Automation of sample preparation for metabolomic analysis using robotic platform

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RESEARCH OBJECTIVE

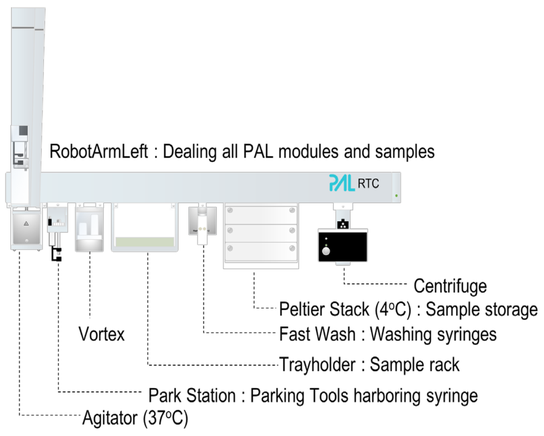
Metabolomic analysis is prone to technical errors at different states of sample preparation (e.g. extraction, purification, derivatization). Therefore, reproducible sample preparation is vital for ensuring reproducible and reliable results. Additionally, sample preparation has historically been a time-consuming challenge and a crucial bottleneck in the whole analytical process. It is desired to develop a method for reproducible, reliable and labor and time-saving sample preparation. Application of robotic platform to automation of sample preparation is a promising approach in this direction. Herein, we developed an automated sample preparation protocol based on a robotic platform PAL RTC (CTC Analytics AG, Zwingen Switzerland), which represent a modified Bligh and Dyer method producing samples for both hydrophilic metabolomics using GC-MS and lipidomics using SFC-MS simultaneously.

INSTRUMENTS & CONDITION

Robotic Platform

CTC Analytics AG PAL RTC

Robotic Tool : PAL RTC (CTC Analytics AG, Zwingen, Switzerland)
Control Software: PAL sample control (CTC Analytics AG)
: Chronos (Axel Semrau, Sprockhövel, Germany)



GC/MS for Hydrophilic Metabolomic Analysis

GC analytical conditions (Agilent GC 7890A)

Column	Agilent 122-5532G （DB-5ms (DuraGuard) : 40 m x 250 µm x 0.25 µm)
Column flow	1.581 mL/min (RTL : d27 Myristic acid (m/z 312.4) set to RT = 16.727 min)
Inlet temperature	250˚C
Oven temperature	60˚C (1 min), 10˚C /min, 325˚C (10 min)
Sample Injection	Split, 10:1

Agilent G1676AA
Fiehn GC/MS Metabolomics RTL Library Method

MS analytical conditions (Agilent MSD 5975C)

Aux temperature	290 ˚C
Ionization	70 eV
Ion source temperature	230 ˚C
Mode	Scan (m/z 50-650)

Derivatization and Sample Injection (PAL RTC)

Derivatization	
Reaction	in Agitator 37˚C
Reagents	on Trayholder (w/o temperature control)
Reagent collection	Tool with 100 µL syringe
Injector	Tool with 10 µL syringe
Injection volume	1 µL

Agilent GC-MS system 7980A-5975C with PAL RTC

SFC/MS/MS for Lipidomic Analysis

SFC analytical conditions (ACQUITY UPC²)

Column	ACQUITY UPC² Torus DEA (3.0mm×100mm, 1.7µm)
Flow rate	1.0 mL/min
Mobile phase	CO_2
Modifier	95% Methanol and 5% Water with 0.1%(w/v) ammonium acetate
Gradient condition	1% (0-1min), 1-65% (1-12min), 65% (12-18min), 65-1% (18-18.1min), 1% (18.1-20min)
Sample volume	1 µL
Pump pressure	103.4 bar
Oven temperature	50 ˚C
Analytical time	20 min
Make up pump	0.2 mL/min

MS analytical conditions (Xevo TQ-S micro)

