Supplementary Material

Organic geochemical methods: Samples were supplied as dried ground powders. Each sample was sequentially extracted with cyclohexane (CH) and dichloromethane (DCM) before acidification (10% hydrochloric acid/methanol; HCl/MeOH) of the residue (to methyl trans-esterify any bound acids) and subsequent recovery of any acid methyl esters in cyclohexane. Extracts recovered in this way were initially screened using high-temperature gas chromatography with flame ionisation detection (HTGC) before analysis using HTGC coupled with Time-of-Flight mass spectrometry (HTGC-ToF-MS).

Cyclohexane (CH) extraction: Around 5 g of each powdered sample was accurately weighed into a vial and extracted with CH (3 × 5 mL) using sonication (40 °C; 5 min). The sample was centrifuged (2500 rpm/5 min), and the supernatant was transferred to a pre-weighed vial through a pre-extracted (DCM; Soxhlet, 8 h) cotton wool filter plug in a Pasteur pipette. Solvent was removed under a gentle stream of nitrogen (N_2/60°C), and the extract was weighed (Table S1). The extract was reconstituted to 1 mg·mL^{-1} in cyclohexane for initial screening using HTGC (Figure S1). Following initial screening, the extracts were transferred to 2 mL GC vials with washings (3 × 100 µL CH), dried (N_2/60 °C), reconstituted in CH to provide an equivalent HTGC response of ca. 100 pA, and analysed using HTGC-ToF-MS (Figures S2–10). Calculated biomarker parameters are presented in Table S2.

Dichloromethane (DCM) extraction: Sample residue from CH extraction was further extracted with DCM (3 × 5 mL) with sonication (5 min). The mixture was centrifuged (2500 rpm/5 min), and the supernatant was transferred to a pre-weighed vial through a pre-extracted (DCM; Soxhlet, 8 h) cotton wool filter plug in a Pasteur pipette. Solvent was removed under a gentle stream of nitrogen (N_2/50 °C), and the extract was weighed (Table S1). Extracts were reconstituted to 1 mg·mL^{-1} in DCM for initial screening using high-temperature gas chromatography (HTGC; Figure S11). Extracted sample residues were dried overnight in a heater block (50 °C).

Methyl esterification and extraction of esters: 10% Conc. HCl/MeOH (v/v; 10 mL) was added to dried sample residues, and vials were left to stand loosely capped for 4 hours until bubbling had ceased. Vials were then capped, taped, and heated in a heater block (60 °C, 1 h; 70 °C, 2 h). After cooling, water (10 mL) was added, and the samples were extracted into CH (1 × 3 mL plus 2 × 2 mL) by mixing and centrifugation (2500 rpm/5 min). The supernatant was transferred to a pre-weighed vial through a pre-extracted (DCM; Soxhlet, 8 h) cotton wool filter plug in a Pasteur pipette. Solvent was removed under a gentle stream of nitrogen (N_2/55 °C), and the extract was weighed (Table S1). Extracts were reconstituted to 1 mg·mL^{-1} in CH for initial screening using high-temperature gas chromatography (HTGC; Figure S12).

Table S1. Gravimetric data from the cyclohexane (CH) and dichloromethane (DCM) extraction of carbonate conduit concretion samples and the extraction of esters following methyl esterification (*no weight recorded but 100 µL solvent added to provide nominal maximum concentration 0.5 mg·mL^{-1}).

Sample	Mass (g)	CH Extract (mg)	DCM extract (mg)	Methyl ester extract (mg)
Procedural blank	0.0000	0.1	0.1	0.1
#5a	5.2569	0.2	0.1	0.3
#5b	5.5259	0.2	0.4	0.1
#5 top	5.4810	0.2	0.2	0.3
#5 bottom	5.3215	0.1	0.4	0.2
#8a	5.2222	0.1	0.3	0.2
#8b	5.5662	0.3	0.1	0.0*
#14	5.1410	0.3	0.3	0.1
#17	5.0228	0.3	0.3	0.7
#20	5.4529	0.1	0.1	0.1
#24	5.2494	0.1	0.5	0.0*
#38 top	5.4179	0.3	0.2	0.1
#38 bottom	5.3897	0.8	0.2	0.1
#42	5.3467	0.3	0.3	0.1

High-temperature gas chromatography with flame ionisation detection: A 0.5 µL sample aliquot was manually injected via a cool-on-column inlet (track oven mode; +3 °C) onto a Vf-5ht Ultimetal column (15 m × 0.25 mm × 0.1 µm; Agilent Technologies Limited, UK) operated with helium carrier gas (constant flow mode; 1 mL·min^{-1}) and the GC oven (HP6890) programmed from 40–430°C at 10 °C·min^{-1} with a 10 minute isothermal hold. The flame ionisation detector (FID; high-temperature jet) was operated at 430 °C with FID gas flows optimised at H_2 40 mL·min^{-1}, air 450 mL·min^{-1}, and N_2 make-up 45 mL·min^{-1}).

High-temperature gas chromatography coupled with Time-of-Flight mass spectrometry: A 0.5 µL sample aliquot was manually injected via a cool-on-column inlet (track oven mode; +3 °C) onto a Vf-5ht Ultimetal column (15 m × 0.25 mm × 0.1 µm; Agilent Technologies Limited, UK) operated with helium carrier gas (constant flow mode; 2 mL min^{-1}) and the GC oven (HP6890) programmed from 40–430 °C at 20 °C·min^{-1} with a 2 minute isothermal hold. The chromatographic column was connected with a Siltite™ mini union via a high-temperature silica transfer capillary (nom. 2 m × 0.18 mm) through a vendor-modified heated (415 °C) transfer line into a BenchToF Select eV (Markes International Ltd, UK) with the ion source at 330 °C. ToF-DS software (Markes International Ltd, UK) was used to operate the mass spectrometer (tuned at 12 eV) and record (*m*/*z* 50–800) and process data.

Squalene (Figures S3 (*m*/*z* 136) and S5) was identified by mass spectral library matching as present in all samples, but it occurs in most organisms and is not diagnostic.

Mass spectra of three (relatively) abundant components (Figures S6–8) all contained a putative molecular ion at *m*/*z* 476, which was used to generate extracted ion chromatograms for these compounds in the sample extracts (Figures S4a,b). These compounds were not present in all samples (absent in samples #17, #24, and #38 top) and the relative abundance of the three peaks appeared to vary in different samples (Figure S9). These data match well with that obtained previously for proximal samples. The identity of these compounds remains uncertain. Previous analysis in our laboratory demonstrated that they were not derivatised by saponification/acidification or trimethylsilylation. In addition, high-resolution mass spectrometry indicated that these compounds may contain two nitrogen atoms ($C_{30}H_{56}N_2O_2$; 3.4 ppm precision). However, other hydrocarbon formulae with less

precisely matching accurate mass also warrant consideration (e.g.$C_{35}H_{56}$, 8 DBE, 12 ppm; $C_{36}H_{44}$, 15 DBE, −185 ppm; $C_{34}H_{68}$, 1 DBE, 209 ppm). Additional experiments in our laboratory suggest that these compounds, measured as $[M+H]^+$ with accurate mass *m*/z477.44543, have the formulae $C_{35}H_{57}$ (−0.048 ppm), indicating the hydrocarbon has the formula $C_{35}H_{56}$ (8 DBE). The late elution and EI mass spectra of these peaks are reminiscent of bacterial sporulenes reported from the cyclisation of regular polyprenes and may be indicative of oxidative stress and an aerobic environment [43,44]. Example extracted ion chromatograms for triterpene and sterane (*m*/*z* 191 and 217, respectively) biomarkers are presented in Figure S10.

