

Supplementary Information

Resolving phytosterols in microalgae using offline two-dimensional reversed phase liquid chromatography × supercritical fluid chromatography coupled to quadrupole time-of-flight mass spectrometry

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Fig. S10 Extracted ion chromatogram for m/z 367.3367 in *Padina pavonica* extract

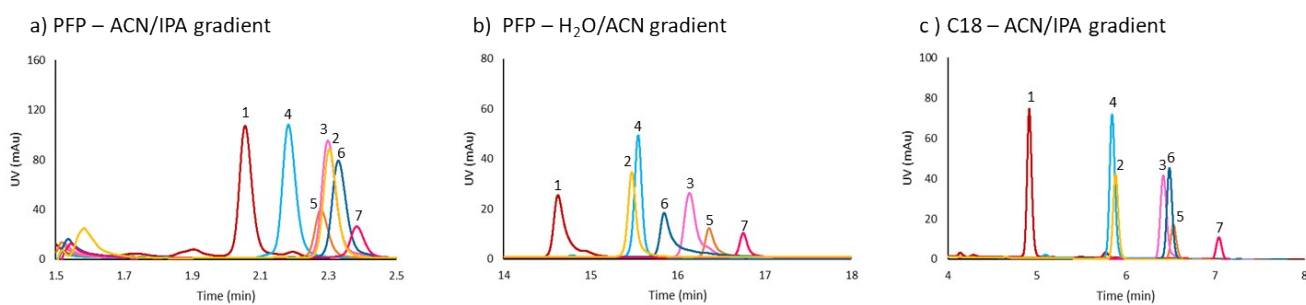


Fig. S1 LC chromatograms of standards using two different columns. a-b) Kinetex F5 PFP and a) ACN/IPA mobile phase with a 0% to 20% IPA gradient and b) ACN/water mobile phase with a 50% to 100% ACN gradient and c) Luna Omega C18 and ACN/IPA mobile phase with a 0% to 50% IPA gradient. Gradient steepness 3%. Individual injection 1% column volume, 1.1 mL/min for a-b and 2.2 mL/min for c. Detection 210 nm

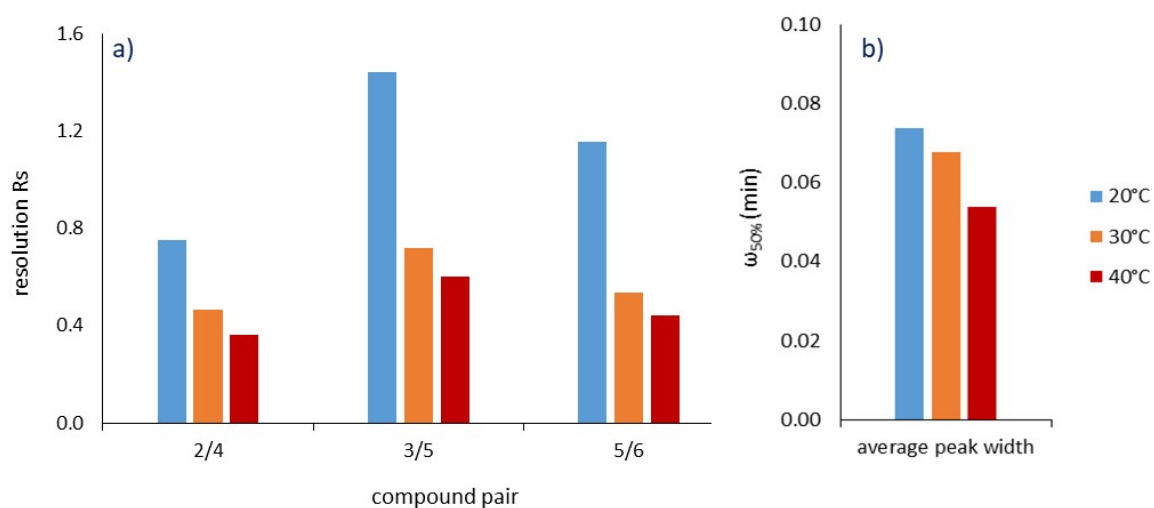


Fig. S2 Influence of temperature on a) the resolution of critical pairs and b) the average peak width in LC analysis. Blue : 20°C, orange : 30°C and red : 40°C