ES+		Extended	
Source voltages		**Source**	
Capillary	3.00 kV	Source Temp	150 ˚C
Cone	60 V	**Collision Cell Lenses**	
Source temperatures		Entrance	1.0
Desolvation Temp	500 ˚C	Exit	1.0
Source Gas Flow			
Desolvation	1000 L/Hr		
Cone	50 L/hr		

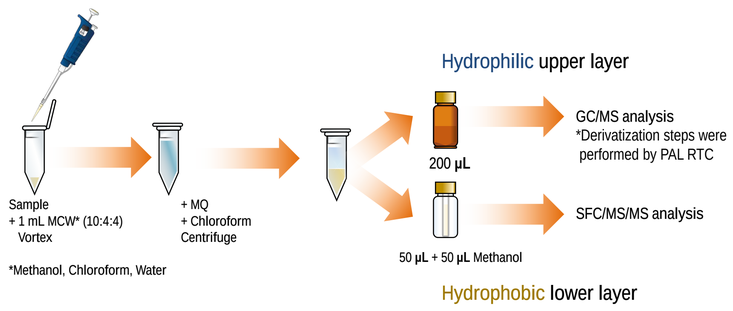
ACQUITY UPC²

Xevo TQ-S micro

METHODS

Conventional Manual Method

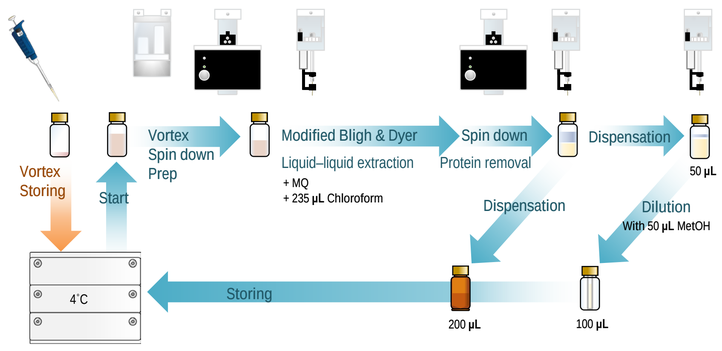
Modified Bligh and Dyer Extraction



Automated Methods using PAL RTC

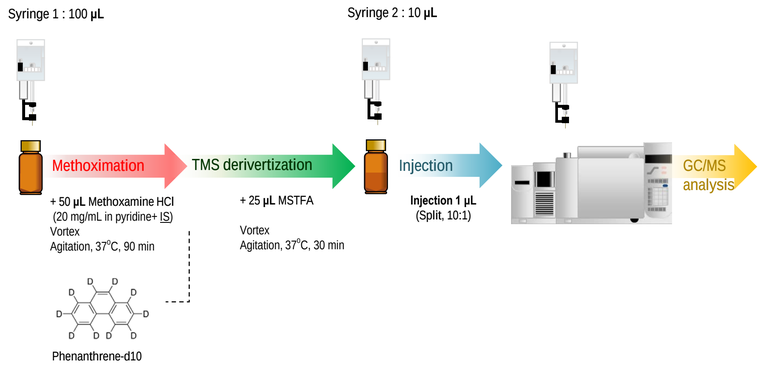
Modified Bligh and Dyer Extraction by PAL RTC

- 10 µL Sample, 10 µL IS-1 (For GC/MS), 10 µL IS_L (For SFC/MS), 1 mL Methanol Chloroform Water (10:4:4)



30 samples can be prepared in over-night (15 h)

Automated Sample Derivatization and Injection



"Just –in-Time" derivatization and injection to GC-MS

RESULTS & DISCUSSION

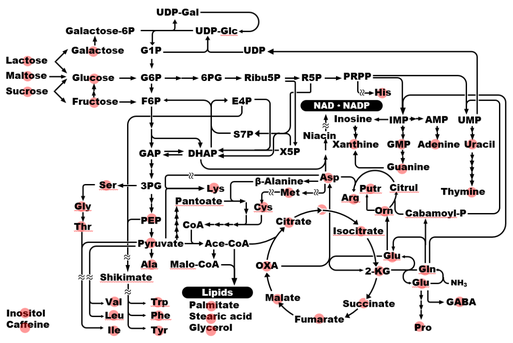
Hydrophilic Metabolomic Analysis using GC/MS

