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Metabolomics Analysis of Viral Therapeutics

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Abstract

Virotherapy, enabled by recent advances in the transdisciplinary field of biotechnology, has emerged as a powerful tool for use in anticancer treatment, gene therapy, immunotherapy, etc. Examining the effects of viruses and virus-derived immune-modulating therapeutics is of great fundamental and clinical interest. Here we describe a sample preparation protocol for metabolite extraction from virus-infected tissue, in addition to liquid chromatography-mass spectrometry conditions essential for subsequent analysis. This metabolomics approach delivers highly sensitive and specific metabolite information on various biospecimens. Such an approach may be adopted to monitor biological changes in over 30 relevant metabolic pathways in response to viral infection and also viral therapeutics.

Keywords

LC-MS/MS; Metabolomics; Sample preparation; Viral vector; Virotherapy

1 Introduction

There is great interest in the use of viruses and virus-derived components as therapeutics. Viral vectors also hold enormous potential to realize the goals of gene therapy [1] and, in the past decade, more than 1700 gene therapy trials have been enabled by the use of viral therapeutics [2]. In addition to their use as a gene therapy delivery vehicle, viral vectors may also be used as an alternative to antibiotics [3] and, recently, have proven useful in DNA vaccination [4]. Utilization of adeno-associated viruses (AAV) for the deliverance of engineered DNA to target cells [5] has led to the development of fit-for-purpose AAV vectors [6]. Further popularizing such therapeutic concepts is the advent of bacteriophage lambda vectors, or RNA-binding proteins of phage origin [7]. These phage vectors have become an attractive vaccine platform given their inexpensive production, biological stability, and suitability to a wide range of applications with minimal development needed between uses [8]. Additionally, virus-derived immune-modulating proteins may also be used for therapeutic purposes. For example, rabies-derived glycoproteins have been shown to facilitate drug delivery to the central nervous system (CNS) [9], while virus-derived anti-inflammatory proteins may improve the human immune response to immune-mediated

diseases and in various cancers through inhibition of protease, chemokine, cytokine, and apoptotic cascades [10].

Since metabolites are sensitive to subtle differences and changes in pathological status and immune response status, metabolomics, the comprehensive study of small-molecular-weight metabolites and their dynamic changes in biological systems [11–15], provides advanced methods to identify changing metabolite levels, and has resulted in the rapid discovery of disease biomarkers during the past decade (*see* Fig. 1) [16–21]. Mass spectrometry (MS)-based metabolic profiling has proven to be a promising tool for analyzing metabolic alterations due to various diseases and, therefore, can provide sensitive and valuable diagnostic information [18, 22, 23], pathogenesis identification [24, 25], and potential therapeutic targets for clinical treatments [26] and disease monitoring [27]. Indeed, previous studies have applied metabolomics to various aspects of viral therapeutics such as the elucidation of metabolites involved in virus infection and pathogenesis [28] and accurate differentiation of vaccination status [29], in addition to characterizing the metabolic profiles of patients with hepatitis B virus-related hepatocellular carcinoma [30], viruses of the gastrointestinal microbiome [31], inflammatory cytokines involved in H1N1 influenza [32], as well as fingerprinting of HIV-1 and -2 infection in macrophages [33].

Herein, we present a sample preparation protocol and associated liquid chromatography-tandem mass spectrometry (LC-MS/MS) conditions for the detection of aqueous metabolites in tissue (*see* Fig. 2). The targeted LC-MS/MS method used here was modeled and developed after those used in a growing number of studies [18, 34–39].

2 Materials

Prepare all solutions using ultrapure water (*see* Note 1). Ensure that all reagents used are at least analytical grade (≥99% purity). Prepare and store all reagents at room temperature unless indicated otherwise. Follow all pertinent waste disposal protocols as they apply. Thaw samples as directed. Check pipettes to confirm readiness for use. This targeted LC-MS/MS protocol does not provide absolute concentrations of detected metabolites.

2.1 Preparation of Tissue Samples

1. 1.5 mL Eppendorf tubes.
2. Bullet beads.
3. 2 mL glass vials.
4. 300 μL glass inserts.
5. 1 mL needle-less syringes.
6. Polyvinylidene fluoride (PVDF) syringe filters.
7. Micropipettes with 50–800 μL extraction capability.
8. 100 mL Methanol (MeOH): 10× dilution with phosphate-buffered saline (PBS).
9. 100 mL PBS:acetonitrile ([ACN] 4:6 dilution).

10. Scientific balance with mg readability.
11. Bullet blender.
12. Vortex mixer.
13. Ultrasonic liquid processor.
14. Centrifuge with speed, time, and temperature control.
15. Vacuum concentrator.

2.2 LC-MS/MS Targeted Analysis

1. Suitable ultra-performance liquid chromatography (UPLC) system.
2. Triple-quadrupole (QQQ)-MS instrument.
3. Electrospray ionization (ESI) source.
4. Hydrophilic interaction chromatography (HILIC) column.
5. Mobile-phase solvents:
   a. 10 mM ammonium acetate, 10 mM ammonium hydroxide in 95% H_2O/5% ACN.
   b. 10 mM ammonium acetate, 10 mM ammonium hydroxide in 95% ACN/5% H_2O.

3 Methods

In an effort to identify and overcome potential issues, please see Subheading 4.

3.1 Preparation of Tissue Samples

1. Weigh out 20 mg ± 1.5 mg of biological sample in a 1.5 mL Eppendorf tube.
2. Homogenize biological sample in 200 μL MeOH:10× diluted PBS in 1.5 mL Eppendorf tube (*see* Note 2).
3. Add ½ spoon of beads and disrupt the sample using bullet blender for 2 min.
4. Centrifuge for 1–2 min to remove liquid from the cap.
5. Add 800 μL MeOH:10× diluted PBS (*see* Note 3).
6. Vortex for 10 s.
7. Store sample at −20 °C for 30 min.
8. Sonicate the mixture in an ice bath for 10 min (*see* Note 4).
9. Centrifuge the mixture at 21,694 × *g* for 15 min at 4 °C (*see* Note 5).
10. Remove 800 μL supernatant and transfer to a new Eppendorf tube (*see* Note 6).
11. Completely dry the sample(s) using a vacuum concentrator at 37 °C for 6 ± 2 h (*see* Note 7).

