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Determination of Differentiating Markers in Coicis Semen From Multi-Sources Based on Structural Similarity Classification Coupled With UPCC-Xevo G2-XS QTOF

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Coicis semen, a medicinal food, is derived from the dried and mature seeds of *Coix lacryma-jobi* L. var. *ma-yuen* (Rom.Caill.) Stapf, a member of the Gramineae family. Lipids are its main constituents. Previous literature reported that coicis semen contains twenty triglycerides and twelve diglycerides. However, we identified thirty-five triglycerides, sixteen diglycerides, four monoglycerides, and two sterols under the preoptimized conditions of UPCC-Xevo G2-XS QTOF combined with a personalized TCM database. Furthermore, we successfully determined glycerol trioleate content to evaluate quality differences. Finally, we identified the fatty acid compositions of seven out of nine differential markers *via* Progenesis QI using principal component analysis, orthogonal projection to latent structures–discriminant analysis, and the LipidMaps database. In addition, we applied a software-based classification, a method that was previously developed by our team, to verify and predict structurally similar compounds. Our findings confirmed that UPCC-Xevo G2-XS QTOF combined with software-based group classification could be used as an efficient method for exploring the potential lipid markers of seed medicine.

Keywords: markers, coicis semen, triglyceride, qualitative and quantitative, MATLAB

INTRODUCTION

Common sense dictates that various natural ingredients exist in TCM. However, most reports on the active components of TCM have focused on polysaccharides, alkaloids, and flavonoids. Fatty oils are widely available ingredients of herbs, and the limited attention that they have received may restrict their further development and application. Fatty oils can be obtained as an active ingredient from animals and plants (Liu et al., 2015; Chen et al., 2016; Hong et al., 2016). A number of TCM contain fatty oils, which are mainly derived from the seeds and fruits of herbs. Diverse fatty oils comprise of glycerol and different types of saturated, monounsaturated, and polyunsaturated fatty acids that each exert therapeutic effects.

Coicis semen (Job's tears seed or adlay), which has been documented in the 2015 edition of the Chinese pharmacopoeia, is the dry and mature seed of *Coix lacryma-jobi* L. var. *ma-yuen* (Rom.Caill.)

Stapf. It is not only a commonly used TCM, but it is also a commonly consumed food. Coicis semen has been proven to have numerous functions, such as detoxification and dampness and arthralgia removal; it also reduces cancer risk (Yu et al., 2011; Bai et al., 2018; Duan, 2018; Xu et al., 2018; Choi et al., 2019; Huang Q. et al., 2019; Huang Y. L. et al., 2019; Li et al., 2019; Liu H. Q. et al., 2019; Liu Y. N. et al., 2019; Manosroi et al., 2019; Qian et al., 2019; Zhang et al., 2019). Moreover, it has a wide range of anti-inflammatory, antioxidation, analgesic, and sedative effects, as well as pharmacological effects against gastric cancer, hepatocellular cancer, Lewis lung cancer, non-small cell lung cancer, pancreatic cancer, and pulmonary cancer ("Fuzheng" category represented by coicis semen oil) (Manosroi et al., 2016a; Manosroi et al., 2016b; Qian et al., 2016; Xi et al., 2016; Wang et al., 2016; Han et al., 2017; Qu et al., 2017a; Schwartzberg et al., 2017; Jang et al., 2018; Kim et al., 2018; Zhang et al., 2018). It has a variety of clinical dosage forms, such as microporous microspheres, microemulsions, and intravenous emulsions (Qu et al., 2016; Qu et al., 2017b; Qu et al., 2017c; Trinh et al., 2017; Chen et al., 2018; Guo et al., 2019). Discrepancies in curative effects can be attributed to the various types, contents, and other nutrients of different fatty oils.

Recent research shows that the variety and content of the active components of different fatty oils remarkably influence human health and have their own specific advantages in treatment. Near-infrared spectroscopy analysis showed that the lipid contents of forty-one polished coicis semen samples range from 5.14% to 9.40%. Coicis semen oils contain seven types of triglycerides (trilinolein, 1,2-linolein-3-olein, 1-palmitin-2-linolein-3-olein, 1-palmitin-2,3-linolein, 1-palmitin-2, 3-olein, triolein, and 1,2-olein-3-linolein). Hou et al. identified twenty triglycerides and twelve diglycerides in the lipid profile of coicis semen and developed a green quantification strategy for simultaneously determining the content of 7 TGs (LLL, LLP, LLO, POL, OOL, OOP, and OOO) by combining core–shell column technology and SSDMCs. Lin et al. found β-sitosterol and stigmasterol in the ethyl acetate fraction of an adlay hull extract. Dong et al. established a rapid and reliable m-SPE approach using magnetic multiwalled carbon nanotubes as the adsorbent for the purification of type A trichothecenes, including T-2 toxins, HT-2 toxins, diacetoxyscirpenol, and neosolaniol, in coicis semen (Dong et al., 2016; Hou et al., 2018a; Hou et al., 2018b; Lin et al., 2019).

However, several components, especially glycerides, of coicis semen oil still require analysis, and the identification of the active ingredients of this material needs in-depth research. Therefore, our team used Acquity UPCC-Xevo G2-XS QTOF coupled with software-based group classification to further excavate, identify, and visually classify active ingredients in coicis semen oils. Coicis semen oils have boundless development prospects and need to be explored in-depth to lay a foundation for its new preparation, development, and extensive clinical application.

