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# **Integrative analytical workflow to enhance comprehensive analysis of organic molecules in extraterrestrial objects.**

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**ABSTRACT:** Molecular identification is a fundamental issue in astrobiology to investigate the routes of emergence of life on our planet involving in particular a potential seeding of extraterrestrial organic matter on the primitive Earth. However, this project encompasses major difficulties due to the low concentration of molecules present in bodies of the Solar System. This work proposes an integrative analytical workflow, no longer based on GC-MS instruments, to enhance comprehensive analysis of organic markers in these objects. Our strategy combines UPLC-HRMS and UPLC-MRM MS methods to bring both a broad molecular mapping and detailed data on indigenous compounds present in any extraterrestrial objects or laboratory analogs. Applied on water extracts from fresh meteorites, our workflow highlights a wide range of free molecules in the non-treated extracts and reveals the wide diversity of amino acid and nucleobase isomers that could lead to misinterpretation as far as the molecular composition of meteorite extracts cannot be anticipated. This strategy, never explored so far, would provide new clues for studying the organic matter in space and should offer new perspectives on its evolution and reactivity.

**KEYWORDS:** Analytical workflow, UPLC-HRMS, UPLC-MRM MS, chirality, organic matter, meteorites.

## 1. Introduction

Laboratory analysis of extraterrestrial objects has substantially deepened our knowledge on the organic compounds available in the Solar System that could have been a source of starting material for the emergence of life on Earth [1]. To investigate molecules in space, gas chromatography coupled to mass spectrometry (GC-MS) has been the state-of-the-art technology so far. With the development of both derivatization agents, enantioselective columns and two-dimensional systems [2–4], GC-MS has allowed the detection of a variety of building blocks of life and many low mass molecules in extraterrestrial samples. However, despite its high resolution and separation efficiency, GC-MS is characterized by a small mass detection range and relatively low sensitivity on the order of a micromolar, thus limiting its investigation field [5].

Ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) has recently been employed to answer astrochemical issues. In this context, hyphenated methods with UPLC separation and fluorescence or time of flight mass spectrometry detection (UPLC-FD-Q-ToF-MS) have been developed, showing sensitivity by up to 1000-fold lower than GC-MS [5–7]. To achieve such performance, researchers generally carry out chemical pretreatments, such as desalting to increase sample purity [8], as well as strong acid hydrolysis and chemical derivatization to improve compounds detectability [9,10]. However these pretreatment steps often rise compounds losses and analytical errors [7,11,12]. Moreover, chemical derivatives, rather instable, can lead to many unknown peaks that cause a high variability within a sample [7,12].

In this context, we propose an integrative workflow only based on UPLC-MS instruments (Q-Orbitrap High Resolution Mass Spectrometry - HRMS and Triple Quadrupole Multiple Reaction Monitoring Mass Spectrometry - TQ MRM MS) to enhance the comprehensive analysis of organics in native meteorite extracts. Our approach starts from a large-scale analysis and ends to an enantioselective analysis of targeted molecules (**Fig. 1**). Since terrestrial residence may impact mineralogy and organo-chemistry of extraterrestrial samples, we opted for fresh chondrites fallen in the five last years. This workflow in hands, we were able to highlight the molecular composition of meteoritic water extracts although they were submitted to neither desalting, acid hydrolysis nor derivatization. Therefore, we do think that this proposal can improve the quality of future astrochemical and astrobiological studies.

## 2. Materials and methods

### 2.1. Meteorite samples and pretreatment

The Museum National d'Histoire Naturelle (Paris, France) provided meteorite fragments (Aguas Zarcas, Mukundpura, Kolang and Tarda). All glassware used in sample processing were pyrolyzed at 500 °C overnight prior to use. One-gram fragments were sonicated with 2 mL of milliQ water (18.2 MΩ.cm<sup>-1</sup>) during 10 min to remove surface contamination. The fragments were then powdered in an Agate mortar and extracted with 2 mL of distilled water at 100 °C for 24 h under vacuum in a sealed evacuated vial. After centrifugation, aqueous supernatants were aliquoted. All aliquots were dried and stored at -20 °C. One aliquot (100 µL for Mukundpura, Kolang and Tarda and 150 µL for Aguas Zarcas) per meteorite was taken for the overall development. Aliquots were dried and resuspended in 50 µL of milliQ water (18.2 MΩ.cm<sup>-1</sup>) for Mukundpura, Kolang and Tarda or 75 µL for Aguas Zarcas. Liquid aliquots were diluted by a 7.5 factor in the different mobile phases to perform the whole UPLC-MS analyses.

The entire procedure was repeated with 2 mL milliQ water (18.2 MΩ.cm<sup>-1</sup>) (procedural blank).

All samples were sonicated for 5 min at 25 °C prior UPLC-MS injection.

## 2.2. Chemicals and solutions

Three 10<sup>-4</sup> mol.L<sup>-1</sup> standard solutions composed of 31 L-amino acids, 23 D-amino acids or the 5 natural nucleobases were prepared in milliQ water (18.2 MΩ.cm<sup>-1</sup>). The entire list of amino acids and nucleobases is listed in supplemental information.