The two large peaks observed to elute between 11-12 minutes, and the peak eluting at 18.2 minutes, in the HTGC chromatograms of most of the DCM extracts (Figure S11) were identified using HTGC-MS as phthalate esters. These are common contaminants in geochemical analysis.

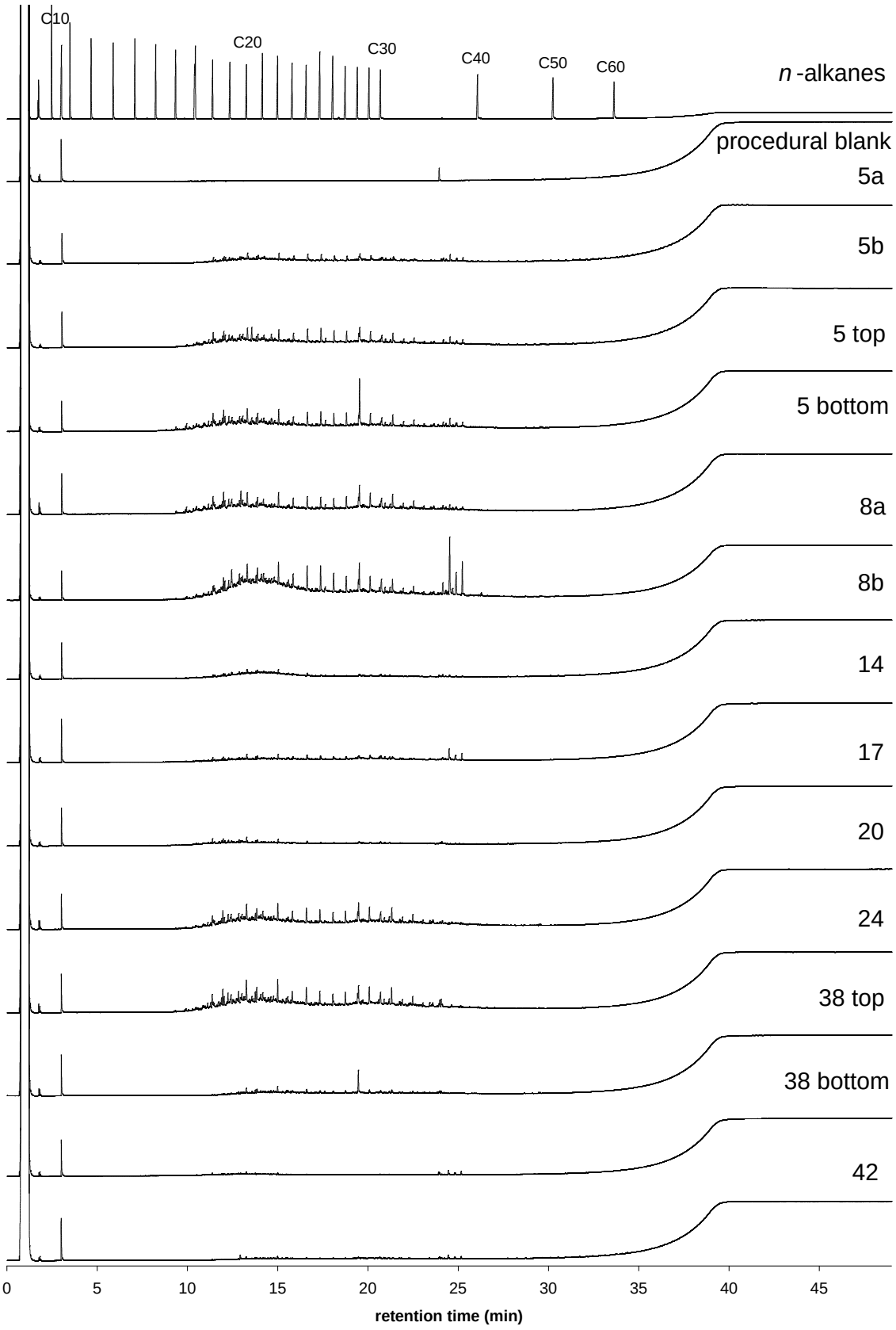

Figure S1. HTGC chromatograms of in-house nC$_{10\text{-}30,40,50,60}$ alkanes standard, and cyclohexane extracts from procedural blank and carbonate conduit concretions.

