# Differential mobility spectrometry resolves isobaric metabolite overlap

***Using SelexION® Technology and QTRAP® System***

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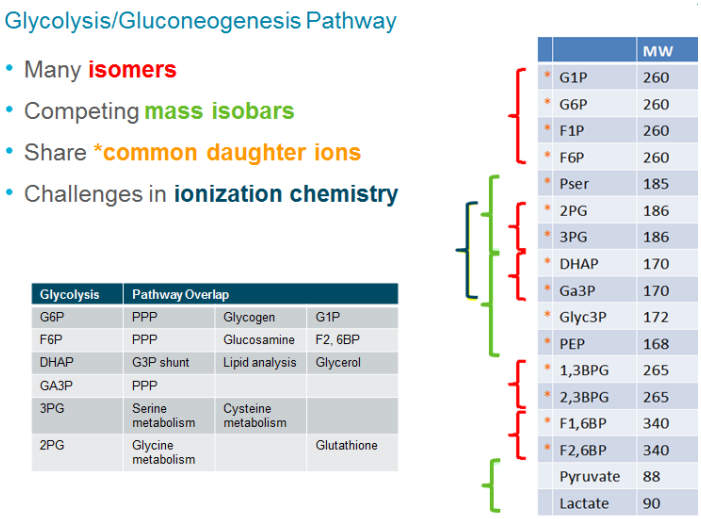
The ability to detect subtle changes in metabolism is key to understand cell homeostasis. While metabolomics offers an instant snapshot of the content of cellular metabolites, it does not provide details on the dynamic interaction between them. Metabolic flux, in contrast, is a measure of the rate of 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 $^{13}C_6$-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.




| 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 |

**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 difficults the analysis of mass flow through these pathways. Because there are several points of intersection between glycolysis/gluconeogensis and other metabolic pathways, the specific detection of these metabolites and their enrichments is of the utmost importance.

## 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 number of $^{13}C$, is characteristic of specific reactions and should be determined for accurate flux measurements.
2. **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 specificity.[1] 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 conditions.[2] With SelexION Technology, 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 Technology is not affected by the presence of $^{13}$C; it does not require additional complex sample preparation.

## Conclusion:

The resolution and quantification of metabolites from the glycolysis and gluconeogenesis pathway using SelexION Technology coupled with the QTRAP System is demonstrated here. The combination of these two technologies is a powerful workflow to resolve complex metabolic information.

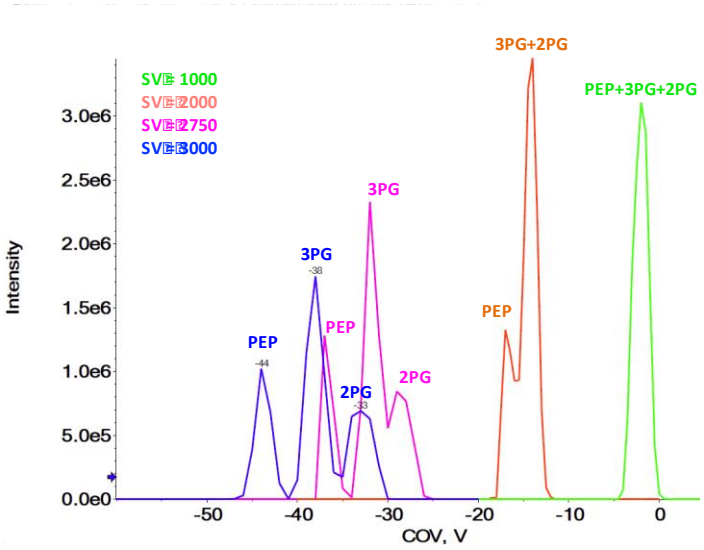



**Figure 2. Differential mobility spectrometry resolves isobaric overlap.** The separation of 3-phosphoglycerate (3PG), 2-phosphoglycerate (2PG) and phosphoenolpyruvate using SelexION Technology was tested using four different separation voltages (SV). Using isopropanol as a modifier reagent, high SV voltages were effective in resolving all metabolites.

## References:

1. Alves TC, Pongratz RL, Zhao X, Yarborough O, Sereda S, Shirihai O, Cline GW, Mason G, Kibbey RG. (2015), Integrated, Step-wise, Mass-Isotopomeric Flux Analysis of the TCA Cycle. *Cell Metab.* **22(5)**: 936-947.

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



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