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Review

Derivatization reactions in the gas–liquid chromatographic analysis of drugs in biological fluids

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Abstract: Alkylation, acylation, silylation and other derivatization reactions applied to the gas chromatographic analysis of drugs in biological matrices are reviewed. Reaction conditions are discussed in relation to reaction mechanisms. Detector-oriented labelling of drugs, and derivatization with chiral reagents for the separation of enantiomers are surveyed. Data on the sample clean-up, derivatization and GLC analysis of more than 300 drugs and related compounds are listed.

Keywords: *Derivatization for gas–liquid chromatography; drug analysis; biological fluids; specific detectors in gas–liquid chromatography; gas–liquid chromatography–mass spectrometry; sample clean-up procedures.*

Introduction

Derivatization of compounds to be analysed by gas–liquid chromatography (GLC) may be desirable to improve their chromatographic properties, to obtain higher sensitivity, or both. Unsatisfactory chromatographic properties of compounds may stem from the low volatility of polar compounds, irreversible adsorption caused by interaction between polar functional groups of the compounds and the free silanol groups of the column-packing material and the glassware, or limited stability at the high temperatures used in GLC, or in the conditions prevailing during pre-chromatographic sample treatment. Derivatization can also serve to decrease the excessive volatility of compounds of very low molecular weight or to improve the separation between certain closely related compounds. A special case of interest is the separation of pairs of enantiomers, which have the same retention properties on non-chiral stationary phases, via the formation of diastereomers. Derivatization reagents with special atoms or molecular features can be used to increase or modify the response of selective and sensitive detectors to the compounds to be analysed.

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A large number of derivatizing reagents are now available, which can be attached to the compounds of interest by different types of reactions. Derivatizations by acylation, silylation or alkylation are most frequently applied, but several other useful reactions have been described. Important criteria valid for all reactions are that the derivative must be formed rapidly and quantitatively, or at least reproducibly, without side-reactions or structural changes of the compounds or their derivatives and with a minimum of manipulation.

The importance of derivatization reactions in GLC (and HPLC) is reflected by the number of recently published books and reviews that are either of a more general nature [1–8] or deal with selected subjects, such as the enhancement of electron capture detector response [9–11], derivatives suitable for GC–MS [12], certain silylating reagents [13], alkylation [14], cyclization [15] or pyrolytic methylation [16].

This review aims to bring together some of the basic principles and reaction mechanisms of derivatization reactions that have been found useful for the GLC analysis of drugs and their metabolites in biological matrices. Mainly the literature of the past 5–7 years has been reviewed. Reaction chromatography (chemical reactions in a GLC pre-column reactor, on the column itself or in a post-column reactor) is not considered except for some on-column flash heater derivatizations.

Alkylation

Many different reagents have been used for the alkylation of drugs and other compounds. All alkylation methods have in common the replacement of one or more of the active hydrogens of the analyte with alkyl groups in compounds with functional groups, such as -OH, -COOH, -SH, -NH, -CONH and $-SO_2NH$. In certain conditions tertiary amines are also known to react with alkylating reagents to yield quaternary ammonium compounds.

(A) *Alkylation by acid-catalysed reactions (esterification)*

Esterification of carboxylic acids with alcohols has been widely applied, particularly for the alkylation of fatty acids. Carboxylic acids are not easily attacked at the carbonyl carbon atom of RCOOH with nucleophiles of the general type $Y^{\ominus}$, as the latter commonly remove protons instead; the resultant carboxylate anion is not susceptible to nucleophilic attack. Weaker nucleophiles such as the alcohols (ROH) do react, but the reaction rates are generally too slow to be useful for derivatization of acids. The reaction rate can be much increased by acid catalysis, which is usually achieved with mineral acids (HCl, H_2SO_4), Lewis acids (BF_3) or organic acid anhydrides (e.g. TFPAA).

Acid-catalysed esterification with primary alcohols of low molecular weight (e.g. methanol, ethanol) are frequently applied in derivatization reactions for GLC, and generally proceed by the bimolecular acyl–oxygen cleavage ($A_{Ac}2$) mechanism [17].

Acid catalysis enhances the carbonyl character of RCOOH by protonation, thus rendering the carbonyl atom more susceptible to nucleophilic attack; it also has the effect of promoting the loss of the leaving group, water, as H_2O is lost more easily than OH^-. Esterification reactions with sulphuric acid have become less popular, because they are rather slow processes. Catalysis with dry hydrogen chloride dissolved in the appropriate alcohol gives much better results. The esterification is usually performed with the appropriate alcohol as the solvent for the acid and the catalyst, but can also proceed in aqueous solutions [18, 19].

$$R-C(=O)-OH \underset{}{\overset{H^{\oplus}}{\rightleftharpoons}} R-\overset{\oplus}{C}(OH)-OH \underset{slow}{\overset{ROH}{\rightleftharpoons}} R-C(OH)(OH)-H\overset{\oplus}{O}R^1$$

$$\rightleftharpoons R-C(OH)(OR^1)-\overset{\oplus}{O}H_2 \underset{}{\overset{-H_2O}{\rightleftharpoons}} R-C(=\overset{\oplus}{O}H)-OR^1 \underset{}{\overset{-H^{\oplus}}{\rightleftharpoons}} R-C(=O)-OR^1$$

The reagent can be prepared either by bubbling hydrogen chloride through the alcohol or by the addition of acetyl chloride or thionyl chloride to the alcohol. Methylation is usually the derivatization reaction of first choice, but in principle a wide range of different esters can be prepared. Halogenated alcohols yield derivatives suitable for electron capture detection; optically active alcohols can be applied to separate the optical isomers of an acid (see below). The reaction will sometimes be completed within a few minutes at room temperature, but usually the mixture must be heated at 60–100°C for up to 2 h. The excess reagents can frequently be evaporated off, but caution is required in the case of low molecular weight and therefore volatile derivatives. Examples of drugs and related compounds esterified by acid catalysis are diclofenac [20] and steroid carboxylic acids [21].

Organic acid anhydrides have also been used successfully in the rapid esterification of a number of compounds. Indomethacin was converted into its pentafluoropropyl ester with pentafluoropropanol and pentafluoropropionic anhydride [22]. These reactions are frequently performed at room temperature, but heating of the derivatization mixture cannot always be avoided. The acid anhydride catalyst can also acylate other functional groups in the analyte molecule, thus allowing derivatization of different functional groups with separate reactions in one derivatization step. In this way homovanillic acid and related compounds were converted into their trifluoroacetylhexafluoroisopropyl ester derivatives by heating the compounds in a mixture of hexafluoroisopropanol and trifluoroacetic anhydride [23]. Excess reagent is usually removed by evaporation. Instead of mineral acids or organic acid anhydride, boron halides (Lewis acids) have also been used as catalysts for the esterification of carboxylic acids with alcohols. The reactions usually proceed at a much higher rate than with mineral acid catalysis. The reaction medium consists of about 15% of boron trifluoride in the appropriate alcohol. A few minutes heating is frequently sufficient for complete derivatization. A typical application is the methylation of fatty acids in methanol with boron trifluoride [e.g. 24].

(B) *Alkylation via nucleophilic substitution at saturated carbon*

Alkylation reactions of this type are nucleophilic substitution (S_N) reactions; a nucleophile (the analyte), Nu or Nu^-, displaces a leaving group, L or L^-, from the substrate (alkylating reagent):

$$Nu + b-C(a)(c)-L \longrightarrow Nu-C(a)(c)-b + L$$

The nucleophile and the leaving group are of the same character; they are anionic or neutral bases with unshared electron pairs. Two mechanistic routes for S_N reactions are discerned, S_N1 and S_N2 [17].

In GLC derivatization reactions the conditions are usually such that the reaction proceeds as a bimolecular one-step direct displacement reaction (S_N2):

$$Nu^- + R{-}L \rightarrow [Nu{\cdots}R{\cdots}L]^- \rightarrow Nu{-}R + L^-.$$

The species in brackets represents the transition state. The S_N2 reaction route is favoured by the use of reagents such as primary alkyl halides, in which the carbon atom under nucleophilic attack carries no bulky groups preventing the formation of the transition state. Alkylation reagents with bulky groups are rarely used in derivatization reactions and S_N2 reactions are the usual reaction type.

Although the ionizing power of the solvent is usually less important for the reaction rate of S_N2 reactions than for S_N1 reactions, S_N2 reactions with nucleophilic anions are best carried out in aprotic solvents, such as acetone, *N,N*-dimethylacetamide and dichloromethane. In the presence of protic solvents such as water and methanol the nucleophile is stabilized via hydrogen bonding with the solvent molecules; consequently an extra energy barrier exists as these hydrogen bonds must be broken before the nucleophile can react with the substrate.

However, the charge type of the nucleophile strongly influences the reaction rate in a given medium. When a neutral base (amines) acts as the nucleophile in an S_N2 type reaction, the reaction rate is reduced in aprotic solvents, because the ionic species formed — a quaternary ammonium ion and the leaving group anion — cannot be efficiently solvated in these solvents. This should be an important consideration in choosing the reaction medium for the alkylation of compounds with acidic groups which also carry an amine group.

Alkylation with alkyl halides. This type of derivatization reaction is almost invariably performed with primary alkyl halides (iodides or bromides) and is therefore predominantly of the S_N2 type. Inductive effects cause the primary *n*-alkyl halides to be less active than methyl iodide, because of the increased electron density at the attacked carbon atom. Resonance effects have a limited effect on the reaction rate; the benzyl halides show about four-fold higher reaction rates than the corresponding methyl halides. The group of alkyl halides with a carbonyl group adjacent to the reactive carbon, e.g. phenacyl bromide, are particularly reactive, apparently because the attacking nucleophile dumps part of its charge on to the carbonyl group as well as to the reaction site. The more polarizable atoms possess better leaving group ability. For halides the following order of decreasing leaving group activity prevails: $I^- > Br^- > Cl^- >> F^-$. Derivatizations are therefore almost exclusively performed with iodides or bromides. Unless an enhanced electron capture detector (ECD) response is aimed at, methyl iodide is usually the reagent of first choice: it reacts more rapidly than the other *n*-alkyl iodides and competing elimination reactions cannot occur due to the absence of a β-hydrogen atom. A number of reagents suitable for enhancement of ECD response are in use of which pentafluorobenzyl bromide (PFBB) is the most prominent one.

Halo-carbonyl compounds, yielding much less volatile derivatives, have occasionally been used. This decrease in volatility can be helpful for the analysis of very volatile compounds. Phenacylbromide has been used for the derivatization of valproic acid

[25–27], and *p*-bromophenacyl bromide for the derivatization of C_1–C_4 fatty acids [28]. Acidic compounds act as nucleophiles in their conjugate base form. Within a series of structurally related compounds nucleophilicity of the anions parallels their basic strength. For anions with oxygen as the attacking atom the following order of decreasing nucleophilicity prevails: $C_2H_5O^- > OH^- > C_6H_5O^- > CH_3COO^-$. Deprotonated aliphatic alcohol groups are very reactive nucleophiles; however, under the mild conditions that normally prevail in derivatization reactions, the very weakly acidic aliphatic alcohols are not deprotonated to any significant extent. Only under very strongly alkaline conditions can alkylation of aliphatic alcohols be achieved. Another example is the difficult alkylation of the second acidic group of phenytoins and related compounds [29, 30]. Some nucleophiles contain more than one atom with active electron pairs and can therefore react in different ways. Under S_N2 conditions the more polarizable and less electronegative atom is preferentially alkylated. Thus the *N*-alkyl derivatives rather than the *O*-alkylderivatives of barbiturates are formed.

Base catalysed reactions. The deprotonation of an acidic compound can be effected by the addition of a base, such as potassium carbonate or tetra-alkylammonium hydroxide. A well-known reaction procedure is the heating of a solution of the acidic compound in acetone or another aprotic solvent, in the presence of powdered potassium carbonate [31]. Many acidic drugs can be derivatized in this way. An example is the pentafluorobenzylation of tetrahydrocannabinol [32]. If necessary, concentration of the sample is easily achieved by evaporation under nitrogen and reconstitution of the residue in a small volume of a suitable solvent, or by partial reflux [31]. Other alkali metal carbonates can be used. The reaction rate in the methylation of carboxylic acids was found to be highest with caesium carbonate [33].

Another approach is the conversion of carboxylic acids into their potassium salts. The dry salts are then reacted with alkyl halide in the presence of a crown ether; the potassium ion of the ion pair, potassium-acidic anion, is complexed by the crown ether and the remaining acidic anion is very reactive in the aprotic solvent [34, 35]. Instead of the potassium salts the strontium salts of carboxylic acids can be used [36]. Heating the reaction mixture for some time is usually necessary for complete derivatization within reasonable time. In view of the volatility of the solvent (acetone) and reagent (e.g. methyl iodide), reactions should be performed in air-tight vessels or in a microreflux apparatus [31].

With methanolic solutions of tetramethylammonium hydroxide or another quaternary ammonium hydroxide as the base catalyst, rapid and usually quantitative alkylation of many acidic compounds can be achieved at room temperature within 5–10 min in *N,N*-dimethylacetamide [37, 38]. Other solvents, e.g. acetone [39], have also been used.

The OH^- ions added in excess for the neutralization of the acid react with the even larger excess of alkyl iodide, also in an S_N2 reaction, to yield the corresponding alkanol and a precipitate of the quaternary ammonium iodide. After centrifugation a few microlitres of supernatant are injected into the GC. Initially the reaction medium is strongly alkaline. However, complete alkylation of very weakly acidic aliphatic alcohol groups is not possible with this method, because of the rapid decrease of hydroxyl ion concentration due to the side-reaction with the reagent. Various groups of acidic compounds have been derivatized by this versatile method, e.g. many barbiturates [38, 40, 41].

A number of other bases in combination with solvents of widely varying polarity have been used to catalyse alkylation reactions [2, 14]. Of special interest is the use of very strong bases in homogeneous solutions for the alkylation of very weak acids, such as the aliphatic alcohols. These can be deprotonated by dissolution in dry dimethyl sulphoxide containing potassium *tert*-butoxide or the strongly basic methylsulphinylcarbanion, obtained by the reaction of sodium hydroxide with dimethylsulphoxide. Other solvents (reacted with sodium hydroxide) can also be used, e.g. *N,N*-dimethylacetamide. Under these conditions permethylation of aliphatic alcohols and other weakly acidic groups has been achieved. After the reaction water is added and the derivatives are extracted with chloroform or another solvent. In this way, for example, 5-fluorouracil [42] and bile acids [43] have been permethylated.

Silver oxide has been used to catalyse alkylation reactions, e.g. of antiepileptic agents by heating in acetone or acetonitrile. With ambident nucleophiles, such as primidone and phenytoin, no single derivatization product is obtained [29].

Phase transfer catalysed alkylation. Deprotonation and subsequent alkylation of acidic drugs can be achieved by transfer of the acidic anion as an ion-pair with a suitable counter ion into an aprotic solvent containing the alkyl halide. This method of liquid–liquid phase transfer catalysis is better known as 'extractive alkylation'. Solid liquid phase transfer has also been described.

Extractive alkylation. The acidic compound is deprotonated in an aqueous buffer system and extracted as an ion-pair with tetraalkylammonium counter ions into an aprotic solvent, usually dichloromethane [44–46]. Due to the poorly solvating properties of the solvent the anions posses high reactivity and are rapidly alkylated with alkyl halide via the S_N2 route. The reaction rate and yield depend on the properties of the ion pair forming ions, the lipophilicity of the ion pair and the properties of the extracting solvent. The most attractive feature of this technique is that it combines the extraction of the drug from aqueous solution (e.g. plasma or urine) with simultaneous conversion of the drug into a derivative with suitable properties for chromatographic separation and/or detection.

