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Organic molecules revealed in Mars's Bagnold Dunes by Curiosity's derivatization experiment

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The wet chemistry experiments on the Sample Analysis at Mars instrument on NASA's Curiosity rover were designed to facilitate gas chromatography mass spectrometry analyses of polar molecules such as amino acids and carboxylic acids. Here we present the results of such a successful wet chemistry experiment on Mars on sand scooped from the Bagnold Dunes with the *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide derivatization agent. No amino-acid derivatives were detected. However, chemically derivatized benzoic acid and ammonia were detected. Mass spectra matching derivatized phosphoric acid and phenol were present, as were several nitrogen-bearing molecules and as yet unidentified high-molecular-weight compounds. The origin of these compounds, including those that may be internal to the Sample Analysis at Mars background, is examined. This derivatization experiment on Mars has expanded the inventory of molecules present in Martian samples and demonstrated a powerful tool to further enable the search for polar organic molecules of biotic or prebiotic relevance.

Since its arrival on Mars in 2012, NASA's Curiosity rover has been exploring the nature and extent of ancient habitable environments in Gale Crater. The search for organic compounds and the characterization of their origins (prebiotic, biotic or abiotic) with the Sample Analysis at Mars (SAM) investigation is a key part of the mission[1]. So far, organic molecules originating from the sediments, including chlorinated hydrocarbons, sulfur-bearing organics, fragments of alkyl and aromatic compounds, and mid-chain alkanes, have been identified in drilled rock samples[2–5]. These molecules were released by pyrolysis at temperatures up to ~850 °C and identified by evolved gas analysis (EGA) and gas chromatography mass spectrometry (GCMS). SAM can also perform wet chemistry experiments using derivatization with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide–dimethylformamide (MTBSTFA-DMF) and thermochemolysis with tetramethylammonium hydroxide (TMAH). In this Article, we report on a nominal derivatization experiment of SAM in which the sample was saturated with MTBSTFA-DMF before heating, designed specifically to aid in the recovery of polar molecules such as carboxylic acids and amino acids.

The derivatization experiment (SAM derivatization experiment and Extended Data Figs. 1 and 2) was conducted on sol 1909 of the mission in December 2017, as Curiosity was exploring Gale Crater's Vera Rubin Ridge. Months earlier, in March 2017, a sample from the Bagnold Dunes (Fig. 1a) given the name Ogunquit Beach (OG) (Mineralogy of Ogunquit Beach and content of volatiles) had been scooped into the Collection and Handling for In-Situ Martian Rock Analysis (CHIMRA) device with a maximum sample collection depth of 3.5 cm (Fig. 1b)[6]. The OG sample was sieved (150 µm) before portioning and subsequent analysis by SAM. Because Curiosity's drill was out of service for much of the Vera Rubin Ridge campaign, the team elected to conduct a SAM derivatization experiment on the OG sample in CHIMRA using two GC columns: MXT-20 and Chirasil-β Dex. Molecules produced from the derivatization experiment were trapped and released from a hydrocarbon (HC) trap to be injected either directly into the MXT-20 column or with an injection trap (IT) in the case of the Chirasil-β Dex. A follow-up analysis was performed after the derivatization experiment, which reproduced the previous GCMS experiment but without a sample.

The Bagnold Dunes had been studied for several months with the full complement of instruments aboard Curiosity. The weathered dune sample had been exposed to ionizing radiation and was not expected to be rich in well preserved ancient organic molecules[7]. However, it enabled not only a test of the derivatization protocol on the surface of Mars, but also a search for organic molecules present in the sample and a survey of those that might have been produced from reactions within the instrument. Here we present the derivatization EGA and GCMS results (including the identifications of derivatized compounds detected in the OG analysis), evaluate their potential sources and describe how studies motivated by these results enable optimization of the derivatization protocols that will continue to be used on Mars. Prior to the derivatization experiment, an OG sample was analysed by pyrolysis-GCMS to assess its contents in organics (see Extended Data Fig. 3).

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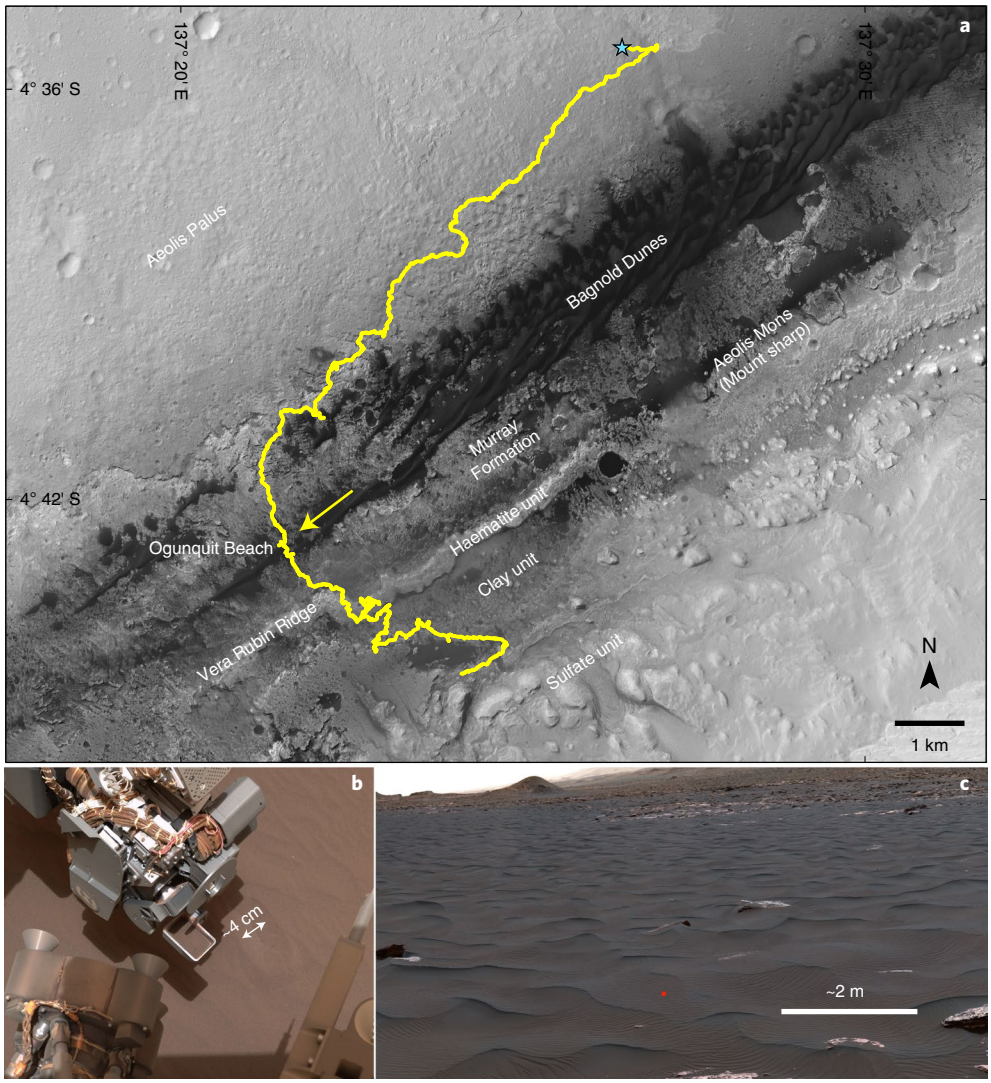

Fig. 1 | The sample acquisition in context. a, Curiosity's traverse (yellow) from the Bradbury landing (blue star) up to August 2021 (sol 3192), overlaid on an image acquired by the HiRISE camera. The yellow arrow indicates the location where the OG sample was scooped from the Bagnold dune field. **b**, The OG scoop sample after collection and before portioning and analysis by SAM, sol 1651 (29 March 2017). **c**, Part of a 360° panorama taken by Mastcam on sol 1646 (between 24 and 25 March 2017), showing the Bagnold dune field on lower Mount Sharp at the OG location. Metre-scale ripples are visible as well as sedimentary textures. The red dot indicates the location of the scooped OG sample. **a**, Credit: MSL/NASA-JPL/USGS/UofA. **c**, Credit: NASA/JPL-Caltech/MSSS.

Overview of results

Results revealed high-molecular-weight compounds in the EGA run (Fig. 2, top) and a complex chromatogram due to detector saturation for some mass-to-charge signals (m/z) during an elution of primary MTBSTFA and DMF by-products for both GC channels (Fig. 2, centre and bottom). The chromatogram obtained with the Chirasil-β Dex column shows the signal saturated by MTBSTFA by-products such as 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane (bi-silylated water derivative (BSW)), unreacted MTBSTFA, DMF and *N*-methyl-2,2,2-trifluoroacetamide (TFMA). The presence of unreacted MTBSTFA demonstrates that it was in excess and did not entirely react with the minerals and inorganic species present in the sample (for example, chemical reactions with hydrated minerals to produce BSW). High-molecular-weight compounds were detected, including masses ranging from m/z 334 to m/z 485 (bands 17 to 22, which represent the total ion current generated by ions in an m/z interval), in EGA and both chromatograms. Derivatized compounds were detected.

To identify the organics detected in the GCMS analyses, SAM mass spectra were compared with hundreds of thousands of mass spectra from the National Institute of Standards and Technology (NIST) library[8]. The measured Mars in situ GC retention times of molecules were compared with retention times of the equivalent molecules analysed in the laboratory under SAM flight conditions (column temperature and inlet pressure) using an identical spare column mounted on a commercial GCMS (Laboratory experiments, Comparison of laboratory and flight retention times and Supplementary Table 1).

One of the main challenges when interpreting the sources of the organics detected in the SAM data is understanding the extent of the contributions from the organic molecules produced within the instrument itself and deconvolving them from indigenous Martian organics. The main sources of SAM internal organics are (1) the MTBSTFA wet chemistry reagent used for derivatization, which decomposes and leads to the formation of by-products, and (2) the Tenax TA used as an adsorbent in the SAM HC trap, whose thermal degradation products

