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Review

Derivatization procedures for gas chromatographic–mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents

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Abstract

The development of low cost MS detectors in recent years has promoted an important increase in the applicability of GC–MS systems to analyze for the presence of foreign substances in the human body. Drugs and toxic agents are in vivo metabolized in such a way that more polar compounds are usually formed. Derivatization of these metabolites is often an unavoidable requirement for gas chromatographic analysis. Application of derivatization methods in recent years has been relevant, especially for silylation, acylation, alkylation and the formation of cyclic or diastereomeric derivatives. Given the relevance of drug of abuse testing in modern toxicology, main derivatization procedures for opiates, cocaine, cannabis, amphetamines, benzodiazepines and LSD have been reviewed. Papers describing the analyses of drugs of abuse in matrixes other than blood, such as hair or sweat, have received especial attention. Advances in derivatization for sports drug testing have been particularly relevant for anabolic steroids, diuretics and corticosteroids. Among the several methodologies applied, the formation of trimethylsilyl, perfluoroacyl or methylated derivatives have probed to be both versatile and extensively used. Further advances in derivatization for GC–MS applications in clinical and forensic toxicology will depend on the one hand on the degree of further use of GC–MS for routine applications and, on the other hand, on the alternative progress made for developments in LC–MS or CE–MS. Last but not least, the appearance of comprehensive libraries in which reference spectra for different derivatives of many drugs and their metabolites are collected will have an important impact on the expansion of derivatization in GC–MS for toxicological applications.

Keywords: Reviews; Xenobiotics; Drugs of abuse; Doping agents

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1. Introduction

The analysis of xenobiotics in biological fluids is of paramount importance in clinical and forensic toxicology. The introduction of gas chromatographic methods in the early sixties allowed a qualitative advance in the potentiality for detection of either the drugs or their marker metabolites. The identifications, which were initially carried out only on the basis of retention times and the use of non-selective detection (such as flame ionization detection), evolved with the introduction of more selective detection methods such as nitrogen–phosphorous or electron-capture detection. Nevertheless, full possibilities for undisputed identification were only achieved with coupling to mass spectrometry (MS). Initial problems for coupling packed columns to the mass spectrometer led to the need for special interfacing devices, which, in turn, prevented the rapid growth of applications of the technique. Nevertheless, the possibility of direct connection of fused-silica capillary columns to the ion source and the possibility of using non-magnetic analyzers in low-priced instruments have made the use of GC–MS a technique of choice in present clinical and forensic toxicology [1–4]. Reference mass spectrometric data easily available either in printed or computer form have given additional power to the approach [5–9], with some data collections being focused on drugs and toxic agents [10–14]. The use of isotopically labeled internal standards [15] has added reliability to quantification by GC–MS. Nevertheless, in spite of GC–MS being so selective, the appearance of interferences must not be neglected [16].

Unfortunately, many drugs or poisons are molecules with polar functional groups. Metabolism in the body (especially in the liver but also in other target organs such as the lung, the gut wall or the kidney, to name a few) is an additional step in the introduction of polar functional groups and the conversion of molecules in less lipophylic compounds. Before being excreted into the urine, the metabolites usually undergo an additional enzymatic process, forming glucuronides, sulfates and other conjugates with extremely high polar and hydrophylic character. Conventional GC is obviously not aimed at the study of polar and hydrophylic compounds, which means that conversion of the target analytes into compounds suitable for analysis by GC is a prerequisite for progress in this field. Luckily, many reagents are useful for ''derivatizing'' polar functional groups and in making the molecule appropriate for GC–MS analysis. Typically, hydroxyl, ketones, carboxylic acids and amines are the functional groups to be derivatized in many drugs of toxicological interest as well as in their metabolites. Specific description of advances in derivatization reagents presented according to the main chemical functional groups targeted is not exactly the focus of this review and can be found elsewhere [17–19].

In addition to the decrease of the polarity and the increase in the volatility of the analytes, some derivatization reagents for GC allow the obtention of very characteristic mass spectra which can be relevant for identification purposes. Usually, the shift of the main fragment ions to high mass ranges (with lower biological background) and the formation of characteristics fragments for a whole family of drugs

are some of the advantages of derivatization of drugs and metabolites in toxicology. In other situations, the identification of the drug or a metabolite is not a problem, but the accurate quantification may be a matter of concern, especially for legal purposes. In such cases, robust derivatization processes, leading to a single derivative without other side reactions, are of preferred use. In all cases, the choice of derivatives to be used in GC–MS must take into account several mass spectrometric aspects such as (a) the ionization mode [20–22], usually electron impact (EI) or chemical ionization (CI), (b) the resolution of the mass spectrometer [23], especially when trying to separate the analyte from various background biological interferences and (c) the possibility of increasing spectrometric selectivity by coupling the first MS detection to a second MS step (tandem MS or MS–MS) [24–26].

The purpose of the present review is to update the developments made in recent years for the derivatization of drugs of forensic interest, taking into account the advantages of some of the aspects indicated above. A general part dealing with the basis of derivatization and updating the main derivatization methods (silylation, acylation, alkylation, formation of cyclic derivatives and chiral derivatizations) is first presented, which is of general applicability to clinical and forensic toxicology. Subsequently, important areas of present toxicological development in derivatization methods are presented, mainly focused on the analysis of drugs of abuse, especially in new biological matrices such as hair, sweat, saliva or meconium, and the control of the misuse of drugs in sport. Groups of drugs of abuse specifically covered are opiates, cocaine, cannabinoids, amphetamines, benzodiazepines and LSD. In regard to drugs in sport, anabolic steroids, diuretics and corticosteroids are specifically addressed. Derivatization for other important groups of drugs misused in sports such as stimulants, narcotics, adrenergic drugs and their metabolites can be found elsewhere [27]. Finally, a brief outline of future needs and perspectives is discussed.

2. Derivatization in GC–MS

Volatility and thermal stability of the compounds is required in GC and GC–MS analysis. Derivatization is mandatory for polar and thermolabile compounds to make them amenable to chromatographic analysis. The reduction in polarity can also improve the gas chromatographic properties of the compounds by minimizing the undesirable and non-specific column adsorption and by, therefore, allowing the obtention of better peak shapes and a reduction in the appearance of ghost peaks. The resolution of closely related compounds not separated in the underivatized form can also be increased by using the appropriate derivative.

The preparation of a derivative may also be performed when the mass spectrum of the underivatized molecule shows poor diagnostic ions. The chemical structure of the substance is changed after derivatization and, in consequence, the fragmentation pattern can be radically altered. Mass spectra with ions of higher m/z ratios and higher abundance can be obtained (see Fig. 1). High-mass-ions have greater diagnostic value, since they are more specific than low-mass-ions, which can be easily affected by interference from the fragment ions of contaminants such as those due to column bleeding. For identification purposes, the monitoring of at least three ions and their abundance ratios is usually required. In quantitative analysis, the monitoring of high abundance high mass ions, less subjected to background interference, is also preferred. An increase in the abundance of the molecular ion or a related ion can also be used for determination of the molecular mass. The preparation of more than one derivative can give helpful additional information to determine the molecular mass.

In GC–MS, derivatization can also be used to enhance the detectability of a compound by introducing groups with high electron affinity, such as halogen atoms, that can produce an increase in the ionization efficiency under negative chemical ionization (NCI) and make possible highly sensitive analyses. Isotopically labeled derivatization reagents can be employed to study the fragmentation pattern of the derivative and, also, to help in structural elucidation [28]. GC–MS can be used for screening analyses of a structurally related group of compounds by monitoring a common and characteristic fragment ion. Derivatization can be used to favour the formation of high stability fragments that can be used for this purpose [27,29].

Side effects can occur during the derivatization

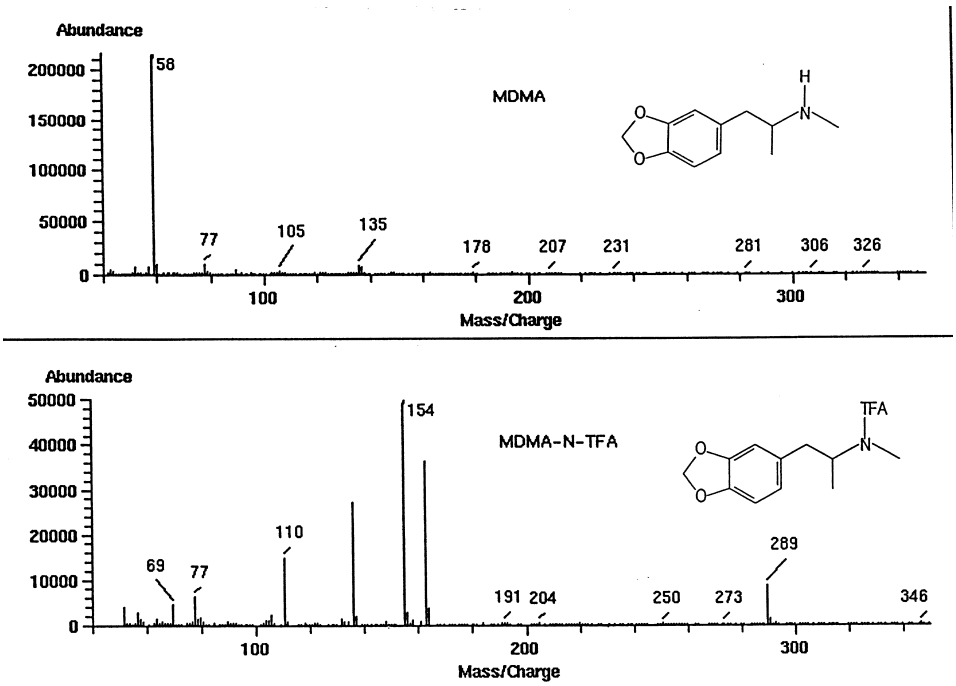

Fig. 1. Changes in mass spectral patterns by means of derivatization: top, mass spectrum of underivatized MDMA (M^+, m/z 193, not observed); bottom, mass spectrum of MDMA-N-TFA (M^+, m/z 289).

reactions. Multiple derivatives can be formed with polyfunctional compounds as a consequence of incomplete derivatization reactions. Uncontrolled formation of unexpected minor derivatives can be produced if the reaction conditions are not well established [30]. Side products of the derivatization reaction can affect the stability of the derivatives formed; i.e., the halogen acids produced during acylation with acyl halides and anhydrides can produce side reactions, such as dehydration or enolization, and neutralization is, subsequently, required. Other side products can affect GC–MS analysis by column contamination, wide solvent fronts, or interference with the detectors. Consequently, elimination of these side products is necessary before GC injection [31,32]. The removal of some derivatization reagents is also often required to avoid secondary derivatization in the injector. In other cases, not removing the excess of reactants prior to GC–MS analysis may be an advantage in terms of time consumption. Also, the usually high temperatures of the injection port may favour the completion of the reaction.

Side effects can sometimes be of interest if they are correctly interpreted. The formation of multiple derivatives produces a reduction in sensitivity, but can be useful for identification purposes if the compound concentration is high. The incomplete methylation of xipamide, a diuretic agent, leads to a mixture of tri- and tetramethyl derivatives. In controlled and reproducible conditions, this side effect can be used by experienced analysts to confirm the presence of xipamide (see Fig. 2).

