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ALKYLATION WITH ALKYL HALIDES AS A DERIVATIZATION METHOD FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF ACIDIC PHARMACEUTICALS

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1. INTRODUCTION

1.1. Derivative formation in quantitative gas–liquid chromatography (GLC)

The use of analytical derivatization in gas chromatographic analysis may be desirable for various reasons. The most important of these are the necessity to increase the volatility of polar compounds; the prevention of irreversible adsorption, caused by hydrogen bonding between polar functional groups of the compounds under investigation and the free silanol groups of the column packing material and the glassware; and the need to increase the sensitivity of detection. Derivatization can also be used to improve the separation between compounds, to prevent decomposition during chromatography or to decrease the excessive volatility of compounds of very low molecular weight.

A large number of derivatization techniques are available. Most frequently applied are derivatizations by acylation, silylation or alkylation, but many other types of reactions have also been used. In an excellent review, Nicholson[1] enumerated a number of criteria to be considered when choosing a method for derivatization. Some important criteria are that the derivative must be formed rapidly and quantitatively, or at least reproducibly, with no side-reactions or structural changes and with a minimum of manipulation.

In the past few years a number of books and review papers have been published, dealing either with most aspects of derivatization in (gas) chromatography[1–8] or with selected subjects, such as silylation reactions[9], the improvement of electron-capture detector response[10–12], pyrolytic methylation in gas chromatography[13] and applications in selected fields of research[14,15].

Among the various alkylation techniques available to the gas chromatographer, the methods using alkyl halides as the alkylating agents have a prominent position. The reason is that reactions with alkyl halides combine a number of desirable properties compared with the other alkylation reactions (these other techniques are discussed briefly in section 1.2). The attractive features are the following: a wide range of derivatives can be prepared; the reaction is usually fairly rapid under mild conditions; direct injection of the reaction mixture into the gas chromatograph is frequently possible; side-reactions are rare and usually one product is formed; the derivatives are comparatively stable; the reagents used are of rather low toxicity.

The aim of this review is to discuss the various types of alkylation reactions with alkyl halides and their application in the gas chromatographic analysis of acidic compounds of pharmaceutical interest. A short treatment of the underlying reaction mechanisms is included for a better understanding of the optimal reaction conditions. Attention is paid to the incorporation of the alkylation reactions in the entire analytical procedure, particularly in relation to the analysis of pharmaceuticals in biological matrices.

1.2. Derivatization by alkylation reactions (other than with alkyl halides)

Apart from the reactions with alkyl halides, on which this review is centred, a number of other alkylation reactions exist[1–3]. All alkylation methods have in common the replacement of the active hydrogens with alkyl groups in compounds

with (mainly) OH, COOH, SH, NH, CONH and SO_2NH groups. Under certain conditions tertiary amine groups can also be alkylated to yield quaternary ammonium compounds. Of particular interest are the compounds with acidic groups (carboxylic acids, phenols, imides), as these show a strong tendency upon gas chromatographic analysis to produce badly tailing peaks in the chromatogram. Alkylation will yield less polar derivatives with better chromatographic properties.

1.2.1. Diazoalkane alkylation

The most important reagent is diazomethane, although other diazoalkanes (*e.g.*, diazoethane and phenyldiazomethane) have also been used. Diazomethane can be prepared from N-methyl-N-nitroso-*p*-toluenesulphonamide or from N-methyl-N-nitroso-N'-nitroguanidine. Diazomethane is toxic and explosive and therefore should be handled with care. The gaseous reagent can be bubbled through a solution of the compound, or a solution of diazomethane in a suitable solvent can be prepared and added to the compound to be methylated. These solutions are, however, not very stable. The methylation reaction with diazomethane is represented by

$$R\text{–}H + CH_2N_2 \rightarrow R\text{–}CH_3 + N_2$$

Excess of reagent can easily be removed by evaporation in a stream of nitrogen. Provided the choice of solvent is correct, the reaction rate can be high, but in some instances, *e.g.*, with phenobarbital, more than one product is formed.

1.2.2. Pyrolytic alkylation

Acidic compounds can react with quaternary ammonium hydroxides to form salts. Upon pyrolysis in the heated injection port (250–350°C) of a gas chromatograph a volatile alkyl derivative of the compound is produced together with a tertiary amine. The reagents that are commonly used for the deprotonation are aqueous or methanolic solutions of tetramethylammonium hydroxide (TMAH), phenyltrimethylammonium hydroxide (PTMAH) and (*m*-trifluoromethylphenyl)trimethylammonium hydroxide. Sometimes reagents such as tetrabutylammonium hydroxide (TBAH) or tetrahexylammonium hydroxide (THAH) are used.

The salt formation of an acid, ROH, with the reagent TMAH and the subsequent degradation reaction at high temperature can be written as

$$R\text{–}OH + (CH_3)_4N^+\ OH^- \xrightarrow{25°C} R\text{–}O^-\ ^+N(CH_3)_4 + H_2O$$

$$R\text{–}O^-\ ^+N(CH_3)_4 \xrightarrow{250\text{–}350°C} R\text{–}O\text{–}CH_3 + N(CH_3)_3$$

Many acidic compounds can thus be rapidly and conveniently alkylated. However, flash alkylation will frequently result in the formation of more than one product; a well known example is the thermal decomposition of phenobarbital under the conditions of high alkalinity and high temperature that are employed[16,17].

1.2.3. Alkylation with N,N-dimethylformamide dialkylacetals

A number of N,N-dimethylformamide dialkylacetals are commercially available, either as the pure reagents or in solution. Methyl, ethyl, propyl and butyl are the most commonly encountered alkyl groups. Complete alkylation can usually be achieved by heating for a short period at 60–100°C in a suitable non-aqueous solvent. If necessary, the excess of reagent can be removed by evaporation under a stream of nitrogen. The alkylation reaction for carboxylic acids is as follows:

$$R\text{–}COOH + (H_3C)_2N\text{–}\underset{\displaystyle OR'}{\overset{\displaystyle OR'}{\overset{|}{\underset{|}{CH}}}} \rightarrow R\text{–}COOR' + R'\text{–}OH + (H_3C)_2N\text{–}CHO$$

The technique has been applied to a wide range of compounds; among these are the amino acids, both the primary amine group and the carboxylic acid group of which are converted simultaneously into an N-dimethylaminomethylene derivative and an alkyl ester, respectively[18]. With this method usually one product is formed, in contrast to the results with, *e.g.*, flash alkylation.

1.2.4. Alkylation with dimethyl sulphate

Derivatization is usually achieved by heating at 60–70°C for 5–10 min the mixture of the compound(s) under investigation, aqueous potassium carbonate (5–10%) and methanol together with a small amount of dimethyl sulphate. Methylation in non-aqueous solutions (acetone) has also been described[19].

The methylation of acidic compounds is based on a base-catalysed nucleophilic substitution reaction of the conjugate base, RO^-, of the acid with the reagent:

$$R\text{–}OH + OH^- \rightarrow R\text{–}O^- + H_2O$$

$$R\text{–}O^- + (CH_3)_2SO_4 \rightarrow R\text{–}OCH_3 + CH_3SO_4^-$$

Isolation of the derivatives before chromatography is usually required. Special care should be taken in all manipulations, because dimethyl sulphate is very toxic.

1.2.5. Acid-catalysed alkylation (esterification) of carboxylic acids

Esterification of carboxylic acids with alcohols of low relative molecular mass is a well known derivatization procedure in the fields of oil and fat chemistry and in biochemistry. The esterification is catalysed by mineral acids and organic acid anhydrides. Catalysis with sulphuric acid has become outdated because of the frequently slow and incomplete reactions. Solutions of dry hydrogen chloride in the appropriate alcohol give much better results. The reagent is 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 chosen as the derivatization reaction, but in principle a wide range of different esters can be prepared. The reaction will sometimes go to completion at room temperature within a short period, but frequently the reaction

mixture has to be heated at 60–100°C for up to 2 h. Isolation of the ester before chromatographic analysis is often necessary.

Organic acid anhydrides, such as trifluoroacetic and heptafluorobutyric anhydride, have been used successfully as catalysts in the rapid esterification of a number of compounds at room temperature.

1.2.6. Other alkylation methods

Closely related to the acid-catalysed esterification is the reaction of carboxylic acids with alcohols under catalytic action of boron trifluoride or boron trichloride. The main advantage of boron trihalide-catalysed esterification over acid-catalysed esterification is its rapidity. Solutions of the gaseous boron trihalides in alcohols are comparatively stable.

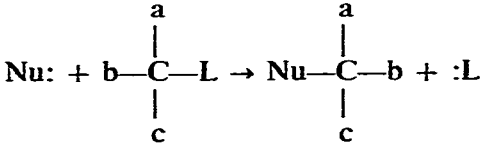
Some less commonly used alkylation reagents are the 3-alkyl-*p*-tolyltriazenes, trialkyloxoniumfluoroborates, alkylsulphonates and alkylfluorosulphonates and O-alkylisourea reagents. Up to now these reagents have found practically no application in the gas chromatographic analysis of pharmaceuticals.

2. ALKYLATION WITH ALKYL HALIDES

2.1. Reaction mechanisms[20–22]

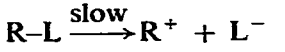
2.1.1. S_N1 and S_N2 reactions

Alkylation reactions with alkyl halides are nucleophilic substitution (S_N) reactions at saturated carbon. A nucleophile, Nu:, displaces a leaving group, :L (halide), from the substrate (the alkyl halide):



Nu: and :L share the same general character, being anionic or neutral bases with unshared electron pairs.

