A rapid and integrated pyramid screening method to classify and identify complex endogenous substances with UPLC/Q-TOF MS-based metabolomics

Yubo Li,[a] Zhenzhu Zhang,[a] Zhiguo Hou,[a] Lei Wang,[a] Xin Wu,[a] Liang Ju,[a] Xiuxiu Zhang[a] and Yanjun Zhang*[a]

[a]Tianjin State Key Laboratory of Modern Chinese Medicine, School of Traditional Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, 312 Anshan west Road, Tianjin 300193, China.

*Author for correspondence:

E-mail: tianjin_tcm001@sina.com.

Tel and Fax number: +86-22-59596223.

Table S1. Summary of methods for the data processing of different classes of substances and corresponding references depending on LC-MS.

Compound classification	Subclass	Parent ion	Diagnostic fragments (m/z)	Diagnostic fragment mass defect range (±10ppm)	Neutral loss (DA)	Mass rang (DA)	Mass defect range	References
Nucleic acids	Purines and Pyrimidines	$[M+H]^+$			17.02(NH_3) 43.00(HNCO)	111.0-181.1	0.0272-0.0702	1-4
	Nucleosides	$[M+H]^+$			116.04($C_5H_8O_3$)[a] 132.04($C_5H_8O_4$)[b]	227.0-311.2	0.0695-0.1230	
	Nucleotides					Contains a phosphate group		
						304.0-368.1	0.0253-0.0682	
						Contains two phosphate groups		
						387.0-443.1	0.0021-0.0346	
						Contains there phosphate groups		
						466.9-524.0	-0.0314-0.0009	
Steroid hormones	Estrogens	$[M-H]^-$	145.06$[C_{10}H_9O]^-$ [c]	0.0639-0.0667		270.1-288.2	0.1412-0.1776	5-7
	Androgens	$[M+H]^+$	97.06$[C_6H_9O]^+$ [d]	0.0643-0.0663		286.1-304.3	0.1725-0.2090	8-11
	Progestagens Glucocorticoids Mineralocorticois		109.06$[C_7H_9O]^+$ [e] 121.06$[C_8H_9O]^+$ [f]	0.0642-0.0664 0.0640-0.0666		312.2-378.3	0.1885-0.2403	
Amino acids		$[M+H]^+$			17.02(NH_3) 46.00(HCOOH) 18.01(H_2O)	75.0-292.2	-0.0081-0.2100	12-14

		[M-H]$^-$			17.02(NH_3) 43.98(CO_2)			
Monosaccharides	Aldoses and Ketoses	[M-H]$^-$			18.01(H_2O)[g] 60.02($C_2H_4O_2$)[g]	150.0-180.1	0.0527-0.0686	15
Organic acids	Carboxylic acids[h]	[M-H]$^-$			43.98(CO_2)	60.0-230.2	0.0003-0.1519	16,17
Phospholipids	Lysophosphatidylcholine (LPC)	[M+H]$^+$	184.07[$C_5H_{15}NO_4P$]$^+$ 104.10[$C_5H_{14}NO$]$^+$	0.0720-0.0758 0.1064-0.1086		412.2-607.5	0.2464-0.4577	18,20,21,24,26
	Phosphatidylcholine (PC)	[M+H]$^+$	184.07[$C_5H_{15}NO_4P$]$^+$ 104.10[$C_5H_{14}NO$]$^+$	0.0720-0.0758 0.1064-0.1086		673.4-957.9	0.4682-0.8126	18,20-22,24,26,27
	Sphingomyelin (SM)	[M+H]$^+$	184.07[$C_5H_{15}NO_4P$]$^+$ 104.10[$C_5H_{14}NO$]$^+$	0.0720-0.0758 0.1064-0.1086		646.5-843.8	0.5049-0.7320	19-22,24
	Lysophosphatidylethanolamine (LPE)	[M+H]$^+$			141.01($C_2H_8NO_4P$)	423.2-565.5	0.2385-0.4108	18,20,21
	Phosphatidylethanolamine (PE)	[M+H]$^+$			141.01($C_2H_8NO_4P$)	631.4-915.8	0.4213-0.7657	18,20-23,26,27
	Lysophosphatidic acid (LPA)	[M-H]$^-$	152.99[$C_3H_6PO_5$]$^-$	0.9937-0.9968		410.2-438.3	0.2433-0.2954	27,28
	Phosphatidic acid (PA)	[M-H]$^-$	152.99[$C_3H_6PO_5$]$^-$	0.9937-0.9968		648.4-700.6	0.4730-0.5251	27,28
	Phosphatidylserine (PS)	[M+H]$^+$			185.00($C_3H_8NO_6P$)	675.4-879.6	0.4111-0.5990	20,22,24,29
		[M-H]$^-$			87.07($C_3H_5NO_2$)			22,25,27,30
	Phosphatidylinositol (PI)	[M+H]$^+$			260.02($C_6H_{13}PO_9$)	806.4-914.6	0.4945-0.5885	20,21,24,26
		[M-H]$^-$	241.01[$C_6H_{10}PO_8$]$^-$	0.0088-0.0138				22,23,27