Interference in GC–MS analysis can be produced as a consequence of the derivatization reaction [16]. False negative results have been described when an interfering drug competes with the targeted drug for the derivatization reagent. The problem can be eliminated by using a greater amount of derivatizing reagent.

The main requirements for a successful derivatization reaction are: a single derivative should be formed for each compound; the derivatization reaction should be simple and rapid, and should occur under mild conditions; the derivative should be formed with a high and reproducible yield and

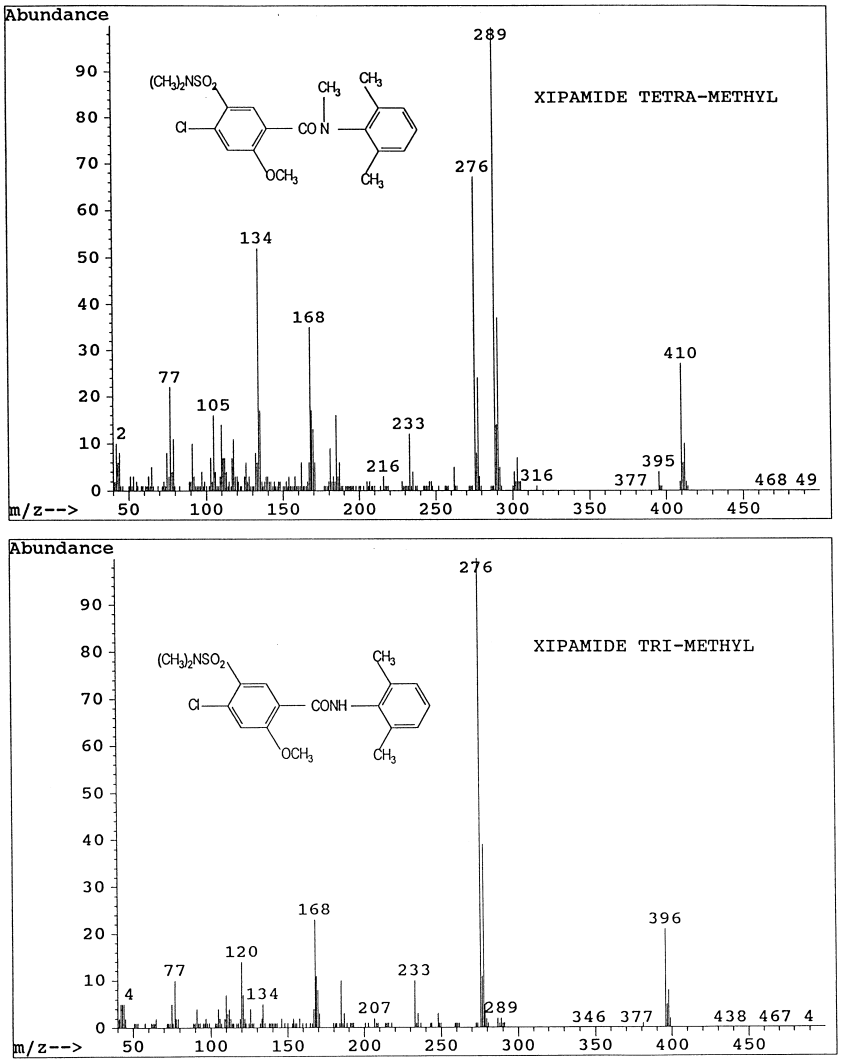

Fig. 2. Multiple derivatives of xipamide: top, mass spectrum and structure of xipamide tetramethyl derivative (M^+, m/z 410); bottom, mass spectrum and structure of xipamide trimethyl derivative (M^+, m/z 396). They are obtained simultaneously when xipamide is derivatized with methyl iodide.

should be stable in the reaction medium; in quantitative analyses, the calibration curve should be linear.

3. Main derivatization methods

3.1. Silylation

Silylation is the most widely used derivatization procedure for GC–MS analyses [18,33,34]. Silyl derivatives are formed when active proton displacement (in –OH, –SH or –NH groups) by an alkylsilyl group occurs. Nearly all protic functional groups present in organic compounds can be converted to silyl ethers or esters. The ability of various functional groups to form silyl derivatives is as follows: alcohols > phenols > carboxylic acids > amines > amides.

The most common silylation procedure is trimethylsilylation. Higher alkyl homologous or

halogen containing analogous have been used to increase hydrolytic stability of the derivative, to improve detectability with some particular detectors, to improve resolution or to obtain mass spectra of higher diagnostic value [34,35]. Trimethylsilyl (TMS) derivatives combine thermal and chemical stability and high volatility. They are easy to prepare, and show excellent GC behaviour. A variety of trimethylsilylating reagents with different properties (such as volatility, reactivity, selectivity, by-product formation, etc.) have been developed including trimethylhalosilanes, TMS-amines, TMS-esters and TMS-amides [34,35]. The TMS amides, *N*,*O*-bis-trimethylsilyl-trifluoroacetamide (BSTFA) [36] and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) [37], are the most commonly employed silylating reagents in analytical work. The high silylating power and high volatility of these reagents and reaction by-products are the main causes of their wide use. MSTFA is the most volatile TMS-amide available. As described below, MSTFA alone has been used to form TMS derivatives of alcohols and phenols followed by acylation of amino groups with MBTFA.

The addition of a catalyst has been used to increase the silylating power of these reagents to derivatize sterically hindered functions or to enhance reaction rates. Trimethylchlorosilane (TMCS), trimethylsilylimidazole (TMSIm), trimethyliodosilane (TMSI), or potassium acetate have been used as a catalysts [38]. BSTFA with 1% TMCS as a catalyst has been widely used to analyze drugs of abuse and their metabolites [39–42].

Trimethylsilylimidazole (TMSIm) also has strong silylation power for hydroxyl and carboxyl groups but does not react with amino groups nor promote enol-TMS ether formation [43]. TMSIm has been used as a catalyst of MSTFA for sterically hindered functional groups, such as tertiary alcohols. Some mixtures of different silylating reagents which provide potent universal silylating activity, such as BSTFA:TMCS:TMSIm, are commercially available.

All silylation reagents and derivatives are sensitive to moisture; for this reason, reactions must be performed under anhydrous conditions. TMS derivatives are more sensitive to hydrolysis than other derivatives containing more sterically crowded alkyl substituents in the silicon atom.

In compounds with ketone and hydroxyl groups, reagents have been used which do not promote enol formation, such as TMSIm, or previous protection of the ketone groups by formation of a methoxime derivative. Formation of methoxime derivatives of ketone groups prior to trimethylsilylation of hydroxyl groups has been widely used to analyze corticosteroids [44–52]. For the quantitative derivatization of ketosteroids as their TMS enol ethers, the use of MSTFA catalyzed by TMSI has been described [53,54].

In general, the EI mass spectra of TMS ethers are characterized by weak or absent molecular ions; the $[M-15]^+$ ion formed by loss of a methyl group bonded to silicon is generally more abundant. This ion can be used to determine the molecular mass. The m/z 73, corresponding to the TMS group, is prominent in nearly all TMS spectra. Other abundant silicon-containing ions are present in the mass spectra of TMS derivatives [34].

tert.-Butyldimethylsilyl (TBDMS) derivatives are used to increase hydrolytic stability and to give useful mass spectrometric fragmentation. TBDMS are reported to be more stable than the corresponding TMS-derivatives. They also are easy to prepare, but have a disadvantage in the difficulty they present for the derivatization of sterically hindered groups. *N*-Methyl-*N*-*tert*.-butyldimethylsilyltrifluoroacetamide (MTBSTFA) is a sylilating reagent which donates TBDMS groups. It is used to derivatize active hydrogens of hydroxyl, carboxyl and thiol groups as well as primary and secondary amines [55–62]. The reaction by-products formed are neutral and volatile. TBDMS derivative mass spectra are characterized by abundant $[M-57]^+$ ions formed by loss of the *tert*.-butyl group; these ions are very suitable for quantitative analysis by SIM, as they usually have high m/z value, and make molecular mass determination possible.

All of these derivatization reagents can be injected directly into the GC–MS system, with the corresponding advantage of shorter sample preparation time.

3.2. Acylation

Acylation is another commonly used derivatization method in GC–MS. It consists of the introduction of

an acyl group in a molecule holding a reactive hydrogen. Acylated derivatives can be obtained from a great variety of functional groups: alcohols, amines, amides, thiols, phenols, enols, sulfonamides, unsaturated compounds and aromatic rings.

Acylation reactions can be performed using three main types of reagents: acyl halides, acid anhydrides or reactive acyl derivatives such as acylated imidazoles. Acyl halides are highly reactive, but a halogen acid is produced during the reaction and a basic acceptor is normally required for neutralization. The elimination of the excess acylating reagent is preferable because its presence may make problems during GC. The reaction with acid anhydrides, at times in the presence of an acidic acceptor such as pyridine, may be preferred because the excess reagent is easier to remove. Acetylation with acetic anhydride (AA) has been used to derivatize biogenic amines and psychotropic drugs [63], β-blockers and their metabolites [64,65] and a broad range of analytes in systematic toxicological analyses [14]. Propionylation of opiates by propionic acid anhydride (PAA) using dimethylaminopyridine as a catalyst has been described [66]. The use of acyl halides and anhydrides can lead to undesirable side reactions (dehydration, enolization, etc.) due to the strongly acidic conditions of the reaction medium. For acid-sensitive compounds, acylation can be performed using reagents that have a high acylation reactivity, such as acylimidazoles, and in which the by-product of the reaction is a basic leaving group.

Haloalkylacyl derivatives are the most popular acyl derivatives. These derivatives increase the electron affinity of the compounds and make possible highly sensitive analyses using NCI-MS. Perfluoroacyl derivatives such as trifluoroacetyl (TFA), pentafluoropropionyl (PFP) and heptafluorobutyryl (HFB), are the most widely used in practice. An additional advantage of the perfluoroacyl derivatives is that the mass spectra frequently have abundant ions of high m/z values. The increments in mass can be adjusted by choice of the derivative and with multifunctional analytes the mass range of the instrument must be taken into account [67].

Perfluoroacyl derivatives can be prepared by reaction with the appropriate acid anhydrides sometimes in the presence of a basic catalyst [67–71] or by reaction with perfluoroacylimidazoles. Imidazole reagents can acylate alcoholic and primary and secondary amino groups. These reagents hydrolyze with moisture and, the excess reagent can thus be removed, when derivatization products are stable enough, by using a wash with an aqueous solution. Perfluoroacylimidazoles have been described to derivatize LSD and metabolites [30,72], and *N*-heptafluorobutyrylimidazole has been used to form HFB derivatives of dihydroethorphine [73,74].

