Supplementary Text S1

The clinical trial protocol of Shaofu Zhuyu formula for treating primary dysmenorrheal

Organizer: Nanjing University of Chinese Medicine

Clinical trial operator: Jiangsu Provincial Hospital of Integrated Traditional and Western Medicine

Date: 2010-10-8~2012-1-10

Study designer: Peijuan Wang

Registration number: ChiCTR-ONC-12002278

Flowchart

stage		**screening**	**Therapy(interview)**			**follow-up**
visit		First visit	First period	Second period	Third period	Fourth period
			First time	Third time	Fifth time	Follow-up
days		Seven days before menstruation	cramp	cramp	cramp	Within 12 hours of menstruation
enrollment		√				
Informed consent sign		√				
Formed medical record			√	√	√	√
Distribute drugs			√			
Lab chemical indicators						
Diagnosis	type-B ultrasonic	√				

indicators	Physical examination	√				
	electrocardiogram	√				
safety indicators	routine blood test	√				
	routine urine test	√				
	ALT BUN Cr	√			√	
Chemical indicators	5-HT, OT, AVP, [S-LP(a)]	√	√	√	√	
	E_2, P, FSH, T, GC	√	√	√	√	
	PGE_2, $PGF_{2\alpha}$, NO , LTB_4, ET-1	√	√	√	√	

	blood coagulation function; blood flow rheometer; routine blood test	√	√	√	√	
Efficacy scores	Pain scores		√	√	√	√
	Pain scale scores		√	√	√	√
	Total symptom severity score of Chinese medicine		√	√	√	√
Evaluation of trial						
Drug combination			√	√	√	
Adverse event			√	√	√	

The clinical protocol of Shaofu Zhuyu formula for treating primary dysmenorrheal

1. Background

Based on the theory of traditional Chinese medicine (TCM), i.e. the philosophy of treating the entire body as a whole, the multi-pathogenic factors of cold - coagulation, qi-stagnation, and blood-insufficiency are considered as the main reasons for PD. According to TCM theory, PD was divided into different disharmony patterns (may be considered as subtypes of disease in Western Medicine) such as the syndrome of cold coagulation and blood stasis (SCB), qi stagnation and blood stasis (SQB), etc.. The prevalence of PD with SCB is estimated to be about 60%.

The Chinese herbal formula, Shaofu Zhuyu (SFZY) decoction, is considered as an effective prescription in treating PD with SCB. This prescription originally came from "*Correction of Errors in Medical Classics*" compiled by Qing-ren Wang in Qing dynasty (A.D. 1830), and which was utilized in clinic to treat blood stasis syndrome of gynecology diseases such as dysmenorrhea, amenorhhea for about 200 years. The efficacy of SFZY decoction for treating PD with SCB was reported to be above 90%. The preparation of Shaofu Zhuyu Formula concentrate-granules (SFZYFG), a modified form of preparation of SFZY decoction, was approved by State Food and Drug Administration of China (SFDA).

The TCM formula that we used in our study was Shaofu Zhuyu Formula concentrated - granule (SFZYFG) produced by Jiangsu Tianjiang Pharmaceutical Co., Ltd. SFZYFG is composed of ten herbal concentrated - granule of Angelicae sinensis

Radix (No. 1008064), Chuanxiong Rhizoma (No. 1007151), Cinnamomi Cortex (No. 1005038), Foeniculi Fructus (No. 0908048), Zingiberis Rhizoma (No. 1007044), Myrrha (No. 1002085), Trogopteri Feces (No. 1005144), Typhae Pollen (No. 1003002), Paeoniae Radix Rubra (No. 1008034), and Corydalis Rhizoma (No. 1008073) in the herbs weight ratio of 3:1:2:1:0.5:2:1:3:1:1. Every herbal concentrate - granule was obtained by refluxing with water for 2 times and the combined filtrates were dried through spraying. In addition, the granule was prepared according to the approved preparation technology.

Our previous study on animals also suggested that SFZY decoction inhibited COX-2 enzyme and its expression in endometrial cell, regulated the ovarian function and hormone secretion, and improved the blood circulation. In this protocol, the study was conducted at Jiangsu Provincial Hospital of Integrated Traditional and Western Medicine, and which was complied with guidelines of Good Clinical Practice.

2. Objective

Blood and urine samples were collected from each subject on the first day of menstrual cycle for continuous 4 periods.

By using metabolomics analysis in plasma and urine of PD patients after intake of SFZYFG, we aimed to: (i). to elucidate the metabolic profiling and phenotype changes between PD patients and healthy volunteers; (ii) to identify the potential biomarkers; and (iii) to explore the intervention effects and action mechanisms.

3. Study design methods

Methods: own control

Sample size: 25

4. Patient enrollment

4.1 Clinical diagnosis criteria of primary dysmenorrhea by Western medicine

Dysmenorrhea, also known as painful menstruation, can be an incapacitating and disruptive condition. Clinically, primary dysmenorrhea (PD) is characterized by painful uterine cramping during the menstrual cycle or before and after menstruation, with no associated pelvic disease. The clinical features are listed as following:

1. PD is prevalent during adolescents and nonporous women.
2. Pain starts within several hours of the onset of menstruation and is most severe on the first day or two of menses.
3. Lower abdominal pain paroxysmal in a convulsive way or pain and tenesmus feeling, even radiation to the lumbosacral, anal, vaginal, and vastus medialis.
4. Some patients are pale, cold sweat, cold limbs and faint.

Gynecological examination (Rectal examination for unmarried women) :

without any organic pathology.

B-ultrasound examination:

without any organic pathology.

4.2 Clinical diagnosis criteria of Primary dysmenorrhea with syndrome of cold coagulation and blood stasis by traditional Chinese medicine (State Administration of TCM approved)

Primary symptoms: Cold and pain of abdomen, Pain reduction with the heat, or with distending pain to press.

Minor symptoms: scanty menstruation or bleeding obstacle, menstruation with red

dark and blood clot, fear of cold, hands and feet are cold, dripping with cold sweat, rectal dropped, nausea and vomiting, red dark tongue and white coat, deep pulse.

The PD patients must meet the all primary symptoms, and at least 2 items of minor symptoms combined tongue and pulse diagnosis.

4.3 Assessment standard of symptoms in traditional Chinese medicine

Observation indicators	**Grades**	**Scores**
Pain during the menstrual cycle	No pain	**0**
	Mild: abdominal pain at fits and intervals, regular work	**3**
	Moderate: frequent abdominal pain, affect work partly	**6**
	Severe: abdominal pain duration, do not work a normal schedule	**9**
Duration time	No pain	**0**
	Mild: abdominal pain last one day	**2**
	Moderate: abdominal pain last two days	**4**
	Severe: abdominal pain last three days	**6**
	Every day added the score added 1	
scanty menstruation	No: period heavy and 5 sanitary towels every day	**0**
	Mild: heavy and 4 or 5 sanitary towels every day	**1**
	Moderate: 2 or 3 sanitary towels every day	**2**
	Severe: period light and less than 1	**3**

	sanitary towels every day	
menstrual colour	red	**0**
	purplish red	**1**
	dark red and some blood clots	**2**
	darkness and many blood clots	**3**
Cold limbs	No	**0**
	Hands and feet not warm	**1**
	Hands and feet cold	**2**
	Hands and feet icy cold	**3**
Fear of cold	No	0
	fear the wind slightly	1
	fear of cold but not add clothes	2
	fear of cold and add clothes	3
Cold sweat	No	0
	Yes	1
Anus pendent swells	No	0
	Yes	1
nausea and vomiting	No	0
	Yes	1

4.4 Clinical Efficacy Assessment standard of Primary Dysmenorrhea

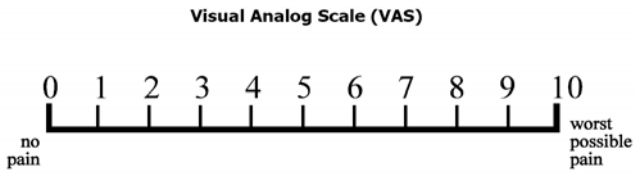
Symptoms	**Scores**
abdominal pain during the menstrual cycle or before and after menstruation	Yes 5; No 0
abdominal pain obvious	Yes 5.5; No 0
Abdominal pain is unbearable	Yes 6; No 0
Abdominal pain is unable to sit down or sleep at ease	Yes 1; No 0
Abdominal pain effects of work and study	Yes 1; No 0
Abdominal pain and need bed rest	Yes 1; No 0
Using the general measures and relief the pain	Yes 0.5; No 0
Using the general measures and afford no relief the pain	Yes 1; No 0
pale	Yes 0.5; No 0
Cold sweat	Yes 1; No 0
Peripheral coldness	Yes 1; No 0
schock	Yes 2; No 0
With the waist ache	Yes 0.5; No 0
With the nausea and vomiting	Yes 0.5; No 0
With anal bulge	Yes 0.5; No 0
Pain duration add one day the score add 0.5	Yes days × 0.5; No 0

Mild <10 scores; moderate 10~20 scores; severe ≥21scores

4.5 Assessment of pain intensity

The visual analog scale (VAS) is a tool widely used to measure pain. Operationally a

VAS is usually a horizontal line, 10 cm in length, anchored by word descriptors at each end, as illustrated in Fig. 1. The patient marks on the line at the point that could represent their perception of their current state. The VAS score is determined by measuring in millimeters from the left hand end of the line to the point that the patient marks. The zero representing one extreme (e.g. no pain) and the 10 representing the other extreme (e.g. " the worst pain possible").



4.6 Criteria for inclusion

(1) accord with the syndrome of cold coagulation and blood stasis (SCB);

(2) dysmenorrhea in the last three periods with an average score higher than 4 on Visual-Analog Scale (VAS) for pain;

(3) regular menstrual cycles for at least 3 months

(4) age 18-40 years

(5) no history of gynecologic disease

(6) informed consent and voluntary participation

4.7 Exclusion criteria

Exclusion criteria for all patients were endometriosis, leiomyomata (fibroids), adenomyosis, pelvic inflammatory disease, and intrauterine device (IUD); age above 40 years; not belonging to the syndrome of cold coagulation and blood stasis; with cardiovascular, liver, kidney, hyperlipidemia, hypertension, or mental diseases through history or evaluation at the screening visit. The level of ALT is higher of

twice as much normal level. The levels of BUN and Cr are higher 20% than normal level. The WBC is under 5% than normal level.

4.8 Rejection criteria

The patients enrolled are rejected if they are misdiagnose or misenrolled or accord with the exclusion criteria or never oral administration of drugs or no medical records or drug combination.

4.9 Shed off criteria

The patients enrolled are considered as shed off cases if they are patients themselves withdraw; failed to be followed up; required withdraw because of severe adverse events, no compliance, and cause other disease.

5. Study design

5.1 Drug administration

All patients were followed to monitor for four menstrual cycles and began receiving treatment with SFZYFG at the first day of the first period, twice daily (200 ml each time) for 5 days. In the second and third menstrual cycle, SFZYFG was administrated with the same dosage for 10 days (beginning 5 days before the menstruation).

5.2 Samples collection

Blood and urine samples were collected from each subject from 7 days before her expected menses and labeled as sample 1. Sample 2 (Pre-2) of blood and urine were collected on the first day of first menstrual cycle before administration of SFZYFG, respectively. Blood and urine collections on the first day of their second, third and

forth periods were labeled as sample 3, 4, and 5 (Post-3, 4, and 5), respectively. Blood for a metabolite profiling was drawn from an antecubital vein. The sodium citrate was adopted as anticoagulant. The twelve urine samples were obtained for metabolomics study after removing the all interfered by menstrual fluid.

5.2 Drug combination

During the protocol carrying out, the drugs including chemicals and Chinese medicines related to curing PD were banned. But at the special conditions of diagnosis other diseases, e.g. stomachache, ranitidine would be used. If the patients are painful with VAS>7, the Ibuprofen capsule will be allowed to oral (1 capsule/time, twice a day). This situation must be record in medical cases and announced the disease, drugs, dosages, and usage.

6. Evaluation Indicators

6.1 Diagnosis

Data of demography: years, height, weight, nation, career

General data: history of present illness, allergic history, past history, drug combination

Physical examination: temperature, heart rate, breathing, blood pressure

Gynaecology test (rectal exam**)**

B-ultrasonic

6.2 Evaluation efficacy

pain intensity; Pain scores; Pain scale scores; Total symptom severity score of Chinese medicine

6.3 Chemical indicators

The therapeutic efficacy was evaluated with the levels of 5-hydroxytryptamine (5-HT), oxytocin (OT), arg^8-Vasopressin (AVP), estradiol (E_2), progesterone (P), follicle-stimulating hormone (FSH), testosterone (T), glucocorticoid (GC), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ apha ($PGF_{2\alpha}$), leukotriene B_4 (LTB_4), nitric Oxide (NO), endothelin-1 (ET-1), and lipoprotein (a) [S-LP(a)] in blood.

6.4 Assessment of Safety

The examined indicators are routine blood test, routine urine test, ALT, BUN, Cr, and electrocardiogram.

Other adverse events and adverse reactions are recorded in medical cases.

7. Study Procedures

7.1 Subject Recruitment and Screening (Visit 1)

Also in this section, list any screening requirements such as laboratory or diagnostic testing necessary to meet any noted inclusion or exclusion criteria. The subjects interview for doctors 7 days before the menstruation will be recruited after the clinical trial had been fully explained to the patients, the written consent was obtained from all subjects. The blood and urine samples will be collected.

7.2 Visit 2

On the first day of the firs period the subjects followed up and administrated SFZYFG treatment after collecting the blood and urine samples. All patients were followed to monitor for four menstrual cycles and began receiving treatment with SFZYFG at the first day of the first period, twice daily (200 ml each time) for 5 days.

7.2 Visit 3

Blood and urine samples were collected on the first day of the second period when the subjects followed up. The clinical medical cases were recorded.

7.2 Visit 4

Blood and urine samples were collected on the third day of the second period when the subjects followed up. The clinical medical cases were recorded.

7.2 Visit 5

Blood and urine samples were collected on the fourth day of the second period when the subjects followed up. The clinical medical cases were recorded. The symptoms and scores were recorded at this time. Filled the forms and evaluated the clinical efficacy.

8. Reporting and recording of adverse events

An adverse event (AE) can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor. During 24 hours, 7 days, and 14 days, these indicators will be retested.

If the unexpected adverse reactions are observed wide spread, the trial would be aborted by the Drug Regulatory Agency or Ethics Committee.

9. Reporting of Serious Adverse Events and Unanticipated Problems

All serious adverse events, including therapy in hospital, prolong the time in

hospital, damaged, effect the live life, and threaten the life or resulted in congenital deformity, are required for reporting. And the participating investigators are treated urgently. Addition to this situation is required to report to ethical committee of the Jiangsu Provincial Hospital of Integrated Traditional and Western Medicine (+86 25 88929115) and Nanjing University of Chinese Medicine.

10. Ethical Considerations

Ethnics committee and informed consent were important organization and method for guarantee the subject's right. Before the clinical trail, clinical trail plan must be discussed and passed by Ethnics committee. During the progress of the clinical trail, any modification of the clinical trial plan must be performed under the certification of ethnic committee.

The regulations require that the following information must be conveyed to each subject: a statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental; a description of any reasonably foreseeable risks or discomforts to the subject; a description of any benefits to the subject or to others which may reasonably be expected from the research; a disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject; a statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained; for research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and,

if so, what they consist of, or where further information may be obtained; an explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and a statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. Informed consent should be signed by subjects and be supplied by inquiry.

11. Study Monitoring Plan

The anticipated plan and finished time

Start time: October, 2010.

End time: January, 2012.

Clinical trial data collection, analysis time: December, 2011.

12. Study Summary

Title	
Short Title	
Protocol Number	
Phase	
Methodology	
Study Duration	
Study Center(s)	
Objectives	
Number of Subjects	
Diagnosis and Main Inclusion Criteria	

Study Product, Dose, Route, Regimen	
Duration of administration	
Reference therapy	
Statistical Methodology	

13. References

http://www.sda.gov.cn/WS01/CL0053/24473.html

http://www.plosone.org/static/policies.action#reporting

http://www.nlm.nih.gov/bsd/uniform_requirements.html.

Draftsman: Peijuan Wang

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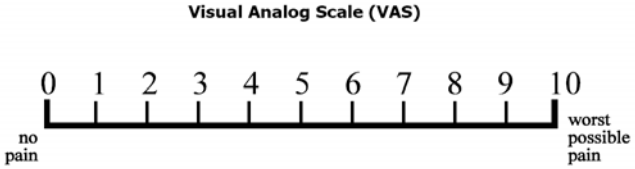
Text S2 Assessment of pain intensity

The visual analog scale (VAS) is a tool widely used to measure pain. Operationally a VAS is usually a horizontal line, 10 cm in length, anchored by word descriptors at each end, as illustrated in Fig. 1. The patient marks on the line at the point that could represent their perception of their current state. The VAS score is determined by measuring in millimeters from the left hand end of the line to the point that the patient marks. The zero representing one extreme (e.g. no pain) and the 10 representing the other extreme (e.g. “ the worst pain possible”).



Text S3

Clinical diagnosis criteria of primary dysmenorrhea by Western medicine

Dysmenorrhea, also known as painful menstruation, can be an incapacitating and disruptive condition. Clinically, primary dysmenorrhea (PD) is characterized by painful uterine cramping during the menstrual cycle or before and after menstruation, with no associated pelvic disease. The clinical features are listed as following:

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4. Some patients are pale, cold sweat, cold limbs and faint.

Gynecological examination (Rectal examination for unmarried women) :

without any organic pathology.

B-ultrasound examination:
without any organic pathology.

