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Session BG 2.5

Quality of stable isotope data - Methods and tools for producing high quality data

High-precision compound-specific carbon isotopic analysis of underivatized amino acids using a multidimensional-HPLC and nano-EA/IRMS

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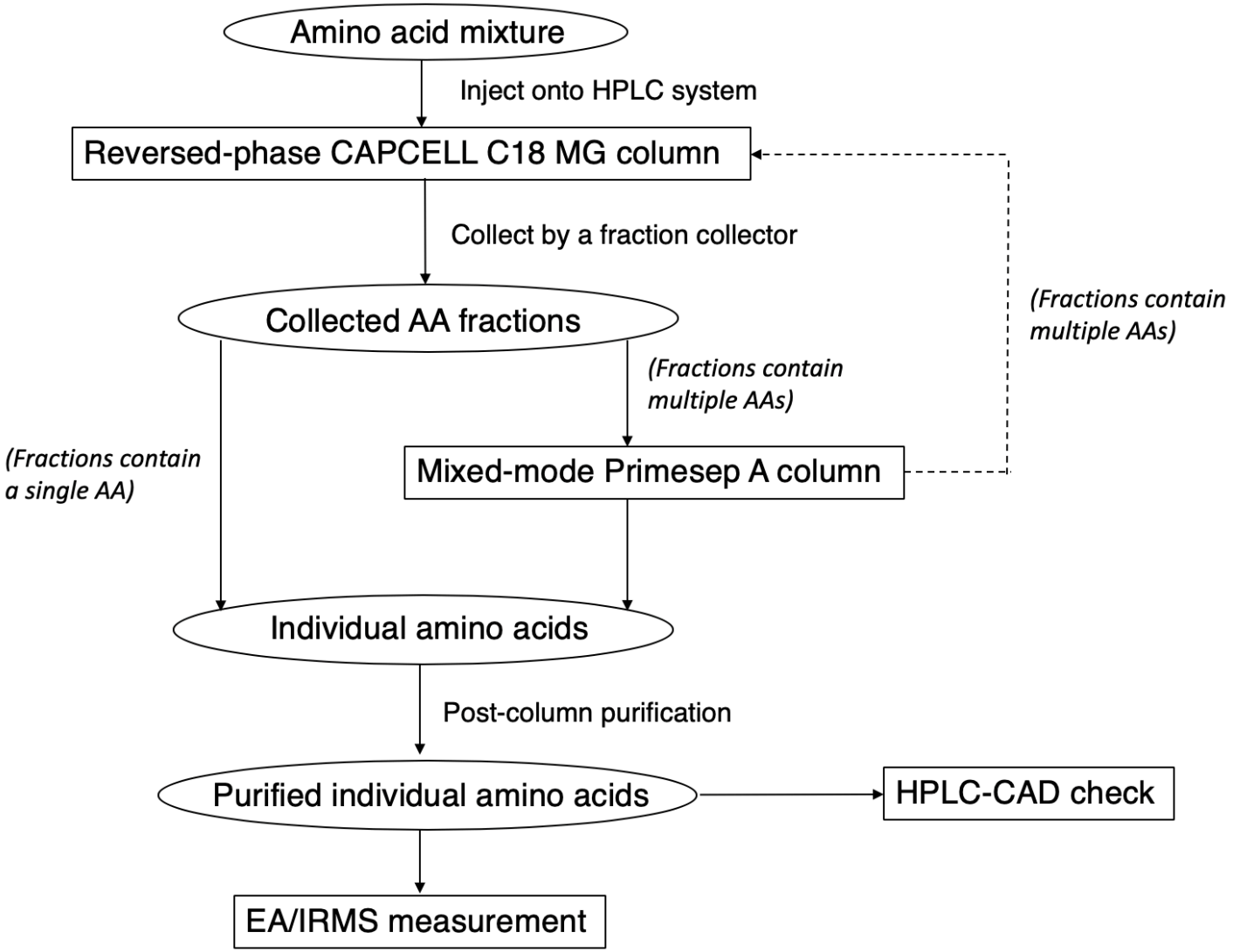
Introduction

To achieve better precision and accuracy for $\delta^{13}C$ analysis of individual amino acids (AAs), we have developed a new analytical method based on multi-dimensional high-performance liquid chromatography (HPLC) and elemental analyzer/isotope ratio mass spectrometry (EA/IRMS).

