Qualitative and Quantitative Analysis of Organic Acids in Urine by Gas Chromatography-High Resolution Mass Spectrometry with Hard Ionization and Soft Ionization

Product: Mass spectrometer (MS)

Introduction

The test of urine organic acids is an essential component of the study of inherited metabolic diseases, such as inborn error of metabolism. Urine contains a variety of organic acids that forms through diverse sources, including normal and abnormal metabolism, drugs and drug metabolism, or even xenobiotics and dietary supplements. The gas chromatography - mass spectrometry (GC-MS), especially GC - single quadrupole MS(GC-QMS) is generally chosen for the analysis of urine organic acids. However, since GC-QMS lacks the ability to the accurate identification of organic acids in urine due to its complicated mixture especially when creating a new method, GC-high resolution MS (GC-HRMS) is a more qualified system instead.

JMS-T2000GC, a GC-HRMS system that incorporates a combination ion source of electron ionization (EI) and field ionization (FI), is capable of switching between the EI and FI modes without breaking the vacuum. The analysis of data acquired by the 2 ionization methods through msFineAnalysis AI software leads to the estimation of chemical structures as well as compositions, which is practical for the determination of compounds in organic acids.

In this work, the qualitative analysis of the chemical composition and structure of organic compounds in urine and the quantitative analysis of target organic acid using JMS-T2000GC with msFineAnalysis AI was executed.

Measurement

The organic acids as sample solutions were extracted from acidified urines by liquid–liquid extraction with ethyl acetate, followed by trimethylsilyl (TMS) derivatization to increase the volatility of organic acids. 1 µL of the sample solution was then injected in JMS-T2000GC by an autosampler. The msFineAnalysis AI software was used for the qualitative analysis and the Escrime™ software was used for quantitative analysis. The detailed GC-HRMS measurement conditions are presented in Table 1.

JMS-T2000GC "AccuTOF™ GC Alpha"

Table 1. Measurement conditions

GC-HRMS	JMS-T2000GC (JEOL Ltd.)
GC inlet mode	Splitless
GC inlet temperature	280 °C
GC Column	DB-5MS, 30m x 0.25mm, 0.25µm
GC Oven	60°C (1min) →10°C/min →300°C (3min)
Carrier gas	He, 1.0mL/min
MS Ionization	EI+: 70eV, 300µA
	FI+: -10 kV with JEOL 5µm emitter (10mA, 6msec)
MS Monitor ion range	*m/z* 45-800
Analysis software	Qualitative: msFineAnalysis AI (JEOL Ltd.) Quantitative: Escrime™ (JEOL Ltd.)

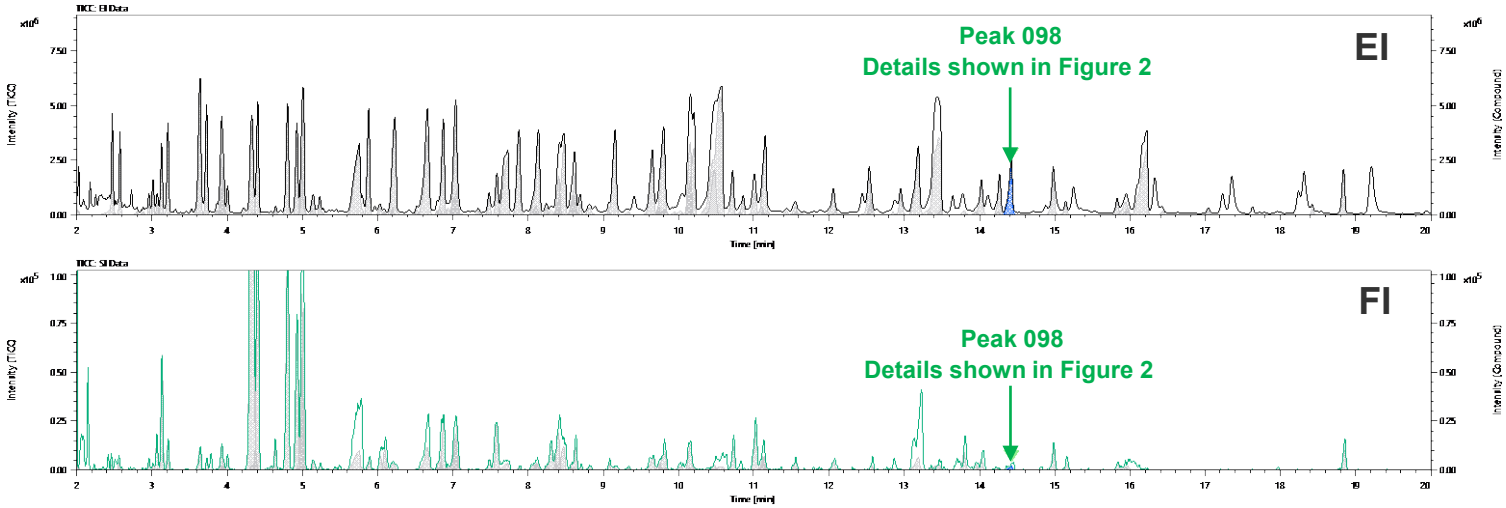

Figure 1. TICCs of a urine organic acid from sample (top: EI, bottom: FI)

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Qualitative Analysis Results

Figure 1 shows both the EI and FI total ion current chromatograms (TICCs) of urine organic acids from the sample, resulting in the detection of 108 compounds including 29 target organic acids, whose msFineAnlaysis AI analysis results are shown in red columns in Table 2. Peak [33] was assigned as 5-Methyl-3-isoxazolepropanoic acid trimethylsilyl ester, a TMS derivative compound from oximated succinyl acetone. Some co-eluted components due to the failure of chromatographically separation such as peak [023] and [024] were however detected separately by the deconvolution function of msFineAnalysis AI.

