Journal of Pharmaceutical Sciences

FEBRUARY 1976

VOLUME 65 NUMBER 2

REVIEW ARTICLE

Derivatization in Gas Chromatography

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Keyphrases □ GC—derivatization methodologies, applications in analysis of pharmaceutical compounds, review □ Derivatization—GC, methodologies, applications in analysis of pharmaceutical compounds, review □ Analysis—derivative formation in GC, review

GC is a valuable technique for analyzing compounds of pharmaceutical interest. The fact that a wide variety of compounds can be analyzed by GC has led to increased use of this technique. However, many pharmaceutical compounds cannot be gas chromatographed unmodified but must be converted into stable and volatile derivatives to achieve successful GC elution and separation. At times, derivatives are also prepared to achieve the desired sensitivity, selectivity, or specificity for a given separation.

GLC of pharmaceutical compounds is generally performed with the following objectives in mind: (*a*) determination of purity and stability of drugs, (*b*) determination of metabolic pathways and toxicology of drugs, (*c*) determination of pharmacokinetics of drugs, and (*d*) determination of drugs in tissues and fluids following overdosage in forensic situations.

All of these studies require that the methodology used should be sensitive and selective for the determination of the compound of interest. Since it is beyond the scope of this article to cover all approaches used for derivatization of pharmaceutical compounds, only those methods that meet the sensitivity and selectivity requirements are discussed. The reader may want to refer to some general references on the subject (1–10).

Pharmaceutical compounds cover a wide variety of different chemical classes and can be neutral, acidic, basic, or amphoteric in character. Furthermore, many of these compounds have high molecular weight and polyfunctional groups. Some functional groups make the compound very polar and reduce its volatility. Such a compound exhibits a long retention time or does not elute from a column. By derivatizing some or all of the functional groups, polarity is decreased, volatility is increased, and the compound can be eluted from the GLC column in a reasonable period.

Derivatization invariably involves a chemical reaction. Hence, it is important to remember that one can obtain an appropriate derivative by selecting suitable reaction conditions; however, the derivative should be sufficiently volatile for GC analysis. The compound must have a vapor pressure of 60 Torr to be carried through the column. The normal range of molecular weights for compounds resolved by GC is 2–500, although derivatives of carbohydrates with molecular weights up to 1800 have been chromatographed (11).

Table I shows the relationship between the structure and volatility or boiling points of different classes of compounds. Even though all of these compounds have essentially the same molecular weight, their boiling points differ. The high boiling points observed for alcohols are due to greater hydrogen bonding in alcohols than in ethers, aldehydes, and hydrocarbons. Furthermore, the hydroxyl group is often responsible for tailing of compounds. The carboxyl, amino, and imino groups also cause some tailing because of an interaction with the GLC column.

Table I—Relationship between Structure and Boiling Point

Compound	Structure	Molecular Weight	Boiling Point
n-Pentane	$CH_3CH_2CH_2CH_2CH_3$	72	36°
n-Butyraldehyde	$CH_3CH_2CH_2CHO$	72	76°
n-Butyl alcohol	$CH_3CH_2CH_2CH_2OH$	74	118°
Ether	$CH_3CH_2OCH_2CH_3$	74	35°

On the other hand, compounds with the carbonyl group show less tailing than those with the above-mentioned groups.

Derivatization can reduce the tailing and improve the separation of two closely related compounds or can make possible a separation that could not be accomplished otherwise. For example, estrone, estradiol, and estriol separate poorly on a nonpolar column, but good separation can be obtained with the silyl derivatives (10). Derivatization also can convert a heat-labile compound to a heat-stable compound or can convert compounds that are too volatile for GC analysis to derivatives that yield good separation.

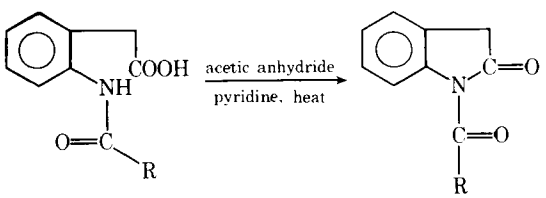
Specific derivatives of a compound can be prepared to increase the sensitivity of the resultant compound for a given detector. The flame-ionization detector in GC can readily detect 10^{-11} g and the electron-capture detector can detect down to 10^{-12} g of many compounds. Halogen derivatives are often used with an electron-capture detector. Phosphorus- or nitrogen-containing compounds also can be employed for the phosphorus or nitrogen detectors, respectively.

Some common derivatization procedures for various functional groups are shown in Table II.

DERIVATIZATION METHODS

For the purpose of this review article, the derivatization procedures of interest fall into eight classes: on-column reactions, reactions with dialkyl acetals, silylation, esterification, acylation, hydrazone formation, ion-pair formation, and derivatization for electron-capture detection. The methodologies, including limitations, are briefly discussed to alert the reader to the pitfalls of these techniques when he/she is investigating new approaches of derivatization.

As mentioned earlier, it is frequently necessary to derivatize some or all of the functional groups in a polyfunctional compound to decrease the polarity and to increase the volatility. Similar results can be obtained by the use of innovative approaches which lead to relatively nonpolar cyclic compounds (12). For example, substituted *o*-amidophenylacetic acid was cyclized readily to yield relatively nonpolar oxindole (Scheme I), which was gas chromatographed



Scheme I

with ease. GC–mass spectrometry was used to confirm that the cyclized product was being chromatographed.

Examples of better known cyclization reactions can be found in some fundamental textbooks of organic chemistry. For example, biguanides undergo the formation of diamine-*s*-triazines when acylated with acid anhydrides (13). Recently, this reaction was used for cyclizing phenformin, buformin, and metformin by reacting them with monochlorodifluoroacetic anhydride (14). This reaction is of interest because it provided a convenient way of introducing a functionality in a group that imparts structural stability, volatility, and sensitivity of detection.

On-Column Reactions—Column interaction or incomplete partition of a compound on the GLC column can cause tailing of chromatographic peaks. Interactions with the solid support or incomplete partition on a GLC column can be eliminated by saturating the carrier gas with the sample vapors. This procedure then leads to negative peaks, when a thermal conductivity detector is used, which can be used to quantitate trace amounts of sample (15).

The addition of formic acid vapor to the carrier gas improved the analysis of fatty acids by GLC (16); similar results were also obtained with barbiturates (17, 18). This effect may be attributed to decreased adsorption of a test substance on the column support by formic acid. The column deactivation can result from the formation of a derivative of the barbiturate with formic acid or the interaction of the formic acid with the support material (19). These results indicate that on-column reactions can be used as a simple means of preventing column interactions.

On-column pyrolysis is another simple approach which can be used for quantitative analysis of pharmaceutical compounds such as menadione sodium bisulfite (20). Differential thermal analysis, mass spectrometry, and GLC retention data were used to prove conclusively the formation of menadione after on-column pyrolysis.

Flash heater methylation of barbiturates (21, 22), *Cannabis* metabolites (23), phenytoin (24), phenylureas (25), and fatty acids (26) with trimethylanilinium hydroxide improves the GLC characteristics of these compounds. The derivatization employing an alkylammonium salt is now accepted as a method of choice for routine urine and serum analyses of barbiturates and related drugs (27).

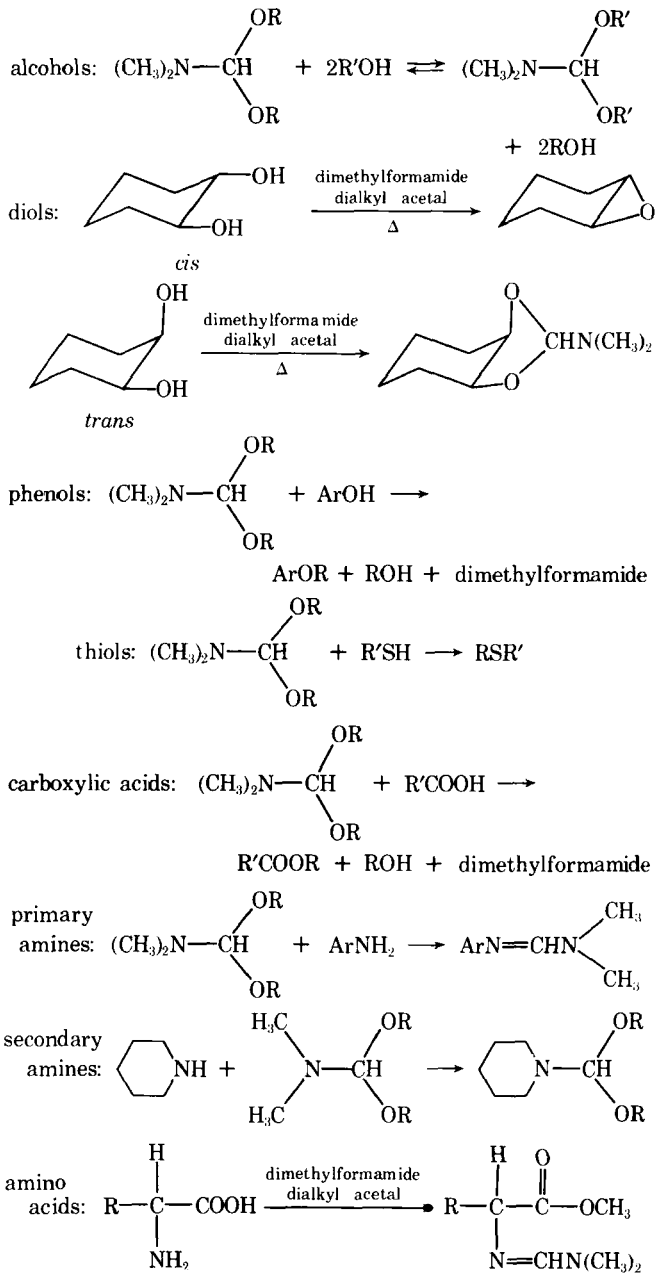
The formation of anisole, a heretofore unknown by-product, was reported in flash heater methylation experiments with trimethylanilinium hydroxide (28). A possible explanation for the formation of anisole is that the strongly alkaline trimethylanilinium hydroxide reagent is vulnerable to nucleophilic attack by the solvent in the injection heater. Since such a nucleophilic displacement should be enhanced by electronegative substituents in the *ortho*- or *para*-position of the aromatic ring, methanolic solutions of *N,N,N*-trimethylnitroanilinium hydroxide and *N,N,N*-trimethylchloroanilinium hydroxide were used for on-column methylation experiments. In these cases, nitroanisole (95%) and chloroanisole (8%) were the re-

Table II—Derivatives Commonly Employed in GC

Group(s)	Derivative
—OH	Alkyl ester Alkyl ether Silyl ether
—COOH	Alkyl ester Silyl ester
—NH_2	Acyl derivative Silyl derivative
=NH	Silyl derivative
—C(=O)—	Hydrazone Oxime
—C(=O)—H	Dimethyl acetal
—C(=O)—NH_2	Acyl derivative *O*-Methoxyl *N*-Alkyl Nitrile
—S(=O)—NH_2	Methyl derivative
—N^+—	Desmethyl derivative
—COOR	Methyl ester (transesterification) Derivative from reductive acylation
—SH	Thioether Silylthioether
—SO_2H	Sulfonyl chloride
—NH_2 and —OH	*n*-Butyl boronate
—NH_2 and —COOH	Acyl ester or amide *N*-Dimethylaminomethylene alkyl ester
—OH and —COOH	*n*-Butyl boronate Acyl ester Diester

action products. Preparations of *N,N,N*-trimethyl-*p*-chloroanilinium hydroxide in ethanol and propanol gave the corresponding ethers. The presence of the respective anisoles sometimes interferes with the GLC analysis of fatty and amino acids. The use of solutions of anilinium hydroxide and dimethylformamide was recommended in these cases (28).