The extraction equilibrium of a deprotonated acid, Y^-, with a tetraalkyl ammonium ion, R_4N^+, can be written as follows:

$$Y^-_{aq} + R_4N^+_{aq} \rightleftarrows R_4N^+Y^-_{org}.$$

The equilibrium constant, K_{Ex}, is defined as:

$$K_{Ex} = \frac{[R_4N^+Y^-]_{org}}{[Y^-]_{aq} \cdot [R_4N^+]_{aq}}.$$

In the absence of side reactions, such as the protonation of Y^-, the distribution ratio D_Y of Y^- is expressed by:

$$D_Y = \frac{[R_4N^+Y^-]_{org}}{[Y^-]_{aq}} = K_{Ex}\,[R_4N^+].$$

Thus the distribution ratio, and therefore the extraction efficiency, are proportional to

K_{Ex} and the concentration of the counter ion, R_4N^+. Higher R_4N^+ concentrations will consequently lead to increased reaction rates. The more lipophilic the ion pair, the higher will be the value of K_{Ex}. In practice, tetraalkyl ammonium ions with alkyl chains shorter than *n*-butyl are not used, as these generally result in insufficient extraction yields. Tetrabutylammonium (TBA^+), tetrapentylammonium (TPA^+) and tetrahexylammonium (THA^+) are most frequently employed. R_4N^+ ions with alkyl chains are able to extract even hydrophilic anions. It is therefore good practice to choose R_4N^+ counterions with the shortest alkyl chain length consistent with a smooth reaction, as this will improve the selectivity of the derivatization procedure.

In general the pH of the aqueous phase should be at least two units above the pK_a of the acidic group. With very lipophilic acids such as the higher fatty acids, a much higher pH is required to prevent extraction of the neutral acid, while with hydrophilic compounds (partition coefficient less than 1) the pH might be lower. Obviously the derivatization procedure is more selective at the lower end of the pH scale. Dichloromethane, chloroform and dichloroethane are good solvents for ion pairs; but a number of other solvents have been used as well.

Optimal conditions for extractive alkylation seldom coincide with the best possible extraction efficiency and reaction rate for a given compound. Apart from the frequent need to choose more selective extraction conditions, the extent of by-product formation and sometimes the excess of reagent may prevent a sensitive and selective determination of the analyte, especially at high pH.

$R_4N^+I^-$ ion pairs in the organic phase can give rise to substantial tailing in the chromatograms. Trans-esterification reactions have been observed, caused by $R_4N^+I^-$, upon injection of the organic phase without purification [47]. A number of methods dealing with the problem of $R_4N^+I^-$ in the extracting solvent have been discussed [see Ref. 14]. Another hazard is the co-extraction of OH^- ions (as ion pairs with R_4N^+), because these OH^- ions may hydrolyse the reagent. The transfer of OH^- ions into the organic phase is enhanced by high aqueous phase pH values and by R_4N^+ ions of increasing lipophilicity.

The conclusion must be that in extractive alkylation procedures one should not extract at higher pH values, or with larger R_4N^+ ions, or with higher concentrations of R_4N^+ ions, than is strictly necessary. The alkyl halide concentration should not be higher than required and the reaction must not be unnecessarily prolonged.

Some reports [48, 49] claim that, without the addition of R_4N^+ counter ions, phenolic compounds such as estradiol can be alkylated in a biphasic system, but that carboxylic acids do not react, thus offering the possibility of enhanced selectivity in the analysis of phenols. No explanation for the mechanism of this procedure has been given.

Solid–liquid phase transfer catalysis. Some compounds, e.g. acetylsalicylic acid, are extremely sensitive to hydrolysis. A derivatization method has been proposed for the alkylation of these compounds under very mild conditions [50, 51]. The compound to be derivatized (HY) is dissolved in a suitable solvent, e.g. dichloromethane, containing tetrabutylammonium hydrogensulphate ($TBA^+HSO_4^-$), and the alkyl halide (R–L). Solid sodium hydrogen carbonate is added. After shaking and centrifugation the organic phase is analysed. A three-step reaction is probably involved. Hydrogen carbonate is transferred into the liquid phase by exchange with hydrogen sulphate at the solid–liquid interface, followed by the (almost) simultaneous deprotonation of HY and the formation of ion-pair TBA^+Y^-; Y^- is subsequently alkylated with R–L.

Diazoalkane alkylation. A number of diazoalkane reagents have been used for the alkylation of drugs, diazomethane is the most important one. The methylation reaction of a compound with active hydrogen, Y–H, proceeds as follows:

$$Y{-}H + [H_2C{=}N^+{=}\bar{\underline{N}}^- \leftrightarrow H_2C^-{-}\overset{+}{N}{\equiv}N|] \rightarrow Y{-}CH_3 + N_2$$

The addition of a proton from the protonated nucleophile causes the immediate elimination of N_2 (an excellent leaving group) and the immediate reaction between the carbenium ion (CH_3^+) and the nucleophile. Diazomethane can be prepared by the reactions with alkali of compounds such as *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide. Diazomethane and related substances are toxic and explosive, and should therefore be handled with care. Recently, a new type of simple, inert unit suitable for methylation of small samples has been developed [52]. The gaseous reagent is bubbled through a solution of the drug, until a persistent yellow colour indicates completeness of methylation, or a solution of diazomethane in a suitable solvent is prepared and added in excess to the analyte solution.

Dry diethyl ether has frequently been used as the solvent. In many cases, however, much higher reaction rates are obtained when *ca* 10% of methanol is added to the solution. Other solvents such as dioxane (with methanol) and tetrahydrofuran have been used. Excess reagent is most easily removed by evaporation in a stream of nitrogen. Ion-pair extraction of the acidic compound, followed by diazomethane methylation, was applied to the analysis of apovincaminic acid [53].

Methylation with diazomethane is usually a smooth and rapid procedure under mild conditions, but side reactions have been reported. Occasionally, other diazoalkanes have been used. Arylmethyl esters of organic acids have been prepared with e.g. phenyldiazomethane and with phenanthryl diazomethane [55]. Trimethylsilyldiazomethane is a stable and safer substitute for diazomethane; it has been used for the methylation of fatty acids prior to GLC analysis [56]. The alkylation rate with the higher diazomethanes can be considerably enhanced by the addition of boron trifluoride to the reaction mixture.

Methylation with dimethyl sulphate. Complete methylation of acidic compounds is usually achieved by heating at 60–70°C for 5–10 min the compound(s) to be derivatized, aqueous potassium carbonate (5–10%) and methanol, with a small amount of dimethyl sulphate. Non-aqueous systems have also been applied. The methylation reaction is a base-catalysed nucleophilic substitution reaction of the deprotonated acid, Y^-, with the reagent:

$$Y{-}H + OH^- \rightarrow Y^- + H_2O$$

$$Y^- + H_3C{-}OSO_2(OCH_3) \rightarrow CH_3Y + CH_3SO_4^-.$$

Isolation of the compound prior to GLC, e.g. by evaporation of the solvent and extraction of the residue with a suitable apolar solvent, is usually required. Continued heating after completion of the reaction may lead to decreased derivatization yields, as was demonstrated with several barbiturates [57]. Instead of dimethylsulphate, bis(2-chloroethyl)sulphate has been used for the alkylation of barbiturates; the derivatives were determined by electron capture detection [58].

Alkylation with N,N-*dimethylformamide dialkylacetals.* *N,N*-Dimethylformamide dialkylacetals (DMF dialkylacetals) are well known alkylating reagents for a large number of acidic drugs. The reaction proceeds by an S_N2 mechanism with degradation of the leaving group:

$$\text{Y—H} + \text{H}_2\overset{\overset{\text{R}}{|}}{\text{C}}\text{—O—}\overset{\overset{\text{O—CH}_2\text{—R}}{|}}{\text{CH}}\text{—N(CH}_3)_2 \longrightarrow \text{Y—CH}_2\text{—R} + (\text{CH}_3)_2\text{N—CH=O} + \text{R—CH}_2\text{OH}$$

Complete alkylation of the acidic compounds of interest, Y–H, is generally achieved by heating for a short period at 60–100°C, either with the pure DMF dialkylacetal or with a suitable solution of the reagent, e.g. in chloroform or acetonitrile. The more commonly encountered reagents possess methyl, ethyl, propyl or butyl groups. Water must be excluded from the derivatization mixture. If necessary, the excess reagent can usually be removed by evaporation under a stream of nitrogen. The alkylation of a number of sulfonamides with various reagents in acetone has been investigated [59]. On-column flash heater derivatization is frequently possible too, with quantitative yields. Examples are the on-column methylation of cannabis constituents [60] and barbiturates [61], and the on-column alkylations of barbiturates [62]. With bifunctional nucleosides such as imides, the *N*-alkyl derivatives are usually obtained. In the case of the barbiturates, however, evidence has been presented that the dialkylacetals at the 2-position are formed [61, 63].

Pyrolytic alkylation. Acidic compounds react with quaternary ammonium hydroxide. Upon injection into the heated port of a gas chromatograph an alkyl derivative of the compound is produced through nucleophilic attack of the acid anion on one of the methyl group carbons of the quaternary ammonium cation. The reactions involved in the methylation of acid compound, YH, with tetramethylammonium hydroxide are:

$$\text{Y-H} + (\text{CH}_3)_4\text{N}^+\text{OH}^- \xrightarrow{25°\text{C}} \text{Y}^-\cdot\text{N}^+(\text{CH}_3)_4 + \text{H}_2\text{O}$$

$$\text{Y}^-\cdot\text{N}^+(\text{CH}_3)_4 \xrightarrow{250\text{–}350°\text{C}} \text{Y-CH}_3 + \text{N(CH}_3)_3$$

$$(\text{CH}_3)_4\text{N}^+\text{OH}^- \xrightarrow{250\text{–}350°\text{C}} \text{CH}_3\text{-OH} + \text{N(CH}_3)_3$$

The leaving group, $N(CH_3)_3$, is the main side-product of this reaction; due to its volatility no serious interference normally occurs with the peak of the methylated analyte. The reagents commonly used are methanolic or aqueous solutions of tetramethylammonium hydroxide (TMAH) and phenyltrimethylammonium hydroxide (TMPAH); leaving group, *N,N*-dimethylaniline. TMPAH has largely supplanted TMAH, because the reaction may proceed smoothly at lower temperatures, due to the better leaving group ability of *N,N*-dimethylaniline. Lower injection port temperatures mean less risk of side reactions. Another methylating agent, (*m*-trifluoromethylphenyl)trimethylammonium hydroxide (TMTFTH), is claimed to be superior to TMPAH. Methanolic or aqueous solutions of these reagents are comparatively stable when stored at 5°C or below. As expected, the reagent with the best leaving group, TMTFTH, is the least stable one.

Sometimes reagents with longer alkyl chains such as tetrabutylammonium hydroxide (TABH) have been used [64]. New types of flash alkylation reagents were introduced by Butte *et al.* [65, 66], who investigated the potential of trialkylsulphonium and trialkylselenium hydroxides for the pyrolytic alkylation of acidic compounds. Many acidic drugs have been derivatized by this simple and rapid procedure. Pyrolytic alkylation has been particularly popular in the GLC analysis of barbiturates [e.g. 67–68] and hydantoins and other cyclic imides and amides [16, 69–72], mainly due to its simplicity.

There are, however, some disadvantages with this technique. A major defect is the occurrence of isomerization or degradation reactions due to the strongly alkaline conditions and high temperatures. A well-known example is phenobarbital, which upon injection as a TMAH or TMPAH solution, generally gives rise to two or more derivatives. One of these is the expected *N,N'*-dimethyl derivative; the other, the so-called 'early phenobarbital', is eluted before the dimethyl derivative and arises from base-catalysed cleavage of the ring structure of phenobarbital [73].

A special reaction which cannot be fitted in either of the derivatization classes discussed above is the permethylation of primary aromatic amines with formaldehyde and sodium borohydride [74].

Acetylation Reactions

Acetylation reactions in derivative formation for GLC generally follow the route:

$$R-\overset{\overset{O}{\|}}{\underset{\underset{Y^{\ominus}}{}}{C}}-X \;\overset{Y^{\ominus}}{\rightleftharpoons}\; R-\overset{\overset{O^{\ominus}}{|}}{\underset{\underset{Y}{|}}{C}}-X \;\overset{-X^{\ominus}}{\rightleftharpoons}\; R-\overset{\overset{O}{\|}}{\underset{\underset{Y}{|}}{C}} + X^{\ominus}$$

The reaction pathway is normally nucleophilic addition/elimination via a tetrahedral intermediate, resulting in overall substitution of nucleophile $Y^{\ominus}$ for leaving group X [17]. Compounds (drugs) containing one or more of a range of different functional groups, usually possessing a replaceable hydrogen atom, can act as the nucleophile $Y^{\ominus}$, e.g. alcohols, enols and phenols, thiols, amines and amides.

Another possibility, much less common in chromatographic derivatization, is the addition of an acylgroup, R–CO–, across a double bond or an aromatic ring. The acylating reagents, R–CO–X, possess different relative reactivities, depending on the electron-donating or withdrawing power of the leaving group towards the carbonyl atom, on the ability of X as a leaving group, and on the electronic and steric effects of R. Generally the type of nucleophile, $Y^{\ominus}$, does not influence the relative reactivity of the acylating reagents. The following types of reagents have been used: acyl chlorides (acylhalides), acid anhydrides, activated acylamides and chloroformates.

Acyl chlorides and acid anhydrides

Because -Cl is a good leaving group, acyl chlorides are extremely reactive and therefore suitable for compounds that are not easily acylated. In polar solvents with good ion-solvating properties the reaction with acyl chloride may proceed via an S_N1 type mechanism, the rate-limiting step being the loss of Cl^-, followed by a rapid reaction of

the acylcarbonium ion, R–C^{+}=0, with the nucleophile. Acyl chlorides are very susceptible to hydrolysis. Acid anhydrides, R–CO–OCOR, are also able to react with comparatively weak nucleophiles such as water and aliphatic alcohols, though less avidly than acyl chloride; RCO^{-}_{2} is a poorer leaving group than Cl^{-}.

Of the reagents with non-halogenated acyl groups, acetic anhydride is the usual first choice. Acyl groups with longer alkyl chains yield increasingly less volatile derivatives and are therefore generally less desirable. Perfluorinated acyl derivatives hardly suffer from loss of volatility with increasing chain length. These reagents are frequently used in combination with electron capture detection (see below). Reactions with acid chlorides or anhydrides can be performed by treating the drug with the pure reagent, or with reagent diluted with a suitable solvent, such as pyridine, tetrahydrofuran, dichloromethane or toluene. The choice of solvent can dramatically influence the derivative yield, as has been shown for the acylation of clonidine with heptafluorobutyric anhydride in ethylacetate or acetonitrile [75]. With acyl chlorides a basic proton acceptor is usually needed in order to neutralize the hydrochloric acid produced in the reaction and thus force the reaction to completion. *N*-Methyl imidazole [76] and 4-dimethylaminopyridine [77] have been used for this purpose in reactions with acid anhydrides. Their catalytic power is much better than that of pyridine.

Quantitative reactions with anhydrides can often be performed without a basic catalyst. Removal of the excess reagent prior to GLC analysis is often necessary, because the reagent may react with active groups of the stationary phase or corrode the chromatograph. Another potential problem is detector overloading by excess reagent, particularly with the electron capture detector. Acid chlorides and anhydrides can frequently be removed by evaporation under nitrogen. Many acylated compounds are very susceptible to hydrolysis, and even nitrogen purging may cause loss of derivative in extreme cases. Storage of the derivatives is therefore best done in organic solvents with excess of the reagent. Some derivatives are more stable when in contact with water, e.g. the heptafluorobutyryl derivatives. Acylation reactions have been performed in aqueous solutions in the presence of a huge excess of reagent. This can be very convenient for the derivatization of highly polar compounds that cannot be extracted into an apolar organic solvent. Examples are the benzoylation of TRIS [78] and the acetylation of phenol [79] and primary and secondary amines [80]. Extractive alkylation in two-phase liquid–liquid systems has been applied, e.g. in the pentafluorobenzoylation of primary and secondary amines [81] and the propionylation of some drugs of abuse [82].

