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Application Data Sheet

No.49

LC-MS
Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of 97 Primary Metabolites By PFPP: Pentafluorophenylpropyl Column

Energy production is essential for every living organism. Energy production at the cellular level is carried out by various metabolic processes, including the glycolytic system and the TCA cycle. In order to investigate the metabolism of living systems, it is important to quantify the amount of each metabolite. However, many primary metabolites are hydrophilic and difficult to analyze by reversed-phase chromatography. Ion pair chromatography is sometimes used for the analysis of such hydrophilic compounds but it is not preferable for LCMS analysis because ion pair reagents cause high background signals and sensitivity deterioration.

A new method using a Pentafluorophenylpropyl (PFPP) column has been developed to overcome these limitations. This data sheet presents the simultaneous analysis of 97 primary metabolites, such as amino acids, organic acids, nucleotides, nucleosides and co-enzymes, using a PFPP column, which uses particles functionalized with a pentafluorophenylpropyl group. It allows not only hydrophobic interaction but also retention of hydrophilic compounds, which is essential for successful analysis of primary metabolites.

Samples were tissue extracts. HPLC and MS experimental conditions include the LC/MS/MS method package for primary metabolites Version 2 and all measurements were done by Shimadzu LCMS-8040 Triple Quadrupole Ultra Fast Mass Spectrometer (UFMS), which features a 5 msec dwell time and 15 msec polarity switching rate.

■ Compound List (Color legend; TCA: ■ Methylation and Transsulfuration: ■ Urea: ■)

2-Aminobutyric acid	Citicoline	Guanosine	Orotic acid
2-Ketoglutaric acid	Citric acid	Guanosine 3',5'-cyclic monophosphate	Oxidized glutathione
2-Morpholinoethanesulfonic acid	Citrulline	Guanosine monophosphate	Pantothenic acid
4-Aminobutyric acid	Creatine	Histamine	Phenylalanine
4-Hydroxyproline	Creatinine	Histidine	Proline
5-Glutamylcysteine	Cystathionine	Homocysteine	Pyruvic acid
Acetylcarnitine	Cysteamine	Homocystine	S-Adenosylhomocysteine
Acetylcholine	Cysteine	Hypoxanthine	S-Adenosylmethionine
Aconitic acid	Cystine	Inosine	Serine
Adenine	Cytidine	Isocitric acid	Serotonin
Adenosine	Cytidine 3',5'-cyclic monophosphate	Isoleucine	Succinic acid
Adenosine 3',5'-cyclic monophosphate	Cytidine monophosphate	Kynurenine	Symmetric dimethylarginine
Adenosine monophosphate	Cytosine	Lactic acid	Taurocholic acid
Adenylsuccinic acid	Dimethylglycine	Leucine	Threonine
Alanine	Dopa	Lysine	Thymidine
Allantoin	Dopamine	Malic acid	Thymidine monophosphate
Arginine	Epinephrine	Methionine	Thymine
Argininosuccinic acid	FAD	Methionine sulfone	Tryptophan
Asparagine	FMN	Methionine sulfoxide	Tyrosine
Aspartic acid	Fumaric acid	NAD	Uracil
Asymmetric dimethylarginine	Glutamic acid	Niacinamide	Uric acid
Carnitine	Glutamine	Nicotinic acid	Uridine
Carnosine	Glutathione	Norepinephrine	Valine
Cholic acid	Glycine	Ophthalmic acid	Xanthine
Choline	Guanine	Ornitine	

HPLC conditions

Column	: Discovery HS F5-3 (2.1 mm I.D. X 150 mm L., 3 µm)
Mobile phase A	: 0.1% formic acid / Water
Mobile phase B	: 0.1% formic acid / Acetonitrile
Time program	: 0% B. (0-2.0 min)→25%B. (5.0 min)→35%B. (11.0 min)→95% B. (15.0-20.0 min)→0% B. (20.1-25.0 min)
Flow rate	: 0.25 mL / min.
Injection volume	: 3 µL
Oven temperature	: 40℃

MS conditions: LCMS-8040

Ionization mode	: ESI (Positive/Negative)
Nebulizer gas	: 2.0L / min.
Drying gas	: 15.0L / min.
DL temperature	: 250℃
Heat block temperature	: 400℃

After excising liver and heart tissues from mice followed by immediate freezing in liquid nitrogen, each frozen sample was weighed and homogenized in methanol containing internal standards. After homogenization, methanol-chloroform extraction was carried out. After collection of hydrophilic metabolites, extracts were concentrated. After preparation, 3 uL of diluted samples were injected on the LCMS-8040. Figure 1 illustrates overlaid MRM chromatograms of metabolites from liver and heart tissue extracts. In each extract, over 80 metabolites were detected. Major peaks and specific metabolites have been highlighted for each tissue.