MATERIALS AND METHODS

Materials and Reagents

Glycerol trioleate with the purity of more than 99.9% as determined *via* HPLC–ELSD was purchased from the Nature Standard Co. Ltd (Shanghai, China). Acetonitrile and methanol (HPLC–MS grade) were purchased from Merck (Darmstadt, Germany). Ammonium formate (HPLC grade) was purchased from Sigma-Aldrich. High-purity CO_2 (99.999%) was purchased from the Shanghai Yizhi Industry Gases Co., Ltd. (Shanghai, China). All other reagents used in sample preparation were of analytical grade. Seven batches of dried coicis semen were purchased from different TCM enterprises in China. The manufacturers and batch numbers of the samples were as follows: batch number 180901 (Zhejiang Chinese Medical University Medical Pieces Co., Ltd., Hangzhou); batch number 190101 (Jirentang Pharmaceutical Co., Ltd., Guiyang); batch number 190105 (Zuoli Baicao Herbal Pieces Co., Ltd., Jiangxi); batch number 181201 (Huadong Herbal Pieces Co., Ltd., Hangzhou); batch number 181206 (Zuoli Baicao Herbal Pieces Co., Ltd., Zhejiang); batch number 190122 (Haiyuan Prepared Slices of Chinese Crude Drugs Co., Ltd., Nanjing); and batch number 190216 (Haichang Chinese Medicine Group Co., Ltd., Nanjing).

Preparation of Reference and Sample Solutions

The appropriate amount of glyceryl trioleate, which was used as the reference substance, was weighed accurately and diluted with n-hexane to prepare a series of working solutions with concentrations of 0.0099, 0.0988, 0.9881, 4.9405, and 9.8810 μg/mL.

All samples were ground and passed through a No. 3 sieve (355 ± 13 μm). The passing rate of the particles was maintained at more than 80%. The sample preparation procedure was as follows:

A total of 50.0 mL of n-hexane was added to 0.6 g of powdered coicis semen. The seed powder was soaked for 2 h and then sonicated (50 kHz, 250 W, KQ-500DB) for 30 min. The supernatant was filtered to obtain the sample solution. The filtrate was diluted with n-hexane 5 times and 100-fold for the analysis of different components of different samples and the quantitative analysis of glyceride trioleate, respectively. A total of 200 μL solution of each batch was mixed together and used as a pooled QC sample solution for the analysis of free fatty acids.

UPCC-Xevo G2-XS QTOF Parameters

On the basis of preliminary experiments, the final experimental conditions were determined as follows: Liquid phase system: ACQUITY UPCC; column: Torus 2-PIC, 3.0 × 100 mm, 1.7 μm; mobile phase A: CO_2, mobile phase B: methanol acetonitrile = 9:1; column temperature: 55°C, ABPR: 2600 psi; compensation solution: methanol solution with 0.5 mM ammonium formate; flow rate: 0.5 mL/min; and injection volume: 0.3 μL. The gradient program was used with a flow rate of 1.0 mL/min and was as follows: 0–2 min (1% B), 2–7 min (1%–5% B), 7–10 min (5% B), and 10–13 min (5%–1% B).

The conditions for mass spectrometry are as follows: Mass spectrometry system: Xevo G2-XS QTOF; ionization method: $ESI^{+/-}$; data acquisition mode: MS^E; MS^E impact energy: low, off, high, 20–50 eV; quantitative ion: m/z 902.8177; collection mass range: 100–1200 Da; capillary voltage: 2.0 kV; cone-hole voltage: 40 V; ion source

temperature: 120°C; atomization temperature: 500°C; cone-hole gas flow rate: 50 L/h; and atomized gas flow rate: 1000 L/h. Data calibration was performed by using an external reference (LockSpray) with the constant infusion of leucine–enkephalin solution (200 pg/μL) at a flow rate of 5 μL/min.

The data processing software included UNIFI 1.9.4 and Masslynx V 4.1.

Data Acquisition and Analysis

Xevo G2-XS QTOF uses the patented LockSpray technology to ensure the accuracy of the collected data in real time. High-accuracy mass numbers can be obtained, and the combination of high-accuracy mass numbers and isotope distribution and secondary fragment information accurately provides molecular formulas. Xevo G2-XS QTOF applies patented MS^E technology to obtain the primary and secondary mass spectral information of the compounds for further structural confirmation with one injection at the same time.

The $ESI^{+/-}$ mode was used for the analysis of glycerides and free fatty acids. The first step involved understanding the fragmentation pattern of glycerides as a whole. In the second step, by combining the fragmentation patterns and consulting related literature, a UNIFI database for the glyceride analysis of coicis semen was established. In the third step, the self-built database was imported into UNIFI in addition to the ChemSpider online database, and the appropriate analysis method was set. Furthermore, the software automatically analyzed primary and secondary mass spectral information. Finally, it quickly screened out the target through a unique workflow.

Our team developed a classification program in the Visual Basic for Applications (VBA; Microsoft, USA) and MATLAB v7.1 (The Mathworks, Natick, USA) environments. The classification program consisted of three parts (Shan et al., 2012).

A total of 2,916 features were introduced into the SIMCA-P 13.5 software (Umetrics, Umeå, Sweden) for principal component analysis (PCA) and orthogonal projection to latent structures–discriminant analysis (OPLS–DA). The corresponding variable importance in the projection value (VIP value) was calculated in the OPLS–DA model. A potential differential marker was selected when its VIP value exceeded 2.00 and its S-Plot exceeded 0.95.

RESULTS AND DISCUSSION

Qualitative Results of Glycerides and Free Fatty Acids in Coicis Semen Oils

In the ESI^+ mode, fifty-six and fifty-seven compounds were identified in five (No. 180901, No. 181201, No. 181206, No. 190101, and No. 190122) and two (No. 190105 and No. 190216) batches of samples, respectively. These compounds were mainly composed of glycerides. The total ion chromatogram of all samples is provided in **Figure 1A**, and the corresponding identified glycerides are listed in **Table 1**. Among them, fifty-six common compounds, including thirty-five triglycerides, fifteen diglycerides, four glycerides, and two sterols, were identified in comparison with the corresponding results of twenty triglycerides and twelve diglycerides (Hou et al., 2018a). However, the OP of diglycerides was identified only in two batches of samples, namely 190105 and 190216, likely because of the different processing technologies of different medical enterprises. The QC sample mentioned in **Figure 1B** was overlaid in the following differential component analysis.

