Quality Management in Lipidomic Extractions to Develop a Lipidomic Integrity Number

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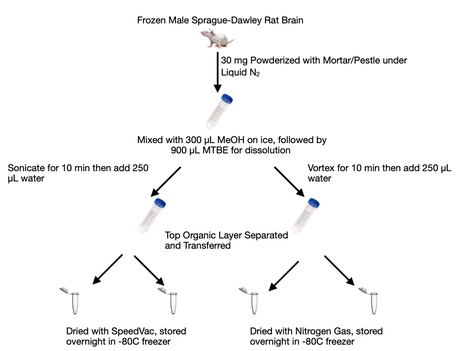
ABSTRACT

Integrity numbers for biomolecules (DNA, RNA, and proteins) are the primary quality control (QC) metrics for Omic sciences/biospecimens.[1, 2, 3] A lipidomic integrity number (LIN) has not been established. LIN will depend on biological (lipases, etc) during the antemortem and postmortem process, while physio-chemical degradation dominates during the tissue extraction process. Phospholipids (PLs) are highly susceptible to hydrolysis and oxidation during drying. This study investigated compared two methods of tissue dissociation during extraction: sonication and vortexing. We found vortexing resulted in about 3% less degradation of major PLs. Two methods of drying extracts were also explored: vacuum drying and drying under N_2 gas. Vacuum drying contributed an additional 19% degradation of PLs. Sample acquisition by vortex mixing and drying under N_2 gas are ideal lipid extraction procedures as factors for determining a LIN become standardized.

INTRODUCTION

- Studying brain diseases relies on donated human brain tissue to understand what's happening with DNA, RNA, proteins, and lipids.
- A catch: biological materials can still degrade after death depending on how long the person was dying (agonal duration), how quickly the brain was harvested and stored, and processing prior to study.
- Relatively, little is understood about effects of chemo-biological factors behind lipid degradation.
- It is hypothesized that phospholipids can be model molecules to track degradation due to it being the most chemically abundant lipid in the brain [phosphatidylcholine (PC) and phosphatidylethanolamine (PE) accounting for ~75%] as well as sensitive to degradation like oxidation and hydrolysis.

METHODS



- Samples were dissolved in 100 µL isopropyl alcohol to prepare for separation via LC-MS.
- Data was collected via LC-MS separation through CompoundDiscoverer and processed through LipidSearch 5.0.
- Raw data from LipidSearch was exported and processed within Excel.

RESULTS

- Out of the ~1750 lipids detected when comparing mixing methods, significant increases in concentration for phosphatidic acid (PA) account for ~3.76% of the total concentration of PA were observed.
- This is reflected in phosphatidylethanolamine (PE)/phosphatidylcholine (PC) degradation, where ~3% of the total concentration of PE and PC are made of statistically significant decreases in PE and PC.
- However, significant increases in concentration for phosphatidic acid (PA) account for ~19.3% of the total concentration of PA were observed when comparing drying by oxidative SpeedVac to anaerobic N_2 drying.
- Limited change (3.84%) in PE/PC degradation occurred with different drying methods.

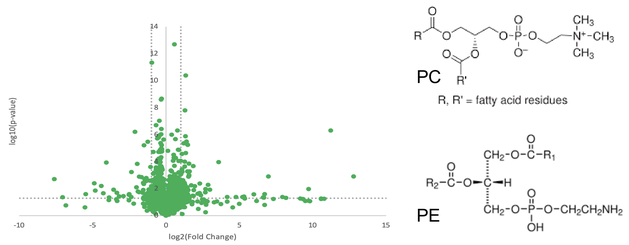


Figure 1: Volcano Plot of Fold Changes in Lipid Concentrations for Sonication versus Vortex Mixing.

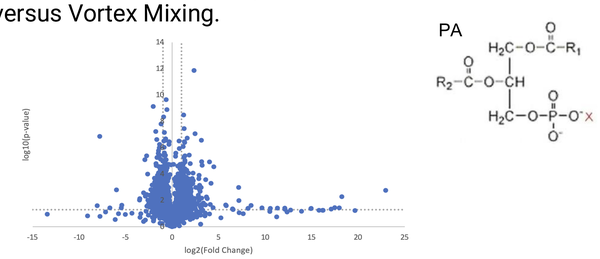


Figure 2: Volcano Plot of Fold Changes in Lipid Concentrations for SpeedVac versus N_2 Gas Drying. Structures of PE, PC, and PA on the right.

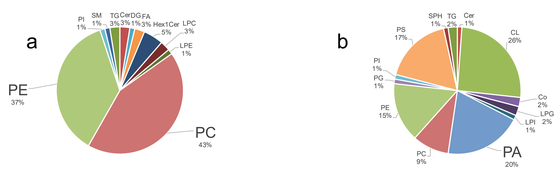


Figure 3: a) Distribution of lipids with decreased lipid concentrations for mixing by sonication. b) Distribution of lipids in with increased lipid concentrations for mixing by sonication.

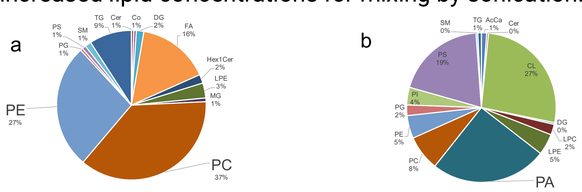
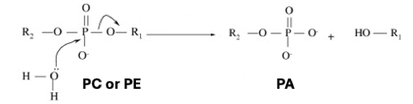


Figure 4: a) Distribution of lipids with decreased lipid concentrations for drying by vacuum drying. b) Distribution of lipids in with increased lipid concentrations for drying by vacuum drying.

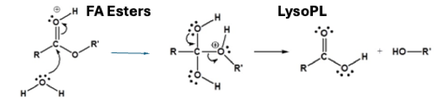
CONCLUSION

- Mixing samples for dissolution via vortexing degrades major PLs at a lesser magnitude compared to sonication. With the slight solubility of water in MTBE PL headgroups as well as FA esters could be hydrolyzed generating lysoPLs, lysoPA and PA (below). In the case of PE with anchimeric assistance of the amine and added energy from ultrasound the nucleophilic attack of the C3 of the amine could make an aziridium ion as a leaving group. Interestingly, LPI and LPC, and LPC and LPE increased with sonication and vacuum drying, respectively. It is unknown with sonication if lysoPL degraded to GPE and GPC since these were not detected.

PE & PC Hydrolysis



FA Ester Hydrolysis



- Presence of oxygen in vacuum drying, when considering the low degradation of PE/PC with high formation of PA, suggests drying under N_2 gas as a gentler method of maintaining lipid integrity.
- To minimize degradation, biospecimens should be mixed with vortexing and dried under anaerobic conditions using N_2 gas.

FUTURE DEVELOPMENT

- Retooling LC-MS data collection, likely with software library used in LipidSearch 5.0, to increase signal to noise ratio.
- Consider using ratio of phospholipids and lysophospholipids as part of a composite metric to evaluate lipid integrity.
- Elucidate potential chemical degradation pathways seen with the chemical changes from drying with methods like vacuum drying.
- Understanding lipase activity during the preanalytic steps of biospecimen processing (see below) on lipidome quality to build a holistic algorithm for determining LIN.
- Incorporate LIN into validating the first penta-omic extraction method from human donors.

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