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Gas chromatography–mass spectrometry with *tert.*-butyldimethylsilyl derivatization: use of the simplified sample preparations and the automated data system to screen for organic acidemias

Takaharu Ohie*, Xiao-wei Fu, Misako Iga, Masahiko Kimura, Seiji Yamaguchi

Department of Pediatrics, Shimane Medical University, 89-1 En-ya-cho, Izumo, Shimane 693-8501, Japan

Abstract

A simplified, sensitive screening method for organic acidemias by gas chromatography–mass spectrometry using urease/direct preparations and *tert.*-butyldimethylsilylation (TBDMS) instead of trimethylsilylation (TMS) is described. We compiled GC–MS data on TBDMS derivatives, including methylene unit values, and quantifying and confirming ions, for use in the automated data system we developed. Quantification using $[M\text{-}57]^+$ ions by mass chromatography was more sensitive, and the coefficient variation was smaller, compared with TMS derivatives. We confirmed the usefulness of this system, analyzing urine specimens from 53 patients with 15 different disorders.

Keywords: Derivatization, GC; Organic acidemias

1. Introduction

Organic acid disorders are clinically characterized by acute or episodic illness which often occurs in neonates or in early infancy [1]. Screening programs for organic acidemias have been attempted throughout the world. There are two approaches for such screening: one is acylcarnitine analysis by tandem mass spectrometry (tandem MS) using blood filter paper [2,3], and the other is organic acid analysis by gas chromatography–mass spectrometry (GC–MS), using liquid urine or dried urine filter paper [4–7].

As to the screening by GC–MS, bench top GC–MS with user-friendly computers and software have been developed recently. A simplified method (direct/urease method) using liquid or filter paper urine is also available [7]. We developed a personal computer-based system for automated metabolic profiling and interpretation of GC–MS results for organic acidemia screening (automated data system) [8,9]. Thus, GC–MS has been increasingly used for screening or for routine laboratory tests.

For derivatization of organic acids, trimethylsilyl (TMS) derivatization is simple and is often used for GC–MS analysis. Mass spectra of TMS derivatives provide information on the chemical structures of compounds with a number of fragment ions, including $[M\text{-}15]^+$ ions specific for each metabolite. However, since ion intensity of specific fragment ions tends to be small, the sensitivity of quantifica-

*Corresponding author. Tel.: +81-853-20-2219; fax: +81-853-20-2215.

E-mail address: tohie@shimane.med.or.jp (T. Ohie).

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tion by mass chromatography may be limited. In another derivatization, *tert.*-butyldimethylsilyl (TBDMS) derivatization, an intense specific ion peak, $[M-57]^+$, is obtained in the mass spectrum of TBDMS derivatives [10]. Mass chromatography with the specific ion of TBDMS derivatives may be more sensitive for quantification, as compared with that of TMS derivatives [11].

We studied the practicability of TBDMS derivatization for organic acidemia screening, using the urease/direct method and GC–MS, and comparisons of recovery, variation and availability between TBDMS and TMS derivatization were made. We compiled GC–MS data on TBDMS derivatives, including methylene unit (MU) values, quantifying (Q-) and confirming (C-) ions, of organic acids and other metabolites such as amino acids, sugars or alcohols, for use in the automated data system; this system requires MU values, Q- and C-ions of each metabolite for identification and quantification of compounds [8]. We analyzed urine specimens from 53 patients with 15 different metabolic disorders, the object being to test the usefulness of the analytical system.

2. Materials and methods

2.1. Reagents

N-Methyl-*N*-(*tert.*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and *N*,*N*-dimethylformamide (DMF) were purchased from Aldrich (Milwaukee, WI, USA); *N*,*O*-bis(trimethylsilyl)-fluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were from Nakalai Tesque (Tokyo, Japan). Acylglycines were generous gifts from Dr P Rinaldo (Mayo Clinic, MI). The other compounds were from the same source, as reported elsewhere [9].

2.2. Preparation

The urease/direct method was according to Matsumoto and Kuhara [7] but with some modifications. Basically 100 μl of aliquots were used, and 20 μg each of margarate (MGA) and tetracosane (C24), and 40 μg of tropate (TA) were added as internal standards. Solvent extraction was performed to acquire GC–MS data of oxime derivatives of 2-ketoacids, using the method reported elsewhere [9].

2.3. Derivatization

In TBDMS derivatization, 50 μl each of MBTBDMS and DMF were added to the dry residues, and the resultant solution was heated at 85°C for 30 min. TMS derivatization was performed, previously described [9].

2.4. GC–MS analysis

The GC–MS equipment used was a Shimadzu QP5000 (Shimadzu, Kyoto, Japan). The capillary column was a fused-silica DB-5, 30 m×0.25 mm I.D., 0.25 μm film thickness, 5% phenylsilicone (J&W, Folsom, CA). Mass spectra were obtained by electron impact ionization mode, scanning every 0.4 s from 50 *m*/*z* to 650 *m*/*z*. The temperature program was as follows: an initial holding at 100°C for 1 min; raising at a rate of 10°C/min to 300°C; and a final holding at 300°C for 15 min (total analysis time, 35 min).

