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# Multicomponent Analysis of Metabolites in Chinese caterpillar fungus using gas chromatography-triple quadrupole mass spectrometry

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## Overview

Smart Metabolites Database registers MRM information of 475 metabolites mainly contained in biological samples such as cells. It enables simultaneous measurement of 475 metabolites using MRM mode. This application presents an analysis of metabolites in Cordyceps sinensis using the MRM methods included in the Smart Metabolites Database.

## Introduction

Chinese caterpillar fungus is a fungus parasitic on the larvae of Lepidoptera and has been considered to be a precious tonic food and herbal medicine since ancient times in China. its special appearance changing in different seasons. In winter, the larvae were infected by the fungi and turned into worms in soil, which is called Dong-Chong. And in summer, the stroma grew out from the head of the larve like grass, which is why being called Xia-Cao. In China, it has been used in medicine for a long history, dated back to Qing dynasty. Researches indicated that C. sinensis contains many bioactive constituents, such as polysaccharide, adenosine, cordycepin, cordycepic acid, ergosterol and minerals. To date, several fatty acids and amino acids, which are very important bio-functional components, were separated or identified from Cordyceps. GC-MS/MS-based Smart Metabolites Database was employed to establish analysis method for determining 475 metabolites in Chinese caterpillar fungus in the absence of target compounds standard using MRM mode. In the pretreatment process, 2-isopropylmalic acid was added as an internal standard to carry out semi quantitative analysis of metabolites. Through this method, 141 kinds of metabolites in Cordyceps sinensis were identified.

## Methods and Materials

Sample preparation for GC-MS/MS analysis: Dried Chinese caterpillar fungus(30 mg) were put into 2 mL centrifugal tube. One milliliter of a single-phase extraction solvent consisting of 2.5:1:1(v/v/v) methanol, distilled water, chloroform was added to extract a wide range of metabolites. As an internal standard, ribitol (50 µL, diluted with deionized water to 0.2 mg/mL) was utilized. The mixture was shaken for 1 minute and then centrifuges for 3 minutes at 4°C and 16000 rpm. The supernatant (900µL) was transferred into 1.5 mL centrifugal tube, diluted the supernatant with 400 mL water. The mixture was vortexed and centrifuged for 3 minutes, then, transferd 400 mL water phase to 1.5 mL plugged centrifugal tube, and finally add 50 µL 2-isopropylmalic acid (0.2 mg/mL, diluted with deionized water to 0.2 mg/mL) as internal standard. The extract was placed in a low temperature drying chamber and dried in vacuum for 120 minutes. The freeze-dried residue was subjected to methyloximation derivatization and trimethylsilyl (TMS) derivatization, and this derivatized sample was used for GC-MS/MS analysis. GC-MS/MS-based Smart Metabolites Database was employed to establish analysis method for simultaneous measurement of 467 metabolites in Chinese caterpillar fungus using MRM mode. Through this method, 142 kinds of metabolites of Cordyceps sinensis were identified.

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High Speed Mass Spectrometer

Ultra Fast Scan Speed
- Max. 20000 amu/sec

Ultra Fast MRM
- Max. 888 transition /sec

Figure 1. GCMS-TQ8050 triple quadrupole mass spectrometer

## Result

| GC-MS/MS conditions | |
|---|---|
| Instrument | : GCMS-TQ8050 (Shimadzu Corporation, Japan) |
| Injection mode | : Split mode, Split ratio:10:1 |
| Column | : DB-5, 30m×0.25mm×1.0μm |
| Column oven temp. | : 100°C (4 min)_4°C/min_320°C(8 min) |
| Carrier gas | : Helium |
| CID gas | : Argon |
| Ionization mode | : EI |
| Detector voltage | : Tuning result+0.5kV |
| Interface temp. | : 280°C |
| Ion source temp. | : 200°C |

The n-alkanes (C9-C33) standard solution was determined by the analysis method of n-alkanes in the metabolites database method file, which was used to predict the retention time of 475 metabolites in the database. The mass chromatogram of n-alkanes is shown in Figure 2.

