An Integrated Ultra-High Resolution FTICR-MS Based Platform for Metabolomics

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Overview

- We introduced an integrated high-throughput FTICR-MS based platform for metabolomics.
- Extraction methods were evaluated using swine serum via FTICR-MS.
- Plasma samples from diabetes-susceptible mice were analyzed via FTICR-MS.

Introduction

Metabolomics aims to characterize diverse classes of small molecules from a variety of sample types. For non-targeted metabolomics, direct injection mass spectrometry (MS) becomes a powerful technique due to its simplicity and high-throughput screening capability. The elimination of a separation step also prevents the introduction of biased and selective retention mechanisms. In this study, we have developed an integrated high-throughput magnetic resonance MS (MRMS), Fourier transform ion cyclotron resonance (FTICR) MS, based platform for metabolomics. The ultra-high resolution, high sensitivity and wide dynamic range of the 12T FTICR mass spectrometer enable the detection of numerous metabolites in a single mass spectrum. Various extraction methods and MeOH:sample ratios were evaluated using swine serum via FTICR-MS. Metabolomic analysis of diabetic mouse plasma samples was also performed. We conclude that this optimized, integrated platform can advance high-throughput screening and discovery approaches.

Methods

FTICR-MS Based Platform for Metabolomics

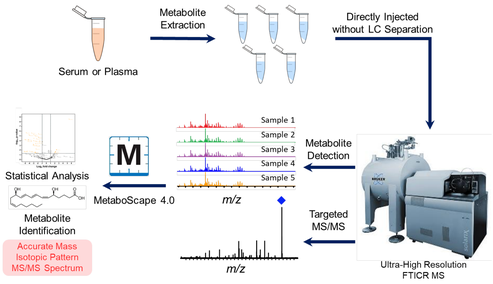


Figure 1. Workflow of FTICR-MS based platform for metabolomics. Metabolite extracts were directly injected into FTICR-MS without LC separation. MS: Bruker solariX 12T FTICR mass spectrometer.

Sample Information

1. Swine serum for extraction method evaluation
2. Plasma from diabetes-susceptible BTBR-Obese mice
   Sample Groups:
   OAP (n=5); OAP-T2D (n=6); OAT-T2D (n=6)
   Obese; Animal Research Facility; Purina or Teklad diet
   T2D: type 2 diabetes
   OAP are obese and severely insulin resistant but not T2D
   Diets are identical in macronutrients but not micronutrients

MetaboScape 4.0 Parameters

Bucket list: T-Rex 2D Algorithm
mzDelta: 0.5 mDa
Max. Charge: 3
Intensity Threshold: 0
Minimum # Features for Results: 5
Positive Mode: +H, +NH_4, +Na, +H-H_2O
Negative Mode: -H, +Cl

Smart Formula Annotation:
Mass Accuracy: Δm/z < 2 ppm; Δm/z < 5 ppm
mSigma* (Δm/z < 2 ppm): < 20; < 50
*Isotopic pattern fit score

Results and Discussion

1. Extraction Method Evaluation

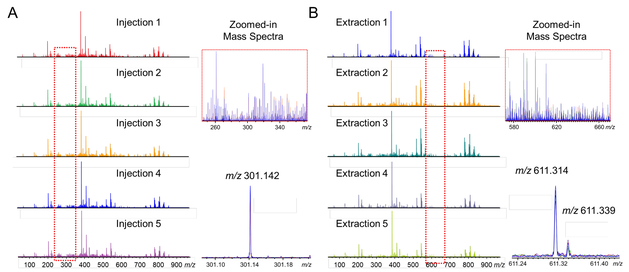


Figure 2. FTICR-MS of swine serum samples. **A.** Mass spectra of 5 injection replicates in positive mode, overlapped zoomed-in mass spectra (m/z 250-370) and a representative feature at m/z 301.142. Median of CV% of the features detected in all 5 injection replicates is 9.1%. **B.** Mass spectra of 5 extraction replicates in positive mode, overlapped zoomed-in mass spectra (m/z 580-670) and two representative features at m/z 611.314 and 611.339. Median of CV% of the features detected in all 5 extraction replicates is 14.6%.

