

1 **Quality evaluation of Semen Platycladi samples from**
2 **different origins by Internal Extractive Electrospray**
3 **Ionization Mass Spectrometry**

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21 **Preparation of standard solutions**

22 **1. Arachidonic acid standard solution**

23 First, 2.2 μL of arachidonic acid standard (density: 0.922 g/mL) was taken and 997.8 μL of
24 methanol was added to prepare 2 mg/mL standard solution. 0.5 mL of 2 mg/mL arachidonic acid
25 standard solution was taken and 0.5 mL of methanol was added to prepare 1 mg/mL standard solution.
26 0.2 mL of 1 mg/mL arachidonic acid standard solution was taken and 0.8 mL of methanol was added to
27 prepare 0.2 mg/mL standard solution. 0.5 mL of 0.2 mg/mL arachidonic acid standard solution was
28 taken and 0.5 mL of methanol was added to prepare 0.1 mg/mL standard solution. 0.1 mL of 0.1
29 mg/mL arachidonic acid standard solution was taken and 0.9 mL of methanol was added to prepare
30 0.01 mg/mL standard solution. 0.1 mL of 0.01 mg/mL arachidonic acid standard solution was taken
31 and 0.9 mL of methanol was added to prepare 0.001 mg/mL standard solution. 0.1 mL of 0.001 mg/mL
32 arachidonic acid standard solution was taken and 0.9 mL of methanol was added to prepare 0.0001
33 mg/mL standard solution. The prepared standard solution was stored in the refrigerator at 4 °C for
34 reserve (Table S1).

35 **2. Linoleic acid standard solution**

36 First, 11.1 μL of linoleic acid standard (density: 0.902 g/mL) was taken and 988.9 μL of methanol
37 was added to prepare 10 mg/mL standard solution. 0.8 mL of 10 mg/mL linoleic acid standard solution
38 was taken and 0.2 mL of methanol was added to prepare 8 mg/mL standard solution. 0.625 mL of 10
39 mg/mL linoleic acid standard solution was taken and 0.375 mL of methanol was added to prepare 5
40 mg/mL standard solution. 0.4 mL of 5 mg/mL linoleic acid standard solution was taken and 0.6 mL of
41 methanol was added to prepare 2 mg/mL standard solution. 0.5 mL of 2 mg/mL linoleic acid standard
42 solution was taken and 0.5 mL of methanol was added to prepare 1 mg/mL standard solution. 0.1 mL
43 of 1 mg/mL linoleic acid standard solution was taken and 0.9 mL of methanol was added to prepare 0.1
44 mg/mL standard solution. 0.1 mL of 0.1 mg/mL linoleic acid standard solution was taken and 0.9 mL
45 of methanol was added to prepare 0.01 mg/mL standard solution. 0.1 mL of 0.01 mg/mL linoleic acid
46 standard solution was taken and 0.9 mL of methanol was added to prepare 0.001 mg/mL standard
47 solution. The prepared standard solution was stored in the refrigerator at 4 °C for reserve (Table S1).

48 **3. Linolenic acid standard solution**

49 First, 11.0 μL of linolenic acid standard (density: 0.914 g/mL) was taken and 989 μL of methanol
50 was added to prepare 10 mg/mL standard solution. 0.8 mL of 10 mg/mL linolenic acid standard
51 solution was taken and 0.2 mL of methanol was added to prepare 8 mg/mL standard solution. 0.625 mL
52 of 10 mg/mL linolenic acid standard solution was taken and 0.375 mL of methanol was added to
53 prepare 5 mg/mL standard solution. 0.4 mL of 5 mg/mL linolenic acid standard solution was taken and
54 0.6 mL of methanol was added to prepare 2 mg/mL standard solution. 0.5 mL of 2 mg/mL linolenic
55 acid standard solution was taken and 0.5 mL of methanol was added to prepare 1 mg/mL standard
56 solution. 0.1 mL of 1 mg/mL linolenic acid standard solution was taken and 0.9 mL of methanol was
57 added to prepare 0.1 mg/mL standard solution. 0.1 mL of 0.1 mg/mL linolenic acid standard solution
58 was taken and 0.9 mL of methanol was added to prepare 0.01 mg/mL standard solution. 0.1 mL of 0.01
59 mg/mL linolenic acid standard solution was taken and 0.9 mL of methanol was added to prepare 0.001
60 mg/mL standard solution. The prepared standard solution was stored in the refrigerator at 4 °C for
61 reserve (Table S1).

62 **4. Palmitic acid standard solution**

63 First, 2 mg of palmitic acid standard was taken and 1 mL of methanol was added to prepare 2
64 mg/mL standard solution. 0.5 mL of 2 mg/mL palmitic acid standard solution was taken and 0.5 mL of

65 methanol was added to prepare 1 mg/mL standard solution. 0.2 mL of 1 mg/mL palmitic acid standard
 66 solution was taken and 0.8 mL of methanol was added to prepare 0.2 mg/mL standard solution. 0.5 mL
 67 of 0.2 mg/mL palmitic acid standard solution was taken and 0.5 mL of methanol was added to prepare
 68 0.1 mg/mL standard solution. 0.1 mL of 0.1 mg/mL palmitic acid standard solution was taken and 0.9
 69 mL of methanol was added to prepare 0.01 mg/mL standard solution. 0.1 mL of 0.01 mg/mL palmitic
 70 acid standard solution was taken and 0.9 mL of methanol was added to prepare 0.001 mg/mL standard
 71 solution. 0.1 mL of 0.001 mg/mL palmitic acid standard solution was taken and 0.9 mL of methanol
 72 was added to prepare 0.0001 mg/mL standard solution. The prepared standard solution was stored in
 73 the refrigerator at 4 °C for reserve (Table S1).

