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Review

Recent advances in non-silylation derivatization techniques for gas chromatography

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Abstract

Analyte derivatization is employed in many analytical methods that employ gas chromatography as the determinative step. Derivatization increases analyte volatility, improves chromatographic characteristics of an analyte by decreasing its polarity and/or increases the detector sensitivity of the target analyte(s). Without the availability of an arsenal of appropriate derivatization reagents, the applicability of gas chromatography in analytical chemistry would be severely curtailed. This review covers recent advances in derivatization techniques. Silylation is not addressed in this review, which covers the use of alkylation, esterification, acylation and condensation reactions which produce volatile and stable GC compatible derivatives. In the selection of examples from a plethora of recent work, emphasis has been placed on recent derivatization techniques that abbreviate sample preparation time or are readily adapted to automated equipment.

Keywords: Reviews; Derivatization, GC; Enantiomer separation; Sulfadimidine; Oxibendazole; Amphetamines

Contents

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1. Introduction

The predominant reasons for derivatization in gas chromatography (GC) are either to increase analyte volatility, to improve chromatographic characteristics of an analyte by decreasing its polarity and/or to increase the detector sensitivity of the target analyte(s). Trimethylsilylation is by far the major derivatization method for increasing the volatility and GC characteristics of a substance. It also has the added advantage in that trimethylsilyl groups increase the total ion current and, therefore, the sensitivity using positive ion MS. It is usually convenient to set aside a specific GC instrument to perform analyses involving trimethylsilylation because build-up of derivatization reagents throughout the chromatographic system can lead to time-dependent in-situ trimethylsilylation of susceptible groups in chromatographic separations where derivatization is not required. The use of trimethylsilyl derivatives in GC has been recently reviewed [1–4] and will not be covered in this paper.

This review focuses on the other derivatization strategies that are employed to improve the detectability and/or ease of quantitation in GC analysis. The publication by Knapp [2], although now 20 years old, provides an excellent overall coverage of derivatization in analytical chemistry and is still available. A recent review focused on derivatization procedures for gas chromatographic–mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents [3]. A Handbook of Derivatives for Chromatography has also appeared [4] and the coupling of derivatization reactions with supercritical fluid extraction has also been recently reviewed [5]. There have been few fundamental additions to the suite of generally useful derivatization reagents since Knapp's 1979 publication, with the exception of the increasingly wide use of chloroformates to effect a variety of derivatization reactions. However, there have been a number of specialized new reagents introduced, such as new methods for chiral differentiation and quantitation, as well as a few significant improvements in various derivatization procedures. Although there have been considerable recent improvements in and abbreviation of analyte isolation, the decreases in overall preparation times for many new methods in which a derivatization step is involved, often hinge on improvements in analyte isolation rather than to advances in derivative preparation procedures.

It would be impossible to provide a comprehensive discussion of all derivatization methods or an exhaustive review of all reagents in the space available. This paper therefore provides only selected recent examples of derivatization reagents discussed in previous reviews [2–4]. Where, in the view of the author, no significant new work has appeared recently, older work is not regurgitated; the reader is directed back to Refs. [2–4]. This review concentrates, in more detail, on examples of newer methods of derivatization, which, in the view of the author, offer advantages or advances in sample preparation time or detection sensitivity. Thus, methods where simultaneous derivatization and extraction is accomplished or derivatization is coupled with GC sample introduction receive prominence.

2. Types of derivatives useful in GC

Most derivatization reagents are employed to replace a labile hydrogen atom, attached to a heteroatom, with a less polar, non-labile group. This results in the elimination of hydrogen bonding and results in a derivative of the original analyte which is both more volatile and produces more symmetrical chromatographic peaks. A number of reagents are also used to convert enolizable groups, such as ketones, into derivatives in which enol formation is avoided. The various commonly used reagent types discussed in this review are listed in Table 1, together with

Table 1
Types of derivatization covered in this review

Type of Reaction	Type of reagent	Typical reaction conditions	Groups derivatized
Alkylation/ esterification	Diazoalkane	Non-polar solvent	Acidic OH and NH
	Alkyl halide–strong base	$CH_3SOCH_2^{(-)}$ in DMSO	Alcohols, amides
	Alkyl halide–weak base	K_2CO_3–acetone	Thiols, phenols, acids, amines, acidic NH
	Alkyl halide–weak base–ionophore	K_2CO_3–18-crown-6-non-protic solvent	Thiols, phenols, acids, amines, acidic NH
	Alkyl chloroformates	Reaction in pyridine with or without second reactant	Phenols, acids
	Alkyl halide–quaternary ammonium salt	Phase transfer	Thiols, phenols, acids, amines, acidic NH
	Quaternary ammonium or sulfonium salt	On-column derivatization	Thiols, phenols, acids, amines, acidic NH, amines
	Amide dialkyl acetals	Dimethylformamide	Acidic OH
	Carbodiimides	Alcohol and carbodiimide in aprotic solvent	Acid
	Alkyl metal halides (Grignard reagents)	Direct reaction, in diethyl ether, with matrix material or with non-protic organic extract	Organometallics
	Alcohol/BX_3, HCl or H_2SO_4	Reaction at room temp.	Carboxylic acids
	Transesterification	Reaction with alcohol/HCl or alcohol/NaOR or Me_3S^+ OH	Esters, glycerides
	Tetraalkyl borate salts	Direct reaction with matrix material or with aqueous organic extracts	Organometallics
Acylation	Acyl anhydride, perfluoroacyl anhydride	Anhydride alone or with organic base; e.g. pyridine	Alcohols, phenols amines, thiols,
	Acyl chloride	Reaction in pyridine or a similar organic base	Alcohols, phenols amines, thiols,
	Perfluoroacylimidazole	Use alone, no acidic by-product	Alcohols, phenols amines, thiols,
	N-methyltrifluoromethyl-trifuoracetamide	Use alone, no acidic by-product	Alcohols, phenols amines, thiols,
	Perfluoroacyl anhydride–alcohol	Combined acylation–esterification	Amino-acids
	Alkyl chloroformate	Chloroformate–pyridine	Amines, alcohols, phenols
Condensation	*O*-alkyloxime	Reaction in alcohol/HCl	Ketones
	Alkylboronic acids	Mix analyte with the boronic acid	Diols, amino-alcohols, hydroxyacids
	Aromatic aldehyde	Heat neat reactants	Amines
	Formaldehyde	Paraldehyde/acid	Diols, amino-alcohols (plus perfluoroacyl derivative)

common functional groups that can be derivatized with a particular reagent.

In general, trimethylsilyl and *tert*.-butyldimethylsilyl derivatives are preferred for GC–MS with positive ion electron impact (EI) detection, because of ease of formation and the observation that such derivatives often enhance detectability. For negative ion chemical ionization mass spectrometry (NICI-MS) and for GC with electron-capture detection (GC–ECD), fluorinated derivatives are preferred.

