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## Highlights

- DMF-DMA fast one-pot derivatization reaction on polar and refractory molecules.
- DMF-DMA enables the molecules' chiral center configuration to be safely preserved.
- Amino acids derivatized by DMF-DMA were analyzed by GC-MS down to a few ppt-ppb.
- More than 70% of DMF-DMA in space capsules will be preserved from space conditions.
- DMF-DMA is slightly sensitive to oxidants and temperature (10% of degradation).

Journal Pre-proof

## Dimethylformamide Dimethyl Acetal Reagent for *in situ* Chiral Analyses of Organic Molecules on Titan with the Dragonfly Mass Spectrometer Space Instrument (*Dragonfly* mission)

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### Abstract

Thanks to the *Cassini-Huygens* space mission between 2004 and 2017, a lot was learned about Titan, the biggest satellite of Saturn, and its intriguing atmosphere, surface, and organic chemistry complexity. However, key questions about the potential for the atmosphere and surface chemistry to produce organic molecules of direct interest for prebiotic chemistry and life did not find an answer. Due to Titan potential as a habitable world, NASA selected the *Dragonfly* space mission to be launched in 2027 to Titan's surface and explore the *Shangri-La* surface region for minimum 3 years. One of the main goals of this mission will be to understand the past and actual abundant prebiotic chemistry on Titan, especially using the Dragonfly Mass Spectrometer (DraMS). Two recently used sample pre-treatments for Gas Chromatography – Mass Spectrometry (GC-MS mode of DraMS) analyses are planned prior analysis to extract refractory organic molecules of interest for prebiotic chemistry and astrobiology. The dimethylformamide dimethylacetal (DMF-DMA) derivatization reaction offers undoubtedly an opportunity to detect biosignatures by volatilizing refractory biological or prebiotic molecules and conserving the chiral carbons' conformation while an enantiomeric excess indicates a chemical feature induced primarily by life (and may be aided on the primitive systems by light polarization). The goal of this study is to investigate the ageing of DMF-DMA in DraMS (and likely MOMA) capsules prior to *in situ* analysis on Titan (or Mars). The main results highlighted by our work on DMF-DMA are first its satisfactory stability for space requirements through time (no significant degradation over a year of storage and less than 30% of lost under thermal stress) to a wide range of temperature (0 °C to 250 °C), or the presence of water and oxidants during the derivatization reaction (between 0 and 10% of DMF-DMA degradation). Moreover, this reagent derivatized very well amines and carboxylic acids in high or trace amounts (ppt to hundreds of ppm), conserving their molecular conformation during the heat at 145 °C for 3 minutes (0 to 4 % in the enantiomeric form change).

**Keywords:** Titan, *Dragonfly* Mass Spectrometer, *in situ* analyses, GC-MS, DMF-DMA reagent robustness and ageing.

## 1. Introduction

### 1.1. Titan, a satellite with potential for prebiotic chemistry

Saturn's moon Titan is a unique exploration target for the investigation of prebiotic chemistry, since it has i) a dense nitrogen and ammonia atmosphere that supports extensive organic chemistry; ii) a solid surface where deposits of organics (analyzed by the landed mass spectrometer) accumulate in dune and inter-dune areas by radar, IR(-Vis) spectroscopy, and surface science package observations; iii) an interior water (and ammonia) ocean under an ice crust; and iv) evidence of past events in which transient liquid water has existed on its surface due to cryovolcanism or impact events (1,2). Much has been learned from previous flyby missions at Titan: *Pioneer 11*, *Voyager 1* and *2*, and most notably *Cassini-Huygens* which spent 13 years in the Saturn system, with over 100 close flybys of Titan (1,3,4). In particular, the *Cassini* orbiter and the atmospheric *Huygens* probe investigated Titan's atmospheric composition and organic chemistry thanks to different instruments mostly based on mass spectrometry (and spectroscopy), such as the Aerosol Collector Pyrolyzer (ACP) experiment linked to a Gas Chromatography – Mass Spectrometer (GC-MS) instrument (and Composite Infrared Spectrometer (CIRS)) (1,5,6). *Cassini-Huygens* had limitations for sampling and detection of such molecules due to the little knowledge scientists had to miniaturize spaceflight mass spectrometer and an absence of heritage to be sent in the outer solar system.

Therefore, the upcoming NASA *Dragonfly* mission addresses questions about the potential for prebiotic chemistry at Titan to address the unsolved *Cassini-Huygens* objectives and arisen questions (7). Indeed, *Dragonfly* will study Titan prebiotic chemistry and the impact of seasons and natural phenomenon on it. *Dragonfly* will be a pioneer and exceptional mission (in terms of technology and analytical approaches) because it is the first rotorcraft lander that will visit multiple locations at Titan's surface in the *Shangri-La* region and the *Selk impact crater* (7,8). Its main goals will be to investigate Titan's prebiotic chemistry, habitability, and search for potential chemical bioindicators (building blocks of life, such as amino acids, fatty acids, etc). To achieve its science goals, the *Dragonfly* scientific payload includes the Dragonfly Mass Spectrometer (DraMS), which features a laser desorption ionization (LD) mode and a gas chromatography (GC) mode connected to an ion trap mass spectrometer (MS).

### 1.2. DraMS Investigation and Processing of Surface Samples

To assess these objectives, a pneumatic sampling system, Dragonfly's Drill for Acquisition of Complex Organics (DrACO) will collect icy-sandy powders for DraMS analysis (9,10). DraMS will then perform measurements of the molecular composition of atmospheric, dune, inter-dune, and water icy samples. These analytical approaches and the DraMS implementation are based on similar instruments developed for surface missions on Mars (11,12), as well as the *Huygens* probe (13).

For GC-MS mode, a sample cup filled with ~ 100-200 mg of surface material will be inserted into an oven where the sample is heated to produce gases from volatile and refractory organic molecules. This analytical step will be performed using either pyrolysis (heating up 600 °C) or employing a wet-chemistry reaction with a derivatization reagent. The chemical derivatizing reagents are stored in capsules that release their contents once the capsules in the oven reach a pre-determined eutectic point (here, 145 °C). The design of the capsules and delivery of the agent to the sample within the oven is based on Mars Organic Molecule Analyzer (MOMA – GC-MS instrument onboard the future ExoMars mission) (12).

Derivatization enables the extraction of specific refractory or polar organic molecules of astrobiological interest detected by GC-MS analysis with a strong heritage from past space applications (14,15). Refractory and/or polar organic compounds, such as amino acids, nucleobases, fatty acids and sugars are not easily volatilizable. Thus, to allow for analysis of these compounds and characterize Titan prebiotic chemistry mechanisms via gas chromatography-mass spectrometry, this additional pre-treatment step (derivatization) need to be investigated. During derivatization, a more labile functional group replaces a polar group on the analyte compounds, raising the molecular volatility and thus allowing the volatile analyte to be entrained in the gas stream. It is currently planned to use dimethylformamide dimethyl acetal (DMF-DMA).

DMF-DMA primarily derivatizes by sequential elimination and addition reactions primary amines and carboxylic acids (Figure 1) (e.g. amino acids that are additionally sensitive to temperatures above 200 °C, nucleobases, sugars). DMF-DMA has also the advantage of preserving the chirality of the targeted molecules that will be separated on the chiral Chirasil-Dex column on DraMS. This can occur in a single derivatization step at 140-145 °C, for three minutes, without degrading the original sample (14). DraMS could thus separate, detect, and quantify organic enantiomers of interest to the astrobiological investigation (16).

