Post Column Epoxidation for Differentiating Polar and Non-polar Lipids Isomers via Liquid Chromatography Co-axial Contained-ESI Mass Spectrometry

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Introduction

- Lipid isomers act as biomarker and pin-pointing C=C position in fatty acyl is crucial for characterizing isomers
- The use of co-axial source induces epoxidation of C=C of fatty acyl groups and thus helps in locating exact C=C position
- This study explores separation using RPLC, identification and characterization of lipids (polar and neutral) without compromising the ionization efficiency at high flow rate.

Goals

- To optimize plasma droplet for the ionization of lipids of different polarities (polar and neutral lipids) at a high flow rate
- To develop and optimize liquid chromatography method for separation of different classes of lipids and their isomers
- To separate and characterize different lipids isomers (C=C positional) using liquid chromatography-Contained co-axial ESI mass spectrometry without significant instrument modification

Approach

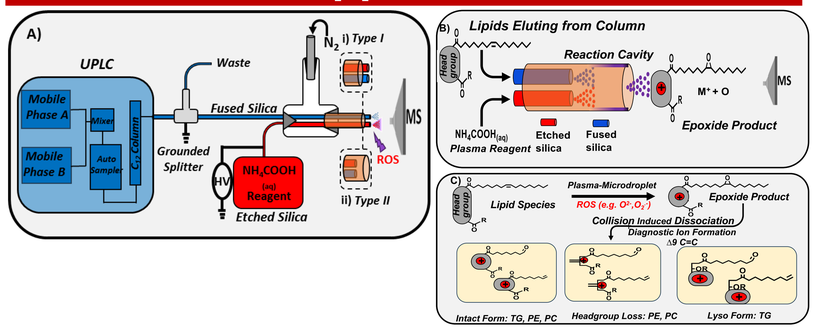

Figure 1. A) Liquid chromatograph combined with Co-axial Contained-ESI mass spectrometer **B)** Reaction cavity showing epoxide formation and **C)** General scheme for diagnostic ions of different lipid class

Experimental

- **MS Optimization parameters :**
  - Ammonium acetate flow rate (0-20 µL/min)
  - Nitrogen Flow rate (0-150 psi)
  - Capillary voltage (0-8 kV)
  - Ammonium acetate concentration (0-80 mM)
  - Cavity size (1-10 mm)
- **Liquid Chromatography parameters:**
  - MP-A: ACN:H_2O (60:40) with 10 mM Ammonium formate
    MP-B: IPA:ACN:H_2O (90:9:1) with 10 mM Amm. Formate
  - Column: Evosphere C12, 100 Å, 1.7 µm, 2.1 x 100 mm Monodisperse Fully Porous Particle UHPLC Column

Results and Discussion

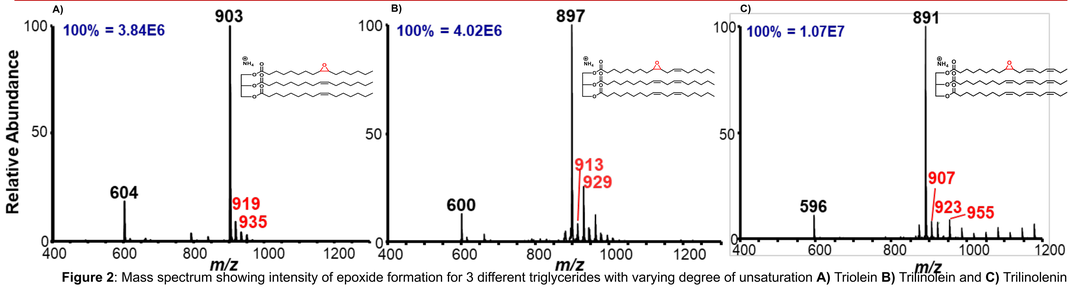

Figure 2: Mass spectrum showing intensity of epoxide formation for 3 different triglycerides with varying degree of unsaturation **A)** Triolein **B)** Trilinolein and **C)** Trilinolenin

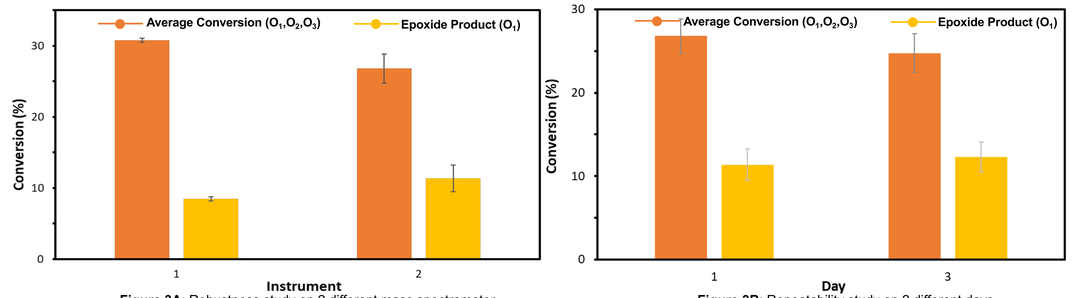

Figure 3A: Robustness study on 2 different mass spectrometer

Figure 3B: Repeatability study on 2 different days

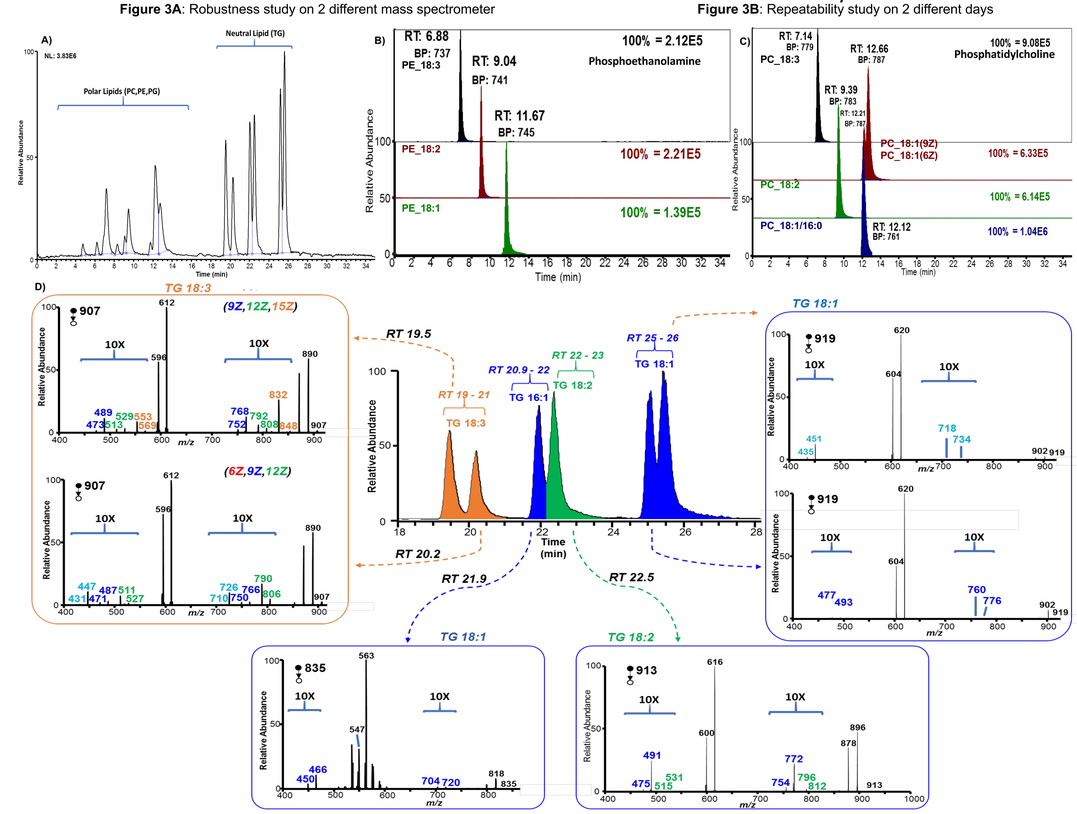

Figure 4. A) XIC of lipids (polar and neutral) standards **B)** EIC of polar lipid (Phosphoethanolamine) **C)** Phosphatidylcholine **D)** Neutral lipid (triglycerides) with isomers

Future Work

- Analysis of lipids in biological and plant extracts and characterization of C=C double bond position of fatty acyl group.

Conclusion

- Parameters were optimized to maintain the plasma formation at high flow rate using:
  - Capillary voltage of 5 kV
  - Nebulizer gas flow rate of 80 psi
  - Co-Axial Source to MS inlet distance of 2 mm
  - Ammonium acetate concentration of 40 mM and flow rate of 11 µL/min
  - Eluent flow rate of 50 µL/min to mass spectrometer
- Epoxide formation was found to be 10-15%
- LC-MS method was developed for fast analysis of both polar and non-polar lipid and determination of C=C position without any instrument modification

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References

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