Text S4

Clinical diagnosis criteria of Primary dysmenorrhea with syndrome of cold coagulation and blood stasis by traditional Chinese medicine

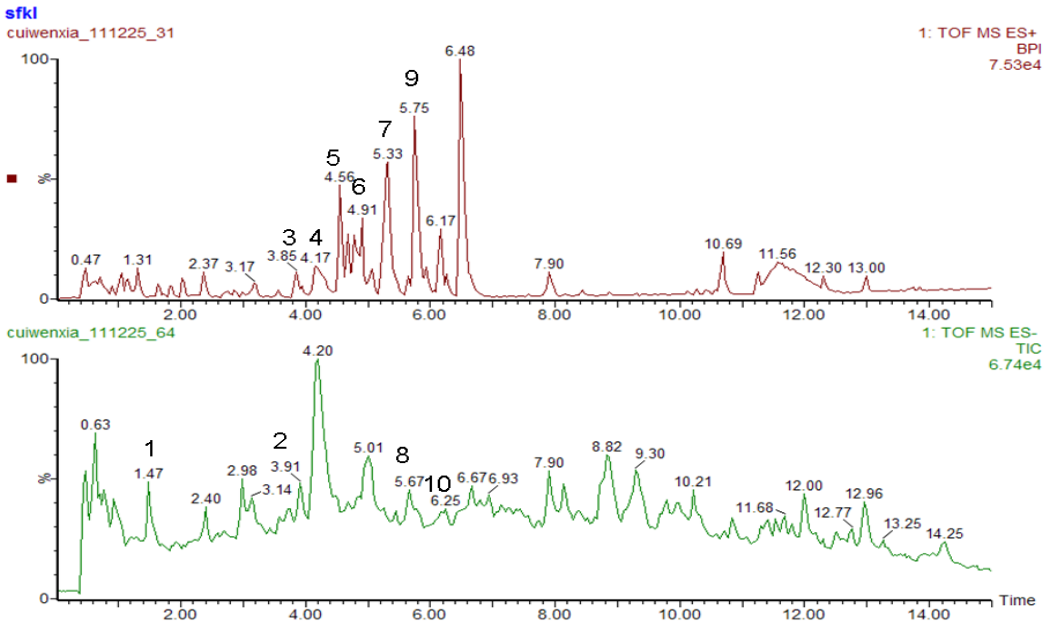
Primary symptoms: Cold and pain of abdomen, Pain reduction with the heat, or with distending pain to press.

Minor symptoms: scanty menstruation or bleeding obstacle, menstruation with red dark and blood clot, fear of cold, hands and feet are cold, dripping with cold sweat, rectal dropped, nausea and vomiting, red dark tongue and white coat, deep pulse.

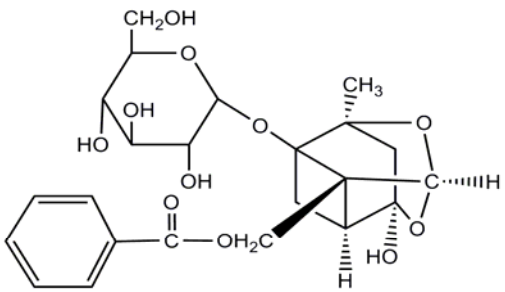
The PD patients must meet the all primary symptoms, and at least 2 items of minor symptoms combined tongue and pulse diagnosis.

Text S5 The quality and quantity analysis of chemical components in SFZYFG.

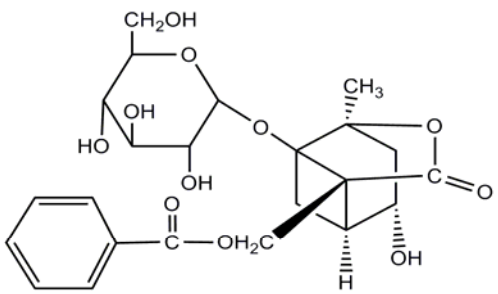
Shaofu Zhuyu Formula concentrate-granule (SFZYFG), a Chinese herbal formula, was produced by Jiangsu Tianjiang Pharmaceutical Co., Ltd., and approved by State Food and Drug Administration of China. The quality of SFZYG is conformed to "Provisional Regulations for Herbal Concentrate-Granule" (National Drug NOTE [2001] No. 325). The quality and quantity of chemical components in SFZYFG were analyzed by UPLC-MS. The results stated that the contents of paeoniflorin **(4)**, albiflorin **(3)**, ferulic acid **(10)**, caffeic acid **(8)**, vanillic acid **(2)**, gallic acid **(9)**, senkyunolide I **(1)**, typhaneosid **(5)**, isohamnetin-3-*O*-neohesperidoside **(6)**, and isohamnetin-3-*O*-rutinosid **(7)** were 12.4%, 1.26%, 2.5%, 1.2%, 1.2%, 1.6%, 1.8%, 1.1%, 0.6%, 0.4%, respectively. The chemical structures of these compounds were listed in Figure.



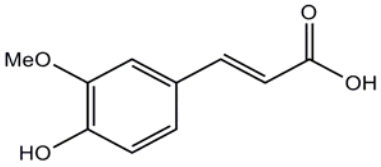
UPLC-MS BPI chromatography of SFZYFG



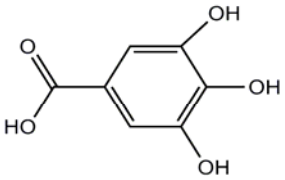
Paeoniflorin



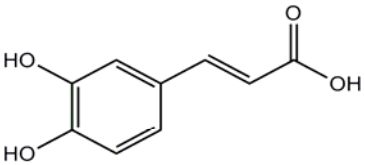
Albiflorin



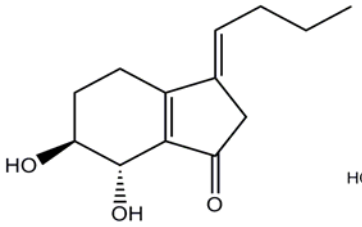
Vanillic acid



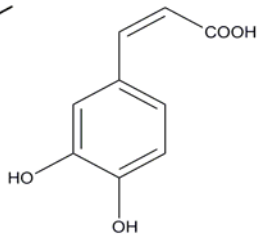
Gallic acid



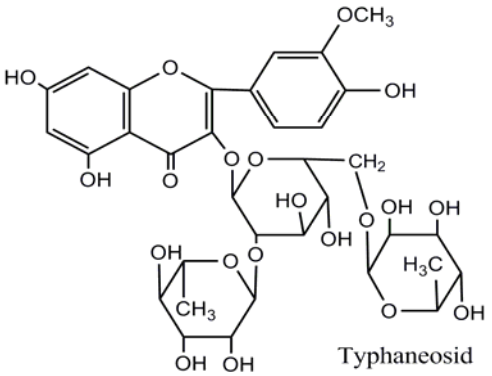
Ferulic acid



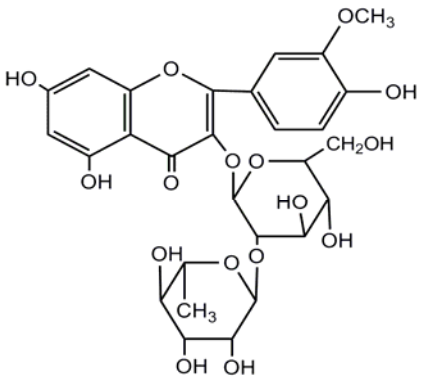
Senkyunolide I



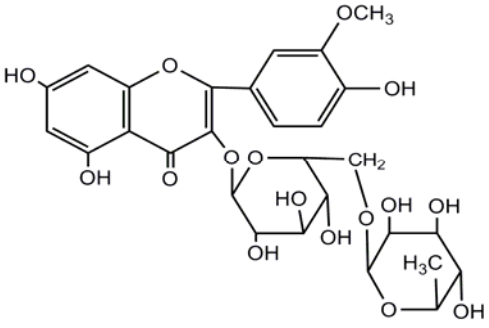
Caffeic acid



Typhaneosid



Isohamnetin-3-*O*-neohesperidoside



Isohamnetin-3-*O*-rutinosid

The chemical structures of 10 compounds in SFZYFG

Text S6 The test methods of clinical biochemical indicators

Total fourteen clinical biochemical indicators were determined in serum of PD patients. The test methods of these indicators were according with the assay procedure of every kit. Bring all reagents to room temperature for at least 30 min prior to opening. All standards and samples should be run in duplicate.

Human Glucocorticoid Elisa kit (BG-HUM11096) was manufactured and distributed by Nova TeinBio, Inc. (Cambridge, MA). Arg^8-Vasopressin EIA Kit (Catalog No. ADI-900-017, 96-well kit); Endothelin-1 EIA Kit (Catalog # ADI-900-020A, 96 well Enzyme Immunoassay kit for use with serum, plasma, and culture fluids); LTB_4 EIA Kit (Catalog No. ADI-900-068, 96=well kit); Serotomin EIA Kit (Catalog # ADI-900-175, 96-well Enzyme Immunoassay kit for use with serum, platelets, plasma, and urine); $PGF_{2\alpha}$ EIA Kit (Catalog No. ADI-900-069, 96 well kit); Nitric Oxide (total), detection kit (Catalog No. ADI-917-020, 192 determinations); PGE_2 EIA Kit (Catalog No. ADI-900-001, 96-well kit); Oxytocin EIA Kit (Catalog No. ADI-900-153, 96-well kit).

