Supplementary Materials

The Therapeutic Effect and Mechanism Study of *Rhodiola wallichianavar. Cholaensis* Injection to Acute Blood Stasis Using Metabolomics Based on UPLC-Q/TOF-MS

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Table S1: Validation of systematic stability and repeatability of UPLC-MS/MS (plasma samples).

Modes	NO	RT/ min	m/z	m/z in Spectrum		RT of Chromatogram	
				Repeatability (RSD%)	Precision (RSD%)	Repeatability (RSD%)	Precision (RSD%)
ESI+	P-1	25.01	284.2935	0.0025	0.0031	0.0010	0.0013
	P-2	27.44	338.3405	0.0026	0.0054	0.0016	0.0021
	P-3	27.14	413.2658	0.0029	0.0041	0.0014	0.0037
	P-4	17.99	496.3424	0.0024	0.0047	0.0000	0.0010
	P-5	20.62	524.3738	0.0019	0.0052	0.0003	0.0007
	P-6	27.12	614.3820	0.0072	0.0016	1.5469	0.3746
	P-7	27.62	786.6028	0.0095	0.0009	0.0012	0.5438
	P-8	27.14	830.5452	0.0013	0.0028	1.3148	0.4056
	P-9	17.99	991.6757	0.0019	0.0007	0.3564	0.4783
	P-10	20.60	1047.7395	0.0034	0.0079	0.5601	0.0013
ESI-	P-1	0.61	215.0349	0.0054	0.0033	0.0021	0.0020
	P-2	24.83	355.2599	0.0051	0.0032	0.0019	0.0052
	P-3	18.03	480.3106	0.0054	0.0025	0.0020	0.0021
	P-4	18.02	540.3315	0.0042	0.0040	0.0019	0.6609
	P-5	20.69	640.2898	0.0039	0.0021	0.9837	0.1702
	P-6	0.53	792.8534	0.0056	0.0031	0.4623	0.0034
	P-7	0.53	860.8389	0.0050	0.0045	0.6138	0.0089

	P-8	0.53	928.8245	0.0059	0.0023	1.2341	0.1918
	P-9	17.02	1083.6509	0.0059	0.0035	0.1985	0.0495
	P-10	17.04	1131.6497	0.0047	0.0030	0.8927	0.2078

m/z, mass to charge ratio, Th; RT, retention time, min, PA, peak area. Precision is intermediate precision, RSD, Relative standard deviation.

Table S2: Validation of systematic stability and repeatability of UPLC-MS/MS (urine samples).

Modes	NO	RT/ min	m/z	m/z in Spectrum		RT of Chromatogram	
				Repeatability (RSD%)	Precision (RSD%)	Repeatability (RSD%)	Precision (RSD%)
ESI+	U-1	22.79	282.2778	0.0008	0.0034	1.3827	2.3819
	U-2	27.43	338.3406	0.0013	0.0053	3.2382	0.1414
	U-3	27.15	413.2659	0.0038	0.0055	2.4864	1.4159
	U-4	26.14	540.5339	0.0024	0.0013	0.9178	2.5259
	U-5	28.05	663.4549	0.0000	0.0073	1.2749	0.0084
	U-6	25.83	758.5719	0.0019	0.0062	2.0198	0.9476
	U-7	25.80	834.6038	0.0003	0.0005	0.8134	1.5849
	U-8	27.78	992.7438	0.0046	0.0025	0.1487	2.0185
	U-9	27.77	1075.7151	0.0027	0.0164	0.9847	0.8915
	U-10	27.23	1194.8242	0.0015	0.0528	1.0941	1.3580
ESI-	U-1	6.43	201.0249	0.0024	0.0004	0.0245	0.2429
	U-2	6.31	297.1016	0.0048	0.0028	0.2594	0.1531
	U-3	8.10	357.1038	0.0014	0.0025	0.9147	0.2563
	U-4	5.00	461.1867	0.1141	0.0059	1.2314	1.0313
	U-5	4.32	567.1711	0.0057	0.0024	0.8357	0.9719
	U-6	8.81	659.3276	0.0012	0.0052	0.1479	0.1972
	U-7	6.26	715.2203	0.0013	0.0002	0.1928	0.9816
	U-8	24.79	802.5512	0.0008	0.0015	1.4862	0.8215
	U-9	28.99	885.5432	0.0014	0.0154	0.5839	0.0015
	U-10	12.27	987.5047	0.0094	0.0015	1.4004	0.0948

m/z, mass to charge ratio, Th; RT, retention time, min, PA, peak area. Precision is intermediate precision, RSD, Relative standard deviation.

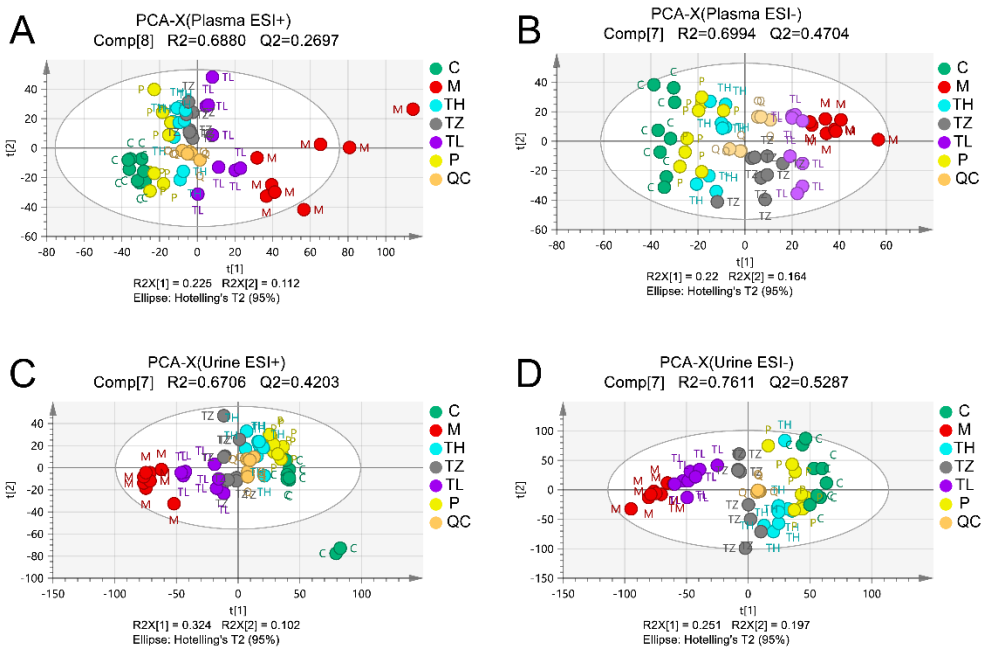


Figure S1: PCA analysis results based on metabolomic data using UPLC -Q/TOF-MS. All plasma samples in ESI$^+$ modes (A) and in ESI$^-$ modes (B). All urine samples in ESI$^+$ modes (C) and in ESI$^-$ modes (D).

Table S3: Quality parameters (R2X, R2Y, Q2) from cross-validation of OPLS-DA model.

OPLS-DA Models	R2X	R2Y	Q2	sample
C vs. T in ESI$^+$ mode	0.541	0.997	0.824	plasma
M vs. C in ESI$^+$ mode	0.601	0.995	0.859	
M vs. T in ESI$^+$ mode	0.587	0.995	0.810	
C vs. T in ESI$^-$ mode	0.647	0.993	0.900	
M vs. C in ESI$^-$ mode	0.731	0.996	0.931	
M vs. T in ESI- mode	0.401	0.684	0.392	
C vs. T in ESI$^+$ mode	0.472	0.986	0.916	urine
M vs. C in ESI$^+$ mode	0.774	0.998	0.929	
M vs. T in ESI$^+$ mode	0.554	0.995	0.733	
C vs. T in ESI$^-$ mode	0.669	0.989	0.957	
M vs. C in ESI$^-$ mode	0.781	0.992	0.965	
M vs. T in ESI$^-$ mode	0.699	0.988	0.678	

Table S4: The pairwise-comparison *P*-values of the post-hoc analysis for multiple comparisons in rats.

No.	C vs. M	M vs. TH	M vs. TM	M vs. TL	C vs. TH	M vs. P
P1	0.0005	0.0058	0.0039	0.0149	0.8195	0.0099
P2	0.0089	0.0039	0.0328	0.0001	0.2874	0.0040
P3	0.0046	0.0071	0.0219	0.0148	0.2924	0.0096
P4	0.0091	0.0033	0.0203	0.0417	0.4628	0.0085
P5	0.0032	0.0015	0.0029	0.0009	0.5278	0.0035

P6	0.0003	0.0009	0.0213	0.0028	0.7821	0.0035
P7	0.0061	0.0037	0.0487	0.0009	0.0819	0.0063
P8	0.0007	0.0081	0.0399	0.0149	0.2869	0.0026
P9	0.0007	0.0040	0.0424	0.0099	0.0991	0.0002
P10	0.0087	0.0014	0.0019	0.0209	0.1490	0.0006
P11	0.0009	0.0018	0.0371	0.0091	0.3003	0.0060
P12	0.0027	0.0004	0.0389	0.0049	0.0802	0.0015
P13	0.0003	0.0019	0.0003	0.0248	0.0932	0.0062
P14	0.0067	0.0014	0.0359	0.0249	0.2949	0.0069
U1	0.0048	0.0004	0.0289	0.0147	0.1097	0.0016
U2	0.0008	0.0014	0.0179	0.0049	0.4901	0.0044
U3	0.0068	0.0068	0.0003	0.0032	0.5987	0.0010
U4	0.0018	0.0037	0.0035	0.0007	0.4272	0.0019
U5	0.0059	0.0057	0.0195	0.0184	0.4329	0.0063
U6	0.0064	0.0094	0.0150	0.0147	0.3841	0.0063
U7	0.0081	0.0026	0.0150	0.0429	0.3916	0.0093
U8	0.0019	0.0024	0.0336	0.0310	0.3386	0.0063
U9	0.0015	0.0048	0.0183	0.0049	0.2558	0.0090
U10	0.0037	0.0002	0.0004	0.0001	0.3424	0.0026
U11	0.0027	0.0093	0.0098	0.0040	0.6915	0.0006
U12	0.0041	0.0002	0.0209	0.0140	0.1964	0.0001
U13	0.0063	0.0049	0.0183	0.0091	0.0919	0.0046
U14	0.0052	0.0038	0.0009	0.0004	0.1974	0.0029
U15	0.0063	0.0040	0.0020	0.0319	0.5296	0.0082
U16	0.0009	0.0092	0.0108	0.0294	0.2781	0.0046
U17	0.0062	0.0004	0.0393	0.0067	0.0991	0.0026
U18	0.0005	0.0048	0.0381	0.0037	0.0819	0.0026
U19	0.0062	0.0090	0.0009	0.0139	0.1975	0.0068
U20	0.0093	0.0003	0.0098	0.0421	0.7815	0.0026
U21	0.0024	0.0047	0.0098	0.0098	0.1498	0.0073
U22	0.0004	0.0048	0.0297	0.0018	0.2845	0.0070
U23	0.0073	0.0060	0.0193	0.0407	0.0614	0.0039
U24	0.0085	0.0084	0.0038	0.0298	0.1040	0.0031
U25	0.0073	0.0093	0.0001	0.0480	0.0701	0.0073
U26	0.0065	0.0027	0.0029	0.0367	0.1958	0.0026
U27	0.0070	0.0027	0.0208	0.0058	0.5901	0.0061
U28	0.0058	0.0003	0.0298	0.0004	0.2904	0.0038
U29	0.0063	0.0049	0.0004	0.0046	0.6714	0.0005
U30	0.0004	0.0094	0.0040	0.0498	0.0881	0.0010
U31	0.0073	0.0024	0.0248	0.0249	0.1789	0.0063
U32	0.0045	0.0049	0.0003	0.0050	0.4195	0.0054
U33	0.0006	0.0004	0.0492	0.0184	0.3904	0.0006

Table S5: The relative information of perturbed metabolic pathways in plasma and urine samples.

Pathway name	Match Status	*P*	-log(*P*)	FDR	Impact
Steroid hormone biosynthesis	21/70	3.0297E-19	42.641	2.454E-17	0.41534
Linoleic acid metabolism	3/5	1.7723E-4	8.6381	0.0071776	1.0
Arachidonic acid metabolism	6/36	2.8367E-4	8.1677	0.0076591	0.42491
Retinol metabolism	4/17	8.4926E-4	7.0711	0.017198	0.37113
Alpha-linolenic acid metabolism	3/9	0.0013803	6.5854	0.022361	1.0
Glycerophospholipid metabolism	1/30	0.56531	0.57039	1.0	0.13889
Tryptophan metabolism	2/41	0.30608	1.1839	1.0	0.09149
Biosynthesis of unsaturated fatty acids	3/42	0.10212	2.2816	1.0	0.0
Steroid biosynthesis	1/35	0.62234	0.47427	1.0	0.0083

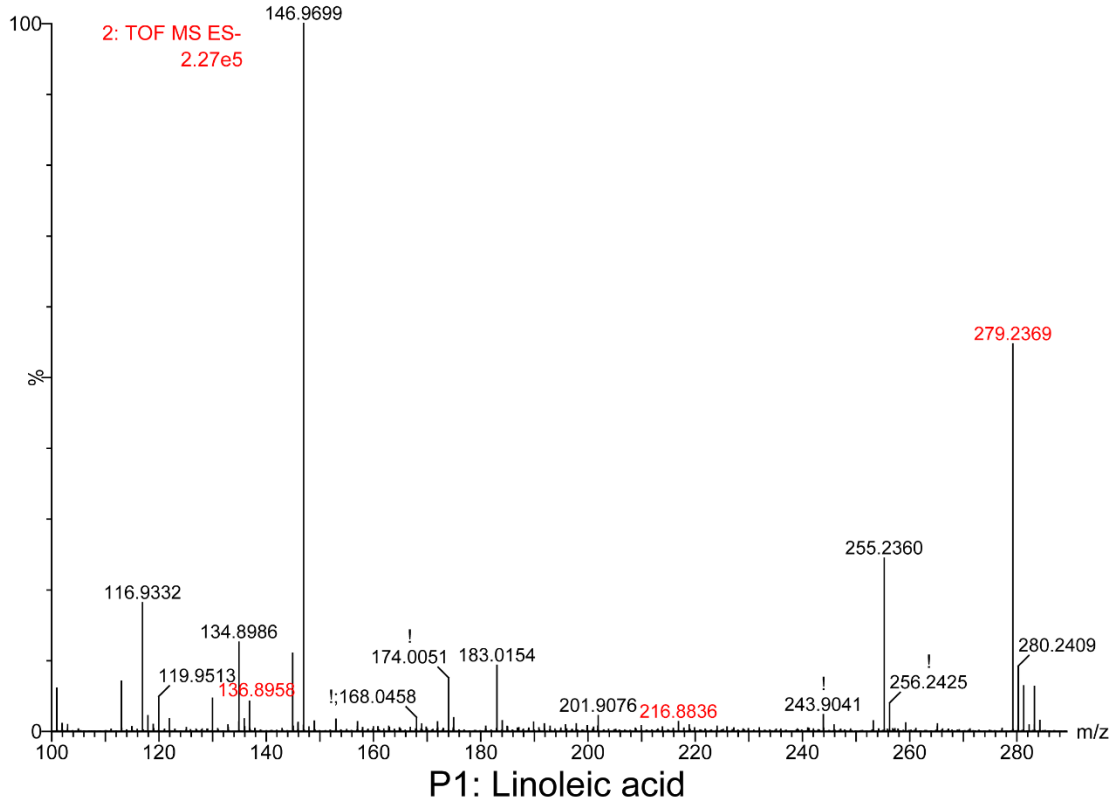


Figure S2: MS/MS spectra of the identified potential marker Linoleic acid in plasma samples.

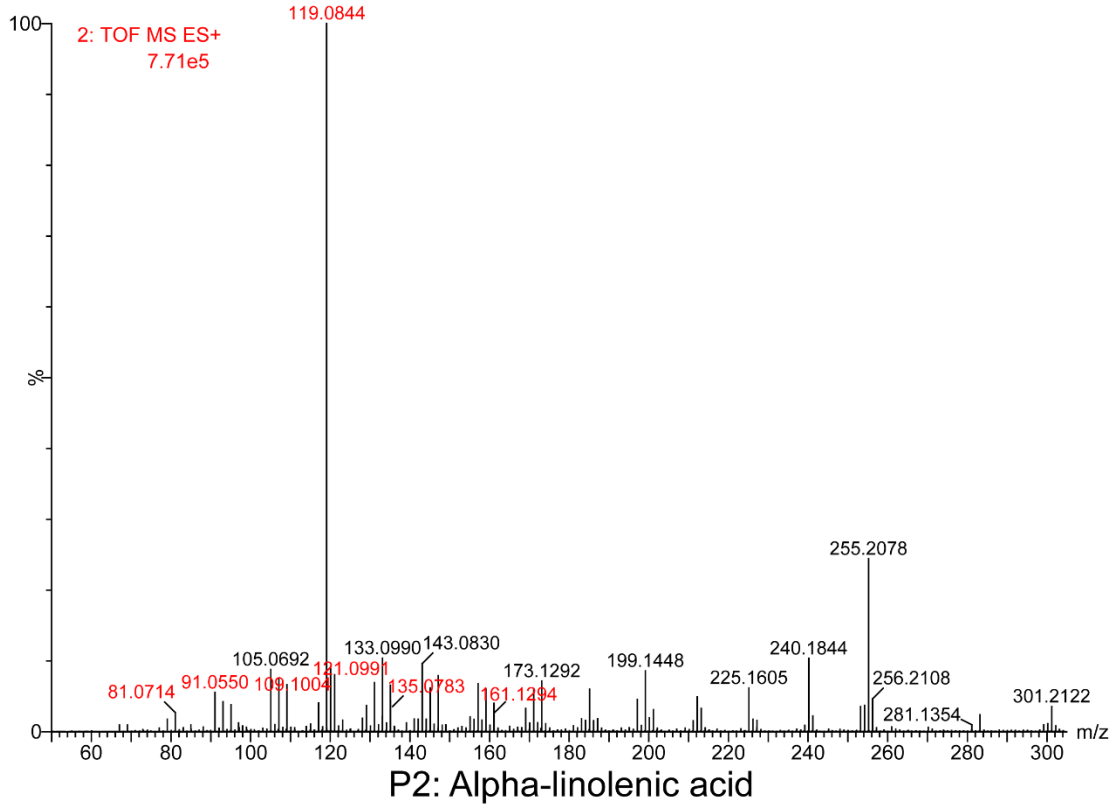


Figure S3: MS/MS spectra of the identified potential marker Alpha-linolenic acid in plasma samples.

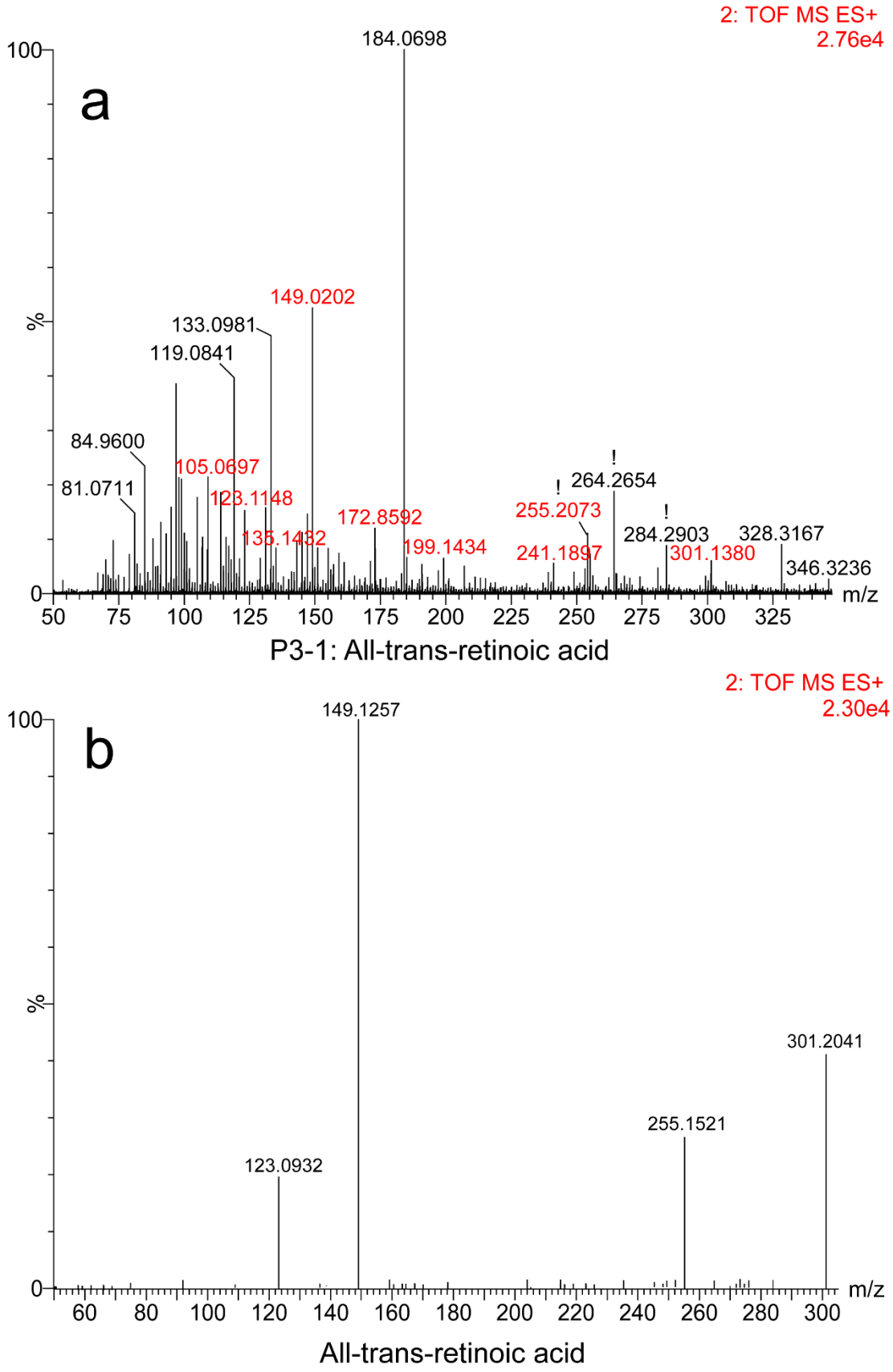


Figure S4: (a) MS/MS spectra of the identified potential marker All-trans-retinoic acid in plasma samples. (b) The reference standard chemical spectra of All-trans-retinoic acid in experimental condition.