12. Once sample(s) is/are completely dried, reconstitute by adding 200 μL of PBS:ACN solution.
13. Vortex the sample for 30 s.
14. Sonicate each sample for 5 min in a room-temperature water bath.
15. Centrifuge the samples at 21,694 × *g* for 10 min at 4 °C (*see* Note 8).
16. Extract 150 μL of the supernatant into a syringe and slowly plunge the sample through the filter and into a new 1.5 mL Eppendorf tube (*see* Note 9).
17. Extract the remaining supernatant from each sample and inject into a single 2 mL tube (larger tubes may be used if needed) for quality control (QC) analysis.
18. Transfer 100 μL of the filtered experimental sample into a glass insert inside a glass LC vial. The leftover may be used for backup.
19. Vortex the QC sample for 10 s and then filter it.
20. Transfer 1 mL of the QC sample into a glass LC vial (*see* Note 10).

3.2 LC-MS/MS Targeted Analysis

1. All experiments should be performed using an UPLC-QQQ-MS platform.
2. Dictate an appropriate worklist such that each sample is injected twice, 10 μL for analysis using negative ionization mode and 4 μL for analysis using positive ionization mode (*see* Note 11).
3. The flow rate is maintained at 0.3 mL/min, auto-sampler temperature is kept at 4 °C, while the column compartment is set to 40 °C.
4. *HILIC gradient parameters*: After an initial 1-min isocratic elution of 90% mobile phase solvent B, decrease the percentage of solvent B to 40% at t = 11 min. Maintain the composition of solvent B at 40% for 4 min (t = 15 min), after which the percentage of solvent B should gradually be returned to 90%, to prepare for the next injection (*see* Note 12). An example of a total ion chromatogram (TIC) can be found in Fig. 3.
5. *MS/MS parameters*: The mass spectrometer should be equipped with an electrospray ionization (ESI) source. Targeted data acquisition should be performed in multiple reaction monitoring (MRM) mode.
6. Table 1 lists LC-MS parameters for the validated detection of 310 metabolites using reference standards. For additional parameter details, *see* Note 13.

4 Notes

1. Render ultrapure water by filtering deionized water.
2. 4:6 dilution of PBS:ACN contains 4:1 (v:v) solution of ^{13}C-lactate and ^{13}C-glutamic acid.
3. Added solutions must be cooled at 4 °C.

4. Prepare ice for use prior to sonication.
5. Ensure that the centrifuge is balanced.
6. Resulting protein pellets may be extracted and saved for protein analysis as needs arise.
7. Drying time is contingent on the number of samples and type of tissue used. It is permitted to dry samples overnight. If the extracted supernatant cannot be dried immediately, samples may be kept at −20 °C for short-term storage or at −80 °C for long-term storage.
8. During this time, you may prepare the vial inserts and syringe filters.
9. It may be necessary to plunge multiple times in order to filter all of the sample. At least 50 μL of filtered sample is required for LC-MS/MS analysis.
10. A glass insert is not needed for analysis of QC sample given sufficient volume of liquid.
11. Both chromatographic separations should be performed in HILIC mode.
12. Targeted data acquisition should be performed in multiple-reaction-monitoring (MRM) mode.
13. MS parameters were optimized using standard references. All LC separation was performed using basic HILIC. The dwell time should be configured to 5 s for each ion signal. Unit resolution is required for both MS1 and MS2.

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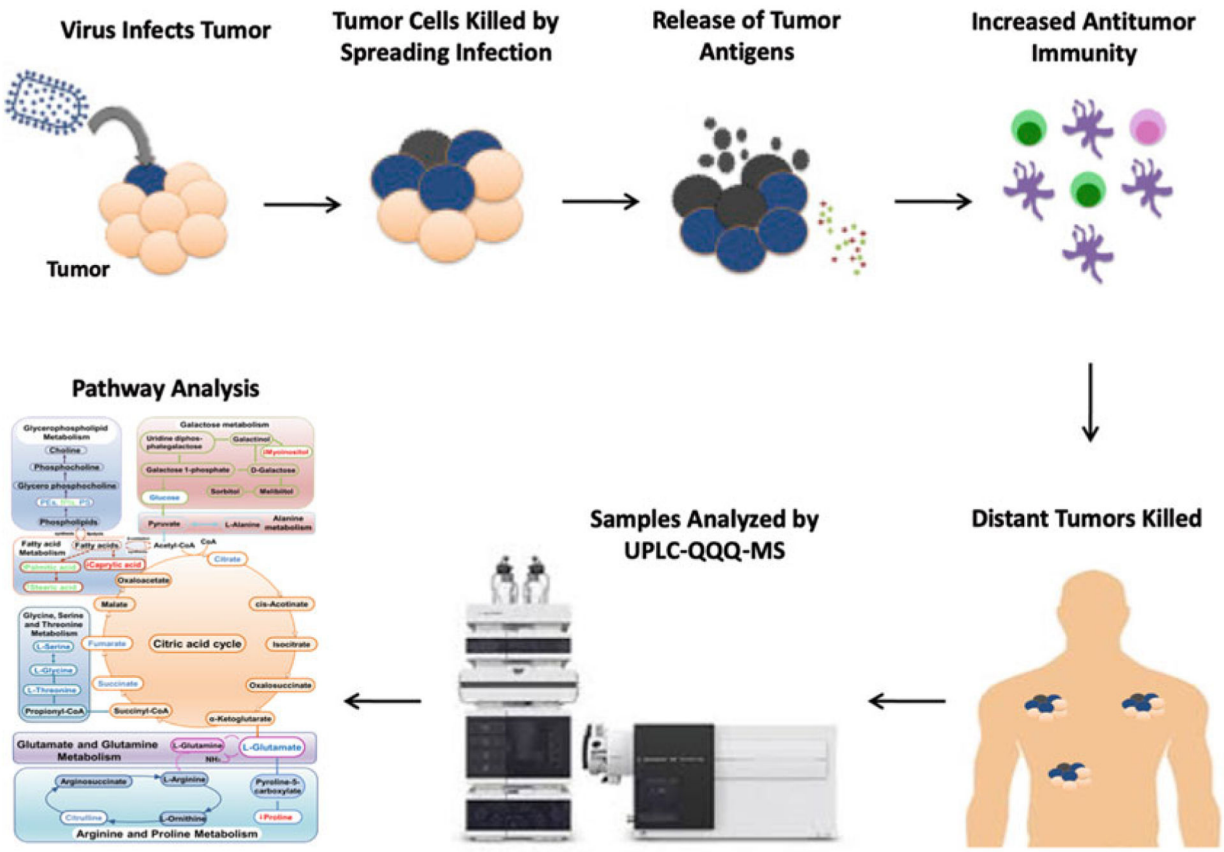

