Automated Cyclic Ion Mobility–Mass Spectrometry MS/MS Workflow and Data Analysis for Shotgun Lipidomics

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720009459EN

Introduction

Recent advances in technology enable sensitive lipid identification down to the single-cell level. Improved MS techniques provide greater precision in characterizing cell-cell interactions, cellular states, and phenotypes based on lipidomic profiles. However, even state-of-the-art lipidomics approaches remain limited in their ability to address the heterogeneity and complexity of cellular lipidomes. Here, the integration of cyclic IM into a shotgun lipidomics workflow is described. In proof-of-principle experiments, this additional dimension of separation improves spectral clarity and facilitates the resolution of isobars and isomers beyond m/z-based discrimination. An automated acquisition and data processing workflow was developed to handle two-dimensional data (*m/z* and drift time) for both MS1 and MS2 of quadrupole-selected precursors.

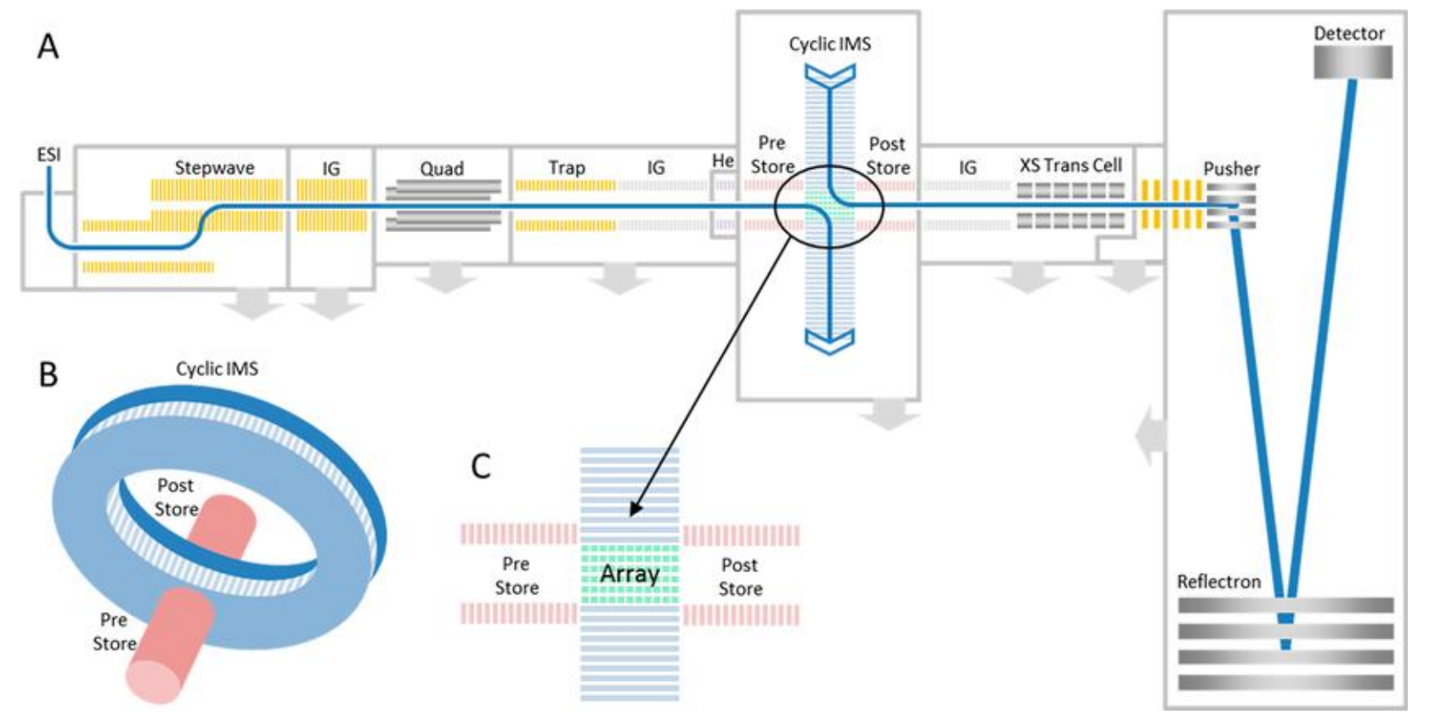

Figure 1. SELECT SERIES™ Cyclic™ IMS [1]. (A) Overall schematic, (B) and (C) cIM array.

