Acids: Derivatization for GC Analysis

Igor G. Zenkevich
Chemical Research Institute, St. Petersburg State University, St. Petersburg, Russia

INTRODUCTION

The class "acids" includes various types of compounds with active hydrogen atoms usually having $pK_a < 7$. The most important group of organic acids is the compounds with carboxyl fragment –COOH. Some other compounds can be classified not only as O-acids [e.g., hydroxamic acids, –CONHOH ⇌ –C(OH)=NOH], but C–H acids [with the presence of structural fragments $-CH(NO_2)_2$, $-CH(CN)_2$, etc.]. Well-known substances of this class for GC analysis are semivolatile fatty acids of triglycerides and lipids, numerous nonvolatile polyfunctional biogenic compounds (including such phenol carboxylic acids like gallic, vanillic, and syringic acid), different acidic herbicides (for example, 2,4-D, 2,4,5-T, MCPB, MCPA, fenoprop, haloxyfop, etc.), and many other substances. Strong inorganic acids like volatile hydrogen halides (HHal) and nonvolatile H_2SO_4, H_3PO_4, etc. can be objects of GC analysis too.

The simplest monofunctional carboxylic acids have boiling points at atmospheric pressure without decomposition and, hence, can be analyzed directly by GC. However, owing to the high polarities of carboxyl compounds, a typical problem of their GC analysis with standard nonpolar phases is the nonlinear sorption isotherm. As a result, these compounds yield broad nonsymmetrical peaks, which leads to poor detection limits and unsatisfactory reproducibility of their retention indices. The recommended stationary phases for direct analysis of free carboxylic acids are polar polyethylene glycols (Carbowax 20M, DBWax, SP-1000, FFAP, etc.). However, these phases have lower thermal stability compared with polydimethyl siloxanes (ca. 225–250 vs. 300–350 °C). This means that the upper limit of GC columns with these polar phases in Retention index (RI) units is not more than 3000–3500 IU. High homologs even of monocarboxylic acids cannot be eluted within this RI window (this is confirmed by the absence of RI data for palmitic acid, $C_{15}H_{31}COOH$, on the mentioned types of polar phases).

Compound	pK_a	T_b, °C	$RI_{nonpolar}$	RI_{polar}
Acetic acid	4.75	118	638 ± 10	1428 ± 30
Palmitic acid	4.9	351.5	1966 ± 7	No data
Benzoic acid	4.2	250	1201 ± 24	2387 ± 5
Phenylacetic acid	4.2	266	1290 ± 44	No data

All RIs with standard deviations are randomized interlaboratory data.

Some dicarboxylic acids can also be distilled without decomposition under reduced pressures. This is at least the theoretical grounds for the possibility of their direct GC analysis. Few successive attempts have been described, but these analytes require "on-column" injection of samples and extremely high inertness of chromatographic systems. Many types of polyfunctional carboxylic acids (hydroxy-, mercapto-, amino-, etc.) cannot be analyzed in free, underivatized form owing to either nonvolatility and/or absence of thermal stability. These features are the principal reasons for the conversion of carboxylic acids before their GC analysis into less polar derivatives without active hydrogen atoms.

METHODS OF ACID DERIVATIZATION

The general method of carboxylic acid derivatization is their esterification with the formation of alkyl (arylalkyl, halogenated alkyl) or silyl esters:[1–4]

$$XCO_2H + RY \rightarrow XCO_2R + YH$$

$$XCO_2H + ZSi(CH_3)_3 \rightarrow XCO_2Si(CH_3)_3 + ZH$$

Some of the most widely used reagents for the synthesis of alkyl carboxylates are listed in Table 1. The general recommendations for the silylation of mono- and polyfunctional carboxylic acids [trimethylsilyl (TMS)[5,6] and more stable *tert*-butyldimethylsilyl (TBDMS) derivatives[7,8]] are the same as those for other hydroxy containing compounds.