Trifluoroacetylation of amine, hydroxyl and thiol groups has been achieved also under mild conditions with *N*-methylbis(trifluoroacetamide (MBTFA) or bis(trifluoroacetamide) (BTFA) [75]. These reagents are highly volatile and do not interfere in the GC analysis, and the reaction mixture alone or with a suitable solvent can be directly analyzed with no adverse effects on GC column performance and lifetime. Selective N-TFA-O-TMS derivative formation has been described for phenolalkylamines, hydroxyamines and amino acids [27,29,76–81]. The trimethylsilylating reagent used was MSTFA, followed by MBTFA as trifluoroacylating agent. These derivatives are very stable in solution and show excellent gas chromatographic properties.

Extractive acylation has also been described using a variety of reagents. A mixture of ether and MBTFA at alkaline pH has been described to form TFA derivatives of primary and secondary amines [82]. Acetic anhydride or pentafluorobenzoyl chloride have been used to perform extractive acylations of amines and phenols [83]. Extractive formation of HFB derivatives of amphetamine and metabolites using heptafluoro-*n*-butyryl chloride has been described [84].

3.3. Alkylation

Alkylation consists of the replacement of an active hydrogen by an alkyl or, at times, an aryl group. Carboxylic acids, alcohols, thiols, phenols, primary and secondary amines, amides and sulfonamides are the main functional groups that can be subjected to alkylation reactions. For GC–MS analysis, alkylation and, even more so, methylation can be of interest for some applications due to the small increase in molecular mass and the volatility of the methyl derivatives. This is especially true when working with multifunctional compounds.

Many reagents and methods to prepare alkyl derivatives have been described. Alkyl halides, mainly the lower-molecular-mass aliphatic bromides and iodides (methyl, ethyl, propyl, isopropyl, etc.) or benzyl and substituted benzyl bromides, are some of the most commonly employed reagents used to obtain alkyl derivatives; silver oxide, barium oxide and sodium hydride have been used as catalysts [14].

Methylation or ethylation has also been accomplished by refluxing a dry acetone solution of the compound and either methyl or ethyl iodide with a mildly basic condensation reagent, such as dry potassium carbonate [85–89]. The reaction mixture can be directly analyzed by GC–MS. A partially automated flow-based method has been described to obtain methyl derivatives of non-steroidal antiinflammatory drugs by reaction with methyl iodide [90].

Alkylation of carboxylic acids can also be achieved by esterification with alcohols. Methanol or ethanol containing an acidic catalyst, such as hydrochloric acid, sulfuric acid or boron trichloride, have been used to form methyl or ethyl esters [91,92]. Higher-molecular-mass alcohols and alcohols containing halogen atoms have been employed to obtain high mass fragment ions [93]. The halogenated esters also have important advantages for special detection techniques such as MS–MS [94]. 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) has been extensively used to derivatize carboxylic functional groups of benzoylecgonine and other cocaine metabolites [95,96].

Diazoalkanes have been used to alkylate moderately acidic functional groups, such as carboxylic and sulfonic acids, phenols and enols. Diazomethane is the diazoalkane most frequently used [14,97,98]. Lewis acids, such as boron trifluoride etherate, have been used as catalysts to promote the alkylation of less reactive hydrogens, such as aliphatic alcohols, but their use is not recommended for extremely acid-labile compounds. Due to the high reactivity and versatility of diazoalkanes as synthetic reagents, the possibility of unexpected side derivatives should always be considered especially when dealing with multifunctional compounds.

N,*N*-Dimethylformamide dialkyl acetals react with carboxylic acids, phenols and thiols to form the corresponding alkyl derivatives. The reagents are sensitive to moisture and the reaction must be performed under dry conditions. The possibility of side-reactions should be considered. The excess of reagent should be removed to avoid interferences in GC analysis.

Extractive alkylation is used to derivatize acidic compounds in the anionic form, such as ionized carboxylic acids and sulfonamides [31,99,100]. The acidic substance is extracted as an ion pair with a quaternary ammonium hydroxide into an appropriate immiscible organic solvent. The alkylation reaction involving nucleophilic displacement with an alkyl halide occurs in the organic phase. Extractive alkylation can be used directly in a biological sample although problems associated with the presence of other anions that compete with the analyte for the phase transfer reagent can limit its direct use. The lower members of the homologous series of alkyl iodides and bromides are normally used as alkylating reagents; pentafluorobenzyl bromide can be used to increase sensitivity in particular detectors. The removal of the resulting tetraalkylammonium halide prior to GC is necessary to avoid problems of column contamination and degradation, secondary derivatization reactions and interferences with the detector response [31,32].

Pyrolytic alkylation consist of the formation of an alkyl derivative from an acidic compound as a result of thermal decomposition of a quaternary alkylammonium salt of the acid in the heated injector port of the gas chromatograph. Tetramethylammonium hydroxide, trimethylanilinium hydroxide or phenyltrimethylammonium hydroxide are usually used to prepare methyl derivatives [101–104]. The injector temperature should be set to 250–300°C. Undesirable side reactions can occur as a consequence of high temperatures and high alkalinity.

3.4. Formation of cyclic derivatives

For polyfunctional compounds, specific reagents can be used to react simultaneously with two proximal reactive groups to form a cyclic derivative. The spatial separation of the involved groups must be adequate for ring formation, and the stability of the resulting ring should be high. In general terms, these compounds contain two functional groups, which can include alcohols, phenols, amines, carboxylic acids and ketones, in alkyl chains at carbon atoms 1,2-, 1,3- or 1,4-, or in aromatic rings in the *ortho*-

position. Rings of five, six or seven atoms are usually formed.

The use of cyclization leads to a derivative of a bifunctional compound with good gas chromatographic properties in a single step; in some cases, single cyclic derivatives are obtained for multifunctional compounds, in comparison with multiple derivatives formed when using reagents for single functional group derivatization [105]. The formation of a ring can increase the stability of sensitive molecules. The higher stability of cyclic groups in relation to mass fragmentation results in mass spectra with high mass and high abundance ions [106–109]. A disadvantage in the formation of cyclic derivatives is that compounds containing further functional groups amenable to derivatization (in addition to the two proximal reactive groups) may form side derivatives.

Reagents used to form cyclic derivatives can be divided into two groups: reagents that can derivatize a broad range of functional groups, and those highly selective for particular functional groups or compounds [110]. The most important derivatives of the first group are cyclic boronates due to their wide range of application, ease of preparation, good GC properties and useful mass spectral characteristics; disadvantages include their sensitivity to moisture. Substituted boronic acids (methylboronic, butylboronic, *tert*.-butylboronic, cyclohexylboronic and phenylboronic) are usually used [105,109,111]; boronic acids with electron-capturing substituents, such as some halogen-containing benzeneboronic acids, can be used for sensitive analysis employing specific detectors. The cyclic anhydride of methylboronic acid, trimethylboroxine, has also been employed to form cyclic methylboronates [108].

Methylboronate derivatives are very volatile and they are useful in MS of high-molecular-mass substances due to the small increment in the molecular mass; butylboronate derivatives are a good compromise between volatility and stability. Reactions occur readily and quickly under mild conditions and usually involve incubation of boronic acid and the substrate in an anhydrous solvent at room temperature for a short period of time; higher temperatures can be required in some cases. Direct GC analysis of the reaction mixture can be performed.

Using dimethylchlorosilane, dimethyldiacetoxysilane or di-*tert*.-butyldichlorosilane, cyclic siliconides can be formed. Incomplete reaction and formation of by-products limit their use in practice. Cyclic siliconides of compounds with β-ethanolamine structure, such as β-adrenergic drugs, have been described using chloromethyldimethylchlorosilane with diethylamine in hexane [107].

Formation of acetal and ketal derivatives with aldehydes and ketones and derivatization of α-ketoacids with 1,2-diaminobenzenes to form cyclic quinoxalinol derivatives are other examples of cyclization reactions [110].

3.5. Chiral derivatization

Several groups of drugs with important pharmacological and toxicological implications are subjected to discrimination in their biological disposition regarding the way the body handles the different enantiomers of the drugs [112–114]. The result is that one of the enantiomers usually accumulates in the body more than the other and, if toxic, it may be responsible for the major effects of overdose [115]. In addition, relatively often, the enantiomer being accumulated is the one having the least beneficial pharmacological effect [116,117]. As a consequence, strong developments in the analytical capability to differentiate between the enantiomers of racemic drugs have been a matter of growing concern [118]. Groups of drugs of toxicological relevance in overdoses such as β-adrenergic agents, anticoagulants, calcium channel blockers, anticancer drugs or nonsteroidal anti-inflammatory drugs are of particular relevance in this regard [119,120].

Liquid chromatography (LC) has been able to comply with many of the requirements for the easy separation of enantiomeric drugs, either directly on chiral columns or after conversion to diastereomers with the suitable chiral reagent [121,122]. Important developments in chiral separations have also been achieved recently by means of capillary electrophoresis (CE) [123,124]. Consequently, GC and GC–MS applications have been relatively rare in the last few years.

Nevertheless, there are situations and specific groups of drugs where chiral separation by GC is interesting. The majority of racemic drugs are relatively well suited to GC–MS analysis. Amphetamine

and several other stimulants with related structures present differences in pharmacology and body disposition between enantiomers and are also well suited to analysis by GC. To name a few, amphetamine, methamphetamine, methylphenidate, fenfluramine or methoxyphenamine are target drugs for enantiomeric separation. As an example, it is interesting to note that *S*-(+)-methamphetamine is considered a strongly restricted drug, while *R*-(−)-methamphetamine can be given as a medication for cold. Examples among narcotic drugs are also relevant as dextropropoxyphene is considered a mild narcotic while levopropoxyphene is nearly devoid of narcotic properties. Similarly, dextromethorphan is a widely used antitusive drug, while its enantiomer is a metabolite of the restricted drug levorphanol. Interpretation of analytical results on selegiline metabolism, which can be converted metabolically to *R*-(−)-amphetamine and *R*-(−)-methamphetamine can be confusing if not properly identified [125].

The procedures used for the chromatographic separation of enantiomers pairs fall into two main categories: conversion into diastereomers by reaction with an optically pure reagent and separation on achiral chromatographic phases; or direct separation on chiral stationary phases without need of chiral derivatization. Only those methodologies corresponding to the first approach (formation of diastereomers) are here considered. A systematic revision of chiral reagents routinely used in GC can be found elsewhere [126] and therefore it will not be included in this review. Nevertheless, it is worthwhile to present some of the reagents introduced or mainly used in recent years to separate these and similar compounds.

Derivatives of fluoroacyl-prolyl chloride [127] have been preferred reagents for many drugs. *S*-(−)-Heptafluorobutyryl prolyl chloride has been used for analyzing stimulants (fenfluramine, methylphenidate and its metabolite ritalinic acid, amphetamine derivatives and β-blockers) [128–133]. Several of these compounds and others such as MDMA and related compounds have also been studied by means of the related reagent *S*-(−)-trifluoroacetyl prolyl chloride [125,134]. Alternatively, the enantiomers of the narcotic drug methadone and the stimulant drug amphetamine have been separated using derivatization with the menthol derivative (−)-menthyl chloroformate [135,136].