Unlike conventional methods using gas chromatography, this approach omits pre-column chemical derivatization, thus reducing systematic errors associated with the isotopic measurement.

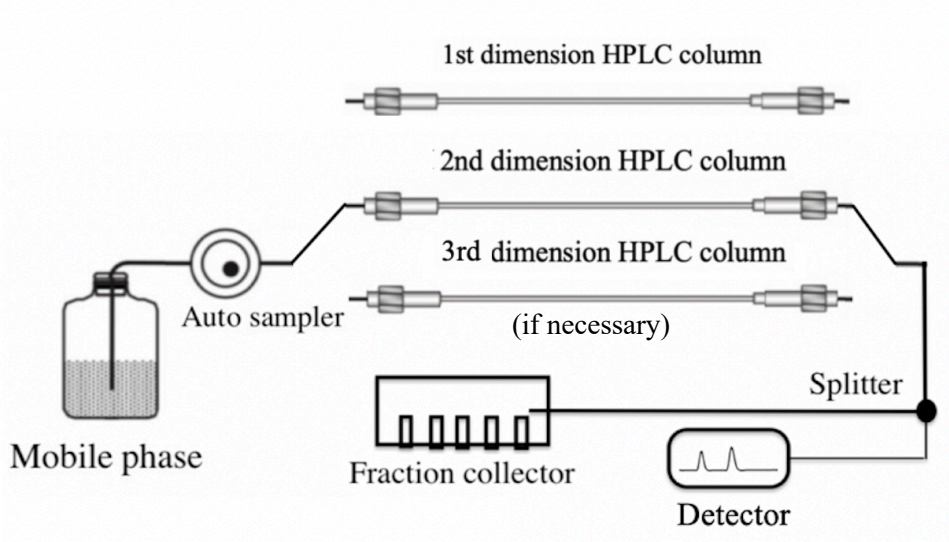
We performed the isolation of individual AAs in a standard mixture containing 15 AAs (Gly, Ser, Asp, Ala, Thr, His, Glu, Arg, Pro, Val, Met, Tyr, Ile, Leu, Phe) and biological samples.

Methods - flow diagram



Methods – multidimensional HPLC

HPLC modules (Agilent 1100) and a Corona Charged Aerosol Detector (CAD) (Thermo Fisher Scientific) are used for the separation and detection of AAs.