A total of thirty free fatty acids were identified in the ESI^- mode, and some unsaturated fatty acids could have had isomers, which needed to be confirmed further by using a reference substance. The total ion chromatogram of the QC sample is given in **Figure 1C**,

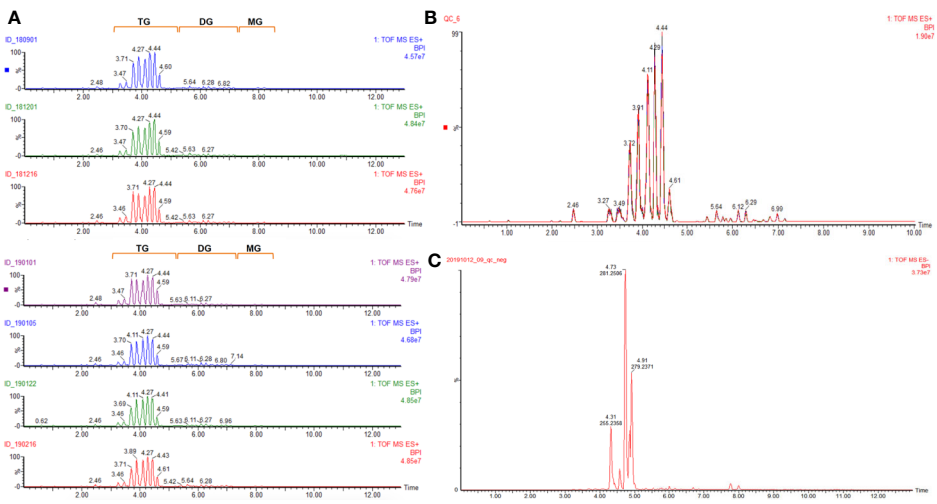

FIGURE 1 | Total ion chromatogram of **(A)** (qualitative analysis of glyceride contents of seven batches of coicis semen oils in ESI^+); **(B)** (overlay of the QC sample for the differential component analysis of different samples in ESI^+); **(C)** (qualitative analysis of free fatty acids of QC sample in ESI^-).

and the corresponding identified free fatty acids are listed in **Table 2**.

As shown in **Figure 2A**, PLO (t_R 3.90 min) provided a precursor ion ($[M+NH_4]^+$) at m/z 874.7874 with a double-bond equivalent. The MS/MS product ions at m/z 601.5184 ($[M-P+H]^+$ palmitic acid), m/z 577.5175 ($[M-L+H]^+$ linoleic acid), and m/z 575.5025 ($[M-O+H]^+$ oleic acid) resulted from the sn-1, sn-2, and sn-3 cleavages of the ester groups, respectively. **Figure 2B** shows the

TABLE 1 | The qualitative results of glycerides in coicis semen oils in ESI^+.

