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Evidence for Proteogenic Peptide-like Sequences in Meteorites Through an Enzyme-Catalysed Stereoselective Hydrolysis Strategy

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In this manuscript, we evidenced for the first time proteogenic like peptide sequences in meteorite thanks to an original stereoselective enzymatic hydrolysis. Within this framework, we have first characterised the amino acids content of two meteorites, Murchison and Allende, after the standard acid hydrolysis protocol currently used in astronomical studies. To reach this goal, we have developed a highly sensitive chiral LC-MS method and we have highlighted new l- and d-enantiomers, never detected before in both meteorites. These primary findings extend the list of amino acids already found in meteorites. We next investigated the presence of proteogenic like peptide sequences. For that, we have compared the amounts of amino acids l- and d-enantiomers released from either the standard acid hydrolysis or our stereoselective peptidase hydrolysis. Thanks to this strategy, we have highlighted the presence of peptide sequences involving proteogenic l-amino acids in the Murchison together with their absence in Allende, which is consistent with the respective organic content of both meteorites. Furthermore, we demonstrated that the peptide sequences were indigenous to the Murchison meteorite.

File list (2)

Evidence for proteogenic peptide-like sequences in meteorites through an enzyme-catalysed stereoselective hydrolysis strategy

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The characterisation of organic molecules that could have been delivered onto the early Earth is of prime importance for our understanding of biomolecules origin. While numerous amino acids were detected in primitive carbonaceous meteorites, the evidence for peptides remains sporadic mostly due to the lack of efficient methods allowing their unambiguous detection. Here, we present the development of a novel strategy, inspired from proteomics, emphasizing the presence of proteogenic peptide-like sequences in meteorites. Murchison and Allende meteorites water extracts were submitted to enzymatic hydrolysis with the stereoselective aminopeptidase M. By monitoring and comparing amino acids enantiomers released by either enzymatic digestion or by the classical HCl hydrolysis method, we showed that at least seven proteogenic L-amino acids are involved in peptide sequences in Murchison while, remarkably, peptides were not detected in Allende. This strategy could be applied with other peptidases to go further in the characterisation of peptide sequences included in extra-terrestrial objects, thereby providing new clues on the molecules that were available in the Solar System for the emergence of life on Earth.

Introduction

Many organic compounds present in comets, asteroids or interplanetary dust particles may have been delivered onto the early Earth and used as chemical precursors to form molecules essential for feeding a prebiotic chemistry that ultimately led to the emergence of life[1]. Within this framework, numerous building blocks of living systems such as amino acids, sugars and nucleobases have been highlighted in various extra-terrestrial environments, including primitive meteorites [2–9]. In contrast, the detection of macromolecules such as peptides, that would represent a major advance for understanding the origin of organised molecular systems on the early Earth, remains challenging.

Several studies reported either the appearance or increase of amino acids signal when solvent extracts of extra-terrestrial samples were submitted to hydrochloric acid (HCl) hydrolysis[10–13], suggesting the presence of molecules with either amide or ester bonds, as well as peptide-like structures[14]. However, the lack of selectivity of this pretreatment does not allow to firmly conclude on the presence of peptides in these objects. By targeting several specific dipeptides using derivatisation-GC-MS analysis, Shimoyama et al.[13] identified confidently glycyl-glycine, the sole dipeptide detected in two meteorites so far (Murchison and Yamato-791198), although di-amino acids were highlighted in Murchison a long time ago[15]. While their direct GC-MS procedure revealed the smallest existing peptide in meteoritic samples, longer peptides cannot be analysed by GC-MS even after proper derivatisation. Mc Geoch et al.[11] also attempted to directly investigate polymer amides in Murchison and Allende meteorites by matrix-assisted laser desorption time-of-flight mass spectrometry. However, given the diversity of possible peptide sequences, their analytical procedure did not allow peptides detection in both meteorites. Thus, since effective and versatile methods enabling peptides evidence are still lacking, their presence in meteorites remains elusive.

Here, we report on a new strategy designed to detect, without ambiguity, proteogenic peptide-like sequences in extra-terrestrial samples. For this purpose, we transposed a proteomics approach currently employed for the sequencing of peptides in biological samples using aminopeptidase M (APM, EC 3.4.11.2[16,17]). In contrast to HCl hydrolysis which induces the release of amino acids from a wide variety of precursors, this exopeptidase catalyses stereoselectivity the stepwise removal of L-α-

amino acids from the *N*-terminal end of peptides[18] (Figure 1). Hence, by submitting meteoritic water extracts to APM digestion, the presence of proteogenic peptide-like sequences can be probed through the generation of free L-amino acids as the result of the selective enzyme-catalysed cleavage of peptide bonds.

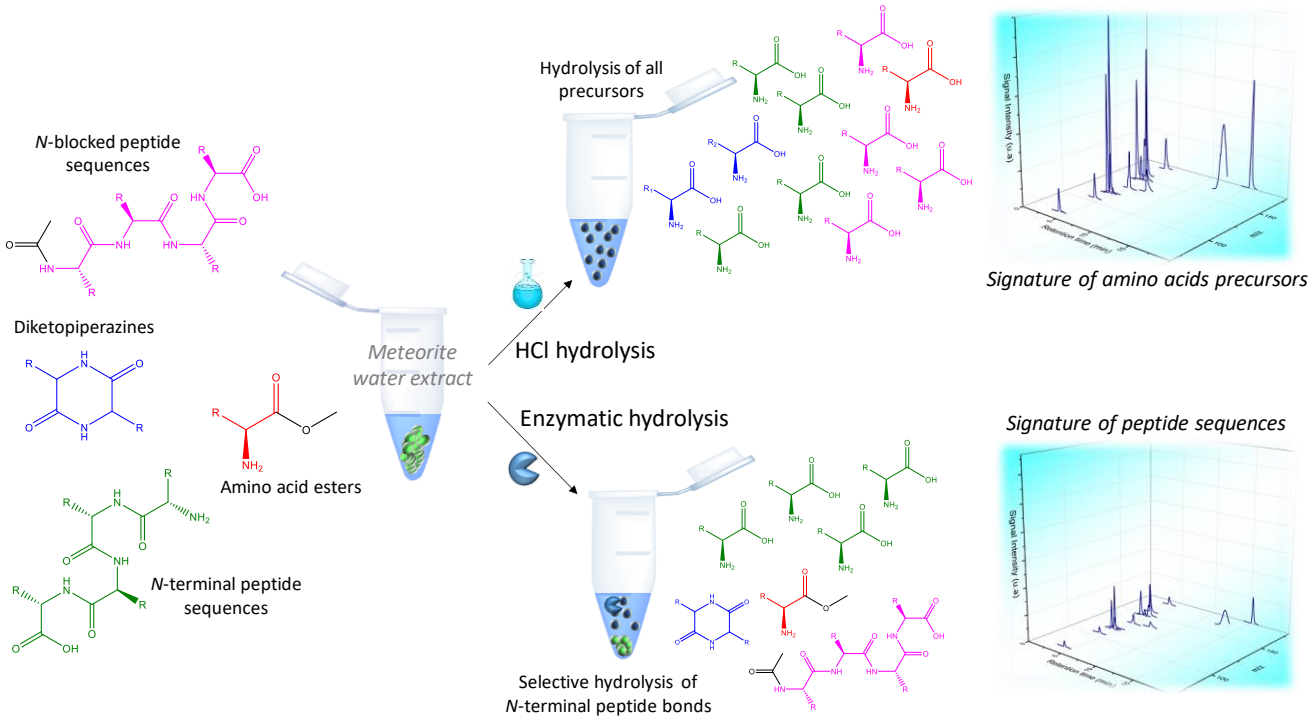


Figure 1. Enzymatic *versus* chemical strategy to evidence peptide sequences in meteorite water extract.

Results and discussion

Amino acids in Murchison and Allende water extracts after the standard HCl-hydrolysis. We started our study by analysing the amino acids present in water extracts of two meteorites, Murchison and Allende, after the standard hydrolysis protocol which implies the use of 6 M HCl at 110 °C during 24 h. For this purpose, we have developed a chiral LC-MRM MS method enabling the separation and detection of a wide range of amino acids (see details in the methods section), and we reveal the presence of glycine, alanine, serine, valine, threonine, leucine, aspartic acid, glutamic acid, phenylalanine, arginine, lysine, proline and histidine in both meteorites (Figure 2 and Table S2). Isoleucine, alloisoleucine, and the non-biogenic isovaline have also been detected in Murchison, but not in the Allende sample.

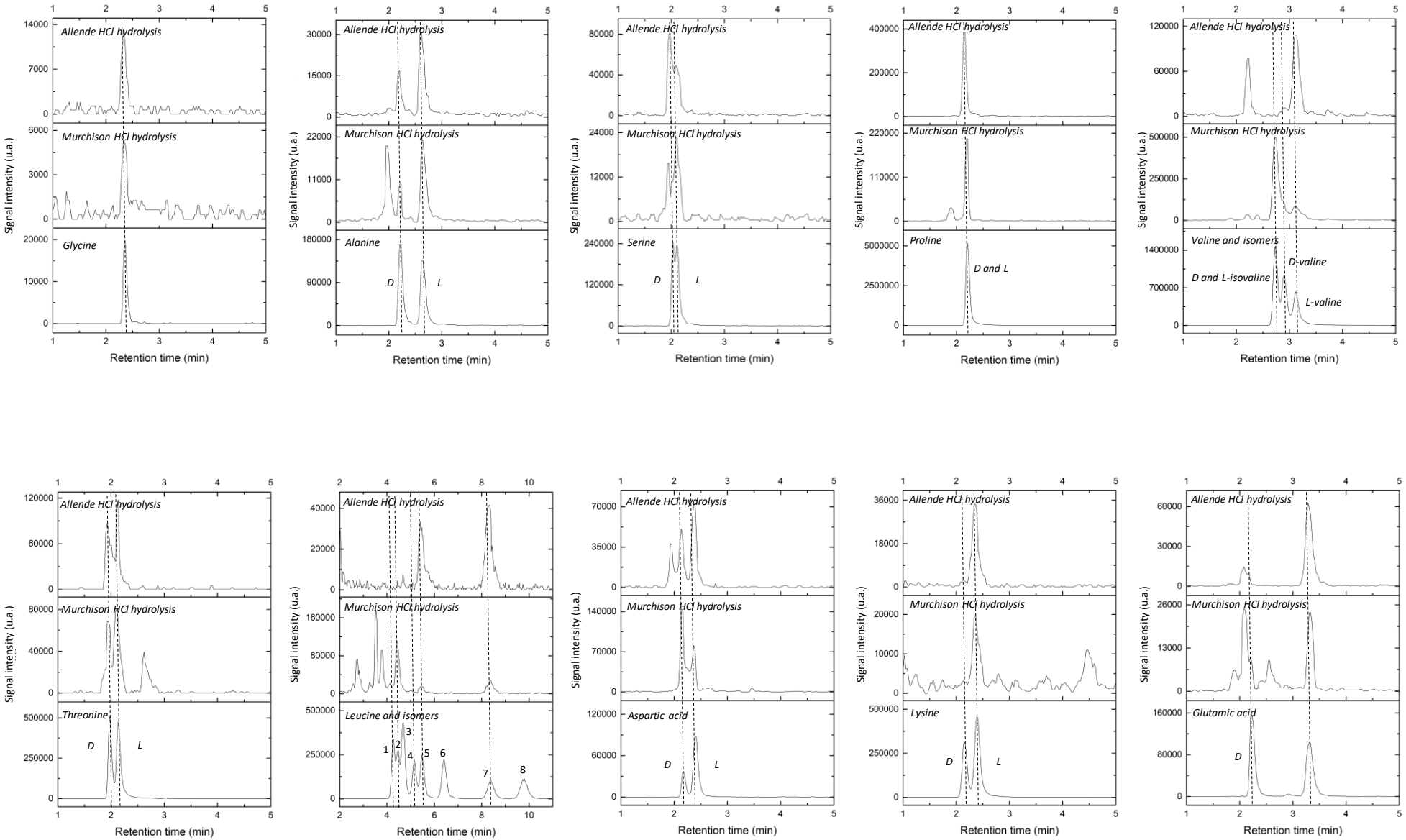
While all these amino acids were previously identified in Murchison after HCl hydrolysis, both D- and L-enantiomers have been evidenced for the first time in this study (Table S2)[20–24]. In the same manner, such amino acid diversity was never highlighted into the interior of Allende to date[25,26] thus suggesting that native compounds (H_2, H_2O, N_2, NH_3, CO, CH_4, CH_3OH, CO_2…) trapped into the core of its parent body during the accretion process could have led to organic molecules when temperatures were decreasing[27]. Furthermore, the presence of D-enantiomers in this sample indicates the indigenous nature of these amino acids. These new findings emphasize the sensitivity of our analytical method which is characterised by a ten to one thousand fold reduced detection limit as compared to the standard derivatisation and GC-MS analysis procedure (Table S1)[22,28,29].

Amino acid concentrations, determined through external standard quantification, were of the same ppb range as reported previously[20–24,30] (Table S2). In contrast to earlier studies, we noted an apparent rather low amount of glycine compared to the other amino acids. This result can be explained by the absence of a chemical derivatisation step[31] in our analytical protocol compared to those described in the literature. Indeed, owing to their physicochemical properties[32,33], we have showed that signals of native glycine and polar amino acids (aspartic acid, glutamic acid, proline, threonine and histidine) are significantly depressed in acid hydrolysed meteorite water extract despite increasing concentration (Figure S1 and Table S3). Apart from glycine, we found however relative distributions of amino acids consistent with an abiotic process of formation (the low mass molecules being the most abundant, while the higher masses present lower quantities, Table S2).

Regarding amino acids enantiomeric excesses (e.e.) in both meteorites, we observed values ranging from 0.6 % to 92 % mostly in favour of the L-enantiomer. While these data could indicate a possible terrestrial contamination, L-enantiomeric enhancements in carbonaceous chondrites have already been shown[20,21,34–36] and plausible hypotheses on the sources of asymmetry induction (e.g. photochemical effects on ices, parity violation or phase changes) are currently studied[37]. Moreover, significant variability is reported for e.e. according to the literature[20–22,30,34,35] and similar values have already been reported (e.g. for Murchison, e.e ranged from 10 to 95 %; reference data shown in Table S2). Nevertheless, the non-ambiguous detection of non-proteogenic residues (i.e. D-enantiomers and isovaline), together with the determination of L/D ratios from 0.5 to 3.3 for alanine, valine, threonine,

leucine, aspartic acid, glutamic acid and arginine confirm the extra-terrestrial origin of at least these seven amino acids in both meteorites.

