Poster Reprint

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# High-Confidence Targeted Data Mining of Untargeted High-Resolution Data for Lipids

Sheher Banu Mohsin, Mark Sartain, Layla Cosovic, Daniel Cuthbertson, and Maozi Liu

Agilent Technologies, Santa Clara, CA 95051

## Introduction

Annotation of lipids is very challenging due to their complex nature and diversity in biological extracts. There is often ambiguity in lipid assignments caused by the presence of isomers and isobars even within a class of lipids. The common practice for increasing the confidence in mass spectrometric assignment is to use standards that match retention times and fragmentation pattern criteria. In the case of lipid identification, especially for complex extracts, it is practically impossible to find standards for thousands of lipids. This requires other strategies for lipid annotation with high degree of confidence.

Our approach was to first leverage a rugged, highly curated, comprehensive TQ method with 763 lipid transitions.[1] We have converted the unit resolution MRM transitions to accurate masses, what we used as precursors in targeted or untargeted experiments on a Q-TOF. The MS/MS spectra generated by the Q-TOF were then exported to the database, which can be used for matching entries based not only on accurate mass and RT but also on MS/MS spectral match.

To further increase confidence in the annotation of lipids, we have also generated CCS (collision cross section) values on the 6560 IM Q-TOF. Searches can now be done with accurate mass, RT, and MS/MS spectral match, as well as CCS filtering, to increase confidence in annotation.

## Experimental

Complete sample preparation, including separation and MS detection were described by Huynh, K, et al.[1]

Briefly, lipid extractions were performed in 1.5 mL microcentrifuge tubes using polypropylene positive-displacement pipettes. Ten microliters of plasma were mixed with 100 µL of extraction solvent, consisting of butanol:methanol (1:1) with 10 mM ammonium formate and the reconstituted mixture of internal standards. Each sample was then vortexed for 5 seconds and subsequently bath-sonicated for 1 hour, with the temperature maintained at 21–25 °C. Samples were then centrifuged (13,000 xg, 10 minutes, 20 °C), and the supernatant transferred into 1.5 mL glass sample vials (5190-9062) with 200 µL glass inserts (5183-2085). Samples were capped using PTFE/S caps (5185-5820) and stored at –80 °C until analysis.

