thermoscientific

Using Ion Chromatography with High Resolution Mass Spectrometry for Metabolomic Profiling of Three Representative Food Diet Samples

Terri Christison[1], Beibei Huang[1], Jeff Rohrer[1], Reiko Kiyonami[2], David Peake[2], and Ralf Tautenhahn[2], Thermo Fisher Scientific, [1]Sunnyvale, CA, USA; [2]San Jose, CA, USA

ABSTRACT

Purpose: Demonstrate the application of metabolomics techniques to the analysis of food samples

Methods: Ionic compounds related to metabolism from three food samples representing different diets were separated by ion exchange chromatography and detected by high resolution mass spectrometry. The data was analyzed with Thermo Fisher Scientific data analysis tools.

Results: Over 1028 features were found in the food samples, of which ~180 small polar compounds were identified using IC-HRAM. The food samples were complex with complex matrices, however the samples had significant differences. Many of these compounds are also TCA metabolites. Only a small portion of the results are shown.

INTRODUCTION

The analysis of small polar metabolites is critical to understanding many of the metabolic disturbances as a result of disease, lifestyle, and diet. In this experimental design, UC Davis Center of Metabolomics generated three food samples representing three different diets: low animal protein (Davis), high fish and vegetables (California), and high beef, high sugar, and high fat (USA). Recently it has been shown that ion chromatography (IC) when combined with HRAM MS can provide superior separations and sensitivity for polar ionic species as compared to other LC methods. These results have been demonstrated using a capillary IC and replicated using a higher throughput IC system.[2,3] In this study these food samples were analyzed using IC-Orbitrap™ MS and processed using Thermo Scientific™ Compound Discoverer™ 2.0 software.

MATERIALS AND METHODS

Sample Preparation
West Coast Metabolomics Center generated the three homogenized, freeze-dried (at -80 °C) food samples. Two mg of the lyophilized samples (equivalent to 20 mg of food) were extracted with 1 mL of degassed, cold 80/20% methanol/deionized water and agitated mechanically for 5 min. The samples were freeze dried at -80 °C and upon receipt, were reconstituted with 100 µL of 10% methanol. Reconstituted in 100 µL of MeOH/H_2O (10:90).

Table 1. Components of three types of food samples.

Davis	California	USA
White rice	Salmon	Hamburger bun
Fried egg	Brown blended rice	Beef
Sesame seeds	Sliced almonds	Cheese
Spicy sauce	Lemon slices	Bacon
Tofu	Steamed carrots	Lettuce
Spinach	Steamed broccoli	Pickles
Bean sprouts	Steamed onions	Tomatoes
Soy sprouts	Steamed cabbage	Ketchup
Carrots	Steamed red bell pepper	French fries
Zucchini	Grapes, blueberries	Baked beans
Radish	Yogurt	2 Chocolate chip cookies
Rice punch	Green tea	Regular Cola

Test Methods
Ion Chromatography
Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ dual IC system with Thermo Scientific™ Dionex™ AS-AP autosampler (see Figure 1).

The IC conditions are shown in Table 2.

Mass Spectrometry
High Field Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer (see Figure 1).

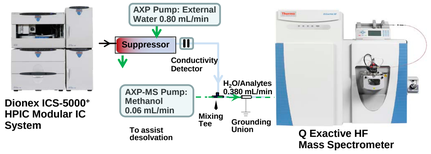
ESI negative mode. Full mass scan: m/z 67-1000, resolution: 120,000 (FWHM) at m/z 200, automatic gain control (AGC) target: 1x10^6 ions, maximum ion injection time (IT): 50 ms. Data dependent MS^2, Top 10, AGC 2e5, IT 100ms, Underfill 1%.

Source ionization parameters: spray voltage: 3.5kV; transfer temp.: 320 °C; S-Lens level: 50; heater temp.: 325 °C; Sheath gas: 36; Aux gas: 5.

Table 2. Ion chromatography conditions.

Columns	Thermo Scientific™ Dionex™ IonPac™ AS11HC-4µm guard and separation, 2 mm i.d
Gradient	25–95 mM KOH in 30 min
Eluent Source	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge with Thermo Scientific™ Dionex™ CR-ATC 500 trap column
Flow	0.38 mL/min
Temp	30 °C
Inj. Vol	2 µL of 5 µL loop
Desalter	Thermo Scientific™ Dionex™ AERS™ 500 suppressor in external water mode (Thermo Scientific™ AXP-MS pump at 0.8 mL/min)
Makeup Solvent	Methanol at 0.06 mL/min (AXP-MS pump) to low volume mixing tee (Idex, P/N P-890). See Figure 1.

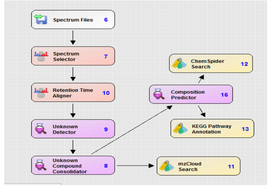
Figure 1. Flow diagram of Dionex ICS-5000⁺ HPIC dual IC to a Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer.



