

Ultra-Fast Analysis of Metabolites in Serum in Under 3 Minutes Using Fast GC/MS/MS

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Introduction

Current situation in metabolic profiling using GC/MS

- Many samples need to be analyzed in metabolomics research.
- Samples should be analyzed within 24 hours of derivatization due to degradation.



Requirement of shortening analysis time in GC-MS.

Problems of shortening analysis time in GC-MS

- Narrow-bore (0.10 and 0.18mm I.D.) columns are not applied to the analysis due to the limited sample capacity.
- Chromatographic resolution of medium-bore columns causes peak overlapping.

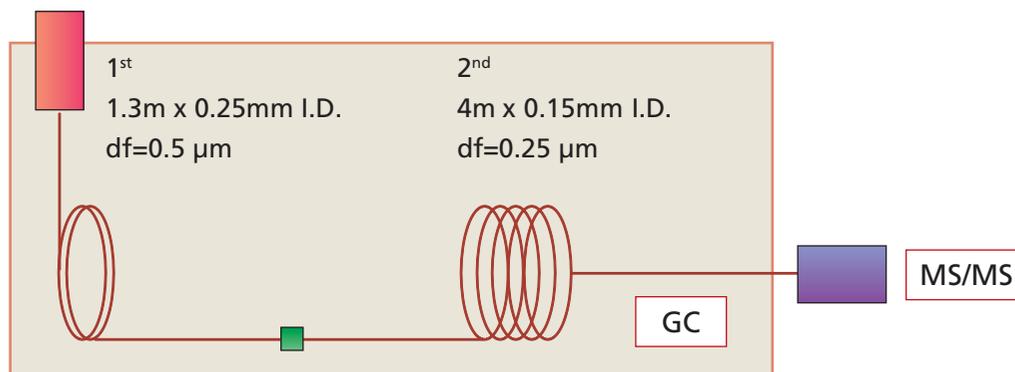
Developed system to solve the problems

Fast GC-MS/MS with a combination of two short columns (tandem column).

Method & Material

Fast-GC/MS/MS System

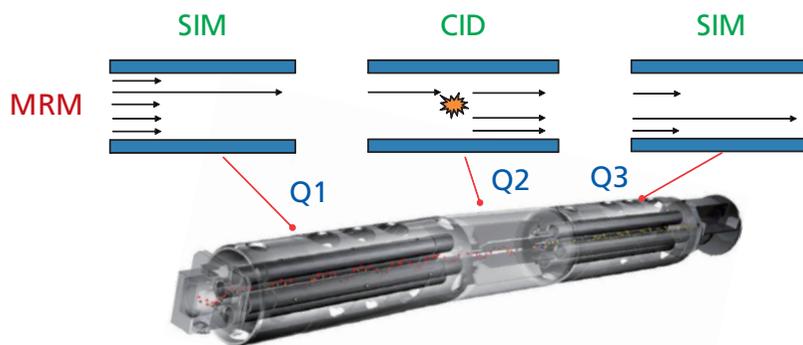
Tandem column



- 1st column : High sample capacity
- 2nd column : High chromatographic separation

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GC-MS/MS (High separation)