For polar UPLC-MS analyses, a mix standard solution containing L-amino acids and nucleobases was prepared at 10<sup>-5</sup> mol.L<sup>-1</sup> in acetonitrile:water (85:15). For chiral UPLC-MRM MS analyses, a second mix standard solution composed of the L- and D-amino acids and the nucleobases at 10<sup>-5</sup> mol.L<sup>-1</sup> was prepared in milliQ water (18.2 MΩ.cm<sup>-1</sup>).

UPLC mobile phases were prepared with LC-MS grade acetonitrile (Sigma-Aldrich), LC-MS grade methanol (Sigma-Aldrich), ultra-pure grade formic acid (Sigma-Aldrich), ultra-pure grade trifluoroacetic acid (Sigma-Aldrich) and ammonium formate salt (Fisher Chemical, Loughborough, UK). Purified water was generated by a Purelab Flex purifier system (Veolia, Paris, France).

Finally, to analyse the compounds by GC-HRMS, amino acids and nucleobases were derivatized with N-methyl-N-(tert.butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) and dimethylformamide which were purchased from Sigma-Aldrich-Fluka (Buchs, Switzerland).

## 2.3. Untargeted analysis of meteorite water extracts

### 2.3.1. Investigation of meteorite organics by UPLC-HRMS

Accela Ultra-Performance Liquid Chromatography (UPLC) pumps are interfaced with a High-Resolution Mass Spectrometer (Q-Exactive, Hybrid Quadrupole-Orbitrap Mass Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA). Compound ionization is performed by ElectroSpray Ionization (ESI). A stack cooler CW (CTC Analytics AG, Zwingen, Switzerland) allows samples storage at 4 °C before analysis. Xcalibur data system (Thermo Fisher Scientific) controls UPLC mobile phases and MS functions. For accurate mass calibration, a LTQ Velos ESI Positive Ion Calibration Solution is injected every day (Pierce Biotechnology Inc., Rockford, IL).

Amino acids and nucleobases were first separated on a Hydrophilic Interaction Liquid Chromatography (HILIC) column. For that, an Acquity UPLC BEH amide analytical column (150 mm x 2.1 mm, 1.7  $\mu\text{m}$  ; Waters Corporation, Milford, MA) fitted with an Acquity UPLC BEH Amide Vanguard guard column (2.1 mm x 5 mm, Waters Corporation) were used. To achieve compound identification, 5  $\mu\text{L}$  of a standard solution containing 31 l-amino acids and the 5 natural nucleobases at  $1.10^{-6} \text{ mol.L}^{-1}$  were injected. Five microliters of meteoritic samples were injected as well. To obtain an efficient separation, samples had to be diluted in the mobile phase (i.e. acetonitrile:water ; 85:15).

Elution was performed at a constant flow of  $300 \mu\text{L.min}^{-1}$  at 30 °C. Acetonitrile:water (85:15) with 0.15 % formic acid was used as mobile phase A and water with 0.15 % formic acid and  $10 \text{ mmol.L}^{-1}$  of ammonium formate as mobile phase B. An elution gradient was optimized for the separation of amino acids and nucleobases. The gradient started with 95 % of A at 0 min and reached 80 % of A in 12.80 min. A second gradient was used to reach 70 % of A in 4 min and then 60 % of A in 1 min. The column was then reconditioned for 2 min with 95 % of A.

Mass spectrometry detection was done in a full scan mode to provide a non-targeted fingerprint of the molecules present in the meteorite extracts (**Fig. 2.a**). The electrospray voltage was set at 4.0 kV, the capillary temperature held at 280 °C and the heater temperature at 300 °C. Sheath, sweep and auxiliary gas flow rates were set at 40, 0 and 30, respectively (arbitrary units). The Q-Exactive worked at 70000 resolution level with an AGC target of  $3.10^6$ , a max IT of 100 ms and a mass range of 50 – 750 amu.

Total Ion Current chromatograms (TICs) were plotted with the Origin software (Origin 9.0.0, OriginLab Corporation, Northampton, USA) (**Fig. 2.a**). Full-scan data were processed by Compound Discoverer (CD) 3.1 software (Thermo Fisher

Scientific) and subjected to a data treatment that involves retention time alignment, normalization, comparison against the procedural blank and statistical analysis (CA - Correspondence Analysis) to explore molecular relationships among meteorites (**Fig. 2.b**). Ions detected after 12 min UPLC-HRMS analysis were not considered due to the broad and noisy peak at 13 min analysis (section 3.1). Mass / charge ( $m/z$ ) ion distribution was examined with CD (**Fig. S1**).

A standard addition procedure was performed to ensure the identification of the molecules listed in **Table S1**. For that, a standard solution was prepared with glycine at  $3.10^{-6}$  mol.L<sup>-1</sup>, and L-glutamic acid, *N*-ethylglycine, L-2-aminobutyric acid, L-3-aminobutyric acid, L-2-aminoisobutyric acid, L-3-aminoisobutyric acid,  $\gamma$ -aminobutyric, sarcosine, L-alanine,  $\beta$ -alanine and uracil at  $3.10^{-7}$  mol.L<sup>-1</sup>. A solution at  $1.5.10^{-7}$  mol.L<sup>-1</sup> containing the 20 remaining amino acids (see the full list in **Table S1**) and the remaining 4 nucleobases was added in the final mix. Once prepared, the standard solution was added in 10  $\mu$ L meteoritic samples. A volume of 5  $\mu$ L of each added sample was injected and analyzed (**Fig. S2**).