While these preliminary experiments highlighted many amino acids in the acidic hydrolysed meteorite extracts, their precursors, either peptidic or not, cannot be determined, given the lack of selectivity of HCl hydrolysis.


Allende HCl hydrolysis
Murchison HCl hydrolysis
Glycine
Alanine
D
L
Serine
Proline
D and L
Valine and isomers
D-valine
D and L-isovaline
L-valine
Threonine
Leucine and isomers
1
2
3
4
5
6
7
8
Aspartic acid
Lysine
Glutamic acid
Signal intensity (u.a.)
Retention time (min)

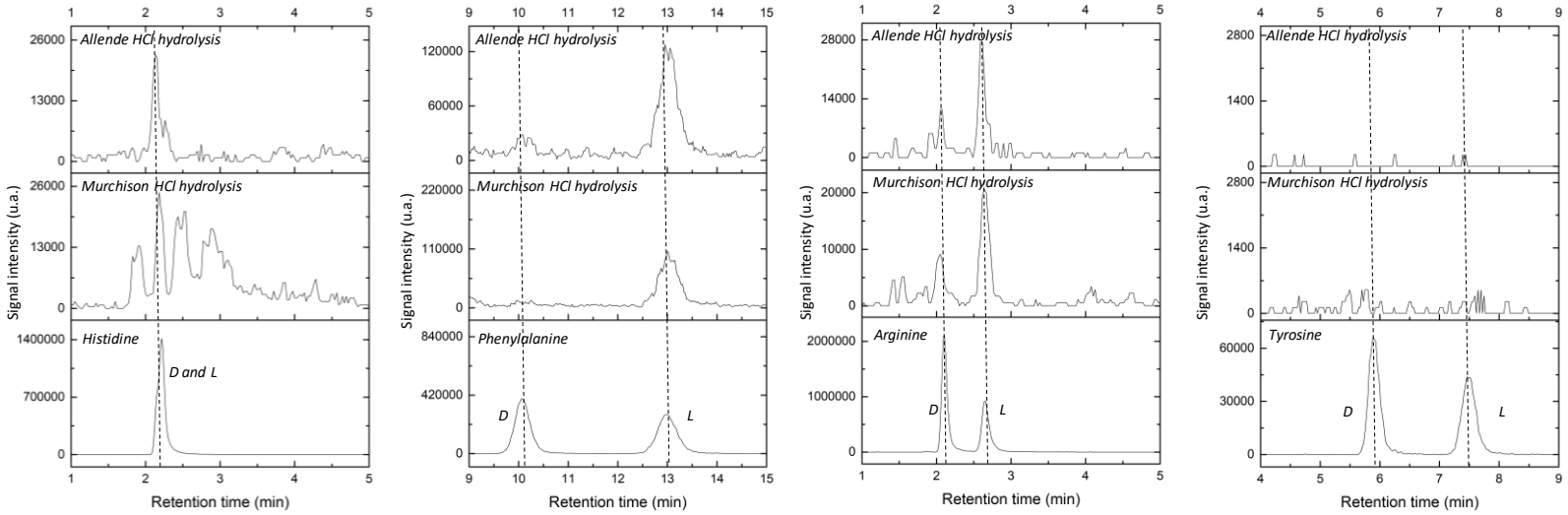


Figure 2. Total Ion Chromatograms of amino acids detected in Murchison and Allende water extracts submitted to strong acidic hydrolysis.

For each amino acid, from bottom to top: Standard; Murchison water extract submitted to 6 M HCl hydrolysis; Allende water extract submitted to 6 M HCl hydrolysis; Leucine isomers: 1. D-allo-isoleucine; 2. D-isoleucine; 3. L-allo-isoleucine; 4. L-isoleucine; 5. D-leucine; 6. D-norleucine; 7. L-leucine; 8. L-norleucine.

APM *versus* HCl selectivity for *N*-terminal peptide bonds. In order to avoid this drawback, we then designed a more selective strategy involving an enzyme, the aminopeptidase M (APM). First, we checked the selectivity of APM for *N*-terminal L-homochiral peptide bonds in comparison to the standard HCl hydrolysis protocol. For that purpose, we evaluated the hydrolysis rate of L-homochiral peptides and other potential amino acid precursors, such as esters and diketopiperazines already reported in meteorite extracts[3,7]. Thus, various amino acid precursors were incubated in phosphate buffers (pH 6.9) for 4 h at 30 °C with or without APM and the mixtures were analysed by LC-MS (Table 1, Figures S2-S3). In parallel, standard solutions were also submitted to 6 M HCl hydrolysis for 24 h. In the absence of the exopeptidase, partial hydrolysis was recorded with the whole tested molecules including peptides of various lengths, the protected peptide Ac-Ala-Ala-Ala-OMe, the piperazine-2,5-dione and the phenylglycine methyl ester (Table 1). When incubated with APM for 4 h, unprotected peptides were almost totally consumed as the result of the enzyme-catalysed peptide bonds cleavage (Table 1, entries 1-5). This result was confirmed by the appearance of significantly higher quantities of free amino acids compared to the experiment in which peptides were not submitted to enzymatic hydrolysis (Figure 3). In contrast, such an increase hydrolysis rate was not observed with the other amino acid precursors when incubated with or without APM (Table 1, entries 6-8). On the other hand, all the compounds were hydrolysed after HCl treatment (Table 1, entries 1-8, Figures S2-S3). This benchmark experiment confirms the selectivity of APM for the hydrolysis of *N*-terminal L-homochiral peptide bonds while the non-selective acid hydrolysis can generate free amino acids from an important variety of precursors.

Table 1. Comparison of HCl and APM selectivity towards diverse amino acid precursors. Percentages of remaining *N*-unsubstituted L-homochiral peptides (entries 1-5), *N*-blocked peptide (entry 6), diketopiperazine (entry 7) and amino acid ester (entry 8) after four hours of incubation in the absence or presence of APM. Comparison to the reference HCl hydrolysis method (detailed experimental results are presented in Figures S2-S3).

Entry	Molecule	t = 4 h, no APM		t = 4 h, APM		APM hydrolysis	t = 24 h, HCl		HCl hydrolysis
1	Gly-Tyr	94	± 1%	12	± 3%	√	0%		√
2	Val-Tyr-Val	97	± 1%	0 %		√	0%		√
3	Tyr-Gly-Gly-Phe-Leu	91	± 2%	0 %		√	0%		√
4	Tyr-Gly-Gly-Phe-Met	96	± 1%	0 %		√	0%		√
5	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	80	± 9%	13	± 3%	√	23	± 3%	√
6	Ac-Ala-Ala-Ala-OMe	92	± 1%	93	± 1%	NO	0 %		√
7	Piperazine-2,5-dione	87	± 3%	93	± 3%	NO	0 %		√
8	Phenylglycine-OMe	67	± 0%	84	± 4%	NO	0 %		√

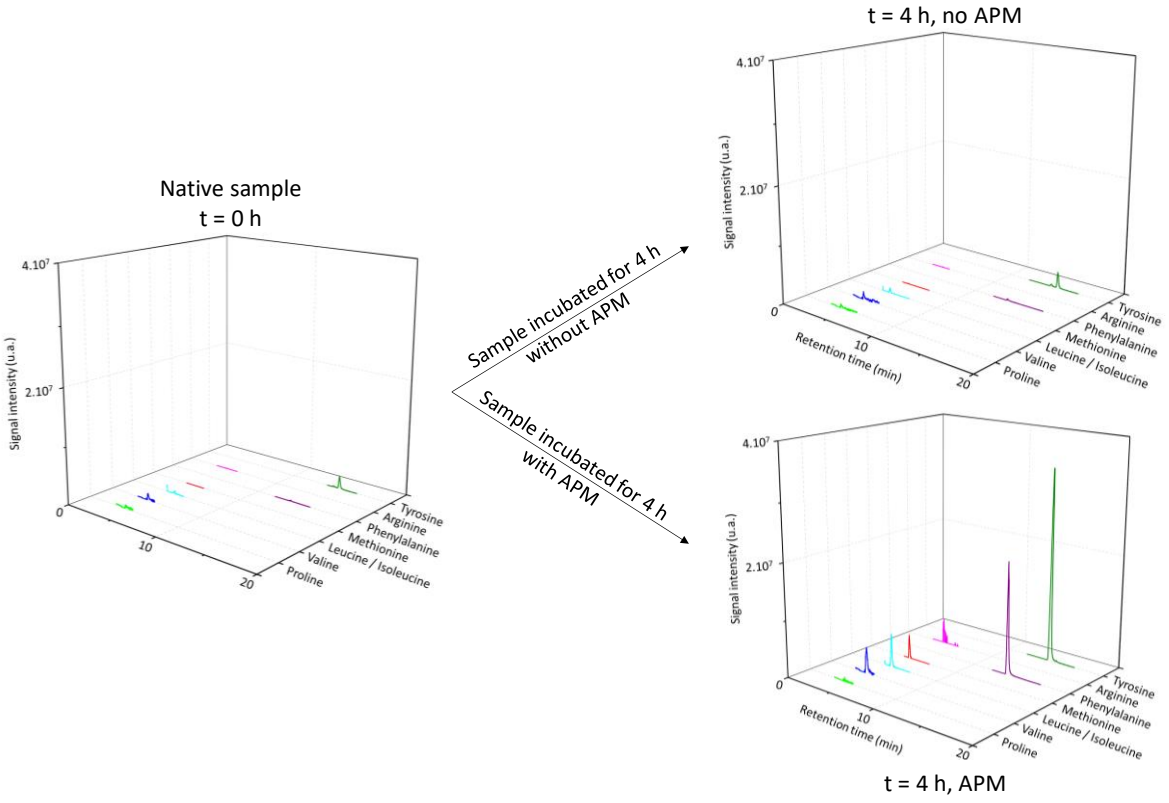


Figure 3. Amino acids released after APM hydrolysis of unsubstituted peptides. Signals of amino acids released from the standard *N*-terminal peptides (entries 1-5 in Table 1) incubated for four hours without (t = 4 h, no APM) or with the enzyme (t = 4 h, APM). The star fitted to arginine meant that its area was multiplied by 10 to be shown on the same scale as the other amino acids.

Investigation of APM stereoselectivity. To get an additional proof of the presence of proteogenic peptide-like sequences in the meteorite extracted samples, we then exploited the stereoselectivity of APM. Indeed, although the enzyme exhibits a broad affinity towards most amino acid residues (except proline which resists to hydrolysis)[16,19], its kinetics of peptide bonds hydrolysis is greatly affected by the absolute configuration of the two *N*-terminal residues. Indeed, APM preferentially cleaves peptide bonds between two consecutive L-amino acids (Figure 4a)[38–40]. Therefore, the treatment of meteoritic extracts with APM should significantly enhance the concentrations of L-amino acids if peptide bonds between two proteogenic amino acids are present in the samples while the amounts of D-enantiomers should only slightly vary.

We first confirmed the stereoselectivity of APM for the hydrolysis of L-homochiral peptide bonds with three of the tri-alanine stereoisomers (L-Ala-L-Ala-L-Ala, D-Ala-D-Ala-D-Ala and L-Ala-D-Ala-L-Ala) used as model peptides. In these experiments, the L-homochiral tri-alanine was indeed totally digested by the exopeptidase within 4 h while the two other isomers were only partially consumed (Figure 4b). Similar results were obtained when L- and D-homochiral penta-alanine were incubated together in isomolar concentration with APM. These results confirm that APM hydrolyses faster *N*-terminal L-homochiral peptide bonds.

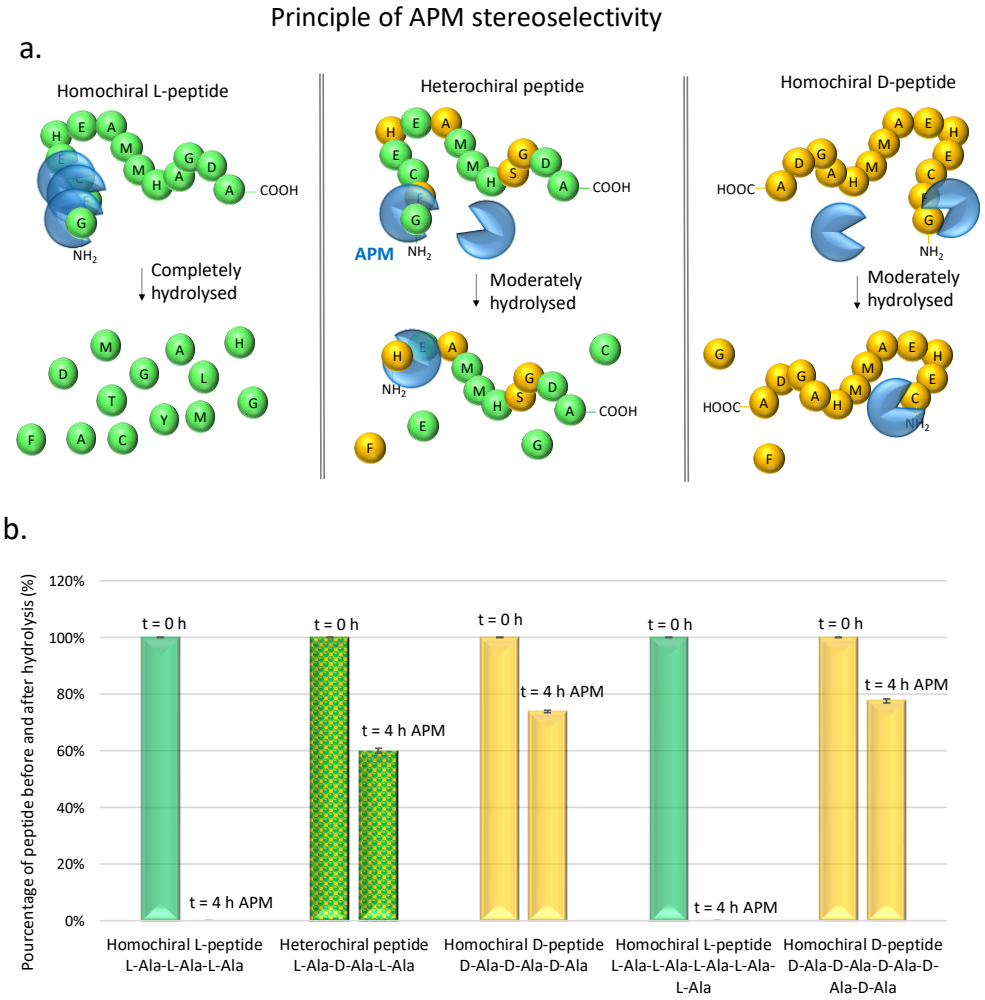


Figure 4. Investigation of APM stereoselectivity. a. Principle of APM stereoselectivity. b. Percentages of remaining homochiral and heterochiral alanine peptides after four hours in the presence of APM. Tri-alanine peptides were incubated separately for hours with APM (experiment run in duplicate). Penta-alanine peptides were mixed together and incubated for hours with APM (experiment run triplicate).

