Analysis of fatty acid content in rice by GC-MS/MS combined with metabolite database

TP 261

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1. Overview

In this paper, Shimadzu GCMS-TQ8040 NX triple quadrupole gas chromatography-mass spectrometer combined with the Smart Metabolites Database was used to establish a method for the analysis of 37 fatty acids in rice.

2. Introduction

The fat content of rice is significantly, positively correlated with the taste quality, and has a greater impact on the taste quality of rice than other quality indicators, which is a direct effect. Therefore, it is of great significance for crop breeders to study the differences of fat and fatty acid components in grains of different rice varieties. Although indicator method and gas chromatography are also widely used to detect fatty acids, these methods have low sensitivity and poor accuracy. In order to overcome these shortcomings, it is urgent to develop a fast and effective analytical method.

3. Methods and Materials

Take 0.1g of rice seed powder, put it into a 15 ml glass tube, add 3 ml of 10% acetyl chloride methanol solution, take a water bath at 80℃ for two hours, transfer the sample solution after reaction to a 50 ml centrifuge tube, wash the glass tube with 3 ml of n-hexane and 3 ml of 6% sodium carbonate solution respectively, and merge it into a 50 ml centrifuge tube, shake and mix it evenly, centrifuge for 10 min at 4000 r/min, and take the supernatant to be tested by GC-MS/MS.

High Speed Mass Spectrometer

Ultra Fast Scan Speed
- Max. 20000 amu/sec

Ultra Fast MRM
- Max. 888 transition /sec

Figure 1. GCMS-TQ8040 NX triple quadrupole mass spectrometer

4. Result

GC-MS/MS conditions

Injection mode: Split mode
Flow control mode: Linear velocity (40cm/sec)
Colum:SH-Rt-2560(100 m ×0.25 mm× 0.2 μm)
Column temperature program：40℃(2 min)_4 ℃/min_240 ℃ (15 min)
Injection port temperature：250℃
Interface temp.: 230℃
Ion source temp.: 300℃
CID gas: Argon

Once the compound retention time have been obtained by analyzing standard solution, multi-compound methods can be created directly in conjunction with the database. The chromatogram of 37 fatty acid methyl ester standard solutions is shown in Figure 2. The interface using database to generate screening method is shown in Fig. 3.

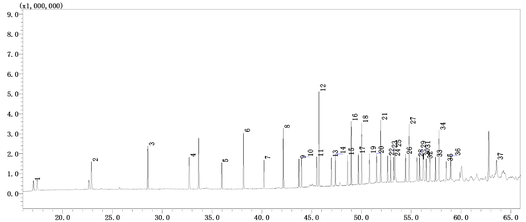


Figure 2. TIC of 37 fatty acid methyl ester standard solutions (1000 μg/L)

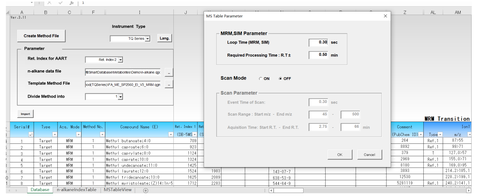
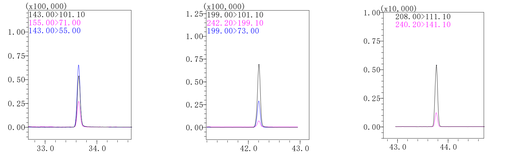


Figure 3 . The interface using database to generate screening method



Methyl Decanoate　　Methyl myristate　　Methyl 9(Z)-tetradecenoate

Figure 4. MC of some fatty acid methyl ester standard solution (100 μg/L)

Prepare 1, 5, 10, 50, 100, 200, 400 μg/L mixed standard solution of fatty acid methyl ester respectively, take 1 μL for injection, use concentration as abscissa and peak area as ordinate to make calibration curve. The detection limits and linear correlation coefficients of each compound are shown in Table 1.