No.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed RT (min)	Response	Adducts
1	β-Sitosterol	$C_{29}H_{50}O$	414.3862	*397.3827*	-0.20	2.65	175317	-H2O+H
2	PPP	$C_{51}H_{98}O_6$	806.7363	*824.7724*	2.20	2.87	76543	+NH4, +Na
3	Stigmasterol	$C_{29}H_{48}O$	412.3705	*413.3782*	0.40	2.98	280236	+H, +Na, -H2O+H
4	PMO	$C_{51}H_{96}O_6$	804.7207	*822.7572*	2.70	3.01	40426	+NH4, +Na
5	PPS	$C_{53}H_{102}O_6$	834.7676	*852.8045*	3.10	3.11	19271	+NH4, +Na
6	PML	$C_{51}H_{94}O_6$	802.7050	*820.7414*	2.50	3.18	31125	+NH4, +Na
7	PPO	$C_{53}H_{100}O_6$	832.7520	*850.7880*	2.20	3.26	4297765	+NH4, +H, +Na, -H2O+H
8	PSS	$C_{55}H_{106}O_6$	862.7989	*880.8372*	4.40	3.38	5232	+NH4, +Na
9	PPL	$C_{53}H_{98}O_6$	830.7363	*848.7720*	1.90	3.46	4668112	+NH4, +H, +Na, -H2O+H
10	POS	$C_{55}H_{104}O_6$	860.7833	*878.8203*	3.20	3.54	1031215	+NH4, +H, +Na
11	PPOL	$C_{53}H_{96}O_6$	828.7207	*846.7580*	3.40	3.64	344524	+NH4, +H, +Na
12	POO	$C_{55}H_{102}O_6$	858.7676	*876.8048*	3.40	3.71	20321224	+NH4, +H, +Na, -H2O+H
13	OPC20:0	$C_{57}H_{108}O_6$	888.8146	*906.8520*	3.60	3.80	323033	+NH4, +H, +Na
14	LLM	$C_{53}H_{94}O_6$	826.7050	*844.7426*	3.70	3.83	49997	+NH4, +H, +Na
15	PLO	$C_{55}H_{100}O_6$	856.7520	*874.7887*	2.90	3.89	21370394	+NH4, +H, +Na, -H2O+H
16	OOS	$C_{57}H_{106}O_6$	886.7989	*904.8357*	2.90	3.96	3378949	+NH4, +H, +Na, -H2O+H
17	OLC17:0	$C_{56}H_{102}O_6$	870.7676	*888.8046*	3.20	4.01	273530	+NH4, +H, +Na
18	OPC22:0	$C_{59}H_{112}O_6$	916.8459	*934.8839*	4.10	4.03	51651	+NH4, +Na
19	LLP	$C_{55}H_{98}O_6$	854.7363	872.7728	2.60	4.06	8367471	+NH4, +H, +Na, -H2O+H
20	OOO	$C_{57}H_{104}O_6$	884.7833	*902.8202*	3.10	4.11	21932960	+NH4, +H, +Na, -H2O+H
21	OOO20:0	$C_{59}H_{110}O_6$	914.8302	*932.8674*	3.30	4.19	590110	+NH4, +H, +Na
22	OOC19:1	$C_{58}H_{106}O_6$	898.7989	*916.8371*	4.30	4.21	28860	+NH4
23	LLPo	$C_{55}H_{96}O_6$	852.7207	*870.7575*	3.00	4.23	335763	+NH4, +H, +Na, -H2O+H
24	OOL	$C_{57}H_{102}O_6$	882.7676	*900.8044*	2.90	4.27	26267878	+NH4, +H, +Na, -H2O+H
25	OLC20:0	$C_{59}H_{108}O_6$	912.8146	930.8517	3.30	4.34	795167	+NH4, +H, +Na, -H2O+H
26	OLC19:1	$C_{58}H_{104}O_6$	896.7833	*914.8206*	3.40	4.37	39779	+NH4, +Na
27	LLO	$C_{57}H_{100}O_6$	880.7520	*898.7884*	2.60	4.43	22285984	+NH4, +H, +Na, -H2O+H
28	LOC20:1	$C_{59}H_{106}O_6$	910.7989	*928.8353*	2.50	4.51	663937	+NH4, +H, +Na
29	LLC19:1	$C_{58}H_{102}O_6$	894.7676	*912.8009*	-0.60	4.53	34245	+NH4
30	LOC22:0	$C_{61}H_{112}O_6$	940.8459	*958.8826*	2.80	4.54	183383	+NH4, +Na
31	OOO24:0	$C_{63}H_{118}O_6$	970.8928	*988.9309*	4.20	4.57	53885	+NH4
32	LLL	$C_{57}H_{98}O_6$	878.7363	*896.7721*	2.00	4.60	8926747	+NH4, +H, +Na, -H2O+H
33	LLC22:0	$C_{61}H_{110}O_6$	938.8302	*956.8666*	2.60	4.69	163391	+NH4, +Na
34	LOO24:0	$C_{63}H_{116}O_6$	968.8772	*986.9141*	3.10	4.73	113189	+NH4, +Na
35	LLLn	$C_{57}H_{96}O_6$	876.7207	*894.7569*	2.40	4.76	375488	+NH4, +H, +Na, -H2O+H
36	LLC24:0	$C_{63}H_{114}O_6$	966.8615	*984.8983*	2.90	4.87	76278	+NH4, +Na
37	OLS	$C_{57}H_{104}O_6$	884.7833	*885.7856*	-4.90	5.21	3683	+H
38	PP	$C_{35}H_{68}O_5$	568.5067	*551.5035*	0.10	5.43	414186	-H2O+H, +Na
39	PS	$C_{37}H_{72}O_5$	596.5380	*579.5348*	0.10	5.63	579793	-H2O+H, +Na
40	PO	$C_{37}H_{70}O_5$	594.5223	*577.5192*	0.10	5.78	924986	-H2O+H, +H, +Na
41	PL	$C_{37}H_{68}O_5$	592.5067	*575.5031*	-0.30	5.95	728564	-H2O+H, +H, +NH4, +Na
42	OS	$C_{39}H_{74}O_5$	622.5536	*605.5500*	-0.30	5.98	150906	-H2O+H, +H, +Na
43	OO	$C_{39}H_{72}O_5$	620.5380	*603.5344*	-0.30	6.11	2014873	-H2O+H, +H, +NH4, +Na
44	PP	$C_{35}H_{68}O_5$	568.5067	*551.5030*	-0.40	6.19	17291	-H2O+H, +Na
45	OL	$C_{39}H_{70}O_5$	618.5223	*601.5188*	-0.20	6.28	2010772	-H2O+H, +H, +NH4, +Na
46	LL	$C_{39}H_{68}O_5$	616.5067	*617.5130*	-1.00	6.48	681857	+H, +NH4, +Na, -H2O+H
47	OP	$C_{37}H_{70}O_5$	594.5223	*617.5130*	1.40	6.48	681857	+Na, +H, +NH4, -H2O+H
48	LP	$C_{37}H_{68}O_5$	592.5067	*575.5037*	0.40	6.67	311434	-H2O+H, +H, +NH4, +Na
49	OS	$C_{39}H_{74}O_5$	622.5536	*645.5432*	0.30	6.70	44689	+Na, +H, +NH4, -H2O+H
50	OO	$C_{39}H_{72}O_5$	620.5380	*603.5350*	0.30	6.81	707855	-H2O+H, +H, +NH4, +Na
51	LO	$C_{39}H_{70}O_5$	618.5223	*601.5193*	0.20	6.98	697567	-H2O+H, +H, +NH4, +Na
52	LL	$C_{39}H_{68}O_5$	616.5067	*639.4962*	0.30	7.13	505275	+Na, +H, +NH4, -H2O+H
53	LLn	$C_{39}H_{66}O_5$	614.4910	*637.4809*	0.60	7.30	13264	+Na, +H, +NH4, -H2O+H
54	MG(16:0/0:0/0:0)	$C_{19}H_{38}O_4$	330.2770	*353.2667*	0.50	7.97	239151	+Na, -H2O+H
55	MG(17:0/0:0/0:0)	$C_{20}H_{40}O_4$	344.2927	*367.2841*	2.20	8.08	632	+Na, -H2O+H
56	MG(18:0/0:0/0:0)	$C_{21}H_{42}O_4$	358.3083	*381.2977*	0.20	8.19	259875	+Na, -H2O+H
57	MG(20:0/0:0/0:0)	$C_{23}H_{46}O_4$	386.3396	*409.3288*	0.00	8.40	2705	+Na, -H2O+H

secondary fragment matching diagram for OOO generated by the UNIFI software.

