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Review

Gas chromatographic–mass spectrometric methods of analysis for detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in biological matrices☆

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ABSTRACT

Gas chromatographic–mass spectrometric methods of analysis for the detection of 11-nor-Δ^9-tetrahydrocinnabinol-9-carboxylic acid, a major metabolite of Δ^9-tetrahydrocannabinol, are reviewed. Emphasis is on analytical methodology including numerous derivatization techniques developed specifically for this analyte. The majority of procedures cited in the literature were developed to detect this metabolite in the blood and urine of man.

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☆ The opinions or assertions contained herein are the private views of the authors and are not to be construed as being official or reflecting the views of the Department of the Air Force or the Department of Defense.

LIST OF ABBREVIATIONS

BSA	N,O-Bis(trimethylsilyl)acetamide
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
CAS	Chemical Abstracts Service
CI-MS	Chemical ionization mass spectrometry
EI-MS	Electron-impact ionization mass spectrometry
GC–MS	Gas chromatography–mass spectrometry
HPLC	High-performance liquid chromatography
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
NICI	Negative-ion chemical ionization
PICI	Positive-ion chemical ionization
RIA	Radioimmunoassay
8-THC	Δ^8-Tetrahydrocannabinol
9-THC	Δ^9-Tetrahydrocannabinol
8-THC-acid	11-Nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid
9-THC-acid	11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid
9-THC-acid-glucuronide	11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-O-β-D-glucuronide
TLC	Thin-layer chromatography

1. INTRODUCTION

Δ^9-Tetrahydrocannabinol (9-THC) is a psychoactive drug found as an ingredient in marijuana (marihuana), hashish, and related drug preparations. It is extensively metabolized in man, one of its major metabolites being 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (9-THC-acid). Trace levels of this metabolite can be detected and conclusively identified only by using a method of analysis possessing high specificity and low detection limits. Commercial immunoassays including radioimmunoassay (RIA), enzyme-multiplied immunoassay technique, and fluorescence polarization immunoassay are rapid and inexpensive, but are subject to chemical interferences. Cannabinoid compounds of related structure are cross-reactive while high concentrations of unrelated substances can adversely affect assay accuracy. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are capable of low detection limits without analyte derivatization. However, the most commonly used detection methods (chemical visualization for TLC, ultraviolet absorption or electrochemical detection for HPLC) are only moderately specific. In contrast, the highly characteristic spectra obtained by gas chromatography–mass spectrometry (GC–MS) practically eliminate the possibility of misidentification.

This chapter presents a review of GC–MS methods for the detection of 9-THC-acid in bi-

ological matrices, primarily in the blood and urine of man. Interested readers are referred to earlier reviews of this subject [1,2] and to more general reviews [3–7].

2. OCCURRENCE AND METABOLISM

9-THC occurs naturally only in plants belonging to the genus *Cannabis*. It is the primary psychoactive ingredient found in *Cannabis*, although numerous structurally similar compounds have also been identified. Drug preparations made from the plant material are generally smoked, but ingestion can also induce drug effects. The purified drug can also be administered intravenously. In man 9-THC is extensively metabolized and subsequently excreted primarily via the feces and urine.

9-THC-acid, the primary urinary metabolite, also appears in the blood and feces of man. In urine 9-THC-acid may be extensively conjugated as its ester glucuronide, although this is highly variable. Conjugation at the phenol position apparently does not occur. In the blood and feces 9-THC-acid exists largely unconjugated. The metabolic conversion of 9-THC to 9-THC-acid has also been observed in numerous animal studies.

This established metabolic pattern provides a basis for detecting use of the drug. The appearance of the 9-THC-acid metabolite in blood or urine is considered conclusive evidence of *Cannabis* use. Detection in other biological specimens is less common. The occurrence of 9-THC-acid in milk has been confirmed by GC–MS in two publications [8,9], and a recent report of 9-THC-acid in human hair must also be attributed to metabolism of 9-THC [10]. Evidence regarding the appearance of 9-THC-acid in saliva is inconclusive [11–13] and awaits further developments.

