Supplementary Appendix

Technical Note: Pine Rosin Identified as a Toxic Cannabis Extract Adulterant

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Materials and Methods

Quantitative NMR: The cannabis extract adulterant (CEA) sample was dissolved in $CDCl_3$ (Cambridge Isotope Laboratories) and acquired at 512 scans, a 6.7 second repetition rate, with a 30° flip angle, and with 64 k data points on a Bruker Avance III 600 MHz NMR spectrometer. Spectra were processed with 0.3 Hz of line broadening with a final data size of 64 k real data points. Quantification was performed using Global Spectral Deconvolution from MestreLab software by comparing analyte peaks to that of a pure standard of caffeine (Sigma Aldrich) as a $CDCl_3$-soluble internal standard. The masses of internal standard and CEA sample added to the NMR tube were then used to calculate an approximate %mass of identified components in the sample.[1]

Semi preparative HPLC: Fractions from the HPLC chromatogram were collected manually using the method in Nilsson *et al.*[2] using an 25 cm x 10 mm, 5 μm Discovery C18 semi-preparative column on a Waters 1525 Binary HPLC Pump and a Waters 2996 Photodiode Array Detector. Product peaks were eluted using an isocratic method consisting of 80 % 95:5 MeOH:H_2O and 20 % 5:95 MeOH:H_2O with 0.05 % formic acid in each with a total flow of 3.5

mL/min. Methanol was removed via rotary evaporation, and product was extracted in dichloromethane.

HPLC-ESIMS: The chromatogram was collected on an Vanquish UHPLC system. 20 µL of CEA in methanol at 930 ng/µL were injected over an Acclaim RSLC Polar Advantage II 3 µm, 120 Å 3.0 x 75 mm column using the following elution program: hold 30 % A for 5 min., ramp to 27 % A until 18 min., hold until 40 min. with a total flow of 0.5 mL/min. Solvent A: 0.05 % formic acid in H_2O, solvent B: 0.05 % formic acid in methanol. MS data was acquired using a high-resolution (35,000) Thermo Scientific Q Exactive Mass Spectrometer with an electrospray ionization source operating in the positive mode. The Orbitrap was externally calibrated prior to data acquisition allowing accurate mass measurements for $[M+H]^+$ to be obtained within 4 ppm. The ionization interface was operated using the following settings: source voltage, 4 kV; sheath and auxiliary gas at 75 and 20 units respectively; capillary temperature, 400 °C. Ionization in the positive mode allowed identification of the fatty acid amide oleamide, but the negative mode would provide higher ionization efficiency for identifying pine rosin components (which are organic acids) at small concentrations.

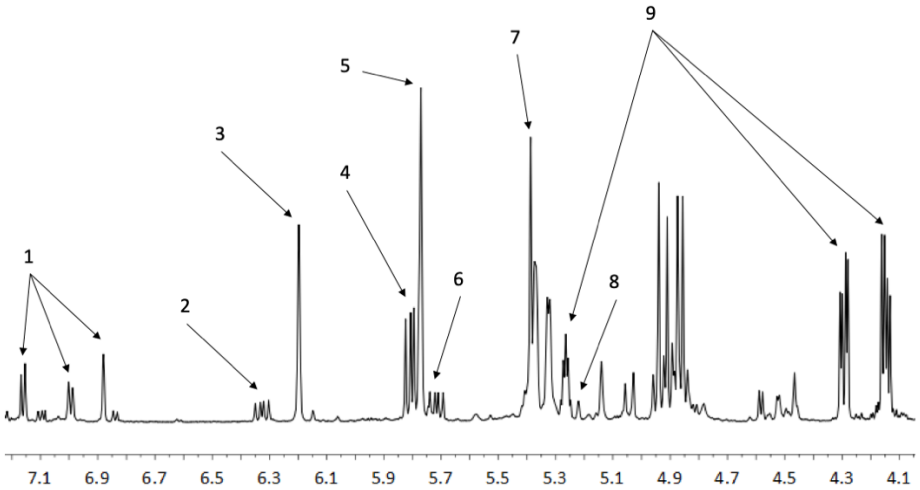

Figure S1: ^{1}H NMR spectrum of CEA showing relevant peaks for (1) dehydroabietic acid, (2) communic acid, (3) neoabietic acid, (4) isopimaric acid, (5) abietic acid, (6) pimaric acid, (7) palustric acid, (9) sandaracopimaric acid, (9) MCT oil.

