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Configurational analysis of chiral acids as *O*-trifluoroacetylated (−)-menthyl esters by achiral dual-capillary column gas chromatography

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Abstract

The simultaneous enantiomeric separation of 30 racemic acids including 24 hydroxy acids in a single analysis is described for the determination of their absolute configurations. It involves the conversion of each enantiomer into diastereomeric *O*-trifluoroacetylated (−)-menthyl ester for the direct separation by gas chromatography on achiral dual-capillary columns of different polarities, with subsequent identification and chiral discrimination by retention index (*I*) library matching. Among the acids studied, the enantiomers of 27 acids were discriminatively resolved on both non-polar DB-5 and the intermediate-polar DB-17 columns with resolution factors in the range of 0.7–7.7 and separation factors in the range of 1.002–1.021. Enantiomers of 3-hydroxybutyric and α-methoxyphenylacetic acids were partially resolved on DB-5 (resolution factor of 0.9), but not resolved on DB-17, while the baseline resolution for 3-hydroxydecanoic acid and the minimal separation on the peak top (resolution factor less than 0.7) for 2-hydroxyglutaric acid were achieved on DB-17 but not on DB-5. The temperature-programmed *I* values measured on both columns were characteristic of each enantiomer and thus simple *I* matching with the reference values was useful in cross-checking for their chemical identification and the chiral discrimination as well. When applied to a clinical urine sample, the present method allowed positive identification of endogenous (*S*)-lactic acid and (*S*)-2-hydroxybutyric acid along with (*R*)-3-hydroxybutyric acid.

Keywords: Enantiomer separation; *O*-Trifluoroacetylated (−)-menthyl esters; Chiral acids; Hydroxy acids

1. Introduction

The absolute configurational analysis of chiral acids [1–5] especially when they are biochemical indicators of inborn errors of metabolism such as D-glyceric aciduria [6], lactic acidemia [7], D-2-hydroxyglutaric aciduria [8], L-2-hydroxyglutaric aciduria [9–11], D-2-hydroxyglutaric acidemia [12], glutaric aciduria type II [13], and respiratory chain disorder [14] has become an important task. It is also crucial to determine absolute configurations of 2- and 3-hydroxy fatty acids composing microbial lipids for the endotoxin detection [15–17]. With the current interest in the chiral recognition of optically active acidic drugs in living systems, the chiral measurement became essential for their optimal therapeutic use [18–21]. Therefore, in recent years, the simultaneous separation of various chiral acids and accurate determination of their absolute configurations in a

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single analysis are increasingly encountered in biomedical and environmental studies [1–5,14–21].

Among the various techniques developed for chiral separations, both direct and indirect methods employing high-resolution gas chromatography (GC) combined with mass spectrometry (MS) require chiral acids to be preconverted into their corresponding volatile derivatives. When considering this prerequisite, indirect enantiomeric separation of the racemic acids as volatile diastereomeric derivatives using conventional achiral stationary phases with high thermal stability and long-term durability appears to be more preferred over the direct approach.

In the literature, indirect separation is widely used because of the simplicity and versatility of the applications [1,2,6,7,13–16,18]. Furthermore, it offers a quicker enantioseparation for the analytes possessing easily derivatizable hydroxyl and carboxyl functional groups. The most widely utilized method of diastereomeric reactions is the esterification of carboxyl groups with chiral alcohol such as (−)-menthol, (*S*)-(+)-3-methyl-2-butanol and (*S*)-(+)-2-butanol [1,2,6,7,14], with subsequent acylation of the remaining hydroxyl or phenolic hydroxyl groups. Another approach is the amidation of carboxyl groups with chiral amines after either ethoxymation [18] or etherification of hydroxyl groups [16].

In our previous work on the enantiomeric separation of 2-hydroxy acids as their *O*-trifluoroacetylated (*S*)-(+)-3-methyl-2-butyl esters [14], the achiral dual-capillary columns of different polarities were found to be useful in achieving a complete resolution of 18 enantiomeric pairs in one analytical run. Moreover, the retention index (*I*) matching with the reference values allowed accurate identification and chiral discrimination of each enantiomer, thus solving the tedious problem of conventional co-chromatographic procedure [1,2,6,7]. Therefore, the overall analysis time was considerably shortened. However, when applied to a clinical urine sample, the chirality of 3-hydroxybutyric acid excreted in large concentrations could not be determined due to the fact that resolution of 3-hydroxy acid enantiomers is not achieved as their (*S*)-(+)-3-methyl-2-butyl esters [1,3].

In continuation of the method development for the enantiomeric discrimination of structurally diverse chiral acids in a single run, the present study was undertaken to combine the simple *I* matching method with the chiral separation of 31 racemic acids including 3-hydroxy acids as their *O*-trifluoroacetylated (−)-menthyl esters. This method was intended to complement our previous method [14] as the confirmation tool for wider range of applications in the configurational analysis.

2. Experimental

2.1. Materials

The following 33 racemic acid standards and 16 enantiomerically pure acid standards were obtained from various vendors such as Sigma–Aldrich (Milwaukee, WI, USA): lactic, 2-hydroxybutyric, 2-hydroxy-2-methylbutyric, 2-hydroxyisovaleric, glyceric, 2-hydroxyvaleric, 3-hydroxybutyric, 2-hydroxyisocaproic, 2-hydroxy-3-methylvaleric, 2-hydroxycaproic, 2-hydroxyoctanoic, mandelic, *m*-hydroxymandelic, *p*-hydroxymandelic, 2-phenylbutyric, 3-phenyllactic, 3-hydroxy-2-phenylpropionic, α-methoxyphenylacetic, 2-hydroxydecanoic, 3-phenylbutyric, *p*-hydroxyphenyllactic, 4-hydroxy-3-methoxymandelic, 3-hydroxydecanoic, 3-hydroxy-4-methoxymandelic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, 2-hydroxytetradecanoic, malic, 2-methylsuccinic, 2-hydroxyglutaric, 3,4-dihydroxymandelic, citramalic, 3-methyladipic, and (*S*)-lactic, (*S*)-2-hydroxyisovaleric, (*S*)-glyceric, (*S*)-3-hydroxybutyric, (*S*)-2-hydroxyisocaproic, (*S*)-2-hydroxy-3-methylvaleric, (*S*)-mandelic, (*S*)-2-phenylbutyric, (*S*)-3-phenyllactic, (*S*)-α-methoxyphenylacetic, (*S*)-3-hydroxydecanoic, (*S*)-3-hydroxydodecanoic, (*S*)-malic, (*S*)-2-methylsuccinic, (*S*)-2-hydroxyglutaric acids, (*S*)-citramalic, and (*S*)-3-methyladipic acids. (−)-Menthol, (*S*)-(+)-3-methyl-2-butanol, acetyl chloride and trifluoroacetic anhydride (TFAA) were purchased from Sigma–Aldrich and *n*-hydrocarbon standards (C_{10}–C_{22}, even numbers only) from Polyscience (Niles, IL, USA). Acetonitrile, toluene, isooctane and ethyl acetate of spectroanalyzed grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade and used as received.

2.2. Acid and hydrocarbon standard solutions

Each standard solution of chiral acids was made up at 10 μg/μl in acetonitrile as their free acid forms. (−)-Menthol solution was prepared at 200 μg/μl in ethyl acetate. Hydrocarbon solution containing *n*-hydrocarbons (C_{10}–C_{22}, even numbers only), each at 1.0 μg/μl in isooctane, was used as the external standard solution for the *I* measurement.

2.3. Derivatization reactions

A mixed solution of acids containing 10 μg of each acid and 100 μl of (−)-menthol solution was evaporated to dryness under a gentle stream of nitrogen at 40°C for the menthylation. To the residue were added toluene (20 μl) and acetyl chloride (1 μl). The mixture was then heated at 100°C for 1 h to form diastereomeric esters. After the removal of excess reagents (under nitrogen stream), the concentrate was reacted with TFAA (20 μl) in the presence of acetonitrile (10 μl) at 60°C for 20 min. The reaction mixture was evaporated to remove excess of reagents and dissolved in toluene (30 μl) for the direct analysis by GC and GC–MS. For the 3-hydroxy acids and non-hydroxy acids, (*S*)-(+)-3-methyl-2-butylation was performed prior to *O*-trifluoroacetylation according to the procedure of the previous work [14].

2.4. Sample preparation

Aliquots (corresponding to 0.25 mg of creatinine) of urine samples without spiking or after spiking with racemic acids (20 μg each) were adjusted to pH 1–2 with conc. sulfuric acid, saturated with sodium chloride and then extracted with diethyl ether (2 ml×3). The ethereal extract was dried over magnesium sulfate and evaporated to dryness, and then subjected to (−)-menthylation with subsequent *O*-trifluoroacetylation as described above.

2.5. Gas chromatography and gas chromatography–mass spectrometry

GC analyses were performed with a Hewlett-Packard HP Model 5890A gas chromatograph series II, equipped with a split–splitless capillary inlet system and two flame ionization detection (FID) systems and interfaced to an HP 3365A GC Chemstation (Hewlett-Packard, Avondale, PA, USA). The injector and detector temperatures were 260 and 280°C, respectively. Samples (ca. 0.5 μl) were injected in the splitless mode with a purge delay time of 0.7 min. The retention index (*I*) measurements were carried out using a dual-capillary column system made of DB-5 (SE-54 bonded phase) and DB-17 (OV-17 bonded phase) fused-silica capillary columns (J&W Scientific, Rancho Corodova, CA, USA; dimensions 30 m×0.25 mm I.D., 0.25 μm film thickness). The two columns were connected to a deactivated fused-silica tubing (1 m×0.25 mm I.D.) as retention gap via a Y-splitter. The inlet pressure of helium as the carrier gas was set to 137.5 kPa. The oven temperature was held at 60°C for 2 min, then programmed at a rate of 30°C/min to 100°C (hold for 2 min), and subsequently programmed to 280°C at a rate of 3°C/min. The GC Chemstation processed the two FID signals simultaneously in dual-channel mode. A standard solution of *n*-hydrocarbons (C_{10}–C_{22}, even numbers only) in isooctane was injected as the external reference and temperature-programmed *I* values were computed via built-in retention index program by linear interpolation between the retention times of adjacent hydrocarbon standards. For the peak identification and chirality determination by computer *I* matching, a database of reference *I* library using *I* sets of 31 chiral acids measured on the dual-columns was built into the GC computer system.

GC analyses for optimization of the diastereomeric reaction conditions were performed by a Younglin M600D Model gas chromatograph (Young Lin Instrument, Kyunggido, South Korea) equipped with a split–splitless capillary inlet system and a FID system interfaced to a Younglin Autochro-WIN data acquisition system. The injector and detector temperatures were 260 and 280°C, respectively. Samples (ca. 1.0 μl) were injected in the splitless mode (purge delay time of 0.7 min) and analyzed on an Ultra-2 (SE-54 bonded phase) capillary column (25 m×0.20 mm I.D., 0.33 μm film thickness; Hewlett-Packard) under the identical oven temperature conditions as above. The inlet pressure of nitrogen used as the carrier gas was set to 28 kPa. All the GC analyses were performed in triplicate.

To obtain mass spectra, an HP 5890A series II gas chromatograph, interfaced to an HP 5970B mass spectrometer (70 eV, electron impact mode), which was on-line to an HP 59940A MS Chemstation was used. Samples were injected into an Ultra-2 (SE-54 bonded phase) capillary column (25 m×0.20 mm I.D., 0.11 μm film thickness) in the split injection mode (10:1) at 260°C, and the oven temperature was initially 120°C for 2 min and then raised to 280°C at 4°C/min. The interface and ion source temperatures were 280 and ca. 250°C, respectively. The mass range scanned was 50–650 u at a rate of 0.99 scan/s.

3. Results and discussion

For the extension of the chiral discrimination method based on simple *I* matching [14] to a wider range of applications, this study was undertaken to resolve enantiomeric pairs of 33 different racemic acids simultaneously in a single run as their *O*-trifluoroacetylated (*O*-TFA) (−)-menthyl esters. In the literature, (−)-menthylation has been used less commonly and attempts were rarely made to apply this method to multi-component analysis [6,7].

Upon (−)-menthylation with subsequent *O*-trifluoroacetylation, all chiral acids studied except for glyceric acid were converted to their corresponding *O*-TFA (−)-menthyl esters. Their structures were confirmed by GC–MS. No serious racemization was observed with authentic enantiopure acid standards of established absolute configuration during the reactions. Unlike (*S*)-3-methyl-2-butylation [14], losses of short-chain hydroxy fatty acids with 3–7 carbons such as lactic acid were minimal during the sample work-up procedure, since *O*-TFA (−)-menthyl esters are much less volatile. (−)-Menthylation was thus more suitable for the short-chain hydroxy fatty acids than those late eluting acids. Moreover, the use of (−)-menthol as chiral reagent is less costly.

Under the present achiral GC conditions, baseline or partial enantiomeric resolution of all the racemic acids studied except for *p*-hydroxyphenyllactic acids, were achieved in one analytical run within 65 min as seen in the dual total chromatograms of 31 racemic acids (Fig. 1). No resolution between 2-hydroxyvaleric and 3-hydroxybutyric acids (peaks 5 and 6), between *p*-hydroxymandelic and 2-phenylbutyric acids (peaks 13 and 14), between 3-phenyllactic, 3-hydroxy-2-phenylpropionic, and α-methoxyphenylacetic acids (peaks 15, 16 and 17), and between 4-hydroxy-3-methoxymandelic and 3-hydroxydecanoic acids (peaks 20 and 21) was achieved on the DB-5 column of low polarity. On the DB-17 column of intermediate polarity, five sets (between peaks 3 and 4, between peaks 7 and 6, between peaks 15 and 21, between peaks 16 and 20,and between peaks 19 and 17) were not resolved. When 31 racemic acids were divided into two groups (groups 1 and 2), 4-hydroxy-3-methoxymandelic and 3-hydroxydecanoic acids (peaks 20 and 21) in group 1 were baseline resolved on DB-17, while 3-hydroxybutyric and 2-hydroxycaproic acids (peaks 6 and 7) in group 1 were resolved only on DB-5.

When acids were individually analyzed, each enantiomeric pair of 27 acids was discriminatively resolved on both DB-5 and DB-17 columns with resolution factors in the range of 0.7–7.7 and separation factors in the range of 1.002–1.021 as listed in Table 1. Enantiomers of 3-hydroxybutyric acid (peak 6) and α-methoxyphenylacetic acid (peak 17) were partially resolved on DB-5 with a resolution factor of 0.9, but not resolved on DB-17, while the baseline resolution for 3-hydroxydecanoic acid (peak 21) and the minimal separation on the peak top (resolution factor less than 0.7) for 2-hydroxyglutaric acid (peak 28) were achieved on DB-17 but not on DB-5. Enantiomeric resolution of *p*-hydroxyphenyllactic acid was not feasible, indicating the need to employ a more polar column such as the SP-1000 as reported for glyceric acid elsewhere [6].

As with the previous 3-methyl-2-butylation [14], the temperature-programmed *I* values measured on both columns were characteristic of each resolved enantiomer as *O*-TFA (−)-menthyl esters (Table 1). And thus simple *I* matching with the reference values was useful in cross-checking for their chemical identification and the chiral discrimination as well.

The elution order of enantiomers as (−)-menthyl esters of *O*-TFA acids turned out to be opposite to those of corresponding (*S*)-(+)-3-methyl-2-butyl esters [14] as shown in Table 2. In cases of menthyl esters of all 2-hydroxy acids and α-methoxyphenyl-

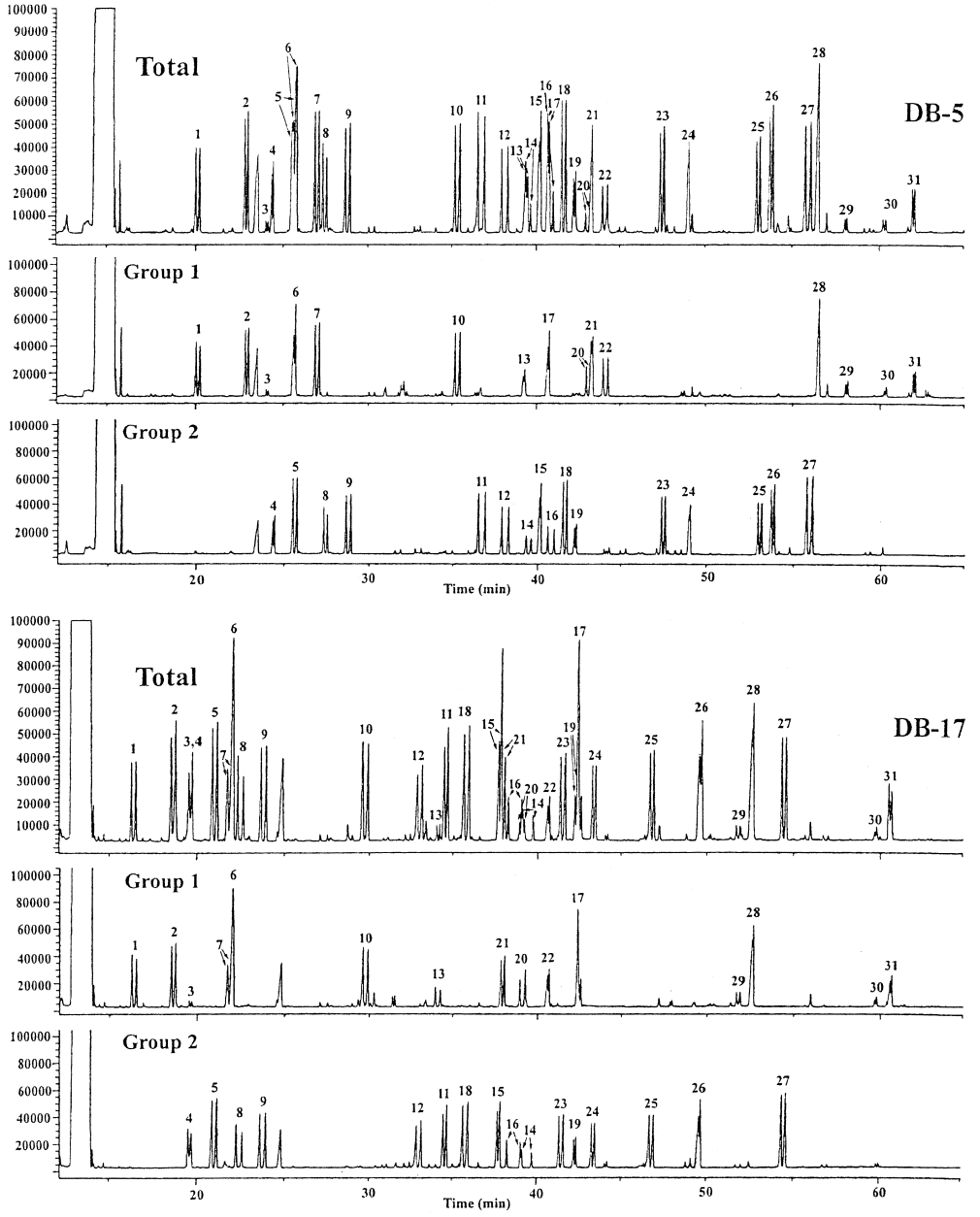

Fig. 1. Dual chromatograms of enantiomers of 31 acids (total), 15 acids (group 1) and 16 acids (group 2) as their diastereomeric *O*-trifluoroacetylated (−)-menthyl esters separated on the DB-5 and DB-17 (both 30 m×0.25 mm I.D., 0.25 μm film thickness) dual-capillary column system. The oven temperature was held at 60°C for 2 min and programmed to 100°C at a rate of 30°C/min, then to 280°C at a rate of 3°C/min. Samples (ca. 0.5 μl) were injected in the splitless mode with a purge delay time of 0.7 min. Peaks: 1=lactic acid; 2=2-hydroxybutyric acid; 3=2-hydroxy-2-methylbutyric acid; 4=2-hydroxyisovaleric acid, 5=2-hydroxyvaleric acid; 6=3-hydroxybutyric acid; 7=2-hydroxyisocaproic acid; 8=2-hydroxy-3-methylvaleric acid; 9=2-hydroxycaproic acid; 10=2-hydroxyoctanoic acid; 11=mandelic acid; 12=*m*-hydroxymandelic acid; 13=*p*-hydroxymandelic acid; 14=2-phenylbutyric acid; 15=3-phenyllactic acid; 16=3-hydroxy-2-phenylpropionic acid; 17=α-methoxyphenylacetic acid; 18=2-hydroxydecanoic acid; 19=3-phenylbutyric acid; 20=4-hydroxy-3-methoxymandelic acid; 21=3-hydroxydecanoic acid; 22=3-hydroxy-4-methoxymandelic acid; 23=2-hydroxydodecanoic acid; 24=3-hydroxydodecanoic acid; 25=2-hydroxytetradecanoic acid; 26=malic acid; 27=2-methylsuccinic acid; 28=2-hydroxyglutaric acid; 29=3,4-dihydroxymandelic acid; 30=citramalic acid; 31=3-methyladipic acid.

Table 1
Gas chromatographic data of 31 acids as diastereomeric *O*-trifluoroacetylated (−)-menthyl esters

No.	Chiral acid	Separation factor (α)[a]		Resolution factor (R)[b]		GC *I* data set[c]	
		DB-5	DB-17	DB-5	DB-17	DB-5	DB-17
1	(*S*)-Lactic	1.010	1.016	2.0	2.5	1251.2	1162.4
	(*R*)-Lactic					1256.3	1166.1
2	2-Hydroxybutyric	1.008	1.013	1.6	1.9	1322.6	1192.3
	2-Hydroxybutyric					1327.1	1198.7
3	2-Hydroxy-2-methylbutyric	1.005	1.007	1.3	1.4	1353.5	1218.9
	2-Hydroxy-2-methylbutyric					1356.5	1223.0
4	(*S*)-2-Hydroxyisovaleric	1.013	1.008	1.1	1.5	1361.9	1218.2
	(*R*)-2-Hydroxyisovaleric					1364.7	1223.4
5	2-Hydroxyvaleric	1.009	1.013	2.2	2.2	1392.2	1253.7
	2-Hydroxyvaleric					1398.4	1261.1
6	(*R*)-3-Hydroxybutyric	1.005	–	0.9	–	1393.9	1286.1
	(*S*)-3-Hydroxybutyric					1397.0	1286.1
7	(*S*)-2-Hydroxyisocaproic	1.009	1.014	2.1	1.6	1413.8	1277.8
	(*R*)-2-Hydroxyisocaproic					1416.9	1286.1
8	(*S*)-2-Hydroxy-3-methylvaleric	1.008	1.015	2.2	3.0	1420.0	1292.9
	(*R*)-2-Hydroxy-3-methylvaleric					1423.1	1302.3
9	2-Hydroxycaproic	1.009	1.013	2.6	2.6	1438.1	1331.2
	2-Hydroxycaproic					1441.8	1340.0
10	2-Hydroxyoctanoic	1.007	1.010	2.4	2.5	1528.0	1452.0
	2-Hydroxyoctanoic					1531.8	1456.5
11	(*S*)-Mandelic	1.011	1.006	3.4	1.7	1546.9	1525.8
	(*R*)-Mandelic					1552.7	1529.1
12	*m*-Hydroxymandelic	1.011	1.009	3.5	2.4	1566.6	1499.8
	m-Hydroxymandelic					1572.6	1504.3
13	*p*-Hydroxymandelic	1.004	1.011	1.0	2.7	1587.2	1517.3
	p-Hydroxymandelic					1590.6	1522.0
14	(*R*)-2-Phenylbutyric	1.007	1.016	2.4	5.5	1588.9	1599.2
	(*S*)-2-Phenylbutyric					1593.1	1609.7
15	(*S*)-3-Phenyllactic	1.002	1.004	0.8	1.3	1600.0	1575.7
	(*R*)-3-Phenyllactic					1601.6	1578.1
16	3-Hydroxy-2-phenylpropionic	1.009	1.021	3.6	7.7	1608.1	1584.5
	3-Hydroxy-2-phenylpropionic					1614.3	1597.7
17	(*S*)-α-Methoxyphenylacetic	1.003	–	0.9	–	1608.4	1655.6
	(*R*)-α-Methoxyphenylacetic					1610.2	1655.6
18	2-Hydroxydecanoic	1.006	1.008	2.2	2.3	1622.5	1543.8
	2-Hydroxydecanoic					1626.4	1548.3
19	3-Phenylbutyric	1.003	1.003	1.1	1.2	1633.4	1652.3
	3-Phenylbutyric					1635.3	1654.7
20	4-Hydroxy-3-methoxymandelic	1.009	1.008	2.6	2.8	1645.1	1595.8
	4-Hydroxy-3-methoxymandelic					1650.6	1600.7

Table 1. Continued

No.	Chiral acid	Separation factor (α)[a]		Resolution factor (R)[b]		GC I data set[c]	
		DB-5	DB-17	DB-5	DB-17	DB-5	DB-17
21	(*R*)-3-Hydroxydecanoic	–	1.005	–	1.7	1651.3	1578.0
	(*S*)-3-Hydroxydecanoic					1651.3	1581.1
22	3-Hydroxy-4-methoxymandelic	1.006	1.003	2.4	1.0	1661.1	1623.1
	3-Hydroxy-4-methoxymandelic					1665.5	1625.0
23	2-Hydroxydodecanoic	1.005	1.006	2.0	2.3	1717.7	1636.9
	2-Hydroxydodecanoic					1721.6	1641.4
24	(*R*)-3-Hydroxydodecanoic	1.002	1.004	0.7	1.6	1744.8	1670.1
	(*S*)-3-Hydroxydodecanoic					1746.0	1673.4
25	2-Hydroxytetradecanoic	1.004	1.005	1.7	1.7	1828.8	1731.8
	2-Hydroxytetradecanoic					1836.7	1736.2
26	(*S*)-Malic	1.003	1.002	1.4	0.8	1857.0	1784.3
	(*R*)-Malic					1863.8	1786.6
27	(*R*)-2-Methylsuccinic	1.005	1.005	2.3	2.0	1933.2	1957.5
	(*S*)-2-Methylsuccinic					1944.5	1967.6
28	(*S*)-2-Hydroxyglutaric	–	1.001	–	<0.7	1973.0	1888.7
	(*R*)-2-Hydroxyglutaric					1973.0	1891.2
29	3,4-Dihydroxymandelic	1.002	1.004	1.2	1.6	2031.3	1850.0
	3,4-Dihydroxymandelic					2041.4	1858.0
30	(*R*)-Citramalic	1.002	1.002	1.1	0.9	2211.7	2360.4
	(*S*)-Citramalic					2221.1	2369.2
31	(*R*)-3-Methyladipic	1.002	1.001	1.2	0.9	2347.4	2440.0
	(*S*)-3-Methyladipic					2358.3	2449.4

[a] Separation factor (α) was the ratio of retention times of two peaks.

[b] Resolution factor (R) was the ratio of separation between two peaks to average width of the two peaks.

[c] Retention index (I) measured on DB-5 and DB-17 (30 m×0.25 mm I.D., 0.25 μm film thickness) dual-capillary columns programmed from 60°C (2 min) to 100°C (2 min) at a rate of 30°C/min, then to 280°C at a rate of 3°C/min.

acetic acid, (*S*)-enantiomers were eluted ahead of (*R*)-enantiomers on both columns, while (*R*)-enantiomers of 3-hydroxy acids and non-hydroxy acids were eluted ahead of (*S*)-enantiomers. These results were in good agreement with the previous findings for lactic, glyceric, 2-hydroxybutyric and 3-hydroxybutyric acids [6,7]. The elution order of 2-hydroxybutyric acid enantiomers was determined by cross-checking our results with the results reported in the literature [7].

As manifested by the resolution factors in Table 2, DB-5 and DB-17 columns together provided enantiomeric resolutions of 30 acids as menthyl esters, while complete separation of 23 enantiomeric pairs as their 3-methyl-2-butyl esters was achieved on both columns with higher resolution factors ($R \geq 1.3$). Compared to separation as 3-methyl-2-butyl esters, seven more chiral acids including 3-hydroxy acids were enantiomerically resolved as a bulkier menthyl group was introduced into the carboxyl function. Therefore, both of the diastereomeric esterification methods appear to be complements each of the other as the confirmation tools in the accurate chiral determination for the 31 chiral acids. However, one of the disadvantages of the methods lies in that the esterification requires 1 h, hence the overall sample work-up takes much longer than other approach such as amidation of carboxyl groups with chiral amines after ethoxymation [18].

As demonstrated in the typical dual chromatographic profiles of spiked normal urine in comparison with urine blank (Fig. 2), the present dual-capillary column method permitted chiral discrimination of 22 spiked racemic acids. The resolution factor

Table 2
Comparison of the two derivatives for the enantioseparation of *O*-trifluoroacetylated chiral acids

Chiral acid	(−)-Menthyl ester			(+)-3-Methyl-2-butyl ester		
	Elution order[a]	Resolution factor		Elution order[a]	Resolution factor	
		DB-5	DB-17		DB-5	DB-17
Lactic	*S*<*R*	2.0	2.5	*R*<*S*	4.4	3.6
2-Hydroxybutyric[b]	*S*<*R*	1.6	1.9	*R*<*S*	3.8	2.7
2-Hydroxy-2-methylbutyric	–	1.3	1.4	–	–	–
2-Hydroxyisovaleric	*S*<*R*	1.1	1.5	*R*<*S*	3.1	1.9
Glyceric	–	–	–	*R*<*S*	1.5	1.8
2-Hydroxyvaleric	–	2.2	2.2	–	4.0	3.4
3-Hydroxybutyric	*R*<*S*	0.9	–	–	–	–
2-Hydroxyisocaproic	*S*<*R*	2.1	1.6	*R*<*S*	4.0	3.4
2-Hydroxy-3-methylvaleric	*S*<*R*	2.2	3.0	*R*<*S*	3.6	3.3
2-Hydroxycaproic	–	2.6	2.6	–	3.7	3.6
2-Hydroxyoctanoic	–	2.4	2.5	–	3.1	3.4
Mandelic	*S*<*R*	3.4	1.7	*R*<*S*	2.9	3.1
m-Hydroxymandelic	–	3.5	2.4	–	2.3	2.7
p-Hydroxymandelic	–	1.0	2.7	–	–	–
2-Phenylbutyric	*R*<*S*	2.4	5.5	*S*<*R*	1.7	1.9
3-Phenyllactic	*S*<*R*	0.8	1.3	*R*<*S*	2.4	2.9
3-Hydroxy-2-phenylpropionic	–	3.6	7.7	–	1.5	2.3
α-Methoxyphenylacetic	*S*<*R*	0.9	–	*R*<*S*	1.3	1.9
2-Hydroxydecanoic	–	2.2	2.3	–	2.7	3.0
3-Phenylbutyric	–	1.1	1.2	–	1.5	1.7
p-Hydroxyphenyllactic	–	–	–	–	2.0	2.9
4-Hydroxy-3-methoxymandelic	–	2.6	2.8	–	–	–
3-Hydroxydecanoic	*R*<*S*	–	1.7	–	–	–
3-Hydroxy-4-methoxymandelic	–	2.4	1.0	–	–	–
2-Hydroxydodecanoic	–	2.0	2.3	–	2.5	3.1
3-Hydroxydodecanoic	*R*<*S*	0.7	1.6	–	–	–
2-Hydroxytetradecanoic	–	1.7	1.7	–	2.1	2.6
Malic	*S*<*R*	1.4	0.8	*R*<*S*	1.4	2.3
2-Methylsuccinic	*R*<*S*	2.3	2.0	*S*<*R*	6.2	4.1
2-Hydroxyglutaric	*S*<*R*	–	0.4	*R*<*S*	2.2	2.0
3,4-Dihydroxymandelic	–	1.2	1.6	–	–	–
Citramalic	*R*<*S*	1.1	0.9	–	–	–
3-Methyladipic	*R*<*S*	1.2	0.9	–	–	–

[a] Elution order was determined by comparing the retention indices of optically pure (*R*)- and (*S*)-enantiomers.
[b] Elution order was determined by cross-checking our results with the results reported in the literature [7].

of 0.9 for the enantiomers of 3-hydroxybutyric acid (peak 6) on DB-5 was found to be sufficient for their discrimination. Compared to the 3-methyl-2-butylation, menthylation suppresses the interference by other endogenous metabolites, yielding relatively clean background in the chromatograms. Intensities of malic acid enantiomers were low, probably due to the incomplete extraction recovery from the urine matrix. When applied to clinical urine specimen from a patient with respiratory chain disorder (RCD), (*S*)-lactic acid was positively detected in large concentrations along with (*S*)-2-hydroxybutyric acid and (*R*)-3-hydroxybutyric acid (Fig. 3). The *R* configuration of 3-hydroxybutyric acid was further validated by the conventional co-injection of racemic acid standard with the sample. In our previous work [14], the chirality of 3-hydroxybutyric acid excreted in large concentrations in urine of a different RCD patient could not be determined due to the fact that no resolution of 3-hydroxy acid enantiomers as their 3-methyl-2-butyl esters is achieved. Presently, our *I* library contains 30 organic acid standards as *O*-TFA

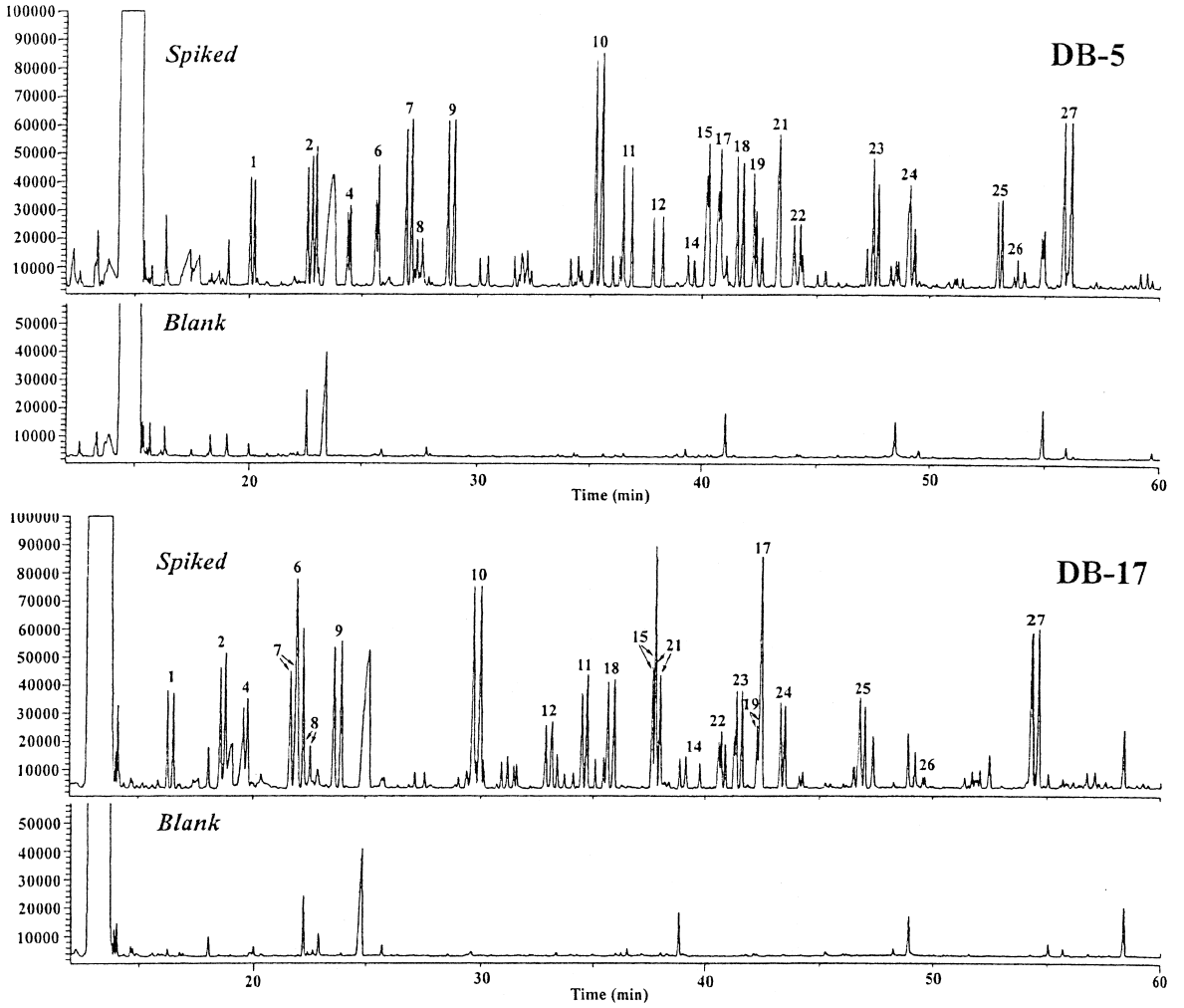

Fig. 2. Dual chromatographic profiles of normal urine specimen without spiking (blank) and after spiking (spiked) with chiral acids separated on the DB-5 and DB-17 (both 30 m×0.25 mm I.D., 0.25 μm film thickness) dual-capillary column system. GC conditions and peak numbers are the same as those in the Fig. 1.

(−)-menthyl esters, which will continue to be expanded to include other chiral acids for a wider range of applications.

4. Conclusions

The present enantiomeric separation on achiral dual-capillary columns of different polarities combined with *I* matching was useful for the chiral discrimination of 30 different chiral acid standards as their *O*-TFA (−)-menthyl esters. When applied to normal urine samples, chirality of each spiked acid enantiomer was accurately determined. From the urine sample of a patient with respiratory chain disorder, endogenous (*S*)-lactic acid and (*S*)-2-hydroxybutyric acid were positively identified along with (*R*)-3-hydroxybutyric acid. An extension of the present chiral discrimination method to more rapid diastereomeric derivatization procedures is under way to be used as the complementary confirmation tool in the configurational analysis.

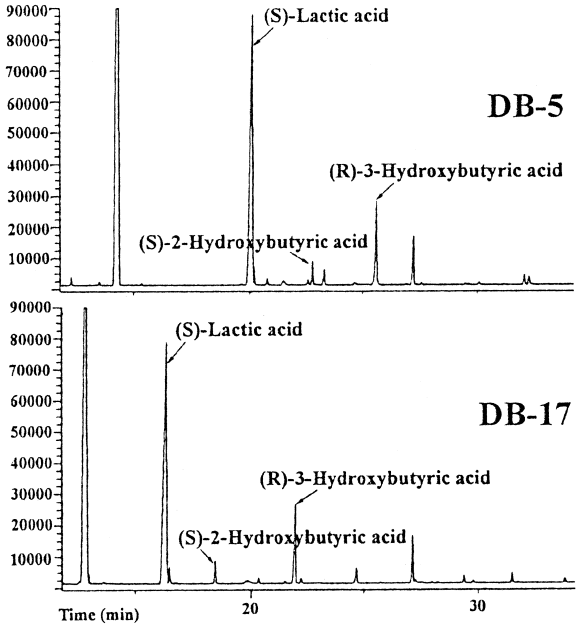

Fig. 3. Dual chromatographic profiles of clinical urine specimen from a patient with respiratory chain disorder separated on the DB-5 and DB-17 (both 30 m×0.25 mm I.D., 0.25 μm film thickness) dual-capillary column system. GC conditions as described in Fig. 1.

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References

[1] W.A. Koenig, I. Benecke, J. Chromatogr. 195 (1980) 292.
[2] J.P. Kamerling, M. Duran, G.J. Gerwig, D. Ketting, L. Bruinvis, J.F.G. Vliegenthart, S.K. Wadman, J. Chromatogr. 222 (1981) 276.
[3] J.P. Kamerling, J.F.G. Vliegenthart, in: Glass Capillary Chromatography in Clinical Medicine and Pharmacology – Determination of the Absolute Configuration of Chiral Hydroxy Acids by GLC, Marcel Dekker, Inc., 1985, p. 371.
[4] A. Kaunzinger, A. Rechner, T. Beck, A. Mosandl, A.C. Sewell, H. Boehles, Enantiomer 1 (1996) 177.
[5] M. Heil, F. Podebrad, T. Beck, A. Mosandl, A.C. Sewell, H. Boehles, J. Chromatogr. B 714 (1998) 119.
[6] J.P. Kamerling, G.J. Gerwig, J.F.G. Vliengenthart, M. Duran, D. Ketting, S.K. Wadman, J. Chromatogr. 143 (1977) 117.
[7] J.P. Kamerling, G.J. Gerwig, M. Duran, D. Ketting, S.K. Wadman, Clin. Chim. Acta 88 (1978) 183.
[8] W.L. Nyhan, G.D. Shelton, C. Jakobs, B. Holmes, C. Bowe, C.J.R. Curry, C. Vance, M. Duran, L. Sweetman, J. Child Neurol. 10 (1995) 137.
[9] G.F. Hoffmann, C. Jakobs, B. Holmes, L. Mitchell, G. Becker, H.P. Hartung, M.L. Nyhan, J. Inherit. Metab. Dis. 18 (1995) 189.
[10] E. Chen, W.L. Nyhan, C. Jakobs, C.M. Greco, A.J. Balkovich, V.A. Cox, S. Packman, J. Inherit. Metab. Dis. 19 (1996) 335.
[11] Y. Geerts, W.O. Renier, J. Bakkeren, J. Dejong, J. Neurol. Sci. 143 (1996) 166.
[12] R.J.A. Wanders, P. Mooger, J. Inherit. Metab. Dis. 18 (1995) 194.
[13] H. Watanabe, S. Yamaguchi, K. Saiki, N. Shimizu, T. Fukao, N. Kondo, T. Orii, Clin. Chim. Acta 238 (1995) 115.
[14] K.-R. Kim, J. Lee, D. Ha, J. Jeon, H.-G. Park, J.H. Kim, J. Chromatogr. A 874 (2000) 91.
[15] H.-W. Wollenweber, E.T. Rietschel, J. Microbiol. Methods 11 (1990) 195.
[16] W. Gradowska, L. Larsson, J. Microbiol. Methods 20 (1994) 55.
[17] Y. Nakagawa, K. Kishida, Y. Kodani, T. Matsuyama, Microbiol. Immunol. 41 (1997) 27.
[18] A. Carlson, O. Gyllenhaal, J. Chromatogr. 508 (1990) 333.
[19] N.R. Srinivas, W.C. Shyu, R.H. Barbhaiya, Biomed. Chromatogr. 9 (1995) 1.
[20] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 18 (1997) 1013.
[21] T. Santa, J. Luo, C.-K. Lim, K. Imai, Biomed. Chromatogr. 12 (1998) 73.