Normal alkyl and acyl derivatives serve for both GC–MS and GC–flame ionization detection (FID) detection where ultimate sensitivity is not a requirement. Some specific derivatives have been developed for special purposes such as Mosher's acid for chiral separations or esters of ferrocenylcarboxylic acid for use in conjunction with an atomic emission detector.

3. General overview of derivatization reagents

Non-silylated derivatization reagents have been divided into three broad categories for convenience. Alkylation and esterification have been combined in that the net result of either process is the replacement of an acidic proton by an alkyl group. Acylation involves the replacement of a non-acidic proton by an acyl group while ''condensation'' comprises the formal replacement of two protons, either in the target analyte or in the derivatization reagent, with the formation of a double bond or cyclic derivative. Table 1 summarizes the main reagent types and the functional groups which these reagents have been used to protect.

4. Alkylation

4.1. Methylation using diazomethane, trimethylsilyldiazomethane and pentafluorophenyldiazomethane

The use of diazomethane for the methylation of a wide variety of acidic substances is rapid, experimentally easy and produces minimal by-products [2]. The major disadvantages to the use of this reagent for sporadic use are its toxicity and the potential dangers in its preparation coupled with a limited reagent storage time. Nevertheless, diazomethane remains the reagent of choice for the preparation of methyl esters when methylation is a very frequently used procedure. The use of deuterodiazomethane to derivatize mixed halophenols and anisoles in cardboard packaging has been described. Detection by GC–MS allows the estimation of a haloanisole and its parent halophenol simultaneously [6]. Sulfonylureas were converted to their dimethyl-derivatives with diazomethane after extraction from water and soil extracts after concentration with C_{18} SPE discs and determination by GC with nitrogen–phosphorus detection (NPD), GC–ECD and GC–MS. It was found that for complete conversion of the sulfonylureas to their dimethyl derivatives it was necessary to use ethyl acetate as solvent and allow 30 min for complete reaction [7,8].

The derivatization of acidic herbicides by a combined solid-phase microextraction (SPME)–gas phase derivatization has recently been published [9]. The herbicides were extracted from aqueous solution by SPME and derivatized directly on the SPME fibre with gaseous diazomethane.

Trimethylsilyldiazomethane is a commercially available safe substitute for diazomethane, available as a 2 *M* solution in hexane. This reagent has been used as a rapid and efficient method for the derivatization of phenoxyacetic acids in a multi-residue method, which used GC–MS determination [10,11]. Trimethylsilyldiazomethane has also been used for the methylation of the carboxyl group of amino acids after prior protection of the amino group with pivaloyl chloride [12]. The use of other diazoalkanes has been sporadic, offering little advantage over diazomethane. An exception is the synthesis and use of two pentafluorophenyldiazomethanes which give derivatives with carboxylic acids that are highly sensitive to ECD and GC–NICI-MS detection and were designed for the sensitive detection of prostanoid derivatives [13].

4.2. Direct alkylation of organic analytes

The use of methyl iodide–potassium carbonate in acetone for *N*- and *O*-methylations has now been largely replaced by phase transfer methods. The determination has been reported of phenoxyherbicides, pentachlorophenol (PCP) and 31 other herbicides, as methyl derivatives, in fatty and non-fatty food. Work-up consisted of a gel permeation chromatographic (GPC) clean-up, methylation with methyl iodide–tetrabutylammonium hydroxide in acetone followed by Florisil chromatography and GC analysis [14]. The speed of extraction, reproducibility and accuracy for the determination of diuretics in human urine by three different methods has been compared [15]. The authors preferred methylation by

methyl iodide and potassium carbonate in acetone over phase transfer methylation and on-column methylation, respectively, but performance characteristics of the three methods are not very different except for the loss of some analytes by the on-column method.

Recent examples of direct formation of pentafluorobenzyl derivatives include the simultaneous determination of chlorophenols, chlorobenzenes and chlorobenzoates in microbial solutions. This was accomplished by extraction of analytes into dichloromethane followed by derivatization with pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile. When necessary, Florisil clean-up preceded GC–ECD determination [16]. The analysis of hydroxy polycyclic aromatic hydrocarbons was performed by derivatization with pentafluorobenzyl bromide in acetone–potassium carbonate–18-crown-6 followed by silica clean-up and determination using GC–ECD or GC–NICI-MS [17]. Phenols have been determined by employing similar derivatization reagents and conditions [18]. Thirty phenoxycarboxylic acids were detected at the ppt level by concentration on a C_{18} solid-phase extraction cartridge followed by derivatization with pentafluorobenzyl bromide followed by GC–MS [19]. The sensitive determination of nitrite and nitrate (after reduction to nitrite with Cd–acetic acid) in human urine and plasma as pentafluorobenzyl derivatives has been described [20]. Derivatives were prepared by diluting urine with four parts acetone and reaction with pentafluorobenzyl bromide, using [^{15}N] nitrite as internal standard and NICI-MS detection.

Supercritical fluid extraction (SFE) has also been used in conjunction with simultaneous derivatization. Phenoxyacetic acids absorbed onto a C_{18} reversed-phase separation cartridge were derivatized in high yield by addition of pentafluorobenzyl bromide and potassium carbonate to the top of the cartridge followed by extraction with supercritical CO_2 (10 min static, 15 min dynamic) [21]. Also, a single step in situ derivatization–extraction of resin and fatty acids from sediments with pentafluorobenzyl bromide and triethylamine has been carried out by SFE [22].

The formation of by-products during direct alkylation with pentafluorobenzyl bromide has been reported by a number of authors, using either potassium carbonate or diisopropylethylamine as basic catalysts [23–25]. Thus, in contrast to the satisfactory derivatization of simple short chain fatty acids with pentafluorobenzyl bromide and acetone–potassium carbonate [26], halogenated acetic and propionic acids afforded a wide variety of products when direct derivatization was attempted under a number of reaction conditions [27]. Indeed, although pentafluorobenzyl derivatives are extremely useful for trace analysis in many applications, a constant requirement during direct derivatization is the frequent need for significant clean-up before chromatography. It is therefore surprising that 3,5-bis(trifluoromethyl)benzyl bromide, which is readily available and appears far less susceptible to side reactions, has received so little attention.

A recent report detailed the use of bromoacetonitrile and iodoacetonitrile as derivatization reagents for acids, phenols and compounds containing acidic N–H groups. All of these compounds form derivatives which are sensitive to GC–NPD [28].

4.3. Direct alkylation of organometallics

For GC analysis, it is necessary to convert the non-volatile ionic organometallic species into volatile derivatives. Two methods which convert ionic species to completely alkylated covalent derivatives suitable for GC analysis are treatment with a Grignard reagent under anhydrous conditions or with sodium alkylborate in aqueous solution.

Determination of organotin species has been performed by alkylation using Grignard reagents, after preliminary isolation of the organotins from matrix material [29–33]. A simpler alternative has recently been reported in which organotin species were derivatized with pentylmagnesium bromide directly in the soils and sediment in which they occur, followed by extraction of the fully derivatized materials from the sediments. By use of this direct derivatization method, the recovery of monobutyl tin was dramatically improved [34]. Preparation of alkyl derivatives using Grignard reagents is a complex procedure restricted to alkylation in an anhydrous organic phase, that may produce interfering compounds.

