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A New HILIC LC/Q-TOF Metabolomics Method with Biologically Important Isomer Separation and Broad Coverage of Metabolite Classes

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Introduction

High resolution accurate mass LC/TOF or Q-TOF MS is routinely used in metabolomics for discovery work. However, some analytical challenges remain including:

1) Retention of ionic metabolites.
2) Reproducibility in presence of salt.
3) Chromatographic separation of biologically relevant isomers.
4) Broad coverage of metabolite classes.

To address these challenges, we have developed a new HILIC LC/Q-TOF MS method with the aim of improving analytical performance for metabolome profiling.

- The preliminary results showed that the reproducibility of ionic and polar metabolites is greatly improved even in the presence of high salt concentration.
- By optimizing the chromatographic conditions, a superior analytical selectivity was achieved for important biological isomers and a broad class of metabolites was detected.
- Thus, it offers a powerful solution for comprehensive metabolomics profiling.

Experimental

Instrument and Supplies

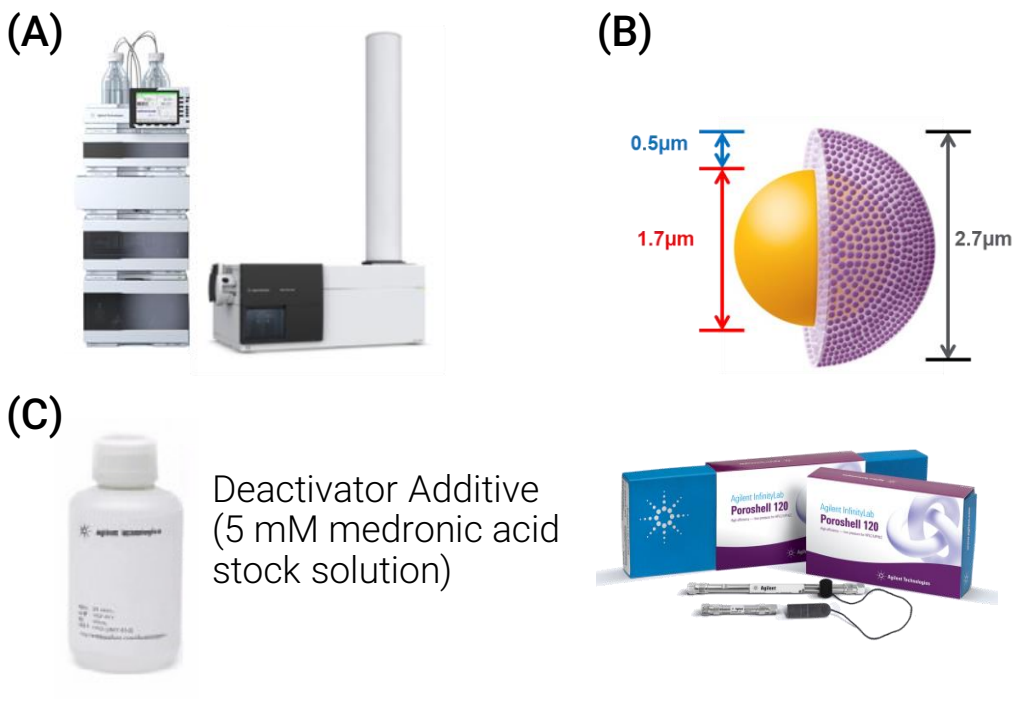

Figure 1. A robust and high performance HILIC LC/Q-TOF MS method was developed to improve the analytical selectivity, reproducibility, and coverage of metabolite for metabolome profiling using (A) 1290 Infinity Binary LC system coupled with 6545 Q-TOF (B) a newly developed HILIC column (p/n 673775-924) coupled with (C) a novel mobile phase additive (InfinityLab Deactivator Additive p/n 5191-4506).

Experimental

LC/MS Analysis

Metabolite standards were separated using Agilent's InfinityLab Poroshell 120 HILIC-Z columns (2.1 x 150 mm) on a 1290 LC system coupled on-line with a 6545 Q-TOF.

Labeled and unlabeled metabolite yeast extracts were obtained from Cambridge Isotope Laboratories.