Reactions with Dialkyl Acetals—Dimethylformamide dialkyl acetals are useful reagents because of the speed of derivatization. The reactions are generally complete as soon as solution is achieved. This one-step procedure can lead to the formation of methyl, ethyl, propyl, *n*-butyl, or *tert*-butyl derivatives. Some examples of the reactions of dimethylformamide dialkyl acetals are shown in Scheme II (29).

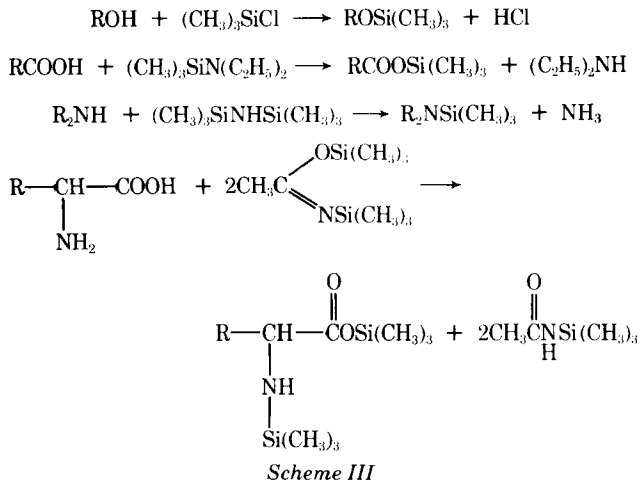


Scheme II

Silylation—A trimethylsilyl group can be readily introduced into a wide variety of organic compounds containing —OH, —COOH, —SH, —NH_2, and =NH groups. Thus, compounds amenable to silylation include alcohols, amides, amines, carbohydrates, carboxylic acids, phenols, steroids, sterols, and many other compounds with polyfunctional groups. It is important to remember that the general order of decreasing ease of silylation of the proton-active acceptor group is: alcohols (primary, secondary, and then tertiary), phenols, carboxylic acids, amines (primary, secondary, and then tertiary), and amides. The replacement of active hydrogen by the silyl group reduces the polarity of the compound and decreases the possibility of hydrogen bonding, with the resultant increase in volatility. Furthermore, stability is enhanced by reduction in the number of reactive sites.

The derivatives are simply and conveniently prepared in a few minutes by the following methods (30): trimethylchlorosilane-based methods, hexamethyldisilazane-based methods, silylimine methods, silylamide methods, and hexamethyldisilthiane or alkylthiotrimethylsilane methods. Some of the applications of silylation are illustrated in Scheme III.

Silylation is often looked upon with too much confidence as an unfailing means of improving GC separations. However, no technique is without hazards. Chambaz *et al.* (31) warned of the formation of enol trimethylsilyl ethers and oxysilylation products from steroidal ketones. When the same gas chromatograph is used for the analysis of phenols after the analysis of samples containing silylation reagent, both the phenol and its trimethylsilyl ether may be eluted (32). For example, when estrone is chromatographed



Scheme III

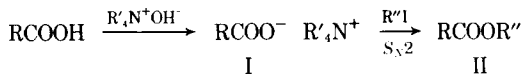
in a gas chromatograph–mass spectrometer 15 min after application of 10 μl of *N,O*-bis(trimethylsilyl)-acetamide to the column, mass spectral scans of the ascending, center, and descending portions of the resulting peaks demonstrate that it has two components: estrone (molecular ion *m/e* 270) and estrone trimethylsilyl derivative (molecular ion *m/e* 342).

Diethylstilbestrol has been commonly chromatographed as its trimethylsilyl derivative (33–36). Diethylstilbestrol occurs nearly exclusively in the *trans*-form. When a sample of *trans*-isomer is dissolved in organic solvent, such as ethyl acetate, and an aliquot is treated with *N,O*-bis(trimethylsilyl)-acetamide after removal of the solvent, GC of the reaction mixture indicates two components. Isomerization of *trans*-diethylstilbestrol to a mixture of the *cis*- and *trans*-isomers is known to occur in a number of common organic solvents (35, 36), and it has also been demonstrated that *cis*-diethylstilbestrol may be formed during trimethylsilylation (6).

Esterification—Esterification is an extremely valuable procedure for the alkylation of acidic compounds to increase volatility for GC analysis and to protect the acidic group during other reactions.

A mixture of a commercially available boron trifluoride etherate and an alcohol functions as an effective reagent in the direct esterification of some aromatic carboxylic acids bearing additional functions (*e.g.*, —OH, —NH_2, >C=O, —O—, and —S—). Unlike the Fischer esterification procedure where strongly acidic conditions prevail, the boron trifluoride etherate–alcohol reagent is unique in that it is both mild and effective. It esterifies the carboxyl group without affecting the other functionality in the molecule or the stability of the acid itself. Furthermore, it satisfactorily meets the esterification requirements of more different classes of carboxylic acids than does any other single reagent (37).

The amount of boron trifluoride etherate to be used has to be determined by the nature and number of functional groups present. Groups such as —OH, —NH_2, and >C=O each require an additional equivalent of boron trifluoride etherate for complex formation. Thus, for 1 mole of sodium *p*-aminosalicylate, 3 moles of boron trifluoride etherate should be employed for good results. When only 1 mole of the reagent is used, the yield of ester drops from 84 to 15%. However, in the case of *o*-aminobenzoic acid, 2 moles of the reagent seems to be sufficient (37).



Scheme IV

A new method of esterification for stearic acid overcomes many problems common to traditional esterification reactions (38). The reaction utilizes extremely mild conditions and nonacidic reagents, is complete for analytical purposes in less than 10 min, and can be used to prepare a variety of esters for an acidic compound. In essence, the reaction employs an organic base such as tetramethylammonium hydroxide and a highly polar solvent system.

When the acidic compound is dissolved in the alkali–solvent system, a soluble salt (I) is immediately formed (Scheme IV). In the solvent system used (*N,N*-dimethylacetamide–methanol), the anion of the soluble salt and any primary alkyl iodide undergo a rapid S_N2 reaction to form the corresponding ester (II). Phenyltrimethylammonium hydroxide was found to be the best organic alkali for GLC derivatization, both because its salts have a high solubility in the organic solvent system and there is no precipitation of the corresponding iodide in the concentration ranges used. The generality of this reaction was demonstrated by reacting a number of alkyl iodides with steric acid salts of tetramethylammonium hydroxide and phenyltrimethylammonium hydroxide. A yield of greater than 98% was observed with each primary iodide, regardless of the alkali employed. This method circumvents the common difficulty of GLC analysis of bile acid methyl esters from a physiological system since interference is caused by massive amounts of cholesterol. Since the bile acid butyl esters prepared by this technique are eluted later than methyl esters, they are easily separated from cholesterol (38).

After comparing various esterification techniques for (2,4-dichlorophenoxy)acetic acid, it was concluded that the reagent of choice for producing a high yield of GLC pure ester is boron trifluoride–alcohol. The diazomethane-based method produces many impurities. Mineral acid catalysis generally produces some impurities. Silyl esters are unstable and are very sensitive to moisture (39).

A reagent mixture of heptafluorobutyric anhydride, pyridine, and ethanol was used to esterify carboxyl groups with the alcohol and to derivatize hydroxyl and amine groups with the anhydride. Diesterification of hydroxy acids was confirmed by mass spectrometry (40).

Acetylation—Direct acetylation is usually possible for drugs having an alcoholic or phenolic hydroxyl group and for drugs that are primary or secondary amines. The acetylating agent is acetic anhydride, and derivatization can be carried out either in final solvent or directly on the GLC column. Acetylation was found to be the technique of choice when analyzing specimens of blood for amphetamines in therapeutic or toxic ranges, since the parent compounds

may not be detectable at these levels. In urine specimens, however, where these drugs are usually more concentrated, both the parent drugs and their acetyl derivatives often can be chromatographed, thus providing the analyst with one more criterion for identification (41). However, care must be exercised in determining the nature of acetylation reactions since anomalous reactions can occur (42). This technique also is discussed under electron-capture detection.

Hydrazone Formation—Carbonyl compounds can be analyzed by GLC as free compounds, as oximes (43), as 2,4-dinitrophenylhydrazones (44, 45), or as phenylhydrazones (46). Korolczuk *et al.* (46) indicated that the determination of carbonyl compounds as their phenylhydrazones seems to be easier than the determination of their 2,4-dinitrophenylhydrazones because the preparation of derivatives is simpler and less time consuming and their separation from aqueous solution by extraction with ether is very easy, rapid, and quantitative. It is possible to calculate by interpolation the exact position of each peak on the chromatogram for *n*-aldehydes, 2-alkanones, and, probably, other carbonyl compounds such as cyclic ketones, branched ketones, and aldehydes.

Ion-Pair Formation—An interesting variation on derivatization was achieved by the use of ion-pair complexes in GC (47). Ion-pair complexes of the base–sulfonic acid type decomposed under GC conditions to give the base. The method was applied to the analysis of five basic drugs: tripelennamine, dimethindene, chlorpromazine, meperidine, and methylphenidate. The principle is also applicable to the extraction and determination of acidic substances.

Ion-pair chromatography also can be used to increase the sensitivity of the method. Chlorthalidone is selectively converted to its tetramethyl derivative by extractive alkylation on the basis of extraction of the ion-pair with the quaternary ammonium ion into methylene chloride in the presence of methyl iodide. The derivative thus formed is very sensitive to electron-capture detection and can be determined down to 2 ng/ml of plasma (48).

Derivatization for Electron-Capture Detection—Silylation in conjunction with a flame-ionization detector can yield detection levels of 2–5 ng for the neuroamines (49); however, the use of electron-capture detection and mass fragmentography has brought the possibility of quantitative evaluation down to the level of a few picograms (50–52). For this purpose, the amines and related products have been converted to trifluoroacetyl (50–52), heptafluorobutyryl (51–53), pentafluoropropionyl (51, 53, 54), and perfluorobenzyl (55) derivatives.

One study compared electron-capture sensitivity with various derivatives (*e.g.*, heptafluorobutyramide, pentafluorobenzamide, and pentafluorobenzylidine) of some clinically important primary and secondary amines (56). The sensitivity and selectivity of the analysis of amines were greatly enhanced by formation of an appropriately halogenated derivative. Generally, the order of electron-capture sensitivity for the derivatives of primary amines is: pentafluorobenzamide > pentafluorobenzylidine > heptafluorobutyramide. The electron-capture sensitivities of various derivatives of amphetamine are shown in Table III.

