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High Sensitivity Analysis of Metabolites in Serum Using Simultaneous SIM and MRM Modes in a Triple Quadrupole GC/MS/MS

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Shuichi Kawana[1], Yukihiko Kudo[2], Kenichi Obayashi[2], Laura Chambers[3], Haruhiko Miyagawa[2]
1 Shimadzu, Osaka, Japan, 2 Shimadzu, Kyoto, Japan, 3 Shimadzu Scientific Instruments, Columbia, MD

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Introduction

Gas chromatography / mass spectrometry (GC–MS) and a gas chromatography-tandem mass spectrometry (GC-MS/MS) are highly suitable techniques for metabolomics because of the chromatographic separation, reproducible retention times and sensitive mass detection.

MRM measurement mode

Some compounds with low CID efficiency produce insufficient product ions for MRM transitions, and the MRM mode is consequently less sensitive than SIM for these compounds.

Our suggestion

SIM, MRM, and simultaneous SIM/MRM modes are evaluated for analysis of metabolites in human serum.

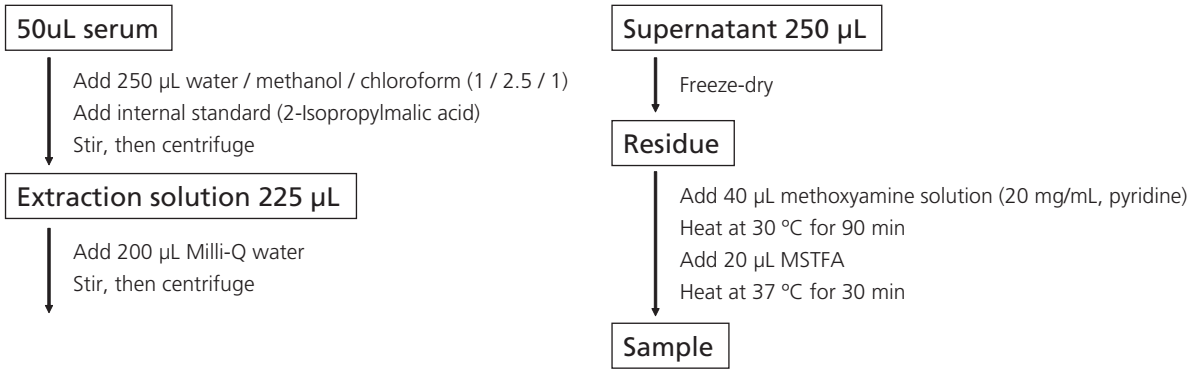
Materials and Method

Sample and Sample preparation

Sample

- Human serum

Sample Preparation[1)]



1) Nishiumi S et. al. Metabolomics. 2010 Nov;6(4):518-528

Instrumentation

GC-MS	: GCMS-TQ8040 (SHIMADZU)
Data analysis	: GCMSsolution Ver.4.2
Database	: GC/MS Metabolite Database Ver.2 (SHIMADZU)
Column	: 30m x 0.25mm I.D., df=1.00µm (5%-Phenyl)-methylpolysiloxane

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Simultaneous SIM and MRM modes in GC/MS/MS

Figure 1 shows the theory of Simultaneous SIM and MRM modes. This analysis mode can measure SIM and MRM data in a single analysis.

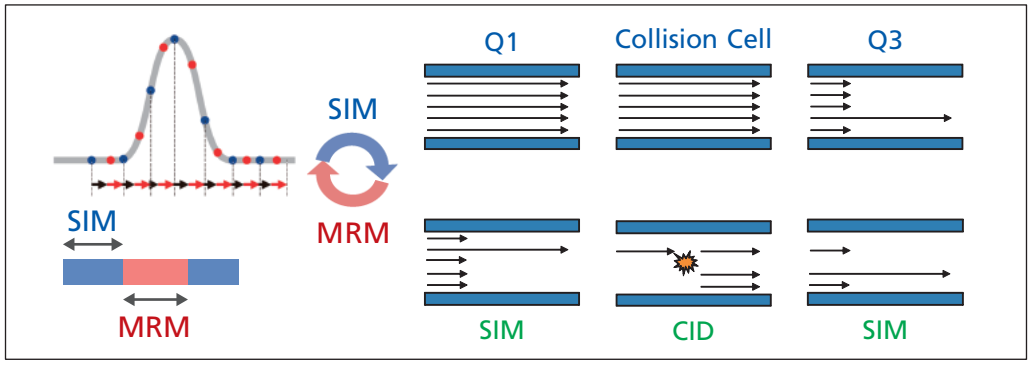


Figure 1 The concept of simultaneous SIM and MRM analysis mode.

