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

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18

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

30

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

32 **1. Introduction**

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

40 Ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) has recently been employed to answer
41 astrochemical issues. In this context, hyphenated methods with UPLC separation and fluorescence or time of flight mass
42 spectrometry detection (UPLC-FD-Q-ToF-MS) have been developed, showing sensitivity by up to 1000-fold lower than
43 GC-MS [5–7]. To achieve such performance, researchers generally carry out chemical pretreatments, such as desalting to
44 increase sample purity [8], as well as strong acid hydrolysis and chemical derivatization to improve compounds detectability
45 [9,10]. However these pretreatment steps often rise compounds losses and analytical errors [7,11,12]. Moreover, chemical
46 derivatives, rather instable, can lead to many unknown peaks that cause a high variability within a sample [7,12].

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

55

56 **2. Materials and methods**

57 2.1. Meteorite samples and pretreatment

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

67 The entire procedure was repeated with 2 mL milliQ water (18.2 MΩ.cm⁻¹) (procedural blank).

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

69

70 2.2. Chemicals and solutions

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

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

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

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

84 2.3. Untargeted analysis of meteorite water extracts

85 2.3.1. Investigation of meteorite organics by UPLC-HRMS

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

92 Amino acids and nucleobases were first separated on a Hydrophilic Interaction Liquid Chromatography (HILIC) column.
93 For that, an Acquity UPLC BEH amide analytical column (150 mm x 2.1 mm, 1.7 µm ; Waters Corporation, Milford, MA)
94 fitted with an Acquity UPLC BEH Amide Vanguard guard column (2.1 mm x 5 mm, Waters Corporation) were used. To
95 achieve compound identification, 5 µL of a standard solution containing 31 l-amino acids and the 5 natural nucleobases at
96 1.10^{-6} mol.L⁻¹ were injected. Five microliters of meteoritic samples were injected as well. To obtain an efficient separation,
97 samples had to be diluted in the mobile phase (i.e. acetonitrile:water ; 85:15).

98 Elution was performed at a constant flow of 300 µL.min⁻¹ at 30 °C. Acetonitrile:water (85:15) with 0.15 % formic acid was
99 used as mobile phase A and water with 0.15 % formic acid and 10 mmol.L⁻¹ of ammonium formate as mobile phase B. An
100 elution gradient was optimized for the separation of amino acids and nucleobases. The gradient started with 95 % of A at 0
101 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
102 min. The column was then reconditioned for 2 min with 95 % of A.

103 Mass spectrometry detection was done in a full scan mode to provide a non-targeted fingerprint of the molecules present in
104 the meteorite extracts (**Fig. 2.a**). The electrospray voltage was set at 4.0 kV, the capillary temperature held at 280 °C and
105 the heater temperature at 300 °C. Sheath, sweep and auxiliary gas flow rates were set at 40, 0 and 30, respectively (arbitrary
106 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
107 of 50 – 750 amu.

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

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

114 A standard addition procedure was performed to ensure the identification of the molecules listed in **Table S1**. For that, a
115 standard solution was prepared with glycine at $3 \cdot 10^{-6}$ mol.L⁻¹, and L-glutamic acid, *N*-ethylglycine, L-2-aminobutyric acid,
116 L-3-aminobutyric acid, L-2-aminoisobutyric acid, L-3-aminoisobutyric acid, γ -aminobutyric, sarcosine, L-alanine, β -alanine
117 and uracil at $3 \cdot 10^{-7}$ mol.L⁻¹. A solution at $1.5 \cdot 10^{-7}$ mol.L⁻¹ containing the 20 remaining amino acids (see the full list in **Table**
118 **S1**) and the remaining 4 nucleobases was added in the final mix. Once prepared, the standard solution was added in 10 μ L
119 meteoritic samples. A volume of 5 μ L of each added sample was injected and analyzed (**Fig. S2**).

120 In parallel, a five-point calibration curve ranging from $5 \cdot 10^{-8}$ to $5 \cdot 10^{-7}$ mol.L⁻¹ was carried out in quadruplicate. Limits of
121 detection (LODs) and quantification (LOQs) were determined as 3 and 10 times signal to noise ratio (**Table S1**).

122

123 2.3.2. Standard analysis method: amino acids derivatization and GC-HRMS analysis

124 To be analyzed by gas chromatography (GC), a standard solution of amino acids was evaporated at 45 °C under N₂ gas
125 stream. Compound derivatization was performed with a 3:1 (v:v) mixture of *N*-methyl-*N*-(tert-
126 butyldimethylsilyl)trifluoroacetamide and dimethylformamide at 75 °C for 30 min.