The results of both integrated analysis and AI structural analysis of peak[098] acquired by msFineAnalysis AI were shown in Figures 2 and 3 respectively. Molecular ion was not detected in the EI mass spectrum of Figure 2 while the $[M+H]^+$ ion was detected in the FI mass spectrum, which implies the importance of the combination analysis of soft ionization methods. The chemical formula was estimated to be $C_{16}H_{34}NO_5Si_2$ from the exact mass of the detected $[M+H]^+$ ion, which did not match the estimated chemical composition from the molecular ion or the candidate compound from the NIST database search results. However, msFineAnalysis AI obtained an AI library that contains plenty of EI mass spectra of compounds which are not registered in the NIST database, and compounds that match the chemical composition estimated from the molecular ion and have a matching EI fragment ions pattern were shown as candidates in the search result of AI library (Figure 3).

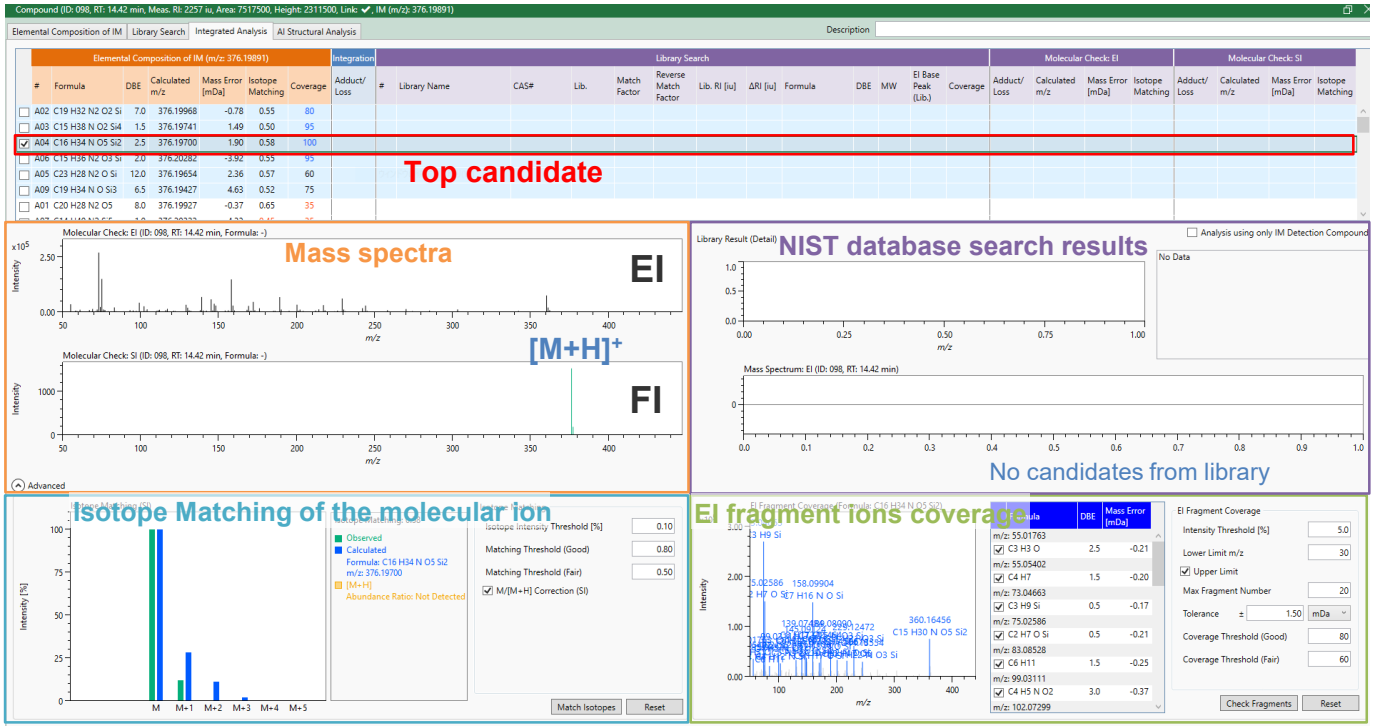

Figure 2. Integrated Analysis results of peak 098

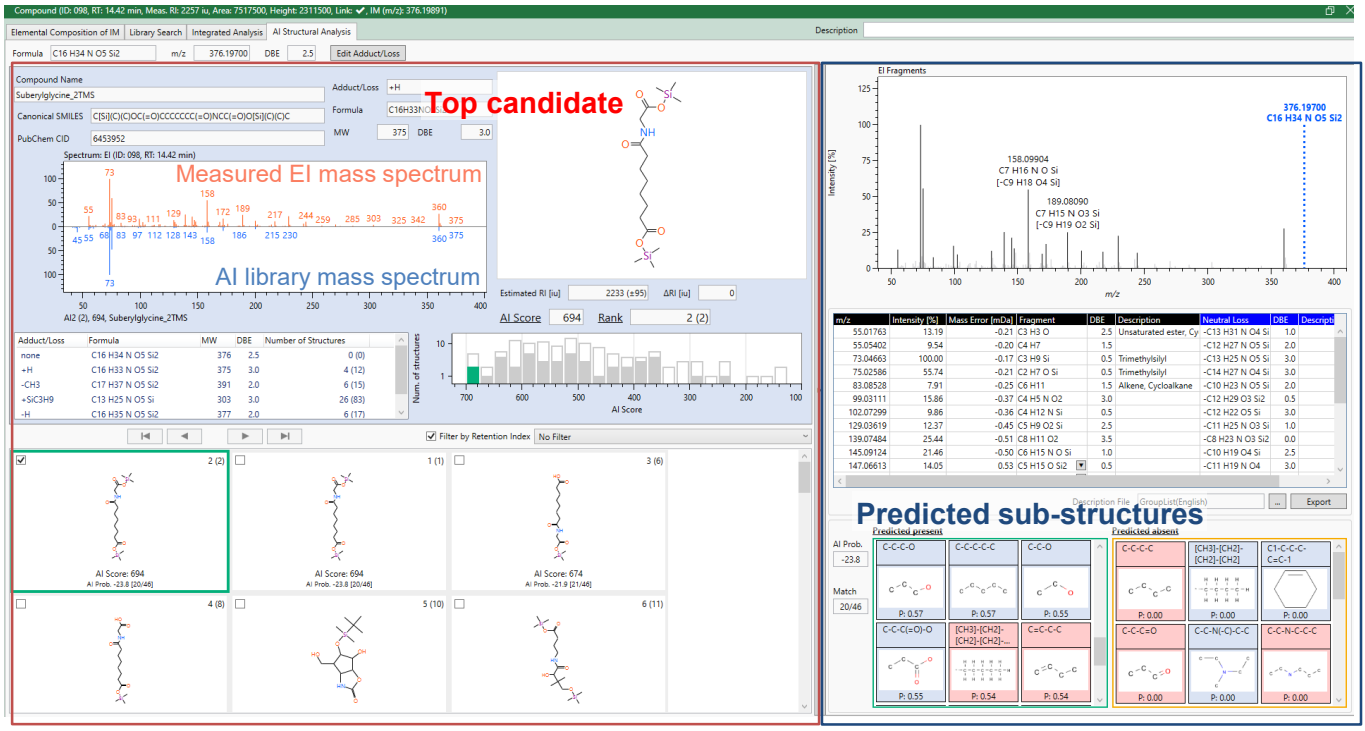

Figure 3. AI Structural Analysis results of peak 098

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Table 2. msFineAnalysis AI results