Mobile phase and gradient conditions

For positive ion mode:

(A) 10 mM ammonium formate in water with 0.1% formic acid

(B) 10 mM ammonium formate in water/acetonitrile 10:90 (v/v) with 0.1% formic acid

- A non-linear gradient from 98% to 5% B in 18 minutes was used with a re-equilibration time of 5 minutes

For negative ion mode:

(A) 10 mM ammonium acetate in water with 2.5 µM medronic acid, pH=9

(B) 10 mM ammonium acetate in water/acetonitrile 15:85 (v/v) with 2.5 µM medronic acid, pH=9

- A non-linear gradient from 96% to 65% B in 24 minutes was used with a re-equilibration time of 5 minutes

Flow rate: 0.25 mL/min

Results and Discussion

Resolving Biologically Important Isomers

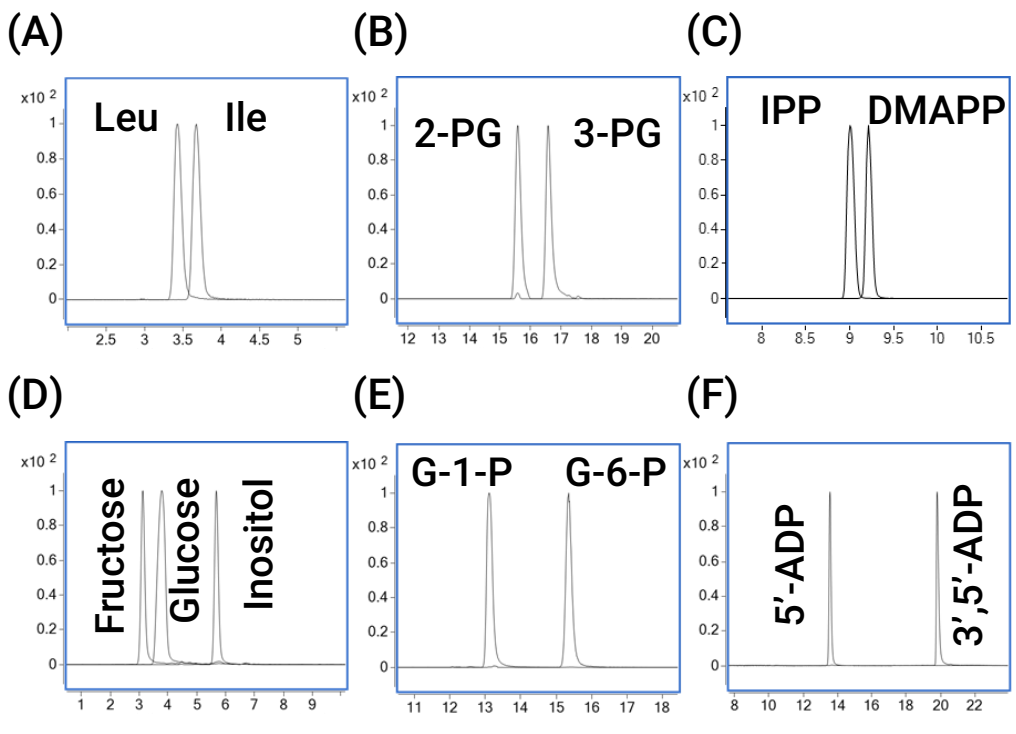

Figure 2. Chromatograms of structural isomers separated by HILIC LC/MS method.

List of Structural Isomers Investigated

Table 1. Separation of structural isomers.

No	Isomer pairs	MW	Polarity	Separation
1	β-Alanine/L-Alanine/L-Sarcosine	89.0477	pos/neg	separated
2	L-α-Amino-n-butyrate/ D, L-β-Aminoisobutyrate	103.0633	pos/neg	separated
3	Maleic acid/Fumaric acid	116.011	neg	separated
4	Methylmalonic acid/Succinic acid	118.0266	neg	separated
5	L-Leucine/L-Isoleucine	131.0946	pos/neg	separated
6	O-acetyl-L-serine/L-Glutamate	147.0532	neg	separated
7	Dihydroxyacetone phosphate/Glyceraldehyde-3-phosphate	169.998	neg	separated
8	Cis-Aconitate/Trans-Aconitate	174.0164	neg	separated
8	Fructose/Glucose or Galactose/Inositol	180.0634	neg	separated
9	Glucose/Galactose	180.0634	neg	Co-eluted
10	2-phosphoglycerate/3-phosphoglycerate	185.9929	neg	separated
11	Citric acid/Iso-citric acid	192.027	neg	Partial
12	IPP/ DMAPP	246.0058	neg	separated
13	α-G-1-P/D-G-6-P	260.0297	neg	separated
14	Fructose-6-phosphate/Fructose-1-phosphate	260.0297	neg	separated
15	α-D-Mannose-1-phosphate/D-Mannose-6-phosphate	260.0297	neg	Co-eluted
16	Fructose-1,6-bisphosphate/Fructose-2,6-bisphosphate	339.996	neg	Co-eluted
17	Maltose/α-Lactose	342.1162	neg	Co-eluted
18	5'-ADP/3'5'-ADP	427.0294	neg	separated
19	Methylmalonyl CoA/Succinyl CoA	867.1313	neg	separated