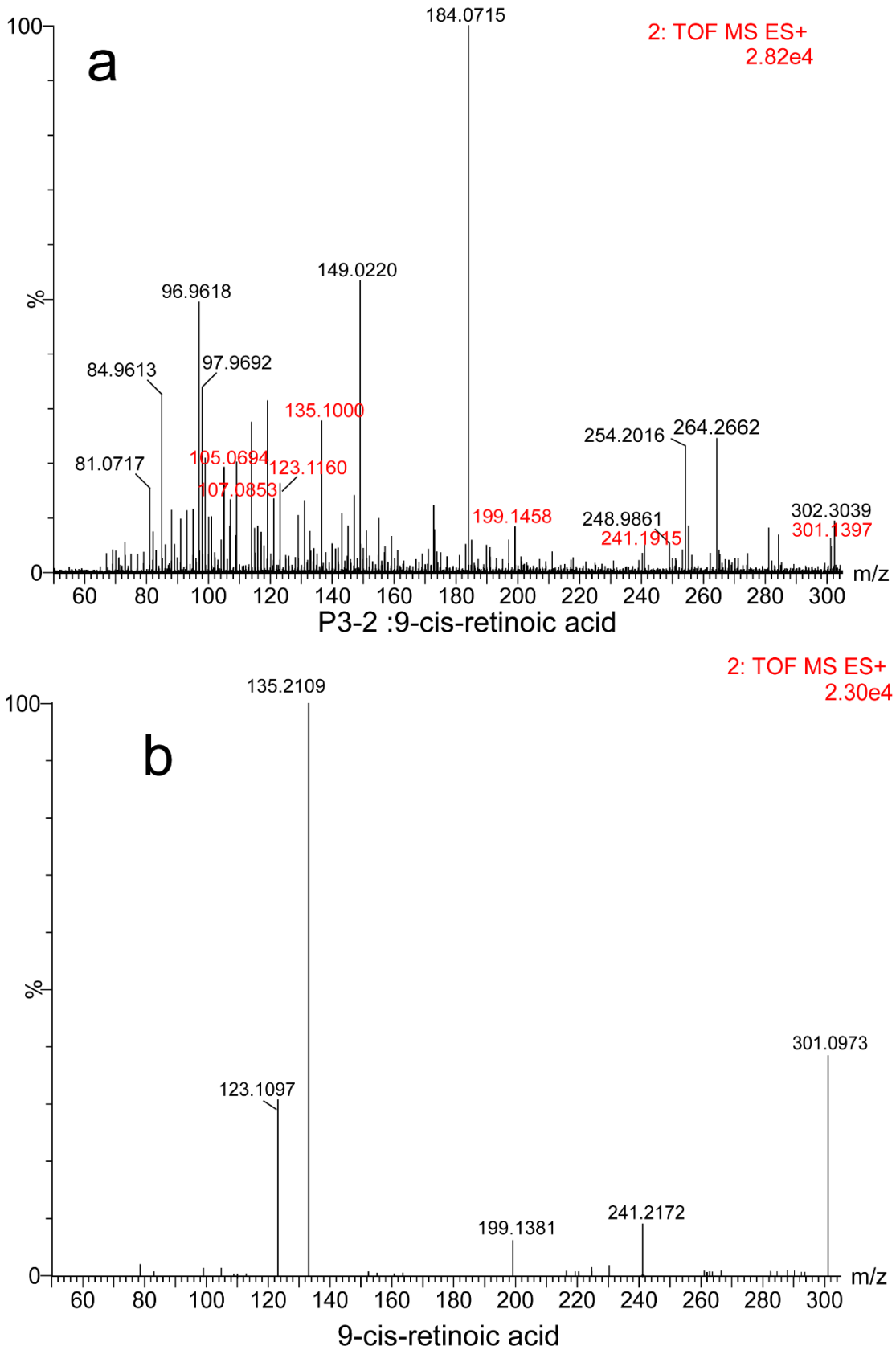


Figure S5: (a) MS/MS spectra of the identified potential marker 9-cis-retinoic acid in plasma samples. (b) The reference standard chemical spectra of 9-cis-retinoic acid in experimental condition.

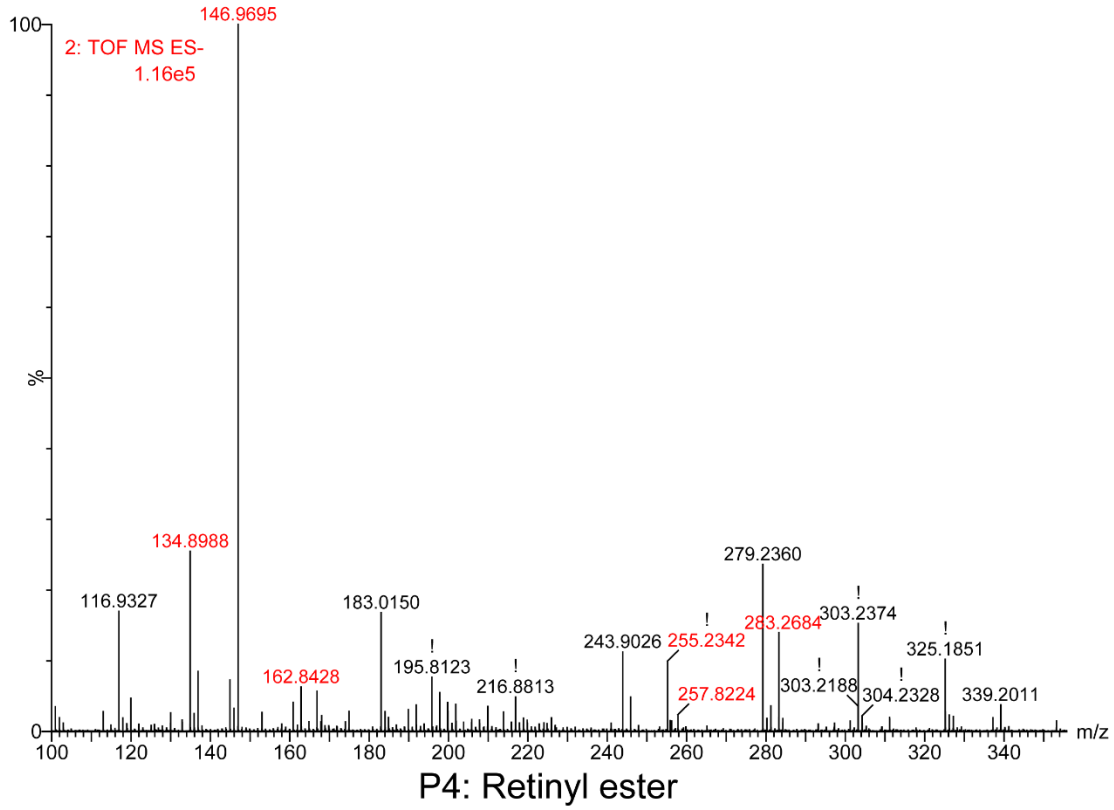


Figure S6: MS/MS spectra of the identified potential marker Retinyl ester in plasma samples.

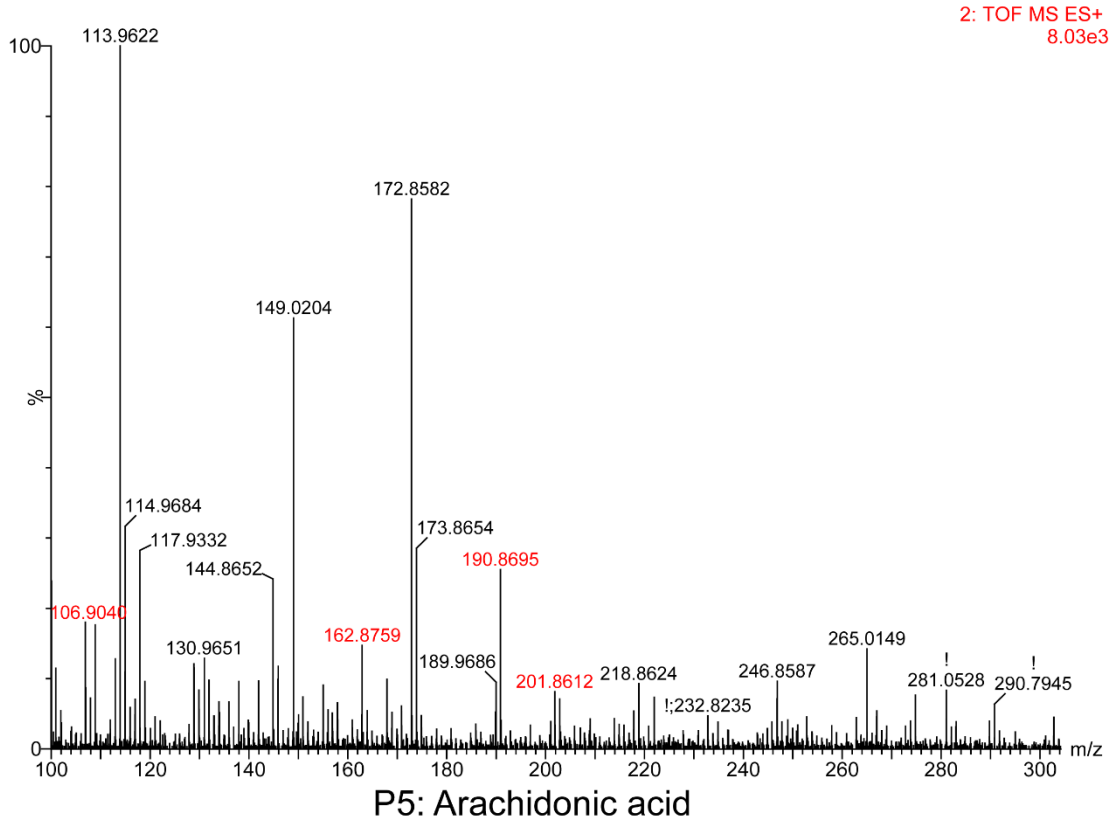


Figure S7: MS/MS spectra of the identified potential marker Arachidonic acid in plasma samples.

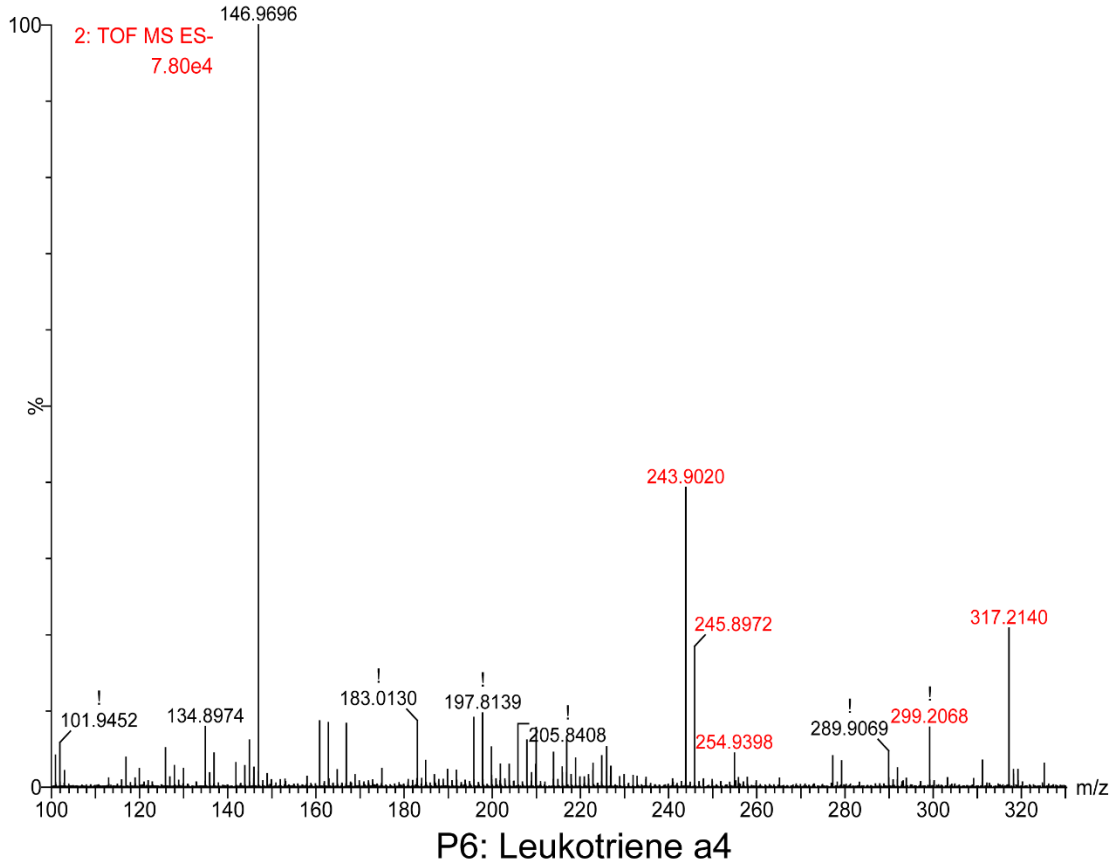


Figure S8: MS/MS spectra of the identified potential marker Leukotriene a4 in plasma samples.

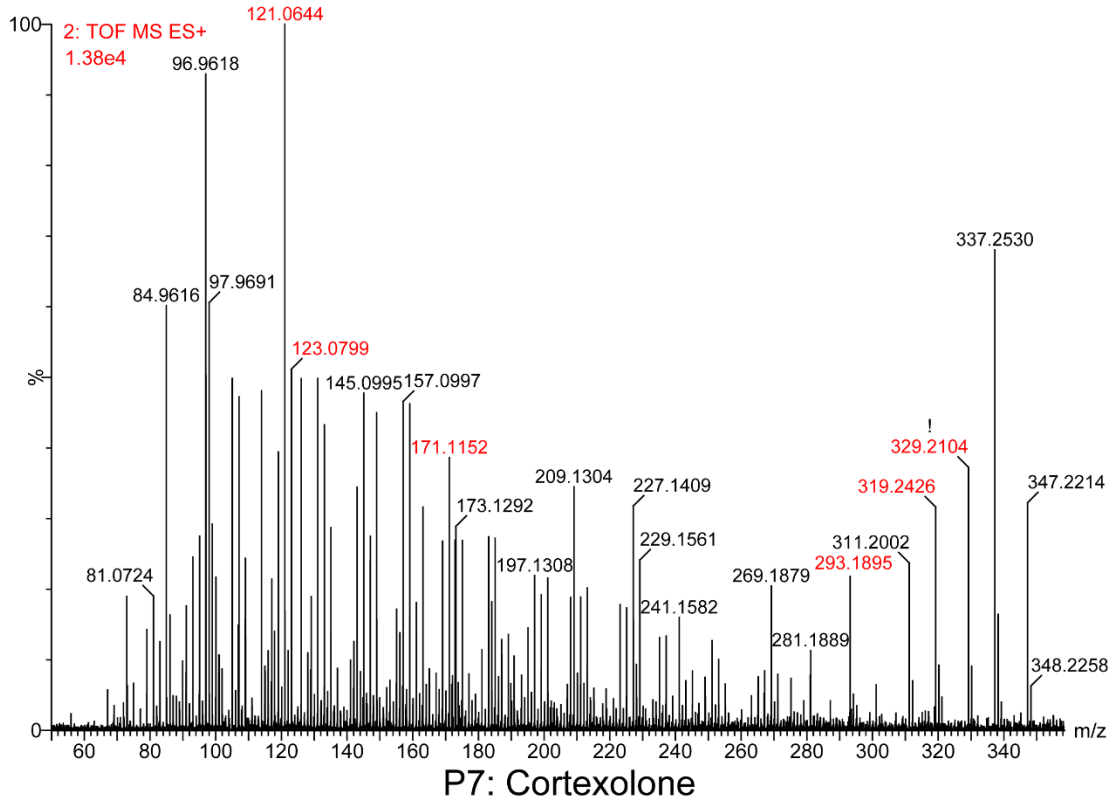


Figure S9: MS/MS spectra of the identified potential marker Cortexolone in plasma samples.

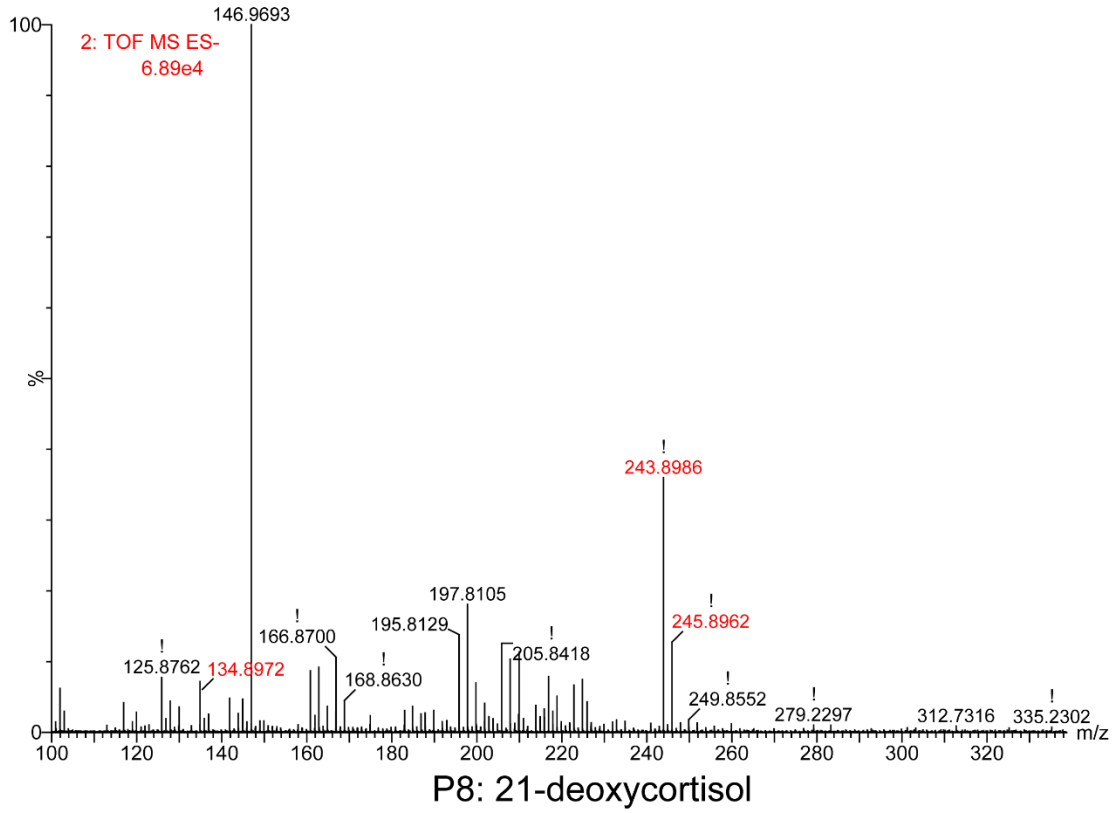


Figure S10: MS/MS spectra of the identified potential marker 21-deoxycortisol in plasma samples.

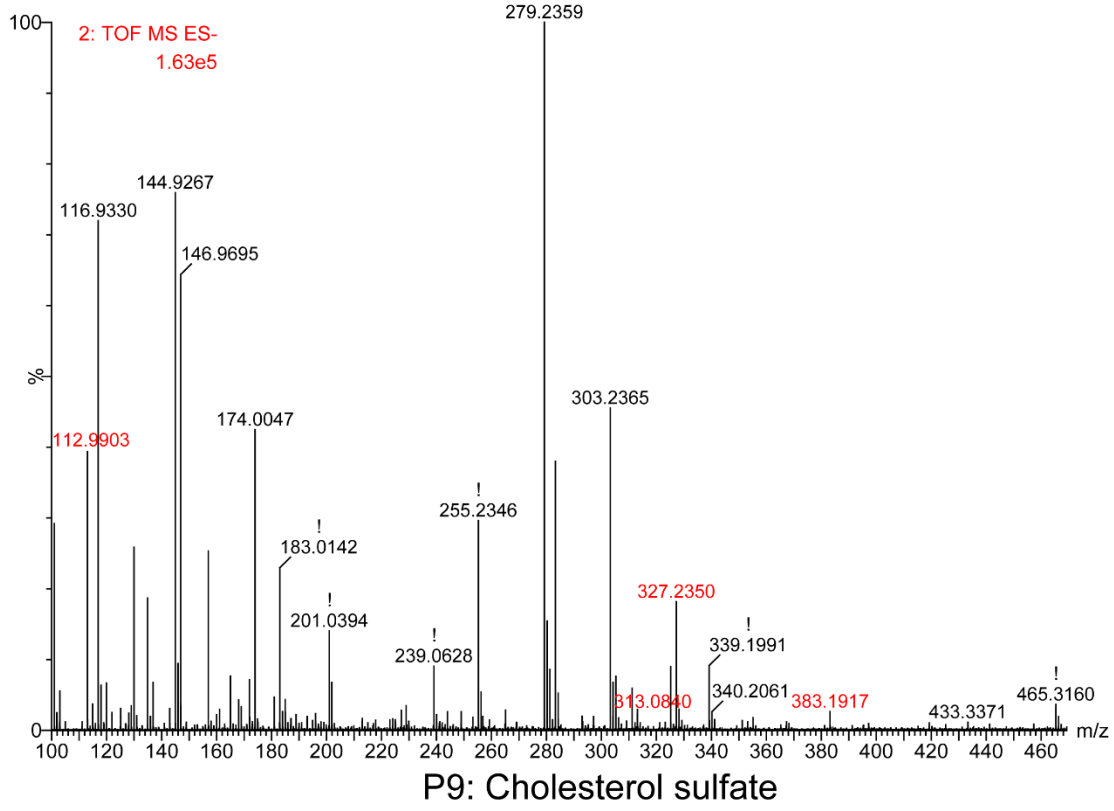


Figure S11: MS/MS spectra of the identified potential marker Cholesterol sulfate in plasma samples.

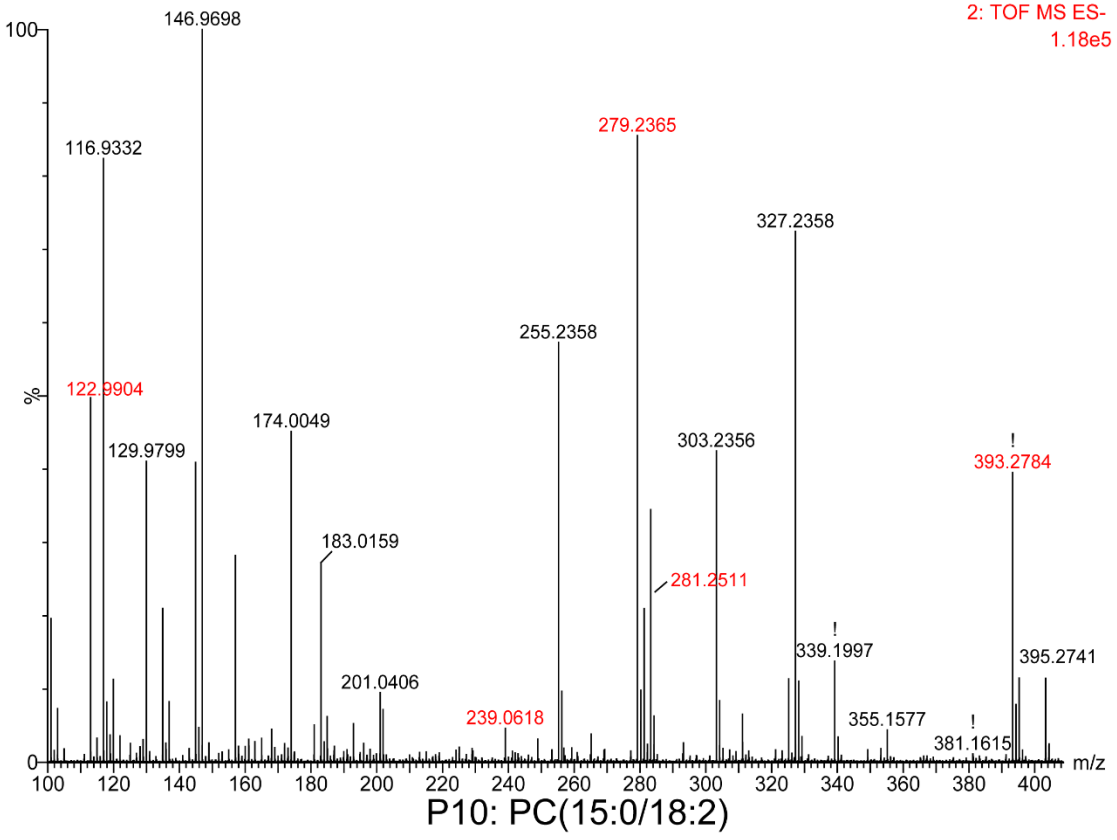


Figure S12: MS/MS spectra of the identified potential marker PC (15:0/18:2) in plasma samples.