A

Extraction	MeOH	IPA	ACN	Acetone	EtOH	ACN:IPA:H_2O 3:3:2	ACN:MeOH 7:3	Acetone:MeOH 7:3
Δm/z < 2 ppm	1470	1391	1032	1500	1484	898	1476	1397
Δm/z < 5 ppm	1567	1492	1095	1591	1552	969	1558	1493
mSigma* < 20	71	51	62	66	41	25	69	66
mSigma* < 50	218	211	148	194	140	76	159	167

* Δm/z < 2 ppm

B

MeOH	IPA	ACN	Acetone	EtOH	ACN:IPA:H_2O 3:3:2	ACN:MeOH 7:3	Acetone:MeOH 7:3	
1567	668	605	860	828	340	861	896	MeOH
	1492	578	628	864	385	751	648	IPA
		1095	721	634	398	649	659	ACN
			1591	816	342	864	947	Acetone
				1552	387	1021	862	EtOH
					969	391	355	ACN:IPA:H_2O 3:3:2
						1558	955	ACN:MeOH 7:3
							1493	Acetone:MeOH 7:3

Table 1. A. Comparison of molecular formula annotation numbers from eight metabolite extraction methods. **B.** Comparison of reproducible features (Δm/z < 5 ppm) shared in common between eight metabolite extraction methods. The annotation numbers represent the total numbers of features detected and assigned in at least 2 of 5 extraction replicates. Overall, MeOH, acetone, EtOH, IPA, acetone:MeOH 7:3 and ACN:MeOH 7:3 show similar performance for metabolite extraction based on the annotation numbers and reproducible features shared.

Ratio	Δm/z < 2 ppm	Δm/z < 5 ppm	mSigma* < 20	mSigma* < 50
1:1	126	135	13	27
2:1	1418	1501	63	161
4:1	537	582	37	148
6:1	346	372	23	77
8:1	346	382	35	85

* Δm/z < 2 ppm

Table 2. Comparison of molecular formula annotation numbers from MeOH extractions at different MeOH:sample ratios. It is very clear that 2:1 MeOH:sample is the most efficient method for metabolite extraction and detection. Thus, we used MeOH extraction at 2:1 MeOH:sample ratio for serum or plasma metabolomic analysis.

2. Ultra-High Resolution and High Mass Accuracy of FTICR-MS

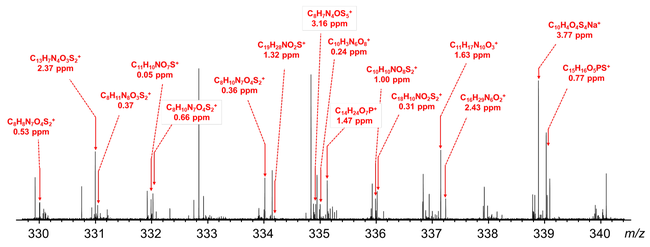


Figure 3. Zoomed-in mass spectrum in the *m/z* range from 330 to 340. In a single mass spectrum, 16 features were assigned in 10 m/z range with Δm/z < 5 ppm. 12 of these features were assigned with Δm/z < 2 ppm.

3. Metabolomic Analysis of Diabetic Mouse Plasma

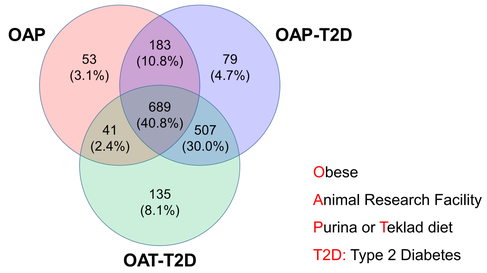


Figure 4. Venn diagram of OAP, OAP-T2D and OAT-T2D plasma samples. The features reported in this study include all features from both positive and negative mode detection after blank reduction. Most shared features were detected between the two diabetic groups.

4. t-Test Analysis

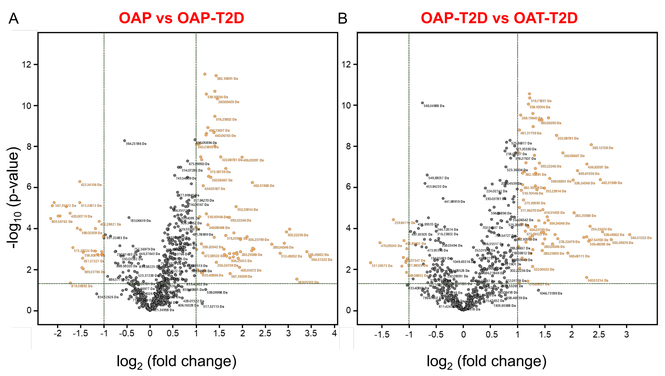


Figure 5. A. Volcano plot of OAP vs. OAP-T2D. 277 metabolic features show statistically significant differences (P < 0.05). **B.** Volcano plot of OAP-T2D vs. OAT-T2D. 231 metabolic features show statistically significant differences (P < 0.05). Orange dots indicate the features have at least 2 fold changes between groups, and black dots indicates the features have at most 2 fold changes between groups.

