# Streamlined Analysis of Polar and Non-Polar Metabolites in Plasma Using Automated Micro-SPE LC/MS

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**COI Statement:** The authors declare a potential conflict of interest. HMG, RP, and GB are employees of CTC Analytics AG, the manufacturer of the PAL System automation platform and (to some extent) consumables used in this study. The study was conducted independently, and the results reflect the authors' objective assessment of the technology.

**Learn more** about µSPE

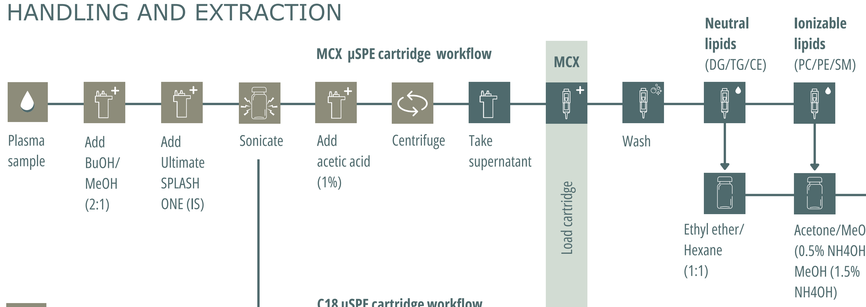
## AT A GLANCE

Here, we present two use cases for streamlined lipid profiling in plasma using automated Micro Solid Phase Extraction (µSPE). We tested the workflows on NIST (SRM 1950) plasma samples, with spiked internal standard (Ultimate SPLASH ONE) and optimized the extraction and elution parameters. Briefly, we focused on two cartridge sorbent types and showcased their usage:

**Mixed-Mode Cation Exchange (MCX)**: Using MCX cartridges, we fractionated lipid groups using different solvent mixtures. This was followed by Reversed-Phase (Kinetex EVO C18; 100 x 2.1 mm, 1.7 µm, Phenomenex) LC/MS analysis. To understand the fractions, we compared the NIST extract with the cartridge extraction qualitatively. After checking the fractions, we created a new LC/MS method to simultaneously analyze the lipid fractions using CID and EAD, enabling quantification as well as lipid characterization in a single run.

**C18 (CEC)**: Using C18 cartridges, we demonstrate an automated dilution scheme for high recovery lipid collection with multiple loadings. Alternatively, this can be directly injected into the LC/MS system or combined with different solvents for lipid group fractionation. To understand the effect of the cartridge on the sample, we measured the lipids by HILIC LC/MS analysis, quantified them (single point calibration) and calculated the recovery within lipid groups, i.e., Sphingomyelins.

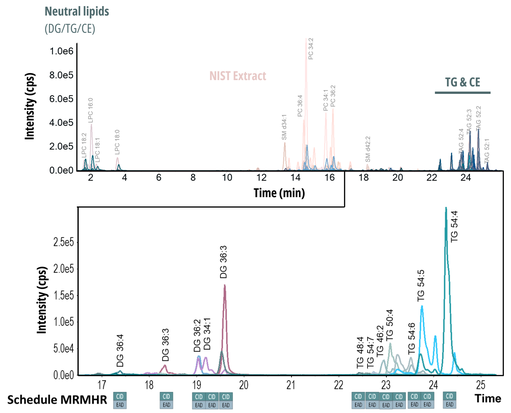
## SAMPLE HANDLING AND EXTRACTION

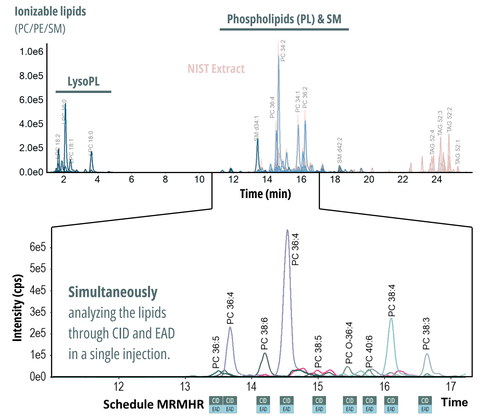


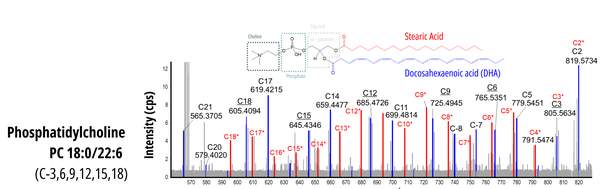

**Work in Progress**
At this stage we performed these first steps manually, however, full automation is possible. Sonication can be replaced by strong vortexing (automated).