Activated amides

In contrast to normal amides, the *N*-acyl imidazoles are comparable to acid anhydrides in their acylating power. This high reactivity stems mainly from the contribution of the lone pair electrons of the ring nitrogen connected with the acyl group to the electron cloud of the imidazole ring, increasing its aromaticity. This prevents delocalization of the lone pair electrons on the nitrogen into the carbonyl group, which is consequently less negatively charged than in normal amides and more susceptible to nucleophilic attack.

Acylation reactions have also been performed with bis(acylamides), such as *N*-methylbis(trifluoroacetamide) [e.g. 83]. Due to the strong electron withdrawing properties of the trifluoracetyl group the lone pair electrons on the nitrogen atom are again less available for delocalization into the carbonyl group.

Acyl imidazoles can carry halogen atoms for better ECD response, e.g. trifluoroacetyl imidazole. *N*-acylimidazole reagents have very different acylating power depending on

the compounds to be derivatized. Thus, the reaction conditions can vary considerably. The reactions are mostly carried out under non-aqueous conditions. The reaction by-product is imidazole. The reaction medium is therefore not acidic as with anhydrides (without base catalyst); the acylimidazoles are thus better suited to the acylation of compounds of which the derivatives undergo degradation in acidic media. Removal of the excess reagent is less easy to perform by evaporation under nitrogen than with the anhydrides because of the lower volatility of the acylamides. Bis(acylamides) are also reacted in organic solvents (e.g. acetonitrile) with the compounds to be derivatized. The by-products formed are volatile and inert acid amides, which can be more easily removed by evaporation than the acylimidazoles.

Chloroformates

Acylation of phenols and amines has been performed with the chloroformates, RO–CO–Cl, in which R can be an alkyl or an aryl moiety and Cl is the leaving group. As expected, the reaction rate increases when R contains electron-withdrawing substituents; the rate constant for the reaction with primary or secondary aliphatic amines was found to be an order of magnitude higher when 2,2,2-trichloroethylformate was used as the reagent instead of ethylchloroformate [84]. Chloroformates can also be used to convert tertiary amines into acylated derivatives (carbamates) according to the following overall reaction

$$RO{-}CO{-}Cl + R_3{-}N\begin{matrix} R_1 \\ R_2 \end{matrix} \longrightarrow RO{-}CO{-}N\begin{matrix} R_1 \\ R_2 \end{matrix} + R_3{-}Cl$$

This reaction is also supposed to be initiated by nucleophilic attack of the non-protonated amine on the carbonyl carbon atom. The intermediate formed yields the carbamate and a by-product, R_3–Cl. It should be borne in mind that carbamate formation may occur with the unintended group of the amine; this happened in the case of promethazine (and a few related compounds), which yielded 10-(2-chloropropyl)-phenothiazine as the side-reaction product after derivatization with chloroformates [85]. Reactions with chloroformates have been performed under varying conditions: in non-polar organic solvents at elevated temperature with a base catalyst [e.g. 86]; in buffered aqueous solution (e.g. aminophenols) [87, 88]; in two-phase systems with a buffered aqueous phase and dichloromethane as the organic solvent [89–91].

A special acylation reaction, in which no side product is formed, has been applied by Farkas *et al.*, who derivatized fatty alcohols with a ketene, generated from acetone by pyrolysis, to yield the acetylated derivatives [92].

On-column acylation

On-column acylation techniques have been applied occasionally, primarily to provide additional data for drug identification. For example amphetamine, methylamphetamine and related compounds have been analysed as their trifluoracetyl derivatives [93]. On-column acylation with acid chlorides and acid anhydrides has the distinct disadvantage that acids are released in the chromatographic system.

N-Acylimidazoles offer considerable advantages over acyl chlorides and acid anhydrides because the by-product of the reaction is the inert imidazole. On the other hand, the acylimidazoles are much less volatile than the corresponding acid chlorides and

anhydrides and will therefore more frequently interfere with the peaks of the analyte derivatives. *N*-(Trifluoroacetyl)imidazole and *N*-(heptafluorobutyryl)imidazole have been used for the quantitative on-column acylation of amphetamine, ephedrine, codeine and morphine [94]. Solutions of the drug were simultaneously injected with the reagent; the derivatives were detected by FID. *N*-(Heptafluorobutyryl)imidazole was used as the derivatizing reagent in the quantitative on-column acylation of morphine and other opiates [95]. The derivatives were detected with an ECD. To prevent detector overloading, the excess reagent was removed with a pre-column venting system.

Acylation reactions do not always result in the formation of the expected derivatives and it is therefore hazardous to assume the nature of a certain acylation product. For example, on acylation of chlordiazepoxide, two acetyl groups are incorporated in the molecule, one through a rearrangement involving the $-CH_2-NO=$ moiety [96].

Silylation

Silylation reactions are the oldest and most popular derivatization reactions for GLC analysis of drugs and other compounds and are, despite some obvious disadvantages, still very much in use in pharmaceutical and biomedical analysis. Silylation of organic compounds is in some respects comparable to alkylation. The active hydrogens of functional groups are replaced by substituted silyl groups (instead of an alkyl group). As in many alkylation reactions the silylation reaction is considered to proceed by an S_N2 mechanism [97].

$$H\text{—}Y + R\text{—}SiR_2\text{—}L \longrightarrow \left[\overset{\delta^+}{HY}\cdots SiR_3 \cdots \overset{\delta^-}{L} \right] \longrightarrow Y\text{—}SiR_2\text{—}R + H\text{—}L$$

Thermostable, volatile derivatives with good GLC properties are usually formed in rapid, one-step reactions.

In general the ease with which functional groups are silanized with a particular reagent follows the order:

$$\text{alcohols} > \text{phenols} > \text{carboxylic acids} > \text{amines} > \text{amides}.$$

Primary alcohols are more reactive than secondary alcohols; secondary alcohols are more easily silylated than tertiary alcohols. Primary amines are easier to derivatize than secondary amines: after the substitution of 1 hydrogen atom of a primary amine for a silyl group, the introduction of a second silyl group is sterically hindered.

The silyl donor ability of the silylating reagents depends to a large extent on the leaving group ability of the active part of the reagent, L. Good leaving groups have the following properties: low basicity, the ability to stabilize a negative charge in the transition state during silyl ether formation, and little or no $(p \rightarrow d)\ \pi$ back-bonding between L and the silicon atom [97]. Most silylating reagents donate trimethylsilyl groups to the analyte molecule. Some of the well-known, frequently used reagents and a few recently developed reagents are listed in Table 1. TMCS and HMDS are unable to stabilize a partial negative charge in the transition state through resonance; consequently their silylating power is very modest compared with the potent silylating reagents TMSI

Table 1
Silylating reagents

Reagent	Abbreviation	Structure
Hexamethyldisilazane	HMDS	$(CH_3)_3Si—NH—Si(CH_3)_3$
N-Trimethylsilyldiethylamine	TMSDEA	$(CH_3)_3Si—N(C_2H_5)_2$
NO-Bis(trimethylsilyl)acetamide	BSA	$CH_3—C(O—Si(CH_3)_3)=N—Si(CH_3)_3$
NO-Bis(trimethylsilyl)trifluoroacetamide	BSTFA	$CF_3—C(O—Si(CH_3)_3)=N—Si(CH_3)_3$
N-Methyl-*N*-trimethylsilyltrifluoroacetamide	MSTFA	$CF_3—C(=O)—N(CH_3)—Si(CH_3)_3$
Trimethylsilylimidazole	TMSI	$(CH_3)_3Si—N$ (imidazole)
tert-Butyldimethylsilylimidazole	TBDMSI	$(CH_3)_3C—Si(CH_3)_2—N$ (imidazole)
Trimethylchlorosilane	TMCS	$(CH_3)_3Si—Cl$
Dimethylchlorosilane	DMCS	$H(CH_3)_2Si—Cl$
Chloromethyldimethylchlorosilane	CMDMCS	$(ClCH_2)(CH_3)_2Si—Cl$
Pentafluorophenyldimethylsilyldiethylamine	Flophemesyldiethylamine	$C_6F_5Si(CH_3)_2—N(C_2H_5)_2$
tert-Butylpentafluorophenylmethylchlorosilane	*tert*-Buflophemesylchloride	$(CH_3)_3C—Si(CH_3)(C_6F_5)—Cl$

and BSA, in which the leaving groups (imidazole, acetamide) can stabilize a partial negative charge. TMSI is an unusual reagent; it is a potent trimethylsilyl donor for hydroxyl groups, comparable in silylating power to BSTFA and BSA, but it does not react at all with aliphatic primary amines. An important consideration in the choice of silylating reagents is the volatility of the reagent and its side-products, the leaving groups formed during derivatization. The more volatile the reagent and its side-product, the less chance of interfering peaks in the chromatograms. BSA, BSTFA and MSTFA have similar silylating potencies, but MSTFA and its reaction by-product, *N*-methyltrifluoroacetamide, are more volatile than BSA and BSTFA and their by-products.

In addition to the much used trimethylsilyl donating reagents other alkyl-substituted silyl groups have been studied. An overview of trialkylsilylether derivatives other than TMS for GLC analysis and mass spectrometry was published by Poole and Zlatkis [13]. Dimethylsilyl derivatives are more volatile than trimethylsilyl derivatives, and may be useful for the derivatization of polyfunctional compounds. On the other hand, the dimethylsilylderivatives are even less resistant to moisture. Halogenated silyl derivatives, such as DMCS, CMDMCS and flophemesyldiethylamine, are mainly used for improved ECD response. A number of reagents carrying donor groups other than TMS have been developed, allowing better separation or more suitable MS fragmentation patterns than the TMS derivatives. Examples are allyldimethylsilyl derivatives of steroids [98]; alkoxydialkylsilyl derivatives of steroids and cannabinoids [99]; dimethylisopropylsilyl derivatives of prostaglandins [100]; and dimethylethylsilyl derivatives of bile acids and cholesterol [10].

It is common practice to use mixtures of silylating reagents. HMDS alone is a weak TMS donor towards alcohols and yields no derivatives with amines. However, with the addition of TMCS (1–10%) the derivatization yields are much improved. HMDS and TMCS in a pyridine solution is a very well-known silylating cocktail. TMCS is also a common additive to other reagents because of its catalytic properties. The mixture BSA/TMS/TMCS (1:1:1, v/v/v) is an extremely potent and general silylating cocktail.

The widespread use of TMS donating reagents is illustrated by two recent reports. In one of these, the retention indices of over 160 metabolically important organic acids, as their TMS derivatives, on OV-1 and OV-17 columns were compiled to help in the diagnosis of metabolic disorders [102]. The second report listed the retention times of 116 drugs, both the parent compounds and their TMS derivatives [103].

The silylating reaction conditions vary considerably and depend on the reactivity of the functional group to be derivatized and the degree of steric hindrance involved in the formation of the transition state. Highly reactive compounds can be derivatized by simply dissolving these in the reagent. If the compound is poorly soluble in the reagent a solvent, e.g. pyridine or *N,N*-dimethyl formamide is added. In the derivatization of bases trifluoroacetic acid has been used as a solvent. Heating of the derivatization mixture is sometimes necessary. Catalysts (other than TMCS) can also be added, e.g. sodium formate in the conversion of testosterone to its 3-enol, 17-bis-*tert*-butyldimethylsilyl ether derivative [104]. On-column silylation techniques have been applied for a number of compounds [e.g. 105, 106].

A group of compounds of special interest are the steroids, because of the markedly different reaction rates of their hydroxyl groups towards silyl reagents and also because steroid keto groups are prone to enol–TMS ether formation in the presence of TMCS catalyst. Many studies have been performed on the silylation reactions of steroids [e.g. 104, 107].

A serious drawback of many silylating reagents and their derivatives is their extreme susceptibility to hydrolysis. Water must generally be rigorously excluded from the reaction mixture and it is frequently not safe to remove the excess of reagent, e.g. by evaporation, prior to GLC analysis. However, some reagents have been developed that appear to be more resistant to hydrolysis. For example, *tert*-butyldimethylsilylated compounds are more stable than their TMS counterparts; *tert*-buflophemesyl derivatives are much more stable towards hydrolysis than the flophemesyl derivatives, allowing partitioning of these compounds in liquid–liquid systems of an organic solvent and an acidic or basic aqueous solution [108]. Silylating reagents and their derivatives

contaminate the FID detectors with silica deposits on the electrodes, changing the sensitivity of the detector or the response factors. BSTFA and other fluorinated silyl reagents appear to be less troublesome in this respect than many other reagents because a more volatile silicon tetrafluoride is produced.

Miscellaneous Methods

Cyclization reactions

Many compounds possess two functional groups in close proximity. With certain reagents these can be reacted to form cyclic derivatives. An extensive review of cyclization reactions in GLC appeared recently [15]. The present overview is therefore restricted to some important examples. Cyclic derivatives can sometimes be formed by the reagents discussed above; examples are the formation of cyclized silyl derivatives of β-hydroxy amines [109], the cyclization of biguanides with carboxylic acid anhydride [110] and the cyclization of clonidine with pentafluorbenzylbromide [111].

Cyclic boronate formation

The boronic acids have been frequently used as cyclization regents, because they cover a wide range of applications; they can react with, for example, diols, α-hydroxy-carboxylic acids, vicinal hydroxy-keto groups and β-hydroxyamines [2]. With suitable solvents, e.g. pyridine or acetone, reactions usually proceed rapidly at room temperature. With diols the reaction is as follows:

$$\begin{matrix} -\overset{|}{C}-OH \\ -\underset{|}{C}-OH \end{matrix} + \begin{matrix} HO \\ HO \end{matrix}\!\!>\!B-R \longrightarrow \begin{matrix} -\overset{|}{C}-O \\ -\underset{|}{C}-O \end{matrix}\!\!>\!B-R + 2H_2O$$

An added advantage of boronic acids can be the improved sensitivity of detection with the thermionic detector and flame photometric detector. Halogenated boronic acids suitable for use with the ECD have been developed [10, 15]. An example of cyclic boronate formation is the analysis of diastereomeric alkanol amines [112]. Other types of cyclization reagents have been used, e.g. pentafluorobenzaldehyde, which forms an oxazolidine derivative with phenylpropanolamine [113]. On-column cyclization with *n*-butyl boronic acid has been used for the cyclization of methylated pyridoxine [114].

Oxime formation

Keto groups do not normally present difficulties in gas chromatography. But it may be desired to block a keto group in order to prevent its participation in other reactions, such as the enolization during silylation of steroid hydroxyl groups. Another motive could be the introduction of ECD-sensitive labels. Both these goals have been realized by the development of pentafluorobenzyloxyamine [115, 116]. The general oxime reaction scheme, as shown below, is an example of a nucleophilic addition of the hydroxylamine derivative with the carbonyl group, followed by the elimination of water:

$$>C{=}\ddot{O}: + H_2\ddot{N}{-}OR \rightleftharpoons -\overset{\bar{O}}{\underset{|}{\overset{|}{C}}}-\overset{+}{N}H_2{-}OR \rightleftharpoons -\overset{OH}{\underset{|}{\overset{|}{C}}}-NH{-}OR$$

$$\rightleftharpoons >C{=}N{-}OR + H_2O$$

Derivatization is frequently carried out in pyridine with a large excess of the desired reagent. Heating may be necessary. Oxime derivatives have been prepared for (low molecular weight) aldehydes and ketones, e.g. as their pentafluorobenzyl oximes [117]. Monoamine oxidase activity is frequently assessed by measuring the hydrogen peroxide formed during the enzymatic reaction. In a new method for H_2O_2 quantitation [118] the hydrogen peroxide is converted into formaldehyde by the enzyme catalase; the formaldehyde is reacted with pentafluorobenzyloxyamine, and the resulting product is assayed by GLC–ECD.

Several other derivatization methods have been described, e.g. the conversion of metoprolol and related compounds into an oxazolidine derivative [119]. These reactions are not necessarily chemical methods; enzymatic conversion of compounds provides a selective method as a step in the discrimination between closely related compounds, such as some steroids [120].

