Comparison of LC-ESI-MS Methods for Targeted and Semi-Targeted Screening of N-acyl Phosphatidylethanolamines in Mouse Brain Tissue

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Introduction

The role of lipids in disease processes is a growing focus of investigation. Targeted acquisition methods provide uncompromised detection sensitivity and straightforward data processing but have limited utility for discovery of unexpected metabolites. For this reason researchers frequently express interest in non-targeted data acquisition and analysis, seeking to evaluate the broadest range of analytes possible. However, they may not appreciate that lower concentration components may be undetectable and effectively excluded by this approach.

N-acyl phosphatidylethanolamines (NAPEs) are implicated in metabolic processes associated with obesity and diabetes. They are present in very low concentrations compared with other lipid classes. Here we compare a non-targeted vs a targeted data acquisition method to illustrate the advantages and limitations for each approach.

Method

Sample Preparation

1. Prepare standard spiking solution in 4mL chloroform
   - 4 uL Avanti Polar Lipid SPLASH mix
   - 1 uL NAPE-STD 50 ug/mL
2. To centrifuge tube with 40 uL mouse brain homogenate (MBH) on ice add:
   - 0.2 mL ice cold methanol and vortex briefly
   - 0.4 mL chloroform ISTD and vortex briefly
3. Incubate the tubes on ice for 20-40 minutes, vortexing occasionally
4. After the 40 minute incubation add 0.12 mL water to separate the organic and aqueous layers
5. Incubate on ice for 10 minutes
6. Centrifuge at 311xg for 5 minutes at 4°C
7. Transfer the lower layer to a clean sample tube
8. Re-extract the aqueous layer with 2:1 chloroform/methanol by adding 0.2 mL methanol then 0.4 mL chloroform and vortexing
9. Dry the combined lower layers under N_2 then reconstitute 50 uL LWS (Lipid Working Solution = chloroform:methanol:ammonium acetate 50:50:10 mM). Transfer to an LC vial with a glass insert. Inject 2-5 uL as needed based on lipid concentration.

Expected concentration of internal standard is ~200 pmol/gram tissue

Instrument Method

Samples were analyzed by LC-ESI-MS/MS on an Agilent 1260 HPLC and Bruker MicrO-TOF-Q II time of flight mass spectrometer in positive ionization mode[1,2]. Solvent A is 60:40:10mM ACN:H2O:ammonium acetate; Solvent B is 90:10:10mM isopropanol:acetonitrile:ammonium acetate. The column is a 2.1x100 mm 2.6 um Phenomenex Kinetex C18, run at 65°C and 0.3 mL/min with the gradient program to the right.

Time (min)	%A	%B
0	85	15
7	52	48
32	18	82
34	1	99
37	1	99

Non-targeted product ion spectrum acquisition: Broad band method composed of two alternating scan functions: one full scan m/z 50-1400 and one scanning the same mass range but applying 35V collision energy, passing all ions to the collision cell.

Targeted data acquisition: Conventional product ion scan in which the NAPE-STD precursor m/z 1024.8 was isolated and fragmented with 35V collision energy.

NAPE-STD and Bovine Heart Extract (BHE) were purchased from Avanti Polar Lipids.

Results and Discussion

Figure 1. NAPE-STD in lipid extracts

A. 500 ppb NAPE-STD spiked into BHE post-extraction, non-targeted. Characteristic ions for the NAPE-STD model compound can be detected with good signal to noise. This spiking level is 10x the expected endogenous concentration.
B. 50 ppb tissue NAPE-STD spiked into BHE post-extraction, non-targeted. The m/z 603 product ion has insufficient signal-to-noise.
C. 200 pmol/g tissue NAPE-STD spiked into MBH pre-extraction, non-targeted. No characteristic ions for NAPE-STD were detected.
D. 200 pmol/g tissue NAPE-STD spiked into MBH pre-extraction, targeted product ions of 1024.8 acquired. Characteristic ions for NAPE-STD are detected. This spiking level is equivalent to expected endogenous concentrations of NAPEs in brain.