Two mechanistic routes have been clearly identified for S_N reactions. One of these is



The ionization to a carbonium ion intermediate is the rate-limiting step. The subsequent reaction of R^+ with the nucleophile is very fast. The reaction rate therefore depends on the concentration of the substrate (alkyl halide) and is independent of the nucleophile concentration; the reaction is unimolecular and is denoted by S_N1. First-

order kinetics are observed. The reaction rate depends heavily on the ionizing power of the solvent. Protic, polar solvents (water, methanol) stabilize the carbonium ion and the displaced leaving group by solvation of the ions. With a suitable substrate (*e.g.*, tertiary alkyl halides) the S_N1 route of reaction is favoured in these solvents. The second mechanism is the one-step direct displacement reaction:

$$Nu^-: + R\text{–}L \rightarrow [Nu \cdots\cdots R \cdots\cdots L]^- \rightarrow Nu\text{–}R + :L^-$$

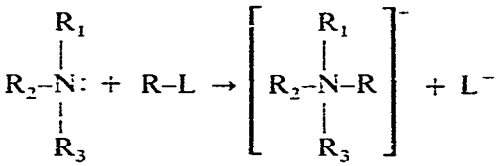
This is a bimolecular process, labeled S_N2. The species in brackets represents the transition state. Overall second-order kinetics are observed. However, derivatization reactions in GLC practice usually show pseudo-first-order kinetics owing to the large excess of the alkylating agent. The reaction is very sensitive to steric hindrance. The nucleophile must be able to approach the carbon atom of the substrate, coming in from the rear side, with its electron-pair orbital on a line with the axis of the orbital bearing the leaving group L. If the substrate (alkyl halide) carries bulky groups at the carbon atom under attack, as in tertiary alkyl halides and many branched alkyl halides, the S_N2 route becomes greatly hindered and the reaction rate will be very low. The influence of the polarity of the solvent is usually not as dramatic as in S_N1 reactions.

S_N1 reaction conditions (tertiary and secondary alkyl halides and protic, polar solvents) are generally unsuitable for alkylation reactions with alkyl halides, because of the high incidence of side-reactions, mainly the elimination of hydrogen halide from the alkyl halide. This results frequently in low yields of derivative products.

In the following sections the four main effects on the rate of S_N2 reactions, usually encountered in derivatization with alkyl halides, are discussed. These are solvent effects, type of alkyl halide, reactivity of the nucleophile and leaving group activity.

2.1.1.1. Solvent effects. In S_N2 reactions between nucleophilic anions of organic acids and alkyl halides the ionizing power of the solvent is usually of less importance than in S_N1 reactions. Aprotic solvents are the best medium for S_N2 reactions. When protic solvents, such as water and methanol, are present, the nucleophile will be stabilized, because the active electron pair of the nucleophile interacts through hydrogen bonding with the solvent molecules; these hydrogen bonds must be broken if the nucleophile is to react with the substrate, thus adding an extra energy barrier. Consequently, S_N2 reactions are favoured in aprotic solvents, such as acetone, N,N-dimethylacetamide and dichloromethane.

It should be borne in mind that not only acidic anions can act as nucleophiles; neutral amines can also be alkylated, yielding quaternary ammonium compounds:



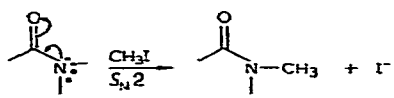
This reaction is strongly inhibited in aprotic solvents such as dichloromethane, because the ionic species which are formed cannot be efficiently solvated in these solvents. This is an important consideration when compounds with acidic groups which also carry an amine group are to be alkylated.

2.1.1.2. Structure of the alkyl halides. The order or reactivity in S_N2 reactions, with respect to the alkyl halide undergoing a nucleophilic attack, is CH_3 > primary > secondary ≫ (tertiary). Inductive effects cause the reactivity of the primary *n*-alkyl halides to be lower than that of methyl halide, because the electron density at the carbon atom under attack is increased. As mentioned above, S_N2 reactions are very subject to steric hindrance; with tertiary substrates the S_N2 displacement is virtually impossible. Resonance effects apparently play a part, although not to any great extent. This is evident from the relative reaction rates of benzyl halides and methyl halides; the benzyl halides show about a four-fold higher reaction rate.

A special case of interest is the group of alkyl halides, such as phenacyl bromide, with a carbonyl α to the reactive carbon. These are often the most reactive substrates in S_N2 reactions, apparently because the attacking nucleophile gives its charge to the carbonyl group as well as to the adjacent reaction site.

2.1.1.3. Relative reactivity of acidic anions. Acidic compounds can be alkylated (with alkyl halides) when they are present in their anionic form, provided that the nucleophilicity of the conjugate base of the acid is sufficient to displace the leaving group. Within a series of structurally related compounds nucleophilicity parallels basic strength. The following order of decreasing nucleophilicity exists for acidic anions with oxygen as the attacking atom: $C_2H_5O^-$ > HO^- > $C_6H_5O^-$ > CH_3COO^-. Although deprotonated aliphatic alcohol groups are very reactive species in nucleophilic substitution reactions, no alkylation of alcohols will take place under the comparatively mild conditions normally used in these reactions, because the aliphatic alcohols are such weak acids that deprotonation to any significant extent does not occur. Only when very strong bases and suitable solvents are used will alkylation of aliphatic alcohols be achieved.

Frequently, nucleophiles contain more than one atom bearing active electron pairs and therefore can react in different ways. An important example is represented by the barbiturates. These compounds usually contain two acidic imide groups, each of which can be alkylated at the N or O atom, depending on the reaction conditions. Under S_N2 conditions the more polarizable (larger) and less electronegative atom is preferentially alkylated. The latter preference can be enhanced by the addition of a protic solvent, which will favour hydrogen bonding of the more electronegative atom, leaving the other site free for the displacement reaction. Therefore, the less electronegative N atom of the imide group of barbiturates is preferentially alkylated under S_N2 conditions (the reverse is true under S_N1 conditions):



Steric hindrance and ion pairing are also factors which can influence the preference for one site over the other.

2.1.1.4. Relative leaving group activity. The more polarizable atoms yield the better leaving groups. For the halides the following decreasing order of leaving group activity exists: $I^- > Br^- > Cl^- \gg F^-$. Derivatizations with alkyl halides are therefore performed almost exclusively with iodides or bromides.

2.1.2. Choice of alkyl halide

Methyl iodide is usually the reagent of first choice. For reasons discussed above only primary, unbranched alkyl halides should be used under S_N2 conditions. Methyl iodide has two advantages over the other *n*-alkyl iodides: it reacts more rapidly and competing elimination reactions cannot occur owing to the absence of a β-hydrogen atom. An important reason for choosing another *n*-alkyl iodide instead of methyl iodide is the possibility of confusion of the derivative with other compounds present. For instance, upon methylation, theophylline is converted into caffeine, which is usually present in human serum and urine samples.

For the enhancement of the electron-capture detector (ECD) response the compounds to be analysed are almost invariably alkylated (in the case of a halide reagent) with pentafluorobenzyl bromide (PFB-Br). PFB derivatives are volatile with good gas chromatographic properties and provide a high response to the ECD. α-Halocarbonyl compounds, such as phenacyl bromide, are frequently used as derivatizing agents in high-performance liquid chromatography (HPLC) to enhance the UV absorption detector response. Their use in GLC has been restricted to a few isolated cases[23,24]. The application of these reagents can be expected to increase owing to the high sensitivity towards electron-capture detection of the phenacyl derivatives[23].

Another sensitive and selective detector that has won wide application in the gas chromatographic analysis of pharmaceuticals is the nitrogen- and phosphorus-sensitive detector (NPD). However, alkyl halides containing phosphorus or nitrogen for the specific aim of enhancing the NPD response have, to our knowledge, not been used.

2.2 Base-catalysed alkylation

The necessary deprotonation of the acidic compound to be alkylated can be effected by the addition of a basic agent, such as potassium carbonate or hydroxyl ions. A number of base-catalysed alkylations with alkyl halides have been described. Two main types of reactions are discernable and will be dealt with in the following sections. Sharp distinctions are not always possible, but some clearly deviating base-catalysed alkylation reactions will be discussed in Section 2.2.3.

2.2.1. Carbonate-catalysed alkylation

Kawahara[25,26] and Dünges and co-workers[19,27] introduced this method, first described by Claisen and Eisleb for preparative purposes at the beginning of the century, into gas chromatographic practice. The acidic compounds are heated in a polar, aprotic solvent (usually acetone) with an alkyl halide in the presence of carbonate. For rapid and complete alkylation the solvent should contain little or no water. The basic catalyst, usually anhydrous potassium carbonate, is almost insoluble in non-aqueous solvents and can therefore be added in large excess, thus providing a

large solid–liquid interface at which the reaction takes place. The reaction between carboxylic acids and methyl iodide, for example, proceeds as follows:

$$2\ R\text{–}COOH + K_2CO_3 \rightarrow 2\ R\text{–}COO^-K^+ + H_2O + CO_2$$

$$R\text{–}COO^-K^+ + CH_3I \rightarrow R\text{–}COOCH_3 + K^+I^-$$

The water produced during the neutralization step is bound by the excess of potassium carbonate. Many acidic compounds can be derivatized in this way: phenols, carboxylic acids and imides are often completely converted into their alkyl derivatives. Aliphatic alcohol groups and amines (except in some special cases) are not derivatized. The derivatives are usually stable in the reaction mixture. The mixture can be injected directly into the gas chromatograph, except when an ECD response-enhancing reagent has been used (PFB-Br). Concentration of the sample before injection is easily achieved by evaporation under nitrogen followed by reconstitution of the residue in a small volume of a suitable solvent, or by concentration under partial reflux[28].