Experimental

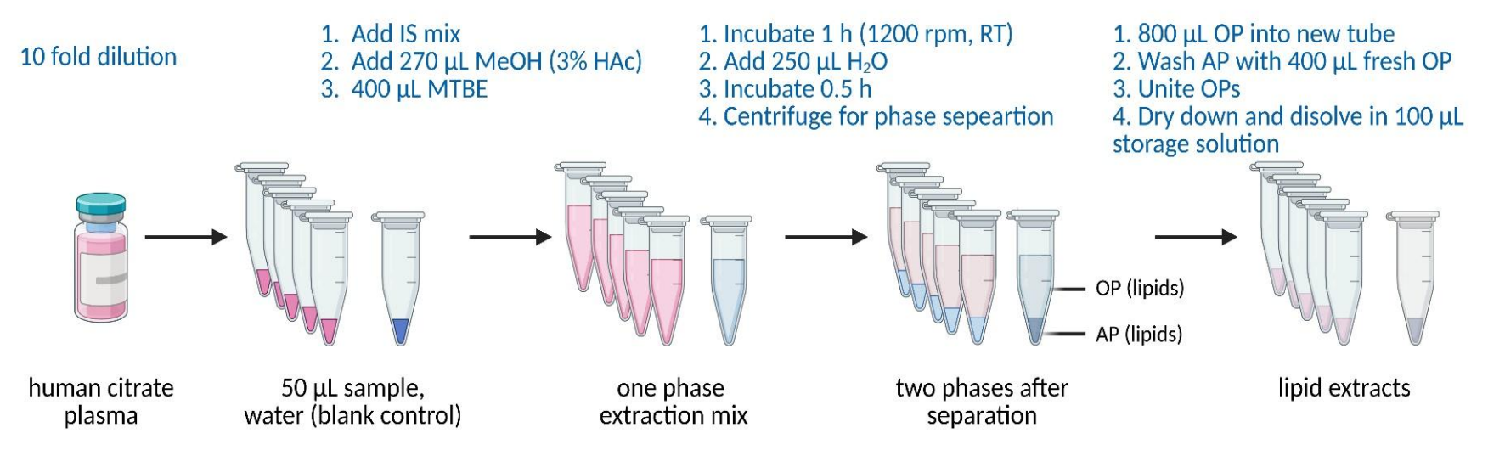

Figure 3. Sample preparation for shotgun lipidomics. Five independent lipid extractions were performed using a customized MTBE protocol [2]. Extracts are dissolved in MS-mix in 1/20 dilution directly before measurements.

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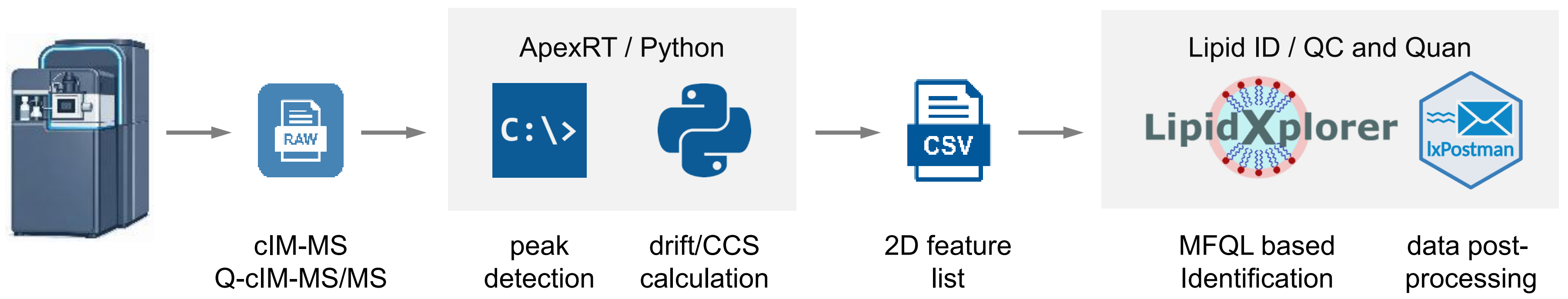
Direct Infusion Mass Spectrometry

