

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

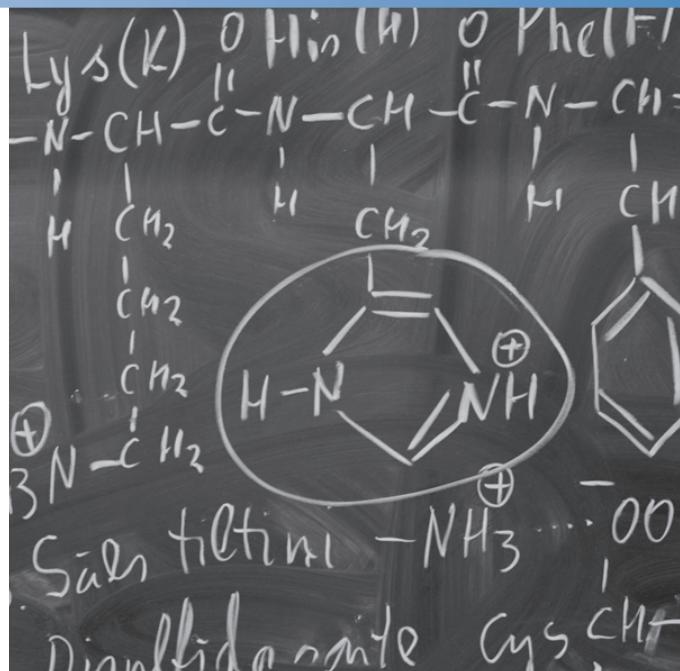
The derivatization of amino acids was achieved using the MSTFA silylation reagent. The TFA derivatized compounds were then analysed on a 5% phenyl methylpolysiloxane column using FID repetition of detection.

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 µL was injected into the GC/FID.

Reagents	Part Number
Thermo Scientific MSTFA 10 x 1 mL ampules	TS-48910
Thermo Scientific acetonitrile silylation grade solvent	TS-20062
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 x 10 mL Reacti-Vials)	TS-18814
Thermo Scientific Reacti-Vial reaction clear glass 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-5 30 m x 0.25 mm x 0.25 µm
Thermo Scientific BT0 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:	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.

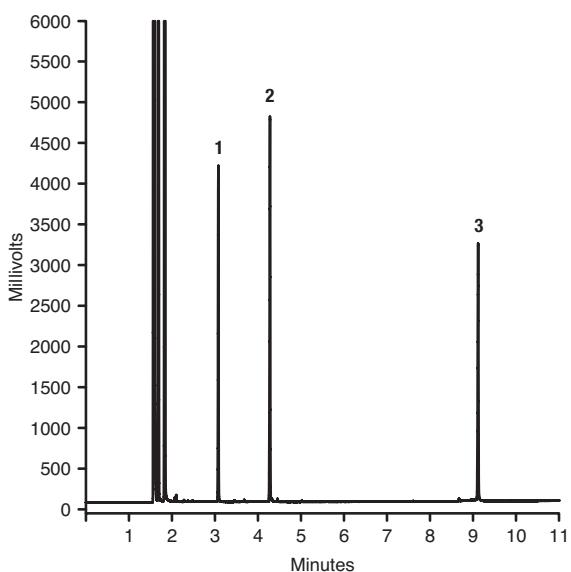


Figure 1: Chromatogram of the separation of the derivatized amino acids

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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