Tolerance to Biological Levels of Salt

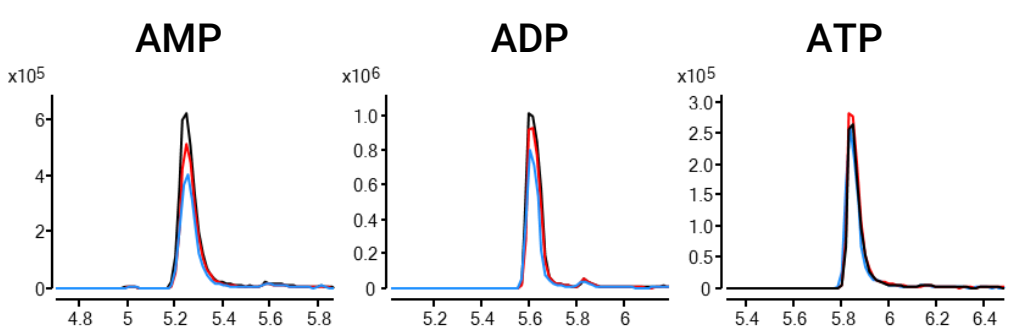

Figure 3. Metabolite standards were spiked with salt that was equivalent to 20% and 40% of salt concentrations found in human urine. Higher salt concentrations (>40%) were not attempted because the salt was not soluble in the sample matrix (80% ACN).

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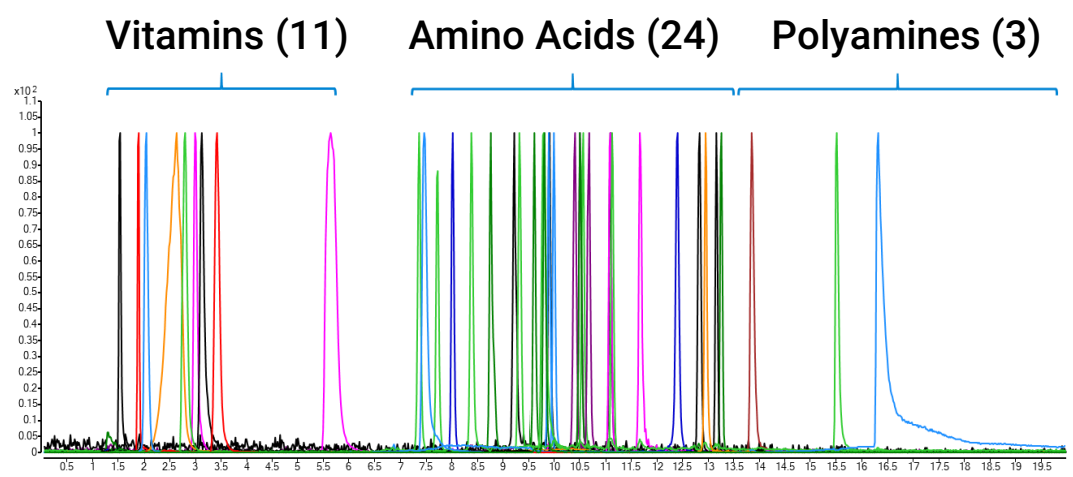
Resolving Near-isobaric Metabolites