- SELECT SERIES Cyclic IMS (Waters Corporation)
- nano-ESI (+) using Triversa NanoMate (Advion) 1.1 kV at 1.0 Psi
- Source temp: 150 ºC
- Acquisition methods: HDMS (cIM-MS), HDMS/MS (Q-cIM-MS/MS) in v-mode

Data processing

- MassLynx™ v4.2 and DriftScope™ 3.0 (data acquisition/review)
- ApexRT 1.17 (peak detection)
- Pandas, numpy Python libraries (drift time calculation)
- Prototype software LipidXplorer 1.5 [3] and lxPostman [4]



cIM-MS
Q-cIM-MS/MS

peak detection

drift/CCS calculation

2D feature list

MFQL based Identification

data post-processing

Figure 2. Semi-supervised prototype data acquisition and processing workflow, illustrating multidimensional peak detection and lipid annotation/ identification, using development software tools, LipidXplorer [3] and lxPostman [4].

Results

Experiment were designed using Cyclic IMS Method Editor, Figure 4. A representative direct-infusion cIM-MS 2D map is shown in Figures 5A and 5B. These data demonstrate the ability of cIM to resolve isobaric, Q-isolated precursor ions, resulting in reduced spectral complexity. Results are shown in Figures 6 to 9.

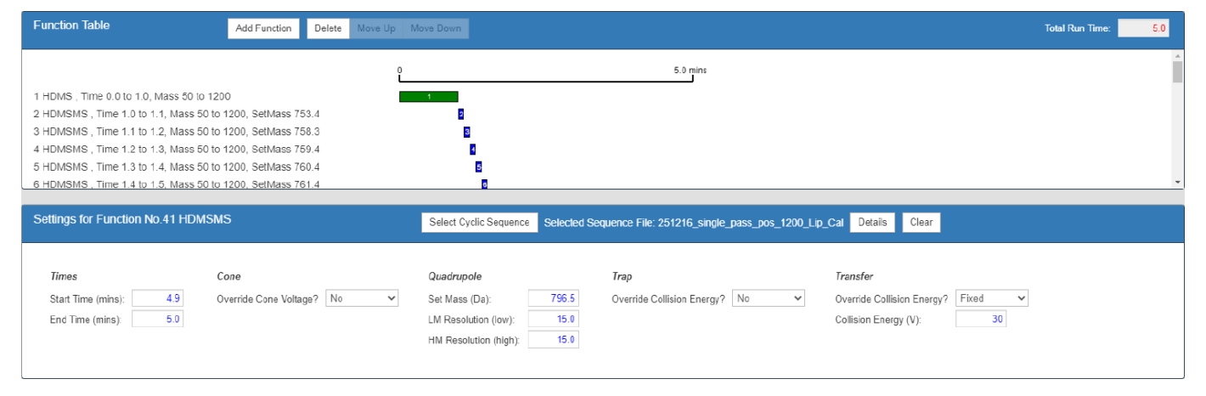


Figure 4. Proof of principle method design for collecting cIM-MS and Q-cIM-MS/MS

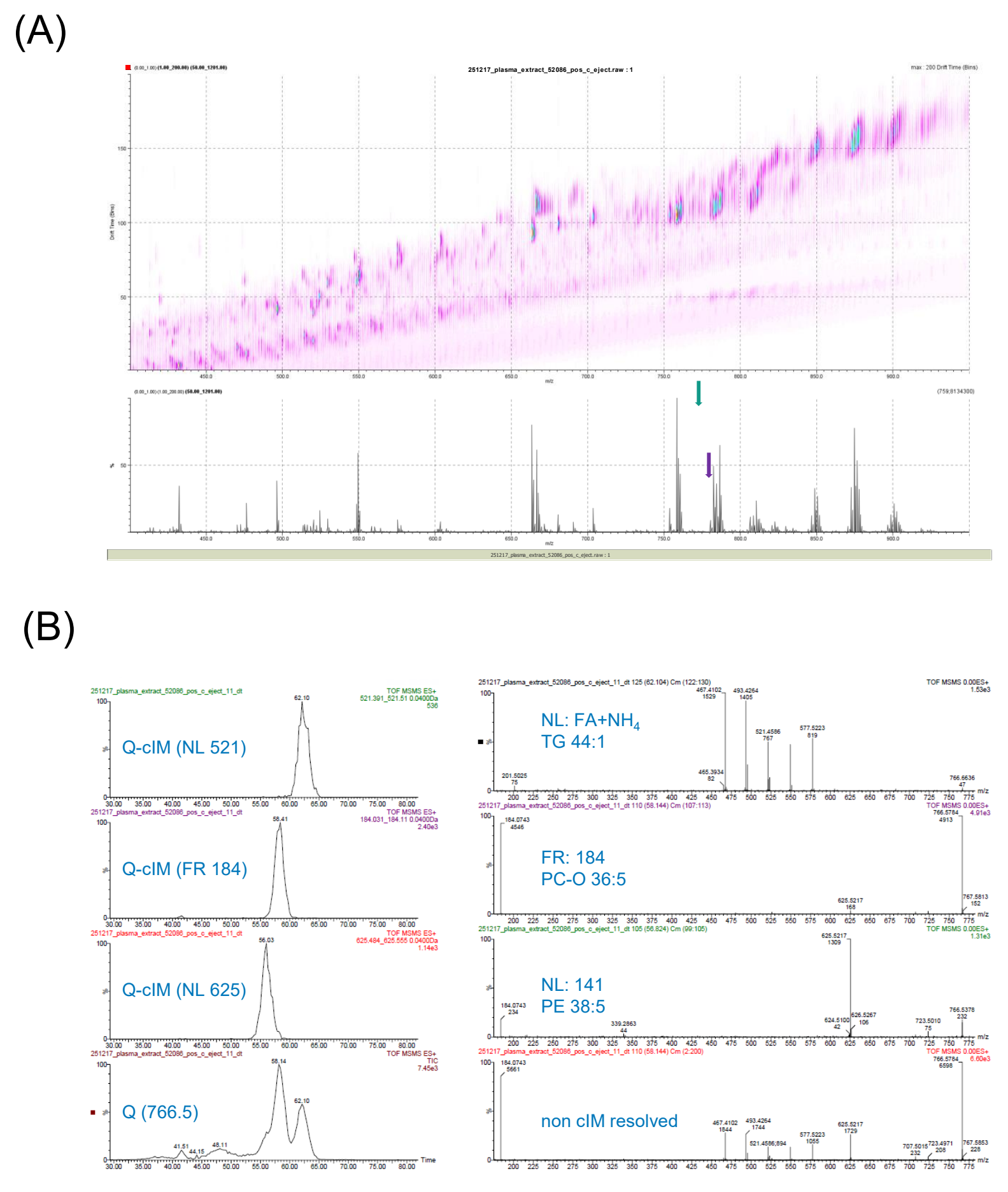


Figure 5. (A) Lipid plasma extract m/z vs. drift time cIM-MS profile, and (B) fragment ATDs of m/z 766.5 (left) and non- and cIM-resolved MS/MS spectra (right), including lipid annotation.

Ion mobility–resolved association of Q-cIM-MS/MS and low-energy cIM-MS enables minimization of signal overlap from isobaric and isomeric lipid species in a shotgun lipidomics approach. The data presented here are based on single-pass experiments.