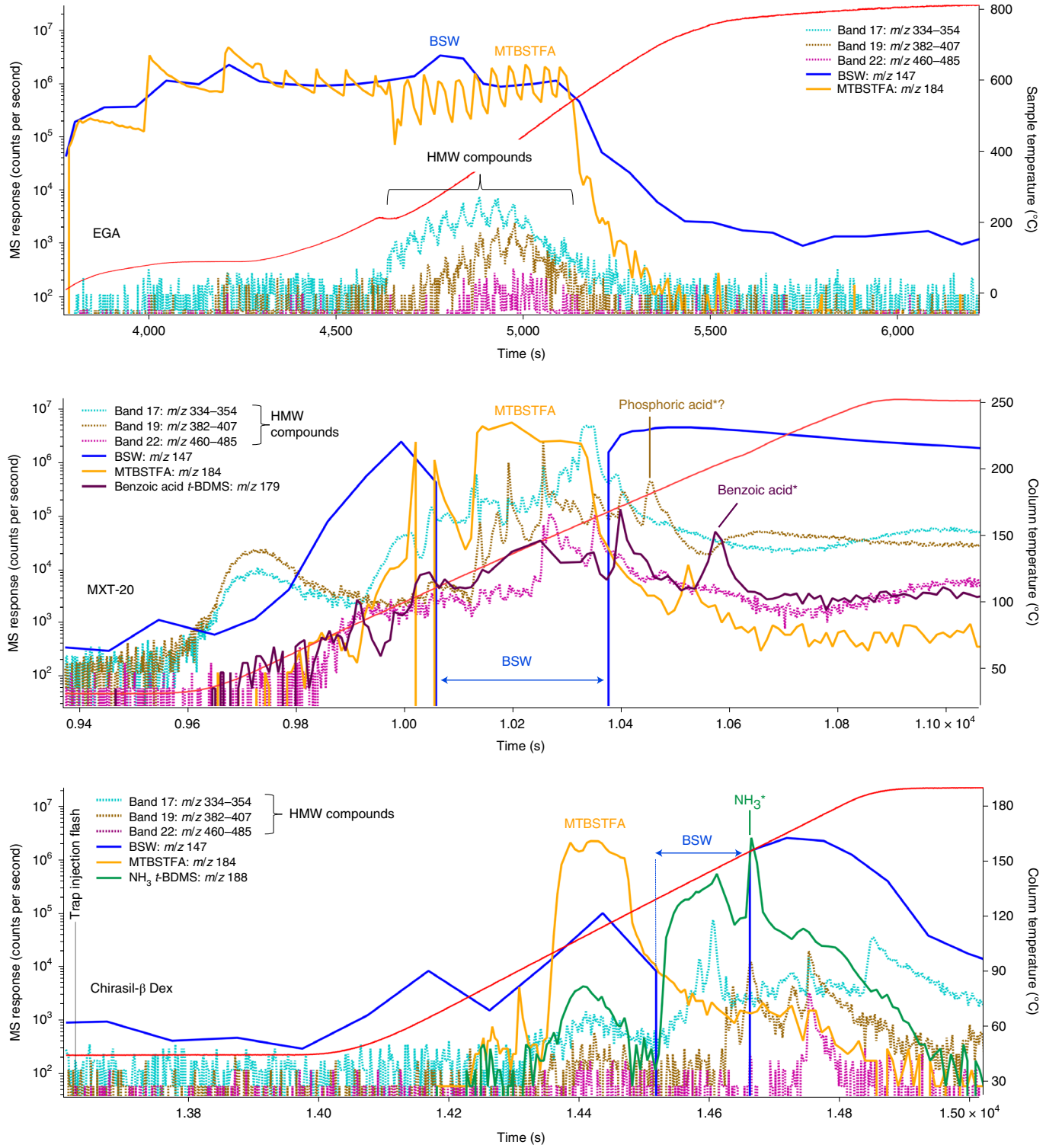

Fig. 2 | Representative traces extracted from the EGA analysis (top) and chromatograms from the MXT-20 (centre) and Chirasil-β Dex (bottom) columns from selected *m/z* values or bands covering a range of masses[1]. Where the detector saturated at high count rates, over 1×10^7 counts per second, the apparent count was reduced to zero. The timeline of the experiment was as follows: it started with the EGA (top) when the power of the instrument was turned on and the MS started collecting data, followed by the MXT-20 analysis (centre), which started when the HC trap was heated to release the compounds into the column, and ended with the Chirasil-β Dex analysis (bottom), which started exactly when the IT was heated to release and inject the compounds into the column defined as 'Trap injection flash'. The sample and column temperatures are plotted in red with the scale on the right-hand axis. The *m/z* values 147 and 184 correspond to BSW and MTBSTFA respectively. TFMA was not displayed in the figure for clarity. *m/z* 179 and *m/z* 188 correspond to derivatized benzoic acid and ammonia. Three bands were plotted including masses from high-molecular-weight (HMW) compounds: band 17, *m/z* 334 to 354; band 19, *m/z* 382 to 407, which includes the major ion of potential phosphoric acid (*m/z* 383); band 22, *m/z* 460 to 485. *Derivatized molecules.

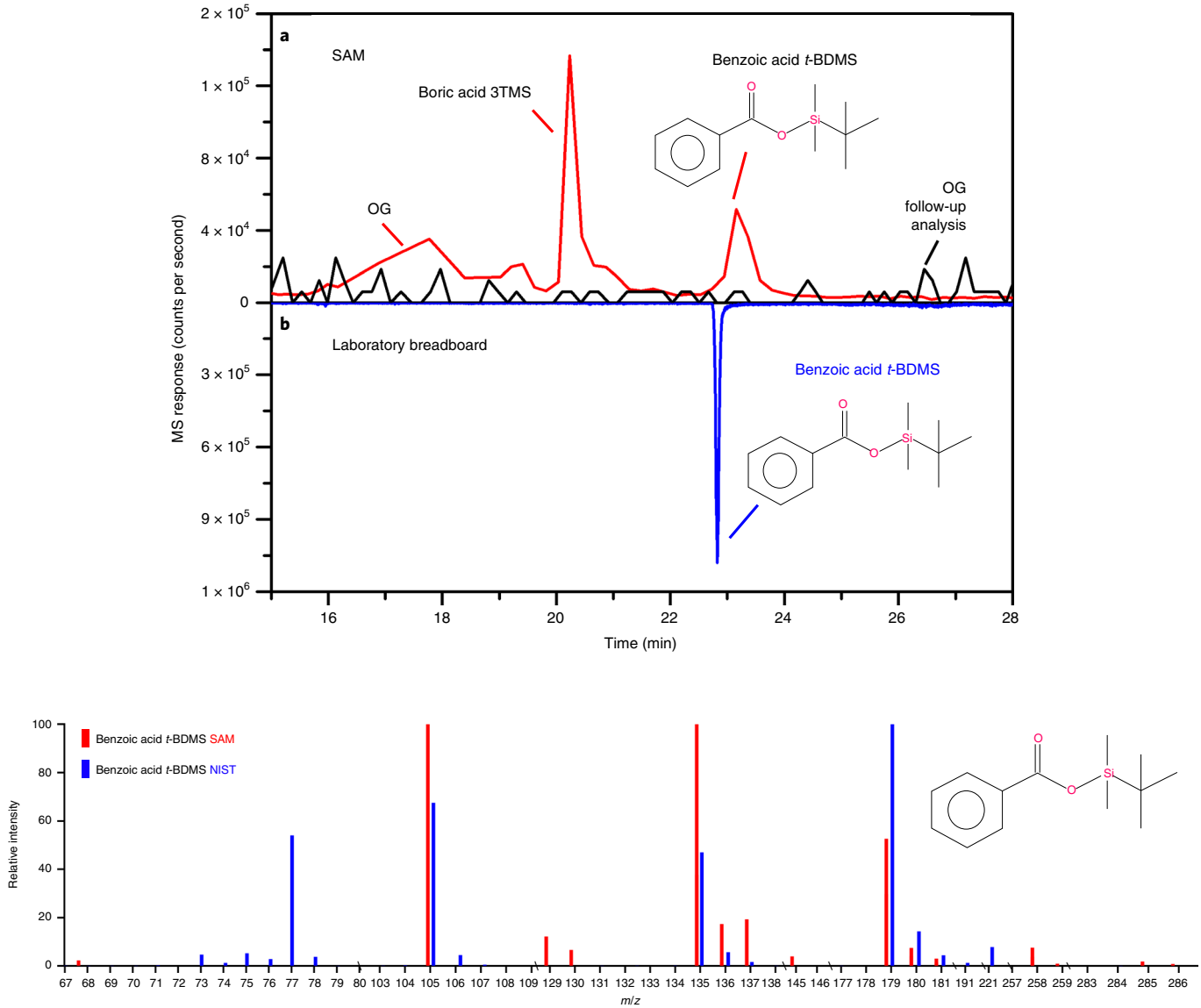

Fig. 3 | SAM GCMS identification of benzoic acid derivatized compared with the SAM-like GC run of benzoic acid derivatized measured in laboratory. Top: GCMS identification of benzoic acid derivatized at 23.2 min in the OG flight chromatogram (**a**) compared with the laboratory GC run of benzoic acid derivatized and analysed in the laboratory in SAM-like operational conditions of the MXT column (**b**) and detected at 22.8 min. The major ion *m/z* 179 was extracted from the total ion current for the OG and OG follow-up chromatograms. The peak at 20.2 min also including *m/z* 179 belongs to the tris(trimethylsilyl)borate derivative MTBSTFA by-product (Boric acid 3TMS). It resulted from the reaction between silica beads from the HC trap in flight and the MTBSTFA-DMF used for derivatization. Bottom: flight mass spectrum of derivatized benzoic acid compared with the mass spectrum extracted from the NIST Mass Spectral Database. The *m/z* 77 ion was not plotted, as it saturates due to its contribution in the MS of other molecules. *m/z* 258, 285 and 286 were also present, and probably belong to a coeluting molecule not yet identified.

include trace levels of aromatic compounds (for example, benzene, toluene) released at each activation. Some of the molecules identified in these experiments may be fully or partially sourced from the SAM instrument itself, as detailed in each section.

Detection of benzoic acid

Derivatized benzoic acid was detected with an abundance of 442 ± 82 pmol (Abundance calculations) in the chromatogram obtained with the MXT-20 column at a retention time of 23.2 min (Fig. 3a). The retention time of 22.8 min of derivatized benzoic acid measured in SAM flight conditions in the laboratory, within 0.4 min of the flight retention time (Fig. 3b), provided a very good match.

There are several possible paths to production of benzoic acid. Non-volatile organics such as benzoic acid or benzenecarboxylate salts—possible products from the oxidation of meteoritic organic matter—have been predicted to accumulate in the upper metre of the Martian surface with concentrations up to 500 ppm by weight[9]. For example, if toluene had been present on Mars, it would be successively oxidized to benzylic alcohol, benzaldehyde and finally benzoic acid. Benzylic alcohol and benzaldehyde were not detected, which would indicate the completion of the oxidation reaction. The most likely oxidizer is the $OH^{\bullet}$ radical, which can be produced via dissociation of H_2O_2 or in the presence of water and iron by the photo-Fenton reactions happening at the surface and in the regolith[9]. Benzoic acid was suggested to be one precursor of the chlorobenzene that was detected on Mars in the drilled Cumberland (CB) mudstone samples[5]. Chlorobenzene can be produced by reactions between benzoic acid and perchlorate at

elevated temperatures[10,11] and was demonstrated to be indigenous to the sample[5]. If benzoic acid had also been present in CB, it could have been entirely consumed by the perchlorates and responsible for a fraction of the chlorobenzene detected. Along with benzoic acid, Benner et al. predicted the formation of other organic acids such as oxalic and formic acids[9]. A recent study also shows the formation of organic acids through radiolysis of macromolecules (for example, kerogens) after high-energy particle radiation exposure (200 MeV)[12]. CO_2 and CO releases from SAM EGA experiments in drilled or scooped Martian samples suggest the presence of organic salts, such as Fe, Ca and Mg oxalates and acetates[13,14]. The detection of benzoic acid may reflect its relative stability to oxidation and radiation processes[12]. A possible contribution to benzoic acid from the SAM Tenax TA adsorbent present in the HC trap is discussed in Methods with Extended Data Fig. 4.

Detection of nitrogen-bearing compounds

Bis(*tert*-butyldimethylsilyl)amine (derivatized ammonia), a N-bearing inorganic compound, was identified by its mass spectrum and retention time in the chromatogram obtained with the Chirasil-β Dex column with an abundance of ~7 ± 1 nmol (Derivatized ammonia and Extended Data Fig. 5). Derivatized ammonia is a commonly observed degradation product of MTBSTFA and/or DMF, but it can also be formed by reaction between MTBSTFA and NH_3 present in the sample or released from N-bearing organic molecules.

The follow-up GCMS analysis revealed no MS saturation. Twelve N-bearing compounds were detected: derivatized ammonia, HCN, derivatized isocyanate, isocyanomethane, trifluoroacetonitrile, acetonitrile, propenenitrile, propanenitrile, isobutyronitrile, butenenitrile, dimethylaminoacetonitrile and benzonitrile (Extended Data Figs. 6 and 7). Among these compounds, an unknown portion of derivatized ammonia, HCN, isocyanomethane, trifluoroacetonitrile and acetonitrile are probably related to the thermal and/or chemical degradation of the MTBSTFA-DMF reagent itself or in the presence of perchlorates as demonstrated in SAM-like pyrolysis laboratory experiments[15]. The other molecules were not detected in laboratory experiments focused on the MTBSTFA-DMF degradation in the presence and absence of perchlorate salts[15,16]. Therefore, these molecules could be degradation products of larger and/or more complex N-bearing organics indigenous to the sample, or products of organic molecules that reacted with the nitrates present in OG during pyrolysis[17,18].

The combination of an elevated abundance of derivatized ammonia and the numerous N-bearing organics detected in the follow-up analysis might indicate the presence of indigenous N-bearing molecules. Organics such as amino acids, amines, amides, nitriles (for example, benzonitrile) and nitrogen-heterocycle compounds (for example, pyrrole) have been detected in Martian meteorites and could have been formed by in situ synthesis or delivered to the surface of Mars by exogenous sources such as carbonaceous chondrites[19–23].

Detection of phenol and potentially phosphoric acid

Mass spectra matching derivatized phosphoric acid and derivatized phenol were identified in the OG and follow-up analyses at 21.2 min and 15.2 min, respectively (Fig. 4). Laboratory experiments conducted under SAM flight conditions did not confirm these identifications, as retention times of 36.0 and 31.2 min were measured for the candidate phosphoric acid and phenol peaks, respectively. However, the in situ mass spectra provided a good match to the mass spectra from the NIST library (Fig. 4), so these compounds could come from a previous experiment or degradation of a more complex molecule. The latter hypotheses are supported by the full-width at half-maximum of the phenol and phosphoric acid peaks in the MXT-20 chromatogram, which are about twice the full-width at half-maximum of the benzoic acid peak. Alternatively, the compounds eluting from the OG sample may be structurally similar to derivatized phenol and phosphoric acid (Other possible compounds with m/z 383 and 425).

