GC-MS analysis of TMS derivatives of plant extracts

Reference: modified from various sources including:
Roessner *et al.* (2000) 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 μm film with 10 m guard column)
MS: EI ionisation (methane CI can be used to confirm MH^+)

GC

Carrier gas: 1 mL min^{-1} He (constant flow)
Purge flow: 5 mL min^{-1}
Injection[1]: 0.5-1 μL splitless (30 second sampling time, 25:1 split ratio), or
Split (5:1 to 20:1 are common split ratios)
Injection temp: 270°C (230 or 250°C can be used if worried about degradation of analytes)
Oven program: 2 min at 70°C
6 or 8°C min^{-1} ramp to 330°C
8 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
Threshold: 100

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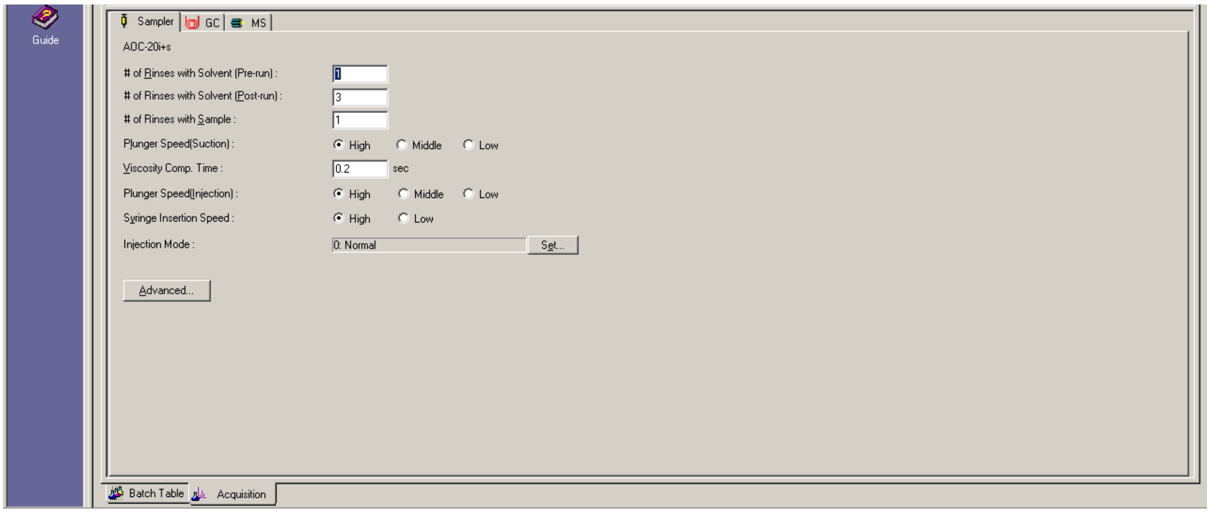
[1] If there is a very wide range in analyte concentrations it may be necessary to analyse everything twice. Once with splitless injection for low abundance analytes, then at 25:1 split for high abundance analytes. To determine if this is necessary check for the tell-tale signs of detector overload.



AOC-20i+s

# of Rinses with Solvent (Pre-run) : 1

# of Rinses with Solvent (Post-run) : 3

# of Rinses with Sample : 1

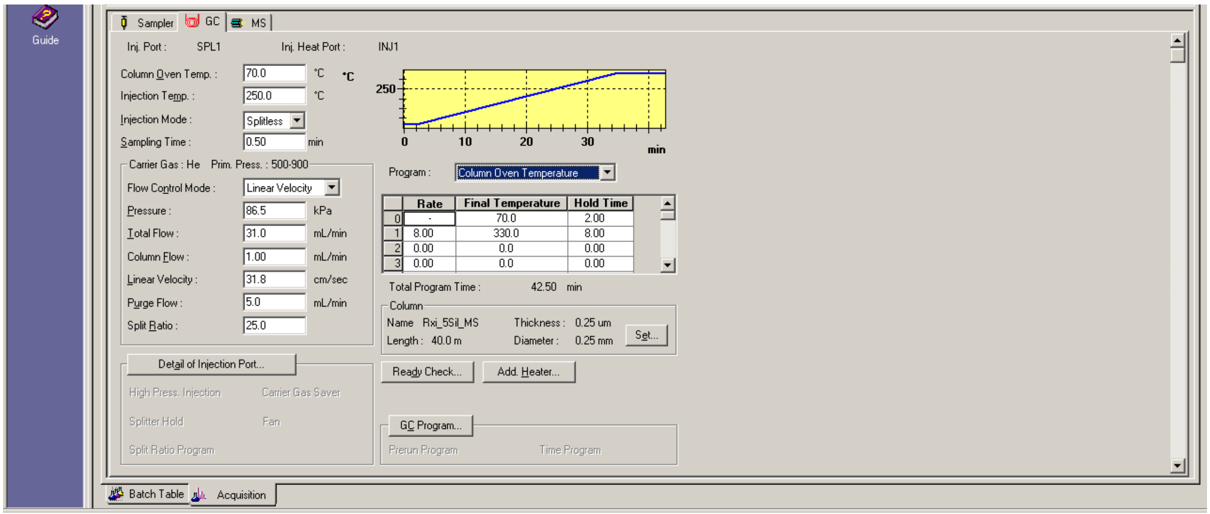
Plunger Speed(Suction) : ● High ○ Middle ○ Low