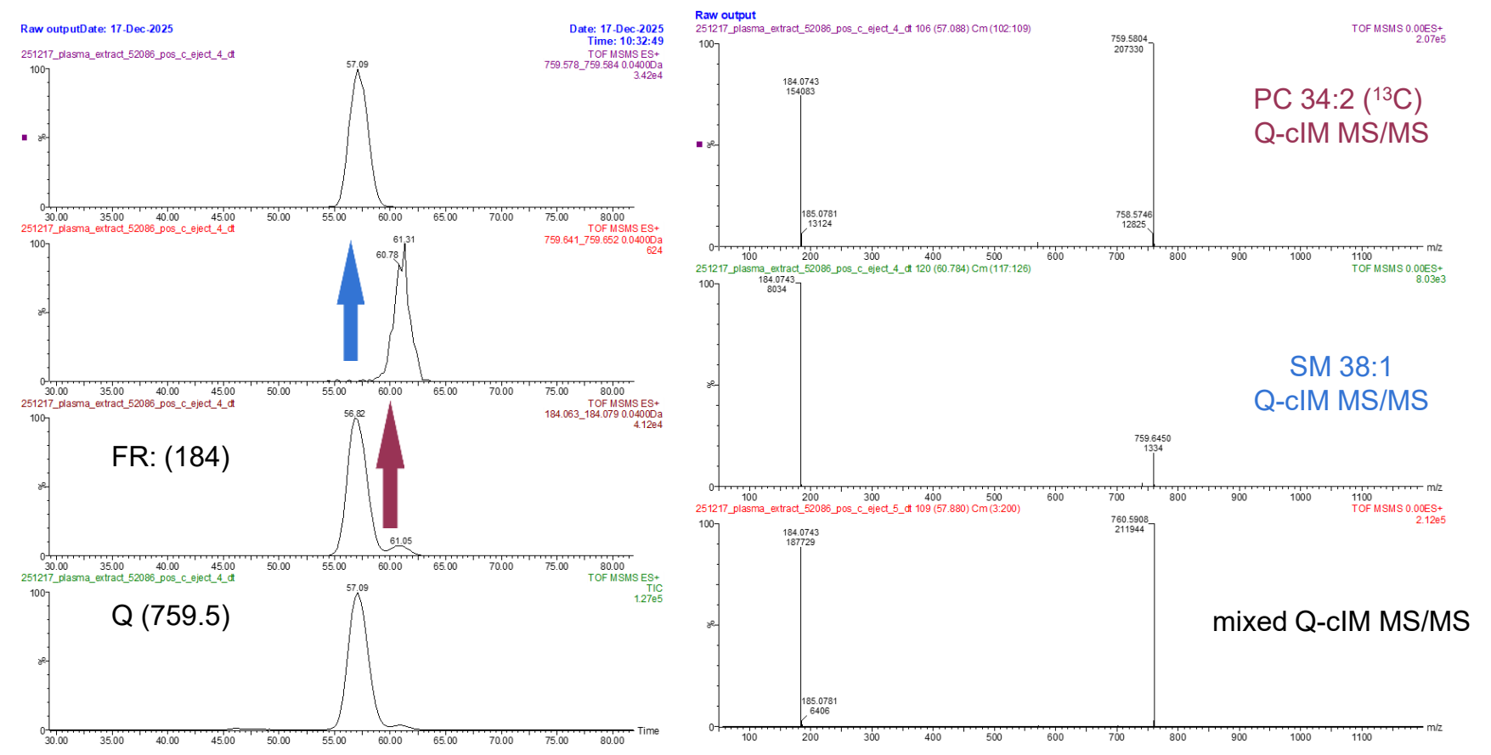


Figure 6. Non-resolved and cIM resolved ATDs of m/z 759.5 (left) and the corresponding MS/MS spectra (right), showing that isomeric overlap of FR: 184.07 can be IM resolved.

