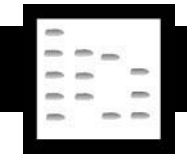
ACIDS



Gas Chromatography

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Introduction

The first separation of acids by gas chromatography (GC) coincides with the inception of GC itself. In 1952 James and Martin pioneered GC by demonstrating the separation of the C_1 to C_{12} aliphatic acids on a stationary phase of silicone oil DC 550 containing stearic acid or H_3PO_4 and quantifying using a special titrimetric detector. Since then, the GC analysis of acids has been extended to a very wide variety of species and samples. To enable ready application of GC, the acids are usually converted to suitable volatile derivatives for resolution on efficient columns. As they are eluted they must be identified by an appropriate technique, the most definitive being mass spectrometry (MS). Various applications are presented in this article.

Derivatization

It was noted early on that separation of free acids is frequently hampered by their relatively low volatility, molecular association and, particularly, their adsorption on the stationary phase support with the resultant tailing, peak distortion and ghosting. Although special columns (FFAP, OV-351, SP-1000) have been developed since then for the separation of short and medium chain free (underivatized) aliphatic acids, the majority of carboxylic acids (especially those containing additional polar substituents) are insufficiently volatile for analysis by GC. Therefore, the carboxyl and other polar groups are usually converted to less polar derivatives to improve their chromatographic properties.

Carboxylic Acids

Both free and bound carboxyl groups are almost exclusively derivatized to volatile esters – predominantly silyl and methyl – by a variety of methods. These employ a number of silylation reagents, acid- and base-catalysed reactions, on-column pyrolysis, diazomethane and other reagents. Each has its advantages, limitations and special applications.

Silyl esters Silylation is now one of the most extensively used techniques for esterifying free acids primarily because of its speed, convenience and the simultaneous derivatization of other polar functional groups containing an active hydrogen (–OH, –SH, $–NH_2$). The trimethylsilyl (TMS) group is the most commonly introduced substituent by the many silylating agents available, of which *N*,*O*-*bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) is the most widely used. It reacts with all the common polar functionalities and yields volatile by-products that are usually eluted with the solvent. Even more volatile by-products are produced by substituted reagents, e.g. *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), which are also more reactive toward the polar functional groups. Although all silylating reagents and their products are sensitive to moisture, considerably greater hydrolytic stability is exhibited by *t*-butyldimethylsilyl (TBDMS) derivatives that are best prepared with *N*-*t*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), which can also serve as its own solvent. It yields excellent results with both volatile and nonvolatile carboxylic acids (**Figure 1**). A limitation of silylation is that bound acids such as lipids (triacylglycerols) are not converted and their derivatization to methyl (or other alkyl) esters is necessary.

Alkyl esters Methyl esters are most frequently prepared by acid-catalysed reactions with methanol. The principal advantage of this method is the concurrent esterification of free acids and the transesterification of bound ones. The most extensively used catalysts are BF_3, HCl and H_2SO_4, usually as 14%, 5% and 2% solutions, respectively. The reaction is fastest with BF_3, requiring the mixture to be boiled for 2 min for free acids and 30–60 min for lipids. With HCl and H_2SO_4 about twice the time is required. The higher concentration of BF_3 used compared to the other catalysts may be responsible not only for the faster reaction, but also for partial degradation of unsaturated acids and reported artefact formation. These problems can be reduced by prior saponification with methanolic KOH, followed by re-esterification of the free acids formed under mild conditions. Several official methods are based on this procedure.

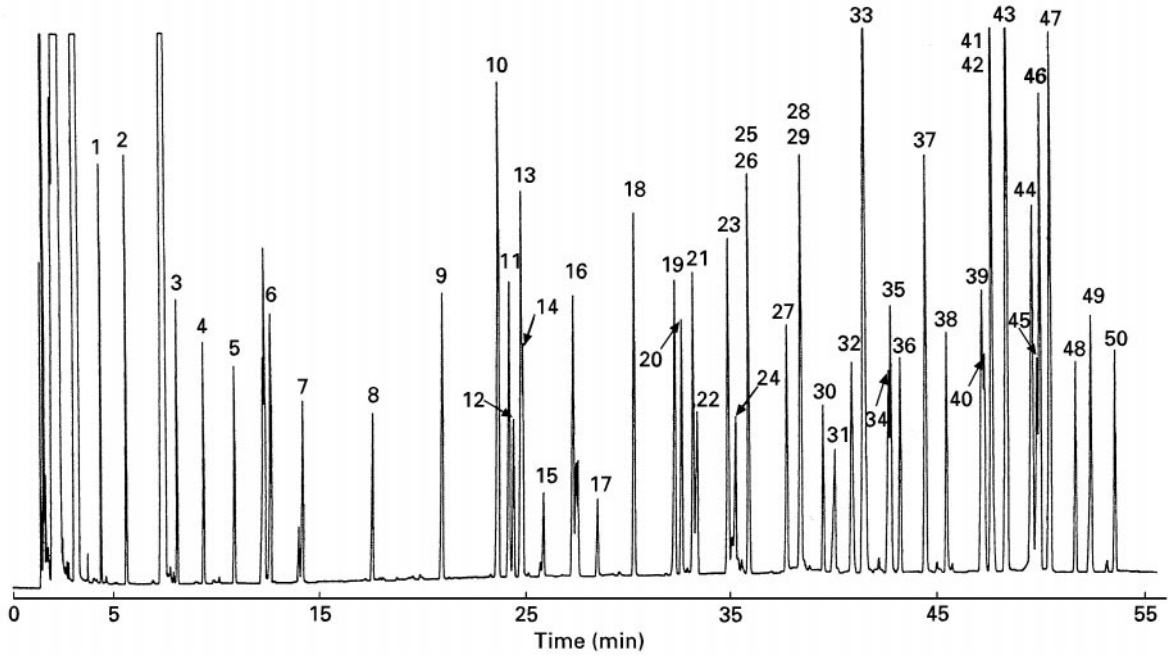

Figure 1 Chromatogram of a mixture of carboxylic acids as the *t*-butyldimethylsilyl derivatives. GC conditions: 30 m × 0.32 mm i.d., DB-1 fused-silica capillary column initially at 60°C for 2 min, then programmed to 280°C at 4°C min^{-1}; 0.8 L sample, injected with split ratio of 15 : 1; both injector and detector temperatures at 300°C; nitrogen as the carrier gas at 0.9 mL min^{-1}. 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, glycol; 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. (Reproduced with permission from Kim KR, Hahn MK, Zlatkis A *et al.* (1989) Simultaneous gas chromatography of volatile and nonvolatile carboxylic acids as *tert*-butyldimethylsilyl derivatives. *Journal of Chromatography* 468: 289.

Substituting microwave irradiation for conventional heating may substantially reduce reaction times and lipid degradation. Thus, using the BF_3-methanol reagent, a reaction time of 30 s sufficed for the transesterification of most lipids to their fatty acid methyl esters (FAMEs) with less oxidation of the unsaturated species.

Base-catalysed reactions are used extensively for the transesterification of lipids because they proceed faster than those in acid media without degradation of the unsaturated fatty acids. However, they do not esterify free fatty acids. The most commonly used reagents are methanolic solutions of $NaOCH_3$ or KOH. Transmethylation of lipids is usually complete in 5 min at room temperature.

Strong organic bases can be used similarly and possess the great advantage of forming salts which, unlike their inorganic analogues, can be pyrolysed to methyl esters at the high temperatures of a GC injection port. This permits simple one-step determination of both free and bound acids. The organic bases that have been recommended for such pyrolytic conversions include (*m*-trifluoromethylphenyl)-trimethylammonium, trimethylphenylammonium and trimethylsulfonium hydroxides. The latter reagent requires the lowest pyrolysis temperature and yields innocuous by-products. It is simply added to the sample solution, mixed and injected.

Esterification of free acids with diazomethane proceeds rapidly in high yield under mild conditions, with minimal side reactions. Special microequipment, reagents and procedures have been developed that allow its relatively safe handling despite its toxic and explosive nature. Other reagents of interest include alkyl chloroformates that can esterify free acids even in the presence of a considerable amount of water (40%). Another reagent, dimethylformamide dimethylacetal, can be simply mixed with the sample of acid and injected into the GC; the reaction occurs in the hot injection port. Silver or potassium salts of acids can be converted to esters with methyl iodide or sulfate. Many other reactions have been reported.

Short chain acids are frequently derivatized to higher esters with butanol or isopropanol and acid

catalysts in order to mitigate losses due to volatility and substantial water solubility. Higher diazoalkanes may also be used if the methyl esters are too volatile.

Enantiomers of optically active carboxylic acids have been separated following acid-catalysed esterification with a chiral alcohol such as *S*(+)-2-butanol, *R*(−)-2-octanol, or (−)-methanol or transesterification with sodium menthylate. Diastereometric esters have also been prepared from optically active acids by reaction with *O*-(−)-menthyl-*N*,*N*-diisopropylisourea.

The above silyl and alkyl esters are most commonly detected by a flame ionization detector (FID). Greater sensitivity, however, can be achieved by forming halogenated silyl esters, e.g. chloromethyldimethylsilyl, and monitoring with an electron-capture detector (ECD). Similarly, very small amounts of volatile acids may be detected via their pentafluorobenzyl (PFB) esters with an ECD. Special derivatives for this detector include the 2-chloroethyl and trichloroethyl esters.

Other derivatives The silyl and alkyl esters described are generally also suitable for detection by MS. However, special derivatives are necessary for unsaturated fatty acids to prevent double-bond migration during fragmentation. The most widely used derivatives are those of 3-hydroxymethylpyridine (picolinyl) and 4,4-dimethyloxazoline (DMOX). Picolinyl esters must be prepared from the acid but DMOX derivatives can be prepared even from their esters.

Amino Acids

For amino acids, derivatization is indispensable for analysis by GC since they all exist in the zwitterion form. Some also contain other polar functionalities, including hydroxyl, thiol and imino groups. The different reactivities of these groups greatly complicate their concurrent derivatization. Silylation offers the best approach for a single-step attachment of the same tag to all these functional groups.

The most successful attempt to generate a single product is by silylation with MTBSTFA to form TBDMS derivatives. Reaction conditions (heating at 150°C for 2.5 h) were developed for the reproducible derivatization of amino acids in high yield. TMS derivatives of the common amino acids, except arginine, can also be prepared with BSTFA under similar conditions.

An alternative method of derivatization of amino acids entails first esterification and then acylation to produce various *N*-acyl alkyl esters (**Figure 2**). The most widely used of these combinations is the *N*-trifluoroacetyl-*n*-butyl ester (TAB) derivative. Esterification is performed by one of the methods described above and acylation by heating the dried product with trifluoroacetic anhydride. The selectivity of the NP detector can be exploited to monitor amino acids in the presence of interfering matrices, particularly lipids.

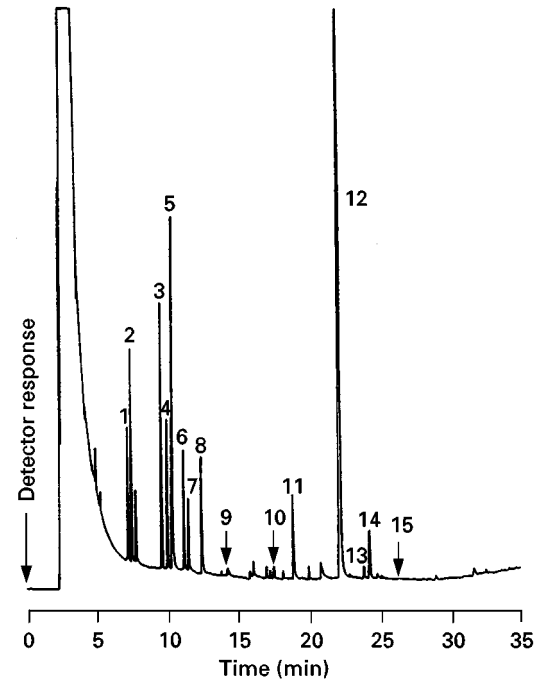

Figure 2 Separation of *N*,*O*-heptafluorobutyryl amino acid isobutyl ester derivatives obtained from silkworm *t*-RNA after deacylation and analysed with FID. GC conditions: 25 m × 0.4 mm i.d. capillary column coated with 5% Chromosorb R and 15% OV-101 SCOT column; carrier gas, hydrogen at a flow rate of 3 mL min^{-1}; make-up gas, nitrogen at a flow rate of 30 mL min^{-1}; hydrogen flow rate, 27 mL min^{-1}; air flow rate, 350 mL min^{-1}; temperatures: detector, 320°C; no inlet heater block; column, 80°C programmed at 4°C min^{-1}. Pulse interval, 15 μs; attenuation, 2×10^2; sample size, 20 μL. Peaks: 1, Alanine; 2, glycine; 3, valine; 4, threonine; 5, serine; 6, leucine; 7, isoleucine; 8, norleucine (I.S.); 9, proline; 10, methionine; 11, aspartic; 12, glutamic acid; 13, lysine; 14, tyrosine; 15, arginine. (Reproduced with permission from Chauhan J and Darbre A (1982) Determination of amino acids by means of glass capillary gas-liquid chromatography with temperature-programmed electron-capture detection. *Journal of Chromatography* 236: 151.

Enantiomeric resolution has been achieved with a chiral aliphatic alcohol and an achiral acylating agent such as *N*-trifluoroacetyl chloride. Alternatively, the amino group has been converted to diastereomeric amides, ureas, thioureas and isoindoles.

Resolution

Since many real samples are complex mixtures of acids (and other components), high efficiency

columns are essential for satisfactory resolution. This requirement has made packed columns effectively obsolete for such samples and use of capillary (or open tubular, OT) columns is becoming routine. The high efficiency of OT columns requires correspondingly less selectivity to gain the necessary separation. Therefore, relatively few different stationary phases in OT columns will adequately separate the majority of mixtures encountered.

Nonpolar stationary phases have the advantages of greater inertness, thermal stability and operation at lower temperatures. Since retention times increase with increasing polarities of the stationary phase and analyte, the least polar column affording the necessary resolution should be selected. Silyl derivatives are usually adequately separated on nonpolar polydimethylsiloxanes (e.g. DB-1, SE-30, OV-101); for greater selectivity somewhat more polar phases such as DB-5, SE-54, OV-17 or even OV-1701 may be used. On the other hand, stationary phases containing hydroxyl groups (such as the polyethylene glycols, PEGs) should be avoided because they react with silylation reagents.

Saturated and unsaturated FAMEs are generally separated on more polar columns because they tend to cluster together on nonpolar phases, with the unsaturated ones preceding the saturated. On polar phases such as PEGs, the unsaturated are eluted after the saturated with minimal overlap of different chain lengths. This shift in retention behaviour is further enhanced on very polar stationary phases such as the cyanosilicones (CP-Sil-88, OV-275, DB-23) which are used for resolving *cis*, *trans* isomers and very complex mixtures.

Relatively nonpolar columns are used for the separation of diastereomeric esters formed from optically active carboxylic and amino acids. As an alternative approach, amino acid enantiomers have been separated as their alkyl *N*-perfluoroacyl derivatives on a chiral column, e.g. Chirasil-Val.

Identification

With conventional GC detectors, such as the FID and ECD, identification of the most commonly encountered acids is based on comparison of the retention times obtained with authentic standards. For unidentified acid peaks in general, retention index values or, for FAMEs, equivalent chain lengths (ECL) from the literature may be helpful. The preferred solution is, however, MS detection in view of the more definitive structural information it provides. Especially for carboxylic acids, the usual data (e.g. molecular weights, fragmentation patterns, isotopic peak patterns) afforded by MS are supplemented by additional structural information, the most useful being the degree of unsaturation.

The presence of a double bond can be deduced from the molecular weight of an ester but its location cannot be ascertained due to migration during fragmentation. Hence, for reliable identification of positional isomers by GC-MS, two methods are employed: the on-site method of fixing the location of the double bond through its chemical modification, or the remote group method in which the carboxylic group is derivatized to a nitrogen-containing product which restricts double-bond migration. The remote group method is more convenient and versatile.

Chemical modification involves the addition of a reagent across the double bond of the acid ester to generate a product which gives diagnostic fragment ions. Dimethyl disulfide is a widely used reagent since it adds to a double bond in a single step at room temperature and enables identification of positional and geometrical isomers after separation on an appropriate column. But the picture is less clear with polyenoic acids, especially when the double bonds are in close proximity, and with acids containing other structural features such as cyclopropane rings. Diels-Alder reactions with cyclopentadiene derivatives can be applied similarly. The double-bond site may also be established by treating the unsaturated acid with OsO_4 and converting the resulting diol to the *bis*-TMS ethers for GC-MS analysis. Although this method is suitable for locating the double-bond sites of polyunsaturated acids, their fragmentation patterns are more complex and careful interpretation is necessary.

In derivatizing the carboxylic group, the picolinyl and DMOX compounds are the most commonly generated nitrogen-containing products. In the mass spectra of these derivatives, the saturated segments of the molecules are indicated by the regular separation of successive peak clusters by 14 amu (corresponding to the cleavage of a CH_2 group), whereas at double-bond sites the gap is only 12 amu. Furthermore, fragmentation on either side of the double bond gives two ions which are separated by 26 amu. In a branched acid derivative, the site of branching is shown by a similar gap of 28 amu.

Geometrical isomers and ring structures are more reliably identified by infrared (IR) spectrometry, which underscores the utility of GC-Fourier transform IR (FTIR)-MS in the structure elucidation of acids. However, the inherently lower sensitivity of IR requires larger sample sizes and columns with a higher load capacity.

Quantitative analysis of acids by GC-MS is carried out most sensitively by selected ion monitoring (SIM) employing an isotopically labelled analogue or a

derivative of a structurally similar acid as internal standard. The desired sensitivity of detection is a critical factor in the choice of the derivative. For increased sensitivity ion currents must be intensified by reducing fragmentation. Hence, TBDMS derivatives are preferred to those of TMS. Moreover, TBDMS derivatizes the amino acids arginine and glutamine, whereas TMS fails to do this. (However, the preferred method for quantification of amino acids involves the butyl perfluoroacyl derivatives.) Fragmentation may also be reduced by increasing molecular stability via cyclic derivatives, as illustrated by quinoxalinol compounds utilized in the GC-MS analysis of 2-oxo-acids. An excellent method of augmenting sensitivity is performing negative ion mass spectrometry via derivatives (e.g. *p*-nitrobenzyl, pentafluorobenzyl) with high electron affinity.

These methods have allowed the determination of a variety of acids by GC-MS at pg levels. Even mixtures of acids can be analysed quantitatively by monitoring several characteristic ions. Programmable SIM, which optimizes the selectivity at various points in a chromatogram and the desired sensitivity of analysis, has been invaluable in this regard.

Applications

Examples of GC analysis of acids in real-world samples are so numerous and diverse as to permit only representative cases from more significant fields to be cited.

Carboxylic acids present at abnormal levels in plasma and urine may indicate various metabolic disorders. Hence, their monitoring is vital for diagnostic purposes. GC has simplified such analysis by expediting the separation and determination of very low concentrations of acids present in these complex matrices (**Figure 3**). For example, C_{27} and C_{29} bile acid levels provide the basis for a screening test for a genetic condition characterized by peroxisomal dysfunction syndrome and are measured by GC-MS as methyl-silyl derivatives. Elevated levels of certain acylcarnitines may signify a potentially lethal condition caused by the deficiency of an enzyme which is essential for β-oxidation of fatty acids. Their quantification by GC-MS has been achieved by the ready conversion to volatile acyloxylactones. Metabolic products of amino acids whose presence in urine at unusually high levels may be symptomatic of

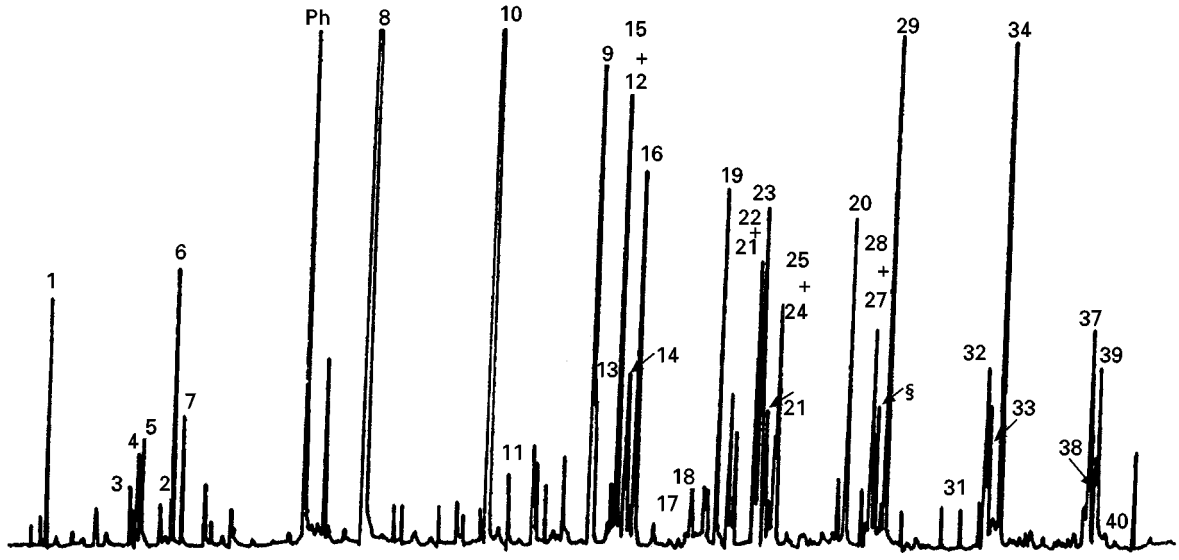

Figure 3 Chromatogram of a 3-hydroxy-dicarboxylic aciduria. GC conditions: 30 m × 0.32 mm i.d. column coated with OV-1701; temperature-programmed from 70 to 270°C at a rate of 5°C min^{-1}. Detector: FID. Some important peaks are indicated: 1, lactic di TMS;2, oxalic di TMS; 3, 3-hydroxy-propionic di TMS; 4, 3-hydroxybutyric di TMS; 5, 3-hydroxy-isobutyric di TMS; 6, 2-methyl-3-hydroxybutyric di TMS; 7, 3-hydroxy-isovaleric di TMS; 8, internal standard; 9, 3-hydroxy-adipic lactone mono TMS; 10, adipic di TMS; 11, hexenedioic di TMS; 12, triglycine mon TMS; 13, 4-hydroxy-phenylacetic di TMS; 14, octenedioic di TMS; 15, 3-hydroxy-adipic tri TMS; 16, suberic di TMS; 17, 3-keto-adipic enol tri TMS; 18, aconitic tri TMS; 19, citric tetra TMS; 20, hippuric mono TMS; 21, decenedioic di TMS; 22, 3-hydroxy-octenedioic tri TMS; 23, 3-hydroxy suberic tri TMS; 24, sebacic di TMS; 25, 4-hydroxy-phenyllactic tri TMS; 27, 3-hydroxy-decendioic tri TMS; 28, 4-hydroxy-phenolpyruvic enol tri TMS; 29, 3-hydroxy-sebacic tri TMS; 31, 3-hydroxy-dodecadienedioic tri TMS; 32, 3-hydroxy-dodecenedioic tri TMS; 33, 3-hydroxydodecenedioic tri TMS; 34, 3-hydroxy-dodecanedioic tri TMS; 37, 3-hydroxy-tetradecadienedioic tri TMS; 38, 39, 3-hydroxy-tetradecentedioic tri TMS; 40, 3-hydroxy-tetradecanedioic tri TMS; Ph = phosphoric tri TMS. (Reproduced with permission from Lefevere MF, Verhaeghe BJ, Declerk DH *et al.* (1989) Metabolic profiling of urinary organic acids by single and multicolumn capillary gas chromatography. *Journal of Chromatographic Science* 27: 23.

metabolic disorders, e.g. hydroxyproline in collagen metabolism, and γ-carboxyglutamate in blood coagulation and bone metabolism. These compounds are converted to *N*-isobutyloxycarbonyl methyl derivatives prior to measurement.

Prostaglandins, which are indicators of several diseases, are a class of acidic biomolecules whose measurement in biomatrices still presents a formidable analytical challenge. They are present in urine at concentrations as low as few pg mL^{-1} and require the quantification of several structurally closely related compounds. The difficulties are further compounded by their extreme sensitivity to acids, bases and oxygen. The determination of prostaglandin E_2 has been achieved by negative ion chemical ionization (NICI)-GC-MS following methylation and derivatization of other functionalities. There are several methods reported for the determination of other prostaglandins by isotope dilution GC-MS. GC-MS has been of immense utility in elucidating the role of γ-aminobutyric acid as a neurotransmitter via its ^{15}N-labelled derivative. Catecholamines and their acidic metabolites such as homovanillic, vandillomandelic, 5-hydroxyindole-3-acetic and phenylacetic acids, are implicated as etiological factors in affective disorders. They have been determined by NICI-GC-MS via acetyl-PFB derivatives and by isotope dilution GC-MS. In clinical research, GC-MS has proved invaluable for pharmacokinetic studies of therapeutic drugs with acidic functionalities. Such studies have been performed on methylphenidate, which is used in the treatment of children suffering from hyperkinesia, and on the butyl ester-trifluoroacetyl derivative of isotopically labelled histidine in investigations of the hereditary metabolic disorder histidinaemia. Another application is the analysis of the anti-inflammatory drug biphenylacetic acid in urine and synovial fluid by NICI-GC-MS-MS via its PFB ester. Some therapeutic drugs can lead to a build-up of toxic metabolites that must be monitored. This is exemplified by GC-MS analysis of patients' urine and plasma for 2-*n*-propyl-4-pentenoic acid, which is a product of the antiepileptic drug valproic acid.

In analytical microbiology, GC of fatty acids provides a basis for microbial chemotaxonomy and a means of identifying genus, species and even strains of microorganisms (**Figure 4**). The compounds profiled may be the nonvolatile C_{10}–C_{20} fatty acids present in cell membranes or the volatile acids which accumulate in the headspace. The extraction of the nonvolatile fatty acids and their derivatization to alkyl esters have been simplified by commercially available automated systems. Fatty acid profiles have permitted identification of pathogenic bacteria and even strains of yeast.

The realization that the enantiomers of a chiral compound may exhibit different bioactivities has prompted pharmaceutical and other industries to

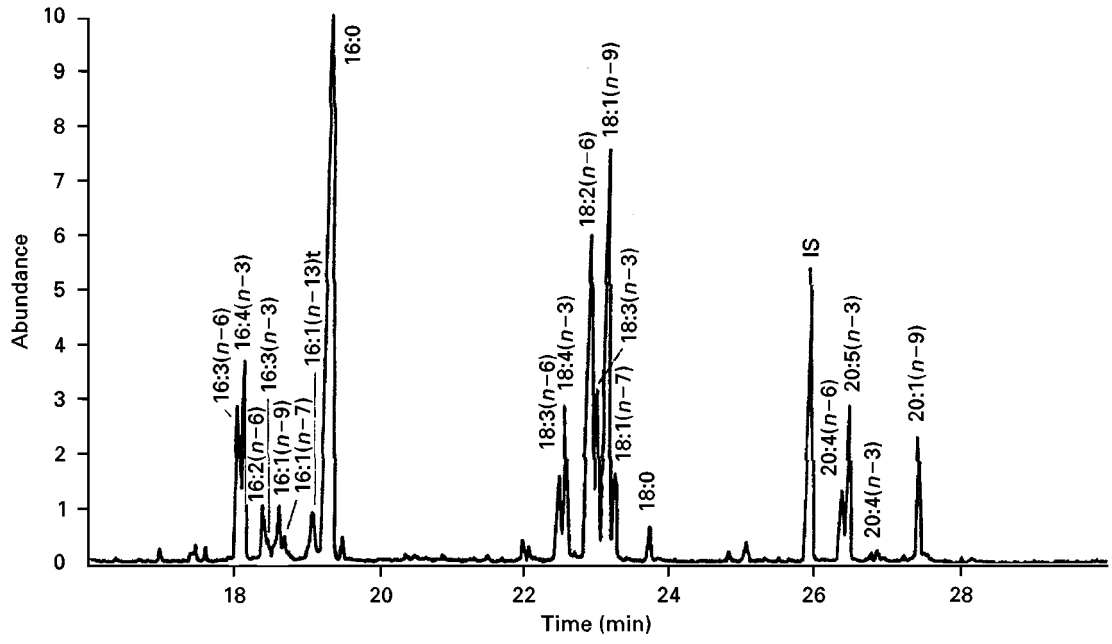

Figure 4 Reconstructed chromatogram of fatty acid methyl esters from the unicellular alga *Tetraselmis suecica* obtained by GC-MS. Chromatographic conditions: 50 m × 0.20 mm i.d. methylsiloxane fused capillary column; column temperature, initially at 40°C for 1 min, increased to 120°C at 30°C min^{-1} and then to 310°C at 4°C min^{-1}; helium carrier gas. (Reproduced with permission from Volkman JK, Jeffrey SW, Nichols PD *et al.* (1989) Fatty acid and lipid composition of ten species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology* 128: 219.