Phosphoric acid has been detected in Mars-analogue samples and simple mixtures of minerals and organic molecules analysed in the laboratory[24,25]. Although it was once interpreted as a biomarker and a degradation product of DNA[24], its detection in simple mineral mixtures implies that it can be easily formed from minerals including phosphorus (for example, phosphates). The close match of the MS fragmentation pattern of *tert*-butyldimethylsilanol (*t*-BDMS) phosphoric acid suggests the presence of an indigenous phosphorus-containing material in the OG sample, since there is no known source of phosphorus in the SAM instrument. This is supported by phosphorus measurements obtained by several instruments on Curiosity. Phosphorus, possibly in the form of fluorapatite, has been identified by the ChemCam and CheMin instruments and could be present as other phosphorus phases below the limit of detection (LOD) of CheMin[26,27]. Phosphorus enrichments associated with diagenetic features were measured in several samples from Gale Crater with the alpha particle X-ray spectrometer (APXS) instrument (for example, phosphorus pentoxide, apatite) and are expected to be present in both amorphous and crystalline phases[28]. In addition, ChemCam detected phosphorus (at the ~5 wt% level) in two Bagnold Dunes targets, indicating that some grains could be rich in phosphorus, as previously shown in the Rocknest sands[26,29,30]. The phosphorus depletion in OG compared with the Martian average in the APXS data may be a consequence of the low phosphorus abundance, below the instrument's LOD[31]. Phosphorus in intermediate oxidation states, such as in hypophosphites and phosphite, is likely on Mars and could result from the accretion of interplanetary dust particles and asteroid impacts[32,33]. During pyrolysis, phosphites release phosphine, which would be detectable by SAM. Recent work showed that the major ions of phosphine, 31, 33 and 34, are indeed present in most SAM data and could be associated with the presence of phosphine[34]. The presence of an unknown compound with a major ion fragment identical to that of *t*-BDMS phosphoric acid (*m*/*z* 383) and not present in our custom MTBSTFA library cannot be excluded.

Derivatized phenol has been detected consistently in the SAM chromatograms, and its abundance seems to correlate with the abundance of MTBSTFA present within the SAM gas processing system. Phenol is one of the known aromatic degradation products of Tenax TA and GR chemical adsorbents contained in both SAM traps—the HC and IT traps located upstream of the Chirasil-β Dex column[35,36]. MTBSFTA may react with the phenol released from the heated trap to produce the derivatized phenol detected in the chromatograms. In related laboratory experiments, Tenax only released phenol when it was heated above 400 °C, after Tenax degradation with time or combined with a high amount of MTBSTFA[36]. Phenol *t*-BDMS is probably present in the OG chromatogram from the Chirasil-β Dex column as well, but its mass spectrum is impossible to extract due to the detector saturation induced by the excess of reagents. Thus, the phenol released from the trap(s) could be the main contributor to the derivatized phenol detected in OG, although the contribution of aromatic hydrocarbon(s) indigenous to the Mars sample cannot be excluded.

MTBSTFA by-products, column bleeding and unknown molecules

HMW compounds (*m*/*z* 300–485) were detected during sample pyrolysis and follow-up analysis (Fig. 2). A thorough analysis of the mass spectra extracted from each chromatographic peak demonstrates that most contained either *m*/*z* 73 and 147, corresponding to MTBSTFA-DMF by-products (10% and ~20% of the total number of compounds from the MXT-20 and Chirasil-β Dex analyses, respectively) (Extended Data Fig. 6), or *m*/*z* 207, 281 and 285, most likely from column bleeding. These results are not surprising given the large excess of MTBSTFA reagent present in the chromatograms that was released after puncturing the derivatization cup (SAM

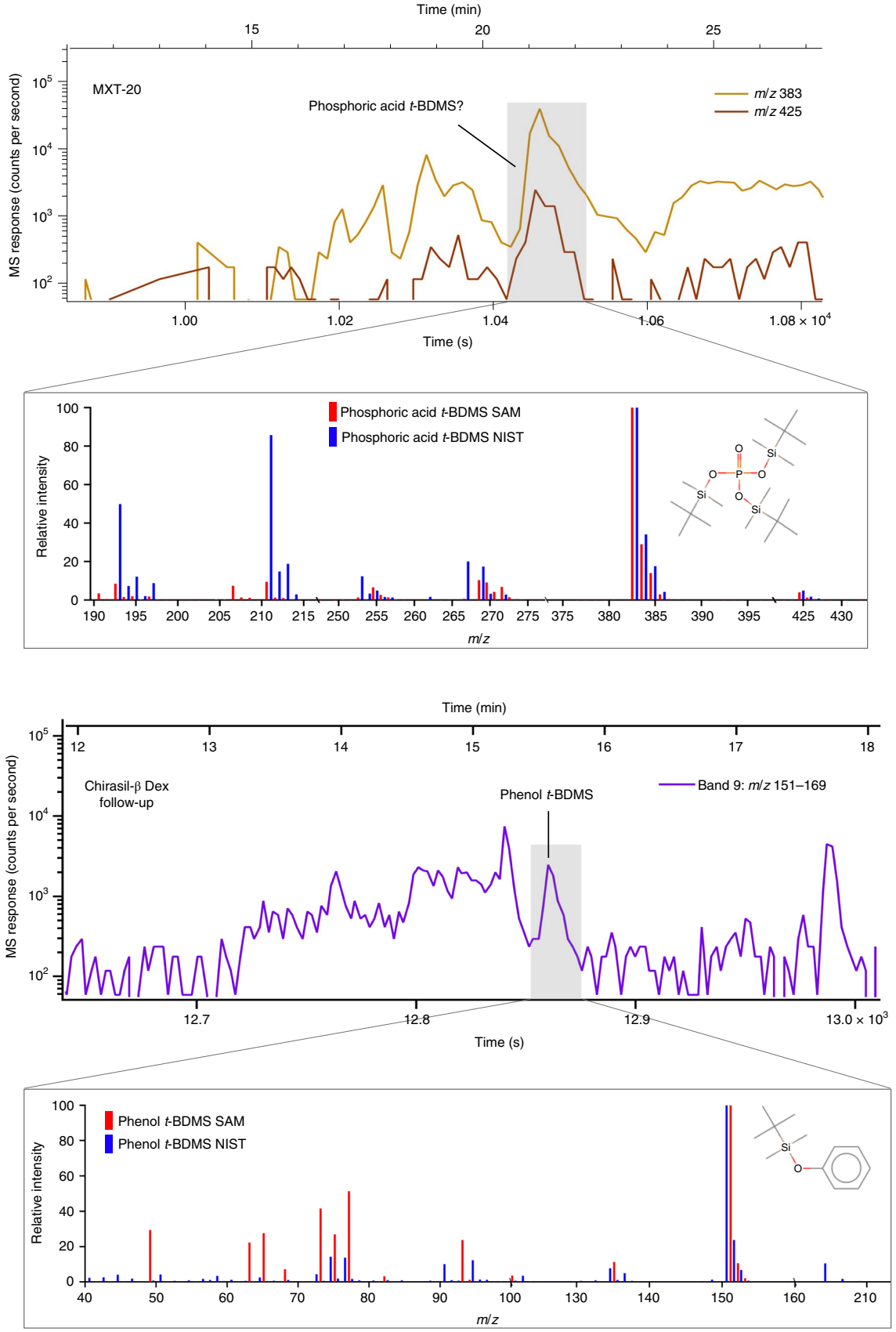

Fig. 4 | First and third panels from top: SAM chromatograms of the MXT-20 and Chirasil-β Dex follow-up analyses. *m/z* 383 and 425 are consistent with the two major ions of phosphoric acid. Band 9 corresponds to *m/z* 151–169 and includes *m/z* 151–153, which are consistent with the major ions of phenol. Second and fourth panels: mass spectra of the potential derivatized phosphoric acid and phenol extracted from the SAM chromatograms of the MXT-20 and the Chirasil-β Dex follow-up runs respectively compared with the mass spectra extracted from the NIST Mass Spectral Database.

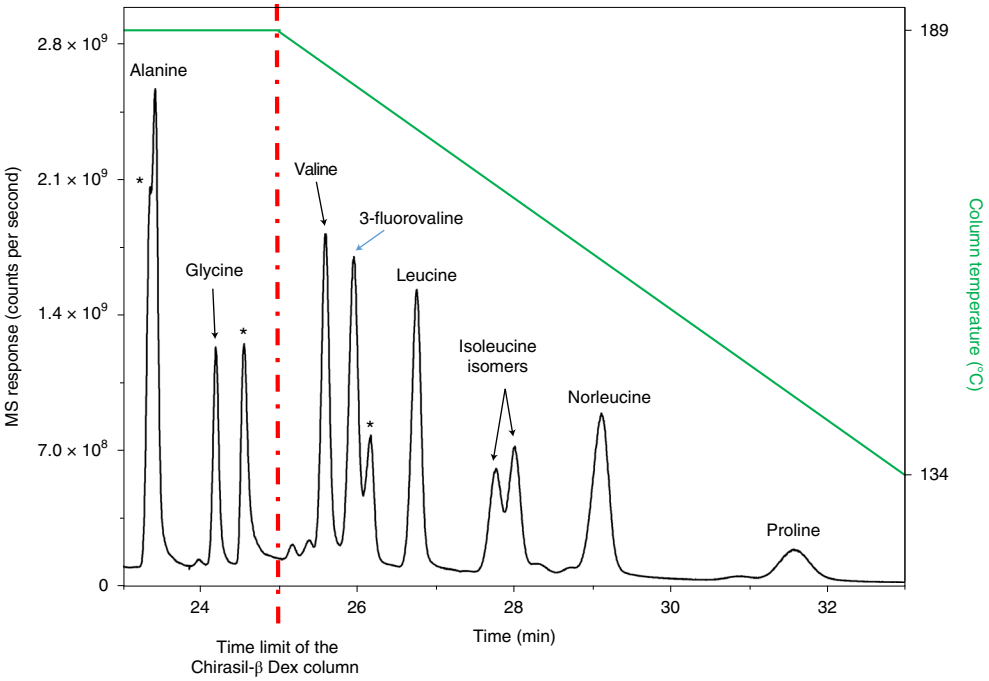

Fig. 5 | 10 min section (23–33 min) of the chromatogram obtained with the spare Chirasil-β Dex column of the standard mixture of 22 amino acids derivatized with MTBSTFA-DMF. As shown above, only derivatized alanine and glycine would be detectable within the standard operating conditions used on Mars for the Chirasil-β Dex column. The derivatized amino acids eluting after 25 min of the run (represented in red dash–dots) include valine, 3-fluorovaline, leucine, isoleucine and allo-isoleucine diastereoisomers, norleucine and proline, none of which are detectable with SAM using the current operating conditions. However, this laboratory experiment shows that these six derivatized amino acids (including the 3-fluoro-DL-valine internal standard used on SAM) would be detectable if the MS signal continued to be recorded while the column cooled down after the end of the nominal run. The temperature of the Chirasil-β Dex column is represented in green. In the flight chromatogram, it was 189 °C at the end of the GC run and ~134 °C at 33 min of the GC run. *MTBSTFA-DMF by-products.

derivatization experiment). MTBSTFA reacts with the organic molecules but also with the inorganic species released either from minerals during pyrolysis or from the analytical components of SAM when they are heated (traps, columns and so on). For example, it is known from laboratory experiments that boric acid is released from the glass bead adsorbent used in the SAM HC trap when it is heated, then reacts with MTBSTFA to produce boric acid derivatives, which are indeed detected in the OG run (Fig. 3). When the column is heated, additional thermal degradation products are released from the stationary-phase polymer (β-cyclodextrin in the case of Chirasil-β Dex). The excess of MTBSFTA-DMF probably increased the degradation of the stationary phase, thereby producing an unusual number of compounds from the column: 15% and 18% of the total number of compounds for MXT-20 and Chirasil-β Dex, respectively (Extended Data Fig. 6).

24 other molecules, which appear unrelated to the MTBSTFA-DMF degradation or column bleed, have been detected in the MXT-20 and Chirasil-β Dex chromatograms. Their mass spectra, sometimes with one single mass, have not been identified because they correspond to many possible molecules and/or do not match published NIST or custom mass spectral libraries. The latter aim to match flight mass spectra with compounds detected in Mars's analogues to gauge the mineral assemblages in which they are detectable, even without having identified these molecules.

Optimization of the derivatization experiment

The non-detection of the derivatized 3-fluoro-DL-valine internal standard, probably eluting beyond the time limit of analysis for the Chirasil-β Dex column in the OG conditions, motivated a series of laboratory experiments to optimize the next derivatization analyses on Mars. A mixture of 22 proteinogenic and non-proteinogenic amino acids were analysed, including 3-fluoro-DL-valine and α-aminoisobutyric acid, rare on Earth and unlikely to be a contaminant but abundant in some carbonaceous meteorites[19]. The amino acids were derivatized before being injected into the commercial GCMS carrying a Chirasil-β Dex spare column, which was heated in an identical manner as the flight column. The following three experiments were conducted to closely simulate the Chirasil-β Dex column flight operating conditions used in the OG run.

1. Injection of the derivatized amino acids separately to measure their retention times within the Chirasil-β Dex flight operating conditions (Comparison of laboratory and flight retention times and Supplementary Table 1).
2. Derivatization of the amino acids with 150 µl of MTBSTFA-DMF, which represents the estimated volume of derivatization mixture sent to the HC trap during the OG run; the liquid mixture was then directly injected through the GC injector (no trapping) to simulate reagent saturation.
3. Derivatization of the amino acids with 20 µl of MTBSTFA-DMF followed by injection of the mixture through the equivalent of the SAM HC trap with flash pyrolysis at 300 °C; this added the efficiency of the HC trap desorption as a parameter, enabling comparison with Experiment 2. We note that because of the design and the volume of the pyrolyser quartz tubes it is impossible to add the 150 µl of MTBSTFA-DMF that was tested in Experiment 2.