### ***1.3. Challenges for using derivatization on DraMS***

There are several challenges to be addressed during the DraMS development to ensure successful use of DMF-DMA derivatization on Titan. First, Titan samples acquired from ice-rich regions may contain a high proportion of water and could potentially contain salts (17). As derivatization reagents are sensitive to the presence of water (18), or salts (19–21), the derivatization efficacy of organic compounds of interest in the analyzed samples may be diminished. Water is a strongly polar molecule, and is typically present in a more significant amount than trace organic molecules, and thus can consume the DMF-DMA derivatization agent (22). For DraMS analyses, it is planned to remove most of the water ice present in the sample prior to proceeding with the derivatization with an evaporation process at 100 °C to prevent water interference during derivatization or pyrolysis process. However, in spite of this sublimation step, water may remain present in amount in the sample due to

hydration of salts (19,20), and adsorption on potential components of the samples. Regarding salts, they increase the ionic force in the aqueous solution, leading to numerous chemical interactions, possibly forming complexes with organics – either with the derivatization reagent or the targeted organic compounds (cage effect in solution below 300 °C (21)) at the high oven temperatures used. Such complex formation would lead to a reduction in the volatilization of the organic molecules as noted in previous studies (21). However, it is not trivial to include a desalting mechanism in the sample processing system due to multiple resource constraints. Therefore, depending on the type of surface from which DrACO will sample, the derivatization of the samples in DraMS may take place with water and salts present.

Therefore, chemical and physical (*e.g.* oxidation and thermal stresses) may happen during storage and cruise prior to the arrival at the target. For example, the derivatization agents need to be loaded into the instrument early in spacecraft integration, thus leading to years-long storage at ambient temperatures. These are warmer conditions than typically commercial advised for these compounds (below 5 °C). During cruise, the temperature and pressure conditions are more optimal, currently estimated to be 2-4 °C and one bar pressure inside the capsule for about seven years. Once landed on Titan, we will perform technical control and Titan sample operational analysis where the different modules and derivatization reagent capsules will be heated up to 60 °C and 150 °C, respectively. Finally, the presence of oxidants (meant as gaseous/liquid oxygen in this study) coming from samples will react first with the reagent that will be degraded before organic molecule derivatization. These environmental constraints might impact the stability and the efficiency of the reagents at the time of use in the planetary environment.

The objective of this article is to address potential concerns about the use of DMF-DMA in an *in situ* spaceflight experiment dedicated to analyze Titan surface samples and demonstrate the usefulness of DMF-DMA derivatization despite the environmental constraints it will see. We have to ensure both that DMF-DMA will reach Titan's surface with the minimum of degradation, and that it will have maximum efficacy once it is released and reacted with organic material inside the DraMS oven. The sub-objectives before Dragonfly launch are: i) The influence of temperature on the DMF-DMA derivatization reagent to evaluate its thermal stability during space cruise and storage; ii) The influence of water and oxidants on the DMF-DMA derivatization efficiency; and iii) The preservation of chirality with regards to the time and temperature of derivatization.

## 2. Material and method

### 2.1. Instrumentation

In this study, experiments were performed with a TRACE GC Ultra Gas Chromatograph (GC) coupled with a DSQ II Quadrupole Mass Spectrometer (Thermo Scientific). Most of the chromatographic analyses were performed with a Zebron ZB-5MSplus capillary column ( $L = 30 \text{ m} \times \text{i.d.} = 0.25 \text{ mm} \times \text{dt} = 0.25 \mu\text{m}$ ) supplied by Phenomenex. This column is capable of separating all amino acids and uses optimized parameters (flux, temperature program) that have been determined in previous studies (14,23). For analyses requiring the separation of organic enantiomers of chiral molecules, a CP Chirasil-Dex capillary column ( $L = 25 \text{ m} \times \text{i.d.} = 0.25 \text{ mm} \times \text{dt} = 0.25 \mu\text{m}$ ) supplied by Agilent was used, again using previously optimized experimental parameters (14). The electronic ionization source energy was 70 eV. The ions produced were analyzed in the  $m/z$  range of 40 to 500 u. Analyses were performed in full scan mode. The transfer line between the chromatograph and the mass spectrometer was set at 300 °C. Helium (purity >99.9995%, Air Liquide) was used as the carrier gas.

The identification of molecules in the GC-MS analyses used comparisons to the National Institute of Standards and Technology (NIST) reference mass spectra library. For each interpretation, we did not take into account matched molecules with a Reversed Search Index (RSI) and Similarity Index (SI) lower than 700. These similarity index and inverse research corresponding factors compare the unknown mass spectrum's GC peaks to those in the NIST library spectra with a scale from 0 to 1000 (based on the correspondence in  $m/z$  value and relative intensity). DMF-DMA was always used in excess compared to amino acids (at least 10 molecules of DMF-DMA for each functional group with labile hydrogens). The analysis of an amino acid mixture derivatized by a fresh DMF-DMA solution helped optimize the GC parameters for amino acid detection and enantiomer separations (ramp temperature for the GC program, retention time with full separation of all compounds, etc.). Then, different tests on DMF-DMA were conducted to assess the potential degradations resulting from different stressors (temperature, oxidation, water, etc.). Therefore, we validated the derivatization efficiency of the remaining percentage of non-degraded DMF-DMA by quantifying the derivatized amino acid mix with the same concentrations as for the control. Comparisons will be made of the retention times, shape, and area of the peaks between the treated DMF-DMA and the reference experiments.

Injections were done through an Optic 4 multimode GC inlet Injector (GL Science). The injections were done in the split mode with a 1:25 ratio. The injector was heated at 270 °C. For both chromatographic columns used, the temperature program started at 70 °C, held for 3 min, and then heated up to 190 °C at 3 °C  $\text{min}^{-1}$ . At 190 °C, the isotherm was held for 5 min. The carrier gas flow rate was set constant to 1.2  $\text{mL min}^{-1}$  in the GC column.



For water titration, we conducted Karl Fischer titration (Radiometer analytica) while we injected 1  $\mu\text{L}$  of pure water for calibration or our samples in a hydranal (Fluka) and we repeated these experiments 6 times for quantification.

## 2.2. Reagents

Derivatization on targeted organic molecules was performed using DMF-DMA reagent from *Sigma-Aldrich* that we will use in spaceflight missions (>95% purity). DMF-DMA is also a solvent. It degrades through time into DMF, which is inert, solvent for organic molecules.

For the characterization of DMF-DMA efficiency, amino acid standards were used as the target of the DMF-DMA derivatization: D-threonine (99%, Fluka), L-2-aminobutyric acid (99%, Fluka), L-alanine (99%, Fluka), glycine (98%, Aldrich), D-valine (99%, Fluka), D-aspartic acid (99%, Fluka), L-aspartic acid (98%, Aldrich), L-cysteine (99,5%, Fluka), and D-phenylalanine (99%, Fluka). We chose nine pure L or D amino acids to observe the conservation of chirality. For aspartic acid, we used both enantiomeric forms to test the enantiomeric separation with the Chirasil-Dex capillary column to be used in DraMS. We selected these amino acids to have the widest range of MS response and types of functions that are derivatized by DMF-DMA to test its efficiency under recommended and space storage conditions. Indeed, threonine and cysteine have a low MS response, on the opposite of phenylalanine, alanine, glycine, and valine. The amino acid that has an intermediate MS response is aspartic acid, which is also the reason we chose it for énantió-separation experiments. L-2-aminobutyric acid is well ionized in the MS source and represent an amino acid that is not essential for life or often found in meteorites. Phenylalanine was chosen for water and oxidative experiments because its response in MS is high compared to all amino acids, is found in extraterrestrial objects, and is essential in prebiotic chemistry (24) as one of the amino acid formed for proteins by primitive cells on Earth (25). Finally, we chose amino for three reasons: i) they represent a model for all organic molecules since they have a carboxylic acid and a primary amine that are the two functions derivatized by DMF-DMA, ii) amino acids represent a bioindicator we are looking for on every extraterrestrial of life to understand prebiotic chemistry with the production of proteins (where metabolisms may have appeared before DNA/RNA for the primitive life) we expect to find on Titan (24,26), and iii) because on Earth the levogyre form has been selected by life on proteinic amino acids and represent via its homochiral character a biosignature that cannot produced on large scale by light polarization since the right and left polarized lights reach the upper atmosphere of Titan. Each enantiomer was diluted into pure milli-Q grade water (TOC lower than 3 ppb) to prepare each amino acid solution. The concentration of each amino acid solution and the mixture used in this study are given in Supplementary Material 1 (Table A). Supplementary Material 1 (Table B) lists the chemical structures of derivatized or non-derivatized amino acids. Methyl Laurate (97% purity, Fluka) diluted into pure ethyl acetate (99.9% purity, Sigma-Aldrich) was used as an internal standard for quantitative comparison and to determine the recovery yield for every amino acid standard.