Fig. 1.
Summary of cancer virotherapy mechanism and LC-MS/MS metabolomics analysis

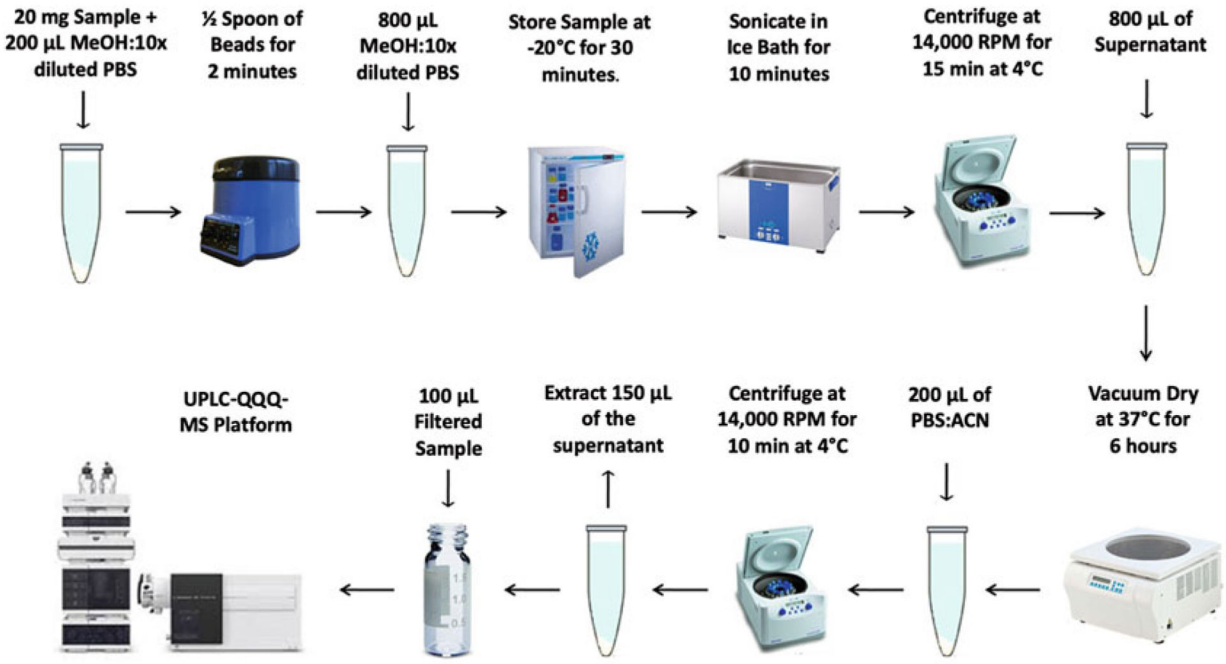

Fig. 2.
Summary of LC-MS/MS sample preparation method

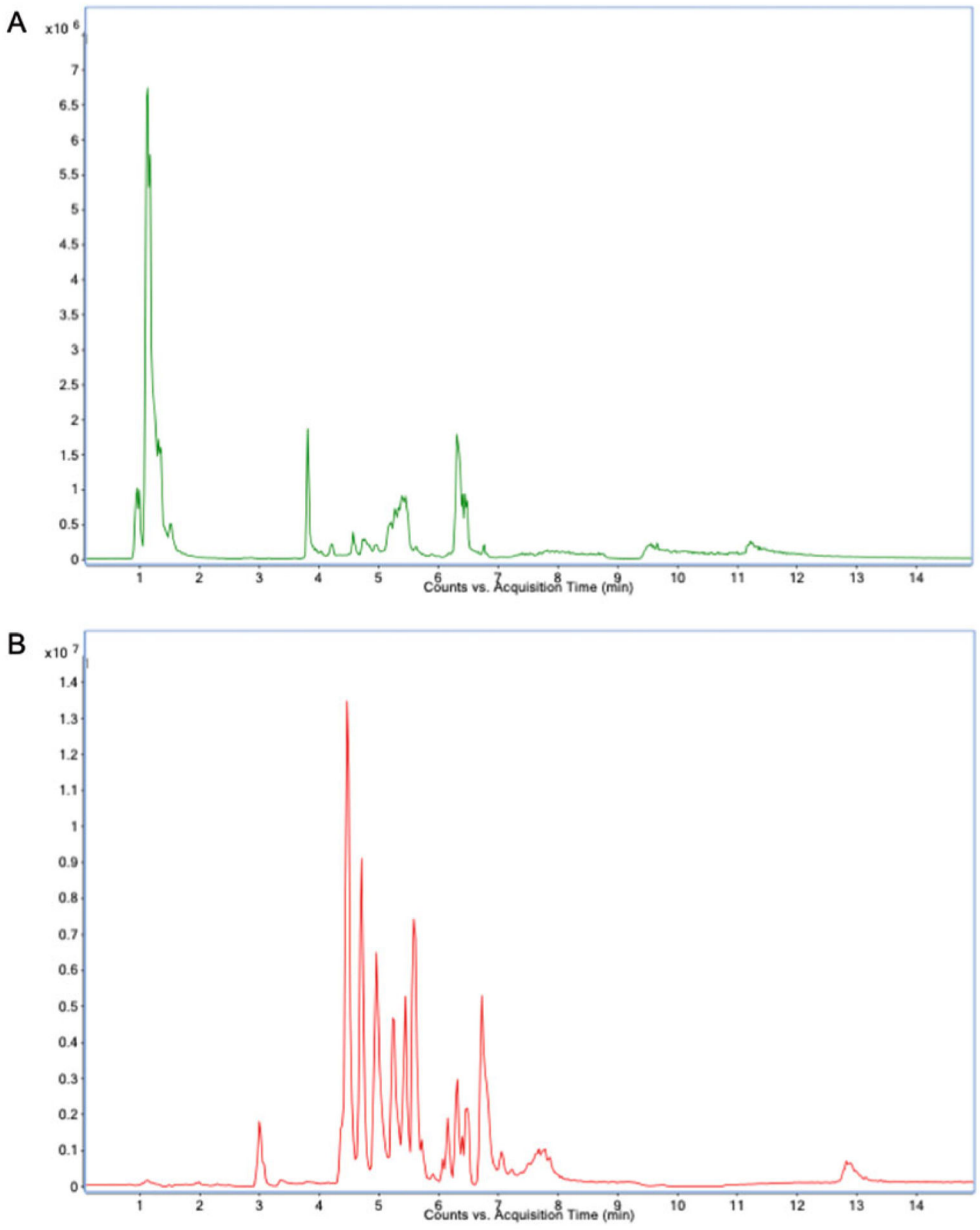

Fig. 3.
LC-MS/MS total ion chromatogram: (**a**) negative and (**b**) positive modes

Table 1

LC-MS parameters for the validated identification and detection of 310 metabolites

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
1	HMDB0031645	Acetamide	60.05	42.80	13	Positive	5.050
2	HMDB0001525	Imidazole	69.05	42.00	21	Positive	1.826
3	HMDB0000119	Glyoxylic acid	72.99	44.90	5	Negative	5.031
4	HMDB0001522	Methylguanidine	74.07	57.00	13	Positive	5.730
5	HMDB0000115	Glycolic acid	75.01	46.90	9	Negative	5.350
6	HMDB0014691	Acetohydroxamic acid	76.04	43.00	9	Positive	6.215
7	HMDB0000123	Glycine	76.04	30.10	13	Positive	6.598
8	HMDB0000925	TMAO	76.08	58.00	21	Positive	6.187
9	HMDB0002039	2-Pyrrolidinone	86.06	44.00	29	Positive	1.620
10	HMDB0000243	Pyruvate	87.00	43.00	5	Negative	5.45
11	HMDB0001873	Isobutyric acid	87.04	87.04	5	Negative	1.948
12	HMDB0000190	Lactate	89.02	43.00	9	Negative	4.1
13	HMDB0001414	Putrescine	89.11	72.00	9	Positive	6.118
14	HMDB0000161	Alanine	90.06	44.00	9	Positive	6.140
15	HMDB0000271	Sarcosine	90.06	44.10	9	Positive	6.184
16	HMDB0001882	Dihydroxyacetone	91.04	91.04	0	Positive	1.734
17	HMDB0000005	2-Ketobutyric acid	101.02	57.00	5	Negative	1.490
18	HMDB0000060	Acetoacetate	101.02	56.90	9	Negative	2.194
19	HMDB0002176	2-Methylbutyric acid	101.06	101.06	5	Negative	1.693
20	HMDB0000718	Isovaleric acid	101.06	101.06	5	Negative	6.064
21	HMDB0000691	Malonic acid	103.00	58.90	9	Negative	4.306
22	HMDB0000008	2-Hydroxybutyric acid	103.04	58.90	9	Negative	5.87
23	HMDB0000357	3-Hydroxybutyric acid	103.04	56.80	9	Negative	4.264
24	HMDB0002322	Cadaverine	103.13	85.90	5	Positive	6.506
25	HMDB0001906	2/3-Aminoisobutyric acid	104.07	57.90	9	Positive	5.295
26	HMDB0000452	2-Aminobutyric acid	104.07	57.90	13	Positive	5.706
27	HMDB0031654	3-Aminobutyric acid	104.07	85.90	5	Positive	5.934
28	HMDB0000112	4-Aminobutyric acid	104.07	87.00	9	Positive	6.596
29	HMDB0000092	Dimethylglycine	104.07	57.90	13	Positive	5.295
30	142-26-7	*N*-Acetylethanolamine	104.07	61.80	5	Positive	1.915
31	HMDB0000097	Choline	104.11	58.00	33	Positive	7.000
32	HMDB0000139	Glyceric acid	105.02	75.00	9	Negative	4.843
33	HMDB0000187	Serine	106.05	60.00	9	Positive	6.824
34	HMDB0001169	4-Aminophenol	110.06	69.00	9	Positive	5.184
35	HMDB0000617	2-Furoic acid	111.01	66.90	5	Negative	1.760
36	HMDB0000630	Cytosine	112.05	94.90	21	Positive	3.445
37	HMDB0000870	Histamine	112.09	95.00	17	Positive	8.673