The cooling of the column during which the GCMS signal was recorded was also simulated, enabling an evaluation of which

organics would elute after the 25 min maximum analysis time and might be missed due to SAM engineering constraints (Fig. 5).

Alanine and glycine were the only amino-acid derivatives detectable within the 25 min limit of the SAM nominal run. Despite their strong separation, heavier amino-acid derivatives, including the 3-fluoro-DL-valine internal standard, eluted beyond the time limit and would not be detectable within the current SAM flight conditions. 3-Fluoro-DL-valine and five additional amino acids—valine, leucine, the isomers of isoleucine, norleucine and proline—elute as the column cools, thus they could potentially be detected if the GCMS signal were recorded on Mars during that time (Fig. 5). On the basis of these experiments, alanine and glycine should have been detected if they were present in the OG sample. The fact they were not indicates that (1) they were not present above the analytical LOD of SAM (~pmol level), which could be due to their low stability under Martian surface conditions, or (2) they were present in the sample but transformed into other products during pyrolysis because of the potential high chemical reactivity of the sample (Search for indigenous amino acids and the 3-fluorovaline internal standard).

To evaluate the possibility of detecting heavier organic molecules (including amino acids that would only elute after nominal sample analysis as conducted so far), we performed a series of follow-up analyses without injecting any compounds to enable all of the molecules to elute from the column (Follow-up analyses in the laboratory and Extended Data Fig. 8).

To prepare and anticipate the results of the future wet chemistry campaigns, organic molecules from various chemical families were analysed in the laboratory within the flight conditions of the MXT-20 and Chirasil-β Dex columns. Chemical families include derivatized amino acids and carboxylic acids and fatty acid methyl esters, which are relevant for the TMAH experiment. These results (Supplementary Table 1) constitute an important database of retention times of molecules expected or suspected to be present on Mars, which will support the search for a greater diversity of polar molecules that may indicate abiotic or biotic origins.

Conclusion

SAM's nominal MTBSFTA derivatization experiment performed in situ on the OG sample was successful and led to the detection of mass spectra from different classes of compounds including derivatized molecules that would have otherwise escaped detection by SAM. MTBSTFA derivatives of the aromatic carboxylic acid benzoic acid and a molecule related to phosphoric acid were detected. Benzoic acid is one precursor of chlorobenzene and could be a contributor to the chlorobenzene detected by SAM in the CB mudstone sample. Further optimization of the derivatization experiment was enabled by laboratory studies motivated by the data from the OG experiment. On the basis of these results, we can surmise why the 3-fluoro-DL-valine internal standard was not detected, and recommend new flight protocols with the Chirasil-β Dex column to improve the detection of heavy organic molecules.

The OG sand was not expected to be organic rich, as it has probably been exposed to ionizing radiation for millions of years[7]. Still, a GCMS search for terrestrial amino acids was conducted and none were detected above the LOD of the SAM instrument, including the 3-fluoro-DL-valine calibrant. However, N-bearing organic molecules and several as yet unidentified organic compounds were present. These results have not only enabled us to optimize the detection of a wider range of organics using SAM-like techniques, such as derivatization with MTBSTFA, but also helped us improve our flight protocols for SAM's TMAH wet chemistry campaign performed in the clay-rich Glen Torridon region[37,38] and the remaining wet chemistry experiments to be utilized in Gale Crater. These results are also relevant for the European Space Agency's Rosalind Franklin rover, soon to carry the Mars Organic Molecule Analyzer (MOMA) instrument, which will implement wet chemistry pyrolysis–GCMS experiments with MTBSTFA, TMAH and dimethylformamide–dimethylacetal dedicated to chiral separation[39].

Deconvolving the source(s) of the organics detected with the SAM instrument is a challenging and continuously evolving process. This derivatization experiment performed on Mars with the MTBSTFA wet chemistry reagent has expanded our understanding of organics that could be present in Martian sands as well as the by-products related to our reagent. The success of this derivatization experiment has demonstrated tools suited for the search for biotic or prebiotic molecules preserved in ancient Martian environments.

Methods

Mineralogy of Ogunquit Beach and content of volatiles. The mineralogy of OG established by CheMin revealed plagioclase feldspar, olivine, augite and pigeonite in decreasing abundance (respectively 47.1, 18.2, 15.7 and 10.2 wt% of the crystalline fraction) as the major mineral phases, with less than 3% magnetite, haematite, anhydrite and quartz[40]. The ~7% phyllosilicate fraction was attributed to likely cross-contamination from material released from a previous sample that had collected on the CheMin funnel. ~40% of the sample was reported to be an X-ray amorphous component[41]. The gases released during SAM EGA included H_2O, CO_2, SO_2, O_2, HCl, H_2S and NO, and revealed this sample to be relatively depleted in volatile compounds compared with previously sampled aeolian sands or sand/dust mixtures[42]. The water release profile is consistent with the presence of hydrated salts or glass inclusions in the sample, while the CO_2 release at high temperature is consistent with the presence of carbonate.

Previous wet chemistry studies. Before the derivatization experiment on Mars, the efficiency of the MTBSTFA extraction and derivatization protocol had been explored with selected Mars-analogue mineralogies[24,43]. Results indicated that some of the organic molecules indigenous to these samples were successfully extracted and derivatized with MTBSTFA. A limited number of silylated amino and carboxylic acids were detected in a desert soil sample from the Atacama desert and in a dolomitic stromatolite from Svalbard using a one-pot one-step MTBSTFA benchtop procedure that approximated the SAM oven and HC trap capabilities[24,43]. Although these samples yielded organics, these studies also highlighted the challenges of the detection of organic molecules by GCMS using the one-pot derivatization approach in the presence of hydrated mineralogies and oxidizing species such as iron oxides and (per)chlorate salts, which can hinder the chemical derivatization reaction. MTBSTFA readily reacts with water and other inorganic species to form *t*-BDMS and 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetram ethyldisiloxanetetramethyldisiloxane (mono- and bi-silylated water respectively) and a wide variety of by-products. The derivatization reagent for the other wet chemistry experiment carried by SAM, TMAH (25% in methanol), is less sensitive to hydrated minerals and oxidizing species and showed an efficient yield of methylated carboxylic and fatty acids in a variety of Mars-analogue samples during laboratory experiments[44].

SAM derivatization experiment. The SAM Sample Manipulation System contains 74 cups, nine of which are foil sealed Inconel metal cups for derivatization. Seven of the nine derivatization cups contain a mixture of MTBSTFA and DMF for derivatization, and the two others are filled with TMAH in methanol for thermochemolysis/methylation[1,44] (Extended Data Fig. 1). Each derivatization cup contains two separate reservoirs. The outer volume (Extended Data Fig. 1c outer) is 0.5 ml of a 4:1 mixture of MTBSTFA-DMF with 24.2 nmol of pyrene (which does not react with MTBSTFA). The inner volume (Extended Data Fig. 1c inner) is a dry internal chemical standard of 36.2 nmol of 3-fluoro-DL-valine, which had been sealed under vacuum inside a separate foil-capped reservoir. 3-fluoro-DL-valine is used to determine the efficiency of the MTBSTFA derivatization reaction of an amino acid after the two metal foils are punctured.

The MTBSFTA derivatization experiment on SAM utilized elements of the standard EGA/GCMS sequence. The 900 °C cup preconditioning step cannot be used with the sealed liquid-containing metal cups, and the derivatization experiment must occur in the same sol. The experimental procedure combined the manifold conditioning at 135 °C, cup puncture, sample delivery and EGA/GCMS analysis into a single experiment to minimize the time between sample delivery and pyrolysis and minimize solvent loss during extraction as detailed below (Extended Data Fig. 2).

The experiment was divided into the following steps: an initial pump down and conditioning of the sample manifold, a cup puncture with sample drop-off, then pyrolysis EGA and finally GCMS. Following the conditioning of the manifolds, QMS background scans were conducted before sample introduction. The sample drop-off moved the chosen sealed cup to the puncture station in the Sample Manipulation System and punctured both foils before moving the cup to the inlet for sample acceptance. The rover delivered a single portion, nominally ~45 mg, to the cup after being sieved to 150 µm, which was then moved and sealed in the SAM pyrolysis oven under ~0.8 sccm helium flow. A cool time of day for the drop-off was selected to help minimize the loss of MTBSFTA to the atmosphere while the

cup was exposed. The cup temperature was then raised and held at 100 °C for 210 s while small portions of the vapour in the manifold were incrementally sampled by the mass spectrometer.

In a standard sequence without a derivatization cup, an empty quartz cup is preconditioned under helium flow in the pyrolysis oven to ~900 °C and then allowed to cool to ambient temperature before delivery of one to three portions (~45 mg per portion) to the cleaned cup, followed by pyrolysis heating for EGA and GCMS analyses. The gases released in a chosen range of oven temperatures, called temperature cuts, are sent to the SAM HC trap for subsequent GCMS analysis. Without derivatization, an EGA/GCMS sequence takes two or three sols whereas derivatization has to be performed in one sol.

The derivatization experiment utilized a linear 35 °C min^{-1} ramp of the punctured cup containing the fluids and the delivered sample up to ~900 °C under He flow. Two techniques were employed to help to mitigate oversaturation of the SAM HC trap with MTBSTFA-DMF during the temperature range below 250 °C, where the majority of the MTBSTFA vapour is expected. First, the SAM HC trap was kept at 80 °C, a temperature that efficiently traps organics while allowing MTBSTFA to pass through. Second, only a portion of the evolved gas from the pyrolysis was captured and sent to the HC trap, with the remaining portion vented to Mars's atmosphere via an exhaust line. As the pyrolysis ramped from ambient temperature to 100 °C, the sample was collected on the SAM trap for 5 s, and vented out the exhaust for 200 s. For the temperature range from 100 to 250 °C, sample was again collected for 5 s, but vented for a shorter 70 s. Finally, from 250 to 900 °C, the sample was collected for 20 s and vented for 20 s (Extended Data Fig. 2). When the cup reached 220 °C, the HC trap was cooled to 0 °C at a ~9 °C min^{-1} rate to effectively collect both derivatized and non-derivatized analytes. After reaching the maximum cup temperature, the manifold was pressurized to 900 mbar, and the HC was heated to 320 °C in 5 min and held at the maximum temperature for 3 min to release the collected gases onto two GC columns for GCMS analysis.

Gas chromatography. The desorbed gases were split into two of the six GC channels of SAM. The first is composed with an MXT-20 (Restek) column, and the second includes a Chirasil-β Dex CB (Agilent) column and an in-line IT (filled with Tenax TA), which is used to focus and inject the analytes as quickly as possible into the column to obtain a high separation power. Both columns are 30 m in length, with an 0.25 mm internal diameter and 0.25 µm film thickness, and are designed to analyse and separate low- to medium-molecular-weight mid-polar organics from 5 to 15 carbon atoms, under the operating conditions used for SAM[1]. During the time of pyrolysis, a portion of the gases released from the sample was sent to the HC trap (~80 °C) in preparation for the GCMS analysis, while a small percentage went directly to the MS for the EGA (Extended Data Fig. 2). The HC trap was then heated to 380 °C and flushed with helium to release the compounds into both columns. The analytes released from the HC trap went directly into the MXT-20 column, immediately starting the analysis. The MXT column was heated to 250 °C at a rate of 10 °C·min^{-1}. During the GC analysis with the MXT-20 column, the remaining analytes were sent to the IT of the Chirasil-β Dex column, which was cooled to ~5 °C to trap the compounds released from the HC trap. Upon completion of the analysis with the MXT-20 column, the IT of the Chirasil-β Dex was rapidly (~5 s) heated to ~305 °C to release and sharply inject the collected gases into the Chirasil-β Dex column, which was heated from 44 (held for 6.5 min) to 190 °C using a 10 °C min^{-1} ramp rate. The exact split ratio between the two columns is unknown. The helium flow rate during the experiment was regulated at ~0.8 sccm. In summary, the timeline of the entire run included the EGA, followed by the MXT-20 analysis and ending with the Chirasil-β Dex analysis.

OG follow-up analysis. After the analysis of OG, a follow-up GCMS analysis was performed. It reproduced the previous experiment without a sample. Follow-up analyses were implemented from the beginning of the mission to clean the transfer lines, traps and columns from eventual condensed/trapped organics and inorganics that would have not come through in the original sample. They also minimized the carry-over of reagents or products to subsequent experiments. Moreover, and especially in derivatization runs, follow-up analyses can provide more scientifically exploitable data compared with the original run, which is saturated by the derivatization reagents and its related by-products.