General						Total Result								
ID	RT [min]	Meas. RI [iu]	Area	IM m/z	IM Ionization	Compound Name	Lib.	Match Factor / AI Score	Lib. RI [iu]	ΔRI [iu]	Formula	DBE	Adduct/ Loss	Calculated m/z
001	2.00	1096	840854	159.10743	EI	trimethylsilyl N-methylpropanimidate	AI	793	929 (±95)	72	C7 H17 N O Si	1.0	none	159.10739
002	2.48	1139	6411786	219.08602	SI	Lactic Acid, 2TMS derivative	mainlib	918	1066	73	C8 H19 O3 Si2	1.5	-CH3	219.08672
003	2.50	1140	153422	233.10237	EI	α-Hydroxyisobutyric acid, 2TMS derivative	replib	717	1071	69	C9 H21 O3 Si2	1.5	-CH3	233.10237
004	2.58	1147	4160738	205.07132	SI	Glycolic acid, 2TMS derivative	replib	953	1080	67	C7 H17 O3 Si2	1.5	-CH3	205.07107
005	2.80	1167	257103	187.15102	EI	2-Octanol, TMS derivative	replib	872	1108	59	C10 H23 O Si	0.5	-CH3	187.15127
006	2.88	1174	250064	143.08833	SI	trimethyl-[(E)-2-methylbut-1-enoxy]silane	AI	738	984 (±95)	95	C7 H15 O Si	1.5	-CH3	143.08867
007	2.96	1181	586136	233.09056	SI	Glyoxylic oxime acid, bis(trimethylsilyl)-	mainlib	785	1135	46	C8 H19 N O3 Si2	2.0	none	233.08980
008	3.02	1186	1871002	307.12302	SI	Oxalic acid, 2TMS derivative	mainlib	767	1134	52	C11 H27 O4 Si3	1.5	+SiC3H9	307.12117
009	3.07	1191	778960	180.09790	SI	p-Cresol, TMS derivative	mainlib	779	1153	38	C10 H16 O Si	4.0	none	180.09649
010	3.13	1196	4614950	247.10678	SI	Pyruvic acid oxime, bis(trimethylsilyl)- deriv.	mainlib	923	1157	39	C9 H21 N O3 Si2	2.0	none	247.10545
011	3.22	1203	6563093	233.10260	SI	3-Hydroxybutyric acid, 2TMS derivative	replib	889	1167	36	C9 H21 O3 Si2	1.5	-CH3	233.10237
012	3.64	1237	13610480	321.13698	SI	Propanedioic acid, 2TMS derivative	replib	931	1216	21	C12 H29 O4 Si3	1.5	+SiC3H9	321.13682
013	3.73	1244	7871900	262.10558	SI	Methylmalonic acid, 2TMS derivative	replib	933	1223	21	C10 H22 O4 Si2	2.0	none	262.10511
014	3.93	1260	6156020	261.13359	SI	2-Hydroxyisocaproic acid, 2TMS derivative	replib	858	1248	12	C11 H25 O3 Si2	1.5	-CH3	261.13367
015	3.95	1262	5469197	233.09926	SI	4-Hydroxybutanoic acid, 2TMS derivative	replib	833	1238	24	C9 H21 O3 Si2	1.5	-CH3	233.10237
016	4.01	1267	1771635	258.09575	SI	2-Propenoic acid, 2-(acetylamino)-_2TMS	AI	700	1302 (±95)	0	C10 H20 N O3 Si2	3.5	-CH3	258.09762
017	4.23	1284	274853	189.08699	EI	Urea, 2TMS derivative	mainlib	771	1246	38	C6 H17 N2 O Si2	1.5	-CH3	189.08739
018	4.33	1292	12007097	315.10364	SI	Silanol, trimethyl-, phosphate (3:1)	mainlib	916	1285	7	C9 H28 O4 P Si3	-0.5	+H	315.10275
019	4.41	1298	9735226	349.16825	SI	Ethylmalonic acid, 2TMS derivative	mainlib	908	1286	12	C14 H33 O4 Si3	1.5	+SiC3H9	349.16812
020	4.64	1317	548152	254.11738	SI	Catechol, 2TMS derivative	mainlib	871	1324	7	C12 H22 O2 Si2	4.0	none	254.11528
021	4.80	1330	11317113	335.15279	SI	Butanedioic acid, 2TMS derivative	mainlib	947	1321	9	C13 H31 O4 Si3	1.5	+SiC3H9	335.15247
022	4.93	1340	7172905	349.16846	SI	Methylsuccinic acid, 2TMS derivative	mainlib	928	1330	10	C14 H33 O4 Si3	1.5	+SiC3H9	349.16812
023	4.99	1345	4213726	329.15392	SI	Uracil, 2TMS derivative	replib	872	1345	0	C13 H29 N2 O2 Si3	3.5	+SiC3H9	329.15313
024	5.02	1347	10038423	395.19272	SI	Glyceric acid, 3TMS derivative	replib	925	1342	5	C15 H39 O4 Si4	0.5	+SiC3H9	395.19199
025	5.12	1355	336850	337.17194	SI	2,3-Dihydroxybutanoic acid tritms	mainlib	714	1362	7	C13 H33 O4 Si3	0.5	+H	337.16812
026	5.14	1357	1678911	337.17750	SI	serine hydroxamate_3TMS	AI	745	1452 (±95)	0	C12 H33 N2 O3 Si3	0.5	+H	337.17935
027	5.24	1364	1227732	409.20702	SI	(R*,S*)-2,3-Dihydroxybutanoic acid, tris(trimethylsilyl) deriv.	mainlib	910	1234 (±381)	0	C16 H41 O4 Si4	0.5	+SiC3H9	409.20764