Multiple derivatization reactions

Molecules with more than one functional group may have to be derivatized by sequential reactions to produce good chromatographic properties. Much work has been done on some important groups of biomolecules, notably the amino acids and the sugars. A well known derivatization procedure for the GLC analysis of amino acids is alkylation of the carboxylic acid function followed by acylation of the amine function. An example is the formation of *N*-heptafluorobutyryl isobutyl derivatives [121]. The conversion of amino acids may be further complicated by the presence of other functional groups. Some drugs also possess primary amino groups and a carboxylic function. Tranexamic acid is a synthetic amino acid with antifibrinolytic properties. An assay based upon arylation of the amino function with 2-nitro,4-trifluoromethyl fluorbenzene in borate buffer pH 9.4, followed by extractive alkylation of the carboxylic group [122], was developed.

Many analytical derivatization reactions have been used in the investigation of prostaglandins and related compounds. These compounds are C_{20} unsaturated carboxylic acid structures with one or more hydroxyl groups and/or keto groups. The multiple functionality and the liability of the prostaglandins make derivatization unavoidable for GLC analysis. An overview of the methods used is given by Knapp [3]. Some recently published studies describe the conversion of prostaglandins into pentafluorobenzyl-trimethylsilyl derivatives [123, 124] or trimethylsilyl-methoxime derivatives [125]. A triple derivatization procedure has been reported involving the alkylation of the carboxylic function, the conversion of the ketofunction into an oxime and the silylation of the hydroxyl groups [126].

Numerous other sequential derivatization reactions have been reported, such as a flash heater derivatization with a double injection technique [127]. Many more can be devised. Sometimes these more involved procedures are necessary, but it should be borne in mind

that each extra step in the analytical procedure enhances the number of manipulations and therefore the risk of systematical errors. If possible, alternative analytical methods (e.g. HPLC) deserve consideration.

Detection-Oriented Derivatization

The primary goal of derivatization is frequently the introduction of a particular label into the compounds of interest, which will allow a more sensitive and selective mode of detection than can be obtained with the parent compounds. Apart from the normal flame ionization detector, the most frequently applied detectors in pharmaceutical and biomedical analysis are the electron capture detector, the thermionic detector and the mass spectrometer. The labelling of compounds to be analysed with these detectors is discussed below, and some recent developments are described.

Electron capture detection

In the previous sections many reagents have been mentioned which confer electron-capture properties upon the molecules to be derivatized, allowing the very sensitive detection of these compounds by the ECD. Although understanding of electron capture processes in the ECD is far from complete, sufficient information has been gathered to provide some general rules, which should be borne in mind when looking for suitable ECD-oriented derivatization reagents [9, 10].

The response of the electron capture detector is markedly temperature-dependent. Electron-absorbing compounds (AB) can be divided into two groups; the first group can attach a thermal electron (resulting from the collision between β-radiation of the source and gas molecules of the carrier gas) without dissociation:

$$AB + e^- \rightleftarrows AB^-.$$

Molecules in the second group are dissociated when capturing an electron:

$$AB + e^- \rightleftarrows A^- + B.$$

Compounds capturing electrons in a non-dissociative manner have their highest detector response at the lowest possible detector temperature, because high temperatures favour the release of the captured electron. On the other hand, compounds capturing electrons in a dissociative manner show their highest detector response at the highest practical detector temperature, because the bond breaking process associated with the electron attachment proceeds more easily at higher temperatures. Optimization of detector temperature is therefore very important when comparing the detector responses of different derivatives. A second important consideration is that the mechanism of electron capture and the optimum temperature of derivatives of different compounds with the same electrophoric tag (e.g. pentafluorophenyl) need not be similar, although this is frequently true for derivatives of a homologous series. For instance, an unexpectedly high detector response is obtained with pentafluorophenyl derivatives when the captured electron-molecule entity can be stabilized by resonance between the pentafluorophenyl ring and some part of the molecular framework of the compound derivatized. The halogens and the nitrogroup are the most suitable electrophores in ECD-oriented reagents. Some of the molecular features which determine the detector

response towards compounds with these electrophores are as follows. A high ECD response is shown by halocarbon compounds, nitroaromatics and some conjugated compounds. The detector response towards the halogen-containing compounds decreases in the order I > Br > Cl > F (which is the reverse order of the volatility) and increases with multiple substitution on the same carbon atom. Perfluorinated halocarbon-containing reagents confer good electron capturing properties upon the derivatized molecules, which show very little increase in boiling point compared to the compounds labelled with the corresponding hydrocarbon-containing reagent. Of the various silylating reagents, the flophemesyl reagents offer the best compromise between derivative volatility and detector sensitivity. The flophemesyl derivatives generally show a dissociative mechanism of electron capture. High detector temperatures are therefore required, and also minimize contamination of the detector by coeluted products from extracts of biological samples. The perfluoroacyl derivatives show retention times similar to those of the TMS ether derivatives on nonpolar stationary phases and possess good GLC peak shape and stability, in contrast to many chloroacetyl and bromoacetyl derivatives. Heptafluorobutyryl derivatives are very prominent in ECD derivatization because of the limited increase in volatility of the derivatized compounds and the presence of the carbonyl group which helps to stabilize the captured electron through resonance. The relative responses of haloacylderivatives can be very different, depending on the nature of the compounds derivatized and the detector temperature [128]. The pentafluorophenyl group can be introduced by several reagents, e.g. pentafluorobenzyl chloride, pentafluorobenzyl bromide and pentafluorobenzyl chloroformate [10]. The mechanism of electron capture of these compounds is assumed to be nondissociative. Halogen-containing boronic acids for the derivatization of some bifunctional compounds have also become available. An example is 4-iodobutane boronic acid [129].

The extremely low detection limits attainable with the electron capture detector are well illustrated by the response obtained with, among others, some derivatized iodothyronines and *N,N*-dipentafluorobenzoylpentafluoroaniline [130]; these compounds showed detection limits at attogram levels. The development of new ECD labels which allow even lower detection limits than those currently obtained may only be a matter of time.

Thermionic detector

The NP-sensitive thermionic detector has gained wide popularity in pharmaceutical analysis. Although it does not possess the same potential for sensitive detection as the ECD, the detection limit for suitable compounds is in the femtomole range. The detector is especially sensitive towards many phosphorus-containing compounds, and a number of phosphorus-carrying labels have been synthesized, such as *o,o'*-dimethyl-α-hydroxyphosphonate, which reacts readily with carboxylic acids to yield the corresponding esters [131]. The reaction is carried out in benzene with pyridine, and with dicyclohexylcarbodiimide as a coupling agent. More recently a reagent has been introduced for the derivatization of bifunctional compounds, ethylphosphonothioic dichloride [132].

NP-detector-oriented detection has not yet gained wide acceptance in pharmaceutical and biomedical analysis, possibly because of the difficulties of removing excess reagent and the inability of the thermionic detector to match the sensitivity of the ECD. A new approach is the conversion of carboxylic acids into secondary amides, which possess acceptable detector response suitable for many applications [133]. The derivatization

reaction is selective, simple and rapid, the excess reagent can be easily removed and the resulting amides are quite stable and show excellent chromatographic behaviour.

Derivatives for GC–MS studies

Obviously many of the same motives apply to GC–MS-oriented derivative formation as for other GLC systems, in that the GLC separation prior to MS detection requires the compounds under investigation to be volatile, stable and to show (nearly) symmetrical peaks in the chromatograms. There are two general ways in which derivatives can be used to affect mass spectra [12]. The first is to introduce mass changes by isotopic or other minor substitution without the aim to alter fragmentation pathways. A well known application is isotope substitution with three or four deuterium atoms to provide a compound for use as an internal standard in the determination of the unlabelled parent compound. These derivatives can be added in large amounts to suppress adsorption of the sample compound which may be present in very low amounts. The second approach aims at the introduction of a particular mode of fragmentation either to obtain more information for structural elucidation or to produce ions of a specific nature (or mass). The parent compounds may produce molecular ions in low abundance (or not at all). Derivatives such as the TMS derivatives of alcohols often yield much increased abundances of their molecular ions; the ions formed by loss of particular label connected radicals, such as $(M-CH_3)^+$, are also indicative of the molecular ion. For instance, the *tert*-butyldimethylsilyl ethers yield prominent ions at *m/e* [M-57] [13, 134].

Miscellaneous

Silicon selective detection following GLC separation has been applied to the determination of salicylic acid in urine [135]. Salicylic acid was extracted from urine and silylated with HMDS in acetone. The derivative was detected by use of the negative response mode of a silicon-selective hydrogen atmosphere flame ionization detector [136]. Another approach is the element specific derivatization for the enhanced detectability of selected compounds or derivatives by the microwave emission detector [137]. An example is the boron-specific detection of diols, derivatized with *n*-butylboronic acid [138].

The Separation of Enantiomers Via Diastereomer Formation

The chromatographic separation of pairs of enantiomers can be approached in two ways: (1) by the use of a chiral stationary phase; (2) by derivatization of the enantiomers with optically active and pure reagents forming diastereoisomers.

In contrast to the situation in HPLC, the derivatization methods in GLC for this purpose have not gained much popularity, mainly because of the difficulty of obtaining optically active and pure reagents of sufficient volatility [139]. An overview of methods and procedures has been provided by Knapp [3]. Some examples suffice to illustrate the types of reagents used. Optically active reagents have been applied for the separation of chiral amines (e.g. with (*S,S*)-*N*-trifluoroacetylproline anhydride [140]), amino acids and arylalkyl amines (as their isopropoxypropionyl derivatives [141]) and sugars (e.g. as their diastereoisomeric dithioacetals [142]). The ephedrine enantiomers have been separated via derivatization with L-α-chloro-isovaleryl-chloride [143]. Diastereomeric urea derivatives were formed between racemic propranolol and *S*-(-)-/-phenylethyl isocyanate [144].

Derivatization as a Part of the Bioanalytical Procedure

Fitting the derivatization step into a complete bioanalytical procedure in a harmonious way is an analytical challenge of the first order. Aqueous biological matrices, such as plasma or serum, urine and saliva, contain a multitude of endogenous compounds that are often present in much higher concentrations than the drug or its metabolites to be analysed, such as the proteins in plasma samples. Many of these endogenous compounds have reactive sites which can participate in derivatization reactions, and can thus interfere with the analysis of the compounds of interest if sufficiently separated by the ensuing GLC procedure. Furthermore, most derivatization reactions are best performed in non-aqueous solvents; the use of an anhydrous medium may even be obligatory. The injection of (semi)aqueous samples into the gas chromatograph is frequently detrimental to the column. These factors necessitate some form of sample clean-up prior to or in conjunction with the derivatization reaction and the GLC analysis. The most direct approach is the combination of an extraction and a derivatization step. Extractive derivatization procedures, particularly extractive alkylation methods, are very popular; the Appendix contains a fair number of procedures under this heading. Frequently, however, extra sample clean-up steps must be included in the procedure to prevent the appearance of interfering peaks in the chromatograms. Examples of these clean-up steps can be the preliminary extraction of the drug from the sample with an organic solvent, or the extraction of the drug from the sample followed by back-extraction into an aqueous phase of suitable pH. Another approach is the extraction of the drug first, followed by a derivatization procedure. Although less direct, this approach has some potential advantages over the extractive derivatization methods. One is the choice of the extracting solvent. The most apolar solvent with which the drugs can be extracted with sufficient efficiency is usually the best one, because of the gain in selectivity of the procedure. Many endogenous compounds are very polar and cannot be extracted with apolar solvents. For difficult separations multiple extractions may be needed. The subsequent derivatization step can sometimes be performed directly in the extracted drug solution, but usually concentration of the sample through evaporation of the organic solvent comes first. The residue is then available for derivatization under optimal conditions. An elegant extraction–concentration procedure suitable for the extraction of many acidic drugs prior to their alkylation consists of the extraction of the drug with toluene followed by back-extraction into a very small volume of a methanolic solution of tetramethylammonium hydroxide solution. Part of this solution can either be injected directly into the gas-chromatograph for flash heater methylation of the drugs, or can be mixed with *N,N*-dimethylacetamide and alkyl iodide in a pre-column alkylation step [38, 40]. Instead of liquid–liquid extractions, the adsorption of the compounds of interest onto a solid surface, such as charcoal, is also sometimes applied. After precipitation of the drug–adsorbant complex the drug is eluted from the adsorbant with a suitable solvent.

Chromatographic sample clean-up procedures have become very popular with the introduction of small sized prefabricated adsorption columns with varying stationary phases, such as silica gel or C_{18} modified silica gel. The choice of the detector and the column must also be compatible with the derivatization procedure. As a general rule, the derivatives must be stable under the prevailing chromatographic conditions. On the other hand, the chromatographic system must not be damaged by the introduction of aggressive chemicals from the derivatization mixture. Clean-up steps following the

derivatization reaction are therefore frequently unavoidable. The removal of excess reagent to prevent excessive detector response is a very general problem and has been mentioned in the previous sections. For the choice of the derivatization mixture clean-up the analyst must be aware of the physico-chemical properties of the constituents of the mixture. Very little is needed to cause appreciable loss of the minute amount of derivative formed in the reaction. The volatility and the stability of the derivatives are of paramount importance here and should be assessed carefully.

The Appendix lists a number of compounds for which derivatization procedures in combination with GLC analysis have been reported. Although the list is necessarily incomplete, it should provide guidance for the analysis of a wide range of drugs.

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Appendix

Under “COMPOUND(S)” the name of the drug or group of drugs for which a given procedure was originally designed is given. In some instances a single compound can be regarded as a model compound for a group of structurally related drug molecules. “SAMPLE” refers to the type and required volume of the biological sample (e.g. blood, plasma, saliva, serum, tissue, urine) for which the method is suitable.

Many differences are found in the clean-up procedures used when analysing drugs in biological materials. It is possible, however, to give an estimate of the type and number of clean-up steps in the pre-chromatographic sample treatment. Simple washings of samples or extracts, in which the analyte does not move into another phase, are not counted. The symbol “a” in the third column (CLEAN UP) denotes some form of protein removal from the biological material by ultrafiltration or by precipitation. The letter “b” stands for a single pre-chromatographic extraction step. Derivatization is performed either directly in the separated organic phase, following solvent extraction of the aqueous sample with an organic solvent, or after evaporation of the organic solvent and reconstitution of the residue in a more suitable solvent. The symbol “c” is used when more than one extraction step is involved, e.g. extraction and back-extraction. The letter “d” denotes some form of chromatographic clean-up step (thin-layer or column chromatography), whereas the letter “e” is used when the initial isolation of the drug is effected by adsorption onto a solid adsorbent. Extractive alkylation procedures with simultaneous extraction and derivatization are denoted by the symbol “f”. When a single extraction step precedes the extractive alkylation procedure, “g” is used, whereas “h” stands for methods in which more than one clean-up step (e.g. extraction and back-extraction) preceding the extractive alkylation is included. Clean-up procedures that do not fit into one of the above categories are denoted by the letter “i”.

Under the heading “DERIVATIZATION” the main features of the derivatization procedure are given: 1, the derivatization reagent; 2, the catalyst; 3, the solvent. A list of the abbreviations in this column is given at the end of the Appendix. The sign “/” is used for “or”. The type of derivatization method is mentioned in the column “METHOD” in which “A” stands for an alkylation reaction, “C” denotes an acylation reaction and “S” stands for a silylation method. All other derivatization methods are summarized by the letter “V” (various).

In the next column the stationary phase is mentioned on which the derivatives can be chromatographed and the symbol “C” denotes the use of a capillary column. Under the heading “DETECTION” the type(s) of detector used in the analysis are given. The last column refers to the original publication in which the method was described.

