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Target and Non-target Analysis of Metabolites in Urine Using Scan/MRM and GC/MS/MS

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Introduction

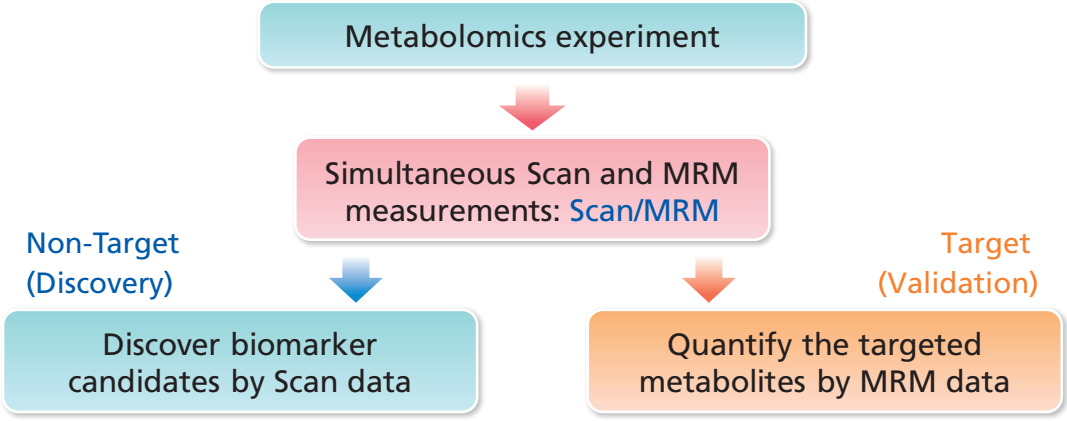
Target and Non-target Analysis in Metabolomics

Item	Target	Non-target
Mode	MRM	Scan
Merit	Accurate Quantitation	Comprehensive analysis
Demerit	Limited compounds	Poor Quantitation

Problem

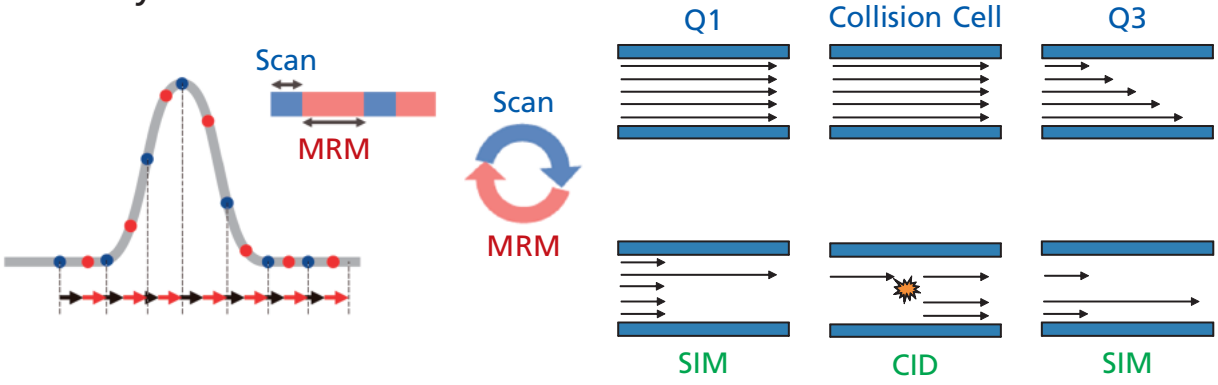
In a conventional system, two injections are needed to collect both results.