028	5.75	1406	16077539	290.16031	SI	Isobutyrylglycine, TMS derivative	mainlib	886	1372	34	C12 H28 N O3 Si2	1.5	+SiC3H9	290.16022
029	5.88	1417	10130214	349.16970	SI	Pentanedioic acid, 2TMS derivative	mainlib	931	1409	8	C14 H33 O4 Si3	1.5	+SiC3H9	349.16812
030	6.03	1429	1000163	362.19938	SI	Glycine, N-(2-methyl-1-oxopropyl)-N-(trimethylsilyl)-, trimethylsilyl ester	mainlib	809	1414	15	C15 H36 N O3 Si3	1.5	+SiC3H9	362.19975
031	6.09	1434	286530	291.14075	SI	bis(trimethylsilyl) 2-methylpentanedioate	AI	718	1406 (±95)	0	C12 H27 O4 Si2	1.5	+H	291.14424
032	6.19	1443	572961	300.14389	SI	trimethylsilyl 3-(3-methyl-1,2-oxazol-5-yl)propanoate	AI	647	1400 (±95)	0	C13 H26 N O3 Si2	3.5	+SiC3H9	300.14457
033	6.23	1446	14930674	227.09678	SI	5-Methyl-3-isoxazolepropanoic acid trimethylsilyl ester	mainlib	789	1344 (±381)	0	C10 H17 N O3 Si	4.0	none	227.09722
034	6.52	1470	431613	365.16214	SI	D-(-)-Citramalic acid, 3TMS derivative	mainlib	729	1487	17	C14 H33 O5 Si3	1.5	+H	365.16303
035	6.66	1482	14654733	232.13616	SI	N-(2-methylbutanoyl)glycine_TMS	AI	725	1492 (±95)	0	C10 H22 N O3 Si	1.5	+H	232.13635
036	6.79	1492	349385	289.13156	SI	bis(trimethylsilyl) 3-methylpent-2-enedioate	AI	662	1474 (±95)	0	C12 H25 O4 Si2	2.5	+H	289.12859
037	6.83	1496	859327	304.17513	SI	N-2-Methylbutyrylglycine, 2TMS derivative	mainlib	737	1491	5	C13 H30 N O3 Si2	1.5	+H	304.17587
038	6.85	1497	242023	355.15705	SI	trimethylsilyl 2-trimethylsilyloxybenzoate	AI	621	1510 (±95)	0	C16 H31 O3 Si3	4.5	+SiC3H9	355.15755
039	6.87	1500	10697228	282.11085	SI	Salicylic acid, 2TMS derivative	replib	940	1522	22	C13 H22 O3 Si2	5.0	none	282.11020
040	6.89	1501	503265	349.16897	SI	trimethyl-[4-trimethylsilyloxy-5-(trimethylsilyloxymethyl)oxolan-2-yl]oxysilane	AI	633	1495 (±95)	0	C14 H33 O4 Si3	1.5	-H	349.16812
041	6.94	1505	907439	236.03786	SI	6-Methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide, TMS	mainlib	811	1523	18	C7 H14 N O4 S Si	2.5	+H	236.04073
042	7.03	1513	12796141	291.14529	SI	Hexanedioic acid, 2TMS derivative	replib	933	1512	1	C12 H27 O4 Si2	1.5	+H	291.14424
043	7.05	1515	4517844	346.16920	SI	DL-Pyroglutamic acid, 2TMS	mainlib	880	1523	8	C14 H32 N O3 Si3	2.5	+SiC3H9	346.16845
044	7.33	1539	360644	289.12803	EI	3-Methyladipic acid, 2TMS derivative	mainlib	822	1544	5	C12 H25 O4 Si2	2.5	-CH3	289.12859
045	7.48	1552	1776015	286.10543	SI	5-Hydroxymethyl-2-furoic acid, 2TMS derivative	mainlib	897	1547	5	C12 H22 O4 Si2	4.0	none	286.10511
046	7.59	1561	3556196	296.07581	SI	1,2-Dithiane-4,5-diol, (Z)-, 2TMS derivative	replib	889	1601	40	C10 H24 O2 S2 Si2	1.0	none	296.07508
047	7.73	1573	12249784	230.11963	SI	Glycine, N-(2-methyl-1-oxo-2-butenyl)-, trimethylsilyl ester, (E)-	mainlib	915	1579	6	C10 H20 N O3 Si	2.5	+H	230.12070
048	7.88	1587	11201975	365.16051	SI	Pentanedioic acid, 2-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	replib	920	1583	4	C14 H33 O5 Si3	1.5	+H	365.16303
049	8.05	1602	456369	296.12605	SI	3-Hydroxyphenylacetic acid, 2TMS derivative	mainlib	755	1618	16	C14 H24 O3 Si2	5.0	none	296.12585
050	8.10	1606	1308053	379.17960	SI	Pentanedioic acid, 3-methyl-3-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	mainlib	739	1614	8	C15 H35 O5 Si3	1.5	+H	379.17868
051	8.14	1610	12976144	305.15935	SI	Pimelic acid, 2TMS derivative	mainlib	909	1610	0	C13 H29 O4 Si2	1.5	+H	305.15989
052	8.24	1619	827860	282.11082	SI	4-Hydroxybenzoic acid, 2TMS derivative	replib	843	1635	16	C13 H22 O3 Si2	5.0	none	282.11020
053	8.31	1625	970706	295.14316	SI	Paracetamol, 2TMS derivative	replib	818	1625	0	C14 H25 N O2 Si2	5.0	none	295.14183