2.2.1.1. The carbonate. Instead of solid potassium carbonate, other carbonates have also been used. Sodium carbonate has sometimes been applied[29–31], as well as sodium hydrogencarbonate[32]. Thio *et al.*[33] investigated the reaction times needed for the methylation of carboxylic acids in acetone (at room temperature) using the anhydrous carbonates of potassium, rubidium and caesium. The reaction rates increased with increasing size of the cation and were highest when caesium carbonate was used. The acetone–caesium carbonate mixture was found to be an excellent reaction mixture also for the derivatization of barbiturates with 2-naphthacyl bromide[34]. For the formation of the PFB derivative of tetrahydrophthalimide, a metabolite of captan, the addition of pyridine together with potassium carbonate was required[32].

Occasionally a small volume of a concentrated potassium carbonate solution in water has been used[35–37]. The introduction of water into the reaction medium will tend to slow down the reaction rate. On the other hand, more carbonate is dissolved in the mixture, which will result in more efficient deprotonation of the acidic compounds.

The addition of a crown ether to the reaction mixture with solid potassium carbonate has been proposed for derivative formation with carboxylic acids and phenols[38,39]. The potassium ion of the ion pair, potassium–acidic anion, is complexed by the crown ether, leaving the acidic anion "naked" and therefore very reactive in the acetone solution.

2.2.1.2. The solvent. Acetone is the most frequently used solvent for the reaction. A number of other solvents or solvent mixtures have been advocated. Ethyl acetate has been reported by Dünges[19,40] to be a suitable solvent for the alkylation of barbiturates (and acetone). Wu and Pearson[41] reported that the use of methanol–acetone–methyl iodide (1:1:1), rather than acetone alone with methyl iodide causes an increase in the reaction rate of the methylation of barbiturates; the reaction was complete within 10 min at 60°C. Acetonitrile[39,42,43] and 2-butanone[44,45] can also be satisfactory solvents. These solvents are less volatile than

acetone, which is helpful if one wants to prevent the loss of solvent when heating the reaction mixture.

Davis[38] investigated the usefulness of acetonitrile, benzene, ethyl acetate, dimethylformamide (DMF) and heptane for PFB derivative formation with carboxylic acids and phenols. Only when the reaction took place in benzene was the yield more than 90%. Unless the use of a strongly alkaline aqueous solution for the deprotonation of acidic compounds is necessary, water should be excluded as far as possible from the reaction mixture.

2.2.1.3. Reaction conditions. Heating the reaction mixture for some time is usually necessary for complete conversion into the derivative. In view of the volatility of the solvent the derivatization is normally performed in an air-tight, closed reaction vial. IJdenberg[42] brought the reaction mixture into a small glass tube; after purging with nitrogen the tube was fused. Alternatively, the reaction mixture can be refluxed; Dünges[46] developed an apparatus, the Microrefluxer, which allows the reflux of microlitre volumes of organic solutions[40,46].

Sometimes the derivatization is allowed to proceed at room temperature[36,47], which will lead to comparatively long reaction times. Thio *et al.*[33] reported that a reduction in reaction time was achieved by ultrasonic treatment while heating the samples at 50°C.

2.2.1.4. The alkyl halides. Methyl iodide and PFB-Br have been extensively used, the latter with the double aim of enhancing the ECD response and improving the chromatographic properties. Ethyl iodide[42,43,48], propyl iodide[42] and butyl iodide[33] have been successfully applied in derivatizations by the carbonate method. With the introduction of the method, Dünges[19] showed that methoxymethyl chloride, allyl iodide and benzyl iodide might also be useful reagents.

2.2.2. Quaternary ammonium hydroxide-catalysed alkylation

A fast and usually quantitative alkylation of acidic compounds under very mild conditions is achieved by the method introduced by Greeley[49,50] in 1974. The acidic compound is dissolved in a polar aprotic solvent (usually N,N-dimethylacetamide, DMA) and deprotonated by tetramethylammonium hydroxide in methanolic solution, or by another quaternary ammonium hydroxide. After addition of the alkyl halide, the reaction mixture is allowed to stand at room temperature; 5–10 min are usually sufficient for complete derivatization. For instance, for carboxylic acids the reaction with *n*-butyl iodide under TMAH catalysis is as follows:

$$R\text{–}COOH + (CH_3)_4N^+ OH^- \rightarrow R\text{–}COO^- {}^+N(CH_3)_4 + H_2O$$

$$R\text{–}COO^- {}^+N(CH_3)_4 + n\text{-}C_4H_9I \rightarrow R\text{–}COOC_4H_9 + (CH_3)_4N^+ I^- \downarrow$$

The alkyl iodide must be added to the reaction mixture after the base, because the hydroxyl ions necessary for the deprotonation of the acid will react immediately, also in an S_N2 reaction, with the alkyl iodide. Consequently, the derivatization of the acidic anion might not go to completion owing to insufficient deprotonation of the

acid. The excess of base is thus removed and at the end of the procedure the reaction mixture is neutral.

$$(CH_3)_4N^+ OH^- + C_4H_9 I \rightarrow (CH_3)_4N^+ I^-\downarrow + C_4H_9 OH$$

Under non-aqueous conditions the TMA iodide precipitates and centrifugation is necessary before the supernatant is injected into the gas chromatograph. The volume of the reaction mixture can be kept very small, so that concentration of the reaction mixture is usually not needed. Separation of the derivative from the solvent may be necessary, if the NPD is to be used as the detector, because a nitrogen-containing solvent such as DMA causes tremendous overload of the detector.

2.2.2.1. The quaternary ammonium hydroxide. Greeley[50] considered PTMAH to be a better organic base than TMAH, because its salts with acidic anions are highly soluble in the organic solvent system and because no precipitation of the corresponding iodide occurs. For practical reasons he chose TMAH as the base for the alkylation reactions: it is available as a concentrated methanolic solution and its solutions are considerably more stable than those of PTMAH. Further, the precipitation of TMA iodide in the derivatization can also be regarded as an advantage, as injections of quaternary ammonium salts into the gas chromatograph may lead to broader "solvent peaks" and can be detrimental to the column packing material. PTMAH has been used by a few investigators[51–53]. Von Minden and D'Amato[52] optimized the reaction conditions for the propylation of benzoylecgonine, the principal metabolite of cocaine. They found that under the prevailing conditions the use of TMAH alone gave rise to hydrolysis, whereas the use of PTMAH alone resulted in long reaction times. A mixture of TMAH and PTMAH proved to be suitable in this instance.

A few workers have reported the use of TBAH[54,55]. McCurdy *et al.*[51] used a solution of tetrabutylammonium hydrogensulphate (TBAHS) and PTMAH in methanol in the propylation reaction of N-desmethyldiazepam; the addition of TBAHS was not motivated by the authors and would appear to be superfluous.

2.2.2.2. The solvent. DMA with methanol has been used as the solvent mixture by most workers. According to Greeley[50], the actual composition of the solvent system is not critical. A solvent containing roughly 80% DMA and 20% methanol was used in his work. Greeley[50] stated that the addition of 10–20% of methanol is necessary to increase to solubility of the intermediate salts. Raisys *et al.*[53], however, used a methanol-free DMA solution to pentylate mephenytoin and desmethylmephenytoin. The methanolic solution of PTMAH was evaporated before use and reconstituted in DMA. The authors did not mention why methanol was excluded.

As usual in S_N2 reactions the presence of water was found to decrease the reaction rate, although the presence of 5% of water did not prevent the butylation of phenobarbital from being quantitative at room temperature[50].

N,N-Dimethylformamide (DMF) can serve as a substitute for DMA[50,56]. Greeley[50] found that acetonitrile was not acceptable as a solvent for the derivatization reactions; Joern[55] used acetonitrile with TBAH for the butylation of phenytoin, but at a higher temperature. The successful use of acetone[57] and butyl acetate[58] has also been reported. These solvents could be helpful when an NPD is to be applied. On the other hand, methanol, ethyl acetate and dichloromethane were found to be less suitable than DMA in the methylation of sulphinpyrazone and its metabolites[59].

2.2.2.3. Reaction conditions. When DMA is used in combination with TMAH (or PTMAH), a period of 5–10 min at room temperature is the usual time needed for the reaction. Menez *et al.*[60] studied the derivatization of barbiturates; the most effective molar ratios between TMAH, alkyl iodide and barbiturate for preparing the dialkyl derivatives proved to be about 21:150:5. Changing the solvent necessitates a change in the reaction conditions, except with DMF. For instance, phenytoin was reacted with butyl iodide in acetonitrile for 20 min at 45°C[55].

2.2.2.4. The alkyl halides. All *n*-alkyl iodides from methyl iodide to *n*-heptyl iodide have been applied in the tetraalkylammonium hydroxide-catalysed alkylation of acidic pharmaceuticals. With branched alkyl reagents such as isopropyl iodide and isobutyl iodide anomalous results were obtained[49,60], and steric hindrance totally prevents the derivatization of phenobarbital with cyclohexyl iodide[60]. We found no reports on derivatization with PFB-Br by this method.

2.2.3. Miscellaneous base-catalysed alkylation reactions

Instead of carbonate or tetraalkylammonium hydroxide a number of other bases, in conjunction with solvents ranging in polarity from very high (ethanol–water) to very low (pentane), have been used to catalyse alkylation reactions of acidic compounds.

Valproic acid has been converted into its phenacyl derivative in an acetonitrile–sodium hydrogencarbonate–crown ether system[23] and in a pentane–triethylamine solvent mixture[24]. *p*-Bromophenacyl bromide and *p*-phenylphenacyl bromide were used as alkylating agents for volatile (C_2–C_{10}) carboxylic acids[61]; the acids were neutralized in aqueous solution with potassium hydroxide and then derivatized by refluxing in ethanol–water.