Abundance calculations. To calculate the abundances of benzoic acid and ammonia from the flight chromatograms, the major ion mass fragments of benzoic acid and ammonia—*m*/*z* 179 and *m*/*z* 146 respectively—were fitted with Gaussian curves using the Igor Pro 8 software (WaveMetrics) and the areas under the curves were measured. The peak areas of each individual *m*/*z* value contributing to the mass spectra of derivatized benzoic acid (41, 45, 51, 73, 75, 77, 105, 135, 180) and derivatized ammonia (188, 130, 147, 116, 189, 73, 142, 132, 148, 100) were calculated from the fragment ions at *m*/*z* 179 and *m*/*z* 146 using the expected ratios from the NIST MS reference library[8]. Abundances were then calculated by summing all *m*/*z* values contributing to both peaks and comparing the total areas with the peak areas from five hexane GCMS measurements that were conducted on the SAM instrument during preflight calibrations[1]. The average electron ionization cross-section of hexane[45–48] of 19.7 Å^2 was used to evaluate the molar response difference between hexane and benzoic acid and ammonia, and we calculated the ionization cross-section of benzoic acid and ammonia using the bond contribution method described previously[45]. Ionization cross-sections of 38.8 Å^2 and 45 Å^2 for benzoic acid and ammonia, respectively, were estimated.

Laboratory experiments. The laboratory experiments to support the interpretation of the flight results were performed on a GC Trace Ultra Chromatograph coupled to an ISQ LT MS from Thermo Fisher. Liquid (~0.1–0.5 µl) and gas (~0.5 ml) injections of the standard molecules were done using a split/splitless injector in split mode with average split ratios between 1:50 and 1:100. The temperature of the injector and the ionization source were set at 250 °C and the GCMS transfer line at 300 °C to prevent condensation of the derivatized molecules in the instrument as they all have lower boiling points. The MS was set to scan the ions produced from the electron impact ionization source (electron energy of 70 eV) in the *m*/*z* 10–535 range, covering all the ions produced from the derivatized compounds targeted. The carrier gas was helium (Air Liquide, purity ≥ 99.9999%) to match the SAM GCMS experiment on Mars. The temperatures of the columns and carrier gas flow conditions used are detailed in the following section.

To support the third part of the optimization experiments, a commercial CDS5100 pyroprobe was coupled to the GCMS. The MTBSTFA-DMF (volume:volume 1:4) liquid was injected onto glass wool packed in an organic-free quartz tube. The quartz tube was then inserted into the interface of the pyrolyser and the sample was flash pyrolysed at 300 °C under helium flow after the sample had gone through the equivalent of the SAM HC.

Comparison of laboratory and flight retention times. To confirm or rule out the identification of the derivatized and non-derivatized molecules in the SAM chromatograms, we compared the flight retention time with the laboratory retention time of equivalent molecules measured in SAM flight operating conditions (temperature and pressure) using a laboratory column (that is, the spare MXT-20 and Chirasil-β Dex columns). A flow restrictor was placed between the injector and the column to allow a SAM-like flow. To make sure that the flight and laboratory retention times were comparable, we adjusted the dead time in the laboratory to the dead time in flight by injecting non-retained compounds into both columns and adjusting the pressure in the laboratory until the two dead times were identical. This method has been used repeatedly in the past to confirm the presence of the chlorohydrocarbons and S-bearing compounds detected in the CB and Mojave samples[2,5,49,50]. It has also been used to build a database of retention times of molecules of astrobiological interest that may be present on Mars and detectable in future flight chromatograms[2,5,49,50] (Supplementary Table 1).

For the spare Chirasil-β Dex column, ~0.5 ml of air was injected to measure the dead time in the laboratory, and the laboratory GC pressure was adjusted until the dead time matched the flight dead time measured for the Chirasil-β Dex column in the SAM flight OG chromatogram. The programme temperature was set to match the flight-temperature programme from 40 °C (6.5 min hold) to 190 °C using a 10 °C min^{-1} ramp.

The MXT-20 column does not include an IT, which makes comparison between the laboratory and flight retention times challenging. For this last column, the laboratory GC pressure had to be adjusted as well as the length of the initial temperature plateau of the column (which is unknown given the absence of an IT flash time). To do so, several retention markers from compounds detected in the MXT-20 flight chromatogram were used, including SO_2, BSW and tristrimethylsilylborate. The latter two molecules are by-products formed from the reaction between MTBSTFA and water, and MTBSTFA and the glass silica beads from the SAM HC trap, respectively. The pressure was adjusted using the SO_2 retention time, and the length of the initial temperature plateau of the MXT-20 column was adjusted by matching the retention times of the two by-products detected in all SAM and laboratory chromatograms including MTBSTFA (Supplementary Table 1). Minor retention time shifts remain due to several factors, including the saturation of the MTBSTFA-DMF in the column and the traps, the slight difference between the flight and laboratory ramp temperatures, and the manufacturing process of both stationary phases[50]. The final temperature program of MXT-20 was a 5 min hold at 31 °C, followed, as in SAM, by a ramp at 10 °C min^{-1} to a final temperature of 250 °C.

The comparison between the flight and laboratory retention times allowed us to confirm the detection of derivatized benzoic acid but did not enable the strict identification of derivatized phenol or phosphoric acid in the OG chromatogram (Supplementary Table 1). Other organic compounds, including carboxylic acids, fatty acids, amino acids, chlorinated compounds and phenol, have also been analysed separately in the laboratory under the same conditions as used in flight for MXT-20 and Chirasil-β Dex, in service of the goal of developing a library of compounds that could potentially be found and detected by the SAM instrument on Mars. Supplementary Table 1 represents a database of retention times of compounds detected and/or suspected in the SAM chromatograms, suspected to be present in the OG chromatogram and/or on Mars, and that could be searched in future SAM analyses.

After the analysis of the chromatogram from the Chirasil-β Dex column, several derivatized molecules were suspected to be detected on the basis of the presence of some ions from the mass spectra, including chlorophenol,

chlorobenzoic acid, undecanoic acid, tridecanoic acid and phthalic acid. However, laboratory retention times measured in SAM flight operating conditions did not match their flight retention times and so detections were ruled out (Supplementary Table 1).

Within the current SAM flight conditions, we would be able to detect up to C_{19} and C_{12} fatty acids with the MXT-20 and Chirasil-β Dex columns, respectively. These data are especially relevant for the TMAH wet chemistry experiment (main text), indicating that some fatty acids, transformed into fatty acid methyl esters through thermochemolysis, would be detectable if present in Martian samples.

Pyro-GCMS analysis of Ogunquit Beach. Before performing the derivatization experiment, three EGA-GCMS analyses of a triple portion of the OG sample were performed to evaluate its potential in terms of content of organics and compare it with the previous samples analysed by SAM. The preliminary analyses of the results from the first EGA-GCMS analyses—OG1 and OG2, which were dropped in the same sample cup (cup 2)—showed either no release of the usual background compounds or a much smaller amount than expected. This was interpreted to be the result of a bad seal of cup 2. Another triple portion of OG was then dropped in a new cup (cup 30), and a new EGA-GCMS was performed. The chromatogram from the OG3 sample is shown in Extended Data Fig. 3 with the main peaks extracted and labelled. Compounds related to the column itself (column bleeding) have not been labelled.

Chlorinated organics, including chloromethane, dichloromethane, trichloromethane, 1- and 3-chloromethylpropene isomers and several MTBSTFA by-products—*tert*-butyldimethylsilylfluorine, TFMA, chloro-*tert*-butyldimethylsilane and BSW—were detected (Extended Data Fig. 3). These compounds were detected in previous SAM analyses and their natures and amounts are consistent with the organics produced within the instrument itself, contributing to the SAM background. The benzene and toluene are known to be released by the IT as it is heated. The chlorohydrocarbons are produced during pyrolysis from reactions between oxychlorine species and MTBSTFA. Other minor organics such as acetonitrile, a volatile nitrogen compound, phenylethyne and styrene aromatics were detected and also associated with SAM background. S-bearing organics including methanethiol and ethanethiol were detected as well as SO_2 (saturated), CS_2 and COS. These results indicate that the S-bearing organics could be indigenous to the sample and/or reaction products of the S inorganic species and parent indigenous organics present in the sample.

The chromatogram extracted from the OG3 sample represents a good background reference for comparison with future samples. The detection of organic molecules, although mostly originating from or produced within the SAM instrument, led us to perform this nominal derivatization experiment to validate the protocol at Mars's surface and provide a chromatogram from a derivatization experiment that will serve as a background reference for future samples to be analysed with the MTBSTFA derivatization.

Tenax TA adsorbent as a possible contributor to benzoic acid. Another possible source of benzoic acid is the Tenax TA adsorbent present in the HC trap used to concentrate in a small volume the gases released by the sample during the ~35 min of pyrolysis. Tenax TA, a polymer made of 2,6-diphenyl-*p*-phenylene oxide, releases aromatic compounds from both thermal and chemical degradation when heated to ≥300 °C (refs. [5,36]). Phenol is one of the major known degradation products that was detected as its *t*-BDMS derivative in several SAM GCMS analyses as well as in laboratory experiments involving Tenax[36]. In the presence of dioxygen, released from the decomposition of oxychlorine compounds in the sample, phenol originating from the Tenax TA adsorbent could be oxidized and transformed into benzoic acid[35]. However, in the reducing environment in which MTBSTFA-DMF fluid wets the sample, orders of magnitude less oxygen is transferred to the Tenax TA adsorbent compared with normal pyrolysis experiments with similar volumes of sample (paragraph below and Extended Data Fig. 4). Moreover, with excess MTBSTFA present on the Tenax TA adsorbent, any phenol released from the trap would be rapidly derivatized, and phenol was indeed detected in the OG chromatogram (main text). In addition, laboratory experiments showed that benzoic acid was detected only when Tenax TA was heated alone and that it was not detected anymore when Tenax TA was heated in the presence of the MTBSTFA-DMF fluid[36]. Therefore, although a fraction of derivatized benzoic acid could be sourced from the Tenax TA, it is unlikely to be the only contributor.

A comparison between the EGA of the OG3 dry (no MTBSTFA-DMF) and the OG4 wet (soaked in MTBSTFA-DMF fluid) samples is shown in Extended Data Fig. 4. Results demonstrated that the abundance of dioxygen detected (and therefore transferred to the trap's Tenax TA adsorbent) was orders of magnitude lower in the OG-wet compared with the dioxygen released from the OG-dry sample. This is probably due to rapid reactions of O_2 in the highly reducing MTBSTFA fluid. Thus, the combustion by O_2 of indigenous organic molecules extracted from the sample or released from the SAM instrument was limited during the derivatization experiment.

Other possible compounds with *m*/*z* 383 and 425. Other compounds matching the same fragmentation pattern as tris[*t*-butyl(dimethyl)silyl] phosphate of either inorganic or organic nature were investigated. One possible compound with the closest mass fragmentation pattern was found to be heneicosanoic acid *t*-BDMS. It has the same *m*/*z* 383 and 425 as the phosphoric acid molecule detected in the SAM data. Heneicosanoic acid is a linear carboxylic acid with the general formula $C_{21}H_{42}O_2$. However, the laboratory retention time measured for this derivatized compound did not match the flight retention time (>25 min). Other compounds that produce the same mass fragments (*m*/*z* 383 and 425) are naphthenic acids *t*-BDMS, a mixture of cyclopentyl and cyclohexyl carboxylic acids represented by the general formulas $C_{21}H_{42}O_2$ (main *m*/*z* 383) and $C_{24}H_{48}O_2$ (main *m*/*z* 425), respectively[51,52]. These latter molecules were not analysed in the laboratory, but their structures and molecular weights imply that they would have similar retention times to phosphoric acid and heneicosanoic acid, beyond the flight-time limitation of the columns (thus they are unlikely to be the molecules detected in the OG analysis).

Search for indigenous amino acids and the 3-fluorovaline internal standard. If present in the OG sample, the *t*-BDMS derivatives of the amino acids alanine and glycine should have eluted from the MXT-20 or Chirasil-β Dex columns within the time period of the GCMS experiment on Mars (main text). However, these amino-acid derivatives were not detected above analytical detection limits. Therefore, either alanine and glycine were not present in the OG sample above the analytical detection limit of SAM (~pmol level), or the chemical reactivity of the radiation-processed sand samples interacting with the hot derivatization fluids transformed these as well as the internal amino-acid standard into other products during this experiment. On the basis of the measured radiolytic constants of pure amino acids exposed to gamma radiation[7], the destruction of amino acids and other organics in the Martian near-surface materials by UV and ionizing radiation may be rapid on geological timescales. For example, Pavlov et al.[53] calculated that the abundances of complex organics with masses of >100 AMU at a depth of ~5 cm below the surface would be reduced by a factor of 1,000 in less than a billion years of ionizing radiation exposure. Thus, the absence of meteoritic or other amino acids in this weathered, UV, ionized and cosmic-ray-processed Martian dune sample should not be surprising.