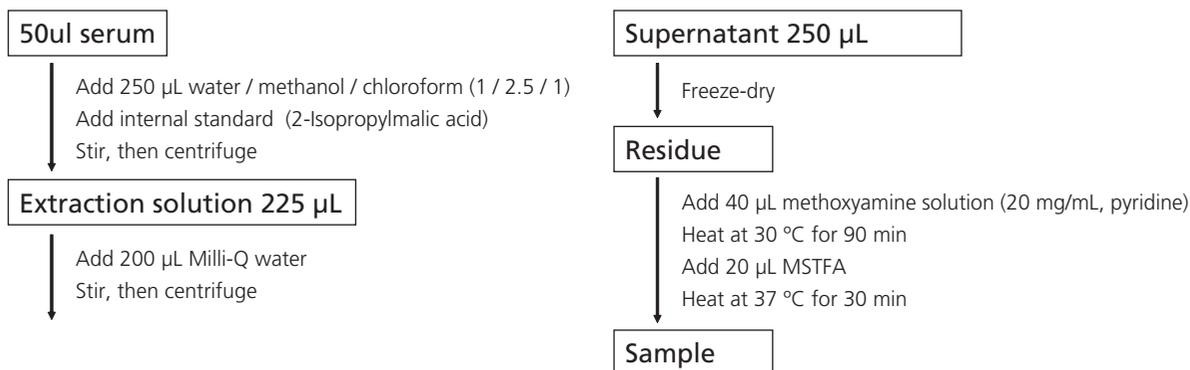


Experimental

Sample

- 25 standard solution (0, 0.05, 0.1, 0.5, 1, 5, 10, 50 ug/ml)
- Human serum

Sample treatments¹⁾



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

Analytical Conditions

GC-MS	: GCMS-TQ8030 (SHIMADZU)
Data analysis	: GCMSsolution Ver. 4
Column	: BPX5 (1.3m x 0.25mm I.D., df=0.5µm) + BPX5 (4m x 0.15mm I.D., df=0.25) (SGE, Australia)

[GC]	
Inj. Temp.	: 280 °C
Column Oven Temp.	: Fast 100 °C (0.35min) → (50 °C/min) → 340 °C (0.35 min)
Flow Control	: Fast 250kPa
Split ratio	: 5, Injection Volume: 1 µL

[MS]	
Interface Temp.	: 280 °C
Ion Source Temp.	: 200 °C
Data acquisition	: Scan or MRM Scan <i>m/z</i> : 50 - 600 (0.06s) 10,000 u/sec

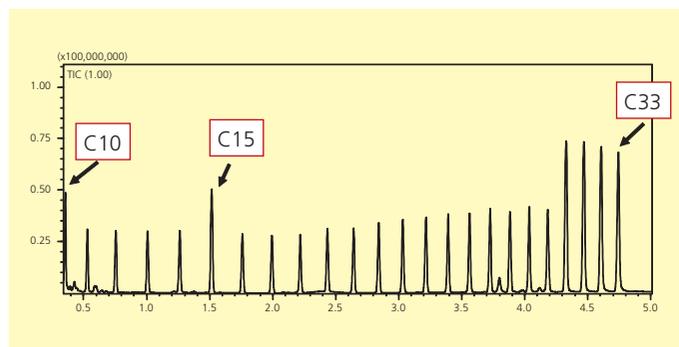
Method creation using Smart MRM™.

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Results

Analytical standard

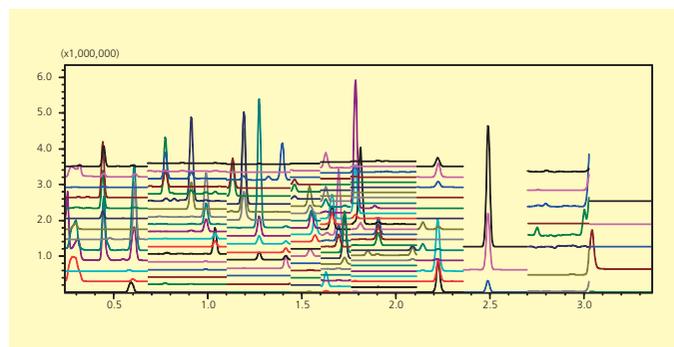
n-alkane analysis with fast-GC/MS



0.3 min

5 min

Analytical standard with fast GC/MS/MS
(25 compounds metabolites (10ug/ml))

