Omics Applications
SelexION® Differential Mobility Spectrometry Technology
RUO-MKT-11-6677-A
SelexION® Technology

A New Dimension in Selectivity

• Differential Mobility Separation (DMS)
• Installation / removal of DMA in < 2mins – no tools required
**Differential Mobility Separation - DMS**

- Planar geometry
- Gas flow towards MS draws ions (transport gas)
- Asymmetric waveform applied which alternates between high field, $K(E)$ and low field, $K(0)$ – separation voltage (SV)
  - Moves charged ion back and forth between plates
  - Ion will have net drift based on its high and low field mobility
- Compensation voltage (COV) is small DC offset between the plates – filtering voltage

**DMS Dimensions**
1x10x30 mm

**Waveform**

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Modified Transport Gases Improve Separations

- Polar modifiers can be added into transport gas (curtain gas)
- Enhances the formation of clusters in field-dependent way, which amplifies the high and low field mobility differences
  - Dynamic cluster – decluster model
- Uniqueness of these cluster interactions
  - Increase selectivity of the separation
  - Decrease low field mobility relative to high field
  - Which increases the compensation voltage (CV) and therefore the peak capacity
Lipidomics Applications
Challenges in Lipidomics Analysis

Isobaric Overlap of Phospholipids

- Lipidomic spectra are very complex
- Problem: The Q1 isolation window during MS/MS is ~1.2 Da, which increases the number of potential isobars.
- MS/MS spectra generated on precursors in zones of isobaric overlap will contain product ions from other isobaric species

Experiment: EMS (−) scan of Bovine Heart Extract (BHE)
Lipidomics Applications

Separation of Phospholipid Classes

• Possible to achieve baseline separation of all six phospholipids without using chromatography.

• As a proof of concept, the phospholipid classes are separable by DMS, which implies elimination of inter-class isobaric interference and improved qualitative and quantitative lipidomic data.

Experiment: MRM (-) scan of 6 phospholipid standards with COV ramp
Lipidomics Applications

Separation of Glycosylceramides (Cerebrosides)

• Isolating individual glycoforms of cerebrosides, such as Galβ1-1'Cer and Glcβ1-1'Cer, difficult to achieve due to the virtually identical structures of these isobaric lipids, whose only difference being the stereochemistry of the 3’-hydroxylgroup.

• Differential Mobility Separation (DMS) effectively resolves these two isomers to enable independent confirmation and quantitation.

Galactosylceramide and glucosylceramide standards were infused individually (top) or together (bottom) and analyzed using SelexION® Technology.
Lipidomics Applications

Resolution of Ether- and Diacyl-Linked Phospholipids

• Ether-linked and diacyl-linked phospholipids are near isobars

• Necessity to resolve ether-linked and diacyl-linked species to characterize their molecular species compositions

• Even with high resolution MS, product ion analysis depends on a low-resolution isolation step that would generate a non-specific, convoluted MS/MS spectrum from the nearly isobaric compounds

DMS allows resolution of these phospholipid subclasses.
Lipidomics Applications

Separation of Iso-Elemental Lipid Species

- Ability to distinguish these iso-elemental species is critical for the characterization, identification and quantitation of lipids

- Using SelexION® Technology on a TripleTOF® 5600 System, a lung lipid extract was infused and the DMS CoV was ramped from -40 to 20V in the negative ion mode.

An XIC of the 750.5446 m/z peak reveals two peaks in the corresponding ionogram. Isopropanol used as DMS modifier.
Lipidomics Applications

Quantitative Lipid Analysis

• Lipid molecular species can have the same MRM transition, which leads to interfering peaks in the LC-MRM chromatogram.

• This can cause significant problems in quantitation.

• An MRM transition for the molecular species PE 40:5 yielded an LC chromatogram with significant isobaric interference (top).

• The target peak is actually a shoulder on an adjacent peak, which prevents peak integration and accurate quantitation.

Pooled human serum lipid extract was analyzed a QTRAP® 5500 LC-MS/MS System equipped with SelexION® Technology for targeted lipid quantitation.
Resolution of Sphingomyelins

- Sphingomyelins (SM) share a common, diagnostic fragment with phosphatidylcholines (PC) - the phosphocholine head group (m/z 184, positive ion mode).
- High resolution mass spectrometry can resolve these two classes of lipids.
- SM and PC are resolved from one another using lipid class-specific compensation voltages (CoV) prior to MS analysis that removes isobaric interference and generates class-homogenous ion spectra.
Lipidomics Applications

Separation of Phospholipids

- The Lipidyzer Platform is equipped with SelexION® technology which allows the separation of phospholipids by their head groups before subsequent MRM analysis.
Lipidomics Applications

Separation of Leukotrienes

- Top: LC-MS/MS analysis of an ethanol extract from peritoneal cells monitoring the SRM transition $m/z$ 335→19519. DMS voltages turned OFF
- Middle and bottom: Analysis of murine peritoneal cell ethanol extracts using LC-MS/MS with DMS voltages turned ON.
- SelexION® Technology allows the resolution of leukotrienes in complex samples

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Another class of biologically relevant isomeric substances we investigated using the SelexION® Technology for was the separation of PD1 and PDX. Direct infusion data for the successful separation of these two isomers is highlighted below.