ANALGESIC (NARCOTIC AND NON-NARCOTIC) AND ANTI-INFLAMMATORY DRUGS

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Acetaminophen	plasma .5 ml	i	PFBB, K_2CO_3, acetone	A	3% SP-2100	ECD	145
	urine .5 ml	i	PFBB, K_2CO_3, acetone	A	3% SP-2100	ECD	145
	serum 1.5 ml	b	TMPAH	A	3.8% SE-30	FID	146
	plasma .1 ml	-	AA, N-methylimidazole, chloroform	C	3% Apolane-87	FID	147
	serum .5 ml	b	TFAA, toluene	C	3% OV-1 / OV-210	FID	148
Acetanilide derivatives	liver	b	alkyl iodide, TMAH, acetone	A	3% XE-60	MS	39
Acetylsalicylic acid	plasma .1 ml	f	TPA, benzyl bromide, DCM	A	1.5% OV-17	MS	149
Alclofenac	plasma 2 ml	b	DMF-dimethylacetal	A	3% OV-17 / Poly A-103	FID	150
	urine 2 ml	b	DMF-dimethylacetal	A	3% OV-17 / Poly A-103	FID	150
Anti-inflammatory analgesics	urine 10 ml	a	AA, pyridine	C	5% OV-101	MS	151
Benzarone	plasma 1 ml	b	TFAA, ACN	C	1% OV-17	MS	152
Benzbromarone	plasma 1 ml	b	TFAA, ACN	C	1% OV-17	MS	152
Bromobenzarone	plasma 1 ml	b	TFAA, ACN	C	1% OV-17	MS	152
Ciramalol	plasma 1 ml	c	PFBC, pyridine, benzene	C	2% OV-101	ECD	153
Diclofenac(M)	urine .001 ml	f	THA, Me-I, DCM	A	C- Carbowax 40M	ECD	154
(M)	urine 1 ml	g	TBA, Me-I, DCM	A	3% OV-17	ECD	155
	plasma .5 ml	c	trifluoroethanol, H_2SO_4/ methanol, H_2SO_4	A	1.5% OV-17	ECD	20
	plasma 1 ml	c	PFPA, hexane	C	3% OV-1	MS	96
Flufenamic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
	plasma 1 ml	b	DAM, ether	A	3% OV-17		156
Flurbiprofen	plasma .5 ml	c	DAM, ether	A	1.5% OV-17	MS	157
	plasma .05 ml	b	BMP, diethylamine, DCM, ACN	V	3% SP-1000	NPD	133
Ibuprofen	serum .1 ml	i	PFBB, K_2CO_3, acetone	A	10% 3-Cyanopropyl-silicone	ECD	158
	serum .2 ml	b	DAM, ether	A	3% OV-17	MS	159
Indomethacin	plasma 1 ml	b	DAM, acetone	A	3% OV-17	ECD	160
	urine 1 ml	b	DAM, acetone	A	3% OV-17	ECD	160
	plasma .5 ml	b	diazopropane, heptane	A	3% OV-1 / XE-60	ECD	161
	plasma 1 ml	b	PFBB, K_2CO_3, acetone	A	2% Dexsil-300	ECD	162
	urine 1 ml	b	PFBB, K_2CO_3, acetone	A	2% Dexsil-300	ECD	162
	plasma .5 ml	g	TBA, Pr-I, $NaHCO_3$, DCM	A	3% OV-1	ECD	161
	plasma .5 ml	g	TPA, Pr-I, DCM	A	3% OV-1/ XE-60	ECD	161
	serum .5 ml	f	THA, Et-I, DCM	A	3% E-350 / SE-52	ECD	163
	plasma .1 ml	b	PFPA, pentafluoropropanol	A	10% SP-2250	ECD	22
	plasma 1 ml	i	TFAA + hexafluoroisopropanol	A	2% OV-1	MS	164
Ketobemidone	plasma 2 ml	c	AA, TEA	C	C- SP-1000	MS	165
Ketoprofen	plasma .4 ml	f	TBA, Me-I, DCM	A	3% OV-17	ECD	166
Levorphanol	plasma 1 ml	b	PFBC, 4-dimethylaminopyridine, chloroform	C	3% Poly S-176	MS	167
Mefenamic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
	serum 2 ml	b	Bu-I, TMAH, DMA	A	3% SP-2250 DA	FID	168
Meperidine(M)	plasma 2 ml	c	TFAA	C	3% Poly I-110	FID	169
	urine .5 ml	b	TFAA	C	3% Poly I-110	FID	169
(M)	plasma 1 ml	c	HFBA, K_2CO_3, toluene	C	2% Carbowax 20M KOH	NPD	170
	urine 1 ml	c	HFBA, K_2CO_3, toluene	C	2% Carbowax 20M KOH	NPD	170
Methylsalicylic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Morphine(M)	plasma 1 ml	c	PFPA	C	C- OV-1	ECD	171
	plasma 1 ml	f	TBA, PFBB, EAC	A	2% OV-17	MS	172
	fluids 2 ml	c	PFPA, EAC	C		MS	173
(M)	urine 10 ml	c	BSA	S	3% OV-17 / OV-225	MS	174
Nalorphine	plasma 1 ml	f	TBA, PFBB, ethylacetate	A	2% OV-17	MS	172
	fluids 2 ml	c	PFPA, EAC			MS	173
Naloxone	plasma 1 ml	h	TBA, PFBB, DCM	A	3% OV-17	ECD	175
Naltrexone	plasma 1 ml	h	TBA, PFBB, DCM	A	3% OV-17	ECD	175
Naproxen	plasma	c	BSA, toluene	S	5% FFAP	MS	176
	plasma .05 ml	b	BMP, diethylamine, DCM, ACN	V	3% SP-1000	NPD	133
Niflumic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
(M)	plasma 1 ml	b	DAM, ethanol	A	3% OV-17	NPD	177
Oxepinac	fluids 1 ml	b	n-propanol, HCl	V	2% OV-17	MS	178
Oxyphenbutazone	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Pentazocine	plasma .2 ml	h	TBA, PFBB, DCM	A	3% Dexsil-300	ECD	179
	urine 1 ml	h	TBA, PFBB, DCM	A	3% Dexsil-300	ECD	179
(Nor)Pethidine	plasma .5 ml	c	trichloroethylchloroformate, TBA, toluene	C	3% OV-17	ECD	180
(Nor)Pethidinic acid	urine 1 ml	i	TPA, PFBB, DCM	A	3% OV-17 / 5%	ECD/ MS	181
Phencyclidine(M)	urine 5 ml	c	HFBA, benzene	C	3% SE-30	MS	182
Phenprofen	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Phenylbutazone	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Pirprofen	plasma 1 ml	b	DAM, ether	A	3% OV-101	ECD/NPD	183
	urine 1 ml	b	DAM, ether	A	3% OV-101	ECD/NPD	183
Probenecid	csf .1 ml	f	TBA, Me-I, DCM	A	5% OV-17	ECD	184
	plasma .1 ml	f	TBA, Me-I, DCM	A	5% OV-17	ECD	184
Salicylates	plasma .1 ml	b	BSTFA, ACN	S	2% OV-225	FID	185
Salicylic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Sulphipyrazone(M)	plasma 1 ml	c	Me-I, TMAH, DMA	A	2% OV-17 / 3% OV-225	ECD/ MS/ NPD	186
	urine 1 ml	c	Me-I, TMAH, DMA	A	2% OV-17 / 3% OV-225	ECD/ MS/ NPD	186
Tolmetin	plasma .05 ml	c	PFBB, K_2CO_3, EAC	A	3% XE-60	ECD	187
Zomepirac	plasma .1 ml	c	PFBB, K_2CO_3, EAC	A	3% OV-17	ECD/ MS	188
	serum	c	dimethylsulfide(on column)	A	OV-17	NPD	189

ANTIDEPRESSANTS

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Amitriptyline(M)	plasma 1 ml	c	TFAA, chloroform	C	3% OV-17	MS	214
(M)	serum 1 ml	b	TFAA, TEA, hexane	C	2% OV-17	MS	215
	plasma 1 ml	a	2,4-dichlorophenylchloroformate	C	3% OV-17	ECD	216
A 643C	plasma .1 ml	c	PFPA, hexane	C	5% OV-225	ECD	217
Desipramine	plasma .5 ml	c	AA, amylacetate	C	5% OV-17	FID	218
	plasma	b	DMF-diethylacetal	A	3% OV-17	MS	219
Desmethyldosepin	plasma	b	DMF-diethylacetal	A	5% OV-17	MS	219
Dothiepin(M)	plasma 1 ml	c	TFAA	C	3% OV-101	MS	220
Doxepin(M)	plasma 1 ml	c	TFAA	C	3% OV-17	MS	221
(M)	plasma 1 ml	b	TFAA, hexane	C	C- OV-17	NPD	222
Fluoxetine	plasma 1 ml	c	PFPA, benzene	C	3% SP-2100	ECD	223
Imipramine(M)	plasma 2 ml	b	($^2H_2$)formaldehyde, $NaBH_3$, methanol	A	1% SP-2250	MS	224
Indeloxazine	plasma .2 ml	c	HFBA, toluene	C	3% OV-225	ECD	225
Maprotiline	plasma 1 ml	c	HFBI	C	3% OV-17	ECD	226
	plasma .5 ml	c	AA, amylacetate	C	5% OV-17	FID	218
(M)	blood 1 ml	c	AA, pyridine	C	3% HI-EFF-8BP	NPD	227
Meclopramide	plasma 1 ml	c	HFBI	C	3% OV-17	ECD	226
Nomifensine	serum 1 ml	c	HFBA, EAC	C	3% OV-17	ECD	228
Nortriptyline	plasma 1 ml	a	2,4-dichlorophenylchloroformate	C	3% OV-17	ECD	216
	plasma	b	DMF-diethylacetal	A	3% OV-17	MS	219
	plasma 1 ml	c	TFAA, chloroform	C	3% OV-17	MS	214
Phenelzine	plasma 2 ml	c	pentafluorobenzaldehyde	C	1% OV-17	MS	229
Protriptyline	plasma	b	DMF-diethylacetal	A	3% OV-17	MS	219
(M)	plasma 1 ml	c	TFAA, chloroform	C	3% OV-17	MS	220
Tranylcypromine	brain	c	AA + PFBC, toluene	C	3% OV-1 / C- OV-101	ECD	230
	plasma 4 ml	c	HFBA, EAC	C	C- OV-225	NPD	231
	urine .4 ml	c	HFBA, EAC	C	C- OV-225	NPD	231
Tricyclic antidepressants	serum 1 ml	b	TFAA, petroleum ether	C	3% OV-17	MS	232
	plasma 2 ml	b	HFBA, EAC	C	3% SP-2230	NPD	233
	plasma 2 ml	b	HFBA, EAC	C	C- SE-30	NPD	233
Trimipramine	serum 1 ml	c	PFB-chloroformate, Na_2CO_3, hexane	C	5% OV-17	ECD	234

ARYLOXYPROPANOLAMINES (β-blocking agents)

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Alprenolol	serum	.2 ml	b	HFBA, EAC	C	3%	OV-1	ECD	235
	plasma	2 ml	b	2,4-dichlorobenzeneboronic acid, 1,3-propanediamine, ACN	V	3%	OV-17	ECD	236
Aryloxypropanolamines	plasma	1 ml	b	n-butylboronic acid, Na-sulphoxide, EAC /	V	2%	OV-17	NPD	237
				phenylboronic acid, Na-sulphoxide, EAC	V	2%	OV-17	NPD	237
Atenolol	plasma	.1 ml	b	TFAA, ether	C	3%	OV-1	ECD	238
Befunolol(M)	urine	2 ml	d	BSTFA, TMSI	S	1.5%	OV-17	FID	239
Betaxolol	blood	.5 ml	c	HFBA, EAC	C	C-	OV-101	ECD	240
	urine	.5 ml	c	HFBA, EAC	C	C-	OV-101	ECD	240
Bevantolol	plasma	.5 ml	c	HFBA, EAC	C	3%	OV-1	ECD	241
Butofilol	plasma	1 ml	c	HFBA, toluene	C	3%	OV-17	ECD	242
	urine	1 ml	c	HFBA, toluene	C	3%	OV-17	ECD	242
Deacetylmetipranolol	serum	1 ml	b	TFAA, ACN	C	3%	SE-30	MS	243
	urine	1 ml	b	TFAA, ACN	C	3%	SE-30	MS	243
FM-24	plasma	1 ml	c	PFAA, EAC	C	3%	OV-17	ECD	244
FM-25	plasma	1 ml	c	PFAA, EAC	C	3%	OV-17	ECD	244
Metoprolol(M)	plasma	1 ml	b	HFBI, benzene	C	3%	OV-1	ECD	245
	plasma	.25 ml	c	TFAA, EAC	C	3%	OV-17	ECD	246
	urine	.25 ml	c	TFAA, EAC	C	3%	OV-17	ECD	246
	plasma	1 ml	c	HFBA, pyridine, hexane	C	3%	OV-1	NPD	247
	plasma	2 ml	b	phosgene, toluene	V	3%	OV-17	NPD	248
						C-	Carbowax 20M		
Moprolol	plasma	1 ml	c	TFAA, ether	C	4%	SE-30	ECD	249
	urine	1.5 ml	c	TFAA, ether	C	4%	SE-30	ECD	249
Oxprenolol	plasma	1 ml	c	HFBA, hexane	C	3%	SE-30	ECD	250
	serum	.2 ml	b	HFBA, EAC	C	3%	OV-1	ECD	235
	plasma	1 ml	c	HFBA, pyridine, hexane	C	3%	OV-1	NPD	247
Pindolol	plasma	2 ml	c	TFAI, TMA	C	2%	OV-17	ECD	251
	urine	2 ml	c	TFAI, TMA	C	2%	OV-17	ECD	251
Prenalterol	plasma	1 ml	c	HFBA, pyridine, ether	C	3%	OV-17	ECD	252
	urine	.05 ml	c	HFBA, pyridine, ether	C	3%	OV-17	ECD	252
	plasma	.1 ml	b	PFPA, toluene	C	3%	OV-17	MS	253
	urine	.1 ml	b	PFPA, toluene	C	3%	OV-17	MS	253
Pronethalol	plasma	1 ml	c	TFAA, ether	C	4%	SE-30	ECD	249
Propranolol	plasma	1 ml	c	HFBA, hexane	C	3%	SE-30	ECD	250
	plasma	.25 ml	c	TFAA, EAC	C	3%	OV-17	ECD	246
	urine	.25 ml	c	TFAA, EAC	C	3%	OV-17	ECD	246
	plasma	2 ml	c	TFAI, TMA	C	2%	OV-17	ECD	251
	urine	2 ml	c	TFAI, TMA	C	2%	OV-17	ECD	251
	plasma	3 ml	b	TFAA, TMA, benzene	C	3%	OV-1 / OV-17 /	ECD/ MS	254
						2%	OV-101		
	plasma	1 ml	c	MSTFA, ACN	S	C-	OV-1	MS	255
	urine	.1 ml	c	MSTFA, ACN	S	C-	OV-1	MS	255
(M)	urine	2 ml	b	MTBSTFA, pyridine	S	C-	SP-2100	MS	256
Salsolinol(M)	csf	5 ml	i	PFPA, EAC	C	1%	OV-17	MS	257
	urine	10 ml	i	PFPA, EAC	C	1%	OV-17	MS	257
Timolol	plasma	.5 ml	i	BSTFA, pyridine	S	C-	SE-30	MS	258
	plasma	1 ml	c	MSTFA, ACN	S	C-	OV-1	MS	255
Tulobuterol	serum	1 ml	c	TFAA, acetic acid	C	2%	OV-1	ECD	259
(M)	urine	4 ml	b	BSA, EAC	S	2%	OV-1 / QF-1	MS	260

BARBITURATES

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Amobarbital	saliva	4 ml	b	bis(2-chloroethyl)sulfate	A	3%	SE-30	ECD	58
Barbital	plasma	1 ml	b	DAM, ether	A	3%	OV-17 / OV-225	FID/ MS	261
Barbiturates	blood	.02 ml	b	Me-I, K_2CO_3, acetone	A	3%	OV-225	FID	262
	blood	2 ml	c	dimethylsulfate, K_2CO_3, methanol	A	10%	SE-30	FID	263
	plasma	1 ml	c	Bu-I, TMAH, DMA	A	3%	OV-17 / OV-1	FID	38, 40
	plasma	.5 ml	i	TBA, Et-I, DCM	A	C-	SE-30	FID	264
	plasma	1 ml	c	TMPAH (on column), CS_2	A	3%	OV-17 / OV-101	FID	265
	blood	.03 ml	b	Pe-I, TMAH, DMA	A	3%	OV-17	NPD	41
	plasma	.1 ml		dimethylsulfate, methanol	A	C-	SP-2100	NPD	266
	serum	.5 ml	i	Et-I, K_2CO_3, acetone	A	C-	SP-2100	NPD	267