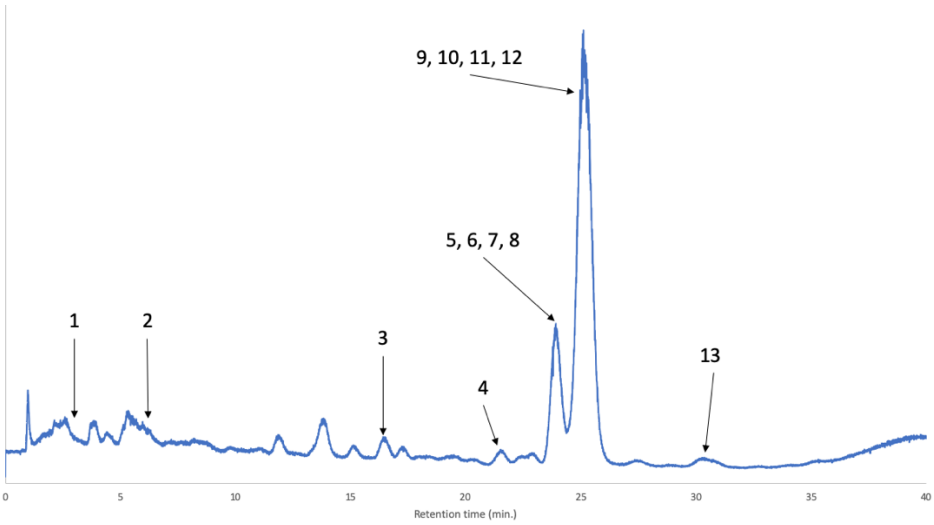

Figure S2: HPLC-ESIMS total ion chromatogram with several peaks of interest highlighted: (1) 15-hydroxyperoxyabietic acid, (2) 12-oxopimaric acid, (3) dehydroabietic acid, (4) communic acid, (5) pimarol, (6) pimaric acid, (7) sandaracopimaric acid, (8) palustric acid, (9) abietic acid, (10) oleamide, (11) neoabietic acid, (12) isopimaric acid, (13) sandaracopimarol.

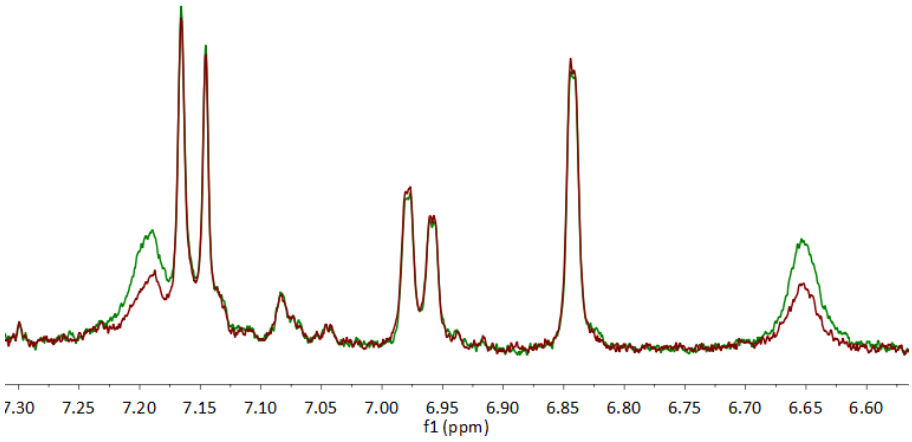

Figure S3: Overlaid ^{1}H NMR spectra of the semi-preparative HPLC band containing oleamide in DMSO-d_6 (maroon), and the same sample spiked with ~100 µg oleamide (green). An increase in the amide N-H proton peaks in the sample without the introduction of new peaks confirms the presence of this compound in CEA.

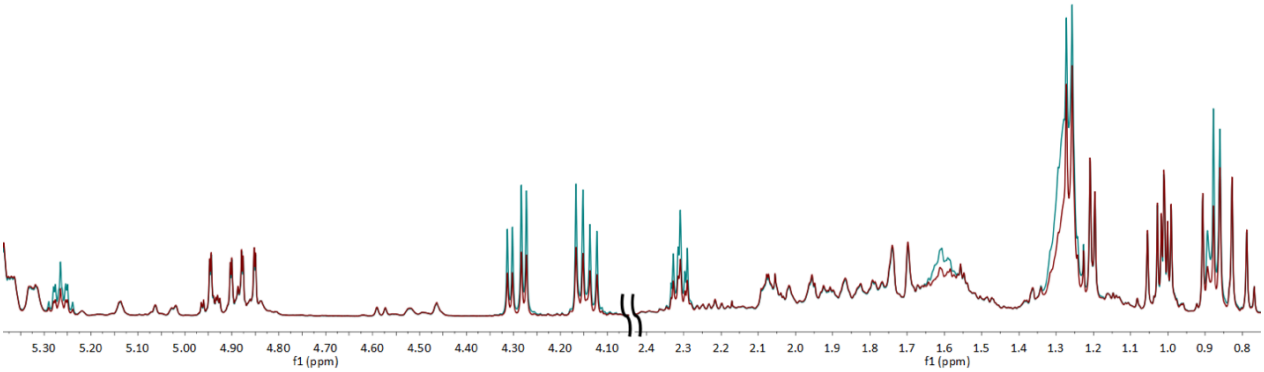

Figure S4: Overlaid ^{1}H NMR spectra of pure CEA in $CDCl_3$ (maroon) and the same sample spiked with ~1.3 mg of MCT oil. An increase in the proton signals at 5.26, 4.31, 4.28, 4.16, 4.13, 2.31, 1.61, 1.26, and 0.88 ppm in the sample without the introduction of new peaks confirms the presence of this substance in CEA.

References

[1] S.K. Bharti, R. Roy, Quantitative 1H NMR spectroscopy, TrAC Trends in Analytical Chemistry 35 (2012) 5-26. https://doi.org/10.1016/j.trac.2012.02.007
[2] U. Nilsson, N. Berglund, F. Lindahl, S. Axelsson, T. Redeby, P. Lassen, A.T. Karlberg, SPE and HPLC/UV of resin acids in colophonium-containing products, J Sep Sci 31(15) (2008) 2784-90. https://doi.org/10.1002/jssc.200800210