Alkylation with tetraalkylborates in aqueous solution is the currently preferred derivatization pro-

cedure. Derivatization of organotin with sodium tetraethylborate (STEB) is simple and easy to perform as long as pH, reagent concentration and reaction time are carefully controlled [35,36]. However, determination of organotin species by simple and rapid aqueous-phase ethylation with STEB using GC–flame photometric detection (FPD) [37,38] and GC–MS [39] quantitation has been reported. Detection of ethylated organotins by GC–atomic emission detection (AED) has been utilized by a number of laboratories [40–42] using propyltin and butylltin compounds as surrogates and internal standards.

Tetrabutylammonium tetraborate has been used to derivatize samples in situ, for the determination of lead in water by atomic absorption spectrometry (AAS) after extraction with pentane–hexane [43]. Methylmercury has been analyzed in water after derivatization with STEB and GC–AAS [44], in fish and river water by GC–MS [45] and in fish by GC–AED [46].

4.4. Indirect alkylation via chloroformates

Chloroformates react with acids to give ester derivatives via decarboxylation but act as acylation reagents with alcohols and amines to yield carbonates and carbamates, respectively. For convenience, all derivatizations by chloroformates are considered together in a single section.

Treatment of acids with an alkyl chloroformate in pyridine yields a mixed anhydride which rapidly decarboxylates to yield an ester. When the reaction is conducted in a mixture of pyridine and an alcohol, two products are formed. The major product arises from the reaction of the alcohol solvent with the mixed anhydride intermediate, while the minor product results from decarboxylation of the mixed anhydride. The ratio of major product to the minor product is dependent on the reactivity of the alcohol. These two competing processes are illustrated in Fig. 1. The reaction of acids, hydroxy acids and amino acids with chloroformates has proved to be a simple, rapid and high yielding method for the preparation of derivatives suited to GC analysis. A judicious choice of the alkyl group of the chloroformate and the alcohol solvent allows the formation of a single derivative in high yield.

A rapid method for the derivatization of fatty acids was reported by Husek et al. in 1990 [47] and was elaborated further to give a method for the derivatization of lipid acids in serum with ethyl choroformate–pyridine in aqueous medium, following deproteination with acetonitrile–ethanol [48]. This work was subsequently expanded to the derivatization of hydroxy acids [49] and amino acids [50,51]. The derivatization of a wide range of chlorophenoxyacetic acid and other acid herbicides has been reported using different alkyl chloroformates in pyridine [52]. Recoveries of 51–98% were reported for 22 acid herbicides as their ester derivatives, with most recoveries being >80%.

Further extension of the use of chloroformates for the derivatization of amino acids in aqueous solutions has been reported by Wang et al. [53]. Addition of alcohols to the aqueous solution of amino acids resulted in the predominant formation of esters containing the alkyl group of the alcohol rather than that of the chloroformate. This led to the postulated mechanism shown in Fig. 1 for chloroformate esterification. However, this mechanism does not explain several other derivatization processes. This is pointed out during detailed discussions of chloroformate

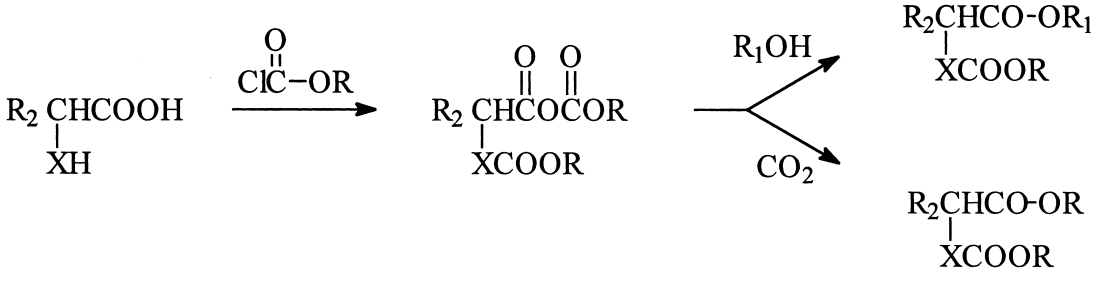

Fig. 1. Alternative possible reaction pathways during alkylation of acids with chloroformates.

reaction mechanisms that are included as part of a comprehensive review of derivatization via chloroformates which has recently appeared [157]. Derivatization of amino, hydroxy and thiol groups proceeds concurrently to produce mixed carbamates, carbonates and thiocarbonates, respectively. Protein [54] and non-protein [55] derived amino acids are conveniently derivatized with ethyl chloroformate, trifluoroethanol and pyridine to yield trifluoro esters of the amino acid *N*-ethylcarbamates. In subsequent work, amino acids and dipeptides were derivatized in aqueous solution using the same reagents, with detection by both positive and negative ion GC–MS. The combined derivatization and analysis time for dipeptides is about 10 min [56]. A number of polar, water soluble substances have been successfully derivatized in aqueous solution with 1-hexyl chloroformate, with catalysis by pyridine or 4-dimethylaminopyridine [57]. An example of the ease of this procedure is the conversion of tartaric acid to a dihexyl carbonate–dihexyl ester derivative. Hydroxycarbamates have also been analyzed by GC–CI-MS after in situ derivatization with *n*-hexyl chloroformate with a limit of detection of 10 ppb [58]. A recent report details an improved procedure for analyte derivatization using derivatization with 1-hexyl chloroformate by sonication followed by addition of dicyclohexycarbodiimide and pyridine [59].

Chloroformates are also useful derivatizing agents for amines, the resulting carbamates possessing superior GC properties to the parent amines. Recent examples are the determination of primary and secondary low-molecular-mass amines from human urine [60], the determination of aliphatic and alicyclic amines in water [61] and the analysis of hexamethylene isocyanate by hydrolysis to the diamine followed by derivatization with trifluoroethyl chloroformate [62]. Trifluoroethyl chloroformate has also been employed as a rapid derivatization reagent for amino acids prior to enantiomeric separation by GC [63]. A rapid method for the GC–MS confirmation of urinary amphetamine and methamphetamine by derivatization with propyl chloroformate has been published [64]. Glyphosate and glufusinate have been analyzed in water by in situ derivatization of the amino function with isopropyl chloroformate followed by extraction of the acidified water and final derivatization of acid functions with diazomethane [65].

4.5. Reductive alkylation

The conversion of secondary amine to tertiary amines with better chromatographic characteristics has been reported [66]. The method, applied to the determination of anabasine and anatabine in the urine of smokers, employed reductive alkylation with sodium borohydride in the presence of propionaldehyde or phenylacetaldehyde. This work was subsequently extended to the analysis of amphetamines, the *N*-propyl derivatives of which had excellent chromatographic properties [67].

