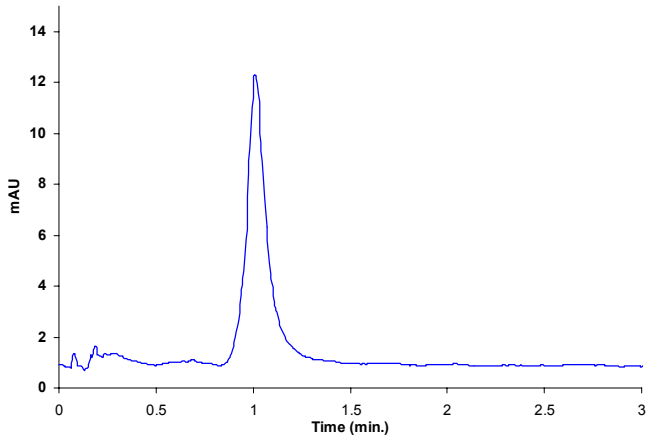
Figure 1. Structure of glyburide.

The routine analysis of glyburide by LC/MS requires a robust method that provides good peak shape, with adequate retention to allow for the separation of the analyte from matrix interferences which interefere with analyte ionization. The extraordinary chemical and physical stability of zirconia-based stationary phases allows temperature to be used as an operating variable in method development. This is particularly useful in this type of routine analysis, where the reduced eluent viscosities encountered at elevated temperatures allow use of higher flow rates and therefore shorter analysis times without appreciable loss in plate count which causes problems when analyses are speeded up by using shorter columns.

Experimental

A sample of glyburide (Sigma-Aldrich) was analyzed at slightly elevated temperature using a narrow-bore ZirChrom®-PBD column. The separation conditions were as follows:

Column:	2.1 mm x 50 mm ZirChrom®-PBD
Mobile Phase:	30/70 ACN/20mM Acetic acid, pH 3.3
Temperature:	70 °C
Injection Vol.:	1 µl
Pressure Drop:	126 bar
Detection:	UV at 240 nm
Flow Rate:	0.80 ml/min



Despite the relatively short retention time of glyburide, under these conditions the retention factor is 4.0 due to the use of high linear velocity. This retention factor provides sufficient separation space to allow for the elution of matrix components well before the glyburide peak, ensuring reliable detection by mass spectrometry.

It is important to note that many temperature-sensitive compounds can benefit from modest increases in temperature, making faster analysis possible. ZirChrom's technical support group has extensive experience in this area, and would be happy to help you with your particular application.

ZirChrom columns combine the high efficiency usually associated with silica columns with complete chemical and thermal stability.

Acknowledgement

Jim Johnson, Acting Director of Bioanalytical Services, PRACS Institute, Fargo, ND

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