The PFB derivatives of theophylline[62], pentobarbital[63] and pseudoephedrine[64] have been prepared in ethanol–water mixtures using aqueous solutions of sodium carbonate or potassium *sec.*-phosphate. Walle[65] also used a very polar solvent, methanol, for PFB derivative formation with barbiturates and phenytoin; triethylamine at a well defined concentration was chosen as the catalyst because hydrolysis of the barbiturate ring was observed with potassium carbonate as the base. Under these conditions only one (of two) acidic group of phenytoin is alkylated. On the other hand, Kogan *et al.*[66] alkylated benzoylecgonine with PFB-Br in the comparatively apolar benzene–dichloromethane mixture with pyridine as the catalyst. The PFB ester of indole-3-acetic acid was prepared in acetone with N-ethylpiperidine[67].

Davis[38] investigated the crown ether-catalysed derivatization of carboxylic acids and phenols with PFB-Br in various solvents, with potassium salts of different basicity. When potassium carbonate was used, the carboxylic acids and the phenols were both derivatized, whereas the weaker bases, potassium hydrogencarbonate, potassium acetate and potassium cyanide, allowed the derivatization of carboxylic acids but not of phenols. Volatile carboxylic acids have been alkylated with benzyl bromide in acetone after conversion of the acids into their tetrabutylammonium salts[68].

The hydantoins phenytoin and desmethylmephenytoin possess two acidic groups; one group has a pK_a value of 8.3 (ref. 69), and the pK_a of the other group is similar to that of an amide. Mephenytoin contains only the very weakly acidic amide group. The peralkylation of these compounds can be accomplished if the pH of the medium is such that even the amide group is deprotonated. Gordos *et al.*[47] added a

buffer solution of pH 13 to the acetone–methyl iodide mixture in order to methylate phenytoin; at lower pH values of the buffer the monomethylated product or no product at all is formed. De Sagher *et al.*[44] found that complete perethylation of the 5,5-disubstituted hydantoins was obtained after heating for 1.5 h at 60°C a mixture of 200 μl of acetone, containing the compound under investigation, 50 μl of ethyl iodide and 5 μl of 5 *N* potassium hydroxide in water. These authors also stated that 2-butanone can be used to replace acetone if loss of solvent during the reaction is a problem. The 2-butanone–ethyl iodide–potassium hydroxide solution system has been used for the ethylation of mephenytoin and its demethylated metabolite[45].

A curious butylation reaction for the alkylation of theophylline has been proposed by Vinet and Zizian[70]. A mixture of butyl iodide, TBAH and methanol is injected, together with a solution of theophylline in dichloromethane–isopropanol, into the injection port of a gas chromatograph at 230°C. Although the experiments performed in this study do not permit a definite conclusion, the resulting on-column butylation of theophylline is probably effected by two simultaneous reactions: the TBAH-catalysed S_N2 alkylation reaction with butyl iodide and the pyrolytic butylation with TBAH alone.

A number of investigators have used solutions of sodium hydride, potassium *tert.*-butoxide or sodium methoxide in dry dimethyl sulphoxide in order to permethylate acidic compounds. The strongly basic methylsulphinyl carbanion which is formed in these solutions deprotonates even very weak acids, which then are able to react with added methyl iodide. The reaction is fast and quantitative in a very short period at room temperature. After the reaction, water is added and the derivatives are isolated from the mixture by extraction with chloroform or another solvent. This procedure has led to successful GLC determinations of bile acids[71], nucleotides[72], insecticides[73], urea herbicides[74], 5-fluorouracil[75], 5-fluorouridine[76] and floxuridine[77]. Thompson[78] showed that the method is not well suited to the methylation of barbiturates.

The silver salts of fatty acids have been methylated in pentane with methyl iodide[79,80]. The concentration of free silver ions in the solvent was found to be important for the result of the derivatization[80].

Silver oxide[42] has been used to catalyse alkylation reactions of antiepileptic agents, such as primidone and barbiturates, by heating in acetone or acetonitrile. Ambident nucleophiles, such as primidone, can be expected to react at least partly via the S_N1 route in the presence of silver ions, resulting in O-alkylation rather than N-alkylation[22].

2.3. Phase transfer catalysis

Deprotonation and subsequent alkylation of acidic compounds can be achieved not only by the addition of (excess) base, but also by the transfer of the acidic anion as an ion pair with a tetraalkylammonium counter ion into a suitable aprotic solvent, such as dichloromethane, containing the alkyl halide. The phase transfer is usually from one solvent to another, and is then called liquid–liquid phase transfer catalysis; this is better known as "extractive alkylation"[81,82]. At the end of the next section, dealing with this technique, two methods deviating from the usual extractive alkylation technique, but still with strong resemblances to this method, will be discussed.

2.3.1. Extractive alkylation

The acidic compound is extracted as the ion pair formed with tetraalkylammonium from an aqueous solution at a suitable pH into an aprotic solvent, usually dichloromethane. The extracting solvent has poorly solvating properties for anions, which therefore possess high reactivity and an S_N2 reaction between the anion and the alkyl halide added to the system can then take place to give the required derivative.

For instance, in the methylation of carboxylic acids upon extraction with TBA^+ ions the reactions are as follows:

$$R\text{–}COOH_{aq} + OH^- \leftrightarrows R\text{–}COO^- + H_2O \quad (1)$$

$$R\text{–}COO^- + TBA^+ \leftrightarrows TBA^+\ R\text{–}COO^-_{org} \quad (2)$$

$$TBA^+\ R\text{–}COO^-_{org} + CH_3I \leftrightarrows R\text{–}COO\text{–}CH_3 + TBA^+I^- \quad (3)$$

Derivative formation is usually achieved by shaking the water–organic solvent system for a certain period of time (from 10 min to many hours) at room temperature.

The speed of the derivatization reaction and the yield of derivative are governed by the efficiency of extraction and the rate of the nucleophilic substitution reaction. The efficiency of extraction of the acidic anion is determined by the properties and the concentrations of the ion pair forming ions, the lipophilicity of the ion pair and the properties of the solvent[83,84].

The extraction equilibrium of an anion, X^-, with a tetraalkylammonium ion, R_4N^+, can be written as

$$X^-_{aq} + R_4N^+_{aq} \leftrightarrows R_4N^+X^-_{org}$$

The equilibrium constant is defined by

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

If no side-reactions occur, such as protonation of X^-, the distribution ratio of X^- is expressed by

$$D_X = \frac{[R_4N^+X^-]_{org}}{[X^-]_{aq}} = K_{Ex}[R_4N^+]$$

The distribution ratio, and therefore the extraction efficiency, is thus determined by K_{Ex} and the concentration of the counter ion, higher R_4N^+ concentrations leading to increased reaction rates[82,84,85]. The percentage of X^- extracted as the ion pair is, of course, also dependent on the phase-volume ratio of the organic extractant and the aqueous layer. The more lipophilic the ion pair, the higher will be the value of K_{Ex}. Lipophilicity is increased with increasing numbers of carbon atoms (in homologous

series). In practice, the use of quaternary ammonium ions with fewer carbon atoms than TBA^+ will generally result in too low extraction efficiencies. TBA^+, TPA^+ (tetrapentylammonium) and THA^+ (tetrahexylammonium) are the most frequently employed counter ions. With hydrophilic anions the use of a larger counter ion is indicated to achieve sufficient extraction of the ion pair. The pK_a and the partition coefficient of the acidic compound determine the minimal pH value, at which partitioning of the compound as the ion pair will occur without significant partitioning of the undissociated acid. The pH of the aqueous phase should be at least two units higher than the pK_a value of the acid, when the partition coefficient of the acid is unity. With very lipophilic acids, such as the higher fatty acids, a much higher pH value is needed; with hydrophilic acids the pH might be kept lower[82,84–86].

K_{Ex} is also very dependent on the nature of the organic extractant, which must be capable of accomodating the ion pair. Chlorinated hydrocarbons, such as chloroform, dichloromethane and dichloroethane are good solvents for ion pairs, but other solvents, such as the alcohols (C_4–C_7), methyl isobutyl ketone, and even less polar solvents such as benzene, toluene and carbon disulphide, can also be effective extractants[82–84,87–89]. The choice of the solvent is, of course, somewhat restricted because of the subsequent S_N2 reaction which has to take place. The alcohols are therefore less suitable extractants; the higher alcohols would also cause problems in concentration steps through evaporation because of their high boiling points. S_N2 alkylation reactions usually proceed rapidly in aprotic solvents such as the halogenated hydrocarbons. Dichloromethane is the most frequently used solvent in extractive alkylation procedures.

As was mentioned in Section 2.1., S_N2 reaction rates are proportional to the nucleophilicities, and consequently also proportional to the base strengths of the conjugate bases of the acids to be alkylated. A linear relationship has been observed between the logarithm of the observed rate constants and the pK_a values of the acids (in water) in some investigations related to the study of extractive alkylation processes[90,91].

The S_N2 reaction rate is also dependent on the structure and concentration of the alkyl halide. The greater reactivity of methyl iodide and PFB-Br compared with that of the *n*-alkyl iodides was confirmed in an investigation on the alkylation of sulphonamides in various solvents[87]. The use of benzyl bromide allowed very fast reactions with acetylsalicylic acid and salicylic acid[92].