Target metabolites of GC/MS analysis

Sample : Rat and mouse serum
Standard substances : GC・GC/MS Metabolomics analysis standard mix (GL science Inc., 1021-58400)
Number of components : 52
Dilution solvent : Methanol and some additives
Concentration : 40 µM (5 premix groups, 200 µM)
Storage : -30°C

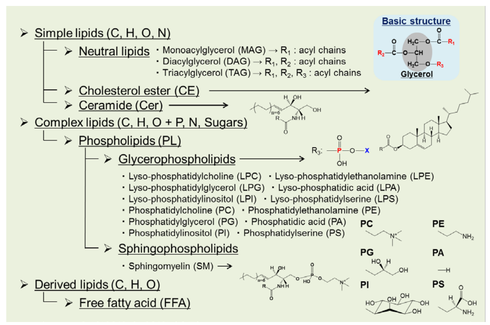
Standard substrates

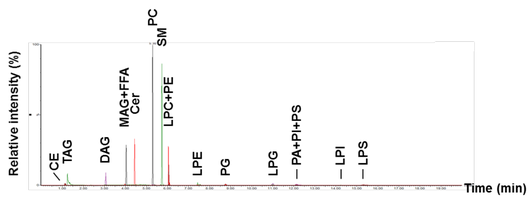
Pyruvic acid	Glycolic acid	Phosphoric acid	Glycerol
Malic acid	4-Aminobutyric acid	α-Ketoglutaric acid	Aconitic acid
Palmitic acid	Stearic acid	Ergosterol	Valine
Proline	Glycine	Serine	Alanine
Aspartic acid	Cysteine	Glutamic acid	Phenylalanine
Ornithine	Glutamine	Lysine	Histidine
Fructose	Glucose	Inositol	Sucrose
Maltose	Raffinose	Uracil	Thymine
Adenine	Xanthine	Guanine	Inosine
Succinic acid	Citiric acid	Leucine	Threonine
Fumaricacid	Isocitric acid	Isoleucine	Methionine
Asparagine	Tyrosine	β-Lactose	Cytosine
Putrescine	Tryptophan	Trehalose	Caffeine