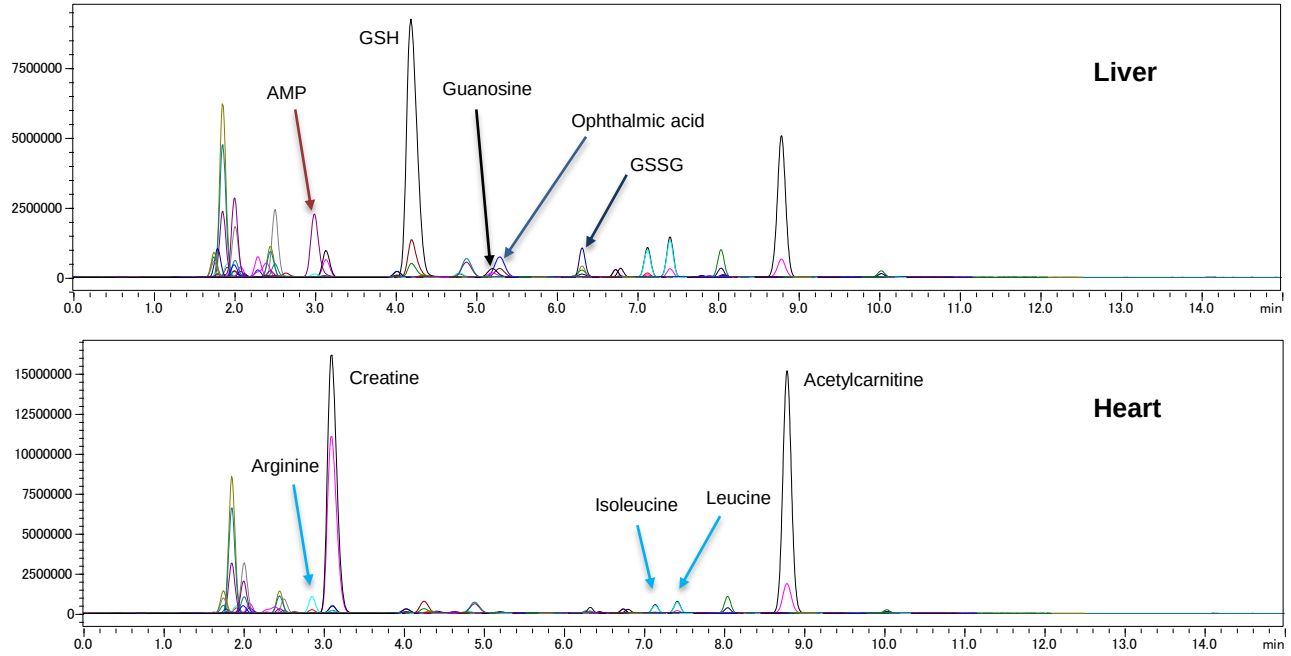
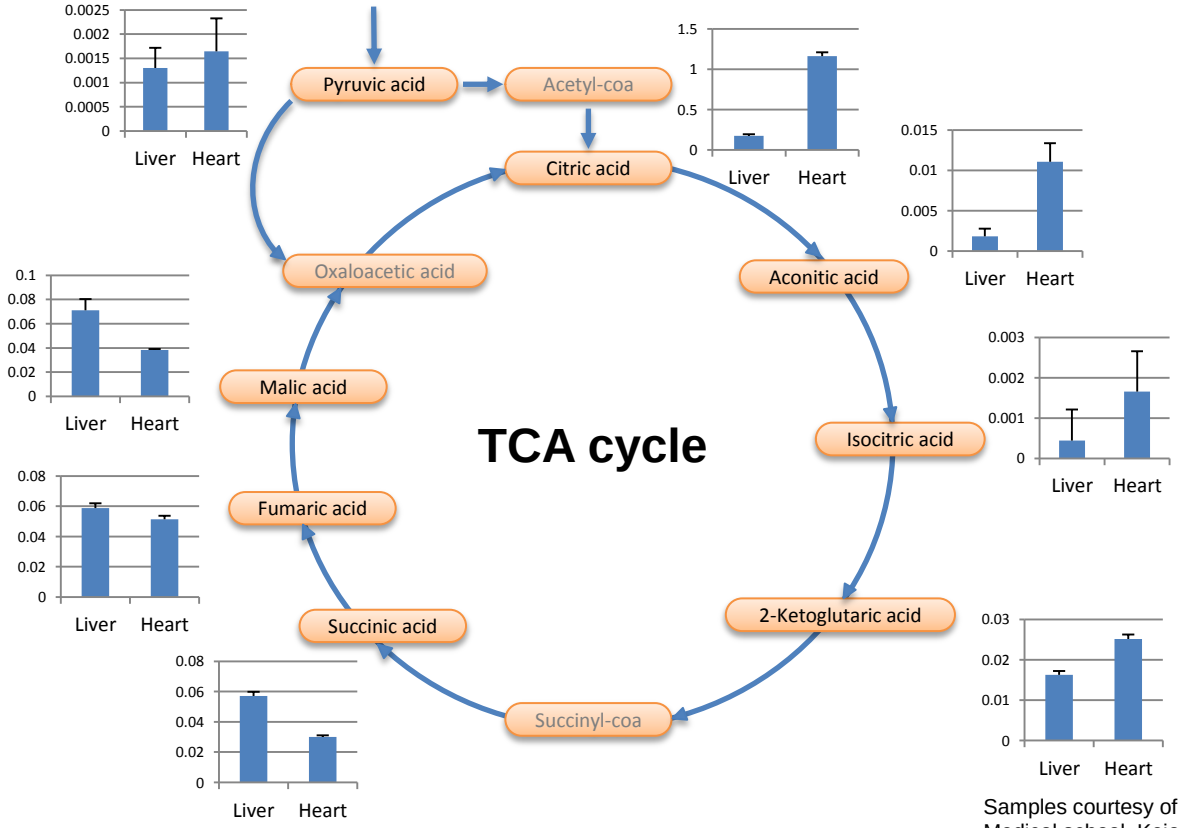


Fig. 1: MRM chromatograms for 97 primary metabolites, Upper: Liver, Lower: Heart

As an example of metabolite comparison on the metabolic pathway, Figure 2 illustrates the area ratio of organic acids in the TCA cycle of liver and heart tissue samples. Each ratio was calculated based on area of internal standard, 2-Morpholinoethanesulfonic acid in triplicate. The PFPP column was especially effective at separating amino and organic acids. The difference of relative ratio for organic acids on TCA cycle in two different tissues was confirmed.



Samples courtesy of Dr. Suematsu, Medical school, Keio University

Fig. 2: Area comparison of organic acids in the TCA cycle between liver and heart tissue

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