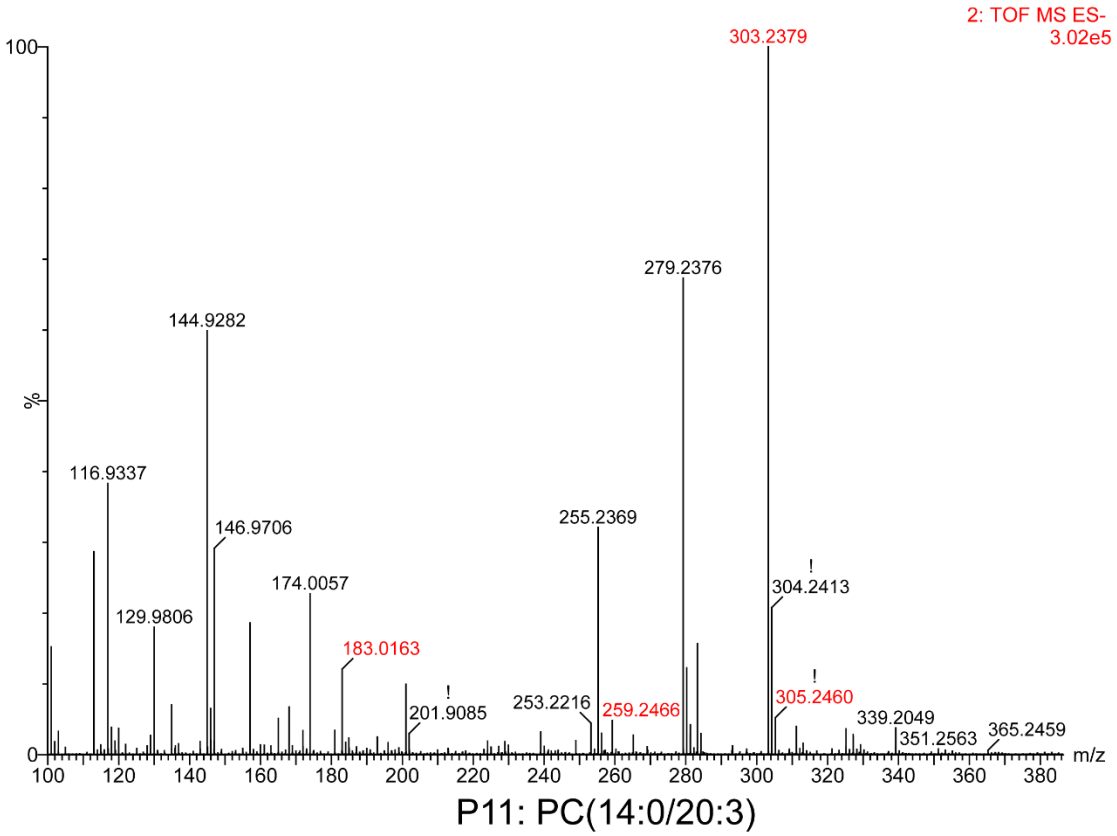


Figure S13: MS/MS spectra of the identified potential marker PC (14:0/20:3) in plasma samples.

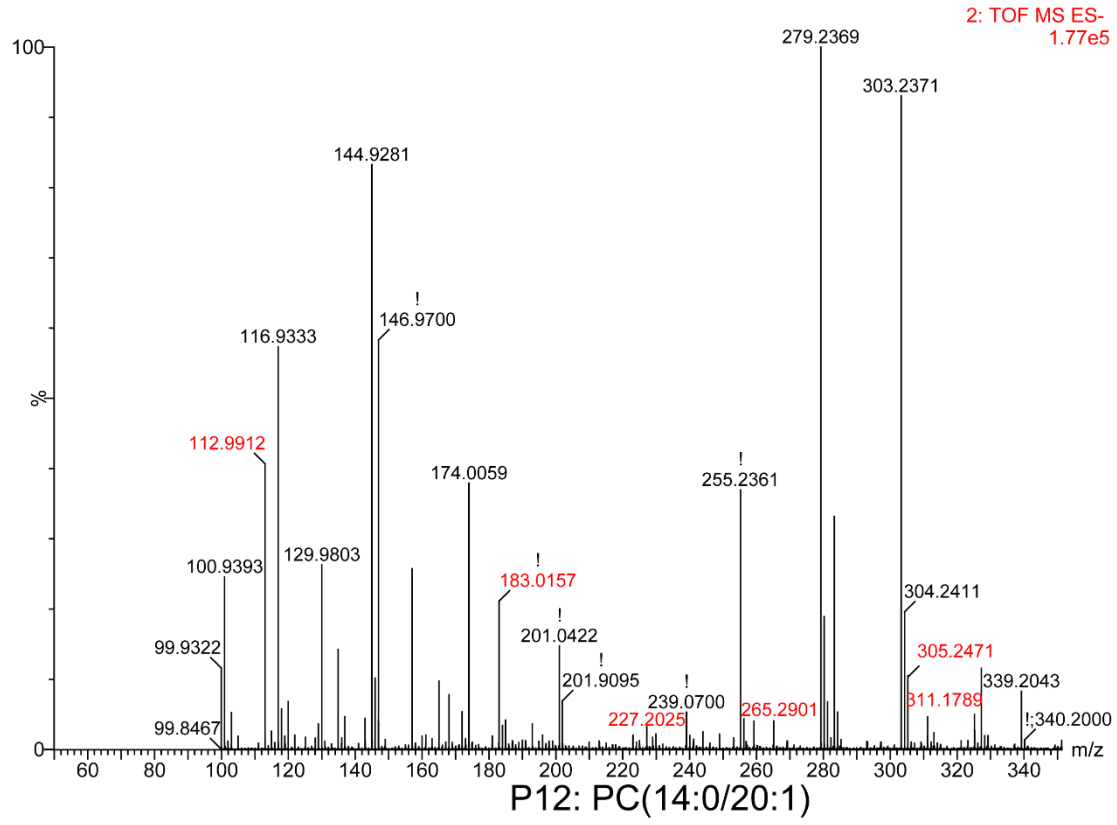


Figure S14: MS/MS spectra of the identified potential marker PC (14:0/20:1) in plasma samples.

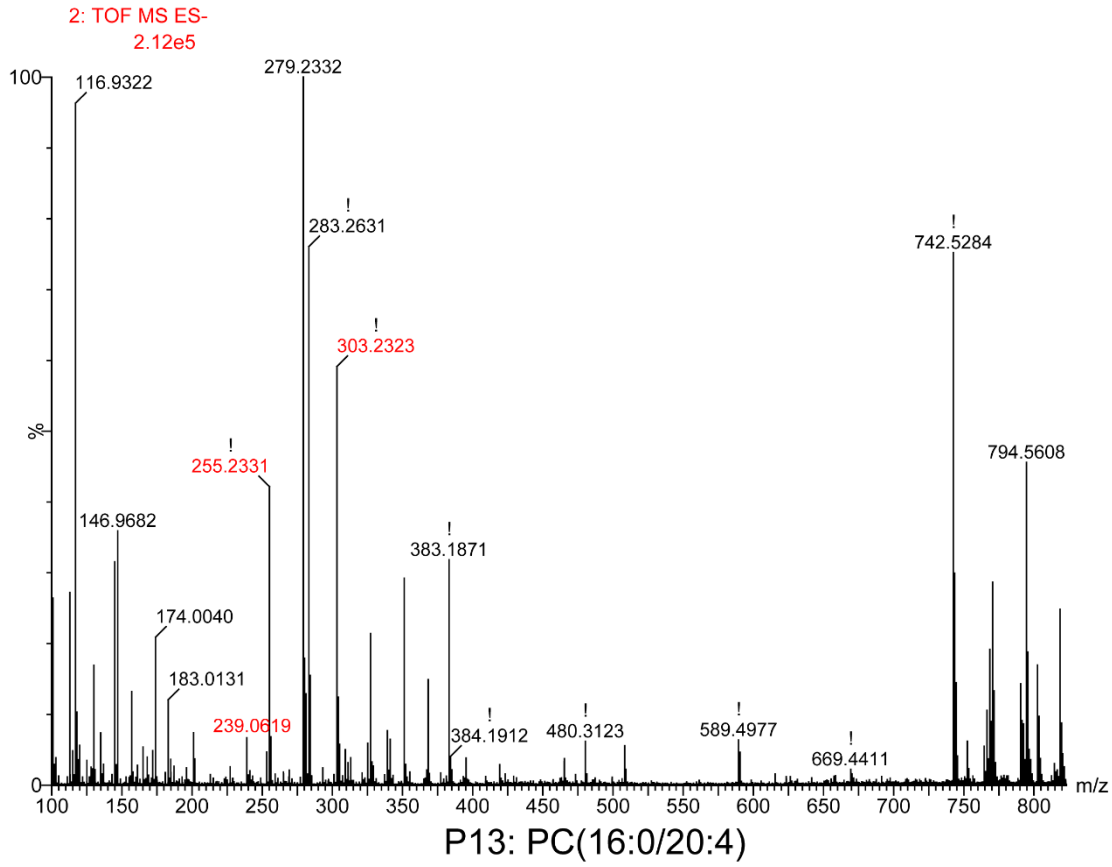


Figure S15: MS/MS spectra of the identified potential marker PC (16:0/20:4) in plasma samples.

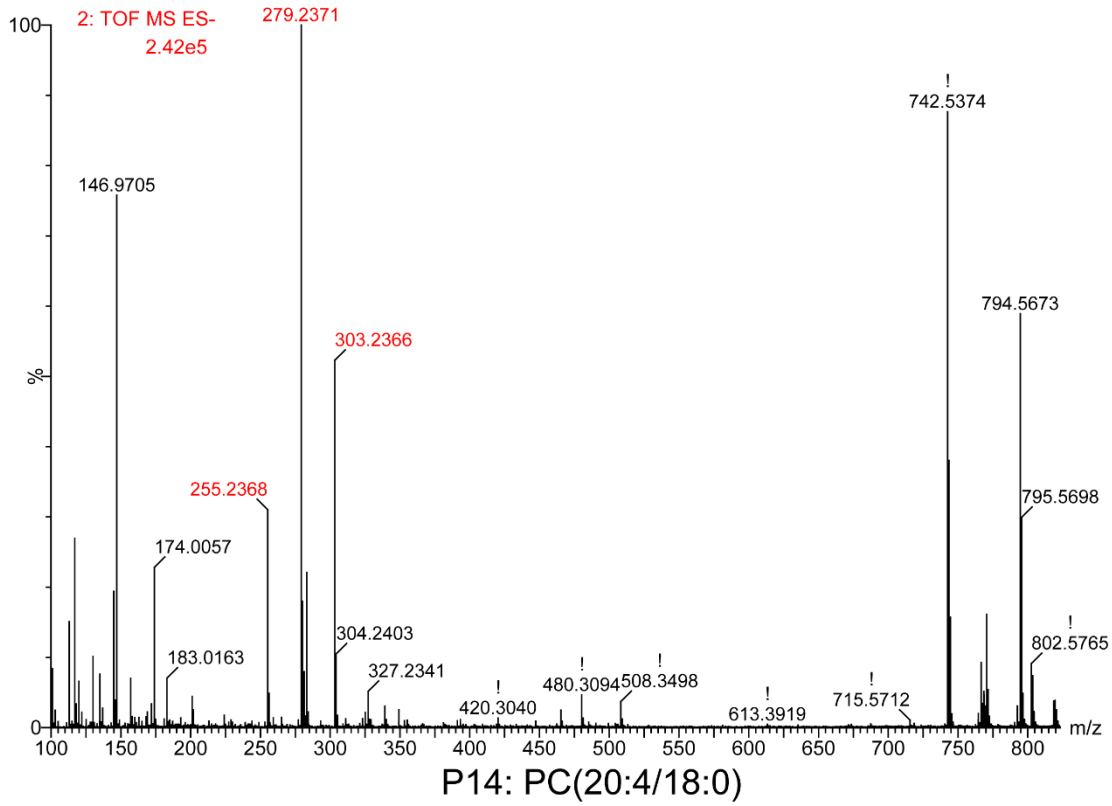


Figure S16: MS/MS spectra of the identified potential marker PC (20:4/18:0) in plasma samples.

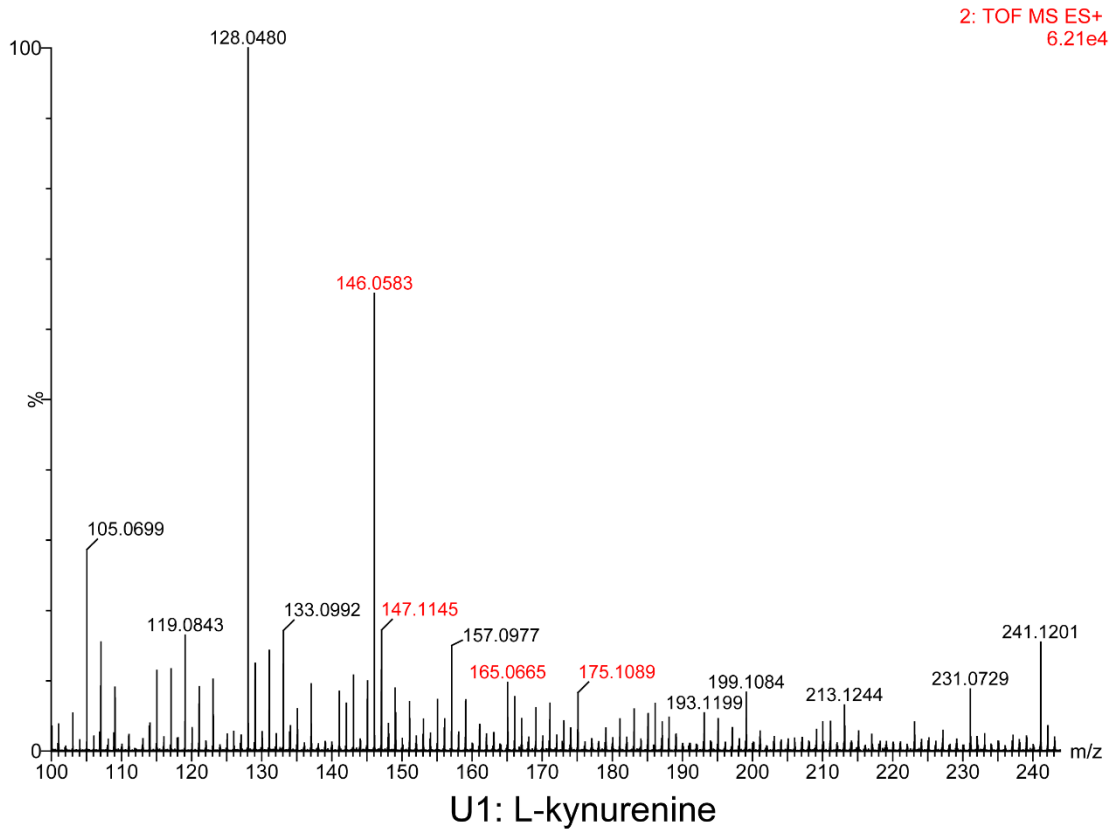


Figure S17: MS/MS spectra of the identified potential marker L-kynurenine in urine samples.

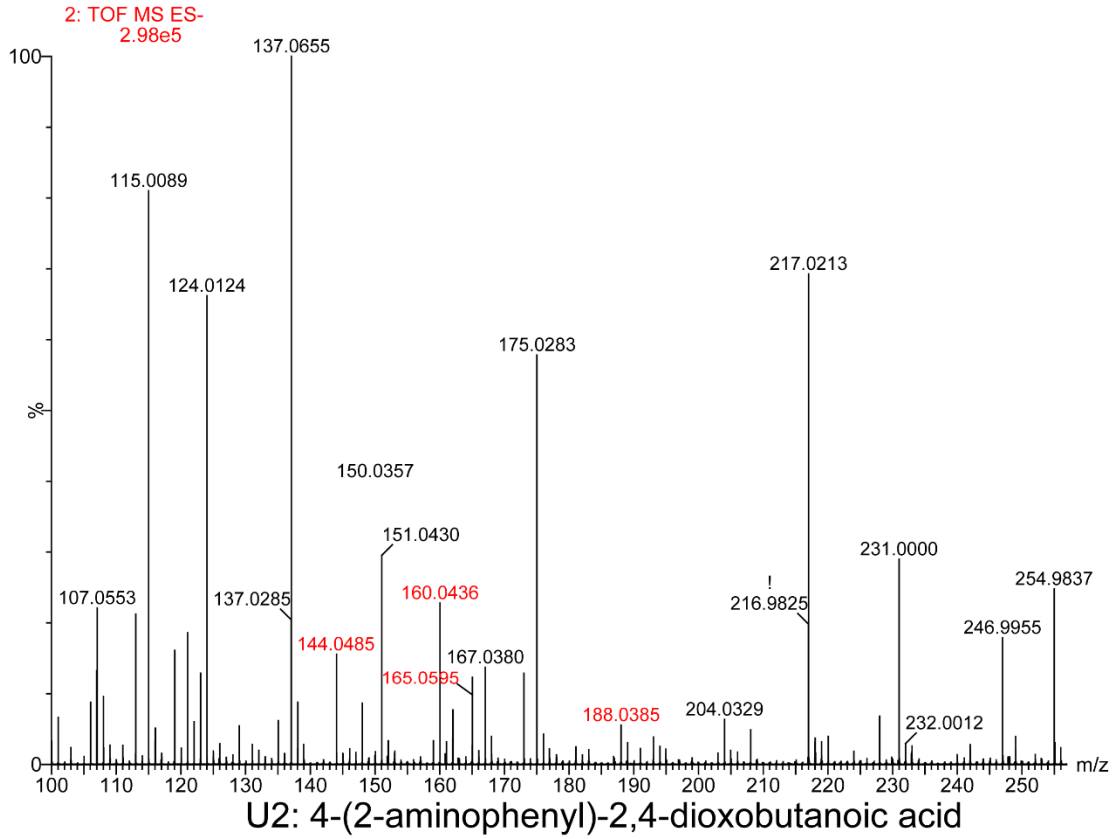


Figure S18: MS/MS spectra of the identified potential marker 4-(2-aminophenyl)-2,4-dioxobutanoic acid in urine samples.

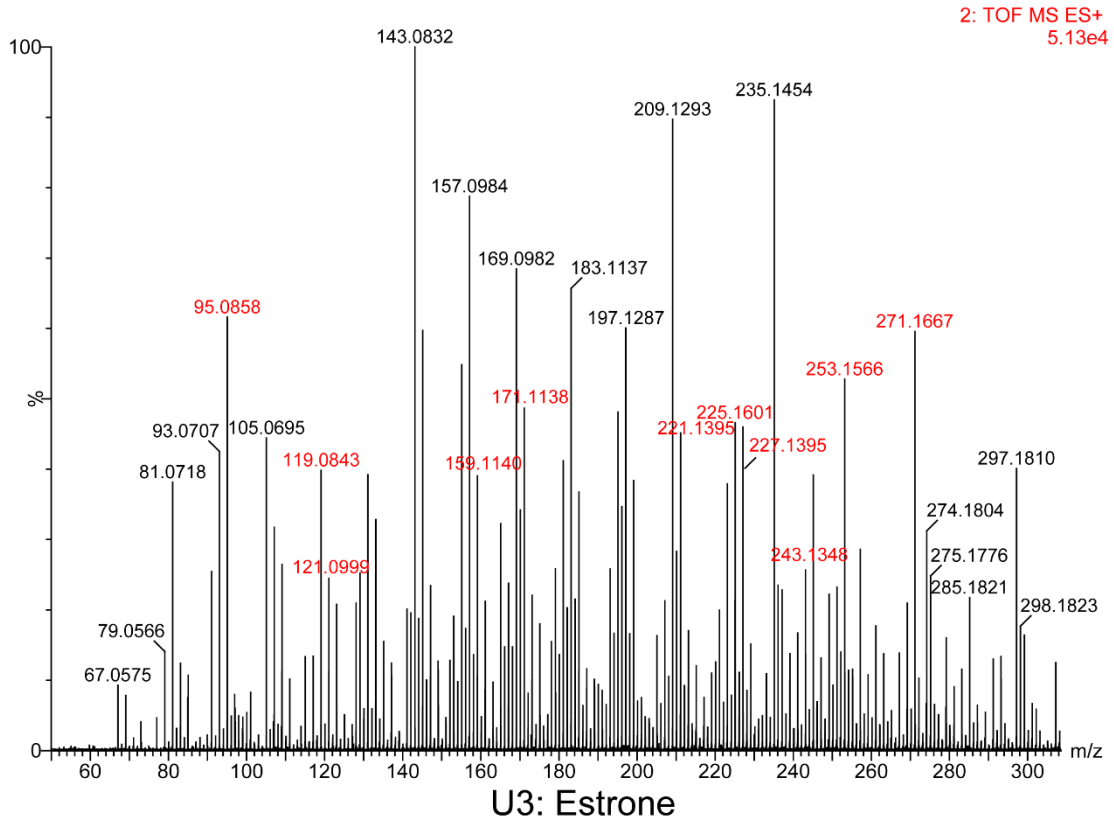


Figure S19: MS/MS spectra of the identified potential marker Estrone in urine samples.