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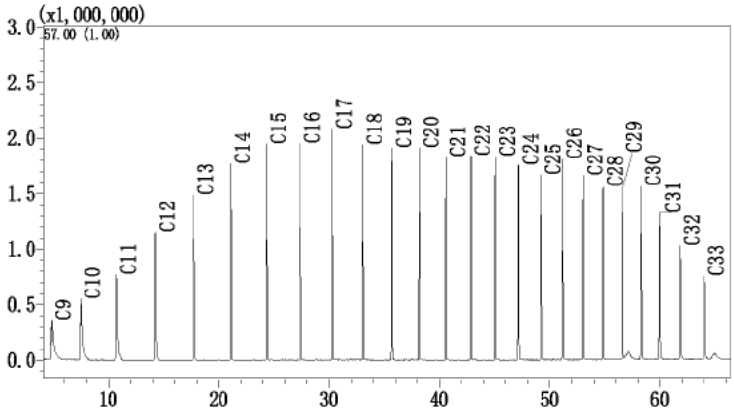
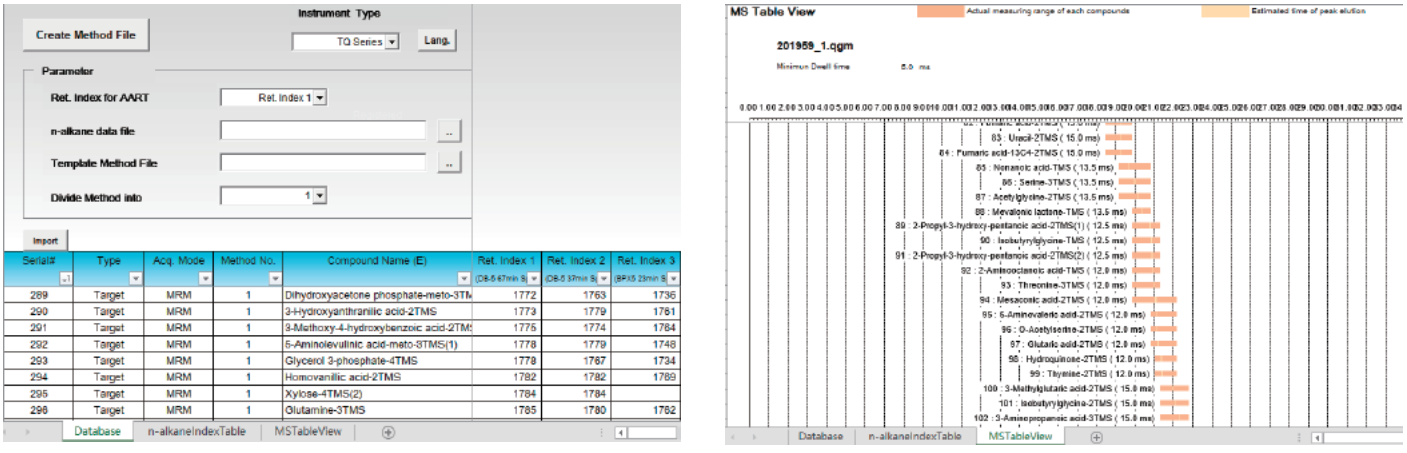



Figure 2. The mass chromatogram of n-alkanes

Then, 475 metabolites MRM method files were established using Smart Metabolites Database and n-alkanes data. Figure 3 shows the Smart Metabolites Database and the picture MRM method has been established.




| Serial# | Type | Acq. Mode | Method No. | Compound Name (E) | Ret. Index 1 | Ret. Index 2 | Ret. Index 3 |
|---|---|---|---|---|---|---|---|
| 289 | Target | MRM | 1 | Dihydroxyacetone phosphate-meto-3TM | 1772 | 1763 | 1736 |
| 290 | Target | MRM | 1 | 3-Hydroxyanthranilic acid-2TMS | 1773 | 1779 | 1761 |
| 291 | Target | MRM | 1 | 3-Methoxy-4-hydroxybenzoic acid-2TMS | 1775 | 1774 | 1784 |
| 292 | Target | MRM | 1 | 5-Aminolevulinic acid-meto-3TMS(1) | 1778 | 1779 | 1748 |
| 293 | Target | MRM | 1 | Glycerol 3-phosphate-4TMS | 1778 | 1767 | 1734 |
| 294 | Target | MRM | 1 | Homovanillic acid-2TMS | 1782 | 1782 | 1789 |
| 295 | Target | MRM | 1 | Xylose-4TMS(2) | 1784 | 1784 | |
| 296 | Target | MRM | 1 | Glutamine-3TMS | 1785 | 1780 | 1762 |

Figure 3. Smart Metabolites Database and the picture MRM method has been established.

The samples were analyzed using GCMS-TQ8050 combined with 475 metabolite derivatives MRM method. The MRM spectrum of metabolite derivatives of Cordyceps sinensis were shown in Figure 4.

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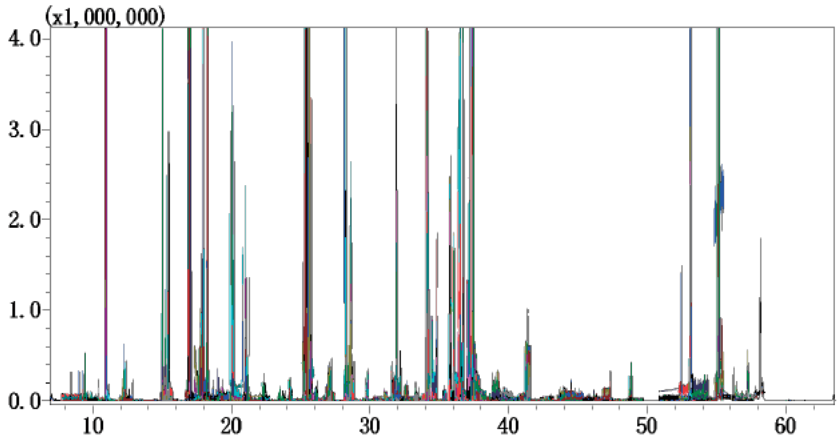


Figure 4. TIC Charts of Metabolites Derivatives in Cordyceps sinensis

The experimental results show 141 metabolites were identified in the absence of target compounds standard. 2-isopropylmalic acid used as internal standard and ribitol as substitutes, the content of metabolites in Cordyceps sinensis can be evaluated by the ratio of peak area of metabolites to peak area of 2-isopropyl malic acid, and the metabolites can be semi-quantitatively analyzed. Because of the large number of metabolite derivatives identified, the MRM spectrum of some components are shown in Figure 5 and the information of some identified metabolites is shown in table 1.