In parallel, a five-point calibration curve ranging from  $5.10^{-8}$  to  $5.10^{-7}$  mol.L<sup>-1</sup> was carried out in quadruplicate. Limits of detection (LODs) and quantification (LOQs) were determined as 3 and 10 times signal to noise ratio (**Table S1**).

### 2.3.2. Standard analysis method: amino acids derivatization and GC-HRMS analysis

To be analyzed by gas chromatography (GC), a standard solution of amino acids was evaporated at 45 °C under N<sub>2</sub> gas stream. Compound derivatization was performed with a 3:1 (v:v) mixture of *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide and dimethylformamide at 75 °C for 30 min.

After cooling, 1  $\mu$ L of the reaction mixture was injected in a GC apparatus (Agilent 7890A, Agilent Technologies, Santa Clara, USA) with electronic impact ionization (ionization energy 70 eV) coupled to a High-Resolution Mass Spectrometer (Quadrupole - Time of flight - Q-ToF; Agilent 7200). The instrument is controlled by the GC-Q-ToF/Mass Hunter software (Agilent, B.06.01.1312) and results were plotted with Origin software.

Amino acids and nucleobases were separated on a fused silica capillary column (Agilent HP-5, 30 m x 0.25 mm, 0.25 mm). Helium was used as the carrier gas with a flow rate at 0.9 mL.min<sup>-1</sup>. The oven temperature started from 60 °C to 300 °C at 5 °C min<sup>-1</sup> (isotherms for 5 min at 180 °C and at 300 °C). Line transfer and source temperature were held at 300 °C and 250 °C. Injector temperature was set at 250 °C in a split mode (20:1). Septum purge flow and gas saver flow were 3 mL.min<sup>-1</sup> and 20 mL.min<sup>-1</sup> respectively. The mass detector was set at 250 °C and operated in full scan mode with a mass range of 50

to 400 amu. A scan rate of 1 scans.s<sup>-1</sup> was used to detect ions. Mass calibration is carried out each day by using FC-43 (Perfluorotributylamine).

A five-point calibration curve ranging from 1.10<sup>-6</sup> to 1.10<sup>-5</sup> mol.L<sup>-1</sup> was performed in duplicate. Limits of detection (LODs) and quantification (LOQs) were determined as 3 and 10 times signal to noise ratio (**Table S1**).

## 2.4.Targeted analysis of meteorite water extracts

### 2.4.1. Analysis of targeted meteorites metabolites by UPLC-MRM MS

To perform UPLC-Multiple reaction monitoring (MRM) MS analyses, a Shimadzu Nexera X2 UPLC system coupled to a 8050 triple quadrupole mass spectrometer (TQ MS) (Shimadzu, Marlborough, MA, USA) was used. Ionization is carried out by an ESI source working in the positive mode. LabSolution data system controlled UPLC gradients and MS function (LabSolution 5.89, Shimadzu). Chromatograms were plotted with Origin software.

To confirm UPLC-HRMS identification, amino acids and nucleobases were analyzed with the same HILIC column (i.e. BEH amide, Waters) which was connected to the TQ MS instrument (**Table S2**). UPLC mobile phases and gradient were the same as exposed before.

TQ-MS parameters were set as follows: capillary voltage 4 kV; interface temperature 300 °C; heat block temperature 500 °C; desolvation line temperature 250 °C, nebulizing gas flow 180 L.h<sup>-1</sup>, heating gas flow 600 L.h<sup>-1</sup> and drying gas flow 600 L.h<sup>-1</sup>. MRM transitions, dwell times and collision energies were optimized (**Table S3**).

During an analysis sequence, a standard solution containing all the L-amino acids and nucleobases at 1.10<sup>-6</sup> mol.L<sup>-1</sup> was prepared and 5 µL injected at the start and end of sequence. In the same manner, 5 µL of meteoritic samples were injected.

To ensure the identification of the molecules, a standard addition procedure was performed as described previously.

To complete our workflow, we finally designed an enantioselective UPLC-MRM MS strategy. Hence, amino acids enantiomers and nucleobases were separated on a chiral column which was a Crownpak CR (+) column (150 mm × 4.0 mm, 5 µm, Chiral Technologies, Europe). In this stationary phase, the chiral separation relies on crown ether as the chiral selector. A guard column Crownpak CR (10 mm × 4.0 mm, 5 µm, Chiral Technologies) was fitted to the analytical column. Elution was carried out at 600 µL.min<sup>-1</sup> with water:methanol (95:5) with 0.5 % trifluoroacetic acid (Sigma-Aldrich). UPLC oven temperature was held at 30 °C.



TQ MS capillary voltage was set at 4 kV, the interface temperature was held at 400 °C and heat block temperature at 500 °C. The desolvation line temperature was held at 250 °C. Nebulizing gas flow, heating gas flow and drying gas flow were set at 180 L.h<sup>-1</sup>, 600 L.h<sup>-1</sup> 600 L.h<sup>-1</sup> respectively. Multiple reaction monitoring (MRM) transitions, dwell times and collision energies for all analytes are summarized in **Table S3**.

During an analysis sequence, 5 µL of both L- and D-standard solution in water at 1.10<sup>-6</sup> mol.L<sup>-1</sup> were injected at the start and end of the sequence. A volume of 5 µL of meteoritic samples were also injected (**Fig. S4**).