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
38	HMDB0000300	Uracil	113.04	71.60	25	Positive	8.707
39	HMDB0000562	Creatinine	114.07	44.10	21	Positive	2.988
40	HMDB0000134	Fumarate	115.00	71.00	5	Negative	2.130
41	HMDB0000176	Maleic acid	115.00	70.90	9	Negative	1.455
42	HMDB0000019	Alpha-ketoisovaleric acid	115.04	70.90	5	Negative	1.455
43	HMDB0000720	Levulinic acid	115.04	70.90	9	Negative	3.250
44	HMDB0000689	4-Methylvaleric acid	115.07	115.07	5	Negative	1.505
45	HMDB0000535	Hexanoic acid	115.07	115.07	5	Negative	1.471
46	HMDB0000162	Proline	116.07	70.00	13	Positive	5.408
47	HMDB0000202	Methylmalonic acid	117.02	73.00	5	Negative	4.622
48	HMDB0000254	Succinate	117.02	73.00	9	Negative	4.293
49	HMDB0000754	Beta-hydroxyisovaleric acid	117.05	71.00	9	Negative	2.132
50	HMDB0000532	Acetylglycine	118.05	75.90	5	Positive	4.335
51	HMDB0000128	Glycocyamine	118.06	42.90	45	Positive	6.344
52	HMDB0000738	Indole	118.07	85.80	13	Positive	5.385
53	HMDB0003355	Amino valerate	118.09	100.90	9	Positive	6.822
54	HMDB0000043	Betaine	118.09	58.00	29	Positive	4.791
55	HMDB0013716	Norvaline	118.09	72.00	5	Positive	5.019
56	HMDB0000883	Valine	118.09	72.00	5	Positive	5.224
57	HMDB0002649	Erythrose	119.03	42.90	17	Negative	4.519
58	HMDB0000719	Homoserine	120.07	73.90	9	Positive	6.343
59	HMDB0000167	Threonine	120.07	73.90	9	Positive	6.343
60	HMDB0011718	4-Hydroxy benzaldehyde	121.03	92.00	25	Negative	1.301
61	HMDB0001870	Benzoic acid	121.03	77.00	9	Negative	1.487
62	HMDB0000574	Cysteine	122.03	80.90	13	Positive	13.454
63	HMDB0001406	Nicotinamide	123.06	79.80	25	Positive	1.746
64	HMDB0001488	Nicotinic acid	124.04	78.10	22	Positive	3.671
65	HMDB0002243	Picolinic acid	124.04	77.70	25	Positive	5.315
66	HMDB0000251	Taurine	126.02	44.10	17	Positive	5.223
67	HMDB0000898	Methylhistamine	126.11	109.10	13	Positive	4.879
68	HMDB0002024	4-Imidazoleacetic acid	127.05	80.90	13	Positive	5.497
69	HMDB0000634	Citraconic acid	129.02	85.10	5	Negative	1.487
70	HMDB0000620	Glutaconic acid	129.02	85.00	5	Negative	4.774
71	HMDB0000491	3-Methyl-2-oxovaleric acid	129.05	129.05	5	Negative	1.351
72	HMDB0000491	Ketoisoleucine	129.05	85.10	5	Negative	1.368
73	HMDB0000695	Ketoleucine	129.05	85.00	5	Negative	1.368
74	HMDB0000267	Pyroglutamic acid	130.05	83.80	9	Positive	4.675
75	HMDB0000070	Pipecolinic acid	130.09	84.00	13	Positive	5.405
76	HMDB0000223	Oxaloacetic acid	131.00	42.00	17	Negative	6.772

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
77	HMDB0000622	Ethylmalonic acid	131.03	87.00	5	Negative	3.282
78	HMDB0000661	Glutaric acid	131.03	86.90	9	Negative	6.179
79	HMDB0001844	Methyl succinate	131.03	98.90	5	Negative	2.011
80	HMDB0000665	Leucic acid	131.07	85.10	9	Negative	1.994
81	HMDB0001432	Agmatine	131.13	71.90	17	Positive	6.912
82	HMDB0001149	5-Aminolevulinic acid	132.07	85.90	13	Positive	6.521
83	HMDB0000725	Hydroxyproline	132.07	85.90	17	Positive	6.113
84	HMDB0000064	Creatine	132.08	44.00	21	Positive	6.204
85	HMDB0000172	Isoleucine	132.10	86.00	9	Positive	4.697
86	HMDB0000557	L-Alloisoleucine	132.10	86.00	9	Positive	4.468
87	HMDB0000687	Leucine	132.10	86.00	9	Positive	4.468
88	HMDB0001645	Norleucine	132.10	86.00	9	Positive	4.468
89	HMDB0000156	Malate	133.01	114.90	9	Negative	5.87
90	HMDB0000168	Asparagine	133.06	73.90	13	Positive	6.813
91	HMDB0003374	Ornithine	133.10	70.00	21	Positive	11.112
92	HMDB0000191	Aspartate	134.05	73.90	13	Positive	6.471
93	HMDB0000209	Phenylacetic acid	135.04	91.00	5	Negative	1.503
94	HMDB0000034	Adenine	136.06	119.10	25	Positive	2.755
95	HMDB0001895	2-Hydroxybenzoic acid	137.02	93.10	21	Negative	1.215
96	HMDB0000710	4-Hydroxybenzoic acid	137.02	93.10	17	Negative	2.316
97	HMDB0014581	Allopurinol	137.05	54.20	29	Positive	2.070
98	HMDB0000157	Hypoxanthine	137.05	54.80	33	Positive	2.938
99	HMDB0001123	Anthranilic acid	138.06	120.00	9	Positive	1.568
100	20989-17-7	2-Phenylglycinol	138.09	103.00	21	Positive	2.549
101	HMDB0000301	Urocanic acid	139.05	92.90	21	Positive	4.444
102	HMDB0002658	6-Hydroxynicotinic acid	140.04	121.90	17	Positive	4.604
103	HMDB0002349	Muconic acid	141.02	97.00	5	Negative	5.684
104	HMDB0000393	3-Hexenedioic acid	143.03	99.00	5	Negative	6.042
105	HMDB0000482	Caprylic acid	143.11	143.11	5	Negative	1.350
106	HMDB0000208	Alpha-ketoglutaric acid	145.01	101.00	5	Negative	4.99
107	HMDB0000422	2-Methylglutaric acid	145.05	101.10	9	Negative	5.500
108	HMDB0000448	Adipic acid	145.05	101.00	9	Negative	6.025
109	HMDB0000895	Acetylcholine	146.12	86.80	9	Positive	3.439