3. STRUCTURE AND NOMENCLATURE

Due to the presence of two chiral centers in its molecular structure 9-THC can potentially exist as any of four possible stereoisomers. The naturally existing configuration is the (3*R*,4*R*)-*trans* isomer whose absolute confirguration was established in 1967 by Mechoulam and Gaoni [14]. The structure of this molecule is shown in Fig. 1. Earlier works [15–18] and even more current publications [19–21] have occasionally shown 9-THC incorrectly as the (3*S*,4*S*)-*trans* isomer. Two metabolic products of 9-THC, 9-THC-acid and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-O-β-D-glucuronide (9-THC-acid-glucuronide), possess the structures shown in Figs. 2 and 3 [22], respectively.

Differing systems of nomenclature and varied names within systems have been used to name the cannabinoids. Harvey [23] stated that five and Ohlsson [24] that four nomenclature systems have been used for cannabinoid compounds, two of these (the dibenzopyran numbering adopted by Chemical Abstracts and the monoterpene numbering) being in current use. Three numbering systems are shown in the work of Thornton and Nakamura [20]. A few references are noted in which dibenzopyran and monoterpene numbering is shown [3,7,21,25,26]. Review of the literature is further complicated by the use of multiple Chemical Abstract Service (CAS) registry numbers for 9-THC-acid depending upon the nomenclature used by authors.

Commonly used names for the acid metabolite of 9-THC include 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (used in this work), 9-carboxy-11-nor-Δ^9-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol-11-oic acid and Δ^1-tetrahydrocannabinol-7-oic acid. The chemical name 6a*R*-*trans*-6a,7,8,10a-tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo[*b*,*d*]pyran-9-carboxylic acid was cited in one publication [1].

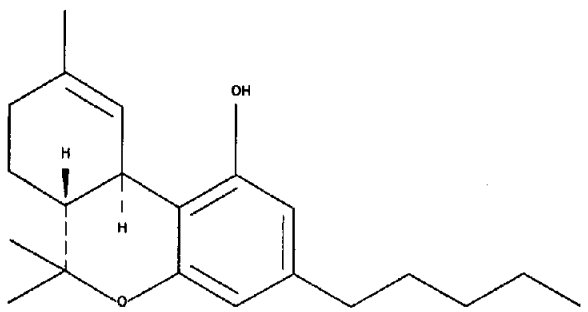

Fig. 1. Structure of (3*R*,4*R*)-Δ^9-tetrahydrocannabinol [14].

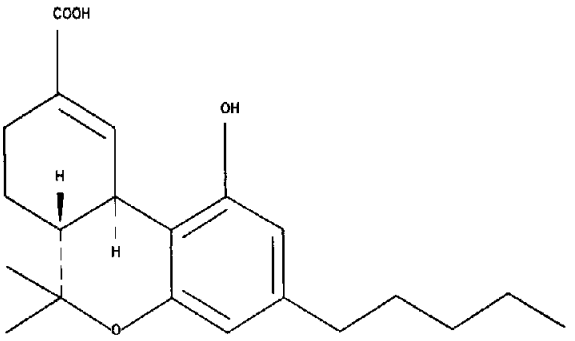

Fig. 2. Structure of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid.

9-THC and 9-THC-acid are often designated as levorotatory compounds, but this is inappropriate without reference to experimental conditions.

4. ANALYTICAL METHODS

Preparation of a biological sample containing 9-THC-acid requires efficient and selective extraction of the analyte from its matrix. Blood, urine and other body fluids are known to contain endogenous material which can adversely affect recovery and also contribute significant "chemical noise" to chromatograms when co-extracted. In many cases assays developed with pure solutions fail to give acceptable results when attempted on real specimens.

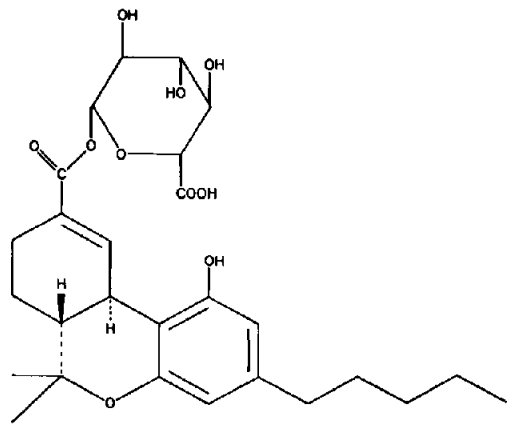

Fig. 3. Structure of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-O-β-D-glucuronide [22].

