Ion chromatography-mass spectrometric analysis

Polar extracts were reconstituted in ultrapure de-ionized water (EMD Millipore, Billerica, MA) in a volume based on tissue wet weight. All analyses were performed on a Dionex ICS-5000+ ion chromatograph interfaced to a Thermo Fusion Orbitrap Tribrid mass spectrometer (IC-FTMS) (Thermo Fisher Scientific, Waltham, MA). Ion-chromatography (Wang et al. 2014, DOI: 10.1021/ac500951v) was performed using an IonPac AS11-HC-4 ⌈m RFIC&HPIC (2x250 mm) column and an IonPac AG11-HC-4 ⌈m guard column (2x50 mm). The column flow rate was kept at 0.38 mL/min with column temperature at 35 °C and 0.06 mL/min methanol added post-column as a make-up solvent to aid vaporization in the Heated Electro-Spray Ionization (HESI) unit. The HESI vaporizer temperature was 400°C with sheath gas set at 35 and auxiliary nitrogen flow at 4. The column was initially equilibrated for 8 min with 1 mM KOH, followed by 1 mM KOH for 2 min after 5 μL of sample was injected. The KOH gradient program used to elute samples included ramping up to 40 mM KOH from 2 to 25 min, and to 100 mM from 25 to 39.1 min, at 100 mM to 50 min, and ramping down to 1 mM KOH at 50.1 min and at 1 mM KOH to 52.5 min. KOH suppression was achieved with a Dionex AERS 500 2 mm suppressor with an external AXP pump supplying regenerant at a flow rate of 0.75 mL/min and injected into the Orbitrap mass spectrometer via HESI. Mass spectra were recorded at a resolution of 450,000 (achieving a resolution of approximately 360,000 @ 400 m/z) from 50 to 750 *m/z* mass scan range, with detection in the negative ion mode voltage using the following settings: HESI = 2800 V; ion transfer tube temperature = 300 °C; Automatic Gain Control (AGC) = $2 \times 10_5$; maximal injection time = 100 ms. Peak areas were integrated and exported to Excel via the Thermo TraceFinder (version 3.3) software package. Peak areas were corrected for natural abundance as previously described (Carreer et al. 2013, DOI:10.3390/metabo3040853).