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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.





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	: El
Detector voltage	: Tuning result+0.5kV
Interface temp.	: 280°C
lon 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.





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.

				Instrument Type				MS Table View	Actual measuring range of each compounds	Estimated time of peak elution
Create	Method File			TQ Series 💌 Lang.				201959_1.qgm		
Paran	neter							Minimun Dwell Sme	5.0 ma	
Ret	Index for AAR	т	Ret.	ndex 1 💌				0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00	100 9:0010 001 002 003 004 005 006 007 008 009 009 001 002 003 0	84.085.026.087.088.089.000.084.082.083.084
n-al	kane dala file			Registered					83: Unexil-2TMS (15.0 ms)	
Ten	nplate Method F	ile							84 : Pumaris acid-1304-2TMS (15.0 ms) 85 : Nenanois acid-TMS (13.5 ms)	
Divi	de Method info			1 -					85 : Serine-3TMS (13.5 ms) 87 : Acety by sine-2TMS (13.5 ms) 88 : Mevalonic lastane-TMS (13.5 ms)	
Import									39 : 2-Propyl-3-hydroxy-pentancic acid-2TMS(1) (12.5 me) 90 : Isoketyrylgiycine-TMS (12.5 ms)	
Serial#	Туре	Acq. Mode	Method No.	Compound Name (E)	Ret. Index 1	Ret. Index 2	Ret. Index 3		31 : 2-Progyl-3-hydroxy-pentancic acid-2TMS(2) (12.5 ms)	
-1					(D8-6 67min Sj 💌	(DB-5 37min S(🛩	(BPX5 23min S 💌		92 : 2-Aminooctanoic acid-TMS (12.0 ms)	
289	Target	MRM	1	Dihydroxyacetone phosphate-meto-3TM	1772	1763	1736		93 : Threonine-3TMS (12.0 ms) 94 : Mesaconic add-2TMS (12.0 ms)	
290	Target	MRM	1	3-Hydroxyanthranilic acid-2TMS	1773	1779	1761		95 : 6-Aminovaleris acid-2TM5 (12.0 ms)	
291	Target	MRM	1	3-Methoxy-4-hydroxybenzoic acid-2TM	1775	1774	1764		96 : O-Acetylserine-2TM5 (12.0 ms)	
292	Target	MRM	1	5-Aminolevulinic acid-meto-3TMS(1)	1778	1779	1748		97 : Glutario acid-2TMS (12.0 ms)	
293	Target	MRM	1	Glycerol 3-phosphate-4TMS	1778	1767	1734		98 : Hydroquinose-2TMS (12.0 ms)	
294	Target	MRM	1	Homovanilic acid-2TMS	1782	1782	1769		99 : Thymine-2TMS (12.0 ms)	
295	Target	MRM	1	Xylose-4TM5(2)	1784	1784			100 : 3-Mathylglutaric acid-2TMS (15.0 ma)	
298	Target	MRM	1	Glutamine-3TMS	1785	1780	1762		101 : isobutyryighjeine-2TMS (15.0 ma)	
	Database	n-alkaneinde	xTable N	ISTableView 🕘			4	 Database n-alka 	neIndexTable MSTableView (+)	

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.



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

Excellence in Science

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

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

Table 1. The information of some identified metabolite derivatives

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-guantitative results can be used to compare the contents of metabolites in different samples.





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