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Application Data Sheet

No.42

LC-MS
Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of Hydrophilic Metabolites Using Triple Quadrupole LC/MS/MS

Many primary metabolites associated with the central metabolic pathway and glycolytic system are hydrophilic compounds, which are difficult to separate using reversed phase chromatography. Ion pairing reagents may be added to the mobile phase to separate and retain polar primary metabolites on reversed phase columns. For instance, in order to measure anions, such as sugar phosphates, nucleotides and organic acids, a cationic ion pair reagent in the mobile phase can be used. In this data sheet, tributylamine was used as an ion pair reagent. It illustrates simultaneous analysis of hydrophilic metabolites using a Shimadzu UFMS triple quadrupole mass spectrometer, the LCMS-8040, with a ready-to-use “LC-MS/MS method package for Primary Metabolites”.

The compound panel is illustrated below. It includes very important metabolites such as amino acids, organic acids, sugar phosphates, nucleotides and co-enzymes whose metabolic changes need to be observed. In simultaneous analysis of these metabolites, it is important to accurately reflect biological variation by preventing analyte degradation during sample preparation as much as possible. We have investigated this effect during sample preparation by comparing an intentionally degraded sample with a properly prepared sample.

■ Compound Panel

Lysine	Fructose-6-phosphate	UDP
Arginine	Tryptophan	3-Phosphoglycerate/2-Phoshoglycerate
Histidine	Glycerol-3-phosphate	NADP
Glycine	Glucose-1-phosphate	Fructose-1,6-bis-phosphate
Serine	Glyceraldehyde-3-phosphate	NADH
Asparagine	Erythrose-4-phosphate	ADP
Alanine	Ribulose-5-phosphate	TDP
Glutamine	Pyruvate	Phosphoenolpyruvate
Threonine	CMP	CTP
Cysteine	NAD	GTP
L-Methionine sulfone	Dihydroxyacetonephosphate	UTP
Methionine	UMP	ATP
Tyrosine	GMP	TTP
MES (2-Morpholinoethanesulfonic acid)	TMP	NADPH
Glutamate	AMP	2,3-bis-Phosphoglycerate
Aspartate	cGMP	Succinyl-CoA
Phenylalanine	cAMP	Acetyl-CoA
Glucose-6-phosphate	CDP	
Ribose-5-phosphate	6-Phosphogluconate	
Sedoheptulose-7-phosphate	GDP	

HPLC conditions

Analytical column	: Mastro C18 (2.0 mmI.D. X 150 mmL., 3 µm)
Mobile phase A	: 15 mmol/L acetic acid, 10 mmol/L tributylamine aq.
Mobile phase B	: Methanol
Time program	: 0% B. (0-0.5min) →25%B. (8 min)→98% B. (12-15 min)→0% B. (15.1-20 min)
Flow rate	: 0.3mL / min.
Injection volume	: 3 µL
Column oven temp.	: 40℃

MS conditions: LCMS-8040

Ionization mode	: ESI (Negative)
Applied voltage	: -3.5kV
Nebulizer gas	: 2.0L / min.
Drying gas	: 10.0L / min.
DL temp.	: 250℃
Heat block temp.	: 400℃

Homogenized mouse liver tissue was used. It was prepared by rapidly freezing with liquid nitrogen immediately after collection. After leaving the weighed tissues for 0, 2 or 10 min at room temperature, samples were treated with methanol-chloroform and then the samples were concentrated. Figure 2 illustrates MRM chromatograms of liver tissue extracts at each time point. Significant changes were observed in several metabolites.