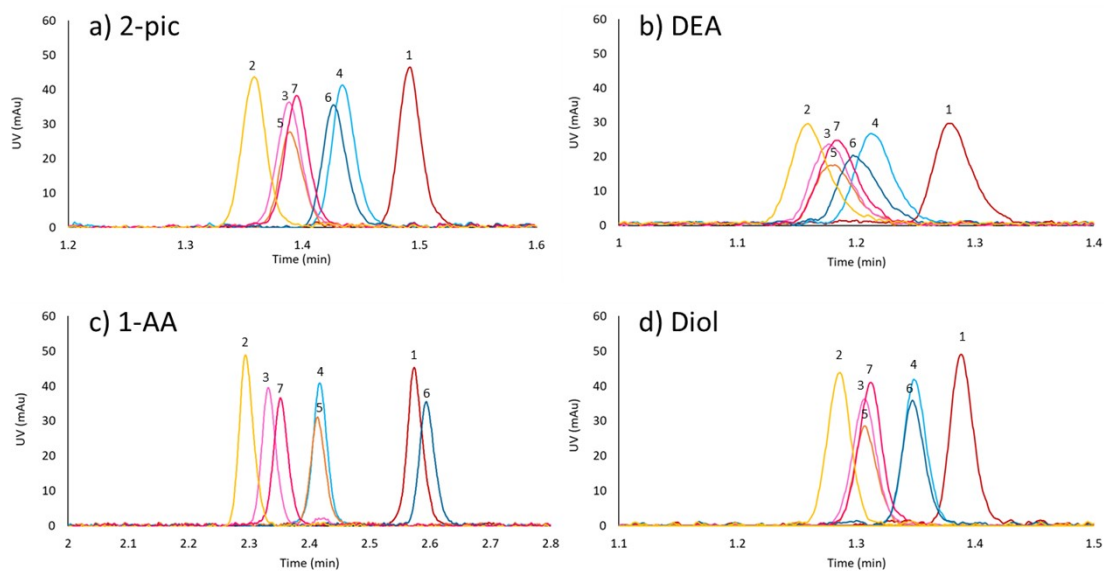


Fig. S3 SFC chromatograms of standards using four different columns at $F=1.2\text{mL}/\text{min}$ corresponding to F_{max} . Co-solvent MeOH gradient 5% - 40 %. Gradient steepness 2.5 %. Individual injections 1% column volume, detection 210 nm. Solute codes as in Table 2

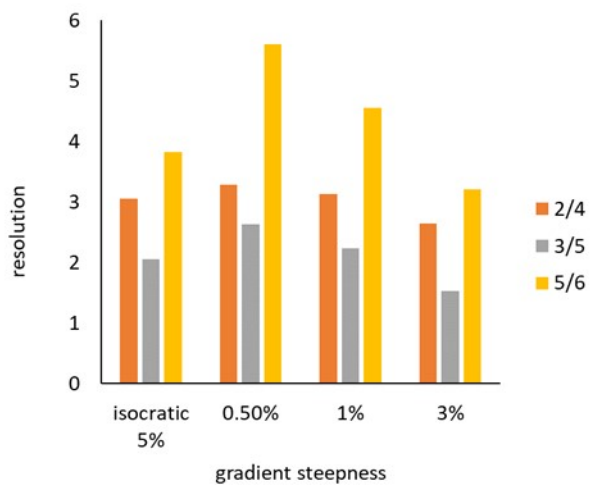


Fig. S4 Impact of gradient steepness on the resolution of critical pairs. Other SFC conditions as in Table 2