Systematic illustration of the HPLC system

HPLC system and CAD

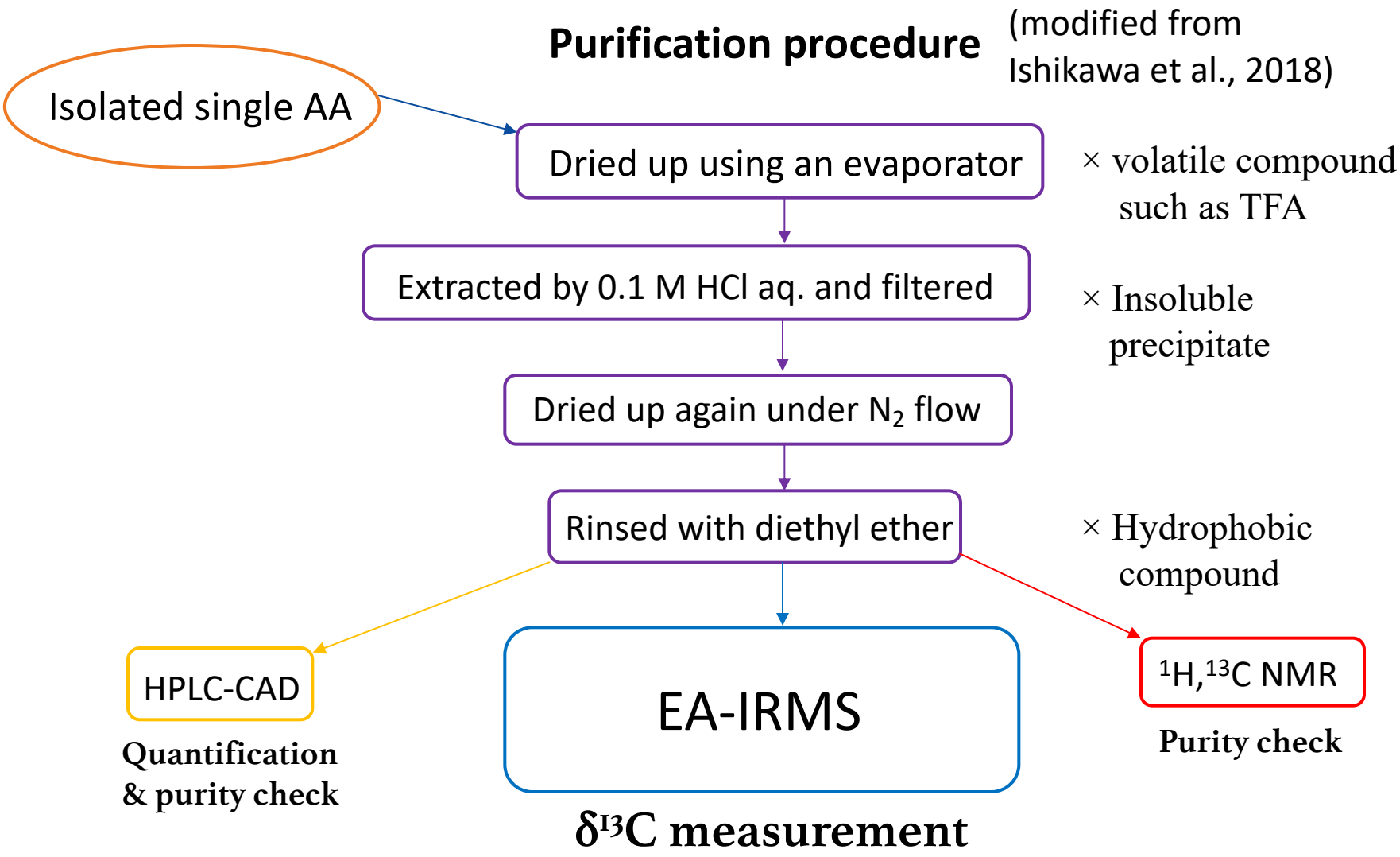
Methods - nano-EA/IRMS

$\delta^{13}C$ measurements are conducted using a nano-EA/IRMS system (Flash EA 1112-Finnigan Delta plus XP IRMS).

< 1 µgC sample is required for reliable analysis (Ogawa et al., 2010)

nano-EA/IRMS system

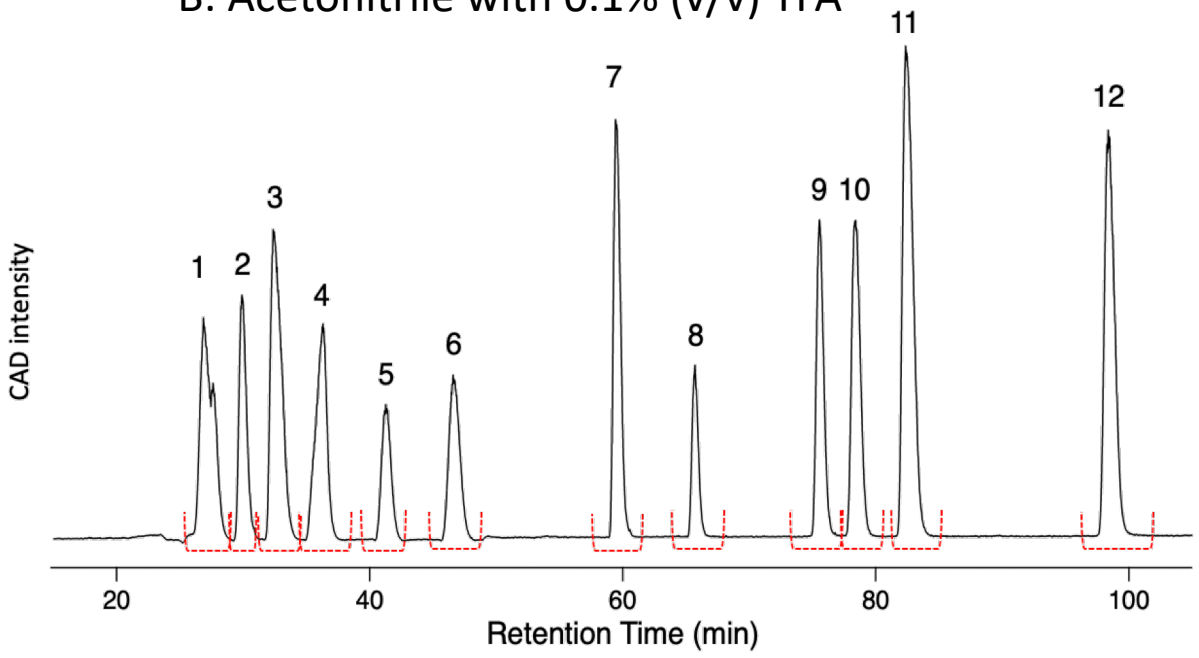
Methods – post-column purification



Results – 1st HPLC column separation

Separation of a 15-AA mixture by a CAPCELL PAK C18 column (5μm, 20mmI.D×250mm).