A five-point calibration curve ranging from 5.10<sup>-8</sup> to 5.10<sup>-7</sup> mol.L<sup>-1</sup> was performed in triplicate. Limits of detection (LODs) and quantification (LOQs) were calculated as 3 and 10 times signal to noise ratio.

#### 2.4.2. Investigation of the accuracy of the chiral UPLC-MRM MS method

To investigate the quantification accuracy of the chiral UPLC-MRM MS method, three standard solutions containing five D- and L-amino acids (D-alanine, L-alanine, D-valine, L-valine, D-leucine, L-leucine, D-glutamic acid, L-glutamic acid, D-isoleucine and L-isoleucine) were prepared with L/D ratios of 52/48, 50/50, 48/52 (1.10<sup>-4</sup> mol.L<sup>-1</sup> final concentration). A volume of 5 µL of these solutions was injected within the chiral UPLC-MRM MS method (three replicates). The signal intensity ratio of both L- and D-enantiomers was determined for each of the combinations (**Fig. S5**).

#### 2.4.3. Compounds identification and quantification

A standard addition procedure was performed to (1) investigate possible meteorite matrix effect on compound signal intensity (**Fig. S6**) and (2) certify identification of targeted amino acids and nucleobases in the four meteorite extracts (**Fig. S7**).

Three added samples per meteorite were prepared. The first one was composed of 10 µL meteorite extract and 5 µL water (encoded SA-0 in **Table S4**). The second and third ones both contained 10 µL meteorite extract and 5 µL of a standard solution containing the 54 amino acids and 5 nucleobases at specific concentrations (encoded SA-1 and SA-2 in **Table S4**). To assess meteorite matrix effect on compound signal intensity, two additional added samples were prepared by replacing the 10 µL meteorite extract by 10 µL of water (**Fig. S6**).

Five microliters of the samples were injected and analysed with the chiral UPLC-MRM MS method.

### 3. Results and discussion

#### 3.1 Non targeted analysis of meteorite extracts

Our analytical development was conducted on extracts of the recently fallen Mukundpura, Aguas Zarcas, Tarda and Kolang meteorites. Whereas amino acids, carboxylic acids and aliphatic amines have been investigated in Mukundpura [14,15] and Aguas Zarcas [16], the organic composition of Tarda and Kolang has not been presented so far. For each of the meteorites, 1 g of pristine and freshly collected fragments was extracted following the standard water extraction procedure [14,16]. A 2 mL extract was obtained and aliquoted. An aliquot of some tens of microliters was taken per meteorite to complete the workflow.

First, we designed a UPLC-HRMS (Q-Orbitrap) method allowing a rapid screening of small polar molecules. Standard analytical procedures usually imply an hydrochloride (HCl) hydrolysis followed by a derivatization reaction to allow compounds detection in either GC-MS or LC-FD/Q-ToF-MS [10,14–16]. However, since chemical derivatization is selective, it does not allow wide molecular screening. Thus, we decided to employ a hydrophilic interaction stationary phase that circumvents compounds derivatization for MS analysis [17] and we compared the overall organic composition of the four meteorites (**Fig. 2**).

Even though samples were not desalted, chromatogram baseline noise was very low (**Fig. 2a**). On the other hand, a chemical background interference was highlighted with a fake peak at 3.8 min corresponding to ammonium formate clusters ( $m/z$  63.0320 in both samples and blank). Organic contaminations were also detected in the blank sample that were aligned with resolved peaks in the four extracts (peaks at 1.3 min and 15 min). To map organics in the meteorites, we thus performed a multivariate data analysis (i.e. Correspondence Analysis or CA) after peaks retention time alignment and subtraction of the ions present in the procedural blank. Because of the presence of an asymmetric and noisy peak at 13 min for all the extracts (peak that can be attributed to mineral interferences from the sample matrices), CA contingency table CA was constructed with ions detected until 12 min analysis to avoid any misinterpretation of the data. As shown in the CA plot (**Fig. 2.b**), Mukundpura and Kolang shared close similarities with many small ions having  $m/z$  values between 100 to 400 as in Aguas Zarcas (**Fig. S1**). Organics distribution in Tarda was substantially different with ions mass range that was no longer in favor of small free molecules (**Fig. S1**). This finding is consistent with petrological and physical differences between the

