GC Analysis of Acylated Sugars

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Key Words

Derivatization reagent, acylation, N-methyl-bis(trifluoroacetamide) (MBTFA) glucose, fructose, sugars, TR-1701

Abstract

Sugars must be derivatized to a volatile form so as to be analyzed by GC. A commonly used derivatization reagent, N-Methyl-bis (trifluoroacetamide) (MBTFA) was used for converting sugars to their volatile forms. In order to achieve separation of TFA sugar derivatives such as fructose and glucose anomers, a mid-polar 14% cyanopropylphenyl polysiloxane phase GC column was used.

Introduction

Sugars such as glucose and fructose are very difficult to analyse by GC, as they decompose in the injector port and "crash" out on the column. The highly polar and involatile nature of the sugars reduces the efficiency of the detection of these molecules. To overcome these problems the sugars can be derivatized to remove the active hydrogens such as -OH, therefore increasing volatility and improving detectability. The most commonly used derivatization method for the analysis of sugars is an acylation reaction. The Thermo Scientific acylation reagent MBTFA is used for derivatizing sugars and it is manufactured to meet the exacting need of sensitive derivatization reactions. This involves converting the active hydrogen into trifluroesters via a carboxylic derivative. The ester in the derivatized sugar improves the volatility, which makes it easier for analysis by GC/FID MBTFA, like the majority of derivatization reagents, produces a by-product. In this case the formation of the byproduct N-methyltrifluoroacetamide does not interfere with the analysis as it elutes earlier in the chromatogram.

In order to achieve separation of fructose and glucose anomers which arise upon derivatization, a mid-polarity 14% cyanophenyl polysiloxane Thermo Scientific TRACE TR-1701 column was used.

Experimental Details

Sample Preparation

5 mg each of glucose and fructose were weighed into a Thermo Scientific Reacti-Vial containing a Reacti-Vial magnetic stirrer. To the Reacti-Vial, 0.5 mL of MBTFA was added followed by 0.5 mL of Thermo Scientific silylation grade solvent pyridine. The Reacti-Vials were then capped and placed in the Thermo Scientific Reacti-Therm Sample Incubation System and stirred for 1 hour at 65 °C. Once dissolved the reaction was complete. The final sample was then transferred to a 2 mL autosampler vial and 1 µL was injected into the GC/FID.

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Reagents	Part Number
Thermo Scientific MBTFA 10 × 1 mL ampules	TS-49700
Thermo Scientific pyridine silylation grade solvent	TS-27530

Sample Handling Equipment	Part Number
Thermo Scientific Reacti-Therm III Heating/Stirring Module	TS-18823
Thermo Scientific Reacti-Vap III Evaporator	TS-18826
Thermo Scientific Reacti-Block Q-1 (Holds 8 × 10 mL Reacti-Vials)	TS-18814
Thermo Scientific Reacti-Vial clear glass reaction vials 10 mL	TS-13225
Thermo Scientific 2 mL amber vial and screw tops	60180-565

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific TRACE GC Ultra	
Column:	TRACE™ TR-1701 30 m × 0.25 mm × 0.25 µm	260Q142P
Thermo Scientific BTO 17 mm septa		31303211
5 mm ID focus split liner, 105 mm long		453T1905
Graphite liner seal		29033406
10 µL, 50 mm needle length gauge 25 Syringe		36500525
Graphite ferrules to fit 0.1-0.25 mm ID columns		29053488
Carrier gas:	Helium	
Split flow:	60 mL/min	
Column flow:	1.2 mL/min, Constant flow	
Split ratio:	50:1	
Oven temperature:	40 °C (1 min), 10 °C/min, 260 °C (5 min)	
Injector type:	Split/Splitless	
Injector mode:	Split	
Injector temperature:	200 °C	
Detector type:	FID	
Detector temperature:	250 °C	
Detector Hydrogen flow:	35 mL/min	
Detector Air flow:	350 mL/min	
Detector Nitrogen flow:	30 mL/min	
Thermo Scientific TriPlus Autosampler		
Injection Volume:	1 µL	

Data Processing	
Software:	Thermo Scientific XCalibur

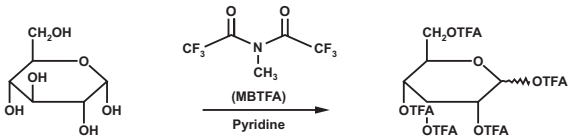

Figure 1: Acylation of glucose with MBTFA and pyridine

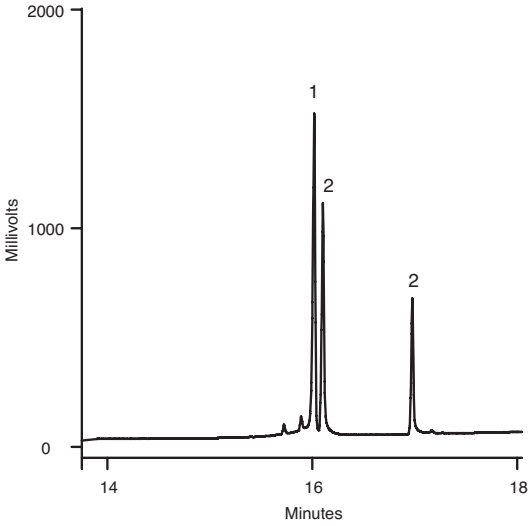

Figure 2: Chromatogram of the separation of derivatized sugars on a TR-1701 column.

Peak Number	Sugar	t_R (min)
1	Fructose	16.0
2	Glucose anomers	16.1 and 17.0

Results

The derivatized glucose produced two peaks, corresponding to the two cyclic forms of glucose existing as anomers (Figure 1) and fructose gave rise to one peak. Good baseline separation between fructose and the two anomers of derivatized glucose was observed (Figure 2) using a 14% cyanophenyl polysiloxane phase column. The stability of the sugars is improved as the acylation reagent protects the unstable groups, aiding separation on the chromatographic column.

Conclusion

MBTFA is an ideal derivatization reagent for increasing the volatility of sugars. This enabled enhanced separation and detection of fructose and glucose anomers using a TRACE TR-1701 GC column.

References

Thermo Scientific reagents, solvents and accessories brochure (Ref: BR20535_E06/12). Available upon request. ACD labs software to draw chemical structures.

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