Two candidate fragments at *m*/*z* 204 and 306 for the 3-fluoro-DL-valine internal standard were present in the Chirasil-β Dex chromatogram at 18.7 min. Laboratory experiments conducted under SAM–Chirasil-β Dex flight operating conditions demonstrated that the retention time of the *t*-BDMS derivative of 3-fluoro-DL-valine is 26.7 min and that it may not have eluted from the Chirasil-β Dex column during the experiment (Fig. 5, main text). Without an IT, the precise elution time from the MXT-20 column is more difficult to quantify, but on the basis of laboratory experiments 3-fluoro-DL-valine should have been eluted before the end of the MXT-20 experiment. However, no spectrum matching 3-fluoro-DL-valine was found in the MXT-20 data. The fact that 3-fluoro-DL-valine was also not detected in the MXT-20 analysis potentially suggests a high chemical reactivity of the OG sample in contact with the derivatization fluids. 3-Fluoro-DL-valine would have been rapidly derivatized with the excess derivatization agent after puncture of the calibrant's foil. However, subsequent reactions with oxychlorine species present in the OG sample—such as perchlorates and chlorates—or with other chemically active compounds may have destroyed the derivatized 3-fluoro-DL-valine . This observation has implications for the detection of other chemically similar compounds that might have been present in this highly exposed and irradiated sand sample.

Derivatized ammonia. Bis(*t*-butyldimethylsilyl)amine was detected in the Chirasil-β Dex analysis at 17.3 min. It is also certainly present in the MXT-20 analysis, but its major ion mass fragments *m*/*z* 146 and 188, also part of the BSW mass spectra, are coeluted with the highly saturated BSW peak. This prevents its deconvolution from the BSW and thus a strict identification by the mass spectrum alone. The laboratory retention time of derivatized ammonia measured with the spare laboratory Chirasil-β Dex column at 18.8 min in the in situ conditions of the Chirasil-β Dex confirmed this identification (Extended Data Fig. 5). The differences between the flight and laboratory retention times, all longer in the laboratory, were observed and explained in a previous study[50]—in short, this is due to the different manufacturing processes for the columns and the environmental conditions of both SAM and the commercial instruments. Moreover, the retention time shift is consistent for all the identified molecules—from 0.4 min for light molecules to 1.5 min for the heaviest ones. In the Chirasil-β Dex follow-up OG analysis, the abundance of derivatized ammonia decreased to background levels at ~0.3 nmol.

MTBSTFA by-products, column bleeding and unknown molecules. The number of compounds extracted from the MXT-20, Chirasil-β Dex and Chirasil-β Dex follow-up chromatograms with the percentages of compounds related to inorganics, column bleeding, MTBSTFA-DMF by-products, organics from the instrument background and unknown molecules is presented in Extended Data Fig. 6. The chromatogram from the follow-up analysis performed after the OG analysis was not saturated, which made the MS extraction and the identification of molecules easier. 85 compounds were extracted, including 10% inorganics (CO_2, CO, N_2, NO, SO_2, COS, H_2S, H_2O, CS_2, derivatized ammonia and derivatized isocyanate), 14% organics from the instrument background (for example, benzene, toluene and other aromatics coming from the traps), ~18% known MTBSTFA-DMF by-products (for example, 2,2,2-trifluoro-*N*-methylacetamide, Extended Data Fig. 7a) and 42% unknown molecules. These molecules were

probably in the lines, traps and/or Chirasil-β Dex column and/or in excess after the analysis of the OG sample. In addition to the known N-bearing MTBSTFA-DMF by-products and derivatized ammonia that were detected in both the Chirasil-β Dex and follow-up analyses (Extended Data Fig. 5), the follow-up analysis included ~14% N-bearing compounds (for example dimethylaminoacetonitrile, Extended Data Fig. 7b), of which most were not present or not detectable in the Chirasil-β Dex chromatogram. The mass spectra of 2,2,2-trifluoro-*N*-methylacetamide, a known MTBSTFA-DMF by-product detected in all three MXT-20, Chirasil-β Dex and follow-up analyses, is shown as an example in Extended Data Fig. 7c.

Follow-up analyses in the laboratory. To evaluate the possibility that heavy amino acids were still trapped into the column after the nominal run, a series of follow-up analyses was performed during which no additional compounds were injected. The GCMS conditions of the flight follow-up analysis were reproduced in the laboratory and differ slightly from the nominal analysis: the GC column was heated from 35 °C for 7.4 min to 189 °C at a 5 °C min^{-1} rate with a final hold of 10 min at 189 °C before the column cooled. In flight, the GC column was heated at 189 °C for only 3.4 min because of thermal constraints related to the use of the MXT-20–Chirasil-β Dex split column analysis. The 10 min hold used in the laboratory represents an ideal hold for the follow-up analysis in the case where the Chirasil-β Dex column is used alone, resulting in a time limit of 49 min for the follow-up analyses. For illustrative purposes, the results of the two first follow-up analyses are presented in Extended Data Fig. 8.

Results indicate that amino acids that were not released in the nominal run (main text, Fig. 5) are indeed released in the subsequent runs (Extended Data Fig. 8.). In the first follow-up analysis, four additional amino acids—methionine, serine, threonine and phenylalanine—were released within the 49 min time limit of the Chirasil-β Dex column and phenylalanine was released as the column cooled. In the second follow-up analysis, two additional amino acids—aspartic acid and hydroxyproline—were released, followed by five additional amino acids—glutamic acid, asparagine, lysine, glutamine and tyrosine—within the five next subsequent column follow-ups, thus enabling us to retrieve 20 of the 22 original amino acids injected. Further laboratory experiments indicate that these compounds were still in the GC columns but they stopped eluting as the column stopped heating. When the column was heated again in the subsequent analyses, molecules were eluted within the time limit of the Chirasil-β Dex column or as the column cooled. The detection of heavier amino acids during the cooling of the column and the subsequent analyses has critical implications for future analyses performed on Mars. Successive analyses would only be performed if interesting molecules (derivatized or not) were detected in the sample analysis and the first follow-up analysis.

Data availability

Data from the SAM experiments are archived in the Planetary Data System (pds.nasa.gov). Data from the laboratory experiments can be found at: https://doi.org/10.17632/fbr8tp38gs.1 Source data are provided with this paper.

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Author contributions

M.M. processed the data, calculated and interpreted GCMS data and wrote most of the manuscript and supplementary material under the supervision of S.S.J. and P.R.M. S.T. and C.A.M. prepared and developed the wet experiment using the SAM Testbed replica located at NASA-Goddard Space Flight Center and performed the SAM flight pyrolysis and derivatization GCMS experiments on Mars. M.M. and A.S. performed SAM-like laboratory analysis in support of flight data. J.C.S. and B.S interpreted EGA data. P.R.M., S.S.J., J.Y.B., A.B., J.P.D., J.L.E., C.F., D.P.G., R.N.-G., C.S., A.J.W., R.H.W. and G.M.W. participated in data processing, discussion and interpretation of results and/or manuscript editing.

Competing interests

The authors declare no competing interests.

Additional information

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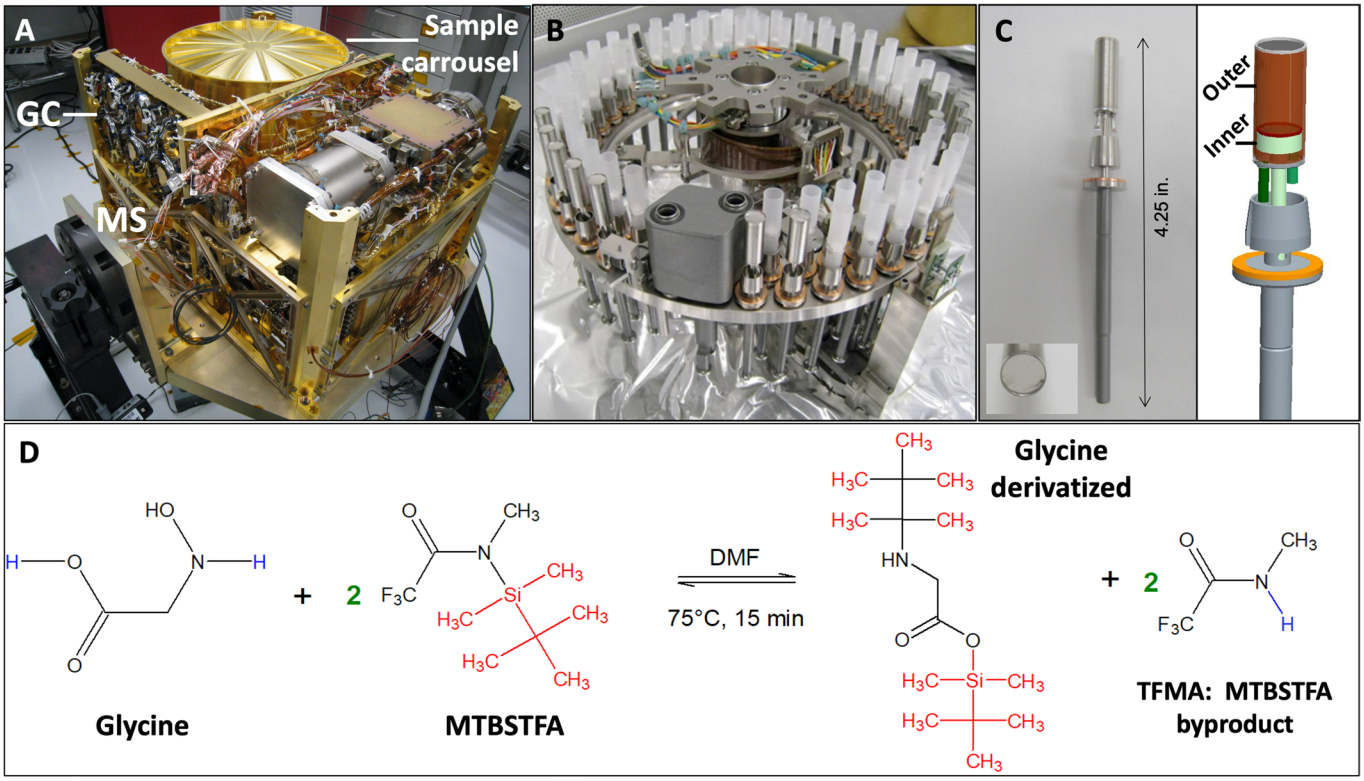
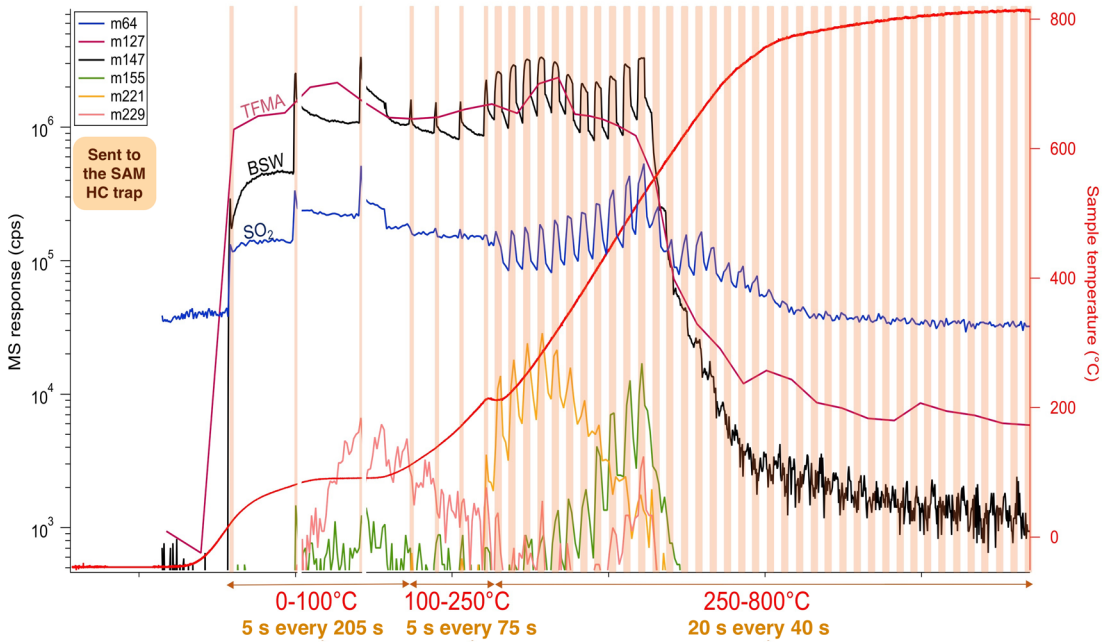
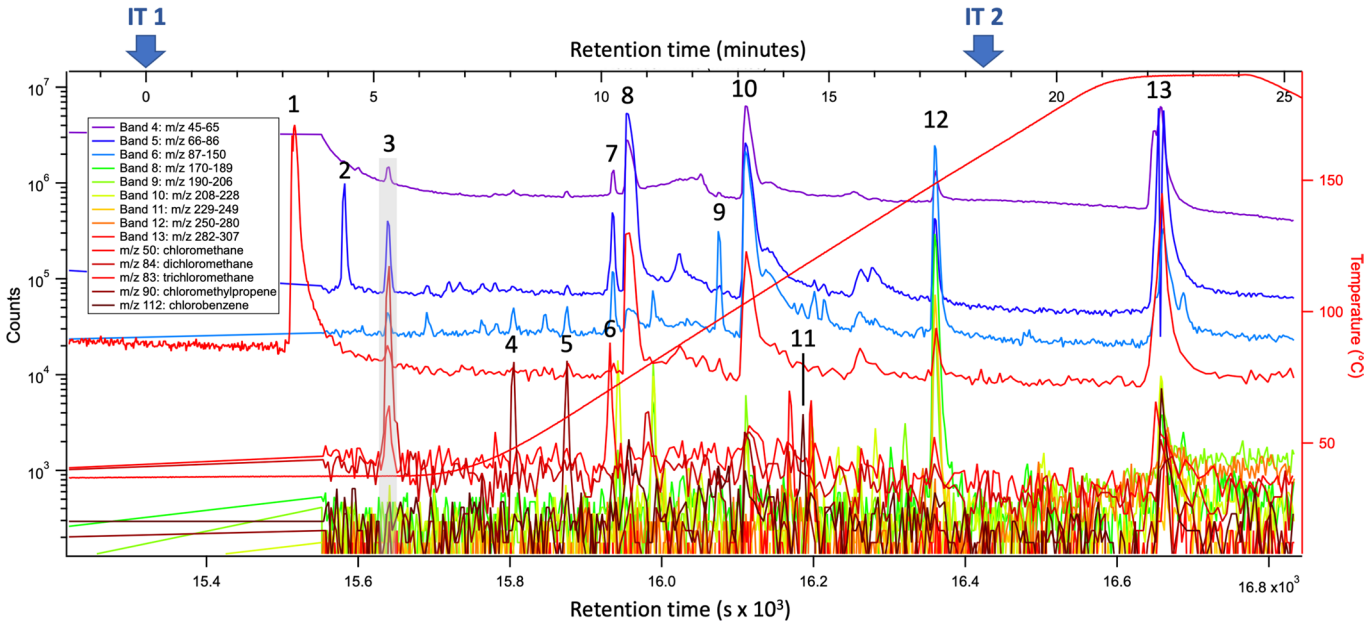


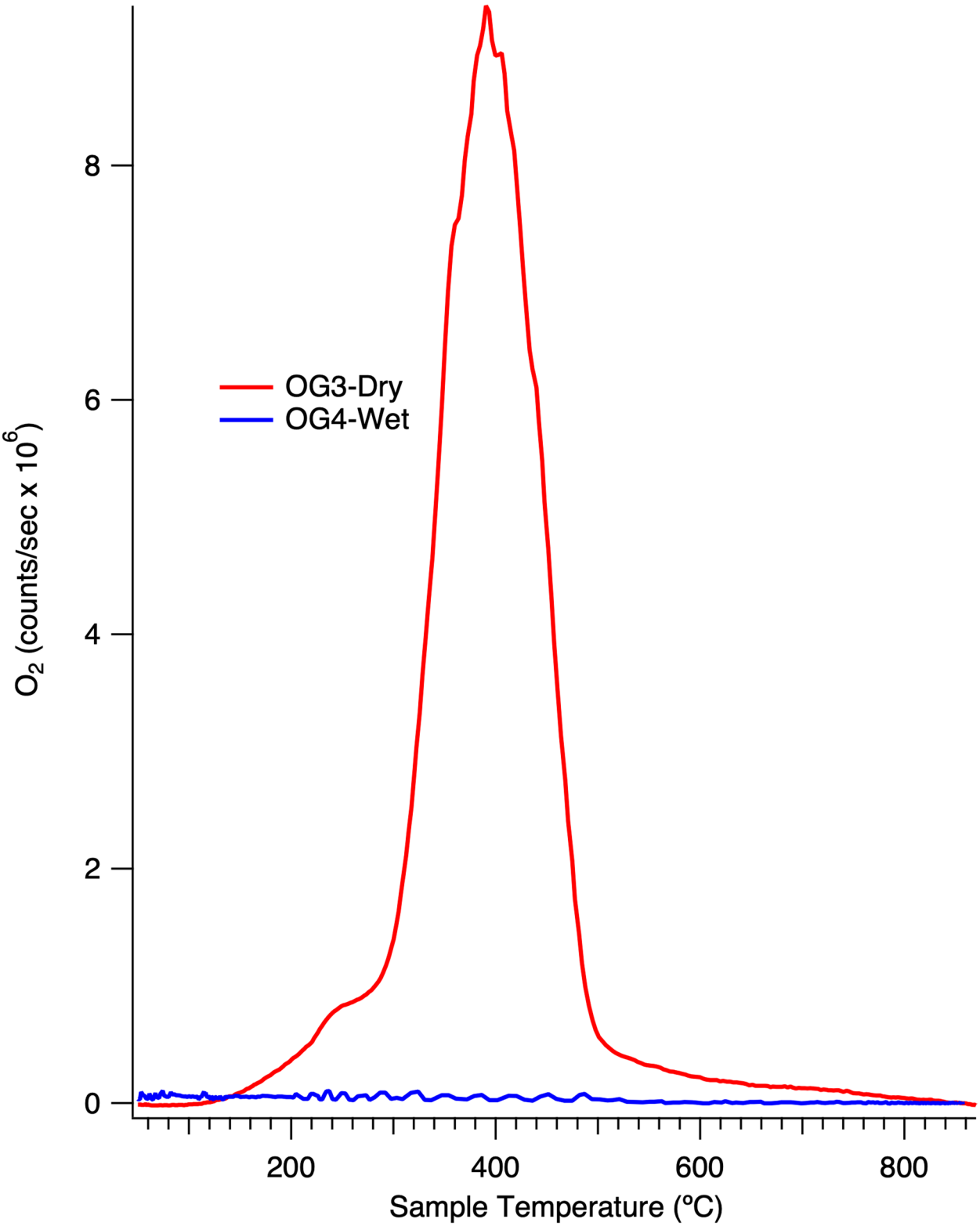
Extended Data Fig. 1 | SAM instrument suite (A), Sample Manipulation System (B), picture of the flight derivatization cup (C) and example of derivatization reaction (D). (**a**) SAM suite showing the gas chromatograph (GC), the quadrupole mass spectrometer (MS) and the sample carousel. (**b**) Sample Manipulation System (SMS) holding the 74 cups in the sample carousel, including both quartz and metal cups. (**c**) Picture of a flight derivatization metal cup (left) and its outer foil cap (bottom left), and a schematic (right) where the outer reservoir containing the MTBSTFA-DMF and pyrene standard and the inner compartment isolated by a welded metal foil containing the dried DL-3-fluorovaline internal standard are labeled. (D) Example of derivatization reaction of an amino acid with MTBSTFA: the labile hydrogen atom of glycine OH function is substituted by the *tert*-butyldimethylsilyl group of the MTBSTFA reagent. The products of the reaction are N,O-bis(dimethyl-t-butylsilyl)-glycine (Glycine, di t-BDMS) and 2,2,2-trifluoromethylacetamide (TFMA). Adapted from Mahaffy et al.[1].



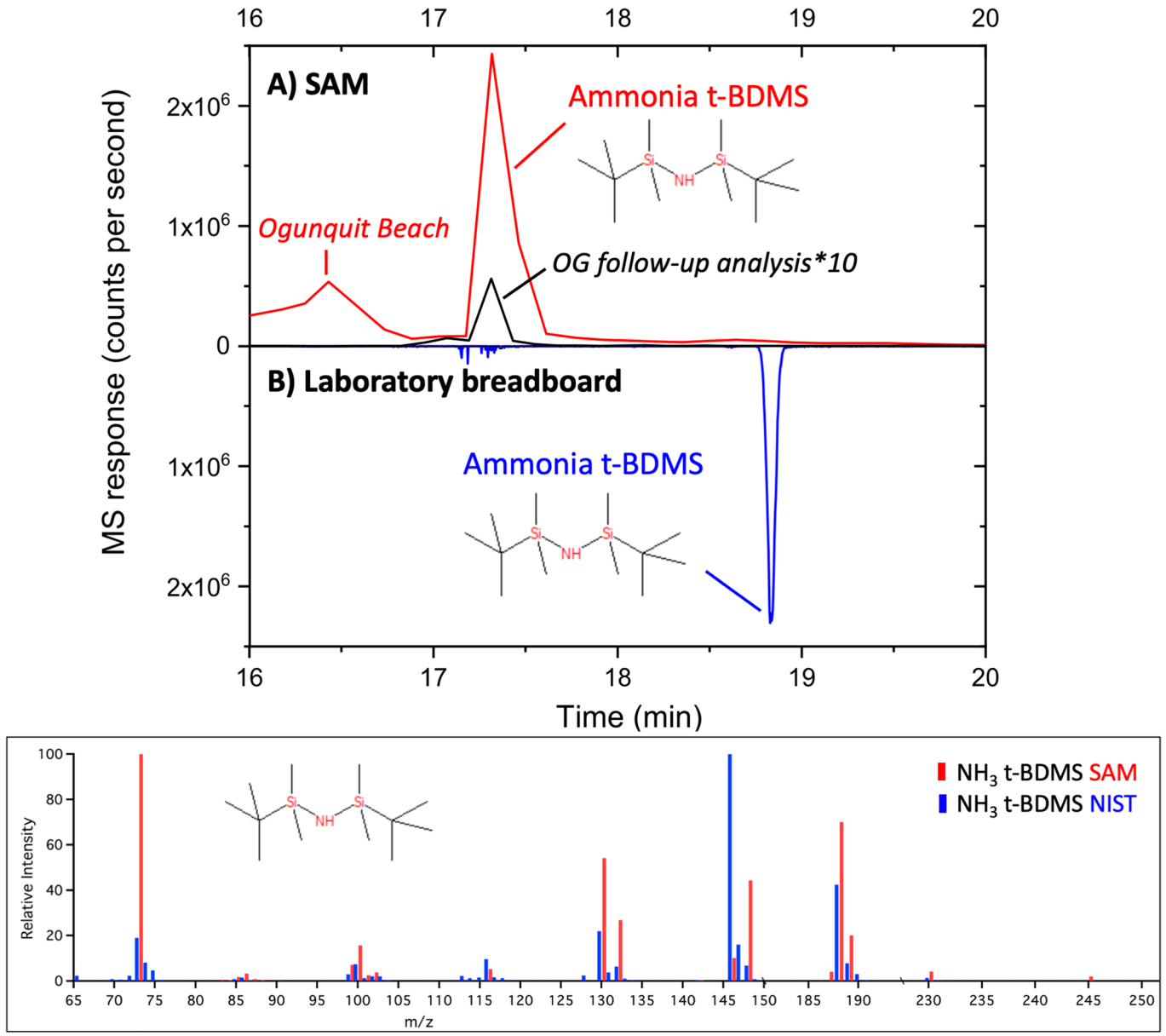
Extended Data Fig. 2 | Temperature cuts sent to the hydrocarbon trap. The orange bands highlight the temperature cuts that were sent to the hydrocarbon (HC) trap during pyrolysis. To avoid the saturation of the MTBSTFA-DMF by-products, illustrated here with bi-silylated water (BSW) and *N*-methyl-2,2,2-trifluoroacetamide (TFMA), 2.5% of the gas was sent from 0–100 °C, 7% from 100–250 °C, and 50% from the remaining portion of the pyrolysis ramp.