4.6. Phase transfer alkylation

There is a large body of literature on phase transfer reactions in which an acidic substance dissolved in an aqueous phase can be reacted with a water-insoluble reagent dissolved in an organic phase (typically dichloromethane or toluene) by adding a tetraalkylammonium salt to the two phase system [2]. The acidic substance forms an ion-pair with the tetraalkylammonium which is soluble in the organic solvent and can therefore now react with the organic soluble reagent to form a product soluble in the organic solvent. Analytical applications of phase transfer catalysis have recently been reviewed [68].

Many recent examples of phase transfer derivatization have been reported and only selected examples are included here. The determination of a large number of the diuretics that are banned in sport was carried out by phase transfer methylation. Methyl iodide–tetrahexylammonium hydrogen sulfate and toluene were utilized, with a clean-up of the methylated derivatives on SM-7 resin to remove small quantities of residual phase transfer reagent before quantitation by GC–EI-MS [69]. The same authors also report the determination of urinary 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid as its dimethyl-derivative [70] and the GC–MS quantitation of urinary buprenorphine and norbuprenorphine by the same process of direct extractive alkylation with methyl iodide [71]. Combined methylation and extraction of phenoxyacetic acids has been accomplished with methyl iodide–tetrabutylammonium hy-

droxide and supercritical CO_2 [72]. Also a liquid-supercritical fluid phase transfer mechanism was established for the methylation and extraction of 2,4-D and 2,4,5-T from aqueous media with methyl iodide–tetrahexyl-ammonium hydrogen sulfate and supercritical CO_2 [73]. In this work it was found that addition of a polar modifier, such as methanol, to the supercritical CO_2 decreased the yield of methylated derivatives and that almost quantitative yields resulted from the use of CO_2 alone.

Ethylene thiourea has been determined down to 50 ppt in water by GC–NICI-MS following a single step derivatization using 3,5-bis(trifluoromethyl)benzyl bromide or iodide and tetrahexylammonium hydrogen sulfate with dichloromethane under phase transfer conditions [74]. A single step extraction and derivatization procedure for chlorophenoxy- and other acidic herbicides has been reported using tetrahexylammoniun hydrogen sulfate, pentafluorobenzyl bromide and dichloromethane followed by GC–ECD or GC–NICI-MS determination [75]. Pentafluorobenzyl bromide has also been used for the phase transfer derivatization of cyanide, iodide, nitrite, sulfide and thiocyanate and determination by GC–MS [76].

Five tri-phase phase transfer catalysts were investigated during the development of a method for the monitoring of dialkyl phosphates by derivatization with pentafluorobenzyl bromide followed by determination using GC–MS [77]. The use of polymer-bound phase transfer catalysts leads to much cleaner derivatization, avoidance of emulsion formation and simple experimental methodology which may well be more widely used for future phase transfer methods. This work has been successfully extended to the derivatization of a series of representative carboxylic acids and phenols. The method could be applied to acetate, benzoate, 2,4,6-trimethylbenzoate, succinate, hippuric acid, L-tryptophan, phenol, 4-nitrophenol, 2,4-dinitrophenol but not to picric acid [78].

4.7. Derivatization of analytes absorbed onto ion-exchange resins

Ion-exchange resins as heterogeneous esterification mediators have been used in synthetic chemistry for many years [79]. In these reactions, the acid was absorbed onto the resin as its anion and displaced as the ester by treatment with an organic halide. Yields were excellent with primary halides. However, applications of this technique in analytical chemistry were delayed by 20 years, although they have received significant recent attention, using both non-aqueous solvents or supercritical fluids as reaction media.

It was reported by Chatfield et al. [80] that macroporous quaternary ammonium anion-exchange resins were a very effective support matrix for the methylation of strongly acidic organic analytes with methyl iodide in either supercritical carbon dioxide or acetonitrile. The chlorophenoxyacetic acid herbicides 2,4-D and 2,4,5-T were readily concentrated from a large volume of basic aqueous solution using AG MP-1 resin in the fluoride form and simply released as the methyl ester after reaction with methyl iodide in supercritical CO_2. This method was found to be applicable to other strong monobasic acids such as pentachlorophenol and quinoxaline-2-carboxylic acid. The advantage of the process was the ability to significantly pre-concentrate ionic analytes from aqueous solutions onto a small quantity of ion-exchange resin and to eliminate non-ionic impurities during the operation. Although methylation was rapid in supercritical CO_2 at 80°C, high yields of methylated products could also be achieved in one hour using acetonitrile at the same temperature.

This concept has been extended by Field and Monohan [81] who have introduced an ‘‘in vial’’ technique using Empore ion-exchange discs in an experimentally elegant method for concentrating the herbicide tetrachloroterephthalic acid from water. The herbicide is first concentrated by filtration through the disc, which is flexible and mechanically stable. After drying, the disc is introduced into a GC auto-injection vial containing acetonitrile and methyl iodide, capped and heated to 80°C for 1 h. The vial is then uncapped and the solvent evaporated and replaced by toluene prior to GC analysis.

Resin-mediated methylation of polyfunctional acids found in fruit juices has also proved successful. Fumaric, succinic, malic, tartaric, isocitric and citric acids, isolated from fruit juices by trapping onto anionic ion-exchange resins, were efficiently converted to methyl esters by reaction with methyl iodide in both supercritical carbon dioxide and

acetonitrile. These esters were readily separated and quantitated by GC and, in contrast to HPLC methods, there were no matrix interferences apparent and determination of acids at concentrations of 10 mg/l was readily achieved by either GC–FID or GC–AED [82]. This technique allowed the detection and quantitation of tartaric acid at levels of 10 ppm in orange juice. This work also provided a method for the determination of isocitric acid in the presence of large amounts of citric acid by a single GC procedure. Comparison of the use of SFE with the use of acetonitrile by ''in vial'' technique of Field and Monohan [81] demonstrated that either method gave comparable recoveries but that SFE provided cleaner extracts.

The isolation and determination of simple volatile aliphatic acids from urine by trapping on ion-exchange resin followed by simultaneous derivatization with pentafluorobenzyl bromide and extraction with supercritical carbon dioxide has also been reported [83]. Recoveries of propanoic acid and higher homologues from urine were more than 50%, with RSDs of less than 8%, at the 20 mg/L level using the simple procedure. A new method for the quantitative determination of the thiouracil thyreostatic agents in bovine muscle was achieved by trapping onto macroporous ion-exchange resin followed by methylation with methyl iodide in either supercritical carbon dioxide or acetonitrile to give dimethyl derivatives which were quantitated by GC–MS [84].

4.8. On-column alkylation reagents

On-column methylation, using either tetraalkyl- or arylalkylammonium hydroxides [85,86] or trialkylsulfonium hydroxides [87] as an alkylation reagent, is a commonly used method for the derivatization of acidic substances for gas chromatographic analysis. The normal procedure is the direct injection of a mixture of analyte and the on-column derivatization reagent in methanol into the hot injection port of the gas chromatograph. This results in the decomposition of the alkylation reagent into an amine or sulfide accompanied by the concomitant alkylation of the analyte in high yield. Phenyltrimethylammonium hydroxide (1), 3-trifluoromethylphenyltrimethylammonium hydroxide (2) and trimethylsulfonium hydroxide are commercially available.[1] General structures of reagents are shown in Fig. 2.