054	8.41	1634	4823139	296.12605	SI	4-Hydroxybenzeneacetic acid, 2TMS derivative	replib	905	1648	14	C14 H24 O3 Si2	5.0	none	296.12585
055	8.48	1641	12537944	377.14935	SI	A-Ketoglutaric acid oxime_3TMS	AI	702	1656 (±95)	0	C14 H31 N O5 Si3	3.0	none	377.15045
056	8.60	1652	3145782	242.08656	SI	trimethylsilyl 2-(furan-2-carbonylamino)acetate	AI	760	1657 (±95)	0	C10 H16 N O4 Si	4.5	+H	242.08431
057	8.62	1653	5215354	318.19184	SI	Hexanoyl glycine, bis(trimethylsilyl)-	mainlib	911	1663	10	C14 H32 N O3 Si2	1.5	+H	318.19152
058	8.69	1660	1029228	378.15546	SI	2-ketoglutaric acid oxime, tri-TMS	mainlib	718	1645	15	C14 H32 N O5 Si3	2.5	+H	378.15828
059	8.81	1671	1069656	237.08284	SI	2-(Formylamino)benzoic acid, TMS derivative	mainlib	855	1693	22	C11 H15 N O3 Si	6.0	none	237.08157
060	9.07	1694	396168	309.12433	SI	2-(Formylamino)benzoic acid, 2TMS derivaitive	mainlib	797	1707	13	C14 H23 N O3 Si2	6.0	none	309.12110
061	9.15	1702	12753036	319.17468	SI	Suberic acid, 2TMS derivative	mainlib	911	1707	5	C14 H31 O4 Si2	1.5	+H	319.17554
062	9.33	1718	376454	310.13944	SI	3-(3-Hydroxyphenyl)propanoic acid, 2TMS derivative	mainlib	811	1738	20	C15 H26 O3 Si2	5.0	none	310.14150
063	9.65	1749	9019293	373.14063	SI	Orotic Acid, 3TMS derivative	replib	947	1757	8	C14 H29 N2 O4 Si3	4.5	+H	373.14296
064	9.73	1757	1138142	312.12172	SI	Vanillic Acid, 2TMS derivative	replib	785	1776	19	C14 H24 O4 Si2	5.0	none	312.12076
065	9.80	1763	13581808	391.14231	SI	Aconitic acid, (Z)-, 3TMS derivative	replib	939	1741	22	C15 H31 O6 Si3	3.5	+H	391.14229
066	9.82	1765	970253	326.13607	EI	Homovanillic Acid, 2TMS derivative	replib	806	1785	20	C15 H26 O4 Si2	5.0	none	326.13641
067	9.94	1777	329413	295.08090	EI	Terephthalic acid, 2TMS derivative	replib	805	1799	22	C13 H19 O4 Si2	6.5	-CH3	295.08164
068	10.02	1784	715977	384.15999	SI	4-Hydroxymandelic acid, 3TMS derivative	mainlib	734	1797	13	C17 H32 O4 Si3	5.0	none	384.16029
069	10.15	1797	10465545	324.14308	SI	Hippuric acid, 2TMS derivative	replib	890	1821	24	C15 H26 N O3 Si2	5.5	+H	324.14457
070	10.21	1802	13628193	405.23020	SI	Azelaic acid, 2TMS derivative	mainlib	876	1806	4	C18 H41 O4 Si3	1.5	+SiC3H9	405.23072
071	10.47	1829	8546357	465.15761	SI	Citric acid, 4TMS derivative	replib	895	1845	16	C17 H37 O7 Si4	3.5	-CH3	465.16109
072	10.55	1837	43236313	251.09668	SI	Hippuric acid, TMS derivative	replib	910	1846	9	C12 H17 N O3 Si	6.0	none	251.09722
073	10.71	1853	4694308	398.17648	SI	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, tris-(O-trimethylsilyl)-	mainlib	934	1839	14	C18 H34 O4 Si3	5.0	none	398.17594
074	10.81	1863	310263	356.14600	EI	trimethylsilyl 3-(2-methoxyphenoxy)-2-trimethylsilyloxypropanoate	AI	515	1858 (±95)	0	C16 H28 O5 Si2	5.0	none	356.14698
075	10.86	1867	1865334	351.17974	EI	2-Hydroxyibuprofen, O,O-bisTMS	mainlib	898	1888	21	C18 H31 O3 Si2	5.5	-CH3	351.18062
076	11.01	1883	3189124	414.17228	SI	Vanillylmandelic acid, 3TMS derivative	replib	852	1900	17	C18 H34 O5 Si3	5.0	none	414.17086
077	11.10	1891	1162987	247.10160	EI	trimethylsilyl 2-indol-1-ylacetate	AI	803	1765 (±95)	31	C13 H17 N O2 Si	7.0	none	247.10231
078	11.12	1893	311628	398.17282	SI	trimethylsilyl 2-trimethylsilyloxy-3-(4-trimethylsilyloxyphenyl)propanoate	AI	614	1895 (±95)	0	C18 H34 O4 Si3	5.0	none	398.17594
079	11.15	1897	10645103	331.17491	EI	Sebacic acid, 2TMS derivative	replib	921	1899	2	C15 H31 O4 Si2	2.5	-CH3	331.17554
080	11.43	1926	390695	351.17962	EI	3-Hydroxyibuprofen_2TMS	AI	730	1940 (±95)	0	C18 H31 O3 Si2	5.5	-CH3	351.18062