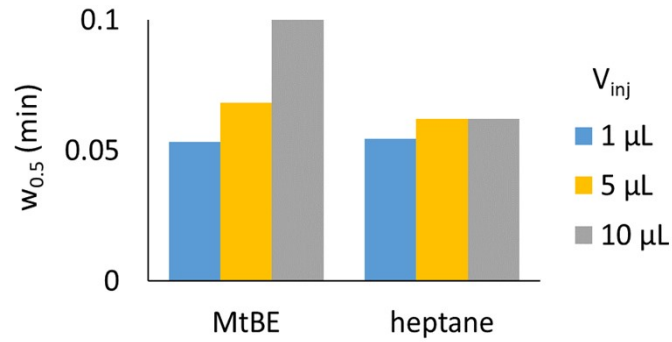


Fig. S5 Impact of injection diluent on peak width of cholesterol standard. SFC conditions as in Table 2

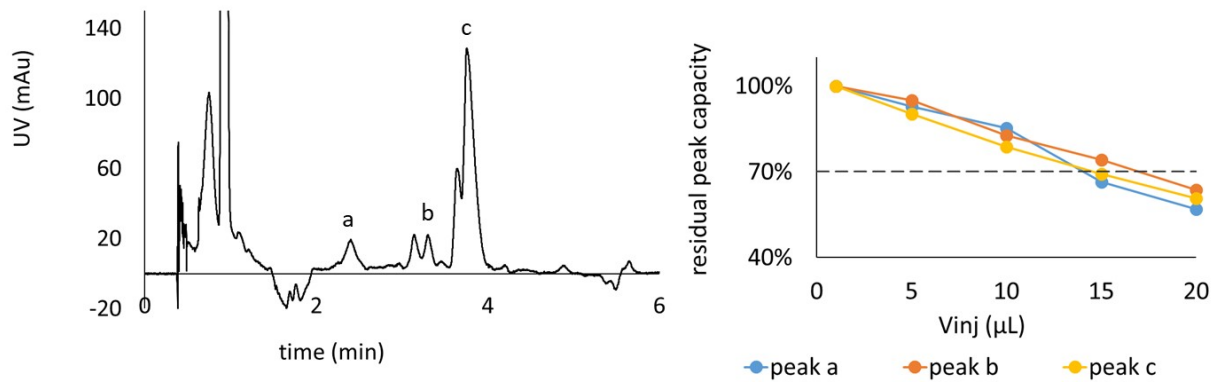


Fig. S6 Impact of injection volume on residual peak capacity of three selected peaks in *Scenedesmus obliquus*. SFC conditions as in Table 1. The peak capacity was evaluated from peak width at half-height of three different peaks noted peak a, peak b and peak c.

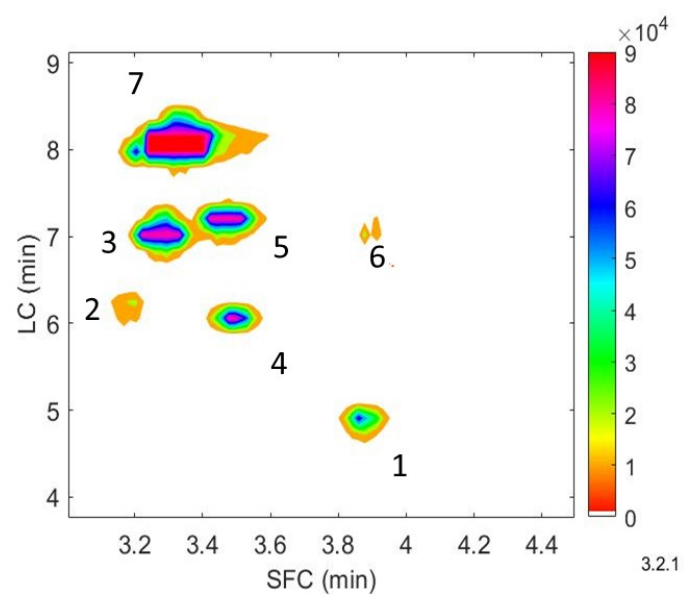
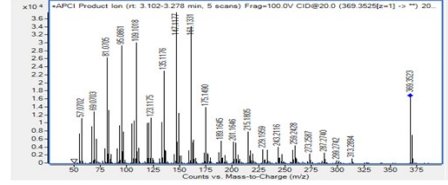
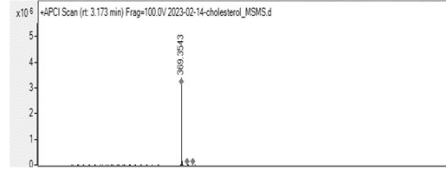
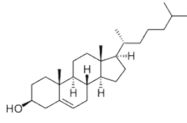