Following extraction, derivatization of the carboxyl moiety of 9-THC-acid is needed prior to GC–MS analysis. Although the parent drug 9-THC is amenable to direct analysis by GC–MS, the higher polarity of 9-THC-acid, together with the tendency of carboxylic acids to decarboxylate at high temperatures, necessitates preparation of derivatives prior to GC. The choice of derivative will, of course, affect the separation of 9-THC-acid from other compounds. Derivatization can also result in improved mass spectral properties, either in terms of creating a greater number of characteristic ions or reducing detection levels. In some cases discrimination against interferences has been achieved by choosing reagents which selectively derivatize one functional group in the 9-THC-acid molecule.

Significant levels of 9-THC-acid in urine may exists as the glucuronide conjugate. Urine specimens, therefore, are routinely hydrolyzed to form free 9-THC-acid prior to extraction. In some cases blood specimens have also been hydrolyzed. Hydrolysis can be effected using either β-glucuronidase or alkaline conditions, although basic hydrolysis is the simpler technique.

4.1. Extraction techniques

The extraction of 9-THC-acid from blood or urine can be accomplished with either liquid–liquid or solid-phase extraction. Blood samples are often treated prior to extraction in order to precipitate interferences. Solid-phase extraction reduces the quantity of chemical waste generated but does have its drawbacks. Many solid-phase cartridges draw solvent by application of vacuum, a method which can make the rate of solvent flow difficult to control. Batch-to-batch variations in the manufacturing process can sometimes cause reproducibility problems for the laboratory. Moisture-sensitive derivatization methods may require special procedures to dry column beds.

The existence of two acidic functionalities on 9-THC-acid makes anion exchange a popular choice in extraction columns. Endogenous acids, however, may be difficult to remove. Reversed-

phase columns have also been employed and many suppliers sell solid-phase columns proprietary in nature. The antibody technique described by Lemm *et al.* [27] is an interesting attempt to obtain highly purified isolates but has not been pursued commercially.

Although many possible solvents and solvent combinations are possible for performing liquid–liquid extractions of 9-THC-acid, not many were found in the review of the literature. Hexane modified with ethyl acetate (typically 7:1) has been reported most often, while some investigators have used diethyl ether.

A common purification process for liquid–liquid extraction of 9-THC-acid involves extraction into organic solvent, extraction into aqueous base, acidification, followed by extraction into organic solvent. A simpler procedure is to adjust the aqueous matrix to a high pH, extract to waste, then extract 9-THC-acid into organic solvent at a low pH. As with anion-exchange column methods, however, endogenous acids may also co-extract.

4.2. Derivatization methods

A number of derivatization techniques for GC–MS detection of 9-THC-acid in blood (whole blood, plasma or serum) and urine have been developed and reported in the literature. In cases where two different reagents are used to derivatize 9-THC-acid it is possible to include a clean-up procedure between reaction steps. Individuals with access to mass spectrometers capable of negative-ion analysis will find methods developed specifically for that purpose.

4.2.1. Esterification–alkylation (formation of alkyl ester–alkyl ethers)

Since first developed by Whiting and Manders in 1982 as a method suitable for GC with flame ionization detection [28] and later modified for GC MS [29], reaction of 9-THC-acid with iodomethane to form the methyl ester–methyl ether derivative has been widely used. The original GC method employed base hydrolysis, extraction from acidified urine into hexane–ethyl acetate (7:1), extraction into aqueous base, extraction into hexane–ethyl acetate (7:1), derivatization with iodomethane using tetramethylammonium hydroxide catalyst, and extraction into iso-octane. The back-extraction was needed to eliminate interferences from compounds endogenous to urine. The researchers were unable to determine the efficacy of the hydrolysis step due to lack of a glucuronide standard. The method was modified for GC–MS by eliminating the back-extraction step [29]. The methyl ester/methyl ether derivative yielded three characteristic high mass fragments by electron-impact ionization (EI) and two by methane positive-ion chemical ionization (PI-CI).

Many other researchers have used this procedure with minor modifications including solid-phase extraction and application to blood, plasma or serum [30–49].

Although Baker *et al.* [49] found the methyl ester–methyl ether derivative of 9-THC-acid gave a lower GC–EI-MS response than either the methyl ester–trimethylsilyl ether or trimethylsilyl ester–trimethylsilyl ether derivatives, Nakamura *et al.* [41] found the trimethylsilyl ester–trimethylsilyl ether no more sensitive than the methyl ester–methyl ether.

Lemm *et al.* [27] achieved lower detection limits for GC–EI-MS detection of the methyl ester–methyl ether using an antibody extraction procedure to achieve a highly pure urine extract.

McCurdy *et al.* [50] compared iodomethane, iodoethane, 1-iodopropane and 1-iodobutane as derivatizing reagents for 9-THC-acid and recommended the propyl derivative based upon favorable retention time and separation from possible interferences. Mulé and Casella [51] also chose the propyl derivative for analysis, while Childs and McCurdy [52] chose the ethyl derivative.

Wall *et al.* [53] used dimethyl sulfate to methylate 9-THC-acid in plasma and urine extracts following purification with HPLC. GC–EI-MS confirmed the presence of 9-THC-acid in the plasma and urine of man following intravenous administration of 9-THC.

Björkman [54] produced the methyl ester–methyl ether of 9-THC-acid on-column using di-

methylformamide dimethylacetal, while Nakamura *et al.* [55] reported on-column methylation using 4:1 methanol–10% tetramethylammonium hydroxide in methanol.

A quite different method of preparing alkyl derivatives has been reported by Rosenfeld and coworkers in two publications [56,57]. Following extraction of 9-THC-acid from acidified plasma onto XAD-2 resin (a polystyrene–divinylbenzene copolymer) the resin was treated with pentafluorobenzyl bromide [56]. The recovered derivative was identified as the pentafluorobenzyl ester–pentafluorobenzyl ether. A 300 pg/ml limit of quantitation was reported for GC–MS analysis utilizing methane–hydrogen negative-ion chemical ionization (NICI). In a later publication employing GC–EI-MS the position of alkylation was found to depend upon reaction conditions [57]. From urine extracts containing 9-THC-acid it was possible to prepare both mono-pentafluorobenzyl derivatives in addition to the pentafluorobenzyl ester–pentafluorobenzyl ether previously reported.

The formation of alkyl ester–alkyl ethers upon reaction of 9-THC-acid with alkyl halides is shown in Fig. 4A.

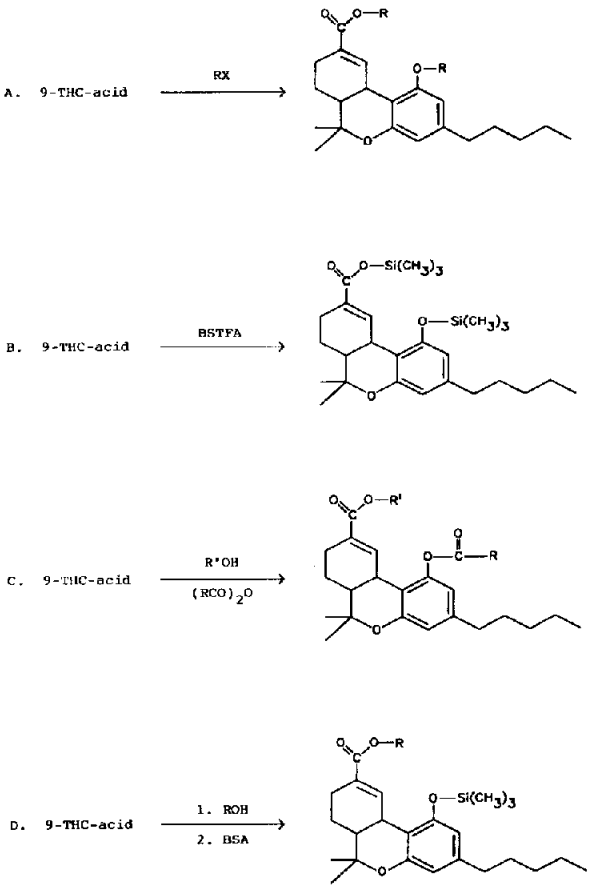

Fig. 4. Representative reactions showing (A) alkylation, (B) silylation, (C) esterification–acylation and (D) esterification–silylation of 9-THC-acid.

