

Differential Mobility Spectrometry Resolves Isobaric Metabolite Overlap for Metabolic Flux Analysis

SelexION® Technology and QTRAP® System Differentiation

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The ability to detect subtle changes in metabolism is key to understand cell homeostasis. While metabolomics offers an instant snapshot of the content of cellular metabolites, it does not provide details on the dynamic interaction between them. Metabolic flux, in contrast, is a measure of the rate of metabolite conversion through the multiple reactions forming a metabolic pathway. Predominant among all metabolic pathways are the ones intersecting the oxidative and anabolic points of mitochondrial metabolism. Glycolysis, gluconeogenesis, glucose/lipid oxidation and TCA cycle are not only common to all living organisms, they are often altered in many disease states like cardiovascular, cancer, inflammation and obesity and diabetes. Stable isotope-labeled tracers, such as $^{13}\text{C}_6$ -glucose, provide a unique window into the study of these metabolic pathways. However, their use in mass spectrometry (MS)-based studies also presents a set of challenges that need to be met for even higher data accuracy and resolution.

The Challenge:

The high sensitivity of modern mass spectrometers coupled to ^{13}C -labeled tracers offers an attractive opportunity to study metabolism. Because the MS detection of metabolites is based on a mass-to-charge ratio (m/z), the extra mass of ^{13}C relative to ^{12}C can be easily detected as increments in m/z . Despite the apparent ease in detection, this approach presents two main challenges:

1. **Obtaining ^{13}C -Positional Information.** With LC-MS, one can assess if one or more carbons of the metabolite of interest were replaced with ^{13}C by determining the deviation from the expected m/z . In a metabolite with mass M and n carbons, the possibility for enrichment varies from $M+1$ to $M+n$. importantly; this approach does not reveal the specific location within the metabolite. When studying metabolism using ^{13}C -tracers, the position of the ^{13}C is extremely important. Distinct reactions can contribute with the same number of ^{13}C but in different positions within the same metabolite. In other words, the pattern of ^{13}C -labeling, but

not the

Glycolysis/Gluconeogenesis Pathway

- Many **isomers**
- Competing **mass isobars**
- Share ***common daughter ions**
- Challenges in **ionization chemistry**

Glycolysis	Pathway Overlap		
G6P	PPP	Glycogen	G1P
F6P	PPP	Glucosamine	F2,6BP
DHAP	G3P shunt	Lipid analysis	Glycerol
GA3P	PPP		
3PG	Serine metabolism	Cysteine metabolism	
2PG	Glycine metabolism		Glutathione

	MW
* G1P	260
* G6P	260
* F1P	260
* F6P	260
* Pser	185
* 2PG	186
* 3PG	186
* DHAP	170
* GA3P	170
* Glyc3P	172
* PEP	168
* 1,3BPG	265
* 2,3BPG	265
* F1,6BP	340
* F2,6BP	340
Pyruvate	88
Lactate	90

Figure 1. Challenges of Isobaric Overlap in Metabolomics. The intermediates common to glycolysis and gluconeogenesis are among the most difficult to resolve using mass spectrometry. While many of these metabolites share the same m/z , even in the absence of mass label, the presence of ^{13}C generates more situations of m/z overlap (isobaric species). In addition, the generation of common daughter ions further difficulties the analysis of mass flow through these pathways. Because there are several points of intersection between glycolysis/gluconeogenesis and other metabolic pathways, the specific detection of these metabolites and their enrichments is of the utmost importance.

- number of ^{13}C , is characteristic of specific reactions and should be determined for accurate flux measurements.
2. **The Presence of ^{13}C Increases the Number of Isobaric Species.** Many of metabolites commonly detected in metabolic studies share the same m/z space. While some are resolved even in nominal mass spectrometers, the addition of ^{13}C can result in overlap of m/z , resulting in artificially altered enrichments. In the absence of chromatographic separation, the overlap of isobaric species

poses a problem for accurate ^{13}C -enrichment calculation and metabolic flux interpretation (Figure 1).

The Solution:

The use of LC-MS/MS (QTRAP[®] system) in combination with SelexION[®] technology permitted the quantitation of a large number of metabolic reactions with an unprecedented high degree of accuracy and specificity¹. The QTRAP[®] system contributes to the resolution of isobaric species by generating and detecting fragments unique to each metabolite. Furthermore, the fragmentation capability of LC-MS/MS enhances the analysis of ^{13}C -label incorporation. The examination of multiple fragments of the same metabolite reveals the pattern of ^{13}C distribution and with it the means to distinguish specific reactions.

The SelexION[®] technology significantly increases the selectivity of the measurements, using differential mobility to transmit only specific metabolites under specific conditions². With SelexION, isobaric and isomeric species are easily separated based on their interaction with a polar modifier in the presence of alternating RF. 3-Phosphoglycerate (3PG) and 2-phosphoglycerate (2PG), two isomers from the glycolysis/gluconeogenesis pathways, cannot be distinguished based on their m/z. However, at increased separation voltage (SV), each isomer has a different compensation voltage (COV) and thus can be separated. A similar separation can be obtained for phosphoenolpyruvate (PEP), another intermediate of the glycolysis/gluconeogenesis pathways (Figure 2). Importantly, the specificity added by SelexION is not affected by the presence of ^{13}C ; it does not require additional complex sample preparation.

Conclusion:

We showed that metabolites from the glycolysis and gluconeogenesis pathway can be easily resolved and quantified using SelexION Technology coupled with the QTRAP system. The combination of these two technologies is a powerful workflow to resolve complex metabolic information.

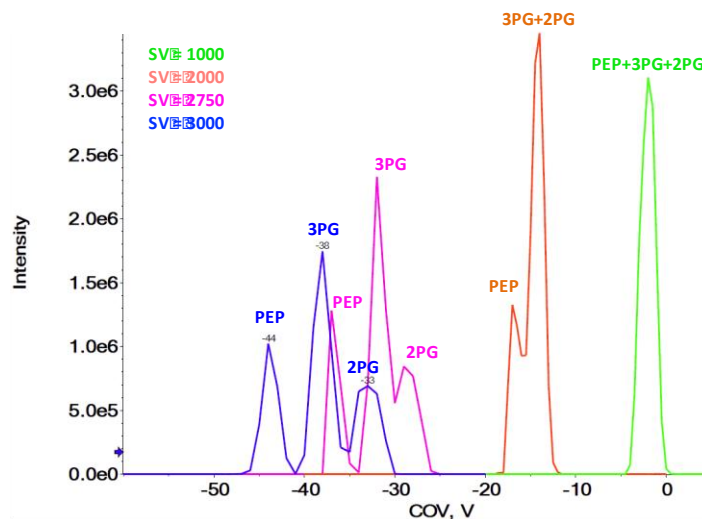


Figure 2. Differential Mobility Spectrometry Resolves Isobaric Overlap. The separation of 3-phosphoglycerate (3PG), 2-phosphoglycerate (2PG) and phosphoenolpyruvate using SelexION technology was tested using four different separation voltages (SV). Using isopropanol as a modifier reagent, high SV voltages were effective in resolving all metabolites.

References:

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2. Campbell JL, Le Blanc JC, Kibbey RG. Differential mobility spectrometry: a valuable technology for analyzing challenging biological samples. *Bioanalysis* (2015), 7(7):853-6

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Document number: RUO-MKT-02-6150-A