214 meteorites. Of note, Tarda is made of low-density stones which are somewhat friable and that can be easily crushed in water.  
 215 Tarda has also a specific O-isotope signature that does not overlap with those of Aguas Zarcas, Kolang and Mukundpura,  
 216 thus explaining its classification apart from the CM (Mighei-like chondrites) group [18].  
 217 Seeing such molecular diversity, we decided to search for amino acids and nucleobases that are considered key markers in  
 218 astrobiology. Thus, we determined the performance of our hydrophilic interaction UPLC-HRMS method towards the  
 219 analysis of these compounds, and we highlighted a sensitivity about 3 to 1000-fold reduced compared to a derivatization-  
 220 GC-HRMS procedure (**Table S1**). Noticeably, we obtained resolute chromatographic peaks (**Fig. S2**) though they did not  
 221 reach the high-level resolution of GC-HRMS analysis (**Fig. S3**). Hence, we were able to separate 23 amino acids and the 5  
 222 natural nucleobases, whereas co-elution was observed for 3-aminobutyric acid/3-aminoisobutyric acid, 2-aminobutyric  
 223 acid/2-aminoisobutyric acid, norleucine/ $\beta$ -leucine and alloisoleucine/isoleucine (**Table S1**).  
 224 Once the method optimized, we analysed the four meteoric samples. To avoid incorrect identification, we performed a  
 225 standard addition procedure and we did detect almost all the targeted molecules in both Aguas Zarcas and Kolang extracts  
 226 (29 and 28 compounds detected respectively), 22 in Mukundpura and 20 in Tarda (**Table S1**). Again, Aguas Zarcas and  
 227 Kolang shared similarities in amino acids and nucleobases composition, which correlates well with the multivariate data  
 228 analysis (**Fig. 2b**).  
 229 In more details, we highlighted glycine, alanine,  $\beta$ -alanine, serine, threonine, proline, valine, isovaline, norvaline, leucine,  
 230 isoleucine/alloisoleucine, aspartic acid, glutamic acid, tyrosine,  $\gamma$ -aminobutyric acid, 3-aminobutyric acid/3-  
 231 aminoisobutyric acid, 2-aminobutyric acid/2-aminoisobutyric acid, and 5-aminovaleric acid in Aguas Zarcas and/or  
 232 Mukundpura (**Table S1**). These results are in accordance with recent studies [14–16] which investigated the organic  
 233 composition of Aguas Zarcas and Mukundpura water extracts, treated or not with HCl, and submitted to a derivatization  
 234 step and GC-MS or LC-FD/Q-ToF-MS analysis. On the other hand, this is the first time that sarcosine, *N*-ethylglycine,  
 235 homoserine, norleucine/ $\beta$ -leucine, lysine, arginine, histidine, and guanine and thymine nucleobases are reported in each of  
 236 these two meteorites.  
 237 Since the molecular composition of Tarda and Kolang have never been studied so far, our large-scale analysis is the first to  
 238 provide a list of free amino acids and nucleobases in both objects. Nevertheless, to confirm such outcomes, we needed to  
 239 develop a targeted analysis method.

### 3.2 Targeted analysis of free amino acids and nucleobases

As we previously demonstrated [19], UPLC-HRMS has certain limitations in testing for the presence of specific molecules in laboratory analogs. In fact, bodies of the Solar System are characterized by a stochastic chemistry producing a wide range of structural isomers that can elute at the same retention time. Therefore, we decided to combine the hydrophilic chromatographic separation with a selective mass spectrometry instrument (UPLC-TQ MRM MS), that searches for the fragmentation fingerprint of a molecule thus offering additional proof of identification.

After method development, we tested for the presence of the 31 amino acids and the 5 nucleobases in the four meteorite extracts and the procedural blank (**Table S2**). Thanks to this targeted method analysis, we were able to indicate additional molecules such as 2-aminobutyric acid/2-aminoisobutyric acid in the extracts. On the other hand, we confirmed only 66 %, 86 %, 54 % and 65 % of the compounds identified by UPLC-HRMS in Aguas Zarcas, Mukundpura, Kolang and Tarda respectively. Proline, arginine and histidine were detected both in the blank and the extracts, suggesting contaminations in all the samples. Moreover, several identifications such as *N*-ethylglycine, homoserine, threonine, lysine, methionine, tyrosine, thymine, were not validated thus suggesting the presence of isomers having the same exact masses as these compounds in the whole extracts (**Table S2**). These results demonstrate the benefits of a selective MS approach to avoid misinterpretation when bodies of the Solar System are to be studied.

### 3.3 Enantioselective targeted analysis

We obtained an efficient separation in a very short time analysis of a wide range of amino acid enantiomers (except L/D-3-aminoisobutyric acid, L/D-isovaline, L/D- $\beta$ -leucine, L/D-proline and L/D-histidine) and constitutional isomers (except some aminobutyric acids,  $\beta$ -alanine/L-alanine and isovaline/D-valine) (**Fig. 3**, **Table S3** and **Fig. S4**). Once we verified that our method ensured the evaluation of very small variations of enantiomer concentrations (**Fig. S5**), we assessed its sensitivity. Remarkably, we showed limits of detection 6-fold to 110-fold reduced compared to chiral GC-MS methodology [21] (**Table S3**). Although our LC-MS strategy is highly sensitive, it can suffer from matrix effects. This effect can be observed either as a loss or increase in ESI-MS response (ion suppression or enhancement), or a shift in LC retention times. Therefore, to validate our method, we needed to evaluate meteorites matrix effect and we decided to perform a standard addition

procedure to (1) investigate possible meteorite matrix effect on compound signal intensity (**Fig. S6**) and (2) certify identification of targeted amino acids and nucleobases (**Fig. S7**).

By doing so, we indicated a negative meteorite matrix effect on the signal intensity of the smallest amino acids, glycine, alanine and isomers (**Fig. S6**). These results could be attributed to variability across meteorite inorganic and organic matrix which can cause the alteration of ionization efficiency of the targeted compounds [10,14,15,18,22], the absence of chemical derivatization in our analytical protocol compared to those described in the literature [23], and the use of liquid chromatography, ion-pairing agent and electrospray ionisation [24,25]. In fact, all these factors could have enhanced the matrix effect on non-derivatized polar molecules present in such complex heterogeneous mixtures.