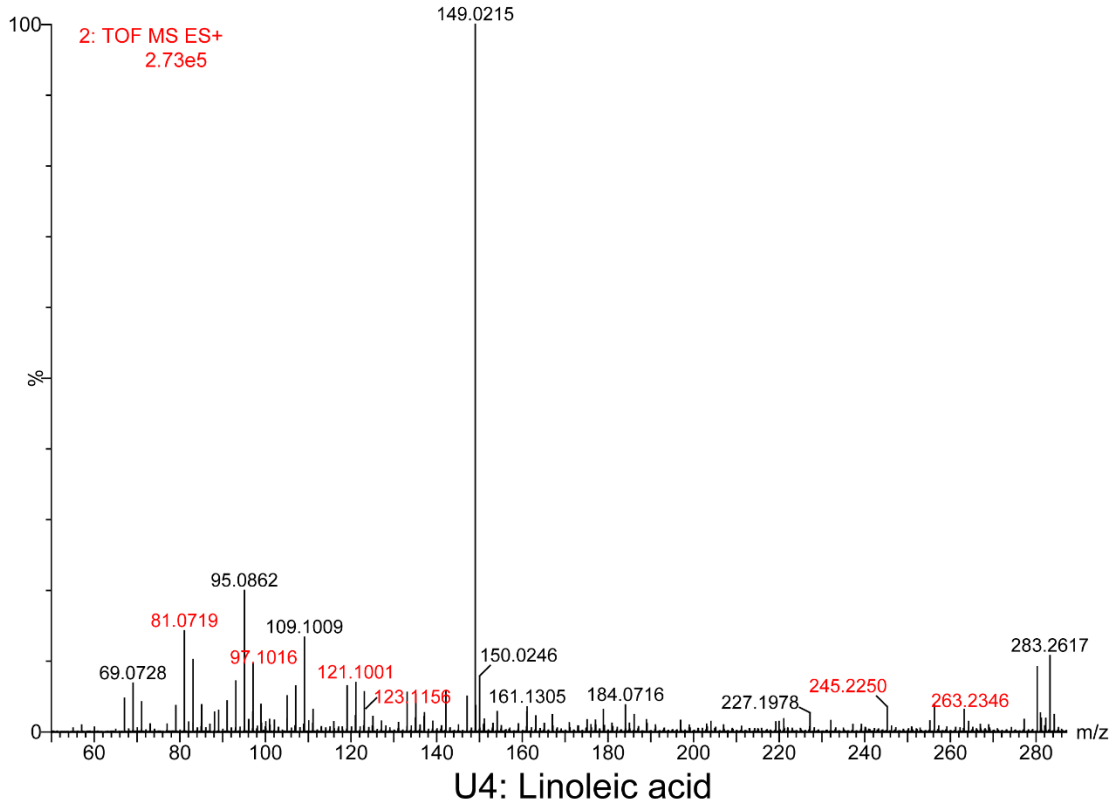


Figure S20: MS/MS spectra of the identified potential marker Linoleic acid in urine samples.

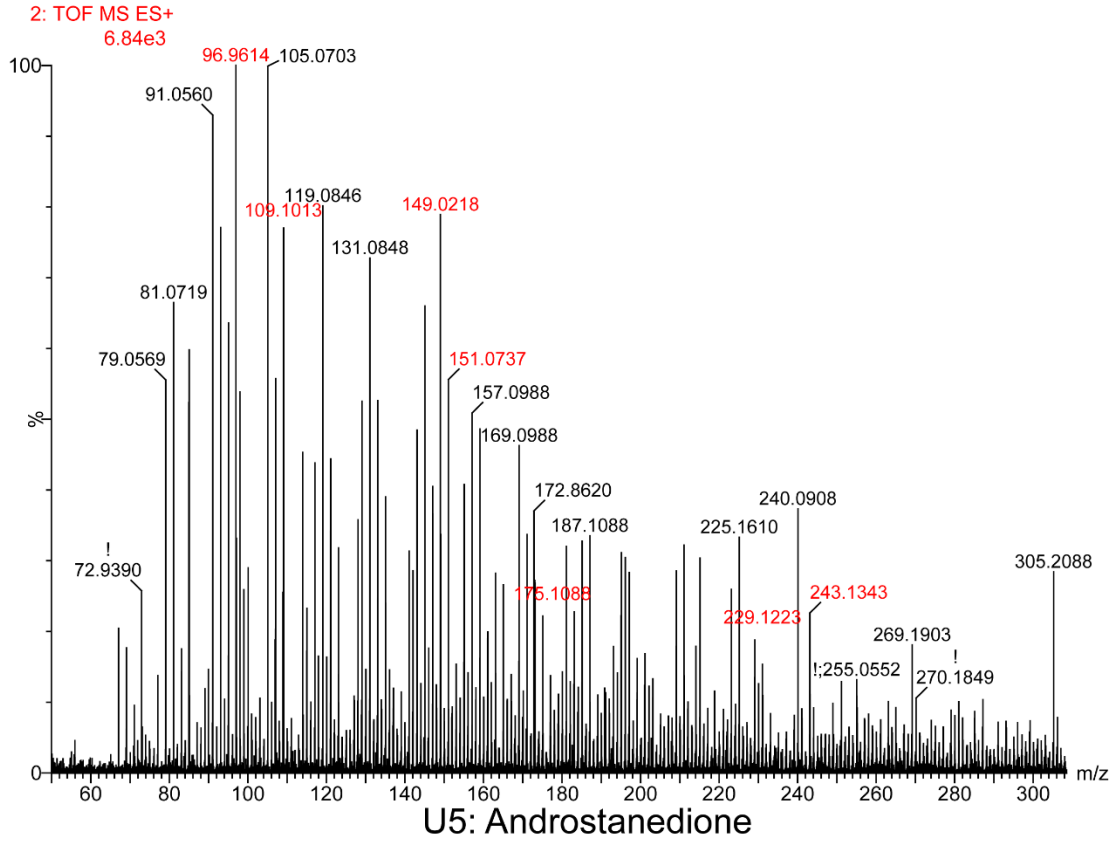


Figure S21: MS/MS spectra of the identified potential marker Androstanedione in urine samples.

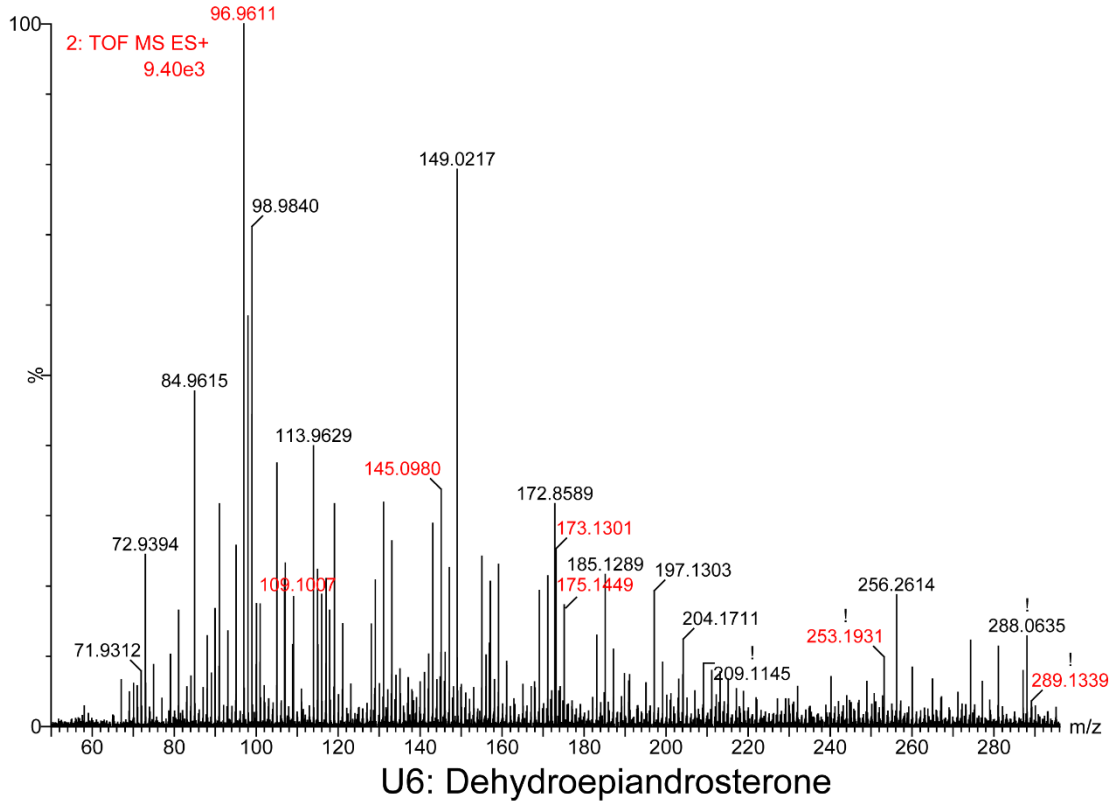


Figure S22: MS/MS spectra of the identified potential marker Dehydroepiandrosterone in urine samples.

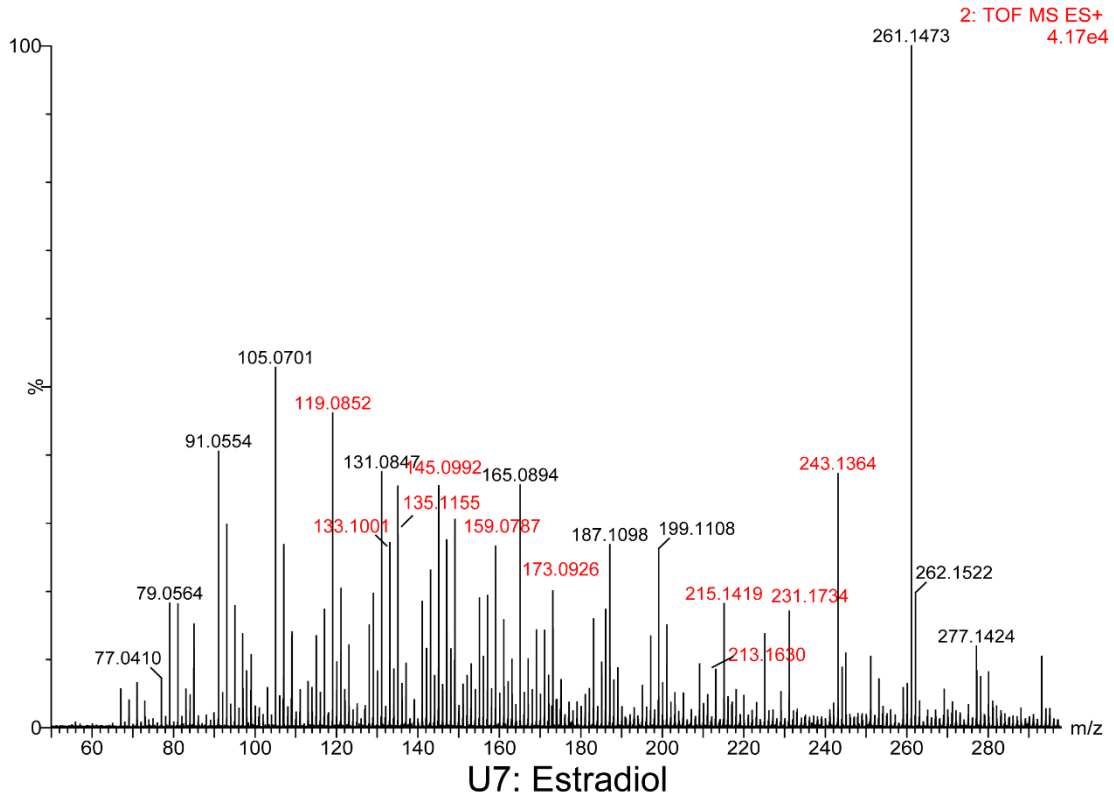


Figure S23: MS/MS spectra of the identified potential marker Estradiol in urine samples.

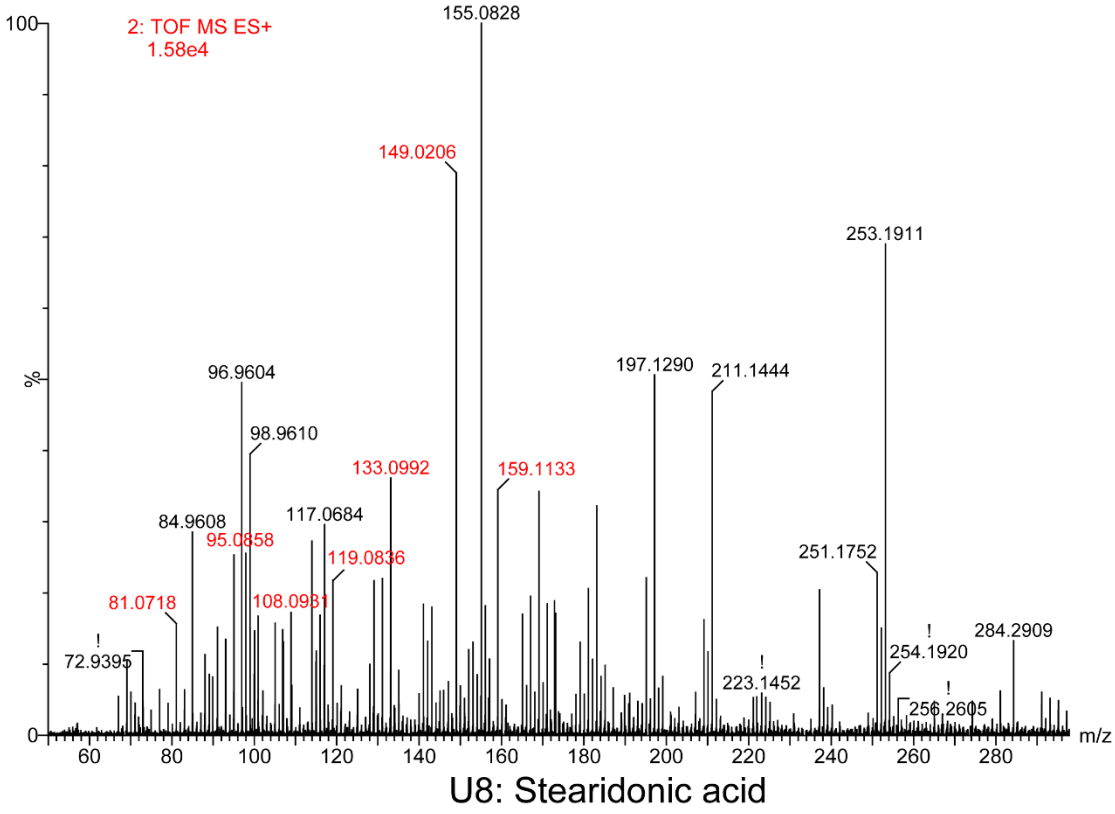


Figure S24: MS/MS spectra of the identified potential marker Stearidonic acid in urine samples.

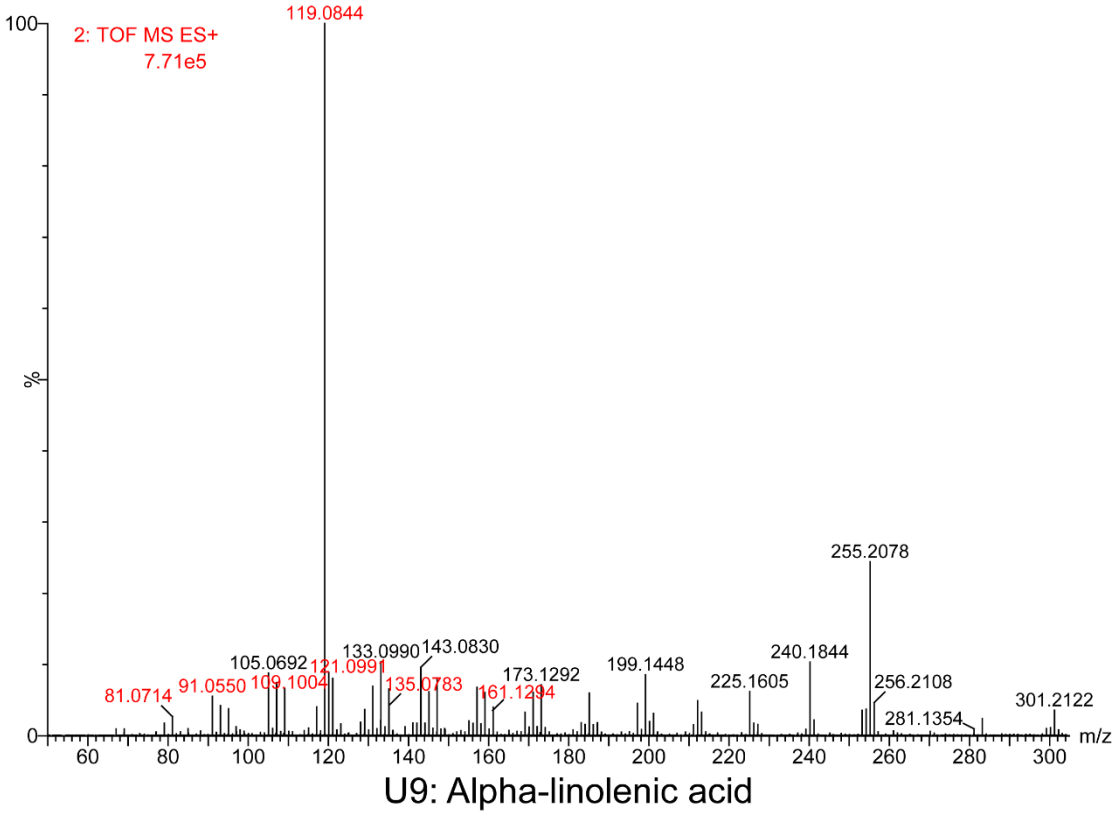


Figure S25: MS/MS spectra of the identified potential marker Alpha-linolenic acid in urine samples.

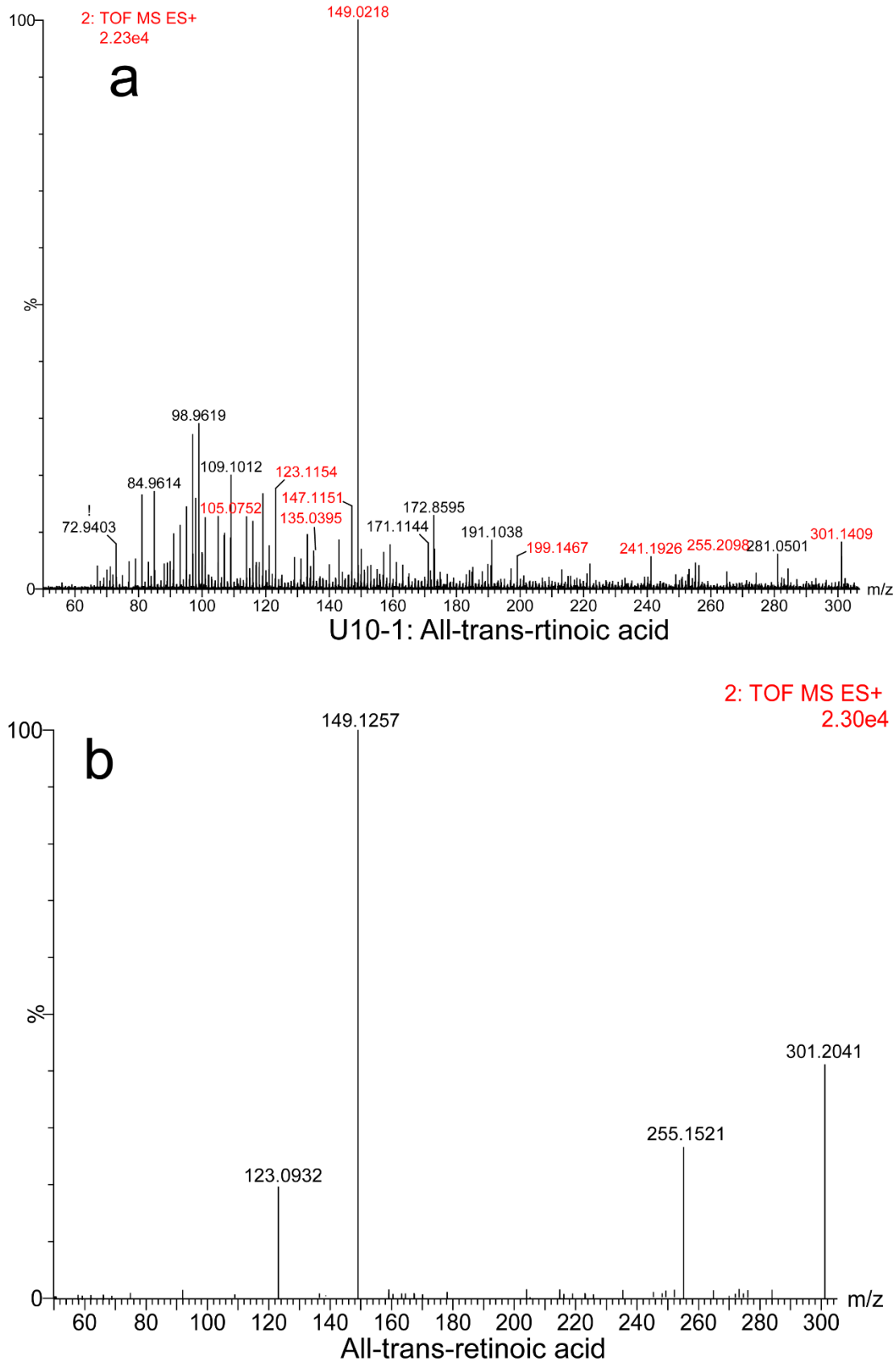


Figure S26: (a) MS/MS spectra of the identified potential marker All-trans-retinoic acid in urine samples. (b) The reference standard chemical spectra of All-trans-retinoic acid in experimental condition.

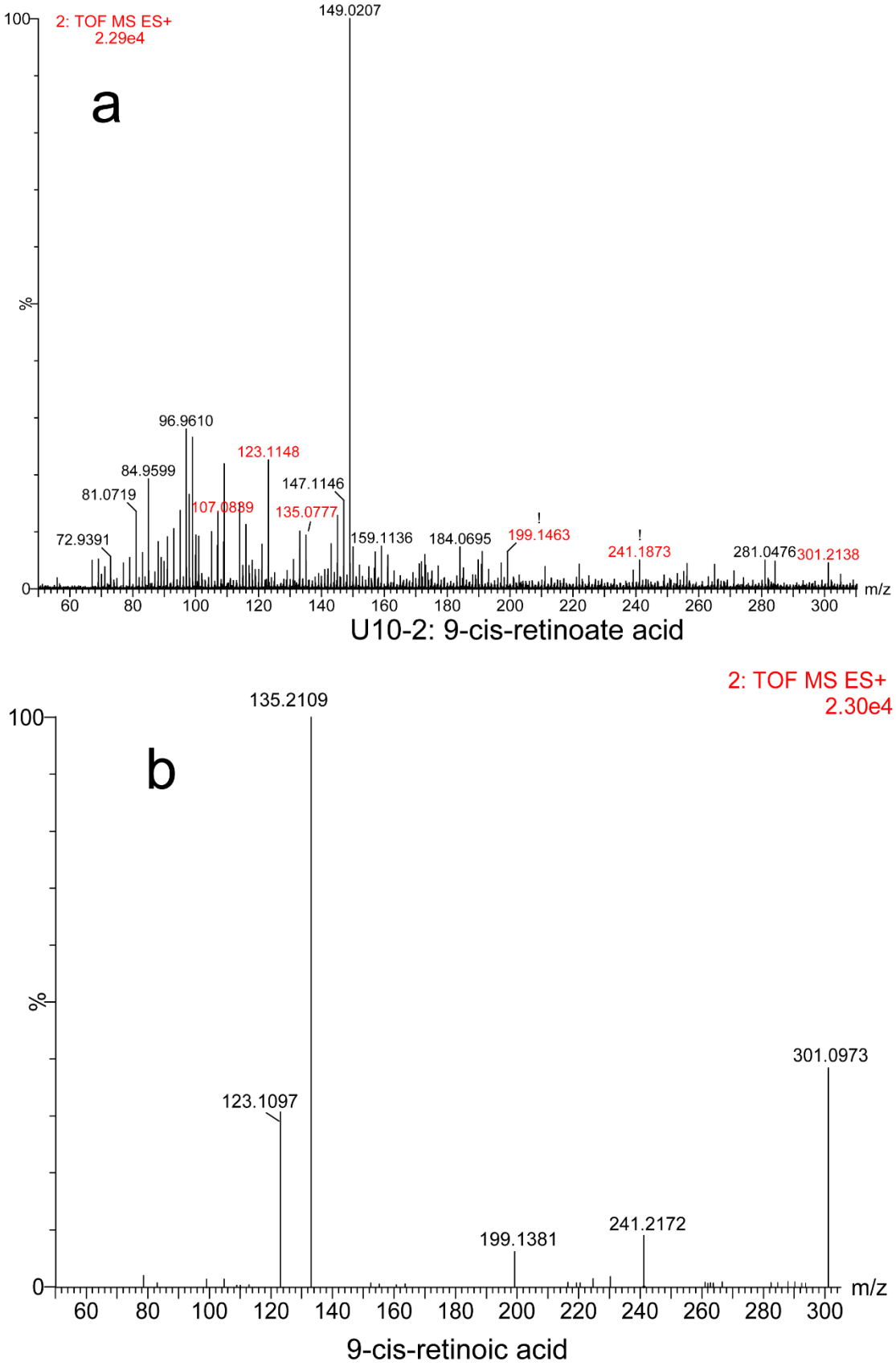


Figure S27: (a) MS/MS spectra of the identified potential marker 9-cis-retinoate acid in urine samples. (b) The reference standard chemical spectra of 9-cis-retinoate acid in experimental

condition.