Eluent A: Distilled water with 0.1% (v/v) TFA
B: Acetonitrile with 0.1% (v/v) TFA



Chromatogram of the 15-AA mixture on the CAPCELL PAK C18 column.
Red dashed lines indicate the collection time of each fraction.
Peak identification: 1. Ser, Gly; 2. Asp; 3. Thr, Ala, His; 4. Glu; 5. Arg; 6. Pro; 7. Val; 8. Met; 9. Tyr; 10. Ile; 11. Leu; 12. Phe.

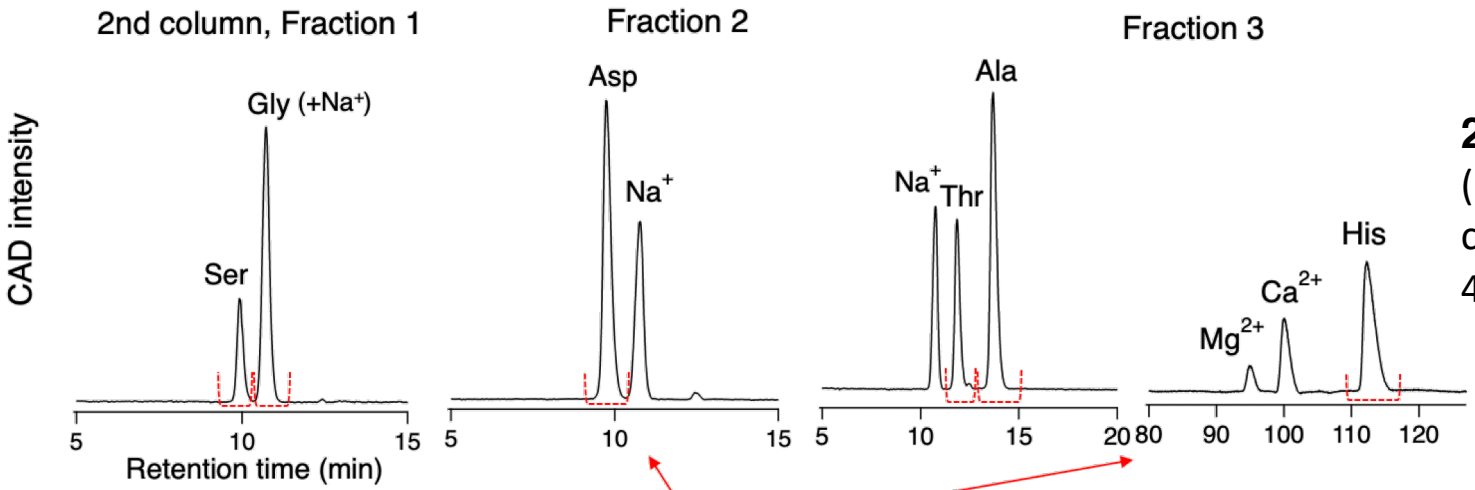
Flow rate: 2 mL/min
Temperature: 30 ℃

Injection amount:
10-60 μg of AAs