Typical examples of the use of on-column methylation are the methylation of phenoxyacetic acids [88] and free fatty acids from cultured bacteria [89] with (1) and the combined *N*-methylation of and transesterification of carbetamide with (1) [90]. Determination of gibberelins as their permethyl derivatives using (1) as on-column derivatization reagent has been reported [91], as has determination of acidic pesticides in water after methylation with trimethylsulfonium hydroxide [92]. The preparation of methyl esters from fatty acids or via the transesterification of triglycerides has been affected with trimethylsulfonium hydroxide [93] or with (2) [94] and the simultaneous determination of lauric acid and ethyl laurate in the palm *S. serrulata* has recently been described [95].

Although these and other previously reported on-column alkylation reagents are effective and efficient, they are all highly caustic and therefore likely to cause rapid column deterioration. During an extensive study of direct on-column methylation, it was reported that tetraalkylammonium fluorides were non-caustic reagents which performed equally to the corresponding hydroxides. Furthermore, the use of tetraalkylammonium cyanides or acetates frequently offered considerable advantages in terms of derivatization selectivity without compromising derivatization efficiency [96]. The utility of selective on-column derivatization reagents has been demonstrated by the selective methylation of a number of veterinary drugs including sulfonamides and benzimidazoles. The results of using a selective reagent are shown in Fig. 3.

On-column methylation of carboxylic acids can be conducted with equal efficiency by reagents (1)–(4) in Fig. 2 and trimethylsulfonium hydroxide. However, there are distinct differences of reactivity between (1)–(4) and trimethylsulfonium salts in the methylation of phenols. Methylation of less acidic phenols (phenol and cresols) is not complete, even at

[1] (1) is available from the Pierce Chemical Co., Rockford, IL, USA as MethElute, TTMA–OH), (2) can be purchased from Alltech, Deerfield IL, USA as MethPrep, while trimethylsulfonium hydroxide is sold by Machery-Nagel.

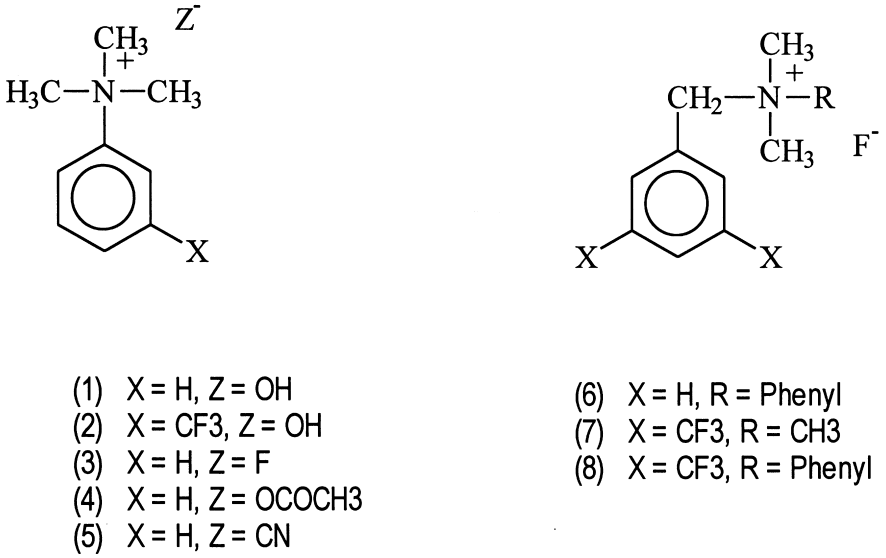

Fig. 2. Structures of various quaternary ammonium on-column derivatization reagents.

high concentrations of trimethylsulfonium salts, whereas only a moderate molar excess of phenyltrimethylammonium salts are required. The methylation of three different concentrations of a standard phenol mixture with the same amount of (4) showed that the more acidic phenols such as pentachlorophenol and 4-nitrophenol are more rapidly and efficiently methylated than less acidic phenols [97].

Higher alkyl derivatives have also been successfully prepared by on-column derivatization. An elegant example is the determination of thiocyanate in plasma [98] using tributylsulfonium perchlorate as a combined ion-pairing reagent for the extraction of thiocyanate and on-column derivatizing agent for the conversion of tributylsulfonium thiocyanate to volatile butyl thiocyanate, which was quantitated using GC–NPD. Theophylline has been determined as its ethyl derivative [99] by on-column derivatization with triethylsulfonium hydroxide.

The success of combined supercritical fluid extraction: on-column butylation of aromatic sulfonic acids by tetrabutylammonium salts [100] was predicated on the legitimate hypothesis that the ion pair between the sulfonic acid and the tetrabutylammonium ion would be sufficiently non-polar to be extracted into supercritical carbon dioxide. In an alternative approach [101], Krueger and Field trapped alkylsulfonic acids on an SAX ion-exchange Empore disc which was then transferred to a GC vial containing $5 \cdot 10^{-3} M$ tetrabutylammonium hydrogen sulfate in chloroform. After an equilibration period of 40 min, the chloroform solution was injected directly into the GC system to give on-column conversion to butyl derivatives. This is another example of the ''in vial'' methylation method discussed earlier [81].

4.9. On-column benzylation reagents

4.9.1. Development of appropriate on-column benzylation reagents

The postulate that the preferential transfer of a benzyl group rather than a methyl or phenyl group during the thermal decomposition of a quaternary ammonium salt in which these groups were present, is due to the greater stability of a benzyl carbonium ion, has been shown to be correct [102]. Indeed, it was found that suitable benzylalkylammonium and benzylalklyphenylammonium salts yielded benzyl esters and ethers exclusively when employed as direct on-column derivatization reagents for acids and phenols. From a large number of potential reagents investigated, benzyldimethylphenylammonium fluoride (6), 3,5-bis(trifluoromethyl)benzyltrimethylammonium fluoride (7) and 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium fluoride