2.5. Compilation of GC–MS data and application to the automated system

To use the automated data system, we compiled GC–MS data, including MU values, Q- and C-ions of TBDMS derivatives of a total of 113 different compounds with 122 peaks, as listed in Table 1. Fig. 1 illustrates mass spectra of TBDMS and TMS derivatives of isovalerylglycine and alanine, respectively. In the TBDMS derivative of these two compounds, the intensity of *m*/*z* 216 and *m*/*z* 260, that corresponds to each $[M-57]^+$ ion is stronger than that of the $[M-15]^+$ ions of TMS derivatives. Hence, the $[M-57]^+$ fragment ion was taken as the Q-ion for most TBDMS derivatives. Data on TBDMS derivatives of nine kinds of 2-ketoacids, after oximation and solvent extraction were also determined. The MU values were calculated with retention times of even-numbered C_{10} to C_{34} in the hydrocarbon mixture solution, as described [12]. Hence, the analytical data on GC–MS of TBDMS derivatives were processed using the automated data system.

Table 1
MU values and Q-ions of TBDMS-derivatives[a]

(No.) Compounds	MU	Q-ion
(1) Lactic-2B	14.92	261
(2) Glycolic-2B	15.09	247
(3) Phenylacetic-1B	15.27	193
(4) Alanine-2B	15.40	260
(5) Oxalic-2B	15.48	261
(6) 2-Hydroxybutyric-3B	15.59	275
(7) Glycine-2B	15.62	246
(8) 3-Hydroxypropionic-3B	15.80	261
(9) Propionylglycine-1B	15.92	188
(10) 3-Hydroxylbutyric-2B	15.94	275
(11) 2-Hydroxyisovaleric-2B	16.08	289
(12) Isobutyrylglycine-1B	16.21	202
(13) Mavalonolactone-1B	16.25	187
(14) 2-Methyl-3-butyric-2B	16.41	289
(15) Malonic-2B	16.45	275
(16) 3-Hydroxyisovaleric-2B	16.52	289
(17) 2-Hydroxyisocaproic-2B	16.54	303
(18) Valine-2B	16.59	288
(19) Methylmalonic-2B	16.60	289
(20) Urea-2B	16.71	231
(21) 4-Hydroxybutyric-2B	16.74	275
(22) Leucine-2B	17.03	302
(23) Isovalerylglycine-1B (1)	17.21	216
(24) Ethylmalonic-2B	17.25	303
(25) Isoleucine-2B	17.48	302
(26) γ-Aminobutyric-2B	17.60	274
(27) Succinic-2B	17.60	289
(28) Uracil-2B	17.65	283
(29) Proline-2B	17.69	286
(30) Methylsuccinic-2B	17.70	303
(31) Fumaric-2B	17.86	287
(32) Maleic-2B	17.89	287
(33) 3-Methylcrotonylglycine-1B	18.01	214
(34) Tiglyglycine-1B	18.12	214
(35) Propionylglycine-2B	18.23	302
(36) Glutaric-2B	18.59	303
(37) Hexanoylglycine-1B	18.72	230
(38) 3-Methylglutaric-2B	18.73	317
(39) Glycerol-3B	18.83	377
(40) Butyrylglycine-2B	18.85	373
(41) 3-Methylglutaconic-2B	18.85	315
(42) Isovalerylglycine-2B	19.10	330
(43) Creatinine-4B	19.41	298
(44) Glyceric-3B	19.56	391
(45) 5-Oxoproline-2B	19.56	300
(46) Adipic-2B	19.66	317
(47) Methionine-2B	19.73	320
(48) 3-Methyladipic-2B	19.90	331
(49) Serine-3B	19.93	390
(50) Threonine-3B	20.29	404
(51) Phenyllactic-2B	20.48	235
(52) Pimelic-2B	20.71	331
(53) Octanoylglycine-1B	20.76	258

Table 1. Continued

(No.) Compounds	MU	Q-ion
(54) Phenylalanine-2B	20.98	336
(55) Hippuric-1B	21.04	236
(56) *N*-Acetylaspartic-2B	21.06	346
(57) 4-Hydroxyphenylacetic-2B	21.15	323
(58) Glutaconic-2B	21.35	301
(59) Octenedioic-2B	21.52	343
(60) Aspartic-3B	21.58	418
(61) Suberic-2B	21.74	345
(62) Mevalonic-3B	22.09	433
(63) 3-Hydroxyglutaric-3B	22.14	433
(64) Cystein-3B	22.15	406
(65) 2-Hydroxyglutaric-3B	22.19	433
(66) Homovanillic-2B	22.46	353
(67) 3-Hydroxy-3-methylglutaric-3B	22.52	447
(68) Vanillic-2B	22.59	339
(69) Glutamic-3B	22.76	432
(70) Azelic-2B	22.78	359
(71) Phenylpropionylglycine-1B	22.79	264
(72) Ornithine-3B	22.85	474
(73) Palmitic-1B	22.90	313
(74) Decenedioic-2B	23.47	371
(75) Sebacic-2B	23.78	373
(76) Lysine-3B	23.81	431
(77) 2-Aminoadipic-3B	23.86	446
(78) Orotic-3B	23.93	441
(79) Glutamine-3B	24.31	431
(80) Glutarylglycine-2B	24.34	360
(81) *t*-Cinnamoylglycine-1B	24.60	262
(82) Stearic-1B	24.89	341
(83) Galactitol (1)	24.90	260
(84) Homogentic-3B	25.07	453
(85) Vanillylmandelic-3B	25.11	483
(86) Galactose (1)	25.16	245
(87) Glucose (1)	25.23	231
(88) 3-Hydroxysuberic-3B	25.26	475
(89) Galactitol (2)	25.36	302
(90) Galactose (2)	25.71	287
(91) Dodecanedioic-2B	25.79	401
(92) Galactose (3)	25.89	287
(93) Glycerol-3-phosphate-1B	25.91	571
(94) Histidine-3B	25.95	440
(95) 4-Hydroxyphenyllactic-3B	26.00	439
(96) Galactitol (4)	26.10	289
(97) Glucose (2)	26.12	315
(98) Citric-4B	26.22	329
(99) Glucose (3)	26.22	591
(100) Isocitric-4B	26.32	591
(101) Tyrosine-3B	26.50	466
(102) Methylcitric-4B (1)	26.65	605
(103) Methylcitric-4B (2)	26.92	605
(104) Tryptophan-4B	26.98	375
(105) Suberylglycine-1B	28.20	402
(106) Ascorbic-4B	28.56	575