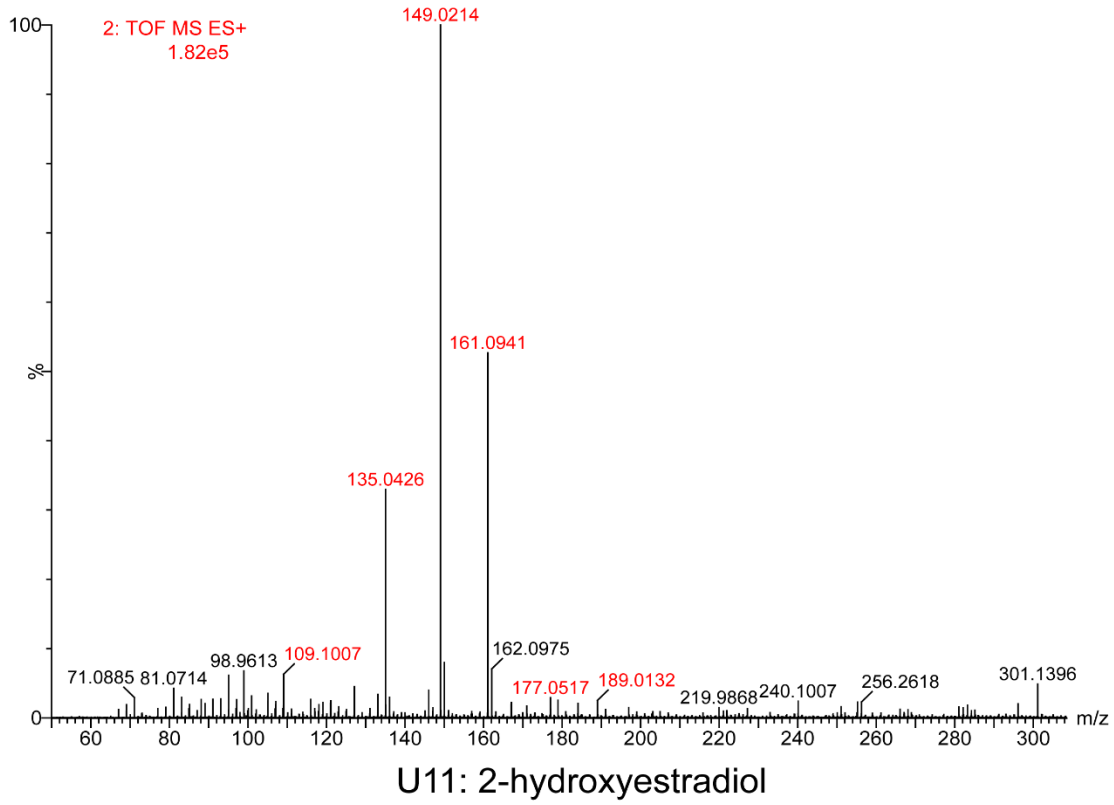


Figure S28: MS/MS spectra of the identified potential marker 2-hydroxyestradiol in urine samples.

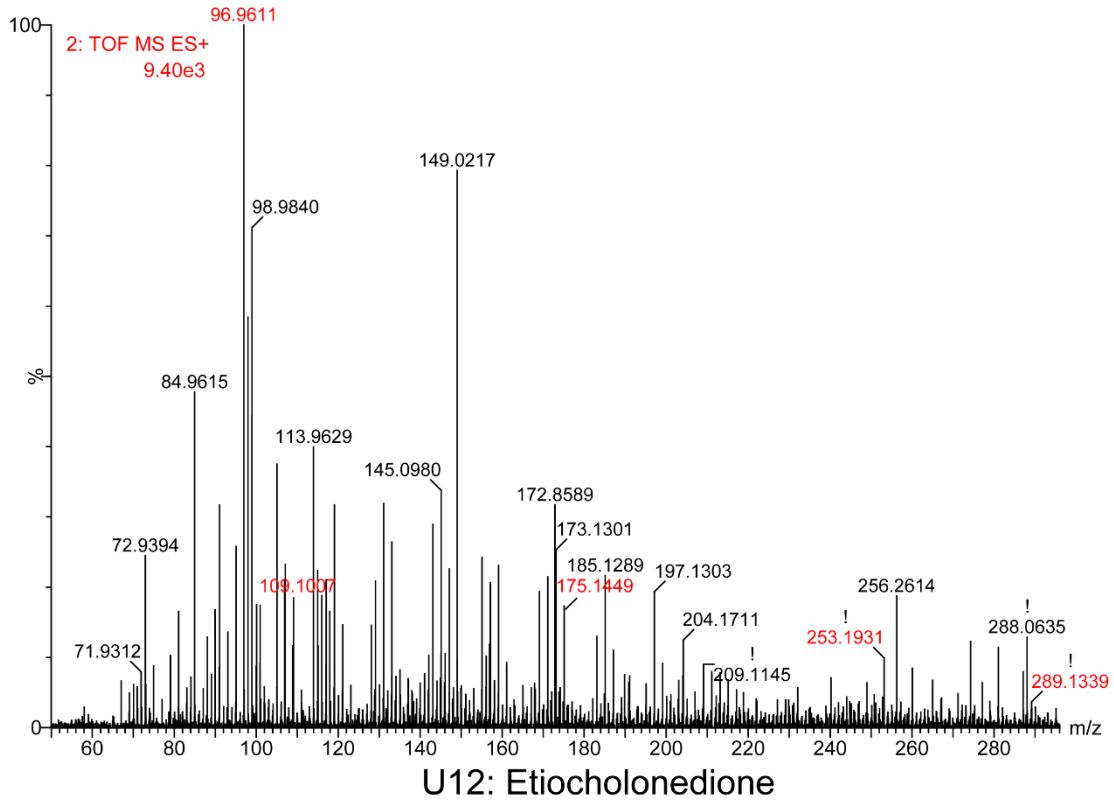


Figure S29: MS/MS spectra of the identified potential marker Etiocholonedione in urine samples.

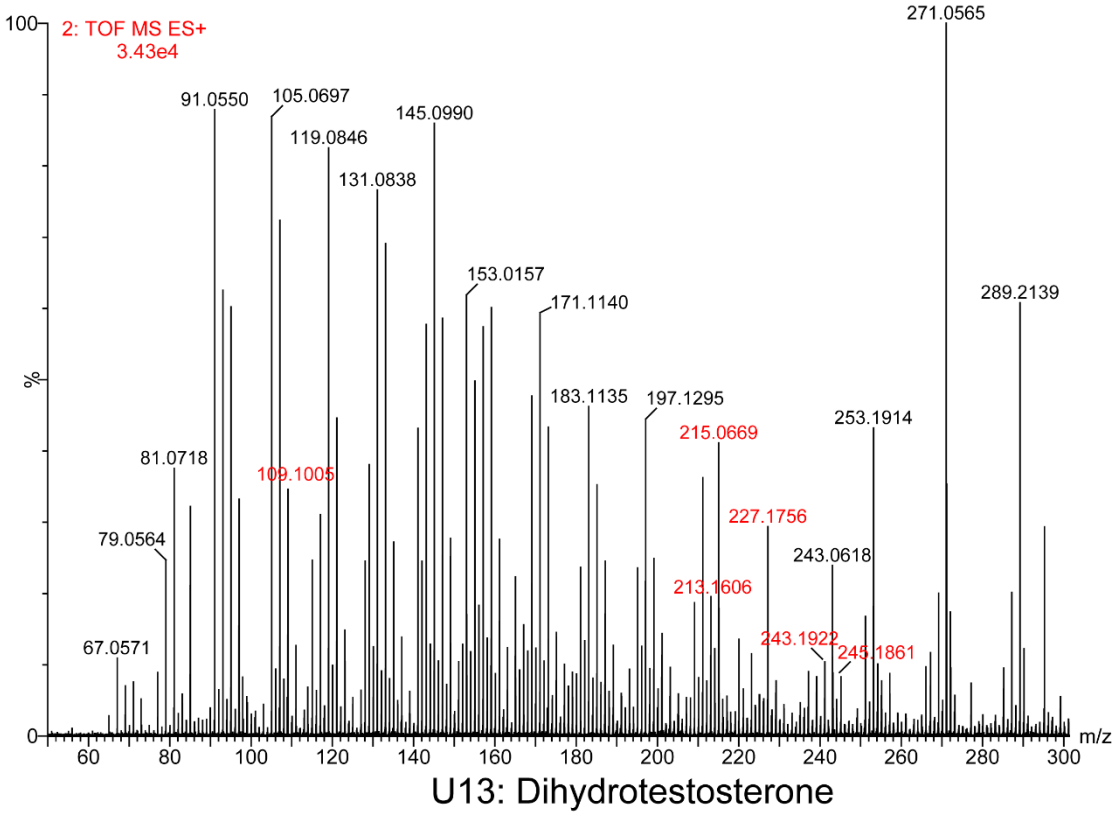


Figure S30: MS/MS spectra of the identified potential marker Dihydrotestosterone in urine samples.

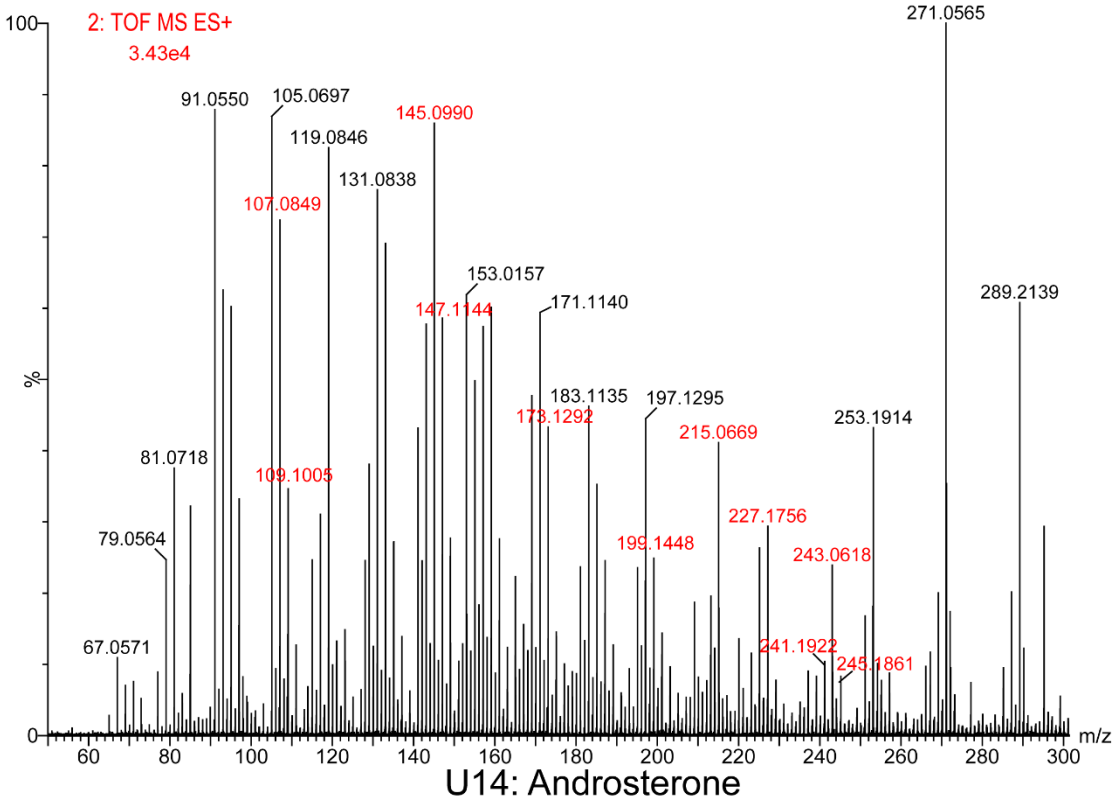


Figure S31: MS/MS spectra of the identified potential marker Androsterone in urine samples.

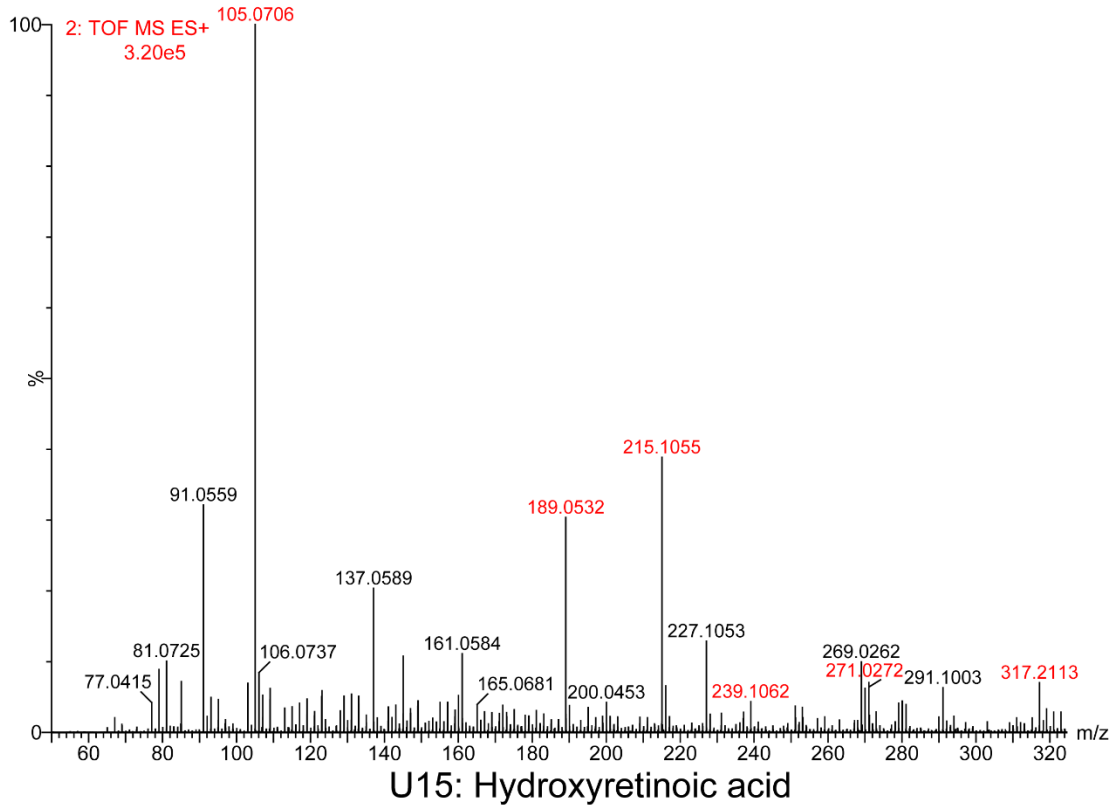


Figure S32: MS/MS spectra of the identified potential marker Hydroxyretinoic acid in urine samples.

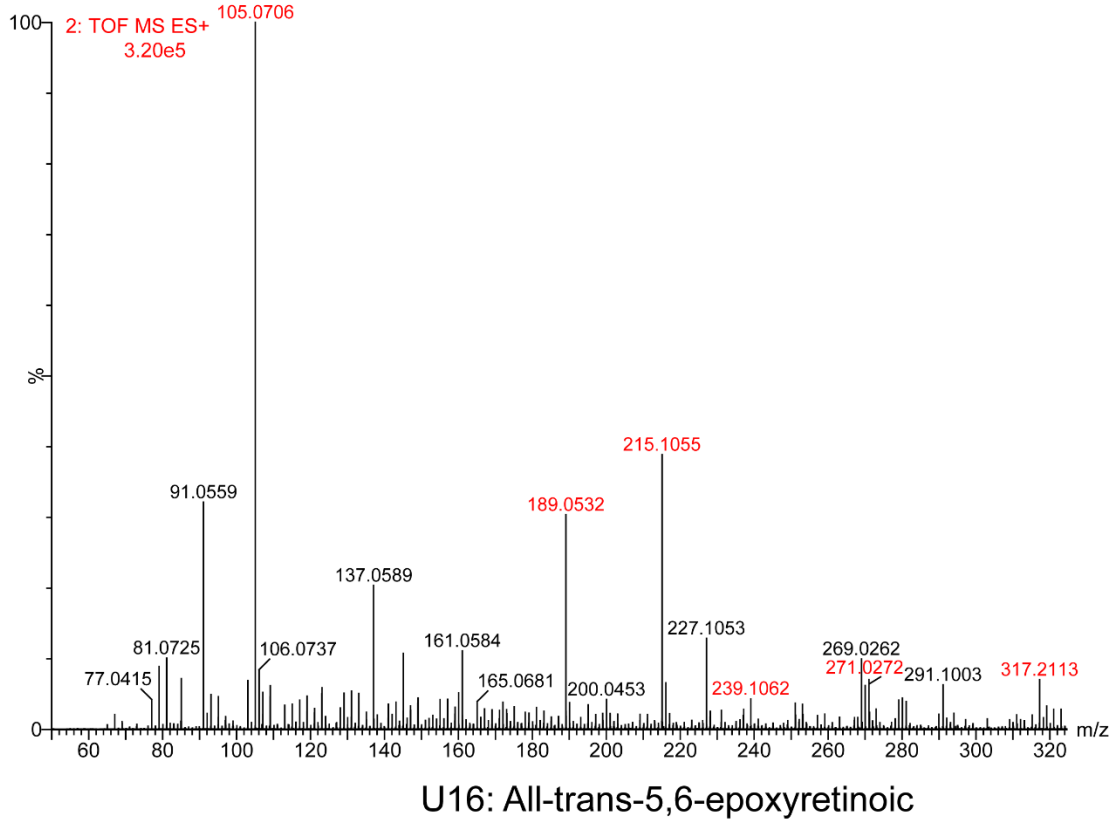


Figure S33: MS/MS spectra of the identified potential marker All-trans-5,6-epoxyretinoic in urine samples.

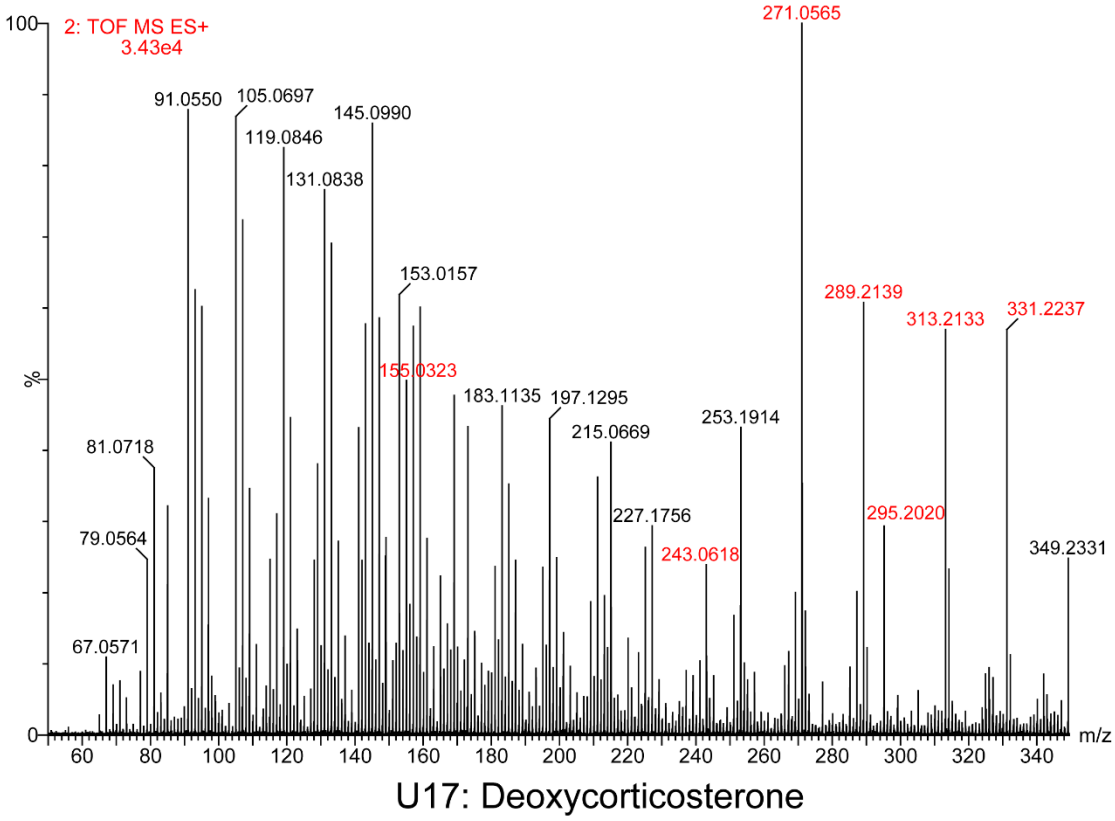


Figure S34: MS/MS spectra of the identified potential marker Deoxycorticosterone in urine samples.

