POSTER NOTE

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

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ABSTRACT

Previous results demonstrated that ion chromatography (IC) had superior sensitivity and separation over other liquid chromatography methods for small polar metabolites when coupled to a high resolution accurate (HRAM) mass spectrometer using Thermo Scientific™ Orbitrap™ technology mass spectrometers.[1] 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. The data was analyzed with Thermo Scientific data analysis tools.

INTRODUCTION

The analysis of small polar metabolites is critical to understanding many of the metabolic disturbances that occur 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. Of the lypholized samples, 2 mg (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.

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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

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).

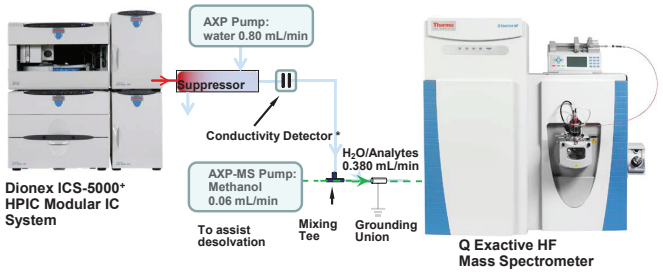
ESI negative mode. Full mass scan: m/z 67-1000, resolution: 120,000 (FWHM) at m/z 200, automatic gain control (AGC) target: $1x10^6$ ions, maximum ion injection time (IT): 50 ms. Data dependent MS^2, Top 10, AGC 2e5, IT 100ms, Underfill 1%.

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 Q Exactive HF MassSpectrometer .



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

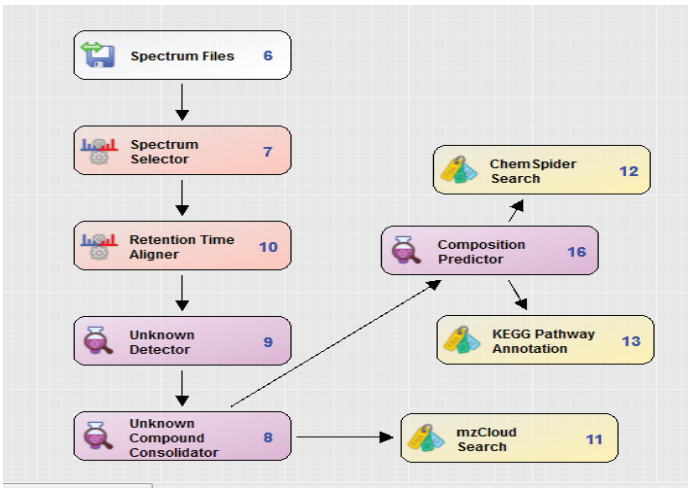


Figure 3B. Volcano plot in Compound Discoverer software. Further analysis is done on red dot (arrow).

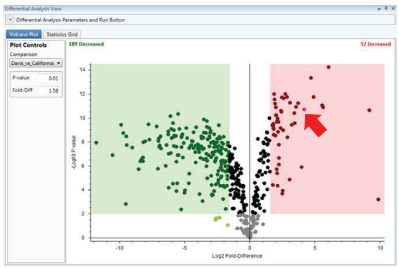


Figure 3C. mzCloud results identify succinic acid.

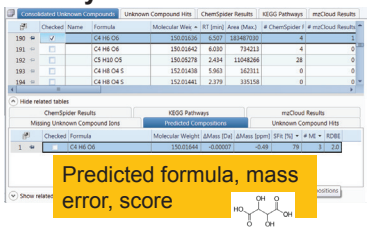


Figure 3D. Compound Discover software compares tartaric acid presence in three diets.

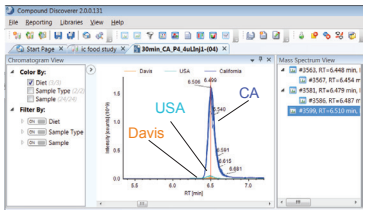


Figure 3E. Compound Discover software query spectrum with mzCloud reference entry.

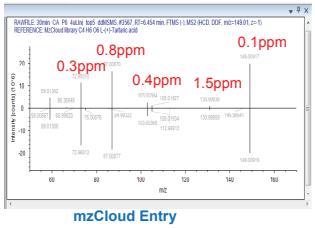
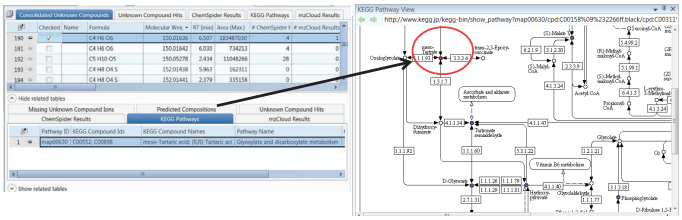
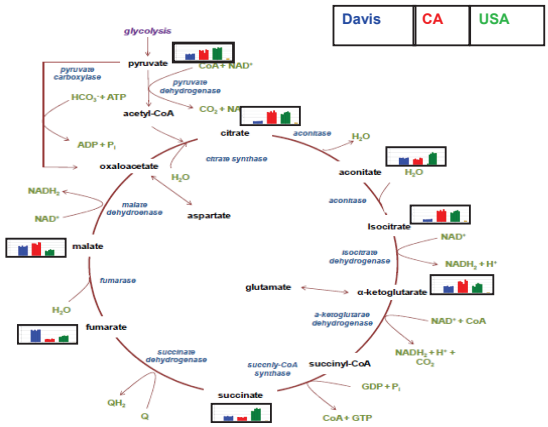


Figure 3F. Compound Discoverer software links metabolite to KEGG pathway.



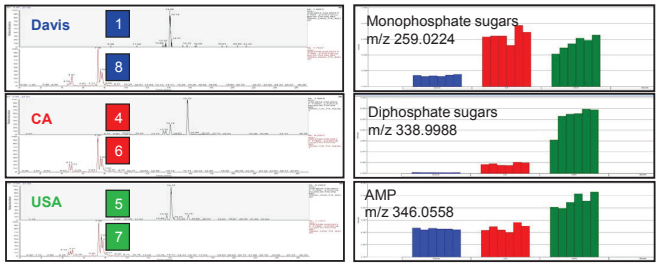
Many of the small polar compounds found would be TCA metabolites. As an illustration of IC-HRAM analysis coverage, these compounds are shown in Figure 4.

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.



CONCLUSIONS

- IC coupled with Orbitrap HRAM of the Q Exactive HF Orbitrap MS instruments 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. AN622. Thermo Fisher Scientific 2015.

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