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

131 Amino acids and nucleobases were separated on a fused silica capillary column (Agilent HP-5, 30 m x 0.25 mm, 0.25 mm).
132 Helium was used as the carrier gas with a flow rate at 0.9 mL.min⁻¹. The oven temperature started from 60 °C to 300 °C at
133 5 °C min⁻¹ (isotherms for 5 min at 180 °C and at 300 °C). Line transfer and source temperature were held at 300 °C and 250
134 °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⁻¹
135 and 20 mL.min⁻¹ respectively. The mass detector was set at 250 °C and operated in full scan mode with a mass range of 50

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

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

140

141 2.4.Targeted analysis of meteorite water extracts

142 2.4.1. Analysis of targeted meteorites metabolites by UPLC-MRM MS

143 To perform UPLC-Multiple reaction monitoring (MRM) MS analyses, a Shimadzu Nexera X2 UPLC system coupled to a
144 8050 triple quadrupole mass spectrometer (TQ MS) (Shimadzu, Marlborough, MA, USA) was used. Ionization is carried
145 out by an ESI source working in the positive mode. LabSolution data system controlled UPLC gradients and MS function
146 (LabSolution 5.89, Shimadzu). Chromatograms were plotted with Origin software.

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

150 TQ-MS parameters were set as follows: capillary voltage 4 kV; interface temperature 300 °C; heat block temperature 500
151 °C; desolvation line temperature 250 °C, nebulizing gas flow 180 L.h⁻¹, heating gas flow 600 L.h⁻¹ and drying gas flow 600
152 L.h⁻¹. MRM transitions, dwell times and collision energies were optimized (**Table S3**).

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

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

156 To complete our workflow, we finally designed an enantioselective UPLC-MRM MS strategy. Hence, amino acids
157 enantiomers and nucleobases were separated on a chiral column which was a Crownpak CR (+) column (150 mm × 4.0
158 mm, 5 µm, Chiral Technologies, Europe). In this stationary phase, the chiral separation relies on crown ether as the chiral
159 selector. A guard column Crownpak CR (10 mm × 4.0 mm, 5 µm, Chiral Technologies) was fitted to the analytical column.

160 Elution was carried out at 600 µL.min⁻¹ with water:methanol (95:5) with 0.5 % trifluoroacetic acid (Sigma-Aldrich). UPLC
161 oven temperature was held at 30 °C.

162 TQ MS capillary voltage was set at 4 kV, the interface temperature was held at 400 °C and heat block temperature at 500
163 °C. The desolvation line temperature was held at 250 °C. Nebulizing gas flow, heating gas flow and drying gas flow were
164 set at 180 L.h⁻¹, 600 L.h⁻¹ 600 L.h⁻¹ respectively. Multiple reaction monitoring (MRM) transitions, dwell times and collision
165 energies for all analytes are summarized in **Table S3**.

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

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

170

171 2.4.2. Investigation of the accuracy of the chiral UPLC-MRM MS method

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

177

178 2.4.3. Compounds identification and quantification

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

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

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

188

189 **3. Results and discussion**

190 3.1 Non targeted analysis of meteorite extracts

191 Our analytical development was conducted on extracts of the recently fallen Mukundpura, Aguas Zarcas, Tarda and Kolang
192 meteorites. Whereas amino acids, carboxylic acids and aliphatic amines have been investigated in Mukundpura[14,15] and
193 Aguas Zarcas [16], the organic composition of Tarda and Kolang has not been presented so far. For each of the meteorites,
194 1 g of pristine and freshly collected fragments was extracted following the standard water extraction procedure [14,16]. A
195 2 mL extract was obtained and aliquoted. An aliquot of some tens of microliters was taken per meteorite to complete the
196 workflow.