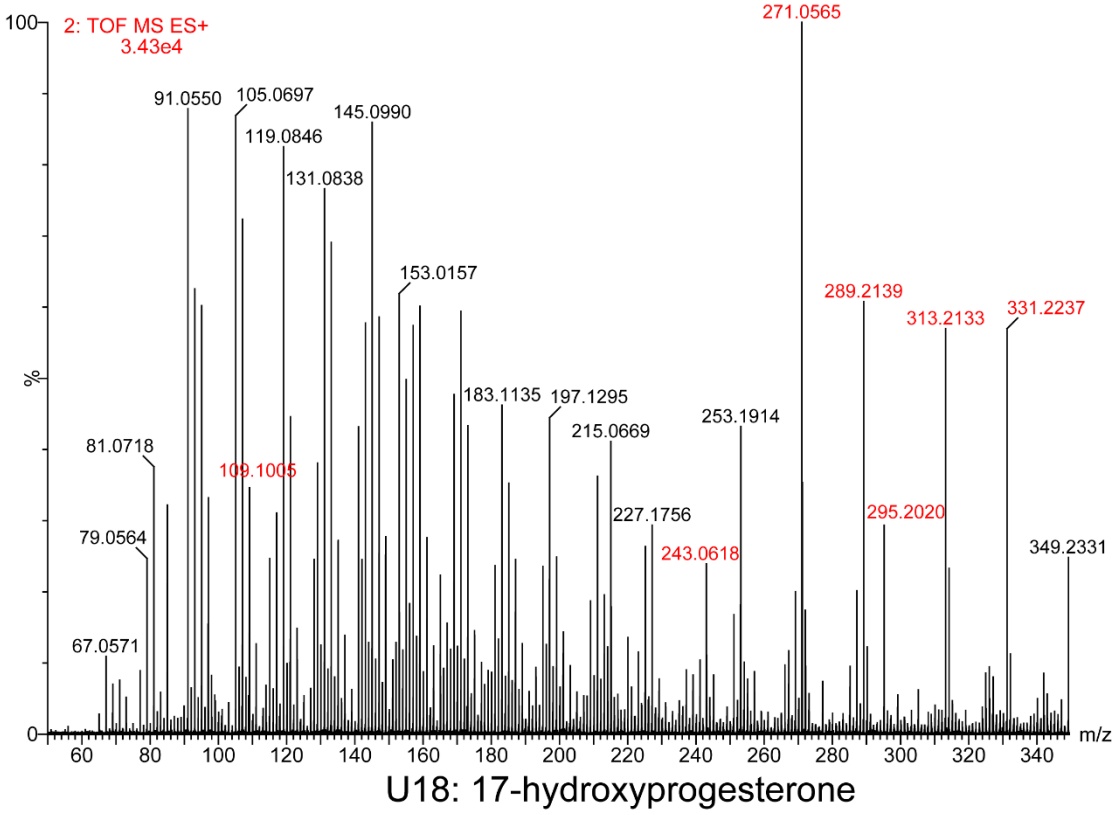


Figure S35: MS/MS spectra of the identified potential marker 17-hydroxyprogesterone in urine

samples.

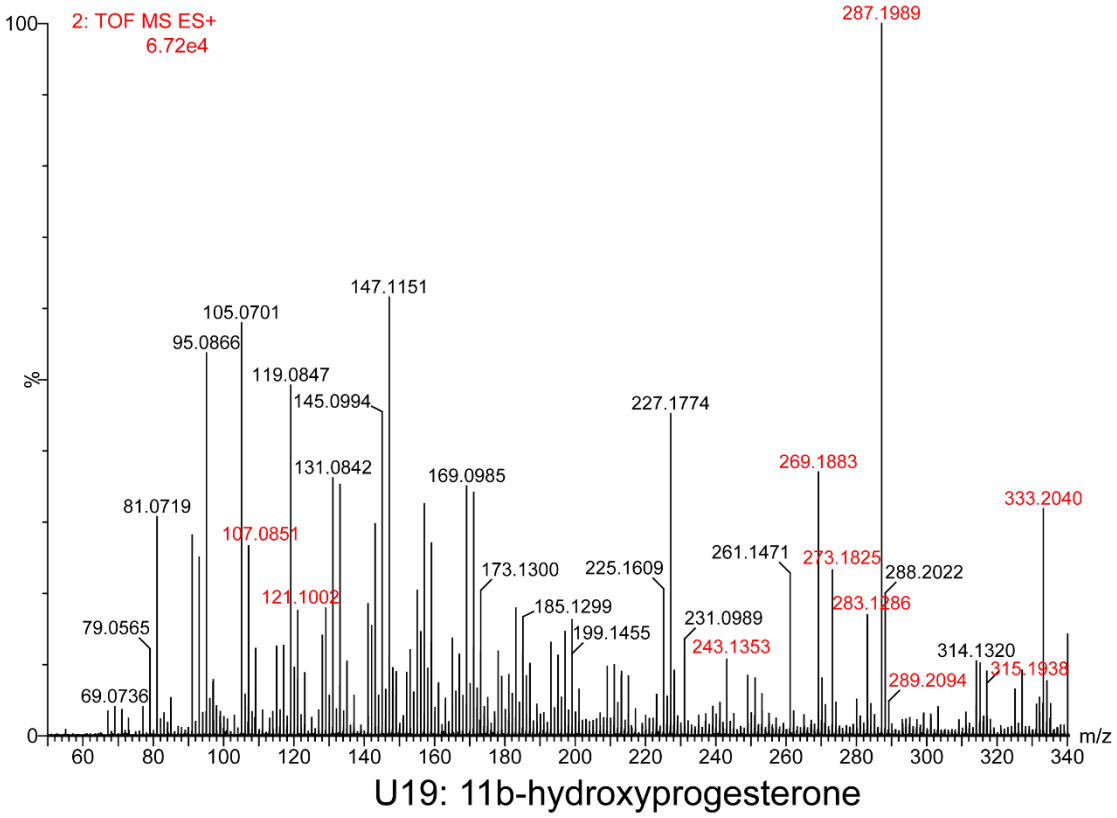


Figure S36: MS/MS spectra of the identified potential marker 11bb-hydroxyprogesterone in urine samples.

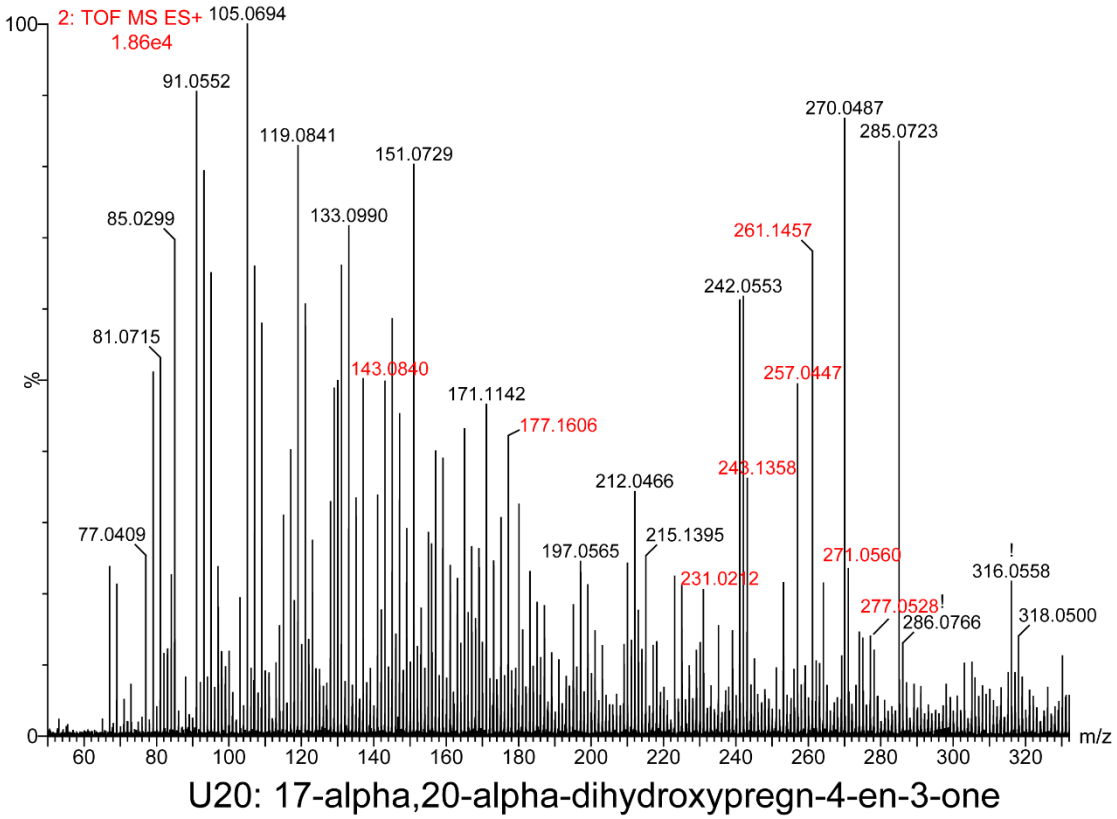


Figure S37: MS/MS spectra of the identified potential marker17-alpha,20-alpha-dihydroxypregn-4-

en-3-one in urine samples.

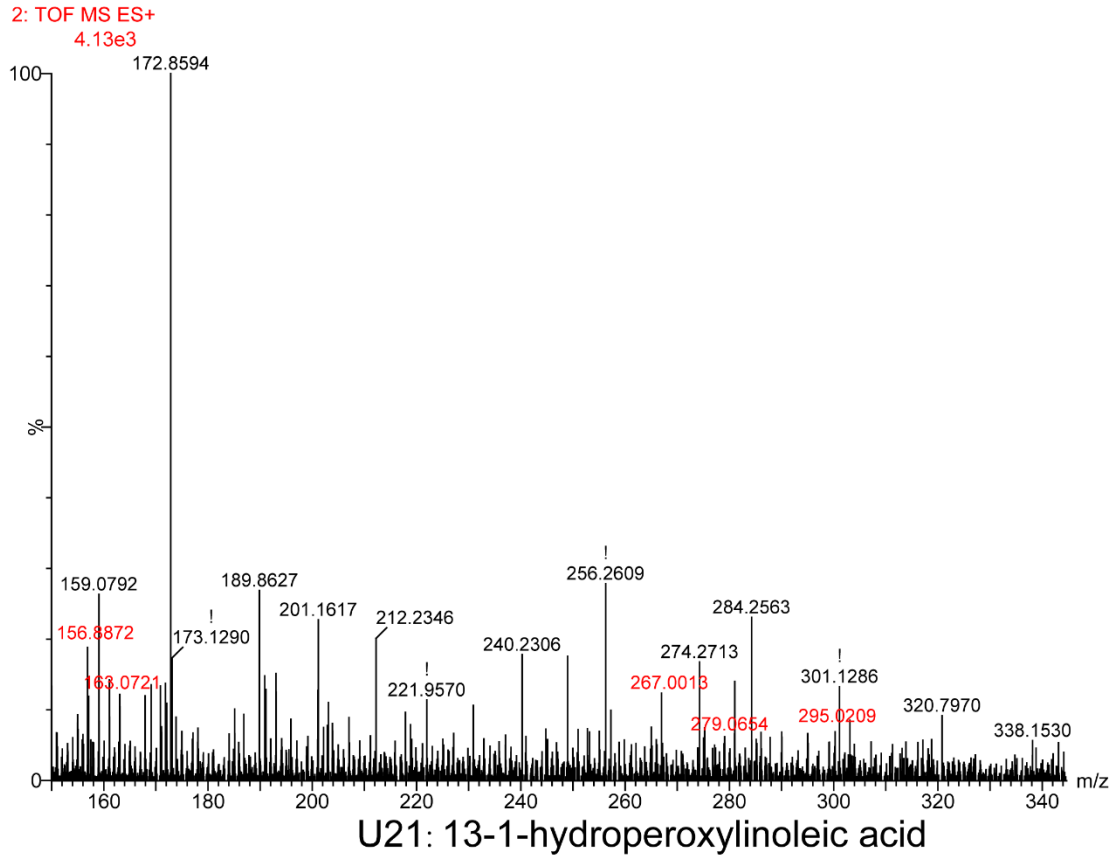


Figure S38: MS/MS spectra of the identified potential marker 13-l-hydroperoxylinoleic acid in urine samples.

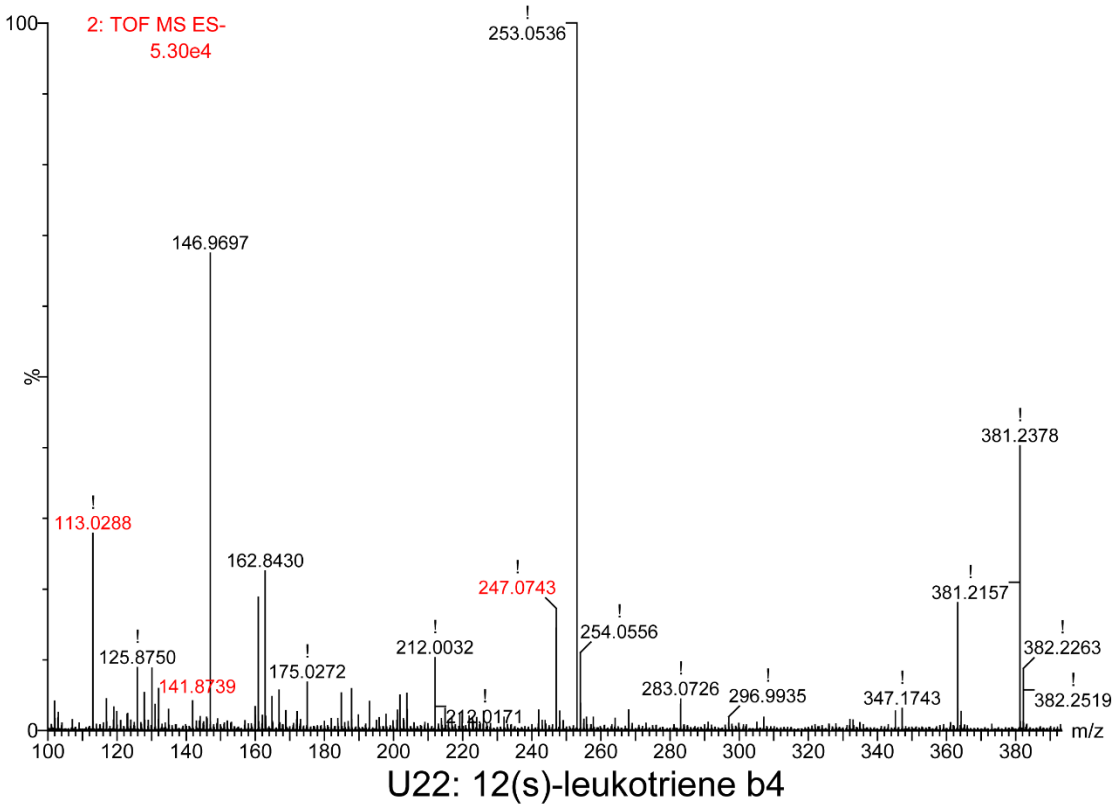


Figure S39: MS/MS spectra of the identified potential marker 12(s)-leukotriene b4 in urine samples.

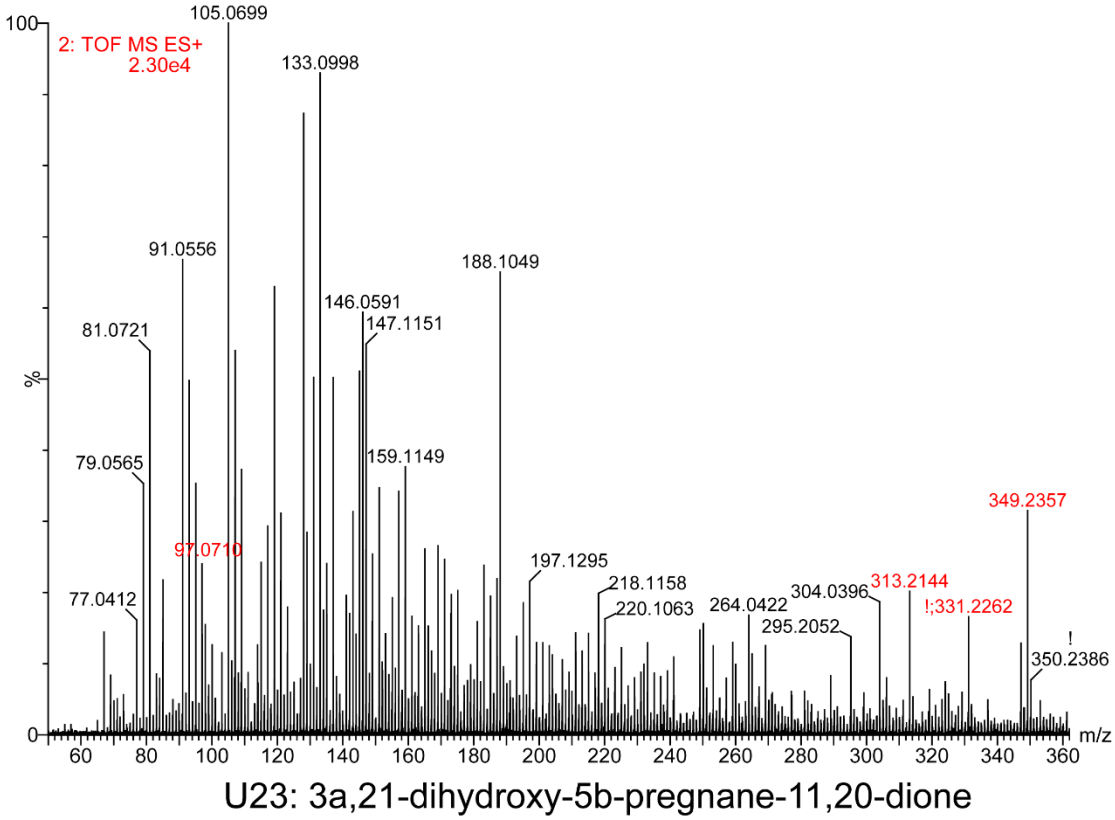


Figure S40: MS/MS spectra of the identified potential marker 3a,21-dihydroxy-5b-pregnane-11,20-dione in urine samples.

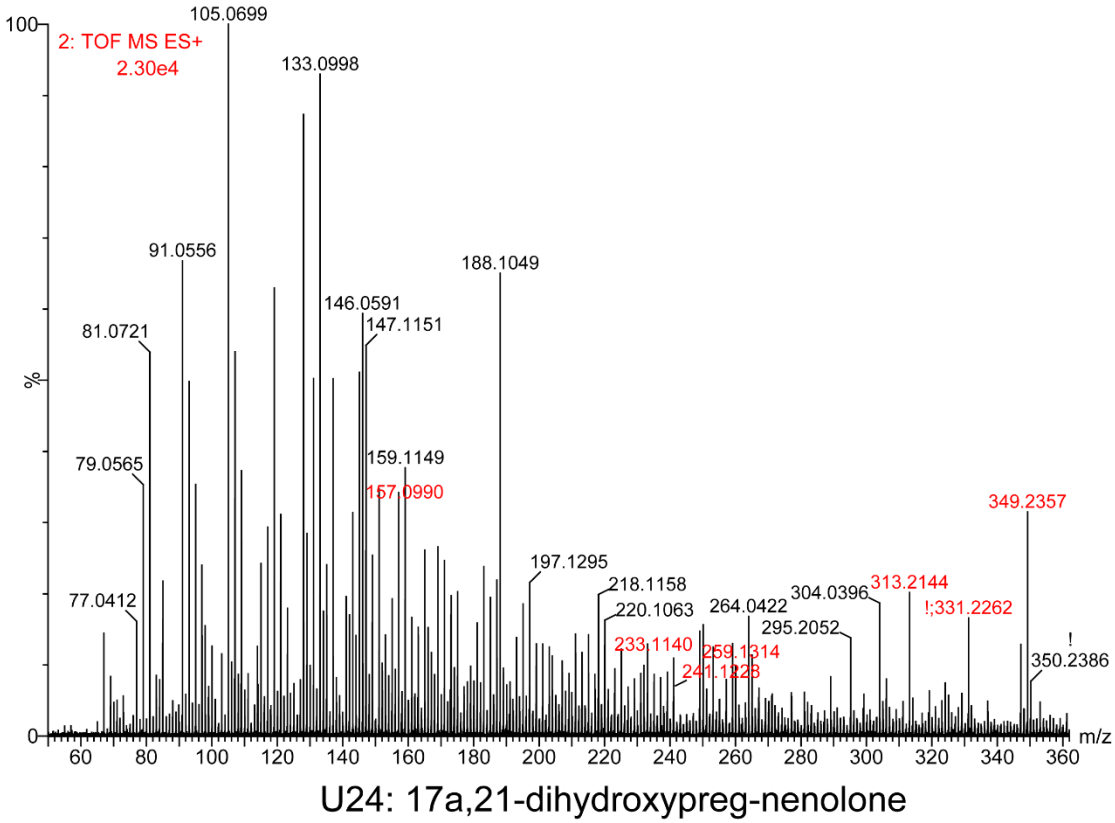


Figure S41: MS/MS spectra of the identified potential marker 17a,21-dihydroxypreg-nenolone in

urine samples.

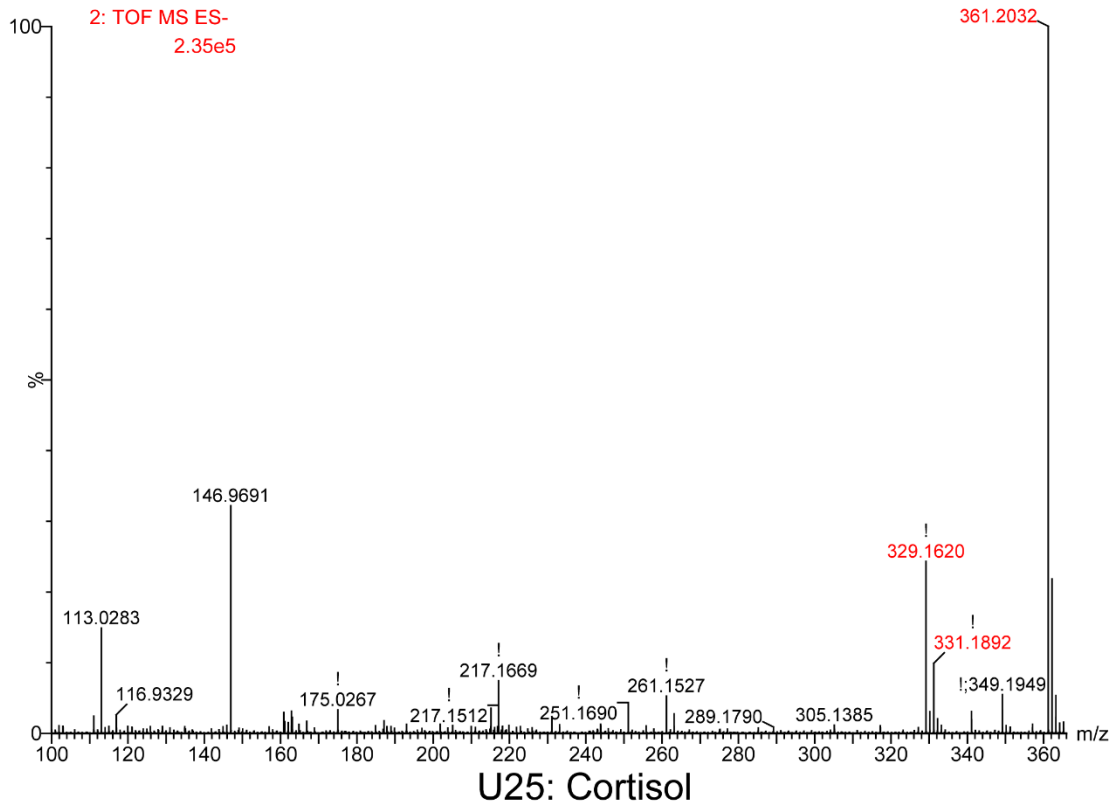


Figure S42: MS/MS spectra of the identified potential marker Cortisol in urine samples.