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
110	HMDB0001257	Spermidine	146.17	72.00	17	Positive	6.499
111	HMDB0059655	2HG	147.03	129.00	5	Negative	5.49
112	HMDB0000641	Glutamine	147.08	84.00	17	Positive	6.673
113	HMDB0000182	Lysine	147.12	83.90	21	Positive	11.749
114	HMDB0003339	Glutamic acid	148.06	84.00	17	Positive	6.39
115	147-73-9	Meso-tartaric acid	149.01	86.90	13	Negative	11.000
116	HMDB0059916	Tartaric acid	149.01	87.00	9	Negative	11.000
117	HMDB0001587	Phenylglyoxylic acid	149.02	77.00	9	Negative	1.315
118	HMDB0000646	L-(+)-Arabinose	149.04	59.00	20	Negative	6.034
119	HMDB0000283	Ribose	149.04	89.00	5	Negative	4.4
120	HMDB0000098	Xylose	149.04	89.00	5	Negative	4.320
121	HMDB0002097	4-Ethylbenzoic acid	149.06	105.10	9	Negative	1.501
122	HMDB0032075	p-Tolylacetic acid	149.06	105.00	5	Negative	1.501
123	HMDB0001878	Thymol	149.09	87.00	13	Negative	11.100
124	HMDB0000696	Methionine	150.06	103.90	9	Positive	4.923
125	HMDB0000669	2-Hydroxy phenylacetic acid	151.04	107.00	13	Negative	1.280
126	HMDB0000440	3-Hydroxyphenylacetic acid	151.04	107.00	5	Negative	1.280
127	HMDB0004815	4-Hydroxy-3-methylbenzoic acid	151.04	107.10	9	Negative	1.923
128	HMDB0000020	4-Hydroxyphenylacetic acid	151.04	107.00	9	Negative	1.907
129	HMDB0000703	Mandelic acid	151.04	107.00	5	Negative	1.907
130	HMDB0000508	Adonitol	151.06	70.80	16	Negative	4.149
131	HMDB0001851	L-(—)-Arabitol	151.06	89.10	8	Negative	4.335
132	HMDB0002917	Xylitol	151.06	59.00	20	Negative	4.390
133	HMDB0000132	Guanine	152.06	135.10	21	Positive	4.009
134	HMDB0000397	2,3-Dihydroxybenzoic acid	153.02	109.00	17	Negative	1.027

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
135	HMDB0000152	Gentisic acid	153.02	108.10	25	Negative	1.415
136	HMDB0001856	Protocatechuic acid	153.02	109.00	13	Negative	1.731
137	HMDB0000292	Xanthine	153.04	109.90	21	Positive	3.415
138	56-17-7	Cystamine	153.05	107.70	5	Positive	9.759
139	HMDB0001476	3-Hydroxyanthranilic acid	154.05	112.90	5	Positive	13.765
140	HMDB0000073	Dopamine	154.09	137.00	9	Positive	4.015
141	HMDB0000177	Histidine	156.08	110.10	13	Positive	7.182
142	HMDB0000555	3-Methyladipic acid	159.06	97.20	9	Negative	5.464
143	HMDB0000678	Isovalerylglycine	160.10	57.00	17	Positive	2.889
144	HMDB0000303	Tryptamine	161.11	144.00	9	Positive	3.433
145	HMDB0000510	2-Aminoadipic acid	162.08	98.00	13	Positive	6.839
146	HMDB0000062	Carnitine	162.12	43.00	25	Positive	6.550
147	HMDB0001713	m-Coumaric acid	163.04	119.00	17	Negative	1.499
148	HMDB0002035	p-Coumaric acid	163.04	119.00	17	Negative	1.849
149	HMDB0000205	Phenylpyruvic acid	163.04	91.00	9	Negative	1.279
150	HMDB0001890	Acetylcysteine	164.04	123.00	9	Positive	2.555
151	HMDB0002107	Phthalic acid	165.02	121.00	5	Negative	2.380
152	HMDB0060256	D-Xylonic acid	165.04	75.00	16	Negative	5.218
153	HMDB0000779	3-Phenyllactic acid	165.05	147.10	9	Negative	1.499
154	HMDB0002072	4-Methoxyphenylacetic acid	165.05	106.00	9	Negative	1.499
155	HMDB0000159	Phenylalanine	166.09	120.20	17	Positive	4.373
156	HMDB0000263	Phosphoenolpyruvic acid	166.97	78.80	33	Negative	6.060
157	HMDB0000130	Homogentisic acid	167.03	108.00	13	Negative	5.765
158	HMDB0000484	Vanillic acid	167.03	152.00	13	Negative	2.000
159	HMDB0000232	Quinolinic acid	168.03	150.00	5	Positive	5.263

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
160	HMDB0001112	D-Glyceraldehyde 3-phosphate	168.99	79.00	33	Negative	5.800
161	HMDB0000289	Urate	169.04	141.00	13	Positive	4.768
162	HMDB0000239	Pyridoxine	170.08	134.10	17	Positive	2.295
163	HMDB0000001	1-Methylhistidine	170.10	95.90	21	Positive	6.930
164	HMDB0000511	Capric acid	171.14	171.14	5	Negative	1.261
165	HMDB0000072	Aconitic acid	173.01	85.00	9	Negative	6.191
166	HMDB0003070	Shikimic acid	173.04	93.10	9	Negative	5.801
167	HMDB0000893	Suberic acid	173.08	111.10	13	Negative	5.004
168	HMDB0000721	Glycylproline	173.09	116.10	13	Positive	6.701
169	HMDB0000044	L-Ascorbic acid	175.02	114.90	9	Negative	3.027
170	HMDB0003357	Acetylornithine	175.11	70.00	25	Positive	6.769
171	HMDB0000517	Arginine	175.12	70.00	25	Positive	11.665
172	HMDB0000197	Indole-3-acetic acid	176.07	158.10	13	Positive	6.193
173	HMDB0000904	Citrulline	176.11	69.90	25	Positive	7.020
174	HMDB0000259	Serotonin	177.10	160.00	9	Positive	4.326
175	HMDB0000707	4-Hydroxy phenylpyruvic acid	179.03	135.00	9	Negative	1.273
176	HMDB0000660	Fructose	179.05	89.00	5	Negative	4.716
177	HMDB0033704	Galactose	179.05	89.10	5	Negative	4.732
178	HMDB0000122	Glucose	179.05	89.00	5	Negative	5.410
179	HMDB0000169	Mannose	179.05	58.90	17	Negative	5.350
180	HMDB0000211	Myoinositol	179.05	135.00	1	Negative	1.497
181	HMDB0000714	Hippuric acid	180.07	104.90	13	Positive	2.499
182	HMDB0006479	Glucosamine	180.09	162.00	5	Positive	6.791
183	HMDB0000118	Homovanillic acid	181.05	136.80	5	Negative	2.268
184	HMDB0003269	Nicotinuric acid	181.06	134.80	17	Positive	4.234