Heptafluorobutyramides have been prepared for a large number of amino drugs. The electron-capture responses of various heptafluorobutyramides are shown in Table IV.

N-Trifluoroacetyl-L-prolyl derivatives have been used to separate the enantiomeric mixtures of various chiral compounds by GLC. Westley and Halpern (57), who separated many enantiomers, rationalized their results in terms of a configuration rule predicting that *N*-trifluoroacetyl-L-prolyl derivatives of the *R* enantiomer usually have a shorter retention time than the corresponding diastereoisomer. A method for GLC separation of some ephedrines and pseudoephedrines by means of their *N*-trifluoroacetyl-L-prolyl derivatives was described (58), and the results confirmed and extended the correlation. The configuration of the carbons (carbon alpha to the reaction nitrogen atom) controls the relative retention time irrespective of the configuration of C-1.

Attempts have been made to improve the GC properties of tertiary amines by derivatization. Thus, amitriptyline was methylated and a Hoffman degradation to an olefin then was performed; better GC separations were obtained (59, 60). *N,N*-Dimethyldibenzo[*b,f*]thiepin-10-methylamine gave, with ethyl chloroformate, the corresponding chloromethylene derivative, which was determined by electron-capture GC (61). A third approach is illustrated in Scheme V (62, 63).

The tertiary amine is transformed to carbamate by reaction with alkyl chloroformate. By hydrolysis the carbamate gives a secondary amine, which is acylated. The resultant heptafluorobutyramide is determined by electron-capture GC with good precision in the nanogram range according to well-established methods (64).

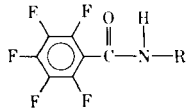
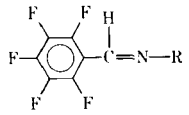
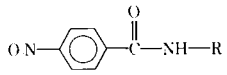
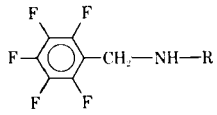
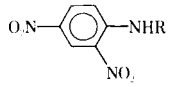
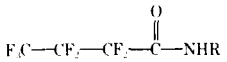
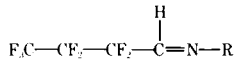
In studies of the reaction of several alkyl chloroformates with tertiary amines, the carbamates were formed in about the same yield irrespective of the alkyl substituent. The carbamates did not exhibit the adsorptive properties of amines. This finding was the basis for determining nanogram amounts of amines (65). A comparison of three benzyl chloroformates—*viz.*, unsubstituted and *p*-nitro and pentafluoro substituted, revealed that pentafluorobenzyl chloroformate was the most useful reagent.

Some commonly employed derivatization procedures used for electron-capture detection are summarized in Table V.

APPLICATIONS

Derivatization GC has been used for analysis of many classes of pharmaceutical compounds. For the purpose of this review article, the pharmaceutical compounds are classified as follows: acids (including amino acids and miscellaneous carboxylic acids); alkaloids; amides, imides, and related compounds; amines; antibiotics; azepines; barbiturates and other

Table III—Electron-Capture Sensitivity of Various Amphetamine Derivatives (56)

Compound	Structure[a]	Response, coulombs x 10^3/mole
N-Pentafluorobenzamide		225
N-Pentafluorobenzylidine		43
N-*p*-Nitrobenzamide		11
N-Pentafluorobenzylamine		3.7
N-2,4-Dinitroaniline		2.8
N-Heptafluorobutyramide		2.2
N-Heptafluorobutyrylidine		2.1
N-Heptafluorobutylamine		0.41

[a] R = $C_6H_5CH_2CH(CH_3)$.

urea derivatives; esters; hydroxy compounds and mercaptans; steroids; vitamins; and miscellaneous compounds. This classification will be helpful in understanding derivatization GC because derivatization is dependent not only on the number, nature, and stereochemical arrangement of the functional groups but also on the size and shape of the molecule (8).

Amino Acids—Derivatization and an analytical technique resembling GC was used in 1906 by Fischer for separation of amino acids as ethyl esters. Some other methods or reagents that have been used over the years with limited applications are (83): triketohydrindene oxidation with GLC of resultant aldehyde, α-chloroamino acid methyl esters, 2,4-dinitrophenylamino acid methyl esters, *N*,*N*-dimethylamino acid methyl esters, *N*-diethylphosphate amino acid methyl esters, alkylidine and alkylamino acid esters, and phenylthiohydantoin amino acids.

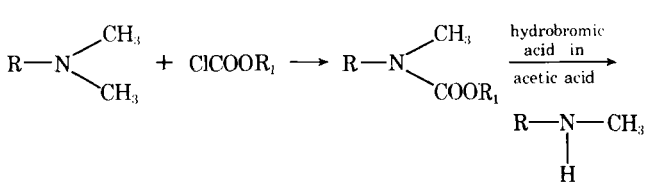
Silylation with several reagents has been used extensively for preparation of volatile derivatives of amino acids (83). *N*,*O*-Bis(trimethylsilyl)acetamide was found to be a useful reagent for quantitative analysis of methyldopa (III) and related amino acid impurities (84). This reagent was used earlier for silylating levodopa (IV) at ambient temperature (85). A silylation procedure has been used for the quantitative determination of liothyronine and thyroxine in dried thyroid (86).

Dual derivatization methods based on esterification of the carboxyl group and acylation of amino and other functional groups have been found to be useful in the analysis of amino acids. Some of these methods are summarized in Table VI.

The ease of reaction depends on the length of the alcohol chain, the amino acid being used, the amount of catalyst, temperature, and the quality of reagents. Methylation takes place in 30 min at room temperature, but increasing time and/or temperature are required through the methanol to pentanol series (91). Acylation reactions are generally carried out with acid anhydrides, often in the presence of pyridine. In addition to the primary amino (or imino) groups, hydroxyl, secondary amino, thiol, imidazole, guanidine, and indole groups can also be acylated.

Table IV—Electron-Capture Response of Various Heptafluorobutyramides (56)

Amine	Response, coulombs x 10^3/mole
β-Phenethylamine	2.0
Amphetamine	2.2
α-Methylbenzylamine	2.5
p-Methoxyphenethylamine	1.9
Mescaline	3.7
Phentermine	1.1
Phenmetrazine	8.8
Mephentermine	2.2
Methamphetamine	4.0
Methoxyphenamine	1.6



Scheme V

Table V—Derivatives Commonly Employed for Electron-Capture Detection (66)

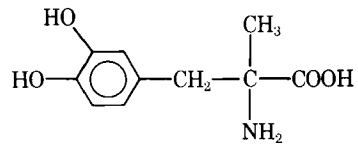
Group	Derivative	Example	Reference
—OH	Heptafluorobutyrate	Perphenazine, digoxin	67, 68
	Trichloroacetate	Warfarin	69
	Trifluoroacetate	Warfarin, testosterone	69, 70
	Pentafluorobenzyl ether	Pentazocine	71
	Monochlorodifluoroacetate	Testosterone	70
$—NH_2$	Trifluoroacetamide	Amphetamine	72
	Pentafluoropropionamide	β-Phenethylamine	73
	Heptafluorobutyramide	Phentermine	56, 74
	Pentadecafluorooctanoate	β-Phenethylamine	73
	Pentafluorobenzylamine	Phenmetrazine	56
	Trichloroacetamide	Amphetamine	75
	Pentafluorobenzylcarbamate	Diphenhydramine	65
$—NH_2$, —OH (aliphatic)	Heptafluorobutyramide, heptafluorobutyrate	Pseudoephedrine	76
	Pentafluorobenzoyl ester	Tryptophol	73
$—NH_2$, —OH (aromatic)	Trifluoroacetamide, trifluoroacetate	Epinephrine	77, 78
	Pentafluorobenzylimine	Dopamine	79
—COOH	Trifluoroacetate	Bile acid	80
	Pentafluorobenzoyl ester	Aliphatic carboxylic acid	81
—C=O	Pentafluorophenyl hydrazone	Estrone	82

Dimethylformamide dialkyl acetals react with both carboxylic and amino groups of amino acids to form *N*-dimethylaminomethylene alkyl ester derivatives. The reaction is rapid and complete at 100°, and hydroxyl groups do not react under these conditions (92). Butler and Darbre (93) found that the relative molar response for the trifluoroacetyl ester of 14 amino acids with a nitrogen-sensitive thermionic detector was much greater than with a flame-ionization detector (Table VII).

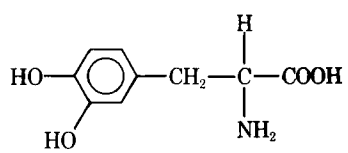
In a weakly alkaline nonaqueous medium, a condensation reaction proceeds between dichlorotetrafluoroacetone and an α-amino acid. A low polarity cyclic derivative results and can be analyzed by GC (Scheme VI) (94). Substitution of X with —NH, —O, or —S resulted in 2,2-bis(chlorodifluoromethyl)-4-substituted 1,3-oxazolidine-5-one (V), -dioxolan-5-one (VI), or -oxathiolan-5-one (VII), respectively.

GC also was found to be the method of choice for analysis of mixtures of nonprotein amino acids (95). Sufficient volatility of the amino acids was obtained by a dual derivatization procedure—*viz.*, esterification of the carboxyl group and acylation of the amino group.

The inner esters of acylamino acids, azalactones, or oxazoline-5-ones provide useful derivatives for analysis. The analysis of α-methylamino acids as the corresponding 2-phenyloxazoline-5-ones, obtained by *N*-benzoylation of the amino groups followed by inner ester formation by dicyclohexylcarbodiimide (Scheme VII), was recently reported (96).



III



IV

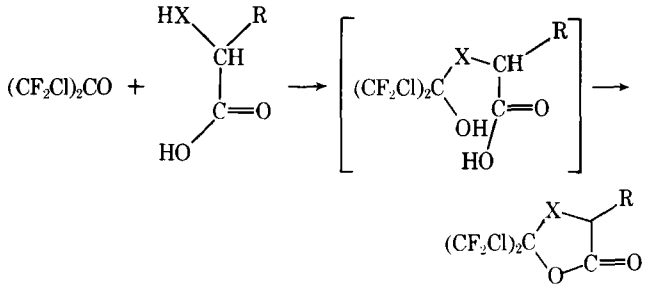
Miscellaneous Carboxylic Acids—Free fatty acids can be determined directly on a phosphoric acid LAC-2R-446 column (97); however, they are most frequently determined as methyl esters. Methylation of carboxylic acids by thermal degradation of their quaternary ammonium salts in the injection port of a gas chromatograph was first accomplished by Robb and Westbrook (98); they injected tetramethylammonium hydroxide solutions of a number of acids and obtained chromatograms of methyl esters. Oakes and Willis (99) found trimethyl(α,α,α-trifluoro-*m*-tolyl)ammonium hydroxide useful for methylation of urinary carboxylic acids.