Phosphatidylglycerol	$[M+H]^+$			172.01($C_3H_9PO_6$)	718.4-826.6	0.4784-0.5724	20,24
(PG)	$[M-H]^-$	152.99$[C_3H_6PO_5]^-$	0.9937-0.9968				27
Ceramide					481.4-679.7	0.4494-0.6843	

[a] Deoxynucleosides produce the neutral fragment of m/z 116.04 ($C_5H_8O_3$) after collision induced dissociation (CID) process in the positive mode.

[b] Nucleosides produce the neutral fragment of m/z 132.04 ($C_5H_8O_4$) after the CID process in the positive mode.

[c] Estrogen that A ring is a phenyl group and the C_3-position that only replaces the phenolic hydroxyl group can generate m/z 145.06 $[C_{10}H_9O]^-$ in the negative mode.

[d] Androgens, progestagens, glucocorticoids, and mineralocorticois that A ring have Δ^4-3-ketone group and C_4-position containing ethylenic bonds generate m/z 97.06 $[C_6H_9O]^+$ in the positive mode, except C_6-position.

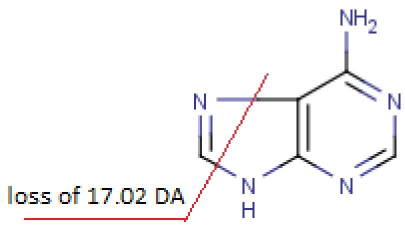
[e] Compound that meets “d” and C_6-position with methyl generate m/z 109.06 $[C_7H_9O]^+$ in the positive mode.

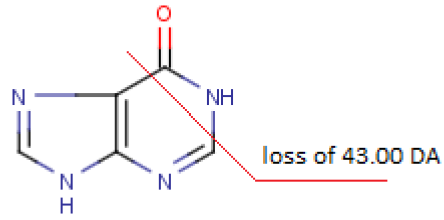
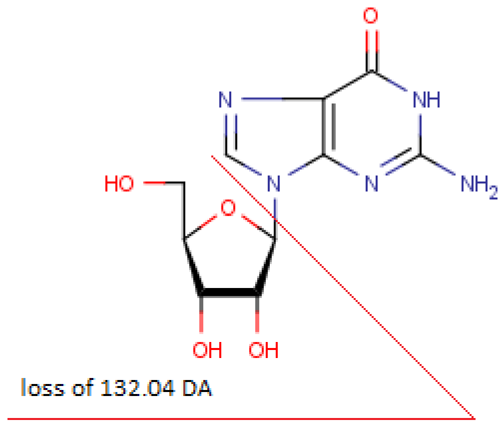
[f] Compound that meets “d” and C_6-position without methyl generate m/z 121.09 $[C_8H_9O]^+$ in the positive mode.

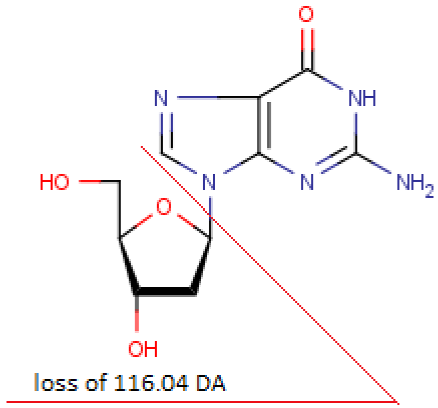
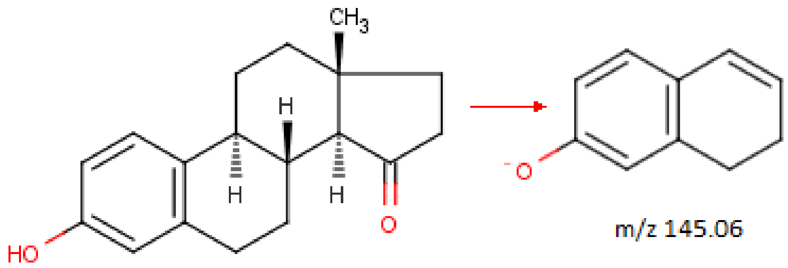
[g] Ketose and aldose (5–6 carbon atoms) can produce m/z 18.01 (H_2O) and m/z 60.02 ($C_2H_4O_2$) in the negative mode.