In practice, the optimal conditions for extractive alkylation seldom coincide with the highest possible extraction efficiency and reaction rate. One reason is that higher selectivity can often be obtained by the judicious choice of pH, type and concentration of R_4N^+ counter ions, etc. Another reason is that the lowest detectable amount of derivative following extractive alkylation is usually not dependent on the signal-to-noise ratio but on the extent of by-product formation, the presence of contaminants and sometimes excess of reagent, causing interferences and/or tailing fronts in the chromatograms. Methyl iodide can contain dimethyl sulphate, which causes long tailing solvent fronts in the gas chromatograms[93]; distillation before use therefore is necessary. The iodide of the R_4N^+ ion formed as a by-product during alkylation can also give rise to long tailing fronts. Furthermore, transesterification reactions caused by $R_4N^+ I^-$ have been observed upon injection of the organic reaction mixture without further purification[93].

$R_4N^+ I^-$ can be removed from the final organic solvent layer after derivatization in different ways. The organic layer can be washed with a solution of silver sulphate[75,93,94]. Another possibility is evaporation of the final organic solvent layer and extraction of the derivative from the residue with a very apolar solvent, usually an *n*-alkane, in which $R_4N^+ I^-$ is insoluble[95–98], or by taking up the residue in toluene and extracting the derivative with *n*-hexane[99]. A successful clean-up after derivatization can also be performed by an additional extraction step with diethyl ether after evaporation of the organic solvent layer and reconstitution of the residue in water[75].

In extractive alkylation procedures it is essential to use conditions that will minimize hydrolysis of the reagent and of acidic anions such as acetylsalicylate[92]. At higher pH values substantial amounts of OH^- ions can be transferred into the organic phase as ion pairs with R_4N^+ counter ions. The OH^- ions will then displace I^- or Br^- from the reagent. In particular with the use of PFB-Br problems can arise owing to the formation of by-products[100]. The OH^- transfer into the organic phase is enhanced by high pH values of the aqueous phase and by the use of R_4N^+ counter ions of increasing lipophilicity. For this reason, when optimizing extractive alkylation procedures, one should not extract at higher pH values or with larger R_4N^+ ions than is strictly necessary. The concentrations of the R_4N^+ ions and of the alkyl halide should not be higher than required and the reaction should not be unduly prolonged.

Another reason for keeping the concentration of ECD response-enhancing alkyl halides in particular as low as possible is the danger of overloading the detector. Usually an extra clean-up step is needed in order to separate the alkyl halide, PFB-Br, from the final organic solvent layer. Possible clean-up steps are evaporation of the excess of PFB-Br[101,102], extraction of the derivative into an aqueous phase[103,104], separation on a silica gel column[105] and coupling of the excess of reagent with an aminophenol (hordenine) to yield a product that can be extracted with water[106]. Another possibility is the use of a pre-column venting system[107].

The adjustment of the pH of the aqueous phase is also important in the extractive alkylation of acids with two (or more) acidic groups, such as many barbiturates and 5-fluorouracil[75,89]. Optimal conditions for ion-pair extraction exist when the acid is present in its monovalent anionic form[108]. The dialkyl derivatives are formed, probably in a stepwise fashion[75]. It seems that the monoalkyl derivative is produced first; this will have a lower pK_a value than the $pK_{a,2}$ value of the underivatized compound (compare mephobarbital and phenobarbital). The monoalkylated product is then extracted into the aqueous solution, back-extracted into the organic phase as an ion pair and subsequently derivatized to yield the dialkylated product. However, the extractive methylation of oxazepam[109] could not be explained by this (hypothetical) mechanism.

2.3.2. *Specific alkylation of phenolic compounds in a biphasic system*

An alkylation procedure closely resembling the extractive alkylation process has been proposed by Rosenfeld and co-workers[110,111] for the specific alkylation of compounds with a phenolic hydroxyl group, such as estradiol[111]. It was observed[110] that these acids can be alkylated in a biphasic system, *without the addition of R_4N^+ counter ions*, with PFB-Br or benzyl bromide. The method is specific for phenolic compounds; carboxylic acids do not react. The kinetic data suggest[111] that the usual S_N2

displacement reaction probably takes place, but there is also some conflicting evidence with respect to the type of reaction mechanism involved. There are indications that phase-transfer catalysis is not the mechanism responsible for alkylation. From a practical point of view, the specificity of the alkylation of phenols is also interesting, because it offers the advantage that one class of substrates (phenols) reacts, whereas a second class of compounds (carboxylic acids) does not.

2.3.3. Alkylation by solid–liquid phase transfer catalysis

Particularly for the alkylation of compounds sensitive to hydrolysis, the following method was found to be useful[112–114]. To dichloromethane (or another suitable solvent such as ketone[114], containing TBA hydrogensulphate, alkyl halide, RX, and the acidic compound (HA) to be alkylated, solid sodium hydrogencarbonate is added. The mixture is shaken for a certain period of time and the organic solvent phase is analysed by GLC. A three-step reaction is probably involved[113]. The hydrogencarbonate is transferred into the liquid phase through exchange with hydrogensulphate anions, followed by the simultaneous deprotonation of the acidic compound and formation of the ion pair; then the alkylation reaction takes place.

$$NaHCO_{3\,solid} + TBA^+ HSO^-_{4\,org} \rightarrow TBA^+ HCO^-_{3\,org} + NaHSO_{4\,solid} \quad (1)$$

$$TBA^+ HCO^-_{3\,org} + HA \rightarrow TBA^+ A^-_{org} + H_2CO_3 \quad (2)$$

$$TBA^+ A^-_{org} + RX \rightarrow RA + TBA^+ X^-_{org} \quad (3)$$

This process has been applied successfully to the analysis of indomethacin[112] and acetylsalicylic acid[113].

3. THE ALKYLATION METHOD IN RELATION TO THE ENTIRE ANALYTICAL PROCEDURE

The derivatization of the compound(s) under investigation is in practice always preceded by some kind of sample pre-treatment and followed by a gas chromatographic separation and detection. When evaluating an analytical procedure for the analysis of compounds that have to be derivatized, these three parts of the procedure should not be treated as separate entities.

The choice of the derivatization method and of the reaction conditions is generally limited. One reason is that the solvent must be compatible with the chromatographic system, and that the derivative must be sufficiently stable at the high temperature usually applied in GLC. Further, there should be a smooth connection between the sample pre-treatment and derivatization procedure, that is, the solution after the final clean-up step must be either directly suitable for the subsequent derivatization reaction or else must be exchanged for another, more suitable solvent with a minimum of manipulations. These aspects are discussed in Sections 3.1 and 3.2.

Finally, the demands made on the analytical procedure as a whole are variable and depend on the field of application of the method. This will, of course, influence the choice of the derivatization technique. For example, in emergency procedures the analysis should be as fast as possible. On-column derivatization methods are then to

be preferred[70]. In some instances the pre-column extractive alkylation technique will also be fast enough for this purpose. When large numbers of samples must be analysed with the help of an automatic sample injection system, the stability at room temperature of the final solution to be injected into the gas chromatograph is an important feature.

3.1. Alkylation method and chromatographic system

A generally accepted criterion for derivatization reactions in GLC is that the alkylated product should be stable in the solution in which it is injected at the elevated temperatures of the injection block and the column. Furosemide has been methylated to its trimethyl derivative before GLC analysis by an extractive alkylation technique[115]. When the residue of the evaporated extraction mixture, containing the furosemide derivative and $THA^+ I^-$, was reconstituted in a solvent in which $THA^+ I^-$ is soluble, one of the methyl groups of trimethyl furosemide was exchanged for a hexyl group after injection; with *n*-hexane as the solvent the degradation of the derivative was prevented. Useful exceptions to the rule of stability of the derivative in the gas chromatographic system are also known. Methylated sulphinpyrazone seems to be degraded in the injection port of the gas chromatograph, because the shape and height of the chromatographic peak change (and improve) at higher temperatures[59]. Above 270°C the peak shape and peak height were found to be constant and reproducible, apparently owing to the quantitative degradation of methylsulphinpyrazone. In fact, this can be regarded as the on-column derivatization of a pre-column derivatized compound.

The most frequently and broadly applied detection methods in the GLC analysis of drugs are FID, NPD and ECD. With FID, extra clean-up steps after the alkylation reaction are generally not needed. There is little danger of overloading the detector, provided that the differences in retention (volatility) between the derivative(s) formed and the solvent with excess of reagent (alkyl halide) are large enough. The FID response for carbon disulphide is very low. Ehrsson[89] described a procedure for the extractive alkylation of barbiturates with carbon disulphide as the organic extractant. Owing to the reduced solvent front compared with those obtained after the injection of the more common organic solvents, the sensitivity of detection could be significantly improved. The FID is an almost universal detector for organic compounds; therefore, the selectivity of GLC–FID procedures depends entirely on the column and the sample pre-treatment. Elaborate sample clean-up procedures are therefore occasionally necessary.

The main goal of derivatization in GLC–FID analysis is the improvement of the chromatographic behaviour of compounds. The gain in sensitivity on introducing very large alkyl groups into the molecules of the acidic compounds is marginal and problems may arise because of the reduced volatility of the derivatives. NPD allows the sensitive and selective detection of nitrogen- and phosphorus-containing compounds. As with FID, the main objective for derivatization in GLC with NPD is the improvement of chromatographic behaviour. To prevent detector overloading the final solution to be injected should not contain large amounts of nitrogen- (or phosphorus-) containing components. The reaction mixture obtained after alkylation in acetone with carbonate catalysis is therefore suitable for direct injection into the

GLC–NPD system. After a $R_4N^+ OH^-$-catalysed alkylation in DMA, however, an extra clean-up step is necessary to remove the DMA and dissolved $R_4N^+ I^-$. The obvious remedy would be to replace DMA with a nitrogen-free solvent, in which the $R_4N^+ I^-$ formed during the reaction is insoluble. No reports on the successful application of such a system in the GLC–NPD analysis of pharmaceuticals could be found.