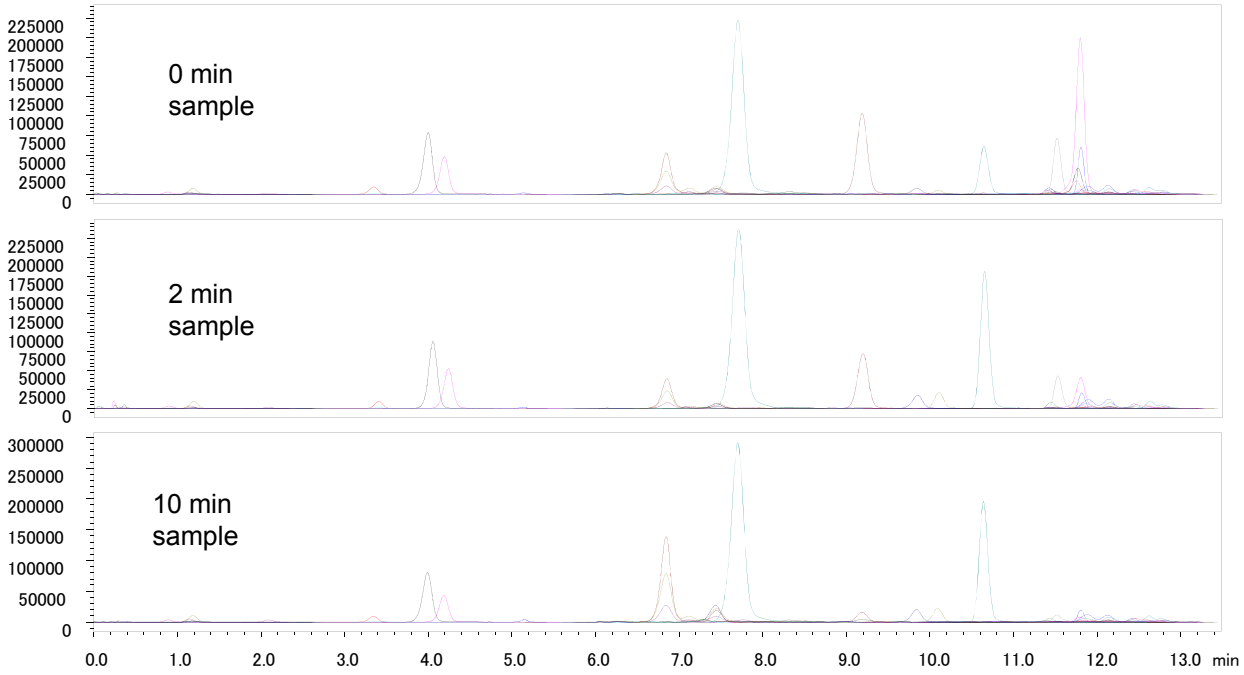

Fig. 1: MRM chromatograms for 55 primary metabolites (liver tissue extracts)
Upper: 0 min, Center: 2 min later, Lower: 10 min later

Fig. 2 illustrates 2-Morpholinoethanesulfonic acid (Internal Standard), along with several amino acids and nucleotides, at each time point in LabSolutions Quant Browser. The peak area of ADP and ATP decreased with time and its degradation product, AMP, increased with time. In contrast, the peak area of glutamate remained constant at each time point. This result indicates prompt and adequate sample preparation is very important to accurately measure the amount of certain metabolites in biological tissues and fluids.

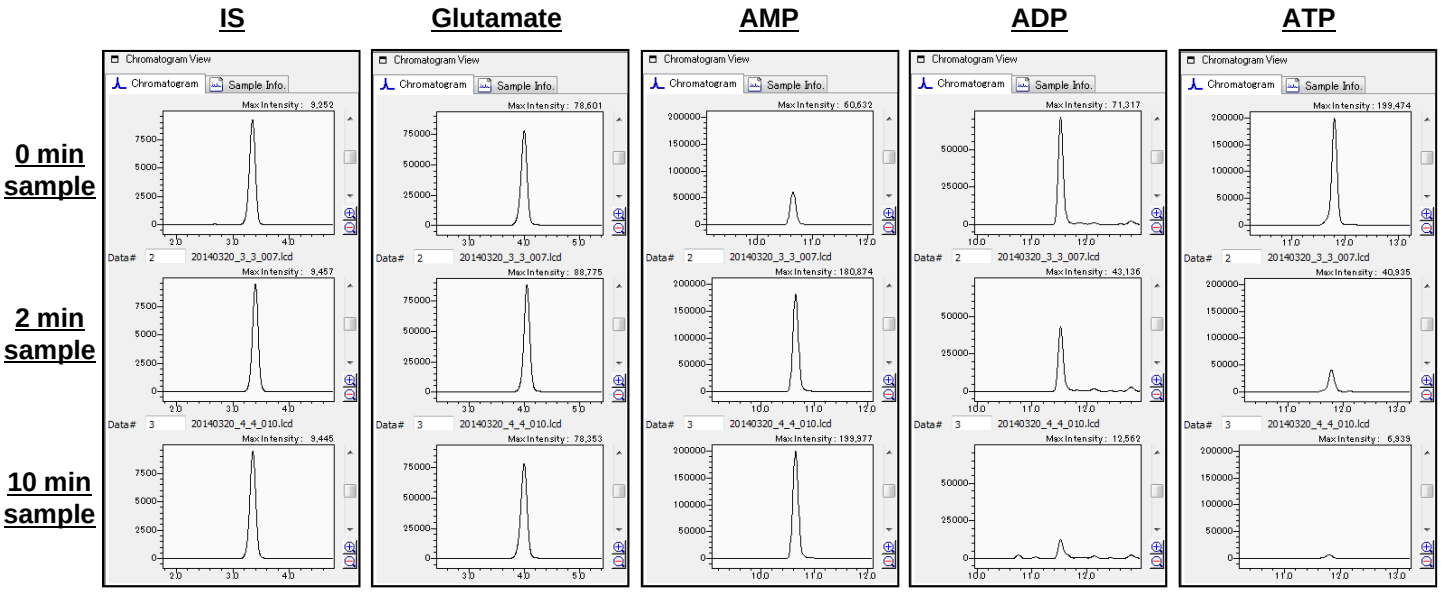

Fig. 2: Quant Browser results for primary metabolites (liver tissue extracts)
Upper: 0 min sample, Center: 2 min sample, Lower: 10 min sample

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