

GC-MS analysis of TMS derivatives of plant extracts

Reference: modified from various sources including:
Roessner *et al.* (200) Plant Journal 23: 131-142.
Lisec *et al.* (2006) Nature Protocols 1: 387-396

Hardware

GC liner: split or splitless depending on analyte concentration
Column: RXi-5Sil-MS (30 metre x 0.25 mm ID x 0.25 μ m film with 10 m guard column)
MS: EI ionisation

GC

Carrier gas: 1 mL min⁻¹ He (constant flow)
Purge flow: 5 mL min⁻¹
Injection¹: 0.5-1 μ L splitless (30 second sampling time, 25:1 split ratio), or
Split (5:1 to 20:1 are common split ratios)
Injection temp: 250°C
Oven program: 2 min at 70°C
8°C min⁻¹ ramp to 330°C
10 minute hold at 330°C

MS

Ion source: 250°C
Interface: 280°C
Solvent delay: 6.0 min
MS program: 6.2 to 42.5 min, scan from 70 to 600 amu at 0.2 sec per scan

¹ If there is a very wide range in analyte concentrations it may be necessary to analyse everything twice. Once with splitless injection for low abundance analytes, then at 25:1 split for high abundance analytes. To determine if this is necessary check for the tell-tale signs of detector overload.

Sampler GC MS
ADC-20+s

of Rinses with Solvent (Pre-run):

of Rinses with Solvent (Post-run):

of Rinses with Sample:

Plunger Speed(Suction): High Middle Low

Viscosity Comp. Time: sec

Plunger Speed(Injection): High Middle Low

Syringe Insertion Speed: High Low

Injection Mode:

Batch Table Acquisition

Sampler GC MS

Inj. Port: SPL1 Inj. Heat Port: INJ1

Column Oven Temp.: °C

Injection Temp.: °C

Injection Mode:

Sampling Time: min

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

Flow Control Mode:

Pressure: kPa

Total Flow: mL/min

Column Flow: mL/min

Linear Velocity: cm/sec

Purge Flow: mL/min

Split Ratio:

Program:

Rate	Final Temperature	Hold Time
0	70.0	2.00
1	8.00	330.0
2	0.00	0.00
3	0.00	0.00

Total Program Time: 42.50 min

Column: Name: Rxi_55i_MS Thickness: 0.25 um Length: 40.0 m Diameter: 0.25 mm

Pre-run Program Time Program

Batch Table Acquisition

Sampler GC MS

GCMS-QP2010

Ion Source Temp.: °C

Interface Temp.: °C

Solvent Cut Time: min

Micro Scan Width: u

Detector Voltage: Relative to the Tuning Result Absolute

kV

Threshold:

Use MS Program: GC Program Time: 42.50 min

Group#1	Event#1	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	Ch9 m/z	Ch10 m/z
1	6.20	42.50	Scan	0.20	3333	70.00	600.00											
2	0.00	0.00	Scan	0.00	0	0.00	0.00											

Batch Table Acquisition

Samples and standards

Each batch of samples should contain at least one blank and one retention index standard

Retention index standards:

- even n-alkanes from C10 to C40
- Run an alkane mix with every batch of samples.
- Update retention index file if necessary

Internal standard:

- 0.2 mg/mL ribitol in MeOH:H₂O (50:50)
- Generally add 5 µL to each sample prior to derivatization

Amino acid, organic acid, sugar and sugar alcohol solutions:

- Make stock solutions at 5 or 1 mg/mL in MeOH:H₂O (50:50).
- Remember to account for HCl, HCl.H₂O and Na 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)
- Take a subsample of the stock solution and dilute to 0.2 mg/mL
- For peak ID confirmation, 1 or 2 µg of standard is appropriate

mass of analyte (µg)	conc (mg/mL)	vol (µL)
1	0.2	5
1	0.1	10
1	0.05	20
1	0.02	50
2	0.2	10
2	0.1	20
2	0.05	40
2	0.02	100

Peak ID and quantification

Peak ID and quantification is based on comparison with retention index and mass spectrum of public (Golm), commercial (NIST, Fiehn) and private libraries (CRW_METAB). Match criteria vary between libraries. For personal library, use $RI \pm 10$ and match $>85\%$. Golm and Fiehn libraries determined RI with similar columns to those used here, and thus RI should match within 50. NIST RIs were determined on different columns and thus don't match particularly well, though mass spectra are good matches.

