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Differences in Metabolic Profiles of Individuals with Heart Failure Using High Resolution GC/Q-TOF

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Introduction

Heart failure (HF) is a clinical condition that significantly affects the quality and duration of life of the individuals and is a major global public health problem affecting 23 million people worldwide [1]. Approximately a half of HF cases are with reduced left ventricular ejection fraction (HFrEF) while another half is characterized by preserved ejection fraction (HFpEF) [2]. Both pathologies have similar morbidity and mortality, however, no effective treatment exists for HFpEF [2]. In this study we have utilized accurate mass GC/MS technique to perform metabolic profiling of individuals with both HFrEF and HFpEF in order to identify underlying mechanisms of this pathology that could be helpful and designing an effective treatment.

Figure 1. Agilent 7250 GC/Q-TOF

Experimental

Blood plasma was collected from subjects from HFrEF and HFpEF groups as well as healthy individuals (10 samples per each group). 30 µl of each blood plasma sample were extracted using 1 mL of acetonitrile:isopropanol:water (3:3:2). 450 µl of extract were dried, derivatized by O-methoximation followed by trimethylsilylation with MSTFA + 1 % TMCS. GC/MS analysis was performed using the 8890 GC coupled to the 7250 Q-TOF (Figure 1). The GC method was retention time locked to d27 myristic acid. Further details of the data acquisition method are shown in Table 1.

GC and MS Conditions:	Q-TOF (7250)
GC	8890
Column	DB-5MS UI, 30 m, 0.25 mm, 0.25 µm, DuraGuard, 10m
Inlet	SSL, 4-mm UI liner single taper
Injection volume	0.2 µL
Injection mode	Splitless
Inlet temperature	280°C
Oven temperature program	50°C for 0.5 min; 10°C/min to 325°C, 10 min hold
Carrier gas	Helium
Column flow	1 mL/min
Transfer line temperature	280°C
Quadrupole temperature	150°C
Source temperature	200°C
Electron energy	70 eV
Emission current	5 µA
Spectral acquisition rate	5 Hz
Mass range	50 to 1200 m/z

Table 1. GC/Q-TOF Acquisition Parameters

The chromatographic deconvolution and library search were performed in the MassHunter Unknowns Analysis 11.1. The accurate mass Metabolomics Personal Compound Database and Library (PCDL), unit mass Fiehn Metabolomics library as well as NIST 20 were used to perform initial compound identification. Retention Indices of all three libraries were utilized to confirm the compound ID. Statistical analysis was performed in Mass Profiler Professional (MPP) 15.1. Structure elucidation was performed using the Molecular Structure Correlator (MSC) 8.2.

Metabolic Profiling

To identify metabolites involved in HFrEF and HFpEF pathophysiology, we have performed an untargeted metabolic plasma profiling of HF subjects as well as healthy individuals using a high-resolution GC/Q-TOF. The chromatographic deconvolution was performed using a highly sensitive and efficient SureMass algorithm based on profile data, specifically optimized for complex high resolution EI spectra. Following the deconvolution and library search in Unknowns Analysis (Figure 2), the components identified in method blanks were automatically subtracted from plasma samples. Accurate mass information as well as both FAMEs and Kovats retention indices (RIs) were used to confirm compound identification. The results were exported as .CEF files for further processing in MPP.

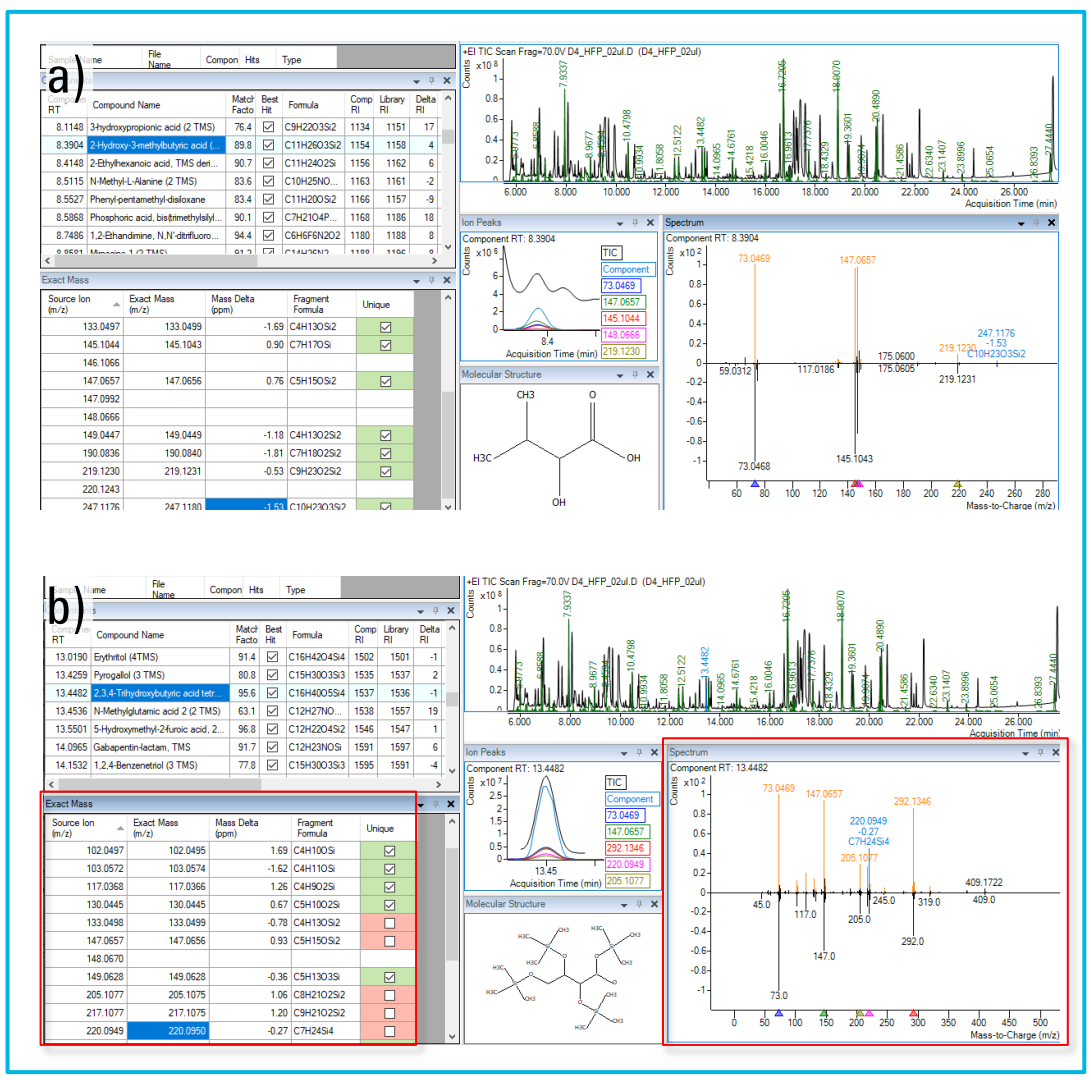

Figure 2. Deconvolution and library search results in Unknowns Analysis using A) Accurate mass Metabolomics PCDL; B) NIST 20 library. ExactMass tool (shown in outlined in red rectangles) helped to eliminate false positives based on accurate mass, this is particularly important when using unit mass libraries such as NIST. Compound ions in mirror plot are highlighted when m/z corresponds to the library hit formula.

Differential Analysis

Statistical analysis was performed in the MPP where the differences between the HF subjects and the healthy controls have been evaluated.

To compare metabolic profiles of HF subjects and healthy individuals, the samples from the subjects of two HF pathologies have been grouped together and formed a distinct cluster from the controls (healthy individuals) as can be seen on the Principal Component Analysis (PCA) plot (Figure 3).