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Butalbital	plasma	b	DAM, ether	A	3% OV-17 / OV-225	FID/ MS	261
Cyclobarbital	plasma 1 ml	b	TMPAH, toluene (on column)	A	10% UC-W98	FID	268
Hexobarbital	plasma 1 ml	c	PFBB, TEA	A	3% OV-17	ECD	269
	plasma 1 ml	b	DMF-dimethylacetal	A	C- OV-1	FID	71
p-Hydroxyphenobarbital	plasma 1 ml	b	Et-I, TMAH, acetone/ Pr-I, TMAH, ACN	A	3% SP-2250DA / 10% SE-30	MS/NPD	270
Mephobarbital	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17	FID	38
	plasma .1 ml	b	Pr-I, TMAH, DMA	A	3% OV-101	MS	271
Methohexital	plasma 1 ml	c	PFBB, TEA	A	3% OV-17	ECD	269
Pentobarbital	saliva 4 ml	b	bis(2-chloroethyl)sulfate	A	3% SE-30	ECD	58
	serum .1 ml	i	PFBB, Na_2CO_3, ethanol	A	3% OV-17	ECD	272
	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-17	FID	40
	serum .1 ml	b	Me-I, Na_2CO_3, acetone	A	2% OV-17	NPD	273
Phenobarbital	saliva 4 ml	b	bis(2-chloroethyl)sulfate	A	3% SE-30 / OV-101	ECD	58
	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17	FID	38
	plasma 1 ml	b	DMF-dimethylacetal	A	C- OV-1	FID	71
	plasma 1 ml	b	TMPAH, toluene (on column)	A	10% UC-W98	FID	268
	plasma 1 ml	b	TMAH (on column)	A	5% OV-17	FID	70
	plasma	b	TMPAH, toluene	A	3% OV-17 (10%)	FID	202,274
	serum .5 ml	b	TMPAH (on column)	A	3% OV-17	FID	203
(M)	plasma 1 ml	b	DAM, ether	A	10% OV-17	MS	275
	urine 1 ml	b	DAM, ether	A	10% OV-17	MS	275
	plasma .1 ml	b	Pr-I, TMAH, DMA	A	3% OV-101	MS	271
	fluids .1 ml	b	Et-I, TMAH, DMA	A	3% OV-17	MS	198
	serum .2 ml	b	TMPAH (on column)	A	3% OV-17	NPD	205
(M)	urine .5 ml	b	TMPAH	A	3% OV-1	NPD	206
Primidone	fluids .2 ml	b	Me-I, methanol, hexane	A	3% OV-1 / OV-17	FID	190
	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
	plasma	b	TMPAH	A	3% OV-17 (5%)	FID	191,192
	serum .5 ml	b	TMPAH (on column)	A	3% OV-17	FID	203
(M)	saliva .01 ml	i	Et-I, TMAH, DMA	A	3% OV-17	MS	198
	serum .2 ml	b	TMPAH (on column)	A	3% OV-17	NPD	205
Thiopental	plasma 1 ml	c	Me-I, Na_2CO_3, acetone	A	3% OV-17	NPD	276

BENZODIAZEPINES

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Bromazepam	plasma .5 ml	b	TBA, Me-I, ether	A	0.5% OV-17	ECD	277
Brotizolam	plasma	c	BSTFA, ACN	S	C- CP Sil-5	ECD	278
Chlordiazepoxide(M)	serum .4 ml	b	TMPAH (on column)	A	5% OV-225	ECD	279
Desmethyldiazepam	plasma .5 ml	b	Bu-I, TBAH, DMA	A	3% OV-17	ECD	280
	blood 2 ml	c	Pr-I, TBA, TMPAH, DMA	A	3% OV-1	NPD	281
Diazepam	plasma .5 ml	b	DAM, ether	A	3% OV-17	FID	282
	urine	b	DAM, ether	A	3% OV-17	FID	282
Estazolam(M)	plasma 1 ml	c	BSTFA, ACN	S	C- CP Sil-5	ECD	283
Flunitrazepam	plasma .5 ml	b	DAM, ether	A	3% OV-17	FID	282
	urine	b	DAM, ether	A	3% OV-17	FID	282
Flurazepam(M)	plasma 3 ml	c	t-BDMS	S	3% OV-17	ECD	284
	plasma .5 ml	c	BSTFA, ACN	S	3% OV-225 C- CP Sil-5	ECD	285
	plasma 2 ml	c	silylation, EAC	S	OV-17	MS	286
Imidazo-1,4-benzodiazepines	blood 1 ml	b	BSA, ACN	S	5% OV-1	ECD	287
(M)	urine .2 ml	b	BSA, ACN	S	5% OV-1	ECD	287
Imidazobenzodiazepine-3-carboxamide	plasma 1 ml	b	PFPA, TEA, chloroform	C	C- CP Sil-8	MS	288
Midazolam(M)	plasma .5 ml	b/c	BSTFA, benzene, acetone, ACN	S	5% OV-101	ECD	289,290
	plasma 1 ml	b	BSA, ACN	S	3% Poly-S-176	MS	291
Oxazepam	serum 2 ml	g	THA, Me-I, DCM	A	3% OV-225	ECD	292,293
Pyrimidobenzazepine(M)	plasma .5 ml	b	BSTFA, ACN	S	5% OV-1	ECD	294
	urine .5 ml	b	BSTFA, ACN	S	5% OV-1	ECD	294
Temazepam	plasma 1 ml	b	BSA, ACN	S	C- .5% PPE-21 + 3% OV-17	ECD	295
Tofizopam(M)	urine 10 ml	b+d	BSTFA, TMCS	S	2% XE-60	FID	296
					2% OV-101	MS	
Triazolam(M)	plasma 1 ml	c	BSTFA, ACN	S	C- CP Sil-5	ECD	283
	urine 1 ml	c	BSTFA, ACN	S	C- CP Sil-5	ECD	283

CYTOSTATICS (ANTINEOPLASTIC AGENTS)

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Anthracycline analogs	bile	b	TMSI, pyridine	S	3% OV-17	MS	297
Busulfan	plasma 1 ml	b	NaI, acetone	A	10% SP-2401	MS	298
Chlorambucil(M)	plasma .5 ml	a	BSTFA, ACN, DCM	S	3% OV-17	MS	299
	urine .5 ml	a	BSTFA, ACN, DCM	S	3% OV-17	MS	299
Cyclophosphamide	plasma .5 ml	b	HFBA	C	3% SE-30	ECD	300
	urine .5 ml	b	HFBA	C	3% SE-30	ECD	300
	plasma .2 ml	b	TFAA, EAC	C	3% SE-30 / Versamid-900	FID/NPD	301
Daunosamine	bile	b	TMSI, pyridine	S	3% OV-17	MS	297
Floxuridine	plasma 1 ml	d	Me-I, K-butoxide, DMSO	A	3% OV-17	NPD	302
5-Fluorouridine	urine 1 ml	i	Me-I, K-butoxide, DMSO	A	C- FFAP	NPD	303
5-Fluorouracil	plasma .5 ml	c	TMPAH, methanol	A	3% OV-17	MS	304
	plasma 1 ml	b	BSA, TMCS, pyridine	S	3% OV-17	MS	305
(M)	plasma	i	BSTFA, pyridine	S	3% OV-17	MS	306
	serum 1 ml	b	DAM, ether	A	3% OV-17	NPD	307
Geranylgeranylacetone	serum 1 ml	i	O-pentafluorobenzyl-hydroxylamine, pyridine	V	3% OV-17	MS	308
Imidazopyrazole	plasma 1 ml	b	PFBB, DCM	A	3% OV-17	NPD	309
Iphosphamide	plasma .5 ml	b	HFBA	C	3% SE-30	ECD	300
	urine .5 ml	b	HFBA	C	3% SE-30	ECD	300
Indicine-N-oxide	urine 1 ml	i	BSTFA, TMSI, TEA, pyridine	S	3% OV-17 / Dexsil-300	MS	310
6-Mercaptopurine	plasma 1 ml	f	TBA, PFBB, DCM	A	C- SE-30	MS	311
	plasma 1 ml	i	THA, Me-I, DCM	A	3% OV-225	MS	312
Procarbazine(M)	plasma	b	AA, toluene	C	3% OV-1	MS	313
Trophosphamide	plasma .2 ml		TFAA, EAC	C	3% SE-30 / Versamid-900	FID/NPD	301

PHENYLETHYLAMINES

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Aliphatic primary hydroxylamines	urine 2.5 ml	b	BSTFA, ACN / TFAA	S/C	3% OV-17 / 2% XE-60	FID	314
Amantadine	fluids	b	trichloroacetyl chloride,	C	5% SE-30	ECD	315
Amphetamine	fluids	b	trichloroacetyl chloride,	C	5% SE-30	ECD	315
	urine .2 ml	b	PFBC, n-pentane	C	2% Thermon-300	ECD	316
	urine 5 ml	b	HFBA, pyridine	C	2% OV-17	ECD	317
Biogenic amines	brain .8 g	b	BCl_3-methanol	A	C- SE-30	MS	318
	tissue	i	PFBC, pyridine, ACN	C	5% OV-17	MS	319
Catecholamines(M)	amniotic 3 ml	b	TFAA, EAC	C	3% OV-17	ECD	320
	urine	d	TFAA, DCM	C	3% OV-17	FID	321
(M)	urine 2 ml	c	t-BDMS	S	C- SE-54	FID	322
(M)	urine 1 ml		BSTFA	S	C- CP Sil-5	FID	323
	plasma .5 ml		methylchloroformate + t-BDMS	C+S	C- OV-1701	MS	324
	urine .5 ml		methylchloroformate + t-BDMS	C+S	C- OV-1701	MS	324
Dopamine(M)	urine 5 ml	b	diazoethane + 2,2-dimethyloxy-propane, n-butylboronic acid	A+V	3% OV-101	FID	325
	brain	e	DNTS + BSTFA	V+S	3% OV-1	ECD	326
(Nor)Ephedrine	urine 1 ml	c	HFBA, pyridine	C	2% OV-17	ECD	317
(Nor)Metanephrine	urine	d	TFAA, DCM	C	3% OV-17	FID	321
	urine 2 ml	i	PFPA, EAC	C	3% OV-1 / 5% OV-101	MS	327,328
Methamphetamine	urine .2 ml	b	PFBC, n-pentane	C	2% Thermon-300	ECD	316
3-Methoxy-4-hydroxy-phenylglycol	urine	i	HFBA, ACN, EAC	C	5% OV-17	FID	329
Norepinephrine(M)	urine 5 ml	b	diazoethane + 2,2-dimethyloxy-propane, n-butylboronic acid	A+V	3% OV-101	FID	325
	brain	e	DNTS + BSTFA	V+S	3% OV-1	ECD	326
Phendimetrazine(M)	plasma 2 ml	c	AA, toluene	C	3% SP-2100	NPD	330
	urine 2 ml	c	AA, toluene	C	3% SP-2100	NPD	330
Phentermine(M)	plasma 2 ml	c	AA, toluene	C	3% SP-2100	NPD	330
	urine 2 ml	c	AA, toluene	C	3% SP-2100	NPD	330
Phenylephrine	urine	i	PFPA, EAC	C	5% OV-101	MS	328
Phenylethylamines	plasma 2 ml	f	PFBC, cyclohexane	C	C- OV-101	ECD	81
	urine 2 ml	f	PFBC, cyclohexane	C	C- OV-101	ECD	81
Pseudoephedrine	serum 1 ml	c	PFBB, K_2HPO_4, ethanol	A	5% OV-225	ECD	331
(M)	plasma .1 ml	b	TFAA, toluene	C	2% SP-2510-DA(GP)	ECD	332
Serotonine	plasma .2 ml	a	PFPA	C	1.5% OV-1	MS	333

STEROIDS

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Alphadolone	plasma	1 ml	b	HFBI	C	2%	Dexsil-300	ECD	334
Cortisol	plasma	.5 ml	b	O-methylhydroxylamine, pyridine + BSTFA, TMCS	V+S	1%	OV-1	MS	335
	fluids	2 ml	b	O-methylhydroxylamine, pyridine + TMSI	V+S	1%	OV-1	MS	336
Diethylstilbestrol	urine	50 ml	i	TFAA, hexane	C	3%	OV-17	ECD	337
Estradiol	serum	.2 ml	f	TBA, Me-I, DCM	A	C-	SP-2250	MS	338
	serum	.2 ml	c	AA, pyridine	C	C-	SP-2250	MS	338
	serum	.5 ml	i	TFAA, EAC	C	1%	OV-1	MS	339
	plasma	2 ml	d	HMDS, TMCS, pyridine	S	C-	SE-30	MS	340
Estriol	serum	.2 ml	f	TBA, Me-I, DCM	A	C-	SP-2250	MS	338
	serum	.2 ml	c	AA, pyridine	C	C-	SP-2250	MS	338
	serum	.5 ml	i	TFAA, EAC	C	1%	OV-1	MS	339
Estrogens	urine	.2 ml	b	BSTFA, pyridine	S	C-	OV-225 / Silar-10C	FID	341
Estrone	serum	.2 ml	f	TBA, Me-I, DCM	A	C-	SP-2250	MS	338
	serum	.2 ml	c	AA, pyridine	C	C-	SP-2250	MS	338
	serum	.5 ml	i	TFAA, EAC	C	1%	OV-1	MS	339
Methandienone(M)	urine	5 ml	i	MSTFA, TMCS, TMSI, pyridine	S	C-	SE-54	MS	342,343
Steroids(M)	urine	2 ml	i/c	O-methylhydroxylamine, pyridine + TMSI	V+S	C-	SE-30	MS	344,345
	urine	5 ml	i	O-methylhydroxylamine + TMSI, BSA, TMCS	V+S	C-	DB-1	MS	346
	urine	20 ml	i	BSTFA, TMCS, pyridine, hexane/ O-methylhydroxylamine, pyridine	S+V	C-	OV-101 / SP-2100	MS	347
Testosterone	plasma	1 ml	b+d	O-methylhydroxylamine, pyridine + t-BDMS, DMF	V+S	1%	OV-1	MS	348

SULPHONAMIDES

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Acetazolamide	serum	.1 ml	f	TPA, Me-I, DCM	A	1%	SE-30	ECD	47
Bendrofluazide	blood	1 ml	h	TBA, Me-I, benzene	A	1%	OV-1	ECD	349
Chlorpropamide	plasma	.1 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	350
Chlorthalidone	plasma	1 ml	f	THA, Me-I, DCM	A	3%	OV-1	ECD	207
	urine	1 ml	f	THA, Me-I, DCM	A	3%	OV-1	ECD	207
	plasma	1 ml	h	THA, Me-I, DCM	A	3%	SE-30	NPD	351
	urine	1 ml	h	THA, Me-I, DCM	A	3%	SE-30	NPD	351
Dichlorphenamide	serum	.5 ml	c	Me-I, TMAH, DMA	A	3%	OV-17	ECD	352
Fenquizone	plasma	1 ml	f	THA, Me-I, DCM	A	30%	OV-101	NPD	353
Glibenclamide	plasma	.5 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	350
Gliclazide	plasma	1 ml	c	DAM, ether + HFBA, pyridine, EAC	A+C	2% / 3%	OV-101 / Xe-60	ECD/FID	354
Glipzide	plasma	.5 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	350
Hydrochlorothiazide	plasma	2 ml	h	THA, Me-I, DCM	A	1%	OV-225	ECD	355
Mefruside(M)	plasma	2 ml	h	THA, Me-I, DCM	A	3%	SE-30	ECD	356
	urine	1 ml	h	THA, Me-I, DCM	A	3%	SE-30	ECD	356
	plasma	2 ml	h	THA, Me-I, DCM	A	3%	SE-30	NPD	357
	urine	1 ml	h	THA, Me-I, DCM	A	3%	SE-30	NPD	357
N-Methylsaccharin	urine		b	DAM	A	4% / 6%	SE-30 / OV-210	FID	358
Saccharin	urine	4 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	359
Sulphadimethoxine	tissue	50 g		DAM, ether + PFPA	A+C	3%	OV-17	MS	360
(Neo)Sulphalepsine(M)	plasma	1 ml	f	THA, Me-I, DCM	A	3%	OV-1	ECD	207
	urine	1 ml	f	THA, Me-I, DCM	A	3%	OV-1	ECD	207
Sulphamerazine	serum	.1 ml	f	TBA, Me-I, DCM	A	5%	OV-17	ECD	361
Sulphapyridine	serum	.1 ml	f	TBA, Me-I, DCM	A	5%	OV-17	ECD	361
Sulphonylureas	plasma	.1 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	350
Tolbutamide	plasma	.1 ml	f	TBA, Me-I, DCM	A	3%	OV-17	ECD	350