Investigation of proteogenic peptide-like sequences in Murchison and Allende water extracts. Murchison and Allende extracts were incubated either with or without APM in phosphate buffer for 4 h and the amounts of D- and L-amino acids were investigated using our chiral analytical method. To avoid misinterpretation, we also verified that the increase in amino acids, which could be observed in the meteorites extracts with the enzyme, was not due to APM auto-hydrolysis (t = 4 h APM, Figures S5 and S6). This control assay showed that, except for arginine which was significantly released through

APM auto-digestion, a rise in amino acid signals would be the consequence of the enzyme-catalysed cleavage of the peptide bonds.

We next focused our attention on the amino acids for which both enantiomers presented resolved chromatographic peaks (Figures S5 and S6). While this condition was completed for several compounds in the soluble-rich chondrite Murchison (alanine, valine, threonine, leucine, aspartic acid, glutamic acid and phenylalanine in Figure S5b), the very low amino acid signals in Allende did not enable the accurate quantification of more than one enantiomer (either D- or L- depending on the compound). These results indicate that, contrary to HCl hydrolysis (Allende HCl hydrolysis in Figure S6), APM had a modest impact on amino acid precursors present in Allende. Thus, in our hydrolysis conditions, peptide sequences were not evidenced in this meteorite. This result is consistent with the organic content of this CV meteorite that consists almost exclusively of an insoluble kerogen-like material likely resulting from its strong thermal alteration[7,27].

We then continued our investigation on the Murchison sample. After having verified that our chiral LC-MRM MS method ensured the measure of very small variations of enantiomeric ratios (Figure S4), the signals of alanine, valine, threonine, leucine, aspartic acid, glutamic acid and phenylalanine obtained under each condition (without APM, with APM, and with HCl) were deconvoluted, quantified, and D- and L-enantiomers concentrations were then compared (Figures 5 and S5b).

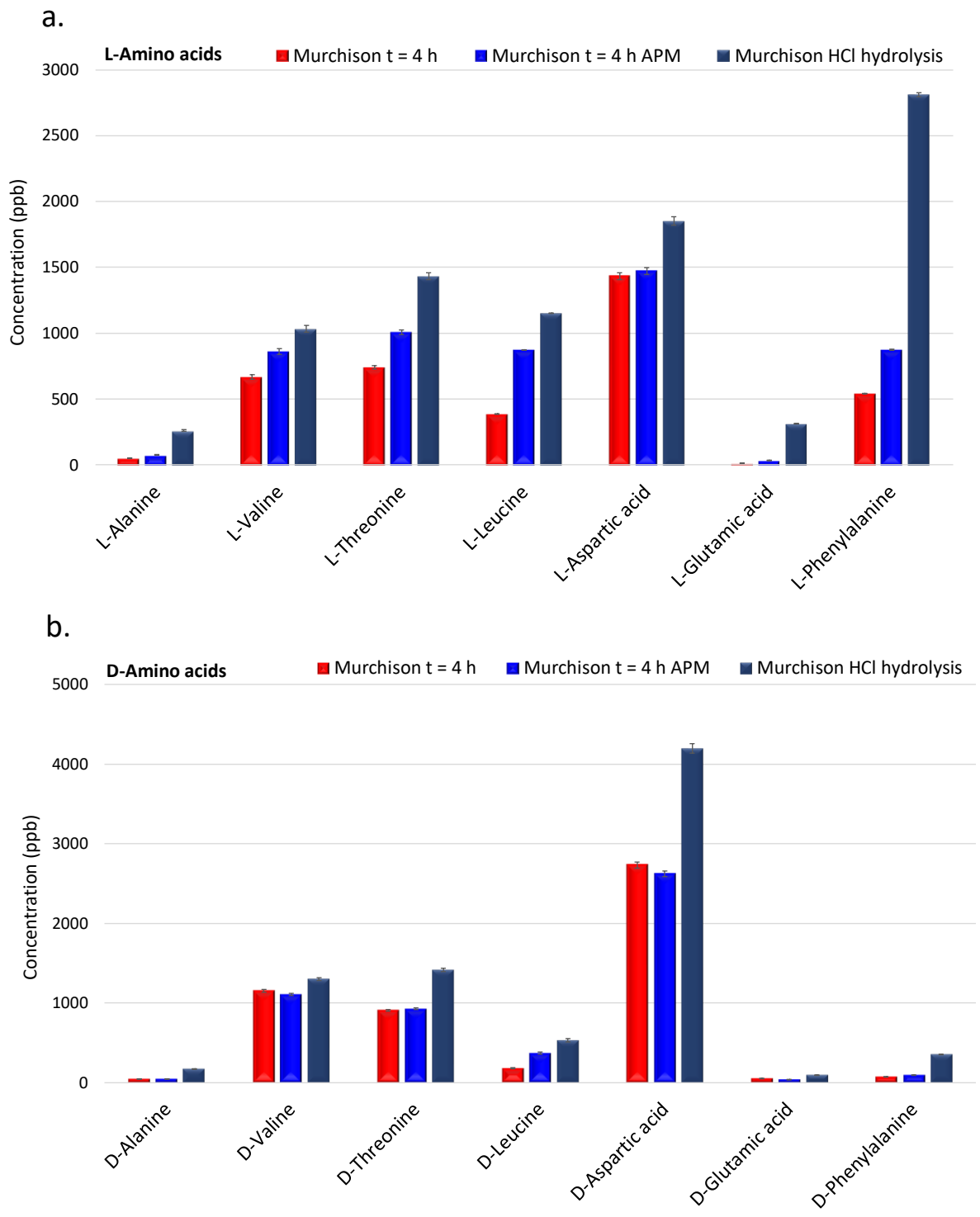


Figure 5. Monitoring of amino acid amounts after HCl and APM hydrolysis. Concentrations of L-amino acids (a) and D-amino acids (b) in Murchison in the absence of APM (Murchison t = 4 h), in the presence of APM (Murchison t = 4 h APM), and after HCl hydrolysis (Murchison HCl hydrolysis) (data from deconvoluted peaks, Figure S5b). For each amino acid enantiomer, error bar was obtained by multiplying its signal value by its variation coefficient calculated from the injection of the 48/52, 50/50, 52/48 standard solutions (Figure S4).

For these seven amino acids, the amounts of both enantiomers increased significantly when Murchison was submitted to HCl hydrolysis in comparison to the non-treated sample (from 1.1-fold for D-valine to 20-fold for L-glutamic acid; Murchison t = 4 h *vs* Murchison HCl hydrolysis). Such a result proves that those D- and L-amino acids were indeed present in the form of precursors, peptidic or not. On the other hand, remarkably, the enzyme generated a rise of the amount of all the L-enantiomers (1.5-fold for L-alanine, 1.3-fold for L-valine, 1.4-fold for L-threonine, 2.2-fold for L-leucine, 1.02-fold for L-aspartic acid, 2.2-fold for L-glutamic acid and 1.6-fold for L-phenylalanine; Murchison t = 4 h *vs* Murchison t = 4 h APM). Hence, our results reveal that these seven amino acids were involved in peptide bonds indigenous to the meteorite extract prior to their APM-catalysed hydrolysis. The quantity of D-alanine, D-valine, D-threonine, D-aspartic acid and D-glutamic acid were similar with or without APM. On the other hand, increases of D-leucine (2.0-fold) and D-phenylalanine (1.3-fold) concentrations were observed in the sample treated with the exopeptidase. While such increases were lower than those recorded for their corresponding L-enantiomers, this result indicates that these two D-amino acids were involved in peptide sequences since their releases have been catalysed by APM. This result shows that the Murchison meteorite also contains extra-terrestrial peptide sequences including non-proteogenic D-amino acids.

In summary, we have developed a novel strategy based on the use of APM to detect proteogenic peptide-like sequences in extra-terrestrial samples. Most of the amino acids resulting from acid hydrolysed meteorite water extracts come from non-peptides precursors. This approach has highlighted for the first time the presence of peptide sequences involving proteogenic L-amino acids in the Murchison meteorite together with their notable absence in Allende. Our methodology opens a new pathway to explore molecules included in minor bodies of the Solar System, for instance through the investigation of other enzymatic systems. Hence, this study should help in deepening the knowledge on the organic matter that may have seeded the early Earth and later triggered the emergence of life on our planet.

Methods

Meteorite cleaning procedure. All chemicals were purchased from Sigma-Aldrich, except hydrochloric acid 37 % (Trace Select) and Dowex 50W-X8 resin which were purchased from Fluka. Purified water was generated by a Purelab UHQ II water purification System (ELGA Lab Water, Veolia, Paris, France). All glassware used in sample processing were pyrolysed at 500 °C in air overnight prior to use.

Meteorite samples were provided by UNIL in collaboration with the Musée Cantonal de Géologie (Lausanne, Switzerland). A compact microcrack-free zone of the 82 g Allende rock was sonicated with pure water (200 mL at 50 °C for 20 h). The resulting solution was isolated. The Allende rock piece was then drilled to a depth of 3 mm with a 10 mm flamed twist drill. The obtained powder was set aside. At the bottom of this hole, a deeper 7 mm wide hole was drilled. The resulting powder (500 mg) of the interior of the Allende rock was then collected and kept in sterile polypropylene tubes until extraction. A Murchison sample (1.5 g) was sonicated with water (2.5 mL respectively) at 50 °C for 20 h. The resulting solution was recovered by centrifugation (5000 g, 10 min) and isolated. An aliquot (approximately 500 mg) was then powdered in an agate mortar.

Meteorite extraction procedure. Meteoritic samples were extracted with water (2 mL) at 100 °C for 20 h in an evacuated and N_2 purged vial. After centrifugation the supernatant was removed, dried down with a speedvac and re-suspended in water (2 mL).

Non-hydrolysed meteorite extracts. One millilitre aliquot of the resuspended sample was desalted through a cation-exchange resin (Dowex 50W-X8 resin, 50–100 mesh, hydrogen form) and the eluates of ammonium hydroxide solution were collected and dried under vacuum. They were stored at -20 °C until use. These extracts were further submitted to the enzymatic digestion process.

Hydrochloric acid (HCl)-hydrolysis of meteoritic water extracts. The other 1 mL aliquot was flame sealed separately in a glass ampoule with 1 mL 6 M HCl at 110 °C for 24 h 00 under argon, dried and desalted through a cation-exchange resin (Dowex 50W-X8 resin, 50–100 mesh, hydrogen form) to

convert amino acid precursors to free amino acids. Both meteorite extracts were stored in a - 20 °C freezer until redissolution in 100 µL water for LC-MRM MS analysis.

Chiral Liquid Chromatography (LC) - Multiple Reaction Monitoring Mass Spectrometry (MRM MS) analysis. Multiple-reaction monitoring (MRM) is a highly selective and sensitive method which focuses on the mass fragmentation pattern of a given molecule, providing thus a better discrimination between isomers as compared to the HRMS analysis[41]. Under this mode, an intact molecule will be selected (named the precursor ion), fragmented and a particular fragment ion (named the product ion) will be isolated.

Analyses were performed on a Shimadzu Nexera X2 UHPLC system coupled to a MS 8050 triple quadrupole mass spectrometer (TQ MS) equipped with an ESI source (Shimadzu, Marlborough, MA, USA). Before injection, samples were stored at 4 °C using a sample cooler (Shimadzu). LC solvent gradients and MS functions were controlled by the LabSolution data system (LabSolution 5.89, Shimadzu). The mobile phase was water / acetonitrile (98/2) with 0.5 % trifluoroacetic acid (Sigma-Aldrich). The D- and L-amino acids were analysed with a Crownpak CR (+) column (150 × 4.0 mm, 5 µm, Chiral Technologies, Europe) containing a chiral crown ether as a chiral selector. A guard column Crownpak CR (10 × 4.0 mm, 5µm, Chiral Technologies) was also used. The LC oven temperature was 30 °C and the elution was performed at a constant flow of 600 µL.min^{-1}. The MS instrument was operated in the MRM mode (Table S1). The parameters of the mass spectrometer were set as following: capillary voltage of 4 Kv; interface temperature of 250 °C; heat block temperature at 300 °C; desolvation line temperature at 250 °C. Nebulizing gas flow, heating gas flow and drying gas flow were set at 180 L.h^{-1}, 300 L.h^{-1} and 900 L.h^{-1}, respectively. MRM transitions, collision energy and dwell time of the targeted compounds are detailed in Table S1. MRM Total Ion Chromatograms were drawn with Origin (Origin 9.0.0, OriginLab Corporation, Northampton, USA) and are shown in Figure 1.