Developed system to solve the problem



Method & Material

Scan/MRM System



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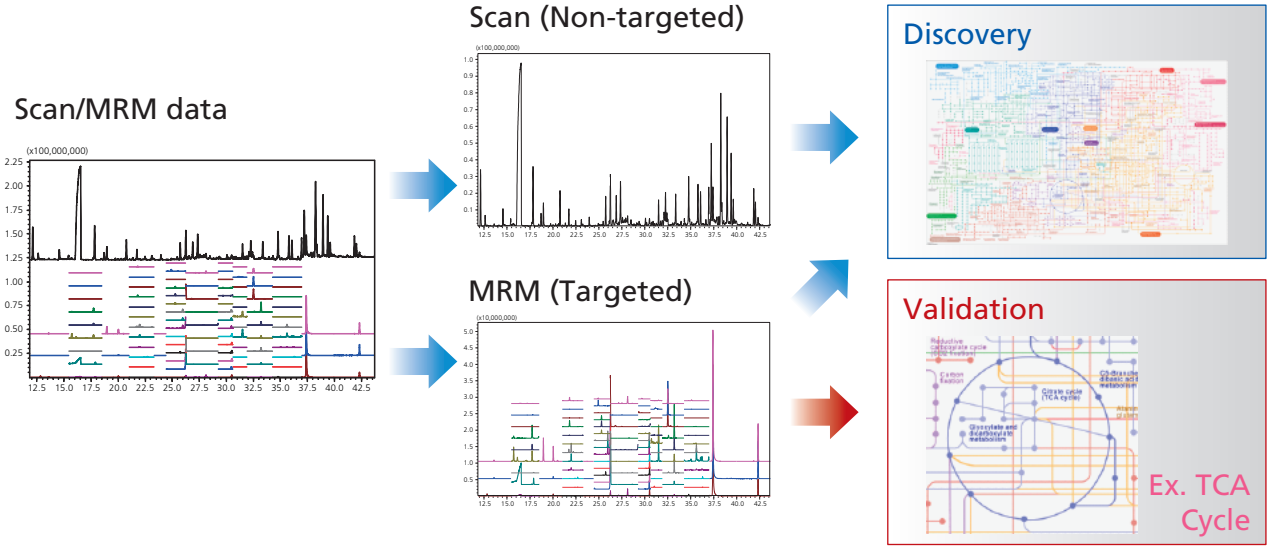


Experimental

Sample

- 15 standard solution (0, 0.5, 1, 5, 1, 5, 10, 50 ug/mL)
- Human urine

Sample treatments[1)]

100 µl urine

↓ Add 1 µg of heptadecanoic acid (I.S.)
Add 1mg of urease at 37 °C
Add 0.9 ml of ethanol and then centrifuge

0.5 mL of supernatant solution

↓ Add 200 µL Milli-Q water
Stir, then centrifuge

Dryness under N2 gas flow at 30 °C

↓ Add 100 µL of BSTFA + 1%TMCS
Heat at 80 °C for 90 min

GC-MS/MS analysis

1) Kuhara T. J Chromatogr B Biomed Sci Anal 2001;758:3-25.

Analytical Conditions

GC-MS	: GCMS-TQ8030 (SHIMADZU)
Data analysis	: MRM data: GCMSsolution Ver. 4, Scan data: Analyzer Pro (SpectralWorks) GC/MS metabolite database Ver.2 (SHIMADZU), NIST11
Column	: DB-5 (Length 30 m, 0.25 mm I.D., df=1.00 µm)

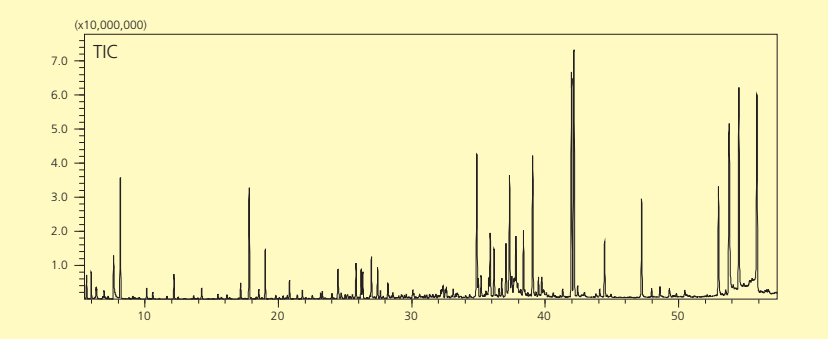
[GC]	
Inj. Temp.	: 280 °C
Column Oven Temp.	: 100 °C (4min) → (4 °C/min) → 320 °C (0 min)
Flow Control	: 39.0 cm/sec
Split ratio	: 10, Injection Volume: 1 µL

