

GC-MS analysis of t-BDMS derivatives of amino acids

Ref: Mawhinney et al. (1986) J Chromatography 358: 231-242

Hardware

GC liner: split or splitless depending on analyte concentration
Column: Rtx-5Sil-MS (30 metre x 0.25 mm ID x 0.25 μ m film)
MS: EI ionisation

GC

Carrier gas: 1 mL min⁻¹ He (constant flow)
Purge flow: 3-5 mL min⁻¹
Injection: 1 μ L splitless or split (5:1 to 20:1 are common split ratios)
Injection temp: 250°C
Oven program: 2 min at 130°C
10°C min⁻¹ ramp to 330°C¹
10 minute hold at 330°C²

MS

Ion source: 200°C
Interface: 250°C
Solvent delay: 4.5 min
MS program: 5 to 32 min, scan from 100 to 600 amu³ at 0.5 sec per scan

Data analysis

All quantification is relative to an internal standard(s), e.g. nor-leucine, and identification is based on retention time and fragment pattern.

Quantification generally based on M-57 ions (mass 57 less than intact molecule) that correspond to loss of the (highly stable) t-butyl group. Arginine is a notable exception. Arg is quantified based on M-188 ion.

¹ Max temp does not need to be > 300 for elution of amino acids

² Long hold at 330 is not necessary for amino acids, but helps reduce background. Omitting hold at high temp can lead to nasty carryover if samples contain "sticky" substances such as carbohydrates

³ SIM can be used to improve detection limits if necessary

Sampler GC MS

Inj. Port: SPL1 Inj. Heat Port: INJ1

Column Oven Temp.: 130.0 °C
 Injection Temp.: 250.0 °C

Injection Mode: Split
 Sampling Time: 1.00 min

Carrier Gas: He Prim. Press.: 500-900

Flow Control Mode: Linear Velocity
 Pressure: 109.7 kPa
 Total Flow: 16.0 mL/min
 Column Flow: 1.00 mL/min
 Linear Velocity: 33.5 cm/sec
 Purge Flow: 5.0 mL/min
 Split Ratio: 10.0

Program: Column Oven Temperature

Rate	Final Temperature	Hold Time
0	130.0	2.00
1	330.0	10.00
2	130.0	0.00
3	0.0	0.00

Total Program Time: 36.00 min

Column Name: Rtx-SMS Thickness: 0.25 um
 Length: 38.0 m Diameter: 0.25 mm

Ready Check... Add Heater...

High Press. Injection Carrier Gas Saver

Batch Table Acquisition

L.Vac. H.Vac.

Ionization Mode

GC Consumables

MS Consumables

Detail...

Sampler GC MS

GDMS-QP2010

Ion Source Temp.: 200 °C
 Interface Temp.: 250 °C
 Solvent Cut Time: 4.5 min
 Micro Scan Width: 0 u

Detector Voltage: Relative to the Tuning Result Absolute
 GC Program Time: 36.00 min

Use MS Program: Set...

Start Time (min)	End Time (min)	Acq. Mode	Event Time(sec)	Scan Speed	Start m/z	End m/z	Ch1 m/z	Ch2 m/z	Ch3 m/z	Ch4 m/z	Ch5 m/z	Ch6 m/z	Ch7 m/z	Ch8 m/z
1	5.00	32.00	Scan	0.50	1111	100.00	600.00							
2	0.00	0.00	Scan	0.00	0	0.00	0.00							

Batch Table Acquisition

L.Vac. H.Vac.

Ionization Mode

GC Consumables

MS Consumables

Detail...

Amino acid standards for *t*-BDMS analysis

Make amino acid stock solutions: 5 mg/mL in MeOH:H₂O (50:50)

Remember to account for HCl and HCl.H₂O salts

Asp, Glu and **Asn** should be dissolved in 0.1 M HCl (MeOH:H₂O)

Tyr should be dissolved in 0.1 M NaOH (MeOH:H₂O)

Make norleucine stock solution: 0.2 mg/mL in MeOH:H₂O (50:50)

Prepare mixed amino acid standard: 0.2 mg/mL and 0.02 mg/mL

Prepare this by mixing aliquots of the individual amino acids (and adding MeOH:H₂O as appropriate to dilute)

Prepare standard curve in GC Vials with glass inserts:

Amino acids (μg)	Amino acids (mg/mL) (assuming 50 μL volume is dried)	Norleucine (internal std)	amino acid mix
12.00	0.240	5 x 0.2 mg/mL	60 x 0.2 mg/mL
8.00	0.160	5 x 0.2 mg/mL	40 x 0.2 mg/mL
4.00	0.080	5 x 0.2 mg/mL	20 x 0.2 mg/mL
2.00	0.040	5 x 0.2 mg/mL	10 x 0.2 mg/mL
1.00	0.020	5 x 0.2 mg/mL	5 x 0.2 mg/mL
0.50	0.010	5 x 0.2 mg/mL	25 x 0.02 mg/mL
0.25	0.005	5 x 0.2 mg/mL	12.5 x 0.02 mg/mL
0.10	0.002	5 x 0.2 mg/mL	5 x 0.02 mg/mL