In general, the simplest methyl esters of carboxylic acids are more stable than TMS-esters to hydrolysis and, hence, they are the preferable derivatives for their GC analysis.[9,10] The most available esterification reagents are the corresponding alcohols, ROH, themselves. Different esters have been used as the analytical derivatives of carboxylic acids: Me, Et, Pr, *iso*-Pr, isomeric Bu (excluding *tert*-Bu esters owing to their poorer synthetic yields), and so forth. This method requires the use of excess of dry alcohol and acid catalysis by BCl_3, BF_3, CH_3COCl, $SOCl_2$, etc. Otherwise, the alcohol used can be saturated by gaseous HCl, which must then be removed by heating the reaction mixtures after completion of the reaction.

Encyclopedia of Chromatography DOI: 10.1081/E-ECHR-120039943

Table 1 Physicochemical and GC properties of some alkylating derivatization reagents

Reagent (abbreviation)	MW	T_b, °C	$RI_{nonpolar}$	By-products ($RI_{nonpolar}$)
Methanol/BCl_3, BF_3, HCl, DCC, etc.	32	64.6	381 ± 15	—
Diazomethane (in ethyl ether solution)	42	−23	None (unstable)	—
Methyl iodide/DMFA, K_2CO_3	142	42.8	515 ± 7	CH_3OH (381 ± 15)
Dimethyl sulfate/*tertiary*-amines	126	188.5	853 ± 22	CH_3OH (381 ± 15)
1-Iodopropane/DMFA, K_2CO_3	170	102	711 ± 11	C_3H_7OH (552 ± 13), $(C_3H_7)_2O$ (680 ± 6)
2-Bromopropane/LiH, DMSO	122	59.4	571 ± 5	$(CH_3)_2CHOH$ (486 ± 9), (*iso*-Pr)$_2$O (598 ± 5)
Methyl chloroformate	94	71	582 ± 17	CH_3OH (381 ± 15)
Ethyl chloroformate	108	—	640 ± 12	C_2H_5OH (452 ± 18)
Butyl chloroformate	136	—	832 ± 10	C_4H_9OH (658 ± 12)
Pentafluorobenzyl bromide (PFB-Br)	260	174–175	991 ± 11[a]	$C_6F_5CH_2OH$ (934 ± 16)[a]
3,5-*bis*-(Trifluoromethyl)benzyl bromide (BTB-Br)	306	—	1103 ± 9[a]	$(CF_3)_2C_6H_3CH_2OH$ (1046 ± 15)[a]
Tetramethylammonium hydroxide (TMAH; in 25% aqueous solution)	74	—	Nonvolatile	$(CH_3)_3N$ (418 ± 9)
Trimethylanilinium hydroxide (TMPAH; in 0.2 M methanol solution)	136	—	Nonvolatile	$C_6H_5N(CH_3)_2$ (1065 ± 9)
3,5-*bis*-(Trifluoromethylbenzyl) dimethylanilinium fluoride (BTBDMA-F)	258	—	Nonvolatile	3,5-$(CF_3)_2C_6H_3CH_2N(CH_3)_2$ (no data), $C_6H_5N(CH_3)_2$ (1065 ± 9)
2-Bromoacetophenone (phenacyl bromide)	198	260	1321 ± 4	$C_6H_5COCH_2OH$ (1118)[b]
Silylating reagents[c]				

[a]Estimated RI values.
[b]Single experimental value.
[c]See *Hydroxy Compounds: Derivatization for GC Analysis in this volume.*

The same procedures are used for the synthesis of 2-chloroethyl ($RCO_2CH_2CH_2Cl$), 2,2,2-trifluoroethyl ($RCO_2CH_2CF_3$), 2,2,2-trichloroethyl ($RCO_2CH_2CCl_3$), and hexafluoroisopropyl esters [$RCO_2CH(CF_3)_2$] for GC analysis with selective detection. Instead of acid catalysis of this reaction, some reagents for the coupling of formed water were recommended, namely 1,1′-carbonyldiimidazole (I) and 1,3-dicyclohexylcarbodiimide (DCC, II):

(I)

(II)

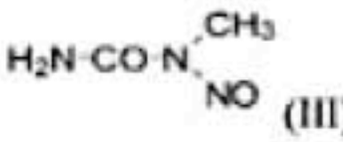
The application of any additive reagents usually leads to the appearance of additional peaks on the chromatograms (including the peaks of by-products, for example imidazole, $RI_{nonpolar}$ 1072 ± 17), which must be reliably identified and excluded from data interpretation. The by-product from compound (II) — 1,3-dicyclohexylurea—is nonvolatile for GC analysis.