081	11.55	1939	1120587	319.14115	SI	trimethylsilyl 2-(1-trimethylsilylindol-3-yl)acetate	AI	846	1973 (±95)	0	C16 H25 N O2 Si2	7.0	none	319.14183
082	11.57	1941	451030	396.14663	EI	2-Hydroxyhippuric acid, 3TMS derivative	replib	715	1941	0	C17 H30 N O4 Si3	6.5	-CH3	396.14771
083	12.05	1991	3375201	380.18232	SI	Carboxyibuprofen, bis(trimethylsilyl) ester	mainlib	849	2003	12	C19 H32 O4 Si2	6.0	none	380.18336
084	12.08	1994	413634	428.18682	EI	3-Hydroxy-3-(4'-hydroxy-3'-methoxyphenyl)propionic acid, tri-TMS	mainlib	795	2012	18	C19 H36 O5 Si3	5.0	none	428.18651
085	12.53	2043	4774829	-	-	Palmitic Acid, TMS derivative	replib	891	2049	6	C19 H40 O2 Si	1.0	-CH3	313.25573
086	12.56	2046	1952910	412.17597	SI	2-Hydroxyhippuric acid, 2TMS derivative	mainlib	862	2086	40	C18 H34 N O4 Si3	5.5	+SiC3H9	412.17901
087	12.85	2077	185405	338.13527	EI	Isoferulic acid, 2TMS	mainlib	817	2080	3	C16 H26 O4 Si2	6.0	none	338.13641
088	12.95	2088	2594812	424.17765	EI	N-acetyltyrosine_3TMS	AI	716	2087 (±95)	0	C19 H34 N O4 Si3	6.5	-CH3	424.17901
089	13.19	2114	11507190	367.16244	SI	N-Acetyltyrosine, 2TMS	replib	951	2143	29	C17 H29 N O4 Si2	6.0	none	367.16296
090	13.34	2132	301329	339.13080	SI	p-hydroxyhippuric acid_2TMS	AI	722	2241 (±95)	14	C15 H25 N O4 Si2	6.0	none	339.13166
091	13.46	2146	23859279	415.34529	SI	Heptadecanoic acid, TMS derivative	replib	828	2142	4	C23 H51 O2 Si2	0.5	+SiC3H9	415.34221
092	13.49	2149	552331	415.33798	SI	16-Methyl-heptadecane-1,2-diol, trimethylsilyl ether	mainlib	703	2250 (±381)	0	C23 H51 O2 Si2	0.5	-CH3	415.34221
093	13.79	2183	525234	335.13782	SI	5-Hydroxyindoleacetic acid, 2TMS derivative	mainlib	892	2224	41	C16 H25 N O3 Si2	7.0	none	335.13675
094	13.95	2201	396373	407.17853	SI	5-Hydroxyindoleacetic acid, 3TMS derivative	replib	906	2239	38	C19 H33 N O3 Si3	7.0	none	407.17627
095	14.03	2210	3987782	339.13140	SI	Glycine, N-[4-[(trimethylsilyl)oxy]benzoyl]-, trimethylsilyl ester	mainlib	899	2233	23	C15 H25 N O4 Si2	6.0	none	339.13166
096	14.24	2236	350950	406.13070	EI	xanthurenic acid_3TMS	AI	661	2290 (±95)	0	C18 H28 N O4 Si3	8.5	-CH3	406.13206
097	14.27	2239	4265781	357.31850	SI	Stearic acid, TMS derivative	mainlib	906	2243	4	C21 H45 O2 Si	0.5	+H	357.31833
098	14.42	2257	7517500	376.19891	SI	Suberylglycine_2TMS	AI	694	2233 (±95)	0	C16 H34 N O5 Si2	2.5	+H	376.19700
099	14.98	2324	4428464	398.18543	SI	10,11-Dihydro-10-hydroxycarbamazepine, N-trimethylsilyl-, trimethylsilyl ether	mainlib	961	2358	34	C21 H30 N2 O2 Si2	10.0	none	398.18403
100	15.15	2345	1171970	369.14082	SI	Vanilloylglycine_2TMS	AI	809	2348 (±95)	0	C16 H27 N O5 Si2	6.0	none	369.14223
101	15.83	2430	1532001	572.24637	SI	p-Tolyl-β-D-glucuronide, tris(trimethylsilyl) ether, trimethylsilyl ester	mainlib	898	2419	11	C25 H48 O7 Si4	6.0	none	572.24716
102	15.94	2444	2688241	409.19614	SI	Phenylacetylglutamine, O-TMS	mainlib	851	2486	42	C19 H33 N2 O4 Si2	6.5	+SiC3H9	409.19734
103	15.97	2448	980568	486.21526	SI	Carbamazepine-10,11-dihydrodiol,3TMS derivative	mainlib	878	2823 (±381)	0	C24 H38 N2 O3 Si3	10.0	none	486.21847
104	16.23	2481	18247983	408.18870	EI	Phenylacetylglutamine, N,O-bis-TMS	mainlib	869	2500	19	C19 H32 N2 O4 Si2	7.0	none	408.18951
105	16.43	2507	397828	375.14626	EI	Phthalic acid, di(2-propylpentyl) ester	mainlib	795	2527	20	C24 H38 O4	6.0	-	-
106	17.04	2588	487023	459.33099	EI	1-Monopalmitin, 2TMS derivative	replib	854	2606	18	C24 H51 O4 Si2	1.5	-CH3	459.33204
107	18.42	2779	535056	487.36193	EI	Glycerol monostearate, 2TMS derivative	replib	846	2768	11	C26 H55 O4 Si2	1.5	-CH3	487.36334
108	18.84	2840	5521808	504.29043	SI	Estriol, 3TMS	mainlib	954	2894	54	C27 H48 O3 Si3	7.0	none	504.29058