Fig. S7 2D chromatograms of sterol standards mixture. Compounds listed in Table 2. Sum of EIC from ions $[M+H - H_2O]^+$

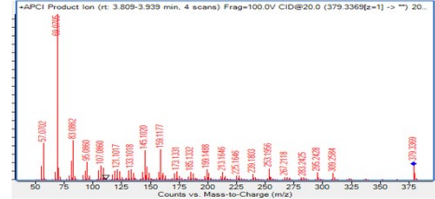
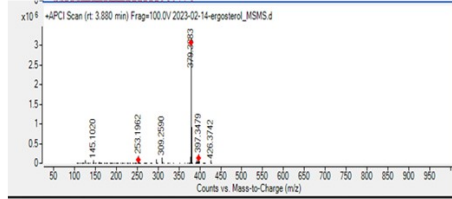
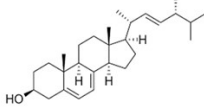
Cholesterol

➤ EIC 369.3516



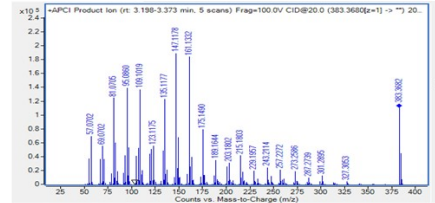
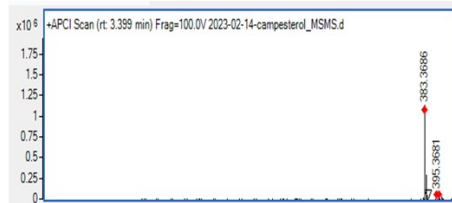
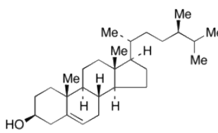
Ergosterol

➤ EIC 379.3359



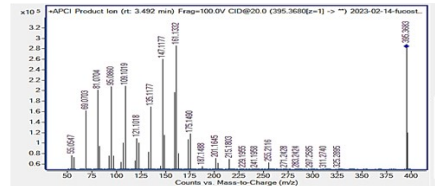
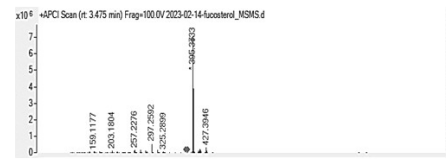
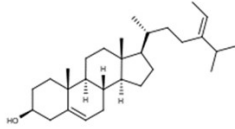
Campesterol

➤ EIC 383.3672



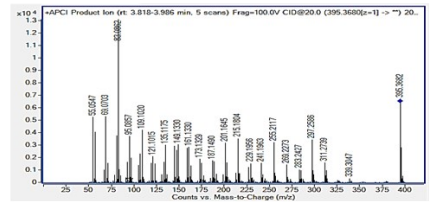
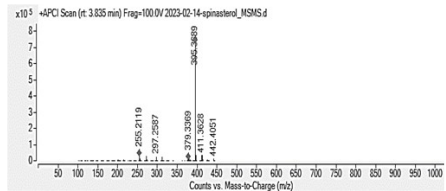
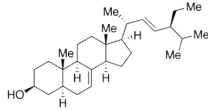
Fucoesterol

➤ EIC 395.3672



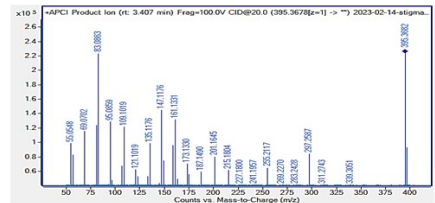
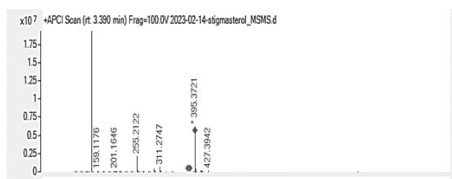
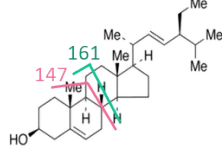
Spinasterol