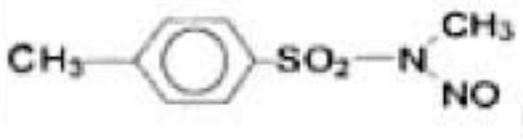
Another class of esterification reagents are halogenated compounds (alkyl iodides, substituted benzyl[11] and phenacyl bromides, etc.), which need basic media for their reaction [K_2CO_3 or DMFA (dimethyl formamide) is used usually for the neutralization of HBr or HCl as acid by-product]. For methylation of carboxylic acids, some tetra-substituted ammonium hydroxides or halides can be used, namely tetramethylammonium hydroxide (in aqueous solutions) or trimethylanilinium hydroxide (in methanol solution). The intermediate ammonium carboxylates are thermally unstable and can produce methylalkanoates during the following heating of reaction mixtures or even their introduction into the hot injector of the gas chromatograph (flash methylation):

$$RCO_2H + XNMe_3^+OH^- \rightarrow [RCO_2^-NMe_3^+] \rightarrow RCO_2Me \quad (X = Me, Ph)$$

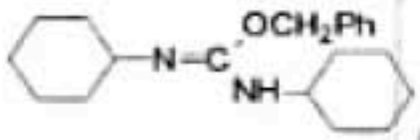
The possible by-products of these reactions are the corresponding amines (Me_3N or $PhNMe_2$). A similar method has been proposed for the butylation of organic acids.[12] If the appearance of any volatile by-products is undesirable, the methylation of carboxylic acids by diazomethane is recommended.

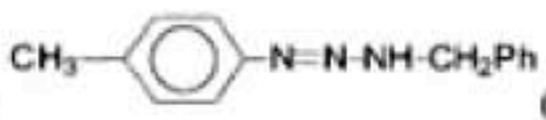
This reagent (*warning*: highly toxic) is synthesized by alkaline cleavage of *N*-methyl-*N*-nitrosourea (III) or *N*-methyl-*N*-nitrosotoluenesulfamide (IV) and owing to its low boiling point (–23 °C) can be used only in diethyl ether solutions prepared immediately before use.


(III)

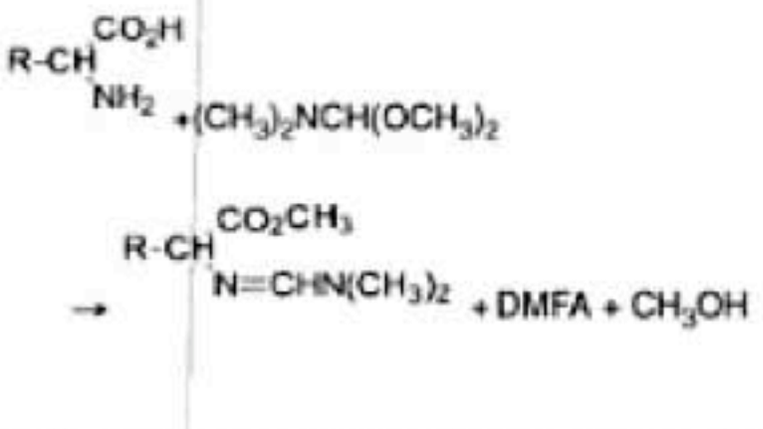

(IV)

In the absence of acid catalysis, diazomethane reacts only with carboxylic acids (pK_a 4–5) and phenols (pK_a 9–10), but has no influence on aliphatic OH– groups. Besides CH_2N_2, some more complex diazocompounds (diazoethane, diazotoluene) have been recommended for the synthesis of other esters (ethyl and benzyl, respectively). For the synthesis of benzyl (or substituted benzyl) esters, some special reagents have also been proposed, for example, *N,N'*-dicyclohexyl-*O*-benzylurea (V) and 1-(4-methylphenyl)-3-benzyltriazene (VI):


(V)


(VI)