4.2.2. Silylation (formation of silyl ester–silyl ethers)

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)acetamide (BSA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) are the most popular silylating reagents for the derivatization of 9-THC-acid when using GC MS detection [9,50,51,58–81]. Reactions employing these reagents often include 1% trimethylchlorosilane as a catalyst. The trimethylsilyl ester–trimethylsilyl ether derivative formed (see Fig. 4B) displays three characteristic high-mass ions under EI conditions. MSTFA, BSA and BSTFA are compatible with GC injectors and columns, and reaction products are typically injected directly into the GC column with no clean-up. Trimethylsilyl derivatives do have the disadvantage of being moisture sensitive. Although longer-chain silylating reagents (trimethyl, triethyl, tripropyl, tributyl and trihexyl) have been used with other cannabinoids [82,83], application to 9-THC-acid has not been extensively pursued.

Urine extracts treated with BSTFA were used by Green *et al.* [84] to study urinary excretion of 9-THC-acid in man following oral administration. The authors reported approximately half the 9-THC-acid present to be non-conjugated.

An improved reaction yield for MSTFA derivatization of 9-THC-acid was reported by Congost *et al.* [64] using the reaction mixture MSTFA trimethyliodosilane–dithioerythritol (1000:2:10).

Harvey and co-workers [69,80,81] employed BSTFA and [$^2H_{18}$]BSA in several studies of cannabinoid metabolism requiring structural identification. The metabolism of non-natural (3*S*,4*S*)-9-THC was studied in the mouse, and

(3*S*,4*S*)-9-THC-acid was identified in the liver [80]. 9-THC-acid was identified in guinea pig liver [81] and rabbit liver [69].

Ahmad and Ahmad [9] used GC–EI-MS to identify 9-THC-acid in buffalo milk and children's urine following derivatization with BSA.

Bourquin and Brenneisen [85] used N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide with 1% *tert.*-butyldimethylsilyl chloride to create a derivative more stable toward hydrolysis. Four high-mass ions were observed in the EI mass spectrum.

Williams and Moffat [22] were able to identify and chromatograph (GC) the glucuronide conjugate of 9-THC-acid obtained from human urine by reacting 9-THC-acid-glucuronide with BSTFA. The parent ion *m/z* value of 880.4268 measured by high-resolution MS agreed well with the theoretical value expected for 9-THC-acid-glucuronide-$(Si(CH_3)_3)_5$.

Methane–ammonia PICI-MS was used by Foltz and co-workers [1,86] to detect 9-THC-acid in blood, plasma and urine following extraction and treatment with BSTFA and in urine following HPLC and treatment with BSTFA. A hydrogen–ammonia PICI mass spectrum was also obtained [66].

4.2.3. Esterification–acylation (formation of alkyl ester–alkyl esters)

Two methods have been reported for the conversion of 9-THC-acid to alkyl ester–alkyl ester derivatives. The first of these employs fluorinated alcohols in conjunction with fluorinated anhydrides (see Fig. 4C). Some researchers have applied these sequentially to effect derivatization, but it is also possible to mix the reagents and use them in combination in a single-step derivatization. An alternative method is to sequentially methylate the carboxylic acid (using diazomethane or methanol–BF_3) to its ester and acylate the phenol by treatment with an anhydride. The variety of fluorinated chemicals suitable as derivatization reagents facilitates development of negative-ion MS methods.

Joern [87] reported a procedure by which urine extracts containing 9-THC-acid were derivatized with pentafluoropropionic acid–2,2,3,3,3-pentafluoro-1-propanol and detected by GC–EI-MS. O'Connor and Rejent [71] used a similar method in which pentafluoropropionic anhydride–hexafluoroisopropanol were used as derivatization reagents.

A comparison of EI, methane PICI and methane NICI GC–MS was made by Karlsson *et al.* [88]. Following extraction of 9-THC-acid from urine and derivatization with pentafluoropropionic anhydride–pentafluoropropanol sensitivities of the three ionization methods were compared. NICI was by far the most sensitive method. However, background peaks from the urine limited the detection limit to approximately 700 pg/ml. In a later publication Karlsson and Roos [89] employed the same GC–NICI-MS method for detecting 9-THC-acid in urine.

