ASSAYS FOR CANNABINOIDS

R. E. Willette, Ph.D.*

SYNOPSIS

Marijuana use pervades every facet of our daily life. However, despite the growing awareness and mounting fear that marijuana is having a serious impact on driving performance and highway safety, routine analysis for the detection of marijuana use is minimal. Part of this reluctance is due to a lack of understanding about the assays, what the assays can and can not do, and what the results mean. Fortunately, intensive research has been conducted over the past 10 years on the metabolism, disposition, and pharmacokinetics of marijuana, and a variety of analytical methods have been developed to detect and measure cannabinoids and/or their metabolites in blood, urine, and other biological materials. In this paper I review these analytical methods and recent studies designed to evaluate their performance, the suitability of cutoff levels, and the compatibility of screening methods and confirmation procedures. Further, I discuss the interpretation of results, the question of intoxication, passive inhalation, and oral ingestion, and describe a scheme used in the workplace, with inexpensive urine tests to select blood specimens.

METABOLISM AND EXCRETION OF CANNABINOIDS

Numerous studies over the past 15 years have demonstrated that the primary psychoactive component in the Cannabis plant and its various preparations is delta-9-tetrahydrocannabinol (THC). Also, these studies have well characterized the absorption, disposition, and metabolism of THC within the body, and its excretion pathways. Although THC is converted into over 30 known metabolites, consisting of a variety of mono- and dihydroxy-THC's and carboxylic acids, analytical efforts have been directed at 2-primary metabolites: 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-9-acid).

Once in the body, THC is very rapidly hydroxylated to the psychoactive 11-OH-THC, which in turn is rapidly oxidized to the inactive THC-9-acid. Appreciable levels of

*Director, Duo Research, Division of Research Designs, Inc., 2 Acton Place, Annapolis, Maryland 21401, U.S.A.
11-OH-THC can be found in blood only following oral consumption of THC. Higher levels are found in feces and in tissues. The THC-9-acid and its glucuronide ester are present in blood and urine in high concentrations, making it the target of most urine assays. THC itself can only be detected in blood and tissue. (Note that nearly two-thirds of the THC taken is excreted unchanged and in the form of its various metabolites in the feces.)

**ANALYTICAL METHODS**

Reviews on the development of analytical methods for the detection and quantification of cannabinoids are available (Foltz, 1984; Foltz et al., 1980; Hawks, 1982; Peat, 1984; Willette, 1976). Methods in common use for urine include immunoassays (EMIT and radioimmunoassays, RIA's; Peat 1984); gas chromatography using various detectors (FID: Jones & ElSohly, 1983; Whiting & Manders, 1982; ECD: ElSohly et al., 1984; MS: Foltz, 1984); high performance or micro-thin layer chromatography; high pressure liquid chromatography (HPLC: ElSohly et al., 1983); and the combination of HPLC and RIA (Law et al., 1984 a). The last named method is especially useful for forensic purposes because it gives a comprehensive profile of the various immunoassays reactive cannabinoids in urine. It is also amenable to blood specimens. Other assays for blood are radioimmunoassays (Peat, 1984) and gas chromatography/mass spectroscopy (GS/MS; Foltz, 1984).

Two of the commercial immunoassays for urine are based on antibodies generated to recognize THC-9-acid and its glucuronide ester preferentially (EMIT and Abuscreen RIA), although these assays also crossreact to various degrees with other THC metabolites. Another commercial assay is directed towards the free acid (Immunalysis). This has often given rise to apparent discrepancies in the semiquantitative concentration of cannabinoids measured by the immunoassays and that of the primary metabolite, THC-9-acid, as measured by one of the chromatographic methods. Various studies have shown that THC-9-acid can account from 5 to 60% of the total urinary metabolites, with an average of about 27% (Halldin et al., 1984). THC-9-acid is excreted into urine almost exclusively as its glucuronide, which is relatively unstable and hydrolyzes upon storage even under refrigeration (Law et al., 1984). Nevertheless, unless the HPLC/RIA method is used, it is essential to hydrolyze urine specimens before chromatography analysis. The preferred method is to treat the specimen with aqueous sodium or potassium hydroxide and heat at 50 to 60°C for 15 minutes or for an hour at room temperature.
The limits of detection or "cutoffs" for cannabinoids in urine varies significantly with each method. It is important to distinguish between properly measured detection limits, that is, the lowest concentration at which the analyte can be distinguished from true negatives with 99.87% confidence (IUPAC definition), and cutoffs that are assigned at some level above the detection limit for a variety of reasons. EMIT is available in 2 instrumental configurations, the portable EMIT-st, which uses a 100 ng/ml cutoff, and the laboratory EMIT-d.a.u., which may use either a 20 or 100 ng/ml cutoff standard. The Abuscreen RIA comes with a 100 ng/ml standard, but may be calibrated down to 25 ng/ml if desired. The Immunalysis RIA claims a useful sensitivity down to 2 ng/ml. Note that all of the immunoassays express concentrations of total crossreacting metabolites in equivalent units of delta-9-THC-9-acid, although the delta-8 acid is used in the calibration standard. This is because the delta-8-acid is considerably less expensive to synthesize and is more stable, thus giving standard solutions a longer shelf life.

Extreme care must be taken in preparing standard solutions of the natural delta-9-THC-9-acid, especially in urine. The acid is relatively insoluble and may precipitate out of solution upon standing or freezing. Care must also be exercised in sampling specimens or standard solutions containing THC-9-acid, since the precipitated acid may cling to the walls of the container or be suspended in any foam that may form if the specimen is shaken. Sonication followed by immediate withdrawal of a sample may help to minimize this problem.

The various chromatographic assays have detection limits for THC-9-acid ranging from 0.1 ng/ml by the negative ion chemical ionization method (Foltz, 1984) to about 20 ng/ml by HPTLC. Most gas chromatographic methods are capable of measuring to 5 ng/ml, although it is common practice to set a cutoff of 10 to 20 ng/ml to eliminate the possibly of identifying passive inhalation levels and to minimize the chance of error.

THC may be determined in blood, plasma, or serum by means of a commercial RIA (Immunalysis), which has a range of 2 to 20 ng/ml, or in research settings by a RIA supplied by the National Institute on Drug Abuse through the Research Triangle Institute. GC/MS methods are used most frequently (Foltz, 1984), with sensitivities for THC down to 0.2 ng/ml. GC/MS methods are also available for 11-OH-THC and THC-9-acid in blood. The combined HPLC/RIA method (Law et al., 1984 a) may also be used to give a complete profile of cannabinoids in blood.
An evaluation of the EMIT and RIA methods and confirmation by means of GC/FID and GC/MS assays has been published (Irving et al., 1984).

**INTERPRETATION OF CANNABINOID ASSAY RESULTS**

Assuming that the collection, handling, and analysis of the specimen in question were properly conducted, we may conclude that a positive finding of THC and/or its metabolites in a biological specimen is proof that THC had somehow entered the person's body. The determination of the levels in themselves will usually not permit an interpretation as to the route of administration. After the first few hours following smoking or oral ingestion of THC, the distribution and elimination of THC and its metabolites is identical. It is only during the first 4 hours or so that blood levels of 11-OH-THC will exceed those of THC after oral intake of THC. Following smoking of marijuana, THC levels rise almost instantly to their peak level and then fall off quite rapidly, dropping below 10 ng/ml usually within 2 hours (Figure 2). At the same time, THC-9-acid levels are rising. There is interest in evaluating the ratio of metabolites to THC in blood as a possible indication of recency of use (Law et al., 1984 b).

The question then often is: what does this level mean? Studies have been conducted correlating the quantitative measurement of THC in a blood specimen, as with alcohol, with performance decrements (Barnett et al., in press). These performance measures were on laboratory tests, although a number of studies have been performed in cars or driving and flying simulators (Reeve et al., this volume). There is far less information available on the possible causative role that marijuana may play in traffic or work-related accidents, but studies have been directed towards gathering this type of information (Owens et al., 1983; Zimmerman et al., 1983).

Many forensic experts have agreed that for marijuana use a concentration exceeding 5 ng/ml of THC in blood or 10 ng/ml in plasma or serum is consistent with being intoxicated. Certainly other factors must be considered, such as eye witness accounts of behavior, to help provide conclusive evidence of intoxication. However, the concentrations in themselves may serve as useful indicators or predictors.
Urinalysis is the most frequent form of drug testing. What do urinary concentrations mean? Controlled experiments and measurements of urinary levels from clinical or other test situations have provided information that can help to assign a certain relevance to urine levels. For example, administration of known amounts of THC to volunteers has shown that certain urinary concentrations of THC metabolites are exceeded for limited periods of time. After smoking a low-to-moderate dose of marijuana, concentrations exceeding 200 ng/ml of total urinary metabolites of THC (as is measured by the immunoassays) or 100 ng/ml of THC-9-acid are usually seen for less than 8 to 12 hours. On the other hand, frequent use of larger amounts of marijuana may produce urinary concentrations exceeding these levels for several days. Regardless of which smoking condition is involved, such high urinary concentrations are consistent with known impairing effects of marijuana. The higher the concentration, the higher the probability that impairing levels of THC remain in the body. However, with our present state of knowledge, such levels can not be used as direct evidence of intoxication, but may serve as presumptive indicators.

An economical testing scenario that can be used if intoxication must be proven is to collect both blood and urine. Assay the urine for the presence of cannabinoids, which can be quickly accomplished with one of the immunoassays. If the urine level is sufficiently high to indicate the possibility of intoxication (e.g., over 100 ng/ml of THC-9-acid or 200 ng/ml of total metabolites) then have the blood assayed for THC. This often provides sufficient information to diagnose with varying degrees of certainty the existence and level of intoxication. The presence of 2 drugs, such as alcohol and THC, which are known to have additive effects, may complicate the interpretation, but at least the additional information may help explain observed behavior.

Concern has been expressed about the possible "passive inhalation" of marijuana smoke to an extent that might cause a positive finding upon blood or urine testing. Published studies have demonstrated, however, that at reasonable exposure levels (e.g., 2 to 4 marijuana cigarettes in a small room or automobile for 1 to 2 hours) produced only 2 urine specimens that were found positive using the EMIT assay with the 20 ng/ml cutoff (Pérez-Reyes et al., 1983). Semiquantitation of these 2 specimens indicated about 30 ng/ml of total crossreacting metabolites. Confirmation of one of them by GC/MS gave 3.9 ng/ml of THC-9-acid. Reports
of more intense levels of exposure gave proportionately higher urinary concentrations, as would be expected. However, no valid report has indicated a concentration exceeding 75 ng/ml of total urinary metabolites.

Recent studies have demonstrated that the oral consumption of THC, in the form of hashish, can give rise to urinary concentrations of total metabolites exceeding 100 ng/ml for up to 4 days (20-mg dose) or for less than 1 day following a sub-pharmacological dose (5 mg; Law et al., 1984 b). Although it has been recognized that it takes larger amounts of THC by the oral route to produce similar pharmacological effects as that produced by smoking, these results clearly indicate that it is possible for someone to innocently ingest THC and produce a positive urine test for cannabinoids. Such defenses are already being offered and will require careful examination of the lapses of time involved between the purported exposure and specimen collection.

REFERENCES


Figure 1. Structures of delta-9-tetrahydrocannabinol and its major metabolites.

Figure 2. Typical plasma concentrations of THC vs time after smoking approximately 16 mg of THC in a marijuana cigarette.