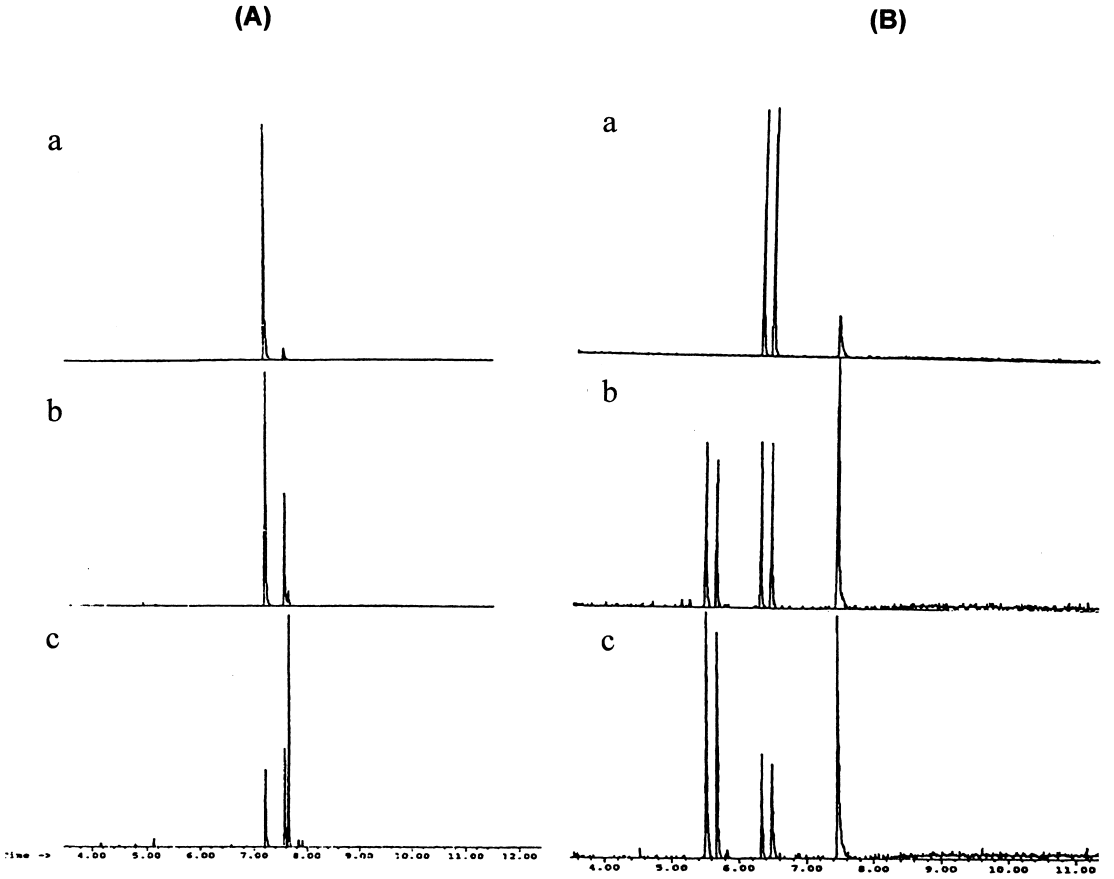

Fig. 3. Variation in product patterns in the methylation of sulfadimidine (A) and with oxibendazole (B) with different reagents (a)=[5], (b)=[4] and (c)=[1].

(8) {see Fig. 2} have been shown to be very useful new derivatization reagents with a variety of uses [102].

In general, moderately acidic substances such as acids and chlorophenols were readily benzylated by all three reagents. However, for less acidic substances such as phenol or the cresols, 3,5-bis (trifluoromethyl)benzyldimethylphenylammonium fluoride (6) proved to be the most efficient reagent for the derivatization and only a modest theoretical excess of reagent was required for complete derivatization.

4.9.2. Practical applications of on-column benzylation of phenols

The benzyl derivatives of phenols, which were produced in high yield, were particularly attractive in that they each produced a significantly higher total ion current in the GC–MS than the corresponding methyl derivatives. A further advantage was that, like acetates, the benzyl derivatives of 3- and 4-cresol were completely separated on a non-polar capillary column that was unable to separate either the parent 3- and 4-cresols or their methyl ethers. Benzylated derivatives of pentachlorophenol could be readily detected at 5 μg/l with NICI, 60 and 20 times lower than that achievable with HPLC–UV and EI, respectively. This sensitivity can be attributed to the ease of fragmentation of benzyl phenol ethers to form a stabilized positive benzyl carbonium ion with the concomitant high yield formation of negative phenylate ions.

There was a considerable benefit of adding the on-column derivatization reagent during sample con-

centration of the eluate from a solid-phase extraction cartridge. It was found that, during evaporation of methanol during the concentration step, there was also some loss of the more volatile phenols and cresols. These were prevented when the on-column benzylation reagent (8), which forms ion-pair complexes with the phenols, was added prior to evaporation.

The methyl esters of lower organic acids are normally unsuitable for routine GC determination because of their volatility. However, 3,5-bis(trifluoromethyl)benzylation has proved useful for on-column derivatization of these acids [103]. The derivatization of a selection of higher-molecular-mass organic acids with (7) yielded 3,5-bis(trifluoromethyl)benzyl esters with high efficiency and with good chromatographic behaviour characteristics in GC and GC–MS [103].

5. Esterification and transesterification

This section will cover the use of alcohols, acetals and orthoesters, with various catalysts, as esterification or transesterification agents. There is an unavoidable overlap with some other sections.

An automated system for the preconcentration and determination of free fatty acids in dairy products has been described [104]. Pre-concentration of the acids was carried out on Amberlite IR-400 resin followed by elution and derivatization with acetyl chloride in methanol. The methyl esters were continuously injected into a GC–FID to prevent losses of the more volatile esters.

Transesterification can be achieved by both basic and acid catalysis. The effect of the derivatization procedure employed (NaOMe, HCl and BF_3 in methanol) in the analysis of human milk and infant formula has been studied. It was found that HCl in methanol gave incomplete transesterification of human milk and infant formula, containing egg lipids and fish oils, and resulted in the wrong absolute values of fatty acids. Therefore, procedures based on the use of NaOMe in methanol, which gave over 90% transesterification, are preferred. For the analysis of free fatty acids in milk, methanol–HCl esterification is the method of choice. [105]. It has also been shown that the use of boron trifluoride–methanol or KOH–methanol for esterification or transesterification gave dimethylacetal derivatives in addition to the required fatty acid esters. Transesterification with trimethylsulfonium hydroxide did not yield these by-products [87].

The GC–ECD determination of permethrin in cattle plasma with a detection limit of 5 ppb has been carried out by cyanide ion transesterification with ethanol using 3-phenoxybenzyl 2-chlorobenzoate as surrogate. [106]. Analysis of carboxylic acids in petroleum by esterification with fluoroalcohols [107] has been reported and the analysis of total free and glucose conjugated pyrethroid acid metabolites present in tea infusions has been published [108]. In this case, derivatization was performed by reaction of pyrethroid isolates with hexafluoroisopropanol and isopropylcarbodiimide in hexane.

The esterification of aminophosphonic acids to give trialkylphosphonate derivatives has been reported. A mixture of a trialkyl orthoformate and trifluoroacetic acid gives two derivatives [109] whereas treatment of the aminophosphonic acid with trifluoracetic anhydride followed by addition of an alkyl orthoformate leads to a single trifluoroacetyl trialkylphosphonate derivative [110] and is to be preferred.

6. Acylation

6.1. O-Acylation

Recent methods for acylation have concentrated on direct acylation from buffered aqueous media followed by solvent, supercritical fluid or solid-phase extraction of the derivatives. Acylation has also been the preferred procedure for derivatization to achieve some chiral separations by GC. The use of fluorinated acyl derivatives for sensitive detection by either ECD or NICI-MS determinations has also proved popular. The examples of acylation that are shown below focus on newer techniques of derivatization or new reagents.