The esterification of carboxylic acids can also be accomplished using synthetic equivalents of acetals of alkanols $RCH(OR')_2$ (by acid catalysis), ortho-esters $RC(OR')_3$ (by acid catalysis), and dialkylcarbonates $CO(OR)_2$ (by base catalysis). The series of bifunctional reagents of this type—dimethylformamide dialkylacetals $(CH_3)_2N–CH(OR)_2$—is commercially available. Besides the esterification of carboxyl groups, these compounds react with primary amino groups used for GC analysis of amino acids:



A "sandwich" technique (flash methylation) can also be used in this case. It implies the injection of the combined sample and reagent in the same syringe into the gas chromatograph, e.g., successively placed 1 mL of derivatization reagent, 1 μL of pyridine with internal standard, and 1 mL of the solution of analytes in the same solvent.

Alkyl chloroformates, $ClCO_2R$ (R = Me, Et, Bu), have been proposed as convenient alkylating reagents for carboxylic acids:[13]

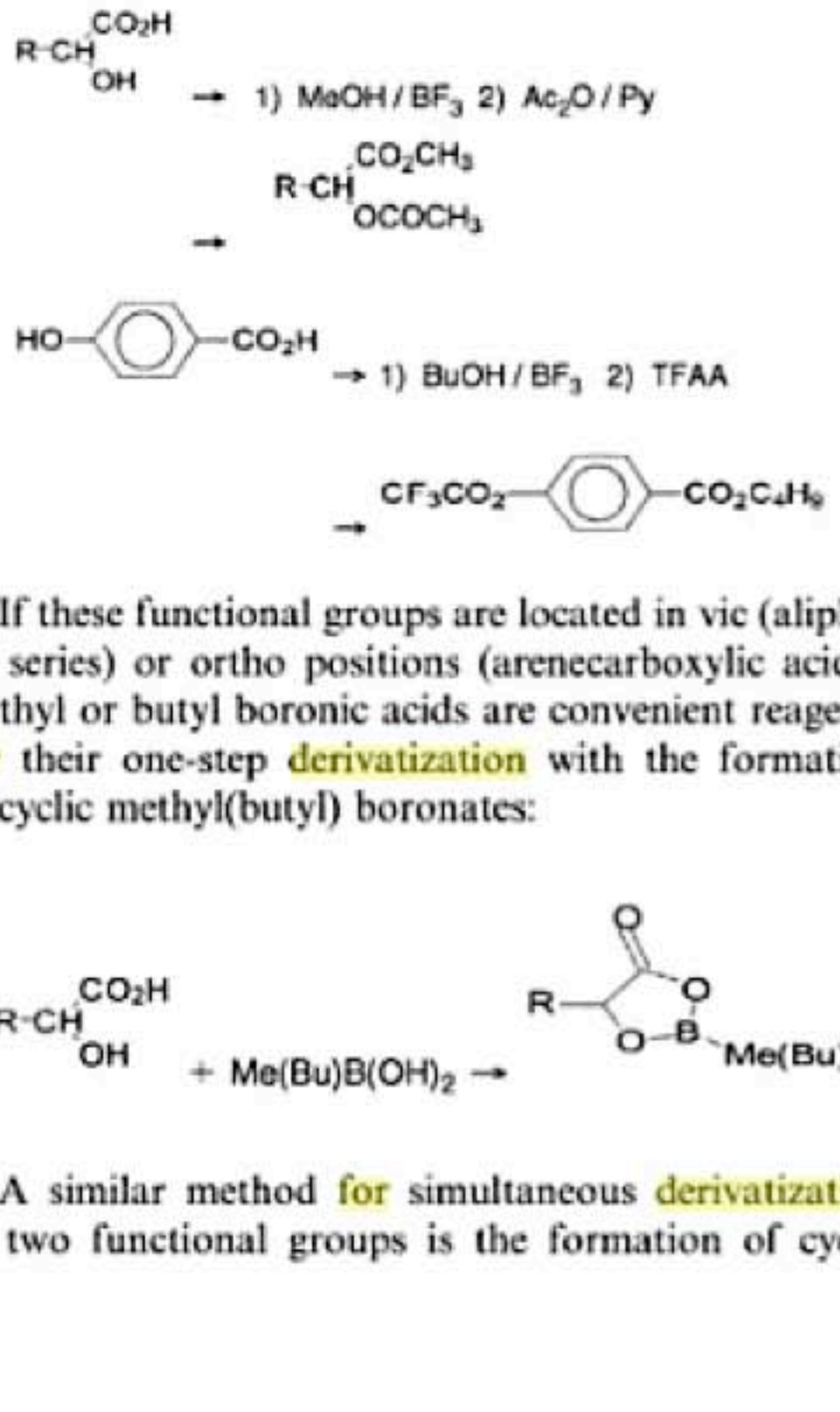
$$RCO_2H + ClCO_2R' + B \rightarrow RCO_2R' + CO_2 + BH^+Cl^-$$