74 **5. Stearic acid standard solution**

75 First, 2 mg of stearic acid standard was taken and 1 mL of methanol was added to prepare 2
 76 mg/mL standard solution. 0.5 mL of 2 mg/mL stearic acid standard solution was taken and 0.5 mL of
 77 methanol was added to prepare 1 mg/mL standard solution. 0.2 mL of 1 mg/mL stearic acid standard
 78 solution was taken and 0.8 mL of methanol was added to prepare 0.2 mg/mL standard solution. 0.5 mL
 79 of 0.2 mg/mL stearic acid standard solution was taken and 0.5 mL of methanol was added to prepare
 80 0.1 mg/mL standard solution. 0.1 mL of 0.1 mg/mL stearic acid standard solution was taken and 0.9
 81 mL of methanol was added to prepare 0.01 mg/mL standard solution. 0.1 mL of 0.01 mg/mL stearic
 82 acid standard solution was taken and 0.9 mL of methanol was added to prepare 0.001 mg/mL standard
 83 solution. 0.1 mL of 0.001 mg/mL stearic acid standard solution was taken and 0.9 mL of methanol was
 84 added to prepare 0.0001 mg/mL standard solution. The prepared standard solution was stored in the
 85 refrigerator at 4 °C for reserve (Table S1).

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88 Table S1. Concentrations of five fatty acid standard solutions.

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Fatty Acid	value/(mg/mL)
arachidonic acid	0.0001, 0.001, 0.01, 0.1, 0.2, 1 ,2
linoleic acid	0.001, 0.01, 0.1, 1, 2, 5, 8, 10
linolenic acid	0.001, 0.01, 0.1, 1 ,2, 5, 8, 10
palmitic acid	0.0001, 0.001, 0.01, 0.1, 0.2, 1, 2
stearic acid	0.0001, 0.001, 0.01, 0.1, 0.2, 1, 2

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101 **Preparation of standard solutions for GC-MS experiments**

102 First, 4.2 μL of triarachidonin standard (density: 0.948 g/mL), 34.6 μL of trilinolein standard
103 (density: 0.925 g/mL), 8.5 μL of trilinolenin standard (density: 0.946 g/mL), 4 mg of tripalmitin
104 standard and 8 mg of tristealin standard was taken and 152.7 μL of n-hexane was added to prepare
105 mixed standard solution. This mixed standard solution is used as a high concentration solution. Take
106 0.05 mL of high concentration solution, add 0.15 mL of n-hexane to dilute as medium concentration
107 solution. Take 0.05 mL of medium concentration solution, add 0.15 mL of n-hexane to dilute as low
108 concentration solution. Specific concentrations are shown in Table S2. The prepared standard solution
109 was stored in the refrigerator at 4 °C for reserve.

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114 Table S2. Concentrations of three fatty acid triglycerides standard solutions

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Fatty acid triglycerides	value/(mg/mL)
triarachidonin	1.25, 5, 20
trilinolein	10, 40, 160
trilinolenin	2.5, 10, 40
tripalmitin	1.25, 5, 20
tristealin	2.5, 10, 40

126 Table S3. Analytical results of spiked samples by GC-MS (n = 6)

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Sample	Sample background value (mg/mL)	Added value (mg/mL)	Measured value (mg/mL)	Recovery rate/%	RSD/%
arachidonic acid	1.14	0.125	1.30	124.3	27.49
		0.5	1.55	81.9	10.31
		2	2.79	82.7	31.67
linoleic acid	0.08	1	0.99	90.8	8.31
		4	5.57	109.9	8.31
		16	19.59	115.1	21.24
linolenic acid	0.11	0.25	0.32	87.2	17.30
		1	1.36	125.8	10.02
		4	3.58	86.8	27.82
palmitic acid	0.09	0.125	0.23	116.0	21.69
		0.5	0.75	131.5	8.28
		2	1.78	84.6	25.64
stearic acid	0.25	0.25	0.49	95.3	20.71
		1	1.41	116.1	7.53
		4	3.39	78.4	26.33

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129 Table S4. Results of actual sample analysis by GC-MS (n=6)

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Origin	arachidonic acid	RSD/ %	linoleic acid	RSD/ %	linolenic acid	RSD/ %	palmitic acid	RSD/ %	stearic acid	RSD/ %
	1.43		0.35		1.11		0.14		0.33	
	1.03		0.32		1.11		0.13		0.38	
Henan	1.27	18.06	0.22	21.69	0.68	23.25	0.13	19.29	0.29	11.90
	0.97		0.21		0.68		0.13		0.28	
	1.30		0.21		0.71		0.08		0.29	
	0.85		0.22		0.73		0.09		0.28	

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