Five microliters of the hydrolysed meteoritic samples were injected, and amino acid concentrations were determined (Table S2). In order to identify D- and L amino acid enantiomers, two standard stock solutions were prepared. The first one contained the L-amino acids (glycine, L-alanine, L-serine, L-proline, L-valine, L-threonine, L-leucine, L-isoleucine, L-aspartic acid, L-lysine, L-glutamic acid, L-

histidine, L-phenylalanine, L-arginine and L-tyrosine) at $2.5.10^{-3}$ mol.L^{-1} in 0.1 mol.L^{-1} hydrochloric acid (≥ 99 %. Sigma-Aldrich). Some L-isomers were also added (L-alloisoleucine, L-norleucine ≥ 98 %, lyophilized powder, Sigma-Aldrich; L-isovaline ≥ 99 %, lyophilized powder, Thermo Fisher Scientific) at $2.5.10^{-3}$ mol.L^{-1} final concentration. The second solution was composed of D-amino acids (D-alanine ≥ 98 %, D-valine ≥ 98 %, D-alloisoleucine ≥ 98 %, D-isoleucine ≥ 98 %, D-norleucine ≥ 98 %, D-lysine ≥ 98 %, D-glutamic acid ≥ 99 %, D-histidine ≥ 98 %, D-arginine ≥ 98 %, D-tyrosine, lyophilized powder, Sigma-Aldrich; D-serine 98 %, D-proline ≥ 99 %, D-isovaline 97 %, D-leucine 99 %, D-aspartic acid 99 %, D-threonine 98 %, lyophilized powder, Thermo Fisher Scientific) at $1.25.10^{-2}$ mol.L^{-1} . They were then mixed and diluted to prepare a standard racemic solution at $1.25.10^{-4}$ mol.L^{-1} of D- and L-amino acids in phosphate buffer. A five-point calibration curve ranging from 1.10^{-8} to 3.10^{-7} mol.L^{-1} was constructed. Limits of detection (LODs) were calculated as 3 times standard deviation of noise signal/slope and linear coefficient of determination (R^2).

A standard addition procedure was also performed to highlight meteorite matrix effect on glycine detection. For that, three samples, each of 10 µL, of the acid-hydrolysed Murchison extract were used. The first one was injected as it, the second one was added with 1 µL of a glycine solution at $2.5.10^{-4}$ M, and the last one with 1 µL of a glycine solution at 5.10^{-4} M. The same procedure was followed by replacing the Murchison extract by 10 µL of water. Five microliters of the samples were analysed (Figure S1 and Table S3).

Gas Chromatography (GC) - Mass Spectrometry (MS) analysis. To be analysed by GC-MS, derivatisation was first performed. A solution containing the amino acids was evaporated at 35 °C by means of a stream of dry nitrogen. N-methyl-N-*(tert-butyldimethylsilyl)* trifluoroacetamide (30 µL) and dimethylformamide (10 µL) were added and the vial was heated at 75 °C for 30 min. After cooling, the reaction mixture (1 µL) was injected directly in a ThermoScientific Gas Chromatography-Mass Spectrometer Trace-DSQ (GC-MS) coupled to a fused silica capillary column (SGE BPX 5 %, 30 m length, 0.25 mm id., 0.25 mm film thickness). The carrier gas was helium and the column head pressure was 15 psi to reach a constant flow rate of 1.2 mL.min^{-1}. The GC was temperature

programmed from 60 to 300 °C at 5 °C min^{-1} (isothermal for 20 min final time). The temperature of the injector was 250 °C. Line transfer and source were held respectively at 290 °C and 200 °C.

Investigation of APM selectivity towards *N*-terminal peptide bonds on standard solutions.

Enzymatic hydrolysis were carried out with a commercial exopeptidase, the aminopeptidase M, APM (EC 3.4.11.2, aminopeptidase from porcine kidney, 24.84 $U.mg^{-1}$ (biuret), suspension in 10 $mmol.L^{-1}$ Tris buffer salts, 3.2 $mol.L^{-1}$ ammonium sulfate and 10 $mmol.L^{-1}$ magnesium chloride, pH 7.5, Sigma-Aldrich). This enzyme liberates amino acids exclusively from the *N*-terminal end of peptides.

To get insights into the specificity of APM, enzymatic hydrolyses of standard solutions were performed. Solutions were prepared in a phosphate buffer composed of 12 $mmol.L^{-1}$ Na_2HPO_4, 8 $mmol.L^{-1}$ KH_2PO_4 in 100 mL H2O (pH 6.9).

First, a solution containing *N*-acetyl-Ala-Ala-Ala-O-methyl ester (Bachem) at 10^{-5} $mol.L^{-1}$ and 1 µL of a commercial mix of five oligopeptides (Gly-Tyr, Val-Tyr-Val, Leu-enkephalin, Met-enkephalin and Angiotensin II, Sigma Aldrich) at 100 $µg.mL^{-1}$ was prepared. It was divided into two samples of 200 µL. One was incubated at 30 °C with APM at an enzyme/peptides ratio of 1/10 (w/w) (7.25 µL of a 0.5 $U.mL^{-1}$ APM solution). The other one did not contain APM. Both samples were then monitored along four hours to ensure quantitative hydrolysis of the compounds. Aliquots (30 µL) were taken at the beginning of the incubation time (t = 0 h), 1 h 00 after (t = 1 h) and finally 4 h 00 after (t = 4 h). In aliquots containing APM, the enzymatic hydrolysis was stopped with formic acid at a 3.8 % (v/v) final concentration. To be representative, formic acid was also added to the aliquots which did not contain APM. The overall experiment was performed in triplicate. In parallel, acid hydrolysis was performed. For this, standards were diluted in 1 mL 6 M HCl solution (final peptides concentration of 10^{-5} $mol.L^{-1}$). The solution was flame sealed separately in a glass ampoule and further hydrolysed under an inert atmosphere at 110 °C for 24 h.

Enzymatic and hydrolysis digestions were also performed on a standard solution containing both the cyclic diglycine and phenyl-glycine methyl ester at a concentration of $1.25.10^{-4}$ $mol.L^{-1}$ following the same procedure.

Analyses were performed with a quaternary Accela LC pump (pump: Accela 1250 pump) interfaced with a Q-Exactive (Hybrid Quadrupole-Orbitrap^{TM}) mass spectrometer equipped with an ESI source (Thermo Fisher Scientific, Waltham, MA, USA). Before injection, samples were stored at 4 °C using a Stack cooler CW (CTC Analytics AG, Zwingen, Switzerland). MS functions and LC solvent gradients were controlled by the Xcalibur data system (Thermo Fisher Scientific). Acetonitrile with 0.1 % formic acid was used as buffer A and water with 0.1 % formic acid as buffer B. Instrument calibration in positive and negative modes was done every day *via* the direct infusion of positive and negative ion calibration solutions. For analysis, 5 µL of samples were injected in LC-Q-Exactive.

To analyse peptides, we developed a specific LC-HRMS method described in Eddhif et al. [42] (encoded method 1 in Table S4). For this analysis, solutions were loaded by an Accela quaternary 1250 pump onto the analytical column (i.e. Hypersil Gold Q C18, 50 mm x 2.1 mm, 1.9 µm, 175 Å; Thermo Fisher Scientific), kept at 40 °C using a column oven 300 (Thermo Fisher Scientific) (method 1). Elution was performed at a constant flow rate of 500 µL.min^{-1}. It started with 5 % buffer A and 95 % buffer B during 1 min and reached 65 % buffer A and 35 % buffer B in 12 min. A second linear gradient was employed to attain 90 % buffer A and 10 % buffer B in 1 min. The column was finally reconditioned at the initial conditions (5 % buffer A and 95 % buffer B) for 2 min.

A second method (method 2 in Table S4) was also developed to discriminate low mass compounds[42]. Amino acids released by the hydrolysis of the peptide solutions, as well as solutions of cyclic diglycine and phenyl-glycine methyl ester, were analysed thanks to this method that is composed of three serially coupled columns. It combined a porous graphitic carbon Hypercarb (50 mm x 2.1 mm, 3 µm, 250 Å; Thermo Fisher Scientific), a Pentafluorophenyl reverse-phase Kinetex F5 (100 mm x 2.1 mm, 1.7 µm, 100 Å, Phenomenex) and a C18 reverse-phase Hypersil Gold Q (50 mm x 1 mm, 1.9 µm, 175 Å, Thermo Fisher Scientific). The LC oven temperature was set at 40 °C. The elution was performed at a constant flow of 100 µL.min^{-1} within the series. The elution gradient started with 5 % buffer B and 95 % buffer A during 3 min. It continued with a slow gradient to reach 35 % buffer B and 65 % buffer A in 12 min. A fast gradient was employed to reach 60 % buffer B in 5 min and then 100 % of buffer in 1 min. The column was rinsed with 100 % buffer B during 2 min and then reconditioned for 6 min with 5 % buffer B and 95 % buffer A.

Mass detection of all compounds was performed in positive ion mode in a full scan mode. Mass parameters associated to each method are summarized in Table S4. The m/z searched for each molecule were as following: Gly-Tyr, m/z 239.1026 [M + H+], Val-Tyr-Val, m/z 380.2180 [M + H+], Tyr-Gly-Gly-Phe-Met m/z 574.2330 [M + H+], Tyr-Gly-Gly-Phe-Leu m/z 556.2766 [M + H+], Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (angiotensin II), m/z 1046.5418 [M + H+], Ac-Ala-Ala-Ala-OMe, m/z 288.1554 [M + H+], cyclic diglycine m/z 115.0502 [M + H+], phenylglycine methyl ester m/z 166.0863 [M + H+], proline, m/z 115.1305 [M + H+], valine, m/z 118.0862 [M + H+], leucine and isoleucine, m/z 132.1019 [M + H+], methionine, m/z 149.2110 [M + H+], histidine, m/z 156.0767 [M + H+], phenylalanine, m/z 166.0862 [M + H+], arginine, m/z 175.1189 [M + H+], tyrosine, m/z 182.0811 [M + H+].
Results are presented in Table 1 and Figure 2, as well as in Figures S2 and S3.

Investigation of APM stereoselective on peptide standard solutions. As a control experiment, the APM hydrolysis kinetics in the presence of homochiral and heterochiral alanine peptides was investigated. Three different solutions, each containing 10^{-3} mol.L^{-1} of D-Ala-D-Ala-D-Ala, or L-Ala-L-Ala-L-Ala or L-Ala-D-Ala-L-Ala were prepared in methanol. Ten microliters of the solutions were evaporated. Peptides were then dissolved in 1000 µL of phosphate buffer. A volume of 200 µL of each solution was incubated at 30 °C in the presence of APM at an enzyme/peptides ratio of 1/10 (w/w) (1.6 µL of a 0.5 U.mL^{-1} APM solution). Aliquots (25 µL) were taken at the beginning of the experiment (t = 0 h) and four hours after (t = 4 h APM). They were added with formic acid at 3.8 % (v/v) final concentration. A volume of 0.5 µL of the aliquots was injected and analysed with the three serially coupled columns method[42] coupled with the TQ MS. The MRM transitions of the tripeptides are shown in Table S5. The experiment was performed in duplicate (Figure 4b).
A solution containing both D-Ala-D-Ala-D-Ala-D-Ala-D-Ala and L-Ala-L-Ala-L-Ala-L-Ala-L-Ala at a concentration of 10^{-3} mol.L^{-1} in 200 µL of phosphate buffer was prepared. It was incubated at 30 °C in the presence of APM at an enzyme/peptides ratio of 1/10 (w/w) (5.3 µL of a 50 U.mL^{-1} APM solution). Aliquots (25 µL) were taken at the beginning of the experiment (t = 0 h), and four hours after (t = 4 h APM). Formic acid was added to reach a 3.8 % (v/v) final concentration. Before analysis, the dipeptide Ala-Gly was added as an internal standard at a concentration $1.25.10^{-4}$ mol.L^{-1}. A volume of 0.5 µL of

the aliquots was injected and analysed with the chiral LC-MRM MS method. The MRM transitions of the pentapeptides are shown in Table S5. The experiment was performed in triplicate (Figure 4b).

Investigation of the accuracy of the chiral LC-MRM MS method. Three standard solutions containing the eight D- and L-amino acids that have been quantified in the meteorite samples were prepared with L/D ratios at 48/52, 50/50, 52/48 ($1.25.10^{-4}$ $mol.L^{-1}$ final concentration in phosphate buffer). A volume of 0.5 µL of these solutions was injected within the chiral LC-MRM MS method (three replicates). The signal intensity ratios of L- and D-enantiomers were significantly different for distinct enantiomeric ratios (Figure S4). This result highlighted the accuracy of our chiral LC-MRM MS method.

Enzymatic hydrolysis of meteoritic water extracts. A Murchison dry extract was dissolved in 150 µL of phosphate buffer. The extract was divided into two samples (each of 60 µL). One was incubated at 30 °C with APM (0.5 µL of a 12.5 $U.mL^{-1}$ APM solution), while no enzyme was added in the second one. Samples were left for four hours at 30 °C. In both samples, an aliquot (25 µL) was taken at the end of the experiment (samples encoded Murchison t = 4 h no APM and Murchison t = 4 h APM). Hydrolysis was stopped with formic acid at 3.8 % (v/v) final concentration. Formic acid was also added in the "Murchison t = 4 h no APM" sample.

The same procedure was applied to Allende. The Allende dry extract was first dissolved in 100 µL of purified water. Eighty microliters were taken and dissolved in 220 µL of phosphate buffer. It was then divided into two samples (each of 135 µL). One was incubated at 30 °C with APM (0.5 µL of a 12.5 $U.mL^{-1}$ APM solution), while no enzyme was added in the second one. In both samples, an aliquot (25 µL) was taken at the end of the experiment (samples encoded Allende t = 4 h no APM and Allende t = 4 h APM). Hydrolysis was stopped with formic acid at 3.8 % (v/v) final concentration. Formic acid was also added in the "Allende t = 4 h no APM" sample.

APM blank hydrolysis was also realised following the same procedure. This experiment provided the enzyme signature after its own hydrolysis (sample encoded t = 4 h APM).

Five microliters of the non-hydrolysed and hydrolysed samples were injected and analysed with the chiral LC-MRM MS method (Figures S5 and S6).

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Author Contributions

P.P. get the idea to transpose a proteomics strategy for peptides detection in extra-terrestrial samples and designed the enzymatic workflow. P.P., C.G.R. and N.K.V.L. supervised the development of the whole analytical methods. P.P. and S.P. get the idea to exploit the L/D ratio of amino acids to confirm unambiguously the presence of peptides in the meteorites. C.G.R. performed meteorite's extraction and HCl hydrolysis. J.L., F.B. and Q.B.R. performed analytical experiments. All enzymatic digestions were conducted by B.E. HCl hydrolysis of standard compounds was carried out by F.B.. J.L., B.E. and A.R. collected all the data and reconstructed the figures. J.L., C.G.R., F.B., L.S.H and P.P. wrote the methods section. P.P., G.D., S.P. and L.S.H wrote the paper with input from all other authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper.

