A Sensitive LC-MRM Method for Analysis of Nucleotides in Cell Extracts

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Overview

- Purpose
  - To develop sensitive LC-MRM methods for analysis of metabolite levels from small number of cells.
- Methods
  - An Ion pairing reverse-phase column for nucleotide analysis.
  - .An Unison UK-Phenyl column for TCA cycle metabolite analysis.
  - An multi-mode ODS column for positive mode metabolite analysis.
- Results
  - 0.1 nM of cAMP, 1 nM of NAD, NADH and NADP, 5 nM of pyruvate, ATP, ADP and AMP, and 250 nM of lactate are detected with10ul injection.
  - Three sensitive LC-MRM methods have been developed to monitor 160 metabolites.
  - The metabolomics of A549 cells has been studied with the treatment of the cancer metabolism modulator aminooxyacetate (AOA).

Introduction

- Rapid cancer cell proliferation suggests that cancer cells engage in a unique metabolic program. Both nucleotide biosynthesis and ATP consumption promote cancer metabolism[1].
- Many commonly used methods fail to resolve ATP, ADP or AMP chromatographically.
- Several LC-MS/MS methods[2-5] have been reported for determining the nucleotides levels. However, the reported lowest limit of detection for ATP is 60 nM with 10 µL of injection[2] and 50 nM with 50 µL of injection[5]
- In this investigation, we developed a sensitive LC-MRM method for nucleotide analysis.
- We also developed a sensitive LC-MRM method for TCA cycle metabolite analysis.

Methods

- Metabolite stock solutions were prepared at 1 mM on ice and stored at -80 °C.
- Working solutions for quality control (QC) and calibration curve were freshly prepared by serially dilution of stock solutions in HPLC grade water.
- The metabolites were extracted from cells using either 60% MeOH/H_2O or 40/40/20 MeCN/MeOH/H_2O, followed by extraction with water.

LC Condition for Nucleotide Analysis

Mobile phase A	97/3 water/methanol +10mM tributylamine + 3mM acetic acid
Mobile phase B	MeOH
Column	Phenomenex Synergi Polar-RP 75 ×2.0 mm column, 4 µm particle size with 80 Å

Table 1. Limits of detection for nine critical metabolites with 100 msec dewell time and 10 µL of injection.

Metabolite	LOD (nM)
ATP	5
ADP	5
AMP	5
cAMP	0.1
NAD	5
NADH	5
NADP	5
pyruvate	5

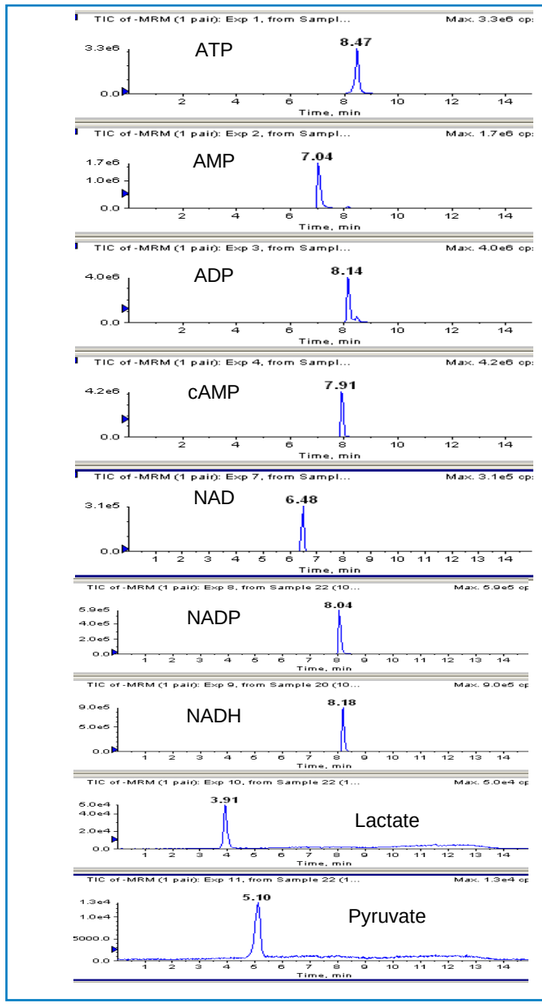


Figure 1. Extracted ion chromatograms for 9 metabolites.

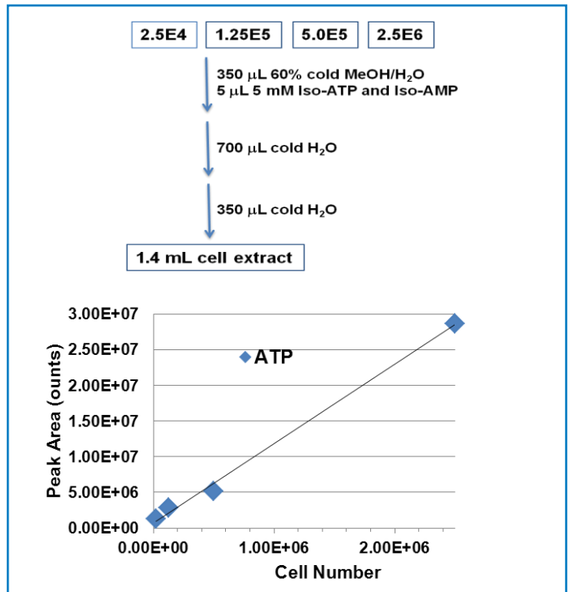


Figure 2. Detected ATP levels in the extracts with the different cell numbers.

Table 2. Three targeted metabolomics methods for monitoring 160 metabolites.

Two negative mode LC-MS methods

Nucleotides 50 metabolites | **Polar organic acids 30 metabolites**

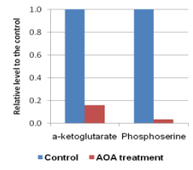
One positive mode LC-MS method

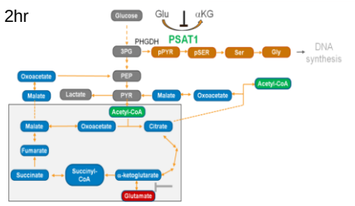
80 metabolites

Pathway	Metabolite	Pathway	Metabolite
Glycolysis/PPP	14	Amino acid biosynthesis	5
TCA	7	Amino acid metabolism	5
Nucleotide	25	Fatty Acid Biosynthesis	7
Deoxynucleotide	6	Fatty Acid Transport	1
Nitrogenous Base	9	Cofactor	5
DNA synthesis	3	ROS	20
Purine Synthesis	3	SAM Pathway	4
Amino Acid	19	other	27

Table 3. Relative levels of metabolites in A549 cells with aminooxyacetate (AOA) treatment relative to control.

Differential Metabolites at 2hr





Conclusions

- LC-MRM methods have been developed to determine the nucleotides and TCA metabolites from the as few as 25,000 cells.
- Two negative and one positive LC-MRM methods identify 50 nucleic acids, 30 polar organic acids, and 80 metabolites; including amino acids and amino acid intermediates, cofactors, nucleic bases and Reactive Oxygen Species-related metabolites.
- Highly sensitive LC-MRM methods were used to monitor the metabolic impact of aminooxyacetate (AOA), a metabolic modulator undergoing evaluation as cancer therapeutic. Profiles provided a metabolic signature including a reduction in TCA intermediates and phosphoserine, suggesting AOA inhibits a-ketoglutarate transaminase (phosphoserine aminotransferase 1 or PSAT1) in A549, a lung cancer cell line.

References

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