

NANOFLOW SEPARATION OF AMINO ACIDS FOR THE ANALYSIS OF COSMIC DUST.

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Introduction: The delivery of amino acids to the early Earth by interplanetary dust particles, comets, and carbonaceous meteorites could have been a significant source of the early Earth's prebiotic organic inventory [1]. Amino acids are central to modern terrestrial biochemistry as major components of proteins and enzymes and were probably vital in the origin of life. A variety of amino acids have been detected in the CM carbonaceous meteorite Murchison [2], many of which are exceptionally rare in the terrestrial biosphere including α -aminoisobutyric acid (AIB) and isovaline. AIB has also been detected in a small percentage of Antarctic micrometeorite grains believed to be related to the CM meteorites [3].

Stratospheric IDPs are small (~10-40 μm) extraterrestrial particles collected at an altitude of ~20 km by high-altitude aircraft. These particles have been shown to contain up to 10% carbon by weight [4]. Previous studies have shown that IDPs contain complex aromatic compounds, carbonyls, and hydrocarbons [3, 5]. Based on the high organic content of IDPs, it is possible that these particles also contain amino acids. In order to evaluate their amino acid content and distinguish it from terrestrial contamination, unprecedented sensitivity and selectivity is required. Assuming Murchison-like amino acid concentrations, a 20 μm sized IDP grain weighing approximately 10 ng would contain $<6 \times 10^{-16}$ moles of the amino acid AIB. Standard liquid chromatography with UV fluorescence and gas chromatography mass spectrometry detection techniques traditionally used for the analysis of amino acids in meteorites are not sensitive enough for the analysis of individual IDPs or Stardust grains.

We report on progress in optimizing a nanoflow liquid chromatography separation system with dual detection via laser-induced-fluorescence time of flight mass spectrometry (nLC-LIF/ToF-MS) for the analysis of *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) labeled amino acids in cosmic dust grains. The very low flow rates ($<3 \mu\text{L}/\text{min}$) of nLC over analytical LC ($>0.1 \text{ mL}/\text{min}$) combined with $<2 \mu\text{m}$ column bead sizes has the potential to produce efficient analyte ionizations and chromatograms with very sharp peaks; both increase sensitivity. The combination of the selectivity (only primary amines are derivatized), sensitivity (>4 orders of magnitude lower than traditional GC-MS techniques), and specificity (compounds identities are determined by both retention time and exact mass) makes this a compelling technique. However, the development of an analytical method to achieve separation of compounds as structurally similar as amino acid monomers and produce the sharp peaks required for maximum sensitivity is challenging.

Analytical Techniques: Work presented here is based on the analysis of laboratory amino acid standards. The standards used are 1. D,L aspartic acid, 2. D,L glutamic acid, 3. D,L serine, 4. glycine, 5. β alanine, 6. γ amino-*n*-butyric acid, 7. D alanine, 8. L alanine, 9. D β amino-*n*-butyric acid, 10. AIB, 11. L β amino-*n*-butyric acid, 12. D,L α amino-*n*-butyric acid, 13. D,L isovaline, 14. L valine, 15. D valine, 16. D,L isoleucine, 17. D,L leucine.

The instruments used are a Waters nanoACQUITY LC, a Picometrics ZETALIF with a 355nm diode pulsed laser LIF detector, and a Waters LCT Premier MS with a Tapertip electrospray inlet. Since LC separation methods change non-linearly under nanoflow conditions, we have taken a step-wise approach in reducing our column internal diameter, particle size and flow rate. The LC phenyl reverse phase columns and flows used in this study are described in Table 1. Several C_{18} columns were also investigated, but showed only modest differences. Surprisingly, the calculated linear velocities for these are substantially different. This demonstrates the non-linear behavior when comparing gradient methods for these columns.

Table 1. Columns investigated with the empirically determined best flow rate. TBD=to be determined.

Bead (μm)	Size (mm)	Flow ($\mu\text{L}/\text{min}$)	Velocity (cm/min)
5	4.6x250	1000	15
1.7	1.2x150	150	33
3.5	0.3x100	1.5	5.3
2.5	0.3x100	1.5	5.3
2.5	0.1x100	TBD	TBD
1.7	0.07x100	TBD	TBD

Results and Discussion: Only through systematic optimizations of methods on an incremental sequence of columns along the axes of diameter and bead size will nLC provide useful results. Initial studies where the method employed on a 4.6 x 250 mm column [6] were ratioed to be applied to a 0.07 x 100 mm column produced no usable data.

Fig 1 compares the separation of the largest and smallest column successfully optimized. It is important to note that the 0.3 mm internal diameter (ID) of the small column is still too large to produce the sharp peaks indicative of nLC. We expect the 0.1 mm ID column around 0.1 $\mu\text{L}/\text{min}$ to begin to show the sharp peaks required to be sensitive enough to analyze IDPs.