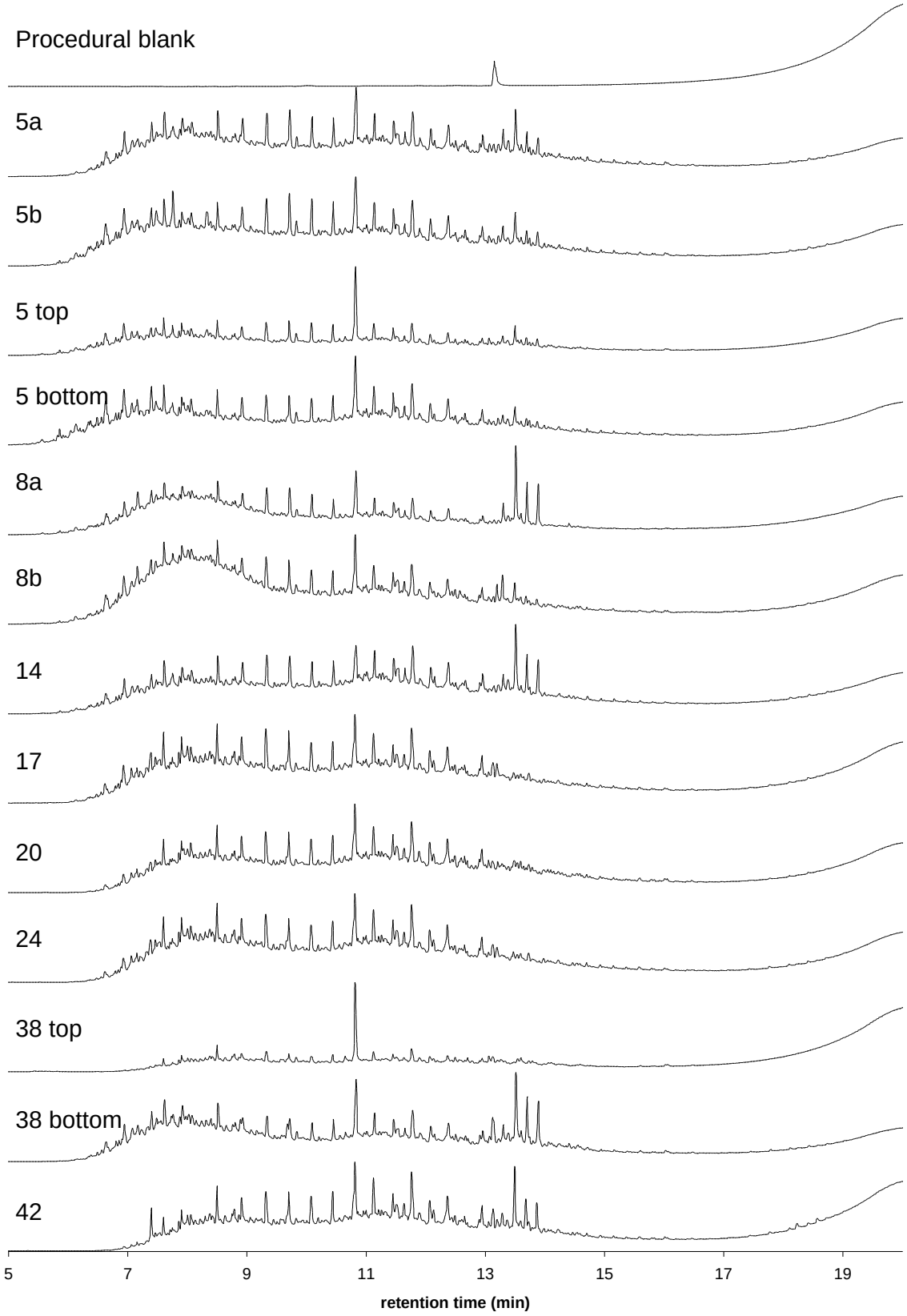

Figure S2. HTGC-ToF-MS (12 eV) total ion chromatograms of the cyclohexane extracts of the procedural blank and carbonate conduit concretions.

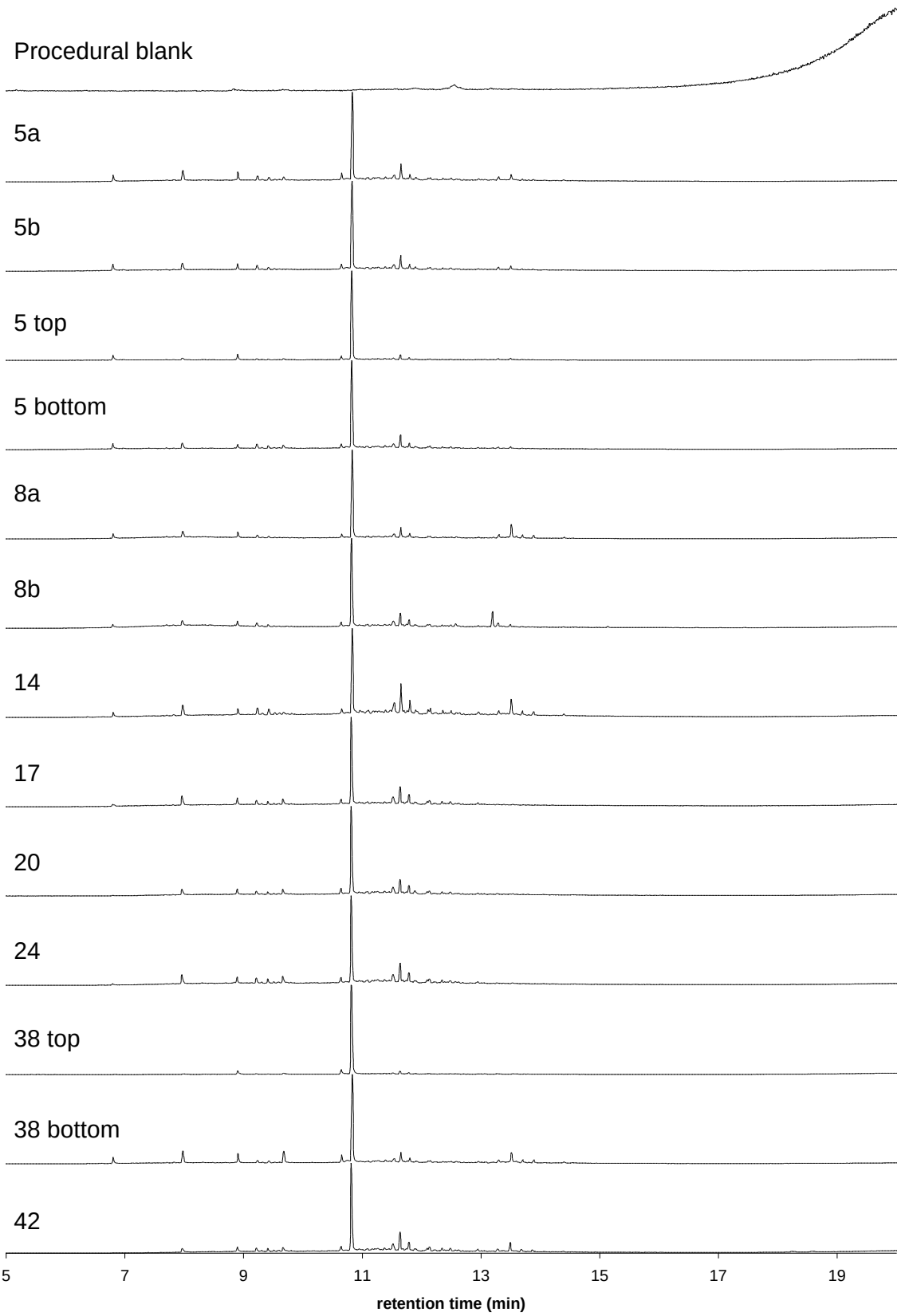

Figure S3. HTGC-ToF-MS (12 eV) extracted ion chromatograms (*m*/*z* 136) of the cyclohexane extracts of the procedural blank and carbonate conduit concretions. Large peak at ~ 11 minutes assigned to squalene.