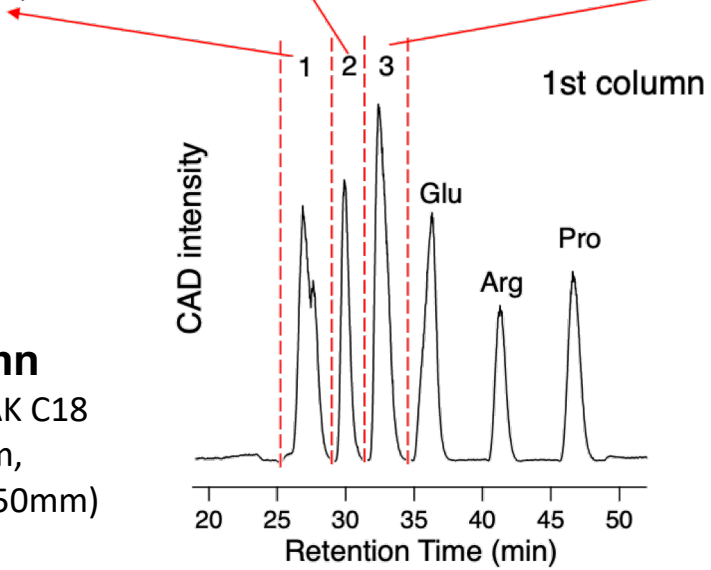
Eluent gradient

Time/ min	B%
0	0
30	10
80	20
125	20

Results – 2nd HPLC column separation



2nd column
(Primesep A column, 5µm, 4.6mmI.D×250mm)



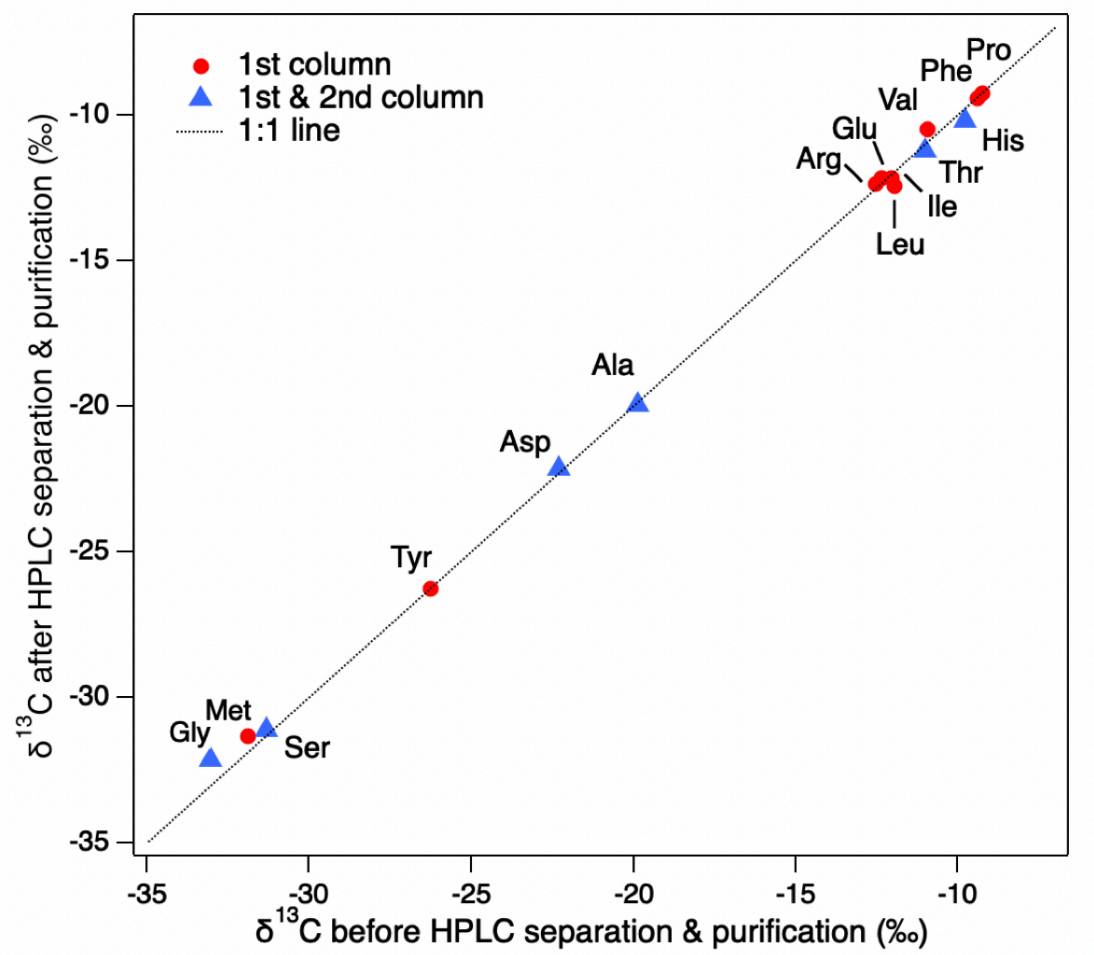
1st column
(CAPCELL PAK C18 column, 5µm, 20mmI.D×250mm)

For the 2nd column:
Flow rate: 1 mL/min
Temperature: 30 °C
Eluent gradient

Time/ min	B%
0	0
20	0
70	25
100	25

Red dashed lines indicate the collection time of each fraction.