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
185	HMDB0000765	D-Mannitol	181.07	101.10	20	Negative	5.213
186	HMDB0000107	Dulcitol	181.07	58.90	24	Negative	5.294
187	HMDB0000247	Sorbitol	181.07	58.70	17	Negative	5.223
188	HMDB0000158	Tyrosine	182.08	90.90	33	Positive	5.284
189	HMDB0000017	4-Pyridoxic acid	184.06	166.10	9	Positive	1.288
190	HMDB0000068	Epinephrine	184.10	166.10	5	Positive	5.366
191	HMDB0000819	Normetanephrine	184.10	166.10	5	Positive	4.462
192	HMDB0000807	3-Phosphoglyceric acid	184.98	79.00	50	Negative	7.013
193	HMDB0000784	Azelaic acid	187.09	125.10	13	Negative	4.020
194	HMDB0006029	Acetyl-L-glutamine	189.09	130.00	13	Positive	4.651
195	HMDB0000715	Kynurenic acid	190.05	144.00	25	Positive	2.338
196	HMDB0002302	3-Indolepropionic acid	190.09	130.10	25	Positive	1.562
197	HMDB0000094	Citrate	191.02	111.00	9	Negative	7
198	HMDB0000193	Isocitrate	191.02	111.00	13	Negative	6.41
199	HMDB0000763	HIAA	192.07	146.10	13	Positive	4.507
200	HMDB0002545	D-Galacturonic acid	193.03	58.70	17	Negative	6.662
201	HMDB0000127	Glucuronic acid	193.03	113.00	9	Negative	6.493
202	HMDB0000954	Ferulic acid	193.05	134.00	13	Negative	1.512
203	HMDB0029965	Methyl alpha-D-glucopyranoside	193.07	102.80	13	Negative	4.320
204	HMDB0029965	Methyl-D-mannopyranoside	193.07	58.80	21	Negative	3.044
205	HMDB0000565	Galactonic acid	195.05	75.00	21	Negative	6.831
206	HMDB0000625	Gluconic acid	195.05	129.00	13	Negative	6.900
207	HMDB0001847	Caffeine	195.09	138.00	17	Positive	1.447
208	HMDB0000609	DOPA	198.08	152.10	5	Positive	5.838
209	HMDB0004063	Metanephrine	198.12	180.10	5	Positive	3.959

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
210	HMDB0000624	Lauric acid	199.17	199.17	5	Negative	1.224
211	HMDB0000792	Sebacic acid	201.11	139.10	17	Negative	3.612
212	HMDB0003334	Dimethylarginine	203.15	70.00	21	Positive	10.306
213	HMDB0060484	Indole-3-pyruvic acid	204.07	163.20	5	Positive	12.162
214	HMDB0000201	Acetylcarnitine	204.13	85.00	17	Positive	5.661
215	HMDB0000929	Tryptophan	205.10	187.90	5	Positive	4.437
216	HMDB0000881	Xanthurenic acid	206.05	131.90	29	Positive	2.245
217	HMDB0000671	Indole-3-lactic acid	206.08	118.10	25	Positive	4.453
218	HMDB0000639	Mucic acid	209.03	84.90	13	Negative	7.078
219	HMDB0000684	Kynurenine	209.09	192.00	5	Positive	4.483
220	HMDB0001511	Phosphocreatine	212.05	45.00	17	Positive	1.674
221	2280-85-5	6-Methyl-DL-tryptophan	219.12	202.10	5	Positive	4.071
222	HMDB0000210	Pantothenic acid	220.12	90.00	9	Positive	4.071
223	HMDB0000472	5-Hydroxytryptophan	221.09	204.00	5	Positive	5.236
224	HMDB0000215	Acetylglucosamine	222.10	138.10	17	Positive	4.505
225	HMDB0000853	*N*-Acetyl-D-galactosamine	222.10	204.10	4	Positive	4.387
226	HMDB0000215	*N*-Acetyl-D-glucosamine	222.10	204.30	0	Positive	4.487
227	HMDB0000742	Homocysteine	223.08	88.00	29	Positive	8.604
228	HMDB0000732	3-Hydroxykynurenine	225.09	207.00	5	Positive	1.102
229	2387-23-7	*N*,*N*-Dicyclohexylurea	225.20	100.10	13	Positive	1.239
230	HMDB0001904	3-Nitrotyrosine	227.07	181.00	5	Positive	4.801
231	HMDB0000033	Carnosine	227.12	110.00	21	Positive	7.655
232	HMDB0000806	Myristic acid	227.20	227.20	5	Negative	1.189
233	HMDB0000014	2-Deoxycytidine	228.10	112.00	9	Positive	3.591
234	4300-28-1	D-Ribose 5-phosphate	229.01	78.80	45	Negative	6.850

