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

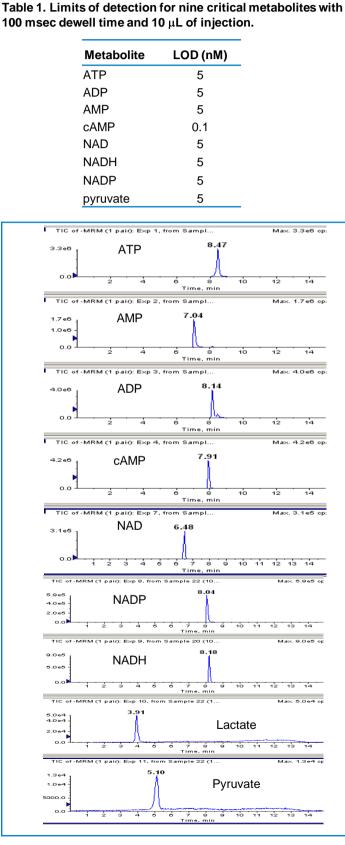
- Rapid cancer cell proliferation suggests that cancer cells engage in a unique metabolic program. Both nucleotide biosynthesis and ATP consumption promote cancer metabolism¹.
- Many commonly used methods fail to resolve ATP, ADP or AMP chromatographically.
- Several LC-MS/MS methods²⁻⁵ have been reported for determining the nucleotides levels. However, the reported lowest limit of detection for ATP is 60 nM with 10 μL of injection² and 50 nM with 50 μL of injection⁵
- 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/H₂O or 40/40/20 MeCN/MeOH/H₂O, 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





	5 μL 5 mM Iso-ATP and Iso-AMP
	700 μL cold H₂O
	350 μL cold H₂O
1.4 mL	cell extract
3.00E+07	
2.50E+07 2.00E+07 1.50E+07 1.00E+07 5.00E+06	◆ATP
0 2.00E+07	
3 1.50E+07	
1.00E+07	
ະ 5.00E+06	
0.00E+00 🚩	
0.00E+00	1.00E+06 2.00E+06 Cell Number
re 2. Detected Al rent cell number	IP levels in the extracts with the

Table 2. Three targeted metabolomics methods formonitoring 160 metabolites.

	Two negative mode LC-MS methods					
	NucleotidesPolar organic acids50 metabolites30 metabolites					
	One p	ositive mode	e LC-MS meth	od		
		80 metal	oolites			
thway	,	Metabolite	Pathway	Metabolite		
lycolysi	is/PPP	14	Amino acid	5		

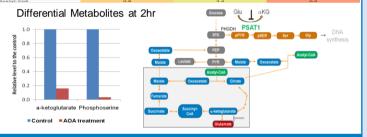
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Pathway	Metabolite	Pathway	Metabolite
Glycolysis/PPP	14	Amino acid biosynthesis	5
ТСА	7	Amino acid metabolism	5
Nucleotide	25	Fatty Acid Biosynthesis	7
Deoxynucleotide	6	Fatty Acid Transport	1
Nitrogenous Base	9	Cofactor	5
DNA synthesis	3	ROS	20
Purine 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.

decrease increas	e 2hr	6hr	24hr
raithing	0.9	1.7	3.4
	4.3	4.2	13
And and an and a second	4.0	4.6	4.3
entesidine	1.3		
tyrasine	1.2	0.9	8.5
EL.			
widized glutathione	1.0	0.6	4.6
anthesine		0.7	4.6
ICAB	0.9 4.1 2.8	0.7 5.7 6.3	4.3 42.5 44.0
aymine	2.8	6.2	4.4.9
envadencine	4.0 2.4		0.8 3.0
racii	0.9 0.7	1.9	2.6
ytasine		0.6	
ytidirae	1.4	1.0 0.6 7.4	2.8
methyluridine		7.4	
aretyl-O-galactesaming	0.9	0.8	3.4
nettrue	0.9	4.8	3.2
roiting	0.6	0.6	10.0
mostrie	0.9	1.2	2.4
enterysteine	0.9	0.8	4.4 4.0 2.6
ating	0.9	4.4	2.6
presine	1.0	1.0	2.3
	1.0	1.3	
henvialanine	1.0	1.0	1.8 2.4
he this is a second sec	4.4	1.1	
eleveine /Leveine Istidine Iveine	1.0	1.3	2.6
lyrine			
lutamine/Lysine	1.0	1.5	3.4 2.0
Strangering	1.1 0.7		4.3 2.5
	1.0	1.3	
radializatione constitutione	1.0	11	4.4
reations	0.9	0.6	1.0
66	0.2	1.0	0.4
anninate	0.4		2.2
aihimate	0.3	4.0	a.s
antethenate			4.6 5.3
EP	0.3	3.0	4.0
-acetyineuraminic acid	0.2		4.5 5.3
lalate	0.3	0.7	0.7
energy GP		3.4	
evenue 10/60	0.8	1.7	2.4
3P/DHAP	4.5	1.3	2.3
Lieurenate		1.1	
ensyrihuse-phosphate	0.5	4.5	2.6
-Rib(ul)ose-S-phosphate			
eetylphesphate	0.5	4.0	2.6
Hydrosyphenylpyruvate	0.7		4.3 4.7 3.6
	0.2	4.4	2.6
TP	0.3		0.7
	0.5	0.4	
UMP			2.0
GNAP	0.7	0.8	0.5
CMP			
COP	0.9	0.6	0.6
S.MP	1.1	0.4	
ADP			0.4
OID .	0.8		
TP MP	0.7	0.3	
DP-D-elucuronate			1.0
DP. D. elucerse DP. D. elucerse DP	1.0	0.4	0.6
narine	0.8	0.5	1.4
ce	0.9	0.3	
ananhunarine	8.2	0.1	
ADDH	4.0 4.1	0.5	
ADP	1.0	3.4	1.4
ADH		0.9	1.0
Net-Intested	1.0	0.8	6.3
alanvi.CoA	0.7	0.3	0.4
ÂD	0.8 1.5 0.8		0.8
furnisar.	0.8	0.6	2.6
De monte de la construcción de l	4.4 3.4		1.4
		0.6	4.7
Te P P P P P P P P P P P P P	0.8		
MP OB	0.9	0.8	5.8 0.4



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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