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Quantitative Analysis Results

Figure 4 shows the EI TICC and extracted ion chromatograms (EICs) of three target organic acids: 2TMS-lactic acid, 2TMS-glycolic acid and 2TMS-pyruvic acid oxime. Multiple peaks were detected in TICC for the complicated components in urine. However, the EICs created by the accurate *m/z* signal manages to detect the target compound while limiting the influence of other compounds to the minimum.

All organic acids, including co-eluted organic acids, show detectable peak shapes for quantitation analysis (partially presented in Figure 4). As an example of quantitative analysis, the quantitative values of target organic acids in several urine samples shown in Table 3 were calculated from the area ratio with reference samples at known concentrations.

Table 3. Quantitative values of target organic acids (Unit: μM)

Target Compounds	Reference sample	Negative control	Sample 1	Sample 2	Sample 3
Lactic Acid, 2TMS derivative	110.39	22.125	47.965	21.408	106.486
Glycolic acid, 2TMS derivative	101.82	21.094	296.258	95.486	68.736
Pyruvic acid oxime, bis(trimethylsilyl)- deriv.	69.32	3.29	68.008	17.624	99.834
3-Hydroxybutyric acid, 2TMS derivative	85.26	24.108	189.822	62.896	140.502
Malonic acid (bis-TMS)	597.39	N.D.	52.678	N.D.	N.D.
Methylmalonic acid, 2TMS derivative	16.71	N.D.	2.226	N.D.	4.476
2-Hydroxyisocaproic acid, 2TMS derivative	26.07	N.D.	N.D.	N.D.	0.947
Ethylmalonic acid, 2TMS derivative	34.06	1.178	6.897	1.399	8.493
Succinic acid (2TMS)	58.99	9.027	11.01	3.012	19.784
Methylsuccinic acid, 2TMS derivative	37.46	0.848	4.966	0.578	3.38
Uracil, 2TMS derivative	120.47	0.366	5.711	N.D.	0.446
Glyceric acid, 3TMS derivative	215.37	N.D.	3.335	N.D.	6.28
Isobutyrylglycine, TMS derivative	32.89	0.056	1.301	0.248	0.581
Glutaric acid (2TMS)	31.39	0.679	3.483	1.149	2.107
5-Methyl-3-isoxazolepropanoic acid trimethylsilyl ester	130.87	0.037	N.D.	N.D.	0.016
2-Methylbutyrylglycine, TMS ester	21.03	0.116	0.755	0.023	0.23
DL-Pyroglutamic acid, 2TMS	101.79	15.708	96.916	16.772	47.851
Adipic acid, (2TMS)	31.54	1.259	12.437	1.074	4.637
Tiglyl glycine, 2TMS derivative	117.44	0.258	32.114	0.315	15.06
2-Hydroxyglutaric acid, tri-TMS	72.16	1.863	13.407	1.767	17.665
3-Hydroxy-3-methylglutaric acid, tri-TMS	124.06	N.D.	9.917	43.703	8.687
Pimelic acid, 2TMS derivative	42.46	1.165	4.868	3.24	7.039
Hexanoyl glycine, bis(trimethylsilyl)-	24.07	N.D.	0.061	N.D.	N.D.
Suberic acid, 2TMS derivative	29.27	0.695	2.989	2.026	5.466
Orotic Acid, 3TMS derivative	48.76	0.108	0.621	0.116	0.902
Azelaic acid, 2TMS derivative	27.62	0.983	4.021	2.833	7.592
Sebacic acid, 2TMS derivative	22.87	0.057	0.355	0.153	0.102
N-Acetyltyrosine, 2TMS	25.41	0.068	0.19	0.012	0.372
Suberylglycine-TMS	12.12	N.D.	N.D.	N.D.	N.D.

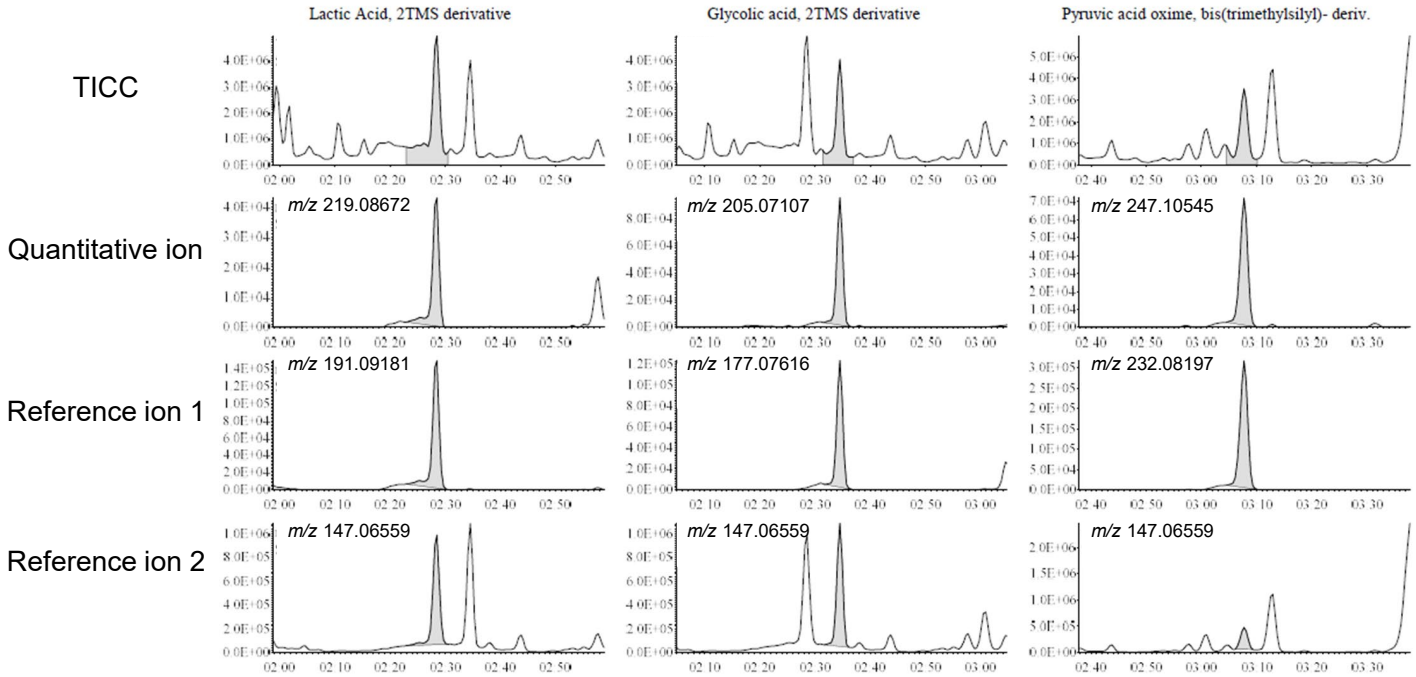

Figure 4. TICC and EICs of extracted urine sample.
(Right: 2TMS-lactic acid, Center: 2TMS-glycolic acid, Left: 2TMS-pyruvic acid oxime)

Summary

The JMS-T2000GC with msFineAnalysis AI is a powerful tool for the identification of the chemical composition and chemical structure of components such as TMS derivatives of organic acids in urine, and for the quantitative analysis by EICs which are created by accurate *m/z*. The JMS-T2000GC with msFineAnalysis AI is expected to have a wide range of applications for metabolomics and clinical analysis in the future.

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