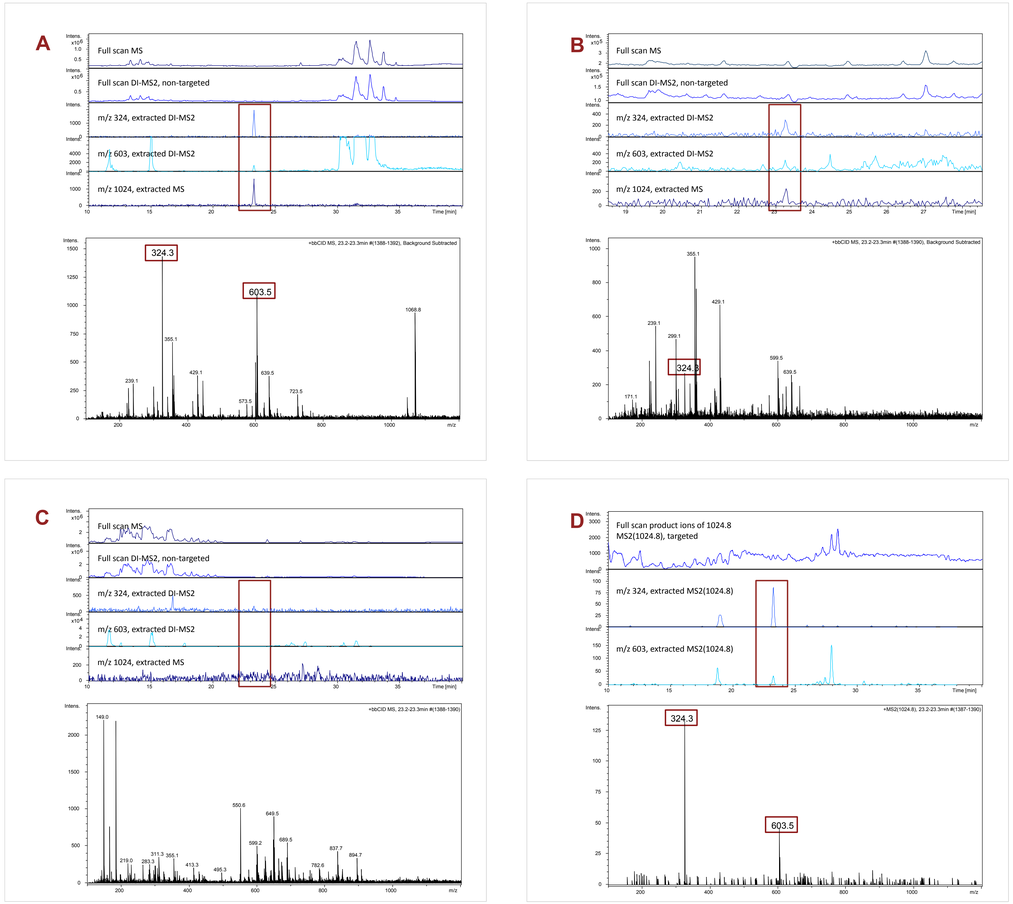

Figure 2. NAPE standard: 18:1 PE-N-19:0

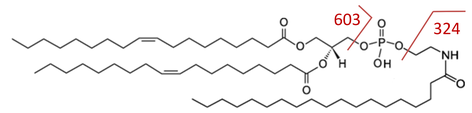

Figure 1-A shows the non-targeted product ion spectrum obtained for NAPE-STD spiked into Bovine Heart Extract at an effective concentration of 500 ppb. The NAPE-STD product ions at m/z 603 and 324 are readily detected with good signal-to-noise. However, the lipid extract is a complex sample, and there are several species co-eluting with the NAPE-STD.

Figure 1-B shows the non-targeted product ion spectrum obtained for NAPE-STD spiked into Bovine Heart Extract spiked with a concentration of 50 ppb. This level corresponds to the expected endogenous concentrations of the more abundant NAPE species in brain as reported by Triebl et al[3]. The signal-to-noise for the product ions at m/z 324 and 603 is below the level needed for reliable detection.

Figure 1-C shows the non-targeted product ion spectrum for NAPE-STD spiked into Mouse Brain Homogenate pre-extraction at an effective concentration of 200 pmol/g tissue, consistent with the upper range of expected endogenous concentrations. Product ions for NAPE-STD are not detected.

Figure 1-D shows the *targeted* product ion spectrum for same sample from Figure 1-C. Here the characteristic product ions for NAPE-STD are readily detected.

Conclusion

Non-targeted product ion acquisition methods can be powerful tools for data collection but may not be appropriate for low-abundance species in complex matrices. Researchers must be aware of the limitations as well as the strengths of this technique when designing their experiments.

References

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