[MS]	
Interface Temp.	: 280 °C
Ion Source Temp.	: 200 °C
Data acquisition	: Scan/MRM
	Scan part *m/z* : 70 - 550 (0.1s)
	MRM part (0.2s)

Method created by Smart MRM™

Results

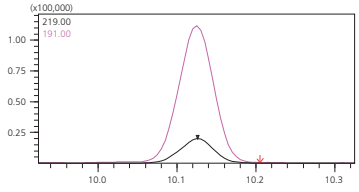
Scan data of Scan/MRM in Urine

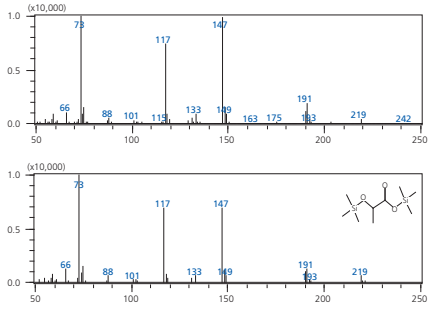


Six replicate analyses of urine

Detected peaks	%RSD < 20%	Identified metabolites
307	147	99

Lactic acid – 2TMS



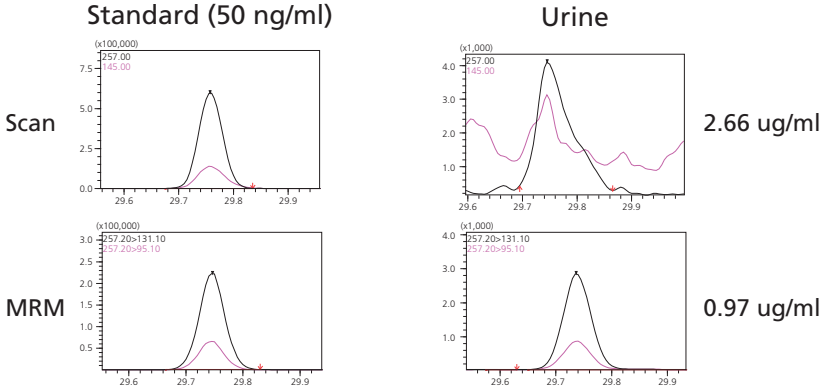


These results were the same as those obtained by Scan alone

MRM data of Scan/MRM in Urine

a. Co-elute contaminants problems

Lauric acid (The quantitation of Scan is 3 times higher than MRM)



The scan result is incorrect due to interfering substances.

b. 15 metabolites were selected from the overlapped peaks in Scan

ID	Compound name	%RSD (n=6)	Con. (ug/ml)	R^2
1	3-Hydroxybutyric-2TMS	6.87	1.23	0.998
2	Octanoic-TMS	5.30	0.88	0.998
3	Glycerol-3TMS	5.65	2.26	0.998
4	3-Methylglutaric-2TMS	4.23	0.42	0.997
5	Decanoic-TMS	6.11	0.24	0.997
6	L-Methionine-2TMS	9.55	0.90	0.997
7	2-Hydroxyphenylacetic-2TMS	6.20	0.40	0.997
8	Pimelic-2TMS	3.10	0.66	0.995
9	3-Hydroxyphenylacetic-2TMS	3.62	0.54	0.995
10	Lauric-TMS	3.52	0.97	0.996
11	Tartaric-4TMS	4.40	0.24	0.993
12	N-Acetylaspartic-3TMS	0.89	2.81	0.994
13	Myristic-TMS	3.36	1.19	0.996
14	Caffeine	5.50	2.04	0.985
15	Sebacic-2TMS	0.96	1.80	0.990

* Calibration curve: 0, 0.5, 1, 5, 10, 50 ug/ml

Conclusion

- Evaluation of a novel metabolomics approach using Scan/MRM through analysis of metabolites in urine
  - 147 peaks (less than 20% %RSD) were measured in urine by scan data.
  - 15 compounds, which were interfered by others in scan, were determined by MRM data.
- Analysis results of Scan/MRM were the same as those obtained by Scan or MRM alone.
- Scan/MRM measurements are expected to find new bio-markers which are not detected by Scan alone in the discovery phase.
- A novel Scan/MRM mode will advance metabolomics studies.

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