Bergman *et al.* [58] reported problems developing an assay designed to detect 9-THC-acid in blood specimens. Derivatization with pentafluoropropionic anhydride–1,1,1,3,3,3-hexafluoroisopropanol followed by GC–EI-MS was unsuccessful due to interference from blood constituents. McBurney *et al.* [90], however, used the same reagents to successfully develop a GC–EI-MS method for 9-THC-acid in plasma and blood. A NICI procedure for urine specimens using this same derivative was reported by Foltz and Sunshine [91].

An interesting application of the anhydride–alcohol reagent mixture has been reported by Hayes *et al.* [10]. Hair extracts derivatized with heptafluorobutyric anhydride–hexafluoroisopropanol were analyzed for 9-THC-acid by GC MS MS using ammonia NICI. A detection limit of 20 pg/g of hair was reported. This study is consistent with a previous report of 9-THC in hair [92].

Cano and Lykissa [93] treated 9-THC-acid in serum extracts with methanol–BF_3 followed by trifluoroacetic anhydride. GC–EI-MS analysis of the methyl ester–trifluoroacetate derivative resulted in higher-mass fragments than could be achieved by silylation.

Foltz *et al.* [94] developed a methane–hydrogen NICI GC–MS method for detection of 9-

THC-acid in whole blood, plasma, serum and urine. After derivatization of the extract with methanol–BF_3 the residue was treated with trifluoroacetic anhydride. The assay had a reported limit for reliable measurement of 100 pg/ml. The authors noted that trifluoroacetate derivatives have the drawback of being easily hydrolyzed. A later publication compared the mass spectra of this derivative obtained by EI, methane PICI, ammonia PICI and methane NICI [2]. The same method of analysis was applied to blood and plasma specimens by Johnson *et al.* [95] and to blood by Hughes and Hoey [96]. The latter investigators achieved an impressive 50:1 signal-to-noise ratio with only 200 pg/ml 9-THC-acid. Blackard and Tennes [97] reported detection of 9-THC-acid in fetal cord blood with this same procedure.

4.2.4. Esterification–silylation (formation of alkyl ester–silyl ethers)

Several authors have reported 9-THC-acid derivatization procedures employing methylation followed by silylation (see Fig. 4D). Diazomethane or methanol (with acid catalyst) have alternatively been used to esterify the carboxylic acid functionality.

Baker *et al.* [49] treated 9-THC-acid in urine extracts with diazomethane followed by either MSTFA or BSTFA. Although the mass spectrometer response (using EI) of the resulting methyl ester–trimethylsilyl ether was greater than the methyl ester–methyl ether and trimethylsilyl ester–trimethylsilyl ether derivatives, the procedure was rejected for safety reasons.

Following administration of 9-THC, Harvey and co-workers were able to identify 9-THC-acid in rabbit liver [69] and guinea pig liver [81] by GC–EI-MS of the methyl ester–trimethylsilyl ether derivative. The metabolism of non-natural (3*S*,4*S*)-9-THC was studied in the mouse, and (3*S*,4*S*)-9-THC-acid was identified in the liver [80].

Foltz and Hidy [66] described a procedure for detecting 9-THC-acid in plasma utilizing ammonia–hydrogen PICI. Extracts were treated with diazomethane followed by BSA. Ammonia–hydrogen reagent gases reportedly gave better quality mass spectra than ammonia–helium. This procedure was briefly described in a previous publication [1].

Norqvist *et al.* [98] analyzed 9-THC-acid extracts from plasma and urine by GC–EI-MS following treatment with diazomethane and BSA. When the silylation step was omitted, the methyl ester derivative was found to partly decompose on-column.

The formation of pentafluorobenzyl ester–trimethylsilyl ether and trimethylsilyl ester–pentafluorobenzyl ether derivatives of 9-THC-acid was described by Rosenfeld *et al.* [57]. 9-THC-acid extracted from urine onto XAD-2 resin was treated with pentafluorobenzyl bromide. Interfering compounds were removed by semi-preparative chromatography prior to reaction with BSTFA. The resulting isolate was substantially free from interferences normally observed in urine extracts. GC EI-MS was used to obtain mass spectra of the 9-THC-acid derivatives.

4.3. Ionization techniques

EI remains the most widely employed GC–MS ionization technique. The relatively low cost and small size of quadrapole mass spectrometers account for their popularity in laboratories engaged in drug testing. EI of 9-THC-acid derivatives typically results in three or more characteristic fragment ions with detection limits falling in the low nanogram per milliliter range.

A few researchers have investigated PICI or NICI as an alternative. Chemical ionization has the advantage of providing a choice of reagent gases to optimize assay capabilities. Commonly used gases such as ammonia, methane and isobutane, however, do not always produce a sufficient number of ions for conclusive identification. NICI is generally capable of providing lower detection limits than either PICI or EI, but biological extract noise levels are often the limiting factor.

No reports of other ionization processes were found.

4.4. Internal standards

Both qualitative and quantitative analyses benefit from the incorporation of internal standards into analytical procedures. The ability of an internal standard to compensate for incomplete derivatization, analyte losses due to decomposition and adsorption, as well as losses occurring during extraction and evaporation facilitates very precise quantitative measurements. Furthermore, recovery of an internal standard verifies method validity with every sample analyzed: failure to detect analyte in the presence of internal standard reduces the risk of a false negative result. To be effective, however, an internal standard must be chemically similar to the analyte. A GC–MS false negative for 9-THC-acid caused by a faulty internal standard was reported by Brunk [30].

Use of an isotopically labeled analyte as its own internal standard is often possible when employing MS detection. Selected-ion monitoring is practical even if only a few internal standard fragment ions incorporate the isotope label. GC–EI-MS full-scan analysis is rarely possible unless the analyte and internal standard are chromatographically separable.

In recent years the most popular internal standard for the GC–MS analysis of 9-THC-acid has been the [5′-$^2H_3$]-9-THC-acid. This compound co-elutes with 9-THC-acid and, therefore, requires analysis by selected-ion monitoring. As the methyl ether–methyl ester derivative this internal standard has the minor drawback of having an ion common to the analyte which limits the linear range of the assay and can affect measurement of relative ion abundances [42,46,99]. The same effect was observed with the pentafluoropropyl–pentafluoropropionyl derivative [88]. Podkowik *et al.* [45] found a ritodrine metabolite and 8-hydroxy-3′,4′,5′-tris-nor-Δ^9-tetrahydrocannabinol-2′-oic acid interfered with use of [$^2H_3$]-9-THC-acid as an internal standard [100]. A hexadeuterated 9-THC-acid in which the deuterium atoms are located in the two methyl groups bonded to the 6-position has been reported by ElSohly *et al.* [101] as an improved internal standard. This labeling reportedly avoids the problems associated with the trideuterated material. Not yet available is a nonadeuterated 9-THC-acid Radian Corporation has listed in its catalogue as "under delopment". One report of trideuterated 9-THC-acid with the deuterium atoms located in one methyl group bonded to the 6-position may be erroneous [44].

Use of [$^2H_3$]-11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid (8-THC-acid-d_3) as an internal standard for 9-THC-acid was noted in three reports [53,98,102]. Wall *et al.* [53] found no difference in precision comparing 8-THC-acid-5′-d_3 and 9-THC-acid-5′-d_3 as internal standards. ElSohly [99] reported 8-THC-acid-d_6 (the deuterium atoms are located in the two methyl groups bonded to the 6-position) as a viable internal standard for the analysis of the methyl ester–methyl ether and the bis(trimethylsilyl) derivatives of 9-THC-acid. None of the major ions are common to both the internal standard and the analyte. In addition, because this deuterated Δ^8 analog is separable from 9-THC-acid by GC it is suitable for full-scan analysis of 9-THC-acid. Use of this material has been reported by others [34,39,47,59]. The major concern in using a 8-THC-acid as an internal standard is the potential conversion of 9-THC-acid to 8-THC-acid (or even 8-THC-acid to 9-THC-acid). Although no problems caused by isomerization were reported in any of the GC–MS procedures reviewed, readers are advised that conflicting reports exist in the literature regarding the facile conversion of 9-THC to 8-THC [14,17,18,103–106].