Supplementary information

Evidence for proteogenic peptide-like sequences in meteorites through enzyme-catalysed stereoselective hydrolysis strategy

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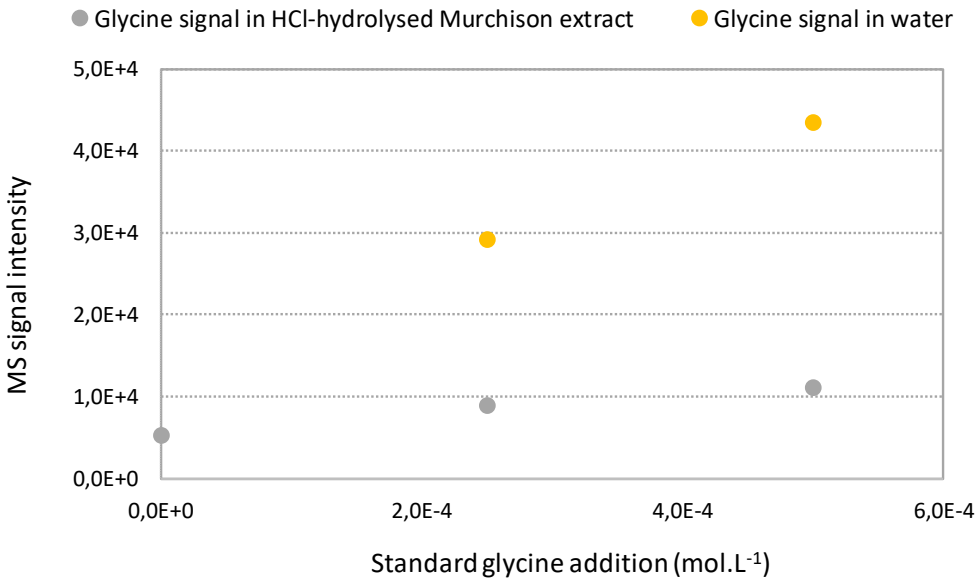


Figure S1. Matrix effect on glycine MS signal. Measurement of MS signal of glycine after standard addition in either HCl-hydrolysed Murchison extract or water.
Glycine signal was significantly reduced in the HCl-hydrolysed Murchison extract compared with water

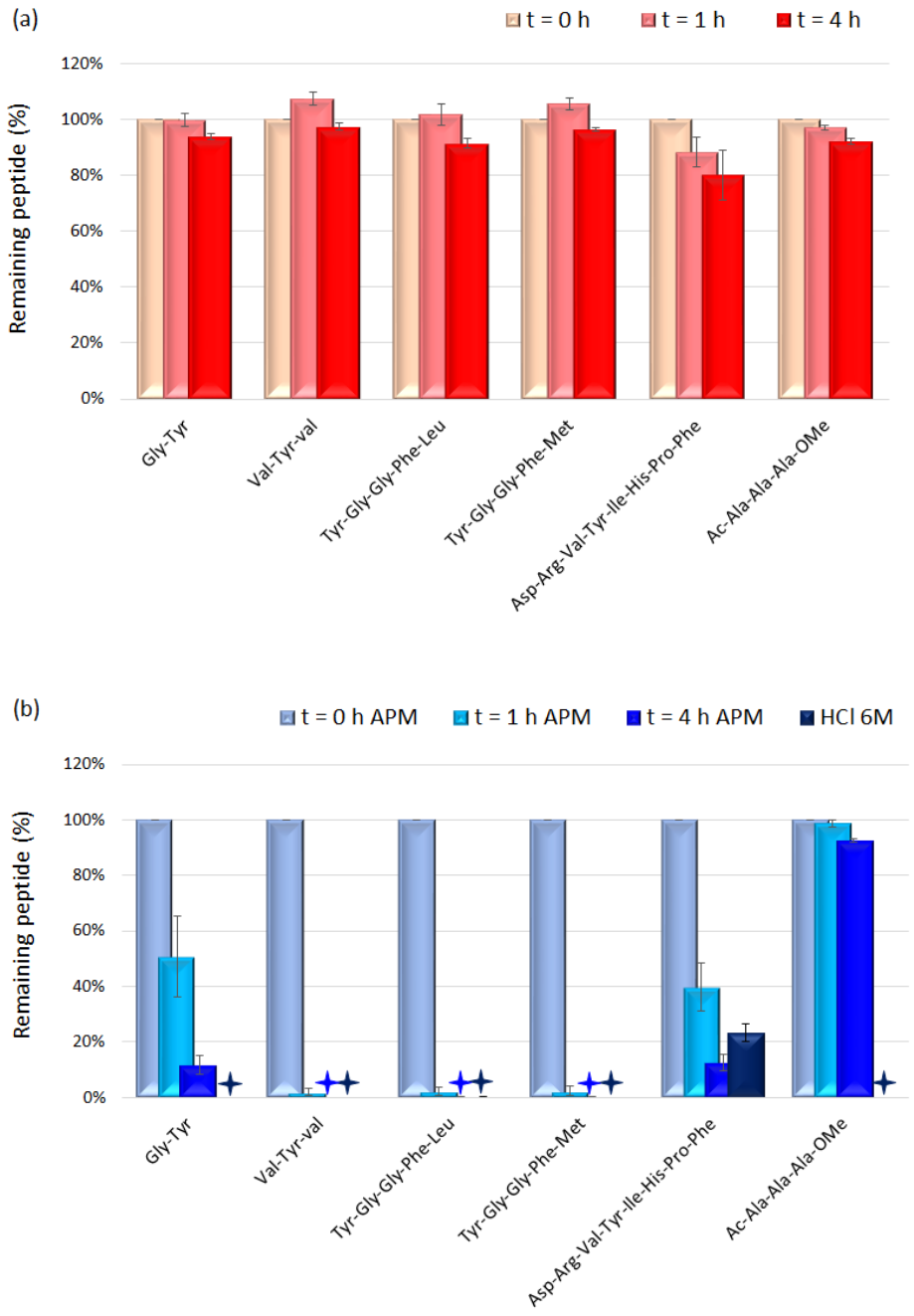


Figure S2. Percentages of peptides that remained in the absence of APM (a), or after APM or HCl digestions (b). For a compound, the percentage was calculated by dividing its area at t = 1 h or t = 4 h to its area at t = 0 h. ✦: APM complete hydrolysis. ✦: HCl complete hydrolysis.

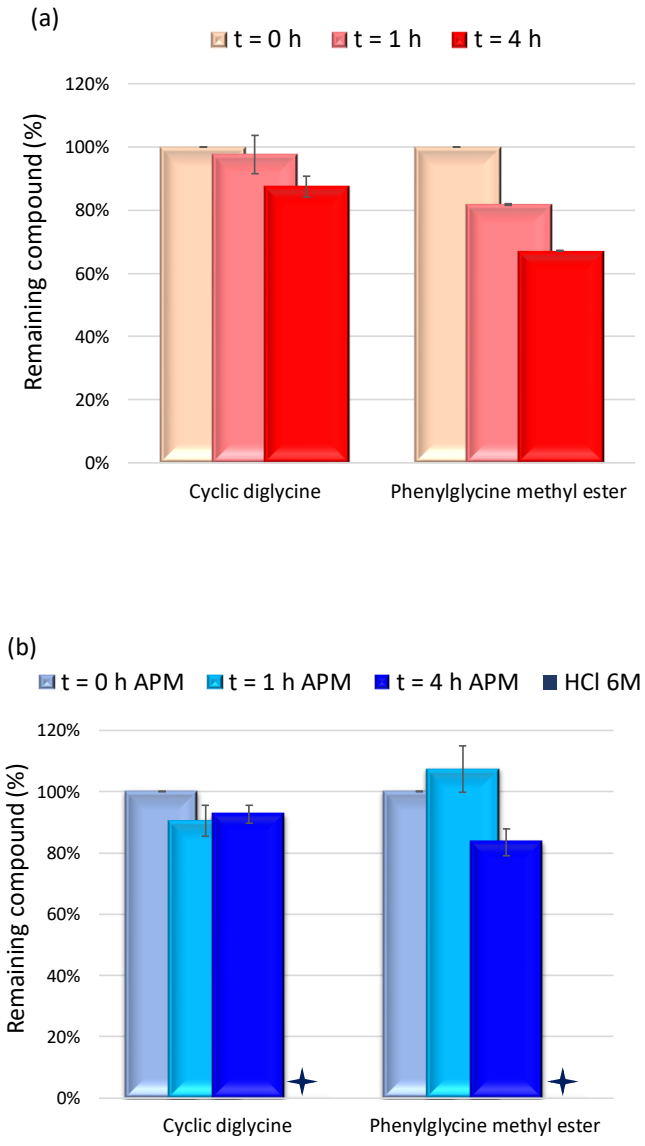


Figure S3. Percentages of cyclic diglycine and phenylglycine that remained in the absence of APM (a) or after APM or HCl digestions (b). For a compound, the percentage was calculated by dividing its area at t = 1 h or t = 4 h to its area at t = 0 h. ✦: HCl complete hydrolysis.