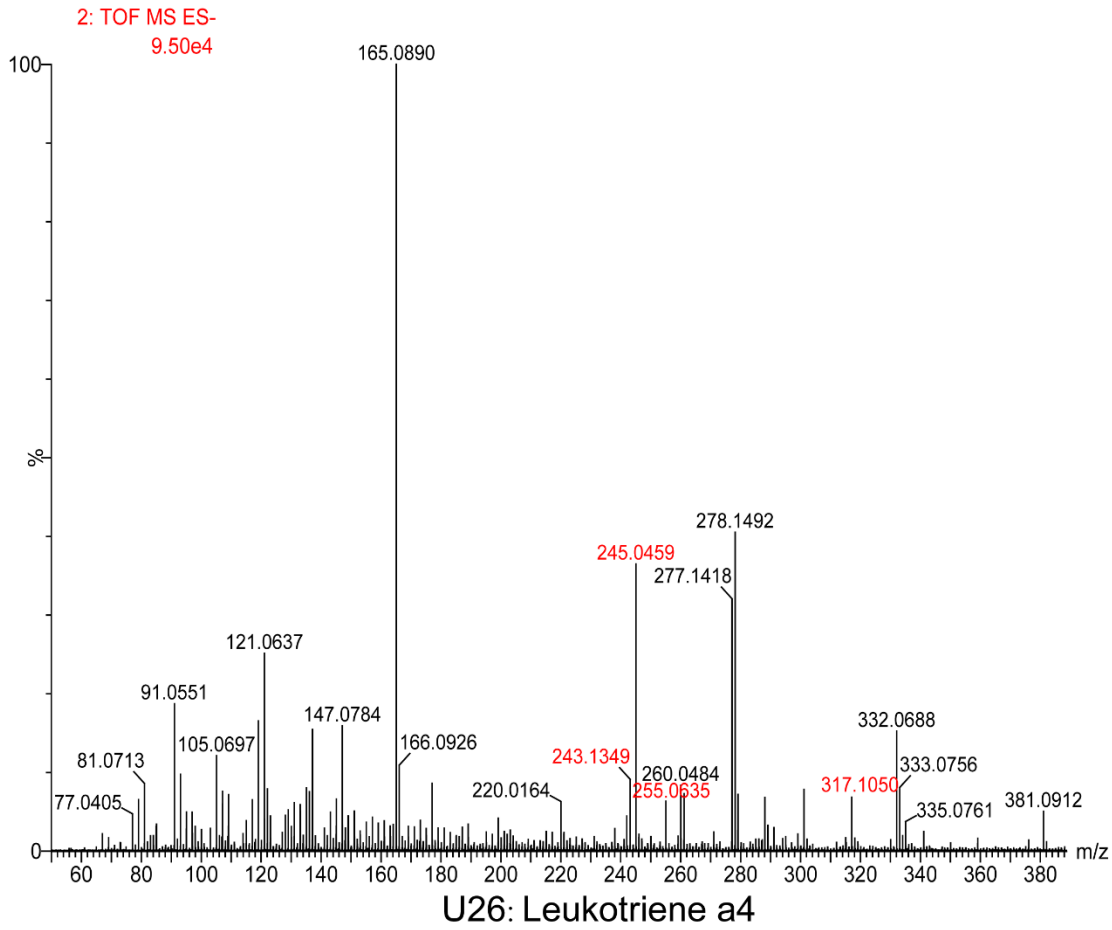


Figure S43: MS/MS spectra of the identified potential marker Leukotriene a4 in urine samples.

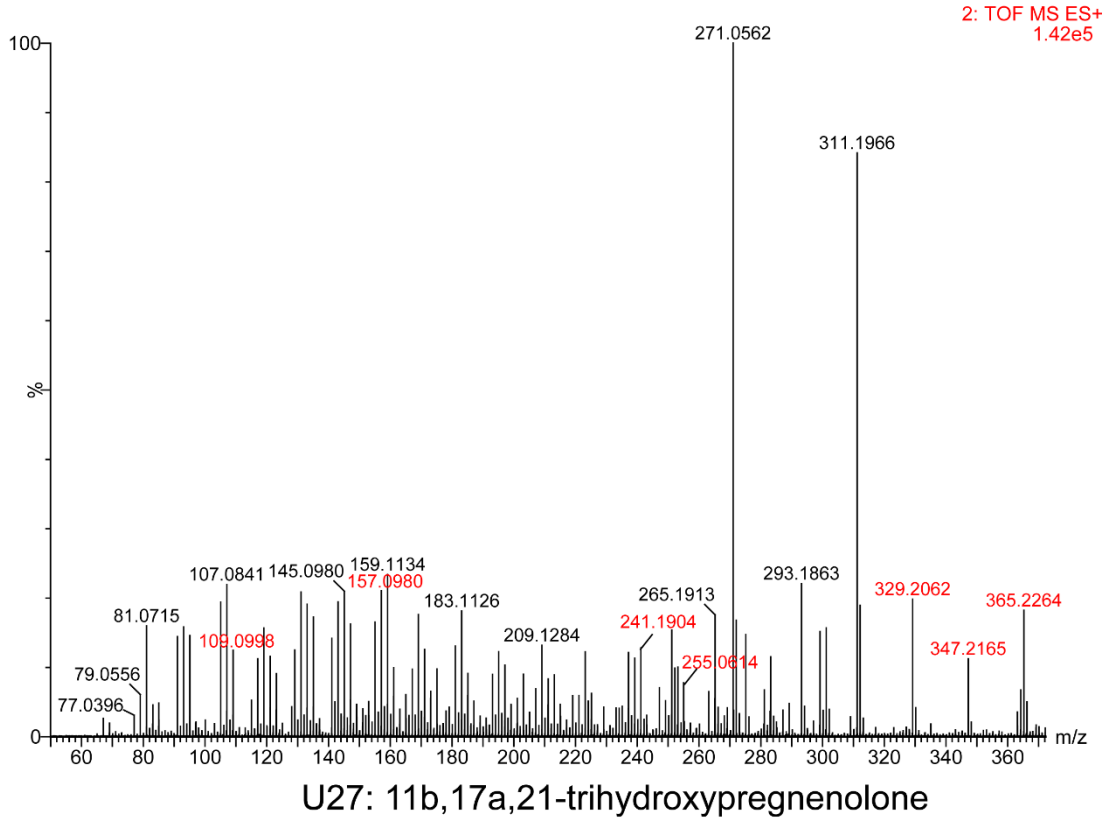


Figure S44: MS/MS spectra of the identified potential marker 11b,17a,21-trihydroxypreg-nenolone in urine samples.

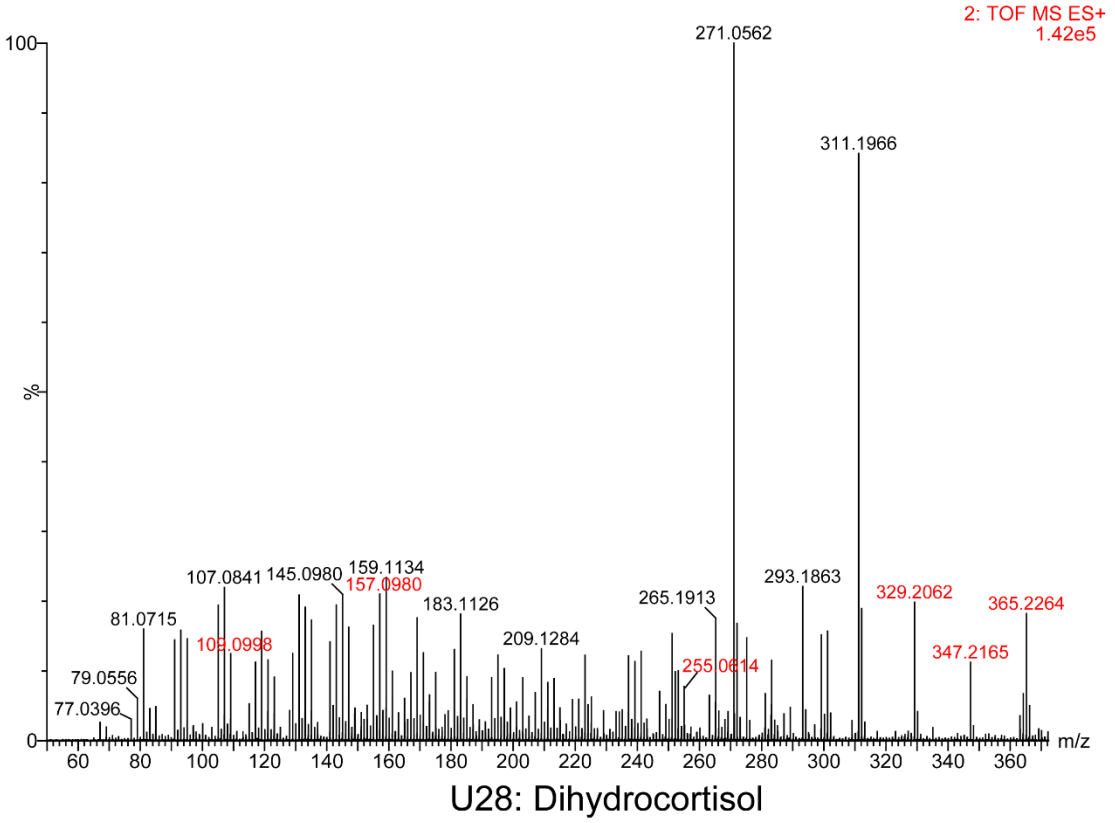


Figure S45: MS/MS spectra of the identified potential marker Dihydrocortisol in urine samples.

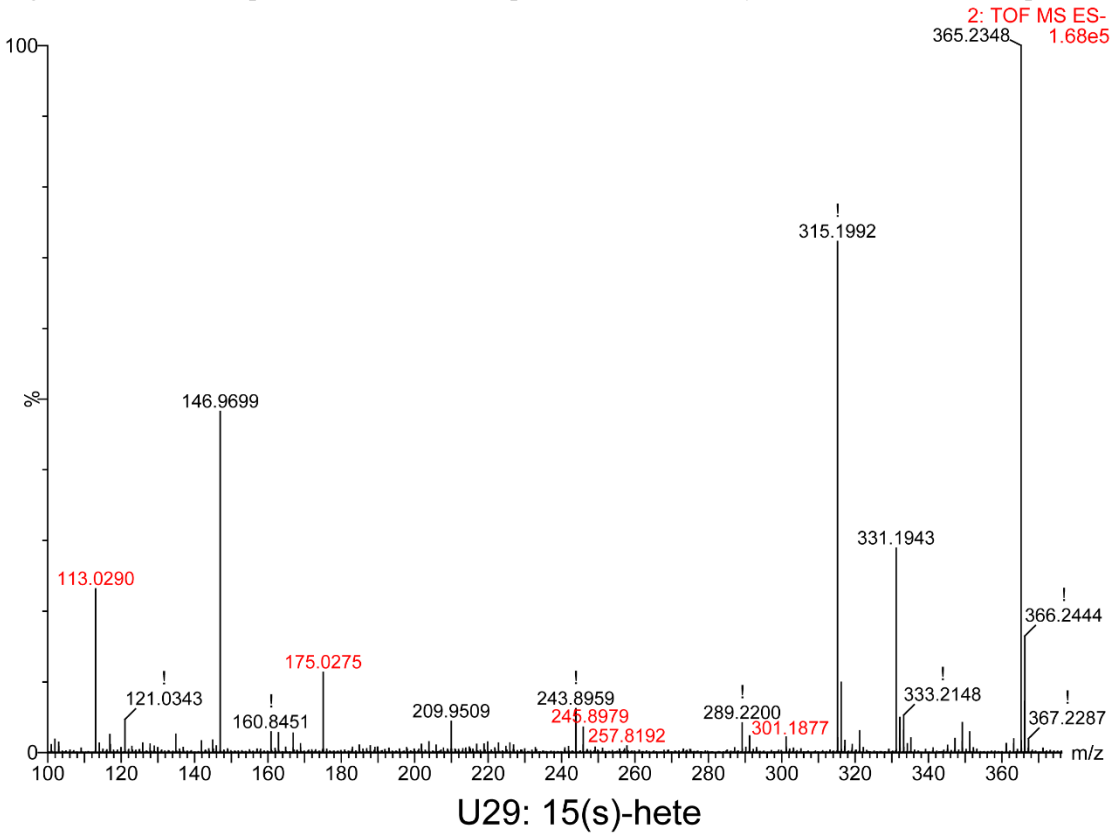


Figure S46: MS/MS spectra of the identified potential marker 15(s)-hete in urine samples.

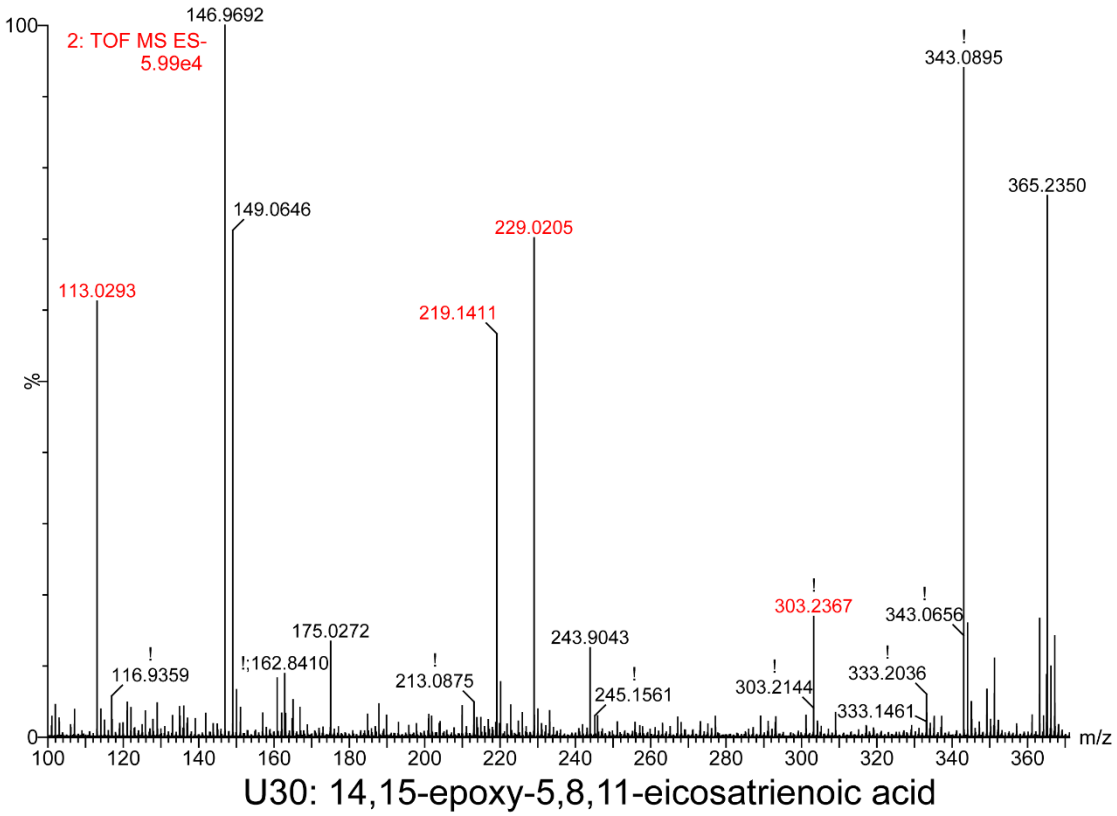


Figure S47: MS/MS spectra of the identified potential marker 14,15-epoxy-5,8,11-eicosatrienoic acid in urine samples.

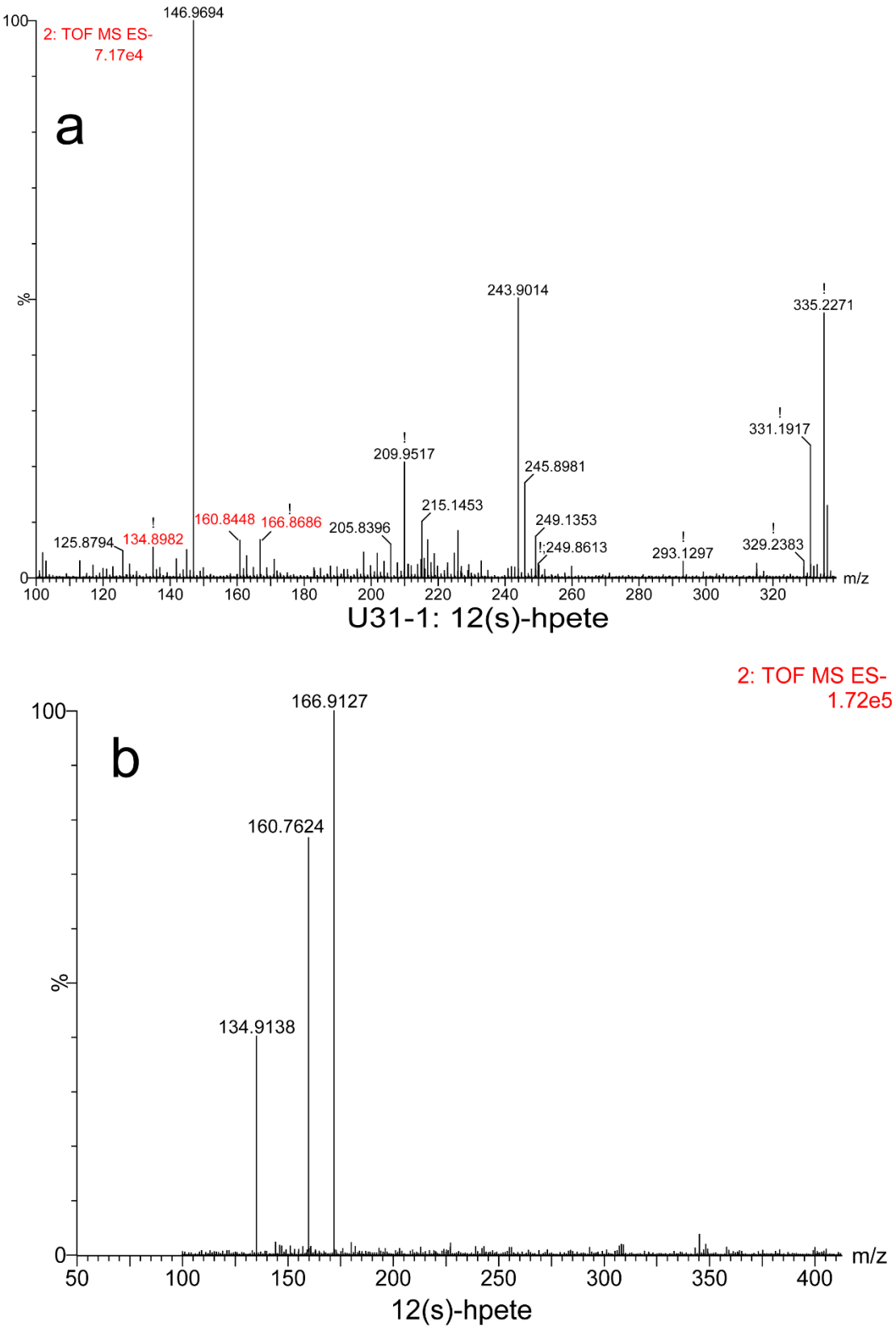


Figure S48: (a) MS/MS spectra of the identified potential marker 12(s)-hpete in urine samples. (b) The reference standard chemical spectra of 12(s)-hpete in experimental condition.

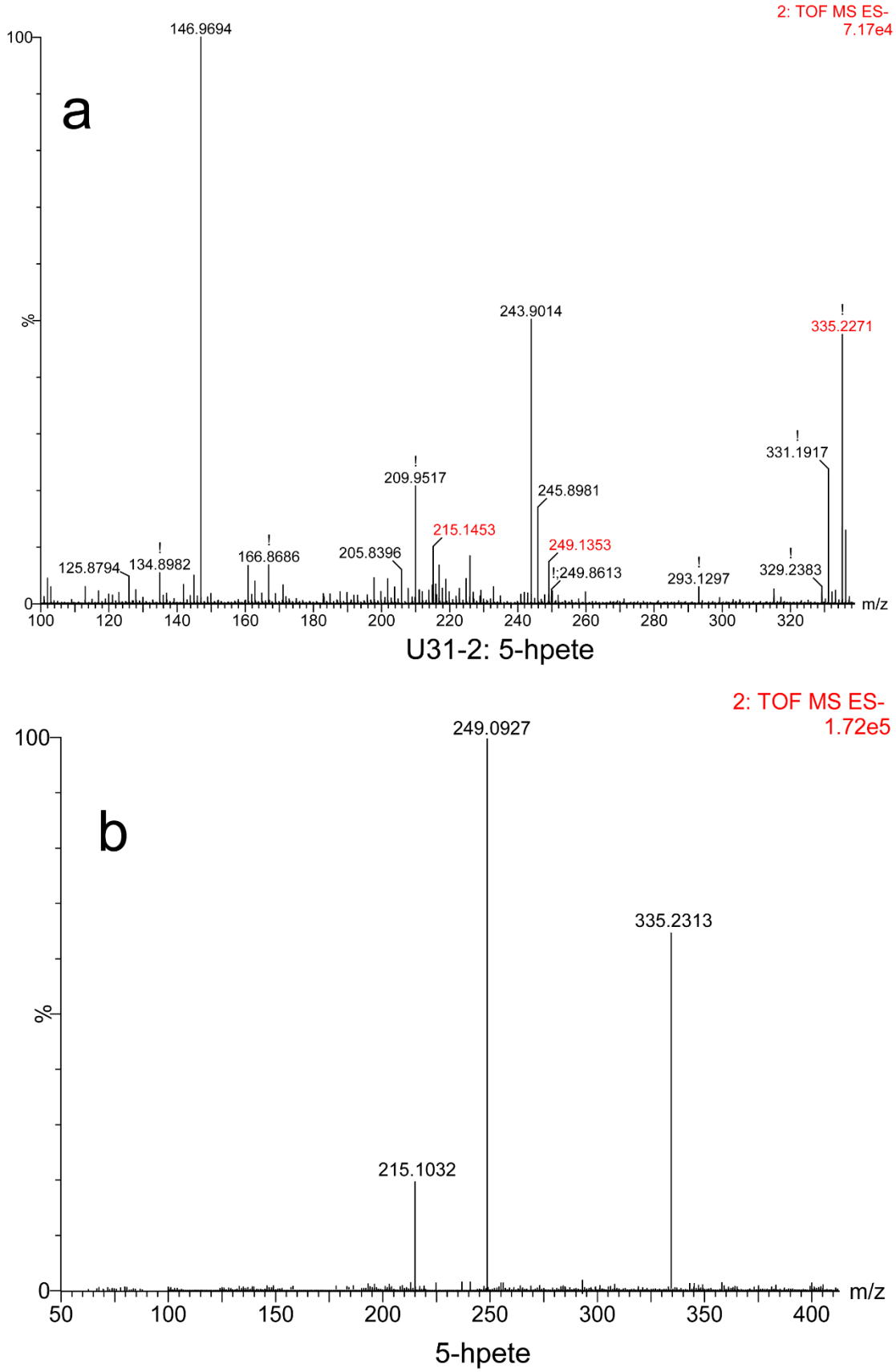


Figure S49: (a) MS/MS spectra of the identified potential marker 5-hpete in urine samples. (b) The reference standard chemical spectra of 5-hpete in experimental condition.