Table 1. Continued

(No.) Compounds	MU	Q-ion
(107) Uric-4B	28.80	567
(108) Xanthurenic-3B	30.04	490
(109) Cystine-4B	31.80	639
Internal standard		
(110) Tropic (TA)-1B	20.33	337
(111) Pentadecanic (PDA)-1B	21.85	299
(112) Margaric (MGA)-1B	23.88	327
(113) Tetracosane (C24)	24.00	99
Oximated 2-ketoacids[b]		
(114) Pyruvic-2B	15.90	274
(115) 2-Ketoisovaleric-2B	16.46	507
(116) Succinylacetone-2B	16.84	344
(117) 2-Keto-3-methylvaleric-2B	17.09	316
(118) 2-Ketoisocaproic-2B	17.26	316
(119) 2-Ketocaproic-2B	17.68	316
(120) 2-Ketoadipic-2B	20.54	360
(121) Phenylpyruvic-2B	21.04	350
(122) 2-Ketoglutaric-2B	21.49	446

[a] The $[M\text{-}57]^+$ was selected as Q-ion in most cases, except for sugars or alcohols. The number next to compound names, such as 2B or 3B, means the number of TBDMS moieties bonded. The number in cases of sugars or alcohols is omitted, because the number of TBDMS moieties could not be precisely identified.

[b] The data of 2-ketoacids were determined after oximation and solvent extraction.

2.6. Standard solution

To compare the recovery and variation of quantification of TBDMS with TMS derivatization, we prepared two kinds of standard solution, A and B, the contents of which are listed in Table 2. Recovery was determined as relative peak areas (RPA, %) of each compound to that an internal standard, tetracosane (C_{24}), on total ion current (TIC) chromatograms, because C_{24} is not affected by either derivatization of TBDMS or TMS. Coefficient variation (CV) values (%), were determined by PRA (%) of Q-ions to that of another internal standard, MGA (m/z 327), on mass chromatograms.

2.7. Analysis of patient urine samples

Urine specimens from 53 Japanese patients with 15 metabolic disorders were analyzed to test the diagnostic usefulness and to compare findings with those of TMS derivatives. Practically, 100 μl of the samples were prepared by the urease/direct method and derivatization of TBDMS or TMS, and analyzed using GC–MS and the same analytical condition, as described above.

3. Results

3.1. Compilation of GC–MS data of TBDMS derivatives

MU values and Q-ions for compounds that we compiled for use in the automated data system are listed in Table 1. The $[M\text{-}57]^+$ ions are the Q-ions for TBDMS derivatives in most metabolites, except for sugars or alcohols. The MU values of compounds determined ranged from 14.92 (lactate (LA)-di TBDMS) to 31.80 (cystine (Cys)-tetra TBDMS). The Q-ions ranged from m/z 188 (propionylglycine-mono TBDMS) to 639 (Cys-tetra TBDMS), respectively. Additionally, data on oximated 2-ketoacids, are also listed in the last part of Table 1, as such may be helpful for analyses in case of solvent extraction after oximation.

3.2. Comparison of recovery between TBDMS and TMS derivatives

Fig. 2 shows TIC chromatograms of TBDMS and TMS of the standard solutions A and B, respectively. Recovery in TIC chromatograms of the standard solution was compared, using RPA (%) to the peak area of C_{24}, that does not undergo derivatization. As illustrated in Fig. 3, the RPA values of TBDMS derivatives were larger in 18 of 25 compounds tested, compared with TMS data. As to the other six compounds, no significant difference was observed.

Recovery of 3-hydroxy-3-methylglutarate (HMG) was much smaller in TMS than in TBDMS. In TMS derivatization, a large part of HMG changed to 3-methylglutaconate, whereas the TBDMS derivative of HMG gave a larger single peak.