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

203 Even though samples were not desalted, chromatogram baseline noise was very low (**Fig. 2a**). On the other hand, a chemical
204 background interference was highlighted with a fake peak at 3.8 min corresponding to ammonium formate clusters (m/z
205 63.0320 in both samples and blank). Organic contaminations were also detected in the blank sample that were aligned with
206 resolved peaks in the four extracts (peaks at 1.3 min and 15 min). To map organics in the meteorites, we thus performed a
207 multivariate data analysis (i.e. Correspondence Analysis or CA) after peaks retention time alignment and subtraction of the
208 ions present in the procedural blank. Because of the presence of an asymmetric and noisy peak at 13 min for all the extracts
209 (peak that can be attributed to mineral interferences from the sample matrices), CA contingency table CA was constructed
210 with ions detected until 12 min analysis to avoid any misinterpretation of the data. As shown in the CA plot (**Fig. 2.b**),
211 Mukundpura and Kolang shared close similarities with many small ions having m/z values between 100 to 400 as in Aguas
212 Zarcas (**Fig. S1**). Organics distribution in Tarda was substantially different with ions mass range that was no longer in favor
213 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/ β -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, β -alanine, serine, threonine, proline, valine, isovaline, norvaline, leucine,
230 isoleucine/alloisoleucine, aspartic acid, glutamic acid, tyrosine, γ -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/ β -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.

240

241 3.2 Targeted analysis of free amino acids and nucleobases

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

247 After method development, we tested for the presence of the 31 amino acids and the 5 nucleobases in the four meteorite
248 extracts and the procedural blank (**Table S2**). Thanks to this targeted method analysis, we were able to indicate additional
249 molecules such as 2-aminobutyric acid/2-aminoisobutyric acid in the extracts. On the other hand, we confirmed only 66 %,
250 86 %, 54 % and 65 % of the compounds identified by UPLC-HRMS in Aguas Zarcas, Mukundpura, Kolang and Tarda
251 respectively. Proline, arginine and histidine were detected both in the blank and the extracts, suggesting contaminations in
252 all the samples. Moreover, several identifications such as *N*-ethylglycine, homoserine, threonine, lysine, methionine,
253 tyrosine, thymine, were not validated thus suggesting the presence of isomers having the same exact masses as these
254 compounds in the whole extracts (**Table S2**). These results demonstrate the benefits of a selective MS approach to avoid
255 misinterpretation when bodies of the Solar System are to be studied.

256

257 3.3 Enantioselective targeted analysis

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

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

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

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

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

292

293 **4. Conclusion**

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

302

303 **Sample CRediT author statement**

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

309

310 **Declaration of competing interest**

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

313

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318

319 **Supplemental information**

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

322

323

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Graphical abstract.

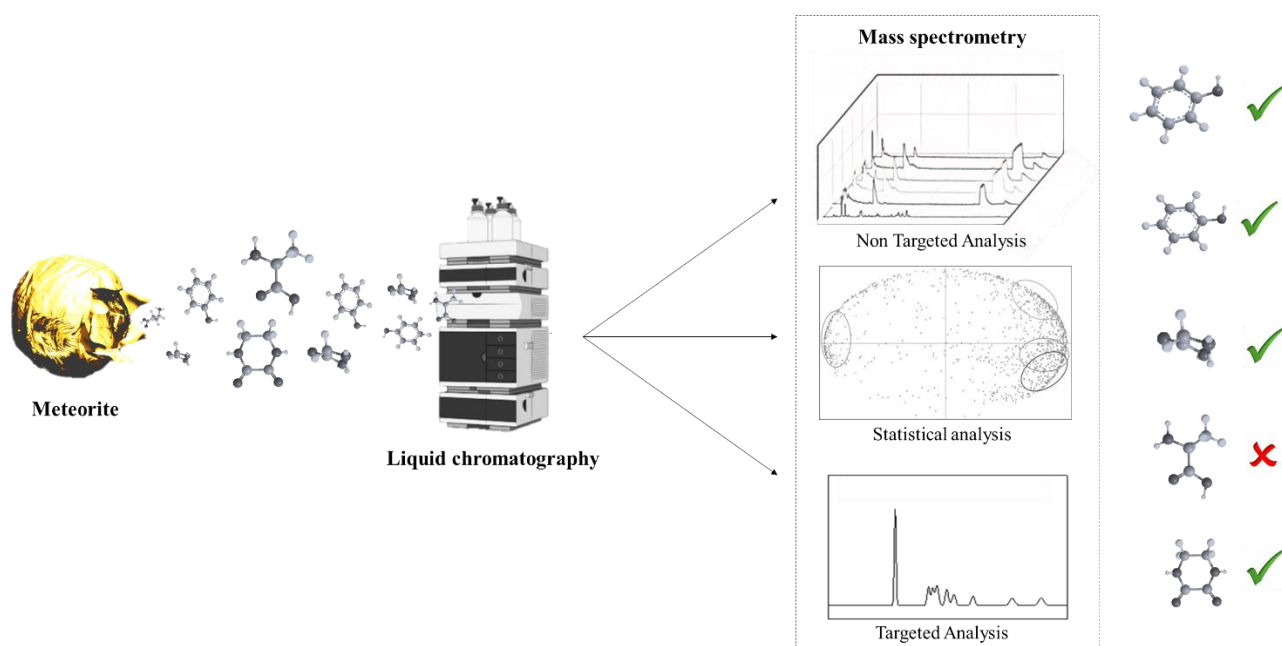


Figure Captions

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/ β -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/ γ -Aminobutyric acid; 8. D-Isovaline/L-Isovaline/D-Valine; 9. L-Valine; 10. D-Norvaline; 11. 5-Aminovaleric acid; 12. L-Norvaline; 13. D- β -Leucine/L- β -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.

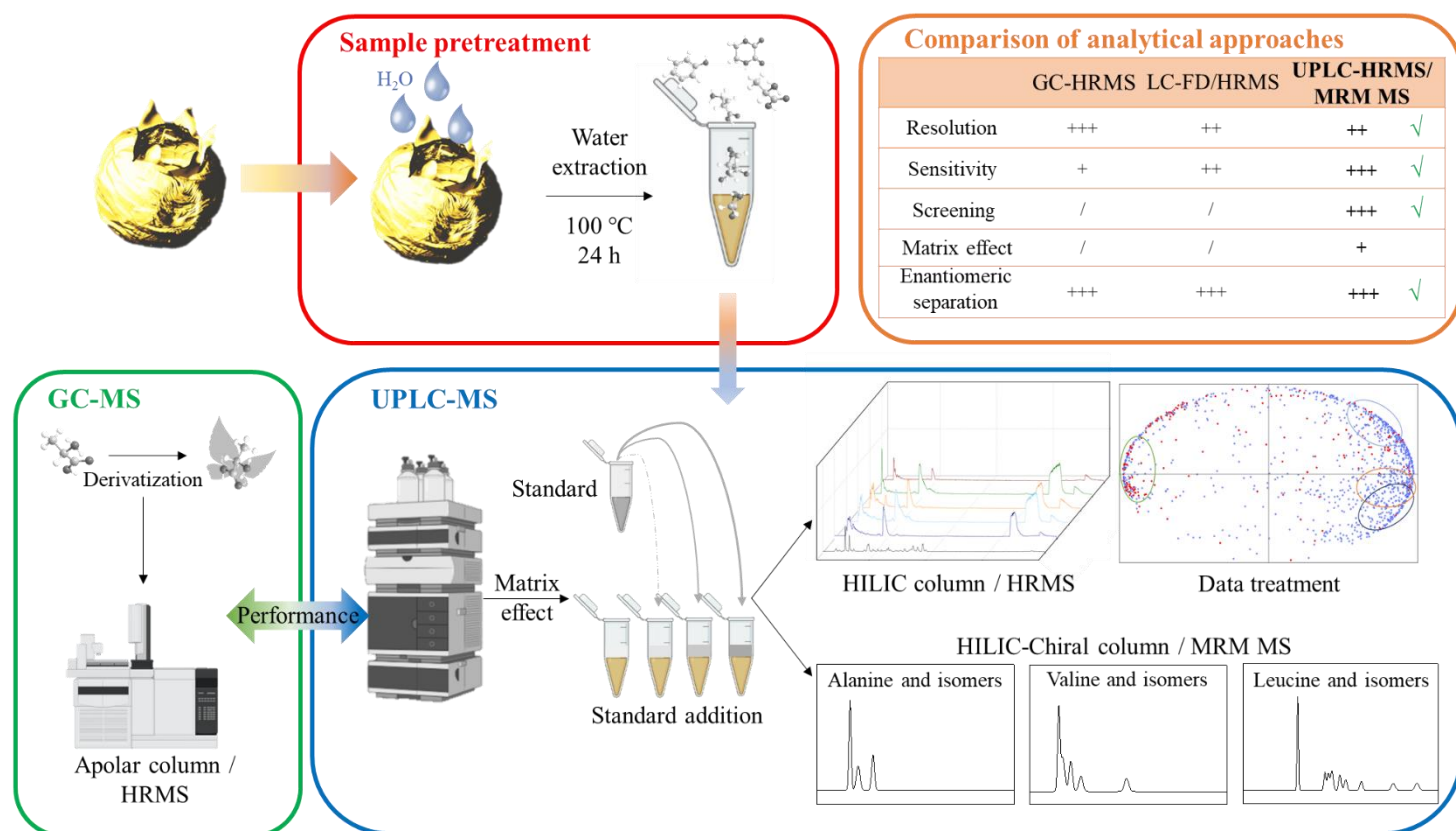


Fig. 2.

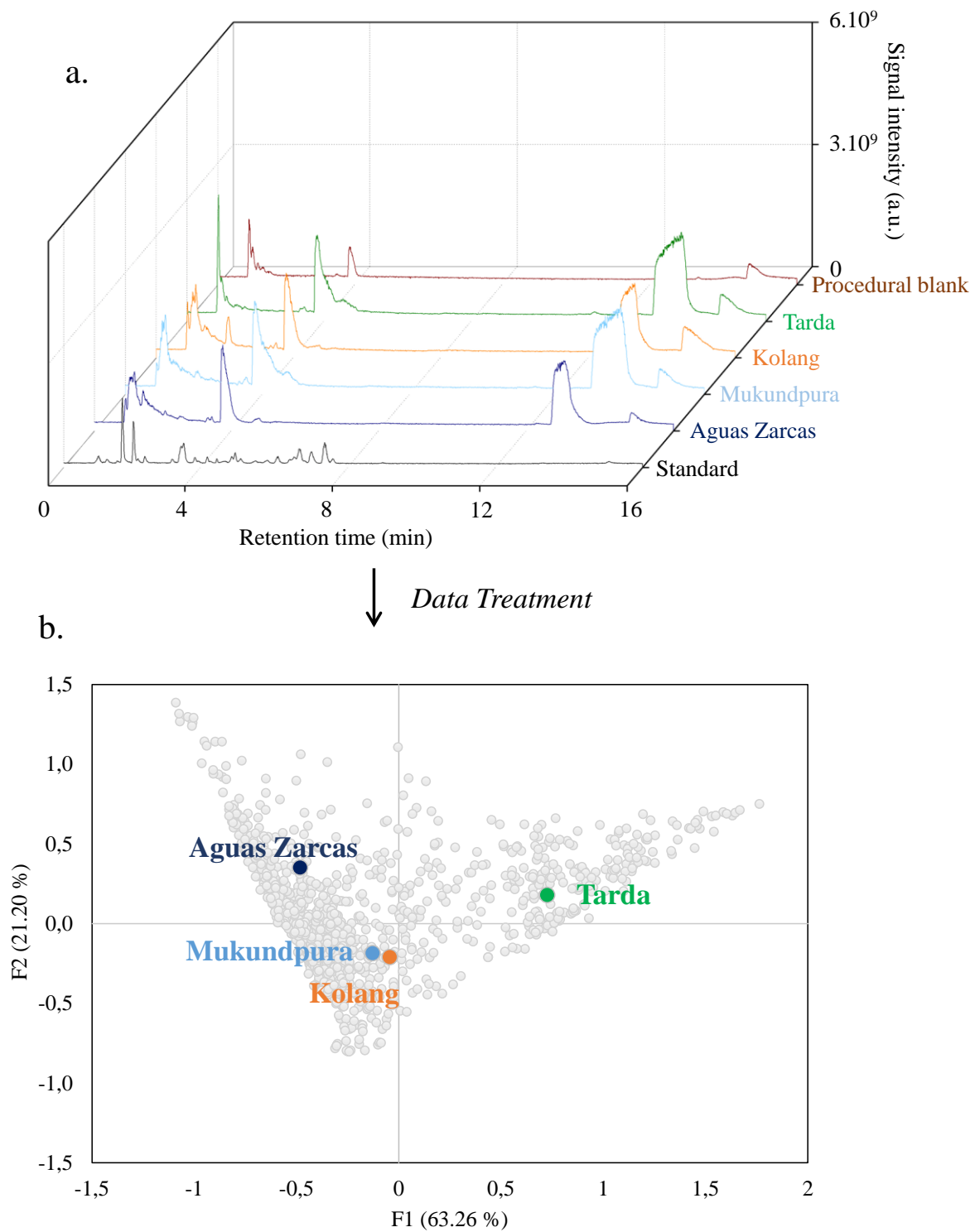


Fig. 3.