Table x. tentative retention indices from private library.

chemical spp	Class	RI
val	amino acid	1081
acetic acid	organic acid	?
Gln? (minor)	amino acid	1093
Ala	amino acid	1101
oxalic acid	organic acid	1125
Pro	amino acid	1172
malonic acid	organic acid	1200
Val	amino acid	1213
Urea		1236
benzoic acid	organic acid	1247
Ser	amino acid	1253
ethanolamine	amine	1264
Leu	amino acid	1269
Ile	amino acid	1271
b-Ala	amino acid	1271
His (minor)	amino acid	1272
glycerol	sugar alcohol	1274
nicotinic acid	organic acid	1288
Ile	amino acid	1291
Thr	amino acid	1291
Pro	amino acid	1295
maleic acid	organic acid	1301
Gly	amino acid	1304
succinic acid	organic acid	1313
glyceric acid	organic acid	1324
maleic acid	organic acid	1349
fumaric acid	organic acid	1349
Ser	amino acid	1356
Thr	amino acid	1380
lactic acid	organic acid	1385
glutaric acid	organic acid	1398
Gly	amino acid	1403
oxalacetic acid	organic acid	1459
Hyp	amino acid	1471
malic acid	organic acid	1488
Asn	amino acid	1497
adipic acid	organic acid	1505
Met	amino acid	1517

Glu	amino acid	1517
Asp	amino acid	1518
Gln	amino acid	1518
hyp	amino acid	1524
Glu	amino acid	1528
GABA	amino acid	1529
Phe	amino acid	1545
trans cinnamic acid	organic acid	1545
Cys	amino acid	1553
a-ketoglutaric acid	organic acid	1570
Asn	amino acid	1590
Glu?	amino acid	1611
maleic acid	organic acid	1613
Glu	amino acid	1616
Phe	amino acid	1624
tartaric acid	organic acid	1634
Xylose(minor)	sugar	1646
Xylose	sugar	1654
Arabinose	sugar	1662
Asn	amino acid	1666
Ribose	sugar	1677
xylitol	sugar alcohol	1706
l-2-aminodipic acid	organic acid	1712
arabitol	sugar alcohol	1719
Ribitol	Sugar alcohol (IS)	1721
Putrescine	amine	1739
2,6 pyridine dicarboxylic acid	organic acid	1749
Orn/Arg	amino acid	1750
Ribitol (degredation/partial deriv ⁿ prod)	Sugar alcohol (IS)	1765
Quercitol	sugar alcohol	1790
shikimic acid	organic acid	1804
Orn/Arg	amino acid	1812
citric acid	organic acid	1813
isocitric acid	organic acid	1815
Orn/Arg	amino acid	1819
Cit	amino acid	1821
Pinitol	sugar alcohol	1829
ascorbic acid	organic acid	1841
Lys	amino acid	1850
quinic acid	organic acid	1856
Fructose	sugar	1870
gluconic acid_1	organic acid	1880
Fructose	sugar	1880
Tyr O TMS	amino acid	1883
Mannose	sugar	1885
Galactose	sugar	1890
Glucose (major)	sugar	1896
Mannose (minor)	sugar	1900
Galactose	sugar	1912
Lys	amino acid	1920
Glucose (minor)	sugar	1925
mannitol	sugar alcohol	1929
Tyr NO TMS	amino acid	1935

sorbitol	sugar alcohol	1935
galactitol	sugar alcohol	1940
gallic acid	organic acid	1950
gluconic acid_2	organic acid	1996
ferulic acid	organic acid	2087
myo-inositol	sugar alcohol	2091
spermidine	amine	2195
Try	amino acid	2197
spermidine(minor)	amine	2253
frc-6-p	sugar phosphate	2301
glc-6-p	sugar phosphate	2313
glc-6-p (minor)	sugar phosphate	2332
6 phospho gluconic	sugar phosphate	2415
Sucrose	sugar	2634
Spermine	amine	2659
Maltose	sugar	2738
Maltose(minor)	sugar	2765
maltitol	sugar alcohol	2838
Raffinose	sugar	
stachyose	sugar	