Ultra High Performance Liquid Chromatography-Triple Quadrupole Mass Spectrometry Detection of Hydroxy Acids in Complex Mixtures from Prebiotic Simulation Experiments

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PREBIOTIC HYDROXY ACIDS

- **Prebiotic simulation experiments: very chemically complex; synthesize α-amino[1]-/hydroxy[2]-acids.**

Figure 1. A) α-amino acid, B) α-hydroxy acid (AHA).

- **Peptide synthesis limited by diketopiperazine[3-5] (DKP), but may be enhanced by AHAs[6].**

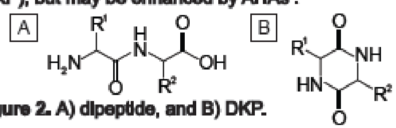

Figure 2. A) dipeptide, and B) DKP.

- **Previous methods were time-intensive[7], used derivatization[8], or had limited detection capabilities[9].**
- **<u>HYPOTHESIS</u>: Triple quadrupole mass spectrometry can provide rapid and sensitive detection of AHAs in model prebiotic experiments.**

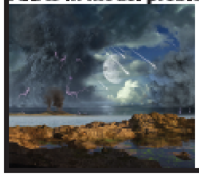

Figure 3. Plausible primordial Earth environment[10] where biomolecule synthesis could have occurred, and from which life may have arisen.

METHOD OPTIMIZATION

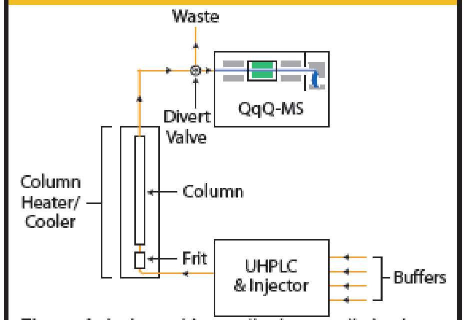

Figure 4. An ion-pairing method was optimized using an Agilent 1290 Infinity ultra high performance liquid chromatograph, and an Agilent 6430 triple quadrupole mass spectrometer (UHPLC/QqQ-MS). A Waters ACQUITY UPLC BEH C_{18} column was used. Mobile phases were A) 5 mM hexylamine and B) 90:10 methanol:10 mM ammonium acetate.

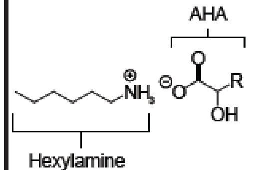

Figure 5. Ionic interaction between hexylamine ion-pairing agent and AHA target analyte helps enhance column retention.

REACTION MONITORING ASSAYS

Table 1. Experimental mass-to-charge (m/z) transitions developed for target AHAs of interest.

AHA	Parent Ion	Product Ion(s)
1) Glycolic	75.0	47.0
2) Lactic	89.02	43.2
3) α-OH-IsoBTA	103.04	40.9, 57.1
4) α-OH-BTA	103.04	57.1
5) α-OH-α-MeBTA	117.05	45.0, 70.9
6) α-OH-Glutaric	147.03	62.1, 129.2
7) Malic	133.01	71.1, 115.0
8) α-OH-Isovaleric	117.05	45.0, 71.2, 94.3
9) α-OH-Isocaproic	131.07	69.0, 85.0,113.2
10) α-OH-Caproic	131.07	45.2, 73.2, 82.9, 85.0

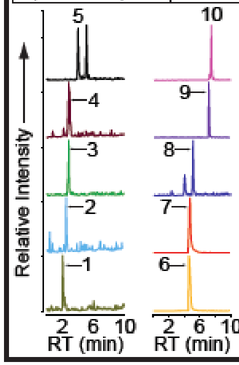

Figure 6. AHA standard mass chromatograms. Peak identity and transitions (LOD in μM):
1 = 75.0 - 47.0 (6.95)
2 = 89.02 - 43.2 (2.20)
3 = 103.04 - 40.9 (1.41)
4 = 103.04 - 57.1 (6.02)
5 = 117.05 - 45.0 (1.00)
6 = 147.03 - 129.2 (0.98)
7 = 133.01 - 115.0 (2.29)
8 = 117.05 - 45.0 (2.13)
9 = 131.07 - 69.0 (1.19)
10 = 131.07 - 82.9 (0.56)

AHAs in PREBIOTIC MIXTURES

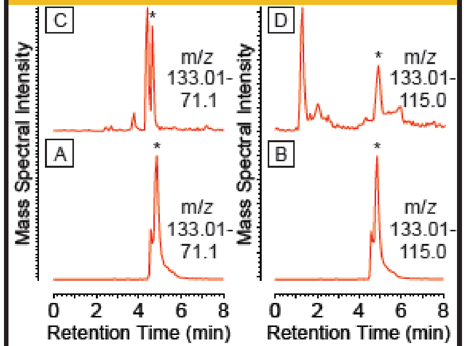

Figure 7. Example mass chromatograms demonstrating malic acid detection in model prebiotic reactions. A) and B) standards, C) spark discharge of N_2/CH_4 atmosphere, and D) repeated cyanamide experiment[10].

REFERENCES

1) Miller, S.L. (1953) Science, 117, 528-529. 2) Miller, S.L. (1955) JACS, 77, 2351-2361. 3) Long, D.A. et al. (1971) Trans. Faraday Soc., 67, 1094-1103. 4) Steinberg, S.M. and Bada, J.L. (1981) Science, 213, 544-545. 5) Qian, Y. et al. (1993) GCA, 57, 3281-3293. 6) Mamajanov, I. et al. (2014) Macromolecules, 47, 1334-1343. 7) Cronin, J.R. and Pizzarello, S. (1997) Science, 275, 951-955. 8) Fransson, B. and Ragnarsson, U. (1998) J. Chromatogr. A, 827, 31-36. 9) Sheng, S. and Reddy, T.M. (2014) J. Agric. Food Chem., 62, 1503-1511. 10) Parker, E.T. et al. (2014) Angew. Chem., Int. Ed., 53, 8132-8136.

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