A 50 ng/mL solution was used for DI experiments with low resolution gas settings and a SV of 4500V.
Lipidomics Applications

Reduction of Background Interference to Enable Retinoic Acid Analysis

**Common Method**
LC-APCI with -MRM
Pooled Liver Extract PA1

Isobaric overlap and high background make quantitation impossible

**LC-DMS-ESI with -MRM**
Pooled Liver Extract PA1
Confident and accurate quantitation
Lipidomics Applications

Isolation of Individual Lipid Classes for Analysis with MS/MS\textsuperscript{ALL}

- Clean MS/MS data without isobaric contamination
- Reduced background
- Improved ID confidence

\[
\text{PC MS/MS}^{\text{ALL}} - \text{CoV} = -6.5
\]
Lipidomics Applications

Removal of Isobaric Interferences of Phospholipids

Experiment: TOFMS analysis of BHE with DMS; 3 separate experiments with PL class-specific CoV values

PC (CoV = -6.7V)
PE (CoV = -4.6V)
PS (CoV = 0.5V)
Correct Identification of PC Lipid Molecular Species

Experiment: Product ion spectrum (TOF MS/MS) of bovine heart extract (m/z 790.6); DMS tuned for PC
Metabolomics Applications
Challenges in Metabolomics Applications

Isobaric Overlap

- The intermediates common to glycolysis and gluconeogenesis are among the most difficult to resolve using mass spectrometry.

Glycolysis/Gluconeogenesis Pathway

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

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<th>Pathway Overlap</th>
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Separation of Glycolysis Pathway Metabolites

- 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.
Metabolomics Applications

Separation of Glycolysis Pathway Metabolites

- DMS parameters were tuned by infusing pooled analyte solutions and ramping separation voltage (SV) and compensation voltage (CoV).
- SelexION® technology enabled accurate quantitation with typical detection levels of <2nmol on column.
Metabolomics Applications

Separation of TCA Cycle Metabolites

- SelexION® technology enabled accurate quantitation with typical detection levels of <2nmol on column.

![Graph showing separation of TCA Cycle Metabolites](image-url)

1. pyruvate
2. lactate
3. taurine
4. succinate/glutamine
5. glutamate/aspartate
6. malate/alpha keto glut
7. citrate
Metabolomics Applications

Separation of TCA Cycle Metabolites

Maleic acid

Fumaric acid

DMS Off

DMS On
Metabolomics Applications

Flux Analysis on TripleTOF® System applying SWATH® Acquisition

- Protection of flux isotope whilst removing background interference
Proteomics Applications
Improving LLOQs of Peptides in Matrix

High Selectivity of Peptides

- Single ion monitoring (SIM) of +6 and +7 charge state of BNP with and without DMS.
- **DMS Off** – Significant interference is observed in blank matrix for the 6+ charge state and background noise for the 7+
- **DMS On** – Background / interference at elution time of peptide significantly drops
- Signals from both charge states at **128** and **640 pg/mL** can now be seen above the noise
- Ion mobility separates the interfering matrix peaks away from the BNP peptide of interest thus providing a better S/N and a substantially improved LLOQ
Improving LLOQs of Peptides in Matrix

Separation and Quantitation of Large Peptides

- Poor fragmentation of large peptides can sometimes limit detection with MRM, making SIM a more desirable approach, although often limited in selectivity
- Top – MRM signal of 30 kDa peptide in plasma at 125 ng/mL
- Middle – SIM signal of same peptide in plasma at 16 ng/mL
- Bottom – DMS + SIM at 16 ng/mL provides large improvement in S/N
- An LLOQ of 4 ng/mL for a 30 amino acid peptide (~4 KDa) was achieved in protein precipitated rat plasma, an ~30x improvement over the MRM analysis strategy
Localization - Di-Phosphorylated Peptides

Separation of \( \text{NpSTLpSEEDYEIK} \) and \( \text{NpSTLSEEDpYIEK} \)

- Isobaric phosphopeptides can be separated by DMS
Localization - Variants of Acetylated Peptides

Separation of KAcQLATKAAR and KQLATKAcAAR

- Acetylated peptides with different acetylated lysine residues can also be successfully separated by SelexION® Technology
Streamlined, Iterative Tuning of Peptide CoV

Three Step On-Column Tuning using Skyline with QTRAP® Systems

- After MRM development and retention time determination, next step is to tune the compensation voltages for each peptide
- 3 step on column tuning strategy for large number of peptides simultaneously

![Graphs showing tuning process](graph.png)
Thank You

It’s Time to Uncover What’s Beyond the Genome
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