Results – EA/IRMS measurement



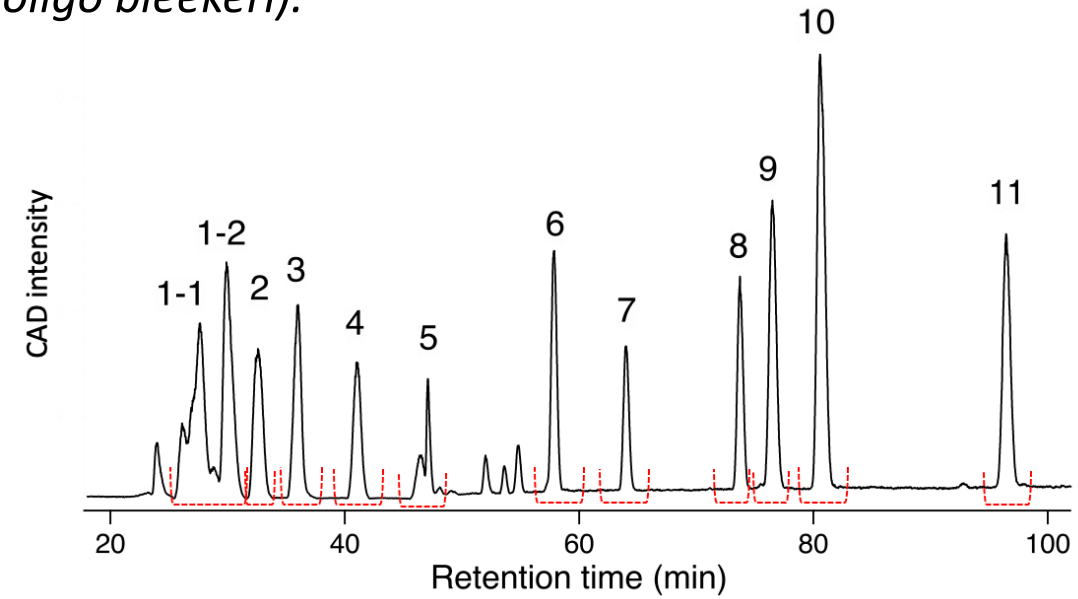
$\delta^{13}C$ values of AAs in the 15-AA standard mixture before and after the HPLC separation and purification. (n=2, ave.)

The $\delta^{13}C$ values of AAs were not changed in the HPLC separation and purification procedures.

Results obtained by this method can correctly reflect the original AA $\delta^{13}C$ values of the measured sample.