3.3. Comparison of CV values between TBDMS- and TMS-derivatives

The CV values (%) in mass chromatographic measurements of the standard solution by 5 times inter-assay are listed in Table 2. The CV values of

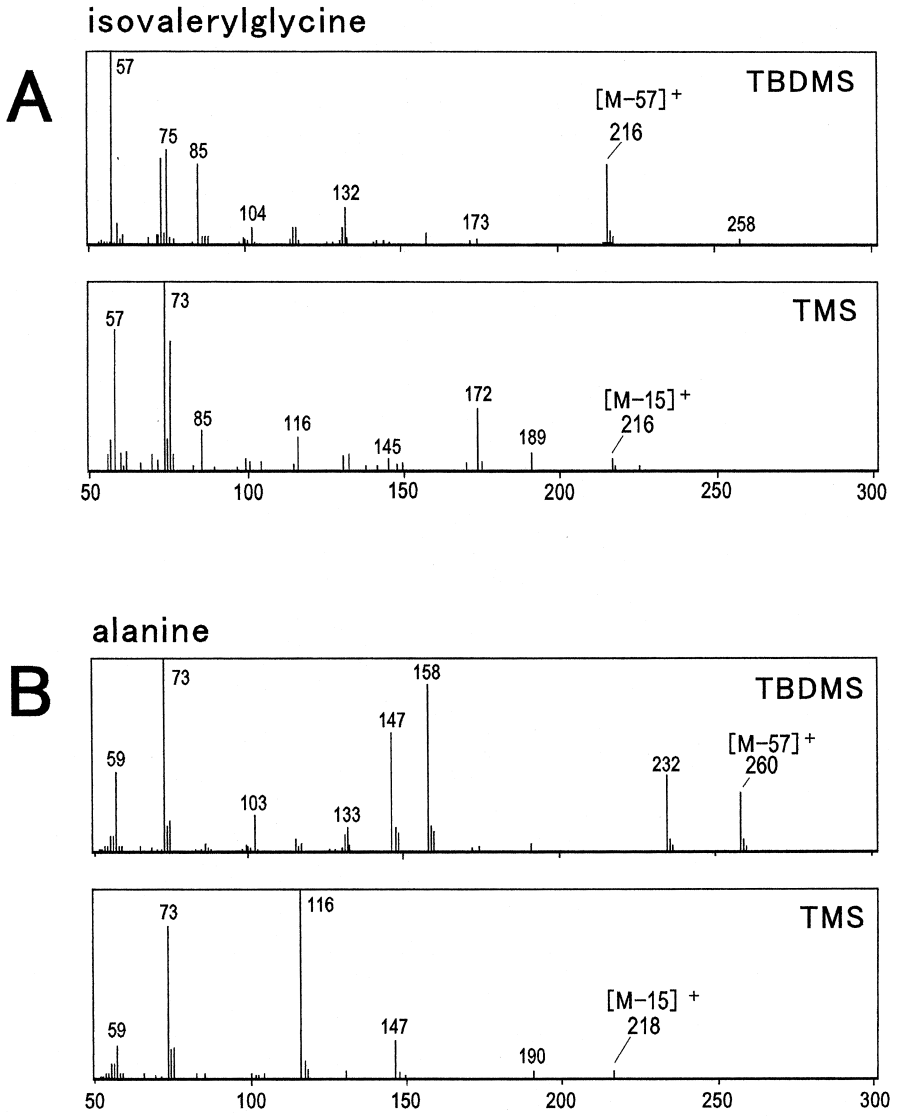

Fig. 1. Mass spectra of TBDMD and TMS derivatives of isovalerylglycine (A) and alanine (B).

TBDMS derivatives of organic acids ranged from 4.3% (glutarate) to 11.0% (methylmalonate), while those of TMS were from 8.8% (adipate) to 16.1% (2-hydroxyisovalerate). For compounds other than organic acids, including amino acids, the CV values of TBDMS derivatives ranged from 2.4% (alanine) to 6.3% (uracil), whereas those of TMS ranged from 6.7% (tyrosine) to 14.2% (methionine). The CV values (%) of all 25 compounds tested were smaller in TBDMS than in TMS derivatives.

3.4. Analysis of samples from patients diagnosed previously

Fig. 4 illustrates TIC chromatograms of a patient with glutaric aciduria type 2, by TBDMS- and TMS-derivatization. The result from the automated data system with TBDMS-derivatization in this case is illustrated in Table 3. In the metabolic profile, diagnostic metabolites, such as ethylmalonate, glutarate, isovalerylglycine and adipate, were automatical-

Table 2
Contents of standard solution and coefficient variation (CV value, %) in quantification[a]

Compounds (abbreviation)	Concentration (μg/ml)	TBDMS			TMS		
		MU	Q-ion (%)	CV	MU	Q-ion (%)	CV
Solution A							
(1) Lactate (LA)	60	14.92	261 (16)	4.4	10.51	219 (1)	11.6
(2) 2-Hydroxyisovalerate (2HIV)	55	16.08	289 (15)	7.4	11.65	219 (3)	16.1
(3) Methylmalonate (MMA)	75	16.60	289 (21)	11.0	12.20	247 (2)	14.8
(4) Isovalerylglycine(1) (IVG)	130	17.21	216 (43)	8.3	14.88	216 (6)	10.1
(5) Thylmalonate (EMA)	75	17.25	303 (18)	7.7	12.83	261 (2)	15.1
(6) Succinate (SA)	90	17.60	289 (23)	4.5	13.14	247 (4)	14.1
(7) Uracil (U)	60	17.65	283 (100)	6.3	13.46	241 (46)	10.9
(8) Glutarate (GA)	60	18.59	303 (26)	4.3	14.07	261 (11)	13.9
(9) Creatinine (Cr)	500	19.41	298 (16)	4.7	15.70	314 (5)	9.9
(10) Glycerate (GCA)	60	19.56	391 (10)	6.3	13.36	261 (7)	12.8
(11) Adipate (AD)	80	19.66	317 (29)	5.1	15.10	275 (4)	8.8
(12) Suberate (SU)	80	21.74	345 (27)	4.4	17.02	303 (6)	12.1
(13) 2-Hydroxyglutarate (2HG)	100	22.19	433 (22)	9.1	15.81	349 (3)	12.7
(14) 3-Hydroxy-3-methylglutarate(HMG)	70	22.52	447 (10)	10.0	16.13	363 (2)	10.7
(15) 4-Hydroxyphenyllactate (PHPL)	70	26.00	439 (10)	4.6	19.19	308 (11)	11.4
Solution B							
(16) Alanine (Ala)	100	15.40	260 (21)	2.4	10.93	218 (1)	8.6
(17) Glycine (Gly)	100	15.62	246 (21)	6.0	11.05	204 (5)	9.7
(18) Valine (Val)	150	16.59	288 (16)	3.5	12.21	246 (1)	7.9
(19) Leucine (Leu)	150	17.03	302 (18)	4.3	12.76	218 (1)	9.4
(20) Isoleucine (Ile)	150	17.48	302 (23)	4.4	13.00	234 (3)	9.1
(21) Proline (Pro)	150	17.69	286 (11)	4.6	13.05	216 (4)	11.8
(22) Methionine (Met)	200	19.73	320 (18)	5.3	14.20	221 (4)	14.2
(23) Serine (Ser)	150	19.93	390 (14)	3.4	13.68	306 (1)	12.1
(24) Phenylalanine (Phe)	200	20.98	336 (13)	4.4	16.40	294 (1)	10.1
(25) Tyrosine (Tyr)	200	26.50	466 (4)	2.5	19.01	310 (3)	6.7
Internal standard							
Tropate (TA)	40	20.33	337 (19)		15.99	280 (18)	
Margarate (MGA)	20	23.88	327 (82)		21.48	327 (24)	
Tetracosane (C24)	20	24.00	99 (11)		24.00	99 (5)	

[a] MU, methylene unit; (%), relative ion intensity (%) to that of the base peak on mass spectra. CV values were calculated by 5× inter-assay.

ly identified as abnormal compounds, and at the bottom of the table, the disease of this patient, glutaric aciduria type 2, is indicated. As to concomitant findings, galactosemia, glyceroluria, ketosis or dicarboxylic aciduria are also indicated. From the result, we could interpret that this patient had glutaric aciduria type 2, and was in a condition complicated by mild galactosemia, glyceroluria or ketosis. Hence, it was confirmed that the TBDMS derivatization was also applicable to the automated data system.

In the same manner, urine specimens from 53 patients with 15 metabolic disorders were analyzed by TBDMS and TMS derivatization. The diagnosis had been made previously using GC–MS analysis of urine samples corresponding to 0.2 mg creatinine with solvent extraction. In this study, 0.1 ml of urine specimens were analyzed. We judged the results as follows: S (successful), in cases that a correct diagnosis was indicated; B (borderline), for cases where the excretion of one or more diagnostic marker metabolites was detected but seemed incomplete; M (missing), that the correct diagnosis was considered to be missed because of absence or too

