Development of a Targeted Metabolomics Method for Environmental Toxicants Using Mass Spectrometry

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Abstract

The aim of this study was to develop a mass spectrometry-based targeted-metabolite quantification method that can be utilized to investigate the metabolic effects of environmental toxicants on organisms. We employed an ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometer (UHPLC-QTOF-MS) to obtain accurate m/z ratios and chromatographic retention times for the target metabolites. Optimization of mobile phase compositions, concentration gradients, additive types, and QTOF-MS source parameters was performed to achieve an optimal chromatographic separation of the metabolites representing different chemical classes and polarities. While 77 metabolites eluted near the void volume during the initial chromatographic run due to their hydrophilic nature, 7 metabolites were retained only on a C18 column. To address the challenges posed by highly polar metabolites, we implemented a hydrophilic interaction liquid chromatography (HILIC) column on which 85 metabolites were detected in electrospray ionization (ESI) positive and negative modes. We observed higher MS signals using a combination of 10 mM ammonium formate, 0.1% ammonium hydroxide, and 0.1 % formic acid as mobile phase modifiers.

Introduction

The impact of environmental toxicants on ecosystems and living organisms remains uncertain due to their complex physicochemical properties and behaviour in the environment. Understanding how exposure to toxicants disrupt biological processes is crucial for environmental research [1]. While untargeted metabolomics approaches are hindered by the intricate identification steps resulting from the diverse chemical properties and metabolite concentrations in biological samples, the targeted approach acts as a valuable complement [2]. In targeted metabolomics, it becomes simple to pinpoint specific metabolites or disrupted metabolic pathways, thereby offering valuable insights into the consequences of environmental toxins on living organisms. In this investigation, we developed targeted metabolomics approach using MS to analyze 85 vital metabolites associated with carbohydrate, amino acid, nucleotide, cofactor, and vitamin metabolism pathways.

Experimental

Fig. 1: Workflow used in this study

Table 1: Chromatographic columns and conditions of mobile phase/buffers tested

Column	Solvents	Modifiers	Concentration
Zorbax Eclipse plus C18 column; (4.6 mm × 100 mm × 3.5 µm)	H_2O	Formic acid (FA)	0.1 %
	ACN:H_2O	Ammonium formate (AmF)	10 mM
Acquity UPLC BEH C18 column; (2.1 mm × 100 mm × 1.7 µm)	H_2O	Formic acid (FA)	0.1 %
	ACN:H_2O	Ammonium formate (AmF)	10 mM
Agilent Poroshell 120 HILIC ; (2.1 mm × 100 mm × 2.7 µm)	H_2O	Formic acid (FA)	0.1 %
	ACN:H_2O	Acetic acid (AA)	0.1 %, 0.5 %
		Ammonium formate (AmF)	10, 20, 100 mM
		Ammonium acetate (AmA)	10, 100 mM
UPLC BEH Amide column; (2.1 mm × 150 mm × 1.7 µm)	H_2O	Formic acid (FA)	0.1 %
	ACN:H_2O	Acetic acid (AA)	0.1 %
		Ammonium formate (AmF)	10 mM
		Ammonium acetate (AmA)	10 mM
		Ammonium hydroxide (AmH)	0.1 %

Time (mins)	Flow rate (mL/min)	Eluent A (%)	Eluent B (%)
0	0.3	5	95
1	0.3	5	95
14	0.3	35	65
16	0.3	60	40
18	0.3	60	40
18.1	0.3	5	95
23	0.3	5	95

Acidic mobile phase

A: 0.1 % Formic acid in 100 % H_2O

B: 0.1 % Formic acid in 100 % ACN

Ion Polarity: Positive

Basic mobile phase

A: 10 mM Ammonium formate + 0.1 % Ammonium hydroxide in 100 % H_2O

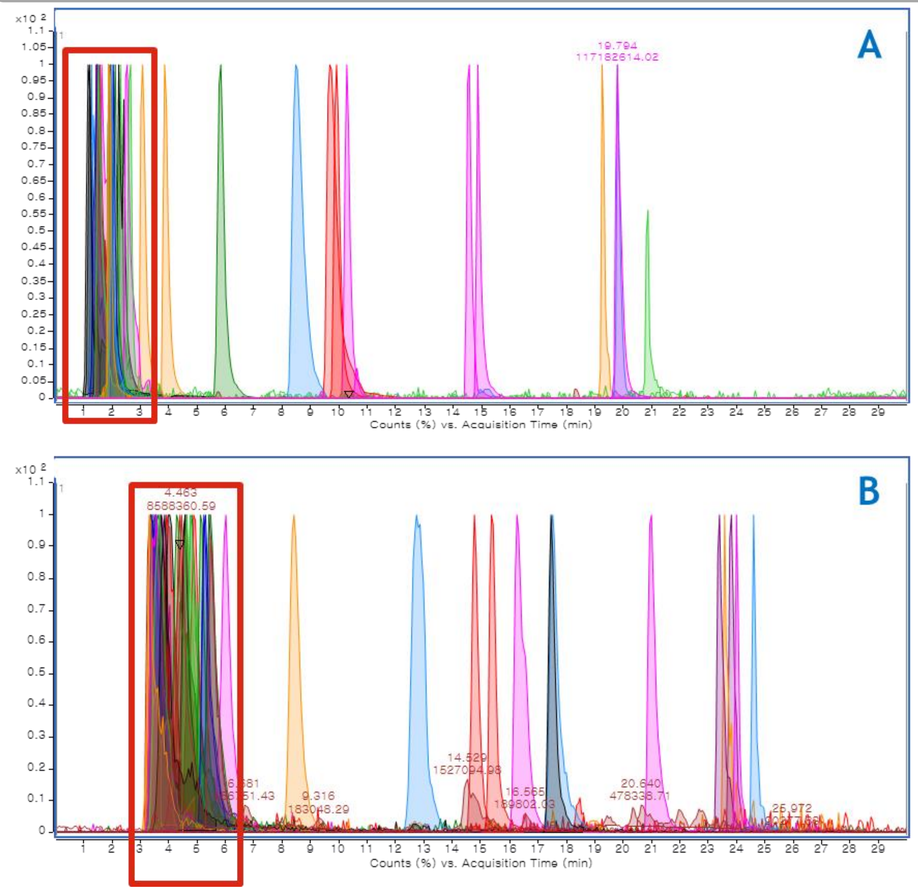
B: 10 mM Ammonium formate + 0.1 % Ammonium hydroxide in 9:1 ACN/H_2O

Ion polarity: Negative

Injection vol: 2 µL

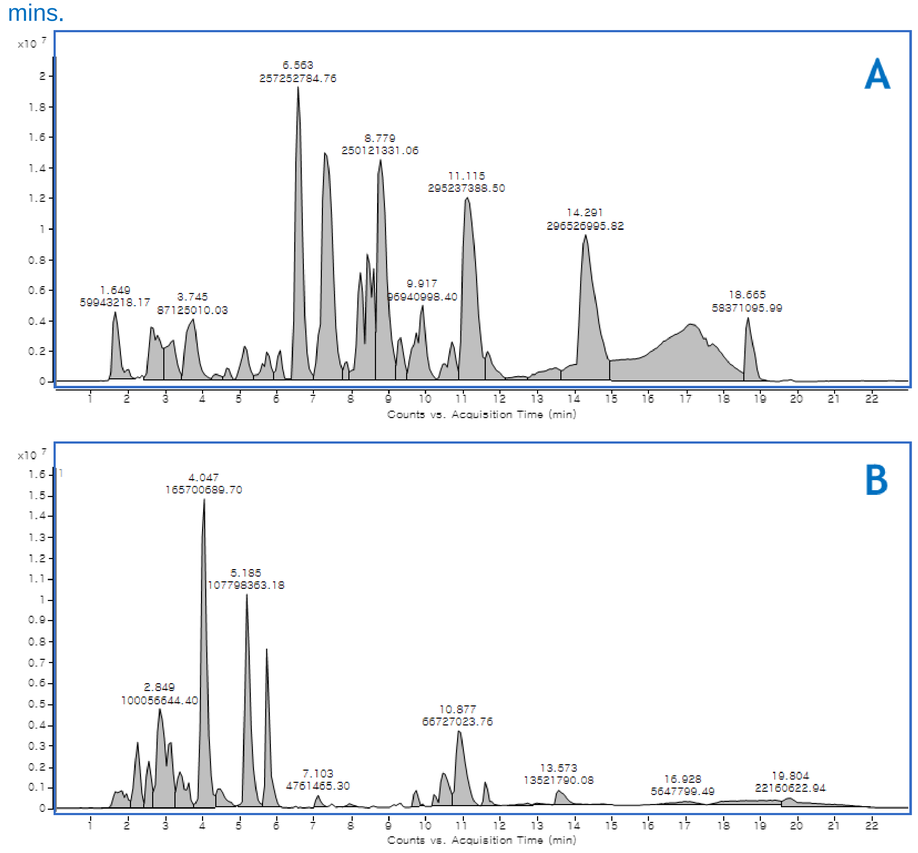
Fig. 2: Optimized LC condition

Results & Discussion



Acidic mobile phase
A: 0.1% FA in 100% Water
B: 0.1% FA in 100% ACN
Basic mobile phase
A: 10 mM AmF in 100% Water
B: 10 mM AmF in ACN/ Water (8:2)

Fig. 3: Normalised Extracted Ion Chromatogram (EIC) of metabolite standards detected in ESI positive mode (A) and negative mode (B) using Acquity UPLC BEH C18 column, (1.7 µm × 2.1 mm × 100 mm)
Unsatisfactory retention and separation of analytes was observed as most of the analytes eluted around 3-6 mins.



Acidic mobile phase
A: 0.1% FA in 100% Water
B: 0.1% FA in 100% ACN
Basic mobile phase
A:10 mM AmF + 0.1 % AmH
B: 10 mM AmF + 0.1 % AmH in ACN/ Water (9:1)

Fig. 4: BPC of metabolite standards detected in ESI positive mode (A) and negative mode (B) using a BEH amide column (2.1 mm × 150 mm × 1.7 µm)
85 compounds (39 in ESI positive mode) and (46 in ESI negative mode) were detected at a retention time range of 1.8 to 18.9 mins.

Conclusions

- ❖An optimized method for targeted metabolomics considers the use of specialized columns, mobile phase compositions, gradient elution strategy and other chromatographic parameters.
- ❖In this study, a suitable chromatographic condition that enabled the separation of 85 target metabolites without any overlap was developed using an ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometer (UHPLC-TOF-MS).
- ❖Subsequently, the sensitivity of the developed method will be enhanced through multiple reaction monitoring mode on a liquid chromatography triple quadrupole mass spectrometer (LC-QqQ-MS).

References

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2. Amer, B., Deshpande, R. R., and Bird, S. S. (2023). Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass Spectrometry-Based Metabolomics. Metabolites, 13(5), 648.

Acknowledgements

We acknowledge support from the National Research Foundation (NRF-2022R1A2C200595512).