

METABOLOMICS IN CANCER RESEARCH – INVESTIGATION OF THE TRICARBOXYLIC ACID CYCLE WITH MASS SPECTROMETRY

**Gábor Tóth^{1,2}, Oliver Ozohanics¹, Norbert Szoboszlai¹, Károly Vékey¹,
László Drahos¹**

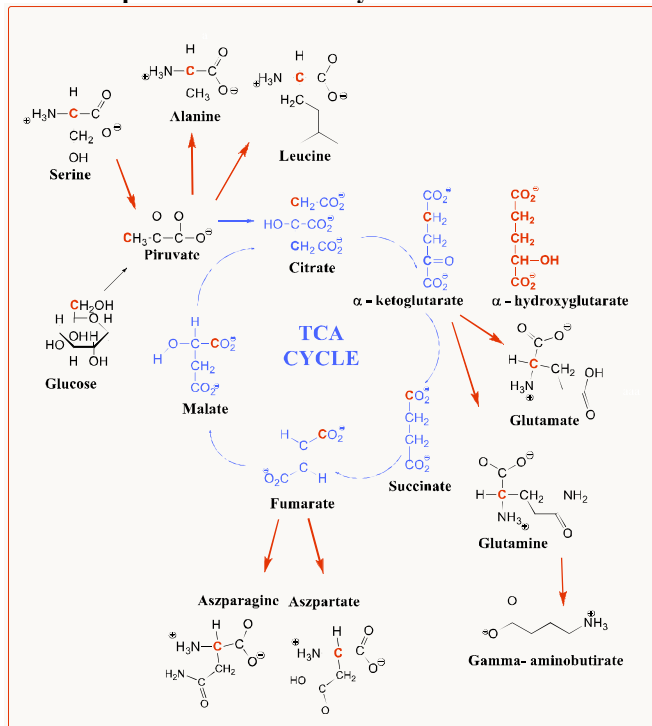
¹ *Research Center for Natural Sciences, Hungarian Academy of Sciences, MS Proteomics
Group, Budapest*

² *Budapest University of Technology and Economics, Budapest*

In medical sciences analytics is constantly gaining importance as it is getting more and more important to know the exact composition of the cells. The tricarboxylic acid (TCA) cycle is one of the main energy production processes of the cells. During the cycle, the energy obtained from the decomposition of glucose is stored in the forms of GTP, NADH and FADH₂.

Tumour cells use up the glucose through the glycolysis pathway 10-50 times faster than normal cells [1]. According to the Warburg's theory the cancer cells take away glucose from normal cells, therefore inhibition of glucose take-up results in cancer cell reproduction slow-down. It implies that the energy production system of the cells has to be investigated. One important marker of the cancer cells is the alpha-hydroxyglutarate [2] which is produced instead of alpha-ketoglutarate in the TCA cycle and this way the cycle is inhibited.

1. Figure: Examined compounds of the TCA cycle with the derivative amino acids



In order to model this process, normal and cancer cells were fed with isotope-marked carbohydrates, thus one can measure the ratio of glucose used up in the different metabolic cycles, respectively.

Undoubtedly, the various chemical properties of metabolites represent a great challenge in their identification in cell cultures. Therefore, a high performance liquid chromatography (HPLC) tandem mass spectrometric (MS/MS) method has been developed for the simultaneous determination of nineteen glycolytic and tricarboxylic acid (TCA) cycle intermediates as well as amino acids.

2. Figure: Limit of quantitation values for the target compounds

Compound	LOQ [pikogram]	Compound	LOQ [pikogram]
<i>Citrate</i>	19.21	<i>Fructose 1,6 bisP</i>	3.40
<i>Hydroxyglutarate</i>	1.48	<i>Glutamate</i>	0.74
<i>Ketoglutarate</i>	2.92	<i>Glutamine</i>	0.73
<i>Malate</i>	2.68	<i>GABA</i>	10.31
<i>Succinate</i>	0.59	<i>Aspartate</i>	0.67
<i>Fumarate</i>	0.58	<i>Asparagine</i>	0.66
<i>Lactate</i>	4.50	<i>Leucine</i>	0.66

<i>Piruvate</i>	0.44	<i>Alanine</i>	0.45
<i>Ribose 5P</i>	2.30	<i>Serine</i>	0.53
<i>Glucose 6P</i>	1.30	<i>Glycine</i>	0.38

These target compounds are highly polar, thus they were separated on a HILIC HPLC column and simultaneously determined by triple quadrupole MS/MS using multiple reaction monitoring. Calibration curves were made for the target compounds in standard stock solutions and the LOQ was calculated.

[1] O. Warburg; *Science*, **1956** (123) 309–314.

[2] Dan Ye, Shenghong Ma, Yue Xiong, Kun-Liang Guan, *Cancer Cell*, **2013** (23) 274–276.