Supplementary Text S7

The mobile phase was composed of A (acetonitrile), and B (0.1% formic acid, v / v). For plasma analysis, the mobile phase with a linear gradient elution was employed: 0 ~ 0.5 min, A: 5%; 0.5 ~ 24 min, A: 5% ~ 100%; 24 ~ 28 min, A: 100%; 28 ~ 30 min, A: 100% ~ 5%. For urine analysis, the mobile phase with a linear gradient elution was employed: 0 ~ 2.5 min, A: 2%; 2.5 ~ 10 min, A: 10% ~ 20%; 10 ~ 11.5 min, A: 20 ~ 40%; 11.5 ~ 16 min, A: 40% ~ 80%; 16 ~ 18 min, A: 80% ~ 2%. The flow rate of the mobile phase was 0.4 mL / min, and the column temperature was maintained at 35 °C.

The instrument was operated by using an electrospray source in positive mode. The ionization source conditions were as follows: capillary voltage of 3.0 kV, source temperature of 120 °C and desolvation temperature of 350 °C. The sampling cone voltage was set at 30 V, extraction cone was 2.0 V (for plasma sample) or 0.7V (for urine sample), trap collision energy was 6.0 V, transfer collision energy 4.0 V, trap gas flow was 1.50 mL / min, ion energy was at 1.0 V. Nitrogen and argon were used as cone and collision gases, respectively. The cone and desolvation gas flow were 50 and 600 L / h, respectively. The scan time of 0.5 s (for plasma sample) or 0.2 s (for urine sample) and with interval scan time of 0.02 s was used throughout and with collision energy of 6 eV. The mass spectrometric data was collected from *m/z* 100 to 1000 in centroid mode. Leucine-enkephalin was used as the lock mass generating an $[M+H]^+$ ion (*m / z* 556.2771) and $[M-H]^-$ ion (*m / z* 554.2615) at a concentration of 200 pg / mL and flow rate of 100 μL / min. Dynamic range enhancement was applied throughout the MS experiment to ensure accurate mass measurement over a wider

dynamic range.

Chromatographic condition of QqQ/MS analysis is the same as UPLC-Q-TOF/MS performed. Mass spectrometry detection was performed on a Xevo Triple Quadrupole MS (Waters Corp., Milford, MA) equipped with an electrospray ionization source (ESI). ESI-MS spectra were acquired in positive ion multiple reaction monitoring (MRM) mode. The conditions of MS analysis were designed as follows: capillary voltage at 2 kV; the desolvation gas flow rate was set to 1,000 L/h at a temperature of 550 ºC; the cone gas flow rate was set at 50 L/h and the source temperature at 150 ºC. The cone voltage (CV) and collision energy (CE) were set depending upon each specific MRM for each biomarker.

Figure S1 Flow diagram showing the progress of subjects at each stage of the clinical trial