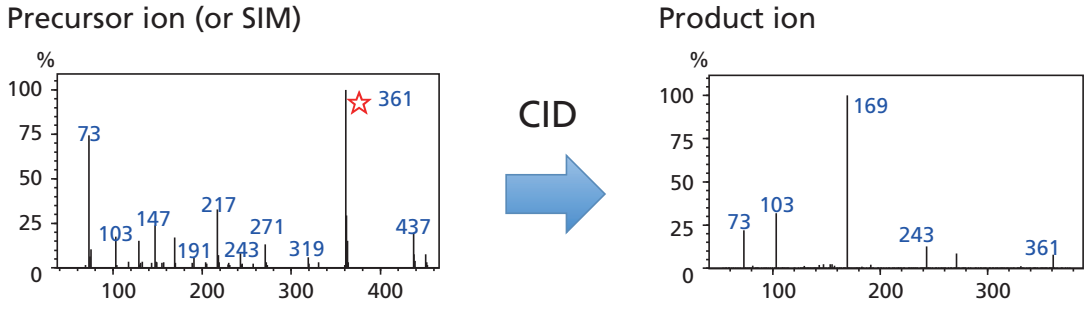


Figure 2 Mass Spectrum of Precursor (or SIM) and Product ion

Poor sensitivity of MRM in some compounds because of low CID efficiency

Method Creation using Database and *SmartMRM*

Figure 3 shows the GC/MS Metabolites Database Ver.2. This database involves conditions of SIM and MRM in 186 metabolites and a method creation function we call ***SmartMRM***. ***SmartMRM*** creates MRM, SIM, SIM/MRM methods from Database automatically.

						MRM Transition					m/z for SIM or Scan		
Serial#	Target		Compound Name	Ret. Index 3	Ret. Time	Ion1					Ion1		
	Flag	Acq.				Typ	m/z	CE	Ratio		Type	m/z	Ratio
3	Target	MRM	Lactic acid-2TMS	1061	8.391		219>191.1	6	100.00		T	219	100.00
4	Target	MRM	Glycolic acid-2TMS	1074	8.645	T	177.1>147.1	6	100.00		T	205	100.00
5	Target	SIM	anine-2TMS	1106	9.286	T	116.1>73	15	100.00		T	190	100.00
6	Target	MRM SIM Scan	Keto-isovaleric acid-meto-TMS	1111	9.375		186.1>170.1	9	100.00		T	186.1	100.00
7	Target		ycine-2TMS	1121	9.553		204.1>176.1	9	100.00		T	204	100.00
8	Target		2-Hydroxybutyric acid-2TMS	1129	9.696		233.1>205.2	6	100.00		T	219	100.00
9	Target	MRM	Oxalic acid-2TMS	1129	9.696		219.1>149.1	3	100.00		T	219	100.00

Figure 3 GC/MS Metabolites Database Ver.2

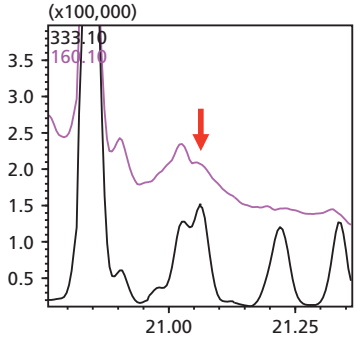
- Select the MRM, SIM and SIM/MRM conditions of 186 TMS derivatization metabolites from GC/MS Metabolites Database Ver.2.
- Select the two transitions (or ions) each metabolite.

Results

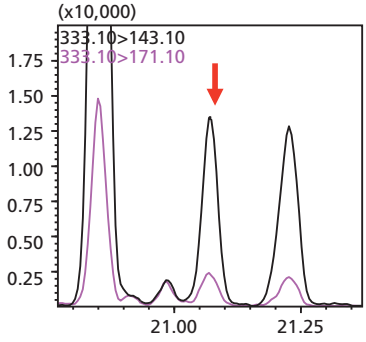
Comparison of the chromatogram between SIM and MRM in human serum

a) Glucuronic acid-meto-5TMS(2)