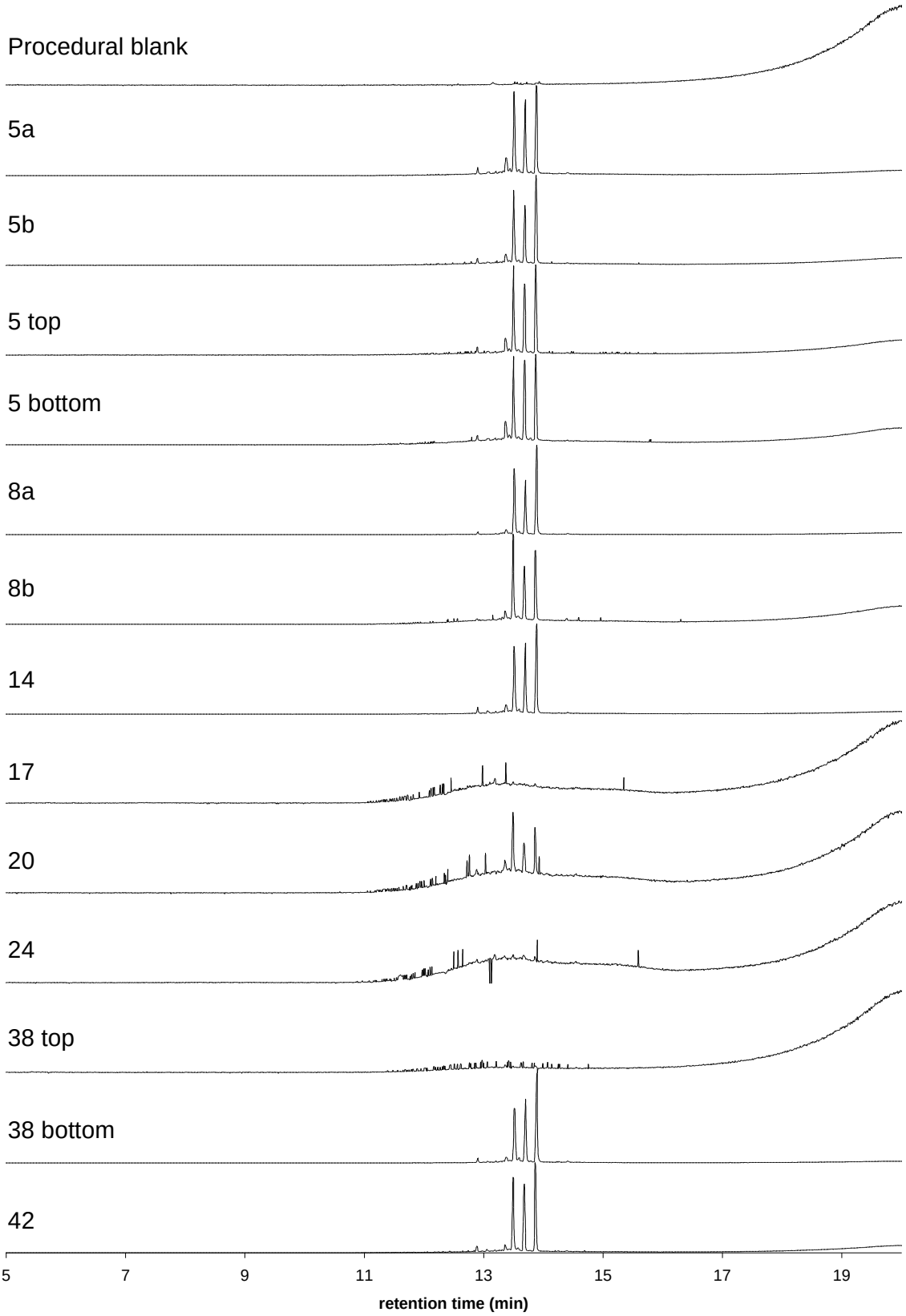

Figure S4a. HTGC-ToF-MS (12 eV) extracted ion chromatograms (*m*/*z* 476) of the cyclohexane extracts of the procedural blank and carbonate conduit concretions.

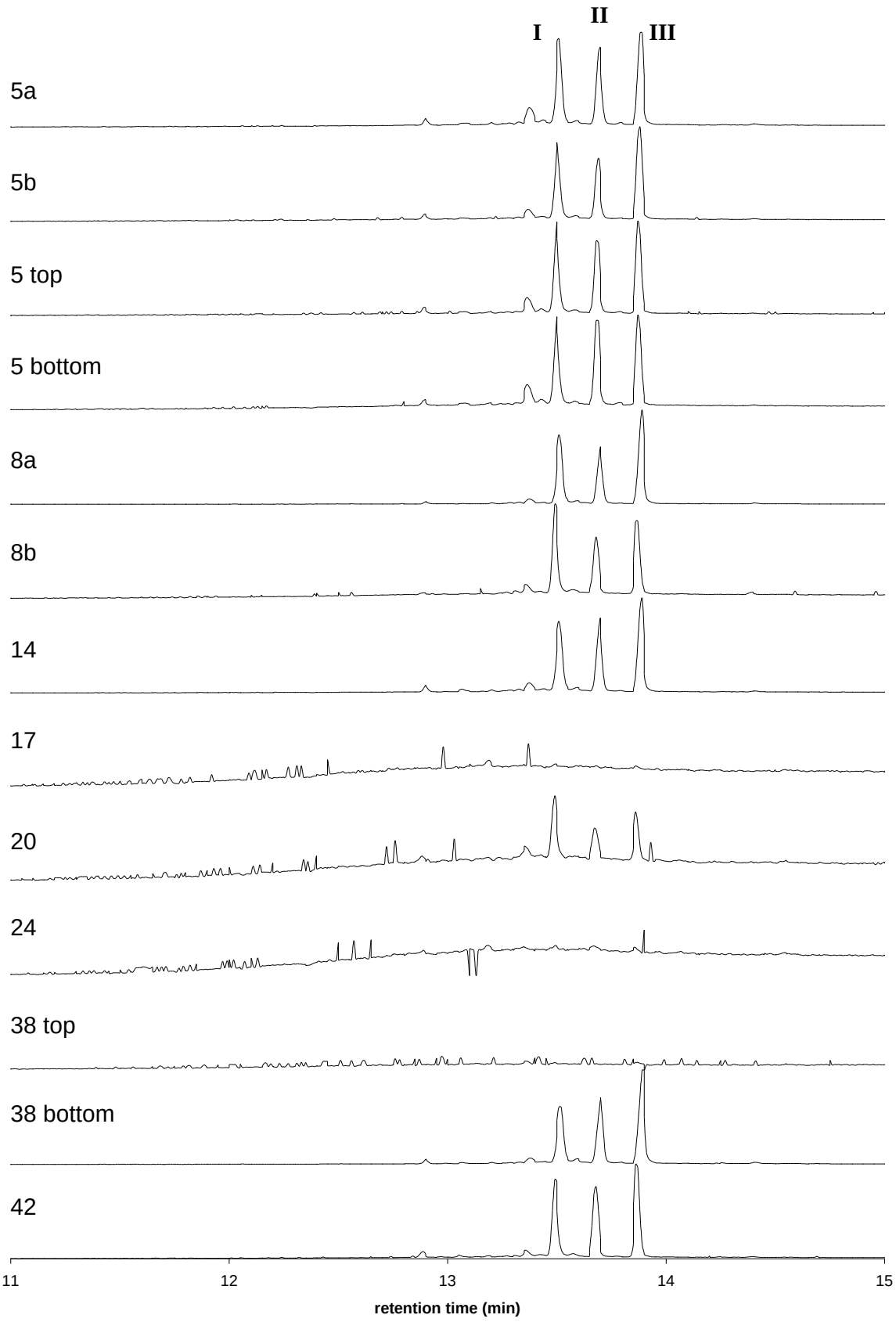

Figure S4b. Zoomed region HTGC-ToF-MS (12 eV) extracted ion chromatograms (*m*/*z* 476) of the cyclohexane extracts of the procedural blank and carbonate conduit concretions.