## Experimental

### Workflow for creating a high-confidence personal compound database for lipids

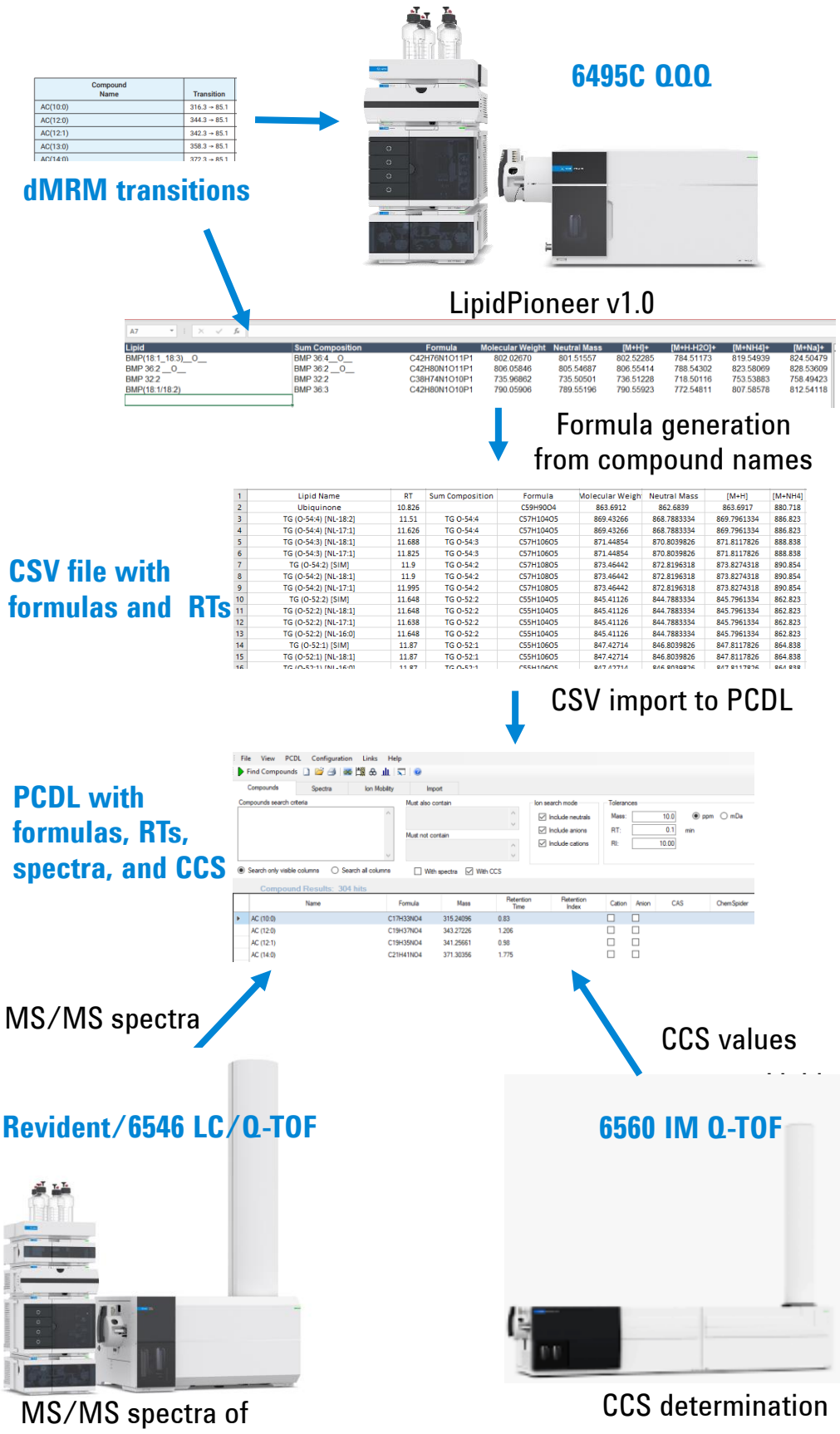


Figure 1. Workflow for creating high-confidence database to use for lipid annotations.

- Step 1. Generate molecular formula from transition list of names with LipidPioneer V1.0.[2]
- Step 2. Create CSV file with lipid formulas and retention times. Export to the database.
- Step 3. Update the database with MS/MS spectra from the Q-TOF.
- Step 4. Update the database with CCS values from the IM Q-TOF.

