GC Analysis of Derivatized Amino Acids

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Key Words

Derivatization reagent, silylation, N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), L-alanine, L-leucine, L-lysine, amino acid

Abstract

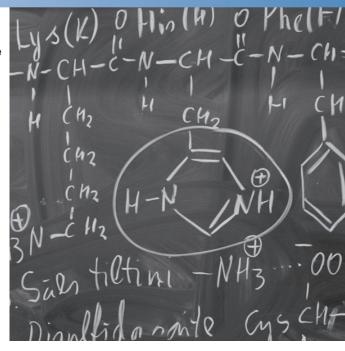
The derivatization of amino acids was achieved using the MSTFA silylation reagent. The TFA derivatized compounds were then analysed on a 5% phenyl methylpolysiloxane column using FID repetition of detection.

Introduction

Amino acids such as L-alanine, L-leucine and L-lysine are very difficult to analyse by GC as they decompose in the injector port and crash out on the column. The highly polar amino acid moiety, aids their solubility in water, but not in organic solvents which reduces the efficiency of their detection. To overcome these problems the amino acids can be derivatized to remove the active hydrogens and improve detectability. One of the most commonly used derivatization methods for the analysis of amino acids is a silylation reaction.

The Thermo Scientific Silylation reagent, N-methyl- N –(trimethylsilyl)trifluoroacetamide (MSTFA) is commonly used for derivatizing amino acids. The main advantage of using MSTFA is the formation of very volatile by-products such as TMS-amide, whilst the reagent itself elutes with the solvent front. MSTFA is manufactured to meet the exacting need for sensitive derivatization reactions. This reagent protects the functional groups present in the molecule aiding separation on the chromatography column. MSTFA involves converting the active hydrogen into a stable and volatile derivative which makes it easier for detection by FID.

In order to achieve separation and highly symmetrical peak shapes of these derivatized amino acids, a Thermo Scientific TRACE TR-5 column was used.



Experimental Details

Sample Preparation

2.5 mg each of L-alanine, L-leucine and L-lysine were weighed into a Thermo Scientific Reacti-Vial containing a Reacti-Vial magnetic stirrer. To the Reacti-Vial, 0.5 mL of MSTFA was added followed by 1 mL of acetonitrile. The Reacti-Vials were capped and placed in the Reacti-Therm Sample Incubation System and stirred for 20 minutes at 70 °C. Once dissolved the reaction was complete. The final sample was then transferred to a 2 mL autosampler vial and 1 μ L was injected into the GC/FID.



Thermo Scientific MSTFA 10 x 1 mL ampules Thermo Scientific acetonitrile silylation grade solvent Sample Handling Equipment Thermo Scientific Reacti-Therm III Heating/Stirring Module Thermo Scientific Reacti-Vap III Evaporator Thermo Scientific Reacti-Block Q-1 (Holds 8 x 10 mL Reacti-Vials) Thermo Scientific Reacti-Vial reaction clear glass vials 10 mL Thermo Scientific 2 mL amber vial and screw tops Separation Conditions Instrumentation: Thermo Scientific TRACE GC Ultra Column: TRACE TR-5 30 m × 0.25 mm × 0.25 μm Thermo Scientific BTO 17 mm septa 5 mm ID focus split liner, 105 mm long Graphite liner seal 10 μL, 50 mm needle length gauge 25 syringe Graphite ferrules to fit 0.1-0.25 mm ID columns Carrier gas: Helium Split flow: 60 mL/min Column flow: 1.2 mL/min, Constant flow Split ratio: 90:1	TS-48910 TS-20062 Part Number TS-18823 TS-18826 TS-18814 TS-13225 60180-565 Part Number 260E142P
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Column flow: 1.2 mL/min, Constant flow	
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Split ratio: 90:1	
Oven temperature: 100 °C, 15 °C/min, 300 °C	
Injector type: Split/Splitless	
Injector mode: Split	
Injector temperature: 240 °C	
Detector type: FID	
Detector temperature: 280 °C	
Detector air flow: 35 mL/min	
Detector Hydrogen flow: 350 mL/min	
Detector nitrogen flow: 30 mL/min	

Thermo Scientific TriPlus Autosampler	
Injection Volume:	1 μL

Data Processing		
Software:	Thermo Scientific XCalibur	

Results

Separation of derivatized amino acids was achieved using a 5% phenyl methylpolysiloxane (TRACE TR-5) column (Figure 1). The stability of the amino acids and enhanced detection is observed following derivatization with MSTFA.

Conclusion

MSTFA increases the volatility and stability of amino acids, allowing for enhanced separation and detection using the TRACE TR-5 GC column.

References

Thermo Scientific reagents, solvents and accessories brochure (Ref: BR20535_E 06/12S). Available upon request.

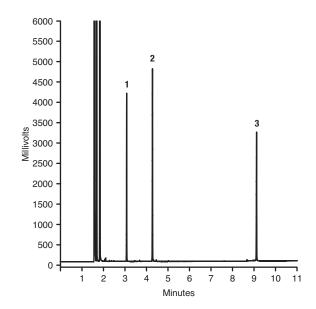
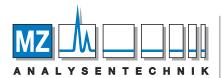


Figure 1: Chromatogram of the separation of the derivatized amino acids

Peak Number	Derivatised Amino acid	t _R (min)
1	L-alanine	3.1
2	L-leucine	4.3
3	L-lysine	9.1



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