A number of publications have reported use of non-isotopically labeled compounds as internal standards for 9-THC-acid including pyrahexyl [20,58], hexahydrocannabinol [58], cannabinol [31,85], 1-pyrenebutyric acid [30], ketoprofen [64], 8-THC-acid [65] and meclofamic acid [41]. Oxyphenbutazone was deemed inadequate as an internal standard [31]. Except 8-THC-acid, none of these compounds possess both carboxylic acid and phenol functionalities and are unsuitable internal standards for 9-THC-acid.

Synthesis of 9-THC-acid homologues in which the pentyl side-chain is altered offers a potential

source of novel internal standards for 9-THC-acid. Although this idea has not been explored for 9-THC-acid, McCallum and co-workers [107,108] have previously used heptyl-Δ^9-tetrahydrocannabinol as an internal standard for pentyl-Δ^9-tetrahydrocannabinol. Straight-chain homologues shorter than pentyl, especially propyl, are known to occur naturally in *Cannabis* and would not be good choices as internal standards.

Recently, [$^2H_3$]-9-THC-acid-glucuronide has become commercially available as an internal standard for 9-THC-acid-glucuronide. This deuterated material available from Alltech–Applied Science corrects for incomplete conversion of the glucuronide metabolite to free 9-THC-acid during hydrolysis. Since 9-THC-acid exists in both free and conjugated forms in biological specimens, however, accurate quantitation of conjugated or total 9-THC-acid from hydrolyzed samples requires use of a differently labeled second internal standard, *e.g.*, [$^2H_6$]-9-THC-acid.

4.5. Reference standards

GC–MS identification and quantitation of an unknown is best accomplished by comparison of mass spectra and GC retention with a pure chemical reference material. 9-THC-acid is commercially available from a number of suppliers in the United States: Research Triangle Institute (Research Triangle Park, NC, USA); The National Institute of Standards and Technology (Gaithersburg, MD, USA); Sigma (St. Louis, MO, USA); Alltech-Applied Science (State College, PA, USA); and Radian Corporation (Austin, TX, USA). It is also available to researchers through the National Institute on Drug Abuse (Rockville, MD, USA). The only known manufacturer of 9-THC-acid, however, is Research Triangle Institute. Nonetheless, the occurrence of biased results traceable to a faulty 9-THC-acid standard was noted in a publication by Peat [109].

Deuterated 9-THC-acid suitable as internal standards are available from Research Triangle Institute, Sigma, Alltech–Applied Science, Radian and ElSohly Labs. (Oxford, MS, USA). Previous analytical work employing 9-THC-acid-glucuronide has required the researchers, themselves, to synthesize the material [22,110]. Only recently has Alltech–Applied Science introduced this metabolite commercially.

4.6. Automation

Sample preparation for GC–MS analysis is a labor-intensive operation. The Prep I automated sample processor, an early semi-automated solid-phase extraction system manufactured by Du Pont, is still used by a number of laboratories for analysis of 9-THC-acid. A description of the Prep system was published by Williams [111]. This processor is no longer being manufactured, however.

A comparison of manual and automated solid-phase extraction of 9-THC-acid from urine was reported by Dino *et al.* [112]. The automated system gave results comparable to manual extraction, but was ten times slower.

The only totally automated sample preparation system on the commercial market is manufactured by Zymark. This robotics system is capable of performing weighing, hydrolysis, extraction, evaporation and derivatization for both urine and blood samples. The purchase cost and limited speed of robotics devices need to be weighed against the benefit in labor savings.

5. CONCLUSIONS

The ability of GC–MS to provide positive identification at trace levels is especially challenging in biological matrices. Most of the methods reviewed are capable of detecting 9-THC-acid in urine and blood in the low nanogram per milliliter range. Detection in the picogram per milliliter range is feasible using NICI-MS. Improvements in specimen clean-up would enhance the methods already developed. Alternatively, use of more sophisticated instrumentation such as two-dimensional GC or tandem MS is possible. Detection of 9-THC-acid by metastable ion monitoring has also been suggested [2]. Reference material is available from a number of commercial suppliers

including Research Triangle Institute, the manufacturer. For GC–EI-MS employing selected-ion monitoring [$^2H_6$]-9-THC-acid is recommended as an internal standard.

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