Excess of a derivatizing agent can cause problems with the chromatography of the derivatized analytes. During a study of suitability of various derivatization reagents for the GC separation of the trichothecene mycotoxins, it was found that derivatives decom-

posed in the presence of excess derivatization reagent, even when the so-called ''neutral reagents'' such as trifluoroacetylimidazole and trifluoromethyl-*N*-methyltrifluoroacetamide were employed. Use of trifluoroacetic anhydride in the presence of sodium bicarbonate gave trifluoroacetyl derivatives, which exhibited excellent chromatographic behaviour with no detectable decomposition [111].

US-Environmental Protection Agency (EPA) method 1653 utilizes in situ derivatization of chlorophenolics with acetic anhydride in water buffered to pH 9–11.5, with acetic anhydride. This has been adapted by Trout [112] as a micro extraction method in which a 1-l sample of water is first acetylated and then extracted in a 1-l volumetric flask with 1 ml of toluene. The toluene layer is allowed to separate and an aliquot is transferred by pipette into a GC vial and measured directly by GC–MS. 4-Fluorophenol served as a surrogate added to the water and d_{10}-anthracene was utilized as an internal standard added to the toluene. The identification and quantitation of mono-, di- and trihydroxybenzenes at trace concentrations in sea water, by direct acetylation of up to 18 l of water and solid-phase extraction [113], employed direct derivatization of the sodium hydrogencarbonate–buffered water samples with acetic anhydride. 2,7-Dihydroxynaphthalene was used as an internal standard. A fully automated method has been reported for the derivatization of phenols and chlorophenols in water with acetic anhydride followed by enrichment and isolation by SPE, elution with an alkyl acetate and determination by GC–FID. The most critical factor in the procedure was the careful buffering of the aqueous samples prior to the addition of acetic anhydride. [114]. 4-Nonylphenol has also been isolated from both effluent and sludge from sewage treatment plants by derivatization with acetic acid and extraction of the acetates [115]. In the case of effluent, an in situ acetylation was performed in much the same manner as in [113] and [114] above but using hexane rather than solid-phase extraction. In the case of sludge, the phenol was derivatized during a supercritical fluid extraction experiment by addition of triethylamine and acetic anhydride before extraction, first static and then dynamic, with supercritical CO_2. The latter technique had been previously reported by the same authors for the isolation and analysis of phenolics as acetates [116]. Pentachlorphenol has also been simultaneously acetylated and extracted from leather with supercritical CO_2 [117].

The effect of microwave and ultrasound accelerated derivatization–extraction is receiving increasing attention. Dasgupta and Banerjee reported the microwave enhanced rapid preparation of acetates and trifluoroacetates of fatty alcohols [118]. Methods to increase acetylation yields of phenol and methyl phenols in soils with a mixture of hexane, acetic anhydride and pyridine have been studied. It was found that ultrasound-assisted acetylation-extraction was 25% lower than microwave assisted recoveries for all analytes [119]. The use of microwave irradiation to shorten derivatization preparation times has also been reported for the preparation of perfluorooctanoyl derivatives of fatty alcohols, which required 30 min at 60°C but which could be accomplished in 1 min with microwave assistance [120]. The same laboratory has extended this work on microwave-induced rapid synthesis to the use of 4-carbethoxyhexafluorobutyryl chloride for the rapid derivatization of fatty alcohols [121].

4-Carbethoxyhexafluorobutyryl chloride has also been employed to derivatize benzyl alcohol in serum and post-mortem blood. The ester derivative is detected by specific ions observed in GC–MS and is very well separated, as this high-molecular-mass derivative, from other interfering substances in serum and blood [122]. The same reagent was also used for the determination of urinary phenols [123]. Similarly, perflurooctanoyl chloride has been used for the derivatization of phenol [124].

Trifluoroacetylation of monoterpene glycosides has been used for GC–NICI-MS determination [125] and twelve anabolic agents were determined in calf urine as their heptafluorobutyroyl derivatives by GC–MS after isolation using octadecylsilyl solid-phase extraction cartridges followed by a two-step clean-up on silica and alumina SPE cartridges [126].

R(−)-2,2,2-Trifluoro-1-(9-anthryl)ethanol has been used as a derivatizing agent for ibuprofen prior to enantiomer separation by GC [127].

6.2. N-Acylation

GC–MS analysis of the pentafluoropropionyl derivative of morphine, using nalorphine as internal

standard, has been used as a highly sensitive method for the quantitation of morphine in serum samples and forms the basis of a method suitable for pharmacokinetic studies [128]. A further example is the analysis of naltrexone and naltrexol as di- and tripentafluoropropionate derivatives using GC–NICI-MS [129]. The determination of polycyclic aromatic nitro-compounds in airborne particulate matter has been carried out by reduction of the nitroaromatics to amines with sodium sulfide followed by reaction with heptafluorobutyric anhydride [130]. Determination of trace amounts of aromatic amines in water after derivatization by the same reagents was also recently described [131]. The simultaneous GC–NPD determination of amphetamine, methamphetamine and their *p*-hydroxylated metabolites in plasma and urine, at the 1–20 ppb level has been carried out using both trifluoroacetyl and heptaflurobutyryl derivatives following extraction with ethyl acetate [132]. Analysis of the amino acid, furosine, using heptafluoroisobutyl derivative has been studied and decomposition of the derivative was found to occur during derivative preparation, even when the most stringent safeguards are observed [133].

A resin-bound pentafluorobenzoylating agent, useful for the derivatization of aliphatic amines, has been developed [134]. The polymeric reagent was prepared by reaction of a polystyrene resin containing a chloroformate function with pentafluorobenzoic acid and triethylamine in tetrahydrofuran. Derivatization reactions were carried out in toluene.

Triacylglycerols have been converted to the pyrrolidide derivatives of the constituent fatty acids directly, by overnight reaction with a 1:1 mixture of pyrrolidine and imidazole at 60°C. The derivatives were analyzed by GC–FID [135].

Lower halogenated aliphatic acids in environmental water samples have been directly derivatized to form haloanilides by a simple procedure in which 2,4-difluoroaniline, dicyclohexyl carbodiimide and a small amount of ethyl acetate were shaken with the acidified water sample [136,137]. Octadeuteronaphthalene was utilized as an internal standard with GC–MS determination. The chiral carboxylic acids, from which the insecticide permethrin, is prepared, have been derivatized with a variety of chiral amines and the resulting diastereoisomeric products separated by capillary GC. Derivatization was effected by stirring a mixture of the requisite acid and carbonyldiimidazole together for a few minutes in dichloromethane and then adding the chiral amine in dichloromethane, with a further 1-h reaction time [138].