## Creating the database

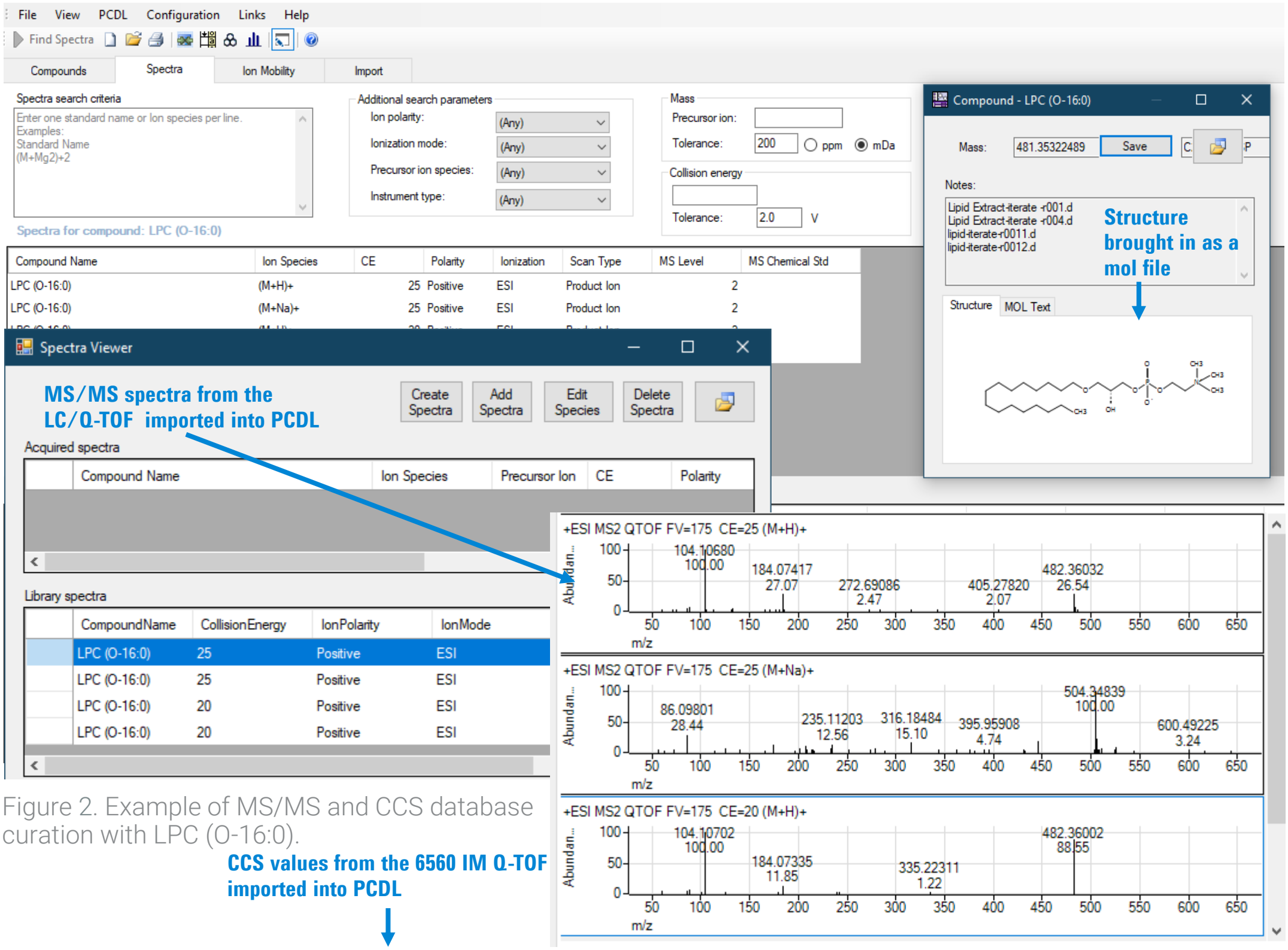


Figure 2. Example of MS/MS and CCS database curation with LPC (O-16:0).

**CCS values from the 6560 IM Q-TOF imported into PCDL**

| Polarity | Ion Species | Z | CCS | CCS FWHM | Conformer | Description | Gas | CCS Chemical Std | m/z | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| Positive | (M+H)+ | 1 | 229.64 | | 1 | | | NIST-1950 Serum Extr... | 482.3605 | Agilent Reference Single Field Method |

## Confidence in the CCS values

Figure 3 shows %RSD for the experimentally determined CCS values for lipids in serum. Most of the lipids have a %RSD <0.2.

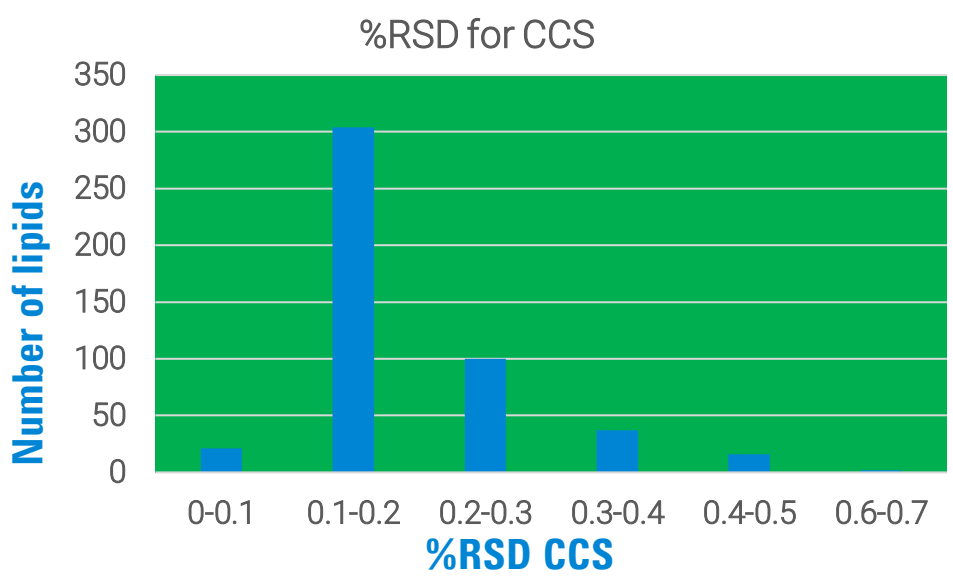


Figure 3. %RSD of CCS from six injections of the NIST 1950 serum lipid extract.