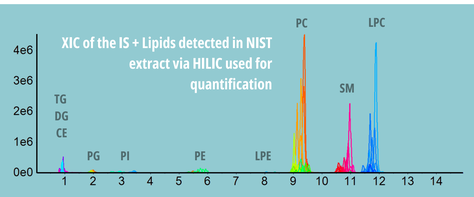
**Fractionation** to improve lipid characterization using **CID/EAD** fragmentation techniques in a single method.

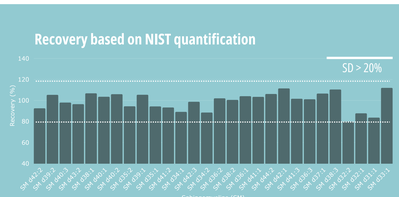
## WORKFLOW RESULTS




### MCX µSPE Cartridge Fractionation

Neutral lipids and Ionizable lipids fractionated with the MCX µSPE cartridge and measured in reverse phase using LC/MS. In red in the background is a NIST extract with all lipid groups to compare to. Also labelled are the lipid groups such as Phospholipids (PL), Lysophospholipids (LysoPL), Sphingomyelins (SM), Triglycerides (TG, also known as Triacylglycerols, TAG), Cholesterol Esters (CE), and Diglycerides (DG), as well as representative lipids like Lysophosphatidylcholines (LPC).

### Fragmentation using CID/EAD

Collision-Induced Dissociation (CID) offers robust, general lipid data, while Electron Activated Dissociation (EAD) provides complementary, detailed structural insights. Our work presents a single method integrating CID and EAD with established chromatography, expanding lipid characterization without altering separation protocols. Optimizing liquid chromatography (LC) to reduce lipid co-elution is a key next step to further enhance EAD's potential.

EAD provides deeper structural details, enabling near-complete lipid characterization in one experiment. For instance, with complex lipids like **PC 18:0/22:6** (containing 18:0 and the highly unsaturated 22:6 DHA omega-3 fatty acid), EAD's electron-driven fragmentation is highly insightful. Irradiating singly charged lipid ions with an electron beam initiates radical-mediated reactions that extensively cleave fatty acyl carbon backbones. This generates a series of diagnostic acyl chain ions, often differing by $CH_2$ units. Their pattern, **especially diminished signals at double bonds**, precisely maps all six double bonds in the 22:6 (DHA) chain (see spectrum). EAD also clearly distinguishes *sn*-positional isomers. It produces key diagnostic fragments (e.g., from glycerol region cleavages) unique to the acyl chain at a specific *sn*-position (like *sn*-2). This identifies if the 18:0 or 22:6 fatty acid is at the *sn*-1 or *sn*-2 glycerol position. This detailed EAD data is crucial for differentiating biologically vital lipid isomers.

## AUTOMATION SETUP

For plasma lipidomic profiling, samples were prepared using a PAL RTC autosampler, ensuring precise automated coupling to the LC system. Analysis was conducted on a SCIEX ZenoTOF® 7600 mass spectrometer.




## TAKE HOME MESSAGES

### Streamlined Plasma Lipidomics

Automated µSPE significantly standardizes plasma lipid analysis workflows, from extraction to injection.

### Flexible Lipid Profiling

µSPE cartridges offer versatile solutions and use cases.

- MCX enables lipid group fractionation for targeted, in-depth structural analysis, i.e., EAD.
- C18 facilitates high-recovery, broad lipidome collection with an automated dilution scheme and potential for online injection.

### Comprehensive Characterization

Integrating automated µSPE with a single LC/MS method using both CID and EAD allows for both quantification and enhanced structural elucidation of lipids in one run.




**Use prep-ahead mode** with overlapping to increase efficiency by performing the online sequential sample preparation and injection while the LC/MS is running. Combine it with direct injections to skip the elution into a separate vial.

Automation is critical in complex lipidomic analyses to minimize variability and enhance data quality.
**Key benefits of automation platforms include:**

- Guarantees uniform sample handling and reagent dispensing, minimizing operator-induced variation/noise.
- Executes repetitive and intricate tasks, such as the automated fractionation of lipid classes using µSPE cartridges.
- Implements sequential, fully automated sample preparation and direct online injections with integrated sample cooling, applying identical, continuous workflows to every sample to protect stability and substantially reduce traditional batch-to-batch variability.
- Facilitates efficient, high-throughput processing of samples (e.g., plasma) while upholding strict analytical standards and ensuring full sample traceability.
- Yields more robust, comparable, and dependable datasets, which are essential for confident and insightful biological interpretation in lipidomics.