Figure 7. (A) Design of a Q-cIM-MS/MS direct infusion experiment, in which 250 Q-cIM-MS/MS were acquired within 6 min. (B) and (C) IM confirmed SM species.

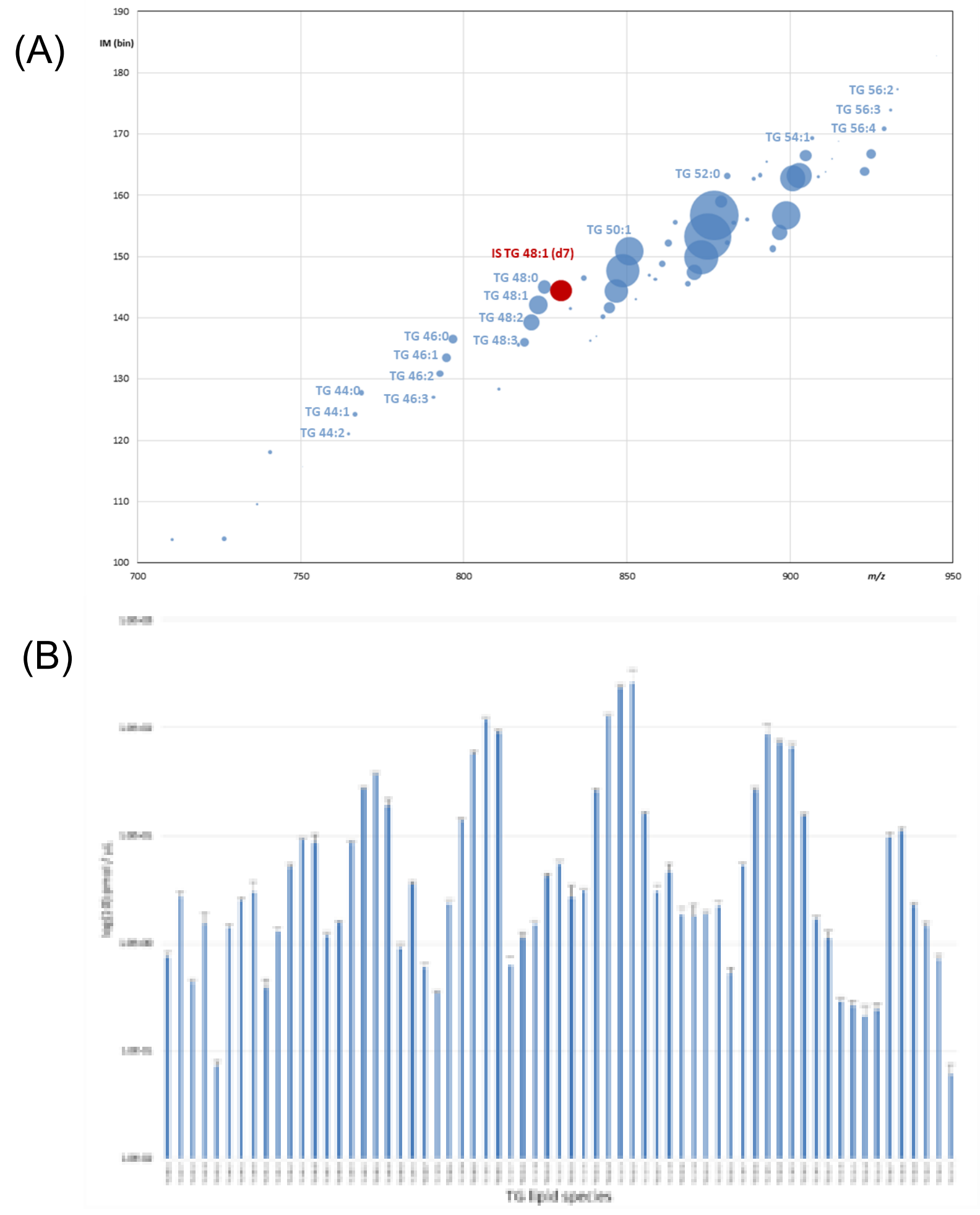


Figure 8. (A) TG species in 2D representation (m/z, IM). (B) Quantitation results.