Software-Based Group Classification of Glycerides

Our teams previously developed a program in VBA and MATLAB for the classification of multiple complex components. This program successfully grouped the constituents in the n-hexane extract of coicis semen. Through the comprehensive analysis of herbal samples, fifty-seven peaks were identified and divided into four groups as shown in **Figures 3**, **4**. Three of these groups consisted of triglycerides and diglycerides. The remaining group was composed of four monoglycerides, two diglycerides, and two sterols. The chemical structures and special MS fragmentation pathways of these compounds indicated that the same group might have similar features, and unknown ingredients could be identified through the comprehensive software-based group classification of these compounds.

Differential Component Analysis of Different Samples

The Progenesis QI omics analysis software was used for differential component research. Before exploring quality markers, the analytical system was first validated for repeatability upon the injection of six QC samples. A total of 2,916 features were extracted and then imported into EZinfo for multivariate statistical analysis. PCA was used to study the variations in the oils of seven batches of coicis semen (**Figure 5A**). The differences between the groups of samples, namely No. 181216 and No.190122, were large. Furthermore, No. 190105 and No. 190101, which showed the largest differences, were subjected to OPLS–DA analysis (**Figure 5B**). These two groups were clearly distinct. Furthermore, we selected compounds with S Plot ≥ 0.95 and VIP ≥ 2 as markers (**Figures 5C, D**), and transferred them back to the QI for identification. Finally, nine markers were found (**Table 3**). Then, the LipidBlast, LipidMaps, and Chemspider databases were searched in QI for further identification. Among the nine markers found, seven were identified (five diglycerides, one triglyceride, and one stigmasterol), and the molecular formulas of the remaining two unknown compounds were estimated using an elemental composition tool. The abundance distribution of the nine markers in all the samples is shown in **Figure 6**. The abundance of markers, except diglycerides (16:0/18:0/0:0), in No. 190105 were remarkably higher than that in No. 190101. This result could be related to the largest differences between No. 190105 and No. 190101 and indicated that massive differences in resources and processing technologies existed among medical enterprises.

TABLE 2 | The qualitative results free fatty acids in coicis semen oils in ESI^-.

No.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	Observed RT (min)	Response	Adducts
1	Oleic acid	$C_{18}H_{34}O_2$	282.2559	*281.2486*	0.0	4.74	7887221	-H, +HCOO
2	Linoleic acid	$C_{18}H_{32}O_2$	280.2402	*279.2330*	0.1	4.92	4629367	-H, +HCOO
3	Palmitic acid	$C_{16}H_{32}O_2$	256.2402	*255.2329*	0.0	4.32	2866760	-H, +HCOO
4	Stearic acid	$C_{18}H_{36}O_2$	284.2715	*283.2642*	-0.1	4.58	940078	-H, +HCOO
5	Arachidic acid	$C_{20}H_{40}O_2$	312.3028	*311.2956*	0.0	4.82	131638	-H, +HCOO
6	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.2559	*281.2484*	-0.2	6.02	126409	-H
7	9, 10- EPOXYOCTADECANOIC ACID	$C_{18}H_{34}O_3$	298.2508	*297.2432*	-0.3	5.79	88969	-H
8	9, 10- EPOXYOCTADECANOIC ACID	$C_{18}H_{34}O_3$	298.2508	*297.2432*	-0.3	5.92	79177	-H
9	11-Eicosenoic acid	$C_{20}H_{38}O_2$	310.2872	*309.2798*	-0.1	4.97	75288	-H
10	Linolenic acid	$C_{18}H_{30}O_2$	278.2246	*277.2172*	-0.1	5.09	61260	-H
11	Lignoceric acid	$C_{24}H_{48}O_2$	368.3654	*367.3579*	-0.2	5.23	58141	-H
12	Behenic acid	$C_{22}H_{44}O_2$	340.3341	*339.3265*	-0.3	5.03	55860	-H
13	Palmitoleic acid	$C_{16}H_{30}O_2$	254.2246	*253.2173*	0.0	4.50	38739	-H
14	Margaric acid	$C_{17}H_{34}O_2$	270.2559	*269.2486*	0.0	4.45	29580	-H
15	Arachidonic acid	$C_{20}H_{32}O_2$	304.2402	*349.2359*	-2.5	4.74	22657	+HCOO
16	TRICOSANOIC ACID	$C_{23}H_{46}O_2$	354.3498	*353.3422*	-0.3	5.13	17977	-H
17	Myristic acid	$C_{14}H_{28}O_2$	228.2089	*227.2017*	0.1	4.05	15915	-H
18	10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268.2402	*267.2327*	-0.3	4.62	11423	-H
19	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302.2246	*347.2200*	-2.8	4.92	11013	+HCOO, -H
20	Hexacosanoic acid	$C_{26}H_{52}O_2$	396.3967	*395.3887*	-0.7	5.42	8914	-H
21	Pentacosanoic acid	$C_{25}H_{50}O_2$	382.3811	*381.3733*	-0.5	5.33	8708	-H
22	Pentadecylic acid	$C_{15}H_{30}O_2$	242.2246	*241.2173*	0.0	4.20	8677	-H
23	NONADECANOIC ACID	$C_{19}H_{38}O_2$	298.2872	*297.2798*	-0.1	4.69	6204	-H
24	HENEICOSANOIC ACID	$C_{21}H_{42}O_2$	326.3185	*325.3111*	-0.1	4.93	5972	-H
25	Octacosanoic acid	$C_{28}H_{56}O_2$	424.4280	*423.4200*	-0.7	5.60	5361	-H
26	Laurie acid	$C_{12}H_{24}O_2$	200.1776	*199.1704*	0.0	3.71	5056	-H
27	Erucic acid	$C_{22}H_{42}O_2$	338.3185	*337.3115*	0.3	5.19	4770	-H
28	Nervonic acid	$C_{24}H_{46}O_2$	366.3498	*411.3476*	-0.4	6.38	3848	+HCOO
29	11-14-Eicosadienoic acid	$C_{20}H_{36}O_2$	308.2715	*307.2643*	0.1	5.14	2012	-H
30	Homo gamma linolenic acid	$C_{20}H_{34}O_2$	306.2559	*351.2511*	-3.0	4.58	1984	+HCOO