To elude matrix effect on UPLC retention times, we carried out a standard addition procedure for ensuring compounds identification (**Table S4**). By doing so, we did detect non-proteogenic D-enantiomers of ten amino acids as expected in these abiotic samples (**Table S5, Fig. S7**). Obviously, we revealed many unknown isomers (**Fig. 3**). This was particularly relevant for valine and leucine which TICs presented several unattributed peaks answering to the same transitions as the standards (orange and red TIC chromatograms respectively; **Fig. 3**. Aguas Zarcas example). Hence, whereas peaks corresponding to leucine, norleucine and alloisoleucine were clearly seen in Mukundpura after hydrophilic interaction UPLC-MS analysis (**Table S2**), none of these three compounds has been confirmed with the chiral UPLC-MS analysis (**Table S5**). This finding underlines the interest of our analytical workflow combining several chromatographic and detection approaches to prove the presence of any organic molecule in meteorites.

Following on, we were able to determine the concentration of 30 amino acid enantiomers in Aguas Zarcas, 25 in Munkundpura, 19 in Kolang and 20 in Tarda (**Table S5**). Guanine was also quantified in Aguas Zarcas, Munkundpura, and Kolang. Although these compounds were accurately detected, almost all the concentrations were close to limits of quantification which allowed us to provide only an estimate. However, our values were in the same ppb range as reported previously in carbonaceous chondrites [14,16,26–28]. Relative distributions were also consistent with an abiotic process of formation (the low mass molecules such as alanine or glycine, being the most abundant; the higher masses such as valine, leucine, aspartic acid and glutamic acid present lower quantities, **Table S5**). Noticeably, concentration values were in average inferior in Tarda compared to those estimated in the three other meteorites. Therefore, thanks to both untargeted and targeted analyses, we were able to emphasize atypic composition of a meteorite sample.

#### 4. Conclusion

In summary, we have designed the first UPLC-MS workflow for cosmochemistry basic research. Our integrative strategy enables both screening and targeted analysis of a wide range of free molecules in native meteorite extracts. Our methods offer good peak resolution, high sensitivity and selectivity, and elude isomers identification without any sample pretreatment as do the standard protocols (i.e. desalting, acid hydrolysis and derivatization). By combining data obtained from different UPLC-MS methods and performing standard addition procedure, our strategy can circumvent sample matrix effects on both identification and quantification of analytes. Therefore, we do think that our workflow represents an important progress in the astrochemistry field and can open new doors for the investigation of organic compounds in interplanetary bodies as well as in returned samples from asteroids and comets.

#### Sample CRediT author statement

**Coline Serra:** Investigation, Methodology, Formal analysis, **Justin Lange:** Investigation, Methodology, Formal analysis, **Quentin Blancart Remaury:** Investigation, Methodology, Formal analysis, **Ramzi Timoumi:** Investigation, Formal analysis, **Grégoire Danger:** Validation, **Boris Laurent:** Resources, Validation, **Laurent Remusat:** Resources, Validation, **Claude Geffroy Rodier:** Investigation, Validation, **Pauline Poinot:** Supervision, Conceptualization, Validation, Writing - Review & Editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplemental information

Supplemental information is provided in the SI appendix. It presents a list of amino acids and nucleobases used for the preparation and analysis of standard samples. It also includes figures and tables summarizing methods performance.

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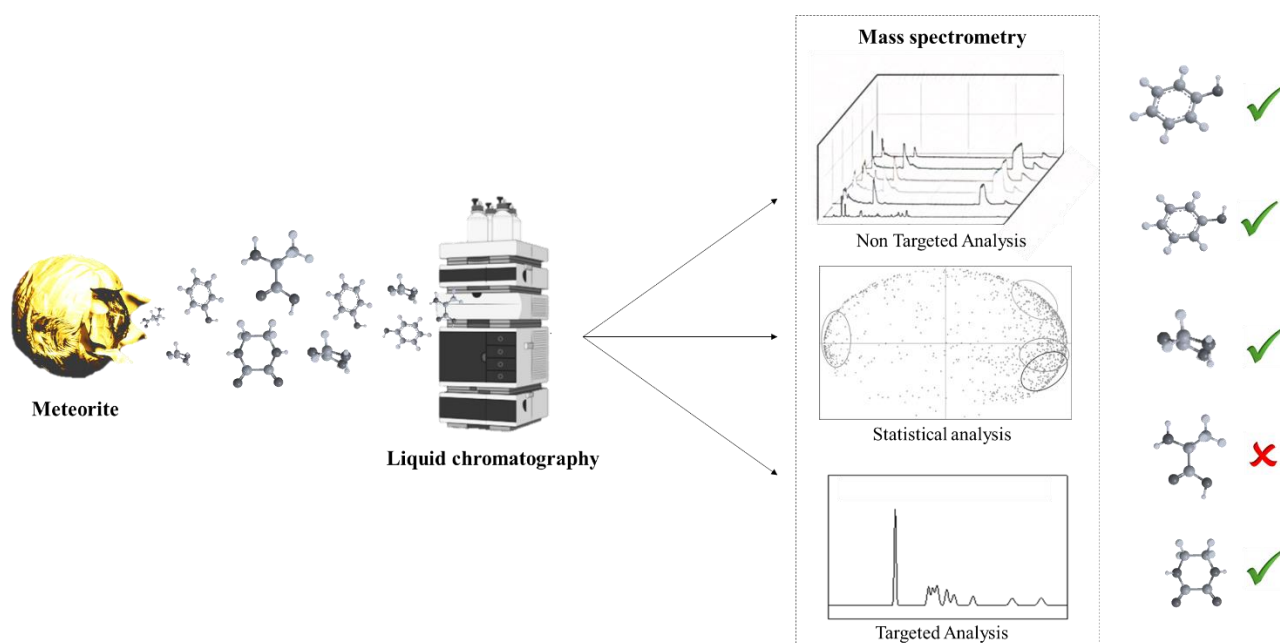
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## Graphical abstract.