Enantiomers of non-steroidal antiinflammatory drugs have also been studied using *S*-(−)-1-(1-naphtyl)ethylamine to form the corresponding amides [137,138]. Carboxylic acids can form diastereomeric esters with enantiomers of alkyl alcohols, such as 2-butanol [139,140], 2-octanol [141], menthol [142] or aryl alcohols (methyl-benzyl-alcohol) [140]. Diastereomeric esters can also be formed with optically active compounds bearing hydroxyl groups, such as triazole fungicides by reaction with enantiomers of carboxylic acids [143]. Similarly, diastereomeric amides can be formed by reaction with enantiomers of phenylethylamine [144,145] or amphetamine [146,147].

4. Derivatization procedures for GC–MS determination of drugs of abuse

4.1. Opiates

Opiates, and especially heroin, are among the most abused drugs. Heroin was first synthesized in 1898 by Dreser. It was obtained from morphine by acetylation with acetic anhydride. Traditionally heroin has been administered intravenously. However, over the last eight years, the use of other administration methods, such as intranasal (snorting) and smoking has increased, which may be due to the fear of AIDS transmission.

Heroin is rapidly metabolized to 6-monoacetylmorphine (6-MAM) and then to morphine. Additionally codeine may be detected, but it is not a metabolite of heroin, and its presence is a consequence of the impurity in heroin street samples. Morphine is further metabolized by conjugation to morphine-3-glucuronide and morphine-6-glucuronide and by *N*-demethylation to normorphine. Codeine is metabolized by conjugation to codeine glucuronide, by *N*-demethylation to norcodeine, and by *O*-demethylation to morphine; the metabolite, morphine, is then metabolized as previously explained. The blood level of 6-MAM is usually very low or not detectable. The detection of 6-MAM in blood or urine shows recent drug use, although the absence of detectable levels of 6-MAM does not exclude heroin consumption since the drug is quickly metabolized. In chronic heroin abuse, 6-MAM can be detected in

the hair, where its concentration is always higher than that of morphine.

Table 1 provides a summary of some GC–MS derivatization methods for the analysis of opiates in biological samples [39,148–157]. Procedures related to the new matrices, especially hair, have been mainly considered. Studies published earlier than 1994 have been reviewed by Goldberger and Cone [4] in a paper on confirmatory GC–MS, including opiates, cocaine, amphetamines, cannabinoids and phencyclidine in urine samples.

In most of the studies, derivatization is performed either with BSTFA or with perfluorinated anhydrides. HFBA has been used by Sachs and Raff [153] to derivatize 6-MAM, morphine and dihydrocodeine in hair samples. Limits of detection (LODs) of 0.03 ng/mg have been achieved. Moeller at al. [151] employed PFPA for the same com-

Table 1
Literature data on derivatization procedures for the analysis of opiates in biological material by GC–MS

Year	Author	Sample	Compound	Derivatization	GC column	Detection mode	LOD	Ref.
1992	Nakahara et al.	Hair	6-MAM Morphine	BSTFA	NB-1	EI-SIM	N.R.	[148]
1993	Cone et al.	Hair	Heroin 6-MAM Morphine Normorphine Codeine Acetylcodeine Norcodeine	BSTFA	HP-1	EI-SIM	50 pg/mg 50 pg/mg 50 pg/mg 500 pg/mg 50 pg/mg 50 pg/mg 500 pg/mg	[149]
1993	Kintz and Mangin	Hair	Morphine	BSTFA	BP-5	EI-SIM	0.1 ng/mg	[150]
1993	Moeller et al.	Hair	6-MAM Morphine Codeine Dihydrocodeine	PFPA	HP-5	EI-SIM	160 pg/ml 40 pg/mg 40 pg/mg 40 pg/mg	[151]
1993	Polettini et al.	Hair	Heroin 6-MAM Morphine Codeine Acetylcodeine	MSTFA	DB-5	MS–MS	N.R.	[152]
1993	Sachs and Raff	Hair	Dihydrocodeine Heroin	HFBA	Ultra-2	EI-SIM	30 pg/mg	[153]
1994	Cone et al.	Sweat	Heroin and metabolites	BSTFA+1% TMCS	Restek 5	EI-SIM	1 ng/patch	[154]
1994	De Giovanni and Strano-Rossi	Urine	Morphine Codeine 6-MAM	BSTFA+1% TMCS	HP-1	EI-SIM	<50 ng/ml	[155]
1994	Wang et al.	Hair Plasma Saliva Urine	Heroin and metabolites	BSTFA+1% TMCS	HP-1	EI-SIM	1–5 ng/ml 0.1–0.3 ng/mg	[39]
1995	Jurado et al.	Hair	6-MAM Morphine Codeine	HFBA/HFIP	HP-1	EI-SIM	20 pg/mg 60 pg/mg 70 pg/mg	[156]
1996	Kintz et al.	Sweat	Codeine	BSTFA+1% TMCS	HP-5 MS	EI-SIM	0.5 ng/patch	[157]

N.R.=Not reported.

pounds; their LODs were 0.04 ng/mg, except for 6-MAM, which was 0.16 ng/mg.

The method developed by Wang et al. [39] for the determination of heroin and six metabolites: 6-MAM, morphine, normorphine, codeine, acetylcodeine and norcodeine in hair, plasma, saliva and urine, included solid-phase extraction and derivatization with 50 μl of BSTFA+1% TMCS at 70°C for 20 min. The LODs were 1 ng/ml, except for norcodeine and normorphine which had LODs of 5 ng/ml. In hair samples, LODs were 0.1 ng/mg, except for norcodeine (0.3 ng/mg) and normorphine (0.5 ng/mg). A similar method was proposed by Cone et al. [149] for hair samples, but the LODs were lower (0.05 ng/mg), except for normorphine and norcodeine with LODs of 0.5 ng/mg. Cone et al. [154] analyzed heroin and metabolites in sweat after derivatization with BSTFA. The LOD obtained was 1 ng/patch; while Kintz et al. [157] were able to detect codeine at the concentration of 0.5 ng/patch, with the same derivatizating agent.

Jenkins et al. [158] compared heroin and metabolite concentrations in saliva and blood after smoking and intravenous administration. TFA derivatives were formed with MBTFA. The LODs and LOQs were approximately 1.0 ng/ml for both analytes.

In agreement with previous papers [148–150,154,155,157], the authors prefer sylilation with BSTFA to acylation with perfluorinated anhydrides for opiate derivatization. Fig. 3 shows the chromatograms of a urine sample spiked with cocaine, benzoylecgonine (BE), morphine, codeine and 6-MAM, and derivatized with BSTFA+1% TMCS (Fig. 3A) or HFBA/HFIP (Fig. 3B). Using cocaine as a common reference (it is not derivatized), the abundances of the TMS derivatives of morphine, codeine and 6-MAM (Fig. 3A) were higher than those of the HFB derivatives (Fig. 3B).

4.2. Cocaine

Illicit cocaine is commonly available either as a hydrochloride salt or as the free base (''crack''). The main administration routes include sniffing, intravenous injection and smoking. The conversion of cocaine to metabolites, BE and ecgonine methyl ester (EME) begins to occur soon after absorption. The coadministration of cocaine and ethanol leads to the formation of ethylbenzoylecgonine (EBE, also known as ''cocaethylene''), a transesterification product, which is hydrolyzed to BE and ecgonine ethyl ester. Other metabolites are norcocaine and benzoylnorecgonine. Anhydroecgonine methyl ester is produced when cocaine is smoked.

The metabolic profiles and detection windows are different depending on the biological matrix. After

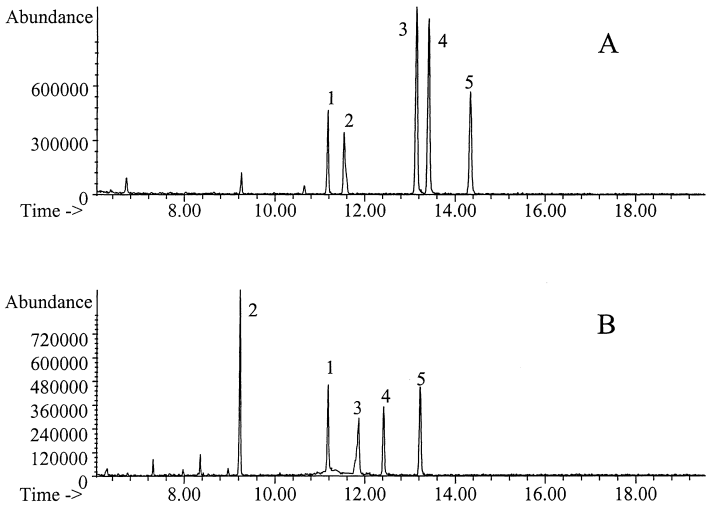

Fig. 3. Selective ion chromatograms (total signal for the acquisition of three ions per compound) of a urine sample containing cocaine (1), BE (2), codeine (3), morphine (4) and 6-MAM (5), after derivatization with BSTFA+1%TMCS (A) or with HFBA/HFIP (B).

cocaine administration, the major compound found in blood and urine is BE; while the parent drug has the highest concentration in other matrices (hair, saliva and sweat). With respect to the detection windows, BE can be detected in blood and saliva for one day; in urine, for several days; in sweat, for two or three weeks; and in hair, for months or years, depending on the length of the hair shaft.

A variety of analytical methods have been reported for the analysis of these compounds. Some of them are summarized in Table 2 [39,40,55,56,95,149,153–155,159–168]. The paper of Goldberger and Cone [4] on workplace confirmation testing by GC–MS also reviewed the procedures for cocaine compounds published before 1994.

In an study to evaluate decontamination procedures in hair analysis, Cone et al. [159] described a method for cocaine and its metabolites. Derivatization was performed with BSTFA+1% TMCS. The LODs were 0.1 ng/mg for all analytes (cocaine, BE, EME, norcocaine, cocaethylene and norcocaethylene). In a posterior study [4], using the same procedure, the same authors reported a LOD of 0.05 ng/mg for cocaine and BE. A similar method was used by Wang et al. [39] for the analysis of cocaine and eight metabolites: anhydroecgonine methyl ester, BE, norcocaine, EME, cocaethylene, benzoylnorecgonine, norcocaethylene, ecgonine ethyl ester in plasma, saliva, urine and hair samples. The LODs were 1 ng/ml with the exception of benzoylnorecgonine (5 ng/ml). In hair, the LODs were 0.1 ng/mg, except for norcodeine (0.3 ng/mg) and benzoylnorecgonine (0.5 ng/mg).

MTBSTFA was used by Henderson et al. [160] and Harkey et al. [55] for the analysis of cocaine, BE and EME in hair samples. Analysis was performed by the NCI-SIM mode with LODs of 0.1 ng/mg for cocaine and BE and 0.5 ng/mg for EME.

Fritch et al. [40] compared RIA and GC–MS techniques for the analysis of cocaine related compounds in hair. Derivatization was performed with BSTFA+1% TMCS at 70°C for 30 min. The LODs were 0.1 ng/mg for cocaine and 0.2 ng/mg for BE and EME.