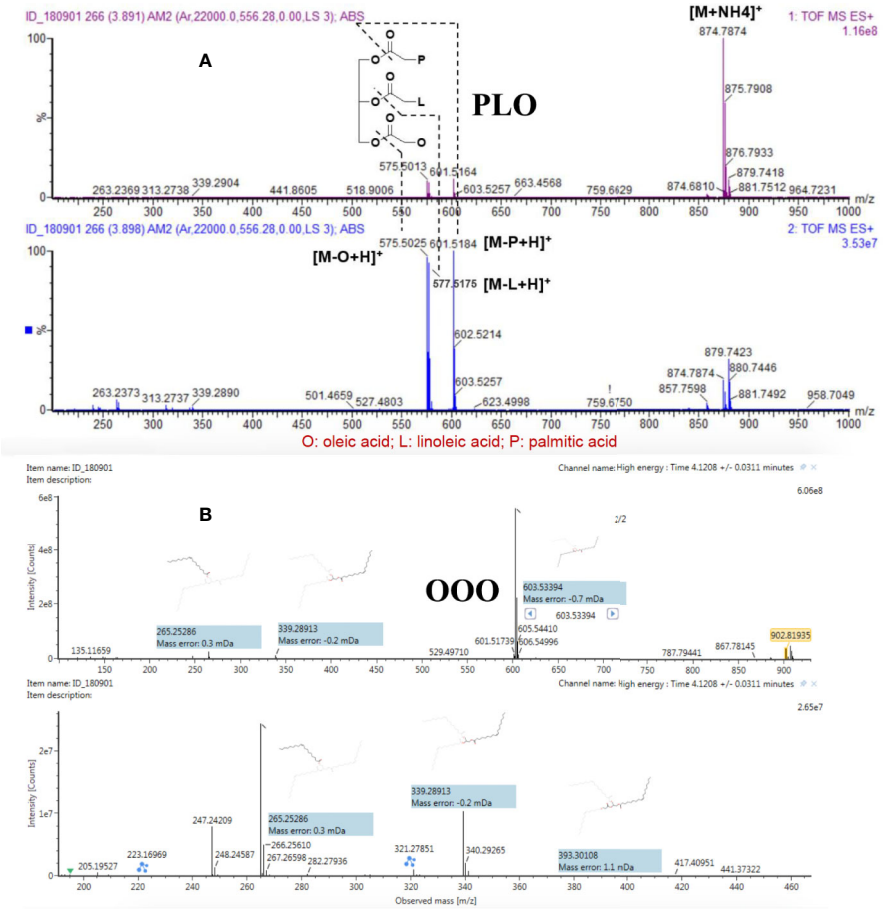

FIGURE 2 | Fragmentation patterns of PLO **(A)** and OOO **(B)** with MS1 and MS2 spectra.

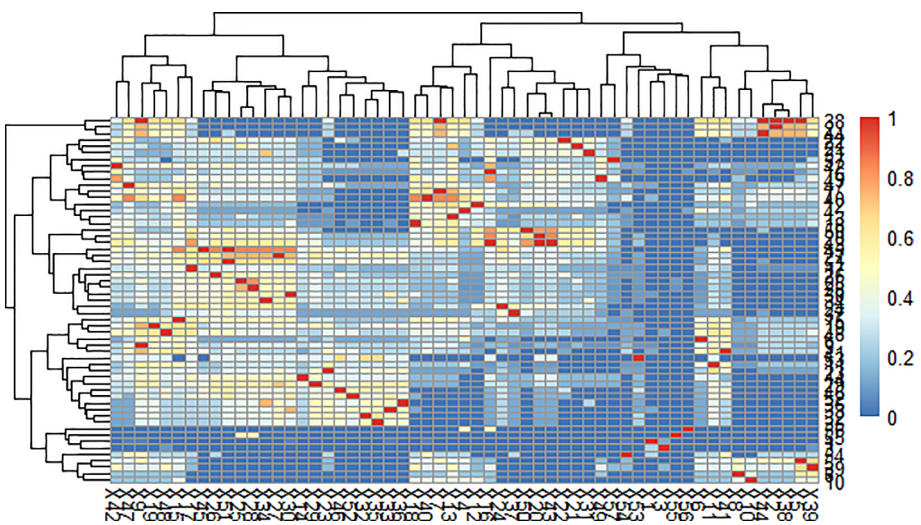

FIGURE 3 | Affinity diagram of the mass spectra of glycerides with software-based group classification.

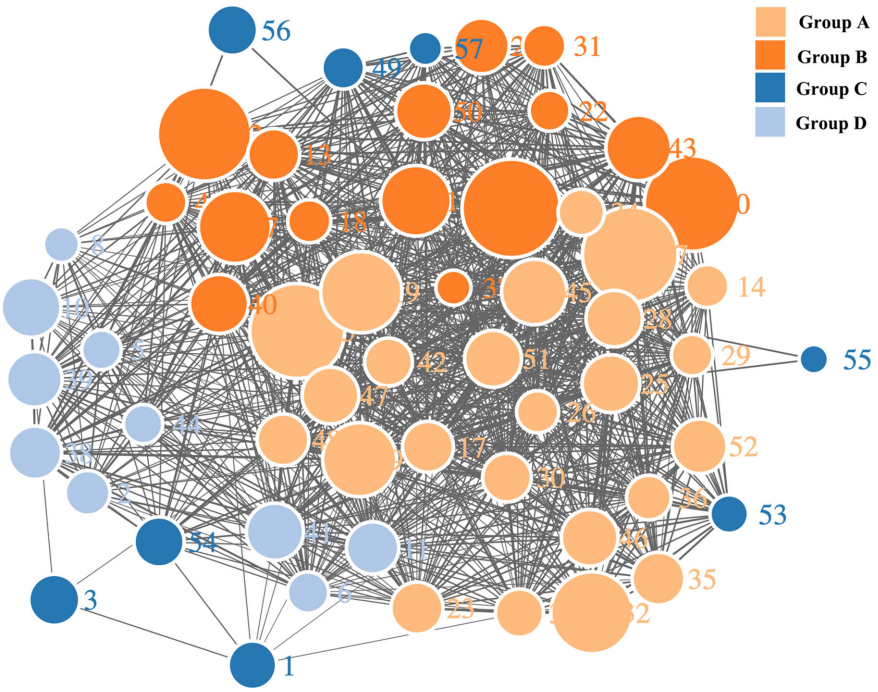

FIGURE 4 | Network diagram of the mass spectra of glycerides with software-based group classification.

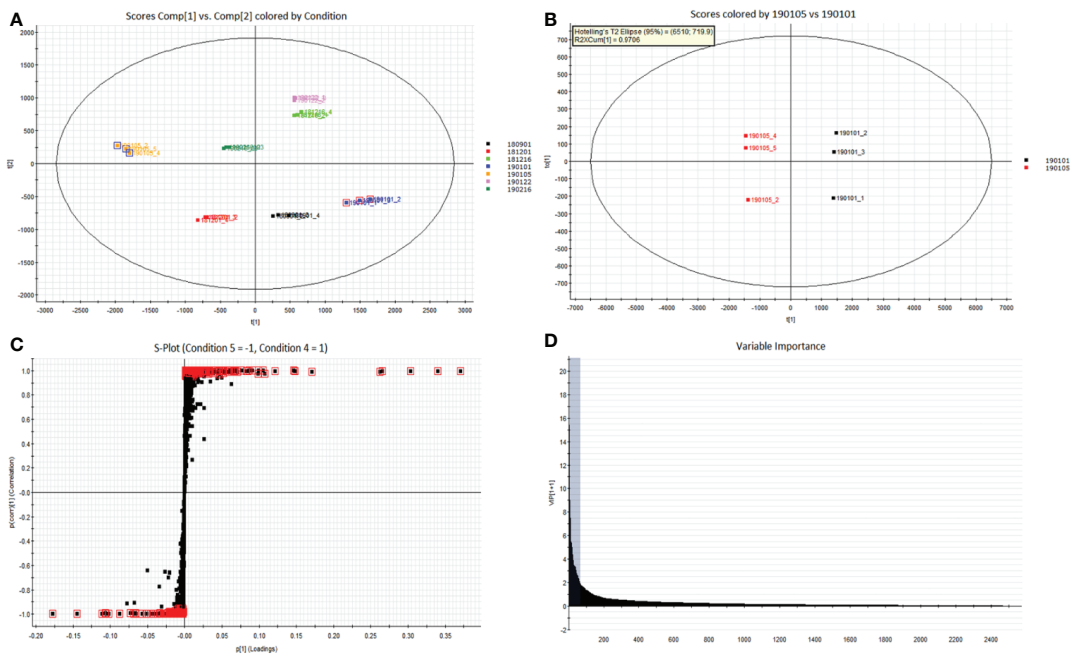

FIGURE 5 | Differential component analysis of different samples. **(A)**: PCA classification of seven batches of samples; **(B)** OPLS–DA analysis of No. 190101 and No. 190105 with significant differences; **(C)** S-Plot of No. 190101 and No. 190105; **(D)** VIP diagram of No. 190101 and No. 190105.

TABLE 3 | The identified results of nine differential markers between No. 190101 and No. 190105.

No.	m/z	Retention time (min)	Identity	Formula	Neutral mass (Da)
1	*413.3781*	2.99	Stigmasterol	$C_{29}H_{48}O$	412.3708
2	*579.5337*	5.64	DG(16:0/18:0/0:0)	$C_{37}H_{72}O_5$	596.5370
3	*963.7297*	5.69	unknown	$C_{53}H_{102}O_{14}$	962.7270
4	*962.7224*	5.69	TG(18:4/20:5/22:6)	$C_{63}H_{92}O_6$	944.6894
5	*961.7175*	6.18	unknown	$C_{53}H_{100}O_{14}$	960.7113
6	*577.5188*	6.52	DG(18:1/16:0/0:0)	$C_{37}H_{70}O_5$	594.5220
7	*603.5345*	6.82	DG(18:1/18:1/0:0)	$C_{39}H_{72}O_5$	620.5378
8	*641.5118*	6.99	DG(18:2/18:1/0:0)	$C_{39}H_{70}O_5$	618.5222
9	*639.4958*	7.14	DG(18:2/18:2/0:0)	$C_{39}H_{68}O_5$	616.5069

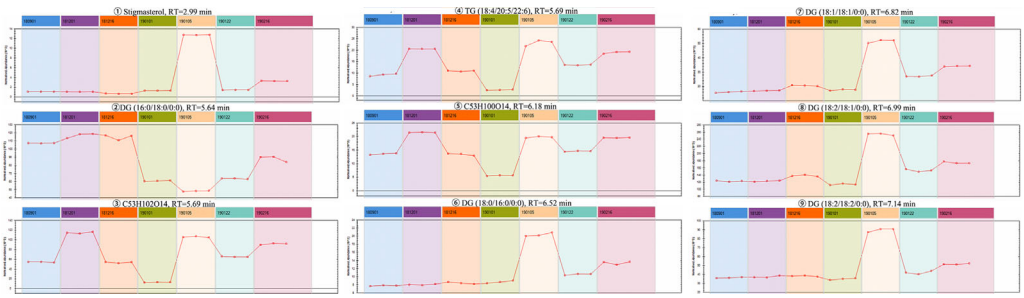

FIGURE 6 | Abundance distribution of nine markers in all samples.