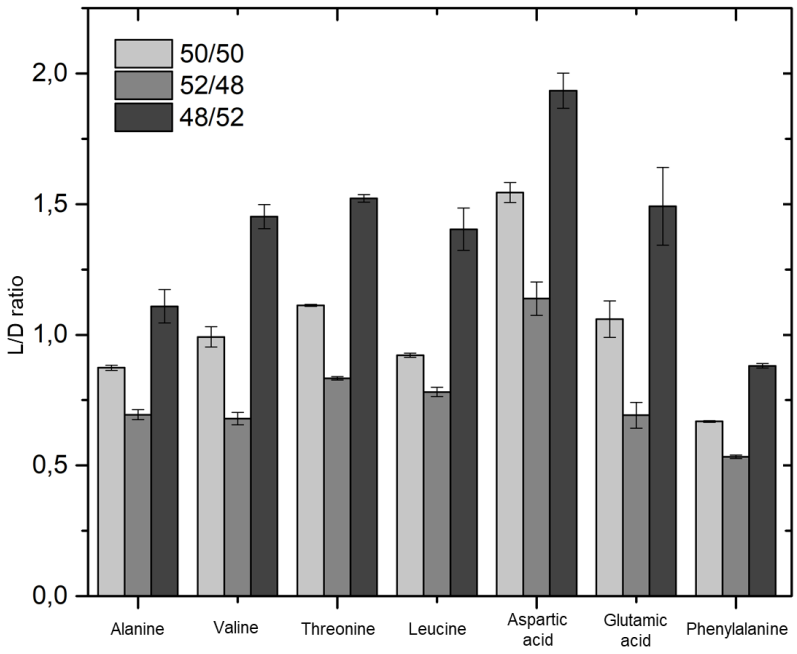
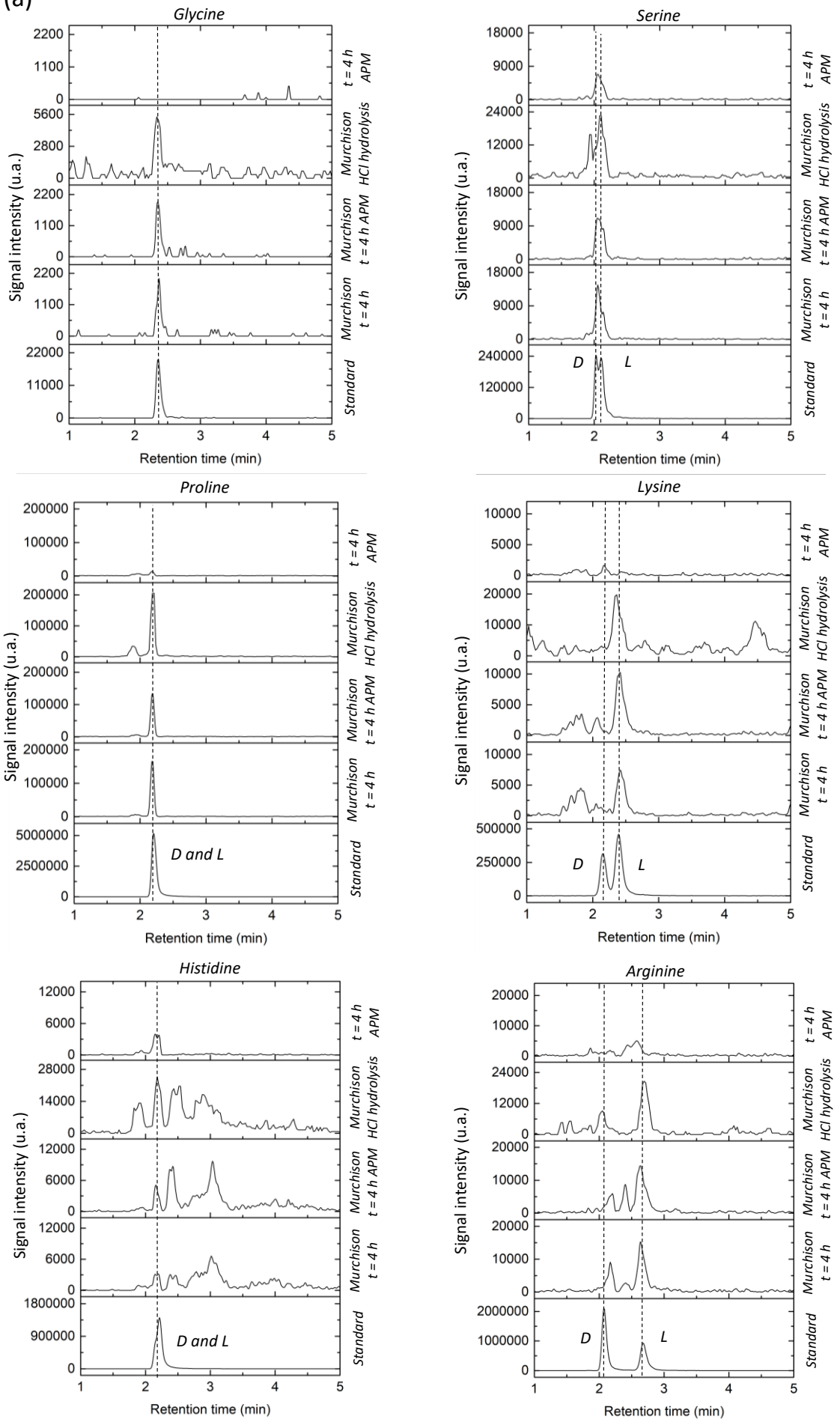
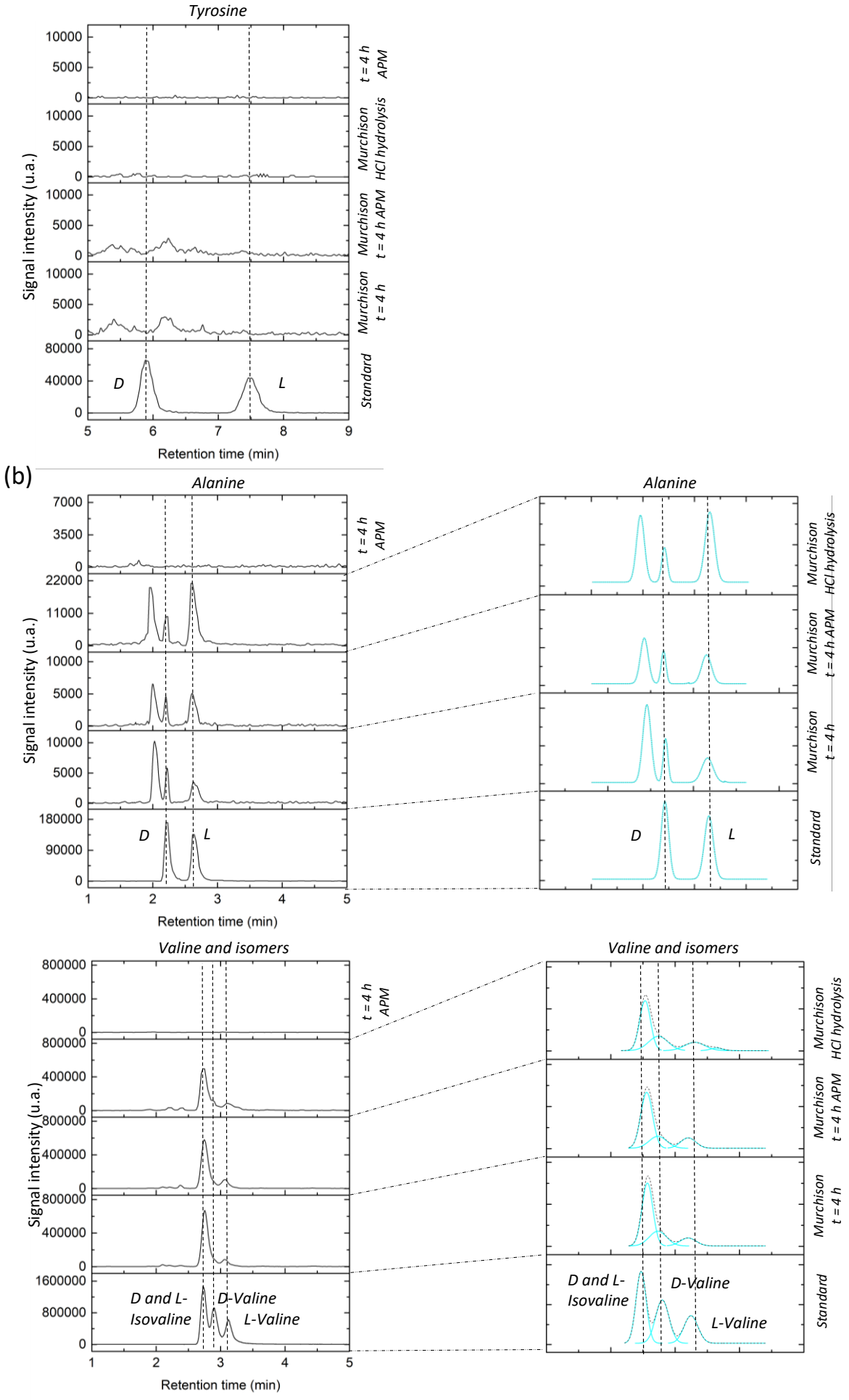
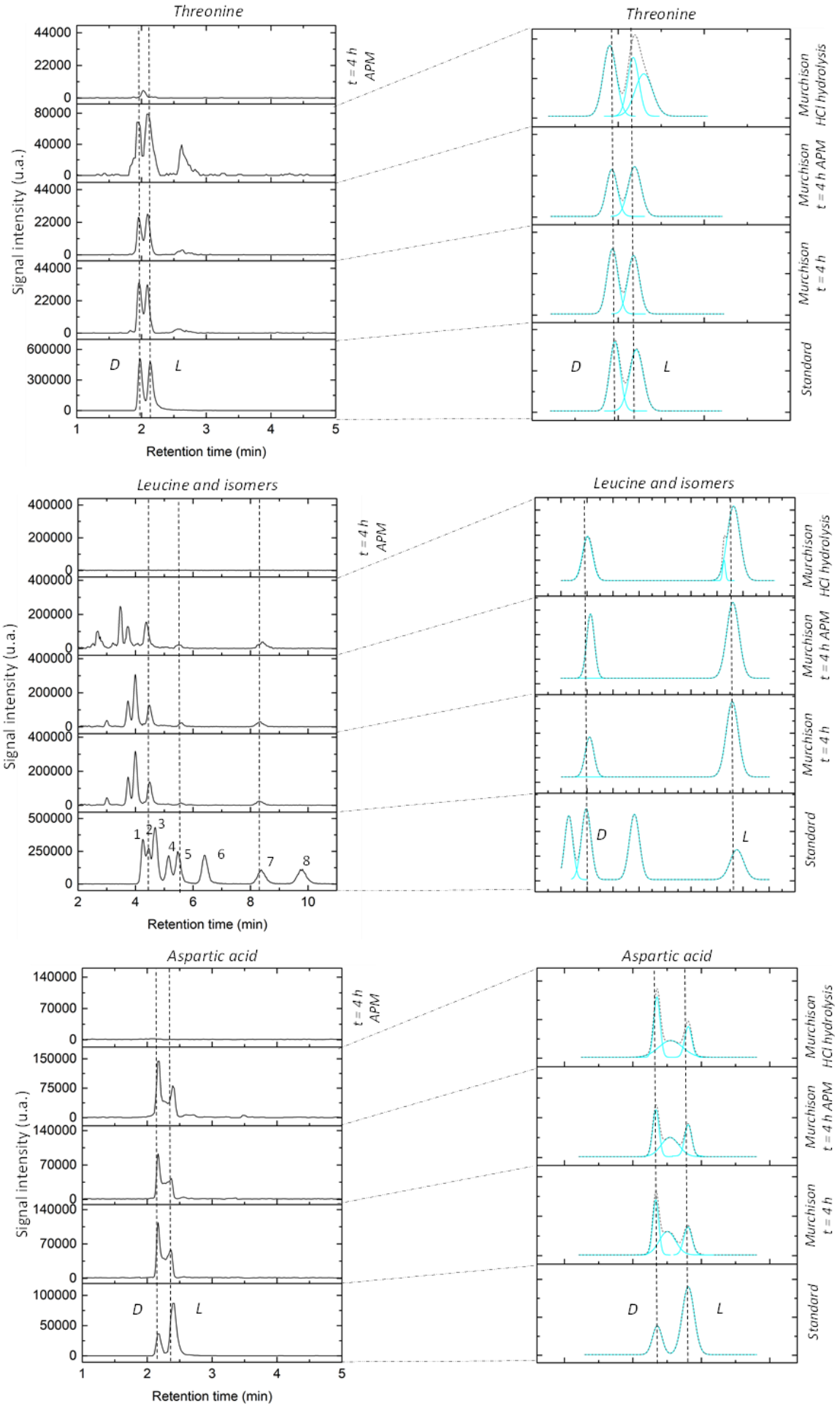


Figure S4. Ratios of L- and D-signal intensities for the seven amino acids quantified in Murchison after APM or HCl hydrolysis.

(a)


Glycine
Serine
Proline
Lysine
Histidine
Arginine
Signal intensity (u.a.)
Retention time (min)
Murchison t = 4 h APM
Murchison HCl hydrolysis
Murchison t = 4 h APM
Murchison t = 4 h
Standard
D
L
D and L


Tyrosine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Murchison HCl hydrolysis
Murchison t = 4 h APM
Murchison t = 4 h
Standard
D
L
(b)
Alanine
Alanine
Murchison HCl hydrolysis
Murchison t = 4 h APM
Murchison t = 4 h
Standard
Valine and isomers
Valine and isomers
D and L-Isovaline
D-Valine
L-Valine


Threonine
Threonine
Signal intensity (u.a.)
Retention time (min)
D
L
t = 4 h APM
Murchison HCl hydrolysis
Murchison t = 4 h APM
Murchison t = 4 h
Standard
Leucine and isomers
Leucine and isomers
1
2
3
4
5
6
7
8
Aspartic acid
Aspartic acid

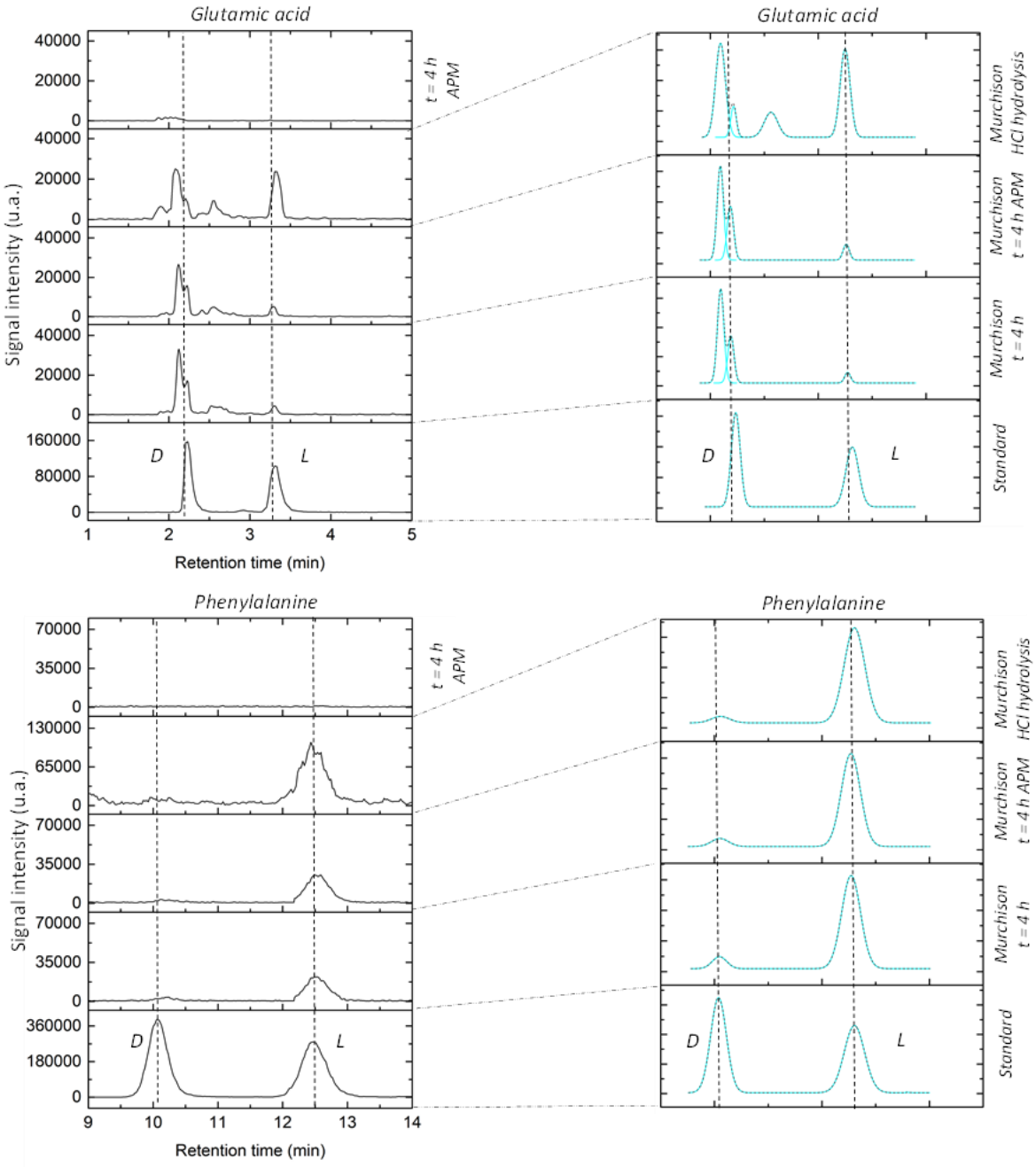
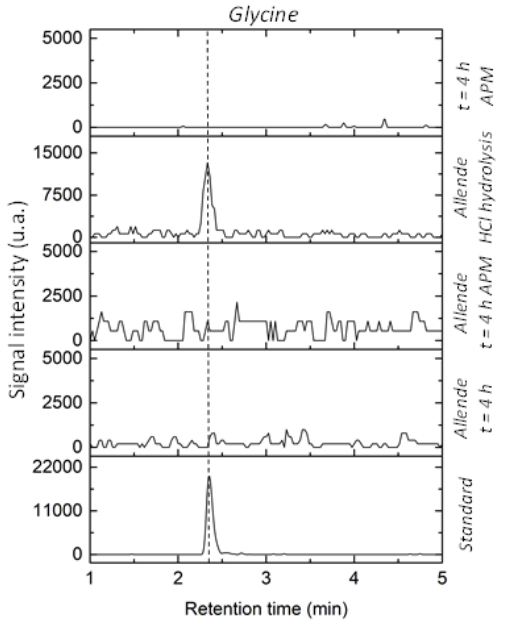
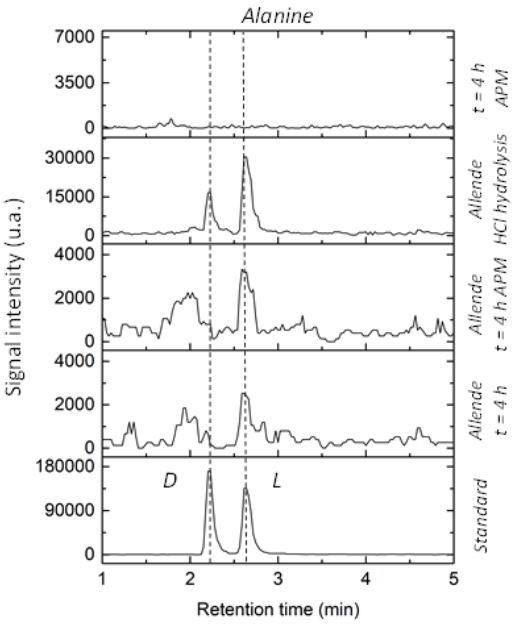
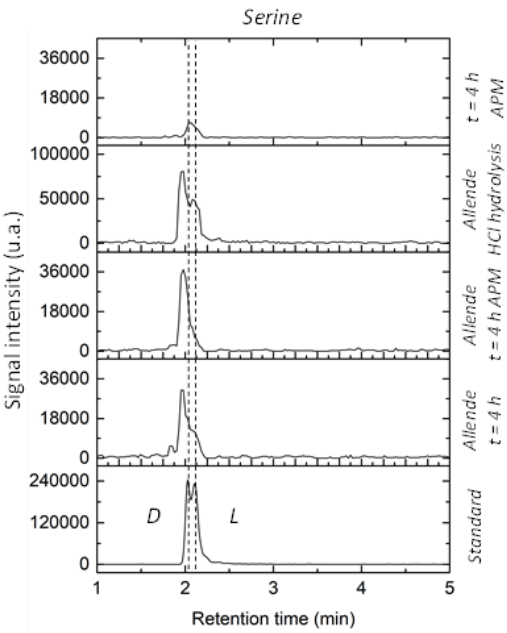
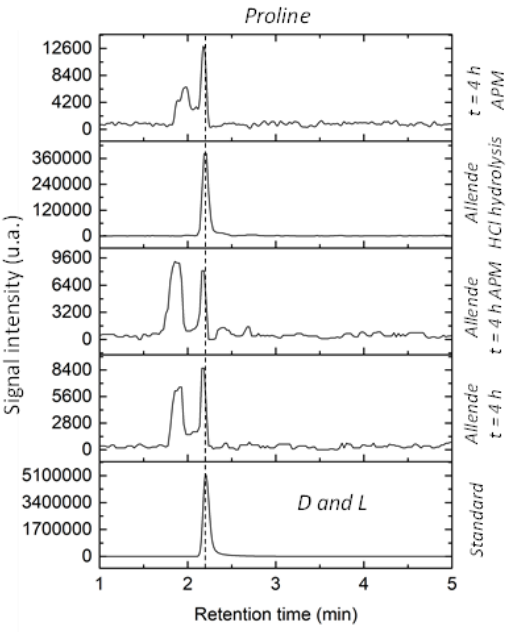