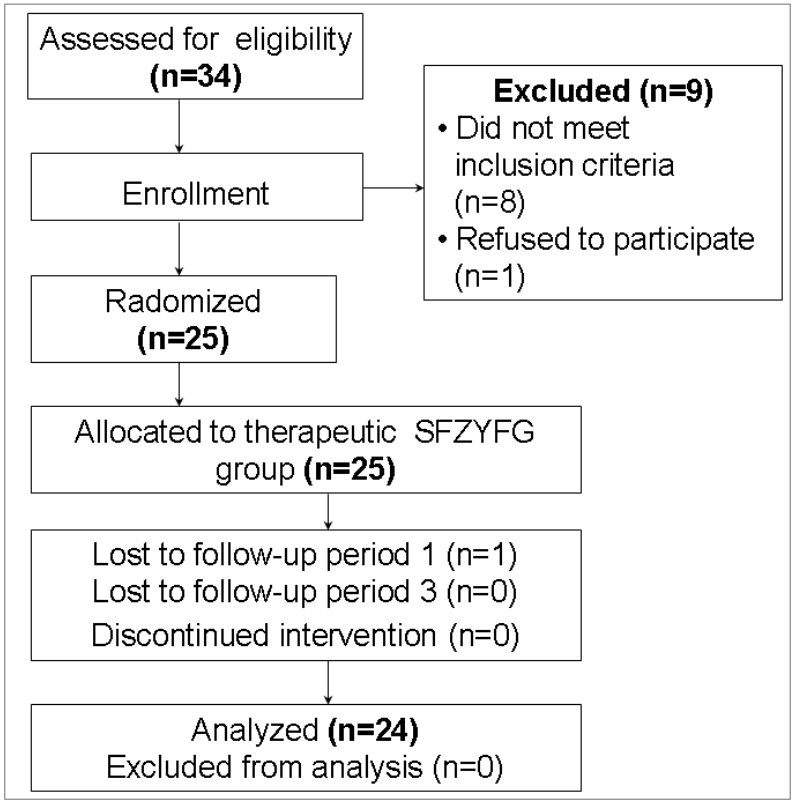
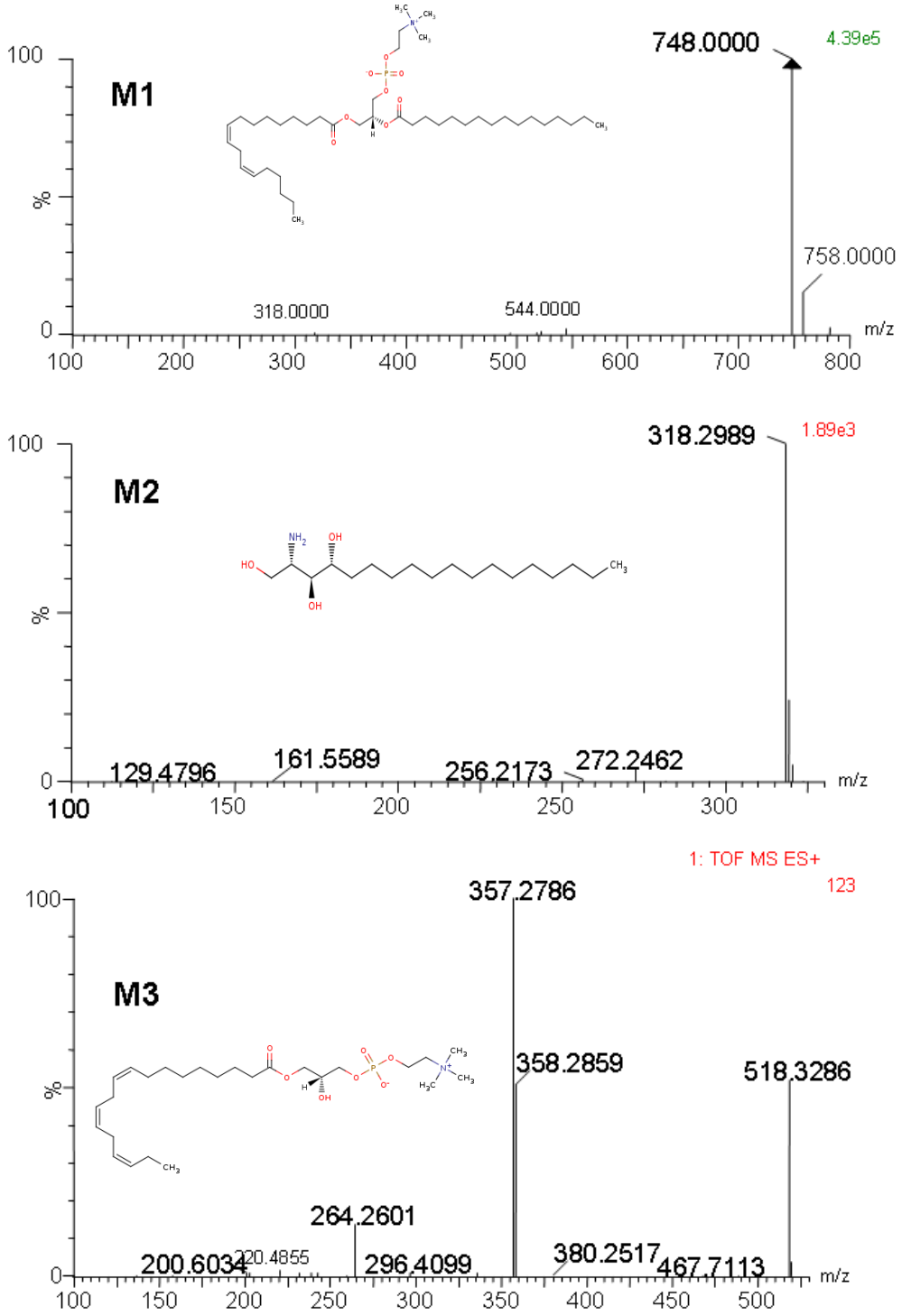
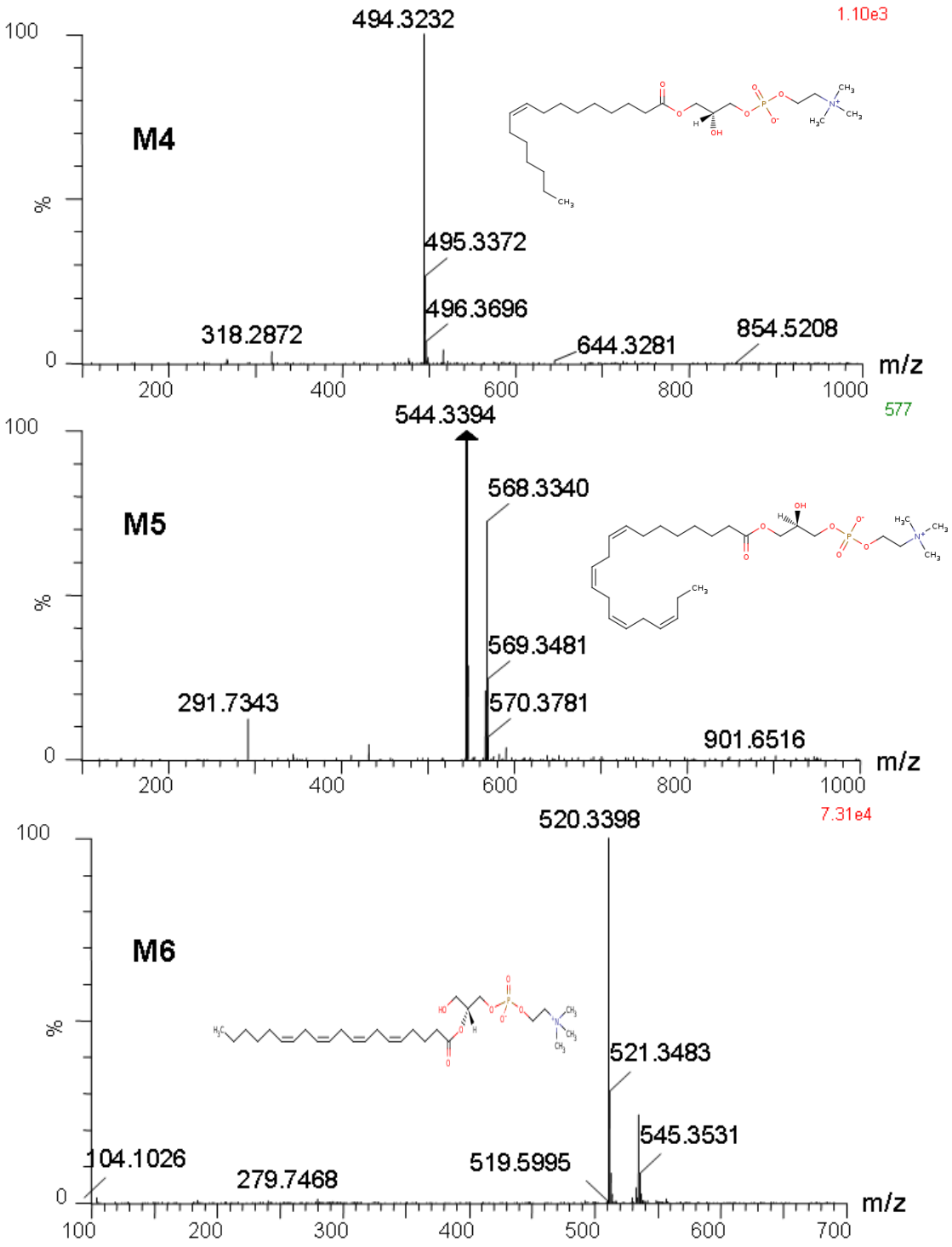
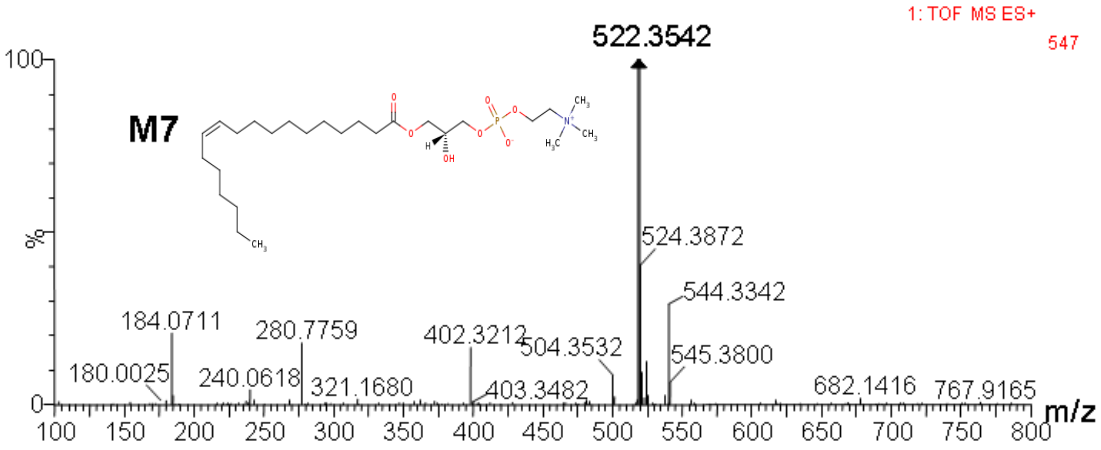
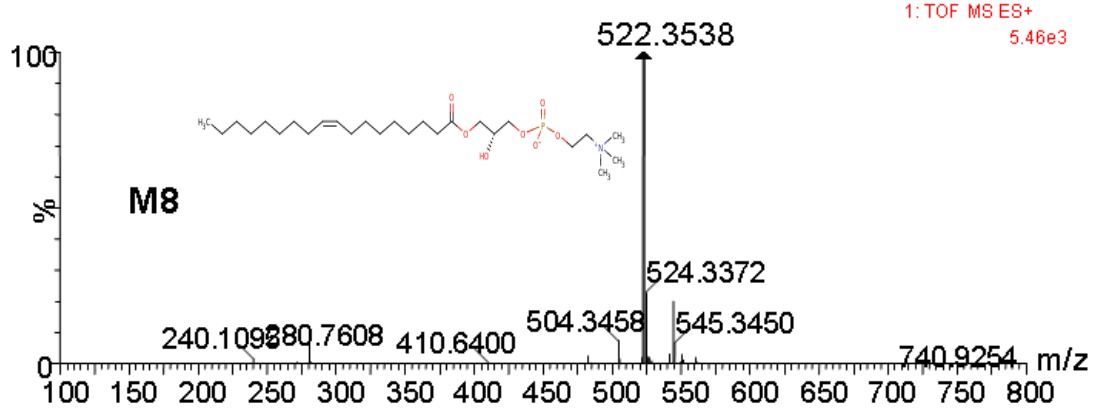


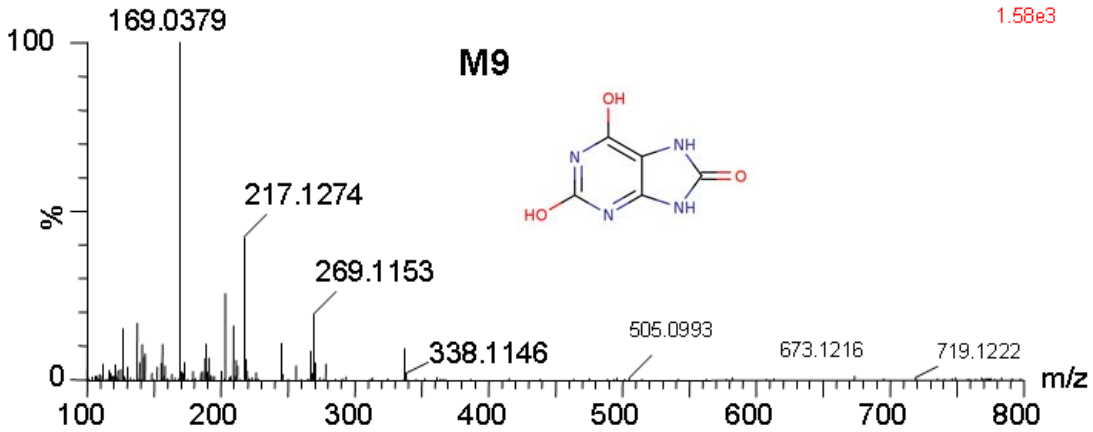
Figure S2 MS spectrum of 24 SFZYFG-regulated promising biomarker candidates