Ester formations using the ketal, 2,2-dimethoxypropane (100), anhydrous methanol–hydrogen chloride (101), and methanol–boron trifluoride (102) have been recommended. Methylation of fatty acids with diazomethane is hazardous (103). However, esterification with diazomethane has been reported by a technique that does not require esterification external to the chromatographic system (104). Thus, distilled diazomethane in ether appears promising as a reagent for esterification and analysis of fatty acids of moderate molecular weight using a precolumn reactor.

The methyl, ethyl, *n*-propyl, *n*-butyl, or *tert*-butyl esters of long chain fatty acids can be prepared quantitatively by reaction with dimethylformamide dialkyl acetals. A GC separation as soon as solution is achieved was reported (105). The mechanism of reaction is believed to involve the carboxylate ion and an alkoxy carbonium ion from the reagent (Scheme VIII) (106).

Table VI—Acyl Esters of Amino Acids

Ester	Acyl Derivative	Reference
Methyl	Acetyl/trifluoroacetyl	87
Ethyl	Acetyl/trifluoroacetyl	88
n-Propyl	Acetyl	88
n-Butyl	Acetyl	89
n-Butyl	Pentafluoropropionyl/ heptafluorobutyryl	90



Scheme VI

The USP XVIII (107) method for analyzing aspirin tablets involves time-consuming column chromatography followed by separate spectrophotometric determination of aspirin and salicylic acid. GC methods that do not involve derivatization (108) show considerable tailing of the salicylic acid peak. Therefore, a method of analysis based on silylation with *N,O*-bis(trimethylsilyl)acetamide for aspirin, salicylic acid, and acetylsalicylsalicylic acid was proposed (109). The last compound was detected in varying amounts in tablets and granulations of different manufacturers.

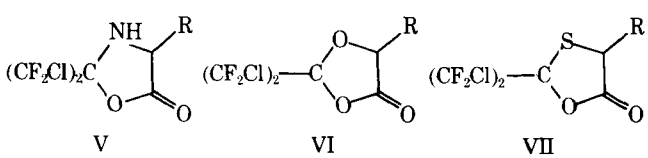
Another method based on methylation was reported for the determination of traces of salicylic acid in aspirin tablets and other preparations containing phenacetin and caffeine (110). A method that utilizes esterification of salicylic acid with diazomethane was claimed to be precise for the estimation of salicylic acid in codeine and propoxyphene-type capsule and tablet analgesic formulations (111).

Aristolochic acid (VIII) can be analyzed by on-column methylation with trimethylanilinium hydroxide with a sensitivity limit of 1–5 μg/ml (112). β-Hydroxybutyric acid can be chromatographed as the methyl ester, and techniques for determining this compound in urine (113) and in blood and milk (114) were reported. Hydroxybutyric acids (α, β, and γ) were resolved after esterification with heptafluorobutyric anhydride on an OV-1 or Tabsorb column. The method is sensitive in the nanomole range (115).

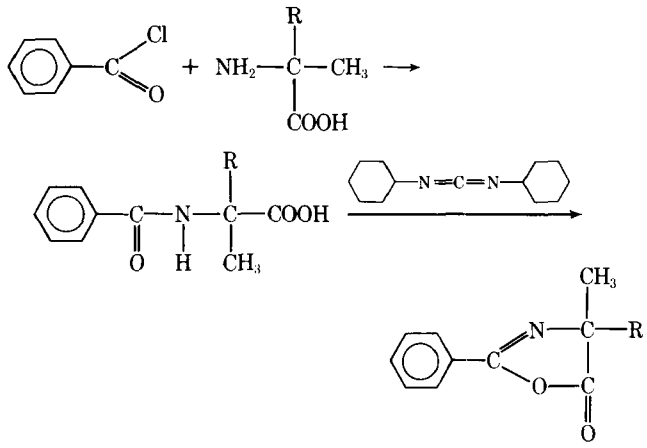
1,1′-Carbonyldiimidazole is a useful reagent for facile esterification of free fatty acids. This reagent was used for almost instantaneous esterification of ibuprofen (IX) with sensitivity down to 0.5 μg/ml of plasma (116). The pentafluorobenzyl ester of flurbiprofen (X) yields a sensitivity of 0.05 μg/ml in plasma by electron-capture GC (117).

Flash heater methylation (118) was used for derivatizing bumetanide (XI) (Scheme IX). The GLC method was found to be accurate at concentrations as low as 0.1 μg/ml of human urine.

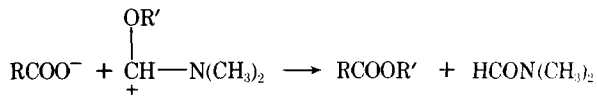
Probenecid (XII) was converted to the methyl ester for GLC analysis by reaction with diazomethane. The sensitivity of the method is 250 ng/ml of plasma (119). Ethylation with diazoethane (120) provided the basis for determination of indomethacin



V VI VII



Scheme VII



Scheme VIII

(XIII) by GC. The ethylation was essentially instantaneous and considerably improved the chromatographic response of indomethacin.

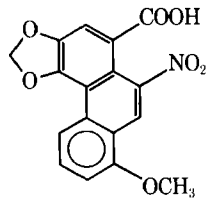
Alkaloids—Windheuser *et al.* (121) noted that the USP XVIII (107) method for the analysis of scopolamine (XIV) is not directly useful when all degradation products of scopolamine must be quantitatively determined. They reported a method based on trimethylsilylation. It can successfully separate and quantitatively determine the drug and its degradation products such as scopoline (XV), atropic acid (XVI), and tropic acid (XVII).

Silylation of the mandelic acid (XVIII) resulting from alkaline hydrolysis of homatropine methylbromide (XIX) was found to be suitable for the analysis of tablets and elixirs containing this compound (122).

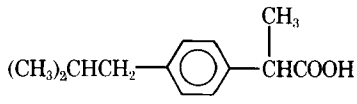
A GC method was reported for opiates using trimethylsilyl derivatives (123). The narcotic analgesics were extracted with organic solvents from urine, blood, and bile and reacted with *N,O*-bis(trimethylsilyl)acetamide or bis(trimethylsilyl)trifluoroacetamide. The detectability of morphine from urine and blood was 0.1 μg/ml. Codeine, hydromorphone, methylhydromorphone, morphine, 3-monoacetylmor-

Table VII—Ratio of GLC Responses of Thermionic Detector (TD) to Flame-Ionization Detector (FID) for Trifluoroacetyl Amino Acid Methyl Esters (93)

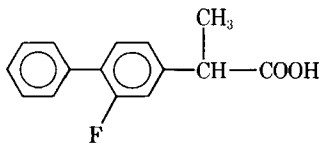
Amino Acid	Ratio of Response, TD/FID
Alanine	16.6
Aspartic acid	9.1
Glutamic acid	5.9
Glycine	21.9
Hydroxyproline	8.3
Isoleucine	4.8
Leucine	5.7
Methionine	4.6
Norleucine	5.0
Phenylalanine	3.5
Proline	8.8
Serine	11.7
Threonine	7.9
Valine	3.5



VIII



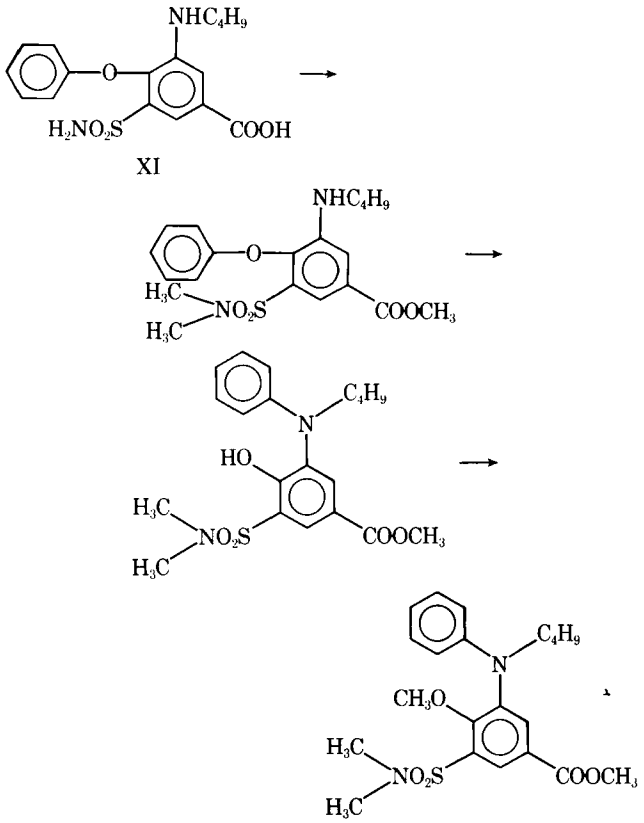
IX



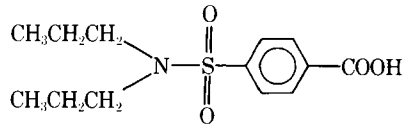
X

phine, 6-monoacetylmorphine, nalorphine, and oxycodone form trimethylsilyl derivatives.

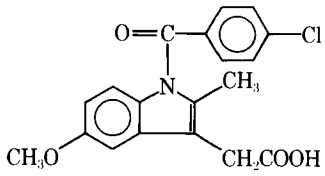
The GC analysis of ergonovine maleate involves derivatization with *N*-trimethylsilyldiethylamine and *N*-trimethylsilylimidazole in pyridine followed by chromatography on a methyl silicone column (124). Ergonovinine, lumiergonovine I, and brucine can be resolved by this method. Procedures for analyzing and studying the biosynthesis of labile hop bitter compounds by means of GC have been developed (125). Derivatives of desoxyhumulones, humulones, and lupulones, obtained by reaction with hexamethyldisilazane in dimethylformamide, can be chromatographed in an all-glass system without any notable



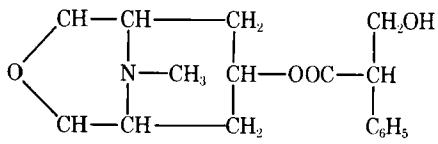
Scheme IX



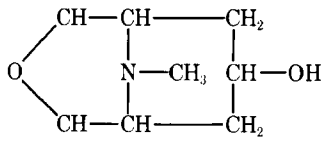
XII



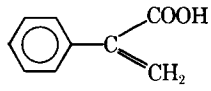
XIII



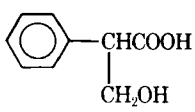
XIV



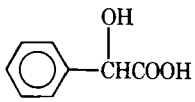
XV



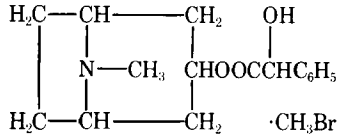
XVI



XVII



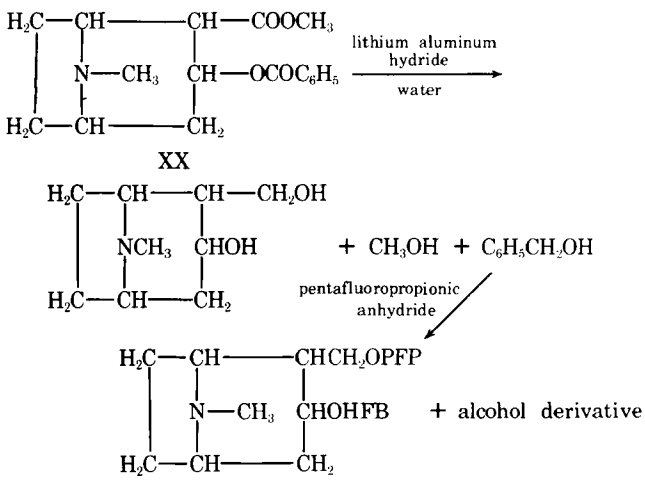
XVIII



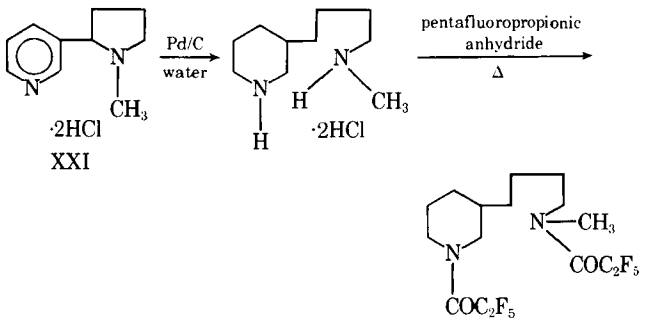
XIX

decomposition. A silylation procedure can be applied for the qualitative determination by GLC of active principles such as cannabichromene and cannabidiol in *Cannabis sativa* L. (126).

A method based on on-column methylation of quinidine with trimethylanilinium hydroxide was reported (127). Detection by flame ionization gives a linear response over the range of 0.2–12.0 μg of quini-



Scheme X



XXI

Scheme XI

dine/ml of plasma, with a 0.05-μg/ml limit of detectability. A method based on reduction and acylation of cocaine (XX) was proposed on the basis of Scheme X (128). With the pentafluoropropionic anhydride derivative, sensitivities of 20–30 ng/ml of sample could be achieved.

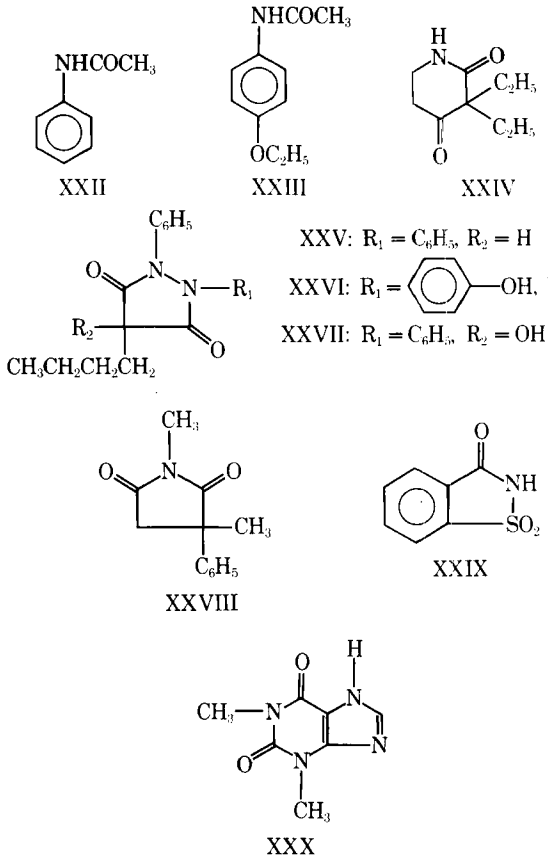
A unique determination method for nicotine in subpicomole quantities is based on catalytic hydrogenation of nicotine (XXI) to yield *N*-methyl-4-(3′-piperidyl)-*n*-butylamine (octahydronicotine). The two secondary amino functions then may be treated with a perfluoro acid anhydride to provide an electron-capture derivative. The dipentafluoropropionyl derivative can be readily quantitated in amounts corresponding to 0.03 pmole of nicotine as prepared by Scheme XI (129).

Amides, Imides, and Related Compounds—A GC method based on perfluoroacylation of amides was described (130). The method was found suitable for the analysis of *N*-decylacetamide, acetanilide (XXII), phenacetin (XXIII), and piperidione (XXIV). The minimum detectable quantity of the pentafluorobenzoyl derivative was 0.1 femtomole/sec by electron-capture detection.

Midha *et al.* (131) extended the GLC method for phenylbutazone (XXV) and its major metabolite, *p*-hydroxyphenylbutazone (oxyphenbutazone) (XXVI), to the estimation of the second major metabolite, γ-hydroxyphenylbutazone (XXVII). The method entails flash heater methylation and is sensitive down to 1.0 μg/ml. Flash heater methylation of γ-hydroxyphenylbutazone with trimethylanilinium hydroxide gives four peaks. Three of these peaks can be ascribed to the methylated breakdown products of γ-hydroxyphenylbutazone. Similarly, *p*-hydroxyphenylbutazone yields two peaks on flash heater methylation.

A GLC procedure based on on-column trimethylsilylation was described for chromatographing methsuximide (XXVIII) and its *N*-demethylated metabolite (132). The same silylation reagent was found useful for chromatographing saccharin (XXIX) in pharmaceutical products (133). Ingredients such as ascorbic acid, nicotinyl alcohol, aprobarbital, dextromethorphan hydrobromide, chlorpheniramine maleate, acetaminophen, and sulfamethoxazole do not interfere with the analysis.

Recently, Dusci *et al.* (134) reported an on-column methylation of theophylline (XXX) which matches the 1-μg/ml plasma sensitivity reported earlier (135). Theophylline was quantitatively converted to caffeine by on-column methylation. This technique was claimed to be more effective than the conventional techniques (136).



XXII XXIII XXIV

XXV: $R_1 = C_6H_5$, $R_2 = H$

XXVI: R_1 = p-hydroxyphenyl, $R_2 = H$

XXVII: $R_1 = C_6H_5$, $R_2 = OH$

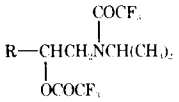
XXVIII XXIX

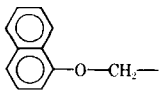
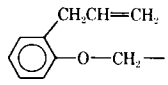
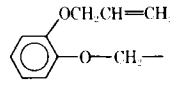
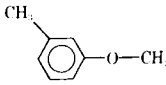
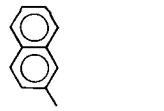
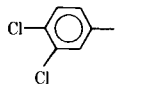
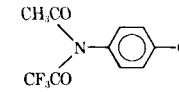
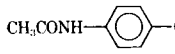
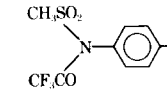
XXX

Amines—Tailing, peak asymmetry, and decomposition are some of the problems encountered in the GLC analysis of amines. Pretreatment of the stationary phase with sodium hydroxide can overcome or eliminate some of them (137, 138). However, derivatization provides a valuable alternative, since both separation and sensitivity can be improved significantly. Specific and sensitive methods are necessary for the determination of biogenic amines of physiological importance. The most commonly used derivatization procedures are based on formation of trimethylsilyl (139, 140), acyl (141), isothiocyanate (142), 2,4-dinitrophenyl (143), and enamine (144) derivatives. With some of these methods, detection levels of 2–5 ng have been achieved (140) using a flame-ionization detector. The use of electron-capture detection and mass fragmentography permits quantitation down to a few picograms (144–146). For this purpose, amines and related products have been converted to trifluoroacetyl (144–146), heptafluorobutyryl (145, 147), pentafluoropropionyl (145, 147, 148), and perfluorobenzoyl (149) derivatives.

The conditions of formation and stability of pentafluorobenzyliminetrimethylsilyl derivatives have been optimized to permit quantitation of catecholamines in the picomole range (150). The highest sensitivity was obtained with a pulsed electron-capture detector, which allows quantitation down to 10 pg of the primary amine of biological interest. A sensitive and specific electron-capture GLC method based on

Table VIII—Detectability for Trifluoroacetyl Derivatives of β-Blocking Agents (151)

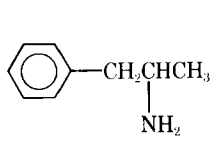


R	Compound	Detectable Amount, pg
	Propranolol	0.5
	Alprenolol	0.7
	Oxprenolol	1.1
	Toliprolol	0.9
	Pronethanol	0.6
	Dichloro-isoproterenol	0.1
	Practolol	0.7
	Practolol	0.7
	Sotalol	0.7

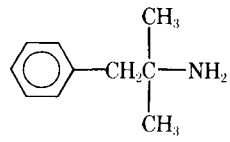
derivatization of both amino and alcoholic groups was reported for β-blocking agents including propranolol, oxprenolol, alprenolol, pronethalol, dichloroisoproterenol, practolol, sotalol, and several of their metabolites (151). The drugs were separated and detected as their trifluoroacetyl derivatives, and minimum detectable amounts ranged from 0.1 to 1.1 pg (Table VIII).

A method was reported involving the use of derivative reagents, such as trifluoroacetic anhydride and pentafluoropropionic anhydride, for the analysis of 1-(2,6-dimethylphenoxy)-2-propylamine (152). The flame-ionization detection of the derivative thus formed offered no advantages over electron-capture detection.

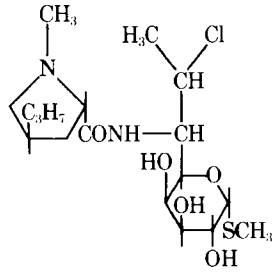
Derivatization of phenylephrine hydrochloride with trifluoroacetic anhydride combined with electron-capture detection permits sensitivities down to 12.5 ng/ml (153). A quantitative GC–mass spectrometry assay was used to determine the plasma and brain levels of amphetamine (XXXI) and phentermine (XXXII) as their *N*-trifluoroacetyl derivatives. The method was sensitive down to the 10-ng level (154).



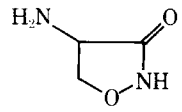
XXXI



XXXII



XXXIII



XXXIV

A GC method was described for the separation and identification of aliphatic amines in blood as their 2,4-dinitrophenyl derivatives (155). The secondary amines investigated (156) eluted more rapidly than the primary amines, even though they had higher molecular weights. This finding may be explained on the basis that the secondary amines have less tendency to form hydrogen bonds than the primary amines.

Antibiotics—GLC has been commonly used for the analysis of lincomycin, spectinomycin, and clindamycin and its esters and is potentially useful for other compounds (Table IX) (157).

Griseofulvin can be chromatographed directly; however, other antibiotics require derivatization. Thus, trimethylsilyl derivatives of lincomycin, spectinomycin, chloramphenicol, neomycins, erythromycins, penicillins, tetracycline, and cycloserine are made prior to analysis, whereas clindamycins are chromatographed as esters. Some derivatives that are frequently prepared for derivatization of antibiotics are summarized in Table IX.

GC–mass spectrometry of trifluoroacetylated clindamycin (XXXIII) indicates that it chromatographs intact as the derivatized free base containing three

Table IX—Derivatization of Antibiotics

Antibiotic	Derivatization Reagent	Reference
Clindamycin hydrochloride	Acetic anhydride	158
Clindamycin phosphate	Trifluoroacetic anhydride	158
Chloramphenicol	Hexamethyldisilazane	159
Cycloserine	*N,O*-Bis(trimethylsilyl)-acetamide, trimethylchlorosilane	160
Erythromycins	*N,O*-Bis(trimethylsilyl)-acetamide, trimethylchlorosilane, trimethylsilylimidazole	161
Kanamycin	Trimethylsilyldiethylamine	162
Lincomycin	Hexamethyldisilazane, trimethylchlorosilane	163, 164
Neomycin	Trimethylsilyldiethylamine	165, 166
Penicillins	Hexamethyldisilazane	167
Tetracyclines	*N,O*-Bis(trimethylsilyl)-acetamide, trimethylchlorosilane	168

Table X—Hydrolytic Products of Benzodiazepines

Benzodiazepine	Hydrolysis Product
Bromazepam	2-Amino-5-bromobenzoylpyridine
Chlordiazepoxide	2-Amino-5-chlorobenzophenone
Clonazepam	2-Amino-2′-chloro-5-nitrobenzophenone
Cloxazolam	2-Amino-7,2′-dichlorobenzophenone
Diazepam	2-Methylamino-5-chlorobenzophenone
Nitrazepam	2-Amino-5-nitrobenzophenone

trifluoroacetyl groups (169). The following four impurities were detected in a research lot of clindamycin hydrochloride: epilincomycin, lincomycin, clindamycin B, and epiclindamycin. Since derivatized clindamycin 2-phosphate could not be chromatographed satisfactorily, it was enzymatically hydrolyzed to clindamycin prior to derivatization and GLC.

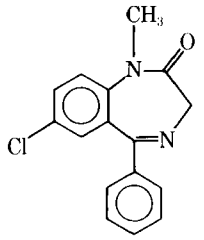
The quantitative determination of cycloserine (XXXIV) in pharmaceutical preparations has been done by the nitroprusside method, titrimetry, UV spectrophotometry, and microbiology. The chemical treatment and solvents required for the assay can cause formation of degradation products which may interfere with the assay (170). The silylation procedure involving the use of *N,O*-bis(trimethylsilyl)-acetamide and trimethylchlorosilane was found to be more specific, because the cycloserine dimer does not chromatograph under these conditions.

Amphotericin B, nystatin, and several members of the aromatic group of heptene antibiotics have been degraded chemically by various methods. Some of the resulting products after derivatization were separated and compared by GC for structure confirmation (171, 172).

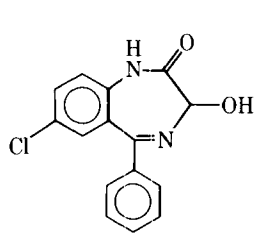
Azepines—Since the early 1960's, 1,4-benzodiazepine drugs have been established as widely used tranquilizers, sleep inducers, and muscle relaxants. With the extensive use of these compounds, there has been a parallel evolution of analytical methodology for their determination. Since they frequently have to be determined at low levels, chromatography is very widely used for determination of benzodiazepines (173). Many benzodiazepines are hydrolyzed by strong acids to give benzophenones, which are then used in chromatographic analysis. Some of the hydrolytic products of benzodiazepines (173) are summarized in Table X.

de Silva *et al.* (174) were the first to use hydrolysis products of diazepam (XXXV) for GC; these derivatives were found to be more volatile and their chromatography was easier than the parent compound. In 1968, Marcucci *et al.* (175) reported the chromatography of intact benzodiazepines; however, de Silva and Puglisi (176) found that incomplete resolution of various compounds in biological extracts was obtained. The formation of the trimethylsilyl derivative with a hexamethyldisilazane–trimethylchlorosilane reagent provided a 10-fold increase in sensitivity along with a shorter analysis time.

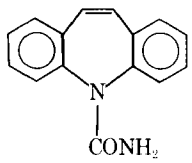
Oxazepam (XXXVI) rapidly loses a molecule of water on the GLC column to yield 6-chloro-4-phenylquinazoline-2-carboxyaldehyde (177, 178). Since the decomposition of oxazepam is rapid and almost quantitative, it can be used as a means of analyzing



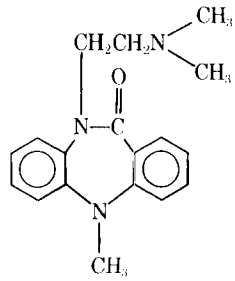
XXXV



XXXVI



XXXVII



XXXVIII

oxazepam by GLC. Similar decomposition occurs for lorazepam, 3-hydroxynitrazepam, and 3-hydroxydesalkylflurazepam (173, 179).

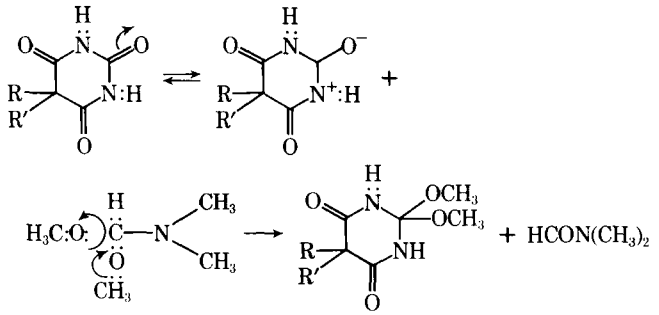
A rapid method of analysis, based on the derivatization of carbamazepine (XXXVII) by dimethylformamide dimethyl acetal, was reported (180). Acetylation followed by GLC was used on a polar column for qualitative and quantitative determination of five *N*-demethylated metabolites of dibenzepin (XXXVIII) (181).

Barbiturates and Other Urea Derivatives—The analysis of barbiturates and related compounds requires derivatization to provide better resolution and peak symmetry than with the free drugs (182). Methylation of barbiturates has been accomplished by reacting them with dimethyl sulfate (183), diazomethane (184), and trimethylanilinium hydroxide for on-column methylation (185).

Venturella *et al.* (186) claimed that dimethylformamide dimethyl acetal is superior for methylation because of its rapid action. They proposed a method for simultaneous determination of amobarbital, secobarbital, hexobarbital, pentobarbital, phenobarbital, aprobarbital, and methyprylon. Scheme XII shows the mode of reaction. The mechanism of methylation using dimethylformamide dimethyl acetal depends on the presence of a rigid keto tautomer to prevent the slower rate reaction of the reagent with latent enols. For this reason, compounds such as amobarbital sodium and secobarbital sodium must be converted to their free acids and extracted prior to reaction.

GC provides a rapid and sensitive method for the determination of plasma urea as its trifluoroacetyl derivative using a gas chromatograph with a nitrogen detection system (187). Trifluoroacylation was also found useful for GLC of monosubstituted and symmetrically disubstituted ureas (188). Acylation was accomplished at room temperature in 2–3 min.

Silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (189) has been used for GLC analysis of fluorouracil (XXXIX). However, Rao *et al.* (190) found it necessary to develop a flash methylation



Scheme XII

technique, using trimethylanilinium hydroxide, for biopharmaceutical studies of fluorouracil.

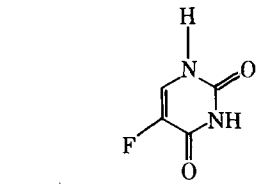
Esters—Various esters can be chromatographed directly. Esterification is frequently used as a means of derivatization to increase the volatility and reduce the column interactions of a compound. However, some esters cannot be chromatographed directly. For example, direct GLC of meprobamate (XL) entails thermal decomposition at the injection port (191). A procedure was proposed for analyzing meprobamate based on the hydrolysis of meprobamate followed by silylation of the hydrolysis product to give the trimethylsilyl derivative for GLC (192).

Trimethylsilylation has also been used for the GLC analysis of chlorphenesin carbamate (XLI) in human serum (193) with sensitivities down to 0.5 μg/ml.

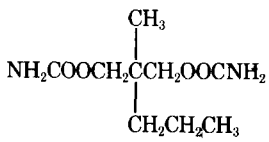
Drugs such as cocaine, atropine, and methylphenidate form sensitive derivatives when subjected to "reductive fragmentation" followed by acylation (194). The mode of reaction for derivatization of methylphenidate (XLII) is shown in Scheme XIII.

The technique of reductive fragmentation can be widely adapted for different drugs having reducible groups (195). Combined with the use of fluorinated derivatizing agents such as heptafluorobutyric anhydride and pentafluoropropionic anhydride, one can increase the sensitivity of the method significantly.

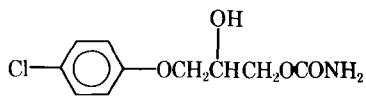
Hydroxy Compounds and Mercaptans—Sorbitol is a naturally occurring, straight-chain hexahydric alcohol commonly incorporated in pharmaceutical products as a humectant and sweetener. Silylation procedures reported in the 1960's (196–198) necessi-



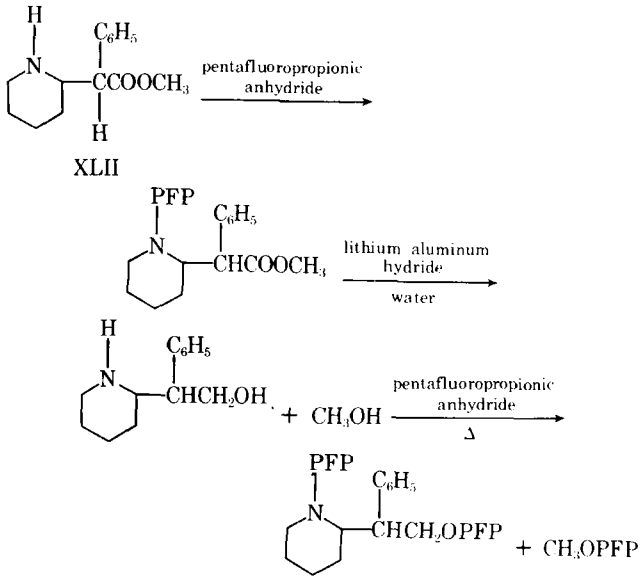
XXXIX



XL



XLI



XLII

PFP = pentafluoropropionic group

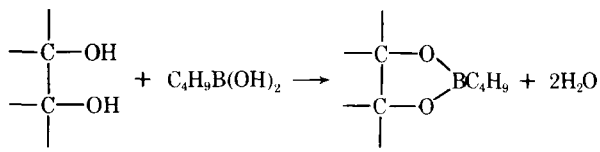
Scheme XIII

tate complete removal of water from the sample before reaction, require extended reaction time, and do not completely separate sorbitol from mannitol and galactitol. Hexaacetate derivatives can be separated; however, this procedure also requires removal of water or residual solvent to assure no tailing of the peak (199, 200). Therefore, a GLC procedure was developed for analyzing sorbitol in solid and aqueous solution forms based on the ability of *n*-butylboronic acid to convert sorbitol quantitatively to the cyclic *n*-butylboronate ester (201). Baseline separations were reported for sorbitol, mannitol, and galactitol. Scheme XIV was proposed for this reaction.