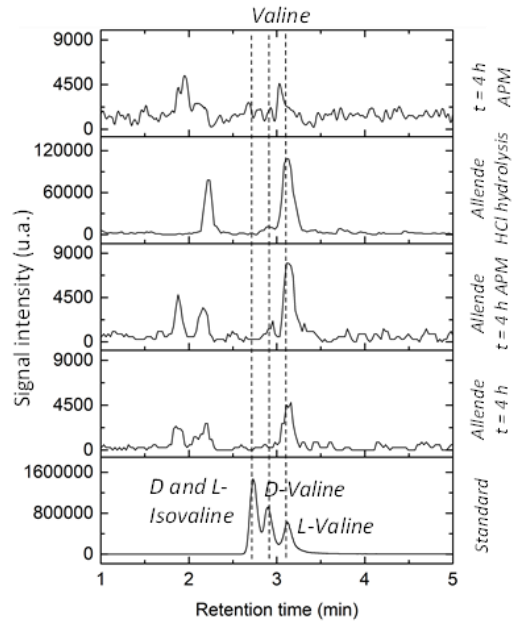
Figure S5. Total Ion Chromatograms of Murchison amino acids enantiomers. (a). Non deconvoluted peaks; (b). Deconvoluted peaks (peaks were deconvoluted when both D- and L-enantiomers were identified confidently; deconvoluted peaks are shown in blue). *For each amino acid. from the bottom to the top: Amino acids standards; Murchison t = 4 h: Murchison extract left for four hours in the phosphate buffer at 30 °C without APM. Murchison t = 4 h APM: Murchison extract left for four hours in the phosphate buffer at 30 °C with APM. Murchison HCl hydrolysis: Murchison extract after HCl hydrolysis. t = 4 h APM: APM left for four hours in the phosphate buffer at 30 °C. Leucine isomers: 1. D-allo-isoleucine; 2. D-isoleucine; 3. L-allo-isoleucine; 4. L-isoleucine; 5. D-leucine; 6. D-norleucine; 7. L-Leucine; 8. L-norleucine.*

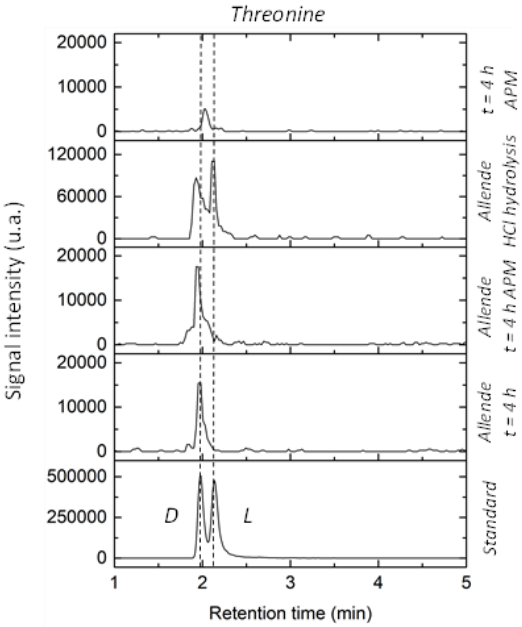

Glycine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard

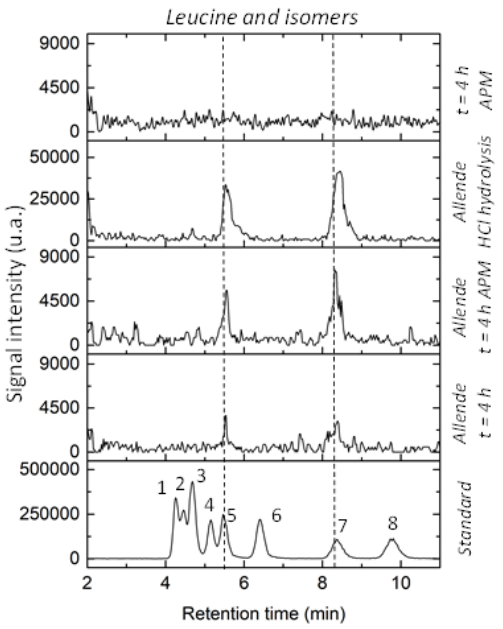

Alanine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L

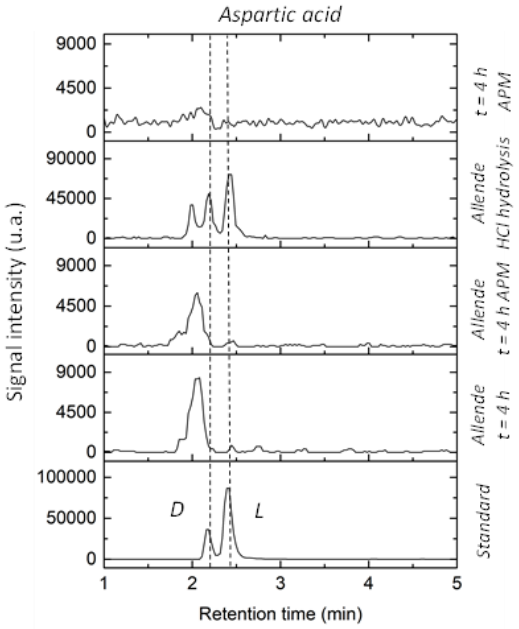

Serine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L

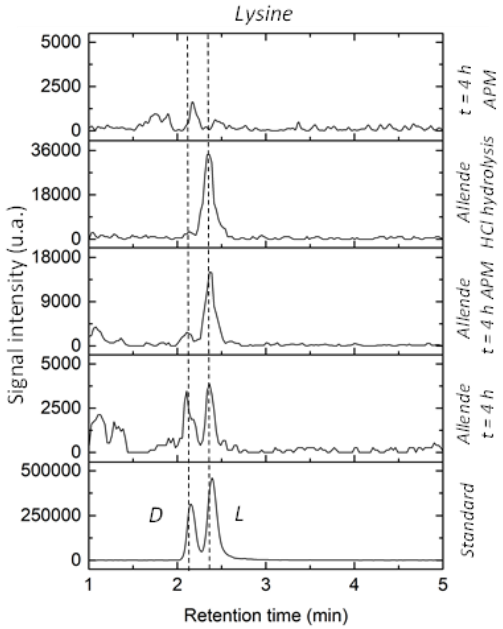

Proline
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D and L

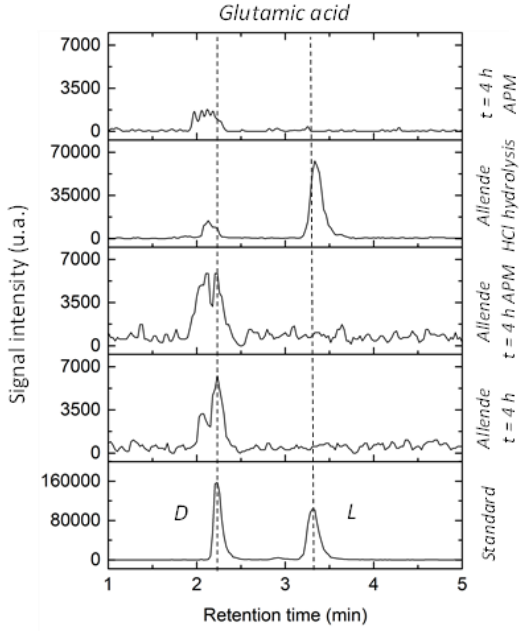

Valine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D and L-Isovaline
D-Valine
L-Valine

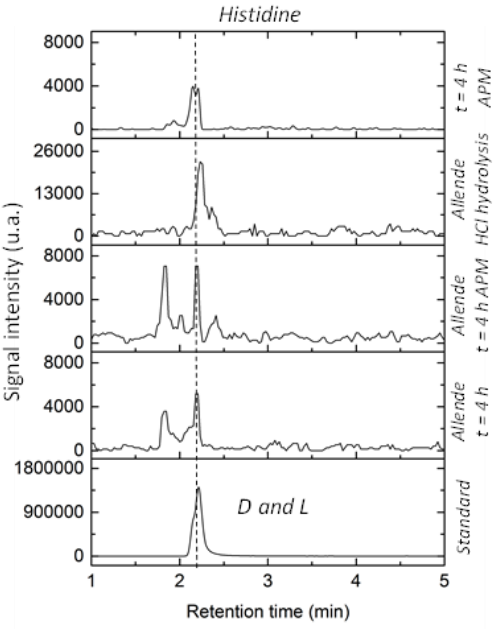

Threonine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L

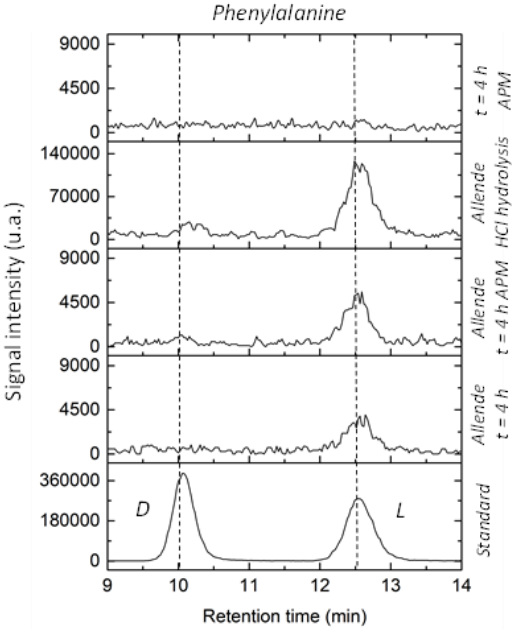

Leucine and isomers
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
1
2
3
4
5
6
7
8


Aspartic acid
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L


Lysine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L


Glutamic acid
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L


Histidine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D and L


Phenylalanine
Signal intensity (u.a.)
Retention time (min)
t = 4 h APM
Allende HCl hydrolysis
Allende t = 4 h APM
Allende t = 4 h
Standard
D
L

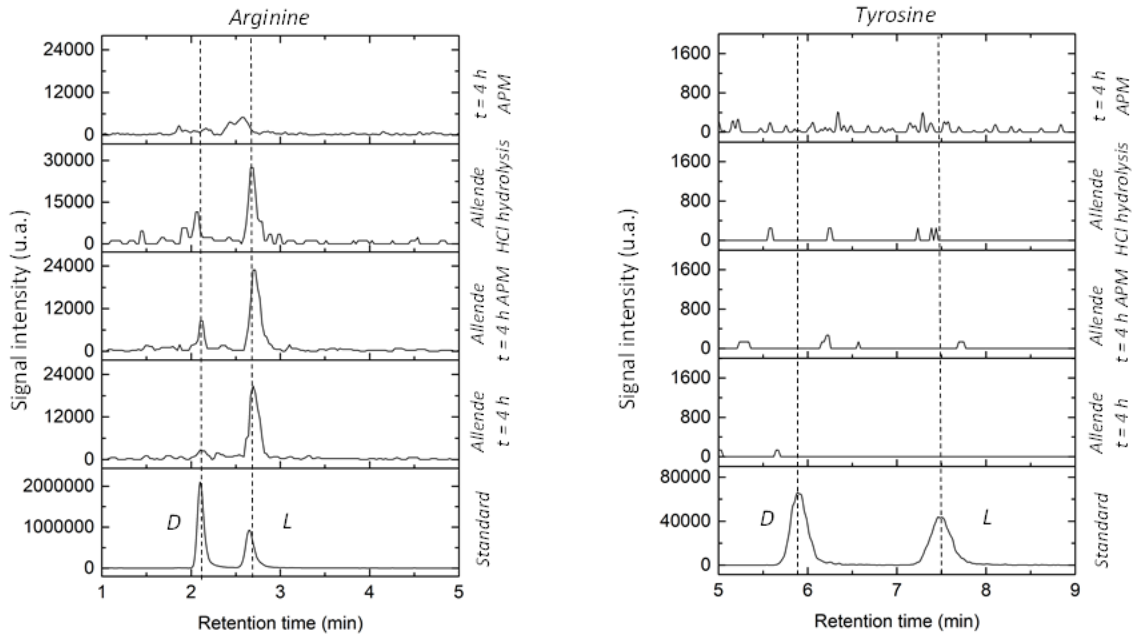


Figure S6. Total Ion Chromatograms of Allende amino acids enantiomers.
For each amino acid. from the bottom to the top: Amino acids standards; Allende t = 4 h no APM: Allende extract left for four hours in the phosphate buffer at 30 °C without APM. Allende t = 4 h APM: Allende extract left for four hours in the phosphate buffer at 30 °C with APM. Allende HCl hydrolysis: Allende extract after HCl hydrolysis. t = 4 h APM: APM left for four hours in the phosphate buffer at 30 °C. Leucine isomers: 1. D-allo-isoleucine; 2. D-isoleucine; 3. L-allo-isoleucine; 4. L-isoleucine; 5. D-leucine; 6. D-norleucine; 7. L-Leucine; 8. L-norleucine.