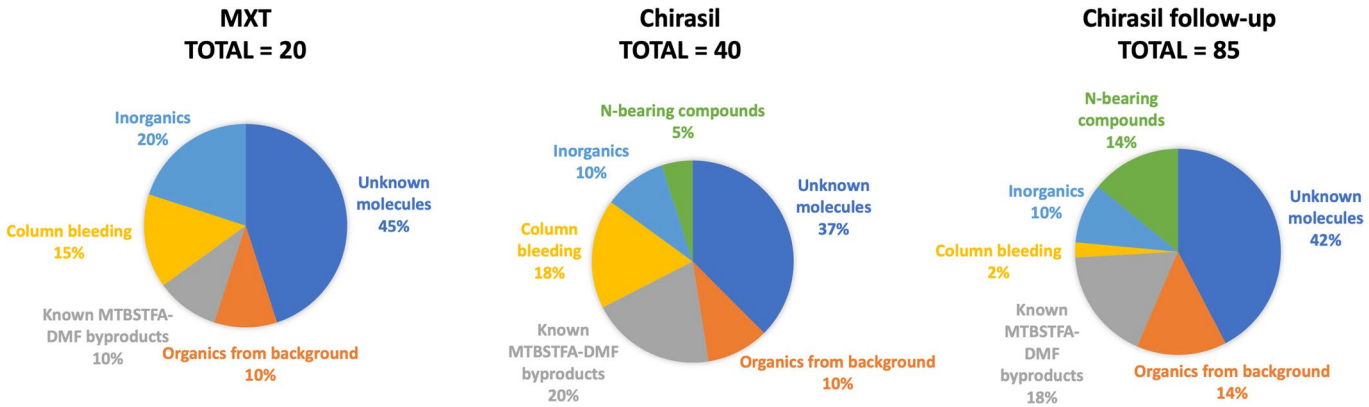
Extended Data Fig. 3 | Chromatogram extracted from the pyrolysis–GCMS analysis of the Ogunquit beach sample (OG3). The two IT flashes: IT 1 and IT 2, are represented by blue arrows at activation times: 0 and 18.3 min, respectively. Bands 4–6 and 8–13 are plotted and correspond to the sum of the ions between the range of given masses (*for example*, band 4 is the sum of the ions from m/z 45 to 65). The major ion mass fragments of the main chlorohydrocarbons detected have also been extracted to facilitate their identification. The main labeled peaks are: 1) m/z 50: chloromethane, 2) CS_2, 3) m/z 84: dichloromethane, 4–5) m/z 90: 1- and 3-chloromethylpropene isomers, 6) m/z 83: trichloromethane, 7) t-BDMS-F, 8) benzene, 9) toluene, 10) TFMA coeluted with Cl-t-BDMS, 11) m/z 112: chlorobenzene, 12) bi-silylated water, and 13) co-elution of all the previous molecules that come out a second time after the second IT flash. SO_2 was also detected and co-elutes with chloromethane at 3.2 minutes but is not represented due to the saturation of its peak.



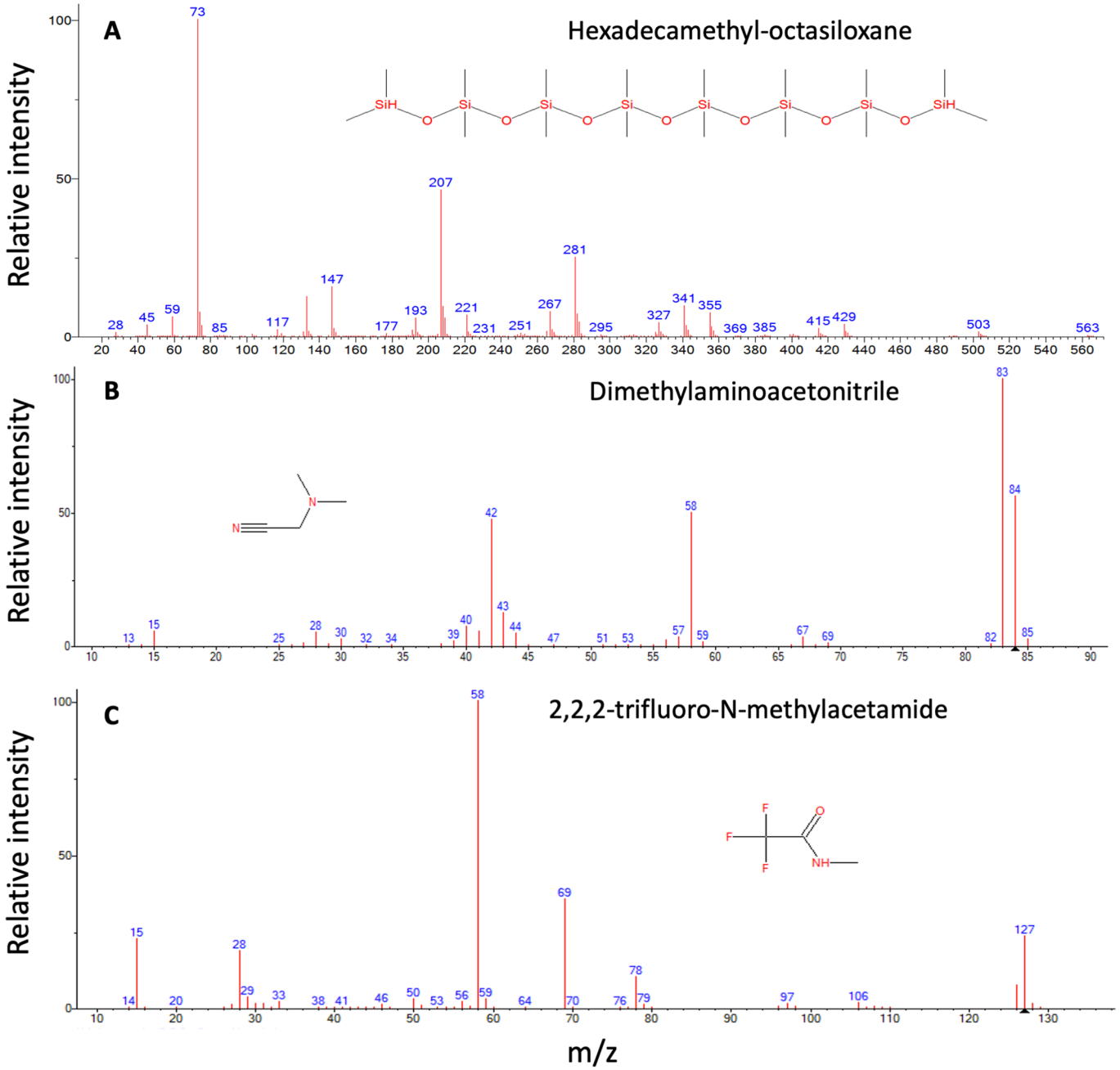
Extended Data Fig. 4 | Dioxygen released from the dry (OG3-red) and wet (OG4-blue) OG sample. Thermograms of evolved gas from the OG sample comparing the dioxygen released from the dry (OG3-red) and wet (OG4-blue) sample.



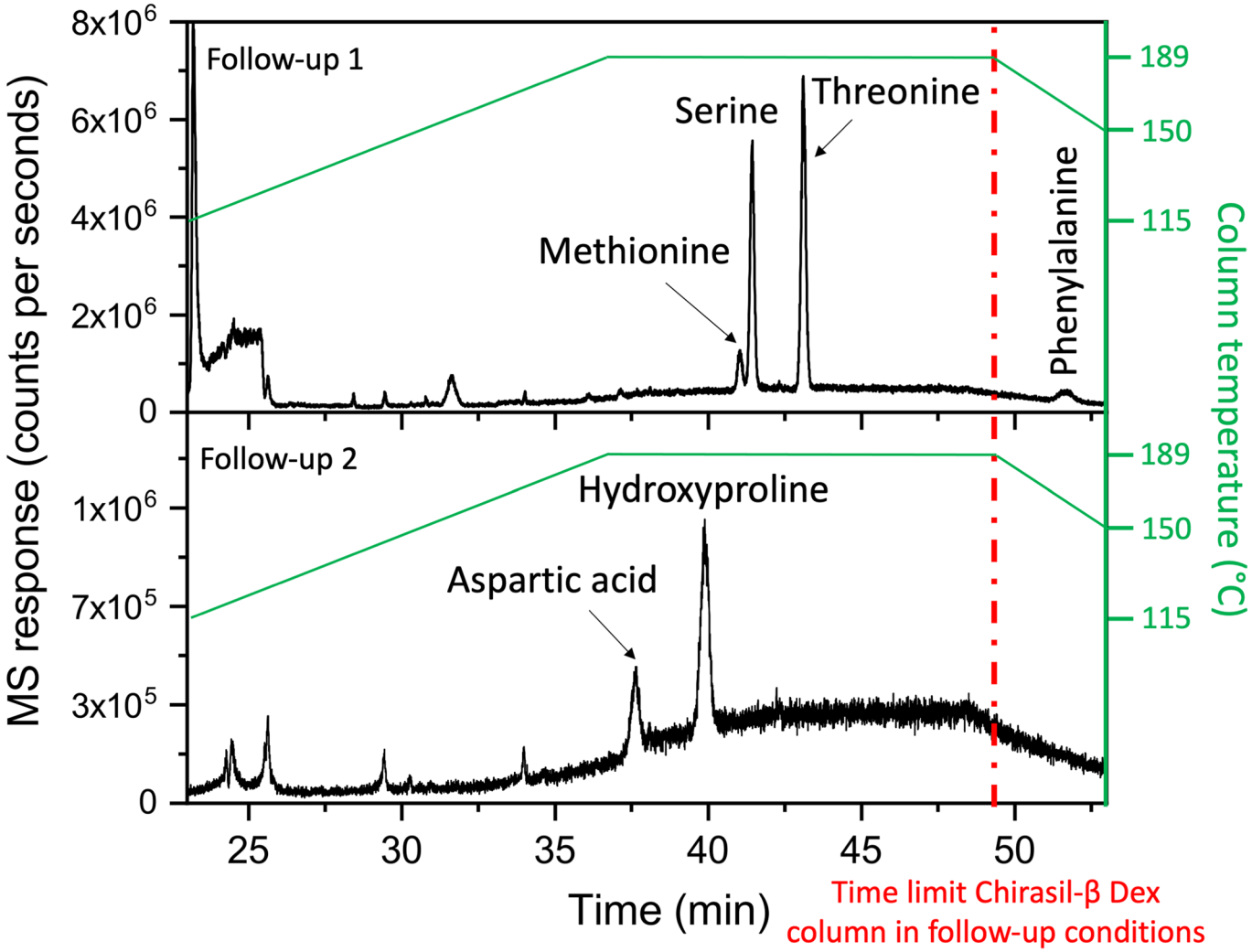
Extended Data Fig. 5 | Derivatized ammonia identified by GCMS. (Top) GCMS identification of derivatized ammonia eluted at 17.3 min in the OG flight chromatogram (**a**) compared to the laboratory GC analysis of derivatized ammonia analysed in SAM-like operational conditions with the Chirasil-β Dex column (**b**) detected at 18.8 min. The OG chromatogram is compared to the follow-up analysis from which the signal was increased by 10. The OG follow-up analysis was performed with a 5 °C/min ramp compared to the 10 °C/min ramp of the OG analysis, thus, t-BDMS ammonia eluted at 25.1 min in the follow-up compared to the 17.3 min in the OG analysis. To allow the comparison of both peaks in one figure, the follow-up chromatogram was shifted of 7.8 min to match both flight-retention times. Because the m/z 146 major ion mass fragment of derivatized NH_3 was under sampled over the course of the peak elution, its intensity is lower compared to the m/z 188 second major ion mass fragment and what it should be from the NIST spectrum. The m/z 188, second major ion mass fragment of derivatized ammonia, was then chosen and extracted from the TIC for all three chromatograms. (Bottom) Mass spectrum of derivatized ammonia extracted from the SAM chromatogram (red) compared to the mass spectrum extracted from the NIST Mass Spectral Database (blue). A fraction of the m/z 73 and 148 from the SAM mass spectra belong to the mass spectra of bi-silylated water (this is why they have higher intensities in flight compared to the NIST intensities).



Extended Data Fig. 6 | Percentages of the main category of compounds that were extracted relative to the total number of compounds that were extracted from the MXT-20, Chirasil-β Dex and Chirasil-β Dex follow-up analyses. These percentages are a rough estimation of the compounds that we were able to extract and with co-elutions represent a lower limit of the molecules present in the GCMS chromatogram. This is especially true for the MXT-20 and Chirasil-β Dex columns where the MTBSTFA-DMF saturation plus related by-products are likely obscuring the signal from other molecules.



Extended Data Fig. 7 | Examples of mass spectra of molecules detected in the OG and follow-up analyses. a. Hexadecamethyl-octasiloxane, a common product from column bleeding was detected in the Chirasil-β Dex chromatogram, **b**. Dimethylaminoacetonitrile, N-bearing organic detected in the Chirasil-β Dex follow-up analysis, **c**. 2,2,2-trifluoro-N-methylacetamide, a common MTBSTFA by-product detected in MXT-20, Chirasil-β Dex and follow-up analyses, **d**. Bis tert-butyldimethylsilyl-ammonia, N-bearing MTBSTFA by-product detected in the Chirasil-β Dex and follow-up analyses.



Extended Data Fig. 8 | A 32 min section (23–55 minutes) of the chromatogram of the follow-up analysis obtained with the spare Chirasil-β Dex column where no amino acids were injected. As shown above, derivatized methionine, serine, threonine, and phenylalanine would be detectable in the first follow-up analysis, and derivatized aspartic acid and hydroxyproline would be detectable in the second follow-up analysis within the standard operating conditions used on Mars for the Chirasil-β Dex column. Derivatized phenylalanine eluting after 49 min of the first follow-up run (represented in red dashed dots) could be detectable if the MS signal continued to be recorded while the column cooled down after the end of the follow-up run. The temperature of the Chirasil-β Dex column is represented in green; it reached ~150 °C at ~56 min of the GC run.