Data acquisition

Data are acquired using the following chromatographic parameters, with more details to be found in Fiehn O. et al. Plant J. 53 (2008) 691–704.
Column: Restek corporation Rtx-5Sil MS (30 m length x 0.25 mm internal diameter with 0.25 µm film made of 95% dimethyl/5%diphenylpolysiloxane)

Mobile phase: Helium
Column temperature: 50-330°C Flow-
rate: 1 mL min^{-1}
Injection volume: 0.5 µL
Injection: 25 splitless time into a multi-baffled glass liner
Injection temperature: 50°C ramped to 250°C by 12°C s^{-1}
Oven temperature program: 50°C for 1 min, then ramped at 20°C min^{-1} to 330°C, held constant for 5 min.

The analytical GC column is protected by a 10 m long empty guard column which is cut by 20 cm intervals whenever the reference mixture QC samples indicate problems caused by column contaminations. We have validated that at this sequence of column cuts, no detrimental effects are detected with respect to peak shapes, absolute or relative metabolite retention times or reproducibility of quantifications. This chromatography method yields excellent retention and separation of primary metabolite classes (amino acids, hydroxyl acids, carbohydrates, sugar acids, sterols, aromatics, nucleosides, amines and miscellaneous compounds) with narrow peak widths of 2–3 s and very good within-series retention time reproducibility of better than 0.2 s absolute deviation of retention times. We use automatic liner exchanges after each set of 10 injections which we could show to reduce sample carryover for highly lipophilic compounds such as free fatty acids.

Mass spectrometry parameters are used as follows: a Leco Pegasus IV mass spectrometer is used with unit mass resolution at 17 spectra s^{-1} from 80-500 Da at -70 eV ionization energy and 1800 V detector voltage with a 230°C transfer line and a 250°C ion source.