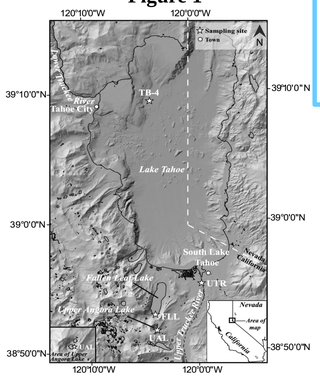
Determining the Amino Acid Composition of Sierra Lake Dissolved Organic Matter (DOM)

Frances Bentley and Lihini I. Aluwihare

Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0244

Introduction: When testing, we found out that that the Lake Tahoe DOM and Fallen Leaf DOM (August 2009) samples showed unusual results. There were abnormally low Carbon: Nitrogen) C:N ratios for terrestrial and/or freshwater DOM, which is more comparable to the results found from the Upper Angora and Upper (Truckee River) samples.
We believe that the reason that we received those results was because the samples were in protein-rich DOM. We decided to use a common amino acid analysis method (Marfey's reagent). Marfey's reagent helps us find the amino acid composition and quantity in each sample. As you read this poster, you will see how the Marfey's method works and how we implemented the reagent.

Figure 1



Goal: By using samples from three dissolved organic compounds (DOM) that were found in three Sierra Lakes in Nevada (look to Figure 1): Lake Tahoe, Fallen Leaf, and Upper Angora, we are looking for the amino acid composition.

Method: The flow chart below describes the major steps in our method scheme. The methods were optimized to suit our analytical equipment and samples.

Dry sample

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Dissolve in 6 N hydrochloric acid, seal and heat or 2 hours at 154 degrees Celsius.

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Dry under nitrogen.

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Re-dissolve in H_2O and adjust the pH to >5 with sodium hydroxide.

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Add Marfey's Reagent (10 mg/mL acetone) + 1M sodium bicarbonate.

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Heat at 40 degrees Celsius for one hour.

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Quench with 1N hydrochloric acid. Dry under nitrogen.

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Re-dissolve in DMSO + H_2O.

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Separate on C 18 HPLC column with acetonitrile (90%): methanol (10%) and 5% acetic acid.

↓

Detect at 354nm via UV-VIS and identify by retention time and mass spectrometry.

Table 1: The C:N ratio of the organic matter samples found in the Lake Tahoe Basin and the Upper Truckee River.

Lake/River ID	Data	Volume (L)	Ambient Dissolved DOC	%C	C:N
Tahoe	May-09	800	36.7	42.4	3.7
	Jun-10	1300	43.6	48	4.5
Fallen Leaf	May-09	660	50	50	16.4
	Aug-09	600	49.1	49	6.6
Upper Angora	Aug-09	540	135.9	50.6	38.8
Upper	Jul-10	200	125	51.2	33.3

Figure 2: Example of how we separated the amino acids: high pressure liquid chromatography (HPLC) run for amino acid standard following Marfey's derivatization. HPLC conditions were as described in the flow chart (to the right). Individual peaks represent UV activity at 354 nm.