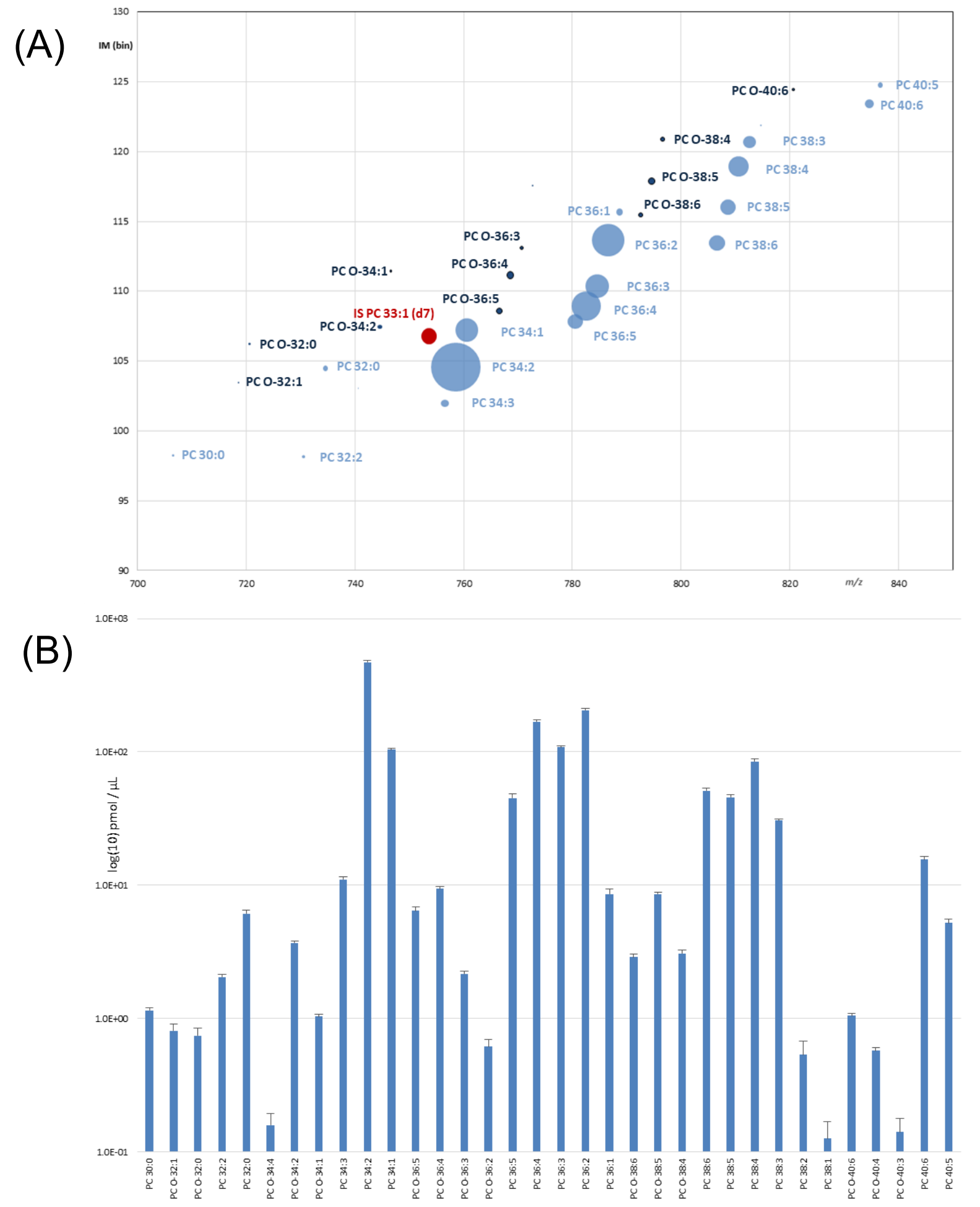
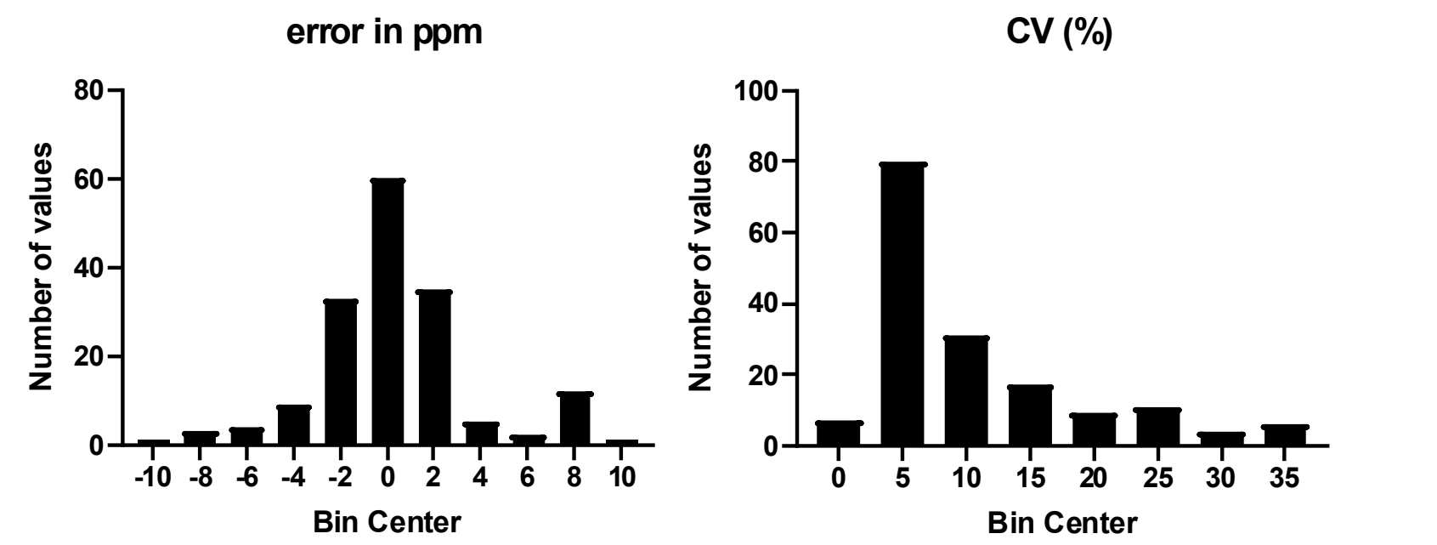


Figure 9. (A) Phosphatidylcholine (PC) and ether-linked phosphatidylcholine (PC-O) are separated in 2D. (B) Overall PC lipid species profile.

Conclusions

- Q-cIM-MS/MS acquisitions produces MS/MS spectra that resolve complex isomeric and isobaric overlaps
- Multidimensional peak detection was performed as part of the data processing pipeline used for both method creation and lipid annotation.
- The presented workflow establishes a framework that can be extended to larger sample cohorts
- A total of 166 lipids from eight classes were quantified using positive-ion shotgun lipidomics in a proof-of-principle experiment



Outlook

- Integration of Q-cIM-MS/MS in positive and negative ion modes
- Increased Q-cIM-MS/MS coverage via inclusion lists
- Automated generation of 2D feature lists
- LipidXplorer updates for multidimensional MasterScan generation and lipid identification

Acknowledgement

Funding for this research in the DS Lab was supported by the German Network for Bioinformatics Infrastructure (de.NBI)

References

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3. *Herzog et al. A novel informatics concept for high-throughput shotgun lipidomics based on the molecular fragmentation query language. Genome Biol . 2011*
4. *https://lifs-tools.org/tools/lxpostman.html*