Table 2. Separation of near-isobaric metabolites

Group	Metabolites	Formula	MW	Polarity	Separation
1	Glutaric acid	C5H8O4	132.0423	neg	separated
	L-Asparagine	C4H8N2O3	132.0535	neg/pos	
	L-Ornithine	C5H12N2O2	132.0899	neg/pos	
2	α-Ketoglutarate	C5H6O5	146.0215	neg	separated
	L-Glutamine	C5H10N2O3	146.0691	neg/pos	
	L-Lysine	C6H14N2O2	146.1055	neg/pos	
3	Cis-Aconitate	C6H6O6	174.0164	neg	separated
	L-Arginine	C6H14N4O2	174.1117	neg/pos	
	N-Acetyl-Ornithine	C7H14N2O3	174.2000	pos	

Developing a Robust HILIC-LC/MS Method to Analyze Polar Metabolites

(A) Positive Ion Mode



(B) Negative Ion Mode

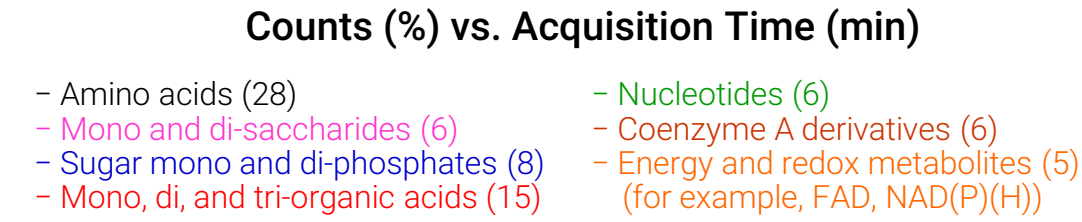


Figure 4. The chromatographic gradient, mobile phase pH, buffer concentration, and column temperature were carefully optimized for the analysis of polar metabolites in both (A) positive and (B) negative ion mode.

Results and Discussion

Optimized AJS Source Conditions for HLIIC LC/MS – Examples of Drying Gas Temperature Optimization