Viscosity Comp. Time : 0.2 sec

Plunger Speed(Injection) : ● High ○ Middle ○ Low

Syringe Insertion Speed : ● High ○ Low

Injection Mode : 0: Normal



Inj. Port : SPL1   Inj. Heat Port : INJ1

Column Oven Temp. : 70.0 °C

Injection Temp. : 250.0 °C

Injection Mode : Splitless

Sampling Time : 0.50 min

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

Flow Control Mode : Linear Velocity

Pressure : 86.5 kPa

Total Flow : 31.0 mL/min

Column Flow : 1.00 mL/min

Linear Velocity : 31.8 cm/sec

Purge Flow : 5.0 mL/min

Split Ratio : 25.0

Program : Column Oven Temperature

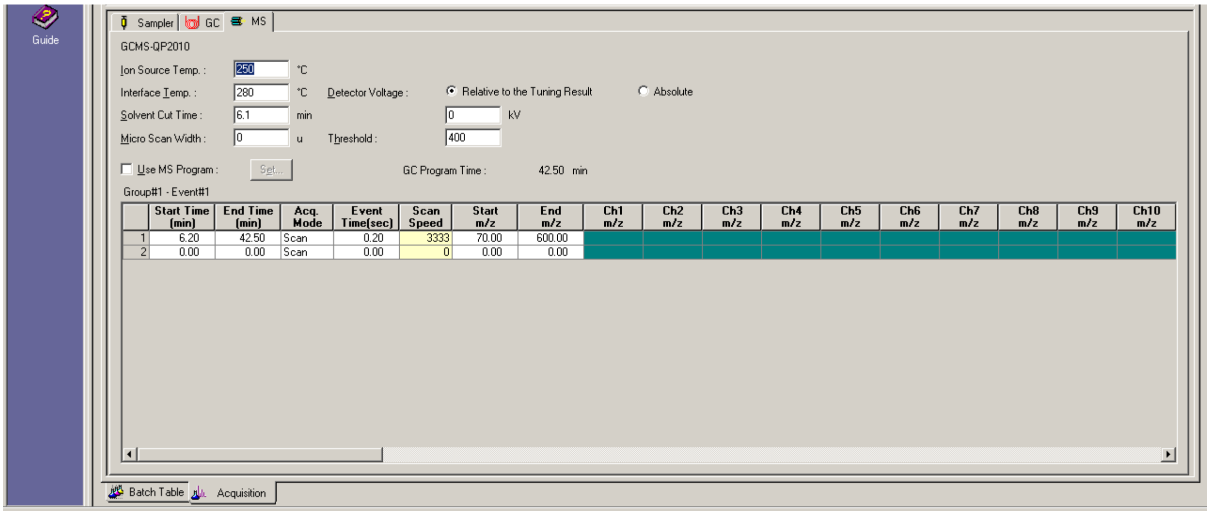
	Rate	Final Temperature	Hold Time
0	-	70.0	2.00
1	8.00	330.0	8.00
2	0.00	0.0	0.00
3	0.00	0.0	0.00

Total Program Time : 42.50 min

Column

Name Rxi_5Sil_MS   Thickness : 0.25 um

Length : 40.0 m   Diameter : 0.25 mm



GCMS-QP2010

Ion Source Temp. : 250 °C

Interface Temp. : 280 °C   Detector Voltage : ● Relative to the Tuning Result ○ Absolute

Solvent Cut Time : 6.1 min   0 kV

Micro Scan Width : 0 u   Threshold : 400

☐ 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										

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:H_2O (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:H_2O (50:50).
- Remember to account for HCl, HCl.H_2O and Na salts
- Asp, Glu and Asn should be dissolved in 0.1 M HCl (MeOH:H_2O)
- Tyr should be dissolved in 0.1 M NaOH (MeOH:H_2O)
- 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 (RI) and mass spectrum of public (Golm), commercial (NIST, Fiehn) and private libraries (CRW_METAB) using instrument software and third-party deconvolution software. As a starting point, for the private library use RI ± 5 and mass spectral match >80%. Note that RI of Golm and Fiehn libraries do not match exactly those determined here (RTx5-Sil_MS with 8℃/min ramp) because they used different columns and temperature ramps. However, there is a very strong and reproducible relationship between experimentally determined RI (RI_CRW) and those quoted in Golm and Fiehn ($R^2 > 0.999$):

RI Golm Quad = 1.0023 RI_CRW + 4.3269; $R^2 = 0.9996$

RI Fiehn = 1.0058 RI_CRW - 331.32; $R^2 = 0.9997$

NIST RIs are normally quoted for isothermal analyses with standard non-polar columns and thus don’t match particularly well with conditions used here. However, in some cases the NIST library has multiple RI entries for the one compound, and thus it may be possible to find a RI determined with a 5% column and similar temperature ramp. Note that the instrument’s software displays only a single entry for NIST RIs. To check for multiple RIs you’ll need to use NIST MS Search V2.0.

Note that the RIs reported here and in the private library are only appropriate for these chromatographic conditions. They will change if you use a different column or temperature ramp or injection method. For example, RIs determined with 1mL/min flow and 8℃/min ramp are up to 10 units different to RIs determined with 2mL/min flow and 15℃/min ramp