Furthermore there are several critical aspects which must be employed to provide useful separations. We have investigated a wide range of column temperatures, pH and ionic strengths for the aqueous buffer, and tested both methanol and acetonitrile organic mobile phases. The best balance of elution qual-

ity and buffer stability is a gradient of 10 mM ammonium formate, pH 8 into methanol. This buffer must be made weekly and filtered the daily or columns are likely to become clogged and ruined.

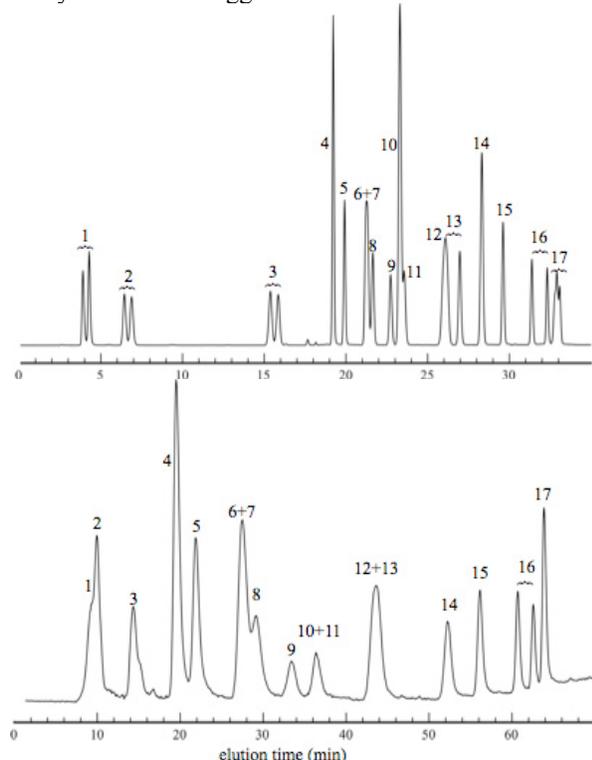


Figure 1. Fluorescence chromatograms of the same amino acid standard derivatized for 15 minutes with OPA/NAC. Top shows the LC separation of 10^{-11} moles on the 4.6 x 250 mm column according to published methods [6]. Bottom shows the nLC separation of 10^{-13} moles achieved with a 2.5 μm bead 0.3 x 100 mm column. In both cases S/N~1000. The bottom separation is adequate for us to proceed to a smaller column.

Our most successful tests have been with standards diluted in mobile phase. This is not useful for the study of very low concentration amines—such as expected in IDPs. In an actual sample, the OPA/NAC derivitization cocktail is >10 times higher ionic strength and pH than the elution buffer. Since the injection volume is large (1 μL) compared to the column volume of the desired column about 15 nL (for a packed 70 μm x 100mm column). Such a large volume of high salt buffer will throw the column out of equilibration and result is essentially no separation. Thus, it is necessary to place an inline trapping column to bind the analyte while flushing the OPA/NAC cocktail.

First, commercially available nLC trapping columns are not pH stable, so a custom column was purchased (Waters Xbridge C₁₈ 5 μm bead 180 μm x 20 mm). Second, the trapping methods used by the proteomics community [7] are not compatible with the methods we are using. Thus, we are developing a

trapping method using a second pump for both injecting and desalting (Fig 2).

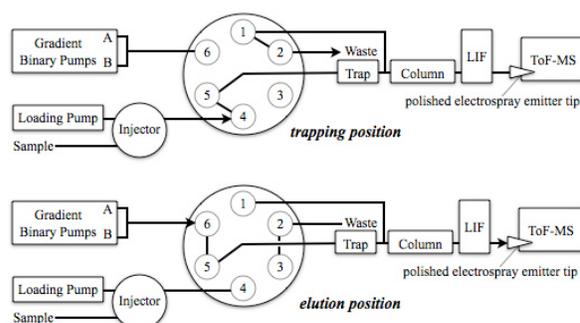


Figure 2. Flow and valve diagram for nLC-LIF/ToF-MS analysis with a trapping column loaded with an auxiliary pump.

An additional discovery was that the use of commercial PEEK connections for user-cut fused silica capillary tubes are adequate do not severely degrade peak-shape. However, it is of prime importance that the fused silica capillary tube acting as the electrospray emitter be polished. Signal to noise ratios worsened by an order of magnitude if a tile-cut fused silica capillary was used as the emitter instead of a polished tapered capillary (Fig 3).

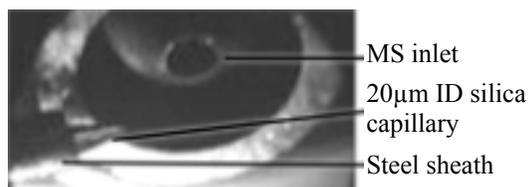


Figure 3. Electrospray tip showing the proximity of the polished fused silica tip to the MS inlet.

Conclusions: The development of nLC methods is a slow process, but the direction of the progress is encouraging. The difficulty of this process should be considered by those interested in developing liquid chromatography for space flight. These instruments are likely to be miniaturized devices which may encounter some of the same complications.

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