Methyl laurate is not derivatized by DMF-DMA, and is not impacted by the chemical process of the derivatization nor is it thermally degraded when heated to 145 °C for 3 minutes.

To quantify the organic recovery and test the reagents' efficiency to each organic, we measured the peak area of each organic molecule in the chromatograms, then divided it by the peak area of the internal standard (IS, or methyl laurate) using Thermo Scientific XCalibur software.

### 2.3. Method applied for the different goals of each experiment

To address the different *Dragonfly* environmental concerns, we conducted experiments under DraMS-like conditions after subjecting the DMF-DMA reagent to relevant conditions for its storage prior its used on Titan and during operational conditions on Titan.

First, we investigated the possible impact of temperature on DMF-DMA. We injected a fresh solution of DMF-DMA into the GC-MS to observe the possible contaminants present in the commercial bottle (purity of 95%). We thus determine whether there are contaminants available to be derivatized or react with DMF-DMA and therefore potentially decreasing the DMF-DMA availability for organic derivatization, or whether contaminants would be significant enough to increase the background and/or coelute with targeted organics.

The fresh DMF-DMA injection was then compared with a DMF-DMA solution heated at 145 °C for 3 minutes (optimal temperature and time for space applications, but also mimicking the heating process of DraMS capsules prior to the eutectic opening) (14). This pre-heated DMF-DMA revealed whether possible trace contaminants in the commercial bottle are subject to the derivatization process. This experiment was carried under either Earth atmosphere or pure nitrogen atmosphere in order to test the impact of oxidants during the derivatization process.

Next, we studied the thermal stability of DMF-DMA through a range of temperatures. Individual aliquots of DMF-DMA (1 mL) were heated at 125 °C, 150 °C, 200 °C, or 250 °C for 2 h; as well as at 200 °C for 12 h or 25 h (temperatures and times of sterilization for spaceflight missions and instruments). After heating, 20 µL of the heated DMF-DMA aliquots was used to derivatize 20 µL of an evaporated amino acid mixture at 145 °C for 3 minutes. We performed controls with a fresh DMF-DMA solution to compare with the different heated DMF-DMA solutions following the same derivatization and GC-MS protocols.

The degradation at ambient temperature, in presence of oxidants, was studied next, mimicking the storage condition in capsules before the launch. DMF-DMA could possibly be oxidized or chemically degraded due to the oxygen present in the atmosphere. The DMF-DMA commercial storage is under air (21% O<sub>2</sub>, and 78% N<sub>2</sub>), or under an inert atmosphere at ambient temperature once introduced into the capsules. This storage step at about 22.5 °C could impact DMF-DMA efficiency for derivatizing organics once *in situ* analyses begin on Titan. This ageing study has been carried out over a year to

observe the possible chemical degradation of DMF-DMA, and results can be extrapolated to the relevant timelines, in which capsules are filled up with reagents one to four years before the launch.

Third, we examined the impact of water on DMF-DMA derivatization. On Titan, water will be present in high abundances in icy or sandy samples (more than 50-90 % in sample volume), and water can be derivatized by DMF-DMA. The aim is to identify a minimum value of water present in samples that is acceptable for spaceflight applications, and in particular the quantity of water that still allows for a full derivatization of the analyte organics that are in the same sample.

Each of these tests with different pre-treatments of DMF-DMA solutions have been conducted with a pure DMF-DMA injection into the GC-MS, and an injection of amino acids derivatized with the different DMF-DMA solutions. Finally, the DMF-DMA is used in spaceflight applications for detecting chiral compounds. So, in addition to derivatization efficiencies we studied the effect of these different pre-treatment on DMF-DMA or derivatization steps on the conservation of the chirality of amino acids after undergoing the derivatization step at 145 °C – 3 minutes.

### **3. Results and discussion**

#### **3.1. Thermal effects on DMF-DMA stability and efficiency**

##### **3.1.1. Degradation pattern and mechanisms of DMF-DMA**

We reported the list of detected by-products (from the six replicated DMF-DMA samples) in Table 1 to note the difference between an unheated and heated (derivatization process at 145 °C for 3 minutes) and the material and method associated to the experiment in Supplementary material 2. The same by-products are observed for the samples stored and heated in the Earth atmosphere and in the pure N<sub>2</sub> atmosphere. Yet, a different quantity of remaining DMF-DMA was measured: 5% loss when heated in a N<sub>2</sub> atmosphere, and 15-20% with the same process under Earth atmosphere. We examined whether this loss of DMF-DMA is acceptable or not for derivatizing abundant organics in standard amino acid samples. The results are presented in Supplementary Material 3 (Figure 1S).

After a year of storage, we did not see any qualitative or quantitative differences in the nature of the degradation products for DMF-DMA, whether stored under laboratory atmosphere, pure N<sub>2</sub>, or with the control. Moreover, the DMF-DMA samples stored at the three different conditions all showed the same derivatization efficiency. This was true for both heated and unheated samples (Table 1).

In Table 1 and Figure 2, it is shown that DMF-DMA is degraded primarily to nitrogen-bearing molecules such as dimethylformamide (DMF), or N,N,N',N'-tetramethyl-methanedi-amine, and to oxygen-bearing molecules, such as methyl ester or acetaldehyde. Figure 2 provides likely chemical schemes for the formation of the observed by-products. It is important to note that these compounds

may interfere with the detection of the molecules of interest that are indigenous to the samples. Thus, this study provides important constraints on the contribution of signal from the degradation of the derivatization agent to compare to what may be observed in a sample. If a compound could originate from both the sample and the derivatization agent, the quantitative study relative to the GC-MS signal over noise (S/N) ratio will estimate the expected contribution coming from DMF-DMA so that one can deduce the amount of the species present in or released by the sample (Table 1).

It is noted that the derivatized forms of DMF-DMA by-products (*i.e.* methyl carbamate dimethylformaminated, dimethylformamidinated pentamine, and dimethylformamidinated hexylamine) are found among the detected compounds, showing that DMF-DMA can be consumed by its own by-products. However, the signal produced by these compounds is close to the detection limit, and so this process will be negligible compared to other reactions degrading or consuming DMF-DMA. Thus, while these products will increase the background level and the limit of detection (LOD) of each organic molecule, they are not a threat to ability to detect the organic compounds on Titan.

Thirteen new degradation products are observed after DMF-DMA was stored for one week at ambient temperature and following pre-heating, as compared to fresh, unheated DMF-DMA injection. No new products are observed after a year under the same conditions, thus there is a short time threshold for degradation. Again, the degradation does not significantly impact the available volume of DMF-DMA to derivatize organics after storage, as at least 70 to 90% of the original amount of DMF-DMA remains. This can be accounted for in the preparation of derivatization capsules.

### 3.1.2. Efficiency of DMF-DMA reagent after a year of storage under laboratory atmosphere

Of a greater potential concern is a reduction in the derivatization efficiency of chiral targeted molecules (*e.g.* amino acids) by the DMF-DMA solutions stored under the different conditions. Here we derivatized the mixture of pure amino acids using the DMF-DMA stored at ambient temperature under laboratory atmosphere for different durations. Figure 3 shows the results when comparing the relative peak area measured for amino acids to the internal standard for solutions stored for up to 1 year (52 weeks). We observed few variations in peak area as compared to the fresh DMF-DMA solution. Most yields show variations of around 10%, except cysteine (L-Cys), phenylalanine (D-Phe), and aspartic acid (Asp Acid) for which the variation is on the order of 20% when compared to the fresh DMF-DMA solution. The derivatization is compound-specific (as is the MS sensitivity), but also shows a dependence with temperature and time. That said, few of the studied amino acids show a consistent trend in yield with time. Therefore, neither the variability in yield over time or the storage and derivatization conditions influence significantly the amino acid derivatization. The remaining percentage of reagent is sufficient and the DMF-DMA by-products are inert towards the derivatized organics. Aspartic acid and cysteine both show the lowest yields as compared with the initial quantity in contact with DMF-DMA (down to 65%), which is consistent with the higher standard deviations for

these species as noted in previous studies (14,22,27) explained by their low MS ionization and chemical instability.