Derivatization with HFBA was performed in hair samples by Jurado et al. [156] for the analysis of cocaine and BE. After evaporation, the dry extracts were derivatized with HFBA/HFIP. The LODs were 30 pg/mg for both compounds. Moeller et al. [161] analyzed the hair of Bolivian coca chewers using derivatization with 100 μl of PFPA and 70 μl of PFPOH for 30 min at 60°C. The LODs were 0.1 ng/mg for cocaine and BE and 1 ng/mg for EME. A similar method was performed by Sachs and Raff [153].

Farré et al. [165] and de la Torre et al. [95] proposed a method for the analysis of cocaine metabolites: BE, EME, EBE and norcocaine in blood and urine samples, respectively. Derivatization was performed with PFPA and HFIP. The tubes were incubated at 60°C for 15 min. After drying, the extracts were redissolved in ethyl acetate. The sensitivity achieved was 1 ng/ml for all compounds except for norcocaine (0.5 ng/ml).

Crouch et al. [56] analyzed tissues, whole blood, plasma and urine samples for cocaine, BE and EME. Derivatization was performed with MTBSTFA, the derivatives were stable and produced mass spectral ions with higher m/z ratios than TMS derivatives. The analysis was performed in the positive chemical ionization (PCI)-SIM mode, and the LODs were 5 ng/ml for all compounds. In addition to the analysis of cocaine, BE, and EME, the method was used to quantify cocaethylene and to identify norcocaine.

Jenkins et al. [158] also analyzed cocaine and eight metabolites in their paper on the comparison of drug concentrations in saliva and blood. After derivatization with BSTFA+1% TMCS, they achieved an LOD of approximately 1.0 ng/ml for each analyte.

As for opiate compounds, the authors compared trimethylsilylation and acylation to derivatize cocaine and BE. In this case, as its shown in Fig. 3, the abundance of BE, with respect to cocaine, was higher when derivatizing with HFBA/HFIP (Fig. 3B) than after derivatization with BSTFA+1% TMCS (Fig. 3A).

4.3. Cannabis

The consumption of hashish and marijuana in Europe and in the United States, respectively, surpasses that of the other illegal psychoactive substances. Its source is *Cannabis sativa*, variety *Indica*, the hemp plant. The main psychoactive agent is Δ^9-tetrahydrocannabinol (THC); its concentration var-

Table 2
Literature data on derivatization procedures for the analysis of cocaine and metabolites in biological material by GC–MS

Year	Author	Sample	Compound	Derivatization	GC column	Detection mode	LOD	Ref.
1991	Cone et al.	Hair	Cocaine BE EME Norcocaine EBE	BSTFA+ 1% TMCS	HP-1	GC–MS	0.1 ng/mg	[159]
1991	Harkey et al.	Hair	Cocaine BE EME	MTBSTFA	HP-1	GC–MS-NCI	0.1 ng/mg 0.1 ng/mg 0.5 ng/mg	[55]
1992	Fritch et al.	Hair	Cocaine BE EME	BSTFA+ 1% TMCS	–	RIA GC–MS	0.1 ng/mg 0.2 ng/mg 0.2 ng/mg	[40]
1992	Henderson et al.	Hair	Cocaine BE EME	BSTFA+ 1% TMCS	HP-1	GC–MS	0.1 ng/mg 0.1 ng/mg 0.5 ng/mg	[160]
1992	Moeller et al.	Hair	Cocaine BE EME	PFPA/ PFPOH	HP-1	GC–MS	0.1 ng/mg 0.1 ng/mg 1 ng/mg	[161]
1992	Nakahara et al.	Hair	Cocaine BE EME	PFPA/ HFIP	HP-1	GC–MS	0.3 ng/mg	[162]
1993	Abusada et al.	Meconium Blood Plasma	Cocaine EME BE EBE	PFPA/ PFPOH	HP-Ultra 2	EI-SIM	6.52–59.41 ng/ml	[163]
1993	Aderjan et al.	Blood Urine	Cocaine BE	PFPA/HFIP	CPSil-5	PCI-SIM	10–20 ng/ml	[164]
1993	Cone et al.	Hair	Cocaine BE	BSTFA	HP-1	EI-SIM	0.05 ng/mg 0.05 ng/mg	[149]
1993	Farré et al.	Blood	Cocaine EBE EME BE Norcocaine	PFPA/HFIP	HP-5	EI-SIM	0.5–1 ng/ml	[165]
1993	Sachs and Raff	Hair	Cocaine BE	PFPA/PFPOH	HP-1	EI-SIM	0.1 ng/mg 0.1 ng/mg 1 ng/mg	[153]
1994	Cone et al.	Sweat	Cocaine and metabolites	BSTFA	Restek 5	EI-SIM	1 ng/patch	[154]
1994	De Giovanni and Strano-Rossi	Urine	Cocaine BE	BSTFA+ 1% TMCS	HP-1	EI-SIM	10 ng/ml	[155]
1994	Wang et al.	Hair Plasma Saliva Urine	Cocaine and metabolites	BSTFA+ 1% TMCS	HP-1	GC–MS	1–5 ng/ml 0.1–0.5 ng/ml	[39]

Table 2. Continued

Year	Author	Sample	Compound	Derivatization	GC column	Detection mode	LOD	Ref.
1995	Bermejo and Strano-Rossi	Hair	Cocaine BE EME	BSTFA		GC–MS	1 ng/mg	[166]
1995	Jurado et al.	Hair	Cocaine BE	HFBA/ HFIP	HP-1	GC–MS	0.03 ng/mg 0.03 ng/mg	[156]
1995	Kintz and Mangin	Hair	Cocaine BE EME EBE	BSTFA+ TMCS	HP-5	EI-SIM	0.1–0.8 ng/mg	[167]
1995	Lewis et al.	Meconium	Cocaine BE	BSTFA	–	EI-SIM	2 ng/g	[168]
1995	de la Torre et al.	Urine	Cocaine EBE EME BE Norcocaine	PFPA/ HFIP	HP-5	EI-SIM	1–2 ng/ml	[95]
1995	Crouch et al.	Tissue Blood Plasma Urine	Cocaine BE EME	MTBSTFA	DB-5	PCI-SIM	5 ng/ml	[56]

ies, depending on the formulation type: marijuana, hashish, bhang, ganja, sinsemilla, etc. Smoking is the main administration route.

THC is metabolized by microsomal hydroxylation to 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), which is subsequently oxidized to 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). Depending on the sample, either THC or its metabolites may be identified. The major compound present in urine samples is THC-COOH, in both conjugated and unconjugated forms; while, in hair, THC is the main cannabinoid detected. In blood samples, THC, THC-COOH and THC-OH are often detected.

Table 3 provides a summary of GC–MS derivatization methods for the analysis of these compounds in different biological matrices [57,58,94,156,169–179]. MTBSTFA was employed as derivatization reagent by Clouette et al. [57] and Moore et al. [58] for the analysis of THC-COOH in urine and meconium samples, respectively. The LODs were 0.9 ng/ml for urine and 2 ng/g for meconium. The advantage of this reagent lies in the formation of unusually stable derivatives of THC-COOH (over a 10-day period). Goodhall and Basteyns [171] developed a method for the analysis of THC, THC-OH and THC-COOH in blood samples. The compounds were isolated by liquid–liquid extraction and the extract was evaporated and derivatized with BSTFA. Analysis was performed with a HP-1 column in the EI-SIM mode. The LODs and LOQs were 2 ng/ml for THC-COOH and 0.2 ng/ml for THC and THC-OH. Kemp et al. [172,173] also employed BSTFA for derivatization of THC and nine metabolites in urine and plasma. The LODs ranged from 0.5 to 2.1 ng/ml.

A comparative study of methods for derivatization of THC-COOH in urine was reported by Szirmai et al. [98]. Five different derivatization agents were studied: (a) diazomethane-BSTFA, esterification of the carboxy group and silylation of the phenol and hydroxyl group were obtained; (b) diazomethane-MBTFA, esterification of the carboxy group and formation of the TFA derivatives of phenolic and hydroxyl groups; (c) BSTFA, silylation of all groups; (d) trifluoroethanol (TFE)-PFPA, to obtain the trifluoroethyl ester derivative of the carboxy group and the pentafluoropropionyl derivative of the phenol and hydroxyl groups; (e) tetramethylammonium hydroxide (TMAH)-methyl iodide, to form methyl derivatives of the carboxy and phenolic groups (the

Table 3
Literature data on derivatization procedures for the analysis of cannabis metabolites in biological material by GC–MS

Year	Author	Sample	Compound	Derivatization	GC column	Detection mode	LOD	Ref.
1993	Clouette et al.	Urine	THC-COOH	MTBSTFA	DB-1	EI-SIM	0.91 mg/ml	[57]
1993	Wu et al.	Urine	THC-COOH	MSTFA	DB-1	EI-SIM	1.1 ng/ml	[169]
1995	Cirimele et al.	Hair	THC THC-COOH	PFPA/ PFPOH	HP-5MS	EI-SIM	0.1 ng/mg 0.1 ng/mg	[170]
1995	Goodhall and Basteyns	Blood	THC THC-OH THC-COOH	BSTFA	HP-1	EI-SIM	0.2–2 ng/ml	[171]
1995	Jurado et al.	Hair	THC THC-COOH	HFBA/ HFIP	HP-1	EI-SIM	0.05 ng/mg 0.04 ng/mg	[156]
1995	Kemp et al.	Urine Plasma	THC and 9 metabolites	BSTFA	HP-5	EI-SIM	0.5–2.1 ng/ml	[172][173]
1995	Kintz et al.	Hair	THC-COOH	PFPA/ PFPOH	HP-1	NCI-SIM	5 pg/mg	[174]
1995	Kudo et al.	Tissues	THC	TBAH	HP-1	EI-SIM	1 ng/g	[175]
1995	Mieczkowski	Hair	THC THC-COOH	HFBA/ HFIP	DB-5	MS–MS	50 fg/mg	[176]
1995	Wilkins et al.	Hair	THC THC-OH THC-COOH	TFAA	Restek Rtx 200-15M	NCI-SIM	0.05 ng/mg 0.5 ng/mg 0.05 ng/mg	[177]
1996	Kauert and Rohrich	Hair	THC	PAA	DB-1	EI-SIM	0.1 ng/mg	[178]
1996	Moore et al.	Meconium	THC-COOH	MTBSTFA	DB-5	EI-SIM	2 ng/g	[58]
1997	Uhl	Hair	THC-COOH	PFPA/ HFIP	DB-5	MS–MS	0.20 pg/mg	[94]

hydroxyl group in the side chain was not derivatized). The more suitable derivatives, according to chromatographic properties, were procedures a, b, and c.

Kudo et al. [175] proposed a method for the analysis of THC in tissue samples, where derivatization was performed with methyl iodide and tetrabutylammonium hydroxide (TBAH) followed by detection in the EI-SIM mode. Under those conditions the LOD was 1 ng/g. Wu et al. [169] developed a solid-phase extraction method on disc, where THC-COOH was simultaneously eluted and derivatized with MSTFA. The LOD was 1.1 ng/ml. The procedure was rapid and did not require organic solvents.

THC-COOH concentrations are very low in hair. For this reason, high sensitivity is required. The analysis of cannabis in hair has usually been performed using derivatization with perfluorinated anhydrides. Jurado et al. [179] described a comparative study for the analysis of THC and THC-COOH in hair samples, where the method proposed by Cirimele et al. [170] was compared with their own method [156]. In both cases, analysis was performed in the EI-SIM mode after basic hydrolysis, followed by liquid–liquid extraction. THC and THC-COOH were derivatized with PFPA/PFPOH [170] and HFBA/HFIP [156]. The LODs were 0.1 ng/mg and 0.05 ng/mg for PFP and HFB derivatives, respectively. The Cirimele et al. method [170] was later improved [174] by changing the detection mode to NCI-SIM. The LOD for THC-COOH was then 0.005 ng/mg.

The LOD for THC-COOH in hair analyses was lowered by using MS–MS. Mieckowski [176] reported a LOD for THC and THC-COOH of 0.05·

10^{-3} ng/mg after derivatizing with HFBA/HFIP, while in Ulh's [94] derivatization was done with PFPA/HFIP; the LOD of THC-COOH was $0.20 \cdot 10^{-3}$ ng/mg.

Following this review and the experience with the analysis of cannabis in the different biological matrices, the authors prefer derivatization with perfluorinated agents. In the new matrices (hair or sweat), where high sensitivity is required, the use of tandem MS or NCI for the analysis of these perfluorinated derivatives would be of great interest.

4.4. Amphetamines

Amphetamine and methamphetamine have been widely available for many years and their abuse has a history as old as the drugs themselves. Amphetamine derivatives, or ''designer drugs'', 3,4-methylenediaxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), are currently abused as psychedelics. Their recreational use has dramatically increased in the USA and Europe during the last decade.

While the metabolism of amphetamine and methamphetamine has been extensively reported, few papers on the metabolism of the designer drugs are available. Maurer [180] studied the metabolism of methylenedioxyphenyl-alkylamines. He found two overlapping metabolic pathways: *O*-dealkylation of the methylenedioxy group followed by methylation of one of the hydroxy groups, and successive degradation of the side chain to *N*-dealkyl and deamino-oxo metabolites. Helmlin et al. [181] investigated the pharmacokinetic behavior of MDMA in humans and also proposed an analytical method for these compounds. Sample extraction and on-disc derivatization with HFBA were performed on solid-phase extraction discs.

Some interesting reviews concerning the analysis of these compounds have been published up to date. In a paper on systematic toxicological analysis of drugs and their metabolites by GC–MS, Maurer [3] reviewed papers on amphetamine and methamphetamine from 1981 to 1991. Cody [120], in a study on methamphetamine enantiomer ratios in urine by GC–MS, reviewed 56 papers on this compound. Goldberger and Cone [4] discussed six papers on the analysis of amphetamine and methamphetamine in urine samples – all of them published earlier than 1994.

With respect to the new matrices, Moeller [182] reviewed eight GC–MS procedures for the analysis of amphetamine and methamphetamine in hair samples, all of them published by Japanese researchers, and using the same derivatizing agent, TFAA. The LODs ranged from 0.01 ng/mg to 0.5 ng/mg, depending on the detection mode, CI or EI. Nakahara [183] reviewed the detection and the incorporation of amphetamine in hair. The derivatizing agents were perfluorinated anhydrides, mainly TFAA.

In addition to these reviews, Table 4 provides a selection of derivatization methods for the analysis of amphetamine and derivatives by GC–MS in biological samples [59,71,136,181,184–189].

Hughes et al. [136] detected amphetamine and methamphetamine in urine as carbamate derivatives following reaction with (−)-menthyl chloroformate. They were able to separate *R*-(−)-methamphetamine from the illicit *S*-(+)-methamphetamine by using an achiral column DB-5. The LODs were 6.7 ng/ml and 9.5 ng/ml for methamphetamine and amphetamine, respectively. Gjerde et al. [184] proposed the derivatization with perfluorooctanoyl chloride. The LODs were 11 ng/ml for amphetamine and 13 ng/ml for methamphetamine. Meatherall [186] described a derivatizing extraction with a mixture of *n*-hexane–chloroform–propylchloroformate to obtain the propylcarbamate derivatives. The LODs were 5 ng/ml for both compounds.

Dallakian et al. [71] compared the analyses of amphetamine and methamphetamine by GC–MS with CI and EI. After solid-phase extraction, the dry extracts were redissolved in pyridine and derivatized with HFBA. The LODs of HFB derivatives were 95 ng/ml for amphetamine and 90 ng/ml for methamphetamine in the CI; they were 10 ng/ml and 9 ng/ml for the same compounds in the EI mode.

Jacob et al. [185] described a reductive alkylation with propionaldehyde and sodium borohydride to produce *N*-propyl derivatives of methamphetamine and its metabolite amphetamine. These derivatives had excellent chromatographic properties and could be carried through acid–base partitioning steps to clean-up and concentrate the extracts.

Melgar and Kelly [59] proposed the use of

Table 4
Literature data on derivatization procedures for the analysis of amphetamines and designer drugs in biological material by GC–MS

Year	Author	Sample	Compound	Derivatization	GC column	Detection mode	LOD	Ref.
1991	Hughes et al.	Urine	Amphetamine Methamphetamine	(−)-Methylchloroformate	DB-5	EI-SIM	6.7–9.5 ng/ml	[136]
1993	Gjerde et al.	Blood	Amphetamine Methamphetamine	Pentafluoro-octanoyl Cl^-	HP-1	EI-SIM	11–13 ng/ml	[184]
1993	Melgar and Kelly	Urine	Amphetamine Methamphetamine	MTBSTFA	HP-1	EI-SIM	5 ng/ml 3 ng/ml	[59]
1995	Jacob III et al.	Urine Plasma	Methamphetamine	Propionaldehyde	HP-1	EI-SIM	10 ng/ml	[185]
1995	Maetherall	Urine	Amphetamine Methamphetamine	Propyl chloroformate	DB-1	EI-SIM	5 ng/ml	[186]
1996	Dallakian et al.	Urine	Amphetamine Methamphetamine	HFBA	SPB-5	EI-SIM	9–10 ng/ml 0.04 ng/mg	[71]
1996	Ensslin et al.	Urine	MDEA and metabolites	AA/pyridine	HP-1	EI-SIM	5 ng/ml	[187]
1996	Helmlin et al.	Plasma Urine	MDMA and metabolites	HFBA	DB-5	EI-SIM	N.R.	[181]
1997	Röhrich and Kauert	Hair	Amphetamine MDA MDMA MDEA	PAA Trifluoroacetic acid	HP-5	EI-SIM	≈0.01 ng/mg	[188]
1997	Kikura et al.	Hair	MDMA and five metabolites	PFPA–ethyl acetate (1:1)	TC-1 methyl silicone	EI-SIM	0.1 ng/mg cut-off	[189]

N.R.=Not reported.

MTBSTFA for amphetamine and methamphetamine analyses. This derivatization agent leads to stable derivatives that are well separated from potential interferences and features high-molecular-mass ions. The LODs obtained in the EI mode were 5 ng/ml and 3 ng/ml for amphetamine and methamphetamine, respectively.

Ensslin et al. [187] described a method for the analysis of MDEA and metabolites in urine samples. They performed an acetylation with AA in pyridine. The LODs were 5 ng/ml for MDEA and 10 ng/ml for its main metabolite, 4-hydroxy-3-methoxyethylamphetamine.

Röhrich and Kauert [188] compared two derivatization methods for the analysis of amphetamine and methylenedioxy-derivatives: MDA, MDMA and MDEA in hair samples. After extraction, the samples were derivatized either with PAA or with trifluoroacetic acid. Although propionyl derivatives were more stable than TFA derivatives, the latter are preferable because they provide more specific mass spectrometric information. With respect to the sensitivity, the LODs for all of the compounds were in the range of 0.01 ng/mg, independently of the derivatization procedure applied.

Kikura et al. [189], in a study to clarify the mechanism of MDMA incorporation into hair, developed a method for the analysis of MDA and five metabolites. Derivatization was performed with PFPA–ethyl acetate (1:1) at 60°C for 20 min.

4.5. Benzodiazepines

In spite of the fact that LC [190,191] and, more recently, CE [192] offer alternatives to the analysis of benzodiazepines, GC and especially GC–MS are

popular methods for the analysis of this class of drugs.

Unlike the GC analysis of benzodiazepines and detection by conventional methods such as nitrogen–phosphorous detection or electron-capture detection, which are carried out mainly without derivatization [103], the analysis by GC–MS is preferred after suitable derivatization. The main reasons are to improve the stability of the compounds and to obtain mass spectra with more structural information. Nevertheless, non-derivatizing approaches for GC–MS analysis of benzodiazepines are also routinely used [193–195].

The analysis of the corresponding benzophenones after acid hydrolysis is a comprehensive method to identify and detect benzodiazepines and their metabolites. Maurer and Pfleger [196] reported differentiation of 29 compounds after acetylation and SIM. Inclusion by other authors of some of the more recently introduced compounds, such as alprazolam, midazolam or triazolam [197], showed that the method is still one of the potential approaches which, if appropriate ions are selected for monitoring, afford useful information.

Alkylation is also a potential derivatization method. Thus, introduction of *N*-methyl groups [198] by use of methyl iodide is possible with the participation of strong reagents such as TMAH. Chromatographic and mass spectral properties are improved if simultaneous acylation of hydroxyl groups is carried out (i.e., propionylation with propionyl chloride) at the same time as *N*-propylation with propyl iodide [199].

By far, the most popular derivatization procedure for benzodiazepines is silylation. Classical formation of TMS ethers has been used by many laboratories, usually employing BSTFA, either alone [200,201] or routinely accompanied with TMCS [41,202–206]. Mass spectral characteristics can be improved by the formation of *tert*.-butyldimethylsilyl ether (by using MTBSTFA), because a base peak with 57 Da less than the molecular ion is usually obtained [60–62]. Derivatization may even be carried out directly on a disk containing an extract of urine [207]. Either EI or NCI may be used, as all benzodiazepines bear nitro or halogen substituents. A higher response may be additionally obtained by forming HFB derivatives [208].

4.6. LSD

Lysergic acid diethylamide (LSD) is a potent psychoactive drug that has been extensively abused. Identification and quantitation of LSD in biological specimens is difficult, due to the extremely low doses ingested (usual oral doses: 20–80 μg) and its extensive metabolism. Additionally, the low volatility of the drug, its thermal instability, and its tendency to undergo adsorptive losses during gas chromatographic analysis contribute to the difficulty of developing methods for confirmation of the drug at the subnanogram per milliliter concentrations normally found in body fluids of LSD users. Chromatographic and mass spectrometric methods for the determination of LSD in biological fluids have been reviewed by Nelson and Foltz [209]. GC–MS was the main method used for routine identification of LSD and metabolites in body fluids.

Determination of LSD in urine by GC–MS was first described by Francom et al. [210]. The N-TMS derivative was formed by treatment with BSTFA. Using EI and SIM, a LOD of 0.5 ng/ml was obtained in urine. This LOD was improved by modification of the extraction procedure and by using deuterated internal standards [211]. These authors stress the importance of maintaining a well-conditioned GC column by injecting derivatization reagent or derivatized urine extracts to reduce undesirable adsorptions that can adversely affect detection sensitivity.

An increase in selectivity and sensitivity was obtained by analysis of the TFA derivatives of LSD and *N*-demethyl-LSD using GC–MS-NCI with methane [30]. The formation of perfluoroacyl derivatives of LSD by reaction with TFAA was not efficient. However, derivatization with trifluoroacetyl imidazoles in the presence of a tertiary amine as a catalysts (1,4-dimethylpiperazine) was successful. The mass spectra contained a very intense molecular anion ideal for SIM analysis. Concentrations of 50 and 30 pg/ml of LSD and *N*-demethyl-LSD were reliably measured in urine. The method was applied to the measurement of the drug in human plasma by Papac and Foltz [72] after a modification of the extraction procedure consisting of the incorporation of additional clean-up steps to eliminate the lipid material present in plasma.

Nelson and Foltz [42] described the determination of LSD, iso-LSD and *N*-demethyl-LSD in urine and blood using GC coupled to MS–MS. TMS and TFA derivatives and sensitivity and specificity of different ionization techniques were compared. CI was used because it generated more intense precursor ions than EI. In PCI, TMS derivatives of LSD, iso-LSD and *N*-demethyl-LSD were used and derivatization was accomplished with BSTFA containing 1% TMCS. Mono-TMS derivatives were obtained for all compounds; bis-TMS derivative of *N*-demethyl-LSD was formed as a minor product. PCI primary mass spectra of the TMS derivatives predominantly showed the protonated molecules. The selected ion monitoring of the resulting CID daughter ions provide a high degree of sensitivity and specificity. LODs of 10 pg/ml were obtained for LSD and iso-LSD in urine. In NCI, TFA derivatization was preferred and it was performed with trifluoroacetylimidazole; mono-TFA derivatives were formed, except for *N*-demethyl-LSD where a bis-TFA derivative was obtained. Primary mass spectra of these derivatives showed predominantly a deprotonated molecular anion. SIM of the product ions resulting from CID allowed the detection of concentrations of 10 pg/ml for *N*-demethyl-LSD, while the GC–MS–MS analysis of LSD was considerably less sensitive (500 pg/ml). The higher sensitivity obtained for *N*-demethyl-LSD than for LSD or iso-LSD was probably due to the greater efficiency of the ionization of the bis-TFA derivative compared to the mono-TFA derivatives of the others.

A mixture of TMSIm, BSTFA and TMCS and heating at 90°C for 1 h to form TMS derivatives of LSD and *N*-demethyl-LSD has been used in the analysis of hair samples [212]. Irreproducible formation of bis-TMS derivative of *N*-demethyl-LSD has also been described.

5. Derivatization procedures for GC–MS determination of dope agents

5.1. Anabolic steroids

Some anabolic steroids and many of their metabolites do not exhibit good chromatographic behaviour mainly because of the presence of hydroxyl and keto groups in their structure. Among the multiple reagents described for the derivatization of hydroxyl groups in anabolic steroids, trimethylsilylation has been particularly useful [34,213,214]. MSTFA has been the reagent of choice, although tertiary alcohols are not easily derivatized only with the reagent alone [38]. Considering the fact that many important anabolic steroids bear a tertiary 17β-hydroxy group, the presence of a catalyst is needed to fully derivatize these compounds. TMSIm is a particularly useful catalyst [215]. Many parent compounds and metabolites of anabolic steroids also have carboxyl groups in the molecule. The possibility of forming their TMS derivatives through the enol form is highly useful in order to increase the molecular mass of the derivatives and to avoid background interferences. The possibility of forming silyl groups for enolic forms is already known by using of potassium acetate [216] or TMSI [53] as catalysts. Recently, the use of ammonium iodide with MSTFA to generate in situ TMSI has been recommended [54]. Addition of reduction agents such as dithioerythritol, ethanethiol or 2-mercaptoethanol minimizes the formation of iodine. In fact, comprehensive methods involving MSTFA plus NH_4I plus reduction agent have been described by several authors [217–221]. Control of the successful derivatization of keto groups may be easily accomplished by monitoring the derivatization of endogenous compounds such as androsterone, present in high amounts in biological samples: if derivatization is successful, detection of the bis-TMS derivative (m/z 434) must be ascertained rather than detection of the mono-TMS derivative (monitoring m/z 272). When analyzing TMS derivatives of anabolic steroids, a careful derivatization of glass liner in the injection port can dramatically affect the chromatographic behavior of some compounds [222]. Some characteristics of the per-TMS spectra of anabolic steroids are the presence of ions corresponding to losses of 90 amu (TMS-OH) as well as the presence of unspecific ions at m/z 73 (TMS) or m/z 147 (2TMS). As in many silyl derivatives, $[M-15]^+$ is a usual peak.

When higher increases in masses are desirable, *tert*.-butylsilyl derivatives can be of use. Applications to testosterone [223], dehydroepiandrosterone [224] or estradiol [225] have been known. In these cases, the loss of fragments of 57 Da is characteris-

tic. With *tert*.-butylsilyl derivatives, each substitution adds 114 amu to the molecular mass, which can compromise the applicability with benchtop instruments having a limited mass range [21]. Also, the derivatization of sterically hindered groups in general and tertiary alcohols in particular is very difficult for these kinds of derivatives. As with TMS, enolization of ketones may be accomplished by using the corresponding alkyl iodosilane (*tert*.-butyldimethyliodo silane) as a catalyst [226]. The compounds thus formed have intense molecular ions. The formation of *tert*.-butylsilyl derivatives has recently been used to analyze various metabolites of methandienone by medium resolution MS, to avoid coelution of the standard TMS derivative of 18-nor-17,17-dimethyl-5-β-androst-1,13-dien-3-α-ol with stearic acid [227]. Alternatively, the use of a higher resolution (ca. 10 000) easily allows such a separation [228].

In some instances, the use of catalysts for enolization of the keto groups needs to be avoided because of serious side reaction such as in the case of trenbolone [229] (a molecule with three double bonds conjugated with the keto group). Alternatively, the protection of the keto group with the formation of alkyloximes can be of use [230–232]. In fact, methyloxime formation and trimethylsilation of hydroxyl groups are used in many confirmatory steps in the analysis of anabolic steroids [233]. Similarly, the formation of hydrazones may be used to isolate anabolic steroids containing the keto group. If the hydrazone is water soluble (i.e., by use of the Girard reagent), its removal by liquid extraction may be used to concentrate only these hydroxyl containing metabolites in the organic phase. Such an approach has been used [234] to prepare pure samples for GC/combustion/isotopic ratio MS in the confirmation of testosterone ingestion. Final acetylation of hydroxy groups renders suitable compounds for $^{13}C/^{12}C$ isotope ratio measurement without much carbon dilution due to derivatization [235].

The use of acyl derivatives is also interesting for some specific anabolic steroids and metabolites containing nitrogen, such as stanozolol. For this compound and its metabolites, bearing a pirazol ring, the formation of tri-, penta- or heptafluoro amides in conjunction with silylation of hydroxyl groups gives stable metabolites [236]. Fluorinated acyl derivatives have also been used for testosterone [237], 17-α-estradiol [238], progesterone [239] and aldosterone [240].

An interesting application of either TMS or perfluoroacyl derivatives has been developed for testosterone esters and applied to blood plasma samples [241]. The direct detection of esters in blood may be one of the best possibilities for the demonstration of testosterone administration in doping control. Thus, enolyzed-mono TMS derivatives of nine different testosterone esters show a base peak corresponding to the molecular ion, which offers low LOD either in GC–MS with SIM or in GC–MS–MS [242]. Alternatively, the formation of enolyzed TFA, PFP or HFB derivatives [241] offers multiple possibilities for structural confirmation and for detection by NCI.

5.2. Diuretics

GC–MS using EI has been widely used to analyze diuretic compounds in biological samples [87]. The direct analysis of substances with diuretic activity by GC is not possible due to the polar nature of the functional groups present in the structure of most of these compounds. Methylation is the derivatization procedure commonly used to analyze diuretics. Three main methylation procedures have been proposed: extractive methylation, pyrolytic methylation and methylation with methyl iodide in acetone. Although some earlier derivatization procedures have been described for GC detection methods such as electron-capture detection or flame ionization detection, they are included in this review because they can also be applied to MS detection.

The same methyl derivatives have been obtained using these procedures with the exception of pyrolytic methylation where tetramethyl derivatives for bumetanide and furosemide have been described [101,243] instead of the trimethyl derivatives normally obtained. In general, mass spectra of high diagnostic value are obtained using EI-MS, and three ions monitoring for each compound is normally used for screening purposes (Table 5). Mass spectra with less fragment ions than in EI conditions, have been obtained for several methylated diuretics using NCI [244,245]. GC–MS using NCI has been employed to analyze the methyl derivative of furosemide, thus allowing for a significative LOD improvement [246].

Table 5
Methyl derivatives obtained for different diuretics using methylation with methyl iodide in acetone, diagnostic ions used in SIM analyses for screening purposes (base ions indicated in italics), and retention times (t_R) and retention times relative to 7-propyltheophylline (t_{RR}) [89]

Compound	Derivative	Diagnostic m/z	t_R (min)	t_{RR}
Acetazolamide	Trimethyl	108, *249*, 264	3.7	1.16
Etacrynic acid	Monomethyl	243, *261*, 316	4.5	1.40
Diclofenamide	Tetramethyl	44, *253*, 360	5.7	1.79
Furosemide	Trimethyl	*81*, 96, 372	7.1	2.22
Chlortalidone	Tetramethyl	176, *287*, 363	7.4	2.32
Bumetanide	Trimethyl	254, 363, *406*	7.5	2.37
Piretanide	Trimethyl	266, *295*, 404	7.8	2.48
Hydrochlorothiazide	Tetramethyl	288, *310*, 353	7.9	2.50
Triamterene	Hexamethyl	307, 322, *336*	8.1	2.54
Canrenone	Underivatized	267, 325, *340*	9.4	2.97
Bendroflumethiazide	Tetramethyl	91, 278, *386*	9.6	3.03

Extractive methylations applied to an aqueous extract of the biological sample and optimized for the detection of particular sulfonamide diuretics have been proposed by different authors [247–253]. Procedures applied directly to the urine sample and allowing the detection of a wide group of diuretic compounds have been described by Fagerlund et al. [254] and Lisi et al. [31,32]. Using methyl iodide in toluene as the methylation reagent and reaction at room temperature, higher derivatization efficacies have been obtained when the hydrophilic nature of the phase-transfer reagent was decreased [31]. The elimination of the phase-transfer reagent before GC analysis has been performed in three ways: by evaporating the organic extract and redissolving the derivatives in a non-polar solvent such as cyclohexane, hexane or mixtures of toluene and hexane [249–251,255]; by washing the organic phase with a saturated silver sulfate solution [31]; or by extracting the organic phase using a solid-phase procedure with a macroreticular acrylic copolymer [32].

Pyrolytic methylation has also been used to derivatize acidic diuretics [102,243,256–259]. The residue obtained from the biological matrix after liquid–liquid extraction of the compounds was dissolved in the methylation reagent (trimethylanilinium hydroxide, TMAH or a mixture of both) and the solution was injected into the gas chromatograph.

Methylation with methyl iodide and dry potassium carbonate allows the methylation of amine functions, such as those of triamterene, in addition to carboxylic acids, sulfonamides and alcohols. For compounds with only carboxylic acid functions methylation can occur without incubation; however, long incubations of the reaction mixture at 60°C are required to derivatize diuretics with sulfonamide or amino groups [28,89,246,260–264].

Comparison of these methylation procedures for the analysis of diuretics in urine revealed that methylation with methyl iodide in acetone is the best compromise for screening purposes due to the fact that it derivatizes a large number of compounds [265]. Extractive and pyrolytic methylation were found to be faster and more effective for some particular compounds and their application for confirmation purposes was suggested.

Other derivatization procedures have been described for particular compounds. Methylation with methanol and hydrochloric acid as a catalyst [91], silylation with BSTFA [266], and reaction with pentafluorobenzyl bromide [267] have been used to analyze etacrynic acid in plasma or urine. Pentafluorobenzyl derivative of etacrynic acid was analyzed by GC–MS under CI conditions [267]. TMS derivatives of amiloride have been formed using methanolysis followed by silylation with MSTFA [268] or using MSTFA alone [89].

5.3. Corticosteroids

As a result of the low therapeutic doses and extensive metabolism, the analysis of corticosteroids in biological samples is difficult due to the low concentrations expected. The direct analysis of corticosteroids by GC–MS is unsuitable owing to the thermal instability of the dihydroxy acetone side

chain at C_{17} which is lost to yield the corresponding 17-oxo steroid. Different derivatization procedures have been proposed for the analysis of natural and synthetic corticosteroids in biological fluids by GC–MS. Trimethylsilylation [43,269–272] and methoximation followed by trimethylsilylation [44–52,273,274] are the most widely employed.

Complete trimethylsilylation of all oxygen functions, including ketone and hydroxyl groups, of triamcinolone, prednisolone, cortisol, corticosterone, dexamethasone and betamethasone has been achieved using a mixture of reagents BSA, TMSIm and TMCS [269]. Only dexamethasone showed incomplete reaction and a mixture of tetra and penta-TMS derivatives was obtained probably because the 16-methyl group in the *cis* position relative to the 17-hydroxyl group is a steric hindrance to the TMS group.

Tetra-TMS derivative of dexamethasone has been formed by reaction with BSTFA or *N*,*O*-bis-trimethylsilylacetamide (BSA) in the presence of a base catalyst such as potassium acetate or sodium acetate [270,271]. The C_{20} ketone group was converted to an enol ether, and all hydroxyl groups were converted to TMS ether groups; no derivatization of the ketone group at the C_3 position was obtained. A single product was obtained, and the tetra-TMS derivative showed good thermal stability and GC behaviour. An intense molecular ion with other fragment ions in the high mass region adequate for identification purposes has been obtained in EI. PCI with methane was found to be more sensitive than EI for the tetra-TMS derivative of dexamethasone.

The tri-TMS derivative of dexamethasone and flumethasone has been formed under soft derivatization conditions consisting of reaction with TMSIm in pyridine and using formamide as a base catalyst [43]. A single product was obtained for each compound as a result of the derivatization of the hydroxyl groups, with good GC behaviour. A highly sensitive analysis was obtained in the SIM mode by using NCI with methane.

Formation of methoxime derivatives of the ketone functions, followed by silylation of the hydroxyl groups, has been extensively employed. Analysis of cortisol and metabolites in urine was performed by oxidation of the analytes to a common product, 11-oxo-aetiocholanolone, with sodium bismuthate in aqueous acetic acid and subsequent conversion of this product to methyloxime TBMS ether derivative by reaction with methoxyamine hydrochloride in pyridine, and *tert*.-butyldimethylchlorosilane/imidazole [44].

Isotope dilution MS has been used for the quantitative determination of cortisol in human plasma [50,52] as dimethoxime-tri-TMS derivative. The cortisol derivatives were obtained by reaction with methoxyamine hydrochloride in pyridine and posterior sylilation with BSA.

Formation of methoxime TMS ether derivatives of natural corticosteroids (cortisol, cortisone, tetrahydrocortisol and tetrahydrocortisone) by reaction with methoxyamine hydrochloride in pyridine and posterior sylilation with a mixture of MSTFA and TMSIm has been employed to analyze these compounds using bench-top GC–MS under EI conditions [274]. EI mass spectra of suitable diagnostic value have been obtained.

Reaction with methoxyamine hydrochloride in pyridine and posterior silylation with TMSIm has also been used to form methoxime-TMS derivatives of methylprednisolone, fluorometholone, betamethasone, prednisone, prednisolone and their metabolites in a series of studies on corticosteroids metabolism [45–48] and for quantitative determination of synthetic corticosteroids (prednisolone, dexamethasone and betamethasone) using isotope dilution GC–MS under NCI conditions [49]. NCI spectra presented higher abundance of diagnostic ions than EI or PCI. NCI mass spectra of methoxime-TMS derivatives of dexamethasone and betamethasone showed abundant diagnostic ions in the high mass region, although molecular anions were not present [51].

Formation of methoxime-TMS derivatives of prednisone, prednisolone, their metabolites and endogenous steroids by reaction with methoxyamine hydrochloride in pyridine and posterior sylilation with BSTFA has been used to screen for these compounds in horse plasma and urine [273]. In general, CI-MS was more sensitive and yielded mass spectra of higher diagnostic value than EI-MS.

Chemical oxidation to the 1,4-androstadiene-3,11,17-trione analogue has been described to determine synthetic corticosteroids, such as dexamethasone, in biological samples using GC–MS under NCI conditions [275,276]. Chemical oxidation trans-

forms dexamethasone to a highly electrophilic species while not significantly affecting the electrophilic character of the biological matrix and, thereby, allowing highly sensitive and selective analyses. Optimization of the oxidation conditions, resulting in a more simple and robust procedure, was achieved by Courtheyn et al. [277] in order to determine dexamethasone in urine and faeces of treated cattle.

Direct GC analysis of dexamethasone and flumethasone was performed using a cool on-column injection port after immunoaffinity chromatographic extraction [278]. Under NCI conditions, the method was able to detect the analyte in equine urine at the subnanogram per milliliter levels required for confirmation purposes. A modification of the procedure including chemical oxidation was also described [279].

Formation of the bismethylenedioxy-3-heptafluoro-*n*-butyryl derivatives of cortisol, cortisone, prednisolone and prednisone by reaction with *p*-formaldehyde in acidic medium and subsequent acylation of the ketone in C_3 position with HFBA has been used to determine these compounds in plasma samples using isotope dilution MS [280].

6. Future perspectives

Extensive developments in derivatization procedures, resulting in significant advances, have been made in the last few decades. Therefore, many new derivatization reagents are not expected to be developed in the years to come although extensive studies of derivatization conditions and further applications of those already existing will undoubtedly take place. As simpler and robust methods are eligible, those developments aiming to reduce the number of variables influencing derivatization performance and the reduction of side effects will be preferred.

Suitable derivatives of drugs and toxic agents to be analyzed by tandem MS (MS–MS), HRMS or NCI will need to be further addressed. Efforts will be directed to obtain compounds containing more electron capturing atoms (i.e., halogen) and with stable molecular or high m/z ions.

Given the developments in the field of molecular biology, toxicology is moving towards the study of high molecular mass toxic agents, mainly peptides and proteins. In this regard, totally new approaches to the study of these molecules will be needed. When using MS, these efforts will be focused mainly on connection between HPLC or CE and the mass analyzer. In this regard, GC–MS may clearly be displaced by separation techniques using liquid mobile phases.

Nevertheless, some areas of study of low-molecular-mass molecules will probably suffer a renewed interest which will make derivatization developments necessary. Clear differentiation between enantiomers of pharmacologically active drugs will promote development in chiral separations, some of them by GC–MS. Also, the power of some reagents to form stable cyclic derivatives with polyfunctional polar metabolites will need to continue progressing for metabolic and toxicokinetic studies.

In spite of the potential growth of LC–MS or CE–MS developments, economic reasons will foment the increasing use of GC–MS systems for many toxicological applications. In fact, the cost of quadrupole or ion trap benchtop systems will result in routine application of GC–MS in situations where up to now only GC with other detectors was being used. This will generate a renewed interest in derivatization to obtain suitable mass fragmentations and identifications. An expected development which will undoubtedly influence to a large extent the possibilities of expansion of derivatization for GC–MS, reference libraries containing a wide range of derivative types will have to be made available. As has been described in this chapter, some paper-based or computer formatted libraries of mass spectral data already exist, but they include a limited number of derivatives (acetyl, silyl and few others; and only for a limited number of drugs). The degree of expansion of these limited data sets to wider collections of derivatives of drugs and toxic agents [281] will be of paramount importance in promoting further developments in derivatization for GC–MS.

7. List of abbreviations

AA	Acetic anhydride
BE	Benzoylecgonine

BSA	*N*,*O*-bis(Trimethylsilyl)acetamide
BSTFA	*N*,*O*-bis(Trimethylsilyl)trifluoroacetamide
BTFA	bis(Trifluoroacetamide)
CE	Capillary electrophoresis
CI	Chemical ionization
CID	Collision induced dissociation
EBE	Ethylbenzoylecgonine
EI	Electron-impact ionization
EME	Ecgonine methyl ester
GC	Gas chromatography
HFB	Heptafluorobutyryl
HFBA	Heptafluorobutyric anhydride
HFIP	1,1,1,3,3,3-Hexafluoroisopropanol
HRMS	High-resolution mass spectrometry
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
LSD	Lysergic acid diethylamide
6-MAM	6-Monoacetylmorphine
MBTFA	*N*-Methyl-bis-trifluoroacetamide
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxyethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MS	Mass spectrometry
MS–MS	Tandem mass spectrometry
MSTFA	*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide
MTBSTFA	*N*-Methyl-*N*-*tert*.-butyldimethylsilyltrifluoroacetamide
NCI	Negative chemical ionization
PCI	Positive chemical ionization
PFP	Pentafluoropropionyl
PFPA	Pentafluoropropionic anhydride
PFPOH	2,2,3,3,3-Pentafluoro-1-propanol
PAA	Propionic acid anhydride
RIA	Radioimmunoassay
SIM	Selected ion monitoring
TBAH	Tetrabutylammonium hydroxide
TBDMS	*tert*.-Butyldimethylsilyl
TFA	Trifluoroacetyl
TFAA	Trifluoroacetic anhydride
TFE	Trifluoroethanol
THC	Δ^9-Tetrahydrocannabinol
THC-COOH	11-nor-Δ^9-Tetrahydrocannabinol-9-carboxylic acid
THC-OH	11-Hydroxy-Δ^9-tetrahydrocannabinol
TMAH	Tetramethylammonium hydroxide
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TMSI	Trimethylsilyl iodide
TMSIm	Trimethylsilylimidazole

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