

Quantitative Lipid Analysis using MRM and Differential Ion Mobility Spectrometry (DMS)

SelexION® Technology

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Triple quadrupole / QTRAP® mass spectrometers using Multiple Reaction Monitoring (MRM) provide the highest sensitivity and specificity for quantitative workflows of biomolecules in complex mixtures. In the field of lipidomics, quantitative analysis of lipid extracts is particularly challenging owing to the extensive isobaric overlap present in the lipidome, and extensive method development is usually required to maintain specificity during analysis.

The Challenge:

In addition to isobaric overlap, complex lipids have many of the same fragment ions due to there only being a discrete number of fatty acid species acylated to the lipid glycerol backbone. As a consequence, different lipid molecular species can have the same MRM transition, which leads to interfering peaks in the LC-MRM chromatogram and can cause significant problems in quantitation. The increasing demand for high throughput analysis with ultra-high-performance liquid chromatography (UHPLC) precludes the extended chromatographic run times needed to separate critical molecules.

The Solution:

SelexION Technology (DMS) can be used to greatly reduce isobaric interferences in lipid analyses. DMS uses a discrete, lipid class-specific compensation voltage (CoV) to filter ions into the mass spectrometer and prevents precursor lipid isobars from contributing to MS/MS spectra. Using reverse phase chromatography and DMS, lipids are separated using LC by their fatty acid composition, and lipid classes are separated using DMS by a class-specific CoV. This results in unparalleled selectivity, a general reduction in noise, which can improve the overall S/N and sensitivity of the targeted lipid assay.

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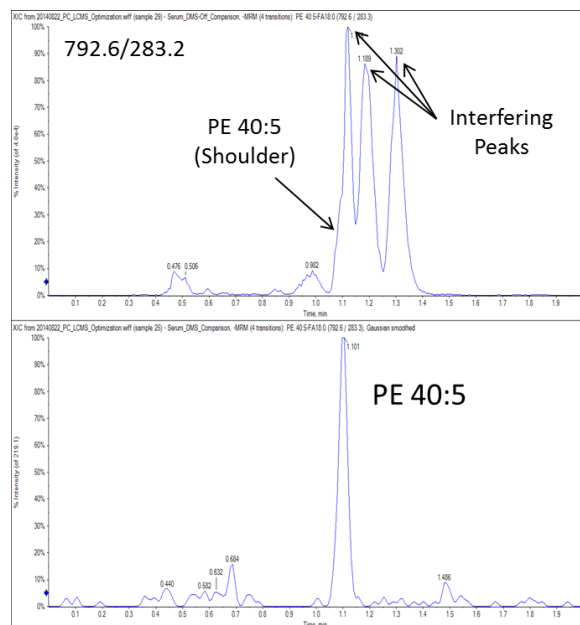


Figure 1. SelexION Technology Removes Isobaric Interferences During MRM Quantitation. Pooled human serum lipid extract was analyzed by LC/MS/MS in negative ion mode. A QTRAP® 5500 System equipped with SelexION™ Technology was used for targeted lipid quantitation using reverse phase UHPLC to partially resolve lipid molecules using a two-minute gradient. An MRM transition for the molecular species PE 40:5 yielded an LC chromatogram with significant isobaric interference (A). The target peak is actually a shoulder on an adjacent peak, which prevents peak integration and accurate quantitation. However, when a PE-specific CoV value (-3.8 V) is associated with this MRM transition, the interfering peaks are removed from the chromatogram, leaving a clear, single component peak.

SelexION Technology removes isobaric interferences during MRM analyses to improve quantitative results in complex mixtures.