Overview

- Ion chromatography (IC) was evaluated to demonstrate its ability to highly retain and separate polar metabolites that are poorly retained and resolved by reversed-phase chromatography.
- Ion chromatography was coupled with mass spectrometry (IC/MS) to quantitate a variety of polar organic acids in mouse muscle.
- The organic acids examined cover several metabolic pathways including glycolysis, the pentose-phosphate pathway, the TCA cycle, nucleotides, and amino acids.

Introduction

- Organic acids (OAs) are crucial metabolites that play a pivotal role in a host of different metabolic and regulatory pathways.
- We have developed a novel analytical method to quantitate 27 different polar OAs using ion chromatography/triple quadrupole mass spectrometry (IC/MS).
- The method was used to quantitate OA differences in quadriceps muscles from sedentary mice compared to mice that underwent a low intensity, long duration (LILD) or high intensity short duration (HISD) forced treadmill exercise regimen.
- This pilot study has demonstrated that IC/MS is a powerful new tool to separate and quantitate low molecular weight, polar metabolites that are difficult to analyze by other techniques.

Methods

- Frozen quadriceps from mice were lyophilized with subsequent powdering and homogenization of 5 mg of powder in 500 µL of 50:50 acetonitrile:0.3% formic acid using a Precellys Evolution homogenizer.
- A 50 µL aliquot of homogenate, spiked with 13C, 2H, or 15N internal standards, was derivatized with 50 µL of 0.2 M benzylhydroxylamine to stabilize keto-acids.
- The sample was extracted with 1 mL of ethyl acetate followed by drying down 900 µL of the organic layer with reconstitution in 100 µL of deionized water for IC/MS analysis.
- Calibration curves, spiked with 13C, 2H, or 15N internal standards, (Cambridge Isotope Laboratories) were prepared as the same tissue samples from 0.1-250 µM or up to 5000 µM for lactic acid.
- A Thermo Scientific ICS-5000 ion chromatography system using a Dionex AS11-HC, 4 µm, 2 x 250 mm anion exchange column was used to separate the OAs using a KOH gradient from 5 mM KOH to 100 mM KOH over 11 min.
- The IC was coupled to a Thermo Scientific Quantiva triple quadrupole mass spectrometer to quantitate the OAs by single reaction monitoring via electrospray ionization in the negative ion mode.

Results

- The anion suppressor neutralizes the KOH eluent, protonates carboxylic acids (HX), and removes potassium cations.
- Hydroxide and potassium ions can contaminate the conductivity detector. Also, potassium salts are not volatile by electrospray ionization.

Conclusions and Future Directions

- Ion chromatography is a complementary separation method to HPLC (i.e. ion-pairing and HILIC) to retain and quantitate polar, water soluble organic acids by mass spectrometry.
- Next Steps: Develop IC/MS methods for nucleotides, glycolysis, and pentose phosphate pathway intermediates.

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