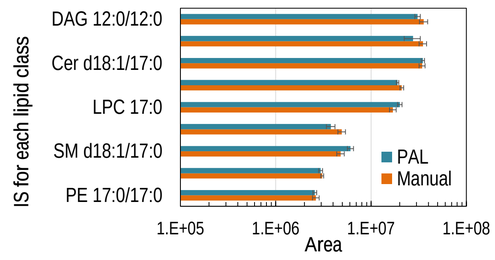
Lipidomic Analysis using SFC/MS

Target lipids of SFC/MS/MS analysis

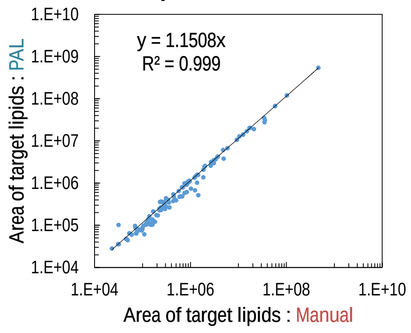




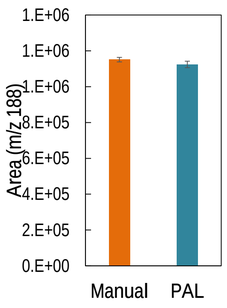
IS Abundances in Serum samples



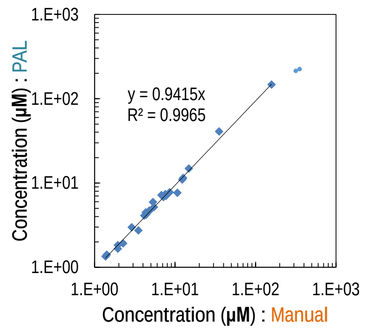
Detected Lipids Abundances



Repeatability of IS

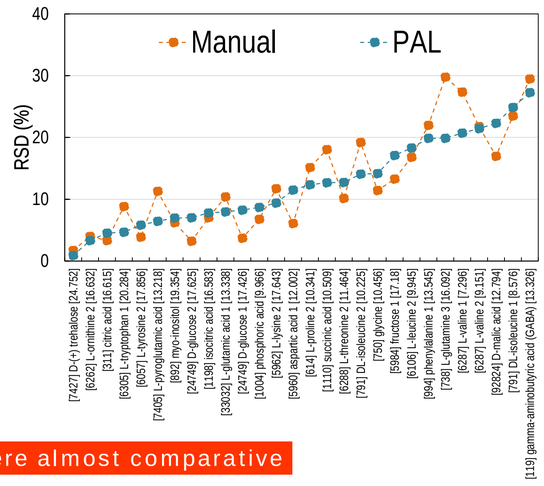


Abundances of Metabolites



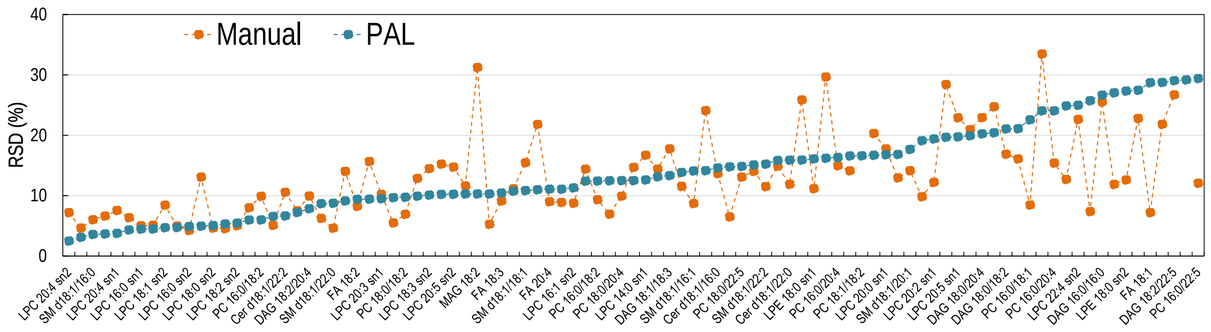
Manual vs PAL

Repeatability of Detected Metabolites



Detected metabolites number, amounts, repeatability were almost comparative

Repeatability in Quantification of Lipids in Serum Pool Samples



Manual vs PAL

Detected lipids number and amounts were same, but repeatabilities were slightly different

Conclusions and Future Works

Conclusions

- ~30 serum samples were extracted and dispensed into hydrophobic and hydrophilic samples automatically.
- Detected metabolites number and abundances were almost comparable between manual and PAL method.
- Repeatability in quantification were almost comparable between manual and PAL method.

Future works

- Under developing : Sample storage condition, scrutiny of extraction efficiency
- Under investigation: reason of difference in reproducibility between PAL and manual procedure

Resource and Other Information

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References

1. Bligh, E. Graham, and W. Justin Dyer. "A rapid method of total lipid extraction and purification." Canadian journal of biochemistry and physiology 37.8 (1959): 911-917.
2. Kind, Tobias, et al. "FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry." Analytical chemistry 81.24 (2009): 10038-10048.
3. Bamba, Takeshi. "Application of supercritical fluid chromatography to the analysis of hydrophobic metabolites." Journal of separation science 31.8 (2008): 1274-1278.