Software and Data Analysis
The instruments are managed by new interface software (Thermo Scientific™ Standard Instrument Integration (SII)) for Xcalibur coupling Thermo Scientific™ Xcalibur™ software and Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System software platform.)

Differential analysis of profiling data and PCA plots were performed using Compound Discoverer 2.0 software (Figure 2).

Figure 2. Data analysis flow path for Compound Discoverer software.



RESULTS

Table 3 and Figures 3A and 3B show the differential analysis of three diets and significant components. In Figure 3C, the data point in Figure 3B (red arrow), is listed in Consolidated Unknown Compounds by Compound Discoverer. Linking automatically to mzCloud (Figures 3C and 3E), tartrate is identified and confirmed by comparing the MS/MS spectra with mzCloud library (Figure 3E). Tartrate results are displayed in each sample and diet (Figure 3D.) Figure 3F uses Compound Discoverer software to link tartrate into the KEGG Pathway.

Table 3. Comparing the features found for all three diets.

Groups (p <0.01)	IC-MS (1028)
Davis / CA	118
Davis / USA	104
CA / Davis	373
USA / Davis	294

Figure 3A. PCA results show significant differences.

Figure 3B. Volcano plot in Compound Discoverer software. Further analysis is done on red dot (arrow).

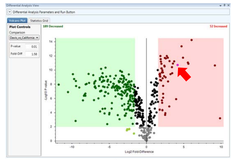


Figure 3C. mzCloud results identify succinic acid.

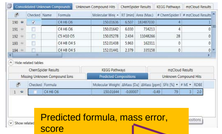


Figure 3D. Compound Discover software compares tartaric acid presence in three diets.

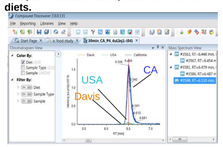


Figure 3E. Compound Discover software query spectrum with mzCloud reference entry.

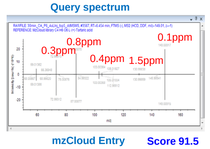
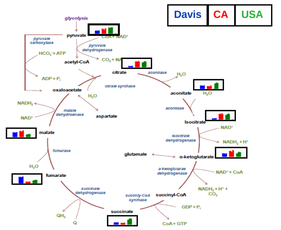


Figure 3F. Compound Discoverer software links metabolite to KEGG pathway.

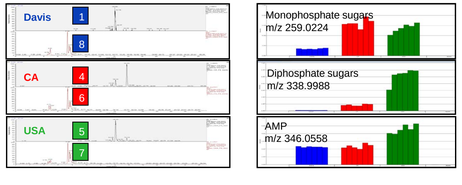
Many of the small polar compounds found would be TCA metabolites. As an illustration of IC-HRAM analysis coverage, these compounds are shown in Figure 4.

Figure 4. Identified features in foods samples that are also TCA metabolites.



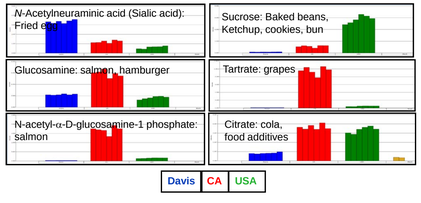
Monophosphate and diphosphate sugars and nucleotides, such as AMP are also well resolved and easily detected by IC-HRAM (Figure 5). These compounds are typically difficult to resolve by HILIC and RP chromatography methods.[1]

Figure 5. Data analysis flow path for Compound Discoverer software.



The three food groups were significantly different. Six examples are shown in Figure 6. For example, a sialic acid compound is attributed to the fried egg in the Davis sample; sucrose is attributed to the sweet items in the USA (Coke typically contains high fructose corn syrup); Tartrate is the characteristic acid in grapes; glucosamine compounds are attributed to the salmon; Citrate is a natural compound and a food additive.

Figure 6. Data analysis flow path for Compound Discoverer software.



CONCLUSIONS

- IC coupled with the HRAM of the Q Exactive HF Hybrid Quadrupole-Orbitrap MS instrument provides a superior method to resolve small polar metabolites.
- The food samples were complex, only a small portion of the results are shown here.
- The food groups had significant differences that could impact metabolomics cycles and impact health.

REFERENCES

1. Wang, J., Christison, T.; et al. *Anal. Chem*., **2014**, *86 (10)*, 5116–5124.
2. Hu, Shen; Wang, J., et al. *Anal. Chem*., **2015** DOI:10.1021/acs.analchem.5b01350
3. Wang, J.; Christison, et al. Thermo Scientific Application Note 622. **2015**.

ACKNOWLEDGEMENTS

We would like to thank Dr. Oliver Fiehn and UC Davis Center of Metabolomics for providing these food samples and developing and managing this comparison study.

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC