EVALUATION OF AQUEOUS-ACETONITRILE BASED MOBILE PHASE FOR UNTARGETED ANALYSIS OF POLAR METABOLOME AND LIPIDOME

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INTRODUCTION

- **Comprehensive analysis of lipidome and metabolome from biological tissues is challenging owing to the structural complexity, polarity differences, diverse functional groups, dissociation, and ionization behavior of the analytes.**
- **Isopropyl alcohol (IPA) is the commonly used organic solvent in reversed - phase chromatographic analysis of lipids due to its high eluotropic strength.**
- **IPA, however, is not suitable for the analysis of polar metabolites.**
- **Here we have investigated various column chemistries to identify a generic aqueous/acetonitrile (ACN) based mobile phase for the untargeted analysis of polar metabolites and lipids from biofluids.**
- **The goal is to develop a single generic mobile phase that can switch between lipid and polar metabolite analysis with an appropriate column using the same LC-system.**

METHODS

Lipid Extraction

A simple plasma sample preparation procedure was adopted using protein precipitation with pre-cooled IPA. A 20 µL of rat plasma or human plasma NIST™ Standard Reference Material™ 1950 was mixed with 80µL pre-cooled IPA. The samples were vortex mixed for one minute and placed at -20 °C for 10 minutes. Samples were vortex mixed again for one minute and placed at 4 °C for two hours to ensure complete protein precipitation. The samples were centrifuged at a maximum of 10,300 g for 10 minutes at 4 °C. The supernatant was transferred and dilute with (IPA/ACN, 50/50) in 1:5 ratio before transferring the supernatant to Waters™ Total Recovery UPLC™ Vials (p/n: 186005669CV) for LC-MS analysis.

LC-MS Conditions

Liquid chromatography-based separation of lipids was performed with various column chemistries (ACQUITY™ Premier CSH™ Phenyl Hexyl Column, CORTECS™ Phenyl Column, CORTECS C8 Column) using water as mobile phase (MP) A and ACN/water (95/5) as MPB with 0.1% formic acid and 1mM ammonium formate as additives on ACQUITY Premier UPLC system. This data was compared against the samples analyzed with ACQUITY Premier CSH C18 Column using ACN-Water-1M aqueous ammonium formate (600:390:10) in 0.1% formic acid as MPA and IPA-ACN-1M aqueous ammonium formate (900:90:10) in 0.1% formic acid as MPB. The columns were maintained at 55 °C and 0.4 mL/min flow rate [1]. The column effluent was monitored in positive and negative ion mode using SYNAPT™ XS mass spectrometer (Figure 1A).

Reversed – phase analysis of moderately polar analytes and drug metabolites was performed on a 2.1 x 100 mm, 2.7 µm CORTECS Premier C8 Column. The column was maintained at 40 °C and eluted with a multilinear gradient using 0.1% (v/v) aqueous formic acid containing 1mM ammonium formate (MPA) and ACN/water (95/5) containing 0.1% formic acid and 1mM ammonium formate (MPB) at a flow rate of 0.6mL/ min.

The HILIC analysis was performed on a 2.1 x 100 mm, 1.7 µm ACQUITY BEH™ Amide Column. The column was maintained at 40 °C and eluted using ACN/water (95/5) containing 0.1% formic acid and 1mM ammonium formate as mobile phase A and 0.1% aqueous formic acid containing 1mM ammonium formate as mobile phase B at a flow rate of 0.6mL/min.

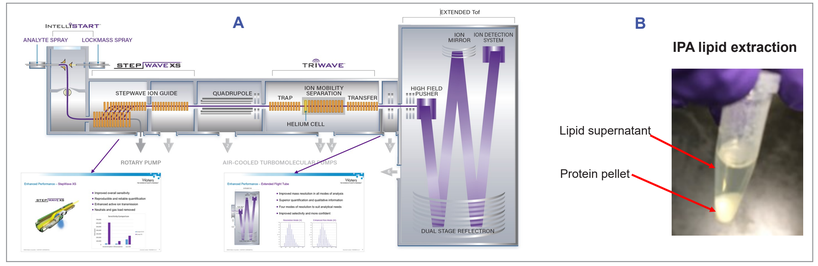

Figure 1. (A) Schematic of the SYNAPT XS mass spectrometer used to collect the lipidomics and metabolomics data. (B) Lipid extraction using isopropyl alcohol showing the supernatant containing all the lipids and protein precipitate.

RESULTS

- Various column screening for NIST plasma lipid separation using the generic aqueous/acetonitrile based MP compared against the gold standard IPA based MP [1].

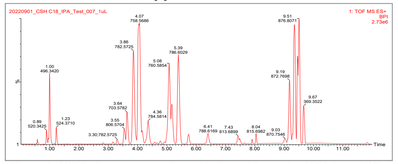

Figure 2. NIST plasma separation with the gold standard IPA base MP using ACQUITY Premier CSH C18 Column (2.1x100mm) [1].

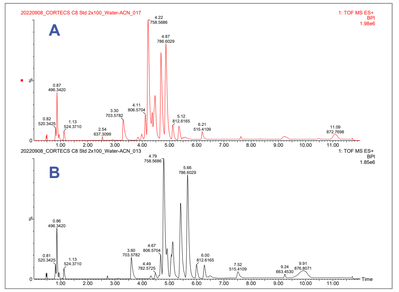

Figure 3. NIST plasma extract separation using ACQUITY CORTECS C8 Column (2.1x100mm) with generic aqueous/acetonitrile MP (A) 6 min gradient 70-99% B at 70 ºC. (B) 8 min gradient 70-99% B at 70 ºC.

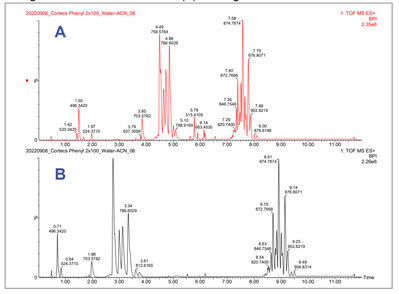

Figure 4. NIST plasma extract using ACQUITY CORTECS Phenyl Hexyl Column (2.1x100mm) with generic aqueous/acetonitrile MP (A) 6 min gradient 70-99% B at 70 ºC. (B) 8 min gradient 70-99% B at 70 ºC.

RESULTS

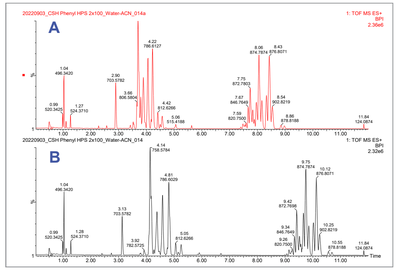

Figure 5. NIST plasma extract separation using ACQUITY Premier CSH Phenyl Hexyl Column (2.1x100mm) with generic aqueous/acetonitrile MP (A) 6 min gradient 70-99% B at 70 ºC. (B) 8 min gradient 70-99% B

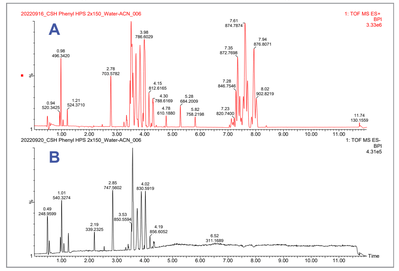

Figure 6. NIST plasma extract separation using ACQUITY Premier CSH Phenyl Hexyl Column (2.1x150mm) with generic aqueous/acetonitrile MP, 6min gradient 70-99% B at 70 ºC and 0.6mL/min flow rate. (A) Positive mode (B) Negative mode.

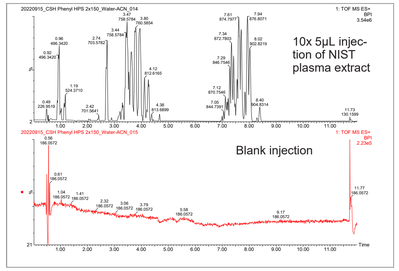

Figure 7. There is no lipid carryover using ACQUITY Premier CSH Phenyl Hexyl Column (2.1x150mm) with the generic aqueous/acetonitrile MP.

RESULTS

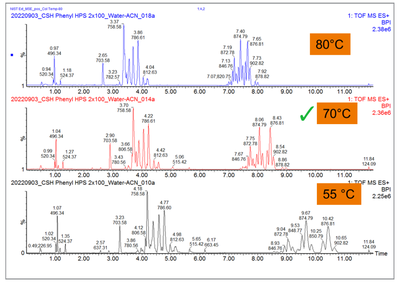

Figure 8. NIST plasma extract separation using ACQUITY Premier CSH Phenyl Hexyl Column (2.1x100mm) at different column temperatures.

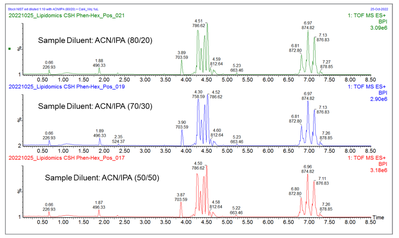

Figure 9. Different sample diluent ratio of ACN/IPA did not have significant influence on the peak shape, retention time and solubility of the lipids (ACQUITY Premier CSH Phenyl Hexyl Column).

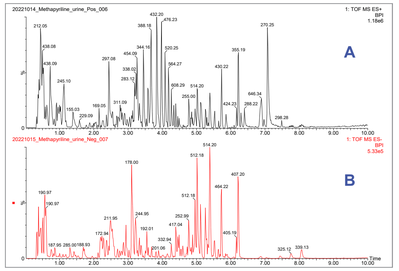

Figure 10. Base peak intensity (BPI) chromatogram of male rat urine for moderately polar metabolites using ACQUITY CORTECS Premier C8 Column (2.1x100mm) with generic aqueous/acetonitrile MP (A) ESI positive mode (B) ESI negative mode.

RESULTS

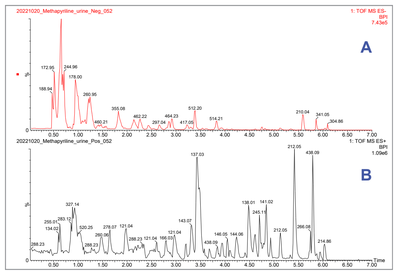

Figure 11. Base peak intensity (BPI) chromatogram of male rat urine for polar metabolites using ACQUITY BEH Amide Column (2.1x100mm) with generic aqueous/acetonitrile MP (A) ESI positive (B) ESI negative.

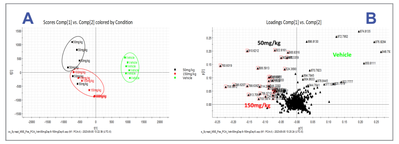

Figure 11. Multivariate analysis of plasma lipid samples from rats fed with an antihistamine compound methapyrilene at 50 and 150mg/kg dose (at day 6) compared to vehicle rat samples. (A) PCA plot (B) Loadings plot with the significant markers highlighted in red.

CONCLUSION

- **A single generic aqueous/acetonitrile mobile phase was developed for the analysis of polar and moderately metabolites and lipids.**
- **The use of a single aqueous/acetonitrile mobile phase composition allowed a switch to polar metabolite analysis (from the lipid analysis) using amide-bonded stationary phase in the same LC system which is a significant time and cost saving.**
- **The lower hydrophobicity of ACQUITY CSH Phenyl-Hexyl Column stationary phase coupled with aqueous/acetonitrile mobile phase enabled the complete elution of extremely hydrophobic neutral lipid classes such as triacylglycerol (TG) and cholesterol ester (CE) in 10 min with excellent peak resolution.**
- **Higher hydrophobicity columns such as ACQUITY CSH C18 and CORTECS C8 Columns did not afford complete elution of the neutral lipids with the aqueous/acetonitrile MP.**
- **The final optimized LC condition for lipid separation is: ACQUITY CSH Phenyl-Hexyl column (2.1x150mm) at 70 ºC with a flow rate of 0.6 mL/min using water as MPA and acetonitrile/ water (95/5) as MP B both with 0.1% formic acid and 1mM ammonium formate as additives using a 70-99% MP B gradient over 6 min. IPA/ACN (50/50) was used as a sample diluent.**
- **No lipid carryover was observed with the developed method.**

References

1. Isaac G., Munjoma N., Gethings L.A., Mullin L. and Plumb R.S. A Robust and Reproducible Reversed-Phase Lipid Profiling Method for Large Sample Sets, Waters Application Note 720006959en. July 2020.

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