Quantitation of Glycerol Trioleate in Coicis Semen Oils

Investigation of Linear Relations

Reference solutions with concentrations of 0.0099, 0.0988, 0.9881, 4.9405, and 9.8810 μg/mL were used to perform three consecutive injections. The results showed that glyceryl trioleate had a good linear relationship in the range of 0.0090–9.8810 μg/mL, $r^2 > 0.9990$.

Quantitative Limit Investigation

The reference solution was diluted stepwise at certain multiples until glyceride trioleate presented S/N ≈ 10. The results showed that the quantitative limit was 4.94 ng/mL.

Instrument Precision Inspection

The low, middle, and high concentrations (0.0099, 0.9881, and 9.8810 μg/mL) of the reference solution on the calibration curve

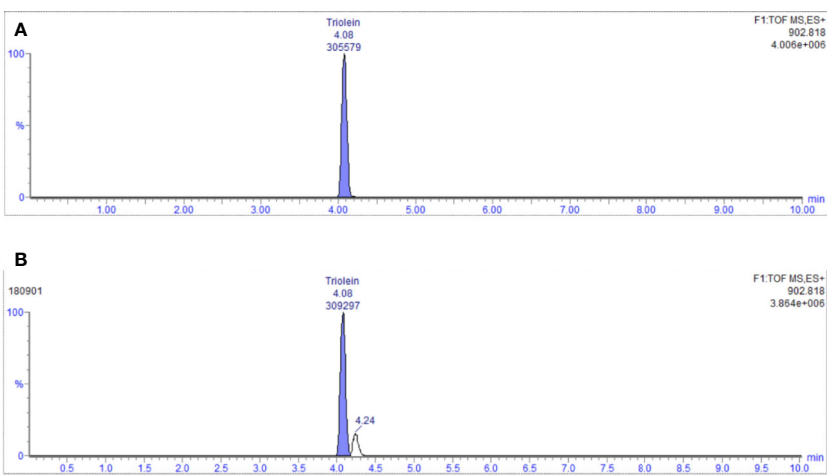

FIGURE 7 | **(A)** Chromatogram of reference (glycerol trioleate); **(B)** EIC diagram of representative sample (No. 180901).

were taken and used in six consecutive injections to check instrument precision. The RSD value was less than 3%, which indicated good precision.

Repeatability Test

Six powder samples (0.6 g each) of the same batch (No. 180901) were weighed and prepared *via* the sample solution preparation method. The average content of glyceryl trioleate was determined and calculated as 0.91%. The results showed that the RSD value was 4.12% (n = 6).

Recovery Rate Test

ine powder samples (0.3 g each) of the same batch of known content were weighed, and then low, medium, and high levels of the three different concentrations of the reference substance were added precisely. The reference substance/sample ratio was controlled at 0.5:1, 1:1, 1.5:1, and each concentration level was tested in triplicate. The results showed a high average recovery of 102.28%.

Sample Measurement Results

The content of glyceryl trioleate in seven batches of the samples was determined using the established method above. The representative chromatogram is shown in **Figure 7**. Each batch was replicated in triplicate. The average content ranged from 0.84% to 1.05%. The RSD value was less than 5%.

CONCLUSION

The established analytical method fully demonstrated that ACQUITY UPCC enabled the fast and efficient chromatographic separation of lipids in coicis semen. Xevo G2-XS QTOF combined with LockSpray real-time external standard mass calibration technology ensured mass accuracy. The data collection method based on MS^E tandem mass spectrometry without content discrimination ensured the full collection of information, and one-shot collection could obtain precursor ion and fragment ion information simultaneously with convenient, fast, and high-throughput characteristics.

By using the ACQUITY UPCC/Xevo G2-XS QTOF system combined with the UNIFI software, fifty-seven compounds of glycerides were identified and divided into four groups on the basis of their similar features *via* software-based group classification in the ESI^+ mode. Moreover, thirty free fatty acids were identified in ESI^-. In addition, QI omics analysis software found nine differential compounds between No. 190101 and No. 190105, and seven of these compounds were identified. Finally, the quantitative analysis of glyceryl trioleate (quality control component in the 2015 Edition of the Chinese Pharmacopoeia) and methodological verification were performed, and the results showed that the linearity, precision, reproducibility, recovery, and other parameters of the method were good. The established quantitative method determined that the glyceryl trioleate contents of the seven batches of samples ranged from 0.84% to 1.05%.

In summary, we identified additional glycerides and free fatty acids in coicis semen oils. Our results could supplement corresponding component research. Furthermore, nine differential components were found to be potential markers of quality for differentiating coicis semen with different origins. Finally, glyceryl trioleate was determined to evaluate its pros and cons. This approach might be useful for assessing the quality of TCM.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

Conceptualization: XW and GC. Data curation: RZ, XX, and QS. Formal analysis: KW. Funding acquisition: GC. Investigation: RZ, XX, and KW. Writing—original draft: RZ and XX. Writing—review and editing: XW and GC. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.549181/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.