Table S1. MRM transitions and limit of detections (LODs) of the amino acids targeted in the meteoritic samples. Comparison to a standard procedure implying a derivatisation and GC-MS analysis of the compounds.

		LC-MRM MS analysis							Derivatisation and GC-MS analysis
					Fragments				
Amino acids	Formulae	Transitions	Collision energy (eV)	Dwell time (ms)	Loss of NH_3	Loss of H_2O	Loss of CO + H_2O	Loss of other fragments	LOD ($mol.L^{-1}$)	LOD ($mol.L^{-1}$)
Glycine	$C_2H_5NO_2$	76.15 > 30.25[1]	13	5			x		$1.45.10^{-7}$	$5.10.10^{-6}$
D-Alanine	$C_3H_7NO_2$	90.05 > 44.25[1]	13	10			x		$8.43.10^{-8}$	$4.21.10^{-6}$
L-Alanine		90.05 > 29.25	40	10				$x(C_2H_6NO)$	$1.07.10^{-7}$	
D-Serine	$C_3H_8NO_3$	106.25 > 60.20[1]	12	5			x		$8.74.10^{-8}$	$4.53.10^{-6}$
L-Serine		106.25 > 88.20[1]	12	5		x				
D-Proline	$C_5H_9NO_2$	116.25 > 70.35[1]	17	10			x		$4.06.10^{-8}$	$5.82.10^{-6}$
L-Proline										
D-Valine	$C_5H_{11}NO_2$	118.30 > 72.25[1]	12	5			x	$x(CH_4NO_2)$	$5.06.10^{-9}$	$4.99.10^{-6}$
L-Valine									$1.42.10^{-9}$	
D-Isovaline		118.30 > 55.25[1]	23	5						
L-Isovaline									$1.83.10^{-8}$	
D-Threonine	$C_4H_9NO_3$	120.25 > 74.25[1]	12	5			x		$9.13.10^{-8}$	$3.91.10^{-6}$
L-Threonine		120.25 > 56.25[1]	16	5				$x(CH_3O_3)$	$2.13.10^{-8}$	
D-Leucine	$C_6H_{13}NO_2$	132.30 > 86.15[1]	11	5			x	$x(C_4H_8O_2)$	$3.78.10^{-8}$	$4.92.10^{-6}$
L-Leucine									$3.46.10^{-8}$	
D-Isoleucine									$2.52.10^{-8}$	
L-Isoleucine									$1.90.10^{-8}$	
D-Norleucine		132.30 > 44.15[1]	22	5					$3.44.10^{-8}$	
L-Norleucine									$1.30.10^{-8}$	
D-Alloisoleucine									$4.64.10^{-8}$	
L-Alloisoleucine									$1.70.10^{-8}$	
D-Aspartic acid	$C_4H_7NO_4$	134.25 > 74.20[1]	15	10				$x(C_2H_3O_2)$	$3.26.10^{-8}$	$3.50.10^{-6}$
L-Aspartic acid		134.25 > 88.20[1]	13	10			x		$3.62.10^{-8}$	
D-Lysine	$C_6H_{14}N_2O_2$	147.30 > 84.25[1]	18	10			x		$1.98.10^{-8}$	$6.80.10^{-6}$
L-Lysine		147.30 > 130.20[1]	15	10	x				$1.40.10^{-8}$	
		147.30 > 56.25[1]	31	10				$x(C_3H_8NO_2)$		
D-Glutamic acid	$C_5H_9NO_4$	148.25 > 84.20[1]	16	10				$x(CH_3O_3)$	$5.93.10^{-8}$	$5.00.10^{-6}$
L-Glutamic acid		148.25 > 56.25[2]	27	10				$x(C_2H_3O_4)$	$2.20.10^{-8}$	
D-Histidine	$C_6H_9N_3O_2$	156.25 > 110.20[1]	15	10			x		$2.61.10^{-8}$	$6.80.10^{-6}$
L-Histidine		156.25 > 93.15[1]	23	10				$x(CH_4NO_2)$		
D-Phenylalanine	$C_9H_{11}NO_2$	166.30 > 103.2[1]	24	10				$x(CH_4NO_2)$	$1.77.10^{-8}$	$4.40.10^{-6}$
L-Phenylalanine		166.30 > 120.25[1]	15	10			x		$4.82.10^{-9}$	
		166.30 > 77.20[1]	39	10				$x(C_3H_6NO_2)$		
D-Arginine	$C_6H_{14}N_4O_2$	175.30 > 70.25[2]	24	5				$x(C_2H_6N_3O_2)$	$4.21.10^{-8}$	$1.20.10^{-5}$
L-Arginine		175.30 > 116.30[1]	16	5				$x(CH_3N_3)$	$7.01.10^{-9}$	
D-Tyrosine	$C_9H_{11}NO_3$	182.30 > 165.20[1]	12	10	x				$6.66.10^{-9}$	$5.00.10^{-6}$
L-Tyrosine									$4.41.10^{-9}$	

*[1] W. van Helmond, C.-J. Kuijpers, E. van Diejen, J. Spiering, B. Maagdelijn, M. de Puit, Anal. Methods **2017**, 9, 5697–5702.*

*[2] K. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A **2000**, 896, 253–263.*

Table S2. Concentrations of the 35 targeted amino acids (ppb) in Murchison and Allende extracts after HCl hydrolysis. Comparison with published works.

Amino acids	Murchison, HCl hydrolysis This study	Mc Geoch et al. 2015[1] Murchison, HCl hydrolysis *Folch extraction* *No amino acids derivatisation* *LC-MRM MS*	Glavin et al. 2010[2] Murchison, HCl hydrolysis *Water extract* *Amino acids derivatisation* *LC-Fluorescence/TOF-MS*	Engel et al. 1982[3] Murchison HCl hydrolysis *Water extract* *Amino acids derivatisation* *GC-MS*	Allende, HCl hydrolysis This study	Burton et al. 2012[4] Allende HCl hydrolysis *Water extract* *Amino acids derivatisation* *LC-Fluorescence/TOF-MS*
Glycine*	942 +/- 0.03	78750	2606 +/- 717	1059	2955 +/- 0.1	851 +/- 87
D-Alanine	174 +/- 3	16198	592 +/- 99	132	594 +/- 9	221 +/- 12
L-Alanine*	262 +/- 6		718 +/- 160	299	816 +/- 20	686 +/- 42
D-Serine	197 +/- 5	68171	n.d	Detected; not quantified	889 +/- 22	120 +/- 19
L-Serine*			n.d			654 +/- 67
D-Proline	156 +/- 0.2	8654	n.d	41	538 +/- 1	n.d
L-Proline*			n.d	350		n.d
D-Valine	1302 +/- 18	8804	160 +/- 5	125	Noise level; difficult to quantify	30 +/- 7
L-Valine*	1035 +/- 26		< 330 (coelution)		1492 +/- 37	370 +/- 15
D-Isovaline	14583 +/- 745		1253 +/- 125	23	n.d	< 0.1
L-Isovaline			2058 +/- 220		n.d	< 0.1
D-Threonine	1416 +/- 19	13328	n.d	n.d	4593 +/- 62	n.d
L-Threonine*	1434 +/- 25		n.d	n.d	4125 +/- 72	n.d
D-Leucine	533 +/- 23	20403	n.d	3	1021 +/- 44	n.d
L-Leucine*	1155 +/- 1		n.d	112	1658 +/- 1	n.d
D-Isoleucine	2439 +/- 202		n.d	Detected; not quantified	n.d	n.d
L-Isoleucine*	Noise level; difficult to quantify		n.d		n.d	n.d
D-Alloisoleucine	Noise level; difficult to quantify		n.d	n.d	n.d	n.d
L-Alloisoleucine	n.d		n.d	n.d	n.d	n.d
D-Norleucine	n.d		n.d	n.d	n.d	n.d
L-Norleucine	n.d		n.d	n.d	n.d	n.d
D-Aspartic acid	4197 +/- 57	41895	189 +/- 14	67	4636 +/- 63	454 +/- 47
L-Aspartic acid*	1851 +/- 32		281 +/- 23	468	4919 +/- 86	801 +/- 233
D-Lysine	Noise level; difficult to quantify	31937	n.d	n.d	Noise level; difficult to quantify	n.d
L-Lysine*	134 +/- 3		n.d	n.d	308 +/- 7	n.d

D-Glutamic acid	96 +/- 0.1	70743	503 +/- 82	176	70 +/- 0.1	587 +/- 128
L-Glutamic acid*	316 +/- 1		819 +/- 113	823	1805 +/- 5	846 +/- 412
D-Histidine	217 +/- 3	15190	n.d	n.d	341 +/- 5	n.d
L-Histidine*			n.d	n.d		n.d
D-Phenylalanine	356 +/- 3	17036	n.d	n.d	1224 +/- 9	n.d
L-Phenylalanine*	2811 +/- 17		n.d	n.d	5834 +/- 35	n.d
D-Arginine	193 +/- 1	38062	n.d	n.d	294 +/- 1	n.d
L-Arginine*	291 +/- 1		n.d	n.d	445 +/- 1	n.d
D-Tyrosine	n.d	23756	n.d	n.d	n.d	n.d
L-Tyrosine*	n.d		n.d	n.d	n.d	n.d

*Values are reported in parts-per-billion (ppb) on a bulk sample basis. *proteogenic amino acids. Uncertainties were calculated from the variation coefficient of standards signals at a concentration of 10^{-5} mol.L^{-1} and injected in triplicate within the same LC-MRM MS method. n.d: not detected.*

*[1] McGeoch Julie E. M., McGeoch Malcolm W., Meteorit. Planet. Sci. **2015**, 50, 1971–1983.*
*[2] D. P. Glavin, J. P. Dworkin, A. Aubrey, O. Botta, J. H. Doty, Z. Martins, J. L. Bada, Meteorit. Planet. Sci. **2010**, 41, 889–902.*
*[3] M. H. Engel, B. Nagy, Nature **1982**, 296, 837–840.*
*[4] A. S. Burton, J. C. Stern, J. E. Elsila, D. P. Glavin, J. P. Dworkin, Chem. Soc. Rev. **2012**, 41, 5459–5472.*

Table S3. Matrix effect on amino acids MS signals. MS signals of amino acids after standard addition in either HCl-hydrolysed Murchison extract or in a water solution.

Amino acids	HCl hydrolysed Murchison No standard addition	HCl hydrolysed Murchison - $2.5.10^{-4}$ M standard addition	HCl hydrolysed Murchison - 5.10^{-4} M standard addition	Water - $2.5.10^{-4}$ M standard addition	Water - 5.10^{-4} M standard addition
Glycine	5226	8920	11003	29121	43353
Alanine	70574	285682	419655	379500	609883
Serine	54316	390248	558498	345423	601592
Proline	283139	944670	1328504	2721078	3988257
Valine	244167	1728336	2321214	1782877	2826343
Isovaline	2052200	3108969	3426951	1951670	2797489
Threonine	44272	143707	192961	437246	616965
Leucine	56544	495291	691510	507506	753654
Isoleucine	44134	949179	1332070	1034176	1563549
Aspartic acid	11027	133023	183388	360851	551402
Lysine	22635	1012269	1411355	1135339	1824915
Glutamic acid	33132	184673	276082	743922	1159907
Histidine	24930	212487	323262	1147553	1901940
Phenylalanine	73951	709866	1006935	750386	1150620
Arginine	6402	930638	1476916	1092279	1919600
Tyrosine	n.d	199043	306095	197887	324938

n.d: not detected.

Table S4. Mass parameters of LC-HRMS methods.

			Method 1	Method 2
Mass parameters	Electrospray voltage (Kv)		4.0	4.0
	Capillary and heater temperatures (°C)		275-300	280-300
	Relative flow rate of the sweep, sheath and auxiliary (nitrogen) (u.a)		10-35-20	0-40-30
	Resolution	Precursor ion	70 000	70 000
		Fragment ions	35 000	17 500
	AGC target	Precursor ion	1.10^6	1.10^6
		Fragment ions	5.10^4	5.10^4
	Max IT (ms)	Precursor ion	200	200
		Fragment ions	50	50
	Normalized collision energy (%)		35	35
	m/z range		100-1500	70-1050

Table S5. MRM transitions, collision energy and dwell time of Ala-Ala-Ala-Ala-Ala and Ala-Ala-Ala.

			Fragments	
Molecules	Formula	Transitions	Collision energy (eV)	Dwell time (ms)
Ala-Ala-Ala-Ala-Ala	$C_{15}H_{27}N_5O_6$	374.30 > 285.25	13	10
		374.30 > 143.15	25	10
		374.30 > 115.25	29	10
Ala-Ala-Ala	$C_9H_{17}N_3O_4$	232.25 > 143.20	9	10
		232.25 >115.20	13	10
		232.25 > 44.20	24	10
		232.25 > 174.20	18	10
		232.25 > 191.05	9	10
		232.25 > 150.05	16	10