5. Heatmap of Top 50 Significant Features

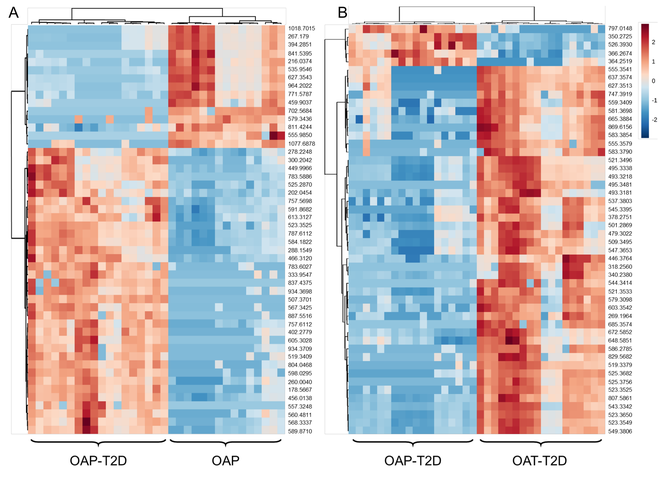


Figure 6. A. Heatmap representing top 50 significant features from t-test analysis of OAP vs. OAP-T2D. **B.** Heatmap representing top 50 significant features from t-test analysis of OAP-T2D vs. OAT-T2D. Three injection replicates were performed for each sample from OAP (non-diabetic with Purina diet, n=5), OAP-T2D (diabetic with Purina diet, n=6) and OAT-T2D (diabetic with Teklad diet, n=6).

6. Representative Metabolites

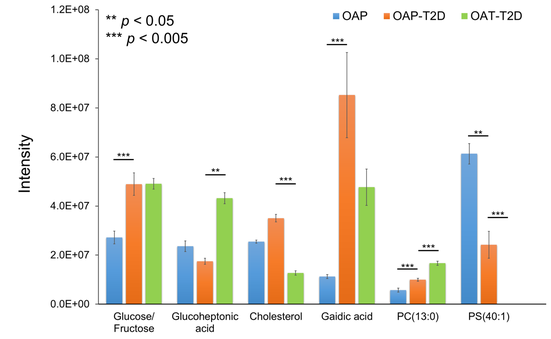


Figure 7. Comparison of representative putative metabolites from OAP, OAP-T2D and OAT-T2D plasma samples. Glucose/fructose level is significantly increased in diabetic groups as expected. Glucoheptonic acid and PC(13:0) are significantly increased in Teklad diet group, whereas cholesterol and PS(40:1) are significantly increased in Purina diet group. Error bars represent the standard error of the mean (SEM).

7. Targeted Metabolite Identification

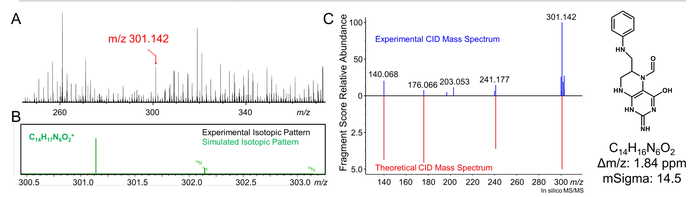
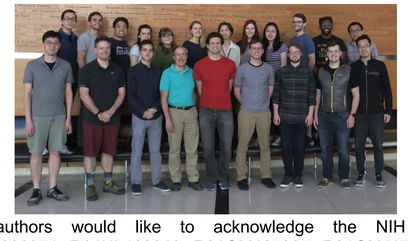


Figure 8. A. Targeted feature found based on highly accurate mass. **B.** Compared with simulated isotopic pattern to confirm the chemical formula. **C.** Structure identification based on MS/MS spectrum matching.

Conclusions

- We introduced an integrated high-throughput FTICR-MS based platform for serum and plasma metabolomics.
- MeOH extraction with a 2:1 MeOH:sample ratio shows the highest efficiency for serum and plasma metabolite extraction and detection.
- Metabolomic analysis of OAP (n=5), OAP-T2D (n=6) and OAT-T2D (n=6) mouse plasma samples were performed via FTICR-MS. Most shared metabolic features were detected between the two diabetic groups.
- 277 metabolic features show statistically significant differences between OAP and OAP-T2D groups. 231 metabolic features show statistically significant differences between OAT-T2D and OAP-T2D groups.
- MS/MS capability increases the confidence of targeted metabolite identification.

Acknowledgements



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