Table1. Linear correlation coefficient, detection limit of each compound

No.	Compound name	Linear correlation coefficient	Detection limit (μg/L)
1	Methyl butanoate;4:0	0.9991	0.02
2	Methyl caproate;6:0	0.9992	0.02
3	Methyl caprylate;8:0	0.9990	0.11
4	Methyl caprate;10:0	0.9994	0.05
5	Methyl undecanoate;11:0	0.9998	0.04
6	Methyl laurate;12:0	0.9997	0.04
7	Methyl tridecanoate;13:0	0.9993	0.12
8	Methyl myristate;14:0	0.9992	0.01
9	Methyl myristoleate;(Z)14:1n-5	0.9995	0.07
10	Methyl pentadecanoate;15:0	0.9994	0.01
11	Methyl cis-10-pentadecenoate;(Z)15:1n-5	0.9992	0.08
12	Methyl palmitate;16:0	0.9991	0.01
13	Methyl palmitoleate;(Z)16:1n-7	0.9995	0.09
14	Methyl margarate;17:0	0.9997	0.01
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	0.9994	0.43
16	Methyl stearate;18:0	0.9998	0.01
17	Methyl elaidate;(E)18:1n-9	0.9995	0.46
18	Methyl oleate;(Z)18:1n-9	0.9993	0.17
19	Methyl linolelaidate;(E)18:2n-6	0.9996	0.78
20	Methyl linoleate;(Z)18:2n-6	0.9998	1.35
21	Methyl arachidate;20:0	0.9994	0.01
22	Methyl gamma-linolenate;(Z)18:3n-6	0.9992	0.07
23	Methyl cis-11-icosenoate;(Z)20:1n-9	0.9996	0.12
24	Methyl linolenate;(Z)18:3n-3	0.9992	0.67
25	Methyl heneicosanoate;21:0	0.9997	0.02
26	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6	0.9993	0.86
27	Methyl behenate;22:0	0.9995	0.01
28	Methyl eicosa-8,11,14-trienoate;20:3n-6	0.9991	0.72
29	Methyl erucate;22:1n-9	0.9998	0.65
30	Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3	0.9990	1.44
31	Methyl tricosanoate;23:0	0.9996	0.09
32	Methyl arachidonate;(Z)20:4n-6	0.9993	5.25
33	Methyl cis-13,16-Docosadienate;(Z)22:2n-6	0.9991	1.79
34	Methyl lignocerate;24:0	0.9998	0.02
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3	0.9995	9.47
36	Methyl nervonate;(Z)24:1n-9	0.9997	3.09
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3	0.9994	5.87

After spiking the blank sample with a concentration of 3mg/kg, proceed with the pre-treatment method mentioned above and test the recovery rate and RSD of three parallel samples.

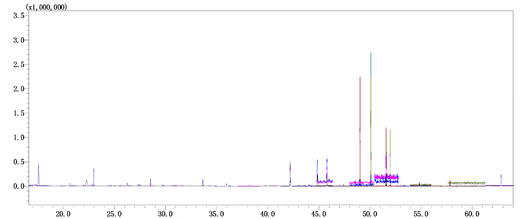


Figure 5. Chromatogram of real rice sample

Table 2. Recovery rate and rice sample determination results of partial compound

No.	Compound name	Added concentration (3mg/kg)		Rice sample (mg/kg)
		Average recovery rate (%)	RSD(%)	
1	Methyl butanoate;4:0	74.54	5.76	0.42
2	Methyl caproate;6:0	81.77	4.94	0.27
3	Methyl caprylate;8:0	87.70	6.09	0.56
4	Methyl caprate;10:0	86.85	7.49	0.05
5	Methyl undecanoate;11:0	71.22	8.04	6.74
6	Methyl laurate;12:0	77.07	9.35	1.31
7	Methyl tridecanoate;13:0	85.08	8.76	N.D
8	Methyl myristate;14:0	74.05	7.50	46.33
9	Methyl myristoleate;(Z)14:1n-5	82.70	6.44	N.D
10	Methyl pentadecanoate;15:0	82.48	8.69	3.03
11	Methyl cis-10-pentadecenoate;(Z)15:1n-5	71.24	3.53	N.D
12	Methyl palmitate;16:0	76.81	4.83	1216.14
13	Methyl palmitoleate;(Z)16:1n-7	67.54	5.42	10.69
14	Methyl margarate;17:0	72.57	11.54	3.16
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	77.48	3.78	1.71
16	Methyl stearate;18:0	83.82	2.98	142.97
17	Methyl elaidate;(E)18:1n-9	75.30	6.47	N.D
18	Methyl oleate;(Z)18:1n-9	67.44	8.73	2488.48
19	Methyl linolelaidate;(E)18:2n-6	72.70	4.52	N.D
20	Methyl linoleate;(Z)18:2n-6	83.38	9.01	3769.72
21	Methyl arachisate;20:0	74.80	3.12	29.65
22	Methyl gamma-linolenate;(Z)18:3n-6	77.43	4.76	N.D
23	Methyl cis-11-icosenoate;(Z)20:1n-9	105.50	7.53	23.41
24	Methyl linolenate;(Z)18:3n-3	109.24	5.00	34.31
25	Methyl heneicosanoate;21:0	76.07	10.16	0.77
26	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6	74.61	2.66	1.22
27	Methyl behenate;22:0	107.14	4.59	17.29
28	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3	69.22	3.63	0.71

5. Conclusions

In this paper, triple quadrupole gas chromatography-mass spectrometry combined with Smart Metabolites Database was used to establish a method for the analysis of 37 fatty acids in rice. In the concentration range of 1~400 μg/L, the linearity of the standard curve was good, the correlation coefficient was above 0.999, and the detection limit of the method was 0.01~9.47 μg/L. 100 μg/L standard solution was continuously injected for 6 times, and the RSD of peak area was less than 3.94%, which shows good precision. The method is simple in operation, fast in analysis, and can be used for analysis of fatty acid content in rice.