➤ EIC 395.3672



Stigmasterol

➤ EIC 395.3672



Sitosterol

➤ EIC 397.3829

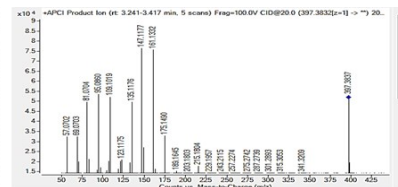
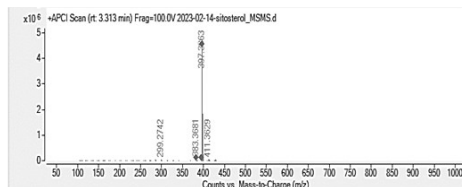
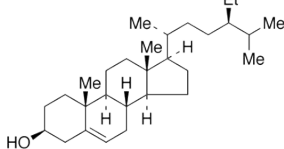


Fig. S8 MS1 and MS2 spectra of sterol standards. SFC-MS conditions in experimental section

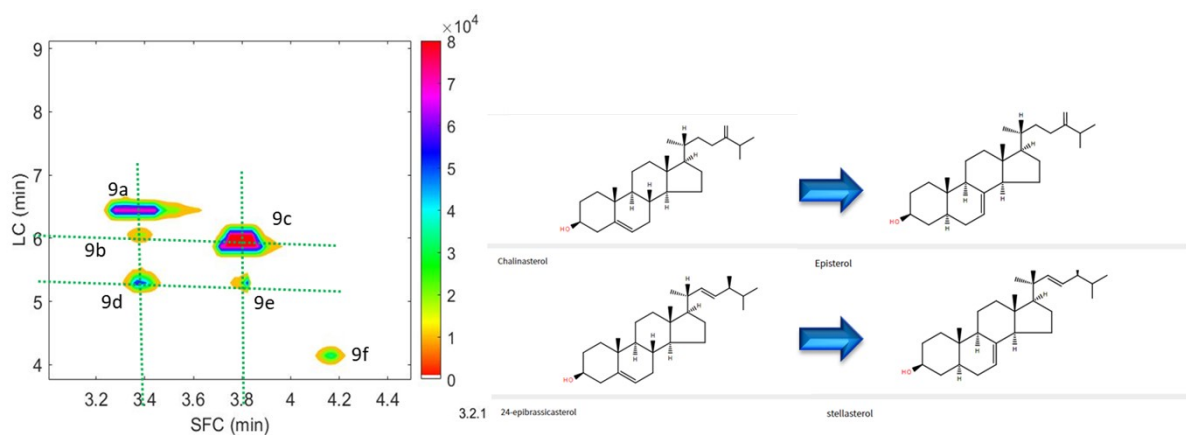


Fig. S9 Extracted ion chromatogram for m/z 381.3516 in *Scenedesmus obliquus* extract and possible structures in Δ^5 and Δ^7 , proposed by MS Finder and sharing the same side chain.

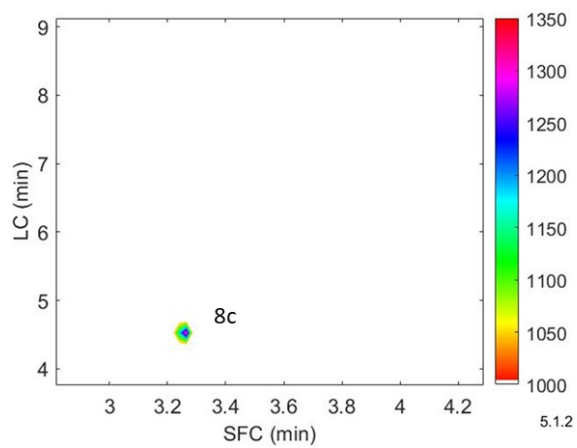


Fig. S10 Extracted ion chromatogram for m/z 367.3367 in *Padina pavonica* extract