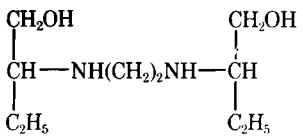
Several GC methods have been reported for separating ethambutol (XLIII) and other antitubercular drugs; however, these methods are not entirely satisfactory because a complex derivatization procedure must be utilized along with a programmed temperature system. A single-step derivatization procedure was proposed followed by isothermal GC for analysis of ethambutol after an investigation of the following six drugs: ethionamide, pyrazinamide, cycloserine, isoniazid, pyridoxine, and ethambutol (202). *N*-Trimethylsilylimidazole derivatized ethambutol immediately and completely. Since *N*-trimethylsilylimidazole reacts only with hydroxyl groups (203, 204), only ethambutol and pyridoxine (XLIV) were derivatized.

Parabens are alkyl hydroxybenzoates. A silylation procedure is described in USP XVIII (107) for the analysis of methyl- and propylparabens (XLV) in various pharmaceutical preparations.

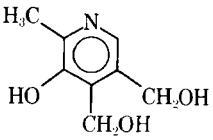
Sensitive methods are required for the toxicological analysis of phenolic compounds. Thiophosphoryl



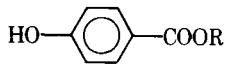
Scheme XIV



XLIII

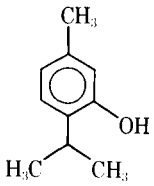


XLIV



XLV

$R = CH_3$ or C_3H_7



XLVI

phenolic derivatives and the use of flame photometric detection were found unsatisfactory for the determination of microgram amounts of phenols (205). A GC method based on formation of diethyl phosphate esters and flame photometric detection was proposed. The maximum sensitivity of detection of the analytically pure thymol (XLVI) phosphate ester was approximately 10 pg. A variety of other phenols such as isoeugenol, *p*-nitrophenol, *p*-chlorophenol, β-naphthol, and 2-methoxy-4-methylphenol were amenable to this method of analysis.

The speed of trimethylsilylation of sterically hindered phenols such as Δ^1-tetrahydrocannabinol can be increased by promoting the reaction with a small amount of trifluoroacetic acid. Trifluoroacetic acid may function as in Scheme XV (206). This reaction may also be of value in promoting silylation of other compounds that are difficult to silylate.

A GLC procedure involving acetylation was reported for the analysis of resorcinol monoacetate in dermatological products (207). The analysis of cream or lotion is performed by the addition of an internal standard, acetylation with acetic anhydride, extraction with benzene, and eventual chromatography of the chloroform solution on a 5% cyanosilicone column.

Gruber *et al.* (208) proposed a rapid GLC analysis based on formation of trimethylsilyl esters of 8-hydroxyquinoline and related halogenated 8-hydroxyquinoline with "significantly greater accuracy and precision" than the USP XVIII procedure. They were successful in resolving 5-chloro-7-iodo-8-hydroxyquinoline from impurities such as 8-hydroxyquinoline, 5-chloro-8-hydroxyquinoline, and 5,7-dichloro-8-hydroxyquinoline.

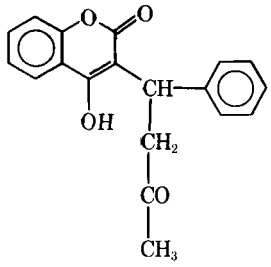
GC of the methyl derivative of warfarin (XLVII) was reported (209). The method was sufficiently sensitive to determine 0.25-μg/ml plasma levels in humans after single doses of 20 mg of warfarin. This sensitivity was extended down to 0.02 μg/ml by forming pentafluorobenzyl derivatives (210).

A method based on trifluoroacylation was developed for quantitative determinations of cyclazocine (XLVIII) and its *N*-dealkylated biotransformation

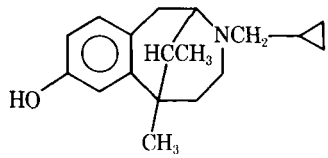
$$RSi(CH_3)_3 + CF_3COOH \rightleftharpoons RH + CF_3COOSi(CH_3)_3$$

$$ArOH + CF_3COOSi(CH_3)_3 \rightleftharpoons ArOSi(CH_3)_3 + CF_3COOH$$

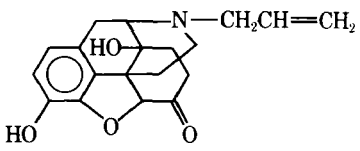
Scheme XV



XLVII



XLVIII



XLIX

product, norcyclazocine, in human urine (211). The method also can be used to estimate the levels of conjugated cyclazocine and norcyclazocine by measurement of these compounds released by acid hydrolysis. Heptafluorobutyric anhydride was used for derivatization of pentazocine and its metabolites followed by electron-capture detection (212). This method is claimed to be sensitive down to 0.1–0.2 μg/ml for pentazocine and its hydrolytic metabolites.

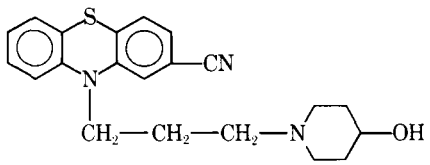
A silylation method was reported for determining naloxone (XLIX) in plasma (213). Enol ester derivatives of naloxone and naltrexate were prepared by reacting them with trifluoroacetic anhydride in the presence of pyridine (214) to achieve a sensitivity of 5 ng/ml in plasma or urine.

Normal and branched mercaptans containing up to eight carbon atoms and their 2,4- and 2,5-dinitrophenyl alkyl thioethers and thioalkylbenzoate derivatives were analyzed by GLC (215). The thioalkylbenzoate derivatives were suitable for resolving these mercaptans on an SE-30 column.

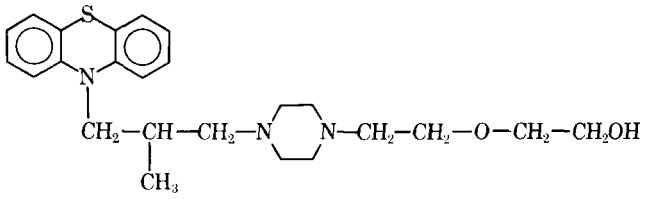
GC represents the most practical means to date for the analysis of phenothiazines and their derivatives. The normal methods of analysis of these drugs and their metabolites in biological media are nonspecific, insensitive, or time consuming in the cleanup and concentration steps. Phenothiazines containing a free primary alcohol group (acetophenazine, dixyrazine, fluphenazine, and perphenazine) cannot be eluted from the chromatographic column even at 280° but chromatograph easily as the acetates (216).

Similar derivatization is necessary for GLC of pericyazine (L) and dixyrazine (LI) (217). Phenothiazine sulfoxides can be readily separated and show chromatographic characteristics similar to those of corresponding free bases. Sulfoxides are prepared by oxidation of the free base in the presence of acetic acid (216).

Steroids—Steroids—natural and hormonal—possess a cyclopentanophenanthrene ring system with a variety of functional groups. The steroid hormones differ from other steroids in that they do not carry a long side chain. The state of oxygenation or dehydrogenation and the presence or absence of a side chain are the distinguishing factors of various hormones. Various derivatives may be used for GLC analysis of



L



LI

steroids depending on the reactive group in the steroid molecule (8): trimethylsilyl ethers, acyl derivatives, phenolic ethers, and cyclic boronates for hydroxyl groups; methoximes and hydrazones for keto groups; and methyl esters for acid groups.

The retention indexes of various steroid derivatives such as acetates, trimethylsilyl ether, trifluoroacetate, heptafluorobutyrate, and chloromethyldimethylsilyl ether derivatives were reported (218).

Trimethylsilyl ethers are widely used in the analysis of steroids on the basis of etherification of the hydroxyl groups. With the presently available silyl reagents, it is possible to etherify selectively different types of steroid hydroxyl groups on the basis of differences in rates of reaction (219). Suitable reaction conditions have been established for the derivatization of even highly hindered and, therefore, normally unreactive groups such as the 14β-hydroxyl of cardenolides (220) and the 17α-hydroxyl of cortol (219).

Gleispach (221) showed that every hydroxyl group in a steroid can be transformed into its silyl ether, depending on the silylating mixture used. Oxo groups in the α-position to a hydroxyl group can react with formation of the enediol trimethylsilyl ether, whereas free oxo groups react under strong conditions with formation of the enol trimethylsilyl derivative. He concluded that hexamethyldisilazane or trimethylchlorosilane alone can be used for partial silylation of 3α-, 3β-, 21-, and 3-phenolic hydroxyl groups. Sterically nonhindered groups can be silylated with *N,O*-bis(trimethylsilyl)acetamide, *N,O*-bis(trimethylsilyl)trimethylsilyldiethylamine, or *N*-methyl-*N*-trimethylsilyltrifluoroacetamide. Silylation of the sterically hindered 17α-hydroxyl group can be carried out with trimethylsilylimidazole, whereas 11β-hydroxyl groups can be silylated with a mixture of trimethylsilyldiethylamine, *N,O*-bis(trimethylsilyl)acetamide, or *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with trimethylchlorosilane. The use of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with an alkaline catalyst such as potassium acetate ensures the formation of the 20,21-enediol trimethylsilyl or 20,21-ene-17,20,21-triol trimethylsilyl derivatives of the glucocorticoids and also the formation of the enol trimethylsilyl ethers of oxo steroids.

Chambaz *et al.* (222) studied the conditions for an alkali-catalyzed silylation procedure using nucleophilic agents. This catalysis leads to preferential formation of enol trimethylsilyl derivatives from steroid keto groups without affecting the silylation rate of hindered hydroxyl groups. The most easily derivatized structures are the ketol and dihydroxyacetone side chains of the corticosteroid metabolites. Trimethylsilylation has also been found to be very useful for characterization of glucuronides and sulfate conjugates of hydroxysteroids (223, 224).

Trifluoropropyl, heptafluoropentyl, and pentafluorophenyl groups substituted into silyl ethers of steroids provide stable and volatile derivatives for GC of steroids. The first two show low sensitivity toward electron-capture detection, but the pentafluorophenyl group is more sensitive to electron-capture detection than the currently used chloromethyl group and is more thermally stable than the heptafluorobutyryl group (225).

A reported GC method (226) used high-resolution open-tubular glass capillary columns for simultaneous estimation of the following neutral steroids as trimethylsilyl ether derivatives: 11-oxoethiocholanolone, pregnanolone, 11β-hydroxyandrosterone, 11β-hydroxyetiocholanolone, allopregnanediol, pregnanediol, Δ^5-pregnanediol, pregnanetriol, and cholesterol.

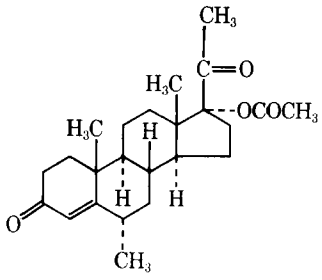
Methoxime trimethylsilyl derivatives were prepared from several steroids isolated from urine (227). The retention indexes were measured for the methoxime trimethylsilyl derivatives of some corticosteroid metabolites at 250° on an SE-30 column using C_{28}–C_{30}, C_{30}–C_{32}, and C_{28}–C_{32} alkane mixtures as standards.