Two-stage single-pot derivatization of carboxylic acids (with intermediate formation of chloroanhydrides with thionyl chloride followed by their conversion into amides) was recommended preferably for HPLC analysis, but the simplest dialkylamides and anilides[14] are volatile enough for GC analysis also (the mixture of Ph_3P and CCl_4 can be used in this reaction instead of $SOCl_2$). Moreover, the same procedure is used for the synthesis of diastereomeric derivatives of enantiomeric carboxylic acids (see below):

$$RCO_2H + SOCl_2 \rightarrow RCOCl + SO_2 + HCl$$

$$RCOCl + R'R''NH + B \rightarrow RCONR'R'' + BH^+Cl^-$$

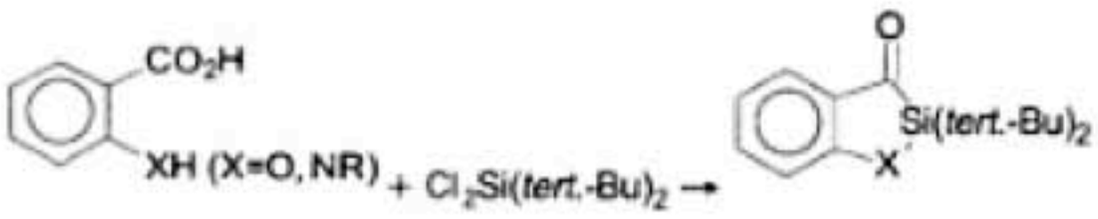
The reactivities of carboxy and hydroxy groups in the polyfunctional hydroxy- and phenol carboxylic acids are different. This indicates the possibility of an independent two-stage derivatization of these compounds, for example:



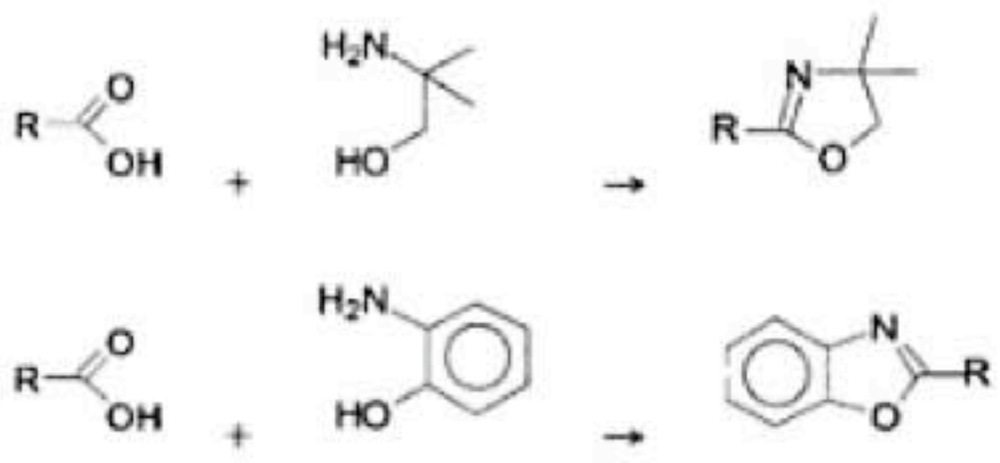
If these functional groups are located in vic (aliphatic series) or ortho positions (arenecarboxylic acids), methyl or butyl boronic acids are convenient reagents for their one-step derivatization with the formation of cyclic methyl(butyl) boronates:

A similar method for simultaneous derivatization of two functional groups is the formation of cyclic

silylene derivatives for the same types of compounds:[15]

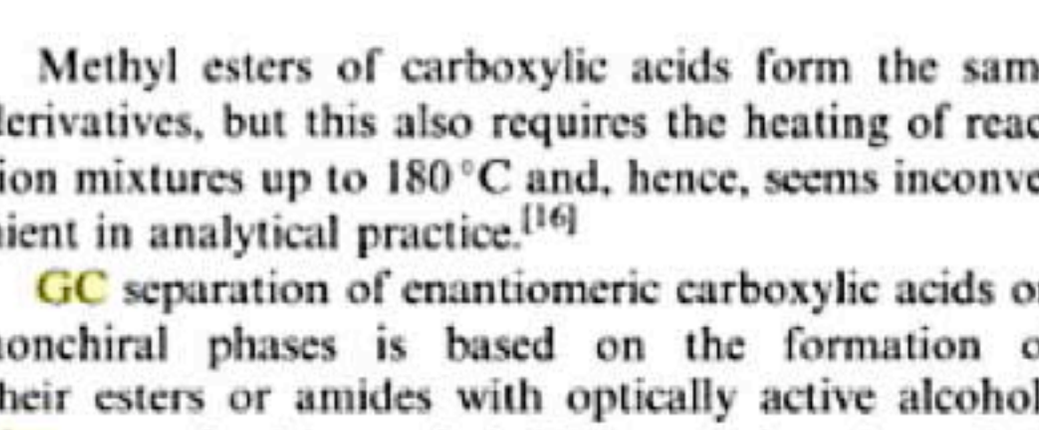


A special type of carbonyl group derivatization is aimed at GC/MS determination of double bond (C=C) positions in unsaturated long-chain acids. The analytical derivatives for the solution of this problem are nitrogen-containing heterocycles. These compounds can be synthesized by high temperature condensation of carboxylic acids with 2-amino-2-methyl-1-propanol (2-substituted 4,4-dimethyloxazolines), 2-aminophenol (2-substituted benzoxazoles), and so forth.



Methyl esters of carboxylic acids form the same derivatives, but this also requires the heating of reaction mixtures up to 180 °C and, hence, seems inconvenient in analytical practice.[16]

GC separation of enantiomeric carboxylic acids on nonchiral phases is based on the formation of their esters or amides with optically active alcohols [for example, (–)-menthol, VII] or amines (α-methylbenzenemethanamine, VIII), usually through the intermediate chloroanhydrides. These diastereomeric products are not so volatile as other acid derivatives but, owing to the presence of two chiral carbon atoms (*) in the molecule, can be separated on nonchiral phases:



A problem closely related to the derivatization of free carboxylic acids is the determination of their composition in biogenic triglycerides, lipids, and so forth. The sample preparation includes the re-esterification (preferably with formation of methyl esters) of these compounds in acid ($MeOH/BF_3$, MeOH/AcCl, etc.) or basic (MeONa, MeOH/KOH, etc.) media. Methyl esters of fatty acids are a group of compounds well characterized by both standard mass spectra and GC retention indices on standard phases. The combination of these analytical parameters provides their reliable identification.

The general method of GC analysis of anions of inorganic acids is their silylation. The values of retention indices on standard nonpolar phases (SE-30) are known for TMS derivatives of the most important among them:[3]

Anion	Volatile derivative for GC analysis	$RI_{nonpolar}$
Borate	$B(OTMS)_3$	1010
Carbonate	$CO(OTMS)_2$	1048
Phosphite	$P(OTMS)_3$	1115
Sulfate	$SO_2(OTMS)_2$	1148
Arsenite	$As(OTMS)_3$	1149
Phosphate	$PO(OTMS)_3$	1273
Vanadate	$VO(OTMS)_3$	1301
Arsenate	$AsO(OTMS)_3$	1353

CONCLUSIONS

Both strong inorganic and weak organic acids usually need derivatization prior to GC analysis. The existence of active hydrogen atoms in the molecules explains the significant contribution of ionic structures, which are responsible for the high polarity and low volatility of these substances.