SIM



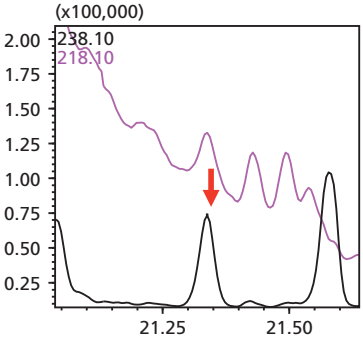
MRM



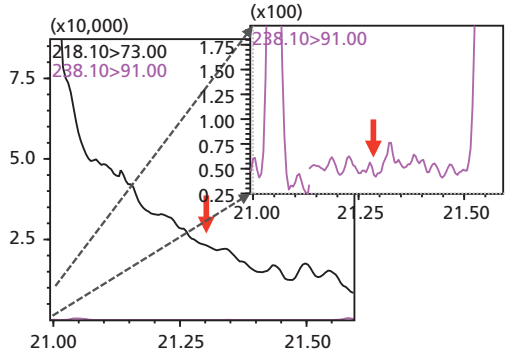
Detected the peak in MRM because of high selectivity

b) S-Benzyl-Cysteine-4TMS

SIM



MRM



Peak was not detected in MRM because of low CID efficiency.

A number of Identification metabolites in serum

Table 1 shows the identification results of metabolites in human serum using SIM, MRM and simultaneous SIM/MRM analysis modes in GC/MS/MS. In SIM/MRM, the metabolites, which were insufficient sensitivity in MRM, were measured by SIM and the other metabolites were measured by MRM.

Table 1 The number of identified metabolites each analysis mode

Modes	A	B	C	Total
SIM	57	51	8	**116**
MRM	131	14	1	**146**
SIM/MRM	133	22	1	**156**

note) A:Target and Confirmation ions were detected.; B: Either Target or Confirmation ion was detected.
Another one was overlapped by contaminants.; C: Either Target or Confirmation ion was detected.

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Fig.4 shows a number of metabolites in each mode can be measured. In metabolites with low CID efficiency, SIM are superior to MRM if there are no interfering substances to the target metabolites.

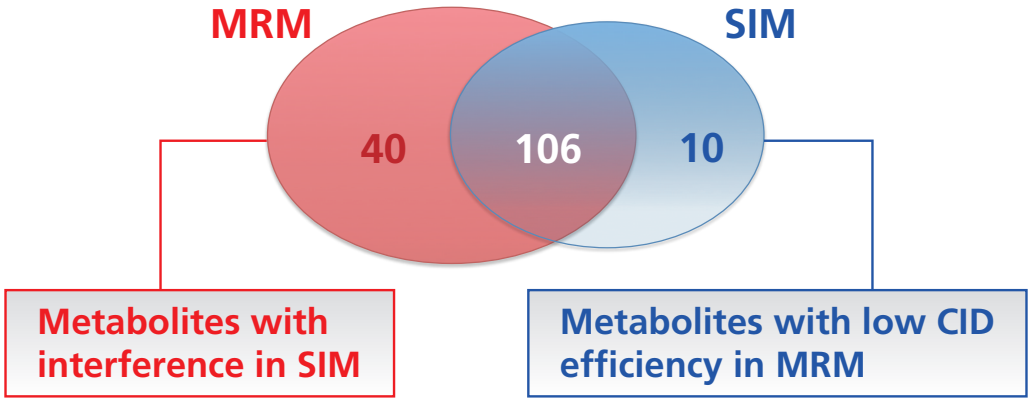

Figure 4 Detected metabolites in human serum each analysis mode.

The reproducibility(n=6) in MRM and SIM/MRM

Table 2 Comparison of the reproducibility results from MRM and SIM/MRM analysis.
A number of detected metabolites involves A, B and C in Table 1.

%RSD	MRM	SIM/MRM	Improvement
- 4.99%	73	76	+3
5 - 9.99%	26	30	+4
10 - 14.99%	8	10	+2
15 - 19.99%	9	10	+1
> 20%	30	30	0
	146	156	+10

Conclusions

- Analytical results from the SIM and MRM modes identified 116 and 146 metabolites, respectively.
- In metabolites with poor CID efficiency, the sensitivity of SIM is more than 10 times higher than MRM.
- Simultaneous SIM and MRM modes in a single analysis (SIM/MRM) improves the sensitivity and reproducibility for analysis of metabolites in human serum compared to MRM alone.
- A novel SIM/MRM expands the utility of a triple quadrupole GC/MS/MS

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