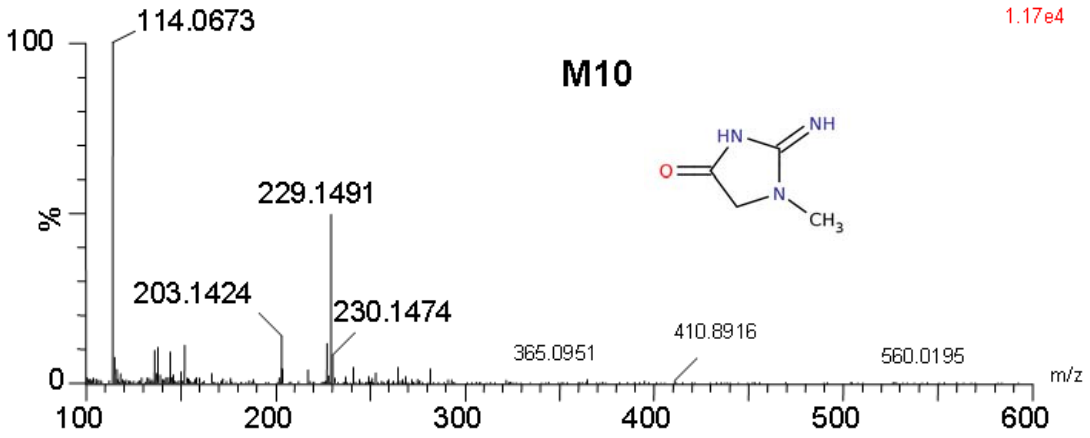


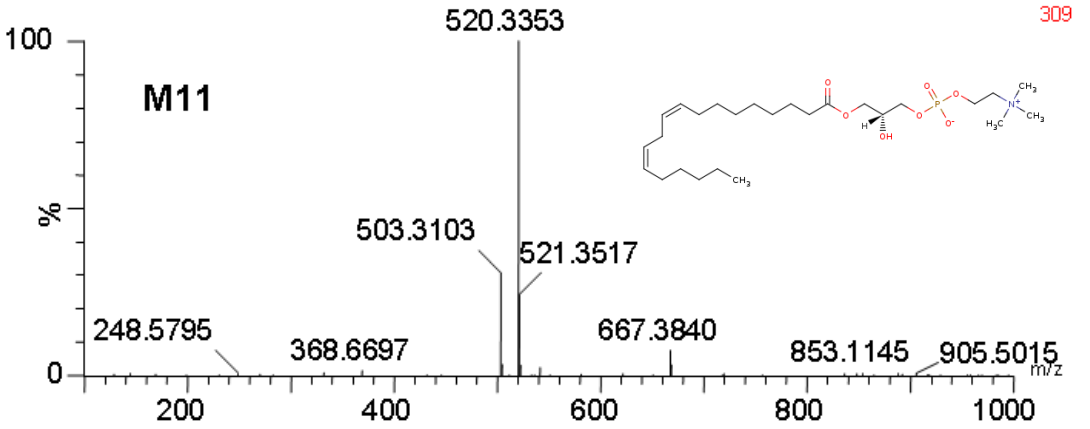

M4
494.3232
1.10e3
495.3372
496.3696
318.2872
644.3281
854.5208
100
%
0
200
400
600
800
1000
m/z
577
M5
544.3394
568.3340
569.3481
570.3781
291.7343
901.6516
7.31e4
M6
520.3398
521.3483
545.3531
104.1026
279.7468
519.5995
100
200
300
400
500
600
700

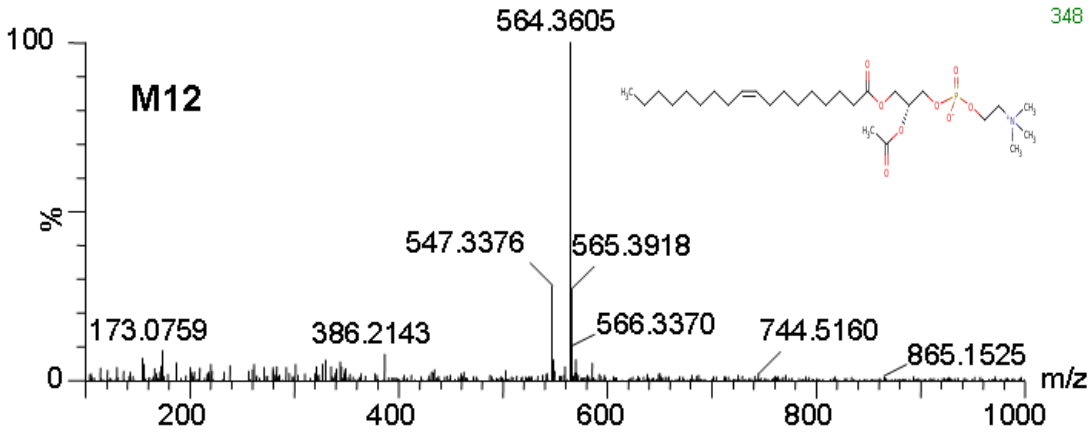

1: TOF MS ES+
547
522.3542
M7
524.3872
544.3342
184.0711
280.7759
402.3212
504.3532
545.3800
180.0025
240.0618
321.1680
403.3482
682.1416
767.9165
%
m/z

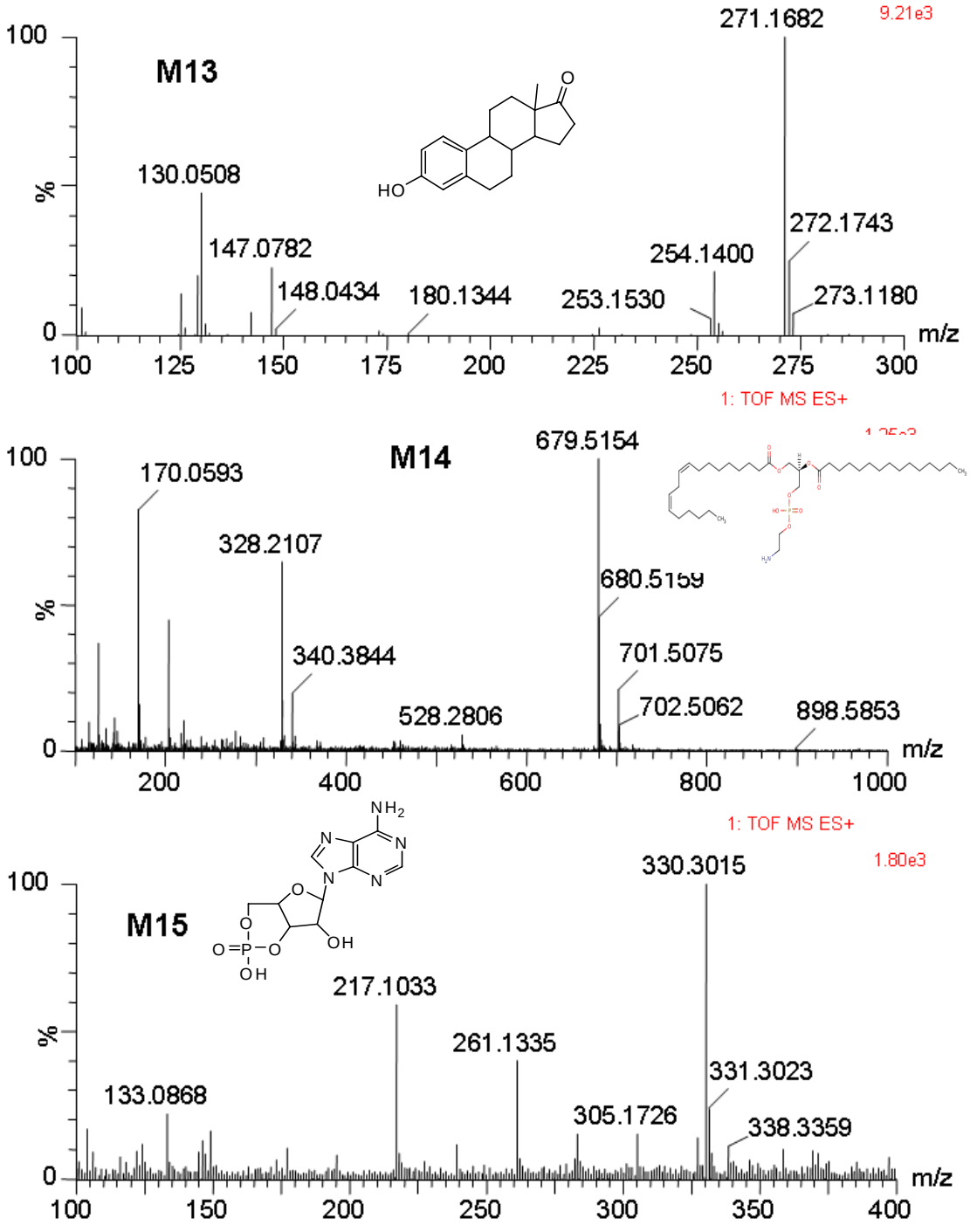

1: TOF MS ES+
5.46e3
522.3538
M8
524.3372
240.1098
280.7608
410.6400
504.3458
545.3450
740.9254
%
m/z