## Results and Discussion

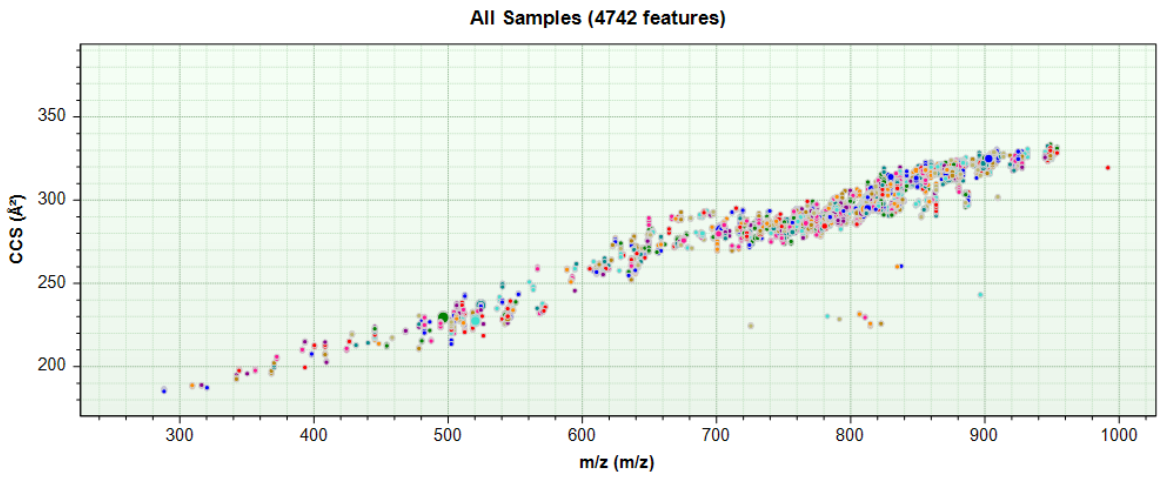


Figure 4. Plot of CCS values for lipids in serum vs. *m/z*.

Untargeted lipidomics analysis detected about 600 lipids across commonly analyzed lipid classes, including phosphatidylcholines, ceramides, diacylglycerols, phosphatidylethanolamines, phosphatidylinositols, sphingomyelins, and triacylglycerols. Figure 4. shows the spread CCS values with *m/z*. Repeat injections (6) of the serum extract shows RSD to be <0.2% for most of the lipids. This increases our confidence in the measurement of CCS and in using CCS values as an additional filter to remove isobaric interferences.

### Parallel reaction monitoring (PRM) method setup leveraging the database

The database has also been used to bring in formulas for parallel reaction monitoring (PRM). Figure 5 below shows the results for 80 lipids.

