Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Evaluation and optimization of biomarkers in primary dysmenorrhea model using ultra performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry combined with support vector machine

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2.Materials and methods

2.1 Animal experiment

On the last day, the blood was drawn from abdominal aorta after anesthetization. Then the blood was separated to made serum and plasma. The newly obtained plasma specimen was immediately centrifuged at 3000 g in 4 ℃ for 15 min. Then stored at -80 ℃ for the subsequent analysis. And the serum specimen was performed for biochemical assay.

3.Results

3.1Biochemical analysis

Figure S1.The standard curve of PGF2α

Independent sample student's *t*-test is used to calculate the difference between the control group and the PD model group according to SPSS software.

Table S1.The level of PGF2α in PD model group and control group.

	PGF2α(pg/mL)					
PD model group	29.23	30.20	24.11	29.14	29.50	30.56
Control group	32.61	36.50	29.77	31.30	31.04	35.60

3.2Metabolic profiling and data processing

Typical base peak intensity (BPI) chromatograms of specimen in positive model

obtained from the control group and the PD model group are displayed in Fig.S2,3

Figure S2. Typical BPI chromatogram of plasma PD model group induced by oestrogen coupled with oxytocin at positive electrospray ionization (ESI) mode.

Figure S3. Typical BPI chromatogram of plasma control group at positive electrospray ionization (ESI) mode.

The relative standard deviations(RSD) of the peak areas and retention times of 20 randomly selected chromatographic peaks were less than 15%, which ensure the method confirm to metabolomics requirements.

Table S2 The results of experimental methodology.

Experiment name	**RSD (Peak area)**
Precision instrument	<14.89%
Method repeatability	<14.31%
Sample stability	<14.90%

TableS3.The value of peak area of biomarkers in PD model group.

	Model1	Model2	Model3	Model4	Model5	Model6
LPC(18:0)	93911.75	217904.26	190875.36	201741.07	161614.2	164922.51

LPC(17:0)	904.39	1019.31	1897.3	1854.18	1450.49	1180.68
sphingosine	41.85	56.12	55.95	27.39	37.45	49.19
LPC(15:0)	1523.23	1877.37	3476.6	2398.99	2370.18	1362.75
progesterone	172.37	189.66	262.32	201.63	76.54	75.21
D-Arginine	288.15	493.18	260.32	225.86	232.87	283.41
L-Tyrosine	194.22	372.98	335.86	261.2	449.89	290.9
LPC(18:2)	5373.29	3239.29	2936.91	2894.31	3343	6349.47

TableS4.The value of peak area of biomarkers in control group.

	Control1	Control2	Control3	Control4	Control5	Control6
LPC(18:0)	231121.75	218338.03	187978.71	362404.59	224935.76	238936.35
LPC(17:0)	2385.79	2309.78	4315.89	2592.29	3245.23	3198.94
sphingosine	96.38	110.52	134.79	189.41	151.1	99.14
LPC(15:0)	4793.18	3159.7	3259.82	5301.01	5153.26	2186.89
progesterone	336.63	397.18	326.19	258.78	462.7	366.46
D-Arginine	1051.92	532.69	741.09	1669.99	407.13	1250.33
L-Tyrosine	554.26	369.62	525.22	651.67	578.08	636.53
LPC(18:2)	1618.92	1308.42	1428.92	1386.54	1766.85	1457.62

3.4Optimization of the biomarkers

Figure S4. 3D view of SVM model of the total eight identified biomarkers (The parameters are described below: Bestc=12.1257, g=0.82469, CV accuracy =100%).