We thus conclude that DMF-DMA can be stored under ambient air in capsules for at least a year, without any observable degradation (qualitatively on the diversity of by-products or quantitatively on their respective derivatization yields). These results can be extrapolated for three years in the case of DraMS with no significant degradation compared to a year at standard conditions ( $P=1$  bar,  $T=22.5$  °C) because the DMF and other DMF-DMA by-products are inert in the solution, and after few weeks at ambient temperature any substantial oxidative species remain in the solution or in the atmosphere in equilibrium with the solution due to their reactivity with DMF-DMA molecules or by-products. Without the addition of energy by heating the solutions or the reaction with non-indigenous metallic inorganic or oxidative organic species, we strengthen the extrapolation even up to seven years corresponding to the *Dragonfly* cruise (considering a temperature below 40 °C). However, it is advised to store and conduct the heated derivatization process under inert atmosphere, since up to 20% of the initial DMF-DMA was observed to be lost to oxidants (such as  $O_2$ ,  $O_3$ ,  $H_2O$ ) prior to the interaction with organics in the sample oven if performed under laboratory or oxidative atmosphere (Supplementary Material 3 – Figure 1S).

A final study on the thermal stressors for DMF-DMA was conducted using a range of temperatures higher than the derivatization heating process, with varying times of exposure. The intent was to simulate possible sterilization steps (not planned for *Dragonfly*, but conducted for missions of interest for astrobiology as *ExoMars* or future missions that can perform *Viking* harsh sterilization conditions). The results from this study are provided in Supplementary Material 4 (Figures 2S and 3S). We concluded that more than 60% to 70% of the original amount of DMF-DMA remains available to derivatize organic molecules regardless of the temperature (115-250 °C) or the duration (2-25 h) of the exposure prior to the derivatization of amino acids.

Thus, neither the sterilization step, nor the capsule storage is expected to significantly degrade the DMF-DMA reagent for the *Dragonfly* mission. An excess of reagent will remain for derivatizing organics present in the sample in high abundance (mmol of organics can interact with the 70-90% of DMF-DMA purity).

### 3.2. Oxidation effects of water on DMF-DMA

#### 3.2.1. Quantification of water in different DraMS samples

When derivatizing with DMF-DMA, the presence of water in the reactive medium can have a significant influence on the detection and quantification of organic molecules present in low abundance. The -OH group in water is able to be derivatized, and thus it can consume some of the DMF-DMA to the detriment of the organic molecules present. Moreover, water usually increases the GC-MS signal background level and degradation of the stationary phase, forming a column-bleeding baseline on GC

chromatograms that can saturate the MS detector. Finally, water can interact with derivatized organics and in some cases reconvert them into their underivatized form. As water is expected to be present in samples collected in extraterrestrial environments, particularly at icy moons including Titan's surface, we investigated the magnitude of the influence of water on the derivatization process. The goal was to determine to what extent water can impact the derivatization yield of other targeted molecules. Water that is present in the derivatization vials and reaction environments may have multiple sources. In the case of the DraMS investigation, the primary sources of water in the reactive medium would be water present in DMF-DMA reagent itself as well as water ice in the surface samples collected on Titan.

In order to assess i) the influence of the amount of water on the DMF-DMA derivatization of amino acids, ii) the influence of water on DMF-DMA storage, and iii) the kinetic factors from the water and DMF-DMA reaction (Figure 4), we quantified the water content using the coulometric Karl Fischer titrations (28) (Figure 5). Karl Fischer allowed the calibration curve of water by the injection of different quantity of pure water we compared with our unknown water quantity in DMF-DMA samples. To validate the results from the Karl Fischer Titration and quantify methanol (coming from the water derivatization), we conducted GC-MS analyses. These quantifications of water and/or methanol are based on calibration curves (of water and methanol) in Karl Fischer and/or GC-MS analyses (see Figure 5 and Supplementary Material 5 – Figures 4S and 5S) and compared to the quantity of water and/or methanol in the different solutions (six replicates have been used for these tests). We thus quantified the amount in a pure water, water and DMF-DMA solution, or a water–amino acids–DMF-DMA solution to estimate the content in fresh before and after storage or derivatization process. For the latter process, we first looked at pure water derivatization to generate the reaction yield and equilibrium constant  $K_1$  of the reaction in Figure 4. We then compared the result with pure water derivatization to that of an aqueous amino acid solution; this served to simulate the efficacy of DMF-DMA to detect amino acids in Titan water-rich samples and to estimate the amount of water that is derivatized at the same time as the amino acids. These results aid in determining the threshold of the organic/water ratio for a successful DMF-DMA derivatization of all organic compounds despite the presence of a high amount of water.

First, to quantify the influence of water on DMF-DMA derivatization, we measured the amount of water in several solutions using Karl Fischer titrations on: fresh DMF-DMA commercial solution, processed DMF-DMA, and derivatized amino acid standard mixture (performed under laboratory atmosphere). The results revealed less than 4-5% of water in fresh DMF-DMA bottle in terms of  $n_{\text{water}}/n_{\text{DMF-DMA}}$ , and around 2% in aged solutions (3-year-old opened laboratory bottles, stored at 5 °C), in agreement with the water quantity calculations using GC-MS calibration curve (and the quantified 2-3% derivatized). Therefore, a storage at 5 °C advised by commercials will not degrade DMF-DMA or add water. However, the content of water increases during the derivatization heating process with a minimum of 10% of water (for pure DMF-DMA or DMF-DMA mixed with amino acids to be derivatized) due to vapor water solubilization. It has been confirmed by GC-MS analysis after Karl

Fischer analysis (Figure 5 and Table C in Supplementary Material 5). Within the GC-MS analysis, we observed the increase in methanol production. Indeed, methanol is produced as by-product during a derivatization. Additionally, DMF-DMA is able to derivatize water which transforms into methanol as we observed in the commercial solutions. On ocean worlds, water is present at more than 10 wt% in samples, it is thus primordial to remove the excess of water before DMF-DMA derivatization because DMF-DMA will interact with the most abundant molecules and water will generally be the solvent. In consequences, organic molecules of interests for prebiotic chemistry and astrobiology purposes might not be derivatized if DMF-DMA is totally consumed by water.

### 3.2.2. Water derivatization by DMF-DMA

Next, studies were performed to estimate the maximum amount of water that could be tolerated for DraMS analyses. According to Titan current data from *Cassini-Huygens* analyses, we assumed that samples will be rich in organic compounds (mmol or 0.1-10% level) (4) that need to be derivatized by 20-60  $\mu\text{L}$  of DMF-DMA (carried within the instrument per sample cup). Both GC-MS and Karl Fischer titration tests were correlated to determine the yield of the water derivatization and obtain water and methanol calibration curves (Figures 5 and 4S in Supplementary Material 5). These calibration curves helped to estimate the equilibrium constant  $K_1$ , for the derivatization of water into the methanol product, where we selected a high abundance of water ( $\sim 12\%$  relative to DMF-DMA), resulting in the synthesis of  $\sim 12\%$  of MeOH (relative to DMF-DMA).

The quantity of methanol plateaus as thermodynamic equilibrium is reached at  $\sim 12\%$ . This is in agreement with the reaction scheme (Figures. 2 and 4) and the equilibrium constant (extracted from Figure 4). The formation of dimethyl ether via Reaction 2 (Figure 4) is the likely explanation for the stagnation in the methanol production, as such a plateau would occur when the system reaches equilibrium between methanol formation in Reaction 1 and its consumption in Reaction 2. Unfortunately, it is not possible to confirm the rate of Reaction 2 using the GC-MS technique in this study, as the chromatographic conditions and the column used for the test do not allow for the quantitative detection of the apolar dimethyl ether component. Thus, no value has been measured or calculated in the case of the derivatization of methanol into dimethyl ether and its associated rate constant  $K_2$ .

The GC-MS calibration curves for DMF, DMF-DMA, and methanol (Figures 4S in Supplementary Material 5) provide calibration equations and the equilibrium and reaction constants for the derivatization of water (Reaction 1, Figure 4). We obtained the following calibration curve equations: for DMF-DMA,  $y = 1.10^6x - 2.10^8$  ( $R^2=0.99$ ); for DMF,  $y = 1.10^6x + 8.10^8$  ( $R^2= 0.95$ ); and for methanol,  $y = 3.10^6x + 5.10^7$  ( $R^2=0.99$ ), where  $x$  is the volume of water injected in  $\mu\text{L}$  and  $y$  the quantity of water in  $\mu\text{mol}$ . Applying these calibration curves to the results from a water derivatization experiment analyzed by GC-MS (mass range 28 - 550  $m/z$ , run in six replicates), the following equilibrium constant was calculated:

$$K_1 = \frac{[DMF]*2[MeOH]}{[H_2O]}, \text{ where DMF-DMA is in excess.}$$

$$K_1 = 3672.91 \text{ mol}^2$$

From this value, we determined the final ratio of water/DMF-DMA that is acceptable for space use and for detecting 1 mmol of organics, which is 0.1.

Second, in Figures 6 and 5S, we introduced water and phenylalanine prior derivatization varying the quantity of water. At low percentages of water (water/DMF-DMA ratio below 0.75), we did not observe any significant influence on the available quantity of DMF-DMA to derivatize the polar amino acids in the sample expected within DraMS cups. Moreover, the ratio  $[DMF]/(\text{initial } [DMF-DMA])$  gets a maximum of 35% due to the initial quantity of impurities (mostly water in the commercial bottle and the sealed Earth-atmosphere) that interact with DMF-DMA and produce DMF. However, while 100% of the initial quantity of DMF-DMA (equivalent to 0.15 mmol) is transformed into DMF, 70-75% of the DMF production is explained by the additional quantity of water we introduced prior the reaction and analysis (Supplementary Material 5 – Figure 5S). The DMF is primarily explained by the amino acid derivatization ( $\mu\text{mol}$  of organics) and in fewer percentage (5-7%) by water derivatization. After derivatizing nmol of phenylalanine, more than 65% of DMF-DMA remain available for further derivatization on additional organics.

### 3.2.3. Efficiency of DMF-DMA to derivatize organic matter in presence of water

Third, one of the aims was to understand whether water can directly influence the derivatization of organics (*e.g.* amino acids), in addition to the indirect effect of reducing the available DMF-DMA available for organic derivatization. Figure 6 shows results from the derivatization of phenylalanine with the addition of water at 0 to 0.33 molar ratio (relative to DMF-DMA amount). A ~90% derivatization yield is achieved at ratios of water/DMF-DMA up to 0.74. This yield drops quickly as water is increased not due to influence of water and methanol by-product on the reverse derivatization reaction, but most likely due to the water derivatization happening before phenylalanine because the quantity of water is more than ten times higher than the quantity of phenylalanine. At a 1.11 molar ratio of water/DMF-DMA, in which the amount of water is slightly higher than the amount of DMF-DMA (0.15 mmol), the derivatized phenylalanine (0.15  $\mu\text{mol}$  initially dissolved in solution) is detected only at trace levels (0.13 nmol). This result is explained by the DMF-DMA no longer being in excess, and so it is preferentially reacting with the excess of water. This result and an equivalent result where we added mmol of phenylalanine and mmol of water (Figure 6S in Supplementary Material 6) revealed that DMF-DMA reacts equally with amino acids that with water. However, in a solution where one organic is in excess, DMF-DMA will spontaneously react with the most abundant organic compound due to a higher probability of interaction with it. This affirmation is also confirmed by the absence of detection of a partially derivatized phenylalanine volatile form. Thus, the remaining phenylalanine was at 1 ppt



derivatized, and the remaining phenylalanine that did not interact at all with DMF-DMA is still in solution and can not be volatilized, thus not detected in GC-MS.

From the results with phenylalanine, it does not appear that water significantly interferes with the derivatization up to 0.74 molar ratio (or 2  $\mu\text{L}$  of water), which represents a maximum for the quantity of water introduced in the DraMS instrument cups after passing the water trap (and evaporation process at 100 °C prior to the DMF-DMA release on Titan samples equivalent to less than 1% of the initial water amount) for ~0.29 mmol of DMF-DMA (40  $\mu\text{L}$ ). Yet it is clear from the data in Figure 6 that the presence of water has a significant indirect effect, by preferentially reacting with the reagent such that there is no longer enough available to derivatize the target molecule.

The amount of water that may be present in the DMF-DMA solutions within the pre-loaded capsules can thus have an effect on the derivatization process. However, we have shown that there is not a high quantity of water in the commercial DMF-DMA solution, and thus the agent itself does not represent a significant threat to the efficient derivatization of amino acids. If possible, it would be preferable to evaporate the water just after the loading of the capsules on Earth before the flight to further minimize this contribution using a water trap. The largest threat is thus from water present in the acquired ice samples if present at similar molar quantities to the derivatization reagent. Thus for these *in situ* missions it will be necessary to use sample desiccation and water traps to minimize the water in the sample prior to releasing the derivatization agent from the capsules.

### 3.3. DMF-DMA extraction efficiency for enantiomers GC separation and MS detection

The purpose of using DMF-DMA in space experiments is its ability to derivatize carboxylic acid and amine functional groups at low-mid temperatures (below 300 °C) while conserving the chirality of the molecule during the reaction, all in a straightforward one-pot reaction (protocol presented in Supplementary Material 2). The DMF-DMA derivatization is compatible with the measurement of potential enantiomeric excess in chiral molecules, which is considered a relevant and high priority measurement as homochirality is a potential biomarker. For calculating the enantiomeric excess, the following equation is used:

$$Ee = 100 \cdot \frac{AAM - AAm}{AAM + AAm},$$

where *AAM*=the homochiral form in excess (in our case the injected one), and *AAm*=the enantiomeric form in minority.

Figures 7 and 7S (in Supplementary Material 7) shows a significant increase of the recovery yield for all the tested amino acids when the temperature or the time of derivatization increases. We also observed for cysteine and phenylalanine that both enantiomers are detected, especially after derivatization at the higher temperature and longer duration (145 °C for 10 minutes). However, it should

be noted that the enantiomeric excess observed is still higher than 97% for the injected pure form, and so the derivatization process only contributes a small amount of uncertainty on the Ee value. Indeed, this would fall within the  $\pm 5\%$  accuracy requirement for an ee measurement as laid out by the Europa Lander Science Definition Team Report (29). Therefore, DMF-DMA fulfills its role of derivatization reagent conserving the chiral conformation of organics even during a heating process (145 °C for 3 minutes) which would generally racemize molecules. For space flight applications, a shorter time of derivatization is favorable, so the recommended procedure of heating for 3 minutes at 145 °C will be maintained, as shown in previous papers (14). Moreover, no differences were noted using 20 or 30  $\mu\text{L}$  of DMF-DMA to derivatize amino acids.

This fast one-pot method revealed similar or better derivatization yield (in presence or absence of oxidants) compared to classical laboratory methods with the advantage to be conducted in 3 minutes at 145 °C for derivatization without modifying the chiral carbon conformation. In addition, DMF-DMA has been primarily selection because it does not induce racemization proving the efficiency of this method for biosignature detection with a likely enantiomeric excess in terrestrial and extraterrestrial samples. In laboratory, the method does not present disadvantage on the opposite of space conditions detailed in (30).

#### 4. Conclusions

Through this stability study, we have addressed concerns regarding the efficacy of DMF-DMA as a derivatization reagent through pre-launch and 7-year cruise requirements for a Titan mission. Within all tested conditions, designed to simulate the prelaunch and *in situ* space conditions during cruise, no significant influence on the efficiency of DMF-DMA was observed. It was shown that at least 80% of DMF-DMA would remain for derivatizing mmol of organics after arrival at Titan, given that there is not a pre-launch sterilization step planned for *Dragonfly*. The “aged” DMF-DMA showed that it could meet all performance requirements including the derivatization and separation of enantiomers, qualitative performance (GC parameters, such as peak shapes) and quantitative performance (efficacy as compared to a fresh DMF-DMA solution derivatization analyzes). However, few by-products of DMF-DMA represent biosignatures (*e.g.* methylated urea, dimethylformated urea), thus it will be primordial to consider these by-products to interpret the prebiotic chemistry on Titan or potential traces of life on Mars.