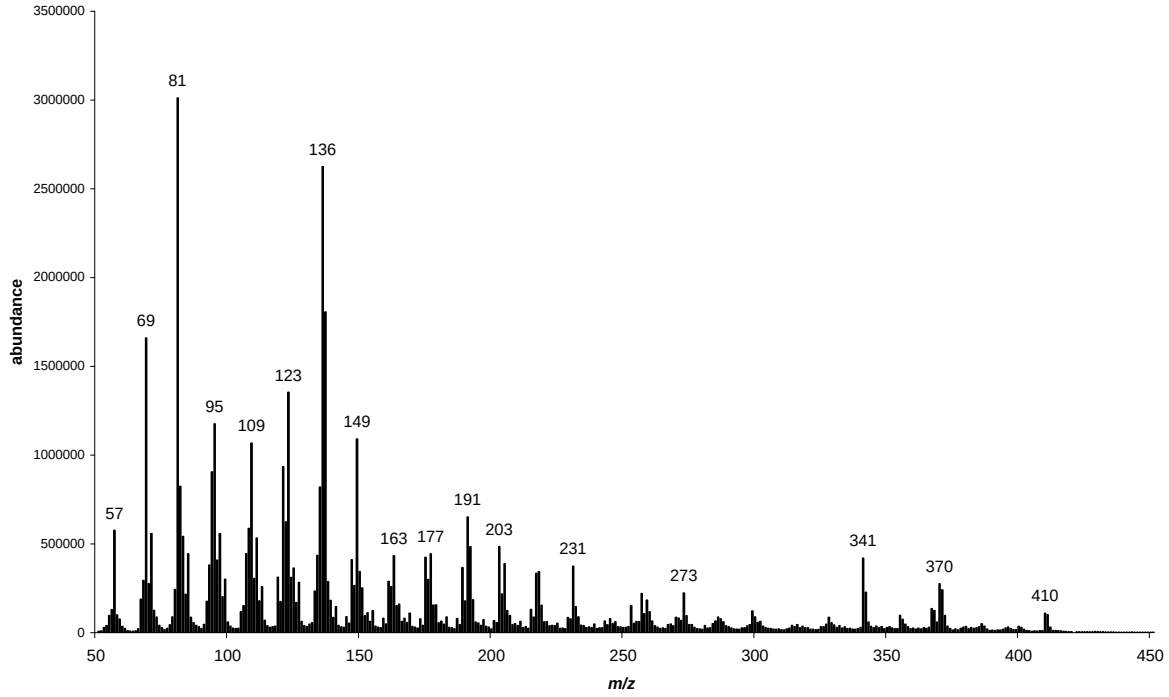

Figure S5a.12eV mass spectrum of peak at 10.81 minutes (Figures 2 and 3; example from sample 20 shown).

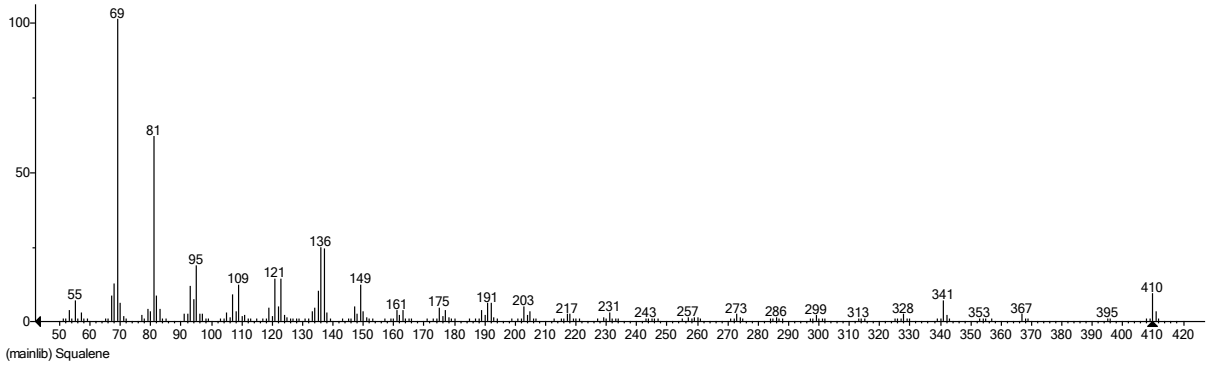

Figure S5b. NIST library mass spectrum (70 eV) of squalene.

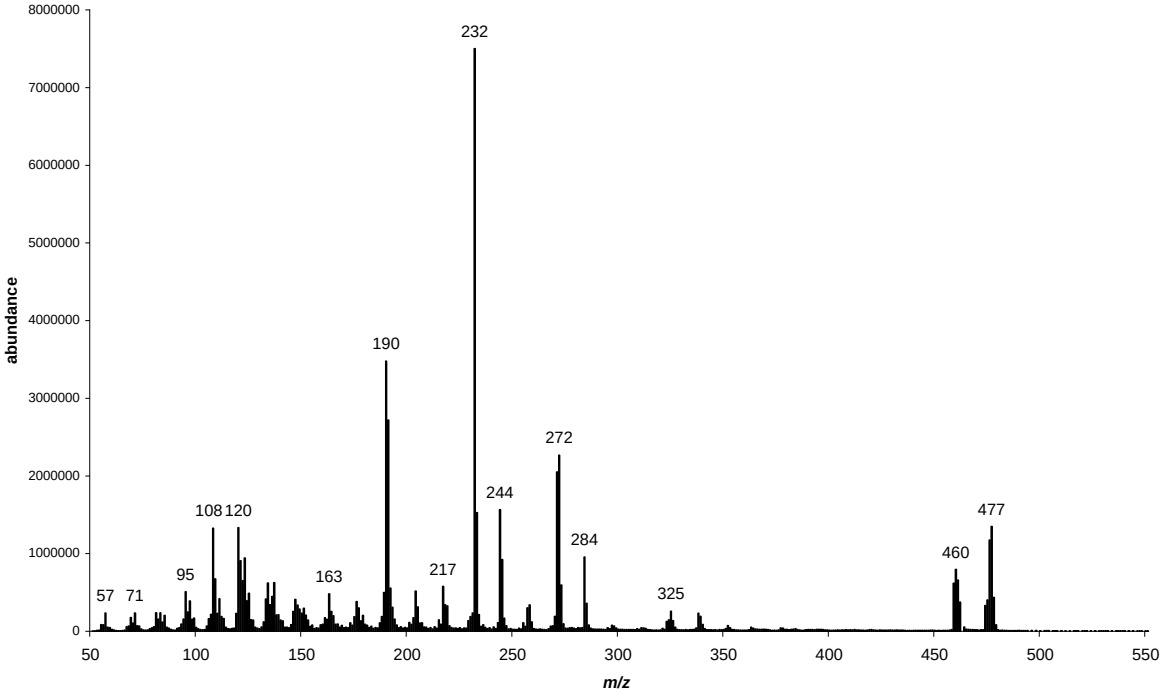

Figure S6. Mass spectrum of peak I at 13.51 minutes (Figures S2 and S4; example from sample 14 shown).

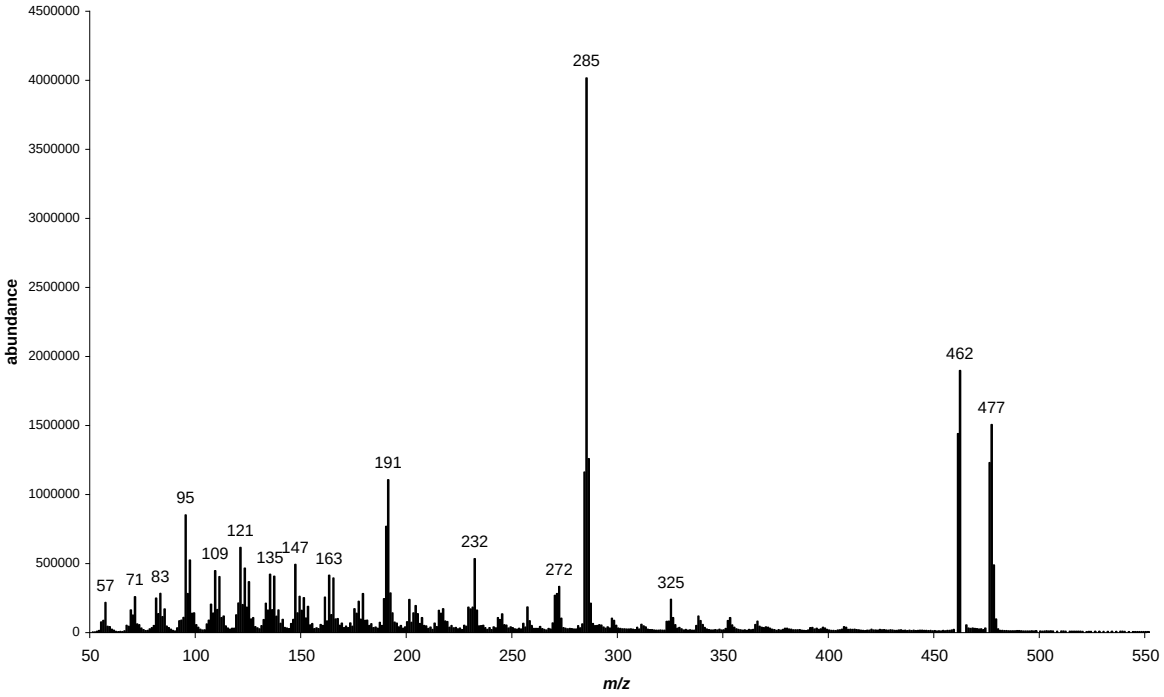

Figure S7. Mass spectrum of peak II at 13.70 minutes (Figures S2 and S4; example from sample 14 shown).