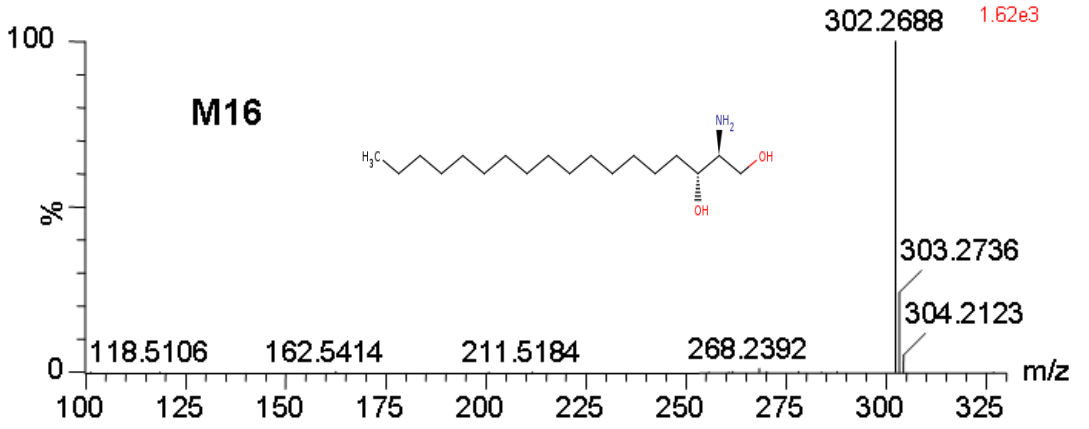

1.58e3
169.0379
M9
217.1274
269.1153
338.1146
505.0993
673.1216
719.1222
%
m/z

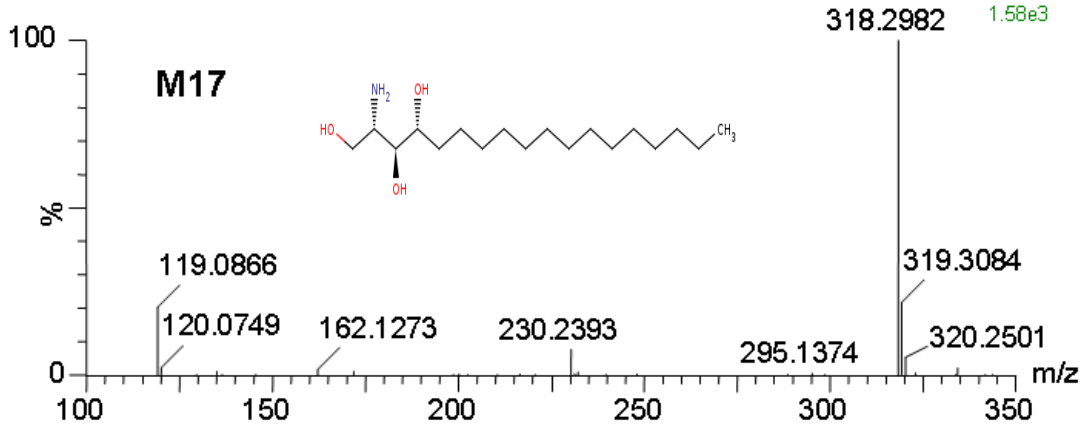

M10
1.17e4
114.0673
229.1491
203.1424
230.1474
365.0951
410.8916
560.0195
100
%
0
m/z
100
200
300
400
500
600
HN
NH
O
N
CH3

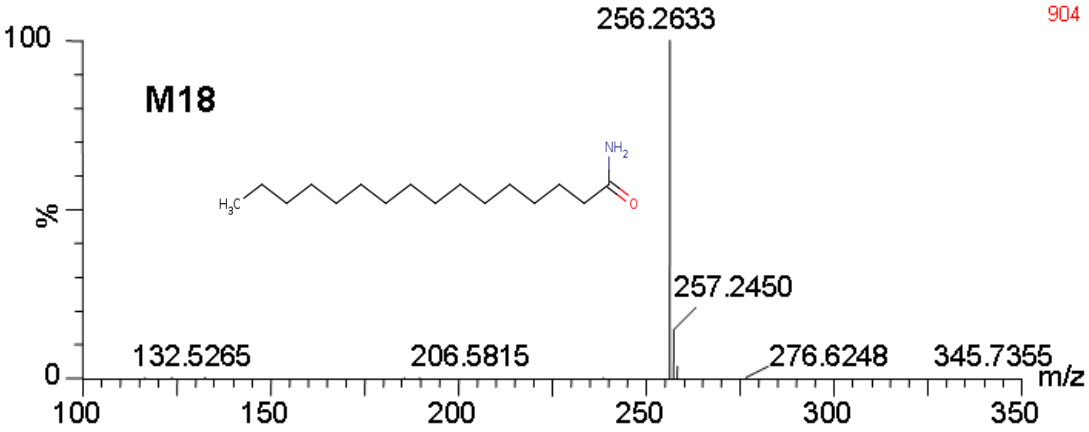

M11
309
520.3353
503.3103
521.3517
248.5795
368.6697
667.3840
853.1145
905.5015
100
%
0
m/z
200
400
600
800
1000

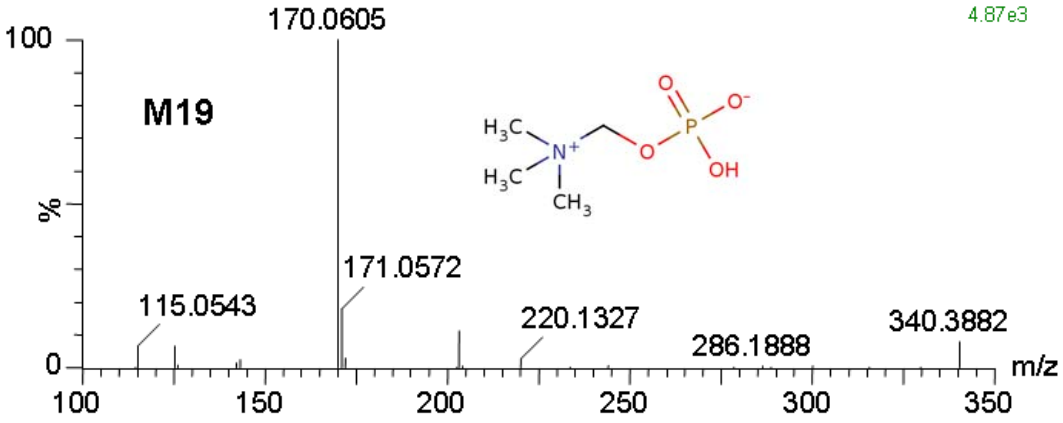

M12
348
564.3605
547.3376
565.3918
173.0759
386.2143
566.3370
744.5160
865.1525
100
%
0
m/z
200
400
600
800
1000

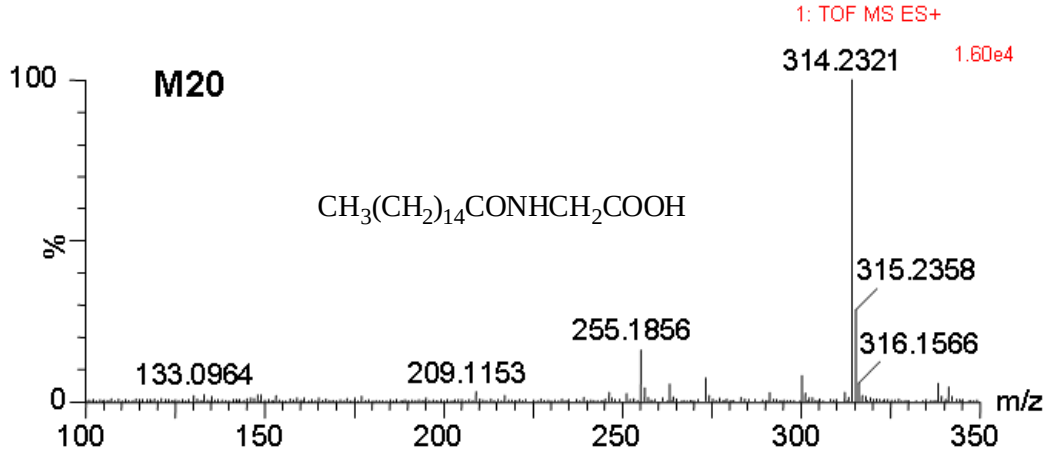

M13
271.1682
9.21e3
130.0508
147.0782
148.0434
180.1344
253.1530
254.1400
272.1743
273.1180
HO
O
100
%
0
100
125
150
175
200
225
250
275
300
m/z
1: TOF MS ES+
M14
679.5154
170.0593
328.2107
680.5159
340.3844
701.5075
528.2806
702.5062
898.5853
100
%
0
200
400
600
800
1000
m/z
1: TOF MS ES+
1.80e3
M15
NH2
N
N
N
N
O
O
O=P−O
OH
OH
330.3015
217.1033
261.1335
331.3023
133.0868
305.1726
338.3359
100
%
0
100
150
200
250
300
350
400
m/z