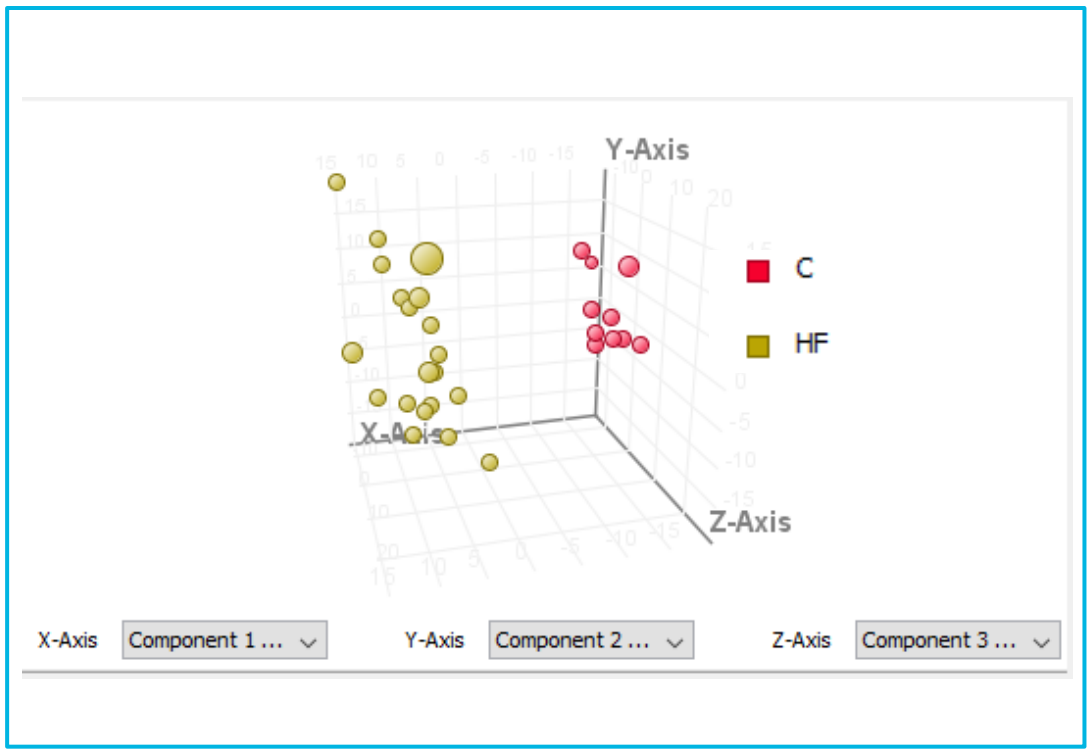

Figure 3. PCA plot showed a clear separation between the clusters of plasma samples from HF (HF) and healthy individuals (C).

Striking differences between metabolic profiles of the HF subjects as compared to the healthy individuals have been observed when using the Fold Change Analysis and visualized on a Volcano plot (Figure 4). Notably, the identified metabolites present at higher concentrations in the control samples were predominantly amino acids, while among metabolites identified at higher concentrations in plasma of HF subjects were organic acids, a few sterols and N-containing compounds, some of which could possibly be metabolites of imidazole-based drugs.

Much smaller differences were observed between the HFrEF and HFpEF groups. One of the metabolites with higher plasma levels in some of HFpEF subjects was identified as iminodiacetic acid.