Water is preferentially derivatized by the DMF-DMA reagent, but the low temperature evaporation step planned for icy samples on DraMS will minimize water contamination during derivatization pre-treatment. All DMF-DMA by-products, whether related to the presence of water in the sample or not, elute early enough that the introduction into the mass spectrometer could be avoided by delaying the MS scanning by a few minutes after trap activation. Such a delay (or “cut off”) would not prohibit the detection of the primary astrobiological products of interests for DraMS as those would have later

retention times and can be anticipated following extensive testing on the breadboard and engineering models. In this case, alternate approaches, such as pyrolysis without derivatization, would be used to identify short and volatile compounds.

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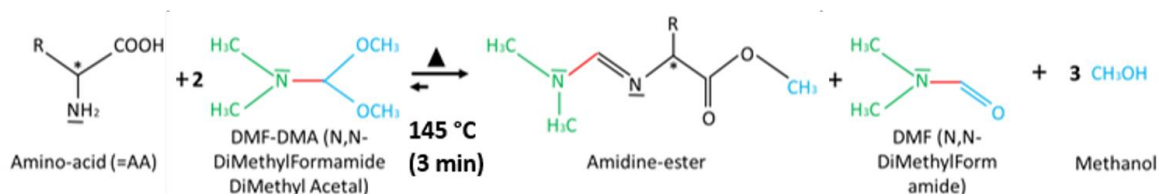
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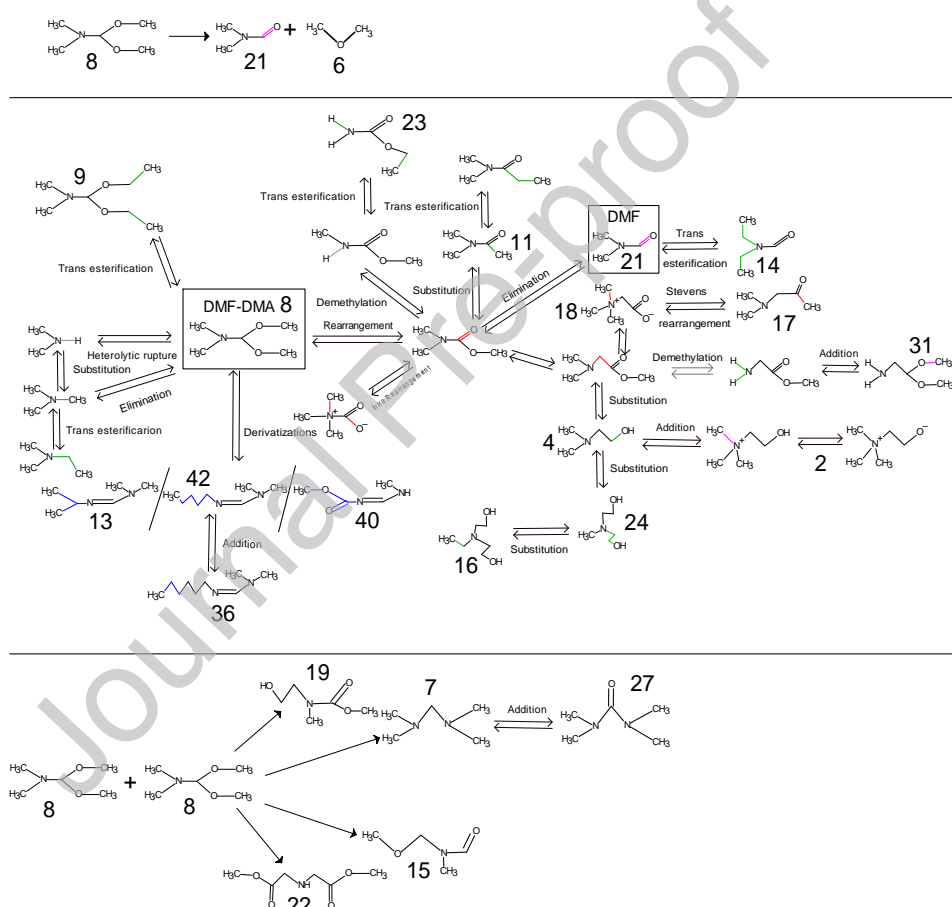
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## Figures in the article body:

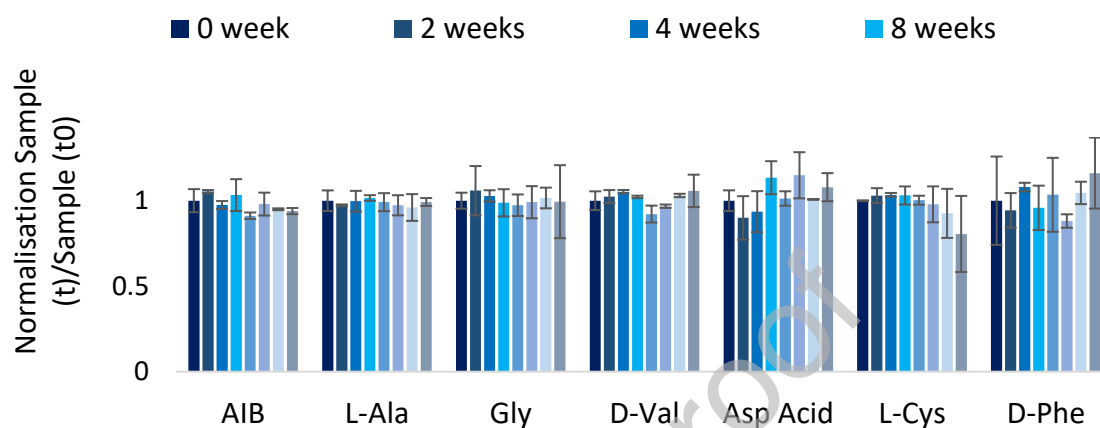


**Figure 1.** Derivatization reaction of amino-acids by DMF-DMA (145 °C – 3 min) (14) that can be reversed, but in very low yield of reaction (and never observed in the present experiments).

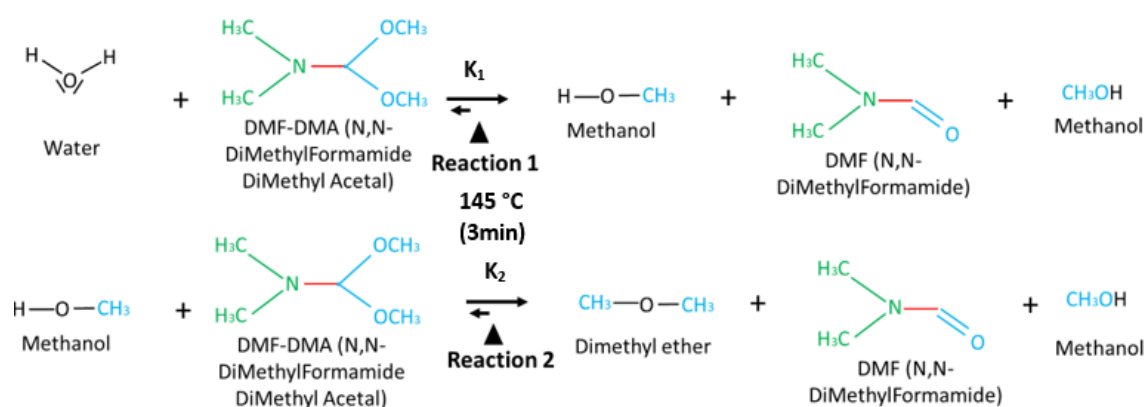


**Figure 2.** Proposed mechanisms for the thermal degradation of the DMF-DMA reagent at temperatures up to 300 °C. These include Hoffman elimination, side chain elimination, random chain scission, heterolytic rupture, interactions between DMF-DMA molecules, or random substitutions of ethyl and ethanol groups. The molecule numbers correspond to those presented in Table 1. Transesterification proposed reaction is explained by the presence of ethanol in the DMF-DMA impurities. These reactions of transesterification could be explained by rearrangement or addition even if these two mechanisms are less probable. The bottom mechanisms implies multiple reactions to produce 7, 15, 19, 22, and 27,

however the intermediate products were not detected in GC-MS, thus we can not conclude on their complete production mechanisms. By-products list is longer in other experimental DraMS conditions presented in details in (30).

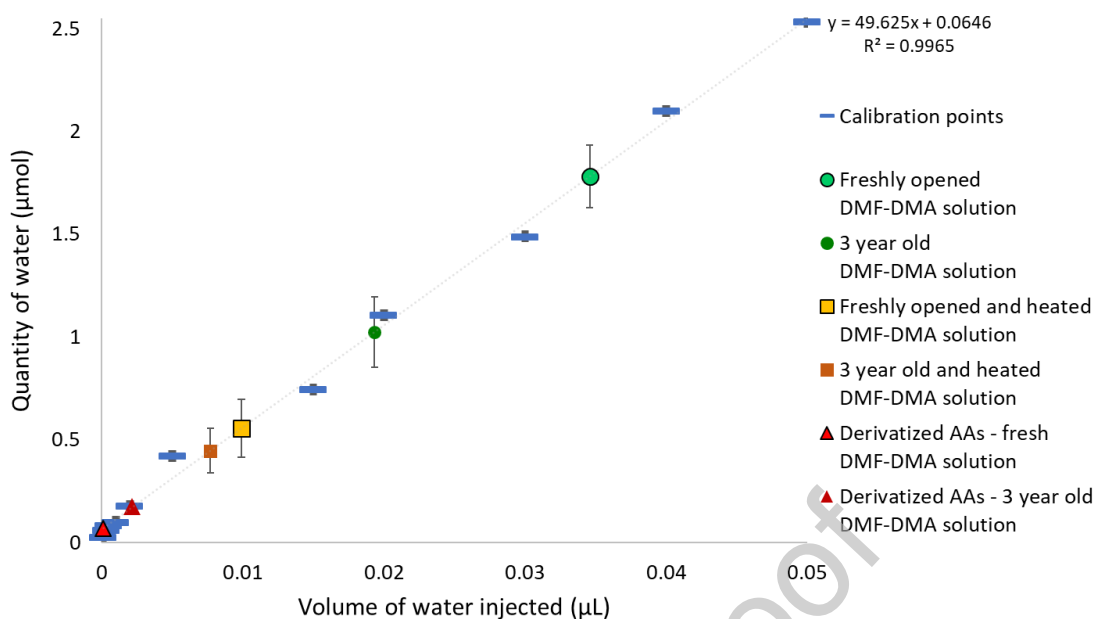


**Figure 3.** Ratio of the relative yield (organic product peak area divided by the internal standard peak area) at  $t$  and  $t_0$  (fresh DMF-DMA solution used before its ambient storage) for each amino acid. The figure represents an efficiency test on DMF-DMA stored for a year at ambient air conditions compared to the derivatization on these pure amino acids by a fresh DMF-DMA solution stored at 2 °C in a hermetically-sealed fridge. The ratios are normalized to the result at  $t_0$ . The instrumental and human error values associated to GC-MS analyses are estimated to  $\pm 0.2$  (20% of  $t_0$ ), which demonstrate a significant difference between storage conditions and the control only if the ratio is lower than 0.8 or higher than 1.2.

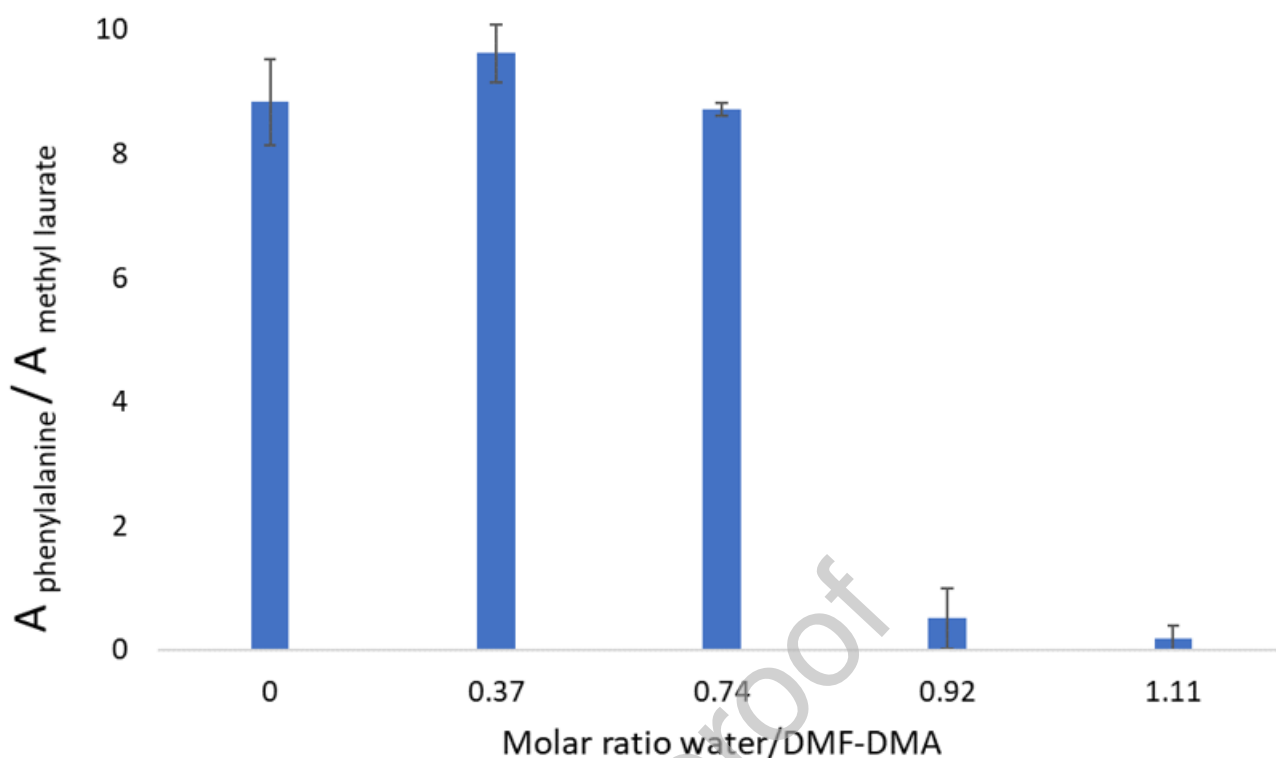


**Figure 4.** Derivatization reaction for water (Reaction 1, where methanol is differentiated between the derivatized water product and the methanol from the DMF-DMA by-product of the derivatization reaction) and further derivatization of the main product (methanol, Reaction 2). Reaction takes place at 145 °C for 3 minutes. K is the equilibrium constant calculated from calibration curves of water (via Karl Fischer Titration), DMF-DMA, DMF, and methanol (via GC-MS).

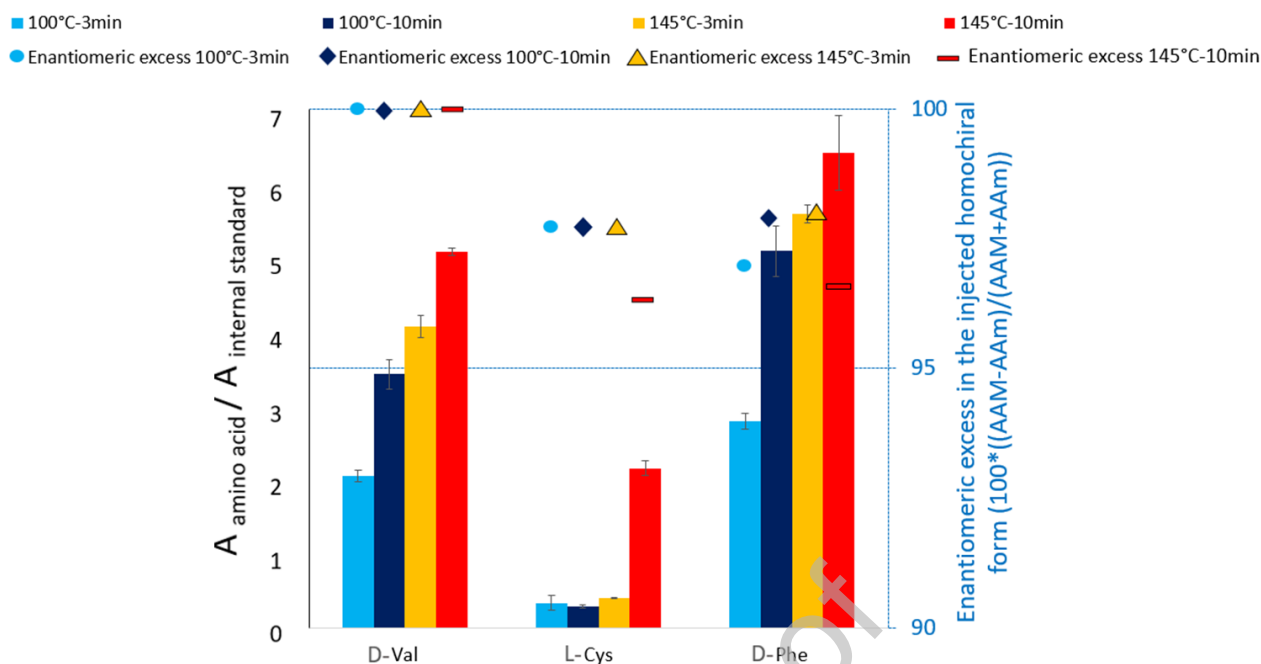




**Figure 5.** Quantification of water within the tested solutions using the calibration curve developed via Karl Fischer Titration test. The calibration points are shown in blue lines, and the equation for the curve is provided. 1 µL DMF-DMA (with or without derivatized amino acid) solutions (red triangles or green circles/orange squares, respectively) were then measured using the same method, and placed at the appropriate location on the curve. The black-bordered and border free points show the comparison between aged (no border) and fresh (bordered) DMF-DMA commercial solutions at 95% purity. The calibration curve is based on six replicates measurements (S.D.<1%) following the methods described in previous work (31).



**Figure 6.** Evolution of the amount of phenylalanine derivatized during the reaction as a function of the amount of water introduced in the initial sample (0.1 to 3  $\mu\text{L}$  or 5.55  $\mu\text{mol}$  to 0.17 mmol compared to DMF-DMA introduced at 0.15 mmol). The ordinate axis is the measure of the peak area of the derivatized form synthesized from a mix of water (variable volume), and phenylalanine (3  $\mu\text{L}$  of a solution at  $5 \cdot 10^{-2}\text{M}$ ) as a function of the molar ratio between water and DMF-DMA reagent.



**Figure 7.** Integrated peak area of amino acids normalized by the peak area of methyl laurate (common internal standard present at the same quantity in all solutions) assessed to (i) compare the efficiency of the DMF-DMA derivatization on different amino acids at different time and temperature of derivatization (histograms) and (ii) to assess the chirality conservation at these derivatization conditions using this reagent and the Chiralil Dex column selected for DraMS (points relative to the second y axis). The second objective was calculated from the formula:  $100 \cdot \frac{A_{AM} - A_{Am}}{A_{AM} + A_{Am}}$ , where AMM=the homochiral form in excess (in our case the injected one), and Amm=the enantiomeric form in minority. The experiments were conducted using six replicates for the relative quantification (to the internal standard). The ee error bars are within less than 5% of RSD with the six replicates. From these analyses, we calculated recovery yield stated as recovery because amino acids cannot be analyzed in GC-MS without prior extraction and derivatization. The chromatogram that accompany this result is presented in Figure 7S (in Supplementary 7).

## Table in the article body:

**Table 1.** List of by-products detected when analyzing pre-heated and unheated DMF-DMA by GC-MS (injection temperature 270 °C) for six replicated solutions stored in ambient air. Note the same by-products were found after analyzing DMF-DMA stored under N<sub>2</sub> atmosphere, and for the control that is fresh DMF-DMA stored at 2 °C in a fridge, which are not described here. The stars (\*) represent the compounds that appeared uniquely in one case. The crosses (X) indicate compounds found in at least two different cases. Each result represents an analysis performed in six replicates, in which by-products were compared. Signal over noise (S/N) ratio indicates if the compounds can be quantified and near by the limit of detection we set at S/N = 3 in GC-MS analysis.

No	Time of retention (min)	By-product names	DMF-DMA unheated	DMF-DMA pre-heated (145 °C - 3 min)	S/N value in TIC mode (LOD: S/N >3 and LOQ: S/N >9.9)
1	1.27	Oxalic acid	*	N.D.	>3 and <9.9
2	1.30	Choline	x	x	>3 and <9.9
3	1.37	Acetaldehyde, O-methyloxime	N.D.	*	>3 and <9.9
4	1.52	N,N-dimethylaminoethanol	x	x	>3 and <9.9
5	1.72	trimethoxy-methane	x	x	>3 and <9.9
6	2.04	Dimethyl ether	x	x	>3 and <9.9
7	2.09	N,N,N',N'-tetramethyl-methanedi-amine	x	x	>9.9
8	2.44	DMF-DMA	x	x	>9.9
9	3.09	N,N-dimethylformamide diethylacetal	x	N.D.	>9.9
11	3.91	N,N-dimethylacetamide	x	N.D.	>3 and <9.9
12	4.01	Propanoic acid, 2-methyl-2-(acetylamino)-, methyl ester	N.D.	*	>3 and <9.9

1 3	4.10	Isopropylamine dimethylformamidinated	*	N.D.	>3 and <9.9
1 4	4.39	N,N-diethyl-formamide	x	N.D.	>9.9
1 5	4.49	N-methoxymethyl-N- methylformamide	x	x	>3 and <9.9
1 6	4.66	Ethyldiethanolamine	N.D.	*	>3 and <9.9
1 7	4.89	4-(dimethylamino)-2-butanone	*	N.D.	>3 and <9.9
1 8	4.99	Betaine	x	x	>9.9
1 9	5.29	Methyl ester N-methyl-N-(2- hydroxyethyl)carbamic acid	*	N.D.	>9.9
2 0	5.39	N-tert-butyl-N',N'-dimethyl- urea	*	N.D.	>3 and <9.9
2 1	5.56	DMF	x	x	>9.9
2 2	5.60	Methyl-2-(methyl- methoxymethyl-amino)acetate	N.D.	*	>3 and <9.9
2 3	5.66	Ethyl-carbamic acid, ethyl ester	*	N.D.	>3 and <9.9
2 4	5.88	Methyl dimethylcarbamate	x	x	>9.9
2 5	5.97	methyldiethanolamine	N.D.	*	>3 and <9.9
2 6	5.99	Tris(dimethylamino)methane	*	N.D.	>3 and <9.9
2 7	6.29	Tetramethyl-urea	x	x	>9.9
2 8	7.02	N,N,N',N'-tetramethyl-1,2- ethanediamine	x	x	>3 and <9.9

2 9	7.29	N,N,N',N'-tetramethyl-1,3-propanediamine	x	x	>3 and <9.9
3 0	7.49	Unknow compound	*	N.D.	>9.9
3 1	7.89	dimethyl acetal aminoacetaldehyde	*	N.D.	>3 and <9.9
3 2	8.61	1,3,5-triazine, hexahydro-1,3,5-trimethyl	N.D.	*	>3 and <9.9
3 3	12.07	2-(methoxycarbonylamino)ethyl ester-N-hydroxy-N ethylcarbamic acid	x	x	>9.9
3 4	12.42	Unknown compound	*	N.D.	>9.9
3 5	12.45	Formyltrimethyl-urea	N.D.	*	>3 and <9.9
3 6	12.53	5-aminovaleric acid	x	x	>9.9
3 7	13.28	Dimethylformamidinated hexylamine	N.D.	*	>3 and <9.9
3 8	13.90	3-buten-2-one, 4-(dimethylamino)	N.D.	*	>3 and <9.9
4 0	15.53	Urea, 1-1-dimethylethyl	N.D.	*	>3 and <9.9
4 1	16.76	Methyl carbamate dimethylformaminated	N.D.	*	>3 and <9.9
4 2	22.61	Urea dimethylated dimethylformaminated	N.D.	*	>3 and <9.9
4 3	24.05	Dimethylformamidinated pentamine	N.D.	*	>9.9
*N.D. = Not Detected					
* S/N = signal over noise					

**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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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