The alkylation of acidic compounds can serve a single or a double purpose when ECD is chosen as the method of detection. With compounds that possess electron-capturing properties, the main objective of derivatization is again improvement of the chromatographic behaviour of the compounds. More often, lowering the detection limit of non-electron-capturing compounds is the principal reason for derivatization, and the necessity to improve the chromatographic properties of the compounds is the second one. In either instance, the excess of alkyl halide, and very often also the organic solvent of the extractive alkylation procedure, must be removed in order to prevent detector overloading. Only when the concentration of the reagent is kept at a minimum and when the greatest sensitivity is not required, can the reaction mixture after derivatization be injected directly into the GLC–ECD system[23]. Some of the problems encountered in the use of GLC–ECD following alkylation reactions, particularly with the extractive alkylation technique with PFB-Br as the alkyl halide, have been discussed in Section 2.3.

3.2. Sample pre-treatment and alkylation method

When the acidic pharmaceutical is present in a solid matrix such as a powder or a tablet, a very simple clean-up will generally be sufficient. The compound(s) of interest can then often be extracted with an organic solvent, in which the derivatization reaction is carried out either straight away or after evaporation of the solvent and reconstitution of the residue in the reaction medium.

Aqueous biological matrices, such as plasma or serum, urine and saliva, contain a multitude of naturally occurring compounds, often in much higher concentrations than the drug or drug metabolite(s) to be analysed. A more involved clean-up procedure of the sample is then very often required. The presence of proteins in plasma and serum samples is another complicating factor. A conceivable approach for the analysis of acidic drugs in urine and saliva would be to derivatize the drug directly by mixing an aliquot of the sample with a sufficiently large volume of a solution of an alkyl iodide in a water-miscible solvent such as acetone or acetonitrile, with the addition of a basic catalyst. No reports on such an approach with protein-free samples were found.

Chan[23] precipitated the proteins in serum samples containing valproic acid with acetonitrile. After centrifugation the acid in the supernatant was directly alkylated with phenacyl bromide.

The extractive alkylation technique in principle allows the simultaneous extraction and alkylation of acidic compounds when dealing with aqueous biological samples; many examples of this approach have been reported (*e.g.*, refs. 92, 93, 97, 107 and 116–120). Frequently, however, extra clean-up steps will have to be included, in order to prevent the appearance of interfering peaks in the chromatograms. These steps can be the preliminary extraction of the acid from the sample with an organic

solvent (*e.g.*, refs. 88, 109, 112 and 121) or the extraction of the acid from the sample followed by back-extraction into an alkaline aqueous phase (*e.g.*, refs. 95, 104, 121 and 122). In some instances a more extensive clean-up procedure proved to be necessary[123,124].

Extraction of the aqueous samples with an organic solvent is the most frequently applied clean-up step prior to base-catalysed alkylation reactions. Dünges and co-workers[28,40] developed an assay procedure that is generally applicable to the gas chromatographic determination of acidic drugs, such as barbiturates, in whole blood. The samples (20 μl) are extracted by stirring with 50-μl portions of acetone–diethyl ether (1:1). The combined extracts are dried with activated molecular sieve and concentrated under partial reflux. After the addition of alkyl iodide and potassium carbonate the alkylation reaction is performed with a microrefluxer. The need for strict adherence to the experimental conditions was stressed by the author[40,28], otherwise unsatisfactory results are obtained.

Double extraction procedures, preceding base-catalysed alkylation reactions, have also been applied frequently. A double extraction–concentration procedure, which is very suitable for a subsequent TMAH-catalysed alkylation, has been reported[125–128]. The acidic drug is extracted with toluene and the toluene layer is back-extracted with a small volume (20–50 μl) of TMAH in methanol. The TMAH layer is then used for the alkylation reaction, which is effected by the addition of DMA and an alkyl iodide. When the acidic drug is not efficiently extracted with toluene, a more polar solvent such as a chloroform–isopropanol mixture can be used; after evaporation of the resulting extract the residue is taken up in a toluene–TMAH in methanol system, etc. Very clean chromatograms from blank plasma samples are thus obtained and the method is applicable to many acidic pharmaceuticals[128].

Occasionally separation of the acid from the aqueous matrix is achieved by the addition of a solid adsorbent, such as charcoal[53,96]. Sample clean-up procedures with thin-layer chromatographic methods[101,129,130] or by column chromatography have also been reported[72,76,77,123].

4. APPLICATIONS OF THE DERIVATIZATION WITH ALKYL HALIDES IN THE ANALYSIS OF ACIDIC PHARMACEUTICALS

The desirable properties of the derivatization with alkyl halides, as mentioned in Section 1.1, have resulted in the widespread use of (the earlier discussed variants of) this alkylation method in GLC practice. In this section an up-to-date survey, in the form of tables, is presented of the manifold applications of the alkylation with alkyl halides in the gas chromatographic analysis of acidic drugs, especially with respect to the quantitative determination of drugs and/or drug metabolites in biological matrices. Tables 1–7 cover the following groups of drugs: 1, barbiturates; 2, anticonvulsants (with the exception of benzodiazepine and barbiturate anticonvulsants); 3, benzodiazepines; 4, xanthines; 5, sulphonamides; 6, analgesic (narcotic and non-narcotic) and anti-inflammatory drugs; and 7, miscellaneous drugs.

4.1. Key to the tables

Under "Compound(s)" the name of the drug or group of drugs is given, for which the procedure referred to was originally designed. 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, serum, urine, saliva) for which the method is suitable.

Many differences are to be found in the details of the clean-up procedures, which are performed when analysing drugs in biological materials. It is possible, however, to give a classification based on the type and number of clean-up steps in the pre-chromatographic sample treatment. Simple washings of samples or extracts, in which the compound of interest does not move into the other phase, are not considered as clean-up steps here.

The symbol "a" in the third column denotes some form of protein removal from the biological material, which precedes any further sample clean-up. Proteins can be removed by ultrafiltration or by precipitation through the addition of some reagents (*e.g.*, sodium tungstate, zinc sulphate, ammonium acetate and ammonium sulphate). One example has been found in which protein denaturation by the addition of acetonitrile forms the sole sample treatment before derivatization.

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 clean-up step is involved, *e.g.*, when the acidic drug molecule is back-extracted from the initial organic extract into an alkaline phase (*e.g.*, methanolic TMAH). Derivatization sometimes can be performed using this alkaline layer after phase separation. In other instances the drug to be derivatized is re-extracted from the alkaline phase with an organic solvent after phase separation and acidification. The symbols "b" and "c" are used only when some form of base-catalysed alkylation follows.

The letter "d" denotes any form of sample clean-up other than solvent extraction (*e.g.*, by thin-layer or column chromatography), whereas the letter "e" is used when the initial isolation of the drugs to be analysed is effected by adsorption on to a solid adsorbent (in the two instances mentioned, charcoal is used as the adsorbent).

In extractive alkylation procedures the simultaneous extraction and derivatization of the compounds under investigation could be regarded as a clean-up step by itself. When this is the only sample clean-up involved, as is the case in the many instances in which extractive alkylation is performed directly on the biological sample, the symbol "f" is used.

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 preceding the extractive alkylation is included. The convenient procedure involving back-extraction of the drugs to be analysed from the initial organic extract with an aqueous alkaline layer, which, after phase separation, is submitted to the extractive alkylation by the addition of a quaternary ammonium ion and the organic extractant containing the alkyl halide, is thus denoted by "h".

TABLE I

BARBITURATES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Based on carbonate method						
Barbiturates	–	–	Acetone or ethyl acetate, alkyl iodide (methyl, allyl or benzyl)/K_2CO_3	3% OV-225	FID	19
Barbiturates	–	–	Acetone, CH_3I/K_2CO_3	3% OV-225	FID	27
Barbiturates	–	–	Acetone, methanol, CH_3I/K_2CO_3	Capillary, 15-m OV-17	FID	41
Barbiturates	Blood, 20 µl	b	Acetone, CH_3I/K_2CO_3	3% OV-225 3% OV-17	FID NPD	28
Phenobarbital, mephobarbital	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Pentobarbital	Serum, 0.1 ml	a + b	Ethanol, PFB-Br, Na_2CO_3 in H_2O	3% OV-17	ECD	63
Pentobarbital	Serum, 0.1 ml	b	Acetone, CH_3I/Na_2CO_3	2% OV-17	NPD	31
Thiopental	Plasma, 1 ml	c	Acetone, CH_3I/Na_2CO_3	3% OV-17	NPD	30
(b) Based on tetra-alkylammonium hydroxide catalysis						
Barbiturates	–	–	DMA, TMAH or PTMAH, alkyl iodide	2% OV-17 1.5% SP-2250	FID	49

Barbiturates	–	–	DMA, TMAH, alkyl iodide	3.08 % OV-101 1.82 % Dexsil 300 GC 3 % SP-2250 1.57 % OV-7	FID	60
Barbiturates	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3 % OV-17	FID	126
Barbiturates	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3 % OV-1 3 % OV-17 3 % SP-1000	FID	128
Pentobarbital	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3 % OV-17	FID	126
Phenobarbital	Serum, 50 µl	c	DMA, TMAH, CH_3I	3 % OV-1	NPD	125
Phenobarbital, mephobarbital, heptobarbital	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3 % OV-1 3 % OV-17 3 % SP-1000	FID	128
Phenobarbital, mephobarbital	Plasma, 0.1–1 ml	b	DMA, TMAH, C_3H_7I	3 % OV-101	MS (SIM)	131
c) Other base-catalysed methods						
Barbiturates	–	–	Methanol, PFB-Br, triethylamine	3 % OV-1 3 % OV-17 3 % NPGSe	ECD	65
d) Extractive alkylation						
Barbiturates (pento- and phenobarbital)	–	–	Buffer pH 10, THA^+/CS_2, CH_3I	3 % SE-30	FID	89
Barbiturates	Plasma, 0.5 ml	e + f	1 *M* NaOH, TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 43-m SE-30	FID	96
Phenobarbital	Saliva, 100 µl	f	Buffer pH 9, TBA^+/CH_2Cl_2, PFB-Br	3 % OV-17	ECD	107

TABLE 2

ANTICONVULSANTS (WITH THE EXCEPTION OF BENZODIAZEPINE AND BARBITURATE ANTICONVULSANTS)

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Hydantoins						
Phenytoin	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Phenytoin	Serum, 50 µl	c	DMA, TMAH, CH_3I	3% OV-1	NPD	125
Phenytoin, mephenytoin	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Mephenytoin, desmethyl-mephenytoin	Serum, 1 ml	c	DMA, PTMAH in DMA, $C_5H_{11}I$	3% OV-225	FID	53
Phenytoin	Plasma, (serum), saliva, 100 µl–1 ml	b	Acetonitrile, TBAH, C_4H_9I	3% OV-17	NPD	55
Phenytoin	–	–	Methanol, PFB-Br, triethylamine	3% OV-1 3% OV-17 3% NPGSe	ECD	65
Phenytoin	Plasma, 100 µl	c	Acetone, CH_3I, buffer pH 13	3% OV-225	FID	47
Phenytoin, mephenytoin, desmethyl-mephenytoin	–	–	Acetone, C_2H_5I, KOH soln.	3% OV-225 capillary, 37-m SE-30	FID	44
Mephenytoin, desmethyl-mephenytoin	Plasma, 1 ml	c	2-Butanone, C_2H_5I, KOH soln.	2% OV-101	MS (SIM)	45
(b) Ethosuximide						
Ethosuximide	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42

Ethosuximide	Plasma, serum, 0.5 ml	c	DMA, TMAH, C_4H_9I	3% OV-1	FID	132
Ethosuximide	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(c) Primidone						
Primidone	–	–	Acetone or acetonitrile, alkyl iodide/Ag_2O	3.8% SE-30	FID	42
Primidone	Serum, 50 µl	c	DMA, TMAH, CH_3I	3% OV-1	NPD	125
Primidone	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Primidone + metabolites	Serum, urine, saliva, breast milk, tissues, 5–100 µl	a + b	DMA, TMAH, C_2H_5I	3% OV-17	MS (SIM)	133
(d) Valproic acid						
Valproic acid	–	–	Acetone or acetonitrile, alkyl iodide/K_2CO_3	3.8% SE-30	FID	42
Valproic acid	Serum, plasma, 50 µl	c	DMA, TMAH, C_4H_9I	3% OV-17	FID	127
Valproic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Valproic acid	Serum, 100 µl	f	Buffer (pH 8), TBA^+/CH_2Cl_2, CH_3I	2% SP-1000	FID	116
Valproic acid	Plasma, 0.25 ml	b	Pentane, phenacyl bromide, triethylamine	3% OV-17	FID	24
Valproic acid	Serum, 100 µl	a	Acetonitrile, phenacyl bromide, crown ether, sat. $NaHCO_3$ solution	3% PC-3210	ECD	23
(e) Other anticonvulsants						
(Neo)sulpha-lepsine + metabolites	Blood, 0.2 ml; plasma, urine, 1 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-101	ECD	122

TABLE 3

BENZODIAZEPINES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Clonazepam	Serum, CSF, 0.2 ml	b	Acetone, C_2H_5I/K_2CO_3	4% OV-101	ECD	48
Bromazepam	Plasma, 0.5–1 ml	b	0.1 *M*. NaOH, TBA^+/diethyl ether, CH_3I*	0.5% OV-17	ECD	134
Desmethyl-diazepam	Plasma, 0.5 ml	b	DMA, TBAH, C_4H_9I	3% OV-17	ECD	54
Desmethyl-diazepam	Blood, 2 ml (bile, tissue)	c	DMA, 0.025 *M* TBAHS in 0.2 *M* PTMAH, C_3H_7I	3% OV-1	NPD	51
Nitrazepam	Plasma, 0.5 ml	g	0.4 *M*. NaOH, TBA^+/benzene, CH_3I	5% OV-17	ECD	88
Clonazepam, nitrazepam	Serum, blood, plasma, 0.1–1 ml	a + g	0.1 *M*. NaOH, TBA^+/benzene-CH_2Cl_2 (9:1), CH_3I	3% OV-17	ECD	121
Demoxepam	Serum, blood, plasma, 0.1–1 ml	h	0.1 *M* NaOH, TBA^+/benzene-CH_2Cl_2 (9:1), CH_3I	3% OV-17	ECD	121
Oxazepam	Serum, 2 ml	g	pH 13, THA^+/CH_2Cl_2, CH_3I	3% OV-225	ECD	109, 135

* The reaction is not an extractive alkylation procedure, because the undissociated bromazepam molecules partition into the other phase; the reaction is probably catalysed by OH^- ions present in the ether layer.

TABLE 4

XANTHINES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Acephylline	Urine, 1 ml	b	Acetonitrile, C_2H_5I/K_2CO_3	3% OV-17	FID	43
Theophylline	Serum, 0.1 ml	b	Ethanol, PFB-Br, Na_2CO_3 in H_2O	5% OV-225	ECD	62
Theophylline	Serum, saliva, 1 ml	c	DMA, TMAH, C_4H_9I	3% SP-2250	FID	136
Theophylline	Serum, 1 ml; saliva, 1 ml	c b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	FID	137
Theophylline	Plasma, serum, saliva, 20 µl	b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	NPD	138
Theophylline	Blood, 0.2–2 ml	a + b	DMA, TMAH, C_4H_9I	3% OV-17	MS	139
Theophylline	Serum, 50 µl	b	DMA, TMAH, $C_5H_{11}I$	3% OV-17	NPD	140
Theophylline	Plasma, 0.05–1 ml	b	DMA, TMAH, $C_5H_{11}I$	Capillary, 70-m SE-30	NPD	141
Theophylline	Serum, 1 ml	c	DMA, TMAH, C_4H_9I	3% SP-2250 DB	FID	142
Xanthines (+ metabolites)	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Theophylline	Serum, 100 µl	b	Methanol, TBAH, C_4H_9I ("on-column")*	3% OV-17	NPD	70
Xanthine, hypoxanthine	Serum, 0.5 ml; urine (10 × diluted)	a + b	DMA, TBAH, C_4H_9I	3% OV-17	MS	143
Theophylline	Plasma, 100 µl; rat brain	h	1 *M* NaOH, TPA^+/CH_2Cl_2, PFB-Br	3% XE-60	ECD	104
Theophylline	Serum, saliva, 25 µl	f	0.1 *M* NaOH, THA^+/CH_2Cl_2, $C_5H_{11}I$	3% OV-17	NPD	97
Theophylline	Plasma, 50 µl	f	Buffer (pH 10), TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 25-m OV-225	MS (MIM)	117

* See Section 2.2.3.

TABLE 5

SULPHONAMIDES

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Sulphonamide diuretics						
Dichlorphenamide	Serum, 0.5 ml; eye liquor	c	DMA, TMAH, CH_3I	3% OV-17	ECD	144
Acetazolamide	Serum, 0.1 ml	f	0.5 *M* NaOH, TPA^+/CH_2Cl_2, CH_3I	1% SE-30	ECD	93
Bendrofluazide	Blood, 1 ml	h	1 *M* NaOH, TBA^+/benzene, CH_3I	1% OV-1	ECD	145
Chlorthalidone	Plasma, serum, urine, 2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	95
Chlorthalidone	Blood, plasma, urine, 1 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-101	ECD	122
Chlorthalidone	Plasma, urine, 1 ml; erythrocytes	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	NPD	146
Furosemide	Plasma, 1 ml	g	0.2 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	115
Hydrochlorothiazide	Plasma, urine 1 ml; blood cells	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% SE-30	ECD	147
Hydrochlorothiazide	Plasma, 2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% OV-225	ECD	98

Mefruside	Blood, urine, plasma, erythrocytes, 1–2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	NPD	148
Mefruside metabolites	Blood, urine, plasma, 1–2 ml	h	0.1 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% SE-30	ECD	149
Sulphonamide-diuretics (as a group)	–	–	0.2 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	1% SE-30 (3% OV-17, 3% QF-1)	ECD	93
(b) Antibacterial sulphonamides						
Sulphonamides (model compounds)	–	–	0.2 *M* NaOH, TBA^+/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD	150
Sulphonamides	–	–	pH 10, TBA^+/different organic solvents and alkylating agents	5% OV-17	ECD	87
Sulphapyridine, N-acetylsulphapyridine	Serum, 0.1 ml	f	Buffer (pH 10), TBA^+/CH_2Cl_2, CH_3I	5% OV-17	ECD	118
(c) Other sulphonamides						
Sulphonylureas	–	–	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
Glipizide	Plasma, 0.5 ml	f	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
Tolbutamide	Plasma, 0.1 ml	f	Buffer (pH 6.9), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	119
(Neo)sulphalepsine			See Table 2, anticonvulsants			122
Saccharin			See Table 7, miscellaneous drugs			151