Results and Discussion

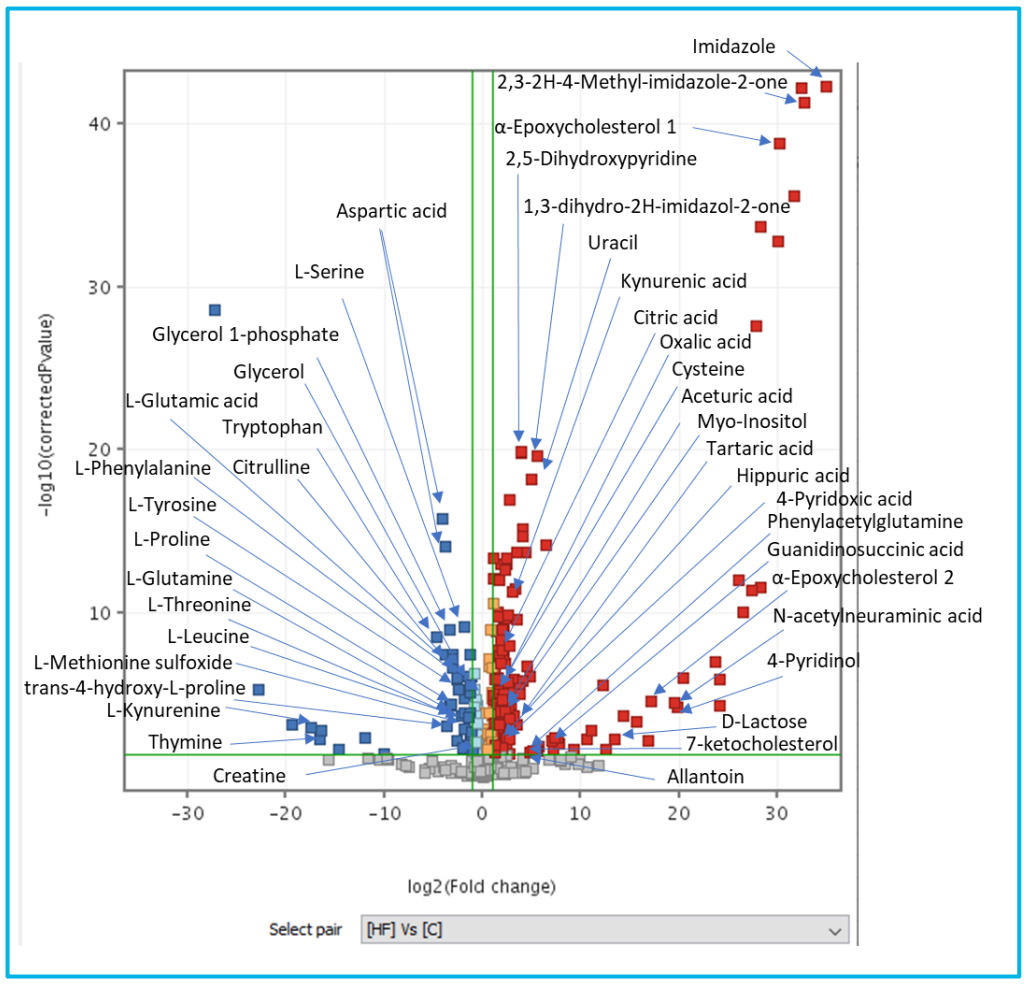

Figure 4. Volcano plot showing log of fold change (cut-off 2) vs. log of p-Value (cut-off 0.05) for HF subjects vs. healthy individuals.

Identification of the Unknown Metabolites

Structure elucidation was performed for one of the unknown metabolites that was present at higher levels in the plasma of HF individuals as compared to the healthy controls. Positive CI using methane (at 20%) as a reagent gas helped to identify the molecular ion of the unknown based on the presence of [M+H]+ and [M+C_2H_5]+ adducts (Figure 5A).

Further, MS/MS was performed in EI at CE = 20V using the molecular ion as a precursor (Figure 5B).

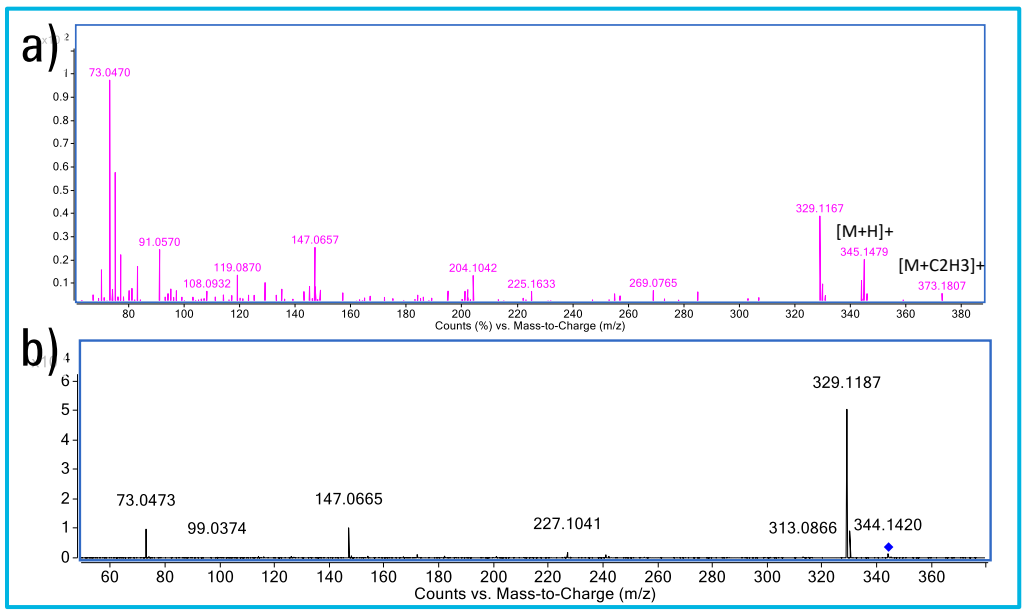

Figure 5. Positive CI (A) and EI MS/MS (B) spectra of the unknown metabolite detected in plasma of HF subjects.