XANTHINES

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Acephylline	urine 1 ml	b	Et-I, K_2CO_3, ACN	A	3% OV-17	FID	362
Hypoxanthine	serum .5 ml	i	Bu-I, TBAH, DMA	A	3% OV-17	MS	363
	urine .5 ml	i	Bu-I, TBAH, DMA	A	3% OV-17	MS	363
Oxpentifylline(M)	plasma 2 ml	b	TFAA, hexane	C	3% OV-225	NPD	364
Theophylline	serum .1 ml	b	PFBB, Na_2CO_3, ethanol	A	5% OV-225	ECD	365
(M)	plasma .05 ml	a	Pr-I, K_2CO_3, DMF	A	3% OV-17	FID	366
	serum 1 ml	c	Bu-I, TMAH, DMA	A	3% SP-2250 DB	FID	367
	blood .2 ml	i	Bu-I, TMAH, DMA	A	3% OV-17	MS	368
	plasma .05 ml	f	TBA, Et-I, DCM	A	C- OV-225	MS	369
	plasma .02 ml	b	TBAH, methanol (on column)	A	3% OV-17	NPD	64
	blood .03 ml	b	Pe-I, TMAH, DMA	A	3% OV-17	NPD	41
	plasma .05 ml	b	Pe-I, TMAH, DMA	A	C- SE-30	NPD	370
	saliva .1 ml	f	THA, Pe-I, DCM	A	3% OV-17	NPD	371
	serum .025 ml	f	THA, Pe-I, DCM	A	3% OV-17	NPD	371
	serum .1 ml	b	Bu-I, TBAH, methanol	A	3% OV-17	NPD	372
	serum .05 ml	b	Pe-I, TMAH, DMA	A	3% OV-17	NPD	373
Xanthines(M)	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
	serum .5 ml	i	Bu-I, TBAH, DMA	A	3% OV-17	MS	363
	urine .5 ml	i	Bu-I, TBAH, DMA	A	3% OV-17	MS	363

ACIDIC COMPOUNDS (and amphoteric compounds with a carboxylic function)

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
p-Aminobenzoic acid(M)	serum 1 ml	b	DAM, ether	A	C- OV-101	NPD	374
	urine 1 ml	b	DAM, ether	A	C- OV-101	NPD	374
Aminobutyric acid	csf 1 ml	f	trichloroethylchloroformate, dioxane + TBA, Me-I, DCM	C+A	C- SE-54	ECD	375
p-Aminohippuric acid(M)	serum 1 ml	b	DAM, ether	A	C- OV-101	NPD	374
	urine 1 ml	b	DAM, ether	A	C- OV-101	NPD	374
Apovincaminic acid	plasma 1 ml	b	DAM, ether	A	C- SP-2100	FID/NPD	53
Benzoic acid	plasma 1 ml	b	PFBB, K_2CO_3, acetone	A	3% OV-17	ECD	376
	urine 1 ml	b	PFBB, K_2CO_3, acetone	A	3% OV-17	ECD	376
	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Bile acids	serum .1 ml	i	HFBA + hexafluoroisopropanol	A	C- OV-17	ECD	377
	serum 1 ml	i	various		1% Hi-EFF 8-BP	MS	378
	bile	i	DAM, ether + AA	A+C	C- SE-30	MS	379
	bile 1 ml	i	DAM, ether + TMSI, ACN	A+S	1.5% SE-70 / Poly-I-110	MS	380
3-(tert.)-Butyl-4-anisole	plasma .1 ml	b	HFBA, pyridine, hexane	C	C- SE-52	MS	381
Cannabinol(diol)	plasma 1 ml	b	PFBB, K_2CO_3, acetone	A	5% OV-225	ECD	32
	serum 1 ml	b	MSTFA, pyridine	S	C- OV-1	MS	382
	urine 5 ml	b	MSTFA, pyridine	S	C- OV-1	MS	382
Captopril	plasma .5 ml	i	TFAA, hexafluoroisopropanol	A	10% Dexsil-300	MS	383
	urine .1 ml	i	TFAA, hexafluoroisopropanol	A	10% Dexsil-300	MS	383
	blood 5 ml	i	BSTFA + methanol, HCl	A	3% OV-17	MS	384
	fluids .1 ml	i	hexafluoroisopropanol, perfluorobutyric acid anhydride,	A	2% OV-210	MS	385
Carboxylic acids(M)	urine 1 ml	b	BSA	S	10% OV-1 / OV-17	FID/ MS	102
	amniotic 5 ml	c	BSTFA, TEA	S	C- CP Sil-5	MS	386
	urine	b	MSTFA, pyridine	S	3% OV-17	MS	387,388
	urine 1.9 ml	b	MSTFA, pyridine	S	C- OV-1701	MS	389
	serum 1 ml	b	O-methylhydroxylamine, EAC + BSTFA	V+S	C- OV-101	MS	390,391
	urine 1 ml	b	O-methylhydroxylamine, EAC + BSTFA	V+S	C- OV-101	MS	390,391
(-)-Threo-chlorocitric acid	plasma 1 ml	c	DAM, ether	A	OV-17	MS	392
Chlorquinaldol	plasma .1 ml	f	TBA, Me-I, DCM	A	3% OV-17	ECD/FID	393
	urine .5 ml	f	TBA, Me-I, DCM	A	3% OV-17	ECD/FID	393
	plasma 1 ml	f	THA, Me-I, DCM	A	3% OV-1	ECD	207
	urine 1 ml	f	THA, Me-I, DCM	A	3% OV-1	ECD	207

COMPOUND(S)	SAMPLE	CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE	DETECTION	REFERENCE
Clioquinol	plasma .1 ml	f	TBA, Me-I, DCM	A	3% OV-17	ECD/FID	393
	urine .5 ml	f	TBA, Me-I, DCM	A	3% OV-17	ECD/FID	393
	plasma 1 ml	b	AA, pyridine, toluene	C	3% OV-101	ECD	394
Diacetoxyscirpenol	plasma 2 ml	i	HFBI, $NaHCO_3$, hexane	C	3% OV-1	ECD	395
	urine .5 ml	i	HFBI, $NaHCO_3$, hexane	C	3% OV-1	ECD	395
Dicarboxylic acids	urine	b	DAM, ether	A	C- OV-17	FID	396
	serum .8 ml	c	BSTFA, TEA	S	C- fused silica	MS	397
Dioxyanthraquinone	urine 15 ml	b	HMDS, TMCS, pyridine	S	3.8% SE-30	MS	398
Ethacrynic acid	plasma 1 ml	b	PFBB, K_2CO_3, ACN	A	1% OV-17	MS	399
2-Ethyl-3-oxohexanoic acid	urine 4 ml	b	O-methylhydroxylamine	V	C- OV-17	MS	400,401
Fatty acids	serum .1 ml	i	BF_3-methanol	A	10% SP-2330	FID	402
	platelets	i	BF_3-methanol	A	C- CP Sil-7	FID	24
	serum .8 ml	i	BF_3-methanol	A	10% CP Sil-10	FID	403
	amniotic .5 ml	b	TMPAH, hexane/BF_3-methanol	A	3% OV-17	FID	404
	plasma .2 ml	c	TMPAH (on column)	A	8% BDS + 15% CP Sil-84	FID	405
	plasma .05 ml	b	Me-I, crown ether, K_2CO_3	A	10% CP Sil	FID	406
	serum .8 ml	i	Me-I, pyridine, DMA	A	10% CP Sil-10	FID	403
	serum .2 ml	b	DAM, ether	A	C- Carbowax-20M	FID	407
	fluids 10 ml	b	BSA, TMCS, TMSI	S	C- CP Sil-88	FID	408
	synovial .1 ml	i	DAM, ether	A	C- SE-30	MS	409
Ferulic acid	plasma 1 ml	i	HFBA, EAC	C	2% OV-17	MS	410
Flucloxacillin	urine	b	HMDS, pyridine	S	1.5% OV-17	MS	411
Fosfomycin	serum .25 ml	a	BSA, TMCS, DCM	S	3% XE-60	MS	412
	urine .1 ml	a	BSA, TMCS, DCM	S	3% XE-60	MS	412
Gemfibrozil	plasma 1 ml	b	TMAH, ether	A	3% OV-22	FID	413
	urine 1 ml	b	TMAH, ether	A	3% OV-22	FID	413
Homopantothenic acid	plasma 1 ml	b	BSTFA, pyridine	S	3% OV-17	MS	414
(Iso)Homovanillic acid	urine .7 ml	b	TFAA, hexafluoroisopropanol	C+A	C- liquid phases	ECD	23
	urine 5 ml	b	BSTFA, TMCS	S	C- SE-54	FID	415
	urine 1 ml	b	BSTFA	S	3% OV-225	FID	416
2-Hydroxy-2-methyllevu-linic acid	serum 1 ml	b	O-methylhydroxylamine + BSTFA	V+S	C- OV-101	MS	417
	urine 1 ml	b	O-methylhydroxylamine + BSTFA	V+S	C- OV-101	MS	417
Itanoscone	plasma 1 ml	b	reduction + DAM, ether	A	3% OV-225	ECD	418
α-Ketocarboxylic acids	plasma 5 ml	-	BSTFA, ACN + o-phenylenediamine	S+V	3% SP-2401	FID	419
	plasma 3 ml	i	BSTFA, pyridine + o-pheny-lenediamine	S+V	C- Dexsil-300	FID/ MS	420
	urine 3 ml	i	BSTFA, pyridine + o-pheny-lenediamine	S+V	C- Dexsil-300	FID/ MS	420
Levodopa	brain	e	DNTS + BSTFA	V+S	3% OV-1	ECD	326
Mandelic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
(M)	urine 3 ml	b	HFBA, hexane	C	C- BSC-SP-2250	MS	421
m-, p-Methylhippuric acid	urine 5 ml	b	DAM, ether	A	2% OV-225	NPD	422
N-Methylimidazoleacetic acid	urine 1 ml	d	acetylchloride, 2-propanol	C	C- SP-2100	NPD	423
Monohydroxy fatty acids	fluids 25 mg	b	DAM, ether + t-BDMS, imidazole, DMF	A+S	3% OV-17	MS	424
Nalidixic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17	FID	38,425
Niclosamide	urine	h	TBA, Me-I, DCM	A	4% OV-101 (5%)	ECD	426
Nicotinic acid	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17 / SP-1000	FID	38
Oxocarboxylic acids	urine 10 ml	i	O-methylhydroxylamine + DAM, ether	V+A	C- UCON 75 H-4000	NPD	427
Oxyphenisatin	urine 15 ml	b	HMDS, TMCS, pyridine	S	3.8% SE-30	MS	398
Palmitic acid	amniotic .5 ml	b	TMPAH, hexane/BF_3-methanol	A	3% OV-17	FID	404
Phenolphthalein	urine	b	HMDS, TMCS, pyridine	S	3.8% SE-30	MS	398
Phenols	plasma 1 ml	b	BSA, TMCS	S	5% TXP	MS	428
	urine 1 ml	b	BSA, TMCS	S	5% TXP	MS	428
Phenprocoumon	plasma 1 ml	c	Bu-I, TMAH, DMA	A	3% OV-1 / OV-17	FID	38
Phentolamine	urine 1 ml	c	HFBI, heptane	C	3% OV-17	ECD	429
Phenylacetic acid	urine 2 ml	b	BSTFA	S	3% OV-17	FID	430
(M)	plasma .5 ml	i	PFPA, hexane + trifluoroethanol	A	C- OV-101	MS	431
2-Phenylbutyric acid	plasma 1.5 ml	b	BSA, heptane	S	2.4% SE-30	FID	432
Procetofenic acid	serum 1 ml	b	Me-I, methanol	A	8% OV-101	ECD	433
Prostaglandins	urine 1 ml	b	O-methylhydroxylamine + PFBB, diisopropylethylamine, ACN + silylimidazole		C- OV-101	ECD	434

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
	plasma	1.5 ml	b	BSTFA, piperidine	S	3%	OV-17 / Dexsil-300	FID	435
	plasma	.5 ml	a	PFBB, diisopropylethylamine, ACN	A	C-	(SCOT)	MS	436
(M)	fluids	5 ml	d	DAM, ether + O-methylhydroxylamine, pyridine	A+V	C-	SE-30	MS	437
	urine	1 ml	c	O-methylhydroxylamine, pyridine, PFBB, diisopropylethylamine, ACN + BSTFA			SE-54 / SP-2100	MS	438
	urine	20 ml	d	DAM, ether + O-methylhydroxylamine, pyridine + BSTFA, pyridine		C-	SE-30	MS	439
	urine	20 ml	c	DAM, ether + O-methylhydroxylamine, pyridine + TMSI, piperidine		2%	SP-2330 /	MS	440
Pyroglutamic acid(M)	urine	1 ml	b	BSTFA, TMCS	S	3%	SE-30	MS	441
Quinolacetic acid(M)	urine	1 ml	b	BSTFA, TMCS	S	3%	SE-30	MS	441
Retinoic acid	plasma	10 ml	b	DMF-dimethylacetal	A	3%	SE-30	MS	442
SA-446	blood	.25 ml	-	DAM, ether + PFBB, ACN	A	0.5%	OV-17	ECD	443
	urine	.25 ml	-	DAM, ether + PFBB, ACN	A	0.5%	OV-17	ECD	443
Sialic acids	urine	5 ml	d	TFAA, ethanediol + HMDS, TMCS, pyridine	A+S	C-	SF-6	FID	444
Tetrahydrocannabidiol	plasma	1 ml	b	PFBB, K_2CO_3, acetone	A	5%	OV-225	ECD	32
Tetrahydrocannabinol	plasma	5 ml	b	HFBA, TMA	C	C-	SE-54	ECD	445
(M)	liver		c	DAM, ether	A	2%	SE-30	FID	446
	plasma	1 ml	b	DAM, ether + BSTFA	A+S	1%	SE-30	MS	447
(M)	urine	3 ml	i	DAM, ether + BSTFA, TMCS, pyridine	A+S	3%	OV-17	MS	448
	serum	1 ml	b	MSTFA, pyridine	S	C-	OV-1	MS	382
	urine	5 ml	b	MSTFA, pyridine	S	C-	OV-1	MS	382
Tetrahydrocannabinol-11-oic acid	urine	1 ml	b	PFPA + pentafluoropropanol	A	C-	SE-54	ECD/ MS	449
Tienilic acid	plasma	1 ml	b	PFBB, K_2CO_3, ACN	A	1%	OV-17	MS	399
Ticrynafen(M)	plasma	1 ml	b	DAM, ether + BSTFA, TMCS, pyridine, ACN	A+S	3%	OV-17	ECD	450
	urine	1 ml	b	DAM, ether + BSTFA TMCS, pyridine, ACN	A+S	3%	OV-17	ECD	450
Tranexamic acid	fluids	.2 ml	f	TBA, Et-I + FNBT, DMSO	V	1%	OV-225	ECD	122
Urochloralic acid	tissues	.5 ml	i	DAM, EAC + TFAA, EAC	A+C	1,5%	OV-17	ECD	451
Vanillylmandelic acid	urine	.7 ml	b	TFAA, hexafluoroisopropanol	C+A	C-	liquid phases	ECD	23
	urine	5 ml	b	BSTFA, TMCS	S		SE-54	FID	415
	urine	1 ml	b	BSTFA	S	3%	OV-225	FID	416
γ-Vinyl-γ-aminobutyric acid	plasma	.1 ml	a	TFAA, DCM / DAM, ether	C/A	C-	L-valine-(tert)-butylamide	MS	452
Warfarin	plasma	1 ml	c	Bu-I, TMAH, DMA	A	3%	OV-1 / OV-17	FID	38