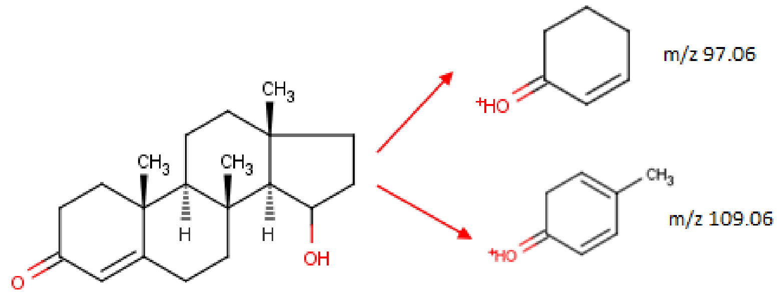
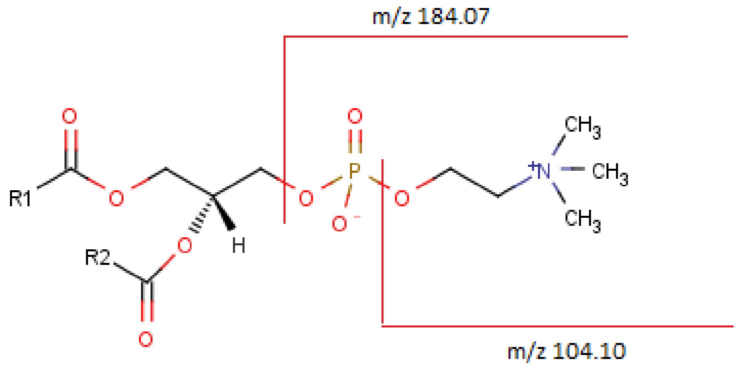
[h] Short-chain and medium-chain of carboxylic acids

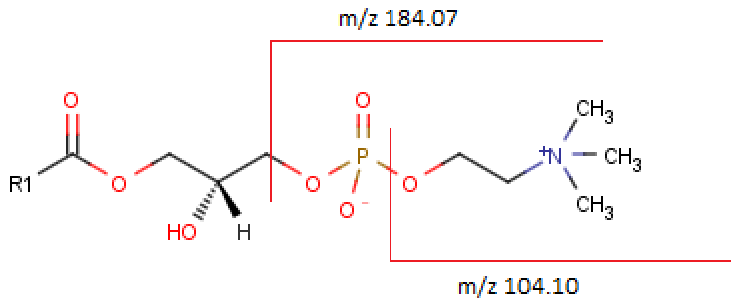
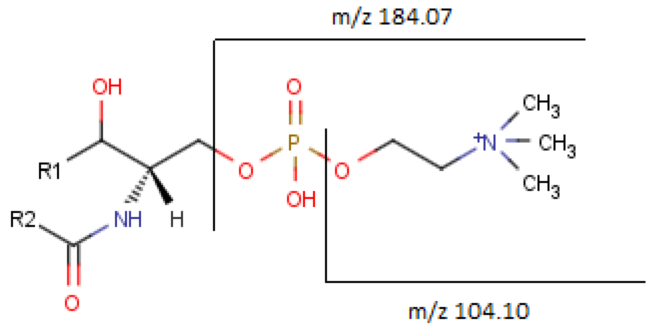
Table S2. Fragmentation rules of different classification of substances.