The optical isomers of a number of amphetamines have been derivatized with *R*(+)-Mosher's acid chloride (2-methoxy-2-phenyl-3,3,3-trifluoropropionyl chloride). The stereospecific derivatization of amphetamines, phenol alkylamines and phenol hydroxyalkylamines and quantitation of the enantiomers by GC–MS has been reported [139]. Alcohol functions were first silylated using a silyl acetamide and the product reacted with Mosher acid chloride to give mixed silyl-amide derivatives which were well separated into enantiomeric pairs by GC. In a subsequent study, amphetamines were derivatized and isolated from urine in a single step. One ml of urine was basified to pH 14 with 6 *M* NaOH, 5 ml of hexane was added followed by 20 μl of a 2% hexane solution of Mosher's acid chloride and shaking for 15 min. Evaporation of the hexane layer was all the work-up required before analysis of GC–MS. Fig. 4 shows the separation of the enantiomeric amides of four racemic amphetamine derivatized by this method [140].

(*S*)-(−)-*N*-(Trifluoroactyl)prolyl chloride has proved to be a useful reagent for the separation of enantiomers of amines. A simple method for the determination of amphetamine optical isomer ratios in urine has been reported. Extraction from basic solution was followed by on-column derivatization with (*S*)-(−)-*N*-(trifluoroactyl)prolyl chloride and GC–MS detection [141]. Two further recent examples are the separation of the enantiomers of α-phenylethylamines [142] and 2-alkylamines [143].

7. Condensation reactions

Derivatization by condensation reactions is often less general in applicability than alkylation or acylation. Many derivatives have applicability to a limited number of functional groups. Only a few of the more recent examples are presented here.

Volatile aldehydes have been converted to their hydrazone derivatives with 2-hydrazino-benzthiazole

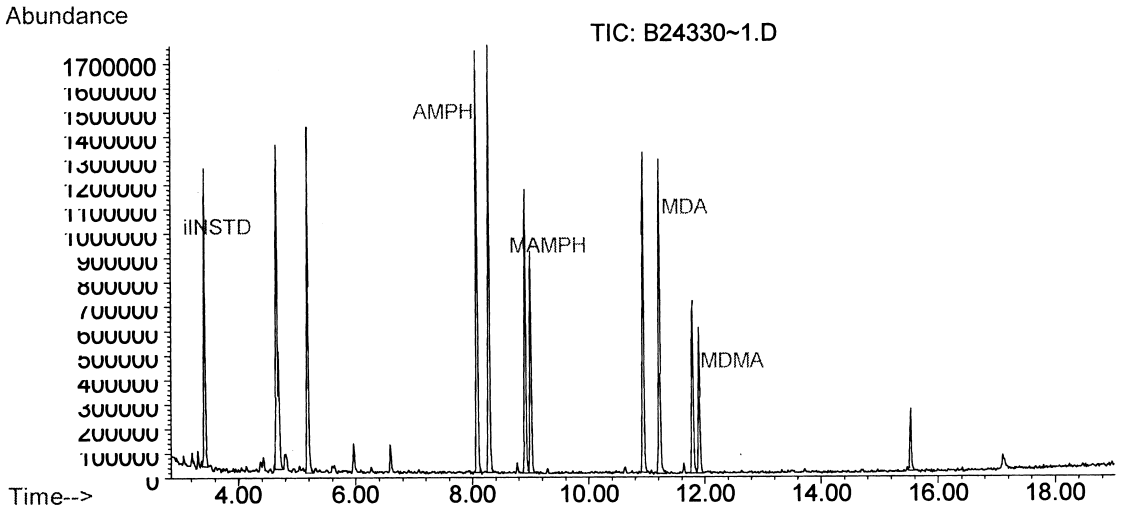

Fig. 4. Separation of enantiomers of amphetamine, methamphetamine, methylenedioxyamphetamine and methylenedioxymethamphetamine from urine fortified at 0.5 μg/l of the racemic drugs after derivatization with Mosher's acid chloride [140].

and analyzed by GC–NPD [144]. Atmospheric aldehydes and ketones were determined by cartridge sampling followed by derivatization with 2,4,6-trichlorophenylhydrazine and GC–ECD [145]. Eighteen aldehydes formed during peroxidation of lipids were converted to their pentafluorobenzylhydrazones and separated by GC. Electron-capture detection gave detection limits down to 10^{-14} mole and MS with selective ion monitoring allowed detection to 10^{-12} moles but was more specific [146]. Aldehydes in beer have been analyzed by GC after derivatization with *O*-pentafluorobenzyloxylamine [147]. Also, a method has been developed for the determination of 4-hydroxy-2-nonenal, a highly reactive product of free radical-stimulated lipid peroxidation, in clinical samples. Derivatization with *O*-pentafluorobenzylhydroxylamine is followed by solid-phase extraction of the derivative and analyzed by GC–NICI-MS. [148]. 3,5-Bis(trifluoromethyl)benzyloxylamine has been synthesized and used for the detection of low levels of steroids such as testosterone by GC–NICI-MS [149].

A three-step derivatization procedure for the GC–MS quantification of thromboxanes, prostacyclin metabolites, prostaglandins and isoprostanes in urine has been developed [150]. It was necessary to protect ketone, alcohol and acidic functions. This was successfully accomplished by firstly protecting ketones as the *O*-methyloxime derivatives with methoxyamine in dimethylformamide followed by alkylation of the carboxylic acids with pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile with a final derivatization of alcohols with bis(trimethylsilyl)trifluoroacetamide. Quantification was carried out using GC–NICI-MS. A further example of ketone protection was the derivatization of the ketone groups in corticosteroids as *O*-methyloxime derivatives prior to trimethylsilylation [151].

Aldehydes in cigarette smoke have been determined by conversion to thiazolidine derivatives with cysteamine at room temperature and neutral pH and analyzed with GC–FPD [152]. In a two-step derivatization procedure, 2,3-butanediol was first oxidized to diacetyl by reaction with aqueous potassium permanganate for 30 min, excess permanganate was destroyed with oxalic acid and the diacetyl reacted with 4,5-dichoro-1,2-diaminobenzene to give a quinoxaline derivative which was determined by GC–ECD [153].

4,4-Dimethyloxazoline derivatives of fatty acids have shown several advantages over other fragment-orienting derivatives for structural studies of mixtures by GC–MS. Free or esterified fatty acids may

be converted into their 4,4-dimethyloxazoline derivatives by heating with 2-amino-2-methylpropanol at 180°C for 18 h [154].

A GC method for the determination of selenium in bovine liver used a digestion procedure to produce Se^{VI}. This is then reduced to Se^{IV} before derivatization with 4-nitro-1,2-diaminobenzene. The selenium heterocycle thus formed may be detected by GC–MS, GC–ECD or GC–NPD with the lowest limit of detection of 25 pg by GC–ECD [155]. In an alternative derivatization approach to the same problem, Se^{IV} was quantitatively derivatized with 4,5-dichloro-1,2-diaminobenzene prior to GC analysis using flame photometric detection [156].

8. Conclusion

Derivatization processes will continue to play a pivotal role in GC analysis. It is probable that future advances in derivatization will focus on abbreviation of sample preparation methods. The present emphasis on methods which automate derivatization or combine it with other work-up procedures will continue to receive attention. GC–MS will become even more predominant as the GC detection method of choice and newer derivatization reagents will be required to accommodate GC–MS detection at low levels.

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