Results – biological sample

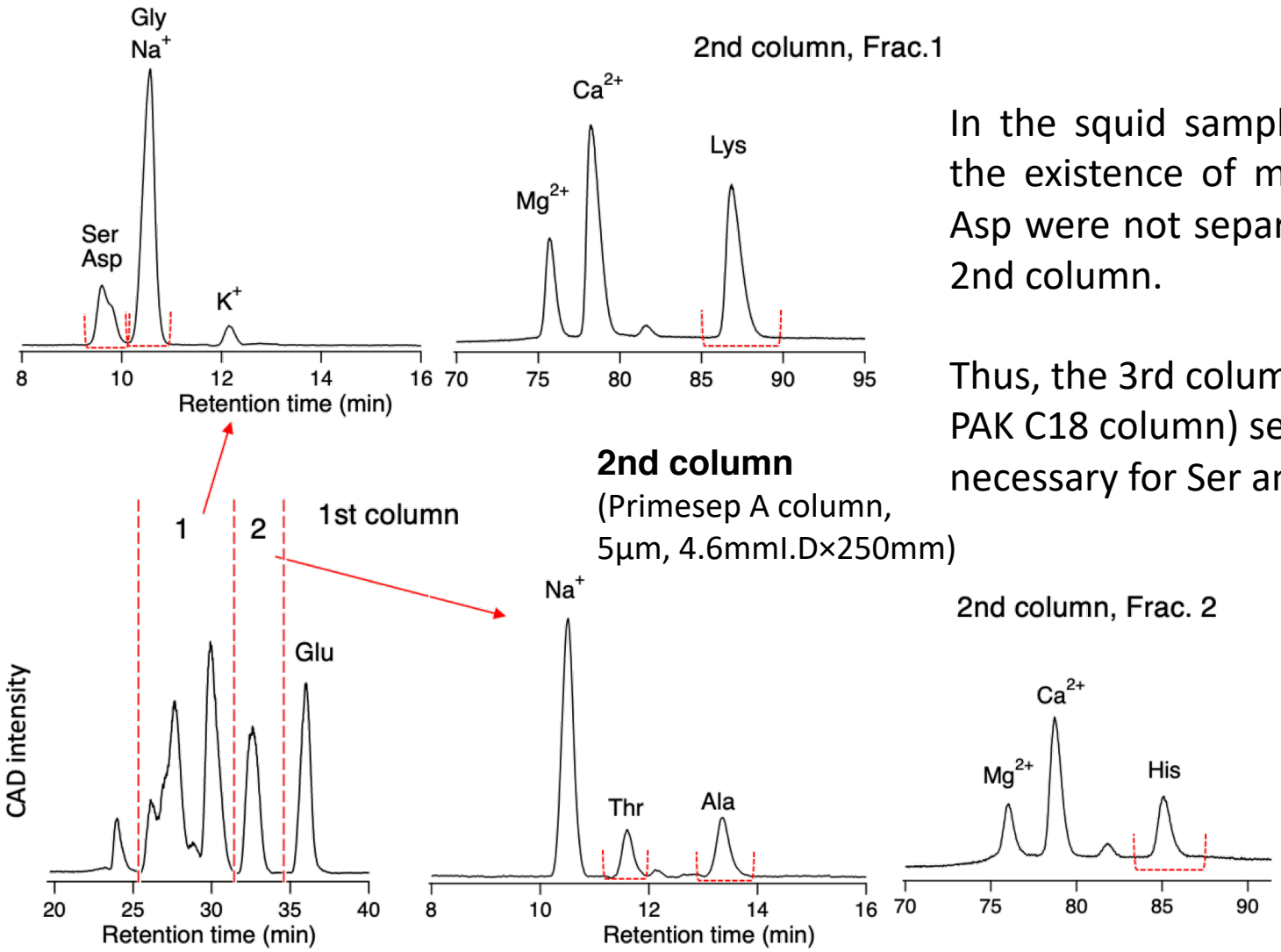
We applied this method to the analysis of several biological samples. Biological samples were hydrolyzed for 12 h with 12 M HCl at 110℃ and defatted before analysis.
Here we show an example of biological samples, spear squid muscle (*Heterololigo bleekeri).*



1st column
(CAPCELL PAK C18 column, 5µm, 20mmI.D×250mm)

Chromatogram of the hydrolyzed squid sample on the CAPCELL PAK C18 column.
Red dashed lines indicate the collection time of each fraction.
Peak identification: 1-1. Ser, Gly; 1-2. Asp; 2. Thr, Ala, His; 3. Glu; 4.Arg; 5. Pro; 6. Val; 7. Met; 8. Tyr; 9. Ile; 10. Leu; 11. Phe.