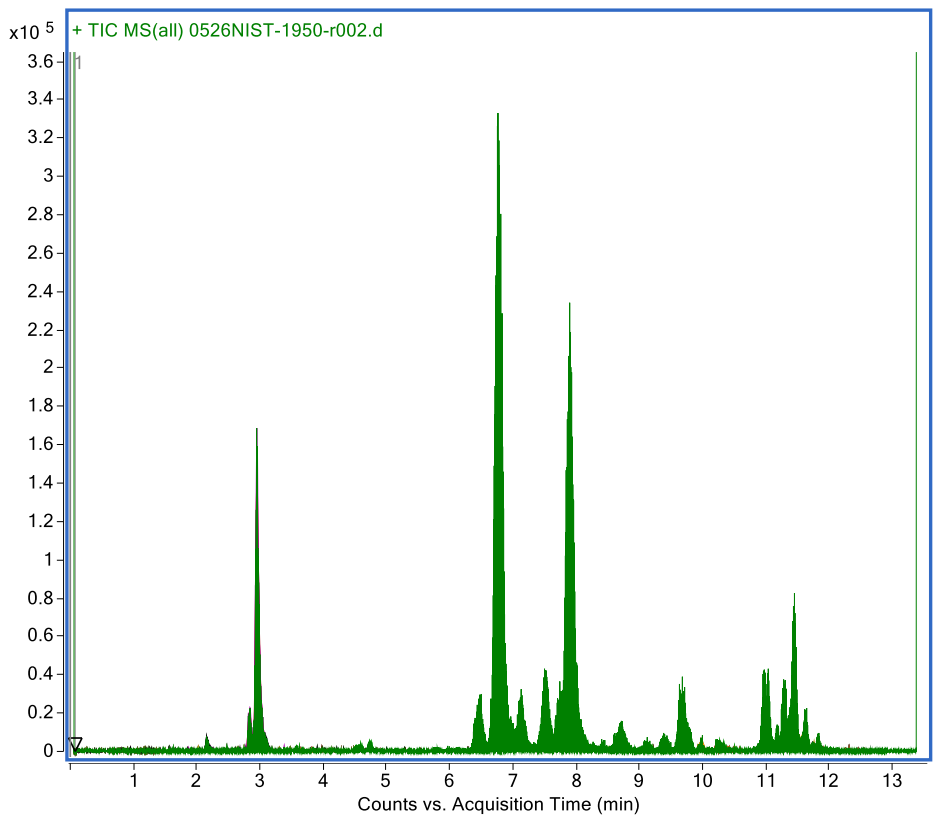


Figure 5. Parallel reaction monitoring of 80 lipids.

### Using PRM to generate high-quality spectra

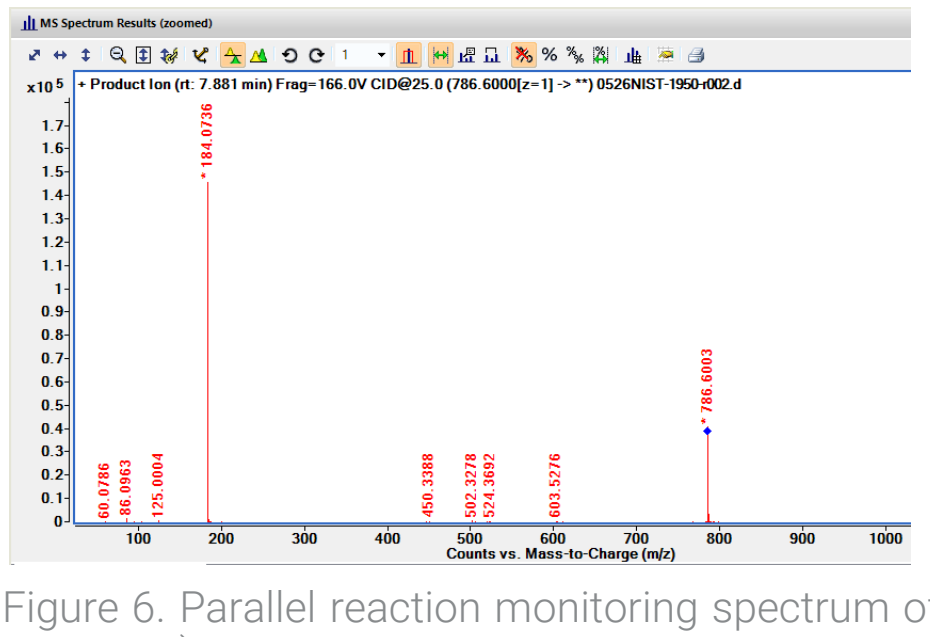


Figure 6. Parallel reaction monitoring spectrum of PC 18:1_18:1).

## Conclusions

- We have created  a high-quality, high-confidence database for the annotation of lipids.
- The database has accurate masses for 763 lipids, including MS/MS spectra, RT, and CCS to increase the confidence of annotation in targeted and untargeted workflows.
- CCS is highly reproducible, with RSDs <0.4% for all the lipids identified. The CCS value improves the accuracy of lipid annotation.
- The database can be leveraged to set up PRM methods.

## References


1. Huynh, K, et al. A Comprehensive, Curated, High-Throughput Method for the Detailed Analysis of the Plasma Lipidome. Agilent Application Note 5994-3747EN, 2021.
2. Ulmer, CZ et al. LipidPioneer: A Comprehensive User-Generated Exact Mass Template for Lipidomics. J Am Soc Mass Spectrom. 2017 March; 28(3): 562-565.


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