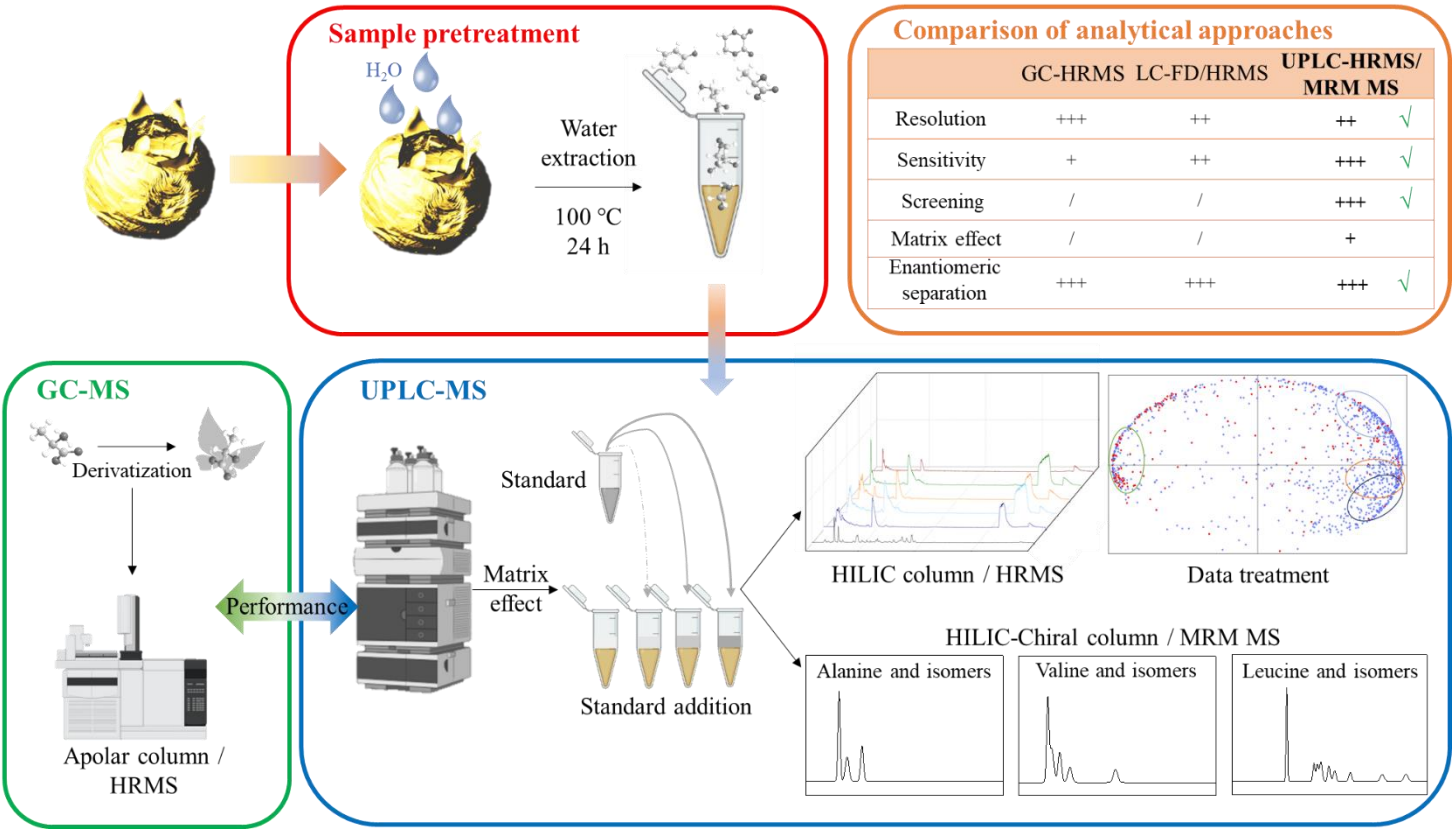
## Figure Captions

**Fig. 1.** Schematic representation of the UPLC-MS workflow.

**Fig. 2.** UPLC-HRMS analysis with an amide phase column (BEH column, Waters). a. Total Ion Current (TIC) chromatograms of a  $10^{-6}$  mol.L $^{-1}$  standard solution, the four meteorite water extracts and their procedural blank. b. ~~Principal–Correspondence~~ **Principal Component Analysis (PCA)** ~~symmetric plot of the loading for representing meteorites and all molecules-ions~~ detected in 50-750 m/z range ~~for 0 – 12 min UPLC-HRMS analysis. Blue ions with m/z value inferior to 350. Red ions with m/z superior to 350. Meteorites points with similar profile are closed on the map.~~