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
235	66768-39-6	D-Xylose 5-phosphate	229.01	78.90	45	Negative	5.200
236	HMDB0000012	2-Deoxyuridine	229.08	113.00	9	Positive	2.289
237	HMDB0001923	Naproxen	229.08	169.00	33	Negative	1.358
238	HMDB0014732	Amiloride	230.06	171.00	13	Positive	4.192
239	HMDB0001389	Melatonin	233.13	174.00	13	Positive	1.307
240	HMDB0000192	Cystine	241.03	74.00	21	Positive	7.980
241	HMDB0000826	Pentadecanoic acid	241.21	241.21	5	Negative	1.189
242	HMDB0000089	Cytidine	244.10	112.00	9	Positive	4.229
243	HMDB0000296	Uridine	245.08	113.00	5	Positive	2.950
244	HMDB0000030	Biotin	245.10	227.00	9	Positive	3.955
245	HMDB0000101	2-Deoxyadenosine	252.11	136.10	13	Positive	2.585
246	HMDB0000845	Neopterin	254.09	206.00	21	Positive	5.621
247	HMDB0000220	Palmitic acid	255.23	255.23	5	Negative	1.189
248	HMDB0000982	5-Methylcytidine	258.11	126.10	9	Positive	3.932
249	HMDB0001078	D-Mannose 6-phosphate	259.02	79.10	52	Negative	7.170
250	HMDB0000124	Fructose 6-phosphate	259.02	78.80	41	Negative	6.87
251	HMDB0001586	Glucose 1-phosphate	259.02	79.10	29	Negative	6.889
252	HMDB0001401	Glucose 6-phosphate	259.02	78.80	41	Negative	7.300
253	HMDB0001254	Glucosamine 6-phosphate	260.06	126.10	9	Positive	7.177
254	HMDB0001849	Propranolol	260.17	116.00	17	Positive	2.516
255	94-24-6	Tetracaine	265.19	176.00	17	Positive	1.534
256	HMDB0000085	2-Deoxyguanosine	268.11	151.90	17	Positive	4.000
257	HMDB0000050	Adenosine	268.11	136.00	13	Positive	3.018
258	HMDB0000195	Inosine	269.09	137.00	21	Positive	3.794
259	HMDB0002259	Heptadecanoic acid	269.25	269.25	5	Negative	1.154

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
260	HMDB0001316	6-Phosphogluconic acid	275.01	78.90	49	Negative	13.882
261	506-24-1	9-Octadecynoic acid	279.23	279.23	5	Negative	1.188
262	HMDB0003331	1-Methyladenosine	282.12	150.00	29	Positive	6.397
263	HMDB0000827	Stearic acid	283.26	283.26	5	Negative	1.137
264	HMDB0000133	Guanosine	284.10	152.10	13	Positive	4.616
265	HMDB0000299	Xanthosine	285.09	153.00	9	Positive	4.844
266	HMDB0060493	*N*-Acetylmuramic acid	292.10	89.00	8	Negative	3.895
267	HMDB0000772	Nonadecanoic acid	297.28	297.28	5	Negative	1.120
268	HMDB0001409	dUMP	307.03	195.10	17	Negative	7.235
269	HMDB0000125	Glutathione reduced	308.09	84.10	25	Positive	6.070
270	HMDB0014950	Phenylbutazone	309.16	76.90	45	Positive	1.030
271	HMDB0000230	*N*-Acetylneuraminic acid	310.12	274.00	5	Positive	6.350
272	HMDB0000651	Decanoylcarnitine	316.25	84.90	25	Positive	2.332
273	HMDB0001227	dTMP	323.07	80.80	21	Positive	6.033
274	HMDB0001058	Fructose 1,6 biphosphate (F16BP)	338.99	78.90	49	Negative	7.9
275	HMDB0003514	Glucose 1,6 biphosphate (G16BP)	338.99	241.10	17	Negative	8.000
276	HMDB0000055	D-(+)-Cellobiose	341.11	161.00	4	Negative	6.869
277	HMDB0005826	Galactinol dihydrate	341.11	179.20	16	Negative	8.318
278	HMDB0000186	Lactose	341.11	161.00	5	Negative	7.065
279	HMDB0000258	Sucrose	341.11	59.00	50	Negative	6.474
280	HMDB0000975	Trehalose	341.11	178.90	9	Negative	7.014
281	HMDB0003559	Gibberellic acid	345.13	143.00	37	Negative	1.559
282	HMDB0001314	cGMP	346.06	152.00	21	Positive	4.63
283	HMDB0000045	AMP	348.10	136.20	17	Positive	6.544
284	HMDB0001220	Prostaglandin E2	351.21	315.20	9	Negative	1.474

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
285	HMDB0000939	Adenosyl-L-homocysteine	385.13	136.00	25	Positive	6.875
286	HMDB0001245	DCDP	388.03	112.00	17	Positive	6.922
287	HMDB0000774	Pregnenolone sulfate	395.19	96.80	45	Negative	1.017
288	HMDB0000295	UDP	402.99	78.90	50	Negative	6.79
289	HMDB0001341	ADP	428.04	136.10	21	Positive	6.900
290	HMDB0000121	Folic acid	442.15	295.00	25	Positive	6.806
291	HMDB0001201	GDP	444.03	152.10	33	Positive	7.255
292	HMDB0001056	Dihydrofolic acid	444.17	297.10	17	Positive	6.783
293	HMDB0000797	SAICAR	455.08	110.00	44	Positive	6.947
294	HMDB0000536	Adenylosuccinate	464.08	252.00	25	Positive	8.036
295	HMDB0000998	dCTP	468.00	111.90	17	Positive	7.5
296	HMDB0001191	dUTP	468.98	80.90	13	Positive	7
297	HMDB0001562	Folinic acid	474.18	327.10	17	Positive	1.464
298	HMDB0003213	Raffinose	503.16	178.90	25	Negative	7.912
299	HMDB0000538	ATP	508.01	136.00	45	Positive	7.350
300	HMDB0001178	ADP ribose	560.10	136.20	21	Positive	6.020
301	HMDB0000290	UDP-GlcNAc	608.09	204.30	9	Positive	7.010
302	HMDB0003337	Glutathione oxidized	613.16	484.00	17	Positive	7.267
303	HMDB0000902	NAD	664.12	136.00	41	Positive	6.767
304	HMDB0003553	Stachyose hydrate	665.21	383.20	40	Negative	8.960
305	HMDB0001487	NADH	666.10	348.10	17	Positive	5.700
306	HMDB0000217	NADP	745.10	604.10	17	Positive	7.200
307	HMDB0000221	NADPH	746.10	746.10	17	Positive	6.500
308	HMDB0001206	Acetyl-CoA	810.14	303.10	37	Positive	6
309	HMDB0001243	Isobutyryl-CoA	838.17	331.20	37	Positive	7.000

No.	HMDB/CAS	Compound name	Precursorion	Production	Collision energy	Polarity	RT (min)
310	HMDB0001166	Hydroxybutyryl coenzyme A	854.16	347.10	37	Positive	6.954