Results – biological sample

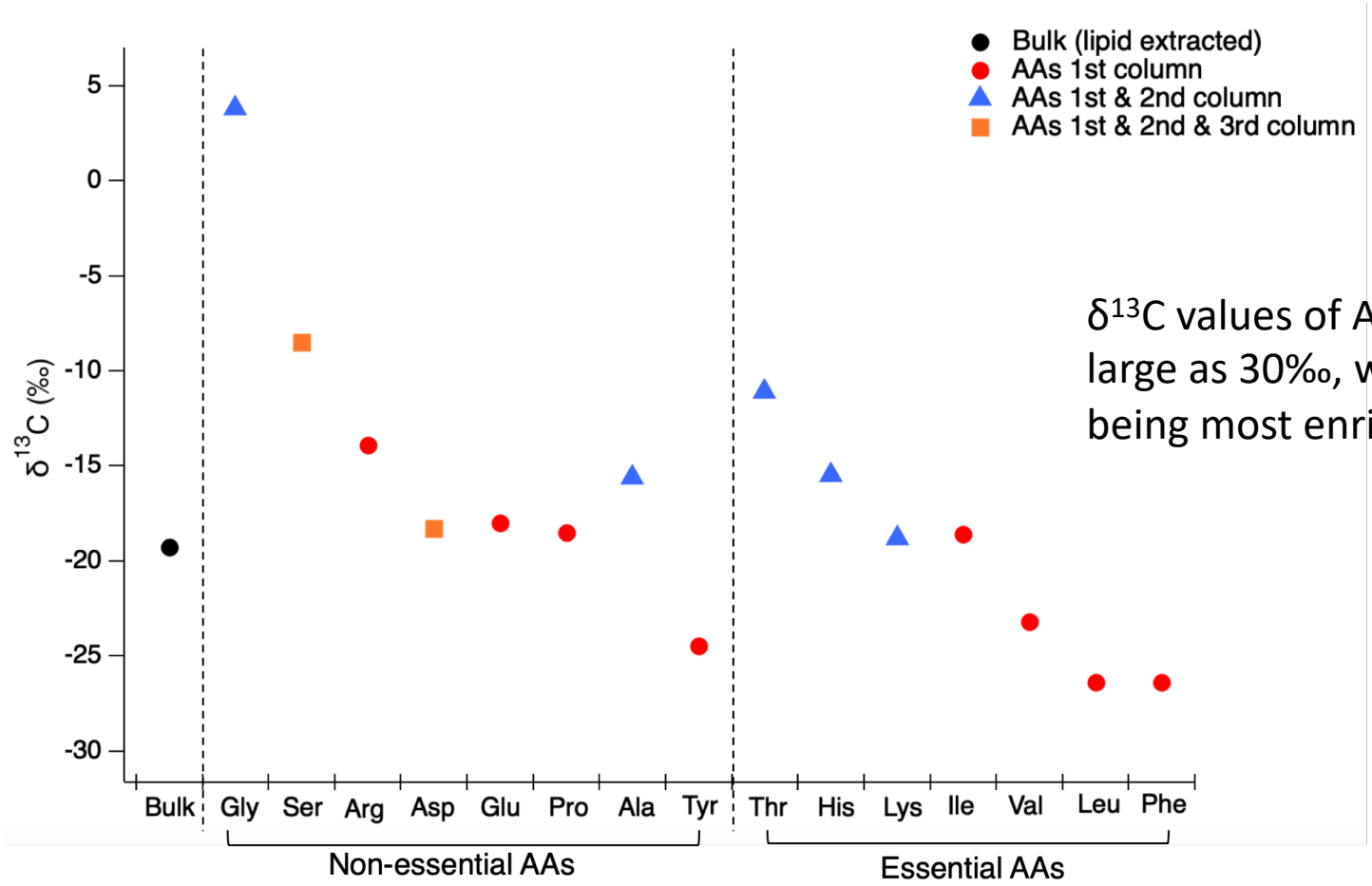


In the squid sample, because of the existence of matrix, Ser and Asp were not separated from the 2nd column.

Thus, the 3rd column (CAPCELL PAK C18 column) separation is necessary for Ser and Asp.

Red dashed lines indicate the collection time of each fraction.

Results – biological sample



$\delta^{13}C$ values of AAs vary as large as 30‰, with Gly being most enriched in ^{13}C.

$\delta^{13}C$ values of AAs isolated from hydrolyzed spear squid muscle.

Summary and Outlook

- We developed a method for compound-specific $\delta^{13}C$ analysis of AAs without the chemical derivatization which potentially affect the $\delta^{13}C$ measurement.

- The analysis of individual AAs isolated from a 15-AA standard mixture was achieved by two-dimensional HPLC separation. The results strongly suggested that the whole experimental procedure did not alter the $\delta^{13}C$ values. The potential of this method for being applied on biological samples has also been confirmed.

- We expect that this method will contribute to a wide range of investigations where $\delta^{13}C$ analysis of AAs is required, for example, studies of trophic relationship in the ecosystem, source and fate of AAs in the sediments and formation processes of AAs in space.

Reference

Ogawa NO, Nagata T, Kitazato H, Ohkouchi N. Ultra-sensitive elemental analyzer/ isotope ratio mass spectrometer for stable nitrogen and carbon isotope analyses. In: Ohkouchi N, Tayasu I, Koba K, eds. *Earth, Life, and Isotopes*; Kyoto: Kyoto University Press; 2010; 339-353.

Ishikawa NF, Itahashi Y, Blattmann TM, et al. An improved method for isolation and purification of underivatized amino acids for radiocarbon analysis. *Analytical Chemistry*. 2018; 90(20):12035-12041.

This work: Sun et al., *Rapid Communication in Mass Spectrometry,* in revision.