Subsequent structure elucidation of the unknown was performed in MSC software using ChemSpider database as a source of the molecular structures (Figure 6).One of the top ranked structures came up as pyrimidine-2,4,6-triol.

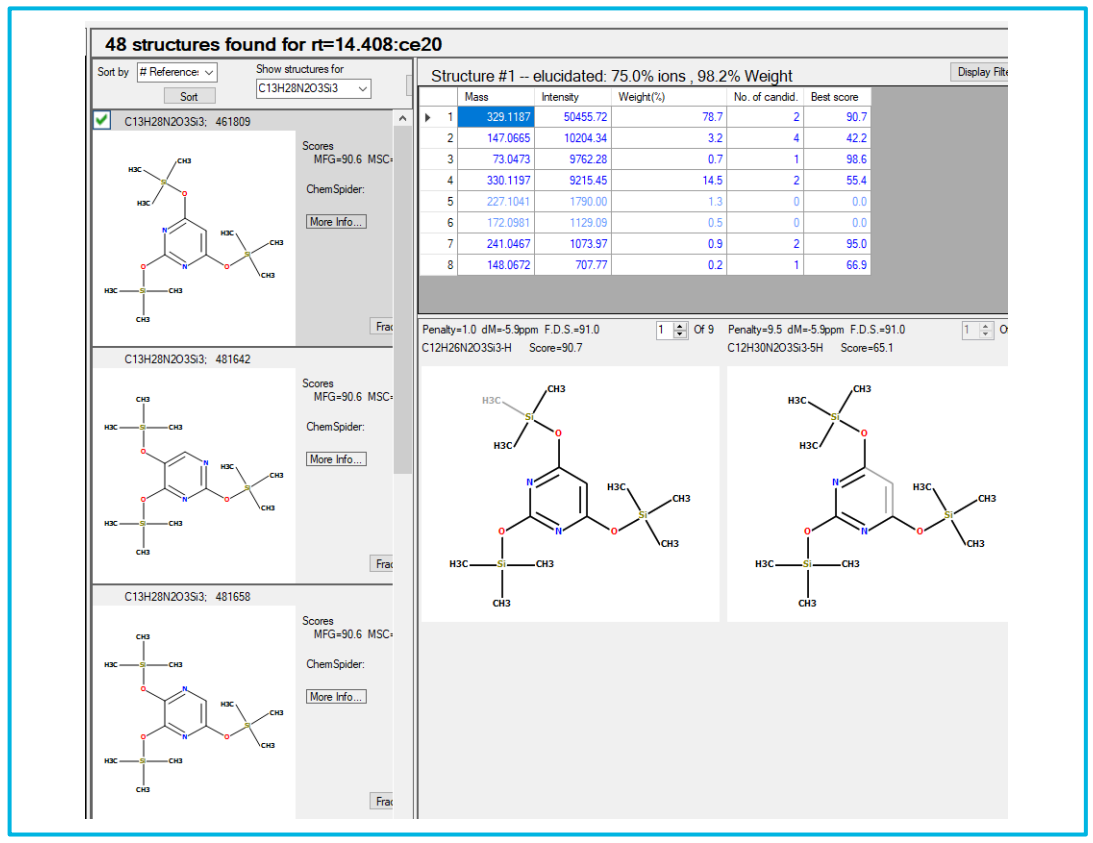

Figure 6. Structure elucidation of the unknown in MSC based on MS/MS data using molecular ion as a precursor.

Conclusions

- Metabolic profiling of heart failure individuals has been conducted using a high-resolution GC/Q-TOF
- Over 40 metabolites have been identified as responsible for the differences between HF and heathy individuals including amino acids, organic acids and sterols
- Iminodiacetic acid was detected at higher levels in some HFpEF as compared to HFrEF individuals

References

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[2]Borlaug B. A.; Paulus W. J.; Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. Eur Heart J. 2011 Mar; 32(6): 670–679.

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