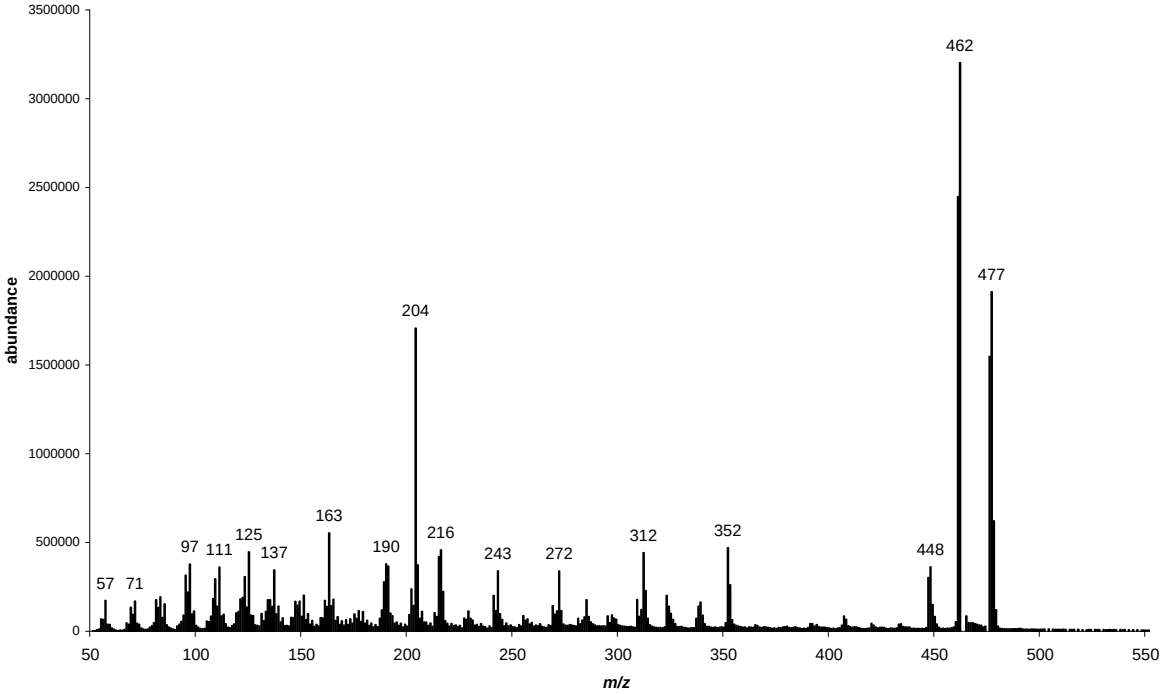

Figure S8. Mass spectrum of peak at 13.89 minutes (Figures S2 and S4; example from sample 14 shown).

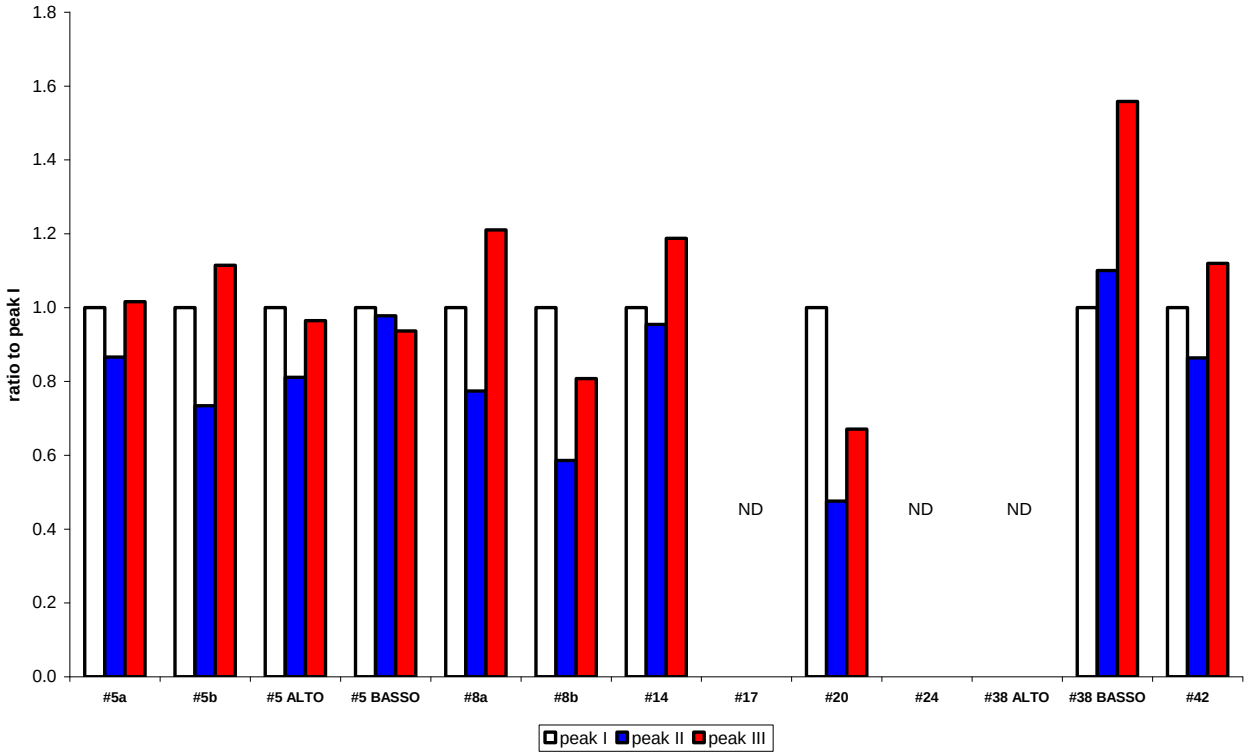

Figure S9. Peak area ratios using *m*/*z* 476 extracted ions for peak II/peak I (Figure S4b: 13.70 minutes, Figure 7/13.51 minutes, Figure S6) and for peak III/peak I (Figure S4b: 13.89 minutes, Figure 8/13.51 minutes, Figure S6).

Table S2. Calculated biomarker parameters.

Sample	C range	CPI[a]	CPI (I)[b]	CPI[c]	OEP (1)[d]	OEP (2)[e]	ACL[f]	PR I[g]	PR II[h]
Procedural blank	ND	--	--	--	--	--	--	ND	ND
#5a	20-55	1.35	1.06	1.04	0.78	1.12	28.26	0.9	1.0
#5b	20-54	1.39	1.13	1.04	0.83	1.17	28.18	0.7	1.1
#5 top	19-50	1.35	1.03	1.05	0.67	1.17	28.17	0.8	1.0
#5 bottom	19-53	1.42	1.12	1.05	0.83	1.20	28.59	1.0	0.9
#8a	20-48	1.41	1.05	1.04	0.69	1.16	27.91	0.8	1.2
#8b	20-46	1.49	1.08	1.05	0.70	1.26	28.24	0.6	0.8
#14	19-52	1.47	1.11	1.04	0.81	1.18	28.46	1.0	1.2
#17	21-48	1.43	1.04	1.04	0.71	1.18	28.34	ND	ND
#20	19-51	1.41	1.05	1.05	0.72	1.22	28.55	0.5	0.7
#24	20-53	1.49	1.06	1.05	0.67	1.22	28.57	ND	ND
#38 top	23-35	1.37	1.08	1.05	0.85	1.12	28.56	ND	ND
#38 bottom	22-51	1.42	1.02	1.05	0.55	1.19	28.54	1.1	1.6
#42	21-52	1.53	1.08	1.05	0.67	1.22	28.64	0.9	1.1

$$CPI^{a} = \frac{\left[\frac{C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{24}+C_{26}+C_{28}+C_{30}+C_{32}} + \frac{C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{26}+C_{28}+C_{30}+C_{32}+C_{34}}\right]}{2}$$

$$CPI(I)^{b} = \frac{2(C_{23} + C_{25} + C_{27} + C_{29})}{[C_{22} + 2(C_{24} + C_{26} + C_{28}) + C_{30}]}$$

$$CPI^{c} = 0.5\left(\frac{C_{27}+C_{28}+C_{29}+C_{30}+C_{31}+C_{32}+C_{33}}{C_{26}+C_{27}+C_{28}+C_{29}+C_{30}+C_{31}+C_{32}}\right) + 0.5\left(\frac{C_{27}+C_{28}+C_{29}+C_{30}+C_{31}+C_{32}+C_{33}}{C_{28}+C_{29}+C_{30}+C_{31}+C_{32}+C_{33}+C_{34}}\right)$$

$$OEP(1)^{d} = \frac{(C_{21} + 6C_{23} + C_{25})}{(4C_{22} + 4C_{24})}$$

$$OEP(2)^{e} = \frac{(C_{25} + 6C_{27} + C_{29})}{(4C_{26} + 4C_{28})}$$

$$ACL^{f} = \frac{25(C_{25}) + 26(C_{26}) + 27(C_{27}) + 28(C_{28}) + 29(C_{29}) + 30(C_{30}) + 31(C_{31}) + 32(C_{32}) + 33(C_{33})}{(C_{25} + C_{26} + C_{27} + C_{28} + C_{29} + C_{30} + C_{31} + C_{32} + C_{33}}$$

CPI[a] and CPI(I)[b][45], OEP(1)[d] and OEP(2)[e] [46]. CPI[c] and ACL[f] [47]. PR I[g] = peak area *m*/*z* 476 at 13.70 minutes/peak area *m*/*z* 476 at 13.51 minutes (Peak II/Peak I e.g. Figure 4a). PR II[h] = peak area *m*/*z* 476 at 13.70 minutes/peak area *m*/*z* 476 at 13.51 minutes (Peak III/Peak I e.g. Figure 4a).

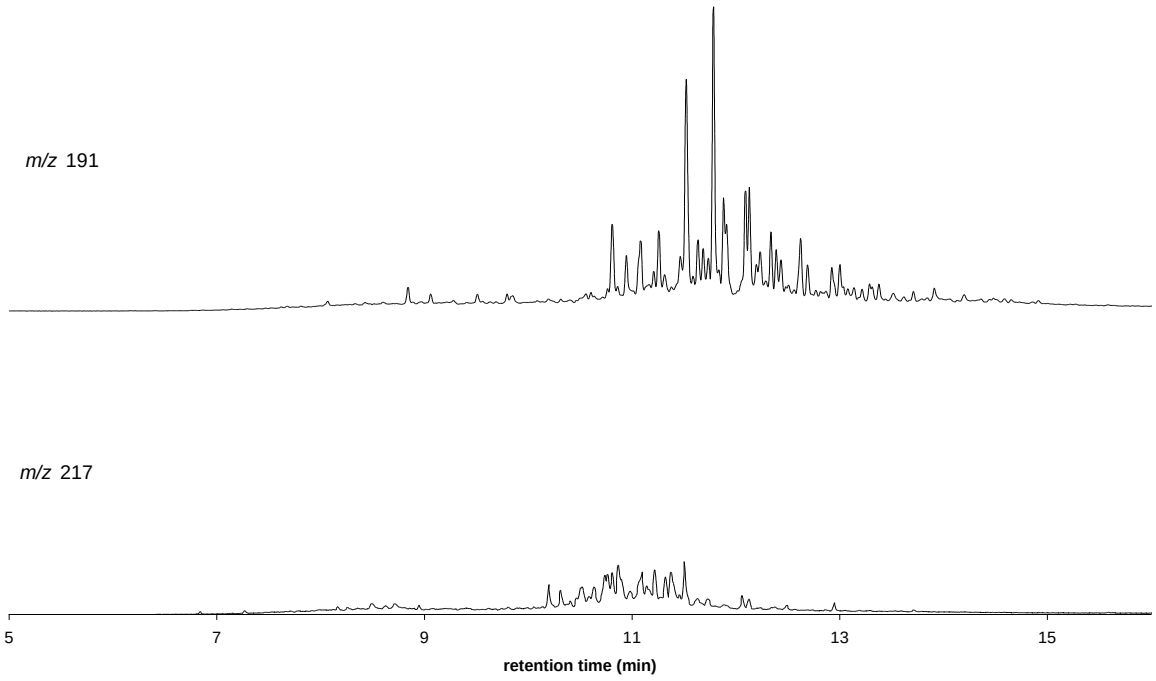

Figure S10. HTGC-MS extracted ion chromatograms (upper *m*/*z* 191; lower *m*/*z* 217) indicating the distribution of triterpanes and sterane biomarkers in sample 24. The ratios of 20R/S steranes and most of the 22R/S triterpene ratios are consistent with mature petroleum rather than the immature hydrocarbons expected in Pleistocene sediments. Traces of less mature indigenous hydrocarbons are observable in only some samples, consistent with the slightly elevated CPI and OEP ratios in some samples (Table S2).

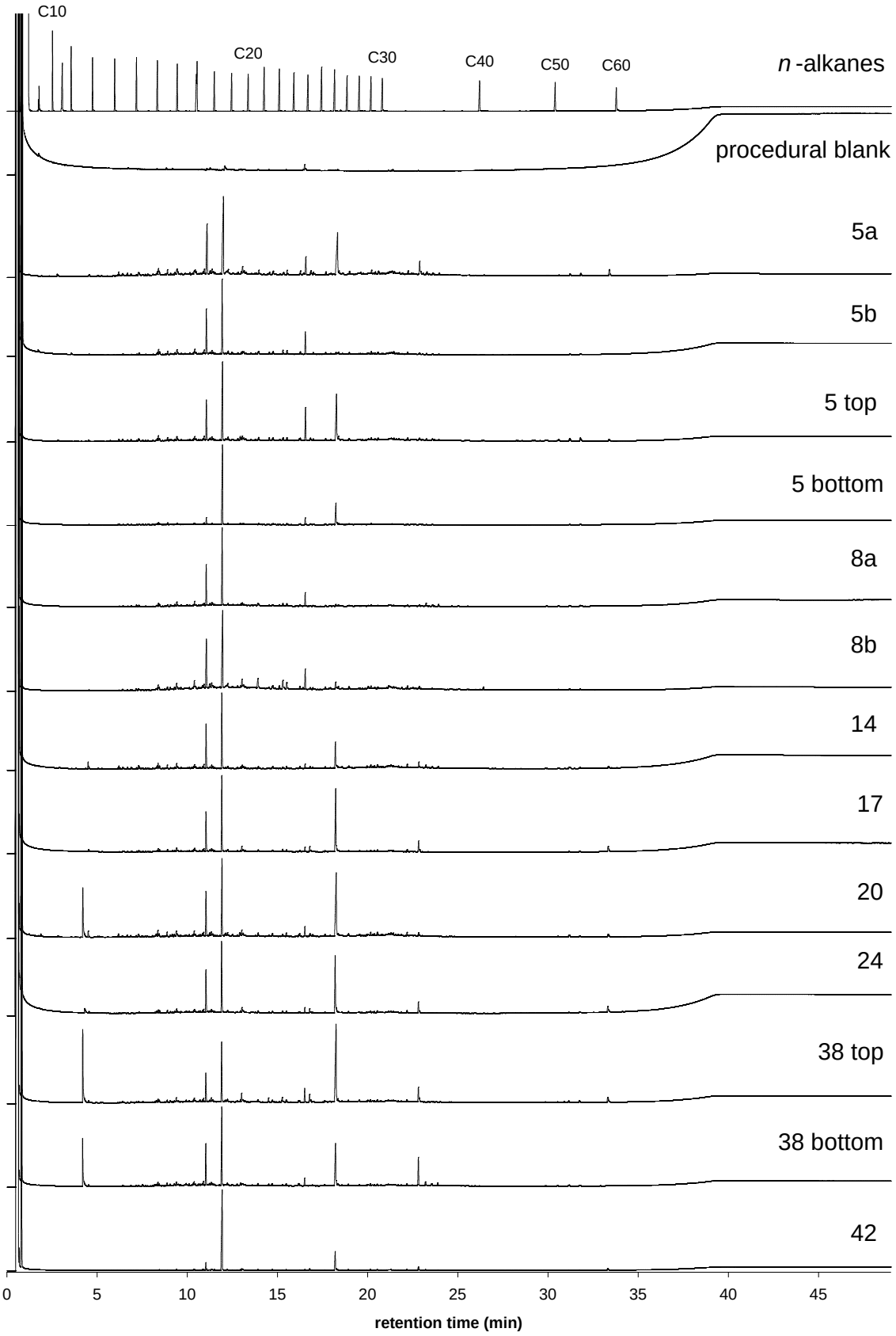

Figure S11. HTGC chromatograms of in-house nC$_{10\text{-}30,40,50,60}$$n$-alkanes standard, and dichloromethane extracts of residues following cyclohexane extraction from procedural blank and carbonate conduit concretions.

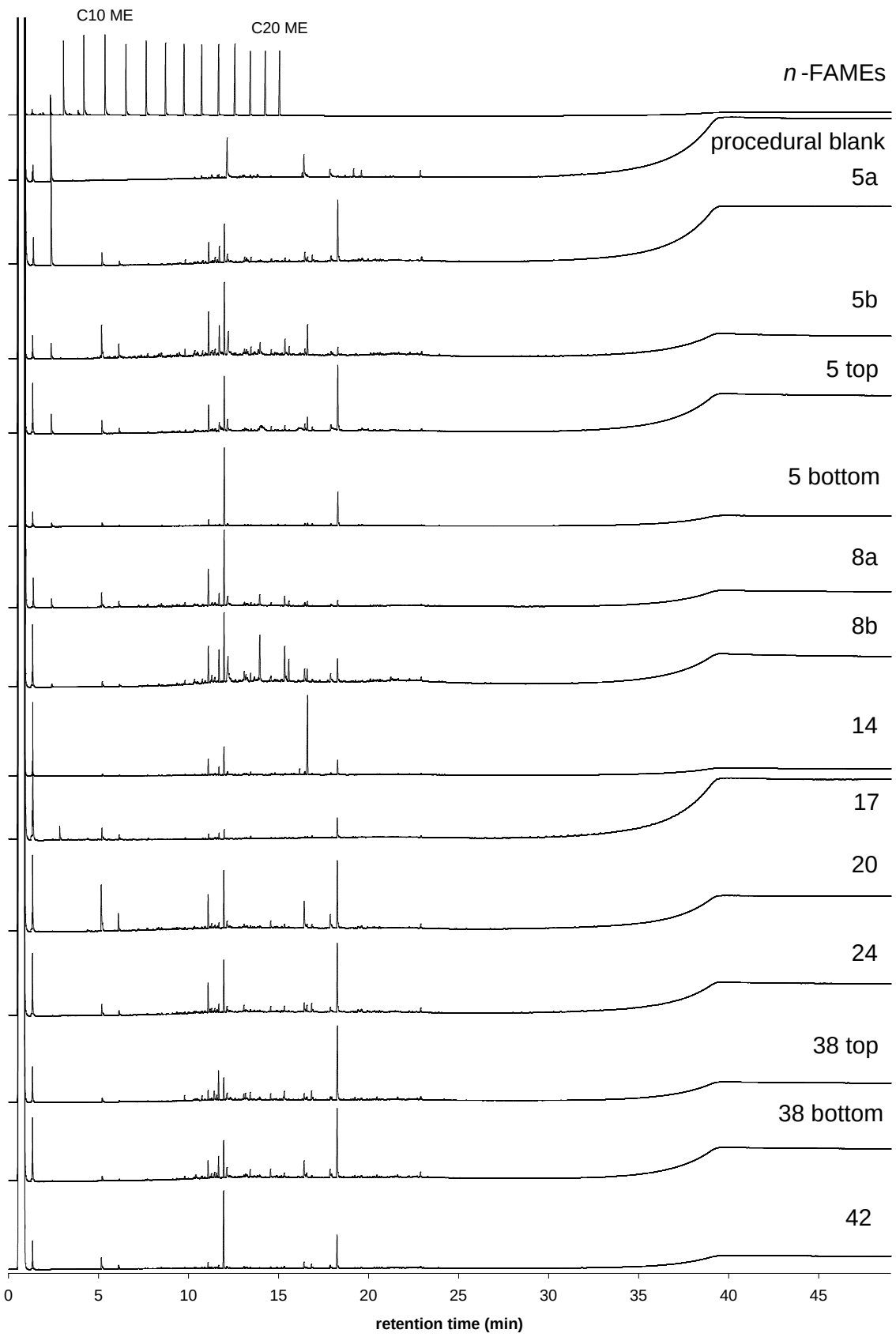

Figure S12. HTGC chromatograms of in-house $C_{8\text{-}20}$ *n*-fatty acid methyl esters (*n*-FAMEs) standard, and cyclohexane (methyl ester) extracts of residues following cyclohexane and dichloromethane extraction from procedural blank and carbonate conduit concretions.

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