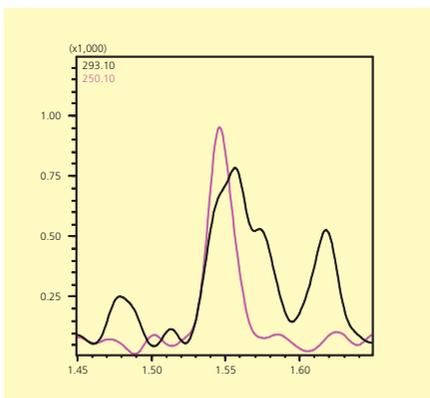


0.3 min

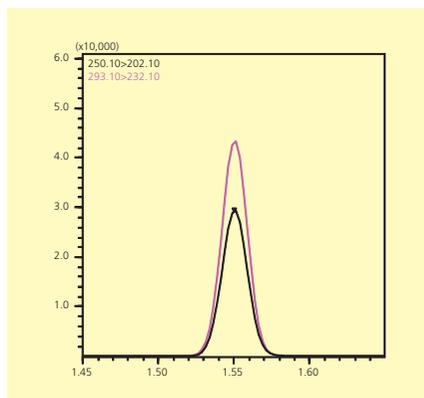
5 min

Serum sample

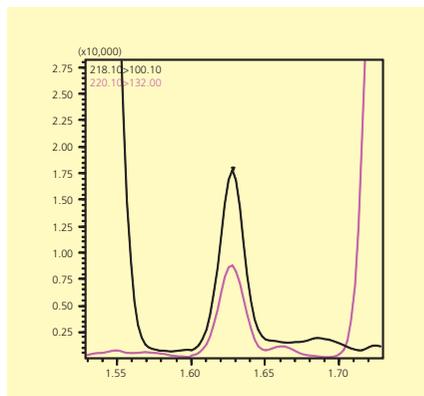
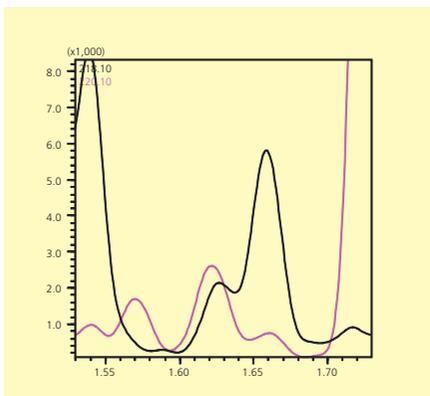
Fast-GC/MS



Fast-GC/MS/MS



Methionine-2TMS



Cysteine-3TMS

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The reproducibility (n=6) and concentration in serum with fast-GC/MS/MS

ID	Compound name	Transition	%RSD	Con. (ug/ml)
1	Lactic acid-2TMS	219.00>191.10	0.77	81.75
2	pyruvate-meto-TMS	174.00>89.00	3.16	0.06
3	Sarcosine-2TMS	190.10>147.10	1.27	13.20
4	Valine-2TMS	218.10>100.10	0.86	9.37
5	Leucine-2TMS	102.10>73.00	0.41	7.89
6	Proline-2TMS	216.10>147.10	0.54	7.54
7	Succinate-2TMS	172.10>128.00	2.95	1.12
8	Fumaric acid-2TMS	245.00>217.10	2.21	0.18
9	Threonine-3TMS	291.00>101.10	0.88	5.98
10	Glutaric acid-2TMS	261.00>147.10	1.32	0.12
11	Malic acid-3TMS	307.00>263.10	3.35	0.30
12	Aspartic acid-3TMS	232.10>188.20	0.48	6.77
13	Methionine-2TMS	250.10>202.10	2.32	1.43
14	Cysteine-3TMS	218.10>100.10	2.87	0.34
15	2-Isopropylmalic acid-2TMS (I.S.)	349.10>259.10	-	-
16	alpha-ketoglutarate-meto-2TMS	198.10>73.00	3.37	0.17
17	Glutamic acid-3TMS	128.10>73.00	0.54	22.75
18	Phenylalanine-2TMS	218.10>100.10	0.70	7.00
19	Asparagine-3TMS	231.10>132.10	3.03	2.29
20	Aconitate-3TMS	285.10>147.10	8.52	0.39
21	Glutamine-3TMS	245.10>156.10	1.55	54.67
22	Citric acid-4TMS	347.10>183.10	1.44	14.87
23	Isocitric acid-4TMS (I.S)	245.10>191.10	1.17	0.39
24	Tyrosine-3TMS	218.10>100.10	0.71	5.55
25	Tryptophan-3TMS	202.10>201.10	0.91	9.49

* Calibration curve: 0, 0.05, 0.1, 0.5, 1, 5, 10 ug/ml

The regression coefficient of the linear calibration curves was higher than 0.994

Conclusion

The fast-GC-MS/MS with the combination of two short columns was developed and evaluated using the example of 25 metabolites (including an internal standard).

- (1) MRM allows the separation of co-eluting peaks.
- (2) The whole analysis cycle time of 10 min achieves the analysis of 144 samples per day.
- (3) The target metabolites in the serum sample were selectively detected and the %RSDs (n=6) were in a range of 0.41 - 8.52%.

Further studies are needed to demonstrate the effectiveness of this system by applying it to more metabolites and different types of samples.

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