BASIC COMPOUNDS

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
4-Aminopyridine	plasma	1 ml	b	TFAA, EAC	C	10%	FFAP	NPD	453
Apomorphine(M)	fluids	1 ml	c	TFAA, pyridine	C	C-	SE-54	ECD/FID	454
	brain		b	BSTFA	S	3%	OV-17	MS	455
Azapetine	plasma	1 ml	-	trichloroethylchloroformate, EAC	C	3%	OV-17	ECD	91
Benzoylecgonine	plasma	.5 ml	b	PFBB, pyridine, benzene, DCM	A	3%	OV-225	ECD	456
	urine	1 ml	b	PFBB, pyridine, benzene, DCM	A	3%	OV-225	ECD	456
	urine	5 ml	b	Pr-I, TMAH, PTMAH, DMA	A	3%	SP-2250 DA	FID	457
	urine	2 ml	f	TMA, Et-I, DCM	A	3%	OV-17	MS	458
Bisacodyl	urine		b	HMDS, TMCS, pyridine	S	3.8%	SE-30	MS	398
β-Blocking agents	plasma	1 ml	b	BSTFA	S	C-	CP Sil-5	FID	459
Bretylium	plasma	1 ml	i	Na-benzenethiolate, EAC	V	4.3%	OV-101	ECD	460
	urine	.1 ml	i	Na-benzenethiolate, EAC	V	4.3%	OV-1-1	ECD	460
Bromhexine	plasma	1 ml	c	TFAA, EAC	C	4%	SE-30	ECD	461
Butailcaine	plasma	2 ml	b	PFBC, cyclohexane	C	3%	OV-17	NPD	462
	urine	2 ml	b	PFBC, cyclohexane	C	3%	OV-17	NPD	462
Butyrophenone(M)	urine	10 ml	b	AA, pyridine	C	20%	UCC-W	MS	463
Carbimazole	plasma	1 ml	f	TBA, PFBB, DCM	A	C-	UCON HB-5100	MS	464
Chinioform(M)	plasma	.1 ml	b	AA, pyridine	C	1%	OV-17	MS	465

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Chloramphenicol	tissue	5 g	b	HMDS, TMCS, pyridine	S	3%	OV-1	ECD	466
Chloroquine(M)	urine	1 ml	b	isobutylchloroformate, DCM	C	3%	OV-17	FID	86
(M)	blood	1 ml	b	PFPA, pyridine	C	C-	OV-1	MS/NPD	467
	plasma	2 ml	c	TFAA, EAC	C	3%	OV-17	NPD	468
Chlorpromazine	plasma	1 ml	c	TFAA, chloroform	C	3%	OV-17	MS	214
Clonidine	plasma	5 ml	c	PFBB, K_2CO_3, acetone	A	C-	OV-17 / SE-30 / SP-2340	ECD	111
Codeine(M)	plasma	1 ml	c	PFPA, EAC	C	C-	OV-1	ECD	171,173
(M)	urine	10 ml	c	BSA	S	3%	OV-17 / OV-225	MS	174
Creatinine	urine			TMPAH	A	5%	Diethyleneglycol succinate	FID	469
Cyclandelate(M)	plasma	1 ml	b	BSTFA, TMCS, pyridine	S	C-	SE-30	FID	470
Dihydralazine	plasma	1 ml	-	nitrous acid, $NaOCH_3$, toluene	A	3%	OV-225	ECD	471
Diltiazem(M)	plasma	1 ml	b	N-methyl-N-(TMS)TFA	S	3%	OV-17	ECD	472
Disopyramide(M)	plasma	1 ml	c	AA, chloroform	C	3%	OV-17 (5%)	FID	473
	urine	9 ml	c	AA, chloroform	C	3%	OV-15 (5%)	FID	473
	serum	2 ml	b	AA	C	3%	OV-17	NPD	474
(M)	plasma	.5 ml	b	TFAA, toluene	C	5%	phenylmethyl silicone	NPD	475
Ethambutol	plasma	.5 ml	b	TFAA, pyridine, benzene	C	2%	OV-17	MS	476,477
Fluphenazine	fluids		b	acetylation	C	3%	OV-17	FID	478
	plasma	5 ml	b	BSTFA	S	3%	OV-17	NPD	479
Gluthethimide	plasma	.5 ml	i	TBA, Et-I, DCM	A	C	SE-30	FID	264
Guanfacine	plasma		b	hexafluoroacetylacetone	C	3%	OV-1	MS	480
Guanidine	urine	5 ml	d	PFPA, EAC	C	3%	OV-1	MS	481
HA-966	plasma	1 ml	a	AA	C	C-	OV-17	NPD	482
Histamine(M)	urine	2 ml	d	BCl_3 + chloroethanol	A	3%	SP-2401-DB	ECD/NPD	483
(M)	plasma	1 ml	i	HFBA, EAC + ethylchloroformate	C	5%	SE-30	MS	484,485
	urine	.1 ml	i	HFBA, EAC + ethylchloroformate	C	5%	SE-30	MS	484,485
(M)	urine	4 ml	b	HFBA, ACN	C	C-	SP-2100	NPD	486
Histidine(M)	urine	.25 ml	d	acetyl chloride, propanol + TFAA, DCM	A+C	C-	pluronic F-68	FID	487
Hydralazine(M)	serum	1 ml	a	formic acid	C	1%	SP-1000	NPD	488
(M)	urine	10 ml	-	acetylacetone	C	10%	OV-17	NPD	489
(M)	urine	10 ml	b	BSA	S	10%	OV-17	NPD	490
3-Hydroxyguanfacine	fluids	2 ml	b	hexafluoroacetylacetone, methanol + TBAH, Me-I	C+A	3%	OV-225	ECD	491
Isoniazid(M)	plasma	1 ml	b	BSTFA	S	1%	OV-17	MS	492
KABI 2128	serum	1 ml	b	TFAA, toluene	C	C-	OV-1 + OV-101	ECD	493
(Nor)Ketamine	plasma	.2 ml	b	HFBA, dimethylaminopropane, toluene	C		Carbowax 20M	NPD	494
	urine	4 ml	d	HFBA, dimethylaminopropane, toluene	C		Carbowax 20M	NPD	494
Loxapine	plasma	1 ml	b	N-methyl-N-(TMS)TFA	S	3%	OV-17	ECD	472
Lidocaine	plasma	1 ml	a	AA	C	C-	OV-17	NPD	482
Mebutamate	plasma	.5 ml	b	benzoylchloride	C	3%	OV-17	FID	495
Mefloquine	plasma	1 ml	b	MSTFA + MBTFA	S+C	3%	SE-30	MS	496
	urine	1 ml	b	MSTFA + MBTFA	S+C	3%	SE-30	MS	496
Meprobamate	plasma	.5 ml	b	benzoylchloride	C	3%	OV-17	FID	495
Methimazole	plasma	1 ml	f	TBA, PFBB, DCM	A	C-	UCON HB-5100	MS	464
Metoclopramide	plasma	.25 ml	c	HFBA, TEA, toluene	C	C-	SE-54	ECD	497
Midazolam(M)	plasma	1 ml	b	BSA, ACN	S	3%	Poly S-176	MS	498
MK-212	fluids		a	TFAA, benzene	S	3%	OV-210	ECD/ MS	499
Moroxydine	fluids	.5 ml	i	chlorodifluoroacetic anhydride, DCM	C	2%	OV-225	ECD	500
Nalorphine	plasma	1 ml	c	PFPA	C	C-	OV-1	ECD	171
Neuroleptics	urine	10 ml	b	AA, pyridine	C	5%	OV-101	MS	501
5-Nitroimidazole	blood	1 ml	b	BSTFA	S	3%	OV-11	ECD	502
Nofedone	serum	1 ml	c	HFBA, toluene, EAC	C	1%	Dexsil-300	ECD	503
Opium alkaloids(M)	urine	5 ml	b	BSA	S	3%	OV-210	MS	504
Oxyphenonium bromide	plasma	1 ml	i	PFBB, DCM	A	3%	OV-17	ECD	505,506
	urine	.1 ml	i	PFBB, DCM	A	3%	OV-17	ECD	505,506
Pemoline	urine	1 ml	b	DAM, ether	A	2%	OV-17	ECD	317
(M)	fluids	2 ml	b	DAM, ether	A	C-	1.0% PPE-21 + 2.6% OV-17	NPD	507
	serum	1 ml	f	TPA, Me-I, DCM	A	5%	FFAP	NPD	508
	urine	1 ml	f	TPA, Me-I, DCM	A	5%	FFAP	NPD	508
	urine	5 ml	b	TMPAH, methanol	A	5%	OV-17	NPD	509

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Perphenazine	serum	1 ml	b	BSA, ACN	S	3%	OV-17	ECD	510
Phenothiazine derivatives	urine	10 ml	b	AA, pyridine	C	5%	OV-101	MS	501
Pipotiazine	plasma	3 ml	c	PFPI, TEA, toluene	C	1%	OV-17	ECD	511
Primaquine	blood	1 ml	b	HFBA, benzene	C	3%	SP-2401	ECD	512
Procaine	urine	4 ml	b	HFBA, pyridine	C	2%	OV-17	ECD	317
Prochlorperazine	plasma	3 ml	c	PFPI, TEA, toluene	C	1%	OV-17	ECD	511
Propafenone	plasma	.1 ml	b	TFAA, toluene	C	3%	OV-101	ECD	513,514
Tertiary amines	serum	1 ml	c	PFB-chloroformate, Na_2CO_3, hexane	C	5%	OV-17	ECD	234
Tiaramide	serum	1 ml	b	BSA, ACN	S	3%	OV-17	ECD	510
Tocainide	plasma	.1 ml	b	HFBA, toluene	C	C-	chiral column	ECD	515
	plasma	.1 ml	b	HFBA, hexane	C	C-	Carbowax 20M	ECD/NPD	477,516
	urine	.1 ml	b	HFBA, hexane	C	C-	Carbowax	ECD/NPD	477,516
	plasma	.1 ml	b	HFBA, toluene	C	3%	OV-17	NPD	515
	plasma	.5 g	b	methylisobutylketon	V	10%	Carbowax 20M	NPD	517
Tolazoline(M)	plasma	1 ml	b	TFAA, TEA, toluene	C	C-	SP-2100	MS	518
Toloxatone	plasma	1 ml	b	BSTFA, EAC	S	3%	OV-101	NPD	519
Trazodone(M)	plasma	.5 ml	b	HFBA, EAC	C	3%	OV-1	NPD	520
Trimethoprim	plasma	.5 ml	b	DAM, ether	A	3%	OV-17	FID	282
Trimetozine(M)	urine	1 ml	i	methylsulfinylcarbanion, Me-I	A	1.5%	OV-17	FID	521
Viloxazine	plasma	.2 ml	c	HFBA, toluene	C	3%	OV-225	ECD	225
YM-09538	urine	1 ml	b	methylboronic acid	A	2%	OV-17	NPD	522

NEUTRAL COMPOUNDS

COMPOUND(S)	SAMPLE		CLEAN-UP	DERIVATIZATION	METHOD	STATIONARY PHASE		DETECTION	REFERENCE
Arildone	plasma	1 ml	b	O-pentafluorobenzylhydroxylamine, acetic acid, ethanol	V	3%	OV-1	ECD	523
	urine	2 ml	b	O-pentafluorobenzylhydroxylamine, acetic acid, ethanol	V	3%	OV-1	ECD	523
N,N-diethyl-m-toluamide (M)	urine	20 ml	b	methylation (on column)	A	C-	OV-101	MS	524
Diols	serum	1 ml	a	p-bromophenylboric acid, EAC	V	C-	Methylsilicone fluid	ECD	525
Galactitol	amniotic	.5 ml	i	AA, pyridine	C	3%	SP-230	FID	526
	plasma	.1 ml	a	n-butylboronic acid, pyridine	V	3%	OV-17	MS	527
Glucose	serum	.5 ml	-	O-pentafluorobenzylhydroxylamine	V	3%	XE-60	FID	528
Glycerol	fluids		a	BSA	S	3.5%	SE-30	FID	529
Guaifenesin	plasma	2 ml	b	2,4-dichlorobenzeneboronic acid, EAC	V	3%	OV-17	ECD/FID	530
Isosorbide(M)	urine	.05 ml	b	HFBA, pyridine	C	C-	OV-101	ECD	531
Mannitol	amniotic	.5 ml	a+d	AA, pyridine	C	3%	OV-17	FID	526
	fluids	1 ml	a	BSA, TMCS, pyridine	S	10%	OV-17	FID	532
	plasma	.1 ml	a	n-butylboronic acid, pyridine	V	3%	OV-17	MS	527
Mephenesin	plasma	2 ml	b	2,4-dichlorobenzeneboronic acid, EAC	V	3%	OV-17	ECD/FID	530
Monosccharides	urine	1 ml	-	TFAA, ethanediol + HMDS, TMCS, pyridine	A+S	C-	SF-96	FID	533
Sobrerol	plasma	1 ml	b	PFPA, benzene	V	10%	SE-30	ECD	534
Sorbitol	plasma	.1 ml	a	n-butylboronic acid, pyridine	V	3%	OV-17	MS	527
Span 20	plasma	1 ml	b	DAM, ether	A	3%	OV-17	FID	535
Sugar alcohols	urine	2 ml		O-methylhydroxylamine, pyridine + AA	V+C	3%	XE-60	FID	536
α-Tocopherol	plasma	.1 ml	b+d	AA, pyridine	C	3%	JXR	FID	537
	tissue	.05 g	-	BSTFA, TMCS, pyridine	S	3%	Silar-10C	MS	538

Explanation of the used abbreviations in the column "DERIVATIZATION"

ACYLATIONS (C): AA- acetic acid anhydride, HFBA- heptafluorobutyric acid anhydride, HFBI- heptafluorobutyril imidazole, MBTFA- N-methyl-bis(trifluoro)acetamide, PFBC- pentafluorobenzoyl chloride, PFPA- pentafluoropropionic acid anhydride, PFPI- pentafluoropropionyl imidazole, TFAA- trifluoroacetic acid anhydride, TFAI- trifluoroacetyl imidazole.

ALKYLATIONS (A): Bu-I- butyl iodide, DAM- diazomethane, Et-I- ethyl iodide, Me-I- methyl iodide, PFBB- pentafluorobenzyl bromide, Pe-I- pentyl iodide, Pr-I- propyl iodide, TBA- tetrabutylammonium cation, TBAH-

tetrabutylammonium hydroxide, THA- tetrahexylammonium cation, THAH- tetrahexylammonium hydroxide, TMAH- tetramethylammonium hydroxide, TPA- tetrapentylammonium cation, TMPAH- trimethylphenylammonium hydroxide, PTMAH- (m-trifluoromethylphenyl) trimethylammonium hydroxide.

SILYLATIONS (S): see table I

VARIOUS (V): BMP- 2-bromo-1-methylpyridinium iodide, DNTS- N-2,6-dinitro-4-trifluoromethylsulfonate, FNBT- 4-fluoro-3-nitro-benzotrifluoride

SOLVENTS: ACN- acetonitrile, DCM- dichloromethane, DMA- dimethylacetamide, DMF- dimethylformamide, DMSO- dimethylsulfoxide, EAC- ethyl acetate, TEA- triethylamine, TMA- trimethylamine