ascertain the optical purity of products and the metabolic fate of each enantiomer. As a result, industries and regulatory bodies have evinced interest in reliable methods for resolving optically active compounds. In the particular case of chiral acids, GC has proved invaluable. This is clearly illustrated by the separation of the optical isomers of the common drug ibuprofen via diastereoisomeric esters, and by a group of anti-inflammatory drugs, arylpropionic acids, which are routinely monitored in biological fluids as their *R*(−)/*S*(+)-amphetamine derivatives.

The differentiation between biogenic and non-biological urinary carboxylic acids is vital in the forensic sciences to establish the use of illicit drugs. Cannabis is the most widely used illicit drug in the world. 11-Nor-Δ-9-tetrahydrocannabinol acid (THCA) is found in urine specimens of cannabis users at few ng mL^{-1} levels as a major metabolite of tetrahydrocannabinol. THCA may be detected in urine 4–6 days after use of marijuana and even up to a month in chronic users: its determination by GC, principally as the TMS derivative, has been the focus of much research. Benzoylecgonine, which is a carboxylic acid produced by de-esterification of cocaine at physiological pH and temperature, and ecgonine methyl ester are the major metabolites that appear in the urine of cocaine users. Both are analysed by either GC-ECD or GC-FID, after converting the acid to the TMS derivative.

Toxic haloacids are environmentally significant and may be present in drinking water and other beverages. They are monitored by GC-MS or GC-ECD as the methyl esters. Low concentrations of pesticide and herbicide residues contaminating fruits and vegetables present another health hazard, e.g. residues of the fungicidal metal salts of alkylene-*bis*-dithiocarbamic acids. These fungicides are first converted to CS_2 for analysis by headspace GC. Traces of some widely used acidic herbicides, such as chlorinated phenoxycarboxylic acids, are quantified in food samples by GC-MS as their methyl esters.

Carboxylic acids and derivatives are important flavour and aroma constituents of foods (**Figure 5**) and beverages. Volatile fatty acids that are present at low concentrations also contribute to organoleptic characteristics and can be determined by headspace GC in

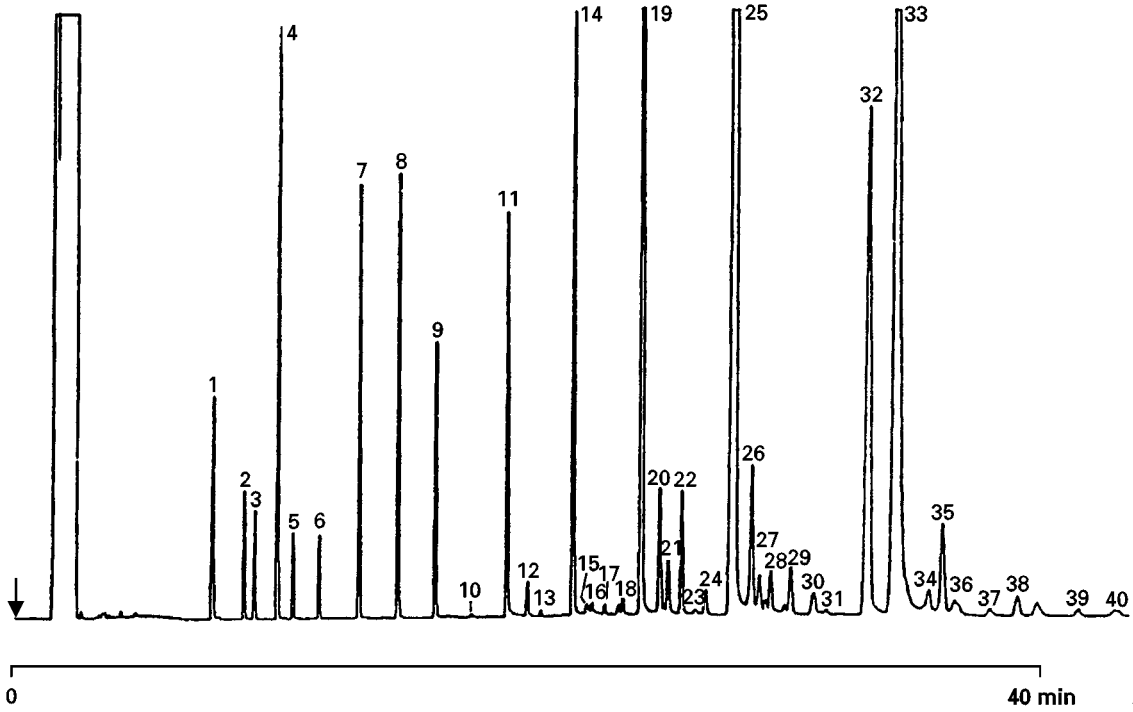

Figure 5 Gas chromatogram of free fatty acids (FFAs) from cheese spiked with an FFA reference mixture and short chain FFA (2:0, 3:0, 2-CH_3-3:0, 5:0, 3-CH_3-4:0 and 7:0). Chromatographic conditions: 25 m × 0.32 mm i.d. fused silica capillary column coated with FFAP-CB; oven temperature-programmed to increase from 65 to 240°C at a rate of 10°C min^{-1}; FID detector; helium carrier gas at a flow rate of 2 mL min^{-1}. Peaks: 1, C2; 2, C3; 3, 2-CH_3-C3; 4, C4; 5, 3-CH_3-C4; 6, C5;7, C6; 8, C7; 9, C8; 10, C9; 11, C10; 12, C10:1; 13, C11; 14, C12:0; 15, C12:1; 16, C13-iso; 17, C13:0; 18, C14-iso; 19, C14:0; 20, C14:1 + C15-iso; 21, C15-anteiso; 22, C15:0; 23, C15:1; 24, C16-iso; 25, C16:0; 26, C16:1; 27, C17-iso; 28, C17-anteiso; 29, C17:0; 30, C17:1; 31, C18-iso; 32, C18:0; 33, C18:1; 34, C18:2; 35, C18:2; 36, C19:0; 37, C18:3; 38, C18:2 conjugated; 39, C20:0; 40, C20:1. (Reproduced with permission from de Jong C and Badings, HT (1990) Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up and capillary gas chromatography. *Journal of High Resolution Chromatography* 13: 94.

underivatized form. Fatty acids containing unusual structural features, such as cyclopropane rings or epoxy groups, are constituents of some edible vegetable oils and are suspected of being health hazards. Hence they have been analysed in foods by capillary GC as FAMEs. Such studies have provided a basis for identifying components in blends of vegetable oils with potential application to detecting adulteration. Similar studies have been carried out to determine brominated acid constituents in vegetable oils that are added to disperse flavouring constituents in citrus-based beverages. Clinical and epidemiological findings of the beneficial effects of fish oils have led to GC methods, effected on polar capillary columns, for determining ω-fatty acids such as eicosapentaenoic and docosahexaenoic acids in foods. *Trans* isomers of fatty acids have a possible link with cardiovascular diseases. Hence the occurrence of *trans* isomers in relatively large concentrations in margarines, shortenings and similar food products has stimulated development of methods for resolving geometrical isomers. The solution of this problem is very difficult by GC alone and has required the use of very long capillary columns and preliminary separation steps. It may be cited as an existing challenge to GC in the analysis of acids.

Conclusion

GC continues to be the method of choice for the analysis of acids because of its speed, efficiency and sensitivity. However, very complex mixtures still pose serious challenges. Future developments may entail use of shorter, narrower capillary columns for greater speed and, in conjunction with routine MS detection, for more definitive identification. Automation of sample preparation, perhaps in conjunction with microwave irradiation in lieu of conventional heating, will shorten derivatization times, relieve the tedium of manual manipulations and reduce total analysis times.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry; Detectors: Selective. **III/Oils, Fats and Waxes:** Supercritical Fluid Chromatography. **Triglycerides:** Liquid Chromatography; Thin Layer (Planar) Chromatography. **Volatile Organic Compounds in Water:** Gas Chromatography.

Further Reading

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Liquid Chromatography

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Introduction

The determination of carboxylic acids is important in many areas of application, including environmental samples, foods and beverages, and pharmaceutical and biological materials. The modes of high performance liquid chromatography (HPLC) used most frequently in the separation of carboxylic acids are ion suppression chromatography, reversed-phase ion interaction chromatography, ion exclusion chromatography and ion exchange chromatography.

In ion suppression chromatography, a buffer of appropriate pH is added to the mobile phase in order to suppress the ionization of the carboxylic acids so that they can be retained on nonpolar stationary phases and eluted in order of increasing hydrophobicity. Ion interaction (or ion pair) chromatography has been used for the separation of carboxylic acids under isocratic or gradient conditions and involves the complete ionization of the solute and the addition to the mobile phase of an ion interaction reagent (IIR), consisting of lipophilic ions of opposite charge to the solute. Ion exclusion chromatography (i.e. the separation of partially ionized carboxylic acids on a cation exchange stationary phase using amperometry, coulometry, ultra-