Steroids such as cortisone with a 17,21-dihydroxy side chain degrade when gas chromatographed unmodified. The cyclic boronates appear to offer advantages over the other derivatives in terms of ease of preparation and stability (228).

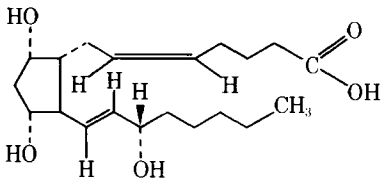
Some perfluorinated esters have been investigated to increase sensitivity by electron-capture detection. Heptafluorobutyrates are extremely volatile compounds and may be chromatographed at the picogram level (229). Esters of secondary alcohols are reasonably stable, but phenol esters are generally not stable. However, pentafluorobenzoates provide stable derivatives for phenols (230).

To study the absorption, metabolism, and excretion of medroxyprogesterone acetate (LII) in animals and humans, a method was developed for measurement of intact drug in plasma based on formation of a 3-enol heptafluorobutyrate ester and quantification with electron-capture detection. The assay was quantitative above 1 ng of medroxyprogesterone acetate/ml of plasma (231). The pentafluorobenzyl ester of dinoprost (LIII) was gas chromatographed as the tris(trimethylsilyl) ether (232). The derivative was stable during GLC, and the lower limit of detection by electron-capture detection was 12.5 pg of the ester injected on-column as the silylated product.

The estrone dimethylphosphinic esters prepared by derivatization of the monohydroxysteroid have good GC properties, and detectability of 10 pg can be achieved with the alkali flame-ionization detector (233). Some steroids that can be derivatized by this approach are: androsterone, dehydroepiandros-



LII



LIII

terone, stanolone, estrone, 3α-etiocholanolone, 3β-etiocholanolone, 3α-pregnanolone, 3α-pregnenolone, and testosterone.

Several methods have been recommended for GC of steroid hormones (Table XI) (234).

Vitamins—GC often has been used for the analysis of water-soluble and fat-soluble vitamins (235). Unmodified polyhydroxy and carboxylic organic compounds such as panthenol (LIV) and pantothenic acid (LV) and its salts are not sufficiently volatile for direct GLC. Furthermore, both LIV and LV tend to decompose at high temperatures. These problems can be easily overcome by converting the compounds to volatile derivatives such as acetates, trimethylsilyl ethers, or trifluoroacetates for GLC analysis.

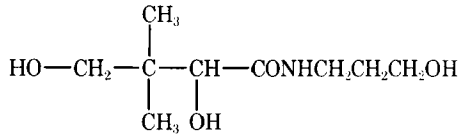
The GLC determination of the acetate derivatives of pantothenic acid ethyl ester, pantothenol acid ethyl ester, and panthenol was described (236). The acetyl derivatives (acetates) were chosen because of their relatively high degree of volatility and stability and their ease of preparation and cleanup. Trimethylsilyl and trifluoroacetate derivatives of pantothenates and panthenol were also analyzed by GC.

A method based on silyl esterification of biotin (LVI) was reported (237); it uses the reaction of biotin with bis(trimethylsilyl)acetamide. The sodium salt of *d*-biotin did not yield a trimethylsilyl derivative. Therefore, Scheme XVI was proposed.

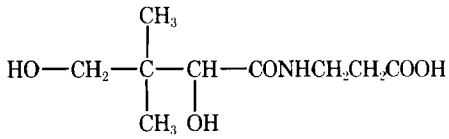
The interference of vitamin A in the determination of D vitamins by GLC is widely recognized. Removal of vitamin A generally necessitates lengthy pre-GLC workup. Tocopherols can also interfere, because they

Table XI—Derivatization GC of Steroid Hormones

Steroid	Sample	Derivative
Estrogens	Urine	Acetate
Estrogens	Blood	Heptafluorobutyrate
Pregnanediol	Urine	Acetate
Progesterone	Blood	Chloroacetate
Testosterone	Blood	Heptafluorobutyrate
17-Ketosteroids	Urine	Trimethylsilyl
Cortisol (hydrocortisone) metabolites	Urine	Trimethylsilyl
Tetrahydroaldosterone	Urine	Monochlorodifluoroacetate



LIV



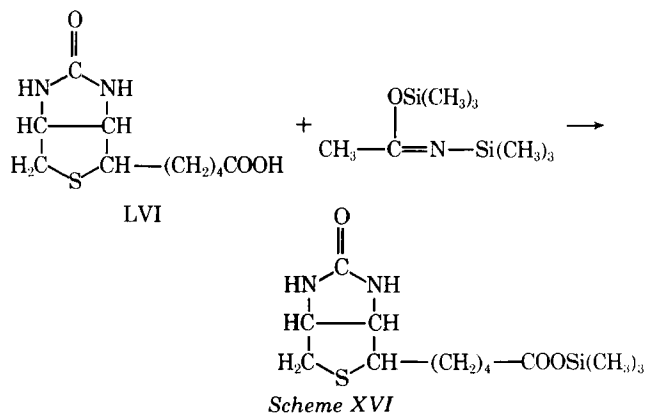
LV

have the same retention times as the D vitamins when the natural vitamins or their trimethylsilyl derivatives are chromatographed. A proposed improved procedure (238) consists of preliminary saponification and extraction steps to remove some interfering materials and then isolation and characterization of vitamins D (ergocalciferol, LVII), E (LVIII), and A (LIX) in their alcohol forms. The mixture so obtained is treated with propionic anhydride to esterify all alcohols to their corresponding propionates. The propionate esters of the following vitamins were prepared and chromatographed: α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, pyrovitamin D_2, isopyrovitamin D_2, pyrovitamin D_3, and isopyrovitamin D_3. Acetic anhydride–pyridine reaction was utilized to determine whether α-tocopherol or α-tocopherol succinate was present in a sample, since the two analogs have similar retention times by GLC (239).

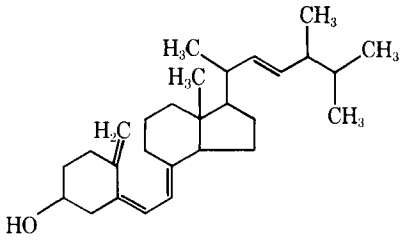
A method for the quantitative GLC determination of niacinamide (LX) is based on its conversion to nicotinonitrile. The dehydration reaction is mediated with trifluoroacetic anhydride and catalyzed by alkali (240).

Miscellaneous Compounds—Determination of diftalone (LXI) and its main metabolite, the 7-hydroxy derivative, is based on acid hydrolysis to isomeric carboxylic acid, followed by esterification with diazomethane for GC (241).

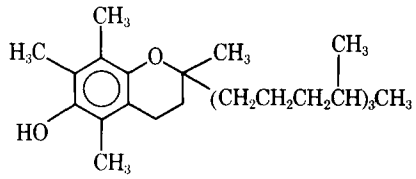
A general method for the analysis of guanidino drugs is based on hydrolysis and trifluoroacetylation of the resulting amines to obtain derivatives with excellent GC properties. Biological samples with levels of guanethidine (LXII) greater than 100 ng/ml can be analyzed easily by GLC with flame-ionization detection. Urine and plasma samples containing 1–100 ng/ml are readily amenable to analysis by GLC and



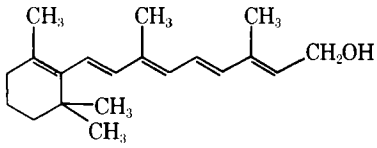
Scheme XVI



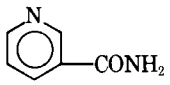
LVII



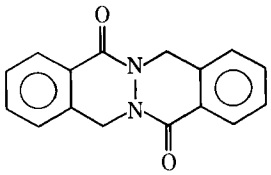
LVIII



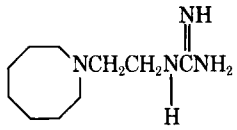
LIX



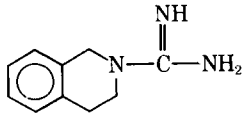
LX



LXI



LXII



LXIII

multiple ion detection (242).

Recently, a hexafluoroacetylacetone derivatization procedure was used for the analysis of debrisoquin (LXIII), guanethidine, and 3,4-dihydro-1-methyl-2-(1*H*)-isoquinolinecarboxamidine (243).

CONCLUSIONS

Derivatization GC permits the analysis of pharmaceutical compounds with great selectivity and sensitivity down to the femtomole level. Furthermore, it is a valuable tool for structure determination, since derivatization GC–mass spectrometry surpasses either technique separately for structural information when only a small amount of sample is available. Anomalous reactions can occur in derivatization GC, and it is important that these reactions are considered in method development.

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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Research and Development Division, Ciba-Geigy Corp., Suffern, NY 10901*

RESEARCH ARTICLES

Influence of Hydrophobic Materials on Dissolution of a Nondisintegrating Hydrophilic Solid (Potassium Chloride)

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Abstract □ A nondisintegrating hydrophilic solid was covered with coatings of hydrophobic materials as a model to study their influence on dissolution rates. Both solution and sublimation techniques were satisfactory for producing coatings of stearic acid. Only a sublimation method was applicable to magnesium stearate, but the resulting coating consisted mainly of a pyrolysis product, stearone. Reductions in dissolution rates in a continuous-flow system could best be interpreted by assuming that they reflected changes in the area of the hydrophilic solid exposed to the solvent. This concept is based on the assumptions that the intrinsic dissolution rate of uncoated potassium chloride did not change and that no dissolution occurred from coated areas. The most important factors controlling the dissolution rate were the strength of attachment of the hydrophobic coat to the surface of the hydrophilic substrate and the thickness of the coat.

Keyphrases □ Dissolution—potassium chloride, a nondisintegrating hydrophilic solid, effect of hydrophobic coating materials □ Potassium chloride disk—effect of hydrophobic coating materials on dissolution

The most common method of ensuring adequate lubrication of the interface between powders and metals during tablet and capsule manufacture is the addition of materials, such as stearic acid and magnesium stearate, in the form of powders. These materials, being hydrophobic in nature, inhibit the penetration of aqueous fluids into the final product (1, 2). This limitation of access results in retarded dissolution rates of tablets (3) and capsules (2–4).

The hydrophobic material, in addition to reducing the rate and extent of penetration of fluid into the preparation, can reduce the dissolution rate of individual particles by covering their surface. In formulated systems, it is difficult to decide which of these two effects is the rate-controlling step in the release of drugs from preparations. For example, liquid penetration of capsule formulations may or may not correlate with drug release (5). Moreover, because the spatial distribution of the lubricant in the final preparation is unknown, the extent and thickness of the hydrophobic covering of the drug particles are also unknown. It is, therefore, difficult to measure the effect of added hydrophobic materials on the dissolution rate of the individual particles within a preparation.

To investigate this problem, it is helpful to consider model systems. The present work describes a model in which the type, thickness, and area of cover