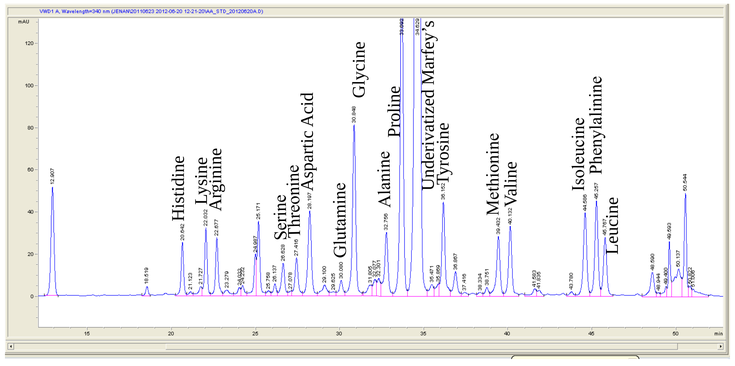
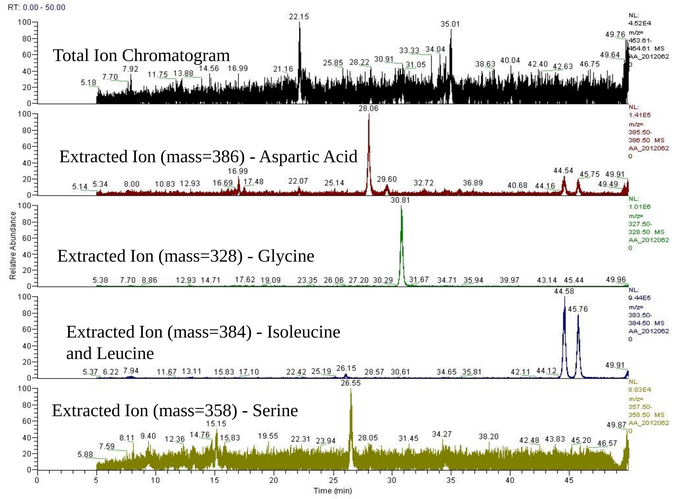


Figure 3: Example of how we identified each amino acid. Electrospray (ES) mass spectrometry (MS) analysis of HPLC separated amino acids. Extracted ions corresponding to amino acid mass (Table 2, column 4) were used to identify each amino acid.



Conclusion:

1. The Marfey's method (shown in the flow chart above) provides adequate separation of the common amino acids.
2. MS identification of amino acids enable low concentrations to be detected.
3. Amino acids are present in some of the Lake Tahoe samples.
4. Individual amino acids had different response factors with UV detection and this has to be taken into account when quantifying each amino acid.
5. Quantitation was hindered by low concentrations. Therefore, samples need to be tested again.

Table 2: Parameters used to identify amino acids. Retention time refers to HPLC elution time (Figure 2) corresponding to conditions shown in the flow cart. Parent Ion Mass is the mass of each amino acid following Marfey's derivatization and is used to identify amino acids by mass spectrometry (figure 3).

Amino Acid	Average Molecular Weight	With Marfey's	Parent Ion Mass	Retention Time
Alanine	89	341	342	32.8
Arginine	174	426	427	22.7
Asparagine	132	384	385	
Aspartic Acid	133	385	386	28.2
Cysteine	121	373	374	
Glutamate Acid	147	399	400	30.1
Glutamine	146	398	399	
Glycine	75	327	328	30.8
Histidine	155	407	408	20.6
Isoleucine	131	383	384	44.6
Leucine	131	383	384	45.8
Lysine	146	398	399	22.0
Methionine	149	401	402	39.4
Phenylaline	165	417	418	45.3
Proline	115	367	368	33.1
Serine	105	357	358	26.6
Threonine	119	371	372	27.4
Tryptophan	204	456	454	
Tyrosine	181	433	434	36.2
Valine	117	369	370	40.1

Figure 4: Relative response factors for individual amino acids in standard mixture calculated based on the area under each peak in Figure 2. All amino acids were initially present ate the same concentration. If response factors were equal, all amino acids would be present at the same relative percent.

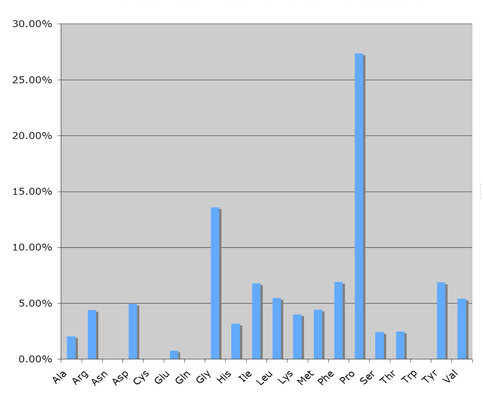


Figure 5: Amino acid composition of Lake Tahoe June 2010 Sample. Amino acids were identified using MS. Examples of presence and absence are shown below. Retention times are different as they were run using a different LC program.