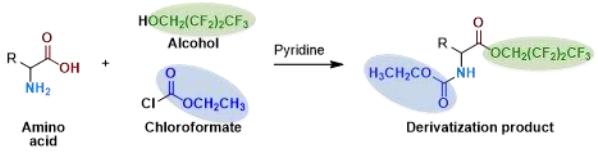
EVALUATING AN ALTERNATE METHOD FOR THE ANALYSIS OF METEORITIC AMINO ACIDS. P. Sen[1], J. C. Aponte[2,3], J. E. Elsila[3], and J. P. Dworkin[3]. [1]University of Texas at Austin, Austin, TX 78712, USA. [2]Catholic University of America, Washington, DC 20064, USA. [3]NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA; e-mail: jose.c.aponte@nasa.gov.

Introduction: Amino acids are protein monomers and essential to life, and among the most studied organic compounds in carbonaceous chondrites. Most amino acids are chiral molecules (having two non-superimposable mirror-image structures). With only a few exceptions, "left-handed" amino acids (L-enantiomers) are biological. Though abiotic processes produce racemic mixtures, some meteoric amino acids have L-enantiomeric excesses. It is possible that the origin of biological homochirality was influenced by carbonaceous chondrites [1,2]. Gas chromatography-mass spectrometry (GC-MS) has been employed for amino acid analyses in meteorites for decades [3], yet current derivatization processes for amino acids could still benefit from improvement for low concentrations, long derivatization procedure times, and simultaneous stable-isotopic measurements. Thus, an optimized derivatization method that operates at meteoric amino acid concentrations could provide a valuable tool for the analysis of amino acids in meteorites.

Methods: We attempted to derivatize 35 different amino acid standards by an esterification reaction (Scheme 1) following previously described methodologies [4,5]. The initial concentrations of amino acid standards in the 20-40 nM range, consistent with quantities of meteoritic abundances [6]; 12 amino acids (10 chiral and 2 achiral) were derivatized successfully (Fig. 1). To optimize the parameters, we varied the reaction times, conditions, and reagents, running a total of 154 reactions on both chiral and non-chiral columns.



Scheme 1. Chlroformate ester derivatization of amino acids.

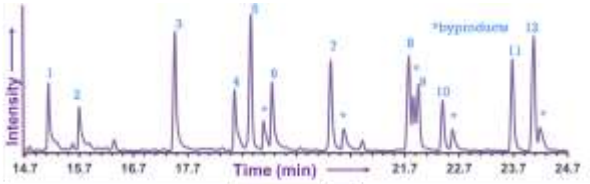


Fig. 1. Non-chiral analysis of 12 derivatized amino acids and their corresponding byproducts using GC-MS (reaction byproducts are shown with *). The concentrations of the standards ranged from 20-36 nM. Analyzed compounds: [1] DL-α-alanine; [2] DL-α-amino-butyric acid; [3] glycine; [4] DL-valine; [5] β-alanine; [6] DL-β-amino-isobutyric acid; [7] DL-norvaline; [8] DL-isoleucine; [9] DL-norleucine; [10] DL-proline; [11] DL-leucine; [12] ε-amino-caproic-acid.

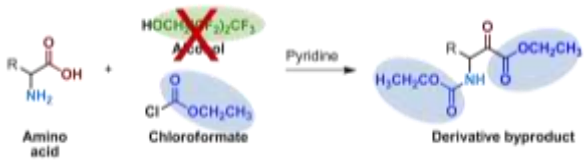
Results: We optimized a room temperature reaction that allows 20 nM analyses and decreases the risk of racemization [5]. A comparison of this method with other popular GC-MS amino acid methods is shown in Table 1. The chloroformate derivatization reaction occurred in 10 seconds and the derivatization products are stable for >72 hours at room temperature, allowing for multiple analyses from a single reaction [7].

Table 1. Comparison of derivitization methods for GC-MS analysis of amino acids.

Derivatization Method	Reaction time	Stability (approx. time)	Chiral separation	Water stable
Chlorofomate ester	10 s	> 72 h	Y	Y
TFAA/IPA	2 h	< 8 h	Y	N
DMF/DMA	5 min	> 24 h	Y	N
MTBSTFA	1 h	< 6 h	N	N
TMS	1 h	< 2 h	N	N

TFAA/IPA: Trifluoroacetic anhydride/isopropanol. **DMF/DMA:** N,N-dimethylformamide dimethylacetal. **MTBSTFA:** N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide. **TMS:** Trimethylsilyl.

Byproduct formation (Scheme 2) was minimized adding the alcohol prior to the addition of chloroformate; even so, byproducts complicate the chromatogram (Fig. 1). This is seen most notably during chiral separation, where it is difficult to differentiate between the products and byproducts. The formation of byproducts may also result in undesirable isotopic fractionation of amino acids [7]. Additionally, this reaction exhibits low reactivity rates with hindered amino acids such as isovaline, serine, and asparctic acid.



Scheme 2. Formation of derivatization byproducts.

Conclusions: The quick reaction time, sensitivity, and product stability indicates that the chloroformate derivatization method investigated could be useful for automated GC-MS analyses.

References: [1] Cronin J. R. and Pizzarello S. (1997) *Science*, *275*, 951-955. [2] Glavin D. P. and Dworkin J. P. (2009) *Proc. Natl. Acad. Sci. USA*, *106*, 5487-5492. [3] Kvenvolden, K. et al. (1971) *Proc. Natl. Acad. Sci. USA*, *68*, 486-490. [4] Zampolli M. et al. (2006) *Chirality*, *18*, 279-295. [5] Myrgorodska I. et al. (2016) *J. Chrom. A*, *1433*, 131-136. [6] Glavin D. P. et al. (2011) *Met. Planet. Sci.*, *45*, 1948-1972. [7] Villas-Bôas et al. (2011) *Metabolites*, *1*, 3-20.