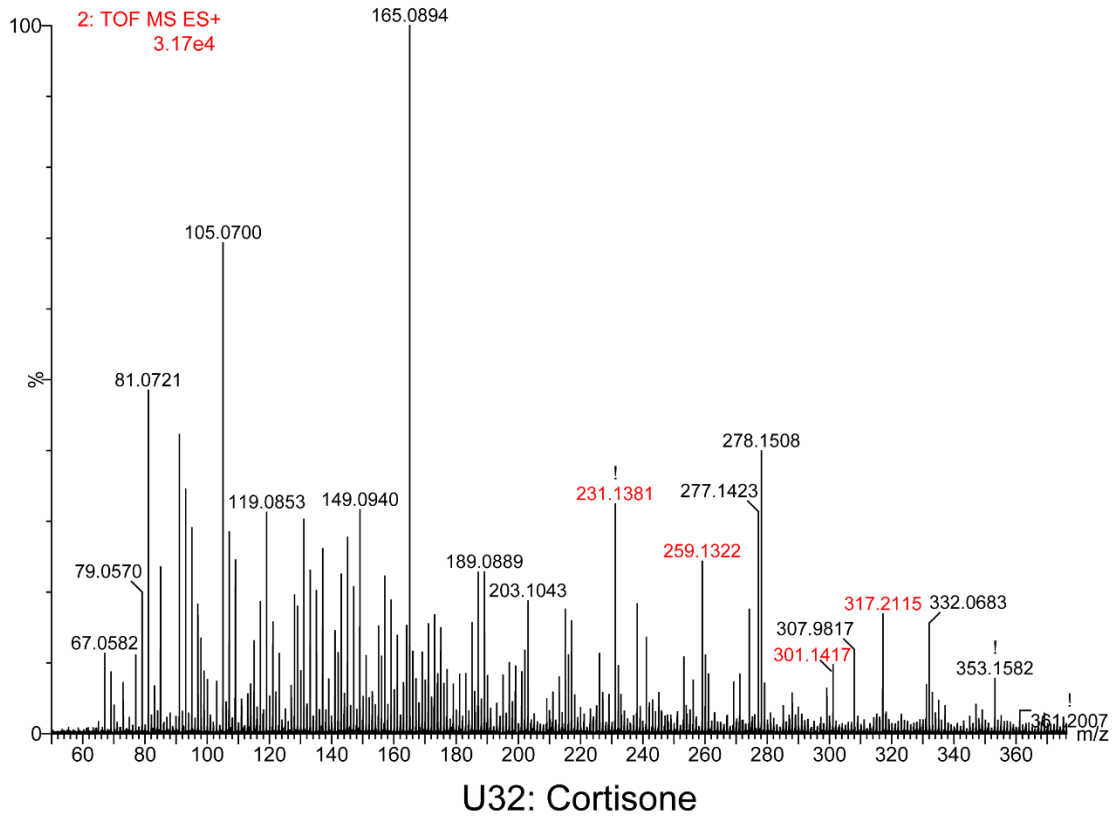


Figure S50: MS/MS spectra of the identified potential marker Cortisone in urine samples.

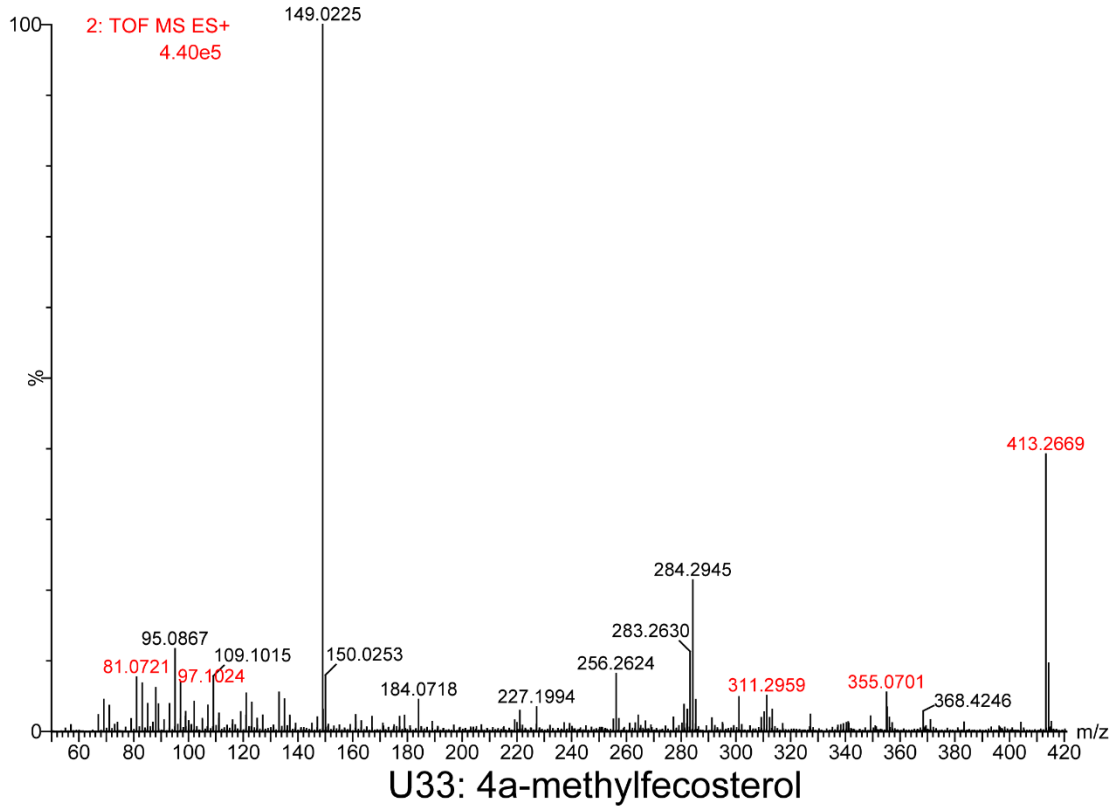


Figure S51: MS/MS spectra of the identified potential marker 4a-methylfecosterol in urine samples.

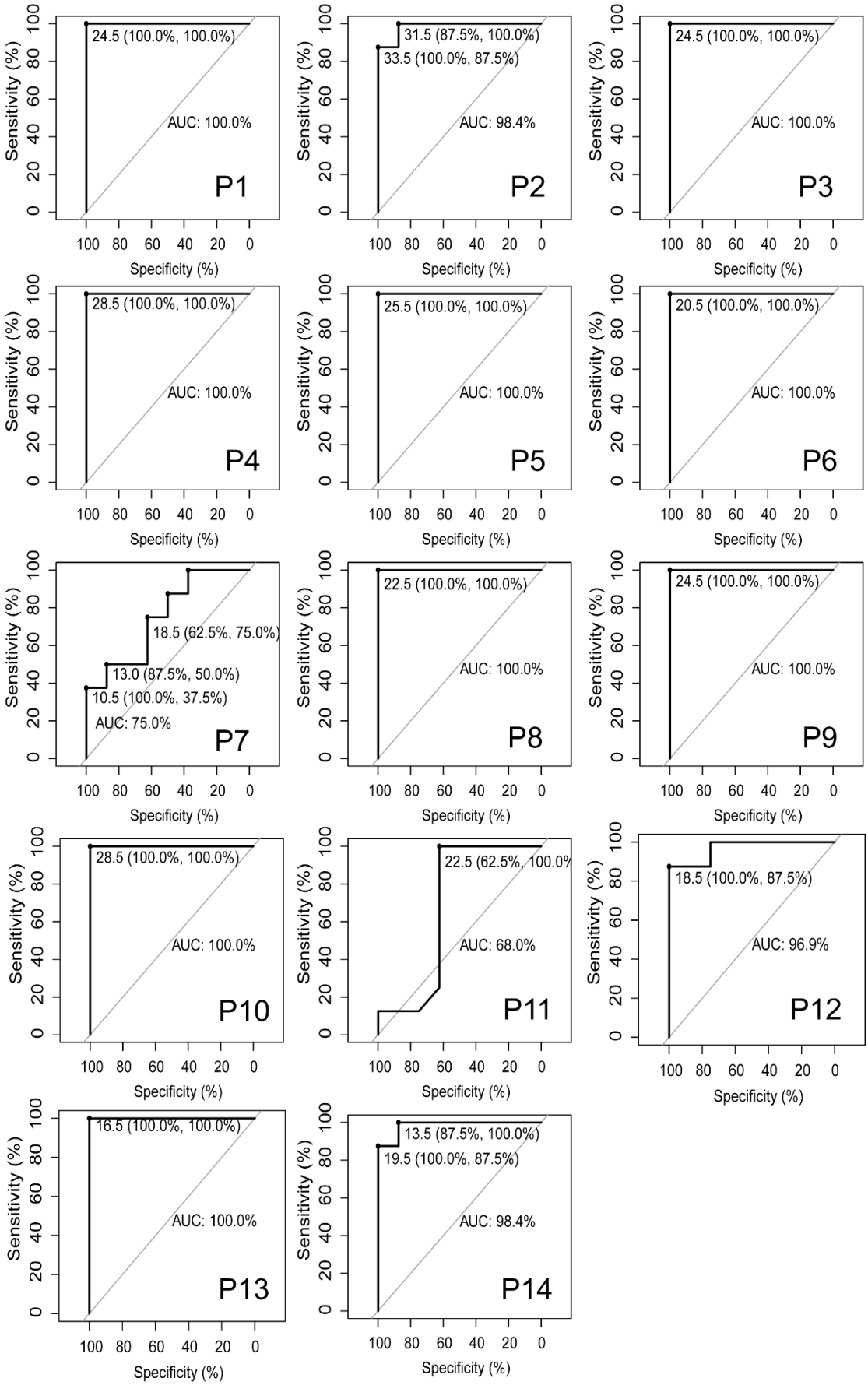
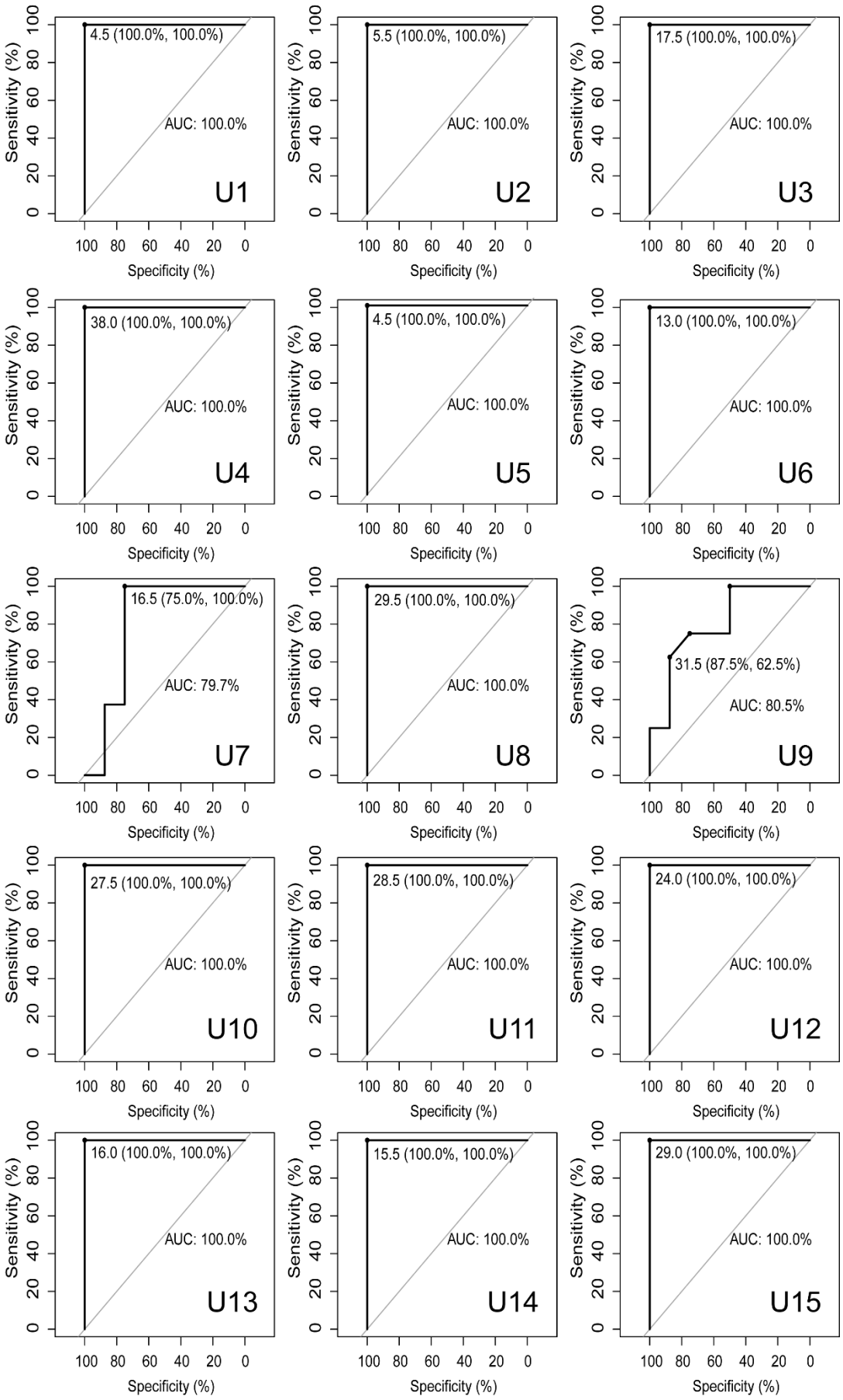
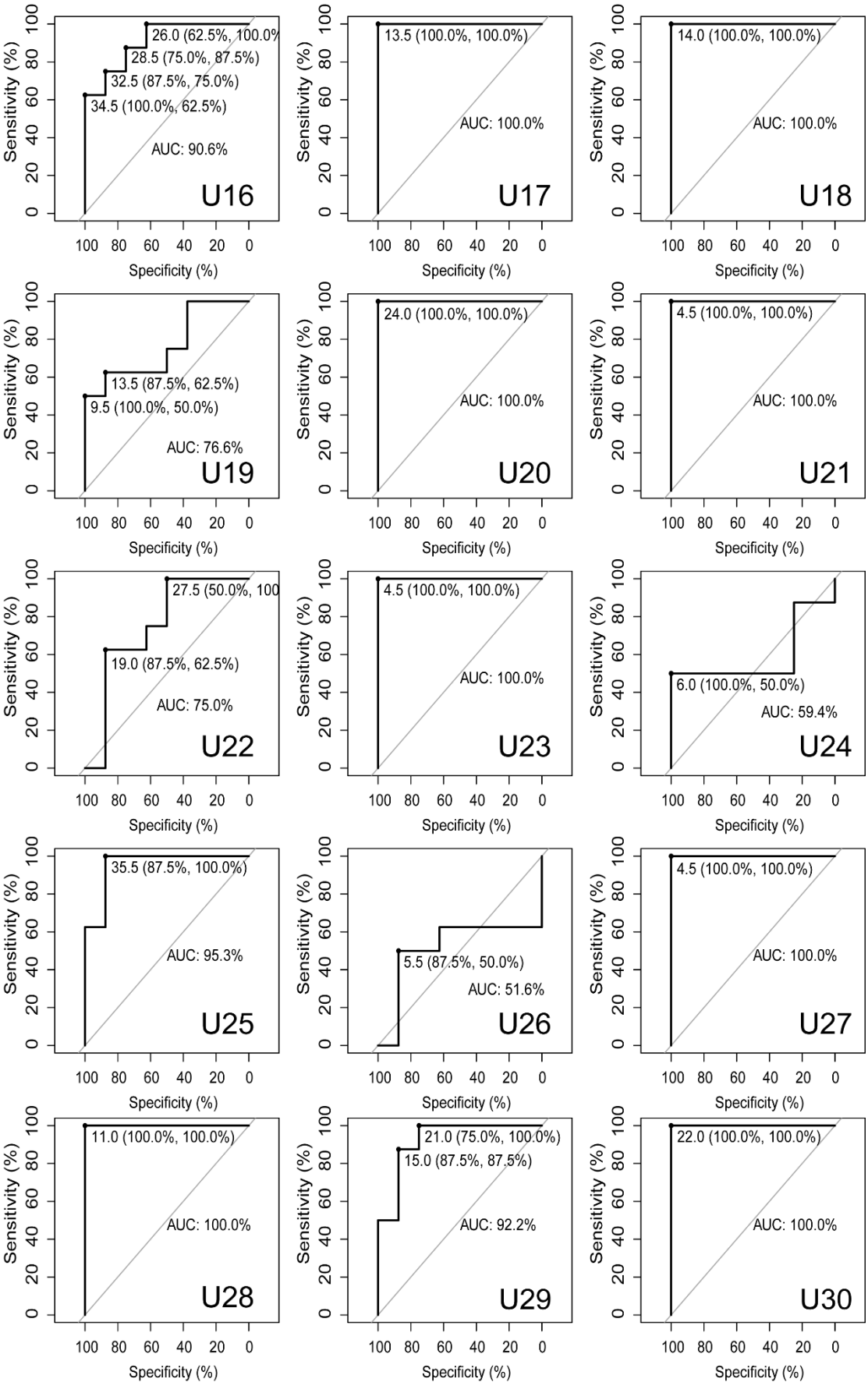

Sensitivity (%)
Specificity (%)
24.5 (100.0%, 100.0%)
AUC: 100.0%
P1
31.5 (87.5%, 100.0%)
33.5 (100.0%, 87.5%)
AUC: 98.4%
P2
24.5 (100.0%, 100.0%)
AUC: 100.0%
P3
28.5 (100.0%, 100.0%)
AUC: 100.0%
P4
25.5 (100.0%, 100.0%)
AUC: 100.0%
P5
20.5 (100.0%, 100.0%)
AUC: 100.0%
P6
18.5 (62.5%, 75.0%)
13.0 (87.5%, 50.0%)
10.5 (100.0%, 37.5%)
AUC: 75.0%
P7
22.5 (100.0%, 100.0%)
AUC: 100.0%
P8
24.5 (100.0%, 100.0%)
AUC: 100.0%
P9
28.5 (100.0%, 100.0%)
AUC: 100.0%
P10
22.5 (62.5%, 100.0%)
AUC: 68.0%
P11
18.5 (100.0%, 87.5%)
AUC: 96.9%
P12
16.5 (100.0%, 100.0%)
AUC: 100.0%
P13
13.5 (87.5%, 100.0%)
19.5 (100.0%, 87.5%)
AUC: 98.4%
P14

Figure S52: Evaluation on the accuracy of all differential metabolites to be biomarkers in plasma samples. All metabolites except P7, P11 have the potential to be biomarkers with high diagnostic accuracy. AUC, Area Under Curve.

a


4.5 (100.0%, 100.0%)
AUC: 100.0%
U1
5.5 (100.0%, 100.0%)
AUC: 100.0%
U2
17.5 (100.0%, 100.0%)
AUC: 100.0%
U3
38.0 (100.0%, 100.0%)
AUC: 100.0%
U4
4.5 (100.0%, 100.0%)
AUC: 100.0%
U5
13.0 (100.0%, 100.0%)
AUC: 100.0%
U6
16.5 (75.0%, 100.0%)
AUC: 79.7%
U7
29.5 (100.0%, 100.0%)
AUC: 100.0%
U8
31.5 (87.5%, 62.5%)
AUC: 80.5%
U9
27.5 (100.0%, 100.0%)
AUC: 100.0%
U10
28.5 (100.0%, 100.0%)
AUC: 100.0%
U11
24.0 (100.0%, 100.0%)
AUC: 100.0%
U12
16.0 (100.0%, 100.0%)
AUC: 100.0%
U13
15.5 (100.0%, 100.0%)
AUC: 100.0%
U14
29.0 (100.0%, 100.0%)
AUC: 100.0%
U15
Sensitivity (%)
Specificity (%)
0 20 40 60 80 100
100 80 60 40 20 0

b


26.0 (62.5%, 100.0%)
28.5 (75.0%, 87.5%)
32.5 (87.5%, 75.0%)
34.5 (100.0%, 62.5%)
AUC: 90.6%
U16
13.5 (100.0%, 100.0%)
AUC: 100.0%
U17
14.0 (100.0%, 100.0%)
AUC: 100.0%
U18
13.5 (87.5%, 62.5%)
9.5 (100.0%, 50.0%)
AUC: 76.6%
U19
24.0 (100.0%, 100.0%)
AUC: 100.0%
U20
4.5 (100.0%, 100.0%)
AUC: 100.0%
U21
27.5 (50.0%, 100
19.0 (87.5%, 62.5%)
AUC: 75.0%
U22
4.5 (100.0%, 100.0%)
AUC: 100.0%
U23
6.0 (100.0%, 50.0%)
AUC: 59.4%
U24
35.5 (87.5%, 100.0%)
AUC: 95.3%
U25
5.5 (87.5%, 50.0%)
AUC: 51.6%
U26
4.5 (100.0%, 100.0%)
AUC: 100.0%
U27
11.0 (100.0%, 100.0%)
AUC: 100.0%
U28
21.0 (75.0%, 100.0%)
15.0 (87.5%, 87.5%)
AUC: 92.2%
U29
22.0 (100.0%, 100.0%)
AUC: 100.0%
U30
Sensitivity (%)
Specificity (%)
100 80 60 40 20 0
0 20 40 60 80 100

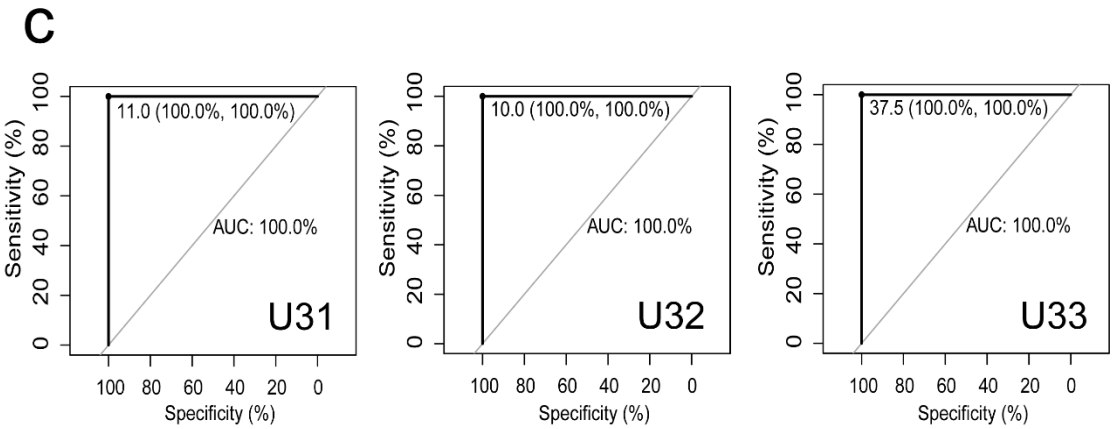


Figure S53: Evaluation on the accuracy of all differential metabolites to be biomarkers in urine samples. (a) 1-13 potential biomarkers in urine samples were evaluated with receiver operating characteristic curve (ROC). (b) 14-30 potential biomarkers in urine samples were evaluated with receiver operating characteristic curve (ROC). (c) 31-33 potential biomarkers in urine samples were evaluated with receiver operating characteristic curve (ROC). All metabolites except U7, U9, U19, U22, U24, U26, U29 have the potential to be biomarkers with high diagnostic accuracy. AUC, Area Under Curve.