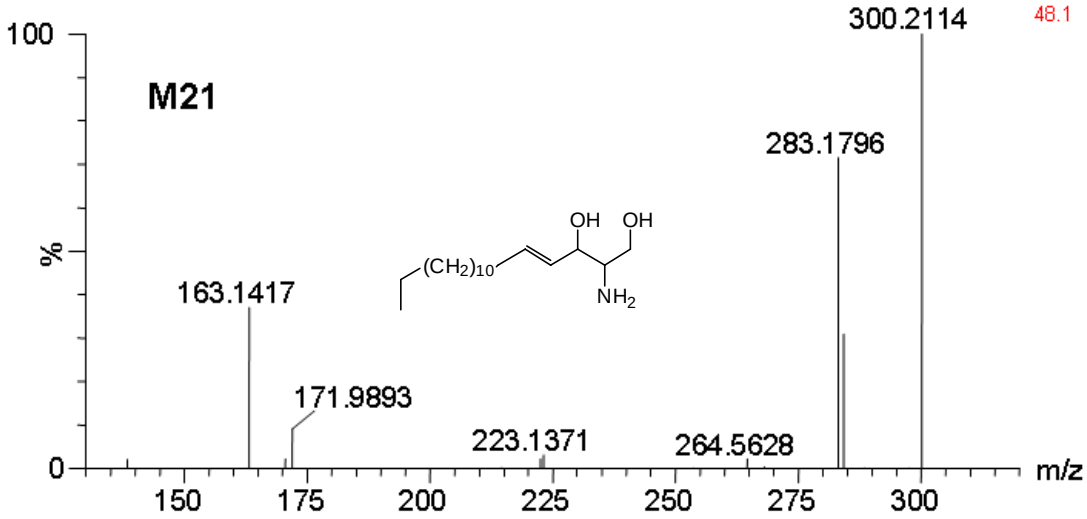

M16
302.2688
1.62e3
303.2736
304.2123
118.5106
162.5414
211.5184
268.2392
m/z
100
%
0
100
125
150
175
200
225
250
275
300
325

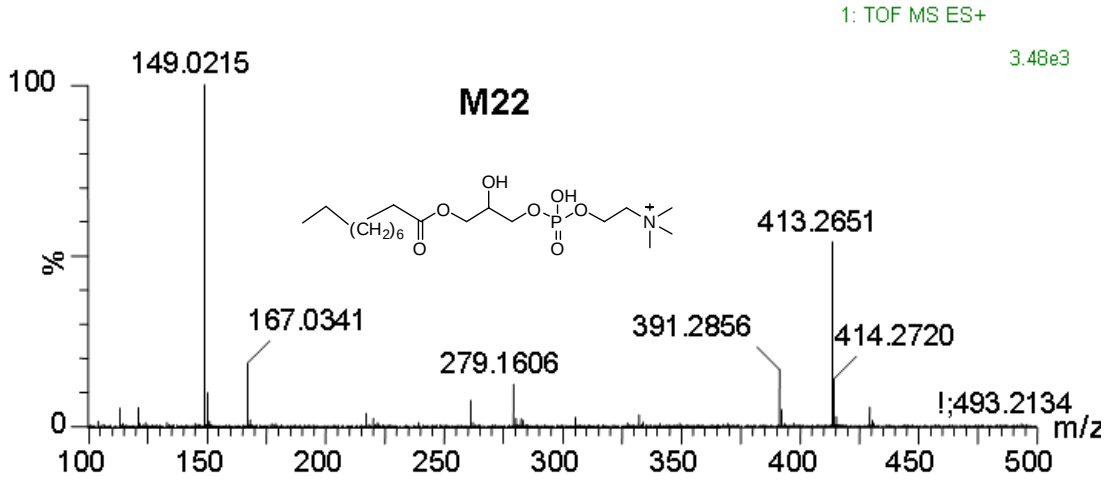

M17
318.2982
1.58e3
319.3084
320.2501
119.0866
120.0749
162.1273
230.2393
295.1374
m/z
100
%
0
100
150
200
250
300
350

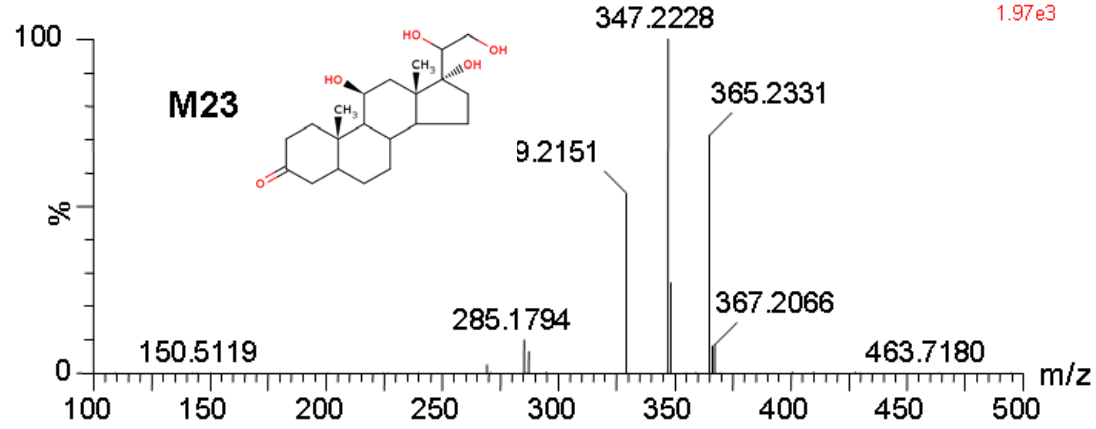

M18
256.2633
904
257.2450
132.5265
206.5815
276.6248
345.7355
m/z
100
%
0
100
150
200
250
300
350


M19
170.0605
4.87e3
171.0572
115.0543
220.1327
286.1888
340.3882
100
%
0
100
150
200
250
300
350
m/z


1: TOF MS ES+
M20
314.2321
1.60e4
CH3(CH2)14CONHCH2COOH
315.2358
316.1566
255.1856
133.0964
209.1153
100
%
0
100
150
200
250
300
350
m/z


M21
300.2114
48.1
283.1796
163.1417
171.9893
223.1371
264.5628
100
%
0
150
175
200
225
250
275
300
m/z


1: TOF MS ES+
3.48e3
M22
149.0215
167.0341
279.1606
391.2856
413.2651
414.2720
!;493.2134
100
%
0
m/z
100
150
200
250
300
350
400
450
500


1.97e3
M23
347.2228
365.2331
9.2151
285.1794
367.2066
150.5119
463.7180
100
%
0
m/z
100
150
200
250
300
350
400
450
500

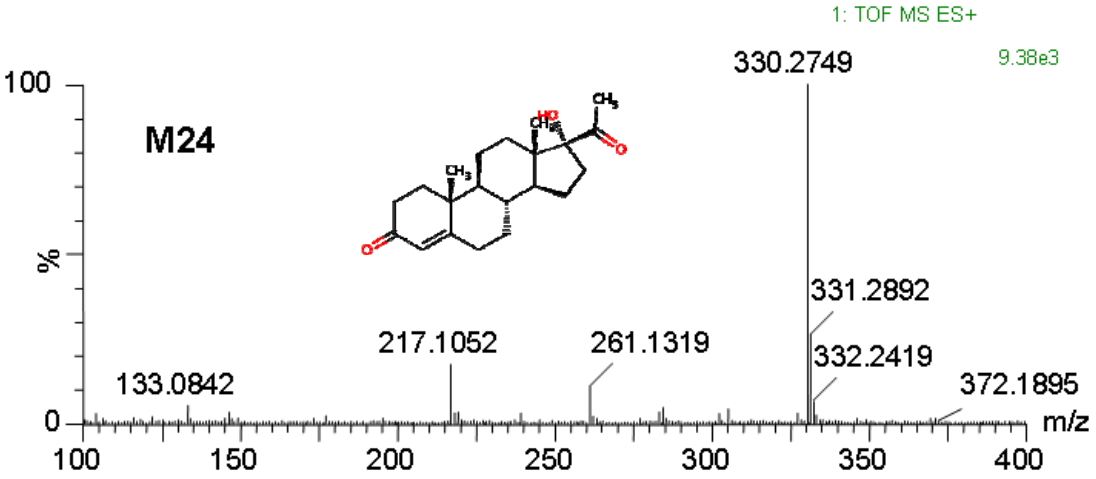

1: TOF MS ES+
9.38e3
M24
330.2749
331.2892
217.1052
261.1319
332.2419
133.0842
372.1895
100
%
0
m/z
100
150
200
250
300
350
400

Table S1 Result from Pathway Analysis with MetPA[a] from plasma

Pathway Name	Total Cmpd	Expected	Hits	Raw p	Holm p	FDR	Impact
Sphingolipid metabolism	25	0.18695	3	7.2922E-4	0.048338	0.048338	0.14974
Glycerophospholipid metabolism	39	0.29165	3	0.0027133	0.21435	0.10853	0.12549

[a] Total is the total number of compounds in the pathway; the hits is the actually matched number from the user uploaded data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis.

Table S2 Result from Pathway Analysis with MetPA [a] from urine

Pathway Name	Total Cmpd	Expected	Hits	Raw p	Holm p	FDR	Impact
Glycerophospholipid metabolism	39	0.25924	4	9.3233E-5	0.0074587	0.0074587	0.18712
Sphingolipid metabolism	25	0.16618	3	5.0737E-4	0.040082	0.020295	0.23081
Steroid hormone biosynthesis	99	0.65808	3	0.025594	1.0	0.6825	0.12791

[a] Total is the total number of compounds in the pathway; the hits is the actually matched number from the user uploaded data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis.