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SIMULTANEOUS GAS CHROMATOGRAPHY OF VOLATILE AND NON-VOLATILE CARBOXYLIC ACIDS AS *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

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SUMMARY

Solid phase extraction with subsequent ion pair formation and silylation was investigated for the simultaneous trace enrichment of volatile and non-volatile carboxylic acids from complex aqueous samples. The solid phase extraction of acids was performed using Chromosorb P as the solid sorbent. The ether eluate was treated with triethylamine. The resulting triethylammonium salts of the acids were converted to stable *tert.*-butyldimethylsilyl (TBDMS) derivatives, which were analyzed by gas chromatography (GC) and GC–mass spectrometry. The characteristic M – 57 ions in the mass spectra of TBDMS derivatives enabled rapid identification of acids. The application of the method to the organic acid profiling of urine and saliva samples is demonstrated.

INTRODUCTION

The organic acids, a group of the most widely occurring compounds in biological samples, are important indicators of a wide variety of biological, physiological and fermentation processes[1–6]. The organic acid fraction of complex biological samples contains a wide range of structural types of acids, including short-chain volatile fatty acids, long-chain non-volatile fatty acids, mono-, and polycarboxylic acids with hydroxy or keto functional groups of both aliphatic and aromatic character. A number of gas chromatographic (GC) methods have been developed to separate one or several classes of these acids. Simultaneous analysis of short-chain volatile fatty acids and various non-volatile carboxylic acids is a commonly encountered problem in the organic acid profiling studies.

Prior to analysis, the organic acids are isolated from complex sample matrices,

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mainly by either solvent extraction[2] or anion-exchange method[6]. In modern analysis, conventional liquid–liquid partitioning extraction is being replaced by liquid–solid adsorption, *i.e.* solid phase extraction (SPE), where compounds of interest are enriched on suitable sorbents, followed by elution with organic solvents. The advantages of SPE methods are well established. Non-polar graphitized carbon black[1], C_{18} bonded silica and XAD-4[4] have been used to enrich organic acids. The anion-exchange approach, being more laborious than solvent extraction procedures, has been reported to have two major drawbacks: the inorganic acid interference and the loss of the more volatile acids[3]. Previously, we reported an efficient two-step SPE method with Chromosorb P as the solid sorbent and diethyl ether as the eluent for enrichment of volatile fatty acids (C_2–C_5) from fermentation media and body fluids[4].

Generally, the carboxyl groups of organic acids are converted to either the alkyl esters (methyl or butyl) or trimethylsilyl esters prior to GC analysis[2–5]. Recently, separation of Krebs cycle and related acids as *tert.*-butyldimethylsilyl (TBDMS) esters and ethers was reported. TBDMS ether derivatives of hydroxyl groups have been widely used, principally because of their high hydrolytic stability and superior GC and mass spectrometric (MS) properties[6], while TBDMS esterification of carboxyl groups has received little attention.

In a previous report[7], we described the GC analysis of volatile fatty acids (C_1–C_7) as their TBDMS esters: the acids were converted to non-volatile triethylammonium (TEA) salts to minimize the loss due to the evaporation during the sample work-up, followed by reaction of the salts with N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to afford TBDMS esters.

The present work was undertaken to examine the SPE method[4,8] and the direct TBDMS derivatization of TEA salts[7] for the simultaneous determination of various volatile and non-volatile carboxylic acids in aqueous samples. The authenticity of the TBDMS derivatives was verified by GC–MS.

EXPERIMENTAL

Materials

Twenty-two saturated fatty acids (C_1–C_{20}) were obtained from Analabs (New Haven, CT, U.S.A.) and Alltech (Deerfield, IL, U.S.A.). The following thirteen aliphatic and sixteen aromatic multifunctional acids were obtained from various commercial vendors such as Sigma (St. Louis, MO, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.): lactic, glycolic, oxalic, malonic, succinic, methylsuccinic, fumaric, adipic, 3-methyladipic, malic, suberic, tartaric, citric, benzoic, phenylacetic, *trans*-cinnamic, 5-phenylvaleric, *p*-aminobenzoic, mandelic, phenyllactic, hippuric, γ-hydroxybenzoic, vanillic, syringic, α-resorcylic, *p*-hydroxymandelic, γ-resorcylic, homogentisic, and protocatechuic acids. TEA was purchased from Aldrich, diethyl ether from Tedia (Fairfield, OH, U.S.A.), MTBSTFA from Pierce (Rockford, IL, U.S.A.), dichloromethane and isooctane from Burdick & Jackson (Muskegon, MI, U.S.A.), methanol from Shinyo (Kyoto, Japan), sodium chloride and sodium bicarbonate from Ishizu (Osaka, Japan), sulfuric acid (98.08%) from Junsei (Tokyo, Japan), and *n*-hydrocarbon standards (C_8–C_{28}) from Polyscience (Niles, IL, U.S.A.). All solvents and reagents were of analytical grade and were used as received, except TEA, which was distilled over potassium hydroxide. Chromosorb P (acid-washed, 80–100 mesh) was

obtained from Supelco (Bellefonte, PA, U.S.A.). A U-shaped glass column (6 mm I.D.) was packed with Chromosorb P (2.3 g), washed successively with methanol, dichloromethane, and diethyl ether, and activated at 200°C overnight prior to being used as a solid phase extraction column.

Acid solutions

Four acid stock solutions and one internal standard solution were prepared for this study as follows: volatile fatty acid mixture (C_1–C_7, 10 μg each per μl in diethyl ether), fatty acid mixture (C_8–C_{20} 10 μg each per μl in dichloromethane), aliphatic multifunctional carboxylic acid mixture (thirteen acids, 50 μg each per μl in methanol), aromatic carboxylic acid (fifteen acids, 50 μg each per μl in methanol) and *trans*-cinnamic acid solution as an internal standard solution (100 μg per μl in methanol).

tert.-Butyldimethylsilylation

Appropriate amounts of carboxylic acid mixtures were diluted to 1.5 ml with diethyl ether, containing 100 μg of *trans*-cinnamic acid as an internal standard. The ether solution was then mixed with 20 μl of triethylamine in a Reacti Vial (Pierce). The ether was reduced to *ca.* 50 μl under a gentle stream of dry nitrogen at room temperature. To the vial were added 20 μl of MTBSTFA and 60 μl of isooctane, and the vial was tightly closed with a PTFE-lined screw cap. The mixture was subjected to GC analysis either directly, or after it was heated at 60°C for 30 min or up to 8 h.

To test the effect of TEA treatment on the recoveries of volatile fatty acids (C_1–C_7), untreated samples were prepared in the same manner except for the addition of TEA.

The samples for calibration were prepared by allowing increasing amounts of acids containing 100 μg of the internal standard to react directly with TEA (20 μl), and MTBSTFA (20 μl) in the presence of isooctane (60 μl) in reacti vials.

Aliquots of the reaction mixtures were examined directly by GC–MS.

Solid phase extraction

To 1 ml of aqueous samples or biological samples 100 μg/ml of the internal standard was added and the solution was made basic by saturating with solid sodium bicarbonate. After extraction with diethyl ether, the ether phase was discarded and the aqueous phase was subjected to SPE, as described in ref. 4. Briefly, following the acidification with concentrated sulfuric acid (0.1 ml) and saturation with sodium chloride (400 mg), the aqueous phase was loaded onto a Chromosorb P column under nitrogen pressure. The packing was wetted up to 80% while the remaining 20% was dry. The organic acids were then eluted from the Chromosorb P with diethyl ether, and the first 1.5 ml of ether eluate was collected in a Reacti Vial containing 20 μl TEA, followed by the evaporation and derivatization, as described above.

Gas chromatography

GC analyses were conducted with a Shimadzu GC-9A gas chromatograph, equipped with a flame ionization detector and interfaced with a Shimadzu C-R2AX data processor, which provided peak area and retention time date (Shimadzu, Kyoto, Japan). A DB-1 (J&W Scientific, Rancho Cordova, CA, U.S.A.) fused-silica capillary

column (30 m × 0.32 mm I.D., 0.25 μm particle size) was used for this study. Nitrogen at a flow-rate of 0.9 ml/min was used as the carrier gas, and 0.8-μl aliquots of samples were injected with a split ratio of 15:1. After an initial hold time of 2 min at 60°C, the oven temperature was programmed to 280°C at a rate of 4°C/min. The injector and detector temperatures were maintained at 300°C. A standard solution of *n*-hydrocarbons (C_8–C_{28}) in isooctane was simultaneously injected with the samples to allow conversion of retention times to retention indices. Samples were analyzed in triplicate.

Gas chromatography–mass spectrometry

A Hewlett Packard (Avondale, PA, U.S.A.) HP 5890 A gas chromatograph, interfaced to an HP 5970 MSD 70-eV electron-impact mode, which was on-line to an HP 35741 Chemstation data system, was used with an HP-1 cross-linked capillary column (16 m × 0.20 mm I.D., 0.33 μm particle size) to obtain mass spectra. Samples were introduced in the split-injection mode (10:1) at 270°C, and the oven temperature was initially 100°C for 2 min, then programmed to 300°C at a rate of 15°C/min. The interface and ion source temperatures were 300°C and 200°C, respectively. The scan-rate of the mass scanning from 50 to 600 a.m.u. was 0.78 scan per s.

RESULTS AND DISCUSSION

In a previous report[8], we established that TEA salts of volatile fatty acids can be converted to their TBDMS esters directly after reaction with MTBSTFA in isooctane. An excess of TEA (20 μl) was used, and the removal of diethyl ether under a stream of nitrogen was stopped when TEA started to evaporate, in order to obtain high yields of TEA salts. The effect of TEA treatment on the recoveries of volatile fatty acids, diluted in ether solution, was evaluated. As shown in Table I, the TEA treatment minimizes the loss of acids and improves the precision of the overall derivatization procedure.

TABLE I

EFFECT OF TRIETHYLAMINE TREATMENT ON THE RECOVERIES OF VOLATILE FATTY ACIDS

A 1.5-ml volume of diethyl ether, containing 70 μg each of C_1 to C_7 volatile fatty acids and 100 μg of *tert.*-cinnamic acid, was subjected to silylation as described in the text (n = 3). C.V. = Coefficient of variation.

Acid	*With TEA treatment*		*Without TEA treatment*	
	Recovery (%)	*C.V. (%)*	*Recovery (%)*	*C.V. (%)*
Formic	91.6	0.1	Trace	–
Acetic	99.8	0.2	Trace	–
Propionic	97.2	<0.1	Trace	–
Isobutyric	100.0	<0.1	2.8	34.5
Butyric	100.0	<0.1	5.6	25.0
Isovaleric	100.0	0.3	8.4	20.7
Valeric	100.0	0.9	20.4	4.9
Caproic	100.0	1.2	44.5	6.4
Enanthic	100.0	0.2	56.0	3.6

The TBDMS esterification of non-sterically hindered carboxyl groups was complete when their TEA salts were mixed with MTBSTFA at room temperature. Even the highly hindered hydroxyl and carboxyl groups of tartaric acid and γ-resorcylic acid as their TEA salts were quantitatively silylated by heating at 60°C for 4 h and 8 h, respectively. However, with *p*-aminobenzoic acid and hippuric acid, N-silylation did not take place, even with excess MTBSTFA for extended periods.

The derivative yields of the carboxylic acids studied were quantitative. The calibration curves were linear, with correlation coefficients (r^2) varying from 0.9944 to 0.9999, over the range 10–80 μg for the fatty acids, and 50–400 μg for the aliphatic and aromatic multifunctional carboxylic acids under the present condition. The quantitative yields of TBDMS derivatives are exemplified by the calibration plots of formic, capric, tridecanoic, arachidic, oxalic, lactic, malic, tartaric, citric, benzoic, *p*-aminobenzoic, phenylacetic, mandelic, and phenyllactic acids in Figs. 1–3.

With the present derivatization procedure, a single chromatographic peak with almost no tailing is obtained for each of the carboxylic acids studied, as illustrated in Fig. 4. Volatile formic, acetic, and propionic acids are well separated from the solvent and reagent peaks. Mandelic acid is eluted with lauric acid, 3-methyladipic acid with tridecanoic acid, and α-resorcylic acid with margaric acid. For their complete separation a more polar phase appears to be needed.

TBDMS derivatives of the carboxylic acids investigated were found to be stable for at least six months when stored at 4°C. The present direct silylation of the TEA salts with MTBSTFA has the following advantages: (1) TEA salts are non-volatile, and this minimizes the loss of volatile fatty acids during the sample work-up; (2) both TEA and TEA salts are soluble in the mixture of MTBSTFA and isooctane; (3) the acids are easily released from TEA salts and silylated quantitatively during the reaction; (4)

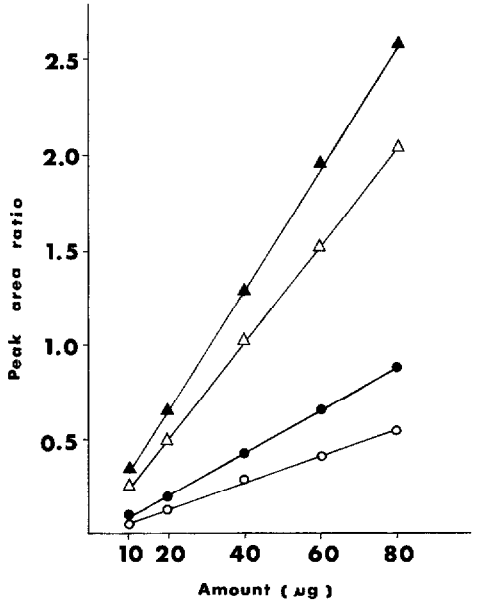

Fig. 1. Calibration curves for formic (@), capric (⅛), tridecanoic (△) and arachidic (⅓) acids.

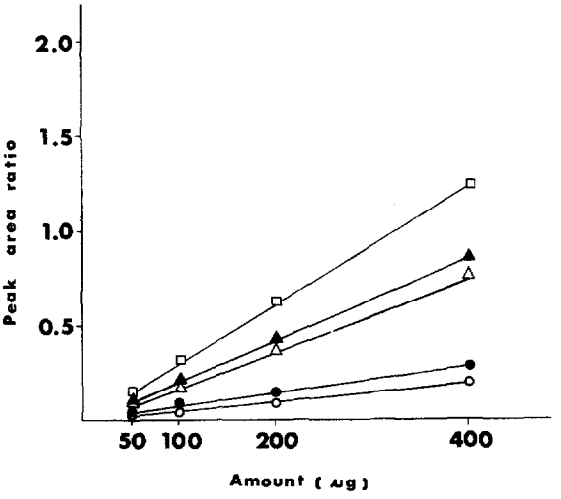

Fig. 2. Calibration curves for oxalic (@), lactic (⅛), malic (△), tartaric (⅓) and citric (□) acids.

aprotic TEA does not undergo silylation; (5) without further processing, the reaction mixtures can be examined directly by GC and GC–MS; (6) TEA is eluted with isooctane, and thus does not interfere with the analysis of volatile acids; (7) hydroxyl and carboxyl functions are quantitatively converted to TBDMS ethers and esters; (8)

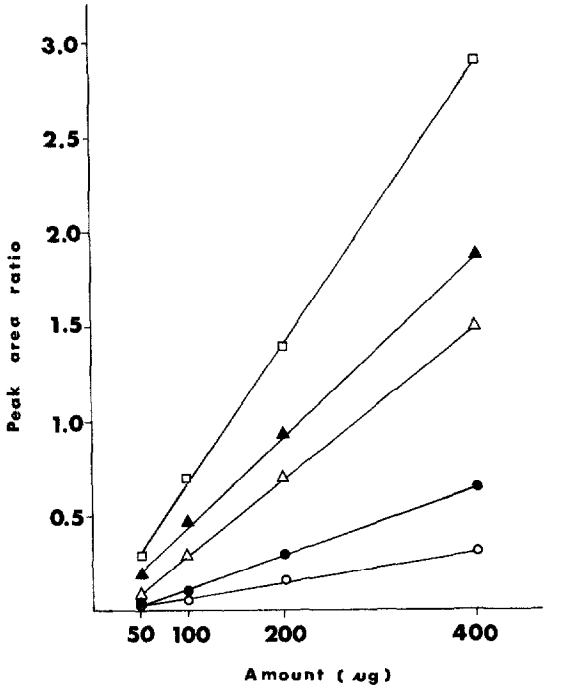

Fig. 3. Calibration curves for benzoic (@), *p*-aminobenzoic (⅛), phenylacetic (△), mandelic (⅓) and phenyllactic (□) acids.

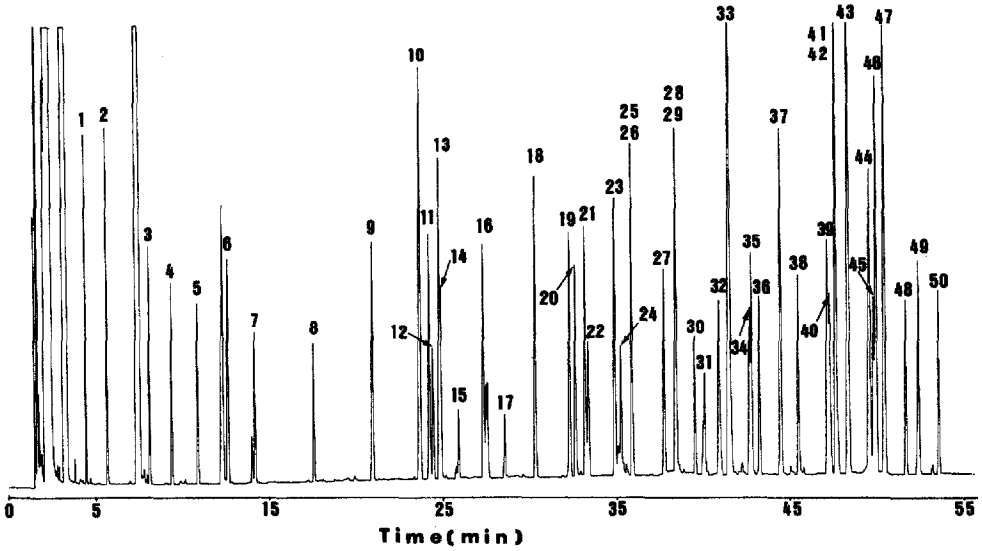

Fig. 4. Chromatogram of a mixture of carboxylic acids as the *tert.*-butyldimethylsilyl derivatives. GC conditions: DB-1 fused-silica capillary column (30 m × 0.32 mm I.D., 0.25 μm), initially at 60°C for 2 min, then programmed to 280°C at 4°C/min; 0.8 μl sample, injected with split ratio of 15:1; both injector and detector temperatures at 300°C; nitrogen as the carrier gas at 0.9 ml/min. Peaks: 1 = formic, 2 = acetic, 3 = propionic, 4 = isobutyric, 5 = butyric, 6 = isovaleric, 7 = valeric, 8 = caproic, 9 = enanthic, 10 = benzoic, 11 = caprylic, 12 = lactic, 13 = phenylacetic, 14 = glycolic, 15 = oxalic, 16 = pelargonic, 17 = malonic, 18 = capric, 19 = succinic, 20 = methylsuccinic, 21 = undecanoic, 22 = fumaric, 23 = 5-phenylvaleric, 24 = *p*-aminobenzoic, 25 = lauric, 26 = mandelic, 27 = adipic, 28 = 3-methyladipic, 29 = tridecanoic, 30 = phenyllactic, 31 = hippuric, 32 = myristic, 33 = *p*-hydroxybenzoic, 34 = malic, 35 = suberic, 36 = pentadecanoic, 37 = vanillic, 38 = palmitic, 39 = syringic, 40 = tartaric, 41 = margaric, 42 = α-resorcylic, 43 = *p*-hydroxymandelic, 44 = γ-resorcylic, 45 = stearic, 46 = homogentisic, 47 = protocatechuic, 48 = nonadecanoic, 49 = citric, 50 = arachidic acid.

amino and amide functions are not capable of being silylated under the present reaction condition.

All the TBDMS derivatives were subjected to GC–MS analysis. The retention index and electron-impact MS data are summarized in Table II. As is characteristic of TBDMS derivatives, the molecular ions and M − 15 ions are absent or of low intensity, but the M − 57 ions, due to the loss of the *tert.*-butyl group function from the molecular ions are very intense, thus enabling rapid identification of acids.

For most of the monocarboxylic acids, M − 57 ions constitute the base peaks, and the ion of high intensity at $m/z = 75$ corresponds to $HOSi(CH_3)_2$, as seen in Fig. 5. The mass spectra of the TBDMS derivatives of lactic, malic, citric, fumaric, and succinic acids do not agree well with those recorded at an electron energy of 22.5 eV. As in the spectra of trimethylsilyl derivatives, the ion at $m/z = 73$, corresponding to $Si(CH_3)_3$, is the base peak for the bisTBDMS derivatives of acids with vicinal hydroxyl groups, two neighboring hydroxyl and carboxyl groups, or dicarboxylic acids, as shown in Fig. 6. Other prominent ions at $m/z = 115$, 157, and 189 correspond to $Si(CH_3)_2C(CH_3)_3$, $(CH_3)_3SiOSi(CH_3)_2$, and $(CH_3)_3C(CH_3)_2$ $SiOSi(CH_3)_2$, respectively.

TABLE II

GAS CHROMATIGRAPHIC AND MASS SPECTRAL DATA OF TBDMS DERIVATIVES OF CARBOXYLIC ACIDS

Retention index (RI) values on a DB-1 capillary column (30 m × 0.32 mm I.D., 0.25-μm) at 60°C (2 min) to 280°C at 4°C/min, and the relative abundance of ions are in parentheses.

Acid	*RI*	*Mol.wt.*	$(M\text{-}57)^+$	*Major ions*				
Formic	858	160	103(100)	75(56)	73(7)	59(5)	57(4)	61(4)
Acetic	916	174	117(91)	75(100)	73(5)	59(3)	57(3)	60(3)
Propionic	990	188	131(80)	75(100)	73(12)	57(8)	59(4)	58(3)
Isobutyric	1029	202	145(82)	75(100)	73(20)	115(7)	59(4)	57(4)
Butyric	1074	202	145(73)	75(100)	73(13)	115(4)	59(4)	57(3)
Isovaleric	1126	216	159(73)	75(100)	73(15)	57(6)	115(4)	59(4)
Valeric	1174	216	159(72)	75(100)	73(13)	57(6)	115(4)	59(4)
Caproic	1283	230	173(83)	75(100)	73(17)	131(13)	115(5)	59(5)
Enanthic	1378	244	187(76)	75(100)	73(17)	131(14)	129(5)	117(5)
Caprylic	1465	258	201(100)	75(94)	131(15)	129(10)	117(7)	57(7)
Pelargonic	1574	272	215(97)	75(100)	73(22)	131(16)	129(16)	117(8)
Capric	1674	286	229(100)	75(73)	73(19)	131(14)	129(14)	55(7)
Undecanoic	1773	300	243(100)	75(78)	73(17)	129(16)	131(12)	55(7)
Lauric	1874	314	257(100)	75(59)	73(15)	129(14)	131(10)	117(8)
Tridecanoic	1973	328	271(100)	75(59)	73(14)	129(14)	131(8)	55(6)
Myristic	2075	342	285(100)	75(45)	129(13)	73(12)	131(8)	55(6)
Pentadecanoic	2174	356	299(100)	75(45)	129(14)	73(13)	117(9)	131(7)
Palmitic	2275	370	313(100)	75(38)	129(13)	73(10)	117(8)	131(6)
Margaric	2373	384	327(100)	75(43)	129(15)	73(13)	117(8)	131(6)
Stearic	2476	398	341(100)	75(28)	129(10)	73(8)	117(6)	131(4)
Nonadecanoic	2576	412	355(100)	75(36)	129(12)	73(12)	117(8)	131(5)
Arachidic	2677	426	369(100)	75(19)	129(7)	73(7)	117(6)	131(3)
Lactic	1483	318	261(24)	73(100)	147(86)	189(25)	133(21)	75(20)
Glycolic	1499	304	247(24)	73(100)	147(51)	189(32)	75(13)	133(12)
Oxalic	1535	318	261(13)	73(100)	147(32)	75(10)	59(9)	133(6)
Malonic	1604	332	275(30)	73(100)	147(27)	75(23)	189(13)	133(12)
Succinic	1743	346	289(51)	73(100)	147(48)	75(36)	133(9)	116(9)
Methylsuccinic	1755	360	303(58)	73(100)	147(60)	75(36)	59(9)	123(8)
Fumaric	1779	344	287(100)	73(48)	75(21)	57(11)	133(8)	84(7)
Adipic	1948	374	317(71)	73(100)	75(79)	111(35)	141(18)	55(15)
3-Methyladipic	1973	388	331(68)	73(100)	75(80)	125(36)	155(17)	69(13)
Malic	2151	476	419(22)	73(100)	147(23)	287(20)	115(15)	75(13)
Suberic	2157	402	345(95)	73(100)	75(89)	129(19)	169(14)	55(13)
Tartaric	2355	606	549(27)	73(100)	147(19)	115(9)	75(7)	417(7)
Citric	2617	648	591(25)	73(100)	459(35)	147(19)	431(14)	357(11)
Benzoic	1460	236	179(100)	105(58)	77(46)	135(30)	51(8)	75(5)
Phenylacetic	1494	250	193(91)	75(100)	91(22)	73(22)	65(9)	137(8)
trans-Cinnamic	1752	262	205(100)	131(49)	103(42)	75(39)	161(32)	77(28)
5-Phenylvaleric	1836	292	235(97)	75(100)	91(29)	73(19)	131(16)	117(15)
p-Aminobenzoic	1850	251	194(90)	120(100)	150(62)	65(30)	92(29)	75(16)
Mandelic	1875	380	323(26)	73(100)	147(39)	221(32)	295(32)	75(14)
Phenyllactic	2016	394	337(36)	73(100)	147(88)	309(27)	75(22)	133(16)
Hippuric	2035	293	236(53)	105(100)	77(31)	73(21)	75(20)	192(10)
p-Hydroxybenzoic	2097	366	309(100)	73(45)	265(33)	235(19)	57(11)	135(8)
Vanillic	2228	396	339(100)	267(42)	73(24)	126(15)	193(12)	295(12)
Syringic	2354	426	369(100)	297(40)	73(20)	149(13)	141(13)	223(9)
α-Resorcylic	2371	496	439(100)	73(70)	267(10)	309(9)	57(9)	147(5)
p-Hydroxymandelic	2400	510	453(29)	73(100)	351(92)	425(45)	147(22)	75(11)
γ-Resorcylic	2467	496	439(100)	73(40)	57(8)	170(5)	397(5)	147(4)
Homogentisic	2486	510	453(43)	73(100)	75(16)	147(14)	327(13)	57(8)
Protocatechuic	2511	496	439(100)	73(61)	193(12)	395(7)	223(5)	267(5)

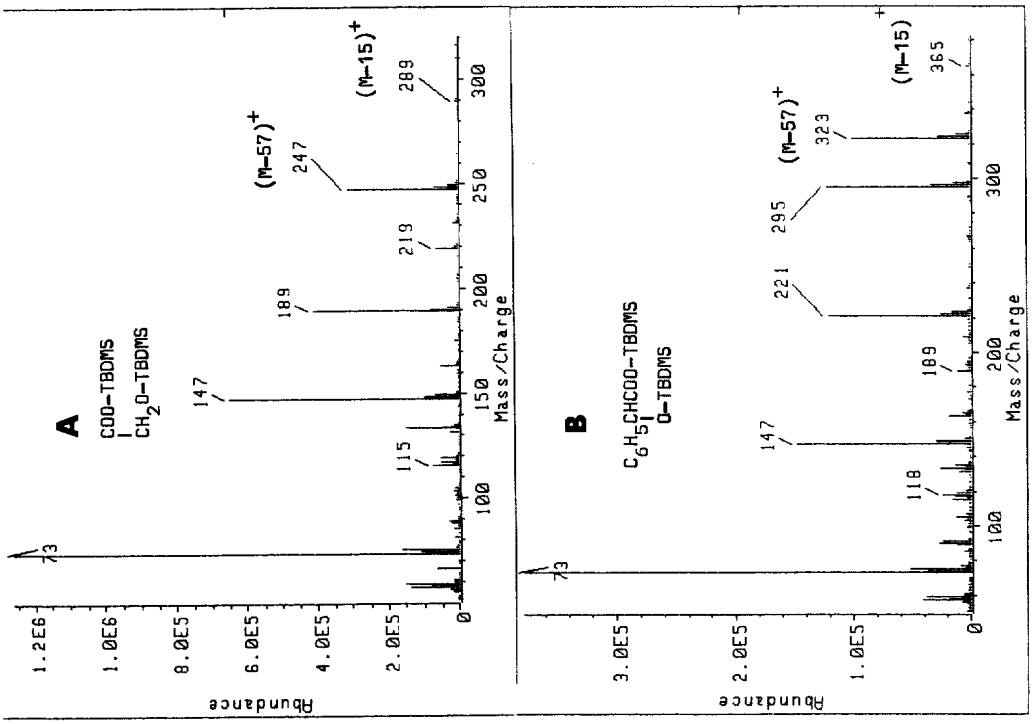

Fig. 5. Electron-impact mass spectra of TBDMS derivatives of formic acid (A) and arachidic acid (B).

Fig. 6. Electron-impact mass spectra of TBDMS derivatives of glycolic acid (A) and mandelic acid (B).

TABLE III

OVERALL EFFICIENCY OF THE COMBINED SOLID PHASE EXTRACTION AND DIRECT SILYLATION OF THE TRIETHYLAMMONIUM SALTS OF FATTY ACIDS

Fatty acid mixture (40 μg each) was added to 1 ml of water, containing 100 μg of *trans*-cinnamic acid and subjected to solid phase extraction and silylation, as described in the text ($n = 3$).

Acid	*Recovery (%)*	*C.V. (%)*	*Acid*	*Recovery (%)*	*C.V. (%)*
Formic	30.8	0.3	Capric	98.0	0.3
Acetic	92.5	1.0	Lauric	96.4	0.7
Propionic	90.1	<0.1	Tridecanoic	98.7	0.1
Isobutyric	88.6	<0.1	Myristic	99.6	0.2
Butyric	93.6	<0.1	Pentadecanoic	98.1	0.2
Isovaleric	92.8	0.6	Palmitic	100.0	0.4
Valeric	90.8	1.1	Margaric	100.0	0.6
Caproic	92.1	1.5	Stearic	100.0	0.6
Enanthic	95.7	1.9	Nonadecanoic	100.0	0.6
Caprylic	98.6	0.2	Arachidic	100.0	0.5
Pelargonic	100.0	<0.1			

TABLE IV

OVERALL EFFICIENCY OF THE COMBINED SOLID PHASE EXTRACTION AND DIRECT SILYLATION OF THE TRIETHYLAMMONIUM SALTS OF ALIPHATIC MULTIFUNCTIONAL ACIDS

Aliphatic acid mixture (100 μg each) was added to 1 ml of water, containing 100 μg of *trans*-cinnamic acid and subjected to solid phase extraction and silylation, as described in the text ($n = 3$).

Acid	*Recovery (%)*	*C.V. (%)*	*Acid*	*Recovery (%)*	*C.V. (%)*
Lactic	81.7	0.4	Adipic	84.4	0.6
Glycolic	88.1	0.6	3-Methyladipic	99.2	0.2
Oxalic	81.4	0.4	Malic	82.0	1.2
Malonic	100.0	0.2	Suberic	91.0	0.1
Succinic	100.0	0.2	Tartaric	81.7	0.9
Methylsuccinic	100.0	0.1	Citric	81.1	0.4
Fumaric	100.0	0.2			

TABLE V

OVERALL EFFICIENCY OF THE COMBINED SOLID PHASE EXTRACTION AND DIRECT SILYLATION OF THE TRIETHYLAMMONIUM SALTS OF AROMATIC MULTIFUNCTIONAL ACIDS

Aromatic acid mixture (100 μg each) was added to 1 ml of water, containing 100 μg of *trans*-cinnamic acid and subjected to solid phase extraction and silylation, as described in the text ($n = 3$).

Acid	*Recovery (%)*	*C.V. (%)*	*Acid*	*Recovery (%)*	*C.V. (%)*
Benzoic	100.0	0.2	Vanillic	99.8	0.3
Phenylacetic	95.9	0.4	Syringic	100.0	0.6
5-Phenylvaleric	99.6	0.1	α-Resorcylic	100.0	0.3
p-Aminobenzoic	99.4	0.2	*p*-Hydroxymandelic	99.5	0.3
Mandelic	99.0	1.0	γ-Resorcylic	99.9	0.5
Phenyllactic	99.4	0.2	Homogentisic	100.0	0.3
Hippuric	100.0	1.5	Protocatechuic	99.6	1.0
p-Hydroxybenzoic	100.0	1.2			

In spite of recent advances in GC, the simultaneous detection of volatile short-chain fatty acids as well as various non-volatile carboxylic acids is still a difficult task. For this purpose, we combined the SPE method, developed for the trace enrichment of organic acids[4], and the direct TBDMS derivatization of their TEA salts[7]. Prior to SPE, aqueous samples were treated with sodium bicarbonate, followed by extraction with diethyl ether to remove the interfering non-carboxylic compounds, such as neutral, basic, and phenolic compounds. The resulting aqueous phase was acidified and loaded onto a Chromosorb P column. Free carboxylic acids were then eluted with diethyl ether, while water, inorganic acids and salts, urea, and other water soluble, polar compounds were held up by the hydrophilic Chromosorb P. Eluted acids were converted to TEA salts, which were then concentrated and directly silylated to TBDMS derivatives.

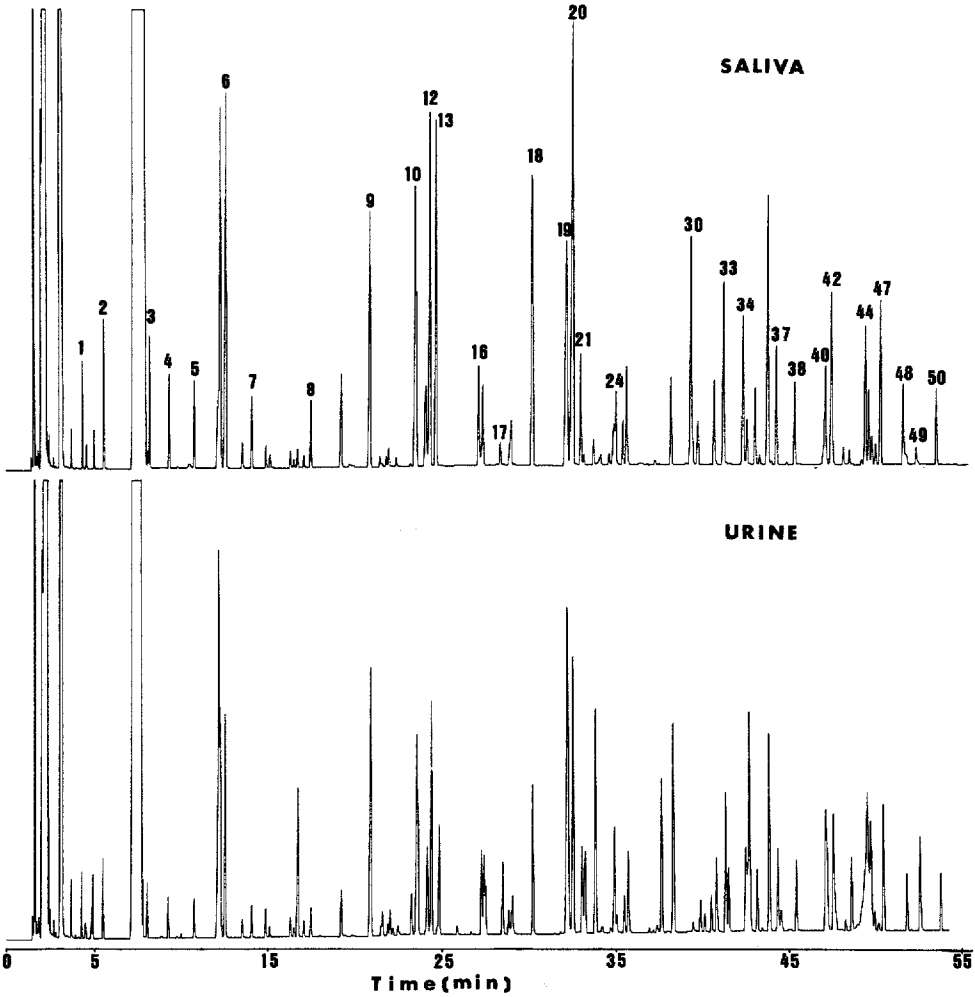

Fig. 7. Chromatograms of organic acids in saliva and urine from a normal person, A. A 1-ml volume of each sample was subjected to combined SPE and direct silylation, as described in the text. GC conditions and peak identities as in Fig. 4.

The recoveries of the three classes of acids from water samples, enriched with the acids at known concentrations, are listed in Tables III, IV, and V, respectively. Most of the acids could be recovered in excellent yields with good precision, except for formic acid and some polar hydroxy carboxylic acids. The exceptionally low recovery rate of formic acid is probably due to its higher affinity for the hydrophilic Chromosorb P.

When applied to body fluids, the present method demonstrated very efficient and highly specific sampling of carboxylic acids from complex aqueous mixtures. More than 40 acids were detected and tentatively identified, both in urine and saliva samples from two normal individuals, as shown in Figs. 7 and 8. Common interfering compounds in biological samples, such as urea, phenols, and phosphate were effectively excluded by the present procedure.

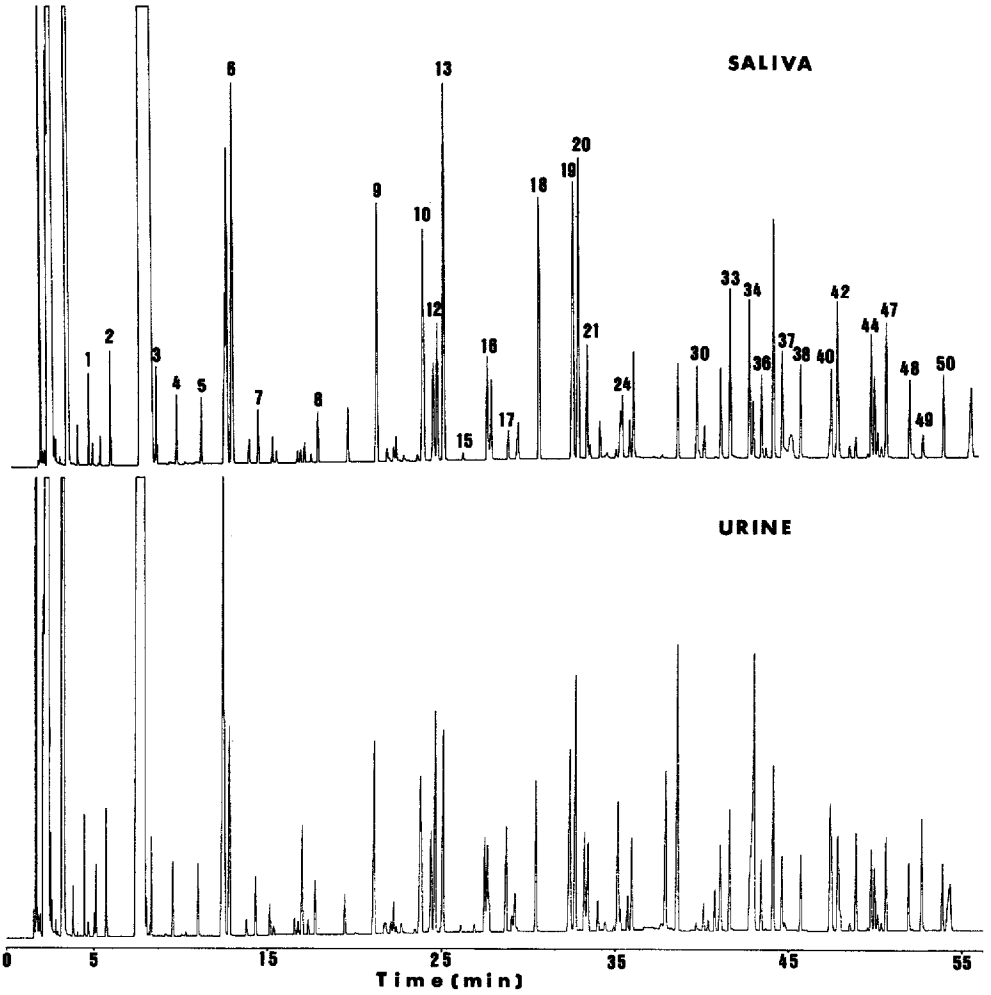

Fig. 8. Chromatograms of organic acids in saliva and urine from a normal person, B. A 1-ml volume of each sample was subjected to combined SPE and direct silylation, as described in the text. GC conditions and peak identities as in Fig. 4.

In conclusion, it can be stated that the present method is simple, rapid and specific as the method of choice for the simultaneous detection of various classes of carboxylic acids in complex sample matrices.

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