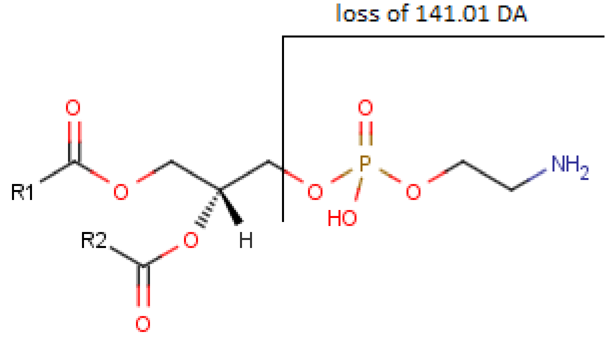
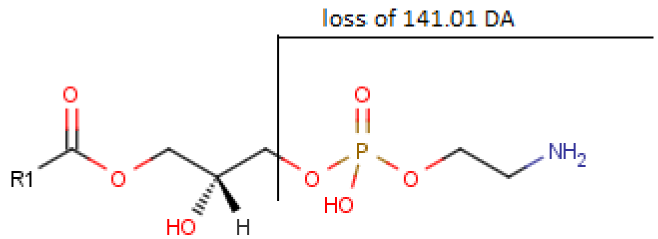
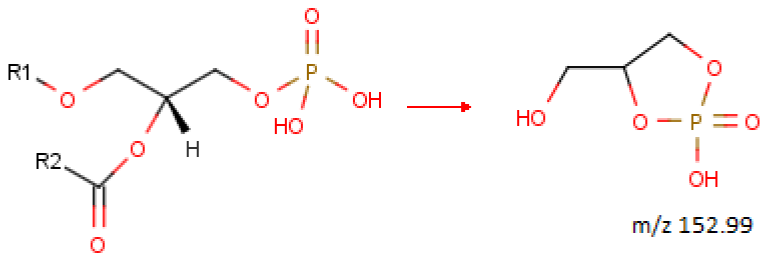
NO.	Endogenous substance	Mode	Structure
1	Adenine	Positive	

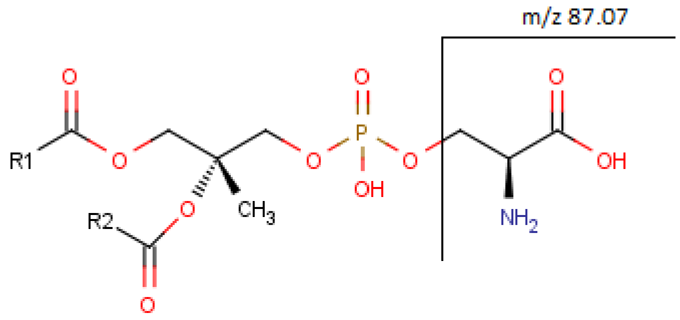
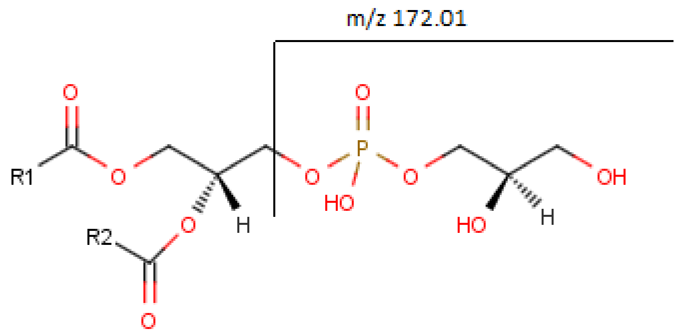
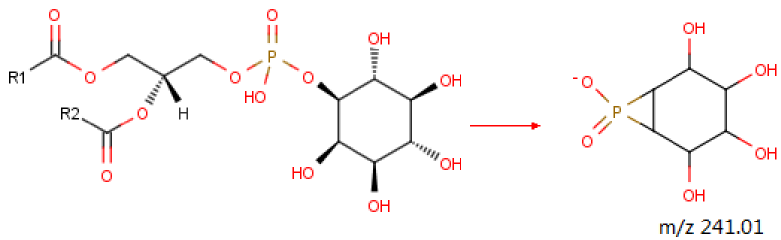
2	Hypoxanthine	Positive	
3	Guanosine	Positive	

4	2’-Deoxyguanosine	Positive	
5	Estrone	Negative	

6	Testosterone	Positive	
7	Phosphatidylcholine (PC)	Positive	

8	Plasmenylcholine (LPC)	Positive	
9	Sphingomyelin (SM)	Positive	

10	Phosphatidylethanolamine (PE)	Positive	
11	Lysophosphatidylethanolamine (LPE)	Positive	
12	Phosphatidic acid (PA)	Negative	

13	Phosphatidylserine (PS)	Negative	
14	Phosphatidylglycerol (PG)	Positive	
15	Phosphatidylinositol (PI)	Negative	

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