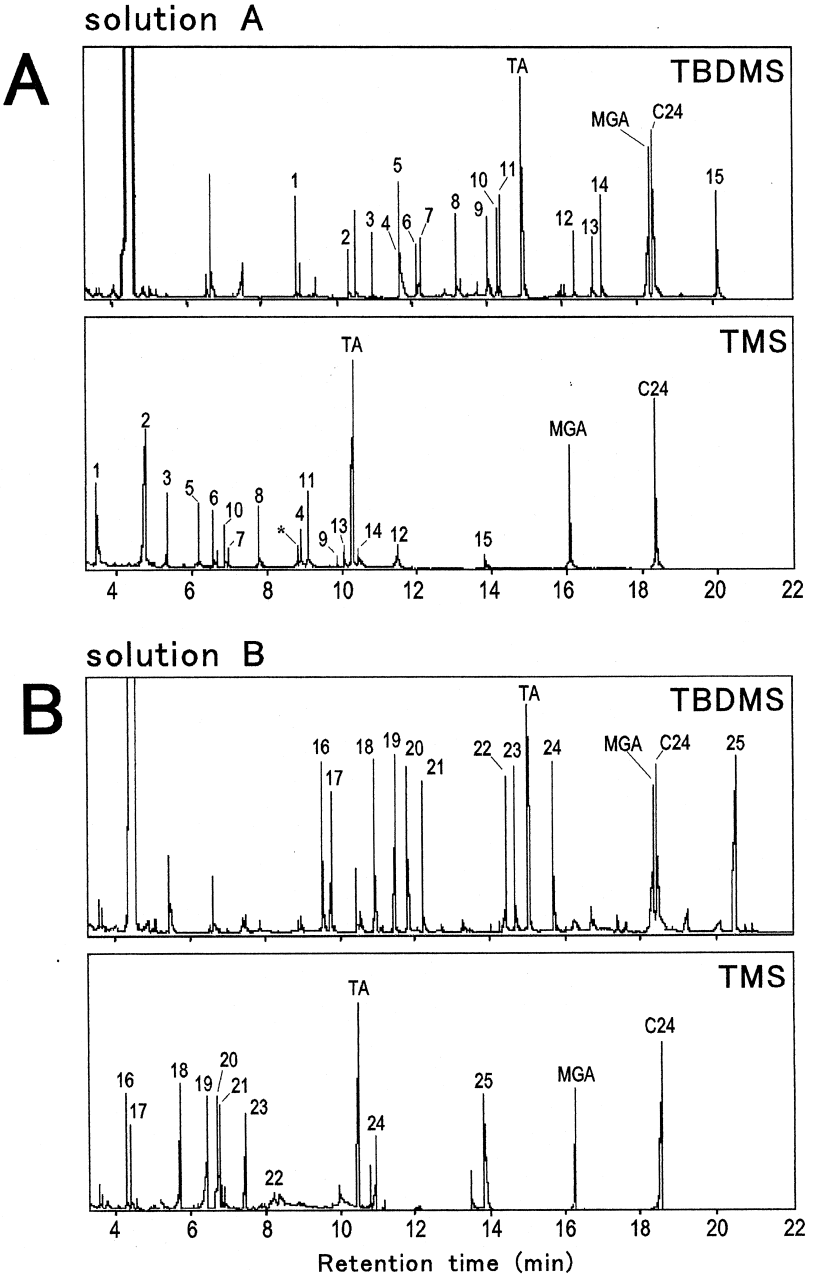

Fig. 2. TIC chromatograms of the standard solution. TIC chromatograms of TBDMS and TMS of solutions A and B are shown in (A) and (B), respectively. Peak identification: 1, lactate; 2, 2-hydroxyisovalerate; 3, methylmalonate; 4, isovalerylglycine; 5, ethylmalonate; 6, succinate; 7, uracil; 8, glutarate; 9, creatinine; 10, glycerate; 11, adipate; 12, suberate; 13, 2-hydroxyglutarate; 14, 3-hydroxy-3-methylglutarate;15, 4-hydroxyphenyllactate; 16, alanine; 17, glycine; 18, leucine; 19, isoleucine; 20, proline; 21, methionine; 22, serine; 23, serine; 24, phenylalanine; and 25, tyrosine. TA, MGA and C_{24} are internal standards, tropate, margarate and tetracosane, respectively.

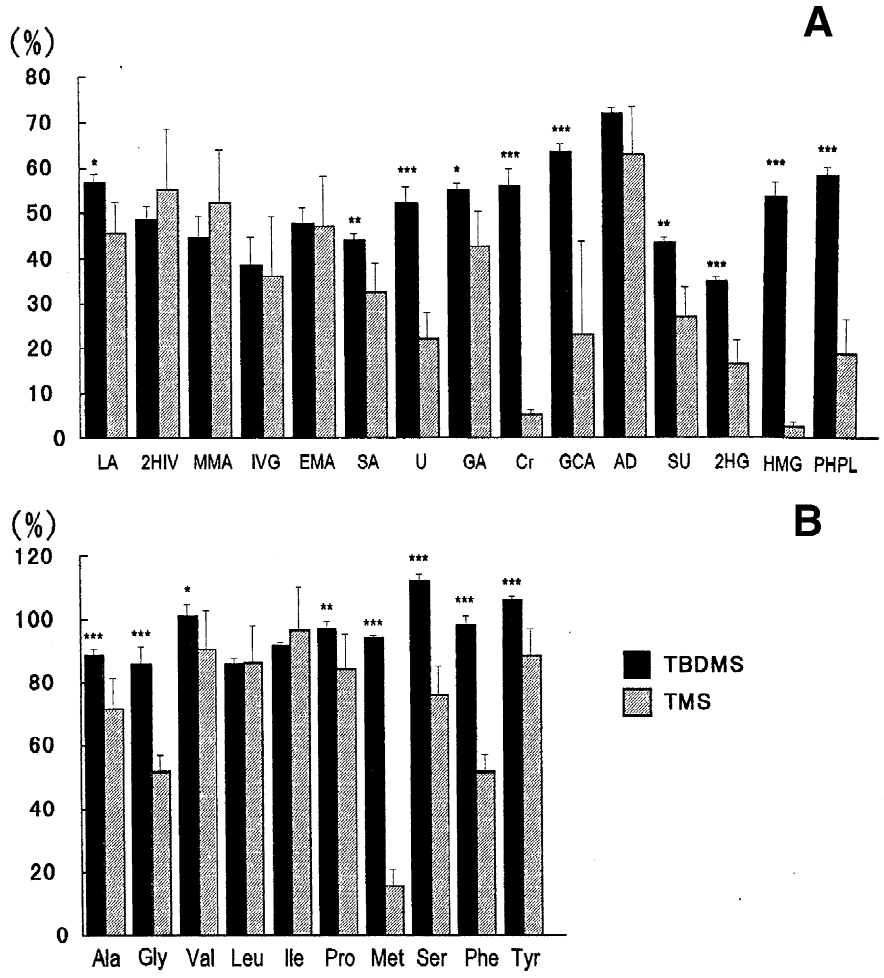

Fig. 3. Comparison of quantification between TBDMS and TMS derivatives. Close bars (■), TBDMS; stripe bars (▨), TMS. *$P<0.05$, **$P<0.01$, ***$P<0.001$. Abbreviations: LA, lactate; 2HIV, 2-hydroxyisovalerate; MMA, methylmalonate; IVG, isovalerylglycine; EMA, ethylmalonate; SA, succinate; U, uracil; GA, glutarate; Cr, creatinine; GCA, glycerate; AD, adipate; SU, suberate; 2HG, 2-hydroxyglutarate; HMG, 3-hydroxy-3-methylglutarate; PHPL, 4-hydroxyphenyllactate; Ala, alanine; Gly, glycine; Leu, leucine; Ile, isoleucine; Pro, proline; Met, methionine; Ser, serine; Phe, phenylalanine; Tyr, tyrosine.