TABLE 6

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

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
(a) Acetanilide derivatives						
Acetaminophen	Serum, saliva, 1 ml	c	DMA, TMAH, $C_7H_{15}I$ (+ flash methylation on nitrogen)	3% OV-17	FID	152
Acetaminophen	Plasma, urine 0.5 ml	a + b	Acetone, PFB-Br/K_2CO_3	3% SP-2100	ECD	153
Acetanilide derivatives	Liver tissue homogenate	b	Acetone, TMAH, alkyl iodide	3% XE-60	MS (SIM)	57
(b) Anthranilic acid derivatives						
Mefenamic acid	Serum, 2 ml	b	DMA, TMAH, C_4H_9I	3% SP-2250 DA	FID	154
Flufenamic acid, mefenamic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(c) Indomethacin						
Indomethacin	Plasma, urine, 1 ml	b	Acetone, PFB-Br/K_2CO_3	2% Dexsil 300	ECD	155
Indomethacin	Plasma, 0.5–1 ml	g	TBAHS, CH_2Cl_2, C_3H_7I/$NaHCO_3$ (SLPT catalysis)	3% OV-1 3% XE-60	ECD	112
Indomethacin	Plasma, 0.5–1 ml	g	pH 7, TPA^+/CH_2Cl_2, C_3H_7I	3% OV-1 3% XE-60	ECD	112
Indomethacin	Serum, 0.5 ml	f	THAHS/CH_2Cl_2, C_2H_5I	3% E-350 (SE-52)	ECD	120
(d) Narcotic analgesics and related drugs						
Morphine, nalorphine	Plasma, 1 ml	f	0.2 *M* NaOH, TBA^+/ethyl acetate, PFB-Br	2% OV-17	MS	156
Naloxone	Blood, plasma, 1 ml	h	NaOH soln., TBA^+/CH_2Cl_2, PFB-Br	3% OV-17	ECD	157
Pentazocine	Plasma, 0.5 ml	h	NaOH soln., TBA^+/CH_2Cl_2, PFB-Br	5% OV-17	ECD	103
Pentazocine	Blood, plasma, urine, 0.2–1 ml	h	NaOH soln., TBA^+/$C_2H_4Cl_2$, PFB-Br	3% Dexsil 300	ECD	158

Pethidinic acid, norpethidinic acid	Urine, 1 ml	i	pH 9.5, TPA$^+$/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD MS	125
(e) Phenylacetic and phenylpropionic acid derivatives						
Diclofenac metabolites	urine, 1 ml	g	2.5 *M* NaOH, TBA$^+$/CH_2Cl_2, CH_3I	3% OV-17	ECD	159
Flurbiprofen	Plasma, 1 ml	b + d	Acetone, PFB-Br/K_2CO_3	3% OV-17	ECD	101
Ibuprofen	Serum, 0.1 ml	b + d	Acetone, PFB-Br/K_2CO_3	10% 3-cyano-propylsilicone	ECD	130
Ketoprofen	Plasma, 400 µl	f	Buffer (pH 7.3), TBA$^+$/CH_2Cl_2, CH_3I	3% OV-17	ECD	160
Phenoprofen	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(f) Pyrazolinone derivatives						
Phenylbutazone, oxyphenbutazone	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Sulphinpyrazone + metabolites	Plasma, serum, urine, 1 ml	c	DMA, TMAH, CH_3I	2% OV-17 3% OV-225	NPD ECD MS	59
(g) Salicylic acid derivatives						
Salicylic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Acetylsalicylic acid	–	–	TBAHS, CH_2Cl_2, alkyl iodide or PFB-Br/$NaHCO_3$ (SLPT catalysis)	3% OV-17	FID ECD	113
Acetylsalicylic acid	Plasma, 100 µl	f	pH 6.5., TPA$^+$/CH_2Cl_2, benzyl bromide	1.5% OV-17	MS	92
Methylsalicylate	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
(h) Miscellaneous						
Niflumic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Tolmetin	Plasma, 25–100 µl	c	Ethyl acetate, PFB-Br/1 *M* K_2CO_3	3% XE-60	ECD	37

TABLE 7

MISCELLANEOUS DRUGS

Compound(s)	*Sample*	*Clean-up*	*Derivatization*	*Stationary phase*	*Detection*	*Ref.*
Acenocoumarin	Plasma, 2 ml	a + b	Acetone, PFB-Br/Na_2CO_3	3% OV-17	ECD	29
Benzoic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Benzoic acid	Plasma, urine, 1 ml	b	Acetone, PFB-Br/K_2CO_3	3% OV-17	ECD	161
Benzoic acid	–	–	TBAHS, CH_2Cl_2, alkyl iodide or PFB-Br/$NaHCO_3$ (SLPT catalysis)	3% OV-17	FID ECD	113 114
Benzoylecgonine	Urine, 5 ml	b	DMA, TMAH + PTMAH, C_3H_7I	3% SP-2250 DA	FID	52
Benzoylecgonine	Plasma, 0.5 ml; urine, 1 ml	b	CH_2Cl_2, PFB-Br, pyridine in benzene	3% OV-225	ECD	66
Benzoylecgonine	Urine, 2 ml	f	NaOH, pH 12, THA^+/CH_2Cl_2, C_2H_5I	3% OV-17	MS	99
p-Chlorophenoxy-isobutyric acid (metabolite of clofibrate)	Serum, 25 µl; saliva, 500 µl	b	DMA, TMAH, C_4H_9I	3% OV-17	FID	162
Chloroquinaldol	Plasma, urine, 0.1–0.5 ml	f	Buffer pH 11, TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	94
Chloroquinaldol	Plasma, 0.1–0.5 ml	f	NaOH soln., THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	122
Clioquinol	Plasma 0.1–0.5 ml	f	NaOH soln., THA^+/CH_2Cl_2, CH_3I	3% JXR	ECD	163
Clioquinol	Plasma, urine 0.1–0.5 ml	f	Buffer pH 11, TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	94
Clonidine	Plasma, 5 ml	c	Acetone, PFB-Br/K_2CO_3	Capillary, *e.g.*, 25-m SE-30, OV-17, SP-1000	ECD	164
Floxuridine	Plasma, 1 ml	d	DMSO, CH_3I, potassium *tert.*-butoxide	3% OV-17	NPD	77
5-Fluorouracil	–	–	(1) pH 10, TPA^+ or THA^+/CH_2Cl_2, CH_3I (2) DMSO, CH_3I, methylsulphinylcarbanion (3) DMA, TMAH, C_4H_9I	2% and 3% SP-2250, 5% XE-60 5% OV-1	FID	75

5-Fluorouridine	Urine, 1 ml	a + d	DMSO, CH_3I, potassium *tert.*-butoxide	Capillary 15-m FFAP	NPD	76
Glutethimide	Plasma, 0.5 ml	e + f	1 *M* NaOH, TBA^+/CH_2Cl_2, C_2H_5I	Capillary, 43-m SE-30	FID	96
Mandelic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
6-Mercaptopurine	Plasma, 1 ml	a + f	0.5 *M* NaOH, THA^+/CH_2Cl_2, CH_3I	3% OV-225	MS (DID)	165
Methimazole	Plasma, 1 ml	f	Buffer (pH 10), TBA^+/CH_2Cl_2, PFB-Br or benzyl chloride	Capillary, 20-m UCON HB 5100	MS (MIM)	166
Nalidixic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	10% OV-17	FID	167
Nalidixic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128
Niclosamide	Urine, 25 ml	h	NaOH soln., TBA^+/CH_2Cl_2, CH_3I	4% and 5% OV-101	ECD	168
Nicotinic acid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Nucleotides (6-mercaptopurine)		a + i	(1) DMSO, $CH_3I/NaOCH_3$ (2) DMSO, CH_3I, methylsulphinylcarbanion	3% OV-17 3% OV-101	MS	72
Oxyphenonium	Plasma, 1 ml	i	pH 7, TPA^+/CH_2Cl_2/PFB-Br	3% OV-17	ECD	124, 169, 170
Palmitic acid (fatty acids)	Serum, 10–50 μl	h	0.2 *M* NaOH, TBA^+/CH_2Cl_2, PFB-Br	3% and 5% OV-17	ECD	106
Pemoline	Serum, urine, 1 ml	f	0.1 *M* NaOH, TPA^+/CH_2Cl_2, CH_3I	5% FFAP	NPD	171
Phenprocoumon	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128
Probenecid	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17 3% SP-1000	FID	128
Pseudo-ephedrine	Serum, 1 ml	c	Ethanol, PFB-Br, K_2HPO_4 soln.	5% OV-225	ECD	64
Saccharin	Urine, 4 ml	f	Buffer (pH 7.4), TBA^+/CH_2Cl_2, CH_3I	3% OV-17	ECD	151
Tranexamic acid	Plasma, 200 μl urine	f	pH 9.5, TBA^+/CH_2Cl_2, C_2H_5I (+ derivatization of amino group)	1% OV-225	ECD	172
Warfarin	Plasma, 1 ml	b + d	Acetone, PFB-Br/K_2CO_3	1% OV-17	ECD	129
Warfarin	Plasma, 1 ml	c	DMA, TMAH, C_4H_9I	3% OV-1 3% OV-17	FID	128

Clean-up procedures that do not fit very well in one of the aforementioned categories are denoted by the letter "i".

Under the heading "Derivatization" the major conditions of the alkylation procedure are given.

The next two columns mention the stationary phases on which the derivatives can be chromatographed and the type(s) of detection used in the analysis, respectively.

The last column refers to the original publication in which the method was presented and where more detailed information concerning the entire analytical procedure can be found.

5. SUMMARY

The various types of alkylation reactions with alkyl halides and their application in the gas chromatographic analysis of acidic compounds of pharmaceutical interest are reviewed. An extensive survey of the use of these methods for the analysis of various (classes of) compounds is given, with special reference to their determination in biological matrices.

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