**Fig. 3.** Chiral UPLC-MRM analysis of some amino acid isomers. a. Standard solution at  $10^{-6}$  mol.L $^{-1}$ . b. Water extract of Aguas Zarcas. A dried Aguas Zarcas water extract was solubilized in the chiral elution phase. Five microliters were injected in a UPLC-TQ MS for targeted analysis of amino acids and nucleobases. 1-21: 1. Sarcosine; 2. D-Alanine; 3. L-Alanine/ $\beta$ -Alanine; 4. *N*-Ethylglycine; 5. 2-Aminoisobutyric acid/D-2-Aminobutyric acid/D-3-Aminobutyric acid/L-3-Aminobutyric acid; 6. L-2-Aminobutyric acid; 7. D-3-Aminoisobutyric acid/L-3-Aminoisobutyric acid/ $\gamma$ -Aminobutyric acid; 8. D-Isovaline/L-Isovaline/D-Valine; 9. L-Valine; 10. D-Norvaline; 11. 5-Aminovaleric acid; 12. L-Norvaline; 13. D- $\beta$ -Leucine/L- $\beta$ -Leucine ; 14. D-Alloisoleucine; 15. D-Isoleucine; 16. L-Alloisoleucine; 17. L-Isoleucine; 18. D-Leucine; 19. D-Norleucine; 20. L-Leucine; 21. L-Norleucine. \* Signals were increased by ten for all to be shown on the same intensity scale.

Fig. 1.



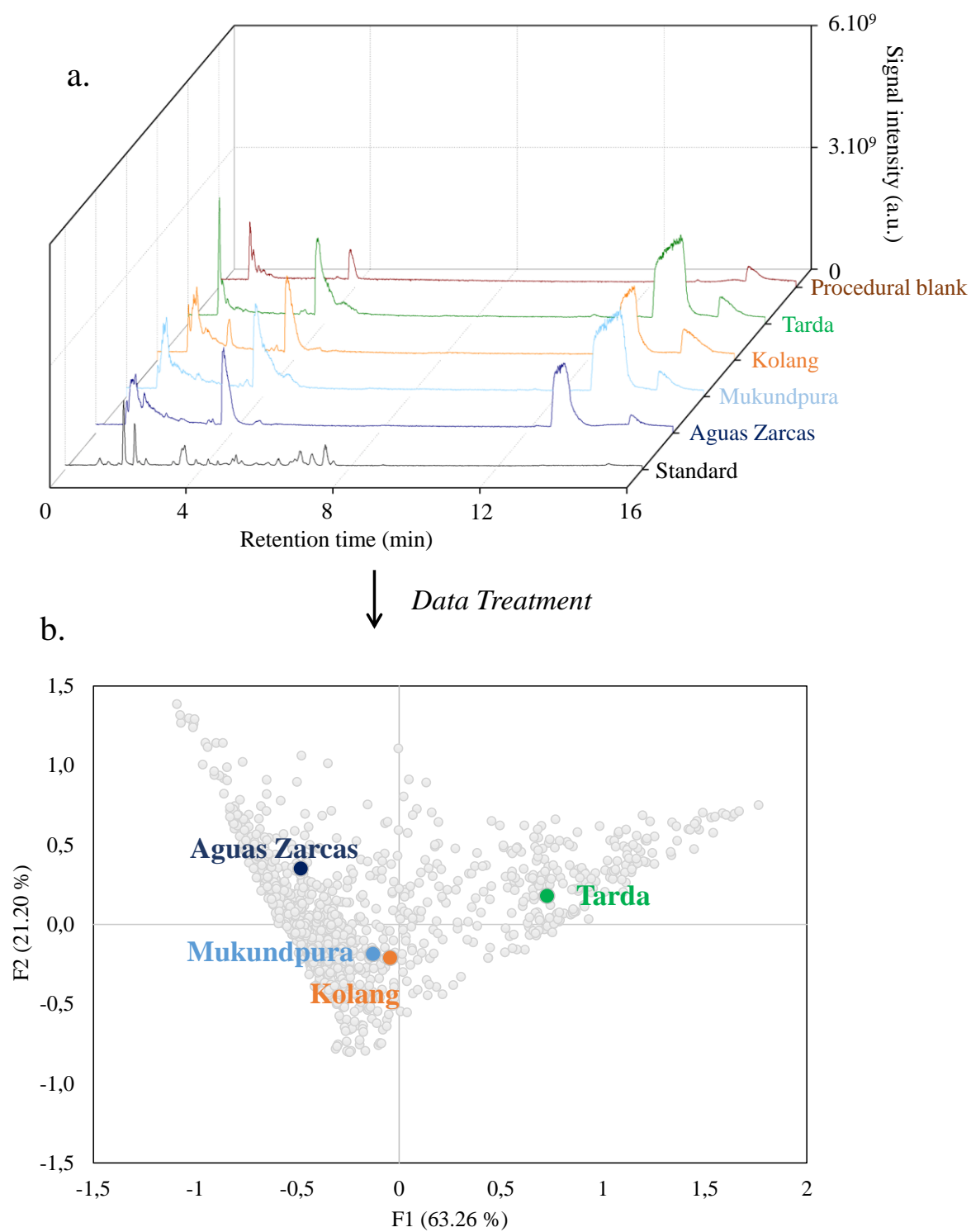
**Fig. 2.**

Fig. 3.