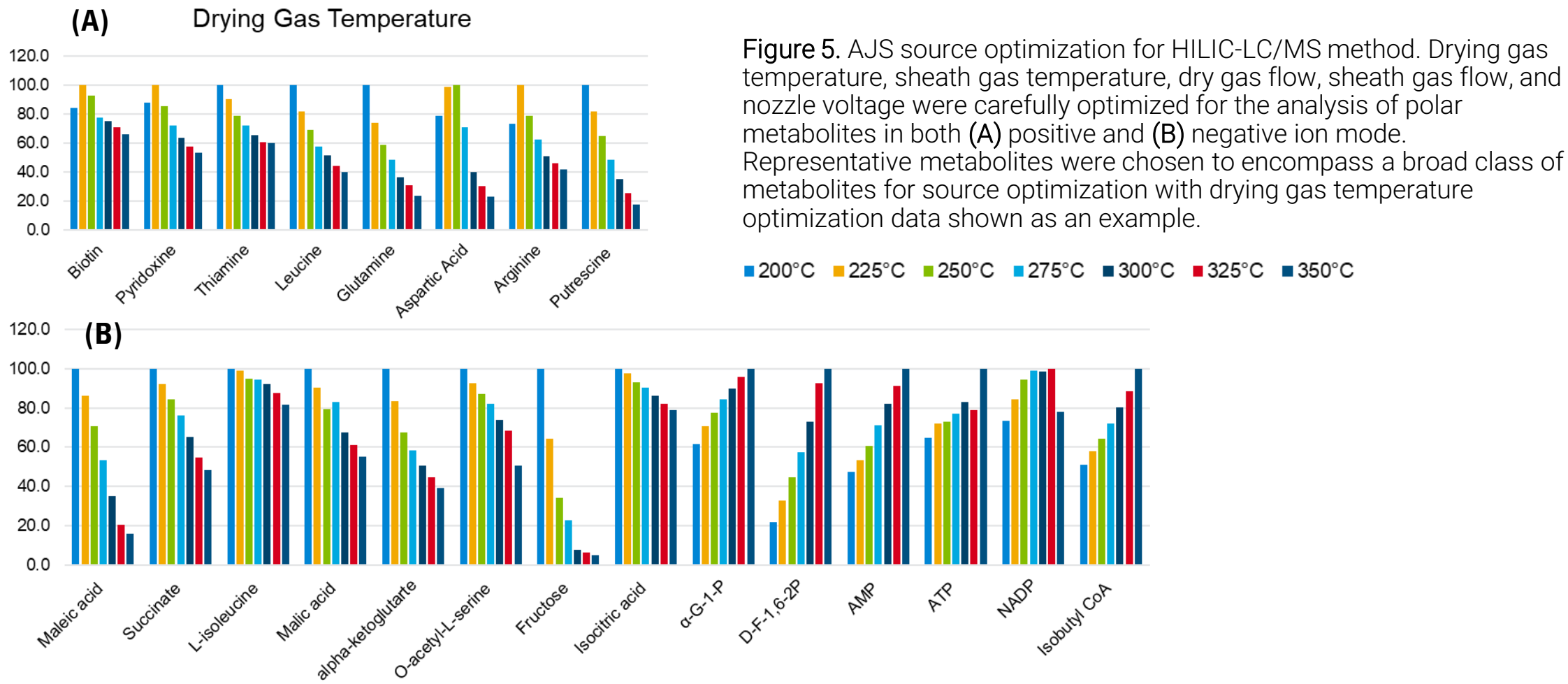


Figure 5. AJS source optimization for HILIC-LC/MS method. Drying gas temperature, sheath gas temperature, dry gas flow, sheath gas flow, and nozzle voltage were carefully optimized for the analysis of polar metabolites in both (A) positive and (B) negative ion mode. Representative metabolites were chosen to encompass a broad class of metabolites for source optimization with drying gas temperature optimization data shown as an example.

Reproducibility Study with Metabolites Extract from Yeast

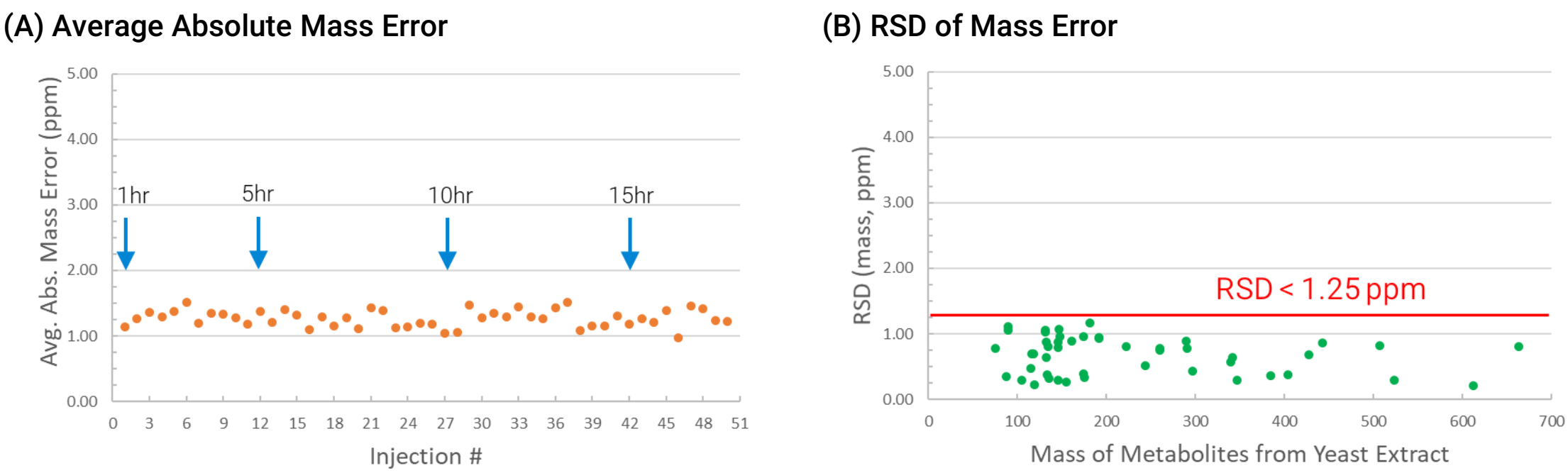


Figure 6. Excellent mass accuracy stability over 18-hour assay time for metabolites extracted from yeast. The (A) average absolute mass error was ≤ 1.5 ppm and (B) RSD of mass error was ≤ 1.25 ppm (n = 47 metabolites) in negative ion mode.

Conclusions

A new HILIC LC/Q-TOF MS method was developed with superior analytical performance:

- Global coverage of metabolites
- Excellent analytical reproducibility with high tolerance for biological levels of salt
- Necessary analytical selectivity for separation of biologically important isomer pairs
- Excellent mass accuracy stability over 18 hours

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