Most universal methods of derivatization of acids are silylation (TMS and TBDMS) and alkylation (the simplest methyl esters are preferable). Other methods have an auxiliary predetermination and can be recommended for the solution of special analytical problems.

ARTICLES OF FURTHER INTEREST

Amines, Amino Acids, Amides, and Imides: Derivatization for GC Analysis, p. 57.
GC System Instrumentation, p. 682.
Hydroxy Compounds: Derivatization for GC Analysis, p. 809.

REFERENCES

1. Blau, K., King, G.S., Eds.; *Handbook of Derivatives for Chromatography;* Heiden: London, 1977; 576.

2. Knapp, D.R. *Handbook of Analytical Derivatization Reactions*; John Wiley & Sons: New York, 1979; 741.
3. Drozd, J. *Chemical Derivatization in Gas Chromatography*; Journal of Chromatography Library; Elsevier: Amsterdam, 1981; Vol. 19, 232.
4. Blau, K., Halket, J.M., Eds. *Handbook of Derivatives for Chromatography*; 2nd Ed.; J. Wiley & Sons: New York, 1993; 369.
5. Wurth, C.; Kumps, A.; Mardens, Y. Urinary organic acids: retention indices on two capillary GC columns. J. Chromatogr. **1989**, *491*, 186–192.
6. Lefevere, M.F.; Verkaeghe, B.J.; Declerk, D.H.; Van Boexlaer, J.F.; De Leenheer, A.P.; De Sagher, R.M. Metabolic profiling of urinary organic acids by single and multicolumn capillary gas chromatography. J. Chromatogr. Sci. **1989**, *27* (1), 23–29.
7. Rodriguez, I.; Quintana, J.B.; Carpinteiro, J.; Carro, A.M.; Lorenzo, R.A.; Cela, R. Determination of acidic drugs in sewage water by GC–MS as *tert.*-butyl dimethylsilyl derivatives. J. Chromatogr. A. **2003**, *985*, 265–274.
8. Crouholm, T.; Norsten, C. Gas chromatography–mass spectrometry of carboxylic acids in tissues as their *tert.*-butyl dimethylsilyl derivatives. J. Chromatogr. B. **1985**, *344*, 1–9.
9. Gonzalez, G.; Ventura, R.; Smith, A.K.; De la Torre, R.; Segura, J. Determination of non-steroidal anti-inflammatory drugs in equine plasma and urine by gas chromatography–mass spectrometry. J. Chromatogr. A. **1996**, *719*, 251–264.
10. Nilsson, T.; Baglio, D.; Galdo-Miquez, I.; Madsen, O.J.; Facchetti, S. Derivatization/solid-phase microextraction followed by GC–MS for the analysis of phenoxy acid herbicides in aqueous samples. J. Chromatogr. A. **1998**, *826*, 211–216.
11. Gabelish, C.L.; Crisp, P.; Schneider, R.P. Simultaneous determination of chlorophenols, chlorobenzenes and chlorobenzoates in microbial solutions using pentafluorobenzyl bromide derivatization and analysis by GC with electron capture detection. J. Chromatogr. A. **1996**, *749*, 165–171.
12. Burke, D.G.; Halpern, B. Quaternary ammonium salts for butylation and mass spectral identification of volatile organic acids. Anal. Chem. **1983**, *55* (6), 822–826.
13. Butz, S.; Stan, H.-J. Determination of chlorophenoxy and other acidic herbicide residues in ground water by capillary GC of their alkyl esters formed by rapid derivatization using various chloroformates. J. Chromatogr. **1993**, *643*, 227–238.
14. Umeh, E.O. Separation and determination of low molecular weight straight chain C_1–C_8 carboxylic acids by gas chromatography of their anilide derivatives. J. Chromatogr. **1970**, *51*, 147–154.
15. Brooks, C.J.W.; Cole, W.T. Cyclic di-*tert.*-butylsilylene derivatives of substituted salicylic acids and related compounds. A study by gas chromatography–mass spectrometry. J. Chromatogr. **1988**, *441*, 13–29.
16. Fay, L.; Richli, U. Location of double bonds in polyunsaturated fatty acids by GC–MS after 4,4-dimethyloxazoline derivatization. J. Chromatogr. **1991**, *541*, 89–98.

A