minute amounts of diagnostic markers. As shown in Table 4, the correct diagnosis was indicated (S) in 52 of a total of 53 cases by TBDMS, and in 49 by TMS. Cases judged 'B' were seen in three cases in TMS. Cases judged 'M' accounted for in one case each in TBDMS and TMS, respectively. There is the possibility that the accurate diagnosis can be missed or is difficult in patients in whom the excretion of diagnostic metabolites is minute, in particular, in cases of samples whose the creatinine concentration is too low, or specimens were preserved under unstable conditions. In such case, detection with TBDMS derivatization may be more sensitive.

4. Discussion

Organic acidemia screening by tandem MS or GC–MS methods has been seriously done. Tandem MS requires only a simple preparation using blood filter papers, and is economical. It can detect fatty acid β-oxidation disorders where the diagnosis is

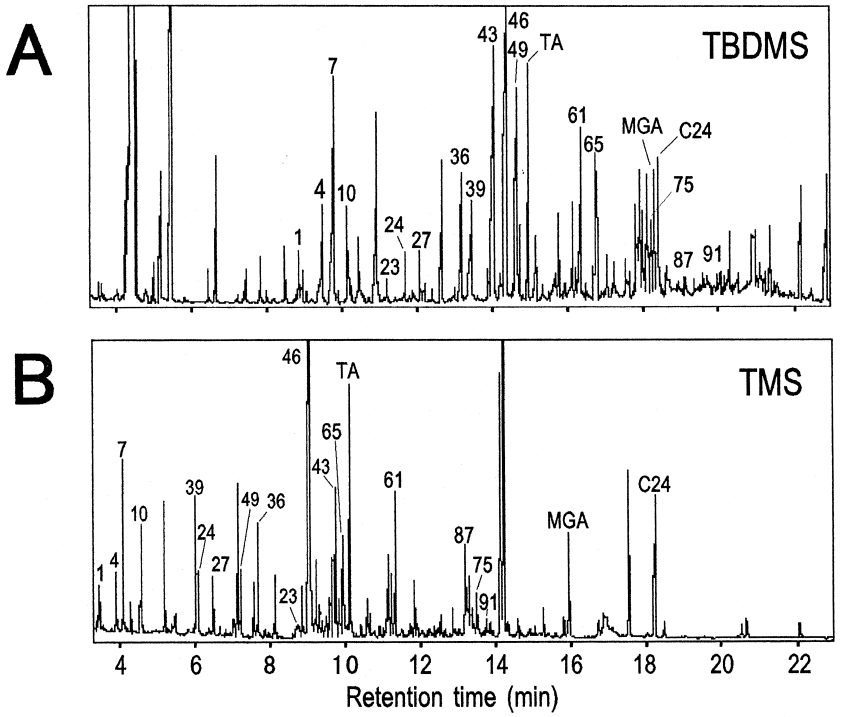

Fig. 4. TIC chromatograms of TBDMS and TMS derivatives of urine specimens from a glutaric aciduria type 2 patient. Peak identification: 1, lactate; 4, alanine; 7, glycine; 10, 3-hydroxylbutyrate; 23, isovalerylglycine; 24, ethylmalonate; 27, succinate; 36, glutarate; 39, glycerol; 43, creatinine; 46, adipate; 61, suberate; 65, 2-hydroxyglutarate; 75, sebacate; 87, glucose (1); 91, galactose (2); TA, MGA and C24 are the same as in Fig. 2.

difficult, using GC–MS alone. On the other hand, there are disorders, the diagnosis of which is possible using GC–MS but not by tandem MS, for example, benign methylmalonic acidemia, glycerolemia or uracil/orotic aciduria. Generally, GC–MS may provide more information on chemical configuration of compounds and on metabolic profiles of patients, compared with tandem MS analysis, although GC–MS requires a more complex sample preparation prior to GC–MS analysis, is a little more expensive and a longer time is needed for analysis.

Since the urease/direct method was developed recently [7], metabolic screening using GC–MS has been simplified and only a small amount of urine specimens is required. In this method, not only organic acids but also amino acids or sugars can be analyzed simultaneously, and more comprehensive information is acquired. TBDMS derivatives give in their mass spectra an intense fragment ion, $[M-57]^+$, specific for each molecule. We studied the practicability of TBDMS derivatization for organic acidemia screening, and we found the sensitivity and reliability in quantification by TBDMS derivatization to be better, compared with the TMS method. Hence, mass chromatographic determination by TBDMS should be more advantageous.

