Characterizing Small Molecules in Biological Extracts using IntelliXtract Data Processing on High Resolution Accurate Mass Time-of-Flight Data

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INTRODUCTION

To detect small molecules in biological extracts, LC/MS performed using high resolution time of flight with accurate mass analysis is a powerful technology. Together with advanced data processing algorithms, accurate mass, and relative isotope abundance measurements, molecular formulae can be determined and compounds identified. Compounds excreted or secreted by organisms can reveal aspects of their normal metabolism or disease states. Illustrative examples include profiling of plasma to investigate the Zucker rat animal model of obesity/diabetes, and probing of bacterial cultures for siderophores—compounds used to scavenge trace metals.

METHODS

Data Acquisition

Plasma samples from lean, fatty, and obese Zucker rats were prepared and analyzed using LC-high resolution mass spectrometry (HRT). Samples were filtered (5000 MWCO, Microcon), then diluted 5x and analyzed. Siderophores were extracted from cell cultures (yersinia) and the isolates analyzed using an LC-HRT. Data were imported to ACD/MS Manager v12.01 software using a new custom developed import filter for HRT data. Molecular formulae could be proposed and the ACD/IntelliXtract COMPARE function was used to process and compare relevant regions of the rat sample chromatograms. Fragmentation algorithms were used to look at in-source dissociation spectra acquired from yersinia samples.

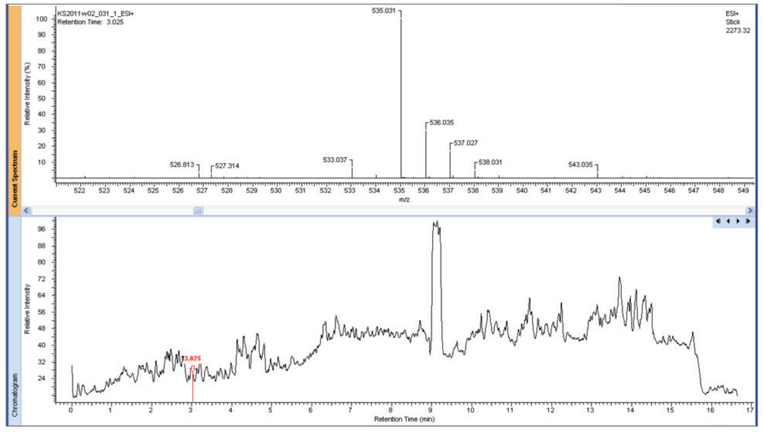

Figure 1. Spectrum of iron siderophore.

RESULTS and DISCUSSION

The three phenotypes of Zucker rat were investigated by LC-HRT analysis of plasma samples, import of the data to ACD/MS Manager software, and then ACD/IntelliXtract COMPARE function processing. Common (similar/different) and unique components were detected with the classification being examined as an approach to distinction. Extracted $[M+H]^+$ ions could be further analyzed to determine molecular formula and compounds consistent with some expected phospholipid and carnitine classes, including carnitine and acyl carnitines C2:0, C3:0, and C4:0.

Formula generation was feasible since measured mass uncertainties of 1 ppm were offered by the LC-HRT operating at R (FWHM) = 50,000 using external calibration and reliable relative isotope abundance. For example, the only elemental formula found within 5 ppm of the C4:0 was the correct one, $C_{11}H_{21}NO_4$, accurate to 4 decimal places. Additional evidence could be provided by in source collision induced dissociation (isCID). The isCID spectra provided fragment ions with accurately measured masses.

For the analysis of bacterial extracts, ACD/IntelliXtract was used. The isotopic envelope of Iron compounds is challenging to detect since the first isotope is only 6% abundant relative to the A+2 isotope and A+1 is dependent solely upon the organic moeity. However, relative isotopic abundance and characteristic mass differences were extremely useful in confidently identifying some metal ion complexes in the mixtures, see Figure 1.

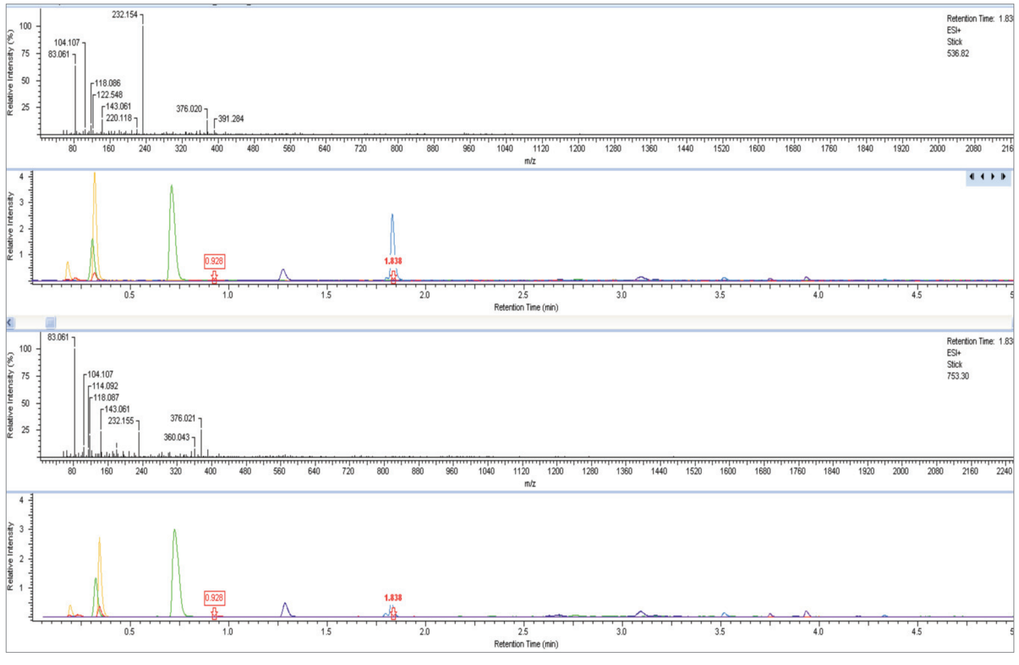

Figure 2. Partial extracted ion chromatograms for acylcarnitines showing differences between obese and lean rats.

CONCLUSIONS

ACD/IntelliXtract COMPARE function processing offers a means to find components that vary between samples. Amino acids and lipids were noted among the components. In the siderophore sample, certain components containing metals gave characteristic isotope patterns. The LC-HRT performance characteristics aid formula generation because of excellent mass accuracy and reliable relative isotope abundance. Further structure information can be gleaned from in source collision induced dissociation (isCID) especially since the isCID spectra provided fragment ions with accurately measured masses.

While it was possible to extract chromatograms, further optimization will be needed to detect iron siderophores reliably since the relatively low abundance of the first two isotopes compared to the third makes them challenging to detect automatically.

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