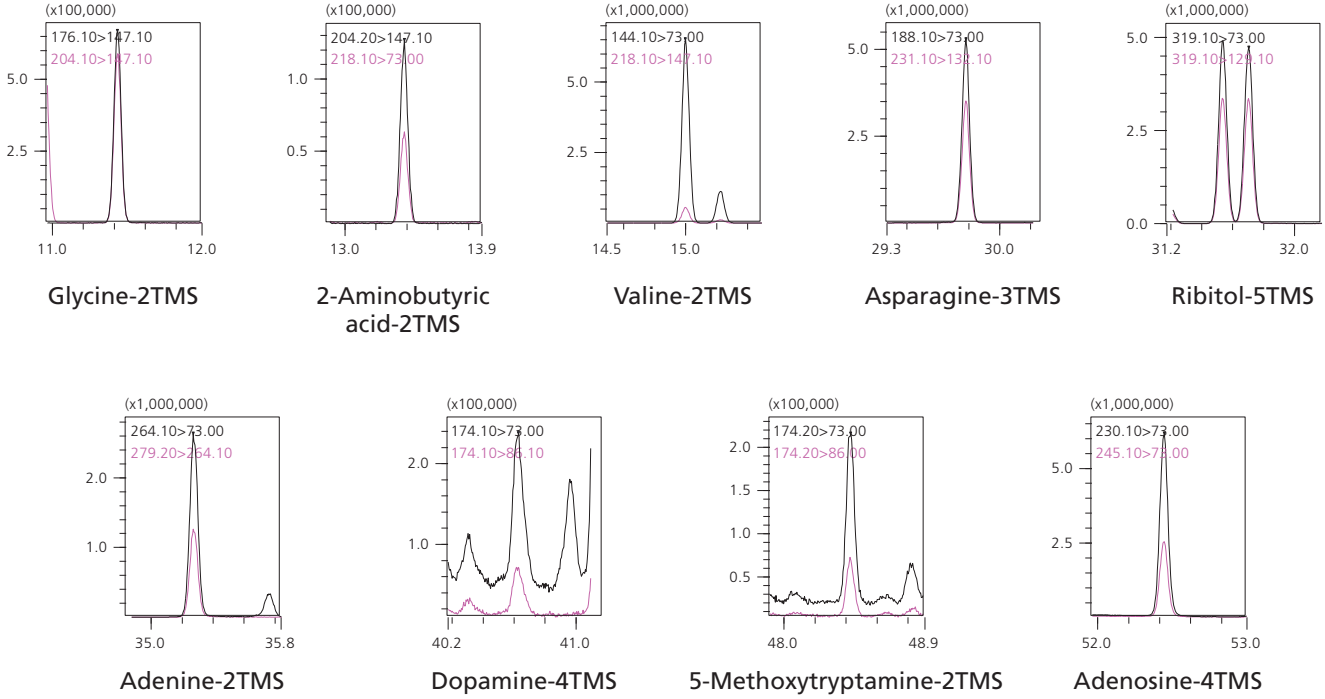


Figure 5. MRM spectrum of some metabolite derivatives identified

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Table 1. The information of some identified metabolite derivatives

| No. | Compound Name | Retention time /min | CAS.No. | Peak-area of target/ Peak-area of ISTD |
|---|---|---|---|---|
| 1 | Glycine-2TMS | 11.42 | 56-40-6 | 0.105 |
| 2 | 2-Aminobutyric acid-2TMS | 13.383 | 2835-81-6 | 0.02 |
| 3 | Valine-2TMS | 14.951 | 72-18-4 | 2.512 |
| 4 | 2-Aminoethanol-3TMS | 16.879 | 141-43-5 | 2.549 |
| 5 | Leucine-2TMS | 16.949 | 61-90-5 | 0.727 |
| 6 | Nicotinic acid-TMS | 17.638 | 59-67-6 | 0.451 |
| 7 | Proline-2TMS | 17.89 | 147-85-3 | 2.807 |
| 8 | Succinic acid-2TMS | 18.207 | 110-15-6 | 0.631 |
| 9 | Fumaric acid-2TMS | 19.291 | 110-17-8 | 0.471 |
| 10 | Serine-3TMS | 20.053 | 56-45-1 | 2.39 |
| 11 | Threonine-3TMS | 20.969 | 72-19-5 | 2.195 |
| 12 | Glutaric acid-2TMS | 21.238 | 110-94-1 | 0.047 |
| 13 | Aspartic acid-3TMS | 25.22 | 56-84-8 | 2.212 |
| 14 | 2-Isopropylmalic acid-3TMS (ISTD) | 27.184 | 3237-44-3 | 1 |
| 15 | Ornithine-3TMS | 28.096 | 70-26-8 | 0.204 |
| 16 | Glutamic acid-3TMS | 28.175 | 56-86-0 | 2.894 |
| 17 | Phenylalanine-2TMS | 28.575 | 150-30-1 | 2.552 |
| 18 | Asparagine-3TMS | 29.782 | 70-47-3 | 0.822 |
| 19 | Arabitol-5TMS | 31.534 | 488-82-4 | 1.008 |
| 20 | Ribitol-5TMS | 31.703 | 488-81-3 | 0.834 |
| 21 | Putrescine-4TMS | 31.841 | 110-60-1 | 2.926 |
| 22 | Glutamine-3TMS | 32.67 | 56-85-9 | 2.694 |
| 23 | Ribonic acid-5TMS | 32.853 | 17812-24-7 | 0.22 |
| 24 | Ornithine-4TMS | 33.965 | 70-26-8 | 2.902 |
| 25 | Citric acid-4TMS | 34.025 | 77-92-9 | 3.293 |
| 26 | Adenine-2TMS | 35.272 | 73-24-5 | 0.459 |
| 27 | Tyramine-3TMS | 36.487 | 51-67-2 | 0.172 |
| 28 | Lysine-4TMS | 36.625 | 56-87-1 | 2.923 |
| 29 | Palmitic acid-TMS | 39.307 | 1957/10/3 | 0.939 |
| 30 | Dopamine-4TMS | 40.627 | 51-61-6 | 0.544 |
| 31 | Uridine-3TMS | 48.808 | 58-96-8 | 1.584 |
| 32 | Adenosine-4TMS | 52.429 | 58-61-7 | 1.088 |

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## Conclusions

GC-MS/MS-based Smart Metabolites Database was employed to establish analysis method for determining 475 metabolites in Chinese caterpillar fungus in the absence of target compounds standard using MRM mode. 2-isopropylmalic acid was used as internal standard for semi-quantitative analysis and ribosol as substitute to evaluate the extraction efficiency of metabolites from Cordyceps sinensis. The experimental results show that 141 metabolites were identified and the semi-quantitative results can be used to compare the contents of metabolites in different samples.

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