In this study, we compiled GC–MS data on TBDMS derivatives for use in the automated data system, and the usefulness in identifying metabolic disorders was apparent. Additionally, we compiled data on several 2-ketoacids with oximation and solvent extraction. MU values are common to the same column, irrespective of analytical conditions, GC–MS apparatus or institutes [12]. MU values are standardized information on retention times which enables identification of metabolites, and are applicable for methods such as solvent extraction. We preliminarily analyzed specimens from several patients with disorders that are complicated with an increased excretion of 2-ketoacids, such as maple

Table 3
Findings with the automated data system after GC–MS analysis with TBDMS derivatization in case of a glutaric aciduria type 2 patient[a]

ID Compound	Value	Normal	Range	Factor
(1) Lactic-2B	0.3134	(0.30	0.00–1.60)	1.04
(4) Alanine-2B	0.6406	(0.90	0.00–5.30)	0.71
(7) Glycine-2B	1.9178	(3.50	0.00–25.70)	0.55
(10) 3-Hydroxylbutyric-2B	0.5728*	(0.00	0.00–0.00)	?
(23) Isovalerylglycine-1B (1)	0.0185*	(0.00	0.00–0.00)	?
(24) Ethylmalonic-2B	0.3228*	(0.00	0.00–0.00)	?
(27) Succinic-2B	0.6047	(1.70	0.40–0.50)	0.40
(36) Glutaric-2B	1.6403*	(0.10	0.00–0.20)	16.40
(39) Glycerol-3B	0.1258*	(0.00	0.00–0.02)	?
(43) Creatinine-4B	6.1151	(2.80	0.00–24.60)	2.18
(46) Adipic-2B	9.7625*	(0.10	0.00–0.20)	97.62
(49) Serine-3B	0.7712	(1.10	0.00–5.60)	0.70
(61) Suberic-2B	2.3464*	(0.00	0.00–0.00)	?
(65) 2-Hydroxyglutaric-3B	0.8743*	(0.00	0.00–0.30)	?
(75) Sebacic-2B	0.7594*	(0.00	0.00–0.20)	?
(87) Glucose (1)	0.0001*	(0.00	0.00–0.00)	?
(91) Galactose (2)	0.0348*	(0.00	0.00–0.00)	?

No. disorders suspected:
(8) Glutaric aciduria type 2
(9) Galactocemia
(13) Glyceroluria
(25) Ketosis
(26) Dicarboxylic aciduria

[a] Upper, automated metabolic profiling. Bottom, automated interpretation by the automated data system. Abbreviations: Value, quantification, relative peak area (RPA, %) of Q-ions for each compound to that of the internal standard (margarate, *m*/*z* 327); Normal and Range, the mean values and ranges of RPA of the age-matched controls; Factor, number of times the normal mean value. Compounds judged as abnormal are indicated with an asterisk (*) next to Value. Automated interpretation, shown in the bottom, was performed based on the combination of abnormal compounds detected in the above profiling.

syrup urine disease, tyrosinemia type 1, or lactic and pyruvic aciduria, using solvent extraction with oximation, TBDMS derivatization and the automated data system. Consequently, the analysis, metabolic profiles and diagnosis of all cases tested went extremely well (data not shown). These data will aid in analysis, not only with the urease/direct method but also for solvent extraction with oximation of 2-ketoacids.

Use of the $[M\text{-}57]^+$ ion of TBDMS derivatives in a stable isotope dilution analysis may be feasible. Rinaldo et al. reported that acylglycine analysis by a stable isotope dilution analysis and chemical ionization (CI) was useful in detection of fatty acid β-oxidation disorders [13]. We also reported the usefulness of analysis of TBDMS derivatives of acylglycines by a stable isotope dilution analysis, without CI [14]. The high mass ion, $[M\text{-}57]^+$, in TBDMS derivatives can be used for quantification in place of the $[M]^+$ or $[M+17]^+$ in the CI mode. Hence, our system with TBDMS may be applicable for not only metabolic screening but also stable isotope dilution analysis.

Regardless of the method in mass screening programs for organic acidemias, tandem MS or GC–MS, to have a system of GC–MS analysis with TBDMS derivatization is important for more precise identification of disorders, or for detailed evaluations of patients mass screened for organic acidemias.

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Table 4
Analysis of samples from patients diagnosed previously[a]

Disorder	*n*	Age	Cr. (mg/dl)	TBDMS			TMS		
				S	B	M	S	B	M
Methylmalonic acidemia	13	2 days–13 years	10.1–81.4	13	0	0	13	0	0
Propionic acidemia	9	7 days–5 years	10.3–88.1	9	0	0	8	1	0
3-Ketothiolase deficiency	1	10 years	65.9	1	0	0	1	0	0
Isovaleric acidemia	3	11 days–8 years	25.4–27.8	3	0	0	3	0	0
3-Methylcrotonylglycemia	1	15 years	53.2	1	0	0	1	0	0
3-Hydroxy-3-methylglutaric acidemia	2	6, 8 months	19.2, 16.2	2	0	0	2	0	0
Multiple carboxylase deficiency	1	7 years	112.4	1	0	0	1	0	0
Glutaric aciduria type 1	5	1 days–1 year	11.4–146.7	5	0	0	5	0	0
Glutaric aciduria type 2	4	1–6 months	12.6–37.4	4	0	0	3	1	0
Ornithine transcarbamylase deficiency	3	11 months–2 years	8.3–31.0	2	0	1	2	0	1
Glyceroluria	5	5 days–4 years	5.1–29.0	5	0	0	5	0	0
Tyrosinemia type 1	2	4, 5 months	7.2, 9.2	2	0	0	1	1	0
Galactosemia	2	7 days, 10 months	2.5–12.4	2	0	0	2	0	0
Maple syrup urine disease	1	12 days	30.7	1	0	0	1	0	0
Phenylketonuria	1	1 month	20.6	1	0	0	1	0	0
Total number	53			52	0	1	49	3	1

[a] Abbreviations: Cr, creatinine; S, the correct diagnosis was made; B, borderline; M, missing.

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